Trace Environmental Quantitative Analysis

Principles, Techniques, and Applications

Paul R. Loconto

© 2006 by Taylor & Francis Group, LLC
Dedication

To my five points of light. Each added a new dimension to my life.

Jennifer Ann
Michelle Ann
Allison Marie
Julia Marie
Elizabeth Marie

In memory of Taylor Renee Hamel
whose light was extinguished much too early.
Preface to the Second Edition

The rapid pace in which trace analysis is changing has warranted the writing of a second edition in a relatively short period. What is new? The second edition attempts to move the reader from the most elementary of principles of trace environmental quantitative analysis (TEQA) to those techniques and applications currently being practiced in analytical laboratories dedicated to trace environmental chemical and trace environmental health quantitative analysis while adding new significant topics.

The increasing importance of mass spectrometry will become apparent to the reader primarily as a low-resolution hyphenated technique. The principles that underlie GC-MS, GC-MS-MS, LC-MS, and ICP-MS can now be found in Chapter 4 on determinative techniques.

Chapter 3 on sample preparation techniques has been enlarged so that even more alternatives to liquid–liquid extraction are introduced. Column chromatographic cleanup, virtually ignored earlier, and gel permeation chromatography have been introduced along with additional applications to biological sample matrices of environmental health and toxicological interest. Matrix solid-phase dispersion as applied to the isolation and recovery of persistent organic pollutants from fish tissue has been added. The prolific growth of SPME as evident in the analytical literature over the past 5 years has warranted an enlarged section on this technique.

More than two dozen new topics not previously discussed in the original book have been added to the second edition.

Any author, upon reviewing the finished product of a first book, has a most immediate desire to rewrite all of it. I have resisted this temptation and have modified only those sections of the original book that I felt enlarge and enhance the environmental analytical chemist’s understanding of TEQA.

Who should read the second edition? Scientists, in addition to analytical chemists, such as organic chemists, biochemists, molecular biologists, geologists, toxicologists, epidemiologists, food scientists, and chemical and environmental engineers will find that the second edition might enhance their understanding of TEQA. Laboratory technicians of various skill and knowledge levels should also find the content of this edition beneficial.

The style for the second edition has remained the same. Section headings continue to be cast in the form of a question. New terms have been italicized when they appear for the first time. Beneath each chapter title is a brief “Chapter at a Glance” so that the reader can more quickly find topics of immediate interest. Figures and tables are both separated and numbered in sequence and integrated in the text without numbering, as before. Digressions from the main topics have also occurred in a manner similar to that in the original book. Graphs are either sketches that I drew to illustrate a principle or carefully drawn from experimental data (I’m a pretty good chemist; an artist, I am not). To reiterate from the preface to the original book,
I have tried very hard to make this text readable, interesting, and relevant, and at the same time, introduce sound principles and practices of TEQA.

I express my gratitude to the Division of Chemistry and Toxicology, Bureau of Laboratories, Michigan Department of Community Health; the Michigan Public Health Institute; and the Biomonitoring Planning Grant, National Center for Environmental Health, Centers for Disease Control and Prevention. These institutions and grants enabled me to find the time to write, edit, and rewrite. Barbara Mathieu and colleagues at Taylor & Francis have painstakingly, for a second time, turned this author’s rough draft into a book. My wife, Priscilla, has graciously endured her husband’s passion for writing. And my motivation to write is rooted in and summarized by the ancient Chinese Proverb:

\[ I \text{ hear and forget; I see and I remember; I write and I understand.}\]
About the Author

Paul R. Loconto is currently a laboratory scientist specialist with the Michigan Department of Community Health, Bureau of Laboratories, Lansing. Dr. Loconto is the author of 24 peer-reviewed publications and 33 oral and poster presentations in trace analysis and chemical education. He combines various work experiences that include teaching at a community college, managing an environmental engineering research laboratory within a large university, and conducting analytical method development for an independent environmental testing laboratory. All have given the author many unique insights over the years into the principles, techniques, and applications of TEQA.
# Table of Contents

1. Introduction to Trace Environmental Quantitative Analysis (TEQA) 1

2. Calibration, Verification, Statistical Treatment of Analytical Data, Detection Limits, and Quality Assurance/Quality Control 37

3. Sample Preparation Techniques to Isolate and Recover Organics and Inorganics 119

4. Determinative Techniques to Measure Organics and Inorganics 323

5. Specific Laboratory Experiments 547

*Appendix A:* Glossary 651

*Appendix B:* QA/QC Illustrated 679

*Appendix C:* TEQA Applied to Drinking Water/Computer Programs for TEQA 689

*Appendix D:* Instrument Designs 705

*Appendix E:* Useful Internet Links for Environmental Analytical Chemists 711

*Appendix F:* Useful Potpourri for Environmental Analytical Chemists 717
APPENDIX B
QA/QC Illustrated

Read not to contradict and confute, nor to believe and take for granted, nor to talk or
discourse, but to weigh and consider.

—Francis Bacon

Trace environmental quantitative analysis (TEQA) requires that a specific QA/QC
document be written and available. This appendix further elaborates on QA/QC as
first introduced in Chapter 2 and first written by the author in support of the National
Institute of Environmental Health Sciences (NIEHS) Basic Superfund Research
Center Analytical Core.

1. WHAT DOES A SPECIFIC QC PLAN LOOK LIKE?

Many of the concepts introduced in Chapter 2 will now be applied in the example
that follows. The standard operating procedure (SOP) and QC document that follow
provide guidelines for the quantitative determination of trace concentrations of either
organochlorine or polychlorinated biphenyl targeted analytes that were routinely
performed in the past in the author’s laboratory. The sample matrix was composed
of rat plasma, and the sample preparation method used consisted of ultrasonic probe
sonification (PS) using acetonitrile, followed by solid-phase extraction in the
reversed-phase mode (RP-SPE) using either an octyl (C₈) chemically bonded silica
or an octadecyl (C₁₈) chemically bonded silica. The analytes were retained on the
RP silica, and the RP silica was dried and then eluted using pesticide residue-grade
iso-octane. After adjustment to a precise 1-mL eluent volume, 1 µL of eluent was
injected into a previously calibrated gas chromatograph.

2. HOW IS A QA/QC PLAN ORGANIZED?

A good QA/QC document tells the client exactly how the analysis will be conducted
and how the analytical data produced by execution of the procedures will be inter-
preted. The document illustrated here is presented in the following sequence:
2.1 **Title:** TRACE ORGANOCHLORINE PESTICIDE (OCs)/POLYCHLORINATED BIPHENYLS (PCBs) Analysis via PS Coupled to RP-SPE Using Capillary Gas Chromatography with Electron-Capture Detection (C-GC-ECD): SOP and QC Protocols

2.2 **Summary of Method**

Samples of biological origin, such as plasma, serum, organ parts, and so forth, that arrive in the laboratory are immediately frozen or refrigerated as recommended by the client. Prior to beginning the analysis, the samples are thawed and brought to ambient temperature. The primary technique used in our laboratory to isolate and recover the target QC or PCB analytes from the biological matrix is ultrasonic probe sonication combined with reversed-phase solid-phase extraction (PS-RP-SPE). A 10-cartridge vacuum manifold is used. This enables up to 10 SPE extractions to be performed simultaneously. In addition to the number of samples that are to be analyzed, one sample must be selected, and two identical aliquots of this sample are needed. The first additional sample is needed to spike (matrix spike), and the second additional sample, identical to the first, is needed to spike again (matrix spike duplicate). If these additional sample matrices are not available for conducting recovery studies, matrix spikes will not be included.

2.3 **Percent Recovery Study**

A percent recovery study will be conducted. A surrogate reference standard consists of a methanolic 2 ppm solution that contains tetrachloro-\textit{m}-xylene (TCMX) and decachlorobiphenyl (DCBP). Surrogates are added to all samples, whereas matrix spikes are added to only selected samples. A matrix spike reference standard consists of a methanolic 24 ppm solution that contains PCB congeners or specific Aroclors. A control sample is prepared by taking the same aliquot of surrogate and matrix spike and placing this in the 1-mL volumetric flask and adjusting the final volume to 1.0 mL. A series of SPE cartridges used to conduct this study are used. In conjunction with a 10-cartridge vacuum manifold (e.g., Vacmaster, International Sorbent Technology, Ltd.), a method development study is listed according to the following table:
A 1-mL eluent is obtained from PS-RP-SPE that is quantitatively transferred to a clean, dry 2-mL GC autosampler glass vial with a crimped-top cap. Percent recoveries are routinely found to be between 75 and 100% for most OCs and PCBs from a biological matrix used to conduct toxicological studies. The appropriate GC method is retrieved from the chromatography software that controls and acquires data from the GC (Turbochrom®, PE-Nelson). The order in which the vials are injected into the G-GC-ECD is called a sequence. A sequence file is created using the Turbochrom chromatography processing software (PE-Nelson). Several QC protocols are used to consider the order in which sample eluents from PS-RP-SPE are injected into a C-GC-ECD. Blanks are run before and after standards. Usually, injection of a standard that has a high concentration of analyte is followed by injection of a blank to minimize carryover. A representative sample sequence is given in the following table:

<table>
<thead>
<tr>
<th>Injection Vial No.</th>
<th>Description of GC Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank (pure iso-octane)</td>
</tr>
<tr>
<td>2</td>
<td>Control (100% recovery)</td>
</tr>
<tr>
<td>3</td>
<td>Blank (pure iso-octane)</td>
</tr>
<tr>
<td>4</td>
<td>SPE method blank</td>
</tr>
<tr>
<td>5</td>
<td>SPE blank spike 1</td>
</tr>
<tr>
<td>6</td>
<td>SPE matrix spike</td>
</tr>
<tr>
<td>7</td>
<td>Blank (pure iso-octane)</td>
</tr>
<tr>
<td>8</td>
<td>SPE sample unspiked 1</td>
</tr>
</tbody>
</table>

Injection vials 1 to 8 represent the first cycle in a series of several cycles in which a vial containing pure iso-octane is first injected to estimate whether there is any carryover from previous injections. Then, a control vial is injected that contains the analyte of interest and represents a 100% recovery. Pure iso-octane is then
injected a second time in this cycle to prevent carryover from the high concentration of analyte in the control. A method blank is then injected that demonstrates that the method itself is free from laboratory and analyst contamination. This is followed by an injection of the blank spike eluent in order to assess the percent recovery of analyte from a relatively clean matrix. This is followed by an injection of the matrix spike eluent in order to assess the percent recovery of analyte from the sample matrix itself. A matrix effect can then be evaluated from these two spiked samples. To prevent carryover again, pure iso-octane is injected. The sample itself, unspiked, is then injected to provide sample analysis. A second cycle is undertaken in a manner similar to that just introduced for the first cycle. If there are sufficient samples, a third, fourth, and even a fifth cycle are also introduced.

2.3.1 Calculation of Analyte Percent Recovery

The integrated peak areas for all targeted analytes over replicate injections of the control and spikes are then averaged and used to calculate a mean percent recovery according to

\[
\%R_i = \frac{\sum_{i} A_i^s / N}{\sum_{i} A_i^c / n} \times 100
\]

where \(A_i^s\) represents the integrated peak area for the \(i\)th component in the spiked sample or spiked blank, \(A_i^c\) represents the integrated peak area for the \(i\)th component in the control sample, \(N\) represents the number of replicate spiked samples or blanks, and \(n\) represents the number of replicate control injections into the GC.

2.4 Calibration, Verification, and Quantitative Analysis

2.4.1 Preparation of the Stock Solution

Weigh 0.0500 g (does not have to be exact, record in notebook) of each OC, PCB congener, or Aroclor. Place the solid or liquid into a 10-mL volumetric flask half filled with methanol (MeOH, use highest purity available). Adjust to a final volume with the bottom of the meniscus horizontally aligned with the calibration mark. This yields a 5000 ppm stock solution. Transfer to a clean, dry glass vial with a Teflon septum and screw cap. Label the vial clearly as “5000 ppm … (MeOH), date.” Store at refrigerated temperatures.

2.4.2 Preparation of the Primary Dilution

Place 100 µL of stock solution into a clean, dry 10-mL volumetric flask that is half filled with MeOH. Adjust to final volume with MeOH, transfer to a storage vial, and label “50 ppm …”
2.4.3 Preparation of the Secondary Dilution

Place 100 µL of secondary dilution into a clean, dry 10-mL volumetric flask that is half filled with MeOH. Adjust to final volume with MeOH, transfer to a storage vial, and label “500 ppb …”

2.4.4 Preparation of the Tertiary Dilution

Place 100 µL of secondary dilution into a clean, dry 10-mL volumetric flask that is half filled with MeOH. Adjust to final volume with MeOH, transfer to a storage vial, and label “5 ppb …”

2.4.5 Preparation of the Working Calibration Standards

Use the following table to prepare a series of working calibration standards using MeOH as the dilution solvent. Transfer the standards to a 2-mL GC vial, cap, and either place on autosampler or store for future use.

<table>
<thead>
<tr>
<th>Standard</th>
<th>5 ppb (µL)</th>
<th>V (total)</th>
<th>Concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>1.0</td>
<td>0.025</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1.0</td>
<td>0.050</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>1.0</td>
<td>0.250</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>1.0</td>
<td>0.500</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>1.0</td>
<td>1.250</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>1.0</td>
<td>2.500</td>
</tr>
</tbody>
</table>

A multipoint calibration curve using the ES mode is established for each OC and PCB congener or total Aroclor using either the secondary or tertiary diluted reference standard (depending on the range of concentration desired). The working calibration standards are prepared originally from either a chemically pure form of the OC or PCB or a commercially available and certified solution. A least square regression analysis is performed using the available software or via in-house computer programs, and the quality of the least squares fit is evaluated in terms of its correlation coefficient and standard deviations in both the slope and intercept. An initial calibration verification (ICV) standard is prepared and injected in triplicate to evaluate the precision and accuracy of the least squares regression. The confidence interval for the ICV is then established. Peak areas for the separated and detected OCs, PCB congeners, or total Aroclors are converted to their corresponding concentration levels expressed in either ppb or ppm of each analyte or as total Aroclor. A confidence interval about the interpolated concentration levels is also obtained. For example, the 3,4,3′,4′-tetrachlorobiphenyl congener present in a sample of rat plasma is reported as 76 ± 5 ppb at the 95% significance level.
2.5 Establishment of the Instrument Detection Limit

The IDL is defined as the minimum concentration or weight of analyte that can be detected at a known confidence level. This limit depends on the ratio of the magnitude of the analytical signal to the size of the statistical fluctuations in the blank signal.

The IDL is then defined as the concentration that corresponds to the ratio of the difference between the minimal distinguishable analytical signal and the mean signal in a blank to the slope of the least squares regression line (calibration sensitivity). Because this minimum distinguishable signal can be defined as being equal to the sum of the mean signal from a blank, \( S_{\text{blank}} \), and 3 or 10 times the standard deviation in the blank signal, \( \text{std dev}_{\text{blank}} \), two definitions must be used: the minimum detectable limit \( C_{\text{IDL}} \) and the limit of quantitation \( C_{\text{LOQ}} \). Expressed mathematically,

\[
S_{\text{IDL}} = \bar{S}_{\text{blank}} + 3s_{\text{blank}}
\]

\[
C_{\text{IDL}} = \frac{S_{\text{IDL}} - S_{\text{blank}}}{\text{Slope}}
\]

\[
S_{\text{LOQ}} = \bar{S}_{\text{blank}} + 10s_{\text{blank}}
\]

\[
C_{\text{LOQ}} = \frac{S_{\text{LOQ}} - S_{\text{blank}}}{\text{Slope}}
\]

Hence, \( C_{\text{IDL}} \) and \( C_{\text{LOQ}} \) yield two levels that must be considered when defining detection limits. Because the sample preparation technique involves the isolation and recovery of analytes into an extract from the original sample, a concentration factor, as well as a consideration of the efficiency of the extraction process as defined by the percent recovery, is involved. Thus, the method detection limit (MDL) will be lower than the IDL.

2.6 Establishment of the MDL

The MDL can be found only after the IDL, the concentration factor \( (F) \), and the percent recovery \( R \) (expressed as its decimal equivalent) are determined for the method. \( F \) is the ratio of sample volume, \( V_s \), to extract volume, \( V_{\text{eluent}} \). The volume of rat plasma, \( V_s \), is usually 1 or 2 mL, whereas \( V_{\text{eluent}} \) is 1.0 mL. Expressed mathematically,

\[
C_{\text{MDL}} = \frac{C_{\text{IDL}}F}{R}
\]

where

\[
F = \frac{V_{\text{eluent}}}{V_s}
\]
2.7 **Definitions of QC Reference Standards**

*Calibration or working standards* — A series of diluted standards prepared from dilutions made from the tertiary dilution standard. These standards are injected directly into the C-GC-ECD and used to calibrate the instrument as well as evaluate the range of ECD linearity. The range of concentrations should cover the anticipated concentration of targeted analytes in the unknown samples.

*Control standard* — Consists of the same representative set of targeted analytes used in the matrix spike, and the same precise aliquot of this solution is added to an aliquot of the elution solvent used in PS-RP-SPE and is used in the calculation of percent recoveries.

*Instrument calibration verification (ICV) standard* — Prepared in a manner similar to that of the working calibration standards, however at a different concentration than any of the working standards. Used to evaluate the precision and accuracy for an interpolation of the least squares regression of the calibration curve.

*Laboratory method blank* — A sample that contains every component used to prepare samples, except for the analyte(s) of interest, via PS-RP-SPE and is taken through the sample preparation process. It is used to evaluate the extent of laboratory contamination for the targeted analytes.

*Matrix spike standard* — Consists of a representative set of the targeted analytes (i.e., OCs or PCBs dissolved in MeOH). A precise aliquot of this solution is added to a sample so that the effect of the sample matrix on the percent recovery can be evaluated.

*Primary dilution standard* — Results from dilution of the stock reference standard.

*Secondary dilution standard* — Results from dilution of the primary dilution standard.

*Stock reference standard* — The highest concentration of target analyte obtained by either dissolving a chemically pure form of the analyte in an appropriate solvent or acquiring it commercially as a certified reference solution.

*Surrogate spiking standard* — Consists of tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCBP) dissolved in MeOH. These two analytes are highly chlorinated and are structurally very similar to OCs and PCB targeted analytes. TCMX elutes prior to and DCBP after the OCs and PCBs, thus eliminating any coelution interferences. A fixed volume (aliquot) of this reference solution is added to every sample, matrix spike, blank, blank spike, and control in the protocol.

*Tertiary dilution standard* — Results from dilution of the secondary dilution standard.
2.8 **QUALITY CONTROL SPECIFICATIONS**

**Minimum Demonstration of Capability**

Before samples are prepared and extracts injected into a GC, the laboratory must demonstrate that the instrumentation is in sound working order and that targeted analytes can be separated and quantitated. GC operating conditions must be established, and reproducibility in the GC retention times, \( t(R) \), for the targeted analytes must be achieved.

**Laboratory Background Contamination**

Before samples are prepared and extracts injected into a GC, the laboratory must demonstrate that the sample preparation bench-top area is essentially free of trace OC and PCB residues. This is accomplished by preparing method blanks using either distilled deionized water or acetonitrile as reagent blank and observing essentially peak-free GC-ECD chromatograms.

**Assessing Targeted and Surrogate Analyte Recovery**

Before samples are prepared and extracts injected into a GC, the laboratory must demonstrate that the surrogate and targeted analytes can be recovered by the method to a reasonable degree. EPA Method 508 suggests that an acceptable range in percent recovery can be found to be \( \pm 30\% \). Typical values for \( R \) range from 65 to 115%.

**Assessing Calibration Curve Linearity**

Multipoint calibration covering one or more orders of magnitude above the 0.1 ppb instrument detection limit (IDL) for C-GC-ECD based on a 1-\( \mu \)L injection volume with a 10:1 split ratio should satisfy a minimum correlation coefficient of 0.9500. Correlation coefficients are calculated within the Turbochrom (PE-Nelson) software or by using a computer program.

**Assessing ICV Precision and Accuracy**

Precision can be evaluated for the triplicate injections of the ICV following establishment of the multipoint calibration by calculating a confidence limit for the interpolated value for the ICV concentration expressed in ppb.

A relative standard deviation expressed as a percent (%RSD) can also be calculated based on peak areas for the \( i \)th component according to

\[
\%\text{RSD}_i = \frac{s_i}{C_i} \times 100
\]

where \( s_i \) is the standard deviation in the interpolated concentration for the \( i \)th targeted component based on a multipoint calibration and triplicate measurements. %RSD is also called the coefficient of variation.
Both the mean concentration and the confidence limits about the mean concentration should be established for the ICV prior to conducting the sample analysis. Accuracy can be evaluated based on a determination of the percent relative error according to

\[
\% \text{ Error} = \left( \frac{\bar{C}_i - C_{i,\text{known}}}{C_{i,\text{known}}} \right) \times 100
\]

Statements of precision and accuracy should be established for the ICV, and as long as subsequent measurements of the ICV remain within the established confidence limits, no new calibration curve need be regenerated.

### 2.9 Reporting Requirements

The client generally dictates what type of reporting format to use. Software for processing raw analytical data into analytical results is usually available on the PC used with the instrument. In the author’s laboratory, a sample-by-sample report can be produced and printed using Turbochrom (PE Nelson). In this sample report, a tabular format shows the peaks found, analyte name, analyte concentration, and analyte retention time, as follows:

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Analyte Name</th>
<th>Concentration (ppb)</th>
<th>(t(R))</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Lindane</td>
<td>50.4</td>
<td>1.84</td>
<td>21,033</td>
</tr>
<tr>
<td>19</td>
<td>Endrin</td>
<td>153.8</td>
<td>4.01</td>
<td>30,163</td>
</tr>
<tr>
<td>24</td>
<td>Methoxychlor</td>
<td>188.1</td>
<td>5.56</td>
<td>17,202</td>
</tr>
</tbody>
</table>

In addition, a summary report is compiled in which the file name for the chromatogram is placed in the first column, the name of the sample analyzed is placed in the second column, and the concentration, retention time, and peak area for the first chromatographically resolved component are placed in the third column:

<table>
<thead>
<tr>
<th>File Name</th>
<th>Sample Name</th>
<th>Concentration (ppb)</th>
<th>(t(R))</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>YA13001</td>
<td>Blank</td>
<td>11.0</td>
<td>3.77</td>
<td>21,245</td>
</tr>
<tr>
<td>YA13003</td>
<td>133 ppb ICV</td>
<td>140.0</td>
<td>3.76</td>
<td>298,906</td>
</tr>
<tr>
<td>YA13009</td>
<td>No. 2</td>
<td>1316.4</td>
<td>3.77</td>
<td>2,832,169</td>
</tr>
</tbody>
</table>
From all the various energies available, is the energy of the creative mind the most valuable and the least to be wasted.

—Wilhelm Ostwald

TEQA utilizes various determinative techniques (Chapter 4) in combination with various sample prep techniques (Chapter 3) that result in analytical data that must be reduced and interpreted (Chapter 2). In this appendix, one specific study performed by the author to quantitatively determine trihalomethanes (THMs) in his drinking water at home is provided. Static headspace sampling automatically coupled to capillary gas chromatography with electron-capture detection was used to generate the analytical data.

Supportive statistical treatment of analytical data not covered in the text is also provided here. The author wrote several computer programs that enable straightforward statistical computation to be accomplished. The reader may find something useful here despite the contemporary proliferation of sophisticated statistical software such as Sigma Plot®. LSQUARES is a computer program developed by the author in GWBASIC. This program was referred to throughout the book and was used to reduce and interpret the THM study discussed earlier. SDMULTI is a program written in GWBASIC that computes a pooled standard deviation for replicate injections over SPEs performed. STAT&REC is a program written in GWBASIC that applies basic statistics to replicate measurement. RSD is a program written in GWBASIC that calculates a simple mean, standard deviation, relative standard deviation, and confidence intervals from replicate measurement. To help facilitate an understanding of SDMULTI, STAT&REC, and RSD, flowcharts are provided.
HOW MANY THMS ARE IN THE AUTHOR’S DRINKING WATER AND DOES THIS RESULT VARY WITH TEMPERATURE?

To illustrate the external standard mode of instrument calibration and subsequent quantitative analysis, a real analysis of the author’s drinking water was undertaken. Samples were obtained at the kitchen faucet and at the refrigerator faucet. Both sampling sites are derived from the same source, municipal drinking water from Meridian Township, Michigan. Both samples were reported to contain all four trihalomethanes. This author was surprised to learn that the concentration of chloroform is higher in the refrigerated drinking water sample. He speculated that the volatile THMs would in some way escape to a greater degree from the warmed water than from the chilled water, choosing to believe that these THMs would tend to remain dissolved to a greater extent in the chilled water. The other three THMs seem to behave as predicted.

The concentration of each THM is normally distributed in the drinking water sample. There exists a population mean concentration for each THM. We can never know this mean concentration, \( \mu \), for each THM from the population; we can, however, by measuring \( L \) replicates of the sample, calculate a mean concentration, \( \bar{x} \), and a standard deviation, \( s \). We really do not know the standard deviation for the population, \( \sigma \). We can make \( L \) replicate measurements and use t statistics to define a confidence interval for the population mean using chloroform as our example:

\[
\text{Conf Int for } \mu_{\text{CHCl}_3} = \bar{x}_{\text{CHCl}_3} \pm \frac{t_{(1-\alpha/2, df)} \cdot s}{\sqrt{L}}
\]

where we use \( t \) such that the probability of making a type I error is \((1 - \alpha/2)\%\) of the time for a number of degrees of freedom, \( df \), where \( df = L - 1 \). The higher the degree of confidence of the mean concentration for chloroform desired, the larger is the value of \( t \) required, and hence the larger the confidence interval.

Calibration data for the four THMs are given below:

<table>
<thead>
<tr>
<th>Concentration of THM (ppb)</th>
<th>PEAKAREA ((\mu)V-sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHCl(_3)</td>
</tr>
<tr>
<td>4</td>
<td>17,235</td>
</tr>
<tr>
<td>8</td>
<td>29,148</td>
</tr>
<tr>
<td>16</td>
<td>53,881</td>
</tr>
<tr>
<td>32</td>
<td>88,720</td>
</tr>
</tbody>
</table>

Upon entering the calibration data above for each of the four THMs, the program titled LSQUARES was used to generate the following statistical parameters. These statistical parameters were used to interpolate the instrument response from the sample and calculate the corresponding concentration, along with a confidence interval at the stated level of significance.

© 2006 by Taylor & Francis Group, LLC
**WHAT IS THE PROGRAM FOR LSQUARES?**

The computer program is listed here for those who would like to use it. The mathematical equations that are programmed into GWBASIC could be easily captured and placed in an EXCEL spreadsheet, and thus bring this program into the more contemporary Windows® environment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHCl₃</th>
<th>CHCl₂Br</th>
<th>CHClBr₂</th>
<th>CHBr₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope (µV-sec/ppb)</td>
<td>2540</td>
<td>10,344</td>
<td>5234</td>
<td>1473</td>
</tr>
<tr>
<td>y intercept (µV-sec)</td>
<td>9143</td>
<td>17,422</td>
<td>12,671</td>
<td>5150</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9960</td>
<td>0.9989</td>
<td>0.9957</td>
<td>0.9931</td>
</tr>
<tr>
<td>Standard deviation in slope</td>
<td>161.7</td>
<td>347</td>
<td>343</td>
<td>123</td>
</tr>
<tr>
<td>Standard deviation in y intercept</td>
<td>2982</td>
<td>6402</td>
<td>6333</td>
<td>2261</td>
</tr>
<tr>
<td>( t(1 - \alpha, df = 3) )</td>
<td>2.353</td>
<td>2.353</td>
<td>2.353</td>
<td>2.353</td>
</tr>
<tr>
<td>( y_c ) (µV-sec)</td>
<td>19,908</td>
<td>40,528</td>
<td>35,528</td>
<td>13,313</td>
</tr>
<tr>
<td>( x_c ) (ppb)</td>
<td>4.2</td>
<td>2.2</td>
<td>4.4</td>
<td>5.5</td>
</tr>
<tr>
<td>( x_0 ) (ppb)</td>
<td>8.2</td>
<td>4.4</td>
<td>8.4</td>
<td>10.6</td>
</tr>
</tbody>
</table>

**Sample**

| Concentration in ppb for kitchen faucet at ~30°C | 24.1 | 16.3 | 15.5 | 3.5   |
| Confidence interval in ppb at 95%               | 5.2  | 2.6  | 5.0  | 7.0   |
| Concentration in ppb for refrigerator faucet at ~4°C | 27.1 | 14.2 | 12.1 | 1.9   |
| Confidence interval in ppb at 95%               | 5.4  | 2.6  | 5.0  | 7.2   |

**Line No.** | **Code in GWBASIC**
---|---
1 | INPUT "ENTER Y (ALL CAPS) FOR WEIGHTED LEAST SQUARES, N IF NOT";U$  
2 | IF US = "Y" THEN GOSUB 700 ELSE 5  
3 | GO TO 323  
5 | REM  
6 | Establishes the calibration curve for \( x \), \( y \) pairs of data using nonweighted least squares regression statistics.  
7 | REM  
8 | Applies basic statistical concepts to find the slope, \( y \) intercept, and correlation coefficient for the least squares regression.  
9 | REM  
10 | Finds the standard deviation in both the slope and \( y \) intercept and uses these parameters to enable an interpolation with confidence intervals specified.  
11 | REM  
<table>
<thead>
<tr>
<th>Line No.</th>
<th>Code in GWBASIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>DIM X(25), Y(25), A(25), Z(25), C(25)</td>
</tr>
<tr>
<td>10</td>
<td>INPUT “HOW MANY DATA POINTS”: N</td>
</tr>
<tr>
<td>20</td>
<td>FOR I = 1 TO N</td>
</tr>
<tr>
<td>25</td>
<td>PRINT</td>
</tr>
<tr>
<td>30</td>
<td>INPUT “WHAT IS THE X VALUE”: X(I)</td>
</tr>
<tr>
<td>40</td>
<td>X = I * X + X(I)</td>
</tr>
<tr>
<td>50</td>
<td>A(I) = A(I) * X(I)</td>
</tr>
<tr>
<td>60</td>
<td>HX = HX + A(I)</td>
</tr>
<tr>
<td>66</td>
<td>PRINT</td>
</tr>
<tr>
<td>70</td>
<td>INPUT “WHAT IS THE Y VALUE”: Y(I)</td>
</tr>
<tr>
<td>80</td>
<td>IY = IY + Y(I)</td>
</tr>
<tr>
<td>90</td>
<td>Z(I) = X(I) * Y(I)</td>
</tr>
<tr>
<td>100</td>
<td>JXY = JXY + Z(I)</td>
</tr>
<tr>
<td>110</td>
<td>C(I) = Y(I) * Y(I)</td>
</tr>
<tr>
<td>120</td>
<td>KY = KY + C(I)</td>
</tr>
<tr>
<td>125</td>
<td>PRINT</td>
</tr>
<tr>
<td>130</td>
<td>NEXT I</td>
</tr>
<tr>
<td>140</td>
<td>LET L = IX / N</td>
</tr>
<tr>
<td>150</td>
<td>LET M = IY / N</td>
</tr>
<tr>
<td>160</td>
<td>LET E = IX * IX</td>
</tr>
<tr>
<td>170</td>
<td>LET F = IY * IY</td>
</tr>
<tr>
<td>180</td>
<td>LET SXX = HX - E / N</td>
</tr>
<tr>
<td>190</td>
<td>LET SXY = JXY - (IX * IY) / N</td>
</tr>
<tr>
<td>200</td>
<td>LET SYY = KY - (IY * IY) / N</td>
</tr>
<tr>
<td>210</td>
<td>LET B = SX Y / SXX</td>
</tr>
<tr>
<td>220</td>
<td>LET A = M - B * L</td>
</tr>
<tr>
<td>230</td>
<td>LET VAR = SYY / (N-2) - SXY^2 / SXX / (N-2)</td>
</tr>
<tr>
<td>240</td>
<td>LET R = SXY / SQR(SXX * SYY)</td>
</tr>
<tr>
<td>250</td>
<td>LET SDB = SQR(VAR) * 1 / SQR(SXX)</td>
</tr>
<tr>
<td>260</td>
<td>LET SDA = SQR(VAR) * SQR(HX / (N * HX - E))</td>
</tr>
<tr>
<td>270</td>
<td>PRINT</td>
</tr>
<tr>
<td>275</td>
<td>PRINT “THE SLOPE OF THE REGRESSION LINE IS”: B</td>
</tr>
<tr>
<td>280</td>
<td>PRINT “THE Y-INTERCEPT OF THE REGRESSION LINE IS”: A</td>
</tr>
<tr>
<td>285</td>
<td>PRINT</td>
</tr>
<tr>
<td>290</td>
<td>PRINT “THE CORRELATION COEFFICIENT FOR THE REGRESSION IS”: R</td>
</tr>
<tr>
<td>295</td>
<td>PRINT</td>
</tr>
<tr>
<td>300</td>
<td>PRINT “THE STANDARD DEVIATION IN THE SLOPE OF THE REGRESSION LINE IS”: SDB</td>
</tr>
<tr>
<td>310</td>
<td>PRINT</td>
</tr>
<tr>
<td>320</td>
<td>PRINT “THE STANDARD DEVIATION IN THE Y-INTERCEPT IS”: SDA</td>
</tr>
</tbody>
</table>
The critical level, \( y_c \) (in response units), the critical limit, \( x_c \) (in concentration or amount units), the instrument detection limit, \( x_d \) (in concentration or amount units), the coordinate that corresponds to \( x_d \), and the revised detection limit, \( x_{dd} \), are all calculated.

```plaintext
323 INPUT "WOULD YOU LIKE TO FIND DETECTION LIMITS FOR WEIGHTED LEAST SQUARES? ENTER Y (ALL CAPS) FOR YES AND N FOR NO"; B$   
324 IF B$ = "Y" THEN 900 ELSE 325   
325 INPUT "WOULD YOU LIKE TO FIND DETECTION LIMITS FOR UNWEIGHTED LEAST SQUARES? ENTER Y (ALL CAPS) FOR YES AND N FOR NO"; B$   
326 PRINT "YC,XC,XD FOR THIS CALIBRATION CURVE ARE:"; YC,XC,XD   
327 PRINT "YD AND XDD FOR THIS CALIBRATION CURVE ARE:"; YD,XDD   
328 INPUT "WOULD YOU LIKE TO FIND XDDD? ENTER Y (ALL CAPS) FOR YES OR N FOR NO"; Z$   
329 IF Z$ = "Y" THEN GOSUB 640 ELSE 335   
330 PRINT "THE INST DET LIMIT ACC TO KOEHN AND ZIMMERMAN, XDD, IS:"; XDD   
331 IF Z$ = "Y" THEN GOSUB 640 ELSE 335   
332 PRINT "WOULD YOU LIKE TO INTERPOLATE? ENTER Y FOR YES OR N FOR NO"; A$   
333 IF A$ = "Y" THEN 345 ELSE 999   
334 INPUT "HOW MANY NON-REPLICATE INSTRUMENT RESPONSES DO YOU HAVE"; X   
335 PRINT   
336 FOR J = 1 TO X   
337 INPUT "IS THE VALUE TO BE INTERPOLATED FROM A SINGLE OR MULTIPLE MEASUREMENT? ENTER S FOR SINGLE AND K FOR MULTIPLE"; D$   
338 IF D$ = "S" THEN GOSUB 400   
339 IF D$ = "K" THEN GOSUB 500   
340 PRINT   
341 PRINT "THE INTERPOLATED VALUE CORRESPONDING TO"; Y(J) "IS"; X(J)   
342 PRINT "THE STANDARD DEVIATION IN"; X(J) "IS"; S(J)   
343 PRINT "THE CONFIDENCE INTERVAL FOR THIS INTERPOLATED VALUE IS"; O(J)   
344 PRINT   
345 NEXT J   
346 GOTO 999   
347 INPUT "ENTER AN INSTRUMENT RESPONSE FOR A SINGLE MEASUREMENT"; Y(J)   
348 INPUT "ENTER THE APPROPRIATE T FOR N-2 DEGREE OF FREEDOM"; T   
349 LET X(J) = (Y(J) - A) / B   
350 LET V(J) = VAR * (1 + 1/N + (Y(J) - M)^2/(B^2*SXX))/B^2   
351 LET S(J) = SQR (V(J))   
352 LET O(J) = T * S(J)   
353 RETURN
```
Trace Environmental Quantitative Analysis, Second Edition

Line No. | Code in GWBASIC
--- | ---
500 | INPUT "ENTER THE NUMBER OF REPLICATE MEASUREMENTS TAKEN":K
505 | INPUT "ENTER THE APPROPRIATE T FOR N-2 DEGREES OF FREEDOM":T
510 | INPUT "ENTER THE AVERAGE OF THESE REPLICATE MEASUREMENTS":Y(J)
520 | LET X(J) = (Y(J) − A) / B
530 | LET V(J) = VAR * (1/K + 1/N + (Y(J) − M)^2/(B^2*SXX)) / B^2
540 | LET S(J) = SQR (V(J))
545 | LET O(J) = T * S(J)
550 | RETURN
600 | REM This subroutine first calculates the decision level, \( x_c \) (in response units), then the decision limit, \( x_s \) (in concentration or amount units), then the detection limit, \( x_d \) (in concentration or amount units).
601 | REM After \( x_d \) is found, the theory of Hubaux and Vos is used to find \( y_d \), where \( y_d = y_0 + p*s + q*s \) and \( s \) is the estimated residual standard deviation.
602 | INPUT "ENTER STUDENT'S T FOR N-2 DEGREES OF FREEDOM": T
603 | LET YC = M − B*L + T*SQR(VAR) + SQR(1 + 1/N + L^2/SXX)
604 | LET XC = (YC − (M − B*L)) / B
605 | LET XD = XC + T * SQR (VAR * (1 + 1/N + (YC − M)^2/(B^2 * SXX)) / B ^2)
610 | LWR Q = T * SQR (1 + 1/N + (XD − L)^2 / SXX)
615 | LET YD = YC + Q * SQR (VAR)
620 | LET XDDD = (YD − A) / B
625 | RETURN
630 | REM The decision limit, \( y_c \), along with the least squares parameters for the found vs. target concentrations, is used to calculate a reporting limit.
631 | REM \( x_{dd} \) is the detection limit according to Koehn and Zimmermann of Shell Development Company and presented at the 1987 EPA Conference on Analysis of Pollutants in the Environment.
640 | LET AA = 1 / (M^2 * T^2 * VAR / SXX)
642 | LET BB = (YC − M)^2/SXX + (1 + 1/N) * (B^2 − T^2*VAR/SXX)
644 | LET XDDDD = L + AA * B * (YC − M) + AA*T*SQR(VAR) * SQR (BB)
645 | PRINT
650 | RETURN
700 | REM This subroutine requires a weight \( w(i) \) for each \( x(i) \), \( y(i) \) entered.
701 | REM This subroutine then finds the least squares regression parameters.
705 | DIM W(25),WX(25),WY(25),WA(25),WZ(25),WC(25)
708 | INPUT "HOW MANY DATA POINTS":N
710 | FOR I = 1 TO N
715 | PRINT
720 | INPUT "WHAT IS THE WEIGHT FACTOR TO BE USED?":W(I)
Line No. | Code in GWBASIC
---|---
725 | PRINT
730 | INPUT “WHAT IS THE X VALUE?”;X(I)
735 | IX = IX + W(I)*X(I)
740 | WA(I) = W(I) * (X(I)) ^2
742 | HX = HX + WA(I)
745 | PRINT
750 | INPUT “WHAT IS THE Y VALUE”;Y(I)
755 | IY = IY + W(I) * W(I)
760 | WZ(I) = W(I) * X(I) * Y(I)
765 | JXY = JXY + WZ(I)
770 | WC(I) = W(I) * (Y(I)) ^2
775 | KY = KY + WC(I)
777 | WW = WW + W(I)
778 | NEXT I
780 | LET F = IY * IY
785 | LET L = IX / WW
790 | LET M = IY / WW
795 | LET E = IX * IX
800 | LET SXX = HX – E / WW
805 | LET SXY = JXY – (IX * IY) WW
810 | LET SYY = KY – (IY * IY) / WW
820 | LET B = SXY / SXX
825 | LET A = M – B * L
830 | LET VAR = SYY / (N-2) – (SXY * SXY) / SXX / (N – 2)
832 | LET SR = SQR (VAR)
835 | LET R = SXY / SQR (SXX * SYY)
840 | LET SDB = ST * 1/SQR(SXX)
850 | PRINT
853 | PRINT “THE STANDARD ERROR OF REGRESSION IS”;SR
854 | PRINT
855 | PRINT “THE SLOPE OF THE WEIGHTED LINE IS”;B
857 | PRINT
860 | PRINT “THE Y-INTERCEPT OF THE WEIGHTED REGRESSION LINE IS”;A
862 | PRINT
865 | PRINT “THE CORRELATION COEFFICIENT FOR THE WEIGHTED LINE IS”;R
867 | PRINT
880 | PRINT “THE STANDARD DEVIATION IN THE SLOPE OF THE WEIGHTED LINE IS”;SDB
882 | PRINT
887 | PRINT
888 | RETURN
900 | REM

This subroutine calculates $y_c$, $x_c$, $x_d$, $y_d$, and $x_{dd}$ using equations from Gibbons et al., *Analytical Chemistry*, 69, 3069–3075, 1997.
WHAT IS THE PROGRAM FOR SDMULTI?

Scheme C.1 is a flowchart that outlines the logic that went into the GWBASIC program for SDMULTI. This is a program that ultimately finds a pooled standard deviation, \( S_{\text{pooled}} \), for \( j \) replicate GC injections of eluent over \( g \) replicate SPEs performed. The program in GWBASIC is given line by line below.

SD Multiprogram

10 REM
This program calculates the standard deviation for replicate injection of an eluent from solid-phase extraction (SPE) over one or more SPEs performed. The author drew on equations from Peters, D. et al., Chemical Separations and Measurements, Saunders, Philadelphia, 1974, and on the pooled standard deviation equation given in Kolthoff, I. et al., Quantitative Chemical Analysis, Macmillan, London, 1969, p. 390. In addition, Student’s t statistics are applied using the computed standard deviation to yield confidence intervals at a stated probability of not committing a type I error, expressed as a percent. Percent relative standard deviations (RSDs) are calculated provided a mean percent recovery is available.

50 DIM A(25), B(25), SX(25), HX(25), AVE(25), Y(25), Z(25), D(25)
100 INPUT “Enter the number of SPEs performed in this series”; M
110 FOR J = 1 TO M
120 INPUT “Enter the number of replicate injections for this SPE”; N
130 FOR I = 1 TO N
140 INPUT “Enter the area count for this injection”; A(I)
150 B(I) = A(I) * A(I)
160 SX(J) = SX(J) + A(I)
170  HX(J) = HX(J) + B(I)
180  NEXT I
190  LET AVE(J) = SX(J)/N
200  LET Y(J) = SX(J)*SX(J)
210  LET Z(J) = Y(J)/N
220  LET D(J) = (HX(J) - Z(J))
230  LET L = L + D(J)
231  PRINT HX(J),Z(J),D(J)
232  PRINT
235  PRINT “The average value for this set is”; AVE(J)
240  NEXT J
260  LET U = T - M
265  LET Q = L/U
270  PRINT
275  LET S = SQR(Q)
280  PRINT “The variance over “;M;” SPEs performed is”;Q
290  PRINT
300  PRINT “The standard deviation for “;M;” SPEs performed is”; S
310  INPUT “Enter the desired confidence level and Student’s t value”; P, ST
320  LET CI = (ST*S) / SQR(T)
330  GO SUB 400
340  LET RSD = (S/MX)*100
345  LET RSD(M) = RSD/SQR(T)
349  PRINT
350  PRINT “The confidence interval at “;P;” percent probability of not committing a type I error is”;CI
359  PRINT
360  PRINT “The relative standard deviation (RSD) in the mean area count is”; RSD
365  PRINT “The rel std dev in the mean of “;T;” measurements is”;RSD(M)
370  GO TO 999
400  REM
This subroutine finds an average of mean values.
405  INPUT “How many sets of mean values do you want averaged?”; X
410  FOR K = 1 TO X
415  INPUT “Enter the mean area count from this set”; AVE(J)
420  LX = LX + AVE(K)
430  NEXT K
440  MX = LX / X
444  PRINT
445  PRINT “The mean area count from “;M;” sets of replicates is”; MX
450  RETURN
999 END
Enter # of SPEs performed in this series, \( M \).

For a given SPE, enter # replicate GC injections \( N \), ith injection is, \( x_i \).

Choose \( t \) based on \( df = (T-1) \) and \( \alpha = 0.05 \) or \( \alpha = 0.01 \)

Print mean area count, \( \bar{x}_g \), for \( j \) replicate injections for each SPE. Print a pooled variance and pooled standard deviation, \( s_{pooled} \), over \( j \) injections over \( g \) SPEs performed. Knowing \( s_{pooled} \) enables a “pooled” confidence interval to be calculated and printed.

\[
\bar{x}_g = \frac{1}{N} \sum_{j=1}^{N} x_j
\]

\[
s_{pooled} = \sqrt{\frac{1}{T-g} \sum_{g=1}^{g} \sum_{j=1}^{j} (x_j - \bar{x})^2}
\]

Conf interval (mean) = \( t(1 - \alpha/2, df)_{pooled} \frac{s_{pooled}}{\sqrt{T}} \)

Enter the total # of replicate measurements, \( T = N^*M \)

Print mean area count over all \( g \) SPEs performed in this series, \( \bar{x}_{SPE} \). From this mean and the pooled standard deviation, \( s_{pooled} \), a relative standard deviation expressed as a percent, RSD = \( s_{pooled} / \bar{x}_{SPE} \times 100 \), and a relative standard deviation in the mean, RSD(m) = RSD/\( \sqrt{T} \) is calculated and printed. In addition, a confidence interval in the mean can be found and printed.

\[
\bar{x}_{SPE} = \sum_{g=1}^{g=M} \bar{x}_g
\]

SCHEME C.1
Scheme C.2 is a flowchart that outlines the logic that went into the GWBASIC program STAT&REC. This program computes a mean and variance from N replicate measurements. A confidence interval in the mean is found from knowing the standard deviation (derived from the variance) at the stated probability (usually 95 or 99%) of not making a type I error for \( N - 1 \) degrees of freedom using Student’s t statistics. If a control standard (for a 100% recovery) or a certified reference value is known, then a percent recovery or percent relative error is calculated. Furthermore, a standard deviation in the mean, a confidence interval in the mean, and a relative standard deviation in the mean can be found. The program in GWBASIC is given line by line below.

**STAT&REC Program**

```
10 REM
   This program finds the mean, variance, standard deviation, relative standard deviation as a percent (%RSD), and confidence interval based on Student’s t statistics at a stated probability for input of one set of replicate data. If a true value is available as input, a percent error in the mean is calculated. If a 100% recovery reference standard is known, a percent recovery can be found. The program also computes a standard deviation in the mean, a confidence interval in the mean, and a %RSD in the mean.
15 DIM A(25), B(25)
20 INPUT “Enter the number of observations”; N
30 INPUT “Enter probability of not committing a type I error as a percent”; P
40 INPUT “Enter Student’s t for (N-1) degrees of freedom (1-α) probability”; T
50 GO SUB 200
75 PRINT “The mean of “;N;” observations is”; AVE
77 PRINT
80 PRINT “The confidence interval at “; P; “ percent is”; CI
85 PRINT
90 PRINT “The relative standard deviation (as a percent) for “; N; “ observations is”; RSD
92 PRINT
95 PRINT “Would you like to find %error and %recovery? Enter Y for yes and N for no”; L$
100 IF L$ = “Y” THEN 110 ELSE 125
110 GO SUB 400
120 PRINT “The percent error for “; AVE; “ is”; A
122 PRINT
124 PRINT
125 PRINT
127 INPUT “Would you like to calculate std dev of mean, conf int of mean and RSD of mean? Enter Y for yes and N for no”; M$
130 IF M$=“Y” THEN 150 ELSE 999
150 GO SUB 600
160 GO TO 999
200 REM
   This subroutine calculates the mean “ave,” variance “V,” standard deviation “SD,” relative standard deviation expressed as a percent “RSD,” and confidence interval “CI” for \( N \) replicate measurements. The data must be obtained under identical experimental conditions to eliminate any systematic error.
```
FOR I = 1 TO N
INPUT "Enter the value"; A(I)
B(I) = A(I)^2
SX = SX + A(I)
HX = HX + B(I)
NEXT I
LET AVE = SX/N
LET Y = SX*SX
LET Z = Y/N
LET V = (HX - Z) / (N-1)
LET SD = SQR(V)
LET RSD = SD/AVE * 100
RETURN

REM
This subroutine calculates the percent error of a mean result if given the expected value. The percent
error is found by taking the absolute error and dividing by the expected value. The percent recovery
is also determined.

PRINT
INPUT "Enter the expected value"; E
PRINT
LET D = ABS(E - AVE)
LET A = D / E * 100
LET R = 100 - A
RETURN
PRINT
PRINT "The percent recovery exceeds 100!"
LET SM = SD/SQR(N)
PRINT
PRINT "The std dev of the mean of ";N;" observations is";SM
LET MD = T * SD/SQR(N)
PRINT
PRINT "The confidence interval for the calculated std dev of mean is";MD
LET RSDM = (SM/AVE)*100
PRINT
PRINT "The rel std dev of the mean is";RSDM
999 END
Enter # of replicate observations, N

Choose t based on df = (N–1) and α = 0.05 or α = 0.01

Input x₁, x₂, ..., xᵢ, ..., xₙ

Print x(ave), confidence interval at probability (1–α)% and %RSD

\[
x = \frac{1}{N} \sum_{i=1}^{N} x_i
\]
\[
\text{var} = \frac{\sum (x_j - \bar{x})}{N - 1}
\]

Find % error or % recovery?

\[
% \text{error} = \frac{|\bar{x} - \tau|}{\tau} \times 100
\]

Find sx(ave), Clx(ave) and %RSDx(ave)?

\[
s_x = \frac{s}{\sqrt{N}}
\]
\[
\text{Cl}_x = \frac{t(1-\alpha/2, df) s}{\sqrt{N}}
\]
\[
%\text{RSD}_x = \frac{s_x}{\bar{x}} \times 100
\]

End

SCHEME C.2
WHAT IS THE PROGRAM FOR RSD?

Scheme C.3 is a flowchart that outlines the logic that went into the GWBASIC program RSD. This program computes a mean and variance from \( N \) replicate measurements. A confidence interval in the mean is found from knowing the standard deviation (derived from the variance) at the stated probability (usually 95 or 99\%) of not making a type I error for \( N - 1 \) degrees of freedom using Student’s \( t \) statistics. The program in GWBASIC is given line by line below.

---

**RSD Program**

```
10 REM This program calculates, for \( M \) different sets of \( N \) replicate analytical data, the mean of \( N \) replicates, standard deviation in the mean, relative standard deviation as a percent, and the confidence interval based on Student’s \( t \) statistics.
25 DIM A(25), B(25), SX(25), HX(25), AVE(25), Y(25), Z(25), Y2(25), SD(25), RSD(25)
30 INPUT “Enter the number of different sets of replicate data”; M
32 FOR J = 1 TO M
33 PRINT
35 GO SUB 100
36 PRINT
38 PRINT “The mean of \( N \);” replicate measurements is”; AVE(J)
39 PRINT
40 PRINT “The standard deviation for \( N \);” replicate measurements is”; SD(J)
41 PRINT
42 PRINT “The relative standard deviation (as a percent) for \( N \);” replicate measurements is”; RSD(J)
43 PRINT
44 PRINT “The confidence interval at \( P \);% probability is”; CI(J)
46 NEXT J
47 PRINT
48 PRINT “This ends your calculations for \( M \); set(s) of replicate measurements”
50 GO TO 999
100 REM This subroutine calculates the mean, standard deviation, relative standard deviation expressed as a percent, and confidence interval for \( N \) replicate measurements. The data must be obtained under identical experimental conditions to eliminate any systematic error. Following the calculation of the statistics for the first set of replicates, the program will take on a second set, then a third set, and so forth.
110 INPUT “Enter the number of replicate measurements”; N
112 INPUT “Enter the value of Student’s \( t \) and the desired probability”; T, P
114 FOR I = 1 TO N
116 INPUT “Enter the parameter”; A(I)
118 B(I) = A(I) * A(I)
120 SX(J) = SX(J) + A(I)
122 HX(J) = HX(J) + B(I)
124 NEXT I
```
SCHEME C.3

For a given set, xᵢ, enter # replicate measurements, N

\[ x = \frac{1}{N} \sum_{i=1}^{N} x_i \]

\[ \text{var} = \frac{\sum_{i} (x_i - \bar{x})^2}{N - 1} \]

Print x(ave), s, %RSD and confidence interval at probability (1 - α)%

Choose t based on df = (N - 1) and \( \alpha = 0.05 \) or \( \alpha = 0.01 \)

Enter # different sets of replicate data, M

Yes

Is M ≥ 1

No

End
APPENDIX D
Instrument Designs

The best education is to be found in gaining the utmost information from the simplest apparatus.

—A.N. Whitehead

TEQA is constantly changing as new technologies enable new analytical methods. This appendix includes photos that show instrumentation and glass apparatus that this author would like to share. Before we get to instruments, let us revisit an old concept.

In Chapter 4, the Craig countercurrent distribution was introduced from a historical perspective as a lead into the topic of chromatography. A complete Craig apparatus is located in the lobby of the Department of Molecular and Cellular Biology at Harvard University. Figure D.1 represents one view of this unique glassware design.

In Chapter 5, the determination of Cr(VI) in a contaminated aquifer experiment was introduced. Figure D.2 depicts various equipment components used to assemble an ion chromatograph with postcolumn reagent delivery and UV detection. Figure D.3 shows the author’s large-volume glass apparatus that was designed to pass a 1-L sample of groundwater through a conditioned RP-SPE sorbent.

Recently introduced analytical instruments are shown in the remaining photos, Figure D.4 through Figure D.10. These photos were taken at and are courtesy of the Michigan Department of Community Health, Bureau of Laboratories, Division of Chemistry and Toxicology.
FIGURE D.1 Craig countercurrent distribution apparatus. (Courtesy of the Department of Molecular and Cellular Biology, Harvard University.)

FIGURE D.2 Instrument configured from different sources to measure trace Cr(VI) via ion chromatography with postcolumn reagent chelation. (Courtesy of the Department of Civil and Environmental Engineering, Michigan State University.)
FIGURE D.3  Large-volume glass apparatus for solid-phase extraction (designed by author).

FIGURE D.4  MPS2® (Gerstel) interfaced to a 6890 GC 5873 MSD® (Agilent Technologies).
FIGURE D.5  1100® HPLC (Agilent) interfaced to an API4000® (Applied Biosystems).

FIGURE D.6  PE SCIEX ELAN 6100 DRC Plus® ICP-MS (PerkinElmer Instruments).
FIGURE D.7 Rapid Trace® SPE Workstation. (Caliper Life Sciences, formerly Zymark Corporation.)

FIGURE D.8 Accelerated Solvent Extractor ASE 300 (Dionex Corporation).
FIGURE D.9 Fourier Transform Infrared Spectrometer Microscope (Smith’s Detection, formerly SensIR).

FIGURE D.10 X-Ray Fluorescence Spectrometer (Innov-X Systems).

A picture is worth 1000 words.

—Anonymous
APPENDIX E

Useful Internet Links for Environmental Analytical Chemists

To research means to see what all people have seen and to think what nobody has thought.

—A. Szent Gyorgyi

TRACE ENVIRO-CHEMICAL WEBSITES
METHODS AND TECHNICAL LITERATURE

   EPA environment test methods and guidelines
   Links to sources of EPA test methods on the Internet
   EPA's Ambient Monitoring Technology Information Center: contains details on monitoring methods and related information
   Home page for EPA Office of Air and Radiation
   EPA Office of Ground Water and Drinking Water: analytical methods for drinking water
   EPA Office of Solid Waste: contains SW-846 online test methods for evaluating solid waste physical/chemical methods
   Detailed summaries and comparison of critical parameters of EPA methods; useful for method selection, method modification, and data comparability
8. http://www/sampleprep.duq.edu
   Sample Prep Web: contains information and advice regarding analytical sample preparation, speciated analysis, trace analysis, and microwave chemistry

   From menu choose Supelco, then Supelco Technical Library: approximately 100 environmental bulletins and application notes can be downloaded and printed

**Certified Reference Materials (CRMs/SRMs)**

   Technical and ordering information for National Institute of Standards and Technology (NIST) SRMs

   CRMs of the Canadian National Research Council

   U.K. organization that acts as a central supply for CRMs from around the world

   Institute for Reference Materials and Measurements: promotes European harmonization and standardization in analytical measurements, quality control in preparation of CRMs

**Environmental/Analytical Conferences and Organizations**

1. http://www.acs-envchem.duq.edu/
   Home page of the American Chemical Society, Division of Environmental Chemistry

   Information concerning the biennial conference EnviroAnalysis; devoted to all aspects of environmental pollution and monitoring

3. http://nemc.us
   Information concerning the annual National Environmental Monitoring Conference (NEMC), formerly WTQA

**Links to Relevant Environmental Information**

   Links to sources of relevant environmental chemistry information

2. http://www.liv.ac.uk/Chemistry/Links/refanal.html
   Contains many links to analytical chemistry sources of information

   ISO/IEC Guides: contains extensive links to international accreditation bodies and standards organizations
TRACE ENVIRO-HEALTH WEBSITES

FEDERAL AND NONFEDERAL INTERNET LINKS

- ATSDR ToxFaqs: www.atsdr.cdc.gov/toxfaq.html or www.atsdr.cdc.gov/toxprofiles
- National Institute for Occupational Safety and Health (NIOSH), Occupational Health and Safety Guidelines for Chemical Hazards: www.cdc.gov/niosh/81-123.html
- National Toxicology Program Report on Carcinogens: http://ehis.niehs.nih.gov/roc
- EPA Integrated Risk-Information System (IRIS): www.epa.gov/iris
- International Programme on Chemical Safety (IPCS): www.who.int/pcs
- Chemfinder: www.chemfinder.com
- Material Safety Data Sheets: www.hazard.com/msds
- International Society of Exposure Analysis: www.iseaweb.org

U.S. GOVERNMENT-RELATED INTERNET LINKS

Centers for Disease Control and Prevention (CDC)

- NIOSH Pocket Guide to Chemical Hazards: www.cdc.gov/niosh/npd/npd0000.html
- Registry of Toxic Effects of Chemical Substances (RTECS): www.cdc.gov/niosh/rtecs.html
- Tobacco Information and Prevention Source: www.cdc.gov/tobacco
- National Center for Health Statistics: www.cdc.gov/nchs
- Childhood Lead Poisoning Prevention Program: www.cdc.gov/ncidod/eid/vol7nol/rose.htm

U.S. Department of Health and Human Services (HHS)

- Environmental Health Policy Committee: http://web.health.gov/environment

U.S. Food and Drug Administration (FDA)

- Center for Devices and Radiological Health: www.fda.gov/cdrh
- Center for Food Safety and Applied Nutrition: www.cfsan.fda.gov
- National Center for Toxicological Research: www.fda.gov.nctr

NATIONAL INSTITUTES OF HEALTH (NIH)

- National Cancer Institute: www.nci.nih.gov
- National Institute of Child Health and Human Development: www.nichd.nih.gov

© 2006 by Taylor & Francis Group, LLC
• National Institute for Environmental Health Sciences: www.niehs.nih.gov
• National Toxicology Program (NTP) Chemical Health and Safety Data: http://ntpserver.niehs.nih.gov/Main_Pages/ChemHS.html

U.S. ENVIRONMENTAL PROTECTION AGENCY (EPA)

• Office of Air and Radiation: www.epa.gov/oar
• Office of Environmental Information (OEI): www.epa.gov/oei
• Office of Prevention, Pesticides, and Toxic Substances (OPPTS): www.epa.gov/opptsmt/index.htm
• Office of Research and Development (ORD): www.epa.gov/ORD
• Office of Water (OW): www.epa.gov/OW
• Office of Pesticide Programs: www.epa.gov/pesticides
• EPA Integrated Risk-Information System (IRIS): www.epa.gov/iris
• EPA Envirofacts: www.epa.gov/OGWDW/dwh/cioc/lead.html

U.S. Department of Agriculture (USDA)

• Food Safety and Inspection Service: http://www.fsis.usda.gov
• USDA, Forest Service Pesticide Fact Sheets: http://svinet2.fs.fed.us/foresthealth/pesticide

U.S. Department of Energy (DOE)

• Office of Environment, Safety and Health: http://tis.eh.doe.gov/portal/home.htm

U.S. Department of Housing and Urban Development (HUD)

• Office of Healthy Homes and Lead-Hazard Control: www.hud.gov/offices/lead

U.S. Consumer Product Safety Commission (CPSC)

• www.cpsc.gov

U.S. Department of Transportation (DOT)

Useful Internet Links for Environmental Analytical Chemists

U.S. Department of Labor, Occupational Safety and Health Administration (OSHA)

• http://www.osha.gov/index.html

OTHER RELATED INTERNET SITES

• American College of Occupational and Environmental Medicine: http://www.acoem.org
• Association of Occupational and Environmental Clinics: http://www.aoec.org
• Association of Public Health Laboratories: http://www.aphl.org

REFERENCES

APPENDIX F

Useful Potpourri for Environmental Analytical Chemists
GRAPHICAL REPRESENTATION OF RELATIVE ISOTOPE PEAK INTENSITIES FOR ANY GIVEN ION CONTAINING THE INDICATED NUMBER OF HALOGENS

Cl  Cl₂  Cl₃  Cl₄  Cl₅  Cl₆
ClBr  Cl₂Br  Cl₃Br  ClBr₂  Cl₂Br₂  Cl₃Br₂
ClBr₃  Cl₂Br₃  Br  Br₂  Br₃  Br₄
Solvent Polarity Chart

Less Polar Solvents

Hexane
Isooctane
Carbon Tetrachloride
Chloroform
Methylene Chloride
Tetrahydrofuran
Ethyl Ether
Ethyl Acetate
Acetone
Acetonitrile
Isopropanol
Methanol
Water

More Polar Solvents

Reversed Phase elution solvents should be less polar than wash solvents. Normal Phase elution solvents should be more polar than wash solvents.

\[ c = \lambda \nu \]
\[ E = h \nu \]
\[ E = mc^2 \]
\[ \nu = \text{frequency} \quad E = \text{energy} \quad m = \text{mass} \quad \lambda = \text{wavelength} \]

The Electromagnetic Spectrum

© 2006 by Taylor & Francis Group, LLC
Strategy for EI mass spectral interpretation

- Attempt to recognize the molecular ion and apply the nitrogen rule
- Estimate the isotopic contributions particularly with respect to carbon and the halogens
- Note changes in the isotope composition between the molecular ion and higher fragment ions; this may indicate loss of certain elements, e.g., Cl vs. Br
- Look for key m/z losses from the molecular ion such as M-15 (CH$_3^-$), M-29 (C$_2$H$_5^-$), M-18 (H$_2$O)
- Check for presence of even m/z values based on McLafferty rearrangements
- Look for common m/z values at lower end of mass axis, e.g., CH$_3$CO$^+$ at m/z 43

The nitrogen rule states:

A molecular ion will have an even m/z value for molecules that contain C, H, O, X provided there is an even (or zero) number of nitrogen atoms in the molecule.

A molecular ion will have an odd m/z value if an odd number of nitrogen atoms are present in the molecule.
Common Contaminants found in GC-MS

<table>
<thead>
<tr>
<th>Ions (m/z)</th>
<th>Compound</th>
<th>Possible Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>18, 28, 32, 44 or 14, 16</td>
<td>H₂O, N₂, O₂, CO₂ or N, O</td>
<td>Residual air and water, air leaks, outgassing from Vespel ferrules</td>
</tr>
<tr>
<td>31</td>
<td>MeOH</td>
<td>Cleaning solvent</td>
</tr>
<tr>
<td>43, 58</td>
<td>Acetone</td>
<td>Cleaning solvent</td>
</tr>
<tr>
<td>78</td>
<td>Benzene</td>
<td>Cleaning solvent</td>
</tr>
<tr>
<td>91, 92</td>
<td>Toluene, xylene</td>
<td>Cleaning solvent</td>
</tr>
<tr>
<td>105, 106</td>
<td>Xylene</td>
<td>Cleaning solvent</td>
</tr>
<tr>
<td>151, 153</td>
<td>TCE</td>
<td>Cleaning solvent</td>
</tr>
<tr>
<td>69</td>
<td>Foreline pump or PFTBA</td>
<td>Foreline pump oil vapor or calibration valve leak</td>
</tr>
<tr>
<td>73, 147, 207, 221, 281, 295, 355, 429</td>
<td>Dimethylpolysiloxane</td>
<td>Septum bleed or methylsilicone column bleed</td>
</tr>
<tr>
<td>77, 94, 115, 141, 168, 170, 262, 354, 446</td>
<td>Diffusion pump fluid and related ions</td>
<td>Diffusion pump fluid</td>
</tr>
<tr>
<td>149</td>
<td>Plasticizer (phthalates)</td>
<td>Vacuum seals (O-rings) damaged by high temperatures, vinyl gloves</td>
</tr>
<tr>
<td>Peaks spaced 14 Da apart</td>
<td>Hydrocarbons</td>
<td>Fingerprints, foreline pump oil</td>
</tr>
</tbody>
</table>

Linear and Volumetric Flow Rates for Capillary GC

Calculation of the capillary column linear carrier gas velocity:

$$ \pi = \frac{100L}{t_0} $$

where

$L = \text{the capillary column length in meters}$
$t_0 = \text{the time it takes after injection for an unretained component to elute in seconds}$

Calculation of the volumetric capillary column flow rate:

$$ F = \frac{0.785d^2L}{t_0} $$

where

$L = \text{the capillary column length in meters}$
$t_0 = \text{the time it takes after injection for an unretained component to elute in seconds}$
$d = \text{the column diameter in mm}$
<table>
<thead>
<tr>
<th>df</th>
<th>t(1 − α)</th>
<th>t(1 − α/2)</th>
<th>t(1 − α)</th>
<th>t(1 − α/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.314</td>
<td>12.706</td>
<td>31.821</td>
<td>63.657</td>
</tr>
<tr>
<td>2</td>
<td>2.920</td>
<td>4.303</td>
<td>6.965</td>
<td>9.925</td>
</tr>
<tr>
<td>3</td>
<td>2.353</td>
<td>3.182</td>
<td>4.541</td>
<td>5.841</td>
</tr>
<tr>
<td>4</td>
<td>2.132</td>
<td>2.776</td>
<td>3.747</td>
<td>4.604</td>
</tr>
<tr>
<td>5</td>
<td>2.015</td>
<td>2.571</td>
<td>3.365</td>
<td>4.032</td>
</tr>
<tr>
<td>6</td>
<td>1.943</td>
<td>2.447</td>
<td>3.143</td>
<td>3.707</td>
</tr>
<tr>
<td>7</td>
<td>1.895</td>
<td>2.365</td>
<td>2.998</td>
<td>3.499</td>
</tr>
<tr>
<td>8</td>
<td>1.860</td>
<td>2.306</td>
<td>2.896</td>
<td>3.355</td>
</tr>
<tr>
<td>9</td>
<td>1.833</td>
<td>2.262</td>
<td>2.821</td>
<td>3.250</td>
</tr>
<tr>
<td>10</td>
<td>1.812</td>
<td>2.228</td>
<td>2.764</td>
<td>3.169</td>
</tr>
<tr>
<td>11</td>
<td>1.796</td>
<td>2.201</td>
<td>2.718</td>
<td>3.106</td>
</tr>
<tr>
<td>12</td>
<td>1.782</td>
<td>2.179</td>
<td>2.681</td>
<td>3.055</td>
</tr>
<tr>
<td>13</td>
<td>1.771</td>
<td>2.160</td>
<td>2.650</td>
<td>3.012</td>
</tr>
<tr>
<td>14</td>
<td>1.761</td>
<td>2.145</td>
<td>2.624</td>
<td>2.977</td>
</tr>
<tr>
<td>15</td>
<td>1.753</td>
<td>2.131</td>
<td>2.602</td>
<td>2.947</td>
</tr>
<tr>
<td>16</td>
<td>1.746</td>
<td>2.120</td>
<td>2.583</td>
<td>2.921</td>
</tr>
<tr>
<td>17</td>
<td>1.740</td>
<td>2.110</td>
<td>2.567</td>
<td>2.898</td>
</tr>
<tr>
<td>18</td>
<td>1.734</td>
<td>2.101</td>
<td>2.552</td>
<td>2.878</td>
</tr>
<tr>
<td>19</td>
<td>1.729</td>
<td>2.093</td>
<td>2.539</td>
<td>2.861</td>
</tr>
<tr>
<td>20</td>
<td>1.725</td>
<td>2.086</td>
<td>2.528</td>
<td>2.845</td>
</tr>
<tr>
<td>25</td>
<td>1.708</td>
<td>2.060</td>
<td>2.485</td>
<td>2.787</td>
</tr>
<tr>
<td>30</td>
<td>1.697</td>
<td>2.042</td>
<td>2.457</td>
<td>2.750</td>
</tr>
<tr>
<td>∞</td>
<td>1.645</td>
<td>1.960</td>
<td>2.326</td>
<td>2.576</td>
</tr>
</tbody>
</table>

### Test for Outliers

#### Values of Q for Rejection of Data

<table>
<thead>
<tr>
<th># Observations</th>
<th>90%</th>
<th>95%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.314</td>
<td>12.706</td>
<td>31.821</td>
</tr>
<tr>
<td>4</td>
<td>2.920</td>
<td>4.303</td>
<td>6.965</td>
</tr>
<tr>
<td>5</td>
<td>2.353</td>
<td>3.182</td>
<td>4.541</td>
</tr>
<tr>
<td>6</td>
<td>2.132</td>
<td>2.776</td>
<td>3.747</td>
</tr>
<tr>
<td>7</td>
<td>2.015</td>
<td>2.571</td>
<td>3.365</td>
</tr>
<tr>
<td>8</td>
<td>1.943</td>
<td>2.447</td>
<td>3.143</td>
</tr>
<tr>
<td>9</td>
<td>1.895</td>
<td>2.365</td>
<td>2.998</td>
</tr>
<tr>
<td>10</td>
<td>1.860</td>
<td>2.306</td>
<td>2.896</td>
</tr>
<tr>
<td>11</td>
<td>1.833</td>
<td>2.262</td>
<td>2.821</td>
</tr>
<tr>
<td>12</td>
<td>1.812</td>
<td>2.228</td>
<td>2.764</td>
</tr>
<tr>
<td>13</td>
<td>1.796</td>
<td>2.201</td>
<td>2.718</td>
</tr>
</tbody>
</table>


### Critical Values of the F Distribution, $\alpha = 0.05$

<table>
<thead>
<tr>
<th>$n_{\text{lower}}$</th>
<th>$n_{\text{upper}}$</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>$\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>19.00</td>
<td>19.16</td>
<td>19.25</td>
<td>19.30</td>
<td>19.50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.55</td>
<td>9.28</td>
<td>9.12</td>
<td>9.01</td>
<td>8.53</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.94</td>
<td>6.59</td>
<td>6.39</td>
<td>6.26</td>
<td>5.63</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.79</td>
<td>5.41</td>
<td>5.19</td>
<td>5.05</td>
<td>4.36</td>
<td></td>
</tr>
<tr>
<td>$\infty$</td>
<td>3.00</td>
<td>2.60</td>
<td>2.37</td>
<td>2.21</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

$n_{\text{upper}}-1$ = # degrees of freedom for the upper variance.

$n_{\text{lower}}-1$ = # degrees of freedom for the lower variance.
### Exact Masses and Natural Isotopic Abundance Ratios

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Exact Mass</th>
<th>Abundance</th>
<th>X+1 Factor</th>
<th>X+2 Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>H</td>
<td>1.007825</td>
<td>99.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D or $^2$H</td>
<td>2.014102</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>$^{12}$C</td>
<td>12.000000</td>
<td>98.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{13}$C</td>
<td>13.003354</td>
<td>1.1</td>
<td>1.1 $n_C$</td>
<td>0.0060 $n_C^2$</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>$^{14}$N</td>
<td>14.003074</td>
<td>99.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{15}$N</td>
<td>15.000108</td>
<td>0.4</td>
<td>0.37 $n_N$</td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>$^{16}$O</td>
<td>15.994915</td>
<td>99.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{17}$O</td>
<td>16.999133</td>
<td>0.04</td>
<td>0.04 $n_O$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{18}$O</td>
<td>17.999160</td>
<td>0.20</td>
<td>0.20 $n_O$</td>
<td></td>
</tr>
<tr>
<td>Fluorine</td>
<td>F</td>
<td>18.998405</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silicon</td>
<td>$^{28}$Si</td>
<td>27.976927</td>
<td>92.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{29}$Si</td>
<td>28.976491</td>
<td>4.7</td>
<td>5.1 $n_{Si}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{30}$Si</td>
<td>29.973761</td>
<td>3.1</td>
<td>3.4 $n_{Si}$</td>
<td></td>
</tr>
<tr>
<td>Phosphorous</td>
<td>P</td>
<td>30.973763</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfur</td>
<td>$^{31}$S</td>
<td>31.972074</td>
<td>95.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{32}$S</td>
<td>32.971461</td>
<td>0.76</td>
<td>0.8 $n_S$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{34}$Si</td>
<td>33.967865</td>
<td>4.22</td>
<td>4.4 $n_{Si}$</td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>$^{35}$Cl</td>
<td>34.968855</td>
<td>75.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{37}$Cl</td>
<td>36.965896</td>
<td>24.23</td>
<td>32.5 $n_{Cl}$</td>
<td></td>
</tr>
<tr>
<td>Bromine</td>
<td>$^{79}$Br</td>
<td>78.918348</td>
<td>50.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{81}$Br</td>
<td>80.916344</td>
<td>49.5</td>
<td>98.0 $n_{Br}$</td>
<td></td>
</tr>
<tr>
<td>Iodine</td>
<td>I</td>
<td>12.904352</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**

1. Assume X = 100%; X represents the relative intensity of the first peak in a cluster of isotope peaks.

2. The factor is multiplied by the number (n) of atoms present to determine the magnitude of the abundance contribution for a given isotope. For example, the fragment ion from electron-impact ionization of the tuning compound, PFTBA at m/z 502 is due to (C$_4$F$_9$)NCF$_2$+, and contains 9 carbons [$n_C = 9$] such that the contribution at X+1 is expected to be [1.1 × 9] = 9.9%.

1 Introduction to Trace Environmental Quantitative Analysis (TEQA)

If you teach a person what to learn, you are preparing him for the past. If you teach him how to learn, you are preparing him for the future.

—Anonymous

CHAPTER AT A GLANCE

Case study from trace enviro-health quantitative analysis .............................................2
Case study from trace enviro-chemical quantitative analysis........................................4
Extent of chemical contaminants in humans ..................................................................7
Analytical chemistry approaches to biomonitoring .........................................................11
Environmental chemistry ..............................................................................................11
EPA regulations ............................................................................................................15
Analytical methods that satisfy EPA regulations ..........................................................20
Physical/chemical basis for EPA’s methods protocols ..................................................32
References ....................................................................................................................35

As we approached the new millennium, the news media and related mass media speculated on what would be different. The 20th century was gone. The 21st century was upon us. The tragic events of 9/11 in the U.S. provided one such answer. Since 9/11, questions such as “If we have a terrorist event, can we measure trace concentration levels of terrorist-related chemical substances and attempt to evaluate exposure over relatively large numbers in the population?” have shifted the dialogue.

Public health laboratories are beginning to respond to this terrorist-related threat. These laboratories are moving toward having a capability in trace environmental health quantitative analysis (also abbreviated TEQA). At the same time, biomonitoring-related initiatives are expanding. Federal laboratories such as the National Center for Environmental Health and the Centers for Disease Control and Prevention (NCEH/CDC) are assisting state labs in the transfer of both bioterrorism- and biomonitoring-related analytical methods. These methods are designed to measure trace
concentration levels of chemical substances that either persist (persistent organic pollutants (POPs)) or are eliminated rather quickly by the body, i.e., nonpersistent organic pollutants (NPOPs).

Bioterrorism and biomonitoring are key initiatives that are currently driving the changing nature of trace quantitative organics and inorganics analysis. The second edition of this book attempts to reflect these changes. This new emphasis, when combined with the more established methods of trace environmental quantitative analysis, has led this author to adopt a new term: *trace enviro-chemical/enviro-health quantitative analysis*, whose acronym is also TEQA. I have tried to add those analytical concepts that are most relevant to conducting trace enviro-health quantitative analysis. Sampling, sample preparation, determinative technique, and data reduction/interpretation are very similar to both trace enviro-chemical and trace enviro-health quantitative analysis. Scheme 1.1 depicts both the enviro-chemical and the enviro-health aspects of trace quantitative organics and inorganics analysis while discerning the similarities and differences in both. One starts with an understanding of the chemical nature of the sample or human or animal specimen received. A client needs to understand just what analytes are to be measured and how these two pathways lead to four steps in the process shown in Scheme 1.1. There is no substitute for effective communication between the client and the analytical laboratory. Sampling (introduced in Chapter 2), sample preparation (introduced in Chapter 3), determinative techniques, often referred to as *instrumental analysis* (introduced in Chapter 4), and data reduction, statistical treatment, and interpretation of analytical data (introduced in Chapter 2) comprise the important aspects of successfully implementing TEQA.

This second edition introduces principles and practices of trace enviro-health quantitative analysis while expanding on the previous treatment of trace enviro-chemical quantitative analysis where the emphasis was placed only on environmental samples.

Two case studies drawn from the recent literature introduce the practice of contemporary TEQA. The first case study demonstrates that a possible endocrine disrupter can be isolated and recovered from human urine.

1. **CAN AN EXAMPLE PROVIDE INSIGHT INTO TRACE ENVIRO-HEALTH QA?**

Yes, we start by briefly introducing results from a published study. Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl)propane, is an industrial chemical used in a variety of plastic materials, some of which are used in packaging, and hence is believed to leach out into consumable items such as food and dental fillings. One way to better assess human exposure to BPA despite the source of exposure being largely unknown is to biomonitor, i.e., to measure, trace BPA in human urine. Brock and coworkers at the NCEH/CDC have developed a quantitative analytical method to determine just how much BPA might be present in a urine sample obtained from a person believed to be exposed to BPA. BPA is apparently excreted either unmetabolized
Urinary BPA glucuronide seems to be a longer-lived biomarker (12 to 48 h). After deglucuronidation using β-glucuronidase, BPA was isolated and recovered by reversed-phase solid-phase extraction. The isolate was converted to its pentafluorobenzyl ether. The pentafluorobenzyl ether of BPA was quantitated using isotope dilution gas chromatography-mass spectrometry (GC-MS). A method detection limit (MDL) was reported to be 120 parts per trillion (ppt). Figure 1.1 shows two different mass spectra for the pentafluorobenzyl ether of BPA that eluted from the gas chromatographic column at ~26.4 min. The top mass spectrum in Figure 1.1 was obtained via electron-impact mass spectrometry and reflects positive fragment ions, while the bottom mass spectrum was obtained via negative chemical ionization mass spectrometry. Pooled human urine samples showed no detectable BPA before the urine was treated, while BPA concentration levels varied from 0.11 to 0.51 parts per trillion.
per billion (ppb) for the treated urine. Molecular structures for Bisphenol A and for pentafluorobenzyl bromide (α-bromo-2,3,4,5,6-pentafluorotoluene) are shown below:

The second case study demonstrates that an emerging pharmaceutical can be isolated and recovered from wastewater.

2. CAN AN EXAMPLE PROVIDE INSIGHT TO TRACE ENVIRO-CHEMICAL QA?

Yes indeed, and we start with a published report on the isolation and recovery of clofibric acid from wastewater. Clofibric acid [2-(4-chlorophenoxy)-2-methyl-propanoic]
acid is the bioactive metabolite of various lipid-regulating prodrugs. Acidic metabolites of pharmaceuticals present one type of analyte that appears in the effluent of many municipal treatment facilities. The isolation and recovery of clofibric acid is consistent with the Environmental Protection Agency’s (EPA) Division of Environmental Sciences, Environmental Chemistry Branch’s mission to study the fate and transport of chemical compounds derived from pharmaceuticals, their metabolites, and personal care products. Patterson and Brumley approached the need to quantitate clofibric acid by comparing two major types of sample preparation, liquid–liquid extraction (LLE) and reversed-phase solid-phase extraction (RP-SPE), using a styrene/divinyl benzene adsorbent. The determinative technique used was electron-impact gas chromatography-mass spectrometry (EI-GC-MS) after conversion of clofibric acid to its methyl ester by derivatizing with trimethyl silyl diazomethane. An internal standard mode of instrument calibration (introduced in Chapter 2) was used to provide a trace quantitative analysis of samples of sewage effluent to determine how much clofibric acid is present. Shown below are the molecular structures for clofibric acid and two organic compounds, 3,4-D and PCB 104 (2,2′,4,6,6′-pentachlorobiphenyl), used by the authors to calibrate the instrument based on the internal standard mode:

Since EI-GC-MS was the only instrumental determinative technique (determinative techniques are introduced in Chapter 4) used, two EI mass spectra are compared in Figure 1.2. The mass spectrum shown on top in Figure 1.2 is for a background-subtracted standard or clofibric acid methyl ester, while the mass spectrum shown below is for a background-subtracted mass spectrum obtained from the effluent
sample extract at the retention time of clofibric acid methyl ester. The disputable fact that both mass spectra are identical demonstrates the unequivocal nature of identification, sometimes referred to in EPA methods as confirmation. Figure 1.2 illustrates trace environmental qualitative analysis. Using all abundant fragment ions or even one or more selected fragment ions with which to build a calibration curve, and from this curve to interpolate and thus to find how much clofibric acid is present in the unknown extract from the waste effluent, nicely illustrates the science of trace environmental quantitative analysis.

Let us summarize some regulatory issues, first from this emerging enviro-health arena. We then complete this introductory chapter with an emphasis on the well-established enviro-chemical arena, largely reviewing the significant environmental regulations. We then show just how the EPA methods fit in. A significant question is before us with respect to enviro-health.
3. TO WHAT EXTENT DO ENVIRONMENTAL CONTAMINANTS ENTER HUMANS?

Table 1.1 (metals) and Table 1.2 (organics) highlight selected analytical results from the first National Report on Human Exposure to Environmental Chemicals, conducted by the CDC. This report provides exposure information about people participating in an ongoing national survey of the general U.S. population — the National Health and Nutrition Examination Survey (NHANES). The survey was conducted by the National Center for Health Statistics of the CDC. This first report presents data for the general U.S. population from the 1999 NHANES. According to the report, this survey was conducted in only 12 locations across the country. Most analyses were conducted in subsamples for the population. More data would be needed to confirm these findings and to allow more detailed analysis to describe exposure levels in population subgroups.  

All the metals determined are listed in Table 1.1, while just those organics that reveal a level above the limit of detection are shown in Table 1.2. The report makes
it very clear that the presence of detectable concentration levels of chemical substances does not indicate that the chemical causes disease. Since 1976, CDC has measured blood Pb levels as part of NHANES. Results presented in Table 1.1 show that the geometric mean blood Pb levels for children aged 1 to 5 have decreased to 2.0 from 2.70 µg/dL, the geometric mean for the period 1991–1994. These decreases in blood Pb levels indicate a success in public health efforts to decrease the exposure of children to Pb.

Not shown in either Table 1.1 or Table 1.2 are the results for reduced exposure of the U.S. population to environmental tobacco smoke (ETS). Cotinine is a metabolite of nicotine that tracks exposure to ETS. Molecular structures for both cotinine and its precursor, nicotine, are shown below:

A decrease in serum cotinine concentration levels from 0.20 ng/mL obtained during the period 1988–1991 to 0.050 ng/mL (obtained in this study) among people aged 3 years and older (a 75% decrease) indicates a dramatic reduction in exposure of the general population to ETS over the past decade.

Table 1.2 reveals some surprising results. CDC scientists measured metabolites of seven major phthalates. Di-2-ethylhexyl phthalate and di-iso-nonyl phthalate are two phthalates produced in greatest quantity; however, metabolites of diethyl and

<table>
<thead>
<tr>
<th>Organic Pesticide or Metabolite</th>
<th>Human Specimen</th>
<th>No. of People Sampled</th>
<th>Units</th>
<th>Geometric Mean (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl phosphate</td>
<td>Blood</td>
<td>703</td>
<td>µg/L</td>
<td>1.84 (1.10–2.59)</td>
</tr>
<tr>
<td>Dimethyl thiophosphate</td>
<td>Blood</td>
<td>703</td>
<td>µg/L</td>
<td>2.61 (1.77–3.45)</td>
</tr>
<tr>
<td>Diethyl phosphate</td>
<td>Blood</td>
<td>703</td>
<td>µg/L</td>
<td>2.55 (1.33–3.78)</td>
</tr>
<tr>
<td>Diethyl thiophosphate</td>
<td>Blood</td>
<td>703</td>
<td>µg/L</td>
<td>0.81 (0.69–0.94)</td>
</tr>
<tr>
<td>Dimethyl dithiophosphate</td>
<td>Blood</td>
<td>703</td>
<td>µg/L</td>
<td>0.51 (0.39–0.62)</td>
</tr>
<tr>
<td>Diethyl dithiophosphate</td>
<td>Blood</td>
<td>703</td>
<td>µg/L</td>
<td>0.19 (0.14–0.23)</td>
</tr>
<tr>
<td>Monobenzyl phthalate</td>
<td>Blood</td>
<td>1029</td>
<td>µg/L</td>
<td>17.4 (14.1–20.7)</td>
</tr>
<tr>
<td>Monobutyl phthalate</td>
<td>Blood</td>
<td>1029</td>
<td>µg/L</td>
<td>26.7 (23.9–29.4)</td>
</tr>
<tr>
<td>Monoethyl phthalate</td>
<td>Blood</td>
<td>1029</td>
<td>µg/L</td>
<td>176.0 (132–220)</td>
</tr>
<tr>
<td>Mono-2-ethyl hexyl phthalate</td>
<td>Blood</td>
<td>1029</td>
<td>µg/L</td>
<td>3.5 (3.0–4.0)</td>
</tr>
</tbody>
</table>

dibutyl phthalate were much higher in the population than levels of metabolites of the most ubiquitous phthalates found in the environment.

Trace enviro-health quantitative analysis, also abbreviated TEQA, is, in this author’s opinion, an evolving subdiscipline of trace environmental quantitative analysis. The Clinical Laboratory Improvement Act of 1988 (CLIA’88) regulates the chemical laboratory and addresses those aspects of traditional clinical chemistry, such as determining the concentration of creatinine in blood. Toxicological chemistry also includes blood alcohol, digoxin, lithium, primidone, and theophylline assays. The concentrations in the blood and urine of these analytes are significantly higher than those that would be considered at a trace level. Our focus in this book is to discuss how environmental pollutants can be quantitatively determined in human specimens. However, environmental priority pollutants found in human specimens may have entered the human domain via the various routes of exposure. Figure 1.3

---

**FIGURE 1.3** Routes of human exposure to environmental contaminants. Types of body fluids as human specimens for biomonitoring.
depicts routes of exposure to environmental priority pollutants along with the possible kinds of body fluids, shown as ovals, that could be defined as suitable human specimens for biomonitoring. Three routes of exposure include inhalation to the respiratory tract, ingestion to the gastrointestinal tract, and absorption through the skin, often termed dermal exposure. The development of so-called biological markers (biomarkers) represents a very active research area involving toxicologists and epidemiologists. TEQA has a vital role to play in this research today. A biomarker can be either cellular, biochemical, or molecular in nature and can be measured analytically in biological media such as tissues, cells, or fluids. A suitable biomarker could be an exogenous substance or its metabolite. It could also be a product of an interaction between the xenobiotic agent and some target molecule. Exposure and dose are two terms that are further elaborated below:

- Exposure is contact of a biological, chemical, or physical agent with the surface of the human body.
- Dose is the time integral of the concentration of the toxicologically active form of the agent at the biological target tissue.
- Dose links exposure to risk of disease.
- Exposure = dose = effect.

The relationship between exposure, dose, and potential health effects is summarized below:

Figure 1.3 depicts a biomonitoring scenario centered with respect to a person’s blood supply. This model does not include adipose or other human tissue. Blood and urine are emerging as the most convenient human specimens to collect and conduct biomonitoring.
Let us return to the concept of a biomarker as a key ingredient in biomonitoring. Biomarkers provide evidence of both exposure and uptake. The concentration level of a given biomarker is directly related to tissue dose. Biomarkers account for all possible routes, as shown in Figure 1.3. Biomarkers account for differences in people. Factors that limit the usefulness of biomarkers include:

- The fact that many biomarkers are still being developed
- The need for standardized protocols in both collection and analysis
- Variability in relationship with exposure
- Timing — each biomarker has a characteristic half-life
- Expense
- Difficulty in interpreting

4. WHAT MIGHT AN ANALYTICAL CHEMISTRY APPROACH TO BIOMONITORING LOOK LIKE?

One such answer to this question can be found in Figure 1.4. The scenario “from human specimen to analytical result” is listed in terms of five essential and sequential steps, each linked by a chain-of-custody protocol. The arrows show that the relationship between steps must include a chain-of-custody protocol. This protocol might take the form of a written document. If, however, a Laboratory Information Management System (LIMS) is in place, the protocol takes the form of an entry into a computer that utilizes a LIMS. Referring to Figure 1.4, the sample prep lab may give to the analyst a complete sample extract along with a signed chain-of-custody form to provide evidence as to where the extract is headed next. This five-step approach to biomonitoring is also applicable to trace enviro-chemical quantitative analysis.

We leave for the moment trace enviro-health quantitative analysis and pick up trace enviro-chemical quantitative analysis. Let us first define what we mean by environmental chemistry.

5. WHAT KIND OF CHEMISTRY IS THIS?

The academic discipline of environmental chemistry is a relatively recent development. Environmental chemistry can be defined as a systematic study of the nature of matter that exists in the air, water, soil, and biomass. This definition could be extended to the plant and animal domains where chemicals from the environment are likely to be found. This discipline, which developed in the late 1960s, requires the knowledge of the traditional branches of organic, inorganic, physical, and analytical chemistry. Environmental chemistry is linked to biotechnology as well as to chemical, environmental, and agricultural engineering practices.

Environmental analytical chemistry can be further defined as a systematic study that seeks to answer two fundamental questions: *What* and *how* much matter exists in the air, water, soil, and biomass? This definition could also be extended to the plant and animal domains just discussed. This discipline, which developed in the
1970s, spearheaded by the first Earth Day in 1970 and the establishment of the U.S. EPA, requires a knowledge of traditional quantitative analysis, contemporary instrumental analysis, and selected topics, such as statistics, electronics, computer software, and experimental skill. Environmental analytical chemistry represents the fundamental measurement science to biotechnology and to chemical, environmental, and agricultural engineering practices. That portion of environmental analytical chemistry devoted to rigorously quantifying the extent to which chemical substances have contaminated the air, water, soil, and biomass is the subject of this book.

In its broadest sense, environmental chemistry might be considered to include the chemistry of everything outside of the synthetic chemist’s flask. The moment that a chemical substance is released to the environment, its physico-chemical properties are subject to change. The kinetics of these changes are a critical part of environmental chemistry, and the analyst must be prepared to measure chemical species both in equilibrium and as they change over time. This book focuses on the methods used to analyze chemical substances in the environment.
properties may have an enormous impact on ecological systems, including humans. Researchers have identified 51 synthetic chemicals that disrupt the endocrine system. Hormone disrupters include some of the 209 polychlorinated biphenyls (PCBs) and some of the 75 dioxins and 135 furans that have a myriad of documented effects (p. 81). The latter half of the 20th century has witnessed more synthetic chemical production than any other period in world history. Between 1940 and 1982, the production of synthetic chemicals increased about 350 times. Billions of pounds of synthetic materials were released into the environment during this period. U.S. production of carbon-based synthetic chemicals topped 435 billion pounds in 1992, or 1600 pounds per capita (p. 137).

The concept of environmental contaminants as estrogenic “mimics” serves to bring attention to the relationship between chemicals and ecological disruption. The structural similarity between DDT and diethyl stilbestrol is striking. The former chemical substance was released into the environment decades ago, whereas the latter was synthesized and marketed to pregnant women during the 1950s and then used as a growth promoter in livestock until it was banned by the Food and Drug Administration (FDA) in 1979.

At levels typically found in the environment, hormone-disrupting chemicals do not kill cells or attack DNA. Their target is hormones, the chemical messengers that move about constantly within the body’s communication. They mug the messengers or impersonate them. They jam signals. They scramble messages. They sow disinformation. They wreak all manner of havoc. Because messages orchestrate many critical aspects of development, from sexual differentiation to brain organization, hormone-disrupting chemicals pose a particular hazard before birth and early in life (pp. 203–204).

A more recent controversy has arisen around the apparent leaching of Bisphenol A from various sources of plastics that are in widespread use among consumers. Earlier, the isolation and recovery of Bisphenol A from human urine was discussed. How could that method be changed to enable Bisphenol A to be isolated and recovered from an environmental matrix such as plastic wrap? Molecular structures for p,p'-DDT and diethyl stilbestrol are shown below. Compare these structures to that shown earlier in this chapter for Bisphenol A. The similarities in molecular structure are striking.

The EPA has released its plan for testing 15,000 chemicals for their potential to disrupt hormone systems in humans and wildlife. These chemicals were chosen because they are produced in volumes greater than 10,000 pounds per year.
One usually hears about environmental catastrophes through the vast resources of the mass media (i.e., radio, television, newspaper, popular magazines, newsletters from special interest organizations, etc.). The mass media usually assigns a name to the disaster that also includes a geographic connotation. Examples include the Valdez Oil Spill in Alaska, Love Canal in New York, Seveso, Italy, and Times Beach, Missouri. What is not so newsworthy, yet may have as profound an impact on the environment, is the ever-so-subtle pollution of the environment day in and day out. Both catastrophic pollution and subtle pollution require the techniques of TEQA to obtain data that enable society to continuously monitor the environment to ensure minimal ecological and toxicological disruption. It is the combination of sophisticated analytical instruments (Chapter 4), sample preparation schemes (Chapter 3), mathematical treatment of analytical data (Chapter 2), and detailed practical procedures (Chapter 5) that enables a student or practicing analyst to effectively conduct TEQA.

This book provides insights and tools that enable an individual who either works in an environmental testing lab or public health lab or anticipates having a career in the environmental science or environmental health field to make a contribution. Individuals are thus empowered and can begin to deal with the problems of monitoring and sometimes finding the extent to which chemicals have contaminated the environment or entered the human body.

6. WHO NEEDS ENVIRONMENTAL TESTING?

It is too easy to answer this question with “everyone.” The industrial sector of the U.S. economy is responsible for the majority of chemical contamination released to the environment. Since the early 1970s, industry has been under state and federal regulatory pressures not to exceed certain maximum contaminant levels (MCLs) for a variety of so-called priority pollutant organic and inorganic chemical substances. However, one of the more poignant examples of small-time pollution is that of dry cleaning establishments located in various shopping plazas throughout the U.S. These small businesses would follow the practice of dumping their dry cleaning fluid into their septic systems. It was not unusual, particularly during the 1980s, for labs to analyze drinking water samples drawn from an aquifer that served the shopping plaza and find parts per billion (ppb) concentration levels of chlorinated volatile organics such as perchloroethylene (PCE).

The necessary sample preparation needed to modify a sample taken from an aquifer that is expected to contain PCE, so as to enable the sample to become compatible with the appropriate analytical instrument, will be described in Chapter 3. The identification and quantitative determination of priority pollutants like PCE in drinking water require sophisticated analytical instrumentation. These so-called determinative techniques will be described in Chapter 4. A laboratory exercise that might introduce a student to the technique involved in sample preparation and instrumental analysis to quantitatively determine the presence or absence of a chlorinated volatile organic like PCE will be described in Chapter 5.
7. WHO REQUIRES INDUSTRY TO PERFORM TEQA?

Demand for trace environmental analysis is largely regulatory driven, with the exception of the research done in methods development by both the private sector and federal, state, and academic labs. The major motivation for a company to conduct TEQA is to demonstrate that its plant’s effluent falls within the MCLs for the kinds of chemical contaminants that are released. There exists a myriad of laws that govern discharges, and these laws also specify MCLs for targeted chemical contaminants. The following outline is a brief overview of the regulations, and it incorporates the abbreviations used by practitioners in this broad category of environmental compliance and monitoring (pp. 1–32).

8. HOW DOES ONE MAKE SENSE OF ALL THE “REGS”? 

The following outline summarizes the federal regulations responsible for environmental compliance:

A. Title 40 Code of Federal Regulations (40 CFR): This is the ultimate authority for environmental compliance. New editions of 40 CFR are published annually and are available on the World Wide Web. This resource includes chapters on air, water, pesticides, radiation protection, noise abatement, ocean dumping, and solid wastes. Superfund, Emergency Planning and Right-to-Know, effluent guidelines and standards, energy policy, and toxic substances are among other topics.

B. Government regulations administered by the Environmental Protection Agency (EPA)
   2. Comprehensive Environmental Response, Compensation, and Liability Act (Superfund) (CERCLA): This authority granted to the EPA enables the agency to take short-term or emergency action to address hazardous situations that affect health. The release of the toxic chemical isocyanate in the Bhopal, India, community that left over 3000 dead might have fallen under CERCLA if it had occurred in the U.S. In addition, the CERCLA contains the authority to force the cleanup of hazardous waste sites that have been identified based on environmental analytical results and placed on the National Priority List. The EPA also has authority to investigate the origins of waste found at these sites and to force the generators and other responsible parties to pay under CER-CLA. Analytical methods that deal with CERCLA are provided.
through the Contract Laboratory Program (CLP). The actual methods are found in various Statements of Work (SOWs) that are distributed to qualified laboratories.

3. Drinking Water and Wastewater: The Safe Drinking Water Act, last amended in 1986, gives EPA the authority to regulate drinking water quality. Two types of chemical compounds, called targeted analytes, are considered in this act. The first is the National Primary Drinking Water Standards. These chemical substances affect human health, and all drinking water systems are required to reduce their presence to below the MCL set for each compound by the federal government. The second is the National Secondary Drinking Water Standards. These analytes include chemical substances that affect the taste, odor, color, and other non-health-related qualities of water. A given chemical compound may appear on both lists at different levels of action. The primary drinking water monitoring contaminants are listed in Table 1.3 to Table 1.5 by category. The secondary drinking water monitoring contaminants are listed in Table 1.6. The MCL is listed as well as the method detection limit (MDL). Note that the MDL should always be less than the MCL. This was not always true historically in the field of TEQA because available technology always serves to limit the MDL, whereas ecological and toxicological considerations govern the decision to estimate what makes for an environmentally acceptable MCL.

### TABLE 1.3
**Primary Drinking Water Monitoring Requirements for Inorganics**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>MCL (mg/L)</th>
<th>MDL (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimony</td>
<td>0.006</td>
<td>0.0008–0.003</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Barium</td>
<td>2</td>
<td>0.001–0.1</td>
</tr>
<tr>
<td>Beryllium</td>
<td>0.004</td>
<td>0.00002–0.0003</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.005</td>
<td>0.0001–0.001</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.1</td>
<td>0.001–0.007</td>
</tr>
<tr>
<td>Copper</td>
<td>1.3</td>
<td>0.001–0.05</td>
</tr>
<tr>
<td>Cyanide</td>
<td>0.2</td>
<td>0.005–0.02</td>
</tr>
<tr>
<td>Fluoride</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.015</td>
<td>0.001</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.002</td>
<td>0.0002</td>
</tr>
<tr>
<td>Nickel</td>
<td>1.1</td>
<td>0.006–0.005</td>
</tr>
<tr>
<td>Nitrate-N</td>
<td>10</td>
<td>0.01–1</td>
</tr>
<tr>
<td>Nitrite-N</td>
<td>1</td>
<td>0.004–0.05</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.05</td>
<td>0.002</td>
</tr>
<tr>
<td>Sodium</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Thallium</td>
<td>0.002</td>
<td>0.0007–0.001</td>
</tr>
</tbody>
</table>
The Clean Water Act, which was last amended in 1987, provides for grants to municipalities to build and upgrade treatment facilities. The act also establishes a permit system known as the National Pollutant Discharge and Elimination System (NPDES) for discharge of natural water bodies by industry and municipalities. Over two thirds of the states have accepted responsibility for administration of the act. The act and its amendments are based on the fact that no one has the right to pollute the navigable waters of the U.S. Permits limit the composition and concentration of pollutants in the discharge. Wastewater effluents are monitored through the NPDES, and this analytical testing has been

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>MCL (mg/L)</th>
<th>MDL (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipates</td>
<td>0.4</td>
<td>0.006</td>
</tr>
<tr>
<td>Alachlor</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Atrazine</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.04</td>
<td>0.009</td>
</tr>
<tr>
<td>Chlordane</td>
<td>0.002</td>
<td>0.0002</td>
</tr>
<tr>
<td>Dalapon</td>
<td>0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Dibromochloropropane</td>
<td>0.0002</td>
<td>0.00002</td>
</tr>
<tr>
<td>2,4-dichlorophenoxy-acetic acid</td>
<td>0.07</td>
<td>0.001</td>
</tr>
<tr>
<td>Dinoseb</td>
<td>0.007</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diquat</td>
<td>0.02</td>
<td>0.0004</td>
</tr>
<tr>
<td>Endothall</td>
<td>0.1</td>
<td>0.009</td>
</tr>
<tr>
<td>Endrin</td>
<td>0.002</td>
<td>0.00001</td>
</tr>
<tr>
<td>Ethylene dibromide</td>
<td>0.0005</td>
<td>0.00001</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>0.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>0.0004</td>
<td>0.0004</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Hexachlorocyclopentadiene</td>
<td>0.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.0002</td>
<td>0.00002</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>0.04</td>
<td>0.0001</td>
</tr>
<tr>
<td>Oxyamyl</td>
<td>0.2</td>
<td>0.002</td>
</tr>
<tr>
<td>PAHs</td>
<td>0.0002</td>
<td>0.00002</td>
</tr>
<tr>
<td>PCBs</td>
<td>0.0005</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>0.001</td>
<td>0.00004</td>
</tr>
<tr>
<td>Phthalates</td>
<td>0.006</td>
<td>0.0006</td>
</tr>
<tr>
<td>Picloram</td>
<td>0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Simazine</td>
<td>0.004</td>
<td>0.00007</td>
</tr>
<tr>
<td>Toxaphene</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>2,3,7,8-TCDD (dioxin)</td>
<td>0.00000003</td>
<td>0.00000005</td>
</tr>
<tr>
<td>2,4,5-TP (Silvex)</td>
<td>0.05</td>
<td>0.0002</td>
</tr>
<tr>
<td>Trichloromethanes (total)</td>
<td>0.1</td>
<td>0.0005</td>
</tr>
</tbody>
</table>
a boon for commercial analytical laboratories. Every industry and wastewater treatment facility that directs into a receiving stream or river has either a federal or state NPDES permit. Methods of analysis are given in 40 CFR 136 for the following:

1. Conventional pollutants such as biological oxygen demand (BOD), chemical oxygen demand (COD), pH, total suspended solids, oil and grease, and fecal coliforms
2. Nonconventional pollutants such as nitrogen, phosphorous, and ammonia
3. The 129 so-called priority pollutants

These pollutants are listed in Table 1.3 to Table 1.5.

4. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA): This act requires the EPA to oversee the manufacture and use of chemical substances that directly terminate insects, fungi, and rodents. Analytical methods are required for the analysis of residues in air, soil, and water. Specifications for labeling and warnings are also required under this act.

5. Food, Drug, and Cosmetic Act: Administered by the Food and Drug Administration (FDA), this act governs all chemicals added to foods, drugs, and cosmetics. These substances are not directly under EPA.
oversight; however, the wastes and by-products generated from the manufacture of these substances are controlled by EPA.

6. Toxic Substance Control Act (TSCA): Since 1976, the EPA has been given the authority to gather information on the toxicity and hazardous nature of individual chemicals. Chemical producers are required to supply information dealing with risk assessment of proposed products 90 days before proposed manufacture or import. Included in this information are chemical fate testing, environmental effects testing, and health effects testing.

7. Superfund Amendments and Reauthorization Act (SARA): Passed in 1986, this act extends the lifetime of the legislation begun in CERCLA and gives the EPA the authority to remediate a site if no responsible parties can be found to pay.

8. Clean Air Act (CAA): The CAA amendments passed in 1990 gave the EPA authority to regulate many hazardous air pollutants (HAPs). Over 100 of these HAPs must be regulated; they include well-known organic compounds such as acetaldehyde, benzene, carbon tetrachloride, 1,4-dioxane, hexane, methyl methacrylate, and so forth. The CAA also requires the EPA to establish permits for the regulation of the maximum amounts of emissions by various industries in a manner similar to that of the NPDES program for wastewater effluents.

As if the number of regulations is not enough, many environmental testing labs also have to satisfy state requirements. Most states run their own private lab certification
programs. Also, some of the method details differ between state and federal programs. One example from the author’s own experience working in an EPA contract lab during the late 1980s involves the implementation of EPA Method 8270 for semivolatile organics in solid waste. EPA Method 3640A, Gel Permeation Chromatography (GPC), is added to any 1 of the 3500 series of sample preparation methods, particularly if soil samples are saturated with oil. To satisfy the New Jersey Department of Environmental Protection requirements when this method is implemented requires that the GPC cleanup step not be used even if some soil samples that arrive to the laboratory are indeed saturated with oil. On the other hand, if similar oil-laden soil samples were analyzed for the New York Department of Environmental Conservation, GPC cleanup is acceptable. What is an analyst to do?

9. WHAT ANALYTICAL METHODS SATISFY THESE REGULATIONS?

Today, myriads of analytical methods exist that the above-cited regulations use to demonstrate compliance. The two parameters cited in Table 1.3 to Table 1.5, namely, the MCL and MDL, are only obtained with good accuracy and precision by applying all of the skills and techniques of TEQA. The analyst who is about to work in the environmental testing laboratory must have access to these written methods in order to become familiar with their content before he or she can effectively execute the methods themselves. The EPA during the past 30 years has operated within two broad mental frameworks or paradigms. The first paradigm, which began in the early

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0000s</td>
<td>Air emission sampling methods from stationary sources</td>
</tr>
<tr>
<td>1000s</td>
<td>Methods to determine hazardous waste characteristics: ignitibility, corrosivity, and reactivity; includes the toxicity characteristic leaching procedure (TCLP)</td>
</tr>
<tr>
<td>2000s</td>
<td>Unused</td>
</tr>
<tr>
<td>3000s</td>
<td>Sample preparation methods for inorganics and organics</td>
</tr>
<tr>
<td>4000s</td>
<td>Soil screening methods by immunoassay</td>
</tr>
<tr>
<td>5000s</td>
<td>Sample preparation methods for volatile organics and miscellaneous sample preparation methods</td>
</tr>
<tr>
<td>6000s</td>
<td>Methods to determine more than one metal at a time</td>
</tr>
<tr>
<td>7000s</td>
<td>Methods to determine one metal at a time</td>
</tr>
<tr>
<td>8000s</td>
<td>Methods to determine organics</td>
</tr>
<tr>
<td>9000s</td>
<td>Miscellaneous test methods; includes cyanides, sulfides, phenols, oil and grease, chlorine, total coliform, potentiometric halides and cyanide, colorimetric chloride and radium-228 isotope</td>
</tr>
</tbody>
</table>

1970s and lasted until the early 1990s, suggested that the methods written by the agency were the only ones that a laboratory could use in order to satisfy the “regs.” This mind-set led to the proverbial “EPA approved” stamp of approval. Instrument manufacturers during the late 1980s would incorporate into their advertisements that their product was EPA approved. During these years, instrument vendors sought collaborations with various EPA offices in an attempt to accelerate the more than 10-year delay between proposal of a new method and its acceptance by the EPA.

For example, the development of an instrumental method that would determine more than one inorganic anion in a drinking water sample was announced in 1975. It took until around 1985 for this technique to become incorporated into EPA Method 300.0 as applied to drinking water. Prior to the development of ion chromatography, individual anionic species such as chloride, nitrate, phosphate, and sulfate were determined individually by applying specific colorimetric methods. Each colorimetric method would require separate sample preparation and determinative steps culminating with the use of a spectrophotometer. The idea that a sample could be injected into some kind of chromatograph and all of the ions separated, detected, and quantitated in a reasonable period represented a bit of a revolutionary concept. It was also thought that there would be a serious matrix effect that would render this new method useless when it came time to analyze “dirty” samples. Today, after some 20 or more years of instrumentation refinement, the advantages and limitations of the method that requires ion chromatography are well understood. This technique has even advanced to the point where one can conduct analyses without having to prepare either an eluent (mobile phase passed through the ion exchange column) or a regenerate (mobile phase passed through the suppressor), thus further simplifying the implementation of this methodology.

Should it take a decade or two for a newly developed method to become EPA approved? The future appears to not be emulating the past, as the second broad mental framework is taking hold; the agency calls it the performance-based method (PBM).

The new paradigm places more of the responsibility for quality assurance on the individual analyst and the laboratory rather than on the method itself. It used to be that as long as an analyst followed the recipes (sounds a lot like cooking) in the various methods, then quality data were assured. The analyst’s input in the process was not required, nor was it requested. This point of view took the analyst out of the picture and served to define the analyst more as a robot-technician than as an involved and motivated professional. This point of view left no room for analyst intervention. If an analyst wished to skip a step here or there in the method, it was assumed that the method was not followed and, therefore, the data could not be assured or, in the parlance of the time, EPA approved. PBMs hopefully have and will change this somewhat myopic view of TEQA and, furthermore, begin to place the responsibility on the analyst to yield quality data that are assured based on method performance, as opposed to blindly adopting the recipe.

For example, instead of an EPA-approved method specifying that a particular gas chromatographic (GC) column be used to measure analyte A in matrix B, the laboratory is free to choose what column to use, as long as an equivalent degree of quality assurance is obtained. In other words, analytical results for the concentration
of analyte A in matrix B from the approved method vs. the PBM approach should be nearly identical. In addition, the laboratory would not have to conduct the side-by-side comparison of a more conventional sample preparation against a proposed alternative in this new paradigm. For example, it was once considered heresy for an analyst to analyze wastewater samples to determine the slew of organochlorine pesticides using anything except a glass column that contained Supelcoport coated with 1.5% SP-2250/1.95% SP-2401 packed into a 1.8-m-long × 4-mm-inner-diameter tube (the essence of Method 608). However, during the late 1980s, labs, including the one this author worked in, began to investigate megabore capillary columns as alternatives to packed columns. Megabore capillary columns made from fused silica could be easily connected to the common ¼-in. injection port used for packed columns via megabore adapter kits. When combined with element-specific detectors, many new organics could be separated and identified using megabore capillary GC columns.13

Performance-based methods are an empowering concept. PBMs enable analysts and the laboratories that they work in to become more creative and encourage attempts to develop alternative methods to perform TEQA. Many of these alternative approaches are much more cost effective while yielding the same outcome. In other words, the performance of the method is the same, deliverable outcome, and the inputs are much reduced in cost and labor. Alternative methods of sample preparation will be discussed in Chapter 3.

10. HOW ARE THE REGULATORY METHODS FOR TEQA IDENTIFIED?

All EPA methods are classified based on a number, whereas other methods are categorized based on the chemical composition of the analytes of interest. It is useful at this point to briefly outline these EPA method numbers while providing a brief description of their origin (pp. 33–36).10 All of the major offices within the EPA have had a role in developing analytical methods that bear a number. Series 1 to 28 are air monitoring methods found in 40 CFR Appendix A, whereas series 101 to 115 are air monitoring methods in 40 CFR Appendix B. Methods that have three digits refer to methods for the chemical analysis of water and wastes and represent some of the earliest EPA methods. The 100 series of methods characterize the physical properties of water and wastes. The 200 series is devoted to metals, and the 300 series deals with inorganics and nonmetals. This is why the ion chromatographic technique discussed earlier is found in this series. The 400 series deals with organics where the emphasis is on nonspecific organics that are primarily found in wastewater. Method 413 for oil and grease, Method 415 for total organic carbon, Method 420 for total recoverable phenols, and Method 425 for methylene blue active surfactants are examples of nonspecific analytical methods that give good indications of whether a waste sample contains organics without the need to be specific. These are workhorse methods that are usually found in most environmental testing labs.

Method 413 has recently become controversial in that the manufacture of the common extraction solvent used in the method, 1,1,2-trichlorotrifluoroethane
(TCTFE), itself a fluorocarbon (Freon 113), is banned. The attributes of TCTFE are such that it enabled this method to move from a purely gravimetric approach (EPA Method 413.1, Gravimetric, Separatory Funnel Extraction) to an instrumental approach, whereby the carbon-to-hydrogen stretching frequency in the infrared spectrum enabled a significant reduction in the MDL to occur. The infrared approach was assigned its own method number, EPA Method 413.2, Spectrophotometric, Infrared. This author’s personal opinion is that the more cost effective infrared approach coupled with the lower MDLs should be maintained. Labs should be strongly encouraged to recycle the waste TCTFE solution so that it can be reused. A simple distillation should suffice to return TCTFE in sufficient purity to use to reextract samples. However, the EPA has chosen to establish Method 1664 for oil and grease analysis. TCTFE has been replaced by hexane, and the determination is by weighing the residue after solvent evaporation.

One good thing about this new method is that it is performance based. The analyst can use an alternative approach to oil and grease isolation and recovery. One approach is to use solid-phase extraction (SPE) to isolate the oil and grease, whereas the other approach is to find a way to eliminate use of the solvent. The recently introduced use of infrared cards by 3M Corporation, whereby the oil and grease remain on a thin film of Teflon, is an example of the latter alternative to the conventional liquid–liquid extraction (LLE). The thin film that now offers a fixed path length is then inserted into the sample holder of a conventional infrared spectrophotometer, and the absorbance of the C–H stretching vibration at 2900 cm⁻¹ is directly related to concentration via the Beer–Lambert law of spectrophotometry, discussed in Chapter 4.

The 500 series methods refer to measurement of trace organics in drinking water. This is a collection of methods first published in the late 1980s and incorporating a number of the more innovative techniques introduced at that time. The 600 series methods describe how to isolate, recover, and measure trace organics in wastewaters. These methods were first promulgated in the 1970s. Both series of methods measure specific organic compounds and require a means to separate, identify, and quantify these organics. Thus, samples that may contain two or more compounds require a chromatographic separation. Hence, most all methods in these two series are GC methods. The scope of both methods is also limited to organics that are amenable to GC. Sample preparation for trace volatile organics (commonly abbreviated VOCs) will be introduced in Chapter 3, and the principles of GC will be introduced in Chapter 4.

The 900 series of methods refer to the measurement of radioactivity in drinking water. These methods were first promulgated in 1980.

The four-digit series, methods 0000 to 9999, involve the analysis of solid wastes. This series was promulgated in the early 1980s and has undergone several major revisions up to the present. This series, when compared to all of the others, seems to be the most dynamic and is considered by the EPA as a general guide. These viewpoints infer that these methods were written within the framework of the second paradigm. In other words, the methods themselves are performance based. The series is periodically updated and revised, and new methods are continuously added. A novice to the field of TEQA would do well to initially focus on this series as a
learning tool. A table of contents for this series of analytical methods, which is collectively known as SW-846, 3rd edition, is given in Table 1.1. To illustrate the dynamic nature of the SW-846 series of analytical methods for TEQA, consider the very recent development of Method 7473 for determining mercury in various solids of environmental interest. A solid sample such as apple leaves, oyster tissue, coal fly ash, or river sediment is dried and thermally decomposed at 750°C in an oxygenated furnace. Mercury vapor is released, swept through, and trapped on a gold amalgamator. The amalgam is thermally desorbed, and the Hg vapor is swept into a cold vapor atomic absorption spectrophotometer, where the absorbance is measured at 254 nm. Again, the Beer–Lambert law is used to relate Hg absorbance to concentration. An instrument detection limit (IDL) is reported to be 0.01 ng of total Hg.

One of the most challenging methods to implement in the environmental testing laboratory is EPA Method 8270. This is a method that separates, identifies, and quantifies over 100 priority pollutant semivolatile and nonvolatile organics in various solid wastes. The method is a determinative one and is used in combination with one of the sample preparation methods found in the 3000 series of SW-846. A brief discussion of this method follows in order to introduce the reader to the challenges of implementing such a method. The most current SW-846 series lists a third revision of this method, identified as 8270C. When Method 8270 was first promulgated, a number of analytes from the targeted list found in the method either did not yield a high recovery or did not chromatograph well. This resulted in the analyst being unable to satisfy the criteria and led to a rejection of the analytical results in the validation process. Method 8270C clearly states at the onset that certain analytes are nondetected by this method or have an unfavorable distribution coefficient (to be discussed in Chapter 3) or adsorb to the inner walls of glassware or hydrolyze during sample preparation. The three revisions of this method serve to illustrate nicely how the EPA has been able to readjust its promulgated methods under the new PBM paradigm to address early flaws. Under the old paradigm, this realization on the part of the EPA was not evident.

The method itself is succinctly summarized as follows:

Method 8270 can be used to quantitate most neutral, acidic or basic organic compounds that are soluble in methylene chloride and capable of being eluted, without derivatization, as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone. Such compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons and pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolines, aromatic nitro compounds and phenols including nitrophenols.

11. WHY IS IT CONSIDERED A CHALLENGE TO IMPLEMENT AN EPA METHOD?

The most tedious and time consuming aspects of Method 8270 are involved in the preparation and organization of the various referenced chemical standards. This is in addition to the use of reference standards that are required in the various sample
preparation methods (i.e., the 3000 series). Method 8270C requires that the following categories of reference standards be prepared, maintained, and replenished, when necessary, by the analyst:

1. Stock reference standards prepared in the laboratory from the dissolution of neat forms of the individual targeted chemical compounds or purchased from various suppliers. The EPA used to provide these from its repository located in Research Triangle Park, NC, but no longer does.

2. Internal standard reference solutions that contain, with the exception of 1,4-dichlorobenzene-\textsubscript{d\textsubscript{8}}, five deuterated polycyclic aromatic hydrocarbons must be prepared. Table 1.8, adapted from the method, shows which of the semivolatile organics are to be quantitated against phenanthrene-\textsubscript{d\textsubscript{10}} as the internal standard. The analytes listed in Table 1.8 all elute from the capillary column over a range of retention times that are close to that of phenanthrene-\textsubscript{d\textsubscript{10}}.

3. GC-MS tuning standard for semivolatiles that contains the tuning compound decafluorotriphenyl phosphine (DFTPP) and also 4,4'-DDT, pentachlorophenol, and benzidine. DFTPP is used to verify that the hardware tune was carried out previously, and the other three analytes are used to verify injection port inertness and GC column performance. Polar compounds like phenols tend to adsorb to contaminated surfaces and result in the complete loss of response or poor peak shape.

4. Dilution of stock solutions to prepare the series of working calibration standards that are injected into the gas chromatograph-mass spectrometer (GC-MS) to calibrate the instrument for each of the 100 or more semivolatile organics. This series of standards should also contain at least one initial calibration verification (ICV) standard to assess the accuracy and precision of the calibration curve.

5. Surrogate standard solutions whose analytes are chemically different from any of the targeted analytes and are added to each sample to assess percent recovery.

| TABLE 1.8 |
| List of Priority Pollutant Semivolatile Organics Quantitated with Respect to the Internal Standard Phenanthrene-\textsubscript{d\textsubscript{10}} |
| 4-Aminobiphenyl | Hexachlorobenzene |
| Anthracene | N-Nitrosodiphenylamine |
| 4-Bromophenyl phenyl ether | Pentachlorophenol |
| Di-\textsubscript{n}-butyl phthalate | Pentachloronitrobenzene |
| 4,6-Dinitro-2-methyl phenol | Phenacetin |
| Diphenylamine | Phenanthrene |
| Fluoranthene | Pronamid |

© 2006 by Taylor & Francis Group, LLC
6. Matrix spike standard solutions and laboratory control standards that are added to one sample per batch to assess matrix effects.
7. System performance check standards and calibration check standards are used to ensure that minimum response factors are met before the calibration curve is used.
8. Blanks that represent standards that contain no targeted analytes, surrogates, or matrix spikes and are used to assess the degree to which a laboratory is contaminated.

All of these standards must be available to the analyst in the laboratory. A most important aspect of an analyst’s job is to keep track of all of these standards.

The requirement that the analytes be quantitated using an internal standard mode of instrument calibration is due to the fact that mass spectrometers are intrinsically unstable; that is, their response factor varies with time when compared to other GC detectors, such as the flame ionization detector (FID). The internal standard technique of instrument calibration is discussed in Chapter 2, and the principles of mass spectrometry are introduced in Chapter 4.

12. WHAT MADE EPA METHOD 625 SO UNIQUE?

EPA Method 625 discusses exactly how to isolate, recover, and quantitate the various priority pollutant VOCs from a wastewater matrix. The 600 series of EPA methods include both sample prep and determinative techniques in contrast to the SW-846 methods. It was realized back in the early 1970s that priority pollutant analyte identification (answers the trace qualitative analysis question) based only on analyte retention time (the time it takes for a given analyte or chemical compound to pass through the chromatographic column after being injected; retention time is usually given the symbol $t_R$) was inadequate for regulatory purposes relying only on gas chromatographs with element-specific or so-called standard detectors. According to Budde (p. 171):17

The proposal of Method 625 for regulatory use was very significant and had a major impact on the subsequent practice of environmental analyses and the development of commercial GC-MS instrumentation. The prospect of using a single analytical method with no required extract fractionation for 83 analytes was attractive even though it required a more costly analytical instrument. The alternative was the implementation of eight different GC columns and detectors. The economic implications were clear to the regulated industries and the emerging environmental testing industry.

Scheme 1.2 introduces the sample preparation rationale that was used to isolate and recover the original 83 priority pollutants from wastewater. Prior to the development of capillary GC columns, the use of packed GC columns necessitated that two fractions be prepared. A base-neutral fraction was prepared and injected into a GC-MS, followed by an acid fraction. The organic phase was further evaluated as shown in Scheme 1.3. Based on how “colored” the extract is, a preliminary screen uses a GC incorporating a flame ionization detector (GC-FID). The FID will be described in detail in Chapter 4, suffice to say here that this detector is universally
SCHEME 1.2

selective for organic compounds whose molecules contain a carbon–hydrogen covalent bond. The aqueous phase from Scheme 1.2 that contains the conjugate base for the organic acids, such as phenols, is acidified, extracted, and also evaluated based on the color of the extract, as shown in Scheme 1.4. Flowcharts will be used throughout this book to briefly describe sample preparation techniques. Their purpose is to help facilitate a quicker understanding of the many approaches to sample
preparation. Budde summarizes and further comments on Method 625 from a contemporary perspective, as follows (p. 173): 17

Method 625 was promulgated in wastewater regulations on 26 October 1984. The method requires the pH adjustments before extraction and specifies two packed GC columns. However, the method does allow the use of capillary GC columns, but does not provide information to assist the analyst in the application of these columns. As of 1999, Method 625 has not been revised to modify the pH adjustments before extraction, provide information about fused silica capillary columns, or incorporate other improvements in analytical techniques.

© 2006 by Taylor & Francis Group, LLC
13. WHAT ABOUT METHODS FOR THE ANALYSIS OF AIR AND OTHER METHODS?

This book clearly focuses on sample matrices drawn from condensed states of matter. However, there is a set of established methods chiefly to monitor targeted VOCs in ambient air. These are the EPA's toxic organics (TO) methods. Table 1.9 lists 11 TO methods along with the required sampling and determinative techniques. These methods are conventional air sampling techniques. A recent and exciting addition to ambient air sampling is to use a solid-phase microextraction fiber as a sampling technique.

© 2006 by Taylor & Francis Group, LLC
Methods IP1A to IP10B are a compendium of methods for determining indoor air pollutants. There is a National Institute for Occupational Safety and Health (NIOSH) Manual of Analytical Methods that covers air analysis. Two methods are found in the Code of Federal Regulations: 40 CFR 50 for lead, ozone, particulates, and NOX and 40 CFR 80 for phosphorous and lead in gasoline. A draft method for air analysis exists under the EPA Contract Laboratory Program (CLP).

Standard Methods for the Examination of Water and Wastewater, published once every 5 years by the American Public Health Association, American Water Works Association, and the Water Environment Federation is a well-regarded "bible" of methods for water and wastewater. As of this writing, the 20th edition of Standard Methods is available. Table 1.10 is a condensed table of contents for the 20th edition and provides a concise overview of its content. There is a strong emphasis on microbiology that is not necessarily found in EPA’s SW-846 series.

Official Methods of AOAC International is the other large compendium of analytical methods that includes discussions pertinent to TEQA. The 17th edition is available as of this writing from the Association of Official Analytical Chemists (AOAC). AOAC has been a pioneer in conducting so-called round-robin studies among participating laboratories as a minimum requirement for a validated analytical method to appear in this collection.

The primary and secondary literature of analytical chemistry are major sources of new methods, techniques, variations on older methods, and innovation. The American Chemical Society (ACS) publishes two premier journals, Analytical Chemistry and Environmental Science and Technology, that should be frequently consulted. The biennial reviews in Analytical Chemistry cover both principles and
applications in alternate years. There exists a host of other very relevant scientific journals and reviews, such as *Chromatographia, International Journal of Environmental Analytical Chemistry, Analytical Chimica Acta, The Analyst, Journal of Chromatography A and B, Journal of Chromatographic Science, Journal of Analytical Toxicology, Trends in Analytical Chemistry*, and *CRC Critical Reviews in Analytical Chemistry*, among others that contain papers pertinent to TEQA.

Trade journals that arrive for free to qualified professionals include the following:

- **LC-GC: Solutions for Separation Scientists** is a must read for the practicing chromatographer, irrespective of whether the reader's interest is environmental, pharmaceutical, industrial, academic, or some combination (as is the case of most of us).
- **American Laboratory** and **American Laboratory News Edition** introduce readers to diverse areas of applied analytical science.
- **Spectroscopy** is focused on the practical aspects of atomic and molecular spectroscopy and is, at times, also pertinent to TEQA.
- **R&D Magazine** includes some of the latest innovations in analytical science.

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Title</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>Introduction</td>
<td>QA; data quality, method development and evaluation; expression of results; collection and preservation of samples; waste minimization and disposal, etc.</td>
</tr>
<tr>
<td>2000</td>
<td>Physical and Aggregate</td>
<td>QC; color; turbidity; oxidation-reduction potential; conductivity; etc.</td>
</tr>
<tr>
<td></td>
<td>Properties</td>
<td></td>
</tr>
<tr>
<td>3000</td>
<td>Metals</td>
<td>QC; preliminary treatment of samples; FI AA; cold vapor AA; GF AA; ICP-AES; ICP-MS; anodic stripping voltammetry; AS; Se via HG AA; individual metals Al to Zn</td>
</tr>
<tr>
<td>4000</td>
<td>Inorganic Nonmetallic</td>
<td>QC; anions by IC; anions by CE; radon; radioactive iodine; strontium 90; etc.</td>
</tr>
<tr>
<td></td>
<td>Constituents</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>Aggregate Organic</td>
<td>BOD; COD; surfactants; UV absorbing organic constituents; total dissolved organic halogen</td>
</tr>
<tr>
<td></td>
<td>Constituents</td>
<td></td>
</tr>
<tr>
<td>6000</td>
<td>Individual Organic</td>
<td>QA/QC; VOCs; THMs; DBPs; BNAs; PCBs; PAHS; etc.</td>
</tr>
<tr>
<td></td>
<td>Compounds</td>
<td></td>
</tr>
<tr>
<td>7000</td>
<td>Radioactivity</td>
<td>QC and statistics; counting instruments</td>
</tr>
<tr>
<td>8000</td>
<td>Toxicity</td>
<td>QA/QC; reporter gene response to dioxin-like organic compounds; etc.</td>
</tr>
<tr>
<td>9000</td>
<td>Microbiological Examination</td>
<td>Sterilization; preparation of culture media; rapid detection methods; membrane filter technique for coliform group; etc.</td>
</tr>
<tr>
<td>10000</td>
<td>Biological Examination</td>
<td>Plankton; nematological examination; etc.</td>
</tr>
</tbody>
</table>
Chromatography consumables suppliers such as Agilent, Alltech, Macherey-Nagel, Phenomenex, Restek, Supelco, Waters, Varian, and others provide timely newsletters, websites, and comprehensive catalogs.

Books on any and all aspects of analytical chemistry should be frequently consulted in addition to this one. In addition to the literature cited in the references in this book, the *Handbook of Water Analysis*, published by Marcel Dekker, is of recent origin and is quite relevant to the principles and practice of TEQA.¹⁸

14. WHAT IS THE PHYSICAL/ CHEMICAL BASIS OF THE EPA’S ORGANICS PROTOCOL?

A fundamental question can be asked to the newcomer to all of this: On what basis do all of the 200 or so priority pollutant organics get organized so that a systematic approach to TEQA can be accomplished? In other words, is there a simple and unifying theme that can be introduced to make some sense of all of this? The answer is yes. A flowchart is developed for the protocol as a whole and is depicted in Scheme 1.5. Any sample matrix obtained from the environment is first categorized as being either water or soil/sediment, and in turn, appropriate methods are implemented based in large part on the degree of volatility of the respective targeted analytes. Ideally, one would wish to screen samples to obtain a preliminary indication as to whether one must dilute the extract before injection into the determinative instrument. For example, EPA Method 8270C fits very nicely into this scheme. A sample of soil taken from a Superfund hazardous waste site would be analyzed according to the path for semivolatile organics. This path assumes that an initial screen is considered, and based on this screen, an appropriate dilution of an extract is prepared from the sample made. The sample preparation techniques of LLE and sample cleanup are used and the quantitative determination accomplished using GC-MS. Scheme 1.6 is a flowchart for this method and directs the analyst in implementing the method. Note that, along the way, there is ample opportunity for analyst intervention and decision making. Chapters 2 to 4 introduce those principles responsible for implementing the flowcharts shown in Scheme 1.5 and Scheme 1.6, not only for this particular method but, in general, for all analytical methods.

Before we plunge into the details of analytical methods to measure environmental contaminants, it is important to first consider just how to interpret analytical data. This topic is usually either placed near the end of most books or added in the form of an appendix.¹⁹ Increasing emphasis on a more rigorous treatment of analytical data is just beginning to emerge.²⁰
# Introduction to Trace Environmental Quantitative Analysis (TEQA)

## Scheme 1.5

**Environmental sample matrix**

- Drinking, ground, surface and waste water
- Soil, sediment, sludge, other solids

### VOCs
- Screen using HS-GC-FID or LLE with Hexadecane/GC-FID
- LLE or RP-SPE & cleanup
- Screen using GC-FID
- GC-MS
- GC-MS

### SVOCs
- Screen using GC-FID
- LLE or RP-SPE & cleanup
- GC-MS

### OCs/PCBs
- LSE or LSE-RP-SPE & cleanup
- GC-ECD

### Soil, sediment, sludge, other solids
- Screen using HS-GC-FID or LLE with Hexadecane/GC-FID
- LLE or RP-SPE & cleanup
- Purge & trap
- LSE or LSE-RP-SPE
- GC-MS

### Acronyms defined that are used in the scheme
- **VOCs**—volatile organics
- **SVOCs**—semi-volatile organics
- **OCs/PCBs**—organochlorine pesticides/polychlorinated biphenyls
- **HS-GC-FID**—static headspace coupled to gas chromatography incorporating flame ionization detection
- **LLE**—liquid-liquid extraction (separatory funnel, countercurrent apparatus)
- **LSE**—liquid-solid extraction (Soxhlet apparatus) or solvent leaching from solid matrices
- **LSE-RP-SPE**—liquid-solid leaching followed by isolation on reversed-phase sorbents
- **GC-ECD**—gas chromatography incorporating electron-capture detection
- **GC-MS**—gas chromatography interfaced to low-resolution quadrupole mass spectrometry

---

© 2006 by Taylor & Francis Group, LLC
SCHEME 1.6

Start

- Prepare sample using appropriate 3500 series method

- If necessary, clean up extract using appropriate 3600 series method

- Establish GC-MS operating conditions; tune to DFTPP; perform initial calibration

- Perform daily calibration verification with SPCCs and CCCs prior to analysis of samples

Screen extract on GC-FID or GC-PID to identify highly contaminated samples; dilute those samples as needed.

Analyze extract by GC-MS

- Does any response exceed initial calibration curve range?
  - Yes
    - Dilute extract
  - No

Identify analyte by comparing the sample and standard mass spectra

Calculate concentration of each individual analyte confirmed present; report results
REFERENCES


© 2006 by Taylor & Francis Group, LLC
2 Calibration, Verification, Statistical Treatment of Analytical Data, Detection Limits, and Quality Assurance/Quality Control

If you can measure that of which you speak, and can express it by a number, you know something of your subject, but if you cannot measure it, your knowledge is meager and unsatisfactory.

—Lord Kelvin

CHAPTER AT A GLANCE

Good laboratory practice ................................................................................................38
Error in laboratory measurement ........................................................................... 41
Instrument calibration and quantification ............................................................... 45
Linear least squares regression .............................................................................. 58
Uncertainty in interpolated linear least squares regression ............................... 64
Instrument detection limits .................................................................................. 68
Limit of quantitation .............................................................................................. 81
Quality control ....................................................................................................... 85
Linear vs. nonlinear least squares regression ...................................................... 91
Electronic interfaces between instruments and PCs .......................................... 104
Sampling considerations ...................................................................................... 112
References ............................................................................................................ 117

Chromatographic and spectroscopic analytical instrumentation are the key determinative tools to quantitate the presence of chemical contaminants in biological fluids and in the environment. These instruments generate electrical signals that are related to the amount or concentration of an analyte of environmental or environmental health significance. This analyte is likely to be found in a sample matrix taken from the environment, or from body fluids. Typical sample matrices drawn from the environment include groundwater, surface water, air, soil, wastewater, sediment, sludge, and so forth. Computer technology has merely aided the conversion of an
analog signal from the transducer to the digital domain. It is the relationship between the analog or digital output from the instrument and the amount or concentration of a chemical species that is discussed in this chapter. The process by which an electrical signal is transformed to an amount or concentration is called instrument calibration. Chemical analysis based on measuring the mass or volume obtained from chemical reactions is stoichiometric. Gravimetric (where the analyte of interest is weighed) and volumetric (where the analyte of interest is titrated) techniques are methods that are stoichiometric. Such methods do not require calibration. Most instrumental determinative methods are nonstoichiometric and thus require instrument calibration.

This chapter introduces the most important aspect of TEQA for the reader. After the basics of what constitutes good laboratory practice are discussed, the concept of instrumental calibration is introduced and the mathematics used to establish such calibrations are developed. The uncertainty present in the interpolation of the calibration is then introduced. A comparison is made between the more conventional approach to determining instrument detection limits and the more contemporary approaches that have recently been discussed in the literature. These more contemporary approaches use least squares regression and incorporate relevant elements from statistics. Quality assurance/quality control principles are then introduced. A contemporary statistical approach toward evaluating the degree of detector linearity is then considered. The principles that enable a detector’s analog signal to be digitized via analog-to-digital converters are introduced. Principles of environmental sampling are then introduced. Readers can compare QA/QC practices from two environmental testing laboratories. Every employer wants to hire an analyst who knows of and practices good laboratory behavior.

1. WHAT IS GOOD LABORATORY PRACTICE?

Good laboratory practice (GLP) requires that a quality control (QC) protocol for trace environmental analysis be put in place. A good laboratory QC protocol for any laboratory attempting to achieve precise and accurate TEQA requires the following considerations:

- Deciding whether an external standard, internal standard, or standard addition mode of instrument calibration is most appropriate for the intended quantitative analysis application.
- Establishing a calibration curve that relates instrument response to analyte amount or concentration by preparing reference standards and measuring their respective instrument responses.
- Performing a least squares regression analysis on the experimental calibration data to evaluate instrument linearity over a range of concentrations of interest and to establish the best relationship between response and concentration.
- Computing the statistical parameters that assist in specifying the uncertainty of the least squares fit to the experimental data points.
- Running one or more reference standards in at least triplicate as initial calibration verification (ICV) standards throughout the calibration range.
ICVs should be prepared so that their concentrations fall to within the mid-calibration range.

- Computing the statistical parameters for the ICV that assist in specifying the precision and accuracy of the least squares fit to the experimental data points.
- Determining the instrument detection limits (IDLs).
- Determining the method detection limits (MDLs), which requires establishing the percent recovery for a given analyte in both a clean matrix and the sample matrix. With some techniques, such as static headspace gas chromatography (GC), the MDL cannot be determined independently from the instrument’s IDL.
- Preparing and running QC reference standards at a frequency of once every 5 or 10 samples. This QC standard serves to monitor instrument precision and accuracy during a batch run. This assumes that both calibration and ICV criteria have been met. A mean value for the QC reference standard should be obtained over all QC standards run in the batch. The standard deviation, $s$, and the relative standard deviation (RSD) should be calculated.
- Preparing the running QC surrogates, matrix spikes, and, in some cases, matrix spike duplicates per batch of samples. A batch is defined in EPA methods to be approximately 20 samples. These reference standard spikes serve to assess extraction efficiency where applicable. Matrix spikes and duplicates are often required in EPA methods.
- Preparing and running laboratory blanks, laboratory control samples, and field and trip blanks. These blanks serve to assess whether samples may have become contaminated during sampling and sample transport.

It has been stated many times by experienced analysts that in order to achieve GLP, close to one QC sample must be prepared and analyzed for nearly each and every real-world environmental sample.

2. **CAN DATA REDUCTION, INTERPRETATION, AND STATISTICAL TREATMENT BE SUMMARIZED BEFORE WE PLUNGE INTO CALIBRATION?**

Yes, indeed. Figure 2.1, adapted and modified, while drawing on recently published International Union of Pure and Applied Chemistry (IUPAC) recommendations, as discussed by Currie, is this author’s attempt to do just that. The true amount that is present in the unknown sample can be expressed as an amount such as a ng analyte, or as a concentration [µg analyte/kg of sample (weight/weight) or µg analyte/L of sample (weight/volume)]. The amount or concentration of true unknown present in either an environmental sample or human/animal specimen and represented by $\tau$ is shown in Figure 2.1 being transformed to an electrical signal $y$. Chapters 3 and 4 describe how the six steps from sampling to transducer are accomplished. The signal $y$, once obtained, is then converted to the reported estimate.
40

Trace Environmental Quantitative Analysis, Second Edition

This chapter describes how the eight steps from calibration to statistical evaluation are accomplished. The ultimate goal of TEQA is then realized, i.e., a reported estimate \( x_0 \) with a calculated uncertainty using statistics in the measurement expressed as \( \pm u \). We can assume that the transduced signal varies linearly with \( x \), where \( x \) is the known analyte amount or concentration of a standard reference. This analyte in the standard reference must be chemically identical to the analyte in the unknown sample represented by its true value \( \tau \). \( x \) is assumed to be known with certainty since it can be traced to accurately known certified reference standards, such as that obtained from the National Institute of Standards and Technology (NIST). We can realize that

\[
y = y_0 + mx + e_y
\]

where

- \( y_0 \) = the \( y \) intercept, the magnitude of the signal in the absence of analyte.
- \( m \) = slope of the best-fit regression line (what we mean by regression will be taken up shortly) through the experimental data points. The slope also defines the sensitivity of the specific determinative technique.
- \( e_y \) = the error associated with the variation in the transduced signal for a given value of \( x \). We assume that \( x \) itself (the amount or concentration of the analyte of interest) is free of error. This assumption is used throughout the mathematical treatment in this chapter and serves to simplify the mathematics introduced.
Referring to Figure 2.1, we can, at best, only estimate \( \tau \) and report a result for the amount or concentration at a trace level, represented by \( x_0 \), with an uncertainty \( u \) such that \( x_0 \) could range from a low of \( x_0 - u \) to a high of \( x_0 + u \). Let us focus a bit more on the concept of error in measurement.

### 2.1 How Is Measurement Error Defined?

Let us digress a bit and discuss measurement error. Each and every measurement includes error. The length and width of a page from this book cannot be measured without error. There is a true length of this page, yet at best we can only estimate its length. We can measure length only to within the accuracy and precision of our measuring device, in this case, a ruler or straightedge. We could increase our precision and accuracy for measuring the length of this page if we used a digital caliper. Currie has defined \( x_0 \) as the statistical estimate derived from a set of observations.

The error in \( x_0 \) represented by \( e \) is shown to consist of two parts, systematic or bias error represented by \( \Delta \) and random error represented by \( \delta \) such that:8

\[
x_0 = \tau + e
\]

\[
\Delta = |\mu - \tau|
\]

\[
\delta = z\sigma
\]

\[
\delta = |x_0 - \mu|
\]

\( \Delta \) is defined as the absolute difference between a population mean represented by \( \mu \) (assuming a Gaussian or normal distribution) and the true value \( \tau \). \( \delta \) is defined as the absolute difference between the estimated analytical result for the unknown sample \( x_0 \) and the population mean \( \mu \). \( \delta \) can also be viewed in terms of a multiple \( z \) of the population standard deviation \( \sigma \), \( \sigma \) being calculated from a Gaussian or normal distribution of \( x \) values from a population.

### 2.2 Are There Laboratory-Based Examples of How \( \Delta \) and \( \delta \) Are Used?

Yes, indeed. Bias, \( \Delta \), reflects systematic error in a measurement. Systematic error may be instrumental, operational, or personal.
Instrumental errors arise from a variety of sources such as:  

- Poor design or manufacture of instruments  
- Faulty calibration of scales  
- Wear of mechanical parts or linkages  
- Maladjustment  
- Deterioration of electrical, electronic, or mechanical parts due to age or location in a harsh environment  
- Lack of lubrication or other maintenance  

Errors in this category are often the easiest to detect. They may present a challenge in attempting to locate them. Use of a certified reference standard might help to reveal just how large the degree of inaccuracy as expressed by a percent relative error really is. The percent relative error (%error), i.e., the absolute difference between the mean or average of a small set of replicate analyses, $x_{\text{ave}}$, and the true or accepted value, $\tau$, divided by $\tau$ and multiplied by 100, is mathematically stated (and used throughout this book) as follows:

\[
\%\text{error} = \left(1 - \frac{x_{\text{ave}}}{\tau}\right) \times 100
\]

It is common to see the expression “the manufacturer states that its instrument’s accuracy is better than 2% relative error.” The analyst should work in the laboratory with a good idea as to what the percent relative error might be in each and every measurement that he or she must make. It is often difficult if not impossible to know the true value. This is where certified reference standards such as those provided by the NIST are valuable. High precision may or may not mean acceptable accuracy.

Operational errors are due to departures from correct procedures or methods. These errors often are time dependent. One example is that of drift in readings from an instrument before the instrument has had time to stabilize. A dependence of instrument response on temperature can be eliminated by waiting until thermal equilibrium has been reached. Another example is the failure to set scales to zero or some other reference point prior to making measurements. Interferences can cause either positive or negative deviations. One example is the deviation from Beer’s law at higher concentrations of the analyte being measured. However, in trace analysis, we are generally confronted with analyte concentration levels that tend toward the opposite direction.

Personal errors result from bad habits and erroneous reading and recording of data. Parallax error in reading the height of a liquid in a buret from titrimetric analysis is a classic case in point. One way to uncover personal bias is to have someone else repeat the operation. Occasional random errors by both persons are to be expected, but a discrepancy between observations by two persons indicates bias on the part of one or both.  

Consider the preparation of reference standards using an analytical balance that reads a larger weight than it should. This could be due to a lack of adjusting the
zero within a set of standard masses. What if an analyst, who desires to prepare a solution of a reference standard to the highest degree of accuracy possible, dissolves what he thinks is 100 mg of standard reference (the solute), but really is only 89 mg, in a suitable solvent using a graduated cylinder and then adjusts the height of the solution to the 10-mL mark? Laboratory practice would suggest that this analyst use a 10-mL volumetric flask. Use of a volumetric flask would yield a more accurate measurement of solution volume. Perhaps 10 mL turns out to be really 9.6 mL when a graduated cylinder is used. We now have inaccuracy, i.e., bias, in both mass and in volume. Bias has direction, i.e., the true mass is always lower or higher. Bias is usually never lower for one measurement and then higher for the next measurement. The mass of solute dissolved in a given volume of solvent yields a solution whose concentration is found from dividing the mass by the total volume of solution. The percent relative error in the measurement of mass and the percent relative error in the measurement of volume propagate to yield a combined error in the reported concentration that can be much more significant than each alone. Here is where the cliché “the whole is greater than the sum of its parts” has some meaning.

Random error, \( \delta \), occurs among replicate measurement without direction. If we were to weigh 100 mg of some chemical substance, such as a reference standard, on the most precise analytical balance available and repeat the weighing of the same mass additional times while remembering to rezero the balance after each weighing, we might get data such as that shown below:

<table>
<thead>
<tr>
<th>Replicate No.</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.98</td>
</tr>
<tr>
<td>2</td>
<td>100.10</td>
</tr>
<tr>
<td>3</td>
<td>100.04</td>
</tr>
<tr>
<td>4</td>
<td>99.99</td>
</tr>
<tr>
<td>5</td>
<td>100.02</td>
</tr>
</tbody>
</table>

Notice that the third replicate weighing yields a value that is less than the second. Had the values kept increasing through all five measurements, systematic error or bias might be evident.

Another example for the systematic vs. random error “defective,” this time using analytical instrumentation, is to make repetitive 1-µL injections of a reference standard solution into a gas chromatograph (GC). A GC with an atomic emission detector (GC-AED) was used by this author to evaluate whether systematic error was evident for triplicate injection of a 20 ppm reference standard containing tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCBP) dissolved in the solvent iso-octane. Both analytes are used as surrogates in EPA organochlorine pesticide/polychlorinated biphenyl (PCB)-related methods such as EPA Methods 608 and 8080. The atomic emission from microwave-induced plasma excitation of chlorine atoms, monitored at a wavelength of 837.6 nm, formed the basis for the transduced electrical signal. Both analytes are separated chromatographically (refer to Chapter 4 for an introduction to the principles underlying chromatographic separations) and
appear in a chromatogram as distinct peaks, each with an instrument response. The emitted intensity is displayed graphically in terms of a peak whose area beneath the curve is given in units of counts-seconds. These data are shown below:

<table>
<thead>
<tr>
<th></th>
<th>TCMX (counts-seconds)</th>
<th>DCBP (counts-seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>48.52</td>
<td>53.65</td>
</tr>
<tr>
<td>2nd</td>
<td>47.48</td>
<td>52.27</td>
</tr>
<tr>
<td>3rd</td>
<td>48.84</td>
<td>54.46</td>
</tr>
</tbody>
</table>

The drop between the first and second injections in the peak area along with the rise between the second and third injections suggests that systematic error has been largely eliminated. A few days before these data were generated a similar set of triplicate injections was made using a somewhat more diluted solution containing TCMX and DCBP into the same GC-AED. The following data were obtained:

<table>
<thead>
<tr>
<th></th>
<th>TCMX (counts-seconds)</th>
<th>DCBP (counts-seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>37.83</td>
<td>41.62</td>
</tr>
<tr>
<td>2nd</td>
<td>38.46</td>
<td>42.09</td>
</tr>
<tr>
<td>3rd</td>
<td>37.67</td>
<td>40.70</td>
</tr>
</tbody>
</table>

The rise between the first and second injections in peak area followed by the drop between the second and third injections suggests again that systematic error has been largely eliminated. One of the classic examples of systematic error, and one that is most relevant to TEQA, is to compare the bias and percent relative standard deviations in the peak area for five identical injections using a liquid-handling autosampler against a manual injection into a graphite furnace atomic absorption spectrophotometer using a common 10-µL glass liquid-handling syringe. It is almost impossible for even the most skilled analyst around to achieve the degree of reproducibility afforded by most automated sample delivery devices.

Good laboratory practice suggests that it should behoove the analyst to eliminate any bias, $\Delta$, so that the population mean equals the true value. Mathematically stated:

$$\Delta = 0 = \mu - \tau$$

$$\therefore \mu = \tau$$

Eliminating $\Delta$ in the practice of TEQA enables one to consider only random errors. Mathematically stated:

$$\delta = \left| x_0 - \mu \right|$$
Random error alone becomes responsible for the absolute difference between the reported estimate $x_0$ and the statistically obtained population mean. Random error can never be completely eliminated. Referring again to Figure 2.1, let us proceed in this chapter to take a more detailed look at those factors that transform $y$ to $x_0$. We focus on those factors that transform $\tau$ to $y$ in Chapters 3 and 4.

3. HOW IMPORTANT IS INSTRUMENT CALIBRATION AND VERIFICATION?

It is very important and the most important task for the analyst who is responsible for operation and maintenance of analytical instrumentation. Calibration is followed by a verification process in which specifications can be established and the analyst can evaluate whether the calibration is verified or refuted. A calibration that has been verified can be used in acquiring data from samples for quantitative analysis. A calibration that has been refuted must be repeated until verification is achieved, e.g., if, after establishing a multipoint calibration for benzene via a gas chromatographic determinative method, an analyst then measures the concentration of benzene in a certified reference standard. The analyst expects no greater than a 5% relative error and discovers to his surprise a 200% relative error. In this case, the analyst must reconstruct the calibration and measure the certified reference standard again. Close attention must be paid to those sources of systematic error in the laboratory that would cause the relative error to greatly exceed the minimally acceptable relative error criteria previously developed for this method.

An analyst who expects to implement TEQA and begins to use any one of the various chromatography data acquisition and processing software packages available in the marketplace today is immediately confronted with several calibration modes available. Most software packages will contain most of the modes of instrumental calibration that appear in Table 2.1. For each calibration mode, the general advantages as well as the overall limitations are given. Area percent and normalization percent (norm%) are not suitable for quantitative analysis at the trace concentration level. This is due to the fact that a concentration of 10,000 ppm is only 1% (parts per hundred), so that a 10 ppb concentration level of, for example, benzene, in drinking water is only 0.000001% benzene in water. Weight% and mole% are subsets of norm% and require response factors for each analyte in units or peak area or peak height per gram or per mole, respectively. Table 2.2 relates each calibration mode with its corresponding quantification equation. Quantification follows calibration and thus achieves the ultimate goal of TEQA, i.e., to perform a quantitative analysis of a sample of environmental or environmental health interest in order to determine the concentration of each targeted chemical analyte of interest at a trace concentration level. Table 2.1 and Table 2.2 are useful as reference guides.

We now proceed to focus on the most suitable calibration modes for TEQA. Referring again to Table 2.1, these calibration modes include external standard (ES), internal standard (IS), to include its more specialized isotope dilution mass spectrometry (IDMS) calibration mode, and standard addition (SA). Each mode will be discussed in sufficient detail to enable the reader to acquire a fundamental understanding.
### TABLE 2.1
**Advantages and Limitations of the Various Modes of Instrument Calibration Used in TEQA**

<table>
<thead>
<tr>
<th>Calibration Mode</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area%</td>
<td>No standards needed; provides for a preliminary evaluation of sample composition; injection volume precision not critical</td>
<td>Need a nearly equal instrument response for all analytes so peak heights/areas all uniform; all peaks must be included in calculation; <strong>not suitable for TEQA</strong></td>
</tr>
<tr>
<td>Norm%</td>
<td>Injection volume precision not critical; accounts for all instrument responses for all peaks</td>
<td>All peaks must be included; calibration standards required; all peaks must be calibrated; <strong>not suitable for TEQA</strong></td>
</tr>
<tr>
<td>ES</td>
<td>Addresses wide variation in GC detector response; more accurate than area%, norm%; not all peaks in a chromatogram of a given sample need to be quantitated; compensates for recovery losses if standards are taken through sample prep in addition to samples; does not have to add any standard to the sample extract for calibration purposes; <strong>ideally suited to TEQA</strong></td>
<td>Injection volume precision is critical; instrument reproducibility over time is critical; no means to compensate for a change in detector sensitivity during a batch run; needs a uniform matrix whereby standards and samples should have similar matrices</td>
</tr>
<tr>
<td>IS</td>
<td>Injection volume precision not critical; instrument reproducibility over time not critical; compensates any variation in detector sensitivity during a batch run; <strong>ideally suited to TEQA</strong></td>
<td>Need to identify a suitable analyte to serve as an IS; bias is introduced if the IS is not added to the sample very carefully; does not compensate for percent recovery losses during sample preparation since IS is usually added after both extraction and cleanup are performed</td>
</tr>
<tr>
<td>IDMS</td>
<td>Same as for IS; injection volume precision not critical; instrument reproducibility over time not critical; compensates for analyte percent recovery losses during sample preparation since isotopes are added prior to extraction and cleanup; eliminates variations in analyte vs. internal standard recoveries; <strong>ideally suited to TEQA</strong></td>
<td>Need to obtain a suitable isotopically labeled analog of each target analyte; isotopically labeled analogs are very expensive; bias is introduced if the labeled isotope is not added to the sample very carefully; needs a mass spectrometer to implement; mass spectrometers are expensive in comparison to element-selective GC detectors or non-MS LC detectors</td>
</tr>
<tr>
<td>SA</td>
<td>Useful when matrix interference cannot be eliminated; applicable where analyte-free matrix cannot be obtained; commonly used to measure trace metals in “dirty” environmental samples</td>
<td>Need two aliquots of same sample to make one measurement; too tedious and time consuming for multiorganics quantitative analysis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode</th>
<th>Quantification Equation for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area%</td>
<td>( C'<em>{\text{unk}} = \frac{A'</em>{\text{unk}}}{\sum_i A_i} \times 100 )</td>
</tr>
<tr>
<td>Norm%</td>
<td>( C'<em>{\text{unk}} = \frac{A'</em>{\text{unk}}RF_i}{\sum_i A'_{\text{unk}}RF_i} \times 100 )</td>
</tr>
<tr>
<td>Weight%/Mole%</td>
<td>( C'<em>{\text{unk}} = \frac{A'</em>{\text{unk}}}{RF_i} )</td>
</tr>
<tr>
<td>ES</td>
<td>( C'<em>{\text{unk}} = \frac{A'</em>{\text{unk}}}{RF_i} )</td>
</tr>
<tr>
<td>IS</td>
<td>( C'<em>{\text{unk}} = \left[ \frac{A'</em>{\text{unk}}}{A_{RF_i}} \right] \left[ \frac{C'_{\text{IS}}}{RRF_i} \right] )</td>
</tr>
<tr>
<td>IDMS</td>
<td>( C'<em>{\text{unk}} = \left[ \frac{C</em>{\text{spike}} - \frac{C_{\text{spike}}}{W_{\text{unk}}} \left( f^{1.1}<em>{\text{spike}} - f^{1.2}</em>{\text{spike}} \right) + f^{1.3}<em>{\text{spike}} - f^{1.4}</em>{\text{spike}}}{f^{1.3}<em>{\text{spike}} - f^{1.4}</em>{\text{spike}}} \right] C_{\text{spike}} )</td>
</tr>
<tr>
<td>SA</td>
<td>( C'<em>{\text{unk}} = \left[ \frac{R'</em>{\text{unk}} - R'<em>{\text{IS-unk}}}{R'</em>{\text{IS-unk}} - R'<em>{\text{IS-unk}}} \right] C</em>{\text{spike}} )</td>
</tr>
</tbody>
</table>

Where:
- \( C'_{\text{unk}} \) — concentration of analyte \( i \) in the unknown sample (the ultimate goal of TEQA)
- \( A'_{\text{unk}} \) — area of \( i \)th peak in unknown sample;
- \( N \) — total number of peaks in chromatogram
- \( RF_i \) — response factors for \( i \)th analyte; peak area or peak height per unit amount (grams or moles)
- \( ES \) — response factor for the \( i \)th analyte; peak area or peak height per unit concentration
- \( IDMS \) — relative response factor for the \( i \)th analyte; peak area or peak height per unit concentration
- \( SA \) — concentration of analyte \( i \) after analyte \( i \) (standard) is added to the unknown sample
- \( R'_{\text{spike}}, R'_{\text{IS}}, R'_{\text{IS-unk}} \) — response of unknown analyte and blank, both associated with unknown sample
- \( R'_{\text{spike}}, R'_{\text{IS}}, R'_{\text{IS-unk}} \) — response of unknown analyte and blank, in spiked or standard added known sample

Refer to text for definition of each term used in the above equation.
of the similarities and differences among all three. Correct execution of calibration on the part of a given analyst on a given instrument is a major factor in achieving GLP.

3.1 HOW DOES THE EXTERNAL MODE OF INSTRUMENT CALIBRATION WORK?

The ES mode uses an external reference source for the analyte whose concentration in an unknown sample is sought. A series of working calibration standards are prepared that encompass the entire range of concentrations anticipated for the unknown samples and may include one or more orders of magnitude. For example, let us assume that a concentration of 75 ppb of a trihalomethane (THM) is anticipated in chlorinated drinking water samples. A series of working calibration standards should be prepared whose concentration levels start from a minimum of 5 ppb to a maximum of 500 ppb each THM. The range for this calibration covers two orders of magnitude. Six standards that are prepared at 5, 25, 50, 100, 250, and 500 ppb for each THM, respectively, would be appropriate in this case. Since these standards will not be added to any samples, they are considered external to the samples, hence defining this mode as ES. The calibration curve is established by plotting the analyte response against the concentration of analyte for each THM.

The external standard is appropriate when there is little to no matrix effect between standards and samples. To illustrate this elimination of a matrix effect, consider the situation whereby an aqueous sample is extracted using a nonpolar solvent. The reference standard used to construct the ES calibration is usually dissolved in an organic solvent such as methanol, hexane, or iso-octane. The analytes of interest are now also in a similar organic solvent. ES is also appropriate when the instrument is stable and the volume of injection of a liquid sample such as an extract can be reproduced with good precision. A single or multipoint calibration curve is usually established when using this mode.

For a single-point calibration, the concept of a response factor, \( R_{fr} \), becomes important. The use of response factors is valid provided that it can be demonstrated that the calibration curve is, in fact, a straight line. If so, the use of \( R_{fr} \) values serves to greatly simplify the process of calibration. \( R_{fr} \) is fixed and is independent of the concentration for its analyte for a truly linear calibration. A response factor for the \( i \)th analyte would be designated as \( R_{fi} \). For example, if 12 analytes are to be calibrated and we are discussing the seventh analyte in this series, \( i \) would then equal 7. The magnitude of \( R_{fi} \) does indeed depend on the chemical nature of the analyte and on the sensitivity of the particular instrument. The definition of \( R_{fr} \) for ES is given as follows (using notation from differential calculus):

\[
\lim_{\Delta C_i \to 0} \frac{\Delta A_{fi}}{\Delta C_i} = R_{fi} \tag{2.1}
\]

A response factor for each analyte (i.e., the \( i \)th analyte) is obtained during the calibration and is found by finding the limit of the ratio of the incremental change in peak area for the \( i \)th analyte, \( \Delta A_{fi} \), to the incremental change in concentration of
the \( i \)th analyte in the reference standard, \( \Delta C_s^i \), as \( \Delta C_s^i \) approaches zero. Quantitative analysis is then carried out by relating the instrument response to the analyte concentration in an unknown sample according to

\[
A^i = R^i_F C_{\text{unknown}}^i
\]  

Equation (2.2) is then solved for the concentration of the \( i \)th analyte, \( C_{\text{unknown}}^i \), in the unknown environmental sample. Refer to the quantification equation for ES in Table 2.2.

Figure 2.2 graphically illustrates the ES approach to multipoint instrument calibration. Six reference standards, each containing Aroclor 1242 (AR 1242), were injected into a gas chromatograph that incorporates a capillary column appropriate to the separation and an electron-capture detector. This instrumental configuration is conveniently abbreviated C-GC-ECD. Aroclor 1242 is a commercially produced mixture of 30 or more polychlorinated biphenyl (PCB) congeners. The peak areas under the chromatographically resolved peaks were integrated and reported as an area count in units of microvolts-seconds (\( \mu \)V-sec). The more than 30 peak areas are then summed over all of the peaks to yield a total peak area, \( A_{1242}^{\text{total}} \), according to
The total peak area is then plotted against the concentration of Aroclor 1242 expressed in units of parts per million (ppm). The experimental data points closely approximate a straight line. This closeness of fit demonstrates that the summation of AR 1242 peak areas varies linearly with AR 1242 concentration expressed in terms of a total Aroclor. These data were obtained in the author’s laboratory and nicely illustrate the ES mode. Chromatography processing software is essential to accomplish such a seemingly complex calibration in a reasonable time frame. This author would not want to undertake such a task armed with only a slide rule.

3.2 **How Does the IS Mode of Instrument Calibration Work and Why Is It Increasingly Important to TEQA?**

The IS mode is most useful when it has been determined that the injection volume cannot be reproduced with good precision. This mode is also preferred when the instrument response for a given analyte at the same concentration will vary over time. Both the analyte response and the IS analyte response will vary to the same extent over time; hence, the ratio of analyte response to IS response will remain constant. The use of an IS thus leads to good precision and accuracy in construction of the calibration curve. The calibration curve is established by plotting the ratio of the analyte to IS response against the ratio of the concentration of analyte to either the concentration of IS or the concentration of analyte. In our THM example, 1,2-dibromopropane (1,2-DBP) is often used as a suitable IS. The molecular formula for 1,2-DBP is similar to each of the THMs, and this results in an instrument response factor that is near to that of the THMs. The concentrations of IS in all standards and samples must be identical so that the calibration curve can be correctly interpolated for the quantitative analysis of unknown samples. Refer to the THM example above and consider the concentrations cited above for the six-point working calibration standards. 1,2-DBP is added to each standard so as to be present at, for example, 200 ppb. This mode is defined as such since 1,2-DBP must be present in the sample or is considered internal to the sample. A single-point or multipoint calibration curve is usually established when using this mode.

The IS mode to instrument calibration has become increasingly important over the past decade as the mass spectrometer (MS) replaced the element-selective detector as the principal detector coupled to gas chromatographs in TEQA. The mass spectrometer is somewhat unstable over time, and the IS mode of GC-MS calibration quite adequately compensates for this instability.

We return now to the determination of clofibric acid (CF) in wastewater. This case study was introduced in Chapter 1 as one example of trace enviro-chemical QA. A plot of the ratio of the CF methyl ester peak area to that of the internal standard 2,2',4,6,6'-pentachlorobiphenyl (22'466'PCBP) against the concentration of CF methyl ester in ppm is shown in Figure 2.3. An ordinary least squares regression line was established and drawn as shown (we will take up least squares regression shortly). The line shows a goodness of fit to the experimental data points.
This plot demonstrates adequate linearity over the range of CF methyl ester concentrations shown. Any instability of the GC-MS instrument during the injection of these calibration standards is not reflected in the calibration. Therein lies the value and importance of IS.

For a single-point calibration approach, a relative response factor is used:

$$\lim_{\Delta \left( \frac{C_i}{C_{IS}} \right) \to 0} \frac{\Delta \left( \frac{A_i}{A_{IS}} \right)}{\Delta \left( \frac{C_i}{C_{IS}} \right)} \equiv RRF_i$$

Quantitative analysis is then carried out by relating the ratio of analyte instrument response for an unknown sample to that of IS instrument response to the ratio of unknown analyte concentration to IS concentration according to

$$\frac{A_{unknown}^i}{A_{IS}^i} = RRF_i \cdot \frac{C_{unknown}^i}{C_{IS}^i}$$

Equation (2.4) is then solved for the concentration of analyte $i$ in the unknown sample, $C_{unknown}^i$. Refer to the quantification equation for IS in Table 2.2.

$A_{unknown}^i$ and $A_{IS}^i$ are allowed to vary with time. This is what one expects when using high-energy detectors such as mass spectrometers. The ratio $A_{unknown}^i/A_{IS}^i$
remains fixed over time. This fact establishes a constant $RR'_{i}$ and hence preserves the linearity of the internal standard mode of instrument calibration. Equation (2.4) suggests that if $RR'_{i}$ is constant, and if we keep the concentration of IS to be used with the $i$th analyte, $C_{IS}$, constant, the ratio $A_{unknown}^{i}/A_{IS}^{i}$ varies linearly with the concentration of the $i$th analyte in the unknown, $C_{unknown}$.

Figure 2.4 graphically illustrates the internal standard approach to multipoint instrument calibration for trichloroethylene (TCE) using perchloroethylene (PCE) (or tetrachloroethylene) as the IS. An automated headspace gas chromatograph incorporating a capillary column and ECD (HS-GC-ECD) was used to generate the data. Figure 2.4 is a plot of $A_{S}^{TCE}/A_{S}^{PCE}$ vs. $C_{S}^{TCE}$ for a four-point calibration obtained in the author’s laboratory. A straight line is then drawn through the experimental data points whose slope is $m$. Rewriting Equation (2.4) gives the mathematical equivalent for this calibration plot:

$$\frac{A_{S}^{TCE}}{A_{S}^{PCE}} = kC_{S}^{TCE}$$
where

\[ k = \frac{m_{\text{PCE}}}{C_{\text{IS}}} \]

Quantitative analysis is accomplished by interpolating the calibration curve. This yields a value for the concentration for TCE expressed in units of ppm. The concentration of TCE in a groundwater sample obtained from a groundwater aquifer that is not known to be contaminated with this priority pollutant volatile organic compound (VOC) can then be found.

The manner in which one uses the IS in sample preparation (Chapter 3) will have a significant impact on the analytical result. Three strategies, shown in Scheme 2.1, have emerged when considering the use of the IS mode of calibration. In the first strategy, internal standards are added to the final extract after sample prep steps.
are complete. The quantification equation for IS shown in Table 2.2 would yield an analytical result for $C_{\text{unknown}}^i$ that is lower than the true concentration for the $i$th analyte in the original sample since percent recovery losses are not accounted for. This strategy is widely used in analytical method development. The second strategy first calibrates the instrument by adding standards and ISs to appropriate solvents, and then proceeds with the calibration. ISs are then added in known amounts to samples prior to extraction and cleanup. According to Budde:10

The measured concentrations will be the true concentrations in the sample if the extraction efficiencies of the analytes and ISs are the same or very similar. This will be true even if the actual extraction efficiencies are low, for example, 50%.

The third strategy depicted in Scheme 2.1 corrects for percent recovery losses. Again, according to Budde:10

The system is calibrated using analytes and ISs in a sample matrix or simulated sample matrix, for example, distilled water, and the calibration standards are processed through the entire analytical method … [this strategy] is sometimes referred to as calibration with procedural standards.

### 3.2.1 What Is Isotope Dilution?

Scheme 2.1 places isotope dilution under the second option for using the IS mode of instrument calibration. The principal EPA methods that require isotope dilution mass spectrometry (IDMS) as the means to calibrate a GC-MS, LC-MS, MS (without a separation technique interfaced), or ICP-MS are shown in Scheme 2.1. Other analytical methods that rely on isotope dilution as the chief means to calibrate and to quantitate are liquid scintillation counting and various radioimmunoassay techniques that are not considered in this book.

TEQA can be implemented using isotope dilution. The unknown concentration of an element or compound in a sample can be found by knowing only the natural isotope abundance (atom fraction of each isotope of a given element) and, after an enriched isotope of this element has been added, equilibrated, and measured, by measuring this altered isotopic ratio in the spiked or diluted mixture. This is the simple yet elegant conceptual framework for isotope dilution as a quantitative tool.

### 3.2.2 Can a Fundamental Quantification Equation Be Derived from Simple Principles?

Yes, indeed, and we proceed to do so now. The derivation begins by first defining this altered and measured ratio of isotopic abundance after the enriched isotope (spike or addition of labeled analog) has been added and equilibrated. Only two isotopes of a given element are needed to provide quantification. Fassett and Paulsen11 showed how isotope dilution is used to determine the concentration at trace levels for vanadium in crude oil, and we use their illustration to develop the principles that appear below.
Let us start by defining $R_m$ as the measured ratio of each of the two isotopes of a given element in the spiked unknown. The contribution made by $^{50}V$ appears in the numerator, and that made by $^{51}V$ appears in the denominator. Fassett and Paulsen obtained this measured ratio from mass spectrometry. Mathematically stated,

$$
R_m = \frac{\text{ amt}^{50}V(\text{unknown}) + \text{ amt}^{50}V(\text{spike})}{\text{ amt}^{51}V(\text{unknown}) + \text{ amt}^{51}V(\text{spike})}
$$

(2.5)

The amount of $^{50}V$ in the unknown sample can be found as a product of the concentration of vanadium in the sample as the $^{50}V$ and the weight of sample. This is expressed as follows:

$$
\text{ amt}^{50}V(\text{unknown}) = \left[ \text{ atomfraction}^{50}V \right] \left[ \text{concV(unknownsample)} \right] \left[ \text{weight(unknownsample)} \right]
$$

(2.6)

The natural isotopic abundances for the element vanadium are 0.250% as $^{50}V$ and 99.750% as $^{51}V$, so that $f^{51} = 0.997512$ for the equations that follow.

Equation (2.6) can be abbreviated and is shown rewritten as follows:

$$
\text{ amt}^{50}V = \left[ f^{50}_{\text{native}} \right] \left[ C^{V}_{\text{unk}} \right] \left[ W_{\text{unk}} \right]
$$

(2.7)

In a similar manner, we can define the amount of the higher isotope of vanadium in the unknown as follows:

$$
\text{ amt}^{51}V = \left[ f^{51}_{\text{native}} \right] \left[ C^{V}_{\text{unk}} \right] \left[ W_{\text{unk}} \right]
$$

(2.8)

Equation (2.7) and Equation (2.8) can also be written in terms of the respective amounts of the 50 and 51 isotopes in the enriched spike. This is shown as follows:

$$
\text{ amt}^{50}V = \left[ f^{50}_{\text{enriched}} \right] \left[ C^{V}_{\text{spike}} \right] \left[ W_{\text{spike}} \right]
$$

(2.9)

$$
\text{ amt}^{51}V = \left[ f^{51}_{\text{enriched}} \right] \left[ C^{V}_{\text{spike}} \right] \left[ W_{\text{spike}} \right]
$$

(2.10)

Equation (2.5) can now be rewritten using the symbolism defined by Equation (2.7) to Equation (2.10) and generalized for the first isotope of the $i$th analyte ($i$, 1) and for the second isotope of the $i$th analyte ($i$, 2) according to

$$
R_m = \left[ f^{i1}_{\text{unk}} \right] \left[ C^{i}_{\text{unk}} \right] \left[ W_{\text{unk}} \right] + \left[ f^{i1}_{\text{spike}} \right] \left[ C^{i}_{\text{spike}} \right] \left[ W_{\text{spike}} \right] + \left[ f^{i2}_{\text{unk}} \right] \left[ C^{i}_{\text{unk}} \right] \left[ W_{\text{unk}} \right] + \left[ f^{i2}_{\text{spike}} \right] \left[ C^{i}_{\text{spike}} \right] \left[ W_{\text{spike}} \right]
$$

(2.11)
where

\[ R_m = \text{isotope ratio (dimensionless number) obtained after an aliquot of the unknown sample has been spiked and equilibrated by the enriched isotope mix. This is measurable in the laboratory using a determinative technique such as mass spectrometry. The ratio could be found by taking the ratio of peak areas at different quantitation ions (quant ions or Q ions) if GC-MS was the determinative technique used.} \]

\[ f_{unk}^{i,1} = \text{natural abundance (atom fraction) of the } i\text{th element of the first isotope in the unknown sample. This is known from tables of isotopic abundance.} \]

\[ f_{unk}^{i,2} = \text{natural abundance (atom fraction) of the } i\text{th element of the second isotope in the unknown sample. This is known from tables of isotopic abundance.} \]

\[ C_{unk}^i = \text{concentration [\(\mu\text{mol/g, } \mu\text{g/g}\) of the } i\text{th element or compound in the unknown sample. This is unknown; the goal of isotope dilution is to find this value.} \]

\[ C_{spike}^i = \text{concentration [\(\mu\text{mol/g, } \mu\text{g/g}\) of the } i\text{th element or compound in the spike. This is known.} \]

\[ W_{unk} = \text{weight of unknown sample in g. This is measurable in the laboratory.} \]

\[ W_{spike} = \text{weight of spike in g. This is measurable in the laboratory.} \]

Equation (2.11), the more general form, can be solved algebraically for \(C_{unk}^i\) to yield the quantification equation:

\[
C_{unk}^i = \left[ \frac{C_{spike}^i W_{spike}}{W_{unk}} \left[ f_{spike}^{i,1} - R_m f_{unk}^{i,2} \right] \right] \tag{2.12}
\]

Equation (2.12) also appears as the quantification equation for IDMS in Table 2.2. We proceed now to consider the use of isotopically labeled organic compounds in IDMS. Returning again to Scheme 2.1, we find the use of IDMS as a means to achieve TEQA when a GC-MS is the determinative technique employed. Methods that determine polychloro-dibenzo-dioxins (PCDDs), polychloro-dibenzo-difurans (PCDFs), and coplanar polychlorinated biphenyls (cp-PCBs) require IDMS. IDMS coupled with the use of high-resolution GC-MS represents the most rigorous and highly precise trace organics analytical techniques designed to conduct TEQA known today.

### 3.2.3 What Is Organics IDMS?

Organics IDMS makes use of \(^2\text{H}-\), \(^{13}\text{C}-\), or \(^{37}\text{Cl}-\)labeled organic compounds. These labeled analogs are added to environmental samples or human specimens. Labeled analogs are structurally identical except for the substitution of \(^2\text{H}\) for \(^1\text{H}\), \(^{13}\text{C}\) for \(^{12}\text{C}\), or \(^{37}\text{Cl}\) for \(^{35}\text{Cl}\). A plethora of labeled analogs are now available for most priority pollutants or persistent organic pollutants (POPs) that are targeted analytes. To
illustrate, the priority pollutant or POP phenanthrene and its deuterated form, i.e., $^2$H, or D, isotopic analog, are shown below:

Polycyclic aromatic hydrocarbons (PAHs), of which phenanthrene is a member, have abundant molecular ions in electron-impact MS. The molecular weight for phenanthrene is 178, while that for the deuterated isotopic analog is 188 (phen-d10). If phenanthrene is diluted with itself, and if an aliquot of this mixture is injected into a GC-MS, the native and deuterated forms can be distinguished at the same retention time by monitoring the mass to charge ratio, abbreviated $m/z$ at 178 and then at 188.

We have seen the use of phen-d10 (Table 1.8) as an internal standard to quantitate all of the analytes listed. Contrast this with IDMS, by which an isotopic label for each and every targeted organic compound is used to quantitate.

### 3.3 HOW DOES THE SA MODE OF INSTRUMENT CALIBRATION WORK?

The SA mode is used primarily when there exists a significant matrix interference and where the concentration of the analyte in the unknown sample is appreciable. SA becomes a calibration mode of choice when the analyte-free matrix cannot be obtained for the preparation of standards for ES. However, for each sample that is to be analyzed, a second so-called standard added or spiked sample must also be analyzed. This mode is preferred when trace metals are to be determined in complex sample matrices such as wastewater, sediments, and soils. If the analyte response is linear within the range of concentration levels anticipated for samples, it is not necessary to construct a multipoint calibration. Only two samples need to be measured, the unspiked and spiked samples.

### 3.3.1 Can We Derive a Quantification Equation for SA?

Yes, indeed, and we proceed to do so now. Assume that $C_{nul}^i$ represents the ultimate goal of TEQA, i.e., the concentration of the $i$th analyte, such as a metal in the
unknown environmental sample or human specimen. Also assume that $C_{\text{spike}}$ represents the concentration of the $i$th analyte in a spike solution. After an aliquot of the spike solution has been added to the unknown sample, an instrument response of the $i$th analyte for the standard added sample, whose concentration must be $C_{\text{SA}}$, $R_{\text{SA}}$, is measured. Knowing only the instrument response for the unknown, $R_{\text{unk}}$, and the instrument response for the standard added, $R_{\text{SA}}$, $C_{\text{unk}}$ can be found. Mathematically, let us prove this. The proportionality constant $k$ must be the same between the concentration of the $i$th analyte and the instrument response, such as a peak area in atomic absorption spectroscopy. The following four relationships must be true:

$$C_{\text{unk}}^i = kR_{\text{unk}}^i \quad (2.13)$$

$$C_{\text{spike}}^i = kR_{\text{spike}}^i \quad (2.14)$$

$$R_{\text{SA}}^i = R_{\text{unk}}^i + R_{\text{spike}}^i \quad (2.15)$$

$$C_{\text{SA}}^i = k \left[ R_{\text{unk}}^i + R_{\text{spike}}^i \right] \quad (2.16)$$

Solving Equation (2.15) for $R_{\text{spike}}^i$ and substituting this into Equation (2.14) leads to the following ratio:

$$\frac{C_{\text{unk}}^i}{C_{\text{spike}}^i} = \frac{R_{\text{unk}}^i}{R_{\text{SA}}^i - R_{\text{unk}}^i} \quad (2.17)$$

Solving Equation (2.17) for $C_{\text{unk}}^i$ yields the quantification equation

$$C_{\text{unk}}^i = \left[ \frac{R_{\text{unk}}^i}{R_{\text{SA}}^i - R_{\text{unk}}^i} \right] C_{\text{spike}}^i \quad (2.18)$$

For real samples that may have nonzero blanks, the concentration of the $i$th analyte in an unknown sample, $C_{\text{unk}}^i$, can be found knowing only the measurable parameters $R_{\text{SA}}^i$ and $R_{\text{unk}}^i$ and instrument responses in blanks along with the known concentration of single standard added or spike concentration $R_{\text{spike}}^i$ according to

$$C_{\text{unk}}^i = \left[ \frac{R_{\text{unk}}^i - R_{\text{bl-unk}}^i}{R_{\text{SA}}^i - R_{\text{unk}}^i - \left( R_{\text{bl-unk}}^i - R_{\text{bl-sp}}^i \right)} \right] C_{\text{spike}}^i \quad (2.19)$$

where $R_{\text{bl-unk}}^i$ represents the instrument response for a blank that is associated with the unknown sample. $R_{\text{bl-sp}}^i$ is the instrument response for a blank associated with
the spike solution and accounts for any contribution that the spike makes to the blank. Equation (2.19) is listed in Table 2.2 as the quantification equation for SA.

If a multipoint calibration is established using SA, the line must be extrapolated across the ordinate (y axis) and terminate on the abscissa (x axis). The value on the abscissa that corresponds to the amount or concentration of unknown analyte yields the desired result. Students are asked to create a multipoint SA calibration to quantitate both Pb and anionic surfactants in Chapter 5. Contemporary software for graphite furnace atomic absorption spectroscopy (GFAA) routinely incorporates SA as well as ES modes of calibration. Autosamplers for GFAA easily can be programmed to add a precise aliquot of a standard solution containing a metal to an aqueous portion of an unknown sample that contains the same metal.

Most comprehensive treatments of various analytical approaches utilizing SA as the principal mode of calibration can be found in an earlier published paper by Bader.13

4. WHAT DOES LEAST SQUARES REGRESSION REALLY MEAN?

Ideally, a calibration curve that is within the linearity range of the instrument’s detector exhibits a straight line whose slope is constant throughout the range of concentration taken. By minimizing the sum of the squares of the residuals, a straight line with a slope \( m \) and a \( y \) intercept \( b \) is obtained. This mathematical approach is called a least squares (LS) fit of a regression line to the experimental data. The degree of fit expressed as a goodness of fit is obtained by the calculation of a correlation coefficient. The degree to which the least squares fit reliably relates detector response and analyte concentration can also be determined using statistics. Upon interpolation of the least squares regression line, the concentration or amount of analyte is obtained. The extent of uncertainty in the interpolated concentration or amount of analyte in the unknown sample is also found. In the next section, equations for the least squares regression will be derived and treated statistically to obtain equations that state what degree of confidence can be achieved in an interpolated value. These concepts are at the heart of what constitutes GLP.

4.1 HOW DO YOU DERIVE THE LEAST SQUARES REGRESSION EQUATIONS?

The concept starts with a definition of a residual for the \( i \)th calibration point. The residual \( Q_i \) is defined to be the square of the difference between the experimental data point \( y_i^e \) and the calculated data point from the best-fit line \( y_i^f \). Figure 2.5 illustrates a residual from the author’s laboratory where a least squares regression line is fitted from the experimental calibration points for \( N,N \)-dimethyl-2-aminoethanol using gas chromatography. Expressed mathematically,

\[
Q_i = \left| y_i^e - y_i^f \right|^2
\]
where $y^c$ is found according to

$$y_i^c = mx_i + b$$

with $m$ being the slope for the best-fit straight line through the data points and $b$ being the $y$ intercept for the best-fit straight line. $x_i$ is the amount of analyte $i$ or the
concentration of analyte \( i \). \( x_i \) is obtained from a knowledge of the analytical reference standard used to prepare the calibration standards and is assumed to be free of error. There are alternative relationships for least squares regression that assume \( x_i \) is not free of error. To obtain the least squares regression slope and intercept, the sum of the residuals over all \( N \) calibration points, defined as \( Q \), is first considered:

\[
Q = \sum_{i}^{N} \left| y_i^c - y_i^f \right|^2
\]

\[
Q = \sum_{i}^{N} \left[ y_i^c - (mx_i + b) \right]^2
\]

The total residual is now minimized with respect to both the slope \( m \) and the intercept \( b \):

\[
\frac{\partial Q}{\partial b} = 0 = -2 \sum_{i}^{N} \left[ y_i^c - (mx_i + b) \right] \quad (2.20)
\]

\[
\frac{\partial Q}{\partial m} = 0 = -2 \sum_{i}^{N} x_i \left[ y_i^c - (mx_i + b) \right] \quad (2.21)
\]

Rearranging Equation (2.20) for \( b \),

\[
b = \frac{1}{N} \left[ \sum_{i}^{N} y_i - m \sum_{i}^{N} x_i \right] \quad (2.22)
\]

Rearranging Equation (2.21) for \( m \),

\[
m = \frac{\sum_{i}^{N} x_i y_i - b \sum_{i}^{N} x_i \sum_{i}^{N} x_i}{\sum_{i}^{N} x_i^2} \quad (2.23)
\]

Next, substitute for \( b \) from Equation (2.22) into Equation (2.23):

\[
m = \frac{\sum_{i}^{N} x_i y_i - (1/N) \left( \sum_{i}^{N} y_i - m \sum_{i}^{N} x_i \right) \sum_{i}^{N} x_i}{\sum_{i}^{N} x_i^2}
\]

Upon simplifying, we obtain

\[
m = \frac{N \sum_{i}^{N} x_i y_i - \sum_{i}^{N} x_i \sum_{i}^{N} y_i}{N \sum_{i}^{N} x_i^2 - \left( \sum_{i}^{N} x_i \right)^2} \quad (2.24)
\]
Recall the definition of a mean:

\[ \bar{x} = \frac{\sum x_i}{N}, \quad \bar{y} = \frac{\sum y_i}{N} \]

Rearranging in terms of summations gives

\[ \sum_i x_i = N\bar{x} \quad (2.25) \]

\[ \sum_i y_i = N\bar{y} \quad (2.26) \]

Upon substituting Equations (2.25) and (2.26) into Equation (2.24), we arrive at an expression for the least squares slope \( m \) in terms of only measurable data points:

\[ m = \frac{\sum_i x_i y_i - N\bar{x}\bar{y}}{\sum_i x_i^2 - N\bar{x}^2} \quad (2.27) \]

Defining the sum of the squares of the deviations in \( x \) and \( y \) calculated from all \( N \) pairs of calibration points gives

\[ SS_{xx} = \sum_i (x_i - \bar{x})^2 \]

\[ SS_{yy} = \sum_i (y_i - \bar{y})^2 \]

The sum of the products of the deviation \( s \) is given by

\[ SS_{xy} = \sum_i (x_i - \bar{x})(y_i - \bar{y}) \]

The slope for the least squares regression can then be expressed as

\[ m = \frac{SS_{xy}}{SS_{xx}} \quad (2.28) \]

and the \( y \) intercept can be obtained by knowing only the slope \( m \) and the mean value of all of the \( x \) data and the mean value of all of the \( y \) data according to

\[ b = \bar{y} - m\bar{x} \quad (2.29) \]
Equations (2.28) and (2.29) enable the best-fit calibration line to be drawn through the experimental \( x, y \) points. Once the slope \( m \) and the \( y \) intercept \( b \) for the least squares regression line are obtained, the calibration line can be drawn by any one of several graphical techniques. A commonly used technique is to use EXCEL®, SIGMA PLOT®, or some other graphics software packages to graphically present the calibration curve. These equations can also be incorporated into computer programs that allow rapid computation.

A quantitative measure of the degree to which the dependent variable (i.e., the analytical signal) depends on the independent variable, the concentration of analyte \( i \), is denoted by the correlation coefficient \( r \) according to

\[
 r = \frac{\sum_i [(x_i - \bar{x})(y_i - \bar{y})]}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}}
\]

Using the previously defined terms, the correlation coefficient can be described as

\[
 r = \frac{SS_{xy}}{\sqrt{SS_{xx}SS_{yy}}} \tag{2.30}
\]

As \( r \) approaches 1, the correlation is said to approach perfection. A good linear calibration plot can achieve values for \( r \) that easily reach and exceed 0.9990, and suggests that good laboratory technique as well as optimum instrument performance have been achieved. A good correlation coefficient should not be confused with the notion of calibration linearity. A curve that upon visual inspection appears to be a nonlinear curve can exhibit a correlation coefficient that is calculated to be quite close to 1. The square of \( r \) is called the coefficient of determination. Several commercially available chromatography software packages, such as Total Chrom (Perkin-Elmer) or ChemStation (Agilent), are programmed to calculate only the coefficient of determination following the establishment of the least squares regression calibration curve. Equation (2.28), which expresses the least squares slope \( m \) in terms of a ratio of sums of the square, can be compared to Equation (2.30). If this is done, it becomes obvious that the true nature of \( r \) is merely a scaled version of the slope (i.e., the slope estimate multiplied by a factor to keep \( r \) always between –1 and +1),

\[
 r = m \frac{S_{xx}}{S_{yy}}
\]

### 4.2 To What Extent Are We Confident in the Analytical Results?

How reliable are these least squares parameters? To answer this question, we first need to find the standard deviation about the least squares best-fit line by summation
over \( N \) calibration points of the square of the difference between experimental and calculated detector responses according to

\[
s_{y/x} = \sqrt{\frac{\sum(y_i' - y_i)^2}{N - 2}}
\]  

(2.31)

where \( s_{y/x} \) represents the standard deviation of the vertical residuals and \( N - 2 \) represents the number of degrees of freedom. \( N \) is the number of \( x, y \) data points used to construct the best-fit calibration line less a degree of freedom used in determining the slope and a second degree of freedom used to determine the \( y \) intercept.

The uncertainty in both the slope and intercept of the least squares regression line can be found using the following equations to calculate the standard deviation in the slope, \( s_m \), and the standard deviation in the \( y \) intercept, \( s_b \):

\[
s_m = \frac{s_{y/x}}{\sqrt{\sum_{i=1}^{N} (x_i - \bar{x})^2}}
\]

\[
s_b = \frac{s_{y/x} \sum_{i=1}^{N} x_i^2}{\sqrt{N \sum_{i=1}^{N} (x_i - \bar{x})^2}}
\]

4.3 How Confident Are We of an Interpolated Result?

For a given instrumental response such as for the unknown, \( y_0 \), the corresponding value \( x_0 \) from interpolation of the best-fit calibration is obtained and the standard deviation in \( x_0 \) can be found according to

\[
s_{x_0} = \frac{s_{y/x}}{m} \sqrt{\frac{1}{L} + \frac{1}{N} + \frac{(y_0 - \bar{y})^2}{m^2 \sum_{i=1}^{N} (x_i - \bar{x})^2}}
\]

where \( s_{x_0} \) represents the standard deviation in the interpolated value \( x_0 \) and \( L \) represents the number of replicate measurements of \( y_0 \) for a calibration having \( N, x, y \) data points.

Upon replacing the summate with \( S_{xx} \), the standard deviation in the interpolated value \( x_0 \) yields a most useful expression:

\[
s_{x_0} = \frac{s_{y/x}}{m} \sqrt{\frac{1}{L} + \frac{1}{N} + \frac{(y_0 - \bar{y})^2}{m^2 S_{xx}}}  \]  

(2.32)
This form enables computer programs, such as the LSQUARES one in Appendix C, to be written that allow for rapid calculation of $s_{y0}$. Equation (2.32) shows that the uncertainty, $s_{x0}$, in the interpolated value, $x_0$, is largely determined by minimizing the ratio of $s_{y0}$ to $m$. A small value for $s_{y0}$ infers good to excellent precision in establishment of the least squares regression line. A large value for $m$ infers good to excellent detector sensitivity. The standard deviation in the interpolated value $x_0$ is also reduced by making $L$ replicate measurements of the sample. The standard deviation can also be reduced by increasing the number, $N$, of calibration standards used to construct the calibration curve.

The determination of $x_0$ for a given detector response, $y_0$, is, of course, the most important outcome of trace quantitative analysis. $x_0$, together with an estimate of its degree of uncertainty, represents the ultimate goal of trace quantitative analysis; that is, it answers the questions, how much of analyte $i$ is present, and how reliable is this number in a given environmental sample? For TEQA, it is usually unlikely that the population mean for this interpolated value $\mu(x_0)$ can ever be known and that the standard deviation in this population mean, $\sigma(x_0)$, can ever be known. TEQA requires the following:

- A determinative technique whereby the concentration of a priority pollutant VOC, such as vinyl chloride, can be measured by an instrument. It can be assumed that for a given analyte such as vinyl chloride, repeatedly injected into a gas chromatograph, $\mu(x_0)$ and $\sigma(x_0)$ are known.
- However, the concentration of vinyl chloride in the environmental sample may involve some kind of sample preparation to get the analyte into the appropriate chemical matrix for the application of the appropriate determinative technique, and we can assume that both $\mu(x_0)$ and $\sigma(x_0)$ are unknown.

These two constraints require that the confidence limits be found when the standard deviation in the population mean is known. This is where the Student’s $t$ statistics have a role to play. Who was “Student”? Anderson has introduced a little history (pp. 70–72):7

Unfortunately, we do not know the true standard deviation of our set; we have only an estimate $s$ based on $L$ observation. Thus, the distribution curve for $\bar{x}$ is an estimate of the true distribution curve. A research chemist, W.S. Gosset, worked out the relationship between these estimates of the distribution curve and the true one so that we can use $s$ in place of $\sigma$. Gosset was employed by a well-known Irish brewery which did not want its competition to know it was using statistical methods. However, he was permitted to publish his work under the pen name “Student,” and the test for differences in averages based on his work is known as Student’s $t$ test. Student’s $t$ test assumes that the average is based on data from a normal population.

Because we know $s_{x0}$ (the standard deviation in the interpolated value $x_0$), we can use the Student’s $t$ statistics to estimate to what extent $x_0$ estimates the population mean $\mu(x_0)$. This can be determined according to
Conf Int for $\mu_{x_0} = x_0 \pm t_{1-\alpha/2,df}s_{x_0}$

where $t_{1-\alpha/2,df}$ represents the value for a two-tailed Student's $t$ at the significance level of $\alpha$ for $N - 2$ degrees of freedom ($df$), where $N$ is the number of $x, y$ data points used to construct the calibration curve. The term $[\pm t_{1-\alpha/2,df}s_{x_0}]$ is called the confidence interval and represents the range of $x$ values within which one can be $(1 - \alpha/2) 100\%$ confident that the mean value for $x_0$ will approximate the population mean $\mu_{x_0}$. Appendix C shows a computer program written in GWBASIC by this author. The program not only finds the least squares regression line through a set of $N$ calibration points, but also finds decision and detection limits, and for a given instrument response, $y_0$, the program finds $x_0$ and the confidence interval for $\mu_{x_0}$.

Figure 2.6 is this author’s attempt to graphically represent the uncertainty present in an interpolated instrument response $y_0$. A segment of what might be a typical ES or IS calibration plot shrouded with its corresponding confidence limits both above and below the regression line is shown. The confidence interval is shown to be equidistant from the regression line. This assumption represents the homoscedastic case, i.e., a regression line having a constant variance, over the range of analyte concentration used to construct the linear regression line. Those sections of the calibration that are highlighted in bold reveal that horizontal movement to either confidence limit at $y_0$ results in equal confidence intervals. The confidence interval

![Figure 2.6 Interpolation of a least squares regression line showing confidence intervals.](image-url)
can be related to the product of Student’s \( t \) and the standard deviation in the interpolated value, \( s_0 \). Mathematically,

\[
\begin{align*}
\left| y_0 - y_{\text{lower}} \right| & \leftrightarrow t_{1-\alpha/2, df} s_0 \\
\left| y_0 - y_{\text{upper}} \right| & \leftrightarrow t_{1-\alpha/2, df} s_0
\end{align*}
\]

**Figure 2.6** is a good starting point from which to consider a derivation proposed earlier by MacTaggert and Farwell.\(^\text{14}\)

The prediction interval about \( x_L \) (or \( x_{\text{lower}} \), as shown in Figure 2.6) can be mathematically defined as follows:

\[
y_L \pm ts_yy_L = b + mx_L \pm t_{(1-\alpha/2, df=n-2)} sy/y \sqrt{1 + \frac{1}{N} + \frac{(x_L - \bar{x})^2}{S_y}}
\]

where

- \( b \) = y intercept of the linear regressed line
- \( m \) = slope of the linear regressed line
- \( t \) = two-tailed value for Student’s \( t \) for \( N - 2 \) degrees of freedom, where \( N \) is the number of \( x, y \) data points used to construct the calibration

Refer again to Figure 2.6. We see that if the value for \( y_L \) is taken and the height of this interval added to it, \( y_0 \) is obtained. This enables two relationships to emerge that can both be set equal to \( y_0 \). These two relationships are given below for \( y_0 \):

\[
y_0 = b + mx_0
\]

\[
y_0 = b + mx_L + ts_yy_L \sqrt{1 + \frac{1}{N} + \frac{(x_L - \bar{x})^2}{S_y}}
\]

Both equations being equal to \( y_0 \) can be set equal to each other such that

\[
b + mx_0 = b + mx_L + ts_yy_L \sqrt{1 + \frac{1}{N} + \frac{(x_L - \bar{x})^2}{S_y}}
\]

Eliminating \( b \), setting this equation equal to zero, squaring both sides, and then solving for \( x_L \) yields the following quadratic equation:

\[
x_L^2 (1 - g) + 2x_L (g\bar{x} - x_0) + x_0^2 - g\bar{x}^2 - gS_y \left( 1 + \frac{1}{N} \right) = 0
\]
Here $g$ is used to collect terms and greatly simplify the expression

$$g = \frac{r^2 s_y^2}{m^2 S_{xy}}$$

Solving the above quadratic equation via the quadratic formula and incorporating the result for $x_0$ yields a *discrimination interval* about the interpolated value for $x_0$:

$$\{x_U, x_L\} = x_0 - g \bar{x} + \frac{ts}{m(1-g)} \sqrt{\frac{1}{1 + \frac{1}{N}}(1-g) + \frac{(x_0 - \bar{x})^2}{S_{xy}}}$$

If $L$ replicates are made of the $y_0$ value, the following equation may be used to define discrimination intervals about the interpolated value for $x_0$ according to

$$\{x_U, x_L\} = \frac{x_0 - g \bar{x}}{1-g} \pm \frac{ts_{xy}}{m(1-g)} \sqrt{\frac{1}{L + \frac{1}{N}}(1-g) + \frac{(x_0 - \bar{x})^2}{S_{xy}}}$$

The expression for $g$ above can be further simplified by substituting for $S_{xy}$ using Equation (2.28) so that

$$g = \frac{r^2 s_y^2}{m^2}$$

This relationship shows that $g$ is a measure of the statistical significance of the slope value. Further use of $g$ can be made to derive a simpler equation for the discrimination interval. Let us assume that $g \ll 1$ such that

$$\{x_U, x_L\} = x_0 \pm \frac{ts_{xy}}{m} \sqrt{\frac{1}{L + \frac{1}{N}} + \frac{(x_0 - \bar{x})^2}{S_{xy}}}$$

This equation shows a symmetrical interval about $x_0$ and is comparable to a prediction interval, as introduced earlier. We have thus mathematically shown that the standard deviation in the vertical residual, $s_{y|x}$, can approximate the discrimination interval. The following quote reinforces this notion: 15

Analytical chemists must always emphasize to the public that the *single most characteristic of any result obtained from one or more analytical measurements is an adequate statement of its uncertainty interval*. Lawyers usually attempt to dispense with uncertainty and try to obtain unequivocal statements; therefore, an uncertainty interval must be clearly defined in cases involving litigation and/or enforcement proceedings. Otherwise, a value of 1.001 without a specified uncertainty, for example, may be viewed as legally exceeding a permissible level of 1.

© 2006 by Taylor & Francis Group, LLC
The concept of a confidence interval around the least squares calibration line will again become important in the calculation of an instrument’s detection limit for a given analyte. In other words, how reliable is the detection limit for a given analyte?

5. HOW DO YOU DERIVE EQUATIONS TO FIND INSTRUMENT DETECTION LIMITS?

The IDL for a specific chemical analyte is defined to be the lowest possible concentration that can be reliably measured. Experimentally, if lower and lower concentrations of a given analyte are measured, the smallest concentration that is barely detectable constitutes a rough estimate of the IDL for that analyte using that particular instrument. For years, EPA has required laboratory analysts to first measure a blank replicate a number of times. The most recent guidelines appeared in 40 Code of Federal Regulations (CFR) Part 136, Appendix B. The steps that are recommended are listed as follows:

1. Prepare a calibration curve for the test with standards.
2. Analyze seven laboratory water blanks.
3. Record the response of the test for the blanks.
4. Prepare the mean and standard deviation of the results from the blanks as above.
5. The IDL is three times the standard deviation on the calibration curve.

Fortunately, a bit more thought has been given to the calculation of the IDL than the meager guidelines given above. The average signal that results from these replicate measurements yields a mean signal, $S_{\text{blank}}$. The analyst then calculates the standard deviation of these replicate blanks, $s_{\text{blank}}$, and finds the sum of the mean signal and a multiple $k$ (often $k = 3$) of the standard deviation to obtain a minimum detectable signal $S_{\text{IDL}}$ according to

$$S_{\text{IDL}} = S_{\text{blank}} + ks_{\text{blank}}$$

If a least squares calibration curve has been previously established, the equation for this line takes on the common form, with $S$ being the instrument response, $m$ being the least squares slope, and $x$ being the concentration according to

$$S = mx + S_{\text{blank}}$$

(2.33)

Solving Equation (2.33) for $x$, denoting the IDL as $x_{\text{IDL}}$, and replacing $S$ with this minimum signal, $S_{\text{IDL}}$, yields

$$x_{\text{IDL}} = \frac{S_{\text{IDL}} - S_{\text{blank}}}{m}$$

(2.34)

Equation (2.34) represents a step up from the meager guidelines introduced earlier; it incorporates the slope of the calibration as a means to address detector
sensitivity. It becomes obvious that minimizing \( x_{\text{IDL}} \) requires that the signal at the detection limit, \( S_{\text{IDL}} \), be maximized while the noise level remains minimized, and that the steepness of the calibration curve be maximized.

The use of Equation (2.34) to quantitatively estimate the IDL for chromatographs and for spectrometers has been roundly criticized over the years. There have been reported numerous attempts to find alternative ways to calculate IDLs. This author will comment on this most controversial topic in the following manner. The approach encompassed in Equation (2.34) clearly lacks a statistical basis for evaluation, and hence is mathematically found to be inadequate. As if this indictment is not enough, IDLs calculated based on Equation (2.34) also ignore the uncertainty inherent in the least squares regression analysis of the experimental calibration, as presented earlier. In other words, what if the analyte is reported to be absent when, in fact, it is present (a false negative)? In the subsections that follow, a more contemporary approach to the determination of IDLs is presented and starts first with the concept of confidence intervals about the regression line.

5.1 CAN WE DERIVE EQUATIONS FOR CONFIDENCE INTERVALS ABOUT THE REGRESSION?

The least squares regression line is seen to be shrouded within confidence intervals, as shown in Figure 2.6. The derivation of the relationship introduced below was first developed by Hubaux and Vos\(^{18}\) over 30 years ago. The upper and lower confidence limits for \( y \) that define the confidence interval for the calibration are obtained for any \( x \) (concentration) and are given as follows:

\[
y = [\bar{y} - m(x - \bar{x})] \pm t\sqrt{V_y}
\]

where \( \bar{x} \) and \( \bar{y} \) are the respective mean values over all \( x \) and \( y \) data points, and \( m \) represents the slope of the linear least squares regression. \( t \) corresponds to a probability of \( 1 - \alpha \) for the upper limit and \( 1 - \beta \) for the lower limit. \( V_y \) represents the variance in the instrument response \( y \) for a normal distribution of instrument responses at a given value of \( x \). This equation represents another way to view the least squares regression line and its accompanying confidence interval. The term in brackets is the calculated response based on least squares regression. The variance in \( y \), \( V_y \), is composed of two contributions. The first is the variance in \( y \) calculated from the least squares regression, and the second is the residual variance \( \sigma^2 \). Expressed mathematically,

\[
V_y = V_{\bar{y} - m(x - \bar{x})} + \sigma^2
\]

and the variance in the least squares calculated \( y \) can be viewed as being composed of a variance in the mean value for \( y \) and a variance in the \( x \) residual according to

\[
V_{\bar{y} - m(x - \bar{x})} = V_{\bar{y}} + V_{m(x - \bar{x})}
\]
The variance in the least squares regression line can further be broken down into the variance in the mean \( \bar{y} \) expressed as \( V_{\bar{y}} \) and the variance in the slope \( m \) expressed in terms of \( V_m \) according to

\[
V_{\bar{y}} = \frac{\sigma^2}{N} \tag{2.37}
\]

\[
V_m = \frac{\sigma^2}{\sum_{i=1}^{N} |x_i - \bar{x}|} \tag{2.38}
\]

Hence, substituting Equations (2.37) and (2.38) into Equation (2.36) gives

\[
V_y = \sigma^2 + \frac{\sigma^2}{N} \sum_{i=1}^{N} |x_i - \bar{x}|^2 \tag{2.39}
\]

Factoring out \( \sigma^2 \) gives

\[
V_y = \sigma^2 \left[ 1 + \frac{1}{N} \frac{|x - \bar{x}|^2}{\sum_{i=1}^{N} |y_i - \bar{y}|^2} \right] \tag{2.40}
\]

The residual variance \( \sigma^2 \) may be replaced by its estimate \( s^2 \), and upon substituting Equation (2.40) into Equation (2.35), it gives

\[
y = [\bar{y} - m(x - \bar{x})] \pm ts \sqrt{1 + \frac{1}{N} \frac{|x - \bar{x}|^2}{\sum_{i=1}^{N} |y_i - \bar{y}|^2}} \tag{2.41}
\]

Equation (2.41) is an important relationship if one desires to plot the confidence intervals that surround the least squares regression line. The upper confidence limit for the particular case where \( x = 0 \) is obtained from Equation (2.41), in which a \( 1 - \alpha \) probability exists that the normal distribution of instrument responses falls to within the mean \( y \) at \( x = 0 \). Mathematically, the instrument response \( y_C \), often termed a critical response, is defined as

\[
y_C = [\bar{y} - m\bar{x}] \pm t_{1-\alpha/2} \sqrt{1 + \frac{1}{N} \frac{x^2}{\sum_{i=1}^{N} |y_i - \bar{y}|^2}} \tag{2.42}
\]
Equation (2.42) can be viewed as being composed of two terms. This is expressed as follows:

\[ y_C = y_0 + [P][s] \]

The y intercept of the least squares regression line is given by

\[ y_0 = \bar{y} - mx \]

The value for \( y_0 \) cannot be reduced because it is derived from the least squares regression; however, the second term, \([P][s]\), may be reduced and hence lead to lower IDLs. \( P \) is defined as

\[ P = t_{1-\alpha} \sqrt{\frac{1}{N} + \frac{\bar{x}^2}{SS_{xx}}} \]

Figure 2.7 provides a graphical view of the terms used to define the decision limit, \( x_C \), and detection limit, \( x_D \). The decision limit, \( x_C \), is a specific concentration level for a targeted analyte above which one may decide whether the result of analytical measurement indicated detection. The detection limit, \( x_D \), is a specific concentration level for a targeted analyte above which one may rely upon to lead to detection. A third limit, known as a determination limit, or using more recent

**FIGURE 2.7** Graphical estimate of the decision limit, \( x_C \), and the detection limit, \( x_D \), for a hypothetical LS regression (not drawn to scale).
jargon from the EPA, the practical quantitation limit, is a specific concentration at
which a given procedure is sufficiently precise to yield satisfactory quantitative
analysis. The determination limit will be introduced in more detail later.

Referring to Figure 2.7, $y_C$ can merely be drawn horizontally until it meets with
the lower confidence limit in which a $1 - \beta$ probability exists that the normal
distribution of instrument responses falls to within the mean at this value of $x$. The
ordinate that corresponds to this value of $x$, $x_{DL}$, can be found according to

$$
y_D = y_C + t_{1-\beta} s \sqrt{1 + \frac{1}{N} + \frac{|x_D - \bar{x}|^2}{\sum_{i=1}^{N} (x_i - \bar{x})^2}}
$$

Equation (2.43) can be viewed as comprising three terms, expressed as follows:

$$
y_D = y_0 + [P][s] + [Q][s]
$$

where

$$Q = t_{1-\beta} s \sqrt{1 + \frac{1}{N} + \frac{|x_D - \bar{x}|^2}{\sum_{i=1}^{N} (x_i - \bar{x})^2}}
$$

The IDL is thus seen to be composed of a fixed value, $y_0$, which is derived from
the nature of the least squares calibration line and the residual standard deviation,
s. Factors $P$ and $Q$ can be varied so as to minimized $y_D$.

Graphically, it is straightforward to interpolate from $y_C$ to the IDL $x_{DL}$. Numeri-
cally, Equations (2.42) and (2.43) can be solved for the critical limit $x_C$ and for the
IDL, $x_{DL}$. The equation for the calibration line can be solved for $x$ as follows:

$$x = \frac{y - y_0}{m}
$$

When the instrument response $y$ is set equal to $y_C$, the concentration that corre-
sponds to a critical concentration limit is defined. This critical level $x_C$, defined
almost 20 years ago, is a decision limit at which one may decide whether the
result of an analysis indicates detection. The critical level has a specifically defined
false positive (type I) error rate, $\alpha$, and an undefined false negative (type II) error
rate, $\beta$; $x_C$ is found according to

$$x_C = \frac{y_C - y_0}{m} = \frac{[P][s]}{m}
$$
In a similar manner, the IDL that satisfies the statistical criteria discussed is obtained according to

\[ x_D = \frac{y_D - y_0}{m} = \frac{[P][s] + [Q][s]}{m} = \frac{s(P + Q)}{m} \]  

(2.45)

The IDL denoted by \( x_D \) and given in Equation (2.45) is the true concentration at which a given analytical procedure may be relied upon to lead to detection. A false negative rate can now be defined. A normal distribution of instrument responses at a concentration of zero and at a concentration \( x_D \) overlaps at \( x_c \) and gives rise to \( \alpha \) and \( \beta \). Figure 2.8 graphically depicts this Gaussian distribution and stems from application of Equation (2.41), whereas confidence intervals can be defined that shroud the linear least squares regression line. The intersection of the upper confidence band with the \( y \) axis at \( y_C \), where \( x = 0 \), corresponds to the highest signal that could be attributed to a blank 100 (1 – \( \alpha \))% of the time. This is represented by a \( t \) distribution with a one-sided tailed \( \alpha \) for \( y \) at \( x = 0 \). The intersection of \( y_C \) with the lower confidence band at \( y_L \) in Figure 2.8 corresponds to the lowest signal that could be attributed to an analyte concentration \( x_D \) 100 (1 – \( \beta \))% of the time. This is represented by the \( t \) distribution with a one-tailed \( \beta \) for \( y \) at \( x = x_D \). The mean for the distribution of signals at \( x_D \) is \( y_D \). Equation (2.45) leads to a quadratic equation whose root is given below:

\[ x_D = \frac{2ts_{sv}}{m(1 - g)} \left[ \frac{1}{L} + \frac{1}{N} + \frac{\hat{x}^2}{S_{sv}} \right] \left[ \frac{2g\hat{x}}{1 - g} \right] \]  

(2.46)
5.2 **WHAT IS WEIGHTED LEAST SQUARES AND HOW DOES THIS INFLUENCE IDLS?**

The previous derivation of the least squares regression equations assumed that the variance about the regressed line was constant throughout the range of analyte concentrations used to construct the calibration and is referred to as *ordinary least squares* (OLS). Many calibration curves, particularly those at concentration levels close to the IDL, have nonconstant variances about the linear regressed line. Chromatographic processing software queries the user as to whether or not a *weighted least squares regression* (WLS) or OLS regression is to be used. It behooves the analyst to have at least a cursory understanding of WLS.

Burdge et al.\(^{19}\) put in this way:

Application of OLS to data with non-constant variance (heteroscedasticity) yields confidence bands and thus detection limits that will not accurately reflect measurement capability. WLS facilitates the determination of realistic detection limits for heteroscedastic data by yielding confidence bands that directly reflect the changing variance….. Application of OLS to significantly heteroscedastic data results in the construction of unnecessarily broad confidence bands at the low end of the calibration curve. The detection limit derived from OLS treatment of such data will not be a fair representation of a measurement method’s detection capability.

Hence, weighting factors or simply weights are used to give more emphasis or weight to calibration data points, usually at the lower end of the calibration. This can be done without changing the raw data. However, implementing WLS requires that we modify the previously developed mathematical relationships used to derive OLS.

Let us return to Equation (2.31) for the standard deviation of the vertical residuals for \( N - 2 \) degrees of freedom, as introduced earlier. A weighted residual would look like the following:

\[
s_{wy/x} = \sqrt{\frac{\sum w_i (y_i^c - y_{wi}^c)}{N - 2}} \tag{2.47}
\]

where \( y_{wi}^c \) is the predicted weighted instrument response for the \( i \)th data point and is obtained from the WLS regressed line according to:

\[
y_{wi}^c = m_w x_{wi} + b_w
\]

The WLS slope can similarly be viewed and compared to Equation (2.28):

\[
m_w = \frac{s_{wyx}}{s_{wx}}
\]
These three relationships according to Zorn et al.\textsuperscript{20} can be used to yield weighted prediction intervals around a predicted response, \( y_{jw}^{c} \), at concentration \( x_{j} \) according to

\[
y_{j} = y_{jw}^{c} \pm t_{(1-\alpha/2,N-p-2)}S_{wy}/w_{j} \left[ \frac{1}{w_{j}} + \frac{1}{\sum w_{j}} + \frac{(x_{j} - x_{w})^{2}}{S_{xw}} \right]^{1/2}
\]  \hfill (2.48)

The weighted parameters have replaced the unweighted parameters \((s, x_{w}, S_{x})\), the sum of the weights has replaced \(N\), and \( t_{(1-\alpha/2,N-p-2)} \) is the \((1-\alpha/2)\) 100 percentage point of the Student’s \(t\) distribution on \(N-p-2\) degrees of freedom (where \(p\) is the number of parameters used to model the weights). Also, the inverse weight \((1/w_{j})\) at \(x_{j}\) has replaced 1 in the unweighted equation [Equation (2.41)]. The reader, at this point, should compare Equation (2.48) with Equation (2.41) while noting similarities and differences between OLS and WLS approaches to instrument calibration.

Three general approaches are introduced to find appropriate weights:

- Define a weight as being inversely proportional to the standard deviation for the \(i\)th calibration data point:
  \[
  w_{i} = \frac{1}{s_{i}}
  \]

- Define a weight as being inversely proportional to the variance for the \(i\)th calibration data point:
  \[
  w_{i} = \frac{1}{S_{i}^{2}}
  \]

- Plot the standard deviation as a function of analyte concentration and fit the data to either a quadric or exponential of a two-component model.

An instrument detection limit based on WLS has been recently reported.\textsuperscript{19}
5.3 IS THERE A DIFFERENCE IN SIMPLIFIED VS. CONTEMPORARY IDLS?

To conclude this discussion on IDLs, it is useful to compare Equations (2.34) and (2.45). Both equations relate \( x_d \) to a ratio of a standard deviation to the slope of the least squares regression line multiplied by a factor. In the simplified case, Equation (2.34), the standard deviation refers to detector noise found in the baseline of a blank reference standard, whereas the standard deviation in the statistical case, Equation (2.45), refers to the uncertainty in the least squares regression itself. This is an important conceptual difference in estimating IDLs. This difference should be understood and incorporated into all future methods.

5.4 HOW DO MDLS DIFFER FROM IDLS?

Obtaining an instrument’s IDL represents only one aspect of the overall estimate of a method’s detection limit. The only situation whereby an IDL is the same as an MDL is when there is no transfer of analyte from the environmental phase to a second phase. Direct aqueous injection of a groundwater sample into a GC in which there is no extraction step involved is one example of where MDLs equal IDLs. The removal of trace organic volatiles from an aqueous sample either by the purge-and-trap technique or by static headspace sampling at elevated temperatures results in nearly 100% efficiency and can also be considered as having MDL equal IDL. Such is not the case for semivolatile to nonvolatile organics in environmental samples when some type of sample extraction is employed. In these situations, the MDL is often much less than the IDL because sample preparation methods are designed to concentrate the analyte from the environmental matrix to the solvent matrix. The efficiency of this concentration must also be taken into account. One cannot always assume that a given sample preparation is 100% efficient. In fact, a key ingredient in GLP is to conduct an efficiency study by adding an accurately known amount of analyte to the sample matrix, and then measure to what extent the method recovers the analyte. The extent of recovery is often called a percent recovery.

5.5 HOW DO I OBTAIN MDLS FOR MY ANALYTICAL METHOD?

The MDL can be found only after the IDL, the concentration factor, i.e., the ratio of final extract or eluent volume (extract, if LLE sample prep techniques were used or eluent, if SPE sample prep techniques were used) \( V_e \) to the sample volume, \( V_s \), and the percent recovery \([% R_i = (100) (R_i)]\), where \( R_i \) is the fraction of the \( i \)th analyte recovered, have been quantitatively determined. For a groundwater sample, \( V_e = 1 \) mL and \( V_s = 1000 \) mL. For a soil sample, \( V_e = 10 \) mL and the sample weight might be 30 g. For serum, the respective volumes might be: \( V_e = 1 \) mL and \( V_s = 1 \) mL.

The important mathematical relationship that answers the question above will now be derived. Mass balance requires that the amount of recovered \( i \)th analyte present in the final extract as measured equal that fraction of \( i \)th analyte recovered originally present in the groundwater, soil, or serum sample using your method. \( R \) must be experimentally measured in the sample matrix in a separate set of experiments called Percent Recovery Studies. Expressed mathematically,
so that

\[
V_e C'_i = V_s C_s \left( \frac{\%R_i}{100} \right)
\]

Solving for \( C'_i \) gives:

\[
C'_i = C_s \left( \frac{V_e}{V_s} \right) \left( \frac{100}{\%R_i} \right)
\]

where

- \( C'_i \) = concentration of \( i \)th analyte in the original sample
- \( C_s \) = concentration of \( i \)th analyte in the final volume of extract (if LLE is used) or final volume of eluent (if SPE is used)
- \( V_e \) = volume of extract or eluent in mL
- \( V_s \) = volume of sample in mL
- \( \%R_i \) = percent recovery for \( i \)th analyte measured independently

If the concentration of \( i \)th analyte in the final extract or eluent, \( C'_i \), is low enough so as to represent the IDL, then the concentration of \( i \)th analyte in the original sample, \( C_s \), would yield the MDL for your method.

The percent recovery (\( \%R \)) can be calculated from measuring the instrument response for the analyte in the spiked matrix denoted by \( A' \), with uncertainty characterized by a standard deviation \( s' \) and a reference control that is known to be 100% recovered, \( A'_c \), with uncertainty \( s'_c \). This is mathematically defined according to

\[
\%R = \left( \frac{A'}{A'_c} \right) \times 100
\]  
(2.49)

The calculation of \( R \) involves dividing \( A' \) by \( A'_c \); this division requires a propagation of error. A relative standard deviation can be calculated by taking into account the propagation of error for random errors using the equation

\[
\frac{s'_R}{R} = \sqrt{\left( \frac{s'}{A'} \right)^2 + \left( \frac{s'_c}{A'_c} \right)^2}
\]  
(2.50)

Equation (2.50) shows that it is relative errors expressed in terms of variances that must be added to enable a determination of the relative error in the percent recovery calculation using Equation (2.49).
The uncertainty in a percent recovery determination is often expressed in terms of a percent relative standard deviation that is also called the coefficient of variation. The coefficient of variation is calculated according to

\[
\text{Coeff Var} = \left( \frac{s_j}{R_i^j} \right) \times 100
\]  

(2.51)

If sufficient replicate analytical data are available, the variance and standard deviation for a percent recovery study can be found by pooling the data. For example, consider a thorough percent recovery study using solid-phase extraction (SPE) for the isolation and recovery of the pesticide methoxychlor from groundwater. If \( j \) replicate injections are made for each of \( g \) SPE extractions conducted, an overall standard deviation for this pooled data, \( s_{\text{pooled}} \), can be calculated according to

\[
s_{\text{pooled}} = \sqrt{\frac{1}{N-g} \sum_{j=1}^{g} \sum_{i=1}^{j} (x_{ij} - \bar{x})^2}
\]  

(2.52)

The analyst is now ready to run samples using the selected method because instrument calibration, instrument verification, and the determination of IDLs and MDLs have all been accomplished.

6. WHY SO MANY REPLICATE MEASUREMENTS?

There is no other measurement science that insists on so many replicate measurements than EPA method-driven TEQA. Earlier, the notion of analyzing seven lab blanks was cited as a guideline. In fact, the number 7 appears quite frequently throughout EPA methods. This author has been known to quip to students that since the Beatles used number 9 in their music, the EPA likes to use number 7 in its methods. The answer goes to the heart of the meaning of the central limit theorem of statistics and attempts to apply its meaning to the above-posed question\(^2\) (a number of texts discuss the basics of probability and statistics; this author continuously refers to this text). How many replicate measurements, \( L \), of a given environmental contaminant such as vinyl chloride are needed to be confident 99% of the time that the estimate of the sample variance is less than three times the true variance (i.e., population variance)? Anderson\(^7\) (pp. 44 and 298) has considered this, and sure enough, in his appendix there exists a table that gives the answer — and lo and behold, the answer is seven.

A few words about the Gaussian or normal distribution are in order at this point, along with the commonly used statistical definitions. Figure 2.9 shows a hypothetical normal distribution with the apex located at the population mean. It is important to distinguish between a confidence level or level of significance and a confidence interval. The statement “to be 95% confident that a single analytical result such as \( x_0 \), in the absence of any systematic error (i.e., 95 of every subsequent 100 measurements),
will fall to within $\pm 1.96\sigma$, where $\sigma$ is the standard deviation in the population mean,” addresses both the confidence level and interval. Confidence intervals are labeled to the left of the Gaussian distribution in Figure 2.9 and indicate width or spread, whereas confidence levels, labeled to the right of the Gaussian distribution, refer to heights. The fraction of a population of observations $dL/L$, whose values lie in the region $x$ to $x + dx$, is given by

$$
\frac{dL}{L} = \frac{1}{2\pi\sigma} e^{-(x-\mu)^2/2\sigma^2} dx
$$

(2.53)

Deviations of individual values of $x$ from the mean are conveniently expressed in terms of a dimensionless unit of standard deviation $z$ according to

$$
z = \frac{x - \mu}{\sigma}
$$

If we differentiate $z$ with respect to $x$ and substitute this into Equation (2.53), the Gaussian equation looks like

$$
\frac{dL}{L} = \frac{1}{2\pi} e^{-z^2} \, dz
$$

The distinction between systematic vs. random error in laboratory measurement was introduced at the beginning of this chapter. The normal distribution shown in Figure 2.9 enables a set of confidence levels to be established in the absence of...
systematic error. Mathematical relationships that enable a confidence interval to be calculated that shrouds the linearly regressed calibration line were also given earlier in the chapter. These relationships enabled a more correct definition of what constitutes an IDL to emerge, as well as to predict an uncertainty in the interpolated value of a concentration $x_0$ taken from the regression.

Here, we consider the basic statistics of replicate measurements. For example, consider making repeated injections of an initial calibration verification (ICV) reference standard using an autosampler atop a gas chromatograph. The degree of automation essentially eliminates any systematic error in the measurement of each analyte that may comprise the ICV reference standard. Confidence levels (refer again to Figure 2.9) are usually selected at 95 or 99% for most TEQA. The true or population mean $\mu$ is fixed for replicate measurement and remains unknown. The normal distribution enables one to set limits such that the measurable sample mean, represented by the $x$ bar below, can be expected to lie within a given degree of probability such that 95% of all measurements lie to within $1.96\sigma$ of the true population mean.

Two approaches emerge. A confidence limit can be found when the sample standard deviation, $s$, is a good approximation of the population standard deviation, $\sigma$, such that for a single measurement, the confidence interval for $\mu$ is

$$\text{Conf Int for } \mu = \bar{x} \pm z\sigma$$

The confidence interval for $\mu$ when a sample mean has been found for $L$ replicate measurements is

$$\text{Conf Int for } \mu = \bar{x} \pm \frac{z\sigma}{\sqrt{L}}$$

When $\sigma$ is unknown, the standard deviation, $s$, of the small number of samples that may have considerable uncertainty requires broader confidence intervals. This is the most likely scenario for conducting TEQA, in that the analyst usually does not know the true value for $\mu$ and has in his possession a very limited number of samples. A two-tailed Student’s $t$ is then substituted for $z$. The value of $t$ selected depends on two factors: the desired degree of confidence and the number of degrees of freedom ($df$), where $df = L - 1$. The confidence interval for the mean of $L$ replicate measurements is found according to

$$\text{Conf Int for } \mu = \bar{x} \pm \frac{t_{(1-\alpha/2,df)}s}{\sqrt{L}}$$

(2.54)

In Appendix C, a short computer program written in BASIC and titled RSD is given. This program applies Equation (2.54). RSD queries the user for a number of replicate measurements and the replicate data itself, and then proceeds to calculate
the mean, standard deviation, relative standard deviation, and confidence interval using \( t \) statistics.

7. **HOW DO I FIND THE LIMIT OF QUANTITATION?**

Again, there are two ways, the simpler and the more contemporary. A recently published text on chemometrics has addressed this difference as follows (p. 66):22

In principle, all performance measures of an analytical procedure ... can be derived from a certain critical signal value, \( y_C \). These performance measures are of special interest in trace analysis. The approaches to estimation of these measures may be subdivided into “methods of blank statistics,” which use only blank measurement statistics, and “methods of calibration statistics,” which in addition take into account calibration confidence band statistics.

The simpler approach, in a manner similar to finding the IDL and discussed earlier in this chapter (and the one suggested by EPA), is to first find the standard deviation in the blank signal, add 10 times this value to the blank signal according to

\[
S_{LOQ} = S_{\text{blank}} + 10s_{\text{blank}} \quad (2.55)
\]

and, in a manner similar to that shown in Equation (2.34), interpolate the concentration from \( S_{LOQ} \) that can be defined at the limit of quantitation or practical limit, \( x_{LOQ} \), according to

\[
x_{LOQ} = \frac{S_{LOQ} - S_{\text{blank}}}{m} \quad (2.56)
\]

The superscript \( LOQ \) is the limit of quantitation. This author has attempted to use the standard deviation in the \( y \) intercept of the least squares regression of a given calibration for a given analyte of interest (refer to Equation (2.31) and the equation for \( s_b \)) to establish the standard deviation for the blank, \( s_{\text{blank}} \), in Equation (2.55). The \( y \) intercept itself, \( b \) from Equation (2.29), is used to represent \( S_{\text{blank}} \). These two parameters are then substituted into Equation (2.55) to yield \( S_{LOQ} \). This elevated signal, \( S_{LOQ} \), can be substituted into Equation (2.56) to find the limit of quantitation.

Oppenheimer et al.3 have proposed, for the case of the unweighted least squares regression, a relationship similar to that shown in Equation (2.42) to calculate \( y_Q \) according to

\[
y_Q = \frac{S_{\text{res}}}{C} \sqrt{\frac{1}{1 + \frac{1}{N} + \frac{\bar{x}^2}{\sum x_i - \bar{x}^2}}} \]

© 2006 by Taylor & Francis Group, LLC
Calibration, Verification, Statistical Treatment

where \( C \) is the ratio of the standard deviation in the signal corresponding to \( x_{LOQ} \) to the signal itself and is usually set equal to 0.10. If \( y_Q \) is substituted for \( S_{LOQ} \) in Equation (2.56), \( x_{LOQ} \) is found. This is a “calibration statistical” approach to finding the limit of quantitation, \( x_{LOQ} \). Zorn et al. have defined \( y_Q \) as equal to 10 times the standard deviation at the critical level, and this term is added to a weighted \( y \) intercept to the weighted least squares regression. In addition, a confidence interval is added to yield an alternative minimum level.20

7.1 IS THERE A WAY TO COUPLE THE BLANK AND CALIBRATION APPROACHES TO FIND \( x_{LOQ} \)?

To illustrate the author’s approach, consider the determination of the element Cr at low ppb concentration levels using a graphite furnace atomic absorption determination technique (determinative techniques are introduced in Chapter 4). The calibration data are as follows:

<table>
<thead>
<tr>
<th>Concentration (ppb)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0134</td>
</tr>
<tr>
<td>5</td>
<td>0.1282</td>
</tr>
<tr>
<td>10</td>
<td>0.2260</td>
</tr>
<tr>
<td>15</td>
<td>0.3276</td>
</tr>
<tr>
<td>20</td>
<td>0.4567</td>
</tr>
</tbody>
</table>

Entering this calibration data into LSQUARES (refer to Appendix C) yields the following statistical parameters:

<table>
<thead>
<tr>
<th>Outcome from LSQUARES</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard deviation in ( y ) intercept, ( s(b) )</td>
<td>0.0101</td>
</tr>
<tr>
<td>Correlation coefficient, ( r )</td>
<td>0.9980</td>
</tr>
<tr>
<td>Student’s ( t ) for one-tail null hypothesis, at a significance level of 95% for three degrees of freedom</td>
<td>2.353</td>
</tr>
<tr>
<td>Critical response level, ( y_C )</td>
<td>0.0377</td>
</tr>
<tr>
<td>Critical concentration level, ( x_C )</td>
<td>1.67 ppb</td>
</tr>
<tr>
<td>Detection concentration level, ( x_D )</td>
<td>3.27 ppb</td>
</tr>
</tbody>
</table>

The measured signal from a blank is 0.0620 absorbance units, and using the author’s program LSQUARES to obtain a value for the standard deviation in the least squares regression line, \( s_b = 0.0101 \). Ten times \( s_b \) added to 0.0620 gives \( S_{LOQ} = 0.163 \). Entering 0.163 into LSQUARES gives \( 7.22 \pm 1.96 \) ppb Cr as the limit of quantitation. The critical concentration was previously found to be 1.67 ppb Cr, whereas the limit of detection, the IDL for the instrument, was previously found to be 3.27 ppb Cr.
Figure 2.10 is a seemingly complex graph that seeks to show how blank statistics and confidence band statistics can be viewed in an attempt to make sense out of all of this (p. 65). The confidence interval that surrounds the least squares regression fit through the experimental calibration points is indicated along with extensions to the various Gaussian distributions that appear to the left of the calibration curve. The possibility of committing a significant type II error, represented by $\beta$ when accepting a detection limit of three times the standard deviation in the blank, $S_{\text{blank}}$, is clearly evident in Figure 2.10. The small type I error at the decision or critical level is evident as well. Note that $y(d)$ is 10 times the standard deviation in the blank ($s_{\text{blank}}$), and this gives a high degree of certainty that neither type I nor II errors will be committed at the limit of quantitation. This concludes our discussion on the statistical treatment of analytical data for TEQA. We now proceed to a discussion of quality in measurement. Before we do this, let us introduce some definitions and clarify some overused words in the lexicon of TEQA.

8. CAN IMPORTANT TERMS BE CLARIFIED?

Yes, they can. Let us start by stating an old adage: Samples are analyzed, constituents in samples are identified (qualitative analysis) or determined (quantitative analysis). According to Erickson (p. 107), the following definitions can be clarified:
Technique: A scientific principle or specific operation (e.g., gas chromatography–electron-capture detector (GC-ECD), Florisil column cleanup, or so-called Webb and McCall quantitation).

Method: A distinct adaptation of a technique for a selected measurement purpose (e.g., a specific GC-ECD operating mode for analysis of PCBs, including column specifications and sample preparation.

Procedure: The written directions to use a method or series of methods and techniques; many authors and agencies blur the distinction between method and procedure.

Protocol: A set of definitive directions that must be followed without deviation to yield acceptable analytical results. Analytical protocols may be found as the cookbooks provided within a laboratory derived from common procedures.

The next topic in this chapter introduces the two tenants of good laboratory practice (GLP): quality assurance and quality control. This is a topic of utmost importance, particularly to laboratory managers and corporate executives. A good lab with a lousy GLP is a sad use of human and facility resources. Each batch of samples analyzed must include, in addition to the samples themselves, a matrix spike, a matrix spike duplicate, and one or more laboratory blanks that verify the cleanliness of the laboratory. For the nonstoichiometric chromatographic and spectroscopic determinative techniques (to be discussed in Chapter 4), chemical reference standards must be available and be in high purity as well. These standards are part of GLP and indicate that a laboratory is implementing a quality assurance program.

9. WHAT DOES IT MEAN FOR A LABORATORY TO HAVE A QUALITY ASSURANCE PROGRAM?

An important aspect of GLP is for each laboratory to have a document titled “Quality Assurance” in its possession.

One of the most confusing issues in TEQA involves the need to clearly define the difference between quality assurance (QA) and quality control (QC). QA is a management strategy. It is an integrated system of management activities involving the following five areas:

1. Planning
2. Implementation
3. Assessment
4. Reporting
5. Quality improvement

This plan ensures that a process, item, or service is of the type and quality needed and expected by the client or customer.

Quality control is defined as an overall system of technical activities that measures the attributes and performance of a process, item, or service against defined
standards to verify that they meet the stated requirements established by the client or customer. QA is a management function, whereas QC can be viewed as a laboratory function. A laboratory must have a written QA document on file, whereas a record of certified reference standards that are properly maintained implies that QC is in place. Much of what has been discussed in this chapter falls under the definition of QA.

Finally, it is necessary to implement an effective QC protocol when all other aspects of the quantitative analysis have been completed. Two topics emerge from a consideration of EPA-related methods. The first comprises a clear set of definitions of QC reference standards to be used in conducting the quantitative analysis, and the second is a set of QC performance criteria and specifications.

10. ARE THERE DEFINITIONS OF QC STANDARDS USED IN THE LAB?

A number of QC standards must be available in the laboratory. These QC standards all have specific roles to play in the QC protocol. The following is a list of the various QC standards, including the role of each QC standard as well.

**Surrogate spiking standard:** A solution that contains one or more reference compounds used to assess analyte recovery in the method chosen. Surrogates cannot be targeted analytes. For example, tetrachloro-\textit{m}-xylene (TCMX) and decachlorobiphenyl (DCBP) dissolved in MeOH are surrogates used in some EPA methods to determine trace organochlorine (OC) pesticide residue analysis. These two analytes are highly chlorinated and are structurally very similar to OC- and PCB-targeted analytes. TCMX elutes prior to and DCBP elutes after the OCs and PCBs, thus eliminating any coelution interferences. A fixed volume (aliquot) of this reference solution is added to every sample, matrix spike, blank, blank spike, and control in the protocol.

**Matrix spike standard:** Consists of a representative set of the targeted analytes whose percent recoveries are evaluated in the sample matrix. For example, if THMs were the targeted analytes, this reference standard would consist of one or more THMs, such as chloroform, bromodichloromethane, and chlorodibromomethane. These compounds would be dissolved in a matrix-compatible solvent such as MeOH. A precise aliquot of this solution is added to a sample so that the effect of the sample matrix on the percent recovery can be evaluated. A second standard, called a **matrix spike duplicate**, is often required in EPA methods and is used to assess matrix recovery precision.

**Control standard:** Consists of the same representative set of targeted analytes used in the matrix spike. The amount of targeted analyte or surrogate is set equal to the amount injected for the surrogate spike and matrix spikes. This amount is dissolved in the exact volume of solvent used in sample
preparation. In this way, a standard that ensures a 100% recovery is obtained. The ratio of the amount of surrogate analyte to the amount of control analyte is used to determine the analyte percent recovery.

**Stock reference standard:** The highest concentration of target analyte either obtained by dissolving a chemically pure form of the analyte in an appropriate solvent or obtained commercially as a certified reference solution.

**Primary dilution standard:** Results from dilution of the stock reference standard.

**Secondary dilution standard:** Results from dilution of the primary dilution standard.

**Tertiary dilution standard:** Results from dilution of the secondary dilution standard.

**Calibration or working standards:** A series of diluted standards prepared from dilutions made from the tertiary dilution standard. These standards are injected directly into the instrument and used to calibrate the instrument as well as to evaluate the range of detector linearity. The range of concentration should cover the anticipated concentration of targeted analytes in the unknown samples.

**Initial calibration verification (ICV) standard:** Prepared in a similar manner as the working calibration standards, however, at a different concentration than any of the working standards. Used to evaluate the precision and accuracy for an interpolation of the least squares regression of the calibration curve. This standard is run immediately after the calibration of the working standards.

**Continuous calibration (CC) standard:** Prepared in a manner similar to the ICV. The CC is run in place of the calibration standards when subsequent batches of samples are anticipated. The initial batch of samples usually includes a series of calibration standards and ICVs. CCs are used to verify that the initial calibration remains analytically viable to quantitate subsequent batches of samples.

**Laboratory method blank:** A sample that contains every component used to prepare samples except for the analyte(s) of interest. The method blank is taken through the sample preparation process. It is used to evaluate the extent of laboratory contamination for the targeted analytes.

**Trip or field blank:** A sample that is obtained in the field or prepared during the trip from the field to the laboratory. This blank should contain no chemical contamination, yet should be obtained in a manner similar to the environmentally significant samples themselves.

### 11. WHAT QC SPECS MUST A LAB MEET?

The following five criteria represent five separate QC specifications that should be provided to each and every client who requests analytical services.
11.1 **Minimum Demonstration of Capability**

Before samples are prepared and analytes quantitatively determined by instrumental analysis, the laboratory must demonstrate that the instrumentation is in sound working order and that targeted analytes can be separated and quantitated. For example, GC operating conditions must be established and reproducibility in the GC retention times, $t_R$, for the targeted analytes must be achieved. Competent analytical scientists coupled with laboratory resources of high quality will greatly help a lab meet this spec.

11.2 **Laboratory Background Contamination**

Before samples are prepared and analytes quantitatively determined by instrumental analysis, the laboratory must demonstrate that the sample preparation bench-top area instrument itself and all reagents and solvents used are essentially free of traces of the targeted analyte of interest. This is accomplished by preparing method blanks using either distilled or deionized water or solvents of ultrahigh purity. This is particularly important for ultratrace analysis (i.e., concentration levels that are down to the low ppb or high ppt range). The use of pesticide residue analysis-grade solvents for conducting trace organics analysis and the use of ultratrace nitric and hydrochloric acids for trace metals analysis are strongly recommended. This author believes that there is a considerable number of organics in organic solvents at concentrations in the low ppt level. Nonzero blanks prevent true IDLs from ever being obtained. Nonzero blanks even affect analyte percent recoveries.

11.3 **Assessing Targeted and Surrogate Analyte Recovery**

Before samples are prepared and quantitatively determined by instrumental analysis, the laboratory must demonstrate that the surrogate and targeted analytes can be recovered to a degree by the method selected. A rugged analytical method will have an established range of percent recoveries. In TEQA, the value of the percent recovery is said to be analyte and matrix specific. For example, if EPA Method 625 is used to isolate and recover phenanthracene from wastewater, the percent recovery is acceptable anywhere between 54 and 120%, whereas that for phenol is between 5 and 112%. These percent recoveries are seen to depend on the chemical nature of the analyte (phenanthracene vs. phenol) and on the matrix, wastewater. Provost and Elder\textsuperscript{24} have put forth a mathematics-based argument stating that the range of percent recoveries that can be expected also depends on the ratio of the amount of added spike to the background concentration present in the environmental sample. Table 2.3 applies several mathematical equations discussed in Provost and Elder\textsuperscript{24} and clearly shows the impact of spike-to-background ratios on the variability in the percent recovery. Using a spike-to-background ratio of 100 does not change the range of percent recoveries that can be expected. This changes as the ratio is reduced and begins to significantly increase this range when the ratio is reduced to 1 and lower.

This author has conducted more percent recovery studies than he cares to remember. A valid percent recovery study incorporates Equation (2.49) and enlarges...
upon it for \( i = 1, 2, \ldots \), up to the maximum number of peaks in a multicomponent separation such as that which can be accomplished by applying capillary GC techniques. Using summation notation, Equation (2.49) is enlarged to encompass \( j \) replicate injections of an extract that contains the \( i \)th analyte from a spiked blank or spiked sample. A control reference standard that contains the \( i \)th analyte for \( k \) replicate injections of this standard is also prepared such that the percent recovery is found according to

\[
\% R_i = \frac{\sum_{j=1}^{L} A_j^i / L}{\sum_{k=1}^{M} A_k^i / M} \times 100
\]  

where \( L \) is the total number of replicate injections for the \( i \)th analyte in the spiked blank or spiked sample whose peak area is \( A_j^i \), and \( M \) is the total number of replicate injections for the \( i \)th analyte in the control reference standard (this defines a 100% recovery) whose peak area is \( A_k^i \).

The nature of the analytical method largely determines whether a percent recovery study can be conducted in the first place. If sample prep is directly interfaced to the determinative technique, i.e., the analytical instrument, without any opportunity for analyst intervention, a control reference standard from which a 100% recovered analyte can be measured cannot be prepared. This is the case for VOCs

---

**Table 2.3**  
Influence of the Spike-to-Background Ratio on Percent Recoveries

<table>
<thead>
<tr>
<th>Spike/Background</th>
<th>Variance in Mean Percent Recovery</th>
<th>Expected Range in Percent Recoveries*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero background</td>
<td>(100(p)) 00*(RSD)</td>
<td>( p = 1.0,) RSD = 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( p = 1.0,) RSD = 0.2</td>
</tr>
<tr>
<td>100</td>
<td>1.02 (100(p))*(RSD)</td>
<td>80, 120</td>
</tr>
<tr>
<td>50</td>
<td>1.04 (100(p))*(RSD)</td>
<td>80, 120</td>
</tr>
<tr>
<td>10</td>
<td>1.22 (100(p))*(RSD)</td>
<td>78, 122</td>
</tr>
<tr>
<td>5</td>
<td>1.48 (100(p))*(RSD)</td>
<td>76, 124</td>
</tr>
<tr>
<td>1</td>
<td>5.00 (100(p))*(RSD)</td>
<td>55, 145</td>
</tr>
<tr>
<td>0.5</td>
<td>13.0 (100(p))*(RSD)</td>
<td>28, 170</td>
</tr>
<tr>
<td>0.1</td>
<td>221 (100(p))*(RSD)</td>
<td>–200, 400</td>
</tr>
</tbody>
</table>

* 95% tolerance interval for percent recoveries with assumed values for \( p \) and RSD; tolerance limits = 100\(p\) ± \(\sqrt{\text{Var}(P)}\).

© 2006 by Taylor & Francis Group, LLC
since sample preparation is most commonly done by purge and trap or static headspace sampling. These sample preparation devices are directly interfaced to the injection port of gas chromatographs. Semivolatile organic compounds (SVOCs) must be extracted via application of phase distribution equilibria (to be introduced in considerable detail in Chapter 3). Some analyte is invariably lost despite the application of excellent lab technique with minimization of systematic error. Percent recoveries for SVOCs are found to be less than 100%. The analyst can prepare a control reference standard in the case of SVOCs by taking a precise aliquot of a more concentrated reference standard and dissolving this aliquot in extracting solvent and adjusting to a precise final volume. This final volume should be identical to the final extract volume used to isolate and recover all spiked blanks, spiked samples, and unspiked samples. Hence, \( A_i \) and \( A_k \) can be compared in Equation (2.57) (i.e., comparing apples to apples) since the same amount of the \( i \)th analyte is added to both sample and control. Both sample and control are brought to the same final extract volume. To illustrate, consider spiking a serum sample with 335 ng of the organochlorine pesticide dieldrin and taking it through an appropriate sample preparation method. This sample preparation approach yields a 1-mL hexane extract that would contain dieldrin. Consider also adding 335 ng of dieldrin to 1.0 mL of a hexane extract. This important technique, used repeatedly by this author over the years to conduct precise and accurate percent recovery studies, is illustrated below:

A calibration of the analytical instrument is not necessary to conduct a percent recovery study.

A second example, this time drawing on the author's own research, involves the calculation of the percent recovery of Aroclor (AR) from rat plasma. AR 1248 consists of 20 to 30 PCB congeners and was manufactured for many years in the U.S. by the Monsanto Chemical Company. Aroclors had numerous uses, ranging from an insulating medium for large electrical capacitors to newspaper ink. This extensive use was due to the stable and nonflammable properties of PCBs. Little was known years ago about the environmental persistence of PCBs coupled to their lipophilic physico-chemical properties. Three spiking scenarios are presented below with the corresponding instrument outputs in terms of a number of counts. The number of counts is proportional to the area under a chromatographically resolved peak. In this study, the integrated area beneath each peak was summed over the entire 20 to 30 fully or partially resolved peaks for AR 1248.
Equation (2.57) addresses only one extraction of one sample. In this case of replicate extractions, the reader should refer back to Equation (2.52). This equation addresses $j$ replicate GC injections for each of $g$ extractions performed.

Once the $\%R_i$ is found, assuming that the IDL, $x_{IDL}$, is known, a method detection limit $x_{MDL}$ can be calculated for the $i$th analyte. This equation requires a percent recovery expressed as a fraction, $R_i$, and a knowledge of the phase ratio, $\beta$. $\beta$ is the ratio of extract volume to sample volume. All of this is summarized below:

$$x_{MDL} = \frac{x_{IDL} \left[ \frac{V_e}{V_S} \right]}{R_i / 100 \beta} \quad (2.58)$$

Equation (2.58) suggests that in order to reach the lowest MDL, analysts should first achieve as low an IDL as possible. We already discussed how to minimize $x_{IDL}$ [Equation (2.34)] or $x_D$ [Equation (2.45)] from a mathematical point of view. A most efficient extraction also serves to maximize $R_i$. However, maximizing the phase ratio, $\beta$, is only possible within the physical constraints of the experiment. One liter of groundwater that winds up as 1 mL of an organic extract yields $\beta = 0.001$, while a 2-mL serum sample that winds up as 1 mL of an organic extract yields $\beta = 0.5$. 
11.4 Assessing Goodness of Fit of the Experimental Calibration Data and the Range of Linearity

A correlation coefficient, \( r \), of 0.9990 is a goal to achieve between instrument response and analyte amount or concentration for chromatographic and spectroscopic determinative techniques. Over how many orders of magnitude in amount or concentration yields \( r = 0.9990 \) defines the range of linearity. This range will differ among determinative techniques. Both linear (or first-order least squares regression) and polynomial (second-order and even third-order least squares regression) calibration covering one or more orders of magnitude above the instrument’s IDL are all useful ways to address the degree of goodness of fit. The least squares slope, called a response factor in some EPA methods, and the uncertainty in the slope are also useful criteria in assessing linearity. The coefficient of determination, \( r^2 \), is a measure of the amount of variation in the dependent variable (instrument response) that is accounted for by the independent variable (analyte amount or concentration) (pp. 118–120).7

Let us delve into the concept of calibration linearity or lack thereof a bit more. The linear dynamic range of an instrument is defined as that range of concentration from the analyte’s limit of quantification \( x_{LOQ} \), on up to where a departure from linearity is observed. This concentration is the analyte’s limit of linearity, \( x_{LOL} \). This concept is best understood graphically as shown below:

The linear dynamic range for most analytical instruments designed to perform TEQA covers at least two orders of magnitude. For example, a range of analyte concentration from 10 to 1000 ppb covers two orders of magnitude and is quite suitable for TEQA. Some methods have a linear dynamic range over five or six orders of magnitude. Nonlinear least squares regression analysis does exist, can be found in most contemporary statistical software packages, and can be applicable to TEQA.
Consider the construction of a seven-point calibration for the determination of lead, Pb, in a human blood specimen. Declines in blood Pb concentration levels in children over the past 20 years represent one of the triumphs in public health in the U.S. Figure 2.11 shows a calibration curve for blood Pb using flame atomic absorption spectroscopy (FlAA). Notice that the concentration levels are in the ppm range. This range is accommodated nicely by FlAA as the appropriate determinative technique (principles of FlAA are introduced in Chapter 4). It becomes almost immediately evident that applying linear least squares fit to the experimental calibration data is not a good fit. Figure 2.12 shows that a quadratic (second order) least squares regression line is a much better fit. Figure 2.13 shows that a cubic (third order) least squares regression line makes a slight improvement over the quadratic fit. All three least squares regressed lines or curves are polynomials that can be described in terms of a set of coefficients, as shown below. The number of degrees of freedom (Rdf) required (Rdf values become important later) is also listed next to each polynomial equation:

<table>
<thead>
<tr>
<th>Polynomial</th>
<th>Type of Fit</th>
<th>Rdf</th>
</tr>
</thead>
<tbody>
<tr>
<td>( y = a_0 + a_1x )</td>
<td>Linear</td>
<td>2</td>
</tr>
<tr>
<td>( y = a_0 + a_1x + a_2x^2 )</td>
<td>Quadratic</td>
<td>3</td>
</tr>
<tr>
<td>( y = a_0 + a_1x + a_2x^2 + a_3x^3 )</td>
<td>Cubic</td>
<td>4</td>
</tr>
</tbody>
</table>

Recall from our previous discussion in this chapter that for the linear fit, \( a_0 \) corresponds to the \( y \) intercept 0 while \( a_1 \) corresponds to the slope of the least squares regression line \( m \). In this section, we wish to compare coefficients; hence, the \( a_i \) symbolism is used throughout the following discussion.
It is instructive to examine the sign of the highest-order coefficients in the linear and quadratic equations introduced above. Consider assigning real numbers to each coefficient and plotting these equations as a function of $x$, $f(x)$ using a graphics calculator. To illustrate, let us ask the following:

**FIGURE 2.12** Quadratic LS fit for Pb in blood.

**FIGURE 2.13** Cubic LS fit for Pb in blood.
What would equations 1, 2, and 3 (shown below) look like if plotted?

Superimposing curves for the first quadrant for all three polynomials clearly shows just what effect a positive or negative coefficient for $x^2$ has on the direction of curves 2 and 3. Most nonlinear regressed quadratic curves plotted in the first quadrant for all positive values for $x$ tend to level off, as shown by curve 3. This curve is quite common for instruments used in TEQA when the linear dynamic range of the detector has been exceeded. A third-order polynomial tends toward more of a sigmoid or slanted curve shape.

Each coefficient in the least squares regression curve has an overall standard error that can be calculated using Sigma Plot or other statistical software packages. In Appendix C, where the author’s LSQUARES program is found, the standard deviation in the least squares slope, $m$ or $a_1$, and the standard deviation in the least squares $y$ intercept, $b$ or $a_0$, is computed based on the derivations that stem from the standard deviation in the residuals using Equation (2.31). Standard deviations for the least squares slope and $y$ intercept can be computed for both second- and third-order polynomials. Please refer to statistical texts that discuss this topic in more detail.

To mathematically conduct a polynomial evaluation of calibration linearity, we draw on guidelines recently published by the National Committee for Clinical Laboratory Standards (NCCLS EP6-A, vol. 21, no. 20, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach: Approved Guideline) and proceed to describe this approach. The evaluation consists of two parts. The first part examines whether a nonlinear polynomial fits the calibration data better than a linear one. The second part, applicable only when a nonlinear polynomial fits the data better than a linear one, assesses whether the difference between the best-fitting nonlinear and linear polynomial is less than the amount of allowable bias for the method, as was previously defined by the analyst.
A statistical software package such as Sigma Plot cannot only find each coefficient, \( a_i \), for the first-, second-, and third-order fits, but also is able to find the standard deviation in each coefficient, \( s_{ai} \). Knowing \( a_i \) and \( s_{ai} \) enables their ratio, \( t_{obs} \), to be calculated. \( t_{obs} \) is compared to the value of \( t \) found in a table of Student’s \( t \) values. The value in the \( t \) table depends on the probability desired or degree of type I error acceptable, \( \alpha \) (\( \alpha \) taken as 0.05), whether this is a one-tailed hypothesis test, and the number of degrees of freedom, \( df \). Selected values for Student’s \( t \) is found in Appendix F. If \( t_{obs} \) exceeds the tabulated value, then the nonlinear coefficients are said to be statistically significant. Only higher-order coefficients (\( a_i \) cannot be less than 2) are applicable since this is a test for nonlinearity. Mathematically,

\[
t_{obs} = \frac{a_i}{s_{ai}}
\]

\( df = (\text{number of calibration levels} \times \text{number of replicates/level}) - \lceil Rdf \rceil \)

The logic is given below:

<table>
<thead>
<tr>
<th>If ( t_{obs} )</th>
<th>Then</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{obs} \geq t_{(1-\alpha/2,df)} )</td>
<td>Nonlinear fit is appropriate; ( a_i ) is significantly different from zero</td>
</tr>
<tr>
<td>( t_{obs} \leq t_{(1-\alpha/2,df)} )</td>
<td>Nonlinear fit is inappropriate and linear fit should be used for quantification</td>
</tr>
</tbody>
</table>

Note the use of absolute values for \( t_{obs} \). The guidelines point out that this is merely a test for statistical significance and indicate only that nonlinearity has been detected.

For the blood Pb calibration between 0.25 and 100 ppm, software packages would generate a table similar to that shown below:

<table>
<thead>
<tr>
<th>Order</th>
<th>Coefficient</th>
<th>( a_i )</th>
<th>( s_{ai} )</th>
<th>( t_{obs} )</th>
<th>( df )</th>
<th>( t_{(1-\alpha/2,df)} )</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>( a_1 )</td>
<td>0.00954</td>
<td>0.0013</td>
<td>1.36</td>
<td>5</td>
<td>—</td>
<td>0.110</td>
</tr>
<tr>
<td>2nd</td>
<td>( a_2 )</td>
<td>-0.0001</td>
<td>0.000006745</td>
<td>-15.4</td>
<td>4</td>
<td>2.776</td>
<td>0.0159</td>
</tr>
<tr>
<td>3rd</td>
<td>( a_3 )</td>
<td>-0.0002</td>
<td>0.000</td>
<td>-10.3</td>
<td>3</td>
<td>3.182</td>
<td>0.0060</td>
</tr>
</tbody>
</table>

The absolute value for the second- and third-order fits greatly exceeds the \( t \) value from the table and implies that a nonlinear fit is appropriate (as if the visual observation of the calibration plot, Figure 2.11, does not give it all away).

Next, the choice of whether a second- or third-order polynomial is the best fit is accomplished by noting the magnitude of the standard error in the estimate. The
table above shows for our example that the third-order fit yields the lowest standard error in the estimate.

A difference from linearity for a given analyte for the $i$th concentration level, denoted by $DL_i$, is found for the polynomial that gave the lowest standard error in the estimate according to

$$DL_i = p(x_i) - (a_0 + a_i x_i)$$

Graphically, this equation tells us that we are to find a difference at each calibration point as shown below:

A percent difference, %diff, can be found as follows:

$$\frac{DL_i}{x_i} \times 100 = \% \text{diff}$$

Examine the %diff at each concentration level and compare this value with the stated quality control criteria previously established by the analyst. To illustrate, consider the calibration data for the blood Pb shown below:

<table>
<thead>
<tr>
<th>$x_i$ (ppm Pb)</th>
<th>%Diff between 3rd and 1st Orders</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>49.4</td>
</tr>
<tr>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>10</td>
<td>0.11</td>
</tr>
<tr>
<td>20</td>
<td>0.34</td>
</tr>
<tr>
<td>25</td>
<td>0.39</td>
</tr>
<tr>
<td>50</td>
<td>0.27</td>
</tr>
<tr>
<td>100</td>
<td>0.10</td>
</tr>
</tbody>
</table>

If the analyst previously set a %diff of 5%, then only the 0.25 ppm Pb level demonstrates a %diff of greater than 5%. This data point in the calibration would
be eliminated from consideration. Eliminating so-called outliers serves to limit the linear dynamic range.

Scheme 2.2 summarizes the NCCLS guidelines using logical flowcharts. These guidelines are but one approach to this topic of nonlinearity. Other regulatory agencies have developed their own approaches as well.

### SCHEME 2.2a

11.5 **Assessing ICV Precision and Accuracy**

Precision can be evaluated for replicate measurement of the ICV following establishment of the multipoint calibration by calculating the confidence interval for the interpolated value for the ICV concentration. Triplicate injection of an ICV ($L = 3$) with a concentration well within the linear region of the calibration range enables a mean concentration for the ICV to be calculated. A standard deviation $s$ can be
calculated. One ICV reference standard can be prepared closer to the low end of the calibration range, and a second ICV reference standard can be prepared closer to the high end of this range. A confidence interval for the mean of both ICVs can then be found as follows:

\[
\text{Conf Int for } \mu_{\text{ICV}} = \bar{x}_{\text{ICV}} \pm \frac{s}{\sqrt{L}}
\]

A relative standard deviation, for the \(i\)th component, expressed as a percent (\%RSD), also called the coefficient of variation, can be calculated based on the ICV instrument responses according to

\[
\%\text{RSD}_{i}^{\text{ICV}} = \left( \frac{s_{i}^{\text{ICV}}}{\bar{x}_{i}^{\text{ICV}}} \right) \times 100
\]

where \(s_{i}\) is the standard deviation in the interpolated concentration for the \(i\)th-targeted component based on a multipoint calibration and \(\bar{x}_{i}\) is the mean concentration from replicate measurements. For example, one can expect a precision for replicate injection into a GC to have a coefficient of variation of about 2%. As another example,
one can expect a precision for replicate solid-phase extractions to have a coefficient of variation of between 10 and 20%. The coefficient of variation itself is independent of the magnitude of the amount or concentration of analyte measured, whereas reporting a mean ICV along with a confidence interval at a level of significance can only be viewed in terms of the amount or concentration measured. The coefficient of variation is thus a more appropriate parameter when comparing the precision between methods.

Accuracy can be evaluated based on a determination of the present relative error provided that a known value is available. A certified reference standard constitutes such a standard. Accuracy is calculated and reported in terms of a percent relative error according to

\[
\% \text{Error} = \frac{|x_{i(\text{unknown})} - x_{i(\text{known})}|}{x_{i(\text{known})}}
\]

Statements of precision and accuracy should be established for the ICV, and as long as subsequent measurements of the ICV remain within the established confidence limits, no new calibration curve needs to be generated.

11.6 Assessing Sample Results

Following the establishment of a calibration and the evaluation of the precision and accuracy for one or more ICVs, we can apply additional statistical assessments on a batch of replicate analytical results from real samples. The Q test can be applied to identify any outliers within a series of sample results. The rejection quotient Q is defined as a ratio of the gap (difference between the questionable value and its nearest neighbor) to the range (difference between questionable value and lowest value in series) (p. 57).\(^2\) For L replicate samples where L = 8 to 10, we have (p. 32):

\[
Q_{\text{calc}} = \frac{x_n - x_{n-1}}{x_n - x_2}
\]

If we have an even larger number of replicate samples such as L = 11 to 13, a different equation for Q is used, as shown below:

\[
Q_{\text{calc}} = \frac{x_n - x_{n-2}}{x_n - x_2}
\]

where \(x_n\) is a questionable result in the set \(x_1, x_2, \ldots, x_n\) that are arranged in order of increasing value such that \(x_1 < x_2 < x_p\), \(x_{n-1}\) designates a result that is nearest \(x_n\), and \(x_{n-2}\) is a result second nearest \(x_p\). \(x_1\) and \(x_2\) are results furthest and second furthest from \(x_p\). \(Q_{\text{calc}}\) is compared to \(Q_{\text{critical}}\) at the 90% confidence level. If \(Q_{\text{calc}}\) exceeds \(Q_{\text{critical}}\), then the result is considered an outlier and can be eliminated from the results. A table of Q values is found in Appendix F.
An initial statistical assessment of replicate samples can be accomplished by calculating a mean analyte concentration from \( L \) replicate samples and a standard deviation using previously stated equations found in this chapter.

For two separate batches of replicate sample results, assumed free of systematic error and drawn from the sample population,

\[
\sigma_A = \sigma_B
\]

The \( F \) test can be used to compare variance from both sets. The \( F \) value is calculated according to

\[
F_{\text{calc}} = \frac{s_A^2}{s_B^2}
\]

where the larger variance should be put in the numerator and the smaller variance in the denominator.

\( F_{\text{calc}} \) is compared to \( F_{\text{crit}} \) from a tabulated list. Refer to the \( F \) table in Appendix F. The number of degrees of freedom for each variance is used to locate the specific \( F_{\text{crit}} \). If \( F_{\text{calc}} < F_{\text{crit}} \), then there is no significant difference in two variances.

If a theoretical or established reference whose mean \( \mu \) is known, we can proceed to find a mean \( \bar{x} \) (average) drawn from \( L \) replicate observations and \( df = L - 1 \) degrees of freedom such that

\[
t_{\text{calc}} = \frac{\bar{x} - \mu}{s/\sqrt{L}}
\]

Depending on the outcome of the \( F \) test, two independent means can be compared. There are two approaches to this. If the \( F \) finds both variances not to be significantly different, then we have

\[
t_{\text{calc}} = \frac{\bar{x}_1 - \bar{x}_2}{s_p \sqrt{\frac{1}{L_1} + \frac{1}{L_2}}}
\]

\[
t_{\text{calc}} = \frac{\bar{x}_1 - \bar{x}_2}{s_p \sqrt{\frac{L_1 L_2}{L_1 + L_2}}}
\]

where \( s_p \) is a pooled standard deviation from both sets and is calculated according to

\[
s_p = \sqrt{\frac{(L_1 - 1)s_1^2 + (L_2 - 1)s_2^2}{(L_1 - 1) + (L_2 - 1)}}
\]

Also,

\[
df = L_1 + L_2 - 2
\]
If the $F$ test finds both variances to be significantly different, then we have

$$t_{\text{calc}} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{L_1} + \frac{s_2^2}{L_2}}}$$

where $df$ is computed as shown below:

$$df = \frac{\left( \frac{s_2^2}{L_1} + \frac{s_2^2}{L_2} \right)^2}{\left( \frac{s_1^2}{L_1} \right)^2 \left( \frac{s_2^2}{L_2} \right)^2 - 2 \left( \frac{s_1^2}{L_1} + \frac{s_2^2}{L_2} \right)}$$

where $x_{1(\text{ave})}$ and $x_{2(\text{ave})}$ are means from respective batches of replicate sample results for $L_1$ replicates having a variance of $s_1^2$ and for $L_2$ replicates having a variance of $s_2^2$. $t_{\text{calc}}$ is compared to $t_{\text{crit}}$ values from a tabulated array of Student’s $t$ values. Part of a Student’s $t$ table is located on the inside rear cover of the book. Both $df$ and the confidence level are necessary when obtaining a value for $t_{\text{crit}}$. In both cases, if $t_{\text{calc}} < t_{\text{crit}}$, then both means that are being compared are not significantly different.

Our journey across the “jungle” of data reduction and interpretation has been completed. Topics whose underlying principles also relate to data reduction and interpretation are now introduced. These include:

- The nature of a trace analysis laboratory
- From analog transducer to spreadsheet
- Chromatographic software peak integration
- Sampling for enviro-health and enviro-chemical trace quantitative analysis

Having discussed what constitutes GLP in terms of QA and QC, let us introduce the very important and not to be neglected people factor in all of this.

12. **HOW IS AN ENVIRONMENTAL TESTING LAB ORGANIZED?**

Laboratories are comprised of people in a workplace using sophisticated technology to generate and report analytical data to clients who have a need for the data. Clearly, people function most effectively under an organization in which each individual knows just what is expected of him or her. Figure 2.14 is a flowchart that depicts the organization of a typical environmental testing laboratory. An individual analyst might work within a lab section that is devoted to the analysis of solid waste samples for the determination of trace levels of semivolatile organics and report to a section...
A manager or supervisor who has had 5 or more years experience in this specialty. A secondary line of responsibility represented by the dashes in Figure 2.14 refers to the QA coordinator’s role in guaranteeing that the lab has adopted a QA plan. For example, our individual analyst may have to report some QC data to the QA coordinator from time to time. The chart in Figure 2.14 facilitates QA and offers the hope that a high level of quality in obtaining analytical data can be maintained. A client can then easily recognize which of two labs, hypothetical Lab X or hypothetical Lab Y, should be offered the contract for environmental monitoring of, perhaps, a hazardous waste site.

**FIGURE 2.14** Typical organization chart of an environmental testing laboratory.
13. WHICH LAB WOULD YOU CHOOSE?

Let us suppose that you are a representative working for an environmental consulting firm and you are asked to choose which company, Lab X or Lab Y, should be hired to analyze your contaminated soil samples from this hypothetical hazardous waste site. Let us also assume that the samples come from a Superfund site and that you are interested in the lab providing you with analytical results and interpretation for semivolatile organics using EPA Method 8270C. You receive analytical results from both companies, as shown in Table 2.4. Based on your interpretation of the reports from both companies, which lab would you choose to contract with for the analysis? It should be obvious.

Lab X lacks any evidence of QC. Although the lab reported concentration levels of three semivolatile organics in ppb, no statement about the precision of the analysis is provided. No correlation coefficient is provided. The lab’s efficiency in extracting the analytes of interest from the environmental sample matrix as shown by a percent recovery is not evident in the report. No other QC is reported for this laboratory. This author would immediately ask upon receiving such a report if the QA document is available for review.

Lab Y, on the other hand, not only reported detectable levels of the three organics, but also gave a confidence interval for each reported concentration. Lab Y demonstrates that reference standards were run to establish a least squares regression line, and the goodness of fit is expressed by the value of the correlation coefficient. The

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Confidence Interval at 95%</th>
<th>Correlation Coefficient</th>
<th>% Recovery</th>
<th>%RSD in the % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Company X Analytical Report for the Hazardous Waste Site</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Chloro-3-methyl phenol</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bis-2-ethyl hexyl phthalate</td>
<td>50</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>QC results: none</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Confidence Interval at 95%</th>
<th>Correlation Coefficient</th>
<th>% Recovery</th>
<th>%RSD in the % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Company Y Analytical Report for the Hazardous Waste Site</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Chloro-3-methyl phenol</td>
<td>56</td>
<td>0.9954</td>
<td>67</td>
<td>15</td>
</tr>
<tr>
<td>Bis-2-ethyl hexyl phthalate</td>
<td>47</td>
<td>0.9928</td>
<td>84</td>
<td>13</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>73</td>
<td>0.9959</td>
<td>73</td>
<td>17</td>
</tr>
<tr>
<td>QC results:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instrument detection limit (IDL) = 10 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method detection limit (MDL) = 10 ppb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory blank &lt; MDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field blank &lt; MDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: NA = not available.
lab also conducted the required percent recovery studies and demonstrated acceptable precision in the reported percent recoveries for each of the three analytes. The lab also reports that it conducted a study of the IDLs and MDLs for the requested method.

Scheme 2.3 sums it up well.

### 14. FROM TRANSDUCERS TO SPREADSHEETS: WHAT DOES THIS MEAN?

Envision a trace analytical laboratory of ~1975 vintage. A wire would run from the lab’s gas chromatograph to a strip-chart recorder. The analog signal from the GC detector (we will address the various detectors used in gas chromatography in Chapter 4) would be recorded on rolled graph paper. The strip-chart recorder’s pen would monitor a GC baseline over time. The distance traveled by the graph paper would be related to chromatographic run time based on the speed of the motor in turning the roll. Since 1975, a revolution in data acquisition as well as instrument control has occurred thanks to advances in solid-state electronics and computer technology.

The contemporary trace analytical laboratory will still have a GC; however, more sophisticated looking cables emanate out of the instrument to a stand-alone personal computer (PC). An RS232 interface cable is necessary for data transmission. PCs were called microcomputers when they first arrived on the scene in the 1980s. Microcomputers were much smaller in size than existing so-called mainframe computers. The GC detector’s analog signal becomes digitized, read, and stored. The GC chromatogram can then be called up from storage, manipulated, interpreted, restored, and printed out in the form of reports. The analog signal emanating from the GC detector, the transducer, must be converted into the digital domain. This is accomplished by adding an analog-to-digital converter (ADC) as an interface
between the instrument and PC. This interface can be found external to the PC as a stand-alone box. This interface can also be a board that inserts into the input/output (I/O) peripheral of the PC. Scheme 2.4 attempts to conceptually outline the inter-domain conversion from the chemical to the physical, and then within the physical domain the evolution from analog to digital electronics. To illustrate, trichloroethylene (TCE), a volatile, priority pollutant organic compound, is suspected to be present in a human serum specimen, possibly due to occupational exposure to organic solvents, and is displayed in the GC chromatogram as a detector or transducer response that takes the shape of a Gaussian peak whose area under the curve is proportional to the concentration of TCE injected into the GC. In 1975, the peak would be displayed as analog output on a strip-chart recorder. Analysts of that era would use a ruler and pencil to measure peak height, triangulation to measure peak area, and cut and weigh to measure peak area, or if fortunate enough, analog integrators. What a contrast from the digitized world that analysts now have to perform their data reduction.

Analytical chemists and technicians are not accustomed to delving into electronics (and this includes the author). However, a bit (no pun intended) of dabbling...
Calibration, Verification, Statistical Treatment

does not hurt as we proceed to introduce those essential principles that take the reader from transducer to spreadsheet. Of the plethora of texts on the subject of electronics, the essential principle for most analysts involves just enough knowledge of the ADC to be able to understand interface specifications and the principle of ADC resolution. This author has chosen to focus on those principles underlying ADCs as deemed most usefully interfaced to gas chromatographs. ADCs are found in most every kind of analytical instrument today. The major challenge for analysts at the bench is to master whatever data processing software is associated with a particular analytical instrument.

14.1 HOW DOES ANALOG BECOME DIGITAL?

A simple form of an ADC is shown in the schematic drawing of Figure 2.15. Malmstadt et al., who have written the seminal text on electronics relative to the interest of scientists, have described a staircase ADC in the following manner:

The conversion cycle begins when a start pulse clears the binary counter. Since $v_{DAC}$ is then less than $v_{IN}$, the comparator goes to 1 and opens the counting gate. Counts are accumulated until $v_{DAC}$ just exceeds $v_{IN}$. At this time, the comparator goes to 0 and closes the counting gate. The parallel digital output of the counter is thus the digital equivalent of the analog input voltage.

Referring again to Figure 2.15, the table below summarizes how a simple ADC works:

<table>
<thead>
<tr>
<th>Voltage to Comparator</th>
<th>What Happens to ADC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{DAC} &lt; v_{IN}$</td>
<td>Logical 1; AND gate opens; clock pulses allowed to pass to counter; $v_{DAC}$ continues to increase</td>
</tr>
<tr>
<td>$v_{DAC} = v_{IN}$</td>
<td>Logical 0; AND gate closes; $v_{DAC}$ ceases to increase; end-of-conversion flag (EOC) is produced at auxiliary output; counter must be reset to zero before starting next measurement</td>
</tr>
</tbody>
</table>

The most common ADCs that are interfaced to gas chromatographs are voltage-to-frequency converters and dual-slope integrators. Both ADCs measure the signal throughout the entire chromatographic run time. Ouchi says it best:

Digital values are then used in data analysis and display and in many cases are stored for further analysis. By graphing the digital values, a computer can generate a reconstructed chromatogram, will be as accurate as the original (analog) chromatogram.... Converting one type of data into another creates opportunities for errors to occur. When an ADC digitizes the analog signal from a detector, the signal’s continuous voltage and time values are transformed into discrete digital values [as shown in Scheme 2.4]. The accuracy of the voltage values is determined by the resolution of the ADC, and the accuracy of the time values is determined by the system’s data acquisition rate.

ADC resolution is defined by either (1) the number of bits available, (2) the dynamic range, or (3) the amount of uncertainty or error for a given full-scale analog...
input signal. Typical resolutions for ADCs are 24, 20, 16, and 12 bits. For an $n$-bit ADC, there are $2^n$ different combinations (configurations or different ways to represent information) of 1s and 0s. To illustrate, three bits can be configured in $2^3$ or 8 different ways, four bits can be configured in $2^4$ or 16 different ways, and five bits can be configured in $2^5$ or 32 different ways. For example, for $n = 3$, we can arrange three bits in the following manner:

\[
\begin{array}{ccc}
000 & 001 & 010 \\
011 & 100 & 101 \\
110 & 111 & \\
\end{array}
\]

Up to eight different values for the analog voltage from the transducer could be digitally measured and stored.

The ADC dynamic range is found from the number of different configurations less one (where zero is excluded), or $2^n - 1$ different ways. The ADC’s resolution, $R_{\text{ADC}}$, is thus calculated for a 1-V full-scale analog output (a 1.0 V signal is typical of a transducer’s full range of output) according to

\[
R_{\text{s-\text{fullADC}}} = \frac{\text{volts (full-scale)}}{2^n - 1}
\]

To illustrate, a 20-bit ADC would have a resolution of 1.0 V divided by 1,048,575 different ways or steps, or 0.954 μV per step. A change of only 0.954 μV is required to register a new configuration for a 20-bit ADC. Contrast this with a 12-bit ADC.
whose resolution for the same 1.0-V output signal is 244 µV per step. A small peak in a chromatogram whose peak height is 150 µV would be picked up by the 20-bit ADC and missed by the 12-bit ADC.

The data acquisition rate (often called a sampling rate) as programmable on most PCs that utilize a chromatographic processing software package defines just how long the analog signal is monitored for each data point used or stored by the computer. As a rule of thumb, between 10 and 20 data points should be taken across a chromatographically resolved Gaussian-shaped peak to achieve the more accurate digitized peak area. Peak widths in capillary gas chromatography (C-GC) are of the order of 1 to 2 sec (1 sec = 0.0166 min) while peak widths in packed column high performance liquid chromatography (HPLC) are of the order of 5 to 10 sec. These requirements indicate that the data acquisition rate for C-GC should be set somewhere between 5 and 10 points/sec, while the rate for HPLC should be set between 1 and 2 points/sec.

Bunching is yet another parameter found in most chromatographic processing software. Instead of just one data point at a time being sampled, digitized, and stored, a number of data points can be bunched, averaged, digitized, and stored. Bunching enables a much higher data acquisition rate to be set, and this increase has certain advantages. For example, increasing a 10 points/sec sampling rate to 60 points/sec while setting the bunching to 6 sums the value for every 6 data points and, in effect, is the equivalent to a data acquisition rate of 10 points/sec. Data bunching enables a filtering out of unwanted noise, particularly noise due to that caused by 60-Hz alternating current.

Let us look at a recent product release for an ADC as advertised over the internet from Laboratory Network (www.laboratorynetwork.com):

The hardware unit interfaces your computer to your detectors, injector, auto-sampler, and/or fraction collection. It contains a high speed analog-to-digital converter and amplifier which accepts two channels of data simultaneously. Each detector input has independent controls and settings … is suitable for use with HPLC, GC, ion chromatography (IC), … capillary electrophoresis and microdialysis systems … record and analyze output from GC, HPLC, IC, size exclusion, and preparative chromatography.

Specifications for this specific ADC directly from the product release is given in the table below:

<table>
<thead>
<tr>
<th>Specification Sheet for a Typical ADC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Input Amplifiers</strong></td>
</tr>
<tr>
<td>Analog inputs</td>
</tr>
<tr>
<td>Input impedance</td>
</tr>
<tr>
<td>Input range</td>
</tr>
<tr>
<td>Frequency response (–3 dB)</td>
</tr>
<tr>
<td>Low-pass filter</td>
</tr>
<tr>
<td>DC offset</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
One can see in this spec sheet how important the concept of ADC resolution really is.

### 14.2 HOW DOES THE 900 SERIES® INTERFACE CONVERT ANALOG SIGNALS TO DIGITAL VALUES?

This author is most familiar with TotalChrom® (upgraded from Turbochrom® PE-Nelson) from PerkinElmer Instruments. TotalChrom is a complete software package for non-mass spectrometric chromatographic data acquisition, processing, and reporting, and for instrument control. Most leading analytical instrument manufacturers have their own complete software packages. Agilent has ChemStation®, etc. Some software packages such as EZ Chrom Elite® (ESA, Inc.) can be downloaded onto a PC, and if this PC can be properly interfaced, the software can be used with a variety of manufacturers’ chromatographs.

According to PerkinElmer Instruments:¹⁰

Each 900 Series Interface can be connected to one or two chromatographic detectors usually on a single instrument. The 900 Series Interface converts an analog voltage signal to a frequency that varies in proportion to the signal voltage. The interface then counts the pulses and records a value every 0.01 second. The count accumulated during the interval is called a time slice. The value of each time slice, or the sum of two or more time slices, becomes a data point on the chromatogram. Because the interface always records a count every 0.01 seconds, its fundamental sampling rate is 100 points per second. However, you can define a lower sampling rate in the method. If you use a rate that is slower than the fundamental rate, the interface sums the appropriate number of slice values taken at the fundamental rate. This integrated value becomes a data point. The number of time slices that are summed to derive a data point depends

---

© 2006 by Taylor & Francis Group, LLC
on the desired sampling rate. For example, if the method calls for a rate of 10 points/sec, the interface sums 10 time slices taken at the fundamental rate.

Nine distinct tasks are identified below if a GC has been downloaded with TotalChrom. Each task is executed starting at the top and proceeding to the bottom.

- Baseline subtraction
- Peak detection
- Peak integration
- Component identification (optional)
- Calibration
- Quantitation
- Report generation (optional)
- Replot generation (optional)
- Postanalysis programs (optional)

Let us focus on peak integration while leaving the other eight topics to the user who must operate a given instrument. This assumes that the software has identified a chromatographically resolved peak in a plot of instrument response against run time, i.e., the time after injection of the sample. A sketch of a chromatographically resolved peak is shown below:

![Sketch of chromatographically resolved peak](image)

Referring to the sketch above, the peak area is found by first dividing the area beginning at a raw data point that corresponds to the peak stop and extending horizontally backward to the data point that corresponds to the peak start. In the above sketch, eight area slices of equal width are shown. The start point of the peak does not contribute to the peak’s area. To integrate the area under this curve, the software first sums the area slices from the peak start to the peak end. Initially, these
slices extend vertically from the level of a data point to the zero-microvolt level. Next, the software corrects this sum of the height of the baseline by subtracting the baseline area. This baseline area is the area of a trapezoid between the baseline and the zero-microvolt level. A correct peak area results. This peak is proportional to the amount of analyte injected into the chromatograph, and this forms the most fundamental basis of TEQA. In the above sketch, note that the peak is somewhat skewed; i.e., the peak is slightly unsymmetrical or is said to be not perfectly Gaussian.

### 14.3 What Are Integration Parameters?

One other aspect of using chromatographic software that the author wishes to address in this chapter is that of integration parameter settings. Every software package has some sort of integration parameter settings protocol. The settings should be established after a preliminary chromatogram has been obtained and reviewed. Much of a chromatogram will have unnecessary and even unwanted noise and peaks. The integration parameter settings assist in eliminating these undesirable portions of the baseline. These parameter settings also assist the analyst who wants to properly integrate a pair of partially chromatographically resolved peaks. Tabulated below are those parameters as found in Agilent’s ChemStation® software.

<table>
<thead>
<tr>
<th>Integration Parameter Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial area reject</td>
</tr>
<tr>
<td>Shoulder detection</td>
</tr>
<tr>
<td>Area reject</td>
</tr>
<tr>
<td>Baseline all valleys off and/or on</td>
</tr>
<tr>
<td>Baseline hold off and/or on</td>
</tr>
<tr>
<td>Baseline now</td>
</tr>
<tr>
<td>Negative peak off and/or on</td>
</tr>
<tr>
<td>Solvent peak off and/or on</td>
</tr>
<tr>
<td>Threshold</td>
</tr>
<tr>
<td>Initial peak width</td>
</tr>
<tr>
<td>Initial threshold</td>
</tr>
<tr>
<td>Area sum off and/or on</td>
</tr>
<tr>
<td>Baseline back</td>
</tr>
<tr>
<td>Baseline next valley</td>
</tr>
<tr>
<td>Integrator off and/or on</td>
</tr>
<tr>
<td>Peak width</td>
</tr>
<tr>
<td>Tangent skim</td>
</tr>
</tbody>
</table>

Integration parameters whose entry includes the word *initial* are default settings and are initiated without user intervention whatsoever. Integrator enables integration to start and stop at preselected time events across the chromatogram. The area sum parameter enables the analyst to sum multiple peaks across a specific time interval in the chromatogram. The user of such software must understand how most of these parameters can be used to yield an analytical method in the software that is efficient as well as to minimize computer memory and disk storage. The manner by which some of these parameters are employed is seen in the sketch below for a simulated chromatogram that reveals undesirable regions along the time axis. The arrows pointing upward represent those timed events that should be programmed in to eliminate unwanted peak integration due to the presence of baseline noise, interfering peaks, and certainly the initial solvent peak (sometimes called a *solvent front*) that is present when more universal GC detectors are used:
14.4 HOW ARE ANALYTICAL RESULTS REPORTED?

The organization of sample results that follows data reduction and interpretation into a tabular format for reporting purposes has evolved significantly over the past 20 years. Laboratory PC software that controls and acquires data all have some type of reporting protocols. The analyst today has numerous options with regard to reporting and presenting analytical results. Reports can be printed out on the accompanying printer right in the laboratory. Various summary versions of sample batches can be printed out in tabular formats. Raw data and results can be transferred to a 3½-in. disk (earlier PCs used floppy disks that were 5¼ in. in length and did indeed flop). The 1.44-MB (megabyte) storage capacity tends to limit this storage device. The compact disk can store GB (gigabytes). Most chromatography data acquisition and control software commercially available have algorithms that enable a specific raw data or result file to be converted to an ASCII (American Standard Code for Information Interchange) file. This ASCII file can be imported into an EXCEL spreadsheet or an ACCESS® database. In recent years, the computer architecture has changed so as to make archiving on hard or portable disk obsolete. The client–server architecture in which the laboratory PC becomes the client will succeed in making even compact disk drives obsolete in the future. Refer to McDowell31 and Dyson32 on the topic of chromatographic integration methods.

15. WHAT DO I NEED TO KNOW ABOUT SAMPLING?

Sampling for trace enviro-chemical QA differs from sampling for trace enviro-health QA due largely to the very different sample matrices. Enviro-chemical samples are drawn from air, surface, ground- and wastewater, soil of various types, sediment from river bottoms, sludge from water treatment plants, industrial effluent, leachate from landfills and hazardous waste sites, etc. On the other hand, enviro-health samples are limited to human and animal specimens such as whole blood, serum, plasma, adipose and various organ tissue, breast milk, urine, saliva, semen, etc. Enviro-health sample volume is limited to anywhere from 0.5 to 10 mL per specimen.
From 100 to 2000 mL is a typical sample volume to conduct trace enviro-chemical QA. Sampling 10 L of groundwater is not unusual. These stark differences in sample volume between enviro-chemical and enviro-health result in differences in MDLs.

Good sampling technique must seek to preserve sample integrity. A groundwater sample that is known to contain trichloroethylene (TCE) at a concentration level of 125 ppb when sampled and then analyzed might yield an analytical result of 2.5 ppb. Analyte loss is significant, especially for those analytes that are volatile organics dissolved in an aqueous matrix such as TCE in groundwater, and is a serious limitation of an improper sampling technique. Analyte loss can also occur during sample storage. Loss due to biological activity can be prevented by:

- Acidifying the sample of pH ~1.5
- Cooling or freezing the sample after collection
- Adding complexing agents
- Adding oxidizing agents such as hydrogen peroxide to destroy organic matter
- Irradiating with ultraviolet light

Since samples obtained from the environment are intrinsically heterogeneous, a large number of samples must be collected and analyzed. A large number of samples can be collected and combined to yield a blend that is reasonably homogeneous. From this composite blend, a sufficient number of subsamples can be analyzed. Provided that the error tolerance and confidence interval are defined, we can view the earlier equation for the absolute difference between a population mean and a sample mean in which systematic error has been eliminated:

$$|\mu - \bar{x}| = \frac{z\sigma_p}{\sqrt{L}}$$

Solving this equation for $L$ and defining a number of samples as $L_s$ gives

$$L_s = \left[\frac{z\sigma_p}{\mu - \bar{x}}\right]^2$$

To illustrate, consider the determination of $N$-methyl carbamates, which is usually accomplished with a direct aqueous injection into a reversed-phase high-performance liquid chromatograph with postcolumn derivatization and fluorescence detection (RP-HPLC-Der-F1). Carbamates are used chiefly in agriculture as insecticides, fungicides, herbicides, nematocides, and sprout inhibitors. Let us assume that surface water runoff from a particular agricultural field in the Midwest U.S. yields a mean concentration level for carbaryl (Sevin®) of 0.1 ppm, with a known standard deviation of 0.025 ppm. If we accept an error tolerance in the mean concentration of 20% (20% of 0.1 ppm carbaryl is 0.02 ppm) at a 95% confidence interval ($z = 1.96$), using the equation above, we find that it is necessary to run six samples. However,
most environmental analyses are done on samples whose mean concentration levels are not known and whose standard deviation for a population is not known as well.\textsuperscript{33}

If the standard deviation among replicate measurements can be obtained, the above equation can be used to estimate the number of replicate samples to take to yield a given confidence interval. Measurement for TEQA consists of applying replicate sample preparations such as SPE and replicate determinative techniques such as repeated injection into a GC. There is random error associated with each of these activities. For example, spiking 100 mL of groundwater with chrysene, isolating and recovering the chrysene from the sample matrix, preparing the extract of chrysene in an organic solvent, and injecting the organic solvent into a GC to measure chrysene can be repeated five times to yield the necessary replicate data in order to estimate a standard deviation in the measurement, $\sigma_m$. Again, if we specify a given error tolerance, $e$, we can use Student’s $t$ to estimate the number of replicate environmental samples, $L_M$, to take through the sample preparation and determination steps according to

$$L_M = \left[ \frac{t(1-p/2, df) \sigma_M}{e} \right]^2$$

\textbf{15.1 How Might We Obtain a Representative Sample from a Large Environmental Location?}

Figure 2.16 is a sketch that briefly shows how a relatively large lot or batch obtained from a contaminated hazardous waste site can be divided into increments. A primary or gross sample can be obtained from these increments and further divided into one or more secondary samples. A laboratory or analytical sample can be taken from this secondary sample.

\textbf{15.2 What Factors Need to Be Considered in Obtaining Representative Samples?}

Eight factors are listed below that answer this question (pp. 95–97):\textsuperscript{22}

- Samples must be replicated within each combination of time, location, or other variables of interest.
- An equal number of randomly allocated replicate samples should be taken.
- Samples should be collected in the presence and absence of conditions of interest in order to test the possible effect of the condition.
- Preliminary sampling provides the basis for the evaluation of sampling design and options for statistical analysis.
- Efficiency and adequacy of the sampling device or method over the range of conditions must be verified.
- Proportional focusing of homogeneous subareas or subspaces is necessary if the entire sampling area has high variability as a result of the environment within the features of interest.
• Sample unit size (representative of the size of the population), densities, and spatial distribution of the objects being sampled must be verified.
• Data must be tested in order to establish the nature of error variation to enable a decision on whether the transformation of the data, the utilization of distribution-free statistical analysis procedures, or the test against simulated zero-hypothesis data is necessary.

An optimized sampling program must take the following aspects into account:

• Place, location, and position of sampling
• Size, quantity, and volume of the sample
• Number of samples
• Date, duration, and frequency of sampling
• Homogeneity of the sample
• Contamination of the sample
• Decontamination of the sample
• Sample conservation and storage

Well, what is left? Analytical chemists must be able to not only process, manipulate, interpret, and report on analytical data and results, but actually acquire the
data. Effectively performed TEQA requires that once sampling is completed, sample preparation techniques must be implemented. This important facet of TEQA comprises the next chapter.

Figures may not lie, but statistics compiled unscientifically and analyzed incompetently are almost sure to be misleading, and when this condition is unnecessarily chronic the so-called statisticians may well be called liars.


REFERENCES

8. The author has drawn on a series of papers in developing these concepts. These papers were written by Lloyd Currie at the NIST and are cited as follows:
Sample Preparation Techniques to Isolate and Recover Organics and Inorganics

Separation methods form the basis of chemistry, and the definition of a pure chemical substance ultimately depends on separative operations.

—Arne Tiselius

CHAPTER AT A GLANCE

Sample prep for trace organics

- Liquid–liquid extraction (LLE) ................................................................. 121
- Separatory funnel .................................................................................... 131
- LLE as cleanup ....................................................................................... 131
- Mini ........................................................................................................ 145
- Micro ...................................................................................................... 145
- Continuous .............................................................................................. 148
- Soxhlet liquid–solid extraction (S-LSE) ................................................ 149
- Conventional Soxhlet ........................................................................ 149
- Automated Soxhlet ............................................................................. 150
- Ultrasonic liquid–solid extraction (U-LSE) .......................................... 153
- Microwave accelerated extraction (MAE) ............................................. 157
- Accelerated solvent extraction (ASE) .................................................... 160

Sample prep for volatile organic compounds (VOCs) .......................... 165

- Mini-LLE ............................................................................................. 165
- Static headspace .................................................................................. 165
- Hexadecane screening via LLE ............................................................ 178
- Purge and trap ...................................................................................... 181

Extract cleanup ........................................................................................ 191

- Adsorption column chromatography ................................................ 192
- SPE adsorption .................................................................................... 194
- Gel permeation chromatography ....................................................... 195

Supercritical fluid extraction (SFE) ......................................................... 200

Reversed-phase solid-phase extraction (RP-SPE) .................................... 211
The importance of sample preparation to TEQA is clearly indicated in the following story. This author was once approached by a student during the era when it became apparent that in the 1970s polychlorinated biphenyls (PCBs) had contaminated the striped bass that migrate up the Hudson River in New York to spawn every spring. Once the student learned that a gas chromatograph (GC) is used to measure the extent that fish are contaminated with PCBs and noticed the instrument on the bench in the corner of the laboratory, the student was curious as to exactly how a fish the size of a striped bass could be put into the injection port of the GC. The diameter of the injection port of the GC was less than 1 mm, which, of course, is miniscule in comparison to the size of the fish. The student thought that all that was necessary was to find a way to get the fish into the injection port and the data, which at that time were displayed on a strip-chart recorder, would indicate the extent of this PCB contamination. The student speculated that it might be easier to cut the fish up and attempt to stuff it into the injection port on the GC. Ah, we see for the first time, in this student, a glimpse into the need for sample preparation.

Indeed, the fish must be transformed in some manner prior to measurement by a determinative technique — in this case, by gas chromatography. Determinative techniques utilize instrumental analysis approaches and are discussed in Chapter 4. The removal of the PCB from fish tissue (known as the sample matrix) to a form that is compatible with the determinative technique or particularly analytical instrument — in this case, the GC — is the basis for sample preparation. The GC requires the introduction of a solvent that contains the dissolved solute — in this case, PCBs. A gas can also be injected into the GC. However, it is much more convenient to get the PCBs from the sample matrix to the liquid state. The liquid is quickly vaporized under the elevated temperature of the GC injection port and undergoes GC separation. The number of molecules of each chemically different substance now present
in the vapor causes a perturbation in the GC detector. This perturbation results in an electrical signal whose magnitude becomes proportional to the number of molecules present in the liquid.

This chapter introduces the various techniques that are commonly used to prepare environmental samples and animal and human specimens and comprises an important component of TEQA. The laboratory approach used to “get the striped bass into the machine” to achieve the utmost goal of TEQA (i.e., to isolate, identify, and quantitate the PCBs in the sample matrix) defines sample preparation. This chapter starts out with the most common and most conceptually simplistic form of sample preparation, whereby a liquid such as water or a solid such as a soil is placed in a beaker or equivalent container. To this container is added an organic solvent that is immiscible with water. The mixture is shaken and allowed to remain stationary for a period, such as 15 min. The analytes originally dissolved in the water or adsorbed onto soil particulates are partitioned into the organic solvent. The organic solvent that now contains the dissolved analyte as a solute is referred to as the extractant. After the principles of liquid–liquid extraction (LLE) are introduced and developed, the practice of LLE in its various forms will be discussed.

In addition to LLE, there are two other major types of analyte isolation and recovery: solid-phase extraction (SPE) and supercritical fluid extraction (SFE). SPE refers to those techniques that isolate the analyte from a sample matrix and partition the analytes of interest onto a chemically bonded silica or polymeric surface. SFE refers to those techniques that isolate the analyte from a sample matrix and partition it into a liquid that has been heated and pressurized beyond its critical temperature and pressure. It is indeed overly simplistic to think that a striped bass can be stuffed into a GC as a means to conduct TEQA.

1. WHAT ARE THE PRINCIPLES UNDERLYING LLE?

A good grounding in the basic principles of LLE is a useful way to begin a chapter that focuses on sample preparation for TEQA. LLE was historically the first sample preparation technique used in analytical chemistry. Organic chemists have used LLE techniques for over 150 years for isolating organic substances from aqueous solutions. A good definition of LLE has been given earlier in the literature and is stated here:

A substance distributes between contacting immiscible liquids — water and a suitable organic solvent, for example — roughly in the ratio of its solubility in each if it does not react with either and if it exits in the same form in both. If, at equilibrium, its concentration is much greater in the organic solvent phase than in the aqueous phase, the distribution behavior may be put to analytical use in concentrating the substance into a small volume of the organic liquid and, more importantly, in separating it from substances that do not distribute similarly.¹

This definition of LLE is concise yet profound in that it covers all ramifications. The first sentence establishes two conditions: compounds that react with the extractant do not obey the rules, and the chemical nature of the compound needs to remain the same throughout the extraction. Mathematical relationships have also been developed to account for the fact that the chemical form may change. This has been called

© 2006 by Taylor & Francis Group, LLC
secondary equilibrium effects, and this topic will also be introduced in this chapter. The second sentence implies that a concentration factor can be realized. The concentrating nature of LLE is most important to TEQA. The fact that different chemical substances will distribute differently between immiscible liquids also forms the theoretical basis for separation among two or more organic substances that might be initially dissolved in the aqueous solution. These differences are exploited in the design of sample preparation schemes as well as provide for the fundamental basis to explain analyte separation by chromatography. Aqueous solutions are of prime importance to TEQA because our sample matrix, if a liquid, consists of drinking water, surface (i.e., rivers) water, groundwater, or wastewater obtained from the environment. The fact that the chemical form can change during the extraction process can be exploited in analytical chemistry toward the development of new methods to separate and isolate the analyte of interest.

To understand the most fundamental concept of liquid–liquid extraction, consider placing 100 mL of an aqueous solution that contains 0.1 \( M \) NaCl and 0.1 \( M \) acetic acid (HOAc) into a piece of laboratory glassware known as a separatory, or commonly abbreviated as a sep funnel. Figure 3.1 shows a conceptually simplified LLE process. Figure 3.1A shows this process just prior to mixing the two immiscible phases. Next, 100 mL of diethyl ether, a moderately polar organic solvent that is largely immiscible with water, is added to the funnel. Indeed, some ether will dissolve in water to the extent of 6.89% at 20°C, while some water dissolves in the ether to the extent of 1.26% at 20°C. Upon shaking the contents of the funnel and allowing some time for the two phases to become stationary, the solute composition of each phase is depicted in Figure 3.1B. The lower layer is removed from the sep funnel, thus physically separating the two phases. Taking an aliquot (portion thereof) of the ether phase and separately taking an aliquot of the water phase while subjecting the aliquot to chemical analysis reveals a concentration of NaCl, denoted as \([\text{NaCl}]\), at \(1.0 \times 10^{-11} M\), and that in water, \([\text{NaCl}]_{\text{aq}}\) = 0.10 \( M \). Analysis of each phase for acetic acid reveals \([\text{HOAc}]_{\text{ether}}\) = \([\text{HOAc}]_{\text{aq}}\) = \(5 \times 10^{-2} M\). Upon combining both phases again, a second chemical analysis of the composition of each phase reveals exactly the same concentration of HOAc and NaCl in each phase. As long as the temperature of the two phases in contact with each other of the sep funnel remain fixed, the concentration of each chemical species in both phases will not change with time. A dynamic chemical equilibrium has been reached. The significant difference in the extent of partitioning of NaCl and HOAc between diethyl ether and water-immiscible phases can be explained by introducing a thermodynamic viewpoint.

2. **DOES THERMODYNAMICS EXPLAIN DIFFERENCES IN NACl VS. HOAc PARTITIONING?**

For spontaneous change to occur, the entropy of the universe must increase. The entropy of the universe continues to increase with each and every spontaneous process. LLE represents an ideally closed thermodynamic system in which solutes originally dissolved in an aqueous sample taken from the environment can diffuse across a solvent–water interface and spontaneously partition into the solvent phase. These concepts are succinctly defined in terms of the change in Gibbs free energy,
Sample Preparation Techniques

123

$G$, for system processes that experience a change in their enthalpy $H$ and a change in the entropy of the system $S$. The criteria for spontaneity requires that the Gibbs free energy, $G$, decrease. In turn, this free-energy change is mathematically related to a system’s enthalpy $H$ and entropy $S$. All three depend on the state of the system and not on the particular pathway, so a change in free energy at constant temperature can be expressed as a difference in the exothermic or endothermic nature of the change and the tendency of the matter in the system to spread according to

$$\Delta G = \Delta H - T \Delta S$$

This equation suggests that for spontaneous physical or chemical change to occur, the process proceeds with a decrease in free energy. As applied to phase distribution, equilibrium is reached when the infinitesimal increase in $G$ per infinitesimal increase in the number of moles of solute $i$ added to each phase becomes equal. Hence, the chemical potential of solute $i$ is defined as

$$\mu = \left( \frac{\partial G}{\partial n_i} \right) _{T,P}$$

The chemical potential can also be expressed in terms of a chemical potential under standard-rate conditions $\mu^0$ and the activity $a$ for a solute in a given phase.
Recognizing that a phase has an activity equal to unity (i.e., \( a = 1 \) defines the standard state at a given temperature and pressure), the equation for the chemical potential \( \mu \) for an activity other than \( a = 1 \) is found according to

\[
\mu = \mu^0 + RT \ln a
\]  

(3.1)

Once equilibrium is reached, the net change in \( \mu \) for the transfer of solute \( i \) between phases must be zero, so that for our example of NaCl or HOAc in the ether/water-immiscible phase illustration, the chemical potentials are equal:

\[
\mu_{\text{ether}}^{\text{NaCl}} = \mu_{\text{aq}}^{\text{NaCl}}
\]  

(3.2)

Hence, upon substituting Equation (3.1) into Equation (3.2) for solute \( i \),

\[
\mu_{\text{ether}}^0 + RT \ln a_{\text{ether}} = \mu_{\text{aq}}^0 + RT \ln a_{\text{aq}}
\]

which rearranges to

\[
RT \ln \left( \frac{a_{\text{ether}}}{a_{\text{aq}}} \right) = \mu_{\text{aq}}^0 - \mu_{\text{ether}}^0
\]  

(3.3)

The change in standard-state chemical potential, \( \Delta \mu^0 \), is usually expressed as the difference between the organic phase and the aqueous phase according to

\[
\Delta \mu^0 = \mu_{\text{ether}}^0 - \mu_{\text{aq}}^0
\]

Solving Equation (3.3) for the ratio of solute activities gives

\[
\frac{a_{\text{ether}}}{a_{\text{aq}}} = e^{-\Delta \mu^0}
\]

Because \( \Delta \mu^0 \) is the difference of two constant standard-state chemical potentials, it must be a constant. The ratio of activities of NaCl or HOAc is fixed provided that the temperature and pressure are held constant.

A thermodynamic approach has just been used to show what is important analytically; that is, LLE enables an analyte to be transferred from the sample to the extracting solvent and remain at a fixed concentration over time in the extractant. This ratio of activities is defined as the thermodynamic distribution constant, \( K^0 \), so that

\[
K^0 \equiv \frac{a_{\text{ether}}}{a_{\text{aq}}}
\]  

(3.4)
3. WHAT ARE SOLUTE ACTIVITIES ANYWAY?

A solute dissolved in a solvent such as water is only partly characterized by its concentration. Solute concentration can be expressed in one of any number of units. The most commonly used units include the following: moles solute per liter solution or molarity (M), moles solute/100 g water or molality (m), and millimoles solute per liter solution or millimolarity (mM). Those units that have greater relevance to TEQA include the following: milligrams of solute per liter solution or parts per million (ppm), micrograms of solute per liter solution or parts per billion (ppb), and picograms of solute per liter solution or parts per trillion (ppt). Note that TEQA relies exclusively on expressing solute concentration in terms of a weight per unit volume basis. The fact that equilibrium constants in chemistry depend not only on solute concentration but also on solute activities serves to explain why any discussion of distribution equilibria must incorporate solute activities. Solute activities are introduced in any number of texts. Activities become important when the concentration of an electrolyte in an aqueous solution becomes appreciable (i.e., at solute concentrations of 0.01 M and higher).

The extent to which a solution whose concentration of solute \( i \) contributes to some physical/chemical property of this solution (i.e., its activity, \( a_i \)) is governed by the solute’s activity coefficient \( \gamma_i \) according to

\[
a_i = \gamma_i c_i
\]

1. Neutral molecules dissolved in water do not affect ionic strength.
2. Very dilute aqueous solutions are most likely found.

However, one aspect of TEQA that is strongly influenced by ionic strength, and hence provides an opportunity for activity coefficients to play a role, is the concept of salting out. The solubility of one chemical substance in another, like \( K^0 \) [Equation (3.4)] in LLE, is also governed by the need for the substance to lower its Gibbs free energy by dissolving in a solvent. Isopropyl alcohol (IPA) or 2-propanol is infinitely soluble in water, as is true for most lower-molecular-weight alcohols. However, for a solution that might consist of 50% IPA and 50% water, the alcohol can be separated out as a separate phase if enough NaCl is added to almost saturate the system. This is a direct influence of ionic strength in an extreme case. The fact that polar solvents can be separated as an immiscible phase opens up new sample preparation opportunities. For example, Loconto and coworkers\(^5\) recently demonstrated that the homologous series of polar 2-aminoethanols could be efficiently partitioned into IPA from an aqueous sample of interest to wood chemists. The sample was saturated with NaCl, then extracted using IPA.

Two important relationships must be discussed that relate activity coefficients to ionic strength. Ionic equilibria are influenced by the presence of all ions in an aqueous solution. The most useful indicator of the total concentration of ions in a

---

\(^*\) The concept of activity and activity coefficients is found in most physical and analytical chemistry texts that consider ionic equilibria. The texts listed in reference 4 are part of the author’s personal library.
solution is the ionic strength, \( I \). The ionic strength can be calculated if the concentration \( C_i \) of an ion whose charge is \( z_i \) is known according to

\[
I = \frac{1}{2} \sum_i C_i z_i^2
\]  

(3.5)

The summation is extended over all ions in solution. For example, consider two aqueous solutions, one containing 0.01 \( M \) NaCl and the other one containing 0.01 \( M \) K\(_2\)SO\(_4\). Using Equation (3.5), the ionic strength for the former solution is calculated to be 0.01 \( M \) and that for the latter is 0.03 \( M \). Assume that a solution is created that consists of 0.01 \( M \) in each salt. The ionic strength of such a mixture is calculated according to Equation (3.5) to be 0.04 \( M \).

Knowledge of a solution’s ionic strength enables a determination of the activity coefficient to be made. This can occur through the application of the Debye–Huckel equation according to

\[
\log \gamma = \frac{-0.51z^2\sqrt{I}}{1 + \alpha \sqrt{I/305}}
\]

where \( \alpha \) refers to the size of the hydrated radius of the ion, and \( z \) is the charge of the ion. This equation gives good approximations for ionic strengths below or equal to 0.1 \( M \). For ionic strengths less than 0.01 \( M \), the following relationship suffices:

\[
\log \gamma = 0.51z^2\sqrt{I}
\]

4. CAN THE DIFFERENCE BETWEEN \( K^0 \) VALUES FOR \( \text{NaCl} \) AND \( \text{HOAc} \) BE SHOWN GRAPHICALLY?

The thermodynamic relationship between standard-state chemical potential differences and the position of chemical equilibrium can be shown graphically. Figure 3.2 illustrates what happens to the Gibbs free energy \( G \) when the solute is partitioned between an aqueous phase in contact with an immiscible organic phase, diethyl ether in this example. The hypothetical plots of \( G \) vs. the mole fraction, denoted by \( X_i \), of solute \( i \) dissolved in the ether phase, are superimposed for comparison. When there is no solute in the ether phase, a standard-state chemical potential, \( \mu^0_{aq} \), can be realized. In the other extreme, when 100% of all of the mass solute is in the ether phase (i.e., having a mole fraction \( X_{\text{ether}} = 1 \)), a standard-state chemical potential, \( \mu^0_{\text{ether}} \), can also be defined. The situation at \( X_{\text{ether}} = 1 \) is a hypothetical one in that for some solutes, 1 mol of solute cannot dissolve to that extent in an organic solvent like ether. This is particularly true for an ionically bonded substance such as sodium chloride. Imagine if this much NaCl could dissolve in ether. The free energy that would be required to dissolve as much as 1 mol NaCl in 1 L of ether would be expected to be extremely large indeed.
Such is not the case when considering the free energy required for the dissolution of 1 mol HOAc in 1 L of ether. The mole fraction of solute partitioned into the ether at equilibrium is that point along the $x$ axis where $G$ is at a minimum, or in other words, the slope of the tangent line (i.e., $dG/dX_i$) is zero. It becomes quite evident when viewing this graphical display that the magnitude of standard-state Gibbs free energies are chiefly responsible for the position along the $x$ axis where $G$ reaches a minimum. At this position, the mole fraction of each solute becomes fixed as defined by Equation (3.3). Figure 3.2 shows that the Gibbs free energy is minimized at equilibrium for NaCl at a much lower mole fraction when compared to the value of the mole fraction for HOAc, where its Gibbs free energy is minimized. In other words, the value of $X_i$ where $dG/dX_i$ is minimized at equilibrium depends entirely on the nature of the chemical compound. If a third solute is added to the original aqueous solution, as depicted in Figure 3.1, it too would exhibit its own $G$-as-a-function-of-$X_i$ plot and reach a minimum at some other point along the $x$ axis. These concepts render Equation (3.3) a bit more meaningful when graphically represented.

5. **CAN WE RELATE $K^0$ TO ANALYTICALLY MEASURABLE QUANTITIES?**

It becomes important to TEQA to relate the thermodynamic distribution constant, $K^0$, to measurable concentration of dissolved solute in both phases. Because the chemical potential for a given solute must be the same in both immiscible phases
that are in equilibrium, Equation (3.2) can be rewritten in terms of activity coefficients and concentration according to

\[ \mu^0_{\text{ether}} + RT \ln C_{\text{ether}} + RT \ln \gamma_{\text{ether}} = \mu^0_{\text{aq}} + RT \ln C_{\text{aq}} + \ln \gamma_{\text{aq}} \]

Upon rearranging and simplifying, we get

\[ RT \ln \frac{C_{\text{ether}}}{C_{\text{aq}}} + RT \ln \frac{\gamma_{\text{ether}}}{\gamma_{\text{aq}}} = -\Delta \mu^0 \]

This equation can be solved for the ratio of measurable concentration of solute in the ether phase to that of the water phase; this is shown by

\[ \frac{C_{\text{ether}}}{C_{\text{aq}}} = \frac{\gamma_{\text{ether}}}{\gamma_{\text{aq}}} e^{-\Delta \mu^0 / RT} \quad (3.6) \]

If we define a partition constant \( K_D \) as a ratio of measurable concentrations of solute in both phases, we get

\[ K_D \equiv \frac{C_{\text{ether}}}{C_{\text{aq}}} \quad (3.7) \]

Upon substituting Equation (3.6) into Equation (3.7), we obtained the relationship between the partition ratio and the thermodynamic distribution constant according to

\[ K_D = \frac{\gamma_{\text{ether}}}{\gamma_{\text{aq}}} K^0 \quad (3.8) \]

Equation (3.8) is the desired outcome. In many cases, with respect to TEQA, the activity coefficients of solutes in both phases are quite close to unity. The partition ratio and thermodynamic distribution constant can be used interchangeably.

For either NaCl or HOAc, or for any other solute distributed between immiscible liquids at a fixed temperature and pressure, provided that the concentration of solute is low (i.e., for the dilute solution case), \( K^0 \) can be set equal to the partition constant \( K_D \) because activity coefficients can be set equal to 1. The partition constant or Nernst distribution constant in our illustration for acetic acid partitioned between ether and water can be defined as

\[ K_D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}}} \]
From the analytical results for measuring the concentration of HOAc in each phase introduced earlier, \( K_D \) can be calculated:

\[
5 \times 10^{-2} M / 5 \times 10^{-2} M = 1
\]

Likewise, from the analytical results for measuring the concentration of NaCl in each phase introduced earlier, \( K_D \) can be calculated:

\[
1 \times 10^{-11} M / 1 \times 10^{-11} M = 1 \times 10^{-10}
\]

6. IS LLE A USEFUL CLEANUP TECHNIQUE?

Two examples of how LLE is used not only to isolate the analyte of interest from possible interferences from the sample matrix but also to provide an important cleanup are now discussed. Both procedures, which were then incorporated into respective methods, yield an extract that is ideally free of interferences that can be used in the determinative step to quantitate the presence of analyte that was originally in the sample.

In the first case, an environmental sample that contains a high concentration of dissolved inorganic salts such as NaCl is suspected to contain trifluoroacetic acid (TFA). TFA is a known by-product from the recently understood persistence of fluorocarbons in the environment. The physical and chemical properties of TFA are well known. When dissolved in water, TFA is a moderately strong carboxylic acid with a pK\(_a\) lower than that of acetic acid. TFA also has an infinite solubility in water. TFA is not directly amenable to detection by GC because it cannot be sufficiently vaporized in the hot-injection port of the GC. It is not good practice to make a direct aqueous injection into a GC that possesses a column that contains the commonly used silicone polymer as a liquid phase. Hence, it is necessary to prepare an analytical reference standard in such a way that (1) TFA can be made amenable to analysis by GC, and (2) extracts that contain TFA must be nonaqueous. TFA could be determined by a direct aqueous injection if a different instrumental technique is used. The options here include either high-performance liquid chromatography (HPLC) in one of its several forms, ion chromatography (IC), or capillary electrophoresis (CE). There is a gain, however, if a sample preparation technique can be developed that concentrates the sample. Wujcik et al.’s\(^7\) group took the following approach to the determination of TFA in environmental waters.

The highly salted aqueous sample that is expected to contain the targeted analyte TFA is initially acidified to suppress the ionization of the acid according to

\[
\text{CF}_3\text{COOH}_{(aq)} \rightleftharpoons \text{H}^+_{(aq)} + \text{CF}_3\text{COO}^-_{(aq)}
\]

where the subscript (aq) refers to the fact that each ionic species is dissolved in water and is surrounded by water dipoles. The triple-head double-arrow denotes that when TFA is initially dissolved in water, a dynamic chemical equilibrium is quickly
established whereby hydronium and trifluoroacetate ions exist in water along with undissociated TFA. Upon acidifying, the extent of this ionization is suppressed and a new equilibrium concentration of hydronium, trifluoroacetate, and TFA is reestablished with significantly higher concentration of TFA and hydronium ion and a much lower concentration of trifluoroacetate. Refer to any number of texts that elaborate on the principles of ionic equilibrium that governs the extent of acid dissociation.

The acidified aqueous environmental water sample is then extracted with a nonpolar solvent such as hexane, iso-octane, dichloromethane (methylene chloride), or some other common water-immiscible solvent. TFA is partitioned into the extractant to an appreciable extent owing to the fact that its ionization has been suppressed in the aqueous phase and the trifluoromethyl moiety gives a hydrophobic character to the molecule. The inorganic salts are left behind in the aqueous phase. Upon physically separating the phases and placing the organic phase in contact with a second aqueous phase that has been made alkaline or basic by the addition of NaOH or KOH, TFA molecules diffuse throughout the bulk of the extractant toward the interfacial surface area where they are ionized according to

$$\text{CF}_3\text{COOH}_{(aq)} \rightleftharpoons \text{CF}_3\text{COO}^-_{(aq)} + H^+_{(aq)}$$

After the rate of TFA transport through to the interface from the bulk extractant and into the alkaline aqueous phase becomes equal to the rate of TFA from the bulk alkaline aqueous phase through to the extractant and equilibrium is reestablished, a new partitioning occurs, with most of the original TFA now in the alkaline aqueous phase. The cleanup has been accomplished because the aqueous phase contains TFA, as it conjugate base, without any dissolved inorganic salts. The alkaline aqueous matrix is then passed through a disk that contains anion exchange sites whereby trifluoroacetate can be retained by the ion exchange interaction. The disk is then placed into a 22-mL headspace vial containing 10% sulfuric acid in methanol and the vial is sealed tightly. Heating at 50°C for a finite period converts TFA to its methyl ester. The headspace, which now contains methyl trifluoracetate, is sampled with a gas-tight GC syringe and injected into a GC. The headspace technique eliminates any solvent interference.

The second case, taken from the author’s own work, uses LLE to initially clean up an aqueous sample taken from the environment that might contain, in addition to the analyte of interest, other organic compounds that may interfere in the determinative step. The analytes of interest are the class of chlorophenoxy acid herbicides (CPHs) and include 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,4,5-trichlorophenoxypropionic acid (Silvex). CPHs are used as herbicides in agricultural weed control, and because of this, CPHs are routinely monitored in drinking water supplies. CPHs are usually produced as their corresponding amine salts or as esters. An initial alkaline hydrolysis of the sample is needed to convert the more complex forms to their corresponding conjugate bases.

---

* In addition to the reference sources cited in reference 4, a number of texts on water chemistry discussing ionic equilibria and a recent book are listed in reference 8.
The sample is then extracted using a nonpolar solvent. This LLE step removes possible organic interferences while leaving the conjugate bases to the CPHs in the aqueous phase. Cleaned-up alkaline aqueous phase results can now be acidified and either reextracted (LLE) or passed through a chemically bonded solid sorbent to isolate the CPHs, and possibly achieve a concentration of the CPHs from that in the original sample. As was true in the first case, the ionizable properties of these analytes can be exploited to yield a clean extract that can be quantitatively determined. Between 95 and 100% recoveries for the three CPHs cited were obtained from water spiked with each CPH. No influence of these high-percentage recoveries upon inserting an initial LLE step was observed.\(^9\)

In contrast, the more conventional approach to trace CPH residue analysis serves to illustrate this difference in approaches to sample preparation. A water sample taken from the environment is initially acidified to preserve its chemical composition prior to sample preparation and analysis. At the onset of sample preparation, the water sample is made alkaline. To this alkaline aqueous phase, nonpolar solvent is added and the immiscible phases are shaken in a glass separatory funnel. Esters of CPHs, being nonpolar themselves, obey the universal principle that like dissolves like and partition into the organic phase. The free CPH acids remain in the aqueous phase. If only the formulated esters of CPHs are of interest, the extract can be cleaned up and analyzed. However, if it is desirable to convert the esters to acids, as is the case in most regulatory methods, a base hydrolysis is conducted on the organic phase that converts these CPH esters to their corresponding salts. The aqueous phase is reacidified and a second LLE is performed. The extracted CPHs are derivatized and converted to their corresponding methyl esters using any of the more common derivatization reagents. Following a cleanup step, the extract is ready for injection into a GC with a chlorine-selective detector such as an electron-capture detector (ECD) or an electrolytic conductivity detector (E1CD). This approach to sample preparation is a good example of the complexity involved in many of the methods of TEQA. If 1 L of an environmental water sample is taken through this method, it is likely that a concentration of 10 ppb 2,4-D originally present in the sample can be separated from other CPHs, identified, detected, and quantified using all of the techniques available in TEQA.

These two examples clearly demonstrate the importance of secondary equilibria phenomena, particularly when the analyte of interest is ionizable in an environmental aqueous sample such as groundwater. Both examples exploit secondary equilibria in developing alternative methods that include LLE in extraction and in cleanup when applied to the complex sample matrices commonly encountered in TEQA. In the next section, the mathematical framework that underlies secondary equilibria will be presented.

7. **HOW DO WE ACCOUNT FOR SECONDARY EQUILIBRIA IN LLE?**

Let us return to the ether/aqueous-immiscible distribution equilibrium model introduced earlier (refer to Figure 3.1). What if the aqueous solution, prior to adding any ether, was made alkaline by the addition of NaOH? We know that the chloride ion
Concentration in the original aqueous solution would not change, but what about the HOAc? We also know that acetic acid is a weak acid and undergoes dissociation to hydronium ions and acetate ions. The extent of this dissociation is governed by the dissociation constant, $K_a$. The triple-head double-arrow notation is used in the following reaction to show that prior to the addition of hydroxide ion to an aqueous solution that contains dissolved acetic acid, the ionic equilibrium is already established.

$$\text{CH}_3\text{COOH} + H_2O \leftrightarrow H_3O^+ + \text{CH}_3\text{COO}^-$$

The effect of the added hydroxide ion is to shift the position of equilibrium to favor the product acetate, and thus to remove HOAc from the aqueous phase. HOAc molecules in the ether phase partition back to the aqueous phase until chemical potentials become equivalent and the magnitude of $K_D$ is restored to the same value that the system had before the addition of the hydroxide ion.

Does this pH adjustment have any effect on the partitioning of HOAc between immiscible phases? By definition, only neutral HOAc can partition between phases. The value for the partition ratio must be preserved based on the thermodynamic arguments put forth earlier. This must mean that the concentrations of HOAc in the ether phase must be reduced due to the pH adjustment because the concentration of undissociated HOAc in the aqueous phase has also been reduced. This is illustrated for the HOAc only, in Figure 3.3. Our model assumes that the only chemical form of acetic acid in the ether phase is HOAc and that only acid dissociation of HOAc occurs in the aqueous phase. Because $K_D$ accounts only for undissociated forms of acetic acid, a new constant is needed to completely account for the undissociated

![Figure 3.3](image-url) Distribution of HOAc between two immiscible phases. The aqueous phase is alkaline.
acetic acid and the acetate ion. This constant is called the distribution ratio, \( D \), and is defined according to

\[
D = \frac{\sum_j [A]_o}{\sum_k [A]_{aq}}
\]

(3.9)

where \([A]_o\) refers to the concentration or activity of the \( j \)th chemical species in the organic or extractant phase, and \([A]_{aq}\) refers to the concentration or activity of the \( k \)th chemical species in the aqueous phase.

The magnitude of \( D \) enables one to understand the extent to which all chemical forms of the analyte of interest are partitioned between two immiscible phases. \( D \) accounts for all secondary equilibrium effects that occur. Let us go back to the concept of acetic acid partitioning between diethyl ether and water while considering the influence of the secondary equilibrium, that of weak acid dissociation due to an adjustment of the pH of the aqueous phase. This discussion will help us enlarge the scope of LLE and set the stage for further insights into the role of secondary equilibrium.

We start by using Equation (3.9) to define the different chemical species that are assumed to be present, and then we proceed to substitute secondary equilibrium expressions governed by acid–base dissociation constants or metal chelate formation constants. In the case of HOAc that is partitioned between ether and water, let us assume that only monomeric forms of HOAc exist in both phases and define the distribution ratio, \( D \), according to

\[
D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}} + [\text{OAc}^-]_{\text{aq}}}
\]

(3.10)

Acetic acid dissociates in pure water to the extent determined by the magnitude of the acid dissociation constant, \( K_a \). Based on the law of mass action, \( K_a \) is defined as

\[
K_a = \frac{[H^+] [\text{OAc}^-]}{[\text{HOAc}]}
\]

(3.11)

Let us solve Equation (3.11) for the acetate ion concentration that is in equilibrium with the hydronium ion, \( H^+ \), and undissociated HOAc:

\[
[\text{OAc}^-] = K_a \frac{[\text{HOAc}]}{[H^+]};
\]

Substituting for \([\text{OAc}^-]\) in Equation (3.10) and simplifying yields a fruitful relationship:
This expression can be further rearranged by factoring out the ratio of both molecular forms of HOAc:

\[
D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}} + K_d \frac{[\text{HOAc}]_{\text{aq}}}{[H^+]} 
\]

This gives an expression for \( D \) in terms of a ratio of concentrations in both phases for the undissociated acid forms, which is exactly our definition of the distribution constant for the partitioning of HOAc between ether and water. Expressing \( D \) in terms of \( K_D \) yields an important relationship:

\[
D = K_D \left[ \frac{1}{1 + K_d /[H^+]} \right] = K_D \frac{[H^+]}{K_a + [H^+]} \tag{3.12}
\]

Equation (3.12) clearly shows the dependence of the distribution ratio on the secondary equilibrium (i.e., the weak acid dissociation) and on the extent of the primary equilibrium (i.e., the partitioning equilibrium of molecular HOAc between two immiscible phases). If Equation (3.12) is rearranged, we get

\[
D = \frac{K_D [H^+]}{K_a + [H^+]} \tag{3.13}
\]

A plot of \( D \) vs. \([H^+]\) is shown in Figure 3.4. The graph is hyperbolic, and upon careful examination, it would appear to resemble the Michaelis–Menten enzymes kinetics found in biochemistry.10 The plot in Figure 3.4 as well as Equation (3.12) show that in the limit as the hydronium ion concentration gets very large, \( K_a \) becomes small in comparison to \([H^+]\), and in the limit of a very large hydronium ion concentration, the following can be stated: in the limit as

\[
[H^+] \to \infty
\]

it is evident that

\[
D \to K_D
\]

The partition constant, \( K_D \), and the acid dissociation constant, \( K_a \), for acetic acid can be found experimentally from a plot of \( D \) vs. \([H^+]\), as shown in Figure 3.4. Let \( D = \frac{1}{2} K_D \) in Equation (3.13) so that
Eliminating $K_D$ and solving this equation for $K_a$ gives

$$K_a = [\text{H}^+]$$

Hence, the acid dissociation constant for HOAc could be calculated. One would need to know experimentally exactly how $D$ varies with the concentration of hydronium ion for this LLE in order to prepare a precise plot. It becomes difficult to estimate $K_D$ from the hyperbolic curve shown in Figure 3.4. Equation (3.13) can be rearranged by taking reciprocals of both sides and rewriting Equation (3.13) in the form of an equation for a straight line of form $y = mx + b$, where $m$ is the slope and $b$ is the $y$ intercept:
A plot of $1/D$ vs. $1/[H^+]$ yields a straight line whose slope $m$ is equal to the ratio $K_a/K_D$, and the $y$ intercept $b$ is equal to $1/K_D$. In this manner, both equilibrium constants can be determined with good precision and accuracy.\(^\text{10}\)

Alternatively, Equation (3.12) can be viewed in terms of the primary equilibrium as represented by $K_D$ and in terms of secondary equilibrium as represented by $\alpha_{\text{HOAc}}$. Let us define $\alpha_{\text{HOAc}}$ as the fraction of neutral or undissociated HOAc present according to

$$\alpha_{\text{HOAc}} = \frac{[\text{HOAc}]_{aq}}{[\text{HOAc}]_{aq} + [\text{OAc}^-]_{aq}}$$

Upon simplifying, it can be shown that Equation (3.12) can be rewritten as

$$D = K_D \alpha_{\text{HOAc}}$$

Upon examination of this relationship among $D$, $K_D$, and $\alpha_{\text{HOAc}}$, it becomes evident that the distribution ratio depends on the extent to which a solute (in our example, acetic acid) distributes itself between two immiscible phases (e.g., ether and water). At the same time, this solute is capable of exhibiting a secondary equilibrium (i.e., that of acid dissociation in the aqueous phase), as determined by the fraction of all acetic acid that remains neutral or undissociated. We will introduce this concept of fractional dissociation as just defined when we discuss LLE involving the chelation of transition metal ions from an aqueous phase to a water-immiscible organic phase.

8. WHAT IF THE CHEMICAL FORM OF HOAc CHANGES IN THE ORGANIC PHASE?

The above formalism assumed that only the monomeric form of HOAc exists in the ether phase. Carboxylic acids are known to dimerize in organic solvents that have a low dielectric constant. Let us assume we have acetic acid forming a dimer in the organic phase. This tendency may be more prominent if HOAc is dissolved in a nonpolar solvent like hexane, as compared to a moderately polar solvent like diethyl ether. The formation of a dimer can be depicted by

$$2 \text{HOAc} \rightleftharpoons K_{\text{dim}} \rightarrow (\text{HOAc})_2$$

The extent to which the dimer is favored over that of the monomer is determined by the magnitude of $K_{\text{dim}}$. This added secondary equilibrium, this time appearing in
the organic phase, is shown in Figure 3.5. The fundamental basis for the partitioning of HOAc between ether and water as introduced by the Nernst law is not violated and still is given by $K_D$. The measurable concentrations $[\text{HOAc}]_{\text{ether}}$ and $[\text{HOAc}]_{\text{aq}}$ will definitely differ with this added dimerization reaction. Let us define $D$ for this distribution equilibrium involving weak acid dissociation of HOAc in the aqueous phase and, at the same time, dimerization of HOAc in the ether phase as follows:

$$D = \frac{[\text{HOAc}]_{\text{ether}} + 2[(\text{HOAc})_2]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}} + [\text{OAc}^-]_{\text{aq}}}$$  \hspace{1cm} (3.14)

The following expressions are applicable to this distribution equilibrium and are defined as follows:

$$K_D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}}}$$

$$K_{\text{dim}} = \frac{[(\text{HOAc})_2]_{\text{ether}}}{[\text{HOAc}]_{\text{ether}}^2}$$

$$K_a = \frac{[\text{H}^+]_{\text{aq}}[\text{OAc}^-]_{\text{aq}}}{[\text{HOAc}]_{\text{aq}}}$$
Substituting the above three definitions into Equation (3.14) and simplifying yields the following relationship:

$$D = \frac{K_D \left[ 1 + 2K_{\text{dim}} [\text{HOAc}]_{\text{ether}} \right]}{1 + K_a / [H^+]_{\text{aq}}}$$

Equation (3.15) shows that the value of the distribution ratio, $D$, depends not only on the equilibrium constants as indicated and the pH, but also on the concentration of HOAc in the ether phase.

It becomes instructive to compare Equations (3.12) and (3.15). The influence of dimerization in the organic phase results in an additional term in the numerator for the distribution ratio, $D$. This additional term depends on the magnitude of $K_{\text{dim}}$ and the concentration of HOAc in this phase. In the case of HOAc, values for $K_{\text{dim}}$ range from a high of 167 for benzene as the solvent to a low of 0.36 for diethyl ether as the solvent. The larger the value for $K_{\text{dim}}$, the larger is the magnitude of $D$ and, as we shall see in the next section, the higher is the percent recovery.

9. IF WE KNOW $D$, CAN WE FIND THE PERCENT RECOVERY?

The discussion so far has focused on first establishing the validity of the partition constant, $K_D$, for LLE and then extending this to the distribution ratio, $D$. We have shown that setting up expressions involving $D$ becomes more useful when secondary equilibria exists. Before we consider other types of secondary equilibria, the importance of knowing how $D$ relates to the percent recovery, $%E$, will be discussed. Percent recovery is an important QC parameter when LLE, SPE, and SFE techniques are used. Most EPA methods discussed in Chapter 1 require that the $%E$ be measured for selected analytes in the same matrix as that for samples. This is particularly important as applied to the EPA methods for trace organics. In Chapter 2, we showed how $%E$ is used in the statistical treatment of experimental data.

The determination of $%E$ is paramount in importance toward establishing an alternative method in TEQA. A method that isolates phenol from wastewater samples using LLE and yields a consistently high $%E$ is preferable to an alternative method that yields a low and inconsistent $%E$. As we showed in Chapter 2, a high $%E$ leads to lower method detection limits (MDLs). However, if the alternative method significantly reduces sample preparation time, then a trade-off must be taken into account: lower MDLs vs. a long sample prep time. A practical question naturally arises here. What does the client want and what degree of trade-off is the client willing to accept?

Let $C_0$ represent the concentration of a particular analyte of interest after being extracted into an organic solvent whose volume is $V_0$ from an aqueous sample whose volume is $V_{\text{aq}}$. Assume also that the concentration of analyte that remains in the aqueous phase after extraction is $C_{\text{aq}}$. Let us define the fraction of analyte extracted, $E$, by
where \( \text{amt}_o \) refers to the amount of analyte extracted into the organic phase and \( \text{amt(total)} \) refers to the total amount of analyte originally present in the aqueous sample. The fraction extracted can be expressed as follows:

\[
E = \frac{\text{amt}_o}{\text{amt(total)}}
\]

Equation (3.16) shows that the fraction extracted and hence the percent recovery depend on two factors. The first is the magnitude of the distribution ratio, which is dependent on the physical/chemical nature of each analyte and the chemical nature of the extractant. The second factor is the phase ratio \( \beta \). The magnitude is usually fixed if the extractant is not changed, whereas the phase ratio can be varied. If, instead of a single-batch LLE, a second and third successive LLE is carried out on the same aqueous solution by removing the extractant and adding fresh solvent, the %\( E \) can be maximized. After allowing time for partition equilibrium to be attained, while keeping the phase ratio constant, it can be shown that a second successive extraction will extract \( E(1 - E) \) while a third successive extraction will extract \( E(1 - E)^2 \). The fraction remaining in the aqueous phase following \( n \) successive LLEs is \( (1 - E)^n \). To achieve at least a 99% recovery, Equation (3.16) suggests that the product \( \beta D \) must be equal to or greater than 100. Even with a product \( \beta D = 10 \), two successive LLEs will remove 99% of the amount of analyte originally in an aqueous environmental sample.\(^{12} \)

10. ARE ORGANICS THE ONLY ANALYTES THAT WILL EXTRACT?

Our examples so far have focused on neutral organic molecules such as acetic acid. The majority of priority pollutant organics of importance to TEQA are neutral molecules in water whose pH values are within the 5 to 8 range. Before we leave the principles that underlie LLE, the answer to the question just posed is yes.
Consider the significant difference in $K_D$ for NaCl vs. HOAc partition constants discussed earlier. Ionic compounds have little to no tendency to partition into a moderate to nonpolar organic solvent. If, however, an ion can be converted to a neutral molecule via chemical change, this ion can exhibit a favorable $K_D$. This is accomplished in two ways: chelation of metal ions and formation of ion pairs. The mathematical development of a metal chelate is discussed in this section.

A number of organic chelating reagents exist that coordinate various metal ions, and the metal chelate that results consists of neutral molecules. This neutral or uncharged metal chelate will have a $K_D$ much greater than 1. Metal ions initially dissolved in an aqueous phase such as a groundwater sample can be effectively removed by metal chelation LLE. Commonly used chelating reagents include four-membered bidentate organic compounds such as dialkyl dithiocarbamates, five-membered bidentates such as 8-hydroxyquinoline and diphenyl thiocarbazone, dithizone, and polydentates such as pyridylazonaphthol. 8-Hydroxyquinoline, commonly called oxine (HOx), is the chelating reagent used in this section to introduce the mathematical relationships for metal chelation LLE. Similar equations can be derived for other chelating reagents.

Figure 3.6 depicts the principal primary and secondary equilibria that would be present if oxine is initially dissolved in an appropriate organic solvent that happens to be less dense than water. If this solution is added to an aqueous solution that contains a metal ion such as copper(II) or Cu$^{2+}$, two immiscible liquid phases persist. The copper(II) oxinate that initially forms in the aqueous phase, oxine, itself is an amphiprotic weak acid and quickly partitions into the organic phase. Being amphiprotic means that oxine itself can accept a proton from an acid and can also donate one to a base. The degree to which oxine either accepts or donates a proton is governed
Sample Preparation Techniques

by the pH of the aqueous solution. The acidic property is the only one considered in the development of the equations considered below. The formation of a Cu oxine chelate can proceed via 1:1 and 1:2 stoichiometry. The fact that it is the 1:2 chelate that is neutral, and therefore the dominant form that partitions into the nonpolar solvent, is important. All of the competing primary and secondary equilibria can be combined to yield a relationship that enables the distribution ratio to be defined in terms of measurable quantities.

The distribution ratio, \( D \), for the immiscible phases and equilibria shown in Figure 3.6 is first defined as the ratio of chelated copper in the organic phase to the concentration of free and chelated copper in the aqueous phase. Expressed mathematically,

\[
D \equiv \frac{[\text{CuOx}_2]_o}{[\text{Cu}^{2+}]_{aq} + [\text{CuOx}_2]_{aq}}
\]

Similar to what was done earlier for HOAc, we can define \( \alpha_{\text{Cu}} \) as the fraction of free \( \text{Cu}^{2+} \) in the aqueous phase: then,

\[
\alpha_{\text{Cu}} = \frac{[\text{Cu}^{2+}]_{aq}}{[\text{Cu}^{2+}]_{aq} + [\text{CuOx}_2]_{aq}}
\]

so that

\[
D = \frac{[\text{CuOx}_2]_o}{[\text{Cu}^{2+}]_{aq}/\alpha_{\text{Cu}}}
\]  

(3.17)

Use of \( \alpha_{\text{Cu}} \) is a simple and convenient way to account for all of the many side reactions involving the metal ion. Substituting the equilibrium expressions into Equation (3.17) yields

\[
D = \frac{K_D^{\text{CuOx}_2} \beta_2 K_{\text{H}}^2 \text{[HOx]}_o^2}{K_D^{\text{HOx}} \text{[H}^+\text{]}_{aq}}
\]  

(3.18)

We have assumed that the protonation of HOx as discussed earlier is negligible. Equation (3.18) states that the distribution ratio for the metal ion chelate LLE depends on the pH of the aqueous phase and on the ligand concentration. \( K_D^{\text{HOx}}, \beta_2, \) and \( \alpha \) are dependent on the particular metal ion. This enables the pH of the aqueous phase to be adjusted such that a selected LLE can occur. One example of this selectivity is the adjustment of the pH to 5 and extraction as their dithizones to selectivity separate \( \text{Cu}^{2+} \) from \( \text{Pb}^{2+} \) and \( \text{Zn}^{2+} \).13

The metal chelate LLE was much more common 25 years ago when it was the principal means to isolate and recover metal ions from aqueous samples of environmental interest. The complexes were quantitated using a visible spectrophotometer.
because most complexes were colored. A large literature exists on this subject. The technological advances in both atomic absorption and inductively coupled plasma-atomic emission spectroscopy have significantly reduced the importance of metal chelate LLE to TEQA. However, metal chelate LLE becomes important in processes whereby selected metal ions can be easily removed from the aqueous phase.

11. CAN ORGANIC CATIONS OR ANIONS BE EXTRACTED?

We have discussed the partitioning of neutral organic molecules from an aqueous phase to a nonpolar organic solvent phase. We have discussed the partitioning of metal ions once they have been converted to neutral metal chelates. In this section, we discuss the partitioning of charged organic cations or charged organic anions. This type of LLE is termed ion pairing. Ion pair LLE is particularly relevant to TEQA, as will be shown below. We start by using equilibrium principles and assume that the only equilibria are the primary ones involving the partitioning of the ion pair between an aqueous phase and a lighter-than-water organic phase. The secondary equilibria consist of formation of the ion pair in the aqueous phase. Also, all cations and anions are assumed not to behave as weak acids or bases. For the formation of the ion pair in the aqueous phase, we have

\[
\text{CA}^+_\text{(aq)} + \text{A}^-_{\text{(aq)}} \rightleftharpoons K_P \text{CA}_{\text{(aq)}}
\]

The ion pair CA, once formed, is then partitioned into an organic solvent that is immiscible with water according to

\[
\text{CA}_{\text{(aq)}} \rightleftharpoons K_D \text{CA}_{\text{(org)}}
\]

The distribution ratio, \(D\), with respect to the anion for IP-LLE, is defined as

\[
D_{A^-} = \frac{[\text{CA}]_{\text{org}}}{[\text{CA}]_{\text{aq}} + [\text{A}^-]_{\text{aq}}}
\]

In a manner similar to that developed earlier, \(D\) can be rewritten as

\[
D_{A^-} = K_D \left( \frac{K_{IP}[C^+]_{\text{aq}}}{1 + K_{IP}[C^+]_{\text{aq}}} \right)
\]

The distribution ratio is seen to depend on the partition coefficient of the ion pair, \(K_D\), to the extent to which the ion pair is formed, \(K_{IP}\), and on the concentration of the cation in the aqueous phase. Equation (3.19) shows some similarity to Equation (3.13).
12. IS THERE AN IMPORTANT APPLICATION OF IP-LLE TO TEQA?

Equation (3.19) suggests that if an ion pair that exhibits a high partition coefficient, $K_D$, forms the ion pair to a great extent (i.e., has a large value for $K_{IP}$), then a large value for $D$ enables an almost complete transfer of a particular anion to the organic phase. Of all the possible ion pair complexes that could form from anions that are present in an environmental sample, the isolation and recovery of anionic surfactants using methylene blue is the most commonly employed IP-LLE technique used in environmental testing labs today. The molecular structure of this ion pair formed a large organic anion that is prevalent in wastewater such as an alkyl benzene sulfonate, a common synthetic detergent, using a large organic cation such as methylene blue, as follows:

![Chemical structures]
This ion pair absorbs visible light strongly at a wavelength of 652 nm. Because a method that might be developed around this ion pair and its high percent recovery into a nonpolar solvent (a commonly used one is chloroform) is nonselective, a cleanup step is usually introduced in addition to the initial LLE step. Of all possible anion surfactants, sodium salts of C_{10} to C_{20} do not form an ion pair with methylene blue, whereas anionic surfactants of the sulfonate and sulfate ester types do. Sulfonate type surfactants contain sulfur covalently bonded to carbon, whereas the sulfate ester type of surfactant contains sulfur covalently bonded to oxygen, which in turn is covalently bonded to sulfur. A good resource on the analysis of surfactants in all of its forms, including some good definitions, was published earlier.\textsuperscript{15} The type of surfactants that form an ion pair and give rise to a high percent recovery are termed methylene blue active substance (MBAS). A microscaled version to the conventional method\textsuperscript{16} for the determination of MBAS in wastewater is introduced as one of the student experiments discussed in Chapter 5.

13. ARE THERE OTHER EXAMPLES OF NONSPECIFIC LLE PERTINENT TO TEQA?

In Chapter 1, the determination of total petroleum hydrocarbons (TPHs) was discussed in relation to EPA method classifications. This method is of widespread interest in environmental monitoring, particularly as this relates to the evaluation of groundwater or wastewater contamination. There are several specific determinations of individual chemical components related to either gasoline, fuel oil, jet fuel, or lubricant oil that involve an initial LLE followed by a GC determinative step. Methods for these require LLE, possible cleanup followed by GC separation, and detection usually via a flame ionization detector (FID). Specific methods are usually required when the type of petroleum hydrocarbon is of interest. There is almost equal interest among environmental contractors for a nonspecific, more universal determination of the petroleum content without regard to chemical specificity. A sample of groundwater is extracted using a nonpolar solvent. The extracted TPHs are then concentrated via evaporation either by use of a rotary evaporator, Kuderna–Danish evaporative concentrator, or via simple distillation to remove the extracting solvent. The residue that remains is usually a liquid, and the weight of this oily residue is obtained gravimetrically. An instrumental technique that represents an alternative to gravimetric analysis involves the use of quantitative infrared (IR) absorption. If the extracting solvent lacks carbon-to-hydrogen covalent bonds in its structure, then the carbon-to-hydrogen stretching vibration could be used to quantitate the presence of TPHs. The most common solvent that emerged was 1,1,2-trichlorotrifluoroethane (TCTFE). With the eventual total phasing out of Freon-based solvents, the EPA has reverted back to the gravimetric determinative approach. It is not possible to measure trace concentrations of TPHs via quantitative IR using a hydrocarbon solvent, due to the strong absorption caused by the presence of carbon-to-hydrogen covalent bonds. The author maintains that labs could recycle and reuse the spent TCTFE without any release of this Freon type solvent to the environment while preserving the quantitative IR determinative method. Only time and politics will determine
which method will dominate in the future. Nevertheless, the technique of LLE to isolate and recover TPHs from water contaminated with oil remains important.

14. CAN LLE BE DOWNSIZED?

We now introduce some recently reported and interesting research that reinforces the basic concepts of LLE. Jeannot and Cantwell\(^\text{17}\) have introduced the concept of a true LLE that has been downsized to a microextraction scale. In the past, the concept of a micro-LLE (μLLE), as introduced by the EPA and promulgated through their 500 series of methods, was designed to conduct TEQA on samples from sources of drinking water. Method 508 required that 35 mL of groundwater or tap water be placed in a 40-mL vial and extracted with exactly 2 mL of hexane. Organochlorine pesticides such as aldrin, alachlor, dieldrin, heptachlor, and so forth, are easily partitioned into the hexane. A 1-μL aliquot is then injected either manually or via autosampler into a GC-ECD to achieve the goal of TEQA. As long as emulsions are not produced, this downsized version of LLE works fine. Wastewater samples are prone to emulsion formation, and this factor limits the scope of samples that can be extracted by this mini-LLE technique.

Cantwell’s group has taken this scale down by a factor of about 20 to the 1-mL and below sample volume levels. Some interesting mathematical relationships that serve to reinforce the principles discussed earlier are introduced here. It does not matter whether an analyst uses a liter of groundwater sample, a milliliter, or even a microliter. The principles remain the same.

The principle of mass balance requires that the amount of a solute that is present in an aqueous sample (e.g., groundwater), \(\text{amt}_{\text{initial}}\), remain mathematically equivalent to the sum of solute in both immiscible phases. Matter cannot escape, theoretically, that is. If the initial amount of a solute is distributed between two immiscible phases, an organic phase, \(o\), and an aqueous phase, \(aq\), mass balance considerations require that

\[
\text{amt}_{\text{initial}} = \text{amt}_{\text{aq}} + \text{amt}_o
\]

We now seek to relate the concentration of solute that remains in the aqueous phase after μLLE to the original concentration of solute, \(C_{\text{initial}}\). For example, a groundwater sample that contains dissolved organochlorine pesticides such as DDT can be mathematically related to the partitioned concentrations in both phases according to

\[
C_{\text{initial}} = \frac{V_{aq}C_{aq} + V_oC_o}{V_{aq}}
\]

We would like to express the concentration of analyte in the organic phase, \(C_o\), in terms of the initial concentration of analyte that would be found in groundwater, \(C_{\text{initial}}\). Dividing through by \(V_{aq}\) and eliminating \(C_{aq}\) for LLE,
Substituting for the phase ratio, $\beta$, dividing the numerator and denominator by $V_{aq}$, and rearranging gives

$$C_{init} = C_o \left[ \frac{1}{K_D} + \frac{\beta}{1} \right]$$

Rearranging and solving for $C_o$ gives

$$C_o = C_{init} \left[ \frac{K_D}{1 + \beta K_D} \right]$$  \hspace{1cm} (3.20)$$

Hence, the concentration of solute present in the organic phase can be directly related to the concentration of solute initially present in the aqueous groundwater sample, $C_{init}$, provided the partition coefficient and phase ratio, $\beta$, are known. The reader should see some similarity between Equations (3.20) and (3.16). Equation (3.20) was derived with the assumption that secondary equilibrium effects were absent. This assumption is valid only for nonionizable organic solutes.

With these mathematical relationships presented, we can now discuss the experimental details. The end of a Teflon® rod was bored to make a cavity. A volume of 8 $\mu$L of a typical organic solvent such as $n$-octane was introduced into the cavity, and a cap and rod were fitted to a 1-mL cylindrical vial with a conical bottom, to which a magnetic stirrer has been placed. After the solvent was placed on top of the aqueous sample, the sample was stirred for a fixed period at a fixed temperature, 25°C. This enables the solute to diffuse into the organic solvent. A 1-$\mu$L aliquot of this extract is taken and injected into a GC for quantitative analysis. This $\mu$LLE yields a $\beta$ of 0.008. As values of $\beta$ get smaller and smaller, the second term in the denominator of Equation (3.20) tends to zero. For a fixed $K_D$ and $C_{init}$, a low value for $\beta$ results in a higher value for $C_o$, and hence a higher sensitivity for this $\mu$LLE technique.

Once a sample preparation method has been established, the analytical methodology, so important to achieving GLP in TEQA, can be sought. The analytical outcomes discussed in Chapter 2 can now be introduced for this $\mu$LLE technique.

An internal standard mode of calibration was used to conduct quantitative analysis using the minivial technique just described. The analyte studied was 4-methyl
acetophenone and the internal standard was \( n \)-dodecane. The slope of the linear calibration was 4.88 L/mmol, with a \( y \) intercept of zero and a coefficient of determination of 0.998.  

15. DOES THE RATE OF MASS TRANSFER BECOME IMPORTANT IN µLLE?

The kinetics of LLE can also be developed. Kinetics become a more important consideration when aqueous and organic phases cannot be afforded maximum contact, as is the case when a large sep funnel is used. It is worthwhile to consider kinetics in the context of the µLLE technique developed by Ma and Cantwell. The general-rate equation for LLE can be written in terms of a differential equation that relates the rate of change of the concentration of analyte in the organic phase, \( C_o \), to a difference in concentration between the aqueous phase, \( C_{aq}(t) \), and the organic phase \( C_o(t) \) according to the following:

\[
\frac{d}{dt} C_o = \frac{A}{V_o} \Gamma_o \left( K_D C_{aq}(t) - C_o(t) \right)
\]

where \( A \) is the interfacial area and \( \Gamma_o \) is the overall mass transfer coefficient with respect to the organic phase (in units of cm/sec). Thus, the time dependence of solute concentration in the organic phase can be seen as

\[
C_o = C_{o,\text{equil}}(1 - e^{-kt})
\]  

(3.21)

\( C_{o,\text{equil}} \) represents the concentration of solute in the organic phase after equilibrium has been reached. \( k \) is the observed rate constant (in units of sec\(^{-1}\)) and is given by

\[
k = \frac{A}{V_o} \Gamma_o [K_D + 1]
\]

Combining Equations (3.20) and (3.21) leads to an expression that is significant to TEQA:

\[
C_{aq,\text{initial}} = C_o(t) \left[ \frac{1 + K_D \beta}{K_D (1 - e^{-kt})} \right]
\]

(3.22)

The term in brackets in Equation (3.22) is usually held constant, and this term is evaluated by extracting a reference aqueous solution where the concentration is known. The concentration must, of course, be in the linear region of the distribution isotherm for both sample and standard.

Cantwell and coworkers have recently extended their µLLE technique to include a back-extraction using a modification of the minivial discussed earlier. An organic
liquid membrane that consists of $n$-octane confined to within a Teflon ring sits on top of 0.5 or 1 mL of an aqueous sample whose pH is approximately 13 and contains an ionizable analyte. If an amine is dissolved in water and the pH adjusted to 13, the amine would remain unprotonated and therefore neutral. A large $K_D$ would be expected, and the amine should partition favorably into the $n$-octane. A 100- or 200-µL acidic aqueous phase with a pH of approximately 2 is placed on top of the liquid membrane. The amine is then protonated and back-extracted into the acidic aqueous phase. A further enhancement utilizes a microliter liquid-handling syringe to suspend a drop of acidic aqueous phase within the $n$-octane phase. The syringe that now contains the back-extracted analyte can be directly inserted into the injection loop of a high-performance liquid chromatograph (HPLC).

16. **IS THERE ANY OTHER WAY TO PERFORM LLE?**

Yes, indeed. There are several alternatives to separatory funnel LLE, mini-LLE, and µLLE (just described). Sep funnels are limited to ~1000 mL or less, while mini-LLEs are limited to the size of ~40 mL (such as a typical screw-top cylindrical vial). For aqueous environmental samples whose volume exceeds 1000 mL, continuous LLE (C-LLE) is often more appropriate and convenient within which to conduct LLE. To illustrate, if a 2-L wastewater effluent sample is to be extracted, C-LLE would be the technique of choice. C-LLE requires a relatively large glass apparatus whereby the receiving pot can vary in size. C-LLE can be performed using a lighter-than-water extractant or a heavier-than-water extractant. Typical lighter-than-water extractants include various lower-molecular-weight alkanes such as $n$-hexane, while typical heavier-than-water extractants include various chlorinated solvents such as methylene chloride (dichloromethane).

The operational procedure for lighter-than-water C-LLE has been described from a manufacturer of C-LLE glassware as follows: The aqueous phase to be extracted and a stirring bar are placed in a 24/40 round-bottom flask. The flask size (up to and including the 5 L) is chosen so that it is not more than 2/3–4/5 full of aqueous phase. The flask is then filled with the lighter-than-water extracting solvent and gentle stirring is started. The extractor and an efficient condenser are put into place and a small flask containing an additional portion of the lighter-than-water extracting solvent is connected to the side-arm and the solvent in the small flask heated above its boiling point. The solvent vapors distill up the side-arm and condense at the condenser. The condensed solvent runs down the center tube where it is passed with stirring, through the aqueous phase. The extracting solvent removes a small amount of material and separates from the water. Since the density of the extracting solvent is less than that of water, the solvent rises past the joint at the top of the flask containing the aqueous phase and, when it reaches the side-arm, it flows back to the distilling flask though the side-arm. The extracted material then remains in the distilling flask while the solvent is distilled, condensed and is used to extract again. “Fresh” solvent is thus used over and over. In this way, by allowing the extractor to operate for long periods, materials only slightly soluble in the organic solvent can be removed from the aqueous phase in very high yields and only a relatively small amount of extracting solvent need be used.
Heavier-than-water C-LLE designs are operated similarly:

Some heavier-than-water extracting solvent and a stirring bar are placed in the flask that contains the aqueous phase to be extracted. A good rule-of-thumb is that the flask should be about 1/5–1/6 full of heavier-than-water extracting solvent. The extractor is put in place and, with the aid of a funnel whose stem extends below the side-arm of the extractor, the aqueous phase to be extracted is added. The aqueous phase will fill the flask and may move up the vigeux column past the lower return tube. A small flask containing the heavier-than-water extracting solvent is then connected to the side-arm of the apparatus and the solvent therein heated above its boiling point.

In TEQA, the sample matrix determines whether LLE involving immiscible solvents is to be used. If the sample is a solid, such as a contaminated soil or sediment, C-LLE gives way to the Soxhlet extraction apparatus. There have also been attempts to modify the conventional Soxhlet via miniaturization or instrumentation that pressurizes and heats the extracting solvent. A recent technique promulgated by the EPA is called pressurized fluid extraction. EPA Method 3545 from Update III of SW-846 has been developed to enable priority pollutant semivolatile organics to be isolated and recovered from soils, clays, sediments, sludges, and other solid waste. The Dionex Corporation has developed what they call accelerated solvent extraction, whereby a much smaller volume of extraction solvent is used. The vial containing the sample and extracting solvent is both heated and pressurized. These extreme temperature and pressure conditions supposedly accelerate the rate at which equilibrium is reached in LLE. The conventional technique for isolating and recovering semivolatile organics from solid matrices is called Soxhlet extraction. Soxhlet extraction as an analytical sample preparation technique has been around for over 100 years. The principle of Soxhlet extraction, abbreviated S-LSE (because it is a solid–liquid extraction technique), will be discussed in the following section.

17. WHAT IS SOXHLET EXTRACTION ANYWAY?

A solid matrix of environmental interest, such as soil that is suspected of containing any of the more than 100 priority pollutant semivolatiles, is placed into a cellulosic thimble. Vapors from heating a volatile organic solvent rise and condense above the thimble. This creates a steady-state condition called reflux. The refluxed solvent condenses into the thimble and fills until it overflows back into the distilling pot. Reflux is a common technique in organic chemistry and serves to bring the S-LSE process to a fixed temperature. Thus, solutes of interest are able to partition between a fixed weight of contaminated soil and the total extractant volume. Usually, a series of six vessels with six separate heaters are available as a single unit whereby the incoming and outgoing water lines for the reflux condensers are connected in series. A large phase ratio is obtained. S-LSE is usually conducted for one sample over a period of 12 to 24 h. An overnight continuous S-LSE is quite common. After the extraction time has ended, the glass S-LSE vessel is cooled to room temperature. The extractant in the thimble is combined with the refluxed extractant in the distilling
pot. The pot is removed, and due to the large volume of solvent required, the analyst may have over 300 mL of extractant. Common solvents used in S-LSE are, in general, those solvents that are moderate to nonpolar and possess relatively low boiling points. Methylene chloride and petroleum ether are the two most commonly used to conduct S-LSE. Due to the low boiling points, both solvents can be easily removed in the next step after S-LSE.

The extractant from S-LSE must be concentrated. If a low boiling solvent such as methylene chloride is used to conduct S-LSE, it is straightforward to remove this solvent by use of either a Kuderna–Danish (K-D) evaporative concentrator or a rotary evaporator. More contemporary K-D designs provide a means to recover the spent solvent. Earlier designs did not include a means to recover the solvent, and because of this, most solvents, such as methylene chloride, were evaporated to the atmosphere usually via a fume hood. A few boiling chips are usually added to the receiving tube so as to prevent “bumping” during the vigorous boiling step. Suspending the K-D vessel above a large boiling water bath where steam can be in contact with the glass surface serves to rapidly remove solvent. Solvent is removed until a volume between 1 and 5 mL is reached. An extractant is obtained whose concentration of the analytes of interest has been greatly increased. The concentrated extract is further cleaned up, depending on what matrix interferences might be present.

Numerous priority pollutant semivolatiles such as PAHs, various substituted phenols, substituted monoaromatics, and other hazardous chemicals are often extracted along with higher-molecular-weight aliphatic hydrocarbons. If these hydrocarbons are not removed prior to the determinative step (e.g., separation and quantitation by GC-MS), the MDL can be significantly increased. A peak in the GC for benzo(a)pyrene might be obscured because the peak might sit on top of an envelope of hydrocarbon.

Most research papers published during the past 10 to 15 years that seek to show the value of alternative approaches to S-LSE start by condemning S-LSE as too labor intensive and consuming too large a volume of extraction solvent. Outside of needing to use approximately 300 mL of volatile solvent, as mentioned earlier, this researcher does not share the view that merely filling six thimbles with sample, reassembling the Soxhlet glassware, and turning on the heaters and coolant supply lines is really that time consuming. After all, once all of this is accomplished, the analyst is free to leave the laboratory and pursue other activities. All the while the sample is continuously refluxed with extractant. Nevertheless, a plethora of research has been done to supplant the classical low-cost Soxhlet, as is discussed next. Before we do that, however, let us cover automated Soxhlet extraction.

EPA Method 3540 uses conventional Soxhlet extraction, S-LSE, to isolate and recover various semivolatile and nonvolatile priority pollutant organic compounds from soil, sediment, sludges, and waste solids. EPA Method 3541 utilizes a unique, three-stage automated Soxhlet extraction, AS-LSE. Arment21 has reviewed those aspects of AS-LSE relevant to TEQA. The fundamental difference between S-LSE and AS-LSE is an improvement in design of the classical Soxhlet glass apparatus. Randall22 developed a Soxhlet apparatus in which the thimble containing the solid sample is immersed into the pot via a sliding rod that extends through the reflux
condenser. The sample can be rinsed of extractant by raising the thimble out of the pot and continuing to reflux as shown in the sketch from EPA Method 3541(23) below:

Several SVOCs of environmental interest are given below, along with mean percent recoveries from spiked clay, using both S-LSE and AS-LSE techniques.\(^{21}\)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean % Recovery (% RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-LSE(^{a})</td>
</tr>
<tr>
<td>δ-BHC (lindane)</td>
<td>65.6 (27.1)</td>
</tr>
<tr>
<td>Endrin</td>
<td>81.0 (3.9)</td>
</tr>
<tr>
<td>(p,p')-DDT(^{c})</td>
<td>73.6 (38.5)</td>
</tr>
</tbody>
</table>

\(^{a}\) Extracted with 1:1 (v/v) hexane–acetone for 16 h; three replicate determinations.

\(^{b}\) Extracted with 1:1 (v/v) hexane–acetone for 60 min of boiling and 60 min of rinsing; four replicate determinations. AS-LSE consists of three distinct steps:

1. **Boiling** — The thimble is immersed in the extracting solvent and refluxed for 60 min.
2. **Rinsing** — The thimble is raised out of the boiling solvent and suspended above it for another 60 min.
3. **Evaporation-preconcentration** — Condensed solvent is redirected away from the sample, and boiling solvent is collected in a condenser or reservoir. This reclaimed solvent could be reused. This step requires 10 to 20 min.
After the evaporation-preconcentration step, ~2 to 20 mL of preconcentrated extract remains in the cup. This extract can be further concentrated via nitrogen blowdown or mini-Kuderna–Danish evaporative concentration. The concentrated extract can be further cleaned up or solvent exchanged as dictated by the particular method or application.

AS-LSE seems ideally suited for the determination of total petroleum hydrocarbons (TPHs), in addition to targeted priority pollutants. To gravimetrically determine TPH content of a contaminated solid waste, the solvent is allowed to evaporate to dryness and the oily residue is weighed.

Several companies manufacture instrumentation to facilitate AS-LSE. These include Buchi, Foss-Tecator, and Gerhardt for the Randall type, while Labconco employs the Goldfisch type.21

The consumption of significantly less solvent and the smaller overall footprint and large sample throughout represent advantages for a laboratory to acquire an AS-LSE capability. Let us now consider alternative sample prep techniques to Soxhlet, classical or otherwise.

18. ARE THERE ALTERNATIVES TO S-LSE?

Yes, and the development of alternatives to conventional S-LSE has occurred during the past 10 years with an emphasis on reducing the extraction solvent volume while maintaining the high efficiency of S-LSE. These newer sample preparation methodologies applied to solid matrices exclusively are variations of S-LSE and include ultrasonic probe liquid–solid extraction (U-LSE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE). These techniques assume that the analytical objective is to isolate and recover semivolatile to nonvolatile organic compounds of interest to TEQA from soils, sediments, sludges, and so forth. Beyond these variations to the classical Soxhlet, much progress has been made in performing extractions of solid matrices using supercritical fluids, a technique commonly called supercritical fluid extraction (SFE).

It would be remiss for this author not to include SFE even though SFE is not the forte of this researcher. SFE can be conducted offline, whereby the solid sample is extracted and the extract is then transported to a solvent or adsorbent. In the case of an extract, the analytes of interest being dissolved in the solvent can be directly injected into a GC or, with a change of matrix, injected into an HPLC to complete the analytical steps that lead to TEQA. In the case of a sorbent that contains adsorbed analytes, the sorbent can be eluted with a solvent in much the same way that solid-phase extraction is conducted (refer to the extensive discussion of this technique in subsequent sections of this chapter). This eluent can then be directly injected into a GC or, with a change of matrix, directly injected into an HPLC in an effort to carry out the determinative step in TEQA. SFE can be interfaced to a chromatograph that uses supercritical fluids as chromatographic mobile phases. The technique is called online supercritical fluid extraction–supercritical fluid chromatography (SFE-SFC). This technique requires the availability of instrumentation to enable the
extracted analytes to be directly injected into the chromatograph, where separation of such analytes takes place.

Supercritical fluid extraction as an alternative sample preparation method applied to solid sample matrices of interest to TEQA became quite popular during the late 1980s and early 1990s. However, SFE requires that instrumentation be purchased. It has also been found that significant matrix dependence exists, and this matrix dependence contributes to differences in percent recoveries. The first generation of SFE instruments also suffered from problems with plugging of the low restrictors that are located after the extraction vessels. SFE will be discussed in more detail later in the chapter. We next discuss the three variations of S-LSE introduced earlier (i.e., U-LSE, MAE, and ASE).

19. WHAT IS ULTRASONIC LIQUID–SOLID EXTRACTION?

Ultrasonic LSE is most applicable to the isolation of semivolatile and nonvolatile organic compounds from solid matrices, such as soil, sediment, clays, sand, coal tar, and other related solid wastes. U-LSE is also very useful for the disruption of biological material such as serum or tissue. U-LSE can be coupled with solid-phase extraction (SPE) to give a very robust sample preparation method at relatively low cost in comparison to MAE and ASE approaches. The author has utilized U-LSE/SPE to isolate and recover 9,10-dimethyl-1,2-benzanthracene from animal bedding. A 89% recovery was obtained for bedding that was spiked with this polycyclic aromatic hydrocarbon (PAH) of interest to toxicologists. An ultrasonic horn and tip are immersed into a mixture of liquid extractant and solid sample and sonicated at some percent of full power for a finite length of time, either continuously or pulsed.

Ultrasonication involves the conversion of a conventional 50/60-Hz alternating-current line power to 20 kHz electrical energy and transformation to mechanical vibration. A lead zirconate titanate electrostrictive (piezoelectric) crystal, when subjected to alternating voltage, expands and contracts. This transducer vibrates longitudinally and transmits this motion to the horn tip. The horn tip is immersed in the liquid slurry and cavitation results. Cavitation is the formation of microscopic vapor bubbles that form and implode, causing powerful shock waves to radiate throughout the sample from the face of the tip. Horns and microtips amplify the longitudinal vibration of the converter and lead to more intense cavitation action and greater disruption. U-LSE dissipates heat, and because of this, a sample should be placed in an ice-water bath. Proper care of the probe is essential. The intensity of cavitation will, after a prolonged period, cause the tip to erode and the power output to decrease without showing up on the power monitor.

Ultrasonic cell disruptors are manufactured by a half dozen or so companies. In the author’s lab, the Model 450 Digital Sonifier (Branson Ultrasonics Corporation) has been in use. It becomes important to retune the generator when a new probe is changed. There are also additional tuning procedures to follow for microtips.

EPA Method 3550B (Revision 2, SW-846, December 1996) is a procedure for extracting semivolatile and nonvolatile organic compounds from solids such as soils,
sludges, and wastes using U-LSE. The method includes two approaches, depending on the expected concentration of contaminants in the sample. In the low-concentration method (<20 mg of each component/kg), a 30-g sample is mixed with anhydrous sodium sulfate to form a free-flowing powder and extracted three times with either 1:1 acetone/methylene chloride or 1:1 acetone/hexane. The use of a mixed solvent serves to adjust the extractant polarity, thereby enabling efficient extraction of some of the more polar analytes as well. In the high-concentration method (>20 mg of each component/kg), a 2-g sample is mixed with anhydrous sodium sulfate, again, to form a free-flowing powder. The extracts are concentrated by evaporating off the solvent using Kuderna–Danish evaporative concentrators. A solvent recovery system is recommended whereby the vaporized solvent can be recycled to prevent escape to the atmosphere. Surrogates and matrix spikes are added to the free-flowing powder once sodium sulfate has been added. Solvent evaporation can be combined with a solvent exchange, depending on which determinative technique is to be used. All solvents used should be pesticide residue grade or equivalent. If further concentration of the extract is needed, either a micro-Snyder column technique or a nitrogen blowdown technique is used.

Percent recoveries for 21 representative semivolatile priority pollutant organic compounds are listed in the method taken from the categories of base (B), neutral (N), and acid (A), the so-called BNAs from the over 100 compounds that are routinely monitored for in EPA contract type work.

Ultrasonic probe sonication utilizing a microtip effectively disrupts cell structures and liberates persistent organic pollutants from ∼1 mL of rat plasma or ∼0.5 g of rat liver homogenate when used with a water-miscible organic solvent such as acetonitrile in a test tube. This author has demonstrated that specimens such as these that are known to contain PCBs such as those found in the commercial product, AR 1248, and can be isolated and recovered in high yield when combined with reversed-phase solid-phase extraction (RP-SPE). Scheme 3.1 (the first of numerous schemes devised by the author to illustrate the steps and logic of sample prep) is a flowchart that utilizes U-LSE, coupled with RP-SPE, for up-front analyte extraction and cleanup. The procedural details outlined in Scheme 3.1 will be discussed in a subsequent section on RP-SPE techniques. A second alternative to S-LSE is microwave-accelerated extraction (MAE).

The realization that the physico-chemical conditions of the extract could be altered led to the development of microwave-accelerated extraction (MAE) and accelerated solvent extraction (ASE), or pressurized fluid extraction, whereby the same solvents used to conduct S-LSE are used. The elevation of the extract temperature and pressure serves to accelerate the mass transfer of analyte from solid matrix to extract, and hence reduce the time it takes to achieve a high percent recovery. The chemical nature of the extractant can also be changed, such as using carbon dioxide as a supercritical fluid. This is accomplished by elevating its temperature to slightly above its critical temperature point while increasing its pressure to slightly above its critical pressure. This sample prep technique, called supercritical fluid extraction (SFE), is another alternative to S-LSE, as applied to samples that are solids. We are going to digress a bit into phase diagrams before we discuss the underlying principles of MAE, ASE, and SFE.
20. CAN PHASE DIAGRAMS HELP TO EXPLAIN THESE ALTERNATIVE SAMPLE PREP TECHNIQUES?

The answer is yes, and we will digress a bit at this point to introduce these concepts, as we did earlier in the chapter. The temperature and pressure conditions that govern physico-chemical behavior of liquids are defined in terms of thermodynamics. The Gibbs phase rule is a direct outcome of the physical chemistry of changes in the state of matter. The phase rule helps to interpret the physico-chemical behavior of solids, liquids, and gases within the framework of the kinetic-molecular theory of phase equilibria.

If there are $c$ distinct chemical species or components, the composition of any one phase is specified by $c - 1$ mole fractions. The composition of the remaining
component is fixed. For \( p \) phases, the number of composition variables that must be independently assigned is

\[
\text{No. of composition variables} = p(c - 1)
\]

In addition to the required composition variables, the two remaining parameters that change the thermodynamic state if varied are temperature and pressure. Hence,

\[
\text{Total no. of variables} = p(c - 1) + 2
\]

Not all of these variables are necessary to define a system; not all of these variables are independent. The fact that the chemical potential for a given component must be the same in every coexisting phase places restrictions on the number of independent variables necessary; hence, for a given component present in three phases (1, 2, and 3), stated mathematically,

\[
\left( \frac{\partial G}{\partial n_1} \right)_{T,P} = \left( \frac{\partial G}{\partial n_2} \right)_{T,P} = \left( \frac{\partial G}{\partial n_3} \right)_{T,P}
\]

Two equations are needed to satisfy the above condition for three phases, or \( p - 1 \) equations or restrictions per component. For \( c \) components, we have

\[
\text{No. of restrictions} = c(p - 1)
\]

The number of parameters that can be independently varied, \( f \), is found from the difference between the total number of variables and the total number of restrictions. Stated mathematically,

\[
\text{Total no. of independent variables} = p(c - 1) + 2 - [c(p - 1)]
\]

The smallest number of independent variables that must be specified in order to describe completely the state of a system is known as the number of degrees of freedom, \( f \). For a fixed mass of a gas, \( f = 2 \), because one can vary any two variables (e.g., pressure and temperature); the third variable is fixed by the equation of state (e.g., volume). Hence, only two properties of a fixed mass of gas are independently variable. Stated mathematically, for a system at equilibrium, the number of degrees of freedom, \( f \), equals the difference between the number of chemical components, \( c \), and the number of phases, \( p \):

\[
f = c - p + 2
\]

This is, in essence, the celebrated Gibbs phase rule. A generalized version of a phase diagram for a one-component system, \( c = 1 \), whereby the pressure exerted by the substance is plotted against the temperature of the substance, is shown in Figure 3.7. This means that different regions of the phase diagram yield different values for \( f \). Regions shown in Figure 3.7 that correspond to a single phase and not a phase transition have \( f = 2 \). This means that both \( P \) and \( T \) can be varied independently. On one of the phase change lines, \( f = 1 \), and this means that if \( T \) were changed, \( P \) could not be changed independently of \( T \) if the two phases are to remain in equilibrium; rather, \( P \) must change in such a way as to keep the point on the line. At the triple point, \( f = 0 \), and there is a unique value for \( P \) and \( T \). At this triple point, \( P \) and \( T \)
can have only one value, and only at this point are solid, liquid, and gas allowed to exist in equilibrium. The phase diagram for most chemically pure substances exhibits a slight tilt to the right for their solid-to-liquid change of phase, the so-called fusion line. One notable exception is that of water, whose fusion line tilts slightly to the left. This enables a skater to apply a large enough pressure onto the surface of ice to enable melting to occur, and thus provide sufficient lubrication. Note the horizontal line that crosses the liquid–gas transition. This line indicates that the temperature of the extractant is being increased. To prevent vaporization from occurring, the vertical line shows that the pressure must be increased to again cross over the liquid–gas transition and keep the extractant as a liquid. This is exactly how MAE and ASE operate, and the phase diagram in Figure 3.7 is so noted. The region beyond the critical temperature and pressure, labeled as the supercritical fluid region, is where SFE is conducted. Phase diagrams for carbon dioxide, the most common substance for performing SFE, are well established. ASE involves conductive heating of extractant, while MAE requires microwave heating. MAE is introduced first, followed by ASE.

21. WHAT IS MICROWAVE-ACCELERATED EXTRACTION?

LeBlanc\textsuperscript{28} has summarized both the key historical developments and the technical details of microwave-accelerated extraction (MAE) as applied to the extraction of
solid matrices to isolate and recover priority pollutant organic compounds. Microwave heating is widely accepted as a replacement for hot-plate acid digestion of soil samples to determine trace metals. This use of microwave heating for inorganics sample prep will be discussed later in this chapter. Microwave heating of a sealed vessel that contains a solid matrix or slurry with an organic solvent is yet another and more contemporary alternative to S-LSE. Closed vessels used for MAE are designed to withstand temperatures as high as 200°C and pressures of 200 psi (14 bars). As Figure 3.7 indicates, an increase in the solvent temperature as well as its pressure in a closed vessel keeps the extractant in the liquid phase while facilitating an increase in mass transfer of analyte from the matrix to the solvent in comparison with S-LSE. Microwave heating conjures up what many of us do every day in our kitchen (i.e., apply microwave energy to warm food). There is great danger in merely placing a soil sample to which an organic solvent has been added and heating in an open vessel such as a beaker. However, nonpolar solvents such as hexane do not heat when exposed to microwave radiation. In this case, an inert fluoro-polymer insert filled with carbon black, a strong microwave absorber, is placed into the solvent–sample mixture. Safety considerations demand a closed vessel and oven technology that is designed to prevent explosions and escape of toxic fumes. The schematic below outlines the essential features of a microwave oven designed for MAE. The sealed vessel is placed in the cavity. The magnetron generates microwave radiation that is propagated down the waveguide into the cavity. The mode stirrer distributes the energy in various directions, and the cavity serves to contain the energy until it is absorbed by the sample. The isolator protects the magnetron from radiation that would reflect back into the magnetron. The isolator acts as a one-way mirror. Microwave radiation generated by the magnetron goes to the cavity and is prevented from returning. A turntable is used to rotate the sample vessels within the cavity to evenly distribute the energy.29
The schematic above also shows important engineered safety features, such as an airflow switch, solvent vapor detector, and vent. A safe microwave oven for MAE should be designed to:

1. Eliminate possible ignition sources
2. Detect solvent leaks
3. Remove solvent vapors

MAE joins ASE, SFE, and SPE in what Lesnik at the EPA calls the collection of green sample prep techniques.

22. HAVE THERE BEEN STUDIES APPLYING MAE TO ENVIRONMENTAL SAMPLES?

Onuska and Terry\textsuperscript{30} are credited with publishing the first data on using MAE to extract pollutants from environmental samples. Lopez-Avila and coworkers\textsuperscript{31} reported on their findings as part of an ongoing EPA evaluation of MAE. The nature and types of organic compounds that were spiked into soil include:

- Semivolatile (SVOCs), such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated phenols, and phthalate esters
- Organochlorine pesticides (OCs)
- Polychlorinated biphenyls (PCBs)
- Organophosphorous pesticides (OPs)

Some of the soil was also aged before it was analyzed. Seventy-seven of the 95 SVOCs that were spiked into topsoil were isolated and recovered within the range of 80 to 120%. The recoveries of 14 compounds were below 80%. Upon spiking the topsoil and allowing 24-h aging at 4°C, and adding water to the spiked soil to ensure good mixing of the target compounds with the matrix, only 47 compounds had recoveries between 80 and 120%.

EPA Method 3546 utilizes closed-vessel MAE to isolate and recover SVOCs, OPs, OCs, chlorinated herbicides, phenoxy acid, substituted phenols, PCBs, and PCDDs/PCDFs.\textsuperscript{32} The extractant is a 1:1 mixture of hexane and acetone, and typical sample matrices include soil, glass fibers, and sand.

LeBlanc\textsuperscript{28} compared MAE against Soxhlet. Given below are several analyte/matrix environmental sample types along with comparative results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$V_{ext}(mL)/t_{ext}(min)$</th>
<th>Concentration</th>
<th>$V_{ext}(mL)/t_{ext}(min)$</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPHs/soil</td>
<td>30/7</td>
<td>943 mg/kg</td>
<td>300/60</td>
<td>773 mg/kg</td>
</tr>
<tr>
<td>OCs/soil</td>
<td>50/7</td>
<td>92.3% recovered</td>
<td>300/1080</td>
<td>83.4% recovered</td>
</tr>
<tr>
<td>PCBs/soil</td>
<td>25/7</td>
<td>47.7 µg/g</td>
<td>250/1080</td>
<td>44.0 µg/g</td>
</tr>
<tr>
<td>CH$_3$Hg/sediment</td>
<td>10/6</td>
<td>80 µg/g</td>
<td>200/150</td>
<td>81 µg/g</td>
</tr>
<tr>
<td>PCDDs/sediments</td>
<td>30/8</td>
<td>565 µg/g</td>
<td>300/1440</td>
<td>542 µg/g</td>
</tr>
<tr>
<td>Organotin/biomaterial</td>
<td>20/5</td>
<td>1.28 µg/g</td>
<td>—</td>
<td>1.3 µg/g</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
The extractant from MAE remains in contact with the sample after the heating period is completed. This requires a filtration step. Instrumentation for MAE is quite expensive. Accelerated solvent extraction (ASE) is yet another alternative to S-LSE.

23. HOW DOES ASE WORK?

A solid sample such as soil, whose priority pollutant content is of interest, is placed in a vessel that is capable of withstanding high pressures and temperatures. The vessel is filled with the extractant and extracted under elevated temperature (50 to 200°C) and pressure (500 to 3000 psi) for relatively short periods (5 to 10 min). The technique was first published by Richter et al., who also discusses the physico-chemical considerations that went into developing ASE. The solubility of organic compounds in organic solvents increases with an increase in solvent temperature. Faster rates of diffusion also occur with an increase in solvent temperature. If fresh extracting solvent is introduced, the concentration gradient is increased, and this serves to increase the mass transfer rate or flux according to Fick’s first law of diffusion. Thermal energy can overcome cohesive (solute–solute) and adhesive (solute–matrix) interactions by decreasing the energy of activation required for the desorption process. Higher extractant temperatures also increase the viscosity of liquid solvents, and thus allow better penetration of matrix particles and enhancing extraction. Increased pressure enables liquids to exist at the elevated temperatures. Increased pressure forces the solvent into areas of the matrix that would not normally be contacted by solvents using atmospheric conditions.

Instrumentation to set up and perform ASE on solid matrices of interest to TEQA has been commercially available for several years from Dionex Corporation. The instrumentation to perform ASE is quite expensive, and for this reason, labs should have a large sample workload so that return on investment can be realized in as short a time frame as possible.

The ASE instrument works as follows. Solvent is pumped into the extraction cell after the cell is filled with the solid sample. The cell is heated and pressurized to enable the extractant to remain as a liquid well above its boiling point. A finite amount of time is taken for LSE to occur. While hot, the cell is flushed one or more times with solvent and the extractant is transferred to a collection vial. The remaining solvent is purged out of the cell with compressed nitrogen. These steps have been recently introduced and are outlined in the flowchart in Scheme 3.2.

24. WHAT SAMPLES HAS ASE BEEN APPLIED TO?

Two studies from the original paper by Richter et al. will now be discussed. The first study involved isolating and recovering total petroleum hydrocarbons (TPHs) from soil using ASE. Four different extraction temperatures were used. TPHs were determined via infrared absorption. The quantitative analysis of oil-laden soils has been introduced in Chapter 1, and the determinative technique for this will be discussed in Chapter 4. The results are shown in Table 3.1 and demonstrate that extractant temperature can significantly influence TPH recoveries. The precision over five replicate ASEs is seen to decrease as the extractant temperature is increased.
The second study involved isolating and recovering the selected polycyclic aromatic hydrocarbons (PAHs) from a certified reference sample of urban dust.

Table 3.2 shows the results and lists the ASE conditions for this study. Nearly 100% recoveries are reported together with good precision (\%RSDs < 7\% for six replicate ASEs) for these 10 priority pollutant PAHs.

Two validation studies conducted in 1994 compared ASE with S-LSE, automated Soxhlet, and wrist-shake or manual LLE for the determination of target analytes.

© 2006 by Taylor & Francis Group, LLC
**TABLE 3.1**  
Effect of Temperature on the Recovery of TPHs from Soil Using ASE

<table>
<thead>
<tr>
<th>Extractant Temperature (°C)</th>
<th>Amount Found (mg) (n = 5)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>974</td>
<td>6.0</td>
</tr>
<tr>
<td>50</td>
<td>1118</td>
<td>5.0</td>
</tr>
<tr>
<td>75</td>
<td>1190</td>
<td>2.0</td>
</tr>
<tr>
<td>100</td>
<td>1232</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Note:* ASE conditions:
- Preheat method
- Perchloroethylene as extractant
- 2500 psi, 5 min in static extraction after equilibration
- 3 g of soil
- 3.5 mL of cell volume
- 4.5 to 5.0 mL of solvent used
- 1200 mg of TPH/kg of soil

---

**TABLE 3.2**  
Recovery of PAHs from Urban Dust (SRM 1649)

<table>
<thead>
<tr>
<th>PAH</th>
<th>Mean % Recovery (n = 6)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenanthrene</td>
<td>113</td>
<td>2.0</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>88.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Pyrene</td>
<td>91.0</td>
<td>5.9</td>
</tr>
<tr>
<td>Ben(a)anthracene</td>
<td>97.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Chrysene</td>
<td>101</td>
<td>4.2</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>115</td>
<td>6.2</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>112</td>
<td>6.7</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>125</td>
<td>6.2</td>
</tr>
<tr>
<td>Benzo(g,h,i)perylene</td>
<td>108</td>
<td>5.8</td>
</tr>
<tr>
<td>Indeno(1,2,3-c,d)pyrene</td>
<td>108</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*Note:* ASE conditions:
- Prefill method
- 100°C
- Methylene chloride/acetone 1:1 (v/v)
- 5 min of equilibration, 5 min of static
- 18-mL final volume
- 2000 psi
ASE proved equivalent to the conventional procedures. The data generated in these two studies resulted in the generation of EPA Method 3545, which received final promulgation in June 1997 as part of SW-846 Method Update III.35

Dionex Corporation published a series of application notes that demonstrate the usefulness of ASE to effectively isolate and recover various persistent organic pollutants (POPs) from a fish tissue homogenate.36 Fish tissue is a biological matrix that is of increasing interest as an indicator of the degree of surface water contamination. Biomagnification of various semivolatile POPs in fish, combined with fish consumption by humans, lends an enviro-health importance to quantitatively determining the concentration of various POPs in fish. Consider using a Model 300 ASE (the model that allows for the largest sample size) whereby 30 g of fish tissue homogenate is weighed and spiked with 50 µL of a series of PCB congeners whose concentration in hexane is in the range of 50 to 250 ppm. After spiking, a concentration of PCB in the sample is in the 80 to 400 ng/g range. Twenty grams of Hydromatrix® (diatomaceous earth from Varian Sample Prep Products) is added and mixed in a mortar using a pestle. The solid mix is loaded into a 100-mL stainless-steel high-pressure extraction cell containing 10 g of alumina and a cellulose filter. ASE conditions as stated in the application note are listed as follows:

<table>
<thead>
<tr>
<th>Compound Class</th>
<th>Matrix</th>
<th>Comparison Technique</th>
<th>Relative Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organochlorine pesticides</td>
<td>Clay, loam, sand</td>
<td>Automated Soxhlet (Method 3541)</td>
<td>97.3</td>
</tr>
<tr>
<td>Semivolatile compounds</td>
<td>Clay, loam, sand</td>
<td>Automated Soxhlet (Method 3541)</td>
<td>99.2</td>
</tr>
<tr>
<td>Organophosphorous pesticides</td>
<td>Clay, loam, sand</td>
<td>Soxhlet (Method 3540)</td>
<td>98.6</td>
</tr>
<tr>
<td>Chlorinated herbicides PCBs</td>
<td>Clay, loam, sand</td>
<td>Manual LLE (Method 8150)</td>
<td>113</td>
</tr>
<tr>
<td>PAHs</td>
<td>Sewage sludge, river sediment, oyster tissue, soil</td>
<td>Various certified reference materials</td>
<td>98.2</td>
</tr>
<tr>
<td>PAHs</td>
<td>Urban dust, marine sediment, soil</td>
<td>Various certified reference materials</td>
<td>105</td>
</tr>
</tbody>
</table>

from solid wastes of interest to TEQA. These results are summarized in Table 3.3. **TABLE 3.3**

Summary of Laboratory Studies Used for Validation of ASE for EPA Method 3545

Extracting solvent Methylene chloride
Extraction temperature 125°C
Extraction pressure 1500 psi (10 MPa)
Heat-up time 5 min
Extracts were dried using anhydrous sodium sulfate, then concentrated to 10 mL under nitrogen blowdown. The concentrated extract was analyzed using gas chromatography with electron-capture detection. According to Dionex, a mean %RSD of 6.1 ($n = 5$) was obtained. Alumina removes coextracted lipid from the extract as it passes from the cell. Alumina will retain ~75 mg of lipid per gram of sample under the ASE conditions described above. For fish tissue with appreciable lipid content, e.g., >5%, additional cleanup of the extract is recommended. Extract cleanup techniques are introduced later in the chapter. Models 200 and 300 are designed for high sample throughput and, as a consequence, are quite expensive. To address those labs that have low sample throughput, the ASE 100 has been developed.37

Fish tissue represents a sample matrix that is relatively high in lipid or fat content. LLE or any of the LSE techniques described earlier in this chapter when applied to fish tissue yield extracts rich in dissolved lipid. The ASE technique permits an adsorbent to be added to the extraction cell that removes significant amounts of lipid. Adsorbents that can be added are called fat retainers. A study, independent of Dionex Corporation, compared the degree to which the most common polar adsorbents, used in conventional sample cleanup, effectively removed lipid from ASE extracts.38

After proving that ASE could effectively extract PCB congeners from a certified reference material, in this case, spiked cod liver oil, various ratios of fat-to-fat retainer were prepared and used to conduct ASE. Plots of the percent retained fat vs. the fat-to-fat retainer ratio, using lard as the source of lipid, showed that the amount of coextracted fat could be reduced to only ~5% using a fat/fat retainer ratio of 0.05. The authors postulate that as much as 40× the amount of retainer is needed to achieve complete removal of lipid. Percent recoveries for three PCB congeners from a naturally contaminated fish meal sample using five different adsorbents are shown below. Two grams of fish meal were weighed using 10 g of fat retainer for each adsorbent below, except where 1 g of fish meal was weighed using 5 g of Florisil® (this adsorbent has a lower density than the others):

<table>
<thead>
<tr>
<th>PCB</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>22′455′PCBP</td>
<td>117</td>
<td>110</td>
<td>112</td>
<td>116</td>
<td>116</td>
</tr>
<tr>
<td>22′44′55′HCBP</td>
<td>107</td>
<td>109</td>
<td>112</td>
<td>110</td>
<td>117</td>
</tr>
<tr>
<td>22′344′55′HCBP</td>
<td>90</td>
<td>95</td>
<td>91</td>
<td>97</td>
<td>96</td>
</tr>
</tbody>
</table>

Note: A = sulfuric acid-impregnated silica gel; B = Florisil; C = basic alumina; D = neutral alumina; E = acidic alumina.
Sulfuric acid-impregnated silica gel emerged from this study as the most favorable fat retainer. A certified reference standard containing various PCB congeners dissolved in cod liver oil was extracted using this fat retainer at a fat-to-fat retainer ratio of 0.022. Percent recoveries for PCB congeners were near 100%, with %RSDs of 2 to 4% for triplicate ASEs performed per sample.

We now turn our attention to sample preparation techniques to quantitatively determine volatile organic compounds (VOCs) in environmental samples, and after this, we return to a discussion of the more recently developed sample prep techniques for SVOCs, namely, SFE, SPE, and SPME. Because we have been discussing LLE and LSE techniques, it is appropriate for us to ask at this point the following question.

25. CAN VOLATILE ORGANICS BE ISOLATED FROM CONTAMINATED WATER USING LLE?

Yes. The original discovery of trihalomethanes (THMs) in the city of New Orleans drinking water was determined by conducting LLE using pentane as the extracting solvent. If the sample contaminants are well known in a particular environmental sample, it is possible to use LLE as the principal sample prep technique. LLE is, however, unselective, and semivolatile and nonvolatile organics are equally likely to be extracted in samples that contain unknown contaminants. Consistent with the EPA protocols introduced in Chapter 1, methods have been developed to remove VOCs either by purging or by sampling the headspace in a sealed vial that contains the contaminated groundwater sample. The former technique will be discussed after we develop the principles behind the latter technique, commonly called static headspace sampling for the determination of trace volatile organics (VOCs), in drinking water, groundwater, surface water, wastewater, or soil.

26. ON WHAT BASIS CAN VOCS BE QUANTITATED USING STATIC HEADSPACE TECHNIQUES?

Liquid–liquid extraction has a counterpart for the determination of VOCs in the technique known as a static or equilibrium headspace sampling. The technique is most often combined with the determinative step and is often referred to as static headspace gas chromatography (HS-GC). The principles that underlie this technique will be outlined in this section. The decision to measure VOCs in the environment by either HS or purge and trap (P&T or dynamic headspace) represents one of the ongoing controversies in the field of TEQA. This author has worked in two different environmental laboratories in which one used P&T as the predominant technique to determine VOCs in the environment and the other used HS-GC.

Let us start by introducing the thermodynamic viewpoint for the distribution of a substance originally dissolved in the sample. The substance exists at room temperature in a closed vessel partitioned between an aqueous phase and a gaseous phase. The gaseous phase in the HS usually consists of air saturated with water vapor. Dissolved gases and volatile organic compounds are the two most common chemical substances that are likely to be found in the headspace. Dissolved gases
Trace Environmental Quantitative Analysis, Second Edition

equilibrate into the HS because they follow Henry’s law. Volatile organic compounds, many of which are classified as priority pollutants by the EPA, behave similarly to fixed gases, and each different VOC has its own Henry’s law constant, \( K_H \). We will consider sulfur dioxide as being illustrative of a dissolved gas and proceed to develop the basis for the equilibrium. We will next use trichloroethylene, as illustrative of a dissolved VOC, and develop the more applied equations that must be considered in order to relate the HS technique to TEQA.

27. WHAT HAPPENS WHEN GASEOUS SO\(_2\) IS DISSOLVED IN WATER, THEN PLACED IN A VESSEL AND SEALED?

Consider a water sample that contains dissolved SO\(_2\). The sample is placed in a cylindrical glass vessel and then sealed. Glass vials with a crimped-top seal and a volume of 22 mL are commonplace. If the pH of the aqueous phase could be measured, the value would indicate that the effect of the dissolved gas acidified the water. It is well known that one molecule of SO\(_2\) combines with one molecule of water to produce sulfurous acid according to

\[
\text{SO}_2(g) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{SO}_3(aq)
\]

Sulfurous acid, in turn, is known to ionize according to

\[
\text{H}_2\text{SO}_3(aq) \rightleftharpoons \text{H}^+(aq) + \text{HSO}_3^-(aq)
\]

Both equations describe what we have been calling secondary equilibrium effects to the primary distribution equilibria, which, in this case, is the distribution of neutral SO\(_2\) molecules between the aqueous phase and the HS, described as follows:

\[
\text{SO}_2(aq) \stackrel{K_H}{\rightleftharpoons} \text{SO}_2(g)
\]

The extent to which SO\(_2\) partitions into the HS is determined by the Henry’s law constant, \( K_H \).

In a manner similar to that developed for LLE [refer to Equations (3.1) and (3.2)], it becomes apparent that in a closed system between two phases, the chemical potentials for SO\(_2\) in each phase become equal once dynamic equilibrium is reached. If this is so, then similar to what was stated earlier, we can mathematically state

\[
\mu_{\text{HS,SO}_2} = \mu_{\text{aq,SO}_2}
\]

The chemical potential for SO\(_2\) in either phase is related to a standard-state chemical potential, \( \mu^0 \), for SO\(_2\) in both the headspace (HS) and the aqueous phase, and a term related to either the pressure exerted by the gas in the HS or the activity in the dissolved state according to

© 2006 by Taylor & Francis Group, LLC
We seek to express the ratio of activity to partial pressure so as to be consistent with a subsequent definition of the partition coefficient. Upon rearranging, we obtain the following relationship:

\[ \mu_{\text{HS}, \text{SO}_2}^0 + RT \ln p_{\text{SO}_2}^0 = \mu_{\text{aq}, \text{SO}_2}^0 + RT \ln a_{\text{aq}}^\text{SO}_2. \]

Upon further rearranging and proceeding to define the Henry’s law constant, we obtain

\[ RT \ln \left( \frac{a_{\text{aq}, \text{SO}_2}}{p_{\text{HS}, \text{SO}_2}} \right) = -\left( \mu_{\text{aq}, \text{SO}_2}^0 - \mu_{\text{HS}, \text{SO}_2}^0 \right) \]

Secondary equilibrium effects are handled by defining the degree of ionization by \( \alpha \), as done previously [refer to Equations (3.17) and (3.18)], and expressing the activity of neutral \( \text{SO}_2 \) in terms of \( \alpha \) and the total activity, \( a_T \), according to

\[ a_{\text{SO}_2} = a_T (1 - \alpha) \]

For example, if 0.7643 mol \( \text{SO}_2 \) is dissolved in 1 kg of water in a closed system, a 0.7643 \( M \) solution results, and with \( \alpha = 0.1535 \), the vapor pressure exerted by \( \text{SO}_2 \) is such that \( K_H \) is 0.813. When 1.273 g of \( \text{SO}_2 \) is dissolved in the same weight of water, with \( \alpha \) this time being 0.1204, the vapor pressure exerted is such that \( K_H \) is again 0.813.40

28. **ON WHAT BASIS CAN WE QUANTITATE TCE IN GROUNDWATER BY HS–GC?**

Consider how the priority pollutant VOC trichloroethylene or trichloroethene, abbreviated TCE, is distributed between a sample of groundwater and the headspace in a sealed HS vial, as shown schematically by

![Diagram showing TCE(g) and TCE(aq) distribution](image)
Let us assume that the temperature is fixed such as would be found in a thermostated automated S analyzer. A consideration of mass balance requires that the original amount of TCE be found in either the headspace or the aqueous sample. Mathematically stated,

\[ \text{amt}_o = \text{amt}_S + \text{amt}_HS \]  

(3.24)

Using principles encompassed by Equation (3.23), we can proceed to define the partition coefficient as

\[ K_{\text{TCE}} = \frac{C_S}{C_{HS}} \]  

(3.25)

It is helpful at this point to define the abbreviations used to develop the equations that lead to Equation (3.26), derived below. Consider the following definitions:

- \( o \): original concentration of TCE in the groundwater sample
- \( S \): sample
- \( HS \): headspace
- \( \text{amt} \): amount of TCE
- \( V_S \): volume of sample placed in the HS vial
- \( V_{HS} \): volume of headspace in the HS vial after \( V_S \) mL have been placed in the HS vial
- \( C \): concentration
- \( \beta \): phase ratio for an HS vial, equals \( V_{HS}/V_S \)

We can proceed from both the mass balance principle and the definition of \( K_{\text{TCE}} \). Let us divide Equation (3.24) by \( V_S \):

\[ \frac{\text{amt}_o}{V_S} = \frac{\text{amt}_S}{V_S} + \frac{\text{amt}_HS}{V_S} \]

\[ C_0 = C_S + \frac{V_{HS}C_{HS}}{V_S} \]

\[ = K_{\text{TCE}}C_{HS} + \frac{V_{HS}}{V_S}C_{HS} \]

\[ = C_{HS} \left[ K_{\text{TCE}} + \frac{V_{HS}}{V_S} \right] \]

\[ = C_{HS} \left[ K_{\text{TCE}} + \frac{V_{HS}}{V_S} \right] \]

(3.26)
Solving Equation (3.26) for $C_{\text{HS}}$ gives a useful relationship:

$$
C_{\text{HS}}^{TCE} = C_o \left[ \frac{1}{K_{\text{TCE}} + \beta} \right]
$$

Equation (3.27) suggests that for a given concentration of TCE in the original sample of groundwater, the concentration of TCE expected to be found in the HS, $C_{\text{HS}}^{TCE}$, depends not only on $C_o$, but also on two factors: $K_{\text{TCE}}$, which is due to the physico-chemical nature of this particular VOC, and $\beta$, which is due to the volumes occupied by HS and sample. $\beta$ might be thought of in terms of the physical characteristics of the HS vial. In practice, however, $K_{\text{TCE}}$ may exhibit some dependence on temperature and sample ionic strength, whereas the range of values that $\beta$ can take on is limited. As we shall see, both factors play off of one another in the consideration of analyte sensitivity in HS techniques.

Equation (3.27) is the basis for TEQA, because for a given VOC and fixed sample volume in a headspace vial (i.e., $K_{\text{HS}}^{VOC}$ and $\beta$), $C_{\text{HS}}^{VOC}$ is directly proportional to the original concentration of VOC in the aqueous sample, $C_o$. The volume of headspace sampled and injected into a GC is usually held fixed so that the area under the curve of a chromatographically resolved GC peak, $A^{VOC}$, is directly proportional to $C_{\text{HS}}^{VOC}$. As discussed in Chapter 2, Equations (2.1) and (2.2), with respect to the external mode of instrument calibration, the sensitivity of the HS-GC technique is related to the magnitude of the response factor, $R^{\text{HS}}_f$, according to

$$
R^{\text{HS}}_f = \frac{A^{VOC}_{\text{HS}}}{C_{\text{HS}}}.
$$

A fixed aliquot of the HS whose concentration of TCE, $C_{\text{HS}}^{TCE}$, is injected into a GC that incorporates a halogen-specific detector, such as an electrolytic conductivity detector (EICD) (refer to EPA Methods 601 and 602, even though these methods employ P&T techniques). Alternatively, this aliquot can be injected into a gas chromatograph-mass spectrometer (GC-MS). The mass spectrometer might be operated in the selective ion monitoring (SIM) mode. The most abundant fragment ions of TCE would then be detected only (refer to EPA Method 624, even though this method employs P&T techniques). Determinative techniques such as GC-EICD and GC-MS are introduced in Chapter 4. Nevertheless, it should be apparent to the reader at this point that the partition coefficient plays an important role in achieving a high sensitivity in HS techniques. We digress from here to discuss this a bit more.

29. **ON WHAT BASIS DOES $K_{\text{TCE}}$ DEPEND?**

Let us approach an equivalent to Equation (3.25) from the perspective of applying the three great laws of phase equilibrium found in most physical chemistry texts: Dalton’s law of partial pressures, Raoult’s law of ideal solutions, and Henry’s law.
for dissolved gases. Applying Dalton’s law enables one to state that the concentration of analyte in the HS is proportional to its partial pressure. The partial pressure exerted by TCE in the HS is independent of all other gases in the HS mixture and is related to the total pressure in the HS as follows:

\[ p_i^{\text{HS}} = p_T X_i^{\text{HS}} \]

This partial pressure exerted by component \( i \) is, in turn, related to the vapor pressure exerted as if \( i \) were pure, \( p_i^o \), and the mole fraction of component \( i \) dissolved in the sample, \( X_i^S \), according to Raoult’s law. An activity coefficient \( \gamma_i \) is included to account for nonideality so that

\[ p_i^{\text{HS}} = p_i^o \gamma_i X_i^S \]

Also, the partial pressure exerted by component \( i \) can be related to the mole fraction of \( i \) dissolved in the sample, \( S \), according to Henry’s law, as follows:

\[ p_i^{\text{HS}} = K_h X_i^S \]

The partial pressure of component \( i \) in the HS can be eliminated so that

\[ p_T X_i^{\text{HS}} = p_i^o \gamma_i X_i^S \]

Rearranging the equation to a ratio of mole fractions yields

\[ \frac{X_i^S}{X_i^{\text{HS}}} = \frac{p_T}{p_i^o \gamma_i} = \frac{C_i^S}{C_i^{\text{HS}}} = K \]

This equation yields an alternative relationship for the partition constant in HS, in which \( K \) is found to be inversely proportional to a product of the partial pressure of component \( i \), if it is pure, and its activity coefficient \( \gamma_i \) according to

\[ K \propto \frac{1}{p_i^o \gamma_i} \quad (3.28) \]

Because Equation (3.25) relates \( K \) to a ratio of concentrations, combining Equations (3.25) and (3.28) leads to the following:

\[ \frac{C_i^S}{C_i^{\text{HS}}} \propto \frac{1}{p_i^o \gamma_i} \quad (3.29) \]
Sample Preparation Techniques

$P_{\text{TCE}}^{o}$ refers to the intrinsic volatility exhibited by a chemically pure substance TCE. TCE is a liquid at room temperature, and if one opens a bottle that contains the pure liquid, one is immediately struck by virtue of the sense of smell with the concept of the vapor pressure of TCE. It is likely that $P_{\text{TCE}}^{o}$ is greater than $P_{\text{PCE}}^{o}$, where PCE refers to perchlorethylene (tetrachloroethene). PCE is also on the EPA's priority pollutant list. One would expect to find that $C_{\text{TCE}}^{\text{HS}}$ is greater than $C_{\text{PCE}}^{\text{HS}}$, assuming all other factors equal. This is largely due to the inverse relationship between $K$ and $p^o$, as shown in Equation (3.28). This equation also suggests that if a matrix effect exists, $K$ is also influenced by differences in the activity coefficient, $\gamma$. Changes in $\gamma$ might be due to changes in the ionic strength due to the sample matrix and, in turn, influence $K$, as shown in Equation (3.28).

30. MUST WE ALWAYS HEAT A SAMPLE IN HS-GC TO CONDUCT TEQA?

Equations (3.27) to (3.29) lay the foundation for a theoretical understanding of what factors are involved in obtaining a sufficiently large value for the concentration of any VOC in the HS (i.e., $C_{\text{VOC}}^{\text{HS}}$). In this section, we look at the effect of increasing temperature of a headspace vial that contains priority pollutant VOC dissolved in water. An example of this might be a sample of groundwater that has been contaminated with priority pollutant VOCs. The static equilibrium HS technique just described would be used to satisfy the criteria for TEQA.

The Clausius–Clapeyron equation, one of the most famous in physical chemistry, is most applicable for this discussion. The equation states that the partial differential with respect to absolute temperature of the logarithm of a pure liquid’s vapor pressure is inversely related to the liquid’s absolute temperature. We again consider the liquid TCE and state the Clausius–Clapeyron equation mathematically:

$$\frac{\partial}{\partial T} \ln p^o = \frac{\Delta H^o}{RT^2}$$

Upon rearranging and expressing this relationship with respect to the VOC that we are considering, TCE, we obtain

$$\ln p_{\text{TCE}}^o = \frac{\Delta H_{\text{TCE}}^o}{R} \int \frac{dT}{T^2}$$

The indefinite integral is then evaluated as

$$-\frac{\Delta H_{\text{TCE}}^o}{RT} + C$$

The vapor pressure exerted by pure TCE can then be expressed as
where $\Delta H_{\text{vapor}}^{\text{TCE}}$ is the molar heat of vaporization for pure TCE and $R$ is the ideal gas constant. Equation (3.30) suggests that a pure liquid's vapor pressure increases exponentially as the temperature of the liquid is increased, until its vapor pressure reaches that exerted by the atmosphere. A plot of Equation (3.30) is shown in Figure 3.8 for three different liquids. For example, if three ClVOCs are plotted, such as for chloroform, TCE, and PCE, their respective vapor pressure/temperature curves would resemble the three shown in Figure 3.8. Because the partition coefficient and the partial pressure of a pure liquid such as TCE are inversely related according to Equation (3.28), we can relate $K$ and $T$ in the following manner:

$$\ln K_{\text{LS}}^{\text{TCE}} = \frac{A}{T} - B$$  \hspace{1cm} (3.31)

where $A$ and $B$ are constants related to TCE. Thus, an increase in $T$ serves to decrease $K_{\text{LS}}^{\text{TCE}}$ and, according to Equation (3.29), partitions a greater percent of more...
TCE molecules into the HS vs. the condensed phase. In other words, the ratio $C_{S}^{TCE}/C_{HS}^{TCE}$ is seen to decrease.

31. IS THERE AN EXAMPLE THAT ILLUSTRATES THE CONCEPT IN EQUATION (3.31)?

One application of these principles is evident in the hypothetical preparation of a mixture of C$_3$ through C$_{20}$ alkanes; each alkane is then placed in an HS vial in equal amounts. Prior to sealing the vial, a 10-$\mu$L aliquot of the liquid is taken and injected into a gas chromatograph with a carbon-selective detector. A chromatogram is generated that shows a separate peak for each of the 16 alkanes. The HS vial is then sealed with a crimped-top cap and septum. The vial is placed inside a cylindrical heater block whose temperature has previously been set to 80°C. A 1.0-cc gas-tight syringe is used to withdraw an aliquot of the headspace, which is directly injected into the same GC. A chromatogram is generated that shows a distribution of the 16 peaks that is different from the first. The lower-carbon-number or lower-molecular-weight alkanes seem to have been enriched in the HS, compared to the direct liquid injection. These results are readily explained by considering Equation (3.26) and the subsequent reexpression of Equation (3.30) in terms of the HS partition coefficient [i.e., Equation (3.31)].

32. WHAT HAPPENS WHEN $K_{HS}^{VOC}$ VALUES VARY SIGNIFICANTLY?

Kolb and Ettre$^{43}$ recently discussed their work on determining values for $K_{HS}^{VOC}$ and report some interesting findings. They observed that over a range of some 15 VOCs that vary in polarity from dioxane to lower-molecular-weight alcohols, through to ketones, and then to acetate type esters, monoaromatics, and polychlorinated ethenes, and finally to C$_6$ alkanes that $K_{HS}^{VOC}$ values vary by over four orders of magnitude.$^{43}$ Those VOCs that exhibit low $K_{HS}^{VOC}$ values (i.e., favor a high concentration in the HS) ($C_{HS}^{VOC}$ is high at equilibrium) are not influenced by an increase in temperature. Those VOCs that exhibit high $K_{HS}^{VOC}$ (i.e., favor a low concentration in the HS) ($C_{HS}^{VOC}$ is low at equilibrium) are strongly influenced by an increase in temperature. Kolb and Ettre chose five solutes as representative of the large number of VOCs that have a wide range of partition coefficients. These are listed below, along with their partition coefficients:

<table>
<thead>
<tr>
<th>Solute</th>
<th>$K_{HS}^{VOC}$ at 40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>1355</td>
</tr>
<tr>
<td>Methyl ethyl ketone</td>
<td>139.5 at 45°C</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.82</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>1.48</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>0.14</td>
</tr>
</tbody>
</table>
As the temperature is increased from 40 to 80°C while $\beta$ is held fixed at 3.46, the partition coefficient for ethanol is observed to increase to the greatest degree, whereas that for methyl ethyl ketone is also increased, but to a lesser degree. Partition coefficients for toluene, tetrachloroethylene, and $n$-hexane do not increase to any extent as the temperature is increased from 40 to 80°C.

This phenomenon is explained by these authors in the following manner. VOCs whose partition coefficients are already low are enriched in the HS at the lower temperature, 40°C, and the percent of remaining dissolved VOCs in the condensed phase that partition into the HS as the temperature is raised to 80°C is not appreciable. Polar solutes, on the other hand, are predominantly dissolved in the condensed phase at 40°C, and the percent that partition into the HS as the temperature is raised to 80°C is much more appreciable.

Let us return to Equation (3.27) and briefly consider the second factor, the phase ratio $\beta$, in the headspace sampling of groundwater to determine trace concentrations of VOCs in the environment. Kolb and Ettre have studied the influence of $\beta$ on HS sensitivity. For a fixed temperature and fixed original concentration of VOC in the aqueous phase, $C_0$, the influence of changing $\beta$ from, for example, 4.00 (only 20% of the total volume of the HS vial contains the sample), to $\beta = 0.250$ (80% of the total volume of the HS vial contains the sample) depends on the magnitude of $K_{\text{HS}}/C_0$.

For nonpolar aliphatic hydrocarbons like $n$-hexane or for chlorinated C1 or C2 aliphatics such as TCE, a change in $\beta$ from 4.00 to 0.250 increases the HS sensitivity by almost a factor of 10. For monoaromatics, the same change in $\beta$ gives an increase in HS sensitivity of about 4. For acetate type esters, ketones, lower-molecular-weight alcohols, and ethers, a change in $\beta$ from 4.00 to 0.250 gives an insignificant increase in HS sensitivity.

The influence of the sample matrix activity coefficient, $\gamma$, represents the third factor that serves to influence HS sensitivity. The theoretical basis for this is encompassed in Equation (3.26) or (3.28). The influence of increasing $\gamma$ by adding salt to an aqueous sample, a well-known technique called salting out, has been shown to have a negligible effect on nonpolar VOCs such as TCE dissolved in water. Polar solutes, however, are more strongly influenced by changes in the sample matrix activity coefficient. It has also been observed that a high concentration of salt must be dissolved in the aqueous sample matrix to have any effect at all. The addition of a high amount of salt increases the volume of the liquid sample and thus serves to decrease $\beta$. There are a number of drawbacks to adding salt in static HS, including increases in sample viscosity and the addition of volatile impurities.

Few comprehensive studies have been published on the effect of the sample matrix on the partitioning of VOCs at trace concentration levels. Friant and Suffet chose four model compounds that are polar and representative of the intramolecular forces of dispersion, dipole orientation, proton-donor capability, and proton-acceptor capability. These were methyl ethyl ketone, nitroethane, $n$-butanol, and $p$-dioxane. The pH of the aqueous sample matrix had no effect at all except for nitroethane. An optimum salt concentration was 3.35 M in sodium sulfate, and an optimum HS sampling temperature of 50°C. For example, the partition coefficient of methyl ethyl ketone increased from 3.90 to 260 when the temperature of the aqueous sample that contained the dissolved ketone was increased from 30 to 50°C, and at the same time,
the concentration of salt increased from zero to 3.35 M. Otson et al. compared static HS, P&T, and LLE techniques for the determination of THMs in drinking water and found that static HS showed the poorest precision and sensitivity. However, a manual HS sampling technique was used back in this era and might possibly have contributed to this loss of precision. LLE was found to be quite comparable to P&T in this study.45

33. WHY IS STATIC HEADSPACE SAMPLING NOT MORE ACCEPTABLE TO THE EPA?

This might be close to the $64,000 question. The answer lies somewhere between “MDLs are not low enough in comparison to those obtained using P&T” and “HS techniques were not developed in EPA labs, while purge and trap techniques were.” Static HS, whose principles have already been introduced in this chapter, requires that analytes be classified as volatile (i.e., having boiling points <125˚C). As also discussed, the fundamental principles behind the static HS technique are only recently becoming understood. Most static HS techniques are performed in automated analyzers. These analyzers are directly interfaced to the injection port of a gas chromatograph via a transfer line. This online instrumental configuration does not allow for the GC’s IDL to be experimentally measured independently, as is done for offline LLE in the determination of semivolatile analytes. Thus, the GC detector that is available is crucial to the notion of HS sensitivity. Because of the low detector response factor, a given VOC can be highly favored to partition into the headspace, yet have an MDL that is less than desirable. In the author’s laboratory, the technique of manual HS sampling is popular among environmental microbiologists who need analytical results almost immediately after they sample the headspace of their bioreactors. These researchers cannot wait for the long equilibration times of commercial automated HS analyzers. P&T techniques would likewise be out of the question as a means to measure trace VOCs, due to the long purging, trapping, and thermal desorbing times involved.

The difficulty that the EPA has had with the static headspace technique might be seen in the comment from Method 3810 (SW-846, third edition). This method was eliminated in the recently published final update (III) and replaced by Method 5021. The authors who wrote Method 3810 state:46

Detection limits for this method may vary widely among samples because of the large variability and complicated matrices of waste samples. The sensitivity of this method will depend on the equilibria of the various compounds between the vapor and dissolved phases. Due to the variability of this method, this procedure is recommended for use only as a screening procedure for other, more accurate determinative methods.

Method 3810 recommends that a sample be heated to 90˚C and 2 mL of headspace gas taken with a gas-tight syringe. The written procedure for this method was published in SW-846 and provided no data on method performance at that time. The publication of a method from a regulatory agency without even a minimum of quality assurance and quality control is inexcusable. This method serves as one example of
neglected GLP. The importance of GLP was introduced in Chapter 1, and this neglect attests to the fact that little to no interest in static HS technique existed in 1986.

34. IS THERE A RECENTLY UPDATED EPA METHOD FOR VOC USING STATIC HS TECHNIQUES?

Yes, there is, and it is Method 5021 from the recently updated SW-846 series of methods published by the Office of Solid Waste at EPA. The method uses the static HS technique to determine VOCs from soil or another solids matrix. This section will focus on some of the details of this method because it includes many of the quality control (QC) features that were absent in the method just discussed. This method also introduced some experimental considerations with respect to trace VOC analyses of soil samples.\(^47\) The method is applicable to a wide range of organic compounds that have sufficiently high volatility to be effectively removed from soil samples using the static HS technique. The method is used in combination with a determinative technique that is described in the 8000 series. The method cautions the user to the fact that solid samples whose organic matter content exceeds 1%, or compounds with high octanol–water partition coefficients, may yield a lower result for the determination of VOCs by static HS than dynamic headspace (P&T). It is recommended to add surrogates to each and every sample to evaluate the so-called matrix effect.

35. HOW SHOULD I PROCEED TO QUANTITATE VOCs?

The EPA's approach to sample preparation to determine trace concentration levels of various VOCs is discussed in this section. We have already introduced the static HS technique from a theoretical point of view. Purge-and-trap (P&T) techniques, in contrast to static HS, involve passing an inert gas through an aqueous sample such as drinking water or groundwater. The purge gas is directed to a *trap*, a term commonly used to describe a packed column that contains an adsorbent that exhibits a high efficiency for VOCs. After sufficient time for purging and trapping has been allowed, the trap is rapidly heated to thermally desorb the VOCs off of the adsorbent and directly into the GC. In this manner, VOCs are removed from the environmental sample without a matrix effect, and the objectives of TEQA can be met.

Purge and trap is dynamic; the analyte is merely transferred from the sample to an organic polymeric matrix that exhibits a large surface area. One point of view assumes that static HS is merely a screen for dynamic HS (P&T) because MDLs for P&T are significantly lower than those for static HS. A second approach to screening for particular priority pollutant VOCs is called hexadecane screening. The logic as to what technique to use is depicted in the flowchart in Scheme 3.3. The sample matrix plays a significant role as to whether one chooses the static or dynamic HS technique for the isolation and recovery of VOCs from environmental samples.

When analyzing environmental samples that are considered grossly contaminated, screening techniques are essential. Screening techniques serve to inform the analyst as to whether a dilution of the original is warranted. Laboratories that have both automatic static HS and automated P&T systems are more likely to use static
HS for the initial screen, followed by P&T for the quantitative analysis. This is particularly true for labs that engage in EPA contract work. Labs that have either static HS or P&T, but not both, often elect to use hexadecane screening prior to the quantitative determination by either static HS or P&T. Labs that analyze predominantly drinking water samples most likely do not have a need to screen samples and usually proceed directly to the available determinative technique.

SCHEME 3.3 Flowchart for decisions involving screening of environmental samples for VOCs.
36. WHAT IS HEXADECANE SCREENING?

Hexadecane screening uses hexadecane (Hxd) as the extractant to perform an initial LLE of a water or wastewater sample that is suspected of containing appreciable levels of VOCs in a complex sample matrix. We thus refer to this technique with the abbreviation Hxd-LLE. Figure 3.9 is a gas chromatogram, GC-gram, of a ground-water sample that has been in contact with gasoline. This is a common occurrence in the environment because gasoline storage tanks are known to corrode with age and leak into the groundwater supply, thus contaminating sources of drinking water. The VOCs, commonly referred to as BTEX components, are clearly evident in the GC-gram. BTEX is an abbreviation of the aromatic VOCs benzene, toluene, ethyl benzene, and one or more of the three isomeric xylenes, ortho, para, and meta. A fourth component is more frequently showing up in groundwater samples. This compound is methyl-\(\text{-}t\)-butyl ether (MTBE). MTBE is added to gasoline as an oxygenated hydrocarbon. A groundwater sample that exhibits a significant concentration of BTEX should be diluted with high-purity water. This diluted sample should be subsequently analyzed to quantitatively determine the concentrations of BTEX and MTBE using either static HS or P&T. If a hydrocarbon of much lower molecular weight, such as \(n\)-hexane, were used to conduct the preliminary assessment of the degree of sample contamination, the instrument response due to the elution of \(n\)-hexane from the GC would interfere with one or more of the peaks due to the presence of BTEX. It is illustrative to compare the physical properties of \(n\)-hexane vs. \(n\)-hexadecane. The following table compares the physical properties of both hydrocarbons:

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>(T_b) (°C)</th>
<th>(\rho^{\text{20°C}}) (g/mL)</th>
<th>(P')</th>
<th>Solubility in Water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)-Hexane</td>
<td>86</td>
<td>68.7</td>
<td>0.659</td>
<td>0.1</td>
<td>0.014</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>226</td>
<td>287</td>
<td>0.773</td>
<td>0.5</td>
<td>—</td>
</tr>
</tbody>
</table>

It is important to use as high a purity of Hxd as can be obtained; otherwise, impurities in this extracting solvent might cause interference with some VOCs. The absence of lower-molecular-weight impurities in Hxd early in the GC-graam greatly contributes to lowering MDLs. A GC that incorporates a flame ionization detector is usually designated as the Hxd-LLE screening instrument. A mini-LLE scale using a phase ratio, \(\beta = V_{\text{Hxd}}/V_{\text{aq}} = 0.057\), such that approximately 35 mL of aqueous sample is extracted once into 2 mL of Hxd is a common technique. The limitations to the effective use of Hxd-LLE lie in the nature of the environmental sample matrix. Samples such as wastewaters that are prone to cause emulsions prevent analysts from quickly injecting a 1-µL aliquot of the extract. Common techniques for breaking emulsions include the following:\textsuperscript{48}

1. Adding salt to the aqueous phase
2. Using a heating–cooling extraction vessel
3. Filtering the emulsion through a glass wool plug
4. Filtering the emulsion through a phase separation filter paper
5. Using a centrifuge
6. Adding a small amount of a different organic solvent
FIGURE 3.9 Capillary gas chromatogram for the separation of BTEX components in a water sample contaminated with gasoline.
If this author is asked to estimate the concentration level of VOCs and static HS or P&T techniques are not available, he would employ Hxd-LLE. EPA Method 3820 from the SW-846 series is a useful starting point for this LLE technique. Seven different approaches to prepare environmental samples to determine VOCs are presented in SW-846. We will discuss each of these with an emphasis on the operational aspects.

37. **WHAT ARE EPA’S APPROACHES TO TRACE VOCs?**

Method 5000 in the SW-846 lists seven methods to prepare samples for the quantitative determination of VOCs. These methods reflect the different samples matrices involved. Water, soil/sediment, sludge, and waste samples that require analysis for VOCs are extracted or introduced into a GC or GC-MS system by the various methods, as discussed below. The following methods are briefly introduced from an EPA point of view, and the advantages and limitations from that viewpoint are discussed.

Method 3585 describes a solvent dilution technique using hexadecane followed by direct injection into a GC-MS instrument for the determination of VOCs in waste oils. Direct injection of an oil waste could lead to instrument contamination problems. However, the method provides for a quick turnaround.

Method 5021 describes the automated static HS technique. Static HS has been introduced in this book from a theoretical viewpoint. A soil sample is placed in a tared septum-sealed vial at the time of sampling. A matrix modifier containing internal or surrogate standards is added. The sample vial is placed into an automated headspace sampler vial, which is placed into an automated equilibrium headspace sample. The vial’s temperature is elevated to a fixed value that does not change over time, and the contents of the vial are mixed by mechanical agitation. A measured volume of headspace is automatically introduced into a GC or GC-MS. The method is automated and downtime is minimal. However, the cost of the automated system is appreciable. Contamination of the instrument is minimal. The MDL for this technique is significantly influenced by the choice of GC detector.

Method 5030 describes the technique of purge and trap for the introduction of purgeable organics into a GC or GC-MS. The method is applicable to aqueous samples such as groundwater, surface water, drinking water, wastewater, and water-miscible extracts prepared by Method 5035. An inert gas is bubbled through the sample and the VOCs are efficiently transferred from the aqueous phase to the vapor phase. The vapor phase is swept through a sorbent trap where the purgeables are trapped. After purging is completed, the trap is heated and back-flushed with the inert gas to desorb the purgeables onto a GC column. P&T is easily automated and provides good precision and accuracy. However, the method is easily contaminated by samples that contain compounds that are present in the sample at the ppm level. Since P&T is an exhaustive removal of VOCs from the environmental sample, some argue that this technique offers the lowest MDLs. Again, the MDL would be strongly influenced by the choice of GC detector. Because this is the most commonly used method to determine VOCs, the method will be elaborated upon later.
Method 5031 describes an azeotropic distillation technique for the determination of nonpurgeable, water-soluble VOCs that are present in aqueous environmental samples. The sample is distilled in an azeotropic distillation apparatus, followed by direct aqueous injection of the distillate into a GC or GC-MS system. The method is not amenable to automation. The distillation is time consuming and is limited to a small number of samples.

Method 5032 describes a closed-system vacuum distillation technique for the determination of VOCs that include nonpurgeable, water-soluble, volatile organics in aqueous samples, solids, and oily waste. The sample is introduced into a sample flask that is in turn attached to the vacuum distillation apparatus. The sample chamber pressure is reduced and remains at approximately 10 torr (the vapor pressure of water) as water is removed from the sample. The vapor is passed over a condenser coil chilled to a temperature of 10°C or less. This results in the condensation of water vapor. The uncondensed distillate is cryogenically trapped on a section of 1/8-in. stainless-steel tubing chilled to the temperature of liquid nitrogen (−196°C). After an appropriate distillation period, the condensate contained in the cryogenic trap is thermally desorbed and transferred to the GC or GC-MS using helium carrier gas. The method very efficiently extracts organics from a variety of matrices. The method requires a vacuum system along with cryogenic cooling and is not readily automated.

Method 5035 describes a closed-system P&T for the determination of VOCs that are purgeable from a solid matrix at 40°C. The method is amenable to soil/sediment and any solid waste sample of a consistency similar to soil. It differs from the original soil method (Method 5030) in that a sample, usually 5 g, is placed into the sample vial at the time of sampling along with a matrix-modifying solution. The sample remains hermetically sealed from sampling through analysis as the closed-system P&T device automatically adds standards and then performs the purge and trap. The method is more accurate than Method 5030 because the sample container is never opened. This minimizes the loss of VOCs through sample handling. However, it does require a special P&T device. Oil wastes can also be examined using this method.

Method 5041 describes a method that is applicable to the analysis of sorbent cartridges from a volatile organic sampling train. The sorbent cartridges are placed in a thermal desorber that is in turn connected to a P&T device.

38. WHAT IS THE P&T TECHNIQUE?

The P&T method to isolate, recover, and quantitate VOCs in various environmental sources of water has and continues to remain the premier technique for this class of environmental contaminants. The technique was developed at the EPA in the early 1970s and remains the method of choice, particularly for environmental testing labs that are regulatory driven. P&T has had the most success with drinking water samples when combined with gas chromatography and element-specific detectors. GC detectors will be discussed in Chapter 4. In this section, we will discuss the EPA methods that use the P&T technique to achieve the goals of TEQA. EPA Method 502.2 summarizes the method as follows:
Highly volatile organic compounds with low water solubility are extracted (purged) from the sample matrix by bubbling an inert gas through a 5 mL aqueous sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated and back-flushed with helium to thermally desorb trapped sample components onto a capillary gas chromatography (GC) column. The column is temperature programmed to separate the method analytes which are then detected with a photoionization detector (PID) and an electrolytic conductivity detector (EICD) placed in series. Analytes are quantitated by procedural standard calibration. Identifications are made by comparison of the retention times of unknown peaks to the retention times of standards analyzed under the same conditions used for samples. Additional confirmatory information can be gained by comparing the relative response from the two detectors. For absolute confirmation, a gas chromatography/mass spectrometry (GC/MS) determination according to EPA Method 524.2 is recommended.

The classical purge vessel is shown in Figure 3.10. Usually, 5 mL of an environmental aqueous sample is placed in the vessel. The sample inlet utilizes a two-way valve. A liquid-handling syringe that can deliver at least 5 mL of sample is connected to this sample inlet, and the sample is transferred to the purge vessel in a way that minimizes the sample’s exposure to the atmosphere. Note that the incoming inert purge gas is passed through a molecular sieve to remove moisture. A hydrocarbon trap can also be inserted prior to the purge vessel to remove traces of organic impurities as well. The vessel contains a fritted gas sparge tube that serves to finely divide and disperse the incoming purge gas. These inert gas bubbles from the purging provide numerous opportunities for dissolved organic solutes to escape to the gas phase. The acceptable dimensions for the purge device are such, according to EPA Method 502.2, that it must accommodate a 5-mL sample with a water column at least 5 cm deep. The headspace above the sample must be kept to a minimum of <15 mL to eliminate dead-volume effects. The glass frit should be installed at the base of the sample chamber, with dispersed bubbles having a diameter of <3 mm at the surface of the frit.

Figure 3.10 also depicts a typical trap to be used in conjunction with the purge vessel. The trap must be at least 25 cm long and have an inside diameter of at least 0.105 in., according to Method 502.2. The trap must contain the following amounts of adsorbents:

- One third of the trap is to be filled with 2,6-diphenylene oxide polymer, commonly called Tenax.
- One third of the trap is to be filled with silica gel.
- One third of the trap is to be filled with coconut charcoal.

It is recommended that 1 cm of methyl silicone-coated packing be inserted at the inlet to extend the life of the trap. Method 5030B from the SW-846 series is more specific than Method 502.2 and recommends a 3% OV-1 on Chromosorb-W, 60/80 mesh, or equivalent. Analysts who do not need to quantitate dichlorodifluoromethane do not need to use the charcoal and can replace this charcoal with more Tenax. If only analytes whose boiling points are above 35°C are to be determined,
both the charcoal and the silica gel can be eliminated and replaced with Tenax. The trap needs to be conditioned at 180°C prior to use and vented to the atmosphere.
instead of the analytical GC column. It is also recommended that the trap be reconditioned on a daily basis at the same temperature. Tenax is a unique polymer and offers the advantage that water is not trapped to any great extent.

Commercially available P&T units are fully automated and consist of a bank of purge vessels with switching valves that enable one vessel to be purged after another. Figure 3.11 is a schematic diagram that clearly depicts the purge, trap, and thermal desorption steps involved in this technique. A six-port valve, placed after the trap and interfaced to the injection port of a GC, provides the needed connection. Referring to Figure 3.11a, the P&T step is shown. Inert gas, usually helium, enters the

© 2006 by Taylor & Francis Group, LLC
purge vessel while the trap outlet is vented to the atmosphere. Meanwhile, GC carrier
gas flows through one side of the six-port valve directly to the GC. The purge vessel
and the trap are generally kept at ambient temperature. Some commercially available
units provide for a heated purge vessel. Referring to Figure 3.11b, the direction of
gas flow during the desorb step is illustrated. The valve is turned and inert gas enters
and passes over the trap in a direction opposite to the P&T step. The trap is rapidly
heated to the required final temperature and the trap outlet is directed to the injection
port of the GC. GCs that are equipped with cryogenic cooling can deposit the VOCs
from the trap to the GC column inlet as a plug. The GC can then be temperature
programmed from the cryogenic temperature to the final temperature, and hence
complete the gas chromatographic separation of all VOCs.

Operating conditions drawn from EPA Method 5030B for the P&T system differ
slightly based on which determinative method is used and are presented in the
following:

<table>
<thead>
<tr>
<th>Analysis Method</th>
<th>8015</th>
<th>8021 or 8260</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purge gas</td>
<td>N₂  or He</td>
<td>N₂  or He</td>
</tr>
<tr>
<td>Purge gas flow rate (mL/min)</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Purge time (min)</td>
<td>15.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Purge temperature (°C)</td>
<td>85</td>
<td>Ambient</td>
</tr>
<tr>
<td>Desorb temperature (°C)</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Back-flush inert gas (mL/min)</td>
<td>20–60</td>
<td>20–60</td>
</tr>
<tr>
<td>Desorb time (min)</td>
<td>1.5</td>
<td>4</td>
</tr>
</tbody>
</table>

39. HOW DOES ONE GO ABOUT CONDUCTING THE P&T TECHNIQUE?

Let us assume that the decisions of whether to screen the sample prior to conducting
P&T have been made and that we are ready to actually perform the technique. We
have available in our laboratory organic-free water, the determinative method has
been defined, and we have reviewed EPA Method 5000 for guidance with respect
to internal and surrogate standards. Environmental samples such as groundwater,
drinking water, wastewater, and so forth, have already been collected, stored in
capped bottles with minimum headspace, free of solvent fumes, and stored at 4°C.
If the sample was found to be improperly sealed, it should be discarded. All samples
should be analyzed within 14 days of receipt at the laboratory. Samples not analyzed
within this period must be noted and data are considered to represent a minimum
value. This is the essence of the so-called holding times. Holdings times, like
detection limits that were discussed in Chapter 2, are a controversial topic between
the regulatory agency and contracting laboratory. Using Method 5030B for guidance,
the sequence of steps needed to carry out the method are as follows:

- Initial calibration: The P&T apparatus is conditioned overnight with an
  inert gas flow rate of at least 20 mL/min and the Tenax trap held at a
temperature of 180°C. The P&T apparatus is connected to the GC, and each purge vessel is filled to its total volume with organic-free water. Methanolic solutions containing VOCs at high enough concentration such that only microliter aliquots need to be added to the water in each purge vessel are added to a series of purge vessels. A blank and set of working calibration standards are prepared right in the purge vessels. Internal standards and surrogates are also added as their methanolic solutions as standard references in a manner similar to that of the analytes to be calibrated. Refer to Chapter 2 for a discussion of the various modes of instrumental calibration.

- Conduct the P&T for the set of blanks and working calibration standards.
- Initial calibration verification (ICV): Prepare one or more purge vessels that contain the ICV. Sometimes, it is advantageous to prepare ICVs in triplicate to enable a preliminary evaluation of the precision and accuracy of the calibration to be made. The ICV criteria are usually given in a determinative method such as in the 8000 series of SW-846. These criteria must be met before real samples can be run.
- Adjust the purge gas flow rate referring to the guidance given in the above table.
- Sample delivery to the purge vessel: Remove the plunger from a 5-mL liquid-handling syringe and attach a closed syringe valve. Open the sample or standard bottle, which has been allowed to come to ambient temperature. Carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. This process of taking an aliquot destroys the validity of the liquid sample for future analysis. If there is only one sample vial, the analyst should fill a second syringe at this time to protect against possible loss of sample integrity. Alternatively, carefully transfer the remaining sample into a 20-mL vial and seal with zero headspace. The second sample is maintained only until such time when the analyst has determined that the first sample has been analyzed properly. In this way, the VOC content of the environmental sample is preserved during the transfer to the purge vessel.

A GC-gram from EPA Method 502.2 that depicts the separated peaks from both the PIDs and EICDs is shown in Figure 3.12. Both chromatograms are overlayed and show the different response for each chromatographically resolved peak between detectors.

40. DO ALL VOCs PURGE WITH THE SAME RATE?

No, they do not, and until recently, little was known about the kinetics of purging for priority pollutant VOCs using conventional P&T techniques. We describe the findings of Lin et al., who demonstrated that first-order kinetics are followed for the removal of VOCs from P&T vessels. The authors make the point that because
FIGURE 3.12 Capillary gas chromatogram for the separation of VOCs using EPA Method 502.2.
EPA methods such as 524.2 and 624 require the use of internal standards to obtain relative response factors, the percent purge efficiency is never considered. Some 28 priority pollutant VOCs were studied. Each VOC, dissolved in methanol, was spiked into high-purity water at concentration levels of 1 and 10 ppb, with a 10 ppb internal standard. Spiked samples were purged for 11 min. A GC-MS was used, and the absolute peak area for the characteristic primary ion was used to quantitate. Experimentally, samples were purged with helium for 11 min and GC-MS data were obtained. These purged samples were then purged a second time for the same 11-min duration under identical conditions. Peak areas were obtained in the same manner as for the first purge.

Let us begin to construct a mathematical view of the kinetics of P&T by first defining the purge ratio, $P_i$, as being the ratio of the mean GC-MS area count for the first purge to the mean GC-MS area count for the second purge according to

$$P_i = \frac{A_i^{(ave)}}{A_i^{(ave)} = \sum_{j}^{5} \frac{A_j^i / 5}{\sum_{j}^{5} A_j^i / 5}}$$

Each peak area for the $i$th analyte was obtained by averaging peak areas over $j$ replicate purges, with $j$ taken from 1 to 5. A selected list of VOCs, along with each VOC retention time, purge ratio, and coefficient of variation (refer to Chapter 2) from their work, is given in the following table:

<table>
<thead>
<tr>
<th>VOC</th>
<th>$t_R$ (min)</th>
<th>$P_i$</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloromethane</td>
<td>1.82</td>
<td>339</td>
<td>36</td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>9.99</td>
<td>2.21</td>
<td>6</td>
</tr>
<tr>
<td>1,1,1,2-Tetrachloroethane</td>
<td>16.98</td>
<td>6.06</td>
<td>9</td>
</tr>
<tr>
<td>1,2,4-Trichlorobenzene</td>
<td>26.30</td>
<td>3.64</td>
<td>8</td>
</tr>
<tr>
<td>Acetone</td>
<td>4.30</td>
<td>1.05</td>
<td>2</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>8.34</td>
<td>1.03</td>
<td>5</td>
</tr>
</tbody>
</table>

$^{a}30 \text{ m } \times 0.53 \text{ mm DB-624 (J&W Scientific); refer to EPA Method 524.2 for GC column temperature program and other GC conditions.}$

The low percent RSDs, except for the very volatile chloromethane, indicate that $P_i$ is constant over the range of concentrations studied. According to these authors, consistent values of $P_i$ would indicate that the purging VOCs from an aqueous solution can be mathematically described by first-order chemical kinetics. How fast any of the 28 VOCs studies are removed from a fixed volume of an aqueous sample such as groundwater in a conventional purge vessel is expressed as $-\frac{dC}{dt}$, where the negative sign indicates that the concentration of a VOC decreases with time. First-order kinetics suggests that the rate is directly proportional to the concentration of each VOC. Expressed mathematically,
where \( C \) is the analyte concentration at any time \( t \) and \( k \) is the first-order rate constant. Differences in the magnitude of \( k \) depend on the chemical nature of the particular VOC and the degree of intermolecular interaction with the sample matrix — in this case, a relatively clean water sample. Equation (3.32) is first rearranged as

\[
\frac{dC}{C} = -k \, dt
\]

Integrating between the initial concentration \( C_1 \) and the concentration after the first purge, \( C_2 \), on the left side, while integrating between time \( t = t_0 \) and time \( t = t_0 + \Delta t \) on the right side is shown below. Identical integration is also appropriate between \( C_2 \) and \( C_3 \). \( \Delta t \) is the time it takes to purge the sample. We then have

\[
\int_{C_1}^{C_2} \frac{dC}{C} = -k \int_{t_0}^{t_0+\Delta t} dt
\]

Evaluating the definite integrals for both sides of the equation yields

\[
\ln(C_2 - C_1) = -k\Delta t
\]

Utilizing a property of logarithms gives

\[
\ln \frac{C_2}{C_1} = -k\Delta t
\]

Expressed in terms of exponents,

\[
\frac{C_2}{C_1} = e^{-k\Delta t}
\]

\[
C_2 = C_1 e^{-k\Delta t}
\]

Equation (3.33) implies that \( C_2 \) can be expressed in terms of \( C_1 \), \( k \), and \( \Delta t \).

The experiment starts with each VOC at a concentration \( C_1 \). After purge 1, each analyte is at concentration \( C_2 \). After purge 2, each analyte is at concentration \( C_3 \). This is depicted by

\[
C_1 \xrightarrow{\text{Purge 1}} C_2 \xrightarrow{\text{Purge 2}} C_3
\]
Because VOCs are continuously removed from a fixed volume of aqueous sample, it is also true that

\[ C_3 < C_2 < C_1 \]

The peak area obtained after the first purge \[ A^1 \] is proportional to the difference in concentration between \( C_1 \) and \( C_2 \); that is,

\[ A^1 \propto C_1 - C_2 \]

Substituting for \( C_2 \) using Equation (3.33) gives

\[ A^1 \propto C_1 (1 - e^{-k\Delta t}) \]

Likewise, the peak area obtained for each analyte after the second purge is proportional to the difference in concentration between \( C_2 \) and \( C_3 \) according to

\[ A^2 \propto C_2 - C_3 \]

Substituting for \( C_3 \) using Equation (3.33) gives

\[ A^2 \propto C_2 (1 - e^{-k\Delta t}) \]

The ratio of peak areas between the first and second purges for a given analyte, \( P \), can be related to these differences in concentration as follows:

\[ P = \frac{C_1 - C_2}{C_2 - C_3} = \frac{C_1}{C_2} \]

Substituting for Equation (3.33) yields

\[ P = \frac{C_1}{C_2 e^{-k\Delta t}} \]

This simplifies to

\[ P = e^{k\Delta t} \quad (3.34) \]

Equation (3.34) suggests that \( P \) is always greater than 1 and is independent of the initial analyte concentration. The development of Equation (3.34) has assumed first-order kinetics. The authors observed good precision over the five replicate experiments for all 28 VOCs studied, with the exception of the very volatile chloromethane.

If \( P \) is determined experimentally, by first obtaining the GC-MS peak area, \( A_1 \), and then obtaining \( A_2 \), Equation (3.34) can be solved for the first-order rate constant, \( k \).
In addition, when \( C_2 = \frac{1}{2} C_1 \), the time it takes for half of the analyte to be removed can be found according to

\[
\frac{C_1}{C_2} = 2 = e^{\frac{kt_1/2}{k}}
\]

\[
\ln 2 = \frac{kt_1/2}{k}
\]

\[
\therefore t_{1/2} = \frac{1}{k} \ln 2
\]

Using Equation (3.35), the \( t_{1/2} \) values given for the three VOCs from the author’s work are shown in the following table:

<table>
<thead>
<tr>
<th>VOC</th>
<th>( t_{1/2} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloromethane</td>
<td>1.3</td>
</tr>
<tr>
<td>1,1,1,2-Tetrachloroethane</td>
<td>4.2</td>
</tr>
<tr>
<td>Acetone</td>
<td>156</td>
</tr>
</tbody>
</table>

A purge time, \( \Delta t = 11 \text{ min} \), is used in indicated EPA methods. For hydrophobic VOCs, the \( t_{1/2} \) seems to be too low, and for hydrophilic VOCs, the \( t_{1/2} \) seems to be too high. Other generalizations emerge with respect to the chemical nature of the VOCs studied. The effect of deuterating 1,2-dichlorobenzene yields values for \( t_{1/2} \) that are quite close to each other, 5.6 and 5.8 (1,2-dichlorobenzene-d₄), respectively. Differences in structural isomers such as 1,1,2,2- vs. 1,1,1,2-tetrachloroethanes are reflected not only in their volatility in water, but also in their gas-phase stability of both neutral and ionic states. It would also appear that the choice of internal standard should reflect the kinetics of purging in addition to GC relative retention time. Choosing internal standards based on relative retention times has been the hallmark of most EPA methods. This work would suggest that \( t_{1/2} \) also be taken into consideration when deciding on which internal standard to use when using P&T techniques.

We leave sample prep for VOCs and return to SVOCs. Conventional sample extract cleanup techniques are introduced followed by SFE and then SPE. We already discussed how LLE is used to remove unwanted neutral interferences when the target analyte of interest is a weak acid and can therefore be ionized. Cleanup introduced here refers to the need to remove neutral polar interferences from neutral targeted SVOC analytes.

### 41. WHAT IS CLEANUP?

It is not the use of detergent to remove dirt. It is the removal of chemical interferences from the extract following any of the extraction techniques discussed earlier. The
dirtier the sample matrix, the greater is the need for extract cleanup. Low pressure or gravity-fed sample extract cleanup is a form of column liquid chromatography (low pressure liquid chromatography (LPLC)). LPLC has a fascinating history.

Meloan lists 16 adsorbents or stationary phases that have been used historically to conduct LPLC. These adsorbents are listed below from most active (polar interferences most strongly retained) to least active (polar interferences weakly retained):

<table>
<thead>
<tr>
<th>Most active</th>
<th>Fuller’s Earth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Charcoal</td>
</tr>
<tr>
<td>Activated alumina</td>
<td></td>
</tr>
<tr>
<td>Magnesium silicate</td>
<td></td>
</tr>
<tr>
<td>Silica gel</td>
<td></td>
</tr>
<tr>
<td>CaO</td>
<td></td>
</tr>
<tr>
<td>MgO</td>
<td></td>
</tr>
<tr>
<td>CaCO₃</td>
<td></td>
</tr>
<tr>
<td>Ca₃(PO₄)₂</td>
<td></td>
</tr>
<tr>
<td>CaCO₃</td>
<td></td>
</tr>
<tr>
<td>K₂CO₃</td>
<td></td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>Least active</td>
<td>Sucrose</td>
</tr>
</tbody>
</table>

EPA Method 3600C from the SW-846 series of solid waste methods provides an overview of sample cleanup techniques. Grossly contaminated solid wastes represent a significant challenge to cleanup techniques. The 3000 series includes eight cleanup methods. Refer to the table below:

<table>
<thead>
<tr>
<th>Method</th>
<th>Method Name</th>
<th>Cleanup Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3610</td>
<td>Alumina Cleanup</td>
<td>Adsorption</td>
</tr>
<tr>
<td>3611</td>
<td>Alumina Cleanup and Separation of Petroleum Wastes</td>
<td>Adsorption</td>
</tr>
<tr>
<td>3620</td>
<td>Florisil Cleanup</td>
<td>Adsorption</td>
</tr>
<tr>
<td>3630</td>
<td>Silica Gel Cleanup</td>
<td>Adsorption</td>
</tr>
<tr>
<td>3640</td>
<td>Gel-Permeation Cleanup (GPC)</td>
<td>Size separation</td>
</tr>
<tr>
<td>3650</td>
<td>Acid-Base Cleanup</td>
<td>Acid–base partitioning</td>
</tr>
<tr>
<td>3660</td>
<td>Sulfur Cleanup</td>
<td>Oxidation/reduction</td>
</tr>
<tr>
<td>3665</td>
<td>Sulfuric Acid/Permanganate Cleanup</td>
<td>Oxidation/reduction</td>
</tr>
</tbody>
</table>

To quote from Method 3600C:

The purpose of applying a cleanup method to an extract is to remove interferences and high boiling material that may result in … errors in quantitation … false positives … false negatives … rapid deterioration of capillary GC columns … instrument downtime due to contamination of detectors, inlets and Ms ion sources.
These techniques may be used individually or in various combinations, depending on the extent and nature of the co-extractives.

Stroll into any pesticide residue analysis laboratory that utilizes LLE and S-LSE sample prep techniques and you will see glass columns of dimensions 1 to 4 cm i.d. × 15 to 60 cm in length. These glass columns contain a retaining frit or glass wool plug at the bottom. The column is packed with one or more of the commercially available and highly purified absorbents listed above. A sketch of a simple glass column used to conduct LPLC is shown below:

Adsorption used to clean up nonpolar extracts is a normal-phase chromatographic phenomenon. Polar interferences are separated from nonpolar targeted analytes of interest to both enviro-chemical and enviro-health analysis. For example, an extract that contains both PAHs and fatty acids upon being passed through a properly deactivated alumina column will separate both compound classes. Deactivation is a term used in adsorption chromatography to mean that the active sites have been “tied up” by the addition of water. PAHs would be weakly retained while fatty acids would be strongly retained owing to the terminal carboxylic acid functional group. The strongly retained and more polar fatty acids will not elute if the mobile phase is of low polarity. Common low-polarity solvents include hexane, heptane, and cyclohexane. The underlying principles of column chromatography are introduced in Chapter 4. Scheme 3.4 places each of the 3000 series cleanup methods in a logical tree structure while adding acid–base hydrolysis. Acid–base hydrolysis is not listed as a method in SW-846. In addition to adsorption, each cleanup method from SW-846 is briefly described in the next paragraph.

“Acid-Base Partitioning,” Method 3650, is an LLE technique that separates organic analytes and interferences from neutral organic compounds of enviro-chemical or enviro-health interest. For example, neutral PAHs are separated from acidic phenols when analyzing a hazardous waste site contaminated with creosote.
and pentachlorophenol. Sulfur Cleanup, Method 3660, is used to eliminate elemental sulfur from sample extracts that could interfere with chromatographic determinative techniques. Sulfuric Acid/Permanganate Cleanup, Method 3665, provides rigorous cleanup of sample extracts prior to the analysis of samples that might contain PCBs. Most OCs, such as Dieldrin and other cyclodiene insecticides, are destroyed chemically by this vigorous oxidation. Size exclusion or Gel Permeation Cleanup (GPC), Method 3640, is the most universal of the cleanup techniques and is based on the principle of size exclusion liquid chromatography. GPC can separate broad classes of SVOCs and pesticides. GPC effectively removes lipids and other
high-molecular-weight interferences from nonpolar sample extracts. GPC is most often applied to cleanup sample extracts with appreciable oil or lipid (fat) content, such as petroleum-contaminated soil or fish tissue and, as we shall see, animal feed. Application of GPC to cleanup will be discussed in more detail shortly. Adsorption chromatography enables another important facet of sample prep to be considered: the separation of compound classes, commonly referred to as fractionation.

42. CAN ADSORPTION CHROMATOGRAPHY BE USED TO ACHIEVE COMPOUND CLASS FRACTIONATION IN ADDITION TO CLEANUP?

Yes, indeed. Both Florisil and silica gel adsorption chromatography, under LPLC conditions, provide opportunities to achieve compound class fractionation. Najam and coworkers at the CDC recently published their fractionation scheme that enabled them to resolve a mixture of OCs and all 209 PCB congeners from human serum. Scheme 3.5 is adapted from this paper and it shows the logic of how both adsorbents were used in sequence to achieve a partial separation of PCBs from OCs. EPA Method 3620C extends adsorption chromatography using larger glass column LPLC and smaller NP-SPE using cartridges to achieve a compound class fractionation. Scheme 3.6 is a flowchart that outlines how a mixture of OCs and PCBs can be separated by the use of binary eluents that are miscible yet of sufficient solvent polarity ($P'$) differences. Principles that underlie $P'$ will be discussed later in this chapter. When oil and grease contaminate an environmental sample or when the lipid content of a biological sample is appreciable, GPC is a good first cleanup approach to the concentrated sample extract.

43. HOW DOES GPC CLEAN UP OILY SAMPLE EXTRACTS?

Gel permeation chromatography (GPC) involves the passage of a mobile phase of low polarity across a polystyrene-divinyl benzene (PS-DVB) or silica stationary phase whose average pore size covers a range from 100 to 1,000,000 Å (silica). Preparative-scale GPC in contrast to analytical-scale GPC is most likely found in laboratories that conduct TEQA. EPA Method 3640 established GPC as the principal means to remove lipid and other higher-molecular-weight coextractives. The method requires a preparative-scale liquid chromatograph with or without an autosampler injector. An ultraviolet absorbance detector equipped with a preparative flow-through cuvette assembly is required for real-time readout. Of course, this analog detector can be interfaced through an A-to-D converter to a PC for complete automated data acquisition and control of the chromatograph. This author first set up a GPC for cleanup of oil-laden soil from Superfund hazardous waste sites back in the mid-1980s. The sole supplier back then of preparative GPC instruments for environmental testing labs to implement EPA Method 3640 was ABC Laboratories, Inc. Several companies today offer preparative GPC instruments configured to provide cleanup of oily or lipid-enriched sample extracts.
The principle of separation via GPC is briefly described as follows. Separation in GPC depends on molecular size, which in turn depends on molecular weight (MW). Figure 3.13, a hypothetical plot of MW against GPC retention volume, \( V_R \), is a classic drawing that explains chromatographic separation by size exclusion very nicely. To illustrate, for all compounds with MWs of <1000, total permeation occurs. This means that the volume of mobile phase required for low-MW compounds to elute from the column is very large. On the other extreme, compounds with MWs of >100,000 do not penetrate the gel and elute with the mobile phase or eluent. This is called size exclusion, and the large MW compounds are said to be size excluded from the gel. Molecules represented by those from peak F are much smaller than...
molecules represented by those from peak C. Compounds represented by bands D, E, and F are separated in the selective permeation region. This region is often referred to as the fractionation region of the packing.

The calibration procedure in Method 3640 recommends that corn oil, bis(2-ethylhexyl)phthalate (bis), and pentachlorophenol (PCP) be separated and collected in the fractionation region. Corn oil consists of higher-MW triglycerides and could represent band E. PCP is typical of many substituted phenols and could represent band F. It is important to know exactly how many milliliters of eluent are required to dump oils while desiring to collect analytes of interest, such as priority

**SCHEME 3.6**

![Scheme 3.6 Diagram](image)

© 2006 by Taylor & Francis Group, LLC
Trace Environmental Quantitative Analysis, Second Edition

FIGURE 3.13 (Top) Plot of hypothetical MW vs. GPC retention volume. (Bottom) A typical GPC chromatogram.

pollutant organic compounds of enviro-chemical or enviro-health interest. A GPC chromatogram is sketched below:
Once it is known how many milliliters of mobile phase is required to elute the analytes of interest while discarding the high-MW fats and oils, extracts from Kuderna–Danish or rotary evaporative concentrative techniques can be injected directly into the 5-mL sample loop of the GPC chromatograph either manually or via autosampler. EPA Method 3640A is a more recently modified method that eliminates PCP in the recommended calibration mixture. Methoxychlor (representative of OCs), perylene (representative of PAHs), and sulfur (common interference in petroleum-contaminated soil) are added to the calibration mixture. The elution order then becomes:

corn oil peak 1 < corn oil peak 2 < bis < methoxychlor < perylene < sulfur

Bringing contemporary GPC capability to a laboratory requires an investment in instrumentation. Hydrolysis as a cleanup technique, shown in Scheme 3.4, although not included in SW-846, becomes useful when the lipid content is appreciable and offers an alternative to GPC. Acid and base hydrolysis can be applied; however, lipids are easily decomposed in the presence of alcoholic potassium hydroxide. The author’s work in isolating PCBs from animal feed nicely illustrates the use of saponification as yet another cleanup tool in the arsenal.

44. HOW IS SAPONIFICATION USED TO CLEAN UP RAT FEED TO ISOLATE PCBS?

This question is answered by focusing on a specific laboratory procedure that this author has used to chemically decompose lipid and release lipophilic PCBs from animal feed (rat ration reported with ~7% lipid as measured gravimetrically). This procedure is adapted from that published earlier by Erickson and is given via a series of steps below:

- To prepare unspiked and spiked control feed samples (a control feed sample is a sample of feed unadulterated by PCBs), place 10 g of control feed into a 40-mL vial, seal and place on a shaker, and label as “unspiked control feed.”
- Place 10 g of control feed into a 40-mL vial and add 500 µL of 10 ppm TCMX (dissolved in MeOH). Place on a shaker and label as “spiked control feed.”
- Shake for 1 h, transfer contents of 40-mL vial to Soxhlet thimble, and place thimble in Soxhlet apparatus. Weigh a 250-mL round-bottom flask, add 230 mL of methylene chloride, and conduct S-LSE for anywhere from 12 to 24 h.
- Remove solvent from the extract via rotary evaporation. This should leave a mass of oily residue at the bottom of the round-bottom flask. Weigh the flask; this gives a gravimetric determination of the percent lipid in the 10 g of control feed.
- Add 25 mL of 2.5% KOH (dissolved in ethanol), add boiling chips to prevent bumping, and connect the flask to the water condenser and reflux for 1 h or until the oily phase disappears.
Cool, then transfer the solution to a 250-mL separatory funnel that already contains 25 mL of distilled deionized water. Rinse the extraction flask with 25 mL of hexane and add this washing to the sep funnel.

Shake the contents of the sep funnel for 1 min. This allows the PCBs to partition into the hexane phase.

Allow phases to separate and withdraw the lower layer (aqueous) into a second sep funnel.

Extract the saponification solution with a second 25-mL aliquot of hexane. After the phases have separated, withdraw the aqueous phase. Add 25 mL of hexane to the aqueous phase and perform a third LLE.

Combine all three hexane extracts and discard the saponified aqueous phase.

Concentrate the hexane extract to 5 mL or less using either a rotary evaporator or a Kuderna–Danish evaporative concentrator.

Transfer the concentrated hexane extract to either a 1-, 2-, or 5-mL volumetric flask, and adjust to the calibration mark of the flask with hexane.

Transfer to a 2-mL GC vial and inject 1 µL via autosampler into a C-GC-ECD or equivalent.

A 99.0% recovery of TCMX and a 107% recovery of AR 1242 (a commercially made mixture of 20 to 30 PCB congeners) was obtained using the procedure as outlined above.59

Consider a move both horizontal and vertical to the so-called supercritical region. We can accomplish similar analytical objectives to those addressed by ASE and those of interest to the practice of TEQA by choosing a fluid whose supercritical pressures and temperatures can be reached in the laboratory. With the availability of carbon dioxide, an inexpensive fluid with relatively low supercritical fluid values (SFE), advanced.

45. HOW DO I GET STARTED DOING SFE?

In principle, all one needs is a means to introduce CO₂ into a high-pressure extraction cell and to collect the extract. You would need a means to both pressurize and elevate the temperature of carbon dioxide. A critical temperature of 31.4°C with a critical pressure of 73 atm is needed to maintain CO₂ in a supercritical state. Consider again the phase diagram shown in Figure 3.7. Unlike liquids, if CO₂ is kept above 31.4°C, it cannot be liquefied no matter how high the pressure. In this supercritical fluid state, the physical properties, such as liquid-like density, intermediate diffusivity, gas-like viscosity, and gas-like surface tension, provide a medium for rapid and selective extraction. SFE as an alternative to S-LSE was, in essence, oversold during the late 1980s and early 1990s. SFE does exhibit a matrix dependence. The use of organic modifiers has helped, however. Like ASE, SFE instrumentation is very expensive, and if only a few samples are to be prepared and analyzed for trace
organics in environmental matrices, is it really worth the cost? In this section, we will discuss how SFE works and then, as we did for ASE, we will delve into some recovery studies.

46. WHAT LIQUIDS AND GASES CAN BE USED TO CONDUCT SFE?

Table 3.4 lists nine fluids together with their respective boiling points, critical temperatures, critical pressures, and critical densities. From this list of nine, several can be eliminated as being either too hazardous or too corrosive to work with, such as ammonia, or as requiring too high a temperature, such as water and methanol. Carbon dioxide, nitrous oxide, xenon, ethane, ethylene, sulfur hexafluoride, and very recently fluoroform, CHF₃, have emerged as the likely candidates. Of these most suitable supercritical fluids (SFs), carbon dioxide emerges as the supercritical fluid of choice because CO₂ is nontoxic and inexpensive and exhibits a relatively low critical temperature and pressure. Its critical density is also appropriate. CO₂ is considered under SF conditions as having a solvent polarity about that equal to hexane — in other words, nonpolar. The need to add polar, so-called matrix modifiers has led to increases in the percent recoveries of some of the more polar priority pollutants. The poor recovery of polar analytes from relatively nonpolar sample matrices has led to the notion of inverse SFE, whereby the sample matrix can be completely removed by SFE while the more polar analyte remains. This has much more relevance to pharmaceutical analysis than to TEQA because sample matrices tend to be more polar than the analyte of interest. In the determination of polar ingredient in an ointment, the sample matrix is extracted by the relatively nonpolar CO₂, leaving the polar-active ingredient in the SFE extraction vessel. Chester⁶⁰ has recently reviewed the eight most likely candidates for use as supercritical fluid chromatography (SFC); this information is also pertinent to SFE.

---

**TABLE 3.4**
Physico-chemical Properties for the Common Supercritical Fluids

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Tₑ (°C)</th>
<th>Tₑ (°C)</th>
<th>Pₑ (atm)</th>
<th>ρₑ (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>−78.5</td>
<td>31.3</td>
<td>72.9</td>
<td>0.448</td>
</tr>
<tr>
<td>NH₃</td>
<td>−33.3</td>
<td>132.4</td>
<td>112.5</td>
<td>0.235</td>
</tr>
<tr>
<td>H₂O</td>
<td>100.0</td>
<td>374.1</td>
<td>218.3</td>
<td>0.315</td>
</tr>
<tr>
<td>N₂O</td>
<td>−88.6</td>
<td>36.5</td>
<td>71.7</td>
<td>0.450</td>
</tr>
<tr>
<td>C₂H₆</td>
<td>−88.6</td>
<td>32.3</td>
<td>48.1</td>
<td>0.203</td>
</tr>
<tr>
<td>Xe</td>
<td>−108.1</td>
<td>16.6</td>
<td>58.4</td>
<td>1.10</td>
</tr>
<tr>
<td>CH₃OH</td>
<td>64.7</td>
<td>240.5</td>
<td>78.9</td>
<td>0.272</td>
</tr>
<tr>
<td>SF₆</td>
<td>63.8 (sublimes)</td>
<td>45.5</td>
<td>7.1</td>
<td>0.740</td>
</tr>
<tr>
<td>C₃H₄</td>
<td>−103.7</td>
<td>9.2</td>
<td>49.7</td>
<td>0.218</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
47. WHAT OTHER PHYSICO-CHEMICAL PROPERTIES ARE OF INTEREST IN SFE?

Supercritical fluids begin to exhibit significant solvent strength when they are compressed to liquid-like densities. Figure 3.14 shows how the density of a pure SF changes as the SF is compressed. The greatest change in SF density, \( \rho \), is seen to occur in the region near to its critical point. An increase in the density of a supercritical fluid has been shown to increase the amount of a chemical compound that will dissolve in the SF.\(^{61} \) Certain other physico-chemical properties give SFs advantages with respect to SFE as well. The more a SF is compressed, the more liquid-like the fluid becomes. Very high density SFs (i.e., \( P >> P_c \)) are very liquid-like fluids. Very low density SFs, where \( T > T_c \) and \( P \sim P_c \), are very gaseous-like. The three most useful physico-chemical properties of fluids, density, viscosity, and diffusion coefficient, are listed for gases, SFs, and liquids in Table 3.5. Interpretation of Table 3.5 clearly shows how SFs are intermediate between gases and liquids with respect to all three properties. It is not surprising that SFs are denser than gases and, in turn, less dense with respect to liquids. SFs are more viscous than gases, yet less viscous by one order of magnitude than liquids. Diffusion coefficients are more

---

**FIGURE 3.14** Influence of compressed gas pressure on the gas density of a supercritical fluid.
important in consideration of SFC; however, it is interesting to compare the range of values of $D$ for SFs vs. liquids. SFs diffuse over 100 times faster than liquids.

The need to add organic modifiers to the supercritical CO$_2$ implies that a second component be considered from the perspective of the Gibbs phase rule. For two components, the number of degrees of freedom, $f$, is found according to $f = 4 - p$. Hence, for a one-phase region in the phase diagram where $p = 1$, three degrees of freedom are needed. In general, in addition to pressure and temperature, composition becomes this third degree of freedom. Therefore, phase diagrams must be viewed in three dimensions. To facilitate a two-dimensional view, in which regions of identical temperature can be plotted, so-called isotherms can be shown. Figure 3.15 is a three-dimensional representation of a pressure–temperature–composition ($P$-$T$-$X$) plot for a binary mixture. Figure 3.15 has been labeled to show the extremes of the composition axis where, using the example of a CO$_2$/MeOH binary mixture,
we start with pure CO₂ on one end and pure methanol on the other. The liquid–vapor equilibrium line is drawn for 0% MeOH (100% CO₂) and for 100% MeOH (0% CO₂). The dashed line connects the locus of mixture critical points. The significance of $P-T-X$ plots for binary SF mixtures is that for values of $P$, $T$, and $X$ values that reside below the curve, two phases are possible depending on the composition. If 10% MeOH is added as an organic modifier when using carbon dioxide as the SF, this plot is useful in knowing what conditions need to be reached to keep the SFE in one phase.

48. **OF WHAT DOES SFE INSTRUMENTATION CONSIST?**

A block diagram of the principal components of an SFE instrument is shown in Figure 3.16. It must be recognized that SFE-SFC-grade CO₂ requires a purity greater than 99.999%. The SF delivery system can be either a high-pressure reciprocating pump or a high-pressure syringe pump. One’s preference as to which type of pump to use depends on choice and cost considerations. It is necessary to cool the pump head when using a reciprocating pump. This need for cooling is unnecessary when using a syringe pump because CO₂ is liquefied by pressure, not temperature. A syringe pump also eliminates the inconsistencies in repressurization that might be present in a reciprocating pump; in other words, it provides a pulseless flow of CO₂. A syringe pump, however, is limited in capacity and must be refilled while a reciprocating pump draws continuously from a large reservoir. A second reciprocating pump is needed if the addition of an organic modifier is needed. This second pump does not need to be cooled. There have been other designs discussed in the literature as well, necessitated by the high cost of having to cool reciprocating pumps.

The sample cell or vessel is usually made of stainless steel of some other inert material and ranges in size from 150 µL to 50 mL. It is most important that the sample vessels are rated for the maximum working pressure of the system. Cell designs are either of a flow-through (dynamic) or headspace sampling (static) type. Flow-through cells enable the SF to continuously pass through the sample. Static headspace sample cells enable the SF to surround the sample while the headspace above the sample is withdrawn. Headspace sampling tends to be used for large

![FIGURE 3.16 Schematic of principal components of an SFE instrument.](image-url)
samples, typically 40 mL. The sketch below is a schematic showing the fundamental difference between these two cell designs.\textsuperscript{62}

The restrictor or depressurization step is very important and, over the years, has been the Achille’s heel of SFE. Plugging of restrictors was a major problem when this device consisted of an unheated fused-silica capillary tube. Restrictor problems have been minimized or even eliminated with the development of automated, variable restrictors.\textsuperscript{63} Variable restrictors use position sensors, motorized gears, and feedback from the CO\textsubscript{2} pump to control the flow set point and make flow adjustments. The restrictor is responsible for maintaining the pressure of the SF above its \( P_c \). The restrictor induces a back-pressure in the sample cell. For a CO\textsubscript{2}-based SFE, the pump delivers the CO\textsubscript{2} in liquid form at a constant flow rate and SF conditions are established in the heated extraction chamber. The smaller the restrictor orifice, the higher the back pressure in the line and the greater the mass flow rate. The final part of SFE instruments is the collection device. Two approaches have been developed for this aspect of SFE instruments, online and offline. Online SFE refers to the coupling of the analyte-containing SF to a chromatographic separation system, and abbreviations are used to indicate which determinative technique is interface. For example, SFE-GC implies that supercritical fluid extraction has been interfaced to a gas chromatograph. Offline SFE refers to the stand-alone instrument whose block diagram is shown in Figure 3.16.

The collection device stores the extracted analyte in one of three ways: (1) via thermal trapping, (2) by sorbent trapping, or (3) by solvent trapping. Thermal trapping is the simplest experimentally and merely involves directing the depressurized SFE effluent into a cooled vessel. Moderately volatile analytes are known to be lost using this type of SFE trapping; therefore, use of thermal trapping is limited to semivolatile and nonvolatile organic analytes. Sorbent trapping incorporates a solid adsorbent whose surface is capable of removing the extracted analytes from the SF effluent. The sorbed analytes are removed from the adsorbent by elution with a solvent. Sorbent trapping affords the opportunity to incorporate a degree of analyte selectivity either by selection of an appropriate sorbent or by choice of elution solvent. Commercially available chemically bonded silicas have been used with success in sorbent trapping. We will have much more to say about the use of these
materials with respect to the sample prep technique called solid-phase extraction later. Solvent trapping is the most widely used method of trapping analytes from the extracted SF effluent and merely involves depressurizing the SF directly into a small vial containing a few milliliters of liquid solvent. Hawthorne reports a tendency toward higher percent recoveries for sand that has been spiked with selected semi-volatile organics such as phenol, nitrobenzene, and benzo(a)pyrene with a change in the chemical nature of the collection solvent. The following solvents were placed in a collection vial, and the order in terms of a percent collection efficiency was hexane < methanol < methylene chloride < methylene chloride kept at 5°C (by using a heating block).64

It has been well established that SF CO₂ is not a good extractant for polar analytes. In addition, some analyte–matrix interactions are sufficiently strong to yield low percent recoveries. The recovery of three PCB congeners was compared against Soxhlet extraction while using four different SFs, including SF CO₂ modified with methanol, shown in Table 3.6.64 The results show that the more polar SFE (CO₂/MeOH) yields as good a recovery as CHClF₂, whose dipole moment is 1.4 Debyes. Pure CO₂ has a dipole moment of 0.0 Debyes, and the addition of a polar modifier like MeOH serves to increase the dipole moment of the SF fluid. This author is not aware if there are any published dipole moment values for polar-modified SF CO₂.

49. IS THERE AN EPA METHOD THAT REQUIRES SFE?
IF SO, PLEASE DESCRIBE.

Yes, there is; it is a recently developed method in the SW-846 series of methods that are most applicable to solid waste samples. It is Method 3562, “SFE of Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides (OCs).”65 Twelve specific PCB congeners of either the tri-, tetra-, penta-, hexa-, or heptachlorobiphenyl variety have been studied in this method. Fourteen of the priority pollutant OCs from aldrin through to heptachlor epoxide have also been studied in this method. SFE is used to extract these analytes from soils, sediments, fly ash, solid-phase extraction media, and other solids materials that are amenable to extraction with conventional solvents. The uniqueness of this method is that it is possible to achieve a class separation (i.e., PCBs from OCs) by differences in SFE conditions. Samples

TABLE 3.6
Comparison of Soxhlet Extraction and Different SFE Fluids for the Recovery of PCB Congeners from River Sediment

<table>
<thead>
<tr>
<th>PCB Congener</th>
<th>Soxhlet</th>
<th>CHClF₂</th>
<th>N₂O</th>
<th>CO₂</th>
<th>CO₂/MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 2', 3, 5'</td>
<td>1070</td>
<td>1158</td>
<td>497</td>
<td>470</td>
<td>855</td>
</tr>
<tr>
<td>2, 3', 4', 5'</td>
<td>510</td>
<td>631</td>
<td>319</td>
<td>382</td>
<td>482</td>
</tr>
<tr>
<td>2, 2', 3, 4', 5'</td>
<td>160</td>
<td>166</td>
<td>145</td>
<td>144</td>
<td>168</td>
</tr>
</tbody>
</table>

to be analyzed for PCBs are subjected to a 10-min static extraction, followed by a 40-min dynamic extraction. Samples for OCs are subjected to a 20-min static extraction, followed by a 30-min dynamic extraction. The analyst must demonstrate that the SFE instrument is free from interferences. This is accomplished by extracting method blanks and determining the background contamination or lack thereof. The method cautions the user about carryover. Reagent blanks should be extracted immediately after analyzing a sample that exhibits a high concentration of analytes of interest. This method does not use a polar organic modifier. Referring again to the schematic of SFE instrumentation, Figure 3.16, Method 3562 specifies the nature of the extraction vessel, restrictor, and collection device. Vessels must be stainless steel with end fittings, with 2 µm first. Fittings must be able to withstand pressures of 400 atm (5878 psi). The method was developed using a continuously variable nozzle restrictor. Sorbent trapping is the collection device used in the method. Florisil with a 30- to 40-µm particle diameter is recommended for PCBs, whereas octadecylsilane is suggested to trap OCs. Solvent trapping is also suggested, with a cautionary remark concerning the loss of volatile analytes. The method requires the use of internal standards and surrogate standards. For sorbent trapping, n-heptane, methylene chloride, and acetone are recommended. The method also requires that a percent dry weight be obtained for each solid sample. The weight loss that accompanies keeping 5 to 10 g of the sample in a drying oven overnight at 105°C is measured. The sample may need to be ground to ensure efficient extraction. To homogenize the sample, at least 100 g of ground sample is mixed with an equal volume of CO₂ solid “snow” prepared from the SFE CO₂ solid. This mixture is to be placed in a food type chopper and ground for 2 min. The chopped sample is then placed on a clean surface, and the CO₂ solid snow is allowed to sublime away. Then, 1 to 5 g of the homogenized sample is weighed and placed in the SFE vessel. If the samples are known to contain elemental sulfur, elemental copper (Cu) powder is added to this homogenized sample in the SFE vessel. No adverse effect from the addition of Cu was observed, and EPA believes that finely divided Cu may enhance the dispersion of CO₂. To selectively extract PCBs, the following SFE conditions using SF CO₂ are recommended:

- Pressure: 4417 psi
- Temperature: 80°C
- Density: 0.75 g/mL
- Static equilibration time: 10 min
- Dynamic extraction time: 40 min
- SFE fluid flow rate: 2.5 mL/min

To extract OCs, the recommended SFE conditions are as follows:

- Pressure: 4330 psi
- Temperature: 50°C
- Density: 0.87 g/mL
- Static equilibration time: 20 min
- Dynamic extraction time: 30 min
- SFE fluid flow rate: 1.0 mL/min

© 2006 by Taylor & Francis Group, LLC
50. HAVE COMPARATIVE STUDIES FOR SFE BEEN DONE?

Yes, there have been comparative studies in which the percent recovery has been measured using not only SFE, but also ASE, as well as comparing these results to percent recoveries from the more conventional Soxhlet extraction (S-LSE). Generally, these studies are done on certified reference samples or glossy contaminated samples. We will discuss two studies from the recent analytical chemistry literature. The first study compared the efficiencies of SFE, high-pressure solvent extraction (HPSE), to S-LSE for removal of nonpesticidal organophosphates from soil. HPSE is very similar to accelerated solvent extraction; however, we will reserve the acronym ASE for use with the commercial instrument developed by Dionex Corporation. HPSE as developed by these authors used parts available in their laboratory. The authors compared S-LSE with SFE and HPSE for extraction of tricresyl phosphate (TCP) and triphenyl phosphate (TPP) from soil. Molecular structures for these two substances are as follows:

![Molecular structures of TCP and TPP](image)

*Ortho-, meta-, and para-*substitution within the phenyl rings can lead to isomeric TCPs. Aryl phosphate esters are of environmental concern due to their widespread use and release. They have in the past been used as fuel and lubricant additives and as flame-retardant hydraulic fluids. Widespread use of TCP associated with military aircraft have led to contamination of soil at U.S. Air Force (USAF) bases. TCP-contaminated soil samples were obtained from a USAF site. The percent water was determined. Spiked oil samples were prepared by adding an ethyl acetate solution containing TPP and TCP to 500 g of a locally obtained soil. This soil was placed in a rotary evaporator whereby the analytes of interest could be uniformly distributed throughout the soil. Methanol was added as the polar modifier directly into the soil in the SFE extraction chamber. It was deemed important to add methanol because TCP and TPP are somewhat polar analytes. The actual SFE consisted of 10 min of static SFE followed by 20 min of dynamic SFE. The sample, which consisted of 1.5 g of soil with 1.0 mL of sand, was placed in the bottom of the SFE vessel. A sorbent trapping technique using glass beads with subsequent washing with ethyl acetate was used to recover the analytes. The equivalent of ASE was conducted in this study, not with a commercial instrument as discussed earlier, but with a combination of a commercially available SFC syringe pump (Model SFC-500 from Isco...
Corp.) and a gas chromatographic oven (Model 1700 GC from Varian Associates). We will use the author’s abbreviation for high-pressure solvent extraction (HPSE). A series of SFE-related method development studies were first undertaken to optimize the effect of the volume of methanol and the effects of temperature and pressure on percent recoveries of TCP from native soil. In a similar manner, extraction conditions for HPSE were optimized by varying temperature and pressure. Optimized conditions arrived at were the following:

SFE: 80°C, 510 atm, and 1250 µL of MeOH
HPSE: 100°C and 136 atm

The determinative technique was capillary gas chromatography equipped with a nitrogen–phosphorous detector. The authors found the percent recoveries to be quite close to S-LSE while reporting that considerable time and solvent consumption could be saved. Next, we consider a second comparative study.

Heemken and coworkers\(^67\) in Germany reported on percent recovery results for the isolation and recovery of PAHs and aliphatic hydrocarbons from marine samples. Results were compared using accelerated solvent extraction (ASE), SFE, ultrasonication (U-LSE), methanolic saponification extraction (MSE), and classical Soxhlet (S-LSE). Both ASE and SFE compared favorably to the more conventional methods in terms of a relative percent recovery against the conventional methods, so that extracted analytes from ASE and SFE were compared to the same extracted analytes from S-LSE and U-LSE. Relative percent recoveries ranged from 96 to 105% for the 23 two through seven (coronene) fused-ring PAHs and methyl-substituted PAHs. To evaluate the percent recoveries, the authors defined a bias, \(D_{\text{rel}}\), according to

\[
D_{\text{rel}} = \frac{X_1 - X_2}{X_1} \times 100\%
\]

where \(X_1\) and \(X_2\) are the extracted yields for one method vs. another. For example, a summation of the amount of nanograms extracted for all 23 PAHs using SFE was 34,346 ng, whereas for S-LSE, it was 33,331 ng. Using the above equation gives a bias of 3.0%. Table 3.7 gives values for \(D_{\text{rel}}\) for PAHs for (1) SFE vs. either S-LSE or U-LSE and (2) ASE vs. either S-LSE or U-LSE. Three different environmental solid samples were obtained. The first was a certified reference marine sediment sample obtained from the National Research Council of Canada. The second sample was a suspended sediment obtained from the Elbe River in Germany using a sedimentation trap. This sample was freeze-dried and homogenized. The third sample was a suspension obtained from the Weser River and collected via a flow-through centrifuge. This sample was air-dried and had a water content of 5.3%. Values of \(D_{\text{rel}}\) were all within 10% among the four methods shown in Table 3.7, and it is safe to conclude that it makes no difference whether SFE or ASE, or S-LSE or U-LSE is used to extract PAHs from the three marine sediment samples. The other criterion used to compare different sample preparation methods is precision. Are SFE and ASE as reproducible as S-LSE and U-LSE? For the certified reference standard, an
overall relative standard deviation for PAHs was found to be 11.5%. For the first sediment, an RSD was found to range from 3.4 to 5.0%, and for the second sediment, RSD ranged from 2.7 to 7.5%. Let us now consider the isolation and recovery of one priority pollutant PAH from this work.

Benzo\((g,h,i)\)perylene (BghiP) is a five-membered fused-phenyl-ring PAH of molecular weight 277 and could be considered quite nonpolar. Its molecular structure is as follows:

\[
\begin{align*}
\text{Benzo (g, h, i) perylene}
\end{align*}
\]

Shown in Table 3.8 are the yields of analyte in amount of nanograms of BghiP per gram of dry sediment. Also included are yields from the MSE approach. MSE as defined in the Heemken paper is essentially a batch LLE in which the sediment is refluxed in alkaline methanol, followed by dilution with water, and then extracted into hexane. The yield of BghiP is similar for all five sample prep methods, as reported by the authors. The other significant outcome was to show that for a nondried marine sediment, the addition of anhydrous sodium sulfate increased the yield of BghiP from 35 to 96 ng, and this result came close to that obtained by the MSE approach.

This completes our digression into alternative sample prep techniques for solid matrices. We now return to aqueous samples and entertain a somewhat detailed discussion of the prime alternative to liquid–liquid extraction that emerged over 20 years ago, namely, liquid–solid extraction using chemically bonded silica gel, commonly called solid-phase extraction.
WHAT IS SOLID-PHASE EXTRACTION?

Solid-phase extraction (SPE) as a means to prepare environmental samples is a relatively recent alternative to LLE. SPE originated during the late 1970s and early 1980s as a means to preconcentrate aqueous samples that might contain dissolved semivolatile analytes that are amenable to analysis by gas chromatographic determinative techniques. SPE was first applied to sample preparation problems involving clinical or pharmaceutical types of samples and only much later evolved as a viable sample preparation method in TEQA.

The concept of column liquid chromatography as a means to perform environmental sample preparation was not immediately evident after the development of high-performance (once called high-pressure) liquid chromatography (HPLC) using reversed-phase silica as the stationary phase in the late 1960s. This technique is termed reversed-phase HPLC (RP-HPLC). It is also referred to as bonded-phase HPLC, and it is estimated that over 75% of HPLC being done today is RP-HPLC. As early as the 1930s, silica, alumina, Florisil, and Kieselguhr or diatomaceous earth were used as solid sorbents primarily for cleanup of nonpolar extractants, and the mechanism of retention was based on adsorption. The early realization that hydrophobic surfaces could isolate polar and nonpolar analytes from environmental aqueous samples such as groundwater came about with the successful use of XAD resins,

### TABLE 3.8
Recovery of BghiP in Amount of ng of BghiP/g of Sediment Using Five Sample Preparation Methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>SFE</th>
<th>ASE</th>
<th>S-LSE</th>
<th>U-LSE</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certified reference marine sediment 1726 ± 720</td>
<td>1483 (5.6), n = 6</td>
<td>1488 (7.4), n = 6</td>
<td>1397 (3.9), n = 3</td>
<td>1349 (3.8), n = 3</td>
<td>1419 (3.4), n = 3</td>
</tr>
<tr>
<td>Free-dried homogenized suspended particulate matter, I</td>
<td>147 (2.3), n = 6</td>
<td>156 (1.6), n = 3</td>
<td>207, n = 1</td>
<td>180 (1.6), n = 3</td>
<td></td>
</tr>
<tr>
<td>Air-dried suspended particulate matter, II</td>
<td>122.5 (1.2), n = 3</td>
<td>129.1 (0.4), n = 3</td>
<td>127.2 (0.1), n = 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II, nondried 56% water</td>
<td>35 (9.0), n = 3</td>
<td>32 (14.1), n = 3</td>
<td>98 (0.6), n = 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II, nondried, anhydrous sodium sulfate</td>
<td>96 (3.7), n = 6</td>
<td>32 (14.1), n = 3</td>
<td>98 (0.6), n = 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: n is the number of replicate extractions. The number in parentheses is the relative standard deviation expressed as a percent.*
whereby as much as 10 L of sample could be passed through the resin. By this time, bonded silicas that contained a hydrocarbon moiety covalently bonded were increasingly predominant in HPLC. One way that this rise in prominence for RP-HPLC can be seen is by perusing the earlier and pioneering text on HPLC and comparing the content in this text to a recently published text by the same authors.

It becomes clear that during this 20+-year period, RP-HPLC dominated the practice of column liquid chromatography. It will also become evident that the demands being made by EPA and state regulatory agencies on environmental testing labs required that sample extracts be made as free of background interferences as possible. Terms began to be used such as “the quality of the chromatogram is only as good as the extract.” A well-used cliché also applied: “Garbage into the GC-MS, garbage out.” All EPA methods during the 1980s that considered aqueous samples required LLE as the sole means to prepare environmental samples for TEQA. LLE was done on a relatively large scale whereby 1000 mL of groundwater or other environmental sample was extracted three times using 60 mL of extracting solvent each time. Because only 1 µL of extract was required, this left almost all of the extractant unused. As MDLs began to go to lower and lower values due in large part to regulatory pressures, it became evident that the 120 mL of extract could be reduced to 5 mL or less via some sort of vaporization of the solvent. This led to the use of lower-boiling solvents such as methylene chloride (dichloromethane), whose boiling point is 34°C. After all of this, the reduced volume of extract still had to be cleaned up so as to obtain a good signal-to-noise ratio, and hence to satisfy the now stringent MDL requirements.

The evolution of RP-SPE came about after the development of RP-HPLC. The concept that the hydrophilic silica gel, as a stationary phase, that had been used to pass a nonpolar mobile phase across it could be transformed to a hydrophobic stationary phase was a significant development. Organic compounds whose polarity ranges from moderate to low could be retained on these hydrophobic sorbents provided that the mobile phase was significantly more polar than that of the stationary phase. If the particle size could be decreased down to the smallest mesh size, a sorbent material with a relatively large surface area would provide plenty of active sites. A surface that was also significantly hydrophobic as well would facilitate removal of moderately polar to nonpolar analytes from water. The stage was set then to bring RP-SPE into the domain of TEQA. This was accomplished throughout the 1980s and led to a plethora of new methods, applications, and SPE suppliers. EPA, however, could not at first envision a role for SPE within its arsenal of methods, and hence largely ignored these developments. It did, however, offer to fund some researchers who were interested in demonstrating the feasibility of applying SPE to environmental samples. It became necessary then for analytical chemists engaged in method development to conduct fundamental studies to determine the percent recovery of numerous priority pollutant semivolatile organics from simulated or real environmental samples. This was all in an attempt to validate methods that would incorporate the SPE technique. This author was one such researcher who, while employed in a contract lab in the late 1980s, received a Phase I Small Business Innovation Research (SBIR) grant to conduct just such studies.
As is true for any emerging and evolving technology, SPE began with a single commercially available product, the Sep-Pak cartridge, developed in 1978 at Waters Associates. These devices, designed to fit to a standard liquid-handling syringe, were at first marketed to the pharmaceutical industry. The focus was on a silica cartridge for what would be considered today as the practice of normal-phase SPE (NP-SPE). The idea that a large volume of sample could exceed 10 mL led to the development in 1979 of the so-called barrel design. Analytichem International, now Varian Sample Preparation Products, was first to introduce the SPE barrel, which in turn could be fitted to a vacuum manifold. This design consisted of a cylindrical geometry that enabled a larger reservoir to be fitted on top while the Luer tip on the bottom of the barrel was tightly fitted to the inlet port of a vacuum manifold. The vacuum manifold resembled the three dimensional rectangular-shaped thin-layer chromatographic development tank. The sorbent bed consisted of chemically bonded silica of irregular particle size with a 40-µm average and was packed between a top and a bottom 20-µm polypropylene frit. This configuration opened the door to the application of SPE techniques to environmental aqueous samples such as drinking water. It was left to researchers to demonstrate that priority pollutant organics dissolved in drinking water could be isolated and recovered to at least the same degree as was well established using LLE techniques. J.T. Baker followed and began to manufacture SPE cartridges and syringe formats in 1982. It is credited with coining the term SPE, as opposed to the EPA's term liquid–solid extraction. Further evolution of the form that SPE would take was sure to follow.

In 1989, the bulk sorbent gave way to a disk format in which 8- to 12-µm C18 silica was impregnated within an inert matrix of polytetrafluoroethylene (PTFE) fibrils in an attempt to significantly increase the volumetric flow rate of water sample that could be passed through the disk. These developments were made by the 3M Corporation. It coined the term Empore Disk. The next major development in SPE design occurred in 1992 as Supelco introduced a device called solid-phase micro-extraction (SPME). This followed the pioneer developments by Pawliszyn and coworkers. The GC capillary column, a topic to be discussed in Chapter 4, was simply inverted. The polydimethyl siloxane polymer coating was deposited on the outer surface of a fused-silica fiber. This fiber is attached to a movable stainless-steel “needle in a barrel” syringe. This design is similar to the 7000 series manufactured by the Hamilton Company for liquid-handling microsyringes. SPME is a solventless variation of SPE in that the coated fiber is immersed within an aqueous phase or in the headspace above the aqueous phase. After a finite period, the fiber is removed from the sample and immediately inserted directly into the hot-injection port of a GC. The analytes are then removed from the fiber by thermal desorption directly into a GC column. We will discuss the principles and practice of SPE and then focus on RP-SPE as applied to TEQA. Some of the work of the author will also be included.

52. HOW IS SPE DONE?

SPE is performed using a variety of consumable items. These items include encapsulated Sep-Pak SPE devices that fit on the end of a handheld plastic disposable
syringe; 47-mm octadecyl or octyl-bonded silica-impregnated PFTE circular disks; and barrel type cartridges packed with bulk sorbent or fitted with bonded silica-impregnated PFTE disks fitted with Luer adapters. Volumes ranging from 1 to 60 cc present the most contemporary barrel cartridge design to conduct up-front reversed-phase (RP-SPE) or normal-phase (NP-SPE) solid-phase extraction. Perusal of catalogs from current suppliers such as Supelco, Alltech, Varian, Waters, and Phenomenex, among others, is the quickest way to become knowledgeable as to what is available. A sketch (not drawn to scale) that depicts the barrel type design for passing an aqueous sample (sample loading) through a conditioned RP-SPE sorbent is shown below:

Barrels and reservoirs have remained the same since their inception; however, some innovation is evident in making the Luer adapter fit more than one barrel mouth size. If an adapter is placed atop the 70-mL polypropylene reservoir and connected to a much larger reservoir, such as an HPLC type container, the vacuum manifold can be modified to pass much larger water samples through the sorbent. SPE as a system has been completely automated. Companies such as Caliper Life Sciences, formerly Zymark, Hamilton, and Gilson, among others, have automated SPE systems commercially available. Automated systems that incorporate a 96-well plate have become a popular adaptation of SPE to pharmaceutical industry interest in recent years.

All forms of SPE generally follow the same four-step procedure. This process comprises (1) sorbent conditioning, (2) passage of the sample through the sorbent, commonly called sample loading, (3) removal of interferences, and (4) elution of the analyte of interest into a receiving vessel. The four steps that are involved in
performing methods that require SPE as the principal means of sample preparation are now discussed:

1. **Sorbent conditioning:** This initial step is particularly important in order to maximize the percent recovery when using RP-SPE with an aqueous sample. Also, once conditioned, the sorbent should not be allowed to dry. If dried, the sorbent should be reconditioned. For RP-SPE, methanol (MeOH) is usually used to condition, whereas for NP-SPE, n-hexane is common. The need for conditioning in RP-SPE has been discussed in the literature. The brush-like nature of the organo-bonded hydrophobic surface is somewhat impermeable. It is as if a thin hydrophobic membrane has been placed on top of the surface. Organics dissolved in water upon passing through this sorbent only partially penetrate this semipermeable membrane. If the sorbent is not conditioned, low and variable percent recoveries from RP-SPE will result. Upon passing MeOH or other polar solvent across the sorbent, the brushes line up and, by interacting with MeOH, become more “wetted.” This hydrophobic membrane becomes permeable, and hence dissolved organics can more effectively penetrate this hypothetical membrane barrier. Conditioning has been thought of in terms of being brush-like. This author has found that by careful adjustment of the level of MeOH by removing trapped air between the reservoir and cartridge in the barrel type SPE column, MeOH can be passed through the sorbent, followed by the water sample, without exposing the sorbent to air. Some analysts, after conditioning, will pass the eluting solvent through the wetted sorbent. The eluting solvent in many cases of RP-SPE is much less polar than MeOH. In this way, the sorbent can be cleaned of organic impurities. This is also a useful technique to recondition and reuse SPE cartridges. This author has reported even slightly higher percent recoveries when conducting RP-SPE on a previously used cartridge.

2. **Sample loading:** The second step in SPE involves the passage of the sample through the previously conditioned sorbent. The sorbent might consist of between 100 mg and 1 g of bonded silica. The two most common hydrophobic bonded silicas are those that contain either a C₈ or a C₁₈ hydrocarbon moiety chemically bonded to 40-μm-particle-size silica gel. Alternatively, a disk consisting of impregnated C₈ or C₁₈ silica in either a Teflon or glass–fiber matrix is used. For RP-SPE, an aqueous water sample is usually passed through the sorbent. Samples that contain particulates or suspended solids are more difficult to pass through the sorbent. Eventually, the top retaining frits will plug. This drastically slows the flow rate, and if the top frit gets completely plugged, there is no more SPE to be done on that particular cartridge. Suspended solids in water samples present a severe limitation to sample loading. A filtration of the sample prior to SPE sample loading will usually correct this problem. Adding the 70-mL reservoir on top of the SPE barrel type cartridge enables a relatively large sample volume to be loaded. Upon frequent refilling of the reservoir, a groundwater sample of volume greater than the 70-mL capacity, up to
perhaps 1000 mL, is feasible. Alternatively, the top of the 70-mL reservoir can be fitted with an adapter and a plastic tube can be connected to a large beaker containing the groundwater sample. The design of the multiport SPE vacuum manifold enables 5 or 10 or more samples, depending on the number of ports, to be simultaneously loaded.

3. **Removal of interferences**: The third step in SPE involves passing a wash solution through the sorbent. The analytes of interest have been retained on the sorbent and should not be removed in this step. In RP-SPE, this wash solution is commonly found to be distilled deionized water. In other cases, particularly when ionizable analytes are of interest, water that has been buffered so that the pH is fixed and known is used as the wash solution. The wash solution should have a solvent strength not much different from that of the sample, so that retained analytes are not prematurely removed from the sorbent. In addition, air can be passed through the sorbent to facilitate moisture removal. It is common to find droplets of water clinging to the inner wall of the barrel type cartridge. Passing air through during this step is beneficial. Also, a Kim-Wipe or other clean tissue can be used to remove surface moisture prior to the elution step. Removal of surface water droplets requires disassembly of the reservoir–adapter–barrel. If a full set of SPE cartridges is used, it is a bit time consuming to complete. At this point in the process, the sorbent could be stored or transported. Too few studies have been done to verify or refute the issue of whether analytes are stable enough to be sampled in the field and then transported to the laboratory.

4. **Elution of retained analyte**: The fourth and last step in SPE involves the actual removal of the analyte of interest; hopefully, the analyte is free of interferences. This is accomplished by passing a relatively small volume of a solvent, called an eluent, whose solvent strength is very strong so that the analyte is removed with as small a volume of eluent as possible. This author has been quite successful, particularly when using barrel type SPE cartridges, in using less than 1 mL of eluent in most cases. A common practice is to make two or three successive elutions of sorbent such that the first elution removes >90% of the retained analyte, and the second or third removes the remaining 10%. If there is evidence of water in the receiving vessel, the analyst can add anhydrous sodium sulfate to the receiving vessel. This is particularly relevant for RP-SPE after passage of a drinking water or groundwater sample. Alternatively, a second SPE cartridge can be placed beneath the first in a so-called piggyback configuration to remove water, remove lipid interferences, and fractionate. As a nonpolar to moderately polar elution solvent or binary solvent is passed through the SPE cartridge while eluting the retained analytes, the eluent is also passed through this second SPE cartridge that contains anhydrous sodium sulfate. A sketch that depicts the piggyback style for the common 3-mL barrel size is shown below.
These four steps, which comprise the contemporary practice of SPE, serve to transform an environmental sample that is, by itself, incompatible with the necessary determinative technique, most commonly gas chromatography. **Scheme 3.7** is a flowchart to assist the environmental analytical chemist in deciding how to approach analytical method development utilizing SPE. The logic used strongly depends on the chemical nature of the semivolatile analyte of interest and on the sample matrix in which the analyte of interest is dissolved. SPE is applicable only to semivolatile to nonvolatile organic analytes. The use of a vacuum to drive the aqueous sample through the sorbent eliminates any applicability that SPE might have in isolating volatile organics.

53. **HOW CAN I LEARN MORE ABOUT SPE TECHNIQUES?**

By keeping abreast of the analytical chemistry literature. Journey through the maze of verboseness to find out how SPE is taken advantage of by asking yourself “How has this author used SPE creatively?” With this in mind, this author looks to some of the journals listed below:

*American Laboratory and American Laboratory News Edition*
*Analyst*
*Analytica Chimica Acta*
SCHEME 3.7 Flowchart for analytical method development using SPE techniques.

Is the analyte moderately polar or non-polar?

- Yes: Proceed to NP-SPE or change matrix.
- No: Consider an alternative sample prep technique.

Is the targeted analyte ionic?

- Yes: Proceed to Ion-exchange SPE.
- No: Is the analyte extremely polar?

- Yes: Use a hydrophobic bonded sorbent, such as C8 or C18 silica or PSDVB resin to isolate analyte from the aqueous matrix.
- No: Proceed to NP-SPE or change matrix.
54. HOW DOES SPE WORK?

Solid-phase extraction works; that is, the bonded silica removes moderately polar to nonpolar solutes from an aqueous matrix based on thermodynamic principles. These principles were introduced early in this chapter as a means to discuss the underlying physical chemistry of LLE. Equation (3.4) can be restated pertinent to SPE as follows:

$$K' = \frac{a_{\text{surface}}}{a_{\text{aq}}}$$

This relationship resulted from the spontaneous tendency for the analyte, initially solubilized in an aqueous matrix, to partition into the surface monolayer of hydrophobic octadecyl- or octyl-bonded silica. Organic compounds as analytes will have a unique value for the thermodynamic distribution constant. One fundamental difference between LLE and SPE stands out. Partition or adsorption of solute molecules onto a solid surface follows the principles of the Langmuir adsorption isotherm, whereas LLE does not. We will briefly develop the principles below. This model assumes that an analyte, A, combines with a site of adsorption, S, in which there is a finite number of such sites according to

$$A + S \rightleftharpoons K_L A - S$$

An equilibrium constant, $K_L$, can be defined in terms of the mole fraction of adsorbed sites, $X_{A,S}$, the mole fraction of unadsorbed sites, $X_S$, and the mole fraction of analyte in equilibrium according to $X_A$: 

© 2006 by Taylor & Francis Group, LLC
A fractional coverage, $\theta$, can be defined whereby

$$\theta = \frac{K^L X_A}{1 + K^L X_A}$$

When the mole fraction of analyte is low, the above relationship can be simplified to

$$\theta = K^L X_A$$

When the mole fraction of analyte is high, $\theta$ approaches 1. These equations can be visualized graphically in Figure 3.17.
An organic compound such as tetrachloro-m-xylene (TCMX, whose structure is shown below), and initially dissolved in water, is thermodynamically unstable.

![Tetrachloro-m-xylene](structure.png)

Upon passage of a water sample containing TCMX through a chemically bonded silica sorbent, the degree of interaction between the sorbent surface and TCMX exceeds the degree of interaction between the aqueous matrix and TCMX; the analyte–sorbent (A–S) interaction is lower than that for the analyte–matrix (A–M) interaction. In other words, the minimum in the free-energy curve, as shown in Figure 3.2, occurs well displaced toward the side of the analyte–sorbent interaction. This concept can be viewed as an eternal triangle, whereby both the sorbent and matrix compete for the analyte:

![Eternal Triangle](triangle.png)

Efficient removal of analyte from the matrix requires that the strength of the A–S interaction be increased while that of the A–M interaction be decreased. This is accomplished chemically by matching the polarity of the sorbent to that of the analyte while mismatching the polarity of the matrix to that of the analyte. Consider TCMX as the analyte and octyl-bonded silica as the sorbent. If TMCX could be dissolved in water (a very small amount of TCMX can actually dissolve in water until a saturated solution is reached), an aqueous sample would be created, and if ultrapure water was used, this sample would be known as a spiked sample. One way to increase the amount of TCMX that can be dissolved in a given volume of water is to spike the water with a methanolic solution. Methanol serves as a molecular interface by enabling TCMX to permeate the hydrogen-bonded matrix. The solubility of TCMX is now significantly increased, and a series of spiked samples can be prepared that cover a wide range of solute concentration.
55. IS SPE A FORM OF COLUMN CHROMATOGRAPHY?

Yes, SPE, as a form of liquid column chromatography, is one of three broad classifications of column chromatography. Historically, SPE would have been called frontal development. The other two categories are elution and displacement chromatography. Elution chromatography is the principal means to conduct GC and HPLC and is of utmost importance to TEQA and as an instrumental technique; it will be discussed in sufficient detail in Chapter 4. In elution chromatography, a mobile phase is continuously passed across a stationary phase and a small plug of sample is injected into this flowing stream. Displacement chromatography is similar to elution, except that the mobile phase is much more strongly retained by the stationary phase than is the sample (p. 129). Displacement chromatography is a rarely used form and will not be considered here.

The principles underlying SPE as an example of frontal chromatography can be more easily understood if the following experiment is designed, the instrument configured, and the results interpreted (pp. 88–92). Figure 3.18 is a schematic of a frontal chromatographic instrumental configuration in which the bonded sorbent is placed into an HPLC column and installed in such a way that the sample is passed through the SPE sorbent and the effluent is also passed through an ultraviolet (UV) absorbance detector via a flow-through microcell. Provided that the total extra column void volume is not large in comparison to the size of the SPE column, a frontal chromatogram can be obtained. The reservoir contains the analyte dissolved in a matrix such as groundwater, where the analyte–matrix (A–M) interaction exists is the frontal chromatogram that would be typical. This assumes that the analyte is a strong absorber in the ultraviolet region of the electromagnetic spectrum. Referring to Figure 3.19, it should be noted that there is a certain volume of sample that can be passed through the sorbent without observing any rise in the absorbance. At $V_b$, a volume that represents the volume of sample that gives an absorbance that is 1% of $A_o$, the first signs of breakthrough are seen to emerge. The inflection point in the curve, denoted by a volume of sample passed through the column, $V_r$, is unique to a given solute. The absorbance is seen to increase sigmoidally as shown in Figure 3.20 and plateaus at $A_r$. This plateau, in essence, is the same one shown by the Langmuir isotherm in Figure 3.17.

![Figure 3.18](image-url)
Let us develop this concept a bit more by assuming that approximately 200 mL of water has been spiked with traces of dimethyl phthalate (DMP). DMP strongly absorbs in the UV, and one can expect a sigmodal curve, as shown in Figure 3.19, to result. If, instead of DMP, dibutyl phthalate (DBP) is added to water, a similar curve is obtained. In this case, a significant increase in $V_b$ and $V_r$ is observed. This is shown in Figure 3.20. If both DMP and DBP were spiked together at the onset to 200 mL of high-purity water and the breakthrough experiment performed, the curve would resemble that shown in Figure 3.21. Much before the commercial development of SPE, a curve such as that shown in Figure 3.21 was already established and the following predictions made (pp. 128–129):\(^{84}\)

Separation results in the formation of fronts … the least retained component emerges first from the bed followed by a mixture of the least and next most strongly retained component…. This technique is useful for concentrating trace impurities.

This author doubts whether these authors could have foreseen the impact of their words on the eventual development of commercial products that rely on frontal development, namely, SPE.
Let us now set aside this spiked aqueous sample that consists of DMP and DBP dissolved in water. This sample easily simulates an environmental sample that contains phthalate esters, since this class of organic compound is commonly found in landfill leachates due to the use of phthalate esters as plasticizers. Let us reconfigure our HPLC to that shown in Figure 3.22, so that ultrapure water is pumped through our SPE column. The results, shown in Figure 3.23, corresponding to elution chromatography, give, instead of the sigmoidal shape, the Gaussian peak profile, with the apex of the peak located at \( V_r \). Because \( V_r \) differs between DMP and DBP, we have a basis of separation. In Chapter 4 we will discuss the theory underlying column elution liquid chromatography as a means to separate and quantitate the presence of organic compounds.

Solid-phase extraction has been called digital chromatography because its chief use is to retain and then remove analytes of interest in an on–off fashion. Outside of conducting breakthrough studies, the purely frontal chromatographic form has little practical value in TEQA. The elution step, in which an eluent’s polarity is near

---

**FIGURE 3.20** Plot of absorbance vs. the volume of sample passed for two analytes injected independently, DMP and DBP.
FIGURE 3.21 Plot of absorbance vs. the volume of sample passed for a mixture of DMP and DBP.

FIGURE 3.22 Schematic of an elution chromatographic instrumental configuration.
to that of the analyte, removes all analytes and can be viewed in a manner similar to that done for the sorption step.

The analyte–matrix interaction becomes so strong that analytes are easily and quickly eluted from the sorbent. The polarity of the matrix now matches the polarity of the analyte.

56. **CAN SPE BREAKTHROUGH BE PREDICTED?**

We saw earlier just how $V_b$ and $V_r$ are obtained experimentally by reconfiguring a high-pressure liquid chromatograph (refer to Figure 3.18). An HPLC column packed with SPE sorbent replaces the conventional HPLC column. A spiked sample is

*FIGURE 3.23* Chromatogram from the elution chromatograph instrumental configuration.
pumped through the SPE column to develop the frontal chromatograms, as shown in Figure 3.19. This configuration is all it takes to conduct the breakthrough study. Is there sufficient theory developed to predict $V_b$ or $V_r$? Yes, there is, and we proceed to consider this below. Before we do, though, we need to introduce certain concepts and definitions that are fundamental to achieving a somewhat deeper understanding of liquid chromatography. We must introduce the important elements from the more contemporary approaches taken to understand exactly what one means by the term solvent polarity. We must also discuss what one means by the term aqueous solubility for an organic compound acting as a solute, and we need to discuss how this important parameter relates to the solute’s octanol–water partition coefficient. We admit to digressing somewhat into the physical chemistry of solution behavior insofar as it aids the reader in understanding the theory that underpins RP-SPE. Introducing these concepts in this chapter on sample preparation will facilitate the discussion of HPLC as a determinative technique in the next chapter.

57. WHAT IS SOLVENT POLARITY?

Solvent polarity is a numerical value that can be assigned to each of the common organic solvents used in TEQA. A solvent polarity index, denoted by $P'$, is defined based on experimental solubility data using test solutes and was originally developed by Rohrschneider and further discussed by Snyder. Table 3.9 lists a number of representative solvents in order of increasing $P'$. The introduction of heteroatoms such as oxygen, sulfur, and nitrogen significantly contributes to solvent polarity. Consider what happens when a test solute such as methanol is added to the very polar solvent water vs. what happens when methanol is added to the very nonpolar solvent hexane. The classic cliché “like dissolves like” certainly applies, whereby methanol, whose $P'$ is 5.1, is infinitely soluble or completely miscible in all proportions with water, whose $P'$ is 10.2. However, methanol, with a density of 0.7915 g/mL at

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$P'$</th>
<th>Solvent</th>
<th>$P'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane</td>
<td>0.0</td>
<td>Tetrahydrofuran</td>
<td>4.0</td>
</tr>
<tr>
<td>1,1,2-TCTFE</td>
<td>0.0</td>
<td>Chloroform</td>
<td>4.1</td>
</tr>
<tr>
<td>Hexane</td>
<td>0.1</td>
<td>Ethyl acetate</td>
<td>4.1</td>
</tr>
<tr>
<td>Iso-octane</td>
<td>0.1</td>
<td>MEK</td>
<td>4.7</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0.1</td>
<td>1,4-dioxane</td>
<td>4.8</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0.2</td>
<td>Acetone</td>
<td>5.1</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>0.5</td>
<td>MeOH</td>
<td>5.1</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.4</td>
<td>Acetonitrile</td>
<td>5.8</td>
</tr>
<tr>
<td>MTBE</td>
<td>2.5</td>
<td>N,N-DMF</td>
<td>6.4</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>2.8</td>
<td>DMSO</td>
<td>7.2</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>3.1</td>
<td>Water</td>
<td>10.2</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>3.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3.9
Polarity of Selected Organic Solvents
20°C, will tend to sink to the bottom when added to hexane, whose density is 0.660 g/mL at the same temperature. The presence of hydroxyl groups in both methanol and water creates an infinite number of hydrogen bonds and is responsible for defining both solvents as associated liquids. The degree to which methanol molecules interact through intramolecular hydrogen bonding is of a similar attractive potential energy, as is the degree to which water molecules interact through intramolecular hydrogen bonding. The energy of interaction is due to hydrogen bonding between methanol and water, the so-called intermolecular hydrogen bonding; this is shown in the following:

Because there is little difference in the potential energy for the interaction intramolecularly and intermolecularly, the tendency for matter to spread (entropy) becomes the driving force between methanol and water. Consider what happens when the two immiscible liquids, methanol whose $P'$ is 5.1 and hexane whose $P'$ is 0.1, are in contact with each other. The weak dispersion forces are present throughout hexane, whereas the much stronger interaction due to hydrogen bonding among methanol molecules exists; this is shown in the following:

It is impossible to overcome the high intermolecular potential energy that exists to afford a true dissolution of hexane into methanol and vice versa. These intramolecular
Sample Preparation Techniques

and intermolecular forces that exist among molecules in liquids, as depicted above, not only serve to explain why hexane and methanol do not mix and remain as two immiscible liquids, but also help in understanding how to go about developing a method that incorporates SPE as the principal sample preparation technique. Hexane is a suitable elution solvent for relatively nonpolar analytes that are sorbed onto and into the reversed-phase material, whereas methanol might not be as suitable in terms of efficient elution from the same SPE sorbent.

58. CAN A SOLUTE'S AQUEOUS SOLUBILITY OR OCTANOL–WATER PARTITION COEFFICIENT BE USED TO PREDICT RP-SPE PERCENT RECOVERIES?

Yes, both solute physico-chemical properties give important clues about the possibility of whether a given organic compound originally dissolved in an aqueous matrix will be effectively recovered by RP-SPE. Again, because SPE is a two-step transfer of solute (sorption from the matrix followed by elution from the sorbent), both factors serve to limit the percent recovery. It is only percent recoveries that can be measured by determinative techniques. There is no direct way to measure the amount of analyte sorbed except through the breakthrough experiments previously discussed. Let us assume then that the analyte, once sorbed, is efficiently removed from the sorbent. The differences in our measured percent recoveries then reflect only the mass transfer of analyte from an aqueous matrix to the chemically bonded sorbent. Solutes that in general exhibit low aqueous solubilities, \( S_{aq} \), and have relatively large octanol–water partition coefficients, \( K_{OW} \), exhibit a strong tendency to sorb on hydrophobic surfaces from an aqueous matrix. The mathematical relationship between \( S_{aq} \) and \( K_{OW} \) has been the focus of extensive study among physical chemists. A nearly inverse and linear relationship exists between two solute parameters. This is stated from one resource as follows:

\[
\log K_{OW} = -\log S_{aq} - \log \gamma_0 - \log V_0 \quad (3.36)
\]

where \( \gamma_0 \) represents the activity coefficient for a water-saturated octanol phase and \( V_0 \) is the molar volume for the water-saturated octanol phase, which is 0.12 L/mol. If \( \log K_{OW} \) is plotted against the values for the aqueous solubility, \( S_{aq} \), for a variety of organic compounds of environmental interest, a linear relationship with a negative slope emerges. The majority of priority pollutant organic compounds have activity coefficients in water-saturated octanol, \( \gamma_0 \), that fall between 1 and 10. This suggests that these solutes exhibit ideal solution behavior and reflects a strongly hydrophobic character.

In general, polar solutes have \( \log K_{OW} \) values less than 1; moderately polar solutes have \( \log K_{OW} \) values between 1 and 3; nonpolar solutes have \( \log K_{OW} \) values greater than 3. To illustrate the influence of various substituents on the \( \log K_{OW} \) for a parent compound such as phenol, consider the following list of phenols:

© 2006 by Taylor & Francis Group, LLC
Clearly, the effect of either chloro or methyl substitution serves to significantly increase the octanol–water partition coefficient. This increase in hydrophobicity is an important consideration in developing an RP-SPE method that is designed to isolate one or more phenols from a groundwater sample. Those substituted phenols with the greater values for $K_{OW}$ would be expected to yield the higher percent recoveries in RP-SPE. The substitution of a trimethyl silyl moiety in place of hydrogen significantly increases the hydrophobicity of the derivatized molecules. This use of a chemical derivative to convert a polar molecule to a relatively nonpolar one has important implications for TEQA and will be discussed in Chapter 4.

### 59. WHAT IS AQUEOUS SOLUBILITY ANYWAY?

The amount of solute that will dissolve in a fixed volume of pure water is defined as that solute’s aqueous solubility, $S_{aq}$. It is a most important concept with respect to TEQA because there is such a wide range of values for $S_{aq}$ among organic compounds. Organic compounds possessing low aqueous solubilities generally have high octanol–water partition coefficients. This leads to a tendency for these compounds to bioaccumulate. The degree to which an organic compound will dissolve in water depends not only on the degree of intramolecular vs. intermolecular interactions, as we saw earlier, but also on the physical state of the substance (i.e., solid, liquid, or gas). The solubility of gases in water is covered by Henry’s law principles, as discussed earlier.

A solute dissolved in a solvent has an activity $a$. This activity is defined as the ratio of the solute’s fugacity (or tendency to escape) in the dissolved solution to that in its pure state. The activity coefficient for the $i$th solute, $\gamma_w^i$, relates solute activity in pure water to the concentration of the solute in water, where the concentration is defined in units of mole fraction, $x_w^i$, according to

\[
x_w^i = \frac{\text{moles}^i}{\sum_i \text{moles}^i} \quad \gamma_w^i \equiv \frac{a_w^i}{x_w^i}
\]

We usually think of aqueous solubilities in terms of the number of moles of the $i$th solute per liter of solution. Alternative units commonly used to express aqueous
solubility include milligrams per liter or parts per million and also micromoles per liter. The concentration in moles per liter can be found by first considering the following definition:

\[ C_i = \frac{x_i \text{(moles/mole total)}}{V_{mix} \text{(L/mole total)}} \]

\( V_w \) is the molar volume of water (0.018 L/mol) and \( V_i \) is the molar volume of a typical solute dissolved. The molar volume of the mixture, \( V_{mix} \), is found by adding the molar volume contributions of the solute and solvent, water, according to

\[ V_{mix,w} = 0.2x_{i,w} + 0.018x_w \]

This expression can be further simplified to

\[ V_{mix,w} = 0.182x_{i,w} + 0.018 \]

Because the mole fraction for solutes dissolved in water is very low, \( x_{i,w} < 0.002 \), which corresponds to about 0.1 \( M \), the molar volume for the mix can be approximated at 0.018, and hence the above equation for the concentration in moles per liter can be expressed as

\[ C_w^\text{sat} = \frac{x_w^\text{sat}}{V_{mix,w}^\text{sat}} \equiv \frac{x_w^\text{sat}}{0.018} \text{(moles/L)} \]

The above equation converts the mole fraction of a solute dissolved in water, up to saturation conditions, \( x_w^\text{sat} \), to the corresponding concentration of that solute in units of moles per liter. By knowing the molecular weight of a particular solute, it is straightforward to obtain the corresponding concentration in milligrams per liter.

For hydrophobic solutes, \( \gamma_w \) is greater than 1. When enough solute has been added to a fixed volume of water until no more can be dissolved, the solution is said to be saturated and a two-phase system results. This is a condition of dynamic equilibrium whereby the solute activity in both phases is equal. Denoting \( o \) as the organic phase, we have

\[ a_w = a_o \]

so that

\[ x_w \gamma_w = x_o \gamma_o \]

For immiscible liquids in water, the mole fraction of solute in itself and its activity coefficient approximate 1, so that

\[ x_w \gamma_w = 1 \]
and the mole fraction, $x_w$, is related to the aqueous activity coefficient by

$$x_w = \frac{1}{\gamma_w}$$

and in terms of logarithms,

$$\log x_w = -\log \gamma_w$$

The purpose of deriving this is to show that attempts to measure the aqueous solubility, $x_w$, are, in essence, attempts to estimate the activity coefficient, $\gamma_w$. This relationship applies only to liquid solutes. Solid or crystalline solutes require an additional term where

$$\log x_w = \log \frac{x_c}{x_{sol}} - \log \gamma_w$$

where $x_c/x_{sol}$ represents the ratio of the solubility of the crystal to that of the hypothetical supercooled liquid. The additional term above is approximated by the term $-0.00989 (T_{mp} - 25)$ for rigid molecules at 25°C and $-[0.01 + 0.002(n - 5)]T_{mp}$ for long-chain molecules, with $n$ monomers per polymer and $T_{mp}$ the melting point of the solid. Octanol–water partition coefficients can also be estimated from solute retention times using, for example, reversed-phase HPLC.

Aqueous solubility for various solutes can be estimated by applying any of the techniques in the following four categories:

1. Methods based on experimental physico-chemical properties, such as partition coefficient, chromatographic retention, boiling point, and molecular volume.
2. Methods based directly on group contributions to measured activity coefficients.
3. Methods based on theoretical calculations from molecular structures, including molecular surface area, molecular connectivity, and parachor.
4. Methods based on combinations of two or more parameters that can be experimentally measured, calculated, or generated empirically. These include the solubility parameter method and the UNIFAC technique (a method based on linear solvation energy relationships and on the use of multivariate statistical methods).

However, for many of the very hydrophobic solutes of interest to TEQA, the aqueous solubility is extremely low. For example, iso-octane, a nonpolar hydrocarbon, will dissolve in water up to 0.0002% at 25°C. Higher-order aliphatic hydrocarbons have such low values for their aqueous solubilities that it is impossible to directly measure this physico-chemical property.
60. WHAT DOES A PLOT OF $K_{ow}$ VS. AQUEOUS SOLUBILITY TELL US?

Figure 3.24 is a qualitative plot of the logarithm of the octanol–water partition coefficient, $K_{ow}$, against the logarithm of the aqueous solubility, $S_{aq}$, of a selected number of organic solutes of environmental interest. This qualitative plot makes it very evident that polar solutes with appreciable aqueous solubility exhibit low values of $K_{ow}$. Hydrophobic solutes that are not soluble in water to any great degree exhibit

\[ \text{Absorbance} \]

\[ V_{\text{DMP}} \]

\[ V_{\text{DBP}} \]

\[ \text{Volume of water passed through SPE column} \]

**FIGURE 3.24** Plot of the logarithm of the octanol–water partition coefficient vs. the logarithm of the aqueous solubility for selected organic compounds of enviro-chem/enviro-health interest. (Adapted from Chiu, G. et al., *Environmental Science and Technology*, 11(5): 475–478, 1977.)
large values of $K_{OW}$. The importance of knowing $K_{OW}$ values as this pertains to the practice of TEQA and to environmental science in general has been stated earlier:

In recent years the octanol/water partition coefficient has become a key parameter in studies of the environmental fate of organic chemicals. It has been found to be related to water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic life. Because of its increasing use in the estimation of these other properties, $K_{OW}$ is considered a required property in studies of new or problematic chemicals.

Much effort has been expended in finding a linear equation that relates octanol–water partition coefficients for any and all solutes to the aqueous solubility in octanol-saturated water. Two useful equations have emerged in which the logarithm of the aqueous solubility, $\log S_{aq}$, is, in general, linearly related to $\log K_{OW}$:

For liquid solutes:

$$\log K_{OW} = 0.8 - \log S_{aq}$$

(3.37)

For crystalline solutes:

$$\log K_{OW} = 0.8 - \log S_{aq} - 0.01(T_{mp} - 25)$$

(3.38)

Yalkowsky and Banerjee have reviewed specific studies and summarized the relationship in Equations (3.37) and (3.38) as applied to liquid organic compounds and crystalline organic solids. The results are shown in Table 3.10 for four classes of liquid hydrocarbons. A plot of $\log K_{OW}$ against $\log S_{aq}$ reveals a slope close to the value of –1 for each of these four classes of hydrocarbons. The differences in the $y$ intercept reflect differences in the activity coefficients as defined in Equation (3.36).

### Table 3.10

<table>
<thead>
<tr>
<th>Class</th>
<th>$\log K_{OW}$ range</th>
<th>$\log S_{aq}$ range</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic hydrocarbons</td>
<td>0.786–1.056</td>
<td>0.995</td>
<td></td>
</tr>
<tr>
<td>Unsaturated hydrocarbons</td>
<td>0.250–0.908</td>
<td>0.993</td>
<td></td>
</tr>
<tr>
<td>Halogenated hydrocarbons</td>
<td>0.323–0.907</td>
<td>0.993</td>
<td></td>
</tr>
<tr>
<td>Normal hydrocarbons</td>
<td>0.467–0.972</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>

61. **CAN WE PREDICT VALUES FOR $K_{OW}$ FROM ONLY A KNOWLEDGE OF MOLECULAR STRUCTURE?**

Yes, and the predictions are quite good. Two different methods emerge from a host of others and are most commonly used to predict octanol–water partition coefficients for the many organic compounds that exist. One approach is to calculate $K_{OW}$ from
a knowledge of structural constants, whereas the second approach requires that a chemical’s partition coefficient be measured between a solvent other than octanol and water, \( K_{SW} \). \( K_{OW} \) can then be calculated from linear regression equations that relate \( \log K_{SW} \) (for a particular solvent) and \( \log K_{OW} \). Two forms of the structural constant approach are most popular: (1) the Hansch \( \pi \) hydrophobic character of substituents approach and (2) the Leo fragment constant approach. The Hansch \( \pi \) approach is based on the assignment of a value for \( \pi_X \) as the difference between octanol–water partition coefficients for a substituted vs. unsubstituted or parent compound. Mathematically, this difference can be stated as follows:

\[
\pi_X = \log K^X_{OW} - \log K^H_{OW}
\]

For example, the \( \log K_{OW} \) for chlorobenzene is 2.84, whereas that for benzene is 2.13, and thus

\[
\pi_X = 2.84 - 2.13 = 0.71
\]

and the \( \pi_X \) for the substituent chlorine on the monoaromatic ring is 0.71. The Hansch \( \pi \) approach has been recently criticized because it ignores hydrogens attached to carbon, and this led to some erroneous values for a few aliphatic hydrocarbons.

The Leo fragment method has emerged as a more powerful way to predict \( \log K_{OW} \). The working mathematical relationship is

\[
\log K_{OW} = \sum_i f_i + \sum_j F_j
\]  

(3.39)

where the logarithm of the octanol–water coefficient for a particular organic compound is found by algebraically summing the contributions due to the \( i \)th structural component \( (f) \) (building block or functional group), overall components, and the algebraic sum of the \( j \)th factor \( (F) \) due to intramolecular interactions caused largely by geometric or electronic effects. For complex molecules, it is desirable to have a known \( \log K_{OW} \) value for a given compound and use the fragment approach to add and subtract structural and geometric/electronic contributions such that

\[
\log K_{OW} \text{ (unknown)} = \log K_{OW} \text{ (known)} - \sum_{\text{removed}} f + \sum_{\text{added}} f - \sum_{\text{removed}} F + \sum_{\text{added}} F
\]  

(3.40)

Thus, two ways emerge to calculate \( \log K_{OW} \) using the Leo fragment method. One is to build the entire molecule from fragments and factors using Equation (3.40), and the other is to calculate \( \log K_{OW} \) from structurally related compounds. To illustrate how one goes about calculating the \( \log K_{OW} \) for a specific organic compound, we return to TCMX. We begin by first using Equation (3.39) to establish the \( \log K_{OW} \) for benzene by using previously published fragment factors. For carbon
that is aromatic, a fragment factor of 0.23 has been established. Hence, because benzene consists of six aromatic carbons that in turn are bonded to six hydrogens,

$$\log K_{\text{OW}}(\text{C}_6\text{H}_6) = 6f_{\text{c(aromatic)}} + 6f_{\text{H}}$$

$$= 6(0.13) + 6(0.23)$$

$$= 2.16$$

(3.41)

This prediction is quite close to the well-established and observed value for benzene, which is 2.13. Using this established value for benzene, we begin to view our molecule of interest, TCMX, as a substituted benzene. Theoretically, we can arrive at TCMX by removing all six aromatic hydrogens and then adding two methyl groups and four chlorine groups. Stated mathematically,

$$\log K_{\text{OW(TCMX)}} = \log K_{\text{OW(C}_6\text{H}_6)} - 6f_{\text{H}} + 2f_{\text{CH}_3} + 4f_{\text{Cl}}$$

(3.42)

The methyl groups are viewed as being composed of an aromatic carbon and three aliphatic bonded hydrogens and a fragment constant. Equation (3.42) is modified as follows:

$$\log K_{\text{OW(TCMX)}} = \log K_{\text{OW(C}_6\text{H}_6)} - 6f_{\text{H}} + 2\left[f_{\text{C}} + 3f_{\text{H}}\right] + 4f_{\text{Cl}}$$

(3.43)

Upon substituting the values for the various fragment constants into Equation (3.43), we get

$$\log K_{\text{OW(TCMX)}} = 2.13 - 6(0.23) + 2[(0.13) + 3(0.23)] + 4(0.19)$$

$$= 6.15$$

Such a large value for the octanol–water partition coefficient for TCMX suggests that the compound exhibits a very low aqueous solubility. Note that the substitution by hydrogen by methyl and chloro groups significantly increases the hydrophobicity of the molecules. The addition of a methyl group is seen to add about 0.5 of a log unit, irrespective of the compound involved. For example, substituting a methyl for hydrogen in benzene contributes the same, as if a methyl were substituted for hydrogen in cyclohexane.

As long as the building blocks lead to a complete molecular structure, only structural fragment constants are necessary, as in the TCMX example. However, for molecules that are more complex, the molecular components begin to exert significant influences on one another through steric, electronic, and resonance intramolecular interactions. These interactions affect both the aqueous and octanol activity coefficients, as ability of groups to rotate about a carbon–carbon single bond, chair/boat confirmation in cyclohexane, and carbon-chain branching in aliphatic structures. Geometric effects increase aqueous solubility, decrease \( K_{\text{OW}} \), and hence their \( F \) factor contributes negativity to Equation (3.40). Electronic effects due to
Sample Preparation Techniques

Electronegativity differences between the elements of both atoms that comprise a polar covalent bond, such as a carbon–chlorine bond, decrease aqueous solubility, increasing $K_{OW}$, and hence their $F$ factor contributes positively to Equation (3.40). Other electronic effects include nearby polyhalogenation on carbon, nearby polar groups, and intramolecular hydrogen bonding. These other factors also contribute in a positive manner to Equation (3.40).

62. **ARE VALUES FOR $K_{OW}$ USEFUL TO PREDICT BREAKTHROUGH IN RP-SPE?**

The capacity factor for an RP-SPE cartridge when pure water is used, $k'_{W}$, discussed earlier, has been found to be closely related to $K_{OW}$ and is related by $^{94,95}$

$$k'_{W} = 0.988 \log K_{OW} + 0.02$$

Hence, merely obtaining octanol–water partition coefficient values from various tabulations in the literature can yield a predictive value for $k'_{W}$ because we have shown that $k'_{W}$ is directly related to the breakthrough volume, $V_r$.

63. **WHERE CAN ONE OBTAIN $K_{OW}$ VALUES?**

The resources cited are available in most university libraries in order to find these values, as well as to obtain good introductions. Leo et al.$^{96}$ published a comprehensive listing and followed this with a more systematic presentation.$^{97}$ Even earlier, Hansch et al.$^{98}$ published their findings. Lyman et al.$^{99}$ have published a handbook on the broad area of physico-chemical property estimations. These references’ sources also provide detailed procedures for calculating and estimating $K_{OW}$.

64. **CAN BREAKTHROUGH VOLUMES BE DETERMINED MORE PRECISELY?**

A more precise way to determine the breakthrough volume for a particular analyte on a given SPE sorbent, $V_r$, is to use reversed-phase HPLC. The SPE sorbent is efficiently packed into a conventional HPLC column. The analyte of interest is injected into the instrument, while the mobile-phase composition is varied. This is accomplished by varying the percent organic modifier, such as acetonitrile or methanol, and measuring the differences in analyte retention time. Extrapolation of a plot of $k'$ vs. percent organic modifier to zero percent modifier yields a value for the capacity factor for that analyte at 100% water. This capacity factor is represented by $k'_{W,SPE,HPLC}$, where the subscript refers to 100% water. The breakthrough volume is related to the capacity factor in 100% water according to

$$V_r = V_0 (1 + k'_{W,HPLC})$$

$V_0$ is the void volume of the SPE column and $k'_{W}$ (SPE, HPLC) is the capacity factor of the analyte eluted by water. This capacity factor should theoretically be
the same for any technique used, as $V_0$ is found by knowing the porosity of the sorbent and the geometric of the SPE column or sorbent bed in the cartridge. The capacity factor of a given analyte, $k'$ (SPE, HPLC), is generally obtained from HPLC. From an HPLC chromatogram, $k'$ (SPE, HPLC) is obtained by taking the ratio of the difference in the retention time between an analyte and its void retention time to the void retention time. Expressed mathematically,

$$k'_{\text{HPLC}} = \frac{t'_R - t'_0}{t'_0}$$

where $t'_R$ represents the retention time for the analyte of interest under the HPLC chromatographic conditions of a fixed mobile-phase composition. $t'_0$ represents the retention time for an analyte that is not retained on the stationary phase. $t'_0$ also relates to the void volume in the column.

Log $k'_{\text{HPLC}}$ is obtained by extrapolation of a plot of log $k'$ vs. percent MeOH to zero. This plot is shown in Figure 3.25. A linear relationship exists between the logarithm of the capacity factor, $k'$ (SPE, HPLC), and the percent MeOH concentration for a specific organic compound in HPLC. Because $k'_{\text{HPLC}}$ has also been shown to be related to the octanol–water partition coefficient, $K_{\text{OW}}$, $k'_{\text{SPE,HPLC}}$, and hence $V_r$, can be obtained for a given RP-SPE sorbent by this approach.

65. HOW DOES SPE RELATE TO TEQA?

Because environmental matrices are largely air, soil, and water of some sort, the objective in TEQA is to isolate and recover a hydrophobic organic substance from
a more polar sample matrix. RP-SPE can directly remove organic contaminants and represents the dominant mode of SPE most relevant to TEQA. Normal-phase SPE (NP-SPE) also has a role to play in TEQA. NP-SPE has served as an important cleanup step following LLE using a nonpolar solvent to remove polar organic interferences. This was accomplished in large-diameter glass columns instead of the familiar barrel type of SPE cartridge. Hydrophobic organic contaminants found in the environment can be easily transferred from the native matrix to the RP-SPE sorbent using procedures that will be discussed below.

Since 1985, the analytical literature has been replete with articles that purport good to excellent recoveries of various organic compounds that have been isolated and recovered by applying RP-SPE techniques. The rise in popularity of RP-SPE between 1985 and the present has served to limit the growth of both LLE and supercritical fluid extraction (SFE). Hydrophobic organic contaminants are partitioned from the environmental matrix to the surface layer of the organo moiety. The contaminants are then eluted off of the sorbent using a solvent with the appropriate polarity. This eluent is then introduced into the appropriate determinative technique, such as a gas or liquid chromatograph.

Reversed-phase SPE is applied principally in two areas of TEQA. The first application is in the determination of organochlorine pesticides (OCs) in connection with the determinative technique of GC using a chlorine-selective detector. The second application is in the determination of priority pollutant semivolatile organics that are recovered from drinking water. Offline RP-SPE coupled to a determinative technique such as GC using an element-selective detector is a very powerful combination with which to achieve the objectives of TEQA. To illustrate, this author’s study of various organophosphorous pesticides (OPs) from spiked water using RP-SPE will now be discussed.

Organophosphorous pesticides are used primarily as insecticides, and in mammals, they act as cholinesterase inhibitors. A number of OPs are listed as priority pollutants. EPA Method 8141A from SW-846, Revision 1, September 1994, is an analytical method that uses LLE coupled to GC with a phosphorous-selective detector and a megabore-capillary column to separate and quantitate some 49 individual OPs. The method is highly detailed and points out numerous challenges to be encountered during implementation. OPs are all structurally related in that a phosphate, thiophosphate, or dithiophosphate has been esterified. Either methyl or ethyl esters occupy two of the three functional groups around central pentavalent phosphorous, whereas a complex organo moiety is esterified to the third functional group. The generalized structure is as follows:

```
O
O
R
Generalized molecular structure
for organophosphorous pesticides
```
This complexity gives rise to a wide range of compound polarities. These polarities are largely governed by the nature of this third esterified functional group.

The multisample nature of the conventional SPE vacuum manifold enabled enough replicate extractions to permit a statistical evaluation to be accomplished. A multi-injection procedure using a liquid GC autosampler enabled enough replicate injections to be made as well. These data were plentiful enough to enable equations first introduced in Chapter 2 to be used. The following flowchart describes part of a multisample/multi-injection study using two dissimilar GC megabore-capillary GC columns while spiked samples are passed through a conditioned octyl-bonded silica, C₈:

© 2006 by Taylor & Francis Group, LLC
Four other replicate SPE extractions/elutions were conducted to yield a total of five replicate SPEs. A similar study was also conducted using an octadecyl-bonded silica, C$_{18}$. Each RP-SPE cartridge contained 200 mg of sorbent and each cartridge was eluted with two successive 500-µL aliquots of methyl-tert-butyl ether (MTBE). The eluent volume was then adjusted with MTBE to a total volume of 4 mL. This enabled triplicate injection with one injection per GC vial to be accomplished. Thus, triplicate injection for each SPE eluent combined with 5 replicate SPEs per sorbent type led to 15 replicate determinations of the percent recovery for each chromatographically resolved OP studied.

Such a plethora of data from the design used above enabled Equation (2.33) to be used to first calculate a pooled standard deviation for triplicate injections made for each of five SPEs conducted. With mean area counts and corresponding standard deviations about the mean area account for each OP, Equation (2.30) can be used next to calculate the mean percent recovery over spiked samples and controls. Relative standard deviations (RSDs) were obtained by dividing the standard deviation by the mean percent recovery. RSDs can be propagated between spiked samples and controls using Equation (2.31) to yield a more realistic RSD and converted to coefficients of variation using Equation (2.32). Table 3.11 is a summary of the mean percent recoveries and the corresponding statistical evaluation via application of Equation (2.57) and calculating measures of precision as discussed in Chapter 2. The C$_{18}$ sorbent gave a larger overall percent recovery when compared to the recoveries using the C$_{8}$ sorbent. One OP, dimethoate, exhibited a relatively low percent recovery. This is not surprising due to the presence of the polar N-methyl-a-mido-esterified functional group. All other OPs studied have nonpolar esterified functional groups, and percent recoveries were found to be more than adequate when compared to Method 8141A. The coefficient of variation in the mean is consistently below 5% among 11 of the 12 OPs selected for study. The application of the Leo fragment method, as discussed earlier, would make for an interesting estimate of the octanol–water partition coefficient for each of these OPs.

We now discuss the author’s earlier experience with RP-SPE using selected OCs. This discussion will serve to both illustrate the technique and close out this aspect of sample preparation.

66. SHOULD I USE RP-SPE TO MEASURE OC IN SOIL AND WATER?

This author’s experience in applying RP-SPE to spiked water answers the question in the affirmative. OCs that exhibit insecticidal properties and were in widespread use early in the 20th century are easily isolated from water and recovered by solvent elution. The majority of OCs that were used as insecticides are relatively high-molecular-weight semivolatile organic molecules having a complex structure. The original approach adopted by the EPA was encompassed in Method 608 as applied to wastewater and required LLE, followed by a NP-SPE cleanup using Florisil and determination using packed-column GC-ECD (electron-capture detector). This method could monitor 17 OCs. With selective use of elution solvents off of the Florisil cleanup column, some fractionation, hence separation, of OCs from multicomponent
OCs such as toxaphene and Aroclors (mixtures of polychlorinated biphenyl congeners commercially manufactured) could be achieved. The replacement of conventional LLE with Florisil cleanup with a direct isolation of OCs from samples of environmental interest using RP-SPE represented a clear alternative to EPA Method 608. When RP-SPE is used in combination with high-resolution capillary GC-ECD, a much more robust and powerful analytical method emerges. Samples can be prepared and analyzed much faster, and the increase in GC resolution afforded by capillary columns enables a greater degree of delineation among all OCs of interest.

This author has always been rather taken back by how efficiently these OCs can be removed from spiked water samples. Of the some dozen or so OCs that are routinely monitored in drinking water (EPA Method 508), wastewater (EPA Method 608), and solid waste (EPA Method 8081A (Revision 1, December 1996)), I chose three that are most representative of all of these OCs: lindane or \( \gamma \)-BHC, endrin, and methoxychlor. Of these three, methoxychlor is still in use.

### TABLE 3.11
Comparison of the Percent Recoveries of Various OCs from Spiked Water between a C\(_8\) and a C\(_{18}\) Chemically Bonded Silica Sorbent

<table>
<thead>
<tr>
<th>OP</th>
<th>% Recovery (\text{(Coefficient of Variation)}) C(_8)</th>
<th>% Recovery (\text{(Coefficient of Variation)}) C(_{18})</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEPT</td>
<td>77.6 (3.9)</td>
<td>94.0 (3.1)</td>
</tr>
<tr>
<td>Dichlorovos</td>
<td>42.0 (3.0)</td>
<td>56.9 (2.6)</td>
</tr>
<tr>
<td>Ethoprop</td>
<td>85.0 (2.1)</td>
<td>92.0 (1.9)</td>
</tr>
<tr>
<td>Phorate</td>
<td>80.1 (2.5)</td>
<td>54.6 (2.2)</td>
</tr>
<tr>
<td>Diazinon</td>
<td>83.5 (2.3)</td>
<td>89.3 (2.4)</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>4.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>89.0 (3.0)</td>
<td>96.3 (2.7)</td>
</tr>
<tr>
<td>Parathion</td>
<td>83.6 (2.5)</td>
<td>94.3 (2.9)</td>
</tr>
<tr>
<td>Tokuthion</td>
<td>41.1 (2.6)</td>
<td>70.4 (3.0)</td>
</tr>
<tr>
<td>Famphur</td>
<td>90.0 (2.3)</td>
<td>99.9 (3.3)</td>
</tr>
<tr>
<td>EPN</td>
<td>61.8 (2.7)</td>
<td>86.3 (3.3)</td>
</tr>
<tr>
<td>Azinphos methyl</td>
<td>92.1 (3.4)</td>
<td>97.2 (3.8)</td>
</tr>
</tbody>
</table>

OCs such as toxaphene and Aroclors (mixtures of polychlorinated biphenyl congeners commercially manufactured) could be achieved. The replacement of conventional LLE with Florisil cleanup with a direct isolation of OCs from samples of environmental interest using RP-SPE represented a clear alternative to EPA Method 608. When RP-SPE is used in combination with high-resolution capillary GC-ECD, a much more robust and powerful analytical method emerges. Samples can be prepared and analyzed much faster, and the increase in GC resolution afforded by capillary columns enables a greater degree of delineation among all OCs of interest. This author has always been rather taken back by how efficiently these OCs can be removed from spiked water samples. Of the some dozen or so OCs that are routinely monitored in drinking water (EPA Method 508), wastewater (EPA Method 608), and solid waste (EPA Method 8081A (Revision 1, December 1996)), I chose three that are most representative of all of these OCs: lindane or \( \gamma \)-BHC, endrin, and methoxychlor. Of these three, methoxychlor is still in use.

### 67. CAN LINDANE, ENDRIN, AND METHOXYCHLOR BE ISOLATED AND RECOVERED USING RP-SPE?

Yes, and the data from one such study by this author is now discussed. Lindane, being a hexachlorocyclohexane, has a higher aqueous solubility than hexachlorobenzene. The gamma isomer, \( \gamma \)-BHC, is 10 times more soluble than the alpha or beta isomer. Lindane (\( \gamma \)-BHC) represent the class of chlorinated aliphatic hydrocarbons.
with an $S_{aq}$ of between 7.5 and 10 ppm. Endrin is a member of the cyclodiene insecticides, possessing the characteristic endomethylene bridge structure. The $S_{aq}$ for endrin is 0.23 ppm, which is similar to that of aldrin, dieldrin, and heptachlor epoxide, but greater than that of heptachlor and chlordane. Endrin is known to partially break down to endrin aldehyde and endrin ketone in packed GC columns.

It is not much of a problem when capillary columns are used. Methoxychlor, a substituted diphenyl trichloroethane similar to DDT, has a $S_{aq}$ of between 0.1 and 0.25 ppm, and it is about 100 times more soluble in water than DDT. Using ethyl acetate as an elution solvent and some of the early chemically bonded silica, of 60 Å pore size and 40-μm irregular particle size (Separalyte, Analytichem International), this author conducted a series of systematic studies of lindane, endrin, and methoxychlor (L, E, M). The work was focused on a sample matrix consisting of distilled deionized water (DDI), and the analysis was performed on a gas chromatograph that used a packed column and electron-capture detector.

One such study is reported here. Nine cartridges were packed with an octyl-bonded silica, C₈ Separalyte. The mass of the sorbent packed in each cartridge varied from a low of 20 mg to a high of 320 mg. In each cartridge, after methanol conditioning, a spiked aqueous sample was passed through the packed bed under a reduced-pressure SPE manifold. Then 8.8 μL of a methanolic reference standard containing L, E, and M, each at a concentration of 100 ng/μL (ppm), was added to approximately 60 mL of DDI. This spiking gave a concentration level in the aqueous sample of 15 pg/μL (ppb). This concentration level is considered low enough to approximate the realm of TEQA. After the sample was passed through the nine replicate RP-SPE cartridges, two 500-μL aliquots of ethyl acetate were used to elute the sorbed analytes into a 1-mL receiver volumetric flask. The contents of the volumetric flask were quantitatively transferred to a 2-mL GC autosampler glass vial, and 2 μL of this eluent was injected into a GC that contained a packed chromatographic column. Only one injection per GC vial was made in this particular study, so that variation in the percent recovery reflects only the random error associated with the RP-SPE process only. Table 3.12 lists the percent recovery for all three OCs from each of the nine cartridges. The concentration of analyte that constituted a 100% recovery was calculated and not actually measured; hence, there is no contribution to the relative standard deviation from the control standard, as shown in Equations (2.30) and (2.31). It is evident from review of the percent recovery results shown in Table 3.12 that breakthrough was not reached even in the case where only 20 mg of sorbent was taken to prepare the packed cartridge.

The mean percent recovery can be found from these nine replicate SPEs. The standard deviation in the mean percent recovery is then found using the fundamental equation for calculating standard deviations. If replicate injections per GC vial were made, a pooled standard deviation relationship, as given in Equation (2.33), would be most appropriate. From the standard deviation, a relative standard deviation or coefficient of variation is obtained, followed by calculation of the corresponding confidence interval. For the percent recoveries shown in Table 3.12, the following statistical evaluation was obtained:
The mean percent recoveries in this replicate series of RP-SPEs are very high and represent a statement of accuracy in the measurement. A relative standard deviation of 8 or 14% among replicate SPEs represents the precision of the method. The confidence interval states that of the next 100 SPEs, 95 of these should fall within the interval specified. For example, if 100 additional percent recoveries using the SPE method could be performed for lindane, one could expect that 95 would fall within 96.8 ± 6.2%.

This high and reproducible percent recovery for the isolation and recovery of lindane from water strongly suggests that RP-SPE is very appropriate as a sample preparation method for this analyte. Lindane is of continued interest to environmental and toxicological scientists. One such study, discussed next, taken from the author’s work, involves the isolation and recovery of lindane from homogenized myometrial tissue suspended in an aqueous matrix.

---

**TABLE 3.12**

Percent Recoveries of OCs Using a C₈-Bonded Silica Sorbent from Spiked Water

<table>
<thead>
<tr>
<th>Sorbent (mg)</th>
<th>Lindane</th>
<th>Endrin</th>
<th>Methoxychlor</th>
</tr>
</thead>
<tbody>
<tr>
<td>115</td>
<td>94.3</td>
<td>111</td>
<td>93.2</td>
</tr>
<tr>
<td>291</td>
<td>97.1</td>
<td>87.3</td>
<td>80.8</td>
</tr>
<tr>
<td>225</td>
<td>106</td>
<td>133</td>
<td>107</td>
</tr>
<tr>
<td>241</td>
<td>100</td>
<td>111</td>
<td>100</td>
</tr>
<tr>
<td>98</td>
<td>100</td>
<td>122</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>106</td>
<td>93.2</td>
</tr>
<tr>
<td>52</td>
<td>77.1</td>
<td>87.3</td>
<td>86.3</td>
</tr>
<tr>
<td>130</td>
<td>97.1</td>
<td>113</td>
<td>93.2</td>
</tr>
<tr>
<td>320</td>
<td>100</td>
<td>95.2</td>
<td>93.2</td>
</tr>
</tbody>
</table>

Note: 8.8 µL of 100 ppm each of lindane, endrin, and methoxychlor, dissolved in methanol, was added to approximately 60 mL of distilled deionized water. The spiked samples were passed through the cartridge, previously conditioned with methanol, and eluted with two 500-µL aliquots of ethyl acetate. The volume of eluent was adjusted to 1.0 mL, and 2 µL of eluent was injected into a GC.
68. WAS LINDANE ISOLATED AND RECOVERED FROM A BIOLOGICAL MATRIX USING RP-SPE? IF SO, HOW?

One hundred microliters of a methanolic solution containing 5 ng/µL (ppm) lindane was placed in a 1-mL volumetric flask half filled with iso-octane, while 100 µL of the same lindane reference standard was added to approximately 70 mL of distilled deionized water (DDI). One microliter of the former solution containing 500 ng of lindane was injected into a GC, and the resulting peak area served to define a control that represents a 100% recovery of lindane. One microliter of the 1-mL eluent from performing the RP-SPE of the spiked DDI was also injected into the same instrument, and the resulting peak area served to define the spiked recovery sample. In this study, quadruplicate injections of the control yielded a mean concentration of 400 ppb lindane from interpolation of the least squares calibration curve. Seven replicates of the eluent from the spiked DDI sample were injected, and a mean concentration of 406 ppb lindane was obtained from interpolation of the same calibration curve. Equation (2.57) was used to find a 106% recovery of lindane. RSDs in the % recovery were found by propagating random error between spiked samples and control reference standards. A complete result can be given by stating both the accuracy, 106% recovery, and the relative standard deviation of 15.6%. Myometrium samples were then prepared and lindane appeared as expected. This work showed that lindane could easily be isolated and recovered from an aqueous matrix and confirmed the earlier work on lindane isolation and recovery, discussed previously. One would be led to believe that this is a robust sample preparation method for lindane because it can be reproduced with confidence. Thus, a subsequent request for additional sample analyses does not require extensive QC and should merely report the analytical results.

69. WHAT DOES A SAMPLE ANALYSIS REPORT LOOK LIKE?

In addition to a tabular format for the determination of lindane in each of the myometrial tissue samples submitted, a method summary should be included. A one-paragraph method summary serves to inform the reader as to how the samples were handled once they arrived in this analyst’s laboratory. The summary should also provide a brief overview of the sample preparation and a brief description of the determination technique used. The following report illustrates these concepts.

**REPORT ON THE QUANTITATIVE DETERMINATION OF LINDANE IN MYOMETRIAL TISSUE SUSPENDED CELLS IN SALINE**

*Summary of Method*

The entire contents (1 mL) of sample that was received by the client were refrigerated upon receipt until the sample was prepared for analysis. Upon thawing, the entire contents of each sample were added to a reservoir that contained approximately 70 mL of distilled deionized water. This aqueous solution was passed across a previously conditioned octadecyl-bonded silica sorbent (C18RPSiO2). The retained analyte was
eluted off of the sorbent with two 500-µL aliquots of pesticides-residue-grade iso-octane. The iso-octane eluent was then passed through a second SPE cartridge. This second cartridge was packed with approximately 0.5 g of anhydrous sodium sulfate. The volume of eluent that now contained the recovered analyte was adjusted to a final volume of 1.0 mL using a volumetric flask. Three microliters of this eluent was injected via autosampler into an Autosystem Gas Chromatograph (PerkinElmer) incorporating an electron-capture detector. A 30 m × 0.32 mm capillary GC column containing DB-5 (J&W Scientific) was used to separate the organics in the eluent. This instrument is abbreviated C-GC-ECD to distinguish it from other gas chromatographs in our laboratory. The column was temperature programmed following injection from 200 to 270°C at a rate of 10°C/min. The C-GC-ECD is connected to a 600 Link (PE-Nelson) interface module. This interface, in turn, is connected to a 386 personal computer. This PC uses Turbochrom® (PE-Nelson) software for data acquisition, processing, and control. A method specific for lindane was written, and one peak was identified within a 3% relative time interval (retention time window). A retention time, \( t[R] \), of 1.8 min was consistently reproduced using autosampler injection.

**Calibration**

A series of calibration or working standards were prepared from the methanolic stock solution containing lindane. This stock solution was prepared by carefully weighing out pure solid lindane on an analytical balance. These standards were injected into the C-GC-ECD from lowest to highest lindane concentration. The peak at 1.8 min was identified as a reference peak in the Turbochrom software, and a narrow \( t[R] \) window was defined around the \( t[R] \) of the apex of the peak. A calibration curve was constructed using a least squares regression algorithm in the software. A correlation coefficient of 0.9990 was obtained.

**Sample Analysis**

The sequence within which the samples were run after calibration was created by using the Sequence File Editor within Turbochrom. Samples are injected via autosampler according to the instructions in the Sequence File. After the sequence is completed, a Summary File is created and the analytical results are reported within this summary format. In the Summary File, for each sample analyzed, the following is given:

(a) The sample number
(b) The concentration of lindane in ppb for 1.0 mL of eluent, interpolated from the external standard mode of instrument calibration
(c) The retention time for lindane, \( t[R] \), in minutes
(d) The integrated peak area, in microvolts-seconds

The reported concentration is that for each eluent and should be multiplied by the eluent volume (in this case, 1.0 mL) to obtain the number of nanograms of lindane found. The number of nanograms found divided by the volume of the aqueous sample used in RP-SPE gives the reported concentration in ppb for lindane. The reported concentration should be divided by the volume of the sample to obtain the reported concentration in ppb for lindane. The reported concentration should also be divided by the percent recovery, expressed as a decimal, in order to find the true and final concentration of lindane in the original sample.
Method Evaluation

Lindane is efficiently recovered from aqueous matrices with percent recoveries between 75 and 100% using RP-SPE. The biological sample closely approximates an aqueous matrix.

70. DOES IT MATTER WHICH ELUTION SOLVENT IS USED IN RP-SPE?

Answers to this can be given as yes and no and a qualifying statement that it might depend on the polarity of the retained analyte. With a series of analytes that have similar octanol–water partition coefficients, differences in eluting solvent polarity may not significantly influence analyte recovery. If breakthrough studies are not performed, as discussed earlier, one never knows whether low percent recoveries are due to poor sorption or to inefficient elution or solvent removal using the RP-SPE technique. This author recently had the opportunity to quantitatively determine trace concentrations of 2,4,6-trichlorobiphenyl (246TCBP) and 4-hydroxy-2,4,6-trichlorobiphenyl (4HO246TCBP) in biological matrices. Molecules from these two organic compounds of environmental interest differ in that a hydroxyl group is substituted for a hydrogen on the phenyl ring para to the second phenyl. These structures are as follows:

![Structures](image)

We were quite surprised to measure a zero percent recovery when we attempted to apply our method for OCs to isolate and recover 4HO246TCBP from spiked water. Our recoveries of 246TCBP using our OC method were very high. Replacing iso-octane with methanol as the eluting solvent resulted in a significant increase in percent recoveries for 4HO246TCBP. This surprising finding led us to conduct a series of systematic RP-SPE recovery studies. An aqueous spiked sample was prepared that contained both PCB congeners, and two elution schemes were used. In Scheme A, the retained analytes were eluted with methanol followed by a separate, second elution with iso-octane. Two receivers were used, and their final volumes were both adjusted to a 1.0-mL total eluent volume. In Scheme B, the retained
analytes were eluted with iso-octane followed by methanol. The integrated peak areas (in units of µV-sec) for the 1-mL eluents are represented in Table 3.13. It is a good assumption that the response factors for both congeners are near one another because the substitution of a hydroxyl for hydrogen in the phenyl ring contributes little or nothing to the ECD response. Eluting first with two 500-µL aliquots of methanol recovers more 4HO246TCBP than 246TCBP, whereas the second elution with iso-octane recovers nearly equal amounts of both congeners. Thus, Scheme A reflects incomplete recovery of the congeners with two 500-µL aliquots of methanol. Eluting first with two 500-µL aliquots of iso-octane recovered even more 246TCBP than MeOH, yet recovered no 4HO246TCBP. This observation was consistent with our preliminary finding, discussed earlier. Upon eluting with MeOH via Scheme B, a relatively small amount of 246TCBP was recovered, whereas a relatively large amount of 4HO246TCBP was recovered. The percent recoveries for 4GO246TCBP were similar between Schemes A and B when MeOH was used to elute off the retained analyte.

Another illustration from the author’s experience is the differences in percent recovery when hexane is used vs. methyl-tert-butyl ether (MTBE) to elute off of a C18 sorbent. Two different aqueous matrices were spiked with the OPs diazinon and malathion. In one series of experiments, two 500-µL aliquots of hexane were used to elute off of the cartridge, followed by two 500-µL aliquots of MTBE eluted off of the same cartridge. The same experiment was performed on a second aqueous matrix that was more acidic by virtue of adding potassium dihydrogen phosphate (KH₂PO₄). The results are shown in Table 3.14. It is clearly evident that the more polar MTBE more effectively removes both OPs from the hydrophobic sorbent than the less polar hexane. This fact has perhaps more to do with the polarity of OPs and the nature of the sorbent–analyte–solvent triangle, as discussed earlier in this chapter.

### Table 3.13
Comparison of Two Sample Preparation Schemes to Isolate and Recover 2,4,6-Trichlorobiphenyl and 4-Hydroxy-2,4,6-Trichlorobiphenyl Using RP-SPE

<table>
<thead>
<tr>
<th>Scheme</th>
<th>246TCBP</th>
<th>4HO246TCBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>137,026</td>
<td>218,526</td>
</tr>
<tr>
<td>Elute with MeOH, mean of 4 SPEs</td>
<td>40,413</td>
<td>58,259</td>
</tr>
<tr>
<td>Elute with iso-octane, mean of 4 SPEs</td>
<td>210,234</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>24,825</td>
<td>194,002</td>
</tr>
<tr>
<td>Elute with iso-octane, mean of 4 SPEs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elute with MeOH, mean of 4 SPEs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

71. **HOW CAN PCBS BE ISOLATED AND RECOVERED FROM SERUM, PLASMA, OR ORGAN FOR TRACE ENVIRONMENTAL HEALTH QA?**

Scheme 3.1 (shown earlier) is a flowchart that summarizes this author’s attempt to use RP-SPE as part of a combination of sample prep techniques that isolate and...
recover AR 1248 with nearly 100% recoveries. PCBs are easily released from a serum, plasma, or tissue homogenate via probe sonification (PC), coagulation using acetonitrile, or other water-miscible organic solvent or salt. The supernatant aqueous phase is easily separated from the coagulated protein via mini-centrifugation. RP-SPE serves in this case, similar to the situation when an environmental water sample is passed through, as an on–off or digital extraction step. The dilute of the aqueous supernatant serves to decrease the analyte–matrix interaction, as discussed earlier, which in turn strengthens the analyte–sorbent interaction. In this case, the combination of PS-RP-SPE with C-GC-ECD as the determinative technique eliminated the need for further cleanup. Such might not be the case if C-GC-MS or another universal determinative technique is used. Cleanup techniques might then need to be considered. Listed below are significant outcomes from the author’s method development study:

Janák and coworkers' have discussed a similar approach to the isolation and recovery of PCBs from whole blood. Details of their approach are outlined via a flowchart in Scheme 3.8. Referring to Scheme 3.8A, formic acid dissolved in isopropyl alcohol (IPA) was used to coagulate protein from 5 g of whole blood prior to bath sonication. After passing the supernatant through a conditioned RP-SPE cartridge, the sorbent is washed with 5% IPA dissolved in water, followed by 10% methanol in water. The sorbent is then washed again with a series of solutions as indicated and dried. Referring to Scheme 3.8B, the sorbent is eluted with methylene chloride (dichloromethane), then evaporated down to 10 μL, and finally reconstituted with enough heptane to yield ~100 μL. This heptane eluent is transferred to a Pasteur

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery as Total PCB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiked acetonitrile 1</td>
<td>102</td>
</tr>
<tr>
<td>Spiked acetonitrile 2</td>
<td>102</td>
</tr>
<tr>
<td>Spiked rat liver 1</td>
<td>115</td>
</tr>
<tr>
<td>Spiked rat liver 2</td>
<td>88.2</td>
</tr>
<tr>
<td>Spiked rat liver 3</td>
<td>77.7</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
pipette filled with mixed adsorbents and previously rinsed with heptane. The cleaned up PCBs are subsequently eluted with a binary solvent eluent consisting of 4:1 heptane to methylene chloride. Mean percent recoveries reported from spiked whole-blood specimens were 78 ± 8%.

RP-SPE as a sample prep technique is low cost, effective, and continues to gain popularity as regulatory restrictions lift over time. For enviro-chemical QA considerations, Font et al. has reviewed developments in water pollution analysis for PCBs and earlier published a review applicable to multiresidue pesticide analysis of water. For enviro-health QA considerations, the comprehensive study involving bovine and human serum conducted by the Centers for Disease Control and Prevention.
A plethora of analytical papers utilizing RP-SPE for up-front extraction have appeared in recent years. We now consider a sample prep technique developed during the late 1980s that relates to SPE, yet is applicable to solid matrices, in particular biological tissue specimens. It is called \textit{matrix solid-phase dispersion}.

\textbf{Scheme 3.8B}

(CDC) is noteworthy. A plethora of analytical papers utilizing RP-SPE for up-front extraction have appeared in recent years. We now consider a sample prep technique developed during the late 1980s that relates to SPE, yet is applicable to solid matrices, in particular biological tissue specimens. It is called \textit{matrix solid-phase dispersion}.

\textbf{72. WHAT IS MATRIX SOLID-PHASE DISPERSION AND IS IT APPLICABLE TO BIOLOGICAL TISSUE?}

In the same manner that S-LSE complements LLE, matrix solid-phase dispersion (MSPD) has emerged as a complement to RP-SPE. MSPD is most applicable to
biological tissue. Biological tissue is easily “ground” into a chemically bonded silica sorbent. Soil may prove to be a more difficult sample matrix to achieve MSPD with. MSPD came about when it was realized that the octadecyl silyl–silica gel sorbent used in RP-SPE could have abrasive properties, while the octadecyl ligates could extract analytes from the biological tissue. Barker, along with coworkers, who published the benchmark paper\textsuperscript{105} on MSPD puts in this way:\textsuperscript{106}

MSPD combines aspects of several techniques for sample disruption while also generating a material that possesses unique chromatographic character for the extraction of compounds from a given sample.

A biological sample matrix such as fish tissue is ground into conventional RP-SPE sorbents such as C\textsubscript{18} silica using a mortar and pestle. The cell structure is disrupted, and organic compounds are released and partitioned into the C\textsubscript{18} silica based on their respective partition coefficients along the same lines as already discussed for RP-SPE. Barker\textsuperscript{107} has compared scanning electron micrographs for bovine liver tissue ground with underivatized silica to those of the same tissue ground with C\textsubscript{18} silica. Although dispersed with both silicas, the underivatized silica shows an in-tact cell structure evenly dispersed over the material.

MSPD is straightforward to perform in the laboratory. A schematic representation of an eight-step process to perform MSPD is shown in Figure 3.26A, which depicts the first four steps of blending the tissue with the bonded silica, while Figure 3.26B depicts the last four steps of solvent elution. A glass or agar mortar and pestle is preferable to the more porous porcelain type to minimize analyte loss. Only gentle blending is recommended. A ratio of 4:1, i.e., 4 g of C\textsubscript{18} silica sorbent material to 1 g of biological tissue, has evolved as the optimum amounts to blend.\textsuperscript{108}

Chemically bonded silicas serve several functions in MSPD. Bonded silicas serve as:\textsuperscript{108}

- An abrasive that promotes cell disruption
- A lipophilic, bound solvent that disrupts and lyses cell membranes
- A sorbent capable of being packed into a column and can be eluted with solvents of differing polarity
- A bonded-phase support that enables sample fractionation

It is all too easy to compare RP-SPE with MSPD. However, after the biological tissue has been blended with the bonded silica sorbent, a new sorbent or stationary-phase results. In RP-SPE, after a sample has passed through a column, most components of the sample accumulate at the top of the column bed. In MSPD, the blended sample suggests that the components are more uniformly distributed throughout the entire sorbent. This creates what Barker\textsuperscript{108} calls a “unique chromatographic phase” whose dynamic interactions are “not completely understood.”

73. WHAT FACTORS INFLUENCE PERCENT RECOVERIES IN MSPD?

A robust sample prep technique ought to yield a decent percent recovery. What have researchers who have sought answers to this question found?\textsuperscript{106}
Sample Preparation Techniques

- General principles of SPE apply.
- A unique chromatographic stationary phase is created when biological tissue is blended with chemically bonded silica.
- Underivatized silanols on the surface and in the pores of the support apparently remove water from the blend, thus yielding a drier support.
- Pore size does not seem to be significant.
- Particle size is relatively important since particles of 3 to 20 µm do not permit flow of elution solvent via gravity, whereas the conventional 40-to 100-µm-diameter particles do permit flow.
- A lipophilic bonded phase is believed to lead to the formation of a new phase that resembles a cell membrane bilayer assembly, giving the MSPD material unique chromatographic properties.
- The percent carbon load does not appear to have an appreciable effect.
- Conditioning of the bonded silica is as essential to MSPD as it is to SPE.

**FIGURE 3.26A** Schematic that shows just how to conduct the first four steps of MSPD.
The matrix has a profound impact on percent recoveries, unlike SPE, due to the fact that the matrix becomes part of the chromatographic phase. Elution yields certain coeluted matrix components with certain analytes of interest that are not well predicted based on SPE principles.

Huang and coworkers\textsuperscript{109} found that percent recoveries of two different PCB (presumably Aroclors)-fortified fish (grass carp) samples utilizing MSPD compared favorably with the more conventional approach of saponification, followed by LLE. Acidified silica gel was added to the elution syringe and provided cleanup of coextractants that resulted in the appearance of a distinct yellow color for the hexane eluent when silica gel was not used. Ling and Huang\textsuperscript{110} followed up their benchmark
Sample Preparation Techniques

paper on isolating and recovering PCBs from fish tissue using MSPD with a second paper related to OCs as well as PCBs. These authors found an optimum cleanup and elution solvent combination following the blending of fish tissue with octadecyl silyl-derivatized silica (ODS), which maximized percent recoveries while minimizing coextractive interferences. For a given ODS, the following adsorbents were tried: Florisil, acidic silica gel (44% H$_2$SO$_4$), and neutral alumina. The mass of fish muscle tissue was obtained by subtracting the mass of the adsorbent-loaded-only column from the mass of the sample-loaded column. Percent recoveries of >90% were realized for most priority pollutant OCs using the Florisil/hexane–acetone (9:1) combination. The authors applied MSPD to a number of fish samples from fish caught from a river that passes through an incineration facility in Taiwan. PCB levels were significantly higher than OC levels. One fish, caught upstream from the facility, showed much lower concentration levels of PCBs.

MSPD provides an alternative to conventional LLE, cleanup, and fractionation sample prep methods as applied to biological tissue. However, laboratories seem slow to adopt MSPD, as evidenced by the discontinuance of the MSPD kit by Varian Sample Preparation Products (the only SPE supplier who ever offered MSPD in kit format). One more offshoot of conventional SPE is now introduced. This sample prep technique was developed in the early 1990s and is called either solventless SPE or, more commonly, solid-phase microextraction.

74. WHAT IS SOLID-PHASE MICROEXTRACTION?

One way to answer this question is to state that solid-phase microextraction (SPME) is to the capillary GC column what SPE is to the HPLC column. For SPE, consider taking a portion of the HPLC column packing, increasing the particle size, and allowing for a wider distribution of the particle size; then pack this material into a cartridge and use this for sample prep. For SPME, on the other hand, coat a fused-silica capillary column on the outside, instead of the inside, and use this for sample prep. Janusz Pawliszyn at the University of Waterloo, who understood the limitations of SFE and SPE, was the first to modify a 7000 Series (Hamilton) liquid-handling microsyringe by coating polydimethyl siloxane on a fine rod such as fused-silica fiber. (See Zhang et al. for two of the pertinent articles published by Pawliszyn and coworkers.) SPME is also solventless in that a sorbed analyte is easily thermally desorbed off of the coated fiber in the injection port of a gas chromatograph. The modified microsyringe of Pawliszyn has given way to the commercial SPME device that incorporates a retractable fiber and is manufactured and marketed by Supelco. The essential design of an SPME sampling device is shown in the following schematic.

The SPME sampling device can be immersed directly into an aqueous sample, such as groundwater, for a finite period, then withdrawn, the fiber and rod retracted back into the needle, brought to the hot-injection port of a gas chromatograph, and then inserted into the septum and the fiber and rod extended into the injection port for a finite period, in which thermal desorption is accomplished. For analysis of aqueous samples for VOCs, the fiber is inserted into the headspace, sampled, retracted, and then injected directly into the injection port. For solids, wastewater,
sludge, etc., this headspace technique is appropriate provided that analytes can partition into the headspace from these dirty sample matrices. The rate of SPME depends on the mass transport from a matrix to the coating. The effectiveness of mass transport depends on the following:

1. Convective transport in air or liquid
2. Desorption rate from particulates that might be present in the sample
3. Diffusion of analytes in the coating itself

For direct SPME sampling with agitation, convective effects can be minimized. In the absence of particulates, the mass transport rate is determined by diffusion of analytes into the coating. For gaseous samples, the rate of mass transport is determined by diffusion of the analyte into the coating, and equilibrium can be achieved in less than 1 min. For aqueous samples, vigorous agitation of the sample is necessary. A common technique is to simply stir the sample with a magnetic stirrer. In this case, it takes much longer for the analyte to diffuse through the static layer of water that surrounds the fiber. Zhang et al.\textsuperscript{115} articulated the challenge for SPME as follows:

For volatile compounds, the release of analytes into the headspace is relatively easy because analytes tend to vaporize once they are dissociated from their matrix. For semivolatile compounds, the low volatility and relatively large molecular size may slow the mass transfer from the matrix to the headspace and, in some cases, the kinetically
controlled desorption or swelling process can also limit the speed of extraction, resulting in a long extraction time. When the matrix adsorbs analytes more strongly than the extracting medium does, the analytes partition poorly into the extraction phase. Because of the limited amount of the extraction phase in SPME (as in SPE), the extraction will have a thermodynamic limitation. In other words, the partition coefficient, $K$, is too small, resulting in poor sensitivity. If the coating has a stronger ability to adsorb analytes than the matrix does, it is only a matter of time for a substantial amount of analytes to be extracted by the fiber coating and only kinetics plays an important role during extraction. One of the most efficient ways to overcome the kinetic limitation is to heat the sample to higher temperatures, which increases the vapor pressure of analytes, provides the energy necessary for analytes to be dissociated from the matrix, and at the same time speeds up the mass transport of analytes.

As was developed for other distribution equilibria, such as LLE [Equation (3.4)] and static HS [Equation (3.25)], we start to discuss the principles that underlie SPME by considering the equilibrium for the $i$th analyte between a sample $S$ and the coated fiber, $f$, once dynamic equilibrium has been reached.

Let us consider this in more detail. We start by defining the partition coefficient, $K'_{f|S}^{i,\text{SPME}}$, for analyte $i$ between the fiber, denoted by $f$, and a sample, denoted by $s$, as follows:

$$
K'_{f|S}^{i,\text{SPME}} = \frac{C_f}{C_s} = \frac{n/V_f}{C_0 - (n/V_f)}
$$

(3.44)

where

- $n$ = amount of analyte $i$ adsorbed on the SPME polymer film (in moles)
- $V_f$ = volume of coating on SPME fiber
- $V_s$ = volume of sample
- $C_0$ = initial concentration of the $i$th analyte in the sample
- $C_f$, $C_s$ = concentration of the $i$th analyte in the fiber and sample, respectively, once equilibrium is reached

Solving Equation (3.44) for $n$ yields

$$
n = \frac{K'_{f|S}^{i,\text{SPME}} V_f V_s}{V_f + K'_{f|S}^{i,\text{SPME}} V_f} C_0
$$

(3.45)

Equation (3.45) presents an opportunity to make two simplifying assumptions. If

$$
K'_{s|0} >> V_s
$$

then

$$
n_0 = V_s C_0.
$$
The amount of analyte sorbed on the fiber is directly proportional to the original concentration of that analyte in the sample. If, however, a large sample volume is used,

\[ K_{f}^{i} V_{f} \ll V_{s} \]

then

\[ n_{0} = K_{f}^{i} V_{f} C_{0}. \]

The amount of analyte sorbed on the fiber is also directly proportional to the original concentration of that analyte in the sample. Both simplifying assumptions lead to the conclusion that the amount of analyte sorbed, after equilibrium is attained, can be related to \( C_{0} \). The term used to describe this is \textit{exhaustive SPME}, and the maximum number of moles of the \( i \)th analyte that can be adsorbed or partitioned into the fiber is denoted by \( n_{0} \).

75. CAN WE QUANTITATE BEFORE WE REACH EQUILIBRIUM IN SPME?

We answer the question by considering the derivation first proposed by Ai.\textsuperscript{116} The assumption is that analyte molecules diffuse from (1) the sample matrix to the surface of the polymer and (2) through the polymer surface to the inner layers. For a steady state, with diffusion as the rate-controlling factor, the mass flow rate of analyte molecules from the sample matrix to the SPME polymer surface would equal the flow rate from the polymer surface to its inner layers. With \( D_{1} \) as the diffusion coefficient of analyte molecules in the sample matrix and \( D_{2} \) as the diffusion coefficient for molecules in the polymer phase whose surface area is denoted by \( A \), and \( C_{s} \) and \( C_{f} \) are concentrations of analyte in the sample matrix and polymer film, respectively, Fick’s first law can be used to give the rate-determining steps governed by

\[
\frac{1}{A} \frac{dn}{dt} = -D_{1} \frac{\partial C_{s}}{\partial x} = -D_{2} \frac{\partial C_{f}}{\partial x}
\]

By assuming that a steady-state mass transfer occurs when agitation is effectively applied, that the diffusion layer is a thin film, and that steady-state diffusion in this thin film is in effect, a simplified normal differential equation can be considered:

\[
\frac{1}{A} \frac{dn}{dt} = \frac{D_{1}}{\delta_{1}} (C_{s} - C'_{s})
\]

\[= \frac{D_{2}}{\delta_{2}} (C_{f} - C'_{f}) \tag{3.46}\]

The terms used in Equation (3.46) are defined as follows:
Sample Preparation Techniques

\(C_s\) = concentration of the analyte in the bulk sample matrix
\(C'_s\) = surface concentration of the analyte in bulk sample matrix
\(\delta_1\) = diffusion layer thickness in the sample matrix
\(\delta_2\) = thickness of polymer film
\(C_f\) = analyte concentration in the polymer film at the surface
\(C'_f\) = analyte concentration in the polymer film in contact with the silica fiber

Shown below is a schematic of the interface of the polymer-coated silica fiber in contact with an aqueous solution. The illustration shows the diffusion layer thickness in the sample matrix and the thickness of the polymer film. The slopes of the concentration gradients are shown at the interface between the bulk sample and polymer film surface. A steady-state diffusion is assumed when the aqueous solution is effectively agitated. The concentration gradient in the SPME film is assumed to be linear:

Equation (3.46) can be rearranged and integrated, and within a set of boundary conditions this ordinary differential equation can be solved for \(n\) to yield\(^{116}\)

\[
n = [1 - e^{-A(t)}] \left[ \frac{KV_f V_s}{KV_f + V_s} \right] C_0
\]  

(3.47)

Equation (3.47) suggests that the number of moles of analyte sorbed depends only on the original concentration of analyte in the sample, \(C_0\), provided that the sampling time, \(t\), and the rates of diffusion remain constant between samples. This diffusion rate is reflected in the slope of the lines between \(C_s\) and \(C'_s\) and \(C_f\) and \(C'_f\), as shown above. As the sampling time gets very large (i.e., \(n\) approaches infinity), Equation (3.47) suggests that \(n\) approaches \(n_0\), where \(n_0\) is the number of moles adsorbed by the coating at equilibrium, so that Equation (3.47) reduces to Equation

© 2006 by Taylor & Francis Group, LLC
Hence, Equation (3.47) can be rewritten in terms of \( n \) as a function of sampling time according to

\[
\frac{n}{n_0} = 1 - e^{-at}
\]  

(3.48)

where \( a \) is a constant that is composed of various mass transfer coefficients, \( K_{fs,SPME} \), \( V'_f \), and \( V_c \). If plots are made of \( n/n_0 \) vs. SPME adsorption or sampling time, curves such as the following one for a given analyte are expected:

In a second paper, Ai\textsuperscript{117} developed a theoretical treatment for HS-SPME. Lakso and Ng\textsuperscript{118} used SPME to isolate nerve gas agents such as Sarin from drinking water and observed similar uptake vs. adsorption time for direct aqueous sampling. A plethora of SPME applications continue to appear in the literature. This author began to use SPME in pursuit of alternative sample prep methods designed to isolate and recover trace concentration levels of specific polychlorinated biphenyl (PCB) congeners and various Aroclors, along with the necessary surrogates, as utilized in EPA Method 8081A.

76. WHAT FACTORS NEED TO BE CONSIDERED WHEN USING SPME TO ISOLATE PCB?

The sample prep considerations are different for SPME from anything we have encountered because this is a solventless technique and requires that we consider the kinetics of sampling. Some factors are as follows:

- Sorption time for the fiber, as discussed earlier
- Thermal desorption temperature of the GC injector port
- Isothermal column temperature and time during thermal desorption
- Chromatographic temperature program to adequately resolve peaks in minimum run time
The molecular structures of the analytes of interest are as follows:

Three chromatograms that were obtained in the author’s laboratory and are overlayed for comparative purposes are shown in Figure 3.27A. The SPME device was used to extract the four polychlorinated organics shown earlier and thermally desorbed into the injection port of an Autosystem® (PerkinElmer) gas chromatograph that incorporated a chlorine-selective electron-capture detector (C-GC-ECD). The top chromatogram was obtained by exposing the fiber into distilled deionized water and constitutes a method blank. The method blank is a useful quality control practice in that a clean blank, as this one shows, demonstrates that the lab and analyst are free of contamination. The absence of any lab contamination suggests that the method used is free of interferences. The center chromatogram was obtained by exposing the fiber to water that had been spiked with the four polychlorinated organics. All four analytes had been extracted out of the sample and thermally desorbed into the injection port of the gas chromatograph.
FIGURE 3.27A Comparison of three SPME/C-GC-ECD chromatograms from a blank, spiked blank, and thermal desorbed blank. (This and the following six panels were taken in the author’s laboratory.)
desorbed into the C-GC-ECD. The separated analytes are given with their abbreviated names and GC retention times ($t_R$) in the following table:

<table>
<thead>
<tr>
<th>Polychloro-organic</th>
<th>Abbreviation</th>
<th>$t_R$ (Minutes after Injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrachloro-$m$-xylene</td>
<td>TCMX</td>
<td>10.29</td>
</tr>
<tr>
<td>2,2',4,4'-Tetrachlorobiphenyl</td>
<td>2244-TCBP</td>
<td>12.90</td>
</tr>
<tr>
<td>3,3',4,4'-Tetrachlorobiphenyl</td>
<td>3344-TCBP</td>
<td>15.32</td>
</tr>
<tr>
<td>Decachlorobiphenyl</td>
<td>DCBP</td>
<td>21.40</td>
</tr>
</tbody>
</table>

The bottom chromatogram was obtained from merely injecting the already spent fiber into the hot-injection port of the C-GC-ECD a second time. Evidence of a tiny trace of each analyte is observed.

Two chromatograms are overlayed in Figure 3.27B. A comparison is shown between a spiked and unspiked aqueous sample using AR 1242. The power of SPME to preconcentrate analytes is shown in Figure 3.27C. The top chromatogram in Figure 3.27C was obtained by injection of 1 µL of a methanolic 10 ppm AR 1242 reference standard, and the bottom chromatogram was obtained by applying the SPME technique to a sample that consisted of 50 µL of methanolic 10 ppm AR 1242 added to 10 mL of distilled deionized water. The sum of the numerous peaks in the chromatogram for AR 1242 provides a quantitative analysis for total Aroclor. A plot of this sum vs. the SPME sorption time is shown in Figure 3.27D for four sampling times. This plot roughly follows the theoretical profile shown earlier for SPME. For a fixed sorption time and magnetic stir rate, a series of calibration standards were prepared and “SPME injected” into our C-GC-ECD. The calibration curve is shown in Figure 3.27E for eight standards. The raw data points are fitted to a third-order polynomial, as shown using Turbochrom (PE-Nelson) software. This author examined the calibration and utilized the program LSQUARES (refer to the program listing in Appendix C). The results of using LSQUARES are as follows:

<table>
<thead>
<tr>
<th>Concentration (ppb)</th>
<th>Sum of Peaks from AR 1242</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7</td>
<td>217,483</td>
</tr>
<tr>
<td>23.3</td>
<td>464,987</td>
</tr>
<tr>
<td>46.6</td>
<td>718,531</td>
</tr>
<tr>
<td>93.2</td>
<td>777,197</td>
</tr>
<tr>
<td>117</td>
<td>976,544</td>
</tr>
<tr>
<td>233</td>
<td>1,510,288</td>
</tr>
<tr>
<td>466</td>
<td>2,243,387</td>
</tr>
<tr>
<td>932</td>
<td>4,666,817</td>
</tr>
</tbody>
</table>
FIGURE 3.27B Overlay of SPME/C-GC-ECD chromatograms for isolating AR 1242 from a blank and a spiked blank.
FIGURE 3.27C Comparison of a direct injection of AR 1242 and AR 1242 isolated and recovered using SPME.

1 ul of 10 ppm AR 1242 (freshly prepared) injected into C-GC-ECD
150 (hold for 2 min) then program to 225 @ 45 deg/min
Program from 225 to 275 @ 6 deg/min

SPME from spiked DDI/50 ul of 10 ppm AR 1242
added to 10 mL DDI in SPME vial
Time of sorption w vigorous magnetic stirring
for 23.5 min/thermal desorption at 325 C for 2.2 min
FIGURE 3.27D Plot of the sum of peak areas for AR 1242 against the sorption time for SPME; a 100 µm thick nonbonded polydimethylsiloxane fused silica fiber was used.

FIGURE 3.27E Plot of the sum of peak areas for AR 1242 against the concentration of AR 1242 in ppb using SPME techniques; superimposed over these points is a third order least squares regression fit. The same fiber was used here as that described for Figure 3.28D.
Applying LSQUARES to this calibration data yields the following:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope of least squares regression line (linear fit)</td>
<td>4548 µV-sec/ppb AR 1242</td>
</tr>
<tr>
<td>y intercept of least squares line</td>
<td>353,887</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9954</td>
</tr>
<tr>
<td>$Y_c$</td>
<td>674,348 µV-sec</td>
</tr>
<tr>
<td>$X_c$</td>
<td>70.4 ppb</td>
</tr>
<tr>
<td>$Y_o$</td>
<td>139.7 ppb</td>
</tr>
<tr>
<td>ICV (low) ± confidence interval at 95%</td>
<td>80.2 ± 87 ppb</td>
</tr>
<tr>
<td>ICV (high) ± confidence interval at 95%</td>
<td>415.4 ± 87 ppb</td>
</tr>
</tbody>
</table>

It is clearly evident that, at least for this first attempt to simultaneously conduct sample prep and calibrate the instrument, good linearity was not achieved. Note that the initial calibration verification (ICV) represented by $x_o$ is much more accurate at the higher concentration level than at the lower level. In addition, the precision determined by the confidence interval at the 95% significance level at the lower level for the ICV is much larger than is the precision for the ICV at the higher concentration level.

A calibration plot for combined SPME/calibration for the surrogate TCMX is shown in Figure 3.27F. Again, there is a tendency for a nonlinear relationship as the concentration of TCMX increases, as shown. A second-order least squares fit gave a correlation coefficient of 0.9999.

77. **MIGHT THE SAMPLE MATRIX INFLUENCE SPME EFFICIENCY?**

Yes, the sample matrix may significantly influence the analyte percent recovery. This became apparent to the author when a request to isolate and recover AR 1242 from adulterated rat feed was made. It became necessary to remove the lipid content (at approximately 7 to 8%), and this was accomplished via base saponification. We attempted to isolate the AR 1242 from this base-saponified matrix, and the three chromatograms shown in Figure 3.27G reveal what success we had using SPME and LLE sample prep techniques. The top chromatogram reveals no recovery of AR 1242 in this base-saponified matrix despite adding salt. The middle chromatogram reveals again no recovery of AR 1242 from a portion of the matrix that was spiked with AR 1242, as shown. However, an LLE of the base-saponified matrix recovered AR 1242, as shown. This observation is difficult to explain because PCBs are neutral and an alkaline matrix should not play such a significant role. Perhaps this is a worthwhile SPME research problem.
78. WHAT IS THIS SOLID-PHASE EXTRACTION THAT USES A STIR BAR?

This author has and continues to be fascinated with the magnetic stirrer/stir-bar device that has been available to laboratories for over 30 years. A Teflon-coated stir bar is dropped into a beaker that is approximately half filled or less with a liquid, the power is turned on, and a vortex is created within the liquid. This swirling vortex greatly facilitates mixing. David and coworkers\(^{120}\) at the University of Ghent in Belgium have recently modified the SPME technique introduced earlier by coating a small stir bar with polydimethyl siloxane (PDMS). They found identical sorptive extraction properties to SPME, thus rendering this new sample prep extraction technique quite suitable for accomplishing the goals of TEQA; they call this new approach stir-bar sorptive extraction (SBSE).\(^{120}\) In a manner similar to that developed for LLE and SPME, we can first define a molecular partition constant \(K_{SBSE}\) such that

\[
K_{SBSE}^i = \frac{C_{PDMS}^i}{C_{aq}^i}
\]

where \(C_{SBSE}^i\) is the concentration for the \(i\)th analyte sorbed on the stir bar with a PDMS coating whose volume is \(V_{PDMS}\). \(C_{aq}^i\) is the concentration for the \(i\)th analyte after equilibrium has been attained for the aqueous or sample phase whose volume.
is \( V_{aq} \). A percent recovery, \( \% E_{SBSE}^i \), for the \( i \)th analyte initially dissolved in the aqueous phase is given by

\[
\% E_{SBSE}^i = \frac{K_{SBSE}^i \beta}{1 + K_{SBSE}^i \beta}
\]

where \( \beta = \frac{V_{PDMS}}{V_{aq}} \) and \( K_{SPME} \sim K_{SBSE}^i \).
Let us consider the actual volumes used in SBSE while comparing these to SPME:

\[ V_{aq} = 10\text{ml}, \ V_{SPME}^{PDMS} = 0.5\mu\text{l} \text{ and } V_{SBSE}^{PDMS} \approx 25 - 125\mu\text{l} \]

\[ \beta_{SPME} < < \beta_{SBSE} \]

Octanol–water partition coefficients, \( K_{OW} \), are good approximations to either \( K_{SPME} \) or \( K_{SBSE} \). \( K_{OW} \) (as introduced earlier) can be used to make predictions about \( %E_i \). Low phase ratio for SPME coupled to low values for \( K_{OW} \) (i.e., more polar analytes) has resulted in low \( %E \) values. Contrast these low percent recoveries with the much higher \( %E \) values using SBSE, due to the significant increase in the phase ratio. To illustrate this difference, a plot of \( %E \) against \( K_{OW} \) is shown below:

The sketch above suggests that SPME is applicable to nonpolar analytes, while SBSE extends down to the more polar analytes as a consequence of an increased phase ratio \( \beta \).

79. WHAT INSTRUMENT ACCESSORIES ARE NEEDED TO CONDUCT SBSE?

Unlike SPME, a gas chromatograph must be outfitted with SBSE-related accessories to successfully conduct the technique. These accessories enable complete automation and are presently available only from Gerstel GmbH. These items include:

- A coated stir bar (Twister®) that consists of a magnetic rod covered by a glass jacket and coated with PDMS
- A thermal desorption tube to place the stir bar in
- A means to thermally desorb analytes off of the stir bar
- A programmed temperature vaporization inlet that enables cryofocusing of the thermally desorbed analytes to become trapped at the head of the wall-coated open tubular (WCOT)
Two systems are commercially available that provide the items just listed: a TDS-A® classic thermal desorption system and a specifically designed Twister desorption unit (both from Gerstel). Both systems can be mounted on GCs equipped with a CIS-4® programmed-temperature vaporizing inlet (Gerstel).120

80. HOW IS SBSE PERFORMED?

The coated stir bar is added to an aqueous or similar liquid sample that has been previously placed in a cylindrical vial, such as a headspace vial or equivalent. To extract solutes from serum or plasma requires dilution with water or buffer, treated similarly to that discussed for RP-SPE. The sample is stirred for anywhere from 30 to 240 min, depending upon sample volume, stir speed, and stir-bar dimensions, and must be optimized for each application. This is accomplished by measuring percent recoveries against SBSE extraction time.

After extraction, the stir bar is removed, dipped on a clean paper tissue to remove water droplets, and placed into an empty glass thermal desorption tube. The stir bar can be rinsed with distilled deionized water to remove adsorbed sugars, proteins, or other interferents. Analytes of enviro-chemical or enviro-health interest are then thermally desorbed, cryofocused, and temperature programmed to generate a chromatogram. Analytes of interest can also be eluted off of the surface of the stir bar with organic solvents in a manner similar to that in RP-SPE. The much larger β for SBSE requires a longer desorption time than SPME.

David and coworkers120 report successful in situ chemical derivatization of polar analytes conducted just prior to SBSE. These analytes range from biological markers such as phenols, hormones, and fatty acids to artificial contaminants such as drugs and plasticizers. Ethyl chloroformate and acetic acid anhydride in an aqueous medium have yielded useful derivatives that can be subsequently extracted via SBSE. To illustrate, the bacteriostate to some dentifrices and mouth rinse products, triclosan, was found in the urine of a male volunteer using SBSE. Dissolving 1 mg of toothpaste containing triclosan and the 0.1% level in 10 mL of water, an analysis of the mixture by SBSE, combined with thermal desorption and GC-MSD, verified its relationship with the dentifrice source.121

81. CAN SPE, SPME, AND SBSE BE AUTOMATED?

Yes, all three sample prep techniques have been automated. The Zymark Corporation (now Caliper Life Sciences, Inc.) was first to automate the barrel type SPE cartridge. Gilson, Hamilton, and other companies also have developed automated SPE, with an emphasis on the 96-well plate SPE design for drug discovery. Gerstel GmbH & Co. and Leap Technologies, Inc., have automated both SPME and SBSE for trace enviro-chemical and enviro-health quantitative analysis within the dual-rail multipurpose robotic technology originally developed by CTC Analytics AG. To better illustrate just how to automate SPE, let us focus on the Rapid Trace® SPE Workstation.
82. **HOW DOES THE RAPID TRACE WORK?**

The conventional SPE vacuum manifold apparatus requires only a source of mild vacuum to enable liquids to pass through SPE sorbent beds by creating a partial vacuum and hence a *pressure drop* across the SPE barrel type cartridge. This pressure drop is enough to facilitate a steady flow rate of sample through the sorbent bed. The Rapid Trace delivers sample to the sorbent bed using positive pressure combined with a series of valves, mixing vessels, tubing, and pumps to achieve the important aspects of automated SPE. A schematic of one Rapid Trace module is shown below along with a description for each important part: 

© 2006 by Taylor & Francis Group, LLC
Laboratories in general obtain the Rapid Trace Workstation in sets, with a set containing either 5 or 10 modules. One 10-module set enables up to 10 samples to be processed sequentially per module (rack), while 10 SPEs can be processed simultaneously. Let us assume that it takes 30 min to process one SPE cartridge; 10 test tubes containing samples, located at position 1 on each rack, across all 10 racks, could be simultaneously processed during the first 30 min. To process all 10 racks that are full of test tubes means that 100 SPEs can be accomplished in 300 min, or 5 h. This is a significant gain in sample prep productivity.

<table>
<thead>
<tr>
<th>Item</th>
<th>Part</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gas valve</td>
<td>Activates gas drying of the column bed if the valve is plumbed into the unit and the dry column step is used in software</td>
</tr>
<tr>
<td>2</td>
<td>Mixing vessel</td>
<td>Creates a mixed reagent when the add reagents to mixing vessel and mix reagents in mixing vessel steps are used in the software</td>
</tr>
<tr>
<td>3</td>
<td>Syringe-drive and liquid sensor</td>
<td>Performs all dispense and aspirate functions; the syringe draws up either sample or reagent through the 12-port valve, as specified in the procedure, and dispenses it back through the 12-port valve to the column plunger or cannula as directed by the procedure</td>
</tr>
<tr>
<td>4</td>
<td>12-port valve</td>
<td>Directs the liquid flow from the syringe to one of the following ports as specified in the procedure:  • Reagents 1–8 (8 different ports)  • Vent  • Cannula  • Column plunger  • Mixing vessel</td>
</tr>
<tr>
<td>5</td>
<td>Thumbwheel switch</td>
<td>Selects a module address of 0–9</td>
</tr>
<tr>
<td>6</td>
<td>Power light</td>
<td>Lights when the power is on</td>
</tr>
<tr>
<td>7</td>
<td>Run light</td>
<td>Lights when the module is running</td>
</tr>
<tr>
<td>8</td>
<td>Error light</td>
<td>Lights when an error occurs</td>
</tr>
<tr>
<td>9</td>
<td>Column plunger</td>
<td>Delivers the sample or reagent to the SPE column; the column plunger will move into the column and place the reagent or sample directly onto the column bed</td>
</tr>
<tr>
<td>10</td>
<td>Start/stop switch</td>
<td>Starts the procedure for a magnetically encoded rack, and stops a procedure while it is running; pressing the start/stop switch while the procedure is running will cause the procedure to stop; when using magnetically encoded racks, modules must be connected to the controller</td>
</tr>
<tr>
<td>11</td>
<td>Cannula</td>
<td>Access the sample test tube to add reagent to the sample, mix the sample, or to draw the sample into the syringe and load it onto the SPE column, as written in the procedure</td>
</tr>
<tr>
<td>12</td>
<td>SPE column turret</td>
<td>Holds the 1- and 3-mL syringe barrel columns; holds up to 10 syringe barrel SPE columns</td>
</tr>
<tr>
<td>13</td>
<td>Service panel</td>
<td>Allows access to shuttle to change internal tubing</td>
</tr>
<tr>
<td>14</td>
<td>Standard rack</td>
<td>Holds up to ten 13 × 100 mm sample tubes and ten 12 × 75 mm collection tubes; holds the magnets for magnetically encoded racks</td>
</tr>
</tbody>
</table>
83. HOW IS THE RAPID TRACE WORKSTATION PROGRAMMED?

It depends on what you want to do. However, the order of conditioning the sorbent bed, loading the sample, purging the cannula, rinsing the sorbent, drying the sorbent to remove surface water, and, finally, eluting the sorbed analytes (collecting) is critical. The table below serves to illustrate RP-SPE and is typical of how the Rapid Trace is programmed:

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Step</th>
<th>Source</th>
<th>Output</th>
<th>mL</th>
<th>mL/min</th>
<th>Liq Sense</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Condition</td>
<td>MeOH</td>
<td>Org W</td>
<td>2</td>
<td>12</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Condition</td>
<td>H₂O</td>
<td>Aq W</td>
<td>2</td>
<td>12</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Load</td>
<td>Sample</td>
<td>Bio W</td>
<td>1</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Rinse</td>
<td>H₂O</td>
<td>Bio W</td>
<td>2</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Purge-cannula</td>
<td>H₂O</td>
<td>Cannula</td>
<td>6</td>
<td>30</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Rinse</td>
<td>Vent</td>
<td>Aq W</td>
<td>0.1</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Dry</td>
<td>—</td>
<td>Time</td>
<td>0.5</td>
<td>Min</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Purge-cannula</td>
<td>MeOH</td>
<td>Cannula</td>
<td>6</td>
<td>30</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Collect</td>
<td>Rin2</td>
<td>Fract 1</td>
<td>2</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Purge-cannula</td>
<td>H₂O</td>
<td>Cannula</td>
<td>6</td>
<td>30</td>
<td>No</td>
</tr>
</tbody>
</table>

Note: W = waste; Org = organic; Aq = aqueous; Bio = biological; Fract = fraction.

Let us explain how to interpret this table.

**Condition — Steps 1 and 2**: Prepare the sorbent to effectively partition or adsorb the hydrophobic analyte of interest from an aqueous sample matrix. Always direct waste to the proper outputs, separating organic (Org W), aqueous (Aq W), and biological fluids (Bio W). **Condition** at a recommended flow rate of 12 mL/min or 0.2 mL/sec.

**Load — Step 3**: To remove the entire liquid content from the test tube that contains sample, use a volume that is 0.2 mL greater than the actual sample volume. **Load** sample at a recommended flow rate of 2 mL/min or 0.03 mL/sec.

**Rinse — Step 4**: Rinse the column with water or alternate aqueous solution and output this rinse water to the biological waste output. Most methods require more than one reagent rinse step. Rinse recommended flow rate is 8 mL/min or 0.15 mL/sec.

**Purge-Cannula — Step 5**: Clean the cannula with water or an aqueous reagent after the first **rinse** step. This will remove any remaining sample matrix from the cannula. Do not use organic solvent when the sample matrix is on the cannula.

**Rinse — Step 6**: Most methods require more than one reagent rinse step at this point.

**Dry — Step 7 (optional)**: To ensure that the **dry** step is effective, set the gas pressure at the tank to 45 psi. Recommended dry time is 0.5 min. Set it
longer as necessary. If your manual SPE method has a dry column step, you must precede the dry step with a rinse step. If it does not have one, use vent as the source. Preceding the dry step with the rinse step positions the column into a waste station.

**Purge-Cannula — Step 8:** Clean the cannula with a reagent (usually MeOH or the elution solvent) to strip remaining analyte from the cannula.

**Collect — Step 9:** Collect the fraction using a slow flow rate. For vacuum manifold users, this is an important elution step. Recommended collect flow rate is 2 mL/min or 0.03 mL/sec.

A schematic of the inner workings of the plunger/cannula is shown below:

---

**84. ARE THERE EXAMPLES OF AUTOMATED SPE OUT THERE?**

Yes there are, but not as many published reports as you might think. Of enviro-health interest is a recent paper from the Centers for Disease Control and Prevention (CDC) on incorporation of the Rapid Trace SPE Workstation as part of a faster sample prep approach to isolating and recovering persistent organic pollutants (POPs) from archived plasma samples. The method consisted of up-front RP-SPE of selected OCs, followed by NP-SPE cleanup using silica gel, with subsequent injection into an analytical HPLC column incorporating an analytical gel permeation column. A fraction is obtained from the GPC column, which is subsequently injected into a GC or GC-MS. Mean recoveries of the $^{13}$C-labeled internal quantification standards ranged from 64 to 123% for the 11 monitored OCs. A semiautomatic high-throughput extraction and cleanup method developed around the use of the Rapid Trace has been recently reported and is a subsequent extension of the work just cited. This paper shows how automated spiking of samples and automated
RP-SPE and NP-SPE can be coupled together while extending the method to polybrominated diphenyl ethers (PBDEs), PBBs, and PCBs in human serum.

85. HOW ARE THE METHODS CATEGORIZED FOR TRACE INORGANICS ANALYSIS?

A recent compilation of EPA methods organizes the numerous analytical methods for inorganics analysis according to the following six major categories:

1. Trace metals identified by flame (FlAA) and by graphite furnace (GFAA) atomic adsorption spectrophotometry
2. Trace metals identified by inductively coupled plasma-atomic emission spectrophotometry (ICP-AES)
3. Trace metals identified by inductively coupled plasma-mass spectrometry (ICP-MS)
4. Mercury identified by cold vapor atomic absorption spectrometry
5. Cyanide (total and amenable)
6. Inorganic carbon (total carbon less organic carbon)

Not listed in these categories are analytical methods for the principal inorganic anions derived from strong acids that are prevalent in groundwater: chloride, bromide, nitrite, nitrate, phosphate, and sulfate. These analytes are currently measured routinely by the application of either ion chromatography (IC) or specific colorimetric procedures following the conversion of the anion to a colored complex. Oxyhalides such as the bromate ion have been found in chlorinated drinking water. IC methods have been developed for various oxyhalide ions in recent years. Water that is free of dissolved organics and heavy metal can be directly injected into ion chromatographs or filtered if particulates are present. Aqueous samples that contained dissolved biomatter or heavy metal ions pose severe challenges to IC because the columns employed in the technique are susceptible to column fouling.

In this chapter, we will discuss the basis of sample preparation for five of the categories listed above and focus on the determinative steps for all six in Chapter 4. Total organic carbon (TOC) is a combustion technology in which aqueous samples can be injected directly without the need for sample preparation; therefore, we will not discuss it any further in this chapter. Let us start with a discussion of the principles of sample prep with respect to trace metals.

86. HOW DO YOU PREPARE AN ENVIRONMENTAL SAMPLE TO MEASURE TRACE METALS?

Sample preparation for the determination of trace concentration levels of the many priority pollutant metals is strongly connected to the nature of the determinative technique. Historically, FlAA was first used to measure metals. The more sensitive GFAA technique followed. Along about the same time as GFAA was being developed,
ICP-AES came along. ICP-AES afforded the opportunity to measure more than one metal in a sample at a time, the so-called multielement approach. In recent years, the development of ICP-MS has carried trace metal analysis to significantly lower IDLs and introduced the opportunity to identify and quantitate the various elemental isotopes.

The metals of greatest interest to TEQA are listed in Table 3.15 along with the author’s comments on the chemical and toxicological nature of each. The design of instrumentation for either FlAA, GFAA, ICP-AES, or ICP-MS requires that the sample be introduced as a liquid. These systems easily accommodate aqueous solutions in contrast to gas chromatographs, which require, for most of the polysiloxane capillary columns, the injection of a nonaqueous liquid such as an organic solvent. Aqueous samples that contain inorganics, in contrast to aqueous samples that contain organics, can be introduced into an FlAA, GFAA, or ICP without removal of the analyte from its sample matrix. Chemically, metals might exist in the environment as ions in one or more oxidation states or partially or wholly chelated to ligands of various sorts. They may be bound or complexed to soil/sediment particulates and therefore cannot be easily released via a liquid–solid extraction or leaching. Some metals form the structural composition of solid matrices derived from the environment, such as aluminosilicates in clay. In these cases, decomposition of the sample removes the organic portion of the matrix. Decomposition methods include the following:

1. Combustion with oxygen with and without fluxes
2. Digestion with acids

Both methods for decomposing the sample matrix require heat, and both have benefited from replacement of resistive heating (e.g., laboratory hot-plate equipment) with microwave heating.127

The following procedure has been used by this author to prepare a sample of fly ash for determination of priority pollutant metals.128 Assume that the ash has been obtained from a previous combustion procedure.

1. Weigh 0.2 g of sample into a 100-mL beaker. Record the weight to the nearest 0.001 g using an analytical balance.
2. Add 5 mL of concentrated nitric acid (HNO₃) and 5 mL of concentrated hydrochloric acid (HCl).
3. Place a watch glass over the beaker and digest at medium heat for 60 min.
4. Evaporate to dryness.
5. Add 5 mL of concentrated HNO₃ and evaporate to dryness.
6. Add 1 mL of concentrated HNO₃ and warm.
7. Add 1 mL of distilled deionized water and warm. Filter into a 25-mL volumetric flask.
8. Cool and dilute to the mark using 1% HNO₃. This gives exactly 25 mL of an aqueous sample containing the solubilized metal ion.
9. Aspirate into a previously calibrated FlAA and record the absorbance.
<table>
<thead>
<tr>
<th>Element</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>No. of Oxidation States</th>
<th>Common Chemical Forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>Mg(2+)</td>
</tr>
<tr>
<td>Al</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>1</td>
<td>Al(3+)</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>9</td>
<td>Cr(3+), Cr(VI)</td>
</tr>
<tr>
<td>Mn</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>11</td>
<td>Mn(IV), Mn(VII)</td>
</tr>
<tr>
<td>Fe</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>9</td>
<td>Fe(2+), Fe(3+)</td>
</tr>
<tr>
<td>Co</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>7</td>
<td>Co(2+)</td>
</tr>
<tr>
<td>Ni</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>7</td>
<td>Ni(2+)</td>
</tr>
<tr>
<td>Cu</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>6</td>
<td>Cu(1), Cu(2+)</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>2</td>
<td>Zn(2+)</td>
</tr>
<tr>
<td>Ga</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>2</td>
<td>Ga(III)</td>
</tr>
<tr>
<td>As</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>3</td>
<td>As(III), As(V)</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>6</td>
<td>Se(IV), Se(VI)</td>
</tr>
<tr>
<td>Mo</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>8</td>
<td>Mo(VI)</td>
</tr>
<tr>
<td>Ag</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>4</td>
<td>Ag(+)</td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>2</td>
<td>Cd(2+)</td>
</tr>
<tr>
<td>In</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>3</td>
<td>In(III)</td>
</tr>
<tr>
<td>Sn</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>2</td>
<td>Sn(II), Sn(IV)</td>
</tr>
<tr>
<td>Sb</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>3</td>
<td>Sb(III), Sb(IV)</td>
</tr>
<tr>
<td>Te</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>7</td>
<td>Te(IV)</td>
</tr>
<tr>
<td>Ba</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>1</td>
<td>Ba(2+)</td>
</tr>
<tr>
<td>Pt</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>Pt(IV)</td>
</tr>
<tr>
<td>Au</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>Au(III)</td>
</tr>
<tr>
<td>Hg</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>Hg(+), Hg(2+)</td>
</tr>
<tr>
<td>Tl</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>Tl(+)</td>
</tr>
<tr>
<td>Pb</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>Pb(2+), Pb(IV)</td>
</tr>
<tr>
<td>Bi</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>Bi(III)</td>
</tr>
</tbody>
</table>

**Note:** Toxicity categories are as follows:
A = major toxic metals with multiple effects  
B = essential metals with potential for toxicity  
C = metals with toxicity related to medical therapy  
D = minor toxic metals

Notes:
1. Be sure to choose the appropriate wavelength and oxidizer gas for each metal of interest. The aqueous solutions used as calibration standards should be prepared in 1% HNO₃.
2. For every batch of samples that has been digested, one blank, one matrix spike, and one matrix spike duplicate should also be prepared. For the matrix spike and matrix duplicate, designate one sample from the batch for these.

Sample matrices from a biological origin such as blood, urine, and serum can be merely diluted with water, dilute nitric acid, or a dilute surfactant such as Triton X-100 and aspirated directly into the flame for FI-AA or placed directly into the graphite tube for GFAA. With respect to GFAA, a plethora of matrix modifiers have been developed over the years to deal with spectral and chemical types of interference. Spectral interferences arise when the absorption of emission of an interfering species either overlaps or lies so close to the analyte absorption or emission that resolution by the monochromator becomes impossible. Chemical interferences result from various chemical processes occurring during atomization that alter the absorption characteristics of the analyte. Spectral interferences refer to the presence of concomitants that affect the quantity of the source light that reaches the detection system, whereas chemical interferences reduce the analyte absorbance signal by interactions with one or more concomitants compared to standards without concomitants.

87. WHAT IS MATRIX MODIFICATION IN GFAA?

Because matrix modification is one aspect of sample preparation, even though it is most often accomplished automatically in GFAA, we will discuss it here. For example, National Institute for Occupational Safety and Health (NIOSH) Method 7105 recommends a matrix modifier that consists of a mixture of ammonium dihydrogen phosphate, magnesium nitrate, and nitric acid for the determination of airborne Pb. Because the graphite furnace can be viewed as a chemical reactor whereby the sample with its matrix is placed on a graphite platform (the L’vov platform, discussed in Chapter 4) and heated to a very high temperature, reactions can take place that involve both the metal analyte of interest and sample matrix components.

The concept of matrix modification, from the sample prep perspective, is to add to the sample a chemical reagent that will cause a desirable chemical reaction or inhibit an undesirable reaction. For metals that tend to volatilize, one can add a modifier that reduces analyte volatility by increasing the volatility of the matrix. Consider the determination of Pb in highly salted aqueous samples such as seawater. Seawater contains appreciably elevated levels of chloride salts. Adding an ammonium ion to the seawater, followed by heating the sample to a high temperature, causes the following reaction to occur:

\[ \text{Cl}^{-}_{\text{aq}} + \text{NH}_4^+_{\text{aq}} \xrightarrow{\text{heat}} \text{NH}_4\text{Cl}_{\text{g}} \]
This reaction removes chloride ions from the sample while minimizing the loss of Pb as the more volatile PbCl₂.

Harris¹³³ discusses the findings of Styris and Redfield,¹³⁴ who studied the effect of magnesium nitrate on the determination of Al. At high temperature, MgO is formed and steadily evaporates. This maintains a steady vapor pressure of MgO in the GFAA tube. The presence of MgO serves to keep Al as the oxide by establishing the following equilibrium:

\[
3\text{MgO} + 2\text{Al}_{(s)} \rightleftharpoons 3\text{Mg}_{(g)} + \text{Al}_2\text{O}_3(\text{s})
\]

When most of the MgO has evaporated, the equilibrium begins to shift to the left as Al₂O₃ is converted to elemental Al. This reaction serves to delay the Al from evaporating until a higher temperature is reached.

Butcher and Sneddon¹³⁵ describe the work of Schlemmer and Welz,¹³⁶ who investigated the use of Pd and Mg nitrates as matrix modifiers in the determination of nine metallic elements. They showed that higher pyrolysis temperatures could be used, compared to no modifier or using other common modifiers. Thus, we have shown how additions to the sample matrix led to improved performance in GFAA.

With respect to FIAA and ICP, the addition of so-called matrix modifiers developed for GFAA serves no useful purpose. FIAA and ICP use nebulization into an oxidizing-reducing high-temperature source to introduce a liquid into the flame and plasma, respectively, and because of this, both techniques require that samples have a low dissolved solids content so as to prevent clogging. ICP is essentially free from most spectral and chemical interferences due to the extremely high temperature of the plasma (8000 to 10,000°C), whereas these interferences are prevalent in FIAA and serve to influence the IDL for a given metal.

88. HOW DO I PREPARE A SOLID WASTE, SLUDGE, SEDIMENT, BIOLOGICAL TISSUE, OR SOIL SAMPLE?

EPA Method 3050 from the SW-846 series of methods involves solubilizing a solid sample with acids and peroxide and removing the insoluble residue by filtration. EPA Method 3051 is a microwave-assisted acid digestion procedure. EPA Method 200.3 is applicable to the preparation of biological tissue samples prior to using atomic spectrometry for quantifications of Al, Sb, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ni, P, K, Se, Ag, Na, Sr, Tl, Th, U, V, and Zn, and an outline of the sample prep procedure is as follows:¹³⁷

- Place up to a 5-g subsample of frozen tissue into a 125-mL Erlenmeyer flask. Any sample-spiking solutions should be added at this time and allowed to be in contact with the sample prior to the addition of acid.
- Add 10 mL of concentrated nitric acid and warm on a hot plate until the tissue is solubilized. Gentle swirling of the sample will aid in this process.
Sample Preparation Techniques

• Increase the temperature to near boiling until the solution begins to turn brown. Cool sample, add an additional 5 mL of concentrated nitric acid, and return to the hot plate until the solution once again begins to turn brown.
• Cool sample, add an additional 2 mL of concentrated nitric acid, return to the hot plate, and reduce the volume to 5 to 10 mL. Cool sample, add 2 mL of 30% hydrogen peroxide, return sample to the hot plate, and reduce the volume to 5 to 10 mL.
• Repeat the previous step until the solution is clear or until a total of 10 mL of peroxide has been added.
• Cool the sample, add 2 mL of concentrated hydrochloric acid, return to the hot plate, and reduce the volume to 5 mL.
• Allow the sample to cool and quantitatively transfer to a 100-mL volumetric flask. Dilute with DDI, mix, and allow any insoluble material to separate. The sample is now ready for either ICP-AES, ICP-MS, or GFAA.

89. WHAT ARE EPA'S MICROWAVE DIGESTION METHODS?

EPA Methods 3015A (applicable to an aqueous sample such as groundwater) and 3051A (applicable to soils, sediments, sludges, and oils) utilize advances in microwave heating technology. Microwave heating significantly reduces the more labor intensive hot-plate techniques described earlier in this chapter. These recently developed methods enable environmental samples to be digested so that a quantitative determination of up to 26 metals can be made. These metals are listed as their chemical symbols as follows:

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>B</th>
<th>Cu</th>
<th>Hg</th>
<th>Ag</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sb</td>
<td>Cd</td>
<td>Fe</td>
<td>Mo</td>
<td>Na</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>Ca</td>
<td>Pb</td>
<td>Ni</td>
<td>Sr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>Cr</td>
<td>Mg</td>
<td>K</td>
<td>Tl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Be</td>
<td>Co</td>
<td>Mn</td>
<td>Se</td>
<td>V</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheme 3.9, adapted for Method 3051A, is a flowchart that outlines the procedure and logic to prepare soil, sediments, sludges, and oils for trace metals analysis. To summarize Method 3052A:

A representative sample of up to 0.5 g is extracted and/or dissolved in 10 mL of concentrated nitric acid or 9 mL of concentrated nitric acid and 3 mL of concentrated hydrochloric acid for 10 min using microwave heating with a suitable laboratory unit. The sample and acid(s) are placed in a fluorocarbon polymer or quartz vessel or vessel liner. The vessel is sealed and heated in a microwave unit. After cooling, the vessel contents are filtered, centrifuged or allowed to settle and then diluted to volume and analyzed by the appropriate determinative method.

Safety considerations are paramount in digestion involving microwave heating technology. It cannot be overemphasized enough that a suitable laboratory microwave
unit must be used. There is a mind-set that a microwave oven designed for ordinary kitchen use is a suitable low-cost substitute. The microwave unit cavity must be corrosion resistant and well ventilated. All electronics must be protected against corrosion for safe operation. The oven exterior, including the door, must be explosion-proof as well as equipped with temperature and pressure sensors and alarms.

SCHEME 3.9A

Does a rigorous reaction occur upon reagent addition?

Yes

Allow sample to pre-digest in upcapped digestion vessel. Add heat if necessary

No

Seal vessel and place in microwave oven

Proceed to Scheme 3.9B
90. WHAT IS DONE TO PREPARE A BLOOD, SERUM, OR URINE SPECIMEN FOR TRACE METALS ANALYSIS?

Public health laboratories have a long history of quantitatively determining Pb in human blood. Some states are required by law to measure blood Pb in children from at-risk environments. Flame atomic absorption and graphite furnace atomic absorption (principles to be introduced in Chapter 4) have and continue to be the principal instrumental analysis approach to quantitatively determine blood Pb. ICP-MS is
rapidly emerging as an instrument of choice for multielement quantitative analysis for enviro-health QA, since whole blood and urine can be (after an appropriate dilution) directly aspirated into the inductively coupled plasma without concomitant interferences.\textsuperscript{139,140}

So far, we have considered destroying or transforming the sample matrix in some way while leaving the metal ion intact. We now consider ways to transfer the metal ion from the sample matrix, and this leads to sample prep techniques that preconcentrate the sample.

**91. WHAT CAN I DO TO PRECONCENTRATE A SAMPLE FOR TRACE METAL ANALYSIS?**

There is a need for methods that can detect ultratrace concentration levels of the priority pollutant metal ions that are present in the environment. It may be desirable to detect and measure the concentration of one or more metals from a matrix that is highly salted, and it is expected that the concentrations of the metals of interest are extremely low (e.g., at parts per trillion (ppt) levels). An aqueous sample can be preconcentrated by evaporating off the water, precipitating the analyte, followed by redissolution of the precipitate, extracting the analyte via cation exchange or after forming an anionic species from the metal ion, via anion exchange, and isolating the metal ion by first forming the neutral metal chelate and sorbing the chelate on a hydrophobic surface, such as a chemically bonded silica, or extracting the metal chelate into a nonpolar solvent via LLE. We will focus on three aspects of preconcentration by considering the following examples:

1. The coprecipitation of Cr(VI) using lead sulfate
2. The mathematics for the general case of LLE involving metal ions and the neutral metal chelates that can be formed
3. The mathematics for isolating and recovering a neutral metal chelate, cadmium(II) oxinate, from a spiked aqueous sample

**92. HOW DO I PRECONCENTRATE CR(VI) FROM A LEACHATE USING COPRECIPITATION?**

In the environment, Cr(VI) is in either the chromate ($\text{CrO}_4^{2-}$) or dichromate ($\text{Cr}_2\text{O}_7^{2-}$) form. Whether Cr(VI) is predominantly one or the other strongly depends on the pH of the aqueous solution within which it is dissolved. The following ionic equilibrium is established between the two:

$$\text{CrO}_4^{2-} + 2\text{H}^+ \rightleftharpoons \text{Cr}_2\text{O}_7^{2-} + \text{H}_2\text{O}$$

Thus, if the aqueous solution that contains $\text{CrO}_4^{2-}$ is made acidic by adding a source of the hydronium ion, the equilibrium adjusts to produce more $\text{Cr}_2\text{O}_7^{2-}$. If the
Sample Preparation Techniques

The hydronium ion is removed by reaction with the hydroxide ion or other base, the equilibrium adjusts to produce more \( \text{CrO}_4^{2-} \). Let us assume that we have an aqueous solution containing Cr(VI) as either chromate or dichromate and CrCl3. The addition of a source of the \( \text{Pb}^{2+} \) ion, such as lead(II) sulfate, will precipitate Cr(VI) as the insoluble \( \text{PbCrO}_4 \) while leaving Cr(III) in the supernatant as \( \text{Cr}^{3+} \). Thus, a speciation of chromium via coprecipitation can be realized. Hence,

\[
\text{CrO}_4^{2-} + \text{Pb}^{2+} \rightarrow \text{PbCrO}_4(s)
\]

The \( \text{PbCrO}_4 \) precipitate is washed clean of occluded \( \text{Cr}^{3+} \) and chemically reduced by the addition of hydrogen peroxide and nitric acid according to

\[
\text{PbCrO}_4(s) + \text{H}_2\text{O}_2 + \text{HNO}_3 \rightarrow \text{Cr}^{3+}(aq) + \text{Pb}^{2+}(aq) + \text{H}_2\text{O}
\]

If it is expected that the concentration is within the range of 1 to 10 mg/L Cr, the dissolved Cr(III) can now be analyzed after adjustment to a precise volume of either FLAA or ICP-AES. If it is expected that the concentration is within the range of 5 to 100 µg/L Cr, the Cr(III) could be injected into the GFAA. A procedure for the possible speciation of chromium that might be found in the environment between Cr(III) and Cr(VI), along with the appropriate sample prep, is found in EPA Method 7195 from the SW-846 series.

Chromium(VI) can be quantitated without coprecipitation by forming a metal chelate. Method 7196A provides a procedure to prepare the diphenyl carbazone complex with Cr(VI) in an aqueous matrix. The method is not sensitive in that it is useful for a range of concentrations between 0.5 and 50 mg/L Cr. A more sensitive colorimetric method converts Cr(VI) to Cr(VI) chelate with ammonium pyrrolidine dithiocarbamate (APDC), followed by LLE into methyl isobutyl ketone (MIBK). The molecular structure for APDC is as follows:

\[
\text{N} \quad \text{S}^2\text{−} \quad \text{S} \quad \text{NH}_4^+
\]

The APDC forms chelates with some two dozen metal ions. The extent of formation of the metal chelate is determined by the magnitude of the formation constant \( \beta \). The efficiency of LLE as defined by a metal chelate’s percent recovery depends on the distribution ratio, \( D \), of the metal chelate in the two-phase LLE. We use the fundamental definition of \( D \) to develop a useful relationship for metal chelate LLE.
93. TO WHAT EXTENT CAN A GIVEN METAL CHELATE BE RECOVERED BY LLE?

Recall the definition of a distribution ratio for a specific chemical species as defined by Equation (3.9). If a chelate itself is a weak acid, secondary equilibrium plays a dominant role. We start by writing down a definition for the distribution ratio that accounts for all chemical species involving a metal ion M:

\[ D = \frac{\sum C_M(\text{organic})}{\sum C_M(\text{aqueous})} \]

This generalization can be reduced to

\[ D = \frac{[\text{ML}_n]_{\text{organic}}}{[\text{ML}_n]_{\text{aqueous}} + [M^{n+}]_{\text{aqueous}}} \]  

(3.49)

Equation (3.49) states that the degree to which a given metal chelate, ML\(_n\), partitions into the organic phase depends on the ratio of the concentration of extracted ML\(_n\) to the concentration of free metal ion, and on the degree to which metal ion remains uncomplexed in the aqueous phase. Equation (3.51) is quite complex, and as it stands, this equation is not too useful in being able to predict the extraction efficiency. Figure 3.28 is a diagrammatic representation of what happens when a metal ion, M\(^{n+}\), forms a metal chelate with a weak acid-chelating reagent, HL. The metal chelate is formed where one metal ion complexes to \(n\) singly charged anionic ligands, L, to form the metal chelate, ML\(_n\). The several equilibria shown set the
Sample Preparation Techniques

94. HOW DO YOU DERIVE A MORE USEFUL RELATIONSHIP FOR METAL CHELATES?

We derive a more useful relationship for the LLE of metal chelates by considering the well-known secondary ionic equilibria described in Figure 3.28. Let us start by assuming that the chelating reagent to be used to complex with our metal ion or environmental interest is, in general, a weak acid. This monoprotic (our assumption) weak acid can ionize only in the aqueous phase and does so according to

\[
HL_{(aq)} \rightleftharpoons H^+ + L^-
\]

The extent of this dissociation is governed by its acid dissociation constant, \(K_a\), and is defined in the case of a monoprotic weak acid, \(HL\), as follows:

\[
K_a = \frac{[H^+][L^-]}{[HL]}
\]

The neutral chelate can also partition into the organic phase according to

\[
HL_{(aq)} \rightleftharpoons HL_{(organic)}
\]
The extent to which HL partitions into the organic phase is governed by its partition coefficient, \( K_{D}^{HL} \), and is defined as

\[
K_{D}^{HL} = \frac{[HL]_{\text{organic}}}{[HL]_{\text{aqueous}}} \tag{3.52}
\]

Chelating reagents that are amphiprotic, such as 8-hydroxyquinoline, HOx, have a more limited pH range within which the distribution ratio for HOx approximates \( K_{D}^{HL} \).

Free metal ions, \( M^{n+} \), and the conjugate base to the weak acid chelate, \( L^{-} \), that are present in the aqueous phase will form the metal chelate by reaction of \( n \) ligands coordinating around a central metal ion. The extent of complexation is governed by the formation complex, \( \beta \), according to

\[
M^{n+} + nL^{-} \rightleftharpoons \beta \rightarrow ML_n
\]

The formation constant of the metal chelate in aqueous solution is defined as

\[
\beta = \frac{[ML_n]_{\text{aqueous}}}{[M^{n+}]_{\text{aqueous}}[L^{-}]^n_{\text{aqueous}}} \tag{3.53}
\]

The partition coefficient for the neutral metal chelate, \( ML_n \), where a relatively nonpolar and water-immiscible solvent is added to an aqueous solution containing the dissolved metal chelate, is given as follows:

\[
K_{D}^{ML_n} = \frac{[ML_n]_{\text{organic}}}{[ML_n]_{\text{aqueous}}} \tag{3.54}
\]

The acid dissociation constant expression, the formation constant expression, and the two expressions for the partition coefficients can be substituted into the defining equation for \( D \) ([Equation (3.49)], rearranged, and simplified.

95. **CAN WE DERIVE A WORKING EXPRESSION FOR THE DISTRIBUTION RATIO?**

Yes, we can, and we utilize all of the above equations to do so. This is an instructive exercise that can be found in a number of analytical chemistry texts that introduce the topic of metal chelate extraction. Usually the derivation itself is not included and only the final equation is given and interpreted. In the derivation that follows, we find that we do not need to add any more simplifying assumptions to those already given to reach the final working equations.
Let us consider eliminating the concentration of free metal ion by solving Equation (3.53) for \([M^{n+}]\) and substituting this expression into Equation (3.50). This gives:

\[
D = \frac{K_D^{ML_n} [ML_n]}{[ML_n] \beta [L^{-}]^n} \alpha_M
\]

The concentration of metal chelate in the aqueous phase cancels, and we obtain the following expression for \(D\):

\[
D = K_D^{ML_n} \beta [L^{-}]^n \alpha_M
\]

This equation can be further simplified by eliminating the ligand concentration term by solving the equation for \(K_a\) given earlier for \([L^{-}]\) and substituting this expression. We get:

\[
D = K_D^{ML_n} \beta \left( \frac{K_a [HL]}{[H^+]^n} \right) \alpha_M
\]

Rearranging this equation gives:

\[
D = \frac{K_D^{ML_n} \beta K_a^n [HL]_{aqueous}^n}{[H^+]^n} \alpha_M
\]

This equation can be further simplified by eliminating the concentration of the undissociated weak acid chelate in the aqueous phase by substituting for \([HL]_{aqueous}\) using Equation (3.52). Upon rearrangement, we have the final working relationship for the distribution ratio of the metal chelate \(ML_n\) when the chelate itself is a monoprotic weak acid HL:

\[
D = \frac{K_D^{ML_n} \beta K_a^n [HL]_{organic}^n}{(K_D^{ML_n})^n [H^+]^n} \alpha_M \quad (3.55)
\]

Equation (3.55) is the generalized relationship. This relationship was developed earlier for a specific metal chelate, as was shown in Equation (3.18). Equation (3.55) shows that the magnitude of the distribution ratio depends on the magnitude of the four equilibrium constants. These constants depend on the particular metal chelate. The distribution ratio can be varied by changing either the concentration of chelate in the organic phase or the pH of the aqueous phase. The number of ligands that bond to the central metal ion, \(n\), is also an important parameter. As shown earlier [Equation (3.16)], once we know \(D\) we can calculate \(E\) if we know or can measure the phase ratio for LLE. Knowing \(E\) enables us to determine the percent recovery and hence to quantitatively estimate the extraction efficiency. Because \(100 \times E\) equals the percent recovery for a given metal chelate, a plot of the percent recovery of a
given metal chelate vs. pH reveals a sigmoid-shaped curve. The inflection point in the curve yields the pH\textsubscript{1/2} for the specific metal. This is the pH at which 50% of a metal is extracted.

Figure 3.29 is a plot of the percent extracted against the solution pH for four metals, Cu(II), Sn(II), Pb(II), and Zn(II), as their respective dithizones. The exact values for each metal’s pH\textsubscript{1/2} are as follows:

<table>
<thead>
<tr>
<th>Metal Dithizone</th>
<th>pH\textsubscript{1/2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td>1.9</td>
</tr>
<tr>
<td>Sn(II)</td>
<td>4.7</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>7.4</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>8.5</td>
</tr>
</tbody>
</table>

It should become clear that pH is a powerful secondary equilibrium effect that can be used to selectively extract a particular metal from a sample that may contain more than one metal.

Day and Underwood\textsuperscript{143} have shown that if the logarithm is taken on both sides of Equation (3.55), we obtain a more useful form. Let us first rewrite Equation (3.55) combining the various equilibrium constants as follows:

\[
D = \frac{K_{ex}[HL]^n_{\text{organic}}}{[H^+]^n_{\text{aqueous}}} \quad (3.56)
\]

where \( K_{ex} \) is substituted for all of the equilibrium constants in Equation (3.55). Upon taking the logarithm of both sides of Equation (3.56), we obtain
This equation can be rewritten in terms of pH as follows:

$$\log D = \log K_{ex} + n \log [HL] - n \log [H^+]$$

A plot of $\log D$ vs. pH should in theory be a straight line whose slope is $n$ and whose intercept on the $\log D$ axis (i.e., when pH = 0) is $\{\log K_{ex} + n \log [HL]\}$. Figure 3.30 shows such a plot in general terms. Note that the two lines drawn correspond to two different values for $[HL]_{organic}$. These straight lines eventually curve and plateau as the pH of the aqueous phase becomes very high. In this case, the $[H^+]$ becomes so low that abundant $L^-$ is made available, which in turn drives the formation of the metal chelate equilibrium to the right. As more aqueous metal chelate becomes available, the partitioning of the metal chelate shifts in favor of the organic metal chelate. Hence, in the absence of any hydroxide, the value for $D$ approaches the value for the molecular partition coefficient for the metal chelate, $K_D^{M^{n+}}$. Metal hydroxide precipitation at a high pH competes for the free metal ion, $M^{n+}$, a factor not taken into account during the development of Equation (3.55).

**96. ARE THERE OTHER WAYS TO PRECONCENTRATE METAL IONS FROM ENVIRONMENTAL SAMPLES?**

Yes, there are, and these are in essence SPE type methods. Both cation and anion exchange resins have been used to preconcentrate inorganic metal cations while removing anionic interferents. A large body of work has been related to the formation...
of the polychloro-anionic complex such as FeCl$_4^-$ and its isolation using anion exchange resins.

Chelating resins, styrene–divinyl benzene copolymer, containing iminodiacetate functional groups have been successfully used to preconcentrate transition metal ions from solutions of high salt concentrations. Selective neutral metal chelates have been found to be isolated and recovered using C$_{18}$ chemically bonded silica gel.$^{144}$

We now digress to some of the author’s findings in this area.

**97. CAN WE ISOLATE AND RECOVER A NEUTRAL METAL CHELATE FROM AN ENVIRONMENTAL SAMPLE USING BONDED SILICAS?**

Neutral metal chelates should behave no differently with respect to the adsorption/partitioning of the species from water to an octadecyl-bonded silica than neutral organics, as discussed earlier. This author has developed mathematical equations for the reaction of cadmium ion, Cd$^{2+}$, with 8-hydroxyquinoline (HOx), also referred to as oxine, to form a series of complexes. If it is assumed that only the neutral 1:2 complex, CdOx$_2^-$, will partition into the bonded sorbent, equations can be derived that relate the distribution ratio to measurable quantities. We now proceed through this derivation and start with a consideration of the secondary equilibria involved. We first need to consider a more expanded concept, enlarging upon that shown for metal chelate LLE (refer back to Figure 3.23). The schematic in Figure 3.31 depicts the bonded silica–aqueous interface, in which only the neutral 1:2 engages in the primary equilibrium, that of partitioning onto or into the monolayer of wetted C$_{18}$ ligates.

![Figure 3.31](image-url)  
*FIGURE 3.31* Various equilibria for the distribution of cadmium oxinate between an aqueous phase and the C$_{18}$ sorbent surface.
The extent to which the free cadmium ion complexes with the oxinate ion to form the 1:1 cation is governed by

\[
\text{Cd}^{2+} + \text{Ox}^- \rightleftharpoons \beta \text{CdOx}^+
\]

The extent to which the cadmium ion complexes with two oxinate anions to form the 1:2 complex is governed by

\[
\text{Cd}^{2+} + 2\text{Ox}^- \rightleftharpoons \beta_2 \text{CdOx}_2
\]

Oxine, itself being amphiprotic, can exist as either the neutral, weak acid or a protonated species. A molecular structure for oxine is as follows:

The hydroxyl group behaves as a weak acid, whereas the aromatic nitrogen can accept a proton and behave as a weak base. The degree to which oxine is protonated, \( \text{H}_2\text{Ox}^+ \), remains neutral, \( \text{HOx} \), or dissociates to \( \text{H}^+ \) and oxinate, \( \text{Ox}^- \), is governed by the pH of the aqueous phase.

For the protonated species, we can write an equilibrium for the acid dissociation:

\[
\text{H}_2\text{Ox}^+ \rightleftharpoons \beta_1 \text{H}^+ \text{HOx}
\]

The extent of dissociation of this weak acid is governed by the first acid dissociation constant, \( K_{a1} \).

For the neutral weak acid, it too dissociates in aqueous media according to

\[
\text{HOx} \rightleftharpoons \beta_2 \text{H}^+ \text{Ox}^-
\]

The extent to which the neutral form of oxine dissociates is governed by the magnitude of the second acid dissociation constant, \( K_{a2} \).
If Cd\(^{2+}\) and oxine are present in the aqueous phase, these four ionic equilibria would be present to the extent determined by their formation constants. Which complex predominates — 1:1 or 1:2? We need to define what we mean by a formation constant for metal complexes, and these definitions we take from well-established chemical concepts. The formation constant for the 1:1 complex is defined as

\[ \beta_1 = \frac{[\text{CdOx}^+]}{[\text{Cd}^{2+}][\text{Ox}^-]} \]  

(3.57)

Likewise, the equilibrium expression for the formation of the 1:2 complex is given by

\[ \beta_2 = \frac{[\text{CdOx}_2^-]}{[\text{Cd}^{2+}][\text{Ox}^-]^2} \]  

(3.58)

The acid dissociation constants for the amphiprotic oxine are

\[ K_{a1} = \frac{[\text{H}^+][\text{HOx}]}{[\text{H}_2\text{Ox}^+]} \]  

(3.59)

\[ K_{a2} = \frac{[\text{H}^+][\text{Ox}^-]}{[\text{HOx}]} \]  

(3.60)

Because our discussion centers around trace Cd analysis, let us consider the fraction of all forms of this metal existing as the free, uncomplexed ion \( \delta_0 \), where this fraction is defined as

\[ \delta_0 = \frac{[\text{Cd}^{2+}]}{C_M} \]  

(3.61)

Likewise, the fraction of cadmium complexed 1:1 is given by

\[ \delta_1 = \frac{[\text{CdOx}^+]}{C_M} \]  

(3.62)

Furthermore, the fraction of cadmium complexed 1:2 is given by

\[ \delta_2 = \frac{[\text{CdOx}_2^-]}{C_M} \]  

(3.63)

We also need to distinguish between the concentration of oxine in the aqueous phase, [HOx], and the total concentration of oxine, \( C_{\text{HOx}} \). This total oxine concentration results from the actual addition of a given amount of the substance to water,
with adjustment of a final solution volume. With all of these definitions in mind, we can now proceed to substitute and manipulate using simple algebra to help us arrive at more useful relationships than those given by just the definitions.

We start by considering the fraction of cadmium complexed as the 1:2 complex. Solving Equation (3.58) for \([\text{CdOx}_2]\) yields

\[
[\text{CdOx}_2] = \beta_2 [\text{Cd}^{2+}] [\text{Ox}^-]^2
\]  

(3.64)

Equation (3.57) can also be solved for the concentration of the 1:1 complex:

\[
[\text{CdOx}^+] = \beta_1 \left[ \text{Cd}^{2+} \right] [\text{Ox}^-]
\]  

(3.65)

Substituting Equation (3.64) into Equation (3.57) gives

\[
\delta_2 = \frac{\beta_2 [\text{Cd}^{2+}] [\text{Ox}^-]^2}{[\text{Cd}^{2+}] + [\text{CdOx}^+] + [\text{CdOx}_2]}
\]

\[
= \frac{\beta_2 [\text{Ox}^-]^2}{1 + [\text{CdOx}^-][\text{Cd}^{2+}] + [\text{CdOx}_2][\text{Cd}^{2+}]}
\]

Upon substituting Equations (3.64) and (3.65) into the denominator in the above expression, we get the following simplified result:

\[
\delta_2 = \frac{\beta_2 [\text{Ox}^-]^2}{1 + \beta_1 [\text{Ox}^-] + \beta_2 [\text{Ox}^-]^2}
\]  

(3.66)

Equation (3.66) is important, but this equation is not the ultimate objective of this derivation. Equation 3.66 states that the fraction of all cadmium can be found in the aqueous phase from knowledge of only the two formation constants and the free oxinate ion concentration. It is difficult analytically to measure this free [Ox\(^-\)]. There is a solution to this dilemma. Let us consider how the chelate, oxine, is distributed in the aqueous phase. We start by writing a mass balance expression for the total concentration of oxine, \(C_{\text{HOx}}\), as follows:

\[
C_{\text{HOx}} = [\text{H}_2\text{Ox}^+] + [\text{HOx}] + [\text{Ox}^-]
\]

We have thus accounted for all forms that the chelate can take. Equations (3.59) and (3.60) can be solved for the undissociated forms and substituted into the mass balance expression to yield

\[
\frac{[\text{H}^+][\text{HOx}]}{K_{a1}} + \frac{[\text{H}^+][\text{Ox}][\text{Ox}^-]}{K_{a2}} + [\text{Ox}^-]
\]
Upon further rearrangement and simplification,

\[
\frac{[H^+]}{K_{a1}} \left( \frac{[Ox^-][H^+]}{K_{a2}} \right) + \frac{[H^+][Ox^-]}{K_{a2}^2} = [Ox^-]
\]

Factoring out \([Ox^-]\) and further rearranging leads to a useful expression:

\[
[Ox^-]\left(1 + \frac{[H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}}\right)
\]

As a result, we now have the total concentration of oxine in the aqueous phase as being equal to the product of the free oxinate ion concentration and a term that is entirely dependent on the magnitude of the two acid dissociation constants for oxine and the hydrogen ion concentration or pH. Let us define this term collectively as \(\alpha\) and define \(\alpha\) as

\[
\alpha = \frac{1 + [H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}}
\]

The total oxine concentration is thus seen as the product of two terms, so that

\[
C_{1Ox} = [Ox^-]\alpha
\] (3.67)

Equation (3.67) can be solved for \([Ox^-]\) and substituted into Equation (3.66) to yield the important outcome whereby the fraction of cadmium as the 1:2 complex can be expressed entirely in terms of known equilibrium constants and the measurable quantities \(C_{1Ox}\) and \(\alpha\):

\[
\delta_2 = \frac{\beta_2C_{1Ox}^2}{\alpha^2 + \beta_2C_{1Ox}^2 + \beta_2^2C_{1Ox}^2}
\] (3.68)

The fraction of cadmium as the 1:1 complex, \(\delta_1\), is defined as

\[
\delta_1 = \frac{[CdOx^-]}{C_M}
\]

\[
\delta_1 = \frac{\beta_i[Ox^-]^i}{1 + \beta_i[Ox^-] + \beta_i^2[Ox^-]^2}
\]

\(\delta_1\) can be expressed in terms of the concentration of free oxinate and is shown as follows. As we did for the 1:2 complex, \(\delta_1\) can be expressed in terms of measurable quantities as follows:

© 2006 by Taylor & Francis Group, LLC
Finally, the fraction of cadmium as the free ion, $\delta_0$, is

$$\delta_0 = \frac{[\text{Cd}^{2+}]}{C_M}$$

$\delta_0$ can be expressed as well in terms of the free oxinate concentration:

$$\delta_0 = \frac{1}{1 + \beta_1 [\text{Ox}^-] + \beta_2 [\text{Ox}^-]^2}$$

$\delta_0$ can be expressed in measurable quantities and is shown as

$$\delta_0 = \frac{1}{1 + \beta_1 \left(\frac{C_{\text{HOx}}}{\alpha}\right) + \beta_2 \left(\frac{C_{\text{HOx}}}{\alpha^2}\right)^2}$$

98. HOW DOES THE FRACTION OF CADMIUM FREE OR COMPLEXED VARY?

Equations (3.68) to (3.70) show that the fraction of cadmium as the 1:2 complex, 1:1 complex, and the free cation depends entirely on the magnitude of $\beta_1$, $\beta_2$, the concentration of total oxine, $C_{\text{HOx}}$, and pH. This is a significant finding. Using the following values for the respective equilibrium constants, the fraction $\delta_0$ is plotted against pH for a given value for $C_{\text{HOx}}$. Likewise, $\delta_1$ and $\delta_2$ can be plotted against pH on the same graph to yield three distribution curves:

$$K_{a1} = 9.84 \times 10^{-6}, \quad \beta_1 = 1.59 \times 10^7$$
$$K_{a2} = 1.23 \times 10^{-10}, \quad \beta_2 = 12.56 \times 10^{13}$$

Figure 3.32 shows how the fraction of free cadmium ion, $\delta_0$, the fraction of 1:1 cadmium oxinate, $\delta_1$, and the fraction of 1:2 cadmium oxinate, $\delta_2$, vary with a change in aqueous phase pH for a $C_{\text{HOx}}$ of 0.5 ppm. As the pH of the aqueous phase is increased, most of the cadmium starts out as Cd$^{2+}$ but gradually is complexed as the 1:1 and 1:2 complexes. The fraction of total cadmium existing as the 1:1 complex reaches a maximum at around 8.8, then decreases, whereas the fraction as the 1:2 complex continuously increases until all of the metal is complexed as the 1:2 complex at around pH = 10. Figure 3.33 is a similar plot, but this time for a $C_{\text{HOx}}$ of 5.0 ppm. Note that the pH in which the 1:1 and 1:2 complexes are formed is lower than that for the lower value of $C_{\text{HOx}}$. Figure 3.34 is a similar plot except that $C_{\text{HOx}}$ is 50 ppm.
FIGURE 3.32 Distribution diagram for the fraction of free, 1:1, and 1:2 cadmium oxinates vs. pH for a fixed concentration of oxine at 0.5 ppm.

FIGURE 3.33 Distribution diagram for the fraction of free, 1:1, and 1:2 cadmium oxinates vs. pH for a fixed concentration of oxine at 5 ppm.
HOx is 400 ppm. At this higher total oxine concentration, the pH at which the complexes form is shifted over one pH unit lower vs. the case for a C\textsubscript{HOx} of 50 ppm. A knowledge of the various δ values enables a relationship to be developed for the distribution ratio.

CAN AN EQUATION BE DERIVED USING THESE δ VALUES TO FIND D?

The distribution ratio for the partitioning of the cadmium oxinate 1:2 complex from an aqueous phase to a C\textsubscript{18}-bonded surface can be defined in terms of a ratio of the concentration of CdOx\textsubscript{2} that would be present on the surface to the sum of all of the forms of the metal in the aqueous phase. This is defined as

\[
D_{\text{CdOx}_2} = \frac{[\text{CdOx}_2]_{\text{surface}}}{[\text{Cd}^{2+}] + [\text{CdOx}^-] + [\text{CdOx}_2]} \tag{3.71}
\]

FIGURE 3.34 Distribution diagram for the fraction of free, 1:1, and 1:2 cadmium oxinates vs. pH for fixed concentration of oxine at 50 ppm.

The pH at which the complexes are formed is further shifted to lower values. Figure 3.35 is a similar plot except that C\textsubscript{HOx} is 400 ppm. At this higher total oxine concentration, the pH at which the complexes form is shifted over one pH unit lower vs. the case for a C\textsubscript{HOx} of 50 ppm. A knowledge of the various δ values enables a relationship to be developed for the distribution ratio.

FIGURE 3.34 Distribution diagram for the fraction of free, 1:1, and 1:2 cadmium oxinates vs. pH for fixed concentration of oxine at 50 ppm.
and the partitioning coefficient for the molecular form of the species can be defined as

$$K_D^{\text{CdOx}_{2}} = \frac{[\text{CdOx}_{2}]_{\text{surface}}}{[\text{CdOx}_{2}]_{\text{aqueous}}}$$  \hspace{1cm} (3.72) \\

given

$$[\text{Cd}^{2+}] = \delta_0 C_M$$  \\
$$[\text{CdOx}^+] = \delta_1 C_M$$  \\
$$[\text{CdOx}_{2}] = \delta_2 C_M$$

Upon eliminating the bracketed concentrations in Equation (3.72), we get

$$D_{\text{CdOx}_{2}} = \frac{K_D^{\text{CdOx}_{2}} \delta_2 C_M}{\delta_0 C_M + \delta_1 C_M + \delta_2 C_M}$$
Upon simplifying and rearranging, we get an important result:

\[
D_{\text{CdOx}_2} = K_{D_{\text{CdOx}_2}} \left[ \frac{\delta_2}{\delta_0 + \delta_1 + \delta_2} \right]
\] (3.73)

Equation (3.73) is an important outcome of our derivation efforts. Let us proceed to interpret this equation as it relates to the cadmium–oxine system. Equation (3.73) states that the distribution ratio for the 1:2 complex between an aqueous phase and a chemically bonded silica such as a C18 sorbent depends on the magnitude of the molecular partition coefficient and the degree to which cadmium is found as the 1:2 complex. Given that Equations (3.68) to (3.70) enable one to calculate $\delta_0$, $\delta_1$, and $\delta_2$, respectively, and that we have already established that these fractions depend only on $C_{\text{HOx}}$ and the pH, we need only to know the pH of the aqueous phase, the molecular partition coefficient from independent studies, and the total oxine concentration in the aqueous phase to calculate $D$. These parameters were incorporated into an EXCEL spreadsheet to calculate $D$ for various values of pH at different $C_{\text{HOx}}$ values. Figure 3.36 is a plot of log $D$ against pH for $C_{\text{HOx}} = 0.5$ ppm; Figure 3.37 is similar, but for $C_{\text{HOx}} = 5.0$ ppm, and in a similar manner for Figure 3.38, for $C_{\text{HOx}} = 50$ ppm. Referring to Figure 3.36, we observe that the curve is linear between a pH of around 6.4 and approximately 8.8, and then the curve levels off, hence becoming independent of pH from about a pH of 9.2 all the way to 14. Figure 3.37

![Log D vs pH for HOX = 0.5 ppm](image.png)

FIGURE 3.36 Plot of the logarithm of the distribution ratio for the 1:2 cadmium oxinates vs. pH for a fixed concentration of oxine at 0.5 ppm.
Let us examine this a bit further. Taking the logarithm of both sides of Equation (3.73) gives the following:

\[
\log D = \log D_{\text{CdO}} + \log K_D^{\text{CdO}} + \log \frac{\delta}{\delta_0 + \delta_1 + \delta_2}
\]

Where the curve crosses the pH axis, \( \log D = 0 \) so that

\[
0 = \log K_D^{\text{CdO}} + \log \frac{\delta}{1}
\]

Hence, at the pH where the curve crosses,

\[
-\log K_D^{\text{CdO}} = \log \delta_2
\]
We have assumed a value for $K_D = 100$ so that $\delta_2 = 0.01$. The curve crosses the pH axis at which the fraction of the 1:2 complex formed is 0.01 and the percent of the 1:2 complex is 1%. The pH at which the $\delta_2$ curve just begins to appear, for example, in Figure 3.32, is about 7.8. This is the same pH value at which the curve in Figure 3.37 crosses the pH axis.
100. HOW GOOD IS THE PREDICTION OF EQUATION (3.73)?

We now discuss the results of studies that show the strong dependence of $D$ on the pH of the aqueous phase. However, we first introduce the experimental procedure used to generate the data. To about 50 mL of distilled deionized water (DDI) is added an aliquot of a 1% solution of oxine dissolved in methanol. An aliquot of a cadmium salt solution is then added. The pH is adjusted to the desired value with 1% ammonia. This spiked sample is passed through a conditioned C$_{18}$-bonded sorbent, and a brightly colored band is seen near the top of the column. The SPE cartridge is subsequently eluted with 5 mL MeOH directly into a 10-mL volumetric flask used as a receiver. The contents of the volumetric flask are brought to the calibration mark with 1% nitric acid in DDI. This is a high-purity acid deemed suitable for atomic absorption spectrophotometric analysis. The contents in the volumetric flask are aspirated into a Model 303 Flame AA (PerkinElmer). The metal, cadmium, is quantitated based on an external standard mode of instrument calibration in 50:50 MeOH:1% HNO$_3$. Ten replicate SPEs were conducted at pH values 6.2 and 8.8. The absorbance ($A$) was measured and the data are shown in Table 3.16. It is evident that at a pH of 6.2, little to no cadmium was recovered, whereas at pH 8.8, almost 30 times as much cadmium was recovered. Two other studies on the isolation and recovery of Cd$^{2+}$ by chelating the ion with oxine and isolating the complex on C$_{18}$ and C$_{6}$ chemically bonded silicas gave similar results.

We end this chapter with a brief discussion of two sample preparation methods. The first is to determine mercury (Hg), and the second method determines cyanide (CN$^-$). Both parameters are important with respect to the evaluation of a sample as a hazardous waste. The presence of either Hg or cyanide renders a sample hazardous due to the acute toxicity of both species. Mercury exists either in elemental form, 

<table>
<thead>
<tr>
<th>SPE Cartridge No.</th>
<th>Weight (mg)</th>
<th>$A$ at pH 6.2</th>
<th>$A$ at pH 8.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>199</td>
<td>0.003</td>
<td>0.217</td>
</tr>
<tr>
<td>2</td>
<td>202</td>
<td>0.035</td>
<td>0.237</td>
</tr>
<tr>
<td>3</td>
<td>272</td>
<td>0.021</td>
<td>0.272</td>
</tr>
<tr>
<td>4</td>
<td>310</td>
<td>0.032</td>
<td>0.236</td>
</tr>
<tr>
<td>5</td>
<td>415</td>
<td>0.007</td>
<td>0.301</td>
</tr>
<tr>
<td>6</td>
<td>415</td>
<td>0.006</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>508</td>
<td>0.009</td>
<td>0.290</td>
</tr>
<tr>
<td>8</td>
<td>517</td>
<td>0.006</td>
<td>0.290</td>
</tr>
<tr>
<td>9</td>
<td>648</td>
<td>0.006</td>
<td>0.348</td>
</tr>
<tr>
<td>10</td>
<td>785</td>
<td>0.004</td>
<td>0.215</td>
</tr>
<tr>
<td>Mean for $n$ replicates</td>
<td>0.01</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Confidence interval at 95% probability</td>
<td>0.009 ($n = 10$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
as the dimer ion Hg$_2^{2+}$, in which the oxidation state of the element is +1, or as the divalent ion Hg$^{2+}$. The elemental form is the familiar liquid silver metal obtained by roasting cinnabar, HgS. The cyanide ion is a moderately strong base derived from the weak acid hydrocyanic acid, HCN. HCN is a gas at room temperature and highly toxic. The fear is that hazardous wastes the contain cyanide, if acidified, could release HCN. Cyanide also forms complexes to many metal ions.

101. HOW DO YOU PREPARE A SAMPLE FOR Hg DETERMINATION?

This author has always been fascinated by the oxidation and reduction reactions that Hg undergoes. Because mercury might be present in environmental samples in any of its three oxidation states, an initial and vigorous oxidation would convert all forms of the element to its highest oxidation state, Hg$^{2+}$. Also, organomercury compounds whereby Hg is in a further reduced state, such as dimethyl mercury, (CH$_3$)$_2$Hg, also would be oxidized to Hg$^{2+}$. The common oxidizing agent used is potassium permanganate, KMnO$_4$. This is diagrammed as follows:

\[
\text{(CH}_3\text{)}_2\text{Hg} \\
\text{Hg}_2^{2+} \xrightarrow{[O]} \text{Hg}_{(aq)}^{2+} \\
\text{Hg}^0
\]

Both EPA Method 7470, applicable to liquid waste, and Method 7471A, applicable to solid waste, use KMnO$_4$. The purple color of the permanganate ion is discharged as this oxidizing agent gets reduced. An excess of MnO$_4^-$ that persists provides visual evidence that oxidation is complete. Further oxidation is afforded by adding potassium persulfate, K$_2$S$_2$O$_8$. Excess MnO$_4^-$ is reduced using hydroxylamine according to

\[
\text{MnO}_4^- \xrightarrow{[\text{NH}_2\text{OH}]} \text{Mn}^{2+}
\]

Tin(II) sulfate is used to reduce all Hg$^{2+}$ to elemental Hg:

\[
\text{Hg}_2^{2+} \xrightarrow{\text{Sn}^{2+}} \text{Hg}^0
\]

The elemental Hg is subsequently purged and swept out of the sample. Figure 3.39 illustrates what accessories are needed to prepare a sample for direct introduction of elemental mercury vapor into a flow-through atomic absorption cell. This modification to the atomic absorption spectrophotometer is called a cold vapor generator.
A typical procedure to determine the concentration of Hg for a drinking water sample is now summarized. This is a routine procedure used in environmental testing labs that perform trace Hg determinations. It reflects EPA Method 245.1 for the determination of Hg in aqueous environmental samples such as surface, saline, and wastewater. A 100-mL sample or equivalent spiked sample is placed into the reaction vessel of the cold vapor generator. A 300-mL biological oxygen demand (BOD) bottle is frequently used for this purpose. To this vessel are added 5 mL of 5% KMnO₄ and 50 mL of DDI. The contents of the container are mixed well and allowed to stand for 15 min. If the purple color is discharged during this time, aliquots of 5% KMnO₄ can be added until the purple coloration persists for 15 min. After this, 8 mL of 5% K₂S₂O₈ is added to the reaction vessel. The vessel is capped and heated in a 95°C heater block or water bath for 2 h. The contents of the reaction vessel are then cooled and 6 mL of a 12% NaCl/12% hydroxylamine hydrogen sulfate (or alternatively hydroxylamine hydrochloride) solution is added. The reaction vessel is immediately connected to the cold vapor generator, and the headspace is purged for 30 sec to remove chlorine and other interferences. Next, 5 mL of a 10% tin(II) chloride solution in 0.5 N sulfuric acid is injected into the reaction vessel. The Hg vapor is swept into the absorption cell of the atomic absorption (AA) spectrometer, where the absorbance at 253.7 nm is measured. Labs generally dedicate one of the AA spectrometers to trace Hg determinations.

FIGURE 3.39 Glass apparatus for sample prep to quantitate elemental mercury.
Cold vapor generators are but one of the four major atomization devices used in AA, the other three being flame (FIAA), electrothermal (GFAA), and hydride generators. The cold vapor generator shown in Figure 3.39 could also be used as a hydride generator. Hydride generators convert the following elements to their gaseous hydrides: Sb, As, Bi, Se, Te, and Sn. A comparison of method detection limits (MDLs) reveals the real advantage of hydride generators. For example, an MDL for direct aspiration of selenium into a flame with measurement as atomic Se gives 100 ppm, whereas measurement as the hydride gives an MDL of 0.03 ppm.148

102. COULD HS-SPME COMBINED WITH AN ELEMENT-SPECIFIC GC BE USED TO SPECIATE Hg?

Yes, indeed. The combination of solid-phase microextraction sampling of the headspace (SPME-HS) with capillary gas chromatography and atomic emission detection (C-GC-AED) enables a speciation of Hg to be achieved. Principles that underlie GC-AED will be introduced in Chapter 4. Organo-Hg compounds such as methyl-Hg and dimethyl-Hg are much more toxic than inorganic Hg. Carro and coworkers149 have recently demonstrated that methyl mercury, ethyl mercury, and phenyl mercury can be isolated, recovered, derivatized, then separated and detected down to a concentration of 100 ppt from seawater using SPME-HS–C-GC-AED. A recent review has also been published that discusses speciation of mercury, tin, and lead using C-GC-AED.150

103. CAN ARSENIC BE SPECIATED?

Yes, indeed. The combination of reversed-phase high-performance liquid chromatography (RP-HPLC) with inductively coupled plasma-mass spectrometry (ICP-MS) has enabled a speciation of As containing species both organic and inorganic.151 Caruso and coworkers152,153 has reviewed the elemental speciation using ICP-MS.

104. HOW IS AN ENVIRONMENTAL SAMPLE PREPARED TO DETERMINE TRACE CYANIDE? WHAT ABOUT PHENOLS?

The tendency of free or bound CN− to become the toxic gas HCN discussed earlier is exploited in the sample prep approach to quantitatively determining cyanide. A wastewater or solid waste sample is placed in (preferably) a three-hole distillation pot. One hole in the pot contains a distilling head and condenser, the second hole is used to introduce purge gas, and the third hole is used for the addition of acid. Following the addition of acid, the pot is sealed. Purge gas is introduced and cyanide is converted to HCN. The HCN is liberated and trapped in a scrubber. The scrubber contains a dilute NaOH solution that converts HCN back to the cyanide ion. The contents of the scrubber now consist of NaCN in excess hydroxide. This completes the sample prep. The sample is now subject to whatever determinative technique is...
FIGURE 3.40 Glass distillation apparatus to prepare wastewater samples to quantitatively determine cyanide and phenolics.

used to quantitate cyanide. Figure 3.40 depicts a schematic for a two-holed distillation and gas scrubber apparatus used to isolate and recover both cyanide and phenols from wastewater samples. The inlet tube, along with the connection to a low-vacuum source, is used to provide purge air. As HCN gas is removed from the distilling flask, it is trapped in the gas scrubber.

Simple distillation is used to prepare the wastewater sample for the determination of total phenols. An acidified aqueous sample is merely simple distilled with the aqueous distillate trapped into a scrubber containing dilute NaOH, as was the case for cyanide. The contents of the scrubber consist of a dilute solution containing sodium phenolate. This solution is subject to whatever determinative technique is applicable to measure trace total phenolics.
105. **COULD WE PREPARE A SAMPLE TO DETECT CYANIDE BY DRIVING HCN INTO THE HEADSPACE?**

Yes, indeed. A sample, be it water, soil (enviro-chemical), or whole blood (enviro-health), is acidified, headspace sampled, and injected via a gas-tight syringe into a dedicated gas chromatograph with a nitrogen-phosphorous detector (NPD). A porous-layer open tabular column is used to provide gas–solid chromatographic retention and separation of HCN (a fixed gas). The high sensitivity afforded by the NPD enables the analyst to measure down to low ppb concentration levels of cyanide in a human specimen. Since static HS is an inherent part of the gas chromatograph, the IDL cannot be independently determined from the MDL, as discussed earlier in this chapter. The sketch below shows a sealed HS vial for this application:

![Diagram of a GC-NPD gas chromatogram](image)

A sketch of what a GC-NPD gas chromatogram would look like after the acidified specimen (spiked with acetonitrile as the internal standard) is shown below:

![Graph showing NPD response over time](image)
A calibration plot using acetonitrile as the internal standard might resemble the sketch shown below:

![Calibration Plot](image)

Prior to acidifying the sample, ascorbic acid is added to whole blood to minimize the loss of cyanide ion due to conversion to thiocyanate ion.

106. WHAT IS CHEMICAL DERIVATIZATION AND WHY IS IT IMPORTANT TO TEQA?

Most priority pollutants (enviro-chemical) or persistent organic pollutants (enviro-health) can be directly injected into a gas chromatograph owing to their physico-chemical properties of being relatively nonpolar, semivolatile, and thermally stable in the hot-injection port of the GC. However, those organic compounds with heteroatom functional groups are polar, nonvolatile, and sometimes thermally labile. Consider Figure 4.1, where the degree of analyte volatility is plotted against the degree of analyte polarity. Polar, nonvolatile analytes are converted to less polar ones, which become semivolatile derivatives. These derivatized organic compounds fall into the realm of GC and are said to be amenable to analysis by GC. Derivatives can also be prepared from analytes that yield a more sensitive means of detection for GC and are of particular importance to HPLC. This author’s first encounter with the need to make a chemical derivative involved the three chlorophenoxy acid herbicides (CPHs) — 2,4-D, 2,4,5-T, and 2,4,5-TP (Silvex) — in drinking water. EPA Methods 515.1 (drinking water) and 8150 (solid waste) require that CPHs and other organic acids be converted to methyl esters. Earlier, boron trifluoride–methanol (BF₃-MeOH) was used to convert carboxylic acids to their corresponding methyl esters (with mixed results from this author’s experience), while more recent methods favor the more vigorous in situ generation of diazomethane gas. EPA Method 8151A also considers that pentafluorobenzyl (PFB) esters of CPHs and other “chlorinated acids of environmental interest” can be made and chromatographed using a GC-ECD.
The PFB moiety in the derivatized ester of the CPH makes the ester extremely sensitive to detection via GC-ECD.

Let us take a broad view of chemical derivatization in analytical chemistry. The flowchart below summarizes how most commercially available derivatization reagents are categorized:

Sililation is the conversion of active hydrogen in a functional group to a trimethyl silyl (TMS) derivative. This was the first means to chemically convert carboxylic acids, alcohols, thiols, and primary and secondary amines to TMS esters. TMS esters are most appropriate where GC-MS is the principal determinative technique. Acylation is the conversion of active hydrogen, as is found in alcohols, phenols, thiols, and amines, into esters, thioesters, and amides by reacting organic compounds that contain these functional groups with fluorinated acid anhydrides. Heptafluorobutyrylimidazole and N-methyl-N-bis(trifluoroacetamide) are particularly effective in converting primary amines to fluorinated amides. Introduction of a perfluoroacyl moiety in the derivative leads to a significant increase in analyte sensitivity when using GC-ECD as the determinative technique. Alkylation is the conversion of active hydrogen by an alkyl or benzyl group to an ester or ether, depending upon whether the functional group in the organic compound is a carboxylic acid or alcohol or phenol, respectively. Diazomethane via in situ generation, BF₃-MeOH, dimethyl formamide–dialkyl acetals, and pentafluorobenzyl bromide are commonly used derivatizing reagents. Enantiomeric purity analysis reagents form diastereomers when reacted with optically active analytes. Diastereomers are easily separated by GC. Commercially available reagents include (−)-methyl chloroformate that reacts with enantio-enriched alcohols and N-TFA-L-prolyl chloride that couples with amines to form diastereomers. Chromotags are derivatizing reagents that add an ultraviolet-absorbing chromophore to an aliphatic carboxylic acid that converts the aliphatic acid to a UV-absorbing derivative to enhance sensitivity in HPLC-UV. Fluorotags convert a minimally fluorescent analyte to a highly fluorescent derivative, and hence enhance sensitivity in HPLC-FL. The reaction of aliphatic carboxylic acids with p-bromophenacyl bromide in the presence of 18-crown-6 under alkaline

© 2006 by Taylor & Francis Group, LLC
conditions to form a strong ultraviolet-absorbing ester, and the conversion of aliphatic carboxylic acids to highly fluorescent 4-bromomethyl-7-methoxycoumarin represent common uses of chromotags and fluorotags.\textsuperscript{155}

Analytes are usually isolated and recovered via any of the extraction and cleanup techniques described in this chapter. The extractant or eluent is evaporated to either dryness or close to dryness in order to concentrate the analyte. The derivatizing reagent, catalysts, acids, or bases, and any other reagents are then added. Heat is applied if necessary to increase the reaction rate. The derivatized analyte is extracted from the product mix and further cleaned up, excess derivatizing reagent is removed if possible, and then the extract is injected into the appropriate chromatographic determinative technique. It is important that the excess derivatizing reagent be chromatographically separated from the derivative(s) to enable quantitative analysis. Let us digress a bit to some specific examples of the use of chemical derivatization to accomplish the goals of TEQA.

\section*{107. HOW DO YOU MAKE A PFB DERIVATIVE OF SOME BUTYRIC ACIDS?}

This author once attempted to prepare PFB esters of \(n\)-butyric, \(i\)-butyric, and 2-methyl butyric acids.\textsuperscript{156,157} Here is what you need to do:

\textbf{To prepare the reagents:}

\begin{itemize}
\item \textit{30\% potassium carbonate:} Dissolve 7.5 g of K\textsubscript{2}CO\textsubscript{3} (anhydrous) in \(\sim\)20 mL of distilled deionized water (DDI). Transfer to a 25-mL volumetric flask and adjust to mark with DDI. Transfer to storage vial and label as “30\% K\textsubscript{2}CO\textsubscript{3}(aq).”
\item \textit{1\% PFBB:} Dissolve 0.25 g of PFBB in \(\sim\)20 mL of acetone. Transfer to a 25-mL volumetric flask and adjust to mark with acetone. Transfer contents to storage vial and label as “1\% PFBB(acetone).”
\item \textit{1000 ppm each carboxylic acid:} Weigh \(\sim\)0.010 g of each acid into a 10-mL volumetric flask and already half filled with DDI. Label as “1000 ppm each acid.”
\end{itemize}

\textbf{To synthesize and extract the PFB ester:}

Into a 22-mL headspace vial with crimp top, place 200 \(\mu\)L of the 100 ppm acid, 200 \(\mu\)L of 1\% PFBB, 50 \(\mu\)L of 30\% K\textsubscript{2}CO\textsubscript{3}, and 4 \(\mu\)L acetone. Shake vigorously and allow the contents of the vial to stand at room temperature for 3 h. Add enough DDI to reach the neck of the headspace vial. Add 2 mL of pesticide-grade iso-octane. Transfer 1.0 mL of extract to a 2-mL GC vial and inject 1 \(\mu\)L of extract into a gas chromatograph incorporating an electron-capture detector (GC-ECD). For a 30 m \(\times\) 0.32 mm DB-5 (J&W Scientific) capillary column, the following temperature program adequately separates the PFB esters of \(C\textsubscript{3}, C\textsubscript{4},\) and \(C\textsubscript{5}\) carboxylic acids. Start at 100\(^\circ\)C and hold for 3 min, then raise the temperature at a rate of 8\(^\circ\)/min to 150\(^\circ\)C, and then hold for 0.5 min. Under these conditions, we found that propionic acid elutes at 3.099 min, \(n\)-butyric at 3.65 min, and valeric at 6.09 min (principles of programmed temperature GC will be considered in Chapter 4). Figure 3.41 shows two chromatograms in a
Sample Preparation Techniques

stacked arrangement for the derivatization of \(i\)-butyric, \(n\)-butyric, and 2-methyl butyric as their PFB esters. A blank (lower chromatogram) and a spiked blank (upper chromatogram) reveal the presence of these PFB esters. Note that a 40 ppb concentration level can easily be reached. After these butyric acids are converted to their respective PFB butyrates, not only are polar acids converted to nonpolar esters, but also significant increases in analyte sensitivity (using a GC-ECD as stated earlier) are realized. Let us consider a second illustration of chemical derivatization, this time for HPLC.

FIGURE 3.41 Two chromatograms for the derivation of \(i\)-butyric, \(n\)-butyric, and 2-methyl butyric as their PFB esters.
108. **HOW DO YOU PREPARE A P-BROMOPHENACYL ESTER OF N-BUTYRIC ACID AS A CHROMOTAG AND CONDUCT A QUANTITATIVE ANALYSIS?**

The following procedure answers this question:

**PREPARATION OF MIXED ALKYLATING REAGENT:**

Weigh 0.47 g of p-bromophenacyl bromide (2,4-dibromoacetophenone) and 0.045 g of 18-crown-6 per every 10 mL using acetone. Dissolve both reagents in enough acetone prior to adjusting to a final volume.

**PREPARATION OF FATTY ACID STOCK REFERENCE STANDARD:**

Prepare an approximately 10,000 ppm stock solution of n-butyric acid (n-BuOOH) in water by weighing out approximately 0.1 g of the acid and dissolving in a beaker filled with approximately 5 to 8 mL of water. Dissolve, then transfer to a 10-mL volumetric flask and adjust to the calibration mark with DDI.

**PREPARATION OF 1 M AQUEOUS KHCO₃:**

Prepare a 1 M solution containing potassium bicarbonate dissolved in DDI by dissolving approximately 10 g of KHCO₃ in enough to reach 100 mL. Transfer to storage bottle.

**TO PREPARE THE POTASSIUM SALT OF BUTYRIC ACID (N-BUOOK):**

To 5 mL of the stock fatty acid reference standard, in a 50-mL beaker, add enough 1 M KOH solution to adjust the pH to 7 to 8. This is best accomplished by filling a buret with the 1 M KHCO₃ solution and titrating to the desired pH. Adjust the acid solution to a precise final volume and record. Transfer to a storage vial and label with a new concentration for the fatty acid.

**PREPARATION OF WORKING CALIBRATION STANDARDS:**

Create a series of working calibration solutions with the same final volume according to the following table. Use a 22-mL headspace vial with crimp top:

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>Alk Rgt (mL)</th>
<th>Acetone (mL)</th>
<th>RCOOK (µL)</th>
<th>V(total) Adjusted with DDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (blank)</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>3</td>
<td>500</td>
<td>5</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
**DERIVATIZATION:**

Place the 22-mL headspace vial or equivalent into a heater block set at 80°C and heat for 30 min. Alternatively, the contents of the vial may be evaporated to dryness and the residue reconstituted in the HPLC-compatible solvent.

**DETERMINATION OF THE ESTER VIA HPLC:**

Inject 5 µL of the content of the GC vial into an HPLC. Use a gradient elution reversed-phase approach as previously developed.

Finally, we consider the use of a fluorescence reagent to convert a carboxylic acid to a highly fluorescent derivative.

109. WHAT IS THE SAMPLE PREP APPROACH TO PLACING A FLUOROTAG ON A CARBOXYLIC ACID?

Scheme 3.10 is a flowchart that outlines the sample prep approach for isolating and recovering perfluorocarboxylic acids from liver homogenate, followed by the preparation of a highly fluorescent derivative using 3-bromoacetyl-7-methoxycoumarin.158 The fact that methoxycoumarins can be used as fluorotags for carboxylic acids has been known for some time.159,160 In this case, shown in Scheme 3.10, the perfluorocarboxylate anion is ion pair extracted into 1:1 ethyl acetate:hexane using a tetrabutyl ammonium cation under alkaline conditions following bath sonication. The extract is evaporated to just dryness and acetonitrile (a polar solvent) is added, followed by the 3-bromoacetyl-7-methoxycoumarin (BrAMC) reagent. The derivatized perfluorocarboxylic acid is subsequently injected into an RP-HPLC-FL, as noted in Scheme 3.10. HPLC-FL as a determinative technique will be introduced in Chapter 4. There are other derivatizing reagents that do not quite fit into the categories described earlier. We will encounter other derivatization concepts as we proceed through Chapter 4.

110. WHAT CAN WE CONCLUDE ABOUT SAMPLE PREP?

An attempt was made to introduce most of the recently developed sample prep techniques as well as provide for the underlying principles of established techniques. The link between true enviro-chemical quantitative analysis and true enviro-health quantitative analysis was attempted from the sample prep perspective. Hopefully, the reader comes away with a deeper appreciation of how samples and specimens are prepared so that these materials can be more properly introduced to the various determinative techniques introduced in the next chapter.

One of the unique features of solvent extraction, particularly for metal ions, is the large variation in distribution ratios and separation factors made possible by controlling the chemical parameters of the system.

—Henry Freiser

© 2006 by Taylor & Francis Group, LLC
REFERENCES

Sample Preparation Techniques


Sample Preparation Techniques

74. Loconto PR. Contract SBIR Study 68-02-4481. U.S. Environmental Protection Agency.
They laughed when they heard Aston say, he would weigh tiny atoms one day. But he had the last laugh — with his mass spectrograph, he “weighed” them a different way.

—Anonymous

### CHAPTER AT A GLANCE

Column chromatographic determinative techniques for trace organics
- Introduction and historical ............................................................... 323
- Differential migration ....................................................................... 327
- Principles of countercurrent distribution ......................................... 330
- Scope of chromatographic separations ............................................ 335
- Theoretical basis of column chromatography ..................................... 339
- Chromatographic resolution ............................................................. 351

Gas Chromatography ................................................................................... 357
- Gas pneumatics and inlets ................................................ 359
- Capillary columns ............................................................. 369
- Programmed column temperature .................................... 386
- Cryogenic techniques ........................................................ 390
- Element selective GC detectors ........................................ 392
- Atomic Emission Detector ............................................... 417

Gas Chromatography-Mass Spectrometry ................................................... 423
- Principles of the quadrupole ............................................. 425
- Principles of the ion-trap .................................................. 433
- Tuning a quadrupole .......................................................... 436
- Principles of Time-of-flight .............................................. 445
- Interpretation ..................................................................... 447
- Tandem strategies and techniques .................................... 449

High Performance Liquid Chromatography ................................................. 452
- Mobile phase/stationary phase considerations ........................... 458
- UV and fluorescence detectors ......................................... 464
- Principles of LC-MS interfaces ........................................... 476
Ion chromatographic determinative technique for trace inorganics

<table>
<thead>
<tr>
<th>Technique</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principles of cation-anion exchange</td>
<td>478</td>
</tr>
<tr>
<td>Principles of suppressed ion chromatography</td>
<td>481</td>
</tr>
</tbody>
</table>

Atomic spectroscopic determinative techniques for trace metals

<table>
<thead>
<tr>
<th>Technique</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction and historical</td>
<td>490</td>
</tr>
<tr>
<td>Choosing among techniques</td>
<td>493</td>
</tr>
<tr>
<td>Inductively-coupled plasma atomic emission</td>
<td>497</td>
</tr>
<tr>
<td>Inductively-coupled plasma-mass spectrometry</td>
<td>506</td>
</tr>
<tr>
<td>Metals speciation</td>
<td>510</td>
</tr>
</tbody>
</table>

Atomic Absorption

<table>
<thead>
<tr>
<th>Technique</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical and Principles</td>
<td>512</td>
</tr>
<tr>
<td>Graphite Furnace</td>
<td>515</td>
</tr>
</tbody>
</table>

Other determinative techniques for trace organics and trace inorganics

<table>
<thead>
<tr>
<th>Technique</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infrared Absorption Spectroscopy</td>
<td>518</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>521</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>525</td>
</tr>
<tr>
<td>Capillary Electrophoresis</td>
<td>528</td>
</tr>
<tr>
<td>Theoretical</td>
<td>530</td>
</tr>
<tr>
<td>Indirect photometric detection</td>
<td>537</td>
</tr>
</tbody>
</table>

References | 540 |

To the observer who does not have the technical background in TEQA and walks into a contemporary environmental testing laboratory, a collection of black boxes (instruments) with cables connecting the black boxes to personal computers and other high-tech devices should be what makes the first impression. This observer will see people, some of whom wear white lab coats, running around, holding various glassware, such as vials, syringes, beakers, test tubes, or whatever else it is lab people handle when at work in the busy lab. Observers will, upon being invited to tour, see different departments within the corporate structure. Some department personnel process analytical data generated by these black boxes; some personnel prepare samples for introduction into the black boxes; and other personnel enter data into a Laboratory Information Management System (LIMS) that reads a bar code label on a given sample and tracks the status of that sample as various analytical methods and instruments are used to generate the data. Some instruments are noisy, some are silent, some incorporate robot-like arms, and some incorporate samples directly, whereas others require sample preparation; all contribute to the last and no less important step in TEQA: determination. Instruments, computers, and accessories all comprise what the Environmental Protection Agency (EPA) refers to as determinative techniques, hence the title of this chapter. In Chapter 2, we discussed the important outcomes of using determinative techniques to perform TEQA. In Chapter 3, we discussed the means by which environmental samples and biological
specimens are made suitable and appropriate for introduction to these instruments (i.e., the science of sample preparation for TEQA). This chapter on determinative techniques therefore completes the thorough discussion of TEQA.

To the sufficiently educated observer, the contemporary environmental testing laboratory is a true testimonial to man’s ingenuity, a high-tech masterpiece. However, unlike a work of art, this observer is quick to discover that this artistic endeavor is a work in progress. This observer may see a robotic arm of an autosampler depositing 5 µL of sample into the graphite tube of a graphite furnace atomic absorption spectrophotometer (GFAA). He may also peer into a monitor that reveals an electron-impact mass spectrum of a priority pollutant, semivolatile organic compound. He will become aware very quickly whether or not this particular instrument is running samples or is still running calibration standards in an attempt to meet the stringent requirements of EPA methods. If this person is interested in the progress made by a particular sample as it makes its way through the maze of methods, he can find this information by peering into the sample status section of the LIMS software.

This chapter takes the reader from the uninformed observer described above to the educated observer who can envision the inner workings of a contemporary environmental testing laboratory. This is the chapter that deals with the determinative step, a term coined by the EPA. Beginning with the SW-846 series of methods, the sample prep portion was separated from the determinative portion. This separation enabled flexibility in conceptualizing the total method objectives of TEQA in the SW-846 series. This author believes that separating the sample prep from the determinative also makes sense in the organization of this book.

Of the plethora of instrumental techniques,3–11 GC, GC-MS (mass spectrometry), HPLC, AA, ICP-AES, and ICP-MS are the principal determinative techniques employed to achieve the objectives of TEQA as applied to both trace organics and trace inorganics analysis. The separation sciences have been coupled to the optical spectroscopic and mass spectrometric sciences to yield very powerful so-called hyphenated instruments. These six techniques are also sensitive enough to give analytical information to the client that is the most relevant to environmental site remediation. For example, one way to clean up a wastewater that is contaminated with polychlorinated volatile organics (ClVOCs) is to purge the wastewater to remove the contaminants, a process known as air stripping. It is important to know that the air-stripped wastewater has a concentration of ClVOCs that meets a regulatory requirement. This requirement is usually at the level of low parts per billion. A determinative technique that can only measure as low as parts per hundred has no place in the arsenal of analytical instruments pertinent to TEQA. Recall from Chapter 2 that techniques relating the acquisition of data directly from analytical instruments provide instrumental detection limits (IDLs), whereas method detection limits (MDLs) combine the sample prep step with the determinative step. This combination serves to significantly lower the overall detection limits and is one of the prime goals of TEQA.

This chapter introduces those six determinative techniques referred to earlier and adds several others. We first discuss those fundamental principles, vital to the practice of both GC and HPLC, that facilitate a more meaningful understanding of column chromatographic separations that are particularly relevant to the quantitative determi-
nation of trace organics. We then introduce the operational aspects of these instruments largely from a user perspective. A strong emphasis is placed on GC-MS, as this has become the dominant determinative technique for organics in TEQA. Ion chromatographic techniques as applied to trace inorganics are then introduced, and this topic provides an important link to the other major class of enviro-chemical/enviro-health chemical contaminants, trace metals, where atomic spectroscopy, as the principal determinative technique, dominates. A link between infrared absorption spectroscopy and TEQA is made through quantitative oil and grease and total organic carbon measurements. Finally, capillary electrophoresis is introduced and applied to the separation, detection, and quantification of trace inorganic anions in surface water via indirect photometric detection.

1. HOW DO YOU KNOW WHICH DETERMINATIVE TECHNIQUE TO USE?

Which determinative technique to use is dictated by the physical and chemical nature of the analyte of interest. The organics protocol flowchart introduced in Chapter 1 serves as a useful guide. Let us consider how we would determine which instrumental technique to use for the following example. Ethylene glycol, 1,2-ethanediol (EG), and 1,2-dichloroethane (1,2-DCA) consist of molecules that contain a two-carbon backbone with either a hydroxyl- or chlorine-terminal functional group. The molecular structures for these are as follows:

\[
\begin{align*}
\text{HOCH}_2 \text{CH}_2 \text{OH} & \quad \text{CICH}_2 \text{CH}_2 \text{Cl} \\
\end{align*}
\]

These two molecules look alike; so, could we use the same instrument and conditions to quantitate the presence of both of these compounds in an environmental sample? Nothing could be farther from the truth. Some relevant physical properties of both compounds are given in Table 4.1. The presence of two hydroxyl groups enables ethylene glycol to extensively hydrogen bond both intramolecularly (i.e., to itself) and intermolecularly (i.e., between molecules) when dissolved in polar solvents such as water and methanol. In stark contrast to this associated liquid, 1,2-DCA interacts intramolecularly through much weaker van der Waals forces and is incapable of interacting intermolecularly with polar solvents while being miscible in

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>Soluble in</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOCH₂CH₂OH</td>
<td>−12.6</td>
<td>197.3</td>
<td>Polar solvents</td>
</tr>
<tr>
<td>CICH₂CH₂Cl</td>
<td>−35.7</td>
<td>83.5</td>
<td>Nonpolar solvents</td>
</tr>
</tbody>
</table>
nonpolar solvents such as chloroform and ether. The boiling point of EG is almost twice as large as that of 1,2-DCA. These significant differences in physical properties would also be reflected in octanol–water partition coefficients. 1,2-DCA can be efficiently partitioned into a nonpolar solvent or to the headspace, whereas any attempt to extract EG from an aqueous solution that contains dissolved EG is useless. Because both compounds are liquids at room temperature, they do exhibit sufficient vapor pressure to be said to be amenable to analysis by gas chromatography. However, it may prove difficult to chromatograph them on the same column. The fundamental differences between a hydroxyl covalently bonded to carbon and a chlorine atom bonded to carbon become evident when one attempts to separate the two. We will continue to use the physical-chemical differences between EG and 1,2-DCA to develop the concept of a separation between the two compounds by differential migration through a hypothetical column and through a series of consecutive stages known as the Craig distribution.

2. WHAT IS DIFFERENTIAL MIGRATION ANYWAY?

Around 100 years ago, Mikhail Tswett, a Russian botanist, demonstrated for the first time that pigments extracted from plant leaves, when introduced into a packed column, whereby a nonpolar solvent is allowed to flow through calcium carbonate, initially separated into green and yellow rings. He called this separation phenomenon chromatography, derived from the Greek roots chroma (color) and graphein (to write). If additional solvent is allowed to pass through, these rings widen and separate more, and further separate into additional rings. In Tswett’s own words:

Like light rays in the spectrum, the different components of a pigment mixture, obeying a law, are resolved on the calcium carbonate column and then can be qualitatively and quantitatively determined. I call such a preparation a chromatogram and the corresponding method the chromatographic method.

His work in establishing the technique of liquid–solid adsorption chromatography would languish for 30 years until resurrected by Edgar Lederer in Germany.

A timeline titled Historica Chromatographica published recently and benchmarks key advances in all of chromatography and serves to recognize those that often go unnoticed; it is summarized in tabular format below:

<table>
<thead>
<tr>
<th>Year</th>
<th>Key Advances</th>
<th>Pioneers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>Persuasive perfusion</td>
<td>PerSeptive Biosystems, part of PerkinElmer, introduces perfusion chromatography, in which samples move both around and through the resin beads</td>
</tr>
<tr>
<td>1985</td>
<td>Superior supression</td>
<td>Dionex researcher Pohl introduces micromembrane suppressors for use in ion chromatography at Pittcon</td>
</tr>
<tr>
<td>1981</td>
<td>Microcolumn SFC</td>
<td>Novotny and Lee, pioneers in microcolumn liquid chromatography, introduce capillary supercritical fluid chromatography (SFC)</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
<table>
<thead>
<tr>
<th>Year</th>
<th>Key Advances</th>
<th>Pioneers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>IC advent</td>
<td>Small, Stevens, and Bauman develop ion chromatography combining a cation exchange column (separator) and strongly basic resin (stripper) to separate cations in dilute HCl</td>
</tr>
<tr>
<td>1974</td>
<td>Capillary zone electrophoresis (CZE) under glass</td>
<td>Virtanen introduces commercial CZE in glass tubes, based largely on pioneering work by Hjerten</td>
</tr>
<tr>
<td>1966</td>
<td>I see HPLC</td>
<td>Horvath and Lipsky develop high-pressure liquid chromatography (HPLC) at Yale University</td>
</tr>
<tr>
<td>1966</td>
<td>Sugar, sugar</td>
<td>Green automates carbohydrate analysis, improving on the earlier efforts of Cohn and Khym, who used a borate-conjugated ion exchange column to separate mono- and disaccharides</td>
</tr>
<tr>
<td>1960</td>
<td>GC’s heart of glass</td>
<td>Desty introduces the glass capillary column for GC, used in his analysis of crude petroleum; the technology was later commercialized by Hupe &amp; Busch and Shimadzu</td>
</tr>
<tr>
<td>1958</td>
<td>Automating AA analysis</td>
<td>Stein, Moore, and Spackman automate amino acid (AA) analysis using ion exchange and Edman degradation</td>
</tr>
<tr>
<td>1955</td>
<td>Going to market</td>
<td>First gas chromatographs were introduced in the U.S. by Burrell Corp., PerkinElmer, and Podbielniai</td>
</tr>
<tr>
<td>1953</td>
<td>Exclusive science</td>
<td>Wheaton and Bauman define ion exclusion chromatography, where one solution ion is excluded from entering the resin beads and passes in the void volume</td>
</tr>
<tr>
<td>1948</td>
<td>Reversing phases</td>
<td>Boldingh develops reversed-phase chromatography when separating the higher fatty acids in methanol against a solid phase of liquid benzene supported on partially vulcanized Hevea rubber</td>
</tr>
<tr>
<td>1945</td>
<td>One small step to GC</td>
<td>Prior describes gas–solid adsorption chromatography when separating O₂ and CO₂ on charcoal column</td>
</tr>
<tr>
<td>1941</td>
<td>Protein pieces</td>
<td>Martin and Synge develop liquid–liquid partition chromatography when separating amino acids through ground silica gel</td>
</tr>
<tr>
<td>1938</td>
<td>Spotting the difference</td>
<td>Izmailov and Shraiber develop drop chromatography on thin horizontal sheets, a precursor to thin-layer chromatography</td>
</tr>
<tr>
<td>1937</td>
<td>The road to white sands</td>
<td>Taylor and Urey use ion exchange chromatography to separate lithium isotopes, work that eventually led to the separation of fissionable uranium for the Manhattan Project</td>
</tr>
<tr>
<td>1922</td>
<td>Clarifying butter</td>
<td>Palmer, who is later recognized for popularizing chromatography’s use, separates carotenoids from butter fat</td>
</tr>
<tr>
<td>1913</td>
<td>Water world</td>
<td>First U.S. use of zeolites in water softening based on earlier work in Germany by Gans</td>
</tr>
<tr>
<td>1906</td>
<td>Our Father …</td>
<td>Tswett develops the concept of chromatography while attempting to purify chlorophylls from plant extracts; this discovery gained him the cognomen “Father of Chromatography”</td>
</tr>
<tr>
<td>1903</td>
<td>Food for thought</td>
<td>Goppelstroeder develops theory of capillary analysis when using paper strips to examine alkaloids, dyes, milk, oils, and wine, improving on the earlier work of his mentor, Schoenbein</td>
</tr>
</tbody>
</table>
If a mixture containing EG and 1,2-DCA is introduced into a column, it is possible to conceive of the notion that the molecules that make up each compound would migrate differentially through the packed bed or stationary phase. Let us assume that this hypothetical column tends to retain the more polar EG longer. This separation of EG from 1,2-DCA is shown as follows:

We observe that the dispersion of the molecules as represented by $\sigma^2$ is found to be proportional to the distance migrated, $z$, according to

$$\sigma^2 = kz$$

where $k$, the constant of proportionality, depends on the system parameters and operating conditions. Because $k$ is a ratio of the degree of spread to migration distance, $k$ can be referred to as a plate height. The resolution, $R_s$, between the separated peaks can be defined in terms of the distance between the apex of the peaks and the broadening of the peak according to

$$R_s = \frac{\Delta z}{\tau \sigma}$$

where $\tau$ is defined as being equal to 4 (p. 109). We will have more to say on this topic later. It also becomes evident that $\Delta z$ is proportional to the migration distance $z$ and $\sigma$ is proportional to the square root of the migration distance. Expressed mathematically, we have

$$\Delta z \propto z$$

and

$$\sigma \propto \sqrt{z}$$

These equations tell us that the distance between zone centers increases more rapidly than the zone widths. From the definition of $R_s$, this suggests that resolution improves with migration distance. We will have more to say about resolution when we take up chromatography. Differences in the rates of analyte migration, however,
do not explain the fundamental basis for separating EG from 1,2-DCA. For this, we begin by discussing the principles that underline the Craig countercurrent extraction experiment.

3. WHAT CAUSES THE BANDS TO SEPARATE?

We just saw that, experimentally, EG and 1,2-DCA differentially migrate through a stationary phase when introduced into a suitable mobile phase, and that chromatography arises when this mobile phase is allowed to pass through a chemically selective stationary phase. It is not sufficient to merely state that EG is retained longer than 1,2-DCA. It is more accurate to state that EG partitions to a greater extent into the stationary phase than does 1,2-DCA, largely based on “like dissolves like.” The stationary phase is more like EG than 1,2-DCA due to similar polarity. This is all well and good, yet these statements do not provide enough rationale to establish a true physical-chemical basis for separation. In Chapter 3, we introduced liquid–liquid extraction (LLE) and also considered successive or multiple LLE. What we did not discuss is what arises when we transfer this immiscible upper or top phase or layer to a second sep funnel (first Craig stage or $n = 1$; see below). Prior to this transfer, the second sep funnel will already contain a fresh lower phase. Equilibration is allowed to occur in the second sep funnel, while the fresh upper phase is brought in contact with the lower phase in the first funnel. What happens if we then transfer the upper phase from this second sep funnel to a third sep funnel that already contains a fresh lower phase? This transfer of the upper phase in each sep funnel to the next stage, with subsequent refill of the original sep funnel with a fresh upper phase, can be continued so that a total of $n$ stages and $n + 1$ sep funnels are used. It becomes very tedious to use sep funnels to conduct this so-called countercurrent extraction. A special glass apparatus developed by L.C. Craig in 1949 provides a means to perform this extraction much more conveniently. Twenty or more Craig tubes are connected in series in what is called a Craig machine. Once connected, up to 1000 tubes previously filled with a lower phase can participate in countercurrent extraction by a mere rotation of the tubes. The following is a schematic diagram of a single Craig tube of 2 mL undergoing rotation:
Determinative Techniques to Measure Organics and Inorganics

It is this rotational motion that removes the extracted organic phase while a fresh organic phase is introduced back into the tube. The phases separate in position A, and after settling, the tube is brought to position B. Then, all of the upper phase flows into decant tube d through c, as the lower phase is at a. When the tube is brought to position C, all of the upper phases in the decant tube are transferred through e into the next tube, and rocking is repeated for equilibration. The tubes are sealed together through the transfer tube, location e in the figure, to form a unit. These units are mounted in series and form a train having the desired number of stages (pp. 111–112).12

The Craig countercurrent extraction enables one to envision the concept of discrete equilibria and helps one understand how differences in partition coefficients among solutes in a mixture can lead to separation of these solutes. Consider a cascade of $n + 1$ stages, each stage containing the same volume of the lower phase. We seek to explain the foreboding-looking Table 4.2. Let us also assume that the total amount of solute is initially introduced into stage 0 (i.e., the first Craig tube in the cascade). The solute is partitioned between the upper and lower phases, as we saw in Equation (3.16), represented here according to

\[ p = \frac{VD}{1 + VD} \]  
\[ q = \frac{1}{1 + VD} \]

| TABLE 4.2 |
| Countercurrent Distribution of a Given Solute in a Craig Apparatus |

<table>
<thead>
<tr>
<th>Stage</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduce solute and equilibrate</td>
<td>$p/q$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>First transfer</td>
<td>$0/q$</td>
<td>$p/0$</td>
<td></td>
<td></td>
<td></td>
<td>$(q + p)^1$</td>
</tr>
<tr>
<td>Equilibrate</td>
<td>$p(q)/q(p)$</td>
<td>$p(p)/q(p)$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second transfer</td>
<td>$0/q(q)$</td>
<td>$p(q)/q(p)$</td>
<td>$p(p)/0$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$q^2$</td>
<td>$2pq$</td>
<td>$p^2$</td>
<td></td>
<td></td>
<td>$(q + p)^2$</td>
</tr>
<tr>
<td>Equilibration</td>
<td>$p(qq)/q(qq)$</td>
<td>$p(2pq)/q(2pq)$</td>
<td>$p(pp)/q(p^2)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third transfer</td>
<td>$0/q^3$</td>
<td>$pq^2/2q^3$</td>
<td>$p$</td>
<td>$2p^2 q(qp^2)$</td>
<td>$p^3/0$</td>
<td>$(q + p)^3$</td>
</tr>
<tr>
<td>Total</td>
<td>$q^3$</td>
<td>$3pq^2$</td>
<td>$3p^2 q$</td>
<td>$p^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equilibrate</td>
<td>$p(q^3)/q(q^3)$</td>
<td>$p(3pq^2)/q(3pq^3)$</td>
<td>$p(3p^2 q)/q(3p^2 q)$</td>
<td>$p(p^3)/q(p^3)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth transfer</td>
<td>$0/q^4$</td>
<td>$pq^3/5q^4$</td>
<td>$p$</td>
<td>$3p^2 q^3/3p^3 q$</td>
<td>$3p^3 q/p^3 q$</td>
<td>$(q + p)^4$</td>
</tr>
<tr>
<td>Total</td>
<td>$q^4$</td>
<td>$4q^3p$</td>
<td>$6p^2 q^2$</td>
<td>$4p^3 q$</td>
<td>$p^4$</td>
<td></td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
In Table 4.2, \( p \) is the fraction of total solute that partitions into the upper phase and \( q \) is the fraction of total solute that partitions into the lower phase. Also, by definition, the following must be true:

\[
p + q = 1
\]

\( V \) is the ratio of the upper phase volume to the lower phase volume and is usually equal to 1 because both volumes in the Craig tubes are usually equal. \( D \) is the distribution ratio and equals \( K_D \), the molecular partition coefficient in the absence of secondary equilibria, as discussed in Chapter 3. If we introduce a mixture of solutes to stage 0, each solute will have its own value for \( D \), and hence a unique value for \( p \) and \( q \). For example, for a mixture containing four solutes, we would realize a fraction \( p \) for the first solute, a fraction \( p' \) for the second solute, and so forth. In a similar manner, we would also realize a fraction \( q \) for the first solute, a fraction \( q' \) for the second solute, and so forth.

We seek now to show how the \( p \) and \( q \) values of Table 4.2 were obtained. We also wish to show how to apply the information contained in Table 4.2. We then extrapolate from the limited number of Craig tubes in Table 4.2 to a much larger number of tubes and see what effect this increase in the number of Craig tubes has on the degree of resolution, \( R_s \).

We start with a realization that once a mixture of solutes, such as our pair, EG and 1,2-DCA, is introduced into the first Craig tube, an initial equilibration occurs and this is shown at stage 0. Again, if \( p \) and \( q \) represent the fraction of EG in each phase, then \( p' \) and \( q' \) represent the fraction of 1,2-DCA in each phase. The first transfer involves moving the upper phase that contains a fraction \( p \) of the total amount of solute to stage 1. A volume of upper phase equal to that in the lower phase is now added to stage 0, and a fraction \( p \) of the total amount of solute in stage 0, \( p \), is partitioned into the upper layer while a fraction \( q \) of the total, \( q \), is partitioned into the lower phase. A fraction \( p \) of the total \( p \) remains in the upper layer in stage 1, and a fraction \( q \) of the total \( p \) is partitioned into the lower phase. The remainder of Table 4.2 is built by partition of a fraction \( p \) of the total after each transfer and equilibration to the upper layer and by partition of a fraction \( q \) of the total into the lower layer. The last column in Table 4.2 demonstrates that if each row labeled “total” is added, this sum is the expansion of a binomial distribution, \((q + p)'\), where \( r \) is the number of transfer. The fraction of solute in each \( n \)th stage after the \( r \)th transfer and corresponding equilibration can then be found using

\[
f = \frac{r!}{n!(r-n)!} p^n q^{r-n}
\]

(4.3)

This fraction represents the sum of the fractions in the upper and lower phases for that stage. For example, suppose we wish to predict the fraction of EG and the fraction of 1,2-DCA in stage 3 after four transfers. Let us assume that the upper phase is the less polar phase. Let us also assume that the distribution ratio for 1,2-DCA into the less polar upper phase is favored and that \( D_{1,2-DCA} = 4 \). Let us also
assume that EG prefers the more polar lower phase and has a distribution ratio that favors the lower phase and that $D_{EG} = 0.1$. We also assume that the volume of upper phase equals that of the lower phase (i.e., $V_{upper} = V_{lower}$) and the volume ratio is therefore 1. Substituting into Equation (4.3) without considering values for $p$ and $q$ yields

$$f^{3,4} = \frac{4!}{3!(4-3)!} p^3 q = 4q^3$$

A comparison of this result with that for the total fraction in the third stage after the fourth transfer (refer to Table 4.2) shows this result to be identical to that shown in the table. Next, we proceed to evaluate $p$ and $q$ for each of the two solutes. Using Equations (4.1) and (4.2), we find the following:

$$p(1,2-DCA) = 0.9, \quad p(EG) = 0.0909$$
$$q(1,2-DCA) = 0.1, \quad q(EG) = 0.909$$

Upon substituting these values for $p$ and $q$ for each of the two solutes in the mixture into Equation (4.3), we obtain:

$$f^{3,4}_{1,2-DCA} = (4)(0.5)^3(0.1) = 0.292$$
$$f^{3,4}_{EG} = (4)(0.0909)^3(0.909) = 0.00273$$

where $f^{3,4}_{1,2-DCA}$ is the fraction of 1,2-DCA present in the third stage after four transfers and $f^{3,4}_{EG}$ is the fraction of EG present in the third stage after four transfers. Hence, after four transfers, we find the fraction of 1,2-DCA in stage 3 (i.e., upper and lower phases) to be 0.292, whereas the fraction of EG in stage 3 is only 0.00273. The fact that these fractions are so different in magnitude is the basis for a separation of 1,2-DCA from EG.

4. **WHAT HAPPENS IF WE REALLY INCREASE THE NUMBER OF CRAIG TUBES?**

We have just examined a relatively small number of Craig countercurrent extractions and seen that differences in $D$ or $K_p$ among solutes result in different distributions among the many Craig tubes. Most distributions are normally distributed. In the absence of systematic error, random error in analytical measurement is normally distributed, and this assumption formed the basis of much of the discussion in Chapter 2. A continuous random variable $x$ has a normal distribution with certain parameters $\mu$ (mean, parameter of location) and $\sigma^2$ (variance, parameter of spread) if its density function is given by: \[13\]
The binomial distribution in Equation (4.3) closely approximates a Gaussian distribution when \( r \) and \( n \) are large. We can write the distribution as a continuous function of the stage number as

\[
f(x; \mu, \sigma) = \frac{1}{\sigma \sqrt{2\pi}} \exp\left(-\frac{1}{2} \frac{(x-\mu)^2}{\sigma^2}\right)
\]

Equation (4.4) is a very good approximation when the total number of stages is larger than 20 or when the product \( rpq \) is greater than or equal to 3. Comparing Equation (4.4) to the above classical relationship for a Gaussian or normal distribution leads to the following observation for the standard deviation, \( \sigma \), of the distribution:

\[
\sigma = \sqrt{rpq}
\]

The mean Craig stage is also that stage with the maximum fraction. This distribution mean is given by

\[
\mu = rp
\]

Equation (4.6) enables a calculation of the mean in this Craig countercurrent distribution, and Equation (4.5) yields an estimate of the standard deviation in the distribution among Craig tubes. For example, let us return to our two solutes, 1,2-DCA and EG. Earlier, we established values for \( p \) and \( q \) from a knowledge of \( D \) or, in the limit of purely molecular partitioning, \( K_D \). Let us find \( \mu \) and \( \sigma \) for both compounds after 100 transfers (i.e., \( r = 100 \)) have been performed:

\[
D(1,2-DCA) = 4, \quad D(EG) = 0.1
\]
\[
p(1,2-DCA) = 0.8, \quad p(EG) = 0.0909
\]
\[
q(1,2-DCA) = 0.2, \quad q(EG) = 0.909
\]

Upon substituting these values into Equation (4.5), we obtain for 1,2-DCA

\[
\sigma_{1,2-DCA} = \sqrt{rpq} = \sqrt{(100)(0.8)(0.2)} = 4
\]

Upon substituting these values into Equation (4.6), we obtain for 1,2-DCA

\[
\mu_{1,2-DCA} = rp = (100)(0.8) = 80
\]
In a similar manner, for EG we obtain:

\[ \sigma_{\text{EG}} = \sqrt{(100)(0.01)(0.91)} = 2.9 \]

\[ \mu_{\text{EG}} = (100)(0.091) = 9.1 \]

It becomes obvious now that differences in \( D \) or \( K_D \) result in different stages in which the maximum fraction appears. The degree of band broadening is also larger for the solute with the larger value of \( \mu \). When the fraction of solute in a given stage is plotted against the stage number, Gaussian-like distributions are produced. The following is a sketch of such a plot:

![Fraction of solute in a given stage vs. stage number](image)

It appears that 100 transfers using a Craig countercurrent apparatus enabled a more than adequate separation of EG and 1,2-DCA. We have therefore found a way to separate organic compounds. Before we leave the countercurrent separation concept, let us discuss the significance of Equations (4.5) and (4.6) a bit further. Equation (4.6) suggests that each solute migrates a distance equal to a constant fraction of the solvent front, and Equation (4.5) suggests that the width of the peak increases with the square root of the number of transfers. Separation is achieved as the number of transfers increases. The distance that each peak travels is proportional to \( r \), and the width of the peak is proportional to the square root of \( r \). It is instructive to compare these findings from countercurrent extraction to those of analyte migration discussed earlier. Differential migration and countercurrent extraction techniques serve to help us to begin thinking about separations. These techniques set the stage for the most powerful of separation methods, namely, chromatography.

5. WHAT IS CHROMATOGRAPHY?

Harris states that chromatography is a “logical extension of countercurrent distribution.”\(^{14}\) Chromatographic separation is indeed the countercurrent extraction taken to a very large number of stages across the chromatographic column. The following quotation is taken from an earlier text:\(^ {15} \)

 Chromatography encompasses a series of techniques having in common the separation of components in a mixture by a series of equilibrium operations that result in the entities being separated as a result of their partitioning (differential sorption) between
two different phases; one stationary with a large surface and the other, a moving phase in contact with the first.

The “inventors” of partition chromatography, Martin and Synge, in 1941 first introduced gas chromatography this way:16

The mobile phase need not be a liquid but may be a vapour…. Very refined separations of volatile substances should therefore be possible in a column in which permanent gas is made to flow over gel impregnated with a non-volatile solvent in which the substances to be separated approximately obey Raoult’s law.

The following excerpt is titled “The King’s Companions — A Chromatographical Allegory”:17

A great and powerful king once ruled in a distant land. One day, he decided he wanted to find the ten strongest men in his kingdom. They would be his sporting companions and would also protect him. In return, the king would give them splendid chambers in his palace and great riches.

But how would these men be found? For surely thousands from his vast lands would seek this promise of wealth and power. From amongst these thousands, how would he find the ten very strongest?

The king consulted his advisors. One suggested a great wrestling tournament, but that would be too time consuming and complicated. A weight-lifting contest was also rejected. Finally, an obscure advisor named Chromos described a plan that pleased the king.

“Your majesty,” said Chromos, “you have in your land a mighty river. Use it for a special contest. At intervals along the river, have your engineers erect poles. The ends of each pole should be anchored on opposite banks so that each pole stretches across the river. The pole must be just high enough above the surface of the river for a man being carried along by the current to reach up and grab hold of it. So strong is the current that he will not be able to pull himself out, but will just be able to hold on until, his strength sapped, the pole will be torn from his grasp. He will be carried downstream until he reaches the next pole which he will also grasp hold of. Of course, the weakest man will be able to hold on to each pole for the shortest length of time, and will be carried downstream fastest. The strongest man will hold on the longest, and will be carried along most slowly by the river. You have only to throw the applicants into the river at one particular place and measure how long it takes each man to get to the finish line downstream (where he will be pulled out). As long as you have enough poles spaced out between the start and finish, the men will all be graded exactly according to their strength. The strongest will be those who take the longest time to reach the finish line.”

So simple and elegant did this method sound, that the king decided to try it. A proclamation promising great wealth and power to the ten strongest men was spread throughout the kingdom. Men came to the river from far and wide to participate in the contest Chromos had devised and the contest was indeed successful. Simply and
quickly, the combination of moving river and stationary poles separated all the applicants from one another according to their strength.

So the king found his ten strongest subjects, and brought them to his palace to be his companions and protectors. He rewarded them all with great wealth. But the man who received the greatest reward was his advisor, Chromos.

Do you see the analogy?

In Chapter 3, we have alluded to chromatographic separation as the most important separation technique to TEQA. Indeed, much of the innovative sample prep techniques for trace organics described in Chapter 3 are designed to enable a sample of environmental interest to be nicely introduced into a chromatograph. A chromatograph is an analytical instrument that has been designed and manufactured to perform either gas or liquid column chromatography.

Chromatography is a separation phenomenon that occurs when a sample is introduced into a system in which a mobile phase is continuously being passed through a stationary phase. Chromatography has a broad scope, in that small molecules can be separated as well as quite large ones. Of interest to TEQA are the separation, detection, and quantification of relatively small molecules. In Chapter 3, we introduced SPE as an example of frontal chromatography. In this chapter, we will discuss elution chromatography exclusively because this form of chromatographic separation lends itself to instrumentation. We will also limit our discussion of chromatography to column methods while being fully aware of the importance of planar chromatography, namely, paper and thin-layer chromatography, because our interest is in trace chromatographic analysis. We will further limit our discussion to the two major types of chromatography most relevant to TEQA: gas chromatography (GC) and high-performance liquid chromatography (HPLC). Figure 4.1 represents an attempt to place the major kinds of chromatographic separation science in various regions of a two-dimensional plot whereby analyte volatility increases from low to high along the ordinate, whereas analyte polarity increases from left to right along the abscissa. The reader should keep in mind that there is much overlap of these various regions and that the focus of the plot is on the use of chromatography as a separation concept without reference to the kinds of detector required. The horizontal lines denote regions where GC is not appropriate. The arrow pointing downward within the GC region serves to point out that the demarcation between volatile analytes and semivolatile ones is not clear-cut. This plot reveals one of the reasons why GC has been so dominant in TEQA, while revealing just how limited GC as a determinative technique really is. This plot also reveals the more universal nature of HPLC in comparison to GC.

6. WHY IS GC SO DOMINANT IN TEQA?

There are several reasons for this, and GC is still the dominant analytical chromatographic determinative technique used in environmental testing labs today. Let us construct a list of reasons why:
Gas chromatography was historically the first instrumented means to separate organic compounds and was first applied in the petroleum industry. Gas chromatography continuously evolved from the packed column, where resolution was more limited to capillary columns that significantly increase chromatographic resolution. The EPA organics protocol referred to in Scheme 1.5 classifies priority pollutant organics based on the degree of volatility, and the volatile and lower-molecular-weight semivolatile regions of Figure 4.1 are predominantly GC. Gas chromatography is simpler to comprehend in contrast to HPLC largely because the mobile phase in GC is chemically inert and contributes nothing to analyte retention and resolution.
• Detectors in GC can be of low, medium, and high sensitivity; highly sensitive GC detectors are of utmost importance to TEQA.
• The price and size of the GC-MS instrument have declined over the past decade, and the instrument has become more sensitive and more robust.

7. **WHY IS HPLC MORE UNIVERSAL IN TEQA?**

There are several reasons for this:

• High-performance liquid chromatography occupies a much larger region of Figure 4.1, and this fact suggests that a much larger range of organic compounds are amenable to analysis by HPLC in contrast to GC.
• High-performance liquid chromatography can take on several different forms depending on the chemical natures of the mobile phase and stationary phase, respectively; this leads to a significant rise in the scope of applications.
• Chemical manipulation of the mobile phase in HPLC enables gradient elution to be conducted.
• The analyte of interest remains dissolved in the liquid phase and can be thermally labile, unlike GC, whereby an analyte must be vaporized and remain thermally stable.

8. **CAN WE VISUALIZE A CHROMATOGRAPHIC SEPARATION?**

Yes, we can. Figure 4.2 is a hypothetical separation of a three-component mixture. Introduction of the mixture is diagrammatically shown in snapshot 1; elution of the mixture with a mobile phase begins in snapshot 2. Snapshots 3 and 4 depict the increase in chromatographic resolution, $R_s$, as the mixture moves through the column. Four chief parameters are used to characterize a chromatographic separation: distribution coefficient, retention or capacity factor, selectivity and column efficiency, and number of theoretical plates. We will spend the next few paragraphs developing the mathematics underlying chromatographic separation.

9. **CAN WE DEVELOP USEFUL MATHEMATICAL RELATIONSHIPS FOR CHROMATOGRAPHY?**

Yes, we can. Let us begin by first recognizing that an analyte that is introduced into a chromatographic column, much like that shown in Figure 4.2, distributes itself between a mobile phase, $m$, and a stationary phase, $s$, based on the amount of analyte distributed instead of the concentration distributed. The fraction of the $i$th analyte in the stationary phase, $\phi_s^{i}$, is defined as

$$\phi_s^i = \frac{\text{amt}^s_i}{\text{amt}^m_i} = \frac{C^s_i V^s}{C^m_i V^m + C^s_i V^s}$$

(4.7)
where \( C_i^m \) and \( C_i^s \) are the \( i \)th analyte concentrations in both phases. \( V^m \) and \( V^s \) are the volumes of both phases. Let us define a molecular distribution constant for this \( i \)th analyte, \( K_i \), as

\[
K_i = \frac{C_i^s}{C_i^m} \quad (4.8)
\]

Equation (4.8) can be substituted into Equation (4.7) to yield the fraction of the \( i \)th analyte in terms of the molecular distribution constant and ratio of phase volumes according to

\[
\phi_i = \frac{K_i V^s / V^m}{1 + K_i V^s / V^m} \quad (4.9)
\]

We now define the capacity factor, \( k' \), a commonly used parameter in all of chromatography, as a ratio of the amount of analyte \( i \) in the stationary phase to the amount of analyte \( i \) in the mobile phase at any one moment:

\[
k' = \frac{\text{amt}_i^s}{\text{amt}_i^m} = \frac{C_i^s V_i^s}{C_i^m V^m} = K_i \left( \frac{V_i^s}{V^m} \right) \quad (4.10)
\]

As we discussed extensively in Chapter 3, when secondary equilibria are involved, \( K \) is replaced by \( D \). Combining Equations (4.9) and (4.10) gives a relationship between the capacity factor and the fraction of analyte \( i \) distributed into the stationary phase:
Determinative Techniques to Measure Organics and Inorganics

\[ \phi_i' = \frac{k'}{1 + k'} \]  

(4.11)

Also, the fraction \( \phi_i^m \) of analyte \( i \) in the mobile phase is

\[ \phi_i^m = \frac{1}{1 + k'} \]  

(4.12)

Equation (4.12) gives the fraction of analyte \( i \), once injected into the flowing mobile phase, as it moves through the chromatographic column. This analyte migrates only when in the mobile phase. The velocity of the analyte through the column, \( v_s \), is a fraction of the mobile phase velocity \( v \) according to

\[ v_s = v \phi_i^m \]  

(4.13)

Equation (4.13) suggests that the analyte does not migrate at all \( (v_s = 0) \) and when \( \phi_i^m = 1 \). It also suggests that the analyte moves with the same velocity as the mobile phase \( (v_s = v) \). The analyte velocity through the column equals the length, \( L \), of the column divided by the analyte retention time, \( t_R \):

\[ v_s = \frac{L}{t_R} \]

The velocity of the mobile phase is given by the length of column divided by the retention time of an unretained component, \( t_0 \), according to

\[ v = \frac{L}{t_0} \]

Substituting for \( v_s \) and \( v \) in Equation (4.13) yields

\[ t_R = \frac{t_0}{\phi_i^m} \]  

(4.14)

The mobile-phase volumetric flow rate, \( F \), expressed in units of cubic centimeters per minute or milliliters per minute, is usually fixed and unchanging in chromatographic systems. Thus, because \( t_R = V_R/F \) and \( t_0 = V_0/F \), Equation (4.14) can be rewritten in terms of retention volumes:

\[ V_R = \frac{V_0}{\phi_i^m} \]
The retention volume, \( V_R \), for a given analyte can be seen as the product of two terms, \( V_0 \) and the reciprocal of \( \phi_m \):

\[
V_R = V_0 \left( \frac{1}{\phi_m} \right) \tag{4.15}
\]

Substituting Equation (4.12) into the above relationship gives

\[
V_R = V_0 \left( \frac{1}{1/1+k'} \right)
\]

Upon rearranging and simplifying,

\[
V_R = V_0 [1 + k'] \tag{4.16}
\]

Equation (4.16) has been called the fundamental equation for chromatography. Each and every analyte of interest that is introduced into a chromatographic column will have its own capacity factor, \( k' \). The column itself will have a volume \( V_0 \). The retention volume for a given analyte is then viewed in terms of the number of column volumes passed through the column before the analyte is said to elute. A chromatogram then consists of a plot of detector response vs. the time elapsed after injection, where each analyte has a unique retention time if sufficient chromatographic resolution is provided. Hence, with reference to a chromatogram, the capacity factor becomes

\[
k' = \frac{t_R - t_0}{t_0} \tag{4.17}
\]

Figure 4.3 is an illustrative chromatogram that defines what one should know when examining either a GC or an HPLC chromatogram. Because the two peaks shown in Figure 4.3 have different retention times, their capacity factors differ according to Equation (4.17). The ratio of two capacity factors, \( \alpha \), relates to the degree that a given chromatographic column is selective:

\[
\alpha = \frac{k'_2}{k'_1} \tag{4.18}
\]

Let us assume that we found a column retains the more polar EG with respect to 1,2-DCA. Equation (4.13) would suggest that the \( \phi_{1,2-DCA}^m \) is much larger than \( \phi_{EG}^m \). The unretained solute peak in the chromatogram, often called the chromatographic dead time or dead volume, might be due to the presence of air in GC or a solvent
Determinative Techniques to Measure Organics and Inorganics

in HPLC. The adjusted retention volume, $V'_R$, and adjusted retention time, $t'_R$, are defined mathematically as

$$ V'_R = V_R - V_0 $$
$$ t'_R = t_R - t_0 $$

We need now to go on and address the issue of peak width. As was pointed out earlier, the longer an analyte is retained in a chromatographic column, the larger is the peak width. This is almost a universal statement with respect to chromatographic separations.

10. **HOW DOES ONE CONTROL THE CHROMATOGRAPHIC PEAK WIDTH?**

The correct answer is to minimize those contributions to peak broadening. These factors are interpreted in terms of contributions to the height equivalent to a theoretical plate (HETP). This line of reasoning leads to the need to define a HETP that in turn requires that we introduce the concept of a theoretical plate in chromatography. So let us get started.

The concept of a theoretical plate is rooted in both the theory of distillation and the Craig countercurrent extraction. A single distillation plate is a location whereby a single equilibration can occur. We already discussed the single equilibration that occurs in a single Craig stage. Imagine an infinite number of stages, and we begin to realize the immense power of chromatography as a means to separate chemical substances. An equilibration of a given analyte between the mobile phase and

© 2006 by Taylor & Francis Group, LLC
stationary phase requires a length of column, and this length can be defined as $H$. A column would then have a length $L$ and a number of these equilibrations denoted by $N$, the number of theoretical plates. Hence, we define the HETP, abbreviated $H$ for brevity here, as follows:

$$H = \frac{L}{N} \tag{4.19}$$

The number of theoretical plates in a given column, $N$, is mathematically defined as the ratio of the square of the retention time, $t_R$, or the retention volume, $V_R$ (note that this is the apex of the Gaussian peak), of a particular analyte of interest over the variance of that Gaussian peak. Expressed mathematically,

$$N = \left(\frac{t_R}{\sigma_t}\right)^2 = \left(\frac{V_R}{\sigma_t}\right)^2 \tag{4.20}$$

The number of theoretical plates can be expressed in terms of the width of the Gaussian peak at the base. This is expressed in units of time, $t_w$, where it is assumed that $t_w$ approximates four standard deviations or, mathematically, $t_w = 4\sigma_t$, so that upon substituting for $\sigma_t$,

$$N = \frac{t_R}{(t_{w}/4)^2} = 16\left(\frac{t_R}{t_w}\right)^2 \tag{4.21}$$

Columns that significantly retain an analyte of interest (i.e., have a relatively large $t_R$) and also have a narrow peak width at base, $t_w$, must have a large value for $N$ according to Equation (4.21). Columns with large values for $N$, such as from 1000 to 10,000 theoretical plates, are therefore considered to be highly efficient. Many manufacturers prefer to cite the number of theoretical plates per meter instead of just the number of theoretical plates. The concept that the number of theoretical plates for a column (be it a GC or an HPLC column) can be calculated from the experimental GC or HPLC chromatogram is an important practical concept. In the realm of GC, when open tubular columns or capillary replaced packed columns, it was largely because of the significant difference in $N$ offered by the former type of column. The second most useful measurement of $N$ is to calculate $N$ from the width of the peak at half height, $t_{w/2}$, using the equation

$$N = 5.55\left(\frac{t_R}{t_{w/2}}\right)^2 \tag{4.22}$$

It is up to the user as to whether Equation (4.21) or (4.22) is used to estimate $N$. Figure 4.3 defines the peak width at half height and the peak width at the base. When a GC or HPLC column is purchased from a supplier, pay close attention to
how the supplier calculates $N$. It is also recommended that a peak be chosen in a chromatogram to calculate $N$ whose $k'$ is around 5. To continue our discussion of peak broadening, we need to return to the height equivalent to a theoretical plate, $H$.

11. IS THERE A MORE PRACTICAL WAY TO DEFINE $H$?

Equation (4.19) defines $H$ in terms of column length and a dimensionless parameter $N$. We will now derive an expression for $H$ in terms of the chromatogram in units of time. We start by considering a chromatographic column of length $L$ to which a sample has been introduced. This sample will experience band broadening as it makes its way through the column. We know that the degree of band broadening denoted by $\sigma$ has units of distance.

$H$ can be defined as the ratio of the variance, in units of distance, over the column length according to

$$H = \frac{\sigma^2}{L} \quad (4.23)$$

This dispersion in distance units can be converted to time units by recognizing that

$$\sigma = \phi^{m} v \sigma_i$$

Upon substituting for $\sigma$ and substituting into Equation (4.23), and doing some algebraic manipulation while recognizing that

$$t_R = \frac{L}{\phi^{m} v}$$

we now have an equation that relates $H$ to the variance of the chromatographically resolved peak in time units according to

$$H = \frac{L \sigma_i^2}{t_R^2} \quad (4.24)$$

Equation (4.24) suggests that the height equivalent to a theoretical plate can be found from a knowledge of the length of the column, the degree of peak broadening as measured by the peak variance, in time units, and the retention time. We now discuss those factors that contribute to $H$ because Equations (4.23) and (4.24) show that $H$ is equal to the product of a constant and a variance. If we can identify those distinct variances, $\sigma_i$, that contribute to the overall variance, $\sigma_{overall}$, then these individual variances can merely be added. Expressed mathematically, for the $i^{th}$
independent contribution to chromatographic peak broadening, the statistics of propagation of error suggest that

$$\sigma^2_{\text{overall}} = \sum_i \sigma^2_i$$

The concept that a rate theory is responsible for contributions to chromatographic peak broadening were first provided by van Deemter, Klinkenberg, and Zuiderweg. The random walk is the simplest molecular model and is due to Giddings.\(^{18}\)

12. WHAT FACTORS CONTRIBUTE TO CHROMATOGRAPHIC PEAK BROADENING?

It is important for the practicing chromatographer to understand the primary reasons why the mere injection of a sample into a chromatographic column will lead to a widening of the peak width. We alluded to peak broadening earlier when we introduced band migration. We will not provide a comprehensive elaboration of peak broadening. Instead, we introduce the primary factors responsible for chromatographic peak broadening. Following this, we introduce and discuss the van Deemter equation. The concept is termed chromatographic rate theory and is adequately elaborated on in the analytical literature elsewhere.\(^{19–21}\)

Equation 4.23 is the starting point for discussing those factors that broaden a chromatographic peak. By the time the solute molecules of a sample that have been injected into a column have traveled a distance \(L\), where \(L\) is the length of the GC or HPLC column, a Gaussian profile emerges. At the end of the column where the GC or HPLC detector is located, the peak has been broadened, whereby one standard deviation has a length defined by \(L - \sigma\) to the left of the peak apex at \(L\), and \(L + \sigma\) to the right of the peak apex at \(L\). \(H\) can now be thought of as the length of column, at the end of the column, that contains a fraction of analyte that lies between \(L - \sigma\) and \(L + \sigma\).\(^{22}\) The fact that there exists a minimum \(H\) in a plot of \(H\) vs. linear flow rate, \(u\), suggests that a complex mathematical relationship exists between \(H\) and \(u\). The following factors have emerged:

- Multiple paths of solute molecules, the \(A\) term, are present only in packed GC and HPLC columns and absent in open tubular GC columns. This term is also called eddy diffusion.
- Longitudinal diffusion, the \(B\) term, is present in all chromatographic columns.
- Finite speed of equilibration and the inability of solute molecules to truly equilibrate in one theoretical plate, the \(C\) term, are present in all chromatographic columns. This term is also called resistance to mass transfer and, in more contemporary versions, consists of two mass transfer coefficients: \(C_{S}\), where \(S\) refers to the stationary phase, and \(C_{M}\), where \(M\) refers to the mobile phase. Equilibrium is established between \(M\) and \(S\) so slowly that a chromatographic column always operates under nonequilibrium conditions.
conditions. Thus, analyte molecules at the front of a band are swept ahead before they have time to equilibrate with S and thus be retained. Similarly, equilibrium is not reached at the trailing edge of a band, and molecules are left behind in S by the fast-moving mobile phase.23

The above three factors broaden chromatographically resolved peaks by contributing a variance for each factor, starting with Equation (4.23), as follows:

\[ H = \frac{1}{L} (\sigma^2) = \frac{1}{L} \left( \sum \sigma_i^2 \right) \]

\[ H = H_L + H_S + H_M \]

where \( H_L \) is the contribution to \( H \) due to longitudinal diffusion, \( H_S \) is the contribution to \( H \) due to resistance to mass transfer to \( S \), and \( H_M \) is the contribution to \( H \) due to resistance to mass transfer to \( M \). We will derive only the case for longitudinal diffusion and state the other two without derivation.

13. HOW DOES LONGITUDINAL DIFFUSION CONTRIBUTE TO \( H \)?

Molecular diffusion of an analyte of environmental interest in the direction of flow is significant only in the mobile phase. Its contribution, \( \sigma_L^2 \), to the total peak variance can be found by substituting the molecular diffusivity and time into the Einstein equation:

\[ \sigma^2 = 2Dt \]

On average, solute molecules spend the time \( t = \frac{L}{u} \) in the mobile phase, so that the variance in the mobile phase is given as

\[ \sigma_L^2 = \frac{2D_M L}{u} \]

where \( D_M \) is the solute diffusion coefficient in the mobile phase. The plate height contribution of longitudinal diffusion, \( H_L \), is then obtained as

\[ H_L = \frac{\sigma^2}{L} = \frac{2yD_M}{u} \]

\[ = \frac{B}{u} \]
where $\gamma$ is an obstruction factor that recognizes that longitudinal diffusion is hindered by the packing or bed structure. $H_L$ is usually only a small contributor to $H$. However, when $D_M$ is large and the mobile-phase velocity, $u$, is small, does $H_L$ become significant? $H_L$ is far more important in GC than in HPLC.

### 14. HOW DOES ALL OF THIS FIT TOGETHER?

Mass transfer into the stationary phase and the mobile-phase contribution to plate height give the terms $C_S$ and $C_M$, respectively, to the total plate height in direct proportion to $u$. This is so because, unlike longitudinal diffusion, molecules diffuse at an angle of 90° with respect to the direction of the mobile-phase flow. The larger the mobile-phase velocity, the greater is the diffusion in this direction. Hence, we have a relationship between $H$ and the linear mobile-phase velocity $u$ according to

$$H = A + \frac{B}{u} + C_S u + C_M u$$  \hspace{1cm} (4.25)

This is a more contemporary van Deemter equation, and this equation takes on different contributions to the terms $A$, $B$, $C_S$, and $C_M$, depending on which form of chromatography is employed. We will introduce specific parameters that comprise both $C$ terms when we discuss GC and HPLC. Equation (4.25) is plotted in Figure 4.4. This plot is obtained by measuring $H$ as the linear velocity of the mobile phase for the solute ethyl acetate dissolved in $n$-hexane and chromatographed on a
normal-phase, silica-based HPLC column. The plot includes the eddy diffusion or the $A$ term and shows the independence of eddy diffusion with respect to the mobile-phase flow rate. The plot shows the inverse relationship between $H$ and $u$ with respect to longitudinal diffusion. The plot also shows the near linear relationship between $H$ and $u$ with respect to mass transfer in both phases. If we differentiate Equation (4.25) with respect to $u$ and set this derivative equal to zero, upon solving for $u$ we find

$$u_{\text{optimum}} = \frac{\sqrt{B}}{\sqrt{C}}$$

In practice, the mobile-phase linear velocity is set slightly higher than $u_{\text{optimum}}$ so as to quicken the chromatographic run time (i.e., the time between injection and separation, and then detection). Figure 4.5 is a plot of $H$ vs. $u$ for a packed GC column and for an open tubular GC column. $H$ is much lower for the open tubular column because multiple flow paths are eliminated. Note also that the curvature in the plot in Figure 4.5 for the open tubular column is much less than that of the packed column. This much wider range of optimal mobile-phase velocities enables the chromatographer to use a much larger range of volumetric flow rates without sacrificing $H$. While we are discussing flow rates, we need to point out the significant difference between linear flow and volumetric flow with respect to column chromatography.

**FIGURE 4.5** Plate height vs. linear carrier gas velocity for a packed vs. capillary GC column.
15. HOW DO WE DISTINGUISH BETWEEN LINEAR AND VOLUMETRIC FLOW RATES?

Note that Equation (4.25) examines column efficiency as a complex function of linear mobile-phase velocity. It is the linear velocity that conducts analytes of interest through a chromatographic column to the detector. Any comparison of van Deemter curves, such as that shown in Figure 4.5, must use linear velocity because the influence of the column radius is eliminated. Jennings has articulated an interesting relationship between linear and volumetric flow rates, $F$, incorporating the column radius, $r_C$, according to

$$u(\text{cm/s}) = \frac{1.67F(\text{cm}^3/\text{min})}{\frac{1}{2}\pi r_C(\text{mm})^2}$$

(4.26)

Figure 4.5 reveals that with respect to the average linear velocity, the optimum linear velocity for a packed GC column is lower than that for an open tubular or capillary GC column. Equation (4.26) is used to calculate $u$ for the five most commonly used GC column diameters, and these results are shown in Table 4.3. The columns are listed from the smallest commercially available diameter to the largest, along with a representative volumetric flow rate passing through the column. It then becomes evident that open tubular columns exhibit linear flow rates that are three to five times higher than packed columns, despite the fact that the user would have to replace compressed gas tanks less frequently. Linear flow rates can also be determined independently of Equation (4.26) by measuring the retention time of an unretained component of the injected sample. In the case of GC, injection of methane or, as this author has done, injection of the butane vapor from a common cigarette lighter using a 10-µL liquid-handling glass syringe gives $t_0$. Knowing the length of the column, $L$, enables the linear velocity to be calculated according to

$$u = \frac{L}{t_0}$$

| TABLE 4.3 |
| (Characteristics of Capillary GC Columns Having Different Internal Diameters) |
| GC Column | $r_C$ (mm) | $F$ (mL/min) | $u$ (cm/sec) |
| Capillary, 0.25 mm i.d. | 0.125 | 0.75 | 35.6 |
| Capillary, 0.32 mm i.d. | 0.16 | 1.5 | 30.7 |
| Capillary, 0.53 mm i.d. | 0.265 | 5.0 | 38.0 |
| Packed, 1/8 in. o.d. | 3.175 | 25 | 1.3 |
| Packed, 1/4 in. o.d. | 6.35 | 75 | 1.0 |
In reversed-phase HPLC with ultraviolet absorption detection (RP-HPLC-UV), this author has used as a source of an unretained component the strongly absorbing and water-miscible solvent acetone. This measured retention time for acetone, commonly termed the void or dead time, can be used to calculate the linear velocity. Our understanding of what causes chromatographic peaks to broaden as they elute has led to the achievement of columns that maximize chromatographic resolution.

16. WHAT IS CHROMATOGRAPHIC RESOLUTION?

With reference to either a GC or HPLC chromatogram, the resolution, $R_s$, is defined as the ratio of the distance between retention times of two separated peaks to the average base peak width, $t_w$, of both peaks, 1 and 2. Expressed mathematically,

$$R_s = \frac{t_R^2 - t_R^1}{\frac{1}{2}(t_w^1 + t_w^2)}$$

(4.27)

Four chromatograms are presented in Figure 4.6. It becomes visually obvious that resolution increases as one goes from top to bottom in the figure. In a manner similar to that used to derive Equation (4.21), we recognize that the peak width at

\[ \text{FIGURE 4.6 Chromatographic resolution and multiples of the peak standard deviation. Not drawn to scale.} \]
the base is equal to four times the standard deviation of the Gaussian peak profile (i.e., $t_w = 4\sigma_t$). We also assume that the variance of both peaks is equal, so that $\sigma_t^2 = \sigma_s^2 = \sigma_z$. Upon substituting this into Equation (4.27), we get

$$R_s = \frac{2(t_2^r - t_1^r)}{8\sigma_z} = \frac{t_2^r - t_1^r}{4\sigma_z}$$ (4.28)

Equation 4.28 shows that the larger the disengagement of a pair of chromatographically separated peaks, the greater the resolution. Equation (4.28) also shows that the smaller the variance of a peak, the greater the resolution. This equation can be viewed as a ratio of a change in retention time to the standard deviation, and different scenarios can be introduced. If we let $\Delta_t$ represent a change in the retention time for a given pair of peaks that have been partly or entirely resolved chromatographically, we can rewrite Equation (4.28) to give

$$R_s = \frac{\Delta t_R}{4\sigma_z}$$ (4.29)

Equation (4.29) suggests that when $R_s = 0.5$, $\Delta t_R = 2\sigma_z$, the distance in time units between both peak apexes equals two standard deviations of the Gaussian peak profile: when $R_s = 0.75$, $\Delta t_R = 3\sigma_z$; when $R_s = 1.0$, $\Delta t_R = 4\sigma_z$; and when $R_s = 1.5$, $\Delta t_R = 6\sigma_z$. Again, refer to Figure 4.6, where each of these four different values for chromatographic resolution is shown. Hence, if we were to calculate a resolution whereby $R_s = 1.0$, Figure 4.6 suggests that baseline resolution has not been attained. Computerized software has enabled a partially resolved chromatographic peak to be accurately quantitated and obviates the need for baseline resolution in many cases. Equations (4.28) and (4.29) are important definitions of $R_s$ for a given situation. These relationships do not, however, show how $R_s$ relates to the fundamental parameters of chromatographic separation. To establish this, we need to derive the fundamental resolution equation in column chromatography.

17. HOW DO YOU DERIVE THIS FUNDAMENTAL EQUATION?

Few elementary treatments of the topic of chromatography take the reader from all that we have developed so far to a consideration of exactly how resolution depends on chromatographic efficiency, selectivity, and capacity factor. We begin to do this here by following a derivation originally presented to the analytical literature by Karger et al.\textsuperscript{25} in their classic text on separation science. Let us start with Equation (4.29), but before we do this, we need to find a way to incorporate $N$ into this equation. Let us consider a separation of peaks 1 and 2 whose peak apexes are separated by $\Delta t_R$. Referring back to Equation (4.20), we can rewrite this equation with reference to peak 2 for a retention time for peak 2 at $t_2$ and standard deviation $\sigma_z$, and solve for $\sigma_z$ to give
\[ \sigma_2 = \frac{t_2}{\sqrt{N}} \]

Upon substituting \( \sigma_2 \) back into Equation (4.29), we obtain

\[ R_S = \frac{\Delta t_R}{4(t_2 / \sqrt{N})} = \frac{\sqrt{N}}{4} \left( 1 - \frac{t_1}{t_2} \right) \]

At this point, we have the resolution in terms of the number of theoretical plates and retention times. We can proceed even further. By combining Equations (4.13) and (4.15) by eliminating \( \phi_m \), we obtain a relationship for retention times in terms of the capacity factor, \( k' \), according to

\[ \frac{t_R}{t_0} = 1 + k' \]

For peak 1, we have \( t_1/h_0 = 1 + k'_1 \), and likewise for peak 2, \( t_2/h_0 = 1 + k'_2 \), so that

\[ \frac{t_1}{t_2} = \frac{1 + k'_1}{1 + k'_2} \]

We can now express resolution in terms of \( N \) and a ratio of capacity factors according to

\[ R_S = \frac{\sqrt{N}}{4} \left( 1 - \frac{1 + k'_1}{1 + k'_2} \right) \]

\[ = \frac{\sqrt{N}}{4} \left( \frac{k'_2 - k'_1}{1 + k'_2} \right) \]

Because \( \alpha = k'_2/k'_1 \), we can solve for \( k'_1 = k'_2/\alpha \) and substitute for \( k'_1 \) above to get

\[ R_S = \frac{\sqrt{N}}{4} \left( \frac{k'_2 - k'_2/\alpha}{1 + k'_2} \right) \]

Upon simplifying, we obtain

\[ R_S = \frac{\sqrt{N}}{4} \left( \frac{k'_2(1 - 1/\alpha)}{1 + k'_2} \right) \]
Upon rearranging terms, we arrive at the fundamental resolution equation:

\[ R_S = \frac{\sqrt{N}}{4} \left( \frac{k'_2}{1 + k'_2} \right) \left( \frac{\alpha - 1}{\alpha} \right) \]  

(4.30)

Equation (4.30) suggests that the degree of chromatographic resolution depends chiefly on three factors: \( N \), \( k' \), and \( \alpha \). \( N \), the number of theoretical plates in a column, relates how efficient a chromatographic column is. \( N \) is independent of the chemical nature of the analyte of interest. \( k'_2 \), the capacity factor of the more retained component in a given pair, depends on the chemical nature of the analyte. \( k' \), in general, is related to the product of the analyte’s partition constant \( K \) and on the phase ratio \( \beta \). The phase ratio in column chromatography is defined as the ratio of the stationary-phase volume, \( V_s \), to the mobile-phase volume, \( V_m \). The stationary-phase volume takes on different values depending on how the stationary phase is defined with respect to the column support. Jennings\(^{26}\) has stated that with respect to GC, \( \beta \) is between 5 and 35 for packed GC columns and between 50 and 1000 for open tubular GC. In any event, the capacity factor for a given analyte can be calculated by knowing the partition constant and phase ratio according to

\[ k' = K \beta = K \frac{V_s}{V_m} \]  

(4.31)

\( \alpha \), the chromatographic separation factor between two adjacent peaks and often called, for simplicity, the column selectivity, relates to the ratio of \( k' \) values for both peaks of interest as introduced earlier. \( N \) can be changed by increasing or decreasing the column length and adjusting the flow rate to minimize \( H \) while maximizing \( N \) for a column of fixed length. Changes in \( \alpha \) and \( k' \) are achieved by selecting different mobile- and stationary-phase chemical compositions or by varying the column temperature or, in some cases, the column pressure. \( k' \) can also be changed for a given component by changing the relative amounts of stationary vs. mobile phase according to Equation (4.31). Equation (4.30) shows that these three factors enter into the resolution equation in a complex manner. We now examine the mathematical nature of each term in Equation (4.30).

18. WHAT IS EQUATION (4.30) REALLY TELLING US?

The first implication of Equation (4.30) is to realize that \( R_S \) approaches zero (i.e., no resolution between chromatographically resolved pairs of peaks) when \( N \) or \( k'_2 \) approaches zero or when \( \alpha \) approaches 1. Note the nature of the third term in Equation (4.30). As the magnitude of \( \alpha \) increases, the contribution of the third term serves to increase \( R_S \). The effect of the \( \alpha \) term is shown in Table 4.4. The most significant gains in chromatographic resolution occur between values of \( \alpha \) that are greater than 1, yet diminishing returns set in if \( \alpha \) is raised above 5. A similar argument can be made for the effect of the second term in Equation (4.30) (i.e., the \( k' \) term).
The effect of the $k'$ term is shown in Table 4.5. It appears that very small values of $k'$ contribute little to increased $R_S$, whereas, again, diminishing returns are evident as $k'$ is increased. The greatest gains are found for a range of $k'$ values between 2 and 5. Equation (4.30) also suggests that resolution varies with the square root of the number of theoretical plates. This is an important feature of Equation (4.30) to remember. A mere doubling of the length of the column will only increase $R_S$ by 1.4.

To summarize the discussion that led to Equation (4.30), Karger et al. have stated it best:

Although each of these three parameters — $N$, $\alpha$, and $k'$ — is important in controlling resolution, we usually do not attempt their simultaneous optimization in an actual separation. Experimental conditions are selected initially that favor large $N$ values, within the practical limits of convenience plus reasonable separation times. Higher $N$ values always provide improved resolution, other factors being equal, and this is true.
of analytical or preparative separations, and simple or complex mixtures. With a reasonable starting value of \( N \), adequate resolution will be attained in most cases if we optimize \( k' \) approximately. In gas chromatography an optimum value of \( k' \) can be achieved by varying the temperature. In liquid chromatography it is more profitable to vary systematically the composition of the mobile phase.

One other relationship is worth mentioning before we discuss the determinative techniques GC and HPLC. Equation (4.30) can be solved algebraically for the number of theoretical plates, and this gives

\[
N_{\text{required}} = 16R_x^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{k' + 1}{k'} \right)^2
\]  

(4.32)

Equation (4.32) is of practical importance in that for a given resolution, the required number of theoretical plates can be found. To illustrate, let us calculate \( N_{\text{required}} \) for the chromatographic separation of two solutes with a given resolution \( R_x = 1.5 \) for two different values for the selectivity, \( \alpha = 1.05 \) and \( \alpha = 1.10 \).27 The results of the application of Equation (4.32) are shown in Table 4.6. For two solutes that have a value for \( k' = 0.01 \), the magnitude of the \( k' \) term in Equation (4.32) is such as to require over a 100 million plates to realize a value for \( \alpha = 1.05 \). For two solutes that have a value for \( k' = 1.0 \), the magnitude of the \( k' \) term in Equation (4.32) is such as to require over 50,000 plates to realize a value for \( \alpha = 1.05 \). For two solutes that have a value for \( k' = 10 \), the magnitude of the \( k' \) term in Equation (4.32) is such as to require over 10,000 plates to realize a value for \( \alpha = 1.05 \).

The fundamental theory of chromatographic separation has been presented in the broadest of terms. We next proceed to discuss the most common determinative technique for measuring trace concentration levels of organics in the environment, gas–liquid or gas–solid chromatography.

<table>
<thead>
<tr>
<th>( k' )</th>
<th>( [(k' + 1)/k']^2 )</th>
<th>( N_{\text{required}} (\alpha = 1.05) )</th>
<th>( N_{\text{required}} (\alpha = 1.10) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>10.201</td>
<td>162,000,000</td>
<td>44,000,000</td>
</tr>
<tr>
<td>0.05</td>
<td>441</td>
<td>7,000,000</td>
<td>1,900,000</td>
</tr>
<tr>
<td>0.10</td>
<td>121</td>
<td>1,900,000</td>
<td>527,000</td>
</tr>
<tr>
<td>0.15</td>
<td>58.8</td>
<td>930,000</td>
<td>256,000</td>
</tr>
<tr>
<td>0.5</td>
<td>9</td>
<td>143,000</td>
<td>39,000</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>63,500</td>
<td>17,500</td>
</tr>
<tr>
<td>3.0</td>
<td>1.8</td>
<td>28,600</td>
<td>7800</td>
</tr>
<tr>
<td>5.0</td>
<td>1.4</td>
<td>22,200</td>
<td>6100</td>
</tr>
<tr>
<td>10</td>
<td>1.1</td>
<td>17,500</td>
<td>4800</td>
</tr>
<tr>
<td>50</td>
<td>1.05</td>
<td>16,700</td>
<td>4600</td>
</tr>
</tbody>
</table>
19. HOW DOES A GC WORK?

A gas chromatograph provides the proper conditions for the separation and detection of trace organic compounds that have been isolated from the environment. A GC works by properly installing the instrument in a laboratory and understanding how to optimize the separation and detection of analytes of interest. After being installed, a GC column that is appropriate to the intended application is installed into the oven of the GC. Figure 4.7 is a generalized schematic diagram of a conventional GC employing a packed column. This schematic might be one that appeared over 30 years ago during the era of packed-column GC. Nevertheless, this schematic serves as a good starting point. We will answer the question posed above by proceeding from left to right across the diagram in Figure 4.7 and point out how each of these instrument components has evolved since the first commercial GC appeared in 1954. Details of how one operates a GC can be found in the appropriate experiment in Chapter 5. Before we proceed to a description of a gas chromatograph, let us view the outcome of injecting a sample into such an instrument.

Figure 4.8 is an actual GC chromatogram and shows the importance of a properly installed and optimized GC as a determinative technique to organics TEQA. A groundwater sample that has been in contact with gasoline, perhaps from a leaking underground storage tank, was placed in a 22-mL headspace vial and sealed. The temperature of the vial was brought to 65°C and kept at that temperature for approximately ½ h. The headspace was sampled using a gas-tight syringe and injected into an Autosystem® GC, manufactured by PerkinElmer Corporation. The
FIGURE 4.8 Separation and detection of BTEX components using static headspace capillary GC with FID.
abbreviation HS-C-GC-FID refers to headspace capillary column–gas chromatograph–flame ionization detection. BTEX refers to benzene, toluene, ethyl benzene, and meta-, para-, and ortho-xylene. These molecular structures are given as follows:

Benzene  Toluene  Ethylbenzene

Meta-xylene  Para-xylene  Ortho-xylene

Note that the order of elution in the GC chromatogram of Figure 4.8 is from lower to higher molecular weight. In other words, the capacity factor, \( k' \), for benzene is much smaller than for ortho-xylene. From Equation (4.31), this suggests that ortho-xylene has a much larger partition constant, \( K \), into the column stationary phase in comparison to benzene. Values of \( k' \) can also be correlated with increases in boiling point for these substituted aromatics. This particular open tubular column lacks the necessary selectivity, \( \alpha \), to separate the meta- from the para-xylene isomer. Also note that resolution is more than adequate for all BTEX compounds except for ethyl benzene from \( m,p \)-xylene. This chromatogram was acquired by sending the analog signal from the FID to an analog-to-digital interface. The digitized data were processed by Turbochrom® software, developed by PE-Nelson. We now return to our journey through Figure 4.7

20. WHAT ARE EXTERNAL GAS PNEUMATICS AND ARE THEY IMPORTANT IN GC?

Any chromatographic separation is such that matter is forced to become more ordered. In other words, its entropy is reduced and is against the spontaneous tendency of matter to spread out. This requires an input of energy, and this input in the case of GC is provided by the potential energy of a compressed gas. Today, the chromatographer has two options with respect to carrier gas: compressed gas cylinders or gas generators. Gas generators require a large investment; however, the return on that investment is the elimination of gas cylinder handling. The annual catalogs of chromatography suppliers, such as Supelco and Alltech, are good sources to learn about gas generators. Carrier gas under pressure enters the GC and provides the necessary mobile phase to enable either a gas–liquid or gas–solid chromatographic separation to occur. Let us assume that compressed gas cylinders are used as the source of carrier gas. A two-stage regulator is necessary to control the gas
pressure delivered to the instrument from the source. It is important to clearly distinguish between a single-stage and two-stage gas regulator. Figure 4.9 depicts schematics for both types. Notice that in the two-stage regulator (Figure 4.9B), the first stage is preset and the compressed gas pressure from the tank gets reduced to 300 to 500 psi (pounds per square inch), while the second stage reduces the pressure to the desired level, which for GC is generally <100 psi. Bartram suggests that “if you use a single-stage regulator at the cylinder for GC, you must constantly adjust the main line pressure as the cylinder pressure decreases.” In multiple-unit GC systems, a single-stage regulator should be incorporated into the branch line to each GC to step down the line pressure to that required by the instrument. For multiple-unit GCs, the use of two-stage and single-stage ensures effective operation by maintaining a minimum 10 to 15 psi pressure differential across all flow- and pressure-controlling devices. This configuration minimizes changes in the mainline pressure that might affect the operation of the individual GC. The sketch below illustrates what a configuration might look like if three GCs are plumbed into a mainline:

Bartram offers these important safety-related guidelines:

Never remove a two-stage regulator from a gas line with high pressure isolated in the first stage — the sudden release of pressure could rupture diaphragms, ruin diaphragms in downstream regulators, and create gaps in a packed column. Always depressurize a two-stage regulator through the second stage or through the GC. If your system has
FIGURE 4.9 Schematic that compares single-stage and two-stage compressed gas regulators. (Courtesy of Scott Specialty Gases.)
a single cylinder gas supply or a gas generator, turn off the GC oven first and let the column cool. Then close the first stage (cylinder side) valve on the regulator and leave the shutoff valve downstream from the regulator open. Leaving this value open will allow the gas remaining in the regulator to pass through the regulator. Vent the pressure through the system (be sure the column is at room temperature) through a vent installed in the gas line or a vent on the regulator itself. Finally, close the downstream pressure-control valve and remove the regulator.

The safe handling of compressed gases is very important.

Figure 4.10 depicts a typical compressed gas cylinder with a two-stage gas pressure regulator. For GC, the most commonly used carrier gases are helium, nitrogen, and hydrogen. Hydrogen is much more commonly found as a GC carrier gas in Europe than in the U.S. due to the significantly higher cost of helium. Hydrogen is also potentially flammable, unlike the other two. Most compressed gas cylinders are factory filled and, upon turning on the cylinder valve, should give an inlet pressure gauge reading of between 2200 and 2500 psi. The outlet pressure gauge is adjusted from 0 to that required, depending on whether the outlet pressure gauge reads 0 to 120 or 0 to 600 psi, by turning the adjusting screw. A constant and reproducible flow rate is essential to be able to reproduce chromatographic retention times, \( t_R \). McNair and Miller state “a compound’s retention time is a useful qualitative tool … two or more compounds may have the same \( t_R \)” External gas pneumatics in GC consist of the following:

- Source of carrier gas and regulation of delivery pressure
- All tubing, fittings, and valves
- All gas-purifying traps

**FIGURE 4.10** Representation of a typical two-stage compressed gas regulator.
All external gas pneumatics must be provided by the laboratory prior to installation of the gas chromatograph. Laboratories vary from simple external gas pneumatics, in which a single set of compressed gas cylinders is interfaced to a single GC, to a more complex configuration, in which a single set of cylinders is interfaced to two or more GCs. External gas pneumatics have been, in general, essentially the same over the past four decades, since compressed gas cylinders were employed. The next question to ask is how pure shall the carrier gas be that is to be delivered to the GC? The early GCs all employed a ¼-in. packed GC column and operated isothermally (i.e., the column temperature remained constant throughout the chromatographic run time). GC detectors such as the thermal conductivity detector (TCD) in those days were much less sensitive than those in use today. Gas purity was not as critical as it is today. Two options are available with respect to providing very high purity to the GC:

1. Purchase the higher-purity gas, 99.9999% research or ultrapure grade, at a significantly high cost.
2. Purchase a high-purity grade, 99.995% only, and purchase and install relatively inexpensive traps between the tank and the GC.

Most labs choose option 2 and install a series of traps that are located external to the GC, unless there is some compelling reason to go with an ultra-high- or research-grade-purity carrier gas. A hydrocarbon trap is first in line. This trap removes traces of saturated and unsaturated hydrocarbons that might be present due to residual oil from regulator diaphragms. Next should come a molecular sieve trap to remove water vapor, followed by an oxygen trap. The oxygen trap is a proprietary material designed to remove traces of oxygen from the carrier gas. The presence of oxygen in the carrier gas is detrimental to GC liquid phases. Some detectors, such as the electron-capture detector (ECD), respond to oxygen in the carrier gas and thus limit instrument sensitivity, and as we know from discussions in Chapter 2, this limits IDLs and MDLs.

**21. WHAT ABOUT GC INLETS? WHAT DO I NEED TO KNOW?**

Flow controllers are the next item in our journey through the schematic in Figure 4.7. In the early days, before GCs controlled carrier gas flow, rotameters were installed between the regulator and the inlet to the instrument. Today, GCs themselves have pressure gauges and very fine needle valves with which to control carrier, makeup, and detector gas flow rates. Contemporary GCs provide computerized control of both the column head pressure (i.e., the gas pressure at the column inlet) and column volumetric flow rate. Contemporary GCs will have internal pneumatics and additional traps. It is not readily apparent that the flow rate decreases as the column temperature is increased. The explanation for this is that the viscosity of the carrier gas increases as the column temperature is increased. A differential flow controller is used to provide a constant mass flow rate by increasing the column head pressure. Electronic pressure control is a relatively recent advance, particularly for split/splitless GC injectors that require a constant pressure. An electronic sensor is used to detect the decrease in flow rate and cause an increase in the pressure to the column,
and hence to maintain a constant flow rate. The chromatographer needs to know the volumetric flow rate of an operating GC. When problems arise, the first question to ask is, “What is the GC flow rate and is it optimized?” A word about pressure settings is in order. The setting at the outlet gauge (Figure 4.10) should be at least 10 to 20 psi higher than the setting at the inlet to the GC. This is particularly important if the sequence of gas purification traps is used, as discussed earlier. The GC was once a single thermostatic unit. Contemporary GCs isolate the injector from the oven and from the detector. The temperature of each is set independent of the other. Injection ports that are usually operated isothermally can be temperature-programmed in the same manner as GC ovens. A GC injector provides the means to introduce the liquid extract (semivolatile or nonvolatile organics) or a volume of gas or headspace (gases or volatile organics) to the chromatographic column. We discussed in detail the inlet interface to the GC for volatile organic compounds (VOCs) analysis in Chapter 3. Injectors have evolved from just a heated cylindrical device that was inserted into a heater block to the split/splitless injection ports used in more contemporary GCs. The objectives of injection in GC are as follows:

1. To rapidly vaporize the liquid, such as an extract, that contains the dissolved analyte of interest to TEQA
2. To introduce all or a portion of the vapor into a GC column
3. To split a portion of the vapor out of the injector to the atmosphere for narrow- and wide-bore capillary columns
4. To continuously clean the septum via a purge flow with carrier gas

Packed-column injection ports that readily accepted a ¼-in.-outer diameter (o.d.) stainless-steel or glass column during the early period are now designed to accommodate ⅛-in.-o.d. injection stainless-steel columns. This injection port can readily accommodate a 0.53-mm megabore (a term introduced by J&W Scientific) capillary provided that an adapter is used. Megabore capillary columns do not require any splitting. This enables packed-column injection ports to easily accommodate megabore capillary columns. Contemporary GCs are dual-injector and dual-detector instruments. One injector can accommodate a ⅛-in. packed column that can be adapted to a megabore capillary column, while the other injector can accommodate a split/splitless narrow- or wide-bore capillary column. The split/splitless injector requires that only a portion of the total sample volume be allowed to enter the narrow- or wide-bore capillary column. The fraction of sample that escapes to the atmosphere vs. the fraction that actually enters the capillary column is determined by the split ratio. Details are given for the Autosystem (PerkinElmer) injectors in Chapter 5.

22. WHAT IS A SPLIT RATIO AND WHAT DOES SPLITLESS REALLY MEAN?

The split ratio is defined as

\[
\text{Split ratio} = \frac{\text{Flow rate (atmosphere)}}{\text{Flow rate (column)}}
\]

© 2006 by Taylor & Francis Group, LLC
A sketch of a split/splitless injector is shown below. By measuring the carrier gas flow rate coming out of the vent and the carrier gas flow rate passing through the capillary column (be sure to turn off the makeup gas to the detector so that an accurate measurement of the column flow rate can be made), the split ratio can be calculated. As with optimum flow rate, the chromatographer should know at all times what the split ratio is and whether the injection was made via split or splitless. As the sketch below reveals, a splitless injection is accomplished by keeping the vent closed for \( t \) seconds after injecting an extract. McNair and Miller\textsuperscript{30} have recommended that splitless injection be preferred over split injection for performing TEQA. These authors clearly discuss the limitations of the splitless technique. Perry\textsuperscript{31} states that injection without splitting produces narrower bands than injection with splitting due to the solvent effect. He explains this phenomenon by quoting the Grobs as follows:\textsuperscript{31}

The vapourized material is transferred on to the column essentially as a mixture. In the first stage of separation, the solvent shifts away from the sample components, leaving them on the back slope of its large peak. Thus, the moving vapour plugs of the sample components meet a liquid phase mixed with retained solvent, whereby the concentration of solvent increases rapidly in the direction of migration. Therefore, the
front of every plug, in contact with stationary liquid containing more solvent, undergoes much stronger retention than the back of the plug. This effect causes the originally very broad bands of sample components to be condensed to a band width which, under properly selected conditions, may become even smaller than that which can be obtained with stream splitting.

Jennings\(^ {32} \) has argued that splitless injection is not really without split and is a term only to be used when comparing the technique to that of split injection. More contemporary GCs enable the user to program a splitless injector (i.e., control of the time that a split vent remains closed). One other concept needs to be addressed before we move to the GC column in our journey through a gas chromatograph: sample size injected. If we are not careful, we might overload the GC column.

**23. WHAT DOES IT MEAN TO OVERLOAD A CHROMATOGRAPHIC COLUMN?**

One of the experiments in Chapter 5 asks the student to devise a way to increase the amount of analyte injected and to observe what happens to chromatographic peak shape. Increasing the amount of analyte to be injected in either GC or HPLC can be accomplished in one of two ways:

1. Inject identical volumes of a series of reference standards of increasing concentration
2. Inject increasing volumes of one reference standard

The result of adding too great an amount of analyte is for the observed peak shape to change from a purely Gaussian peak shape to a seriously tailed peak. Figure 4.11 illustrates this loss of peak symmetry. The amount of analyte that can be injected regardless of peak shape is limited by the linear dynamic range, \( R_L \), of a given detector. Increasing the split ratio also helps to maintain good peak shape. Peak distortion is a column problem, whereas \( R_L \) is GC detector dependent. Perry\(^ {13} \) has developed a relationship between the concentration, \( C_{\text{max}} \), corrected retention volume, \( V'_R \), and weight of analyte injected, \( w_r \), according to

\[
C_{\text{max}} = \frac{\sqrt{N}}{V'_R} w_r \sqrt{2\pi}
\]

Using this equation, Perry has shown that if \( w_D \) is the maximum weight of a given analyte with an adjusted retention time \( t'_R \) that is injected into a GC whose column contains \( N \) theoretical plates having a detector sensitivity \( S \) and a linear dynamic range \( R_L \), then

\[
w_D = 0.4 \frac{SR_t t'_R}{\sqrt{N}}
\]
For example, assume that we are using a GC with a FID. Let us assume that the FID used has a detector sensitivity $S = 1 \times 10^{-12}$ g/sec and a linear dynamic range $R_L = 1 \times 10^7$ and a column with 10,000 plates. For an analyte of interest whose adjusted retention time is 500 sec, we find

$$w_d = 0.4 \frac{(1 \times 10^{-12})(10^7 \text{ g/s})(5 \times 10^5 S)}{\sqrt{1 \times 10^4}} = 2 \times 10^{-5} \text{ g}$$

This equation applies only to isothermal operation. In TEQA, our interests tend to be directed toward the lower end of $R_L$, although it is good to be aware of such limitations on the high end. The fact that increasing the amount of analyte injected leads to an unsymmetrical or non-Gaussian peak shape is more formally described as moving from *linear elution to nonlinear elution* column chromatography. Unsymmetrical peaks are further categorized as tailing or fronting. These correspond to
convex or concave distribution isotherms whereby the distribution constant $K$ becomes dependent upon analyte concentration. The sketches below serve to illustrate how these sorption isotherms relate to observed chromatographic peak shapes:34

A convex isotherm exhibits peak tailing, while a concave isotherm exhibits peak fronting, as shown above. Equation 4.16 and Equation 4.31 can be combined to yield an expression that relates a solute’s (or analyte’s) retention volume, $V_R$, to its partition constant, $K$, between the stationary ($S$) and mobile ($M$) phases. For the partition constant defined as

$$K \equiv C_s/C_M$$

the solute’s retention volume is

$$V_R = V_0 + KV_s$$

Since $K$ will decrease with increasing solute concentration for a convex isotherm, $V_R$ also decreases. Also, $V_R$ increases with solute concentration for the concave isotherm. At the very low solute concentration, both nonlinear isotherms tend toward the linear isotherm. Consider the convex isotherm; the band center will tend to migrate more rapidly than the band extremities due to the smaller value for $K$ at large solute concentrations. Hence, the injected plug of solute starts out at the column inlet as Gaussian and gradually becomes unsymmetrical as it migrates through the column. This results in the development of a sharp front and an extended tail. Tailed peaks become even more skewed as the amount of solute injected increases, as is
shown in Figure 4.11. Unsymmetrical peaks make it nearly impossible to accurately quantitate, while some isotherm nonlinearity can be tolerated in semipreparative scale separations.

We now continue in our journey through a GC by moving on to what many chromatographers call the heart of the chromatograph: the GC column.

### 24. WHAT IS SO IMPORTANT ABOUT GC COLUMNS?

In a human, the heart is the most important organ in the body. The reasons for this rest in the field of human physiology. So too, in a GC, the chromatographic column is where separations take place, and the purpose of doing GC is to separate components in a mixture. Historically, packed GC columns dominated gas chromatographic separations up until around 1980. At that time, any work with capillary columns was accomplished using glass. This required that the ends of the coiled and brittle glass columns be heated until softened to enable an installation into the GC to occur. It took a significant level of patience and skill to install a glass capillary GC column at that time. Open tubular columns owe their origins to Marcel Golay and their present robustness from the development of fused-silica capillaries at Hewlett-Packard.

### 25. WHAT GC COLUMNS ARE USED IN TEQA?

The significantly large values of $N$ for open tubular columns when compared to packed columns have established capillary columns as essential for TEQA. For example, a 60-m capillary with 3000 to 5000 plates/m yields 180,000 to 300,000 plates, whereas a 2-m packed column with 2000 plates/m yields only 4000 plates. This difference in column efficiency was largely ignored by industry and regulatory government agencies during the 1959–1980 era, until the patent for open tubular columns expired. It was not until the introduction of the 0.53-mm megabore column by J&W Scientific that the EPA “allowed” the abandonment of the 6 ft × $\frac{1}{4}$-in.-o.d. packed glass column for the determination of organochlorine pesticides (OCs) via EPA Method 608. This author even heard it said in those days that “you could not connect a cap column to an ECD.” The EPA was slow to abandon the packed-column mixed-liquid phase consisting of 1.5% OV-17 and 1.95% OV 210 coated on Chromosorb. This stationary phase served the regulatory agency very well when all that was needed was to measure trace residues for some dozen OCs. Three GC chromatograms from the earlier use of a packed column to separate and detect OCs are shown in Figure 4.12. During the packed-column days, where $N$ was limited, column selectivity, $\alpha$, took on much more importance and numerous liquid phases became available. The open tubular column with its very large value of $N$ does not require as large a value for $\alpha$. For this reason, the number of capillary columns with different stationary phases is much more limited. In fact, it can be argued that, today, only two different cap columns are needed to handle almost all of the volatile and semivolatile priority pollutant organic analytes. McNair and Miller present the basics of the stationary phase, packed columns, and capillary columns, and this source or an equivalent should be consulted for these topics. Narrow-, wide-, and
megabore cap columns are commonplace in environmental testing labs today. A wall-coated open tubular (WCOT) column, such as a DB-624 (J&W Scientific), or equivalent is usually a good first choice for trace volatile organics analysis, whereas a WCOT such as DB-5 (J&W Scientific) is a good first choice for trace semivolatile

FIGURE 4.12 Comparison of chromatograms for the separation and detection of priority pollutant organochlorine pesticides for three different packed GC columns.
organics analysis. Perusal of GC consumable supply houses, such as Alltech and Supelco, are good starting points. Reviewing the appropriate EPA methods is also good practice in developing an understanding of applying GC to environment-related problems. Whether one is interested in broad-spectrum environmental monitoring or target-specific analysis is also an important consideration. To understand which columns to use for a given application, Farwell has considered four basic criteria:

1. Polarity of analytes to be gas chromatographed
2. Polarity of the cap column stationary phase
3. Selectivity of the cap column stationary phase
4. Stationary-phase stability

A knowledge of the basics of organic chemistry is very helpful in satisfying the first criterion. A scale of some kind to satisfy the second criterion would be most helpful. Satisfying the third criterion requires contributions from both the analyte and stationary phase, and criterion 4 refers to the physical attributes of the stationary phase. Figure 4.1 serves as a reminder of the limited scope of gas chromatography. Organic compounds of environmental interest must be vaporizable and, at the same time, be thermally stable at injector temperatures of 200 to 300°C. These two criteria are met only for those analytes that are neutral, relatively nonpolar, and consisting of aliphatic and aromatic carbon classes. As oxygen begins to get incorporated into the molecule, the ability of a molecule to meet these two criteria becomes more limited. Consider this series of aromatics, starting with benzene, then moving to toluene by substituting a methyl group for hydrogen. We then replace a hydrogen in the methyl group with a hydroxy group and get benzyl alcohol. We proceed to partially oxidize this benzyl group to an aldehydic group and obtain benzaldehyde. Finally, we fully oxidize the aldehydic functional group to a carboxylic group and obtain benzoic acid. The structures are as follows:

![FIGURE 4.12](continued)
The first four monoaromatics in this series are liquids at room temperature, and benzoic acid is a solid. It is possible to gas chromatograph benzoic acid; however, peak distortion and lowered sensitivity result. The polarity of these compounds takes us from the volatile, nonpolar benzene out to the semivolatile, polar benzoic acid. Benzoic acid can be moved back across the domain to the more volatile and nonpolar region by conversion of this carboxylic acid to its methyl ester by chemical derivatization techniques. A more exact definition of solute polarity was developed by Kovats, who stated that “polarity cannot be expressed with one simple parameter.”

26. WHAT IS THE KOVATS RETENTION INDEX?

The Kovats retention index for a specific organic compound (i.e., the analyte of interest on a specific GC column) is nothing more than a relative retention time. The index is in relation to aliphatic hydrocarbons. For example, the hydrocarbon iso-octane, or 2,2,4-trimethyl pentane if gas chromatographed, would probably have a retention time between that of the straight-chained alkanes \( n \)-octane \( C_8 \) and \( n \)-nonane \( C_9 \). The Kovats index for \( n \)-octane is 800, and that for \( n \)-nonane is 900. Iso-octane would then have a Kovats index somewhere in between, such as 860. The Kovats index is calculated from a GC chromatogram using the retention volume for the analyte whose index is sought, \( V_R^{\text{unknown}} \), and the retention volumes for the alkane with \( n \) carbon atoms per molecule, \( V_R^n \), and for the alkane with \( n + 1 \) carbon atoms per molecule, \( V_R^{n+1} \), using the following relationship:

\[
I = \left[ \frac{\log V_R^{\text{unknown}} - \log V_R^n}{\log V_R^{n+1} - \log V_R^n} \right] + 100n \tag{4.33}
\]

Note that the Kovats retention index is independent of the chemical nature of the column. If we were to chromatograph the five monoaromatics cited above on a purely nonpolar stationary phase such as Squalane (a \( C_{30} \) aliphatic hydrocarbon), we would generate a series of \( I \) values for each compound. If we then chromatographed these same compounds on a more polar stationary phase such as diethylene glycolsuccinate (DEGS), we would obtain an entirely different set of \( I \) values. We calculate the difference in Kovats indices as follows:
Thus, stationary phases can have their polarities compared for a given test probe, such as benzene or benzyl alcohol. In an attempt to reduce the number of liquid phases used in packed-column GC, McReynolds\textsuperscript{41} published an extensive listing of Kovats retention indices using 10 probes for hundreds of liquid phases at that time. Rohrschneider\textsuperscript{42} considered only five probes: benzene, ethanol, 2-butane or MEK, nitromethane, and pyridine. McReynolds\textsuperscript{43} expanded this to 10 probes and proved his assumption that liquid phases had, indeed, proliferated because many liquid phases possessed “McReynolds constants” that differed little. The reasons for choosing which organic compounds are suitable probes are outlined in Table 4.7.\textsuperscript{44} Usually only the first five probes are cited. Table 4.8 lists most of the commonly used liquid phases along with recommended operating temperatures and corresponding values for McReynolds constants. Characterizations of GC liquid phases is an ongoing research area. Li and coworkers\textsuperscript{45} have developed a new set of GC-based solute parameters using solvatochromic linear solvation energy relationships. McNair and Miller\textsuperscript{46} considered a thermodynamic view of the interaction between a solute and a stationary phase and devised a definition of the separation factor or selectivity $\alpha$ between solutes A and B according to

$$
\alpha = \frac{K_B}{K_A} = \frac{p_A \gamma_A}{p_B \gamma_B}
$$

where $p^\circ$ is the vapor pressure of the solute as if it were a pure liquid and $\gamma$ is the activity coefficient for a specific solute. This equation shows that the extent of separation between solutes A and B depends not only on the ratio of the vapor pressure of the pure solutes, but also on the ratio of their activity coefficients. This explains why two analytes with very similar boiling points (i.e., similar pure solute vapor pressures, $p^\circ$) have significantly different retention times.

27. WHY DO I NOT SEE MCREYNOLDS/ROHRSCHNEIDER CONSTANTS IN CURRENT CHROMATOGRAPHY CATALOGS?

A perusal of a current catalog of one of the largest suppliers of chromatography and related products in the U.S. makes minimal mention of McReynolds constants and no mention of Rohrschneider constants. Perusal of the current catalog of a second large supplier of chromatography and related products does not even mention these constants. Perhaps this reflects the commercial maturity of GC, in that GC has been around for some 40 years and applications have become well known. The popularity...
of open tubular columns has reduced the need for numerous liquid phases; hence, there is little need to distinguish liquid phases anymore. The most commonly used liquid phases for TEQA are the family of silicone polymers (also called polysiloxanes). As introduced in Chapter 1, the EPA organics protocol is divided into volatile

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Test Compound</th>
<th>Basic Molecular Interactions</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>X'</td>
<td>Benzene</td>
<td>Dispersion with some weak proton-acceptor properties</td>
<td>Aromatics, olefins</td>
</tr>
<tr>
<td>Y'</td>
<td>Butanol</td>
<td>Orientation properties with both proton-donor and proton-acceptor capabilities</td>
<td>Alcohols, nitriles, acids</td>
</tr>
<tr>
<td>Z'</td>
<td>2-Pentanone</td>
<td>Orientation properties with proton-acceptor capabilities</td>
<td>Ketone, ethers, aldehydes, esters, epoxides, dimethylamino derivatives</td>
</tr>
<tr>
<td>U'</td>
<td>Nitropropane</td>
<td>Dipole orientation properties</td>
<td>Nitro and nitrile derivatives</td>
</tr>
<tr>
<td>S'</td>
<td>Pyridine</td>
<td>Weak dipole orientation with strong proton-acceptor capability</td>
<td>Aromatic bases</td>
</tr>
<tr>
<td>H'</td>
<td>2-Methyl-2-pentanol</td>
<td></td>
<td>Branched alcohols</td>
</tr>
<tr>
<td>L'</td>
<td>1,4-Dioxane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M'</td>
<td>cis-Hydrindane</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liquid Phase</th>
<th>Commercially Available Name</th>
<th>Range of Operating WCOT Temperatures</th>
<th>Range of McReynolds Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl polysiloxanes (gum, oil)</td>
<td>DB-1, BP-1, SP-1, OV-1,</td>
<td>−60 to 280 (oil)</td>
<td>220–229</td>
</tr>
<tr>
<td></td>
<td>OV-101, SE-30, SP-2100</td>
<td>−60 to 325 (gum)</td>
<td></td>
</tr>
<tr>
<td>5% phenyl methyl polysiloxane</td>
<td>DB-5, BP-5, SPB-5, SE-52,</td>
<td>−60 to 325</td>
<td>334–337</td>
</tr>
<tr>
<td></td>
<td>OV-73, RT-5, HP-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% phenyl methyl polysiloxane</td>
<td>DB-17, HP-17, OV-17, GC-17,</td>
<td>40 to 280</td>
<td>884</td>
</tr>
<tr>
<td></td>
<td>SP-2250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% trifluoropropyl methyl polysiloxane</td>
<td>DB-210, OV-210, QF-1, SP-2401</td>
<td>40 to 240</td>
<td>1520–1550</td>
</tr>
<tr>
<td>25% cyanopropyl methyl polysiloxane</td>
<td>DB-225, OV-225, SP-2300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(ethylene glycol) gum</td>
<td>DB-WAX, HP-20M, SP-1000,</td>
<td>50 to 220</td>
<td>2301–2309</td>
</tr>
<tr>
<td></td>
<td>Carbowax 20M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Determinative Techniques to Measure Organics and Inorganics

Organic compounds (VOCs) and semivolatile to nonvolatile organic compounds (SVOCs). Either a 30 or 60 m × 0.53 or 0.75 mm cap column with a stationary phase specifically designed for the separation of VOCs is commonly used. The chemical nature of these phases is considered proprietary bonded phases by most suppliers. For example, a 75 m × 0.53 mm i.d. with a 3-µm film thickness is sold by Supelco as the SPB-624, VOCOL cap column to separate some 93 VOCs without subambient temperatures in less than 20 min. The 624 designation refers to the EPA method number as applied to wastewater analysis. The 105 m × 0.53 mm i.d. with a 3-µm film thickness developed in the early 1980s by Restek (called the Rtx-502.2 column) is based on a diphenyl/dimethyl polysiloxane that provides low bleed and thermal stability up to 270°C. The 502.2 designation refers to the EPA method number as applied to drinking water analysis. SVOCs are commonly separated by using a WCOT column that contains a 5% phenyl methyl/dimethyl polysiloxane. The column length for adequately separating SVOCs is generally 30 m. Narrow-bore and wide-bore columns are used with a split/splitless injector, and a megabore cap column is installed on a 1/8-in. packed-column injector without split. SVOCs can be further subclassified into the 100 or so priority pollutant base, neutral, acids (BNAs), 30 or more organochlorine pesticides (OCs), polychlorinated biphenyls (PCB), and a miscellaneous category for many other analytes of environmental concern. VOCs can be subclassified as polychlorinated ethylenes and ethanes (CIVOCs), monoaromatics from fuel contamination of groundwater (BTEX), trihalomethanes from chlorine disinfection of drinking water (THMs), and a miscellaneous category for many other analytes of environmental concern. This classification scheme is shown in a flowchart in Figure 4.13. This author believes that a good silicone WCOT, if properly maintained and if appropriate sample prep techniques are always used, will last for years. Two other types of cap columns used in GC are support-coated open tubular (SCOT) and porous-layer open tubular (PLOT). SCOT columns are no longer widely used, whereas PLOT columns are essential for the separation of permanent gases (i.e., Ne, Ar, O₂, N₂, CO₂, Kr, Xe, and lower-molecular-weight aliphatic hydrocarbons). Let us consider these fused-silica WCOTs in more detail.

![Flowchart for choosing the appropriate WCOT column](image)

**FIGURE 4.13** Flowchart for choosing the appropriate WCOT column.
28. WHAT ARE FUSED-SILICA POLYSILOXANE WCOTs?

One of this author’s earliest laboratory experiences involved placing a piece of glass tubing into a burner flame and observing the softening of the center portion of the tubing. At some point in the heating, upon pulling on both ends of the unheated portions of the tubing, fine capillary tubing can be produced. This is still the principle of cap column fabrication. Figure 4.14 depicts a capillary drawing machine and some detail of the actual coating of the outside sheath.47 These schematics are based on the original design of Desty et al.48 A good discussion of the fabrication and technical challenges of polysiloxane WCOTs has been written by one of the founders of such cap columns.49 One of the more detailed reviews of liquid phases can be found in a series of three articles by Yancey.50 Polysiloxanes are chemically bonded to the fused silica using proprietary surface treatment techniques. Jennings51 has summarized these approaches as follows:

Peroxides may be added to the coating solution, and static coating procedures are generally employed to deposit a film of vinyl-containing stationary-phase oligomers on the interior wall of the tubing. Heating causes peroxide decomposition, which yields free radicals and initiates cross-linking (and, if the surface has been properly prepared, surface bonding) when the column is heated. The preferred peroxide is generally dicumyl peroxide, which decomposes to form volatile products that are dissipated during the heating step.

Let us focus on the WCOT columns used to implement EPA Method 507, “Determination of Nitrogen- and Phosphorus-Containing Pesticides in Water by GC with a Nitrogen-Phosphorous Detector.” The method is summarized as follows:52

A measured volume of drinking water of approximately 1 L is extracted with methylene chloride by shaking in a separatory funnel or mechanical tumbling in a bottle. The methylene chloride extract is isolated, dried and concentrated to a volume of 5 mL during a solvent exchange to methyl tert-butyl ether, MTBE. Chromatographic conditions are described that permit the separation and measurement of the analytes in the extract by capillary column GC with a nitrogen-phosphorous detector, NPD.

A primary WCOT is required to separate and detect N- and P-containing pesticides that may be present in drinking water. The separated N and P pesticides must be confirmed by injecting the MTBE extract into a confirmatory WCOT. The primary WCOT is DB-5 from J&W Scientific or an equivalent; the confirmatory WCOT is DB-1701 from J&W Scientific or an equivalent. The molecular structure for each of these polysiloxanes is shown in Figure 4.15. The liquid phases are similar in the $n$ repeating monomer, whereas they differ in the substitution in the $m$ repeating monomer unit. The DB-5 is a 5% phenyl/dimethyl polysiloxane, and the DB-1701 is a 14% cyanopropyl/phenyl/dimethyl polysiloxane. The introduction of a cyano group increases the polarity of this WCOT, and hence shifts the retention time for the N and P pesticides that are to be monitored. An MTBE extract from an unknown drinking water sample, if injected onto both columns, will give values for $t_R$ that differ by a fixed number of minutes. These retention times would have been previously
determined by injecting standards onto both columns. The only other alternative to dual WCOT identification and confirmation is to use single WCOT and both an element-specific detector, such as a nitrogen-phosphorus detector (NPD), and a mass spectrometric detector. Table 4.9 is a sample from the list of some 46 individual N and P pesticides and herbicides that are to be monitored in drinking water by this
Diphenyldimethylpolysiloxane (e.g. DB-5)

- non-polar
- excellent general purpose column
- wide range of applications
- low bleed
- high temperature limit
- bonded and cross-linked
- solvent rinsable
- wide range of column dimensions available
- equivalent to USP Phase G27

Cyanopropylphenylmethylpolysiloxane (e.g. DB 1301,1701)

- low to mid polarity
- bonded and cross-linked
- solvent rinsable
- equivalent to USP Phase G43

**FIGURE 4.15** Two different liquid phases used in capillary GC. (From J&W Scientific, Inc., 1998 catalog.)

**TABLE 4.9**

<table>
<thead>
<tr>
<th>N/P Pesticide</th>
<th>Classification</th>
<th>t(R) on DB-5 (min)</th>
<th>t(R) on DB-1701 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alachlor</td>
<td>Substituted acetanilide herbicide</td>
<td>35.96</td>
<td>34.10</td>
</tr>
<tr>
<td>Atrazine</td>
<td>Triazine herbicide</td>
<td>31.77</td>
<td>31.23</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>Organophosphorus pesticide</td>
<td>16.54</td>
<td>15.35</td>
</tr>
<tr>
<td>Hexazinone</td>
<td>Triazine herbicide</td>
<td>46.58</td>
<td>47.80</td>
</tr>
<tr>
<td>Simazine</td>
<td>Triazine herbicide</td>
<td>31.49</td>
<td>31.32</td>
</tr>
<tr>
<td>Triphenyl phosphate</td>
<td>Internal standard</td>
<td>47.00</td>
<td>45.40</td>
</tr>
</tbody>
</table>
method. Alachlor is a substituted acetanilide herbicide in widespread use today, and atrazine and simazine are examples of triazine herbicides also in widespread use. Dichlorovos and hexazinone are illustrative of organophosphorus pesticides. Triphenyl phosphate is used as an internal standard in the method. Note the 1- to 2-min or so shift in retention time for each N and P pesticide and herbicide listed in Table 4.9.

The differences in capacity factors among different polysiloxane WCOT columns are made quite evident in Figure 4.16. In this figure, two GC chromatograms are

**FIGURE 4.16** Comparison of the GC chromatograms upon injecting an Aroclor into a DB-5 vs. DB-1 WCOT.
stacked using Turbochrom (PE-Nelson) software and obtained on gas chromatographs in the author’s laboratory. A 100 ppm reference standard consisting of AR 1260 dissolved in acetone is taken and 1 µL of this solution is injected into a GC that contains DB-5 (J&W Scientific) to yield the top chromatogram, and the same solution is injected into a GC that contains DB-1 (J&W Scientific) to yield the bottom chromatogram. The liquid-phase DB-1 is a dimethyl polysiloxane.

If methyl groups are substituted for the phenyl groups in the DB-5 structure shown in Figure 4.15, a DB-1 structure is obtained. Commercially available equivalents to DB-1 include HP-1, HP-101, Ultra-1, SPB-1, Rtx-1, OV-1, SP-2100, and SE-30, among others. The influence of the phenyl groups in DB-5 creates additional aromatic group intermolecular interactions that influence the partitioning constant, and hence according to Equation (4.31), the capacity factor, \(k'\). Use of these two WCOT columns provides acceptable identification and confirmation for this complex mixture of polychlorinated biphenyl congeners. Two other significant concepts with respect to WCOTs and their use in TEQA are film thickness, \(d_f\), and the influence of column temperature on \(k'\).

### 29. WHY IS WCOT FILM THICKNESS IMPORTANT?

Because it influences the \(C\) term in the Golay equation. The Golay equation as applied to WCOT is similar to that developed earlier [e.g., Equation (4.25)]:

\[
H = \frac{B}{\bar{u}} + (C_M + C_S)\bar{u} \tag{4.34}
\]

Note the absence of the \(A\) term and the separate resistance-to-mass transfer in the mobile phase, \(C_M\), and in the stationary phase, \(C_S\). This equation can be written even more specifically:

\[
H = \frac{2D_M}{\bar{u}} + \left(\frac{1 + 6k' + 11k'^2}{24(1 + k')^2}\right)\left(\frac{r_C}{D_M}\right)\bar{u} + \left(\frac{2k'}{3(1 + k')^2}\right)\left(\frac{d_f^2}{D_S}\right)\bar{u} \tag{4.35}
\]

The Golay equation shows a dependence of \(H\) on the square of \(d_f\) in the \(C_s\) term. While we are discussing this equation, note the dependence of \(H\) on the square of the column radius, \(r_C\), in the \(C_M\) term. The choice of GC carrier gas through the WCOT influences the diffusion of analyte in the mobile phase, \(D_M\), and the diffusion of analyte in the stationary phase, \(D_S\). Figure 4.17 is a Golay plot of \(H\) vs. the average linear carrier gas velocity (in cm/sec) for nitrogen, helium, and hydrogen using a WCOT with a 25 m × 0.25 mm i.d. and \(d_f = 0.4 \mu m\) thickness. Nitrogen as the carrier gas yields the lowest optimized \(H\), whereas the lighter gases, He and H₂, do not have as severe a resistance to the mass transfer term and can be operated at a much higher linear velocity. Figure 4.17 should always be kept in mind when considering which gas to use in GC.
The \( B \) term, the contribution to \( H \) due to longitudinal diffusion, becomes evident as the two chromatograms are compared in Figure 4.18. The top GC chromatogram shows an optimized carrier gas flow rate and a separation of the two surrogate compounds required for the determination of various OCs and PCBs in such methods as EPA Method 8080. The molecular structures of both surrogates are as follows:

These highly chlorinated aromatics have \( k' \) values that serve to bracket the \( k' \) values for the GC elution of all OCs and PCBs. The bottom chromatogram in Figure 4.18 is at approximately half the volumetric flow rate of the top chromatogram. Both peaks are tailing, while their retention times have increased somewhat. A much stronger ECD signal is also evident. These surrogate compounds are so
FIGURE 4.18 Comparison of GC chromatograms under optimized carrier gas head pressure and reduced head pressure conditions.

Separation of tcmx and dcbp

190°C (hold for 2 min) then to 275 @ 20 deg/min, hold for 5 min

Optimized carrier gas head pressure

Carrier head pressure reduced to one-half of that above

© 2006 by Taylor & Francis Group, LLC
significantly different in their $k'$ values that it would require a much more elevated column temperature to elute decachlorobiphenyl (DCBP). A fully chlorinated biphenyl is as nonpolar and nonvolatile as one can get. In fact, as Figure 4.18 demonstrates, it requires keeping the column temperature at 275°C. This is the recommended maximum operating temperature of the WCOT to finally elute DCBP from the column. Column temperature can significantly influence the magnitude of an analyte capacity factor, $k'$. All broad-spectrum monitoring EPA methods using WCOTs use GC with a column temperature that varies with time after sample injection.

We know that the retention time of any organic compound is influenced by the column length, the linear velocity of the carrier gas, and the magnitude of the compound’s capacity factor, $k'$. Revisiting Equation (4.17), rewriting it, and solving for $t_R$, we get

$$t_R = t_0[(1 + k') = \frac{L}{u} (1 + k')$$

In Equation (4.10), a chromatographic capacity factor, $k'$, was defined in terms of the product of a partition constant and a phase ratio denoted by $\beta$. For a WCOT, $\beta$ is related to film thickness, $d_f$, and column diameter, $d_c$, according to53

$$\beta = \frac{d_c}{4d_f}$$

For example, a 250-µm-i.d. WCOT column with a film thickness of 0.2 µm has a $\beta = 26.7$. Recall that $k' = K/\beta$, and if $K$, the partition constant for a compound between the mobile and stationary phases, is held constant, an increase in $d_f$ reduces $\beta$ while increasing $k'$ and, of course, $t_R$. If $d_c$ is increased, for the same film thickness, $d_f$, then a higher $\beta$ results. This higher phase ratio for a fixed value of the partition constant, $K$, serves to decrease $k'$ and results in a shorter retention time. In general, higher $\beta$ values, such as $\beta > 100$, are more suitable for SVOCs, whereas lower $\beta$ values, such as $\beta < 100$, are better for VOCs.53

30. HOW DOES COLUMN TEMPERATURE INFLUENCE $k'$?

Once a WCOT column is chosen, only the flow rate and column temperature can be varied so as to maximize chromatographic resolution, $R_s$. We have discussed the influence of flow rate on $R_s$ earlier; what remains is to discuss the effect of column temperature, $T_c$, on the separation factor, $\alpha$, and on $R_s$. Figure 4.19 shows how both $k'$ and $R_s$ depend on $T_c$. Injection of a solution that contains these four aliphatic hydrocarbons at $T_c = 45°C$ yields the top chromatogram shown in Figure 4.19. The peaks are all adequately resolved; however, the run time is excessive, over 40 min. Increasing the column temperature to 75°C reduces chromatographic run time, but
also decreases chromatographic resolution. A further increase to \( T_c = 95^\circ C \) further decreases \( R_s \) to unacceptable levels. The optimum \( T_c \) that maximizes \( R_s \) for all pairs of closely spaced peaks would be somewhere between 45 and 75\(^\circ\)C. Note the change in elution order as peak 1 is retained longer than peak 2.

If the log \( K \) is plotted against the reciprocal of absolute temperature, \( 1/T \), for a given organic compound, a straight line is established. The slope of the line varies somewhat with the nature of the solute. At certain temperatures, the lines can cross, and this explains the observed reversal in elution order, as shown in Figure 4.19. Such a plot is shown in Figure 4.20. At \( T_c = T_{A\alpha} \), peak 1 elutes before peak 2, whereas at \( T_c = T_{B\beta} \), peak 2 elutes before peak 1. Differences in the slope are caused by the difference in analyte–stationary-phase interactions. From Equation (4.30), we know that \( R_s \) depends on the separation factor, \( \alpha \), so that for a pair of peaks, it is of interest to know just how \( \alpha \) varies with increasing the WCOT column temperature.

**FIGURE 4.19** Separation of four hydrocarbons at different temperatures. Support-coated open tubular column (45 m \( \times \) 0.5 mm i.d.) coated with squalane. Peak identification: (1) methyl cyclohexane, (2) 2,5-dimethyl hexane, (3) 2,4-dimethyl hexane, and (4) 2,2,3-trimethyl pentane.
The Clausius–Clapeyron equation in physical chemistry states

\[
\log p^\circ = -\frac{\Delta H_v}{2.3RT} + C
\]

where \( p^\circ \) is the vapor pressure exerted by a substance if it were pure at an absolute temperature \( T \). \( R \) is the ideal gas constant, \( C \) is a constant, and \( \Delta H_v \) is the change in the substance’s enthalpy in moving from a liquid to a vapor and is also called the heat of vaporization. If \( T_c \) is increased, the following happens:\(^{54}\)

1. \( k' \) and \( t_r \) both decrease.
2. \( \alpha \) can either rise; peak out, then fall; or fall, bottom out, then rise or steadily decrease.\(^ {55}\)
3. \( N \) slightly increases.

**FIGURE 4.20** Plot of the logarithm of an analyte’s chromatographic distribution constant between mobile and stationary phases and column temperature for two different chemical compounds.
In the case of our two surrogates discussed earlier (TCMX and DCBP), their respective \( p^o \) values (alternatively, their respective boiling points) differ by such a wide range that if we set the \( T_c \) to a fixed value (e.g., 225°C), we would be able to elute TCMX but not DCBP. If the GC is capable, increasing the \( T_c \) after injection of the sample ensures that both surrogates will be eluted within a reasonable chromatographic run time, as was done in Figure 4.19. One says then that the way to elute both surrogates is to temperature program. For this reason, programmed-temperature gas chromatography (PTGC) is one of the most powerful features of contemporary GC instrument design. According to Perry:\(^{56}\)

PTGC also brought about direct control of carrier gas flow rate. Before PTGC, carrier gas flow was adjusted by pressure control. However, because gas viscosity and therefore the column pressure drop, \( \Delta P \), increases with increasing \( T_c \), the carrier gas flow tends to decrease during PTGC. Such a decrease is quickly reflected in the deviating baseline signal from a single, flow-sensitive detector. To hold the carrier gas flow constant during PTGC, and thus also the retention times (from run to run) and particularly the baseline (during each run), flow controllers were introduced. They became standard, expected components in gas chromatographs … the prime characteristics of both GCs and stationary phases became those relevant to the demands of programmed temperature. The GC had to be able to bring about sharp but well-controlled changes in column temperature, and yet record a gas chromatogram; the stationary phases had to withstand these changes without decomposing or vaporizing to a prohibitive degree, and yet retain the selectivity relevant to each … to permit rapid changes in column temperature, high-velocity, high-power, low-mass column ovens were introduced. PTGC or no, the column oven must allow easy column installation and interchange; therefore air had to remain the heat-exchange medium. In the newer designs, however, the air would be moved in much greater quantity and velocity, by and over much more powerful fans and heaters. Further, the column oven would become low-mass. A light inner aluminum shell of low thermal mass, insulated from the more substantial walls of the outer oven.

31. WHAT IS PTGC ANYWAY?

Just after a sample is introduced by either manual or autosampler syringe into the GC injection port, the oven temperature (and hence the column temperature) is increased via a previously programmed series of increases in \( T_c \) at a constant rate of change (i.e., \( dT_c/dt \) is fixed), along with periods of time where \( T_c \) does not change. These periods of time are called holds or isothermal steps. GCs can be temperature programmed either in one step or in a series of steps, each step having a different value for the ramp or, as defined earlier, \( dT_c/dt \). Each step can be viewed as consisting of an initial temperature with its hold time, a ramp at a fixed value, and a final temperature with its own hold time. Contemporary GCs are temperature programmed via commercially available chromatography processing software with a precision to 0.02 min or less. This aspect of the GC oven has advanced considerably over the past 40 years to accommodate PTGC. Earlier instruments used clock motor-driven potentiometers to change the column oven set point at a constant rate. Instruments
of the early 1980s vintage had electronic temperature programmers that used plug-in resistors to set one or more programming rates. This author’s experience with thumbwheel settings was obtained using the Model 3700 (Varian Associates). As a rule of thumb, retention times decrease by about one half for each 15 to 20°C increase in $T_c$. McNair and Miller have pointed out that for a homologous series, analyte retention times are logarithmic, whereas under isothermal conditions, analyte $t_R$ values are linear when temperature programmed. Onuska and Karasek have pointed out in their classic text on environmental analysis that the Kovats retention index [Equation (4.33)] can be replaced with the following relationship to give a linear scale:

$$I_{^{PGC}} = 100 \left( \frac{t_R^h - t_R^n}{t_R^{11} - t_R^h} \right) + 100n$$

### 32. CAN WE FIND HOW RETENTION TIME VARIES WITH $T_c$?

Yes, Perry has considered the relationship between a solute’s specific retention volume, $V_r$ (the net retention volume, $V_n$, per gram of liquid phase) and the column temperature, $T_c$. Perry’s approach is not seen in most other GC texts, so let us derive it here. Before we present his approach, we need to shore up some fundamentals first. One of the few chromatographic relationships to really commit to memory is the relationship among solute retention volume, $V_r$, column void volume, $V_m$, and the solute chromatographic capacity factor, $k'$, according to

$$V_r = V_m (1 + k') \quad (4.36)$$

The net retention volume, $V_n$, is defined as the difference between the solute retention volume and the void volume according to

$$V_n = V_r - V_m = k'V_m = \left( \frac{K}{\beta} \right) V_m$$

Upon eliminating the phase ratio, $\beta$, we get

$$V_n = K \left( \frac{V_r}{V_m} \right) V_m = KV_L$$
where $V_L$ is the volume of the liquid phase. This is another of those important chromatographic relationships that help in understanding all this. If you double the volume of the liquid phase, you can expect a doubling of the solute net retention volume. Perry has shown earlier that $V_N$ can be related to $V_N'$. He defines $V_N'$ as the net retention volume per gram of stationary phase of density $\rho_L$ and volume $V_L$ in the column, with the temperature, $T_c$, of the column corrected to 0°C in the following way:

$$V_N = \left( \frac{T_c}{273} \right) V_L \rho_L V_g$$

Eliminating $V_N'$ between these two equations gives

$$K = \left( \frac{T_c}{273} \right) \rho_L V_g$$

Taking the common logarithms of both sides of this equation yields

$$\log K = \log V_g + \log \frac{\rho_L T_c}{273} \tag{4.37}$$

The Clausius–Clapeyron equation introduced earlier can be differentiated with respect to temperature, and the temperature term itself manipulated to yield

$$\frac{d}{d(1/T)} (\ln p^*) = -\frac{\Delta H_v}{R}$$

An analogous statement for the temperature rate of change of the Henry’s law constant, $K_p$, discussed in Chapter 3, can be found in texts on chemical thermodynamics. The differential molar heat of vaporization of a solute from an infinitely dilute solution, $\Delta H_v$, can be related to the Henry’s law constant according to

$$\frac{d}{d(1/T)} (\ln K_p) = -\frac{\Delta H_v}{R}$$

Upon elimination of the term $d(1/T)$ from both of these differential equations, we can rewrite

$$\frac{d(\ln K_p)}{d(\ln p^*)} = \frac{\Delta H_v}{\Delta H_v} = a$$
so that
\[ d(\ln K_H) = ad(\ln p^\circ) \]

Henry’s law constant is essentially the increase of the chromatographic partition coefficient, \( K = 1/K_H \), and this allows us to restate this equation as
\[ d(\ln K) = -ad(\ln p^\circ) \quad (4.38) \]

Combining Equations (4.37) and (4.38) while eliminating \( \ln K \) yields a relationship between \( V_g \) and the vapor pressure of the pure solute, \( p^\circ \). Laub and Pecsok\(^6\)I have also arrived at this relationship:
\[ \log V_g = -a \log p^\circ + \text{const} \quad (4.39) \]

Returning to the Clausius–Clapeyron equation and integrating, we obtain
\[ \ln p^\circ = -\frac{\Delta H_v}{RT} + \text{const} \]

Upon multiplying through by \( a \) and converting to common logarithms, we get
\[ a \log p^\circ = -a \left( \frac{\Delta H_v}{2.3RT} \right) + \text{const} \]

Substituting for the term “\( a \log p^\circ \)” in Equation (4.39) gives
\[ \log V_g = -\left( \frac{-a \Delta H_v}{2.3RT} + \text{const} \right) + \text{const} \]

Because \( \Delta H_s = a \Delta H_v \), we can now arrive at the ultimate objective of Perry’s derivation:
\[ \log V_g = -\frac{\Delta H_s}{2.3RT_c} + \text{const} \]

The specific retention volume, a chromatographic property of a given solute of interest, is related to the column temperature such that plots of \( \log V_g \) vs. \( 1/T_c \) should
yield a straight line whose slope is related to either the heat of vaporization of the solute at infinite dilution or, if $a = 1$ (if Raoult’s law holds), the heat of vaporization of the pure solute. Studies of column efficiency via a consideration of the effect of $T_c$ on $H (N = H/L)$ have shown that an optimum $T_c$ can be found that minimizes $H$ or maximizes $N$. The interested reader can refer to the excellent text by Harris and Habgood on PTGC. Hinshaw has offered some additional insights into PTGC.

33. THIS IS ALL WELL AND GOOD, BUT DO I NOT FIND TEMPERATURE PROGRAMS ALREADY PROVIDED IN EPA METHODS?

Yes, you do if you are implementing the broad-spectrum monitoring of these methods. For a shorter list of analytes of environmental interest, knowledge of the effect of $T_c$ on various GC conditions is important. Also, be aware that there is a finite amount of time needed to cool the instrument down from the maximum $T_c$ reached in a given temperature program. Experience in the operational aspects of PTGC using different reference standards is a great way to gain the necessary hands-on knowledge that will pay great dividends in any future laboratory work involving GCs. We would not know that we have achieved an optimized temperature program if it were not for the ability to detect the eluted analyte of interest to TEQA. The lower limit for PTGC can be extended by applying cryogenic GC techniques.

34. WHAT ROLE DOES CRYOGENICS PLAY IN GC?

An important one when it is desirable to either start the initial column temperature of a GC run below ambient temperature or minimize peak broadening due to the extra dead-volume effects caused by interfaces with purge-and-trap or static headspace sampling techniques as applied to VOCs. Let us digress a bit on cryogenics then and discuss the more contemporary approaches to GC today that involve cryofocusing techniques.

Listed below are the boiling points of the most commonly used gases as cryogens:

<table>
<thead>
<tr>
<th>Cryogen</th>
<th>$T_{bp}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$</td>
<td>−78.5</td>
</tr>
<tr>
<td>CH$_4$</td>
<td>−161.4</td>
</tr>
<tr>
<td>O$_2$</td>
<td>−183.0</td>
</tr>
<tr>
<td>Ar</td>
<td>−185.7</td>
</tr>
<tr>
<td>F$_2$</td>
<td>−187.0</td>
</tr>
<tr>
<td>N$_2$</td>
<td>−195.8</td>
</tr>
<tr>
<td>He</td>
<td>−269.9</td>
</tr>
</tbody>
</table>
Liquefied carbon dioxide and liquefied nitrogen are the two most common cryogens used with gas chromatographs. If the entire oven is to be cooled down, do not introduce the coolant until the oven has been air-cooled to ~60°C. Heat pumps based on thermoelectricity (Peltier effect) and evaporation based on adiabatic expansion (Joule–Thompson effect) represent alternatives to cryogenic cooling. Cryogenic liquids are pumped into GC ovens with the use of solenoid switching valves. It is feasible to cool the GC oven to well below ambient temperature and to start a PTGC run at this subambient temperature provided that the column, usually a WCOT or PLOT, is stable at these subambient temperatures. Working with cryogenic liquids requires an increased awareness of the risks involved. It is imperative to use liquefied CO₂ with equipment designed for liquefied CO₂, and liquefied N₂ with equipment designed for liquefied N₂. Heavy insulation should be placed on all transfer lines and plumbing to prevent a buildup of dry ice snow around the lab. Contact between skin and a cryogenic liquid will lead to serious burns.

Cryogenic focusing, or cryofocusing, uses the cold temperatures of cryogenic coolants to focus the sample into a plug at or near the head of the GC column to improve chromatographic peak shape. Cryogenic trapping, or cryotrapping, is used to concentrate trace amounts of components rather than focusing them onto the analytical column. Cryofocusing traps are commercially available units that cool the first segment of a cap column with subsequent rapid heating of the trap to 400°C at 800°C/min. Let us revisit Equation (4.10) or (4.31). The chromatographic capacity factor \( k' \) determines the speed of the solute through the column. Temperature influences the partition constant \( K \), as we saw earlier. Changes in \( K \) influence \( k' \), and hence the relative speeds of the front and rear tailing with respect to peak apex. This tightening of the tailings leads to a narrowing of the solute plug at the onset of the chromatographic run. This cryofocusing might be analogous to the narrower chromatographically resolved peaks that result from manual syringe injection of a solvent that contains a dissolved solute. A firm and rapid thrust of the syringe plunger usually results in narrow peaks, whereas a light and slow thrust usually yields much broader peaks.

The sketch below shows a GC Cryo-Trap® (Scientific Instruments Services), which enables a GC to be converted to take advantage of cryofocusing. The Cryo-Trap consists of a small heating and cooling chamber that surrounds the first 5 in. of the GC capillary column. The unit is installed inside the GC column oven just under the injection port. A separate digital dual-temperature range controller regulates as well as measures chamber temperature. The system can be used either manually to switch between the cooling and heating cycles or automatically via an input signal from a controlling device or GC signal switch. Trace VOCs from either purge-and-trap or static headspace (Chapter 3) can be trapped at temperatures down to −70°C when liquefied CO₂ is used, or down to −180°C when liquefied N₂ is used. To release the VOCs from the Cyro-Trap, a heater coil inside the unit rapidly heats the capillary column to temperatures up to 400°C. The released VOCs are subsequently temperature programmed through the GC column.
35. WHAT ARE THE COMMON GC DETECTORS AND HOW DO THEY WORK?

We continue now on our journey across the GC schematic as shown in Figure 4.7 to the topic of GC detectors. An overview of the common detectors along with their commonly used abbreviations is found in Table 4.10. We will discuss only those detectors of relevance to TEQA and mention only some of the more recently developed GC detectors. This author was recently confronted with the notion of removing an existing detector from a GC and replacing it with another type. This is not as straightforward as one might think. The one universal concept here is that all GC detectors produced an analog signal related to the analyte that has just eluted the chromatographic column. The magnitude of this signal, whether positive or negative, should always be viewed with respect to the noise that is also generated by the
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Selectivity</th>
<th>Flow Dependence</th>
<th>Destructive or Not</th>
<th>Detector Stability</th>
<th>Linear Dynamic Range</th>
<th>Sensitivity</th>
<th>Importance to TEQA</th>
<th>Used by Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCD</td>
<td>Universal</td>
<td>C</td>
<td>N</td>
<td>Good</td>
<td>&gt;10^9</td>
<td>&gt;10 ppm</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>FID</td>
<td>Selective for C</td>
<td>M</td>
<td>D</td>
<td>Good</td>
<td>&gt;10^7</td>
<td>&gt;10 ppm</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>PID</td>
<td>Selective for aromatics</td>
<td>C</td>
<td>N</td>
<td>Moderate</td>
<td>&gt;10^7</td>
<td>&gt;1 ppb</td>
<td>Much</td>
<td>Yes</td>
</tr>
<tr>
<td>ElCD</td>
<td>Selective for X, S, N</td>
<td>C</td>
<td>D</td>
<td>Moderate</td>
<td>&gt;1 ppb</td>
<td>Much</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>ECD</td>
<td>Selective for X</td>
<td>C</td>
<td>N</td>
<td>Moderate</td>
<td>&gt;10^4</td>
<td>&gt;1 ppb</td>
<td>Much</td>
<td>Yes</td>
</tr>
<tr>
<td>TSD</td>
<td>Selective for N, P</td>
<td>M</td>
<td>D</td>
<td>Good</td>
<td>&gt;10^7</td>
<td>&gt;1 ppb</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>FPD</td>
<td>Selective for N, P, S</td>
<td>C</td>
<td>D</td>
<td>Good</td>
<td>&gt;10</td>
<td>&gt;1 ppb</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>MSD</td>
<td>Universal and selective</td>
<td>M</td>
<td>D</td>
<td>Good</td>
<td>&gt;10^6</td>
<td>&gt;10 ppb</td>
<td>Much</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Note: C = concentration; N = nondestructive; M = mass; D = destructive.*

© 2006 by Taylor & Francis Group, LLC
detector and the processing electronics. Refer to the topics involving the signal-to-noise ratio discussed in Chapter 2. One experiment in Chapter 5 asks the student to compare organic compound specificity for a FID vs. ECD.

The GC detector is the last major instrument component to discuss. The GC detector appears in Figure 4.7 as the box to which the column outlet is connected. Evolution in GC detector technology has been as great as any other component of the gas chromatograph during the past 40 years. Among all GC detectors, the photoionization (PID), electrolytic conductivity (EICD), electron-capture (ECD), and mass-selective detector (MSD) (or quadrupole mass filter) have been the most important to TEQA. The fact that an environmental contaminant can be measured in some cases down to concentration levels of parts per trillion (ppt) is a direct tribute to the success of these very sensitive GC detectors and to advances in electronic amplifier design. GC detectors manufactured during the packed-column era were found to be compatible with WCOTs. In some cases, makeup gas must be introduced, such as for the ECD. Before we discuss these GC detectors and their importance to TEQA, let us list the most common commercially available GC detectors and then classify these detectors from several points of view.

Most GC detectors in use today, along with the primary criteria used to compare such detectors, are listed in Table 4.10. It seems that the development of new GC detectors over the past 10 years is rare. This reflects more the maturity of gas chromatography as an analytical determinative technique rather than the lack of innovative ideas. We will discuss two of the more recent commercially developed GC detectors after we discuss the most common ones that have been around for 30 or more years. The following question is appropriate at this point: If you can detect down to ppt levels and can do this with a good signal-to-noise ratio, why change to a newly introduced detector? Each GC detector listed in the table can be further categorized as either universal or selective, concentration or mass flow dependent, and destructive or nondestructive to the analyte as it elutes from the chromatographic column. Additional questions must be asked. Does the detector have a baseline that is stable over time? Over what range of concentration or mass will the detector give a linear signal? Just how sensitive is the detector, or with respect to an entire instrument such as a GC, how low can it measure (the IDL; refer to Chapter 2)? Is the detector very important to TEQA? Has this author ever operated such a detector, or in other words, can he write from experience?

Ewing 68 has defined a concentration-dependent GC detector as one that gives a response proportional to the concentration of sample, expressed in mole fraction. He also defines a mass-dependent GC detector as one that depends upon the rate at which the sample is delivered to the sensing element, but the extent of dilution by carrier gas is irrelevant. 68 The distinction between a concentration and mass-dependent GC detector has to do with understanding just what is fundamentally responsible for the signal that originates from such a detector. A Gaussian profile of a chromatographically resolved peak is shown in Figure 4.21. The nature of the signal that is responsible for any rise in the detector response when an analyte passes through is viewed in terms of an infinitesimal area, \( dA \). For a concentration-dependent detector such as a thermal conductivity detector (TCD) or an electron-capture detector (ECD)
having a mole fraction $x_S$ that can be defined in terms of the velocity of solute, $v_S$, and a carrier gas velocity, $v_C$, we can define $dA$ as follows:

$$dA = x_S \, dt = \left( \frac{v_S}{v_S + v_C} \right) \, dt$$

Let us assume that as the analyte of interest elutes from the column into the detector, the total gas velocity can be composed of contributions from the solute as well as from the carrier gas. Mathematically stated, $v = v_S + v_C$, so that upon integration over the entire peak, we can show that

$$A = \int x_S \, dt = \int \frac{v_S}{v} \, dt$$

The solute velocity is the time rate of change of mass, $v_S = dmdt$, and we can express the integral as
The peak area is directly related to the mass of solute passing through it. A concentration-dependent detector does not necessarily respond to the presence of a substance in the carrier gas, but rather to a difference or change in the concentration of that substance. Hence, a TCD would have its sensitivity defined as the smallest number of microvolt-seconds per ppm, whereas the sensitivity of a flame ionization detector (FID) might be defined as the smallest number of microvolt-seconds per nanogram. Recall from the discussions in Chapter 2 that sensitivity refers to the magnitude of the slope of a plot of detector response vs. analyte concentration or analyte mass, and the detection limit of the GC detector, or as part of an instrument (IDL), refers to a signal-to-noise ratio measurement. This author believes it to be very important that practicing analysts and lab technicians have some understanding as to how GC detectors work. It helps to solve a lot of problems later. The TCD has been around for a long time. Aside from the TCD being used in environmental testing labs to measure fixed gases and any organic compounds that are present at low parts per hundred (ppm) levels or higher, this detector does not have a role in TEQA. The FID, however, has a somewhat limited role in TEQA, largely due to a FID detection limit in the 10 ppm realm. The FID is suggested in some EPA methods for SVOCs screening procedures. The basic design of the FID is often a starting point for explaining how other GC detectors work. For these reasons, we delve into the details of this GC detector.

36. HOW DOES A FID WORK?

The FID is a micro hydrogen/oxygen burner that continuously maintains a hydrogen flame. A collector electrode is set at +300 V relative to the flame tip. When an organic compound is eluted from the GC column, it is ionized and a small current in generated. It is this current that forms the basis for the analog signal from the FID whose magnitude is proportional to the mass of compound being detected.
Carrier gas, being chemically inert, does not cause the formation of ions. An igniter is provided to start the hydrogen flame burning. Hence, the FID looks like a chimney with two side arms. One side arm is for the electrical connections for the igniter; the other side arm provides for the polarizing voltage and analog signal output. Lighting a FID is one of the more memorable analytical lab type experiences one might confront. Recently, this author, who is accustomed to checking to see whether the FID is lit by holding the lens on his glasses above the outer barrel of the detector, changed his glasses from glass to plastic lenses. An accumulation of moisture on the lens surface is indicative of a lighted FID. A small area on the surface of the plastic lens, instead of accumulating water, melted, much to the surprise and chagrin of the author. Figure 4.22 shows a schematic of a FID. Carrier gas (either He, N₂, or H₂) enters from beneath, being joined by hydrogen and then air, and continues up through the cylinder to the jet tip. Above the jet tip is a collector cup/electrode arrangement. Coaxial cables lead out from the side of the FID and connect to instrument electronics.

The operating characteristics of the FID serve as a good frame of reference from which to view all other GC detectors. It is for this reason that we discuss these FID operating characteristics. We will then discuss the ECD, PID, EI, TCD, FPD, and MSD in terms of how each of these compares to the FID. This author has had numerous requests over the years to use the FID to measure down to low ppb, such

© 2006 by Taylor & Francis Group, LLC
as 10 to 100 ppt of an aliphatic or aromatic organic compound by direct injection of a solution that contains the dissolved compound. This it cannot do. A gas chromatograph incorporating a cap column and a FID, abbreviated C-GC-FID, does not have an IDL of 1 ppb or lower. Prospective clients are surprised to learn this. Let us take a look at these FID operating characteristics.

37. WHAT ARE THE OPTIMUM GAS FLOW RATES AND OPERATING CHARACTERISTICS OF A FID?

Compounds that do not contain organic carbon do not burn and are insensitive to the FID. These include water, inert gases, and nonmetal hydrides such as ammonia and hydrogen sulfide. The greater the number of carbon atoms per molecule in an organic compound, the greater is the FID sensitivity. For example, injection of 1 µL of a 50 ppm solution containing \( n \)-hexane dissolved in acetone would yield a signal that is strong, yet less than the signal from injection of 1 µL of a 50 ppm solution containing \( n \)-nonane dissolved in the same solvent. One says that \( n \)-nonane has a greater FID relative response factor (RRF) than \( n \)-hexane. Aromatic hydrocarbons have a greater RRF than aliphatic hydrocarbons having the same number of carbon atoms per molecule. As shown in one of the GC experiments in Chapter 5, a student can keep the carrier gas flow rate and the FID airflow rate fixed while varying the FID hydrogen flow rate and deduce that 30 to 50 cm\(^3\)/min for a conventional FID maximizes the GC response. Keeping the carrier and FID hydrogen flow rates fixed while varying the FID airflow rate reveals that beyond 300 cm\(^3\)/min, no further increase in GC response is observed.

McNair and Miller\(^{69} \) have characterized the FID based on the following criteria. The FID is selective for all carbon-containing compounds. The FID has a minimal detectable amount of \( 10^{-11} \) g. A linear dynamic range of \( 10^6 \) enables, for a 1-µL injection volume, a detector response that is linear from 1 g of hydrocarbon all the way down to 1 µg of hydrocarbon. The next criterion is that of good baseline stability over time, and this translates into minimal baseline drift. Also, the effect of a change in flow rate or temperature on baseline stability is an important characteristic of FIDs. Finally, it is important that a GC detector be thermally stable because the analyte must remain in the gas phase. The FID can be operated up to a maximum temperature setting of 400°C. The GC chromatogram shown for the separation of BTEX components in gasoline-contaminated groundwater (Figure 4.8) was obtained using a FID. The EPA’s Contract Laboratory Program utilizes a preliminary method known as “Hexadecane Screening of Water and Soil Screening for Volatiles.” This method comprises a preliminary evaluation of a hazardous waste sample prior to implementing the determinative GC-MS technique in EPA Method 8270. In the author’s laboratory, a C-GC-FID is a very useful instrument for conducting preliminary examinations for the presence of trace organic contaminants in drinking or wastewater samples. For example, if gasoline-contaminated drinking water is suspected, a mini-LLE (liquid–liquid extraction) using hexadecane, with a subsequent injection of 1 µL of the hexadecane extract into the C-GC-FID, will give the analyst a quick, yet informative indication of the extent of the contamination.
More than 20 years ago, the need to separate and detect VOCs was accomplished by placing a PID in series with an EICD, in which aromatic VOCs could be detected on the PID and ClVOCs could be detected on the EICD. This was the principal basis for EPA Methods 501 and 502 for drinking water and 601 and 602 for wastewater. The lowering of GC IDLs from the low ppm level afforded by the FID to the low ppb level was first accomplished on a routine basis with the PID, EICD, and ECD. An instrument that incorporated a cap column and ECD, C-GC-ECD, first detected DDT in the environment, and a cap column with a PID, C-GC-PID, first found ppb levels of BTEX in contaminated groundwater.

38. HOW DO A PID AND AN EICD WORK?

Configuring both the PID and EICD in tandem has proven to be a powerful duo for relatively clean water samples. EPA Method 8021B in the most recent SW-846 series recommends that both detectors be used in tandem or individually. The PID is an aromatics-specific detector, and the EICD is a halogen-specific detector. When placed in tandem, both GC detectors provide for a large number of VOCs that can be separated and detected using WCOTs. The PID consists of a deuterium lamp that provides the necessary 10.2 eV of energy to ionize an aromatic molecule such as benzene, while unable to ionize many other hydrocarbons whose ionization potential is greater than 10.2 V. A neutral molecule, RH, absorbs a photon of ultraviolet (UV) energy and dissociates into a parent ion and an electron according to

\[ \text{RH} + \text{hv} \rightarrow \text{RH}^+ + e^- \]

The energy of the photon must be of a frequency such that this energy exceeds the ionization potential of the organic compound for photoionization to occur. The PID is equipped with a sealed UV light source that emits photons that pass through a UV transparent window made of LiF, MgF₂, NaF, or sapphire into an ionization chamber, where photons are absorbed by the eluted analyte. A positively biased high-voltage electrode accelerates the resulting ions to a collector electrode. The current produced by the ion flow is measured by the electrometer and is proportional to analyte concentration in the gas phase. The PID is a concentration-dependent, nondestructive detector. Other lamps are available that enable a wider range of hydrocarbons to be as sensitive as aromatics. A schematic of the PID is shown in Figure 4.23. The PID requires only carrier gas. Routine maintenance includes lamp window cleaning, lamp replacement, lamp window seal replacement, and positioning. With a detection limit in the picogram range coupled to a linear dynamic range of 10⁷, the PID complements other detectors. The PID has also been configured in series before a FID to provide selective aliphatic hydrocarbon as well as aromatic hydrocarbon detection.

It is instructive to view the schematic of an EICD, shown in Figure 4.24, from the perspective of the analyte of interest as it emerges from the outlet of the PID and enters the reaction tube. The analyte is pyrolyzed in the reactor. In the sulfur mode, the analyte is oxidized using oxygen reaction gas to sulfur dioxide. In the
halogen and nitrogen modes, the analyte is reduced using hydrogen as a reaction gas to form HCl, HBr, HF, or NH₃. The reaction tube acts as a catalyst to hasten reaction. The chemically converted analytes of interest are swept through a transfer line into a conductivity cell. In the cell, the ionized analyte is dissolved in a flowing solvent and the change in conductivity is measured. To obtain a good response from the conductivity cell, the solvent flow and pH must be optimized. The sensitivity of the ElCD is inversely related to solvent flow rate. The pH of the solvent is controlled by passing it through an ion exchange resin located in the solvent reservoir. The proper resin mixture will provide the correct pH for the solvent. The halogen and sulfur modes are acidic and require an acidic solvent; the nitrogen mode is basic and requires a basic solvent. Let us consider what happens to CIVOCs as they enter...
Determinative Techniques to Measure Organics and Inorganics

The reactor is made of nickel and is maintained at a temperature of 850 to 1000°C. The CIVOCs are reduced to haloacids by mixing them with hydrogen reaction gas. Nonhalogenated VOCs are reduced to methane, which is nonionic. The haloacids are dissolved in n-propanol and the change in solvent conductivity is measured in the cell. The EICD is a bit more difficult to operate and maintain than other GC detectors. The EICD in the halogen mode has one chief competitor, the ECD.

39. WHAT IS AN ECD, HOW DOES IT WORK, AND WHY IS IT SO IMPORTANT TO TEQA?

The ECD introduced by Lovelock and Lipsky and reviewed in two parts by Lovelock and Watson is aptly described by Ewing in the following manner:

For many years GC made use of TCDs almost exclusively, but with the advent of capillary columns, which are limited to smaller samples, greater sensitivity was required. One method of detection that can give greater sensitivity is a modification of the ionization chamber long used for radiation detection. The effluent from the chromatographic column is allowed to flow through such a chamber, where it is subjected to a constant flux of beta ray electrons from a permanently installed radioisotope.

The inner wall of a cylindrically shaped cavity is lined with 63Ni foil, and a voltage is imposed between a pair of electrodes. This generates a standing current, \( I_b \). When nitrogen or 95% Ar/5% methane gas is passed through, \( I_b \) is approximately \( 1 \times 10^{-8} \) A. The 63Ni is the source of beta radiation, and thermal electrons are produced that do not recombine with either positive ions or neutral carrier gas molecules. Molecules of the analyte of interest that elute from the chromatographic column and contain electronegative heteroatoms, such as chlorine, capture electrons and become negatively charged. These negatively charged gas-phase ions quickly combine with any positive ions present. A decrease in \( I_b \) results. As molecules of the electron-capturing analyte elute and are swept through the ECD, a negative peak results. The peak is inverted by the signal processing electronics to yield a positive peak like that obtained from all other GC detectors. The ECD requires about 30 cm³/min of gas flow. Because a typical WCOT flow rate is more than 10 times lower, makeup gas is required. More contemporary GCs provide the necessary makeup gas. This author has used the inlet from a second GC injection port as a source of makeup gas when makeup is not “plumbed in.” The advantage of 63Ni over 3H (tritium was an earlier choice of beta radiation) lies in the much elevated temperature enjoyed by this isotope. A 63Ni ECD can be safely taken to \( T_{ECD} = 400°C \). The following reactions help to explain the electron-capturing phenomena:

\[
N_2 + \beta \rightarrow N_2^+ + e^- + N_2^+
\]

\[
CCl_4 + e^- \longrightarrow CCl_4^- \text{ (nondissociative capture)}
\]

\[
CCl_4 + e^- \longrightarrow Cl + CCl_3^- \text{ (dissociative capture)}
\]
Many of the operational problems of ECD instability that plagued these detectors for so long have been overcome with advances in design. Contemporary GCs that have ECDs enable either $\text{N}_2$ or $\text{Ar–CH}_4$ to be used as carrier or makeup gas. The low-bleed WCOTs available today are a major contributor to the good stability of ECDs. Contamination even in this era of low-bleed WCOTs and well-designed ECDs is still a problem in the day-to-day laboratory operation. It is a wrong assumption to believe that all GCs that have ECDs are ready to use all of the time to meet the objectives of TEQA. This statement is consistent with this author’s experience. Onuska and Karasek$^{74}$ have identified the sources of ECD contamination that take the form of deposition of liquid phase and of dirt on the ECD electrodes. A contaminated ECD leads to loss of sensitivity, trailing peaks, and erratic baselines. The major sources of ECD contamination include the following:

1. Column bleed  
2. Contaminated sample inlet, including a dirty glass insert  
3. Oxygen in carrier gas or a dirty carrier gas  
4. Lack of a tight WCOT connection, thus allowing oxygen to enter

Figure 4.25 is a schematic for an ECD. EPA methods in general tend to require this detector for the determination of semivolatile to nonvolatile organochlorine analytes such as OCs and PCBs, whereby solvent extracts are introduced into the C-GC-ECD, and water has therefore been completely eliminated.

![FIGURE 4.25 Schematic of the electron-capture GC detector.](image-url)
40. HOW RESPONSIVE ARE ECDS?

Relative response factors (RRFs) for various organic compounds of interest to TEQA reveal an answer to this question. Listed in Table 4.11 are RRFs for most of the functional groups that one might encounter.74 The factor of 100 differences in RRFs between a trichloro-organic and a tetrachloro-organic is vividly demonstrated by a Gas Chromatograph (PerkinElmer) that is interfaced to an HS-40 (PerkinElmer) automated headspace analyzer. The response of carbon tetrachloride ($\text{CCl}_4$) is significantly higher than either chloroform ($\text{CHCl}_3$) or trichloroethylene ($\text{TCE}$). We close out our discussion of the ECD by showing two GC chromatograms from a sample extract from the Soxhlet extraction of a fiberglass insulation sample. The envelope of peaks shows the presence of this particular commercial source of polychlorinated biphenyls (PCBs) in the insulation. This clear and almost interference-free chromatogram was obtained with no additional sample cleanup and shows the real value of a element-specific GC detector. Imagine what this chromatogram would look like if it were injected into a C-GC-FID.

41. ARE THERE OTHER ELEMENT-SPECIFIC DETECTORS?

Yes, indeed, and we make brief mention of these. We must ask, however, the following question: What other chemical elements are incorporated into organic molecules that are of interest to TEQA? Priority pollutant organic compounds of environmental interest that incorporate the elements nitrogen, phosphorus, and sulfur answer this question. GC detectors developed for these elements are of a more recent vintage and include thermionic, flame photometric, and chemiluminescence. In addition, the atomic emission spectroscopic detector (AES) has been recently developed commercially for organometallics. The AES can be tuned to a specific emission

---

**TABLE 4.11**

<table>
<thead>
<tr>
<th>RRF</th>
<th>Electron-Capturing Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Aliphatic hydrocarbons, aromatic hydrocarbons</td>
</tr>
<tr>
<td>101</td>
<td>Esters, ethers</td>
</tr>
<tr>
<td>102</td>
<td>Monochloro, monofluoro, aliphatic alcohols, ketones</td>
</tr>
<tr>
<td>103</td>
<td>Dichloro, difluoro, monobromo derivatives</td>
</tr>
<tr>
<td>104</td>
<td>Trichloro, anhydrides</td>
</tr>
<tr>
<td>105</td>
<td>Monoiodo, dibromo, nitro derivatives</td>
</tr>
<tr>
<td>106</td>
<td>Diiodo, tribromo, polychlorinated aromatics</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
FIGURE 4.26 Overlay static headspace capillary HS-GC-ECD chromatograms for the separation and detection of chloroform, carbon tetrachloride, and trichloroethylene. Calibration standards ranging from 0.3 to 3.0 ppb each in water were overlayed.

© 2006 by Taylor & Francis Group, LLC
Determinative Techniques to Measure Organics and Inorganics

wavelength for a particular metal. Development of C-GC-AES techniques has advanced the analytical chemistry of trace metal speciation.

The thermionic-specific detector (TSD), also called the alkali flame ionization detector, is really a FID with a bead of an alkali metal salt such as Rb or Cs. A schematic is shown in Figure 4.28. The TSD shows enhanced sensitivity for organic compounds that contain the elements nitrogen and phosphorus. This author has used a GC that incorporated a TSD to measure the extent of contamination of groundwater in an aquifer somewhere in Maine with $N,N$-dimethyl formamide (DMF). Figure 4.28
406  Trace Environmental Quantitative Analysis, Second Edition

shows that the bead can be heated to a red glow and the heating of the bead has led to increased TSD stability. A negative polarizing voltage is applied to the collector. The ion current generated by the thermionic emission can be related to the mass of, for example, DMF eluted from the WCOT.

The flame photometric detector (FPD) operates on the principle that phosphorus- and sulfur-containing organics compounds eluted from the GC column emit characteristic green and blue colorations, respectively, in hydrogen-rich H\textsubscript{2}–air flames. A schematic of a dual FPD is shown in Figure 4.29. This design, first developed at Varian Associates, burns the organic solute during passage through the lower flame and then excites the free radical HPO species and causes it to emit at 526 nm for phosphorus-containing organic compounds, or S\textsubscript{2} at 394 nm for sulfur-containing organic compounds in the higher flame.\footnote{These species emit characteristic wavelengths through an optical window through various cutoff filters to a photomultiplier tube, as shown in Figure 4.29. Hydrogen gas enters from the side and mixes with air and carrier gas to sustain a hydrogen flame. The design criteria in the dual-flame FPD is designed, for example, to maximize the flame photometric emission of HPO while minimizing hydrocarbon emissions and interferences due to S\textsubscript{2}.}

This author was once fortunate to have access to a gas chromatograph (Model 3400, Varian) that incorporated both a TSD and an FPD. This instrumental configuration enabled this author to conduct a comprehension study of the isolation and...

FIGURE 4.28 Schematic of the thermionic GC detector.
recovery of representative OPs via reversed-phase solid-phase extraction (SPE). 76

Figure 4.30 is a schematic of the single-injector/dual-column/dual-detector GC configuration used to conduct the study. In order to provide the necessary makeup carrier gas to both detectors, stainless-steel tubing from the inlet of the first injector was connected to the detector inlets as shown in Figure 4.30. A dual GC chromatogram, shown in Figure 4.31, was obtained using the dual GC just described. A DB-5 and a DB-608 WCOT column were used to separate a mixture that contains seven OPs and the internal standard, triphenyl phosphate. Note that the elution order

FIGURE 4.29 Schematic of the flame photometric GC detector.
changes somewhat between both columns. The instrumentation used and the instru-
mental conditions employed in this study are given in Table 4.12. The specific 
operational parameters shown in this table reveal much about how to operate both 
the TSD and FPD GC detectors. The instrumentation was of a late 1980s vintage 
and reveals the aspects of GC instrumentation that have changed during the past 
15 years. The fact that these OPs could be isolated, recovered, and detected at trace 
concentration levels is most significant and cannot change.

42. WHAT ABOUT ANY RECENTLY DEVELOPED 
ELEMENT-SPECIFIC GC DETECTORS?

Two such detectors will be discussed here, the pulsed-discharge detector (PDD), 
manufactured by VICI (Valco Instruments Company, Inc.), and the chemilumines-
cence detector, manufactured by Sievers Instruments. Both detectors are of a more 
recent vintage. The PDD has the potential of replacing either the ECD or FID, and 
also the PID, depending on the application to TEQA. The PDD is a nonradioactive 
pulsed-discharge ionization detector and was designed for ease of installation to the 
Model 6890 GC (Hewlett-Packard, now Agilent Technologies). A stable, low-power, 
pulsed DC discharge in helium is the source of ionization. Solutes eluting from the 
cap column flowing in the opposite direction to that of the flow of helium from the 
discharge zone are ionized by photons from the helium discharge above. Electrons 
from this discharge are focused toward the collector electrode by the two bias 
electrodes. A schematic of the PDD is shown in Figure 4.32. Photoionization by 
radiation is the principal mode of ionization and arises from the transition of diatomic 
helium to the dissociated monatomic He ground state. In the electron-capture mode, 
the PDD is a selective detector for monitoring high-electron-affinity compounds 
such as CIVOCs, OCs, PCBs, and so forth. GCs that incorporate the PDD would 
be capable of IDLs down to low ppb concentration levels. In this mode, helium and 
methane are introduced just upstream from the column exit. In the helium photoion-
ization mode, the PDD is a universal, nondestructive, highly sensitive detector. In
FIGURE 4.31 Dual chromatograms from a single injection of a reference standard containing organophosphorus pesticides.

**Peaks:**
1. TEPT (triethyl phosphothioate)
2. Phorate
3. Disulfoton
4. Dimethoate
5. Meparathion
6. Parathion
7. Famphur
8. Triphenyl phosphate (int. std.)

**Conditions:**
- Column dimensions: 30 m long × 0.53 mm i.d., fused silica
- Column phase: DB-5 (J&W scientific) bonded phenyl (5%) methyl silicone, 1.0 µm film
- Column temperature: held initially for 0.5 min at 100°C, then programmed at 10°C/min to 280°C
- Carrier gas: helium, 10 cm/s
- Sample: standard mixture of 8 organophosphorous pesticides
- Sample introduction: splitless injection
- Detector: TSD (thermionic detector)
**FIGURE 4.31** (continued).

**Organophosphorous pesticides**

dual wide-bore capillary/FPD

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 TEPT (triethyl phosphothioate)</td>
<td>Column dimensions: 30 m long × 0.53 mm i.d.,</td>
</tr>
<tr>
<td>2 Phorate</td>
<td>fused silica</td>
</tr>
<tr>
<td>3 Dimethoate</td>
<td>Column phase: DB-608 (J&amp;W scientific)</td>
</tr>
<tr>
<td>4 Disulfoton</td>
<td>bonded cyanopropyl phanyl silicone</td>
</tr>
<tr>
<td>5 Meparathion</td>
<td>Column temperature: held initially for 0.5 min at 100°C,</td>
</tr>
<tr>
<td>6 Parathion</td>
<td>then programmed at 10°C/min to 280°C</td>
</tr>
<tr>
<td>7 Famphur</td>
<td>Carrier gas: helium, 10 cm/s</td>
</tr>
<tr>
<td>8 Triphenyl phosphate (int. Std.)</td>
<td>Sample: standard mixture of 8 organophosphorous pesticides</td>
</tr>
<tr>
<td></td>
<td>Sample introduction: splitless injection</td>
</tr>
<tr>
<td></td>
<td>Detector: FPD (flame photometric detector)</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
Determinative Techniques to Measure Organics and Inorganics

this mode, the PDD is a suitable replacement for a conventional FID. When a dopant is added to the discharge gas, the PDD also functions as a selective photoionization detector. Suitable dopants include Ar for organic compounds, Kr for unsaturated compounds, and Xe for polynuclear aromatics. A drawing of the PDD as it might appear when being installed into a 6890 GC is shown in Figure 4.33. This is a good example of how existing gas chromatographs can be retrofitted with more newly developed GC detectors as the need arises.

FIGURE 4.32 Schematic of the pulsed-discharge GC detector.

TABLE 4.12
GC Operation Conditions Used to Conduct Dual Megabore Capillary GC-NP Detection

<table>
<thead>
<tr>
<th>Description</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas chromatograph</td>
<td>Varian 3400 with Varian 8034 autosampler</td>
</tr>
<tr>
<td>Megabore splitter</td>
<td>Supelco direct injector tee, silanized with appropriate fittings</td>
</tr>
<tr>
<td>Data station</td>
<td>Varian 604 with Varian IIM interface using the dual-column software option</td>
</tr>
<tr>
<td>Printer</td>
<td>Hewlett-Packard Think Jet printer</td>
</tr>
<tr>
<td>WCOT</td>
<td>30 m × 0.53 mm DB-5 and 30 m × 0.53 mm DB-608 from J&amp;W Scientific</td>
</tr>
<tr>
<td>TSD and flow characteristics</td>
<td>300°C, air at 175 cm³/min, H₂ at 4.5 cm³/min, carrier (N₂) at 5 cm³/min, makeup (N₂) at 25 cm³/min</td>
</tr>
<tr>
<td>FPD and flow characteristics</td>
<td>300°C, air 1 at 80 cm³/min, air 2 at 170 cm³/min, H₂ at 140 cm³/min, carrier (N₂) at 5 cm³/min, makeup (N₂) at 25 cm³/min</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
Because ECDs require radioactive sources, the pulsed-discharge electron-capture detector (PDECD) is a relatively recent alternative. A cross section of a PDECD that incorporates methane as a dopant is shown in Figure 4.34. A detailed description of the design of this detector is available. Cai et al. have described their recent developments while studying the influence of discharge current, bias and collecting voltages, and the chemical nature of the dopant gas while comparing conventional ECDs to the PDECD. The pulsed discharge in pure helium produces high-energy photons according to

\[
\text{He}_2 \longrightarrow 2\text{He} + \text{hv}
\]

If methane is added downstream from the discharge, free electrons are produced. The dopant also thermalizes the electrons, making them more capturable by analytes. Analytes capture these thermalized electrons and reduce the standing current \(I_b\) according to

\[
\frac{I_b - I_c}{I_c} = K_{\text{EC}} [\text{AB}]
\]
where $I_b$ is the standing current without analyte, $I_e$ is the current with the electron-capturing analyte AB present, $K_{EC}$ is a capture coefficient for AB, and $[AB]$ represents the concentration of AB. This equation was first developed by Wentworth et al.\textsuperscript{78} for an ECD model. Thermal electrons are first formed in the discharge zone as methane undergoes photoionization according to

\[
\text{CH}_4 + h\nu \rightarrow \text{P}^+ + e^-
\]

These primary electrons from photoionization become thermalized:

\[
e^- + \text{CH}_4 \rightarrow e^-_{\text{thermal}} + \text{CH}_4
\]

Some electrons and positive ion species, P$^+$, recombine to form neutral species according to
The dissociative and nondissociative mechanisms discussed earlier for the ECD also apply to the PDECD. The only difference in kinetic models is that of the first step of electron formation. The capture coefficient $K_{EC}$ can then be defined in terms of the various rate constants for these various reactions. The collection of thermal electrons constitutes $I_b$ for the PDECD. This standing current decreases during passage of an electron-capturing analyte through the detector and is responsible for the PDECD signal. The use of a pulsed discharge rather than a continuous discharge serves to impart a higher energy to the cell because energy is dissipated during a very short period. The density of ions should be kept low for the charged species to be sustained in the reaction region. Pulsing also keeps the average potential to near zero, thus allowing what Wentworth et al. call a field-free condition to be realized shortly after the discharge takes place.

A comparison of the relative response factors (RRFs) for a selective number of ClVOCs that have been discussed earlier is given in Table 4.13. The PDECD is as responsive as, if not more responsive than, the $^{63}$Ni-ECD for aliphatic ClVOCs; however, the PDECD becomes somewhat less responsive for mono- and dichlorobenzenes. A comparison was also made between a conventional $^{63}$Ni-ECD and the PDECD with respect to satisfying the criteria posed in EPA Method 608 for OCs. GC chromatograms for the injection of a multicomponent OC standard, including the TCMX and DCBP surrogates discussed earlier, are shown for a standard sample and matrix spike for both detectors in Figure 4.35. Note that the authors plot the ratio $(I_b - I_e)/I_e$ vs. chromatographic run time in the figure. The difference in WCOT column polarity is responsible for the differences in elution order. It would appear that the PDECD is a viable alternative to the ECD. One other element-specific GC detector needs to be discussed that involves chemiluminescence.

![Chemical Reaction](e^- + P^+ → Neutrals)

**TABLE 4.13**
Comparison of RRFs for PDECD and $^{63}$Ni-ECD

<table>
<thead>
<tr>
<th>ClVOC</th>
<th>RRF (PDECD)</th>
<th>RRF ($^{63}$Ni-ECD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl₄</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>PCE</td>
<td>5000</td>
<td>4000</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>660</td>
<td>470</td>
</tr>
<tr>
<td>TCE</td>
<td>570</td>
<td>380</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>13</td>
<td>45</td>
</tr>
<tr>
<td>1,3-Dichlorobenzene</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Note:* PCE = tetrachloroethene; TCE = trichloroethene.
Chemiluminescence can be defined as the spontaneous emission of light by chemical reaction. In the sulfur chemiluminescence detector (SCD), organosulfur compounds that elute from a WCOT are combusted in a hydrogen-rich flame to produce sulfur monoxide (SO), among other products. This is the same process that occurs in a FID. These combustion products are collected and removed from the flame using a ceramic sampling tube (probe) interface and transferred under a vacuum through a flexible tube to the reaction chamber of the SCD. Sulfur monoxide is detected by an ozone/SO chemiluminescent reaction to form electronically excited sulfur dioxide (SO$_2$), which relaxes with emission of light in the blue and ultraviolet regions of the electromagnetic spectrum according to

$$\text{SO} + \text{O}_3 \rightarrow \text{SO}_2 + \text{O}_2 + h\nu$$

One model of an SCD is manufactured by Ionics Instruments (formerly Sievers Instruments) and readily adapts to existing GCs. A more recent development from Sievers is the nitrogen chemiluminescence detector (CD). Organonitrogen compounds that elute from a WCOT enter a ceramic combustion tube in a stainless-steel burner. The hydrogen and oxygen plasma in the combustion tubes convert all organonitrogen compounds to nitric oxide at temperatures greater than 1800°C according to

---

See FIGURE 4.35 Chromatograms that compare the response of the PDECD to a conventional $^{63}$Ni-ECD under the same conditions.

43. OK, BUT WHAT ABOUT CHEMILUMINESCENCE?
Nitric oxide reacts with ozone to form electronically excited nitrogen dioxide according to

$$\text{NO} + \text{O}_3 \rightarrow \text{N}_2^* \rightarrow \text{NO}_2 + h\nu$$

Excited NO$_2$ emits light in the red and infrared regions of the electromagnetic spectrum, from 600 to 3200 nm, when it relaxes to its ground state. The light emitted is directly proportional to the amount of nitrogen in the sample.$^{81}$ Figure 4.36 depicts a schematic diagram for either an SCD or an NCD connected to a GC. Note that a burner head is attached to the existing detector port much like a FID. The combustion products are directed to a reaction cell where ozone can be generated and added. A photomultiplier tube, not shown in the schematic, is incorporated into the detector. Comparisons have been made between the NCD and TSD. Element-specific GC detectors are in general very sensitive and are quite useful for target-specific determinations; however, the most widely used GC detector and the one that satisfies the most EPA methods is the mass spectrometer (MS) and the success that has been achieved in interfacing a GC with a “mass spec.” This interface was the first successful hyphenated method, a term coined by Hirshfeld to describe how two established instruments could be married such that the whole is greater than the sum of the parts (Hirshfeld, T. Anal Chem 52: 297A, 1980). Before we delve into the mass spectrometer as a GC detector, let us digress to yet another spectroscopic GC detector, namely, the atomic emission detector (AED).

**FIGURE 4.36** Schematic diagram of a sulfur or nitrogen chemiluminescence GC detector.
44. ATOMIC SPECTROMETRIC EMISSION AS A GC DETECTOR, WHAT IS IT?

Ionization-based GC detectors have just been discussed (FIDs, ECDs, PIDs, etc.). Organic compounds that are chromatographically resolved have been introduced into a miniaturized helium microwave-induced plasma (MIP) discharge. The topic of atomic emission spectroscopy will be introduced as a determinative technique for trace metals analysis later in this chapter. Organic compounds entering the hot plasma are atomized and electrons are excited to higher quantized potential energy levels. Consider TCMX, a common surrogate used in the determination of various organochlorine pesticides. TCMX is retained on a WCOT column such as a DB-5. The analyte elutes from the column and enters the MIP. Look at what happens when TCMX enters the MIP:

![Diagram of atomic emission process]

In general, the emitted intensity is independent of the number of atoms of a given element in the molecular formula of the organic compound. Another way to express this is to say that there is an equal molar response for each element. In other words, 1 mole of TCMX with four chlorines per molecule gives the same signal response as would 1 mole of hexachlorobenzene with six chlorines per molecule. The physico-chemical basis of this equimolar signal response is shown by use of the double-arrow notation. The double arrow symbolizes a dynamic equilibrium being set up within the plasma. This equimolar response is in stark contrast to, for example, the ECD, as is demonstrated in Table 4.11, and has enabled users of C-GC-AED to accomplish compound-independent calibration (CIC). Software (AED ChemStation®, Agilent) has been written to enable CIC to be performed.

In addition to analytically useful atomic emission lines, there exists molecular emission bands known as broad bands and quite narrow atomic emission bands that act as interferences. A handful of elemental atomic emission lines found most useful in the practice of GC-AED are provided below:

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-193</td>
<td>C-496</td>
</tr>
<tr>
<td>S-181</td>
<td>Cl-479</td>
</tr>
<tr>
<td>N-174</td>
<td>Br-478</td>
</tr>
<tr>
<td>P-178 requires hydrogen</td>
<td>O-171 (CO*)</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
One of the organophosphorous pesticides that contains several heteroatoms is chlorpyrifos, whose molecular structure is shown below:

\[
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{S} \\
\text{P} \\
\text{Cl} \\
\text{Cl}
\end{array}
\]

Chlorpyrifos

With carbon and four heteroatoms in a molecule of chlorpyrifos, C, Cl, S, P, and N, it should be expected that injection of a standard that contains chlorpyrifos with the appropriate software configuration should yield a peak for all five channels at the same retention time. This is indeed the case. This author, who is fortunate to have access to a GC-AED (6890GC interfaced to a G2350A® AED, Agilent Technologies) instrument, generated the series of GC chromatograms shown in Figure 4.37. One microliter of a solution containing 1.6 ppm chlorpyrifos dissolved in iso-octane was injected into this instrument. A method within the AED ChemStation (Agilent) was developed in such a way that five different atomic emission wavelengths (called element-specific channels) are monitored simultaneously. To interpret the chromatograms in Figure 4.37, be sure to view the height of each peak with respect to the corresponding response scale.

Figure 4.38 is a GC-AED chromatogram generated in the author’s laboratory. This chromatogram shows a separation of the two surrogates TCMX and DCBP monitored only at the 479-nm emission wavelength for the element chlorine. Since both compounds are polychlorinated aromatics, signals should be observed on one or more carbon channels, as well as one or more chlorine channels.

A block diagram for the GC-AED is depicted in Figure 4.39. This schematic should convey the impression that operating the instrument properly requires a number of compressed gas sources. Note that the 6890 GC pneumatically controls the reagent gas pressure and flow rates via Agilent Technologies’ electronic pressure control (EPC). In the author’s experience, helium carrier gas and nitrogen purge gas are the two most frequently replaced compressed gases in practice. A N₂ Dewar is the only practical source (short of a nitrogen generator) to use as the source for the N₂ purge. Figure 4.40 depicts the relationships between the AED cavity that incorporates a WCOT column interfaced to a capillary discharge tube, the plasma itself, and the exit through a conical aperture through an optical lens and into the monochromator, where a fixed photodiode array with the established atomic emission wavelengths for the various elements is shown that scans from 690 down to 171 nm. This fixed photodiode array for the newer model stands in contrast to the earlier model design, whereby the grating was fixed and the array moved. It is important in practice to maintain a cavity pressure of ~1.5 psi, which leads to a stable plasma. Indeed, the MIP is quite stable, and this leads to a very stable AED in practice and, subsequently, a stable baseline in the chromatogram.

© 2006 by Taylor & Francis Group, LLC
FIGURE 4.37 Multichannel GC-AED chromatogram for injection of 1 µL of 1.6 ppm chloropyrifos dissolved in iso-octane. Chromatogram generated in the author’s laboratory.
The AED has witnessed a rise and fall as a popular GC detector over the past decade. The GC-AED arose from early experimental work to hyphenate GC and elemental spectroscopy. The miniaturization of the plasma was demonstrated for GC-MIP in 1965. A commercial unit was developed in Great Britain, followed by the successful development by Peter Uden at the University of Massachusetts–Amherst. Agilent Technologies (formerly Hewlett-Packard) developed the first commercial instrument, HP5921A®, in 1989. The second-generation instrument, HPG2350A®, came out in 1996, and this model remains the contemporary version found most often in laboratories today. Agilent decided to cease manufacture of

**FIGURE 4.38** Single-channel GC-AED chromatogram for TCMX and DCBP (surrogates for OCs and PCBs).
FIGURE 4.39 Schematic for the 6890GC-G2350A GC-AED system. (Courtesy of Diablo Analytical, Inc. With permission.)

FIGURE 4.40 Schematic of the AED cavity, lens, and Czerny–Turner monochromator with diode array detection. (Courtesy of Diablo Analytical, Inc. With permission.)

© 2006 by Taylor & Francis Group, LLC
the G2350A in 2002. The Model G2350A incorporates a number of unique features, including:

- Beeneker MIP design
- Water-cooled discharge tube
- Czerny–Turner spectrometer design
- Fixed photodiode array detector
- Utilization of EPC controlled reagent gas delivery
- Interfaces to the 5890 and 6890 GCs (Agilent)
- Computer controlled within the Windows NT® platform

45. IS IT POSSIBLE TO COUPLE SAMPLE PREP WITH GC-AED?

Yes, indeed. The true potential for multielement quantitative analysis for TEQA may lie in the development of analytical methods that focus on the element selectivity afforded by GC-AED combined with an innovative sample prep approach. We illustrate this by describing the author’s attempt to couple RP-SPE with GC-AED as the determinative technique for selected polychloro-organics, and then discuss some recent literature on the use of SPME coupled to GC-AED to speciate Hg.

This author recently conducted a preliminary percent recovery study for the isolation and recovery of TCMX and DCBP from spiked drinking water using RP-SPE conditions published earlier. The table below compares the percent recoveries of TCMX and DCBP from two different suppliers (vendors A and B) of chemically bonded silicas. Reversed-phase solid-phase extraction and capillary gas chromatography with atomic emission detection using a chlorine channel, $\lambda = 837.6$ nm, were employed to conduct the study whose findings are reported below:

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Supplier</th>
<th>% Recoveries (Mean of Triplicate SPEs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₈ (new)</td>
<td>A</td>
<td>70.9 87.1</td>
</tr>
<tr>
<td>C₃ (new)</td>
<td>A</td>
<td>51.7 90.5</td>
</tr>
<tr>
<td>C₁₈ (old)</td>
<td>A</td>
<td>74.2 96.6</td>
</tr>
<tr>
<td>C₄ (new)</td>
<td>B</td>
<td>89.6 94.6</td>
</tr>
<tr>
<td>C₁₈ (new)</td>
<td>B</td>
<td>81.9 80.6</td>
</tr>
</tbody>
</table>

The procedure is summarized below:

A ~70-mL tap water sample was acidified and spiked with 50 µL of 200 ppm TCMX/DCBP in acetone. This sample was passed through a previously conditioned sorbent under reduced pressure. The cartridge was eluted with 2 × 500 µL 75:25 acetone:ethyl acetate. The eluent was transferred to a 1-mL volumetric flask and the volume adjusted to the mark with the eluent. One microliter of this eluent was injected into the C-GC-AED using a previously developed method written within the Agilent
ChemStation. A control is prepared that represents 100% recovery of the surrogates by adding 50 µL of 200 ppm TCMX/DCBP to a previously half-filled 1-mL volumetric flask, then the volume adjusted to the mark with the eluent. The ratio of the analyte peak area in the recovered sample from SPE to the corresponding peak area of the same analyte in the control ×100 gives the percent recovery for that analyte.

Percent recoveries for TCMX and DCBP vary widely among C18, C8, and C2 chemically bonded silica sorbents obtained from the same supplier. Note that the smaller the organic moiety, the higher the recovery of the larger DCBP. A C18 SPE sorbent obtained over 5 years ago (old) actually yielded a significantly higher percent recovery than a C18 SPE sorbent from a different supplier and obtained recently (new). Nevertheless, these preliminary experiments do demonstrate the importance of coupling RP-SPE with C-GC-AED toward accomplishing TEQA.

The combination of solid-phase microextraction sampling of the headspace (SPME-HS) coupled to capillary gas chromatography and atomic emission detection (C-GC-AED) enables a speciation of Hg to be achieved. Organo-Hg compounds such as methyl-Hg and dimethyl-Hg are much more toxic than inorganic Hg. Carro and coworkers have recently demonstrated that methyl mercury, ethyl mercury, and phenyl mercury can be derivatized, volatilized, then separated and detected down to a concentration of 100 ppt levels from seawater using SPME-HS-C-GC-AED. A recent review has also been published that discusses speciation of mercury, tin, and lead using C-GC-AED. Methods that couple SPME techniques with determinative techniques such as C-GC-AED using a Fiber Holder for Manual Sampling (Supelco) can lead to trace metal speciation of other metallic elements in addition to Hg. This may give new life to the GC-AED instrument with respect to TEQA. It would be utterly irresponsible for this author to leave the topic of trace organics analysis without diving into those developments that joined GC with mass spectrometry (MS). Gas chromatography-mass spectrometry (GC-MS) has profoundly influenced the practice of TEQA. We complete our discussion of GC with a focus on MS as the most significant of GC detectors yet devised.

46. OF THE PLETHORA OF MASS SPECS, WHICH IS MOST USEFUL TO TEQA?

Because volumes have been written concerning mass spectrometry over the past 40 years, our approach here is to focus on the type of mass spec instrumentation required to perform EPA methods, and those systems can be described as being low resolution in nature and the most affordable. The quadrupole mass filter or mass-selective detector (MSD) and quadrupole ion trap mass spectrometer (ITD) fit this criteria and will be the only mass specs discussed. Onuska and Karasek have given a good definition and description of the importance of gas chromatography-mass spectrometry (GC-MS) to TEQA:

In its simplest form the mass spectrometer performs three basic functions as a GC detector. These functions are to deliver a sample into the ion source at a pressure of $1 \times 10^{-5}$ torr to produce ions from neutral molecules, and to separate and record a
spectrum of ions according to their mass-to-charge ratios (m/z) and relative abundance. If a WCOT column represents the heart of the system, the mass spectrometric detector is the brain at the highest intelligence level. It is capable of providing both qualitative and quantitative data by means of spectral interpretation procedures developed to identify and quantify individual components in a mixture or of measuring a specific compound or group of positional isomers by means of selective ion monitoring (SIM).

For a more complete description, as well as ease of comprehension of the broad field of mass spectrometry, including mass spectral interpretation, please refer to Watson. A somewhat oversimplified schematic of a quadrupole MSD is shown in Figure 4.41 and depicts the trajectory of a resonant ion that makes it through the four rods of the quadrupole and a nonresonant ion that does not. The effluent from a narrow-bore WCOT can be fitted in such a way that the column outlet is positioned just in front of the electron beam. This beam is produced by “boiling off” electrons from a heated filament and accelerating the electrons to the collector. Molecular and fragment ions that are produced from this electron impact are accelerated through a series of electric fields and enter the center of the four rods. Diagonally opposite rods are connected together electrically and to radio frequency (RF) and DC voltage sources, as indicated in Figure 4.42. The applied voltages on one set of quadrupole rods are 180° out of phase with the applied voltages on the other set of rods. The quadrupole MSD is scanned by increasing the magnitude of the RF amplitude and DC voltages while maintaining a fixed ratio of the two. It is this ratio of the RF to the DC voltage that determines the resolving power of the quadrupole MSD. Sweeping the voltages from a preestablished minimum to a maximum value, while keeping the ratio of RF to DC voltage constant, will provide a mass spectrum of resolution $R = 1$ for the molecular ion, if any, and any and all mass fragment ions from electron impact of the chromatographically resolved component in a mixture. This scan occurs very fast and enables a large number of scans to be recorded as the chromatographically resolved component enters the MSD. A plot of ion abundance vs. $m/z$ gives a mass spectrum and serves to confirm the presence of a particular analyte.

**FIGURE 4.41** Schematic diagram of a quadrupole mass-selective filter as found in typical environmental testing labs. Not drawn to scale.
C-GC-MS therefore serves to confirm the identity of an analyte and thus complements C-GC with element-specific detection. This is one of the most powerful trace analytical instrumentation concepts devised in the 20th century.

Miller and Denton have likened the quadrupole MSD filter to a “tunable variable bandpass mass filter” and contrasted this with “true mass spectrometers” that resolve ions by dispersing them in either space, such as a magnetic sector instrument, or time, such as that of a time-of-flight instrument. These authors suggest that the combination of a time-independent DC and a time-dependent RF potential applied to the four rods in essence act like a mass band filter. With reference to Figure 4.43 and with the center of the quadrupoles at the origin of a set of Cartesian coordinates, the rods act as a low-pass mass filter for ions in the $x-z$ plane, while acting as a high-pass mass filter in the $y-z$ plane. An ion must remain stable in both the $x-z$ and $y-z$ planes to make it from the ion source to the detector. It must be light enough so as not to be eliminated by the low-pass filter operating in the $x-z$ plane, yet not so light as to be eliminated by the high-pass mass filter that operates in the $y-z$ plane. This condition of mutual stability allows a narrow mass range to make it through.

47. CAN WE PREDICT WHAT M/Z VALUES ARE STABLE THROUGH THE QUADRUPOLE RODS?

Yes, we can. However, we must delve into the physics of the quadrupole. For the classical hyperbolic mass filter, the potential $\Phi$ for any time $t$ can be described by

$$\Phi = [V_{DC} + V_{RF} \cos(\omega t)] \frac{x^2 - y^2}{2\sigma_0^2}$$
FIGURE 4.43 Conceptual framework to help explain the mass filtering properties of the quadrupole mass spectrometer. (From Miller, P. and Denton, M., J. Chem. Educ., 63, 617–622, 1986.)
where $V_{DC}$ is the time-independent DC potential, $V_{RF}$ is the magnitude of the applied RF amplitude, $\omega$ is the angular frequency (where $\omega = 2\pi f$, with $f$ being the RF frequency), $x$ and $y$ are Cartesian coordinates defined with respect to Figure 4.39, and $r_0$ is the distance above the $z$ axis. The RF frequency is typically around 1.5 MHz. The partial derivative of this potential gives the magnitude of the electric field in all three directions: $x$, $y$, and $z$. The electric field in the $x$ direction is obtained by taking the partial derivative with respect to $x$ of the potential $\Phi$, as is shown by:

$$E_x = -\frac{\partial \Phi}{\partial x} = -[V_{DC} + V_{RF} \cos(\omega t)] \frac{x}{r_0^2}$$

The electric field in the $y$ direction is obtained similarly as follows:

$$E_y = -\frac{\partial \Phi}{\partial y} = V_{DC} + V_{RF} \cos(\omega t)) \frac{y}{r_0^2}$$

The electric field in the $z$ direction is obtained in a similar manner as well; however, because there is no dependence of the potential in this direction, we get

$$E_z = -\frac{\partial \Phi}{\partial z} = 0$$

The force, $F_x$, exerted on a charged particle in the $x$ direction by the magnitude of the electric field is equal to the product of the electric field $E$ and the charge $e$ such that

$$F_x = -[V_{DC} + V_{RF} \cos(\omega t)] \frac{ex}{r_0^2}$$

The force $F_y$ exerted in the $y$ direction is likewise found:

$$F_y = [V_{DC} + V_{RF} \cos(\omega t)] \frac{ey}{r_0^2}$$

There is no force exerted in the $z$ direction, so

$$F_z = 0$$
The force exerted on the ion is also equal to the product of its mass and its acceleration, expressed in terms of the $x$ coordinate; we obtain

$$F_x = ma = m\frac{d^2x}{dt^2}$$

Rearranging this equation, we have

$$m\frac{d^2x}{dt^2} - F_x = 0$$

Substituting for $F_x$, we then have

$$m\frac{d^2x}{dt^2} - \left[V_{DC} + V_{RF}\cos(\omega t)\right]x^x = 0$$

We can develop similar relationships for the $y$ direction, and upon substituting for $F_y$, we have

$$m\frac{d^2y}{dt^2} - \left[V_{DC} + V_{RF}\cos(\omega t)\right]y^y = 0$$

By letting $\xi = \omega t/2$ and designating any $x$, $y$, or $z$ coordinate as $u$, the derivation as presented by March and Hughes enables us to arrive at the canonical form of the Mathieu equation:

$$\frac{d^2u}{d\xi^2} + [a_u + 2q_u \cos(2\xi)]u = 0$$

where the terms $a_u$ and $q_u$ are defined as follows:

$$a_u = \frac{8V_{DC}}{\omega^2 I_0^2 (m/e)}$$

$$q_u = \frac{4V_{RF}}{\omega^2 I_0^2 (m/e)}$$
Solutions to the Mathieu equation completely describe the trajectory of an ion in terms of each ion’s initial conditions. Without any force acting along the $z$ axis, the position and velocity of an ion along its $z$ axis are unaffected by any potential applied to the rods. The use of rods of a hyperbolic cross section leads to equations of motion that contain no cross-coordinate terms.\(^{90}\)

Solutions to the Mathieu equation reveal regions of $a$–$q$ space whereby the ion is bound, and therefore the ion is able to remain stable within the region between the four rods and make its way through to the conversion dynodes and electron multiplier detector. This is the so-called stability region. Note that parameters $a$ and $q$ are, in essence, reduced parameters and enable one to simplify the concepts. For a given mass-to-charge ratio $m/e$, a fixed distance $r_0$, and a fixed RF frequency $\omega$, $a$ and $q$ depend only on $V_{\text{DC}}$ and $V_{\text{RF}}$ respectively. In practice, quadrupoles are usually operated in a manner such that the values of parameters $a$ and $q$ are always related by a simple ratio. This condition is established by ensuring that the applied DC potential is always some fraction of the applied AC potential. The ratio is held constant, irrespective of the magnitude of $V_{\text{DC}}$ or $V_{\text{RF}}$. Holding the ratio of $V_{\text{DC}}$ to $V_{\text{RF}}$ constant defines a set of points in $a$–$q$ space called an operating line or mass scan line. If $a$ is divided by $q$ for a given $m/e$, a ratio equal to $2(V_{\text{DC}}/V_{\text{RF}})$ is obtained.

Resolution is increased by slightly changing the slope of the mass scan lines such that it is possible to achieve a desired resolution. Let us review what we mean by mass spectrometric resolution so that we do not confuse this term with chromatographic resolution.

### 48. WHAT IS MASS SPECTROMETRIC RESOLUTION ANYWAY?

Mass spectrometric resolution is defined as the ratio of a given mass $m$ to the smallest discernable difference in mass, $\Delta m$, that can be measured. Mathematically, we have

$$R = \frac{m}{\Delta m}$$

A numerical expression can be obtained from this ratio where $m$ and $\Delta m$ are $m/z$ values of two adjacent peaks in the mass spectrum.\(^{91}\) Table 4.14 suggests, for a given mass and resolution, to what extent one can resolve close $m/z$ values.

In practice, $V_{\text{DC}}$ can be varied from $-250$ to $+250$ V, and $V_{\text{RF}}$ can vary from $-1500$ to $+1500$ V. The magnitude of $V_{\text{RF}}$ is approximately six times that of $V_{\text{DC}}$. The mass scan line is swept across the bandpass region by changing $V_{\text{DC}}$ and $V_{\text{RF}}$ while keeping their ratio constant. A voltage increase is equivalent to sliding the mass scale shown in Figure 4.44 upward and to the right along the mass scan line. Sweeping the voltage applied to the rods thus provides a convenient method of scanning the bandpass region of the MSD.
FIGURE 4.44 Stability diagram of a–q space showing regions where a stable ion trajectory exists.

### TABLE 4.14
Applied $V_{DC}$ and $V_{RF}$ to the Pair of Quadrupole Rods for Six Snapshots in Time

<table>
<thead>
<tr>
<th>Snapshot No.</th>
<th>$V(\text{DC}) + V(\text{RF}) \cos(\omega t)$</th>
<th>Rods 1 and 4</th>
<th>Rods 2 and 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_0$</td>
<td>$+20$</td>
<td>$-20$</td>
<td></td>
</tr>
<tr>
<td>$t_1$</td>
<td>$+140$</td>
<td>$-140$</td>
<td></td>
</tr>
<tr>
<td>$t_2$</td>
<td>$+20$</td>
<td>$-20$</td>
<td></td>
</tr>
<tr>
<td>$t_3$</td>
<td>$-100$</td>
<td>$+100$</td>
<td></td>
</tr>
<tr>
<td>$t_4$</td>
<td>$+20$</td>
<td>$-20$</td>
<td></td>
</tr>
<tr>
<td>$t_5$</td>
<td>$+140$</td>
<td>$-140$</td>
<td></td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
49. THE MATH IS OK, BUT WHAT REALLY HAPPENS TO M·+ WHEN IT IS PROPELLED INTO THE CENTER OF THE QUADRUPOLE?

The mathematics just discussed provide a theoretical basis to understand how the quadrupole MSD selects specific \( m/z \) values with the desired degree of mass spectrometric resolution. Two books \(^{92,93}\) provide sufficient detail for the reader who desires a more in-depth treatment on theory. To answer the question, let us consider “riding the back” of a molecule that has lost its valence electron due to electron impact. This molecule ion can be abbreviated M·+, and taking this viewpoint will enable us to look at how this species gets knocked around by varying the applied voltages to the rods. This pedagogical approach was first used by the Finnigan MAT Institute as part of its training program. \(^{94}\)

Figure 4.45 is a plot of the magnitude of the \( V_{DC} \) and \( V_{RF} \) voltages applied to the rods of a MSD. The dashed line shows how the combined DC and AC voltages vary on the first set of diagonally opposite rods, labeled 1 and 4, while the solid line represents the combined DC and AC voltages applied to the other set of rods, labeled 3 and 4. Our M·+ begins at time \( t_0 \), and we follow four subsequent time frames or “snapshots.” The applied voltages for each of six snapshots are given in Table 4.15 for this exercise. The snapshots are shown in Figure 4.46. Three mass fragment ions of \( m/z \) 4, 100, and 500 are propelled into the center of the four rods.

At time \( t_0 \) with rods 1 and 4 at 20 V and rods 2 and 3 at −20 V, all three fragment ions are attracted to rods 2 and 3, while being repelled by rods 1 and 2. At time \( t_1 \), rods 1 and 4 have become even more positive, while rods 2 and 3 have become even more negative. This change in the rod voltages accelerates all three ions to rod 2. The lightest ion, that with \( m/z \) 4, crashes into rod 2. At time \( t_2 \), rod 2 is suddenly made quite positive and ions of \( m/z \) 100 and 500 are repelled, as shown. At time \( t_3 \), rod 2 is made quite negative and \( m/z \) 500 is too heavy to change direction away from the rod and crashes into it. The ion with \( m/z \) 100 is now heading toward rod

---

**TABLE 4.15**

<table>
<thead>
<tr>
<th>( m )</th>
<th>( R )</th>
<th>( m - \Delta m )</th>
<th>( m )</th>
<th>( m + \Delta m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>90</td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>9.9</td>
<td>10.0</td>
<td>10.1</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>99</td>
<td>100</td>
<td>101</td>
</tr>
<tr>
<td>10</td>
<td>1000</td>
<td>9.99</td>
<td>10.00</td>
<td>10.01</td>
</tr>
<tr>
<td>100</td>
<td>1000</td>
<td>99.9</td>
<td>100.0</td>
<td>100.1</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
and is suddenly repelled by this rod, whose voltage is +140, and is steered toward the center of the rods, as shown. Figure 4.47 shows the somewhat helical path taken by the ion whose $m/z$ is 100 through the quadrupole and to the detector. This ion would be considered bound and resides in $a-q$ space. Beyond this more or less oversimplified concept, in practice, the operation can make minor changes to optimize performance. These changes with respect to operating a Finnigan Incos MSD include the following:

1. Adjusting the low resolution by keeping the $V_{DC}/V_{RF}$ ratio, and hence the slope, constant, but changing the magnitude of the RF envelope
2. Adjusting the high resolution by slightly changing the $V_{DC}/V_{RF}$ ratio

© 2006 by Taylor & Francis Group, LLC
3. Adjusting the offset digital-to-analog converter by changing the zero reference of the RF start points
4. Adjusting the offset program by changing slopes, while starting points are unaffected

50. **IS THE MSD THE ONLY MASS SPEC USED IN TEQA?**

No, a mass spec opposite in concept to the MSD, yet utilizing similar principles, is that of the quadrupole ion trap mass spectrometer (ITD). The ITD is often found in environmental testing labs. Theoretically, it is the three-dimensional analog of the MSD. It is generally less expensive than the MSD and is of a more recent development.

If little to no DC voltage is applied to a quadrupole, ions with any \( \frac{m}{z} \) will remain in the \( a-q \) stability region. The ion trap is depicted schematically in Figure 4.48.

The distance from the center to the end cap, \( z_0 \), is called the axial distance. The distance from the center to the ring electrode, \( r_0 \), is called the radial distance. It helps to imagine that the plane of this paper contains the \( x, y \) coordinates and that this plane cuts through the ITD at its center. The \( z \) axis is then viewed as being above the \( x-y \) plane, and the radial distance, \( r \), rests within this plane. Ion traps are compact, with the filament positioned just above the upper end cap, in contrast to MSDs, whereby the ion source is separated. A variable RF voltage is applied to the ring electrode while the two end cap electrodes are grounded or set at either a positive

---

**FIGURE 4.46** Behavior of three ions of different \( m/z \) ratios in the space between the four quadrupole rods.

© 2006 by Taylor & Francis Group, LLC
or negative voltage. As the RF voltage is increased, the heavier ions remain trapped while the lighter ions become destabilized and exit the trap through the bottom end cap. This bottom cap has openings drilled into the stainless steel to allow these ions to escape. A good introduction to ion trap mass spectrometry, including some interesting historical facts, can be found in the article written by Cooks et al.\textsuperscript{95}

With reference to Figure 4.48, a symmetrical electrical field allows consideration of only a radial and $z$ displacement. The equations of motion of an ion in the three-dimensional quadrupole field are derived as in the case of the quadrupole mass filter:

\[
\begin{align*}
\frac{d^2z}{dt^2} &= \frac{4}{(m/e)\epsilon_0^2}[V_{DC} - V_{RF} \cos(\omega t)]z = 0 \\
\frac{d^2r}{dt^2} &= \frac{2}{(m/e)\epsilon_0^2}[V_{DC} - V_{RF} \cos(\omega t)]r = 0
\end{align*}
\]

with the following substitutions:

\[
\begin{align*}
a_z &= -2a_r = -\frac{16V_{DC}}{(m/e)\epsilon_0^2 \beta_{RF}^2} \\
a_r &= -2a_z = -\frac{8V_{RF}}{(m/e)\epsilon_0^2 \beta_{RF}^2}
\end{align*}
\]

Let $u$ represent either $r$ or $z$ and let $\xi = \omega t/2$.

In a manner similar to that derived for the MSD, the canonical form of the Mathieu equation results. Note that the stability parameters for the $z$ and $r$ directions differ by a factor of $-2$. According to March and Hughes,\textsuperscript{96}

The stability regions defining the $a, q$ values corresponding to solutions of the Mathieu equation that are stable in the $z$ direction. The regions corresponding to solutions that are stable in the $r$ direction must be thought of as being twice the size of the $z$ direction and then rotated about the $q$ axis to account for the minus sign. The region of simultaneous stability comprises the intersection of these two regions.
Radial stability expressed in terms of $a_r$ and $q_r$ must also be maintained with $z$ direction stability. Ions of a given $m/e$ are stable in the trap under operating conditions given in $a-q$ space. Figure 4.48 depicts a small section of the $a-q$ stability region very near to the origin for a typical ion trap. This figure graphically demonstrates how three ions of different $m/e$ values can be moved along the $q_z$ axis by simply increasing $V_{RF}$ until the ions are sequential, according to increasing $m/e$ ejected from the trap into the instability region of $a-q$ space. This occurs at a value of $q_z = 0.908$.

51. AGAIN, THE MATH IS OK, BUT THIS TIME, WHAT ABOUT SOME APPLICATIONS OF C-GC-MS(ITD)?

This author has maintained a C-GC-MS(ITD) instrument for several years. One of the more interesting requests that not only utilized this instrument, but also involved some sample preparation, came from the forestry area. The homologous series of 2-aminoethanols (2-AEs) are organic compounds of importance to wood preservative
Few methods, if any, can be found in the analytical literature that describe the qualitative and quantitative analyses of samples that contain any of the three 2-AEs. This series begins with 2-aminoethanol, followed by N-methyl-2-AE and, upon further substitution, N,N-dimethyl-2-AE. These compounds lack a UV-absorbing chromophore, and hence are not directly amenable to analysis using HPLC. The 2-AEs are quite polar, owing to both the presence of an amino functionality and being an alcohol. The C-GC-MS(ITD) that was configured in the author’s laboratory is shown in Figure 4.49. An Autosystem GC (PerkinElmer) was interfaced to a 800 Series (Finnigan, ThermoQuest) ITD. The WCOT column was passed through the transfer line into the ITD itself, as is shown. The three 2-AEs were easily separated using a DB-624 WCOT (J&W Scientific). An analytical method was developed that included novel sample preparation techniques coupled to the separation by GC, identification, and quantitative analysis by the ITD.97

52. I HEAR THAT A MASS SPEC MUST BE TUNED, HOW IS THIS DONE?

We are most familiar with the word tuning as it relates to what we do each day with a radio or television. Indeed, good laboratory practice (GLP) requires that the mass spectrometer interfaced to either a GC or LC be tuned to a specific set of criteria (tuning specifications) before the instrument can be used as a determinative technique to conduct TEQA. EPA methods require that the mass spec be tuned using decafluorotriphenyl phosphine (DFTPP) if SVOCs are to be determined and 4-bromofluorobenzene (BFB) if VOCs are to be determined. Other methods require that the mass spec be tuned to a set of criteria only for the tuning compound intrinsic to the instrument. In most instruments, this compound is perfluorotributyl amine (PFTBA), whose molecular structure is shown below (showing only one perfluorobutyl moiety for clarity):

![PFTBA molecular structure](image)

The three most abundant fragment ions in the EI mass spectrum for PFTBA are used for tuning. Loss of \(-\text{CF}_2\text{-CF}_2\text{-CF}_3\) with the charge remaining on nitrogen, \((\text{C}_3\text{F}_9)\text{N-CF}_2^+\), yields a fragment ion whose \(m/z\) is 502. Heteroatom cleavage of the N to F covalent bond, with the charge residing on the carbon, \(\text{C}_6\text{F}_{12}^+\), yields a
FIGURE 4.49 Configuration of a capillary GC-MS (ion trap) developed in the author’s lab.
fragment ion whose $m/z$ is 219. Cleavage of the C–C bond at the terminal carbon, with the charge residing on the carbon, CF$_3^+$, yields a fragment ion whose $m/z$ is 69. Use of perfluoro-organic compounds as reference standards for mass spec tuning purposes eliminates isotopic abundances due to hydrogen since fluorine does not have an isotope. The M + 1 abundance is due only to the 1.1% of all carbon that naturally exists as the $^{13}$C isotope. This feature serves to simplify the EI mass spectrum of PFTBA. The $m/z$ 69 fragment should show a $^{13}$C isotopic abundance of ~1.1%, owing to a fragment containing only one carbon; the $m/z$ 219 fragment should show a $^{13}$C isotopic abundance of ~4.4%, due to this fragment having four carbons; and the $m/z$ 502 should show a $^{13}$C isotopic abundance of ~9.9% for the same reason. Because there are three N–F covalent bonds, and three C–CF$_3$ covalent bonds in each molecule of PFTBA, it would be expected that the $m/z$ 69 and $m/z$ 219 fragment ions should be the most abundant masses found, and this is indeed the case.

The Model 5973 MSD® (Agilent Technologies) is considered the most widely used low-resolution mass spectrometer for single quadrupole operation in contemporary enviro-chemical and enviro-health testing labs. Under high vacuum, the ion source volume depicted in the sketch below creates, accelerates, and focuses the intact molecular ion and fragments of the molecular ion for entrance into the quadrupole region, where mass selection occurs. Ions whose stable trajectories pass through the four rods hit the high-energy conversion dynode (HED) operating at −10,000 V, whereby electrons are created and attracted to the less negatively charged electron multiplier (EM). The EM amplifies the signal output by ~100,000. The sketch below (side view of a conventional single quadrupole mass spec) shows where the various devices are located. Values assigned to these devices represent the parameters that can be adjusted in the tuning process:

Referring to the above sketch, the ion source contains two filaments. This provides backup in case the filament in use burns out. The filament’s emission current can be set by the user; however, it is recommended that the default setting be used. The electron energy can be set on the 5973 MSD. The optimum setting is 70 electron-volts (eV). This value yields the most vigorous ionization that results in the most abundant fragmentation patterns for organic molecules. A 70-eV setting has been
used to generate most of the reference electron-impact (EI) mass spectra that comprise the large libraries available.

Referring again to the sketch above, a positive voltage applied to the repeller pushes these newly generated positively charged molecular ions out of the ion source. There exists an optimum repeller voltage that guarantees that sufficient ion abundance is achieved without an overly high ion velocity being created and leaving the ion source prematurely. The ion focus lens serves to narrow the stream of positive ions, and without this voltage, poor mass response is evident at the higher end of the mass range. The entrance lens minimizes the fringing field of the quadrupole. Increasing the entrance lens voltage increases the abundance at high mass and decreases the abundance at low mass. AMU gain and AMU offset parameters affect the $V_{\text{DC}}/V_{\text{RF}}$ ratio of the mass filter, while the mass axis gain and mass axis offset serve to recalibrate the mass axis scale. Table 4.16 lists each major tuning parameter, possible range for programmable values, and just what influence or effect a change in a given tune setting has on PFTBA’s mass spectrum for a 5973 MSD.

We now proceed to outline the three major steps necessary to complete a manual tune adjustment for the 5973 MSD. The manual tune should follow an autotune performed by the ChemStation software. Perform a manual tune when you must meet criteria perhaps established in an analytical method being implemented.

**Step 1:** Minimize the peak width of PFTBA that is allowed to leak into the mass spec by adjusting the AMU gain. The $m/z$ 502 is influenced more strongly than are the lower masses, 69 and 219. AMU offset moves the scan line up or down. Decreasing the offset leads to wider peaks and loss

---

**TABLE 4.16**

<table>
<thead>
<tr>
<th>Tuning Parameter</th>
<th>5973 MSD</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filament</td>
<td>70 eV 300 µV emission</td>
<td>Energy of electron beam; number of electrons generated</td>
</tr>
<tr>
<td>Repeller</td>
<td>0–42.7 V</td>
<td>Pushes ions out of source</td>
</tr>
<tr>
<td>Draw out</td>
<td>Ground potential</td>
<td>Entrance aperture of lens stack</td>
</tr>
<tr>
<td>Ion focus</td>
<td>0–242.0 V</td>
<td>Relative abundance</td>
</tr>
<tr>
<td>Entrance lens</td>
<td>0–128 mV/amu</td>
<td>Relative abundance</td>
</tr>
<tr>
<td>Entrance lens offset</td>
<td>0–127.5 V</td>
<td>Relative abundance</td>
</tr>
<tr>
<td>AMU gain</td>
<td>0–4095</td>
<td>Peak width</td>
</tr>
<tr>
<td>AMU offset</td>
<td>0–255</td>
<td>Peak width</td>
</tr>
<tr>
<td>X-ray HED</td>
<td></td>
<td>Optimizes sensitivity for a particular EM voltage setting</td>
</tr>
<tr>
<td>EM</td>
<td>0–3000 V</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Mass axis gain</td>
<td>±2047</td>
<td>Mass assignment</td>
</tr>
<tr>
<td>Mass axis offset</td>
<td>±499</td>
<td>Mass assignment</td>
</tr>
</tbody>
</table>
of mass spectrometric resolution, while increasing the offset leads to narrow
peaks and higher resolution. The Mathieu stability diagram (Figure 4.44)
is resketches below showing all three mass-to-charge ratios for PFTBA:

AMU gain affects the $V_{DC}/V_{RF}$ ratio of the mass filter. This controls the
width of the mass peaks. Increasing the gain increases the slope of the
scan line (see above) and more profoundly influences the 502 mass while
having a much smaller effect on masses 69 and 219. AMU offset also
affects the $V_{DC}/V_{RF}$ ratio of the mass filter. This also controls the width of
the mass peaks. A high offset yields narrower peaks, and this effect applies
equally across the mass range.

**Step 2:** Perform a mass axis calibration by adjusting the mass axis gain or
mass axis offset. The tuning algorithm calibrates the mass axis to within
±0.2 amu. When PFTBA is allowed to leak into the mass spec, one would
expect the $m/z$ for 69 to read 69.00. If 69.34 appears, the mass axis gain
and offset can be adjusted to bring the mass axis back to 69.00.

**Step 3:** Adjust the EM voltage to match the $m/z$ abundance criteria in terms
of counts. Meeting this criteria, such as requiring the $m/z$ 69 peak to exhibit
an abundance of ~500,000 ± 50,000 counts, provides the requisite sensi-
tivity that is required for a given method.

ChemStation provides a half-dozen different tune algorithms. The particular
model of MSD will determine which tunes are available. Listed below are the various
tunes available and a brief description of each.

© 2006 by Taylor & Francis Group, LLC
A properly tuned MSD yields the following relative abundance ratios for one fragment ion relative to another and for carbon isotopic abundances for PFTBA:

<table>
<thead>
<tr>
<th>m/z</th>
<th>Relative Abundance Criteria for Proper PFTBA Tune</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>100% (base peak)</td>
</tr>
<tr>
<td>70/69</td>
<td>0.5% ≤ x ≤ 1.6%</td>
</tr>
<tr>
<td>219/69</td>
<td>70% ≤ x ≤ 250%</td>
</tr>
<tr>
<td>220/219</td>
<td>3.2% ≤ x ≤ 5.4%</td>
</tr>
<tr>
<td>502/69</td>
<td>3% ≤ x</td>
</tr>
<tr>
<td>503/502</td>
<td>7.9% ≤ x ≤ 12.3%</td>
</tr>
</tbody>
</table>

53. **WHAT'S THE DIFFERENCE BETWEEN SO-CALLED SOFT VS. HARD MASS SPEC?**

GC-MS using a single quadrupole mass spectrometer can provide both electron-impact (EI) and chemical ionization (CI) mass spectra. Often CI complements EI. EI bombards neutral organic molecules with highly energetic (70-eV) electrons. These electrons are boiled off from a hot filament wire and accelerated. Energy is available from the 70-eV electrons not only to ionize the molecule, but also to cause the molecule to break apart or fragment. For the most part, the molecule fragments in well-understood ways. Ionization using CI is also achieved without the transfer of excessive energy, and this soft ionization yields a molecular adduct. Watson98 has articulated this difference as follows:

CI differs from EI in that molecules of the compound of interest are ionized by interaction or collision with ions of a reagent gas rather than with electrons. If alkyl reagent gases such as methane are employed, CI generally effects protonation of the molecule of interest. The site of protonation is most likely on a heteroatom of greatest proton affinity; some fragmentation of this protonated molecular ion may involve elimination of the heteroatom. Because fission of C-Cs bonds is rarely involved, this
type of CI produces little fragmentation, which provides an insight to molecular structure.

Consider an analyte symbolized by using the letter A being introduced into an EI ion source of a GC-MS or stand-alone MS. Also consider analyte A being introduced into a second instrument, this instrument being configured with a CI ion source. Two different fragmentation pathways occur:

\[
\text{EI} \quad e^-(70 \text{ eV}) + A \quad \xrightarrow{2e^-} A^+ \quad \xrightarrow{F_1, F_2, F_3} F_1, F_2, F_3
\]

The possibility exists for fragmentation to occur as \( A^+ \) disposes of excess energy. The degree of fragmentation depends upon stabilities of various charged species. A typical EI mass spectrum that reflects the above gas-phase reaction is depicted below:

![EI Mass Spectrum](image)

The predominant species is a protonated molecule with the possibility of adducts. The degree to which an analyte molecule such as A gets protonated to \( \text{AH}^+ \) is governed by the analyte’s proton affinity. These values are tabulated. A typical CI mass spectrum is depicted below:

\[
\text{CI} \quad \text{CH}_4 + e^-(70 \text{ eV}) \quad \xrightarrow{2e^-} \text{CH}_4^+
\]

\[
\text{CH}_4 + \text{CH}_4^+ \quad \xrightarrow{} \text{CH}_5^+ + \text{CH}_3^+
\]

\[
\text{CH}_5^+ + A \quad \xrightarrow{} \text{AH}^+ + \text{CH}_4
\]

The predominant species is a protonated molecule with the possibility of adducts. The degree to which an analyte molecule such as A gets protonated to \( \text{AH}^+ \) is governed by the analyte’s proton affinity. These values are tabulated. A typical CI mass spectrum is depicted below:
54. HOW DO NEGATIVE IONS GET DETECTED IN GC-MS THAT BENEFITS TEQA?

By moving away from EI conditions, reducing energetic (70-eV) electrons to thermal (0- to 15-eV) electrons, and introducing CI conditions into the ion source. What this does is to enable the elevated gas pressure in the ion source to form secondary electrons with energies low enough (so-called thermal electrons) to yield resonance electron capture and dissociative electron capture. Reactions can be summarized as follows, where M-X represents an organic compound, with M being that moiety in the molecule that would under positive EI conditions yield a molecular ion M⁺ (discussed earlier) and a more electronegative moiety such as Cl, F, NO₂, CN, or OH.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-X + e² (&gt;20 eV) M⁺ X⁻</td>
<td>ion pair formation (observed with highly fluorinated-, polynitro-, and some B-, P-, S-, and metal-containing compounds)</td>
<td></td>
</tr>
<tr>
<td>M-X + e⁻ (-0–1 eV)</td>
<td>MX⁻</td>
<td>resonance capture or resonance electron capture or simply electron capture (most useful to TEQA)</td>
</tr>
<tr>
<td>M-X + e⁻ (-0–15 eV)</td>
<td>M⁻ + X⁻</td>
<td>dissociative resonance capture (usually minimized or avoided in TEQA)</td>
</tr>
</tbody>
</table>

Budde⁹⁹ has articulated the importance of resonance electron-capture negative ion GC-MS in the following manner:

Electron capture ionization is the most important negative-ion technique used in chemical analysis. High purity methane or isobutane are the standard reagent gases used to generate the plasma of thermal electrons needed for electron capture. The ionization technique has the advantages of simplicity, high sensitivity for some analytes, high selectivity, and often little or no fragmentation of the M⁻ ion, which allows determination of molecular weight.

55. HOW DOES GC-MS USING A SINGLE QUADRUPOLE RELATE TO TEQA?

It is beyond the scope of this book to digress into the interpretation of mass spectra for qualitative purposes. Several books on the interpretation of EI spectra are available.¹⁰⁰
The most abundant molecular ion or fragment ion in the mass spectrum of a given compound is usually selected for quantification. Tables at the end of each EPA method that requires GC-MS as the determinative technique will usually list these quant or Q ions for each of the analytes to be quantitated. TEQA is carried out by using an internal standard (IS) mode of instrument calibration, provided that a suitable IS can be found. If isotopically labeled organic compounds, otherwise identical to the analyte of interest, are available, the isotope dilution calibration mode is employed (Chapter 2).

We next focus on selective mass spectra themselves. Figure 4.50 shows EI mass spectra drawn from the National Institute of Standards and Technology (NIST) mass spectral library, available within the GC-MSD ChemStation software, for two of the most notorious and toxic organic compounds known: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF). Their molecular structures are shown below:

Referring to Figure 4.50, and without any rules for EI mass spectral interpretation, we compare both mass spectra. The molecular ions (denoted by a cluster around m/z 322 and 306, respectively) in both spectra are the most abundant for each molecule, and the mass differs between molecules by 16 atomic mass units (amu) or Daltons (Da). This difference is explained by noting that the furan contains one less oxygen atom than the dioxin. The multiplicity of ions surrounding the molecular ion indicates polychloro substitution. The m/z value at 257 Da in the dioxin spectrum is attributed to loss of a COCl from the molecular ion. The ratio of the abundances of m/z 320 and 322, which are the molecular ions $^{35}$Cl$_4$-2378–TCDD and $^{35}$Cl$_3^{37}$Cl-2378–TCDD,
must be within 10% of the value expected from the natural abundances of $^{35}\text{Cl}$ and $^{37}\text{Cl}$.\textsuperscript{101} This completes our discussion of the single quad mass spec, which represents a type of \textit{scanning} mass spec. Let us digress a bit and pick up the physics that underlie one of several \textit{batch} mass specs, namely, the time-of-flight mass spectrometer.

56. \textbf{HOW DOES A TIME-OF-FLIGHT MASS SPECTROMETER WORK?}

Watson\textsuperscript{102} describes a time-of-flight (TOF) mass spec this way:

The operating principle of the TOF mass spectrometer involves measuring the time for an ion to travel from the ion source to the detector. This process requires producing a discrete “bunch” of ions in a region near the ion source and then, through a series of synchronized events, accelerating them toward and measuring their time of arrival at

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{library EI mass spectra of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran}
\caption{Library EI mass spectra of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran.}
\end{figure}
a detector located 1 to 2 m from the source. All the ions receive the same kinetic energy
during acceleration (e.g., 3000V), but because they have different masses, they separate
into groups according to velocity (and hence mass) as they traverse the field-free region
between the ion source and detector. The \( \frac{m}{z} \) value of an ion is determined by its time
of arrival at the detector. Ions of low mass reach the detector before those of high mass,
because these heavier ions have a lower velocity.

A schematic for a TOF mass spec is shown below:\textsuperscript{102}

The derivation below is adapted from Berry et al.\textsuperscript{103}

A molecular ion or fragment ion leaves the acceleration region with an average
kinetic energy \( T \) of mass \( m_j \), whose \( x \) component has a velocity \( v_{xj} \) according to

\[
T = \frac{1}{2} m_j v_{xj}^2
\]

This same molecular ion or fragment ion acquires a kinetic energy \( T \) whose
charge is \( q_j \) under an electric field in the \( x \) direction \( E_x \) across a distance \( d \) such that

\[
T = q_j E_x d
\]

Eliminating \( T \) between both equations and solving for \( v_{xj} \) gives

\[
v_{xj} = \sqrt{\frac{2q_j E_x d}{m_j}}
\]

We also know that an object with a fixed velocity in the field-free drift tube of
length \( D \) will arrive at the detector at a time \( t_j \) (the time of flight) according to

\[
v_{xj} = D \cdot t_j
\]

Eliminating \( v_{xj} \) from both equations above yields an expression for the time of
flight \( t_j \) according to

\[
t_j = D \sqrt{\frac{m_j}{2q_j E_x d}}
\]

© 2006 by Taylor & Francis Group, LLC
or

\[ t_j = D \sqrt{\frac{1}{2E_i d} \left( \frac{m_j}{q_j} \right)} \]

The above equation, the principal outcome of this derivation, proves that the time of flight is directly proportional to the mass-to-charge ratio. Two different masses, \( m_i \) and \( m_j \), for a single charge would arrive at the detector in a ratio dictated by their ratio of masses, as follows:

\[ \frac{t_i}{t_j} = \frac{m_i}{m_j} \]

Interfacing a TOF with a GC gives another powerful hyphenated determinative technique that is currently finding its way into enviro-chemical and enviro-health laboratories. Let us digress a bit and contemplate just how GC-MS adds a third dimension to the GC chromatogram.

57. WHAT ARE WE REALLY SEEING WHEN WE PEER INTO A COMPUTER SCREEN WHILE ACQUIRING GC-MS DATA?

We see a three-dimensional chromatogram in two-dimensions! The sketch below (adapted from a training manual from Agilent Technologies) is a 3-D plot of ion abundance vs. mass vs. chromatographic run time (i.e., time after GC injection). When the abundances at each instant of time (across the mass spectrum for each chromatographically resolved analyte) are added together, a *Reconstructed Ion Chromatogram* (RIC) is observed. GC-MS software will give you mass chromatograms as well as RICs. A mass chromatogram can be viewed in the drawing below as being represented by a plane that slices through a specific m/z value. A plot of ion abundance for a selected m/z against chromatographic run time yields a mass chromatogram.
In other words, data from a GC-MS needs to be interpreted in three dimensions even though the analyst is seeing on the PC screen only two dimensions at any moment. Cartesian coordinates are defined below:

The RIC is viewed in the $x$–$z$ plane by taking a slice at $y = 0$. A mass chromatogram is represented in the $x$–$z$ plane by taking a slice at $y > 0$. Across a given chromatographically resolved peak, such as the peak that elutes near 6 min in the previous sketch, a mass spectrum at the apex of this peak is analogous in the $y$–$z$ plane by taking a slice with $x > 0$.

58. **HOW ARE THESE QUANT IONS FOUND?**

TEQA using GC-MS is, in most cases, based on selecting the base peak from the mass spectrum of a chromatographically resolved analyte. The base peak is defined as the most abundant ion. In some organic compounds, the base peak corresponds to the molecular ion, while in other compounds, a prominent fragment ion is the base peak. To illustrate, let us focus again on the ubiquitous trihalomethanes (THMs). These volatile organics are found in municipal drinking water supplies due to disinfection processes involving chlorine and chlorine-containing chemicals. EPA Method 524.1 combines a sample prep technique (purge and trap, introduced in Chapter 3) with a determinative technique (C-GC-MS). At the end of the method, a table of molecular weights and quantitation ions for all VOCs considered in the method is found. Let us look at the four THMs tabulated from this method:

<table>
<thead>
<tr>
<th>THM</th>
<th>Molecular Formula</th>
<th>MW</th>
<th>Primary Q Ion</th>
<th>Secondary Q Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromodichloromethane</td>
<td>CHCl₂Br</td>
<td>162</td>
<td>83</td>
<td>85, 127</td>
</tr>
<tr>
<td>Bromoform</td>
<td>CHBr₃</td>
<td>250</td>
<td>173</td>
<td>175, 252</td>
</tr>
<tr>
<td>Chloroform</td>
<td>CHCl₃</td>
<td>118</td>
<td>83</td>
<td>85</td>
</tr>
<tr>
<td>Dibromochloromethane</td>
<td>CHClBr₂</td>
<td>206</td>
<td>129</td>
<td>127</td>
</tr>
</tbody>
</table>
Bromodichloromethane fragments under EI conditions as follows:

\[
\begin{align*}
\text{CHCl}_2\text{Br} & \quad \text{e}^- (70 \text{ eV}) \quad \text{CHCl}_2\text{Br}^+ \\
\text{Loss of } 2e^- & \quad \text{m/z } 163 \\
\text{--Cl} & \quad \text{m/z } 128 \\
\text{--Br} & \quad \text{m/z } 83 \\
\text{CHBrCl}^+ & \\
\text{CHCl}_2^+ & 
\end{align*}
\]

The other three THMs undergo heteroatom cleavage in a similar manner. The fragment ion of m/z 83 is the parent ion in the EI mass spectrum and comprises the primary quant ion. It is this ion abundance that is proportional to the concentration of bromodichloromethane in the original drinking water sample, and this fact leads to quantification. The secondary quant ions of m/z 85 and m/z 127 reflect the significant natural isotopic contributions of chlorine and bromine, respectively. What happens when single quadrupoles are placed in tandem?

59. WHAT IS TANDEM MASS SPECTROMETRY AND WHAT ROLE DOES IT PLAY IN TEQA?

Budde (p. 229) answers the question this way:

Tandem mass spectrometry is the linking together in space or time of two independently operating m/z analyzers. Ions are formed in an ion source, separated in the first analyzer, undergo ion-molecule reactions in a collision cell, and the ionic products of these reactions are measured in the second analyzer.

Tandem MS techniques enhance analyte selectivity as well as analyte sensitivity. A fragment ion from the first m/z analyzer can undergo collision-induced dissociation (CID) with an inert gas such as He or Ar to yield product ions. The collision cell is often a quadrupole analyzer operated in the RF-only mode, thereby trapping (in a radial sense) the residual parent ions and all of the daughters in an appropriate concentration of collision gas. The second m/z analyzer or third quadrupole provides a means of analyzing all of the products of CID. A block diagram of a tandem-in-space MS is shown below:
Figure 4.51 illustrates the essence of the tandem MS technique. A molecule leaves the ion source after being ionized and fragmented. The fragment at $m/z$ 129 is selected after passing through the first $m/z$ analyzer and undergoes decomposition in the collision cell. The fragment at $m/z$ 129 is further fragmented by CID and enters the second $m/z$ analyzer to yield the product ion mass spectrum as shown.

Benchmark papers that established tandem-in-space techniques first explored double-focusing instruments consisting of magnetic and electrostatic sectors. This was followed by connecting multiple quadrupole analyzers. Benchmark papers that first explored single ion storage type analyzers, such as a Fourier transform mass spectrometer or an ion trap by a sequence of timed ion storage, isolation, reaction, and measurement events, represent tandem-in-time concepts and will not be pursued further in this book.

It is important when introducing concepts that underlie the use of tandem MS to summarize the data acquisition strategies that we have available to maximize this enhanced selectivity over that of a single quadrupole analyzer. This is best shown in the flowchart below:
In the product ion scan strategy, a molecular ion or other fragment ion is selected by setting the first $m/z$ analyzer to operate in the SIM mode. After CID, the second $m/z$ analyzer is set to scan mode and a mass spectrum is generated. The chromatogram for a product ion scan would yield a series of peaks originating from a specific $m/z$ selected to yield a spectra of products.

In the selected reaction monitoring (SRM) strategy, a specific transformation such as that shown

\[
M + \rightarrow B^+ 
\]

provides a highly selective measure of the original molecule $M$. Budde (p. 233) cites the prime advantage of SRM as follows:  

The advantage of SRM is tremendous selectivity for a specific substance and the exclusion of any potential interferences including chemical noise and coeluting substances with the same integer masses. Although coeluting compounds may produce multiple $M^+$, and these ions will be injected into the collision cell, it is unlikely that substances other than the target analyte will produce the $B^+$ fragment ion by CID. SRM is the most widely used MS/MS technique for quantitative analysis of very complex samples.

In the precursor or parent ion scan strategy, the reverse of product (daughter) ion scan occurs. In this case, the second $m/z$ analyzer sits on a specific mass via SIM and the chromatogram yields a series of peaks that correspond to molecules that give precursor ions that undergo CID to produce the specific ion.

In the neutral loss scan strategy, both analyzers are operated in the scan mode with a constant time delay between the scans from both. This delay or mass difference corresponds to a specific mass difference.

60. **IS GC-MS-MS USED IN TEQA?**

Yes, indeed. To illustrate an application, consider recent work in enviro-health QA that utilizes a GC-MS-MS. Driskell and coworkers at the CDC’s National Center for Environmental Health published a method to quantitatively determine the significant organophosphonate nerve agent metabolites (VX acid, GB acid, GA acid, GD acid, and GF acid) in human urine via isotope dilution (a deuterated methyl group is available) of the protonated daughter ion of each nerve agent metabolite.
under chemical ionization and SRM. Let us focus on Sarin, the nerve agent released in an apartment located in Matsumoto, Japan, in 1994. The other nerve agents behave in a similar manner. Sarin was then released in a subway in Tokyo in 1995. Sarin is known to hydrolyze in the environment to the GB acid form. The body metabolizes Sarin to the GB acid form as well. The analytical method converts the GB acid to a methyl phosphonate ester by reacting the GB acid with diazomethane according to:

\[
\begin{align*}
 &\text{OH} \quad \text{CH}_2\text{N}_2 \quad \text{P} \\
 \text{O} \quad \text{O} &\quad \text{O} \\
 &\text{O} \quad \text{OH} \\
 \text{O} &\quad \text{P}
\end{align*}
\]

Under CI conditions using isobutane as the reagent gas and Argon as the collision gas for CID, the methyl phosphonate ester loses the isopropyl group and yields a daughter ion for both the native and deuterated isotopes as shown below:

\[
\begin{align*}
 &\text{H}^+ \quad \text{CID} \quad -(\text{CH}_3)_2\text{CH} \\
 &\text{P} \quad \text{O} \\
 \text{OH} &\quad \text{H}^+
\end{align*}
\]

This approach is just one of several approaches to isolating, recovering, and quantitating organophosphonate nerve agent metabolites. We now move out of GC and GC-MS. Next, we introduce the other cornerstone to column chromatography deemed essential to meet the goals of TEQA: high-performance liquid chromatography (HPLC).

### 61. WHERE DOES HPLC PLAY A ROLE IN TEQA?

This author was astonished one day back in the 1980s when he obtained a sample of an aqueous phase from a Superfund waste site that had been extracted (refer to the discussions of LLE in Chapter 3) with methylene chloride. The remaining extracted aqueous phase was sent to waste. The organic extract was analyzed using EPA Method 8270. This is a C-GC-MS(MSD) determinative method that separates, identifies, and quantitates about 90 priority pollutant SVOCs. The author injected about 10 µL of this discarded aqueous phase into an HPLC in a reversed-phase mode using a UV absorbance detector. Over a half-dozen large peaks in the HPLC
chromatogram were observed, and the retention times for these peaks closely matched those of phenol and some substituted phenols. This author exclaimed, “I thought all of the priority pollutant organics were extracted out!” Upon further thought, if the percent recoveries of these phenols is low, then it is reasonable to assume that these polar analytes would remain in the aqueous phase due to the appreciable hydrogen bonding between the hydroxy group on the phenol and the oxygen end of the water molecule. In Chapter 3, we discussed at length the fact that significant differences in partition coefficients between polar and nonpolar solutes lead to differences in percent recoveries. According to Figure 4.1, if there remained residual phenols, then we should expect the complementary determinative technique of HPLC to detect these polar solutes.

Current EPA methods requiring HPLC as the principal determinative technique found in SW-846 to quantitate semi- and non-volatile analytes of enviro-chemical interest are listed, along with their annotated titles, below:

- **Method 8310**: Polycyclic Aromatic Hydrocarbons (PAHs) using High Performance Liquid Chromatography (RP-HPLC-UV/FL)
- **Method 8315A**: Determination of Carbonyl Compounds by High Performance Liquid Chromatography (RP-HPLC-UV)
- **Method 8316**: Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (RP-HPLC-UV)
- **Method 8318**: N-Methylcarbamates by High Performance Liquid Chromatography (RP-HPLC-FL with post column reaction and derivatization)
- **Method 8321A**: Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection
- **Method 8325**: Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry (HPLC/PB/MS)
- **Method 8330**: Nitroaromatics and Nitramines by High Performance Liquid Chromatography (RP-HPLC-UV)
- **Method 8331**: Tetrazene by High Performance Liquid Chromatography (RP-HPLC-UV)
- **Method 8332**: Nitroglycerine by High Performance Liquid Chromatography (RP-HPLC-UV)
- **EP Method 8323**: Determination of Organotins by Micro-Liquid Chromatography-Electrospray Ion Trap Mass Spectrometry

EPA Method 8323 is the most recent addition to SW-846 and reflects perhaps what the future will hold as LC-MS determinative techniques become increasingly accepted in the regulatory arena. Principles of LC-MS are introduced later in this chapter. Comments to this new method, found at EPA’s SW-846 web site, are given below:113

Method 8323 is the first product of an EPA Office of Solid Waste (OSW) project to develop a series of class-specific Electrospray HPLC/MS methods to replace the obsolete
Thermospray interface currently used in Method 8321. When the project is complete, OSW will issue a single integrated Electrospray HPLC/MS method. Method 8323 covers the use of solid-phase extraction (SPE) discs, solvent extractions (for biological tissues) as sample preparation methods, and micro-liquid chromatography (LC) coupled with Electrospray ion trap mass spectrometry (ES-ITMS) [this technique would also be applicable to ES-quadrupole mass spectrometry (ES-MS)] for the determination of organotins (as the cation) in waters and biological tissues.

Let us focus here on determining the various carbonyl compounds. Figure 4.52 shows an HPLC chromatogram for the separation and detection of the first six homologous series of aldehydes as their 2,4-dinitrophenyl hydrazone derivatives, accomplished in the author’s laboratory. The reaction of aldehydes and ketones with 2,4-dinitrophenyl hydrazine under mildly acidic conditions in water to form stable hydrazones is a well-known reaction in organic chemistry. The reaction of C₃ (pro-pionaldehyde) with 2,4-dinitrophenyl hydrazine to yield the derivative is illustrated below:

```
O
\[\text{Propionaldehyde} \quad + \quad \begin{array}{c}
\text{2,4-dinitrophenylhydrazine}
\end{array} \]
\[\rightarrow \]
\[\begin{array}{c}
\text{2,4-dinitrophenylhydrazone of propionaldehyde}
\end{array}\]
```

This method nicely illustrates the importance of HPLC to environmental testing labs and forms the basis for EPA Method 8315A, “Determination of Carbonyl Compounds by HPLC.”¹¹³ Aldehydes and ketones are, in general, too polar to be isolated from aqueous matrices and are too thermally labile or thermally reactive for the GC determinative technique. Note the wavelength setting on the UV detector to 365 nm in Figure 4.52. This wavelength is sufficiently removed from the crowded region between 200 and 300 nm, and therefore can be considered somewhat unique for 2,4-diphenyl hydrazones. Method 8315A combines both sample prep and determinative techniques and offers both LLE and SPE to isolate and recover the 2,4-diphenyl hydrazones from an aqueous or soil matrix. The method suggests that gaseous stack samples are to be collected by Method 0011 and indoor air samples.
FIGURE 4.52 RP-HPLC separation and detection of the homologous series of aldehydes as their 2,4-dinitrophenyl hydrazones.

Homologous series of aldehydes
As their 2,4-dinitrophenylhydrazone
Derivatives; separated by RP-HPLC-UV
Wavelength @ 365 nm

Mobile phase: 80% ACN/20% water
Flow rate @ 1.0 mL/min
Column: C18 silica
Trace Environmental Quantitative Analysis, Second Edition

are to be collected by Method 0100. We now introduce the basics of the HPLC determinative technique.

62. WHAT IS HPLC?

Some nomenclature with respect to the broad field of liquid chromatography is in order. The concept of HPLC should be called high-performance liquid chromatography because column efficiency is so greatly improved when columns contain a stationary phase whose particle size has been reduced to 10 µm, or to 5 µm, and even as low as 3 µm. This reduced particle size from that used in column liquid chromatography necessitates that the mobile phase be pumped through these columns. The pressure drop across these low-particle-size columns required to achieve a flow rate that minimizes the height equivalent to a theoretical plate [refer to Equations (4.19) and (4.23)] exceeds 1000 psi, and therefore necessitates pumping systems that can push liquids against this relatively high back-pressure. Instrumentation took what once was called low-pressure column chromatography and developed it into HPLC. However, the instrument itself is a high-pressure liquid chromatograph, aptly abbreviated HPLC, hence the source of confusion in describing this important determinative technique.

A block diagram of a high-pressure liquid chromatograph is shown in Figure 4.53. This diagram is a useful way to understand how an HPLC works and shows how HPLC is clearly a modular instrument. This modular nature of HPLC enables a number of options to be considered, as introduced in Figure 4.53. The reservoir can be either left at atmospheric pressure or pressurized with inert gas. However, a mobile phase that is not pressurized must be degassed; otherwise, air bubbles are continuously squeezed out of the mobile phase and accumulate in the micro-flow cell of the detector. These trapped air bubbles prevent the analyst from obtaining a stable detector baseline. This is particularly true for a UV absorbance detector. Pump systems can be either those that deliver essentially a constant flow rate or those that deliver a constant pressure. Reciprocating piston pumps deliver a constant flow rate and are manufactured with single, dual, or even triple pump heads. Pumps designed for HPLC must be able to withstand fluid pressure in excess of 6000 psi. A common safety feature built in to most HPLC pumps is a maximum pressure sensor. In practice, this value is set at approximately 4000 psi. If the back-pressure at the column head exceeds this value, the pump will shut down automatically. The addition of a pressure transducer and a pulse dampener completes the necessary accessories for a good pump. Gradient elution HPLC is analogous to temperature programming in GC and is achieved by varying the percent organic modifier in the mobile phase after the sample has been injected. There are two different designs for performing gradient elution. One is to mix two or more different solvents at low pressure, and the other is to mix them at high pressure.

One of the great developments that led to an increase in the scope of HPLC occurred with the preparation of chemically bonded silicas. In addition, it was the realization that a reduction in particle size along with an increase in particle size homogeneity would lead to highly efficient columns. Together, these bonded-phase
silicas led to the development of reversed-phase HPLC (RP–HPLC), and thus greatly expanded the scope of this instrumental technique. Figure 4.1 shows that for non-volatile organics that are somewhat polar, RP-HPLC is most appropriate for their retention and separation. The fact that the analyst can make a direct aqueous injection into an HPLC that is operated under reversed-phase conditions enabled RP-HPLC to take on a major role as a determinative technique in TEQA. Snyder and Kirkland, in their classic book on HPLC, commented on how “modern LC arose” by stating the following:

Modern LC is based on developments in several areas: equipment, special columns and column packings, and theory. High-pressure, low-dead-volume equipment with sensitive detectors plays a vital role. The new column packings that have been developed specifically for modern LC are also essential for rapid, high-performance separations. Theory has played a much more important role in the development of modern LC than for preceding chromatographic innovations. Fortunately, the theory of LC is not very different from that of GC, and a good understanding of the fundamentals of GC had evolved by the early 1960s. This GC theory was readily extended to include LC, and this in turn led directly to the development of the first high-performance column packings for LC and the design of the first model LC units…. The potential advantages of modern LC first came to the attention of a wide audience in early 1969…. 

---

| Mobile phase: polar for RP, RP-IP; non-polar for NP; aqueous for IC |
| Inert gas for pressurized headspace |
| Large (50 μm), small (5 μm) or pellicular particles |
| uv/vis; fluorescence; RI; LSED; conductivity; amperometric; APCI or ES/MS or MS/MS |
| Fixed or variable loop size; manual or automatic injection |
| Column: RP, RP-IP or NP, ion-exchange for IC |
| Sample |
| Injector |
| Pump |
| Reservoir |
| PC that acquires data and/or controls autosampler, pump |
| Detector |
| Waste |

**FIGURE 4.53** Generalized block diagram of a high-performance liquid chromatograph.
However, modern LC had its beginnings in the late 1950s, with the introduction of automated amino acid analysis by Spackman, Stein, and Moore and this was followed by the pioneering work of Hamilton and Giddings on the fundamental theory of high-performance LC in columns, the work of C.D. Scott at Oak Ridge on high-pressure ion exchange chromatography, and the introduction of gel permeation chromatography by J.C. Moore and Waters Associates in the mid-1960s.

63. HOW DID THE NAME REVERSED-PHASE HPLC COME ABOUT?

A nonpolar mobile phase passing through a packed column that contains a polar stationary phase defines normal-phase HPLC (NP-HPLC). For example, if n-hexane comprises the mobile phase and silica gel is used for the stationary phase, separations of nonpolar organic analytes, as shown in Figure 4.1, are accomplished. With respect to neutral organic compounds, the polar and ionic domains cannot be reached by NP-HPLC. NP-HPLC was the first high-pressure form of liquid chromatography to be developed. If the stationary phase could be made hydrophobic by chemical treatment and the mobile phase made more polar, a reversal of mobile-/stationary-phase polarities could be achieved. Like it or not, we are stuck with this nomenclature. RP-HPLC has certainly extended the range of analyte polarity that can be separated and detected. Reversed-phase ion pair HPLC (RP-IP-HPLC) has enabled organic cations such as quaternary ammonium salts and organic anions such as anionic surfactants to be retained, separated, and quantitated, thus extending the range of analyte polarity even further. To begin to realize the influence of mobile-phase composition on HPLC resolution, let us return to the fundamental resolution equation.

64. WHY DOES THE MOBILE PHASE EXERT SO MUCH INFLUENCE ON $R_S$?

We discussed Equation (4.30) earlier with respect to GC theory. This equation applies equally well to HPLC. Unlike GC, the HPLC mobile phase exerts considerable control over the chromatogram, and this is evident in the chromatograms shown in Figure 4.54. Consider an initial injection of two poorly resolved peaks such as in Figure 4.54A. The capacity factor can be varied as shown in Figure 4.54B, and the result either decreases $R_S$ when $k'$ is decreased or increases $R_S$ when $k'$ is increased, as shown. However, sometimes an increase in $k'$ also leads to increased peak broadening, and hence to no greater advantage with respect to $R_S$, as shown. The column can be made more efficient by changing the stationary phase, as shown in Figure 4.54C. The chemical nature of the stationary phase can be changed to make the column more selective, and thus serve to increase $\alpha$, as is shown in Figure 4.54D. To answer the question, the magnitude of the partition coefficient, $K$, for a neutral organic compound between a liquid mobile phase and a given stationary phase can be significantly changed by a change in the percent organic modifier. Equation (4.31) suggests that significant changes in $K$ lead to differences in $k'$. As is shown in Figure 4.55, an increase in the percent acetonitrile (solvent B by convention) from 43% in distilled deionized water (solvent A) to 50% acetonitrile in acetate buffer at pH 5.0 not only
reduced $k'$ for 3-(trifluoromethyl)-p-nitrophenol (TFM), but also improved peak shape. A well-known relationship in RP-HPLC relates the capacity factor in a given organic/aqueous mobile-phase $k$ to the capacity factor in 100% water, $k_w$, and the volume fraction of organics in the mobile-phase $\phi$, where $\phi = \%B/100$ according to$^{115}$

$$\log k = \log k_w - S \phi$$

For low-molecular-weight compounds, $S = 4$. Thus, $k$ increases by a factor of 2 to 3 for a decrease of 10% B. Three RP-HPLC chromatograms from the author’s laboratory are shown in Figure 4.56. The software enables all three chromatograms to be stacked, as shown. The top chromatogram shows a column that has deteriorated beyond its useful life. The middle chromatogram illustrates how a change in the column can radically improve HPLC column efficiency. The isocratic test mix available from Supelco consists of a homologous series of substituted parabens (esters of $p$-hydroxybenzoic acid). The bottom chromatogram shows how the injection of the same mixture using the same column looks when the %B is lowered. When viewing Figure 4.56, note that the timescales have not been aligned.

FIGURE 4.54 The effect on chromatographic resolution of changes in $k'$, $N$, or $\alpha$. 

© 2006 by Taylor & Francis Group, LLC
FIGURE 4.55 Effect of mobile phase on $R_s$, $k'$, and peak shape in RP-HPLC.
**FIGURE 4.56** Comparison of the RP-HPLC chromatograms for a deteriorated column vs. an efficient column.
FIGURE 4.57  Comparison of the RP-HPLC chromatograms between two C\textsubscript{18} columns from two different manufacturers.

© 2006 by Taylor & Francis Group, LLC
65. ARE ALL OCTADECYL-BONDED SILICA HPLC COLUMNS THE SAME?

The stacked HPLC chromatograms shown in Figure 4.57 should provide the answer to this question. Both chromatograms were obtained on the same instrument, using the same mobile phase; however, the octadecyl-bonded silica (ODS) columns used were obtained from two different suppliers, as indicated. Note that the column dimensions differ slightly between both columns. Interpretation of the top chromatogram indicates that this column exhibits a much higher $k'$ than the column used to generate the lower chromatogram. A narrower peak width is, however, evident in the bottom chromatogram. The number of theoretical plates for an HPLC column can be calculated using either Equation (4.21) or (4.22), depending on how the peak width is measured. When stating how good a column is, Snyder et al.\textsuperscript{116} have listed the following requirements:

- Plate number $N$ for a given value of $k'$
- Peak asymmetry
- Selectivity, $\alpha$, for two different solutes
- Column back-pressure
- Retention, $k'$, reproducibility
- Bonded-phase concentration
- Column stability

They also suggest that the following equation be used to estimate the column plate number for small molecules under optimum conditions for a column length $L$ and a particle diameter $d_p$, according to\textsuperscript{116}

$$N = \frac{3500L(\text{cm})}{d_p(\mu\text{m})}$$

As is quite evident from interpreting the chromatograms in Figure 4.56, RP-HPLC columns have a finite lifetime. It is good practice for the analyst to keep a record of $N$ (as calculated using the above equation) vs. either time or number of injected samples in an attempt to continuously monitor column performance. Because HPLC reciprocating pumps maintain constant flow rate, a continuous observation of the back-pressure or pressure buildup at the front on the HPLC column is an important parameter to monitor. Making sure that there are no leaks in an operating HPLC is also very important.

Another useful equation that is used to predict the back-pressure or pressure drop, $\Delta P$, for well-packed columns having similar operating conditions, with the mobile-phase viscosity $\eta$ in centipoises for a dead time $t_0$ for columns packed with spherical particles, can be found from\textsuperscript{116}

$$\Delta P(\text{psi}) = \frac{3000L(\text{cm}) \eta(\text{cP})}{t_0 d_p^2(\mu\text{m})}$$

Columns packed with irregular particles might yield back-pressures that are higher. A new spherical particle column should have a $\Delta P$ no greater than about
30% in excess of that predicted by the above equation. Let us return to Figure 4.53 and take a brief look at some HPLC detectors.

66. HOW DO HPLC DETECTORS WORK?

Depicted in Figure 4.53 are the names of the major types of HPLC detectors. It has been said that the FID is to the GC what the UV-vis-absorbance detector is to the HPLC. However, unlike GC, there is no universal HPLC non-mass spectrometric detector. The recent advances in LC-MS have made this hyphenated technique a universal one. We will focus on two detectors designed for HPLC that are in current use in the author’s laboratory.

The first is a UV-vis-absorption or absorbance spectrophotometric detector. (The phenomenon responsible for the signal is absorption; however, what is actually measured is absorbance, UV.) The second is a molecular fluorescence luminescence spectrophotometric detector (commonly called fluorescence, FL). Other non-mass spectrometric detectors designed for HPLC include refractive index (RI), electrochemical (EC), and, of a more recent vintage, the light-scattering evaporative detector (LSED). RI and LSED HPLC detectors are not sensitive enough to meet the needs of TEQA. Electrochemical HPLC detectors have the required sensitivity, but due to frequent fouling of electrode surfaces, they have not really found a place in TEQA. This author knows of no EPA methods as of yet that incorporate EC HPLC detectors. For this reason, EC HPLC detectors will be not considered.

Organic compounds that possess an ultraviolet- or visible-absorbing chromophore obey the Beer–Lambert or Beer–Bouguer law of spectrophotometry. In what is generally termed molecular absorption spectrophotometry, a cuvette (in the case of stand-alone UV-vis spectrophotometers) or a micro-flow cell (in the case of flow-through HPLC UV-vis detectors) is used. We now proceed to derive the fundamental equation that relates absorbance as measured on a UV-vis HPLC detector to concentration, because this relationship is important to the practice of TEQA. Similar approaches have been shown in two of the most popular textbooks in analytical chemistry. Let us start by considering what happens when UV radiation impinges onto a cuvette of path length b:
Ultraviolet or visible radiation incident on the infinitesimal volume of area \( A \) and thickness \( dx \) experiences a decrease in transmitted intensity or power \( dP \) that is proportional to the incident power \( P \), to the number of absorbing molecules \( N_c \), where \( N \) is Avogadro’s number and \( c \) is the concentration of absorbing species in moles per liter, and to the thickness \( dx \) according to

\[
dP = -\beta PN_c Adx
\]

where \( \beta \) is a proportionality constant. This equation can be rearranged and integrated as follows:

\[
-\frac{dP}{P} = \beta c \, dx
\]

\[
-\int_{P_0}^{P} \frac{dP}{P} = \beta c \int_{0}^{b} dx
\]

At \( x = 0 \), \( P = P_0 \), and at \( x = b \), \( P = P \), and we have the limits of integration that are necessary to evaluate these integrals. Rearranging and removing the negative sign yields the desired outcome:

\[
\ln \left( \frac{P_0}{P} \right) = \beta cb
\]

Converting from the natural to the common logarithm while substituting \( \ln x = (\ln 10)(\log x) \) yields

\[
\log \left( \frac{P_0}{P} \right) = \left( \frac{\beta}{\ln 10} \right) cb
\]

The absorbance, \( A \), is defined as the logarithm of the ratio of the incident intensity, \( P_0 \), to the transmitted intensity, \( P \), so that

\[
A = \varepsilon bc
\]

This equation states that the absorbance is directly proportional to solute concentration for a given solute/solvent, (i.e., \( \varepsilon \), the molar absorptivity) and for a fixed length \( b \). It must be remembered that the absorbance and the molar absorptivity are dependent on the wavelength. It becomes important in practice for an analyst to know how the molar absorptivity varies with wavelength, \( \lambda \). The percent transmittance, \( %T = 100T \), a common term used with stand-alone spectrophotometers, can be related to the absorbance by manipulating the above definition for \( A \) according to

\[
A = \log \left( \frac{P_0}{P} \right) = -\log \left( \frac{P}{P_0} \right)
\]
It is seen from this relationship that when no radiation is transmitted, \( \%T = 0 \) and \( A = 2 \), whereas if all incident radiation is transmitted through the cuvette or micro-flow cell, \( \%T = 100 \), all radiation is transmitted, and \( A = 0 \). The simple proportion between \( A \) and \( c \) forms the basis for TEQA using UV-vis-absorption HPLC detectors.

67. HOW DO YOU GO ABOUT DOING QUANTITATIVE ANALYSIS USING HPLC WITH UV DETECTION?

Because a solute’s absorbance, \( A \), and its molar absorptivity, \( \varepsilon \), depend on the wavelength of the incident UV radiation, it is necessary that a wavelength be found that maximizes \( \varepsilon \). It becomes important, then, to either know the UV absorption spectrum for the analyte of interest or have some means to record the UV absorption spectrum. This can be accomplished using a stand-alone scanning UV-vis spectrophotometer. An absorption spectrum is a plot of absorbance against wavelength across either the UV alone (200 to 400 nm) or the UV and visible (200 to 700 nm). Ultraviolet absorption of UV photons is enough not only to excite electrons from the ground electronic state to the first excited state, but also to excite rotational and vibrational quantized levels within each electronic state. This yields UV absorption that covers a wide range of wavelengths, and hence leads to large absorption bands. An experiment in Chapter 5 provides a number of practical details related to molecular absorption spectra. The chlorophenoxy acids provide a good illustration. These organic acids are used as herbicides and are likely found in the environment. Dicamba, 3,6-dichloro-2-methoxybenzoic acid, although not a phenoxy acid, is included along with 2,4-dichlorophenoxyacetic acid (2,4-D) and two structurally very similar chlorophenoxy acids, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichloro)-propionic acid, commonly called Silvex. Molecular structures for all four compounds are as follows:
The UV absorption spectra were recorded at the apex of the chromatographically resolved peak using RP-HPLC with a photodiode array UV detector. This detector, unlike a fixed-wavelength detector, can record all absorbances throughout the UV region of interest simultaneously. All four compounds yield UV absorption spectra that show intense absorbance below 250 nm, while having an absorption peak maximum between 270 and 295 nm. The other factor to consider in choosing the most sensitive wavelength for trace analysis is the UV cutoff of the organic modifiers used in the mobile phase. A selected list of solvents is given in Table 4.17. The UV cutoff is the wavelength above which useful quantitative analysis can be obtained.

The solvent viscosity, boiling point, and solvent polarity are all important factors in arriving at the optimum organic solvent to be used in the mobile phase. For example, acetone is a low-viscosity liquid with a boiling point that is appropriate for RP-HPLC and a polarity that, when mixed with water, yields a mobile phase with sufficient solvent strength that would enable moderately polar solutes to have $k'$ values between 2 and 10. However, its UV cutoff is so high as to render the solvent useless for TEQA based on molecular absorption in the low-UV region where most organic compounds absorb. Acetonitrile, on the other hand, has similar physical properties combined with a low-UV cutoff. Cyclohexane has a favorable UV cutoff, viscosity, and boiling point. However, its polarity is so low that it is not even miscible in water. Cyclohexane is useless as an organic modifier for RP-HPLC, yet is appropriate as a mobile-phase additive in the normal phase (NP-HPLC) due to the extremely low solvent polarity. Acetonitrile, methanol, and tetrahydrofuran are the most commonly used organic modifiers for the laboratory practice of RP-HPLC, whereas cyclohexane and $n$-hexane are the most common solvents for NP-HPLC. Let us consider the inner workings of a typical UV absorbance HPLC detector. NP-HPLC is of limited value to TEQA and will not be considered any further. Because this book emphasizes quantitative measurement, we next focus on the HPLC-UV detector.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>UV Cutoff (nm)</th>
<th>Viscosity (cP)</th>
<th>Boiling Point (°C)</th>
<th>Polarity $P'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>330</td>
<td>0.36</td>
<td>56.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>190</td>
<td>0.38</td>
<td>81.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>200</td>
<td>1.0</td>
<td>80.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>256</td>
<td>0.45</td>
<td>77.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Hexane</td>
<td>195</td>
<td>0.31</td>
<td>68.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Methanol</td>
<td>205</td>
<td>0.55</td>
<td>64.7</td>
<td>5.1</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>233</td>
<td>0.44</td>
<td>39.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>212</td>
<td>0.55</td>
<td>66.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Water</td>
<td>190</td>
<td>1.00</td>
<td>100.0</td>
<td>10.2</td>
</tr>
</tbody>
</table>
68. HOW DOES THE ABSORBANCE OF A SOLUTE GET MEASURED?

The optics for the Model 2487® Dual λ Absorbance Detector (Waters) are shown in Figure 4.58. A deuterium lamp is the source of UV radiation, and the ellipsoidal mirror collects light from the lamp and focuses it through the filter wheel onto the entrance slit. The spherical mirror directs light toward the grating. Another part of the spherical mirror focuses dispersed light of a particular wavelength, determined by the grating angle, onto the entrance of the flow cell. Prior to the flow cell, a portion of the beam is split to a reference photodiode while the remaining incident intensity is transmitted through the flow cell to the sample photodiode. The pre-amplifier board integrates and digitizes the currents from the photodiodes for processing by the signal processing electronics and output to a computer. When a new wavelength is entered through the front panel, the detector rotates the grating to the appropriate position. The difference between a conventional flow cell and the TaperSlit® is shown in Figure 4.59. According to Waters, the TaperSlit flow cell renders the detector insensitive to changes in mobile-phase refractive index. This is significant in gradient elution HPLC where the mobile-phase composition changes with time. As shown in Figure 4.59, this tapered design minimizes the amount of radiation that is reflected off of the inner wall, thus allowing a greater UV light throughput. This is not the only non-mass spectrometric HPLC detector useful to TEQA. Let us consider measuring the UV-induced fluorescence, instead of measuring UV absorption.
69. WHAT IS AN HPLC FLUORESCENCE DETECTOR?

The fluorescence HPLC detector (HPLC-FL) is similar to the UV absorption HPLC detector (HPLC-UV) in that a source of UV radiation is made incident to a micro-flow cell in which the chromatographically resolved analyte passes through. However, a photomultiplier tube (PMT) and associated optics are positioned at right angles relative to the incident UV radiation. Lakowicz\textsuperscript{119} has articulated just what molecular luminescence is:

Luminescence is the emission of photons from electronically excited states. Luminescence is divided into two types, depending upon the nature of the ground and the excited states. In a singlet excited state, the electron in the higher-energy orbital has the opposite spin orientation as the second electron in the lower orbital. These two electrons are said to be paired. In a triplet state, these electrons are unpaired — that is, their spins have the same orientation. Return to the ground state from an excited singlet state does not require an electron to change its spin orientation. A change in spin orientation is needed for a triplet state to return to the singlet ground state. Fluorescence is the emission that results from the return to the lower orbital of the paired electron. Such transitions are quantum mechanically “allowed.” ... Phosphorescence is the emission that results from transition between states of different multiplicity, generally a triplet state returns to a singlet ground state.

Ewing\textsuperscript{120} defines molecular luminescence that includes fluorescence, phosphorescence, and Raman spectroscopies as methods whereby radiation absorbed by molecular species is reemitted with a change in wavelength.

Both organic and inorganic compounds exhibit fluorescence. Molecules that display significant fluorescence possess delocalized electrons due to conjugated
double bonds. A fluorescence spectra is a plot of FL emission intensity vs. wavelength of the emitted radiation. A simplified Joblonski diagram provides a qualitative explanation of how fluorescence (FL) and phosphorescence (PHOS) spectra originate:

There exists within molecules a myriad of quantized vibrational levels in both the ground and first excited electronic states. Not shown in the diagram are the myriad of rotational quantized levels within each vibrational level. Transitions, as shown in the diagram, yield absorption and fluorescence spectra that are quite broad. The fact that these spectra are obtained for solutes that are dissolved in solvents is also a contributor to broad bands. Photons emitted due to \( h\nu \) (FL) are on the order of nanoseconds, whereas photons emitted due to \( h\nu \) (PHOS) range from milliseconds to seconds.

Fluorescence quenching is the term used to explain any decrease in the analyte-emitted fluorescence intensity due to the influence of the sample matrix. Quenching can also be attributed to the analyte itself and is called self-quenching. Ewing\(^{121}\) discusses the effect of quenching when the concentration of phenol dissolved in water is increased beyond 10 ppm, with a maximum in the emitted intensity at around 75 ppm, followed by a gradual loss in intensity due to self-quenching. A quenching agent, if present, contributes to a loss in the emitted intensity. A good example is the quenching of PAHs by dissolved oxygen.

70. HOW IS IT THAT FLUORESCENCE CAN BE QUANTITATED?

For a single fluorophore, as would be the case for a chromatographically resolved compound in the HPLC chromatogram, the fluorescence is related to the incident UV radiation of power \( P_0 \) according to

\[
F = P_0 K (1 - 10^{-A})
\]

where \( A \) is the UV absorbance and \( K \) is a constant for a given system and instrument and is related to the quantum yield. This expression can be made more useful by considering a Taylor’s series expansion for the exponential term:

\[
F = P_0 K \left( 2.30 A - \frac{(2.30A)^2}{2!} + \frac{(2.30A)^3}{3!} + \ldots \right)
\]
To a first approximation, for solutions of low absorbance that are most likely the case for TEQA, the higher-order terms can be neglected so that

\[ F = P_0 K(2.30)A \]

We see that a direct proportionality exists between the emitted fluorescence intensity denoted by \( F \) and the sample absorbance denoted by \( A \). If a standalone spectrofluorimeter is used to conduct a quantitative analysis, the error introduced by neglecting the \( A^2 \) term should be considered. However, for a fluorescence HPLC detector where a set of working calibration standards are used (refer to Chapter 2) and where the concentration of fluorophore is relatively low, it is sufficient to ignore the higher-order terms. Upon substituting for \( A \), we obtain

\[ F \equiv P_0 K(2.30)(\varepsilon bc) = K'\varepsilon bc = K'' \varepsilon \]

The linear dynamic range of this detector is limited at the high end by fluorescence quenching due to self-absorption. These equations demonstrate that a fluorescence HPLC detector is intrinsically more sensitive than a UV absorption HPLC detector because for dilute solution, the PMT senses a faint light against a dark background. For a UV absorption detector, the PMT or photodiode compares a slightly lower transmitted intensity \( P \) against a higher-incident intensity \( P_0 \), and this small difference is related to analyte concentration.

Instrumentation to measure the fluorescence of a substance can be as simple as a filter fluorimeter or as complex as a dual-excitation/emission spectrofluorimeter. A schematic of the Model 474® (Waters) dual monochromatic fluorescence HPLC detector is shown in Figure 4.60A. A xenon lamp is the source of UV radiation, and this light reflects off of a mirror to an excitation grating through a narrow entrance slit. This monochromator enables a narrow band of excitation wavelengths to be selected. The selected UV photons are passed through a beam splitter, whereby a portion of this excitation radiation is detected by a photodiode. This photodiode enables corrections to the lamp output to be made. The collection mirror is positioned at a right angle with respect to the direction of the excitation light, as shown in Figure 4.60A. The fluorescence radiation is collimated and reflected through a narrow emission slit where it is diffracted off of the grating. This emission monochromator selects a narrow band of fluorescence wavelengths whose intensity reaches a PMT. The output of the PMT is amplified and fed to an analog-to-digital (A/D) converter, and then fed into a PC for processing and quantitative analysis. Contemporary fluorescence HPLC detectors like the Model 474 are completely microprocessor controlled; this is best shown in Figure 4.60B.

71. HOW DO UV ABSORPTION AND FLUORESCENCE EMISSION HPLC CHROMATOGRAMS COMPARE?

The answer lies in the nature of the chemical compounds that are of interest. For example, there is little difference in HPLC detector sensitivity between UV absorption
FIGURE 4.60 (a) Schematic of the Model 474® (Waters) fluorescence HPLC detector. (b) Schematic of a fluorescence HPLC detector showing microprocessor control and analog output.
and fluorescence emission for phenol, but a great difference exists for naphthalene. Naphthalene is a fused aromatic and allows for extensive delocalization of electrons and is quite sensitive when measured by RP-HPLC-FL. For both analytes of environmental concern being dissolved in water or dissolved in the RP-HPLC mobile phase, a vivid illustration of this difference in HPLC detector sensitivity is shown in Figure 4.61. An RP-HPLC-UV chromatogram is shown at the top and an RP-HPLC-FL chromatogram is shown at the bottom. The chromatograms are stacked, and both the ordinate and abscissa axes are aligned. Effluent from a reversed-phase column was fed to the Model 2478 UV absorption detector, and the effluent from this detector was made the influent to the Model 474 fluorescence detector, as shown in Figure 4.62. Analog signals from both detectors are sent through an A/D converter to a central PC workstation that uses Turbochrom (PE-Nelson) to acquire the data and to generate the chromatograms. The peaks in both chromatograms of Figure 4.61 are due to the injection of a multicomponent standard of the 16 priority pollutant PAHs. They elute in the order shown in Table 4.18. Before leaving our discussion on HPLC, let us introduce LC-MS.

72. CAN A MASS SPEC BE USED AS A DETECTOR FOR HPLC?

Yes, indeed. Over the past 25 years, major advances in hyphenating both single and tandem quadrupole mass spectrometers to liquid chromatographs have occurred. Of particular interest, and of a more difficult undertaking, has been to interface RP-HPLC with mass spectrometers. RP-HPLC uses aqueous and water-miscible organic solvents, while NP-HPLC uses water-immiscible organic solvents. It is much easier to deal with nonpolar organic solvents within an interface. Nevertheless, the successful interface technology for RP-HPLC and quadrupole-based mass spectrometry has led to new terms, such as LC-MS for single quadrupole operation and LC-MS-MS for tandem operation. In addition, the type of LC-MS interface also appears in the acronym LC-APCI-MS, where APCI refers to atmospheric pressure chemical ionization, and LC-ES-MS, where ES refers to electrospray. Connection of a liquid–solid column chromatographic separation technique to a mass spectrometer that operates in a high vacuum requires an interface that is capable of meeting the goals of TEQA. Budde\textsuperscript{122} defines interfaces such as LC-MS this way:

\[
\text{as the device that receives the condensed-phase flow from the separation system and converts this flow into gas phase analyte molecules or ions suitable for injection into the high vacuum of the MS.}
\]

Henion et al.\textsuperscript{123} have articulated the interface in another manner:

We suggest that atmospheric pressure ionization mass spectrometry is a preferred way to simplify the coupling of liquid inlet systems such as HPLC, CE and ion pair chromatography to mass spectrometry. A key feature of this approach is that liquid effluent from a particular separation science is not directly introduced into the mass spectrometer vacuum system … the effluent is “sprayed” in the vicinity of an in sampling orifice…. Gas phase ions are formed in this region by either electrospray or
FIGURE 4.61 Comparison of RP-HPLC chromatograms for the separation and detection of a 1 ppm reference standard containing the 16 priority pollutant PAHs between a UV absorbance and fluorescence HPLC detector.
Determinative Techniques to Measure Organics and Inorganics

475

atmospheric pressure chemical ionization. These ions are sampled through the ion sampling orifice into the vacuum system for mass analysis. Large excesses of solvent from the effluent do not enter the vacuum system.

A truly universal MS detector for HPLC would clearly widen the scope of separations (refer again to Figure 1.4). Commercially developed LC-MS interfaces over the past 30 years include:

- Direct liquid and fluid introduction
- Moving belt interface developed by McFadden at Finnigan Corp. in the mid-1970s
- Particle beam interface developed by Willoughby and Browner in the mid-1980s

© 2006 by Taylor & Francis Group, LLC

FIGURE 4.62 Schematic for the configuration in the author’s lab of the single HPLC column and dual UV absorbance and fluorescence detectors placed in series showing the relationship to a PC.

TABLE 4.18
Summary of the Number of Fused Rings Associated with the Priority Pollutant PAHs

<table>
<thead>
<tr>
<th>No.</th>
<th>No. of Rings</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Naphthalene</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Acenaphthylene</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Fluorene</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Acenaphthene</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Phenanthracene</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>Anthracene</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>Fluoranthene</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>Pyrene</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>Benzo(a)anthracene</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>Chrysene</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>Benzo(b)fluoranthene</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>Benzo(k)fluoranthene</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>Benzo(a)pyrene</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>Dibenzo(a,h)anthracene</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>Indeno(1,2,3-c,d)pyrene</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>Benzo(g,h,i)perylene</td>
</tr>
</tbody>
</table>

A/D Wast A/D PC
• Thermospray developed by Vestal in the early 1980s
• APCI developed by Horning (1970s), Thomson at SCIEX (1980s)
• ES developed by Thomson at SCIEX (1990s), Fenn at Yale University,\textsuperscript{125} and pneumatically assisted ES LC-MS interface by Henion\textsuperscript{126} at Cornell University

A schematic of an APCI-LC-MS is shown below:

73. IS LC-MS USED IN TEQA?

Yes, indeed. We discuss one application related to trace enviro-health quantitative analysis. Blount and coworkers\textsuperscript{127} at CDC have developed an analytical method that quantitatively determines eight phthalate metabolites in human urine. Enzymatic deconjugation is used to release the monoalkyl phthalate esters. Molecular structures for an environmentally ubiquitous dialkyl phthalate and its monoalkyl phthalate metabolite are shown below:

A glucuronide moiety is then covalently bonded to the hydroxy oxygen. Selective use of $\beta$-glucuronidase enzyme to hydrolyze glucuronide metabolites allows for quantification for both free and glucuronidated forms of each phthalate metabolite.

The esters are isolated and recovered using RP-SPE techniques. $^{13}$C\textsubscript{4}-labeled standards such as monoethyl-$^{13}$C\textsubscript{4} and others are commercially available and enable quantification via isotope dilution (Chapter 2). Negative ion APCI was used to form
negatively charged analyte ions. Daughter ions were formed in the collision cell using argon as the collision gas at 2.0 millitorr. The method was applied to 289 human urine samples to establish baseline levels for phthalate exposure in the American population. Detectable levels of some phthalate monoesters were found in all urine samples tested.

We have completed our discussion of the determinative techniques for the quantitative determination of organic compounds that are of concern to TEQA. What is left? Well, let us return to Figure 4.1 and consider that region in the chromatography world that deals with the analysis of ionic substances. We want to be able to identify ionically bonded chemical substances that are of concern to TEQA and to consider what determinative technique one would find in trace environmental testing labs today. We must then discuss how ion chromatography (IC) became the dominant determinative technique to separate, identify, and detect trace concentrations of inorganic ions in aqueous samples of environmental interest.

74. HOW DID IC MAKE IT TO THE FOREFRONT IN TRACE INORGANICS ANALYSIS?

To answer in brief, by evolving from a low-pressure large-column-diameter (>1 cm) ion exchange chromatography (IEC) technique to a high-pressure, narrow-column-diameter (~5 mm) high-performance IEC technique. The use of glass columns packed with either a strong or weak anion or cation exchange resin is routine in many chemical laboratories. However, the development of IC as an additional determinative technique very useful to TEQA requires that we discuss these principles. But first, as a way to review some principles of conventional IEC, let us discuss an ingenious use of two chemically different ion exchange resins and the simple experiment that can illustrate very nicely the principle of ion exchange (IE). One of the most useful IE resins is Chelex-100, 1% cross-linked, 50 to 100 mesh, Na⁺ form (Sigma). Chelex-100 is a styrene–divinyl benzene resin containing iminodiacetate groups. Its structure is shown as follows:
Conventional cation exchange resin such as AG 50W-X8, 8% cross-linked, 20 to 50 mesh, H\(^+\) form (Bio-Rad), whose IE capacity is 5.1 mEq/g, is a sulfonic acid type. The structure of this resin is:

![Resin Structure](image)

Chelex-100 in its Na\(^+\) form can exchange other metal ions such as Fe\(^{2+}\), whereas AG 50W-X8 in the H\(^+\) form exchanges other metal ions such as Na\(^+\) for H\(^+\). The author recently packed two conventional glass columns, one containing Chelex-100 and the other containing AG 50W-X8, as shown in Figure 4.63. Suppose that we pass an aqueous solution containing Fe\(^{2+}\) through the first column in Figure 4.63. If we then take the effluent from the first column and place it on top of the second column, we discover, after enough aqueous solution has been passed through this column, that the effluent is quite acidic (i.e., its pH is <4). This solution can be titrated against a standardized base such as 0.02 M NaOH to a Bromocresol endpoint, and from the number of milliliters of titrant required, the number of millimoles of H\(^+\) can be obtained. This many millimoles is also the number of millimoles of Na\(^+\) that was exchanged for H\(^+\). For every millimole of Fe\(^{2+}\) exchanged on the first column, 2 mmol of Na\(^+\) ions must have been released. Hence, the number of milliliters of 0.02 M NaOH can be indirectly related to the number of millimoles of iron(II) present in the original sample. The stoichiometry for this series of chemical reactions is as follows:

**CHEMICAL REACTIONS USED IN THIS DEMONSTRATION**

Column to the left:

\[
Fe^{2+} + 2\text{CheR}^+\text{Na}^+ \rightarrow (\text{CheR})_2\text{Fe} + 2\text{Na}^+
\]
FIGURE 4.63 Schematic of two gravity-fed low-pressure LC columns packed with ion exchange resins.

Column to the right:

\[
\text{Na}^+ + \text{RSO}_3\text{H}^+ \rightarrow \text{RSO}_3\text{Na}^+ + \text{H}^+
\]
At the buret:

\[
H^+ + OH^- \rightarrow H_2O
\]

Bromocresol Green turns yellow when the pH is below 4.0 and blue when the pH is above 5.6.

The appearance of a gradual darkening of the head of the packed column that contains Chelex-100 is observed at first. This amount of darkening of the white resin gets larger and larger as successive aliquots of the iron(II) solution are added on top of the column. The brownish AG 50W-X8 does not change color during these repetitive additions of sodium ion to the top of this column. Let us return to our development of the principles of IC.

Two achievements that led to the acceptance of IC as an important determinative technique in TEQA are now discussed. The first was the development of surface-agglomerated low-capacity anion and cation exchange resins with particle sizes appropriate for preparing high-performance IC columns. The second was the realization that detecting trace concentration levels in a background of highly conducting IC eluent required a chemical suppression of the eluent conductivity. The research group led by Small\textsuperscript{128} at Dow Chemical in the early 1970s is credited with the pioneer development of suppressed IC, and a portion of their abstract to this benchmark paper follows:

Ion exchange resins have a well known ability to provide excellent separation of ions, but the automated analysis of the eluted species is often frustrated by the presence of the background electrolyte used for elution. By using a novel combination of resins, we have succeeded in neutralizing or suppressing this background without significantly affecting the species being analyzed which in turns permit the use of a conductivity cell as a universal and very sensitive monitor of all ionic species either cationic or anionic.

This benchmark publication was followed by a series of developments that continues to this day in the evolution of IC as a trace determinative technique. The Dionex Corporation was formed out of these developments from Dow Chemical in the mid-1970s, and this company has been quite an innovator in the manufacture of instrumentation for suppressed IC. From the earlier model, such as the 10, to the 2000I to the DX 500, and from the cation exchange column to the hollow-fiber cation suppressor to the micromembrane cation suppressor to the self-regenerate cation suppression for anion analysis and now to its “just add water” slogan, Dionex Corporation has made considerable advances in IC technology. A nonsuppressed form of IC also developed during the 1970s. To quote from the author’s abstract of this benchmark paper:\textsuperscript{129}

The anions are separated on a column containing a macroporous anion exchange resin which has a very low exchange capacity of 0.007–0.07 milli-equivalents per gram. Because of the low resin capacity, only a very dilute solution, \(1 \times 10^{-4} M\) of an aromatic organic acid salt, is needed as the eluent. The eluent conductance is sufficiently low that a suppressor column is not needed, and the separated anions can be detected with a simple conductance detector.
Unfortunately, nonsuppressed IC has not been developed commercially and remains an interesting determinative technique for academic labs. It took, however, over 10 years for the EPA to approve of suppressed IC to quantitatively determine the common inorganic anions derived from moderate to strong acids such as fluoride, chloride, nitrite, phosphate, and sulfate in drinking water. Years of effort have resulted in EPA Method 300.1, “The Determination of Inorganic Anions in Drinking Water by Ion Chromatography.” This method considers two different sets of inorganic anions in drinking water, reagent, surface water, and groundwater. The first set is the classical one listed earlier, and the second set consists of analytes derived from the so-called disinfection by-products (DBPs). This second set includes bromate, bromide, chloride, and chlorate (oxyhalides). The method is replete with sufficient quality control, something that was seriously lacking in the earlier versions of Method 300.

75. HOW DOES AN IC WORK?

An ion chromatograph using a suppressed IC mode of operation can be viewed as a moderate-pressure-performance liquid chromatograph. A schematic of the essential components of an IC is shown in Figure 4.64. Because the trace concentration levels of various inorganic anions is of most interest to TEQA, we will focus our discussions on how anions are measured. Alkali and alkaline–earth metal ions and the ammonium ion are common applications of IC. In fact, \( \text{NH}_4^+ \) can only be measured chromatographically by cation IC. The transition metal ions can also be separated and detected; however, atomic spectroscopy, to be discussed after we complete IC, is the predominant TEQA determinative technique. We will not discuss the separation and detection of cations here.

Referring to Figure 4.64 and focusing on trace anion analysis, the simplest ion chromatograph can be viewed as consisting of four modes:

1. A delivery mode that uses a bicarbonate/carbonate buffer as the mobile-phase eluent and a single-piston reciprocating pump with check valves and pressure gauge/dampener accessory; a means to introduce a fixed volume of sample
2. A separation mode that consists of a column packed with a low-capacity anion exchange resin
3. A detection mode that includes a suppressor device and a micro-flow conductivity cell
4. A data mode that could be either a strip-chart recorder, an electronic integrator (ones made by either Spectra-Physics or Hewlett-Packard were popular at one time), or a PC via an A/D converter

In the author’s laboratory, the analog signal from the conductivity cell is connected to a 900 interface box (PE-Nelson). The interface is connected to the input of a PC using an IEEE cable. The PC utilizes Turbochrom (PE-Nelson) chromatography data acquisition software in a Windows disk operating environment.
It was critical to the development of IC to achieve the IE separation of the common inorganic anions in a reasonable length of time (e.g., within 20 min of the \( SO_4^{2-} \) eluting) and with sufficient chromatographic resolution. It appeared inevitable that advances in HPLC made in the early 1970s would simultaneously advance the chromatographic separation of ions or ionizable compounds. The drastic lowering of particle size, predicted by the contributions to plate height and the realization that pellicular materials minimize the stationary-phase thickness, leads to a more chromatographically efficient IC column. Ways to lower the IE capacity were pursued, and these efforts resulted in more efficient columns and significantly lower values for \( k' \).

To achieve this increase in IC column efficiency, IE resins had to be developed that differed significantly from those of conventional resins. Table 4.19, taken from an earlier article, compares the properties of conventional IE resins to those required for IC.\(^{130}\) A decrease in IE capacity by about two orders of magnitude permitted the use of eluents for IC whose concentrations are three orders of magnitude lower than those used in conventional IE analysis. Eluent concentrations in IC are routinely

---

\( \text{FIGURE 4.64 Schematic diagram for a generalized view of a typical suppressed ion chromatograph.} \)

© 2006 by Taylor & Francis Group, LLC
preparing at the millimolar level. The favorable IE properties were realized with the development of surface-agglomerated pellicular resins. Such a pellicular particle is shown in Figure 4.65. A surface-sulfonated particle is shown agglomerated with an anion latex particle. The illustration shows the bicarbonate and carbonate ions, the most common eluent ions used in anion IC, as attached to the latex particle. The chemistry involved in separating chloride from sulfate and being able to quantitate these common ions found in groundwaters is shown schematically in Figure 4.66. The eluent used in this schematic is NaOH, so that the counterion is hydroxide ion, as shown as the analyte ion symbolized as \( X^- \) is chromatographed through the separator column. As the eluent and analyte ions reach the suppressor, prior to the conductivity detector, as shown in Figure 4.66, the hydroxide ion eluent is converted to the minimally conducting water and the analyte ion is converted to the highly

<table>
<thead>
<tr>
<th>TABLE 4.19</th>
<th>Comparison of Conventional to Surface-Agglomerated Ion Exchange Resin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional Resin</strong></td>
<td><strong>Surface-Agglomerated Resin</strong></td>
</tr>
<tr>
<td>Capacity: 3–5 mEq/g</td>
<td>Capacity: 0.02 mEq/g</td>
</tr>
<tr>
<td>Large degree of swelling</td>
<td>Very little swelling</td>
</tr>
<tr>
<td>Many diffusion paths</td>
<td>Limited diffusion paths</td>
</tr>
<tr>
<td>Separation by ion size</td>
<td>Little separation by ion size</td>
</tr>
<tr>
<td>Not easily poisoned</td>
<td>Easily poisoned but can be regenerated</td>
</tr>
<tr>
<td>High sample concentrations</td>
<td>Low sample concentrations</td>
</tr>
<tr>
<td>High eluent strength</td>
<td>Low eluent strength</td>
</tr>
</tbody>
</table>


FIGURE 4.65 Surface-agglomerated pellicular particles used in low-capacity anion exchange columns for IC.

© 2006 by Taylor & Francis Group, LLC
conducting strong acids HCl and H$_2$SO$_4$. It is well known that H$^+$ and OH$^-$ are significantly more conductive than are any of the common anions.

76. IS THERE A THEORETICAL BASIS FOR CONDUCTIVITY MEASUREMENTS?

Let us digress a moment and discuss the relationships involving conductivity.$^{131}$ An aqueous solution containing dissolved inorganic salts or electrolytes conducts electric current. The extent of this conductance, denoted by $G$, is defined in terms of the electrical resistance of the electrolytic solution. The reciprocal of this resistance is the conductance such that $G = 1/R$, and the conductance is expressed in units of $\Omega^{-1}$ or siemen, denoted by S. An older unit is the mho, which is ohm spelled backward. Conductance is a bulk property and encompasses both the solute and solvent that pass through a conductivity detector. A solution has a specific conductivity $k$ in units of siemens per centimeter for a typical conductivity cell. This cell consists of a pair of identical electrodes, each having a surface area $A$ and spaced 1 cm apart. An AC voltage is applied across the electrodes and the resistance

---

© 2006 by Taylor & Francis Group, LLC

**FIGURE 4.66** Schematic of IC showing the ion exchange chemistry.
measured. A cell constant \( k \) can be defined as the ratio of the spacing distance to surface area \( (l/A) \), and all of this gives

\[
\kappa = \frac{Gl}{A} = Gk
\]

Conductivity as an analyte-specific property can be considered by first defining the equivalent limiting conductivity, denoted by \( \Lambda \), for a typical inorganic salt, \( MX_n \), where \( M^n+ \) is a cation of positive charge \( n \) and \( X \) is a monatomic anion (the selection of a monatomic anion serves to simplify the equations). \( \Lambda \) is the conductance if enough of the salt was available to equal 1 g-equivalent weight in a liter of solution. Hypothetically, this would require very large electrode surface areas while maintaining the same spacing between electrodes at 1 cm. This is summarized in the following relationship:

\[
\Lambda = \frac{1000k}{C}
\]

For a concentration \( C \) of the salt \( MX_n \) dissolved in water (in equivalents per liter), \( \Lambda \) has units of siemens-square centimeters per equivalent. By substitution and rearranging, the conductance \( G \) can be related to the equivalent limiting conductivity and \( \Lambda \). The use of \( \Lambda \) enables one to consider the separate contributions of cation and anion. The conductance can then be related to the concentration of the salt, the separate limiting conductances of the individual ions, and the cell constant \( k \) as follows:

\[
G = \frac{CA}{1000k} = \frac{C_{MX}}{1000k} \left( \lambda_M + \lambda_X \right)
\]

We see from the equation that each cation, \( M^n+ \), and each anion, \( X^- \), can be given its own value for \( \lambda \).

We now proceed to relate the bulk property \( G \) to the analyte concentration, and thus connect instrumental IC to TEQA. We focus only on anion exchange IC; however, these relationships can also be derived from the perspective of the cation using cation exchange principles. We consider any monatomic anion \( X \), where \( X \) could be chloride, nitrate, and so forth, in a background of eluent such as \( E^+E^- \), where \( E^+ \) might be Na\(^+\) and \( E^- \) might be CO\(_3\)^{2-}. Let us assume that the concentration of anion is \( C_X \) and the degree of ionization of this anion is \( \alpha_X \). Because the anion displaces the anions in the eluent as it passes from the column to the detector, we can state that the concentration of eluent is

\[
C_E = C_X \alpha_X
\]
Let us consider the nature of the conductance \( G \) during chromatographic elution of the salt \( MX_n \) such that the following relationship holds:

\[
G_{\text{total}} = G_{\text{eluent}} + G_{\text{anion}}
\]

Upon substituting the equation that relates limiting equivalent conductances for separate ions to the conductance of a solution, we have

\[
G_{\text{total}} = \frac{(\lambda_{E^+} + \lambda_{E^-})(C_E - C_X \alpha_X)\alpha_E + (\lambda_{E^+} + \lambda_{X^-})C_X\alpha_X}{1000k}
\]

At the moment the anion is detected, there is a change in the total conductance such that

\[
\Delta G = G_{\text{total}} - G_{\text{backgd}}
\]

The background conductance can be expressed in terms of the eluent cation and anion, respectively, and when subtracted from the total conductance, leads to an important relationship according to

\[
\Delta G = \frac{(\lambda_{E^+} + \lambda_{X^-})\alpha_X - (\lambda_{E^+} + \lambda_{E^-})\alpha_E\alpha_X}{1000k} C_X
\]

This equation is the most general of all and accounts for both suppressed and nonsuppressed IC. Note that the degree of eluent ionization, \( \alpha_E \), has a significant effect on the magnitude of \( \Delta G \). In the case of suppressed IC, the eluent cation, \( E^+E^- \), is converted to \( H^+E^- \), while the analyte salt \( MX_n \) is converted to highly conductive \( H^+X^- \). The creative idea here was to recognize that if \( E^+E^- \) could be made from a moderate to strong base, such as NaOH, Na₂CO₃, or NaHCO₃, then \( H^+E^- \) that is formed in the suppressor is rendered minimally conductive.

If the eluent and anions are fully ionized, the above equation can be further simplified to give us a more direct relationship between the change in conductance and anion concentration:

\[
\Delta G = \frac{(\lambda_{X^-} - \lambda_{E^-})C_X}{1000k}
\]

We thus see that the magnitude of the change in conductance depends not only on anion concentration, but also on the difference between the limiting equivalent conductances of the anion and the eluent ion.¹³¹

The idea behind eluent suppression is to convert the moderately conductive \( Na^+ \) in the eluent to the weakly conductive \( H_2CO_3 \) while converting the low-conducting
Determinative Techniques to Measure Organics and Inorganics

analyte NaX to the high-conducting HX. Limiting equivalent conductivities, denoted by $\lambda$, of some ions in aqueous solution at 25°C are as follows:\textsuperscript{132}

<table>
<thead>
<tr>
<th>Anion/Cation</th>
<th>$\lambda$ (mho-cm²/Eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H\textsuperscript{+}</td>
<td>350</td>
</tr>
<tr>
<td>OH\textsuperscript{-}</td>
<td>198</td>
</tr>
<tr>
<td>SO\textsubscript{4}\textsuperscript{2–}</td>
<td>80</td>
</tr>
<tr>
<td>Cl\textsuperscript{-}</td>
<td>76</td>
</tr>
<tr>
<td>K\textsuperscript{+}</td>
<td>74</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>73</td>
</tr>
<tr>
<td>Pb</td>
<td>71</td>
</tr>
</tbody>
</table>

It is this author's opinion that the development of the micromembrane suppressor was the key advance that stabilized the background conductivity. A schematic of this suppressor is shown in Figure 4.67. The suppressor can be viewed as a "sandwich," whereby the "bread" represents eluent and regenerant flow in opposite directions and the "meat" is a semipermeable cation exchange membrane. This membrane allows H\textsuperscript{+} to pass through and neutralize the carbonate eluent by converting it to the weakly conductive H\textsubscript{2}CO\textsubscript{3}. Figure 4.68 shows several ion chromatograms obtained by the author during the late 1980s. The top IC chromatogram shows the separation and detection of all seven of the common inorganic anions in less than 7 min using an AS-4 anion exchange column (Dionex) with an eluent consisting of 17 mM NaHCO\textsubscript{3}/18 mM Na\textsubscript{2}CO\textsubscript{3}, with a flow rate of 2.0 mL/min. Referring back to Figure 4.64, this Model 2000I (Dionex) was connected to an electronic integrator at the time to generate the printout shown in Figure 4.68. The bottom IC chromatogram is that of a typical groundwater sample that identifies chloride and sulfate. These ions are very common in groundwater, revealing an unidentified peak at a retention time of 4.06 min. In the chromatogram adjacent to this one, a second groundwater sample that happened to be preserved with sulfuric acid is shown. This IC chromatogram indicates that preserving aqueous samples from the environment for subsequent analysis by IC with H\textsubscript{2}SO\textsubscript{4} is a real mistake.
IF IC IS NOT THE PRINCIPAL DETERMINATIVE TECHNIQUE TO MEASURE METALS IN THE ENVIRONMENT, WHAT IS?

Atomic emission spectroscopy (AES) and atomic absorption spectroscopy (AAS) are. In a manner similar to our discussion of molecular spectroscopy, where we compared UV absorption with UV excitation and subsequent fluorescence, these two determinative approaches are the principal ways to identify and quantitate trace concentration levels of metal contamination in the environment. As the need developed to quantitate increasing numbers of chemical elements in the periodic table, so too came advances in instrumentation that enabled this to be achieved at lower and lower IDLs. AES and AAS techniques are both complementary and competitive. Atomic fluorescence spectroscopy (AFS) is a third approach to trace metals analysis. However, instrumentation for this has not yet become widespread in environmental testing labs, and it is unlikely that one would see atomic, or what has become useful x-ray atomic, fluorescence spectroscopy. Outside of a brief mention of the configuration for AFS, we will not cover it here.

We begin this section of Chapter 4 with a discussion of spectroscopy, and it is useful for us to briefly survey all of molecular and atomic spectroscopy from the perspective of the electromagnetic spectrum. The entire electromagnetic spectrum is shown in Figure 4.69, along with those regions in the spectrum that are associated with different types of radiation. The various regions of the electromagnetic spectrum and their respective interactions with matter are cited below each region. The wavelength region starting from the near-UV (~200 nm) through to the near-infrared (~800 nm) is used in atomic spectroscopy, and it involves transitions of electrons between ground and excited electronic states. Spectroscopy, in general, has been defined as that branch of physical chemistry that considers the interaction of electromagnetic radiation with matter. Harris\(^\text{133}\) has described what it is that atomic spectroscopy does:

In atomic spectroscopy, samples are vaporized at very high temperatures and the concentrations of selected atoms are determined by measuring absorption and emission at their characteristic wavelengths. Because of its high sensitivity and the ease with which many samples can be examined, atomic spectroscopy has become one of the principal tools of analytical chemistry.
The quantitative determination of trace concentration levels of the priority pollutant metals is a major activity in environmental testing laboratories. Instruments are designed to accept aqueous samples directly and convert these samples to aerosols or fine mists that are aspirated into flames or plasmas. Solids must be solubilized by vigorous use of strong acids and hydrogen peroxide. This oxidation results in an acidic/aqueous sample that can be subsequently aspirated into a flame or plasma or placed in a resistively heated graphite tube. A logic flowchart is shown in Figure 4.70, and the decision as to which determinative instrumental technique to use largely depends on the required IDLs, or in more familiar terms, “how low you can go.”

**FIGURE 4.68** Actual IC readouts from an integrator obtained by the author. IC conditions; column; AS-4® (Dionex); flow rate, 2 mL/min; eluent, 0.0017 M HCO$^-$/0.0018 M CO$_3^{2-}$; attenuation, at 6; sensitivity, 300 µS full scale. Samples: (A) 6 ppm inorganic anions standard, (B) typical groundwater sample showing significant concentration levels for chloride and sulfate, and (C) groundwater sample fixed with sulfuric acid.
The logic presented in Figure 4.70 should enable the reader to make the right decision given the available instrumentation. If reaching the IDLs as indicated in Figure 4.70 is not necessary, there exists a plethora of analytical methods for metal ions in aqueous solution based on metal chelation with subsequent quantitative analysis using a stand-alone UV-vis spectrophotometer. As introduced in Chapter 3, LLE or alternative column chromatographic techniques for sample prep are available to isolate or preconcentrate metal chelates from complex sample matrices. The reference work by Sandell and Onishi\textsuperscript{134} on the UV-vis spectrophotometry of metals is the most complete work of which this author is aware. One experiment in Chapter 5 shows how one can quantitate the two oxidation states of the element iron, namely, Fe(II) and Fe(III), by forming a metal chelate and using a stand-alone UV-vis spectrophotometer.

78. **HOW DO I CHOOSE WHICH ATOMIC SPECTRAL METHOD TO USE?**

Most alkali and alkaline–earth metal ions that are found dissolved in water are readily quantitated by flame atomic emission spectrometry (FI-AES). This determinative technique has been in the past termed flame photometry. A simple photometer uses
Determinative Techniques to Measure Organics and Inorganics

Cutoff filters to isolate the wavelength, denoted by $\lambda$, with the strongest emission intensity that usually originates or terminates from a resonance line. Resonance transitions are those originating from or terminating to the ground electronic state. For example, the intensity of the 589/590-nm resonance atomic emission doublet for sodium is used to quantitate Na in aqueous samples. Contemporary atomic absorption spectrophotometers incorporate a monochromator and are used in the Fl-AES mode by merely not using the hollow-cathode lamp. The transition or so-called heavy metal ions dissolved in water are more amenable to quantitative analysis using flame atomic absorption (FIAA), graphite furnace atomic absorption (GFAA), inductively coupled plasma atomic emission (ICP-AES), or ICP integrated to a quadrupole mass filter. This relatively recent development is termed ICP-MS. Table 4.20 lists current IDLs for selective metals of interest to TEQA. The IDLs cited make it quite clear as to which atomic spectroscopic determinative technique is more appropriate.

**FIGURE 4.70** Logical flowchart for choosing which atomic spectrometric determinative technique to use. FIAA (flame AA), ICP-AES (inductively coupled plasma atomic emission), GFAA (graphic furnace AA), HGAA (hydride generation AA), and ICP-MS (inductively coupled plasma mass spectrometry).
The following example illustrates how one can decide among these several options. Consider a request from an environmental engineering firm involving the evaluation of a landfill that is suspected to contain the toxic element arsenic (As). This element is part metal and part nonmetal owing to its position in the periodic table. Atoms of the element As are usually found covalently bonded to oxygen either in a trivalent, denoted by As(III), or in a pentavalent, denoted by As(V), oxidation.
state. Arsenic speciation (i.e., the quantitative analysis of environmental samples) for both As(III) and As(V) is an important and current area of research interest. We will have more to say about metal speciation and its growing importance to TEQA later in this chapter. AES and AAS determinative techniques yield total As without regard to speciation. As is difficult to measure down to low IDLs by FlAA. The drinking water regulatory limit for As is set at 50 ppb As. The IDLs listed in Table 14.9 for As suggest that of the several determinative techniques available, either GFAA with an IDL of 0.5 ppb, HGAA with an IDL of 0.03 ppb, or ICP-MS with an IDL of 0.006 ppb, if available, is the recommended determinative technique. Recently, considerations such as these for As have become critical in the author’s laboratory. The decision as to which atomic spectroscopic instrument to choose in TEQA, as outlined in Figure 4.70, is analogous to the choice of which type of instrumental chromatographic separation determinative technique to use, as outlined in Figure 4.1. In contrast to the way this author introduced column chromatographic principles and techniques earlier in this chapter, the early flame AES observations that led to principles are discussed first. Next, the ICP as the principal replacement for the flame is introduced. Following this, the principles that underlie the more recently developed ICP-MS determinative technique are discussed. Finally, we wrap up the topic of how to do trace metals QA with an excursion into the principles and practice of AAS.

79. WHAT HAPPENS WHEN VARIOUS INORGANIC SALTS ARE THRUST INTO FLAMES?

We start with the observations of Kirchoff and Bunsen in Germany in 1859. They observed the bright-line spectra for many alkali and alkaline–earth metal-based salts and are credited with the discovery of spectrochemical analysis. The so-called principal atomic emission series for the common alkali metals is shown in Figure 4.71.

**FIGURE 4.71** Principal atomic emission lines for the alkali metals.
Note that these older atomic spectra are calibrated in terms of wave number, denoted by \( \nu \), of the emitted radiation, whose units are in reciprocal centimeters (denoted by \( \text{cm}^{-1} \)).

Contemporary discussions express the wavelength in units of nanometers (denoted by nm). Frequency is expressed in units of cycles per second or Hertz (denoted by Hz). Frequency can also be viewed in terms of the number of wavelengths per second as \( \nu = c/\lambda \). Can we convert between the two? From Planck’s law, we know that the energy carried by these emitted photons is inversely related to the frequency \( \lambda \), directly related to \( \nu \), and directly related to \( \nu \); this is summarized as

\[
E = h\nu = h\nu \nu
\]

A rule of thumb between wavelength (in nm) and wave number (cm\(^{-1}\)) is given by

\[
\lambda(\text{nm}) = \frac{1 \times 10^7}{\nu(\text{cm}^{-1})}
\]

We introduce the term wave number at this point because it relates directly to energy, and wave number is the principal unit used for the mid-infrared region of the electromagnetic spectrum. In Figure 4.71, the Na doublet resonance emission line occurs at \( \nu \approx 17,000 \text{ cm}^{-1} \). Applying the above equation gives \( \nu = 588 \text{ nm} \), and this value is quite close to the 589/590-nm doublet.

The alkali metals all have a lone electron in an outermost \( s \) atomic orbital. The transition from the higher \( p \) atomic orbital in the excited state to this \( s \) orbital corresponds to an energy difference \( \Delta E \) that can be attributed to release of a photon whose energy is \( h\nu \). Quantum mechanical selection rules allow a change in the azimuthal quantum number \( \Delta l = +1 \) or \( \Delta l = -1 \). Each transition gives rise to a doublet because the spin \( s = 1/2 \) may couple either as \( 1 + s = 3/2 \) or \( 1 - s = 1/2 \) in the \( p \) state. The separation of the doublet of Na and K has been exaggerated in Figure 4.71. One of the more fascinating observations in the introductory general chemistry laboratory is to observe the various colors emitted when the salts NaCl, LiCl, KCl, CaCl\(_2\), and SrCl\(_2\) are thrust into a lab burner flame. Bright-line spectra result when this luminescence is directed through a prism or transmission grating such that the bright light is dispersed into its component \( \lambda \) values.

A partial energy-level diagram is shown in Figure 4.72 for the element Na. Note the two closely spaced levels for \( 3p \) electrons that account for the most intense 589/590-nm doublet. AE results from a relaxation of the electron from the excited \( 3p \) atomic orbital to the ground-state \( s \) atomic orbital, and this process is depicted as

\[
3p \rightarrow 3s
\]

The energy emitted as photons according to Planck’s law is depicted as
Provided sodium vapor is in the path of this incident visible radiation, these emitted photons are absorbed and a ground-state electron residing in an $s$ atomic orbital is promoted to a $3p$ excited atomic orbital and the transition for this is depicted as

$$E_{3p} - E_{3s} = \Delta E = \hbar \nu$$

This energy is absorbed and corresponds to

$$E_{3p} - E_{3s} = \Delta E = \hbar \nu$$
This transition is also the most intense for Na among its other emission lines. In contrast to molecular spectra in solution, atomic spectra in the gas phase consist of very discreet and narrow wavelengths having very narrow bandwidths. For example, Zn has a \( \lambda \) at 213.9 nm and \( \Delta \lambda = 0.002 \) nm.

The AES and AAS determinative techniques can be furthered classified according to the excitation temperatures attained. The following is this classification for most optical, atomic spectral methods of elemental analysis:

<table>
<thead>
<tr>
<th>Atomization</th>
<th>Temperature (°C)</th>
<th>Basis</th>
<th>Acronym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame</td>
<td>1700–3150</td>
<td>Absorption</td>
<td>AAS</td>
</tr>
<tr>
<td>Electrothermal</td>
<td>1200–3000</td>
<td>Emission</td>
<td>AES</td>
</tr>
<tr>
<td>Inductively coupled argon plasma</td>
<td>6000–8000</td>
<td>Emission</td>
<td>ICP-AES</td>
</tr>
<tr>
<td>Direct-current argon plasma</td>
<td>6000–10,000</td>
<td>Emission</td>
<td>DCP</td>
</tr>
<tr>
<td>Electric arc</td>
<td>4000–5000</td>
<td>Emission</td>
<td>Arc-source emission</td>
</tr>
<tr>
<td>Electric spark</td>
<td>40,000(?)</td>
<td>Emission</td>
<td>Spark-source emission</td>
</tr>
</tbody>
</table>

Of the six AES and AAS determinative instrumental techniques cited, the first three are most likely to be found in environmental testing labs, and for this reason, we will consider only these. Of the three, the two most important to TEQA are ICP-AES and GFAA. FI-AAS is still important; however, its relatively high IDLs, particularly for the priority pollutant metals, serve to limit its usefulness. FI-AES remains the analytical determinative technique of choice for the quantification of alkali and alkaline–earth metal ions found in aqueous samples. The degree to which a groundwater sample is categorized as hard is quantitatively determined by measuring the concentration of Ca and Mg via FI-AAS as introduced in a student experiment in Chapter 5.

80. HOW DOES THE DESIGN OF AA, AE, AND AF INSTRUMENTS FUNDAMENTALLY DIFFER?

Figure 4.73 depicts the simplest instrument configurations for AE (top), AA (middle), and AF (bottom). Note how the positions of the flame source, optics, and detector differ among all three. The analyst should have this simple schematic in mind when approaching atomic spectroscopic instrumentation. With respect to the top schematic, instrumentation has been advanced that has sought to maximize the emitted intensity of a particular wavelength while minimizing the background emission at that wavelength. For FI-AES or ICP-AES, it is the emitted line intensity of a particular wavelength against this background emitted intensity that forms the basis of TEQA. This includes the analysis of environmental samples for priority pollutant metal ions that follows the process of calibration, verification of the calibration (i.e., running sufficient ICVs; see Chapter 2), interpolation of the calibration curve (quantitative analysis), and establishing the IDL for a given metal analyte. We proceed now to discuss ICP-AES.
81. WHAT IS ICP-AES?

This author has been searching for a good description of the ICP for this book. A recently written book, published by those affiliated with the PerkinElmer Corporation, at the time describe the ICP very well:\textsuperscript{135}

The ICP discharge used today for optical emission spectrometry is very much the same in appearance as the one described by Velmer Fassel in the early 1970's. Argon gas is directed through a torch consisting of three concentric tubes made of quartz or some other suitable material. A copper coil, the load coil, surrounds the top end of the torch and is connected to a radio frequency (RF) generator. When RF power, typically 700–1500 watts, is applied to the load coil, an alternating current moves back and forth
within the coil, or oscillates, at a rate corresponding to the frequency of the generator. In most ICP instruments this frequency is either 27 or 40 MHz. This RF oscillation of the current in the load coil causes RF electric and magnetic fields to be set up in the area at the top of the torch. With argon gas being swirled through the torch, a spark is applied to the gas causing some electrons to be stripped from their argon atoms. These electrons are then caught up in the magnetic field and accelerated by them. Adding energy to the electrons by the use of a coil in this manner is known as inductive coupling. These high-energy electrons in turn collide with other argon atoms, stripping off still more electrons. This collisional ionization of the argon continues in a chain reaction, breaking down the gas into a plasma consisting of argon atoms, electrons, and argon ions, forming what is known as an inductively coupled plasma discharge. The ICP discharge is then sustained within the torch and load coil as RF energy is continually transferred to it through the inductive coupling process. The ICP discharge appears as a very intense, brilliant white, teardrop-shaped discharge…. At the base, the discharge is toroidal, or “doughnut shaped” because the sample-carrying nebulizer flow literally punches a hole through the center of the discharge. The body of the “doughnut” is called the induction region because this is the region in which the inductive energy transfer from the load coil to the plasma takes place … the area from which most of the white light, the induction region, and into the center of the plasma gives the ICP many of its unique analytical capabilities.

Stanley Greenfield\textsuperscript{136} in England first published a report in 1964 on the use of an atmospheric pressure ICP for elemental analysis, whereas Velmer Fassel and colleagues made the earliest refinements, including nebulization of the aqueous sample. The ICP-AES determinative method appeared in the early 1980s as EPA Method 200.7 for the determination of metals in drinking water. Figure 4.74 is a schematic from

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{fig4_74.jpg}
\caption{Schematic of the original Fassel design for ICP.}
\end{figure}
the original Fassel design and shows the three possible pathways up through the concentric cylindrical quartz ICP torch. An aqueous sample converted to an aerosol is taken up into the plasma via aerosol formation with Ar, and any atomic emission due to the metals in the sample is observed atop the torch, as shown. In an EPA contract lab that this author worked in during the 1980s, the ICP-AES instrument was operated continuously. A large tank containing liquefied Ar serves well when continuous operation of the ICP torch is anticipated. In the 1990s, in academia, this author used a compressed tank of Ar because of only intermittent use of the ICP-AES instrument. Figure 4.75 shows a simple schematic of a double-monochromator optical system that might be typical of mid-1980s technology for ICP-AES. The emitted radiation from the ICP is directed through a lens, L, and reflected off of a mirror, M₁. This reflected light enters slit S₁ and goes to mirror M₂, which reflects the light to a diffracting grating, G, off of M₂ a second time. This reflected light exits through slit S₂. A schematic for a typical ICP-AES is shown in Figure 4.76.

82. HOW HAVE ICP-AES INSTRUMENTS EVOLVED?

Advances in design of ICP-AES instruments have led to systems that now incorporate an Echelle grating and a charged-couple detector as shown in Figure 4.77.²⁷ A comparison of the major instrumental components for an ICP-AES for instruments manufactured in the 1980s vs. those made in the 1990s, particularly during the latter part of the decade, is shown in Table 4.21.

83. WHAT HAPPENS TO Ar AND TO A METAL ANALYTE IN THE PLASMA?

Electrons ionize the plasma gas according to

\[ e^- + Ar \rightarrow Ar^+ + 2e^- \]

In addition, recombination can occur according to

\[ e^- + Ar^+ \rightarrow Ar^* + hv \]

with the formation of excited Ar atoms, symbolized by Ar*, and a background emission at resonance lines of 104.8 and 106.7 nm, respectively.

The plasma source serves two roles in ICP-AES:

- To atomize the sample so as to free the metal analyte, usually in its ground state
- To partially ionize the metal analyte with excitation of both atoms and ions
FIGURE 4.75 Schematic of a double monochromator for ICP-AES and emission spectrum of a mercury source.
These processes are summarized as follows, where Ar\textsuperscript{m} represents a metastable atom:\textsuperscript{141}

<table>
<thead>
<tr>
<th>Chief Ionization Processes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge transfer</td>
<td>$\text{Ar}^+ + \text{M} \rightarrow \text{M}^++ \text{Ar}$</td>
</tr>
<tr>
<td>Electron-impact ionization</td>
<td>$e^-(\text{fast}) + \text{M} \rightarrow \text{M}^+ + 2e^-$ (slow)</td>
</tr>
<tr>
<td>Penning ionization</td>
<td>$\text{Ar}^m + \text{M} \rightarrow \text{M}^++ \text{Ar}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chief Excitation Processes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron-impact excitation</td>
<td>$e^- + \text{M} \rightarrow \text{M}^+ + e^-$</td>
</tr>
<tr>
<td>Ion–electron radiative recombination</td>
<td>$\text{M}^+ + e^- \rightarrow \text{M}^+ + h\nu$</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
When the sum of the metal analyte’s ionization and excitation energies is below the ionization potential for Ar (i.e., ~16 eV), ionic lines become the most sensitive. The following table summarizes those elements that yield the most sensitive lines:\(^{137}\)

**TABLE 4.21**
Comparison of ICP-AES Components between Decades

<table>
<thead>
<tr>
<th>Component</th>
<th>1980s</th>
<th>1990s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample introduction</td>
<td>Pneumatic</td>
<td>Pneumatic, frit, ultrasonic, direct insert, thermospray, electrospray</td>
</tr>
<tr>
<td>RF generator</td>
<td>Piezoelectric</td>
<td>Solid state</td>
</tr>
<tr>
<td>Torch</td>
<td>Greenfield or Fassel type</td>
<td>Modified Fassel type</td>
</tr>
<tr>
<td>Plasma view</td>
<td>Side-on (radial)</td>
<td>Side-on (radial), end-on (axial), dual</td>
</tr>
<tr>
<td>Monochromator</td>
<td>Czerny–Turner or Ebert</td>
<td>Echelle, dispersion of (\lambda) values/prism, dispersion of diffraction orders</td>
</tr>
<tr>
<td>Detector</td>
<td>Photomultiplier</td>
<td>Solid state, charge transfer device</td>
</tr>
<tr>
<td>Signal processing</td>
<td>Analog, analog-to-digital, signal, then background</td>
<td>Analog-to-digital with storage of signal and background simultaneously</td>
</tr>
</tbody>
</table>

**FIGURE 4.77** The Echelle grating used in contemporary ICP-AES instruments.
84. CAN THE INTENSITY OF AN ATOMIC EMISSION LINE BE PREDICTED?

Yes, but we need to discuss relationships previously derived from statistical mechanics that relate to the population of atoms and ions among the various quantized electronic energy levels. Unless a solid sample is being directly introduced into the plasma by one of the direct insertion techniques, a metal ion dissolved in water, $M_{(aq)}^{n+}$, is most likely to be found. This hydrated metal ion becomes dehydrated in the plasma, atomized, and then undergoes excitation or ionization at the extremely high temperature of the plasma. The use of double arrows below depicts the various equilibria occurring in the plasma:

$$
\begin{align*}
M_{(aq)}^{n+} & \rightleftharpoons M_{(g)}^{n+} \rightleftharpoons M^{n+} \\
M_{(g)} & \rightleftharpoons M_{(g)}
\end{align*}
$$

The high temperature achieved in the ICP favors formation of analyte ions over formation of analyte atoms (90% conversion to ions), as indicated by the thicker arrow.

The ratio of the number of metal atoms that have their outermost electrons in the excited quantized energy level ($N_{\text{ground}}$) and how this ratio is influenced by the temperature in the plasma are governed by the Boltzmann distribution. This well-known equation from statistical mechanics is

$$
\frac{N_{\text{excited}}}{N_{\text{ground}}} = \left( \frac{g_{\text{excited}}}{g_{\text{ground}}} \right) e^{-\Delta E/kT}
$$

where $g_{\text{excited}}$ is the degeneracy of the excited state and $g_{\text{ground}}$ is the degeneracy of the ground state. Degeneracy is the number of states available at each quantized energy level and $g = 2J + 1$, where $J$ is the total angular momentum quantum number. $\Delta E$ is the energy-level spacing, $k$ is Boltzmann’s constant ($1.38 \times 10^{-23} \text{ J/K}$) or, in
terms of wave number, \( k = 0.695 \text{ cm}^{-1} \), and \( T \) is the absolute temperature in degrees Kelvin. The total population among all quantized atomic energy levels is given by

\[
N = n_0 + n_1 + n_2 + \ldots + n_i + \ldots
\]

The total number of metal analyte atoms can be considered as being distributed among or partitioned into the various quantized energy states according to a partition function denoted by \( Z \) and can be written as

\[
Z = g_0 + g_1 e^{-E_1/kT} + g_2 e^{-E_2/kT} + \ldots + g_i e^{-E_i/kT} + \ldots
\]

The fraction of \( M \) atoms whose electrons are in the \( j \)th excited state becomes

\[
\frac{n_j}{N} = \left( \frac{g_j}{Z} \right) e^{-E_j/kT}
\]

The extent that \( M \) atoms are ionized in the plasma can be predicted from applying the Saha equation, and after manipulation of the basic relationship, it can be written as follows:\textsuperscript{138}

\[
\frac{N_j N_e}{N_{aj}} = \left( \frac{(2\pi m_i kT)^{3/2} 2Z_{ij}}{\hbar^3 Z_{aj}} \right) e^{-E_i/kT}
\]

These parameters are defined as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N_j )</td>
<td>Number of ionic species ( j )</td>
</tr>
<tr>
<td>( N_{aj} )</td>
<td>Number of atomic species ( j )</td>
</tr>
<tr>
<td>( E_i )</td>
<td>Ionization potential of atomic species ( j )</td>
</tr>
<tr>
<td>( Z_{ij} )</td>
<td>Partition function of ionic species ( j )</td>
</tr>
<tr>
<td>( Z_{aj} )</td>
<td>Partition function of atomic species ( j )</td>
</tr>
<tr>
<td>( N_e )</td>
<td>Number of free electrons</td>
</tr>
<tr>
<td>( M_e )</td>
<td>Mass of electron</td>
</tr>
<tr>
<td>( h )</td>
<td>Planck’s constant</td>
</tr>
<tr>
<td>( k )</td>
<td>Boltzmann’s constant</td>
</tr>
</tbody>
</table>

Consider the transition between a ground-state atom whose potential energy is \( E_0 \) and an excited state \( j \) whose potential energy is \( E_j \). The intensity of an emitted wavelength in the plasma depends on the following factors:\textsuperscript{139}

- The difference \( E_j - E_0 = h\nu = \Delta E \), the number of metal atoms in the excited state, \( n_j \)
- The number of possible transitions between \( E_j \) and \( E_0 \) per unit time
• The Einstein transition probability $A_j$ such that the emitted intensity $I$ is proportional to the product of $\Delta E$ and $A_j$ such that

$$I \propto \Delta E A_j$$

Solving the Boltzmann distribution for the number of atoms in the excited state, $N_{aj}$, and substituting for $N_{aj}$ leads to the outcome of this section and answers the question posed:

$$I = \Phi \left( \frac{h c g_j A_j N}{4\pi \lambda Z} \right) e^{-\Delta E/kT}$$

where $\Phi$ is a coefficient to account for emission being isotropic over a solid angle of $4\pi$ steradians. For the AE intensity to be directly proportional to the number of ground-state atoms, $N$, as shown above, all other terms in the above equation must remain constant. Therefore,

$$I \propto \text{conc}$$

and this relationship makes it possible to conduct TEQA.

We assume for purposes of TEQA that a certified reference standard such as for Cr when aspirated into the plasma exhibits nearly identical parameters as that for Cr in the environmental sample. This requires good stability of the source characteristics (i.e., forward power and gas flow rates). A calibration plot for Cr is shown in Figure 4.78; this plot was obtained using a Plasma 2000 ICP spectrometer (Perkin-Elmer) from the author’s laboratory. The upper plot shows the raw calibration data using the common 267-nm line for Cr, and the lower plot shows how good these data points fit to a least squares regression line using the trend line in EXCEL. The results shown below this plot were obtained from entering the calibration data into a computer program written by the author. IDLs were determined using principles discussed in Chapter 2.

85. WHY DO THE AE LINES BROADEN?

Two factors contribute to AE band broadening. The first is due to the motion of $M$ atoms in the plasma, a so-called Doppler effect, and the second is broadening due to collisions. Doppler broadening, symbolized by $\Delta \lambda_{dp}$, depends on the wavelength chosen, the kinetic temperature, and the molecular weight of the metal of interest and can be predicted as follows:

$$\Delta \lambda_{dp} = 7.16 \times 10^{-7} \lambda \sqrt{\frac{T_{\text{min}}}{M}}$$
Values for $\Delta \lambda_D$ are low for heavy elements (e.g., Au(II) at 200.08 nm has a value of 0.8 pm), whereas $\Delta \lambda_B$ values are high for light elements, such as Be(II) at 313.11 nm that has a value of 5.9 pm. Collisional broadening results from collisions among analyte ions, atoms, and neutral Ar atoms and has also been called pressure broadening. Doppler broadening is dominant near the center of the band, whereas collision broadening dominates near the tails. AE line widths dictate what resolution is needed to resolve one AE emission line from another. For each transition metal, there is a plethora of lines to consider.

**FIGURE 4.78** Calibration plots for the determination of chromium by ICP-AES showing (A) raw data and (B) a least squares regression fit to the raw data. A Plasma 2000® (Perkin-Elmer) was used to generate this data.

© 2006 by Taylor & Francis Group, LLC

**86. WHAT IS ICP-MS AND HOW DOES THIS DETERMINATIVE TECHNIQUE COMPLEMENT AND COMPETE WITH ICP-AES?**

The coupling of the ICP source with a single quadrupole mass spec via developments in the design of a suitable interface between sample introduction, the ICP torch itself, and a quadrupole mass spec “opened the flood gates” while expanding the scope of trace metals analysis. Laboratories that already have ICP-AES capability are looking to expand their analytical services to include ICP-MS. ICP-MS offers a significant increase in sensitivity (refer again to Table 4.20), as well as providing isotopic abundance data. The ICP torch is rotated to the horizontal position that enables an aqueous aerosol to be introduced into the torch in the conventional way; however, instead of energy from the plasma being used to excite ground-state electronic levels that lead to atomic emission spectra, metal ions enter a tiny orifice
Determinative Techniques to Measure Organics and Inorganics

via a sampler/skimmer and enter the quadrupole rods, where rapid scanning enables mass-selective detection. Skoog and Leary\cite{140} have introduced the principle in this manner:

Positive metal ions, produced in a conventional ICP torch, are sampled through a differentially pumped interface linked to a quadrupole spectrometer. The spectra produced in this way, which are remarkably simple compared with conventional ICP spectra, consist of a simple series of isotope peaks. These spectra are used for quantitative measurements based upon calibration curves, often with an internal standard. Analysis can also be performed by the isotope dilution technique.

The benchmark papers for early investigations of the ICP as an ion source for mass spec were published in the late 1970s.\cite{141} A more detailed treatment of this topic can be found elsewhere.\cite{142}

Figure 4.79 is a schematic diagram of a commercially available ICP-MS, with the exception of the mass spec. This drawing shows the various ways to introduce a sample into an ICP-MS instrument. Note that a GC, SFC, LC, IC, and CE can serve as important up-front separation techniques. This is in addition to the more conventional flow injection approach to sample introduction. Note also how compressed argon gas not only is used to sustain the plasma, as discussed earlier, but also is used as the source for nebulizer gas. Note also that the sample aerosol that enters the plasma gets introduced to the mass spec via a sampler under conditions of atmospheric pressure. The region between the sampler (closest to the torch) and the skimmer (closest to the mass spec) is a partially evacuated chamber in which 99.9\% of unwanted species are removed to the rough pump. Figure 4.80 depicts just how an HPLC could be interfaced to an ICP-MS. This instrument was used to conduct trace arsenic speciation.\cite{143} The drawing includes important terms used to describe the more significant features of the instrument. Budde\cite{144} has articulated sample introduction to the ICP-MS in the following manner:

FIGURE 4.79 Schematic of the many options available that interface to an ICP-MS to accomplish metals speciation as well as total metals quantitative analysis.
Liquids are injected first into a nebulizer, which produces an aerosol spray into a spray chamber. Large droplets and condensate are removed in the spray chamber, and the fine aerosol particles are transported by the flowing Ar gas into the ICP torch. The spray chamber is similar to the desolvation chamber used with the particle beam LC interface. Efficiencies of transport of analytes from the sample to the ICP torch are only in the 1–5% range. If a low flow rate separation column is used, for example, a microbore LC column or CE column, a supplemental flow of liquid into the nebulizer can be used or the spray chamber can be by-passed and the nebulizer spray injected directly into the ICP torch.

ICP-MS cannot (in contrast to ICP-AES) simultaneously detect and quantitate all the metal ions in a sample. However, ICP-MS provides for fast sequential acquisition using either scan or SIM modes (refer to earlier discussions). Mass spectra of metal ions are much simpler to interpret than the numerous lines in atomic emission spectra. Isotopic abundances for each element enable correct identifications to be made, and this advantage of ICP-MS has the potential to further widen the scope of trace metals analysis to, for example, the geological sciences. In addition to single quadrupole operation, both high-resolution MS (HRMS) and tandem MS are used with ICP ionization, which leads to gains in selectivity, exact m/z data, and structural elucidation. EPA Methods 6020A and 6800 provide useful insights to the scope and application of conducting TEQA using ICP-MS in the enviro-chemical QA arena, while the Nixon and Moyer show applicability to enviro-health QA.
87. **WHAT INTERFERENCES ARE PRESENT AND HOW ARE THESE OVERCOME?**

Ions from the plasma gas, matrix components, or the solvent acid used to dissolve the sampler have been recognized as the major spectral interferences generated in the practice of ICP-MS. The interferences are termed *isobaric* since they have the same \( m/z \) as the analyte of interest. Thomas provides examples of these interferences:

<table>
<thead>
<tr>
<th>Plasma Gas Isobaric Interference</th>
<th>Interferes with a Determination of</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{40}\text{Ar}^{16}\text{O})</td>
<td>(^{56}\text{Fe})</td>
</tr>
<tr>
<td>(^{38}\text{ArH})</td>
<td>(^{39}\text{K})</td>
</tr>
<tr>
<td>(^{40}\text{Ar})</td>
<td>(^{40}\text{Ca})</td>
</tr>
<tr>
<td>(^{40}\text{Ar}^{35}\text{Cl})</td>
<td>(^{30}\text{Se})</td>
</tr>
<tr>
<td>(^{35}\text{Cl}^{16}\text{O})</td>
<td>(^{75}\text{As})</td>
</tr>
<tr>
<td>(^{40}\text{Ar}^{12}\text{C})</td>
<td>(^{52}\text{Cr})</td>
</tr>
<tr>
<td>(^{35}\text{Cl}^{16}\text{O})</td>
<td>(^{51}\text{V})</td>
</tr>
</tbody>
</table>

Development of collision/reactor cells placed between the interface and quadrupole rods for the most part eliminated many of these spectral interferences. Interferences can be removed in ICP-MS by discriminating based on either kinetic energy or mass. For kinetic energy discrimination, a low reactive collision gas such as \( \text{H}_2 \) or \( \text{He} \) is bled into the cell. The cell consists of a quadrupole, hexapole, or octapole, usually operated in the RF-only mode. The *RF-only* field does not enable a mass selection to occur in contrast to a quadrupole. This difference enables a focusing of ions prior to collision with reaction gas. For mass discrimination, dynamic reaction cell (DCR) technology has been developed. Thomas has described the DCR this way:

In DCR technology, a quadrupole is used instead of a hexapole or octapole. A highly reactive gas, such as ammonia or methane, is bled into the cell, which is a catalyst for ion molecule chemistry to take place. By a number of different reaction mechanisms, the gaseous molecules react with the interfering ions to convert them either into an innocuous species different from the analyte mass or a harmless neutral species. The analyte mass then emerges from the DCR free of its interference and is then steered into the analyzer quadrupole for conventional mass separation. The advantage of using a quadrupole in the reaction cell is that the stability regions are much better defined than a hexapole or octapole, so it is relatively straightforward to operate the quadrupole inside the reaction cell as a mass or bandpass filter, and not just an ion-focusing guide. Therefore, by careful optimization of the quadrupole electric fields, unwanted reactions between the gas and the sample matrix or solvent are prevented. Everytime an analyte and interfering ions enter the DCR, the bandpass of the quadrupole can be optimized for that specific problem and then changed on-the-fly for the next one.

Two DCR reactions are shown below that serve to eliminate an isobaric interference. In the first, the \(^{40}\text{Ar}^+\) interference is eliminated in the determination of \(^{40}\text{Ca}^+\), while in the second, the \(^{56}\text{ArO}^+\) isobaric interference is eliminated in the determination of \(^{56}\text{Fe}\). These reactions are shown below:
We now seek to get a bit beyond the use of ICP-MS to quantitate metals in terms of a total metal content to the more recent developments in trace metals speciation.

### 88. HOW IS ICP-MS USED IN TRACE METALS SPECIATION?

The hyphenation of various HPLC techniques with ICP-MS, as shown previously in Figure 4.80, has made the largest impact. A summary of metal speciation is given below, where SRM refers to the NIST’s standard reference material:

<table>
<thead>
<tr>
<th>Metal</th>
<th>Type of HPLC</th>
<th>Species and Sample Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Anion exchange</td>
<td>As$^{3+}$, As$^{5+}$, dimethyl arsonate (DMA), monomethyl arsonate (MMA) in urine</td>
</tr>
<tr>
<td>As</td>
<td>Anion pairing</td>
<td>As$^{3+}$, As$^{5+}$, DMA, MMA, arsenobetaine in dogfish SRM</td>
</tr>
<tr>
<td>As</td>
<td>Micellar</td>
<td>As$^{3+}$, As$^{5+}$, DMA, MMA, As–betaene, As–choline in dogfish SRM</td>
</tr>
<tr>
<td>As</td>
<td>Anion exchange and reversed phase</td>
<td>As$^{3+}$, As$^{5+}$, DMA in urine</td>
</tr>
<tr>
<td>Cr</td>
<td>Anion exchange</td>
<td>Cr(III), Cr(VI)</td>
</tr>
<tr>
<td>Cr</td>
<td>Ion pairing</td>
<td>Cr(III), Cr(VI)</td>
</tr>
<tr>
<td>Sn</td>
<td>Reversed phase and micellar</td>
<td>Trimethyl tin chloride, trimethyl tin bromide, tripropyl tin chloride, monomethyl tin trichloride, dimethyl tin dichloride, trimethyl tin chloride standards</td>
</tr>
<tr>
<td>Sn</td>
<td>Ion pairing and ion exchange</td>
<td>Trimethyl tin chloride, tributyl tin chloride, triphenyl tin acetate standards</td>
</tr>
<tr>
<td>Hg</td>
<td>Reversed phase</td>
<td>Methyl mercury acetate, ethyl mercury chloride and Hg(II) chloride in tuna fish SRM, thimerosal in vaccines</td>
</tr>
<tr>
<td>Cd</td>
<td>Size exclusion</td>
<td>Cd in pig kidney</td>
</tr>
<tr>
<td>Pb</td>
<td>Reversed phase, cation exchange, ion pairing, size exclusion</td>
<td>Inorganic lead, tetraalkyl lead, tri- and dialkyl lead</td>
</tr>
<tr>
<td>P, S</td>
<td>Ion pairing</td>
<td>P and S in inorganic phosphates and sulfates, oranic phosphates and amino acids</td>
</tr>
<tr>
<td>V, Ni</td>
<td>Anion exchange</td>
<td>V(III), V(V), Ni(II)</td>
</tr>
<tr>
<td>Au</td>
<td>Ion pairing and size exclusion</td>
<td>Dicyanogold(I) in blood and urine</td>
</tr>
<tr>
<td>I</td>
<td>Reversed phase</td>
<td>Iodide ion and free iodo amino acids</td>
</tr>
</tbody>
</table>

Figure 4.81 shows three chromatograms that illustrate the successful metal speciations obtained with HPLC-ICP-MS. In Figure 4.81A a separation of free Zn from zinc protoporphyrin from the whole blood of a lead poisoned patient using
RP-HPLC-ICP-MS. In Figure 4.81B, IP-RP-HPLC-ICP-MS was used to separate Hg and Pb species. A microbore PEEK® column packed with C18 chemically bonded silica was used. The mobile phase consisted of 5 mM sodium pentanesulfonate in 20:80 v/v acetonitrile-water at 1000 µL/min. Pb was detected at 0.2 pg and Hg at 7 pg. In Figure 4.81C, a separation of four arsenic standards again using IP-RP-HPLC-ICP-MS was achieved. This method has been demonstrated to speciate arsenic in urine. Emon’s group in Germany report on successful speciation of As and Sb via (1) interfacing of HPLC with hydride generation and atomic absorption spectroscopy and (2) HPLC interfaced to ICP-MS.\textsuperscript{149} Those interested in more details on speciation are referred elsewhere.\textsuperscript{150} In most laboratories today, one or more atomic absorption spectrophotometers “dot the landscape.” EPA contract lab requirements are that graphite furnace atomic absorption spectrophotometry is used to quantitatively determine As, Se, Tl, and Pb, while ICP-AES is used to determine all the rest. Enviro-health labs continue to quantitate trace Pb via GFAA. Many state public health labs quantitate the presence of Pb in from 5000 to 10,000 blood specimens annually. For this reason, we delve into atomic absorption.
WHAT IS ATOMIC ABSORPTION?

It is the phenomenon by which resonance atomic emission lines are absorbed by atoms in the vapor phase; the phenomenon adheres to Beer’s law, and hence forms the basis for TEQA involving environmental samples that contain heavy metal residues. Kirchoff in the mid-1800s is credited with conceiving and performing the classical experiment that first observed atomic absorption. A simple schematic of the experiment is shown in Figure 4.82. The yellow light emitted when a NaCl-coated nichrome wire is thrust into the flame was directed to a screen with a small hole. Observations of the brightness of the yellow light, reflected off of a solid back screen that disappears when elemental sodium is heated in a watch glass above a second burner, must have surprised Kirchoff. The resonance emission of the characteristic 589-nm yellow light was completely absorbed by the Na vapor, explaining the absence of light at the detector, and was dubbed atomic absorption. Contemporary sources from resonance atomic emission lines of many elements emit in the colorless UV region of the electromagnetic spectrum. The photomultiplier tube (PMT) can sense this colorless UV radiation, enabling atomic absorption to “cast a wide net” to include most metals and metalloids, like As and Se, in the periodic table.

Atomic absorption as an analytical technique remained dormant until Walsh in Australia and Alkemade and Milatz in the Netherlands conceived the notion and published their findings in the same year, 1955. Varian Associates licensed the technology from Walsh, and to this day, Varian manufactures its AA instruments in Australia. The original experiment of Kirchoff is repeated every time an analyst performs a quantitative analysis of specific metal using an AA spectrophotometer. Significant modifications to the classical experiment have been made:

- The source of the resonance line has been replaced by hollow-cathode lamps (HCLs) or, in some cases, electrodeless discharge lamps (EDLs).
- The second burner has been replaced by a concentric flow nebulizer with an acetylene–nitrous oxide burner.
- The wavelength for the resonance line from the source is isolated from extraneous wavelengths due to interferences by a monochromator.
- The back screen and eyeball have been replaced by the photomultiplier tube and amplifying electronics.
This author has used AA spectrophotometers made by either PerkinElmer or Varian over the years. The advances in ICP-AES during the late 1970s and 1980s relegated FI-AAS to the back burner. The low IDLs enjoyed by contemporary GFAAs have kept this technology relevant to the goals of TEQA. Before we focus our discussion on how GFAA is used to perform TEQA, let us compare AA instruments in general.

90. WHAT ELEMENTS OF THE AA SPECTROPHOTOMETER DO I NEED TO KNOW MORE ABOUT?

Single- vs. double-beam design and the necessity for an HCL is the answer. Figure 4.83 is a schematic of a single-beam AA configuration showing the single beam of resonance line radiation emanating from an HCL, passing through a flame atomizer and into the entrance slit of a monochromator. Figure 4.84 shows how this basic configuration is modified to accommodate a double-beam configuration. A rotating chopper is a semitransparent device that directs a portion of the HCL resonance line radiation to become diverted, pass through the atomizer and on to the entrance slit to the monochromator, and comprise the sample beam while the transparent portion bypasses the atomizer directly to the monochromator. The PMT in the double case alternatively sees sample intensity/reference intensity. This comparison yields a very stable noise level over time. When an aqueous solution is aspirated into the flame, or when a microdrop is placed in the furnace, the transmitted power decreases in comparison to the reference power and a good signal-to-noise

FIGURE 4.83 Schematic of a single-beam AA instrument. The dashed line represents a modulated signal from the radiation source, and the solid line represents direct-current emission from the atomizer.

FIGURE 4.84 Light path in a double-beam AA design.
ratio can be realized. For this reason, double-beam AA instruments are preferred, but are also more expensive. Figure 4.85 compares the major components of both a FlAA and a GFAA.

It would seem that an ordinary deuterium lamp that emits a continuum spectrum would make a low-cost radiation source for AA, provided the source is passed through a monochromator whereby a narrow bandpass of wavelengths can be selected. However, in order for Beer’s law to be obeyed, the bandwidth at half height, $\Delta \lambda_{1/2}$, for the monochromator should be less than $\Delta \lambda_{1/2}$ for the analyte of interest —
in this case, the atoms in the vapor phase of metallic elements. Robinson (p. 320) has articulated the problem this way:

With the use of slits and a good monochromator, the band falling on the detector can be reduced to about 0.2 nm. If a band 0.002 nm wide were absorbed from this, the signal would be reduced 1%. Since this is about the absorption line width of atoms, even with complete absorption of radiation by atoms, the total signal would change by only 1%. This would result in an insensitive analytical procedure of little practical use. The problem of using such narrow absorption lines was solved by adopting a hollow cathode as the radiation source.

91. WHY IS THE GFAA A SUITABLE TRACE DETERMINATIVE TECHNIQUE?

The relatively low IDLs listed in Table 4.20 suggest that GFAA is an ideal determinative technique for TEQA involving most metals. Contemporary GFAA instrumentation incorporates an autosampler that delivers a microdrop of sampler directly into the center of a cylindrically shaped graphite tube via a predrilled hole midway up the length of the tube. Because the total amount of sample is available and when combined with the ability of the instrument to emit the resonance line associated with the HCL or EDL and then absorb this narrow band of UV radiation across a path length of a few centimeters, an appreciable absorbance is measured from a very low analyte concentration. This is in contrast to the concentric flow nebulizer used in FIAA, whereby most of the sampler volume (~90%) is drawn to waste. For the highest-quality analytical determination, it is suggested that pyrolytically coated graphite tubes be used. Prior to use, a L’vov (after the Russian scientist) platform is inserted into the graphite tube in the manner shown in Figure 4.86. The purpose of using a L’vov platform along with the additional expense is to isolate the sample from the tube walls to allow more reproducible atomization of the sample through indirect heating. The platform enables a higher atomization temperature to be reached, as is shown in Figure 4.87, and this produces more free atoms, which reduces interferences. Pyro-coated tubes can also be purchased with the platform already built in.

![Atomic absorption spectroscopy](image)

**FIGURE 4.86** The L’vov platform used in GFAA.
92. WHAT DETERMINES THE STRENGTH OF ABSORPTION FOR A GIVEN METAL ANALYTE?

In a manner similar to our discussion of atomic emission, the Boltzmann distribution again needs to be considered, and the ratio of the number of atoms in an excited state to the number of atoms in the ground state is reiterated as follows:

\[
\frac{N_{aj}}{N_0} = \frac{g_j}{g_0} e^{-E_{aj}/kT}
\]

The maximum sensitivity obtained when 100% of all atoms are in their ground state is \(N_{aj}/N_0 \sim 0\); this ratio is low when the temperature is low and \(E_{aj}\) is low. Examples are as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>(\lambda) (nm)</th>
<th>(N_{aj}/N_0) at 3000 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cs</td>
<td>852.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Zn</td>
<td>213.8</td>
<td>(1 \times 10^{-10})</td>
</tr>
<tr>
<td>Na</td>
<td>590</td>
<td>(4 \times 10^{-4})</td>
</tr>
</tbody>
</table>

Because most atoms are in their ground state, the atomic absorption signal depends on the number of ground-state atoms, \(N_0\), and \(T\) has minimal influence, unlike AES, where changes in \(T\) cause significant changes in \(N_{aj}/N_0\), as discussed earlier. Temperature-dependent chemical reactions may occur that influence the number of gaseous atoms formed, and this leads to chemical interferences.

We proceed now to take the notion of atomic absorption for GFAA a bit further. (Benchmark papers are provided in Walsh.151) A consideration of Einstein probability coefficients for the simple concept of a transition from the ground electronic state...
to the first excited state results in a relationship between the absorbance $A$ and more fundamental parameters, assuming that $\Delta \lambda_{1/2}$ for the monochromator is less than $\Delta \lambda_{1/2}$ for the analyte of interest:

$$A = \frac{3.83 \times 10^{-13} f_{01} b N_0(t)}{\Delta \lambda_{\text{eff}}}$$

where $f_{01}$ is the oscillator strength, $b$ is the path length, $N_0(t)$ is the number of ground-state atoms in the atomic vapor at time $t$, and $\Delta \lambda_{\text{eff}}$ is the width of a rectangular absorption profile that has the same $k$ area and peak value as the light sources. Oscillator strengths for selected metals are as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>$\lambda$ (nm)</th>
<th>$f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>422.7</td>
<td>1.75</td>
</tr>
<tr>
<td>Cr</td>
<td>357.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Cu</td>
<td>324.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Na</td>
<td>589</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>589.6</td>
<td>0.655</td>
</tr>
<tr>
<td>Tl</td>
<td>276.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**93. WHY IS IT IMPORTANT TO CORRECT FOR BACKGROUND ABSORPTION?**

To achieve good precision and accuracy (see Chapter 2) in quantitating trace concentration levels of various metals of interest. Robinson (pp. 338–341)\textsuperscript{152} has suggested that unknown samples, whose residual metal content is of interest to TEQA, the calibration standards, and the initial calibration verifications (ICVs) should be as “similar as possible.” To achieve this similarity, the following considerations to both FIAA and GFAA become important:

- Use identical sample matrices for samples and standards.
- Introduce a predominant anion, such as sulfate or chloride, at the same concentration in the matrix.
- For FIAA, use the same fuel–oxidant mixture, sample pressure, and flame height.
- Use the same type of background correction technique.

The metallic analyte is converted to atomic vapor in four steps:
Butcher and Sneddon describe the conditions for GFAA in a most interesting manner:

A graphite tube is typically 20 to 30 mm in length and 3 to 6 mm in diameter. The tube is surrounded by argon to prevent combustion in air at elevated temperatures. The sample is introduced into the furnace through a dosing hole (1–3 mm in diameter). In many cases, chemical compounds called chemical modifiers are also added to improve the sensitivity or accuracy for a given analyte. The temperature of the tube can be controlled from ambient up to approximately 2700°C, with heating rates up to 1500°C/sec. It has been shown to be beneficial for many elements to insert a platform into the tube onto which the sample is placed … some metals may vaporize as a compound. The first step involves the relatively straightforward removal of solvent (usually water) from the sample. The remaining steps include chemical/physical surface processes, such as homogenous or heterogeneous solid–solid interactions, solid-phase nucleation and diffusion into graphite; heterogeneous gas–solid interactions, that is, adsorption/desorption and reaction of molecules with the wall to form atoms; homogeneous gas-phase reactions; and processes by which analyte leaves the furnace.

The purpose of background correction in AAS is to accurately measure the background and subtract this absorbance value from the uncorrected signal (signal + background) to give a background-corrected signal. This is accomplished in AAS instrumentation in one of three major ways:

- By continuum source background correction, pioneered in 1965 by Koirtyohann and Pickett (see benchmark papers listed in Koirtyohann and Pickett)
- By self-reversal, developed by Smith and Hieftje
- By exploiting the discovery by Zeeman that an intense magnetic field causes atomic energy levels to split, causing atomic lines to split into two or more components

Several monographs give more comprehensive discussions of these three distinct approaches to background correction and are listed in Reference 155.

To conclude our discussion on GFAA, as we did for ICP-AES, a calibration plot for the element Cr (as total chromium) is presented in Figure 4.88, taken from the author’s laboratory. We also close our atomic spectroscopy discussion by summarizing in Table 4.22 the four major techniques to measure trace levels of metals from environmental samples, and how to handle interferences. The presence of Pb in drinking water is quantitatively determined by measuring the concentration of Pb via GFAA in a student experiment shown in Chapter 5. We now turn to two remaining determinative techniques of relevance to TEQA: infrared absorption spectroscopy and capillary electrophoresis.

94. IN WHAT WAYS DOES IR ABSORPTION CONTRIBUTE TO TEQA?

Two principal applications of infrared (IR) absorption spectroscopy are useful to achieve the goals of TEQA:

© 2006 by Taylor & Francis Group, LLC
Determinative Techniques to Measure Organics and Inorganics

The quantitative determination of the extent to which soil, groundwater, surface water, and wastewater are contaminated with oil and grease

The quantitative determination of the extent to which an aqueous sample or aqueous soil leachate contains dissolved organic and inorganic forms of the element carbon

The hyphenated instrumental technique in which a GC was interfaced to a Fourier-transform infrared (FTIR) spectrometer, via a gold-lined “light pipe,” and given the acronym GC-FTIR gained widespread acceptance in analytical chemistry during the 1980s. IDLs for the GC-FTIR determinative technique are just not low enough to be of much use to TEQA. We will only discuss the principles of IR absorption spectroscopy insofar as this instrumental technique pertains to the two applications cited above. We will also not address the use of IR absorption spectroscopy in air analysis. A stand-alone IR spectrometer using a conventional dispersive monochromator or an interferometer will be discussed in light of these important

FIGURE 4.88 Calibration plots for the determination of chromium by GFAA showing (A) raw data and (B) a least squares regression fit to the raw data and statistical summary using LSQUARES. A 3110<sup>n</sup> GFAA Spectrophotometer (PerkinElmer Instruments) with a deuterium background correction was used to generate these data.

- The quantitative determination of the extent to which soil, groundwater, surface water, and wastewater are contaminated with oil and grease
- The quantitative determination of the extent to which an aqueous sample or aqueous soil leachate contains dissolved organic and inorganic forms of the element carbon

© 2006 by Taylor & Francis Group, LLC
determinative techniques. Nondispersive IR instruments for both oil and grease and for total organic carbon are quite common and will be briefly introduced.

Both determinative techniques cited above absorb radiation in the mid-IR region of the electromagnetic spectrum. The electromagnetic spectrum shown in Figure 4.69 places IR absorption with the longer wavelength, lower frequency, and lower wave number, in comparison to the visible region. IR radiation spans from just beyond the visible, known as the near-IR, beginning at $\lambda = 0.78 \, \mu m (\nu = 13,000 \, cm^{-1})$, through the mid-IR, 2.5 to 50 $\mu m (\nu = 4000 \sim 200 \, cm^{-1})$, and finally to the far-IR, beginning at $\lambda = 40 \, \mu m (\nu = 250 \, cm^{-1})$ and ending at $\lambda = 40 \, \mu m (\nu = 250 \, cm^{-1})$.

Historically, mid-range IR absorption spectroscopy has been a valuable qualitative instrumental technique and has served the science of organic chemistry very well. The mid-IR spectrum is a qualitative property of an organic compound and provides important structural information. For example, if you want to distinguish between an aliphatic and an aromatic hydrocarbon, a mere record of their respective IR spectra, particularly around 3000 cm$^{-1}$, reveals the difference. Those interested in a more in-depth introduction to IR spectrometry from the organic chemist’s viewpoint should consult Shriner et al.$^{156}$ or other equivalent books.

Photons whose $\lambda$ values fall to within the mid-IR region of the electromagnetic spectrum are absorbed by organic molecules in both the solid and liquid phases and yield a characteristic absorption spectrum. If the photons of emitted IR radiation

---

### TABLE 4.22
**Summary of Physical and Chemical Interferences in Atomic Absorption and Emission Spectroscopy**

<table>
<thead>
<tr>
<th>Atomic Spectral Technique</th>
<th>Type of Interference</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame AA</td>
<td>Ionization</td>
<td>Add a buffer to suppress</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>Add a releasing agent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use a C$_2$H$_2$–N$_2$O flame</td>
</tr>
<tr>
<td></td>
<td>Physical</td>
<td>Dilute the sample, match the matrix, and use the standard addition mode of instrument calibration</td>
</tr>
<tr>
<td>Graphite furnace AA</td>
<td>Physical</td>
<td>Adjust furnace temperature conditions</td>
</tr>
<tr>
<td></td>
<td>Molecular absorption</td>
<td>Zeeman or D$_2$ background correction</td>
</tr>
<tr>
<td></td>
<td>Spectral</td>
<td>Zeeman background correction</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Spectral</td>
<td>Use alternative AE line or subtract out background</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>Use the internal standard mode of instrument calibration</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Spectral</td>
<td>Use a collision/reactor cell with low reactivity gases</td>
</tr>
<tr>
<td></td>
<td>Spectral</td>
<td>Use DCR technology involving high reactivity gases</td>
</tr>
</tbody>
</table>
match the quantized energy-level spacing between the ground-state vibrational level denoted by $v_0$ and the first excited vibrational level denoted by $v_1$, a fundamental absorption is said to have occurred at that wavelength or wave number. IR absorption from the sun by glass windows in a closed automobile is a common example of how infrared radiation heats the atmosphere, and this phenomenon has helped coin the term greenhouse effect. Herschel performed a similar experiment in 1800 when he directed sunlight through a glass prism to a blackened thermometer as a detector, and now is credited with the discovery of IR.

95. HOW DO I QUANTITATE OIL AND GREASE IN WASTEWATER USING IR ABSORPTION?

We already discussed the nature of sample preparation for this straightforward, yet elegant quantitative determination in Chapter 3. Both aliphatic and aromatic hydrocarbons (HCs) containing many carbon-to-hydrogen covalent bonds are present in oil and grease. Suppose some oil is found dispersed into water due to some type of spill. Let us assume that a sample of this wastewater has undergone either an LLE or an SPE and the recovered HCs are now dissolved in a solvent that lacks the C–H bond, such as 1,1,2-trichlorotrifluoroethane (TCTFE). The C–H bond possesses a dipole moment due to the slight difference in electronegativity between the elements carbon and hydrogen that changes upon absorbing IR radiation between 3200 and 2750 cm$^{-1}$ in the mid-IR region. One such mode of vibration that absorbs IR radiation in this region is known as symmetric stretching and is depicted as follows:

$$\Delta E = h\nu = E_{v1} - E_{v0}$$

Where $\nu = 2960$ cm$^{-1}$

The energy-level spacing, $\Delta E$, between the ground-state vibrational level and first excited-state vibrational level is depicted as follows, along with a typical IR absorption band for the symmetric stretch just discussed:
The fact that numerous quantized rotational levels exist within each vibrational level serves to help explain the broad-band nature of infrared spectra, along with the fact that samples are in condensed phases.

In contrast to UV-vis stand-alone spectrophotometers, dispersive IR spectrometers are designed so that the sample is positioned immediately after the radiation source and just before the dispersive device. This is not the case for a FTIR spectrometer. To assist in distinguishing between dispersive and FTIR instruments, a schematic of the two is presented in Figure 4.89. In a review article in 1968, Whetsel declared that “as far as instrumentation is concerned, I know of no major new developments on the horizon.” Then came the revolutionary development of
FTIR spectroscopy based on the Michelson interferometer (he received the 1907 Nobel Prize for this invention). FTIR designs have all but displaced dispersive designs in infrared spectral technology today. However, the analyst is likely to find dispersive IR instruments that are dedicated to trace oil and grease determinations in environmental testing labs today. Quantitative IR analysis, as is true for UV-vis and atomic spectroscopy, is based on Beer’s law. The experiment introduced in Chapter 5 provides sufficient detail to quantitatively determine the concentration of oil and grease in an environmental sample. Several texts provide good introductory discussions on IR absorption spectroscopy, including FTIR. This author has available to him a Model 1600 FTIR spectrometer (PerkinElmer), and this instrument utilizes software developed by PerkinElmer consistent with ASTM Method D 3921 and similar to EPA Method 431.2 (“Oil and Grease, Total Recoverable”) and Method 418.1 (“Petroleum Hydrocarbons, Total Recoverable”). The idea that a nonpolar extracting solvent lacks the C–H stretch in the mid-IR originated from early work published by Gruenfeld at EPA. That portion of the IR spectrum between 3200 and 2750 cm\(^{-1}\) of iso-octane dissolved in TCTFE is shown in Figure 4.90. This portion is sufficiently well resolved that distinct C–H stretching absorption bands can be seen for this group frequency. Almost 95% of all absorption in the region between 3100 and 2700 cm\(^{-1}\) is due to C–H stretching and includes the following:

- Asymmetrical stretching of a C–H bond in both a methyl and a methylene group. Both are present in iso-octane and centered at 2962 cm\(^{-1}\).
- Symmetrical stretching of a C–H bond in a methyl group centered at 2872 cm\(^{-1}\).
- Asymmetrical C–H stretching present in a methylene group at 2926 cm\(^{-1}\).
- Symmetrical C–H stretching present in a methylene group at 2853 cm\(^{-1}\).

A horizontal baseline is drawn, as shown above, to define a 100% T. The %T for an absorption as shown above is measured at the minimum of the absorption band. Beer’s law is used in a manner similar to that for UV-vis spectroscopy, and the measured %T is related to the absorbance according to

\[
\%T = 100 \times \left(1 - e^{-\varepsilon c l}ight)
\]

FIGURE 4.90 The IR spectrum over the wave number range from 3200 to 2750 cm\(^{-1}\) for iso-octane dissolved in TCTFE from a Model 1600 (PerkinElmer) FTIR spectrometer.

© 2006 by Taylor & Francis Group, LLC
Quantitative IR therefore requires a cuvette of fixed path length \( b \). It so happens that the standard rectangular quartz cuvette absorbs everywhere in the mid-IR except in the C–H stretching region. This author, using a Model 567 Grating IR spectrometer (PerkinElmer) and a 10-mm rectangular quartz cuvette, developed the data and external calibration for petroleum hydrocarbons, as shown in Figure 4.91, using data shown in Table 4.23. We have used the same EXCEL programming to generate the raw data calibration points in a manner similar to what we did for the GFAA and ICP-AES data discussed earlier. The analytical results for the unknown samples are real and were reported at the time. Note the influence of the spiked Soil A in comparison to the unspiked Soil A. Results for Soils B and C are given for comparison and serve to illustrate the utility of IR absorption spectroscopy toward achieving yet another goal of TEQA. One other determinative technique that utilizes IR absorption needs to be addressed: the determination of total organic carbon (TOC).

![Raw data for HCs calib](image1)

![Least squares regression](image2)

**FIGURE 4.91** Calibration plots for the determination of petroleum hydrocarbons by FTIR showing raw data and a least squares regression fit to the raw data.
96. WHAT IS TOC AND HOW IS IR ABSORPTION USED?

The TOC content of a sample of water is an important and non-compound-specific parameter. It is measured by oxidizing the dissolved organic carbon (DOC) and quantitating the evolved carbon dioxide. One way to do this is to sweep the released CO₂ into a nondispersive IR (NDIR) photometer that has been tuned to measure CO₂. This is accomplished by measuring the absorbance of the characteristic C–O stretching vibration.

Two analytical approaches to the quantitative determination of TOC for aqueous samples that contain DOC have emerged over the years: oxidation of carbon to CO₂ via high-temperature catalyzed combustion and persulfate–ultraviolet irradiation. Teledyne-Tekmar (formerly Tekmar-Dohrmann) manufactures (among others) TOC analyzers that perform both types of oxidations. Its most current models are the focus of this discussion. A TOC measurement involves oxidizing organic carbon in an aqueous sample, detecting and quantifying the oxidized carbon as CO₂, and presenting the results in terms of the mass of carbon per unit volume of the aqueous sample. Let us clarify some of the terms used (drawn from Teledyne-Tekmar literature) in this important and nonselective determinative technique:

**Total carbon (TC)** is the measure of all the carbon in the sample, both inorganic and organic, as a single parameter. Generally, the measurement is made by placing the sample directly into the analyzer without pretreatment.
Total organic carbon (TOC) is the sum of all the organic carbon in the sample. TOC can be measured in one of two ways:

**TOC measurement directly** requires that inorganic carbon be removed by acidification and sparging. The DOC that remains is measured as TOC. Inorganic carbon and purgeable organic compounds (POCs) are lost. POCs are generally present at 1% or less of total carbon.

**TOC measurement by difference** requires two quantitative determinations: one to measure TC and one to measure inorganic carbon. The difference between these two measurements is rigorously TOC.

Inorganic carbon (IC) includes carbonate, bicarbonate, and dissolved carbon dioxide. IC is determined in aqueous samples by acidifying with an inorganic acid to pH 3 or lower, and then sparging with a stream of inert gas. The acidification converts carbonates and bicarbonates to CO₂, which is then removed along with dissolved CO₂ by the gas stream and measured to provide an IC value.

Purgeable organic carbon (POC) is defined as the sum of volatile and semi-volatile organic compounds sparged from an aqueous sample. However, these compounds are generally less than 1% of TC in an environmental aqueous sample.

**97. HOW DO THE TWO TYPES OF TOC ANALYZERS WORK?**

UV-promoted persulfate oxidation involves exposing an aqueous sample to persulfate ions and UV radiation. This produces highly reactive sulfate and hydroxyl free radicals. The CO₂ produced from the persulfate oxidation reactions is swept by a stream of inert carrier gas, such as nitrogen, to the NDIR detector. IC and POCs are removed by acidification and sparging in the IC sparger. An aliquot of the sparged sample is then transferred to the UV reactor and persulfate reagent is added to oxidize the organic carbon based on the following chemical reactions:

\[
\begin{align*}
S_2O_8^{2-} & \overset{\text{uv}}{\longrightarrow} 2SO_4^- \\
H_2O & \overset{\text{uv}}{\longrightarrow} H^+ + OH^- \\
SO_4^{2-} + H_2O & \overset{\text{uv}}{\longrightarrow} SO_4^{2-} + OH^- + H^+ \\
R (\text{organics}) & \overset{\text{uv}}{\longrightarrow} R^* \\
R^* + SO_4^- + OH & \overset{\text{uv}}{\longrightarrow} n\ CO_2 + \ldots \ldots
\end{align*}
\]

These reactions show how persulfate ion and UV radiation combine to generate sulfate and hydroxyl free radicals that oxidize UV-excited organic compounds (R*) to CO₂. The UV-promoted persulfate oxidation approach to sample introduction, as
developed for the Apollo 8000® TOC Analyzer, is shown in the schematic below. The aqueous sample is transferred to the IC sparger device, where inorganic carbon is initially removed. The sample is then moved to the UV reactor, where oxidation to CO₂ occurs and this gas product is swept into the NDIR detector.

The *high-temperature combustion* (HTC) technique uses heat (680°C or higher), in the presence of a titanium dioxide-based platinum catalyst, with a stream of hydrocarbon free compressed air or oxygen to oxidize organic carbon. DOC and particulates that contain carbon fully oxidize to CO₂ under these conditions. Following IC removal, an aliquot of the sparged aqueous sample is transferred to the combustion furnace to oxidize the organic carbon to form CO₂. The catalytic combustion oxidation products are continuously swept through the NDIR detector, which is selective to CO₂ and whose analog output signal is proportional to the concentration of CO₂ in the carrier gas, and thus in the original sample. The HTC approach, whose sketch is shown below, also shows an IC sparger device and similar sample introduction technology (Apollo 9000®, Teledyne-Tekmar). The sparged sample is
introduced into the injection port of a combustion furnace. An inert carrier gas continuously sweeps the CO₂ out of the furnace to the NDIR detector.

98. WHAT IS CAPILLARY ELECTROPHORESIS AND WHAT ROLE DOES IT HAVE IN TEQA?

We next consider a determinative technique introduced during the late 1980s and commercialized during the 1990s to the arsenal of instrumental analysis relevant to TEQA — capillary electrophoresis (CE). Our discussion of CE forces us to return to the separation sciences. CE encompasses a number of distinct modes:

- Capillary zone electrophoresis (CZE)
- Capillary gel electrophoresis
- Micellar electrokinetic capillary chromatography (MEKC)
- Capillary electrochromatography (CEC)
- Capillary isoelectric focusing
- Capillary isotachophoresis
Li$^{160}$ has provided a good definition of CE:

In electrophoresis, a mixture of different substances in solution is introduced, usually as a relatively narrow zone, into the separating system, and induced to move under the influence of an applied potential. Due to differences in the effective mobilities (and hence migration velocities) of different substances under the electric field, the mixture then separates into spatially discrete zones of individual substances after a certain time.

Our focus will be on CZE in this book, although MEKC holds great potential for selective organic priority pollutant trace analysis. After we introduce the underlying principles of CZE, we will cite this author’s efforts in developing a method to separate inorganic anions.

It is useful to compare CE with the two established instrumental separation techniques already discussed: HPLC and GC. The three schematics shown in Figure 4.92 provide a means to compare and contrast this third contributor to separation science. Moving from left to right across all three schematics reveals the following:

- All three techniques require a source of high potential energy. For CE, a high-voltage power supply is required; for HPLC, a fluid at high pressure is required; for GC, a compressed gas at high pressure is required.
- All three techniques require a column within which the separation of specific chemical compounds can be achieved. For CE, an uncoated narrow-bore cap column is used; for HPLC, a packed column; for GC, a coated-cap column or packed column.
- All three techniques require some means to detect and quantitate the separated chemical compounds. For CE, a UV absorbance detector was first used; for HPLC, several detectors, as already discussed; for GC, several detectors, as already discussed.

Electrophoresis has been known since 1886, when Lodge observed the migration of protons, H$^+$, in a tube of phenolphthalein dissolved in a gel; Hjerten in 1967 first used a high electric field in solution electrophoresis using a 3-mm-i.d. capillary. However, Jorgensen and Lukacs$^{161}$ are credited with writing the benchmark paper that demonstrated highly efficient separations using narrow-bore (<100-µm-i.d.) capillaries. Figure 4.93 vividly depicts, in a somewhat oversimplified manner, the basic components of a CE instrument. A CE instrument can be built from simple components, as shown in the figure, and consists of two beakers joined by a filled capillary column. A high-voltage power supply that is capable of providing 30,000 V is impressed onto Pt electrodes that are placed in each beaker. Due to this extremely high voltage requirement, an open configuration, such as is shown in Figure 4.93, is very dangerous. Safety considerations have led to the development of instruments that provide the necessary protection so that CE can be safely performed. As has been true in the historical development of GC and HPLC, the number of manufacturers of CE instruments rose and fell during the late 1980s and early 1990s, and today, only a few manufacturers remain. We digress briefly to introduce the basic theory underlying CE by asking the following question.
99. WHAT FACTORS INFLUENCE SEPARATIONS IN CE?

Applying such a large voltage across a capillary tube that is filled with electrolytes actually moves the fluid from anode to cathode, and this phenomenon is termed electro-osmosis. CE opens up the possibility to quantitate the presence of analytes of interest to TEQA that are either cationic, anionic, or neutral. Cations migrate to the cathode, ahead or behind the electro-osmotic velocity, depending on the polarity of the electrodes. Anions migrate to the anode, ahead or behind, and neutrals migrate...
Determinative Techniques to Measure Organics and Inorganics

with the electro-osmotic force. Electro-osmotic flow originates from the negative charges on the inner wall of the capillary tube due to deprotonizing surface silanol groups in neutral or alkaline medium. Cations from the buffer move toward this negatively charged surface. It is the water dipoles that surround the cation that get dragged when the electric field is applied.

To better explain electro-osmosis from the perspective of the analyte, consider the following analogy. What happens to the velocity (i.e., from physics, speed, and direction) of an individual when he or she rides the horizontal escalator, like those found in airport terminals? The velocity of the escalator is analogous to what is called the electro-osmotic mobility, symbolized by $\mu_{\text{EO}}$, and the velocity of the individual riding the escalator is analogous to the electrophoretic mobility, symbolized by $\mu_{\text{EP}}$. When you walk in a direction that is in the same direction as that of the horizontal escalator, your speed relative to a stationary observer standing on the floor next to the moving escalator is greatly increased. If you walk in a direction 180° opposite the direction of the escalator, you will still be carried in the direction of the escalator with respect to the stationary observer. You will, however, arrive at the end of the ride at a much later time. The vector notation for the mobilities of cations and anions is as follows:

© 2006 by Taylor & Francis Group, LLC
The net effect to the detector (our stationary observer) is as follows:

\[ + \rightarrow \mu_{EO} \rightarrow + \]

\[ 0 \rightarrow \mu + \]

\[ \mu - \rightarrow - \]

Ions originating from a buffer also have an electrophoretic mobility, symbolized by \( \mu_{ion} \). It should also be appreciated that the fluid flow profile through a tube is flat when the driving force is an electric field vs. the parabolic profile for flow created by a pressure difference.

The derivation shown below is adapted from that introduced earlier. The electro-osmotic velocity, \( v_{EO} \), is defined as the product of the electro-osmotic mobility, \( \mu_{EO} \), and the applied electric field, \( E \). For an applied voltage \( V \) and for a length of capillary \( L \), we can write

\[
v_{EO} = \mu_{EO} E = \mu_{EO} \frac{V}{L}
\]

Similarly, the electrophoretic velocity, \( v_{EP} \), is related to the electrophoretic mobility, \( \mu_{EP} \), according to

\[
v_{EP} = \mu_{EP} \frac{V}{L}
\]

The magnitude of \( \mu \) is related to the charge-to-size ratio of the analyte ion. The time it takes for an analyte, once introduced into the capillary inlet, to be detected is known as the solute migration time \( t \). The solute migration time is unique to a given analyte, and much like the solute retention time, \( r_R \), in GC and HPLC, \( t \) is defined as follows and, in turn, can be written in terms of mobilities:

\[
t = \frac{L}{v_{EP} + v_{EO}} = \frac{L^2}{(\mu_{EP} + \mu_{EO})V}
\]

The above equation demonstrates that the CE solute migration time depends on the following:
• Solute charge-to-size ratio (i.e., $\mu_{EP}$)
• Applied voltage, $V$
• Capillary wall surface chemistry (i.e., $\mu_{EO}$)
• Buffer composition and concentration
• Length of column from inlet to detector, $L$

The only factor contributing to band broadening is, unlike HPLC, longitudinal diffusion. The spread in the axial direction down the center of the capillary, for ions and molecules introduced as a tight plug at the inlet to the column, can be described by the Einstein equation:

$$\sigma^2 = 2Dt$$

where $D$ is the molecular diffusion coefficient. The column efficiency, defined in terms of the number of theoretical plates, $N$, as in chromatography, is related to the capillary length, $L$, and the spread of molecules due to longitudinal diffusion of analyte through the capillary column, denoted by $\sigma_L$:

$$N = \frac{L^2}{\sigma^2_L} = \frac{(\mu_{EP} + \mu_{EO})V}{2D}$$

The above equation suggests that the efficiency in CZE is related to the applied voltage and not capillary column length. Maximum efficiency and a reduction in analysis times are obtained using high voltages and short columns. This can be accomplished only by efficient heat dissipation. Control of column temperature in CE becomes very important.

To answer the question posed, as we did for GC and HPLC, we must consider those factors that influence the electrophoretic resolution, $R_s$. $R_s$ can be related to the column efficiency, $N$, and the relative velocity difference between two zones, $(\Delta v/v)$, and this relationship is

$$R_s = \frac{1}{4} \sqrt{N} \left( \frac{\Delta v}{v} \right)$$

This equation can be restated in terms of electrophoretic and electro-osmotic mobilities as follows:

$$R_s = \frac{1}{4} \sqrt{N} \left( \frac{\mu_{EP2} - \mu_{EP1}}{\bar{\mu}_{EP}} \right)$$

where a mean electrophoretic mobility between separated peaks 1 and 2 is given by a simple average ($\bar{\mu}_{EP}$) over the mobility of each analyte according to
\[ \bar{\mu}_{EP} = \frac{1}{2}(\mu_{EP1} + \mu_{EP2}) \]

Eliminating \( N \) in the above equation in terms of electrophoretic ion mobilities yields

\[ R_s = 0.18(\mu_{EP2} - \mu_{EP1}) \left( \frac{V}{D(\bar{\mu}_{EP} + \mu_{EO})} \right)^{1/2} \]

This equation can be further simplified to yield

\[ R_s = \frac{1}{4} \left( \frac{V}{2D} \right)^{1/2} \frac{\Delta\mu_{EP}}{(\bar{\mu}_{EP} + \mu_{EO})^{1/2}} \]

Electrophoretic resolution, \( R_s \), is found to depend on the following:

- \( \Delta\mu_{EP} \), the difference in electrophoretic mobility between zones 1 and 2
- \( V \), the applied voltage
- \( \mu_{EO} \), the electro-osmotic mobility
- \( \bar{\mu}_{EP} \), the mean electrophoretic mobility of peaks 1 and 2

The practice of CZE is largely influenced by the following factors:

- The chemical nature, pH, and concentration of the buffer
- Ionic strength
- Temperature, quite sensitive, needed to keep constant
- Viscosity
- Dielectric constant
- Applied voltage
- Length of capillary between the inlet and detection
- Capillary diameter, significant factor with respect to Joule heating; column inner diameters of <100 \( \mu \)m minimize Joule heating
- Chemical nature of buffer additives, such as the percent organic modifier, buffer ions of different \( \mu_{EO} \)
- Injection time
- Internal capillary wall surface chemistry

100. **HOW DOES ELECTRODE POLARITY INFLUENCE CE?**

Commercially available CE instruments are capable of placing either a positive or negative charge on the Pt wire at the injector, and at the same time placing a negative or positive charge on the Pt wire at the detector. A positive or negative potential can be programmed via a microprocessor. Depicted below is a schematic showing the
various cation, anion, and neutral solutes introduced into a capillary, along with the anticipated electropherograms (to the right) for impressing +30 kV. In case 1, a neutral organic compound that absorbs UV radiation (this assumes that the CE instrument incorporates a UV absorption detector) is introduced into the injector end of a hypothetical column. This neutral marker will migrate with the same velocity as that of the electro-osmotic force and arrive at the detector end with a migration time that corresponds to the electro-osmotic velocity. Any anions that are present reach the detector after the neutral marker, depending on the magnitude of their anion electrophoretic mobility, as shown in cases 2 and 3. Cations that are present in the sample migrate ahead of the neutral marker, depending on their respective cation mobilities, as shown in cases 4 and 5. Cases 4 and 5 are illustrated in Figure 4.94, in which three protonated peptides are separated. This electropherogram was obtained in the author’s lab using a Model 270A-HT (Applied Biosystems). These schematics are as follows:

Impressing −30 kV to the injector/detector Pt wires reverses the polarity and drastically alters the order of ion migration, as shown in the second scenario depicted below. The detector is now anodic, and the injector becomes cathodic. Case 1 shows the migration time for a neutral marker. Cases 2 and 3 depict two cations of different \( \mu_{EO} \) values. Cases 4 and 5 show the expected migration times for two anions with
different values of $\mu_{ep}$. To illustrate the use of a reversed polarity, consider the electropherogram, also obtained in the author’s lab, shown in Figure 4.95. A mixture of the sodium or potassium salts of the common inorganic anions was introduced by hydrodynamic injection into the Model 270A-HT. A potential of $-25$ kV with a thermostatically controlled temperature of $30^\circ$C in a $25$ mM phosphate buffer was used. The order of ion migration is shown in Figure 4.95 and differs from the ion chromatographic elution order discussed earlier. The peaks are actually negative peaks because a UV-absorbing chromophore has been added to the buffer. The lead wires from the detector were reversed and then connected to the interface. The schematic for this scenario is as follows:

FIGURE 4.94 Capillary electropherogram for the separation and detection of three peptides from a Model 270A-HT® (Applied Biosystems).
101. WHAT IS VACANCY OR INDIRECT UV ABSORPTION DETECTION?

This technique has been used in HPLC to detect those analytes that either lack a UV chromophore in their molecular structure or have a weak molar absorptivity, \( \varepsilon \). A strong UV-absorbing chromophore is added to the mobile phase in HPLC and to
the buffer in CE.\textsuperscript{163} For the separation, detection, and quantification of the common inorganic anions, vacancy or indirect UV photometric detection (IPD) is the only means that exists to quantitate all of the common anions. The detector wavelength is usually set at that $\lambda$ where a maximum in the absorption spectrum for the chromophore is located. A research group at Waters Associates has made significant progress in method development for CE-IPD.\textsuperscript{164} The methodology developed by this group has been recently applied in this author’s laboratory to adapt the CE-IPD determinative technique to environmental-geochemical-related problems.\textsuperscript{165} We close our discussion of CE by examining the influence of buffer pH on CE migration time and migration order.

102. HOW IMPORTANT IS BUFFER PH IN CE?

“Quite important” is the answer; however, it depends on the secondary equilibrium characteristics of the analyte of interest. Consider the three isomeric pyridinium carboxylates listed in Table 4.24, along with a neutral marker, mesityl oxide (MO).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Analyte & Structure & $pK_{a1}$ & $pK_{a2}$ \\
\hline
Mesityl oxide & & — & — \\
\hline
PA & \includegraphics[width=1cm]{PA_structure} & 1.06 & 5.37 \\
\hline
INA & \includegraphics[width=1cm]{INA_structure} & 1.70 & 4.89 \\
\hline
NA & \includegraphics[width=1cm]{NA_structure} & 2.07 & 4.73 \\
\hline
\end{tabular}
\caption{Name, Structure, and First and Second Acid Dissociation Constants for Compounds Discussed in Figure 4.96}
\end{table}
The electropherograms were developed using a 25 mM phosphate buffer and an applied potential of +25 kV. A plot of the migration time vs. the buffer pH is shown in Figure 4.96. Note that the migration time for the neutral marker is not influenced by pH, in contrast to the migration times for all three isomeric pyridinium carboxylates. PA, INA, and NA all exhibit faster migrations time than the MO at pH 2. A decrease in their migration times at increasing pH becomes evident as the pH reaches the crossover pH at around 3, as indicated in the plot. The order of migration actually reverses itself after this crossover pH has been passed, as indicated in Figure 4.96.

103. WHAT DOES THE FUTURE HOLD FOR DETERMINATIVE TECHNIQUES?

That these techniques will forever be evolving, albeit ever so slightly in the future. The need for high sensitivity and higher selectivity coupled to operational stability and ease of use serves to ensure a higher degree of precision and accuracy in trace analytical measurement. It is recognized today that attainment of high precision and accuracy is intrinsically tied to efficient sample preparation techniques, as introduced in Chapter 3. The term garbage in, garbage out reflects this recognized synergy between sample prep and analytical instrumentation. In the future, expect to see:

- A greater role for hyphenated instruments in environmental testing labs
- More automated sample prep techniques interfaced to existing GC-MS and LC-MS instrumentation
- Elemental speciation techniques that become more routine
- Faster GC and HPLC
- More convenient use of IC techniques
- Greater throughput from the transducer through to a LIMS database

This chapter has strongly emphasized GC determinative techniques and closes with the following quotation:

FIGURE 4.96 Plot of CE relative migration time vs. buffer pH.
Gas chromatography is a volatility phenomenon in which solutes elute in an order that is mandated by the net vapor pressures of the solutes under a particular combination of operational parameters.

—Walter Jennings, Founder of J&W Scientific

REFERENCES

Determinative Techniques to Measure Organics and Inorganics

Determinative Techniques to Measure Organics and Inorganics


131. Haddard P, P Jandik. In J Tarter, Ed. *Ion Chromatography*. New York: Marcel Dekker, 1987, pp. 88–91. (This is one of the more consistent treatments of the mathematics of conductance theory as applied to ion chromatography.)
Determinative Techniques to Measure Organics and Inorganics


5 Specific Laboratory Experiments

Theory guides, experiment decides.

—I.M. Kolthoff

CHAPTER AT A GLANCE

Identifying the ubiquitous phthalate esters in the environment ........................................ 551
Determination of polycyclic aromatic hydrocarbons in contaminated soil ............... 556
Data acquisition and control software, introduction to HPLC ............................................. 561
Determination of organochlorine pesticides, comparison of LLE and SPE techniques ................................................................. 566
Determination of trifluralin in chemically treated lawns ................................................. 571
Determination of VOCs in gasoline-contaminated groundwater ................................. 576
Screening for BTEX in wastewater .................................................................................. 582
Introduction to GC .............................................................................................................. 586
Comparison of soil types via quantitative determination of chromium ...................... 591
Determination of ultratrace lead in drinking water ......................................................... 594
Determination of degree of hardness in groundwater ..................................................... 599
Determination of oil and grease in wastewater using SPE ........................................... 604
Comparison of UV and IR absorption spectra of chemically similar organic compounds ................................................................................................................. 609
Determination of anionic surfactants in wastewater ..................................................... 613
Visible spectrophotometric determination of trace iron in groundwater .................... 617
Spectrophotometric determination of phosphorous in eutrophicated surface water ............................................................................................................................... 621
Introduction to the visible spectrophotometer ................................................................. 623
Determination of inorganic anions in drinking water using IC ...................................... 628
Determination of Cr(VI) in a contaminated aquifer ......................................................... 636
Introduction to pH measurement, estimating the degree of purity of snow ................ 641
How to weigh the right way ............................................................................................... 645
References .......................................................................................................................... 646

This chapter provides a series of laboratory experiments that attempt to show some examples of how to conduct trace environmental quantitative analysis (TEQA) in light of what has been discussed so far. These experiments were written by the author before the first four chapters were created. The impetus for writing these experiments...
was in support of a graduate-level course titled “Environmental Analytical Chemistry Laboratory.” This course began in the mid-1990s, and the instruction followed the installation of a teaching analytical laboratory coordinated by the author at Michigan State University in the Department of Civil and Environmental Engineering.

There are several options that an instructor can use to design a laboratory program that gives students the opportunity to measure environmentally significant chemical analytes. It is this author’s opinion that it does not really matter which analytes are to be quantitated as long as an appropriate mix of sample prep and instrumental techniques is applied. One laboratory schedule that was used during the 1995–1996 academic year is now considered.

1. **WHAT MIGHT A TYPICAL LABORATORY SCHEDULE LOOK LIKE?**

Listed below is the laboratory program implemented by the author for a course in TEQA. Under each experiment title is a statement about what outcomes the student will realize. The degree to which the instructor makes the course more or less rigorous is determined by the curriculum objectives. An experimental course in TEQA can consist of a series of experiments with everything set up for the student at the less rigorous level or of the same experiments whereby the student does everything. Some compromise between these two extremes might be the most appropriate.

A series of actual student experiments given as individual handouts follows this laboratory course outline.

<table>
<thead>
<tr>
<th>Project No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 6 weeks</td>
<td>Orientation to laboratory discussion of outcomes and what is expected; definition of and assignment to workstations; safety requirements; waste disposable regulations. Descriptive introductory information.</td>
</tr>
<tr>
<td>1</td>
<td>Introduction to visible spectrophotometry and determination of Fe(III)/Fe(II) in groundwater or determination of PO₄³⁻ in surface waters. Quantitative analysis; emphasis on standards preparation techniques; statistical treatment of data; environmental sampling techniques; learning to operate the UV-vis spectrophotometer; learning to operate the flame atomic absorption (AA) spectrophotometer; no write-up required</td>
</tr>
<tr>
<td>2</td>
<td>Determination of anionic surfactants by micro-liquid–liquid extraction (µLLE) using ion pairing with methylene blue. Quantitative analysis; emphasis on sample preparation, unknown sample analysis; write-up required</td>
</tr>
<tr>
<td>3</td>
<td>Ultraviolet absorption spectroscopy or infrared absorption spectroscopy or fluorescence spectroscopy. Qualitative analysis; introduction to molecular spectroscopic instrumentation; sampling techniques; write-up required</td>
</tr>
<tr>
<td>4</td>
<td>Determination of the degree of hardness in groundwater using flame atomic absorption spectroscopy: measuring Ca, Mg, and Fe. Quantitative analysis; calibration using external standard mode; spiked recovery; no write-up required</td>
</tr>
</tbody>
</table>
Specific Laboratory Experiments

<table>
<thead>
<tr>
<th>Project No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Determination of lead in drinking water using graphite furnace atomic absorption spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Quantitative analysis; learning to use the WinLab software for furnace atomic absorption spectroscopy; calibration based on standard addition; no write-up required</td>
</tr>
<tr>
<td>6</td>
<td>Comparison of soil types via a quantitative determination of the chromium content using visible spectrophotometry and flame atomic absorption spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Quantitative analysis; use of two instrumental methods to determine the Cr (III) and Cr (VI) oxidation states; digestion techniques applied to soils; write-up required</td>
</tr>
</tbody>
</table>

Next 7 weeks

<table>
<thead>
<tr>
<th>Project No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>An introduction to data acquisition and control using Turbochrom and an introduction to high-performance liquid chromatograph (HPLC): evaluating those experimental parameters that influence instrument performance</td>
</tr>
<tr>
<td></td>
<td>Qualitative analysis; emphasis on learning to operate the HPLC and the Turbochrom software; no write-up required; answer questions in lab notebook</td>
</tr>
<tr>
<td>8</td>
<td>Identifying the ubiquitous phthalate esters in the environment using HPLC, photodiode array detection (PDA), and possible confirmation by gas chromatography-mass spectrometry (GC-MS)</td>
</tr>
<tr>
<td></td>
<td>Qualitative analysis; interpretation of chromatograms, UV absorption spectra, mass spectra; experience with GC-MS; write-up required</td>
</tr>
<tr>
<td>9</td>
<td>An introduction to gas chromatography: evaluating experimental parameters that affect gas chromatographic performance</td>
</tr>
<tr>
<td></td>
<td>Qualitative analysis; emphasis on learning to operate the GC; measurement of split ratio; no write-up required; answer questions in lab notebook</td>
</tr>
<tr>
<td>10</td>
<td>Determination of priority pollutant volatile organic compounds (VOCs) in wastewater: comparison of sample preparation methods — µLLE vs. static headspace sampling</td>
</tr>
<tr>
<td></td>
<td>Quantitative analysis; unknown sample analysis; statistical treatment of data; write-up required</td>
</tr>
<tr>
<td>11</td>
<td>Determination of the herbicide residue trifluralin in soil from lawn treatment by gas chromatography using solid-phase extraction (SPE) methods</td>
</tr>
<tr>
<td></td>
<td>Quantitative analysis; calibration based on internal standard mode; unknown sample analysis; statistical treatment of data; write-up required</td>
</tr>
<tr>
<td>12</td>
<td>Determination of priority pollutant nonvolatile organochlorine pesticides in contaminated groundwater: comparison of sample preparation methods — µLLE vs. solid-phase extraction techniques a</td>
</tr>
<tr>
<td></td>
<td>Quantitative analysis; emphasis on sample preparation, unknown sample analysis; calibration based on internal mode; statistical treatment of data; write-up required</td>
</tr>
<tr>
<td>13</td>
<td>Determination of selected priority pollutant polycyclic aromatic hydrocarbons in oil-contaminated soil using LLE-RP-HPLC-PDA; determination of oil and grease in contaminated soil via quantitative Fourier-transform infrared spectrophotometry</td>
</tr>
<tr>
<td></td>
<td>Quantitative analysis; sample preparation; write-up required</td>
</tr>
</tbody>
</table>

a Projects are considered extra credit and thus not required. Students must make arrangements with the laboratory instructor in order to perform these experiments.
This is a very ambitious one-semester laboratory schedule. To effectively educate students while delivering the course content requires a dedicated support staff, a committed faculty, sufficient laboratory glassware and accessories, and expensive analytical instrumentation, including interface of each instrument to a PC that operates chromatography or spectroscopy software. Each lab session requires a minimum of 4 h and a maximum of 8 h. Students must be taught not only how to prepare environmental samples for trace analysis, but also how to operate sophisticated analytical instrumentation. The intensity of the lab activities starts from an initial and less rigorous laboratory session, with rigor increasing as each session unfolds.

2. HOW IS THE INSTRUCTIONAL LABORATORY CONFIGURED?

When the laboratory experiments that follow were developed, the author had just completed coordinating the installation and start-up of four student workstations. Each workstation consisted of:

1. One Autosystem® (PerkinElmer Instruments) gas chromatograph incorporating dual capillary columns (one for VOCs and one for SVOCs) and dual detectors (FID and ECD).
2. One HPLC that included a 200 Series® LC binary pump, a manual injector (Rheodyne), a reversed-phase column and guard column, and a LC250® photodiode array (PDA) ultraviolet absorption detector.
3. One Model 3110® (PerkinElmer Instruments) atomic absorption spectrophotometer with flame and graphite furnace capability with deuterium background correction.
4. One personal computer (PC) that enabled all three instruments above to be interfaced. For GC and HPLC, Turbochrom® (PE-Nelson) Chromatography Processing Software (now called TotalChrom; PerkinElmer Instruments) was used for the data acquisition via the 600 LINK® (PE-Nelson) interface that was external to the PC. For AA, Winlab® (PerkinElmer Instruments) software was used via an interface board that was installed into the PC console.
5. A UV-vis spectrophotometer Genesys 5® (Spectronic Instruments) was used. If another spectrophotometer is used, an infrared phototude is necessary to quantitate in that experiment.

In addition, a Model 2000® (Dionex) ion chromatograph interfaced to the PC via a 900® interface (PE-Nelson) and a Model 1600® FTIR Spectrophotometer (PerkinElmer Instruments) were available for all students to use in the instructional laboratory. Individual university and college departments will have their own unique laboratory configurations. In order to carry out all of the experiments introduced in this chapter, instructional laboratories must have, at a minimum, the following analytical instruments: GC-FID, GC-ECD, HPLC-UV, FlAA and GFAA, IC, and a UV-vis spectrophotometer (stand-alone). Accessories for sample preparation, as listed in each of the subsequent experiments, are also needed.
Each experiment that follows was written as independent of the others in the collection as possible. For ease of access, references drawn from each experiment have been collected at the end of the chapter and consecutively numbered. Safety tips appear in each experiment as poignant reminders to students and instructors alike of the perils associated with laboratory work. Instructors can pick and choose to use a given experiment as written here or modify it to fit their unique laboratory situations. Several experiments make reference to the computer programs written by the author in GWBASIC, found in Appendix C. Instructors can decide whether they desire their students to use these programs. If they want to use these programs, they will have to manually enter the code into MSDOS, along with an execution program for GWBASIC. The reader will notice that some information in each experiment duplicates topics covered in Chapters 2, 3, and 4. This duplication is by intent, and the author hopes revisiting certain key concepts in this chapter reinforces reader comprehension.

IDENTIFYING THE UBIQUITOUS PHTHALATE ESTERS IN THE ENVIRONMENT USING HPLC, PHOTODIODE ARRAY DETECTION, AND CONFIRMATION BY GC–MS

BACKGROUND AND SUMMARY OF METHOD

The most commonly found organic contaminant in landfills and hazardous waste sites has proven to be the homologous series of aliphatic esters of phthalic acid. This author has found phthalate esters in almost every Superfund waste site sample that he personally analyzed during the period 1986–1990 while employed in an environmental testing laboratory in New York.

The molecular structures for two representative phthalate esters are drawn below.\(^1\) Dimethyl phthalate (DMP) and bis(2-ethyl hexyl)phthalate (bis) represent examples of a lower-molecular-weight phthalate ester to a higher-molecular-weight ester. DMP and the higher homologs, diethyl phthalate (DEP), di-n-propyl (DPP), and di-n-phthalate (DBP), are the focus of this exercise.

![Molecular structures of phthalate esters](image)

The photodiode array UV absorption detector provides both spectral peak matching and, if desired, peak purity determinations. This is nicely illustrated in Figure 5.1 and Figure 5.2.\(^2\) In Figure 5.1, the UV absorption spectrum from the peak at or near 39 min in the HPLC chromatogram is retrieved from a stored library file. The UV spectrum for the peak and that for a reference standard are compared.

Figure 5.2 overlays UV absorption spectra for three points along the Gaussian chromatographically resolved peak and uses an algorithm to calculate a purity match.
Note the difference between the overlayed UV absorption spectra for the impure vs. the pure peak. You will not be using the peak purity algorithm in this exercise.

**Analytical Method Development Using HPLC**

Analytical method development in HPLC usually involves changing the composition of the mobile phase until the desired degree of separation of the targeted organic compounds has been achieved. One starts with a mobile phase that has a high solvent strength and moves downward in solvent strength to where a satisfactory resolution
Specific Laboratory Experiments

553

can be achieved. Recall the key relationship for chromatographic resolution from Chapter 4:

\[ R_s = \frac{1}{4} (\alpha - 1)(N)^{1/2} \left( \frac{k'}{1 + k'} \right) \]

A useful illustration of the effects of selectivity, plate count, and capacity factor follows:

HPLC chromatogram (A) shows a partial separation of two organic compounds, e.g., DMP from DEP. This degree of resolution, \( R_s \), could be improved by changing \( k' \), \( N \), or \( \alpha \). In (B), \( k' \) is increased, which changes the retention times and shows a slight improvement in \( R_s \). Increasing \( N \) significantly increases \( R_s \), as shown in (C); the greatest increase in \( R_s \) is obtained by increasing \( \alpha \), as shown in (D). Refer to Chapter 4 or an appropriate monograph on HPLC to enlarge on these concepts.

**GC-MS Using a Quadrupole Mass Spectrometer**

In a manner similar to obtaining specific UV absorption spectra for chromatographically separated peaks, as in HPLC-PDA, GC-MS also provides important identification of organic compounds first separated by gas chromatography. The mass spectrometer that you will use consists of four rods arranged to form parallel sides of a rectangle,
as shown below. The beam from the ion source is directed through the quadrupole section, as shown below.

The quadrupole rods are excited with a large DC voltage superimposed on a radio frequency (RF) voltage. This creates a three-dimensional, time-varying field in the quadrupole. An ion traveling through this field follows an oscillatory path. By controlling the ratio of RF to DC voltage, ions are selected according to their mass-to-charge ratio. Continuously sweeping the RF/DC ratio will bring different \( m/z \) ratios across the detector. An oversimplified sketch of a single quadrupole MS, as shown in Chapter 4, appears below:

**OF WHAT VALUE IS THIS EXPERIMENT?**

The goal of this experiment is to provide an opportunity for students to engage in analytical method development by identifying an unknown phthalate ester provided to them. This is an example of qualitative analysis. The reference standard solution consists of a mixture of the four phthalate esters: DMP, DEP, DPP, and DBP. Each group will be given an unknown that contains one or more of these phthalate esters. A major objective would be to use available instrumentation to achieve the goal. Students will have available to them an HPLC in the reversed-phase mode (RP-HPLC) and access to the department’s gas chromatograph-mass spectrometer system.

Students must first optimize the separation of the esters using RP-HPLC, record and store the ultraviolet absorption spectra of the separated esters, and compare the spectrum of the unknown against the stored UV spectra. In addition, staff will be available to conduct the necessary GC-MS determination of the unknown. A hard copy of the chromatogram and mass spectrum will be provided so that the student will have additional confirmatory data from which to make a successful identification of the unknown phthalate ester.

**EXPERIMENTAL**

Preparation of Chemical Reagents

Note: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

Accessories to Be Used with the HPLC per Student or Group

1. HPLC syringe. This syringe incorporates a blunt end; use of a beveled-end GC syringe would damage inner seals to the Rheodyne HPLC injector.
2. Four-component phthalate ester standard. Check the label for concentration values.
3. Unknown sample that contains one or more phthalate esters. Be sure to record the code for the unknown assigned.

Procedure

Unlike previous exercises, no methods have been developed for this exercise. Consult with your lab instructor regarding the details for developing a general strategy. You will be introduced to Turboscan®, software that will allow you to store and retrieve UV absorption spectra.

First, find the mobile phase solvent strength that optimizes the separation of the four phthalate esters. Second, retrieve the UV absorption spectrum for each of the four and build a library. Third, inject the unknown sample and retrieve its UV spectrum. Fourth, make arrangements with the staff to get your unknown analyzed using GC-MS.

FOR THE REPORT

Include your unknown phthalate ester identification code along with the necessary laboratory data and interpretation of results to support your conclusions.

Please address the following in your report:

1. Compare the similarities and differences for the homologous series of phthalate esters on both UV absorption spectra and mass spectra from your data.
2. Explain what you would have to do if you achieved the optimum resolution and suddenly ran out of acetonitrile. Assume that you have only methanol available in the lab. Would you use the same mobile-phase composition in this case?
3. This exercise introduces you to the quadrupole mass filter. Briefly describe how the mass spectrum is obtained, and if you so desire, attempt to provide a brief mass spectral interpretation. You may want to review a text that introduces GC-MS or review Chapter 4.
DETERMINATION OF PRIORITY POLLUTANT POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN CONTAMINATED SOIL USING RP-HPLC-PDA WITH WAVELENGTH PROGRAMMING

BACKGROUND AND SUMMARY OF METHOD

In 1979, the EPA proposed Method 610, which, if properly implemented, would determine the 16 priority pollutant PAHs in municipal and industrial discharges. The method was designed to be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System (NPDES). The assumption used was that a high expectation of finding some, if not all, of the PAHs was likely. The method incorporated packed-column GC in addition to HPLC, and because of the inherent limitation of packed columns, they were unable to resolve four pairs of compounds (e.g., anthracene from phenanthrene). Because RP-HPLC could separate all 16 PAHs, it became the method of choice. The method involved extracting a 1-L sample of wastewater using methylene chloride, use of Kuderna–Danish evaporative concentrators to reduce the volume of solvent, cleanup using a silica gel microcolumn, and a solvent exchange to acetonitrile prior to an injection into an HPLC system. The method requires that a UV absorbance detector and a fluorescence detector be connected in series to the column outlet. This affords maximum detection sensitivity because some PAHs (e.g., naphthalene, phenanthrene, fluoranthene, among others) are much more sensitive when detected by fluorescence than by UV absorption.

In most laboratories today, PAHs are routinely monitored under EPA Method 8270 and comprise the majority of neutrals under the base, neutral, acid (BNAs) designation of the method. This is a liquid–liquid extraction method with determination by gas chromatography-mass spectrometry (GC-MS). Careful changes in pH of the aqueous phase enables a selective extraction of bases and neutrals from acidic compounds. Examples of priority pollutant organic bases include aniline and substituted anilines. Examples of priority pollutant organic acids include phenol and substituted phenols. The most popular method of recent years has been EPA Method 525, which incorporates SPE techniques and is applicable to PAHs in drinking water.

The most common wavelength, \( \lambda \), for use with aromatic organic compounds is generally 254 nm because almost all molecules that incorporate the benzene ring in their structure absorb at this wavelength. This wavelength may or may not be the most sensitive wavelength for most PAHs.

Figure 5.3 compares RP-HPLC chromatograms for the 16 priority pollutant PAHs in a reference standard mixture and from a soil extract. In the lower chromatogram of each figure, \( \lambda \) was held fixed at 255 nm, whereas for the upper chromatogram of each figure, \( \lambda \) was changed during the run so as to demonstrate how the wavelength influences peak height. The wavelength-programmed HPLC chromatogram shows much less background absorbance and hence increased sensitivity. This information should be used in developing the wavelength-programmed HPLC method.
The exercise affords the student an opportunity to build a new HPLC method using the chromatography data-handling software. The method will also incorporate the concept of wavelength programming, whose objective is to maximize detector sensitivity for a given analyte and which can only be performed using a PDA detector and accompanying digital electronics. The following table summarizes the detection limits for $\lambda = 255$ and 280 and for UV programming during the chromatographic run:

**FIGURE 5.3** Comparison of UV detection at 255 nm with programmed wavelength for PAH standards and for soil extracts that contain PAHs.

**OF WHAT VALUE IS THIS EXPERIMENT?**

© 2006 by Taylor & Francis Group, LLC
EXPERIMENTAL

High-performance liquid chromatograph that incorporates a UV absorption detector under reversed-phase conditions.

Preparation of Chemical Reagents

*Note*: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

Accessories to Be Used with the HPLC per Group

1. HPLC syringe. This syringe incorporates a blunt end; use of a beveled-end GC syringe would damage inner seals to the Rheodyne injector.
2. sixteen-component PAH standard. Check the label for concentration values.

Procedure

Be sure to record your observations in your laboratory notebook.

*Creating the Wavelength Program Method*

Again, you will first find the HPLC instrument in the off position; use “hands on” to activate the instrument and allow at least 15 min for the detector to warm up and stabilize. Ask your laboratory instructor for assistance if necessary. Observe the
variability in baseline absorbance. Absorbance should not vary much above a \( \Delta A \) of 0.0100. Significant variability is most often due to trapped air bubbles because of insufficient degassing of the mobile phase. Inform your instructor if this baseline absorbance variation is significant.

Once the baseline is stable, retrieve the method titled “PAH255” and download it. This method is one previously created by the instructional staff and is a fixed wavelength (\( \lambda \) at 255 nm). Fill the 5-\( \mu \)L injection loop with the PAH standard and observe the chromatogram. The method separates the PAHs based on gradient elution. The method incorporates a one-point calibration.

Use the above tabular information and modify this method to incorporate wavelength programming as discussed earlier. Save the modified method as “PAHWP,” where WP stands for “wavelength programmed.” Ask your laboratory instructor for assistance in developing this software capability. Fill the 5-\( \mu \)L injection loop with the PAH standard. Using the “chromatograms” section in the main menu, proceed to retrieve both HPLC chromatograms that you just generated. Use the overlay capability to compare both chromatograms and print the overlay. Update the one-point calibration with this standard. You should not have a new method with an updated calibration prior to injecting the extract from the soil discussed below.

**Extraction Procedure for Soil**

Weigh approximately 2.0 g of contaminated soil into a 50- or 125-mL glass beaker. Add 20 mL of methylene chloride and use a glass stirring rod to facilitate mixing. Let the contents of the mixture stand for at least 10 min. Decant the extract into a second beaker. It may be necessary to filter this extract if particulates become a problem. This will depend on the type of sample. Pipette 1.0 mL of the methylene chloride extract into a clean, dry 10-mL volumetric flask. Adjust to the calibration mark with acetonitrile. Fill the injection loop with this diluted extract. It may be necessary to use a 0.45-\( \mu \)m syringe filter to remove particulates from the diluted extract. Fill the HPLC syringe with about five times the loop volume to ensure a reproducible injection volume. The peak area that is found refers to the concentration of a given PAH in the diluted extract. You will be given assistance on how to allow Turbochrom to calculate the concentration of each PAH in the original contaminated soil. If time permits, make a second injection of the diluted extract. **Discard the excess methylene chloride extract and CH2Cl2/ACN diluted extract into a waste receptacle when finished.**

**Calculation of the ppm of Each PAH in Contaminated Soil**

Let us assume that upon injection of the diluted soil extract, a concentration of 225 ppm dibenzo(\( a,h \))anthracene in the diluted soil extract was obtained based on a correctly calibrated instrument.

What would the original concentration of dibenzo(\( a,h \))anthracene be in the contaminated soil?

- 225 ppm means 225 \( \mu \)g/mL of diluted soil extract
- Thus, 225 \( \times \) 10 = 2250 \( \mu \)g/mL in the original 20 mL of extract before dilution
- One says that the dilution factor DF is 10

© 2006 by Taylor & Francis Group, LLC
(20 mL extract)(2250 µg/mL dibenzo(a,h)anthracene) = 45,000 µg total from 2 g of soil
45,000 µg total/2.0 g soil = 22,500 µg/g or ppm dibenzo(a,h)anthracene in contaminated soil

Upon properly completing the sequence file within Turbochrom, the final result, 22,500 ppm, will be directly obtained in the “peak report” for that sample.

FOR THE REPORT

Include the overlay comparison and calibration results and list the concentration of each PAH in the contaminated soil sample. If a second sample result is available, estimate the precision of the method. Comment on the advantage of using a PDA to increase sensitivity.

Address the following:

1. Explain the elution order for the 16 PAHs using chemical principles.
2. The method detection limit using a UV absorption detector for some of the 16 priority pollutant PAHs could be improved if a different detector could be used. Explain.
3. Explain why this method is considered quick. Are there limitations to the use of quick methods, and if so, what are some of these?

Some representative PAHs are as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>M_r</th>
<th>Molecular Formula</th>
<th>Molecular Structure</th>
<th>Aqueous Solubility</th>
<th>Log(K_ow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>NA</td>
<td>128</td>
<td>C_{10}H_{8}</td>
<td><img src="image" alt="Structure" /></td>
<td>31.7</td>
<td>3.36</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>ACY</td>
<td>152</td>
<td>C_{12}H_{8}</td>
<td><img src="image" alt="Structure" /></td>
<td>16.1</td>
<td>3.94</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>ACE</td>
<td>154</td>
<td>C_{12}H_{10}</td>
<td><img src="image" alt="Structure" /></td>
<td>3.93</td>
<td>4.03</td>
</tr>
<tr>
<td>Fluorene</td>
<td>FLE</td>
<td>166</td>
<td>C_{13}H_{10}</td>
<td><img src="image" alt="Structure" /></td>
<td>1.98</td>
<td>4.47</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>PH</td>
<td>178</td>
<td>C_{14}C_{10}</td>
<td><img src="image" alt="Structure" /></td>
<td>1.29</td>
<td>4.57</td>
</tr>
<tr>
<td>Anthracene</td>
<td>AN</td>
<td>178</td>
<td>C_{14}H_{10}</td>
<td><img src="image" alt="Structure" /></td>
<td>0.073</td>
<td>4.54</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>FLA</td>
<td>202</td>
<td>C_{16}H_{10}</td>
<td><img src="image" alt="Structure" /></td>
<td>0.260</td>
<td>5.22</td>
</tr>
</tbody>
</table>
AN INTRODUCTION TO DATA ACQUISITION AND CONTROL USING TURBOCHROM AND AN INTRODUCTION TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC): EVALUATING THOSE EXPERIMENTAL PARAMETERS THAT INFLUENCE SEPARATIONS

BACKGROUND AND SUMMARY OF METHOD

Contemporary analytical instrumentation is said to be interfaced to computers. These developments commenced in the early to mid-1980s and took hold with Windows-based software environments in the 1990s. This can be illustrated as follows:

![Diagram showing data acquisition and control](https://via.placeholder.com/150)

Interfaces can be either stand-alone or installed into the console of the PC. Instruments can be controlled and data acquired from a PC, or if a control is not available, only data acquisition is obtained. In our laboratory, both types of interfaces are used. With appropriate software, the control and data acquisition tasks are easily performed. If a means can be acquired to enable automatic sampling to be controlled as well, a totally automated system results. This was accomplished in our laboratory.

The HPLC within each workstation is PC controlled, and the photodiode array detector (PDA) is interfaced to the same PC, thus enabling real-time data acquisition.
Students are first asked to study the present architecture so as to gain an appreciation of contemporary HPLC-PDA-DS (data system) technology.

This experiment is designed to take you through an initial hands-on experience with the HPLC-PDA-DS from a first sample injection to a simple quantitative analysis. A quick method is first necessary for the system to recognize something. This is followed by optimizing the initial method, conducting a calibration, creating a customized report format, and evaluating the initial calibration verification (ICV).

Following completion of the initial experiment, the focus shifts to the separation of the test mixture or organic compounds using the HPLC instrument. The effect of solvent strength on \( k' \) and the effect of mobile-phase flow rate on \( R_s \) will be considered by retrieving previously developed Turbochrom methods and making manual injections.

### HPLC and Trace Environmental Analysis

High-performance liquid chromatography followed GC in the early development of instrumental column chromatographic techniques that could be applied to trace environmental analysis. HPLC most always complements and sometimes duplicates GC. For example, polycyclic aromatic hydrocarbons (PAHs) can be separated and quantitated by both techniques; however, \( N \)-methyl carbamate pesticides can be determined by HPLC only as a result of the thermal instability in a GC injection port. HPLC has become the dominant instrumental analysis method for the pharmaceutical industry, yet continues to take a secondary role in the environmental field. Samples that contain the more polar and thermally labile analytes are much more amenable to analysis by HPLC rather than by GC. A major contaminant in a lake in California went undetected until State Department of Health chemists identified a sulfonated anionic surfactant as the chief cause of the pollution. This pollutant was found using HPLC techniques. HPLC encompasses a much broader range of applicability in terms of solute polarity and molecular-weight range when compared with GC. (Review Figure 4.1.)

To illustrate how these different kinds of HPLC might aid the analyst in the environmental testing laboratory, consider the request from an engineering firm that wishes to evaluate the degree of phthalate ester contamination from leachate emanating from a hazardous waste site. Reversed-phase HPLC is an appropriate choice for the separation of lower-molecular-weight phthalate esters (e.g., dimethyl from diethyl from dibutyl). Attempts to elute higher-molecular-weight and much more hydrophobic (lipophilic) phthalate esters (e.g., dioctyl, bis(2-ethyl hexyl)) under reversed-phase conditions are unsuccessful. The separation of these under normal-phase HPLC conditions is successful.

### Flow-Through Packed Columns

High-performance liquid chromatography requires that liquid be pumped across a packed bed within a tubular configuration. Snyder and Kirkland \(^7\) have used the Hagen–Poiseuille equation for laminar flow through tubes and Darcy’s law for fluid flow through packed beds and derived the following relationship: 

\[ \frac{Q}{A} = \frac{12}{T} \]
where $t_0$ is the retention time of an unretained solute (the time it takes after injection for an unretained solute to pass through the column and reach the detector), $L$ is the length of the column, $\eta$ is the viscosity of the mobile phase, $\Delta P$ is the pressure drop across the column, $d_p$ is the particle size of the stationary-phase packing, and $f$ is an integer and is 1 for irregular porous, 2 for spherical porous, and 4 for pellicular packings.

The importance of stationary-phase particle size is reflected in the dependence of the void retention volume $V_0 = F(t_0)$, where $F$ is the mobile-phase flow rate in, for example, cm$^3$/min, on the inverse square of $d_p$. Recall that the retention volume of a retained solute whose capacity factor is given by $k'$ is

$$V_R = V_0(1 + k')$$

Hence, the smaller the $d_p$, the larger is $V_0$ and, consequently, $V_R$. A smaller $d_p$ also contributes in a significant manner to a larger $N$ (refer to theoretical equations found in Chapter 4).

**High-Pressure Liquid Chromatograph**

It is quite useful to view the instrumentation for HPLC in terms of zones according to the following schematic:

**Zone 1** — Low-pressure zone prior to pump. This is a noncritical area served by Teflon tubing. A fritted filter is placed at the inlet to prevent particulates from entering the column.

**Zone 2** — High-pressure zone between pump and injector. This is a noncritical area served by standard stainless-steel (SS) tubing usually 1/16 in. in outer diameter (o.d.). A high-surface-area 0.5-μm filter can be placed here to prevent particulates from reaching the column.
Zone 3 — High-pressure area surrounding injector and column. This is a critical area where the sample is introduced to the separation system. The volume must be well swept and minimized. Special fittings are 0.25-mm-inner diameter (i.d.) SS tubing.

Zone 4 — Low-pressure area between column and detector. In this critical area, separation achieved in the column can be lost prior to detection. The volume must be well swept and minimized. Special fittings and 0.25-mm SS or plastic tubing are required. The critical zone extends to all detectors or fraction collectors in series or parallel connection.

Zone 5 — Low-pressure area leading to waste collector. This noncritical area is served by Teflon tubing. Most labs fail to fit the waste vessel with a vent line to the hood or exhaust area.

Experimental

High-performance liquid chromatograph incorporating a UV absorption detector under reversed-phase conditions.

Preparation of Chemical Reagents

Note: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

Accessories to Be Used with the HPLC per Group

1. HPLC syringe. This syringe incorporates a blunt-end; use of a beveled-end GC syringe would damage inner seals to the Rheodyne injector.
2. 10-mL two-component mix at 1000 ppm each. Prepare the mixture by dissolving 10 mg of phthalic acid (PhtA) and 10 mg of dimethyl phthalate (DMP) in about 5 mL of 50:50 ACN:H₂O in a 50-mL beaker. After dissolution, transfer to a 10-mL volumetric flask and adjust to the final mark with the 50:50 solution.

Procedure

Be sure to record your observations in your laboratory notebook.

Initial Observations of a Computer-Controlled High-Performance Liquid Chromatograph

Upon approaching the HPLC-PDA-DS, conduct the following:

1. Identify each of the five zones discussed above.
2. Locate the following hardware components:
   a. The IEEE-488 cable to the LINK interface
   b. The start/stop line from the Rheodyne injector to the LINK
   c. The data acquisition line from the PDA to the LINK
   d. The keying and master key
Creating a QuickStart Method, Acquiring Data, Optimizing, Calibrating, and Conducting Analysis Using the QuickStart Method

Proceed with the Turbochrom 4 Tutorial and create a method using QuickStart. Inject a 100 ppm test mix reference standard. Optimize the method using the Graphic Editor. Develop the calibration and report format sections of your method. Establish a three-point calibration for DMP only between 10 and 100 ppm (inject from low concentration to high) and prepare an ICV. Run the ICV in triplicate.

Effect of Solvent Strength on $k'$

A good practice when beginning to use an RP-HPLC instrument is to initially pass a mobile phase that contains 100% acetonitrile (ACN) so as to flush out of the reversed-phase column any nonpolar residue that might have been retained from previously running the instrument. Retrieve the Turbo method titled “100%ACN” and download if not already set up. Download within “setup” using the “method” approach.

Retrieve the method from Turbochrom or equivalent software titled “80%ACN” and proceed to use “Setup in the method mode” to enable you to operate the HPLC with a mobile-phase composition of 80% ACN and 20% aqueous. The use of “Setup” is called downloading the method and sequence file so that data acquisition can begin. The aqueous mobile phase consists of 0.05% phosphoric acid dissolved in distilled deionized water (DDI). Carefully fill the 5-$\mu$L injection loop (the injector arm should be in the “load” position with the evaluation test mix with the HPLC syringe). Inject by moving the injector arm from the “load” position to the “inject” position. Observe the chromatogram that results. Note the retention times of the components in the mixture. Give all members in the group the opportunity to make this initial injection.

Retrieve the method titled “60%ACN,” download it, and then proceed to repeat the injection procedure discussed above. Observe the chromatogram that results and note retention times.

Retrieve the method titled “40%ACN,” download it, and then proceed to repeat the injection discussed earlier. Observe the chromatogram that results and note retention times.

Retrieve the method titled “20%ACN,” download it, and then proceed to repeat the injection procedure discussed earlier. Observe the chromatogram that results and note retention times.

Effect of Mobile-Phase Flow Rate on Resolution

The mobile-phase flow rate will be varied and its influence on chromatographic resolution will be evaluated.

Retrieve the method titled “FlowHi,” download it, and then proceed to use “Setup” as you did during the variation of solvent strength experiments. Allow sufficient equilibration time at this elevated mobile-phase flow rate. Notice what happens to the column back-pressure when a high flow rate is in operation. Inject the test mix and observe the chromatogram that results.

Retrieve the method titled “FlowLo,” download it, and then proceed to repeat the injection procedure discussed earlier. Observe the chromatogram that results.
FOR THE LAB NOTEBOOK

The following empirical relationship has been developed for RP-HPLC. Refer to Chapter 4 or to a more specialized monograph.\textsuperscript{9}

\[ \log k' = \log k_w - S\Phi \]

where \( k' \) is the capacity factor for a retained peak, \( k_w \) is the capacity factor (extrapolated) \( k' \) for pure water, \( \Phi \) is the volume fraction of the organic solvent in the mobile phase, and \( S \) is a constant that is approximately proportional to solute molecular size or surface area.

Choose one component in the evaluation test mix and determine whether the above equation is consistent with your observations.

Address the following:

1. Among the three major parameters upon which resolution \( R_s \) depends, which of the three is influenced by changes in mobile-phase flow rate? Explain.

2. Mr. Everett Efficient believes that he can conserve resources by operating his HPLC using a mobile phase that consists only of a 0.01 \( M \) aqueous solution containing sodium dihydrogen phosphate. Discuss what is seriously deficient in Mr. Efficient’s fundamental assumption.

3. Assume that you could change HPLC columns in this exercise and that you installed a column that contained 3-\( \mu \)m particle size silica. Assume that you used the same mobile-phase composition that you used for the reversed-phase separations that you observed. Explain what you would expect to find if the reversed-phase test mix were injected into this HPLC configuration.

4. Explain why DMP is retained longer (i.e., has the higher \( k' \)) than phthalic acid given the same mobile-phase composition.

DETERMINATION OF PRIORITY POLLUTANT SEMIVOLATILE ORGANOCHLORINE PESTICIDES: A COMPARISON OF MICRO-LIQUID–LIQUID AND SOLID-PHASE EXTRACTION TECHNIQUES

BACKGROUND AND SUMMARY OF METHOD

Organochlorine pesticides (OCs) were used widely in agriculture during the first half of the 20th century in the U.S. and were subsequently banned from use during the 1970s. Unfortunately, some of the OCs are still in widespread use around the world. Their persistence in the environment was not apparent until Lovelock introduced the electron-capture detector (ECD) in 1960.\textsuperscript{10} When combined with high-resolution capillary gas chromatography and appropriate sample preparation methods, the ECD provides the analytical chemist with the most sensitive means by which to identify...
and quantitate OCs in environmental aqueous and soil/sediment samples. As analytical chemists were seeking to identify and quantitate OCs during the early 1970s, it became apparent that many additional chromatographically resolved peaks were appearing. What were considered as unknown interfering peaks in the chromatogram were then subsequently found to be polychlorinated biphenyls (PCBs).

The OCs and PCBs were first determined in wastewaters using EPA Method 608. This method originally required packed columns, and because of this, it necessitated extensive sample preparation and cleanup techniques, which included liquid–liquid extraction and low-pressure column liquid chromatography. Capillary GC-ECD, when combined with more contemporary methods of sample preparation, provides for rapid and cost-effective trace environmental analysis. Over the past 10 years, there has been dramatic improvements in sample preparation techniques as they relate to semivolatile and nonvolatile trace analyses.

In addition to external standard and standard addition, the last principal mode of calibration is called internal standard. This mode of calibration should be used when there exists variability in sample injection volume, when there is concern about the lack of instrument stability, and when there is unavoidable sample loss. Instrumental response then becomes related to the ratio of the unknown analyte X to that for the internal standard S, instead of related only to the unknown analyte. If some X is lost, one can assume that some S would be lost as well. This preserves the ratio [X]/[S]. For extraction methods, the internal standard (IS) is added to the final extract just prior to adjusting the final volume. Selecting a suitable IS is not trivial. It should possess similar physical and chemical properties to the analyte of interest and not interfere with the elution of any of the analytes that need to be identified and quantitated. The IS should be within the same concentration range as for the calibration standards and at a fixed concentration. The following is a calibration curve for the IS mode:

![Calibration Curve](https://example.com/calibration_curve.png)

This exercise introduces the student to solid-phase extraction (SPE) techniques. The µLLE method will also be implemented. The two methods will be compared. SPE in the reversed-phase (RP) mode of operation involves passing an aqueous
sample over a previously conditioned sorbent that contains a chemically bonded silica gel held in place with polyethylene frits within a column configuration. A typical RP-SPE sequence follows:

For RP-SPE, methanol is used to condition or wet the sorbent surface, thereby activating the octadecyl moiety and hence forcing it to be receptive to a van der Waals type of intermolecular interaction between the analyte and the C\textsubscript{18} moiety. This phenomenon is shown below for the isolation of n-butyl phthalate on a C\textsubscript{18} chemically bonded sorbent\textsuperscript{12}.

The OCs studied in this exercise are lindane, endrin, and methoxychlor. Lindane (γ-BHC) is synthesized via chlorination of benzene in the presence of ultraviolet light. This forms a mixture of BHC isomers that are identified as α, β, γ, δ, and ε. Selective crystallization isolates the γ isomer, whose aqueous solubility is 7.3 to 10.0 ppm and is the most soluble of the BHC isomers. Endrin is a member of the cyclodiene insecticides and is synthesized using Diels–Alder chemistry. Methoxychlor belongs to the \textit{p,\textit{p}′}-DDT category and structurally differs from DDT in substitution of a methoxy group in place of a chloro group \textit{para} to the central carbon. Methoxychlor’s aqueous solubility is 0.1 to 0.25 ppm and exceeds that of \textit{p,\textit{p}′}-DDT.
by a factor of 100. Molecular structures and correct organic nomenclature of these three representative OCs are shown in the following:

**Gamma-BHC**

[Gamma-BHC structure]

**Endrin**

[Endrin structure]

**γ-1,2,3,4,5,6-Hexachlorocyclohexane**

1,2,3,4,10,10-Hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8-dimethanonaphthalene

**P,P′-Methoxychlor**

1,1,1-Trichloro-2, bis (p-methoxyphenyl) ethane

**Experimental**

Gas chromatograph that incorporates an electron-capture detector.

**Preparation of Chemical Reagents**

*Note: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.*

**Chemicals/Reagents Needed per Group**

1. 1000 ppm each of lindane, endrin, and methoxychlor stock standard solution dissolved in iso-octane. This is a solvent available in ultrahigh purity, which is an important requirement in trace environmental analysis.
2. 20 ppm of an internal standard. Available candidates include 4-hydroxy-2,4,6-trichlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl, 1,2-dibromo-3-chloropropane, and β-BHC.
3. Vial containing approximately 30 mL of methanol for conditioning the RP-SPE sorbent.
4. Vial containing approximately 30 mL of iso-octane.

**Preliminary Planning**

Because there are two sample preparation methods to be implemented, assemble as a group at the beginning of the laboratory session and decide who does what. Once all results are obtained, the group should reassemble and share all analytical data.
Selection of a Suitable Internal Standard

The most appropriate IS needs to be selected from the above list of candidates. Consult with your laboratory instructor and proceed to inject one or more ISs and base your decision on an interpretation of the chromatogram.

Procedure for Calibration and Quantitation of the GC-ECD

1. Prepare the necessary primary and secondary dilution standards. The range of concentration levels for the unknowns is between 100 and 1000 pg/µL (ppb). For example, take a 100-µL aliquot of the 1000 ppm stock and add to a 10-mL volumetric flask previously half filled with iso-octane. Adjust to the calibration mark and label “10 ppm L,E,M (iso-octane), primary dilution.” A 1:10 dilution of this primary dilution standard gives a secondary dilution, which should be labeled “1 ppm L,E,M (iso-octane), secondary dilution.” Use the secondary dilution to prepare a series of working calibrations that cover the range of concentrations in the ppb domain, as discussed above.

2. Prepare a set of working calibration standards to include an ICV that brackets the anticipated range for the unknowns. To each calibration standard, add 50-µL of 20 ppm IS so that the concentration of IS in each calibration standard is identical and at 1.0 ppm.

3. Retrieve the method from Turbochrom or other equivalent software titled “LEMIS,” which stands for lindane, endrin, methoxychlor, internal standard mode of calibration; allow sufficient instrument equilibration time. Write a sequence encompassing the calibration standards, ICV, and unknowns. Save the sequence as a file with the name “G#0317” (group #, March 17th), for example. Begin to inject a 1-µL aliquot of each working standard. Initially inject iso-octane, then inject in the order of lowest to highest concentration level. This order is important because it prevents carryover from one standard to the next.

4. Update the calibration for the LEMIS method and check with your instructor as to the acceptability of the calibration. If found acceptable, proceed to the analysis once samples have been prepared using both extraction methods. Be sure to add the same amount of IS to each unknown extract, as was done for the calibration standards. Because the instrument has been calibrated and updated, the report will include an accurate readout of concentration in a tabular format.

Procedure for Performing µLLE and RP-SPE

5. Place exactly 40 mL of unknown sample into a 42-mL vial and extract using 2 mL of iso-octane in a manner similar to that for the BTEX/THMs exercise. This time, however, add twice the amount of IS that you added for the preparation of the calibration standards so that the concentration of IS remains identical to that for all other standards and samples.
6. Place exactly 40 mL of unknown sample into the 70-mL SPE reservoir, which sits atop a previously conditioned C<sub>18</sub> sorbent, according to specific instructions given to you by your laboratory instructor. Add distilled deionized water (DDI) to the reservoir so as to fill to near capacity. Pass the aqueous sample through the cartridge, which contains approximately 200 mg of C<sub>18</sub> chemically bonded silica gel. Use a wash bottle that contains DDI to rinse the residual sample from both the reservoir and the cartridge. Place a second SPE cartridge that is filled with anhydrous sodium sulfate beneath the sorbent cartridge. The second SPE cartridge containing anhydrous Na<sub>2</sub>SO<sub>4</sub> is used to remove residual moisture from the eluent. Into the manifold place a 1.0-mL volumetric flask as an eluent receiver and elute with two successive 500-µL aliquots of iso-octane. Add the same amount of IS as used for the calibration standards, then adjust to a final volume of 1.0 mL. Transfer to a separate container if necessary.

7. Inject a 1-µL aliquot of the sample extract that also contains the IS into the GC-ECD. At this point, the LEMIS method should have had its calibration updated.

8. Continue to make injections into the calibrated GC-ECD until all samples have been completed. You may want to make replicate injections of a given sample extract.

FOR THE REPORT

Include all calibration plots and calculate the correlation coefficient for the calibration plot. Calculate the precision and accuracy for the ICV, which should have been injected in triplicate. Report on the concentration of each unknown sample. Construct a table that shows the respective concentrations for the unknowns for each of the two methods. Recall that the final extract volume from µLLE was 2 mL, and that from SPE was 1 mL. Take this into account when comparing the two methods. Which sample preparation method is preferable? Give reasons for your preference and support this with analytical data.

DETERMINATION OF THE HERBICIDE RESIDUE TRIFLURALIN IN CHEMICALLY TREATED LAWN SOIL BY GAS CHROMATOGRAPHY USING SOLID-PHASE EXTRACTION TECHNIQUES

BACKGROUND AND SUMMARY OF METHOD

The persistence of trace residue levels of pesticides and herbicides in the environment has been cause for continued concern since the early 1960s, when it became apparent that these residues were detrimental to wildlife and possibly to human health. The benefits of using DDT gradually gave way to the increasing risk of continued use and led to the banning of its use. Herbicides, however, do not appear to present such a high risk to the environment and continue to find widespread use. The chlorophenoxy acid herbicides are not directly amenable to GC and must first be chemically
converted to their more volatile methyl esters prior to analysis using GC. Trifluralin or, according to International Union of Pure and Applied Chemistry (IUPAC) organic nomenclature, \(\alpha,\alpha,\alpha\text{-trifluoro-2,6-dinitro-}N,N\text{-dipropyl-}p\text{-toluidine,}\) is commonly one of the active preemergent herbicide ingredients in some lawn treatment formulations. Consider the molecular structure of trifluralin:

![Molecular structure of trifluralin](image)

With reference to the molecular structure for trifluralin, the presence of electronegative heteroatoms, such as fluorine combined with two nitro substituents on the benzene ring, would make the organic compound highly sensitive to the electron-capture detector (ECD), provided that the substance is sufficiently vaporizable and therefore amenable to GC. With a boiling point of 139°C, trifluralin is appropriately classified as a semivolatile, neutral organic and could be isolated by conventional sample preparation techniques such as liquid–liquid extraction (LLE).\textsuperscript{14,15}

The assay for the commercially available formulation that was dispersed over the lawn whose soil beneath has been sampled is given as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen</td>
<td>20</td>
<td>Chlorine (not more than)</td>
<td>3</td>
</tr>
<tr>
<td>Trifluralin ((N,N\text{-dipropyl}))</td>
<td>0.82</td>
<td>Ammonical nitrogen</td>
<td>1.17</td>
</tr>
<tr>
<td>Urea nitrogen</td>
<td>18.83</td>
<td>Trifluralin((N\text{-butyl,}N\text{-ethyl}))</td>
<td>0.43</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.2</td>
<td>Soluble potash</td>
<td>3</td>
</tr>
<tr>
<td>Available phosphate</td>
<td>3</td>
<td>Inert</td>
<td>98.5</td>
</tr>
</tbody>
</table>

**Solid-Phase Extraction**

Solid-phase extraction (SPE) techniques provide an alternative to LLE whereby a chemically bonded silica gel is packed into microcolumns or impregnated into disks and used to isolate and recover semivolatile organic contaminants from various environmental samples.\textsuperscript{16,17} A chemically neutral organic compound originally dissolved in water is thermodynamically unstable, and if an aqueous solution containing this compound is allowed to contact a hydrophobic surface, a much stronger van der Waals type of intermolecular interaction causes the molecules of the analyte to stick to the surface, and thus effectively be removed from the aqueous media. A relatively small volume of a nonpolar or sometimes polar solvent provides enough hydrophobic interaction to then remove (elute in a chromatographic sense) the
analyte molecules. The following is a schematic for the interaction of analyte molecules 2-naphthylamine and hexyl benzene sulfonate, with a C₈-bonded silica:

The SPE technique is performed in a stepwise manner as follows:

**Conditioning**
Conditioning the sorbent prior to sample application ensures reproducible retention of the compound of interest (the isolate).

**Retention**
- Adsorbed isolate
- Undesired matrix constituents
- Other undesired matrix components

**Rinse**
- Rinse the columns to remove undesired matrix components

**Elution**
- Undesired components remain
- Purified and concentrated isolate ready for analysis
Trifluralin, which might be present in lawn-treated soil, will be initially extracted into methanol. The methanol extract will be diluted with distilled deionized water (DDI), and the aqueous solution transferred to a 70-mL SPE reservoir on top of a conditioned C18-bonded silica sorbent. The sorbent cartridge will be eluted with high-purity iso-octane. The iso-octane eluent is dried by passing it through a second SPE cartridge directly into a 1.0-mL volumetric receiver. An internal standard is then added and the eluent brought to a final volume of 1.0 mL. A 1- to 2-µL aliquot of the eluent can then be directly injected in a C-GC-ECD (Autosystem GC). The concentration of trifluralin in the eluent can be determined following establishment and verification of the instrument calibration.

**Internal Standard Mode of Calibration**

In addition to external standard and standard addition, the last principal mode of calibration is the internal standard. This mode of calibration should be used when there exists variability in sample injection volume, when there is concern about the lack of instrument stability, and when there is unavoidable sample loss. Instrumental response becomes related then to the ratio of the unknown analyte X to that for the internal standard S, instead of related only to the unknown analyte. If some X is lost, one can assume that some S would be lost as well. This preserves the ratio [X]/[S]. For extraction methods, the internal standard (IS) is added to the final extract just prior to adjusting the final volume. Selecting a suitable IS is not trivial. It should possess similar physical and chemical properties to the analyte of interest and not interfere with the elution of any of the analytes that need to be identified and quantitated. The IS should be within the same concentration range as for the calibration standards and at a fixed concentration. A calibration curve for the IS mode is shown as follows:18
**EXPERIMENTAL**

**Preparation of Chemical Reagents**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

**Chemicals/Reagents/Accessories Needed per Group**

- 1 10 mL of iso-octane, suitable for trace pesticide residue analysis
- 1 100 mL of methanol, suitable for trace pesticide residue analysis
- 10 SPE cartridges packed with approximately 200 mg of C\textsubscript{18}-bonded silica
- 10 Empty SPE cartridges loosely packed with approximately 500 mg of anhydrous sodium sulfate
- 1 SPE vacuum manifold connected to a vacuum pump via a water trap
- 1 1.0-mL glass volumetric flasks with ground-glass stoppers
- 1 10-µL syringe (Hamilton Co.) for injection into the GC
- 1 10 ppm trifluralin reference stock standard dissolved in iso-octane
- 1 10 ppm 1,2,4-trichlorobenzene (IS) dissolved in MeOH or methyl-tert-butyl ether (MTBE)

**Preparation of the Working Calibration Standards**

From the reference stock solution of trifluralin in iso-octane, prepare a series of working calibration standards that cover the range of concentration levels between 10 and 1000 ppb of trifluralin in high-purity iso-octane. Each working standard should also contain the IS at a concentration level that should fall within the range of concentrations for the calibration standards. This level should be identical among all standards and sample extracts. Use the table below to guide you in preparing your calibration standards.

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>10 ppm Trifluralin (µL)</th>
<th>10 ppm IS (µL)</th>
<th>V(T) (mL)</th>
<th>Concentration of Trifluralin (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>50</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>50</td>
<td>1.0</td>
<td>250</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>50</td>
<td>1.0</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>50</td>
<td>1.0</td>
<td>1000</td>
</tr>
<tr>
<td>ICV</td>
<td>40</td>
<td>50</td>
<td>1.0</td>
<td>400</td>
</tr>
</tbody>
</table>

**Establishing the Calibration**

*Retrieve* the method from Turbochrom or other equivalent software titled “Tri-flu.mth,” *create* a sequence file, and *download* the sequence. *Turn off* the nitrogen makeup gas to the ECD and *measure the split ratio*. *Adjust to a ratio of between 15 and 20 to 1.* *Turn the makeup on* after you make the split ratio measurements.
**Trace Environmental Quantitative Analysis, Second Edition**

Inject approximately 1 µL of each working calibration standard and inject the ICV in triplicate using the 10-µL liquid-handling syringe. Update the method titled “Triflu.mth” with the new calibration standards data using the Graphic Editor. The precision and accuracy data for the ICV can be obtained by retrieving the Graphic Editor and bringing up the raw file for each ICV. Print the tabular formatted report for that particular sample.

**Isolating Trifluralin from Lawn-Treated Soil Using SPE Techniques**

A SPE vacuum manifold, which should be connected to a vacuum pump via a water trap, should be available at the workbench for each of the four workstations. Condition the C$_{18}$ sorbent by passing 2 mL of MeOH through it. Attach a 70-mL polypropylene reservoir to the top of the SPE cartridge and fill with DDI to approximately two thirds full.

Place 0.25 g of lawn-treated soil into each of three 50-mL beakers, add 10 mL of methanol (pesticide residue grade) to each beaker, and use a glass stirring rod or plastic magnetic stirring bar to stir this mixture for 5 min. Let stand for another 5 min; then decant the supernatant liquid through a Pasteur pipette, which contains nonsilanized glass wool to remove large particulates, into a clean beaker. Transfer the liquid to the 70-mL reservoir. Turn on the vacuum pump and pass the contents of the reservoir through the C$_{18}$ sorbent cartridge. After the contents of the reservoir have passed through the sorbent, rinse the reservoir and cartridge with DDI.

Remove the surface moisture with a tissue or equivalent and attach a second SPE cartridge that contains anhydrous sodium sulfate beneath the C$_{18}$ sorbent cartridge that contains the retained trifluralin. Elute the sorbent with two 500-µL aliquots of iso-octane into a 1.0-mL volumetric flask. Remove the receiving volumetric flask from the apparatus and adjust to the calibration mark on the flask with iso-octane.

Inject 1 µL of the dried iso-octane eluent into the C-GC-ECD. Repeat for the other two samples.

**FOR THE REPORT**

Include all calibration plots, correlation coefficients, and precision and accuracy estimates of the ICV, and report on the concentration of trifluralin in the soil in mg/kg (ppm).

**DETERMINATION OF PRIORITY POLLUTANT VOLATILE ORGANIC COMPOUNDS (VOCs) IN GASOLINE-CONTAMINATED GROUNDWATER USING STATIC HEADSPACE (HS) AND SOLID-PHASE MICROEXTRACTION HEADSPACE (SPME-HS) AND GAS CHROMATOGRAPHY**

**BACKGROUND AND SUMMARY METHOD**

Benzene, toluene, ethyl benzene, para-, meta-, and ortho-xylenes, collectively referred to as BTEX, constitute some of the most environmentally detrimental
organic compounds that have made their way into groundwater, primarily due to gasoline spills and underground storage tank leakage. In preparing for this experiment, this author has contaminated groundwater with gasoline and has observed not only the six BTEX components, but a large and early eluting peak that matches the retention time of methyl-tert-butyl ether (MTBE), a gasoline additive and a known groundwater contaminant. BTEX compounds comprise around 20 to 30% of gasoline and have an appreciable solubility in water in contrast to aliphatic hydrocarbons. EPA Methods 601 and 602 comprise the real workhorse approaches to trace VOCs analyses in wastewaters. These methods use dynamic headspace sampling coupled to GC with electrolytic conductivity (601) and photoionization (602) detection.\(^{19}\) EPA Method 502.2 is a high-resolution capillary column method with both detectors cited above connected in series and provides monitoring capabilities for over 60 VOCs that could be found in municipal drinking water supplies.\(^{20}\) An alternative to dynamic headspace (commonly referred to as purge and trap) is static headspace capillary gas chromatographic (HS-C-GC) techniques, as introduced in Chapter 3 and elsewhere.\(^{21}\)

Static HS techniques take advantage of the volatility exhibited by VOCs whereby the air remaining in a sealed vial above a liquid (defined as the headspace (HS)) is sampled with a gas-tight syringe and directly injected into the GC-FID for carbon-containing VOCs. This technique is called manual HS-GC, as distinguished from automated HS-GC techniques. A complement to static HS is SPME-HS. A fiber coated with a polymer such as polydimethyl siloxane is inserted through the septum and into the HS. VOCs partition from the HS to the polymer film. The principles underlying SPME in general are discussed in Chapter 3. The SPME syringe–fiber assembly is removed from the vial and inserted directly into the injection port of a gas chromatograph. VOCs are thermally desorbed off of the fiber and on to the head of a wall-coated open tubular (WCOT), where the VOCs are chromatographed. Varian Instruments manufactures a GC autosampler that has been modified to perform SPME-HS. CTC Analytics offers an HS syringe mounted on a robotic head that moves horizontally (known as a rail). This mount can accommodate either a gas-tight syringe to conduct HS or an SPME syringe holder (Supelco) to conduct SPME-HS. The robotic autosampler is controlled through software provided by either LEAP Technologies or Gerstel. A second rail provides robotic automated reagent delivery to the HS vial. Figure 3.10 and Figure 4.8 show chromatograms for static HS-C-FID of spiked aqueous samples for BTEX components obtained in the author’s lab.

**OF WHAT VALUE IS THIS EXPERIMENT?**

Students will have an opportunity in this experiment to quantitatively determine the concentration level of various BTEX compounds from gasoline-contaminated groundwater using static HS and SPME-HS techniques. Both sampling/sample prep techniques will be performed manually. This experiment affords students an opportunity to operate a conventional gas chromatograph. This GC is interfaced to a personal computer that utilizes Turbochrom software or the equivalent for data acquisition. Hence, a student must become familiar with the sampling/sample prep
trace environmental quantitative analysis, second edition

The method titled “BTEX.mth” will be retrieved from Turbochrom or other chromatography processing software available in the lab. External standard calibration curves will be generated using HS-C-GC-FID (headspace capillary gas chromatography with flame ionization detection). An aqueous environmental sample that has been contaminated with gasoline will be available and analyzed for traces of BTEX. Since two different analytical methods are applied to the same standards and samples, students will have the opportunity to apply t statistics to compare the analytical results from both methods.

**Use of t Statistics**

Comparison of two dependent averages is a statistical procedure that helps to determine whether two different analytical methods give the same average result for a given sample. If one analyzes each of a series of samples, which could include calibration standards, ICVs, blanks, and unknowns, by the two methods, a pair of results for each sample will be obtained. The difference between these two results for each pair will reflect only the difference in the methods. The following equation is used for the t test on paired data:

\[ t_{calc} = \frac{\bar{d}}{S_d} \sqrt{n} \]

\[ S_d = \frac{\sum d^2 - \left( \sum d \right)^2}{n - 1} \]

where

- \( d = \) difference in each pair of values
- \( \bar{d} = \) average absolute difference in the pairs of values
- \( n = \) number of pairs of values
- \( df = \) degrees of freedom associated with a given value for t
- \( s_d = \) standard deviation of the differences between the pairs of observations

A comparison of the calculate value, \( t_{calc} \), with that from a table of Student’s t values is then made. If \( t_{calc} > t \) (from table at the desired level of significance), then both methods do not give the same result. If \( t_{calc} < t \) (from table at the desired level of significance), then it is statistically valid to assume that both methods are equivalent.
EXPERIMENTAL

Preparation of Chemical Reagents

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

**Chemicals/Reagents Needed per Group**
- 1 Neat benzene
- 1 Neat toluene
- 1 Neat ethyl benzene
- 1 Neat xylene: ortho, meta, and para
- 1 2000 ppm BTEX, certified reference standard, dissolved in MeOH
- 1 40 mL of gasoline-contaminated groundwater for BTEX determination
- 1 40 mL of an unknown sample prepared by the staff for BTEX determination

**Items/Accessories Needed per Student or per Group**
- 10 22-mL glass headspace vials with PTFE/silicone septa and aluminum crimp-top caps.
- 1 Crimping tool for headspace vials.
- 1 0.5- or 1.0-cc capacity gas-tight syringe for headspace sampling and direct injection (Precision Sampling Corp., SGE, or Hamilton).
- 1 Heating block assembly that accepts a 22-mL HS vial and allows for measurement of the block temperature.
- 1 SPME fiber holder for manual sampling (Supelco).
- 1 Manual SPME sampling stand (Supelco) or equivalent, including mini stir bars. This apparatus is optional; the heating block assembly can be used to conduct SPME-HS.
- 1 100 µm of polydimethyl siloxane (PDMS) fiber for use with the SPME holder. Instructions for installing the PDMS fiber into the SPME fiber holder is available from the manufacturer (Supelco).

**Preliminary Planning**

At the onset of the laboratory period, assemble as a group and decide who is going to do what. Assign specific tasks to each member of the group. Once all results are obtained, the group should reassemble and share all analytical data.

**Procedure for BTEX Instrumental Analysis HS Techniques**

Using the 2000 ppm BTEX stock reference solution dissolved in MeOH, prepare a series of calibration standards in which the BTEX is present in a final volume $V_f = 10$ mL of DDI, which is contained in a 22-mL HS vial with PTFE/silicone septa.
and aluminum crimp-top caps. Refer to the calibration table below for reference as you prepare a series of working calibration standards for HS-GC analysis. Following the development of a calibration curve, inject the ICV (only one injection per vial is acceptable in HS-GC) and then one or more of the gasoline-contaminated aqueous samples.

- Prepare a series of working calibration standards and ICVs according to the following table:

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>2000 ppm BTEX (MeOH) (µL)</th>
<th>Concentration of BTEX (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>16</td>
</tr>
<tr>
<td>ICV 1</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>ICV 2</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>ICV 3</td>
<td>30</td>
<td>6</td>
</tr>
</tbody>
</table>

- Place the indicated aliquot of 2000 ppm BTEX (MeOH) into a 22-mL HS vial containing 10 mL of DDI and seal promptly using the crimping tool. Place the vial into the heated block, whose temperature should be elevated above ambient. Maintain this temperature through the experiment.

- Retrieve the method BTEX from Turbochrom or equivalent software. Open a new sequence file and name the raw data file according to the following example: “G116” (group 1, 16th of the month). Save the sequence file and name it in a manner similar to that in the following example: “G10316” (group 1, March 16th). Download the method (BTEX.mth) and the sequence file (e.g., G10316.seq).

- Make manual injections of approximately 0.25 cc of headspace using a gas-tight syringe (refer to the technique section below). After the three calibration standards have been run, update the calibration method within Turbochrom or equivalent software. Ask your lab instructor for help in updating the calibration within the method. Observe the calibration curve and note the value of the square of the correlation coefficient. Discuss with your instructor whether this calibration is acceptable.

- After the instrument has been properly calibrated, proceed to inject the headspace for the three ICVs, a method blank, and unknown samples, as assigned. Your instructor may give you a sample whose concentration is unknown. Record the code on the vial label.

- Obtain the interpolated values from the least squares regression for your three ICVs, method blank, and any and all samples. Obtain assistance from staff in getting a hard copy of your results.

© 2006 by Taylor & Francis Group, LLC
Specific Laboratory Experiments

Technique to Conduct a Manual Headspace Sampling and Direct Injection using a Gas-Tight Sampling Syringe

If a heater block is available, place the sealed and capped 22-mL headspace vial into the block and allow time for the vial to equilibrate before sampling. A water bath, i.e., a large beaker that is half filled with water, could serve as a constant-temperature environment for headspace sampling. Insert the gas-tight syringe with the valve in the “on” position (if a Precision Sampling type syringe is used) by penetrating the septum seal and withdraw a 0.25 cc aliquot of headspace. Be careful not to withdraw any liquid. Immediately close the on–off valve to the syringe while positioned within the headspace. Position the syringe into the injection port, open the syringe valve, and transfer the 0.5-cc aliquot into the GC.

Technique to Conduct an SPME Headspace Sampling and Injection/Thermal Desorption Using an SPME Syringe/Fiber Assembly

Install the 100-μm PDMS fiber into the SPME holder if this has not already been done by your instructor. Follow directions for installing the fiber. Clean the fiber by inserting the SPME holder into an unused GC injection port whose temperature is ~250°C for about ½ hour. To a sealed HS vial that contains either spiked water or aqueous unknown sample, add a stir bar and begin magnetic stirring. Insert the retracted fiber holder through the septum. Expose the fiber by depressing the plunger and lock it in the bottom position by turning it clockwise. The PDMS fused-silica fiber that is attached to a stainless-steel rod is now exposed to the HS. The fiber should remain above the height of the liquid level. Allow the extraction to take place for ~2 min. Retract the fiber back into the needle and pull the device out of the vial. Insert the needle of the SPME device into the injection port of the GC. This must be done carefully since SPME needles tend to be of a thinner gauge. Start the analysis by depressing the plunger and locking it in position. After 30 sec withdraw the fiber back into the needle, and pull the needle out of the injector. When the separation is completed, repeat the analysis to determine fiber carryover. Repeat this technique for every calibration standard, ICV, blank, and sample in the same general manner introduced above for the static HS technique.

For the Report

For each sample prep technique, include:

1. A three-point external calibration plot for each chromatographically resolved BTEX analyte with corresponding correlation coefficient. Note that Turbochrom finds the square of the correlation coefficient, known as a coefficient of determination.
2. A table that includes results for all calibration standards, ICVs, and samples from an interpolation of the regressed calibration curve. This table is to be used for the statistical comparison between both methods.
3. The coefficient of variation for the ICVs.
4. The relative error for the ICVs.
5. A representative gas chromatogram for the separation.

How do both techniques compare? Apply the comparison of two dependent averages to all data. Write several paragraphs using your findings to address this question. Identify those sources of error that might compromise accuracy and precision for both techniques. How might the calibration procedure be modified if an internal standard mode of instrument calibration were used? If an isotope dilution approach were used?

SCREENING FOR THE PRESENCE OF BTEX IN WASTEWATER USING LIQUID–LIQUID EXTRACTION (LLE) AND GAS CHROMATOGRAPHY; SCREENING FOR THMS IN CHLORINE-DISINFECTED DRINKING WATER USING STATIC HEADSPACE (HS) GAS CHROMATOGRAPHY

BACKGROUND AND SUMMARY OF METHOD

Screening for hydrocarbon-containing VOCs using hexadecane LLE to detect the presence of BTEX and screening for organochlorine-containing VOCs using static HS-GC-ECD to detect the presence of trihalomethanes (THMs) are introduced in this experiment. Figure 3.10 and Figure 4.8 show HS-C-GC-FID chromatograms for BTEX. THMs consist of chloroform, dichlorobromomethane, dibromochloromethane, and bromoform, and these analytes have been found in drinking water that has been disinfected using chlorine. Refer to Scheme 3.3, which shows a logical flowchart for VOCs screening.

We will take a more simplified approach to trace VOCs analysis, which utilizes our limited sample preparation and instrumentation capabilities in the instructional laboratory. If a suitable extraction solvent can be found, i.e., one that does not interfere with the VOCs to be identified and quantitated by gas chromatography, then the analytes of interest can be isolated and concentrated from the environmental sample matrix via a mini-LLE technique. A 40-mL sample of wastewater that might contain BTEX is extracted with 2 mL of a suitable organic solvent. The organic solvent, being less dense than water, conveniently occupies the neck of the 40-mL vial. A 2-µL aliquot of the extract is injected into a C-GC-FID to determine BTEX. The C-GC-FID must be previously optimized to separate most BTEX compounds. In a separate experiment, 40 mL of chlorine-disinfected drinking water is placed in a sealed HS vial and heated, and 0.5 cc of the headspace is sampled and injected directly into a C-GC-ECD. The C-GC-ECD must be previously optimized to separate the four THMs. Typical levels of BTEX contamination for wastewater are in the low parts per million (ppm) concentration range. Typical levels of THM contamination for chlorine-disinfected drinking water are typically between 10 and 100 ppb for each THM. A severe limitation to LLE techniques is the possible formation of emulsions when applied to wastewaters that could have an appreciable surfactant.
concentration level. HS-C-GC-ECD is a very selective approach for screening chlorine-disinfected drinking water samples for THMs.

**OF WHAT VALUE IS THIS EXPERIMENT?**

This exercise affords to the student an opportunity to further utilize gas chromatography, this time as a screening tool. Two different sample preparation approaches to screening are introduced for two very different sample matrices. If a method involves phase distribution equilibria either for screening or for quantification, some analyte will always be lost between phases. Volatility losses can be considerable when VOCs are dissolved in water, while these losses are not so critical for semivolatile organics dissolved in water.

A previously created method will be retrieved from Turbochrom or other chromatography processing software available in the lab. It is possible for your instructor to turn this qualitative screening experiment into a quantitative one. If so, external standard calibration curves must be generated.

**EXPERIMENTAL**

**Preparation of Chemical Reagents**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

**Chemicals/Reagents Needed per Group**

- Neat benzene
- Neat toluene
- Neat ethyl benzene
- Neat xylene
- Neat hexane to evaluate as a suitable screening extractant
- Neat hexadecane to evaluate as a suitable screening extractant
- Neat dichloromethane to evaluate as a suitable screening extractant
- Approximately 5000 ppm stock BTEX standard (refer to actual label for exact values)
- 40 mL of a wastewater sample for screening for BTEXs
- 40 mL of a chlorine-disinfected drinking water sample for screening for THMs
- 500 ppm reference stock standard containing THMs in MeOH

**Items/Accessories Needed per Student or per Group**

- 42-mL glass vial with screw caps and PTFE/silicone septa
- 22-mL glass headspace vial with PTFE/silicone septa and crimp-top caps
- Crimping tool for headspace vials
- Liquid-handling syringe whose capacity is 10 µL with Chaney adapter (Hamilton or other manufacturer) for injection of liquid extracts

© 2006 by Taylor & Francis Group, LLC
1. 0.5- or 1.0-cc capacity gas-tight syringe for headspace sampling and direct injection (Precision Sampling, SGE, and Hamilton, among others, manufacture such syringes)
1. Heating block assembly that accepts a 22-mL HS vial and allows for measurement of the block temperature (VWR or other supply house)

**Preliminary Planning**

At the onset of the laboratory period, assemble as a group and decide who is going to do what. Assign specific tasks to each member of the group. Once all results are obtained, the group should reassemble and share all analytical data.

**Procedure for BTEX Instrumental Analysis Using LLE Techniques**

*Selecting the Most Suitable Extraction Solvent*

1. Place one small drop of each of the neat BTEX liquids into approximately 10 mL of hexane. Inject 1 µL into the GC-FID and interpret the resulting chromatogram. Repeat for dichloromethane and then for hexadecane. Methods must be previously created on Turbochrom or equivalent software. Recall, the most suitable solvent is the one that does not interfere with the GC elution of BTEXs. From these observations, select the most appropriate extraction solvent, then proceed to prepare calibration standards.

*Preparation of the Primary Dilution Standard and Working Calibration Standards*

2. Using a clean and dry glass pipette (volumetric), transfer 1.0 mL of the 5000 ppm BTEX to a 10-mL volumetric flask that has been previously half filled with the most suitable solvent that you chose earlier. Adjust to the calibration mark with this solvent and label as “500 ppm BTEX,” for example. This is what EPA methods call a primary dilution standard, since it is the first dilution that the analyst prepares from a given source. In this case, a 1:10 dilution has been made.
3. Prepare a series of working calibration standards according to the following table:

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>500 ppm BTEX (mL)</th>
<th>Final Volume (mL)</th>
<th>Concentration of BTEX (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>10</td>
<td>400</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>—</td>
<td>500</td>
</tr>
<tr>
<td>ICV</td>
<td>5</td>
<td>10</td>
<td>250</td>
</tr>
</tbody>
</table>
Specific Laboratory Experiments

For example, to prepare standard 3, 4 mL of 500 ppm BTEX (MeOH) added to a 10-mL volumetric flask half filled with MeOH is added to bring the menicus to the mark of the volumetric flask. This yields a calibration standard whose concentration is 200 ppm BTEX (MeOH).

4. Retrieve the method BTEX from Turbo, open a new sequence file, and name the raw data file according to the following example: “G116” (group 1, 16th of the month). Save the sequence file and name it in a manner similar to the following example: “G10316” (group 1, March 16th).

5. Inject 1-µL aliquots of all calibration standards and inject the ICV in triplicate. Update the calibration method within Turbo. Ask you lab instructor for help in updating the calibration within the method. Observe the calibration curve and note the value of the square of the correlation coefficient. Discuss with your instructor whether this calibration is acceptable.

6. After the instrument has been properly calibrated, proceed to inject the extracts from the LLE method from the unknown contaminated samples (refer to “Procedure to Conduct a Screen …,” below). Obtain the interpolated values from the external standard mode of calibration.

**Procedure for THM Instrumental Analysis Using HS Techniques**

Using the 500 ppm THM stock reference solution, prepare a series of calibration standards in which the THMs are present in 10 mL of DDI, which is contained in a 22-mL HS vial with PTFE/silicone septa and aluminum crimp-top caps. Refer to the BTEX calibration for guidance as you prepare a series of working calibration standards for HS-GC analysis. Ask your instructor to review your calibration table for correctness. Following the development of a calibration curve, inject the ICV (only one injection per sample is acceptable in HS-GC), then inject the headspace above the aqueous samples. Following the development of a calibration curve, inject the ICV and the chlorine-disinfected drinking water samples.

**Procedure to Conduct a Screen for BTEXs via LLE and Subsequent Injection into a GC-FID**

Once the most appropriate extraction solvent has been selected, the wastewater sample that contains dissolved BTEX can be extracted. To a clean 42-mL glass vial with a PTFE/silicone septum and screw cap, add 40 mL of aqueous sample. Pipette 2.0 mL of extraction solvent, and place the septum and cap in place. Shake for 1 min and let stand for at least 5 min until both phases clearly separate. Using a glass transfer pipette, remove approximately 75% of the extract and place in a small test tube or vial. Inject 1 µL of extract into the GC-FID. Discard the contents of the 42-mL glass vial into the waste receptacles that are located in the laboratory.

**Procedure to Conduct Manual Headspace Sampling and Direct Injection into a GC-ECD**

If a heater block is available, place the sealed and capped 22-mL headspace vial into the block and allow time for the vial to equilibrate before sampling. A water
bath, i.e., a large beaker that is half filled with water, could serve as a constant-
temperature environment for headspace sampling. Insert the gas-tight syringe with
the valve in the “on” position (applicable to syringes made by Precision Sampling)
by penetrating the septum seal and withdraw a 0.5-cc aliquot of headspace. Be
careful not to withdraw any liquid. Immediately close the on–off valve to the syringe
while positioned within the headspace. Position the syringe into the injection port,
open the syringe valve, and transfer the 0.5-cc aliquot into the GC.

FOR THE REPORT

Since this experiment involves screening only, quantification of the wastewater and
chlorine-disinfected samples is unnecessary unless your instructor asks you to quan-
titate. If you find BTEX or THMs from the screens, discuss how you might conduct
a quantitative analysis of these samples. If these samples identified additional comp-
ounds that were not BTEX or THM compounds, suggest ways that the identity of
these unknown compounds could be revealed. Explain the basis on which you chose
the screening extractant. Refer to that section of VOCs screening in Chapter 3 for
some help here.

AN INTRODUCTION TO GAS CHROMATOGRAPHY:
EVALUATING EXPERIMENTAL PARAMETERS THAT
INFLUENCE GAS CHROMATOGRAPHIC PERFORMANCE

BACKGROUND AND SUMMARY OF METHOD

Gas chromatography (GC) is the most widely used instrumental technique for the
determination of trace concentrations of organic pollutants found in environmental
samples today. Its origins stem from the pioneering work of Martin and Synge in
1941 to the development of open tubular gas chromatographic columns advanced
by Golay to the fabrication by Dandeneau at Hewlett-Packard of the flexible fused-
silica capillary column. It must be recognized, however, that approximately 20%
of all of the organic compounds that exist and could possibly make their way into
the environment are amenable to GC techniques without prior chemical modification
of the sample. Despite this limitation, over 60 organic compounds classified as
volatile organic compounds (VOCs) have been found in drinking water, groundwater,
surface water, and wastewater and are routinely monitored. Over 100 semivolatile
organic compounds have also been found, which include phenols, polycyclic aromatic
hydrocarbons, mono-, di-, and trichloro aromatics and aliphatics, nitro aromatics,
polychlorinated biphenyls, organochlorine pesticides, organophosphorus pesticides,
triazine herbicides, and phthalate esters, among others.

The theoretical principles that underlie GC are presented in Chapter 4 and in
numerous texts and monographs. Specific methods that incorporate GC as the
determinative instrumental technique are to be found in a plethora of analytical
methods published by the Environmental Protection Agency (EPA), the American
Public Health Association/American Water Works Association/Water Pollution

© 2006 by Taylor & Francis Group, LLC
This exercise introduces the student to those experimental GC parameters that exert a major influence on GC performance. These include (1) detector selectivity, (2) injection volume vs. chromatographic peak shape, (3) the effect of changing the carrier gas flow rate on column efficiency, and (4) the effect of column temperature on chromatographic resolution and analysis time. This exercise affords the student the opportunity to vary these parameters and assess the outcomes. This is a qualitative analysis exercise only and involves making and recording observations and doing some calculations from information found in the chromatograms.

**BRIEF DESCRIPTION OF GAS CHROMATOGRAPHS IN THE HAZARDOUS WASTE ANALYSIS LAB AT MICHIGAN STATE UNIVERSITY**

The PerkinElmer Autosystem GC consists of a dual-injector port, dual-capillary-column configuration, and dual detectors (flame ionization (FID), electron-capture (ECD)) connected via an analog-to-digital (A/D) interface to a central personal computer workstation, which is driven by the Turbochrom (PE-Nelson) chromatography data processing software. You will encounter two types of A/D interfaces in the laboratory. The 600 LINK interface provides for both data acquisition and instrument control. The 900 interface provides for data acquisition only.

The *front injector* consists of a split/splitless capillary column type and is connected to a 0.25 mm (i.d.) × 30 m (length) capillary column (referred to as a narrow-bore type column). The column is coated with a DB-5 liquid phase (5% phenyl dimethyl siloxane) that is chemically bonded to the inner tubing wall. This type of liquid phase is appropriate for the separation of semivolatile or nonvolatile organic compounds whose boiling points are much greater than 100°C. The optimum volumetric flow rate (i.e., the flow rate that gives a minimum in the van Deemter curve) is between 1 and 3 cm³/min. To obtain such a low flow rate, a split vent is required to remove most of the gas. Refer to the instruction manual for setting the split ratio. Typical split ratios are 1:25, 1:50, or 1:100, and this ratio refers to the ratio of gas flow through the column to that through the vent. The outlet end of this column is connected to the inlet to the ECD. This detector requires an additional source of inert gas, commonly called makeup. The flow rate for the makeup should be approximately 30 cm³/min. Using the digital flow check (refer to the instruction manual), measure the initial flow rate, then adjust to the optimum for operation of a narrow-bore capillary column.

The *rear injector* consists of a packed column adapted for connection to a 0.53 mm (i.d.) × 30 m (length) capillary column (referred to as a megabore column). The column is coated with a cyanopropyl dimethyl polysiloxane liquid phase that is chemically bonded to the inner tubing wall. This type of liquid phase is appropriate for the separation of volatile organic compounds (VOCs). The optimum volumetric flow rate is between 5 and 15 cm³/min. The column outlet is connected to the inlet to the FID. This detector does not require makeup gas. The FID, however, requires a 10:1 ratio of airflow to hydrogen flow. Conventional flow rates are 300 cm³/min.
for air and 30 cm³/min for H₂. Once the air/fuel ratio has been established, the FID can be ignited. Sometimes, a slightly fuel-rich ratio is necessary to ignite the FID.

**PRINCIPLE OF SEPARATION IN GC**

When two compounds migrate at the same rate through a chromatographic column, no separation is possible. Two compounds that differ in retention times, \( t(R) \), or capacity factor, \( k' \), and appear to separate, do so because of differences in their equilibrium distribution constants, denoted by \( K_D \). If \( K_D \) is independent of sample size, Gaussian elution bands (i.e., symmetrical peaks) are observed. This is the case of linear elution chromatography. In other words, a plot of the concentration of analyte in the stationary phase to the concentration in the mobile phase yields a straight line whose slope equals \( K_D \). If the amount of analyte increases either by injecting equal volumes of solutions whose concentrations are increasing or by injecting increasing volumes of a solution whose concentration is fixed, nonsymmetrical chromatographic peaks result. \( K_D \) is now dependent on the amount of solute, and either peak tailing or peak fronting results. This is the case of nonlinear elution chromatography. Gaussian or symmetrical peak shape is a chief objective when GC is used to perform trace quantitative analysis. The following equations relate the parameters discussed above:

\[
\begin{align*}
k' &= \frac{t_R - t_0}{t_0} \\
K_D &= \beta k'
\end{align*}
\]

where \( \beta \) is the volume (mobile phase)/volume (stationary phase), \( t_R \) is the retention time for a retained peak, and \( t_0 \) is the retention time for an unretained peak.

**EXPERIMENTAL**

Gas chromatograph interfaced to a PC that is loaded with chromatographic software. In our lab, an Autosystem (PerkinElmer) is interfaced to a PC workstation that utilizes Turbochrom (PE-Nelson) for data acquisition, processing, and readout.

**Preparation of Chemical Reagents**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

**Accessories to Be Used with the GC per Group**

1. Digital flow check meter.
2. GC syringe with a beveled end that includes a Chaney adapter. Do not confuse with the blunt-end syringe used for HPLC.
3. GC test mix for each of the studies discussed below. Refer to the summary at the end of this handout.

© 2006 by Taylor & Francis Group, LLC
Specific Laboratory Experiments

589

TABLE 5.1
Summary of Turbochrom Methods to Be Used in This Experiment

<table>
<thead>
<tr>
<th>Order</th>
<th>Turbo Method</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FLOWRATE</td>
<td>Near-ambient column temperature Measure flow rate, split ratio</td>
</tr>
<tr>
<td>2</td>
<td>DETSENS</td>
<td>Neat acetone — FID Neat methylene chloride — ECD</td>
</tr>
<tr>
<td>3</td>
<td>INJECID</td>
<td>Inject increasing amounts of 10 ppm 1,2,4-trichlorobenzene INJFID</td>
</tr>
<tr>
<td>4</td>
<td>PLATES</td>
<td>Temperature program: 265 (0.1) to 285 (10.0) at 6°/min; multicomponent OC mixture</td>
</tr>
<tr>
<td>5</td>
<td>TMAX</td>
<td>Isothermal at 285°C</td>
</tr>
<tr>
<td></td>
<td>TMIN</td>
<td>Isothermal at 200°C</td>
</tr>
</tbody>
</table>

Procedure

Refer to Table 5.1 for a definition of each of the Turbochrom methods created in support of this experiment. These previously created methods are illustrative of how chromatography-based software can be used to teach fundamental principles of GC.

Measurement and Adjustment of Carrier Gas Flow Rate and Split Ratio

As you approach the gas chromatograph, you will find it in an operational mode, with carrier gas flowing through both capillary columns. If not already set up, retrieve the Turbo file titled “FLOWRATE” and download this method. Your first task will be to measure the flow rate of the carrier gas through both capillary columns with the makeup gas off. After turning the makeup gas on, measure the split ratio through the capillary injector using the digital flow check meter. Record flow rate data in your lab notebook.

Once the optimum carrier flow rates have been established, the dual detector method titled “DETSENS” can be retrieved from the Turbochrom software, then transferred to the instrument via the interface (a process known as download) and the comparison of detector sensitivity can be undertaken. Ignite the FID (refer to the operator’s manual for the Autosystem GC from PerkinElmer for the specific procedure).

Comparison of the FID vs. the ECD Sensitivity

Allow time for the GC to equilibrate at the column temperature set in the method. Using the manual injection syringe (Hamilton), inject equal microliter aliquots of acetone into both injectors. Observe the appearance of a retained chromatographic peak found in both chromatograms. You cannot assume that \( t_R \) will be identical on both columns. Compare the peak heights from both chromatograms. Inject equal microliter aliquots into both injectors, as earlier, of the specific chlorinated hydrocarbon that is available. Record your observations and compare the peak heights as done previously. Each member of the group should have an opportunity to make
these sample injections so as to gain some experience with manual syringe injection of organic solvents.

**Injection Volume vs. GC Peak Shape**
Retrieve the Turbo file titled “INJEC” and download. Inject a series of increasing microliter aliquots of a reference solution labeled “10 ppm 1,2,4-trichlorobenzene” into the front capillary injection port. *Observe and record the changes in chromatographic peak shape as the amount of analyte is increased.* Retrieve the Turbo file titled “INJFID” and download. Repeat the series of injections as before using the reference solution labeled “hexadecane” and make these injections into the rear injector. *Observe and record the changes in chromatographic peak shape as the amount of analyte is increased.*

**Flow Rate vs. Capillary Column Efficiency**
A column’s efficiency is determined in a quantitative manner from the chromatogram by measuring the number of theoretical plates, \( N \). The effect of carrier flow rate on capillary column efficiency is significant in GC and will be examined under isothermal conditions (i.e., at a fixed and unchanging column temperature). Retrieve the Turbo file “PLATES” and program this method for a high flow rate by increasing the head pressure. Save this change in the method and download the method. Turn off the makeup gas and adjust the actual pressure so as to nearly match that which is set in the method and *measure* the flow rate. Turn the makeup back on. Inject 1 \( \mu \)L of the test mix and *observe* the chromatogram. Retrieve the method a second time and reprogram the head pressure to a much lower value. Turn off the makeup, decrease the carrier head pressure, *measure* the new flow rate, turn the makeup gas back on, and then make a second injection using the same volume.

For the carrier gas flow rate that exhibited the highest efficiency, *calculate* the number of theoretical plates using equations from your text. In addition, for the optimum carrier flow rate, choose any pair of peaks and calculate the resolution for that pair.

**Column Temperature vs. Capacity Factor**
Retrieve the Turbo file titled “TMIC” and download the method. Inject approximately 1 \( \mu \)L of the CIHCs test mix at this column temperature of 200°C. *Observe the degree of separation among organochlorine analytes and record your qualitative comments.*

Retrieve the Turbo file titled “TMAX” and download the method. Inject the same volume of CIHCs test mix and *observe* the chromatogram when the column temperature has been increased to 285°C.

**FOR THE LAB NOTEBOOK**
Review relevant sections of Chapter 4. Use these concepts to write a brief discussion on how your experimental observations connect to the theoretical relationships discussed in the text.
Address the following:
1. Explain why different GC detectors have different instrument detection limits.
2. If you operated a GC at significantly reduced carrier gas flow rates, predict what you would observe in a gas chromatogram for the injection of organic compounds. What would be the principal cause for these observations?
3. Explain why a symmetrical peak shape is important in gas chromatography.
4. What happens to \( K_p \) for a given organic compound when column temperature is varied?
5. How efficient is your GC column? That is, what is the number of theoretical plates? How many plates per meter do you have?
6. How is the phase ratio, \( \beta \), determined for capillary GC columns?

A COMPARISON OF SOIL TYPES VIA A QUANTITATIVE DETERMINATION OF THE CHROMIUM CONTENT USING VISIBLE SPECTROPHOTOMETRY AND FLAME ATOMIC ABSORPTION SPECTROSCOPY

BACKGROUND AND SUMMARY OF METHOD

Chromium exists in three oxidation states, of which Cr(III) and Cr(VI) are the most stable. Hexavalent Cr is classified as a known human carcinogen via inhalation, and Cr(III) is an essential dietary element for humans and other animals. Certain soils that exhibit a strong chemically reducing potential have been shown to convert Cr(VI) to Cr(III). It is possible for the analysis of soils from a hazardous waste site to reveal little to no Cr(VI) via the colorimetric method because this method is selective for Cr(VI) only.29,30 Because atomic absorption spectrophotometric methods yield total Cr, the difference between analytical results from both methods should be indicative of the Cr(III) content of a given soil type. Both methods will be implemented in this lab exercise and applied to one or more soil types.

This exercise affords students the opportunity to use several instrumental techniques to which they have previously been introduced in the laboratory in order to conduct a comparison of soil types with respect to determining the ratio of Cr(III) to Cr(VI). The exercise includes pH measurement, calibration of a UV-vis spectrophotometer, calibration of an atomic absorption spectrophotometer in the flame mode (FlAA), and sample preparation techniques.

Cr(VI) in its dichromate form, \( \text{Cr}_2\text{O}_7^{2-} \), reacts selectively with diphenyl carbazide, \( \text{C}_6\text{H}_5\text{NHNHCONHNHC}_6\text{H}_5 \), in acidic media to form a red–violet color of unknown composition. This selectivity for Cr occurs in the absence of interferences such as molybdenum, vanadium, and mercury. The colored complex has a very high molar absorptivity at 540 nm. This gives the method a very low detection limit (MDL) for Cr(VI) using a UV-vis spectrophotometer. Flame atomic absorption spectroscopy is also a very sensitive technique for determining total Cr, with instrument detection limits (IDLs) as low as 3 ppb.31
A modification to EPA Method 7196 has been published and will be implemented in this lab exercise. The method uses a hot alkaline solution (pH 12) to solubilize chromates that are to be found in soils obtained from hazardous waste sites. One portion of the aqueous sample would then be aspirated into the FIAA for a determination of total Cr, whereas diphenyl carbazide dissolved in acetone will be added to another portion, and the absorbance of the red–violet complex will be measured at 540 nm using a visible spectrometer. In this manner, both total Cr and Cr(VI) can be determined on the same sample. Thus, the ratio of the concentration of Cr(III) to the concentration of Cr(VI) in a soil sample can be calculated from the data generated in this experiment.

**EXPERIMENTAL**

**Chemical Reagents Needed per Student or Group**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

Potassium dichromate stock solution: Dissolve 141.4 mg of dried K₂Cr₂O₇ in distilled deionized water (DDI) and dilute to 1 L (1 mL = 50 µg of Cr).

Potassium dichromate standard solution: Dilute 10.00 mL of stock solution to 100 mL (1 mL = 5 µg of Cr).

Sulfuric acid, 10% (v/v): Dilute 10 mL of concentrated H₂SO₄ to 100 mL with DDI. Also, 1.8 M H₂SO₄ is needed. An aliquot of 10% H₂SO₄ could be used to prepare this solution.

Diphenyl carbazide (DPC) solution: Dissolve 250 mg of 1,5-diphenyl-carbazide in 50 mL of acetone. Store in an amber bottle. Discard when the solution becomes discolored.

Acetone, CH₃COCH₃: Use the highest purity available.

Alkaline digestion reagent, 0.28 M Na₂CO₃/0.5 M NaOH: Use your knowledge of chemical stoichiometry to calculate the amount of each base needed to prepare a solution of the molarity desired.

Concentrated nitric acid, HNO₃.

**Procedure for Alkaline Digestion**

1. Place 2.5 g of a given soil type into a 250-mL beaker. Add 50 mL of the alkaline digestion reagent. Stir at room temperature for at least 5 min and then heat on a hot plate to maintain the suspensions at 90 to 95°C, with constant stirring for about 1 h. Repeat for all other soil types to be studied whose Cr content is to be determined in this experiment. *Note:* Heating on a hot plate can cause bumping and lead to splatter, and thus loss of analyte.

2. Cool the digestates to room temperature, then filter through 0.45-µm cellulose or polycarbonate membrane filters.

3. Adjust the pH to 7.5 using concentrated HNO₃ and dilute with DDI to a final volume of 100 mL. You now have 100 mL of digestate.
Procedure for Conducting Visible Spectrophotometric Analysis

1. Prepare six working standards from careful dilutions of the 5 µg/mL Cr standard. The range of concentrations should be from 0 to 2 µg/mL Cr. You should prepare 100 mL of each standard.

2. Prepare an initial calibration verification (ICV) standard, which should have its concentration approximately near the mid-range of the calibration. You should prepare 100 mL of the ICV.

3. Prepare a matrix spike and a matrix spike duplicate. The amount of spike should double the concentration found in the original sample. The spike recovery must be between 85 and 115% in order to verify the method.

4. To 45 mL of DDI (this is the method blank), standard, ICV, and digestate, add 1 mL of DPC solution, followed by the addition of 1.8 M H₂SO₄ until the pH reaches approximately 2. This should be done in a 125-mL beaker with stirring and immersion of the glass electrode until the desired pH is attained. After cessation of effervescence, dilute the mixture with DDI to 50 mL. Allow the solution to stand from 5 to 10 min. If the solution appears turbid after the addition of DPC, filter through a 0.45-µm membrane. Store the remaining samples and standards in properly labeled bottles. Use if it is necessary to repeat this analysis.

5. Set the spectrophotometer at 540 nm; correctly set the 0 and 100% transmittance settings. Transfer an aliquot of the 50-mL sample to a cuvette. Measure the absorbance of all blanks, standards, and samples. Construct a table in your notebook to facilitate the entry of data.

Procedure for Atomic Absorption Spectrophotometric Analysis or ICP-AES

Refer to SW-846 Methods 7000A (“Atomic Absorption Methods”) and 7190 (“Chromium, Atomic Absorption, Direct Aspiration”) or Method 6010B (“Inductively Coupled Plasma–Atomic Emission Spectrometry”) (ICP-AES) for the quantitative determination of chromium at total Cr. In the lab, proceed to prepare calibration standards and ICVs and aspirate these into the flame using the Model 3110 (PerkinElmer) atomic absorption spectrophotometer or the Model 2000 (PerkinElmer) plasma ICP-AES. Use the remaining digestates from step 3 and determine total Cr. The FIAA may need to be set up from its present configuration. Refer to previous exercises and training manuals for the necessary information.

For the Report

Include all calibration data, ICVs, and sample unknowns for both instrumental methods. Perform a statistical evaluation in a manner that is similar to that in previous experiments. Use EXCEL or LSQUARES (refer to Appendix C) or other computer programs to conduct a least squares regression analysis of the calibration data. Calculate the accuracy (expressed as a percent relative error for the ICV) and the precision (relative standard deviation for the ICV) from both instrumental methods.
Calculate the percent recovery for the matrix spike and matrix spike duplicate. Report on the concentration of Cr in the unknown soil samples. Be aware of all dilution factors and concentrations as you perform calculations.

Find the ratio of the concentration of Cr(III) to that of Cr(VI) in each of the soil samples analyzed and present this value at the end of your report.

DETERMINATION OF LEAD IN DRINKING WATER USING GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROSCOPY (GFAA): EXTERNAL STANDARD VS. STANDARD ADDITION CALIBRATION MODE

BACKGROUND AND SUMMARY OF METHOD

Unfortunately, among the so-called heavy metals that have made their way into the environment, lead (Pb) is considered highly toxic and its presence must be identified and its concentration measured in air, water, and soil. Two principal historical uses in which Pb was contained were in coatings (e.g., house paint) and in gasoline (e.g., as alkyl lead compounds added to boost octane ratings). The extreme toxicity of Pb has required that instrumental analytical techniques that offer the lowest possible detection limits be used. Pb joins elements such as Hg, Cd, As, and Tl as requiring determinative techniques with the lowest instrument detection limits (IDLs) possible. It is important to recognize the difference between method detection limits (MDLs) and IDLs possible. The MDL incorporates the IDL and is equal to the IDL if and only if there is no sample preparation involved. Sample preparation is more common in trace organics analysis in contrast to trace metals analysis. For example, the IDL for Pb using flame AA is 0.1 ppm, whereas the IDL for Pb using graphite furnace atomic absorption spectroscopic (GFAA) methods is 1 ppb.33

When using the furnace technique, a representative aliquot of a sample is placed in the graphite tube in the furnace, evaporated to dryness, charred, and atomized. A greater percentage of available analyte atoms is vaporized and dissociated for absorption in the tube in contrast to the flame. It becomes possible to use smaller sample volumes. Radiation from a given excited element is passed through the vapor containing ground-state atoms of that element. The intensity of the transmitted radiation decreases in proportion to the amount of the ground-state element in the vapor. The metal atoms to be measured are placed in the beam of radiation, which is nearly monochromatic (from a hollow-cathode tube), by increasing the temperature of the furnace. A monochromator isolates the wavelength of the transmitted radiation and a photosensitive device measures the attenuated intensity. Beer’s law of spectrophotometry applies, as was the case for UV-vis absorption spectrophotometry, and the concentration of the specific element is determined by various modes of analyte calibration in a manner similar to that for UV absorption spectrophotometry. A schematic of an electrothermal atomizer follows:
The tube is usually coated with pyrolytic graphite, which is made by heating the tube in a methane atmosphere. Pyrolytic graphite exhibits a low gas permeability and good resistance to chemical attack, and this lengthens the lifetime (i.e., the number of successful firings) of the tube. There is, however, a finite lifetime for each tube in GFAA.

It is generally believed that the atomization mechanism for Pb involves reduction of the solid oxide on the graphite surface according to

\[ \text{MO}_{(s)} + \text{C}_{(s)} \rightarrow \text{M}_{(g)} \rightarrow \text{M}_{(g)} + 0.5\text{M}_{2(g)} \]

Most commercial electrothermal atomizers based on the L’vov furnace, as simplified by Massman, undergo vigorous changes in tube temperature. The analyte atoms volatilized from the tube wall come into a cooler gas, so that molecular species that are not detected are formed. This leads to what is termed matrix interferences. For example, equal concentrations of Pb in a matrix of deionized water vs. one of high chloride content would yield different absorbances. One way to remove this chloride matrix interference is to use a matrix modifier. The addition of an ammonium salt to the chloride-containing matrix would cause volatilization of NH₄Cl with removal of chloride from the sample matrix.

Each end of the furnace tube is connected to a high-current, programmable power supply through water-cooled contacts. The power supply controlling the furnace can be programmed to dry, ash, and atomize the sample at the appropriate temperatures. The temperature and duration of each of these steps can be controlled over a wide range. The optimization of the operating conditions is very important in developing methods using GFAA. A description of the three major temperature program changes, known as steps, is important in understanding GFAA operation. In the first step (drying), the solvent is evaporated at a temperature just above its boiling point. For aqueous solutions, the temperature is held at 110°C for about 30 sec. In the second step (ashing), the temperature is raised to remove organic matter and as many volatile components from the sample matrix as possible without analyte loss. The ashing temperature varies from 350 to 1200°C and is maintained for about 45 sec. The last step (atomization) occurs between 2000 and 3000°C and
lasts for just a few seconds. The element of interest is atomized and the absorption of the source radiation by the atomic vapor is measured. The furnace is then cleaned by heating the atomizer to the maximum temperature for a short period. Finally, the temperature is decreased to room temperature using water coolant and inert gas flow (argon). This process is depicted graphically below. Note that the graph includes the overall transmitted intensity $I_t$, the furnace temperature $T$, and the net absorption after background correction. It becomes important to have a means to subtract out this background. The PerkinElmer Model 3110 AA uses a deuterium background correction technique.36

Of the three modes of calibration used in instrumental analysis, external, internal, and standard addition, the latter provides for the most accurate analytical results for samples that exhibit a matrix interference. The external mode of calibration is used to convert instrumental response to concentration when matrix interferences are not considered a factor. A series of standard solutions that contain the metal of interest are prepared from precise dilution of a certified standard stock solution. The calibration curve is obtained and a least squares regression is performed on the $x$, $y$ data points. The best-fit line is used to establish the calibration curve. Samples that contain the metal at an unknown concentration level in a sample matrix nearly identical to that used to prepare the serial standards can be run and the data interpolated to give the concentration. In contrast, the standard addition mode of calibration requires that calibration and analysis be performed on the sample itself. Standard addition can be used provided that (1) a linear relationship exists between the physical parameter measured and the concentration of analyte, (2) the sensitivity of the method is not changed by the additions, and (3) the method blank can be corrected for. A typical standard addition calibration follows:
This exercise affords students an opportunity to operate a PerkinElmer Model 3110 GFAA with the objective of determining the concentration of Pb at ppb levels by calibrating the instrument using two of the three modes: external standard and standard addition. Following establishment of the working calibration curve, an instrument calibration verification (ICV) standard containing Pb will be prepared, run in triplicate, and the precision determined. At least one unknown sample will be provided for students to determine the concentration of Pb. Students are asked to bring in a sample of drinking water to determine the concentration of Pb once the GFAA has been successfully calibrated and the precision and accuracy for the ICV have been found to be acceptable.

**Experimental**

**Preparation of Chemical Reagents**

*Note: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.*

**Reagents Needed per Student or Group**

1. 40 ppb reference standard containing Pb in 1% spectrograde nitric acid.
2. Blank reference standard in 1% spectrograde nitric acid.
4. Unknown sample containing Pb. Be sure to record the code.
5. Drinking water from any source.
Procedure

The setup and operation of the GFAA involves the following sequence of activities:

1. Install and align the hollow-cathode lamp (HCL).
2. Align the furnace in the spectrophotometer.
3. Install and condition a new tube.
4. Set up an element parameter file within the WinLab software.
5. Align the autosampler.
6. Place blanks, modifiers, and the standard in appropriate locations in the autosampler carousel.
7. Run the calibration according to the programmable sequence within the WinLab software.
8. Run any and all samples, provided that the calibration and ICV are acceptable.

Using the WinLab® Software

You should find the WinLab (PerkinElmer) software used to control and acquire GFAA data already downloaded. Retrieve and enter the software via the keyboard. The Pb HCL should turn on as well. A stable signal is necessary in order to continue. Proceed to autozero the detector, and if not satisfied, go to the “realign lamps” screen and adjust the physical position of the Pb HCL so as to bring the energy throughput near to that for the deuterium lamp. Set up a sequence to run a lab blank, a series of calibration standards for Pb, and one or more ICVs.

Evaluate the quality of the calibration and, if satisfactory, run the ICV under the “run samples.” Retrieve the weight/ID screen and choose an autosampler location and run in triplicate. If precision is acceptable, set up the weight/ID screen with the fortified Pb sample and any other samples of interest.

Preparation of the Stock Reference Pb Standard and Start of the Autosampler

To prepare the calibration standards for Pb, obtain the certified Pb stock standard, which should be 1000 ppm as Pb. Using an appropriate liquid syringe, take 40 µL of the 1000 ppm Pb stock and place this aliquot into a 10-mL volumetric flask already half filled with 1% spectrograde nitric acid. Adjust to final volume using the 1% acid. Transfer this solution to a storage container and label “4 ppm Pb.” Take 100 µL of the 4 ppm Pb and place this aliquot into a clean 10-mL volumetric flask. Adjust to final volume and transfer to a clean storage container and label “40 ppb Pb.” Transfer about a 1-mL aliquot of the 40 ppb Pb reference standard to a plastic GFAA autosampler vial and place in location 38. Place a clean vial filled with 1% nitric in location 36. Place a clean vial filled with matrix modifier in location 37. The programming for the autosampler is found under “element parameters\calib”; retrieve this and the entries into the table in your lab notebook.
Using the 40 ppb Pb standard and the WinLab software, implement an external mode of calibration. Retrieve or create a method title for this and conduct the calibration and quantitation. Evaluate whether the calibration is free from systematic error. If so, inject the ICV in triplicate. Record the code for the unknown and inject in triplicate. Run one or more drinking water samples.

Proceed on to the implementation of the standard addition mode of calibration using the 40 ppb Pb standard and the WinLab software. Retrieve the method and conduct the calibration and quantitation. Evaluate whether the calibration is free from systematic error. Inject the ICV in triplicate. Record the code for the unknown and inject in triplicate. Run one or more drinking water samples. Rerun one or more samples, provided the calibration is linear and the precision and accuracy for the ICV are acceptable.

FOR THE NOTEBOOK

Include in your notebook data from both modes of calibration for each of the two metals; calculate the confidence limits at 95% probability using the Student’s t statistics for the ICV for both calibration modes. Report on the concentration of Pb in the coded unknown provided to you. Report on the Pb concentration of any unknown drinking water sample that you analyzed. Two statistical computer programs, RSD and LSQUARES, written in BASIC, are available for your use on the laboratory PCs. These programs are found in Appendix C. Proceed to calculate the means, standard deviations, relative standard deviations, and confidence intervals at a given probability. Calculate the relative error between the mean result for the ICV and the expected result for both types of calibration modes (i.e., external standard and standard addition). Comment on the effect of the external vs. standard addition mode of GFAA calibration on the precision and accuracy in the ultratrace determination of Pb in drinking water.

Discuss why background correction is necessary in AA. Distinguish between physical and chemical interferences in AA. Explain what is meant by the term Smith–Hieftje background correction and discuss how it differs from the kind of correction employed in the PerkinElmer Model 3110 atomic absorption spectrophotometer.

DETERMINATION OF THE DEGREE OF HARDNESS IN VARIOUS SOURCES OF GROUNDWATER USING FLAME ATOMIC ABSORPTION SPECTROSCOPY

BACKGROUND AND SUMMARY OF METHOD

The extent to which groundwater has been rendered hard has been defined as the concentration of dissolved bicarbonates containing calcium and magnesium ions present in the sample. Hardness can be quantitatively measured by finding some way to measure these two alkaline–earth metal ions. The classical method that still finds widespread use is titration with EDTA. We are going to approach the problem
by measuring the concentration of Ca$^{2+}$ by flame atomic absorption spectroscopy (FAAS) and by a mere change of hollow-cathode lamps, by measuring the concentration of Mg$^{2+}$. Several sources of groundwater will be obtained, and the Ca/Mg content will be used to estimate the degree of water hardness.

Recall that an analytical method’s precision is a measure of the degree to which it can be reproduced or repeated and is evaluated by calculating the standard deviation for the method’s instrument calibration verification (ICV) standard following establishment of a single-point or multipoint calibration. A method’s accuracy is a measure of how close the results are to an established or authoritative value and is evaluated by calculating the percent relative error.

Flame atomic absorption spectrometry requires a means by which an aqueous solution containing metal ions can be aspirated into a reducing flame environment by which atomic Mg or Ca vapor is formed. Photons from the characteristic Mg emission of a hollow-cathode lamp (HCL) are absorbed by ground-state Mg atoms present in the approximately 2300°C air-acetylene flame. The amount of radiant energy absorbed as a function of concentration of an element in the flame is the basis of AA and follows Beer’s law. In contrast to molecular absorption in solution, atomic spectra consist of lines and originate from either atomic absorption or atomic emission processes, which are depicted schematically below (Sawyer et al.,38 chap. 9):

Instrument detection limits (IDLs) for most metals by FAAS are in the low-ppm realm in contrast to graphite furnace AA (GFAA). The conventional premixed chamber type nebulizer burner is common. The sample is drawn up through the capillary by the decreased pressure created by the expanding oxidant gas at the end of the capillary, and a spray of fine droplets is formed. The droplets are turbulently mixed with additional oxidant and fuel and pass into the burner head and the flame. Large droplets deposit and pass down the drain; 85 to 90% of the sample is discarded in this way. Figures 10 to 15 in Skoog and Leary39 (pp. 216–218) provide a good schematic of the laminar flow burner.

Flame atomic absorption spectrometry was developed in 1955 independently by Walsh in Australia40 and by Alkemade and Milatz41 in the Netherlands. Because
electrons in quantized energy states for atoms of alkali metal elements can easily be raised to excited states (see above), flame emission spectroscopy is a more appropriate instrumental technique, whereas plasma sources are needed for atoms of most other elements. Atomic absorption spectroscopy is unique in that it uses a flame to create the atomic vapor within which the absorption of radiation from a HCL can occur. This enables the quantitative determination of some 65 elements (Sawyer et al.,38 p. 245), provided a line source can be used. The source of light for AA must produce a narrow band of adequate intensity and stability for prolonged periods. An ordinary monochromator is incapable of yielding a band of radiation as narrow as the peak width of an atomic absorption line. Hollow-cathode lamps satisfy these criteria (Skoog and Leary,39 pp. 211–214). Refer to the following schematic:

Many models of atomic absorption spectrophotometers are in use in environmental testing laboratories today. Because of this, the type of readouts that one may get might differ. Older instruments most often used percent absorption, whereas more contemporary instruments might read out in absorbance or percent transmittance. The following schematic relates all three types of AA readouts (Sawyer et al.,38 p. 247):
Trace Environmental Quantitative Analysis, Second Edition

The exercise introduces students to trace metals analysis for determining the concentration of Ca and Mg using FlAA techniques. The PerkinElmer Model 3110 AA needs to be set up in the flame mode of operation. The FlAA will be calibrated and the calibration verified. Various sources of groundwater need to be obtained.

**EXPERIMENTAL**

**Preparation of Chemical Reagents**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

**Chemicals/Reagents Needed per Student or Group**

- 5 mL 1% HNO₃ (use spectrograde nitric acid only). If unavailable, prepare by placing 3.6 mL of concentrated HNO₃ into a 250-mL volumetric flask previously half filled with distilled deionized (DDI) water, adjust to the calibration mark with DDI, transfer to a plastic storage bottle, and label “1% HNO₃, spectrograde.”
- 5 mL 1000 ppm Mg (certified stock solution).
- 5 mL 1000 ppm Ca (certified stock solution).

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>% Transmittance</th>
<th>% Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0.045</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>0.097</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>0.155</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>0.229</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>0.301</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>0.398</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>0.523</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>0.699</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>1.00</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>∞</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

The table and diagram illustrate the relationship between absorbance, transmittance, and concentration.
Specific Laboratory Experiments 603

FlAA Operating Analytical Requirement

<table>
<thead>
<tr>
<th>Specification</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum range of concentration (minimum)</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Optimum range of concentration (maximum)</td>
<td>7.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>422.7</td>
<td>285.2</td>
</tr>
<tr>
<td>Sensitivity (ppm)</td>
<td>0.08</td>
<td>0.007</td>
</tr>
<tr>
<td>Instrument detection limit (IDL) (ppm)</td>
<td>0.01</td>
<td>0.001</td>
</tr>
</tbody>
</table>

If a wavelength of 202 nm is used for Mg, a wider linear dynamic range is available (i.e., from 0 to 10 ppm). The sensitivity and IDL will be different.

Preparation of the Calibration Curve

As an illustration only, working calibration standards could be prepared as suggested below (this scheme assumes that the 1000 ppm certified stock reference standard solution has been carefully diluted to 3 ppm).

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>3 ppm (mL)</th>
<th>Final Volume (mL)*</th>
<th>Concentration of Mg (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4.15</td>
<td>50</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>50</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>50</td>
<td>1.5</td>
</tr>
<tr>
<td>ICV</td>
<td>10</td>
<td>50</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Use 1% HNO₃ for all dilutions

Procedure

Detailed instructions on how to set up and operate an FlAA using the Model 3110 AA will be made available in the laboratory. The burner–nebulizer attachment will need to be installed and the energy throughput optimized using either the Ca or Mg HCL. Proceed to aspirate the working calibration standards. An instrument calibration verification (ICV) standard should be prepared whose concentration should be approximately between the low and high standards. It should be sufficiently aspirated so that triplicate determinations of the absorbance from the same ICV solution can be made. If the precision and accuracy for the calibration and ICV are found acceptable, then any and all available unknowns can be run. A fortified sample containing both Ca and Mg should be available. This sample may have to be diluted if the absorbance is found to significantly exceed the linear dynamic range of the instrument. Be sure to record the name or the correct code for each unknown sample. Establish the IDL experimentally for your instrument for each of the two metals.
This is accomplished by obtaining seven replicate absorbance measurements of your blank standard.

**FOR THE LAB NOTEBOOK (NO REPORT NECESSARY)**

Conduct a determination of the IDL for each metal by measuring the signal due to a blank that is repeatedly aspirated into the flame seven times. Apply the principles discussed in Reference 39 (pp. 7–8) and calculate the IDL assuming \( k = 3 \). Establish a least squares regression fit to the experimental calibration data points for both metals. Calculate the standard deviation in both the slope and \( y \) intercept for the calibration curve for both metals. Calculate the standard deviation in the measurement of the ICV for triplicate absorbance measurements. Use equations presented in Chapter 2 or obtain via a computer program. LSQUARES, a routine written in GWBASIC, may be available on the laboratory PCs for your use. Calculate the confidence limits for the ICV for each metal. Calculate a percent error in the ICV for each metal. Report the concentrations for both Ca and Mg in the fortified sample and for one or more environmental samples. Comment on the differences in IDLs for Ca and Mg using FlAA.

---

**DETERMINATION OF OIL AND GREASE AND OF TOTAL PETROLEUM HYDROCARBONS IN WASTEWATER VIA REVERSED-PHASE SOLID-PHASE EXTRACTION TECHNIQUES (RP-SPE) AND QUANTITATIVE FOURIER-TRANSFORM INFRARED (FTIR) SPECTROSCOPY**

**BACKGROUND AND SUMMARY OF METHOD**

One of the most informative and straightforward analytical methods involves the quantitative analysis of environmental samples that have been contaminated with what can be collectively termed oil and grease. The term *oil and grease* refers to any and all hydrocarbons and includes lipids, high-molecular-weight fatty acids, triglycerides, higher olefinic hydrocarbons, alkanes, alkenes, monocyclic and polycyclic aromatics, and so forth. The total petroleum hydrocarbon (TPH) content of this oil and grease contamination can also be quantitatively determined by this method if the extracted sample is placed in contact with silica gel. The software that will be used with the FTIR spectrophotometer distinguishes between these two definitions. The absorption in the infrared region of the electromagnetic spectrum due to the presence of aliphatic and aromatic carbon to hydrogen-stretching vibrations occurs in the 3100 to 2900 cm\(^{-1}\) range. These concepts form the physical-chemical basis for the instrumental measurement.

The method is adapted from Method 5520, “Oil and Grease,” in *Standard Methods for the Examination of Water and Wastewater*\(^{44}\) and follows from EPA Method 413.2.\(^{45}\) A recent revision of the procedures has appeared as EPA Method 1664 and calls for a replacement of the infrared absorption measurement by a gravimetric technique following distillation.\(^{46}\) The FTIR technique will be used here, and we will responsibly recover the solvent whose waste disposal is the subject of
current environmental concern and debate. Software developed at PerkinElmer Corporation during the late 1980s has been downloaded to the Model 1600 FTIR spectrophotometer and will be used to provide for the quantitative analysis. The original concept was first published by Gruenfeld at the EPA in the early 1970s.

An aqueous sample whose oil and grease content is to be determined is first acidified, then extracted using 1,1,2-trichloro-1,2,2-trifluoroethane (TCTFE). Molecules of this solvent lack a C–H covalent bond, and thus serve to provide an excellent background infrared spectrum because no absorption in the 3100 to 2900 cm\(^{-1}\) region is found. The extract is isolated from the aqueous matrix, and an aliquot (a portion thereof) is transferred to a 1-cm (path length) quartz cuvette. The FTIR absorbance vs. wavelength or wave number is graphically displayed on the screen. This absorbance, which is initially related to the concentration of oil and grease in standards, yields the concentration as oil and grease in the unknown sample via interpolation of the least squares regression fit. The common concentration unit used is mg of oil and grease/100 mL of solution (oil and grease dissolved in TCTFE).

A soil/sediment or contaminated sludge with a very high solids content can be extracted via Soxhlet extraction techniques or, more conveniently, via ultrasonic probe sonication into a mixture of TCTFE and a carefully weighed amount of soil/sediment/sludge.

**EXPERIMENTAL**

**Preparation of Chemical Reagents**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

**Reagents Needed per Student or Group of Students**

- 0.1 g of hexadecane, \(C_{16}H_{34}\). Alternatively, dodecane, \(C_{12}H_{26}\), can be substituted.
- 0.1 g of iso-octane (2,2,4-trimethyl pentane), \((CH_3)3CCH_2CH(CH_3)2\).
- 0.1 g of benzene, \(C_6H_6\).
- 250 mL of TCTFE.
- 100 mL of MeOH (methanol). Use as high a purity as is available.
- 5 g of silica gel, needed only if TPHs are to be determined in addition to oil and grease.
- 5 g of sodium sulfate anhydrous (\(Na_2CO_3\)).
- 20 mL of 1:1 hydrochloric acid.

Mix equal volumes of acid and deionized water. Remember, always add acid to water.

**Apparatus Needed per Group**

- Vacuum manifold to conduct solid-phase extraction (SPE)
- Water trap and associated vacuum tubing to be used with the Vacmaster
Suction vacuum pump connected to the water trap
Accessories for use of the SPE vacuum manifold, including a receiving rack
SPE cartridges packed with C\textsubscript{18}-bonded silica
70-mL sample reservoirs
10-mL glass volumetric flasks

**Procedure**

Refer to the oil and grease analysis method of Reference 44 and implement the appropriate procedure. For wastewater samples, follow the procedure outlined below. If a percent recovery study is required, the procedure that immediately follows provides guidance for this study.

**Percent Recovery Study**

To isolate and recover TPHs from water by RP-SPE combined with quantitative FTIR:

- Spike approximately 200 mL of high-purity laboratory water that has been *acidified with 1:1 HCl* with approximately 2 mg of dodecane; note that most of the dodecane will float on top. This sample is called a *matrix spike*, using terms first developed at the EPA. Spike a second 200-mL portion of *acidified* water with approximately 2 mg of dodecane. This sample is called a *matrix spike duplicate*. Leave a third 200-mL portion of water unspiked and *acidify*. This sample is called a *method blank*.
- Set up three C\textsubscript{18} SPE cartridges and condition with MeOH.
- Pass the method blank, matrix spike, and matrix spike duplicate samples through the cartridges under vacuum.
- Remove water droplets with a Kim-Wipe; apply more vacuum to remove water from the sorbent.
- Elute off the cartridge with two 500-µL portions of TCTFE into a 1-mL volumetric as receiver; use glass syringe that is available near the SPE manifold.
- Transfer contents of the 1-mL receiver to the quartz cuvette and add exactly 1.0 mL of TCTFE using a glass pipette.
- Call previous calibration standards from disk on Model 1600 FTIR.
- Run each of the eluents from SPE. Transfer each eluent to a quartz cuvette and measure the absorbance. For the sample ID, enter any number. For the initial mass of sample, enter “200,” and for the volume of sample after extraction, enter “2.”

For the preparation of a control (i.e., a 100% recovered sample), weigh approximately 2 mg of dodecane into a 1-mL volumetric flask half filled with TCTFE and adjust to the mark with TCTFE. Transfer to a quartz cuvette, add 1 mL of TCTFE, and measure the absorbance using the Model 1600 FTIR.
Probe Sonication: Liquid–Solid Extraction

It is first necessary to estimate to what extent the solid sample is laden with oil and grease. This minimizes the necessity to dilute the extract so that the absorbance will remain on scale. This can be accomplished by taking 0.5-, 5-, and 15-g samples and using identical extraction volumes. Once the optimum sample weight has been estimated, proceed to the next step.

Calibration of the FTIR Spectrophotometer

Calibrate the Model 1600 FTIR (PerkinElmer) or equivalent instrument by first preparing a series of working calibration standards. The table below serves as a useful guide and yields concentrations that are compatible with the software that operates the instrument.

Prepare Blend A by obtaining a total weight of approximately 0.10 g for the pure form of the oil that is to be defined as the reference. For example, if hexadecane, iso-octane, and benzene are to be used and mixed, add approximately 0.033 g of each to obtain the desired weight. Transfer the oil to a clean, dry 10-mL volumetric flask. Add about 5 mL of TCTFE to dissolve the oil, then adjust to the calibration mark. Transfer to a clean, dry glass vial with a Teflon/silicone septum and screw cap. Label this solution “Blend A, 1000 mg Oil and Grease/100 mL (in TCTFE)”; prepare Blends B through F according to the following table:

<table>
<thead>
<tr>
<th>Blend</th>
<th>Blend A (mL)</th>
<th>Extract Volume (mL)²</th>
<th>Concentration (mg/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.01</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td>E</td>
<td>0.1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>0.2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>0.3</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>0.4</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>ICVᵇ</td>
<td>0.25</td>
<td>10</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

² Use 10-mL glass volumetric flasks and TCTFE to adjust to final volume.

ᵇ ICV = instrument calibration verification standard; run as if it were a sample; enter a “1” for sample weight and “1” for extract volume.

When you are ready to perform the calibration, retrieve under method “og & ph” (oil, grease, and petroleum hydrocarbons) and exercise one of the six options. It is important to obtain a fresh background by placing TCTFE into a clean 1-cm quartz cuvette. If the blank reveals a large absorbance, the quartz cuvettes must be cleaned with detergent. Contamination of the surface of the quartz cuvettes represents a major source of error with this method.
Isolation of Oil and Grease from Wastewater Samples

- Place approximately 200 mL of wastewater sample into a clean, dry 250-mL beaker using a graduated cylinder. Record the volume of sample in your lab notebook.
- Acidify to a pH of approximately 2 by adding sufficient 1:1 HCl.
- Set up the SPE vacuum manifold and condition the sorbent with MeOH; the sorbent surface should be wet with MeOH prior to passing the wastewater sample through the cartridge.
- Connect the 70-mL polyethylene sample reservoir to the cartridge via the adapter.
- Pass 200 mL of sample through under vacuum; observe that the sample actually flows through the SPE sorbent and watch for plugging.
- Remove the reservoir; remove the water droplets on the inner wall of the cartridge barrel.
- Elute with two 500-µL of TCTFE directly into a 1.0-mL volumetric flask.
- Add enough anhydrous sodium sulfate, if necessary, to remove residual water in the eluent and stir.
- Transfer the contents of the volumetric to the quartz cuvette, add, with a pipette, 1 mL of additional TCFFE, and measure the absorbance using the Model 1600 FTIR.
- The printer will give you a hard-copy output after a certain number of FTIR scans have been acquired. Record all absorbance data in your laboratory notebook. Transfer all waste TCTFE to a properly labeled waste receptacle for recovery purposes.
- If the absorbance is too large, make the appropriate dilution. Repeat the dilution step if necessary. Transfer all waste TCTFE to a properly labeled waste receptacle for recovery purposes.
- Prior to each day’s FTIR measurements, the ICV standard should be measured and a log kept of its daily interpolated concentration. If the ICV value becomes a statistical outlier, rerun the calibration.

Calculations

The printout for a correctly measured TCTFE extract gives a direct value for the concentration of oil and grease in a solid sample, provided the weight of sample is entered in grams and the volume of extract is entered in milliliters. Because we are measuring sample volume, instead of weight, substitute for the weight with 200 mL and use an extract volume of 2 mL. The calculation is based on the following:

\[
\frac{(2 \text{ mL})(\text{mg oil and grease/100 mL})(\text{DF})}{200 \text{ mL sample}} \times \frac{1000 \text{ mL}}{1 \text{ L}} = \frac{\text{mg}}{\text{L}}
\]

where DF is the dilution factor. If no dilution, assume DF = 1. If a 1:25 dilution is made, DF would equal 25.
COMPARISON OF ULTRAVIOLET AND INFRARED ABSORPTION SPECTRA OF CHEMICALLY SIMILAR ORGANIC COMPOUNDS

BACKGROUND AND SUMMARY OF METHOD

Because most organic compounds known to pollute the environment are colorless, it would be close to impossible to identify them and therefore to quantitate if only a visible spectrophotometer were available. Do not despair. The ultraviolet (UV) spectrophotometer enables the full UV region to be used, and these organic contaminants can be identified. The UV region is defined to be those wavelengths from 190 to 400 nm. We know that the internal energies of atoms and molecules are quantized; that is, only certain discrete energy levels are possible, and the atoms and molecules must exist at all times in one or the other of these allowed energy states. For absorption of radiation to occur, a fundamental requirement is that the energy of the photon absorbed must match exactly the energy difference between initial and final energy states within the atom or molecule. Consideration of atoms falls within the realm of atomic absorption and atomic emission, whereas molecular absorption involves transitions between electronic (ultraviolet-visible absorption), vibrational (mid-infrared absorption), and rotational (microwave absorption). The following diagram depicts quantized energy states for organic molecules that are dissolved in a solvent:49

The molecular absorption phenomenon can only be accurately measured provided that the ratio of transmitted intensity, \( I \), of the UV radiation to that of the incident intensity, \( I_0 \), is due to the presence of the dissolved solute and not to
scattering of the incident beam. If the optical windows (see below) absorb UV radiation, then the absorbance that is related to the logarithm of the ratio $I/I_0$ would cause an increase in sample absorbance; hence, this would lead to an erroneous result. The student will encounter two types of optical window material. One consists of glass and is said to have a UV cutoff (UV wavelengths below this would absorb) of 300 nm (near UV), and the other consists of quartz with a UV cutoff of 190 nm. The rectangular cuvette is depicted as follows:

UV-Vis Absorption Spectroscopy

The light source for molecular absorption spectroscopy in the visible and UV regions (i.e., 750 nm down to 350 nm) is a tungsten filament lamp, which radiates as a black body at about 2800 K. Below 350 nm, the hydrogen or deuterium gas discharge lamp is preferred. The reciprocal linear dispersion (refer to Reference 51 or other equivalent text for explanation) is around 1 nm/mm for diffraction grating spectrophotometers and between 0.5 and 5 nm/mm for prism spectrophotometers. (Note: Dispersion depends on wavelength for prism.) Air absorbs UV light of wavelengths shorter than about 180 nm, so studies at wavelengths shorter than 180 nm require the use of an evacuated spectrometer. This region is thus termed the vacuum ultraviolet.50

The role of the solvent becomes critical in obtaining accurate UV absorption spectra. A solvent considered suitable for use in the UV-vis region must itself exhibit a low absorbance as well as dissolve the solute whose spectrum is sought experimentally. Fortunately, most common solvents are highly transparent to visible light, but all begin to absorb at some wavelength in the UV. It is not essential that the solvent have 100% transmittance, although it is desirable that as large a fraction as possible of the incident radiant energy be available for absorption by the solute. The following table lists the approximate absorption cutoffs for several widely used solvents. The cutoff defines a practical short-wavelength limit for the useful range of a solvent. Below this wavelength, the absorbance of the solvent, if placed in a 1-cm cell, exceeds 1.0 absorbance unit full scale (AUFS).
Different solutes exhibit different UV absorptivities. Recall that what is measured in a spectrophotometer is absorbance, and absorbance $A$ is related to absorptivity $\varepsilon$ via

$$A = \varepsilon bc$$

Two chemically different solutions that contain solutes at identical concentrations in a suitable solvent using the same cuvette would not be expected to have the same absorbance due to differences in absorptivity (earlier terms included molar absorptivity and extinction coefficient). If logarithms are taken on both sides of the above equation, we have

$$\log A = \log \varepsilon + \log bc$$

Because the $\log bc$ term is independent of wavelength, $\log A$ will vary only as a function of absorptivity. Thus, a plot of $\log A$ vs. spectrophotometer wavelength setting will be the same even though concentrations and path lengths for individual samples may differ. In this way, a comparison of the different curves can be made.

**Mid-Infrared Absorption Spectroscopy**

The mid-infrared region of the electromagnetic spectrum starts on the higher energy at 2.5 $\mu$m (4000 cm$^{-1}$) and ends on the lower energy at 6 $\mu$m (650 cm$^{-1}$). Dispersive type infrared absorption spectrometers have given way to Fourier-transform infrared (FTIR) spectrometers. Chapter 12 of Reference 51, or other equivalent text, should be reviewed to better understand the principles underlying infrared spectroscopy.

**EXPERIMENTAL**

This exercise affords students an opportunity to measure actual UV and FTIR absorption spectra of several organic solutes while comparing overall differences in UV and FTIR spectra for chemically similar and dissimilar organic solutes. The laboratory experiment focuses on the influence of delocalization of electron density.
and the nature of UV absorption spectra. The experiment also focuses on the relationship of organic functional group analysis and FTIR absorption spectra. A review of an appropriate text that reviews the theory of UV-vis and FTIR absorption spectroscopy and molecular structure gives an appreciation of what will be observed in the laboratory.

Two sets of illustrative solutions are to be studied in this exercise. The first set consists of organosulfonates and enables a comparison of UV absorption spectra of alkane vs. aromatic sulfonates that are dissolved in water. The second set consists of two representative organic esters that differ in their carbon backbone. The student is to engage both the UV spectrophotometer and the FTIR spectrometer in accomplishing the experimental aspects of this exercise.

**Items/Accessories Needed per Student or Group**

1. Pair of matching rectangular quartz cuvettes that transmit between 200 and 350 nm
2. Polyethylene (PE) disposable IR card (Type 61, 3M)
3. Polytetrafluoroethylene (PTFE) disposable IR card (Type 62, 3M)
4. Model 160-UV (Shimadzu) UV-vis scanning absorption spectrophotometer or other equivalent scanning instrument
5. Model 1600 Fourier-transform infrared spectrometer (PerkinElmer) or other equivalent instrument

**Preparation of Chemical Reagents**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

**Procedure to Obtain UV Absorption Spectra for Two Sets of Chemically Similar Organic Compounds: (1) An Alkane Sulfonate vs. Alkyl Sulfate and (2) Two Esters with Different Carbon Backbones**

Prepare two aqueous solutions that contain approximately 1000 ppm each of 1-octanesulfonic acid or sodium dodecyl sulfate (use whichever is available) and p-toluene sulfonic acid dissolved in distilled deionized water (DDI). Transfer a portion of this solution into a quartz rectangular cuvette and record the UV absorption spectrum for both organic compounds. Prepare a 100 ppm solution that contains methyl methacrylate and a 100 ppm solution that contains ethyl glycolate. You will need to use the wavelength cutoff guide to choose a suitable solvent because these organic compounds do not dissolve to any great extent in water. Record the UV absorption spectrum between 200 and 350 nm. Compare the spectra when a solvent is placed in the reference beam of the dual-beam instrument.
**Procedure to Obtain FTIR Absorption (Transmission) Spectra for Various Organic Compounds**

Disks that are partially to fully transparent in the infrared are available to easily prepare samples. An aqueous solution containing the anionic surfactants can be deposited directly onto either the PTFE or PE disk. Allow sufficient time for the solvent to evaporate off of the disk. Conduct a survey scan and take 16 FTIR scans. Plot the spectra. Consult the staff for assistance with the Model 1600 FTIR (PerkinElmer). FTIR absorption (transmission) spectra for both sulfonate surfactants and for both esters can be obtained using this technique.52

**FOR THE REPORT**

This exercise has introduced selected principles of molecular UV and infrared spectroscopy for qualitative chemical analysis. Interpret the significance of these spectra in terms of molecular structure. You should have obtained hard-copy printouts of UV and FTIR spectra. Please include all relevant spectra in your report.

Suppose that a client sent to you a mixture that was prepared from the ethyl benzene and styrene solutions that you used in the lab. Would benzene be a suitable solvent to use in obtaining UV spectra for ethyl benzene and styrene? Discuss how you would design a quantitative analysis to determine the concentration of ethyl benzene and of styrene in the client’s sample. Assume that you have available pure ethyl benzene and pure styrene (chemist’s refer to these as neat forms of the liquids).

**DETERMINATION OF ANIONIC SURFACTANTS BY MINI-LIQUID–LIQUID EXTRACTION (mini-LLE) IN AN INDUSTRIAL WASTEWATER EFFLUENT USING ION PAIRING WITH METHYLENE BLUE**

**BACKGROUND AND SUMMARY OF METHOD**

Synthetic detergent formulations make their way into the environment via industrial waste effluent. It is important that levels of these surfactants or surface-active substances be monitored. The classical analytical method that utilizes a visible spectrophotometer involves a consideration of the chemical nature of the surfactants. Surfactants are classified as either anionic, nonionic, or cationic, depending on the nature of the organic moiety when the substance is dissolved in water.

The miniscale extraction method utilizes the ability of anionic surfactants to form ion pair complexes with cationic dyes such as methylene blue. These complexes behave as if they are neutral organic molecules. These ion pairs are easily extracted into a nonpolar solvent, thus imparting a color to the extract. The intensity of the color becomes proportional to the concentration of the surfactants in accordance with the Beer–Lambert law of spectrophotometry.
EXPERIMENTAL

This exercise introduces many of the techniques that are required to identify and quantitate an environmental pollutant while facilitating an understanding of the relationship between chemical principles and instrumental analysis.

Preparation of Chemical Reagents

Note: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

*Methylene Blue (MB)*
Dissolve 0.05 g of MB in 50 mL of distilled deionized water (DDI).

*3M Sulfuric Acid*
Add 16.7 mL of concentrated sulfuric acid to a 100-mL volumetric flask half filled with DDI. Adjust to the calibration mark with DDI.

*To Prepare a 0.5 M Sulfuric Acid Solution*
Add approximately 4 mL of the 3 M sulfuric acid to a 25-mL volumetric flask half filled with DDI, then adjust to the final volume. Transfer to a storage vial and label.

*To Prepare a 0.1 M Sodium Hydroxide Solution*
Weigh approximately 0.1 g of NaOH pellets into a 50-mL beaker that contains approximately 20 mL of DDI. Dissolve with stirring. Transfer the contents of the beaker to a 25-mL volumetric flask and adjust to the final volume. Transfer to a vial and label.

*To Prepare the Wash Solution*
To a 1000-mL volumetric flask half filled with DDI, add 41 mL of 3 M sulfuric acid to 5 g of Na$_2$HPO$_4$·H$_2$O. Adjust to the mark with DDI.

*To Prepare the MB Reagent*
To a 500-mL volumetric flask half filled with DDI, add the following:

- 15 mL of MB
- 20.5 mL of 3 M sulfuric acid
- 25 g of Na$_2$HPO$_4$·H$_2$O

Shake until dissolved. Then adjust to the calibration mark with DDI. Transfer contents of volumetric flask to a glass storage bottle and label “MB Reagent.”
Specific Laboratory Experiments

615

Operation and Calibration of the Orion SA 720A pH Meter

The pH meter must be set up and calibrated with two buffers. Use buffer solutions whose pH values are 7 and 10, as these buffers have pH values near those required in this method. Refer to the detailed instructions available at your workbench.

Preparation of the 100 ppm Surfactant Stock Solution

Dissolve approximately 0.01 g of sodium lauryl sulfate in between 5 and 10 mL of MeOH in a 50-mL beaker. Transfer the contents of the beaker to a 10-mL volumetric flask and adjust to the mark with MeOH. This yields a stock solution whose concentration is 1000 ppm. Transfer 1 mL using a glass pipette and pipette pump to a 10-mL volumetric flask. Adjust to the mark with DDI. This yields a primary dilution reference standard whose concentration is 100 ppm.

It becomes important to know the chemical nature of the particular surfactant to be used to prepare the stock standard for construction of the calibration table (Table 5.2), so that the number of parts per million can be related to the concentration of anionic sulfonate actually taken and extracted as an ion pair. For example, for \( p \)-toluene sulfonic acid, 100 mg as the \( p \)-toluene sulfonate ion is only 100.6 mg as the acid; however, 100 mg as the \( p \)-toluene sulfonate ion is 113.4 mg as its sodium salt. A stock solution is stable for no more than 1 week. Working solutions such as the 100 ppm surfactant should be prepared fresh daily. Keep in mind that approximately 0.1 g of any pure solid dissolved in enough solvent to prepare 10 mL of solution yields a standard whose concentration is approximately 10,000 ppm.

Procedure for mini-LLE

1. Using a clean 25-mL graduated cylinder, place 10 mL of an aqueous sample whose anionic surfactant concentration is to be determined into a 50-mL beaker. The aqueous sample could be a blank (i.e., a sample with all reagents added except for the analyte of interest), calibration standard, ICV, fortified (i.e., spiked) sample, or an actual unknown wastewater effluent sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>100 ppm Surfactant (µL)</th>
<th>Surfactant Added (µg)</th>
<th>MB Reagent (µL)</th>
<th>DDI (µL)</th>
<th>CH₂Cl₂ (total) (µL)</th>
<th>Concentration Original Sample (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Std. 1</td>
<td>100</td>
<td>10</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td>Std. 2</td>
<td>250</td>
<td>25</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>Std. 3</td>
<td>500</td>
<td>50</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>5.0</td>
</tr>
<tr>
<td>Std. 4</td>
<td>1000</td>
<td>100</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>ICV</td>
<td>400</td>
<td>40</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>4.0</td>
</tr>
</tbody>
</table>

© 2006 by Taylor & Francis Group, LLC
2. Neutralize the sample to a pH between 7 and 8 by dropwise addition of either 1 M NaOH or 0.5 M H₂SO₄.

3. Add 2.5 mL of the MB reagent and swirl; then transfer the contents of the beaker to a 30-mL glass separatory funnel. Be sure the stopcock is closed.

4. Add 2 mL of methylene chloride (dichloromethane) or equivalent solvent. Because methylene chloride is much denser than water, it will comprise the lower layer after the two phases separate.

5. Stopper the separatory funnel with the ground-glass top, invert the funnel, and shake. Be sure to vent the vapor. This should be done in the fume hood.

6. Withdraw the lower layer into a second clean beaker.

7. Extract the remaining aqueous phase two more times with 2-mL portions of methylene chloride. Then combine the methylene chloride extracts. Approximately 6 mL of organic solvent should be obtained at this point.

8. Wash the combined extracts with wash solution. To do this, transfer the 6 mL of extract (MeCl₂ + dissolved ion pair) to a clean 30-mL separatory funnel. Add approximately 10 mL of wash solution, shake, allow time for the two phases to separate, and then remove the lower layer directly into a clean 50-mL beaker.

9. Transfer the washed extract (approximately 6 mL) to a 10-mL volumetric flask and adjust to a final volume.

10. Transfer a portion of the 10-mL methylene chloride extract to a standard spectrophotometric cuvette and measure the absorbance against methylene chloride as a blank in the reference cell at 652 nm. Record and repeat steps 1 through 10 for all standards and samples.

11. Discard the waste extract into the hazardous waste receptacle located in the laboratory.

FOR THE REPORT (A WRITTEN LABORATORY REPORT DUE ON THIS EXPERIMENT)

Use a spreadsheet program such as EXCEL or equivalent to construct a four-point calibration plot. The plot should show absorbance values on the ordinate and concentration of surfactant in ppm on the abscissa. Calibration data and the least squares regression plotted in EXCEL for an external standard mode (Figure 5.4) and the standard addition mode (Figure 5.5) for the quantitative analysis of anionic surfactants using the methylene blue colorimetric method, as shown. Use a least squares regression to fit the experimental points and calculate the correlation coefficient. Calculate and report on the percent error and the confidence interval at 95% probability for the ICV. A computer program written in BASIC is available for you to use to obtain the statistical results (refer to the program listing in Appendix C). Report on the concentrations of any unknown environmental samples to which this method was applied. Discuss what you learned from this environmental analysis method drawing on your previous experience with spectrophotometric methods. Comment on the precision and accuracy afforded by the analytical method.
Visible Spectrophotometric Determination of Trace Levels of Iron in Groundwater

Background and Summary of Method

Iron (Fe), a metal in great abundance in the Earth, is a common contaminant in groundwaters in its oxidized forms, ferric ion or iron(III) and ferrous ion or iron(II). Common rust consists of ferric oxides and persists in groundwaters as either solubilized or particulate matter. For environmental analytical purposes, one must distinguish between total Fe and dissolved Fe that could be present in groundwaters. It is also of interest to determine the degree of metal speciation (i.e., the concentration...
of Fe(III) to that of Fe(II)). The hexaquo Fe(III) itself behaves as a weak acid and ionizes in water according to

$$\text{Fe(H}_2\text{O)}^3_6+ \leftrightarrow \text{Fe(H}_2\text{O)}_3\text{H}^{2+} + \text{H}^+$$

thus contributing to groundwater acidity. Pure solutions of salts that contain the hexaquo Fe(III) are distinctly acidic, having a pH from about 2 to 4, depending on concentration. If either dissolved Fe or Fe(II) is to be determined, the sample must be analyzed as soon as possible after collection. If only total Fe is to be determined, the sample should be immediately acidified with hydrochloric acid.

Fe(II), once formed by reduction, forms an intensely colored complex ion with 1,10-phenanthroline according to the following reactions:

$$\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$$

Only the Fe(II) oxidation state for iron forms the colored complex. Hence, this selectivity provides the basis for quantitatively determining the ferric ferrous ratio that characterizes the dissolved Fe portion of the iron analysis. Several organic compounds that are readily soluble in water and easily reduce Fe(III) to Fe(II) are available and include hydroquinone, ascorbic acid, and hydroxylamine hydrochloride, among others.

This exercise affords an opportunity to introduce good laboratory practices when conducting an analysis using a spectrophotometer. Each student is given at least one unknown groundwater sample with which to measure the concentrations of Fe(III) and Fe(II).

You will need to consider how both oxidation states of iron can be quantitatively determined using the complexation with 1,10-phenanthroline method only. For a given volume of groundwater, the amount of Fe(III) and the amount of Fe(II) should approximate the amount of total Fe found independently by flame atomic absorption spectrophotometry (FAAS).

**Experimental**

**Volumetric Glassware Needed per Student**

- 1 Volumetric flask (100 mL)
- 1 Pipette (5 mL)
Specific Laboratory Experiments

1 Pipette (10 mL)
1 Volumetric flask (500 mL)
1 10-mL pipette calibrated in 1/10-mL increments (10 mL)

Gravity Filtration Setup

1 Glass funnel and standard circular filter paper

Chemical Reagents Needed per Student or Group

Note: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

- 15 mL of 1.0% hydroxyamine hydrochloride. Dissolve 10 g in every 100 mL of solution.
- 15 mL of 0.1% 1,10-phenanthroline. Dissolve 0.1 g in enough acetone until completely solubilized, then add DDI for every 100 mL of solution.
- 5 mL of concentrated hydrochloric acid, HCl.
- 0.5 g of ferrous ammonium sulfate, Fe$_2$(NH$_4$)$_3$(SO$_4$_)$_3$. Dissolve 0.35 g in a 500-mL volumetric flask half filled with DDI, then add 5 mL of concentrated HCl and adjust to the calibration mark with DDI. This gives a solution that is 100 ppm as Fe.
- 100 mL of saturated sodium acetate solution. Dissolve enough NaOAc until crystal formation is observed.

Spectrophotometer

A stand-alone UV-vis spectrophotometer such as a GENESYS® (Spectronic Instruments) or equivalent is suitable.

An atomic absorption spectrophotometer or an inductively coupled plasma spectrophotometer should be available if the instructor chooses to include these instruments in this exercise.

Procedure

1. Turn on the spectrophotometer and allow at least a 15-min warm-up time. Set the wavelength to 508 nm.
2. Prepare the calibration standards by pipetting 0- (this is called the reagent blank), 1-, 2-, 3-, 4-, and 5-mL aliquots (portions thereof) of the 100 ppm Fe stock solution into a 100-mL volumetric flask. Also prepare an instrument calibration verification (ICV) standard by pipetting 2.5 mL of the 100 ppm Fe stock solution into a 100-mL volumetric flask. Measure the ICV’s absorbance after developing the color below in triplicate. The ICV is used to evaluate the precision and accuracy of any instrumental method via interpolation of the calibration curve and is essential to maintaining
good quality control. *Hint:* To minimize contamination due to carryover when using only one volumetric, prepare standards from low to high concentration.

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>100 ppm Fe (mL)</th>
<th>V (total)</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>100</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>100</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>100</td>
<td>5.0</td>
</tr>
<tr>
<td>ICV</td>
<td>2.5</td>
<td>100</td>
<td>2.5</td>
</tr>
</tbody>
</table>

3. To 20 mL of each reference standard solution, add 10 mL of saturated NaOAc and 10 mL of 1% hydroxylamine hydrochloride. Wait 5 min and add 10 mL of the 0.1% 1,10-phenanthroline solution. Allow 10 min, then dilute to the calibration mark of the 100-mL volumetric beaker with DDI.

4. Carefully filter approximately 35 mL of the unknown groundwater sample if necessary. Pipette 20 mL of sample and place into a clean 100-mL volumetric beaker. Add reagents as done previously for the calibration standard preparation and adjust to the final volume with DDI.

5. Set the zero control and 100% transmittance controls according to the operating manual instructions using DDI. Record transmittance values for the blank calibration standards, ICV measured in triplicate, and unknown sample.

**Determination of Total Fe by FlAA or ICP-AES**

You may have an opportunity to use either FlAA or ICP-AES to quantitatively measure the total iron in the same groundwater samples that were used above. The same set of calibration standards that you prepared can be used and directly aspirated into either the flame or the plasma. If you choose FlAA, you may need to install an Fe hollow-cathode lamp.

**FOR THE NOTEBOOK**

Use a spreadsheet program such as EXCEL or its equivalent to construct a six-point calibration plot. The plot should show absorbance values on the ordinate and concentration as ppm Fe on the abscissa. Use a least squares regression to fit the experimental points and calculate the correlation coefficient. Calculate the percent error and the confidence interval at 95% probability for the ICV. Interpolate the calibration plot and obtain a concentration for Fe(III) and Fe(II) from the visible spectrophotometer. Obtain the concentration, $C$, for Fe(total) from the AA spectrophotometer. From these results, compare the calculated $C_{Fe(II)}/C_{Fe(III)}$ ratio from the colorimetric method against the AA method (i.e., conduct a mass balance).
computer program written in BASIC is available for use to obtain the statistical results. Refer to Appendix C for the statistical treatment.

SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHORUS IN EUTROPHICATED SURFACE WATER

BACKGROUND AND SUMMARY OF METHOD

The persistence of phosphates in lakes, rivers, and streams due to domestic and industrial pollution has led to elevated levels and is regarded as largely responsible for lake eutrophication. This was an even more serious problem up until some 20 years ago, prior to the ban on phosphate-containing detergents. In considering an environmental sample, one must distinguish between several chemical forms that contain the element phosphorus. Separation into dissolved and total recoverable phosphorus depends on filtration through a 0.45-µm membrane filter. Dissolved forms of phosphorus (P) include meta-, pyro-, and tripolyphosphates. The visible spectrophotometric procedure requires that all forms of P be chemically converted to the water-soluble orthophosphate ion, PO$_4^{3-}$. Hence, an acid hydrolysis step must be included in the method, which should account for all hydrolyzable P. A more rigorous conversion is necessary to include organophosphorous compounds and involves an acid–persulfate digestion of the sample. The following flowchart summarizes the analytical scheme to differentiate the various chemical forms of phosphorus:\textsuperscript{57}
Orthophosphate forms a complex with the molybdate ion in the presence of a reducing agent such as hydrazine sulfate, amino-naphthol-sulfonic acid, tin(II) chloride, or ascorbic acid, which is commonly called heteropoly blue and has the molecular formula \( \text{H}_3\text{PO}_4(\text{MoO}_3)_{12.58} \). There are two wavelengths that can be used: one between 625 and 650 nm and a more sensitive one at 830 nm. The Spectronic 21 DUV is capable of measuring up to 1000 nm, whereas the Spectronic 20\(^{a}\) can go only up to 600 nm with the standard phototube. An infrared phototube is necessary to widen the wavelength of this instrument.

This exercise provides an opportunity to reinforce the principles of spectrophotometry and its relationship to environmental analysis. To minimize laboratory time, the acid hydrolysis step will be eliminated, and thus only dissolved orthophosphate will be measured. In addition to determining the phosphorus content of a surface water sample, each student will be given an unknown sample by the instructor whose phosphorus concentration has been previously determined by anionic ion chromatography.

**EXPERIMENTAL**

**Preparation of Chemical Reagents**

*Note:* All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

5\( M \) Sulfuric Acid

Use an appropriate size graduated cylinder and add 70.25 mL of concentrated sulfuric acid to a 250-mL volumetric flask that has been previously half filled with distilled deionized water (DDI). This solution will release heat. Allow to cool; then fill to the calibration mark with DDI. Label as appropriate. The unused 5\( M \) sulfuric could be saved, diluted, and used in the exercise “Determination of Anionic Surfactants…”

Molybdate Reagent

Dissolve 12.5 g of ammonium molybdate in approximately 100 mL of DDI in a 500-mL volumetric flask. Add 150 mL of 5\( M \) sulfuric acid. Fill to the calibration mark with DDI. Label as appropriate.

1\% Ascorbic Acid

Dissolve 1 g of ascorbic acid into a 100-mL volumetric flask half filled with DDI. Adjust to the calibration mark with DDI. Label as appropriate.

**Preparation of Stock Phosphorus Standards**\(^{57}\)

Dissolve 0.2197 g of potassium dihydrogen phosphate, \( \text{KH}_2\text{PO}_4 \), that has been dried for 1 h at 104°C in approximately 200 mL of DDI in an appropriately sized beaker. You will need use of a correctly calibrated analytical balance. After dissolution is complete, transfer the solution to a 1-L volumetric flask and adjust to a final volume with DDI. Label as “1.0 mL = 0.05 mg P.” Carefully pipette 50 mL of the stock solution into a 1-L volumetric flask half filled with DDI. Adjust to the calibration mark with DDI. Label as “1.0 mL = 0.0025 mg P.”
### Procedure

A 5-mL aliquot of sample is taken and transferred to a 50-mL volumetric flask. Add 5 mL of the molybdate reagent and 3 mL of the reducing agent. The mixture is diluted to volume, and after waiting 6 min for color development (time for development for standards and unknown should be the same), the absorbance is determined at 830 nm using the Spectronic 21 DUV or equivalent spectrophotometer. The ICV should be prepared in triplicate and its absorbance measured three times. Follow good laboratory practices (GLP), as discussed in Chapter 2.

### FOR THE NOTEBOOK

Use a spreadsheet program such as EXCEL or its equivalent to construct a five-point calibration plot. The plot should show absorbance values on the ordinate and concentration as ppm P on the abscissa. Use a least squares regression to fit the experimental points and calculate the correlation coefficient. Calculate the percent error and the confidence interval at 95% probability for the ICV. Report on the unknown ppm P and estimate the confidence interval at 95% probability for both the surface water sample and the sample given to you by the instructor; be sure to include the code for this sample. Review this method in a resource such as Standard Methods for the Examination of Water and Wastewater or other sources of the colorimetric determination of phosphorus and discuss the effect of matrix interferences on the precision and accuracy for determining P in environmental samples using the visible spectrophotometric method. A computer program written in BASIC (refer to Appendix C) is available for use to obtain the statistical results. Refer to the presentation in Chapter 2 to assist you in developing the statistical treatment of the data.

### INTRODUCTION TO THE VISIBLE SPECTROPHOTOMETER

### BACKGROUND AND SUMMARY OF METHOD

The simple visible spectrophotometer has been an important instrument in trace environmental analysis for many years. Colorless environmental contaminants must
be chemically converted to a species that appears colored to the eye. The colored species will then exhibit regions of the visible electromagnetic spectrum where it will absorb photons. This absorption can be related to the Beer–Lambert or Beer–Bouguer law of spectrophotometry according to

$$A = \varepsilon bc$$

where $A$ is the absorbance measured in absorbance units (AUs), and in turn is related to the logarithm of the ratio of incident to transmitted intensity; $\varepsilon$ is the molar absorptivity, a unique property of a chemical substance, viewed as a constant in the equation; $b$ is the path length in either millimeters or centimeters, assumed to be a constant (see cuvette matching below); and $c$ is the concentration in either molarity or weight/unit volume, if known. This forms the basis for calibration of UV-vis and atomic absorption spectrophotometers.

Recall that the color of a solution is the complement of the color of light that it absorbs. For example, the red cobalt chloride solution you will be using to conduct the cuvette matching (see below) actually absorbs the complement to red, which is green at a wavelength of 510 nm. The term spectrophotometer succinctly and completely describes the instrument. Spectro refers to the visible spectrum and the ability of the instrument to select a wavelength or, more accurately, a range of wavelengths. Photo refers to light, and meter implies a measurement process. Some instruments lack the capability of selecting a wavelength and should be called photometers. A good question to ask when using a spectrophotometer is: What is the precision in nanometers when I set the wavelength? The answer should be as follows: It depends on the effective bandpass of the monochromator. The effective bandpass is a measure of the band of wavelengths allowed to pass through the spectrophotometer for a given width of the exit slit of the monochromator. The bandpass is given by the product of the slit width and the reciprocal linear dispersion. The theory of light dispersion on gratings and prisms and subsequent effects on monochromator resolution has been well developed.60,61

The major limitation on the use of visible spectrophotometric or colorimetric methods is the fact that the analyte must absorb within the visible domain of the electromagnetic spectrum. Many analytes of environmental interest are colorless. Many can be chemically converted to a colored product by direct chemical change of the analyte, formation of a metal chelate, or formation of an ion pair. An example of a chemical conversion of the analyte of interest is the colorimetric determination

of nitrile–nitrogen via reaction of the nitrite ion with sulfanilic acid in acidic media to form the diazonium salt, with subsequent reaction with chromotropic acid to form a highly colored azo dye.

An example of metal chelation can be found if one refers to *Standard Methods for the Examination of Water and Wastewater* for the recommended methods for determining the toxic metal cadmium. An atomic absorption method is listed along with a dithizone method. CD\(^{2+}\) combines with dithizone (HDz) in aqueous media to form the neutral colored molecule Cd(Dz)\(_2\). This neutral molecule can be subsequently extracted into a nonpolar solvent, thus providing an increase in the method detection limit (MDL). The extent to which a given metal ion can be chelated with HDz and subsequently extracted is defined as the distribution ratio, \(D_M\), for a given metal ion and depends on the formation constant of the complex, the overall extraction constant, the initial concentration of HDz in the organic phase, the fraction of the total metal concentration present as the divalent cation, and, most importantly, the pH of the aqueous solution. For the equilibrium

\[
M\text{\textsubscript{(aq)}\(^{n+}\)} + n\text{HDz\textsubscript{(aq)}} \leftrightarrow M\text{Dz\textsubscript{n(aq)}} + nH\text{\textsubscript{aq}}
\]

the distribution ratio is expressed mathematically as (refer back to Equation 3.57)

\[
D_M = \frac{\beta_M K_{\alpha \beta} [\text{HDz}\textsubscript{n}\textsuperscript{\alpha}]}{[H^\text{\textsuperscript{+}}]^n \alpha_M}
\]

An example of a chelated ion association pair that when formed is subsequently extracted into a nonpolar solvent is that of the iron(II)-\(o\)-phenanthroline cation, which efficiently partitions into chloroform, provided a large counterion such as perchlorate is present in the chloroform.

**Experimental**

A possible major source of error in spectrophotometric measurement occurs when the path length differs from one sample to the next (i.e., the value for \(b\) (see above)
is different). Hence, two solutions having identical concentrations of a colored analyte (i.e., both \( a \) and \( c \) are equal) could exhibit different absorbances due to differences in path length \( b \). To minimize this source of systematic error, a cuvette-matching exercise is introduced. Cuvette tubes are matched by placing a solution of intermediate absorbance in each and comparing absorbance readings. One tube is chosen arbitrarily as a reference, and others are selected that have the same reading to within 1%. Tubes should always be matched in a separate operation before any spectrophotometric experiments are begun.

**Glassware Needed per Student or Group**

Seven to 10 13 \( \times \) 100 nm glass test tubes.

**Chemical Reagents Needed per Student or Group**

Ten milliliters of a 2% cobalt chloride solution; dissolve 2 g of CoCl\(_2\)·6H\(_2\)O in approximately 100 mL of 0.3 \( M \) HCl. This solution is recommended because it is stable, has a broad absorption band at about the center of the visible region, and transmits about 50% in a 1-cm cell. Each group should prepare this solution and share among members.

**Miscellaneous Item Needed per Student or Group**

One piece of chalk that nicely fits into a 13 \( \times \) 100 mm test tube.

**Spectrophotometer**

Any stand-alone UV-vis absorption spectrophotometer can be used to perform this exercise. In college laboratories, the Spectronic 20 is commonly found. In recent years, the series of GENESYS instruments from Spectronic Instruments, Inc., is finding increasing use in analytical labs.

**Procedure**

Obtain a supply of 13 \( \times \) 100 mm test tubes that are clean, dry, and free of scratches. Half-fill each tube with the 2% CoCl\(_2\) solution. Set the wavelength to 510 nm on the spectrophotometer, then zero the readout. Choose one tube as a reference, place a vertical index mark near the top of the tube, and insert the tube into the sample compartment. Adjust the light control so that the meter reads 90% transmittance (\( T \)). Insert each of the other tubes and record their transmittances. If the \( %T \) is within 1% of the reference tube, place an index mark so that the tube can be inserted in the same position every time. If it is not within 1%, rotate the tube to see whether it can be brought into range. In future measurements, insert each tube in the same position relative to its index mark. Choose a set of seven tubes that have less than
1% variation in reading. Retain these tubes for subsequent photometric work and return the remainder. To compensate for variations between instruments, use the same instrument for both tube matching and experimental work.

To show that as the wavelength cam is manually rotated, different wavelengths are passed across the exit slit by the monochromator, place a piece of chalk into a test tube and insert into the sample compartment while leaving the cover open. Starting at 400 nm, scan the visible range up to 700 nm and observe the exit-slit image by looking straight down into the cell compartment as you rotate the cam.

**FOR THE REPORT**

Because this is an introductory exercise, there is no report.

**USING THE PROGRAM LSQUARES TO ESTABLISH THE LEAST SQUARES REGRESSION LINE FOR VISIBLE SPECTROPHOTOMETRIC DETERMINATIONS**

Typical calibration data obtained from a visible spectrophotometer for aqueous solutions that contain dissolved cobalt chloride, CoCl₂, are as follows:

<table>
<thead>
<tr>
<th>Concentration (mg/L or ppm)</th>
<th>Absorbance (absorbance units, AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCl₂ (aq)</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>0.100</td>
</tr>
<tr>
<td>600</td>
<td>0.235</td>
</tr>
<tr>
<td>700</td>
<td>0.415</td>
</tr>
<tr>
<td>800</td>
<td>0.610</td>
</tr>
<tr>
<td>900</td>
<td>0.795</td>
</tr>
<tr>
<td>1000</td>
<td>1.010</td>
</tr>
</tbody>
</table>

Use LSQUARES to find the best straight line fit through the experimental \( x, y \) data points.

At the wavelength used in the measurement, a portion of a solution whose concentration is unknown is added to a cuvette, placed in the spectrophotometer, and the absorbance measured. Assuming that the unknown solute responsible for the color (in this case the aquated cobalt(II) ion) is the same chemical species in which the previously prepared calibration plot was made, find the concentration in mg/L or ppm from the interpolated least squares regression plot if the absorbance measured is 0.755 AU.

The following graph is the visible absorption spectrum for Co²⁺. What wavelength should be used to conduct this quantitative analysis?
DETERMINATION OF INORGANIC ANIONS USING ION CHROMATOGRAPHY (IC): ANION EXCHANGE IC WITH SUPPRESSED CONDUCTIVITY DETECTION

BACKGROUND

An aqueous sample obtained from the environment that may be expected to contain dissolved inorganic salts can be injected into a properly installed and optimized ion chromatograph. The concentration of the most common inorganic anions and corresponding cations can be quantitatively determined. The instrumentation available in our laboratory is that manufactured by the Dionex Corporation in the early to mid-1980s.

Ion chromatography (IC) is a low- to moderate-pressure liquid chromatographic (LC) technique and should be clearly distinguished from that of high-pressure LC (HPLC). IC as a determinative technique has been developed to separate both cations and anions. The instrument available to the student utilizes anion exchange IC with suppressed conductivity detection. This technique can separate the common inorganic anions — fluoride, chloride, bromide, nitrite, nitrate, phosphate, and sulfate — under the IC conditions used here. A second set of related anions of environmental interest has emerged in recent years, and these are collectively called disinfection by-products and include bromate, bromide, chlorite, and chlorate. EPA Method 300.1 has been recently revised, and this method addresses both sets of...
analytes. Prior to the development of the micromembrane suppressor, a suppressor column was used to reduce the background conductivity of the mobile-phase eluent. The following is a schematic drawing of the classical ion chromatograph:

For anion exchange IC, the eluent must be a moderate to strong base (e.g., carbonate or hydroxide). Carbonate or hydroxide ions displace the analyte ion through the anion exchange resin in the separator column. The separator must contain a low-capacity anion exchange resin so that the analyte ions can make it through the column in a reasonable length of time after injection. Because the conductivity detector responds to all ions, a strong signal due to the eluent would be observed, thus “swamping out” the contribution due to the analyte ions. These eluent ions can be chemically removed while the analyte ions elute from the suppressor in a low-conductivity background. This is done in the suppressor, and the conductivity of the eluent is said to be chemically suppressed. The suppressor and conductivity cell comprise the IC detector, as shown above. The suppressor must contain a cation exchange substrate whereby H\(^+\) from the regenerant migrates across the membrane (which is itself a cation exchanger) and neutralizes the carbonate or hydroxide to form neutral carbonic acid or water. The micromembrane suppressor requires a continuous supply of regenerant solution that consists of 0.025 N sulfuric acid. The regenerant used in our laboratory utilizes mid-1980s’ technology and consists of a reservoir that contains the dilute H\(_2\)SO\(_4\). This solution is made to flow into and out of the micromembrane suppressor by means of positive air pressure. More contemporary designs self-generate the H\(_2\)SO\(_4\) electrochemically.

A typical ion chromatogram for separation and detection of a reference standard that contains all seven common anions follows:
HOW DO I OPERATE A TYPICAL ION CHROMATOGRAPH?

To operate the ion chromatograph, read the operator’s manual. Alternatively, your instructor may have a written procedure. For purposes of illustration, we list below a procedure used in our lab. A Model 2000 (Dionex) instrument interfaced to a PC via a 900 (PE-Nelson) interface is available:

1. Turn the compressed air valve on and adjust the main pressure gauge to 80 psi. This provides a head pressure to the eluent reservoir.
2. Adjust the small pressure gauge located adjacent to the ion chromatograph to approximately 10 psi. This provides a head pressure to the regenerant reservoir.
3. Turn on the power to the chromatography module/SP via a switch located in the rear of the module. This will start the single-piston reciprocating pump on the Model 2000.
4. Turn the conductivity detector cell display to “on” and monitor the output. This is located on the detector module. A reading between 10 and 20 μS with a tolerance of <1 μS represents a stable baseline. This implies that the ion chromatograph is sufficiently equilibrated across the micromembrane suppressor. At times, the regenerate flow rate may need to be increased or decreased by adjusting the head pressure. If regenerate is not flowing through the micromembrane suppressor, the conductivity value will skyrocket.
5. At the PC workstation, retrieve the appropriate method from Turbochrom. The document “Anion.mth” is available if a more specific method has not been created.
6. Either create a sequence or use the method under “setup” and proceed to download the method. This enables the instrument and PC to communicate.
7. Once the workstation PC reaches a ready status, press inject to remove the lit LED on the Dionex Automation Interface. Fill the 50-μL injection

---

**Anion standard**

**Conditions**
- Separator: AS4A
- Suppressor: AMMS
- Eluant: 2 mM Na₂CO₃, 0.75 mM NaHCO₃
- Flow rate: 2.5 mL/min

**Concentrations**

<table>
<thead>
<tr>
<th>Anion</th>
<th>PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>F⁻</td>
<td>1</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>2</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>3</td>
</tr>
<tr>
<td>Br⁻</td>
<td>10</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>10</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>10</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>15</td>
</tr>
</tbody>
</table>
Specific Laboratory Experiments

loop, which is located at the sample port on the chromatography/SP, with a filtered, aqueous sample. Press the “inject” and immediately proceed to the 900 interface box (PE-Nelson) and press “start.”

8. After the chromatographic run is complete, repeat step 7 by first depressing the “inject” to remove the LED.

**Is There a Need for Sample Prep?**

Aqueous samples that are free of suspended matter and contain dissolved inorganic salts are the only type of sample matrix that is suitable for direct injection into the IC. Wastewater samples that contain suspended solids must be filtered prior to injection into the IC. Wastewater samples that have a dissolved organic content (i.e., an appreciable total organic carbon (TOC) level should be passed through a previously conditioned reversed-phase solid-phase extraction (RP-SPE) cartridge to attempt to remove the dissolved organic matter prior to injection into the IC. Keep in mind that these RP-SPE cartridges have a finite capacity. If this capacity is exceeded, contaminants will merely pass through. If samples come from a bioreactor, proteins and other high-molecular-weight solutes must also be removed prior to injection into the IC.

After implementing the operations procedure, the eluent should be pumping through the separator column and the micromembrane suppressor, while the regent should be flowing in the opposite direction to the eluent flow, through the suppressor under building-supplied compressed air.

Also included in this experiment are specific procedures to prepare the bicarbonate/carbonate eluent, the preparation of the mixed anion reference standards, and weights of various salts to be used to prepare stock reference standard solution for all analytes of interest.

**How Do I Prepare a Reference Stock Standard for Each Anion?**

The weight of each salt that can be used to prepare a 1000 ppm as the anion portion of the salt without any reference to a cation is listed in the following table:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Grams to Dissolve to Prepare 1 L of a 1000 ppm as X</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF</td>
<td>2.210</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.646</td>
</tr>
<tr>
<td>KBr</td>
<td>1.488</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>1.500</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>1.371</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>1.814</td>
</tr>
<tr>
<td>KBrO₃</td>
<td>1.315</td>
</tr>
<tr>
<td>KClO₃</td>
<td>1.467</td>
</tr>
<tr>
<td>NaClO₂</td>
<td>1.341</td>
</tr>
</tbody>
</table>

*Note: Where X = F, Cl, NO₂, etc.*

© 2006 by Taylor & Francis Group, LLC
**HOW DO I PREPARE THE BICARBONATE/CARBONATE ELUENT FROM SCRATCH?**

Dissolve 0.571 g of NaHCO₃ and 0.763 g of Na₂CO₃ in approximately 250 mL of distilled deionized water (DDI). Transfer this solution to a 200-mL graduated cylinder. Adjust to the mark with DDI and transfer this solution to the IC reservoir. Add 2000 mL more of DDI to the IC reservoir for a total of 4 L. Label the IC reservoir as “1.7 mM HCO₃/1.8 mM CO₃” eluent. This eluent is used with either a AS4A (Dionex) or equivalent anion exchange IC separator column.

**HOW DO I PREPARE A MIXED ANION STOCK STANDARD FOR IC?**

The following table outlines one approach to prepare a mixed-stock reference standard:

<table>
<thead>
<tr>
<th>1000 ppm Stock (mL)</th>
<th>Anion</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>Chloride</td>
<td>3.0</td>
</tr>
<tr>
<td>1.0</td>
<td>Nitrite</td>
<td>1.0</td>
</tr>
<tr>
<td>10.0</td>
<td>Bromide</td>
<td>10</td>
</tr>
<tr>
<td>2.0</td>
<td>Nitrate</td>
<td>2.0</td>
</tr>
<tr>
<td>20.0</td>
<td>Phosphate</td>
<td>20.0</td>
</tr>
<tr>
<td>10.0</td>
<td>Sulfate</td>
<td>10.0</td>
</tr>
</tbody>
</table>

A 1000-mL volumetric flask is used. Pipette the indicated aliquot into a flask that is approximately half filled with DDI.

**HOW DO I PREPARE A FOUR-LEVEL SET OF CALIBRATION STANDARDS FOR IC?**

Using the stock solution, proceed using the following table as a guide to prepare a set of working calibration standards:

<table>
<thead>
<tr>
<th>Stock (mL)</th>
<th>V (mL)</th>
<th>Cl</th>
<th>NO₂</th>
<th>Br</th>
<th>NO₃</th>
<th>HPO₄</th>
<th>SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>25</td>
<td>0.6</td>
<td>0.2</td>
<td>2.0</td>
<td>0.4</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>10.0</td>
<td>25</td>
<td>1.2</td>
<td>0.4</td>
<td>4.0</td>
<td>0.8</td>
<td>8.0</td>
<td>4.0</td>
</tr>
<tr>
<td>20.0</td>
<td>25</td>
<td>2.4</td>
<td>0.8</td>
<td>8.0</td>
<td>1.6</td>
<td>16.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Neat</td>
<td>—</td>
<td>3.0</td>
<td>1.0</td>
<td>10.0</td>
<td>2.0</td>
<td>20.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

**WHAT DO THE DATA LOOK LIKE?**

Four ion chromatograms are included in this experiment that were run on the Model 2000 instrument. Figure 5.6 shows a separation of six of the seven common anions only 5 min after the instrument was turned on. Note the gradual rise in the baseline during development of the chromatogram. This same reference standard of six anions was injected long after the baseline had stabilized and is shown in Figure 5.7.
Specific Laboratory Experiments

stable baseline is essential for reproducible peak areas, and hence good precision and accuracy for TEQA. The removal of bromide ion from a three-anion mix when passed through a conditioned resin coated with silver nitrate (SPE cartridges manufactured by Alltech) is evident when ion chromatograms shown in Figure 5.8 and Figure 5.9 are compared. It is evident that bromide is partially removed, whereas nitrite and nitrate (anions that do not form insoluble precipitates with Ag⁺) are not.

**PROCEDURE**

Prepare the four calibration standards according to the guidelines given earlier. Prepare the ICV. After the ion chromatograph has been operating and the baseline has stabilized, inject samples in the following order:

![FIGURE 5.6 Direct injection only 5 min after instrument was turned on.](image-url)

© 2006 by Taylor & Francis Group, LLC
1. Blank, such as distilled deionized water
2. Calibration standards from lowest concentration to highest
3. Blank
4. ICV, injected in triplicate
5. Blank
6. Unknown samples

FOR THE REPORT

Establish a four-point calibration for all anions separated and detected by IC. Use Turbochrom or other software to establish the best-fit line through the experimental data points. Interpolate the detector response for the triplicate ICV injections,
Specific Laboratory Experiments

calculate a mean concentration, and report a relative standard deviation. Interpolate the detector response for each of the unknown samples, report the concentration, and include the code for each unknown with your report. LSQUARES (Appendix C) can be used to find the slope of the least squares regression, $y$ intercept, interpolated values for ICVs, and unknowns. The IDL for each anion could also be found.

Injection of a typical groundwater sample reveals significant concentration levels of the inorganic anions chloride and sulfate. These levels are so high as to cause interference in quantitating trace concentration levels of fluoride, nitrite, nitrate, and phosphate. Suggest a sample preparation approach that might serve to minimize or even eliminate these matrix interferences if these anions are to be quantitated. Hint: peruse the Alltech catalog.
How has Dionex Corporation’s approach to the practice of IC changed over the years? Where might you go to answer this question?

**DETERMINATION OF CR(VI) IN A CONTAMINATED AQUIFER USING ANION EXCHANGE IC WITH POSTCOLUMN REACTION AND DETECTION BY VISIBLE PHOTOMETRY**

**BACKGROUND AND SUMMARY OF METHOD**

Another experiment in this collection showed one approach to Cr speciation. This experiment uses anion exchange IC while eliminating most of the tedious sample prep inherent in the other approach. This experiment requires some ingenuity on the
part of the staff. The instrumentation required to achieve the objectives is not usually found in academic labs. This author assembled a mix of different manufacturers’ equipment not in use at the time. This instrumentation included an isocratic pump (Gilson), an HPLC autosampler (Alcott), and an electrically actuated HPLC injector valve (VICI) that enabled autoinjection into a guard/analytical column (Dionex). This column is designed to retain polyvalent anions such as CrO$_4^{2-}$. The column outlet was connected to a Post Column Reaction (PCR) module (Dionex) that in turn was connected to a visible spectrophotometer (Vis) incorporating a flow-through cell (Dionex). The analog output was interfaced to a PC using a 900 series interface (PE-Nelson) utilizing Turbochrom software for data acquisition and postrun processing. A photo of this instrument as assembled by the author is shown in Appendix D. Figure 5.10 depicts a schematic for the IC-PCR-Vis instrument.

The selective formation of a red–violet colored complex from reduction of chromate and subsequent formation of the 1,5-diphenyl carbazone/Cr(III) was first reported by Bose.64 This trace chromatographic method, first developed by Dionex65 and first reported in the chromatographic literature by Arar and Pfaff,66 forms the basis for this experiment. A round-robin study recommended a range of concentration levels of no more than two orders of magnitude between 1 and 100 ppb.67 This experiment also draws from EPA Method 7199, and if possible, this method should be reviewed by students prior to beginning the lab work.68 Figure 5.11 shows three stacked IC-PCR-Vis chromatograms obtained using the instrument depicted in Figure 5.10.

**OF WHAT VALUE IS THIS EXPERIMENT?**

Students will have an opportunity to observe how liquid chromatographic techniques can be interfaced to postcolumn selective reagent addition and photometric detection to achieve ultratrace quantitative analysis of environmental samples originating from chromium-contaminated groundwater aquifers. This experiment may answer the question “How much Cr present is due to Cr(VI)?”

**UV-Vis Absorption Spectrum of 1,5-Diphenyl Carbazone/Cr(III)**

The red–violet colored complex, once prepared, was placed in a cuvette and a UV-vis absorption spectrum was recorded. This spectrum is shown below:
It is evident that beyond the 254-nm region where one expects to find aromatic compounds, the complex strongly absorbs in the visible region with $\lambda_{\text{max}} = 540$ nm. A spectrophotometer with a flow-through cuvette that is set to 540 nm will detect only the 1,5-DPC/Cr(III) complex with excellent sensitivity. Figure 5.11 shows three
IC-PCR-Vis chromatograms that are typical of an instrument that has been optimized for this application. The bottom chromatogram shown is from a real aquifer.

EXPERIMENTAL

An ion chromatograph with postcolumn reagent delivery interfaced to a visible spectrophotometer with flow cell.
Preparation of Chemical Reagents

Note: All reagents used in this analytical method contain hazardous chemicals. Wear appropriate eye protection, gloves, and protective attire. Use of concentrated acids and bases should be done in the fume hood.

Chemicals/Reagents Needed per Group

Ammonium hydroxide — Concentrated, specific gravity = 0.902.
Ammonium sulfate, \((\text{NH}_4)_2\text{SO}_4\).
1,5-Diphenyl carbazide.
Methanol — HPLC grade.
Sulfuric acid — Concentrated, specific gravity = 1.84.
Reagent water — This usually corresponds to the highest purity water available in the laboratory, often referred to as distilled deionized water (DDI).
Cr(VI) stock solution — 1000 mg of Cr/L. Dissolve 4.501 g of \(\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}\) in reagent water and dilute to 1 L. Transfer and store in a polypropylene storage container.
Eluent — Dissolve 33 g of ammonium sulfate in 500 mL of reagent water and add 6.5 mL of ammonium hydroxide. Dilute to 1 L with reagent water. Degas the solution with helium for 5 to 10 min prior to use.
Postcolumn reagent — Dissolve 0.5 g of 1,5-diphenyl carbazide in 100 mL of HPLC-grade methanol in a 100-mL volumetric flask. In a separate container, add 28 mL of concentrated sulfuric acid into 500 mL of reagent water, mix, and degas with helium for 5 to 10 min prior to adding the diphenyl carbazide solution. Dilute to volume with reagent water. Reagent is stable for 4 to 5 days, but should only be prepared in 1-L quantities as needed.
Buffer solution — Dissolve 33 g of ammonium sulfate in 75 mL of reagent water and add 6.5 mL of ammonium hydroxide. Dilute to 100 mL with reagent water. Degas the solution with helium for 5 to 10 min prior to use.
Dilution water — A batch of reagent-grade water must be prepared by adjusting the pH to within the range of 9.0 to 9.5 using the buffer solution. Use this solution for diluting working standards and high-level samples.

Accessories to Be Used with the IC-PCR-Vis per Group

Guard column — A column placed before the analytical column that removes organics and particulates that would damage the more expensive analytical column. IonPac NG1 (Dionex) or equivalent.
Analytical column — A column packed with a high-capacity anion exchange resin capable of resolving \(\text{CrO}_4^{2-}\) from other interferences. IonPac AS7 (Dionex) or equivalent.
Postcolumn reactor — Mixing tee, or membrane reactor, with reaction coil. Must be compatible with flow rates from 0 to 2 mL/min.
Volumetric glassware — All reusable glassware, including sample containers, should be soaked overnight in laboratory-grade detergent and water, rinsed
with water, and soaked for 4 h in a mixture of dilute nitric and hydrochloric acid (1 + 2 +9), followed by rinsing with tap water and reagent water. This applies to volumetric flasks, calibrated pipettes, and graduated cylinders.  

**pH meter** — To read pH between 0 and 14 with an accuracy of ±0.03.  

**Syringe filters** — 0.45 μm.

**Preparation of Calibration Standards**

The 1000 ppm Cr(VI) stock solution must be serially diluted down to 10 ppb Cr(VI). This can be accomplished by first preparing a 1:100 dilution of the stock to bring the concentration to 10 ppm. A 1:100 dilution of 10 ppm brings the concentration to 100 ppb. A 1:10 dilution of 100 ppb gives the 10 ppb needed. Use minimum aliquots to make these primary, secondary, and tertiary dilutions so as to conserve resources and minimize waste.

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>10 ppb Cr(VI) (mL)</th>
<th>V₁ (mL)</th>
<th>Concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.25</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>5</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>5</td>
<td>8.0</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>10.0</td>
</tr>
<tr>
<td>ICV, low</td>
<td>0.75</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>ICV, medium</td>
<td>3.0</td>
<td>5</td>
<td>6.0</td>
</tr>
<tr>
<td>ICV, high</td>
<td>4.5</td>
<td>5</td>
<td>9.0</td>
</tr>
</tbody>
</table>

**FOR THE REPORT**

Establish a calibration and apply a linear least squares regression without weighting through the data points. Verify the curve by finding the uncertainty in the ICVs. Inject samples from the contaminated aquifer and report all analytical results in terms of number ppb as Cr(VI) in each sample.

Explain why a conventional low-capacity anion exchange column such as a AS-4A (Dionex) would not be suitable for this method.

**AN INTRODUCTION TO PH MEASUREMENT:**

**ESTIMATING THE DEGREE OF PURITY OF SNOW;**

**MEASURING SOIL PH; ION CHROMATOGRAPHY**

**BACKGROUND AND SUMMARY OF METHOD**

The laboratory will focus on the operational aspects of pH measurement. It is appropriate that we start this course with pH because this parameter is so fundamental to the physical-chemical phenomenon that occurs in aqueous solutions. The pH of
a solution that contains a weak acid determines the degree of ionization of that weak acid. Of environmental importance is an understanding of the acidic properties of carbon dioxide. The extent to which gaseous CO$_2$ dissolves in water and equilibrates is governed by the Henry’s law constant for CO$_2$. We are all familiar with the carbonation of beverages. The equilibrium is

$$\text{CO}_2(g) \leftrightarrow \text{CO}_2(aq)$$

When CO$_2$ is dissolved in water, a small fraction of the dissolved gas exists as carbonic acid, H$_2$CO$_3$. The acid–base character of CO$_2$ may be described by the following reactions and equilibrium constants:

\[
\begin{align*}
\text{CO}_2(aq) + \text{H}_2\text{O} &\leftrightarrow \text{H}_3\text{O}^+ + \text{HCO}_3^- \\
K_{a1} &= \frac{[\text{H}_3\text{O}^+][\text{HCO}_3^-]}{[\text{CO}_2]} = 4.45 \times 10^{-7} \quad (5.1) \\
pK_{a1} &= 6.35 \\
\text{HCO}_3^-(aq) &\leftrightarrow \text{H}_3\text{O}^+ + \text{CO}_3^{2-} \\
pK_{a2} &= 10.33
\end{align*}
\]

For example, the molar concentration of CO$_2$ in water saturated with this gas at a pressure of 1 atm at 25°C is $3.27 \times 10^{-2}$ M. The pH, using Equation 5.1, can be calculated as 3.92. Environmental water samples generally have less than the saturated molarity value and yield a pH of approximately 5.9. Thus, the difference between ultrapure water, with a theoretical pH of 7, and water exposed to the atmosphere, with a measurable pH of 5.9, is attributed to dissolved CO$_2$ and its formation of carbonic acid, which in turn dissociates hydronium, bicarbonate, and carbonate ions. The degree to which carbon dioxide remains in its molecular form or exists as either bicarbonate or carbonate depends upon the pH. The fraction, $\alpha$, of each of these species of the total is mathematically defined as

\[
\begin{align*}
\alpha_{\text{CO}_2} &= \frac{[\text{CO}_2]}{[\text{CO}_2]+[\text{HCO}_3^-]+[\text{CO}_3^{2-}]} \quad (5.3) \\
\alpha_{\text{HCO}_3^-} &= \frac{[\text{HCO}_3^-]}{[\text{CO}_2]+[\text{HCO}_3^-]+[\text{CO}_3^{2-}]} \quad (5.4) \\
\alpha_{\text{CO}_3^{2-}} &= \frac{[\text{CO}_3^{2-}]}{[\text{CO}_2]+[\text{HCO}_3^-]+[\text{CO}_3^{2-}]} \quad (5.5)
\end{align*}
\]
Equations (5.3) to (5.5) can be reworked in terms of acid dissociation constants and hydronium ion concentrations to yield

\[
\alpha_{\text{CO}_2} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{a1}[\text{H}^+]+K_{a1}K_{a2}}
\]  \hspace{1cm} (5.6)

\[
\alpha_{\text{HCO}_3^-} = \frac{K_{a1}[\text{H}^+]}{[\text{H}^+]^2 + K_{a1}[\text{H}^+]+K_{a1}K_{a2}}
\]  \hspace{1cm} (5.7)

\[
\alpha_{\text{CO}_3^-} = \frac{K_{a1}K_{a2}}{[\text{H}^+]^2 + K_{a1}[\text{H}^+]+K_{a1}K_{a2}}
\]  \hspace{1cm} (5.8)

Equations (5.6) to (5.8) can be used to construct what are called distribution of species diagrams, which are plots of the fraction of each species as a function of solution pH. For the CO$_3$/HCO$_3$/CO$_2$ system, the distribution of species diagram is as follows:

![Distribution of species diagram](image)

The amount of acidic solutes or alkaline solutes present in a given volume of a groundwater, surface water, or wastewater sample is determined by conventional acid–base titration. Refer to Standard Methods for the Examination of Water and Wastewater for specific procedures. Titrations are performed in these methods to a specified pH. Be sure to distinguish among the concepts of acidity, alkalinity, and pH when considering the nature of environmental samples. The pH will need to be
measured and adjusted prior to conducting the ion pair liquid–liquid extraction for
determining methylene blue active surfactants.

The pH was originally measured by judicious choice of acid–base indicator dyes
and has in recent years given way to potentiometric methods due to the inherent
limitations of color. A dye serves no useful purpose when the wastewater sample is
light brown. Advances in both the instrument and the glass electrode have taken the
measurement of pH very far since the early days, when in 1935 Arnold Beckman
was first asked to measure the pH of a lemon.

EXPERIMENTAL

Glassware Needed per Student

1. 250- or 500-mL beaker to sample snow
2. 50-mL beaker
3. Stirring rod

Chemical Reagents Needed per Student

Each pH measurement station should consist of the following:

1. Three buffer solutions of pH 4, 7, and 10, respectively
2. One squeeze bottle containing distilled deionized water (DDI)
3. One waste beaker
4. Training guide for operating the Orion SA 720 pH/ISE Direct Readout
   Meter

Procedure

Use the guide located at each of four pH measurement stations and familiarize
yourself with the operation of the pH meter. Each student should calibrate the meter
using the pH 4 and 7 buffer solutions that are available by implementing the
autocalibration. An ATC probe will not be available; therefore, samples and standards
should be at the same temperature. Use a stirring rod to continuously stir the solution
in the 50-mL beaker while a pH measurement is being taken.

Obtain at least three samples of snow. One sample should be obtained close to
a walkway or roadway and appear visibly dirty. One sample should be obtained far
away from pollutant sources and appear visibly clean. Use a relatively large beaker
to collect snow and allow the snow to melt. Record pH values in your notebook.
Draw conclusions about your observations and write these in your laboratory note-
book.

Obtain one or more soil samples from a hazardous waste site. Alternatively, your
instructor may have a series of fortified soils available in the laboratory. An illus-
tration of how a series of laboratory-fortified acidic, neutral, and alkaline soils can
be prepared and given to students as sample unknowns is shown in Table 5.3.
Implement EPA Method 9045C while reviewing its details. Assume that the unknown
soil is noncalcareous. Notice the use of flowcharts in helping to understand the
Specific Laboratory Experiments

TABLE 5.3

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Soil Type</th>
<th>Chemical Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tappan B</td>
<td>Salicylic acid</td>
</tr>
<tr>
<td>2</td>
<td>Tappan B</td>
<td>Sodium carbonate</td>
</tr>
<tr>
<td>3</td>
<td>Tappan B</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Hudson River sediment, naturally contaminated with PCBs</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Unknown sandy soil</td>
<td>Citric acid</td>
</tr>
<tr>
<td>6</td>
<td>Unknown sandy soil</td>
<td>Potassium hydrogen phthalate</td>
</tr>
<tr>
<td>7</td>
<td>Unknown sandy soil</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>Unknown sandy soil</td>
<td>$p$-Nitrophenol</td>
</tr>
<tr>
<td>9</td>
<td>Unknown sandy soil</td>
<td>Tetrabutyl ammonium hydroxide</td>
</tr>
</tbody>
</table>

procedural aspects of the method. Repeat the pH measurement for the four other samples and record your results in your laboratory notebook. The chemical nature of the contaminated soil samples will be revealed to you after you have completed your pH measurements. Rationalize the observed pH value for each soil based on knowledge of the chemical used to contaminate the soil. Write your comments in your lab notebook.

As an additional feature to this experiment, the environmental engineering staff will have a Dionex 2000i Ion Chromatograph in operation. This instrument will separate and detect inorganic anions such as chloride, nitrate, and sulfate. Are these chemical species present in the various snow samples? In what manner do inorganic anions contribute to snow acidity?

You have generated hazardous waste from your soil pH measurements. Dispose of properly in accordance with the Office of Radiation, Chemical and Biological Safety (ORCBS) guidelines.

SUGGESTED READINGS RELATED TO THIS EXPERIMENT

Many of the more lucid presentations on pH are found in general chemistry texts, whereas instrumental concepts of pH measurement are found in analytical chemistry texts. The most definitive text at an advanced level is R. Bates’s Determination of pH (New York: John Wiley & Sons, 1964), located in most chemistry libraries.


HOW TO WEIGH THE RIGHT WAY

The analytical balance has been and continues to be the most profound instrument in the enviro-chemical or enviro-health analytical lab. The precision and accuracy of the most sophisticated analytical instrument are limited by the precision and accuracy inherent in the weight of the neat form of a certified reference standard.
The analytical balance must be properly maintained, calibrated, and used properly. Some helpful hints on developing proper weighing skills are given below.

- Before weighing, ensure that your balance is leveled correctly.
- Periodically check, clean, and calibrate the balance.
- Zero out or tare the balance display prior to weighing.
- Minimize the use of hands to place tare weights or samples in the weigh chamber. Use appropriately sized and shaped tweezers or tongs to handle weighing vessels.
- Use vessels of an appropriate size and material for samples, e.g., a 500 mL glass beaker is too large for 500 mg of powder.
- Weigh to the same side each and every time. This will maximize repeatability.
- When placing items to be weighed on to the weigh pan, open only the draft shield door on the side on which you are weighing; e.g., if you are right handed, open the right door.
- Understand how the balance indicates a stable weight, i.e., gives a weight that can be safely trusted. All electronic balances give a visual indication of weight stability.
- Zero out or tare weight as carefully as done for the sample. Using tweezers, place the tare weight onto the weigh pan, close all doors, press the tare button, and wait for the balance to give a stable zero.
- Introduce the sample into the weighing vessel using a long spatula, spoon, scoop, or tweezers as appropriate.
- Be aware of how the balance is affected by the working environment. Modern, busy labs are not ideal places for the four- or five-decimal-place balances that we need to put in them. It may be difficult to stabilize. It may take as long as 60 sec for the balance to reach stability.
- Aim for the same location on the balance weigh pan; try to aim for the center of the pan each time.
- After finishing weighing, check that the weigh chamber is clean and free of any spills. This is not just a courtesy to others, or conformance to regulations; it is helping to keep your balance working accurately by eliminating unwanted ingression that could damage the internal mechanics of the balance.

REFERENCES


10. Perry J. Introduction to Analytical Gas Chromatography. New York: Marcel Dekker, 1981, pp. 164–175. This is one of the better discussions of the principles behind the operation of the ECD up through technological development of that time.


This appendix consists of a glossary of terms used in TEQA as well as the regulatory realm. Terms that appear in the literature of statistical evaluation of analytical data, sample preparation, and instrumental analysis are also included. The objective here is to present a wide and diverse glossary that is not unduly large. In addition to a few terms added by the author, the following sources were used and the language adapted to achieve some degree of uniformity in presentation:

- Erickson M. *Analytical Chemistry of PCBs*, 2nd ed. Chelsea, MI: Lewis Publishers, 1997, Appendix H. (Terms related to the determination of PCBs.)
- The Exposure Terminology Subcommittee of the IPCS Harmonization Project. (Refer to its website: www.ipcsharmonize.org.)
- Varian, Inc. Consumable products. (Formerly Varian Sample Preparation Products; SPE terms.)
- User’s manual, ChemStation for GC-MS. Agilent Technologies. (GC-MS terms.)

**α** — Separation factor of two adjacent chromatographically resolved peaks: \( \alpha = \frac{k_2}{k_1} \).

**Absorption** — The partitioning of a solute into a solvent or penetration of a solute into the surface layer of a sorbent surface.

**Absorption barrier** — Any exposure that may allow diffusion of an agent into a target. Examples of absorption barriers are the skin, lung tissue, and gastrointestinal tract wall. See *exposure surface*.
Accuracy — A measure of how close a measured value is to a known true value. Accuracy is assessed by means of reference samples and percent recoveries of spiked samples.

Acute exposure — A contact between an agent and a target occurring over a short time, generally less than a day.

Adsorption — Refers to the clinging of solute molecules to the surface of a sorbent.

Aliquot — A measured portion of a sample taken for analysis.

Alternative hypothesis — Usually comes about from the logic of statistical testing. One example of an alternative hypothesis (refer to the definition of a null hypothesis) is to state that the precision of population A is not equal to that of population B. If the two variances are not equal, the nonequality may be stated mathematically in three ways: $H_A$: (1) $\sigma_A^2 \neq \sigma_B^2$, (2) $\sigma_A^2 < \sigma_B^2$, and (3) $\sigma_A^2 > \sigma_B^2$. The first inequality is two-sided, meaning that the inequality can be approached in either direction, whereas the second and third inequalities are one-sided. If the first inequality is of interest, a two-tailed F test (for variances) or a two-tailed t test (for means) is most appropriate. For the second or third inequalities, a one-tailed F or t test is most appropriate.

Alumina — A highly purified aluminum oxide used chiefly in sample prep cleanup steps; in GC, a gas-solid adsorbent stationary phase.

Analysis — The ascertainment of the identity or the concentration of the constituents or components of a sample. Analysis is often used incorrectly in place of determination. Only samples can be analyzed; constituents or components are determined. Examples of correct usage are analysis of fish for PCBs and determination of PCBs in fish.

Analyte — The chemical element or compound an analyst seeks to determine; the chemical element of interest; the compounds of interest to be isolated from the sample matrix.

Analytical batch — The basic unit of analytical quality control, defined as samples that are analyzed together with the same method sequence and the same host of reagents, and with the manipulations common to each sample within the same time period or in continuous sequential time periods. Samples in each batch should be of similar composition, e.g., groundwater, sludge, ash.

Analytical sample — Any solution or medium introduced into an instrument on which an analysis is performed, excluding instrument calibration, initial calibration verification, initial calibration blank, and continuing calibration blank. The following are all analytical samples: undiluted and diluted samples, predigestion spike samples, duplicate samples, serial dilution samples, analytical spike samples, postdigestion spike samples, interference check samples, laboratory control samples, preparation or method blank, and linear range analysis samples.

Appendix VIII — This list in 40 CFR 261 contains 355 compounds and classes of compounds shown to be toxic, carcinogenic, mutagenic, or teratogenic in reputable scientific studies.

Appendix IX — This list in 40 CRF 264 replaced Appendix VIII with regard to the groundwater monitoring regulations found in 40 CRF 264 and 40 CFR 270.
**Glossary**

**Area units** — A term used in gas chromatography that indicates the peak area of a compound exiting a chromatographic column. The size or area of the peak is proportional to the amount of analyte in the sample.

**Aroclor** — Trade name (Monsanto, St. Louis, MO) for a series of commercial PCB and polychlorinated terphenyl mixtures marked in the U.S.; AR 1242 refers to numerous $C_{12}$ polychlorinated biphenyls that are 42% chlorine by weight.

**Askarel** — A general term for a group of nonflammable, synthetic, chlorinated, aromatic hydrocarbons used as electrical insulating media.

**Atomic absorption spectrophotometry (AAS)** — A technique for analyzing environmental samples or human specimens for trace concentrations of metal using an element-specific lamp that emits a characteristic light spectrum. A sample is heated in a flame or graphite furnace and the light beam is passed through it. When the sample absorbs light, an energy loss is detected and is translated into a concentration of metal in the sample. This technique detects one metal at a time.

**Audit** — A systematic check to determine the quality of some function or activity. Two basic types are performance audits and system audits. Performance audits involve a quantitative comparison of the laboratory’s results to those of a proficiency sample containing known concentrations of analytes. A system audit is a qualitative evaluation that normally consists of an on-site review of a lab’s quality assurance system and physical facilities.

**β** — Phase ratio. In GC, the ratio of mobile- to stationary-phase volumes. Thicker stationary-phase films yield longer retention times and higher peak capacities. For open tubular columns of diameter $d_c$ and stationary-phase film thickness $d_f$, $\beta = \frac{V_G}{V_L} = \frac{d_c}{\pi d_f}$.

**Backflush** — Occurs when peaks at the end of a chromatogram are flushed from a column to vent or to another column by flow reversal in GC.

**Background correction** — A technique usually employed relative to metals analysis, which compensates for variable background contribution to the instrument signal in the determination of trace elements.

**Background level** — The amount of an agent in a medium (e.g., water, soil) that is not attributed to the source(s) under investigation in an exposure assessment. Can be naturally occurring or anthropogenic. Natural background is the concentration of an agent in a medium that occurs naturally or is not the result of human activities.

**Bakeout** — The process of removing contaminants from a column in GC by operation at elevated temperatures, which should not exceed a column’s maximum allowable operating temperature.

**Ballshmitter number** — Serial numbering system for PCBs from 1 to 209, starting with 2-monochlorobiphenyl and ending with decachlorobiphenyl; also termed IUPAC number.

**Band broadening** — Several processes that cause solute profiles to broaden as they migrate through a column in both GC and HPLC.

**Batch** — See analytical batch.

**Bed volume** — For a given mass of sorbent in SPE packed in a cylindrical bed, the volume of solvent required to fill all pores of the sorbent bed and
interstitial spaces between the sorbent particles. This value is approximately 1.25 mL/g of sorbent for the most common (40-µm) silica-based sorbents.

**Bioassay** — The use of living organisms to measure the effect of a substance, factor, or condition by comparing before and after data. The term is often used to mean cancer bioassays.

**Bioavailability** — The degree to which an agent is capable of being absorbed by an organism and available for metabolism or interaction with biologically significant receptors. Bioavailability involves both release from a medium (if present) and absorption by an organism.

**Biochemical oxygen demand (BOD)** — A measure of the amount of oxygen consumed in the biochemical processes that break down organic matter in water. A larger BOD value indicates a greater degree of organic pollution. A related term $\text{BOD}_5$ is the amount of dissolved oxygen consumed in 5 days.

**Biomarker/biological marker** — Indicator of changes or events in biological systems. Biological markers of exposure refer to cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells, or fluids and are indicative of exposure to an agent.

**Blank** — An artificial sample designed to monitor the introduction of artifacts into the measurement process. For aqueous samples, reagent water is used as a blank matrix. A universal matrix does not exist for solid samples: therefore, no matrix is routinely used. There are several types of blanks, which monitor a variety of processes:
- A **laboratory blank** is taken through sample preparation and analysis only. It is a test for contamination in sample preparation and analyses.
- A **holding blank** is stored and analyzed with samples at the laboratory. It is a test for contamination in sample storage, as well as sample preparation and analysis.
- A **trip blank** is shipped to and from the field with the sample containers. It is not opened in the field, and therefore provides a test for contamination from sample preparation, site conditions, and transport, as well as sample storage, preparation, and analysis. It is most commonly used for volatile organics.
- A **field blank** is opened in the field and tests for contamination from the atmosphere as well as those activities listed under trip blank.

**Bleed** — The loss of material from a column in GC or septum caused by high-temperature operation. Bleed can result in ghost peaks and increased detector baseline offset and noise.

**BNA** — Base, neutral, and acid extractable compounds. The terms *base*, *neutral*, and *acid* refer to the pH condition of the sample undergoing extract more efficiently from water under acidic conditions. Such compounds are often referred to as “acid extractables.”

**Bonded phase** — A stationary phase in GC that has been chemically bonded to the inner column wall.

**Breakthrough** — Undesired elution of an isolate in SPE from a sorbent bed, which occurs when the isolate is too weakly retained or sorbent capacity is exceeded.
4-Bromofluorobenzene (BFB) — A compound utilized in EPA gas chromatography-mass spectrometry (GC-MS) volatile methods to establish mass spectral instrument performance. It is also used as a surrogate for volatile organic analysis.

BTEX — Benzene, toluene, ethyl benzene, and the three isomeric xylenes; environmental contaminants in groundwater from leaking underground gasoline storage tanks.

Buffer — An aqueous solution containing a weak acid and its conjugate base (or a weak base and its conjugate acid) that will maintain its pH upon addition of moderate amounts of either strong bases or strong acids.

Calibration — The systematic determination of the relationship of the responses of the measurement system to the concentration of the analyte of interest. Instrument calibration performed before any samples are analyzed is called the initial calibration. Subsequent checks on the instrument calibration performed throughout analysis are called continuing calibration.

California list — Created by the state of California, this list is used to determine those wastes that are restricted from land disposal. The list was incorporated by RCRA.

Capacity — The total mass of isolates or interferences in SPE that a specific sorbent mass can retain in a given solvent environment.

Capacity factor — In GC and HPLC, provides a quantitative measure of the degree to which a given analyte is retained on a stationary phase; given the symbol \( k \) or \( k' \); \( k' = \frac{(t_R - t_0)}{t_0} \).

Capillary column — A long, coiled optical fiber that is the principal GC separation medium. Lengths are typically between 30 and 60 m, with column diameters defined as follows:
- Ultra-narrow bore — 180 \( \mu \)m
- Narrow bore — 250 \( \mu \)m
- Wide bore — 320 \( \mu \)m
- Megabore — 530 \( \mu \)m
The column consists of (1) an outer polyimide protective coating, (2) an inner fused-silica wall, and (3) a stationary phase such as various polydimethyl siloxanes either coated or chemically bonded to the inner fused-silica wall. Capillary columns are used extensively in GC and CE.

Carrier gas linear velocity — In GC, the average speed at which a molecule of carrier gas passes through a column in GC, expressed in units of centimeters per second and usually given the symbol \( \dot{u} \). For a column of length \( L \) in meters and a time in seconds for an unretained compound to pass through the column after injection, \( t_0 \), the average linear carrier gas velocity can be found according to

\[
\dot{u} = \frac{100L}{t_0}
\]

This author uses a butane lighter to measure \( \dot{u} \) as follows. A 10-\( \mu \)L liquid-handling glass syringe is inserted into the orifice of the lighter, the valve
depressed, and ∼5 µL of butane gas is removed and injected directly into the injection port of a GC-MS. The MSD is set to m/z 58, and the time it takes for the ion abundance to maximize represents t₀.

**CAS registry number** — Unique number assigned by the Chemical Abstracts Service to each chemical compound.

**CERCLA** — The Comprehensive Environmental Response, Compensation and Liability Act, also known as Superfund. Enacted December 11, 1980, and amended thereafter, CERCLA provides for identification and cleanup of hazardous materials released over the land and into the air, waterways, and groundwater. It covers areas affected by newly released materials and older leaking or abandoned dump sites. CERCLA established the Superfund, a trust fund, to help pay for cleanup of hazardous materials sites. The EPA has authority to collect cleanup costs from those who release the waste material. Cleanup funds come from fines and penalties, taxes on chemical/petrochemical feed stocks, and the U.S. Department of the Treasury. A separate fund collects taxes on active disposal sites to finance monitoring after they close.

**Chain of custody** — Procedures and associated documents designed to trace the custody of a sample from the point of origin to final disposition, with the intent of legally demonstrating that custody remained intact and that tampering or substitutions were precluded.

**Chemical ionization MS** — An alternative to electron-impact MS; uses a reagent gas such as methane to promote collision of analyte molecules with ions from this reagent gas. The result is minimum molecular fragmentation, with a heteroatom of greatest proton affinity becoming protonated. Because little fragmentation occurs, this technique provides molecular structure information.

**Chemical oxygen demand (COD)** — A measure of the oxygen required to oxidize all compounds in water, both organic and inorganic.

**Chlorinated hydrocarbons** — Organic compounds containing one or more chlorine atoms. These include a class of persistent, broad-spectrum insecticides that linger in the environment and accumulate in the food chain. Among them are DDT, aldrin, dieldrin, heptachlor, chlordane, lindane, endrin, mirex, hexachlorobenzene, and toxaphene. Another example is trichloroethene (TCE), an industrial solvent.

**Chronic exposure** — A continuous or intermittent long-term contact between an agent and a target.

**Clean Water Act (CWA)** — Regulates the discharge of nontoxic and toxic pollutants into surface waters. The CWA (Public Law 92-500) became effective November 18, 1972, and has been amended significantly since then. Its ultimate goal is to eliminate all discharges into surface waters usable for fishing, swimming, and other beneficial uses. EPA and the Army Corps of Engineers have jurisdiction. EPA sets guidelines, and state agencies issue permits, e.g., National Pollutant Discharge Elimination system permits, specifying the types of control equipment and allowable discharges for each facility.

Cold injection — An injection in GC that occurs at temperatures lower than the final oven temperature, usually at or below the solvent boiling point.

Colorimetric — Analysis based on the measurement of the color that develops during the test-specific reaction. The intensity of color development is usually measured at a specified wavelength on a spectrophotometer.

Compound-independent calibration (CIC) — An instrument calibration model where the compound used to calibrate the instrument is not necessarily the analyte of interest. For the atomic emission detector interfaced to a gas chromatograph (GC-AED), the intensity of the spectral emission line of an element is calibrated to the concentration of the element. The analyte of interest must contain the element for which the instrument is to be calibrated, but it need not be the compound with which the instrument is calibrated. The source of the element for calibration is thus independent of the analyte of interest. This definition is adapted from EPA Method 8085. CIC is unique to GC-AED. All other GC detectors are compound dependent.

Comprehensive GC (GC × GC) — Two-dimensional technique in which all compounds experience the selectivity of two columns connected in series by a retention modulation device, thereby generating much higher resolution than that attainable with any single column.

Compressibility correction factor \( (j) \) — This factor compensates for the expansion of a carrier gas in GC as it moves along the column from the entrance at the inlet pressure, \( P_i \), to the column exit, at the outlet pressure, \( P_o \).

Confirmation — In GC, an unknown compound in a sample is identified on the basis of its retention time on a specific chromatographic column. Because several compounds may exhibit that exact same retention time on a given column, a secondary confirmation on a different column or detector is often recommended for additional confidence in the compound identification. This additional confirmation is often referred to as dual-column or dual-detector confirmation.

Congeners — Compounds containing different numbers and positions of chlorination (or other single-atom substituents) on the same base structure. For example, dioxin congeners all contain the same dibenzo-p-dioxin nucleus, but are chlorinated to different levels and in different ring positions. There are 209 PCB congeners.

Continuous liquid–liquid extraction — An extraction technique that involves boiling the extraction solvent in a flask and condensing the solvent above the aqueous sample. The condensed solvent drips through the sample and extracts the sample and the compounds of interest from the aqueous phase.

Contract Laboratory Program (CLP) — A program coordinated through the EPA to provide a wide range of analytical services by commercial laboratories in support of investigation, remediation, and enforcement actions at Superfund sites. Laboratories participating in this program are under contract to the EPA and must follow very specific analytical protocols during analyses and data delivery, as specified in the statement of work associated with the contract.
Coplanar — Those PCB congeners lacking or containing only one chlorine in the ortho positions. Chlorine substituents are larger than hydrogens and thus constrain rotation about the carbon–carbon bond bridging the two rings in the biphenyl. PCBs with zero or one ortho-chlorines can form a coplanar conformation more readily than those congeners with two, three, and four chlorines in the ortho positions. The mono-ortho-chloro congeners may or may not be included in the term coplanar, depending on the authors of the study.

Counterion — An ionic species associated with the ionic functional group of opposite charge on the sorbent surface.

Cross-linked phase — A stationary phase in GC that includes cross-linked polymer chains. Usually, it also is bonded to the column inner wall.

Data quality objective (DQO) — During the planning phase of a project requiring laboratory support, the data user must establish the quality of data required from the investigation. Such statements of data quality are known as DQOs. Qualitative and quantitative statements about the data required to support specific decisions or regulatory actions, DQOs must take into account sampling considerations as well as analytical protocols.

Data validation — A systematic effort to review data for identification of errors, thereby deleting or flagging suspect values to ensure the validity of the data for the user. This process may be done by manual or computer methods.

Dead volume — Extra volume experiences by solutes as they pass through a chromatographic system. Excessive dead volume causes additional peak broadening.

Decafluorotriphenyl phosphine (DFTPP) — An organic compound utilized in several EPA GC-MS methods to establish proper mass spectral instrument performance for semivolatile analyses.

Deflagration — An incident involving the sudden combustion of a substance. The deflagration results in a subsonic shock wave.

DEGS — Diethylene glycol succinate; used in GC as a stationary phase.

 Determination — The ascertainment of the quantity or concentration of a specific substance in a sample.

Detonation — An incident involving a violent explosion. Detonation results in a supersonic shock wave. For example, acetylene can detonate upon exposure to pure copper, nickel, mercury, or gold.

Digestion log — An official record of the sample preparation procedures used in processing a sample prior to instrumental analysis. This is most often associated with digestion of samples utilizing various acids prior to analysis for metals.

Diol sorbent — A polar sorbent, typically used for polar extractions from nonpolar solvents.

Direct aqueous injection (DAI) — Injection of between 5 and 10 µL of an aqueous solution containing the analyte of interest; this is generally done using a packed GC column.

Direct injection — Occurs in GC when sample enters an inlet and is swept into a column by carrier gas flow. No sample splitting or venting occurs during or after the injection.
Dissolved metals — Metallic elements determined on a water sample that has been passed through a 0.45-µm filter.

Dissolved oxygen (DO) — The oxygen freely available in water. DO is vital to fish and other aquatic life and for the prevention of odors. Traditionally, the level of DO has been accepted as the single most important indicator of a water body’s ability to support desirable aquatic life. Secondary and advanced waste treatment are generally designed to protect DO in waste-receiving waters.

Dissolved solids — Disintegrated organic and inorganic material contained in water. Excessive amounts make water unfit to drink or use in industrial processes.

DMCS — Dimethyl chlorosilane; used for silanizing glass GC parts.

Dose — The amount of agent that enters a target by crossing an exposure surface. If the exposure surface is an absorption barrier, the dose is an absorbed dose/uptake dose (see uptake); otherwise, it is an intake dose.

Dose rate — Dose per unit time.

Dry weight — The weight of a sample based on a percent solids. Also, the weight of a sample after drying in an oven at a specified temperature.

Efficiency — The ability of a column in GC or HPLC to produce sharp, well-defined peaks. More efficient columns have more theoretical plates N and smaller theoretical plate heights H.

Effluent — Treated or untreated wastewater that flows out of a treatment plant, sewer, or industrial outfall. Generally refers to wastes that are discharged into surface waters and are regulated under the Clean Water Act. Effluent limitations are restrictions on quantities, rates, and concentrations of wastewater discharges that are established by a state or EPA.

Electrolytic conductivity detector (ElCD) — A GC detector that catalytically reacts halogen-containing solutes with hydrogen (reductive mode) to produce strong acid by-products that are dissolved in a working fluid. The acids dissociate, and the detector measures increased electrolytic conductivity. Other operating modes modify the chemistry for response to nitrogen- or sulfur-containing substances.

Electron-capture detector (ECD) — A GC detector that ionizes solutes by collision with metastable carrier gas molecules produced by beta emission from a radioactive source such as 60Ni. The ECD is one of the most sensitive detectors, and it responds strongly to halogenated solutes and others with high electron-capture cross sections.

Electron-impact mass spectrometry (EIMS) — Analyte molecules collide with 70-eV electrons boiled off of a hot wire filament and accelerated and are fragmented; this fragmentation follows well-established patterns; low-resolution mass spectrometry operated in the electron-impact ionization mode.

Elution — Removal of a chemical species from a sorbent in SPE by changing the solvent or matrix chemistry to disrupt the analyte/sorbent interaction.

Emulsion — A stable dispersion of one liquid in a second immiscible liquid. Intractable emulsions are detrimental to efficient isolation and recovery of analytes in LLE.
**Exposure** — Contact between an agent and a target. Contact takes place at an exposure point or exposure surface over an exposure interval. For inhalation and ingestion routes, exposure is expressed as a function of exposure concentration; for the dermal route, exposure is expressed as a function of exposure loading. Related terms are cited as follows:

*Exposure assessment* — The process of estimating or measuring the intensity, frequency, and duration of exposure to an agent. Ideally, it describes the sources, pathways, routes, magnitude, duration, and pattern of exposure; the characteristics of the population exposed; and the uncertainties in the assessment.

*Exposure concentration* — The amount of agent present in the contact volume divided by the contact volume. For example, the amount of agent collected in a personal air monitor divided by volume sampled.

*Exposure duration* — The total period over which contacts occur between an agent and a target. For example, if an individual is in contact with an agent for 10 min a day, for 300 days over a 1-year period, the exposure duration is 1 year.

*Exposure frequency* — The number of exposure intervals in an exposure duration.

*Exposure interval* — A period of continuous contact between an agent and a target.

*Exposure loading* — The amount of agent present in the contact volume divided by the exposure surface area. For example, a dermal exposure measurement based on a skin wipe sample, expressed as a mass of residue per skin surface area, is an exposure loading.

*Exposure mass* — The amount of agent present in the contact volume. For example, the total mass of residue collected with a skin wipe sample is an exposure mass.

*Exposure model* — A conceptual or mathematical representation of exposure.

*Exposure pathway* — The course an agent takes from the source to the target.

*Exposure route* — The way an agent enters a human or animal after contact (e.g., by ingestion, inhalation, or dermal absorption).

*Exposure scenario* — A set of facts, assumptions, and inferences about how exposure takes place. Scenarios are often created to aid exposure assessors in estimating exposure.

*Exposure surface* — A surface on a target where an agent is present. Examples of locations of exposure surfaces include the lining of the stomach wall, the lung surface, the exterior of an eyeball, the skin surface, and a conceptual surface over the open mouth. Exposure surfaces can be absorptive or nonabsorptive.

**External standards** — A method of quantifying chromatographic data in which standards of known concentrations are analyzed prior to unknown samples. The chromatographic peak area (or height) of a sample component is compared to a calibration curve of a peak area constructed from the standard
data for that component. This comparison allows the concentration of the component in the sample to be determined.

**Extraction** — Distribution of an analyte of interest between two immiscible liquids; transfer of a chemical species from one phase into another; LLE obeys the Nernst distribution law.

**Fast GC** — Gas chromatography that uses a small-diameter capillary column (≤100 µm i.d.), hydrogen carrier gas, and a fast oven temperature ramp rate to dramatically decrease chromatographic run time.

**Fast HPLC** — High-performance liquid chromatography that uses high flow rates and increased column temperature to maximize overall chromatographic efficiency and optimize the instrument for rapid throughput.

**Fecal coliform bacteria** — Bacteria found in mammals’ intestinal tracts. Their presence in water or sludge is an indicator of pollution and possible contamination by pathogens.

**Field screening** — An investigative technique utilizing analytical chemistry at or near a work site to rapidly determine the presence or absence of environmental contaminants and the approximate concentration of specific target compounds.

**Flame ionization detection (FID)** — In GC where column effluent gas is mixed with hydrogen and burned in air or oxygen. The ions and electrons produced in the flame generate an electric current proportional to the amount of material in the detector. The FID responds to nearly all organic compounds, but it does not respond to air and water, which makes it exceptionally suited to environmental samples.

**Flame photometric detection (FPD)** — In GC where column effluent burns heteroatomic solutes in a hydrogen–air flame. This visible-range atomic emission spectrum is filtered through an interference filter and detected with a photomultiplier tube. Different interference filters can be selected for sulfur, tin, or phosphorous emission lines. The FPD is sensitive and selective.

**Flammable** — A substance that when mixed with air, oxygen, or other oxidant, burns upon ignition. Each flammable gas has a concentration range in air within which the gas may be ignited.

**Flammable limits** — The concentrations of vapor in air or oxygen in which a flame propagates on contact with a source of ignition. It is usually expressed in terms of percentage by volume of gas or vapor in air. The lower explosive limit (LEL) or lower flammable limit (LFL) is the minimum concentration of vapor below which a flame does not propagate. The upper explosive limit (UEL) is the maximum concentration above that a flame does not propagate. A change in temperature or pressure will vary the flammable limits.

**Flash point** — The lowest temperature at which a flammable liquid gives off sufficient vapor to form an ignitable mixture with air near its surface or within a vessel. Combustion does not continue.

**Florisil** — Magnesium silicate; very polar in nature and ideal for the isolation of polar compounds from nonpolar matrices; commonly used as a sample prep cleanup adsorbent.
Flow rate (F) — The column outlet flow rate in GC in units of cubic centimeters per minute and corrected to room temperature and pressure; for example, the flow rate as measured by a flow meter. An estimate for $F$ in cc/min can be calculated by knowing the column diameter in mm, $d_c$, the column length in m, $L$, and the time in minutes to elute an unretained chemical compound to $t_0$ according to

$$F = \frac{0.785d_c^2L}{t_0}$$

Functional group — The reactive portion of an organic molecule; a group of atoms on a chemical species having properties that can be exploited in SPE for retention or sorbents. For example, the functional group for an aliphatic carboxylic acid whose general molecular formula is RCOOH is $\text{−COOH}$.

Gas chromatograph (GC) — An analytical instrument for detecting organic compounds by using their physical and chemical properties to separate a mixture. The compounds are identified and quantified with various types of detectors as they exit the chromatograph. Selection of detectors is dependent on the particular compounds of interest.

Gas chromatography-mass spectrometry (GC-MS) — An analytical instrument in which sample organic compounds of interest are first separated by GC and then enter the mass spectrometer. Molecules are bombarded with electrons as they exit a GC column and are fragmented into characteristic ion patterns. The mass spectrometer is the detector. It can determine which fragments are present and therefore the identity of the compounds.

Gas–liquid chromatography (GLC) — In this determinative technique, solutes partition between a gaseous mobile phase and a liquid stationary phase. Selective interactions between the solutes and the liquid phase cause different retention times in the column.

Gas–solid chromatography (GSC) — In this determinative technique, solutes partition between a gaseous mobile phase and a solid stationary phase. Selective interactions between the solutes and the solid phase cause different retention times in the column.

Ghost peaks — Peaks not present in the original sample. Ghost peaks can be caused by septum bleed, solute decomposition, or carrier gas contamination.

GLP — Good laboratory practice.

Graphite furnace atomic absorption spectrophotometry (GFAA) — A technique used for the analysis of samples that contain metals. An AA spectrophotometer heats the sample within a graphite tube using an electrical current. It is also commonly called a flameless furnace and generally provides greater sensitivity for certain metals than flame or inductively coupled argon plasma techniques.

Gravimetric — Analyses based on the direct or indirect weighing of the analyte in question. This technique usually requires the use of an analytical balance with a sensitivity of 0.1 mg or better.
Hall electrolytic conductivity detection — Accomplished using an element-selective GC detector, primarily intended for trace analysis of organic compounds containing chlorine, nitrogen, or sulfur. In operation, this detector pyrolyzes the column effluent gas into soluble electrolytes that are dissolved in a stream of deionized liquid. The observed change in electrical conductivity, proportional to the amount of material present, is measured.

Hazardous ranking system (HRS) — The principal screening tool used by EPA to evaluate risks to public health and the environment associated with abandoned or uncontrolled hazardous waste sites. The HRS calculates a score based on the potential for hazardous substances to spread from the site through the air, surface water, or groundwater and on other factors, such as nearby population. This score is the primary factor in deciding if the site should be on the National Priorities List and, if so, what ranking it should have there.

Hazardous substance — Any material that poses a threat to human health or the environment. Typical hazardous substances are toxic, corrosive, ignitable, explosive, or chemically reactive.

Hazardous waste — Waste regulated under RCRA that can pose a substantial or potential hazard to human health or the environment when improperly managed. Such wastes possess at least one of four characteristics (ignitability, corrosivity, reactivity, or toxicity) or appear on special EPA hazardous waste lists. The term is not interchangeable with hazardous substance or material.

Headspace — Any area in a container not completely filled by the sample in which gases can collect.

Heart cut — In GC, a technique in which two or more partially resolved peaks that are eluted from one column are directed onto another column of different polarity or at a different temperature for improved resolution.

Heavy metals — Metallic elements that reside in the transition metal section of the periodic table. These elements exhibit relatively high atomic weights; examples include mercury, chromium, cadmium, arsenic, and lead. They can damage the health of plants and animals at low concentrations and tend to accumulate in the food chain. Most are considered to be priority pollutants and some persist in the environment and in humans.

Height equivalent to a theoretical plate (HETP or H) — The distance along the column occupied by one theoretical plate: $H = L/N$, where $L$ is the column length and $N$ is the number of theoretical plates.

Heteroatoms — Organic compounds that contain atoms of elements other than carbon, hydrogen, and oxygen. This includes bromine, chlorine, fluorine, iodine, nitrogen, phosphorous, and sulfur.

Holding time — The storage time allowed between sample collection and sample analysis when the designated preservation and storage technique are employed.

Homolog — One of the 10 degrees of chlorination of PCBs ($C_{12}H_9Cl$ through $C_{12}Cl_{10}$) or other group of compound varying by systematic addition of a substituent.

Hydrocarbons (HCs) — Chemical compounds that consist entirely of carbon and hydrogen.
ICP — Inductively coupled argon plasma (also referred to as ICAP). An instrument used for determining the trace concentrations of various metals in environmental and human specimens. Because the temperature of the plasma is considerably higher (10,000 K) than the temperature of a flame AA spectrophotometer, it is especially useful for refractory metals. Some instruments are also capable of performing simultaneous multielement analysis. Two distinct instrument configurations incorporate the ICP: ICP-AES and ICP-MS.

IgnitableView — Capable of burning or causing a fire.

Inert — A substance that does not react chemically with most material. Some gases, such as argon and helium, are inert, but can displace oxygen in air to cause asphyxiatiom. Nitrogen is a relatively inert gas that is responsible for many deaths by asphyxiation each year.

Inorganic chemicals — Chemical substances of mineral origin, unlike organic chemicals whose structures rely on carbon atoms.

Instrument detection limit (IDL) — The lowest concentration of an analyst of interest that is detectable within the statistical parameters defined. According to the EPA, the IDL is three times the standard deviation obtained for the analysis of a standard solution (each analyte in reagent water) at a concentration of three to five times that of the IDL on three nonconsecutive days, with seven consecutive measurements per day.

Instrument tuning — A technique used in GC-MS procedures to verify that the instrument is properly calibrated to produce reliable mass spectral information.

Interaction — Attraction or repulsion between two chemical species in a specific chemical environment.

Interferences — Undesired components in the sample matrix.

Internal standards — Compounds added to every standard, blank, matrix spike, matrix spike duplicate, sample (for volatile organics), and sample extract (for semivolatiles) at a known concentration, prior to analysis. Internal standards are used as the basis for quantification of the target compound.

Ion exchange — Involves the interaction of an ionic isolate functional group with an ionic functional group of opposite charge on the sorbent surface.

Ionization — Utilized in mass spectrometry to fragment analyte molecules into smaller segments. These smaller mass segments are then separated and plotted to form a mass spectrum, which is used to identify the parent molecule. Electron impact is one example of ionization used in mass spectrometry. In more technical terms, ionization is the process by which neutral atoms or groups of atoms become electrically charged, either positively or negatively, by the loss or gain of electrons.

Ion trap detector — A mass spectrometric detector that uses an ion trap device to generate mass spectra.

Isomer — Chemical compounds with the same molecular weight and atomic composition, but differing molecular structure, e.g., n-pentane and 2-methyl butane.

j — Carrier gas compressibility correction factor GC.

k or k' — Chromatographic retention factor.
K — Partition coefficient. The relative concentration of solute in the mobile and stationary phases.

Keeper — A high boiling solvent used to keep analyte in solution during sample evaporation. In GC methods, typically tetradecane or similar hydrocarbon.

Land ban — The 1984 Resource Conservation and Recovery Act amendments mandated that by May 1990, all untreated hazardous waste must be banned from land disposal. The treatment standard and concentration levels were implemented in thirds beginning in November 1986.

Leachate — A liquid that results from water collecting contaminants as it trickles through wastes, agricultural pesticides, or fertilizers. Leaching may occur in farming areas, feedlots, and landfills, and may result in hazardous substances entering surface water, groundwater, or soil.

Library search — A technique in which an unknown mass spectrum of a compound is compared to the mass spectra of compounds contained in a computer library in an effort to identify the compound. Compounds identified in this manner are referred to as tentatively identified compounds (TICs).

Limit of decision (critical limit) $x_c$ — Lowest concentration of analyte of interest above which indicates detection. Lowest concentration that corresponds to the critical instrumental response $y_c$ such that the probability of not committing a type I error, $\alpha$, at the $y$ intercept, $y_0$, of the least squares regression (i.e., a false positive) is 95%. Represents $3 \times$ the standard deviation in the blank. Refer to Figure 2.9 for a graphical illustration.

Limit of detection (instrumental detection limit) $x_{LD}$ — Lowest concentration of analyte of interest above which leads to detection. Corresponds to an instrumental detection response $y_D$ such that the probability of not committing a type II error at $y_c$ (i.e., a false negative) is 95%. Represents $6 \times$ the standard deviation in the blank. Refer to Figure 2.9 for a graphical illustration.

Limit of quantitation $x_{LOQ}$ — Lowest concentration of analyte of interest above which leads to quantification. Corresponds to an instrumental response $y_D$ such that the probability of not committing a type II error at $y_D$ (i.e., a false negative) is 99%. Represents $10 \times$ the standard deviation in the blank. Refer to Figure 2.9 for a graphical illustration.

Linear dynamic range — The range of solute concentrations or amount beyond which a GC or HPLC detector is not directly proportional to solute concentration.

Linear velocity ($u$) — The speed at which the carrier gas moves through the column, usually expressed as the average carrier gas linear velocity ($\bar{u}$).

Liquid chromatography (LC) — A chromatographic separation technique in which the substance to be analyzed is dissolved in a solvent and, using the same or different solvent, is eluted through a solid adsorbent exhibiting differential adsorption for the components of the substance. The technique is analogous to GC except that heat is not necessarily required for the separations to take place.

Liquid phase — In GC, a stationary liquid layer coated on the inner column wall (WCOT) or on a support (packed SCOT column) that selectively interacts with different solutes as they are eluted from the column.

© 2006 by Taylor & Francis Group, LLC
Listed waste — Any waste listed as hazardous under the Resource Conservation and Recovery Act, but which has not been subjected to the Toxic Characteristics Listing Process because the dangers it presents are considered self-evident.

Log-in — The receipt and initial management of an environmental sample. It generally includes identifying who sent the sample, maintaining chain of custody, checking report and invoice information, recording analyses requested, including methodology and special instructions, and assigning a discreet in-lab identification, usually a number or bar code.

Mass spectrometric detector (MS, MSD) — A detector that records mass spectra of solutes (ion abundance vs. m/z); if solutes are eluted from a column such as in GC-MS, retention time becomes a third dimension.

Mass spectrum — A plot of ion mass/charge ratio, m/z, vs. intensity or ion abundance. A fragmentation pattern results from the impact upon a given molecule of a beam of electrons. The impact produces a family of charged molecular species whose mass distribution is characteristic of the parent molecule. Qualitative information is provided by a mass spectrum.

Material Safety Data Sheet (MSDS) — A compilation of information required under the OSHA Communication Standard on the identity of hazardous chemicals and their associated health and physical hazards, exposure limits, and precautions.

Matrix — The physical characteristics or state of a sample (e.g., water, soil, sludge). The sample environment from which the analyte is to be extracted.

Matrix interference — The influence of the sample matrix or sample components upon the ability to qualitatively identify and quantitatively measure compounds in environmental samples.

Matrix modifiers — Chemicals added to samples for metals analysis that are used to lessen the effects of chemical interferences, viscosity, and surface tension.

Matrix spike — Aliquot of a sample fortified (spiked) with known quantities of specific compounds and subjected to the entire analytical procedure in order to indicate the appropriateness of the method for the matrix by measuring recovery of the spike.

Matrix spike duplicate — A second aliquot of the same matrix as the matrix spike that is spiked to determine the precision of the method.

Maximum allowable operating temperature — In GC, the highest continuous column operating temperature that will not damage a column, if the carrier gas is free of oxygen and other contaminants. Slightly higher temperatures are permissible for short periods during column bakeouts.

Maximum contaminant level (MCL) — The maximum permissible level of a contaminant in water delivered to any user of a public water system. MCLs are enforceable standards.

Mechanism — The nature of the chemistry leading to analyte or interference retention and elution.

Method detection limit (MDL) — The minimum concentration of a compound that can be measured using a specific method and reported with 95 or 99% confidence that the value is above zero.
Minimum detectable quantity (MDQ) — The amount of solute that produces a signal twofold that of the noise level.

Mobile phase — In GC, the carrier gas; in HPLC, a pure liquid or mixture of liquids. Both permeate a stationary phase under pressure. A mobile phase passing across a stationary-phase forms a fundamental definition of GC and HPLC.

Monolithic HPLC columns — Consists of a single plug (instead of the traditional beads) of an organic polymer or of silica of sufficient porosity to allow liquid throughput under pressure.

MS scan parameters — The terms listed below comprise this (adapted from Agilent ChemStation):

   Start time — The time in minutes after the start of the run at which to activate the scan parameters defined by the entries in each row of the table. A maximum of three scan ranges can be active during a run.

   Mass range — Enter the low and high masses (amu) to specify the range to be scanned by the MSD. The larger the range, the lower the number of scans per second.

   Threshold — Only ions with an abundance equal to or greater than this value will be retained in the mass spectrum of each scan. A threshold of 500 is typical.

   Sampling — The value entered here is used to calculate the number of times the abundance of each mass is recorded before going on to the next mass. A value of 2 is suitable for most analyses. The resulting number is reported in samples and calculated as $2^N$. The recommended value is 2 (giving a sample value of 4). Range is 0 to 7.

   Scans/sec — An approximate value calculated from the mass range and sampling values you have entered. It does not take into account the overhead time needed to process the timed events.

   MS window 1/MS window 2 mass range — Enter the ion mass range to be plotted in real time. These fields are active only when the extracted ion plot type is selected in the real-time plot parameters section of the dialog box for the corresponding window.

Multidimensional — Separations performed with two or more columns in which peaks are selectively directed onto or removed from at least one of the columns by a timed valve system.

$N$ — The number of theoretical plates for a given chromatographic column in GC or HPLC.

$N_{eff}$ — The number of effective plates. This term is an alternate measurement of theoretical plate height that compensates for the nonpartitioning nature of an unretained peak.

$N_{req}$ — The number of theoretical plates required to yield a particular resolution ($R_s$) at a specific peak separation ($\alpha$) and retention factor ($k$).

Narrative — In an analytical report, descriptive documentation of any problems encountered in processing the samples, along with corrective action taken and problem resolution.
National Pollutant Discharge Elimination System (NPDES) — A provision of the Clean Water Act that prohibits discharge of pollutants into waters within the U.S. unless a special permit is issued by EPA, a state (where delegated), or a tribal government on an Indian reservation.

Nitrogen-phosphorous detector (NPD) — The NPD catalytically ionizes nitrogen- and phosphorous-containing solutes on a heated rubidium or cesium surface in a reductive atmosphere. The NPD is highly selective and provides sensitivity that is somewhat better than that of a FID.

Nonpolar — Inter- and intramolecular interactions that occur between nonpolar molecules based on London dispersive forces.

Normal-phase HPLC — High-performance liquid chromatography that uses a polar stationary phase and a nonpolar mobile phase.

Null hypothesis — Usually comes about from the logic of statistical testing. One example of a null hypothesis is to state that the precision of population A is equal to that of population B. Given a symbol \( H_0 \) and stated mathematically as \( \sigma_A^2 = \sigma_B^2 \), To answer the question “Are my two variances really different?” is rephrased statistically as “Are the two variances equally precise?”

Nutrient — Any substance assimilated by living things that promotes growth. The term is generally applied to nitrogen and phosphorous in wastewater, but is also applied to other essential and trace elements.

Octadeyl-bonded silica sorbent — A silica gel with C\(_{18}\) moieties chemically bonded to its surface; generally consists of 40-µm particle size with irregular shapes and used predominantly to conduct reversed-phase SPE.

On-column injection — In GC, sample enters the column directly from the syringe and does not contact other surfaces. On-column injection usually signifies cold injection for capillary columns.

Organic — Generally, any compound that contains carbon bonded to a hydrogen or halogen atom.

Oxidant — A gas that is required for combustion of flammable materials. In some cases, the oxidant may initiate combustion. Materials that burn in air burn more vigorously or explosively in the presence of oxygen and another oxidant.

Oxidation — The process in chemistry whereby electrons are removed from a molecule.

PAH — Poly (polycyclic or polynuclear) aromatic hydrocarbon.

Part per billion — One part of solute in \( 10^9 \) parts of solution. For a low concentration of analyte in an aqueous sample, a weight/volume (w/v) basis is most commonly used; 1 ppb = 1 µg/L. For nonaqueous liquids and solids, a weight/weight (w/w) basis is most commonly used; 1 ppb = 1 µg/kg.

Part per million — One part of solute in \( 10^6 \) parts of solution. For a low concentration of analyte in an aqueous sample, a weight/volume (w/v) basis is most commonly used; 1 ppm = 1 mg/L. For nonaqueous liquids and solids, a weight/weight (w/w) basis is most commonly used; 1 ppm = 1 mg/kg.

Part per quadrillion — One part of solute in \( 10^{15} \) parts of solution. For a low concentration of analyte in an aqueous sample, a weight/volume (w/v) basis is most commonly used; 1 ppq = 1 pg/L. For nonaqueous liquids and solids, a weight/weight (w/w) basis is most commonly used; 1 ppt = 1 pg/kg.
Part per trillion — One part of solute in 10^{12} parts of solution. For a low concentration of analyte in an aqueous sample, a weight/volume (w/v) basis is most commonly used; 1 ppt = 1 ng/L. For nonaqueous liquids and solids, a weight/weight (w/w) basis is most commonly used; 1 ppt = 1 ng/kg.

PCBs — Polychlorinated biphenyls, a group of toxic and persistent chemicals once used in transformers and capacitors for insulating purposes and in gas pipeline systems as a lubricant. Sale of PCBs for new uses was banned by law in 1979.

Peak capacity — In GC or HPLC, the amount of solute that can be injected without a significant loss of column efficiency.

Peak overload — In GC or HPLC, when too much of any one solute is injected, its peak can be distorted into a triangular shape.

PEG — Polyethylene glycol.

PEL — Permissible exposure limit. This is the permissible amount of exposure for an 8-h workday, 40 h per week. The PEL does not address cancer, neurological, or reproductive issues. Ten to 15% of the population may suffer acute effects at the PEL.

Percent recovery — The extent to which an analyte of interest is isolated and recovered from a given matrix by the specific analytical method employed. Percent recoveries are measured in the laboratory by using various forms of spiking.

Performance audit — A quantitative evaluation of a measurement system that involves the analysis of standard reference samples or materials that are certified as to their chemical composition or physical characteristics.

Petrochemicals — Chemicals derived from the refining of hydrocarbons (oil and natural gas). Plastics are created through processing of petrochemicals, making them valuable as a fuel in waste-to-energy incineration facilities.

Petroleum hydrocarbon fingerprinting — A method that identifies sources of oil and allows spills to be traced back to their source.

pH — A numerical designation of relative acidity and alkalinity. A pH of 7.0 indicates precise neutrality. Progressively higher values indicate increasing alkalinity and lower values increasing acidity. Mathematically, a solution’s pH is defined as the negative logarithm of the hydrogen ion concentration in aqueous solution.

Photoionization detector (PID) — The PID ionizes solute molecules with photons in the UV energy range. The PID is a selective detector that responds to aromatic compounds and olefins when operated in the 10.2-eV photon range, and it can respond to other compounds with a more energetic light source.

pK_a — For an acidic functional group or the cation of a basic functional group, the pH at which half of the functional groups in solution are charged and half are neutral.

Polar — Inter- and intramolecular interactions that occur between polar molecules based on dipole–dipole or hydrogen bonding forces.

Pollutant — Generally, any substance introduced into the environment that adversely affects the usefulness of a resource.
Porous-layer open tubular (PLOT) column — A capillary column in GC with a modified inner wall that has been etched or otherwise treated to increase the inner surface area or to provide GSC retention behavior. Stationary phases for contemporary plot columns include:
- 5 Å molecular sieve, zeolite
- aluminum oxide, deactivated
- polyester-divinyl benzene
- bonded silica
- bonded monolithic carbon

Porous polymer — A stationary-phase material that retains solutes by selective adsorption or molecular size interaction.

Potentially responsible party (PRP) — Any individual or company, including owners, operators, transporters, or generators, potentially responsible for, or contributing to, the contamination problems at a Superfund site. Whenever possible, EPA requires PRPs through administrative and legal actions, to clean up sites they have contaminated.

Practical quantitation limit (PQL) — The lowest level that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions.

Precision — A measure of the ability to reproduce analytical results. It is generally determined through the analysis of replicate samples. Standard deviation is commonly used to measure the degree of precision.

Precut — In GC, peaks at the beginning of a chromatogram are removed to vent or directed onto another column of different temperature for improved resolution.

Preservative — A chemical or reagent added to a sample to prevent or slow decomposition or degradation of a target analyte or a physical process. Physical and chemical preservation may be used in tandem to prevent sample deterioration.

Primary Drinking Water Regulation — Applies to public water systems and specifies a contaminant level that, in the judgment of the EPA administrator, will have no adverse effect on human health.

Procedure — The written directions necessary to use a method or series of methods and techniques.

Programmed-temperature GC (PTGC) — The column temperature changes in a controlled manner as peaks are eluted.

Programmed-temperature injection — A cold injection technique where the inlet temperature is specifically programmed from the gas chromatograph.

Programmed-temperature vaporizer — In GC, an inlet system designed to perform programmed-temperature injection.

Propagation of random error — In the computation of the percent recovery of the ith analyte, %Ri: using the first equation shown below whose standard deviation, si, is found by propagating relative standard deviations for the ith analyte and for the control reference standard as shown in the second equation shown below. A relative standard deviation, RSD, for the percent recovery can then be found using the third equation shown below.
Protocol — A sampling or analysis procedure that is highly specific and from which few or no deviations are allowed.

Purgeable organic — An organic compound that is generally less that 2% soluble in water and has a boiling point at or below 200°C. A volatile organic. An organic compound is generally considered to be purgeable if it can be removed from water using the purging process.

Purge and trap — A technique used in the analysis of volatile organics where analytes are purged from a sample by means of an inert gas and trapped on a sorbent column. The sorbent is then flash heated, and the analytes are transferred onto a GC column for separation and identification.

Pyrolysis GC — Sample is pyrolyzed (decomposed) in the inlet before GC analysis.

Qualitative — Having to do with establishing the presence or identity of a compound.

Quality assurance (QA) — All those planned and systematic actions necessary to provide adequate confidence in laboratory results.

Quality assurance program plan — A written assembly of management policies, objectives, principles, and general procedures that outlines how the laboratory intends to generate data of known and accepted quality.

Quality control (QC) — Those quality assurance actions that provide a means to control and measure the characteristics of measurement equipment and processes in order to meet established requirements.

Quantitation limit — The minimum concentration of a compound that can be reliably quantified; very dependent upon the sample matrix. See practical quantitation limit (PQL).

Quantitative — Having to do with measuring the amount or concentration of a compound in a sample.

Quantitative analysis — Describes the laboratory operations designed to quantitatively determine the amount or concentration of a targeted chemical substance.

Reactivity — The tendency of a chemical to explode under normal management conditions, to react violently when mixed with water, or to generate toxic gases.

Reagent water — Water in which an interference is not observed at or above the minimum quantitation limit of the parameters of interest.
Reconstructed ion chromatogram (RIC) — A mass spectral graphical representation of the separation achieved by a GC indicating total ion current vs. retention time. All the abundances in the mass spectrum are summed and then reconstructed at the start of a chromatographically resolved peak.

Relative response factor (RRF) — A ratio of the response factor of the ith analyte to its corresponding internal standard (IS).

Relative retention time (RRT) — Retention time of a compound on a chromatographic system, relative to an internal standard; unitless number.

Resolution — The degree of separation between peaks eluting from a chromatographic column. Sufficient resolution between peaks is required for proper quantitation of unknown analytes.

Resource Conservation and Recovery Act (RCRA) — A federal law that established a regulatory system to track hazardous substances from the time of generation to disposal. The law requires safe and secure procedure to be used in treating, transporting, storing, and disposing of hazardous substances. RCRA is designed to prevent new and uncontrolled hazardous waste sites.

Retention gap — In GC, a short piece of deactivated but uncoated column placed between the inlet and the analytical column. A retention gap often helps relieve solvent flooding. It also contains nonvolatile sample contaminants from on-column injection.

Retention index — Systematic, unitless measure of a compound’s chromatographic retention as compared to a homologous series of standards, usually the n-alkanes.

Retention time — A term used in GC and HPLC describing the time elapsed from sample injection until the specific compound elutes or exits the chromatographic column at the detector. Each compound has a characteristic retention time on a specific column; therefore, this information is used to qualitatively identity the compound in the sample.

Reversed-phase HPLC — High-performance liquid chromatography uses a non-polar stationary phase and a polar mobile phase.

RSD — Relative standard deviation; the ratio of the standard deviation in the mean to the mean value of replicate measurements.

Sandwich technique — An injection technique in GC in which a sample plug is placed between two solvent plugs in the syringe to wash the syringe needle with solvent and obtain better sample transfer into the inlet.

Secondary Drinking Water Regulations — Unenforceable regulations that apply to public water systems and specify contamination levels that, in the judgment of EPA, are required to protect the public welfare. These regulations apply to any contaminants that may adversely affect the odor or appearance of such water and consequently may cause people served by the system to discontinue its use.

Selectivity — The fundamental ability of a stationary phase to retain analytes selectively based upon their chemical characteristics, including vapor pressure and polarity.

Sensitivity — The degree of detector response to a specified solute amount per unit time or per unit volume.
Glossary

Separation number of Trennzahl — A measurement of the number of peaks that could be placed with baseline resolution between two sequential peaks $z$ and $z+1$ in a homologous series such as two hydrocarbons.

Septum — Silicone or other elastomeric material used in GC that isolates inlet carrier flow from the atmosphere and permits syringe penetration for injection.

Septum purge — Occurs when carrier gas in GC is swept across the septum face to a separate vent so that material emitted from the septum does not enter the column.

Signal-to-noise ratio (S/N) — The ratio of the peak height to the noise level.

Silica gel — Common adsorbent used in sample prep cleanup techniques; composed of a network of silicon–oxygen covalent bonds with surface silanols. Used as a support to which organic moieties can be chemically bonded through these surface silanol groups. Silica is considered to be the most polar sorbent available. In SPE, base silica is 40-µm irregularly shaped silica with a mean porosity of 60 Å.

Simulated distillation (SIMDIS) — A boiling point separation technique in GC that simulates physical distillation of petroleum products.

Skinner List — Created by John Skinner of the EPA Office of Solid Waste, a list of those compounds most often found in petroleum refining wastes.

Solid wastes — Nonliquid, nonsoluble materials, ranging from municipal garbage to industrial wastes, that contain complex and sometimes hazardous substances. Solid wastes include sewage, sludge, agricultural refuse, demolition wastes, mining residues, and even liquids and gases in containers.

Solvation — The process that prepares a sorbent for sample application.

Solvent — A substance, usually liquid, capable of dissolving or dispersing one or more other substances. The liquid phase involved in sorbent extraction.

Solvent effect — A solute profile sharpening technique used in GC with splitless and on-column injection. Condensed solvent in the column during and shortly after injection traps volatile solutes into a narrow band.

Sorbent — The porous, chemically modified silica used for selective extraction of chemical species from liquids.

Sorbent bed — Sorbent packed into a configuration such that solvents and liquid samples can be passed through the sorbent.

Split injection — The sample size is adjusted to suit capillary column requirements by splitting off a major fraction of sample vapors in the inlet so that as little as 0.1% enters the column. The rest is vented.

Splitless injection — A derivative of split injection. During the first 0.4 to 4 min of sampling, the sample is not split and enters only the column. Splitting is restored afterward to purge the sample remaining in the inlet. As much as 99% of the sample enters the column.

Split ratio — The ratio of the sample amount that is vented in GC to the sample amount that enters the column during split injection. Higher split ratios place less sample on the column. The split ratio is measured as the ratio of total inlet flow to column flow; e.g., a split ratio of 40:1 suggests that for every molecule that enters the capillary column, 40 molecules are split off and exit to the atmosphere via the split vent valve.
Standard curve — A curve that plots concentrations of known analyte standards vs. the instrument response to the analyte. Calibration standards are prepared by diluting the stock analyte solution in graduated amounts that cover the expected range of the samples being analyzed. The calibration standards must be prepared by using the same type of acid or solvent at the same concentration as for the samples following sample preparation. This is applicable to organic and inorganic chemical analyses.

Standard deviation — A statistical measure, usually symbolized by $s$, of spread among replicate measurements assuming a Gaussian or normal distribution. The three equations below all yield the same standard deviation, $s$ for $N$ replicate measurements, and facilitate computation of this important statistical parameter using computer programs or calculators:

\[
s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N-1}}
\]

\[
s = \sqrt{\frac{\left( \sum x_i \right)^2}{N(N-1)}}
\]

\[
s = \sqrt{\frac{N \left( \sum x_i^2 \right) - \left( \sum x_i \right)^2}{N(N-1)}}
\]

Standard operating procedure (SOP) — A written quality assurance document that describes the way an organization typically conducts a routine activity. May address instrumental operation, instrument maintenance, application of a laboratory technique, data review, or management oversight. A detailed written description of how a laboratory executes a particular procedure or method, intended to standardize its performance.

Stationary phase — Liquid or solid materials coated inside a column in GC that selectively retain solutes.

Statistical testing — Proposing a null hypothesis and an alternative hypothesis. The logic of statistical testing as discussed by Anderson is as follows:

- Formulate a null hypothesis and an alternative hypothesis.
- Define the population (or universe) and state any assumptions.
- Plan the experiment.
- Define the basis for decision making.
- Run the experiment as planned.
• Evaluate the data.
• State the conclusion.

Our decision to accept or reject a null hypothesis may be a correct one (i.e., we may accept a true null hypothesis or reject a false null hypothesis) or a wrong one. Rejecting a true null hypothesis is called committing a type I error. Accepting as true a false hypothesis is called committing a type II error. These possibilities can be summarized in a truth table.

**Statistically significant** — When the difference between a predicted and an observed value is so large that it is improbable it could be attributed to chance.

**Subchronic exposure** — A contact between an agent and a target of intermediate duration between acute and chronic.

**Sulfur chemiluminescence detector (SCD)** — A SCD responds to sulfur-containing compounds by generating and measuring the light from chemiluminescence.

**Superfund** — The Response Trust Fund, established by CERCLA as a mechanism for the federal government to take emergency or remedial action to clean up both abandoned and existing disposal sites when there is a release, or potential threat of a release, of a hazardous substance presenting imminent and substantial danger to public health and welfare. See CERCLA.

**Support-coated open tubular (SCOT)** — A capillary column in GC in which the stationary phase is coated onto a support material that is distributed over the column inner wall. A SCOT column generally has a higher peak capacity than a WCOT column with the same average film thickness.

**Surrogate** — An organic compound similar to the analyte of interest in chemical composition, extraction, and chromatography, but not normally found in environmental samples. Primarily used in chromatography techniques, the surrogate standard is spiked into quality control blanks, calibration and check standards, samples (including duplicates and QC reference samples), and spiked samples before analysis. A percent recovery is calculated for each surrogate.

**Suspected solids** — Small pollutant particles that float on the surface of, or are suspended in, sewage or other liquids. They resist removal by conventional means.

**t₀** — Unretained peak holdup time. The time required for one column volume ($V_o$) of carrier gas to pass through a column.

**Target** — A physical, biological, or ecological object. Examples of targets are humans, human organs, and animals.

**Target Compound List (TCL)** — A list of organic compounds that are determined during Superfund site remediations. Created by EPA for use in the Contract Laboratory Program, this list was formerly referred to as the Hazardous Substance List (HSL).

**Target compounds** — Specific compounds that are to be quantified in a sample, based on a standard list of potential compounds.

**Technique** — Scientific principle or specific operation; a skill, accomplished in the laboratory.

**Tentatively identified compounds (TICs)** — Compounds detected in samples that are not target compounds, internal standards, system monitoring compounds,
or surrogates. TICs usually consist of up to 30 peaks that are greater than 10% of the peak areas, or heights, of the nearest internal standard. They are subjected to mass spectral library searches for tentative identification. A client may specify the number of unknown peaks in its samples that it wishes the laboratory to tentatively identify.

**Theoretical plate** — A hypothetical entity inside a GC or HPLC column that exists by analogy to a multiple-plate distillation column. As solutes migrate through a column, they partition between the stationary phase and the mobile phase. Although this process is continuous, chromatographers often visualize a step-wise model. One step corresponds roughly to a theoretical plate.

**Thermal conductivity detector (TCD)** — A TCD measures the differential thermal conductivity of the carrier and reference gas flows. Solutes emerging from a column change the carrier gas thermal conductivity and produce a response. TCD is a universal detection technique with moderate sensitivity.

**Thermionic-specific detector (TSD)** — See nitrogen-phosphorous detector.

**Threshold limit value time-weighted average (TLV-TWA)** — The time-weighted average airborne concentration of substances for a normal 8-h workday or 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. This is being replaced by the PEL. The TLV-TWA is subject to the same limitations as the PEL.

**Time-averaged exposure** — The time-integrated exposure divided by the exposure duration. An example is the daily average exposure of an individual to carbon monoxide.

**Time-integrated exposure** — The integral of instantaneous exposures over the exposure duration. An example is the area under a daily time profile of personal air monitor readings, with units of concentration multiplied by time.

**TMS** — Trimethyl silyl, a chemical derivative.

**Toxic** — A substance that may chemically produce injurious or lethal effects. The degree of toxicity and its effects vary with the compound and time of exposure.

**Total metals** — Metallic elements that have been digested prior to analysis.

**Toxic equivalency factor (TEF)** — Toxicity values given to several of the halogenated aryl hydrocarbons relative to the most congener, 2,3,7,8-tetrachloridibenzo-p-dioxin (2,3,7,8-TCDD).

**Toxic equivalent concentration (TEQ)** — A toxicity-weighted concentration that accounts for both the concentration of individual congeners and their different TEF values. Calculates as $\text{TEQ} = \sum (\text{congener concentration} \times \text{congener TEF})$ for all congeners having assigned TEF values.

**TPH** — Total petroleum hydrocarbons.

$t_R$ — Chromatographic retention time. The time required for a peak to pass through a column.

$t'_R$ — Adjusted retention time; $t'_R = t_R - t_0$.

**Trennzahl (TZ)** — See separation number of Trennzahl.

**Trihalomethane (THM)** — One of a family of organic compounds that are derivatives of methane. THMs are generally by-products of the chlorination of drinking water that contained dissolved humic acids or other types of organic substances.
Trust Fund — A fund set up under the Comprehensive Environmental Response, Compensation and Liability Act (or equivalent state Superfund law) to help pay for cleanup of hazardous waste sites and for legal action to force those responsible for them to clean them up.

Truth table (related to the logic of statistical testing) — The probability \( p \) of making a type I error is \( \alpha \), and thus the probability of making a correct decision when the null hypothesis is true is \( 1 - \alpha \). Similarly, the probability of making a type II error when the null hypothesis is false is \( \beta \), and the probability of making a correct decision is \( 1 - \beta \). This logic is summarized in the truth table shown below:

<table>
<thead>
<tr>
<th>What the Analyst Thinks</th>
<th>What the Scientific Facts Suggest</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_0 ) is true</td>
<td>( \alpha )</td>
</tr>
<tr>
<td>Correct</td>
<td>Type II error</td>
</tr>
<tr>
<td>( p = 1 - \alpha )</td>
<td>( p = \beta )</td>
</tr>
<tr>
<td>( H_0 ) is false</td>
<td>Type I error</td>
</tr>
<tr>
<td>( p = \beta )</td>
<td>Correct</td>
</tr>
<tr>
<td>( p = 1 - \beta )</td>
<td></td>
</tr>
</tbody>
</table>

\( \bar{u} \) — Average linear carrier gas velocity; in GC, \( \bar{u} = \frac{L}{t_0} \).

\( u_0 \) — Carrier gas velocity at the column outlet; \( u_0 = \bar{u}j \).

\( u_{opt} \) — Optimum linear gas velocity. The carrier gas velocity corresponding to the minimum theoretical plate height, ignoring stationary-phase contributions to band broadening.

Underground storage tank — A chemical tank located entirely or partially underground. Usually associated with gasoline storage.

Uptake — The process by which an agent crosses an absorption barrier. See dose.

\( V_G \) — In GC, the volume of carrier gas contained in a column. For open tubular columns of length \( L \) whose diameter is \( d_i \), ignoring the stationary-phase film thickness, \( d_f \): \( V_G = L(\pi d_i^2)/4 \).

\( V_L \) — In GC, volume of stationary phase contained in a column.

Vadose zone — Unsaturated soil, i.e., above the water table.

Vapor pressure — The pressure in mmHg characteristic of a vapor in equilibrium with its liquid or solid form. The vapor pressure of a chemical relates to its speed of evaporation. For example, since xylene has a higher vapor pressure than propylene glycol, it will evaporate faster. If equal quantities of xylene and propylene glycol were spilled, the airborne concentrations of xylene would be higher than those of propylene glycol.

VOA — Volatile organic analysis; also refers to volatile organic acids.

VOA bottle — A vial used to contain samples for volatile organic analysis.

Volatile compounds — Compounds amenable to analysis by purge and trap. Synonymous with purgeable compounds.

Volatile organic compound (VOC) — Any organic compound that participates in atmospheric photochemical reactions, except for those designated by the EPA administrator as having negligible photochemical reactivity. A subset of
VOCs that include one or more chlorine atoms covalently bonded to carbon are abbreviated, at least in this book, as ClVOCs. Examples of ClVOCs are vinyl chloride, trichloroethene (TCE), and perchloroethylene or tetrachloroethylene (PCE).

\( w_b \) and \( w_h \) — In GC, the peak width at its base and the peak width at half height (both are measured in seconds).

**Wall-coated open tubular (WCOT)** — In GC, a capillary column in which the stationary phase is coated directly on the column wall. WCOT columns are the most widely used in contemporary practice of GC.

**Wet chemistry** — Procedures that involve distillations, colorimetric determinations, and titrimetric measurements. Examples of analytes that are routinely quantitatively determined by wet chemical methods include cyanides, methylene blue active surfactants, and biological and chemical oxygen demands (BOD and COD).

**Wide-bore open tubular** — Open tubular (capillary) column with a nominal inner diameter of 320 \( \mu \text{m} \); was once associated with 530 \( \mu \text{m} \) until J&W Scientific coined the term *megabore*. For a full description, see *capillary column*. 