

# Sample Preparation Techniques in Analytical Chemistry

Edited by

**SOMENATH MITRA**

Department of Chemistry and Environmental Science  
New Jersey Institute of Technology



**WILEY-INTERSCIENCE**

**A JOHN WILEY & SONS, INC., PUBLICATION**



# Sample Preparation Techniques in Analytical Chemistry

# CHEMICAL ANALYSIS

A SERIES OF MONOGRAPHS ON ANALYTICAL CHEMISTRY  
AND ITS APPLICATIONS

*Editor*

**J. D. WINEFORDNER**

**VOLUME 162**

A complete list of the titles in this series appears at the end of this volume.

# Sample Preparation Techniques in Analytical Chemistry

Edited by

**SOMENATH MITRA**

Department of Chemistry and Environmental Science  
New Jersey Institute of Technology



**WILEY-INTERSCIENCE**

**A JOHN WILEY & SONS, INC., PUBLICATION**

Copyright © 2003 by John Wiley & Sons, Inc. All rights reserved.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.

Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400, fax 978-750-4470, or on the web at [www.copyright.com](http://www.copyright.com). Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, e-mail: [permreq@wiley.com](mailto:permreq@wiley.com).

**Limit of Liability/Disclaimer of Warranty:** While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services please contact our Customer Care Department within the U.S. at 877-762-2974, outside the U.S. at 317-572-3993 or fax 317-572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print, however, may not be available in electronic format.

***Library of Congress Cataloging-in-Publication Data:***

Sample preparation techniques in analytical chemistry / edited by Somenath Mitra.

p. cm. — (Chemical analysis; v. 162)

Includes index.

ISBN 0-471-32845-6 (cloth : acid-free paper)

1. Sampling. 2. Chemistry, Analytic—Methodology. I. Mitra, S.

(Somenath), 1959— II. Series.

QD75.4.S24S26 2003

543—dc21

2003001379

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

To the hands in the laboratory  
and  
the heads seeking information





# CONTENTS

<b>CONTRIBUTORS</b>	<b>xvii</b>
<b>PREFACE</b>	<b>xix</b>
<b>CHAPTER 1    SAMPLE PREPARATION: AN ANALYTICAL PERSPECTIVE</b>	<b>1</b>
<i>Somenath Mitra and Roman Brukh</i>	
1.1. The Measurement Process	1
1.1.1. Qualitative and Quantitative Analysis	3
1.1.2. Methods of Quantitation	4
1.2. Errors in Quantitative Analysis: Accuracy and Precision	6
1.2.1. Accuracy	6
1.2.2. Precision	6
1.2.3. Statistical Aspects of Sample Preparation	10
1.3. Method Performance and Method Validation	12
1.3.1. Sensitivity	13
1.3.2. Detection Limit	14
1.3.3. Range of Quantitation	15
1.3.4. Other Important Parameters	15
1.3.5. Method Validation	16
1.4. Preservation of Samples	17
1.4.1. Volatilization	19
1.4.2. Choice of Proper Containers	19
1.4.3. Absorption of Gases from the Atmosphere	20
1.4.4. Chemical Changes	20
1.4.5. Preservation of Unstable Solids	20

1.5.	Postextraction Procedures	21
1.5.1.	Concentration of Sample Extracts	21
1.5.2.	Sample Cleanup	22
1.6.	Quality Assurance and Quality Control during Sample Preparation	25
1.6.1.	Determination of Accuracy and Precision	28
1.6.2.	Statistical Control	29
1.6.3.	Matrix Control	31
1.6.4.	Contamination Control	32
	References	35

## **SECTION A      EXTRACTION AND ENRICHMENT IN SAMPLE PREPARATION**

### **CHAPTER 2      PRINCIPLES OF EXTRACTION AND THE EXTRACTION OF SEMIVOLATILE ORGANICS FROM LIQUIDS      37**

*Martha J. M. Wells*

2.1.	Principles of Extraction	37
2.1.1.	Volatilization	38
2.1.2.	Hydrophobicity	43
2.1.3.	Acid–Base Equilibria	50
2.1.4.	Distribution of Hydrophobic Ionogenic Organic Compounds	57
2.2.	Liquid–Liquid Extraction	57
2.2.1.	Recovery	60
2.2.2.	Methodology	66
2.2.3.	Procedures	68
2.2.4.	Recent Advances in Techniques	72
2.3.	Liquid–Solid Extraction	74
2.3.1.	Sorption	75
2.4.	Solid-Phase Extraction	78
2.4.1.	Sorbents in SPE	81
2.4.2.	Sorbent Selection	96
2.4.3.	Recovery	99
2.4.4.	Methodology	108

2.4.5.	Procedures	111
2.4.6.	Recent Advances in SPE	113
2.5.	Solid-Phase Microextraction	113
2.5.1.	Sorbents	116
2.5.2.	Sorbent Selection	118
2.5.3.	Methodology	119
2.5.4.	Recent Advances in Techniques	124
2.6.	Stir Bar Sorptive Extraction	125
2.6.1.	Sorbent and Analyte Recovery	125
2.6.2.	Methodology	127
2.6.3.	Recent Advances in Techniques	129
2.7.	Method Comparison	130
	References	131

### **CHAPTER 3    EXTRACTION OF SEMIVOLATILE ORGANIC COMPOUNDS FROM SOLID MATRICES** **139**

*Dawen Kou and Somenath Mitra*

3.1.	Introduction	139
3.1.1.	Extraction Mechanism	140
3.1.2.	Preextraction Procedures	141
3.1.3.	Postextraction Procedures	141
3.2.	Soxhlet and Automated Soxhlet	142
3.2.1.	Soxhlet Extraction	142
3.2.2.	Automated Soxhlet Extraction	143
3.2.3.	Comparison between Soxtec and Soxhlet	145
3.3.	Ultrasonic Extraction	145
3.3.1.	Selected Applications and Comparison with Soxhlet	147
3.4.	Supercritical Fluid Extraction	148
3.4.1.	Theoretical Considerations	148
3.4.2.	Instrumentation	152
3.4.3.	Operational Procedures	153
3.4.4.	Advantages/Disadvantages and Applications of SFE	154
3.5.	Accelerated Solvent Extraction	155



4.4.1.	SPME Method Development for Volatile Organics	201
4.4.2.	Choosing an SPME Fiber Coating	204
4.4.3.	Optimizing Extraction Conditions	206
4.4.4.	Optimizing SPME–GC Injection	207
4.5.	Liquid–Liquid Extraction with Large-Volume Injection	208
4.5.1.	Large-Volume GC Injection Techniques	208
4.5.2.	Liquid–Liquid Extraction for Large-Volume Injection	211
4.6.	Membrane Extraction	212
4.6.1.	Membranes and Membrane Modules	215
4.6.2.	Membrane Introduction Mass Spectrometry	217
4.6.3.	Membrane Extraction with Gas Chromatography	218
4.6.4.	Optimization of Membrane Extraction	222
4.7.	Conclusions	223
	References	223

## **CHAPTER 5 PREPARATION OF SAMPLES FOR METALS ANALYSIS 227**

*Barbara B. Kebbekus*

5.1.	Introduction	227
5.2.	Wet Digestion Methods	230
5.2.1.	Acid Digestion—Wet Ashing	231
5.2.2.	Microwave Digestion	234
5.2.3.	Comparison of Digestion Methods	235
5.2.4.	Pressure Ashing	237
5.2.5.	Wet Ashing for Soil Samples	237
5.3.	Dry Ashing	240
5.3.1.	Organic Extraction of Metals	241
5.3.2.	Extraction with Supercritical Fluids	244
5.3.3.	Ultrasonic Sample Preparation	245

5.4.	Solid-Phase Extraction for Preconcentration	245
5.5.	Sample Preparation for Water Samples	248
5.6.	Precipitation Methods	251
5.7.	Preparation of Sample Slurries for Direct AAS Analysis	251
5.8.	Hydride Generation Methods	252
5.9.	Colorimetric Methods	254
5.10.	Metal Speciation	255
5.10.1.	Types of Speciation	257
5.10.2.	Speciation for Soils and Sediments	258
5.10.3.	Sequential Schemes for Metals in Soil or Sediment	259
5.10.4.	Speciation for Metals in Plant Materials	260
5.10.5.	Speciation of Specific Elements	262
5.11.	Contamination during Metal Analysis	263
5.12.	Safe Handling of Acids	264
	References	264

## **SECTION B     SAMPLE PREPARATION FOR NUCLEIC ACID ANALYSIS**

### **CHAPTER 6     SAMPLE PREPARATION IN DNA ANALYSIS     271**

*Satish Parimoo and Bhama Parimoo*

6.1.	DNA and Its Structure	271
6.1.1.	Physical and Chemical Properties of DNA	274
6.1.2.	Isolation of DNA	276
6.2.	Isolation of DNA from Bacteria	278
6.2.1.	Phenol Extraction and Precipitation of DNA	278
6.2.2.	Removal of Contaminants from DNA	282
6.3.	Isolation of Plasmid DNA	283
6.3.1.	Plasmid DNA Preparation	284
6.3.2.	Purification of Plasmid DNA	285
6.4.	Genomic DNA Isolation from Yeast	287

6.5.	DNA from Mammalian Tissues	288
6.5.1.	Blood	288
6.5.2.	Tissues and Tissue Culture Cells	289
6.6.	DNA from Plant Tissue	290
6.7.	Isolation of Very High Molecular Weight DNA	290
6.8.	DNA Amplification by Polymerase Chain Reaction	291
6.8.1.	Starting a PCR Reaction	291
6.8.2.	Isolation of DNA from Small Real-World Samples for PCR	294
6.9.	Assessment of Quality and Quantitation of DNA	296
6.9.1.	Precautions for Preparing DNA	296
6.9.2.	Assessment of Concentration and Quality	296
6.9.3.	Storage of DNA	299
	References	299

## **CHAPTER 7    SAMPLE PREPARATION IN RNA ANALYSIS    301**

*Bhama Parimoo and Satish Parimoo*

7.1.	RNA: Structure and Properties	301
7.1.1.	Types and Location of Various RNAs	303
7.2.	RNA Isolation: Basic Considerations	306
7.2.1.	Methods of Extraction and Isolation of RNA	307
7.3.	Phenol Extraction and RNA Recovery: Basic Principles	309
7.3.1.	Examples of RNA Isolation Using Phenol Extraction	310
7.4.	Guanidinium Salt Method	313
7.4.1.	Examples of RNA Isolation Using Guanidinium Salts	313
7.5.	Isolation of RNA from Nuclear and Cytoplasmic Cellular Fractions	317

7.6.	Removal of DNA Contamination from RNA	317
7.7.	Fractionation of RNA Using Chromatography Methods	318
7.7.1.	Fractionation of Small RNA by HPLC	318
7.7.2.	mRNA Isolation by Affinity Chromatography	319
7.8.	Isolation of RNA from Small Numbers of Cells	323
7.9.	In Vitro Synthesis of RNA	324
7.10.	Assessment of Quality and Quantitation of RNA	326
7.11.	Storage of RNA	328
	References	329

## **CHAPTER 8    TECHNIQUES FOR THE EXTRACTION, ISOLATION, AND PURIFICATION OF NUCLEIC ACIDS** **331**

*Mahesh Karwa and Somenath Mitra*

8.1.	Introduction	331
8.2.	Methods of Cell Lysis	333
8.2.1.	Mechanical Methods of Cell Lysis	335
8.2.2.	Nonmechanical Methods of Cell Lysis	339
8.3.	Isolation of Nucleic Acids	342
8.3.1.	Solvent Extraction and Precipitation	344
8.3.2.	Membrane Filtration	345
8.4.	Chromatographic Methods for the Purification of Nucleic Acids	346
8.4.1.	Size-Exclusion Chromatography	347
8.4.2.	Anion-Exchange Chromatography	348
8.4.3.	Solid-Phase Extraction	351
8.4.4.	Affinity Purification	352
8.5.	Automated High-Throughput DNA Purification Systems	355
8.6.	Electrophoretic Separation of Nucleic Acids	360



8.6.1.	Gel Electrophoresis for Nucleic Acids Purification	360
8.6.2.	Techniques for the Isolation of DNA from Gels	362
8.7.	Capillary Electrophoresis for Sequencing and Sizing	364
8.8.	Microfabricated Devices for Nucleic Acids Analysis	366
8.8.1.	Sample Preparation on Microchips	370
	References	373

## **SECTION C      SAMPLE PREPARATION IN MICROSCOPY AND SPECTROSCOPY**

### **CHAPTER 9      SAMPLE PREPARATION FOR MICROSCOPIC AND SPECTROSCOPIC CHARACTERIZATION OF SOLID SURFACES AND FILMS**

*Sharmila M. Mukhopadhyay*

9.1.	Introduction	377
9.1.1.	Microscopy of Solids	378
9.1.2.	Spectroscopic Techniques for Solids	381
9.2.	Sample Preparation for Microscopic Evaluation	382
9.2.1.	Sectioning and Polishing	382
9.2.2.	Chemical and Thermal Etching	385
9.2.3.	Sample Coating Techniques	387
9.3.	Specimen Thinning for TEM Analysis	389
9.3.1.	Ion Milling	391
9.3.2.	Reactive Ion Techniques	393
9.3.3.	Chemical Polishing and Electropolishing	394
9.3.4.	Tripod Polishing	396
9.3.5.	Ultramicrotomy	398
9.3.6.	Special Techniques and Variations	399
9.4.	Summary: Sample Preparation for Microscopy	400

9.5.	Sample Preparation for Surface Spectroscopy	402
9.5.1.	Ion Bombardment	407
9.5.2.	Sample Heating	408
9.5.3.	In Situ Abrasion and Scraping	408
9.5.4.	In Situ Cleavage or Fracture Stage	408
9.5.5.	Sample Preparation/Treatment Options for In Situ Reaction Studies	409
9.6.	Summary: Sample Preparation for Surface Spectroscopy	409
	References	410
<b>CHAPTER 10</b>	<b>SURFACE ENHANCEMENT BY SAMPLE AND SUBSTRATE PREPARATION TECHNIQUES IN RAMAN AND INFRARED SPECTROSCOPY</b>	<b>413</b>
	<i>Zafar Iqbal</i>	
10.1.	Introduction	413
10.1.1.	Raman Effect	413
10.1.2.	Fundamentals of Surface-Enhanced Raman Spectroscopy	415
10.1.3.	Attenuated Total Reflection Infrared Spectroscopy	420
10.1.4.	Fundamentals of Surface-Enhanced Infrared Spectroscopy	421
10.2.	Sample Preparation for SERS	423
10.2.1.	Electrochemical Techniques	423
10.2.2.	Vapor Deposition and Chemical Preparation Techniques	424
10.2.3.	Colloidal Sol Techniques	425
10.2.4.	Nanoparticle Arrays and Gratings	427
10.3.	Sample Preparation for SEIRA	431
10.4.	Potential Applications	433
	References	436
<b>INDEX</b>		<b>439</b>

## CONTRIBUTORS

**Roman Brukh**, Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ 07102

**Zafar Iqbal**, Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, New Jersey 07102

**Mahesh Karwa**, Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ 07102

**Barbara B. Kebbekus**, Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ 07102

**Dawen Kou**, Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ 07102

**Somenath Mitra**, Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ 07102

**Sharmila M. Mukhopadhyay**, Department of Mechanical and Materials Engineering, Wright State University, Dayton, OH 45435

**Bhama Parimoo**, Department of Pharmaceutical Chemistry, Rutgers University College of Pharmacy, Piscataway, NJ 08854

**Satish Parimoo**, Aderans Research Institute, Inc., 3701 Market Street, Philadelphia, PA 19104

**Gregory C. Slack**, Department of Chemistry, Clarkson University, Potsdam, NY 13676

**Nicholas H. Snow**, Department of Chemistry and Biochemistry, Seton Hall University, South Orange, NJ 07079

**Martha J. M. Wells**, Center for the Management, Utilization and Protection of Water Resources and Department of Chemistry, Tennessee Technological University, Cookeville, TN 38505



## PREFACE

There has been unprecedented growth in measurement techniques over the last few decades. Instrumentation, such as chromatography, spectroscopy and microscopy, as well as sensors and microdevices, have undergone phenomenal developments. Despite the sophisticated arsenal of analytical tools, complete noninvasive measurements are still not possible in most cases. More often than not, one or more pretreatment steps are necessary. These are referred to as *sample preparation*, whose goal is enrichment, cleanup, and signal enhancement. Sample preparation is often the bottleneck in a measurement process, as they tend to be slow and labor-intensive. Despite this reality, it did not receive much attention until quite recently. However, the last two decades have seen rapid evolution and an explosive growth of this industry. This was particularly driven by the needs of the environmental and the pharmaceutical industries, which analyze large number of samples requiring significant efforts in sample preparation.

Sample preparation is important in all aspects of chemical, biological, materials, and surface analysis. Notable among recent developments are faster, greener extraction methods and microextraction techniques. Specialized sample preparations, such as self-assembly of analytes on nanoparticles for surface enhancement, have also evolved. Developments in high-throughput workstations for faster preparation–analysis of a large number of samples are impressive. These use 96-well plates (moving toward 384 wells) and robotics to process hundreds of samples per day, and have revolutionized research in the pharmaceutical industry. Advanced microfabrication techniques have resulted in the development of miniaturized chemical analysis systems that include microscale sample preparation on a chip. Considering all these, sample preparation has evolved to be a separate discipline within the analytical/measurement sciences.

The objective of this book is to provide an overview of a variety of sample preparation techniques and to bring the diverse methods under a common banner. Knowing fully well that it is impossible to cover all aspects in a single text, this book attempts to cover some of the more important and widely used techniques. The first chapter outlines the fundamental issues relating to sample preparation and the associated quality control. The

remainder of the book is divided into three sections. In the first we describe various extraction and enrichment approaches. Fundamentals of extraction, along with specific details on the preparation of organic and metal analytes, are presented. Classical methods such as Soxhlett and liquid–liquid extraction are described, along with recent developments in widely accepted methods such as SPE, SPME, stir-bar microextraction, microwave extraction, supercritical extraction, accelerated solvent extraction, purge and trap, headspace, and membrane extraction.

The second section is dedicated to the preparation for nucleic acid analysis. Specific examples of DNA and RNA analyses are presented, along with the description of techniques used in these procedures. Sections on high-throughput workstations and microfabricated devices are included. The third section deals with sample preparation techniques used in microscopy, spectroscopy, and surface-enhanced Raman.

The book is intended to be a reference book for scientists who use sample preparation in the chemical, biological, pharmaceutical, environmental, and material sciences. The other objective is to serve as a text for advanced undergraduate and graduate students.

I am grateful to the New Jersey Institute of Technology for granting me a sabbatical leave to compile this book. My sincere thanks to my graduate students Dawen Kou, Roman Brukh, and Mahesh Karwa, who got going when the going got tough; each contributed to one or more chapters.

*New Jersey Institute of Technology*  
*Newark, NJ*

SOMENATH MITRA

## CHAPTER

### 1

## SAMPLE PREPARATION: AN ANALYTICAL PERSPECTIVE

SOMENATH MITRA AND ROMAN BRUKH

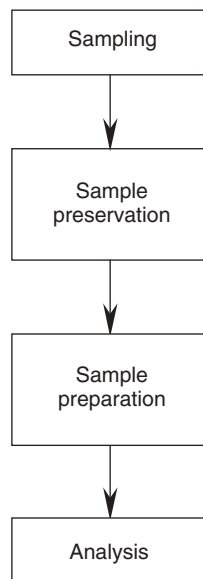
*Department of Chemistry and Environmental Science,  
New Jersey Institute of Technology, Newark, New Jersey*

### 1.1. THE MEASUREMENT PROCESS

The purpose of an analytical study is to obtain information about some object or substance. The substance could be a solid, a liquid, a gas, or a biological material. The information to be obtained can be varied. It could be the chemical or physical composition, structural or surface properties, or a sequence of proteins in genetic material. Despite the sophisticated arsenal of analytical techniques available, it is not possible to find every bit of information of even a very small number of samples. For the most part, the state of current instrumentation has not evolved to the point where we can take an instrument to an object and get all the necessary information. Although there is much interest in such noninvasive devices, most analysis is still done by taking a part (or portion) of the object under study (referred to as the *sample*) and analyzing it in the laboratory (or at the site). Some common steps involved in the process are shown in Figure 1.1.

The first step is *sampling*, where the sample is obtained from the object to be analyzed. This is collected such that it represents the original object. Sampling is done with variability within the object in mind. For example, while collecting samples for determination of  $\text{Ca}^{2+}$  in a lake, it should be kept in mind that its concentrations can vary depending on the location, the depth, and the time of year.

The next step is *sample preservation*. This is an important step, because there is usually a delay between sample collection and analysis. Sample preservation ensures that the sample retains its physical and chemical characteristics so that the analysis truly represents the object under study. Once

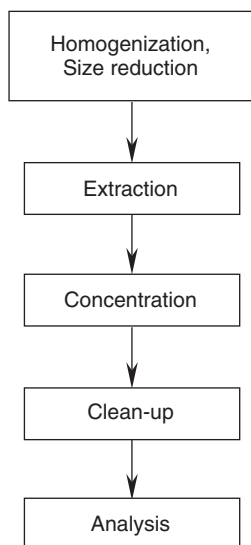


**Figure 1.1.** Steps in a measurement process.

the sample is ready for analysis, *sample preparation* is the next step. Most samples are not ready for direct introduction into instruments. For example, in the analysis of pesticides in fish liver, it is not possible to analyze the liver directly. The pesticides have to be extracted into a solution, which can be analyzed by an instrument. There might be several processes within sample preparation itself. Some steps commonly encountered are shown in Figure 1.2. However, they depend on the sample, the matrix, and the concentration level at which the analysis needs to be carried out. For instance, trace analysis requires more stringent sample preparation than major component analysis.

Once the sample preparation is complete, the analysis is carried out by an instrument of choice. A variety of instruments are used for different types of analysis, depending on the information to be acquired: for example, chromatography for organic analysis, atomic spectroscopy for metal analysis, capillary electrophoresis for DNA sequencing, and electron microscopy for small structures. Common analytical instrumentation and the sample preparation associated with them are listed in Table 1.1. The sample preparation depends on the analytical techniques to be employed and their capabilities. For instance, only a few microliters can be injected into a gas chromatograph. So in the example of the analysis of pesticides in fish liver, the ultimate product is a solution of a few microliters that can be injected into a gas chromatograph. Sampling, sample preservation, and sample preparation are





**Figure 1.2.** Possible steps within sample preparation.

all aimed at producing those few microliters that represent what is in the fish. It is obvious that an error in the first three steps cannot be rectified by even the most sophisticated analytical instrument. So the importance of the prior steps, in particular the sample preparation, cannot be understressed.

### 1.1.1. Qualitative and Quantitative Analysis

There is seldom a unique way to design a measurement process. Even an explicitly defined analysis can be approached in more than one ways. Different studies have different purposes, different financial constraints, and are carried out by staff with different expertise and personal preferences. The most important step in a study design is the determination of the purpose, and at least a notion of the final results. It should yield data that provide useful information to solve the problem at hand.

The objective of an analytical measurement can be qualitative or quantitative. For example, the presence of pesticide in fish is a topic of concern. The questions may be: Are there pesticides in fish? If so, which ones? An analysis designed to address these questions is a *qualitative analysis*, where the analyst screens for the presence of certain pesticides. The next obvious question is: How much pesticide is there? This type of analysis, *quantitative analysis*, not only addresses the presence of the pesticide, but also its concentration. The other important category is *semiquantitative analysis*. Here

**Table 1.1. Common Instrumental Methods and the Necessary Sample Preparation Steps Prior to Analysis**

Analytes	Sample Preparation	Instrument <sup>a</sup>
Organics	Extraction, concentration, cleanup, derivatization	GC, HPLC, GC/MS, LC/MS
Volatile organics	Transfer to vapor phase, concentration	GC, GC-MS
Metals	Extraction, concentration, speciation	AA, GFAA, ICP, ICP/MS
Metals	Extraction, derivatization, concentration, speciation	UV-VIS molecular absorption spectrophotometry, ion chromatography
Ions	Extraction, concentration, derivatization	IC, UV-VIS
DNA/RNA	Cell lysis, extraction, PCR	Electrophoresis, UV-VIS, fluorescence
Amino acids, fats, carbohydrates	Extraction, cleanup	GC, HPLC, electrophoresis
Microstructures	Etching, polishing, reactive ion techniques, ion bombardments, etc.	Microscopy, surface spectroscopy

<sup>a</sup>GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectroscopy; AA, atomic absorption; GFAA, graphite furnace atomic absorption; ICP, inductively coupled plasma; UV-VIS, ultraviolet–visible molecular absorption spectroscopy; IC, ion chromatography.

the concern is not exactly how much is there but whether it is above or below a certain threshold level. The prostate specific antigen (PSA) test for the screening of prostate cancer is one such example. A PSA value of 4 ng/L (or higher) implies a higher risk of prostate cancer. The goal here is to determine if the PSA is higher or lower than 4 ng/L.

Once the goal of the analyses and target analytes have been identified, the methods available for doing the analysis have to be reviewed with an eye to accuracy, precision, cost, and other relevant constraints. The amount of labor, time required to perform the analysis, and degree of automation can also be important.

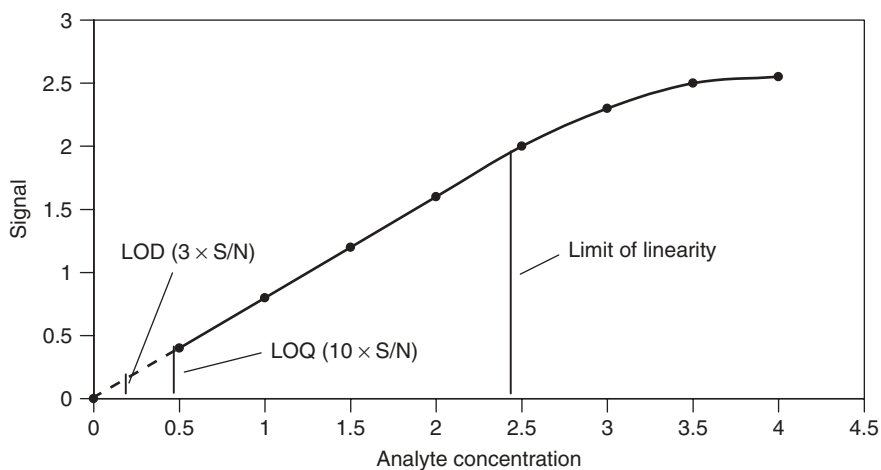
### 1.1.2. Methods of Quantitation

Almost all measurement processes, including sample preparation and analysis, require calibration against chemical standards. The relationship between a detector signal and the amount of analyte is obtained by recording

the response from known quantities. Similarly, if an extraction step is involved, it is important to add a known amount of analyte to the matrix and measure its recovery. Such processes require standards, which may be prepared in the laboratory or obtained from a commercial source. An important consideration in the choice of standards is the matrix. For some analytical instruments, such as x-ray fluorescence, the matrix is very important, but it may not be as critical for others. Sample preparation is usually matrix dependent. It may be easy to extract a polycyclic aromatic hydrocarbon from sand by supercritical extraction but not so from an aged soil with a high organic content.

### *Calibration Curves*

The most common calibration method is to prepare standards of known concentrations, covering the concentration range expected in the sample. The matrix of the standard should be as close to the samples as possible. For instance, if the sample is to be extracted into a certain organic solvent, the standards should be prepared in the same solvent. The calibration curve is a plot of detector response as a function of concentration. A typical calibration curve is shown in Figure 1.3. It is used to determine the amount of analyte in the unknown samples. The calibration can be done in two ways, best illustrated by an example. Let us say that the amount of lead in soil is being measured. The analytical method includes sample preparation by acid extraction followed by analysis using atomic absorption (AA). The stan-



**Figure 1.3.** Typical calibration curve.

dards can be made by spiking clean soil with known quantities of lead. Then the standards are taken through the entire process of extraction and analysis. Finally, the instrument response is plotted as a function of concentration. The other option assumes quantitative extraction, and the standards are used to calibrate only the AA. The first approach is more accurate; the latter is simpler. A calibration method that takes the matrix effects into account is the method of standard addition, which is discussed briefly in Chapter 4.

## 1.2. ERRORS IN QUANTITATIVE ANALYSIS: ACCURACY AND PRECISION

All measurements are accompanied by a certain amount of error, and an estimate of its magnitude is necessary to validate results. The error cannot be eliminated completely, although its magnitude and nature can be characterized. It can also be reduced with improved techniques. In general, errors can be classified as random and systematic. If the same experiment is repeated several times, the individual measurements cluster around the mean value. The differences are due to unknown factors that are stochastic in nature and are termed *random errors*. They have a Gaussian distribution and equal probability of being above or below the mean. On the other hand, *systematic errors* tend to bias the measurements in one direction. Systematic error is measured as the deviation from the true value.

### 1.2.1. Accuracy

*Accuracy*, the deviation from the true value, is a measure of systematic error. It is often estimated as the deviation of the mean from the true value:

$$\text{accuracy} = \frac{\text{mean} - \text{true value}}{\text{true value}}$$

The true value may not be known. For the purpose of comparison, measurement by an established method or by an accredited institution is accepted as the true value.

### 1.2.2. Precision

*Precision* is a measure of reproducibility and is affected by random error. Since all measurements contain random error, the result from a single measurement cannot be accepted as the true value. An estimate of this error is necessary to predict within what range the true value may lie, and this is done

by repeating a measurement several times [1]. Two important parameters, the *average value* and the *variability of the measurement*, are obtained from this process. The most widely used measure of average value is the arithmetic mean,  $\bar{x}$ :

$$\bar{x} = \frac{\sum x_i}{n}$$

where  $\sum x_i$  is the sum of the replicate measurements and  $n$  is the total number of measurements. Since random errors are normally distributed, the common measure of variability (or precision) is the standard deviation,  $\sigma$ . This is calculated as

$$\sigma = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n}} \quad (1.1)$$

When the data set is limited, the mean is often approximated as the true value, and the standard deviation may be underestimated. In that case, the unbiased estimate of  $\sigma$ , which is designated  $s$ , is computed as follows:

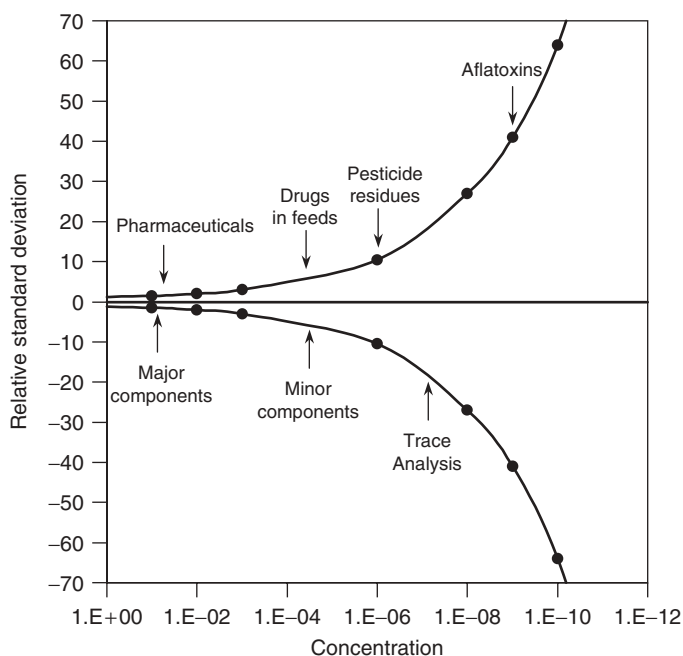
$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}} \quad (1.2)$$

As the number of data points becomes larger, the value of  $s$  approaches that of  $\sigma$ . When  $n$  becomes as large as 20, the equation for  $\sigma$  may be used. Another term commonly used to measure variability is the *coefficient of variation* (CV) or *relative standard deviation* (RSD), which may also be expressed as a percentage:

$$\text{RSD} = \frac{s}{\bar{x}} \quad \text{or} \quad \% \text{ RSD} = \frac{s}{\bar{x}} \times 100 \quad (1.3)$$

Relative standard deviation is the parameter of choice for expressing precision in analytical sciences.

Precision is particularly important when sample preparation is involved. The variability can also affect accuracy. It is well known that reproducibility of an analysis decreases disproportionately with decreasing concentration [2]. A typical relationship is shown in Figure 1.4, which shows that the uncertainty in trace analysis increases exponentially compared to the major and minor component analysis. Additional deviations to this curve are expected if sample preparation steps are added to the process. It may be prudent to assume that uncertainty from sample preparation would also increase with decrease in concentration. Generally speaking, analytical



**Figure 1.4.** Reproducibility as a function of concentration during analytical measurements. (Reproduced from Ref. 3 with permission from LC-GC North America.)

instruments have become quite sophisticated and provide high levels of accuracy and precision. On the other hand, sample preparation often remains a rigorous process that accounts for the majority of the variability. Going back to the example of the measurement of pesticides in fish, the final analysis may be carried out in a modern computer-controlled gas chromatograph/mass spectrograph (GC-MS). At the same time, the sample preparation may involve homogenization of the liver in a grinder, followed by Soxhlett extraction, concentration, and cleanup. The sample preparation might take days, whereas the GC-MS analysis is complete in a matter of minutes. The sample preparation also involves several discrete steps that involve manual handling. Consequently, both random and systematic errors are higher during sample preparation than during analysis.

The relative contribution of sample preparation depends on the steps in the measurement process. For instance, typically two-thirds of the time in an analytical chromatographic procedure is spent on sample preparation. An example of the determination of olanzapine in serum by high-performance liquid chromatography/mass spectroscopy (HPLC-MS) illustrates this point [3]. Here, samples were mixed with an internal standard and cleaned up in a