Sample Preparation Techniques in Analytical Chemistry

Edited by

SOMENATH MITRA

Department of Chemistry and Environmental Science
New Jersey Institute of Technology

WILEY-INTERSCIENCE

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AND ITS APPLICATIONS

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To the hands in the laboratory
and
the heads seeking information

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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.

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CHAPTER

1

SAMPLE PREPARATION: AN ANALYTICAL PERSPECTIVE

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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 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

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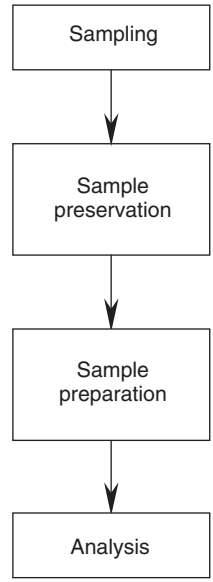

Figure 1.1. Steps in a measurement process.

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

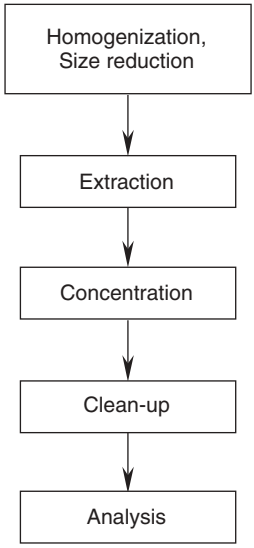

Figure 1.2. Possible steps within sample preparation.

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 *semiqualitative analysis*. Here

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

Analytes	Sample Preparation	Instrument[a]
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, florescence
Amino acids, fats carbohydrates	Extraction, cleanup	GC, HPLC, electrophoresis
Microstructures	Etching, polishing, reactive ion techniques, ion bombardments, etc.	Microscopy, surface spectroscopy

[a]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 then 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-

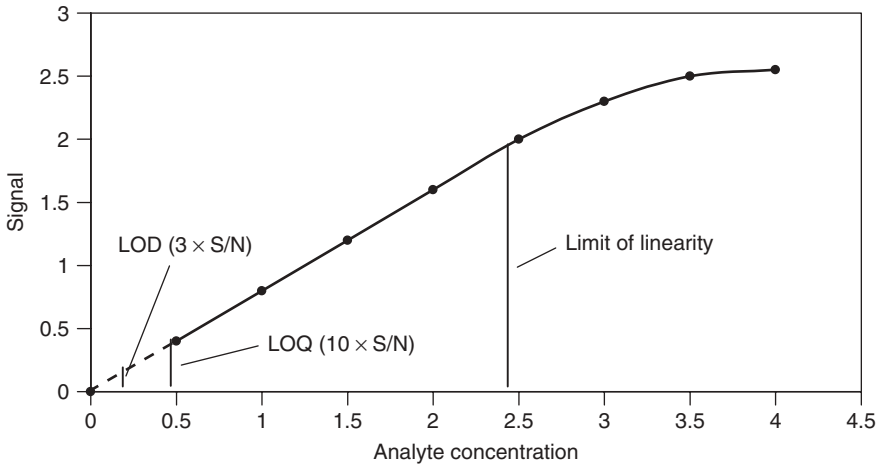

Figure 1.3. Typical calibration curve.

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, σ. This is calculated as

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

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 σ, which is designated s, is computed as follows:

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

As the number of data points becomes larger, the value of s approaches that of σ. When n becomes as large as 20, the equation for σ 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:

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

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

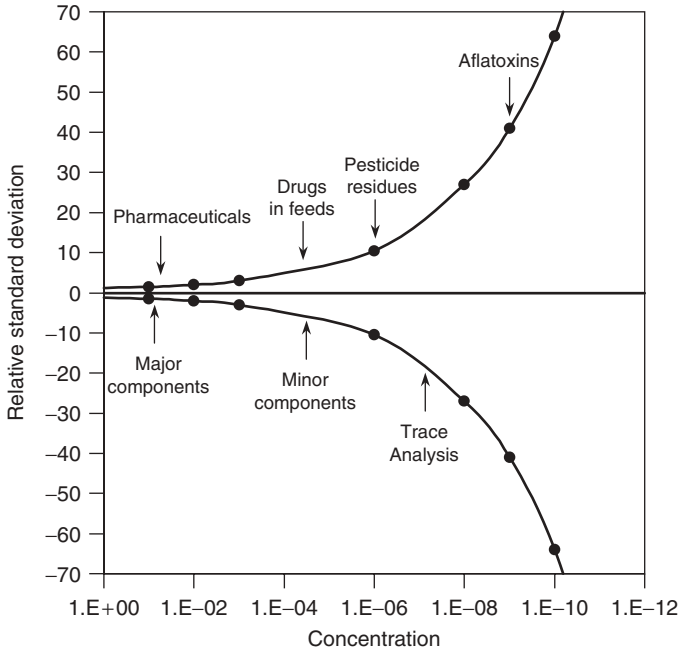

Figure 1.4. Reproducibility as a function of concentration during analytical measurements. (Reproduced from Ref. 3 with permission from LC-GC North America.)

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