

# Chapter 3

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## The Vocabulary of Analytical Chemistry

### Chapter Overview

- 3A Analysis, Determination, and Measurement
- 3B Techniques, Methods, Procedures, and Protocols
- 3C Classifying Analytical Techniques
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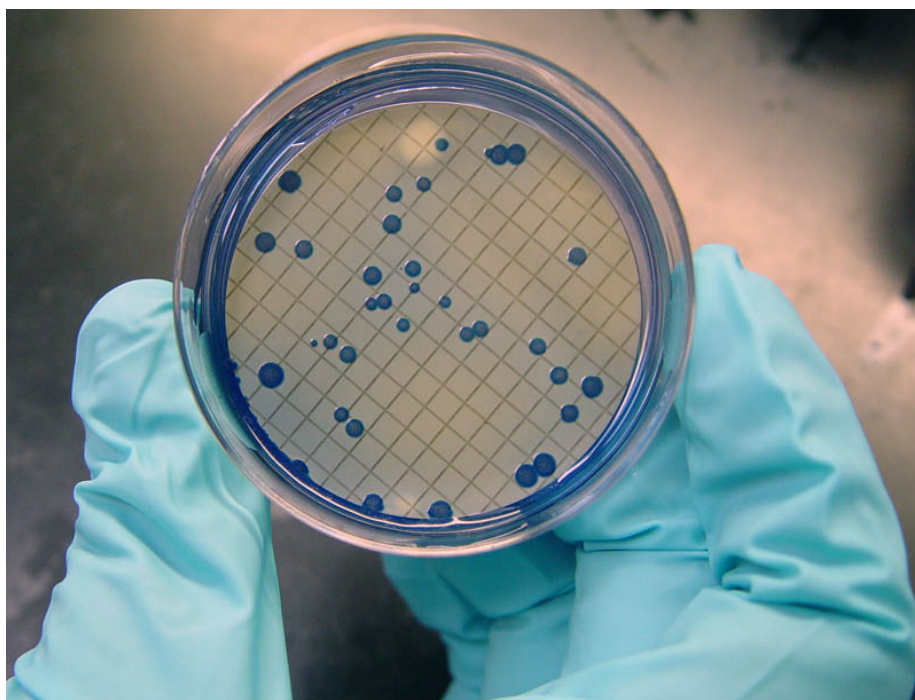
If you browse through an issue of the journal *Analytical Chemistry*, you will discover that the authors and readers share a common vocabulary of analytical terms. You probably are familiar with some of these terms, such as accuracy and precision, but other terms, such as analyte and matrix, are perhaps less familiar to you. In order to participate in any community, one must first understand its vocabulary; the goal of this chapter, therefore, is to introduce some important analytical terms. Becoming comfortable with these terms will make the chapters that follow easier to read and to understand.

### 3A Analysis, Determination and Measurement

The first important distinction we will make is among the terms analysis, determination, and measurement. An analysis provides chemical or physical information about a sample. The component in the sample of interest to us is called the **ANALYTE**, and the remainder of the sample is the **MATRIX**. In an analysis we determine the identity, the concentration, or the properties of an analyte. To make this determination we measure one or more of the analyte's chemical or physical properties.

An example will help clarify the difference between an **ANALYSIS**, a **DETERMINATION** and a **MEASUREMENT**. In 1974 the federal government enacted the Safe Drinking Water Act to ensure the safety of the nation's public drinking water supplies. To comply with this act, municipalities monitor their drinking water supply for potentially harmful substances, such as fecal coliform bacteria. Municipal water departments collect and analyze samples from their water supply. To determine the concentration of fecal coliform bacteria an analyst passes a portion of water through a membrane filter, places the filter in a dish that contains a nutrient broth, and incubates the sample for 22–24 hrs at  $44.5\text{ }^{\circ}\text{C} \pm 0.2\text{ }^{\circ}\text{C}$ . At the end of the incubation period the analyst counts the number of bacterial colonies in the dish and reports the result as the number of colonies per 100 mL (Figure 3.1). Thus, a municipal water department analyzes samples of water to determine the concentration of fecal coliform bacteria by measuring the number of bacterial colonies that form during a carefully defined incubation period.

A fecal coliform count provides a general measure of the presence of pathogenic organisms in a water supply. For drinking water, the current maximum contaminant level (MCL) for total coliforms, including fecal coliforms is less than 1 colony/100 mL. Municipal water departments must regularly test the water supply and must take action if more than 5% of the samples in any month test positive for coliform bacteria.



**Figure 3.1** Colonies of fecal coliform bacteria from a water supply. Source: Susan Boyer. Photo courtesy of ARS–USDA ([www.ars.usda.gov](http://www.ars.usda.gov)).

### 3B Techniques, Methods, Procedures, and Protocols

Suppose you are asked to develop an analytical method to determine the concentration of lead in drinking water. How would you approach this problem? To provide a structure for answering this question, it is helpful to consider four levels of analytical methodology: techniques, methods, procedures, and protocols.<sup>1</sup>

A **TECHNIQUE** is any chemical or physical principle that we can use to study an analyte. There are many techniques for that we can use to determine the concentration of lead in drinking water.<sup>2</sup> In graphite furnace atomic absorption spectroscopy (GFAAS), for example, we first convert aqueous lead ions into free atoms—a process we call atomization. We then measure the amount of light absorbed by the free atoms. Thus, GFAAS uses both a chemical principle (atomization) and a physical principle (absorption of light).

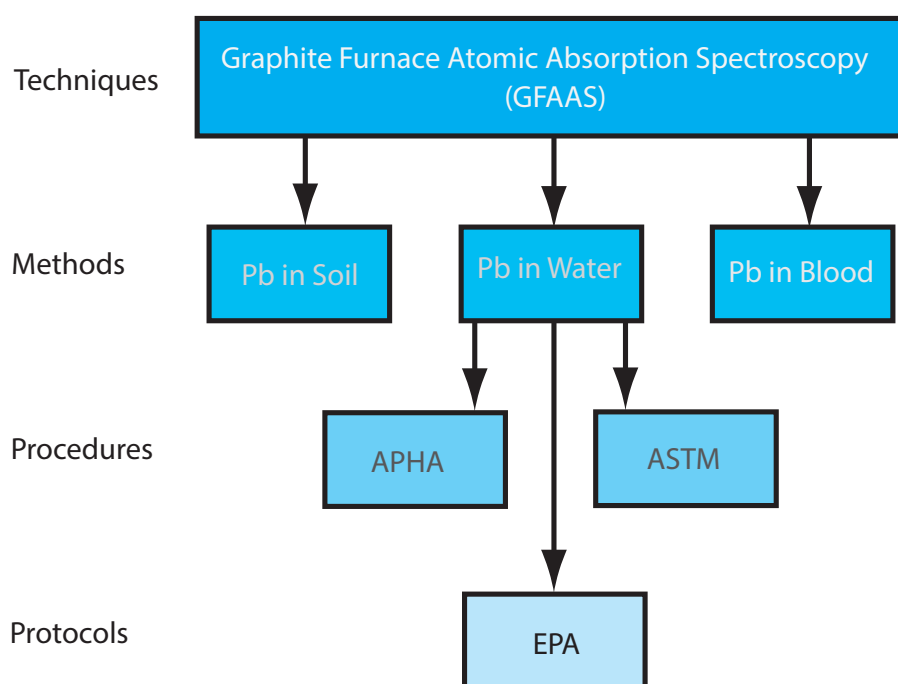
A **METHOD** is the application of a technique for a specific analyte in a specific matrix. As shown in Figure 3.2, the GFAAS method for determining the concentration of lead in water is different from that for lead in soil or blood.

A **PROCEDURE** is a set of written directions that tell us how to apply a method to a particular sample, including information on how to collect the sample, how to handle interferences, and how to validate results. A method may have several procedures as each analyst or agency adapts it to a specific need. As shown in Figure 3.2, the American Public Health Agency and

See Chapter 10 for a discussion of graphite furnace atomic absorption spectroscopy. Chapters 8–13 provide coverage for a range of important analytical techniques.

<sup>1</sup> Taylor, J. K. *Anal. Chem.* **1983**, *55*, 600A–608A.

<sup>2</sup> Fitch, A.; Wang, Y.; Mellican, S.; Macha, S. *Anal. Chem.* **1996**, *68*, 727A–731A.



**Figure 3.2** Chart showing the hierarchical relationship between a technique, methods that use the technique, and procedures and protocols for a method. The abbreviations are APHA: American Public Health Association, ASTM: American Society for Testing Materials, EPA: Environmental Protection Agency.

the American Society for Testing Materials publish separate procedures for determining the concentration of lead in water.

Finally, a **PROTOCOL** is a set of stringent guidelines that specify a procedure that an analyst must follow if an agency is to accept the results. Protocols are common when the result of an analysis supports or defines public policy. When determining the concentration of lead in water under the Safe Drinking Water Act, for example, the analyst must use a protocol specified by the Environmental Protection Agency.

There is an obvious order to these four levels of analytical methodology. Ideally, a protocol uses a previously validated procedure. Before developing and validating a procedure, a method of analysis must be selected. This requires, in turn, an initial screening of available techniques to determine those that have the potential for monitoring the analyte.

### 3C Classifying Analytical Techniques

The analysis of a sample generates a chemical or physical **SIGNAL** that is proportional to the amount of analyte in the sample. This signal may be anything we can measure, such as volume or absorbance. It is convenient to divide analytical techniques into two general classes based on whether the signal is proportional to the mass or moles of analyte, or is proportional to the analyte's concentration.

Consider the two graduated cylinders in Figure 3.3, each of which contains a solution of 0.010 M  $\text{Cu}(\text{NO}_3)_2$ . Cylinder 1 contains 10 mL, or  $1.0 \times 10^{-4}$  moles of  $\text{Cu}^{2+}$ , and cylinder 2 contains 20 mL, or  $2.0 \times 10^{-4}$  moles of  $\text{Cu}^{2+}$ . If a technique responds to the absolute amount of analyte in the sample, then the signal due to the analyte,  $S_A$ , is

$$S_A = k_A n_A \quad 3.1$$

where  $n_A$  is the moles or grams of analyte in the sample, and  $k_A$  is a proportionality constant. Because cylinder 2 contains twice as many moles of  $\text{Cu}^{2+}$  as cylinder 1, analyzing the contents of cylinder 2 gives a signal twice as large that for cylinder 1.

A second class of analytical techniques are those that respond to the analyte's concentration,  $C_A$

$$S_A = k_A C_A \quad 3.2$$

Since the solutions in both cylinders have the same concentration of  $\text{Cu}^{2+}$ , their analysis yields identical signals.

A technique that responds to the absolute amount of analyte is a **TOTAL ANALYSIS TECHNIQUE**. Mass and volume are the most common signals for a total analysis technique, and the corresponding techniques are gravimetry (Chapter 8) and titrimetry (Chapter 9). With a few exceptions, the signal for a total analysis technique is the result of one or more chemical reactions, the stoichiometry of which determines the value of  $k_A$  in equation 3.1.



**Figure 3.3** Two graduated cylinders, each containing 0.010 M  $\text{Cu}(\text{NO}_3)_2$ . Although the cylinders contain the same concentration of  $\text{Cu}^{2+}$ , the cylinder on the left contains  $1.0 \times 10^{-4}$  mol  $\text{Cu}^{2+}$  and the cylinder on the right contains  $2.0 \times 10^{-4}$  mol  $\text{Cu}^{2+}$ .

Historically, most early analytical methods used a total analysis technique. For this reason, total analysis techniques are often called “classical” techniques.

Spectroscopy (Chapter 10) and electrochemistry (Chapter 11), in which an optical or an electrical signal is proportional to the relative amount of analyte in a sample, are examples of **CONCENTRATION TECHNIQUES**. The relationship between the signal and the analyte's concentration is a theoretical function that depends on experimental conditions and the instrumentation used to measure the signal. For this reason the value of  $k_A$  in [equation 3.2](#) is determined experimentally.

Since most concentration techniques rely on measuring an optical or electrical signal, they also are known as “instrumental” techniques.

### 3D Selecting an Analytical Method

A method is the application of a technique to a specific analyte in a specific matrix. We can develop an analytical method to determine the concentration of lead in drinking water using any of the techniques mentioned in the previous section. A gravimetric method, for example, might precipitate the lead as  $\text{PbSO}_4$  or as  $\text{PbCrO}_4$ , and use the precipitate's mass as the analytical signal. Lead forms several soluble complexes, which we can use to design a complexation titrimetric method. As shown in [Figure 3.2](#), we can use graphite furnace atomic absorption spectroscopy to determine the concentration of lead in drinking water. Finally, lead's multiple oxidation states ( $\text{Pb}^0$ ,  $\text{Pb}^{2+}$ ,  $\text{Pb}^{4+}$ ) makes feasible a variety of electrochemical methods.

Ultimately, the requirements of the analysis determine the best method. In choosing among the available methods, we give consideration to some or all the following design criteria: accuracy, precision, sensitivity, selectivity, robustness, ruggedness, scale of operation, analysis time, availability of equipment, and cost.

#### 3D.1 Accuracy

**ACCURACY** is how closely the result of an experiment agrees with the “true” or expected result. We can express accuracy as an absolute error,  $e$

$$e = \text{obtained result} - \text{expected result}$$

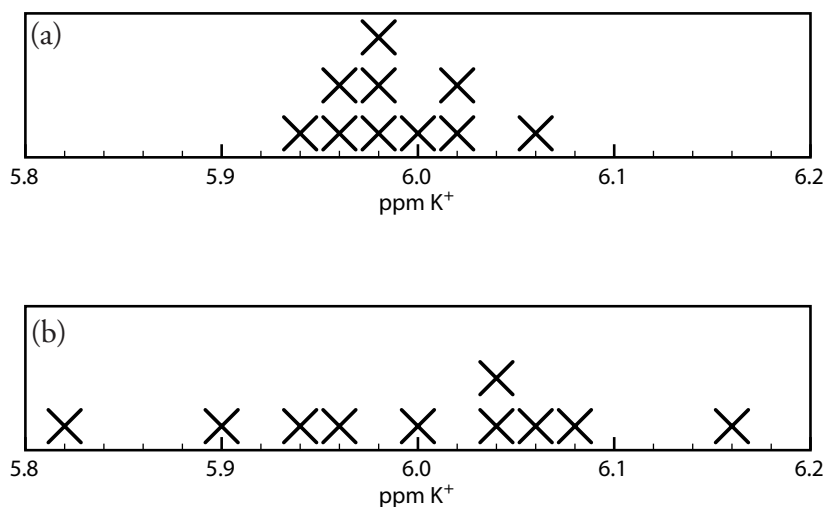
or as a percentage relative error,  $\%e_r$ ,

$$\%e_r = \frac{\text{obtained result} - \text{expected result}}{\text{expected result}} \times 100$$

A method's accuracy depends on many things, including the signal's source, the value of  $k_A$  in [equation 3.1](#) or [equation 3.2](#), and the ease of handling samples without loss or contamination. A total analysis technique, such as gravimetry and titrimetry, often produce more accurate results than does a concentration technique because we can measure mass and volume with high accuracy, and because the value of  $k_A$  is known exactly through stoichiometry.

Because it is unlikely that we know the true result, we use an expected or accepted result to evaluate accuracy. For example, we might use a standard reference material, which has an accepted value, to establish an analytical method's accuracy.

You will find a more detailed treatment of accuracy in Chapter 4, including a discussion of sources of errors.



**Figure 3.4** Two determinations of the concentration of  $K^+$  in serum, showing the effect of precision on the distribution of individual results. The data in (a) are less scattered and, therefore, more precise than the data in (b).

### 3D.2 Precision

When a sample is analyzed several times, the individual results vary from trial-to-trial. **PRECISION** is a measure of this variability. The closer the agreement between individual analyses, the more precise the results. For example, the results shown in Figure 3.4(a) for the concentration of  $K^+$  in a sample of serum are more precise than those in Figure 3.4(b). It is important to understand that precision does not imply accuracy. That the data in Figure 3.4(a) are more precise does not mean that the first set of results is more accurate. In fact, neither set of results may be accurate.

A method's precision depends on several factors, including the uncertainty in measuring the signal and the ease of handling samples reproducibly. In most cases we can measure the signal for a total analysis technique with a higher precision than is the case for a concentration method. Precision is covered in more detail in Chapter 4.

### 3D.3 Sensitivity

The ability to demonstrate that two samples have different amounts of analyte is an essential part of many analyses. A method's **SENSITIVITY** is a measure of its ability to establish that such a difference is significant. Sensitivity is often confused with a method's **DETECTION LIMIT**, which is the smallest amount of analyte we can determine with confidence.

Sensitivity is equivalent to the proportionality constant,  $k_A$ , in [equation 3.1](#) and [equation 3.2](#).<sup>3</sup> If  $\Delta S_A$  is the smallest difference we can measure

Confusing accuracy and precision is a common mistake. See Ryder, J.; Clark, A. *U. Chem. Ed.* **2002**, *6*, 1–3, and Tomlinson, J.; Dyson, P. J.; Garratt, J. *U. Chem. Ed.* **2001**, *5*, 16–23 for discussions of this and other common misconceptions about the meaning of error.

You will find a more detailed treatment of precision in Chapter 4, including a discussion of sources of errors.

Confidence, as we will see in Chapter 4, is a statistical concept that builds on the idea of a population of results. For this reason, we will postpone our discussion of detection limits to Chapter 4. For now, the definition of a detection limit given here is sufficient.

<sup>3</sup> IUPAC Compendium of Chemical Terminology, Electronic version, <http://goldbook.iupac.org/S05606.html>.



between two signals, then the smallest detectable difference in the absolute amount or the relative amount of analyte is

$$\Delta n_A = \frac{\Delta S_A}{k_A} \quad \text{or} \quad \Delta C_A = \frac{\Delta S_A}{k_A}$$

Suppose, for example, that our analytical signal is a measurement of mass using a balance whose smallest detectable increment is  $\pm 0.0001$  g. If our method's sensitivity is 0.200, then our method can conceivably detect a difference in mass of as little as

$$\Delta n_A = \frac{\pm 0.0001 \text{ g}}{0.200} = \pm 0.0005 \text{ g}$$

For two methods with the same  $\Delta S_A$ , the method with the greater sensitivity—that is, the method with the larger  $k_A$ —is better able to discriminate between smaller amounts of analyte.

### 3D.4 Specificity and Selectivity

An analytical method is specific if its signal depends only on the analyte.<sup>4</sup> Although **SPECIFICITY** is the ideal, few analytical methods are free from interferences. When an **INTERFERENT** contributes to the signal, we expand [equation 3.1](#) and [equation 3.2](#) to include its contribution to the sample's signal,  $S_{\text{samp}}$

$$S_{\text{samp}} = S_A + S_I = k_A n_A + k_I n_I \quad 3.3$$

$$S_{\text{samp}} = S_A + S_I = k_A C_A + k_I C_I \quad 3.4$$

where  $S_I$  is the interferent's contribution to the signal,  $k_I$  is the interferent's sensitivity, and  $n_I$  and  $C_I$  are the moles (or grams) and the concentration of interferent in the sample, respectively.

**SELECTIVITY** is a measure of a method's freedom from interferences.<sup>5</sup> A method's selectivity for an interferent relative to the analyte is defined by a **SELECTIVITY COEFFICIENT**,  $K_{A,I}$

$$K_{A,I} = \frac{k_I}{k_A} \quad 3.5$$

which may be positive or negative depending on the sign of  $k_I$  and  $k_A$ . The selectivity coefficient is greater than +1 or less than -1 when the method is more selective for the interferent than for the analyte.

Determining the selectivity coefficient's value is easy if we already know the values for  $k_A$  and  $k_I$ . As shown by [Example 3.1](#), we also can determine  $K_{A,I}$  by measuring  $S_{\text{samp}}$  in the presence of and in the absence of the interferent.

Although  $k_A$  and  $k_I$  usually are positive, they can be negative. For example, some analytical methods work by measuring the concentration of a species that remains after it reacts with the analyte. As the analyte's concentration increases, the concentration of the species that produces the signal decreases, and the signal becomes smaller. If the signal in the absence of analyte is assigned a value of zero, then the subsequent signals are negative.

<sup>4</sup> (a) Persson, B-A; Vessman, J. *Trends Anal. Chem.* **1998**, *17*, 117–119; (b) Persson, B-A; Vessman, J. *Trends Anal. Chem.* **2001**, *20*, 526–532.

<sup>5</sup> Valcárcel, M.; Gomez-Hens, A.; Rubio, S. *Trends Anal. Chem.* **2001**, *20*, 386–393.

If you are unsure why the signal in the presence of zinc is 100.5, note that the percentage relative error for this problem is given by

$$\frac{\text{obtained result} - 100}{100} \times 100 = +0.5\%$$

Solving gives an obtained result of 100.5.

### Example 3.1

A method for the analysis of  $\text{Ca}^{2+}$  in water suffers from an interference in the presence of  $\text{Zn}^{2+}$ . When the concentration of  $\text{Ca}^{2+}$  is 100 times greater than that of  $\text{Zn}^{2+}$ , an analysis for  $\text{Ca}^{2+}$  has a relative error of +0.5%. What is the selectivity coefficient for this method?

#### SOLUTION

Since only relative concentrations are reported, we can arbitrarily assign absolute concentrations. To make the calculations easy, we will let  $C_{\text{Ca}} = 100$  (arbitrary units) and  $C_{\text{Zn}} = 1$ . A relative error of +0.5% means the signal in the presence of  $\text{Zn}^{2+}$  is 0.5% greater than the signal in the absence of  $\text{Zn}^{2+}$ . Again, we can assign values to make the calculation easier. If the signal for  $\text{Ca}^{2+}$  in the absence of  $\text{Zn}^{2+}$  is 100 (arbitrary units), then the signal in the presence of  $\text{Zn}^{2+}$  is 100.5.

The value of  $k_{\text{Ca}}$  is determined using [equation 3.2](#)

$$k_{\text{Ca}} = \frac{S_{\text{Ca}}}{C_{\text{Ca}}} = \frac{100}{100} = 1$$

In the presence of  $\text{Zn}^{2+}$  the signal is given by [equation 3.4](#); thus

$$S_{\text{samp}} = 100.5 = k_{\text{Ca}} C_{\text{Ca}} + k_{\text{Zn}} C_{\text{Zn}} = (1 \times 100) + k_{\text{Zn}} \times 1$$

Solving for  $k_{\text{Zn}}$  gives its value as 0.5. The selectivity coefficient is

$$K_{\text{Ca,Zn}} = \frac{k_{\text{Zn}}}{k_{\text{Ca}}} = \frac{0.5}{1} = 0.5$$

### Practice Exercise 3.1

Wang and colleagues describe a fluorescence method for the analysis of  $\text{Ag}^+$  in water. When analyzing a solution that contains  $1.0 \times 10^{-9}$  M  $\text{Ag}^+$  and  $1.1 \times 10^{-7}$  M  $\text{Ni}^{2+}$ , the fluorescence intensity (the signal) was +4.9% greater than that obtained for a sample of  $1.0 \times 10^{-9}$  M  $\text{Ag}^+$ . What is  $K_{\text{Ag,Ni}}$  for this analytical method? The full citation for the data in this exercise is Wang, L.; Liang, A. N.; Chen, H.; Liu, Y.; Qian, B.; Fu, J. *Anal. Chim. Acta* **2008**, 616, 170-176.

Click [here](#) to review your answer to this exercise.

A selectivity coefficient provides us with a useful way to evaluate an interferent's potential effect on an analysis. Solving [equation 3.5](#) for  $k_I$

$$k_I = K_{A,I} \times k_A \quad 3.6$$

substituting in [equation 3.3](#) and [equation 3.4](#), and simplifying gives

$$S_{\text{samp}} = k_A \{n_A + K_{A,I} \times n_I\} \quad 3.7$$

$$S_{\text{samp}} = k_A \{C_A + K_{A,I} \times C_I\} \quad 3.8$$



An interferent will not pose a problem as long as the term  $K_{A,I} \times n_I$  in [equation 3.7](#) is significantly smaller than  $n_A$ , or if  $K_{A,I} \times C_I$  in [equation 3.8](#) is significantly smaller than  $C_A$ .

### Example 3.2

Barnett and colleagues developed a method to determine the concentration of codeine in poppy plants.<sup>6</sup> As part of their study they evaluated the effect of several interferents. For example, the authors found that equimolar solutions of codeine and the interferent 6-methoxycodeine gave signals, respectively of 40 and 6 (arbitrary units).

- What is the selectivity coefficient for the interferent, 6-methoxycodeine, relative to that for the analyte, codeine.
- If we need to know the concentration of codeine with an accuracy of  $\pm 0.50\%$ , what is the maximum relative concentration of 6-methoxycodeine that we can tolerate?

#### SOLUTION

- The signals due to the analyte,  $S_A$ , and the interferent,  $S_I$ , are

$$S_A = k_A C_A \quad S_I = k_I C_I$$

Solving these equations for  $k_A$  and for  $k_I$ , and substituting into [equation 3.6](#) gives

$$K_{A,I} = \frac{S_I/C_I}{S_A/C_A}$$

Because the concentrations of analyte and interferent are equimolar ( $C_A = C_I$ ), the selectivity coefficient is

$$K_{A,I} = \frac{S_I}{S_A} = \frac{6}{40} = 0.15$$

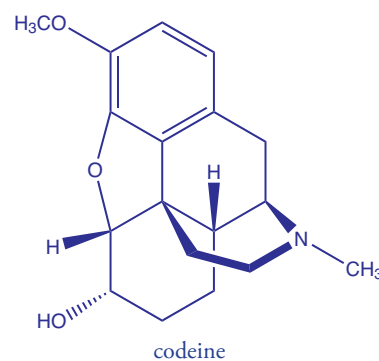
- To achieve an accuracy of better than  $\pm 0.50\%$  the term  $K_{A,I} \times C_I$  in [equation 3.8](#) must be less than 0.50% of  $C_A$ ; thus

$$K_{A,I} \times C_I \leq 0.0050 \times C_A$$

Solving this inequality for the ratio  $C_I/C_A$  and substituting in the value for  $K_{A,I}$  from part (a) gives

$$\frac{C_I}{C_A} \leq \frac{0.0050}{K_{A,I}} = \frac{0.0050}{0.15} = 0.033$$

Therefore, the concentration of 6-methoxycodeine must be less than 3.3% of codeine's concentration.



When a method's signal is the result of a chemical reaction—for example, when the signal is the mass of a precipitate—there is a good chance that the method is not very selective and that it is susceptible to an interference.

<sup>6</sup> Barnett, N. W.; Bowser, T. A.; Geraldi, R. D.; Smith, B. *Anal. Chim. Acta* **1996**, 318, 309–317.

Look back at [Figure 1.1](#), which shows Fresenius' analytical method for the determination of nickel in ores. The reason there are so many steps in this procedure is that precipitation reactions generally are not very selective. The method in [Figure 1.2](#) includes fewer steps because dimethylglyoxime is a more selective reagent. Even so, if an ore contains palladium, additional steps are needed to prevent the palladium from interfering.

### Practice Exercise 3.2

Mercury (II) also is an interferent in the fluorescence method for  $\text{Ag}^+$  developed by Wang and colleagues (see [Practice Exercise 3.1](#) for the citation). The selectivity coefficient,  $K_{\text{Ag,Hg}}$  has a value of  $-1.0 \times 10^{-3}$ .

- What is the significance of the selectivity coefficient's negative sign?
- Suppose you plan to use this method to analyze solutions with concentrations of  $\text{Ag}^+$  no smaller than 1.0 nM. What is the maximum concentration of  $\text{Hg}^{2+}$  you can tolerate if your percentage relative errors must be less than  $\pm 1.0\%$ ?

Click [here](#) to review your answers to this exercise.

Problems with selectivity also are more likely when the analyte is present at a very low concentration.<sup>7</sup>

### 3D.5 Robustness and Ruggedness

For a method to be useful it must provide reliable results. Unfortunately, methods are subject to a variety of chemical and physical interferences that contribute uncertainty to the analysis. If a method is relatively free from chemical interferences, we can use it to analyze an analyte in a wide variety of sample matrices. Such methods are considered **ROBUST**.

Random variations in experimental conditions introduces uncertainty. If a method's sensitivity,  $k$ , is too dependent on experimental conditions, such as temperature, acidity, or reaction time, then a slight change in any of these conditions may give a significantly different result. A **RUGGED** method is relatively insensitive to changes in experimental conditions.

### 3D.6 Scale of Operation

Another way to narrow the choice of methods is to consider three potential limitations: the amount of sample available for the analysis, the expected concentration of analyte in the samples, and the minimum amount of analyte that will produce a measurable signal. Collectively, these limitations define the analytical method's scale of operations.

We can display the scale of operations visually ([Figure 3.5](#)) by plotting the sample's size on the  $x$ -axis and the analyte's concentration on the  $y$ -axis.<sup>8</sup> For convenience, we divide samples into macro ( $>0.1$  g), meso (10 mg–100 mg), micro (0.1 mg–10 mg), and ultramicro ( $<0.1$  mg) sizes, and we divide analytes into major ( $>1\%$  w/w), minor (0.01% w/w–1% w/w), trace ( $10^{-7}\%$  w/w–0.01% w/w), and ultratrace ( $<10^{-7}\%$  w/w) components. Together, the analyte's concentration and the sample's size provide a characteristic description for an analysis. For example, in a microtrace analysis the

<sup>7</sup> Rodgers, L. B. *J. Chem. Educ.* **1986**, *63*, 3–6.

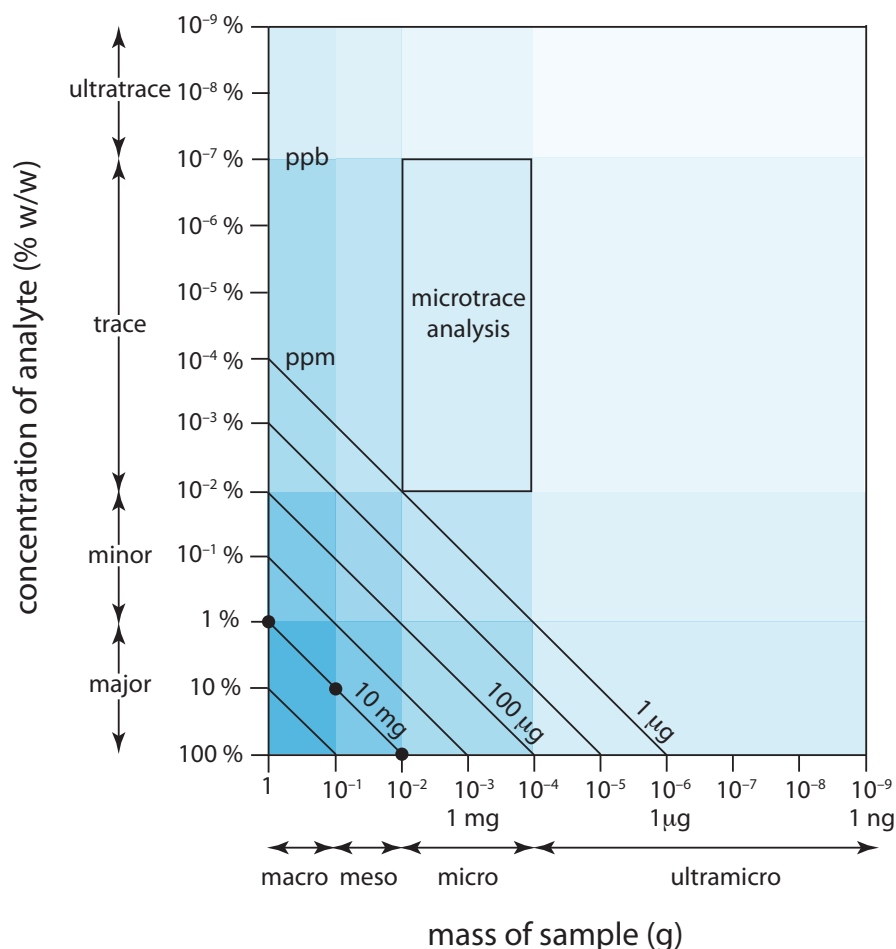
<sup>8</sup> (a) Sandell, E. B.; Elving, P. J. in Kolthoff, I. M.; Elving, P. J., eds. *Treatise on Analytical Chemistry*, Interscience: New York, Part I, Vol. 1, Chapter 1, pp. 3–6; (b) Potts, L. W. *Quantitative Analysis—Theory and Practice*, Harper and Row: New York, 1987, pp. 12.

sample weighs between 0.1 mg and 10 mg and contains a concentration of analyte between  $10^{-7}\%$  w/w and  $10^{-2}\%$  w/w.

The diagonal lines connecting the axes show combinations of sample size and analyte concentration that contain the same absolute mass of analyte. As shown in Figure 3.5, for example, a 1-g sample that is 1% w/w analyte has the same amount of analyte (10 mg) as a 100-mg sample that is 10% w/w analyte, or a 10-mg sample that is 100% w/w analyte.

We can use Figure 3.5 to establish limits for analytical methods. If a method's minimum detectable signal is equivalent to 10 mg of analyte, then it is best suited to a major analyte in a macro or meso sample. Extending the method to an analyte with a concentration of 0.1% w/w requires a sample of 10 g, which rarely is practical due to the complications of carrying such a large amount of material through the analysis. On the other hand, a small sample that contains a trace amount of analyte places significant restrictions on an analysis. For example, a 1-mg sample that is  $10^{-4}\%$  w/w in analyte contains just 1 ng of analyte. If we isolate the analyte in 1 mL of solution, then we need an analytical method that reliably can detect it at a concentration of 1 ng/mL.

It should not surprise you to learn that a total analysis technique typically requires a macro or a meso sample that contains a major analyte. A concentration technique is particularly useful for a minor, trace, or ultratrace analyte in a macro, meso, or micro sample.



**Figure 3.5** Scale of operations for analytical methods (adapted from references 8a and 8b).

The shaded areas define different types of analyses. The boxed area, for example, represents a microtrace analysis.

The diagonal lines show combinations of sample size and analyte concentration that contain the same mass of analyte. The three filled circles (●), for example, indicate analyses that use 10 mg of analyte.

### 3D.7 Equipment, Time, and Cost

Finally, we can compare analytical methods with respect to their equipment needs, the time needed to complete an analysis, and the cost per sample. Methods that rely on instrumentation are equipment-intensive and may require significant operator training. For example, the graphite furnace atomic absorption spectroscopic method for determining lead in water requires a significant capital investment in the instrument and an experienced operator to obtain reliable results. Other methods, such as titrimetry, require less expensive equipment and less training.

The time to complete an analysis for one sample often is fairly similar from method-to-method. This is somewhat misleading, however, because much of this time is spent preparing samples, preparing reagents, and gathering together equipment. Once the samples, reagents, and equipment are in place, the sampling rate may differ substantially. For example, it takes just a few minutes to analyze a single sample for lead using graphite furnace atomic absorption spectroscopy, but several hours to analyze the same sample using gravimetry. This is a significant factor in selecting a method for a laboratory that handles a high volume of samples.

The cost of an analysis depends on many factors, including the cost of equipment and reagents, the cost of hiring analysts, and the number of samples that can be processed per hour. In general, methods that rely on instruments cost more per sample than other methods.

### 3D.8 Making the Final Choice

Unfortunately, the design criteria discussed in this section are not mutually independent.<sup>9</sup> Working with smaller samples or improving selectivity often comes at the expense of precision. Minimizing cost and analysis time may decrease accuracy. Selecting a method requires carefully balancing the various design criteria. Usually, the most important design criterion is accuracy, and the best method is the one that gives the most accurate result. When the need for a result is urgent, as is often the case in clinical labs, analysis time may become the critical factor.

In some cases it is the sample's properties that determine the best method. A sample with a complex matrix, for example, may require a method with excellent selectivity to avoid interferences. Samples in which the analyte is present at a trace or ultratrace concentration usually require a concentration method. If the quantity of sample is limited, then the method must not require a large amount of sample.

Determining the concentration of lead in drinking water requires a method that can detect lead at the parts per billion concentration level. Selectivity is important because other metal ions are present at significantly higher concentrations. A method that uses graphite furnace atomic absorption spectroscopy is a common choice for determining lead in drinking

<sup>9</sup> Valcárcel, M.; Ríos, A. *Anal. Chem.* **1993**, *65*, 781A–787A.

water because it meets these specifications. The same method is also useful for determining lead in blood where its ability to detect low concentrations of lead using a few microliters of sample is an important consideration.

### 3E Developing the Procedure

After selecting a method, the next step is to develop a procedure that accomplish our goals for the analysis. In developing a procedure we give attention to compensating for interferences, to selecting and calibrating equipment, to acquiring a representative sample, and to validating the method.

#### 3E.1 Compensating for Interferences

A method's accuracy depends on its selectivity for the analyte. Even the best method, however, may not be free from interferents that contribute to the measured signal. Potential interferents may be present in the sample itself or in the reagents used during the analysis.

When the sample is free of interferents, the total signal,  $S_{total}$ , is a sum of the signal due to the analyte,  $S_A$ , and the signal due to interferents in the reagents,  $S_{reag}$ ,

$$S_{total} = S_A + S_{reag} = k_A n_A + S_{reag} \quad 3.9$$

$$S_{total} = S_A + S_{reag} = k_A C_A + S_{reag} \quad 3.10$$

Without an independent determination of  $S_{reag}$  we cannot solve equation 3.9 or 3.10 for the moles or concentration of analyte.

To determine the contribution of  $S_{reag}$  in equations 3.9 and 3.10 we measure the signal for a **METHOD BLANK**, a solution that does not contain the sample. Consider, for example, a procedure in which we dissolve a 0.1-g sample in a portion of solvent, add several reagents, and dilute to 100 mL with additional solvent. To prepare the method blank we omit the sample and dilute the reagents to 100 mL using the solvent. Because the analyte is absent,  $S_{total}$  for the method blank is equal to  $S_{reag}$ . Knowing the value for  $S_{reag}$  makes it is easy to correct  $S_{total}$  for the reagent's contribution to the total signal; thus

$$(S_{total} - S_{reag}) = S_A = k_A n_A$$

$$(S_{total} - S_{reag}) = S_A = k_A C_A$$

A method blank also is known as a reagent blank.

When the sample is a liquid, or is in solution, we use an equivalent volume of an inert solvent as a substitute for the sample.

By itself, a method blank cannot compensate for an interferent that is part of the sample's matrix. If we happen to know the interferent's identity and concentration, then we can be add it to the method blank; however, this is not a common circumstance and we must, instead, find a method for separating the analyte and interferent before continuing the analysis.

Methods for effecting this separation are discussed in Chapter 7.

Chapter 7 provides a more detailed discussion of sampling, including strategies for obtaining representative samples.

**Figure 3.6** Example of a calibration curve. The filled circles (●) are the results for five standard samples, each with a different concentration of analyte, and the line is the best fit to the data determined by a linear regression analysis. See Chapter 5 for a further discussion of calibration curves and an explanation of linear regression.

### 3E.2 Calibration

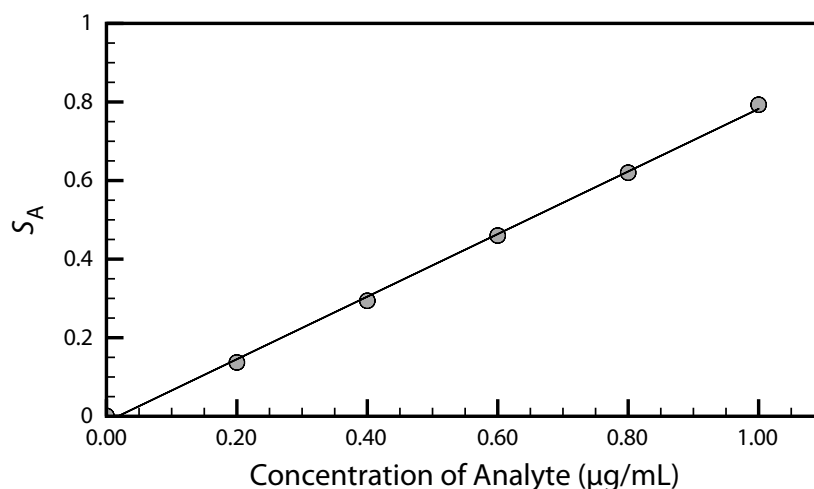
A simple definition of a quantitative analytical method is that it is a mechanism for converting a measurement, the signal, into the amount of analyte in a sample. Assuming we can correct for interferences, a quantitative analysis is nothing more than solving [equation 3.1](#) or [equation 3.2](#) for  $n_A$  or for  $C_A$ .

To solve these equations we need the value of  $k_A$ . For a total analysis method usually we know the value of  $k_A$  because it is defined by the stoichiometry of the chemical reactions responsible for the signal. For a concentration method, however, the value of  $k_A$  usually is a complex function of experimental conditions. A **CALIBRATION** is the process of experimentally determining the value of  $k_A$  by measuring the signal for one or more standard samples, each of which contains a known concentration of analyte. With a single standard we can calculate the value of  $k_A$  using [equation 3.1](#) or [equation 3.2](#). When using several standards with different concentrations of analyte, the result is best viewed visually by plotting  $S_A$  versus the concentration of analyte in the standards. Such a plot is known as a **CALIBRATION CURVE**, an example of which is shown in Figure 3.6.

### 3E.3 Sampling

Selecting an appropriate method and executing it properly helps us ensure that our analysis is accurate. If we analyze the wrong sample, however, then the accuracy of our work is of little consequence.

A proper sampling strategy ensures that our samples are representative of the material from which they are taken. Biased or nonrepresentative sampling, and contaminating samples during or after their collection are two examples of sampling errors that can lead to a significant error in accuracy. It is important to realize that sampling errors are independent of errors in the analytical method. As a result, we cannot correct a sampling error in the laboratory by, for example, evaluating a reagent blank.





### 3E.4 Validation

If we are to have confidence in our procedure we must demonstrate that it can provide acceptable results, a process we call **VALIDATION**. Perhaps the most important part of validating a procedure is establishing that its precision and accuracy are appropriate for the problem we are trying to solve. We also ensure that the written procedure has sufficient detail so that different analysts or laboratories will obtain comparable results. Ideally, validation uses a standard sample whose composition closely matches the samples we will analyze. In the absence of appropriate standards, we can evaluate accuracy by comparing results to those obtained using a method of known accuracy.

You will find more details about validating analytical methods in Chapter 14.

### 3F Protocols

Earlier we defined a protocol as a set of stringent written guidelines that specify an exact procedure that we must follow if an agency is to accept the results of our analysis. In addition to the considerations that went into the procedure's design, a protocol also contains explicit instructions regarding internal and external quality assurance and quality control (QA/QC) procedures.<sup>10</sup> The goal of internal QA/QC is to ensure that a laboratory's work is both accurate and precise. External QA/QC is a process in which an external agency certifies a laboratory.

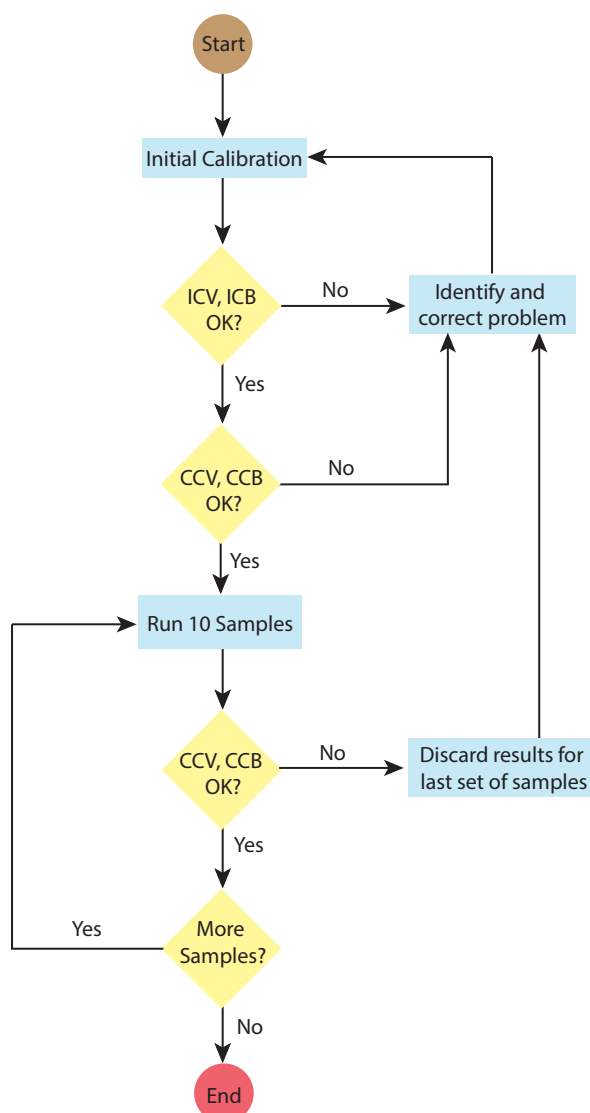
As an example, let's outline a portion of the Environmental Protection Agency's protocol for determining trace metals in water by graphite furnace atomic absorption spectroscopy as part of its Contract Laboratory Program (CLP). The CLP protocol (see [Figure 3.7](#)) calls for an initial calibration using a method blank and three standards, one of which is at the detection limit. The resulting calibration curve is verified by analyzing initial calibration verification (ICV) and initial calibration blank (ICB) samples. The lab's result for the ICV sample must fall within  $\pm 10\%$  of its expected concentration. If the result is outside this limit the analysis is stopped and the problem identified and corrected before continuing.

After a successful analysis of the ICV and ICB samples, the lab re verifies the calibration by analyzing a continuing calibration verification (CCV) sample and a continuing calibration blank (CCB). Results for the CCV also must be within  $\pm 10\%$  of its expected concentration. Again, if the lab's result for the CCV is outside the established limits, the analysis is stopped, the problem identified and corrected, and the system recalibrated as described above. Additional CCV and the CCB samples are analyzed before the first sample and after the last sample, and between every set of ten samples. If the result for any CCV or CCB sample is unacceptable, the results for the last set of samples are discarded, the system is recalibrated, and the samples reanalyzed. By following this protocol, each result is bound by successful

<sup>10</sup> (a) Amore, F. *Anal. Chem.* **1979**, *51*, 1105A–1110A; (b) Taylor, J. K. *Anal. Chem.* **1981**, *53*, 1588A–1593A.

**Figure 3.7** Schematic diagram showing a portion of the EPA's protocol for determining trace metals in water using graphite furnace atomic absorption spectrometry.

The abbreviations are ICV: initial calibration verification; ICB: initial calibration blank; CCV: continuing calibration verification; CCB: continuing calibration blank.



checks on the calibration. Although not shown in Figure 3.7, the protocol also contains instructions for analyzing duplicate or split samples, and for using spike tests to verify accuracy.

### 3G The Importance of Analytical Methodology

The importance of the issues raised in this chapter is evident if we examine environmental monitoring programs. The purpose of a monitoring program is to determine the present status of an environmental system, and to assess long term trends in the system's health. These are broad and poorly defined goals. In many cases, an environmental monitoring program begins before the essential questions are known. This is not surprising since it is difficult to formulate questions in the absence of results. Without careful planning, however, a poor experimental design may result in data that has little value.

These concerns are illustrated by the Chesapeake Bay Monitoring Program. This research program, designed to study nutrients and toxic pollutants in the Chesapeake Bay, was initiated in 1984 as a cooperative venture between the federal government, the state governments of Maryland, Virginia, and Pennsylvania, and the District of Columbia. A 1989 review of the program highlights the problems common to many monitoring programs.<sup>11</sup>

At the beginning of the Chesapeake Bay monitoring program, little attention was given to selecting analytical methods, in large part because the eventual use of the data was not yet specified. The analytical methods initially chosen were standard methods already approved by the Environmental Protection Agency (EPA). In many cases these methods were not useful because they were designed to detect pollutants at their legally mandated maximum allowed concentrations. In unpolluted waters, however, the concentrations of these contaminants often are well below the detection limit of the EPA methods. For example, the detection limit for the EPA approved standard method for phosphate was 7.5 ppb. Since the actual phosphate concentrations in Chesapeake Bay were below the EPA method's detection limit, it provided no useful information. On the other hand, the detection limit for a non-approved variant of the EPA method, a method routinely used by chemical oceanographers, was 0.06 ppb, a more realistic detection limit for their samples. In other cases, such as the elemental analysis for particulate forms of carbon, nitrogen and phosphorous, EPA approved procedures provided poorer reproducibility than nonapproved methods.

### 3H Key Terms

accuracy	analysis	analyte
calibration	calibration curve	concentration techniques
detection limit	determination	interferent
matrix	measurement	method
method blank	precision	procedure
protocol	QA/QC	robust
rugged	selectivity	selectivity coefficient
sensitivity	signal	specificity
technique	total analysis techniques	validation

### 3I Chapter Summary

Every discipline has its own vocabulary and your success in studying analytical chemistry will improve if you master this vocabulary. Be sure you understand the difference between an analyte and its matrix, between a technique and a method, between a procedure and a protocol, and between a total analysis technique and a concentration technique.

<sup>11</sup> D'Elia, C. F.; Sanders, J. G.; Capone, D. G. *Environ. Sci. Technol.* **1989**, *23*, 768–774.

In selecting an analytical method we consider criteria such as accuracy, precision, sensitivity, selectivity, robustness, ruggedness, the amount of available sample, the amount of analyte in the sample, time, cost, and the availability of equipment. These criteria are not mutually independent, and often it is necessary to find an acceptable balance between them.

In developing a procedure or protocol, we give consideration to compensating for interferences, calibrating the method, obtaining an appropriate sample, and validating the analysis. Poorly designed procedures and protocols produce results that are insufficient to meet the needs of the analysis.

### 3J Problems

1. When working with a solid sample, often it is necessary to bring the analyte into solution by digesting the sample with a suitable solvent. Any remaining solid impurities are removed by filtration before continuing with the analysis. In a typical total analysis method, the procedure might read

After digesting the sample in a beaker using approximately 25 mL of solvent, remove any solid impurities that remain by passing the solution the analyte through filter paper, collecting the filtrate in a clean Erlenmeyer flask. Rinse the beaker with several small portions of solvent, passing these rinsings through the filter paper and collecting them in the same Erlenmeyer flask. Finally, rinse the filter paper with several portions of solvent, collecting the rinsings in the same Erlenmeyer flask.

For a typical concentration method, however, the procedure might state

After digesting the sample in a beaker using 25.00 mL of solvent, remove any solid impurities by filtering a portion of the solution containing the analyte. Collect and discard the first several mL of filtrate before collecting a sample of 5.00 mL for further analysis.

Explain why these two procedures are different.

2. A certain concentration method works best when the analyte's concentration is approximately 10 ppb.
  - (a) If the method requires a sample of 0.5 mL, about what mass of analyte is being measured?
  - (b) If the analyte is present at 10% w/v, how would you prepare the sample for analysis?
  - (c) Repeat for the case where the analyte is present at 10% w/w.

- (d) Based on your answers to parts (a)–(c), comment on the method's suitability for the determination of a major analyte.
3. An analyst needs to evaluate the potential effect of an interferent,  $I$ , on the quantitative analysis for an analyte,  $A$ . She begins by measuring the signal for a sample in which the interferent is absent and the analyte is present with a concentration of 15 ppm, obtaining an average signal of 23.3 (arbitrary units). When she analyzes a sample in which the analyte is absent and the interferent is present with a concentration of 25 ppm, she obtains an average signal of 13.7.
- (a) What is the sensitivity for the analyte?
- (b) What is the sensitivity for the interferent?
- (c) What is the value of the selectivity coefficient?
- (d) Is the method more selective for the analyte or the interferent?
- (e) What is the maximum concentration of interferent relative to that of the analyte if the error in the analysis is to be less than 1%?
4. A sample is analyzed to determine the concentration of an analyte. Under the conditions of the analysis the sensitivity is  $17.2 \text{ ppm}^{-1}$ . What is the analyte's concentration if  $S_{\text{total}}$  is 35.2 and  $S_{\text{reag}}$  is 0.6?
5. A method for the analysis of  $\text{Ca}^{2+}$  in water suffers from an interference in the presence of  $\text{Zn}^{2+}$ . When the concentration of  $\text{Ca}^{2+}$  is 50 times greater than that of  $\text{Zn}^{2+}$ , an analysis for  $\text{Ca}^{2+}$  gives a relative error of  $-2.0\%$ . What is the value of the selectivity coefficient for this method?
6. The quantitative analysis for reduced glutathione in blood is complicated by many potential interferents. In one study, when analyzing a solution of 10.0 ppb glutathione and 1.5 ppb ascorbic acid, the signal was 5.43 times greater than that obtained for the analysis of 10.0 ppb glutathione.<sup>12</sup> What is the selectivity coefficient for this analysis? The same study found that analyzing a solution of  $3.5 \times 10^2$  ppb methionine and 10.0 ppb glutathione gives a signal that is 0.906 times less than that obtained for the analysis of 10.0 ppb glutathione. What is the selectivity coefficient for this analysis? In what ways do these interferents behave differently?
7. Oungpipat and Alexander described a method for determining the concentration of glycolic acid (GA) in a variety of samples, including physiological fluids such as urine.<sup>13</sup> In the presence of only GA, the signal is

12 Jiménez-Prieto, R.; Velasco, A.; Silva, M; Pérez-Bendito, D. *Anal. Chem. Acta* **1992**, 269, 273–279.

13 Oungpipat, W.; Alexander, P. W. *Anal. Chim. Acta* **1994**, 295, 36–46.

$$S_{\text{samp},1} = k_{\text{GA}} C_{\text{GA}}$$

and in the presence of both glycolic acid and ascorbic acid (AA), the signal is

$$S_{\text{samp},2} = k_{\text{GA}} C_{\text{GA}} + k_{\text{AA}} C_{\text{AA}}$$

When the concentration of glycolic acid is  $1.0 \times 10^{-4}$  M and the concentration of ascorbic acid is  $1.0 \times 10^{-5}$  M, the ratio of their signals is

$$\frac{S_{\text{samp},1}}{S_{\text{samp},2}} = 1.44$$

- (a) Using the ratio of the two signals, determine the value of the selectivity ratio  $K_{\text{GA,AA}}$ .
  - (b) Is the method more selective toward glycolic acid or ascorbic acid?
  - (c) If the concentration of ascorbic acid is  $1.0 \times 10^{-5}$  M, what is the smallest concentration of glycolic acid that can be determined such that the error introduced by failing to account for the signal from ascorbic acid is less than 1%?
8. Ibrahim and co-workers developed a new method for the quantitative analysis of hypoxanthine, a natural compound of some nucleic acids.<sup>14</sup> As part of their study they evaluated the method's selectivity for hypoxanthine in the presence of several possible interferents, including ascorbic acid.
- (a) When analyzing a solution of  $1.12 \times 10^{-6}$  M hypoxanthine the authors obtained a signal of  $7.45 \times 10^{-5}$  amps. What is the sensitivity for hypoxanthine? You may assume the signal has been corrected for the method blank.
  - (b) When a solution containing  $1.12 \times 10^{-6}$  M hypoxanthine and  $6.5 \times 10^{-5}$  M ascorbic acid is analyzed a signal of  $4.04 \times 10^{-5}$  amps is obtained. What is the selectivity coefficient for this method?
  - (c) Is the method more selective for hypoxanthine or for ascorbic acid?
  - (d) What is the largest concentration of ascorbic acid that may be present if a concentration of  $1.12 \times 10^{-6}$  M hypoxanthine is to be determined within 1.0%?
9. Examine a procedure from *Standard Methods for the Analysis of Waters and Wastewaters* (or another manual of standard analytical methods) and identify the steps taken to compensate for interferences, to cali-

<sup>14</sup> Ibrahim, M. S.; Ahmad, M. E.; Temerk, Y. M.; Kaucke, A. M. *Anal. Chim. Acta* **1996**, *328*, 47–52.



brate equipment and instruments, to standardize the method, and to acquire a representative sample.

### 3K Solutions to Practice Exercises

#### Practice Exercise 3.1

Because the signal for  $\text{Ag}^+$  in the presence of  $\text{Ni}^{2+}$  is reported as a relative error, we will assign a value of 100 as the signal for  $1 \times 10^{-9} \text{ M Ag}^+$ . With a relative error of +4.9%, the signal for the solution of  $1 \times 10^{-9} \text{ M Ag}^+$  and  $1.1 \times 10^{-7} \text{ M Ni}^{2+}$  is 104.9. The sensitivity for  $\text{Ag}^+$  is determined using the solution that does not contain  $\text{Ni}^{2+}$ ; thus

$$k_{\text{Ag}} = \frac{S_{\text{Ag}}}{C_{\text{Ag}}} = \frac{100}{1 \times 10^{-9} \text{ M}} = 1.0 \times 10^{11} \text{ M}^{-1}$$

Substituting into equation 3.4 values for  $k_{\text{Ag}}$ ,  $S_{\text{sample}}$ , and the concentrations of  $\text{Ag}^+$  and  $\text{Ni}^{2+}$

$$104.9 = (1.0 \times 10^{11} \text{ M}^{-1}) \times (1.0 \times 10^{-9} \text{ M}) + k_{\text{Ni}} \times (1.1 \times 10^{-7} \text{ M})$$

and solving gives  $k_{\text{Ni}}$  as  $4.5 \times 10^7 \text{ M}^{-1}$ . The selectivity coefficient is

$$K_{\text{Ag,Ni}} = \frac{k_{\text{Ni}}}{k_{\text{Ag}}} = \frac{4.5 \times 10^7 \text{ M}^{-1}}{1.0 \times 10^{11} \text{ M}^{-1}} = 4.5 \times 10^{-4}$$

Click [here](#) to return to the chapter.

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#### Practice Exercise 3.2

(a) A negative value for  $K_{\text{Ag,Hg}}$  means that the presence of  $\text{Hg}^{2+}$  decreases the signal from  $\text{Ag}^+$ .

(b) In this case we need to consider an error of -1%, since the effect of  $\text{Hg}^{2+}$  is to decrease the signal from  $\text{Ag}^+$ . To achieve this error, the term  $K_{A,I} \times C_I$  in equation 3.8 must be less than -1% of  $C_A$ ; thus

$$K_{\text{Ag,Hg}} \times C_{\text{Hg}} = -0.01 \times C_{\text{Ag}}$$

Substituting in known values for  $K_{\text{Ag,Hg}}$  and  $C_{\text{Ag}}$ , we find that the maximum concentration of  $\text{Hg}^{2+}$  is  $1.0 \times 10^{-8} \text{ M}$ .

Click [here](#) to return to the chapter.

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