Chapter 3

The Vocabulary of Analytical Chemistry

Chapter Overview

If you leaf through an issue of the journal *Analytical Chemistry*, you will soon discover that the authors and readers share a common vocabulary of analytical terms. You are probably familiar with some of these terms, such as accuracy and precision, but other terms, such as analyte and matrix may be less familiar to you. In order to participate in the community of analytical chemists, you must first understand its vocabulary. The goal of this chapter, therefore, is to introduce you to some important analytical terms. Becoming comfortable with these terms will make the material in the chapters that follow easier to read and 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 of interest in the sample is called the ANALYTE, and the remainder of the sample is the MATRIX. In an analysis we determine the identity, concentration, or properties of an analyte. To make this determination we measure one or more of the analyte's chemical or physical properties.

An example helps 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 public drinking water supplies. To comply with this act, municipalities regularly monitor their drinking water supply for potentially harmful substances. One such substance is fecal coliform bacteria. Municipal water departments collect and analyze samples from their water supply. They determine the concentration of fecal coliform bacteria by passing a portion of water through a membrane filter, placing the filter in a dish containing a nutrient broth, and incubating for 22–24 hr at 44.5 °C ± 0.2 °C. At the end of the incubation period they count the number of bacterial colonies in the dish and report the result as the number of colonies per 100 mL (Figure 3.1). Thus, municipal water departments analyze 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.

Fecal coliform counts provide 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.

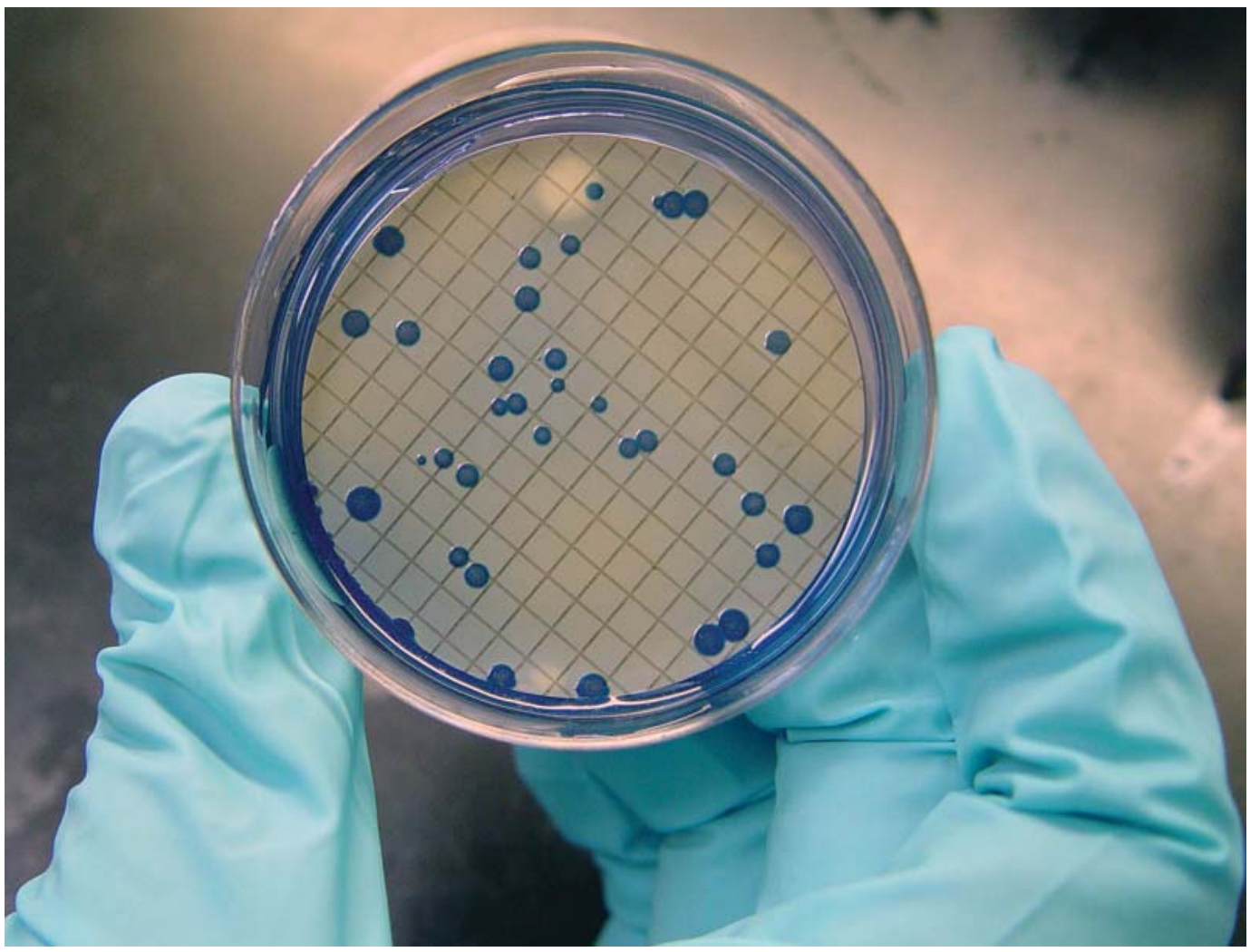


Figure 3.1 Colonies of fecal coliform bacteria from a water supply. Source: Susan Boyer. Photo courtesy of ARS–USDA (www.ars.usda.gov).

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 let's draw a distinction among four levels of analytical methodology: techniques, methods, procedures, and protocols.[1]

A TECHNIQUE is any chemical or physical principle we can use to study an analyte. There are many techniques for determining the concentration of lead in drinking water.[2] In graphite furnace atomic absorption spectroscopy (GFAAS), for example, we first convert aqueous lead ions into a free atom state—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).

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

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 lead in water is different from that for lead in soil or blood.

A PROCEDURE is a set of written directions telling us how to apply a method to a particular sample, including information on obtaining samples, handling interferents, and validating 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 the American Society for Testing Materials publish separate procedures for determining the concentration of lead in water.

1 Taylor, J. K. *Anal. Chem.* **1983**, *55*, 600A–608A.
2 Fitch, A.; Wang, Y.; Mellican, S.; Macha, S. *Anal. Chem.* **1996**, *68*, 727A–731A.

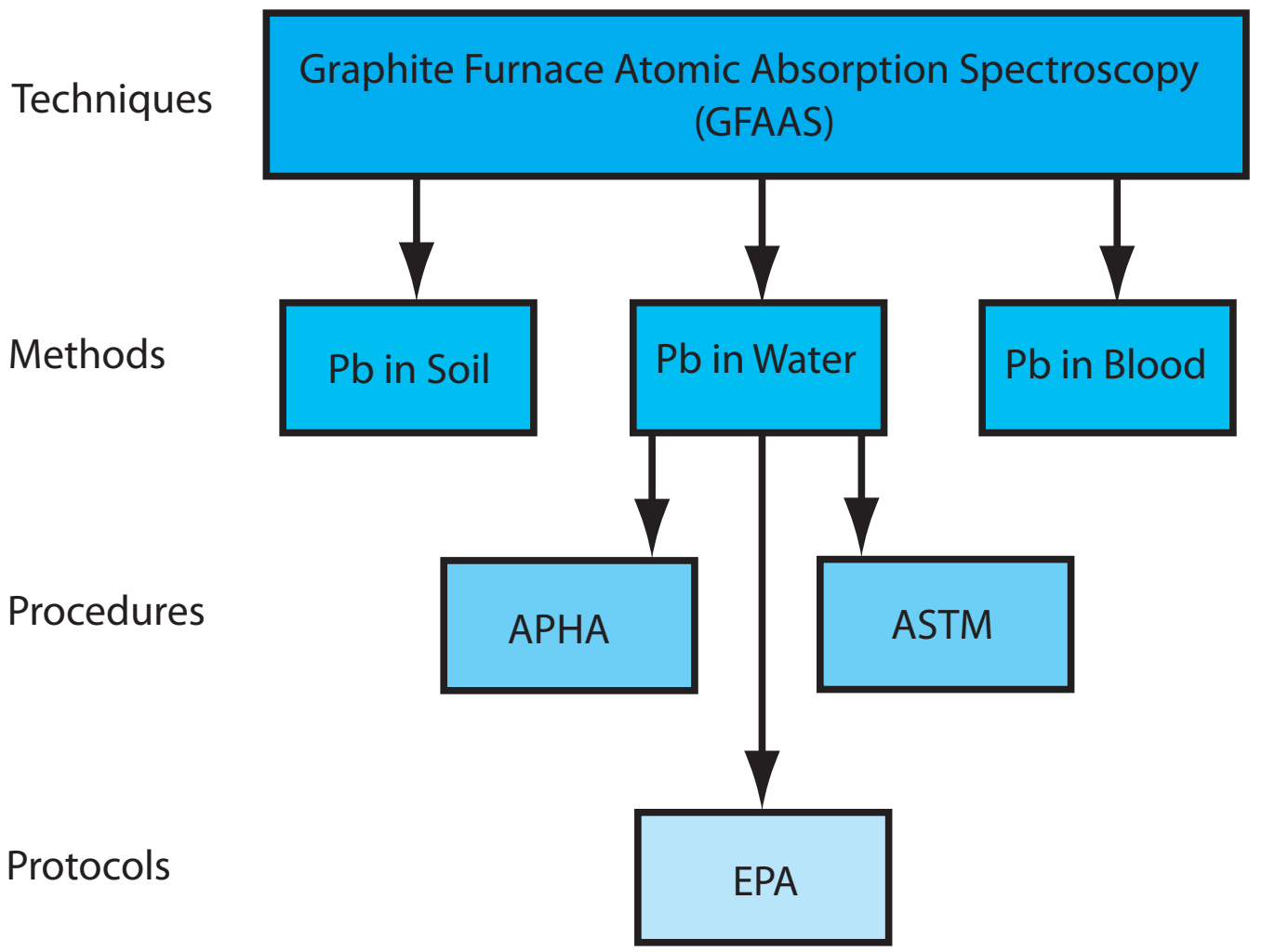


Figure 3.2 Chart showing the hierarchical relationship among a technique, methods using that technique, and procedures and protocols for one method.

The abbreviations are APHA: American Public Health Association, ASTM: American Society for Testing Materials, EPA: Environmental Protection Agency.

Figure 3.3 Graduated cylinders containing 0.10 M $Cu(NO_3)_2$. Although the cylinders contain the same concentration of Cu^{2+}, the cylinder on the left contains 1.0×10^{-4} mol Cu^{2+} and the cylinder on the right contains 2.0×10^{-4} mol Cu^{2+}.

Finally, a PROTOCOL is a set of stringent guidelines specifying a procedure that must be followed 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, labs 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

Analyzing 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 mass or absorbance. It is convenient to divide analytical techniques into two general classes depending on whether the signal is proportional to the mass or moles of analyte, or to the analyte's concentration.

Consider the two graduated cylinders in Figure 3.3, each containing a solution of 0.010 M $Cu(NO_3)_2$. Cylinder 1 contains 10 mL, or 1.0×10^{-4} moles of Cu^{2+}, and cylinder 2 contains 20 mL, or 2.0×10^{-4} moles of 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

$$S_A = k_A n_A \qquad 3.1$$

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

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

$$S_A = k_A C_A \qquad 3.2$$

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

A technique responding 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 involving the analyte. These reactions may involve any combination of precipitation, acid–base, complexation, or redox chemistry. The stoichiometry of the reactions determines the value of k_A in equation 3.1.

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 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 must be 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 for determining 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 $PbSO_4$ or $PbCrO_4$, 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, the availability of multiple oxidation states (Pb^0, Pb^{2+}, Pb^{4+}) makes electrochemical methods feasible.

The requirements of the analysis determine the best method. In choosing a method, consideration is given 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, $\%e_r$

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

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

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. In general, methods relying on total analysis techniques, such as gravimetry and titrimetry, produce results of higher accuracy because we can measure mass and volume with high accuracy, and because the value of k_A is known exactly through stoichiometry.

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

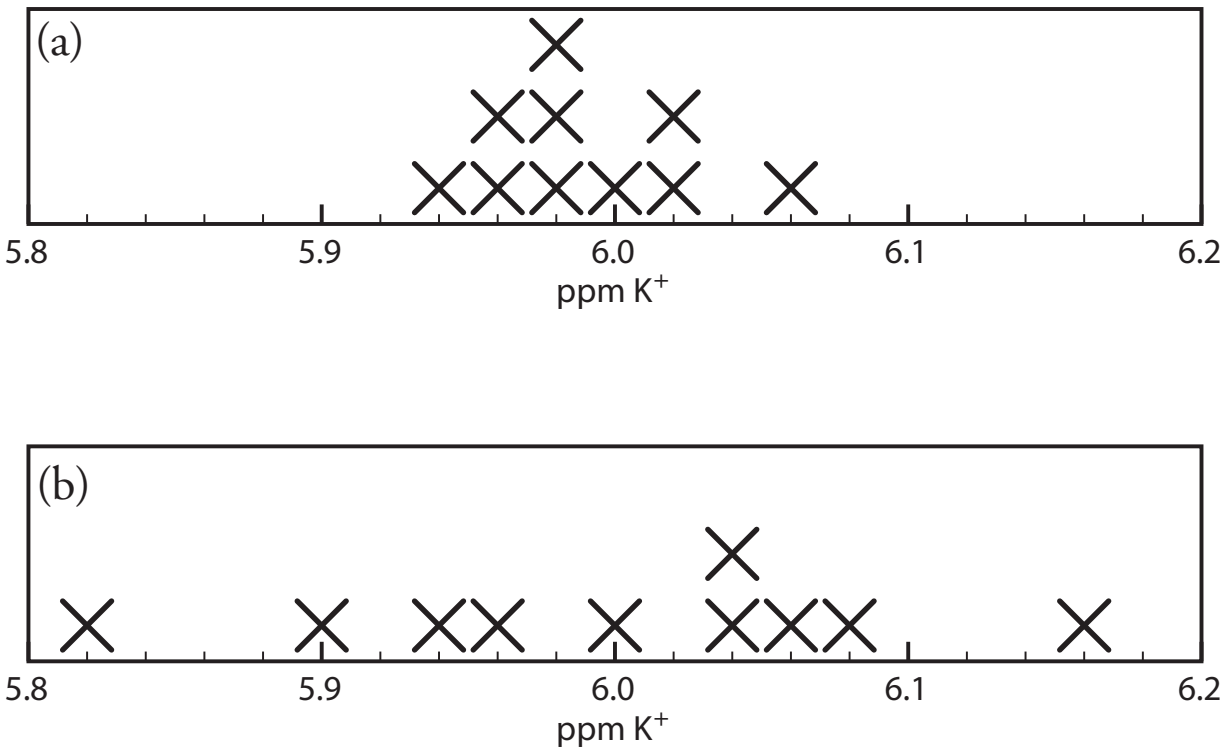


Figure 3.4 Two determinations of the concentration of K^+ in serum, showing the effect of precision on the distribution of individual results. The data in (a) are less scattered and, therefore, more precise than the data in (b).

3D.2 Precision

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.

When a sample is analyzed several times, the individual results are rarely the same. Instead, the results are randomly scattered. PRECISION is a measure of this variability. The closer the agreement between individual analyses, the more precise the results. For example, in determining the concentration of K^+ in serum the results shown in Figure 3.4(a) 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 method with a higher precision than the corresponding signal for a concentration method. Precision is covered in more detail in Chapter 4.

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

3D.3 Sensitivity

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.

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 differences are significant. Sensitivity is often confused with a method's DETECTION LIMIT, which is the smallest amount of analyte that we can determine with confidence.

Sensitivity is equivalent to the proportionality constant, k_A, in equation 3.1 and equation 3.2.[3] If ΔS_A is the smallest difference that we can measure between two signals, then the smallest detectable difference in the absolute amount or relative amount of analyte is

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

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

Suppose, for example, that our analytical signal is a measurement of mass using a balance whose smallest detectable increment is ±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 ΔS_A, the method with the greater sensitivity—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.[4] Although SPECIFICITY is the ideal, few analytical methods are completely free from the influence of interfering species. 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_{samp}

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

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

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 concentration of the interferent in the sample.

SELECTIVITY is a measure of a method's freedom from interferences.[5] The selectivity of a method for the interferent relative to the analyte is defined by a SELECTIVITY COEFFICIENT, $K_{A,I}$

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

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_{samp} in the presence of and in the absence of the interferent.

Although k_A and k_I are usually positive, they also may be negative. For example, some analytical methods work by measuring the concentration of a species that reacts with the analyte. As the analyte's concentration increases, the concentration of the species producing 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.

4 (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.

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

Example 3.1

A method for the analysis of Ca^{2+} in water suffers from an interference in the presence of Zn^{2+}. When the concentration of Ca^{2+} is 100 times greater than that of Zn^{2+} the analysis for Ca^{2+} gives 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 $C_{Ca} = 100$ (arbitrary units) and $C_{Zn} = 1$. A relative error of +0.5% means that the signal in the presence of Zn^{2+} is 0.5% greater than the signal in the absence of zinc. Again, we can assign values to make the calculation easier. If the signal in the absence of zinc is 100 (arbitrary units), then the signal in the presence of zinc is 100.5.

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.

The value of k_{Ca} is determined using equation 3.2

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

In the presence of zinc the signal is given by equation 3.4; thus

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

Solving for k_{Zn} gives a value of 0.5. The selectivity coefficient is

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

Practice Exercise 3.1

Wang and colleagues describe a fluorescence method for the analysis of Ag^+ in water. When analyzing a solution containing 1.0×10^{-9} M Ag^+ and 1.1×10^{-7} M Ni^{2+} the fluorescence intensity (the signal) was +4.9% greater than that obtained for a sample of 1.0×10^{-9} M Ag^+. What is $K_{Ag,Ni}$ 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.

The 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

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

substituting in equation 3.3 and equation 3.4, and simplifying gives

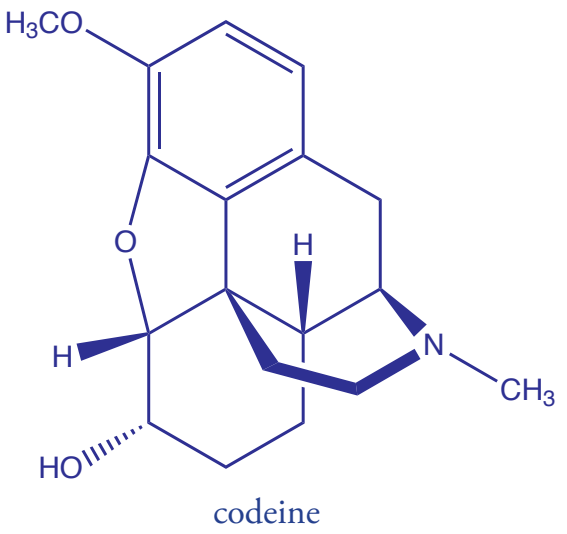
$$S_{samp} = k_A \left\{ n_A + K_{A,I} \times n_I \right\} \qquad 3.7$$

$$S_{samp} = k_A \left\{ C_A + K_{A,I} \times C_I \right\} \qquad 3.8$$

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 for determining the concentration of codeine in poppy plants.[6] As part of their study they determined the method's response to codeine in the presence of several interferents. For example, the authors found that the method's signal for 6-methoxycodeine was 6 (arbitrary units) when that for an equimolar solution of codeine was 40.



codeine

(a) What is the value of the selectivity coefficient when 6-methoxycodeine is the interferent and codeine is the analyte.

(b) If the concentration of codeine must be known with an accuracy of ±0.50%, what is the maximum relative concentration of 6-methoxycodeine (i.e. [6-methoxycodeine]/[codeine]) that can be present?

Solution

(a) The signals due to the analyte, S_A, and the interferent, S_I, are

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

Solving these equations for k_A and k_I, and substituting into equation 3.6 gives

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

Since the concentrations of analyte and interferent are equimolar ($C_A = C_I$), we have

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

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

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

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

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 can not exceed 3.3% of codeine's concentration.

Practice Exercise 3.2

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

(a) What is the significance of the selectivity coefficient's negative sign?

(b) Suppose you plan to use this method to analyze solutions with concentrations of Ag^+ that are no smaller than 1.0 nM . What is the maximum concentration of Hg^{2+} you can tolerate to ensure that your percentage relative errors are less than ±1.0%?

Click here to review your answers to this exercise.

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 will be needed to prevent the palladium from interfering.

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 interferences. Problems with selectivity also are more likely when the analyte is present at a very low concentration.[7]

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. When a method is relatively free from chemical interferences, we can use it on many analytes in a wide variety of sample matrices. Such methods are considered ROBUST.

Random variations in experimental conditions also 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 ana-

7 Rodgers, L. B. *J. Chem. Educ.* **1986**, *63*, 3–6.

lyte that produces a measurable signal. Collectively, these limitations define the analytical method's scale of operations.

We can display the scale of operations graphically (Figure 3.5) by plotting the sample's size on the *x*-axis and the analyte's concentration on the *y*-axis.[8] 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. The analyte's concentration and the sample's size provide a characteristic description for an analysis. For example, in a microtrace analysis the sample weighs between 0.1 mg–10 mg and contains a concentration of analyte between 10^{-2}% w/w–10^{-7}% w/w.

Diagonal lines connecting the axes show combinations of sample size and analyte concentration containing the same 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.

8 (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.

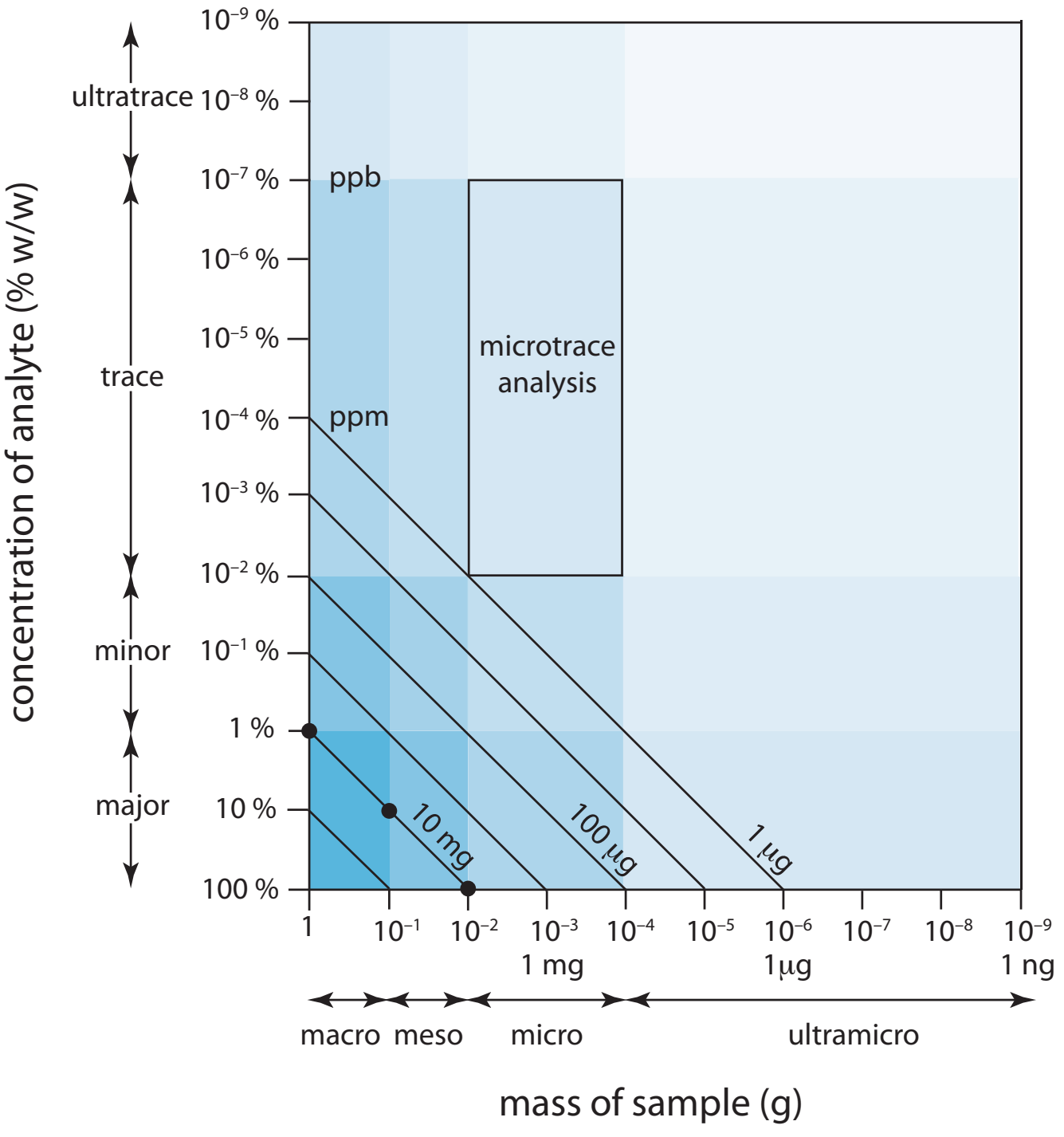


Figure 3.5 Scale of operations for analytical methods (adapted from references 8a and 8b).

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 containing the same mass of analyte. The three filled circles (●), for example, indicate analyses using 10 mg of analyte.

It should not surprise you to learn that total analysis methods typically require macro or meso samples containing major analytes. Concentration methods are particularly useful for minor, trace, and ultratrace analytes in macro, meso, and micro samples.

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 is rarely practical due to the complications of carrying such a large amount of material through the analysis. On the other hand, small samples containing trace amounts of analyte place significant restrictions on an analysis. For example, 1-mg sample with an analyte present at 10^{-4}% w/w contains just 1 ng of analyte. If we can isolate the analyte in 1 mL of solution, then we need an analytical method that can reliably detect it at a concentration of 1 ng/mL.

3D.7 Equipment, Time, and Cost

Finally, we can compare analytical methods with respect to equipment needs, the time to complete an analysis, and the cost per sample. Methods relying 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 is often fairly similar from method to method. This is somewhat misleading, however, because much of this time is spent preparing solutions and gathering together equipment. Once the solutions and equipment are in place, the sampling rate may differ substantially from method to method. Additionally, some methods are more easily automated. 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 relying on instruments cost more per sample then other methods.

3D.8 Making the Final Choice

Unfortunately, the design criteria discussed in this section are not mutually independent.[9] 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 design criteria. Usually, the most important design criterion is accuracy, and the best method is the one giving the most accurate result. When the need for results is urgent, as is often the case in clinical labs, analysis time may become the critical factor.

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

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 using graphite furnace atomic absorption spectroscopy is a common choice for determining lead in drinking 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 are important considerations.

3E Developing the Procedure

After selecting a method the next step is to develop a procedure that will accomplish the goals of our analysis. In developing the procedure attention is given 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},

$$S_{total} = S_A + S_{reag} = kn_A + S_{reag} \qquad 3.9$$

$$S_{total} = S_A + S_{reag} = kC_A + S_{reag} \qquad 3.10$$

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

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.

$$\left(S_{\text{total}} - S_{\text{reag}}\right) = S_{\text{A}} = kn_{\text{A}}$$

$$\left(S_{\text{total}} - S_{\text{reag}}\right) = S_{\text{A}} = kC_{\text{A}}$$

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. A more common approach is to find a method for separating the analyte and interferent by removing one from the sample. Once the separation is complete, we can proceed with the analysis using equation 3.9 or equation 3.10.

Methods for effecting this separation are discussed in Chapter 7.

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 that we can correct for the method blank and that we can compensate for interferents, a quantitative analysis is nothing more than solving equation 3.1 or equation 3.2 for n_{A} or C_{A}.

To solve these equations we need the value of k_{A}. For a total analysis method we usually know the value of k_{A} because it is defined by the stoichiometry of the chemical reactions generating 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 containing 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.

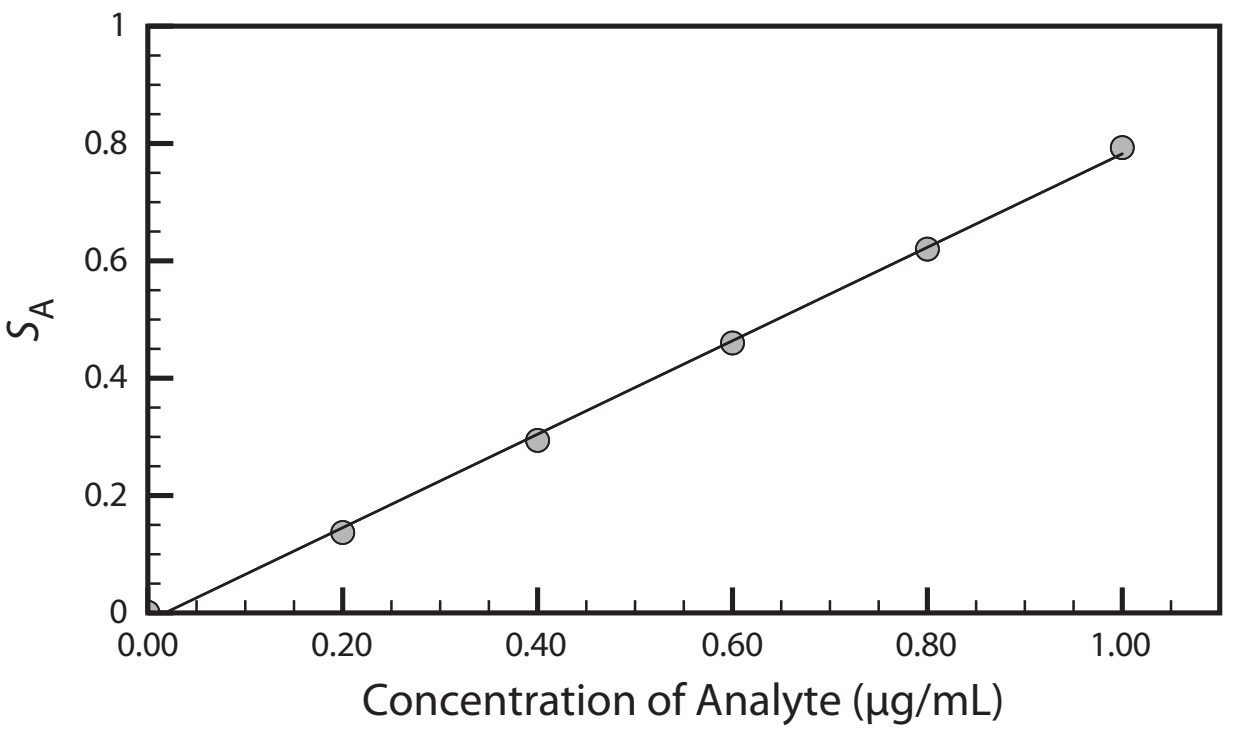


Figure 3.6 Example of a calibration curve. The filled circles (●) are the individual results for the standard samples and the line is the best fit to the data determined by a linear regression analysis. See Chapter 5 for a further discussion of calibration curves and an explanation of linear regression.

3E.3 Sampling

Selecting an appropriate method and executing it properly helps us ensure that our analyses are accurate. If we analyze the wrong samples, 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 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, sampling errors can not be corrected by evaluating a reagent blank.

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

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 under investigation. 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 that will be analyzed. 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 specifying an exact procedure that must be followed before an agency will accept the results of an analysis. In addition to the considerations taken into account when designing a procedure, a protocol also contains explicit instructions regarding internal and external quality assurance and quality control (QA/QC) procedures.[10] 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 ±10% of its expected

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

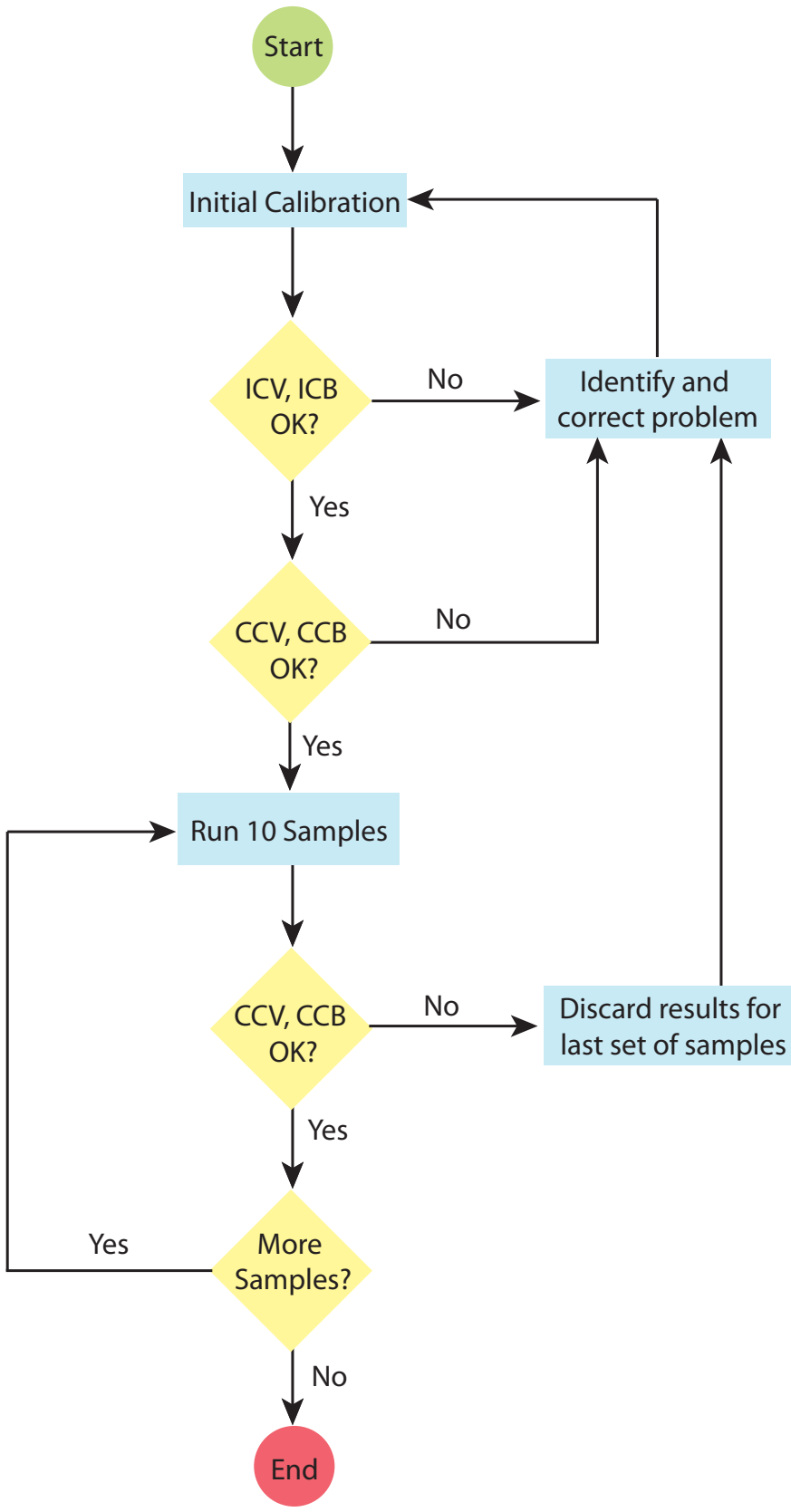


Figure 3.7 Schematic diagram showing a portion of the EPA's protocol for determining trace metals in water using graphite furnace atomic absorption spectrometry.

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

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 reverifies the calibration by analyzing a continuing calibration verification (CCV) sample and a continuing calibration blank (CCB). Results for the CCV also must be within ±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, every result is bound by successful 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 of spike tests to verify accuracy.

3G The Importance of Analytical Methodology

The importance of analytical methodology 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 any 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.[11]

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 monitoring data had yet to be 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 are often 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 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. 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

As you review this chapter, try to define a key term in your own words. Check your answer by clicking on the key term, which will take you to the page where it was first introduced. Clicking on the KEY TERM there, will bring you back to this page so that you can continue with another key term.

11 D'Elia, C. F.; Sanders, J. G.; Capone, D. G. *Envrion. Sci. Technol.* **1989**, *23*, 768–774.

rugged	selectivity	selectivity coefficient
sensitivity	signal	specificity
technique	total analysis techniques	validation

3I Chapter Summary

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

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 it often 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, it often 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, remove any solid impurities by passing the solution containing 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, 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 approximately 5 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 analyzing 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 (i.e. [interferent]/[analyte]), if the error in the analysis is to be less than 1%?

4. A sample was analyzed to determine the concentration of an analyte. Under the conditions of the analysis the sensitivity is 17.2 ppm^{-1}. What is the analyte's concentration if S_{total} is 35.2 and S_{reag} is 0.6?

5. A method for the analysis of Ca^{2+} in water suffers from an interference in the presence of Zn^{2+}. When the concentration of Ca^{2+} is 50 times greater than that of Zn^{2+}, an analysis for 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 the presence of many potential interferents. In one study, when analyzing a solution of 10 ppb glutathione and 1.5 ppb ascorbic acid, the signal was 5.43 times greater than that obtained for the analysis of

10 ppb glutathione.[12] What is the selectivity coefficient for this analysis? The same study found that when analyzing a solution of 350 ppb methionine and 10 ppb glutathione the signal was 0.906 times less than that obtained for the analysis of 10 ppb glutathione. What is the selectivity coefficient for this analysis? In what way 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.[13] In the presence of only GA, the signal is given as

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

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 1.0×10^{-4} M and the concentration of ascorbic acid is 1.0×10^{-5} M, the ratio of the two signals was found to be

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

(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 1.0×10^{-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.[14] As part of their study they evaluated the method's selectivity for hypoxanthine in the presence of several possible interferents, including ascorbic acid.

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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.

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

(a) When analyzing a solution of 1.12×10^{-6} M hypoxanthine the authors obtained a signal of 7.45×10^{-5} amps. What is the sensitivity for hypoxanthine? You may assume that the signal has been corrected for the method blank.

(b) When a solution containing 1.12×10^{-6} M hypoxanthine and 6.5×10^{-5} M ascorbic acid was analyzed a signal of 4.04×10^{-5} amps was 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 1.12×10^{-6} M hypoxanthine is to be determined within ±1%?

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

3K Solutions to Practice Exercises

Practice Exercise 3.1

Since the signal for Ag^+ in the presence of Ni^{2+} is given as a relative error, the fact that are not given absolute signals is of no consequence. Instead, we will assign a value of 100 as the signal for 1×10^{-9} M Ag^+. With a relative error of +4.9%, the signal for the solution of 1×10^{-9} M Ag^+ and 1.1×10^{-7} M Ni^{2+} is 104.9. The sensitivity for Ag+ is determined using the solution that does not contain Ni^{2+}.

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

Substituting into equation 3.4 values for k_{Ag}, S_{samp} for the solution containing Ag^+ and Ni^{2+}, and the concentrations of Ag^+ and Ni^{2+}

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

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

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

Click here to return to the chapter.

Practice Exercise 3.2

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

(b) In this case we need to consider an error of –1%, since the effect of Hg^{2+} is to decrease the signal from 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

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

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

Click here to return to the chapter.