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, $\%e_r$

$$\%e_r = ((\text{obtained result} - \text{expected result}) / \text{expected result}) \times 100$$

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. 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.

3D.2 Precision

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.

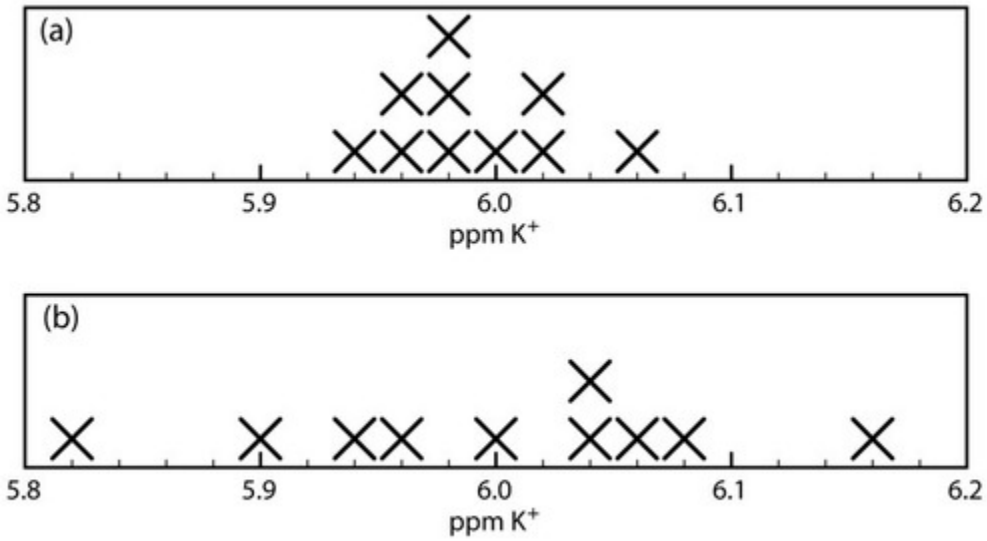


Figure 3.4 Two determinations of the concentration of K^+ in serum, showing the effect of precision on the distribution of individual results. The data in (a) are less scattered and, therefore, more precise than the data in (b).

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.

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 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 DSA 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

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

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

$$\Delta n_A = (\pm 0.0001 \text{ g} / 0.200) = \pm 0.0005 \text{ g}$$

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}

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

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

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**, $K_{A,I}$

$$K_{A,I} = k_I / k_A$$

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.

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 th 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 $C_{Ca} = 1$ (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 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 t presence of zinc is 100.5.

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

$$k_{Ca} = S_{Ca} / C_{Ca} = 100/100 = 1$$

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

$$K_{Ca,Zn} = k_{Zn} / k_{Ca} = 0.5 / 1 = 0.5$$

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

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

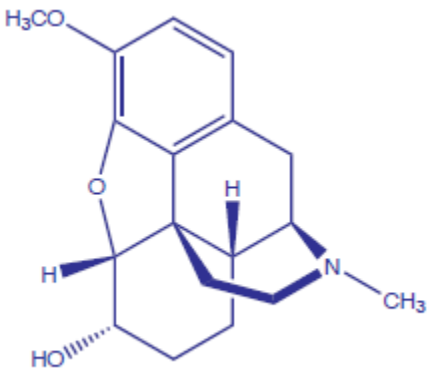
substituting in equation 3.3 and equation 3.4, and simplifying gives

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

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

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



codeine

Barnett and colleagues developed a method for determining the concentration of codeine in pop 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 fou that the method's signal for 6-methoxycodeine was 6 (arbitrary units) when that for an equimolar solution of codeine was 40.

(a) What is the value of the selectivity coefficient when 6-methoxycodeine is the interferent and codeine is the analy
(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 [6-methoxycodeine]/[codeine]) that can be present?

Solution

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

$$S_A = k_A C_A$$

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

$$K_{A,I} = (S_I / C_I) / (S_A / C_A)$$

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

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

(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

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

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

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

Therefore, the concentration of 6-methoxycodeine can not exceed 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 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 analyte 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.

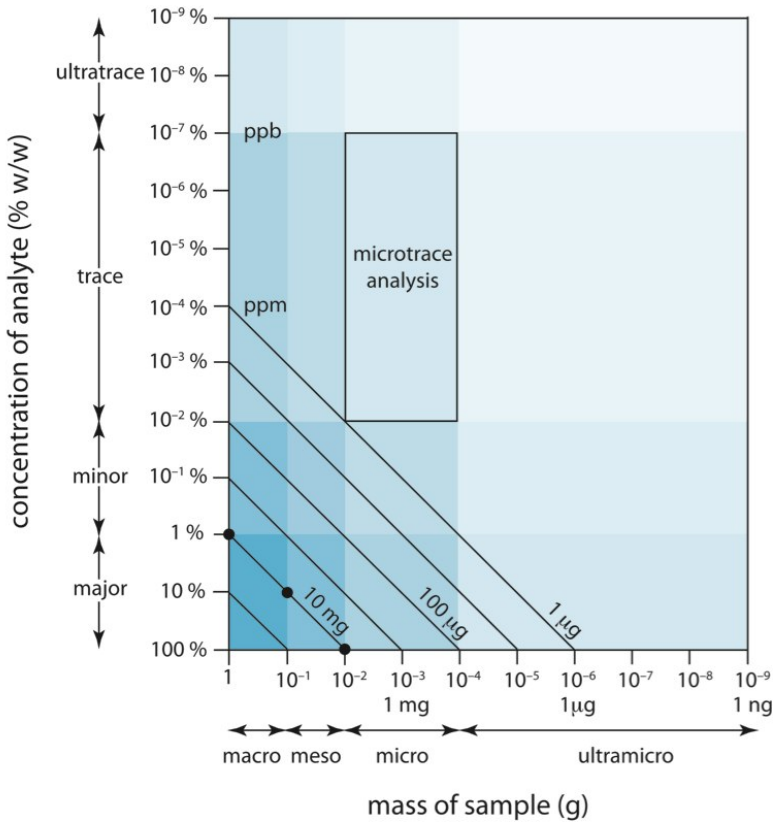


Figure 3.5 Scale of operations for analytical methods (adapted from references 8a and 8b).
The shaded areas define different types of analyses. The boxed area, for example, represents a microtrace analysis. The diagonal lines show combinations of sample size and analyte concentration containing the same mass of analyte. The three filled circles (•), for example, indicate analyses using 10 mg of analyte.

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.

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.

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Analytical_Chemistry_2.0/03_The_Vocabulary_of_Analytical_Chemistry/3D_Selecting_an_Analytical_Method