

USD CHEM 220 FALL 2022



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CHAPTER OVERVIEW

1: Introduction to Analytical Chemsitry

1.1: Introduction to Analytical Chemistry

1.2: Common Analytical Problems

1.3: Selecting an Analytical Method

1.4: Developing the Procedure

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1.1: Introduction to Analytical Chemistry

What is analytical chemistry?

According to the American Chemical Society, analytical chemistry is defined as "the science of obtaining, processing and communicating information about the composition and structure of matter"[1] In particular, this means understanding the ways in which chemical measurements are made, and learning how to communicate about those measurement. When an analytical chemist communicates about a measurement, it is critical that both the result and the uncertainty in that result are reported together. There are no exact measurements, and there is no solutions manual in real life.

So, this class will focus on not only the tools and techniques used to make chemical measurements, but also the tools and techniques used to quantify and communicate uncertainty. We will focus on several main measurement techniques:

1. Titration
2. Spectroscopy
3. Electrochemistry
4. Chromatography

and we will learn some fundamental statistics that can be used with all of these techniques. Additionally, we'll combine our knowledge of the chemical measurements and the statistical analysis to consider how we design and validate new measurements.

[1] <https://www.acs.org/careers/chemical...chemistry.html>

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1.2: Common Analytical Problems

Many problems in analytical chemistry begin with the need to identify what is present in a sample. This is the scope of a **qualitative analysis**, examples of which include identifying the products of a chemical reaction, screening an athlete's urine for a performance-enhancing drug, or determining the spatial distribution of Pb on the surface of an airborne particulate. An early challenge for analytical chemists was developing simple chemical tests to identify inorganic ions and organic functional groups. The classical laboratory courses in inorganic and organic qualitative analysis, still taught at some schools, are based on this work.

See, for example, the following laboratory textbooks: (a) Sorum, C. H.; Lagowski, J. J. *Introduction to Semimicro Qualitative Analysis*, 5th Ed.; Prentice-Hall: Englewood, NJ, 1977; (b) Shriner, R. L.; Fuson, R. C.; Curtin, D. Y. *The Systematic Identification of Organic Compounds*, 5th Ed.; John Wiley and Sons: New York, 1964.

Modern methods for qualitative analysis rely on instrumental techniques, such as infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS). Because these qualitative applications are covered adequately elsewhere in the undergraduate curriculum, typically in organic chemistry, they receive no further consideration in this text.

Perhaps the most common analytical problem is a **quantitative analysis**, examples of which include the elemental analysis of a newly synthesized compound, measuring the concentration of glucose in blood, or determining the difference between the bulk and the surface concentrations of Cr in steel. Much of the analytical work in clinical, pharmaceutical, environmental, and industrial labs involves developing new quantitative methods to detect trace amounts of chemical species in complex samples. Most of the examples in this text are of quantitative analyses.

Another important area of analytical chemistry, which receives some attention in this text, are methods for characterizing physical and chemical properties. The determination of chemical structure, of equilibrium constants, of particle size, and of surface structure are examples of a **characterization analysis**.

The purpose of a qualitative, a quantitative, or a characterization analysis is to solve a problem associated with a particular sample. The purpose of a **fundamental analysis**, on the other hand, is to improve our understanding of the theory that supports an analytical method and to understand better an analytical method's limitations.

A good resource for current examples of qualitative, quantitative, characterization, and fundamental analyses is *Analytical Chemistry's* annual review issue that highlights fundamental and applied research in analytical chemistry. Examples of review articles in the 2015 issue include "Analytical Chemistry in Archaeological Research," "Recent Developments in Paper-Based Microfluidic Devices," and "Vibrational Spectroscopy: Recent Developments to Revolutionize Forensic Science."

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1.3: 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 PbSO_4 or as 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 3.2.1](#), we can use graphite furnace atomic absorption spectroscopy to determine the concentration of lead in drinking water. Finally, lead's multiple oxidation states (Pb^0 , Pb^{2+} , 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.

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 3.3.1](#) or [Equation 3.3.2](#), 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.

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 the upper half of [Figure 3.4.1](#) for the concentration of K^+ in a sample of serum are more precise than those in the lower half of [Figure 3.4.1](#). It is important to understand that precision does not imply accuracy. That the data in the upper half of [Figure 3.4.1](#) are more precise does not mean that the first set of results is more accurate. In fact, neither set of results may be accurate.

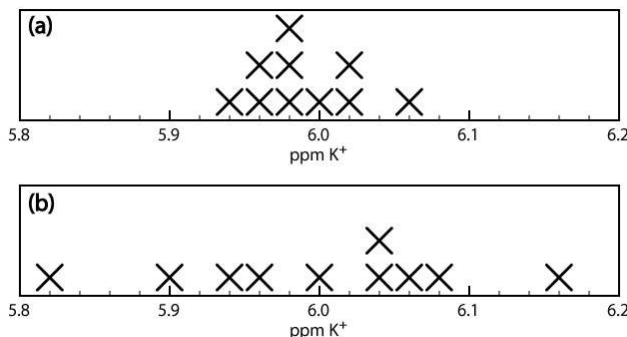


Figure 3.4.1 : Two determinations of the concentration of K^+ in serum, showing the effect of precision on the distribution of individual results. The data in (a) are less scattered and, therefore, more precise than the data in (b).

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 technique with a higher precision than is the case for a concentration method.

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.

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.

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.

Sensitivity is equivalent to the proportionality constant, k_A , in [Equation 3.3.1](#) and [Equation 3.3.2](#) [IUPAC Compendium of Chemical Terminology, Electronic version]. If ΔS_A is the smallest difference we can measure 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 ± 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—that is, the method with the larger k_A —is better able to discriminate between smaller amounts of analyte.

Specificity and Selectivity

An analytical method is specific if its signal depends only on the analyte [Persson, B-A; Vessman, J. *Trends Anal. Chem.* **1998**, 17, 117–119; Persson, B-A; Vessman, J. *Trends Anal. Chem.* **2001**, 20, 526–532]. Although **specificity** is the ideal, few analytical methods are free from interferences. When an **interferent** contributes to the signal, we expand [Equation 3.3.1](#) and [Equation 3.3.2](#) to include its contribution to the sample's signal, S_{samp}

$$S_{\text{samp}} = S_A + S_I = k_A n_A + k_I n_I \quad (1.3.1)$$

$$S_{\text{samp}} = S_A + S_I = k_A C_A + k_I C_I \quad (1.3.2)$$

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 [Valcárcel, M.; Gomez-Hens, A.; Rubio, S. *Trends Anal. Chem.* **2001**, 20, 386–393]. A method's selectivity for an interferent relative to the analyte is defined by a **selectivity coefficient**, $K_{A,I}$

$$K_{A,I} = \frac{k_I}{k_A} \quad (1.3.3)$$

which may be positive or negative depending on the signs 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.

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.

Determining the selectivity coefficient's value is easy if we already know the values for k_A and k_I . As shown by Example 3.4.1, we also can determine $K_{A,I}$ by measuring S_{samp} in the presence of and in the absence of the interferent.

✓ Example 3.4.1

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+} , an analysis for 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 Zn^{2+} is 0.5% greater than the signal in the absence of Zn^{2+} . Again, we can assign values to make the calculation easier. If the signal for Ca^{2+} in the absence of Zn^{2+} is 100 (arbitrary units), then the signal in the presence of Zn^{2+} is 100.5.

The value of k_{Ca} is determined using Equation 3.3.2

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

In the presence of Zn^{2+} the signal is given by Equation 3.4.2; 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_{Zn} gives its value as 0.5. The selectivity coefficient is

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

If you are unsure why, in the above example, 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.

? Exercise 3.4.1

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

Answer

Because the signal for Ag^+ in the presence of 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 Ag^+ is determined using the solution that does not contain 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 1.3.2 values for k_{Ag} , S_{samp} , and the concentrations of Ag^+ and Ni^{2+}

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

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

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

A selectivity coefficient provides us with a useful way to evaluate an interferent's potential effect on an analysis. Solving Equation 1.3.3 for k_I

$$k_I = K_{A,I} \times k_A \quad (1.3.4)$$

and substituting in Equation 1.3.1 and Equation 1.3.2, and simplifying gives

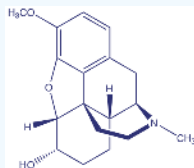
$$S_{\text{samp}} = k_A \{n_A + K_{A,I} \times n_I\} \quad (1.3.5)$$

$$S_{\text{samp}} = k_A \{C_A + K_{A,I} \times C_I\} \quad (1.3.6)$$

An interferent will not pose a problem as long as the term $K_{A,I} \times n_I$ in Equation 1.3.5 is significantly smaller than n_A , or if $K_{A,I} \times C_I$ in Equation 1.3.6 is significantly smaller than C_A .

✓ Example 3.4.2

Barnett and colleagues developed a method to determine the concentration of codeine (structure shown below) in poppy plants [Barnett, N. W.; Bowser, T. A.; Geraldi, R. D.; Smith, B. *Anal. Chim. Acta* **1996**, 318, 309–317]. 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-methoxy-codeine 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 1.3.4 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 1.3.6 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-methoxycodine 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.

? Exercise 3.4.2

Mercury (II) also is an interferent in the fluorescence method for Ag^+ developed by Wang and colleagues (see [Practice Exercise 3.4.1](#)). The selectivity coefficient, $K_{\text{Ag,Hg}}$ has a value of -1.0×10^{-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 Ag^+ no smaller than 1.0 nM. What is the maximum concentration of Hg^{2+} you can tolerate if your percentage relative errors must be less than $\pm 1.0\%$?

Answer

- A negative value for $K_{\text{Ag,Hg}}$ means that the presence of Hg^{2+} decreases the signal from Ag^+ .
- 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 1.3.6 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_{Ag} , we find that the maximum concentration of Hg^{2+} is 1.0×10^{-8} M.

Problems with selectivity also are more likely when the analyte is present at a very low concentration [Rodgers, L. B. *J. Chem. Educ.* **1986**, 63, 3–6].

Look back at [Figure 1.1.1](#), 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 1.1.2](#) 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.

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.

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 3.4.2) by plotting the sample's size on the x-axis and the analyte's concentration on the y-axis. 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 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.

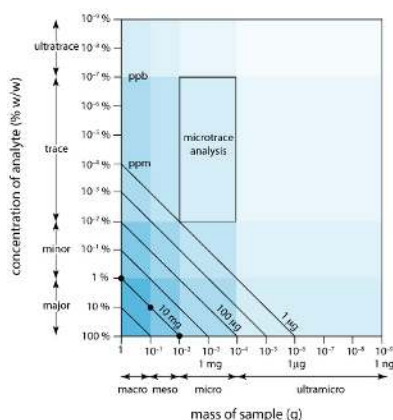


Figure 3.4.2 : Scale of operations for analytical methods. The shaded areas define different types of analyses. The boxed area, for example, represents a microtrace analysis. The diagonal lines show combinations of sample size and analyte concentration that contain the same mass of analyte. The three filled circles (•), for example, indicate analyses that use 10 mg of analyte. See 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 for more details.

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 3.4.2, 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 3.4.2 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.

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.

Making the Final Choice

Unfortunately, the design criteria discussed in this section are not mutually independent [Valcárcel, M.; Ríos, A. *Anal. Chem.* **1993**, 65, 781A–787A]. 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 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.

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1.4: 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.

Compensating for Interferences

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

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

$$S_{total} = S_A + S_{reag} = k_A n_A + S_{reag} \quad (1.4.1)$$

$$S_{total} = S_A + S_{reag} = k_A C_A + S_{reag} \quad (1.4.2)$$

Without an independent determination of S_{reag} we cannot solve Equation 1.4.1 or 1.4.2 for the moles or concentration of analyte.

To determine the contribution of S_{reag} in Equations 1.4.1 and 1.4.2 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

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

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

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 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.

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.

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 3.3.1 or Equation 3.3.2 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 3.3.1 or Equation 3.3.2. 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 3.5.1 .

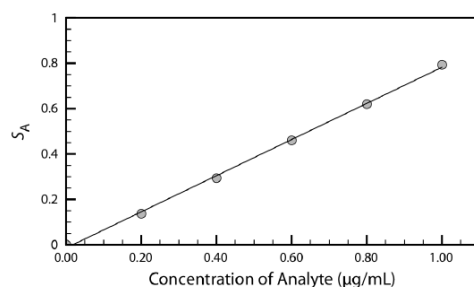


Figure 3.5.1 : Example of a calibration curve. The filled circles (•) are the results for five standard samples, each with a different concentration of analyte, and the line is the best fit to the data determined by a linear regression analysis. See [Chapter 5](#) for a further discussion of calibration curves and an explanation of linear regression.

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.

[Chapter 7](#) provides a more detailed discussion of sampling, including strategies for obtaining representative samples.

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](#).

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CHAPTER OVERVIEW

2: Descriptive Statistics

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2.1: Measures of Center

Both graphical and numerical methods of summarizing data make up the branch of statistics known as descriptive statistics. This section introduces numerical measurements to describe sample data.

This section focuses on measures of central tendency. Many times, you are asking what to expect “on average.” Such as when you pick a career, you would probably ask how much you expect to earn in that field. If you are trying to buy a home, you might ask how much homes are selling for in your area. If you are planting vegetables in your garden, you might want to know how long it will be until you can harvest. These questions, and many more, can be answered by knowing the center of the data set. The three most common measures of the “center” of the data are called the mode, mean, and median.

3.1.1 Mode

To find the mode, you count how often each data value occurs, and then determine which data value occurs most often.

The **mode** is the data value that occurs the most frequently in the data.

There may not be a mode at all, or you may have more than one mode. If there is a tie between two values for the greatest number of times then both values are the mode and the data is called bimodal (two modes). If every data point occurs the same number of times, there is no mode. If there are more than two numbers that appear the most times, then usually we write there is no mode. When looking at grouped data in a frequency distribution or a histogram then the largest frequency is called the **modal class**.

Below is a dotplot showing the height of some 3-year-old children in cm and we would like to answer the question, “How tall are 3-year-olds?”

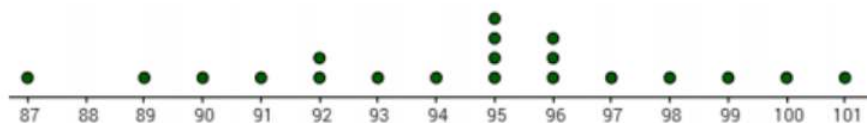


Figure 3-1

From the graph, we can see that the most frequent value is 95 cm. This is not exactly the middle of the distribution, but it is the most common height and is close to the middle in this case. We call this most frequent value the mode.

For larger data sets, use software to find the mode or at least sort the data so that you can see grouping of numbers. Excel reports a mode at the first repetitive value, so be careful in Excel with bimodal data or data with many multiples that would really have no mode at all.

Note that zero may be the most frequent value in a data set. The mode = 0 is not the same as “no mode” in the data set.

The mode is the observation that occurs most often.

- **Example 3-1:** -5 4 8 3 4 2 0 mode = 4
- **Example 3-2:** 3 -6 0 1 -2 1 0 5 0 mode = 0
- **Example 3-3:** 18 25 15 32 10 27 no mode (Excel writes N/A)
- **Example 3-4:** 15 23 18 15 24 23 17 modes = 15, 23 (bimodal)
- **Example 3-5:** 100 125 100 125 130 140 130 140 no mode (Excel gives 100)

Summation Notation

Throughout this course, we will be using **summation notation**, also called sigma notation. The capital Greek letter Σ “sigma” means to add. For example, Σx means to sum up all of the x values where X is the variable name.

A random sample of households had the following number of children living at home 4, -3, 2, 1, and 3. Calculate Σx .

Solution

Let $x_1 = 4$, $x_2 = -3$, $x_3 = 2$, $x_4 = 1$, $x_5 = 3$. Start with the first value $i = 1$ up to the n th value $i = 5$ to get $\sum_{i=1}^n x_i = 4 + -3 + 2 + 1 + 3 = 7$.

To make things simpler we will drop the subscripts and write $\sum_{i=1}^n x_i$ as Σx_i or Σx .

The order of operations is important in summation notation.

For example, $\Sigma x^2 = (4)^2 + (-3)^2 + (2)^2 + (1)^2 + (3)^2 = 39$.

When we insert parentheses $(\Sigma x)^2 = (4 + -3 + 2 + 1 + 3)^2 = (7)^2 = 49$.

Note that $\Sigma x^2 \neq (\Sigma x)^2$.

“‘One of the interesting things about space,’ Arthur heard Slartibartfast saying to a large and voluminous creature who looked like someone losing a fight with a pink duvet and was gazing raptly at the old man’s deep eyes and silver beard, ‘is how dull it is.’

‘Dull?’ said the creature, and blinked her rather wrinkled and bloodshot eyes.

‘Yes,’ said Slartibartfast, ‘staggeringly dull. Bewilderingly so. You see, there’s so much of it and so little in it. Would you like me to quote some statistics?’

‘Er, well...’

‘Please, I would like to. They, too, are quite sensationally dull.’” (Adams, 2002)

3.1.2 Mean

The **mean** is the arithmetic average of the numbers. This is the center that most people call the average.

Distinguishing between a population and a sample is very important in statistics. We frequently use a representative sample to generalize about a population.

A statistic is any characteristic or measure from a sample. A parameter is any characteristic or measure from a population. We use sample statistics to make inferences about population parameters.

The sample mean $= \bar{x}$ (pronounced “x bar”) of a sample of n observations $x_1, x_2, x_3, \dots, x_n$ taken from a population, is given by the formula:

$$\bar{x} = \frac{\Sigma x}{n} = \frac{x_1 + x_2 + x_3 + \dots + x_n}{n}$$

The population mean $= \mu$ (pronounced “mu”) is the average of the entire population, is given by the formula:

$$\mu = \frac{\Sigma x}{N} = \frac{x_1 + x_2 + x_3 + \dots + x_N}{N}$$

Most cases, you cannot find the population parameter, so you use the sample statistic to estimate the population parameter. Since μ cannot be calculated in most situations, the value for $\bar{x}$ is used to estimate μ . You should memorize the symbol μ and what it represents for future reference.

Find the mean for the following sample of house prices (\$1,000): 325, 375, 385, 395, 420, and 825.

Solution

Before starting any mathematics problem, it is always a good idea to define the unknown in the problem. In this case, you want to define the variable. The symbol for the variable is x . The variable is x = price of a house in \$1,000.

$$\bar{x} = \frac{\Sigma x}{n} = \frac{325 + 375 + 385 + 395 + 420 + 825}{6} = 454.1\bar{6}$$

The sample mean house price is \$454,166.67.

3.1.3 Median

Another statistic that measures the center of a distribution is the median.

The **median** is the data value in the middle of the ordered data that has 50% of the data below that point and 50% of the data above that point. The median is also referred to as the 50th percentile and is the midpoint of a distribution.

To find the median:

1. Arrange the observations from smallest to largest.
2. If the number of observations n is odd, the middle observation is the median.
3. If the number of observations n is even, the mean of the two middle observations is the median.

Find the median for the following sample of ages: 15, 23, 18, 15, 24, 23, and 17.

Solution

First, sort the data: 15, 15, 17, 18, 23, 23, and 24. The sample size is odd so the median will be the middle number. Use your fingers to cover outside numbers, one pair at a time until you get to 18. Median = 18 years old.

Find the median for the following sample of house prices (in \$1,000): 325, 375, 385, 395, 420, and 825.

Solution

The data is already ordered from smallest to largest. The sample size is even so take the average of the two middle values $\frac{385+395}{2} = 390$. The median house price is \$390,000.

We can use technology to find the median. Directions for the TI calculators are in the next section. In Excel the median is found using the cell function MEDIAN(array). For this example, we can type the data into column A and then in a blank cell =MEDIAN(A1:A6).

Recall that the sample mean house price is \$454,167. Note that the median is much lower than the mean for this example. The observation of 825 is an outlier and is very large compared to the rest of the data. The sample mean is sensitive to unusual observations, i.e. outliers. The median is resistant to outliers.

3.1.4 Outliers

An **outlier** is a data value that is very different from the rest of the data and is far enough from the center. If there are extreme values in the data, the median is a better measure of the center than the mean. The mean is not a resistant measure because it is moved in the direction of the outlier. The median and the mode are resistant measures because they are not affected by extreme values.

As a consumer, you need to be aware that people choose the measure of center that best supports their claim. When you read an article in the newspaper and it talks about the “average,” it usually means the mean but sometimes it refers to the median. Some articles will use the word “median” instead of “average” to be more specific. If you need to make an important decision and the information says “average,” it would be wise to ask if the “average” is the mean or the median before you decide.

As an example, suppose that a company administration wants to use the mean salary as the average salary for the company. This is because the high salaries of the administration will pull the mean higher. The company can say that the employees are paid well because the average is high. However, the employees’ union wants to use the median since it discounts the extreme values of the administration and will give a lower value of the average. This will make the salaries seem lower and that a raise is in order.

Why use the mean instead of the median? When multiple samples are taken from the same population, the sample means tend to be more consistent than other measures of the center. The sample mean is the more reliable measure of center.

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2.2: Measures of Spread

Variability is an important idea in statistics. If you were to measure the height of everyone in your classroom, every student gives you a different value. That means not every student has the same height. Thus, there is variability in people's heights. If you were to take a sample of the income level of people in a town, every sample gives you different information. There is variability between samples too. Variability describes how the data are spread out. If the data are very close to each other, then there is low variability. If the data are very spread out, then there is high variability. How do you measure variability? It would be good to have a number that measures it. This section will describe some of the different measures of variability, also known as variation.

Numerical statistics for variation can show how spread out data is. The variation of data is relative, and is usually used when comparing two sets of similar data. When we are making inferences about an average, we can make better estimates when there is less variation in the data. The four most common measures of the "spread" of data are called the range, variance, standard deviation, and coefficient of variation.

A sample of house prices (in \$1,000): 325, 375, 385, 395, 420, and 825, found the mean house price of \$454,167. How much does this tell you about the price of all houses? Can you tell if most of the prices were close to the mean or were the prices really spread out? What is the highest price and the lowest price? All you know is that the center of the price is \$454,167. What if you were approved for only \$400,000 for a home loan, could you buy a home in this area? You need more information.

3.2.1 Range

The range of a set of data is the difference between the highest and the lowest data values (or maximum and minimum values). Note in statistics we only report a single number which represents the spread from the lowest to highest value.

$$\text{Range} = \text{Max} - \text{Min.}$$

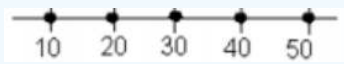
Look at the following three sets of data. Find the mean, median and range of each of data set.

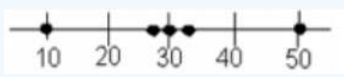
1. 10, 20, 30, 40, 50

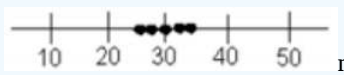
2. 10, 29, 30, 31, 50

3. 28, 29, 30, 31, 32

Solution

1.  mean = 30, median = 30, range = $50 - 10 = 40$

2.  mean = 30, median = 30, range = $50 - 10 = 40$

3.  mean = 30, median = 30, range = $32 - 28 = 4$

Based on the mean, median, and range, the first two distributions are the same, but you can see from the graphs that they are distributed differently. In part 1, the data are spread out equally. In part 2, the data has a clump in the middle and a single value at each end. The mean and median are the same for part 3, but the range is much smaller. All the data is clumped together in the middle.

3.2.2 Variance & Standard Deviation

The range does not really provide a very detailed picture of the variability. A better way to describe how the data is spread out is needed. Instead of looking at the distance as the highest value from the lowest, how about looking at the distance each value is from the mean? This spread is called the deviation.

Suppose a vet wants to analyze the weights of cats. The weights (in pounds) of five cats are 6.8, 8.2, 7.5, 9.4, and 8.2. Compute the deviation for each of the data values. The deviation is how far each data point is from the mean. To be consistent always subtract the data point minus the mean.

Solution

Variable: X = weight of a cat. First, find the mean for the data set. The mean is $\bar{x} = \frac{\Sigma(x)}{n} = \frac{(6.8+8.2+7.5+9.4+8.2)}{5} = 8.02$ pounds. Subtract the mean from each data point to get the deviations.

x	$x - \bar{x}$
6.8	$6.8 - 8.02 = -1.22$
8.2	$8.2 - 8.02 = 0.18$
7.5	$7.5 - 8.02 = -0.52$
9.4	$9.4 - 8.02 = 1.38$
8.2	$8.2 - 8.02 = 0.18$

Figure 3-11

Now average the deviations. Add the deviations together.

x	$x - \bar{x}$
6.8	$6.8 - 8.02 = -1.22$
8.2	$8.2 - 8.02 = 0.18$
7.5	$7.5 - 8.02 = -0.52$
9.4	$9.4 - 8.02 = 1.38$
8.2	$8.2 - 8.02 = 0.18$
Total	0

Figure 3-12

The average distance from the mean cannot be zero. The reason the deviations add to 0 is that there are some positive and negative values. The sum of the deviations from the mean will always be zero.

To get rid of the negative signs square each deviation.

x	$x - \bar{x}$	$(x - \bar{x})^2$
6.8	$6.8 - 8.02 = -1.22$	1.4884
8.2	$8.2 - 8.02 = 0.18$	0.0324
7.5	$7.5 - 8.02 = -0.52$	0.2704
9.4	$9.4 - 8.02 = 1.38$	1.9044
8.2	$8.2 - 8.02 = 0.18$	0.0324
Total	0	3.728

Figure 3-13

Then average the total of the squared deviations. The only thing is that in statistics there is a strange average here. Instead of dividing by the number of data values, you divide by the number of data values minus one. This $n - 1$ is called the degrees of freedom and will be discussed more later in the text. When we divide by the degrees of freedom, this gives an unbiased statistic. In this case, you would have the following:

$$s^2 = \frac{(x - \bar{x})^2}{n - 1} = \frac{3.728}{5 - 1} = \frac{3.728}{4} = 0.932 \text{ pounds}^2$$

Notice that this statistic is denoted as s^2 . This statistic is called the sample variance and it is a measure of the average squared distance from the mean. If you now take the square root, you will get the average distance from the mean. The square root of the variance is called the sample standard deviation, and is denoted with the letter s .

$$s = \sqrt{0.932} = 0.9654 \text{ pounds}$$

The standard deviation is the average (mean) distance from a data point to the mean. It can be thought of as how much a typical data point differs from the mean.

The **sample variance** formula: $s^2 = \frac{\sum (x - \bar{x})^2}{n-1}$.

Where $\bar{x}$ is the sample mean, n is the sample size, and Σ means to find the sum.

The **sample standard deviation** formula: $s = \sqrt{s^2} = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$.

The $n - 1$ in the denominator has to do with a concept called degrees of freedom (df). Dividing by the df makes the sample standard deviation a better approximation of the population standard deviation than dividing by n .

We rarely will find a population variance or standard deviation, but you will need to know the symbols.

The **population variance** formula: $\sigma^2 = \frac{\sum (x - \mu)^2}{N}$.

The population standard deviation formula: $\sigma = \sqrt{\frac{\sum (x - \mu)^2}{N}}$.

The lower-case Greek letter σ pronounced “sigma” and σ^2 represents the population variance, μ is the population mean, and N is the size of the population.

Note: the sum of the deviations should always be zero. Try not to round too much in the calculations for standard deviation since each rounding causes a slight error.

Suppose that a manager wants to test two new training programs. They randomly select 5 people for each training type and measures the time it takes to complete a task after the training. The times for both trainings are in table below. Which training method is more consistent?

Time to Finish Task in Minutes					
Training 1	56	75	48	63	59
Training 2	60	58	66	59	58

Solution

It is important that you define what each variable is since there are two of them.

Variable 1: X_1 = productivity from training 1

Variable 2: X_2 = productivity from training 2

The units and scale are the same for both groups. To answer which training method better, first you need some descriptive statistics. Start with the mean for each sample.

$$\bar{x}_1 = \frac{56 + 75 + 48 + 63 + 59}{5} = 60.2 \text{ minutes}$$

$$\bar{x}_2 = \frac{60 + 58 + 66 + 59 + 58}{5} = 60.2 \text{ minutes}$$

Since both means are the same values, you cannot answer the question about which is better.

Now calculate the standard deviation for each sample.

Squared Deviations for Training 1

x_1	$x_1 - \bar{x}_1$	$(x_1 - \bar{x}_1)^2$
56	-4.2	17.64
75	14.8	219.04
48	-12.2	148.84
63	2.8	7.84
59	-1.2	1.44
Total	0	394.8

Figure 3-14

Squared Deviations for Training 2

x_2	$x_2 - \bar{x}_2$	$(x_2 - \bar{x}_2)^2$
60	-0.2	0.04
58	-2.2	4.84
66	5.8	33.64
59	-1.2	1.44
58	-2.2	4.84
Total	0	44.8

Figure 3-15

The variance for each sample is:

$$s_1^2 = \frac{394.8}{4} = 98.7 \text{ minutes}^2$$

$$s_2^2 = \frac{44.8}{4} = 11.2 \text{ minutes}^2$$

The standard deviations are: $s_1 = \sqrt{98.7} = 9.9348$ minutes

$$s_2 = \sqrt{11.2} = 3.3466 \text{ minutes.}$$

Comparing the standard deviations, the second training method seemed to be the better training since the data is less spread out. This means it is more consistent. It would be better for the managers in this case to have a training program that produces more consistent results so they know what to expect for the time it takes to complete the task.

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2.3: Confidence Intervals

Up to this point, we have learned how to estimate the mean using sample data and a sample statistic. We have also seen that all statistics have sampling error and that the value we find for the sample mean will bounce around based on the people in our sample, simply due to random chance. Thinking about estimation from this perspective, it would make more sense to take that error into account rather than relying just on our point estimate. To do this, we calculate what is known as a confidence interval.

A confidence interval starts with our point estimate then creates a range of scores considered plausible based on our standard deviation, our sample size, and the level of confidence with which we would like to estimate the parameter. This range, which extends equally in both directions away from the point estimate, is called the margin of error. We calculate the margin of error by multiplying our two-tailed critical value by our standard error:

$$\text{Margin of Error} = t^*(s/\sqrt{n}) \quad (2.3.1)$$

One important consideration when calculating the margin of error is that it can only be calculated using the critical value for a two-tailed test. This is because the margin of error moves away from the point estimate in both directions, so a one-tailed value does not make sense.

The critical value we use will be based on a chosen level of confidence, which is equal to $1 - \alpha$. Thus, a 95% level of confidence corresponds to $\alpha = 0.05$. Thus, at the 0.05 level of significance, we create a 95% Confidence Interval. How to interpret that is discussed further on.

Once we have our margin of error calculated, we add it to our point estimate for the mean to get an upper bound to the confidence interval and subtract it from the point estimate for the mean to get a lower bound for the confidence interval:

$$\begin{aligned} \text{Upper Bound} &= \bar{X} + \text{Margin of Error} \\ \text{Lower Bound} &= \bar{X} - \text{Margin of Error} \end{aligned} \quad (2.3.2)$$

Or simply:

$$\text{Confidence Interval} = \bar{X} \pm t^*(s/\sqrt{n}) \quad (2.3.3)$$

To write out a confidence interval, we always use soft brackets and put the lower bound, a comma, and the upper bound:

$$\text{Confidence Interval} = (\text{Lower Bound}, \text{Upper Bound}) \quad (2.3.4)$$

Let's see what this looks like with some actual numbers by taking our oil change data and using it to create a 95% confidence interval estimating the average length of time it takes at the new mechanic. We already found that our average was $\bar{X} = 53.75$ and our standard error was $s_{\bar{X}} = 6.86$. We also found a critical value to test our hypothesis, but remember that we were testing a one-tailed hypothesis, so that critical value won't work. To see why that is, look at the column headers on the t -table. The column for one-tailed $\alpha = 0.05$ is the same as a two-tailed $\alpha = 0.10$. If we used the old critical value, we'd actually be creating a 90% confidence interval ($1.00 - 0.10 = 0.90$, or 90%). To find the correct value, we use the column for two-tailed $\alpha = 0.05$ and, again, the row for 3 degrees of freedom, to find $t^* = 3.182$.

Now we have all the pieces we need to construct our confidence interval:

$$\begin{aligned} 95\%CI &= 53.75 \pm 3.182(6.86) \\ \text{Upper Bound} &= 53.75 + 3.182(6.86) \\ UB &= 53.75 + 21.83 \\ UB &= 75.58 \\ \text{Lower Bound} &= 53.75 - 3.182(6.86) \\ LB &= 53.75 - 21.83 \\ LB &= 31.92 \\ 95\%CI &= (31.92, 75.58) \end{aligned}$$

So we find that our 95% confidence interval runs from 31.92 minutes to 75.58 minutes, but what does that actually mean? The range (31.92, 75.58) represents values of the mean that we consider reasonable or plausible based on our observed data. It includes

our point estimate of the mean, $\bar{X} = 53.75$, in the center, but it also has a range of values that could also have been the case based on what we know about how much these scores vary (i.e. our standard error).

It is very tempting to also interpret this interval by saying that we are 95% confident that the true population mean falls within the range (31.92, 75.58), but this is not true. The reason it is not true is that phrasing our interpretation this way suggests that we have firmly established an interval and the population mean does or does not fall into it, suggesting that our interval is firm and the population mean will move around. However, the population mean is an absolute that does not change; it is our interval that will vary from data collection to data collection, even taking into account our standard error. The correct interpretation, then, is that we are 95% confident that the range (31.92, 75.58) brackets the true population mean. This is a very subtle difference, but it is an important one.

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2.4: Statistical Methods for Normal Distributions

The most common distribution for our results is a normal distribution. Because the area between any two limits of a normal distribution curve is well defined, constructing and evaluating significance tests is straightforward.

Comparing $\bar{X}$ to μ

One way to validate a new analytical method is to analyze a sample that contains a known amount of analyte, μ . To judge the method's accuracy we analyze several portions of the sample, determine the average amount of analyte in the sample, $\bar{X}$, and use a significance test to compare $\bar{X}$ to μ . Our null hypothesis is that the difference between $\bar{X}$ and μ is explained by indeterminate errors that affect the determination of $\bar{X}$. The alternative hypothesis is that the difference between $\bar{X}$ and μ is too large to be explained by indeterminate error.

$$H_0: \bar{X} = \mu$$

$$H_A: \bar{X} \neq \mu$$

The test statistic is t_{exp} , which we substitute into the confidence interval for μ given by [Equation 4.4.5](#)

$$\mu = \bar{X} \pm \frac{t_{\text{exp}} s}{\sqrt{n}} \quad (2.4.1)$$

Rearranging this equation and solving for t_{exp}

$$t_{\text{exp}} = \frac{|\mu - \bar{X}| \sqrt{n}}{s} \quad (2.4.2)$$

gives the value for t_{exp} when μ is at either the right edge or the left edge of the sample's confidence interval (Figure 2.4.1 a)

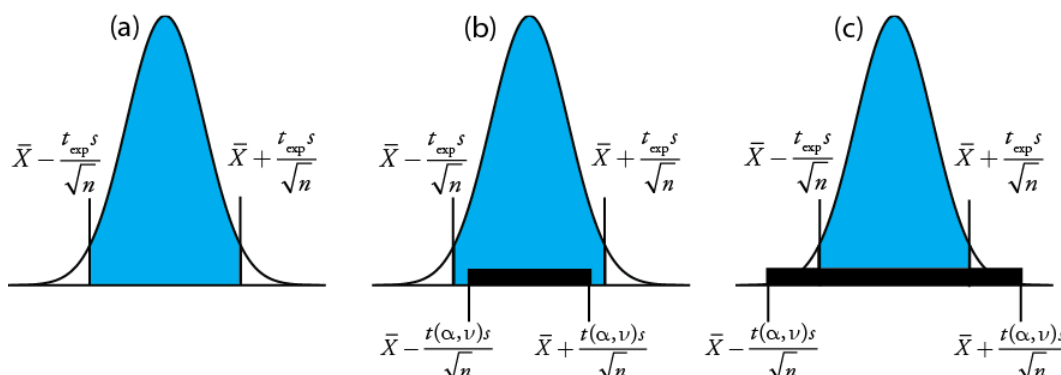


Figure 2.4.1 : Relationship between a confidence interval and the result of a significance test. (a) The shaded area under the normal distribution curve shows the sample's confidence interval for μ based on t_{exp} . The solid bars in (b) and (c) show the expected confidence intervals for μ explained by indeterminate error given the choice of α and the available degrees of freedom, ν . For (b) we reject the null hypothesis because portions of the sample's confidence interval fall outside the confidence interval explained by indeterminate error. In the case of (c) we retain the null hypothesis because the confidence interval explained by indeterminate error completely encompasses the sample's confidence interval.

To determine if we should retain or reject the null hypothesis, we compare the value of t_{exp} to a critical value, $t(\alpha, \nu)$, where α is the confidence level and ν is the degrees of freedom for the sample. The critical value $t(\alpha, \nu)$ defines the largest confidence interval explained by indeterminate error. If $t_{\text{exp}} > t(\alpha, \nu)$, then our sample's confidence interval is greater than that explained by indeterminate errors (Figure 2.4.1 b). In this case, we reject the null hypothesis and accept the alternative hypothesis. If $t_{\text{exp}} \leq t(\alpha, \nu)$, then our sample's confidence interval is smaller than that explained by indeterminate error, and we retain the null hypothesis (Figure 2.4.1 c). Example 2.4.1 provides a typical application of this significance test, which is known as a **t-test** of $\bar{X}$ to μ .

You will find values for $t(\alpha, \nu)$ in [Appendix 4](#), or we can look them up in Python, using the following code:

```
import scipy.stats as stats
```

$t = \text{stats.t.ppf}(1-\alpha/2, \text{dof})$

Copy that code into a blank Jupyter notebook, define α and dof as explained above, and compare to the values in Appendix 4. The results should be exactly the same!

Another name for the t -test is Student's t -test. Student was the pen name for William Gossett (1876-1927) who developed the t -test while working as a statistician for the Guinness Brewery in Dublin, Ireland. He published under the name Student because the brewery did not want its competitors to know they were using statistics to help improve the quality of their products.

✓ Example 2.4.1

Before determining the amount of Na_2CO_3 in a sample, you decide to check your procedure by analyzing a standard sample that is 98.76% w/w Na_2CO_3 . Five replicate determinations of the %w/w Na_2CO_3 in the standard gave the following results

98.71% 98.59% 98.62% 98.44% 98.58%

Using $\alpha = 0.05$, is there any evidence that the analysis is giving inaccurate results?

Solution

The mean and standard deviation for the five trials are

$$\bar{X} = 98.59 \quad s = 0.0973$$

Because there is no reason to believe that the results for the standard must be larger or smaller than μ , a two-tailed t -test is appropriate. The null hypothesis and alternative hypothesis are

$$H_0: \bar{X} = \mu \quad H_A: \bar{X} \neq \mu$$

The test statistic, t_{exp} , is

$$t_{\text{exp}} = \frac{|\mu - \bar{X}|\sqrt{n}}{s} = \frac{|98.76 - 98.59|\sqrt{5}}{0.0973} = 3.91$$

The critical value for $t(0.05, 4)$ from Appendix 4 is 2.78. Since t_{exp} is greater than $t(0.05, 4)$, we reject the null hypothesis and accept the alternative hypothesis. At the 95% confidence level the difference between $\bar{X}$ and μ is too large to be explained by indeterminate sources of error, which suggests there is a determinate source of error that affects the analysis.

There is another way to interpret the result of this t -test. Knowing that t_{exp} is 3.91 and that there are 4 degrees of freedom, we use Appendix 4 to estimate the α value corresponding to a $t(\alpha, 4)$ of 3.91. From Appendix 4, $t(0.02, 4)$ is 3.75 and $t(0.01, 4)$ is 4.60. Although we can reject the null hypothesis at the 98% confidence level, we cannot reject it at the 99% confidence level. For a discussion of the advantages of this approach, see J. A. C. Sterne and G. D. Smith "Sifting the evidence—what's wrong with significance tests?" *BMJ* **2001**, 322, 226–231.

? Exercise 2.4.1

To evaluate the accuracy of a new analytical method, an analyst determines the purity of a standard for which μ is 100.0%, obtaining the following results.

99.28% 103.93% 99.43% 99.84% 97.60% 96.70% 98.02%

Is there any evidence at $\alpha = 0.05$ that there is a determinate error affecting the results?

Answer

The null hypothesis is $H_0: \bar{X} = \mu$ and the alternative hypothesis is $H_A: \bar{X} \neq \mu$. The mean and the standard deviation for the data are 99.26% and 2.35%, respectively. The value for t_{exp} is

$$t_{\text{exp}} = \frac{|100.0 - 99.26|\sqrt{7}}{2.35} = 0.833$$

and the critical value for $t(0.05, 6)$ is 2.477. Because t_{exp} is less than $t(0.05, 6)$ we retain the null hypothesis and have no evidence for a significant difference between $\bar{X}$ and μ .

Earlier we made the point that we must exercise caution when we interpret the result of a statistical analysis. We will keep returning to this point because it is an important one. Having determined that a result is inaccurate, as we did in Example 2.4.1, the next step is to identify and to correct the error. Before we expend time and money on this, however, we first should examine critically our data. For example, the smaller the value of s , the larger the value of t_{exp} . If the standard deviation for our analysis is unrealistically small, then the probability of a type 2 error increases. Including a few additional replicate analyses of the standard and reevaluating the t -test may strengthen our evidence for a determinate error, or it may show us that there is no evidence for a determinate error.

Comparing s^2 to σ^2

If we analyze regularly a particular sample, we may be able to establish an expected variance, σ^2 , for the analysis. This often is the case, for example, in a clinical lab that analyze hundreds of blood samples each day. A few replicate analyses of a single sample gives a sample variance, s^2 , whose value may or may not differ significantly from σ^2 .

We can use an F -test to evaluate whether a difference between s^2 and σ^2 is significant. The null hypothesis is $H_0: s^2 = \sigma^2$ and the alternative hypothesis is $H_A: s^2 \neq \sigma^2$. The test statistic for evaluating the null hypothesis is F_{exp} , which is given as either

$$F_{\text{exp}} = \frac{s^2}{\sigma^2} \text{ if } s^2 > \sigma^2 \text{ or } F_{\text{exp}} = \frac{\sigma^2}{s^2} \text{ if } \sigma^2 > s^2 \quad (2.4.3)$$

depending on whether s^2 is larger or smaller than σ^2 . This way of defining F_{exp} ensures that its value is always greater than or equal to one.

If the null hypothesis is true, then F_{exp} should equal one; however, because of indeterminate errors F_{exp} usually is greater than one. A critical value, $F(\alpha, \nu_{\text{num}}, \nu_{\text{den}})$, is the largest value of F_{exp} that we can attribute to indeterminate error given the specified significance level, α , and the degrees of freedom for the variance in the numerator, ν_{num} , and the variance in the denominator, ν_{den} . The degrees of freedom for s^2 is $n - 1$, where n is the number of replicates used to determine the sample's variance, and the degrees of freedom for σ^2 is defined as infinity, ∞ . Critical values of F for $\alpha = 0.05$ are listed in [Appendix 5](#) for both one-tailed and two-tailed F -tests.

✓ Example 2.4.2

A manufacturer's process for analyzing aspirin tablets has a known variance of 25. A sample of 10 aspirin tablets is selected and analyzed for the amount of aspirin, yielding the following results in mg aspirin/tablet.

254 249 252 252 249 249 250 247 251 252

Determine whether there is evidence of a significant difference between the sample's variance and the expected variance at $\alpha = 0.05$.

Solution

The variance for the sample of 10 tablets is 4.3. The null hypothesis and alternative hypotheses are

$$H_0: s^2 = \sigma^2 \quad H_A: s^2 \neq \sigma^2$$

and the value for F_{exp} is

$$F_{\text{exp}} = \frac{\sigma^2}{s^2} = \frac{25}{4.3} = 5.8$$

The critical value for $F(0.05, \infty, 9)$ from [Appendix 5](#) is 3.333. Since F_{exp} is greater than $F(0.05, \infty, 9)$, we reject the null hypothesis and accept the alternative hypothesis that there is a significant difference between the sample's variance and the expected variance. One explanation for the difference might be that the aspirin tablets were not selected randomly.

Comparing Variances for Two Samples

We can extend the F -test to compare the variances for two samples, A and B , by rewriting Equation 2.4.3 as

$$F_{\text{exp}} = \frac{s_A^2}{s_B^2}$$

defining A and B so that the value of F_{exp} is greater than or equal to 1.

✓ Example 2.4.3

Table 4.4.1 shows results for two experiments to determine the mass of a circulating U.S. penny. Determine whether there is a difference in the variances of these analyses at $\alpha = 0.05$.

Solution

The standard deviations for the two experiments are 0.051 for the first experiment (A) and 0.037 for the second experiment (B). The null and alternative hypotheses are

$$H_0: s_A^2 = s_B^2 \quad H_A: s_A^2 \neq s_B^2$$

and the value of F_{exp} is

$$F_{\text{exp}} = \frac{s_A^2}{s_B^2} = \frac{(0.051)^2}{(0.037)^2} = \frac{0.00260}{0.00137} = 1.90$$

From Appendix 5, the critical value for $F(0.05, 6, 4)$ is 9.197. Because $F_{\text{exp}} < F(0.05, 6, 4)$, we retain the null hypothesis. There is no evidence at $\alpha = 0.05$ to suggest that the difference in variances is significant.

? Exercise 2.4.2

To compare two production lots of aspirin tablets, we collect and analyze samples from each, obtaining the following results (in mg aspirin/tablet).

Lot 1: 256 248 245 245 244 248 261

Lot 2: 241 258 241 244 256 254

Is there any evidence at $\alpha = 0.05$ that there is a significant difference in the variances for these two samples?

Answer

The standard deviations are 6.451 mg for Lot 1 and 7.849 mg for Lot 2. The null and alternative hypotheses are

$$H_0: s_{\text{Lot 1}}^2 = s_{\text{Lot 2}}^2 \quad H_A: s_{\text{Lot 1}}^2 \neq s_{\text{Lot 2}}^2$$

and the value of F_{exp} is

$$F_{\text{exp}} = \frac{(7.849)^2}{(6.451)^2} = 1.480$$

The critical value for $F(0.05, 5, 6)$ is 5.988. Because $F_{\text{exp}} < F(0.05, 5, 6)$, we retain the null hypothesis. There is no evidence at $\alpha = 0.05$ to suggest that the difference in the variances is significant.

Comparing Means for Two Samples

Three factors influence the result of an analysis: the method, the sample, and the analyst. We can study the influence of these factors by conducting experiments in which we change one factor while holding constant the other factors. For example, to compare two analytical methods we can have the same analyst apply each method to the same sample and then examine the resulting means. In a similar fashion, we can design experiments to compare two analysts or to compare two samples.

It also is possible to design experiments in which we vary more than one of these factors. We will return to this point in [Chapter 14](#).

Before we consider the significance tests for comparing the means of two samples, we need to make a distinction between **unpaired data** and **paired data**. This is a critical distinction and learning to distinguish between these two types of data is important. Here are two simple examples that highlight the difference between unpaired data and paired data. In each example the goal is to compare two balances by weighing pennies.

- Example 1: We collect 10 pennies and weigh each penny on each balance. This is an example of paired data because we use the same 10 pennies to evaluate each balance.
- Example 2: We collect 10 pennies and divide them into two groups of five pennies each. We weigh the pennies in the first group on one balance and we weigh the second group of pennies on the other balance. Note that no penny is weighed on both balances. This is an example of unpaired data because we evaluate each balance using a different sample of pennies.

In both examples the samples of 10 pennies were drawn from the same population; the difference is how we sampled that population. We will learn why this distinction is important when we review the significance test for paired data; first, however, we present the significance test for unpaired data.

One simple test for determining whether data are paired or unpaired is to look at the size of each sample. If the samples are of different size, then the data must be unpaired. The converse is not true. If two samples are of equal size, they may be paired or unpaired.

Unpaired Data

Consider two analyses, A and B with means of $\bar{X}_A$ and $\bar{X}_B$, and standard deviations of s_A and s_B . The confidence intervals for μ_A and for μ_B are

$$\mu_A = \bar{X}_A \pm \frac{ts_A}{\sqrt{n_A}} \quad (2.4.4)$$

$$\mu_B = \bar{X}_B \pm \frac{ts_B}{\sqrt{n_B}} \quad (2.4.5)$$

where n_A and n_B are the sample sizes for A and for B . Our null hypothesis, $H_0: \mu_A = \mu_B$, is that any difference between μ_A and μ_B is the result of indeterminate errors that affect the analyses. The alternative hypothesis, $H_A: \mu_A \neq \mu_B$, is that the difference between μ_A and μ_B is too large to be explained by indeterminate error.

To derive an equation for t_{exp} , we assume that μ_A equals μ_B , and combine Equation 2.4.4 and Equation 2.4.5

$$\bar{X}_A \pm \frac{t_{\text{exp}} s_A}{\sqrt{n_A}} = \bar{X}_B \pm \frac{t_{\text{exp}} s_B}{\sqrt{n_B}}$$

Solving for $|\bar{X}_A - \bar{X}_B|$ and using a propagation of uncertainty, gives

$$|\bar{X}_A - \bar{X}_B| = t_{\text{exp}} \times \sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}} \quad (2.4.6)$$

Finally, we solve for t_{exp}

$$t_{\text{exp}} = \frac{|\bar{X}_A - \bar{X}_B|}{\sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}} \quad (2.4.7)$$

and compare it to a critical value, $t(\alpha, \nu)$, where α is the probability of a type 1 error, and ν is the degrees of freedom.

Problem 9 asks you to use a propagation of uncertainty to show that Equation 2.4.6 is correct.

Thus far our development of this t -test is similar to that for comparing $\bar{X}$ to μ , and yet we do not have enough information to evaluate the t -test. Do you see the problem? With two independent sets of data it is unclear how many degrees of freedom we have.

Suppose that the variances s_A^2 and s_B^2 provide estimates of the same σ^2 . In this case we can replace s_A^2 and s_B^2 with a pooled variance, s_{pool}^2 , that is a better estimate for the variance. Thus, Equation 2.4.7 becomes

$$t_{\text{exp}} = \frac{|\bar{X}_A - \bar{X}_B|}{s_{\text{pool}} \times \sqrt{\frac{1}{n_A} + \frac{1}{n_B}}} = \frac{|\bar{X}_A - \bar{X}_B|}{s_{\text{pool}}} \times \sqrt{\frac{n_A n_B}{n_A + n_B}} \quad (2.4.8)$$

where s_{pool} , the pooled standard deviation, is

$$s_{\text{pool}} = \sqrt{\frac{(n_A - 1)s_A^2 + (n_B - 1)s_B^2}{n_A + n_B - 2}} \quad (2.4.9)$$

The denominator of Equation 2.4.9 shows us that the degrees of freedom for a pooled standard deviation is $n_A + n_B - 2$, which also is the degrees of freedom for the t -test. Note that we lose two degrees of freedom because the calculations for s_A^2 and s_B^2 require the prior calculation of $\bar{X}_A$ and $\bar{X}_B$.

So how do you determine if it is okay to pool the variances? Use an F -test.

If s_A^2 and s_B^2 are significantly different, then we calculate t_{exp} using Equation 2.4.7. In this case, we find the degrees of freedom using the following imposing equation.

$$\nu = \frac{\left(\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}\right)^2}{\frac{\left(\frac{s_A^2}{n_A}\right)^2}{n_A + 1} + \frac{\left(\frac{s_B^2}{n_B}\right)^2}{n_B + 1}} - 2 \quad (2.4.10)$$

Because the degrees of freedom must be an integer, we round to the nearest integer the value of ν obtained using Equation 2.4.10.

Equation 2.4.10, which is from Miller, J.C.; Miller, J.N. *Statistics for Analytical Chemistry*, 2nd Ed., Ellis-Horward: Chichester, UK, 1988. In the 6th Edition, the authors note that several different equations have been suggested for the number of degrees of freedom for t when s_A and s_B differ, reflecting the fact that the determination of degrees of freedom an approximation. An alternative equation—which is used by statistical software packages, such as R, Minitab, Excel—is

$$\nu = \frac{\left(\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}\right)^2}{\frac{\left(\frac{s_A^2}{n_A}\right)^2}{n_A - 1} + \frac{\left(\frac{s_B^2}{n_B}\right)^2}{n_B - 1}} = \frac{\left(\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}\right)^2}{\frac{s_A^4}{n_A^2(n_A - 1)} + \frac{s_B^4}{n_B^2(n_B - 1)}}$$

For typical problems in analytical chemistry, the calculated degrees of freedom is reasonably insensitive to the choice of equation.

Regardless of whether we calculate t_{exp} using Equation 2.4.7 or Equation 2.4.8, we reject the null hypothesis if t_{exp} is greater than $t(\alpha, \nu)$ and retain the null hypothesis if t_{exp} is less than or equal to $t(\alpha, \nu)$.

✓ Example 2.4.4

Table 4.4.1 provides results for two experiments to determine the mass of a circulating U.S. penny. Determine whether there is a difference in the means of these analyses at $\alpha = 0.05$.

Solution

First we use an F -test to determine whether we can pool the variances. We completed this analysis in Example 2.4.3, finding no evidence of a significant difference, which means we can pool the standard deviations, obtaining

$$s_{\text{pool}} = \sqrt{\frac{(7-1)(0.051)^2 + (5-1)(0.037)^2}{7+5-2}} = 0.0459$$

with 10 degrees of freedom. To compare the means we use the following null hypothesis and alternative hypotheses

$$H_0: \mu_A = \mu_B \quad H_A: \mu_A \neq \mu_B$$

Because we are using the pooled standard deviation, we calculate t_{exp} using Equation 2.4.8.

$$t_{\text{exp}} = \frac{|3.117 - 3.081|}{0.0459} \times \sqrt{\frac{7 \times 5}{7+5}} = 1.34$$

The critical value for $t(0.05, 10)$, from Appendix 4, is 2.23. Because t_{exp} is less than $t(0.05, 10)$ we retain the null hypothesis. For $\alpha = 0.05$ we do not have evidence that the two sets of pennies are significantly different.

✓ Example 2.4.5

One method for determining the %w/w Na_2CO_3 in soda ash is to use an acid–base titration. When two analysts analyze the same sample of soda ash they obtain the results shown here.

Analyst A: 86.82% 87.04% 86.93% 87.01% 86.20% 87.00%

Analyst B: 81.01% 86.15% 81.73% 83.19% 80.27% 83.93%

Determine whether the difference in the mean values is significant at $\alpha = 0.05$.

Solution

We begin by reporting the mean and standard deviation for each analyst.

$$\bar{X}_A = 86.83\% \quad s_A = 0.32\%$$

$$\bar{X}_B = 82.71\% \quad s_B = 2.16\%$$

To determine whether we can use a pooled standard deviation, we first complete an F -test using the following null and alternative hypotheses.

$$H_0: s_A^2 = s_B^2 \quad H_A: s_A^2 \neq s_B^2$$

Calculating F_{exp} , we obtain a value of

$$F_{\text{exp}} = \frac{(2.16)^2}{(0.32)^2} = 45.6$$

Because F_{exp} is larger than the critical value of 7.15 for $F(0.05, 5, 5)$ from Appendix 5, we reject the null hypothesis and accept the alternative hypothesis that there is a significant difference between the variances; thus, we cannot calculate a pooled standard deviation.

To compare the means for the two analysts we use the following null and alternative hypotheses.

$$H_0: \bar{X}_A = \bar{X}_B \quad H_A: \bar{X}_A \neq \bar{X}_B$$

Because we cannot pool the standard deviations, we calculate t_{exp} using Equation 2.4.7 instead of Equation 2.4.8

$$t_{\text{exp}} = \frac{|86.83 - 82.71|}{\sqrt{\frac{(0.32)^2}{6} + \frac{(2.16)^2}{6}}} = 4.62$$

and calculate the degrees of freedom using Equation 2.4.10

$$\nu = \frac{\left(\frac{(0.32)^2}{6} + \frac{(2.16)^2}{6} \right)^2}{\frac{\left(\frac{(0.32)^2}{6} \right)^2}{6+1} + \frac{\left(\frac{(2.16)^2}{6} \right)^2}{6+1}} - 2 = 5.3 \approx 5$$

From [Appendix 4](#), the critical value for $t(0.05, 5)$ is 2.57. Because t_{exp} is greater than $t(0.05, 5)$ we reject the null hypothesis and accept the alternative hypothesis that the means for the two analysts are significantly different at $\alpha = 0.05$.

? Exercise 2.4.3

To compare two production lots of aspirin tablets, you collect samples from each and analyze them, obtaining the following results (in mg aspirin/tablet).

Lot 1: 256 248 245 245 244 248 261

Lot 2: 241 258 241 244 256 254

Is there any evidence at $\alpha = 0.05$ that there is a significant difference in the variance between the results for these two samples? This is the same data from [Exercise 2.4.2](#).

Answer

To compare the means for the two lots, we use an unpaired t -test of the null hypothesis $H_0: \bar{X}_{\text{Lot 1}} = \bar{X}_{\text{Lot 2}}$ and the alternative hypothesis $H_A: \bar{X}_{\text{Lot 1}} \neq \bar{X}_{\text{Lot 2}}$. Because there is no evidence to suggest a difference in the variances (see [Exercise 2.4.2](#)) we pool the standard deviations, obtaining an s_{pool} of

$$s_{\text{pool}} = \sqrt{\frac{(7-1)(6.451)^2 + (6-1)(7.849)^2}{7+6-2}} = 7.121$$

The means for the two samples are 249.57 mg for Lot 1 and 249.00 mg for Lot 2. The value for t_{exp} is

$$t_{\text{exp}} = \frac{|249.57 - 249.00|}{7.121} \times \sqrt{\frac{7 \times 6}{7+6}} = 0.1439$$

The critical value for $t(0.05, 11)$ is 2.204. Because t_{exp} is less than $t(0.05, 11)$, we retain the null hypothesis and find no evidence at $\alpha = 0.05$ that there is a significant difference between the means for the two lots of aspirin tablets.

Paired Data

Suppose we are evaluating a new method for monitoring blood glucose concentrations in patients. An important part of evaluating a new method is to compare it to an established method. What is the best way to gather data for this study? Because the variation in the blood glucose levels amongst patients is large we may be unable to detect a small, but significant difference between the methods if we use different patients to gather data for each method. Using paired data, in which we analyze each patient's blood using both methods, prevents a large variance within a population from adversely affecting a t -test of means.

Typical blood glucose levels for most non-diabetic individuals ranges between 80–120 mg/dL (4.4–6.7 mM), rising to as high as 140 mg/dL (7.8 mM) shortly after eating. Higher levels are common for individuals who are pre-diabetic or diabetic.

When we use paired data we first calculate the difference, d_i , between the paired values for each sample. Using these difference values, we then calculate the average difference, $\bar{d}$, and the standard deviation of the differences, s_d . The null hypothesis, $H_0: d = 0$, is that there is no difference between the two samples, and the alternative hypothesis, $H_A: d \neq 0$, is that the difference between the two samples is significant.

The test statistic, t_{exp} , is derived from a confidence interval around $\bar{d}$

$$t_{\text{exp}} = \frac{|\bar{d}| \sqrt{n}}{s_d}$$

where n is the number of paired samples. As is true for other forms of the t -test, we compare t_{exp} to $t(\alpha, \nu)$, where the degrees of freedom, ν , is $n - 1$. If t_{exp} is greater than $t(\alpha, \nu)$, then we reject the null hypothesis and accept the alternative hypothesis. We retain the null hypothesis if t_{exp} is less than or equal to $t(\alpha, \nu)$. This is known as a **paired t -test**.

✓ Example 2.4.6

Marecek et. al. developed a new electrochemical method for the rapid determination of the concentration of the antibiotic monensin in fermentation vats [Marecek, V.; Janchenova, H.; Brezina, M.; Betti, M. *Anal. Chim. Acta* **1991**, 244, 15–19]. The standard method for the analysis is a test for microbiological activity, which is both difficult to complete and time-consuming. Samples were collected from the fermentation vats at various times during production and analyzed for the concentration of monensin using both methods. The results, in parts per thousand (ppt), are reported in the following table.

Sample	Microbiological	Electrochemical
1	129.5	132.3
2	89.6	91.0
3	76.6	73.6
4	52.2	58.2
5	110.8	104.2
6	50.4	49.9
7	72.4	82.1
8	141.4	154.1
9	75.0	73.4
10	34.1	38.1
11	60.3	60.1

Is there a significant difference between the methods at $\alpha = 0.05$?

Solution

Acquiring samples over an extended period of time introduces a substantial time-dependent change in the concentration of monensin. Because the variation in concentration between samples is so large, we use a paired t -test with the following null and alternative hypotheses.

$$H_0: \bar{d} = 0 \quad H_A: \bar{d} \neq 0$$

Defining the difference between the methods as

$$d_i = (X_{\text{elect}})_i - (X_{\text{micro}})_i$$

we calculate the difference for each sample.

sample	1	2	3	4	5	6	7	8	9	10	11
d_i	2.8	1.4	-3.0	6.0	-6.6	-0.5	9.7	12.7	-1.6	4.0	-0.2

The mean and the standard deviation for the differences are, respectively, 2.25 ppt and 5.63 ppt. The value of t_{exp} is

$$t_{\text{exp}} = \frac{|2.25|\sqrt{11}}{5.63} = 1.33$$

which is smaller than the critical value of 2.23 for $t(0.05, 10)$ from [Appendix 4](#). We retain the null hypothesis and find no evidence for a significant difference in the methods at $\alpha = 0.05$.

? Exercise 2.4.4

Suppose you are studying the distribution of zinc in a lake and want to know if there is a significant difference between the concentration of Zn^{2+} at the sediment-water interface and its concentration at the air-water interface. You collect samples from six locations—near the lake's center, near its drainage outlet, etc.—obtaining the results (in mg/L) shown in the table. Using this data, determine if there is a significant difference between the concentration of Zn^{2+} at the two interfaces at $\alpha = 0.05$. Complete this analysis treating the data as (a) unpaired and as (b) paired. Briefly comment on your results.

Location	Air-Water Interface	Sediment-Water Interface
1	0.430	0.415
2	0.266	0.238
3	0.457	0.390
4	0.531	0.410
5	0.707	0.605
6	0.716	0.609

Complete this analysis treating the data as (a) unpaired and as (b) paired. Briefly comment on your results.

Answer

Treating as Unpaired Data: The mean and the standard deviation for the concentration of Zn^{2+} at the air-water interface are 0.5178 mg/L and 0.1732 mg/L, respectively, and the values for the sediment-water interface are 0.4445 mg/L and 0.1418 mg/L, respectively. An F -test of the variances gives an F_{exp} of 1.493 and an $F(0.05, 5, 5)$ of 7.146. Because F_{exp} is smaller than $F(0.05, 5, 5)$, we have no evidence at $\alpha = 0.05$ to suggest that the difference in variances is significant. Pooling the standard deviations gives an s_{pool} of 0.1582 mg/L. An unpaired t -test gives t_{exp} as 0.8025. Because t_{exp} is smaller than $t(0.05, 11)$, which is 2.204, we have no evidence that there is a difference in the concentration of Zn^{2+} between the two interfaces.

Treating as Paired Data: To treat as paired data we need to calculate the difference, d_i , between the concentration of Zn^{2+} at the air-water interface and at the sediment-water interface for each location, where

$$d_i = ([\text{Zn}^{2+}]_{\text{air-water}})_i - ([\text{Zn}^{2+}]_{\text{sed-water}})_i$$

The mean difference is 0.07333 mg/L with a standard deviation of 0.0441 mg/L. The null hypothesis and the alternative hypothesis are

$$H_0: \bar{d} = 0 \quad H_A: \bar{d} \neq 0$$

and the value of t_{exp} is

$$t_{\text{exp}} = \frac{|0.07333|\sqrt{6}}{0.0441} = 4.073$$

Because t_{exp} is greater than $t(0.05, 5)$, which is 2.571, we reject the null hypothesis and accept the alternative hypothesis that there is a significant difference in the concentration of Zn^{2+} between the air-water interface and the sediment-water interface.

The difference in the concentration of Zn^{2+} between locations is much larger than the difference in the concentration of Zn^{2+} between the interfaces. Because our interest is in studying the difference between the interfaces, the larger standard deviation when treating the data as unpaired increases the probability of incorrectly retaining the null hypothesis, a type 2 error.

One important requirement for a paired t -test is that the determinate and the indeterminate errors that affect the analysis must be independent of the analyte's concentration. If this is not the case, then a sample with an unusually high concentration of analyte

will have an unusually large d_i . Including this sample in the calculation of $\bar{d}$ and s_d gives a biased estimate for the expected mean and standard deviation. This rarely is a problem for samples that span a limited range of analyte concentrations, such as those in Example 2.4.6 or Exercise 2.4.4. When paired data span a wide range of concentrations, however, the magnitude of the determinate and indeterminate sources of error may not be independent of the analyte's concentration; when true, a paired t -test may give misleading results because the paired data with the largest absolute determinate and indeterminate errors will dominate $\bar{d}$. In this situation a regression analysis, which is the subject of the next chapter, is more appropriate method for comparing the data.

Outliers

Earlier in the chapter we examined several data sets consisting of the mass of a circulating United States penny. Table 2.4.1 provides one more data set. Do you notice anything unusual in this data? Of the 112 pennies included in Table 4.4.1 and Table 4.4.3, no penny weighed less than 3 g. In Table 4.6.1, however, the mass of one penny is less than 3 g. We might ask whether this penny's mass is so different from the other pennies that it is in error.

Table 2.4.1 : Mass (g) for Additional Sample of Circulating U. S. Pennies

3.067	2.514	3.094
3.049	3.048	3.109
3.039	3.079	3.102

A measurement that is not consistent with other measurements is called outlier. An **outlier** might exist for many reasons: the outlier might belong to a different population (Is this a Canadian penny?); the outlier might be a contaminated or otherwise altered sample (Is the penny damaged or unusually dirty?); or the outlier may result from an error in the analysis (Did we forget to tare the balance?). Regardless of its source, the presence of an outlier compromises any meaningful analysis of our data. There are many significance tests that we can use to identify a potential outlier, three of which we present here.

Grubb's Test

Although Dixon's Q -test is a common method for evaluating outliers, it is no longer favored by the International Standards Organization (ISO), which recommends the **Grubb's test**. There are several versions of Grubb's test depending on the number of potential outliers. Here we will consider the case where there is a single suspected outlier.

For details on this recommendation, see International Standards ISO Guide 5752-2 "Accuracy (trueness and precision) of measurement methods and results—Part 2: basic methods for the determination of repeatability and reproducibility of a standard measurement method," 1994.

The test statistic for Grubb's test, G_{exp} , is the distance between the sample's mean, $\bar{X}$, and the potential outlier, X_{out} , in terms of the sample's standard deviation, s .

$$G_{\text{exp}} = \frac{|X_{\text{out}} - \bar{X}|}{s}$$

We compare the value of G_{exp} to a critical value $G(\alpha, n)$, where α is the probability that we will reject a valid data point and n is the number of data points in the sample. If G_{exp} is greater than $G(\alpha, n)$, then we may reject the data point as an outlier, otherwise we retain the data point as part of the sample. Table 2.4.3 provides values for $G(0.05, n)$ for a sample containing 3–10 values. A more extensive table is in Appendix 7. Values for $G(\alpha, n)$ assume an underlying normal distribution.

Table 2.4.3 : Grubb's Test

n	$G(0.05, n)$
3	1.115
4	1.481
5	1.715
6	1.887

7	2.020
8	2.126
9	2.215
10	2.290

You should exercise caution when using a significance test for outliers because there is a chance you will reject a valid result. In addition, you should avoid rejecting an outlier if it leads to a precision that is much better than expected based on a propagation of uncertainty. Given these concerns it is not surprising that some statisticians caution against the removal of outliers [Deming, W. E. *Statistical Analysis of Data*; Wiley: New York, 1943 (republished by Dover: New York, 1961); p. 171].

You also can adopt a more stringent requirement for rejecting data. When using the Grubb's test, for example, the ISO 5752 guidelines suggests retaining a value if the probability for rejecting it is greater than $\alpha = 0.05$, and flagging a value as a "straggler" if the probability for rejecting it is between $\alpha = 0.05$ and $\alpha = 0.01$. A "straggler" is retained unless there is compelling reason for its rejection. The guidelines recommend using $\alpha = 0.01$ as the minimum criterion for rejecting a possible outlier.

On the other hand, testing for outliers can provide useful information if we try to understand the source of the suspected outlier. For example, the outlier in [Table 2.4.1](#) represents a significant change in the mass of a penny (an approximately 17% decrease in mass), which is the result of a change in the composition of the U.S. penny. In 1982 the composition of a U.S. penny changed from a brass alloy that was 95% w/w Cu and 5% w/w Zn (with a nominal mass of 3.1 g), to a pure zinc core covered with copper (with a nominal mass of 2.5 g) [Richardson, T. H. *J. Chem. Educ.* **1991**, 68, 310–311]. The pennies in [Table 2.4.1](#), therefore, were drawn from different populations.

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2.5: Python Basics for Analytical Chemists

In this class, we will rely on Python code for a lot of our data processing. Python is preferable to Excel in many ways, especially as data sets become larger and more unwieldy.

Operators:

Python can be used as essentially a calculator, and because we will work with a lot of mathematical data, it is important to know the basic operators in Python. Below, you'll see examples of the syntax for basic math functions like addition (+), subtraction (-), multiplication (*) and division (/). Additionally, note that exponents use the notation `x**y` to denote x raised to the y power. Also note that some operations are not limited to numbers!

To show the results of the operations shown here, we need to instruct the computer to output the information stored in each variable. We will use the `print()` command to do that.

```
print(1+2)
print(6-4)
print(7*8)
print(42/7)
print(4**2)
print('hello ' + 'world')
```

runrestartrestart & run all

Variables:

To store information, we can define variables. Variables come in several types; the four most important for us will be integers, floats and strings:

Integers:

Integers are whole number values. A number entered without a decimal place will default to an integer value unless otherwise specified.

```
a = 6259

print(a)
print(type(a))
```

runrestartrestart & run all

Float:

Float is the name Python uses for decimal values (because they saved as something called a "floating point value"). Any value which is entered with a decimal place will automatically be a float. Additionally, the division operator will return a float if the result of the division step is not an integer. Note that all of the numbers below return a variable type of float:

```
p = 2.0
q = 2.3
r = 4.3

print(p,q,r)
print(type(p))
print(type(q))
print(type(r))
```

runrestartrestart & run all

Strings:

Strings are what Python calls words or other text that you would like to save as a variable. They are useful in a variety of contexts, but we will mostly use them to keep track of detailed information in our print statements, and for things like figure captions and axis labels when we're working with graphs.

When working with strings and numbers together, Python has a particularly useful function called F printing. You will see this construction a lot in our lab activities!

To let Python know that we are mixing numbers and strings, you will start your print statement with an F and then place the entire statement you want to make inside single quotes, inside parenthesis. Any time we wish to express a variable in that sequence, we use curly brackets. The general format is `print(f' words {variables} more words.')` See below for an example

```
pi = 3.14
b = 2
print(f'The value stored for the variable pi is {pi} and it is currently recorded to
```

run restart restart & run all

Rounding with F Printing:

If you would like to round the variable number in a F print statement, a small addition is needed inside the curly brackets. For example, add `":3f"` to round to the third decimal place, or `":3"` to round to 3 digits. Be careful - Python is not always great at counting *significant digits*!.

```
pi = 3.1415926535

print(F"If I want to print pi to three digits, type {pi:.3}")
print(F"If I want to print pi to three decimal places, it looks just a little bit dif
```

run restart restart & run all

Lists:

We often want to work with many numbers (or strings, etc.) in a large data set. There are several ways to organize groups of variables in Python, but the one that we will see most frequently is the list. Lists allow us to take a bunch of data of the same type and store it together in a single variable. Here is a simple data set for us to work some example problems with, stored in a list called "data1"

```
# here is a set of endpoint volumes from different trials of a titration, stored in a
data1 = [12.10, 12.12, 12.08, 12.09, 12.16] # mL

# we can display the data outside of the coding window using the print command:

print (data1)
# we can also ask for the data type (as seen above)
print(type(data1))
```

run restart restart & run all

If we want to call an individual data point out of the list, we use an index number which corresponds to its place in the list. All computer programming languages start counting at zero, so calling each data point in the list and printing them out looks like this:

```
# this will print the first number in the list data 1:
print ("the first number in 'data1' is:")
print (data1[0])

# this will print the second number in data 1:
```

```
print ("the second number in 'data1' is: ")
print (data1[1])

# this will print the third number in data 1:
print ("the third number in 'data1' is: ")
print (data1[2])

# print the 4th number
print ("the fourth number in 'data1' is: ")
print (data1[3])

# and the fifth number
print ("the fifth number in 'data1' is: ")
print (data1[4])
# press RUN to see the output
```

run restart restart & run all

Iteration:

Iteration allows us to repeat repetitive tasks over and over again without having to copy and paste or rewrite the same code over and over again. In Python, there are a number of ways to iterate over repetitive processes, but we will primarily use for loops. The general format of a for loop is

for *variable* in *list*:

 Do things

```
fruit = ['apples', 'oranges', 'grapes', 'pears']
for x in fruit:
    print(x)
```

run restart restart & run all

Conditionals:

Conditionals allow us to tell the computer that we need it to make a decision. If you can frame the statement in terms of "if" something is true "then" do something, we can teach the computer how to do it! This is particularly valuable for statistical tests, but here is a simple example which will tell me whether I have enough change to buy a candy bar today.

The general format is always to start with if and define your first statement. In this case, the question is whether I have enough cash to buy a candy bar, so I want to know if my amount of change adds up to greater than or equal to the price of the candy bar. Note that the syntax for greater than (>) is different from greater than or equal to (>=). If I want to check if numbers are exactly equal, I will use '==' to designate that.

Run this code, and then try changing the number of quarters, dimes or nickels until the message changes. Can you make each different message appear?

```
# price of snickers bar in Shiley vending machine
price = 1.50

# money in my desk drawer right now
quarters = 3
dimes = 2
nickels = 1

# Do I have enough for a candy bar?
cash = (quarters*.25)+(dimes*0.1)+(nickels*0.05)
```

```
if cash > price:
    print (F'yes, you have {cash} dollars, which is more than enough to buy a candy bar')
if cash == price:
    print ("You have exactly enough cash to buy a candy bar")
if cash < price:
    print(F'no, {cash} dollars is not enough for a candy bar')
```

run

restart

restart & run all

no, 1.0 dollars is not enough for a candy bar

Functions:

We use a function to call some pre-written code that will perform a task for us. We can write our own functions, but we can also take advantage of the many functions already built in to python, or defined in extra packages! Here are several examples of functions that will use over and over again in this class:

- `len(list)` returns the length of a list. We use this function regularly to get `n` (i.e. the number of data points in a data set)
- `max(list)` returns the maximum value in the list
- `min(list)` returns the minimum value in the list
- `print("strings")` outputs words
 - "F printing" allows us to mix strings and variables, and round within the print statement. The format when F printing is `print(F "words here {variables} and then more words and {variable:.n} rounded to the n decimal place")`

Other useful functions may need to be imported, so we will often start out code with "import math", "import numpy as np" and "import statistics as stat" so that we can use these functions.

Important functions from the math package:

- `math.log10(x)` takes the logarithm of `x`
- `math.sqrt(x)` takes the square root of `x`

Important Functions from the numpy package:

- `np.mean(array)` returns the average, or mean, of an array
- `np.std(array, ddof = 1)` returns the sample standard deviation of an array. Omitting the `ddof = 1` portion of this code will return the population standard deviation, which may be helpful in other contexts, but will not be used in this class

Important Functions from the statistics package:

- `stats.t.ppf(1-alpha/2, dof)` returns the `t` value for a confidence level of `1-alpha`, with `dof` degrees of freedom. See confidence interval class example for the use of this code.
- `stats.f.ppf(q=1-alpha, dfn=x, dfd=x)` returns the `F` value for a confidence level of `1-alpha`, with `dfn` and `dfd` representing the degrees of freedom of each data set being compared.

Other functions, especially related to graphing and linear regressions will be addressed in later chapters!

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2.6: Chapter 3 Formulas

Sample Mean: $\bar{x} = \frac{\sum x}{n}$	Population Mean: $\mu = \frac{\sum x}{N}$
Weighted Mean: $\bar{x} = \frac{\sum(xw)}{\sum w}$	Range = Max – Min
Sample Standard Deviation: $s = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$	Population Standard Deviation = σ
Sample Variance: $s^2 = \frac{\sum(x-\bar{x})^2}{n-1}$	Population Variance = σ^2
Coefficient of Variation: $CVar = \left(\frac{s}{\bar{x}} \cdot 100\right) \%$	Z-Score: $z = \frac{x-\bar{x}}{s}$
Percentile Index: $i = \frac{(n+1)p}{100}$	Interquartile Range: $IQR = Q_3 - Q_1$
Empirical Rule: $z = 1, 2, 3 \Rightarrow 68\%, 95\%, 99.7\%$	Outlier Lower Limit: $Q_1 - (1.5 \cdot IQR)$
Chebyshev's Inequality: $\left(\left(1 - \frac{1}{(z)^2}\right) \cdot 100\right) \%$	Outlier Upper Limit: $Q_3 + (1.5 \cdot IQR)$

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2.7: Chapter 3 Exercises

? Chapter 3 Exercises

1. A sample of eight cats found the following weights in kg.

4.0	4.1	3.2	4.0	3.8	3.6	3.7	3.4
-----	-----	-----	-----	-----	-----	-----	-----

a) Compute the mode.

b) Compute the median.

c) Compute the mean.

2. Cholesterol levels in milligrams (mg) of cholesterol per deciliter (dL) of blood, were collected from patients two days after they had a heart attack (Ryan, Joiner & Ryan, Jr, 1985).

270	236	210	142	280	272	160
220	226	242	186	266	206	318
294	282	234	224	276	282	360
310	280	278	288	288	244	236

Retrieved from <http://www.statsci.org/data/general/cholest.html>.

a) Compute the mode.

b) Compute the median.

c) Compute the mean.

3. The lengths (in kilometers) of rivers on the South Island of New Zealand that flow to the Tasman Sea are listed below.

River	Length (km)	River	Length (km)
Hollyford	76	Waimea	48
Cascade	64	Motueka	108
Arawhata	68	Takaka	72
Haast	64	Aorere	72
Karangarua	37	Heaphy	35
Cook	32	Karamea	80
Waiho	32	Mokihinui	56
Whataroa	51	Buller	177
Wanganui	56	Grey	121
Waitaha	40	Taramakau	80
Hokitika	64	Arahura	56

Data from <http://www.statsci.org/data/oz/nzrivers.html>.

a) Compute the mode.

b) Compute the median.

c) Compute the mean.

4. A university assigns letter grades with the following 4-point scale: A = 4.00, A- = 3.67, B+ = 3.33, B = 3.00, B- = 2.67, C+ = 2.33, C = 2.00, C- = 1.67, D+ = 1.33, D = 1.00, D- = 0.67, F = 0.00. Calculate the grade point average (GPA) for a student who took in one term a 4 credit math course and received a B+, a 1 credit seminar course and received an A, a 3 credit history course and received an A- and a 5 credit writing course and received a D.

5. A university assigns letter grades with the following 4-point scale: A = 4.00, A- = 3.67, B+ = 3.33, B = 3.00, B- = 2.67, C+ = 2.33, C = 2.00, C- = 1.67, D+ = 1.33, D = 1.00, D- = 0.67, F = 0.00. Calculate the grade point average (GPA) for a student

who took in one term a 3 credit biology course and received a C+, a 1 credit lab course and received a B, a 4 credit engineering course and received an A- and a 4 credit chemistry course and received a C+.

6. An employee at Clackamas Community College (CCC) is evaluated based on goal setting and accomplishments toward the goals, job effectiveness, competencies, and CCC core values. Suppose for a specific employee, goal 1 has a weight of 30%, goal 2 has a weight of 20%, job effectiveness has a weight of 25%, competency 1 has a weight of 4%, competency 2 has weight of 3%, competency 3 has a weight of 3%, competency 4 has a weight of 3%, competency 5 has a weight of 2%, and core values has a weight of 10%. Suppose the employee has scores of 3.0 for goal 1, 3.0 for goal 2, 2.0 for job effectiveness, 3.0 for competency 1, 2.0 for competency 2, 2.0 for competency 3, 3.0 for competency 4, 4.0 for competency 5, and 3.0 for core values. Compute the weighted mean score for this employee. If an employee has a score less than 2.5, they must have a Performance Enhancement Plan written. Does this employee need a plan?

7. A statistics class has the following activities and weights for determining a grade in the course: test 1 worth 15% of the grade, test 2 worth 15% of the grade, test 3 worth 15% of the grade, homework worth 10% of the grade, semester project worth 20% of the grade, and the final exam worth 25% of the grade. If a student receives an 85 on test 1, a 76 on test 2, an 83 on test 3, a 74 on the homework, a 65 on the project, and a 61 on the final, what grade did the student earn in the course? All the assignments were out of 100 points.

8. A statistics class has the following activities and weights for determining a grade in the course: test 1 worth 15% of the grade, test 2 worth 15% of the grade, test 3 worth 15% of the grade, homework worth 10% of the grade, semester project worth 20% of the grade, and the final exam worth 25% of the grade. If a student receives a 25 out of 30 on test 1, a 20 out of 30 on test 2, a 28 out of 30 on test 3, a 120 out of 140 points on the homework, a 65 out of 100 on the project, and a 31 out of 35 on the final, what grade did the student earn in the course?

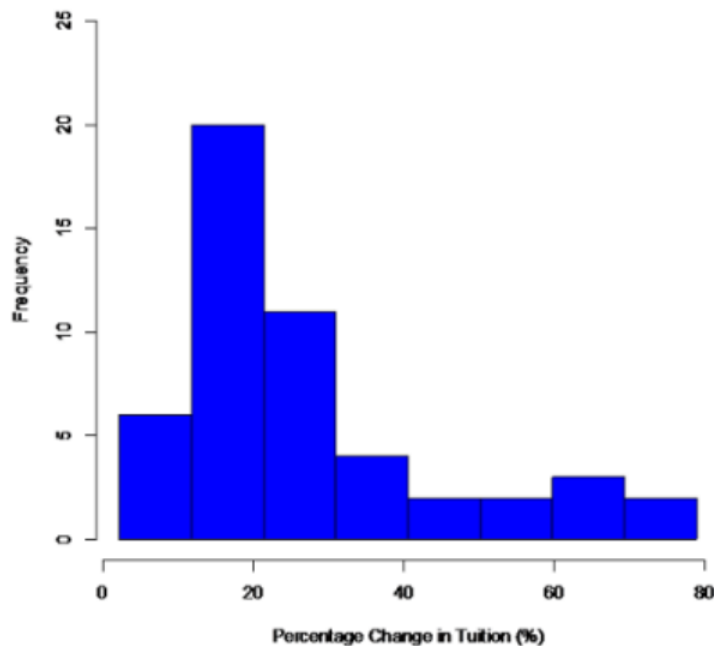
9. A sample of eight cats found the following weights in kg.

3.7	4.1	3.2	4.0	3.8	3.6	3.7	3.4
-----	-----	-----	-----	-----	-----	-----	-----

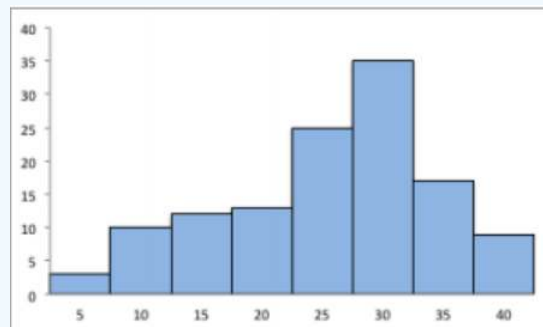
- Compute the range.
- Compute the variance.
- Compute the standard deviation.

10. The following data represents the percent change in tuition levels at public, four-year colleges (inflation adjusted) from 2008 to 2013 (Weissmann, 2013). To the right is the histogram. What is the shape of the distribution?

Percentage Change in Tuition Levels (Inflation Adjusted) 2008 to 2013



11. The following is a histogram of quiz grades.



- What is the shape of the distribution?
- Which is higher, the mean or the median?

12. Cholesterol levels were collected from patients two days after they had a heart attack (Ryan, Joiner & Ryan, Jr, 1985). Retrieved from <http://www.statsci.org/data/general/cholest.html>.

270	236	210	142	280	272	160
220	226	242	186	266	206	318
294	282	234	224	276	282	360
310	280	278	288	288	244	236

- Compute the range.
- Compute the variance.
- Compute the standard deviation.

13. Suppose that a manager wants to test two new training programs. The manager randomly selects five people for each training type and measures the time it takes to complete a task after the training. The times for both trainings are in table below. Which training method is more variable?

Training 1	56	75	48	63	59
Training 2	60	58	66	59	58

14. The lengths (in kilometers) of rivers on the South Island of New Zealand that flow to the Tasman Sea are listed below.

River	Length (km)	River	Length (km)
Hollyford	76	Waimea	48
Cascade	64	Motueka	108
Arawhata	68	Takaka	72
Haast	64	Aorere	72
Karangarua	37	Heaphy	35
Cook	32	Karamea	80
Waiho	32	Mokihinui	56
Whataroa	51	Buller	177
Wanganui	56	Grey	121
Waitaha	40	Taramakau	80
Hokitika	64	Arahura	56

Data from <http://www.statsci.org/data/oz/nzrivers.html>.

- Compute the range.
- Compute the variance.
- Compute the standard deviation.

15. Here are pulse rates before and after exercise. Which group has the larger range?

Before	Pulse Rates	After
9 8 8 7 6 5 2	6	
9 8 8 8 6 5 5 5 1 1 0 0	7	
8 8 7 5 4 2	8	5 6 6 7 8 9
4 0	9	0 1 1 2 3 4 5 5 6 8
4	10	0 1 4 6 7
	11	6 7
	12	4 5 7

16. A midterm in a statistics course had a mean score of 70 with a standard deviation 5. A quiz in a biology course had an average of 20 with a standard deviation of 5.

- Compute the coefficient of variation for the statistics midterm exam.
- Compute the coefficient of variation for the biology quiz.
- Aaliyah scored a 75 on the statistics midterm exam. Compute Aaliyah's z-score.
- Viannie scored a 25 on the biology quiz. Compute Viannie's z-score.
- Which student did better on their respect test? Why? f) Was there more variability for the midterm or the quiz scores? Justify your answer with statistics.

17. The following is a sample of quiz scores.

Score	17	44.5	16.1	37.2	42.8	37.5	19.5	28.2
-------	----	------	------	------	------	------	------	------

- Compute $\bar{x}$.
- Compute s^2 .

- c) Compute the median.
- d) Compute the coefficient of variation.
- e) Compute the range.

18. The time it takes to fill an online order was recorded and the following descriptive statistics were found using Excel. What is the coefficient of variation?

<i>Time</i>	
Mean	7
Standard Error	0.8085
Median	6.5
Standard Deviation	3.43
Sample Variance	11.7647
Range	12
Minimum	3
Maximum	15
Sum	126
Count	18

19. The following is the height and weight of a random sample of baseball players.

Height (inches)	Weight (pounds)
76	212
76	224
72	180
74	210
75	215
71	200
77	235
78	235
77	194
76	185
72	180
72	170
75	220
74	228
73	210
72	180
70	185
73	190
71	186
74	200
74	200
75	210
78	240
72	208
75	180

- a) Compute the coefficient of variation for both height and weight.
- b) Is there more variation in height or weight? a) Compute the coefficient of variation for both height

and weight.

20. A midterm in a statistics course had a mean score of 70 with a standard deviation 5. A quiz had an average of 20 with a standard deviation of 5. A student scored a 73 on their midterm and 22 on their quiz. On which test did the student do better on compared to the rest of the class? Justify your answer with statistics.

21. The length of a human pregnancy is normally distributed with a mean of 272 days with a standard deviation of 9.1 days. William Hunnicut was born in Portland, Oregon, at just 181 days into his gestation. What is the zscore for William Hunnicut's gestation? Retrieved from: http://digitalcommons.georgefox.edu/cgi/viewcontent.cgi?article=1149&context=gfc_life.

22. Arm span (sometimes referred to as wingspan) is the physical measurement of the length of an individual's arms from fingertip to fingertip. The average arm span of a man is 70 inches with a standard deviation of 4.5 inches. The Olympic gold medalists Michael Phelps has an arm span of 6 feet 7 inches, which is three inches more than his height. What is the z-score for Michael Phelps arm span?

23. The average time to run the Pikes Peak Marathon 2017 was 7.44 hours with a standard deviation of 1.34 hours. Rémi Bonnet won the Pikes Peak Marathon with a run time of 3.62 hours. Retrieved from: <http://pikespeakmarathon.org/results/ppm/2017/>.

The Tevis Cup 100-mile one-day horse race for 2017 had an average finish time of 20.38 hours with a standard deviation of 1.77 hours. Tennessee Lane won the 2017 Tevis cup in a ride time of 14.75 hours. Retrieved from: <https://aerc.org/rpts/RideResults.aspx>.

- Compute the z-score for Rémi Bonnet's time.
- Compute the z-score for Tennessee Lane's time.
- Which competitor did better compared to their respective events?

24. Cholesterol levels were collected from patients two days after they had a heart attack (Ryan, Joiner & Ryan, Jr, 1985). Retrieved from <http://www.statsci.org/data/general/cholest.html>.

270	236	210	142	280	272	160
220	226	242	186	266	206	318
294	282	234	224	276	282	360
310	280	278	288	288	244	236

- Compute the 25th percentile.
- Compute the 90th percentile.
- Compute the 5th percentile.
- Compute Q_3 .

25. A sample of eight cats found the following weights in kg. Compute the 5-number summary.

3.7	4.1	3.2	4.0	3.8	3.6	3.7	3.4
-----	-----	-----	-----	-----	-----	-----	-----

26. The following data represent the grade point averages for a sample of 15 PSU students.

GPA		
3.37	2.61	2.02
3.33	2.93	2.98
3.5	3.81	3.77
2.91	2.51	3.33
3.11	3.32	3.87

- Compute the lower and upper limits.
- Identify if there are any outliers.
- Draw a modified box-and-whisker plot.

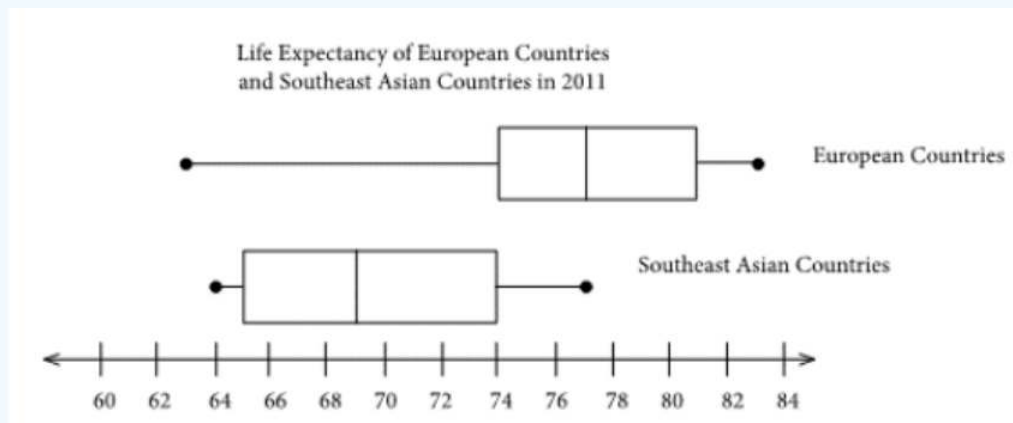
27. The lengths (in kilometers) of rivers on the South Island of New Zealand that flow to the Tasman Sea are listed below.

River	Length (km)	River	Length (km)
Hollyford	76	Waimea	48
Cascade	64	Motueka	108
Arawhata	68	Takaka	72
Haast	64	Aorere	72
Karangarua	37	Heaphy	35
Cook	32	Karamea	80
Waiho	32	Mokihinui	56
Whataroa	51	Buller	177
Wanganui	56	Grey	121
Waitaha	40	Taramakau	80
Hokitika	64	Arahura	56

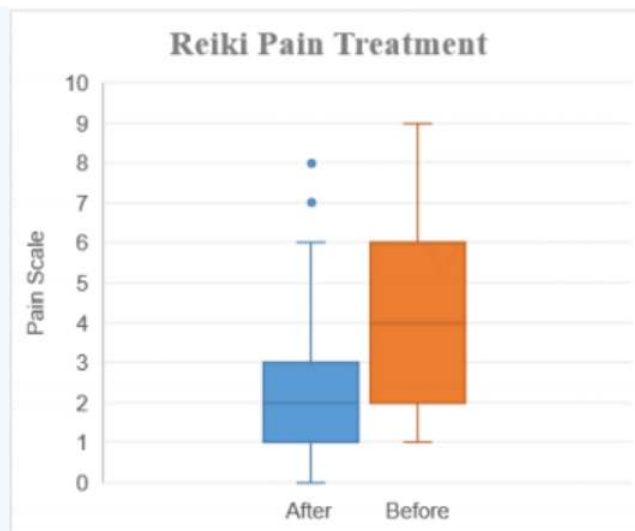
Data from <http://www.statsci.org/data/oz/nzrivers.html>.

- Compute the 5-number summary.
- Compute the lower and upper limits and any outlier(s) if any exist.
- Make a modified box-and-whisker plot.

28. The following are box-and-whiskers plot for life expectancy for European countries and Southeast Asian countries from 2011. What is the distribution shape of the European countries' life expectancy?



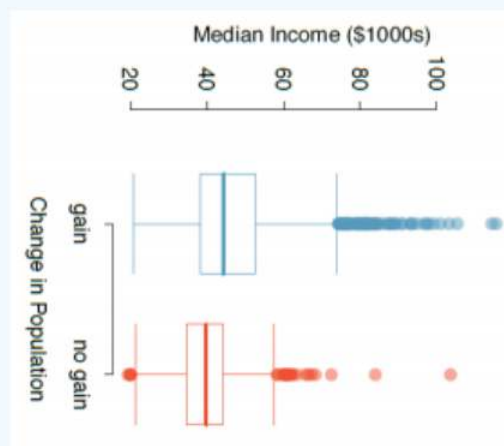
29. To determine if Reiki is an effective method for treating pain, a pilot study was carried out where a certified second-degree Reiki therapist provided treatment on volunteers. Pain was measured using a visual analogue scale (VAS) immediately before and after the Reiki treatment (Olson & Hanson, 1997). Higher numbers mean the patients had more pain.



a) Use the box-and-whiskers plots to determine the IQR for the before treatment measurements.

b) Use the box-and-whiskers plots of the before and after VAS ratings to determine if the Reiki method was effective in reducing pain.

30. The median household income (in \$1,000s) from a random sample of 100 counties that gained population over 2000-2010 are shown on the left. Median incomes from a random sample of 50 counties that had no population gain are shown on the right.



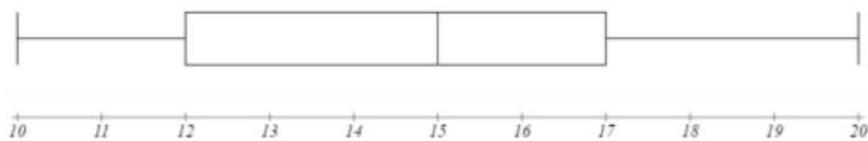
(OpenIntro Statistics, 2016)

What is the distribution shape for the counties with no population gain?

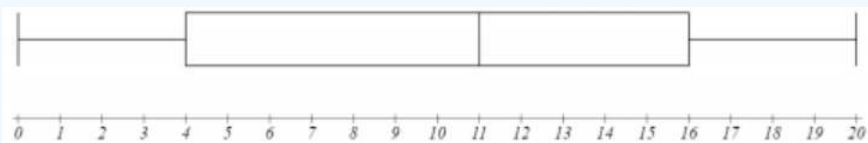
31. Match the correct descriptive statistics to the letter of the corresponding histogram and boxplot. Choose the correct letter for the corresponding histogram and Roman numeral for the corresponding boxplot. You should only use the visual representation, the definition of standard deviation and measures of central tendency to match the graphs with their respective descriptive statistics.

	Mean	Median	Standard Deviation	Histogram Letter	Boxplot Number
1	10.4	11	6.2		
2	16	15.8	1.9		
3	14.8	15	3.1		

i.



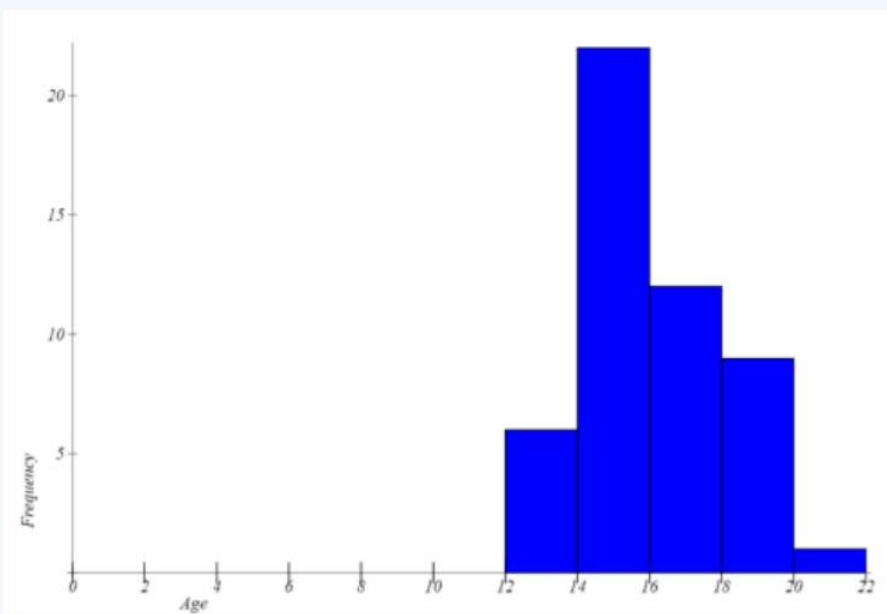
ii.



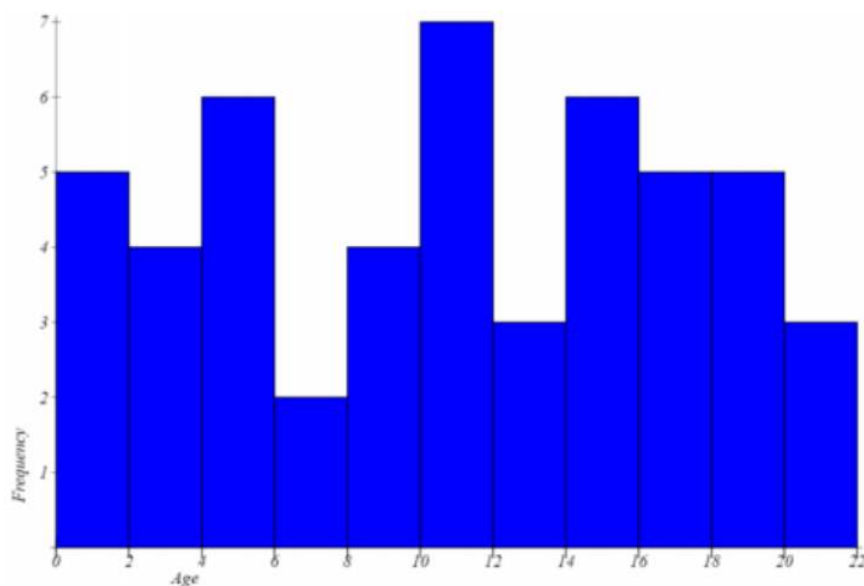
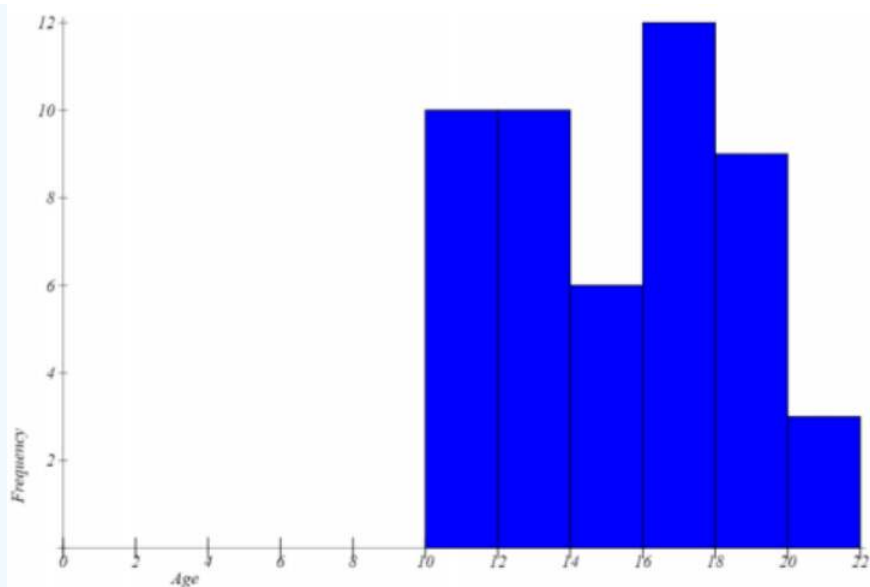
iii.



a)



b)



c)

32. A sample has the following statistics: Minimum = 10, Maximum = 50, Range = 40, $\bar{x}$ = 20, mode = 32, Q_1 = 30, Q_2 = 35, Q_3 = 38, standard deviation = 5 and IQR = 8.

a) According to Chebyshev's Inequality, what percentage of data would fall within the values 12.5 and 27.5?

b) If this sample were bell-shaped what percentage of the data would fall within the values 10 and 30?

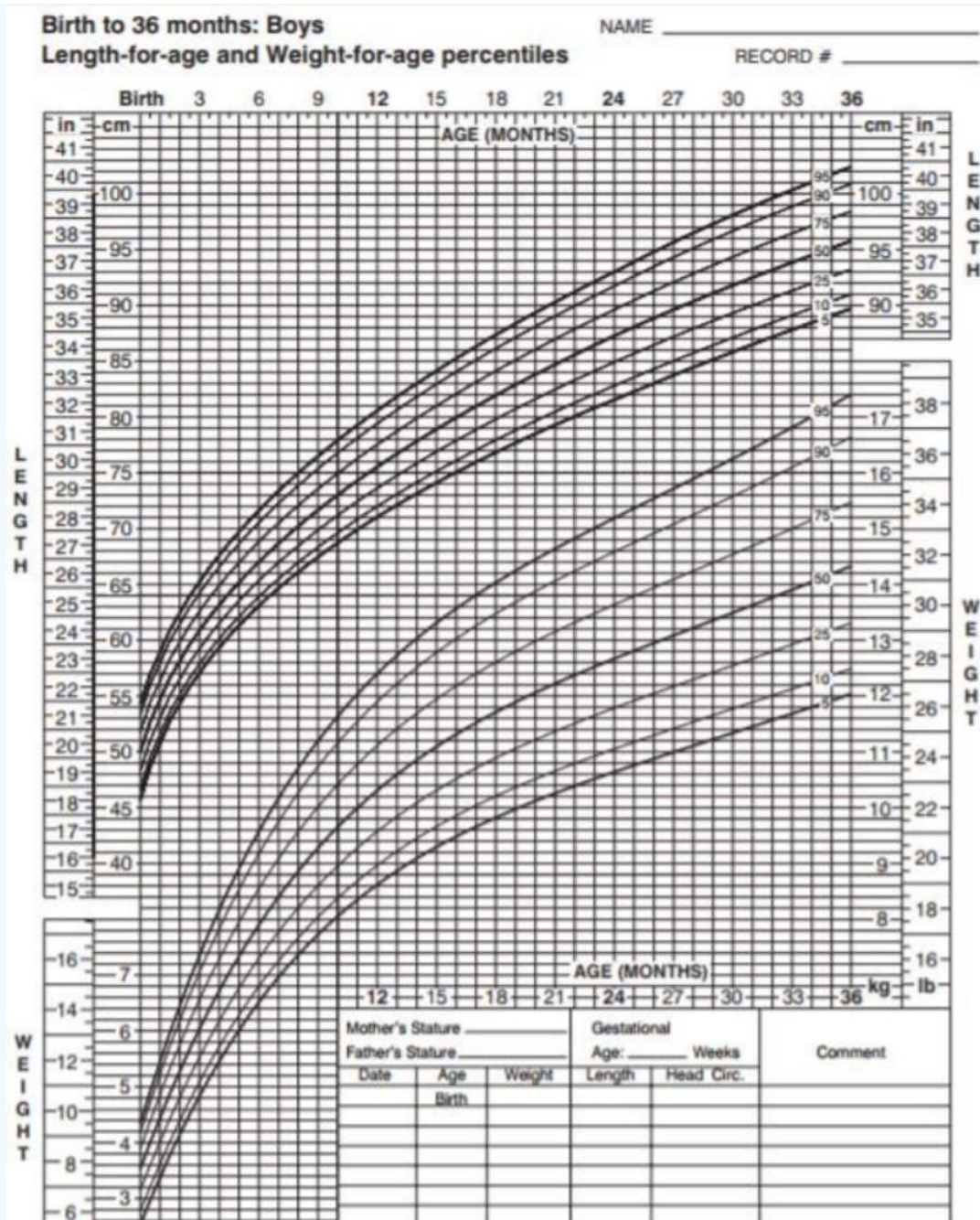
33. The length of a human pregnancy is bell-shaped with a mean of 272 days with a standard deviation of 9 days (Bhat & Kushtagi, 2006). Compute the percentage of pregnancies that last between 245 and 299 days.

34. Arm span is the physical measurement of the length of an individual's arms from fingertip to fingertip. A man's arm span is approximately bell-shaped with mean of 70 inches with a standard deviation of 4.5 inches. What percent of men have an arm span between 61 and 79 inches?

35. The size of fish is very important to commercial fishing. A study conducted in 2012 found the length of Atlantic cod caught in nets in Karlskrona to have a mean of 49.9 cm and a standard deviation of 3.74 cm (Ovegard, Berndt & Lunneryd, 2012).

a) According to Chebyshev's Inequality, at least what percent of Atlantic cod should be between 44.29 and 55.51 cm?

- b) Assume the length of Atlantic cod is bell-shaped. Approximately what percent of Atlantic cod are between 46.16 and 53.64 cm?
- c) Assume the length of Atlantic cod is bell-shaped. Approximately what percent of Atlantic cod are between 42.42 and 57.38 cm?
36. Scores on the SAT for a certain year were bell-shaped with a mean of 1511 and a standard deviation of 194.
- a) What two SAT scores that separated the middle 68% of SAT scores for that year?
- b) What two SAT scores that separated the middle 95% of SAT scores for that year?
- c) How high did a student need to score that year to be in the top 2.5%?
37. In a mid-size company, the distribution of the number of phone calls answered each day by each of the 12 employees is bell-shaped and has a mean of 59 and a standard deviation of 10. Using the empirical rule, what is the approximate percentage of daily phone calls numbering between 29 and 89?
38. The number of potholes in any given 1 mile stretch of pavement in Portland has a bell-shaped distribution. This distribution has a mean of 54 and a standard deviation of 5. Using the empirical rule, what is the approximate percentage of 1-mile long roadways with potholes numbering between 44 and 59?
39. A company has a policy of retiring company cars; this policy looks at number of miles driven, purpose of trips, style of car and other features. The distribution of the number of months in service for the fleet of cars is bellshaped and has a mean of 42 months and a standard deviation of 3 months. Using the Empirical Rule, what is the approximate percentage of cars that remain in service between 48 and 51 months?
40. The following is an infant weight percentile chart. What is the 50th percentile height in cm for a 10-month old boy?

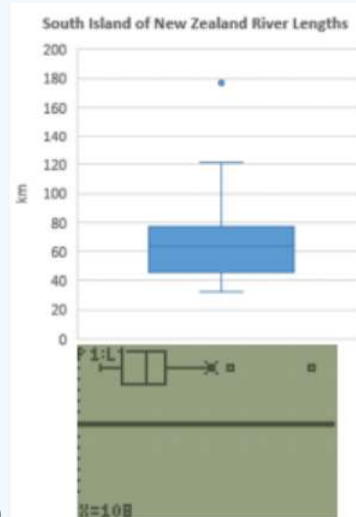


Retrieved from: <https://www.cdc.gov/growthcharts/data/set1clinical/cj411017.pdf>.

Answer to Odd Numbered Exercises

- 1 a) mode = 4 b) median = 3.75 c) $\bar{x} = 3.725$
- 3) a) 56 & 64 b) 64 c) 67.6818
- 5) 2.833
- 7) 72.25
- 9) a) Range = 0.9 b) $s^2 = 0.0870$ c) $s = 0.2949$
- 11) a) Negatively skewed b) The median is higher.
- 13) $s_1 = 9.9348$, $s_2 = 3.3466$; Training 1 is more variable

- 15) Before range = 42, after range = 42, both groups have the same range.
- 17) a) 30.35 b) 136.7 c) 32.7 d) 38.52% e) 28.4
- 19) a) CVheight = 3.04%; CVweight = 9.84% b) Weight, because it has a higher coefficient of variation.
- 21) -10
- 23) a) -2.8507 b) -3.1808 c) Tennessee Lane
- 25) Min = 3.2, $Q_1 = 3.45$ (TI: 3.55), $Q_2 = 3.7$, $Q_3 = 3.95$ (TI: 3.85), Max = 4.1
- 27) a) Min = 32, $Q_1 = 46$ (TI: 48), $Q_2 = 64$, $Q_3 = 77$ (TI: 76), Max = 177 b) lower limit = -0.5 (TI: 6), upper limit = 123.5



(TI: 118), outliers = 177 (TI: 121 & 177) c)

29) a) 4 b) Yes, the treatment was effective.

31) 1. c. ii; 2. a. iii; 3. b. i

33) 99.7%

35) a) 55.56% b) 68% c) 95%

37) 99.7%

39) 2.35%

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CHAPTER OVERVIEW

3: Basic Analytical Tools

3.1: Concentration

3.2: Basic Equipment

3.3: Preparing Solutions

3.4: Calibration Methods

3.5: Linear Regression and Calibration Curves

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3.1: Concentration

Concentration is a general measurement unit that reports the amount of solute present in a known amount of solution

$$\text{concentration} = \frac{\text{amount of solute}}{\text{amount of solution}} \quad (3.1.1)$$

Although we associate the terms “solute” and “solution” with liquid samples, we can extend their use to gas-phase and solid-phase samples as well. Table 3.1.1 lists the most common units of concentration.

Table 3.1.1 : Common Units for Reporting Concentration

Name	Units	Symbol
molarity	$\frac{\text{moles solute}}{\text{liters solution}}$	M
formality	$\frac{\text{moles solute}}{\text{liters solution}}$	F
normality	$\frac{\text{equivalents solute}}{\text{liters solution}}$	N
molality	$\frac{\text{moles solute}}{\text{kilograms solvent}}$	m
weight percent	$\frac{\text{grams solute}}{100 \text{ grams solution}}$	% w/w
volume percent	$\frac{\text{mL solute}}{100 \text{ mL solution}}$	% v/v
weight-to-volume percent	$\frac{\text{grams solute}}{100 \text{ mL solution}}$	% w/v
parts per million	$\frac{\text{grams solute}}{10^6 \text{ grams solution}}$	ppm
parts per billion	$\frac{\text{grams solute}}{10^9 \text{ grams solution}}$	ppb

An alternative expression for weight percent is

$$\frac{\text{grams solute}}{\text{grams solution}} \times 100$$

You can use similar alternative expressions for volume percent and for weight-to-volume percent.

Molarity and Formality

Both molarity and formality express concentration as moles of solute per liter of solution; however, there is a subtle difference between them. **Molarity** is the concentration of a particular chemical species. **Formality**, on the other hand, is a substance’s total concentration without regard to its specific chemical form. There is no difference between a compound’s molarity and formality if it dissolves without dissociating into ions. The formal concentration of a solution of glucose, for example, is the same as its molarity.

For a compound that ionizes in solution, such as CaCl_2 , molarity and formality are different. When we dissolve 0.1 moles of CaCl_2 in 1 L of water, the solution contains 0.1 moles of Ca^{2+} and 0.2 moles of Cl^- . The molarity of CaCl_2 , therefore, is zero since there is no undissociated CaCl_2 in solution; instead, the solution is 0.1 M in Ca^{2+} and 0.2 M in Cl^- . The formality of CaCl_2 , however, is 0.1 F since it represents the total amount of CaCl_2 in solution. This more rigorous definition of molarity, for better or worse, largely is ignored in the current literature, as it is in this textbook. When we state that a solution is 0.1 M CaCl_2 we understand it to consist of Ca^{2+} and Cl^- ions. We will reserve the unit of formality to situations where it provides a clearer description of solution chemistry.

Molarity is used so frequently that we use a symbolic notation to simplify its expression in equations and in writing. Square brackets around a species indicate that we are referring to that species' molarity. Thus, $[\text{Ca}^{2+}]$ is read as "the molarity of calcium ions."

For a solute that dissolves without undergoing ionization, molarity and formality have the same value. A solution that is 0.0259 M in glucose, for example, is 0.0259 F in glucose as well.

Normality

Normality is a concentration unit that no longer is in common use; however, because you may encounter normality in older handbooks of analytical methods, it is helpful to understand its meaning. **Normality** defines concentration in terms of an equivalent, which is the amount of one chemical species that reacts stoichiometrically with another chemical species. Note that this definition makes an equivalent, and thus normality, a function of the chemical reaction in which the species participates. Although a solution of H_2SO_4 has a fixed molarity, its normality depends on how it reacts. You will find a more detailed treatment of normality in [Appendix 1](#).

One handbook that still uses normality is *Standard Methods for the Examination of Water and Wastewater*, a joint publication of the American Public Health Association, the American Water Works Association, and the Water Environment Federation. This handbook is one of the primary resources for the environmental analysis of water and wastewater.

Molality

Molality is used in thermodynamic calculations where a temperature independent unit of concentration is needed. Molarity is based on the volume of solution that contains the solute. Since density is a temperature dependent property, a solution's volume, and thus its molar concentration, changes with temperature. By using the solvent's mass in place of the solution's volume, the resulting concentration becomes independent of temperature.

Weight, Volume, and Weight-to-Volume Percent

Weight percent (% w/w), **volume percent** (% v/v) and **weight-to-volume percent** (% w/v) express concentration as the units of solute present in 100 units of solution. A solution that is 1.5% w/v NH_4NO_3 , for example, contains 1.5 gram of NH_4NO_3 in 100 mL of solution.

Parts Per Million and Parts Per Billion

Parts per million (ppm) and **parts per billion** (ppb) are ratios that give the grams of solute in, respectively, one million or one billion grams of sample. For example, a sample of steel that is 450 ppm in Mn contains 450 μg of Mn for every gram of steel. If we approximate the density of an aqueous solution as 1.00 g/mL, then we can express solution concentrations in ppm or ppb using the following relationships.

$$\text{ppm} = \frac{\mu\text{g}}{\text{g}} = \frac{\text{mg}}{\text{L}} = \frac{\mu\text{g}}{\text{mL}} \quad \text{ppb} = \frac{\text{ng}}{\text{g}} = \frac{\mu\text{g}}{\text{L}} = \frac{\text{ng}}{\text{mL}}$$

For gases a part per million usually is expressed as a volume ratio; for example, a helium concentration of 6.3 ppm means that one liter of air contains 6.3 μL of He.

You should be careful when using parts per million and parts per billion to express the concentration of an aqueous solute. The difference between a solute's concentration in mg/L and ng/g, for example, is significant if the solution's density is not 1.00 g/mL. For this reason many organizations advise against using the abbreviation ppm and ppb (see section 7.10.3 at www.nist.gov). If in doubt, include the exact units, such as 0.53 $\mu\text{g Pb}^{2+}/\text{L}$ for the concentration of lead in a sample of seawater.

Converting Between Concentration Units

The most common ways to express concentration in analytical chemistry are molarity, weight percent, volume percent, weight-to-volume percent, parts per million and parts per billion. The general definition of concentration in Equation 3.1.1 makes it is easy to convert between concentration units.

✓ Example 3.1.1

A concentrated solution of ammonia is 28.0% w/w NH_3 and has a density of 0.899 g/mL. What is the molar concentration of NH_3 in this solution?

Solution

$$\frac{28.0 \text{ g NH}_3}{100 \text{ g soln}} \times \frac{0.899 \text{ g soln}}{\text{ml soln}} \times \frac{1 \text{ mol NH}_3}{17.03 \text{ g NH}_3} \times \frac{1000 \text{ mL}}{\text{L}} = 14.8 \text{ M}$$

✓ Example 3.1.2

The maximum permissible concentration of chloride ion in a municipal drinking water supply is 2.50×10^2 ppm Cl^- . When the supply of water exceeds this limit it often has a distinctive salty taste. What is the equivalent molar concentration of Cl^- ?

Solution

$$\frac{2.50 \times 10^2 \text{ mg Cl}^-}{\text{L}} \times \frac{1 \text{ g}}{1000 \text{ mg}} \times \frac{1 \text{ mol Cl}^-}{35.453 \text{ g Cl}^-} = 7.05 \times 10^{-3} \text{ M}$$

? Exercise 3.1.1

Which solution—0.50 M NaCl or 0.25 M SrCl_2 —has the larger concentration when expressed in mg/mL?

Answer

The concentrations of the two solutions are

$$\begin{aligned} \frac{0.50 \text{ mol NaCl}}{\text{L}} \times \frac{58.44 \text{ g NaCl}}{\text{mol NaCl}} \times \frac{10^6 \mu\text{g}}{\text{g}} \times \frac{1 \text{ L}}{1000 \text{ mL}} &= 2.9 \times 10^4 \mu\text{g/mL NaCl} \\ \frac{0.25 \text{ mol SrCl}_2}{\text{L}} \times \frac{158.5 \text{ g SrCl}_2}{\text{mol SrCl}_2} \times \frac{10^6 \mu\text{g}}{\text{g}} \times \frac{1 \text{ L}}{1000 \text{ mL}} &= 4.0 \times 10^4 \mu\text{g/mL SrCl}_2 \end{aligned}$$

The solution of SrCl_2 has the larger concentration when it is expressed in $\mu\text{g/mL}$ instead of in mol/L.

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3.2: Basic Equipment

The array of equipment available for making analytical measurements and working with analytical samples is impressive, ranging from the simple and inexpensive, to the complex and expensive. With three exceptions—the measurement of mass, the measurement of volume, and the drying of materials—we will postpone the discussion of equipment to later chapters where its application to specific analytical methods is relevant.

Equipment for Measuring Mass

An object's mass is measured using a digital electronic analytical balance (Figure 2.4.1). An electromagnet levitates the sample pan above a permanent cylindrical magnet. When we place an object on the sample pan, it displaces the sample pan downward by a force equal to the product of the sample's mass and its acceleration due to gravity. The balance detects this downward movement and generates a counterbalancing force by increasing the current to the electromagnet. The current needed to return the balance to its original position is proportional to the object's mass. A typical electronic balance has a capacity of 100–200 g, and can measure mass to the nearest ± 0.01 mg to ± 1 mg.



Figure 2.4.1 : The photo shows a typical digital electronic balance capable of determining mass to the nearest ± 0.1 mg. The sticker inside the balance's wind shield is its annual calibration certification. For a review of other types of electronic balances, see Schoonover, R. M. *Anal. Chem.* **1982**, 54, 973A-980A.

Although we tend to use interchangeably, the terms “weight” and “mass,” there is an important distinction between them. Mass is the absolute amount of matter in an object, measured in grams. Weight, W , is a measure of gravitational acceleration, g , acting on that mass, m :

$$W = m \times g$$

An object has a fixed mass but its weight depends upon the acceleration due to gravity, which varies subtly from location-to-location.

A balance measures an object's weight, not its mass. Because weight and mass are proportional to each other, we can calibrate a balance using a standard weight whose mass is traceable to the standard prototype for the kilogram. A properly calibrated balance gives an accurate value for an object's mass; see [Appendix 9](#) for more details on calibrating a balance.

If the sample is not moisture sensitive, a clean and dry container is placed on the balance. The container's mass is called the **tare** and most balances allow you to set the container's tare to a mass of zero. The sample is transferred to the container, the new mass is measured and the sample's mass determined by subtracting the tare. A sample that absorbs moisture from the air is treated differently. The sample is placed in a covered weighing bottle and their combined mass is determined. A portion of the sample is removed and the weighing bottle and the remaining sample are reweighed. The difference between the two masses gives the sample's mass.

Several important precautions help to minimize errors when we determine an object's mass. To minimize the effect of vibrations, the balance is placed on a stable surface and in a level position. Because the sensitivity of an analytical balance is sufficient to measure the mass of a fingerprint, materials often are handled using tongs or laboratory tissues. Volatile liquid samples must be weighed in a covered container to avoid the loss of sample by evaporation. To minimize fluctuations in mass due to air currents, the balance pan often is housed within a wind shield, as seen in Figure 2.4.1 . A sample that is cooler or warmer than the surrounding air will create a convective air current that affects the measurement of its mass. For this reason, bring your samples to room temperature before determining their mass. Finally, samples dried in an oven are stored in a desiccator to prevent them from reabsorbing moisture from the atmosphere.

Equipment for Measuring Volume

Analytical chemists use a variety of glassware to measure volume, including graduated cylinders, volumetric pipets, and volumetric flasks. The choice of what type of glassware to use depends on how accurately and how precisely we need to measure the volume and whether we are interested in containing or delivering the sample.

A **graduated cylinder** is the simplest device for delivering a known volume of a liquid reagent (Figure 2.4.2). The graduated scale allows you to deliver any volume up to the cylinder's maximum. Typical accuracy is $\pm 1\%$ of the maximum volume. A 100-mL graduated cylinder, for example, is accurate to ± 1 mL.



Figure 2.4.2 : An example of a 250-mL graduated cylinder.

A **volumetric pipet** provides a more accurate method for delivering a known volume of solution. Several different styles of pipets are available, two of which are shown in Figure 2.4.3 . Transfer pipets provide the most accurate means for delivering a known volume of solution. A transfer pipet delivering less than 100 mL generally is accurate to the hundredth of a mL. Larger transfer pipets are accurate to a tenth of a mL. For example, the 10-mL transfer pipet in Figure 2.4.3 will deliver 10.00 mL with an accuracy of ± 0.02 mL.

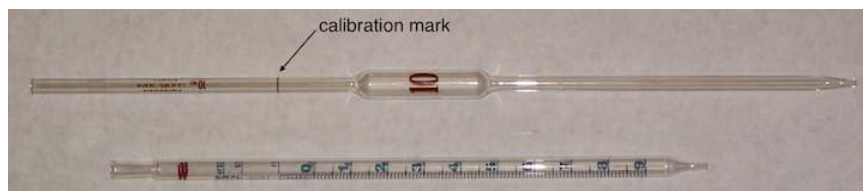


Figure 2.4.3 : Two examples of 10-mL volumetric pipets. The pipet on the top is a transfer pipet and the pipet on the bottom is a Mohr measuring pipet. The transfer pipet delivers a single volume of 10.00 mL when filled to its calibration mark. The Mohr pipet has a mark every 0.1 mL, allowing for the delivery of variable volumes. It also has additional graduations at 11 mL, 12 mL, and 12.5 mL.

Scientists at the Brookhaven National Laboratory used a germanium nanowire to make a pipet that delivers a 35 zeptoliter (10^{-21} L) drop of a liquid gold-germanium alloy. You can read about this work in the April 21, 2007 issue of *Science News*.

To fill a transfer pipet, use a rubber suction bulb to pull the solution up past the calibration mark (*Never use your mouth to suck a solution into a pipet!*). After replacing the bulb with your finger, adjust the solution's level to the calibration mark and dry the outside of the pipet with a laboratory tissue. Allow the pipet's contents to drain into the receiving container with the pipet's tip touching the inner wall of the container. A small portion of the liquid remains in the pipet's tip and is not be blown out. With some measuring pipets any solution remaining in the tip must be blown out.

Delivering microliter volumes of liquids is not possible using transfer or measuring pipets. Digital micropipets (Figure 2.4.4), which come in a variety of volume ranges, provide for the routine measurement of microliter volumes.



Figure 2.4.4 : A set of two digital micropipets. The pipet on the top delivers volumes between 0.5 mL and 10 μ L and the pipet on the bottom delivers volumes between 10 mL and 100 μ L.

Graduated cylinders and pipets deliver a known volume of solution. A **volumetric flask**, on the other hand, contains a specific volume of solution (Figure 2.4.5). When filled to its calibration mark, a volumetric flask that contains less than 100 mL generally is accurate to the hundredth of a mL, whereas larger volumetric flasks are accurate to the tenth of a mL. For example, a 10-mL volumetric flask contains $10.00 \text{ mL} \pm 0.02 \text{ mL}$ and a 250-mL volumetric flask contains $250.0 \text{ mL} \pm 0.12 \text{ mL}$.



Figure 2.4.5 : A collection of volumetric flasks with volumes of 10 mL, 50 mL, 100 mL, 250 mL, and 500 mL.

Because a volumetric flask contains a solution, it is used to prepare a solution with an accurately known concentration. Transfer the reagent to the volumetric flask and add enough solvent to bring the reagent into solution. Continue adding solvent in several portions, mixing thoroughly after each addition, and then adjust the volume to the flask's calibration mark using a dropper. Finally, complete the mixing process by inverting and shaking the flask at least 10 times.

If you look closely at a volumetric pipet or a volumetric flask you will see markings similar to those shown in Figure 2.4.6 . The text of the markings, which reads

10 mL T. D. at $20^\circ\text{C} \pm 0.02 \text{ mL}$

indicates that the pipet is calibrated to deliver (T. D.) 10 mL of solution with an uncertainty of $\pm 0.02 \text{ mL}$ at a temperature of 20°C . The temperature is important because glass expands and contracts with changes in temperatures; thus, the pipet's accuracy is less than $\pm 0.02 \text{ mL}$ at a higher or a lower temperature. For a more accurate result, you can calibrate your volumetric glassware at the temperature you are working by weighing the amount of water contained or delivered and calculating the volume using its temperature dependent density.



Figure 2.4.6 : Close-up of the 10-mL transfer pipet from Figure 2.4.3 .

A volumetric flask has similar markings, but uses the abbreviation T. C. for “to contain” in place of T. D.

You should take three additional precautions when you work with pipets and volumetric flasks. First, the volume delivered by a pipet or contained by a volumetric flask assumes that the glassware is clean. Dirt and grease on the inner surface prevent liquids from draining evenly, leaving droplets of liquid on the container’s walls. For a pipet this means the delivered volume is less than the calibrated volume, while drops of liquid above the calibration mark mean that a volumetric flask contains more than its calibrated volume. Commercially available cleaning solutions are available for cleaning pipets and volumetric flasks.

Second, when filling a pipet or volumetric flask the liquid’s level must be set exactly at the calibration mark. The liquid’s top surface is curved into a meniscus, the bottom of which should align with the glassware’s calibration mark (Figure 2.4.7). When adjusting the meniscus, keep your eye in line with the calibration mark to avoid parallax errors. If your eye level is above the calibration mark you will overfill the pipet or the volumetric flask and you will underfill them if your eye level is below the calibration mark.

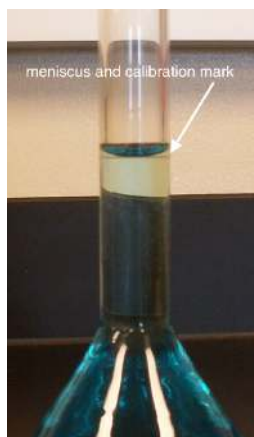


Figure 2.4.7 : Proper position of the solution’s meniscus relative to the volumetric flask’s calibration mark.

Finally, before using a pipet or volumetric flask rinse it with several small portions of the solution whose volume you are measuring. This ensures the removal of any residual liquid remaining in the pipet or volumetric flask.

Equipment for Drying Samples

Many materials need to be dried prior to their analysis to remove residual moisture. Depending on the material, heating to a temperature between 110 °C and 140 °C usually is sufficient. Other materials need much higher temperatures to initiate thermal decomposition.

Conventional drying ovens provide maximum temperatures of 160 °C to 325 °C, depending on the model. Some ovens include the ability to circulate heated air, which allows for a more efficient removal of moisture and shorter drying times. Other ovens provide a tight seal for the door, which allows the oven to be evacuated. In some situations a microwave oven can replace a conventional laboratory oven. Higher temperatures, up to as much as 1700 °C, require a muffle furnace (Figure 2.4.8).



Figure 2.4.8 : Example of a muffle furnace.

After drying or decomposing a sample, it is cooled to room temperature in a desiccator to prevent the readsorption of moisture. A **desiccator** (Figure 2.4.9) is a closed container that isolates the sample from the atmosphere. A drying agent, called a **desiccant**, is placed in the bottom of the container. Typical desiccants include calcium chloride and silica gel. A perforated plate sits above the desiccant, providing a shelf for storing samples. Some desiccators include a stopcock that allows them to be evacuated.



Figure 2.4.9 : Example of a desiccator. The solid in the bottom of the desiccator is the desiccant, which in this case is silica gel.

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3.3: Preparing Solutions

Preparing a solution of known concentration is perhaps the most common activity in any analytical lab. The method for measuring out the solute and the solvent depend on the desired concentration and how exact the solution's concentration needs to be known. Pipets and volumetric flasks are used when we need to know a solution's exact concentration; graduated cylinders, beakers, and/or reagent bottles suffice when a concentrations need only be approximate. Two methods for preparing solutions are described in this section.

Preparing Stock Solutions

A **stock solution** is prepared by weighing out an appropriate portion of a pure solid or by measuring out an appropriate volume of a pure liquid, placing it in a suitable flask, and diluting to a known volume. Exactly how one measure's the reagent depends on the desired concentration unit. For example, to prepare a solution with a known molarity you weigh out an appropriate mass of the reagent, dissolve it in a portion of solvent, and bring it to the desired volume. To prepare a solution where the solute's concentration is a volume percent, you measure out an appropriate volume of solute and add sufficient solvent to obtain the desired total volume.

✓ Example 2.5.1

Describe how to prepare the following three solutions: (a) 500 mL of approximately 0.20 M NaOH using solid NaOH; (b) 1 L of 150.0 ppm Cu^{2+} using Cu metal; and (c) 2 L of 4% v/v acetic acid using concentrated glacial acetic acid (99.8% w/w acetic acid).

Solution

(a) Because the desired concentration is known to two significant figures, we do not need to measure precisely the mass of NaOH or the volume of solution. The desired mass of NaOH is

$$\frac{0.20 \text{ mol NaOH}}{\text{L}} \times \frac{40.0 \text{ g NaOH}}{\text{mol NaOH}} \times 0.50 \text{ L} = 4.0 \text{ g NaOH}$$

To prepare the solution, place 4.0 grams of NaOH, weighed to the nearest tenth of a gram, in a bottle or beaker and add approximately 500 mL of water.

(b) Since the desired concentration of Cu^{2+} is given to four significant figures, we must measure precisely the mass of Cu metal and the final solution volume. The desired mass of Cu metal is

$$\frac{150.0 \text{ mg Cu}}{\text{L}} \times 1.000 \text{ M} \times \frac{1 \text{ g}}{1000 \text{ mg}} = 0.1500 \text{ g Cu}$$

To prepare the solution, measure out exactly 0.1500 g of Cu into a small beaker and dissolve it using a small portion of concentrated HNO_3 . To ensure a complete transfer of Cu^{2+} from the beaker to the volumetric flask—what we call a **quantitative transfer**—rinse the beaker several times with small portions of water, adding each rinse to the volumetric flask. Finally, add additional water to the volumetric flask's calibration mark.

(c) The concentration of this solution is only approximate so it is not necessary to measure exactly the volumes, nor is it necessary to account for the fact that glacial acetic acid is slightly less than 100% w/w acetic acid (it is approximately 99.8% w/w). The necessary volume of glacial acetic acid is

$$\frac{4 \text{ mL CH}_3\text{COOH}}{100 \text{ mL}} \times 2000 \text{ mL} = 80 \text{ mL CH}_3\text{COOH}$$

To prepare the solution, use a graduated cylinder to transfer 80 mL of glacial acetic acid to a container that holds approximately 2 L and add sufficient water to bring the solution to the desired volume.

? Exercise 2.5.1

Provide instructions for preparing 500 mL of 0.1250 M KBrO_3 .

Answer

Preparing 500 mL of 0.1250 M KBrO_3 requires

$$0.5000 \text{ L} \times \frac{0.1250 \text{ mol KBrO}_3}{\text{L}} \times \frac{167.00 \text{ g KBrO}_3}{\text{mol KBrO}_3} = 10.44 \text{ g KBrO}_3$$

Because the concentration has four significant figures, we must prepare the solution using volumetric glassware. Place a 10.44 g sample of KBrO_3 in a 500-mL volumetric flask and fill part way with water. Swirl to dissolve the KBrO_3 and then dilute with water to the flask's calibration mark.

Preparing Solutions by Dilution

Solutions are often prepared by diluting a more concentrated stock solution. A known volume of the stock solution is transferred to a new container and brought to a new volume. Since the total amount of solute is the same before and after dilution, we know that

$$C_o \times V_o = C_d \times V_d \quad (3.3.1)$$

where C_o is the stock solution's concentration, V_o is the volume of stock solution being diluted, C_d is the dilute solution's concentration, and V_d is the volume of the dilute solution. Again, the type of glassware used to measure V_o and V_d depends on how precisely we need to know the solution's concentration.

Note that Equation 3.3.1 applies only to those concentration units that are expressed in terms of the solution's volume, including molarity, formality, normality, volume percent, and weight-to-volume percent. It also applies to weight percent, parts per million, and parts per billion if the solution's density is 1.00 g/mL. We cannot use Equation 3.3.1 if we express concentration in terms of molality as this is based on the mass of solvent, not the volume of solution. See Rodríguez-López, M.; Carrasquillo, A. *J. Chem. Educ.* **2005**, *82*, 1327-1328 for further discussion.

✓ Example 2.5.2

A laboratory procedure calls for 250 mL of an approximately 0.10 M solution of NH_3 . Describe how you would prepare this solution using a stock solution of concentrated NH_3 (14.8 M).

Solution

Substituting known values into Equation 3.3.1

$$14.8 \text{ M} \times V_o = 0.10 \text{ M} \times 250 \text{ mL}$$

and solving for V_o gives 1.7 mL. Since we are making a solution that is approximately 0.10 M NH_3 , we can use a graduated cylinder to measure the 1.7 mL of concentrated NH_3 , transfer the NH_3 to a beaker, and add sufficient water to give a total volume of approximately 250 mL.

Although usually we express molarity as mol/L, we can express the volumes in mL if we do so both for both V_o and V_d .

? Exercise 2.5.2

To prepare a standard solution of Zn^{2+} you dissolve a 1.004 g sample of Zn wire in a minimal amount of HCl and dilute to volume in a 500-mL volumetric flask. If you dilute 2.000 mL of this stock solution to 250.0 mL, what is the concentration of Zn^{2+} , in $\mu\text{g/mL}$, in your standard solution?

Answer

The first solution is a stock solution, which we then dilute to prepare the standard solution. The concentration of Zn^{2+} in the stock solution is

$$\frac{1.004 \text{ g Zn}^{2+}}{500.0 \text{ mL}} \times \frac{10^6 \mu\text{g}}{\text{g}} = 2008 \mu\text{g Zn}^{2+}/\text{mL}$$

To find the concentration of the standard solution we use Equation 3.3.1

$$\frac{2008 \mu\text{g Zn}^{2+}}{\text{mL}} \times 2.000 \text{ mL} = C_d \times 250.0 \text{ mL}$$

where C_d is the standard solution's concentration. Solving gives a concentration of $16.06 \mu\text{g Zn}^{2+}/\text{mL}$.

As shown in the following example, we can use Equation 3.3.1 to calculate a solution's original concentration using its known concentration after dilution.

✓ Example 2.5.3

A sample of an ore was analyzed for Cu^{2+} as follows. A 1.25 gram sample of the ore was dissolved in acid and diluted to volume in a 250-mL volumetric flask. A 20 mL portion of the resulting solution was transferred by pipet to a 50-mL volumetric flask and diluted to volume. An analysis of this solution gives the concentration of Cu^{2+} as $4.62 \mu\text{g/mL}$. What is the weight percent of Cu in the original ore?

Solution

Substituting known values (with significant figures appropriate for pipets and volumetric flasks) into Equation 3.3.1

$$(C_{\text{Cu}})_o \times 20.00 \text{ mL} = 4.62 \mu\text{g/mL Cu}^{2+} \times 50.00 \text{ mL}$$

and solving for $(C_{\text{Cu}})_o$ gives the original concentration as $11.55 \mu\text{g/mL Cu}^{2+}$. To calculate the grams of Cu^{2+} we multiply this concentration by the total volume

$$\frac{11.55 \mu\text{g Cu}^{2+}}{\text{mL}} \times 250.0 \text{ mL} \times \frac{1 \text{ g}}{10^6 \mu\text{g}} = 2.888 \times 10^{-3} \text{ g Cu}^{2+}$$

The weight percent Cu is

$$\frac{2.888 \times 10^{-3} \text{ g Cu}^{2+}}{1.25 \text{ g sample}} \times 100 = 0.231\% \text{ w/w Cu}^{2+}$$

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3.4: Calibration Methods

To standardize an analytical method we also must determine the analyte's sensitivity, k_A , in Equation 5.3.1 or Equation 5.3.2 .

$$S_{total} = k_A n_A + S_{reag} \quad (3.4.1)$$

$$S_{total} = k_A C_A + S_{reag} \quad (3.4.2)$$

In principle, it is possible to derive the value of k_A for any analytical method if we understand fully all the chemical reactions and physical processes responsible for the signal. Unfortunately, such calculations are not feasible if we lack a sufficiently developed theoretical model of the physical processes or if the chemical reaction's evince non-ideal behavior. In such situations we must determine the value of k_A by analyzing one or more standard solutions, each of which contains a known amount of analyte. In this section we consider several approaches for determining the value of k_A . For simplicity we assume that S_{reag} is accounted for by a proper reagent blank, allowing us to replace S_{total} in Equation 3.4.1 and Equation 3.4.2 with the analyte's signal, S_A .

$$S_A = k_A n_A \quad (3.4.3)$$

$$S_A = k_A C_A \quad (3.4.4)$$

Equation 3.4.3 and Equation 3.4.4 essentially are identical, differing only in whether we choose to express the amount of analyte in moles or as a concentration. For the remainder of this chapter we will limit our treatment to Equation 3.4.4. You can extend this treatment to Equation 3.4.3 by replacing C_A with n_A .

Single-Point versus Multiple-Point Standardizations

The simplest way to determine the value of k_A in Equation 3.4.4 is to use a single-point standardization in which we measure the signal for a standard, S_{std} , that contains a known concentration of analyte, C_{std} . Substituting these values into Equation 3.4.4

$$k_A = \frac{S_{std}}{C_{std}} \quad (3.4.5)$$

gives us the value for k_A . Having determined k_A , we can calculate the concentration of analyte in a sample by measuring its signal, S_{samp} , and calculating C_A using Equation 3.4.6.

$$C_A = \frac{S_{samp}}{k_A} \quad (3.4.6)$$

A single-point standardization is the least desirable method for standardizing a method. There are two reasons for this. First, any error in our determination of k_A carries over into our calculation of C_A . Second, our experimental value for k_A is based on a single concentration of analyte. To extend this value of k_A to other concentrations of analyte requires that we assume a linear relationship between the signal and the analyte's concentration, an assumption that often is not true [Cardone, M. J.; Palmero, P. J.; Sybrandt, L. B. *Anal. Chem.* **1980**, 52, 1187–1191]. Figure 5.3.1 shows how assuming a constant value of k_A leads to a determinate error in C_A if k_A becomes smaller at higher concentrations of analyte. Despite these limitations, single-point standardizations find routine use when the expected range for the analyte's concentrations is small. Under these conditions it often is safe to assume that k_A is constant (although you should verify this assumption experimentally). This is the case, for example, in clinical labs where many automated analyzers use only a single standard.

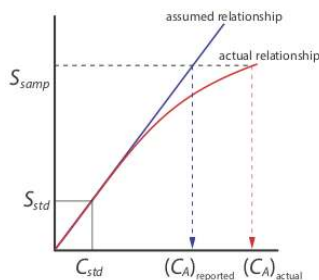


Figure 5.3.1 : Example showing how a single-point standardization leads to a determinate error in an analyte's reported concentration if we incorrectly assume that k_A is constant. The **assumed** relationship between S_{smp} and C_A is based on a single standard and is a straight-line; the **actual** relationship between S_{smp} and C_A becomes curved for larger concentrations of analyte.

The better way to standardize a method is to prepare a series of standards, each of which contains a different concentration of analyte. Standards are chosen such that they bracket the expected range for the analyte's concentration. A **multiple-point standardization** should include at least three standards, although more are preferable. A plot of S_{std} versus C_{std} is called a **calibration curve**. The exact standardization, or calibration relationship, is determined by an appropriate curve-fitting algorithm.

Linear regression, which also is known as the method of least squares, is one such algorithm. Its use is covered in Section 5.4.

There are two advantages to a multiple-point standardization. First, although a determinate error in one standard introduces a determinate error, its effect is minimized by the remaining standards. Second, because we measure the signal for several concentrations of analyte, we no longer must assume k_A is independent of the analyte's concentration. Instead, we can construct a calibration curve similar to the "actual relationship" in Figure 5.3.1.

External Standards

The most common method of standardization uses one or more **external standards**, each of which contains a known concentration of analyte. We call these standards "external" because they are prepared and analyzed separate from the samples.

Appending the adjective "external" to the noun "standard" might strike you as odd at this point, as it seems reasonable to assume that standards and samples are analyzed separately. As we will soon learn, however, we can add standards to our samples and analyze both simultaneously.

Single External Standard

With a single external standard we determine k_A using Equation 3.4.5 and then calculate the concentration of analyte, C_A , using Equation 3.4.6.

✓ Example 5.3.1

A spectrophotometric method for the quantitative analysis of Pb^{2+} in blood yields an S_{std} of 0.474 for a single standard for which the concentration of lead is 1.75 ppb. What is the concentration of Pb^{2+} in a sample of blood for which S_{smp} is 0.361?

Solution

Equation 3.4.5 allows us to calculate the value of k_A using the data for the single external standard.

$$k_A = \frac{S_{std}}{C_{std}} = \frac{0.474}{1.75 \text{ ppb}} = 0.2709 \text{ ppb}^{-1}$$

Having determined the value of k_A , we calculate the concentration of Pb^{2+} in the sample of blood is calculated using Equation 3.4.6.

$$C_A = \frac{S_{smp}}{k_A} = \frac{0.361}{0.2709 \text{ ppb}^{-1}} = 1.33 \text{ ppb}$$

Multiple External Standards

Figure 5.3.2 shows a typical multiple-point external standardization. The volumetric flask on the left contains a reagent blank and the remaining volumetric flasks contain increasing concentrations of Cu^{2+} . Shown below the volumetric flasks is the resulting calibration curve. Because this is the most common method of standardization, the resulting relationship is called a **normal calibration curve**.

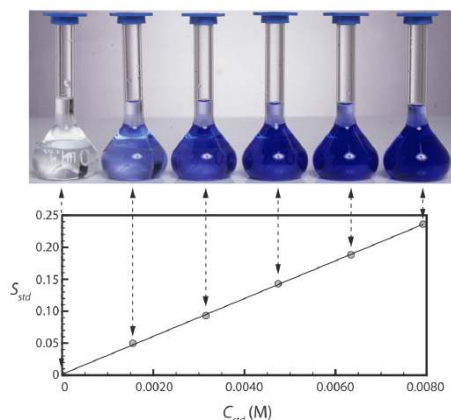


Figure 5.3.2 : The photo at the top of the figure shows a reagent blank (far left) and a set of five external standards for Cu^{2+} with concentrations that increase from left-to-right. Shown below the external standards is the resulting normal calibration curve. The absorbance of each standard, S_{std} , is shown by the filled circles.

When a calibration curve is a straight-line, as it is in Figure 5.3.2, the slope of the line gives the value of k_A . This is the most desirable situation because the method's sensitivity remains constant throughout the analyte's concentration range. When the calibration curve is not a straight-line, the method's sensitivity is a function of the analyte's concentration. In Figure 5.3.1, for example, the value of k_A is greatest when the analyte's concentration is small and it decreases continuously for higher concentrations of analyte. The value of k_A at any point along the calibration curve in Figure 5.3.1 is the slope at that point. In either case, a calibration curve allows to relate S_{samp} to the analyte's concentration.

✓ Example 5.3.2

A second spectrophotometric method for the quantitative analysis of Pb^{2+} in blood has a normal calibration curve for which

$$S_{std} = (0.296 \text{ ppb}^{-1} \times C_{std}) + 0.003$$

What is the concentration of Pb^{2+} in a sample of blood if S_{samp} is 0.397?

Solution

To determine the concentration of Pb^{2+} in the sample of blood, we replace S_{std} in the calibration equation with S_{samp} and solve for C_A .

$$C_A = \frac{S_{samp} - 0.003}{0.296 \text{ ppb}^{-1}} = \frac{0.397 - 0.003}{0.296 \text{ ppb}^{-1}} = 1.33 \text{ ppb}$$

It is worth noting that the calibration equation in this problem includes an extra term that does not appear in Equation 3.4.6. Ideally we expect our calibration curve to have a signal of zero when C_A is zero. This is the purpose of using a reagent blank to correct the measured signal. The extra term of +0.003 in our calibration equation results from the uncertainty in measuring the signal for the reagent blank and the standards.

? Exercise 5.3.1

Figure 5.3.2 shows a normal calibration curve for the quantitative analysis of Cu^{2+} . The equation for the calibration curve is

$$S_{std} = 29.59 \text{ M}^{-1} \times C_{std} + 0.015$$

What is the concentration of Cu^{2+} in a sample whose absorbance, S_{samp} , is 0.114? Compare your answer to a one-point standardization where a standard of $3.16 \times 10^{-3} \text{ M } \text{Cu}^{2+}$ gives a signal of 0.0931.

Answer

Substituting the sample's absorbance into the calibration equation and solving for C_A give

$$S_{\text{samp}} = 0.114 = 29.59 \text{ M}^{-1} \times C_A + 0.015$$

$$C_A = 3.35 \times 10^{-3} \text{ M}$$

For the one-point standardization, we first solve for k_A

$$k_A = \frac{S_{\text{std}}}{C_{\text{std}}} = \frac{0.0931}{3.16 \times 10^{-3} \text{ M}} = 29.46 \text{ M}^{-1}$$

and then use this value of k_A to solve for C_A .

$$C_A = \frac{S_{\text{samp}}}{k_A} = \frac{0.114}{29.46 \text{ M}^{-1}} = 3.87 \times 10^{-3} \text{ M}$$

When using multiple standards, the indeterminate errors that affect the signal for one standard are partially compensated for by the indeterminate errors that affect the other standards. The standard selected for the one-point standardization has a signal that is smaller than that predicted by the regression equation, which underestimates k_A and overestimates C_A .

An external standardization allows us to analyze a series of samples using a single calibration curve. This is an important advantage when we have many samples to analyze. Not surprisingly, many of the most common quantitative analytical methods use an external standardization.

There is a serious limitation, however, to an external standardization. When we determine the value of k_A using Equation 3.4.5, the analyte is present in the external standard's matrix, which usually is a much simpler matrix than that of our samples. When we use an external standardization we assume the matrix does not affect the value of k_A . If this is not true, then we introduce a proportional determinate error into our analysis. This is not the case in Figure 5.3.3, for instance, where we show calibration curves for an analyte in the sample's matrix and in the standard's matrix. In this case, using the calibration curve for the external standards leads to a negative determinate error in analyte's reported concentration. If we expect that matrix effects are important, then we try to match the standard's matrix to that of the sample, a process known as **matrix matching**. If we are unsure of the sample's matrix, then we must show that matrix effects are negligible or use an alternative method of standardization. Both approaches are discussed in the following section.

The matrix for the external standards in Figure 5.3.2, for example, is dilute ammonia. Because the $\text{Cu}(\text{NH}_3)_4^{2+}$ complex absorbs more strongly than Cu^{2+} , adding ammonia increases the signal's magnitude. If we fail to add the same amount of ammonia to our samples, then we will introduce a proportional determinate error into our analysis.

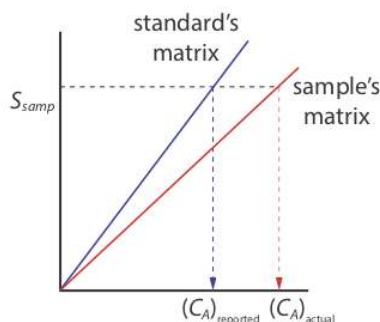


Figure 5.3.3 : Calibration curves for an analyte in the standard's matrix and in the sample's matrix. If the matrix affects the value of k_A , as is the case here, then we introduce a proportional determinate error into our analysis if we use a normal calibration curve.

Standard Additions

We can avoid the complication of matching the matrix of the standards to the matrix of the sample if we carry out the standardization in the sample. This is known as the **method of standard additions**.

Single Standard Addition

The simplest version of a standard addition is shown in Figure 5.3.4 . First we add a portion of the sample, V_o , to a volumetric flask, dilute it to volume, V_f , and measure its signal, S_{samp} . Next, we add a second identical portion of sample to an equivalent volumetric flask along with a spike, V_{std} , of an external standard whose concentration is C_{std} . After we dilute the spiked sample to the same final volume, we measure its signal, S_{spike} .

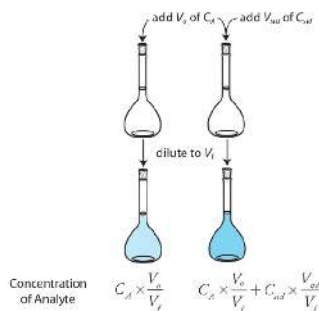


Figure 5.3.4 : Illustration showing the method of standard additions. The volumetric flask on the left contains a portion of the sample, V_o , and the volumetric flask on the right contains an identical portion of the sample and a spike, V_{std} , of a standard solution of the analyte. Both flasks are diluted to the same final volume, V_f . The concentration of analyte in each flask is shown at the bottom of the figure where C_A is the analyte's concentration in the original sample and C_{std} is the concentration of analyte in the external standard.

The following two equations relate S_{samp} and S_{spike} to the concentration of analyte, C_A , in the original sample.

$$S_{samp} = k_A C_A \frac{V_o}{V_f} \quad (3.4.7)$$

$$S_{spike} = k_A \left(C_A \frac{V_o}{V_f} + C_{std} \frac{V_{std}}{V_f} \right) \quad (3.4.8)$$

As long as V_{std} is small relative to V_o , the effect of the standard's matrix on the sample's matrix is insignificant. Under these conditions the value of k_A is the same in Equation 3.4.7 and Equation 3.4.8. Solving both equations for k_A and equating gives

$$\frac{S_{samp}}{C_A \frac{V_o}{V_f}} = \frac{S_{spike}}{C_A \frac{V_o}{V_f} + C_{std} \frac{V_{std}}{V_f}} \quad (3.4.9)$$

which we can solve for the concentration of analyte, C_A , in the original sample.

✓ Example 5.3.3

A third spectrophotometric method for the quantitative analysis of Pb^{2+} in blood yields an S_{samp} of 0.193 when a 1.00 mL sample of blood is diluted to 5.00 mL. A second 1.00 mL sample of blood is spiked with 1.00 mL of a 1560-ppb Pb^{2+} external standard and diluted to 5.00 mL, yielding an S_{spike} of 0.419. What is the concentration of Pb^{2+} in the original sample of blood?

Solution

We begin by making appropriate substitutions into Equation 3.4.9 and solving for C_A . Note that all volumes must be in the same units; thus, we first convert V_{std} from 1.00 mL to 1.00×10^{-3} mL.

$$\begin{aligned} \frac{0.193}{C_A \frac{1.00 \text{ mL}}{5.00 \text{ mL}}} &= \frac{0.419}{C_A \frac{1.00 \text{ mL}}{5.00 \text{ mL}} + 1560 \text{ ppb} \frac{1.00 \times 10^{-3} \text{ mL}}{5.00 \text{ mL}}} \\ \frac{0.193}{0.200 C_A} &= \frac{0.419}{0.200 C_A + 0.3120 \text{ ppb}} \\ 0.0386 C_A + 0.0602 \text{ ppb} &= 0.0838 C_A \\ 0.0452 C_A &= 0.0602 \text{ ppb} \end{aligned}$$

$$C_A = 1.33 \text{ ppb}$$

The concentration of Pb^{2+} in the original sample of blood is 1.33 ppb.

It also is possible to add the standard addition directly to the sample, measuring the signal both before and after the spike (Figure 5.3.5). In this case the final volume after the standard addition is $V_o + V_{std}$ and Equation 3.4.7, Equation 3.4.8, and Equation 3.4.9 become

$$S_{\text{samp}} = k_A C_A$$

$$S_{\text{spike}} = k_A \left(C_A \frac{V_o}{V_o + V_{std}} + C_{std} \frac{V_{std}}{V_o + V_{std}} \right) \quad (3.4.10)$$

$$\frac{S_{\text{samp}}}{C_A} = \frac{S_{\text{spike}}}{C_A \frac{V_o}{V_o + V_{std}} + C_{std} \frac{V_{std}}{V_o + V_{std}}} \quad (3.4.11)$$

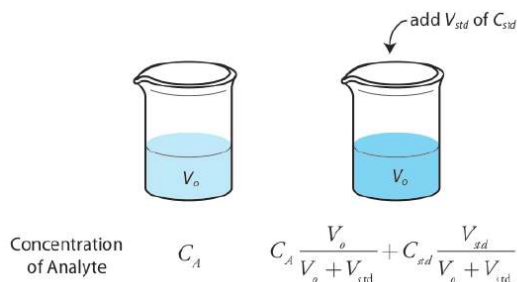


Figure 5.3.5 : Illustration showing an alternative form of the method of standard additions. In this case we add the spike of external standard directly to the sample without any further adjust in the volume.

✓ Example 5.3.4

A fourth spectrophotometric method for the quantitative analysis of Pb^{2+} in blood yields an S_{samp} of 0.712 for a 5.00 mL sample of blood. After spiking the blood sample with 5.00 mL of a 1560-ppb Pb^{2+} external standard, an S_{spike} of 1.546 is measured. What is the concentration of Pb^{2+} in the original sample of blood?

Solution

$$\frac{0.712}{C_A} = \frac{1.546}{C_A \frac{5.00 \text{ mL}}{5.005 \text{ mL}} + 1560 \text{ ppb} \frac{5.00 \times 10^{-3} \text{ mL}}{5.005 \text{ mL}}}$$

$$\frac{0.712}{C_A} = \frac{1.546}{0.9990 C_A + 1.558 \text{ ppb}}$$

$$0.7113 C_A + 1.109 \text{ ppb} = 1.546 C_A$$

$$C_A = 1.33 \text{ ppb}$$

The concentration of Pb^{2+} in the original sample of blood is 1.33 ppb.

Multiple Standard Additions

We can adapt a single-point standard addition into a multiple-point standard addition by preparing a series of samples that contain increasing amounts of the external standard. Figure 5.3.6 shows two ways to plot a standard addition calibration curve based on Equation 3.4.8. In Figure 5.3.6 a we plot S_{spike} against the volume of the spikes, V_{std} . If k_A is constant, then the calibration curve is a straight-line. It is easy to show that the x -intercept is equivalent to $-C_A V_o / C_{std}$.

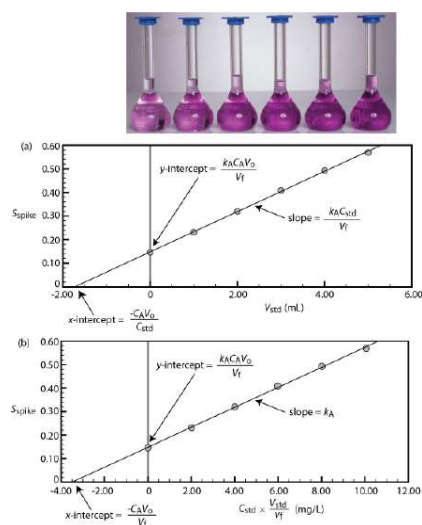


Figure 5.3.6 : Shown at the top of the figure is a set of six standard additions for the determination of Mn^{2+} . The flask on the left is a 25.00 mL sample diluted to 50.00 mL with water. The remaining flasks contain 25.00 mL of sample and, from left-to-right, 1.00, 2.00, 3.00, 4.00, and 5.00 mL spikes of an external standard that is 100.6 mg/L Mn^{2+} . Shown below are two ways to plot the standard additions calibration curve. The absorbance for each standard addition, S_{spike} , is shown by the filled circles.

✓ Example 5.3.5

Beginning with Equation 3.4.8 show that the equations in Figure 5.3.6 a for the slope, the y-intercept, and the x-intercept are correct.

Solution

We begin by rewriting Equation 3.4.8 as

$$S_{\text{spike}} = \frac{k_A C_A V_o}{V_f} + \frac{k_A C_{\text{std}}}{V_f} \times V_{\text{std}}$$

which is in the form of the equation for a straight-line

$$y = y\text{-intercept} + \text{slope} \times x\text{-intercept}$$

where y is S_{spike} and x is V_{std} . The slope of the line, therefore, is $k_A C_{\text{std}}/V_f$ and the y-intercept is $k_A C_A V_o/V_f$. The x-intercept is the value of x when y is zero, or

$$0 = \frac{k_A C_A V_o}{V_f} + \frac{k_A C_{\text{std}}}{V_f} \times x\text{-intercept}$$

$$x\text{-intercept} = -\frac{k_A C_A V_o/V_f}{k_A C_{\text{std}}/V_f} = -\frac{C_A V_o}{C_{\text{std}}}$$

? Exercise 5.3.2

Beginning with Equation 3.4.8 show that the Equations in Figure 5.3.6 b for the slope, the y-intercept, and the x-intercept are correct.

Answer

We begin with Equation 3.4.8

$$S_{\text{spike}} = k_A \left(C_A \frac{V_o}{V_f} + C_{\text{std}} \frac{V_{\text{std}}}{V_f} \right)$$

rewriting it as

$$S_{spike} = \frac{k_A C_A V_o}{V_f} + k_A \left(C_{std} \frac{V_{std}}{V_f} \right)$$

which is in the form of the linear equation

$$y = y\text{-intercept} + \text{slope} \times x\text{-intercept}$$

where y is S_{spike} and x is $C_{std} \times V_{std}/V_f$. The slope of the line, therefore, is k_A , and the y -intercept is $k_A C_A V_o/V_f$. The x -intercept is the value of x when y is zero, or

$$x\text{-intercept} = -\frac{k_A C_A V_o/V_f}{k_A} = -\frac{C_A V_o}{V_f}$$

Because we know the volume of the original sample, V_o , and the concentration of the external standard, C_{std} , we can calculate the analyte's concentrations from the x -intercept of a multiple-point standard additions.

✓ Example 5.3.6

A fifth spectrophotometric method for the quantitative analysis of Pb^{2+} in blood uses a multiple-point standard addition based on Equation 3.4.8. The original blood sample has a volume of 1.00 mL and the standard used for spiking the sample has a concentration of 1560 ppb Pb^{2+} . All samples were diluted to 5.00 mL before measuring the signal. A calibration curve of S_{spike} versus V_{std} has the following equation

$$S_{spike} = 0.266 + 312 \text{ mL}^{-1} \times V_{std}$$

What is the concentration of Pb^{2+} in the original sample of blood?

Solution

To find the x -intercept we set S_{spike} equal to zero.

$$S_{spike} = 0.266 + 312 \text{ mL}^{-1} \times V_{std}$$

Solving for V_{std} , we obtain a value of -8.526×10^{-4} mL for the x -intercept. Substituting the x -intercept's value into the equation from Figure 5.3.6 a

$$-8.526 \times 10^{-4} \text{ mL} = -\frac{C_A V_o}{C_{std}} = -\frac{C_A \times 1.00 \text{ mL}}{1560 \text{ ppb}}$$

and solving for C_A gives the concentration of Pb^{2+} in the blood sample as 1.33 ppb.

? Exercise 5.3.3

Figure 5.3.6 shows a standard additions calibration curve for the quantitative analysis of Mn^{2+} . Each solution contains 25.00 mL of the original sample and either 0, 1.00, 2.00, 3.00, 4.00, or 5.00 mL of a 100.6 mg/L external standard of Mn^{2+} . All standard addition samples were diluted to 50.00 mL with water before reading the absorbance. The equation for the calibration curve in Figure 5.3.6 a is

$$S_{std} = 0.0854 \times V_{std} + 0.1478$$

What is the concentration of Mn^{2+} in this sample? Compare your answer to the data in Figure 5.3.6 b, for which the calibration curve is

$$S_{std} = 0.425 \times C_{std} (V_{std}/V_f) + 0.1478$$

Answer

Using the calibration equation from Figure 5.3.6 a, we find that the x -intercept is

$$x\text{-intercept} = -\frac{0.1478}{0.0854 \text{ mL}^{-1}} = -1.731 \text{ mL}$$

If we plug this result into the equation for the x -intercept and solve for C_A , we find that the concentration of Mn^{2+} is

$$C_A = -\frac{x\text{-intercept} \times C_{std}}{V_o} = -\frac{-1.731 \text{ mL} \times 100.6 \text{ mg/L}}{25.00 \text{ mL}} = 6.96 \text{ mg/L}$$

For Figure 5.3.6 b, the x -intercept is

$$x\text{-intercept} = -\frac{0.1478}{0.0425 \text{ mL/mg}} = -3.478 \text{ mg/mL}$$

and the concentration of Mn^{2+} is

$$C_A = -\frac{x\text{-intercept} \times V_f}{V_o} = -\frac{-3.478 \text{ mg/mL} \times 50.00 \text{ mL}}{25.00 \text{ mL}} = 6.96 \text{ mg/L}$$

Since we construct a standard additions calibration curve in the sample, we can not use the calibration equation for other samples. Each sample, therefore, requires its own standard additions calibration curve. This is a serious drawback if you have many samples. For example, suppose you need to analyze 10 samples using a five-point calibration curve. For a normal calibration curve you need to analyze only 15 solutions (five standards and ten samples). If you use the method of standard additions, however, you must analyze 50 solutions (each of the ten samples is analyzed five times, once before spiking and after each of four spikes).

Using a Standard Addition to Identify Matrix Effects

We can use the method of standard additions to validate an external standardization when matrix matching is not feasible. First, we prepare a normal calibration curve of S_{std} versus C_{std} and determine the value of k_A from its slope. Next, we prepare a standard additions calibration curve using Equation 3.4.8, plotting the data as shown in Figure 5.3.6 b. The slope of this standard additions calibration curve provides an independent determination of k_A . If there is no significant difference between the two values of k_A , then we can ignore the difference between the sample's matrix and that of the external standards. When the values of k_A are significantly different, then using a normal calibration curve introduces a proportional determinate error.

Internal Standards

To use an external standardization or the method of standard additions, we must be able to treat identically all samples and standards. When this is not possible, the accuracy and precision of our standardization may suffer. For example, if our analyte is in a volatile solvent, then its concentration will increase if we lose solvent to evaporation. Suppose we have a sample and a standard with identical concentrations of analyte and identical signals. If both experience the same proportional loss of solvent, then their respective concentrations of analyte and signals remain identical. In effect, we can ignore evaporation if the samples and the standards experience an equivalent loss of solvent. If an identical standard and sample lose different amounts of solvent, however, then their respective concentrations and signals are no longer equal. In this case a simple external standardization or standard addition is not possible.

We can still complete a standardization if we reference the analyte's signal to a signal from another species that we add to all samples and standards. The species, which we call an **internal standard**, must be different than the analyte.

Because the analyte and the internal standard receive the same treatment, the ratio of their signals is unaffected by any lack of reproducibility in the procedure. If a solution contains an analyte of concentration C_A and an internal standard of concentration C_{IS} , then the signals due to the analyte, S_A , and the internal standard, S_{IS} , are

$$S_A = k_A C_A$$

$$S_{IS} = k_{IS} C_{IS}$$

where k_A and k_{IS} are the sensitivities for the analyte and the internal standard, respectively. Taking the ratio of the two signals gives the fundamental equation for an internal standardization.

$$\frac{S_A}{S_{IS}} = \frac{k_A C_A}{k_{IS} C_{IS}} = K \times \frac{C_A}{C_{IS}} \quad (3.4.12)$$

Because K is a ratio of the analyte's sensitivity and the internal standard's sensitivity, it is not necessary to determine independently values for either k_A or k_{IS} .

Single Internal Standard

In a single-point internal standardization, we prepare a single standard that contains the analyte and the internal standard, and use it to determine the value of K in Equation 3.4.12

$$K = \left(\frac{C_{IS}}{C_A} \right)_{std} \times \left(\frac{S_A}{S_{IS}} \right)_{std} \quad (3.4.13)$$

Having standardized the method, the analyte's concentration is given by

$$C_A = \frac{C_{IS}}{K} \times \left(\frac{S_A}{S_{IS}} \right)_{samp}$$

✓ Example 5.3.7

A sixth spectrophotometric method for the quantitative analysis of Pb^{2+} in blood uses Cu^{2+} as an internal standard. A standard that is 1.75 ppb Pb^{2+} and 2.25 ppb Cu^{2+} yields a ratio of $(S_A/S_{IS})_{std}$ of 2.37. A sample of blood spiked with the same concentration of Cu^{2+} gives a signal ratio, $(S_A/S_{IS})_{samp}$, of 1.80. What is the concentration of Pb^{2+} in the sample of blood?

Solution

Equation 3.4.13 allows us to calculate the value of K using the data for the standard

$$K = \left(\frac{C_{IS}}{C_A} \right)_{std} \times \left(\frac{S_A}{S_{IS}} \right)_{std} = \frac{2.25 \text{ ppb } Cu^{2+}}{1.75 \text{ ppb } Pb^{2+}} \times 2.37 = 3.05 \frac{\text{ppb } Cu^{2+}}{\text{ppb } Pb^{2+}}$$

The concentration of Pb^{2+} , therefore, is

$$C_A = \frac{C_{IS}}{K} \times \left(\frac{S_A}{S_{IS}} \right)_{samp} = \frac{2.25 \text{ ppb } Cu^{2+}}{3.05 \frac{\text{ppb } Cu^{2+}}{\text{ppb } Pb^{2+}}} \times 1.80 = 1.33 \text{ ppb } Pb^{2+}$$

Multiple Internal Standards

A single-point internal standardization has the same limitations as a single-point normal calibration. To construct an internal standard calibration curve we prepare a series of standards, each of which contains the same concentration of internal standard and a different concentrations of analyte. Under these conditions a calibration curve of $(S_A/S_{IS})_{std}$ versus C_A is linear with a slope of K/C_{IS} .

Although the usual practice is to prepare the standards so that each contains an identical amount of the internal standard, this is not a requirement.

✓ Example 5.3.8

A seventh spectrophotometric method for the quantitative analysis of Pb^{2+} in blood gives a linear internal standards calibration curve for which

$$\left(\frac{S_A}{S_{IS}} \right)_{std} = (2.11 \text{ ppb}^{-1} \times C_A) - 0.006$$

What is the ppb Pb^{2+} in a sample of blood if $(S_A/S_{IS})_{samp}$ is 2.80?

Solution

To determine the concentration of Pb^{2+} in the sample of blood we replace $(S_A/S_{IS})_{std}$ in the calibration equation with $(S_A/S_{IS})_{samp}$ and solve for C_A .

$$C_A = \frac{\left(\frac{S_A}{S_{IS}} \right)_{samp} + 0.006}{2.11 \text{ ppb}^{-1}} = \frac{2.80 + 0.006}{2.11 \text{ ppb}^{-1}} = 1.33 \text{ ppb } Pb^{2+}$$

The concentration of Pb^{2+} in the sample of blood is 1.33 ppb.

In some circumstances it is not possible to prepare the standards so that each contains the same concentration of internal standard. This is the case, for example, when we prepare samples by mass instead of volume. We can still prepare a calibration curve, however, by plotting $(S_A/S_{IS})_{std}$ versus C_A/C_{IS} , giving a linear calibration curve with a slope of K .

You might wonder if it is possible to include an internal standard in the method of standard additions to correct for both matrix effects and uncontrolled variations between samples; well, the answer is yes as described in the paper “Standard Dilution Analysis,” the full reference for which is Jones, W. B.; Donati, G. L.; Calloway, C. P.; Jones, B. T. *Anal. Chem.* **2015**, *87*, 2321-2327.

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3.5: Linear Regression and Calibration Curves

In a single-point external standardization we determine the value of k_A by measuring the signal for a single standard that contains a known concentration of analyte. Using this value of k_A and our sample's signal, we then calculate the concentration of analyte in our sample (see [Example 5.3.1](#)). With only a single determination of k_A , a quantitative analysis using a single-point external standardization is straightforward.

A multiple-point standardization presents a more difficult problem. Consider the data in Table 3.5.1 for a multiple-point external standardization. What is our best estimate of the relationship between S_{std} and C_{std} ? It is tempting to treat this data as five separate single-point standardizations, determining k_A for each standard, and reporting the mean value for the five trials. Despite its simplicity, this is not an appropriate way to treat a multiple-point standardization.

Table 3.5.1 : Data for a Hypothetical Multiple-Point External Standardization

C_{std} (arbitrary units)	S_{std} (arbitrary units)	$k_A = S_{std}/C_{std}$
0.000	0.00	—
0.100	12.36	123.6
0.200	24.83	124.2
0.300	35.91	119.7
0.400	48.79	122.0
0.500	60.42	122.8
		mean $k_A = 122.5$

So why is it inappropriate to calculate an average value for k_A using the data in Table 3.5.1 ? In a single-point standardization we assume that the reagent blank (the first row in Table 3.5.1) corrects for all constant sources of determinate error. If this is not the case, then the value of k_A from a single-point standardization has a constant determinate error. Table 3.5.2 demonstrates how an uncorrected constant error affects our determination of k_A . The first three columns show the concentration of analyte in a set of standards, C_{std} , the signal without any source of constant error, S_{std} , and the actual value of k_A for five standards. As we expect, the value of k_A is the same for each standard. In the fourth column we add a constant determinate error of +0.50 to the signals, $(S_{std})_e$. The last column contains the corresponding apparent values of k_A . Note that we obtain a different value of k_A for each standard and that each apparent k_A is greater than the true value.

Table 3.5.2 : Effect of a Constant Determinate Error on the Value of k_A From a Single-Point Standardization

C_{std}	S_{std} (without constant error)	$k_A = S_{std}/C_{std}$ (actual)	$(S_{std})_e$ (with constant error)	$k_A = (S_{std})_e/C_{std}$ (apparent)
1.00	1.00	1.00	1.50	1.50
2.00	2.00	1.00	2.50	1.25
3.00	3.00	1.00	3.50	1.17
4.00	4.00	1.00	4.50	1.13
5.00	5.00	1.00	5.50	1.10
		mean k_A (true) = 1.00	mean k_A (apparent) = 1.23	

How do we find the best estimate for the relationship between the signal and the concentration of analyte in a multiple-point standardization? Figure 3.5.1 shows the data in Table 3.5.1 plotted as a normal calibration curve. Although the data certainly appear to fall along a straight line, the actual calibration curve is not intuitively obvious. The process of determining the best equation for the calibration curve is called linear regression.

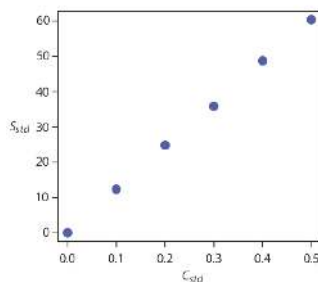


Figure 3.5.1 : Normal calibration curve data for the hypothetical multiple-point external standardization in Table 3.5.1 .

Linear Regression of Straight Line Calibration Curves

When a calibration curve is a straight-line, we represent it using the following mathematical equation

$$y = \beta_0 + \beta_1 x \quad (3.5.1)$$

where y is the analyte's signal, S_{std} , and x is the analyte's concentration, C_{std} . The constants β_0 and β_1 are, respectively, the calibration curve's expected y -intercept and its expected slope. Because of uncertainty in our measurements, the best we can do is to estimate values for β_0 and β_1 , which we represent as b_0 and b_1 . The goal of a **linear regression** analysis is to determine the best estimates for b_0 and b_1 . How we do this depends on the uncertainty in our measurements.

Unweighted Linear Regression with Errors in y

The most common method for completing the linear regression for Equation 3.5.1 makes three assumptions:

1. that the difference between our experimental data and the calculated regression line is the result of indeterminate errors that affect y
2. that indeterminate errors that affect y are normally distributed
3. that the indeterminate errors in y are independent of the value of x

Because we assume that the indeterminate errors are the same for all standards, each standard contributes equally in our estimate of the slope and the y -intercept. For this reason the result is considered an **unweighted linear regression**.

The second assumption generally is true because of the central limit theorem, which we considered in Chapter 4. The validity of the two remaining assumptions is less obvious and you should evaluate them before you accept the results of a linear regression. In particular the first assumption always is suspect because there certainly is some indeterminate error in the measurement of x . When we prepare a calibration curve, however, it is not unusual to find that the uncertainty in the signal, S_{std} , is significantly larger than the uncertainty in the analyte's concentration, C_{std} . In such circumstances the first assumption is usually reasonable.

How a Linear Regression Works

To understand the logic of a linear regression consider the example shown in Figure 3.5.2 , which shows three data points and two possible straight-lines that might reasonably explain the data. How do we decide how well these straight-lines fit the data, and how do we determine the best straight-line?

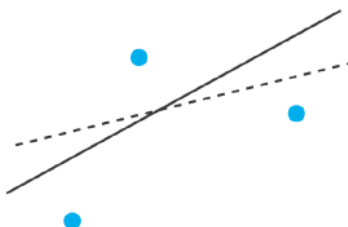


Figure 3.5.2 : Illustration showing three data points and two possible straight-lines that might explain the data. The goal of a linear regression is to find the mathematical model, in this case a straight-line, that best explains the data.

Let's focus on the solid line in Figure 3.5.2 . The equation for this line is

$$\hat{y} = b_0 + b_1 x \quad (3.5.2)$$

where b_0 and b_1 are estimates for the y-intercept and the slope, and $\hat{y}$ is the predicted value of y for any value of x . Because we assume that all uncertainty is the result of indeterminate errors in y , the difference between y and $\hat{y}$ for each value of x is the residual error, r , in our mathematical model.

$$r_i = (y_i - \hat{y}_i)$$

Figure 3.5.3 shows the residual errors for the three data points. The smaller the total residual error, R , which we define as

$$R = \sum_{i=1}^n (y_i - \hat{y}_i)^2 \quad (3.5.3)$$

the better the fit between the straight-line and the data. In a linear regression analysis, we seek values of b_0 and b_1 that give the smallest total residual error.

The reason for squaring the individual residual errors is to prevent a positive residual error from canceling out a negative residual error. You have seen this before in the equations for the sample and population standard deviations. You also can see from this equation why a linear regression is sometimes called the method of least squares.

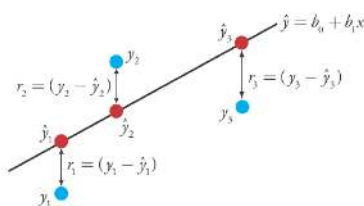


Figure 3.5.3 : Illustration showing the evaluation of a linear regression in which we assume that all uncertainty is the result of indeterminate errors in y . The points in blue, y , are the original data and the points in red, $\hat{y}_i$, are the predicted values from the regression equation, $\hat{y} = b_0 + b_1 x$. The smaller the total residual error (Equation 3.5.3), the better the fit of the straight-line to the data.

Finding the Slope and y-Intercept

Although we will not formally develop the mathematical equations for a linear regression analysis, you can find the derivations in many standard statistical texts [See, for example, Draper, N. R.; Smith, H. Applied Regression Analysis, 3rd ed.; Wiley: New York, 1998]. The resulting equation for the slope, b_1 , is

$$b_1 = \frac{n \sum_{i=1}^n x_i y_i - \sum_{i=1}^n x_i \sum_{i=1}^n y_i}{n \sum_{i=1}^n x_i^2 - (\sum_{i=1}^n x_i)^2} \quad (3.5.4)$$

and the equation for the y-intercept, b_0 , is

$$b_0 = \frac{\sum_{i=1}^n y_i - b_1 \sum_{i=1}^n x_i}{n} \quad (3.5.5)$$

Although Equation 3.5.4 and Equation 3.5.5 appear formidable, it is necessary only to evaluate the following four summations

$$\sum_{i=1}^n x_i \quad \sum_{i=1}^n y_i \quad \sum_{i=1}^n x_i y_i \quad \sum_{i=1}^n x_i^2$$

Many calculators, spreadsheets, and other statistical software packages are capable of performing a linear regression analysis based on this model. To save time and to avoid tedious calculations, learn how to use one of these tools (and see Section 5.6 for details on completing a linear regression analysis using Excel and R.). For illustrative purposes the necessary calculations are shown in detail in the following example.

Equation 3.5.4 and Equation 3.5.5 are written in terms of the general variables x and y . As you work through this example, remember that x corresponds to C_{std} , and that y corresponds to S_{std} .

✓ Example 3.5.1

Using the data from Table 3.5.1, determine the relationship between S_{std} and C_{std} using an unweighted linear regression.

Solution

We begin by setting up a table to help us organize the calculation.

x_i	y_i	$x_i y_i$	x_i^2
0.000	0.00	0.000	0.000
0.100	12.36	1.236	0.010
0.200	24.83	4.966	0.040
0.300	35.91	10.773	0.090
0.400	48.79	19.516	0.160
0.500	60.42	30.210	0.250

Adding the values in each column gives

$$\sum_{i=1}^n x_i = 1.500 \quad \sum_{i=1}^n y_i = 182.31 \quad \sum_{i=1}^n x_i y_i = 66.701 \quad \sum_{i=1}^n x_i^2 = 0.550$$

Substituting these values into Equation 3.5.4 and Equation 3.5.5, we find that the slope and the y-intercept are

$$b_1 = \frac{(6 \times 66.701) - (1.500 \times 182.31)}{(6 \times 0.550) - (1.500)^2} = 120.706 \approx 120.71$$

$$b_0 = \frac{182.31 - (120.706 \times 1.500)}{6} = 0.209 \approx 0.21$$

The relationship between the signal and the analyte, therefore, is

$$S_{std} = 120.71 \times C_{std} + 0.21$$

For now we keep two decimal places to match the number of decimal places in the signal. The resulting calibration curve is shown in Figure 3.5.4.

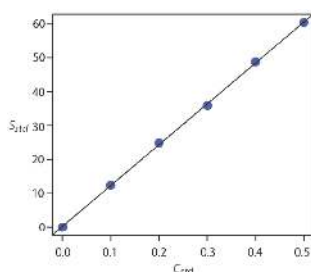


Figure 3.5.4 : Calibration curve for the data in Table 3.5.1 and Example 3.5.1.

Uncertainty in the Regression Analysis

As shown in Figure 3.5.4, because indeterminate errors in the signal, the regression line may not pass through the exact center of each data point. The cumulative deviation of our data from the regression line—that is, the total residual error—is proportional to the uncertainty in the regression. We call this uncertainty the **standard deviation about the regression**, s_r , which is equal to

$$s_r = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n - 2}} \quad (3.5.6)$$

where y_i is the i^{th} experimental value, and $\hat{y}_i$ is the corresponding value predicted by the regression line in Equation 3.5.2. Note that the denominator of Equation 3.5.6 indicates that our regression analysis has $n - 2$ degrees of freedom—we lose two degrees of freedom because we use two parameters, the slope and the y-intercept, to calculate $\hat{y}_i$.

Did you notice the similarity between the standard deviation about the regression (Equation 3.5.6) and the standard deviation for a sample (Equation 4.1.1)?

A more useful representation of the uncertainty in our regression analysis is to consider the effect of indeterminate errors on the slope, b_1 , and the y-intercept, b_0 , which we express as standard deviations.

$$s_{b_1} = \sqrt{\frac{ns_r^2}{n \sum_{i=1}^n x_i^2 - (\sum_{i=1}^n x_i)^2}} = \sqrt{\frac{s_r^2}{\sum_{i=1}^n (x_i - \bar{x})^2}} \quad (3.5.7)$$

$$s_{b_0} = \sqrt{\frac{s_r^2 \sum_{i=1}^n x_i^2}{n \sum_{i=1}^n x_i^2 - (\sum_{i=1}^n x_i)^2}} = \sqrt{\frac{s_r^2 \sum_{i=1}^n x_i^2}{n \sum_{i=1}^n (x_i - \bar{x})^2}} \quad (3.5.8)$$

We use these standard deviations to establish confidence intervals for the expected slope, β_1 , and the expected y-intercept, β_0

$$\beta_1 = b_1 \pm ts_{b_1} \quad (3.5.9)$$

$$\beta_0 = b_0 \pm ts_{b_0} \quad (3.5.10)$$

where we select t for a significance level of α and for $n - 2$ degrees of freedom. Note that Equation 3.5.9 and Equation 3.5.10 do not contain a factor of $(\sqrt{n})^{-1}$ because the confidence interval is based on a single regression line.

✓ Example 3.5.2

Calculate the 95% confidence intervals for the slope and y-intercept from Example 3.5.1.

Solution

We begin by calculating the standard deviation about the regression. To do this we must calculate the predicted signals, $\hat{y}_i$, using the slope and y-intercept from Example 3.5.1, and the squares of the residual error, $(y_i - \hat{y}_i)^2$. Using the last standard as an example, we find that the predicted signal is

$$\hat{y}_6 = b_0 + b_1 x_6 = 0.209 + (120.706 \times 0.500) = 60.562$$

and that the square of the residual error is

$$(y_i - \hat{y}_i)^2 = (60.42 - 60.562)^2 = 0.2016 \approx 0.202$$

The following table displays the results for all six solutions.

x_i	y_i	$\hat{y}_i$	$(y_i - \hat{y}_i)^2$
0.000	0.00	0.209	0.0437
0.100	12.36	12.280	0.0064
0.200	24.83	24.350	0.2304
0.300	35.91	36.421	0.2611
0.400	48.79	48.491	0.0894
0.500	60.42	60.562	0.0202

Adding together the data in the last column gives the numerator of Equation 3.5.6 as 0.6512; thus, the standard deviation about the regression is

$$s_r = \sqrt{\frac{0.6512}{6-2}} = 0.4035$$

Next we calculate the standard deviations for the slope and the y-intercept using Equation 3.5.7 and Equation 3.5.8. The values for the summation terms are from Example 3.5.1 .

$$s_{b_1} = \sqrt{\frac{6 \times (0.4035)^2}{(6 \times 0.550) - (1.500)^2}} = 0.965$$

$$s_{b_0} = \sqrt{\frac{(0.4035)^2 \times 0.550}{(6 \times 0.550) - (1.500)^2}} = 0.292$$

Finally, the 95% confidence intervals ($\alpha = 0.05$, 4 degrees of freedom) for the slope and y-intercept are

$$\beta_1 = b_1 \pm ts_{b_1} = 120.706 \pm (2.78 \times 0.965) = 120.7 \pm 2.7$$

$$\beta_0 = b_0 \pm ts_{b_0} = 0.209 \pm (2.78 \times 0.292) = 0.2 \pm 0.80$$

where $t(0.05, 4)$ from Appendix 4 is 2.78. The standard deviation about the regression, s_r , suggests that the signal, S_{std} , is precise to one decimal place. For this reason we report the slope and the y-intercept to a single decimal place.

Minimizing Uncertainty in Calibration Model

To minimize the uncertainty in a calibration curve's slope and y-intercept, we evenly space our standards over a wide range of analyte concentrations. A close examination of Equation 3.5.7 and Equation 3.5.8 help us appreciate why this is true. The denominators of both equations include the term $\sum_{i=1}^n (x_i - \bar{x})^2$. The larger the value of this term—which we accomplish by increasing the range of x around its mean value—the smaller the standard deviations in the slope and the y-intercept. Furthermore, to minimize the uncertainty in the y-intercept, it helps to decrease the value of the term $\sum_{i=1}^n x_i$ in Equation 3.5.8, which we accomplish by including standards for lower concentrations of the analyte.

Obtaining the Analyte's Concentration From a Regression Equation

Once we have our regression equation, it is easy to determine the concentration of analyte in a sample. When we use a normal calibration curve, for example, we measure the signal for our sample, S_{samp} , and calculate the analyte's concentration, C_A , using the regression equation.

$$C_A = \frac{S_{samp} - b_0}{b_1} \quad (3.5.11)$$

What is less obvious is how to report a confidence interval for C_A that expresses the uncertainty in our analysis. To calculate a confidence interval we need to know the standard deviation in the analyte's concentration, s_{C_A} , which is given by the following equation

$$s_{C_A} = \frac{s_r}{b_1} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{S}_{samp} - \bar{S}_{std})^2}{(b_1)^2 \sum_{i=1}^n (C_{std_i} - \bar{C}_{std})^2}} \quad (3.5.12)$$

where m is the number of replicate we use to establish the sample's average signal, S_{samp} , n is the number of calibration standards, S_{std} is the average signal for the calibration standards, and C_{std_i} and $\bar{C}_{std}$ are the individual and the mean concentrations for the calibration standards. Knowing the value of s_{C_A} , the confidence interval for the analyte's concentration is

$$\mu_{C_A} = C_A \pm ts_{C_A}$$

where μ_{C_A} is the expected value of C_A in the absence of determinate errors, and with the value of t is based on the desired level of confidence and $n - 2$ degrees of freedom.

Equation 3.5.12 is written in terms of a calibration experiment. A more general form of the equation, written in terms of x and y , is given here.

$$s_x = \frac{s_r}{b_1} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{Y} - \bar{y})^2}{(b_1)^2 \sum_{i=1}^n (x_i - \bar{x})^2}}$$

A close examination of Equation 3.5.12 should convince you that the uncertainty in C_A is smallest when the sample's average signal, $\bar{S}_{\text{samp}}$, is equal to the average signal for the standards, $\bar{S}_{\text{std}}$. When practical, you should plan your calibration curve so that S_{samp} falls in the middle of the calibration curve. For more information about these regression equations see (a) Miller, J. N. *Analyst* **1991**, 116, 3–14; (b) Sharaf, M. A.; Illman, D. L.; Kowalski, B. R. *Chemometrics*, Wiley-Interscience: New York, 1986, pp. 126-127; (c) Analytical Methods Committee "Uncertainties in concentrations estimated from calibration experiments," [AMC Technical Brief](#), March 2006.

✓ Example 3.5.3

Three replicate analyses for a sample that contains an unknown concentration of analyte, yield values for S_{samp} of 29.32, 29.16 and 29.51 (arbitrary units). Using the results from [Example 3.5.1](#) and [Example 3.5.2](#), determine the analyte's concentration, C_A , and its 95% confidence interval.

Solution

The average signal, $\bar{S}_{\text{samp}}$, is 29.33, which, using Equation 3.5.11 and the slope and the y-intercept from [Example 3.5.1](#), gives the analyte's concentration as

$$C_A = \frac{\bar{S}_{\text{samp}} - b_0}{b_1} = \frac{29.33 - 0.209}{120.706} = 0.241$$

To calculate the standard deviation for the analyte's concentration we must determine the values for $\bar{S}_{\text{std}}$ and for $\sum_{i=1}^2 (C_{\text{std}_i} - \bar{C}_{\text{std}})^2$. The former is just the average signal for the calibration standards, which, using the data in [Table 3.5.1](#), is 30.385. Calculating $\sum_{i=1}^2 (C_{\text{std}_i} - \bar{C}_{\text{std}})^2$ looks formidable, but we can simplify its calculation by recognizing that this sum-of-squares is the numerator in a standard deviation equation; thus,

$$\sum_{i=1}^n (C_{\text{std}_i} - \bar{C}_{\text{std}})^2 = (s_{C_{\text{std}}})^2 \times (n - 1)$$

where $s_{C_{\text{std}}}$ is the standard deviation for the concentration of analyte in the calibration standards. Using the data in [Table 3.5.1](#) we find that $s_{C_{\text{std}}}$ is 0.1871 and

$$\sum_{i=1}^n (C_{\text{std}_i} - \bar{C}_{\text{std}})^2 = (0.1872)^2 \times (6 - 1) = 0.175$$

Substituting known values into Equation 3.5.12 gives

$$s_{C_A} = \frac{0.4035}{120.706} \sqrt{\frac{1}{3} + \frac{1}{6} + \frac{(29.33 - 30.385)^2}{(120.706)^2 \times 0.175}} = 0.0024$$

Finally, the 95% confidence interval for 4 degrees of freedom is

$$\mu_{C_A} = C_A \pm t s_{C_A} = 0.241 \pm (2.78 \times 0.0024) = 0.241 \pm 0.007$$

Figure 3.5.5 shows the calibration curve with curves showing the 95% confidence interval for C_A .

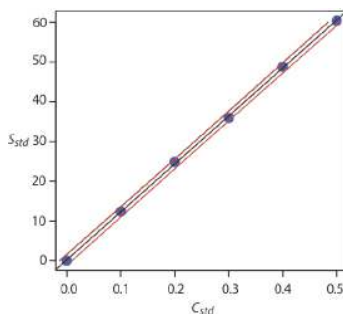


Figure 3.5.5 : Example of a normal calibration curve with a superimposed confidence interval for the analyte's concentration. The points in blue are the original data from Table 3.5.1 . The black line is the normal calibration curve as determined in Example 3.5.1 . The red lines show the 95% confidence interval for C_A assuming a single determination of S_{samp} .

In a standard addition we determine the analyte's concentration by extrapolating the calibration curve to the x-intercept. In this case the value of C_A is

$$C_A = x\text{-intercept} = \frac{-b_0}{b_1}$$

and the standard deviation in C_A is

$$s_{C_A} = \frac{s_r}{b_1} \sqrt{\frac{1}{n} + \frac{(\bar{S}_{std})^2}{(b_1)^2 \sum_{i=1}^n (C_{std_i} - \bar{C}_{std})^2}}$$

where n is the number of standard additions (including the sample with no added standard), and $\bar{S}_{std}$ is the average signal for the n standards. Because we determine the analyte's concentration by extrapolation, rather than by interpolation, s_{C_A} for the method of standard additions generally is larger than for a normal calibration curve.

? Exercise 3.5.1

Figure 3.5.2 shows a normal calibration curve for the quantitative analysis of Cu^{2+} . The data for the calibration curve are shown here.

$[\text{Cu}^{2+}] \text{ (M)}$	Absorbance
0	0
1.55×10^{-3}	0.050
3.16×10^{-3}	0.093
4.74×10^{-3}	0.143
6.34×10^{-3}	0.188
7.92×10^{-3}	0.236

Complete a linear regression analysis for this calibration data, reporting the calibration equation and the 95% confidence interval for the slope and the y-intercept. If three replicate samples give an S_{samp} of 0.114, what is the concentration of analyte in the sample and its 95% confidence interval?

Answer

We begin by setting up a table to help us organize the calculation

x_i	y_i	$x_i y_i$	x_i^2
0.000	0.000	0.000	0.000
1.55×10^{-3}	0.050	7.750×10^{-5}	2.403×10^{-6}

x_i	y_i	$x_i y_i$	x_i^2
3.16×10^{-3}	0.093	2.939×10^{-4}	9.986×10^{-6}
4.74×10^{-3}	0.143	6.778×10^{-4}	2.247×10^{-5}
6.34×10^{-3}	0.188	1.192×10^{-3}	4.020×10^{-5}
7.92×10^{-3}	0.236	1.869×10^{-3}	6.273×10^{-5}

Adding the values in each column gives

$$\sum_{i=1}^n x_i = 2.371 \times 10^{-2} \quad \sum_{i=1}^n y_i = 0.710 \quad \sum_{i=1}^n x_i y_i = 4.110 \times 10^{-3} \quad \sum_{i=1}^n x_i^2 = 1.378 \times 10^{-4}$$

When we substitute these values into Equation 3.5.4 and Equation 3.5.5 we find that the slope and the y-intercept are

$$b_1 = \frac{6 \times (4.110 \times 10^{-3}) - (2.371 \times 10^{-2}) \times 0.710}{6 \times (1.378 \times 10^{-4}) - (2.371 \times 10^{-2})^2} = 29.57$$

$$b_0 = \frac{0.710 - 29.57 \times (2.371 \times 10^{-2})}{6} = 0.0015$$

and that the regression equation is

$$S_{std} = 29.57 \times C_{std} + 0.0015$$

To calculate the 95% confidence intervals, we first need to determine the standard deviation about the regression. The following table helps us organize the calculation.

x_i	y_i	$\hat{y}_i$	$(y_i - \hat{y}_i)^2$
0.000	0.000	0.0015	2.250×10^{-6}
1.55×10^{-3}	0.050	0.0473	7.110×10^{-6}
3.16×10^{-3}	0.093	0.0949	3.768×10^{-6}
4.74×10^{-3}	0.143	0.1417	1.791×10^{-6}
6.34×10^{-3}	0.188	0.1890	9.483×10^{-6}
7.92×10^{-3}	0.236	0.2357	9.339×10^{-6}

Adding together the data in the last column gives the numerator of Equation 3.5.6 as 1.596×10^{-5} . The standard deviation about the regression, therefore, is

$$s_r = \sqrt{\frac{1.596 \times 10^{-5}}{6 - 2}} = 1.997 \times 10^{-3}$$

Next, we need to calculate the standard deviations for the slope and the y-intercept using Equation 3.5.7 and Equation 3.5.8

$$s_{b_1} = \sqrt{\frac{6 \times (1.997 \times 10^{-3})^2}{6 \times (1.378 \times 10^{-4}) - (2.371 \times 10^{-2})^2}} = 0.3007$$

$$s_{b_0} = \sqrt{\frac{(1.997 \times 10^{-3})^2 \times (1.378 \times 10^{-4})}{6 \times (1.378 \times 10^{-4}) - (2.371 \times 10^{-2})^2}} = 1.441 \times 10^{-3}$$

and use them to calculate the 95% confidence intervals for the slope and the y-intercept

$$\beta_1 = b_1 \pm t s_{b_1} = 29.57 \pm (2.78 \times 0.3007) = 29.57 \text{ M}^{-1} \pm 0.84 \text{ M}^{-1}$$

$$\beta_0 = b_0 \pm ts_{b_0} = 0.0015 \pm (2.78 \times 1.441 \times 10^{-3}) = 0.0015 \pm 0.0040$$

With an average S_{samp} of 0.114, the concentration of analyte, C_A , is

$$C_A = \frac{S_{\text{samp}} - b_0}{b_1} = \frac{0.114 - 0.0015}{29.57 \text{ M}^{-1}} = 3.80 \times 10^{-3} \text{ M}$$

The standard deviation in C_A is

$$s_{C_A} = \frac{1.997 \times 10^{-3}}{29.57} \sqrt{\frac{1}{3} + \frac{1}{6} + \frac{(0.114 - 0.1183)^2}{(29.57)^2 \times (4.408 \times 10^{-5})}} = 4.778 \times 10^{-5}$$

and the 95% confidence interval is

$$\begin{aligned} \mu &= C_A \pm ts_{C_A} = 3.80 \times 10^{-3} \pm \{2.78 \times (4.778 \times 10^{-5})\} \\ \mu &= 3.80 \times 10^{-3} \text{ M} \pm 0.13 \times 10^{-3} \text{ M} \end{aligned}$$

Evaluating a Linear Regression Model

You should never accept the result of a linear regression analysis without evaluating the validity of the model. Perhaps the simplest way to evaluate a regression analysis is to examine the residual errors. As we saw earlier, the residual error for a single calibration standard, r_i , is

$$r_i = (y_i - \hat{y}_i)$$

If the regression model is valid, then the residual errors should be distributed randomly about an average residual error of zero, with no apparent trend toward either smaller or larger residual errors (Figure 3.5.6 a). Trends such as those in Figure 3.5.6 b and Figure 3.5.6 c provide evidence that at least one of the model's assumptions is incorrect. For example, a trend toward larger residual errors at higher concentrations, Figure 3.5.6 b, suggests that the indeterminate errors affecting the signal are not independent of the analyte's concentration. In Figure 3.5.6 c, the residual errors are not random, which suggests we cannot model the data using a straight-line relationship. Regression methods for the latter two cases are discussed in the following sections.

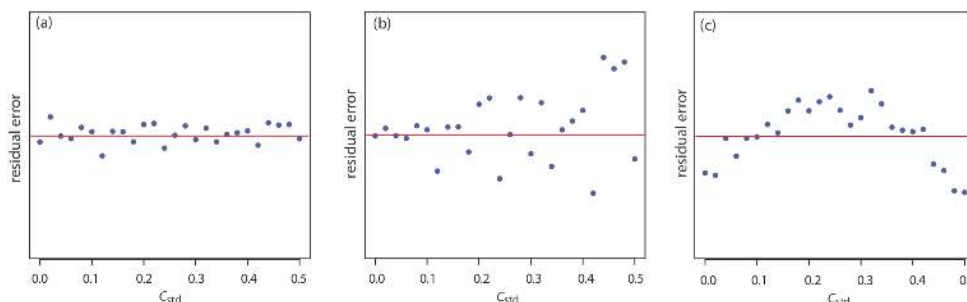


Figure 3.5.6 : Plots of the residual error in the signal, S_{std} , as a function of the concentration of analyte, C_{std} , for an unweighted straight-line regression model. The red line shows a residual error of zero. The distribution of the residual errors in (a) indicates that the unweighted linear regression model is appropriate. The increase in the residual errors in (b) for higher concentrations of analyte, suggests that a weighted straight-line regression is more appropriate. For (c), the curved pattern to the residuals suggests that a straight-line model is inappropriate; linear regression using a quadratic model might produce a better fit.

? Exercise 3.5.2

Using your results from [Exercise 3.5.1](#), construct a residual plot and explain its significance.

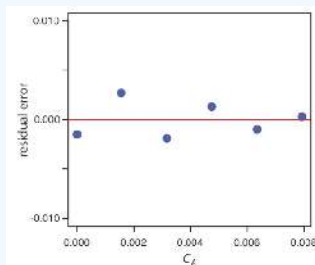
Answer

To create a residual plot, we need to calculate the residual error for each standard. The following table contains the relevant information.

x_i	y_i	$\hat{y}_i$	$y_i - \hat{y}_i$
0.000	0.000	0.0015	-0.0015

1.55×10^{-3}	0.050	0.0473	0.0027
3.16×10^{-3}	0.093	0.0949	-0.0019
4.74×10^{-3}	0.143	0.1417	0.0013
6.34×10^{-3}	0.188	0.1890	-0.0010
7.92×10^{-3}	0.236	0.2357	0.0003
9.51×10^{-3}	0.286	0.2857	0.0003

The figure below shows a plot of the resulting residual errors. The residual errors appear random, although they do alternate in sign, and that do not show any significant dependence on the analyte's concentration. Taken together, these observations suggest that our regression model is appropriate.



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CHAPTER OVERVIEW

4: Equilibrium and Activity

4.1: Solving Equilibrium Problems

4.2: Buffer Solutions

4.3: Activity Effects

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4.1: Solving Equilibrium Problems

In this section we will learn how to set-up and solve equilibrium problems. We will start with a simple problem and work toward more complex problems.

A Simple Problem: The Solubility of $\text{Pb}(\text{IO}_3)_2$

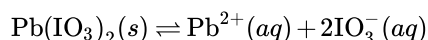
If we place an insoluble compound such as $\text{Pb}(\text{IO}_3)_2$ in deionized water, the solid dissolves until the concentrations of Pb^{2+} and IO_3^- satisfy the solubility product for $\text{Pb}(\text{IO}_3)_2$. At equilibrium the solution is saturated with $\text{Pb}(\text{IO}_3)_2$, which means simply that no more solid can dissolve. How do we determine the equilibrium concentrations of Pb^{2+} and IO_3^- , and what is the molar solubility of $\text{Pb}(\text{IO}_3)_2$ in this saturated solution?

When we first add solid $\text{Pb}(\text{IO}_3)_2$ to water, the concentrations of Pb^{2+} and IO_3^- are zero and the reaction quotient, Q_r , is

$$Q_r = [\text{Pb}^{2+}] [\text{IO}_3^-]^2 = 0$$

As the solid dissolves, the concentrations of these ions increase, but Q_r remains smaller than K_{sp} . We reach equilibrium and “satisfy the solubility product” when $Q_r = K_{sp}$.

We begin by writing the equilibrium reaction and the solubility product expression for $\text{Pb}(\text{IO}_3)_2$.



e

$$K_{sp} = [\text{Pb}^{2+}] [\text{IO}_3^-]^2 = 2.5 \times 10^{-13} \quad (4.1.1)$$

As $\text{Pb}(\text{IO}_3)_2$ dissolves, two IO_3^- ions form for each ion of Pb^{2+} . If we assume that the change in the molar concentration of Pb^{2+} at equilibrium is x , then the change in the molar concentration of IO_3^- is $2x$. The following table helps us keep track of the initial concentrations, the change in concentrations, and the equilibrium concentrations of Pb^{2+} and IO_3^- .

concentrations	$\text{Pb}(\text{IO}_3)_2(s)$	$\rightleftharpoons$	$\text{Pb}^{2+}(aq)$	+	$2\text{IO}_3^-(aq)$
initial	solid		0		0
change	solid		$+x$		$+2x$
equilibrium	solid		x		$2x$

Because a solid, such as $\text{Pb}(\text{IO}_3)_2$, does not appear in the solubility product expression, we do not need to keep track of its concentration. Remember, however, that the K_{sp} value applies only if there is some solid $\text{Pb}(\text{IO}_3)_2$ present at equilibrium.

Substituting the equilibrium concentrations into Equation 4.1.1 and solving gives

$$\begin{aligned} (x)(2x)^2 &= 4x^3 = 2.5 \times 10^{-13} \\ x &= 3.97 \times 10^{-5} \end{aligned}$$

Substituting this value of x back into the equilibrium concentration expressions for Pb^{2+} and IO_3^- gives their concentrations as

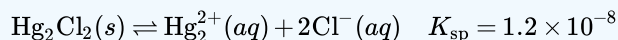
$$[\text{Pb}^{2+}] = x = 4.0 \times 10^{-5} \text{ M and } [\text{IO}_3^-] = 2x = 7.9 \times 10^{-5}$$

Because one mole of $\text{Pb}(\text{IO}_3)_2$ contains one mole of Pb^{2+} , the molar solubility of $\text{Pb}(\text{IO}_3)_2$ is equal to the concentration of Pb^{2+} , or $4.0 \times 10^{-5} \text{ M}$.

We can express a compound's solubility in two ways: as its molar solubility (mol/L) or as its mass solubility (g/L). Be sure to express your answer clearly.

? Exercise 4.1.1

Calculate the molar solubility and the mass solubility for Hg_2Cl_2 , given the following solubility reaction and K_{sp} value.



Answer

When Hg_2Cl_2 dissolves, two Cl^- are produced for each ion of Hg_2^{2+} . If we assume x is the change in the molar concentration of Hg_2^{2+} , then the change in the molar concentration of Cl^- is $2x$. The following table helps us keep track of our solution to this problem.

concentration	$\text{Hg}_2\text{Cl}_2(s)$	$\rightleftharpoons$	$\text{Hg}_2^{2+}(aq)$	+	$\text{Cl}^-(aq)$
initial	solid		0		0
change	solid		$+x$		$+2x$
equilibrium	solid		x		$2x$

Substituting the equilibrium concentrations into the K_{sp} expression for Hg_2Cl_2 gives

$$K_{\text{sp}} = [\text{Hg}_2^{2+}] [\text{Cl}^-]^2 = (x)(2x)^2 = 4x^3 = 1.2 \times 10^{-8}$$

$$x = 6.69 \times 10^{-7}$$

Substituting x back into the equilibrium expressions for Hg_2^{2+} and Cl^- gives their concentrations as

$$[\text{Hg}_2^{2+}] = x = 6.7 \times 10^{-7} \text{ M} \quad [\text{Cl}^-] = 2x = 1.3 \times 10^{-6} \text{ M}$$

The molar solubility is equal to $[\text{Hg}_2^{2+}]$, or $6.7 \times 10^{-7} \text{ mol/L}$.

A More Complex Problem: The Common Ion Effect

Calculating the solubility of $\text{Pb}(\text{IO}_3)_2$ in deionized water is a straightforward problem because the solid's dissolution is the only source of Pb^{2+} and IO_3^- . But what if we add $\text{Pb}(\text{IO}_3)_2$ to a solution of $0.10 \text{ M Pb}(\text{NO}_3)_2$? Before we set-up and solve this problem algebraically, think about the system's chemistry and decide whether the solubility of $\text{Pb}(\text{IO}_3)_2$ will increase, decrease, or remain the same. Beginning a problem by thinking about the likely answer is a good habit to develop. Knowing what answers are reasonable will help you spot errors in your calculations and give you more confidence that your solution to a problem is correct. Because the solution already contains a source of Pb^{2+} , we can use Le Châtelier's principle to predict that the solubility of $\text{Pb}(\text{IO}_3)_2$ is smaller than that in our previous problem.

We begin by setting up a table to help us keep track of the concentrations of Pb^{2+} and IO_3^- as this system moves toward and reaches equilibrium.

concentrations	$\text{Pb}(\text{IO}_3)_2(s)$	$\rightleftharpoons$	$\text{Pb}^{2+}(aq)$	+	$2\text{IO}_3^-(aq)$
initial	solid		0.10		0
change	solid		$+x$		$+2x$
equilibrium	solid		$0.10 + x$		$2x$

Substituting the equilibrium concentrations into Equation 4.1.1

$$(0.10 + x)(2x)^2 = 2.5 \times 10^{-13}$$

and multiplying out the terms on the equation's left side leaves us with

$$4x^3 + 0.40x^2 = 2.5 \times 10^{-13} \quad (4.1.2)$$

This is a more difficult equation to solve than that for the solubility of $\text{Pb}(\text{IO}_3)_2$ in deionized water, and its solution is not immediately obvious. We can find a rigorous solution to Equation 4.1.2 using computational software packages and spreadsheets, some of which are described in Chapter 6.10.

There are several approaches to solving cubic equations, but none are computationally easy using just paper and pencil.

How might we solve Equation 4.1.2 if we do not have access to a computer? One approach is to use our understanding of chemistry to simplify the problem. From Le Châtelier's principle we know that a large initial concentration of Pb^{2+} will decrease significantly the solubility of $\text{Pb}(\text{IO}_3)_2$. One reasonable assumption is that the initial concentration of Pb^{2+} is very close to its equilibrium concentration. If this assumption is correct, then the following approximation is reasonable

$$[\text{Pb}^{2+}] = 0.10 + x \approx 0.10$$

Substituting this approximation into Equation 4.1.1 and solving for x gives

$$(0.10)(2x)^2 = 0.4x^2 = 2.5 \times 10^{-13}$$

$$x = 7.91 \times 10^{-7}$$

Before we accept this answer, we must verify that our approximation is reasonable. The difference between the actual concentration of Pb^{2+} , which is $0.10 + x$ M, and our assumption that the concentration of Pb^{2+} is 0.10 M is 7.9×10^{-7} , or 7.9×10^{-4} % of the assumed concentration. This is a negligible error. If we accept the result of our calculation, we find that the equilibrium concentrations of Pb^{2+} and IO_3^- are

$$[\text{Pb}^{2+}] = 0.10 + x \approx 0.10 \text{ M and } [\text{IO}_3^-] = 2x = 1.6 \times 10^{-6} \text{ M}$$

$$\begin{aligned} \% \text{ error} &= \frac{\text{actual} - \text{assumed}}{\text{assumed}} \times 100 \\ &= \frac{(0.10 + x) - 0.10}{0.10} \times 100 \\ &= \frac{7.91 \times 10^{-7}}{0.10} \times 100 \\ &= 7.91 \times 10^{-4} \% \end{aligned}$$

The molar solubility of $\text{Pb}(\text{IO}_3)_2$ is equal to the additional concentration of Pb^{2+} in solution, or 7.9×10^{-4} mol/L. As expected, we find that $\text{Pb}(\text{IO}_3)_2$ is less soluble in the presence of a solution that already contains one of its ions. This is known as the **common ion effect**.

As outlined in the following example, if an approximation leads to an error that is unacceptably large, then we can extend the process of making and evaluating approximations.

One “rule of thumb” when making an approximation is that it should not introduce an error of more than $\pm 5\%$. Although this is not an unreasonable choice, what matters is that the error makes sense within the context of the problem you are solving.

✓ Example 4.1.1

Calculate the solubility of $\text{Pb}(\text{IO}_3)_2$ in 1.0×10^{-4} M $\text{Pb}(\text{NO}_3)_2$.

Solution

If we let x equal the change in the concentration of Pb^{2+} , then the equilibrium concentrations of Pb^{2+} and IO_3^- are

$$[\text{Pb}^{2+}] = 1.0 \times 10^{-4} + x \text{ and } [\text{IO}_3^-] = 2x$$

Substituting these concentrations into Equation 4.1.1 leaves us with

$$(1.0 \times 10^{-4} + x)(2x)^2 = 2.5 \times 10^{-13}$$

To solve this equation for x , let's make the following assumption

$$[\text{Pb}^{2+}] = 1.0 \times 10^{-4} + x \approx 1.0 \times 10^{-4} \text{ M}$$

Solving for x gives its value as 2.50×10^{-5} ; however, when we substitute this value for x back, we find that the calculated concentration of Pb^{2+} at equilibrium

$$[\text{Pb}^{2+}] = 1.0 \times 10^{-4} + x = 1.0 \times 10^{-4} + 2.50 \times 10^{-5} = 1.25 \times 10^{-4} \text{ M}$$

is 25% greater than our assumption of $1.0 \times 10^{-4} \text{ M}$. This error is unreasonably large.

Rather than shouting in frustration, let's make a new assumption. Our first assumption—that the concentration of Pb^{2+} is $1.0 \times 10^{-4} \text{ M}$ —was too small. The calculated concentration of $1.25 \times 10^{-4} \text{ M}$, therefore, probably is a too large, but closer to the correct concentration than was our first assumption. For our second approximation, let's assume that

$$[\text{Pb}^{2+}] = 1.0 \times 10^{-4} + x = 1.25 \times 10^{-4} \text{ M}$$

Substituting into Equation 4.1.1 and solving for x gives its value as 2.24×10^{-5} . The resulting concentration of Pb^{2+} is

$$[\text{Pb}^{2+}] = 1.0 \times 10^{-4} + 2.24 \times 10^{-5} = 1.22 \times 10^{-4} \text{ M}$$

which differs from our assumption of $1.25 \times 10^{-4} \text{ M}$ by 2.4%. Because the original concentration of Pb^{2+} is given to two significant figure, this is a more reasonable error. Our final solution, to two significant figures, is

$$[\text{Pb}^{2+}] = 1.2 \times 10^{-4} \text{ M and } [\text{IO}_3] = 4.5 \times 10^{-5} \text{ M}$$

and the molar solubility of $\text{Pb}(\text{IO}_3)_2$ is $2.2 \times 10^{-5} \text{ mol/L}$. This iterative approach to solving the problems is known as the **method of successive approximations**.

? Exercise 4.1.2

Calculate the molar solubility for Hg_2Cl_2 in 0.10 M NaCl and compare your answer to its molar solubility in deionized water (see Exercise 4.1.1).

Answer

We begin by setting up a table to help us keep track of the concentrations Hg_2^{2+} and Cl^- as this system moves toward and reaches equilibrium.

concentration	$\text{Hg}_2\text{Cl}_2 (\text{s})$	$\rightleftharpoons$	$\text{Hg}_2^{2+} (\text{aq})$	+	$\text{Cl}^- (\text{aq})$
initial	solid		0		0.10
change	solid		+x		+2x
equilibrium	solid		x		0.1 + 2x

Substituting the equilibrium concentrations into the K_{sp} expression for Hg_2Cl_2 leaves us with a difficult to solve cubic equation.

$$K_{\text{sp}} = [\text{Hg}_2^{2+}] [\text{Cl}^-]^2 = (x)(0.10 + 2x)^2 = 4x^3 + 0.40x^2 + 0.010x$$

Let's make an assumption to simplify this problem. Because we expect the value of x to be small, let's assume that

$$[\text{Cl}^-] = 0.10 + 2x \approx 0.10$$

This simplifies our problem to

$$K_{\text{sp}} = [\text{Hg}_2^{2+}] [\text{Cl}^-]^2 = (x)(0.10)^2 = 0.010x = 1.2 \times 10^{-18}$$

which gives the value of x as 1.2×10^{-16} M. The difference between the actual concentration of Cl^- , which is $(0.10 + 2x)$ M, and our assumption that it is 0.10 M introduces an error of 2.4×10^{-13} %. This is a negligible error. The molar solubility of Hg_2Cl_2 is the same as the concentration of Hg_2^{2+} , or 1.2×10^{-16} M. As expected, the molar solubility in 0.10 M NaCl is less than 6.7×10^{-7} mol/L, which is its solubility in water (see solution to [Exercise 4.1.1](#)).

A Systematic Approach to Solving Equilibrium Problems

Calculating the solubility of $\text{Pb}(\text{IO}_3)_2$ in a solution of $\text{Pb}(\text{NO}_3)_2$ is more complicated than calculating its solubility in deionized water. The calculation, however, is still relatively easy to organize and the simplifying assumptions are fairly obvious. This problem is reasonably straightforward because it involves only one equilibrium reaction and one equilibrium constant.

Determining the equilibrium composition of a system with multiple equilibrium reactions is more complicated. In this section we introduce a systematic approach to setting-up and solving equilibrium problems. As shown in Table 4.1.1, this approach involves four steps.

Table 4.1.1 . Systematic Approach to Solving Equilibrium Problems

Step 1
Write all relevant equilibrium reactions and equilibrium constant expressions.
Step 2
Count the unique species that appear in the equilibrium constant expressions; these are your unknowns. You have enough information to solve the problem if the number of unknowns equals the number of equilibrium constant expressions. If not, add a mass balance equation and/or a charge balance equation. Continue adding equations until the number of equations equals the number of unknowns.
Step 3
Combine your equations and solve for one unknown. Whenever possible, simplify the algebra by making appropriate assumptions. If you make an assumption, set a limit for its error. This decision influences your evaluation of the assumption.
Step 4
Check your assumptions. If any assumption proves invalid, return to the previous step and continue solving. The problem is complete when you have an answer that does not violate any of your assumptions.

In addition to equilibrium constant expressions, two other equations are important to this systematic approach to solving an equilibrium problem. The first of these equations is a **mass balance equation**, which simply is a statement that matter is conserved during a chemical reaction. In a solution of acetic acid, for example, the combined concentrations of the conjugate weak acid, CH_3COOH , and the conjugate weak base, CH_3COO^- , must equal acetic acid's initial concentration, $C_{\text{CH}_3\text{COOH}}$.

$$C_{\text{CH}_3\text{COOH}} = [\text{CH}_3\text{COOH}] + [\text{CH}_3\text{COO}^-]$$

You may recall from [Chapter 2](#) that this is the difference between a formal concentration and a molar concentration. The variable C represents a formal concentration.

The second equation is a **charge balance equation**, which requires that the total positive charge from the cations equal the total negative charge from the anions. Mathematically, the charge balance equation is

$$\sum_{i=1}^n (z^+) _i [C^{z^+}] _i = - \sum_{j=1}^m (z^-) _j [A^{z^-}] _j$$

where $[C^{z^+}] _i$ and $[A^{z^-}] _j$ are, respectively, the concentrations of the i^{th} cation and the j^{th} anion, and $(z^+) _i$ and $(z^-) _j$ are the charges for the i^{th} cation and the j^{th} anion. Every ion in solution, even if it does not appear in an equilibrium reaction, must appear in the charge balance equation. For example, the charge balance equation for an aqueous solution of $\text{Ca}(\text{NO}_3)_2$ is

$$2 \times [\text{Ca}^{2+}] + [\text{H}_3\text{O}^+] = [\text{OH}^-] + [\text{NO}_3^-]$$

Note that we multiply the concentration of Ca^{2+} by two and that we include the concentrations of H_3O^+ and OH^- .

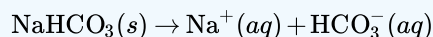
A charge balance is a conservation of a charge. The minus sign in front of the summation term on the right side of the charge balance equation ensures that both summations are positive. There are situations where it is impossible to write a charge balance equation because we do not have enough information about the solution's composition. For example, suppose we fix a solution's pH using a buffer. If the buffer's composition is not specified, then we cannot write a charge balance equation.

✓ Example 4.1.2

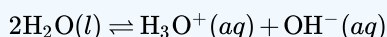
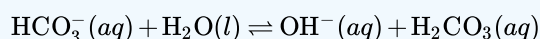
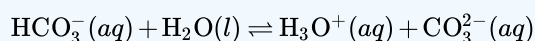
Write mass balance equations and a charge balance equation for a 0.10 M solution of NaHCO_3 .

Solution

It is easier to keep track of the species in solution if we write down the reactions that define the solution's composition. These reactions are the dissolution of a soluble salt



and the acid–base dissociation reactions of HCO_3^- and H_2O



The mass balance equations are

$$0.10\text{M} = [\text{H}_2\text{CO}_3] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]$$

$$0.10\text{ M} = [\text{Na}^+]$$

and the charge balance equation is

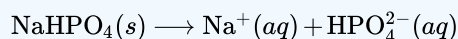
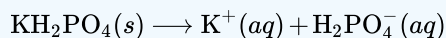
$$[\text{Na}^+] + [\text{H}_3\text{O}^+] = [\text{OH}^-] + [\text{HCO}_3^-] + 2 \times [\text{CO}_3^{2-}]$$

? Exercise 4.1.3

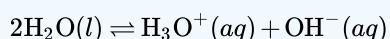
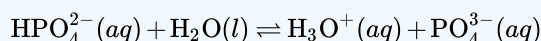
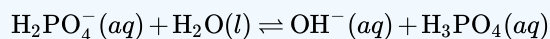
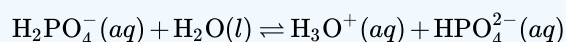
Write appropriate mass balance and charge balance equations for a solution containing 0.10 M KH_2PO_4 and 0.050 M Na_2HPO_4 .

Answer

To help us determine what ions are in solution, let's write down all the reaction needed to prepare the solutions and the equilibrium reactions that take place within these solutions. These reactions are the dissolution of two soluble salts



and the acid–base dissociation reactions for H_2PO_4^- , HPO_4^{2-} , and H_2O .



Note that we did not include the base dissociation reaction for HPO_4^{2-} because we already accounted for its product, H_2PO_4^- , in another reaction. The mass balance equations for K^+ and Na^+ are straightforward

$$[\text{K}^+] = 0.10\text{ M and } [\text{Na}^+] = 0.10\text{ M}$$

but the mass balance equation for phosphate takes a bit more thought. Both H_2PO_4^- and HPO_4^{2-} produce the same ions in solution. We can, therefore, imagine that the solution initially contains 0.15 M KH_2PO_4 , which gives the following mass balance equation.

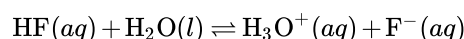
$$[\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}] = 0.15 \text{ M}$$

The charge balance equation is

$$[\text{H}_3\text{O}^+] + [\text{K}^+] + [\text{Na}^+] = [\text{H}_2\text{PO}_4^-] + 2 \times [\text{HPO}_4^{2-}] + 3 \times [\text{PO}_4^{3-}] + [\text{OH}^-]$$

pH of a Monoprotic Weak Acid

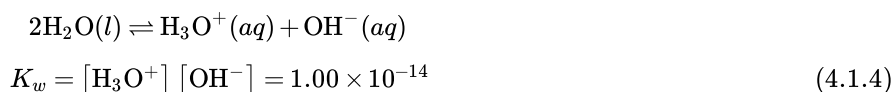
To illustrate the systematic approach to solving equilibrium problems, let's calculate the pH of 1.0 M HF. Two equilibrium reactions affect the pH. The first, and most obvious, is the acid dissociation reaction for HF



for which the equilibrium constant expression is

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{F}^-]}{[\text{HF}]} = 6.8 \times 10^{-4} \quad (4.1.3)$$

The second equilibrium reaction is the dissociation of water, which is an obvious yet easily neglected reaction



Counting unknowns, we find four: $[\text{HF}]$, $[\text{F}^-]$, $[\text{H}_3\text{O}^+]$, and $[\text{OH}^-]$. To solve this problem we need two additional equations. These equations are a mass balance equation on hydrofluoric acid

$$C_{\text{HF}} = [\text{HF}] + [\text{F}^-] = 1.0 \text{ M} \quad (4.1.5)$$

and a charge balance equation

$$[\text{H}_3\text{O}^+] = [\text{OH}^-] + [\text{F}^-] \quad (4.1.6)$$

With four equations and four unknowns, we are ready to solve the problem. Before doing so, let's simplify the algebra by making two assumptions.

Assumption One. Because HF is a weak acid, we know that the solution is acidic. For an acidic solution it is reasonable to assume that

$$[\text{H}_3\text{O}^+] \gg [\text{OH}^-]$$

which simplifies the charge balance equation to

$$C_{\text{HF}} = [\text{HF}] = 1.0 \text{ M} \quad (4.1.7)$$

Assumption Two. Because HF is a weak acid, very little of it dissociates to form F^- . Most of the HF remains in its conjugate weak acid form and it is reasonable to assume that

$$[\text{HF}] \gg [\text{F}^-]$$

which simplifies the mass balance equation to

$$C_{\text{HF}} = [\text{HF}] = 1.0 \text{ M} \quad (4.1.8)$$

For this exercise let's accept an assumption if it introduces an error of less than $\pm 5\%$.

Substituting Equation 4.1.7 and Equation 4.1.8 into Equation 4.1.3, and solving for the concentration of H_3O^+ gives us

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{F}^-]}{[\text{HF}]} = \frac{[\text{H}_3\text{O}^+][\text{H}_3\text{O}^+]}{C_{\text{HF}}} = \frac{[\text{H}_3\text{O}^+]^2}{C_{\text{HF}}} = 6.8 \times 10^{-4}$$

$$[\text{H}_3\text{O}^+] = \sqrt{K_a C_{\text{HF}}} = \sqrt{(6.8 \times 10^{-4})(1.0)} = 2.6 \times 10^{-2}$$

Before accepting this answer, we must verify our assumptions. The first assumption is that $[\text{OH}^-]$ is significantly smaller than $[\text{H}_3\text{O}^+]$. Using Equation 4.1.4, we find that

$$[\text{OH}^-] = \frac{K_w}{[\text{H}_3\text{O}^+]} = \frac{1.00 \times 10^{-14}}{2.6 \times 10^{-2}} = 3.8 \times 10^{-13}$$

Clearly this assumption is acceptable. The second assumption is that $[\text{F}^-]$ is significantly smaller than $[\text{HF}]$. From Equation 4.1.7 we have

$$[\text{F}^-] = 2.6 \times 10^{-2} \text{ M}$$

Because $[\text{F}^-]$ is 2.60% of C_{HF} , this assumption also is acceptable. Given that $[\text{H}_3\text{O}^+]$ is $2.6 \times 10^{-2} \text{ M}$, the pH of 1.0 M HF is 1.59.

How does the calculation change if we require that the error introduced in our assumptions be less than $\pm 1\%$? In this case we no longer can assume that $[\text{HF}] \gg [\text{F}^-]$ and we cannot simplify the mass balance equation. Solving the mass balance equation for $[\text{HF}]$

$$[\text{HF}] = C_{\text{HF}} - [\text{F}^-] = C_{\text{HF}} - [\text{H}_3\text{O}^+]$$

and substituting into the K_a expression along with Equation 4.1.7 gives

$$K_a = \frac{[\text{H}_3\text{O}^+]^2}{C_{\text{HF}} - [\text{H}_3\text{O}^+]}$$

Rearranging this equation leaves us with a quadratic equation

$$[\text{H}_3\text{O}^+]^2 + K_a [\text{H}_3\text{O}^+] - K_a C_{\text{HF}} = 0$$

which we solve using the quadratic formula

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where a , b , and c are the coefficients in the quadratic equation

$$ax^2 + bx + c = 0$$

Solving a quadratic equation gives two roots, only one of which has chemical significance. For our problem, the equation's roots are

$$\begin{aligned} x &= \frac{-6.8 \times 10^{-4} \pm \sqrt{(6.8 \times 10^{-4})^2 - (4)(1)(-6.8 \times 10^{-4})}}{(2)(1)} \\ x &= \frac{-6.8 \times 10^{-4} \pm 5.22 \times 10^{-2}}{2} \\ x &= 2.57 \times 10^{-2} \text{ or } -2.64 \times 10^{-2} \end{aligned}$$

Only the positive root is chemically significant because the negative root gives a negative concentration for H_3O^+ . Thus, $[\text{H}_3\text{O}^+]$ is $2.57 \times 10^{-2} \text{ M}$ and the pH is 1.59.

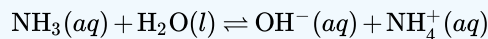
You can extend this approach to calculating the pH of a monoprotic weak base by replacing K_a with K_b , replacing C_{HF} with the weak base's concentration, and solving for $[\text{OH}^-]$ in place of $[\text{H}_3\text{O}^+]$.

? Exercise 4.1.4

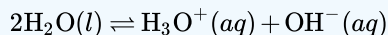
Calculate the pH of 0.050 M NH_3 . State any assumptions you make in solving the problem, limiting the error for any assumption to $\pm 5\%$. The K_b value for NH_3 is 1.75×10^{-5} .

Answer

To determine the pH of 0.050 M NH_3 , we need to consider two equilibrium reactions: the base dissociation reaction for NH_3



and water's dissociation reaction.



These two reactions contain four species whose concentrations we need to consider: NH_3 , NH_4^+ , H_3O^+ , and OH^- . We need four equations to solve the problem—these equations are the K_b equation for NH_3

$$K_b = \frac{[\text{NH}_4^+][\text{OH}^-]}{[\text{NH}_3]} = 1.75 \times 10^{-5}$$

the K_w equation for H_2O

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$$

a mass balance equation on ammonia

$$C_{\text{NH}_3} = 0.050 \text{ M} = [\text{NH}_3] + [\text{NH}_4^+]$$

and a charge balance equation

$$[\text{H}_3\text{O}^+] + [\text{NH}_4^+] = [\text{OH}^-]$$

To solve this problem, we will make two assumptions. Because NH_3 is a base, our first assumption is

$$[\text{OH}^-] \gg [\text{H}_3\text{O}^+]$$

which simplifies the charge balance equation to

$$[\text{NH}_4^+] = [\text{OH}^-]$$

Because NH_3 is a weak base, our second assumption is

$$[\text{NH}_3] \gg [\text{NH}_4^+]$$

which simplifies the mass balance equation to

$$C_{\text{NH}_3} = 0.050 \text{ M} = [\text{NH}_3]$$

Substituting the simplified charge balance equation and mass balance equation into the K_b equation leave us with

$$K_b = \frac{[\text{NH}_4^+][\text{OH}^-]}{[\text{NH}_3]} = \frac{[\text{OH}^-][\text{OH}^-]}{C_{\text{NH}_3}} = \frac{[\text{OH}^-]^2}{C_{\text{NH}_3}} = 1.75 \times 10^{-5}$$

$$[\text{OH}^-] = \sqrt{K_b C_{\text{NH}_3}} = \sqrt{(1.75 \times 10^{-5})(0.050)} = 9.35 \times 10^{-4}$$

Before we accept this answer, we must verify our two assumptions. The first assumption is that the concentration of OH^- is significantly greater than the concentration of H_3O^+ . Using K_w , we find that

$$[\text{H}_3\text{O}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.00 \times 10^{-14}}{9.35 \times 10^{-4}} = 1.07 \times 10^{-11}$$

Clearly this assumption is acceptable. Our second assumption is that the concentration of NH_3 is significantly greater than the concentration of NH_4^+ . Using our simplified charge balance equation, we find that

$$[\text{NH}_4^+] = [\text{OH}^-] = 9.35 \times 10^{-4}$$

Because the concentration of NH_4^+ is 1.9% of C_{NH_3} , our second assumption also is reasonable. Given that $[\text{H}_3\text{O}^+]$ is 1.07×10^{-11} , the pH is 10.97.

pH of a Polyprotic Acid or Base

A more challenging problem is to find the pH of a solution that contains a polyprotic weak acid or one of its conjugate species. As an example, consider the amino acid alanine, whose structure is shown in Figure 4.1.1. The ladder diagram in Figure 4.1.2 shows alanine's three acid-base forms and their respective areas of predominance. For simplicity, we identify these species as H_2L^+ , HL , and L^- .

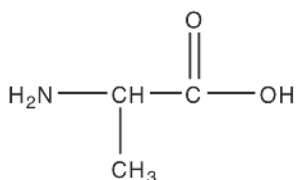


Figure 4.1.1. Structure of the amino acid alanine, which has pK_a values of 2.348 ($-\text{COOH}$) and 9.867 ($-\text{NH}_2$).

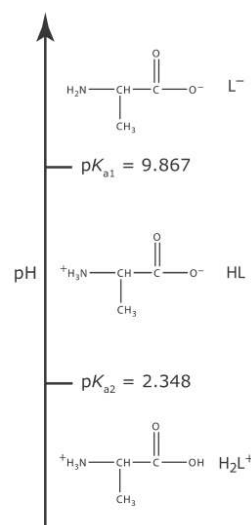


Figure 4.1.2. Ladder diagram for alanine.

pH of 0.10 M Alanine Hydrochloride (H_2L^+)

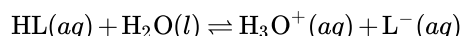
Alanine hydrochloride is the salt of the diprotic weak acid H_2L^+ and Cl^- . Because H_2L^+ has two acid dissociation reactions, a complete systematic solution to this problem is more complicated than that for a monoprotic weak acid. The ladder diagram in Figure 4.1.2 helps us simplify the problem. Because the areas of predominance for H_2L^+ and L^- are so far apart, we can assume that a solution of H_2L^+ will not contain a significant amount of L^- . As a result, we can treat H_2L^+ as though it is a monoprotic weak acid. Calculating the pH of 0.10 M alanine hydrochloride, which is 1.72, is left to the reader as an exercise.

pH of 0.10 M Sodium Alaninate (L^-)

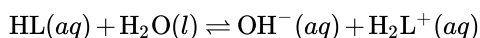
The alaninate ion is a diprotic weak base. Because L^- has two base dissociation reactions, a complete systematic solution to this problem is more complicated than that for a monoprotic weak base. Once again, the ladder diagram in Figure 4.1.2 helps us simplify the problem. Because the areas of predominance for H_2L^+ and L^- are so far apart, we can assume that a solution of L^- will not contain a significant amount of H_2L^+ . As a result, we can treat L^- as though it is a monoprotic weak base. Calculating the pH of 0.10 M sodium alaninate, which is 11.42, is left to the reader as an exercise.

pH of 0.10 M Alanine (HL)

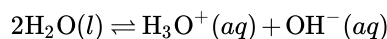
Finding the pH of a solution of alanine is more complicated than our previous two examples because we cannot ignore the presence of either H_2L^+ or L^- . To calculate the solution's pH we must consider alanine's acid dissociation reaction



and its base dissociation reaction



and, as always, we must also consider the dissociation of water



This leaves us with five unknowns— $[\text{H}_2\text{L}^+]$, $[\text{HL}]$, $[\text{L}^-]$, $[\text{H}_3\text{O}^+]$, and $[\text{OH}^-]$ —for which we need five equations. These equations are K_{a2} and K_{b2} for alanine

$$K_{a2} = \frac{[\text{H}_3\text{O}^+][\text{L}^-]}{[\text{HL}]}$$

$$K_{b2} = \frac{K_w}{K_{a1}} = \frac{[\text{OH}^-][\text{H}_2\text{L}^+]}{[\text{HL}]}$$

the K_w equation

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$$

a mass balance equation for alanine

$$C_{\text{HL}} = [\text{H}_2\text{L}^+] + [\text{HL}] + [\text{L}^-]$$

and a charge balance equation

$$[\text{H}_2\text{L}^+] + [\text{H}_3\text{O}^+] = [\text{OH}^-] + [\text{L}^-]$$

Because HL is a weak acid and a weak base, it seems reasonable to assume that little of it will dissociate and that

$$[\text{HL}] \gg [\text{H}_2\text{L}^+] + [\text{L}^-]$$

which allows us to simplify the mass balance equation to

$$C_{\text{HL}} = [\text{HL}]$$

Next we solve K_{b2} for $[\text{H}_2\text{L}^+]$

$$[\text{H}_2\text{L}^+] = \frac{K_w[\text{HL}]}{K_{a1}[\text{OH}^-]} = \frac{[\text{H}_3\text{O}^+][\text{HL}]}{K_{a1}} = \frac{C_{\text{HL}}[\text{H}_3\text{O}^+]}{K_{a1}}$$

and solve K_{a2} for $[\text{L}^-]$

$$[\text{L}^-] = \frac{K_{a2}[\text{HL}]}{[\text{H}_3\text{O}^+]} = \frac{K_{a2}C_{\text{HL}}}{[\text{H}_3\text{O}^+]}$$

Substituting these equations for $[\text{H}_2\text{L}^+]$ and $[\text{L}^-]$, and the equation for K_w , into the charge balance equation give us

$$\frac{C_{\text{HL}}[\text{H}_3\text{O}^+]}{K_{a1}} + [\text{H}_3\text{O}^+] = \frac{K_w}{[\text{H}_3\text{O}^+]} + \frac{K_{a2}C_{\text{HL}}}{[\text{H}_3\text{O}^+]}$$

which we simplify to

$$\begin{aligned} [\text{H}_3\text{O}^+] \left(\frac{C_{\text{HL}}}{K_{a1}} + 1 \right) &= \frac{1}{[\text{H}_3\text{O}^+]} (K_w + K_{a2}C_{\text{HL}}) \\ [\text{H}_3\text{O}^+]^2 &= \frac{(K_{a2}C_{\text{HL}} + K_w)}{\frac{C_{\text{HL}}}{K_{a1}} + 1} = \frac{K_{a1}(K_{a2}C_{\text{HL}} + K_w)}{C_{\text{HL}} + K_{a1}} \\ [\text{H}_3\text{O}^+] &= \sqrt{\frac{(K_{a1}K_{a2}C_{\text{HL}} + K_{a1}K_w)}{C_{\text{HL}} + K_{a1}}} \end{aligned}$$

We can further simplify this equation if $K_{a1}K_w \ll K_{a1}K_{a2}C_{\text{HL}}$, and if $K_{a1} \ll C_{\text{HL}}$, leaving us with

$$[\text{H}_3\text{O}^+] = \sqrt{K_{a1}K_{a2}}$$

For a solution of 0.10 M alanine the $[\text{H}_3\text{O}^+]$ is

$$[\text{H}_3\text{O}^+] = \sqrt{(4.487 \times 10^{-3})(1.358 \times 10^{-10})} = 7.806 \times 10^{-7} \text{ M}$$

or a pH of 6.11.

? Exercise 4.1.5

Verify that each assumption in our solution for the pH of 0.10 M alanine is reasonable, using $\pm 5\%$ as the limit for the acceptable error.

Answer

In solving for the pH of 0.10 M alanine, we made the following three assumptions: (a) $[\text{HL}] \gg [\text{H}_2\text{L}^+] + [\text{L}^-]$; (b) $K_{a1}K_w \ll K_{a1}K_{a2}C_{\text{HL}}$; and (c) $K_{a1} \ll C_{\text{HL}}$. Assumptions (b) and (c) are easy to check. The value of K_{a1} (4.487×10^{-3}) is 4.5% of C_{HL} (0.10), and $K_{a1}K_w$ (4.487×10^{-17}) is 0.074% of $K_{a1}K_{a2}C_{\text{HL}}$ (6.093×10^{-14}). Each of these assumptions introduces an error of less than $\pm 5\%$.

To test assumption (a) we need to calculate the concentrations of H_2L^+ and L^- , which we accomplish using the equations for K_{a1} and K_{a2} .

$$[\text{H}_2\text{L}^+] = \frac{[\text{H}_3\text{O}^+][\text{HL}]}{K_{a1}} = \frac{(7.807 \times 10^{-7})(0.10)}{4.487 \times 10^{-3}} = 1.74 \times 10^{-5}$$

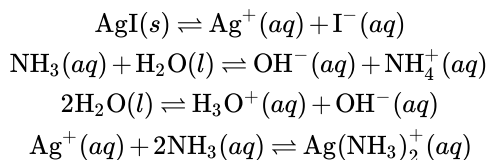
$$[\text{L}^-] = \frac{K_{a2}[\text{HL}]}{[\text{H}_3\text{O}^+]} = \frac{(1.358 \times 10^{-10})(0.10)}{7.807 \times 10^{-7}} = 1.74 \times 10^{-5}$$

Because these concentrations are less than $\pm 5\%$ of C_{HL} , the first assumption also is acceptable.

Effect of Complexation on Solubility

One method for increasing a precipitate's solubility is to add a ligand that forms soluble complexes with one of the precipitate's ions. For example, the solubility of AgI increases in the presence of NH_3 due to the formation of the soluble $\text{Ag}(\text{NH}_3)_2^+$ complex. As a final illustration of the systematic approach to solving equilibrium problems, let's calculate the molar solubility of AgI in 0.10 M NH_3 .

We begin by writing the relevant equilibrium reactions, which includes the solubility of AgI, the acid-base chemistry of NH_3 and H_2O , and the metal-ligand complexation chemistry between Ag^+ and NH_3 .



This leaves us with seven unknowns— $[\text{Ag}^+]$, $[\text{I}^-]$, $[\text{NH}_3]$, $[\text{NH}_4^+]$, $[\text{OH}^-]$, $[\text{H}_3\text{O}^+]$, and $[\text{Ag}(\text{NH}_3)_2^+]$ —and a need for seven equations. Four of the equations we need to solve this problem are the equilibrium constant expressions

$$K_{\text{sp}} = [\text{Ag}^+][\text{I}^-] = 8.3 \times 10^{-17} \quad (4.1.9)$$

$$K_{\text{b}} = \frac{[\text{NH}_4^+][\text{OH}^-]}{[\text{NH}_3]} = 1.75 \times 10^{-5} \quad (4.1.10)$$

$$K_{\text{w}} = [\text{H}_3\text{O}^+][\text{OH}^-] = 1.00 \times 10^{-14} \quad (4.1.11)$$

$$\beta_2 = \frac{[\text{Ag}(\text{NH}_3)_2^+]}{[\text{Ag}^+][\text{NH}_3]^2} = 1.7 \times 10^7 \quad (4.1.12)$$

We still need three additional equations. The first of these equations is a mass balance for NH_3 .

$$C_{\text{NH}_3} = [\text{NH}_3] + [\text{NH}_4^+] + 2 \times [\text{Ag}(\text{NH}_3)_2^+] \quad (4.1.13)$$

In writing this mass balance equation we multiply the concentration of $\text{Ag}(\text{NH}_3)_2^+$ by two since there are two moles of NH_3 per mole of $\text{Ag}(\text{NH}_3)_2^+$. The second additional equation is a mass balance between iodide and silver. Because AgI is the only source of I^- and Ag^+ , each iodide in solution must have an associated silver ion, which may be Ag^+ or $\text{Ag}(\text{NH}_3)_2^+$; thus

$$[\text{I}^-] = [\text{Ag}^+] + [\text{Ag}(\text{NH}_3)_2^+] \quad (4.1.14)$$

Finally, we include a charge balance equation.

$$[\text{Ag}^+] + [\text{Ag}(\text{NH}_3)_2^+] + [\text{NH}_4^+] + [\text{H}_3\text{O}^+] = [\text{OH}^-] + [\text{I}^-] \quad (4.1.15)$$

Although the problem looks challenging, three assumptions greatly simplify the algebra.

Assumption One. Because the formation of the $\text{Ag}(\text{NH}_3)_2^+$ complex is so favorable (β_2 is 1.7×10^7), there is very little free Ag^+ in solution and it is reasonable to assume that

$$[\text{Ag}^+] \ll [\text{Ag}(\text{NH}_3)_2^+]$$

Assumption Two. Because NH_3 is a weak base we may reasonably assume that most uncomplexed ammonia remains as NH_3 ; thus

$$[\text{NH}_4^+] \ll [\text{NH}_3]$$

Assumption Three. Because K_{sp} for AgI is significantly smaller than β_2 for $\text{Ag}(\text{NH}_3)_2^+$, the solubility of AgI probably is small enough that very little ammonia is needed to form the metal–ligand complex; thus

$$[\text{Ag}(\text{NH}_3)_2^+] \ll [\text{NH}_3]$$

As we use these assumptions to simplify the algebra, let's set $\pm 5\%$ as the limit for error.

Assumption two and assumption three suggest that the concentration of NH_3 is much larger than the concentrations of either NH_4^+ or $\text{Ag}(\text{NH}_3)_2^+$, which allows us to simplify the mass balance equation for NH_3 to

$$C_{\text{NH}_3} = [\text{NH}_3] \quad (4.1.16)$$

Finally, using assumption one, which suggests that the concentration of $\text{Ag}(\text{NH}_3)_2^+$ is much larger than the concentration of Ag^+ , we simplify the mass balance equation for I^- to

$$[\text{I}^-] = [\text{Ag}(\text{NH}_3)_2^+] \quad (4.1.17)$$

Now we are ready to combine equations and to solve the problem. We begin by solving Equation 4.1.9 for $[\text{Ag}^+]$ and substitute it into β_2 (Equation 4.1.12), which leaves us with

$$\beta_2 = \frac{[\text{Ag}(\text{NH}_3)_2^+] [\text{I}^-]}{K_{\text{sp}} [\text{NH}_3]^2} \quad (4.1.18)$$

Next we substitute Equation 4.1.16 and Equation 4.1.17 into Equation 4.1.18 obtaining

$$\beta_2 = \frac{[\text{I}^-]^2}{K_{\text{sp}} (C_{\text{NH}_3})^2} \quad (4.1.19)$$

Solving Equation 4.1.19 for $[\text{I}^-]$ gives

$$[\text{I}^-] = C_{\text{NH}_3} \sqrt{\beta_2 K_{\text{sp}}} = (0.10) \sqrt{(1.7 \times 10^7) (8.3 \times 10^{-17})} = 3.76 \times 10^{-6} \text{ M}$$

Because one mole of AgI produces one mole of I^- , the molar solubility of AgI is the same as the $[\text{I}^-]$, or $3.8 \times 10^{-6} \text{ mol/L}$.

Before we accept this answer we need to check our assumptions. Substituting $[\text{I}^-]$ into Equation 4.1.9, we find that the concentration of Ag^+ is

$$[\text{Ag}^+] = \frac{K_{\text{sp}}}{[\text{I}^-]} = \frac{8.3 \times 10^{-17}}{3.76 \times 10^{-6}} = 2.2 \times 10^{-11} \text{ M}$$

Substituting the concentrations of I^- and Ag^+ into the mass balance equation for iodide (Equation 4.1.14), gives the concentration of $\text{Ag}(\text{NH}_3)_2^+$ as

$$[\text{Ag}(\text{NH}_3)_2^+] = [\text{I}^-] - [\text{Ag}^+] = 3.76 \times 10^{-6} - 2.2 \times 10^{-11} = 3.76 \times 10^{-6} \text{ M}$$

Our first assumption that $[\text{Ag}^+]$ is significantly smaller than the $[\text{Ag}(\text{NH}_3)_2^+]$ is reasonable.

Substituting the concentrations of Ag^+ and $\text{Ag}(\text{NH}_3)_2^+$ into Equation 4.1.12 and solving for $[\text{NH}_3]$, gives

$$[\text{NH}_3] = \sqrt{\frac{[\text{Ag}(\text{NH}_3)_2^+]}{[\text{Ag}^+] \beta_2}} = \sqrt{\frac{3.76 \times 10^{-6}}{(2.2 \times 10^{-11})(1.7 \times 10^7)}} = 0.10 \text{ M}$$

From the mass balance equation for NH_3 (Equation 4.1.12) we see that $[\text{NH}_4^+]$ is negligible, verifying our second assumption that $[\text{NH}_4^+]$ is significantly smaller than $[\text{NH}_3]$. Our third assumption that $[\text{Ag}(\text{NH}_3)_2^+]$ is significantly smaller than $[\text{NH}_3]$ also is reasonable.

Did you notice that our solution to this problem did not make use of Equation 4.1.15, the charge balance equation? The reason for this is that we did not try to solve for the concentration of all seven species. If we need to know the reaction mixture's complete composition at equilibrium, then we will need to incorporate the charge balance equation into our solution.

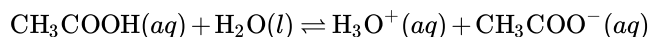
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4.2: Buffer Solutions

Adding as little as 0.1 mL of concentrated HCl to a liter of H₂O shifts the pH from 7.0 to 3.0. Adding the same amount of HCl to a liter of a solution that 0.1 M in acetic acid and 0.1 M in sodium acetate, however, results in a negligible change in pH. Why do these two solutions respond so differently to the addition of HCl?

A mixture of acetic acid and sodium acetate is one example of an acid–base **buffer**. To understand how this buffer works to limit the change in pH, we need to consider its acid dissociation reaction



and its corresponding acid dissociation constant

$$K_a = \frac{[\text{CH}_3\text{COO}^-][\text{H}_3\text{O}^+]}{[\text{CH}_3\text{COOH}]} = 1.75 \times 10^{-5} \quad (4.2.1)$$

Taking the negative log of the terms in Equation 4.2.1 and solving for pH leaves us with the result shown here.

$$\begin{aligned} \text{pH} &= \text{p}K_a + \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} \\ \text{pH} &= 4.76 + \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} \end{aligned} \quad (4.2.2)$$

You may recall that we developed these same equations in [Chapter 6.6](#) when we introduced ladder diagrams.

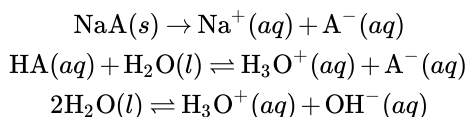
Buffering occurs because of the logarithmic relationship between pH and the concentration ratio of acetate and acetic acid. Here is an example to illustrate this point. If the concentrations of acetic acid and acetate are equal, the buffer's pH is 4.76. If we convert 10% of the acetate to acetic acid, by adding a strong acid, the ratio $[\text{CH}_3\text{COO}^-]/[\text{CH}_3\text{COOH}]$ changes from 1.00 to 0.818, and the pH decreases from 4.76 to 4.67—a decrease of only 0.09 pH units.

The ratio $[\text{CH}_3\text{COO}^-]/[\text{CH}_3\text{COOH}]$ becomes $0.9/1.1 = 0.818$ and the pH becomes

$$\text{pH} = 4.76 + \log(0.818) = 4.67$$

Systematic Solution to Buffer Problems

Equation 4.2.2 is written in terms of the equilibrium concentrations of CH₃COOH and of CH₃COO[−]. A more useful relationship relates a buffer's pH to the initial concentrations of the weak acid and the weak base. We can derive a general buffer equation by considering the following reactions for a weak acid, HA, and the soluble salt of its conjugate weak base, NaA.



Because the concentrations of Na⁺, A[−], HA, H₃O⁺, and OH[−] are unknown, we need five equations to define the solution's composition. Two of these equations are the equilibrium constant expressions for HA and H₂O.

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}]} \quad (4.2.3)$$

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$$

The remaining three equations are mass balance equations for HA and Na⁺

$$C_{\text{HA}} + C_{\text{NaA}} = [\text{HA}] + [\text{A}^-] \quad (4.2.4)$$

$$C_{\text{NaA}} = [\text{Na}^+] \quad (4.2.5)$$

and a charge balance equation

$$[\text{H}_3\text{O}^+] + [\text{Na}^+] = [\text{OH}^-] + [\text{A}^-] \quad (4.2.6)$$

Substituting Equation 4.2.5 into Equation 4.2.6 and solving for $[\text{A}^-]$ gives

$$[\text{A}^-] = C_{\text{NaA}} - [\text{OH}^-] + [\text{H}_3\text{O}^+] \quad (4.2.7)$$

Next, we substitute Equation 4.2.7 into Equation 4.2.4, which gives the concentration of HA as

$$[\text{HA}] = C_{\text{HA}} + [\text{OH}^-] - [\text{H}_3\text{O}^+] \quad (4.2.8)$$

Finally, we substitute Equation 4.2.7 and Equation 4.2.8 into Equation 4.2.3 and solve for pH to arrive at a general equation for a buffer's pH.

$$\text{pH} = \text{p}K_{\text{a}} + \log \frac{C_{\text{NaA}} - [\text{OH}^-] + [\text{H}_3\text{O}^+]}{C_{\text{HA}} + [\text{OH}^-] - [\text{H}_3\text{O}^+]}$$

If the initial concentrations of the weak acid, C_{HA} , and the weak base, C_{NaA} , are significantly greater than $[\text{H}_3\text{O}^+]$ and $[\text{OH}^-]$, then we can simplify the general equation to the **Henderson–Hasselbalch equation**.

$$\text{pH} = \text{p}K_{\text{a}} + \log \frac{C_{\text{NaA}}}{C_{\text{HA}}} \quad (4.2.9)$$

As outlined below, the Henderson–Hasselbalch equation provides a simple way to calculate the pH of a buffer, and to determine the change in pH upon adding a strong acid or strong base.

Lawrence Henderson (1878-1942) first developed a relationship between $[\text{H}_3\text{O}^+]$, $[\text{HA}]$, and $[\text{A}^-]$ while studying the buffering of blood. Kurt Hasselbalch (1874-1962) modified Henderson's equation by transforming it to the logarithmic form shown in Equation 4.2.9. The assumptions that lead to Equation 4.2.9 result in a minimal error in pH ($<\pm 5\%$) for larger concentrations of HA and A^- , for concentrations of HA and A^- that are similar in magnitude, and for weak acid's with $\text{p}K_{\text{a}}$ values closer to 7. For most problems in this textbook, Equation 4.2.9 provides acceptable results. Be sure, however, to test your assumptions. For a discussion of the Henderson–Hasselbalch equation, including the error inherent in Equation 4.2.9, see Po, H. N.; Senozan, N. M. "The Henderson–Hasselbalch Equation: Its History and Limitations," *J. Chem. Educ.* **2001**, 78, 1499–1503.

✓ Example 6.8.1

Calculate the pH of a buffer that is 0.020 M in NH_3 and 0.030 M in NH_4Cl . What is the pH after we add 1.0 mL of 0.10 M NaOH to 0.10 L of this buffer?

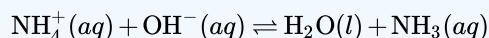
Solution

The acid dissociation constant for NH_4^+ is 5.70×10^{-10} , which is a $\text{p}K_{\text{a}}$ of 9.24. Substituting the initial concentrations of NH_3 and NH_4Cl into Equation 4.2.9 and solving, we find that the buffer's pH is

$$\text{pH} = 9.24 + \log \frac{0.020}{0.030} = 9.06$$

With a pH of 9.06, the concentration of H_3O^+ is 8.71×10^{-10} and the concentration of OH^- is 1.15×10^{-5} . Because both of these concentrations are much smaller than either C_{NH_3} or $C_{\text{NH}_4\text{Cl}}$, the approximations used to derive Equation 4.2.9 are reasonable.

Adding NaOH converts a portion of the NH_4^+ to NH_3 following reaction



Because this reaction's equilibrium constant is so large (it is equal to $(K_{\text{b}})^{-1}$ or 5.7×10^4), we may treat the reaction as if it goes to completion. The new concentrations of NH_4^+ and NH_3 are

$$C_{\text{NH}_4^+} = \frac{\text{mol NH}_4^+ - \text{mol OH}^-}{V_{\text{total}}}$$

$$C_{\text{NH}_4^+} = \frac{(0.030 \text{ M})(0.10 \text{ L}) - (0.10 \text{ M})(1.0 \times 10^{-3} \text{ L})}{0.10 \text{ L} + 1.0 \times 10^{-3} \text{ L}} = 0.029 \text{ M}$$

$$C_{\text{NH}_3} = \frac{\text{mol NH}_3 + \text{mol OH}^-}{V_{\text{total}}}$$

$$C_{\text{NH}_3} = \frac{(0.020 \text{ M})(0.10 \text{ L}) + (0.10 \text{ M})(1.0 \times 10^{-3} \text{ L})}{0.10 \text{ L} + 1.0 \times 10^{-3} \text{ L}} = 0.021 \text{ M}$$

Substituting these concentrations into the equation 6.60 gives a pH of

$$\text{pH} = 9.24 + \log \frac{0.021}{0.029} = 9.10$$

Note that adding NaOH increases the pH from 9.06 to 9.10. As we expect, adding a base makes the pH more basic. Checking to see that the pH changes in the right direction is one way to catch a calculation error.

? Exercise 6.8.1

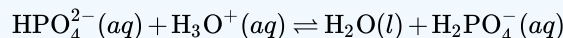
Calculate the pH of a buffer that is 0.10 M in KH_2PO_4 and 0.050 M in Na_2HPO_4 . What is the pH after we add 5.0 mL of 0.20 M HCl to 0.10 L of this buffer. Use [Appendix 11](#) to find the appropriate K_a value.

Answer

The acid dissociation constant for H_2PO_4^- is 6.32×10^{-8} , or a $\text{p}K_a$ of 7.199. Substituting the initial concentrations of H_2PO_4^- and HPO_4^{2-} into Equation 4.2.9 and solving gives the buffer's pH as

$$\text{pH} = 7.199 + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = 7.199 + \log \frac{0.050}{0.10} = 6.898 \approx 6.90$$

Adding HCl converts a portion of HPO_4^{2-} to H_2PO_4^- as a result of the following reaction



Because this reaction's equilibrium constant is so large (it is 1.59×10^7), we may treat the reaction as if it goes to completion. The new concentrations of H_2PO_4^- and HPO_4^{2-} are

$$C_{\text{H}_2\text{PO}_4^-} = \frac{\text{mol H}_2\text{PO}_4^- + \text{mol HCl}}{V_{\text{total}}}$$

$$C_{\text{H}_2\text{PO}_4^-} = \frac{(0.10 \text{ M})(0.10 \text{ L}) + (0.20 \text{ M})(5.0 \times 10^{-3} \text{ L})}{0.10 \text{ L} + 5.0 \times 10^{-3} \text{ L}} = 0.105 \text{ M}$$

$$C_{\text{HPO}_4^{2-}} = \frac{\text{mol HPO}_4^{2-} - \text{mol HCl}}{V_{\text{total}}}$$

$$C_{\text{HPO}_4^{2-}} = \frac{(0.05 \text{ M})(0.10 \text{ L}) - (0.20 \text{ M})(5.0 \times 10^{-3} \text{ L})}{0.10 \text{ L} + 5.0 \times 10^{-3} \text{ L}} = 0.0381 \text{ M}$$

Substituting these concentrations into Equation 4.2.9 gives a pH of

$$\text{pH} = 7.199 + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = 7.199 + \log \frac{0.0381}{0.105} = 6.759 \approx 6.76$$

As we expect, adding HCl decreases the buffer's pH by a small amount, dropping from 6.90 to 6.76.

We can use a multiprotic weak acid to prepare buffers at as many different pH's as there are acidic protons, with the Henderson-Hasselbalch equation applying in each case. For example, for malonic acid ($\text{p}K_{a1} = 2.85$ and $\text{p}K_{a2} = 5.70$) we can prepare buffers with pH values of

$$\text{pH} = 2.85 + \log \frac{C_{\text{HM}^-}}{C_{\text{H}_2\text{M}}}$$

$$\text{pH} = 5.70 + \log \frac{C_{\text{M}^{2-}}}{C_{\text{HM}^-}}$$

where H_2M , HM^- and M^{2-} are malonic acid's different acid–base forms.

Although our treatment of buffers is based on acid–base chemistry, we can extend buffers to equilibria that involve complexation or redox reactions. For example, the Nernst equation for a solution that contains Fe^{2+} and Fe^{3+} is similar in form to the Henderson–Hasselbalch equation.

$$E = E_{\text{Fe}^{3+}/\text{Fe}^{2+}}^\circ - 0.05916 \log \frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]}$$

A solution that contains similar concentrations of Fe^{2+} and Fe^{3+} is buffered to a potential near the standard state reduction potential for Fe^{3+} . We call such solutions redox buffers. Adding a strong oxidizing agent or a strong reducing agent to a redox buffer results in a small change in potential.

Representing Buffer Solutions with Ladder Diagrams

A ladder diagram provides a simple way to visualize a solution's predominate species as a function of solution conditions. It also provides a convenient way to show the range of solution conditions over which a buffer is effective. For example, an acid–base buffer exists when the concentrations of the weak acid and its conjugate weak base are similar. For convenience, let's assume that an acid–base buffer exists when

$$\frac{1}{10} \leq \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} \leq \frac{10}{1}$$

Substituting these ratios into the Henderson–Hasselbalch equation

$$\text{pH} = \text{p}K_{\text{a}} + \log \frac{1}{10} = \text{p}K_{\text{a}} - 1$$

$$\text{pH} = \text{p}K_{\text{a}} + \log \frac{10}{1} = \text{p}K_{\text{a}} + 1$$

shows that an acid–base buffer works over a pH range of $\text{p}K_{\text{a}} \pm 1$.

Using the same approach, it is easy to show that a metal–ligand complexation buffer for ML_n exists when

$$\text{pL} = \log K_n \pm 1 \text{ or } \text{pL} = \log \beta_n \pm \frac{1}{n}$$

where K_n or β_n is the relevant stepwise or overall formation constant. For an oxidizing agent and its conjugate reducing agent, a redox buffer exists when

$$E = E^\circ \pm \frac{1}{n} \times \frac{RT}{F} = E^\circ \pm \frac{0.05916}{n} \text{ (at } 25^\circ\text{C)}$$

Figure 6.8.1 shows ladder diagrams with buffer regions for several equilibrium systems.

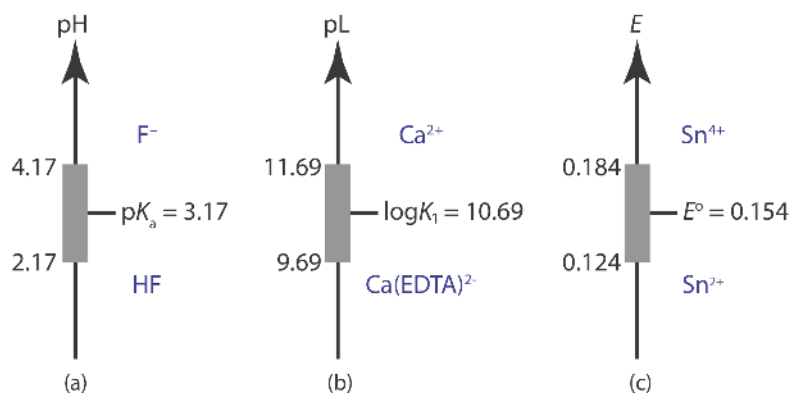


Figure 6.8.1 . Ladder diagrams showing buffer regions shaded in grey for (a) an acid–base buffer of HF and F^- ; (b) a metal–ligand complexation buffer of Ca^{2+} and $Ca(EDTA)^{2-}$; and (c) an oxidation–reduction (redox) buffer of Sn^{4+} and Sn^{2+} .

Preparing a Buffer

Buffer capacity is the ability of a buffer to resist a change in pH when we add to it a strong acid or a strong base. A buffer's capacity to resist a change in pH is a function of the concentrations of the weak acid and the weak base, as well as their relative proportions. The importance of the weak acid's concentration and the weak base's concentration is obvious. The more moles of weak acid and weak base a buffer has, the more strong base or strong acid it can neutralize without a significant change in its pH.

Although a higher concentration of buffering agents provides greater buffer capacity, there are reasons for using smaller concentrations, including the formation of unwanted precipitates and the tolerance of biological systems for high concentrations of dissolved salts.

The relative proportions of a weak acid and a weak base also affects how much the pH changes when we add a strong acid or a strong base. A buffer that is equimolar in weak acid and weak base requires a greater amount of strong acid or strong base to bring about a one unit change in pH. Consequently, a buffer is most effective against the addition of strong acids or strong bases when its pH is near the weak acid's pK_a value.

Buffer solutions are often prepared using standard “recipes” found in the chemical literature [see, for example, (a) Bower, V. E.; Bates, R. G. *J. Res. Natl. Bur. Stand. (U. S.)* **1955**, 55, 197– 200; (b) Bates, R. G. *Ann. N. Y. Acad. Sci.* **1961**, 92, 341–356; (c) Bates, R. G. *Determination of pH*, 2nd ed.; Wiley-Interscience: New York, 1973]. In addition, there are computer programs and on-line calculators to aid in preparing buffers [(a) Lambert, W. J. *J. Chem. Educ.* 1990, 67, 150–153; (b) <http://www.bioinformatics.org/JaMBW/5/4/index.html>]. Perhaps the simplest way to make a buffer, however, is to prepare a solution that contains an appropriate conjugate weak acid and weak base, measure its pH, and then adjust the pH to the desired value by adding small portions of either a strong acid or a strong base.

A good “rule of thumb” when choosing a buffer is to select one whose reagents have a pK_a value close to your desired pH.

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4.3: Activity Effects

Careful measurements on the metal–ligand complex $\text{Fe}(\text{SCN})^{2+}$ suggest its stability decreases in the presence of inert ions [Lister, M. W.; Rivington, D. E. *Can. J. Chem.* **1995**, 33, 1572–1590]. We can demonstrate this by adding an inert salt to an equilibrium mixture of Fe^{3+} and SCN^- . Figure 6.9.1 a shows the result of mixing together equal volumes of 1.0 mM FeCl_3 and 1.5 mM KSCN , both of which are colorless. The solution's reddish–orange color is due to the formation of $\text{Fe}(\text{SCN})^{2+}$.

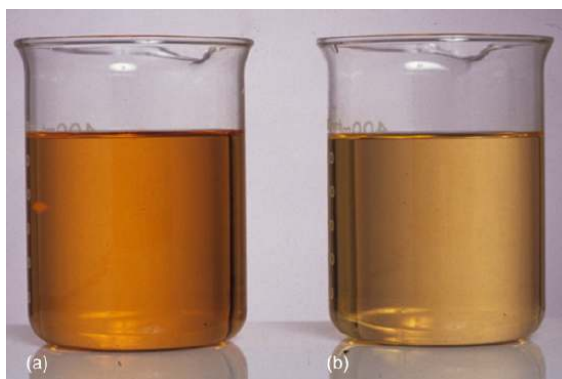
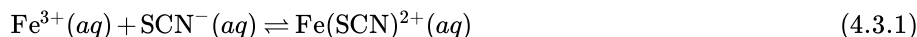


Figure 6.9.1 . The effect of a inert salt on a reaction's equilibrium position is shown by the solutions in these two beakers. The beaker on the left contains equal volumes of 1.0 mM FeCl_3 and 1.5 mM KSCN . The solution's color is due to the formation of the metal–ligand complex $\text{Fe}(\text{SCN})^{2+}$. Adding 10 g of KNO_3 to the beaker on the left produces the result shown on the right. The lighter color suggests that there is less $\text{Fe}(\text{SCN})^{2+}$ as a result of the equilibrium in reaction 4.3.1 shifting to the left.

Adding 10 g of KNO_3 to the solution and stirring to dissolve the solid, produces the result shown in Figure 6.9.1 b. The solution's lighter color suggests that adding KNO_3 shifts reaction 4.3.1 to the left, decreasing the concentration of $\text{Fe}(\text{SCN})^{2+}$ and increasing the concentrations of Fe^{3+} and SCN^- . The result is a decrease in the complex's formation constant, K_1 .

$$K_1 = \frac{[\text{Fe}(\text{SCN})^{2+}]}{[\text{Fe}^{3+}][\text{SCN}^-]} \quad (4.3.2)$$

Why should adding an inert electrolyte affect a reaction's equilibrium position? We can explain the effect of KNO_3 on the formation of $\text{Fe}(\text{SCN})^{2+}$ if we consider the reaction on a microscopic scale. The solution in Figure 6.9.1 b contains a variety of cations and anions: Fe^{3+} , SCN^- , K^+ , NO_3^- , H_3O^+ , and OH^- . Although the solution is homogeneous, on average, there are slightly more anions in regions near the Fe^{3+} ions, and slightly more cations in regions near the SCN^- ions. As shown in Figure 6.9.2, each Fe^{3+} ion and each SCN^- ion is surrounded by an ionic atmosphere of opposite charge (δ^- and δ^+) that partially screen the ions from each other. Because each ion's apparent charge at the edge of its ionic atmosphere is less than its actual charge, the force of attraction between the two ions is smaller. As a result, the formation of $\text{Fe}(\text{SCN})^{2+}$ is slightly less favorable and the formation constant in Equation 4.3.2 is slightly smaller. Higher concentrations of KNO_3 increase δ^- and δ^+ , resulting in even smaller values for the formation constant.

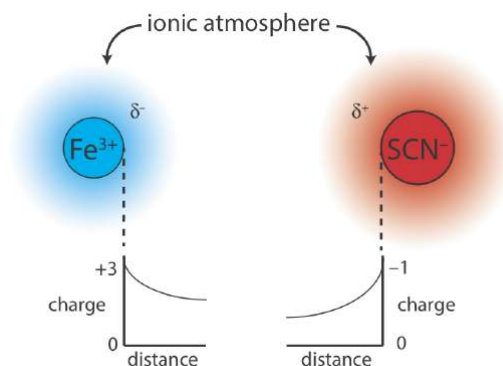


Figure 6.9.2 . Ions of Fe^{3+} and SCN^- are surrounded by ionic atmospheres with net charges of δ^- and δ^+ . Because of these ionic atmospheres, each ion's apparent charge at the edge of its ionic atmosphere is less than the ion's actual charge.

Ionic Strength

To factor the concentration of ions into the formation constant for $\text{Fe}(\text{SCN})^{2+}$, we need a way to express that concentration in a meaningful way. Because both an ion's concentration and its charge are important, we define the solution's **ionic strength**, μ as

$$\mu = \frac{1}{2} \sum_{i=1}^n c_i z_i^2$$

where c_i and z_i are the concentration and charge of the i^{th} ion.

✓ Example 6.9.1

Calculate the ionic strength of a solution of 0.10 M NaCl. Repeat the calculation for a solution of 0.10 M Na_2SO_4 .

Solution

The ionic strength for 0.10 M NaCl is

$$\begin{aligned}\mu &= \frac{1}{2} \{ [\text{Na}^+] \times (+1)^2 + [\text{Cl}^-] \times (-1)^2 \} \\ \mu &= \frac{1}{2} \{ (0.10) \times (+1)^2 + (0.10) \times (-1)^2 \} = 0.10 \text{ M}\end{aligned}$$

For 0.10 M Na_2SO_4 the ionic strength is

$$\begin{aligned}\mu &= \frac{1}{2} \{ [\text{Na}^+] \times (+1)^2 + [\text{SO}_4^{2-}] \times (-2)^2 \} \\ \mu &= \frac{1}{2} \{ (0.20) \times (+1)^2 + (0.10) \times (-2)^2 \} = 0.30 \text{ M}\end{aligned}$$

In calculating the ionic strengths of these solutions we are ignoring the presence of H_3O^+ and OH^- , and, in the case of Na_2SO_4 , the presence of HSO_4^- from the base dissociation reaction of SO_4^{2-} . In the case of 0.10 M NaCl, the concentrations of H_3O^+ and OH^- are 1.0×10^{-7} , which is significantly smaller than the concentrations of Na^+ and Cl^- . Because SO_4^{2-} is a very weak base ($K_b = 1.0 \times 10^{-12}$), the solution is only slightly basic (pH = 7.5), and the concentrations of H_3O^+ , OH^- , and HSO_4^- are negligible. Although we can ignore the presence of H_3O^+ , OH^- , and HSO_4^- when we calculate the ionic strength of these two solutions, be aware that an equilibrium reaction can generate ions that might affect the solution's ionic strength.

Note that the unit for ionic strength is molarity, but that a salt's ionic strength need not match its molar concentration. For a 1:1 salt, such as NaCl, ionic strength and molar concentration are identical. The ionic strength of a 2:1 electrolyte, such as Na_2SO_4 , is three times larger than the electrolyte's molar concentration.

Activity and Activity Coefficients

Figure 6.9.1 shows that adding KNO_3 to a mixture of Fe^{3+} and SCN^- decreases the formation constant for $\text{Fe}(\text{SCN})^{2+}$. This creates a contradiction. Earlier in this chapter we showed that there is a relationship between a reaction's standard-state free energy, ΔG° , and its equilibrium constant, K .

$$\Delta G^\circ = -RT \ln K$$

Because a reaction has only one standard-state, its equilibrium constant must be independent of solution conditions. Although ionic strength affects the *apparent* formation constant for $\text{Fe}(\text{SCN})^{2+}$, reaction 4.3.1 must have an underlying *thermodynamic* formation constant that is independent of ionic strength.

The apparent formation constant for $\text{Fe}(\text{SCN})^{2+}$, as shown in Equation 4.3.2, is a function of concentrations. In place of concentrations, we define the true thermodynamic equilibrium constant using activities. The activity of species A, a_A , is the product of its concentration, $[A]$, and a solution-dependent activity coefficient, γ_A

$$a_A = [A] \gamma_A$$

The true thermodynamic formation constant for $\text{Fe}(\text{SCN})^{2+}$, therefore, is

$$K_1 = \frac{a_{\text{Fe}(\text{SCN})^{2+}}}{a_{\text{Fe}^{3+}} \times a_{\text{SCN}^-}} = \frac{[\text{Fe}(\text{SCN})^{2+}] \gamma_{\text{Fe}(\text{SCN})^{2+}}}{[\text{Fe}^{3+}] \gamma_{\text{Fe}^{3+}} [\text{SCN}^-] \gamma_{\text{SCN}^-}}$$

Unless otherwise specified, the equilibrium constants in the appendices are thermodynamic equilibrium constants.

A species' **activity coefficient** corrects for any deviation between its physical concentration and its ideal value. For a gas, a pure solid, a pure liquid, or a non-ionic solute, the activity coefficient is approximately one under most reasonable experimental conditions.

For a gas the proper terms are fugacity and fugacity coefficient, instead of activity and activity coefficient.

For a reaction that involves only these species, the difference between activity and concentration is negligible. The activity coefficient for an ion, however, depends on the solution's ionic strength, the ion's charge, and the ion's size. It is possible to estimate activity coefficients using the extended **Debye-Hückel equation**

$$\log \gamma_A = \frac{-0.51 \times z_A^2 \times \sqrt{\mu}}{1 + 3.3 \times \alpha_A \times \sqrt{\mu}} \quad (4.3.3)$$

where z_A is the ion's charge, α_A is the hydrated ion's effective diameter in nanometers (Table 6.2), μ is the solution's ionic strength, and 0.51 and 3.3 are constants appropriate for an aqueous solution at 25°C. A hydrated ion's effective radius is the radius of the ion plus those water molecules closely bound to the ion. The effective radius is greater for smaller, more highly charged ions than it is for larger, less highly charged ions.

Table 6.9.1 . Effective Diameters (α) for Selected Ions

ion	effective diameter (nm)
H ₃ O ⁺	0.9
Li ⁺	0.6
Na ⁺ , IO ₃ ⁻ , HSO ₃ ⁻ , HCO ₃ ⁻ , H ₂ PO ₄ ⁻	0.45
OH ⁻ , F ⁻ , SCN ⁻ , HS ⁻ , ClO ₃ ⁻ , ClO ₄ ⁻ , MnO ₄ ⁻	0.35
K ⁺ , Cl ⁻ , Br ⁻ , I ⁻ , CN ⁻ , NO ₂ ⁻ , NO ₃ ⁻	0.3
Cs ⁺ , Tl ⁺ , Ag ⁺ , NH ₄ ⁺	0.25
Mg ²⁺ , Be ²⁺	0.8
Ca ²⁺ , Cu ²⁺ , Zn ²⁺ , Sn ²⁺ , Mn ²⁺ , Fe ²⁺ , Ni ²⁺ , Co ²⁺	0.6
Sr ²⁺ , Ba ²⁺ , Cd ²⁺ , Hg ²⁺ , S ²⁻	0.5
Pb ²⁺ , SO ₄ ²⁻ , SO ₃ ²⁻	0.45
Hg ₂ ²⁺ , SO ₄ ²⁻ , S ₂ O ₃ ²⁻ , CrO ₄ ²⁻ , HPO ₄ ²⁻	0.40
Al ³⁺ , Fe ³⁺ , Cr ³⁺	0.9
PO ₄ ³⁻ , Fe(CN) ₆ ³⁻	0.4
Zr ⁴⁺ , Ce ⁴⁺ , Sn ⁴⁺	1.1
Fe(CN) ₆ ⁴⁻	0.5

Source: Kielland, J. J. *Am. Chem. Soc.* **1937**, 59, 1675–1678.

Several features of Equation 4.3.3 deserve our attention. First, as the ionic strength approaches zero an ion's activity coefficient approaches a value of one. In a solution where $\mu = 0$, an ion's activity and its concentration are identical. We can take advantage of this fact to determine a reaction's thermodynamic equilibrium constant by measuring the apparent equilibrium constant for several increasingly smaller ionic strengths and extrapolating back to an ionic strength of zero. Second, an activity coefficient is smaller, and the effect of activity is more important, for an ion with a higher charge and a smaller effective radius. Finally, the extended Debye-Hückel equation provides a reasonable estimate of an ion's activity coefficient when the ionic strength is less than 0.1.

Modifications to Equation 4.3.3 extend the calculation of activity coefficients to higher ionic strengths [Davies, C. W. *Ion Association*, Butterworth: London, 1962].

Including Activity Coefficients When Solving Equilibrium Problems

Earlier in this chapter we calculated the solubility of $\text{Pb}(\text{IO}_3)_2$ in deionized water, obtaining a result of 4.0×10^{-5} mol/L. Because the only significant source of ions is from the solubility reaction, the ionic strength is very low and we can assume that $\gamma \approx 1$ for both Pb^{2+} and IO_3^- . In calculating the solubility of $\text{Pb}(\text{IO}_3)_2$ in deionized water, we do not need to account for ionic strength. But what if we need to know the solubility of $\text{Pb}(\text{IO}_3)_2$ in a solution that contains other, inert ions? In this case we need to include activity coefficients in our calculation.

✓ Example 6.9.2

Calculate the solubility of $\text{Pb}(\text{IO}_3)_2$ in a matrix of 0.020 M $\text{Mg}(\text{NO}_3)_2$.

Solution

We begin by calculating the solution's ionic strength. Since $\text{Pb}(\text{IO}_3)_2$ is only sparingly soluble, we will assume we can ignore its contribution to the ionic strength; thus

$$\mu = \frac{1}{2} \{ (0.020)(+2)^2 + (0.040)(-1)^2 \} = 0.060 \text{ M}$$

Next, we use Equation 4.3.3 to calculate the activity coefficients for Pb^{2+} and IO_3^- .

$$\log \gamma_{\text{Pb}^{2+}} = \frac{-0.51 \times (+2)^2 \times \sqrt{0.060}}{1 + 3.3 \times 0.45 \times \sqrt{0.060}} = -0.366$$

$$\gamma_{\text{Pb}^{2+}} = 0.431$$

$$\log \gamma_{\text{IO}_3^-} = \frac{-0.51 \times (-1)^2 \times \sqrt{0.060}}{1 + 3.3 \times 0.45 \times \sqrt{0.060}} = -0.0916$$

$$\gamma_{\text{IO}_3^-} = 0.810$$

Defining the equilibrium concentrations of Pb^{2+} and IO_3^- in terms of the variable x

Concentrations	$\text{Pb}(\text{IO}_3)_2$ (s)	$\rightleftharpoons$	Pb^{2+} (aq)	+	2IO_3^- (aq)
initial	solid		0		0
change	solid		+x		+2x
equilibrium	solid		x		2x

and substituting into the thermodynamic solubility product for $\text{Pb}(\text{IO}_3)_2$ leaves us with

$$K_{\text{sp}} = a_{\text{Pb}^{2+}} \times a_{\text{IO}_3^-}^2 = \gamma_{\text{Pb}^{2+}} [\text{Pb}^{2+}] \times \gamma_{\text{IO}_3^-}^2 [\text{IO}_3^-]^2 = 2.5 \times 10^{-13}$$

$$K_{\text{sp}} = (0.431)(x)(0.810)^2(2x)^2 = 2.5 \times 10^{-13}$$

$$K_{\text{sp}} = 1.131x^3 = 2.5 \times 10^{-13}$$

Solving for x gives 6.0×10^{-5} and a molar solubility of 6.0×10^{-5} mol/L for $\text{Pb}(\text{IO}_3)_2$. If we ignore activity, as we did in our earlier calculation, we report the molar solubility as 4.0×10^{-5} mol/L. Failing to account for activity in this case underestimates the molar solubility of $\text{Pb}(\text{IO}_3)_2$ by 33%.

The solution's equilibrium composition is

$$\begin{aligned}[\text{Pb}^{2+}] &= 6.0 \times 10^{-5} \text{ M} \\ [\text{IO}_3^-] &= 1.2 \times 10^{-4} \text{ M} \\ [\text{Mg}^{2+}] &= 0.020 \text{ M} \\ [\text{NO}_3^-] &= 0.040 \text{ M}\end{aligned}$$

Because the concentrations of both Pb^{2+} and IO_3^- are much smaller than the concentrations of Mg^{2+} and NO_3^- our decision to ignore the contribution of Pb^{2+} and IO_3^- to the ionic strength is reasonable.

How do we handle the calculation if we can not ignore the concentrations of Pb^{2+} and IO_3^- when calculating the ionic strength. One approach is to use the method of successive approximations. First, we recalculate the ionic strength using the concentrations of all ions, including Pb^{2+} and IO_3^- . Next, we recalculate the activity coefficients for Pb^{2+} and IO_3^- using this new ionic strength and then recalculate the molar solubility. We continue this cycle until two successive calculations yield the same molar solubility within an acceptable margin of error.

? Exercise 6.9.1

Calculate the molar solubility of Hg_2Cl_2 in 0.10 M NaCl, taking into account the effect of ionic strength. Compare your answer to that from [Exercise 6.7.2](#) in which you ignored the effect of ionic strength.

Answer

We begin by calculating the solution's ionic strength. Because NaCl is a 1:1 ionic salt, the ionic strength is the same as the concentration of NaCl; thus $\mu = 0.10 \text{ M}$. This assumes, of course, that we can ignore the contributions of Hg_2^{2+} and Cl^- from the solubility of Hg_2Cl_2 .

Next we use Equation 4.3.3 to calculate the activity coefficients for Hg_2^{2+} and Cl^- .

$$\begin{aligned}\log \gamma_{\text{Hg}_2^{2+}} &= \frac{-0.51 \times (+2)^2 \times \sqrt{0.10}}{1 + 3.3 \times 0.40 \times \sqrt{0.10}} = -0.455 \\ \gamma_{\text{Hg}_2^{2+}} &= 0.351 \\ \log \gamma_{\text{Cl}^-} &= \frac{-0.51 \times (-1)^2 \times \sqrt{0.10}}{1 + 3.3 \times 0.3 \times \sqrt{0.10}} = -0.12 \\ \gamma_{\text{Cl}^-} &= 0.75\end{aligned}$$

Defining the equilibrium concentrations of Hg_2^{2+} and Cl^- in terms of the variable x

concentrations	$\text{Hg}_2\text{Cl}_2 (\text{s})$	$\rightleftharpoons$	$\text{Hg}_2^{2+} (\text{aq})$	+	$2\text{Cl}^- (\text{aq})$
initial	solid		0		0.10
change	solid		+x		+2x
equilibrium	solid		x		0.10 + 2x

and substituting into the thermodynamic solubility product for Hg_2Cl_2 , leave us with

$$K_{\text{sp}} = a_{\text{Hg}_2^{2+}} (a_{\text{Cl}^-})^2 = \gamma_{\text{Hg}_2^{2+}} [\text{Hg}_2^{2+}] (\gamma_{\text{Cl}^-})^2 [\text{Cl}^-]^2 = 1.2 \times 10^{-18}$$

Because the value of x likely is small, let's simplify this equation to

$$(0.351)(x)(0.75)^2(0.1)^2 = 1.2 \times 10^{-18}$$

Solving for x gives its value as 6.1×10^{-16} . Because x is the concentration of Hg_2^{2+} and $2x$ is the concentration of Cl^- , our decision to ignore their contributions to the ionic strength is reasonable. The molar solubility of Hg_2Cl_2 in 0.10 M NaCl is

6.1×10^{-16} mol/L. In [Exercise 6.7.2](#), where we ignored ionic strength, we determined that the molar solubility of Hg_2Cl_2 is 1.2×10^{-16} mol/L, a result that is $5\times$ smaller than its actual value.

As Example 6.9.2 and Exercise 6.9.1 show, failing to correct for the effect of ionic strength can lead to a significant error in an equilibrium calculation. Nevertheless, it is not unusual to ignore activities and to assume that the equilibrium constant is expressed in terms of concentrations. There is a practical reason for this—in an analysis we rarely know the exact composition, much less the ionic strength of aqueous samples or of solid samples brought into solution. Equilibrium calculations are a useful guide when we develop an analytical method; however, it only is when we complete an analysis and evaluate the results that can we judge whether our theory matches reality. In the end, work in the laboratory is the most critical step in developing a reliable analytical method.

This is a good place to revisit the meaning of pH. In [Chapter 2](#) we defined pH as

$$\text{pH} = -\log [\text{H}_3\text{O}^+]$$

Now we see that the correct definition is

$$\begin{aligned}\text{pH} &= -\log a_{\text{H}_3\text{O}^+} \\ \text{pH} &= -\log \gamma_{\text{H}_3\text{O}^+} [\text{H}_3\text{O}^+]\end{aligned}$$

Failing to account for the effect of ionic strength can lead to a significant error in the reported concentration of H_3O^+ . For example, if the pH of a solution is 7.00 and the activity coefficient for H_3O^+ is 0.90, then the concentration of H_3O^+ is 1.11×10^{-7} M, not 1.00×10^{-7} M, an error of +11%. Fortunately, when we develop and carry out an analytical method, we are more interested in controlling pH than in calculating $[\text{H}_3\text{O}^+]$. As a result, the difference between the two definitions of pH rarely is of significant concern.

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CHAPTER OVERVIEW

5: Titrations and Working with Standards

5.1: Analytical Signals

5.2: Overview of Titrimetry

5.3: Acid–Base Titrations

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5.1: Analytical Signals

To standardize an analytical method we use standards that contain known amounts of analyte. The accuracy of a standardization, therefore, depends on the quality of the reagents and the glassware we use to prepare these standards. For example, in an acid–base titration the stoichiometry of the acid–base reaction defines the relationship between the moles of analyte and the moles of titrant. In turn, the moles of titrant is the product of the titrant’s concentration and the volume of titrant used to reach the equivalence point. The accuracy of a titrimetric analysis, therefore, is never better than the accuracy with which we know the titrant’s concentration.

See [Chapter 9](#) for a thorough discussion of titrimetric methods of analysis.

Primary and Secondary Standards

There are two categories of analytical standards: primary standards and secondary standards. A **primary standard** is a reagent that we can use to dispense an accurately known amount of analyte. For example, a 0.1250-g sample of $\text{K}_2\text{Cr}_2\text{O}_7$ contains 4.249×10^{-4} moles of $\text{K}_2\text{Cr}_2\text{O}_7$. If we place this sample in a 250-mL volumetric flask and dilute to volume, the concentration of $\text{K}_2\text{Cr}_2\text{O}_7$ in the resulting solution is 1.700×10^{-3} M. A primary standard must have a known stoichiometry, a known purity (or assay), and it must be stable during long-term storage. Because it is difficult to establishing accurately the degree of hydration, even after drying, a hydrated reagent usually is not a primary standard.

Reagents that do not meet these criteria are secondary standards. The concentration of a **secondary standard** is determined relative to a primary standard. Lists of acceptable primary standards are available (see, for instance, Smith, B. W.; Parsons, M. L. *J. Chem. Educ.* **1973**, 50, 679–681; or Moody, J. R.; Green- burg, P. R.; Pratt, K. W.; Rains, T. C. *Anal. Chem.* **1988**, 60, 1203A–1218A). [Appendix 8](#) provides examples of some common primary standards.

NaOH is one example of a secondary standard. Commercially available NaOH contains impurities of NaCl, Na_2CO_3 , and Na_2SO_4 , and readily absorbs H_2O from the atmosphere. To determine the concentration of NaOH in a solution, we titrate it against a primary standard weak acid, such as potassium hydrogen phthalate, $\text{KHC}_8\text{H}_4\text{O}_4$.

Other Reagents

Preparing a standard often requires additional reagents that are not primary standards or secondary standards, such as a suitable solvent or reagents needed to adjust the standard’s matrix. These solvents and reagents are potential sources of additional analyte, which, if not accounted for, produce a determinate error in the standardization. If available, reagent grade chemicals that conform to standards set by the American Chemical Society are used [Committee on Analytical Reagents, *Reagent Chemicals*, 8th ed., American Chemical Society: Washington, D. C., 1993]. The label on the bottle of a reagent grade chemical (Figure 5.1.1) lists either the limits for specific impurities or provides an assay for the impurities. We can improve the quality of a reagent grade chemical by purifying it, or by conducting a more accurate assay. As discussed later in the chapter, we can correct for contributions to S_{total} from reagents used in an analysis by including an appropriate blank determination in the analytical procedure.



Figure 5.1.1 : Two examples of packaging labels for reagent grade chemicals. The label on the bottle on the right provides the manufacturer's assay for the reagent, NaBr. Note that potassium is flagged with an asterisk (*) because its assay exceeds the limit established by the American Chemical Society (ACS). The label for the bottle on the left does not provide an assay for impurities; however it indicates that the reagent meets ACS specifications by providing the maximum limits for impurities. An assay for the reagent, NaHCO₃, is provided.

Preparing a Standard Solution

It often is necessary to prepare a series of standards, each with a different concentration of analyte. We can prepare these standards in two ways. If the range of concentrations is limited to one or two orders of magnitude, then each solution is best prepared by transferring a known mass or volume of the pure standard to a volumetric flask and diluting to volume.

When working with a larger range of concentrations, particularly a range that extends over more than three orders of magnitude, standards are best prepared by a serial dilution from a single stock solution. In a **serial dilution** we prepare the most concentrated standard and then dilute a portion of that solution to prepare the next most concentrated standard. Next, we dilute a portion of the second standard to prepare a third standard, continuing this process until we have prepared all of our standards. Serial dilutions must be prepared with extra care because an error in preparing one standard is passed on to all succeeding standards.

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5.2: Overview of Titrimetry

In **titrimetry** we add a reagent, called the **titrant**, to a solution that contains another reagent, called the **titrand**, and allow them to react. The type of reaction provides us with a simple way to divide titrimetry into four categories: acid–base titrations, in which an acidic or basic titrant reacts with a titrand that is a base or an acid; complexometric titrations, which are based on metal–ligand complexation; redox titrations, in which the titrant is an oxidizing or reducing agent; and precipitation titrations, in which the titrand and titrant form a precipitate.

We will deliberately avoid the term analyte at this point in our introduction to titrimetry. Although in most titrations the analyte is the titrand, there are circumstances where the analyte is the titrant. Later, when we discuss specific titrimetric methods, we will use the term analyte where appropriate.

Despite their difference in chemistry, all titrations share several common features. Before we consider individual titrimetric methods in greater detail, let's take a moment to consider some of these similarities. As you work through this chapter, this overview will help you focus on the similarities between different titrimetric methods. You will find it easier to understand a new analytical method when you can see its relationship to other similar methods.

Equivalence Points and End Points

If a titration is to give an accurate result we must combine the titrand and the titrant in stoichiometrically equivalent amounts. We call this stoichiometric mixture the **equivalence point**. Unlike precipitation gravimetry, where we add the precipitant in excess, an accurate titration requires that we know the exact volume of titrant at the equivalence point, V_{eq} . The product of the titrant's equivalence point volume and its molarity, M_T , is equal to the moles of titrant that react with the titrand.

$$\text{moles titrant} = M_T \times V_{eq}$$

If we know the stoichiometry of the titration reaction, then we can calculate the moles of titrand.

Unfortunately, for most titration reactions there is no obvious sign when we reach the equivalence point. Instead, we stop adding the titrant at an **end point** of our choosing. Often this end point is a change in the color of a substance, called an **indicator**, that we add to the titrand's solution. The difference between the end point's volume and the equivalence point's volume is a determinate **titration error**. If the end point and the equivalence point volumes coincide closely, then this error is insignificant and is safely ignored. Clearly, selecting an appropriate end point is of critical importance.

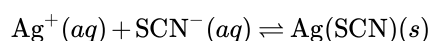
Volume as a Signal

Instead of measuring the titrant's volume, we may choose to measure its mass. Although generally we can measure mass more precisely than we can measure volume, the simplicity of a volumetric titration makes it the more popular choice.

Almost any chemical reaction can serve as a titrimetric method provided that it meets the following four conditions. The first condition is that we must know the stoichiometry between the titrant and the titrand. If this is not the case, then we cannot convert the moles of titrant used to reach the end point to the moles of titrand in our sample. Second, the titration reaction effectively must proceed to completion; that is, the stoichiometric mixing of the titrant and the titrand must result in their complete reaction. Third, the titration reaction must occur rapidly. If we add the titrant faster than it can react with the titrand, then the end point and the equivalence point will differ significantly. Finally, we must have a suitable method for accurately determining the end point. These are significant limitations and, for this reason, there are several common titration strategies.

Depending on how we are detecting the endpoint, we may stop the titration too early or too late. If the end point is a function of the titrant's concentration, then adding the titrant too quickly leads to an early end point. On the other hand, if the end point is a function of the titrand's concentration, then the end point exceeds the equivalence point.

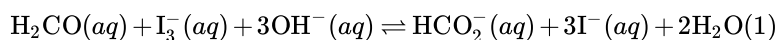
A simple example of a titration is an analysis for Ag^+ using thiocyanate, SCN^- , as a titrant.



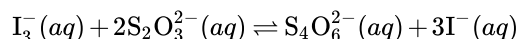
This reaction occurs quickly and with a known stoichiometry, which satisfies two of our requirements. To indicate the titration's end point, we add a small amount of Fe^{3+} to the analyte's solution before we begin the titration. When the reaction between Ag^+ and SCN^- is complete, formation of the red-colored $\text{Fe}(\text{SCN})^{2+}$ complex signals the end point. This is an example of a **direct titration** since the titrant reacts directly with the analyte.

This is an example of a precipitation titration. You will find more information about precipitation titrations [later](#) in this chapter.

If the titration's reaction is too slow, if a suitable indicator is not available, or if there is no useful direct titration reaction, then an indirect analysis may be possible. Suppose you wish to determine the concentration of formaldehyde, H_2CO , in an aqueous solution. The oxidation of H_2CO by I_3^-



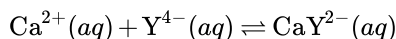
is a useful reaction, but it is too slow for a titration. If we add a known excess of I_3^- and allow its reaction with H_2CO to go to completion, we can titrate the unreacted I_3^- with thiosulfate, $\text{S}_2\text{O}_3^{2-}$.



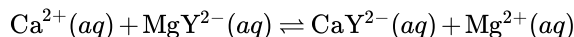
The difference between the initial amount of I_3^- and the amount in excess gives us the amount of I_3^- that reacts with the formaldehyde. This is an example of a **back titration**.

This is an example of a redox titration. You will find more information about redox titrations [later](#) in this chapter.

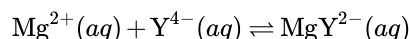
Calcium ions play an important role in many environmental systems. A direct analysis for Ca^{2+} might take advantage of its reaction with the ligand ethylenediaminetetraacetic acid (EDTA), which we represent here as Y^{4-} .



Unfortunately, for most samples this titration does not have a useful indicator. Instead, we react the Ca^{2+} with an excess of MgY^{2-}



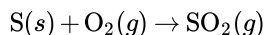
releasing an amount of Mg^{2+} equivalent to the amount of Ca^{2+} in the sample. Because the titration of Mg^{2+} with EDTA



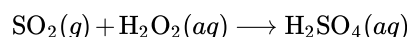
has a suitable end point, we can complete the analysis. The amount of EDTA used in the titration provides an indirect measure of the amount of Ca^{2+} in the original sample. Because the species we are titrating was displaced by the analyte, we call this a **displacement titration**.

MgY^{2-} is the Mg^{2+} -EDTA metal-ligand complex. You can prepare a solution of MgY^{2-} by combining equimolar solutions of Mg^{2+} and EDTA. This is an example of a complexation titration. You will find more information about complexation titrations [later](#) in this chapter.

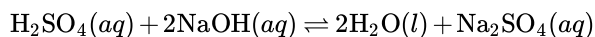
If a suitable reaction with the analyte does not exist it may be possible to generate a species that we can titrate. For example, we can determine the sulfur content of coal by using a combustion reaction to convert sulfur to sulfur dioxide



and then convert the SO_2 to sulfuric acid, H_2SO_4 , by bubbling it through an aqueous solution of hydrogen peroxide, H_2O_2 .



Titration of H_2SO_4 with NaOH



provides an indirect determination of sulfur.

This is an example of an acid–base titration. You will find more information about acid–base titrations [later](#) in this chapter.

Titration Curves

To find a titration's end point, we need to monitor some property of the reaction that has a well-defined value at the equivalence point. For example, the equivalence point for a titration of HCl with NaOH occurs at a pH of 7.0. A simple method for finding the equivalence point is to monitor the titration mixture's pH using a pH electrode, stopping the titration when we reach a pH of 7.0. Alternatively, we can add an indicator to the titrand's solution that changes color at a pH of 7.0.

Why a pH of 7.0 is the equivalence point for this titration is a topic we will cover later in the section on acid–base titrations.

Suppose the only available indicator changes color at a pH of 6.8. Is the difference between this end point and the equivalence point small enough that we safely can ignore the titration error? To answer this question we need to know how the pH changes during the titration.

A **titration curve** provides a visual picture of how a property of the titration reaction changes as we add the titrant to the titrand. The titration curve in Figure 9.1.1, for example, was obtained by suspending a pH electrode in a solution of 0.100 M HCl (the titrand) and monitoring the pH while adding 0.100 M NaOH (the titrant). A close examination of this titration curve should convince you that an end point pH of 6.8 produces a negligible titration error. Selecting a pH of 11.6 as the end point, however, produces an unacceptably large titration error.

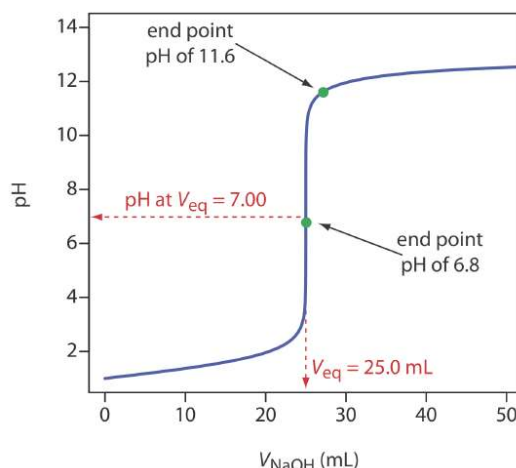


Figure 9.1.1 . Typical acid–base titration curve showing how the titrand's pH changes with the addition of titrant. The titrand is a 25.0 mL solution of 0.100 M HCl and the titrant is 0.100 M NaOH. The titration curve is the solid blue line, and the equivalence point volume (25.0 mL) and pH (7.00) are shown by the dashed red lines. The green dots show two end points. The end point at a pH of 6.8 has a small titration error, and the end point at a pH of 11.6 has a larger titration error.

For the titration curve in Figure 9.1.1, the volume of titrant to reach a pH of 6.8 is 24.99995 mL, a titration error of $-2.00 \times 10^{-4}\%$ relative to the equivalence point of 25.00 mL. Typically, we can read the volume only to the nearest ± 0.01 mL, which means this uncertainty is too small to affect our results. The volume of titrant to reach a pH of 11.6 is 27.07 mL, or a titration error of +8.28%. This is a significant error.

The shape of the titration curve in Figure 9.1.1 is not unique to an acid–base titration. Any titration curve that follows the change in concentration of a species in the titration reaction (plotted logarithmically) as a function of the titrant's volume has the same general sigmoidal shape. Several additional examples are shown in Figure 9.1.2.

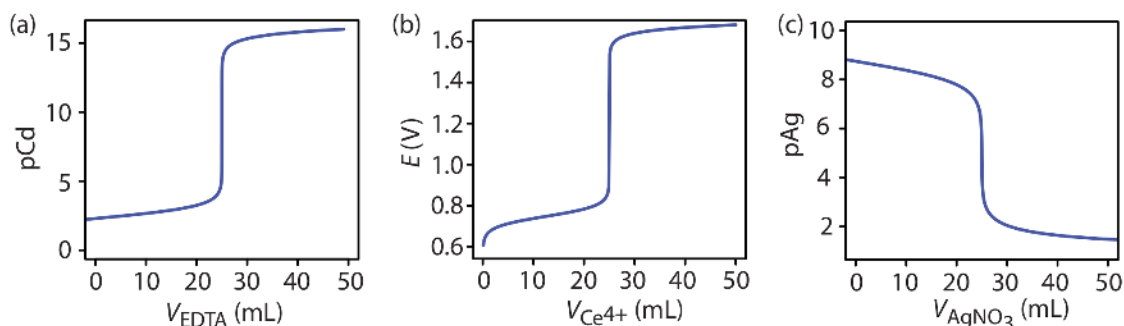


Figure 9.1.2 . Additional examples of titration curves. (a) Complexation titration of 25.0 mL of 1.0 mM Cd^{2+} with 1.0 mM EDTA at a pH of 10. The y-axis displays the titrand's equilibrium concentration as pCd. (b) Redox titration of 25.0 mL of 0.050 M Fe^{2+} with 0.050 M Ce^{4+} in 1 M HClO_4 . The y-axis displays the titration mixture's electrochemical potential, E , which, through the Nernst equation is a logarithmic function of concentrations. (c) Precipitation titration of 25.0 mL of 0.10 M NaCl with 0.10 M AgNO_3 . The y-axis displays the titrant's equilibrium concentration as pAg.

The titrand's or the titrant's concentration is not the only property we can use to record a titration curve. Other parameters, such as the temperature or absorbance of the titrand's solution, may provide a useful end point signal. Many acid–base titration reactions, for example, are exothermic. As the titrant and the titrand react, the temperature of the titrand's solution increases. Once we reach the equivalence point, further additions of titrant do not produce as exothermic a response. Figure 9.1.3 shows a typical **thermometric titration curve** where the intersection of the two linear segments indicates the equivalence point.

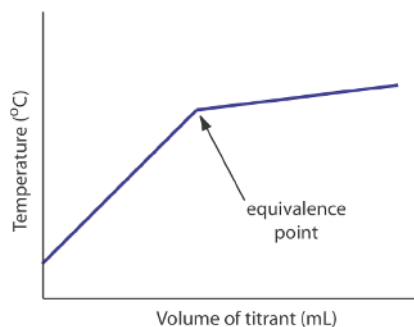


Figure 9.1.3 . Example of a thermometric titration curve showing the location of the equivalence point.

The Buret

The only essential equipment for an acid–base titration is a means for delivering the titrant to the titrand's solution. The most common method for delivering titrant is a **buret** (Figure 9.1.4), which is a long, narrow tube with graduated markings and equipped with a stopcock for dispensing the titrant. The buret's small internal diameter provides a better defined meniscus, making it easier to read precisely the titrant's volume. Burets are available in a variety of sizes and tolerances (Table 9.1.1), with the choice of buret determined by the needs of the analysis. You can improve a buret's accuracy by calibrating it over several intermediate ranges of volumes using the method described in Chapter 5 for calibrating pipets. Calibrating a buret corrects for variations in the buret's internal diameter.

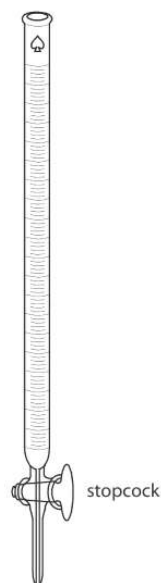


Figure 9.1.4 . A typical volumetric buret. The stopcock is shown here in the open position, which allows the titrant to flow into the titrand's solution. Rotating the stopcock controls the titrant's flow rate.

Table 9.1.1 . Specifications for Volumetric Burets

volume (mL)	class	subdivision (mL)	tolerance ($\pm$)
5	A	0.01	± 0.01
	B	0.01	± 0.01
10	A	0.02	± 0.02
	B	0.02	± 0.04
25	A	0.1	± 0.03
	B	0.1	± 0.06
50	A	0.1	± 0.05
	B	0.1	± 0.10
100	A	0.2	± 0.10
	B	0.2	± 0.20

An automated titration uses a pump to deliver the titrant at a constant flow rate (Figure 9.1.5). Automated titrations offer the additional advantage of using a microcomputer for data storage and analysis.

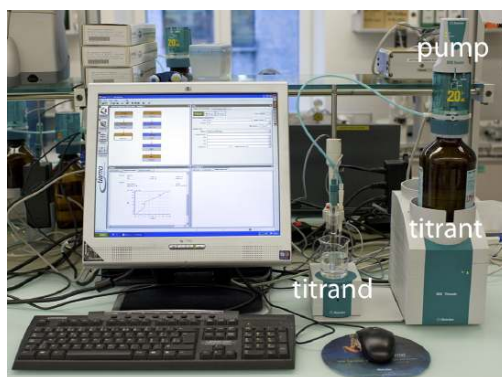


Figure 9.1.5 . Typical instrumentation for an automated acid–base titration showing the titrant, the pump, and the titrand. The pH electrode in the titrand’s solution is used to monitor the titration’s progress. You can see the titration curve in the lower-left quadrant of the computer’s display. Modified from: Datamax ([commons. Wikipedia.org](https://commons.wikimedia.org/wiki/File:Titration_setup.jpg)).

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5.3: Acid–Base Titrations

Before 1800, most **acid–base titrations** used H_2SO_4 , HCl , or HNO_3 as acidic titrants, and K_2CO_3 or Na_2CO_3 as basic titrants. A titration's end point was determined using litmus as an indicator, which is red in acidic solutions and blue in basic solutions, or by the cessation of CO_2 effervescence when neutralizing CO_3^{2-} . Early examples of acid–base titrimetry include determining the acidity or alkalinity of solutions, and determining the purity of carbonates and alkaline earth oxides.

The determination of acidity and alkalinity continue to be important applications of acid–base titrimetry. We will take a closer look at these applications later in this section.

Three limitations slowed the development of acid–base titrimetry: the lack of a strong base titrant for the analysis of weak acids, the lack of suitable indicators, and the absence of a theory of acid–base reactivity. The introduction, in 1846, of NaOH as a strong base titrant extended acid–base titrimetry to the determination of weak acids. The synthesis of organic dyes provided many new indicators. Phenolphthalein, for example, was first synthesized by Bayer in 1871 and used as an indicator for acid–base titrations in 1877.

Despite the increased availability of indicators, the absence of a theory of acid–base reactivity made it difficult to select an indicator. The development of equilibrium theory in the late 19th century led to significant improvements in the theoretical understanding of acid–base chemistry, and, in turn, of acid–base titrimetry. Sørensen's establishment of the pH scale in 1909 provided a rigorous means to compare indicators. The determination of acid–base dissociation constants made it possible to calculate a theoretical titration curve, as outlined by Bjerrum in 1914. For the first time analytical chemists had a rational method for selecting an indicator, making acid–base titrimetry a useful alternative to gravimetry.

Acid–Base Titration Curves

In the overview to this chapter we noted that a titration's end point should coincide with its equivalence point. To understand the relationship between an acid–base titration's end point and its equivalence point we must know how the titrand's pH changes during a titration. In this section we will learn how to calculate a titration curve using the equilibrium calculations from [Chapter 6](#). We also will learn how to sketch a good approximation of any acid–base titration curve using a limited number of simple calculations.

Titration Strong Acids and Strong Bases

For our first titration curve, let's consider the titration of 50.0 mL of 0.100 M HCl using a titrant of 0.200 M NaOH . When a strong base and a strong acid react the only reaction of importance is



Although we have not written reaction [5.3.1](#) as an equilibrium reaction, it is at equilibrium; however, because its equilibrium constant is large—it is $(K_w)^{-1}$ or 1.00×10^{14} —we can treat reaction [5.3.1](#) as though it goes to completion.

The first task is to calculate the volume of NaOH needed to reach the equivalence point, V_{eq} . At the equivalence point we know from reaction [5.3.1](#) that

$$\begin{aligned} \text{moles HCl} &= \text{moles NaOH} \\ M_a \times V_a &= M_b \times V_b \end{aligned}$$

where the subscript 'a' indicates the acid, HCl , and the subscript 'b' indicates the base, NaOH . The volume of NaOH needed to reach the equivalence point is

$$V_{eq} = V_b = \frac{M_a V_a}{M_b} = \frac{(0.100 \text{ M})(50.0 \text{ mL})}{(0.200 \text{ M})} = 25.0 \text{ mL}$$

Before the equivalence point, HCl is present in excess and the pH is determined by the concentration of unreacted HCl . At the start of the titration the solution is 0.100 M in HCl , which, because HCl is a strong acid, means the pH is

$$\text{pH} = -\log[\text{H}_3\text{O}^+] = -\log[\text{HCl}] = -\log(0.100) = 1.00$$

After adding 10.0 mL of NaOH the concentration of excess HCl is

$$[\text{HCl}] = \frac{(\text{mol HCl})_{\text{initial}} - (\text{mol NaOH})_{\text{added}}}{\text{total volume}} = \frac{M_a V_a - M_b V_b}{V_a + V_b}$$

$$[\text{HCl}] = \frac{(0.100 \text{ M})(50.0 \text{ mL}) - (0.200 \text{ M})(10.0 \text{ mL})}{50.0 \text{ mL} + 10.0 \text{ mL}} = 0.0500 \text{ M}$$

and the pH increases to 1.30.

At the equivalence point the moles of HCl and the moles of NaOH are equal. Since neither the acid nor the base is in excess, the pH is determined by the dissociation of water.

$$K_w = 1.00 \times 10^{-14} = [\text{H}_3\text{O}^+] [\text{OH}^-] = [\text{H}_3\text{O}^+]^2$$

$$[\text{H}_3\text{O}^+] = 1.00 \times 10^{-7}$$

Thus, the pH at the equivalence point is 7.00.

For volumes of NaOH greater than the equivalence point, the pH is determined by the concentration of excess OH^- . For example, after adding 30.0 mL of titrant the concentration of OH^- is

$$[\text{OH}^-] = \frac{(\text{mol NaOH})_{\text{added}} - (\text{mol HCl})_{\text{initial}}}{\text{total volume}} = \frac{M_b V_b - M_a V_a}{V_a + V_b}$$

$$[\text{OH}^-] = \frac{(0.200 \text{ M})(30.0 \text{ mL}) - (0.100 \text{ M})(50.0 \text{ mL})}{30.0 \text{ mL} + 50.0 \text{ mL}} = 0.0125 \text{ M}$$

To find the concentration of H_3O^+ we use the K_w expression

$$[\text{H}_3\text{O}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.00 \times 10^{-14}}{0.0125} = 8.00 \times 10^{-13} \text{ M}$$

to find that the pH is 12.10. Table 9.2.1 and Figure 9.2.1 show additional results for this titration curve. You can use this same approach to calculate the titration curve for the titration of a strong base with a strong acid, except the strong base is in excess before the equivalence point and the strong acid is in excess after the equivalence point.

Table 9.2.1 . Titration of 50.0 mL of 0.100 M HCl with 0.200 M NaOH

volume of NaOH (mL)	pH	volume of NaOH (mL)	pH
0.00	1.00	26.0	11.42
5.00	1.14	28.0	11.89
10.0	1.30	30.0	12.10
15.0	1.51	35.0	12.37
20.0	1.85	40.0	12.52
22.0	2.08	45.0	12.63
24.0	2.57	50.0	12.70
25.0	7.00		

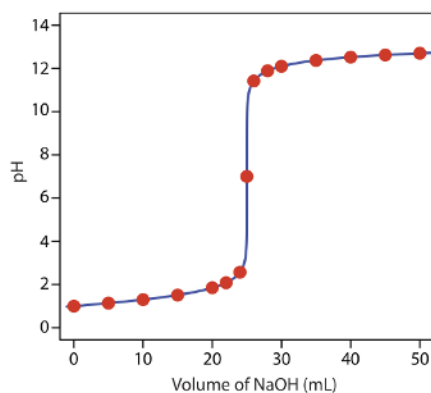


Figure 9.2.1 . Titration curve for the titration of 50.0 mL of 0.100 M HCl with 0.200 M NaOH. The red points correspond to the data in Table 9.2.1 . The blue line shows the complete titration curve.

? Exercise 9.2.1

Construct a titration curve for the titration of 25.0 mL of 0.125 M NaOH with 0.0625 M HCl.

Answer

The volume of HCl needed to reach the equivalence point is

$$V_{eq} = V_a = \frac{M_b V_b}{M_a} = \frac{(0.125 \text{ M})(25.0 \text{ mL})}{(0.0625 \text{ M})} = 50.0 \text{ mL}$$

Before the equivalence point, NaOH is present in excess and the pH is determined by the concentration of unreacted OH^- . For example, after adding 10.0 mL of HCl

$$[\text{OH}^-] = \frac{(0.125 \text{ M})(25.0 \text{ mL}) - (0.0625 \text{ M})(10.0 \text{ mL})}{25.0 \text{ mL} + 10.0 \text{ mL}} = 0.0714 \text{ M}$$

$$[\text{H}_3\text{O}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.00 \times 10^{-14}}{0.0714 \text{ M}} = 1.40 \times 10^{-13} \text{ M}$$

the pH is 12.85.

For the titration of a strong base with a strong acid the pH at the equivalence point is 7.00.

For volumes of HCl greater than the equivalence point, the pH is determined by the concentration of excess HCl. For example, after adding 70.0 mL of titrant the concentration of HCl is

$$[\text{HCl}] = \frac{(0.0625 \text{ M})(70.0 \text{ mL}) - (0.125 \text{ M})(25.0 \text{ mL})}{70.0 \text{ mL} + 25.0 \text{ mL}} = 0.0132 \text{ M}$$

giving a pH of 1.88. Some additional results are shown here.

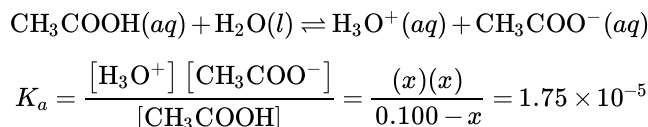
volume of HCl (mL)	pH	volume of HCl (mL)	pH
0	13.10	60	2.13
10	12.85	70	1.88
20	12.62	80	1.75
30	12.36	90	1.66
40	11.98	100	1.60
50	7.00		

Titration of a Weak Acid with a Strong Base

For this example, let's consider the titration of 50.0 mL of 0.100 M acetic acid, CH_3COOH , with 0.200 M NaOH. Again, we start by calculating the volume of NaOH needed to reach the equivalence point; thus

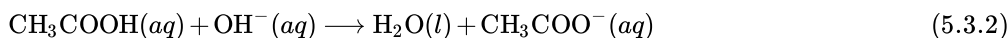
$$\begin{aligned}\text{mol CH}_3\text{COOH} &= \text{mol NaOH} \\ M_a \times V_a &= M_b \times V_b \\ V_{eq} = V_b &= \frac{M_a V_a}{M_b} = \frac{(0.100 \text{ M})(50.0 \text{ mL})}{(0.200 \text{ M})} = 25.0 \text{ mL}\end{aligned}$$

Before we begin the titration the pH is that for a solution of 0.100 M acetic acid. Because acetic acid is a weak acid, we calculate the pH using the method outlined in [Chapter 6](#)



finding that the pH is 2.88.

Adding NaOH converts a portion of the acetic acid to its conjugate base, CH_3COO^- .



Because the equilibrium constant for reaction [5.3.2](#) is quite large

$$K = K_a / K_w = 1.75 \times 10^9$$

we can treat the reaction as if it goes to completion.

Any solution that contains comparable amounts of a weak acid, HA, and its conjugate weak base, A^- , is a buffer. As we learned in [Chapter 6](#), we can calculate the pH of a buffer using the Henderson–Hasselbalch equation.

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

Before the equivalence point the concentration of unreacted acetic acid is

$$[\text{CH}_3\text{COOH}] = \frac{(\text{mol CH}_3\text{COOH})_{\text{initial}} - (\text{mol NaOH})_{\text{added}}}{\text{total volume}} = \frac{M_a V_a - M_b V_b}{V_a + V_b}$$

and the concentration of acetate is

$$[\text{CH}_3\text{COO}^-] = \frac{(\text{mol NaOH})_{\text{added}}}{\text{total volume}} = \frac{M_b V_b}{V_a + V_b}$$

For example, after adding 10.0 mL of NaOH the concentrations of CH_3COOH and CH_3COO^- are

$$\begin{aligned}[\text{CH}_3\text{COOH}] &= \frac{(0.100 \text{ M})(50.0 \text{ mL}) - (0.200 \text{ M})(10.0 \text{ mL})}{50.0 \text{ mL} + 10.0 \text{ mL}} = 0.0500 \text{ M} \\ [\text{CH}_3\text{COO}^-] &= \frac{(0.200 \text{ M})(10.0 \text{ mL})}{50.0 \text{ mL} + 10.0 \text{ mL}} = 0.0333 \text{ M}\end{aligned}$$

which gives us a pH of

$$\text{pH} = 4.76 + \log \frac{0.0333 \text{ M}}{0.0500 \text{ M}} = 4.58$$

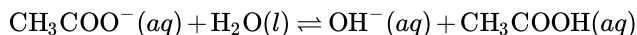
At the equivalence point the moles of acetic acid initially present and the moles of NaOH added are identical. Because their reaction effectively proceeds to completion, the predominate ion in solution is CH_3COO^- , which is a weak base. To calculate the pH we first determine the concentration of CH_3COO^-

$$[\text{CH}_3\text{COO}^-] = \frac{(\text{mol NaOH})_{\text{added}}}{\text{total volume}} = \frac{(0.200 \text{ M})(25.0 \text{ mL})}{50.0 \text{ mL} + 25.0 \text{ mL}} = 0.0667 \text{ M}$$

Alternatively, we can calculate acetate's concentration using the initial moles of acetic acid; thus

$$[\text{CH}_3\text{COO}^-] = \frac{(\text{mol CH}_3\text{COOH})_{\text{initial}}}{\text{total volume}} = \frac{(0.100 \text{ M})(50.0 \text{ mL})}{50.0 \text{ mL} + 25.0 \text{ mL}} = 0.0667 \text{ M}$$

Next, we calculate the pH of the weak base as shown earlier in [Chapter 6](#)



$$K_b = \frac{[\text{OH}^-][\text{CH}_3\text{COOH}]}{[\text{CH}_3\text{COO}^-]} = \frac{(x)(x)}{0.0667 - x} = 5.71 \times 10^{-10}$$

$$x = [\text{OH}^-] = 6.17 \times 10^{-6} \text{ M}$$

$$[\text{H}_3\text{O}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.00 \times 10^{-14}}{6.17 \times 10^{-6}} = 1.62 \times 10^{-9} \text{ M}$$

finding that the pH at the equivalence point is 8.79.

After the equivalence point, the titrant is in excess and the titration mixture is a dilute solution of NaOH. We can calculate the pH using the same strategy as in the titration of a strong acid with a strong base. For example, after adding 30.0 mL of NaOH the concentration of OH^- is

$$[\text{OH}^-] = \frac{(0.200 \text{ M})(30.0 \text{ mL}) - (0.100 \text{ M})(50.0 \text{ mL})}{30.0 \text{ mL} + 50.0 \text{ mL}} = 0.0125 \text{ M}$$

$$[\text{H}_3\text{O}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.00 \times 10^{-14}}{0.0125} = 8.00 \times 10^{-13} \text{ M}$$

giving a pH of 12.10. Table 9.2.2 and Figure 9.2.2 show additional results for this titration. You can use this same approach to calculate the titration curve for the titration of a weak base with a strong acid, except the initial pH is determined by the weak base, the pH at the equivalence point by its conjugate weak acid, and the pH after the equivalence point by excess strong acid.

Table 9.2.2 . Titration of 50.0 mL of 0.100 M Acetic Acid with 0.200 M NaOH

volume of HCl (mL)	pH	volume of HCl (mL)	pH
0.00	2.88	26.0	11.43
5.00	4.16	28.0	11.89
10.0	4.58	30.0	12.10
15.0	4.94	35.0	12.37
20.0	5.36	40.0	12.52
22.0	5.63	45.0	12.63
24.0	6.14	50.0	12.70
25.0	8.79		

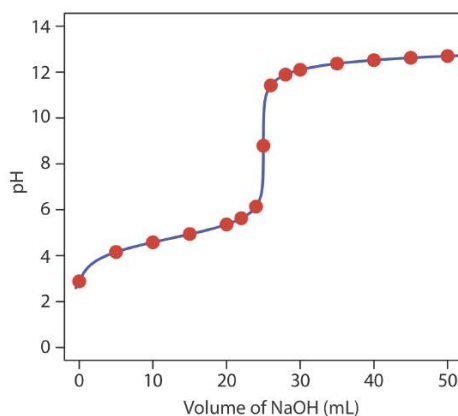


Figure 9.2.2 . Titration curve for the titration of 50.0 mL of 0.100 M CH_3COOH with 0.200 M NaOH. The red points correspond to the data in Table 9.2.2 . The blue line shows the complete titration curve.

? Exercise 9.2.2

Construct a titration curve for the titration of 25.0 mL of 0.125 M NH_3 with 0.0625 M HCl.

Answer

The volume of HCl needed to reach the equivalence point is

$$V_{aq} = V_a = \frac{M_b V_b}{M_a} = \frac{(0.125 \text{ M})(25.0 \text{ mL})}{(0.0625 \text{ M})} = 50.0 \text{ mL}$$

Before adding HCl the pH is that for a solution of 0.100 M NH_3 .

$$K_b = \frac{[\text{OH}^-][\text{NH}_4^+]}{[\text{NH}_3]} = \frac{(x)(x)}{0.125 - x} = 1.75 \times 10^{-5}$$

$$x = [\text{OH}^-] = 1.48 \times 10^{-3} \text{ M}$$

$$[\text{H}_3\text{O}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.00 \times 10^{-14}}{1.48 \times 10^{-3} \text{ M}} = 6.76 \times 10^{-12} \text{ M}$$

The pH at the beginning of the titration, therefore, is 11.17.

Before the equivalence point the pH is determined by an $\text{NH}_3/\text{NH}_4^+$ buffer. For example, after adding 10.0 mL of HCl

$$[\text{NH}_3] = \frac{(0.125 \text{ M})(25.0 \text{ mL}) - (0.0625 \text{ M})(10.0 \text{ mL})}{25.0 \text{ mL} + 10.0 \text{ mL}} = 0.0714 \text{ M}$$

$$[\text{NH}_4^+] = \frac{(0.0625 \text{ M})(10.0 \text{ mL})}{25.0 \text{ mL} + 10.0 \text{ mL}} = 0.0179 \text{ M}$$

$$\text{pH} = 9.244 + \log \frac{0.0714 \text{ M}}{0.0179 \text{ M}} = 9.84$$

At the equivalence point the predominate ion in solution is NH_4^+ . To calculate the pH we first determine the concentration of NH_4^+

$$[\text{NH}_4^+] = \frac{(0.125 \text{ M})(25.0 \text{ mL})}{25.0 \text{ mL} + 50.0 \text{ mL}} = 0.0417 \text{ M}$$

and then calculate the pH

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{NH}_3]}{[\text{NH}_4^+]} = \frac{(x)(x)}{0.0417 - x} = 5.70 \times 10^{-10}$$

obtaining a value of 5.31.

After the equivalence point, the pH is determined by the excess HCl. For example, after adding 70.0 mL of HCl

$$[\text{HCl}] = \frac{(0.0625 \text{ M})(70.0 \text{ mL}) - (0.125 \text{ M})(25.0 \text{ mL})}{70.0 \text{ mL} + 25.0 \text{ mL}} = 0.0132 \text{ M}$$

and the pH is 1.88. Some additional results are shown here.

volume of HCl (mL)	pH	volume of HCl (mL)	pH
0	11.17	60	2.13
10	9.84	70	1.88
20	9.42	80	1.75
30	9.07	90	1.66
40	8.64	100	1.60
50	5.31		

We can extend this approach for calculating a weak acid–strong base titration curve to reactions that involve multiprotic acids or bases, and mixtures of acids or bases. As the complexity of the titration increases, however, the necessary calculations become more time consuming. Not surprisingly, a variety of algebraic and spreadsheet approaches are available to aid in constructing titration curves.

The following papers provide information on algebraic approaches to calculating titration curves: (a) Willis, C. J. *J. Chem. Educ.* **1981**, 58, 659–663; (b) Nakagawa, K. *J. Chem. Educ.* **1990**, 67, 673–676; (c) Gordus, A. A. *J. Chem. Educ.* **1991**, 68, 759–761; (d) de Levie, R. *J. Chem. Educ.* **1993**, 70, 209–217; (e) Chaston, S. *J. Chem. Educ.* **1993**, 70, 878–880; (f) de Levie, R. *Anal. Chem.* **1996**, 68, 585–590.

The following papers provide information on the use of spreadsheets to generate titration curves: (a) Currie, J. O.; Whiteley, R. V. *J. Chem. Educ.* **1991**, 68, 923–926; (b) Breneman, G. L.; Parker, O. J. *J. Chem. Educ.* **1992**, 69, 46–47; (c) Carter, D. R.; Frye, M. S.; Mattson, W. A. *J. Chem. Educ.* **1993**, 70, 67–71; (d) Freiser, H. *Concepts and Calculations in Analytical Chemistry*, CRC Press: Boca Raton, 1992.

Sketching an Acid–Base Titration Curve

To evaluate the relationship between a titration’s equivalence point and its end point we need to construct only a reasonable approximation of the exact titration curve. In this section we demonstrate a simple method for sketching an acid–base titration curve. Our goal is to sketch the titration curve quickly, using as few calculations as possible. Let’s use the titration of 50.0 mL of 0.100 M CH_3COOH with 0.200 M NaOH to illustrate our approach. This is the same example that we used to develop the calculations for a weak acid–strong base titration curve. You can review the results of that calculation in [Table 9.2.2](#) and in [Figure 9.2.2](#).

We begin by calculating the titration’s equivalence point volume, which, as we determined earlier, is 25.0 mL. Next we draw our axes, placing pH on the y-axis and the titrant’s volume on the x-axis. To indicate the equivalence point volume, we draw a vertical line that intersects the x-axis at 25.0 mL of NaOH. Figure 9.2.3 a shows the first step in our sketch.

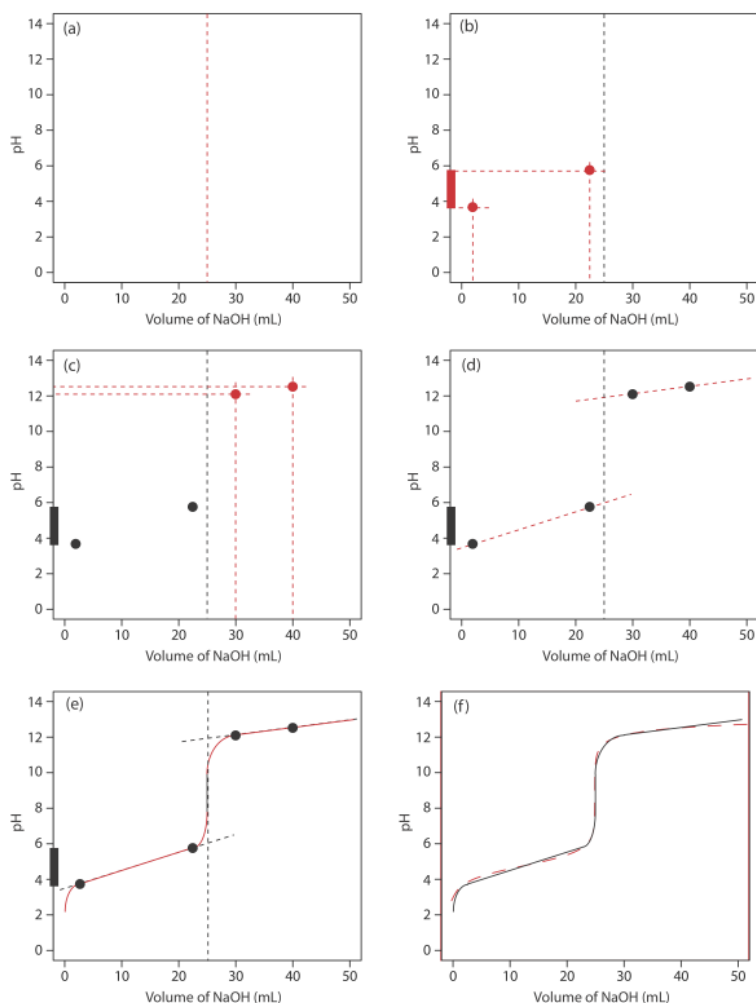


Figure 9.2.3 . Illustrations showing the steps used to sketch an approximate titration curve for the titration of 50.0 mL of 0.100 M CH_3COOH with 0.200 M NaOH: (a) locating the equivalence point volume; (b) plotting two points before the equivalence point; (c) plotting two points after the equivalence point; (d) preliminary approximation of titration curve using straight-lines; (e) final approximation of titration curve using a smooth curve; (f) comparison of approximate titration curve (solid black line) and exact titration curve (dashed red line). See the text for additional details.

Before the equivalence point the titrand's pH is determined by a buffer of acetic acid, CH_3COOH , and acetate, CH_3COO^- . Although we can calculate a buffer's pH using the Henderson–Hasselbalch equation, we can avoid this calculation by making a simple assumption. You may recall from [Chapter 6](#) that a buffer operates over a pH range that extends approximately ± 1 pH unit on either side of the weak acid's $\text{p}K_a$ value. The pH is at the lower end of this range, $\text{pH} = \text{p}K_a - 1$, when the weak acid's concentration is $10\times$ greater than that of its conjugate weak base. The buffer reaches its upper pH limit, $\text{pH} = \text{p}K_a + 1$, when the weak acid's concentration is $10\times$ smaller than that of its conjugate weak base. When we titrate a weak acid or a weak base, the buffer spans a range of volumes from approximately 10% of the equivalence point volume to approximately 90% of the equivalence point volume.

The actual values are 9.09% and 90.9%, but for our purpose, using 10% and 90% is more convenient; that is, after all, one advantage of an approximation!

Figure 9.2.3 b shows the second step in our sketch. First, we superimpose acetic acid's ladder diagram on the y-axis, including its buffer range, using its $\text{p}K_a$ value of 4.76. Next, we add two points, one for the pH at 10% of the equivalence point volume (a pH of 3.76 at 2.5 mL) and one for the pH at 90% of the equivalence point volume (a pH of 5.76 at 22.5 mL).

The third step is to add two points after the equivalence point. The pH after the equivalence point is fixed by the concentration of excess titrant, NaOH. Calculating the pH of a strong base is straightforward, as we saw earlier. Figure 9.2.3 c includes points (see

Table 9.2.2) for the pH after adding 30.0 mL and after adding 40.0 mL of NaOH.

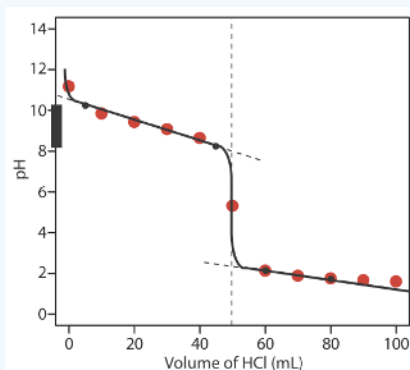
Next, we draw a straight line through each pair of points, extending each line through the vertical line that represents the equivalence point's volume (Figure 9.2.3 d). Finally, we complete our sketch by drawing a smooth curve that connects the three straight-line segments (Figure 9.2.3 e). A comparison of our sketch to the exact titration curve (Figure 9.2.3 f) shows that they are in close agreement.

? Exercise 9.2.3

Sketch a titration curve for the titration of 25.0 mL of 0.125 M NH_3 with 0.0625 M HCl and compare to the result from Exercise 9.2.2 .

Answer

The figure below shows a sketch of the titration curve. The black dots and curve are the approximate sketch of the titration curve. The points in red are the calculations from Exercise 9.2.2 . The two black points before the equivalence point ($V_{\text{HCl}} = 5 \text{ mL}$, $\text{pH} = 10.24$ and $V_{\text{HCl}} = 45 \text{ mL}$, $\text{pH} = 8.24$) are plotted using the pK_a of 9.244 for NH_4^+ . The two black points after the equivalence point ($V_{\text{HCl}} = 60 \text{ mL}$, $\text{pH} = 2.13$ and $V_{\text{HCl}} = 80 \text{ mL}$, $\text{pH} = 1.75$) are from the answer to Exercise 9.2.2 .



As shown in the following example, we can adapt this approach to any acid–base titration, including those where exact calculations are more challenging, including the titration of polyprotic weak acids and bases, and the titration of mixtures of weak acids or weak bases.

✓ Example 9.2.1

Sketch titration curves for the following two systems: (a) the titration of 50.0 mL of 0.050 M H_2A , a diprotic weak acid with a pK_{a1} of 3 and a pK_{a2} of 7; and (b) the titration of a 50.0 mL mixture that contains 0.075 M HA, a weak acid with a pK_a of 3, and 0.025 M HB, a weak acid with a pK_a of 7. For both titrations, assume that the titrant is 0.10 M NaOH.

Solution

Figure 9.2.4 a shows the titration curve for H_2A , including the ladder diagram for H_2A on the y-axis, the two equivalence points at 25.0 mL and at 50.0 mL, two points before each equivalence point, two points after the last equivalence point, and the straight-lines used to sketch the final titration curve. Before the first equivalence point the pH is controlled by a buffer of H_2A and HA^- . An $\text{HA}^-/\text{A}^{2-}$ buffer controls the pH between the two equivalence points. After the second equivalence point the pH reflects the concentration of excess NaOH.

Figure 9.2.4 b shows the titration curve for the mixture of HA and HB. Again, there are two equivalence points; however, in this case the equivalence points are not equally spaced because the concentration of HA is greater than that for HB. Because HA is the stronger of the two weak acids it reacts first; thus, the pH before the first equivalence point is controlled by a buffer of HA and A^- . Between the two equivalence points the pH reflects the titration of HB and is determined by a buffer of HB and B^- . After the second equivalence point excess NaOH determines the pH.

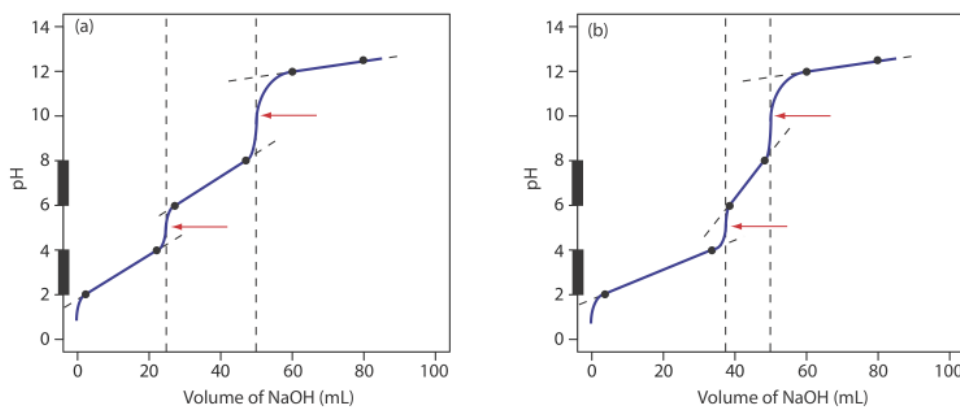


Figure 9.2.4 . Titration curves for Example 9.2.1 . The solid black dots show the points used to sketch the titration curves (shown in blue) and the red arrows show the locations of the equivalence points.

? Exercise 9.2.4

Sketch the titration curve for 50.0 mL of 0.050 M H_2A , a diprotic weak acid with a $\text{p}K_{\text{a}1}$ of 3 and a $\text{p}K_{\text{a}2}$ of 4, using 0.100 M NaOH as the titrant. The fact that $\text{p}K_{\text{a}2}$ falls within the buffer range of $\text{p}K_{\text{a}1}$ presents a challenge that you will need to consider.

Answer

The figure below shows a sketch of the titration curve. The titration curve has two equivalence points, one at 25.0 mL ($\text{H}_2\text{A} \rightarrow \text{HA}^-$) and one at 50.0 mL ($\text{HA}^- \rightarrow \text{A}^{2-}$). In sketching the curve, we plot two points before the first equivalence point using the $\text{p}K_{\text{a}1}$ of 3 for H_2A

$$V_{\text{HCl}} = 2.5 \text{ mL, pH} = 2 \text{ and } V_{\text{HCl}} = 22.5 \text{ mL, pH} = 4$$

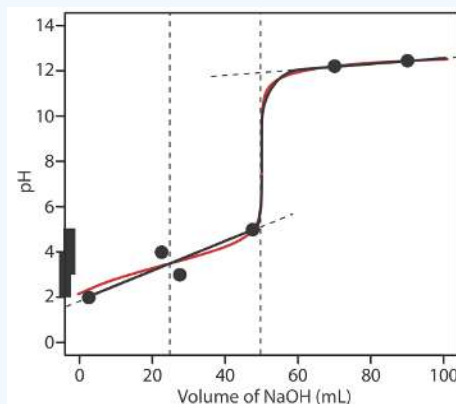
two points between the equivalence points using the $\text{p}K_{\text{a}2}$ of 5 for HA^-

$$V_{\text{HCl}} = 27.5 \text{ mL, pH} = 3, \text{ and } V_{\text{HCl}} = 47.5 \text{ mL, pH} = 5$$

and two points after the second equivalence point

$$V_{\text{HCl}} = 70 \text{ mL, pH} = 12.22 \text{ and } V_{\text{HCl}} = 90 \text{ mL, pH} = 12.46$$

Drawing a smooth curve through these points presents us with the following dilemma—the pH appears to increase as the titrant's volume approaches the first equivalence point and then appears to decrease as it passes through the first equivalence point. This is, of course, absurd; as we add NaOH the pH cannot decrease. Instead, we model the titration curve before the second equivalence point by drawing a straight line from the first point ($V_{\text{HCl}} = 2.5 \text{ mL, pH} = 2$) to the fourth point ($V_{\text{HCl}} = 47.5 \text{ mL, pH} = 5$), ignoring the second and third points. The results is a reasonable approximation of the exact titration curve.



Selecting and Evaluating the End Point

Earlier we made an important distinction between a titration's end point and its equivalence point. The difference between these two terms is important and deserves repeating. An equivalence point, which occurs when we react stoichiometrically equal amounts of the analyte and the titrant, is a theoretical not an experimental value. A titration's end point is an experimental result that represents our best estimate of the equivalence point. Any difference between a titration's equivalence point and its corresponding end point is a source of determinate error.

Where is the Equivalence Point?

Earlier we learned how to calculate the pH at the equivalence point for the titration of a strong acid with a strong base, and for the titration of a weak acid with a strong base. We also learned how to sketch a titration curve with only a minimum of calculations. Can we also locate the equivalence point without performing any calculations. The answer, as you might guess, often is yes!

For most acid–base titrations the inflection point—the point on a titration curve that has the greatest slope—very nearly coincides with the titration's equivalence point. The red arrows in [Figure 9.2.4](#), for example, identify the equivalence points for the titration curves in [Example 9.2.1](#). An inflection point actually precedes its corresponding equivalence point by a small amount, with the error approaching 0.1% for weak acids and weak bases with dissociation constants smaller than 10^{-9} , or for very dilute solutions [Meites, L.; Goldman, J. A. *Anal. Chim. Acta* **1963**, 29, 472–479].

The principal limitation of an inflection point is that it must be present and easy to identify. For some titrations the inflection point is missing or difficult to find. [Figure 9.2.5](#), for example, demonstrates the affect of a weak acid's dissociation constant, K_a , on the shape of its titration curve. An inflection point is visible, even if barely so, for acid dissociation constants larger than 10^{-9} , but is missing when K_a is 10^{-11} .

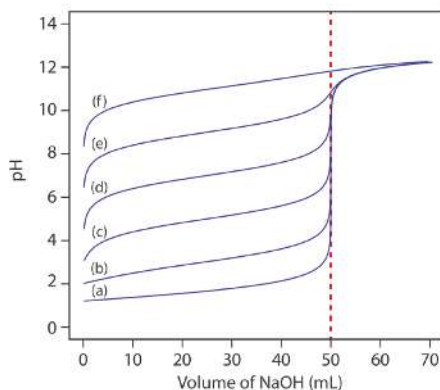
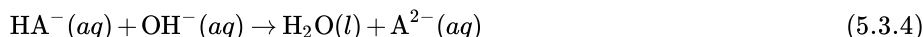
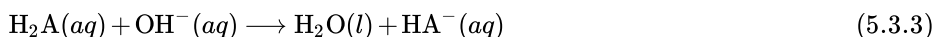


Figure 9.2.5. Weak acid–strong base titration curves for the titration of 50.0 mL of 0.100 M HA with 0.100 M NaOH. The pK_a values for HA are (a) 1, (b) 3, (c) 5, (d) 7, (e) 9, and (f) 11. The dashed red line shows the equivalence point, which is 50.0 mL for all six analytes.

An inflection point also may be missing or difficult to see if the analyte is a multiprotic weak acid or weak base with successive dissociation constants that are similar in magnitude. To appreciate why this is true let's consider the titration of a diprotic weak acid, H_2A , with NaOH. During the titration the following two reactions occur.



To see two distinct inflection points, reaction [5.3.3](#) must essentially be complete before reaction [5.3.4](#) begins.

[Figure 9.2.6](#) shows titration curves for three diprotic weak acids. The titration curve for maleic acid, for which K_{a1} is approximately $20000 \times$ larger than K_{a2} , has two distinct inflection points. Malonic acid, on the other hand, has acid dissociation constants that differ by a factor of approximately 690. Although malonic acid's titration curve shows two inflection points, the first is not as distinct as the second. Finally, the titration curve for succinic acid, for which the two K_a values differ by a factor of only $27 \times$, has only a single inflection point that corresponds to the neutralization of $HC_2H_4O_4^-$ to $C_2H_4O_4^{2-}$. In general, we can detect separate inflection points when successive acid dissociation constants differ by a factor of at least 500 (a ΔK_a of at least 2.7).

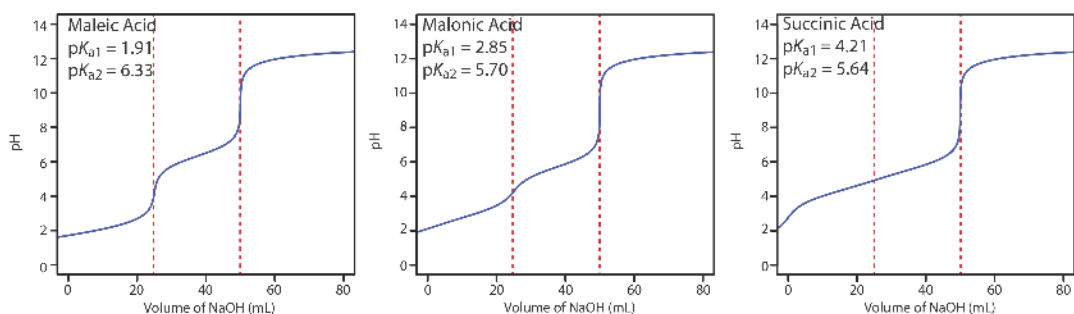


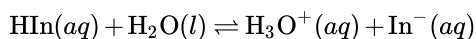
Figure 9.2.6 . Titration curves for the diprotic weak acids maleic acid, malonic acid, and succinic acid. Each titration curve is for 50.0 mL of 0.0500 M weak acid using 0.100 M NaOH as the titrant. Although each titration curve has equivalence points at 25.0 mL and 50.0 mL of NaOH (shown by the dashed red lines), the titration curve for succinic acid shows only one inflection point.

The same holds true for mixtures of weak acids or mixtures of weak bases. To detect separate inflection points when titrating a mixture of weak acids, their pK_a values must differ by at least a factor of 500.

Finding the End Point with an Indicator

One interesting group of weak acids and weak bases are organic dyes. Because an organic dye has at least one highly colored conjugate acid–base species, its titration results in a change in both its pH and its color. We can use this change in color to indicate the end point of a titration provided that it occurs at or near the titration’s equivalence point.

As an example, let’s consider an indicator for which the acid form, HIn , is yellow and the base form, In^- , is red. The color of the indicator’s solution depends on the relative concentrations of HIn and In^- . To understand the relationship between pH and color we use the indicator’s acid dissociation reaction



and its equilibrium constant expression.

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{In}^-]}{[\text{HIn}]} \quad (5.3.5)$$

Taking the negative log of each side of Equation 5.3.5, and rearranging to solve for pH leaves us with a equation that relates the solution’s pH to the relative concentrations of HIn and In^- .

$$\text{pH} = \text{p}K_a + \log \frac{[\text{In}^-]}{[\text{HIn}]} \quad (5.3.6)$$

If we can detect HIn and In^- with equal ease, then the transition from yellow-to-red (or from red-to-yellow) reaches its midpoint, which is orange, when the concentrations of HIn and In^- are equal, or when the pH is equal to the indicator’s $\text{p}K_a$. If the indicator’s $\text{p}K_a$ and the pH at the equivalence point are identical, then titrating until the indicator turns orange is a suitable end point. Unfortunately, we rarely know the exact pH at the equivalence point. In addition, determining when the concentrations of HIn and In^- are equal is difficult if the indicator’s change in color is subtle.

We can establish the range of pHs over which the average analyst observes a change in the indicator’s color by making two assumptions: that the indicator’s color is yellow if the concentration of HIn is $10\times$ greater than that of In^- and that its color is red if the concentration of HIn is $10\times$ smaller than that of In^- . Substituting these inequalities into Equation 5.3.6

$$\begin{aligned} \text{pH} &= \text{p}K_a + \log \frac{1}{10} = \text{p}K_a - 1 \\ \text{pH} &= \text{p}K_a + \log \frac{10}{1} = \text{p}K_a + 1 \end{aligned}$$

shows that the indicator changes color over a pH range that extends ± 1 unit on either side of its $\text{p}K_a$. As shown in Figure 9.2.7, the indicator is yellow when the pH is less than $\text{p}K_a - 1$ and it is red when the pH is greater than $\text{p}K_a + 1$. For pH values between $\text{p}K_a - 1$ and $\text{p}K_a + 1$ the indicator’s color passes through various shades of orange. The properties of several common acid–base indicators are listed in Table 9.2.3.

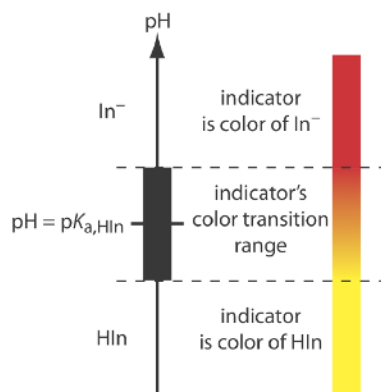


Figure 9.2.7 . Diagram showing the relationship between pH and an indicator's color. The ladder diagram defines pH values where HIn and In^- are the predominate species. The indicator changes color when the pH is between $\text{p}K_{\text{a}} - 1$ and $\text{p}K_{\text{a}} + 1$.

Table 9.2.3 . Properties of Selected Acid–Base Indicators

indicator	acid color	base color	pH range	$\text{p}K_{\text{a}}$
cresol red	red	yellow	0.2–1.8	—
thymol blue	red	yellow	1.2–2.8	1.7
bromothymol blue	yellow	blue	3.0–4.6	4.1
methyl orange	red	yellow	3.4–4.4	3.7
Congo red	blue	red	3.0–5.0	—
bromocresol green	yellow	blue	3.8–5.4	4.7
methyl red	red	yellow	4.2–6.3	5.0
bromocresol purple	yellow	purple	5.2–6.8	6.1
litmus	red	blue	5.0–8.0	—
bromothymol blue	yellow	blue	6.0–7.6	7.1
phenol red	yellow	blue	6.8–8.4	7.8
cresol red	yellow	red	7.2–8.8	8.2
thymol blue	yellow	red	8.0–9.6	8.9
phenolphthalein	colorless	red	8.3–10.0	9.6
alizarin yellow R	yellow	orange-red	10.1–12.0	—

You may wonder why an indicator's pH range, such as that for phenolphthalein, is not equally distributed around its $\text{p}K_{\text{a}}$ value. The explanation is simple. Figure 9.2.7 presents an idealized view in which our sensitivity to the indicator's two colors is equal. For some indicators only the weak acid or the weak base is colored. For other indicators both the weak acid and the weak base are colored, but one form is easier to see. In either case, the indicator's pH range is skewed in the direction of the indicator's less colored form. Thus, phenolphthalein's pH range is skewed in the direction of its colorless form, shifting the pH range to values lower than those suggested by Figure 9.2.7 .

The relatively broad range of pHs over which an indicator changes color places additional limitations on its ability to signal a titration's end point. To minimize a determinate titration error, the indicator's entire pH range must fall within the rapid change in pH near the equivalence point. For example, in Figure 9.2.8 we see that phenolphthalein is an appropriate indicator for the titration of 50.0 mL of 0.050 M acetic acid with 0.10 M NaOH. Bromothymol blue, on the other hand, is an inappropriate indicator because its change in color begins well before the initial sharp rise in pH, and, as a result, spans a relatively large range of volumes. The

early change in color increases the probability of obtaining an inaccurate result, and the range of possible end point volumes increases the probability of obtaining imprecise results.

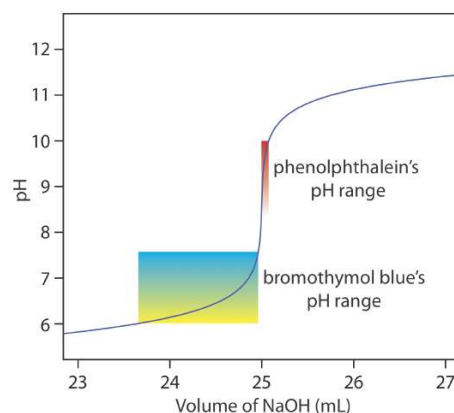


Figure 9.2.8 . Portion of the titration curve for 50.0 mL of 0.050 M CH_3COOH with 0.10 M NaOH, highlighting the region that contains the equivalence point. The end point transitions for the indicators phenolphthalein and bromothymol blue are superimposed on the titration curve.

? Exercise 9.2.5

Suggest a suitable indicator for the titration of 25.0 mL of 0.125 M NH_3 with 0.0625 M NaOH. You constructed a titration curve for this titration in [Exercise 9.2.2](#) and [Exercise 9.2.3](#) .

Answer

The pH at the equivalence point is 5.31 (see [Exercise 9.2.2](#)) and the sharp part of the titration curve extends from a pH of approximately 7 to a pH of approximately 4. Of the indicators in [Table 9.2.3](#) , methyl red is the best choice because its pK_a value of 5.0 is closest to the equivalence point's pH and because the pH range of 4.2–6.3 for its change in color will not produce a significant titration error.

Finding the End Point by Monitoring pH

An alternative approach for locating a titration's end point is to monitor the titration's progress using a sensor whose signal is a function of the analyte's concentration. The result is a plot of the entire titration curve, which we can use to locate the end point with a minimal error.

A pH electrode is the obvious sensor for monitoring an acid–base titration and the result is a **potentiometric titration curve**. For example, Figure 9.2.9 a shows a small portion of the potentiometric titration curve for the titration of 50.0 mL of 0.050 M CH_3COOH with 0.10 M NaOH, which focuses on the region that contains the equivalence point. The simplest method for finding the end point is to locate the titration curve's inflection point, which is shown by the arrow. This is also the least accurate method, particularly if the titration curve has a shallow slope at the equivalence point.

See [Chapter 11](#) for more details about pH electrodes.

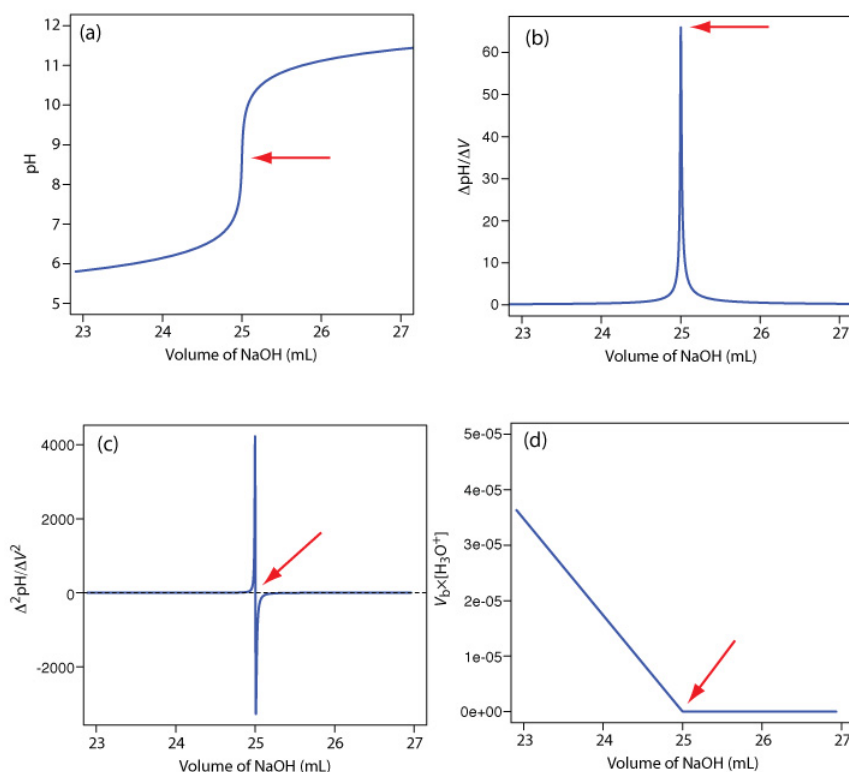


Figure 9.2.9 . Titration curves for the titration of 50.0 mL of 0.050 M CH_3COOH with 0.10 M NaOH : (a) normal titration curve; (b) first derivative titration curve; (c) second derivative titration curve; (d) Gran plot. The red arrows show the location of each titration's end point.

Another method for locating the end point is to plot the first derivative of the titration curve, which gives its slope at each point along the x-axis. Examine Figure 9.2.9 a and consider how the titration curve's slope changes as we approach, reach, and pass the equivalence point. Because the slope reaches its maximum value at the inflection point, the first derivative shows a spike at the equivalence point (Figure 9.2.9 b). The second derivative of a titration curve can be more useful than the first derivative because the equivalence point intersects the volume axis. Figure 9.2.9 c shows the resulting titration curve.

Suppose we have the following three points on our titration curve:

volume (mL)	pH
23.65	6.00
23.91	6.10
24.13	6.20

Mathematically, we can approximate the first derivative as $\Delta\text{pH}/\Delta V$, where ΔpH is the change in pH between successive additions of titrant. Using the first two points, the first derivative is

$$\frac{\Delta\text{pH}}{\Delta V} = \frac{6.10 - 6.00}{23.91 - 23.65} = 0.385$$

which we assign to the average of the two volumes, or 23.78 mL. For the second and third points, the first derivative is 0.455 and the average volume is 24.02 mL.

volume (mL)	ΔpH
23.78	0.385
24.02	0.455

We can approximate the second derivative as $\Delta(\Delta\text{pH}/\Delta V)/\Delta V$, or $\Delta^2\text{pH}/\Delta V^2$. Using the two points from our calculation of the first derivative, the second derivative is

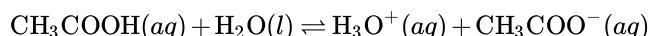
$$\frac{\Delta^2\text{pH}}{\Delta V^2} = \frac{0.455 - 0.385}{24.02 - 23.78} = 0.292$$

which we assign to the average of the two volumes, or 23.90 mL. Note that calculating the first derivative comes at the expense of losing one piece of information (three points become two points), and calculating the second derivative comes at the expense of losing two pieces of information.

Derivative methods are particularly useful when titrating a sample that contains more than one analyte. If we rely on indicators to locate the end points, then we usually must complete separate titrations for each analyte so that we can see the change in color for each end point. If we record the titration curve, however, then a single titration is sufficient. The precision with which we can locate the end point also makes derivative methods attractive for an analyte that has a poorly defined normal titration curve.

Derivative methods work well only if we record sufficient data during the rapid increase in pH near the equivalence point. This usually is not a problem if we use an automatic titrator, such as the one seen earlier in [Figure 9.1.5](#). Because the pH changes so rapidly near the equivalence point—a change of several pH units over a span of several drops of titrant is not unusual—a manual titration does not provide enough data for a useful derivative titration curve. A manual titration does contain an abundance of data during the more gently rising portions of the titration curve before and after the equivalence point. This data also contains information about the titration curve's equivalence point.

Consider again the titration of acetic acid, CH_3COOH , with NaOH . At any point during the titration acetic acid is in equilibrium with H_3O^+ and CH_3COO^-



for which the equilibrium constant is

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

Before the equivalence point the concentrations of CH_3COOH and CH_3COO^- are

$$[\text{CH}_3\text{COOH}] = \frac{(\text{mol CH}_3\text{COOH})_{\text{initial}} - (\text{mol NaOH})_{\text{added}}}{\text{total volume}} = \frac{M_a V_a - M_b V_b}{V_a + V_b}$$

$$[\text{CH}_3\text{COO}^-] = \frac{(\text{mol NaOH})_{\text{added}}}{\text{total volume}} = \frac{M_b V_b}{V_a + V_b}$$

Substituting these equations into the K_a expression and rearranging leaves us with

$$K_a = \frac{[\text{H}_3\text{O}^+](M_b V_b) / (V_a + V_b)}{\{M_a V_a - M_b V_b\} / (V_a + V_b)}$$

$$K_a M_a V_a - K_a M_b V_b = [\text{H}_3\text{O}^+](M_b V_b)$$

$$\frac{K_a M_a V_a}{M_b} - K_a V_b = [\text{H}_3\text{O}^+] V_b$$

Finally, recognizing that the equivalence point volume is

$$V_{eq} = \frac{M_a V_a}{M_b}$$

leaves us with the following equation.

$$[\text{H}_3\text{O}^+] \times V_b = K_a V_{eq} - K_a V_b$$

For volumes of titrant before the equivalence point, a plot of $V_b \times [\text{H}_3\text{O}^+]$ versus V_b is a straight-line with an x-intercept of V_{eq} and a slope of $-K_a$. [Figure 9.2.9 d](#) shows a typical result. This method of data analysis, which converts a portion of a titration curve into a straight-line, is a **Gran plot**.

Values of K_a determined by this method may have a substantial error if the effect of activity is ignored. See [Chapter 6.9](#) for a discussion of activity.

Finding the End Point by Monitoring Temperature

The reaction between an acid and a base is exothermic. Heat generated by the reaction is absorbed by the titrand, which increases its temperature. Monitoring the titrand's temperature as we add the titrant provides us with another method for recording a titration curve and identifying the titration's end point ([Figure 9.2.10](#)).

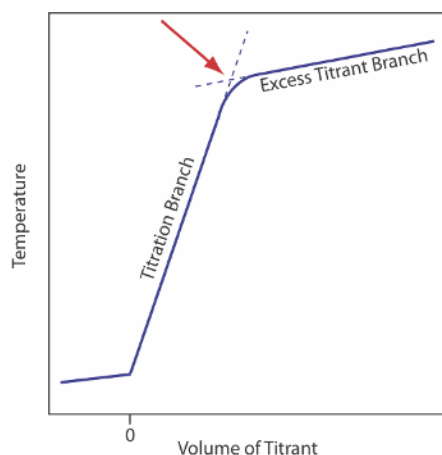


Figure 9.2.10 . Typical thermometric titration curve. The endpoint, shown by the red arrow, is found by extrapolating the titration branch and the excess titration branch.

Before we add the titrant, any change in the titrand's temperature is the result of warming or cooling as it equilibrates with the surroundings. Adding titrant initiates the exothermic acid–base reaction and increases the titrand's temperature. This part of a thermometric titration curve is called the titration branch. The temperature continues to rise with each addition of titrant until we reach the equivalence point. After the equivalence point, any change in temperature is due to the titrant's enthalpy of dilution and the difference between the temperatures of the titrant and titrand. Ideally, the equivalence point is a distinct intersection of the titration branch and the excess titrant branch. As shown in [Figure 9.2.10](#) , however, a thermometric titration curve usually shows curvature near the equivalence point due to an incomplete neutralization reaction or to the excessive dilution of the titrand and the titrant during the titration. The latter problem is minimized by using a titrant that is 10–100 times more concentrated than the analyte, although this results in a very small end point volume and a larger relative error. If necessary, the end point is found by extrapolation.

Although not a common method for monitoring an acid–base titration, a thermometric titration has one distinct advantage over the direct or indirect monitoring of pH. As discussed earlier, the use of an indicator or the monitoring of pH is limited by the magnitude of the relevant equilibrium constants. For example, titrating boric acid, H_3BO_3 , with NaOH does not provide a sharp end point when monitoring pH because boric acid's K_a of 5.8×10^{-10} is too small ([Figure 9.2.11 a](#)). Because boric acid's enthalpy of neutralization is fairly large, -42.7 kJ/mole, its thermometric titration curve provides a useful endpoint ([Figure 9.2.11 b](#)).

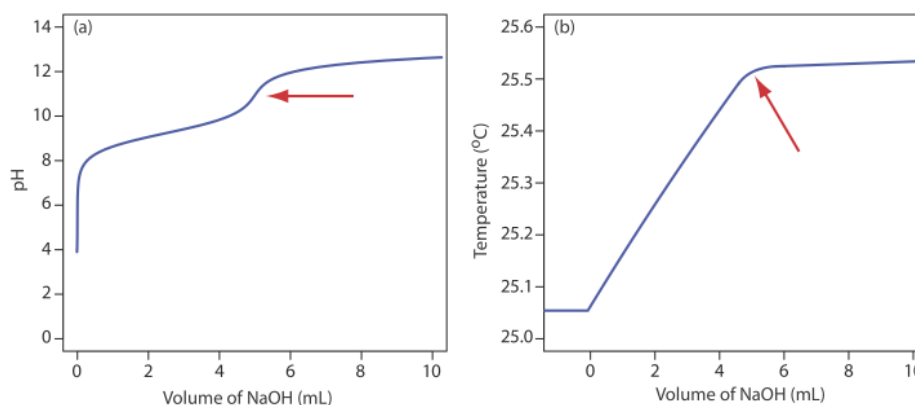
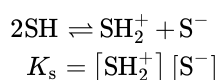


Figure 9.2.11 . Titration curves for the titration of 50.0 mL of 0.050 M H_3BO_3 with 0.50 M NaOH obtained by monitoring (a) pH and (b) temperature. The red arrows show the end points for the titrations.

Titration in Nonaqueous Solvents

Thus far we have assumed that the titrant and the titrand are aqueous solutions. Although water is the most common solvent for acid–base titrimetry, switching to a nonaqueous solvent can improve a titration’s feasibility.

For an amphoteric solvent, SH, the autoprotolysis constant, K_s , relates the concentration of its protonated form, SH_2^+ , to its deprotonated form, S^-



and the solvent’s pH and pOH are

$$\text{pH} = -\log[\text{SH}_2^+]$$

$$\text{pOH} = -\log[\text{S}^-]$$

You should recognize that K_w is just specific form of K_s when the solvent is water.

The most important limitation imposed by K_s is the change in pH during a titration. To understand why this is true, let’s consider the titration of 50.0 mL of 1.0×10^{-4} M HCl using 1.0×10^{-4} M NaOH as the titrant. Before the equivalence point, the pH is determined by the untitrated strong acid. For example, when the volume of NaOH is 90% of V_{eq} , the concentration of H_3O^+ is

$$[\text{H}_3\text{O}^+] = \frac{M_a V_a - M_b V_b}{V_a + V_b} = \frac{(1.0 \times 10^{-4} \text{ M})(50.0 \text{ mL}) - (1.0 \times 10^{-4} \text{ M})(45.0 \text{ mL})}{50.0 \text{ mL} + 45.0 \text{ mL}} = 5.3 \times 10^{-6} \text{ M}$$

and the pH is 5.3. When the volume of NaOH is 110% of V_{eq} , the concentration of OH^- is

$$[\text{OH}^-] = \frac{M_b V_b - M_a V_a}{V_a + V_b} = \frac{(1.0 \times 10^{-4} \text{ M})(55.0 \text{ mL}) - (1.0 \times 10^{-4} \text{ M})(50.0 \text{ mL})}{55.0 \text{ mL} + 50.0 \text{ mL}} = 4.8 \times 10^{-6} \text{ M}$$

and the pOH is 5.3. The titrand’s pH is

$$\text{pH} = \text{p}K_w - \text{pOH} = 14.0 - 5.3 = 8.7$$

and the change in the titrand’s pH as the titration goes from 90% to 110% of V_{eq} is

$$\Delta\text{pH} = 8.7 - 5.3 = 3.4$$

If we carry out the same titration in a nonaqueous amphiprotic solvent that has a K_s of 1.0×10^{-20} , the pH after adding 45.0 mL of NaOH is still 5.3. However, the pH after adding 55.0 mL of NaOH is

$$\text{pH} = \text{p}K_s - \text{pOH} = 20.0 - 5.3 = 14.7$$

In this case the change in pH

$$\Delta\text{pH} = 14.7 - 5.3 = 9.4$$

is significantly greater than that obtained when the titration is carried out in water. Figure 9.2.12 shows the titration curves in both the aqueous and the nonaqueous solvents.

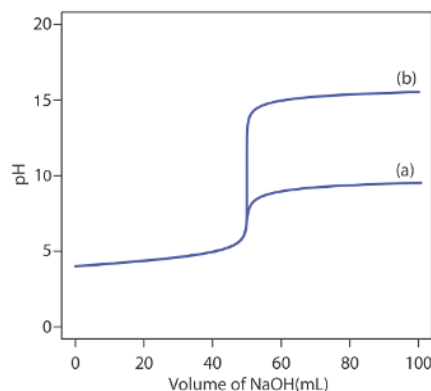
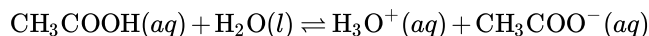


Figure 9.2.12 . Titration curves for 50.0 mL of 1.0×10^{-4} M HCl using 1.0×10^{-4} M NaOH in (a) water, $K_w = 1.0 \times 10^{-14}$, and (b) a nonaqueous amphiprotic solvent, $K_s = 1.0 \times 10^{-20}$.

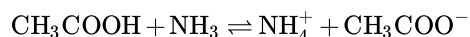
Another parameter that affects the feasibility of an acid–base titration is the titrand’s dissociation constant. Here, too, the solvent plays an important role. The strength of an acid or a base is a relative measure of how easy it is to transfer a proton from the acid to the solvent or from the solvent to the base. For example, HF, with a K_a of 6.8×10^{-4} , is a better proton donor than CH_3COOH , for which K_a is 1.75×10^{-5} .

The strongest acid that can exist in water is the hydronium ion, H_3O^+ . HCl and HNO_3 are strong acids because they are better proton donors than H_3O^+ and essentially donate all their protons to H_2O , leveling their acid strength to that of H_3O^+ . In a different solvent HCl and HNO_3 may not behave as strong acids.

If we place acetic acid in water the dissociation reaction



does not proceed to a significant extent because CH_3COO^- is a stronger base than H_2O and H_3O^+ is a stronger acid than CH_3COOH . If we place acetic acid in a solvent that is a stronger base than water, such as ammonia, then the reaction



proceeds to a greater extent. In fact, both HCl and CH_3COOH are strong acids in ammonia.

All other things being equal, the strength of a weak acid increases if we place it in a solvent that is more basic than water, and the strength of a weak base increases if we place it in a solvent that is more acidic than water. In some cases, however, the opposite effect is observed. For example, the $\text{p}K_b$ for NH_3 is 4.75 in water and it is 6.40 in the more acidic glacial acetic acid. In contradiction to our expectations, NH_3 is a weaker base in the more acidic solvent. A full description of the solvent’s effect on the $\text{p}K_a$ of weak acid or the $\text{p}K_b$ of a weak base is beyond the scope of this text. You should be aware, however, that a titration that is not feasible in water may be feasible in a different solvent.

Representative Method 9.2.1: Determination of Protein in Bread

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical acid–base titrimetric method. Although each method is unique, the following description of the determination of protein in bread provides an instructive example of a typical procedure. The description here is based on Method 13.86 as published in *Official Methods of Analysis*, 8th Ed., Association of Official Agricultural Chemists: Washington, D. C., 1955.

Description of the Methods

This method is based on a determination of %w/w nitrogen using the Kjeldahl method. The protein in a sample of bread is oxidized to NH_4^+ using hot concentrated H_2SO_4 . After making the solution alkaline, which converts NH_4^+ to NH_3 , the ammonia is distilled into a flask that contains a known amount of HCl. The amount of unreacted HCl is determined by a back titration using a standard strong base titrant. Because different cereal proteins contain similar amounts of nitrogen—on average there are 5.7 g protein for

every gram of nitrogen—we multiply the experimentally determined %w/w N by a factor of 5.7 gives the %w/w protein in the sample.

Procedure

Transfer a 2.0-g sample of bread, which previously has been air-dried and ground into a powder, to a suitable digestion flask along with 0.7 g of a HgO catalyst, 10 g of K_2SO_4 , and 25 mL of concentrated H_2SO_4 . Bring the solution to a boil. Continue boiling until the solution turns clear and then boil for at least an additional 30 minutes. After cooling the solution below room temperature, remove the Hg^{2+} catalyst by adding 200 mL of H_2O and 25 mL of 4% w/v K_2S . Add a few Zn granules to serve as boiling stones and 25 g of NaOH. Quickly connect the flask to a distillation apparatus and distill the NH_3 into a collecting flask that contains a known amount of standardized HCl. The tip of the condenser must be placed below the surface of the strong acid. After the distillation is complete, titrate the excess strong acid with a standard solution of NaOH using methyl red as an indicator (Figure 9.2.13).

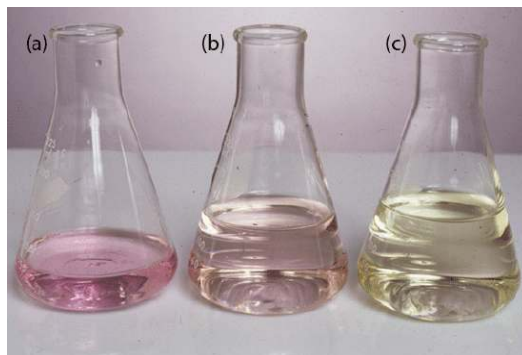


Figure 9.2.13 . Methyl red's endpoint for the titration of a strong acid with a strong base; the indicator is: (a) red prior to the end point; (b) orange at the end point; and (c) yellow after the end point.

Questions

1. Oxidizing the protein converts all of its nitrogen to NH_4^+ . Why is the amount of nitrogen not determined by directly titrating the NH_4^+ with a strong base?

There are two reasons for not directly titrating the ammonium ion. First, because NH_4^+ is a very weak acid (its K_a is 5.6×10^{-10}), its titration with NaOH has a poorly-defined end point. Second, even if we can determine the end point with acceptable accuracy and precision, the solution also contains a substantial concentration of unreacted H_2SO_4 . The presence of two acids that differ greatly in concentration makes for a difficult analysis. If the titrant's concentration is similar to that of H_2SO_4 , then the equivalence point volume for the titration of NH_4^+ is too small to measure reliably. On the other hand, if the titrant's concentration is similar to that of NH_4^+ , the volume needed to neutralize the H_2SO_4 is unreasonably large.

2. Ammonia is a volatile compound as evidenced by the strong smell of even dilute solutions. This volatility is a potential source of determinate error. Is this determinate error negative or positive?

Any loss of NH_3 is loss of nitrogen and, therefore, a loss of protein. The result is a negative determinate error.

3. Identify the steps in this procedure that minimize the determinate error from the possible loss of NH_3 .

Three specific steps minimize the loss of ammonia: (1) the solution is cooled below room temperature before we add NaOH; (2) after we add NaOH, the digestion flask is quickly connected to the distillation apparatus; and (3) we place the condenser's tip below the surface of the HCl to ensure that the NH_3 reacts with the HCl before it is lost through volatilization.

4. How does K_2S remove Hg^{2+} , and why is its removal important?

Adding sulfide precipitates Hg^{2+} as HgS . This is important because NH_3 forms stable complexes with many metal ions, including Hg^{2+} . Any NH_3 that reacts with Hg^{2+} is not collected during distillation, providing another source of determinate error.

Quantitative Applications

Although many quantitative applications of acid–base titrimetry have been replaced by other analytical methods, a few important applications continue to find use. In this section we review the general application of acid–base titrimetry to the analysis of

inorganic and organic compounds, with an emphasis on applications in environmental and clinical analysis. First, however, we discuss the selection and standardization of acidic and basic titrants.

Selecting and Standardizing a Titrant

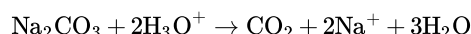
The most common strong acid titrants are HCl, HClO₄, and H₂SO₄. Solutions of these titrants usually are prepared by diluting a commercially available concentrated stock solution. Because the concentration of a concentrated acid is known only approximately, the titrant's concentration is determined by standardizing against one of the primary standard weak bases listed in Table 9.2.4.

The nominal concentrations of the concentrated stock solutions are 12.1 M HCl, 11.7 M HClO₄, and 18.0 M H₂SO₄. The actual concentrations of these acids are given as %w/v and vary slightly from lot-to-lot.

Table 9.2.4 . Selected Primary Standards for Standardizing Strong Acid and Strong Base Titrants

titrant type	primary standard	titration reaction	comment
strong acid	Na ₂ CO ₃	$\text{Na}_2\text{CO}_3 + 2\text{H}_3\text{O}^+ \rightarrow \text{H}_2\text{CO}_3 + 2\text{Na}^+ + 2\text{H}_2\text{O}$	a
strong acid	(HOCH ₂) ₃ CNH ₂	$(\text{HOCH}_2)_3\text{CNH}_2 + \text{H}_3\text{O}^+ \rightarrow (\text{HOCH}_2)_3\text{CNH}_3^+ + \text{H}_2\text{O}$	
strong acid	Na ₂ B ₄ O ₇	$\text{Na}_2\text{B}_4\text{O}_7 + 2\text{H}_3\text{O}^+ + 3\text{H}_2\text{O} \rightarrow 2\text{Na}^+ + 4\text{H}_3\text{BO}_3$	
strong base	KHC ₈ H ₄ O ₄	$\text{KHC}_8\text{H}_4\text{O}_4 + \text{OH}^- \rightarrow \text{K}^+ + \text{C}_8\text{H}_4\text{O}_4^- + \text{H}_2\text{O}$	c
strong base	C ₆ H ₅ COOH	$\text{C}_6\text{H}_5\text{COOH} + \text{OH}^- \rightarrow \text{C}_6\text{H}_5\text{COO}^- + \text{H}_2\text{O}$	d
strong base	KH(IO ₃) ₂	$\text{KH}(\text{IO}_3)_2 + \text{OH}^- \rightarrow \text{K}^+ + 2\text{IO}_3^- + \text{H}_2\text{O}$	

(a) The end point for this titration is improved by titrating to the second equivalence point, boiling the solution to expel CO₂, and retitrating to the second equivalence point. The reaction in this case is



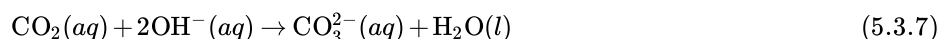
(b) *Tris*-(hydroxymethyl)aminomethane often goes by the shorter name of TRIS or THAM.

(c) Potassium hydrogen phthalate often goes by the shorter name of KHP.

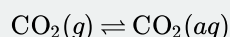
(d) Because it is not very soluble in water, dissolve benzoic acid in a small amount of ethanol before diluting with water.

The most common strong base titrant is NaOH, which is available both as an impure solid and as an approximately 50% w/v solution. Solutions of NaOH are standardized against any of the primary weak acid standards listed in Table 5.3. [4]

Using NaOH as a titrant is complicated by potential contamination from the following reaction between dissolved CO₂ and OH⁻.



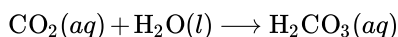
Any solution in contact with the atmosphere contains a small amount of CO₂(aq) from the equilibrium



During the titration, NaOH reacts both with the titrand and with CO₂, which increases the volume of NaOH needed to reach the titration's end point. This is not a problem if the end point pH is less than 6. Below this pH the CO₃²⁻ from reaction 5.3.7 reacts with H₃O⁺ to form carbonic acid.

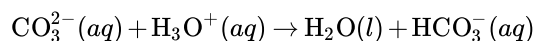


Combining reaction 5.3.7 and reaction 5.3.8 gives an overall reaction that does not include OH⁻.

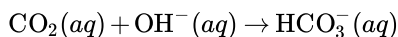


Under these conditions the presence of CO₂ does not affect the quantity of OH⁻ used in the titration and is not a source of determinate error.

If the end point pH is between 6 and 10, however, the neutralization of CO₃²⁻ requires one proton



and the net reaction between CO_2 and OH^- is



Under these conditions some OH^- is consumed in neutralizing CO_2 , which results in a determinate error. We can avoid the determinate error if we use the same end point pH for both the standardization of NaOH and the analysis of our analyte, although this is not always practical.

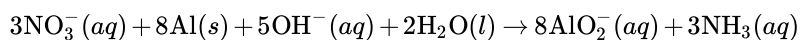
Solid NaOH is always contaminated with carbonate due to its contact with the atmosphere, and we cannot use it to prepare a carbonate-free solution of NaOH. Solutions of carbonate-free NaOH are prepared from 50% w/v NaOH because Na_2CO_3 is insoluble in concentrated NaOH. When CO_2 is absorbed, Na_2CO_3 precipitates and settles to the bottom of the container, which allow access to the carbonate-free NaOH. When preparing a solution of NaOH, be sure to use water that is free from dissolved CO_2 . Briefly boiling the water expels CO_2 ; after it cools, the water is used to prepare carbonate-free solutions of NaOH. A solution of carbonate-free NaOH is relatively stable if we limit its contact with the atmosphere. Standard solutions of sodium hydroxide are not stored in glass bottles as NaOH reacts with glass to form silicate; instead, store such solutions in polyethylene bottles.

Inorganic Analysis

Acid–base titrimetry is a standard method for the quantitative analysis of many inorganic acids and bases. A standard solution of NaOH is used to determine the concentration of inorganic acids, such as H_3PO_4 or H_3AsO_4 , and inorganic bases, such as Na_2CO_3 are analyzed using a standard solution of HCl.

If an inorganic acid or base that is too weak to be analyzed by an aqueous acid–base titration, it may be possible to complete the analysis by adjusting the solvent or by an indirect analysis. For example, when analyzing boric acid, H_3BO_3 , by titrating with NaOH, accuracy is limited by boric acid's small acid dissociation constant of 5.8×10^{-10} . Boric acid's K_a value increases to 1.5×10^{-4} in the presence of mannitol, because it forms a stable complex with the borate ion, which results in a sharper end point and a more accurate titration. Similarly, the analysis of ammonium salts is limited by the ammonium ion's small acid dissociation constant of 5.7×10^{-10} . We can determine NH_4^+ indirectly by using a strong base to convert it to NH_3 , which is removed by distillation and titrated with HCl. Because NH_3 is a stronger weak base than NH_4^+ is a weak acid (its K_b is 1.58×10^{-5}), the titration has a sharper end point.

We can analyze a neutral inorganic analyte if we can first convert it into an acid or a base. For example, we can determine the concentration of NO_3^- by reducing it to NH_3 in a strongly alkaline solution using Devarda's alloy, a mixture of 50% w/w Cu, 45% w/w Al, and 5% w/w Zn.



The NH_3 is removed by distillation and titrated with HCl. Alternatively, we can titrate NO_3^- as a weak base by placing it in an acidic nonaqueous solvent, such as anhydrous acetic acid, and using HClO_4 as a titrant.

Acid–base titrimetry continues to be listed as a standard method for the determination of alkalinity, acidity, and free CO_2 in waters and wastewaters. **Alkalinity** is a measure of a sample's capacity to neutralize acids. The most important sources of alkalinity are OH^- , HCO_3^- , and CO_3^{2-} , although other weak bases, such as phosphate, may contribute to the overall alkalinity. Total alkalinity is determined by titrating to a fixed end point pH of 4.5 (or to the bromocresol green end point) using a standard solution of HCl or H_2SO_4 . Results are reported as mg CaCO_3/L .

Although a variety of strong bases and weak bases may contribute to a sample's alkalinity, a single titration cannot distinguish between the possible sources. Reporting the total alkalinity as if CaCO_3 is the only source provides a means for comparing the acid-neutralizing capacities of different samples.

When the sources of alkalinity are limited to OH^- , HCO_3^- , and CO_3^{2-} , separate titrations to a pH of 4.5 (or the bromocresol green end point) and a pH of 8.3 (or the phenolphthalein end point) allow us to determine which species are present and their respective concentrations. Titration curves for OH^- , HCO_3^- , and CO_3^{2-} are shown in Figure 9.2.14. For a solution that contains OH^- alkalinity only, the volume of strong acid needed to reach each of the two end points is identical (Figure 9.2.14 a). When the only source of alkalinity is CO_3^{2-} , the volume of strong acid needed to reach the end point at a pH of 4.5 is exactly twice that needed to

reach the end point at a pH of 8.3 (Figure 9.2.14 b). If a solution contains HCO_3^- alkalinity only, the volume of strong acid needed to reach the end point at a pH of 8.3 is zero, but that for the pH 4.5 end point is greater than zero (Figure 9.2.14 c).

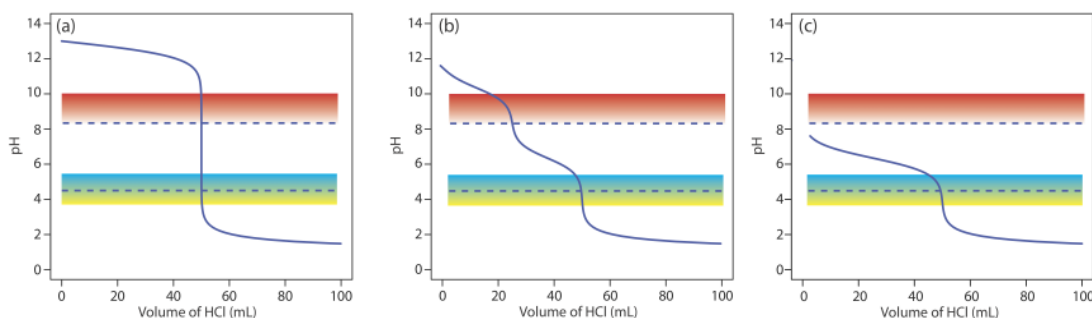


Figure 9.2.14. Titration curves for 50.0 mL of (a) 0.10 M NaOH, (b) 0.050 M Na_2CO_3 , and (c) 0.10 M NaHCO_3 using 0.10 M HCl as a titrant. The dashed lines indicate the fixed pH end points of 8.3 and 4.5. The color gradients show the phenolphthalein (red-to-colorless) and the bromocresol green (blue-to-green) endpoints. When titrating to the phenolphthalein endpoint, the titration continues until the last trace of red is lost.

A mixture of OH^- and CO_3^{2-} or a mixture of HCO_3^- and CO_3^{2-} also is possible. Consider, for example, a mixture of OH^- and CO_3^{2-} . The volume of strong acid to titrate OH^- is the same whether we titrate to a pH of 8.3 or a pH of 4.5. Titrating CO_3^{2-} to a pH of 4.5, however, requires twice as much strong acid as titrating to a pH of 8.3. Consequently, when we titrate a mixture of these two ions, the volume of strong acid needed to reach a pH of 4.5 is less than twice that needed to reach a pH of 8.3. For a mixture of HCO_3^- and CO_3^{2-} the volume of strong acid needed to reach a pH of 4.5 is more than twice that needed to reach a pH of 8.3. Table 9.2.5 summarizes the relationship between the sources of alkalinity and the volumes of titrant needed to reach the two end points.

A mixture of OH^- and HCO_3^- is unstable with respect to the formation of CO_3^{2-} . Problem 15 in the end-of-chapter problems asks you to explain why this is true.

Table 9.2.5 . Relationship Between End Point Volumes and Sources of Alkalinity

source of alkalinity	relationship between end point volumes
OH^-	$V_{\text{pH } 4.5} = V_{\text{pH } 8.3}$
CO_3^{2-}	$V_{\text{pH } 4.5} = 2 \times V_{\text{pH } 8.3}$
HCO_3^-	$V_{\text{pH } 4.5} > 0; V_{\text{pH } 8.3} = 0$
OH^- and CO_3^{2-}	$V_{\text{pH } 4.5} < 2 \times V_{\text{pH } 8.3}$
CO_3^{2-} and HCO_3^-	$V_{\text{pH } 4.5} > 2 \times V_{\text{pH } 8.3}$

Acidity is a measure of a water sample's capacity to neutralize base and is divided into strong acid and weak acid acidity. Strong acid acidity from inorganic acids such as HCl, HNO_3 , and H_2SO_4 is common in industrial effluents and in acid mine drainage. Weak acid acidity usually is dominated by the formation of H_2CO_3 from dissolved CO_2 , but also includes contributions from hydrolyzable metal ions such as Fe^{3+} , Al^{3+} , and Mn^{2+} . In addition, weak acid acidity may include a contribution from organic acids.

Acidity is determined by titrating with a standard solution of NaOH to a fixed pH of 3.7 (or the bromothymol blue end point) and to a fixed pH of 8.3 (or the phenolphthalein end point). Titrating to a pH of 3.7 provides a measure of strong acid acidity, and titrating to a pH of 8.3 provides a measure of total acidity. Weak acid acidity is the difference between the total acidity and the strong acid acidity. Results are expressed as the amount of CaCO_3 that can be neutralized by the sample's acidity. An alternative approach for determining strong acid and weak acid acidity is to obtain a potentiometric titration curve and use a Gran plot to determine the two equivalence points. This approach has been used, for example, to determine the forms of acidity in atmospheric aerosols [Ferek, R. J.; Lazrus, A. L.; Haagenson, P. L.; Winchester, J. W. *Environ. Sci. Technol.* **1983**, 17, 315–324].

As is the case with alkalinity, acidity is reported as mg CaCO_3/L .

Water in contact with either the atmosphere or with carbonate-bearing sediments contains free CO_2 in equilibrium with $\text{CO}_2(g)$ and with aqueous H_2CO_3 , HCO_3^- and CO_3^{2-} . The concentration of free CO_2 is determined by titrating with a standard solution of NaOH to the phenolphthalein end point, or to a pH of 8.3, with results reported as $\text{mg CO}_2/\text{L}$. This analysis essentially is the same as that for the determination of total acidity and is used only for water samples that do not contain strong acid acidity.

Free CO_2 is the same thing as $\text{CO}_2(aq)$.

Organic Analysis

Acid–base titrimetry continues to have a small, but important role for the analysis of organic compounds in pharmaceutical, biochemical, agricultural, and environmental laboratories. Perhaps the most widely employed acid–base titration is the **Kjeldahl analysis** for organic nitrogen. Examples of analytes determined by a Kjeldahl analysis include caffeine and saccharin in pharmaceutical products, proteins in foods, and the analysis of nitrogen in fertilizers, sludges, and sediments. Any nitrogen present in a -3 oxidation state is oxidized quantitatively to NH_4^+ . Because some aromatic heterocyclic compounds, such as pyridine, are difficult to oxidize, a catalyst is used to ensure a quantitative oxidation. Nitrogen in other oxidation states, such as nitro and azo nitrogens, are oxidized to N_2 , which results in a negative determinate error. Including a reducing agent, such as salicylic acid, converts this nitrogen to a -3 oxidation state, eliminating this source of error. Table 9.2.6 provides additional examples in which an element is converted quantitatively into a titratable acid or base.

Table 9.2.6 . Selected Elemental Analyses Based on an Acid–Base Titration

element	convert to...	reaction producing titratable species	titration details
N	$\text{NH}_3(g)$	$\text{NH}_3(aq) + \text{HCl}(aq) \rightarrow \text{NH}_4^+(aq) + \text{Cl}^-(aq)$	add HCl in excess and back titrate with NaOH
S	$\text{SO}_2(g)$	$\text{SO}_3(g) + \text{H}_2\text{O}_2(aq) \rightarrow \text{H}_2\text{SO}_4(aq)$	titrate H_2SO_4 with NaOH
C	$\text{CO}_2(g)$	$\text{CO}_2(g) + \text{Ba}(\text{OH})_2(aq) \rightarrow \text{BaCO}_3(s) + \text{H}_2\text{O}(l)$	add excess $\text{Ba}(\text{OH})_2$ and back titrate with HCl
Cl	HCl (g)	—	titrate HCl with NaOH
F	$\text{SiF}_4(g)$	$3\text{SiF}_4(aq) + 2\text{H}_2\text{O}(l) \rightarrow 2\text{H}_3\text{SiF}_6(aq) + \text{SiO}_2(s)$	titrate H_2SiF_6 with NaOH

the species that is titrated is shown in **bold**

Several organic functional groups are weak acids or weak bases. Carboxylic ($-\text{COOH}$), sulfonic ($-\text{SO}_3\text{H}$) and phenolic ($-\text{C}_6\text{H}_5\text{OH}$) functional groups are weak acids that are titrated successfully in either aqueous or non-aqueous solvents. Sodium hydroxide is the titrant of choice for aqueous solutions. Nonaqueous titrations often are carried out in a basic solvent, such as ethylenediamine, using tetrabutylammonium hydroxide, $(\text{C}_4\text{H}_9)_4\text{NOH}$, as the titrant. Aliphatic and aromatic amines are weak bases that are titrated using HCl in aqueous solutions, or HClO_4 in glacial acetic acid. Other functional groups are analyzed indirectly following a reaction that produces or consumes an acid or base. Typical examples are shown in Table 9.2.7 .

Table 9.2.7 . Selected Acid–Base Titrimetric Methods for Organic Functional Groups Based on the Production or Consumption of Acid or Base

functional group	reaction producing titratable species	titration details
ester	$\text{RCOOR}'(aq) + \text{OH}^-(aq) \rightarrow \text{RCOO}^-(aq) + \text{HOR}'(aq)$	titrate OH^- with HCl
carbonyl	$\text{R}_2\text{CO}(aq) + \text{NH}_4\text{OH} \cdot \text{HCl}(aq) \rightarrow \text{R}_2\text{CNOH}(aq) + \text{HCl}(aq) + \text{H}_2\text{O}(l)$	titrate HCl with NaOH
alcohol	[1]: $(\text{CH}_3\text{CO})_2\text{O} + \text{ROH} \rightarrow \text{CH}_3\text{COOR} + \text{CH}_3\text{COOH}$ [2]: $(\text{CH}_3\text{CO})_2 + \text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH}$	titrate CH_3COOH with NaOH; a blank titration of acetic anhydride, $(\text{CH}_3\text{CO})_2\text{O}$, corrects for the contribution of reaction [2]

functional group	reaction producing titratable species	titration details
the species that is titrated is shown in bold		
for alcohols, reaction [1] is carried out in pyridine to prevent the hydrolysis of acetic anhydride by water. After reaction [1] is complete, water is added to convert any unreacted acetic anhydride to acetic acid (reaction [2])		

Many pharmaceutical compounds are weak acids or weak bases that are analyzed by an aqueous or a nonaqueous acid–base titration; examples include salicylic acid, phenobarbital, caffeine, and sulfanilamide. Amino acids and proteins are analyzed in glacial acetic acid using HClO_4 as the titrant. For example, a procedure for determining the amount of nutritionally available protein uses an acid–base titration of lysine residues [(a) Molnár-Perl, I.; Pintée-Szakács, M. *Anal. Chim. Acta* **1987**, 202, 159–166; (b) Barbosa, J.; Bosch, E.; Cortina, J. L.; Rosés, M. *Anal. Chim. Acta* **1992**, 256, 177–181].

Quantitative Calculations

The quantitative relationship between the titrand and the titrant is determined by the titration reaction's stoichiometry. If the titrand is polyprotic, then we must know to which equivalence point we are titrating. The following example illustrates how we can use a ladder diagram to determine a titration reaction's stoichiometry.

✓ Example 9.2.2

A 50.00-mL sample of a citrus drink requires 17.62 mL of 0.04166 M NaOH to reach the phenolphthalein end point. Express the sample's acidity as grams of citric acid, $\text{C}_6\text{H}_8\text{O}_7$, per 100 mL.

Solution

Because citric acid is a triprotic weak acid, we first must determine if the phenolphthalein end point corresponds to the first, second, or third equivalence point. Citric acid's ladder diagram is shown in Figure 9.2.15 a. Based on this ladder diagram, the first equivalence point is between a pH of 3.13 and a pH of 4.76, the second equivalence point is between a pH of 4.76 and a pH of 6.40, and the third equivalence point is greater than a pH of 6.40. Because phenolphthalein's end point pH is 8.3–10.0 (see Table 9.2.3), the titration must proceed to the third equivalence point and the titration reaction is



To reach the equivalence point, each mole of citric acid consumes three moles of NaOH; thus

$$\begin{aligned} (0.04166 \text{ M NaOH})(0.01762 \text{ L NaOH}) &= 7.3405 \times 10^{-4} \text{ mol NaOH} \\ 7.3405 \times 10^{-4} \text{ mol NaOH} \times \frac{1 \text{ mol C}_6\text{H}_8\text{O}_7}{3 \text{ mol NaOH}} &= 2.4468 \times 10^{-4} \text{ mol C}_6\text{H}_8\text{O}_7 \\ 2.4468 \times 10^{-4} \text{ mol C}_6\text{H}_8\text{O}_7 \times \frac{192.1 \text{ g C}_6\text{H}_8\text{O}_7}{\text{mol C}_6\text{H}_8\text{O}_7} &= 0.04700 \text{ g C}_6\text{H}_8\text{O}_7 \end{aligned}$$

Because this is the amount of citric acid in a 50.00 mL sample, the concentration of citric acid in the citrus drink is 0.09400 g/100 mL. The complete titration curve is shown in Figure 9.2.15 b.

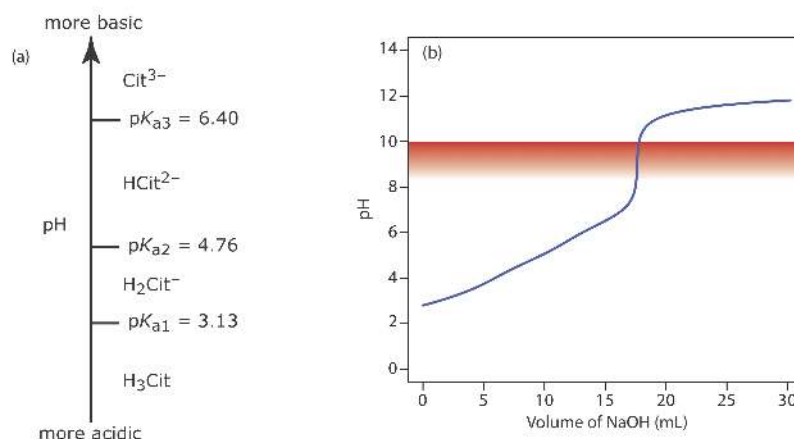


Figure 9.2.15 . (a) Ladder diagram for citric acid; (b) Titration curve for the sample in Example 9.2.2 showing phenolphthalein's pH transition region.

? Exercise 9.2.6

Your company recently received a shipment of salicylic acid, $\text{C}_7\text{H}_6\text{O}_3$, for use in the production of acetylsalicylic acid (aspirin). You can accept the shipment only if the salicylic acid is more than 99% pure. To evaluate the shipment's purity, you dissolve a 0.4208-g sample in water and titrate to the phenolphthalein end point, using 21.92 mL of 0.1354 M NaOH. Report the shipment's purity as %w/w $\text{C}_7\text{H}_6\text{O}_3$. Salicylic acid is a diprotic weak acid with $\text{p}K_a$ values of 2.97 and 13.74.

Answer

Because salicylic acid is a diprotic weak acid, we must first determine to which equivalence point it is being titrated. Using salicylic acid's $\text{p}K_a$ values as a guide, the pH at the first equivalence point is between 2.97 and 13.74, and the second equivalence point is at a pH greater than 13.74. From Table 9.2.3, phenolphthalein's end point is in the pH range 8.3–10.0. The titration, therefore, is to the first equivalence point for which the moles of NaOH equal the moles of salicylic acid; thus

$$\begin{aligned} (0.1354 \text{ M})(0.02192 \text{ L}) &= 2.968 \times 10^{-3} \text{ mol NaOH} \\ 2.968 \times 10^{-3} \text{ mol NaOH} \times \frac{1 \text{ mol C}_7\text{H}_6\text{O}_3}{\text{mol NaOH}} \times \frac{138.12 \text{ g C}_7\text{H}_6\text{O}_3}{\text{mol C}_7\text{H}_6\text{O}_3} &= 0.4099 \text{ g C}_7\text{H}_6\text{O}_3 \\ \frac{0.4099 \text{ g C}_7\text{H}_6\text{O}_3}{0.4208 \text{ g sample}} \times 100 &= 97.41 \% \text{w/w C}_7\text{H}_6\text{O}_3 \end{aligned}$$

Because the purity of the sample is less than 99%, we reject the shipment.

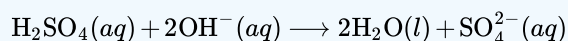
In an indirect analysis the analyte participates in one or more preliminary reactions, one of which produces or consumes acid or base. Despite the additional complexity, the calculations are straightforward.

✓ Example 9.2.3

The purity of a pharmaceutical preparation of sulfanilamide, $\text{C}_6\text{H}_4\text{N}_2\text{O}_2\text{S}$, is determined by oxidizing the sulfur to SO_2 and bubbling it through H_2O_2 to produce H_2SO_4 . The acid is titrated to the bromothymol blue end point using a standard solution of NaOH. Calculate the purity of the preparation given that a 0.5136-g sample requires 48.13 mL of 0.1251 M NaOH.

Solution

The bromothymol blue end point has a pH range of 6.0–7.6. Sulfuric acid is a diprotic acid, with a $\text{p}K_{a2}$ of 1.99 (the first K_a value is very large and the acid dissociation reaction goes to completion, which is why H_2SO_4 is a strong acid). The titration, therefore, proceeds to the second equivalence point and the titration reaction is



Using the titration results, there are

$$(0.1251 \text{ M NaOH})(0.04813 \text{ L NaOH}) = 6.021 \times 10^{-3} \text{ mol NaOH}$$

$$6.012 \times 10^{-3} \text{ mol NaOH} \times \frac{1 \text{ mol H}_2\text{SO}_4}{2 \text{ mol NaOH}} = 3.010 \times 10^{-3} \text{ mol H}_2\text{SO}_4$$

$$3.010 \times 10^{-3} \text{ mol H}_2\text{SO}_4 \times \frac{1 \text{ mol S}}{\text{mol H}_2\text{SO}_4} \times \frac{1 \text{ mol C}_6\text{H}_4\text{N}_2\text{O}_2\text{S}}{\text{mol S}} \times \frac{168.17 \text{ g C}_6\text{H}_4\text{N}_2\text{O}_2\text{S}}{\text{mol C}_6\text{H}_4\text{N}_2\text{O}_2\text{S}} = 0.5062 \text{ g C}_6\text{H}_4\text{N}_2\text{O}_2\text{S}$$

produced when the SO_2 is bubbled through H_2O_2 . Because all the sulfur in H_2SO_4 comes from the sulfanilamide, we can use a conservation of mass to determine the amount of sulfanilamide in the sample.

$$\frac{0.5062 \text{ g C}_6\text{H}_4\text{N}_2\text{O}_2\text{S}}{0.5136 \text{ g sample}} \times 100 = 98.56 \% \text{w/w C}_6\text{H}_4\text{N}_2\text{O}_2\text{S}$$

? Exercise 9.2.7

The concentration of NO_2 in air is determined by passing the sample through a solution of H_2O_2 , which oxidizes NO_2 to HNO_3 , and titrating the HNO_3 with NaOH . What is the concentration of NO_2 , in mg/L, if a 5.0 L sample of air requires 9.14 mL of 0.01012 M NaOH to reach the methyl red end point

Answer

The moles of HNO_3 produced by pulling the sample through H_2O_2 is

$$(0.01012 \text{ M})(0.00914 \text{ L}) \times \frac{1 \text{ mol HNO}_3}{\text{mol NaOH}} = 9.25 \times 10^{-5} \text{ mol HNO}_3$$

A conservation of mass on nitrogen requires that each mole of NO_2 produces one mole of HNO_3 ; thus, the mass of NO_2 in the sample is

$$9.25 \times 10^{-5} \text{ mol HNO}_3 \times \frac{1 \text{ mol NO}_2}{\text{mol HNO}_3} \times \frac{46.01 \text{ g NO}_2}{\text{mol NO}_2} = 4.26 \times 10^{-3} \text{ g NO}_2$$

and the concentration of NO_2 is

$$\frac{4.26 \times 10^{-3} \text{ g NO}_2}{5 \text{ L air}} \times \frac{1000 \text{ mg}}{\text{g}} = 0.852 \text{ mg NO}_2 \text{ L air}$$

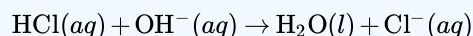
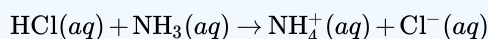
For a back titration we must consider two acid–base reactions. Again, the calculations are straightforward.

✓ Example 9.2.4

The amount of protein in a sample of cheese is determined by a Kjeldahl analysis for nitrogen. After digesting a 0.9814-g sample of cheese, the nitrogen is oxidized to NH_4^+ , converted to NH_3 with NaOH , and the NH_3 distilled into a collection flask that contains 50.00 mL of 0.1047 M HCl . The excess HCl is back titrated with 0.1183 M NaOH , requiring 22.84 mL to reach the bromothymol blue end point. Report the %w/w protein in the cheese assuming there are 6.38 grams of protein for every gram of nitrogen in most dairy products.

Solution

The HCl in the collection flask reacts with two bases



The collection flask originally contains

$$(0.1047 \text{ M HCl})(0.05000 \text{ L HCl}) = 5.235 \times 10^{-3} \text{ mol HCl}$$

of which

$$(0.1183 \text{ M NaOH})(0.02284 \text{ L NaOH}) \times \frac{1 \text{ mol HCl}}{\text{mol NaOH}} = 2.702 \times 10^{-3} \text{ mol HCl}$$

react with NaOH. The difference between the total moles of HCl and the moles of HCl that react with NaOH is the moles of HCl that react with NH_3 .

$$5.235 \times 10^{-3} \text{ mol HCl} - 2.702 \times 10^{-3} \text{ mol HCl} = 2.533 \times 10^{-3} \text{ mol HCl}$$

Because all the nitrogen in NH_3 comes from the sample of cheese, we use a conservation of mass to determine the grams of nitrogen in the sample.

$$2.533 \times 10^{-3} \text{ mol HCl} \times \frac{1 \text{ mol NH}_3}{\text{mol HCl}} \times \frac{14.01 \text{ g N}}{\text{mol NH}_3} = 0.03549 \text{ g N}$$

The mass of protein, therefore, is

$$0.03549 \text{ g N} \times \frac{6.38 \text{ g protein}}{\text{g N}} = 0.2264 \text{ g protein}$$

and the % w/w protein is

$$\frac{0.2264 \text{ g protein}}{0.9814 \text{ g sample}} \times 100 = 23.1 \% \text{w/w protein}$$

? Exercise 9.2.8

Limestone consists mainly of CaCO_3 , with traces of iron oxides and other metal oxides. To determine the purity of a limestone, a 0.5413-g sample is dissolved using 10.00 mL of 1.396 M HCl. After heating to expel CO_2 , the excess HCl was titrated to the phenolphthalein end point, requiring 39.96 mL of 0.1004 M NaOH. Report the sample's purity as %w/w CaCO_3 .

Answer

The total moles of HCl used in this analysis is

$$(1.396 \text{ M})(0.01000 \text{ L}) = 1.396 \times 10^{-2} \text{ mol HCl}$$

Of the total moles of HCl

$$(0.1004 \text{ M NaOH})(0.03996 \text{ L}) \times \frac{1 \text{ mol HCl}}{\text{mol NaOH}} = 4.012 \times 10^{-3} \text{ mol HCl}$$

are consumed in the back titration with NaOH, which means that

$$1.396 \times 10^{-2} \text{ mol HCl} - 4.012 \times 10^{-3} \text{ mol HCl} \\ = 9.95 \times 10^{-3} \text{ mol HCl}$$

react with the CaCO_3 . Because CO_3^{2-} is dibasic, each mole of CaCO_3 consumes two moles of HCl; thus

$$9.95 \times 10^{-3} \text{ mol HCl} \times \frac{1 \text{ mol CaCO}_3}{2 \text{ mol HCl}} \times \\ \frac{100.09 \text{ g CaCO}_3}{\text{mol CaCO}_3} = 0.498 \text{ g CaCO}_3 \\ \frac{0.498 \text{ g CaCO}_3}{0.5143 \text{ g sample}} \times 100 = 96.8 \% \text{w/w CaCO}_3$$

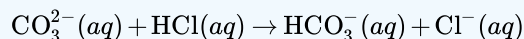
Earlier we noted that we can use an acid–base titration to analyze a mixture of acids or bases by titrating to more than one equivalence point. The concentration of each analyte is determined by accounting for its contribution to each equivalence point.

✓ Example 9.2.5

The alkalinity of natural waters usually is controlled by OH^- , HCO_3^- , and CO_3^{2-} , present singularly or in combination. Titrating a 100.0-mL sample to a pH of 8.3 requires 18.67 mL of 0.02812 M HCl. A second 100.0-mL aliquot requires 48.12 mL of the same titrant to reach a pH of 4.5. Identify the sources of alkalinity and their concentrations in milligrams per liter.

Solution

Because the volume of titrant to reach a pH of 4.5 is more than twice that needed to reach a pH of 8.3, we know from [Table 9.2.5](#), that the sample's alkalinity is controlled by CO_3^{2-} and HCO_3^- . Titrating to a pH of 8.3 neutralizes CO_3^{2-} to HCO_3^-

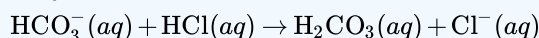
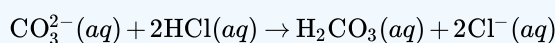


but there is no reaction between the titrant and HCO_3^- (see [Figure 9.2.14](#)). The concentration of CO_3^{2-} in the sample, therefore, is

$$(0.02812 \text{ M HCl})(0.01867 \text{ L HCl}) \times \frac{1 \text{ mol CO}_3^{2-}}{\text{mol HCl}} = 5.250 \times 10^{-4} \text{ mol CO}_3^{2-}$$

$$\frac{5.250 \times 10^{-4} \text{ mol CO}_3^{2-}}{0.1000 \text{ L}} \times \frac{60.01 \text{ g CO}_3^{2-}}{\text{mol CO}_3^{2-}} \times \frac{1000 \text{ mg}}{\text{g}} = 315.1 \text{ mg/L}$$

Titrating to a pH of 4.5 neutralizes CO_3^{2-} to H_2CO_3 and neutralizes HCO_3^- to H_2CO_3 (see [Figure 9.2.14](#)).



Because we know how many moles of CO_3^{2-} are in the sample, we can calculate the volume of HCl it consumes.

$$5.250 \times 10^{-4} \text{ mol CO}_3^{2-} \times \frac{2 \text{ mol HCl}}{\text{mol CO}_3^{2-}} \times \frac{1 \text{ L HCl}}{0.02812 \text{ mol HCl}} \times \frac{1000 \text{ mL}}{\text{L}} = 37.34 \text{ mL HCl}$$

This leaves 48.12 mL–37.34 mL, or 10.78 mL of HCl to react with HCO_3^- . The amount of HCO_3^- in the sample is

$$(0.02812 \text{ M HCl})(0.01078 \text{ L HCl}) \times \frac{1 \text{ mol HCO}_3^-}{\text{mol HCl}} = 3.031 \times 10^{-4} \text{ mol HCO}_3^-$$

The sample contains 315.1 mg CO_3^{2-} /L and 185.0 mg HCO_3^- /L

? Exercise 9.2.9

Samples that contain a mixture of the monoprotic weak acids 2-methylanilinium chloride ($\text{C}_7\text{H}_{10}\text{NCl}$, $\text{pK}_a = 4.447$) and 3-nitrophenol ($\text{C}_6\text{H}_5\text{NO}_3$, $\text{pK}_a = 8.39$) can be analyzed by titrating with NaOH. A 2.006-g sample requires 19.65 mL of 0.200 M NaOH to reach the bromocresol purple end point and 48.41 mL of 0.200 M NaOH to reach the phenolphthalein end point. Report the %w/w of each compound in the sample.

Answer

Of the two analytes, 2-methylanilinium is the stronger acid and is the first to react with the titrant. Titrating to the bromocresol purple end point, therefore, provides information about the amount of 2-methylanilinium in the sample.

$$(0.200 \text{ M NaOH})(0.01965 \text{ L}) \times \frac{1 \text{ mol C}_7\text{H}_{10}\text{NCl}}{\text{mol NaOH}} \times \frac{143.61 \text{ g C}_7\text{H}_{10}\text{NCl}}{\text{mol C}_7\text{H}_{10}\text{NCl}} = 0.564 \text{ g C}_7\text{H}_{10}\text{NCl}$$

$$\frac{0.564 \text{ g C}_7\text{H}_{10}\text{NCl}}{2.006 \text{ g sample}} \times 100 = 28.1 \% \text{w/w C}_7\text{H}_{10}\text{NCl}$$

Titrating from the bromocresol purple end point to the phenolphthalein end point, a total of 48.41 mL – 19.65 mL = 28.76 mL, gives the amount of NaOH that reacts with 3-nitrophenol. The amount of 3-nitrophenol in the sample, therefore, is

$$(0.200 \text{ M NaOH})(0.02876 \text{ L}) \times \frac{1 \text{ mol C}_6\text{H}_5\text{NO}_3}{\text{mol NaOH}} \times \frac{139.11 \text{ g C}_6\text{H}_5\text{NO}_3}{\text{mol C}_6\text{H}_5\text{NO}_3} = 0.800 \text{ g C}_6\text{H}_5\text{NO}_3$$

$$\frac{0.800 \text{ g C}_6\text{H}_5\text{NO}_3}{2.006 \text{ g sample}} \times 100 = 39.8 \% \text{w/w C}_6\text{H}_5\text{NO}_3$$

Qualitative Applications

Example 9.5 shows how we can use an acid–base titration to determine the forms of alkalinity in waters and their concentrations. We can extend this approach to other systems. For example, if we titrate a sample to the methyl orange end point and the phenolphthalein end point using either a strong acid or a strong base, we can determine which of the following species are present and their concentrations: H_3PO_4 , H_2PO_4^- , HPO_4^{2-} , PO_4^{3-} , HCl , and NaOH . As outlined in Table 9.2.8, each species or mixture of species has a unique relationship between the volumes of titrant needed to reach these two end points. Note that mixtures containing three or more these species are not possible.

Use a ladder diagram to convince yourself that mixtures containing three or more of these species are unstable.

Table 9.2.8 . Relationship Between End Point Volumes for Mixtures of Phosphate Species with HCl and NaOH

solution composition	relationship between end point volumes with strong base titrant	relationship between end point volumes with strong acid titrant
H_3PO_4	$V_{\text{PH}} = 2 \times V_{\text{MO}}$	—
H_2PO_4^-	$V_{\text{PH}} > 0; V_{\text{MO}} = 0$	—
HPO_4^{2-}	—	$V_{\text{MO}} > 0; V_{\text{PH}} = 0$
PO_4^{3-}	—	$V_{\text{MO}} = 2 \times V_{\text{PH}}$
HCl	$V_{\text{PH}} = V_{\text{MO}}$	—
NaOH	—	$V_{\text{MO}} = V_{\text{PH}}$
HCl and H_3PO_4	$V_{\text{PH}} < 2 \times V_{\text{MO}}$	—
H_3PO_4 and H_2PO_4^-	$V_{\text{PH}} > 2 \times V_{\text{MO}}$	—
H_2PO_4^- and HPO_4^{2-}	$V_{\text{PH}} > 0; V_{\text{MO}} = 0$	$V_{\text{MO}} > 0; V_{\text{PH}} = 0$
HPO_4^{2-} and PO_4^{3-}	—	$V_{\text{MO}} > 2 \times V_{\text{PH}}$
PO_4^{3-} and NaOH	—	$V_{\text{MO}} < 2 \times V_{\text{PH}}$

V_{PH} and V_{MO} are, respectively, the volume of titrant at the phenolphthalein and methyl orange end points
when no information is provided, the volume at each end point is zero

Characterization Applications

In addition to a quantitative analysis and a qualitative analysis, we also can use an acid–base titration to characterize the chemical and physical properties of matter. Two useful characterization applications are the determination of a compound's equivalent weight and the determination of its acid dissociation constant or its base dissociation constant.

Equivalent Weights

Suppose we titrate a sample of an impure weak acid to a well-defined end point using a monoprotic strong base as the titrant. If we assume the titration involves the transfer of n protons, then the moles of titrant needed to reach the end point is

$$\text{moles titrant} = \frac{n \text{ moles titrant}}{\text{moles analyte}} \times \text{moles analyte}$$

If we know the analyte's identity, we can use this equation to determine the amount of analyte in the sample

$$\text{grams analyte} = \text{moles titrant} \times \frac{1 \text{ mole analyte}}{n \text{ moles analyte}} \times FW \text{ analyte}$$

where FW is the analyte's formula weight.

But what if we do not know the analyte's identity? If we titrate a pure sample of the analyte, we can obtain some useful information that may help us establish its identity. Because we do not know the number of protons that are titrated, we let $n = 1$ and replace the analyte's formula weight with its equivalent weight (EW)

$$\text{grams analyte} = \text{moles titrant} \times \frac{1 \text{ equivalent analyte}}{1 \text{ mole analyte}} = EW \text{ analyte}$$

where

$$FW = n \times EW$$

✓ Example 9.2.6

A 0.2521-g sample of an unknown weak acid is titrated with 0.1005 M NaOH, requiring 42.68 mL to reach the phenolphthalein end point. Determine the compound's equivalent weight. Which of the following compounds is most likely to be the unknown weak acid?

acid	formula	formula weight (g/mol)	type
ascorbic acid	$C_8H_8O_6$	176.1	monoprotic
malonic acid	$C_3H_4O_4$	104.1	diprotic
succinic acid	$C_4H_6O_4$	118.1	diprotic
citric acid	$C_6H_8O_7$	192.1	triprotic

Solution

The moles of NaOH needed to reach the end point is

$$(0.1005 \text{ M NaOH})(0.04268 \text{ L NaOH}) = 4.289 \times 10^{-3} \text{ mol NaOH}$$

The equivalents of weak acid are the same as the moles of NaOH used in the titration; thus, the analyte's equivalent weight is

$$EW = \frac{0.2521 \text{ g}}{4.289 \times 10^{-3} \text{ equivalents}} = 58.78 \text{ g/equivalent}$$

The possible formula weights for the weak acid are 58.78 g/mol ($n = 1$), 117.6 g/mol ($n = 2$), and 176.3 g/mol ($n = 3$). If the analyte is a monoprotic weak acid, then its formula weight is 58.78 g/mol, eliminating ascorbic acid as a possibility. If it is a diprotic weak acid, then the analyte's formula weight is either 58.78 g/mol or 117.6 g/mol, depending on whether the weak acid was titrated to its first or its second equivalence point. Succinic acid, with a formula weight of 118.1 g/mole is a possibility, but malonic acid is not. If the analyte is a triprotic weak acid, then its formula weight is 58.78 g/mol, 117.6 g/mol, or 176.3 g/mol. None of these values is close to the formula weight for citric acid, eliminating it as a possibility. Only succinic acid provides a possible match.

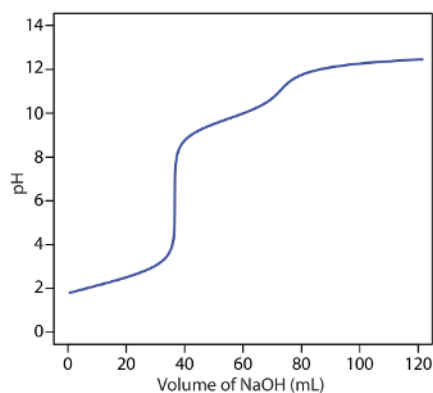


Figure 9.2.16 . Titration curve for Exercise 9.2.10 .

? Exercise 9.2.10

Figure 9.2.16 shows the potentiometric titration curve for the titration of a 0.500-g sample an unknown weak acid. The titrant is 0.1032 M NaOH. What is the weak acid's equivalent weight?

Answer

The first of the two visible end points is approximately 37 mL of NaOH. The analyte's equivalent weight, therefore, is

$$(0.1032 \text{ M NaOH})(0.037 \text{ L}) \times \frac{1 \text{ equivalent}}{\text{mol NaOH}} = 3.8 \times 10^{-3} \text{ equivalents}$$

$$EW = \frac{0.5000 \text{ g}}{3.8 \times 10^{-3} \text{ equivalents}} = 1.3 \times 10^2 \text{ g/equivalent}$$

Equilibrium Constants

Another application of acid–base titrimetry is the determination of a weak acid's or a weak base's dissociation constant. Consider, for example, a solution of acetic acid, CH_3COOH , for which the dissociation constant is

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

When the concentrations of CH_3COOH and CH_3COO^- are equal, the K_a expression reduces to $K_a = [\text{H}_3\text{O}^+]$, or $\text{pH} = \text{p}K_a$. If we titrate a solution of acetic acid with NaOH, the pH equals the $\text{p}K_a$ when the volume of NaOH is approximately $1/2V_{eq}$. As shown in Figure 9.2.17 , a potentiometric titration curve provides a reasonable estimate of acetic acid's $\text{p}K_a$.

Recall that $\text{pH} = \text{p}K_a$ is a step on a ladder diagram, which divides the pH axis into two regions, one where the weak acid is the predominate species, and one where its conjugate weak base is the predominate species.

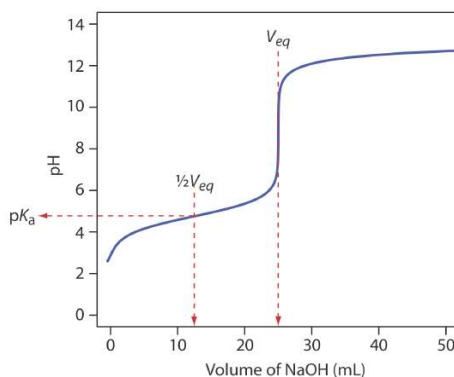


Figure 9.2.17 . Estimating acetic acid's $\text{p}K_a$ using its potentiometric titration curve.

This method provides a reasonable estimate for a weak acid's pK_a if the acid is neither too strong nor too weak. These limitations are easy to appreciate if we consider two limiting cases. For the first limiting case, let's assume the weak acid, HA, is more than 50% dissociated before the titration begins (a relatively large K_a value); in this case the concentration of HA before the equivalence point is always less than the concentration of A^- and there is no point on the titration curve where $[HA] = [A^-]$. At the other extreme, if the acid is too weak, then less than 50% of the weak acid reacts with the titrant at the equivalence point. In this case the concentration of HA before the equivalence point is always greater than that of A^- . Determining the pK_a by the half-equivalence point method overestimates its value if the acid is too strong and underestimates its value if the acid is too weak.

? Exercise 9.2.11

Use the potentiometric titration curve in [Figure 9.2.16](#) to estimate the pK_a values for the weak acid in [Exercise 9.2.10](#).

Answer

At $1/2V_{eq}$, or approximately 18.5 mL, the pH is approximately 2.2; thus, we estimate that the analyte's pK_a is 2.2.

A second approach for determining a weak acid's pK_a is to use a Gran plot. For example, earlier in this chapter we derived the following equation for the titration of a weak acid with a strong base.

$$[H_3O^+] \times V_b = K_a V_{eq} - K_a V_b$$

A plot of $[H_3O^+] \times V_b$ versus V_b for volumes less than the equivalence point yields a straight line with a slope of $-K_a$. Other linearizations have been developed that use the entire titration curve or that require no assumptions [(a) Gonzalez, A. G.; Asuero, A. G. *Anal. Chim. Acta* **1992**, 256, 29–33; (b) Papanastasiou, G.; Ziogas, I.; Kokkindis, G. *Anal. Chim. Acta* **1993**, 277, 119–135]. This approach to determining an acidity constant has been used to study the acid–base properties of humic acids, which are naturally occurring, large molecular weight organic acids with multiple acidic sites. In one study a humic acid was found to have six titratable sites, three which were identified as carboxylic acids, two which were believed to be secondary or tertiary amines, and one which was identified as a phenolic group [Alexio, L. M.; Godinho, O. E. S.; da Costa, W. F. *Anal. Chim. Acta* **1992**, 257, 35–39].

Values of K_a determined by this method may have a substantial error if the effect of activity is ignored. See [Chapter 6.9](#) for a discussion of activity.

Evaluation of Acid–Base Titrimetry

Scale of Operation

In an acid–base titration, the volume of titrant needed to reach the equivalence point is proportional to the moles of titrand. Because the pH of the titrand or the titrant is a function of its concentration, the change in pH at the equivalence point—and thus the feasibility of an acid–base titration—depends on their respective concentrations. [Figure 9.2.18](#), for example, shows a series of titration curves for the titration of several concentrations of HCl with equimolar solutions NaOH. For titrand and titrant concentrations smaller than 10^{-3} M, the change in pH at the end point is too small to provide an accurate and a precise result.

Acid–base titrimetry is an example of a total analysis technique in which the signal is proportional to the absolute amount of analyte. See [Chapter 3](#) for a discussion of the difference between total analysis techniques and concentration techniques.

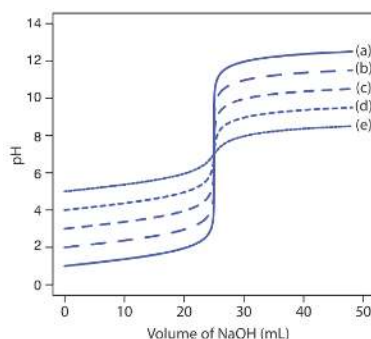


Figure 9.2.18 . Titration curves for 25.0 mL of (a) 10^{-1} M HCl, (b) 10^{-2} M HCl, (c) 10^{-3} M HCl, (d) 10^{-4} M HCl, and (e) 10^{-5} M HCl. In each case the titrant is an equimolar solution of NaOH.

A minimum concentration of 10^{-3} M places limits on the smallest amount of analyte we can analyze successfully. For example, suppose our analyte has a formula weight of 120 g/mol. To successfully monitor the titration's end point using an indicator or a pH probe, the titrand needs an initial volume of approximately 25 mL. If we assume the analyte's formula weight is 120 g/mol, then each sample must contain at least 3 mg of analyte. For this reason, acid–base titrations generally are limited to major and minor analytes. We can extend the analysis of gases to trace analytes by pulling a large volume of the gas through a suitable collection solution.

We need a volume of titrand sufficient to cover the tip of the pH probe or to allow for an easy observation of the indicator's color. A volume of 25 mL is not an unreasonable estimate of the minimum volume.

One goal of analytical chemistry is to extend analyses to smaller samples. Here we describe two interesting approaches to titrating μL and pL samples. In one experimental design (Figure 9.2.19), samples of 20–100 μL are held by capillary action between a flat-surface pH electrode and a stainless steel sample stage [Steele, A.; Hieftje, G. M. *Anal. Chem.* **1984**, 56, 2884–2888]. The titrant is added using the oscillations of a piezoelectric ceramic device to move an angled glass rod in and out of a tube connected to a reservoir that contains the titrant. Each time the glass tube is withdrawn an approximately 2 nL microdroplet of titrant is released. The microdroplets are allowed to fall onto the sample, with mixing accomplished by spinning the sample stage at 120 rpm. A total of 450 microdroplets, with a combined volume of 0.81–0.84 μL , is dispensed between each pH measurement. In this fashion a titration curve is constructed. This method has been used to titrate solutions of 0.1 M HCl and 0.1 M CH_3COOH with 0.1 M NaOH. Absolute errors ranged from a minimum of +0.1% to a maximum of –4.1%, with relative standard deviations from 0.15% to 4.7%. Samples as small as 20 μL were titrated successfully.

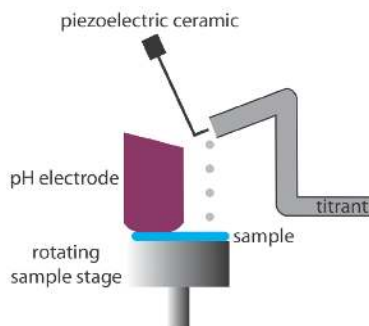


Figure 9.2.19 . Experimental design for a microdroplet titration apparatus.

Another approach carries out the acid–base titration in a single drop of solution [(a) Gratzl, M.; Yi, C. *Anal. Chem.* **1993**, 65, 2085–2088; (b) Yi, C.; Gratzl, M. *Anal. Chem.* **1994**, 66, 1976–1982; (c) Hui, K. Y.; Gratzl, M. *Anal. Chem.* **1997**, 69, 695–698; (d) Yi, C.; Huang, D.; Gratzl, M. *Anal. Chem.* **1996**, 68, 1580–1584; (e) Xie, H.; Gratzl, M. *Anal. Chem.* **1996**, 68, 3665–3669]. The titrant is delivered using a microburet fashioned from a glass capillary micropipet (Figure 9.2.20). The microburet has a 1–2 μm tip filled with an agar gel membrane. The tip of the microburet is placed within a drop of the sample solution, which is suspended in heptane, and the titrant is allowed to diffuse into the sample. The titration's progress is monitored using an acid–base indicator and the time needed to reach the end point is measured. The rate of the titrant's diffusion from the microburet is determined by a prior

calibration. Once calibrated the end point time is converted to an end point volume. Samples usually consist of picoliter volumes (10^{-12} liters), with the smallest sample being 0.7 pL. The precision of the titrations is about 2%.

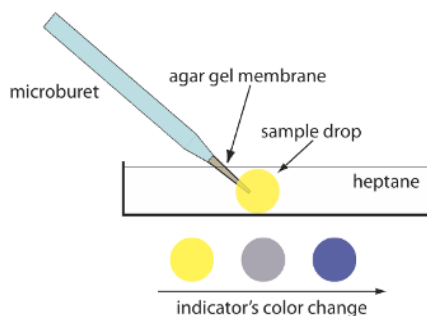


Figure 9.2.20 . Experimental set-up for a diffusional microtitration. The indicator is a mixture of bromothymol blue and bromocresol purple.

Titrations conducted with microliter or picoliter sample volumes require a smaller absolute amount of analyte. For example, diffusional titrations have been conducted on as little as 29 femtomoles (10^{-15} moles) of nitric acid. Nevertheless, the analyte must be present in the sample at a major or minor level for the titration to give accurate and precise results.

Accuracy

When working with a macro-major or a macro-minor sample, an acid-base titration can achieve a relative error of 0.1–0.2%. The principal limitation to accuracy is the difference between the end point and the equivalence point.

Precision

An acid-base titration's relative precision depends primarily on the precision with which we can measure the end point volume and the precision in detecting the end point. Under optimum conditions, an acid-base titration has a relative precision of 0.1–0.2%. We can improve the relative precision by using the largest possible buret and by ensuring we use most of its capacity in reaching the end point. A smaller volume buret is a better choice when using costly reagents, when waste disposal is a concern, or when we must complete the titration quickly to avoid competing chemical reactions. An automatic titrator is particularly useful for titrations that require small volumes of titrant because it provides significantly better precision (typically about $\pm 0.05\%$ of the buret's volume).

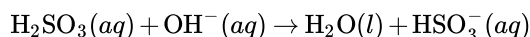
The precision of detecting the end point depends on how it is measured and the slope of the titration curve at the end point. With an indicator the precision of the end point signal usually is ± 0.03 – 0.10 mL. Potentiometric end points usually are more precise.

Sensitivity

For an acid-base titration we can write the following general analytical equation to express the titrant's volume in terms of the amount of titrand

$$\text{volume of titrant} = k \times \text{moles of titrand}$$

where k , the sensitivity, is determined by the stoichiometry between the titrand and the titrant. Consider, for example, the determination of sulfurous acid, H_2SO_3 , by titrating with NaOH to the first equivalence point



At the equivalence point the relationship between the moles of NaOH and the moles of H_2SO_3 is

$$\text{mol NaOH} = \text{mol H}_2\text{SO}_3$$

Substituting the titrant's molarity and volume for the moles of NaOH and rearranging

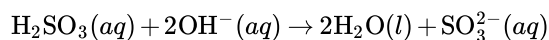
$$M_{\text{NaOH}} \times V_{\text{NaOH}} = \text{mol H}_2\text{SO}_3$$

$$V_{\text{NaOH}} = \frac{1}{M_{\text{NaOH}}} \times \text{mol H}_2\text{SO}_3$$

we find that k is

$$k = \frac{1}{M_{\text{NaOH}}}$$

There are two ways in which we can improve a titration's sensitivity. The first, and most obvious, is to decrease the titrant's concentration because it is inversely proportional to the sensitivity, k . The second approach, which applies only if the titrand is multiprotic, is to titrate to a later equivalence point. If we titrate H_2SO_3 to its second equivalence point



then each mole of H_2SO_3 consumes two moles of NaOH

$$\text{mol NaOH} = 2 \times \text{mol H}_2\text{SO}_3$$

and the sensitivity becomes

$$k = \frac{2}{M_{\text{NaOH}}}$$

In practice, however, any improvement in sensitivity is offset by a decrease in the end point's precision if a larger volume of titrant requires us to refill the buret. For this reason, standard acid–base titrimetric procedures are written to ensure that a titration uses 60–100% of the buret's volume.

Selectivity

Acid–base titrants are not selective. A strong base titrant, for example, reacts with all acids in a sample, regardless of their individual strengths. If the titrand contains an analyte and an interferent, then selectivity depends on their relative acid strengths. Let's consider two limiting situations.

If the analyte is a stronger acid than the interferent, then the titrant will react with the analyte before it begins reacting with the interferent. The feasibility of the analysis depends on whether the titrant's reaction with the interferent affects the accurate location of the analyte's equivalence point. If the acid dissociation constants are substantially different, the end point for the analyte can be determined accurately. Conversely, if the acid dissociation constants for the analyte and interferent are similar, then there may not be an accurate end point for the analyte. In the latter case a quantitative analysis for the analyte is not possible.

In the second limiting situation the analyte is a weaker acid than the interferent. In this case the volume of titrant needed to reach the analyte's equivalence point is determined by the concentration of both the analyte and the interferent. To account for the interferent's contribution to the end point, an end point for the interferent must be available. Again, if the acid dissociation constants for the analyte and interferent are significantly different, then the analyte's determination is possible. If the acid dissociation constants are similar, however, there is only a single equivalence point and we cannot separate the analyte's and the interferent's contributions to the equivalence point volume.

Time, Cost, and Equipment

Acid–base titrations require less time than most gravimetric procedures, but more time than many instrumental methods of analysis, particularly when analyzing many samples. With an automatic titrator, however, concerns about analysis time are less significant. When performing a titration manually our equipment needs—a buret and, perhaps, a pH meter—are few in number, inexpensive, routinely available, and easy to maintain. Automatic titrators are available for between \$3000 and \$10 000.

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CHAPTER OVERVIEW

6: Atomic Emission Spectroscopy

[6.1: Overview of Spectroscopy](#)

[6.2: Emission Spectroscopy](#)

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6.1: Overview of Spectroscopy

The focus of this chapter is on the interaction of ultraviolet, visible, and infrared radiation with matter. Because these techniques use optical materials to disperse and focus the radiation, they often are identified as optical spectroscopies. For convenience we will use the simpler term spectroscopy in place of optical **spectroscopy**; however, you should understand we will consider only a limited piece of what is a much broader area of analytical techniques.

Despite the difference in instrumentation, all spectroscopic techniques share several common features. Before we consider individual examples in greater detail, let's take a moment to consider some of these similarities. As you work through the chapter, this overview will help you focus on the similarities between different spectroscopic methods of analysis. You will find it easier to understand a new analytical method when you can see its relationship to other similar methods.

What is Electromagnetic Radiation?

Electromagnetic radiation—light—is a form of energy whose behavior is described by the properties of both waves and particles. Some properties of electromagnetic radiation, such as its refraction when it passes from one medium to another (Figure 10.1.1), are explained best when we describe light as a wave. Other properties, such as absorption and emission, are better described by treating light as a particle. The exact nature of electromagnetic radiation remains unclear, as it has since the development of quantum mechanics in the first quarter of the 20th century [Home, D.; Gribbin, J. *New Scientist* 1991, 2 Nov. 30–33]. Nevertheless, this dual model of wave and particle behavior provide a useful description for electromagnetic radiation.



Figure 10.1.1 . The Golden Gate bridge as seen through rain drops. Refraction of light by the rain drops produces the distorted images. Source: Brocken Inaglory (commons. Wikipedia.org).

Wave Properties of Electromagnetic Radiation

Electromagnetic radiation consists of oscillating electric and magnetic fields that propagate through space along a linear path and with a constant velocity. In a vacuum, electromagnetic radiation travels at the speed of light, c , which is 2.99792×10^8 m/s. When electromagnetic radiation moves through a medium other than a vacuum, its velocity, v , is less than the speed of light in a vacuum. The difference between v and c is sufficiently small ($<0.1\%$) that the speed of light to three significant figures, 3.00×10^8 m/s, is accurate enough for most purposes.

The oscillations in the electric field and the magnetic field are perpendicular to each other and to the direction of the wave's propagation. Figure 10.1.2 shows an example of plane-polarized electromagnetic radiation, which consists of a single oscillating electric field and a single oscillating magnetic field.

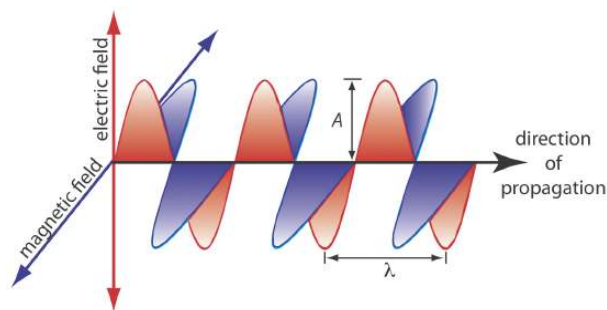


Figure 10.1.2 . Plane-polarized electromagnetic radiation showing the oscillating electric field in blue and the oscillating magnetic field in red. The radiation's amplitude, A , and its wavelength, λ , are shown. Normally, electromagnetic radiation is unpolarized, with oscillating electric and magnetic fields present in all possible planes perpendicular to the direction of propagation.

An electromagnetic wave is characterized by several fundamental properties, including its velocity, amplitude, frequency, phase angle, polarization, and direction of propagation [Ball, D. W. *Spectroscopy* **1994**, 9(5), 24–25]. For example, the amplitude of the oscillating electric field at any point along the propagating wave is

$$A_t = A_e \sin(2\pi\nu t + \Phi)$$

where A_t is the magnitude of the electric field at time t , A_e is the electric field's maximum **amplitude**, ν is the wave's **frequency**—the number of oscillations in the electric field per unit time—and Φ is a **phase angle** that accounts for the fact that A_t need not have a value of zero at $t = 0$. The identical equation for the magnetic field is

$$A_t = A_m \sin(2\pi\nu t + \Phi)$$

where A_m is the magnetic field's maximum amplitude.

Other properties also are useful for characterizing the wave behavior of electromagnetic radiation. The **wavelength**, λ , is defined as the distance between successive maxima (see Figure 10.1.2). For ultraviolet and visible electromagnetic radiation the wavelength usually is expressed in nanometers ($1 \text{ nm} = 10^{-9} \text{ m}$), and for infrared radiation it is expressed in microns ($1 \text{ mm} = 10^{-6} \text{ m}$). The relationship between wavelength and frequency is

$$\lambda = \frac{c}{\nu}$$

Another useful unit is the **wavenumber**, $\bar{\nu}$, which is the reciprocal of wavelength

$$\bar{\nu} = \frac{1}{\lambda}$$

Wavenumbers frequently are used to characterize infrared radiation, with the units given in cm^{-1} .

When electromagnetic radiation moves between different media—for example, when it moves from air into water—its frequency, ν , remains constant. Because its velocity depends upon the medium in which it is traveling, the electromagnetic radiation's wavelength, λ , changes. If we replace the speed of light in a vacuum, c , with its speed in the medium, v , then the wavelength is

$$\lambda = \frac{v}{\nu}$$

This change in wavelength as light passes between two media explains the refraction of electromagnetic radiation shown in Figure 10.1.1 .

✓ Example 10.1.1

In 1817, Josef Fraunhofer studied the spectrum of solar radiation, observing a continuous spectrum with numerous dark lines. Fraunhofer labeled the most prominent of the dark lines with letters. In 1859, Gustav Kirchhoff showed that the D line in the sun's spectrum was due to the absorption of solar radiation by sodium atoms. The wavelength of the sodium D line is 589 nm. What are the frequency and the wavenumber for this line?

Solution

The frequency and wavenumber of the sodium D line are

$$\begin{aligned}\nu &= \frac{c}{\lambda} = \frac{3.00 \times 10^8 \text{ m/s}}{589 \times 10^{-9} \text{ m}} = 5.09 \times 10^{14} \text{ s}^{-1} \\ \bar{\nu} &= \frac{1}{\lambda} = \frac{1}{589 \times 10^{-9} \text{ m}} \times \frac{1 \text{ m}}{100 \text{ cm}} = 1.70 \times 10^4 \text{ cm}^{-1}\end{aligned}$$

? Exercise 10.1.1

Another historically important series of spectral lines is the Balmer series of emission lines from hydrogen. One of its lines has a wavelength of 656.3 nm. What are the frequency and the wavenumber for this line?

Answer

The frequency and wavenumber for the line are

$$\nu = \frac{c}{\lambda} = \frac{3.00 \times 10^8 \text{ m/s}}{656.3 \times 10^{-9} \text{ m}} = 4.57 \times 10^{14} \text{ s}^{-1}$$

$$\bar{\nu} = \frac{1}{\lambda} = \frac{1}{656.3 \times 10^{-9} \text{ m}} \times \frac{1 \text{ m}}{100 \text{ cm}} = 1.524 \times 10^4 \text{ cm}^{-1}$$

Particle Properties of Electromagnetic Radiation

When matter absorbs electromagnetic radiation it undergoes a change in energy. The interaction between matter and electromagnetic radiation is easiest to understand if we assume that radiation consists of a beam of energetic particles called photons. When a **photon** is absorbed by a sample it is “destroyed” and its energy acquired by the sample [Ball, D. W. *Spectroscopy* 1994, 9(6) 20–21]. The energy of a photon, in joules, is related to its frequency, wavelength, and wavenumber by the following equalities

$$E = h\nu = \frac{hc}{\lambda} = hc\bar{\nu}$$

where h is Planck’s constant, which has a value of $6.626 \times 10^{-34} \text{ Js}$.

✓ Example 10.1.2

What is the energy of a photon from the sodium D line at 589 nm?

Solution

The photon’s energy is

$$E = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34} \text{ Js}) (3.00 \times 10^8 \text{ m/s})}{589 \times 10^{-7} \text{ m}} = 3.37 \times 10^{-19} \text{ J}$$

? Exercise 10.1.2

What is the energy of a photon for the Balmer line at a wavelength of 656.3 nm?

Answer

The photon’s energy is

$$E = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34} \text{ Js}) (3.00 \times 10^8 \text{ m/s})}{656.3 \times 10^{-9} \text{ m}} = 3.03 \times 10^{-19} \text{ J}$$

The Electromagnetic Spectrum

The frequency and the wavelength of electromagnetic radiation vary over many orders of magnitude. For convenience, we divide electromagnetic radiation into different regions—the **electromagnetic spectrum**—based on the type of atomic or molecular transitions that gives rise to the absorption or emission of photons (Figure 10.1.3). The boundaries between the regions of the electromagnetic spectrum are not rigid and overlap between spectral regions is possible.

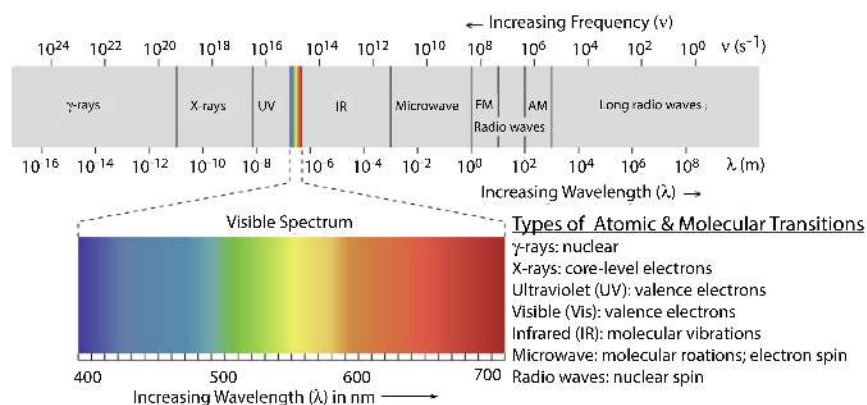


Figure 10.1.3 . The electromagnetic spectrum showing the boundaries between different regions and the type of atomic or molecular transitions responsible for the change in energy. The colored inset shows the visible spectrum. Source: modified from Zedh (www.commonswikipedia.org).

Photons as a Signal Source

In the previous section we defined several characteristic properties of electromagnetic radiation, including its energy, velocity, amplitude, frequency, phase angle, polarization, and direction of propagation. A spectroscopic measurement is possible only if the photon's interaction with the sample leads to a change in one or more of these characteristic properties.

We will divide spectroscopy into two broad classes of techniques. In one class of techniques there is a transfer of energy between the photon and the sample. Table 10.1.1 provides a list of several representative examples.

Table 10.1.1 . Examples of Spectroscopic Techniques That Involve an Exchange of Energy Between a Photon and the Sample

type of energy transfer	region of electromagnetic spectrum	spectroscopic technique
absorption	γ -ray	Mossbauer spectroscopy
	X-ray	X-ray absorption spectroscopy
	UV/Vis	<i>UV/Vis spectroscopy</i>
	IR	<i>infrared spectroscopy</i>
	microwave	raman spectroscopy
	radio wave	electron spin resonance nuclear magnetic resonance
emission (thermal excitation)	UV/Vis	<i>atomic emission spectroscopy</i>
photoluminescence	X-ray	X-ray fluorescence
	UV/Vis	<i>fluorescence spectroscopy</i> <i>phosphorescence spectroscopy</i> atomic fluorescence spectroscopy
chemiluminescence	UV/Vis	chemiluminescence spectroscopy

techniques discussed in this chapter are shown in *italics*

In absorption spectroscopy a photon is absorbed by an atom or molecule, which undergoes a transition from a lower-energy state to a higher-energy, or excited state (Figure 10.1.4). The type of transition depends on the photon's energy. The electromagnetic spectrum in Figure 10.1.3 , for example, shows that absorbing a photon of visible light promotes one of the atom's or molecule's valence electrons to a higher-energy level. When an molecule absorbs infrared radiation, on the other hand, one of its chemical bonds experiences a change in vibrational energy.

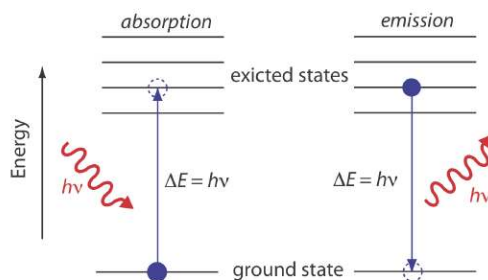


Figure 10.1.4 . A simplified energy diagram that shows the absorption and emission of a photon by an atom or a molecule. When a photon of energy $h\nu$ strikes the atom or molecule, absorption may occur if the difference in energy, ΔE , between the ground state and the excited state is equal to the photon's energy. An atom or molecule in an excited state may emit a photon and return to the ground state. The photon's energy, $h\nu$, equals the difference in energy, ΔE , between the two states.

When it absorbs electromagnetic radiation the number of photons passing through a sample decreases. The measurement of this decrease in photons, which we call **absorbance**, is a useful analytical signal. Note that each energy level in Figure 10.1.4 has a well-defined value because each is quantized. Absorption occurs only when the photon's energy, $h\nu$, matches the difference in energy, ΔE , between two energy levels. A plot of absorbance as a function of the photon's energy is called an **absorbance spectrum**. Figure 10.1.5 , for example, shows the absorbance spectrum of cranberry juice.

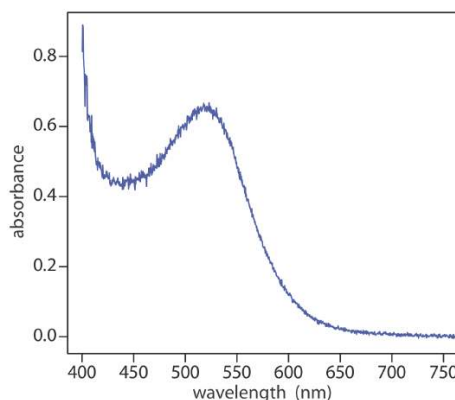


Figure 10.1.5 . Visible absorbance spectrum for cranberry juice. The anthocyanin dyes in cranberry juice absorb visible light with blue, green, and yellow wavelengths (see Figure 10.1.3). As a result, the juice appears red.

When an atom or molecule in an excited state returns to a lower energy state, the excess energy often is released as a photon, a process we call **emission** (Figure 10.1.4). There are several ways in which an atom or a molecule may end up in an excited state, including thermal energy, absorption of a photon, or as the result of a chemical reaction. Emission following the absorption of a photon is also called **photoluminescence**, and that following a chemical reaction is called **chemiluminescence**. A typical emission spectrum is shown in Figure 10.1.6 .

Molecules also can release energy in the form of heat. We will return to this point later in the chapter.

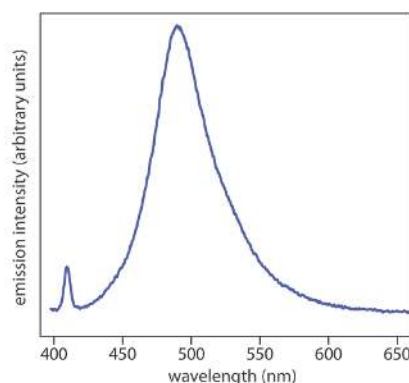


Figure 10.1.6 . Photoluminescence spectrum of the dye coumarin 343, which is incorporated in a reverse micelle suspended in cyclohexanol. The dye's absorbance spectrum (not shown) has a broad peak around 400 nm. The sharp peak at 409 nm is from the laser source used to excite coumarin 343. The broad band centered at approximately 500 nm is the dye's emission band. Because the dye absorbs blue light, a solution of coumarin 343 appears yellow in the absence of photoluminescence. Its photoluminescent emission is blue-green. Source: data from Bridget Gourley, Department of Chemistry & Biochemistry, DePauw University.

In the second broad class of spectroscopic techniques, the electromagnetic radiation undergoes a change in amplitude, phase angle, polarization, or direction of propagation as a result of its refraction, reflection, scattering, diffraction, or dispersion by the sample. Several representative spectroscopic techniques are listed in Table 10.1.2 .

Table 10.1.2 . Examples of Spectroscopic Techniques That Do Not Involve an Exchange of Energy Between a Photon and the Sample

region of electromagnetic spectrum	type of interaction	spectroscopic technique
X-ray	diffraction	X-ray diffraction
UV/Vis	refraction	refractometry
	scattering	<i>nephelometry</i> <i>turbidimetry</i>
	dispersion	optical rotary dispersion

techniques discussed in this chapter are shown in *italics*

Basic Components of Spectroscopic Instruments

The spectroscopic techniques in Table 10.1.1 and Table 10.1.2 use instruments that share several common basic components, including a source of energy, a means for isolating a narrow range of wavelengths, a detector for measuring the signal, and a signal processor that displays the signal in a form convenient for the analyst. In this section we introduce these basic components. Specific instrument designs are considered in later sections.

You will find a more detailed treatment of these components in the [additional resources](#) for this chapter.

Sources of Energy

All forms of spectroscopy require a source of energy. In absorption and scattering spectroscopy this energy is supplied by photons. Emission and photoluminescence spectroscopy use thermal, radiant (photon), or chemical energy to promote the analyte to a suitable excited state.

Sources of Electromagnetic Radiation. A source of electromagnetic radiation must provide an output that is both intense and stable. Sources of electromagnetic radiation are classified as either continuum or line sources. A **continuum source** emits radiation over a broad range of wavelengths, with a relatively smooth variation in intensity (Figure 10.1.7). A **line source**, on the other hand, emits radiation at selected wavelengths (Figure 10.1.8). Table 10.1.3 provides a list of the most common sources of electromagnetic radiation.

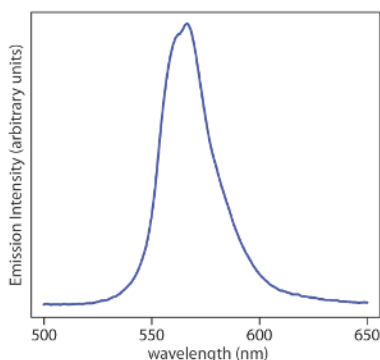


Figure 10.1.7 . Spectrum showing the emission from a green LED, which provides continuous emission over a wavelength range of approximately 530–640 nm.

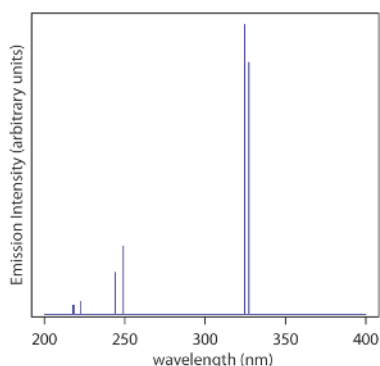


Figure 10.1.8 . Emission spectrum from a Cu hollow cathode lamp. This spectrum consists of seven distinct emission lines (the first two differ by only 0.4 nm and are not resolved at the scale shown in this spectrum). Each emission line has a width of approximately 0.01 nm at 1/2 of its maximum intensity.

Table 10.1.3 . Common Source of Electromagnetic Radiation

source	wavelength region	useful for...
H ₂ and D ₂ lamp	continuum source from 160–380 nm	molecular absorption
tungsten lamp	continuum source from 320–2400 nm	molecular absorption
Xe arc lamp	continuum source from 200–1000 nm	molecular fluorescence
nernst glower	continuum source from 0.4–20 μ m	molecular absorption
globalar	continuum source from 1–40 μ m	molecular absorption
nichrome wire	continuum source from 0.75–20 μ m	molecular absorption
hollow cathode lamp	line source in UV/Vis	atomic absorption
Hg vapor lamp	line source in UV/Vis	molecular fluorescence
laser	line source in UV/Vis/IR	atomic and molecular absorption, fluorescence, and scattering

Sources of Thermal Radiation. The most common sources of thermal energy are flames and plasmas. A flame source uses a combustion of a fuel and an oxidant to achieve temperatures of 2000–3400 K. Plasmas, which are hot, ionized gases, provide temperatures of 6000–10000 K.

Chemical Sources of Energy. Exothermic reactions also may serve as a source of energy. In chemiluminescence the analyte is raised to a higher-energy state by means of a chemical reaction, emitting characteristic radiation when it returns to a lower-energy state. When the chemical reaction results from a biological or enzymatic reaction, the emission of radiation is called bioluminescence. Commercially available “light sticks” and the flash of light from a firefly are examples of chemiluminescence and bioluminescence.

Wavelength Selection

In Nessler's original colorimetric method for ammonia, which was described at the beginning of the chapter, the sample and several standard solutions of ammonia are placed in separate tall, flat-bottomed tubes. As shown in Figure 10.1.9, after adding the reagents and allowing the color to develop, the analyst evaluates the color by passing ambient light through the bottom of the tubes and looking down through the solutions. By matching the sample's color to that of a standard, the analyst is able to determine the concentration of ammonia in the sample.

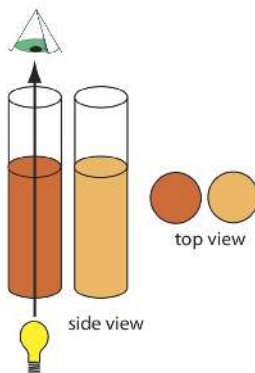


Figure 10.1.9 . Nessler's original method for comparing the color of two solutions. Natural light passes upwards through the samples and standards and the analyst views the solutions by looking down toward the light source. The top view, shown on the right, is what the analyst sees. To determine the analyte's concentration, the analyst exchanges standards until the two colors match.

In Figure 10.1.9 every wavelength of light from the source passes through the sample. This is not a problem if there is only one absorbing species in the sample. If the sample contains two components, then a quantitative analysis using Nessler's original method is impossible unless the standards contain the second component at the same concentration it has in the sample.

To overcome this problem, we want to select a wavelength that only the analyte absorbs. Unfortunately, we can not isolate a single wavelength of radiation from a continuum source, although we can narrow the range of wavelengths that reach the sample. As seen in Figure 10.1.10, a wavelength selector always passes a narrow band of radiation characterized by a **nominal wavelength**, an **effective bandwidth**, and a maximum throughput of radiation. The effective bandwidth is defined as the width of the radiation at half of its maximum throughput.

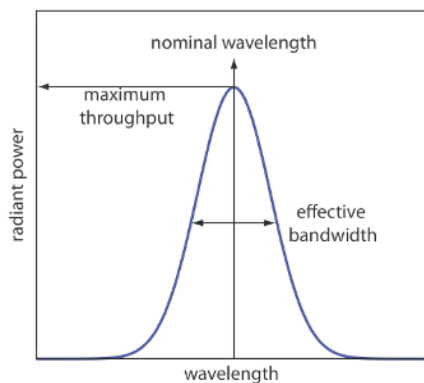


Figure 10.1.10 . Radiation exiting a wavelength selector showing the band's nominal wavelength and its effective bandwidth.

The ideal wavelength selector has a high throughput of radiation and a narrow effective bandwidth. A high throughput is desirable because the more photons that pass through the wavelength selector, the stronger the signal and the smaller the background noise. A narrow effective bandwidth provides a higher **resolution**, with spectral features separated by more than twice the effective bandwidth being resolved. As shown in Figure 10.1.11, these two features of a wavelength selector often are in opposition. A larger effective bandwidth favors a higher throughput of radiation, but provide less resolution. Decreasing the effective bandwidth improves resolution, but at the cost of a noisier signal [Jiang, S.; Parker, G. A. *Am. Lab.* **1981**, October, 38–43]. For a qualitative analysis, resolution usually is more important than noise and a smaller effective bandwidth is desirable; however, in a quantitative analysis less noise usually is desirable.

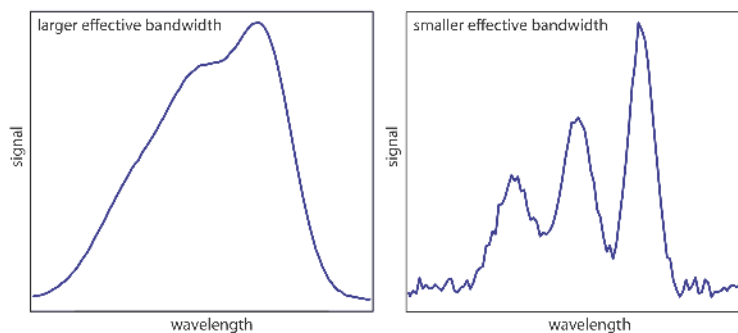


Figure 10.1.11 . Example showing the effect of the wavelength selector's effective bandwidth on resolution and noise. The spectrum with the smaller effective bandwidth (on the right) has a better resolution, allowing us to see the presence of three peaks, but at the expense of a noisier signal. The spectrum with the larger effective bandwidth (on the left) has less noise, but at the expense of less resolution between the three peaks.

Wavelength Selection Using Filters. The simplest method for isolating a narrow band of radiation is to use an absorption or interference **filter**. Absorption filters work by selectively absorbing radiation from a narrow region of the electromagnetic spectrum. Interference filters use constructive and destructive interference to isolate a narrow range of wavelengths. A simple example of an absorption filter is a piece of colored glass. A purple filter, for example, removes the complementary color green from 500–560 nm.

Commercially available absorption filters provide effective bandwidths of 30–250 nm, although the throughput at the low end of this range often is only 10% of the source's emission intensity. Interference filters are more expensive than absorption filters, but have narrower effective bandwidths, typically 10–20 nm, with maximum throughputs of at least 40%.

Wavelength Selection Using Monochromators. A filter has one significant limitation—because a filter has a fixed nominal wavelength, if we need to make measurements at two different wavelengths, then we must use two different filters. A **monochromator** is an alternative method for selecting a narrow band of radiation that also allows us to continuously adjust the band's nominal wavelength.

The construction of a typical monochromator is shown in Figure 10.1.12 . Radiation from the source enters the monochromator through an entrance slit. The radiation is collected by a collimating mirror, which reflects a parallel beam of radiation to a diffraction grating. The diffraction grating is an optically reflecting surface with a large number of parallel grooves (see insert to Figure 10.1.12). The diffraction grating disperses the radiation and a second mirror focuses the radiation onto a planar surface that contains an exit slit. In some monochromators a prism is used in place of the diffraction grating.

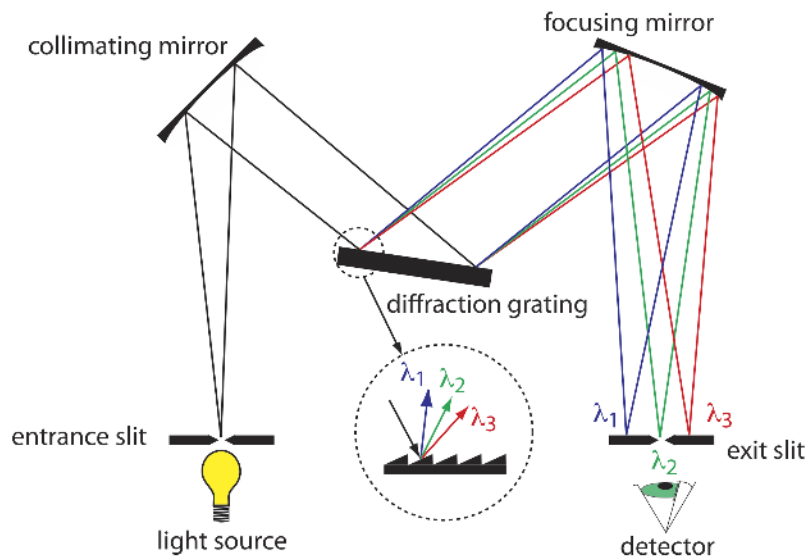


Figure 10.1.12 . Schematic diagram of a monochromator that uses a diffraction grating to disperse the radiation.

Radiation exits the monochromator and passes to the detector. As shown in Figure 10.1.12 , a monochromator converts a **polychromatic** source of radiation at the entrance slit to a **monochromatic** source of finite effective bandwidth at the exit slit. The choice of which wavelength exits the monochromator is determined by rotating the diffraction grating. A narrower exit slit provides a smaller effective bandwidth and better resolution than does a wider exit slit, but at the cost of a smaller throughput of radiation.

Polychromatic means many colored. Polychromatic radiation contains many different wavelengths of light. Monochromatic means one color, or one wavelength. Although the light exiting a monochromator is not strictly of a single wavelength, its narrow effective bandwidth allows us to think of it as monochromatic.

Monochromators are classified as either fixed-wavelength or scanning. In a fixed-wavelength monochromator we manually select the wavelength by rotating the grating. Normally a fixed-wavelength monochromator is used for a quantitative analysis where measurements are made at one or two wavelengths. A scanning monochromator includes a drive mechanism that continuously rotates the grating, which allows successive wavelengths of light to exit from the monochromator. A scanning monochromator is used to acquire spectra, and, when operated in a fixed-wavelength mode, for a quantitative analysis.

Interferometers. An **interferometer** provides an alternative approach for wavelength selection. Instead of filtering or dispersing the electromagnetic radiation, an interferometer allows source radiation of all wavelengths to reach the detector simultaneously (Figure 10.1.13). Radiation from the source is focused on a beam splitter that reflects half of the radiation to a fixed mirror and transmits the other half to a moving mirror. The radiation recombines at the beam splitter, where constructive and destructive interference determines, for each wavelength, the intensity of light that reaches the detector. As the moving mirror changes position, the wavelength of light that experiences maximum constructive interference and maximum destructive interference also changes. The signal at the detector shows intensity as a function of the moving mirror's position, expressed in units of distance or time. The result is called an **interferogram** or a time domain spectrum. The time domain spectrum is converted mathematically, by a process called a Fourier transform, to a spectrum (a frequency domain spectrum) that shows intensity as a function of the radiation's energy.

The mathematical details of the Fourier transform are beyond the level of this textbook. You can consult the chapter's additional resources for additional information.

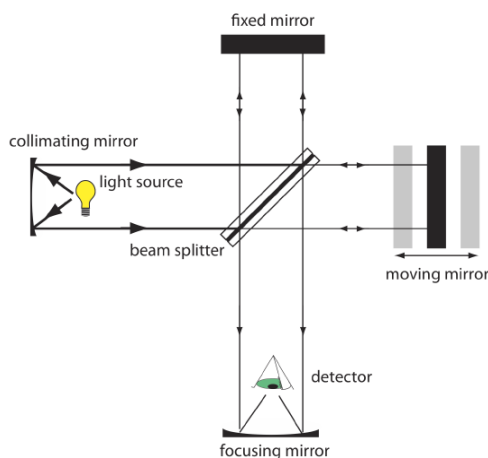


Figure 10.1.13 . Schematic diagram of an interferometers.

In comparison to a monochromator, an interferometer has two significant advantages. The first advantage, which is termed **Jacquinot's advantage**, is the greater throughput of source radiation. Because an interferometer does not use slits and has fewer optical components from which radiation is scattered and lost, the throughput of radiation reaching the detector is $80 - 200\times$ greater than that for a monochromator. The result is less noise. The second advantage, which is called **Fellgett's advantage**, is a savings in the time needed to obtain a spectrum. Because the detector monitors all frequencies simultaneously, a spectrum takes approximately one second to record, as compared to 10–15 minutes when using a scanning monochromator.

Detectors

In Nessler's original method for determining ammonia (Figure 10.1.9) the analyst's eye serves as the detector, matching the sample's color to that of a standard. The human eye, of course, has a poor range—it responds only to visible light—and it is not particularly sensitive or accurate. Modern detectors use a sensitive **transducer** to convert a signal consisting of photons into an easily measured electrical signal. Ideally the detector's signal, S , is a linear function of the electromagnetic radiation's power, P ,

$$S = kP + D$$

where k is the detector's sensitivity, and D is the detector's **dark current**, or the background current when we prevent the source's radiation from reaching the detector.

There are two broad classes of spectroscopic transducers: thermal transducers and photon transducers. Table 10.1.4 provides several representative examples of each class of transducers.

Transducer is a general term that refers to any device that converts a chemical or a physical property into an easily measured electrical signal. The retina in your eye, for example, is a transducer that converts photons into an electrical nerve impulse; your eardrum is a transducer that converts sound waves into a different electrical nerve impulse.

Table 10.1.4 . Examples of Transducers for Spectroscopy

transducer	class	wavelength range	output signal
phototube	photon	200–1000 nm	current
photomultiplier	photon	110–1000 nm	current
Si photodiode	photon	250–1100 nm	current
photoconductor	photon	750–6000 nm	change in resistance
photovoltaic cell	photon	400–5000 nm	current or voltage
thermocouple	thermal	0.8–40 μm	voltage
thermistor	thermal	0.8–40 μm	change in resistance
pneumatic	thermal	0.8–1000 μm	membrane displacement
pyroelectric	thermal	0.3–1000 μm	current

Photon Transducers. Phototubes and photomultipliers use a photosensitive surface that absorbs radiation in the ultraviolet, visible, or near IR to produce an electrical current that is proportional to the number of photons reaching the transducer (Figure 10.1.14). Other photon detectors use a semiconductor as the photosensitive surface. When the semiconductor absorbs photons, valence electrons move to the semiconductor's conduction band, producing a measurable current. One advantage of the Si photodiode is that it is easy to miniaturize. Groups of photodiodes are gathered together in a linear array that contains 64–4096 individual photodiodes. With a width of 25 μm per diode, a linear array of 2048 photodiodes requires only 51.2 mm of linear space. By placing a **photodiode array** along the monochromator's focal plane, it is possible to monitor simultaneously an entire range of wavelengths.

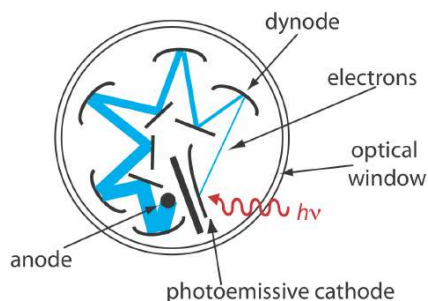


Figure 10.1.14 . Schematic of a photomultiplier. A **photon** strikes the photoemissive cathode producing **electrons**, which accelerate toward a positively charged dynode. Collision of these electrons with the dynode generates additional electrons, which accelerate toward the next dynode. A total of 10^6 – 10^7 electrons per photon eventually reach the anode, generating an electrical current.

Thermal Transducers. Infrared photons do not have enough energy to produce a measurable current with a photon transducer. A thermal transducer, therefore, is used for infrared spectroscopy. The absorption of infrared photons increases a thermal transducer's temperature, changing one or more of its characteristic properties. A pneumatic transducer, for example, is a small tube of xenon gas with an IR transparent window at one end and a flexible membrane at the other end. Photons enter the tube and are absorbed by a blackened surface, increasing the temperature of the gas. As the temperature inside the tube fluctuates, the gas expands and contracts and the flexible membrane moves in and out. Monitoring the membrane's displacement produces an electrical signal.

Signal Processors

A transducer's electrical signal is sent to a **signal processor** where it is displayed in a form that is more convenient for the analyst. Examples of signal processors include analog or digital meters, recorders, and computers equipped with digital acquisition boards. A signal processor also is used to calibrate the detector's response, to amplify the transducer's signal, to remove noise by filtering, or to mathematically transform the signal.

If the retina in your eye and the eardrum in your ear are transducers, then your brain is the signal processor.

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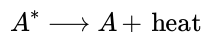
6.2: Emission Spectroscopy

An analyte in an excited state possesses an energy, E_2 , that is greater than its energy when it is in a lower energy state, E_1 . When the analyte returns to its lower energy state—a process we call relaxation—the excess energy, ΔE

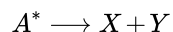
$$\Delta E = E_2 - E_1$$

is released. [Figure 10.1.4](#) shows a simplified picture of this process.

The amount of time an analyte, A , spends in its excited state—what we call the excited state's *lifetime*—is short, typically 10^{-5} – 10^{-9} s for an electronic excited state and 10–15 s for a vibrational excited state. Relaxation of the analyte's excited state, A^* , occurs through several mechanisms, including collisions with other species in the sample, photochemical reactions, and the emission of photons. In the first process, which we call vibrational relaxation or nonradiative relaxation, the excess energy is released as heat.



Relaxation by a photochemical reaction may involve simple decomposition

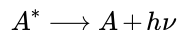


or a reaction between A^* and another species



In both cases the excess energy is used up in the chemical reaction or released as heat.

In the third mechanism, the excess energy is released as a photon of electromagnetic radiation.



The release of a photon following thermal excitation is called emission and that following the absorption of a photon is called photoluminescence. In chemiluminescence and bioluminescence, excitation results from a chemical or a biochemical reaction, respectively. Spectroscopic methods based on photoluminescence are the subject of the next section and atomic emission is covered in [Chapter 10.7](#).

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6.3: Atomic Emission Spectroscopy

The focus of this section is on the emission of ultraviolet and visible radiation following the thermal excitation of atoms. Atomic emission spectroscopy has a long history. Qualitative applications based on the color of flames were used in the smelting of ores as early as 1550 and were more fully developed around 1830 with the observation of atomic spectra generated by flame emission and spark emission [Dawson, J. B. *J. Anal. At. Spectrosc.* **1991**, 6, 93–98]. Quantitative applications based on the atomic emission from electric sparks were developed by Lockyer in the early 1870 and quantitative applications based on flame emission were pioneered by Lundegardh in 1930. Atomic emission based on emission from a plasma was introduced in 1964.

For an on-line introduction to much of the material in this section, see [Atomic Emission Spectroscopy \(AES\)](#) by Tomas Spudich and Alexander Scheeline, a resource that is part of the Analytical Sciences Digital Library.

Atomic Emission Spectra

Atomic emission occurs when a valence electron in a higher energy atomic orbital returns to a lower energy atomic orbital. Figure 10.7.1 shows a portion of the energy level diagram for sodium, which consists of a series of discrete lines at wavelengths that correspond to the difference in energy between two atomic orbitals.

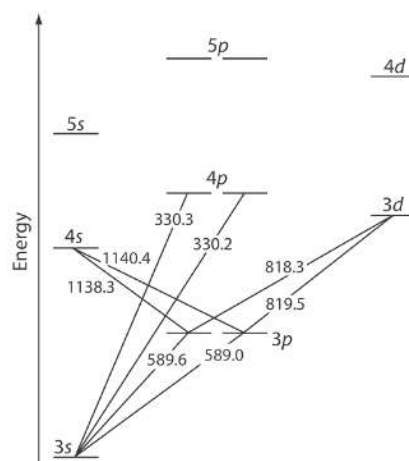


Figure 10.7.1 . Valence shell energy level diagram for sodium. The wavelengths corresponding to several transitions are shown. Note that this is the same energy level diagram as [Figure 10.2.5](#).

The intensity of an atomic emission line, I_e , is proportional to the number of atoms, N^* , that populate the excited state,

$$I_e = kN^* \quad (6.3.1)$$

where k is a constant that accounts for the efficiency of the transition. If a system of atoms is in thermal equilibrium, the population of excited state i is related to the total concentration of atoms, N , by the Boltzmann distribution. For many elements at temperatures of less than 5000 K the Boltzmann distribution is approximated as

$$N^* = N \left(\frac{g_i}{g_0} \right) e^{-E_i/kT} \quad (6.3.2)$$

where g_i and g_0 are statistical factors that account for the number of equivalent energy levels for the excited state and the ground state, E_i is the energy of the excited state relative to a ground state energy, E_0 , k is Boltzmann's constant (1.3807×10^{-23} J/K), and T is the temperature in Kelvin. From Equation 6.3.2 we expect that excited states with lower energies have larger populations and more intense emission lines. We also expect emission intensity to increase with temperature.

Equipment

An atomic emission spectrometer is similar in design to the instrumentation for atomic absorption. In fact, it is easy to adapt most flame atomic absorption spectrometers for atomic emission by turning off the hollow cathode lamp and monitoring the difference between the emission intensity when aspirating the sample and when aspirating a blank. Many atomic emission spectrometers,

however, are dedicated instruments designed to take advantage of features unique to atomic emission, including the use of plasmas, arcs, sparks, and lasers as atomization and excitation sources, and an enhanced capability for multielemental analysis.

Atomization and Excitation

Atomic emission requires a means for converting into a free gaseous atom an analyte that is present in a solid, liquid, or solution sample. The same source of thermal energy used for atomization usually serves as the excitation source. The most common methods are flames and plasmas, both of which are useful for liquid or solution samples. Solid samples are analyzed by dissolving in a solvent and using a flame or plasma atomizer.

Flame Sources

Atomization and excitation in flame atomic emission is accomplished with the same nebulization and spray chamber assembly used in atomic absorption (Figure 10.4.1). The burner head consists of a single or multiple slots, or a Meker-style burner. Older atomic emission instruments often used a total consumption burner in which the sample is drawn through a capillary tube and injected directly into the flame.

A Meker burner is similar to the more common Bunsen burner found in most laboratories; it is designed to allow for higher temperatures and for a larger diameter flame.

Plasma Sources

A **plasma** is a hot, partially ionized gas that contains an abundant concentration of cations and electrons. The plasma used in atomic emission is formed by ionizing a flowing stream of argon gas, producing argon ions and electrons. A plasma's high temperature results from resistive heating as the electrons and argon ions move through the gas. Because a plasma operates at a much higher temperature than a flame, it provides for a better atomization efficiency and a higher population of excited states.

A schematic diagram of the inductively coupled plasma source (ICP) is shown in Figure 10.7.2. The ICP torch consists of three concentric quartz tubes, surrounded at the top by a radio-frequency induction coil. The sample is mixed with a stream of Ar using a nebulizer, and is carried to the plasma through the torch's central capillary tube. Plasma formation is initiated by a spark from a Tesla coil. An alternating radio-frequency current in the induction coil creates a fluctuating magnetic field that induces the argon ions and the electrons to move in a circular path. The resulting collisions with the abundant unionized gas give rise to resistive heating, providing temperatures as high as 10000 K at the base of the plasma, and between 6000 and 8000 K at a height of 15–20 mm above the coil, where emission usually is measured. At these high temperatures the outer quartz tube must be thermally isolated from the plasma. This is accomplished by the tangential flow of argon shown in the schematic diagram.

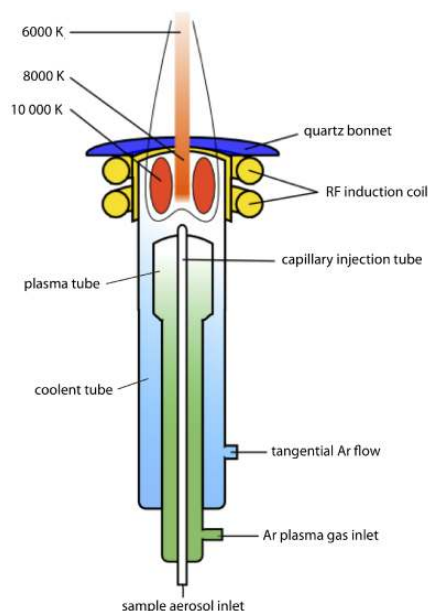


Figure 10.7.2 . Schematic diagram of an inductively coupled plasma torch.

Multielemental Analysis

Atomic emission spectroscopy is ideally suited for a multielemental analysis because all analytes in a sample are excited simultaneously. If the instrument includes a scanning monochromator, we can program it to move rapidly to an analyte's desired wavelength, pause to record its emission intensity, and then move to the next analyte's wavelength. This sequential analysis allows for a sampling rate of 3–4 analytes per minute.

Another approach to a multielemental analysis is to use a multichannel instrument that allows us to monitor simultaneously many analytes. A simple design for a multichannel spectrometer, shown in Figure 10.7.3, couples a monochromator with multiple detectors that are positioned in a semicircular array around the monochromator at positions that correspond to the wavelengths for the analytes.

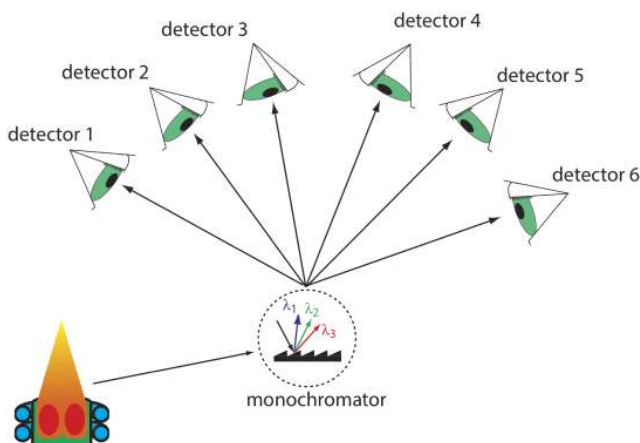


Figure 10.7.3 . Schematic diagram of a multichannel atomic emission spectrometer for the simultaneous analysis of several elements. Instruments may contain as many as 48–60 detectors.

Quantitative Applications

Atomic emission is used widely for the analysis of trace metals in a variety of sample matrices. The development of a quantitative atomic emission method requires several considerations, including choosing a source for atomization and excitation, selecting a wavelength and slit width, preparing the sample for analysis, minimizing spectral and chemical interferences, and selecting a method of standardization.

Choice of Atomization and Excitation Source

Except for the alkali metals, detection limits when using an ICP are significantly better than those obtained with flame emission (Table 10.7.1). Plasmas also are subject to fewer spectral and chemical interferences. For these reasons a plasma emission source is usually the better choice.

Table 10.7.1. Detection Limits for Atomic Emission

element	detection limit (µg/mL): flame emission	detection limit (µg/mL): ICP
Ag	2	0.2
Al	3	0.2
As	2000	2
Ca	0.1	0.0001
Cd	300	0.07
Co	5	0.1
Cr	1	0.08
Fe	10	0.09
Hg	150	1

element	detection limit ($\mu\text{g/mL}$): flame emission	detection limit ($\mu\text{g/mL}$): ICP
K	0.01	30
Li	0.001	0.02
Mg	1	0.02
Mn	1	0.01
Na	0.01	0.1
Ni	10	0.2
Pb	0.2	1
Pt	2000	0.9
Sn	100	3
Zn	1000	0.1

Source: Parsons, M. L.; Major, S.; Forster, A. R.; App. Spectrosc. 1983, 37, 411–418.

Selecting the Wavelength and Slit Width

The choice of wavelength is dictated by the need for sensitivity and the need to avoid interferences from the emission lines of other constituents in the sample. Because an analyte's atomic emission spectrum has an abundance of emission lines—particularly when using a high temperature plasma source—it is inevitable that there will be some overlap between emission lines. For example, an analysis for Ni using the atomic emission line at 349.30 nm is complicated by the atomic emission line for Fe at 349.06 nm.

A narrower slit width provides better resolution, but at the cost of less radiation reaching the detector. The easiest approach to selecting a wavelength is to record the sample's emission spectrum and look for an emission line that provides an intense signal and is resolved from other emission lines.

Preparing the Sample

Flame and plasma sources are best suited for samples in solution and in liquid form. Although a solid sample can be analyzed by directly inserting it into the flame or plasma, they usually are first brought into solution by digestion or extraction.

Minimizing Spectral Interferences

The most important spectral interference is broad, background emission from the flame or plasma and emission bands from molecular species. This background emission is particularly severe for flames because the temperature is insufficient to break down refractory compounds, such as oxides and hydroxides. Background corrections for flame emission are made by scanning over the emission line and drawing a baseline (Figure 10.7.4). Because a plasma's temperature is much higher, a background interference due to molecular emission is less of a problem. Although emission from the plasma's core is strong, it is insignificant at a height of 10–30 mm above the core where measurements normally are made.

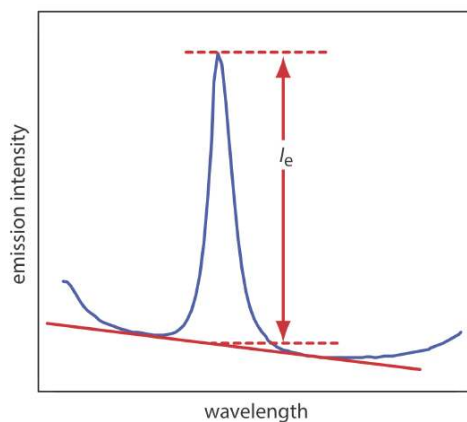


Figure 10.7.4 . Method for correcting an analyte's emission for the flame's background emission.

Minimizing Chemical Interferences

Flame emission is subject to the same types of chemical interferences as atomic absorption; they are minimized using the same methods: by adjusting the flame's composition and by adding protecting agents, releasing agents, or ionization suppressors. An additional chemical interference results from **self-absorption**. Because the flame's temperature is greatest at its center, the concentration of analyte atoms in an excited state is greater at the flame's center than at its outer edges. If an excited state atom in the flame's center emits a photon, then a ground state atom in the cooler, outer regions of the flame may absorb the photon, which decreases the emission intensity. For higher concentrations of analyte self-absorption may invert the center of the emission band (Figure 10.7.5).

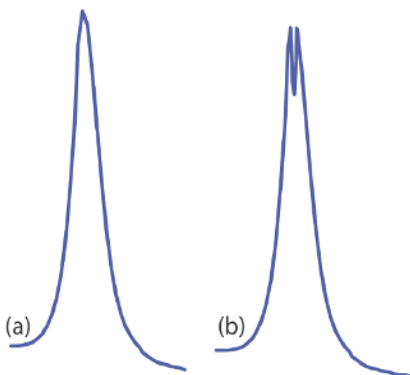


Figure 10.7.5 . Atomic emission lines for (a) a low concentration of analyte, and (b) a high concentration of analyte showing the effect of self-absorption.

Chemical interferences when using a plasma source generally are not significant because the plasma's higher temperature limits the formation of nonvolatile species. For example, PO_4^{3-} is a significant interferent when analyzing samples for Ca^{2+} by flame emission, but has a negligible effect when using a plasma source. In addition, the high concentration of electrons from the ionization of argon minimizes ionization interferences.

Standardizing the Method

From Equation 6.3.1 we know that emission intensity is proportional to the population of the analyte's excited state, N^* . If the flame or plasma is in thermal equilibrium, then the excited state population is proportional to the analyte's total population, N , through the Boltzmann distribution (Equation 6.3.2).

A calibration curve for flame emission usually is linear over two to three orders of magnitude, with ionization limiting linearity when the analyte's concentrations is small and self-absorption limiting linearity at higher concentrations of analyte. When using a plasma, which suffers from fewer chemical interferences, the calibration curve often is linear over four to five orders of magnitude and is not affected significantly by changes in the matrix of the standards.

Emission intensity is affected significantly by many parameters, including the temperature of the excitation source and the efficiency of atomization. An increase in temperature of 10 K, for example, produces a 4% increase in the fraction of Na atoms in

the $3p$ excited state, an uncertainty in the signal that may limit the use of external standards. The method of internal standards is used when the variations in source parameters are difficult to control. To compensate for changes in the temperature of the excitation source, the internal standard is selected so that its emission line is close to the analyte's emission line. In addition, the internal standard should be subject to the same chemical interferences to compensate for changes in atomization efficiency. To accurately correct for these errors the analyte and internal standard emission lines are monitored simultaneously.

Representative Method 10.7.1: Determination of Sodium in a Salt Substitute

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of sodium in salt substitutes provides an instructive example of a typical procedure. The description here is based on Goodney, D. E. *J. Chem. Educ.* **1982**, 59, 875–876.

Description of Method

Salt substitutes, which are used in place of table salt for individuals on low-sodium diets, replaces NaCl with KCl. Depending on the brand, fumaric acid, calcium hydrogen phosphate, or potassium tartrate also are present. Although intended to be sodium-free, salt substitutes contain small amounts of NaCl as an impurity. Typically, the concentration of sodium in a salt substitute is about 100 $\mu\text{g/g}$. The exact concentration of sodium is determined by flame atomic emission. Because it is difficult to match the matrix of the standards to that of the sample, the analysis is accomplished by the method of standard additions.

Procedure

A sample is prepared by placing an approximately 10-g portion of the salt substitute in 10 mL of 3 M HCl and 100 mL of distilled water. After the sample has dissolved, it is transferred to a 250-mL volumetric flask and diluted to volume with distilled water. A series of standard additions is prepared by placing 25-mL portions of the diluted sample into separate 50-mL volumetric flasks, spiking each with a known amount of an approximately 10 mg/L standard solution of Na^+ , and diluting to volume. After zeroing the instrument with an appropriate blank, the instrument is optimized at a wavelength of 589.0 nm while aspirating a standard solution of Na^+ . The emission intensity is measured for each of the standard addition samples and the concentration of sodium in the salt substitute is reported in $\mu\text{g/g}$.

Questions

1. Potassium ionizes more easily than sodium. What problem might this present if you use external standards prepared from a stock solution of 10 mg Na/L instead of using a set of standard additions?

Because potassium is present at a much higher concentration than is sodium, its ionization suppresses the ionization of sodium. Normally suppressing ionization is a good thing because it increases emission intensity. In this case, however, the difference between the standard's matrix and the sample's matrix means that the sodium in a standard experiences more ionization than an equivalent amount of sodium in a sample. The result is a determinate error.

2. One way to avoid a determinate error when using external standards is to match the matrix of the standards to that of the sample. We could, for example, prepare external standards using reagent grade KCl to match the matrix to that of the sample. Why is this not a good idea for this analysis?

Sodium is a common contaminant in many chemicals. Reagent grade KCl, for example, may contain 40–50 $\mu\text{g Na/g}$. This is a significant source of sodium, given that the salt substitute contains approximately 100 $\mu\text{g Na/g}$.

3. Suppose you decide to use an external standardization. Given the previous questions, is the result of your analysis likely to underestimate or to overestimate the amount of sodium in the salt substitute?

The solid black line in Figure 10.7.6 shows the ideal calibration curve, assuming we match the standard's matrix to the sample's matrix, and that we do so without adding any additional sodium. If we prepare the external standards without adding KCl, the emission for each standard decreases due to increased ionization. This is shown by the lower of the two dashed red lines. Preparing the standards by adding reagent grade KCl increases the concentration of sodium due to its contamination. Because we underestimate the actual concentration of sodium in the standards, the resulting calibration curve is shown by the other dashed red line. In both cases, the sample's emission results in our overestimating the concentration of sodium in the sample.

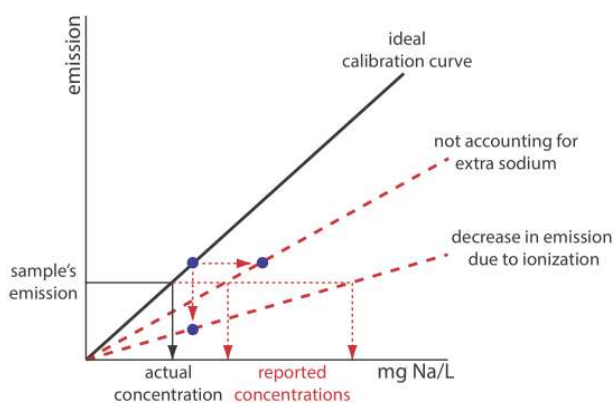


Figure 10.7.6 . External standards calibration curves for the flame atomic emission analysis of Na in a salt substitute. The solid black line shows the ideal calibration curve assuming we match the matrix of the samples and the standards using pure KCl. The lower of the two dashed red lines shows the effect of failing to add KCl to the external standards, which decreases emission. The other dashed red line shows the effect of using KCl that is contaminated with NaCl, which causes us to underestimate the concentration of Na in the standards. In both cases, the result is a positive determinate error in the analysis of samples.

4. One problem with analyzing salt samples is their tendency to clog the aspirator and burner assembly. What effect does this have on the analysis?

Clogging the aspirator and burner assembly decreases the rate of aspiration, which decreases the analyte's concentration in the flame. The result is a decrease in the emission intensity and a negative determinate error.

✓ Example 10.7.1

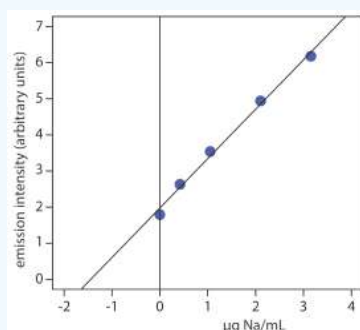
To evaluate the method described in Representative Method 10.7.1, a series of standard additions is prepared using a 10.0077-g sample of a salt substitute. The results of a flame atomic emission analysis of the standards is shown here [Goodney, D. E. *J. Chem. Educ.* **1982**, 59, 875–876].

added Na ($\mu\text{g/mL}$)	I_e (arb. units)
0.000	1.79
0.420	2.63
1.051	3.54
2.102	4.94
3.153	6.18

What is the concentration of sodium, in $\mu\text{g/g}$, in the salt substitute.

Solution

Linear regression of emission intensity versus the concentration of added Na gives the standard additions calibration curve shown below, which has the following calibration equation.



$$I_e = 1.97 + 1.37 \times \frac{\mu\text{g Na}}{\text{mL}}$$

The concentration of sodium in the sample is the absolute value of the calibration curve's x-intercept. Substituting zero for the emission intensity and solving for sodium's concentration gives a result of 1.44 $\mu\text{gNa/mL}$. The concentration of sodium in the salt substitute is

$$\frac{\frac{1.44 \mu\text{g Na}}{\text{mL}} \times \frac{50.00 \text{ mL}}{25.00 \text{ mL}} \times 250.0 \text{ mL}}{10.0077 \text{ g sample}} = 71.9 \mu\text{g Na/g}$$

Evaluation of Atomic Emission Spectroscopy

Scale of Operation

The scale of operations for atomic emission is ideal for the direct analysis of trace and ultratrace analytes in macro and meso samples. With appropriate dilutions, atomic emission can be applied to major and minor analytes.

Accuracy

When spectral and chemical interferences are insignificant, atomic emission can achieve quantitative results with accuracies of 1–5%. For flame emission, accuracy frequently is limited by chemical interferences. Because the higher temperature of a plasma source gives rise to more emission lines, accuracy when using plasma emission often is limited by stray radiation from overlapping emission lines.

Precision

For samples and standards in which the analyte's concentration exceeds the detection limit by at least a factor of 50, the relative standard deviation for both flame and plasma emission is about 1–5%. Perhaps the most important factor that affect precision is the stability of the flame's or the plasma's temperature. For example, in a 2500 K flame a temperature fluctuation of ± 2.5 K gives a relative standard deviation of 1% in emission intensity. Significant improvements in precision are realized when using internal standards.

Sensitivity

Sensitivity is influenced by the temperature of the excitation source and the composition of the sample matrix. Sensitivity is optimized by aspirating a standard solution of analyte and maximizing the emission by adjusting the flame's composition and the height from which we monitor the emission. Chemical interferences, when present, decrease the sensitivity of the analysis. Because the sensitivity of plasma emission is less affected by the sample matrix, a calibration curve prepared using standards in a matrix of distilled water is possible even for samples that have more complex matrices.

Selectivity

The selectivity of atomic emission is similar to that of atomic absorption. Atomic emission has the further advantage of rapid sequential or simultaneous analysis of multiple analytes.

Time, Cost, and Equipment

Sample throughput with atomic emission is rapid when using an automated system that can analyze multiple analytes. For example, sampling rates of 3000 determinations per hour are possible using a multichannel ICP, and sampling rates of 300 determinations per hour when using a sequential ICP. Flame emission often is accomplished using an atomic absorption spectrometer, which typically costs between \$10,000–\$50,000. Sequential ICP's range in price from \$55,000–\$150,000, while an ICP capable of simultaneous multielemental analysis costs between \$80,000–\$200,000. Combination ICP's that are capable of both sequential and simultaneous analysis range in price from \$150,000–\$300,000. The cost of Ar, which is consumed in significant quantities, can not be overlooked when considering the expense of operating an ICP.

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CHAPTER OVERVIEW

7: Molecular Spectroscopy

7.1: Spectroscopy Based on Absorption

7.2: UV/Vis and IR Spectroscopy

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7.1: Spectroscopy Based on Absorption

In absorption spectroscopy a beam of electromagnetic radiation passes through a sample. Much of the radiation passes through the sample without a loss in intensity. At selected wavelengths, however, the radiation's intensity is attenuated. This process of attenuation is called absorption.

Absorption Spectra

There are two general requirements for an analyte's absorption of electromagnetic radiation. First, there must be a mechanism by which the radiation's electric field or magnetic field interacts with the analyte. For ultraviolet and visible radiation, absorption of a photon changes the energy of the analyte's valence electrons. A bond's vibrational energy is altered by the absorption of infrared radiation.

Figure 10.1.3 provides a list of the types of atomic and molecular transitions associated with different types of electromagnetic radiation.

The second requirement is that the photon's energy, $h\nu$, must exactly equal the difference in energy, ΔE , between two of the analyte's quantized energy states. Figure 10.1.4 shows a simplified view of a photon's absorption, which is useful because it emphasizes that the photon's energy must match the difference in energy between a lower-energy state and a higher-energy state. What is missing, however, is information about what types of energy states are involved, which transitions between energy states are likely to occur, and the appearance of the resulting spectrum.

We can use the energy level diagram in Figure 10.2.1 to explain an absorbance spectrum. The lines labeled E_0 and E_1 represent the analyte's ground (lowest) electronic state and its first electronic excited state. Superimposed on each electronic energy level is a series of lines representing vibrational energy levels.

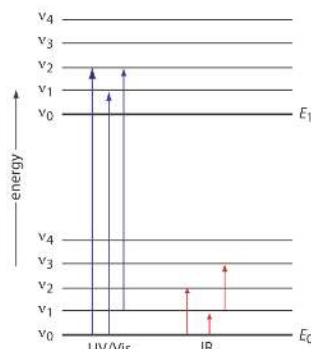


Figure 10.2.1 . Diagram showing two electronic energy levels (E_0 and E_1), each with five vibrational energy levels ν_0 – ν_4 . Absorption of ultraviolet and visible radiation (shown by the blue arrows) leads to a change in the analyte's electronic energy levels and, possibly, a change in vibrational energy as well. A change in vibrational energy without a change in electronic energy levels occurs with the absorption of infrared radiation (shown by the red arrows).

Infrared Spectra for Molecules and Polyatomic Ions

The energy of infrared radiation produces a change in a molecule's or a polyatomic ion's vibrational energy, but is not sufficient to effect a change in its electronic energy. As shown in Figure 10.2.1 , vibrational energy levels are quantized; that is, a molecule or polyatomic ion has only certain, discrete vibrational energies. The energy for an allowed vibrational mode, E_ν , is

$$E_\nu = \nu + \frac{1}{2} h\nu_0$$

where ν is the vibrational quantum number, which has values of 0, 1, 2, ..., and ν_0 is the bond's fundamental vibrational frequency. The value of ν_0 , which is determined by the bond's strength and by the mass at each end of the bond, is a characteristic property of a bond. For example, a carbon-carbon single bond (C–C) absorbs infrared radiation at a lower energy than a carbon-carbon double bond (C=C) because a single bond is weaker than a double bond.

At room temperature most molecules are in their ground vibrational state ($\nu = 0$). A transition from the ground vibrational state to the first vibrational excited state ($\nu = 1$) requires absorption of a photon with an energy of $h\nu_0$. Transitions in which $\Delta\nu = \pm 1$ give rise to the fundamental absorption lines. Weaker absorption lines, called overtones, result from transitions in which $\Delta\nu$ is ± 2 or ± 3 . The number of possible normal vibrational modes for a linear molecule is $3N - 5$, and for a non-linear molecule is $3N - 6$, where N is the number of atoms in the molecule. Not surprisingly, infrared spectra often show a considerable number of absorption bands. Even a relatively simple molecule, such as ethanol ($\text{C}_2\text{H}_6\text{O}$), for example, has $3 \times 9 - 6$, or 21 possible normal modes of vibration, although not all of these vibrational modes give rise to an absorption. The IR spectrum for ethanol is shown in Figure 10.2.2.

Why does a non-linear molecule have $3N - 6$ vibrational modes? Consider a molecule of methane, CH_4 . Each of methane's five atoms can move in one of three directions (x , y , and z) for a total of $5 \times 3 = 15$ different ways in which the molecule's atoms can move. A molecule can move in three ways: it can move from one place to another, which we call translational motion; it can rotate around an axis, which we call rotational motion; and its bonds can stretch and bend, which we call vibrational motion. Because the entire molecule can move in the x , y , and z directions, three of methane's 15 different motions are translational. In addition, the molecule can rotate about its x , y , and z axes, accounting for three additional forms of motion. This leaves $15 - 3 - 3 = 9$ vibrational modes. A linear molecule, such as CO_2 , has $3N - 5$ vibrational modes because it can rotate around only two axes.

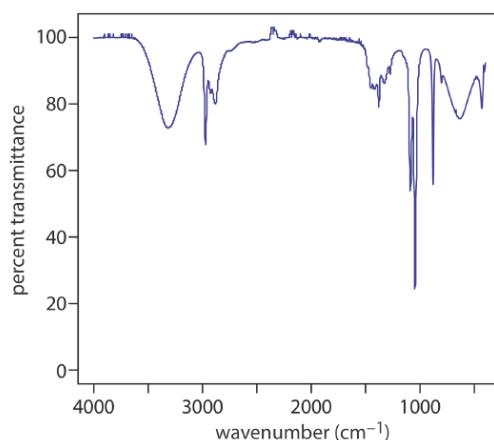


Figure 10.2.2 . Infrared spectrum of ethanol.

UV/Vis Spectra for Molecules and Ions

The valence electrons in organic molecules and polyatomic ions, such as CO_3^{2-} , occupy quantized sigma bonding (σ), pi bonding (π), and non-bonding (n) molecular orbitals (MOs). Unoccupied sigma antibonding (σ^*) and pi antibonding (π^*) molecular orbitals are slightly higher in energy. Because the difference in energy between the highest-energy occupied MOs and the lowest-energy unoccupied MOs corresponds to ultraviolet and visible radiation, absorption of a photon is possible.

Four types of transitions between quantized energy levels account for most molecular UV/Vis spectra. Table 10.2.1 lists the approximate wavelength ranges for these transitions, as well as a partial list of bonds, functional groups, or molecules responsible for these transitions. Of these transitions, the most important are $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ because they involve important functional groups that are characteristic of many analytes and because the wavelengths are easily accessible. The bonds and functional groups that give rise to the absorption of ultraviolet and visible radiation are called **chromophores**.

Table 10.2.1 . Electronic Transitions Involving n , σ , and π Molecular Orbitals

transition	wavelength range	examples
$\sigma \rightarrow \sigma^*$	<200 nm	C—C, C—H
$n \rightarrow \sigma^*$	160–260 nm	H_2O , CH_3OH , CH_3Cl
$\pi \rightarrow \pi^*$	200–500 nm	C=C, C=O, C=N, C≡C

transition	wavelength range	examples
$n \rightarrow \pi^*$	250–600 nm	C=O, C=N, N=N, N=O

Many transition metal ions, such as Cu^{2+} and Co^{2+} , form colorful solutions because the metal ion absorbs visible light. The transitions that give rise to this absorption are valence electrons in the metal ion's d -orbitals. For a free metal ion, the five d -orbitals are of equal energy. In the presence of a complexing ligand or solvent molecule, however, the d -orbitals split into two or more groups that differ in energy. For example, in an octahedral complex of $\text{Cu}(\text{H}_2\text{O})_6^{2+}$ the six water molecules perturb the d -orbitals into the two groups shown in Figure 10.2.3. The resulting $d \rightarrow d$ transitions for transition metal ions are relatively weak.

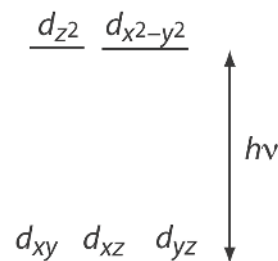
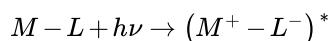


Figure 10.2.3. Splitting of the d -orbitals in an octahedral field.

A more important source of UV/Vis absorption for inorganic metal–ligand complexes is charge transfer, in which absorption of a photon produces an excited state in which there is transfer of an electron from the metal, M , to the ligand, L .



Charge-transfer absorption is important because it produces very large absorbances. One important example of a charge-transfer complex is that of *o*-phenanthroline with Fe^{2+} , the UV/Vis spectrum for which is shown in Figure 10.2.4. Charge-transfer absorption in which an electron moves from the ligand to the metal also is possible.

Why is a larger absorbance desirable? An analytical method is more sensitive if a smaller concentration of analyte gives a larger signal.

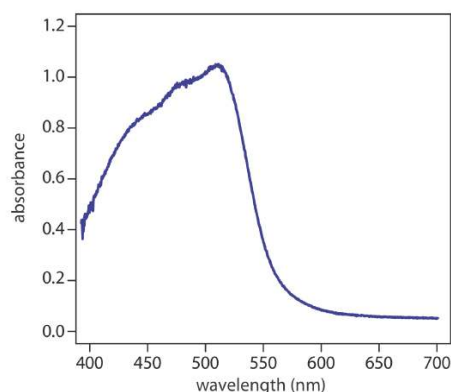


Figure 10.2.4. UV/Vis spectrum for the metal–ligand complex $\text{Fe}(\text{phen})_3^{2+}$, where phen is the ligand *o*-phenanthroline.

Comparing the IR spectrum in Figure 10.2.2 to the UV/Vis spectrum in Figure 10.2.4 shows us that UV/Vis absorption bands are often significantly broader than those for IR absorption. We can use Figure 10.2.1 to explain why this is true. When a species absorbs UV/Vis radiation, the transition between electronic energy levels may also include a transition between vibrational energy levels. The result is a number of closely spaced absorption bands that merge together to form a single broad absorption band.

UV/Vis Spectra for Atoms

The energy of ultraviolet and visible electromagnetic radiation is sufficient to cause a change in an atom's valence electron configuration. Sodium, for example, has a single valence electron in its 3s atomic orbital. As shown in Figure 10.2.5, unoccupied,

higher energy atomic orbitals also exist.

The valence shell energy level diagram in Figure 10.2.5 might strike you as odd because it shows that the 3*p* orbitals are split into two groups of slightly different energy. The reasons for this splitting are unimportant in the context of our treatment of atomic absorption. For further information about the reasons for this splitting, consult the chapter's additional resources.

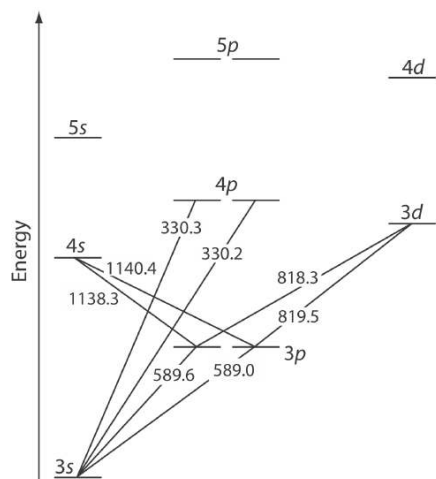


Figure 10.2.5 . Valence shell energy level diagram for sodium. The wavelengths (in wavenumbers) corresponding to several transitions are shown.

Absorption of a photon is accompanied by the excitation of an electron from a lower-energy atomic orbital to an atomic orbital of higher energy. Not all possible transitions between atomic orbitals are allowed. For sodium the only allowed transitions are those in which there is a change of ± 1 in the orbital quantum number (*l*); thus transitions from *s* $\rightarrow$ *p* orbitals are allowed, but transitions from *s* $\rightarrow$ *s* and from *s* $\rightarrow$ *d* orbitals are forbidden.

The atomic absorption spectrum for Na is shown in Figure 10.2.6 , and is typical of that found for most atoms. The most obvious feature of this spectrum is that it consists of a small number of discrete absorption lines that correspond to transitions between the ground state (the 3*s* atomic orbital) and the 3*p* and the 4*p* atomic orbitals. Absorption from excited states, such as the 3*p* $\rightarrow$ 4*s* and the 3*p* $\rightarrow$ 3*d* transitions included in Figure 10.2.5 , are too weak to detect. Because an excited state's lifetime is short—an excited state atom typically returns to a lower energy state in 10^{-7} to 10^{-8} seconds—an atom in the excited state is likely to return to the ground state before it has an opportunity to absorb a photon.

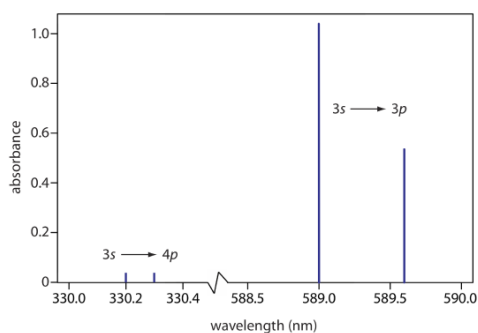


Figure 10.2.6 . Atomic absorption spectrum for sodium. Note that the scale on the x-axis includes a break.

Another feature of the atomic absorption spectrum in Figure 10.2.6 is the narrow width of the absorption lines, which is a consequence of the fixed difference in energy between the ground state and the excited state, and the lack of vibrational and rotational energy levels. Natural line widths for atomic absorption, which are governed by the uncertainty principle, are approximately 10^{-5} nm. Other contributions to broadening increase this line width to approximately 10^{-3} nm.

Transmittance and Absorbance

As light passes through a sample, its power decreases as some of it is absorbed. This attenuation of radiation is described quantitatively by two separate, but related terms: transmittance and absorbance. As shown in Figure 10.2.7 a, transmittance is the ratio of the source radiation's power as it exits the sample, P_T , to that incident on the sample, P_0 .

$$T = \frac{P_T}{P_0} \quad (7.1.1)$$

Multiplying the transmittance by 100 gives the percent transmittance, % T , which varies between 100% (no absorption) and 0% (complete absorption). All methods of detecting photons—including the human eye and modern photoelectric transducers—measure the transmittance of electromagnetic radiation.

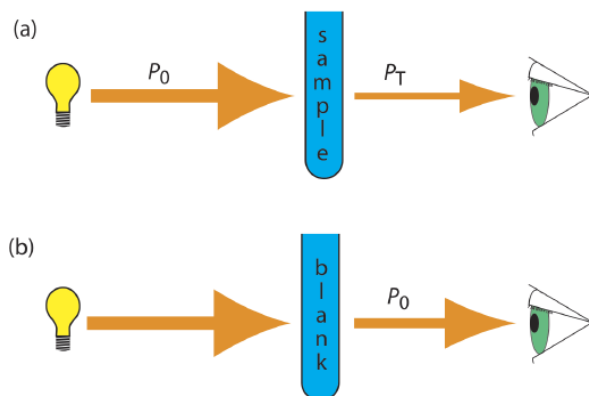


Figure 10.2.7 . (a) Schematic diagram showing the attenuation of radiation passing through a sample; P_0 is the source's radiant power and P_T is the radiant power transmitted by the sample. (b) Schematic diagram showing how we redefine P_0 as the radiant power transmitted by the blank. Redefining P_0 in this way corrects the transmittance in (a) for the loss of radiation due to scattering, reflection, absorption by the sample's container, and absorption by the sample's matrix.

Equation 7.1.1 does not distinguish between different mechanisms that prevent a photon emitted by the source from reaching the detector. In addition to absorption by the analyte, several additional phenomena contribute to the attenuation of radiation, including reflection and absorption by the sample's container, absorption by other components in the sample's matrix, and the scattering of radiation. To compensate for this loss of the radiation's power, we use a method blank. As shown in Figure 10.2.7 b, we redefine P_0 as the power exiting the method blank.

An alternative method for expressing the attenuation of electromagnetic radiation is absorbance, A , which we define as

$$A = -\log T = -\log \frac{P_T}{P_0} \quad (7.1.2)$$

Absorbance is the more common unit for expressing the attenuation of radiation because it is a linear function of the analyte's concentration.

We will show that this is true in the next section when we introduce Beer's law.

✓ Example 10.2.1

A sample has a percent transmittance of 50%. What is its absorbance?

Solution

A percent transmittance of 50.0% is the same as a transmittance of 0.500. Substituting into Equation 7.1.2 gives

$$A = -\log T = -\log(0.500) = 0.301$$

? Exercise 10.2.1

What is the % T for a sample if its absorbance is 1.27?

Answer

To find the transmittance, T , we begin by noting that

$$A = 1.27 = -\log T$$

Solving for T

$$\begin{aligned} -1.27 &= \log T \\ 10^{-1.27} &= T \end{aligned}$$

gives a transmittance of 0.054, or a %T of 5.4%.

Equation 7.1.1 has an important consequence for atomic absorption. As we learned from Figure 10.2.6, atomic absorption lines are very narrow. Even with a high quality monochromator, the effective bandwidth for a continuum source is $100 - 1000\times$ greater than the width of an atomic absorption line. As a result, little radiation from a continuum source is absorbed when it passes through a sample of atoms; because $P_0 \approx P_T$ the measured absorbance effectively is zero. For this reason, atomic absorption requires that we use a line source instead of a continuum source.

Absorbance and Concentration: Beer's Law

When monochromatic electromagnetic radiation passes through an infinitesimally thin layer of sample of thickness dx , it experiences a decrease in its power of dP (Figure 10.2.8).

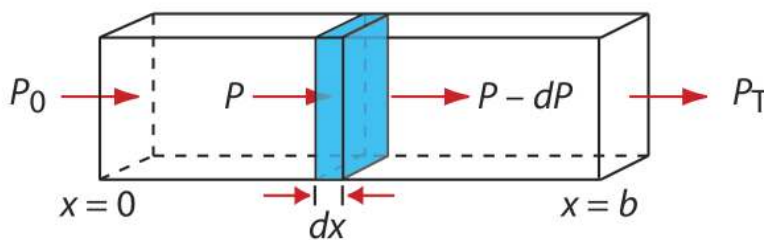


Figure 10.2.8. Factors used to derive the Beer's law.

This fractional decrease in power is proportional to the sample's thickness and to the analyte's concentration, C ; thus

$$-\frac{dP}{P} = \alpha C dx \quad (7.1.3)$$

where P is the power incident on the thin layer of sample and α is a proportionality constant. Integrating the left side of Equation 7.1.3 over the sample's full thickness

$$\begin{aligned} -\int_{P=P_0}^{P=P_T} \frac{dP}{P} &= \alpha C \int_{x=0}^{x=b} dx \\ \ln \frac{P_0}{P_T} &= \alpha b C \end{aligned}$$

converting from $\ln$ to $\log$, and substituting into Equation 7.1.2, gives

$$A = abC \quad (7.1.4)$$

where a is the analyte's **absorptivity** with units of $\text{cm}^{-1} \text{conc}^{-1}$. If we express the concentration using molarity, then we replace a with the **molar absorptivity**, ϵ , which has units of $\text{cm}^{-1} \text{M}^{-1}$.

$$A = \epsilon b C \quad (7.1.5)$$

The absorptivity and the molar absorptivity are proportional to the probability that the analyte absorbs a photon of a given energy. As a result, values for both a and ϵ depend on the wavelength of the absorbed photon.

✓ Example 10.2.2

A 5.00×10^{-4} M solution of analyte is placed in a sample cell that has a pathlength of 1.00 cm. At a wavelength of 490 nm, the solution's absorbance is 0.338. What is the analyte's molar absorptivity at this wavelength?

Solution

Solving Equation 7.1.5 for ϵ and making appropriate substitutions gives

$$\epsilon = \frac{A}{bC} = \frac{0.338}{(1.00 \text{ cm})(5.00 \times 10^{-4} \text{ M})} = 676 \text{ cm}^{-1} \text{ M}^{-1}$$

? Exercise 10.2.2

A solution of the analyte from Example 10.2.2 has an absorbance of 0.228 in a 1.00-cm sample cell. What is the analyte's concentration?

Answer

Making appropriate substitutions into Beer's law

$$A = 0.228 = \epsilon bC = (676 \text{ M}^{-1} \text{ cm}^{-1})(1 \text{ cm})C$$

and solving for C gives a concentration of 3.37×10^{-4} M.

Equation 7.1.4 and Equation 7.1.5, which establish the linear relationship between absorbance and concentration, are known as Beer's law. Calibration curves based on Beer's law are common in quantitative analyses.

As is often the case, the formulation of a law is more complicated than its name suggests. This is the case, for example, with Beer's law, which also is known as the Beer-Lambert law or the Beer-Lambert-Bouguer law. Pierre Bouguer, in 1729, and Johann Lambert, in 1760, noted that the transmittance of light decreases exponentially with an increase in the sample's thickness.

$$T \propto e^{-b}$$

Later, in 1852, August Beer noted that the transmittance of light decreases exponentially as the concentration of the absorbing species increases.

$$T \propto e^{-C}$$

Together, and when written in terms of absorbance instead of transmittance, these two relationships make up what we know as Beer's law.

Beer's Law and Multicomponent Samples

We can extend Beer's law to a sample that contains several absorbing components. If there are no interactions between the components, then the individual absorbances, A_i , are additive. For a two-component mixture of analyte's X and Y , the total absorbance, A_{tot} , is

$$A_{tot} = A_X + A_Y = \epsilon_X bC_X + \epsilon_Y bC_Y$$

Generalizing, the absorbance for a mixture of n components, A_{mix} , is

$$A_{mix} = \sum_{i=1}^n A_i = \sum_{i=1}^n \epsilon_i bC_i \quad (7.1.6)$$

Limitations to Beer's Law

Beer's law suggests that a plot of absorbance vs. concentration—we will call this a Beer's law plot—is a straight line with a y-intercept of zero and a slope of ab or ϵb . In some cases a Beer's law plot deviates from this ideal behavior (see Figure 10.2.9), and such deviations from linearity are divided into three categories: fundamental, chemical, and instrumental.

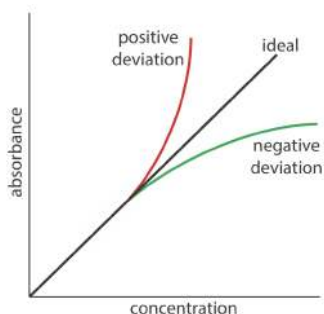


Figure 10.2.9 . Plots of absorbance vs. concentration showing **positive** and **negative** deviations from the ideal Beer's law relationship, which is a straight line.

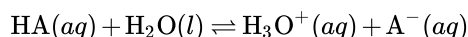
Fundamental Limitations to Beer's Law

Beer's law is a limiting law that is valid only for low concentrations of analyte. There are two contributions to this fundamental limitation to Beer's law. At higher concentrations the individual particles of analyte no longer are independent of each other. The resulting interaction between particles of analyte may change the analyte's absorptivity. A second contribution is that an analyte's absorptivity depends on the solution's refractive index. Because a solution's refractive index varies with the analyte's concentration, values of a and ϵ may change. For sufficiently low concentrations of analyte, the refractive index essentially is constant and a Beer's law plot is linear.

Chemical Limitations to Beer's Law

A chemical deviation from Beer's law may occur if the analyte is involved in an equilibrium reaction. Consider, for example, the weak acid, HA. To construct a Beer's law plot we prepare a series of standard solutions—each of which contains a known total concentration of HA—and then measure each solution's absorbance at the same wavelength. Because HA is a weak acid, it is in equilibrium with its conjugate weak base, A^- .

In the equations that follow, the conjugate weak base A^- is written as A as it is easy to mistake the symbol for anionic charge as a minus sign.



If both HA and A^- absorb at the selected wavelength, then Beer's law is

$$A = \epsilon_{HA} b C_{HA} + \epsilon_A b C_A \quad (7.1.7)$$

Because the weak acid's total concentration, C_{total} , is

$$C_{total} = C_{HA} + C_A$$

we can write the concentrations of HA and A^- as

$$C_{HA} = \alpha_{HA} C_{total} \quad (7.1.8)$$

$$C_A = (1 - \alpha_{HA}) C_{total} \quad (7.1.9)$$

where α_{HA} is the fraction of weak acid present as HA. Substituting Equation 7.1.8 and Equation 7.1.9 into Equation 7.1.7 and rearranging, gives

$$A = (\epsilon_{HA} \alpha_{HA} + \epsilon_A - \epsilon_A \alpha_{HA}) b C_{total} \quad (7.1.10)$$

To obtain a linear Beer's law plot, we must satisfy one of two conditions. If ϵ_{HA} and ϵ_A have the same value at the selected wavelength, then Equation 7.1.10 simplifies to

$$A = \epsilon_A b C_{total} = \epsilon_{HA} b C_{total}$$

Alternatively, if α_{HA} has the same value for all standard solutions, then each term within the parentheses of Equation 7.1.10 is constant—which we replace with k —and a linear calibration curve is obtained at any wavelength.

$$A = kbC_{\text{total}}$$

Because HA is a weak acid, the value of α_{HA} varies with pH. To hold α_{HA} constant we buffer each standard solution to the same pH. Depending on the relative values of α_{HA} and α_{A} , the calibration curve has a positive or a negative deviation from Beer's law if we do not buffer the standards to the same pH.

Instrumental Limitations to Beer's Law

There are two principal instrumental limitations to Beer's law. The first limitation is that Beer's law assumes that radiation reaching the sample is of a single wavelength—that is, it assumes a purely monochromatic source of radiation. As shown in Figure 10.1.10, even the best wavelength selector passes radiation with a small, but finite effective bandwidth. Polychromatic radiation always gives a negative deviation from Beer's law, but the effect is smaller if the value of ϵ essentially is constant over the wavelength range passed by the wavelength selector. For this reason, as shown in Figure 10.2.10, it is better to make absorbance measurements at the top of a broad absorption peak. In addition, the deviation from Beer's law is less serious if the source's effective bandwidth is less than one-tenth of the absorbing species' natural bandwidth [(a) Strong, F. C., III *Anal. Chem.* **1984**, 56, 16A–34A; Gilbert, D. D. *J. Chem. Educ.* **1991**, 68, A278–A281]. When measurements must be made on a slope, linearity is improved by using a narrower effective bandwidth.

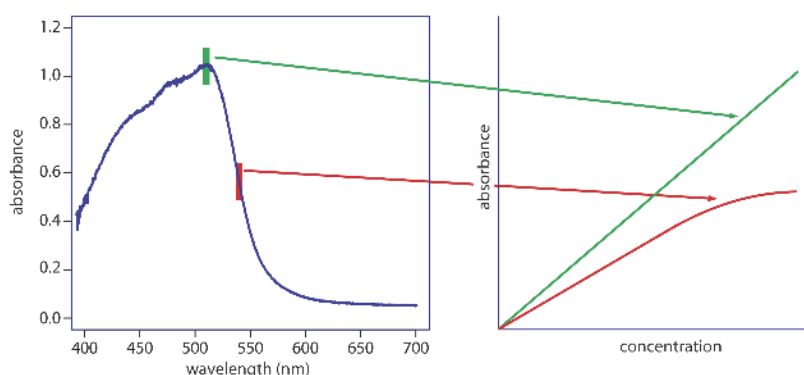


Figure 10.2.10 . Effect of wavelength selection on the linearity of a Beer's law plot. Another reason for measuring absorbance at the top of an absorbance peak is that it provides for a more sensitive analysis. Note that the green Beer's law plot has a steeper slope—and, therefore, a greater sensitivity—than the red Beer's law plot. A Beer's law plot, of course, is equivalent to a calibration curve.

Stray radiation is the second contribution to instrumental deviations from Beer's law. Stray radiation arises from imperfections in the wavelength selector that allow light to enter the instrument and to reach the detector without passing through the sample. Stray radiation adds an additional contribution, P_{stray} , to the radiant power that reaches the detector; thus

$$A = -\log \frac{P_{\text{T}} + P_{\text{stray}}}{P_0 + P_{\text{stray}}}$$

For a small concentration of analyte, P_{stray} is significantly smaller than P_0 and P_{T} , and the absorbance is unaffected by the stray radiation. For higher concentrations of analyte, less light passes through the sample and P_{T} and P_{stray} become similar in magnitude. This results in an absorbance that is smaller than expected, and a negative deviation from Beer's law.

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7.2: UV/Vis and IR Spectroscopy

In [Figure 10.1.9](#) we examined Nessler's original method for matching the color of a sample to the color of a standard. Matching colors is a labor intensive process for the analyst and, not surprisingly, spectroscopic methods of analysis were slow to find favor. The 1930s and 1940s saw the introduction of photoelectric transducers for ultraviolet and visible radiation, and thermocouples for infrared radiation. As a result, modern instrumentation for absorption spectroscopy routinely became available in the 1940s—further progress has been rapid ever since.

Instrumentation

Frequently an analyst must select from among several instruments of different design, the one instrument best suited for a particular analysis. In this section we examine several different instruments for molecular absorption spectroscopy, with an emphasis on their advantages and limitations. Methods of sample introduction also are covered in this section.

Instrument Designs for Molecular UV/Vis Absorption

Filter Photometer. The simplest instrument for molecular UV/Vis absorption is a **filter photometer** ([Figure 10.3.1](#)), which uses an absorption or interference filter to isolate a band of radiation. The filter is placed between the source and the sample to prevent the sample from decomposing when exposed to higher energy radiation. A filter photometer has a single optical path between the source and detector, and is called a **single-beam** instrument. The instrument is calibrated to 0% T while using a shutter to block the source radiation from the detector. After opening the shutter, the instrument is calibrated to 100% T using an appropriate blank. The blank is then replaced with the sample and its transmittance measured. Because the source's incident power and the sensitivity of the detector vary with wavelength, the photometer is recalibrated whenever the filter is changed. Photometers have the advantage of being relatively inexpensive, rugged, and easy to maintain. Another advantage of a photometer is its portability, making it easy to take into the field. Disadvantages of a photometer include the inability to record an absorption spectrum and the source's relatively large effective bandwidth, which limits the calibration curve's linearity.

The percent transmittance varies between 0% and 100%. As we learned from [Figure 10.2.7](#), we use a blank to determine P_0 , which corresponds to 100%T. Even in the absence of light the detector records a signal. Closing the shutter allows us to assign 0%T to this signal. Together, setting 0% T and 100%T calibrates the instrument. The amount of light that passes through a sample produces a signal that is greater than or equal to 0%T and smaller than or equal to 100%T.

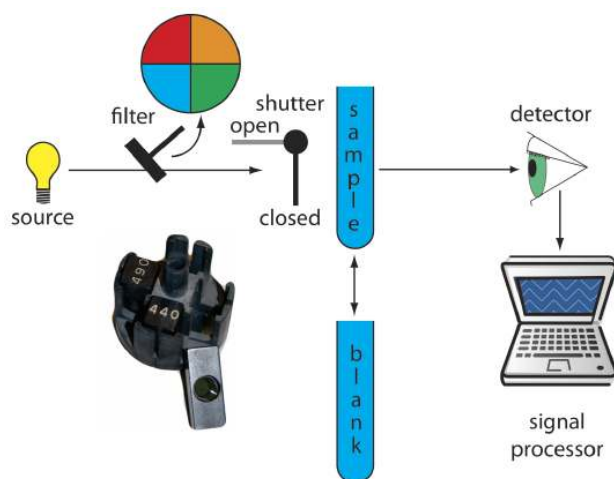


Figure 10.3.1 . Schematic diagram of a filter photometer. The analyst either inserts a removable filter or the filters are placed in a carousel, an example of which is shown in the photographic inset. The analyst selects a filter by rotating it into place.

Single-Beam Spectrophotometer. An instrument that uses a monochromator for wavelength selection is called a **spectrophotometer**. The simplest spectrophotometer is a single-beam instrument equipped with a fixed-wavelength monochromator ([Figure 10.3.2](#)). Single-beam spectrophotometers are calibrated and used in the same manner as a photometer. One example of a

single-beam spectrophotometer is Thermo Scientific's Spectronic 20D+, which is shown in the photographic insert to Figure 10.3.2. The Spectronic 20D+ has a wavelength range of 340–625 nm (950 nm when using a red-sensitive detector), and a fixed effective bandwidth of 20 nm. Battery-operated, hand-held single-beam spectrophotometers are available, which are easy to transport into the field. Other single-beam spectrophotometers also are available with effective bandwidths of 2–8 nm. Fixed wavelength single-beam spectrophotometers are not practical for recording spectra because manually adjusting the wavelength and recalibrating the spectrophotometer is awkward and time-consuming. The accuracy of a single-beam spectrophotometer is limited by the stability of its source and detector over time.

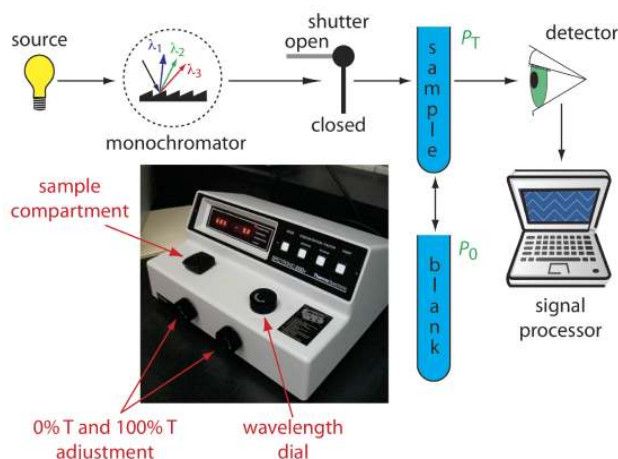


Figure 10.3.2. Schematic diagram of a fixed-wavelength, single-beam spectrophotometer. The photographic inset shows a typical instrument. The shutter remains closed until the sample or blank is placed in the sample compartment. The analyst manually selects the wavelength by adjusting the wavelength dial. Inset photo modified from: Adi (www.commons.Wikipedia.org).

Double-Beam Spectrophotometer. The limitations of a fixed-wavelength, single-beam spectrophotometer is minimized by using a **double-beam** spectrophotometer (Figure 10.3.3). A chopper controls the radiation's path, alternating it between the sample, the blank, and a shutter. The signal processor uses the chopper's speed of rotation to resolve the signal that reaches the detector into the transmission of the blank, P_0 , and the sample, P_T . By including an opaque surface as a shutter, it also is possible to continuously adjust 0%T. The effective bandwidth of a double-beam spectrophotometer is controlled by adjusting the monochromator's entrance and exit slits. Effective bandwidths of 0.2–3.0 nm are common. A scanning monochromator allows for the automated recording of spectra. Double-beam instruments are more versatile than single-beam instruments, being useful for both quantitative and qualitative analyses, but also are more expensive and not particularly portable.

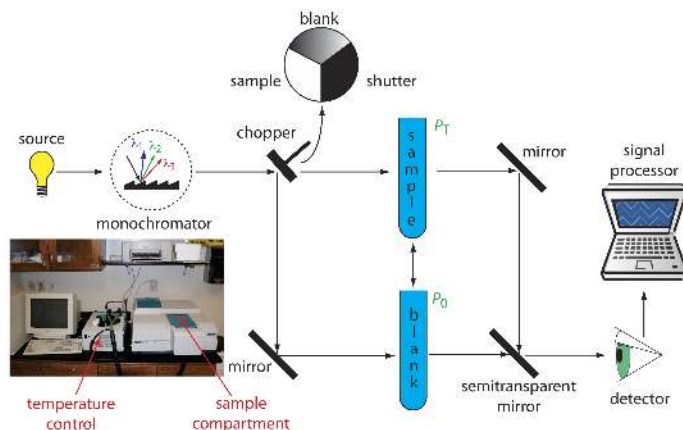


Figure 10.3.3. Schematic diagram of a scanning, double-beam spectrophotometer. A chopper directs the source's radiation, using a transparent window to pass radiation to the sample and a mirror to reflect radiation to the blank. The chopper's opaque surface serves as a shutter, which allows for a constant adjustment of the spectrophotometer's 0%T. The photographic inset shows a typical instrument. The module in the middle of the photo is a temperature control unit that makes it possible to heat or cool the sample to a constant temperature.

Diode Array Spectrometer. An instrument with a single detector can monitor only one wavelength at a time. If we replace a single photomultiplier with an array of photodiodes, we can use the resulting detector to record a full spectrum in as little as 0.1 s. In a diode array spectrometer the source radiation passes through the sample and is dispersed by a grating (Figure 10.3.4). The

photodiode array detector is situated at the grating's focal plane, with each diode recording the radiant power over a narrow range of wavelengths. Because we replace a full monochromator with just a grating, a diode array spectrometer is small and compact.

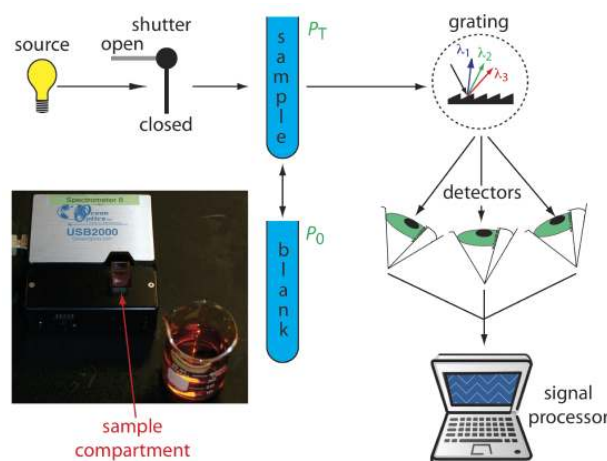


Figure 10.3.4 . Schematic diagram of a diode array spectrophotometer. The photographic insert shows a typical instrument. Note that the 50-mL beaker provides a sense of scale. Because the spectrometer is small and compact, it is easy to transport into the field.

One advantage of a diode array spectrometer is the speed of data acquisition, which allows us to collect multiple spectra for a single sample. Individual spectra are added and averaged to obtain the final spectrum. This **signal averaging** improves a spectrum's signal-to-noise ratio. If we add together n spectra, the sum of the signal at any point, x , increases as nS_x , where S_x is the signal. The noise at any point, N_x , is a random event, which increases as $\sqrt{n}N_x$ when we add together n spectra. The **signal-to-noise ratio** after n scans, $(S/N)_n$ is

$$\left(\frac{S}{N}\right)_n = \frac{nS_x}{\sqrt{n}N_x} = \sqrt{n} \frac{S_x}{N_x}$$

where S_x/N_x is the signal-to-noise ratio for a single scan. The impact of signal averaging is shown in Figure 10.3.5 . The first spectrum shows the signal after one scan, which consists of a single, noisy peak. Signal averaging using 4 scans and 16 scans decreases the noise and improves the signal-to-noise ratio. One disadvantage of a photodiode array is that the effective bandwidth per diode is roughly an order of magnitude larger than that for a high quality monochromator.

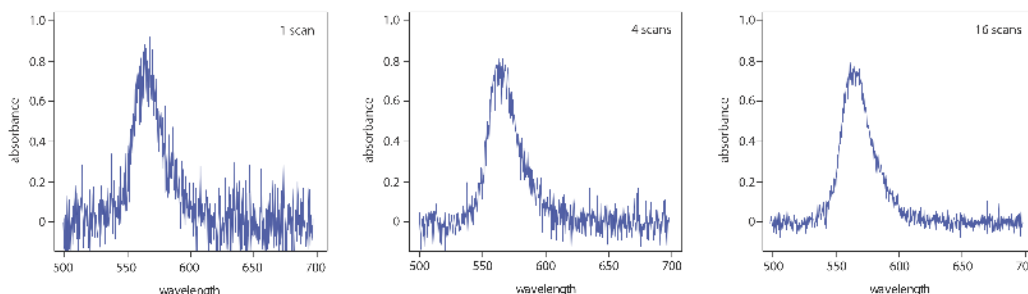


Figure 10.3.5 . Effect of signal averaging on a spectrum's signal-to-noise ratio. From top to bottom: spectrum for a single scan; average spectrum after four scans; and average spectrum after adding 16 scans.

For more details on signals and noise, see Introduction to Signals and Noise by Steven Petrovic, an on-line resource that is part of the Analytical Sciences Digital Library.

Sample Cells. The sample compartment provides a light-tight environment that limits stray radiation. Samples normally are in a liquid or solution state, and are placed in cells constructed with UV/Vis transparent materials, such as quartz, glass, and plastic (Figure 10.3.6). A quartz or fused-silica cell is required when working at a wavelength <300 nm where other materials show a significant absorption. The most common pathlength is 1 cm (10 mm), although cells with shorter (as little as 0.1 cm) and longer pathlengths (up to 10 cm) are available. Longer pathlength cells are useful when analyzing a very dilute solution or for gas

samples. The highest quality cells allow the radiation to strike a flat surface at a 90° angle, minimizing the loss of radiation to reflection. A test tube often is used as a sample cell with simple, single-beam instruments, although differences in the cell's pathlength and optical properties add an additional source of error to the analysis.



Figure 10.3.6 . Examples of sample cells for UV/Vis spectroscopy. From left to right (with path lengths in parentheses): rectangular plastic cuvette (10.0 mm), rectangular quartz cuvette (5.000 mm), rectangular quartz cuvette (1.000 mm), cylindrical quartz cuvette (10.00 mm), cylindrical glass cuvette with quartz windows (100.0 mm). Cells often are available as a matched pair, which is important when using a double-beam instrument.

If we need to monitor an analyte's concentration over time, it may not be possible to remove samples for analysis. This often is the case, for example, when monitoring an industrial production line or waste line, when monitoring a patient's blood, or when monitoring an environmental system, such as stream. With a **fiber-optic probe** we can analyze samples *in situ*. An example of a remote sensing fiber-optic probe is shown in Figure 10.3.7 . The probe consists of two bundles of fiber-optic cable. One bundle transmits radiation from the source to the probe's tip, which is designed to allow the sample to flow through the sample cell. Radiation from the source passes through the solution and is reflected back by a mirror. The second bundle of fiber-optic cable transmits the nonabsorbed radiation to the wavelength selector. Another design replaces the flow cell shown in Figure 10.3.7 with a membrane that contains a reagent that reacts with the analyte. When the analyte diffuses into the membrane it reacts with the reagent, producing a product that absorbs UV or visible radiation. The nonabsorbed radiation from the source is reflected or scattered back to the detector. Fiber optic probes that show chemical selectivity are called optrodes [(a) Seitz, W. R. *Anal. Chem.* **1984**, 56, 16A–34A; (b) Angel, S. M. *Spectroscopy* **1987**, 2(2), 38–48].

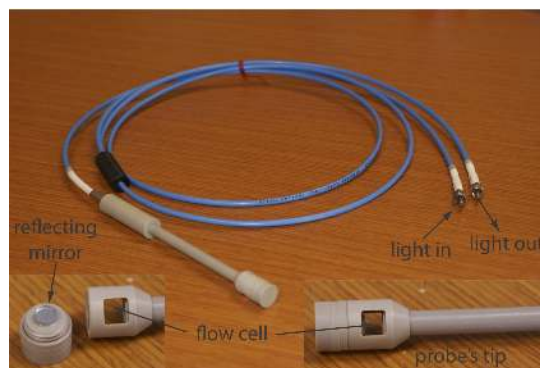


Figure 10.3.7 . Example of a fiber-optic probe. The inset photographs at the bottom of the figure provide close-up views of the probe's flow cell and the reflecting mirror.

Instrument Designs for Infrared Adsorption

Filter Photometer. The simplest instrument for IR absorption spectroscopy is a filter photometer similar to that shown in Figure 10.3.1 for UV/Vis absorption. These instruments have the advantage of portability and typically are used as dedicated analyzers for gases such as HCN and CO.

Double-beam spectrophotometer. Infrared instruments using a monochromator for wavelength selection use double-beam optics similar to that shown in Figure 10.3.3 . Double-beam optics are preferred over single-beam optics because the sources and detectors for infrared radiation are less stable than those for UV/Vis radiation. In addition, it is easier to correct for the absorption of infrared radiation by atmospheric CO₂ and H₂O vapor when using double-beam optics. Resolutions of 1–3 cm⁻¹ are typical for most instruments.

Fourier transform spectrometer. In a Fourier transform infrared spectrometer, or FT-IR, the monochromator is replaced with an interferometer (Figure 10.1.13). Because an FT-IR includes only a single optical path, it is necessary to collect a separate spectrum

to compensate for the absorbance of atmospheric CO_2 and H_2O vapor. This is done by collecting a background spectrum without the sample and storing the result in the instrument's computer memory. The background spectrum is removed from the sample's spectrum by taking the ratio the two signals. In comparison to other instrument designs, an FT-IR provides for rapid data acquisition, which allows for an enhancement in signal-to-noise ratio through signal-averaging.

Sample Cells. Infrared spectroscopy routinely is used to analyze gas, liquid, and solid samples. Sample cells are made from materials, such as NaCl and KBr, that are transparent to infrared radiation. Gases are analyzed using a cell with a pathlength of approximately 10 cm. Longer pathlengths are obtained by using mirrors to pass the beam of radiation through the sample several times.

A liquid sample may be analyzed using a variety of different sample cells (Figure 10.3.8). For non-volatile liquids a suitable sample is prepared by placing a drop of the liquid between two NaCl plates, forming a thin film that typically is less than 0.01 mm thick. Volatile liquids are placed in a sealed cell to prevent their evaporation.

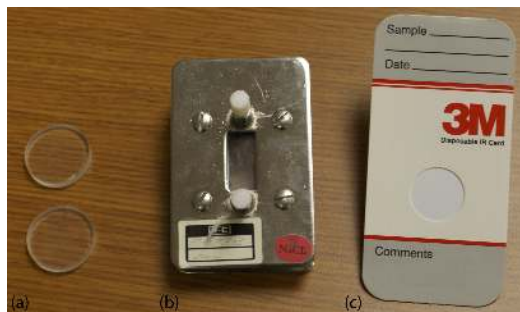


Figure 10.3.8 . Three examples of IR sample cells: (a) NaCl salts plates; (b) fixed pathlength (0.5 mm) sample cell with NaCl windows; (c) disposable card with a polyethylene window that is IR transparent with the exception of strong absorption bands at 2918 cm^{-1} and 2849 cm^{-1} .

The analysis of solution samples is limited by the solvent's IR absorbing properties, with CCl_4 , CS_2 , and CHCl_3 being the most common solvents. Solutions are placed in cells that contain two NaCl windows separated by a Teflon spacer. By changing the Teflon spacer, pathlengths from 0.015–1.0 mm are obtained.

Transparent solid samples are analyzed by placing them directly in the IR beam. Most solid samples, however, are opaque, and are first dispersed in a more transparent medium before recording the IR spectrum. If a suitable solvent is available, then the solid is analyzed by preparing a solution and analyzing as described above. When a suitable solvent is not available, solid samples are analyzed by preparing a mull of the finely powdered sample with a suitable oil. Alternatively, the powdered sample is mixed with KBr and pressed into an optically transparent pellet.

The analysis of an aqueous sample is complicated by the solubility of the NaCl cell window in water. One approach to obtaining an infrared spectrum of an aqueous solution is to use **attenuated total reflectance** instead of transmission. Figure 10.3.9 shows a diagram of a typical attenuated total reflectance (ATR) FT-IR instrument. The ATR cell consists of a high refractive index material, such as ZnSe or diamond, sandwiched between a low refractive index substrate and a lower refractive index sample. Radiation from the source enters the ATR crystal where it undergoes a series of internal reflections before exiting the crystal. During each reflection the radiation penetrates into the sample to a depth of a few microns, which results in a selective attenuation of the radiation at those wavelengths where the sample absorbs. ATR spectra are similar, but not identical, to those obtained by measuring the transmission of radiation.

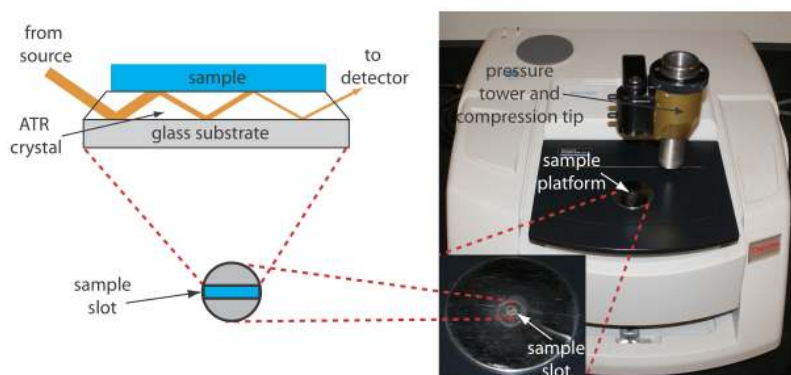


Figure 10.3.9 . FT-IR spectrometer equipped with a diamond ATR sample cell. The inserts show a close-up photo of the sample platform, a sketch of the ATR's sample slot, and a schematic showing how the source's radiation interacts with the sample. The pressure tower is used to ensure proper contact of a solid sample with the ATR crystal.

Solid samples also can be analyzed using an ATR sample cell. After placing the solid in the sample slot, a compression tip ensures that it is in contact with the ATR crystal. Examples of solids analyzed by ATR include polymers, fibers, fabrics, powders, and biological tissue samples. Another reflectance method is diffuse reflectance, in which radiation is reflected from a rough surface, such as a powder. Powdered samples are mixed with a non-absorbing material, such as powdered KBr, and the reflected light is collected and analyzed. As with ATR, the resulting spectrum is similar to that obtained by conventional transmission methods. Further details about these, and other methods for preparing solids for infrared analysis can be found in this chapter's [additional resources](#).

Quantitative Applications

The determination of an analyte's concentration based on its absorption of ultraviolet or visible radiation is one of the most frequently encountered quantitative analytical methods. One reason for its popularity is that many organic and inorganic compounds have strong absorption bands in the UV/Vis region of the electromagnetic spectrum. In addition, if an analyte does not absorb UV/Vis radiation—or if its absorbance is too weak—we often can react it with another species that is strongly absorbing. For example, a dilute solution of Fe^{2+} does not absorb visible light. Reacting Fe^{2+} with *o*-phenanthroline, however, forms an orange-red complex of $\text{Fe}(\text{phen})_3^{2+}$ that has a strong, broad absorbance band near 500 nm. An additional advantage to UV/Vis absorption is that in most cases it is relatively easy to adjust experimental and instrumental conditions so that Beer's law is obeyed.

A quantitative analysis based on the absorption of infrared radiation, although important, is encountered less frequently than with UV/Vis absorption. One reason is the greater tendency for instrumental deviations from Beer's law when using infrared radiation. Because an infrared absorption band is relatively narrow, any deviation due to the lack of monochromatic radiation is more pronounced. In addition, infrared sources are less intense than UV/Vis sources, which makes stray radiation more of a problem. Differences between the pathlengths for samples and for standards when using thin liquid films or KBr pellets are a problem, although an internal standard can correct for any difference in pathlength. Finally, establishing a 100%T ($A = 0$) baseline often is difficult because the optical properties of NaCl sample cells may change significantly with wavelength due to contamination and degradation. We can minimize this problem by measuring absorbance relative to a baseline established for the absorption band. Figure 10.3.10 shows how this is accomplished.

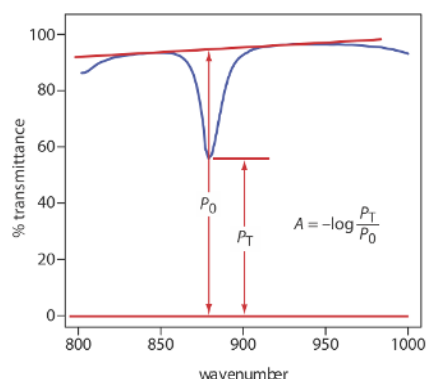


Figure 10.3.10 . Method for determining absorbance from an IR spectrum.

Another approach is to use a cell with a fixed pathlength, such as that shown in [Figure 10.3.8 b](#).

Environmental Applications

The analysis of waters and wastewaters often relies on the absorption of ultraviolet and visible radiation. Many of these methods are outlined in Table 10.3.1 . Several of these methods are described here in more detail.

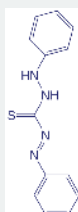
Table 10.3.1 . Examples of Molecular UV/Vis Analysis of Waters and Wastewaters

analyte	method	λ (nm)
trace metals		
aluminum	react with Eriochrome cyanide R dye at pH 6; forms red to pink complex	535
arsenic	reduce to AsH_3 using Zn and react with silver diethyldithiocarbamate; forms red complex	535
cadmium	extract into CHCl_3 containing dithizone from a sample made basic with NaOH; forms pink to red complex	518
chromium	oxidize to Cr(VI) and react with diphenylcarbazide; forms 540 red-violet product	540
copper	react with neocuprine in neutral to slightly acid solution and extract into $\text{CHCl}_3/\text{CH}_3\text{OH}$; forms yellow complex	457
iron	reduce to Fe^{2+} and react with o-phenanthroline; forms orange-red complex	510
lead	extract into CHCl_3 containing dithizone from sample made basic with $\text{NH}_3/\text{NH}_4^+$ buffer; forms cherry red complex	510
manganese	oxidize to MnO_4^- with persulfate; forms purple solution	525
mercury	extract into CHCl_3 containing dithizone from acidic sample; forms orange complex	492
zinc	react with zincon at pH 9; forms blue complex	620
inorganic nonmetals		
ammonia	reaction with hypochlorite and phenol using a manganous salt catalyst; forms blue indophenol as product	630
cyanide	react with chloroamine-T to form CNCl and then with a pyridine-barbituric acid; forms a red-blue dye	578
fluoride	react with red Zr-SPADNS lake; formation of ZrF_6^{2-} decreases color of the red lake	570

analyte	method	λ (nm)
chlorine (residual)	react with leuco crystal violet; forms blue product	592
nitrate	react with Cd to form NO_2^- and then react with sulfanilamide and <i>N</i> -(1-naphthyl)-ethylenediamine; forms red azo 543 dye	543
phosphate	react with ammonium molybdate and then reduce with SnCl_2 ; forms molybdenum blue	690
organics		
phenol	react with 4-aminoantipyrine and $\text{K}_3\text{Fe}(\text{CN})_6$; forms yellow antipyrine dye	460
anionic surfactants	react with cationic methylene blue dye and extract into CHCl_3 ; forms blue ion pair	652

Although the quantitative analysis of metals in waters and wastewaters is accomplished primarily by atomic absorption or atomic emission spectroscopy, many metals also can be analyzed following the formation of a colored metal–ligand complex. One advantage to these spectroscopic methods is that they easily are adapted to the analysis of samples in the field using a filter photometer. One ligand used for the analysis of several metals is diphenylthiocarbazone, also known as dithizone. Dithizone is not soluble in water, but when a solution of dithizone in CHCl_3 is shaken with an aqueous solution that contains an appropriate metal ion, a colored metal–dithizonate complex forms that is soluble in CHCl_3 . The selectivity of dithizone is controlled by adjusting the sample's pH. For example, Cd^{2+} is extracted from solutions made strongly basic with NaOH , Pb^{2+} from solutions made basic with an $\text{NH}_3/\text{NH}_4^+$ buffer, and Hg^{2+} from solutions that are slightly acidic.

The structure of dithizone is shown below. See [Chapter 7](#) for a discussion of extracting metal ions using dithizone.

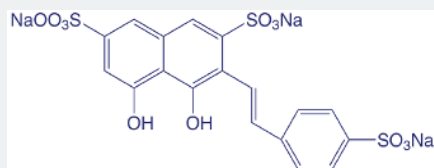


When chlorine is added to water the portion available for disinfection is called the chlorine residual. There are two forms of chlorine residual. The free chlorine residual includes Cl_2 , HOCl , and OCl^- . The combined chlorine residual, which forms from the reaction of NH_3 with HOCl , consists of monochloramine, NH_2Cl , dichloramine, NHCl_2 , and trichloramine, NCl_3 . Because the free chlorine residual is more efficient as a disinfectant, there is an interest in methods that can distinguish between the total chlorine residual's different forms. One such method is the leuco crystal violet method. The free residual chlorine is determined by adding leuco crystal violet to the sample, which instantaneously oxidizes to give a blue-colored compound that is monitored at 592 nm. Completing the analysis in less than five minutes prevents a possible interference from the combined chlorine residual. The total chlorine residual (free + combined) is determined by reacting a separate sample with iodide, which reacts with both chlorine residuals to form HOI . When the reaction is complete, leuco crystal violet is added and oxidized by HOI , giving the same blue-colored product. The combined chlorine residual is determined by difference.

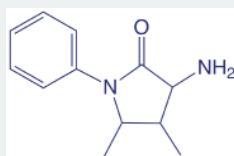
In Chapter 9 we explored how the total chlorine residual can be determined by a redox titration; see [Representative Method 9.4.1](#) for further details. The method described here allows us to divide the total chlorine residual into its component parts.

The concentration of fluoride in drinking water is determined indirectly by its ability to form a complex with zirconium. In the presence of the dye SPADNS, a solution of zirconium forms a red colored compound, called a lake, that absorbs at 570 nm. When fluoride is added, the formation of the stable ZrF_6^{2-} complex causes a portion of the lake to dissociate, decreasing the absorbance. A plot of absorbance versus the concentration of fluoride, therefore, has a negative slope.

SPADNS, the structure of which is shown below, is an abbreviation for the sodium salt of 2-(4-sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid, which is a mouthful to say.



Spectroscopic methods also are used to determine organic constituents in water. For example, the combined concentrations of phenol and ortho- and meta-substituted phenols are determined by using steam distillation to separate the phenols from nonvolatile impurities. The distillate reacts with 4-aminoantipyrine at $\text{pH } 7.9 \pm 0.1$ in the presence of $\text{K}_3\text{Fe}(\text{CN})_6$ to a yellow colored antipyrine dye. After extracting the dye into CHCl_3 , its absorbance is monitored at 460 nm. A calibration curve is prepared using only the unsubstituted phenol, $\text{C}_6\text{H}_5\text{OH}$. Because the molar absorptivity of substituted phenols generally are less than that for phenol, the reported concentration represents the minimum concentration of phenolic compounds.



4-aminoantipyrine

Molecular absorption also is used for the analysis of environmentally significant airborne pollutants. In many cases the analysis is carried out by collecting the sample in water, converting the analyte to an aqueous form that can be analyzed by methods such as those described in Table 10.3.1. For example, the concentration of NO_2 is determined by oxidizing NO_2 to NO_3^- . The concentration of NO_3^- is then determined by first reducing it to NO_2^- with Cd, and then reacting NO_2^- with sulfanilamide and *N*-(1-naphthyl)-ethylenediamine to form a red azo dye. Another important application is the analysis for SO_2 , which is determined by collecting the sample in an aqueous solution of HgCl_4^{2-} where it reacts to form $\text{Hg}(\text{SO}_3)_2^{2-}$. Addition of *p*-rosaniline and formaldehyde produces a purple complex that is monitored at 569 nm. Infrared absorption is useful for the analysis of organic vapors, including HCN, SO_2 , nitrobenzene, methyl mercaptan, and vinyl chloride. Frequently, these analyses are accomplished using portable, dedicated infrared photometers.

Clinical Applications

The analysis of clinical samples often is complicated by the complexity of the sample's matrix, which may contribute a significant background absorption at the desired wavelength. The determination of serum barbiturates provides one example of how this problem is overcome. The barbiturates are first extracted from a sample of serum with CHCl_3 and then extracted from the CHCl_3 into 0.45 M NaOH ($\text{pH} \approx 13$). The absorbance of the aqueous extract is measured at 260 nm, and includes contributions from the barbiturates as well as other components extracted from the serum sample. The pH of the sample is then lowered to approximately 10 by adding NH_4Cl and the absorbance remeasured. Because the barbiturates do not absorb at this pH, we can use the absorbance at pH 10, $A_{\text{pH } 10}$, to correct the absorbance at pH 13, $A_{\text{pH } 13}$

$$A_{\text{barb}} = A_{\text{pH } 13} - \frac{V_{\text{samp}} + V_{\text{NH}_4\text{Cl}}}{V_{\text{samp}}} \times A_{\text{pH } 10}$$

where A_{barb} is the absorbance due to the serum barbiturates and V_{samp} and $V_{\text{NH}_4\text{Cl}}$ are the volumes of sample and NH_4Cl , respectively. Table 10.3.2 provides a summary of several other methods for analyzing clinical samples.

Table 10.3.2. Examples of the Molecular UV/Vis Analysis of Clinical Samples

analyte	method	λ (nm)
total serum protein	react with NaOH and Cu^{2+} ; forms blue-violet complex	540
serum cholesterol	react with Fe^{3+} in presence of isopropanol, acetic acid, and H_2SO_4 ; forms blue-violet complex	540
uric acid	react with phosphotungstic acid; forms tungsten blue	710
serum barbituates	extract into CHCl_3 to isolate from interferences and then extract into 0.45 M NaOH	260
glucose	react with <i>o</i> -toluidine at 100°C ; forms blue-green complex	630
protein-bound iodine	decompose protein to release iodide, which catalyzes redox reaction between Ce^{3+} and As^{3+} ; forms yellow colored Ce^{4+}	420

Industrial Applications

UV/Vis molecular absorption is used for the analysis of a diverse array of industrial samples including pharmaceuticals, food, paint, glass, and metals. In many cases the methods are similar to those described in [Table 10.3.1](#) and in [Table 10.3.2](#). For example, the amount of iron in food is determined by bringing the iron into solution and analyzing using the *o*-phenanthroline method listed in [Table 10.3.1](#).

Many pharmaceutical compounds contain chromophores that make them suitable for analysis by UV/Vis absorption. Products analyzed in this fashion include antibiotics, hormones, vitamins, and analgesics. One example of the use of UV absorption is in determining the purity of aspirin tablets, for which the active ingredient is acetylsalicylic acid. Salicylic acid, which is produced by the hydrolysis of acetylsalicylic acid, is an undesirable impurity in aspirin tablets, and should not be present at more than 0.01% w/w. Samples are screened for unacceptable levels of salicylic acid by monitoring the absorbance at a wavelength of 312 nm. Acetylsalicylic acid absorbs at 280 nm, but absorbs poorly at 312 nm. Conditions for preparing the sample are chosen such that an absorbance of greater than 0.02 signifies an unacceptable level of salicylic acid.

Forensic Applications

UV/Vis molecular absorption routinely is used for the analysis of narcotics and for drug testing. One interesting forensic application is the determination of blood alcohol using the Breathalyzer test. In this test a 52.5-mL breath sample is bubbled through an acidified solution of $\text{K}_2\text{Cr}_2\text{O}_7$, which oxidizes ethanol to acetic acid. The concentration of ethanol in the breath sample is determined by a decrease in the absorbance at 440 nm where the dichromate ion absorbs. A blood alcohol content of 0.10%, which is above the legal limit, corresponds to 0.025 mg of ethanol in the breath sample.

Developing a Quantitative Method for a Single Component

To develop a quantitative analytical method, the conditions under which Beer's law is obeyed must be established. First, the most appropriate wavelength for the analysis is determined from an absorption spectrum. In most cases the best wavelength corresponds to an absorption maximum because it provides greater sensitivity and is less susceptible to instrumental limitations. Second, if the instrument has adjustable slits, then an appropriate slit width is chosen. The absorption spectrum also aids in selecting a slit width by choosing a width that is narrow enough to avoid instrumental limitations to Beer's law, but wide enough to increase the throughput of source radiation. Finally, a calibration curve is constructed to determine the range of concentrations for which Beer's law is valid. Additional considerations that are important in any quantitative method are the effect of potential interferences and establishing an appropriate blank.

Representative Method 10.3.1: Determination of Iron in Water and Wastewater

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of iron in water and wastewater provides an instructive example of a typical procedure. The description here is based on Method 3500-Fe B as published in *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., American Public Health Association: Washington, D. C., 1998.

Description of Method

Iron in the +2 oxidation state reacts with *o*-phenanthroline to form the orange-red $\text{Fe}(\text{phen})_3^{2+}$ complex. The intensity of the complex's color is independent of the solution's acidity between a pH of 3 and 9. Because the complex forms more rapidly at lower pH levels, the reaction usually is carried out within a pH range of 3.0–3.5. Any iron present in the +3 oxidation state is reduced with hydroxylamine before adding *o*-phenanthroline. The most important interferents are strong oxidizing agents, polyphosphates, and metal ions such as Cu^{2+} , Zn^{2+} , Ni^{2+} , and Cd^{2+} . An interference from oxidizing agents is minimized by adding an excess of hydroxylamine, and an interference from polyphosphate is minimized by boiling the sample in the presence of acid. The absorbance of samples and standards are measured at a wavelength of 510 nm using a 1-cm cell (longer pathlength cells also may be used). Beer's law is obeyed for concentrations of within the range of 0.2–4.0 mg Fe/L.

Procedure

For a sample that contains less than 2 mg Fe/L, directly transfer a 50-mL portion to a 125-mL Erlenmeyer flask. Samples that contain more than 2 mg Fe/L are diluted before acquiring the 50-mL portion. Add 2 mL of concentrated HCl and 1 mL of hydroxylamine to the sample. Bring the solution to a boil and continue boiling until the solution's volume is reduced to between 15 and 20 mL. After cooling to room temperature, transfer the solution to a 50-mL volumetric flask, add 10 mL of an ammonium acetate buffer, 2 mL of a 1000 ppm solution of *o*-phenanthroline, and dilute to volume. Allow 10–15 minutes for color development before measuring the absorbance, using distilled water to set 100% T. Calibration standards, including a blank, are prepared by the same procedure using a stock solution that contains a known concentration of Fe^{2+} .

Questions

1. Explain why strong oxidizing agents are interferents and why an excess of hydroxylamine prevents the interference.

A strong oxidizing agent will oxidize some Fe^{2+} to Fe^{3+} . Because $\text{Fe}(\text{phen})_3^{3+}$ does not absorb as strongly as $\text{Fe}(\text{phen})_3^{2+}$, the absorbance is smaller than expected, which produces a negative determinate error. The excess hydroxylamine reacts with the oxidizing agents, removing them from the solution.

2. The color of the complex is stable between pH levels of 3 and 9. What are some possible complications at more acidic or at more basic pH's?

Because *o*-phenanthroline is a weak base, its conditional formation constant for $\text{Fe}(\text{phen})_3^{2+}$ becomes smaller at more acidic pH levels, where *o*-phenanthroline is present in its protonated form. The result is a decrease in absorbance and a less sensitive analytical method. When the pH is greater than 9, competition between OH^- and *o*-phenanthroline for Fe^{2+} also decreases the absorbance. In addition, if the pH is sufficiently basic there is a risk that the iron will precipitate as $\text{Fe}(\text{OH})_2$.

3. Cadmium is an interferent because it forms a precipitate with *o*-phenanthroline. What effect does the formation of precipitate have on the determination of iron?

Because *o*-phenanthroline is present in large excess (2000 μg of *o*-phenanthroline for 100 μg of Fe^{2+}), it is not likely that the interference is due to an insufficient amount of *o*-phenanthroline being available to react with the Fe^{2+} . The presence of a precipitate in the sample cell results in the scattering of radiation, which causes an apparent increase in absorbance. Because the measured absorbance increases, the reported concentration is too high. Although scattering is a problem here, it can serve as the basis of a useful analytical method. See [Chapter 10.8](#) for further details.

4. Even high quality ammonium acetate contains a significant amount of iron. Why is this source of iron not a problem?

Because all samples and standards are prepared using the same volume of ammonium acetate buffer, the contribution of this source of iron is accounted for by the calibration curve's reagent blank.

Quantitative Analysis for a Single Sample

To determine the concentration of an analyte we measure its absorbance and apply Beer's law using any of the standardization methods described in [Chapter 5](#). The most common methods are a normal calibration curve using external standards and the method of standard additions. A single point standardization also is possible, although we must first verify that Beer's law holds for the concentration of analyte in the samples and the standard.

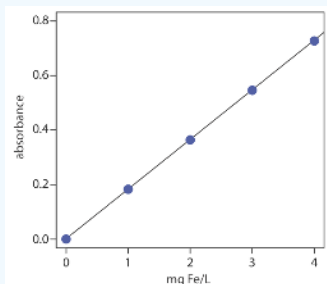
✓ Example 10.3.1

The determination of iron in an industrial waste stream is carried out by the *o*-phenanthroline described in [Representative Method 10.3.1](#). Using the data in the following table, determine the mg Fe/L in the waste stream.

mg Fe/L	absorbance
0.00	0.000
1.00	0.183
2.00	0.364
3.00	0.546
4.00	0.727
sample	0.269

Solution

Linear regression of absorbance versus the concentration of Fe in the standards gives the calibration curve and calibration equation shown here



$$A = 0.0006 + (0.1817 \text{ mg}^{-1}\text{L}) \times (\text{mgFe/L})$$

Substituting the sample's absorbance into the calibration equation gives the concentration of Fe in the waste stream as 1.48 mg Fe/L

? Exercise 10.3.1

The concentration of Cu^{2+} in a sample is determined by reacting it with the ligand cuprizone and measuring its absorbance at 606 nm in a 1.00-cm cell. When a 5.00-mL sample is treated with cuprizone and diluted to 10.00 mL, the resulting solution has an absorbance of 0.118. A second 5.00-mL sample is mixed with 1.00 mL of a 20.00 mg/L standard of Cu^{2+} , treated with cuprizone and diluted to 10.00 mL, giving an absorbance of 0.162. Report the mg Cu^{2+} /L in the sample.

Answer

For this standard addition we write equations that relate absorbance to the concentration of Cu^{2+} in the sample before the standard addition

$$0.118 = \epsilon b \left[C_{\text{Cu}} \times \frac{5.00 \text{ mL}}{10.00 \text{ mL}} \right]$$

and after the standard addition

$$0.162 = \epsilon b \left(C_{\text{Cu}} \times \frac{5.00 \text{ mL}}{10.00 \text{ mL}} + \frac{20.00 \text{ mg Cu}}{\text{L}} \times \frac{1.00 \text{ mL}}{10.00 \text{ mL}} \right)$$

in each case accounting for the dilution of the original sample and for the standard. The value of ϵb is the same in both equation. Solving each equation for ϵb and equating

$$\frac{0.162}{C_{\text{Cu}} \times \frac{5.00 \text{ mL}}{10.00 \text{ mL}} + \frac{20.00 \text{ mg Cu}}{\text{L}} \times \frac{1.00 \text{ mL}}{10.00 \text{ mL}}} = \frac{0.118}{C_{\text{Cu}} \times \frac{5.00 \text{ mL}}{10.00 \text{ mL}}}$$

leaves us with an equation in which C_{Cu} is the only variable. Solving for C_{Cu} gives its value as

$$\begin{aligned} \frac{0.162}{0.500 \times C_{\text{Cu}} + 2.00 \text{ mg Cu/L}} &= \frac{0.118}{0.500 \times C_{\text{Cu}}} \\ 0.0810 \times C_{\text{Cu}} &= 0.0590 \times C_{\text{Cu}} + 0.236 \text{ mg Cu/L} \\ 0.0220 \times C_{\text{Cu}} &= 0.236 \text{ mg Cu/L} \\ C_{\text{Cu}} &= 10.7 \text{ mg Cu/L} \end{aligned}$$

Quantitative Analysis of Mixtures

Suppose we need to determine the concentration of two analytes, X and Y , in a sample. If each analyte has a wavelength where the other analyte does not absorb, then we can proceed using the approach in Example 10.3.5. Unfortunately, UV/Vis absorption bands are so broad that frequently it is not possible to find suitable wavelengths. Because Beer's law is additive the mixture's absorbance, A_{mix} , is

$$(A_{\text{mix}})_{\lambda_1} = (\epsilon_x)_{\lambda_1} b C_X + (\epsilon_Y)_{\lambda_1} b C_Y \quad (7.2.1)$$

where λ_1 is the wavelength at which we measure the absorbance. Because Equation 7.2.1 includes terms for the concentration of both X and Y , the absorbance at one wavelength does not provide enough information to determine either C_X or C_Y . If we measure the absorbance at a second wavelength

$$(A_{\text{mix}})_{\lambda_2} = (\epsilon_x)_{\lambda_2} b C_X + (\epsilon_Y)_{\lambda_2} b C_Y \quad (7.2.2)$$

then we can determine C_X and C_Y by solving simultaneously Equation 7.2.1 and Equation 7.2.2. Of course, we also must determine the value for ϵ_X and ϵ_Y at each wavelength. For a mixture of n components, we must measure the absorbance at n different wavelengths.

✓ Example 10.3.2

The concentrations of Fe^{3+} and Cu^{2+} in a mixture are determined following their reaction with hexacyanoruthenate (II), $\text{Ru}(\text{CN})_6^{4-}$, which forms a purple-blue complex with Fe^{3+} ($\lambda_{\text{max}} = 550 \text{ nm}$) and a pale-green complex with Cu^{2+} ($\lambda_{\text{max}} = 396 \text{ nm}$) [DiTusa, M. R.; Schlitt, A. A. *J. Chem. Educ.* **1985**, 62, 541–542]. The molar absorptivities ($\text{M}^{-1} \text{ cm}^{-1}$) for the metal complexes at the two wavelengths are summarized in the following table.

analyte	ϵ_{550}	ϵ_{396}
Fe^{3+}	9970	84
Cu^{2+}	34	856

When a sample that contains Fe^{3+} and Cu^{2+} is analyzed in a cell with a pathlength of 1.00 cm, the absorbance at 550 nm is 0.183 and the absorbance at 396 nm is 0.109. What are the molar concentrations of Fe^{3+} and Cu^{2+} in the sample?

Solution

Substituting known values into Equation 7.2.1 and Equation 7.2.2 gives

$$A_{550} = 0.183 = 9970C_{\text{Fe}} + 34C_{\text{Cu}}$$

$$A_{396} = 0.109 = 84C_{\text{Fe}} + 856C_{\text{Cu}}$$

To determine C_{Fe} and C_{Cu} we solve the first equation for C_{Cu}

$$C_{\text{Cu}} = \frac{0.183 - 9970C_{\text{Fe}}}{34}$$

and substitute the result into the second equation.

$$\begin{aligned} 0.109 &= 84C_{\text{Fe}} + 856 \times \frac{0.183 - 9970C_{\text{Fe}}}{34} \\ &= 4.607 - (2.51 \times 10^5) C_{\text{Fe}} \end{aligned}$$

Solving for C_{Fe} gives the concentration of Fe^{3+} as 1.8×10^{-5} M. Substituting this concentration back into the equation for the mixture's absorbance at 396 nm gives the concentration of Cu^{2+} as 1.3×10^{-4} M.

Another approach to solving Example 10.3.2 is to multiply the first equation by 856/34 giving

$$4.607 = 251009C_{\text{Fe}} + 856C_{\text{Cu}}$$

Subtracting the second equation from this equation

$$\begin{aligned} 4.607 &= 251009C_{\text{Fe}} + 856C_{\text{Cu}} \\ -0.109 &= 84C_{\text{Fe}} + 856C_{\text{Cu}} \end{aligned}$$

gives

$$4.498 = 250925C_{\text{Fe}}$$

and we find that C_{Fe} is 1.8×10^{-5} . Having determined C_{Fe} we can substitute back into one of the other equations to solve for C_{Cu} , which is 1.3×10^{-5} .

? Exercise 10.3.2

The absorbance spectra for Cr^{3+} and Co^{2+} overlap significantly. To determine the concentration of these analytes in a mixture, its absorbance is measured at 400 nm and at 505 nm, yielding values of 0.336 and 0.187, respectively. The individual molar absorptivities ($\text{M}^{-1} \text{cm}^{-1}$) for Cr^{3+} are 15.2 at 400 nm and 0.533 at 505 nm; the values for Co^{2+} are 5.60 at 400 nm and 5.07 at 505 nm.

Answer

Substituting into Equation 7.2.1 and Equation 7.2.2 gives

$$A_{400} = 0.336 = 15.2C_{\text{Cr}} + 5.60C_{\text{Co}}$$

$$A_{505} = 0.187 = 0.533C_{\text{Cr}} + 5.07C_{\text{Co}}$$

To determine C_{Cr} and C_{Co} we solve the first equation for C_{Co}

$$C_{\text{Co}} = \frac{0.336 - 15.2C_{\text{Cr}}}{5.60}$$

and substitute the result into the second equation.

$$0.187 = 0.533C_{\text{Cr}} + 5.07 \times \frac{0.336 - 15.2C_{\text{Cr}}}{5.60}$$

$$0.187 = 0.3042 - 13.23C_{\text{Cr}}$$

Solving for C_{Cr} gives the concentration of Cr^{3+} as 8.86×10^{-3} M. Substituting this concentration back into the equation for the mixture's absorbance at 400 nm gives the concentration of Co^{2+} as 3.60×10^{-2} M.

To obtain results with good accuracy and precision the two wavelengths should be selected so that $\epsilon_X > \epsilon_Y$ at one wavelength and $\epsilon_X < \epsilon_Y$ at the other wavelength. It is easy to appreciate why this is true. Because the absorbance at each wavelength is dominated by one analyte, any uncertainty in the concentration of the other analyte has less of an impact. Figure 10.3.11 shows that the choice of wavelengths for Practice Exercise 10.3.2 are reasonable. When the choice of wavelengths is not obvious, one method for locating the optimum wavelengths is to plot ϵ_X/ϵ_Y as function of wavelength, and determine the wavelengths where ϵ_X/ϵ_Y reaches maximum and minimum values [Mehra, M. C.; Rioux, J. J. *Chem. Educ.* **1982**, 59, 688–689].

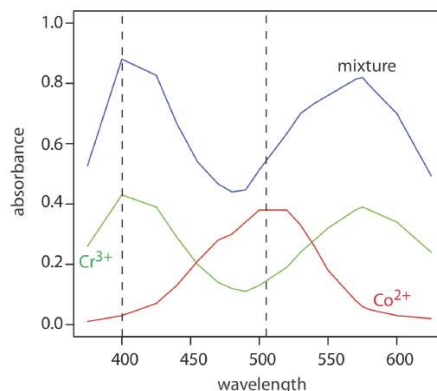


Figure 10.3.11 . Visible absorption spectra for 0.0250 M Cr^{3+} , 0.0750 M Co^{2+} , and for a mixture of Cr^{3+} and Co^{2+} . The two wavelengths used to analyze the mixture of Cr^{3+} and Co^{2+} are shown by the dashed lines. The data for the two standards are from Brewer, S. *Solving Problems in Analytical Chemistry*, John Wiley & Sons: New York, 1980.

When the analyte's spectra overlap severely, such that $\epsilon_X \approx \epsilon_Y$ at all wavelengths, other computational methods may provide better accuracy and precision. In a multiwavelength linear regression analysis, for example, a mixture's absorbance is compared to that for a set of standard solutions at several wavelengths [Blanco, M.; Iturriaga, H.; Maspocho, S.; Tarin, P. J. *Chem. Educ.* **1989**, 66, 178–180]. If A_{SX} and A_{SY} are the absorbance values for standard solutions of components X and Y at any wavelength, then

$$A_{SX} = \epsilon_X b C_{SX} \quad (7.2.3)$$

$$A_{SY} = \epsilon_Y b C_{SY} \quad (7.2.4)$$

where C_{SX} and C_{SY} are the known concentrations of X and Y in the standard solutions. Solving Equation 7.2.3 and Equation 7.2.4 for ϵ_X and for ϵ_Y , substituting into Equation 7.2.1, and rearranging, gives

$$\frac{A_{\text{mix}}}{A_{SX}} = \frac{C_X}{C_{SX}} + \frac{C_Y}{C_{SY}} \times \frac{A_{SY}}{A_{SX}}$$

To determine C_X and C_Y the mixture's absorbance and the absorbances of the standard solutions are measured at several wavelengths. Graphing A_{mix}/A_{SX} versus A_{SY}/A_{SX} gives a straight line with a slope of C_Y/C_{SY} and a y-intercept of C_X/C_{SX} . This approach is particularly helpful when it is not possible to find wavelengths where $\epsilon_X > \epsilon_Y$ and $\epsilon_X < \epsilon_Y$.

The approach outlined here for a multiwavelength linear regression uses a single standard solution for each analyte. A more rigorous approach uses multiple standards for each analyte. The math behind the analysis of such data—which we call a multiple linear regression—is beyond the level of this text. For more details about multiple linear regression see Brereton, R. G. *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*, Wiley: Chichester, England, 2003.

✓ Example 10.3.3

Figure PageIndex10.11 shows visible absorbance spectra for a standard solution of 0.0250 M Cr^{3+} , a standard solution of 0.0750 M Co^{2+} , and a mixture that contains unknown concentrations of each ion. The data for these spectra are shown here.

λ (nm)	A_{Cr}	A_{Cu}	A_{mix}	λ (nm)	A_{Cr}	A_{Cu}	A_{mix}
375	0.26	0.01	0.53	520	0.19	0.38	0.63
400	0.43	0.03	0.88	530	0.24	0.33	0.70

425	0.39	0.07	0.83	540	0.28	0.26	0.73
440	0.29	0.13	0.67	550	0.32	0.18	0.76
455	0.20	0.21	0.54	570	0.38	0.08	0.81
470	0.14	0.28	0.47	575	0.39	0.06	0.82
480	0.12	0.30	0.44	580	0.38	0.05	0.79
490	0.11	0.34	0.45	600	0.34	0.03	0.70
500	0.13	0.38	0.51	625	0.24	0.02	0.49

Use a multiwavelength regression analysis to determine the composition of the unknown.

Solution

First we need to calculate values for A_{mix}/A_{SX} and for A_{SY}/A_{SX} . Let's define X as Co^{2+} and Y as Cr^{3+} . For example, at a wavelength of 375 nm A_{mix}/A_{SX} is 0.53/0.01, or 53 and A_{SY}/A_{SX} is 0.26/0.01, or 26. Completing the calculation for all wavelengths and graphing A_{mix}/A_{SX} versus A_{SY}/A_{SX} gives the calibration curve shown in Figure 10.3.12. Fitting a straight-line to the data gives a regression model of

$$\frac{A_{\text{mix}}}{A_{SX}} = 0.636 + 2.01 \times \frac{A_{SY}}{A_{SX}}$$

Using the y-intercept, the concentration of Co^{2+} is

$$\frac{C_X}{C_{SX}} = \frac{[\text{Co}^{2+}]}{0.0750\text{M}} = 0.636$$

or $[\text{Co}^{2+}] = 0.048\text{ M}$; using the slope the concentration of Cr^{3+} is

$$\frac{C_Y}{C_{SY}} = \frac{[\text{Cr}^{3+}]}{0.0250\text{M}} = 2.01$$

or $[\text{Cr}^{3+}] = 0.050\text{ M}$.

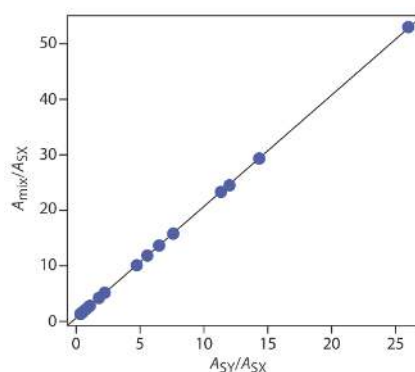


Figure 10.3.12. Multiwavelength linear regression analysis for the data in Example 10.3.3.

? Exercise 10.3.3

A mixture of MnO_4^- and $\text{Cr}_2\text{O}_7^{2-}$, and standards of 0.10 mM KMnO_4 and of 0.10 mM $\text{K}_2\text{Cr}_2\text{O}_7$ give the results shown in the following table. Determine the composition of the mixture. The data for this problem is from Blanco, M. C.; Iturriaga, H.; Maspoch, S.; Tarin, P. J. *Chem. Educ.* **1989**, 66, 178–180.

λ (nm)	A_{Mn}	A_{Cr}	A_{mix}
266	0.042	0.410	0.766

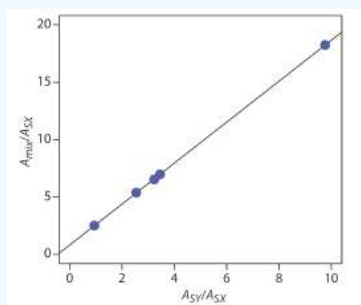
λ (nm)	A_{Mn}	A_{Cr}	A_{mix}
288	0.082	0.283	0.571
320	0.168	0.158	0.422
350	0.125	0.318	0.672
360	0.036	0.181	0.366

Answer

Letting X represent MnO_4^- and letting Y represent $Cr_2O_7^{2-}$, we plot the equation

$$\frac{A_{mix}}{A_{SX}} = \frac{C_X}{C_{SX}} + \frac{C_Y}{C_{SY}} \times \frac{A_{SY}}{A_{SX}}$$

placing A_{mix}/A_{SX} on the y -axis and A_{SY}/A_{SX} on the x -axis. For example, at a wavelength of 266 nm the value A_{mix}/A_{SX} of is 0.766/0.042, or 18.2, and the value of A_{SY}/A_{SX} is 0.410/0.042, or 9.76. Completing the calculations for all wavelengths and plotting the data gives the result shown here



Fitting a straight-line to the data gives a regression model of

$$\frac{A_{mix}}{A_{SX}} = 0.8147 + 1.7839 \times \frac{A_{SY}}{A_{SX}}$$

Using the y -intercept, the concentration of MnO_4^- is

$$\frac{C_X}{C_{SX}} = 0.8147 = \frac{[MnO_4^-]}{1.0 \times 10^{-4} \text{ M } MnO_4^-}$$

or $8.15 \times 10^{-5} \text{ M } MnO_4^-$, and using the slope, the concentration of $Cr_2O_7^{2-}$ is

$$\frac{C_Y}{C_{SY}} = 1.7839 = \frac{[Cr_2O_7^{2-}]}{1.00 \times 10^{-4} \text{ M } Cr_2O_7^{2-}}$$

or $1.78 \times 10^{-4} \text{ M } Cr_2O_7^{2-}$.

Qualitative Applications

As discussed in [Chapter 10.2](#), ultraviolet, visible, and infrared absorption bands result from the absorption of electromagnetic radiation by specific valence electrons or bonds. The energy at which the absorption occurs, and the intensity of that absorption, is determined by the chemical environment of the absorbing moiety. For example, benzene has several ultraviolet absorption bands due to $\pi \rightarrow \pi^*$ transitions. The position and intensity of two of these bands, 203.5 nm ($\epsilon = 7400 \text{ M}^{-1} \text{ cm}^{-1}$) and 254 nm ($\epsilon = 204 \text{ M}^{-1} \text{ cm}^{-1}$), are sensitive to substitution. For benzoic acid, in which a carboxylic acid group replaces one of the aromatic hydrogens, the two bands shift to 230 nm ($\epsilon = 11600 \text{ M}^{-1} \text{ cm}^{-1}$) and 273 nm ($\epsilon = 970 \text{ M}^{-1} \text{ cm}^{-1}$). A variety of rules have been developed to aid in correlating UV/Vis absorption bands to chemical structure. Similar correlations are available for infrared absorption bands. For example a carbonyl's C=O stretch is sensitive to adjacent functional groups, appearing at 1650 cm^{-1} for acids, 1700 cm^{-1} for

ketones, and 1800 cm^{-1} for acid chlorides. The interpretation of UV/ Vis and IR spectra receives adequate coverage elsewhere in the chemistry curriculum, notably in organic chemistry, and is not considered further in this text.

With the availability of computerized data acquisition and storage it is possible to build digital libraries of standard reference spectra. The identity of an unknown compound often can be determined by comparing its spectrum against a library of reference spectra, a process known as **spectral searching**. Comparisons are made using an algorithm that calculates the cumulative difference between the sample's spectrum and a reference spectrum. For example, one simple algorithm uses the following equation

$$D = \sum_{i=1}^n |(A_{\text{sample}})_i - (A_{\text{reference}})_i|$$

where D is the cumulative difference, A_{sample} is the sample's absorbance at wavelength or wavenumber i , $A_{\text{reference}}$ is the absorbance of the reference compound at the same wavelength or wavenumber, and n is the number of digitized points in the spectra. The cumulative difference is calculated for each reference spectrum. The reference compound with the smallest value of D is the closest match to the unknown compound. The accuracy of spectral searching is limited by the number and type of compounds included in the library, and by the effect of the sample's matrix on the spectrum.

Another advantage of computerized data acquisition is the ability to subtract one spectrum from another. When coupled with spectral searching it is possible to determine the identity of several components in a sample without the need of a prior separation step by repeatedly searching and subtracting reference spectra. An example is shown in Figure 10.3.13 in which the composition of a two-component mixture is determined by successive searching and subtraction. Figure 10.3.13 a shows the spectrum of the mixture. A search of the spectral library selects cocaine•HCl (Figure 10.3.13 b) as a likely component of the mixture. Subtracting the reference spectrum for cocaine•HCl from the mixture's spectrum leaves a result (Figure 10.3.13 c) that closely matches mannitol's reference spectrum (Figure 10.3.13 d). Subtracting the reference spectrum for mannitol leaves a small residual signal (Figure 10.3.13 e).

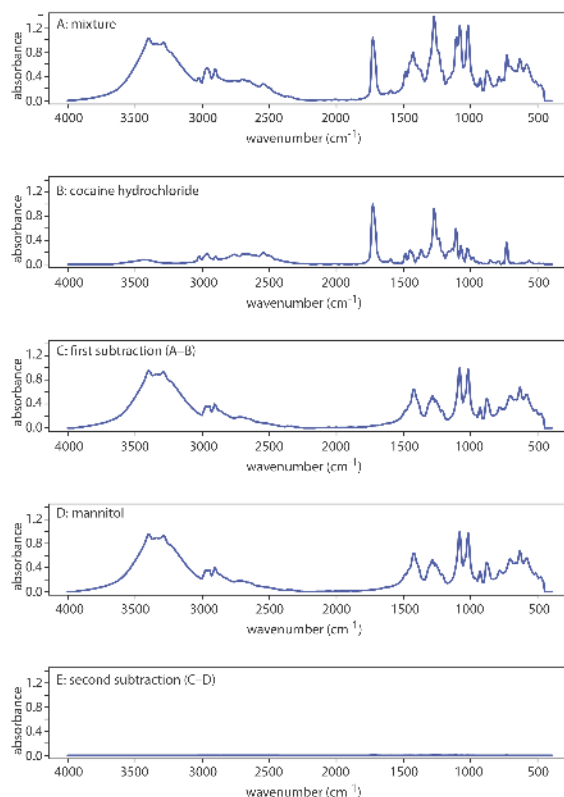


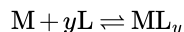
Figure 10.3.13 . Identifying the components of a mixture by spectral searching and subtracting. (a) IR spectrum of the mixture; (b) Reference IR spectrum of cocaine•HCl; (c) Result of subtracting the spectrum of cocaine•HCl from the mixture's spectrum; (d) Reference IR spectrum of mannitol; and (e) The residual spectrum after removing mannitol's contribution to the mixture's spectrum. IR spectra traditionally are displayed using percent transmittance, %T, along the y-axis (for example, see [Figure 10.2.2](#)). Because absorbance—not percent transmittance—is a linear function of concentration, spectral searching and spectral subtraction, is easier to do when displaying absorbance on the y-axis.

Characterization Applications

Molecular absorption, particularly in the UV/Vis range, has been used for a variety of different characterization studies, including determining the stoichiometry of metal–ligand complexes and determining equilibrium constants. Both of these examples are examined in this section.

Stoichiometry of a Metal-Ligand Complex

We can determine the stoichiometry of the metal–ligand complexation reaction



using one of three methods: the method of continuous variations, the mole-ratio method, and the slope-ratio method. Of these approaches, the **method of continuous variations**, also called Job's method, is the most popular. In this method a series of solutions is prepared such that the total moles of metal and of ligand, n_{total} , in each solution is the same. If $(n_M)_i$ and $(n_L)_i$ are, respectively, the moles of metal and ligand in solution i , then

$$n_{\text{total}} = (n_M)_i + (n_L)_i$$

The relative amount of ligand and metal in each solution is expressed as the mole fraction of ligand, $(X_L)_i$, and the mole fraction of metal, $(X_M)_i$,

$$(X_L)_i = \frac{(n_L)_i}{n_{\text{total}}}$$

$$(X_M)_i = 1 - \frac{(n_L)_i}{n_{\text{total}}} = \frac{(n_M)_i}{n_{\text{total}}}$$

The concentration of the metal–ligand complex in any solution is determined by the limiting reagent, with the greatest concentration occurring when the metal and the ligand are mixed stoichiometrically. If we monitor the complexation reaction at a wavelength where only the metal–ligand complex absorbs, a graph of absorbance versus the mole fraction of ligand has two linear branches—one when the ligand is the limiting reagent and a second when the metal is the limiting reagent. The intersection of the two branches represents a stoichiometric mixing of the metal and the ligand. We use the mole fraction of ligand at the intersection to determine the value of y for the metal–ligand complex ML_y .

$$y = \frac{n_L}{n_M} = \frac{X_L}{X_M} = \frac{X_L}{1 - X_L}$$

You also can plot the data as absorbance versus the mole fraction of metal. In this case, y is equal to $(1 - X_M)/X_M$.

✓ Example 10.3.4

To determine the formula for the complex between Fe^{2+} and *o*-phenanthroline, a series of solutions is prepared in which the total concentration of metal and ligand is held constant at 3.15×10^{-4} M. The absorbance of each solution is measured at a wavelength of 510 nm. Using the following data, determine the formula for the complex.

X_L	absorbance	X_L	absorbance
0.000	0.000	0.600	0.693
0.100	0.116	0.700	0.809
0.200	0.231	0.800	0.693
0.300	0.347	0.900	0.347
0.400	0.462	1.000	0.000
0.500	0.578		

Solution

A plot of absorbance versus the mole fraction of ligand is shown in Figure 10.3.14 . To find the maximum absorbance, we extrapolate the two linear portions of the plot. The two lines intersect at a mole fraction of ligand of 0.75. Solving for y gives

$$y = \frac{X_L}{1 - X_L} = \frac{0.75}{1 - 0.75} = 3$$

The formula for the metal–ligand complex is $\text{Fe}(\text{phen})_3^{2+}$.

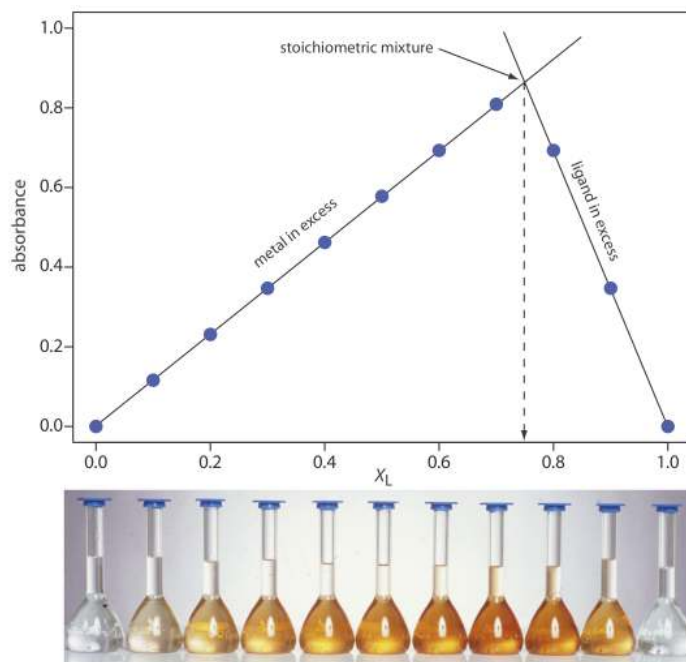


Figure 10.3.14 . Continuous variations plot for Example 10.3.4 . The photo shows the solutions used to gather the data. Each solution is displayed directly below its corresponding point on the continuous variations plot. To prepare these solutions I first prepared a solution of 3.15×10^{-4} M Fe^{2+} and a solution of 3.15×10^{-4} M o -phenanthroline. Because the two stock solutions have the same concentration, diluting a portion of one solution with the other solution gives a mixture in which the combined concentration of o -phenanthroline and Fe^{2+} is 3.15×10^{-4} M. Because each solution has the same volume, each solution also contains the same total moles of metal and ligand.

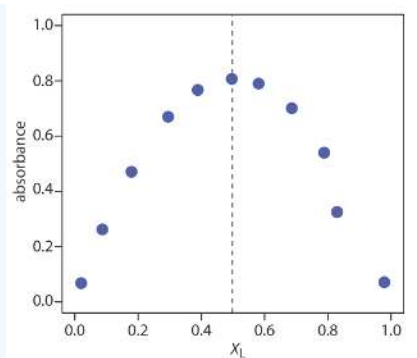
? Exercise 10.3.4

Use the continuous variations data in the following table to determine the formula for the complex between Fe^{2+} and SCN^- . The data for this problem is adapted from Meloun, M.; Havel, J.; Högfeltdt, E. *Computation of Solution Equilibria*, Ellis Horwood: Chichester, England, 1988, p. 236.

X_L	absorbance	X_L	absorbance	X_L	absorbance	X_L	absorbance
0.0200	0.068	0.2951	0.670	0.5811	0.790	0.8923	0.324
0.0870	0.262	0.3887	0.767	0.6860	0.701	0.9787	0.071
0.1792	0.471	0.4964	0.807	0.7885	0.540		

Answer

The figure below shows a continuous variations plot for the data in this exercise. Although the individual data points show substantial curvature—enough curvature that there is little point in trying to draw linear branches for excess metal and excess ligand—the maximum absorbance clearly occurs at $X_L \approx 0.5$. The complex's stoichiometry, therefore, is $\text{Fe}(\text{SCN})_2^{2+}$.



Several precautions are necessary when using the method of continuous variations. First, the metal and the ligand must form only one metal–ligand complex. To determine if this condition is true, plots of absorbance versus X_L are constructed at several different wavelengths and for several different values of n_{total} . If the maximum absorbance does not occur at the same value of X_L for each set of conditions, then more than one metal–ligand complex is present. A second precaution is that the metal–ligand complex’s absorbance must obey Beer’s law. Third, if the metal–ligand complex’s formation constant is relatively small, a plot of absorbance versus X_L may show significant curvature. In this case it often is difficult to determine the stoichiometry by extrapolation. Finally, because the stability of a metal–ligand complex may be influenced by solution conditions, it is necessary to control carefully the composition of the solutions. When the ligand is a weak base, for example, each solutions must be buffered to the same pH.

In the **mole-ratio method** the moles of one reactant, usually the metal, is held constant, while the moles of the other reactant is varied. The absorbance is monitored at a wavelength where the metal–ligand complex absorbs. A plot of absorbance as a function of the ligand-to-metal mole ratio, n_L/n_M , has two linear branches that intersect at a mole–ratio corresponding to the complex’s formula. Figure 10.3.15 a shows a mole-ratio plot for the formation of a 1:1 complex in which the absorbance is monitored at a wavelength where only the complex absorbs. Figure 10.3.15 b shows a mole-ratio plot for a 1:2 complex in which all three species—the metal, the ligand, and the complex—absorb at the selected wavelength. Unlike the method of continuous variations, the mole-ratio method can be used for complexation reactions that occur in a stepwise fashion if there is a difference in the molar absorptivities of the metal–ligand complexes, and if the formation constants are sufficiently different. A typical mole-ratio plot for the step-wise formation of ML and ML_2 is shown in Figure 10.3.15 c.

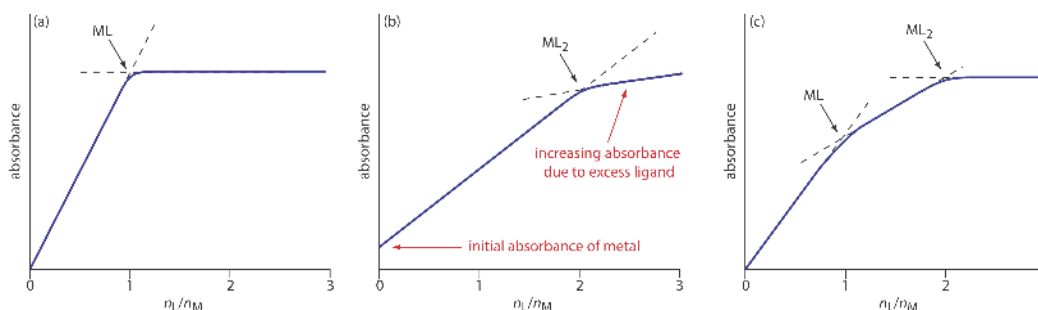


Figure 10.3.15 . Mole-ratio plots for: (a) a 1:1 metal–ligand complex in which only the complex absorbs; (b) a 1:2 metal–ligand complex in which the metal, the ligand, and the complex absorb; and (c) the stepwise formation of a 1:1 and a 1:2 metal–ligand complex.

For both the method of continuous variations and the mole-ratio method, we determine the complex’s stoichiometry by extrapolating absorbance data from conditions in which there is a linear relationship between absorbance and the relative amounts of metal and ligand. If a metal–ligand complex is very weak, a plot of absorbance versus X_L or n_L/n_M becomes so curved that it is impossible to determine the stoichiometry by extrapolation. In this case the slope-ratio is used.

In the **slope-ratio method** two sets of solutions are prepared. The first set of solutions contains a constant amount of metal and a variable amount of ligand, chosen such that the total concentration of metal, C_M , is much larger than the total concentration of ligand, C_L . Under these conditions we may assume that essentially all the ligand reacts to form the metal–ligand complex. The concentration of the complex, which has the general form M_xL_y , is

$$[M_xL_y] = \frac{C_L}{y}$$

If we monitor the absorbance at a wavelength where only M_xL_y absorbs, then

$$A = \epsilon b [M_xL_y] = \frac{\epsilon b C_L}{y}$$

and a plot of absorbance versus C_L is linear with a slope, s_L , of

$$s_L = \frac{\epsilon b}{y}$$

A second set of solutions is prepared with a fixed concentration of ligand that is much greater than a variable concentration of metal; thus

$$\begin{aligned} [M_xL_y] &= \frac{C_M}{x} \\ A &= \epsilon b [M_xL_y] = \frac{\epsilon b C_M}{x} \\ s_M &= \frac{\epsilon b}{x} \end{aligned}$$

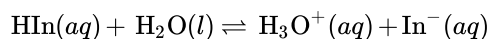
A ratio of the slopes provides the relative values of x and y .

$$\frac{s_M}{s_L} = \frac{\epsilon b/x}{\epsilon b/y} = \frac{y}{x}$$

An important assumption in the slope-ratio method is that the complexation reaction continues to completion in the presence of a sufficiently large excess of metal or ligand. The slope-ratio method also is limited to systems in which only a single complex forms and for which Beer's law is obeyed.

Determination of Equilibrium Constants

Another important application of molecular absorption spectroscopy is the determination of equilibrium constants. Let's consider, as a simple example, an acid-base reaction of the general form



where HIn and In^- are the conjugate weak acid and weak base forms of an acid-base indicator. The equilibrium constant for this reaction is

$$K_a = \frac{[H_3O^+][A^-]}{[HA]}$$

To determine the equilibrium constant's value, we prepare a solution in which the reaction is in a state of equilibrium and determine the equilibrium concentration for H_3O^+ , HIn , and In^- . The concentration of H_3O^+ is easy to determine by measuring the solution's pH. To determine the concentration of HIn and In^- we can measure the solution's absorbance.

If both HIn and In^- absorb at the selected wavelength, then, from Beer's law, we know that

$$A = \epsilon_{HIn} b [HIn] + \epsilon_{In^-} b [In^-] \quad (7.2.5)$$

where ϵ_{HIn} and ϵ_{In^-} are the molar absorptivities for HIn and In^- . The indicator's total concentration, C , is given by a mass balance equation

$$C = [HIn] + [In^-] \quad (7.2.6)$$

Solving Equation 7.2.6 for $[HIn]$ and substituting into Equation 7.2.5 gives

$$A = \epsilon_{HIn} b (C - [In^-]) + \epsilon_{In^-} b [In^-]$$

which we simplify to

$$\begin{aligned} A &= \epsilon_{HIn} b C - \epsilon_{HIn} b [In^-] + \epsilon_{In^-} b [In^-] \\ A &= A_{HIn} + b [In^-] (\epsilon_{In^-} - \epsilon_{HIn}) \end{aligned} \quad (7.2.7)$$

where A_{HIn} , which is equal to $\epsilon_{\text{HIn}} bC$, is the absorbance when the pH is acidic enough that essentially all the indicator is present as HIn. Solving Equation 7.2.7 for the concentration of In^- gives

$$[\text{In}^-] = \frac{A - A_{\text{HIn}}}{b(\epsilon_{\text{In}} - \epsilon_{\text{HIn}})} \quad (7.2.8)$$

Proceeding in the same fashion, we derive a similar equation for the concentration of HIn

$$[\text{HIn}] = \frac{A_{\text{In}} - A}{b(\epsilon_{\text{In}} - \epsilon_{\text{HIn}})} \quad (7.2.9)$$

where A_{In} , which is equal to $\epsilon_{\text{In}} bC$, is the absorbance when the pH is basic enough that only In^- contributes to the absorbance. Substituting Equation 7.2.8 and Equation 7.2.9 into the equilibrium constant expression for HIn gives

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{In}^-]}{[\text{HIn}]} = [\text{H}_3\text{O}^+] \times \frac{A - A_{\text{HIn}}}{A_{\text{In}} - A} \quad (7.2.10)$$

We can use Equation 7.2.10 to determine K_a in one of two ways. The simplest approach is to prepare three solutions, each of which contains the same amount, C , of indicator. The pH of one solution is made sufficiently acidic such that $[\text{HIn}] \gg [\text{In}^-]$. The absorbance of this solution gives A_{HIn} . The value of A_{In} is determined by adjusting the pH of the second solution such that $[\text{In}^-] \gg [\text{HIn}]$. Finally, the pH of the third solution is adjusted to an intermediate value, and the pH and absorbance, A , recorded. The value of K_a is calculated using Equation 7.2.10

✓ Example 10.3.5

The acidity constant for an acid–base indicator is determined by preparing three solutions, each of which has a total concentration of indicator equal to 5.00×10^{-5} M. The first solution is made strongly acidic with HCl and has an absorbance of 0.250. The second solution is made strongly basic and has an absorbance of 1.40. The pH of the third solution is 2.91 and has an absorbance of 0.662. What is the value of K_a for the indicator?

Solution

The value of K_a is determined by making appropriate substitutions into 10.20 where $[\text{H}_3\text{O}^+]$ is 1.23×10^{-3} ; thus

$$K_a = (1.23 \times 10^{-3}) \times \frac{0.662 - 0.250}{1.40 - 0.662} = 6.87 \times 10^{-4}$$

? Exercise 10.3.5

To determine the K_a of a merocyanine dye, the absorbance of a solution of 3.5×10^{-4} M dye was measured at a pH of 2.00, a pH of 6.00, and a pH of 12.00, yielding absorbances of 0.000, 0.225, and 0.680, respectively. What is the value of K_a for this dye? The data for this problem is adapted from Lu, H.; Rutan, S. C. *Anal. Chem.*, **1996**, 68, 1381–1386.

Answer

The value of K_a is

$$K_a = (1.00 \times 10^{-6}) \times \frac{0.225 - 0.000}{0.680 - 0.225} = 4.95 \times 10^{-7}$$

A second approach for determining K_a is to prepare a series of solutions, each of which contains the same amount of indicator. Two solutions are used to determine values for A_{HIn} and A_{In} . Taking the log of both sides of Equation 7.2.10 and rearranging leave us with the following equation.

$$\log \frac{A - A_{\text{HIn}}}{A_{\text{In}} - A} = \text{pH} - \text{p}K_a \quad (7.2.11)$$

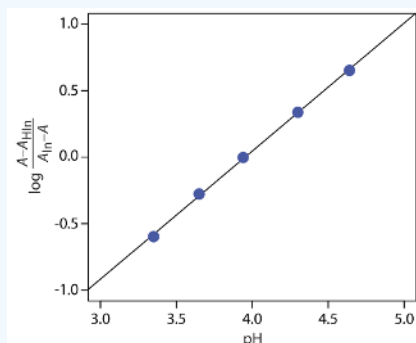
A plot of $\log[(A - A_{\text{HIn}})/(A_{\text{In}} - A)]$ versus pH is a straight-line with a slope of +1 and a y-intercept of $-\text{p}K_a$.

? Exercise 10.3.6

To determine the K_a for the indicator bromothymol blue, the absorbance of each a series of solutions that contain the same concentration of bromothymol blue is measured at pH levels of 3.35, 3.65, 3.94, 4.30, and 4.64, yielding absorbance values of 0.170, 0.287, 0.411, 0.562, and 0.670, respectively. Acidifying the first solution to a pH of 2 changes its absorbance to 0.006, and adjusting the pH of the last solution to 12 changes its absorbance to 0.818. What is the value of K_a for bromothymol blue? The data for this problem is from Patterson, G. S. *J. Chem. Educ.*, **1999**, 76, 395–398.

Answer

To determine K_a we use Equation 7.2.11, plotting $\log[(A - A_{\text{HIn}})/(A_{\text{In}} - A)]$ versus pH, as shown below.

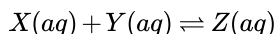


Fitting a straight-line to the data gives a regression model of

$$\log \frac{A - A_{\text{HIn}}}{A_{\text{In}} - A} = -3.80 + 0.962\text{pH}$$

The y-intercept is $-\text{p}K_a$; thus, the $\text{p}K_a$ is 3.80 and the K_a is 1.58×10^{-4} .

In developing these approaches for determining K_a we considered a relatively simple system in which the absorbance of HIn and In^- are easy to measure and for which it is easy to determine the concentration of H_3O^+ . In addition to acid–base reactions, we can adapt these approaches to any reaction of the general form



including metal–ligand complexation reactions and redox reactions, provided we can determine spectrophotometrically the concentration of the product, Z, and one of the reactants, either X or Y, and that we can determine the concentration of the other reactant by some other method. With appropriate modifications, a more complicated system in which we cannot determine the concentration of one or more of the reactants or products also is possible [Ramette, R. W. *Chemical Equilibrium and Analysis*, Addison-Wesley: Reading, MA, 1981, Chapter 13].

Evaluation of UV/Vis and IR Spectroscopy

Scale of Operations

Molecular UV/Vis absorption routinely is used for the analysis of trace analytes in macro and meso samples. Major and minor analytes are determined by diluting the sample before analysis, and concentrating a sample may allow for the analysis of ultratrace analytes. The scale of operations for infrared absorption is generally poorer than that for UV/Vis absorption.

Accuracy

Under normal conditions a relative error of 1–5% is easy to obtained with UV/Vis absorption. Accuracy usually is limited by the quality of the blank. Examples of the type of problems that are encountered include the presence of particulates in the sample that scatter radiation, and the presence of interferents that react with analytical reagents. In the latter case the interferent may react to form an absorbing species, which leads to a positive determinate error. Interferents also may prevent the analyte from reacting, which leads to a negative determinate error. With care, it is possible to improve the accuracy of an analysis by as much as an order of magnitude.

Precision

In absorption spectroscopy, precision is limited by indeterminate errors—primarily instrumental noise—which are introduced when we measure absorbance. Precision generally is worse for low absorbances where $P_0 \approx P_T$, and for high absorbances where P_T approaches 0. We might expect, therefore, that precision will vary with transmittance.

We can derive an expression between precision and transmittance by applying the propagation of uncertainty as described in Chapter 4. To do so we rewrite Beer's law as

$$C = -\frac{1}{\epsilon b} \log T \quad (7.2.12)$$

Table 4.3.1 in Chapter 4 helps us complete the propagation of uncertainty for Equation 7.2.12; thus, the absolute uncertainty in the concentration, s_C , is

$$s_C = -\frac{0.4343}{\epsilon b} \times \frac{s_T}{T} \quad (7.2.13)$$

where s_T is the absolute uncertainty in the transmittance. Dividing Equation 7.2.13 by Equation 7.2.12 gives the relative uncertainty in concentration, s_C/C , as

$$\frac{s_C}{C} = \frac{0.4343 s_T}{T \log T}$$

If we know the transmittance's absolute uncertainty, then we can determine the relative uncertainty in concentration for any measured transmittance.

Determining the relative uncertainty in concentration is complicated because s_T is a function of the transmittance. As shown in Table 10.3.3, three categories of indeterminate instrumental error are observed [Rothman, L. D.; Crouch, S. R.; Ingle, J. D. Jr. *Anal. Chem.* **1975**, 47, 1226–1233]. A constant s_T is observed for the uncertainty associated with reading %T on a meter's analog or digital scale. Typical values are ± 0.2 – 0.3% (a k_1 of ± 0.002 – 0.003) for an analog scale and $\pm 0.001\%$ (a k_1 of ± 0.00001) for a digital scale.

Table 10.3.3 . Effect of Indeterminate Errors on Relative Uncertainty in Concentration

category	sources of indeterminate error	relative uncertainty in concentration
$s_T = k_1$	%T readout resolution noise in thermal detectors	$\frac{s_C}{C} = \frac{0.4343 k_1}{T \log T}$
$s_T = k_2 \sqrt{T^2 + T}$	noise in photon detectors	$\frac{s_C}{C} = \frac{0.4343 k_2}{\log T} \sqrt{1 + \frac{1}{T}}$
$s_T = k_3 T$	positioning of sample cell fluctuations in source intensity	$\frac{s_C}{C} = \frac{0.4343 k_3}{\log T}$

A constant s_T also is observed for the thermal transducers used in infrared spectrophotometers. The effect of a constant s_T on the relative uncertainty in concentration is shown by curve A in Figure 10.3.16. Note that the relative uncertainty is very large for both high absorbances and low absorbances, reaching a minimum when the absorbance is 0.4343. This source of indeterminate error is important for infrared spectrophotometers and for inexpensive UV/Vis spectrophotometers. To obtain a relative uncertainty in concentration of ± 1 – 2% , the absorbance is kept within the range 0.1–1.

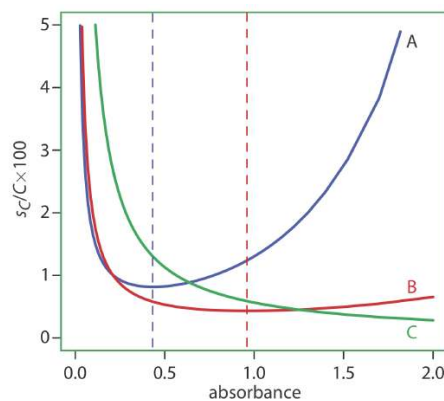


Figure 10.3.16 . Percent relative uncertainty in concentration as a function of absorbance for the categories of indeterminate errors in Table 7.2.10.3. A: $k_1 = \pm 0.0030$; B: $k_2 = \pm 0.0030$; and C: $k_3 = \pm 0.0130$. The dashed lines correspond to the minimum uncertainty for curve A (absorbance of 0.4343) and for curve B (absorbance of 0.963).

Values of s_T are a complex function of transmittance when indeterminate errors are dominated by the noise associated with photon detectors. Curve B in Figure 10.3.16 shows that the relative uncertainty in concentration is very large for low absorbances, but is smaller at higher absorbances. Although the relative uncertainty reaches a minimum when the absorbance is 0.963, there is little change in the relative uncertainty for absorbances between 0.5 and 2. This source of indeterminate error generally limits the precision of high quality UV/Vis spectrophotometers for mid-to-high absorbances.

Finally, the value of s_T is directly proportional to transmittance for indeterminate errors that result from fluctuations in the source's intensity and from uncertainty in positioning the sample within the spectrometer. The latter is particularly important because the optical properties of a sample cell are not uniform. As a result, repositioning the sample cell may lead to a change in the intensity of transmitted radiation. As shown by curve C in Figure 10.3.16, the effect is important only at low absorbances. This source of indeterminate errors usually is the limiting factor for high quality UV/Vis spectrophotometers when the absorbance is relatively small.

When the relative uncertainty in concentration is limited by the %T readout resolution, it is possible to improve the precision of the analysis by redefining 100% T and 0% T. Normally 100% T is established using a blank and 0% T is established while preventing the source's radiation from reaching the detector. If the absorbance is too high, precision is improved by resetting 100% T using a standard solution of analyte whose concentration is less than that of the sample (Figure 10.3.17 a). For a sample whose absorbance is too low, precision is improved by redefining 0% T using a standard solution of the analyte whose concentration is greater than that of the analyte (Figure 10.3.17 b). In this case a calibration curve is required because a linear relationship between absorbance and concentration no longer exists. Precision is further increased by combining these two methods (Figure 10.3.17 c). Again, a calibration curve is necessary since the relationship between absorbance and concentration is no longer linear.

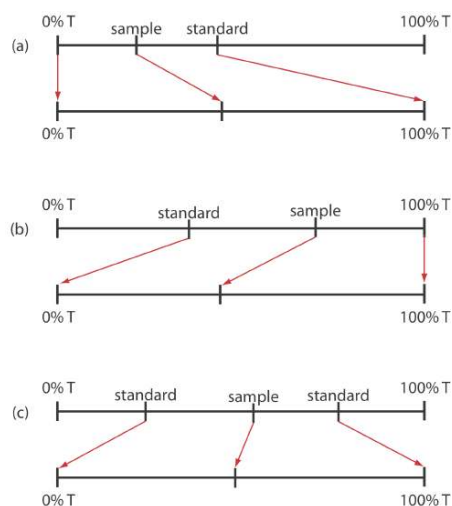


Figure 10.3.17 . Methods for improving the precision of absorption methods: (a) high-absorbance method; (b) low-absorbance method; (c) maximum precision method.

Sensitivity

The sensitivity of a molecular absorption method, which is the slope of a Beer's law calibration curve, is the product of the analyte's absorptivity and the pathlength of the sample cell (ϵb). You can improve a method's sensitivity by selecting a wavelength where absorbance is at a maximum or by increasing pathlength.

See [Figure 10.2.10](#) for an example of how the choice of wavelength affects a calibration curve's sensitivity.

Selectivity

Selectivity rarely is a problem in molecular absorption spectrophotometry. In many cases it is possible to find a wavelength where only the analyte absorbs. When two or more species do contribute to the measured absorbance, a multicomponent analysis is still possible, as shown in [Example 10.3.2](#) and [Example 10.3.3](#).

Time, Cost, and Equipment

The analysis of a sample by molecular absorption spectroscopy is relatively rapid, although additional time is required if we need to convert a nonabsorbing analyte into an absorbing form. The cost of UV/Vis instrumentation ranges from several hundred dollars for a simple filter photometer, to more than \$50,000 for a computer-controlled, high-resolution double-beam instrument equipped with variable slit widths, and operating over an extended range of wavelengths. Fourier transform infrared spectrometers can be obtained for as little as \$15,000–\$20,000, although more expensive models are available.

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CHAPTER OVERVIEW

8: Electrochemistry

[8.1: Overview of Electrochemistry](#)

[8.2: Voltammetric and Amperometric Methods](#)

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8.1: Overview of Electrochemistry

The focus of this chapter is on analytical techniques that use a measurement of potential, current, or charge to determine an analyte's concentration or to characterize an analyte's chemical reactivity. Collectively we call this area of analytical chemistry **electrochemistry** because it originated from the study of the movement of electrons in an oxidation–reduction reaction.

Despite the difference in instrumentation, all electrochemical techniques share several common features. Before we consider individual examples in greater detail, let's take a moment to consider some of these similarities. As you work through the chapter, this overview will help you focus on similarities between different electrochemical methods of analysis. You will find it easier to understand a new analytical method when you can see its relationship to other similar methods.

Five Important Concepts

To understand electrochemistry we need to appreciate five important and interrelated concepts: (1) the electrode's potential determines the analyte's form at the electrode's surface; (2) the concentration of analyte at the electrode's surface may not be the same as its concentration in bulk solution; (3) in addition to an oxidation–reduction reaction, the analyte may participate in other chemical reactions; (4) current is a measure of the rate of the analyte's oxidation or reduction; and (5) we cannot control simultaneously current and potential.

The material in this section—particularly the five important concepts—draws upon a vision for understanding electrochemistry outlined by Larry Faulkner in the article “Understanding Electrochemistry: Some Distinctive Concepts,” *J. Chem. Educ.* **1983**, 60, 262–264. See also, Kissinger, P. T.; Bott, A. W. “Electrochemistry for the Non-Electrochemist,” *Current Separations*, **2002**, 20:2, 51–53.

The Electrode's Potential Determines the Analyte's Form

In Chapter 6 we introduced the ladder diagram as a tool for predicting how a change in solution conditions affects the position of an equilibrium reaction. Figure 11.1.1, for example, shows a ladder diagram for the $\text{Fe}^{3+}/\text{Fe}^{2+}$ and the $\text{Sn}^{4+}/\text{Sn}^{2+}$ equilibria. If we place an electrode in a solution of Fe^{3+} and Sn^{4+} and adjust its potential to +0.500 V, Fe^{3+} is reduced to Fe^{2+} but Sn^{4+} is not reduced to Sn^{2+} .

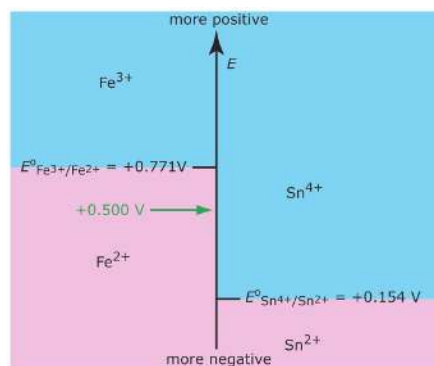


Figure 11.1.1. Redox ladder diagram for $\text{Fe}^{3+}/\text{Fe}^{2+}$ and for $\text{Sn}^{4+}/\text{Sn}^{2+}$ redox couples. The areas in blue show the potential range where the oxidized forms are the predominate species; the reduced forms are the predominate species in the areas shown in pink. Note that a more positive potential favors the oxidized forms. At a potential of +0.500 V (green arrow) Fe^{3+} reduces to Fe^{2+} , but Sn^{4+} remains unchanged. You may wish to review the earlier treatment of oxidation–reduction reactions in Chapter 6.4 and the development of ladder diagrams for oxidation–reduction reactions in Chapter 6.6.

Interfacial Concentrations May Not Equal Bulk Concentrations

In Chapter 6 we introduced the Nernst equation, which provides a mathematical relationship between the electrode's potential and the concentrations of an analyte's oxidized and reduced forms in solution. For example, the Nernst equation for Fe^{3+} and Fe^{2+} is

$$E = E_{\text{Fe}^{3+}/\text{Fe}^{2+}} - \frac{RT}{nF} \ln \frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} = \frac{0.05916}{1} \log \frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} \quad (8.1.1)$$

where E is the electrode's potential and $E_{\text{Fe}^{3+}/\text{Fe}^{2+}}^{\circ}$ is the standard-state reduction potential for the reaction $\text{Fe}^{3+}(\text{aq}) \rightleftharpoons \text{Fe}^{2+}(\text{aq}) + e^{-}$. Because it is the potential of the electrode that determines the analyte's form at the electrode's surface, the concentration terms in Equation 8.1.1 are those of Fe^{2+} and Fe^{3+} at the electrode's surface, not their concentrations in bulk solution.

This distinction between a species' surface concentration and its bulk concentration is important. Suppose we place an electrode in a solution of Fe^{3+} and fix its potential at 1.00 V. From the ladder diagram in Figure 11.1.1, we know that Fe^{3+} is stable at this potential and, as shown in Figure 11.1.2 a, the concentration of Fe^{3+} is the same at all distances from the electrode's surface. If we change the electrode's potential to +0.500 V, the concentration of Fe^{3+} at the electrode's surface decreases to approximately zero. As shown in Figure 11.1.2 b, the concentration of Fe^{3+} increases as we move away from the electrode's surface until it equals the concentration of Fe^{3+} in bulk solution. The resulting concentration gradient causes additional Fe^{3+} from the bulk solution to diffuse to the electrode's surface.

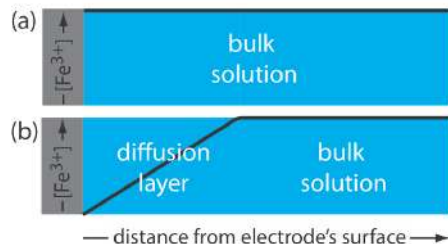


Figure 11.1.2. Concentration of Fe^{3+} as a function of distance from the electrode's surface at (a) $E = +1.00$ V and (b) $E = +0.500$ V. The electrode is shown in gray and the solution in blue.

We call the region of solution that contains this concentration gradient in Fe^{3+} the diffusion layer. We will have more to say about this in Chapter 11.4.

The Analyte May Participate in Other Reactions

Figure 11.1.1 and Figure 11.1.2 shows how the electrode's potential affects the concentration of Fe^{3+} and how the concentration of Fe^{3+} varies as a function of distance from the electrode's surface. The reduction of Fe^{3+} to Fe^{2+} , which is governed by Equation 8.1.1, may not be the only reaction that affects the concentration of Fe^{3+} in bulk solution or at the electrode's surface. The adsorption of Fe^{3+} at the electrode's surface or the formation of a metal-ligand complex in bulk solution, such as $\text{Fe}(\text{OH})^{2+}$, also affects the concentration of Fe^{3+} .

Current is a Measure of Rate

The reduction of Fe^{3+} to Fe^{2+} consumes an electron, which is drawn from the electrode. The oxidation of another species, perhaps the solvent, at a second electrode is the source of this electron. Because the reduction of Fe^{3+} to Fe^{2+} consumes one electron, the flow of electrons between the electrodes—in other words, the current—is a measure of the rate at which Fe^{3+} is reduced. One important consequence of this observation is that the current is zero when the reaction $\text{Fe}^{3+}(\text{aq}) \rightleftharpoons \text{Fe}^{2+}(\text{aq}) + e^{-}$ is at equilibrium.

The rate of the reaction $\text{Fe}^{3+}(\text{aq}) \rightleftharpoons \text{Fe}^{2+}(\text{aq}) + e^{-}$ is the change in the concentration of Fe^{3+} as a function of time.

We Cannot Control Simultaneously Both the Current and the Potential

If a solution of Fe^{3+} and Fe^{2+} is at equilibrium, the current is zero and the potential is given by Equation 8.1.1. If we change the potential away from its equilibrium position, current flows as the system moves toward its new equilibrium position. Although the initial current is quite large, it decreases over time, reaching zero when the reaction reaches equilibrium. The current, therefore, changes in response to the applied potential. Alternatively, we can pass a fixed current through the electrochemical cell, forcing the reduction of Fe^{3+} to Fe^{2+} . Because the concentrations of Fe^{3+} decreases and the concentration of Fe^{2+} increases, the potential, as given by Equation 8.1.1, also changes over time. In short, if we choose to control the potential, then we must accept the resulting current, and we must accept the resulting potential if we choose to control the current.

Controlling and Measuring Current and Potential

Electrochemical measurements are made in an electrochemical cell that consists of two or more electrodes and the electronic circuitry needed to control and measure the current and the potential. In this section we introduce the basic components of electrochemical instrumentation.

The simplest electrochemical cell uses two electrodes. The potential of one electrode is sensitive to the analyte's concentration, and is called the **working electrode** or the **indicator electrode**. The second electrode, which we call the **counter electrode**, completes the electrical circuit and provides a reference potential against which we measure the working electrode's potential. Ideally the counter electrode's potential remains constant so that we can assign to the working electrode any change in the overall cell potential. If the counter electrode's potential is not constant, then we replace it with two electrodes: a **reference electrode** whose potential remains constant and an **auxiliary electrode** that completes the electrical circuit.

Because we cannot control simultaneously the current and the potential, there are only three basic experimental designs: (1) we can measure the potential when the current is zero, (2) we can measure the potential while we control the current, and (3) we can measure the current while we control the potential. Each of these experimental designs relies on **Ohm's law**, which states that the current, i , passing through an electrical circuit of resistance, R , generates a potential, E .

$$E = iR$$

Each of these experimental designs uses a different type of instrument. To help us understand how we can control and measure current and potential, *we will describe these instruments as if the analyst is operating them manually*. To do so the analyst observes a change in the current or the potential and manually adjusts the instrument's settings to maintain the desired experimental conditions. It is important to understand that modern electrochemical instruments provide an automated, electronic means for controlling and measuring current and potential, and that they do so by using very different electronic circuitry than that described here.

This point bears repeating: It is important to understand that the experimental designs in Figure 11.1.3, Figure 11.1.4, and Figure 11.1.5 do not represent the electrochemical instruments you will find in today's analytical labs. For further information about modern electrochemical instrumentation, see this chapter's additional resources.

Potentiometers

To measure the potential of an electrochemical cell under a condition of zero current we use a **potentiometer**. Figure 11.1.3 shows a schematic diagram for a manual potentiometer that consists of a power supply, an electrochemical cell with a working electrode and a counter electrode, an ammeter to measure the current that passes through the electrochemical cell, an adjustable, slide-wire resistor, and a tap key for closing the circuit through the electrochemical cell. Using Ohm's law, the current in the upper half of the circuit is

$$i_{\text{upper}} = \frac{E_{\text{PS}}}{R_{ab}}$$

where E_{PS} is the power supply's potential, and R_{ab} is the resistance between points a and b of the slide-wire resistor. In a similar manner, the current in the lower half of the circuit is

$$i_{\text{lower}} = \frac{E_{\text{cell}}}{R_{cb}}$$

where E_{cell} is the potential difference between the working electrode and the counter electrode, and R_{cb} is the resistance between the points c and b of the slide-wire resistor. When $i_{\text{upper}} = i_{\text{lower}} = 0$, no current flows through the ammeter and the potential of the electrochemical cell is

$$E_{\text{cell}} = \frac{R_{cb}}{R_{ab}} \times E_{\text{PS}} \quad (8.1.2)$$

To determine E_{cell} we briefly press the tap key and observe the current at the ammeter. If the current is not zero, then we adjust the slide wire resistor and remeasure the current, continuing this process until the current is zero. When the current is zero, we use Equation 8.1.2 to calculate E_{cell} .

Using the tap key to briefly close the circuit through the electrochemical cell minimizes the current that passes through the cell and limits the change in the electrochemical cell's composition. For example, passing a current of 10^{-9} A through the electrochemical cell for 1 s changes the concentrations of species in the cell by approximately 10^{-14} moles. Modern potentiometers use operational amplifiers to create a high-impedance voltmeter that measures the potential while drawing a current of less than 10^{-9} A.

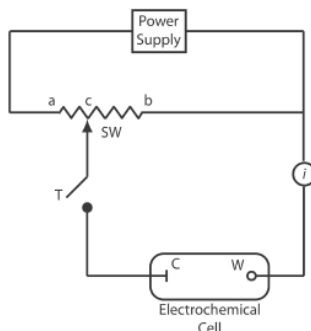


Figure 11.1.3 . Schematic diagram of a manual potentiometer: C is the counter electrode; W is the working electrode; SW is a slide-wire resistor; T is a tap key and i is an ammeter for measuring current.

Galvanostats

A **galvanostat**, a schematic diagram of which is shown in Figure 11.1.4 , allows us to control the current that flows through an electrochemical cell. The current from the power supply through the working electrode is

$$i = \frac{E_{PS}}{R + R_{cell}}$$

where E_{PS} is the potential of the power supply, R is the resistance of the resistor, and R_{cell} is the resistance of the electrochemical cell. If $R \gg R_{cell}$, then the current between the auxiliary and working electrodes

$$i = \frac{E_{PS}}{R} \approx \text{constant}$$

maintains a constant value. To monitor the working electrode's potential, which changes as the composition of the electrochemical cell changes, we can include an optional reference electrode and a high-impedance potentiometer.

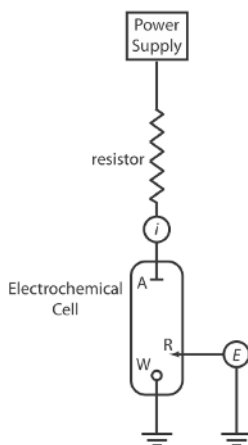


Figure 11.1.4 . Schematic diagram of a galvanostat: A is the auxiliary electrode; W is the working electrode; R is an optional reference electrode, E is a high-impedance potentiometer, and i is an ammeter. The working electrode and the optional reference electrode are connected to a ground.

Potentiostats

A **potentiostat**, a schematic diagram of which is shown in Figure 11.1.5 , allows us to control the working electrode's potential. The potential of the working electrode is measured relative to a constant-potential reference electrode that is connected to the working electrode through a high-impedance potentiometer. To set the working electrode's potential we adjust the slide wire resistor that is connected to the auxiliary electrode. If the working electrode's potential begins to drift, we adjust the slide wire resistor to return the potential to its initial value. The current flowing between the auxiliary electrode and the working electrode is measured with an

ammeter. Modern potentiostats include waveform generators that allow us to apply a time-dependent potential profile, such as a series of potential pulses, to the working electrode.

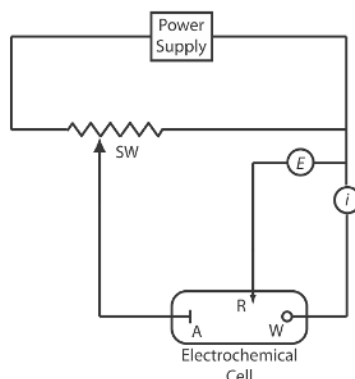


Figure 11.1.5 . Schematic diagram for a manual potentiostat: W is the working electrode; A is the auxiliary electrode; R is the reference electrode; SW is a slide-wire resistor; E is a high-impedance potentiometer; and i is an ammeter.

Interfacial Electrochemical Techniques

Because interfacial electrochemistry is such a broad field, let's use Figure 11.1.6 to organize techniques by the experimental conditions we choose to use (Do we control the potential or the current? How do we change the applied potential or applied current? Do we stir the solution?) and the analytical signal we decide to measure (Current? Potential?).

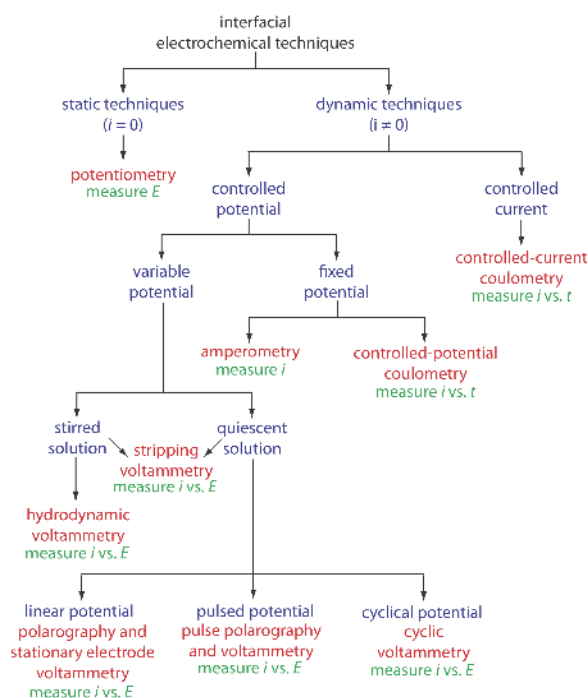


Figure 11.1.6 . Family tree that highlights the similarities and differences between a number of interfacial electrochemical techniques. The specific techniques are shown in red, the experimental conditions are shown in blue, and the analytical signals are shown in green.

At the first level, we divide interfacial electrochemical techniques into static techniques and dynamic techniques. In a static technique we do not allow current to pass through the electrochemical cell and, as a result, the concentrations of all species remain constant. Potentiometry, in which we measure the potential of an electrochemical cell under static conditions, is one of the most important quantitative electrochemical methods and is discussed in detail in Chapter 11.2.

Dynamic techniques, in which we allow current to flow and force a change in the concentration of species in the electrochemical cell, comprise the largest group of interfacial electrochemical techniques. Coulometry, in which we measure current as a function of time, is covered in Chapter 11.3. Amperometry and voltammetry, in which we measure current as a function of a fixed or variable potential, is the subject of Chapter 11.4.

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8.2: Voltammetric and Amperometric Methods

In **voltammetry** we apply a time-dependent potential to an electrochemical cell and measure the resulting current as a function of that potential. We call the resulting plot of current versus applied potential a **voltammogram**, and it is the electrochemical equivalent of a spectrum in spectroscopy, providing quantitative and qualitative information about the species involved in the oxidation or reduction reaction [Maloy, J. T. *J. Chem. Educ.* **1983**, 60, 285–289]. The earliest voltammetric technique is polarography, developed by Jaroslav Heyrovsky in the early 1920s—an achievement for which he was awarded the Nobel Prize in Chemistry in 1959. Since then, many different forms of voltammetry have been developed, a few of which are highlighted in [Figure 11.1.6](#). Before examining these techniques and their applications in more detail, we must first consider the basic experimental design for voltammetry and the factors influencing the shape of the resulting voltammogram.

For an on-line introduction to much of the material in this section, see [Analytical Electrochemistry: The Basic Concepts](#) by Richard S. Kelly, a resource that is part of the Analytical Sciences Digital Library.

Voltammetric Measurements

Although early voltammetric methods used only two electrodes, a modern voltammeter makes use of a three-electrode potentiostat, such as that shown in [Figure 11.1.5](#). In voltammetry we apply a time-dependent potential excitation signal to the working electrode—changing its potential relative to the fixed potential of the reference electrode—and measure the current that flows between the working electrode and the auxiliary electrode. The auxiliary electrode generally is a platinum wire and the reference electrode usually is a SCE or a Ag/AgCl electrode.

[Figure 11.1.5](#) shows an example of a manual three-electrode potentiostat. Although a modern potentiostat uses very different circuitry, you can use [Figure 11.1.5](#) and the accompanying discussion to understand how we can control the potential of working electrode and measure the resulting current.

For the working electrode we can choose among several different materials, including mercury, platinum, gold, silver, and carbon. The earliest voltammetric techniques used a mercury working electrode. Because mercury is a liquid, the working electrode usual is a drop suspended from the end of a capillary tube. In the **hanging mercury drop electrode**, or HMDE, we extrude the drop of Hg by rotating a micrometer screw that pushes the mercury from a reservoir through a narrow capillary tube ([Figure 11.4.1 a](#)).

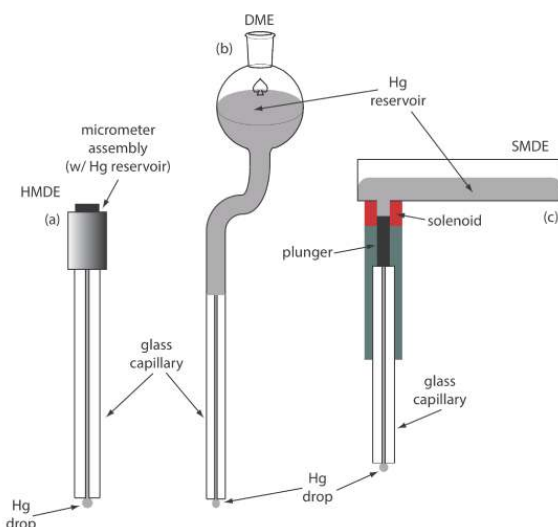


Figure 11.4.1 . Three examples of mercury electrodes: (a) hanging mercury drop electrode, or HMDE; (b) dropping mercury electrode, or DME; and (c) static mercury drop electrode, or SMDE.

In the **dropping mercury electrode**, or DME, mercury drops form at the end of the capillary tube as a result of gravity ([Figure 11.4.1 b](#)). Unlike the HMDE, the mercury drop of a DME grows continuously—as mercury flows from the reservoir under the influence of gravity—and has a finite lifetime of several seconds. At the end of its lifetime the mercury drop is dislodged, either

manually or on its own, and is replaced by a new drop. The **static mercury drop electrode**, or SMDE, uses a solenoid driven plunger to control the flow of mercury (Figure 11.4.1 c). Activation of the solenoid momentarily lifts the plunger, allowing mercury to flow through the capillary, forming a single, hanging Hg drop. Repeated activation of the solenoid produces a series of Hg drops. In this way the SMDE may be used as either a HMDE or a DME. There is one additional type of mercury electrode: the **mercury film electrode**. A solid electrode—typically carbon, platinum, or gold—is placed in a solution of Hg^{2+} and held at a potential where the reduction of Hg^{2+} to Hg is favorable, depositing a thin film of mercury on the solid electrode's surface.

Mercury has several advantages as a working electrode. Perhaps its most important advantage is its high overpotential for the reduction of H_3O^+ to H_2 , which makes accessible potentials as negative as -1 V versus the SCE in acidic solutions and -2 V versus the SCE in basic solutions (Figure 11.4.2). A species such as Zn^{2+} , which is difficult to reduce at other electrodes without simultaneously reducing H_3O^+ , is easy to reduce at a mercury working electrode. Other advantages include the ability of metals to dissolve in mercury—which results in the formation of an **amalgam**—and the ability to renew the surface of the electrode by extruding a new drop. One limitation to mercury as a working electrode is the ease with which it is oxidized. Depending on the solvent, a mercury electrode can not be used at potentials more positive than approximately -0.3 V to $+0.4$ V versus the SCE.

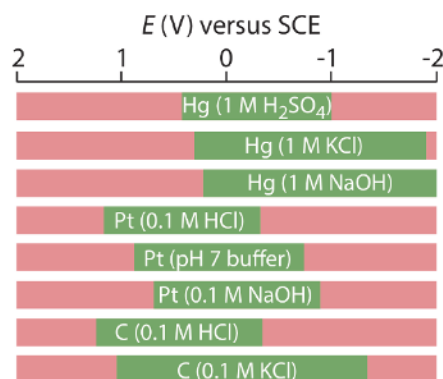


Figure 11.4.2 . Approximate potential windows for mercury, platinum, and carbon (graphite) electrodes in acidic, neutral, and basic aqueous solvents. The useful potential windows are shown in green; potentials in red result in the oxidation or the reduction of the solvent or the electrode. Compiled from Adams, R. N. *Electrochemistry at Solid Electrodes*, Marcel Dekker, Inc.: New York, 1969 and Bard, A. J.; Faulkner, L. R. *Electro- chemical Methods*, John Wiley & Sons: New York, 1980.

Solid electrodes constructed using platinum, gold, silver, or carbon may be used over a range of potentials, including potentials that are negative and positive with respect to the SCE (Figure 11.4.2). For example, the potential window for a Pt electrode extends from approximately $+1.2$ V to -0.2 V versus the SCE in acidic solutions, and from $+0.7$ V to -1 V versus the SCE in basic solutions. A solid electrode can replace a mercury electrode for many voltammetric analyses that require negative potentials, and is the electrode of choice at more positive potentials. Except for the carbon paste electrode, a solid electrode is fashioned into a disk and sealed into the end of an inert support with an electrical lead (Figure 11.4.3). The carbon paste electrode is made by filling the cavity at the end of the inert support with a paste that consists of carbon particles and a viscous oil. Solid electrodes are not without problems, the most important of which is the ease with which the electrode's surface is altered by the adsorption of a solution species or by the formation of an oxide layer. For this reason a solid electrode needs frequent reconditioning, either by applying an appropriate potential or by polishing.

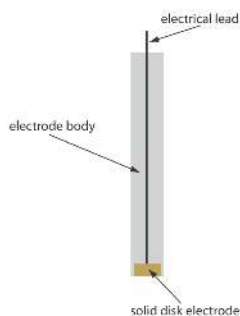


Figure 11.4.3 . Schematic showing a solid electrode. The electrode is fashioned into a disk and sealed in the end of an inert polymer support along with an electrical lead.

A typical arrangement for a voltammetric electrochemical cell is shown in Figure 11.4.4 . In addition to the working electrode, the reference electrode, and the auxiliary electrode, the cell also includes a N_2 -purge line for removing dissolved O_2 , and an optional

stir bar. Electrochemical cells are available in a variety of sizes, allowing the analysis of solution volumes ranging from more than 100 mL to as small as 50 μL .

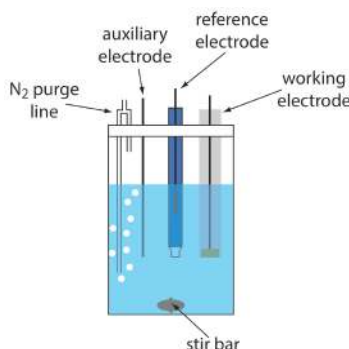


Figure 11.4.4 . Typical electrochemical cell for voltammetry.

Current In Voltammetry

When we oxidize an analyte at the working electrode, the resulting electrons pass through the potentiostat to the auxiliary electrode, reducing the solvent or some other component of the solution matrix. If we reduce the analyte at the working electrode, the current flows from the auxiliary electrode to the cathode. In either case, the current from the redox reactions at the working electrode and the auxiliary electrodes is called a **faradaic current**. In this section we consider the factors affecting the magnitude of the faradaic current, as well as the sources of any non-faradaic currents.

Sign Conventions

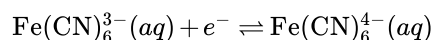
Because the reaction of interest occurs at the working electrode, we describe the faradaic current using this reaction. A faradaic current due to the analyte's reduction is a **cathodic current**, and its sign is positive. An **anodic current** results from the analyte's oxidation at the working electrode, and its sign is negative.

Influence of Applied Potential on the Faradaic Current

As an example, let's consider the faradaic current when we reduce $\text{Fe}(\text{CN})_6^{3-}$ to $\text{Fe}(\text{CN})_6^{4-}$ at the working electrode. The relationship between the concentrations of $\text{Fe}(\text{CN})_6^{3-}$, the concentration of $\text{Fe}(\text{CN})_6^{4-}$, and the potential is given by the Nernst equation

$$E = +0.356 \text{ V} - 0.05916 \log \frac{[\text{Fe}(\text{CN})_6^{4-}]_{x=0}}{[\text{Fe}(\text{CN})_6^{3-}]_{x=0}}$$

where +0.356V is the standard-state potential for the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ redox couple, and $x = 0$ indicates that the concentrations of $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$ are those at the surface of the working electrode. We use surface concentrations instead of bulk concentrations because the equilibrium position for the redox reaction



is established at the electrode's surface.

Let's assume we have a solution for which the initial concentration of $\text{Fe}(\text{CN})_6^{3-}$ is 1.0 mM and that $\text{Fe}(\text{CN})_6^{4-}$ is absent. Figure 11.4.5 shows the ladder diagram for this solution.

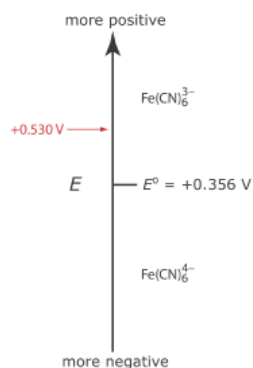


Figure 11.4.5 . Ladder diagram for the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ redox half-reaction.

If we apply a potential of +0.530 V to the working electrode, the concentrations of $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$ at the surface of the electrode are unaffected, and no faradaic current is observed. If we switch the potential to +0.356 V some of the $\text{Fe}(\text{CN})_6^{3-}$ at the electrode's surface is reduced to $\text{Fe}(\text{CN})_6^{4-}$ until we reach a condition where

$$[\text{Fe}(\text{CN})_6^{3-}]_{x=0} = [\text{Fe}(\text{CN})_6^{4-}]_{x=0} = 0.50 \text{ mM}$$

This is the first of the five important principles of electrochemistry outlined in [Chapter 11.1](#): the electrode's potential determines the analyte's form at the electrode's surface.

If this is all that happens after we apply the potential, then there would be a brief surge of faradaic current that quickly returns to zero, which is not the most interesting of results. Although the concentrations of $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$ at the electrode surface are 0.50 mM, their concentrations in bulk solution remains unchanged.

This is the second of the five important principles of electrochemistry outlined in [Chapter 11.1](#): the analyte's concentration at the electrode may not be the same as its concentration in bulk solution.

Because of this difference in concentration, there is a concentration gradient between the solution at the electrode's surface and the bulk solution. This concentration gradient creates a driving force that transports $\text{Fe}(\text{CN})_6^{4-}$ away from the electrode and that transports $\text{Fe}(\text{CN})_6^{3-}$ to the electrode (Figure 11.4.6). As the $\text{Fe}(\text{CN})_6^{3-}$ arrives at the electrode it, too, is reduced to $\text{Fe}(\text{CN})_6^{4-}$. A faradaic current continues to flow until there is no difference between the concentrations of $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$ at the electrode and their concentrations in bulk solution.

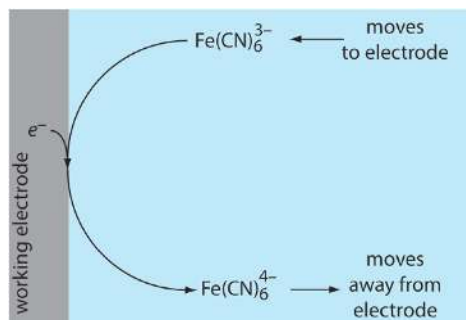


Figure 11.4.6 . Schematic diagram showing the transport of $\text{Fe}(\text{CN})_6^{4-}$ away from the electrode's surface and the transport of $\text{Fe}(\text{CN})_6^{3-}$ toward the electrode's surface following the reduction of $\text{Fe}(\text{CN})_6^{3-}$ to $\text{Fe}(\text{CN})_6^{4-}$.

Although the potential at the working electrode determines if a faradaic current flows, the magnitude of the current is determined by the rate of the resulting oxidation or reduction reaction. Two factors contribute to the rate of the electrochemical reaction: the rate at which the reactants and products are transported to and from the electrode—what we call **mass transport**—and the rate at which electrons pass between the electrode and the reactants and products in solution.

This is the fourth of the five important principles of electrochemistry outlined in [Chapter 11.1](#): current is a measure of rate.

Influence of Mass Transport on the Faradaic Current

There are three modes of mass transport that affect the rate at which reactants and products move toward or away from the electrode surface: diffusion, migration, and convection. **Diffusion** occurs whenever the concentration of an ion or a molecule at the surface of the electrode is different from that in bulk solution. If we apply a potential sufficient to completely reduce $\text{Fe}(\text{CN})_6^{3-}$ at the electrode surface, the result is a concentration gradient similar to that shown in Figure 11.4.7. The region of solution over which diffusion occurs is the **diffusion layer**. In the absence of other modes of mass transport, the width of the diffusion layer, δ , increases with time as the $\text{Fe}(\text{CN})_6^{3-}$ must diffuse from an increasingly greater distance.

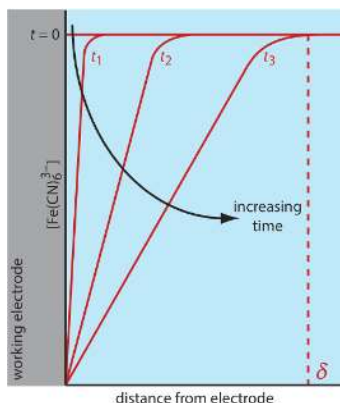


Figure 11.4.7. Concentration gradients (in red) for $\text{Fe}(\text{CN})_6^{3-}$ following the application of a potential that completely reduces it to $\text{Fe}(\text{CN})_6^{4-}$. Before we apply the potential ($t = 0$) the concentration of $\text{Fe}(\text{CN})_6^{3-}$ is the same at all distances from the electrode's surface. After we apply the potential, its concentration at the electrode's surface decreases to zero and $\text{Fe}(\text{CN})_6^{3-}$ diffuses to the electrode from bulk solution. The longer we apply the potential, the greater the distance over which diffusion occurs. The dashed red line shows the extent of the diffusion layer at time t_3 . These profiles assume that convection and migration do not contribute significantly to the mass transport of $\text{Fe}(\text{CN})_6^{3-}$.

Convection occurs when we mix the solution, which carries reactants toward the electrode and removes products from the electrode. The most common form of convection is stirring the solution with a stir bar; other methods include rotating the electrode and incorporating the electrode into a flow-cell.

The final mode of mass transport is **migration**, which occurs when a charged particle in solution is attracted to or repelled from an electrode that carries a surface charge. If the electrode carries a positive charge, for example, an anion will move toward the electrode and a cation will move toward the bulk solution. Unlike diffusion and convection, migration affects only the mass transport of charged particles.

The movement of material to and from the electrode surface is a complex function of all three modes of mass transport. In the limit where diffusion is the only significant form of mass transport, the current in a voltammetric cell is equal to

$$i = \frac{nFAD(C_{\text{bulk}} - C_{x=0})}{\delta} \quad (8.2.1)$$

where n the number of electrons in the redox reaction, F is Faraday's constant, A is the area of the electrode, D is the diffusion coefficient for the species reacting at the electrode, C_{bulk} and $C_{x=0}$ are its concentrations in bulk solution and at the electrode surface, and δ is the thickness of the diffusion layer.

For Equation 8.2.1 to be valid, convection and migration must not interfere with the formation of a diffusion layer. We can eliminate migration by adding a high concentration of an inert supporting electrolyte. Because ions of similar charge equally are attracted to or repelled from the surface of the electrode, each has an equal probability of undergoing migration. A large excess of an inert electrolyte ensures that few reactants or products experience migration. Although it is easy to eliminate convection by not stirring the solution, there are experimental designs where we cannot avoid convection, either because we must stir the solution or because we are using an electrochemical flow cell. Fortunately, as shown in Figure 11.4.8, the dynamics of a fluid moving past an electrode results in a small diffusion layer—typically 1–10 μm in thickness—in which the rate of mass transport by convection drops to zero.

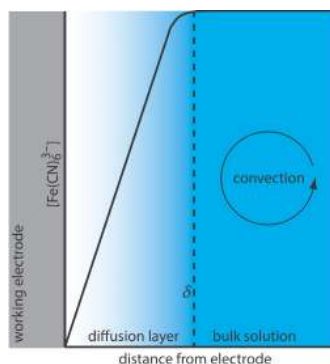


Figure 11.4.8 . Concentration gradient for $\text{Fe}(\text{CN})_6^{3-}$ when stirring the solution. Diffusion is the only significant form of mass transport close to the electrode's surface. At distances greater than δ , convection is the only significant form of mass transport, maintaining a homogeneous solution in which the concentration of $\text{Fe}(\text{CN})_6^{3-}$ at δ is the same as its concentration in bulk solution.

Effect of Electron Transfer Kinetics on the Faradaic Current

The rate of mass transport is one factor that influences the current in voltammetry. The ease with which electrons move between the electrode and the species that reacts at the electrode also affects the current. When electron transfer kinetics are fast, the redox reaction is at equilibrium. Under these conditions the redox reaction is **electrochemically reversible** and the Nernst equation applies. If the electron transfer kinetics are sufficiently slow, the concentration of reactants and products at the electrode surface—and thus the magnitude of the faradaic current—are not what is predicted by the Nernst equation. In this case the system is **electrochemically irreversible**.

Charging Currents

In addition to the faradaic current from a redox reaction, the current in an electrochemical cell includes other, nonfaradaic sources. Suppose the charge on an electrode is zero and we suddenly change its potential so that the electrode's surface acquires a positive charge. Cations near the electrode's surface will respond to this positive charge by migrating away from the electrode; anions, on the other hand, will migrate toward the electrode. This migration of ions occurs until the electrode's positive surface charge and the negative charge of the solution near the electrode are equal. Because the movement of ions and the movement of electrons are indistinguishable, the result is a small, short-lived **nonfaradaic current** that we call the **charging current**. Every time we change the electrode's potential, a transient charging current flows.

The migration of ions in response to the electrode's surface charge leads to the formation of a structured electrode-solution interface that we call the **electrical double layer**, or EDL. When we change an electrode's potential, the charging current is the result of a restructuring of the EDL. The exact structure of the electrical double layer is not important in the context of this text, but you can consult this chapter's additional resources for additional information.

Residual Current

Even in the absence of analyte, a small, measurable current flows through an electrochemical cell. This **residual current** has two components: a faradaic current due to the oxidation or reduction of trace impurities and a nonfaradaic charging current. Methods for discriminating between the analyte's faradaic current and the residual current are discussed later in this chapter.

Shape of Voltammograms

The shape of a voltammogram is determined by several experimental factors, the most important of which are how we measure the current and whether convection is included as a means of mass transport. As shown in Figure 11.4.9 , despite an abundance of different voltammetric techniques, several of which are discussed in this chapter, there are only three common shapes for voltammograms.

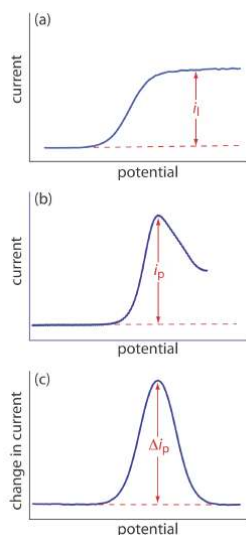


Figure 11.4.9 . The three common shapes for voltammograms. The dashed red line shows the residual current.

For the voltammogram in Figure 11.4.9 a, the current increases from a background residual current to a **limiting current**, i_l . Because the faradaic current is inversely proportional to δ (Equation 8.2.1), a limiting current occurs only if the thickness of the diffusion layer remains constant because we are stirring the solution (see Figure 11.4.8). In the absence of convection the diffusion layer increases with time (see Figure 11.4.7). As shown in Figure 11.4.9 b, the resulting voltammogram has a **peak current** instead of a limiting current.

For the voltammograms in Figure 11.4.9 a and Figure 11.4.9 b, we measure the current as a function of the applied potential. We also can monitor the change in current, Δi , following a change in potential. The resulting voltammogram, shown in Figure 11.4.9 c, also has a peak current.

Quantitative and Qualitative Aspects of Voltammetry

Earlier we described a voltammogram as the electrochemical equivalent of a spectrum in spectroscopy. In this section we consider how we can extract quantitative and qualitative information from a voltammogram. For simplicity we will limit our treatment to voltammograms similar to Figure 11.4.9 a.

Determining Concentration

Let's assume that the redox reaction at the working electrode is



where O is the analyte's oxidized form and R is its reduced form. Let's also assume that only O initially is present in bulk solution and that we are stirring the solution. When we apply a potential that results in the reduction of O to R , the current depends on the rate at which O diffuses through the fixed diffusion layer shown in Figure 11.4.7 . Using Equation 8.2.1, the current, i , is

$$i = K_O ([O]_{\text{bulk}} - [O]_{x=0}) \quad (8.2.3)$$

where K_O is a constant equal to $nFAD_O/\delta$. When we reach the limiting current, i_l , the concentration of O at the electrode surface is zero and Equation 8.2.3 simplifies to

$$i_l = K_O [O]_{\text{bulk}} \quad (8.2.4)$$

Equation 8.2.4 shows us that the limiting current is a linear function of the concentration of O in bulk solution. To determine the value of K_O we can use any of the standardization methods covered in Chapter 5. Equations similar to Equation 8.2.4 can be developed for the other two types of voltammograms shown in Figure 11.4.9 .

Determining the Standard-State Potential

To extract the standard-state potential from a voltammogram, we need to rewrite the Nernst equation for reaction 8.2.2

$$E = E_{O/R}^{\circ} - \frac{0.05916}{n} \log \frac{[R]_{x=0}}{[O]_{x=0}} \quad (8.2.5)$$

in terms of current instead of the concentrations of O and R . We will do this in several steps. First, we substitute Equation 8.2.4 into Equation 8.2.3 and rearrange to give

$$[O]_{x=0} = \frac{i_l - i}{K_O} \quad (8.2.6)$$

Next, we derive a similar equation for $[R]_{x=0}$, by noting that

$$i = K_R ([R]_{x=0} - [R]_{\text{bulk}})$$

Because the concentration of $[R]_{\text{bulk}}$ is zero—remember our assumption that the initial solution contains only O —we can simplify this equation

$$i = K_R [R]_{x=0}$$

and solve for $[R]_{x=0}$.

$$[R]_{x=0} = \frac{i}{K_R} \quad (8.2.7)$$

Now we are ready to finish our derivation. Substituting Equation 8.2.7 and Equation 8.2.6 into Equation 8.2.5 and rearranging leaves us with

$$E = E_{O/R}^{\circ} - \frac{0.05916}{n} \log \frac{K_O}{K_R} - \frac{0.05916}{n} \log \frac{i}{i_l - i} \quad (8.2.8)$$

When the current, i , is half of the limiting current, i_l ,

$$i = 0.5 \times i_l$$

we can simplify Equation 8.2.8 to

$$E_{1/2} = E_{O/R}^{\circ} - \frac{0.05916}{n} \log \frac{K_O}{K_R} \quad (8.2.9)$$

where $E_{1/2}$ is the half-wave potential (Figure 11.4.10). If K_O is approximately equal to K_R , which often is the case, then the half-wave potential is equal to the standard-state potential. Note that Equation 8.2.9 is valid only if the redox reaction is electrochemically reversible.

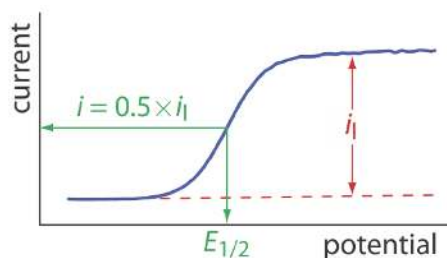


Figure 11.4.10 . Determination of the limiting current, i_l , and the half-wave potential, $E_{1/2}$, for the voltammogram in Figure 11.4.9 a.

Voltammetric Techniques

In voltammetry there are three important experimental parameters under our control: how we change the potential applied to the working electrode, when we choose to measure the current, and whether we choose to stir the solution. Not surprisingly, there are many different voltammetric techniques. In this section we consider several important examples.

Polarography

The first important voltammetric technique to be developed—**polarography**—uses the dropping mercury electrode shown in Figure 11.4.1 b as the working electrode. As shown in Figure 11.4.11, the current is measured while applying a linear potential ramp.

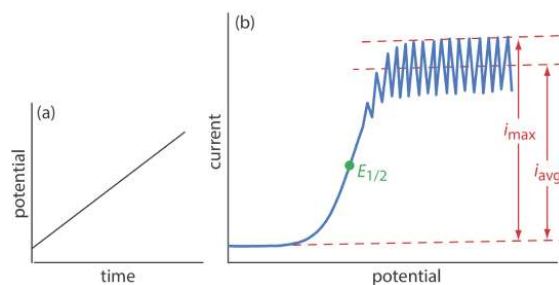


Figure 11.4.11 . Details of normal polarography: (a) the linear potential-excitation signal, and (b) the resulting voltammogram.

Although polarography takes place in an unstirred solution, we obtain a limiting current instead of a peak current. When a Hg drop separates from the glass capillary and falls to the bottom of the electrochemical cell, it mixes the solution. Each new Hg drop, therefore, grows into a solution whose composition is identical to the bulk solution. The oscillations in the current are a result of the Hg drop's growth, which leads to a time-dependent change in the area of the working electrode. The limiting current—which also is called the diffusion current—is measured using either the maximum current, i_{max} , or from the average current, i_{avg} . The relationship between the analyte's concentration, C_A , and the limiting current is given by the Ilkovic equations

$$i_{max} = 706nD^{1/2}m^{2/3}t^{1/6}C_A = K_{max}C_A$$

$$i_{avg} = 607nD^{1/2}m^{2/3}t^{1/6}C_A = K_{avg}C_A$$

where n is the number of electrons in the redox reaction, D is the analyte's diffusion coefficient, m is the flow rate of Hg, t is the drop's lifetime and K_{max} and K_{avg} are constants. The half-wave potential, $E_{1/2}$, provides qualitative information about the redox reaction.

Normal polarography has been replaced by various forms of **pulse polarography**, several examples of which are shown in Figure 11.4.12 [Osteryoung, J. J. *Chem. Educ.* **1983**, 60, 296–298]. Normal pulse polarography (Figure 11.4.12 a), for example, uses a series of potential pulses characterized by a cycle of time τ , a pulse-time of t_p , a pulse potential of ΔE_p , and a scan rate of $\Delta E/\Delta t$. Typical experimental conditions for normal pulse polarography are $\tau \approx 1$ s, $t_p \approx 50$ ms, and $\Delta E/\Delta t \approx 2$ mV/s. The initial value of $\Delta E_p \approx 2$ mV, and it increases by ≈ 2 mV with each pulse. The current is sampled at the end of each potential pulse for approximately 17 ms before returning the potential to its initial value. The shape of the resulting voltammogram is similar to Figure 11.4.11, but without the current oscillations. Because we apply the potential for only a small portion of the drop's lifetime, there is less time for the analyte to undergo oxidation or reduction and a smaller diffusion layer. As a result, the faradaic current in normal pulse polarography is greater than in the polarography, resulting in better sensitivity and smaller detection limits.

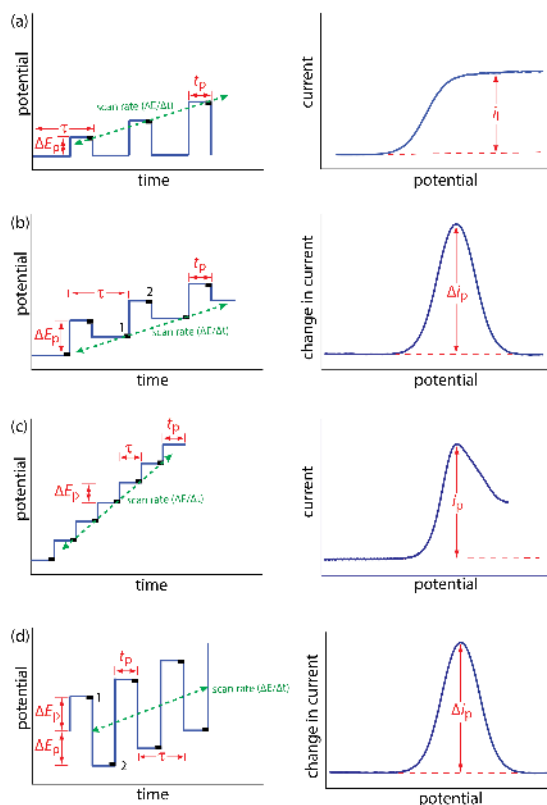


Figure 11.4.12 . Potential-excitation signals and voltammograms for (a) normal pulse polarography, (b) differential pulse polarography, (c) staircase polarography, and (d) square-wave polarography. The current is sampled at the time intervals shown by the black rectangles. When measuring a change in current, Δi , the current at point 1 is subtracted from the current at point 2. The symbols in the diagrams are as follows: τ is the cycle time; ΔE_p is a fixed or variable pulse potential relative to the beginning of the cycle; and t_p is the pulse time. The scan rate (change in potential per unit time) is shown by the dashed arrow.

In differential pulse polarography (Figure 11.4.12 b) the current is measured twice per cycle: for approximately 17 ms before applying the pulse and for approximately 17 ms at the end of the cycle. The difference in the two currents gives rise to the peak-shaped voltammogram. Typical experimental conditions for differential pulse polarography are $\tau \approx 1$ s, $t_p \approx 50$ ms, $\Delta E_p \approx 50$ mV, and $\Delta E/\Delta t \approx 2$ mV/s.

The voltammogram for differential pulse polarography is approximately the first derivative of the voltammogram for normal pulse polarography. To see why this is the case, note that the change in current over a fixed change in potential, $\Delta i/\Delta E$, approximates the slope of the voltammogram for normal pulse polarography. You may recall that the first derivative of a function returns the slope of the function at each point. The first derivative of a sigmoidal function is a peak-shaped function.

Other forms of pulse polarography include staircase polarography (Figure 11.4.12 c) and square-wave polarography (Figure 11.4.12 d). One advantage of square-wave polarography is that we can make τ very small—perhaps as small as 5 ms, compared to 1 s for other forms of pulse polarography—which significantly decreases analysis time. For example, suppose we need to scan a potential range of 400 mV. If we use normal pulse polarography with a $\Delta E/\Delta t$ of 2 mV/cycle and a τ of 1 s/cycle, then we need 200 s to complete the scan. If we use square-wave polarography with a $\Delta E/\Delta t$ of 2 mV/cycle and a τ of 5 ms/cycle, we can complete the scan in 1 s. At this rate, we can acquire a complete voltammogram using a single drop of Hg!

Polarography is used extensively for the analysis of metal ions and inorganic anions, such as IO_3^- and NO_3^- . We also can use polarography to study organic compounds with easily reducible or oxidizable functional groups, such as carbonyls, carboxylic acids, and carbon-carbon double bonds.

Hydrodynamic Voltammetry

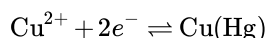
In polarography we obtain a limiting current because each drop of mercury mixes the solution as it falls to the bottom of the electrochemical cell. If we replace the DME with a solid electrode (see Figure 11.4.3), we can still obtain a limiting current if we mechanically stir the solution during the analysis, using either a stir bar or by rotating the electrode. We call this approach **hydrodynamic voltammetry**.

Hydrodynamic voltammetry uses the same potential profiles as in polarography, such as a linear scan (Figure 11.4.11) or a differential pulse (Figure 11.4.12 b). The resulting voltammograms are identical to those for polarography, except for the lack of current oscillations from the growth of the mercury drops. Because hydrodynamic voltammetry is not limited to Hg electrodes, it is useful for analytes that undergo oxidation or reduction at more positive potentials.

Stripping Voltammetry

Another important voltammetric technique is **stripping voltammetry**, which consists of three related techniques: anodic stripping voltammetry, cathodic stripping voltammetry, and adsorptive stripping voltammetry. Because anodic stripping voltammetry is the more widely used of these techniques, we will consider it in greatest detail.

Anodic stripping voltammetry consists of two steps (Figure 11.4.13). The first step is a controlled potential electrolysis in which we hold the working electrode—usually a hanging mercury drop or a mercury film electrode—at a cathodic potential sufficient to deposit the metal ion on the electrode. For example, when analyzing Cu^{2+} the deposition reaction is



where Cu(Hg) indicates that the copper is amalgamated with the mercury. This step serves as a means of concentrating the analyte by transferring it from the larger volume of the solution to the smaller volume of the electrode. During most of the electrolysis we stir the solution to increase the rate of deposition. Near the end of the deposition time we stop the stirring—eliminating convection as a mode of mass transport—and allow the solution to become quiescent. Typical deposition times of 1–30 min are common, with analytes at lower concentrations requiring longer times.

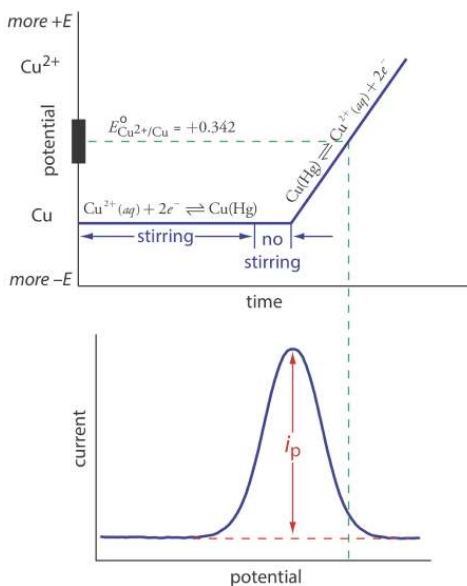
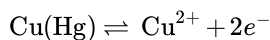


Figure 11.4.13 . Potential-excitation signal and voltammogram for anodic stripping voltammetry at a hanging mercury drop electrode or a mercury film electrode. Note the ladder diagram for copper in the upper figure.

In the second step, we scan the potential anodically—that is, toward a more positive potential. When the working electrode's potential is sufficiently positive, the analyte is stripped from the electrode, returning to solution in its oxidized form.



Monitoring the current during the stripping step gives a peak-shaped voltammogram, as shown in Figure 11.4.13 . The peak current is proportional to the analyte's concentration in the solution. Because we are concentrating the analyte in the electrode, detection limits are much smaller than other electrochemical techniques. An improvement of three orders of magnitude—the equivalent of parts per billion instead of parts per million—is routine.

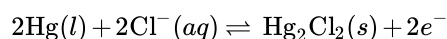
Anodic stripping voltammetry is very sensitive to experimental conditions, which we must carefully control to obtain results that are accurate and precise. Key variables include the area of the mercury film or the size of the hanging Hg drop, the deposition time, the rest time, the rate of stirring, and the scan rate during the stripping step. Anodic stripping voltammetry is particularly useful for metals that form amalgams with mercury, several examples of which are listed in Table 11.4.1 .

Table 11.4.1 . Representative Examples of Analytes Determined by Stripping Voltammetry

anodic stripping voltammetry	cathodic stripping voltammetry	adsorptive stripping voltammetry
Bi^{3+}	Br^-	bilirubin
Cd^{2+}	Cl^-	codeine
Cu^{2+}	I^-	cocaine
Ga^{3+}	mercaptans (RSH)	digitoxin
In^{3+}	S^{2-}	dopamine
Pb^{2+}	SCN^-	heme
Tl^+		monesin
Sn^{2+}		testosterone
Zn^{2+}		

Source: Compiled from Peterson, W. M.; Wong, R. V. *Am. Lab.* November 1981, 116–128; Wang, J. *Am. Lab.* May 1985, 41–50.

The experimental design for cathodic stripping voltammetry is similar to anodic stripping voltammetry with two exceptions. First, the deposition step involves the oxidation of the Hg electrode to Hg_2^{2+} , which then reacts with the analyte to form an insoluble film at the surface of the electrode. For example, when Cl^- is the analyte the deposition step is



Second, stripping is accomplished by scanning cathodically toward a more negative potential, reducing Hg_2^{2+} back to Hg and returning the analyte to solution.

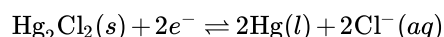
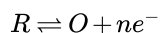


Table 11.4.1 lists several analytes analyzed successfully by cathodic stripping voltammetry.

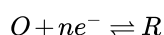
In adsorptive stripping voltammetry, the deposition step occurs without electrolysis. Instead, the analyte adsorbs to the electrode's surface. During deposition we maintain the electrode at a potential that enhances adsorption. For example, we can adsorb a neutral molecule on a Hg drop if we apply a potential of -0.4 V versus the SCE, a potential where the surface charge of mercury is approximately zero. When deposition is complete, we scan the potential in an anodic or a cathodic direction, depending on whether we are oxidizing or reducing the analyte. Examples of compounds that have been analyzed by adsorptive stripping voltammetry also are listed in Table 11.4.1 .

Cyclic Voltammetry

In the voltammetric techniques consider to this point we scan the potential in one direction, either to more positive potentials or to more negative potentials. In **cyclic voltammetry** we complete a scan in both directions. Figure 11.4.14 a shows a typical potential-excitation signal. In this example, we first scan the potential to more positive values, resulting in the following oxidation reaction for the species R .



When the potential reaches a predetermined switching potential, we reverse the direction of the scan toward more negative potentials. Because we generated the species O on the forward scan, during the reverse scan it reduces back to R .



Cyclic voltammetry is carried out in an unstirred solution, which, as shown in Figure 11.4.14 b, results in peak currents instead of limiting currents. The voltammogram has separate peaks for the oxidation reaction and for the reduction reaction, each characterized by a peak potential and a peak current.

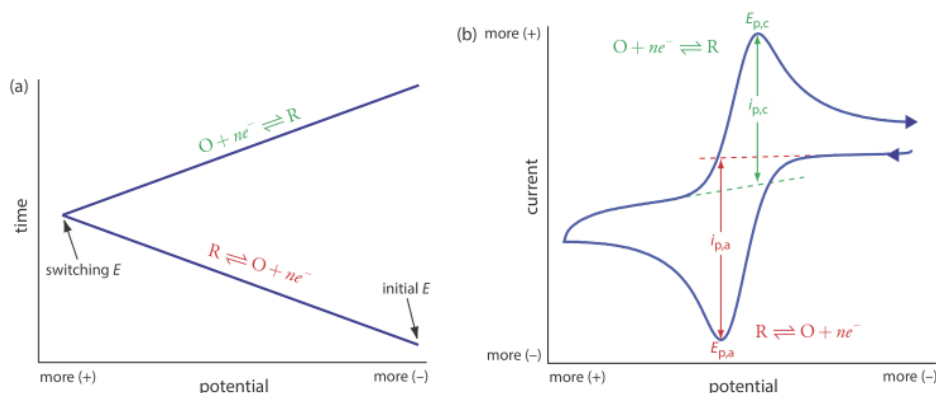


Figure 11.4.14 . Details for cyclic voltammetry. (a) One cycle of the triangular potential-excitation signal showing the initial potential and the switching potential. A cyclic voltammetry experiment can consist of one cycle or many cycles. Although the initial potential in this example is the negative switching potential, the cycle can begin with an intermediate initial potential and cycle between two limits. (b) The resulting cyclic voltammogram showing the measurement of the peak currents and peak potentials.

The peak current in cyclic voltammetry is given by the Randles-Sevcik equation

$$i_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} \nu^{1/2} C_A$$

where n is the number of electrons in the redox reaction, A is the area of the working electrode, D is the diffusion coefficient for the electroactive species, ν is the scan rate, and C_A is the concentration of the electroactive species at the electrode. For a well-behaved system, the anodic and the cathodic peak currents are equal, and the ratio $i_{p,a}/i_{p,c}$ is 1.00. The half-wave potential, $E_{1/2}$, is midway between the anodic and cathodic peak potentials.

$$E_{1/2} = \frac{E_{p,a} + E_{p,c}}{2}$$

Scanning the potential in both directions provides an opportunity to explore the electrochemical behavior of species generated at the electrode. This is a distinct advantage of cyclic voltammetry over other voltammetric techniques. Figure 11.4.15 shows the cyclic voltammogram for the same redox couple at both a faster and a slower scan rate. At the faster scan rate, 11.4.15 a, we see two peaks. At the slower scan rate in Figure 11.4.15 b, however, the peak on the reverse scan disappears. One explanation for this is that the products from the reduction of R on the forward scan have sufficient time to participate in a chemical reaction whose products are not electroactive.

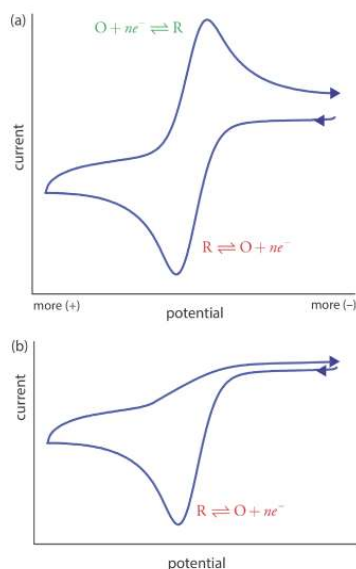
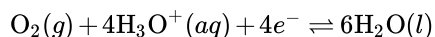


Figure 11.4.15 . Cyclic voltammograms for R obtained at (a) a faster scan rate and at (b) a slower scan rate. One of the principal uses of cyclic voltammetry is to study the chemical and electrochemical behavior of compounds. See this chapter's additional resources for further information.

Amperometry

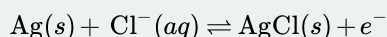
The final voltammetric technique we will consider is **amperometry**, in which we apply a constant potential to the working electrode and measure current as a function of time. Because we do not vary the potential, amperometry does not result in a voltammogram.

One important application of amperometry is in the construction of chemical sensors. One of the first amperometric sensors was developed in 1956 by L. C. Clark to measure dissolved O_2 in blood. Figure 11.4.16 shows the sensor's design, which is similar to a potentiometric membrane electrode. A thin, gas-permeable membrane is stretched across the end of the sensor and is separated from the working electrode and the counter electrode by a thin solution of KCl. The working electrode is a Pt disk cathode, and a Ag ring anode serves as the counter electrode. Although several gases can diffuse across the membrane, including O_2 , N_2 , and CO_2 , only oxygen undergoes reduction at the cathode



with its concentration at the electrode's surface quickly reaching zero. The concentration of O_2 at the membrane's inner surface is fixed by its diffusion through the membrane, which creates a diffusion profile similar to that in Figure 11.4.8 . The result is a steady-state current that is proportional to the concentration of dissolved oxygen. Because the electrode consumes oxygen, the sample is stirred to prevent the depletion of O_2 at the membrane's outer surface.

The oxidation of the Ag anode is the other half-reaction.



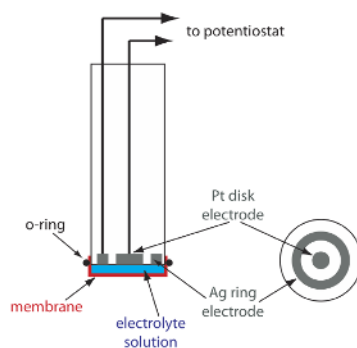
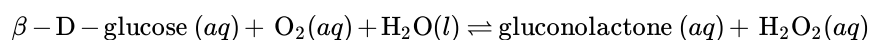


Figure 11.4.16 . Clark amperometric sensor for determining dissolved O_2 . The diagram on the right is a cross-section through the electrode, which shows the Ag ring electrode and the Pt disk electrode.

Another example of an amperometric sensor is a glucose sensor. In this sensor the single membrane in Figure 11.4.16 is replaced with three membranes. The outermost membrane of polycarbonate is permeable to glucose and O_2 . The second membrane contains an immobilized preparation of glucose oxidase that catalyzes the oxidation of glucose to gluconolactone and hydrogen peroxide.



The hydrogen peroxide diffuses through the innermost membrane of cellulose acetate where it undergoes oxidation at a Pt anode.

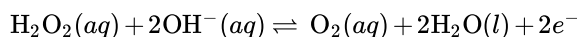


Figure 11.4.17 summarizes the reactions that take place in this amperometric sensor. FAD is the oxidized form of flavin adenine nucleotide—the active site of the enzyme glucose oxidase—and $FADH_2$ is the active site's reduced form. Note that O_2 serves a mediator, carrying electrons to the electrode.

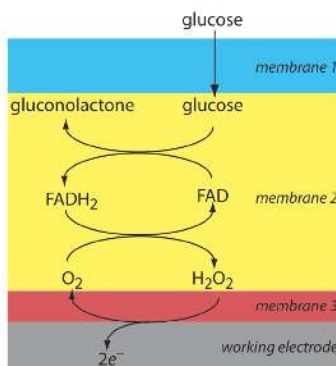


Figure 11.4.17 . Schematic showing the reactions by which an amperometric biosensor responds to glucose.

By changing the enzyme and mediator, it is easy to extend to the amperometric sensor in Figure 11.4.17 to the analysis of other analytes. For example, a CO_2 sensor has been developed using an amperometric O_2 sensor with a two-layer membrane, one of which contains an immobilized preparation of autotrophic bacteria [Karube, I.; Nomura, Y.; Arikawa, Y. *Trends in Anal. Chem.* **1995**, *14*, 295–299]. As CO_2 diffuses through the membranes it is converted to O_2 by the bacteria, increasing the concentration of O_2 at the Pt cathode.

Quantitative Applications

Voltammetry has been used for the quantitative analysis of a wide variety of samples, including environmental samples, clinical samples, pharmaceutical formulations, steels, gasoline, and oil.

Selecting the Voltammetric Technique

The choice of which voltammetric technique to use depends on the sample's characteristics, including the analyte's expected concentration and the sample's location. For example, amperometry is ideally suited for detecting analytes in flow systems, including the *in vivo* analysis of a patient's blood or as a selective sensor for the rapid analysis of a single analyte. The portability of amperometric sensors, which are similar to potentiometric sensors, also make them ideal for field studies. Although cyclic

voltammetry is used to determine an analyte's concentration, other methods described in this chapter are better suited for quantitative work.

Pulse polarography and stripping voltammetry frequently are interchangeable. The choice of which technique to use often depends on the analyte's concentration and the desired accuracy and precision. Detection limits for normal pulse polarography generally are on the order of 10^{-6} M to 10^{-7} M, and those for differential pulse polarography, staircase, and square wave polarography are between 10^{-7} M and 10^{-9} M. Because we concentrate the analyte in stripping voltammetry, the detection limit for many analytes is as little as 10^{-10} M to 10^{-12} M. On the other hand, the current in stripping voltammetry is much more sensitive than pulse polarography to changes in experimental conditions, which may lead to poorer precision and accuracy. We also can use pulse polarography to analyze a wider range of inorganic and organic analytes because there is no need to first deposit the analyte at the electrode surface.

Stripping voltammetry also suffers from occasional interferences when two metals, such as Cu and Zn, combine to form an intermetallic compound in the mercury amalgam. The deposition potential for Zn is sufficiently negative that any Cu^{2+} in the sample also deposits into the mercury drop or film, leading to the formation of intermetallic compounds such as CuZn and CuZn_2 . During the stripping step, zinc in the intermetallic compounds strips at potentials near that of copper, decreasing the current for zinc at its usual potential and increasing the apparent current for copper. It is possible to overcome this problem by adding an element that forms a stronger intermetallic compound with the interfering metal. Thus, adding Ga^{3+} minimizes the interference of Cu when analyzing for Zn by forming an intermetallic compound of Cu and Ga.

Correcting the Residual Current

In any quantitative analysis we must correct the analyte's signal for signals that arise from other sources. The total current, i_{tot} , in voltammetry consists of two parts: the current from the analyte's oxidation or reduction, i_A , and a background or residual current, i_r .

$$i_{\text{tot}} = i_A + i_r$$

The residual current, in turn, has two sources. One source is a faradaic current from the oxidation or reduction of trace interferents in the sample, i_{int} . The other source is the charging current, i_{ch} , that accompanies a change in the working electrode's potential.

$$i_r = i_{\text{int}} + i_{\text{ch}}$$

We can minimize the faradaic current due to impurities by carefully preparing the sample. For example, one important impurity is dissolved O_2 , which undergoes a two-step reduction: first to H_2O_2 at a potential of -0.1 V versus the SCE, and then to H_2O at a potential of -0.9 V versus the SCE. Removing dissolved O_2 by bubbling an inert gas such as N_2 through the sample eliminates this interference. After removing the dissolved O_2 , maintaining a blanket of N_2 over the top of the solution prevents O_2 from reentering the solution.

The cell in [Figure 11.4.4](#) shows a typical N_2 purge line.

There are two methods to compensate for the residual current. One method is to measure the total current at potentials where the analyte's faradaic current is zero and extrapolate it to other potentials. This is the method shown in [Figure 11.4.9](#). One advantage of extrapolating is that we do not need to acquire additional data. An important disadvantage is that an extrapolation assumes that any change in the residual current with potential is predictable, which may not be the case. A second, and more rigorous approach, is to obtain a voltammogram for an appropriate blank. The blank's residual current is then subtracted from the sample's total current.

Analysis for Single Components

The analysis of a sample with a single analyte is straightforward using any of the standardization methods discussed in [Chapter 5](#).

✓ Example 11.4.1

The concentration of As(III) in water is determined by differential pulse polarography in 1 M HCl. The initial potential is set to -0.1 V versus the SCE and is scanned toward more negative potentials at a rate of 5 mV/s. Reduction of As(III) to As(0) occurs at a potential of approximately -0.44 V versus the SCE. The peak currents for a set of standard solutions, corrected for the residual current, are shown in the following table.

[As(III)] (μM)	i_p (μA)
1.00	0.298
3.00	0.947
6.00	1.83
9.00	2.72

What is the concentration of As(III) in a sample of water if its peak current is 1.37 μA?

Solution

Linear regression gives the calibration curve shown in Figure 11.4.18, with an equation of

$$i_p = 0.0176 + 3.01 \times [\text{As(III)}]$$

Substituting the sample's peak current into the regression equation gives the concentration of As(III) as 4.49 μM.

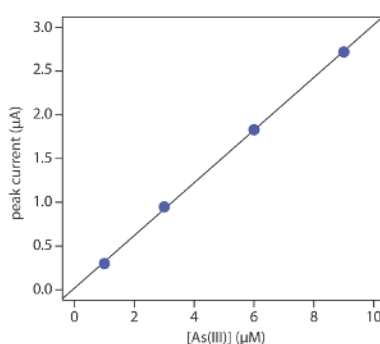


Figure 11.4.18. Calibration curve for the data in Example 11.4.1.

? Exercise 11.4.1

The concentration of copper in a sample of sea water is determined by anodic stripping voltammetry using the method of standard additions. The analysis of a 50.0-mL sample gives a peak current of 0.886 μA. After adding a 5.00-μL spike of 10.0 mg/L Cu²⁺, the peak current increases to 2.52 μA. Calculate the μg/L copper in the sample of sea water.

Answer

For anodic stripping voltammetry, the peak current, i_p , is a linear function of the analyte's concentration

$$i_p = K \times C_{\text{Cu}}$$

where K is a constant that accounts for experimental parameters such as the electrode's area, the diffusion coefficient for Cu²⁺, the deposition time, and the rate of stirring. For the analysis of the sample before the standard addition we know that the current is

$$i_p = 0.886 \mu\text{A} = K \times C_{\text{Cu}}$$

and after the standard addition the current is

$$i_p = 2.52 \mu\text{A} = K \left\{ C_{\text{Cu}} \times \frac{50.00 \text{ mL}}{50.005 \text{ mL}} + \frac{10.00 \text{ mg Cu}}{\text{L}} \times \frac{0.005 \text{ mL}}{50.005 \text{ mL}} \right\}$$

where 50.005 mL is the total volume after we add the 5.00 μL spike. Solving each equation for K and combining leaves us with the following equation.

$$\frac{0.886 \mu\text{A}}{C_{\text{Cu}}} = K = \frac{2.52 \mu\text{A}}{C_{\text{Cu}} \times \frac{50.00 \text{ mL}}{50.005 \text{ mL}} + \frac{10.00 \text{ mg Cu}}{\text{L}} \times \frac{0.005 \text{ mL}}{50.005 \text{ mL}}}$$

Solving this equation for C_{Cu} gives its value as $5.42 \times 10^{-4} \text{ mg Cu}^{2+}/\text{L}$, or $0.542 \mu\text{g Cu}^{2+}/\text{L}$.

Multicomponent Analysis

Voltammetry is a particularly attractive technique for the analysis of samples that contain two or more analytes. Provided that the analytes behave independently, the voltammogram of a multicomponent mixture is a summation of each analyte's individual voltammograms. As shown in Figure 11.4.19, if the separation between the half-wave potentials or between the peak potentials is sufficient, we can determine the presence of each analyte as if it is the only analyte in the sample. The minimum separation between the half-wave potentials or peak potentials for two analytes depends on several factors, including the type of electrode and the potential-excitation signal. For normal polarography the separation is at least $\pm 0.2\text{--}0.3 \text{ V}$, and differential pulse voltammetry requires a minimum separation of $\pm 0.04\text{--}0.05 \text{ V}$.

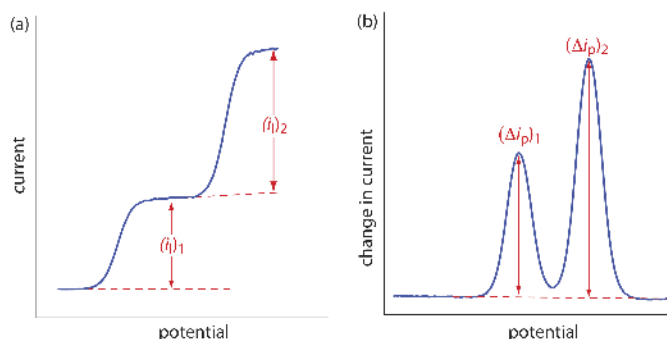


Figure 11.4.19. Voltammograms for a sample that contains two analytes showing the measurement of (a) limiting currents, and (b) peak currents.

If the voltammograms for two analytes are not sufficiently separated, a simultaneous analysis may be possible. An example of this approach is outlined in the following example.

✓ Example 11.4.2

The differential pulse polarographic analysis of a mixture of indium and cadmium in 0.1 M HCl is complicated by the overlap of their respective voltammograms [Lanza P. *J. Chem. Educ.* **1990**, 67, 704–705]. The peak potential for indium is at -0.557 V and that for cadmium is at -0.597 V . When a 0.800-ppm indium standard is analyzed, Δi_p (in arbitrary units) is 200.5 at -0.557 V and 87.5 at -0.597 V relative to a saturated Ag/AgCl reference electrode. A standard solution of 0.793 ppm cadmium has a Δi_p of 58.5 at -0.557 V and 128.5 at -0.597 V . What is the concentration of indium and cadmium in a sample if Δi_p is 167.0 at a potential of -0.557 V and 99.5 at a potential of -0.597 V .

Solution

The change in current, Δi_p , in differential pulse polarography is a linear function of the analyte's concentration

$$\Delta i_p = k_A C_A$$

where k_A is a constant that depends on the analyte and the applied potential, and C_A is the analyte's concentration. To determine the concentrations of indium and cadmium in the sample we must first find the value of k_A for each analyte at each potential. For simplicity we will identify the potential of -0.557 V as E_1 , and that for -0.597 V as E_2 . The values of k_A are

$$\begin{aligned} k_{\text{In}, E_1} &= \frac{200.5}{0.800 \text{ ppm}} = 250.6 \text{ ppm}^{-1} \\ k_{\text{In}, E_2} &= \frac{87.5}{0.800 \text{ ppm}} = 109.4 \text{ ppm}^{-1} \\ k_{\text{Cd}, E_1} &= \frac{58.5}{0.793 \text{ ppm}} = 73.8 \text{ ppm}^{-1} \\ k_{\text{Cd}, E_2} &= \frac{128.5}{0.793 \text{ ppm}} = 162.0 \text{ ppm}^{-1} \end{aligned}$$

Next, we write simultaneous equations for the current at the two potentials.

$$\Delta i_{E_1} = 167.0 = 250.6 \text{ ppm}^{-1} \times C_{\text{In}} + 73.8 \text{ ppm}^{-1} \times C_{\text{Cd}}$$

$$\Delta i_{E_2} = 99.5 = 109.4 \text{ ppm}^{-1} \times C_{\text{In}} + 162.0 \text{ ppm}^{-1} \times C_{\text{Cd}}$$

Solving the simultaneous equations, which is left as an exercise, gives the concentration of indium as 0.606 ppm and the concentration of cadmium as 0.205 ppm.

Environmental Samples

Voltammetry is one of several important analytical techniques for the analysis of trace metals in environmental samples, including groundwater, lakes, rivers and streams, seawater, rain, and snow. Detection limits at the parts-per-billion level are routine for many trace metals using differential pulse polarography, with anodic stripping voltammetry providing parts-per-trillion detection limits for some trace metals.

One interesting environmental application of anodic stripping voltammetry is the determination of a trace metal's chemical form within a water sample. Speciation is important because a trace metal's bioavailability, toxicity, and ease of transport through the environment often depends on its chemical form. For example, a trace metal that is strongly bound to colloidal particles generally is not toxic because it is not available to aquatic lifeforms. Unfortunately, anodic stripping voltammetry can not distinguish a trace metal's exact chemical form because closely related species, such as Pb^{2+} and PbCl^+ , produce a single stripping peak. Instead, trace metals are divided into "operationally defined" categories that have environmental significance.

Operationally defined means that an analyte is divided into categories by the specific methods used to isolate it from the sample. There are many examples of operational definitions in the environmental literature. The distribution of trace metals in soils and sediments, for example, often is defined in terms of the reagents used to extract them; thus, you might find an operational definition for Zn^{2+} in a lake sediment as that extracted using 1.0 M sodium acetate, or that extracted using 1.0 M HCl.

Although there are many speciation schemes in the environmental literature, we will consider one proposed by Batley and Florence [see (a) Batley, G. E.; Florence, T. M. *Anal. Lett.* **1976**, 9, 379–388; (b) Batley, G. E.; Florence, T. M. *Talanta* **1977**, 24, 151–158; (c) Batley, G. E.; Florence, T. M. *Anal. Chem.* **1980**, 52, 1962–1963; (d) Florence, T. M., Batley, G. E.; *CRC Crit. Rev. Anal. Chem.* **1980**, 9, 219–296]. This scheme, which is outlined in Table 11.4.2, combines anodic stripping voltammetry with ion-exchange and UV irradiation, dividing soluble trace metals into seven groups. In the first step, anodic stripping voltammetry in a pH 4.8 acetic acid buffer differentiates between labile metals and nonlabile metals. Only labile metals—those present as hydrated ions, weakly bound complexes, or weakly adsorbed on colloidal surfaces—deposit at the electrode and give rise to a signal. Total metal concentration are determined by ASV after digesting the sample in 2 M HNO_3 for 5 min, which converts all metals into an ASV-labile form.

Table 11.4.2 . Operational Speciation of Soluble Trace Metals

method	speciation of soluble metals						
ASV	labile metals			nonlabile or bound metals			
Ion-Exchange	removed	not removed		removed		not removed	
UV Irradiation		released	not released	released	not released	released	not released
Groups	I	II	III	IV	V	VI	VII
Group I: free metal ions; weaker labile organic complexes and inorganic complexes							
Group II: stronger labile organic complexes; labile metals absorbed on organic solids							
Group III: stronger labile inorganic complexes; labile metals absorbed on inorganic solids							
Group IV: weaker nonlabile organic complexes							
Group V: weaker nonlabile inorganic complexes							
Group VI: stronger nonlabile organic complexes; nonlabile metals absorbed on organic solids							
Group VII: stronger nonlabile inorganic complexes; nonlabile metals absorbed on inorganic solids							

method	speciation of soluble metals
Operational definitions of speciation from (a) Batley, G. E.; Florence, T. M. <i>Anal. Lett.</i> 1976 , <i>9</i> , 379–388; (b) Batley, G. E.; Florence, T. M. <i>Talanta</i> 1977 , <i>24</i> , 151–158; (c) Batley, G. E.; Florence, T. M. <i>Anal. Chem.</i> 1980 , <i>52</i> , 1962–1963; (d) Florence, T. M., Batley, G. E.; <i>CRC Crit. Rev. Anal. Chem.</i> 1980 , <i>9</i> , 219–296.	

A Chelex-100 ion-exchange resin further differentiates between strongly bound metals—usually metals bound to inorganic and organic solids, but also those tightly bound to chelating ligands—and more loosely bound metals. Finally, UV radiation differentiates between metals bound to organic phases and inorganic phases. The analysis of seawater samples, for example, suggests that cadmium, copper, and lead are present primarily as labile organic complexes or as labile adsorbates on organic colloids (Group II in Table 11.4.2).

Differential pulse polarography and stripping voltammetry are used to determine trace metals in airborne particulates, incinerator fly ash, rocks, minerals, and sediments. The trace metals, of course, are first brought into solution using a digestion or an extraction.

Amperometric sensors also are used to analyze environmental samples. For example, the dissolved O₂ sensor described earlier is used to determine the level of dissolved oxygen and the biochemical oxygen demand, or BOD, of waters and wastewaters. The latter test—which is a measure of the amount of oxygen required by aquatic bacteria as they decompose organic matter—is important when evaluating the efficiency of a wastewater treatment plant and for monitoring organic pollution in natural waters. A high BOD suggests that the water has a high concentration of organic matter. Decomposition of this organic matter may seriously deplete the level of dissolved oxygen in the water, adversely affecting aquatic life. Other amperometric sensors are available to monitor anionic surfactants in water, and CO₂, H₂SO₄, and NH₃ in atmospheric gases.

Clinical Samples

Differential pulse polarography and stripping voltammetry are used to determine the concentration of trace metals in a variety of clinical samples, including blood, urine, and tissue. The determination of lead in blood is of considerable interest due to concerns about lead poisoning. Because the concentration of lead in blood is so small, anodic stripping voltammetry frequently is the more appropriate technique. The analysis is complicated, however, by the presence of proteins that may adsorb to the mercury electrode, inhibiting either the deposition or stripping of lead. In addition, proteins may prevent the electrodeposition of lead through the formation of stable, nonlabile complexes. Digesting and ashing the blood sample minimizes this problem. Differential pulse polarography is useful for the routine quantitative analysis of drugs in biological fluids, at concentrations of less than 10⁻⁶ M [Brooks, M. A. “Application of Electrochemistry to Pharmaceutical Analysis,” Chapter 21 in Kissinger, P. T.; Heinemann, W. R., eds. *Laboratory Techniques in Electroanalytical Chemistry*, Marcel Dekker, Inc.: New York, 1984, pp 539–568.]. Amperometric sensors using enzyme catalysts also have many clinical uses, several examples of which are shown in Table 11.4.3.

Table 11.4.3 . Representative Amperometric Biosensors

analyte	enzyme	species detected
choline	choline oxidase	H ₂ O ₂
ethanol	alcohol oxidase	H ₂ O ₂
formaldehyde	formaldehyde dehydrogenase	NADH
glucose	glucose oxidase	H ₂ O ₂
glutamine	glutaminase, glutamine oxidase	H ₂ O ₂
glycerol	glycerol dehydrogenase	NADH, O ₂
lactate	lactate oxidase	H ₂ O ₂
phenol	polyphenol oxidase	quinone
inorganic phosphorous	nucleoside phosphoylase	O ₂

Source: Cammann, K.; Lemke, U.; Rohen, A.; Sander, J.; Wilken, H.; Winter, B. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 516–539.

Miscellaneous Samples

In addition to environmental samples and clinical samples, differential pulse polarography and stripping voltammetry are used for the analysis of trace metals in other sample, including food, steels and other alloys, gasoline, gunpowder residues, and pharmaceuticals. Voltammetry is an important technique for the quantitative analysis of organics, particularly in the pharmaceutical industry where it is used to determine the concentration of drugs and vitamins in formulations. For example, voltammetric methods are available for the quantitative analysis of vitamin A, niacinamide, and riboflavin. When the compound of interest is not electroactive, it often can be derivatized to an electroactive form. One example is the differential pulse polarographic determination of sulfanilamide, which is converted into an electroactive azo dye by coupling with sulfamic acid and 1-naphthol.

Representative Method 11.4.1: Determination of Chlorpromazine in a Pharmaceutical Product

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of chlorpromazine in a pharmaceutical product provides an instructive example of a typical procedure. The description here is based on a method from Pungor, E. *A Practical Guide to Instrumental Analysis*, CRC Press: Boca Raton, FL, 1995, pp. 34–37.

Description of Method

Chlorpromazine, also is known by its trade name Thorazine, is an antipsychotic drug used in the treatment of schizophrenia. The amount of chlorpromazine in a pharmaceutical product is determined voltammetrically at a graphite working electrode in a unstirred solution, with calibration by the method of standard additions.

Procedure

Add 10.00 mL of an electrolyte solution consisting of 0.01 M HCl and 0.1 M KCl to the electrochemical cell. Place a graphite working electrode, a Pt auxiliary electrode, and a SCE reference electrode in the cell, and record the voltammogram from 0.2 V to 2.0 V at a scan rate of 50 mV/s. Weigh out an appropriate amount of the pharmaceutical product and dissolve it in a small amount of the electrolyte. Transfer the solution to a 100-mL volumetric flask and dilute to volume with the electrolyte. Filter a small amount of the diluted solution and transfer 1.00 mL of the filtrate to the voltammetric cell. Mix the contents of the voltammetric cell and allow the solution to sit for 10 s before recording the voltammogram. Return the potential to 0.2 V, add 1.00 mL of a chlorpromazine standard and record the voltammogram. Report the %w/w chlorpromazine in the formulation.

Questions

1. Is chlorpromazine undergoing oxidation or reduction at the graphite working electrode?

Because we are scanning toward more positive potentials, we are oxidizing chlorpromazine.

2. Why does this procedure use a graphite electrode instead of a Hg electrode?

As shown in [Figure 11.4.2](#), the potential window for a Hg electrode extends from approximately -0.3 V to between -1 V and -2 V, depending on the pH. Because we are scanning the potential from 0.2 V to 2.0 V, we cannot use a Hg electrode.

3. Many voltammetric procedures require that we first remove dissolved O_2 by bubbling N_2 through the solution. Why is this not necessary for this analysis?

Dissolved O_2 is a problem when we scan toward more negative potentials, because its reduction may produce a significant cathodic current. In this procedure we are scanning toward more positive potentials and generating anodic currents; thus, dissolved O_2 is not an interferent and does not need to be removed.

4. What is the purpose of recording a voltammogram in the absence of chlorpromazine?

This voltammogram serves as a blank, which provides a measurement of the residual current due to the electrolyte. Because the potential window for a graphite working electrode (see [Figure 11.4.2](#)) does not extend to 2.0 V, there is a measurable anodic residual current due to the solvent's oxidation. Having measured this residual current, we can subtract it from the total current in the presence of chlorpromazine.

5. Based on the description of this procedure, what is the shape of the resulting voltammogram. You may wish to review the three common shapes shown in [Figure 11.4.9](#).

Because the solution is unstirred, the voltammogram will have a peak current similar to that shown in [Figure 11.4.9 b](#).

Characterization Applications

In the previous section we learned how to use voltammetry to determine an analyte's concentration in a variety of different samples. We also can use voltammetry to characterize an analyte's properties, including verifying its electrochemical reversibility, determining the number of electrons transferred during its oxidation or reduction, and determining its equilibrium constant in a coupled chemical reaction.

Electrochemical Reversibility and Determination of n

Earlier in this chapter we derived a relationship between $E_{1/2}$ and the standard-state potential for a redox couple (Equation 8.2.9), noting that a redox reaction must be electrochemically reversible. How can we tell if a redox reaction is reversible by looking at its voltammogram? For a reversible redox reaction Equation 8.2.8, which we repeat here, describes the relationship between potential and current for a voltammetric experiment with a limiting current.

$$E = E_{O/R}^{\circ} - \frac{0.05916}{n} \log \frac{K_O}{K_R} - \frac{0.05916}{n} \log \frac{i}{i_l - i}$$

If a reaction is electrochemically reversible, a plot of E versus $\log(i/i_l - i)$ is a straight line with a slope of $-0.05916/n$. In addition, the slope should yield an integer value for n .

✓ Example 11.4.3

The following data were obtained from a linear scan hydrodynamic voltammogram of a reversible reduction reaction.

E (V vs. SCE)	current (μA)
-0.358	0.37
-0.372	0.95
-0.382	1.71
-0.400	3.48
-0.410	4.20
-0.435	4.97

The limiting current is $5.15 \mu\text{A}$. Show that the reduction reaction is reversible, and determine values for n and for $E_{1/2}$.

Solution

Figure 11.4.20 shows a plot of E versus $\log(i/i_l - i)$. Because the result is a straight-line, we know the reaction is electrochemically reversible under the conditions of the experiment. A linear regression analysis gives the equation for the straight line as

$$E = -0.391\text{V} - 0.0300 \log \frac{i}{i_l - i}$$

From Equation 8.2.8, the slope is equivalent to $-0.05916/n$; solving for n gives a value of 1.97, or 2 electrons. From Equation 8.2.8 and Equation 8.2.9, we know that $E_{1/2}$ is the y -intercept for a plot of E versus $\log(i/i_l - i)$; thus, $E_{1/2}$ for the data in this example is -0.391 V versus the SCE.

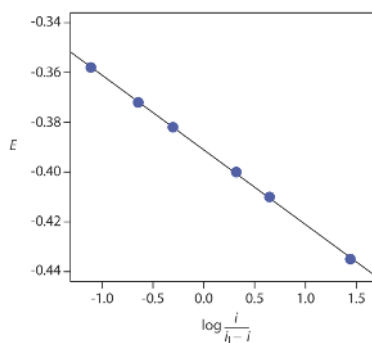


Figure 11.4.20 . Determination of electrochemical reversibility for the data in Example 11.4.3 .

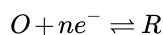
We also can use cyclic voltammetry to evaluate electrochemical reversibility by looking at the difference between the peak potentials for the anodic and the cathodic scans. For an electrochemically reversible reaction, the following equation holds true.

$$\Delta E_p = E_{p,a} - E_{p,c} = \frac{0.05916 \text{ V}}{n}$$

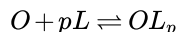
As an example, for a two-electron reduction we expect a ΔE_p of approximately 29.6 mV. For an electrochemically irreversible reaction the value of ΔE_p is larger than expected.

Determining Equilibrium Constants for Coupled Chemical Reactions

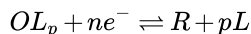
Another important application of voltammetry is determining the equilibrium constant for a solution reaction that is coupled to a redox reaction. The presence of the solution reaction affects the ease of electron transfer in the redox reaction, shifting $E_{1/2}$ to a more negative or to a more positive potential. Consider, for example, the reduction of O to R



the voltammogram for which is shown in Figure 11.4.21 . If we introduce a ligand, L , that forms a strong complex with O , then we also must consider the reaction



In the presence of the ligand, the overall redox reaction is



Because of its stability, the reduction of the OL_p complex is less favorable than the reduction of O . As shown in Figure 11.4.21 , the resulting voltammogram shifts to a potential that is more negative than that for O . Furthermore, the shift in the voltammogram increases as we increase the ligand's concentration.

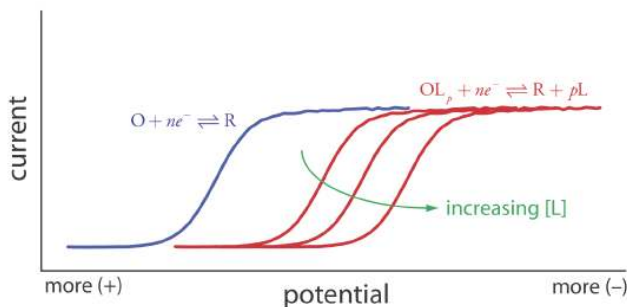


Figure 11.4.21 . Effect of a metal-ligand complexation reaction on a voltammogram. The voltammogram in blue is for the reduction of O in the absence of ligand. Adding the ligand shifts the potentials to more negative potentials, as shown by the voltammograms in red.

We can use this shift in the value of $E_{1/2}$ to determine both the stoichiometry and the formation constant for a metal-ligand complex. To derive a relationship between the relevant variables we begin with two equations: the Nernst equation for the reduction of O

$$E = E_{O/R}^{\circ} - \frac{0.05916}{n} \log \frac{[R]_{x=0}}{[O]_{x=0}} \quad (8.2.10)$$

and the stability constant, β_p for the metal-ligand complex at the electrode surface.

$$\beta_p = \frac{[OL_p]_{x=0}}{[O]_{x=0} [L]_{x=0}^p} \quad (8.2.11)$$

In the absence of ligand the half-wave potential occurs when $[R]_{x=0}$ and $[O]_{x=0}$ are equal; thus, from the Nernst equation we have

$$(E_{1/2})_{nc} = E_{O/R}^{\circ} \quad (8.2.12)$$

where the subscript “nc” signifies that the complex is not present.

When ligand is present we must account for its effect on the concentration of O . Solving Equation 8.2.1 for $[O]_{x=0}$ and substituting into the Equation 8.2.10 gives

$$E = E_{O/R}^{\circ} - \frac{0.05916}{n} \log \frac{[R]_{x=0} [L]_{x=0}^p \beta_p}{[OL_p]_{x=0}} \quad (8.2.13)$$

If the formation constant is sufficiently large, such that essentially all O is present as the complex OL_p , then $[R]_{x=0}$ and $[OL_p]_{x=0}$ are equal at the half-wave potential, and Equation 8.2.13 simplifies to

$$(E_{1/2})_c = E_{O/R}^{\circ} - \frac{0.05916}{n} \log [L]_{x=0}^p \beta_p \quad (8.2.14)$$

where the subscript “c” indicates that the complex is present. Defining $\Delta E_{1/2}$ as

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_{nc} \quad (8.2.15)$$

and substituting Equation 8.2.12 and Equation 8.2.14 and expanding the log term leaves us with the following equation.

$$\Delta E_{1/2} = -\frac{0.05916}{n} \log \beta_p - \frac{0.05916p}{n} \log [L] \quad (8.2.16)$$

A plot of $\Delta E_{1/2}$ versus $\log[L]$ is a straight-line, with a slope that is a function of the metal-ligand complex's stoichiometric coefficient, p , and a y-intercept that is a function of its formation constant β_p .

✓ Example 11.4.4

A voltammogram for the two-electron reduction ($n = 2$) of a metal, M , has a half-wave potential of -0.226 V versus the SCE. In the presence of an excess of ligand, L , the following half-wave potentials are recorded.

$[L]$ (M)	$(E_{1/2})_c$ (V vs. SCE)
0.020	-0.494
0.040	-0.512
0.060	-0.523
0.080	-0.530
0.100	-0.536

Determine the stoichiometry of the metal-ligand complex and its formation constant.

Solution

We begin by calculating values of $\Delta E_{1/2}$ using Equation 8.2.15, obtaining the values in the following table.

$[L]$ (M)	$\Delta E_{1/2}$ (V vs. SCE)
0.020	-0.268

$\log[L]$	$\Delta E_{1/2}$ (V vs SCE)
0.080	-0.286
0.080	-0.304
0.040	-0.286
0.100	-0.310
0.060	-0.297
0.080	-0.304
0.100	-0.310

Figure 11.4.22 shows the resulting plot of $\Delta E_{1/2}$ as a function of $\log[L]$. A linear regression analysis gives the equation for the straight line as

$$\Delta E_{1/2} = -0.370\text{V} - 0.0601 \log [L]$$

From Equation 8.2.16 we know that the slope is equal to $-0.05916p/n$. Using the slope and $n = 2$, we solve for p obtaining a value of $2.03 \approx 2$. The complex's stoichiometry, therefore, is ML_2 . We also know, from Equation 8.2.16, that the y-intercept is equivalent to $-(0.05916/n)\log\beta_p$. Solving for β_2 gives a formation constant of 3.2×10^{12} .

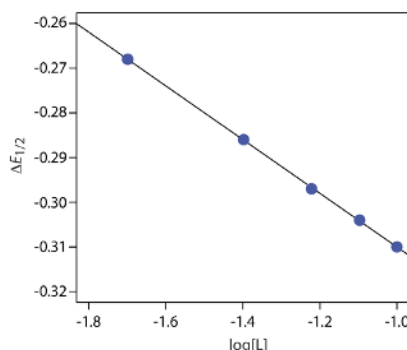


Figure 11.4.22 . Determination of the stoichiometry and formation constant for a metal-ligand complex using the data in Example 11.4.4 .

? Exercise 11.4.2

The voltammogram for 0.50 mM Cd^{2+} has an $E_{1/2}$ of -0.565 V versus an SCE. After making the solution 0.115 M in ethylenediamine, $E_{1/2}$ is -0.845 V, and $E_{1/2}$ is -0.873 V when the solution is 0.231 M in ethylenediamine. Determine the stoichiometry of the Cd^{2+} –ethylenediamine complex and its formation constant. The data in this problem comes from Morinaga, K. “Polarographic Studies of Metal Complexes. V. Ethylenediamine Complexes of Cadmium, Nickel, and Zinc,” *Bull. Chem. Soc. Japan* **1956**, 29, 793–799.

Answer

For simplicity, we will use *en* as a shorthand notation for ethylenediamine. From the three half-wave potentials we have a $\Delta E_{1/2}$ of -0.280 V for 0.115 M en and a $\Delta E_{1/2}$ of -0.308 V for 0.231 M en. Using Equation 8.2.16 we write the following two equations.

$$\begin{aligned} -0.280 &= -\frac{0.05916}{2} \log \beta_p - \frac{0.05916p}{2} \log(0.115) \\ -0.308 &= -\frac{0.05916}{2} \log \beta_p - \frac{0.05916p}{2} \log(0.231) \end{aligned}$$

To solve for the value of p , we first subtract the second equation from the first equation

$$0.028 = -\frac{0.05916p}{2} \log(0.115) - \left\{ -\frac{0.05916p}{2} \log(0.231) \right\}$$

which eliminates the term with β_p . Next we solve this equation for p

$$0.028 = (2.778 \times 10^{-2}) \times p - (1.882 \times 10^{-2}) \times p = (8.96 \times 10^{-3}) \times p$$

obtaining a value of 3.1, or $p \approx 3$. Thus, the complex is $\text{Cd}(\text{en})_3$. To find the formation complex, β_3 , we return to Equation 8.2.16 using our value for p . Using the data for an en concentration of 0.115 M

$$\begin{aligned}
 -0.280 &= -\frac{0.05916}{2} \log \beta_3 - \frac{0.05916 \times 3}{2} \log(0.115) \\
 -0.363 &= -\frac{0.05916}{2} \log \beta_3
 \end{aligned}$$

gives a value for β_3 of 1.92×10^{12} . Using the data for an en concentration of 0.231 M gives a value of 2.10×10^{12} .

As suggested by [Figure 11.4.15](#), cyclic voltammetry is one of the most powerful electrochemical techniques for exploring the mechanism of coupled electrochemical and chemical reactions. The treatment of this aspect of cyclic voltammetry is beyond the level of this text, although you can consult this chapter's additional resources for additional information.

Evaluation

Scale of Operation

Detection levels at the parts-per-million level are routine. For some analytes and for some voltammetric techniques, lower detection limits are possible. Detection limits at the parts-per-billion and the part-per-trillion level are possible with stripping voltammetry. Although most analyses are carried out in conventional electrochemical cells using macro samples, the availability of microelectrodes with diameters as small as 2 μm , allows for the analysis of samples with volumes under 50 μL . For example, the concentration of glucose in 200- μm pond snail neurons was monitored successfully using an amperometric glucose electrode with a 2 mm tip [Abe, T.; Lauw, L. L.; Ewing, A. G. *J. Am. Chem. Soc.* **1991**, *113*, 7421–7423].

Accuracy

The accuracy of a voltammetric analysis usually is limited by our ability to correct for residual currents, particularly those due to charging. For an analyte at the parts-per-million level, an accuracy of ± 1 –3% is routine. Accuracy decreases for samples with significantly smaller concentrations of analyte.

Precision

Precision generally is limited by the uncertainty in measuring the limiting current or the peak current. Under most conditions, a precision of ± 1 –3% is reasonable. One exception is the analysis of ultratrace analytes in complex matrices by stripping voltammetry, in which the precision may be as poor as $\pm 25\%$.

Sensitivity

In many voltammetric experiments, we can improve the sensitivity by adjusting the experimental conditions. For example, in stripping voltammetry we can improve sensitivity by increasing the deposition time, by increasing the rate of the linear potential scan, or by using a differential-pulse technique. One reason that potential pulse techniques are popular is that they provide an improvement in current relative to a linear potential scan.

Selectivity

Selectivity in voltammetry is determined by the difference between half-wave potentials or peak potentials, with a minimum difference of ± 0.2 –0.3 V for a linear potential scan and ± 0.04 –0.05 V for differential pulse voltammetry. We often can improve selectivity by adjusting solution conditions. The addition of a complexing ligand, for example, can substantially shift the potential where a species is oxidized or reduced to a potential where it no longer interferes with the determination of an analyte. Other solution parameters, such as pH, also can be used to improve selectivity.

Time, Cost, and Equipment

Commercial instrumentation for voltammetry ranges from <\$1000 for simple instruments to >\$20,000 for a more sophisticated instrument. In general, less expensive instrumentation is limited to linear potential scans. More expensive instruments provide for more complex potential-excitation signals using potential pulses. Except for stripping voltammetry, which needs a long deposition time, voltammetric analyses are relatively rapid.

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CHAPTER OVERVIEW

9: Chromatography

9.1: Overview of Analytical Separations

9.2: General Theory of Column Chromatography

9.3: Optimizing Chromatographic Separations

9.4: High-Performance Liquid Chromatography

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9.1: Overview of Analytical Separations

In [Chapter 7](#) we examined several methods for separating an analyte from potential interferents. For example, in a liquid–liquid extraction the analyte and interferent initially are present in a single liquid phase. We add a second, immiscible liquid phase and thoroughly mix them by shaking. During this process the analyte and interferents partition between the two phases to different extents, effecting their separation. After allowing the phases to separate, we draw off the phase enriched in analyte. Despite the power of liquid–liquid extractions, there are significant limitations.

Two Limitations of Liquid-Liquid Extractions

Suppose we have a sample that contains an analyte in a matrix that is incompatible with our analytical method. To determine the analyte's concentration we first separate it from the matrix using a simple liquid–liquid extraction. If we have several analytes, we may need to complete a separate extraction for each analyte. For a complex mixture of analytes this quickly becomes a tedious process. This is one limitation to a liquid–liquid extraction.

A more significant limitation is that the extent of a separation depends on the distribution ratio of each species in the sample. If the analyte's distribution ratio is similar to that of another species, then their separation becomes impossible. For example, let's assume that an analyte, *A*, and an interferent, *I*, have distribution ratios of, respectively, 5 and 0.5. If we use a liquid–liquid extraction with equal volumes of sample and extractant, then it is easy to show that a single extraction removes approximately 83% of the analyte and 33% of the interferent. Although we can remove 99% of the analyte with three extractions, we also remove 70% of the interferent. In fact, there is no practical combination of number of extractions or volumes of sample and extractant that produce an acceptable separation.

From [Chapter 7](#) we know that the distribution ratio, *D*, for a solute, *S*, is

$$D = \frac{[S]_{\text{ext}}}{[S]_{\text{samp}}}$$

where $[S]_{\text{ext}}$ is its equilibrium concentration in the extracting phase and $[S]_{\text{samp}}$ is its equilibrium concentration in the sample. We can use the distribution ratio to calculate the fraction of *S* that remains in the sample, q_{samp} , after an extraction

$$q_{\text{samp}} = \frac{V_{\text{samp}}}{DV_{\text{ext}} + V_{\text{samp}}}$$

where V_{samp} is the volume of sample and V_{ext} is the volume of the extracting phase. For example, if $D = 10$, $V_{\text{samp}} = 20$, and $V_{\text{ext}} = 5$, the fraction of *S* remaining in the sample after the extraction is

$$q_{\text{samp}} = \frac{20}{10 \times 5 + 20} = 0.29$$

or 29%. The remaining 71% of the analyte is in the extracting phase.

A Better Way to Separate Mixtures

The problem with a liquid–liquid extraction is that the separation occurs in one direction only: from the sample to the extracting phase. Let's take a closer look at the liquid–liquid extraction of an analyte and an interferent with distribution ratios of, respectively, 5 and 0.5. Figure 12.1.1 shows that a single extraction using equal volumes of sample and extractant transfers 83% of the analyte and 33% of the interferent to the extracting phase. If the original concentrations of *A* and *I* are identical, then their concentration ratio in the extracting phase after one extraction is

$$\frac{[A]}{[I]} = \frac{0.83}{0.33} = 2.5$$

A single extraction, therefore, enriches the analyte by a factor of $2.5\times$. After completing a second extraction (Figure 12.1.1) and combining the two extracting phases, the separation of the analyte and the interferent, surprisingly, is less efficient.

$$\frac{[A]}{[I]} = \frac{0.97}{0.55} = 1.8$$

Figure 12.1.1 makes it clear why the second extraction results in a poorer overall separation: the second extraction actually favors the interferent!

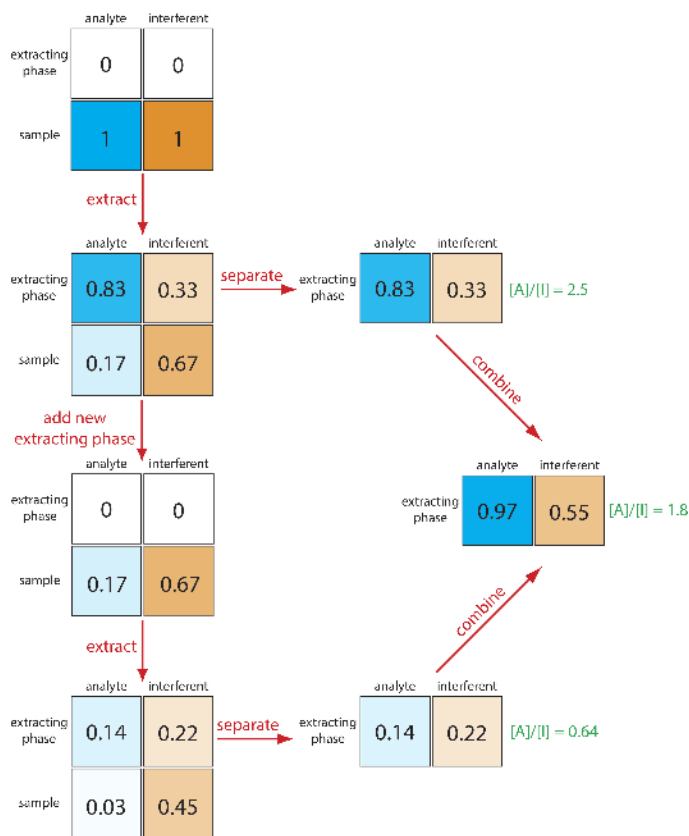


Figure 12.1.1 . Progress of a traditional liquid–liquid extraction using two identical extractions of a single sample using fresh portions of the extractant. The numbers give the fraction of **analyte** and **interferent** in each phase assuming equal volumes of sample and extractant and distribution ratios of 5 and 0.5 for the **analyte** and the **interferent**, respectively. The opacity of the colors equals the fraction of **analyte** and **interferent** present.

We can improve the separation by first extracting the solutes from the sample into the extracting phase and then extracting them back into a fresh portion of solvent that matches the sample's matrix (Figure 12.1.2). Because the analyte has the larger distribution ratio, more of it moves into the extractant during the first extraction and less of it moves back to the sample phase during the second extraction. In this case the concentration ratio in the extracting phase after two extractions is significantly greater.

$$\frac{[A]}{[I]} = \frac{0.69}{0.11} = 6.3$$

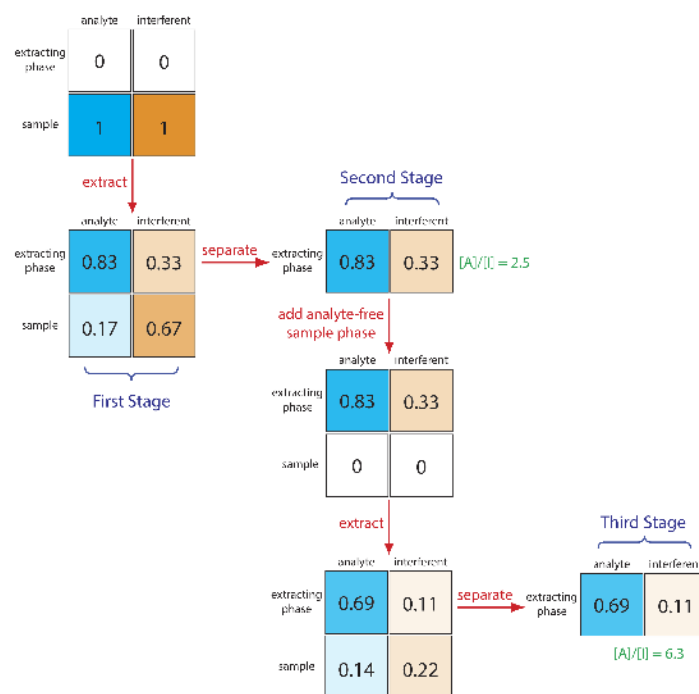


Figure 12.1.2 . Progress of a liquid–liquid extraction in which we first extract the solutes into the extracting phase and then extract them back into an analyte-free portion of the sample’s phase. The numbers give the fraction of **analyte** and **interferent** in each phase assuming equal volumes of sample and extractant and distribution ratios of 5 and 0.5 for the **analyte** and the **interferent**, respectively. The opacity of the colors equals the fraction of **analyte** and **interferent** present.

Not shown in Figure 12.2 is that we can add a fresh portion of the extracting phase to the sample that remains after the first extraction (the bottom row of the first stage in Figure 12.2, beginning the process anew. As we increase the number of extractions, the analyte and the interferent each spread out in space over a series of stages. Because the interferent’s distribution ratio is smaller than the analyte’s, the interferent lags behind the analyte. With a sufficient number of extractions—that is, a sufficient number of stages—a complete separation of the analyte and interferent is possible. This process of extracting the solutes back and forth between fresh portions of the two phases, which we call a **countercurrent extraction**, was developed by Craig in the 1940s [Craig, L. C. *J. Biol. Chem.* **1944**, 155, 519–534]. The same phenomenon forms the basis of modern chromatography.

See [Appendix 16](#) for a more detailed consideration of the mathematics behind a countercurrent extraction.

Chromatographic Separations

In **chromatography** we pass a sample-free phase, which we call the **mobile phase**, over a second sample-free **stationary phase** that remains fixed in space (Figure 12.1.3). We inject or place the sample into the mobile phase. As the sample moves with the mobile phase, its components partition between the mobile phase and the stationary phase. A component whose distribution ratio favors the stationary phase requires more time to pass through the system. Given sufficient time and sufficient stationary and mobile phase, we can separate solutes even if they have similar distribution ratios.

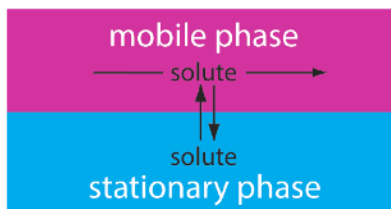


Figure 12.1.3 . In chromatography we pass a mobile phase over a stationary phase. When we inject a sample into the mobile phase, the sample’s components both move with the mobile phase and partition into the stationary phase. The solute that spends the most time in the stationary phase takes the longest time to move through the system.

There are many ways in which we can identify a chromatographic separation: by describing the physical state of the mobile phase and the stationary phase; by describing how we bring the stationary phase and the mobile phase into contact with each other; or by describing the chemical or physical interactions between the solute and the stationary phase. Let's briefly consider how we might use each of these classifications.

We can trace the history of chromatography to the turn of the century when the Russian botanist Mikhail Tswett used a column packed with calcium carbonate and a mobile phase of petroleum ether to separate colored pigments from plant extracts. As the sample moved through the column, the plant's pigments separated into individual colored bands. After effecting the separation, the calcium carbonate was removed from the column, sectioned, and the pigments recovered. Tswett named the technique chromatography, combining the Greek words for "color" and "to write." There was little interest in Tswett's technique until Martin and Synge's pioneering development of a theory of chromatography (see Martin, A. J. P.; Synge, R. L. M. "A New Form of Chromatogram Employing Two Liquid Phases," *Biochem. J.* **1941**, 35, 1358–1366). Martin and Synge were awarded the 1952 Nobel Prize in Chemistry for this work.

Types of Mobile Phases and Stationary Phases

The mobile phase is a liquid or a gas, and the stationary phase is a solid or a liquid film coated on a solid substrate. We often name chromatographic techniques by listing the type of mobile phase followed by the type of stationary phase. In gas–liquid chromatography, for example, the mobile phase is a gas and the stationary phase is a liquid film coated on a solid substrate. If a technique's name includes only one phase, as in gas chromatography, it is the mobile phase.

Contact Between the Mobile Phase and the Stationary Phase

There are two common methods for bringing the mobile phase and the stationary phase into contact. In **column chromatography** we pack the stationary phase into a narrow column and pass the mobile phase through the column using gravity or by applying pressure. The stationary phase is a solid particle or a thin liquid film coated on either a solid particulate packing material or on the column's walls.

In **planar chromatography** the stationary phase is coated on a flat surface—typically, a glass, metal, or plastic plate. One end of the plate is placed in a reservoir that contains the mobile phase, which moves through the stationary phase by capillary action. In paper chromatography, for example, paper is the stationary phase.

Interaction Between the Solute and the Stationary Phase

The interaction between the solute and the stationary phase provides a third method for describing a separation (Figure 12.1.4). In **adsorption chromatography**, solutes separate based on their ability to adsorb to a solid stationary phase. In **partition chromatography**, the stationary phase is a thin liquid film on a solid support. Separation occurs because there is a difference in the equilibrium partitioning of solutes between the stationary phase and the mobile phase. A stationary phase that consists of a solid support with covalently attached anionic (e.g., $-\text{SO}_3^-$) or cationic (e.g., $-\text{N}(\text{CH}_3)_3^+$) functional groups is the basis for ion-exchange chromatography in which ionic solutes are attracted to the stationary phase by electrostatic forces. In size-exclusion chromatography the stationary phase is a porous particle or gel, with separation based on the size of the solutes. Larger solutes are unable to penetrate as deeply into the porous stationary phase and pass more quickly through the column.

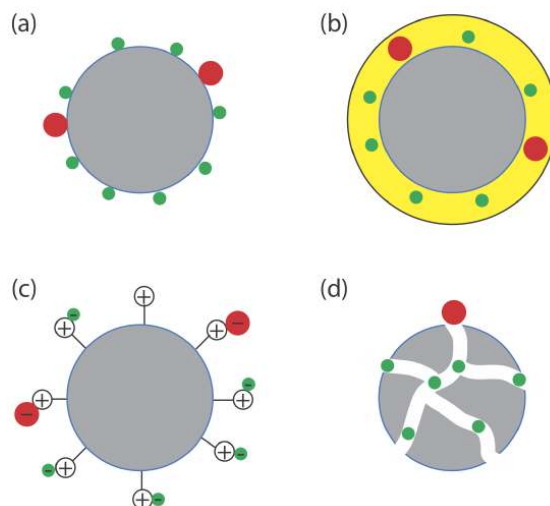


Figure 12.1.4 . Four examples of interactions between a solute and the stationary phase: (a) adsorption on a solid surface, (b) partitioning into a liquid phase, (c) ion-exchange, and (d) size exclusion. For each example, the smaller, green solute is more strongly retained than the larger, red solute.

There are other interactions that can serve as the basis of a separation. In affinity chromatography the interaction between an antigen and an antibody, between an enzyme and a substrate, or between a receptor and a ligand forms the basis of a separation. See this chapter's [additional resources](#) for some suggested readings.

Electrophoretic Separations

In chromatography, a separation occurs because there is a difference in the equilibrium partitioning of solutes between the mobile phase and the stationary phase. Equilibrium partitioning, however, is not the only basis for effecting a separation. In an electrophoretic separation, for example, charged solutes migrate under the influence of an applied potential. A separation occurs because of differences in the charges and the sizes of the solutes (Figure 12.1.5).

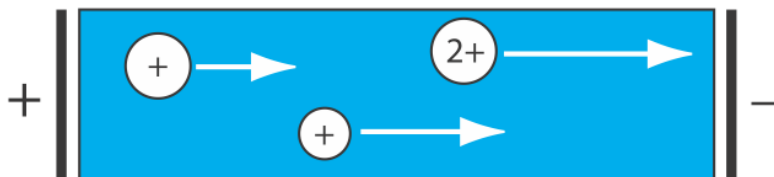


Figure 12.1.5 . Movement of charged solutes under the influence of an applied potential. The lengths of the arrows indicate the relative speed of the solutes. In general, a larger solute moves more slowly than a smaller solute of equal charge, and a solute with a larger charge move more quickly than a solute with a smaller charge.

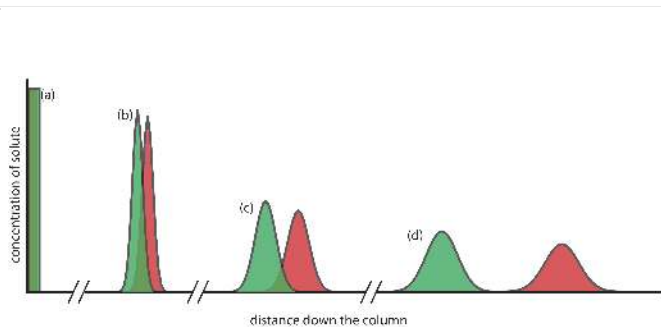
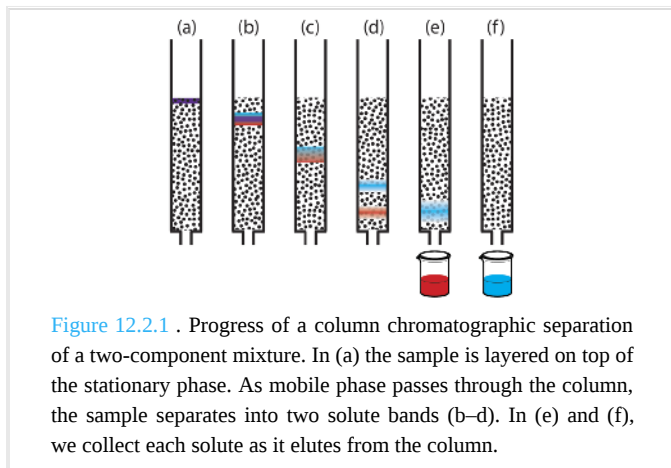
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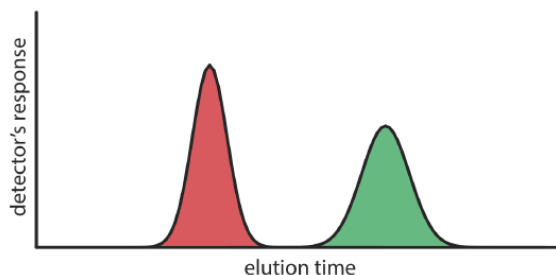
9.2: General Theory of Column Chromatography

Of the two methods for bringing the stationary phase and the mobile phases into contact, the most important is column chromatography. In this section we develop a general theory that we may apply to any form of column chromatography.

Figure 12.2.1 provides a simple view of a liquid–solid column chromatography experiment. The sample is introduced as a narrow band at the top of the column. Ideally, the solute's initial concentration profile is rectangular (Figure 12.2.2 a). As the sample moves down the column, the solutes begin to separate (Figure 12.2.1 b,c) and the individual solute bands begin to broaden and develop a Gaussian profile (Figure 12.2.2 b,c). If the strength of each solute's interaction with the stationary phase is sufficiently different, then the solutes separate into individual bands (Figure 12.2.1 d and Figure 12.2.2 d).



We can follow the progress of the separation by collecting fractions as they elute from the column (Figure 12.2.1 e,f), or by placing a suitable detector at the end of the column. A plot of the detector's response as a function of elution time, or as a function of the volume of mobile phase, is known as a **chromatogram** (Figure 12.2.3), and consists of a peak for each solute.



There are many possible detectors that we can use to monitor the separation. Later sections of this chapter describe some of the most popular.

We can characterize a chromatographic peak's properties in several ways, two of which are shown in Figure 12.2.4 . **Retention time**, t_r , is the time between the sample's injection and the maximum response for the solute's peak. A chromatographic peak's **baseline width**, w , as shown in Figure 12.2.4 , is determined by extending tangent lines from the inflection points on either side of the peak through the baseline. Although usually we report t_r and w using units of time, we can report them using units of volume by multiplying each by the mobile phase's velocity, or report them in linear units by measuring distances with a ruler.

For example, a solute's retention volume, V_r , is $t_r \times u$ where u is the mobile phase's velocity through the column.

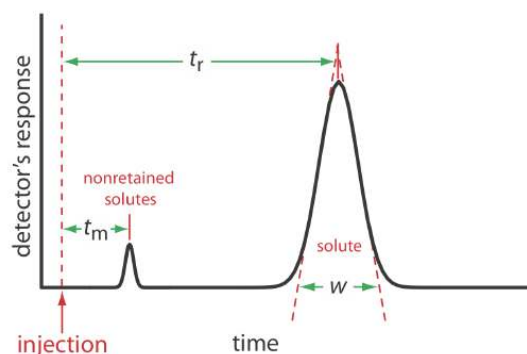


Figure 12.2.4 . Chromatogram showing a solute's retention time, t_r , and baseline width, w , and the column's void time, t_m , for nonretained solutes.

In addition to the solute's peak, Figure 12.2.4 also shows a small peak that elutes shortly after the sample is injected into the mobile phase. This peak contains all **nonretained solutes**, which move through the column at the same rate as the mobile phase. The time required to elute the nonretained solutes is called the column's **void time**, t_m .

Chromatographic Resolution

The goal of chromatography is to separate a mixture into a series of chromatographic peaks, each of which constitutes a single component of the mixture. The **resolution** between two chromatographic peaks, R_{AB} , is a quantitative measure of their separation, and is defined as

$$R_{AB} = \frac{t_{r,B} - t_{r,A}}{0.5(w_B + w_A)} = \frac{2\Delta t_r}{w_B + w_A} \quad (9.2.1)$$

where B is the later eluting of the two solutes. As shown in Figure 12.2.5, the separation of two chromatographic peaks improves with an increase in R_{AB} . If the areas under the two peaks are identical—as is the case in Figure 12.2.5—then a resolution of 1.50 corresponds to an overlap of only 0.13% for the two elution profiles. Because resolution is a quantitative measure of a separation's success, it is a useful way to determine if a change in experimental conditions leads to a better separation.

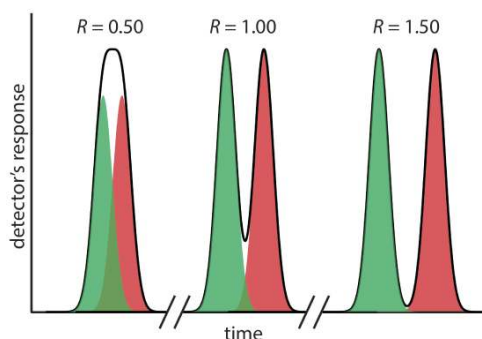


Figure 12.2.5 . Three examples that show the relationship between resolution and the separation of a two component mixture. The green peak and the red peak are the elution profiles for the two components. The chromatographic peak—which is the sum of the two elution profiles—is shown by the solid black line.

✓ Example 12.2.1

In a chromatographic analysis of lemon oil a peak for limonene has a retention time of 8.36 min with a baseline width of 0.96 min. γ -Terpinene elutes at 9.54 min with a baseline width of 0.64 min. What is the resolution between the two peaks?

Solution

Using Equation 9.2.1 we find that the resolution is

$$R_{AB} = \frac{2\Delta t_r}{w_B + w_A} = \frac{2(9.54 \text{ min} - 8.36 \text{ min})}{0.64 \text{ min} + 0.96 \text{ min}} = 1.48$$

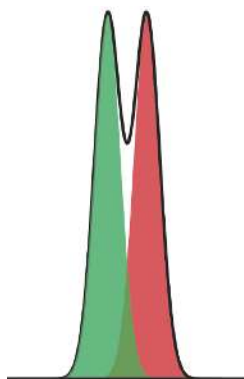


Figure 12.2.6 . Chromatogram for Exercise 12.2.1 .

? Exercise 12.2.1

Figure 12.2.6 shows the separation of a two-component mixture. What is the resolution between the two components? Use a ruler to measure Δt_r , w_A , and w_B in millimeters.

Answer

Because the relationship between elution time and distance is proportional, we can measure Δt_r , w_A , and w_B using a ruler. My measurements are 8.5 mm for Δt_r , and 12.0 mm each for w_A and w_B . Using these values, the resolution is

$$R_{AB} = \frac{2\Delta t_r}{w_A + w_B} = \frac{2(8.5 \text{ mm})}{12.0 \text{ mm} + 12.0 \text{ mm}} = 0.70$$

Your measurements for Δt_r , w_A , and w_B will depend on the relative size of your monitor or printout; however, your value for the resolution should be similar to the answer above.

Equation 9.2.1 suggests that we can improve resolution by increasing Δt_r , or by decreasing w_A and w_B (Figure 12.2.7). To increase Δt_r we can use one of two strategies. One approach is to adjust the separation conditions so that both solutes spend less time in the mobile phase—that is, we increase each *solute's retention factor*—which provides more time to effect a separation. A second approach is to increase *selectivity* by adjusting conditions so that only one solute experiences a significant change in its retention time. The baseline width of a solute's peak depends on the solutes movement within and between the mobile phase and the stationary phase, and is governed by several factors that collectively we call *column efficiency*. We will consider each of these approaches for improving resolution in more detail, but first we must define some terms.

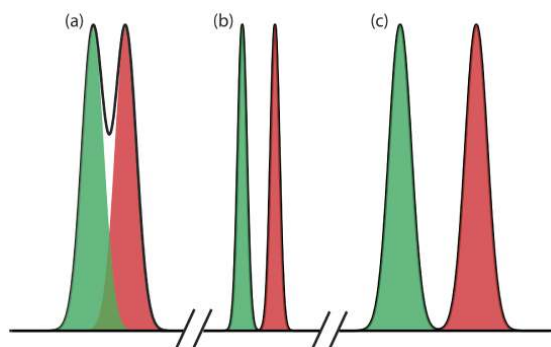
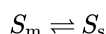


Figure 12.2.7 . Two method for improving chromatographic resolution: (a) original chromatogram; (b) chromatogram after decreasing w_A and w_B by $4\times$; and (c) chromatogram after increasing Δt_r by $2\times$.

Solute Retention Factor

Let's assume we can describe a solute's distribution between the mobile phase and stationary phase using the following equilibrium reaction



where S_m is the solute in the mobile phase and S_s is the solute in the stationary phase. Following the same approach we used in [Chapter 7.7](#) for liquid–liquid extractions, the equilibrium constant for this reaction is an equilibrium partition coefficient, K_D .

$$K_D = \frac{[S_s]}{[S_m]}$$

This is not a trivial assumption. In this section we are, in effect, treating the solute's equilibrium between the mobile phase and the stationary phase as if it is identical to the equilibrium in a liquid–liquid extraction. You might question whether this is a reasonable assumption. There is an important difference between the two experiments that we need to consider. In a liquid–liquid extraction, which takes place in a separatory funnel, the two phases remain in contact with each other at all times, allowing for a true equilibrium. In chromatography, however, the mobile phase is in constant motion. A solute that moves into the stationary phase from the mobile phase will equilibrate back into a different portion of the mobile phase; this does not describe a true equilibrium.

So, we ask again: Can we treat a solute's distribution between the mobile phase and the stationary phase as an equilibrium process? The answer is yes, if the mobile phase velocity is slow relative to the kinetics of the solute's movement back and forth between the two phase. In general, this is a reasonable assumption.

In the absence of any additional equilibrium reactions in the mobile phase or the stationary phase, K_D is equivalent to the distribution ratio, D ,

$$D = \frac{[S_0]}{[S_m]} = \frac{(\text{mol } S)_s / V_s}{(\text{mol } S)_m / V_m} = K_D \quad (9.2.2)$$

where V_s and V_m are the volumes of the stationary phase and the mobile phase, respectively.

A conservation of mass requires that the total moles of solute remain constant throughout the separation; thus, we know that the following equation is true.

$$(\text{mol } S)_{\text{tot}} = (\text{mol } S)_m + (\text{mol } S)_s \quad (9.2.3)$$

Solving Equation 9.2.3 for the moles of solute in the stationary phase and substituting into Equation 9.2.2 leaves us with

$$D = \frac{\{(\text{mol } S)_{\text{tot}} - (\text{mol } S)_m\} / V_s}{(\text{mol } S)_m / V_m}$$

Rearranging this equation and solving for the fraction of solute in the mobile phase, f_m , gives

$$f_m = \frac{(\text{mol } S)_m}{(\text{mol } S)_{\text{tot}}} = \frac{V_m}{DV_s + V_m} \quad (9.2.4)$$

which is identical to the result for a liquid-liquid extraction (see [Chapter 7](#)). Because we may not know the exact volumes of the stationary phase and the mobile phase, we simplify Equation 9.2.4 by dividing both the numerator and the denominator by V_m ; thus

$$f_m = \frac{V_m/V_m}{DV_s/V_m + V_m/V_m} = \frac{1}{DV_s/V_m + 1} = \frac{1}{1 + k} \quad (9.2.5)$$

where k

$$k = D \times \frac{V_s}{V_m} \quad (9.2.6)$$

is the solute's **retention factor**. Note that the larger the retention factor, the more the distribution ratio favors the stationary phase, leading to a more strongly retained solute and a longer retention time.

Other (older) names for the retention factor are capacity factor, capacity ratio, and partition ratio, and it sometimes is given the symbol k' . Keep this in mind if you are using other resources. Retention factor is the approved name from the IUPAC Gold Book.

We can determine a solute's retention factor from a chromatogram by measuring the column's void time, t_m , and the solute's retention time, t_r (see Figure 12.2.4). Solving Equation 9.2.5 for k , we find that

$$k = \frac{1 - f_m}{f_m} \quad (9.2.7)$$

Earlier we defined f_m as the fraction of solute in the mobile phase. Assuming a constant mobile phase velocity, we also can define f_m as

$$f_m = \frac{\text{time spent in the mobile phase}}{\text{time spent in the stationary phase}} = \frac{t_m}{t_r}$$

Substituting back into Equation 9.2.7 and rearranging leaves us with

$$k = \frac{1 - \frac{t_m}{t_r}}{\frac{t_m}{t_r}} = \frac{t_r - t_m}{t_m} = \frac{t'_r}{t_m} \quad (9.2.8)$$

where t'_r is the **adjusted retention time**.

✓ Example 12.2.2

In a chromatographic analysis of low molecular weight acids, butyric acid elutes with a retention time of 7.63 min. The column's void time is 0.31 min. Calculate the retention factor for butyric acid.

Solution

$$k_{\text{but}} = \frac{t_r - t_m}{t_m} = \frac{7.63 \text{ min} - 0.31 \text{ min}}{0.31 \text{ min}} = 23.6$$

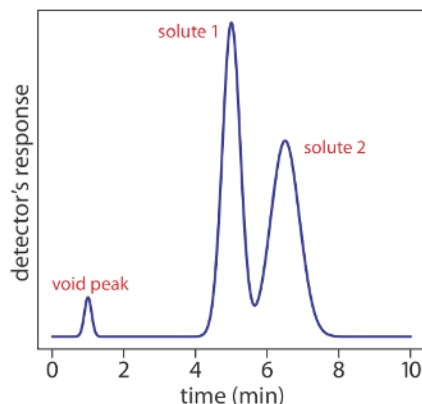


Figure 12.2.8 . Chromatogram for Exercise 12.2.2 .

? Exercise 12.2.2

Figure 12.2.8 is the chromatogram for a two-component mixture. Determine the retention factor for each solute assuming the sample was injected at time $t = 0$.

Answer

Because the relationship between elution time and distance is proportional, we can measure t_m , $t_{r,1}$, and $t_{r,2}$ using a ruler. My measurements are 7.8 mm, 40.2 mm, and 51.5 mm, respectively. Using these values, the retention factors for solute A and solute B are

$$k_1 = \frac{t_{r1} - t_m}{t_m} = \frac{40.2 \text{ mm} - 7.8 \text{ mm}}{7.8 \text{ mm}} = 4.15$$

$$k_2 = \frac{t_{r2} - t_m}{t_m} = \frac{51.5 \text{ mm} - 7.8 \text{ mm}}{7.8 \text{ mm}} = 5.60$$

Your measurements for t_m , $t_{r,1}$, and $t_{r,2}$ will depend on the relative size of your monitor or printout; however, your value for the resolution should be similar to the answer above.

Selectivity

Selectivity is a relative measure of the retention of two solutes, which we define using a selectivity factor, α

$$\alpha = \frac{k_B}{k_A} = \frac{t_{r,B} - t_m}{t_{r,A} - t_m} \quad (9.2.9)$$

where solute A has the smaller retention time. When two solutes elute with identical retention time, $\alpha = 1.00$; for all other conditions $\alpha > 1.00$.

✓ Example 12.2.3

In the chromatographic analysis for low molecular weight acids described in [Example 12.2.2](#), the retention time for isobutyric acid is 5.98 min. What is the selectivity factor for isobutyric acid and butyric acid?

Solution

First we must calculate the retention factor for isobutyric acid. Using the void time from [Example 12.2.2](#) we have

$$k_{\text{iso}} = \frac{t_r - t_m}{t_m} = \frac{5.98 \text{ min} - 0.31 \text{ min}}{0.31 \text{ min}} = 18.3$$

The selectivity factor, therefore, is

$$\alpha = \frac{k_{\text{but}}}{k_{\text{iso}}} = \frac{23.6}{18.3} = 1.29$$

? Exercise 12.2.3

Determine the selectivity factor for the chromatogram in [Exercise 12.2.2](#).

Answer

Using the results from [Exercise 12.2.2](#), the selectivity factor is

$$\alpha = \frac{k_2}{k_1} = \frac{5.60}{4.15} = 1.35$$

Your answer may differ slightly due to differences in your values for the two retention factors.

Column Efficiency

Suppose we inject a sample that has a single component. At the moment we inject the sample it is a narrow band of finite width. As the sample passes through the column, the width of this band continually increases in a process we call **band broadening**. Column efficiency is a quantitative measure of the extent of band broadening.

See [Figure 12.2.1](#) and [Figure 12.2.2](#). When we inject the sample it has a uniform, or rectangular concentration profile with respect to distance down the column. As it passes through the column, the band broadens and takes on a Gaussian concentration profile.

In their original theoretical model of chromatography, Martin and Synge divided the chromatographic column into discrete sections, which they called theoretical plates. Within each theoretical plate there is an equilibrium between the solute present in the stationary phase and the solute present in the mobile phase [Martin, A. J. P.; Synge, R. L. M. *Biochem. J.* **1941**, 35, 1358–1366]. They described column efficiency in terms of the number of **theoretical plates**, N ,

$$N = \frac{L}{H} \quad (9.2.10)$$

where L is the column's length and H is the height of a theoretical plate. For any given column, the column efficiency improves—and chromatographic peaks become narrower—when there are more theoretical plates.

If we assume that a chromatographic peak has a Gaussian profile, then the extent of band broadening is given by the peak's variance or standard deviation. The height of a theoretical plate is the peak's variance per unit length of the column

$$H = \frac{\sigma^2}{L} \quad (9.2.11)$$

where the standard deviation, σ , has units of distance. Because retention times and peak widths usually are measured in seconds or minutes, it is more convenient to express the standard deviation in units of time, τ , by dividing σ by the solute's average linear velocity, $\bar{u}$, which is equivalent to dividing the distance it travels, L , by its retention time, t_r .

$$\tau = \frac{\sigma}{\bar{u}} = \frac{\sigma t_r}{L} \quad (9.2.12)$$

For a Gaussian peak shape, the width at the baseline, w , is four times its standard deviation, τ .

$$w = 4\tau \quad (9.2.13)$$

Combining Equation 9.2.11, Equation 9.2.12, and Equation 9.2.13 defines the height of a theoretical plate in terms of the easily measured chromatographic parameters t_r and w .

$$H = \frac{Lw^2}{16t_r^2} \quad (9.2.14)$$

Combining Equation 9.2.14 and Equation 9.2.10 gives the number of theoretical plates.

$$N = 16 \frac{t_r^2}{w^2} = 16 \left(\frac{t_r}{w} \right)^2 \quad (9.2.15)$$

✓ Example 12.2.4

A chromatographic analysis for the chlorinated pesticide Dieldrin gives a peak with a retention time of 8.68 min and a baseline width of 0.29 min. Calculate the number of theoretical plates? Given that the column is 2.0 m long, what is the height of a theoretical plate in mm?

Solution

Using Equation 9.2.15, the number of theoretical plates is

$$N = 16 \frac{t_r^2}{w^2} = 16 \times \frac{(8.68 \text{ min})^2}{(0.29 \text{ min})^2} = 14300 \text{ plates}$$

Solving Equation 9.2.10 for H gives the average height of a theoretical plate as

$$H = \frac{L}{N} = \frac{2.00 \text{ m}}{14300 \text{ plates}} \times \frac{1000 \text{ mm}}{\text{m}} = 0.14 \text{ mm/plate}$$

? Exercise 12.2.4

For each solute in the chromatogram for Exercise 12.2.2, calculate the number of theoretical plates and the average height of a theoretical plate. The column is 0.5 m long.

Answer

Because the relationship between elution time and distance is proportional, we can measure $t_{r,1}$, $t_{r,2}$, w_1 , and w_2 using a ruler. My measurements are 40.2 mm, 51.5 mm, 8.0 mm, and 13.5 mm, respectively. Using these values, the number of theoretical plates for each solute is

$$N_1 = 16 \frac{t_{r,1}^2}{w_1^2} = 16 \times \frac{(40.2 \text{ mm})^2}{(8.0 \text{ mm})^2} = 400 \text{ theoretical plates}$$

$$N_2 = 16 \frac{t_{r,2}^2}{w_2^2} = 16 \times \frac{(51.5 \text{ mm})^2}{(13.5 \text{ mm})^2} = 233 \text{ theoretical plates}$$

The height of a theoretical plate for each solute is

$$H_1 = \frac{L}{N_1} = \frac{0.500 \text{ m}}{400 \text{ plates}} \times \frac{1000 \text{ mm}}{\text{m}} = 1.2 \text{ mm/plate}$$

$$H_2 = \frac{L}{N_2} = \frac{0.500 \text{ m}}{233 \text{ plates}} \times \frac{1000 \text{ mm}}{\text{m}} = 2.15 \text{ mm/plate}$$

Your measurements for $t_{r,1}$, $t_{r,2}$, w_1 , and w_2 will depend on the relative size of your monitor or printout; however, your values for N and for H should be similar to the answer above.

It is important to remember that a theoretical plate is an artificial construct and that a chromatographic column does not contain physical plates. In fact, the number of theoretical plates depends on both the properties of the column and the solute. As a result, the number of theoretical plates for a column may vary from solute to solute.

Peak Capacity

One advantage of improving column efficiency is that we can separate more solutes with baseline resolution. One estimate of the number of solutes that we can separate is

$$n_c = 1 + \frac{\sqrt{N}}{4} \ln \frac{V_{\max}}{V_{\min}} \quad (9.2.16)$$

where n_c is the column's **peak capacity**, and $V_{\min}$ and $V_{\max}$ are the smallest and the largest volumes of mobile phase in which we can elute and detect a solute [Giddings, J. C. *Unified Separation Science*, Wiley-Interscience: New York, 1991]. A column with 10 000 theoretical plates, for example, can resolve no more than

$$n_c = 1 + \frac{\sqrt{10000}}{4} \ln \frac{30 \text{ mL}}{1 \text{ mL}} = 86 \text{ solutes}$$

if $V_{\min}$ and $V_{\max}$ are 1 mL and 30 mL, respectively. This estimate provides an upper bound on the number of solutes and may help us exclude from consideration a column that does not have enough theoretical plates to separate a complex mixture. Just because a column's theoretical peak capacity is larger than the number of solutes, however, does not mean that a separation is feasible. In most situations the practical peak capacity is less than the theoretical peak capacity because the retention characteristics of some solutes are so similar that a separation is impossible. Nevertheless, columns with more theoretical plates, or with a greater range of possible elution volumes, are more likely to separate a complex mixture.

The smallest volume we can use is the column's void volume. The largest volume is determined either by our patience—the maximum analysis time we can tolerate—or by our inability to detect solutes because there is too much band broadening.

Asymmetric Peaks

Our treatment of chromatography in this section assumes that a solute elutes as a symmetrical Gaussian peak, such as that shown in Figure 12.2.4 . This ideal behavior occurs when the solute's partition coefficient, K_D

$$K_D = \frac{[S_s]}{[S_m]}$$

is the same for all concentrations of solute. If this is not the case, then the chromatographic peak has an asymmetric peak shape similar to those shown in Figure 12.2.9 . The chromatographic peak in Figure 12.2.9 a is an example of **peak tailing**, which occurs when some sites on the stationary phase retain the solute more strongly than other sites. Figure 12.2.9 b, which is an example of **peak fronting** most often is the result of overloading the column with sample.

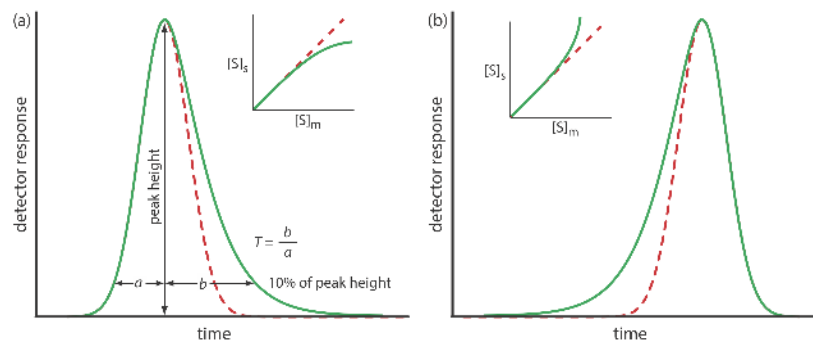


Figure 12.2.9 . Examples of asymmetric chromatographic peaks showing (a) peak tailing and (b) peak fronting. For both (a) and (b) the green chromatogram is the asymmetric peak and the red dashed chromatogram shows the ideal, Gaussian peak shape. The insets show the relationship between the concentration of solute in the stationary phase, $[S]_s$, and its concentration in the mobile phase, $[S]_m$. The dashed red lines show ideal behavior (K_D is constant for all conditions) and the green lines show nonideal behavior (K_D decreases or increases for higher total concentrations of solute). A quantitative measure of peak tailing, T , is shown in (a).

As shown in Figure 12.2.9 a, we can report a peak's asymmetry by drawing a horizontal line at 10% of the peak's maximum height and measuring the distance from each side of the peak to a line drawn vertically through the peak's maximum. The asymmetry factor, T , is defined as

$$T = \frac{b}{a}$$

The number of theoretical plates for an asymmetric peak shape is approximately

$$N \approx \frac{41.7 \times \frac{t_r^2}{(w_{0.1})^2}}{T + 1.25} = \frac{41.7 \times \frac{t_r^2}{(a+b)^2}}{T + 1.25}$$

where $w_{0.1}$ is the width at 10% of the peak's height [Foley, J. P.; Dorsey, J. G. *Anal. Chem.* **1983**, 55, 730–737].

Asymmetric peaks have fewer theoretical plates, and the more asymmetric the peak the smaller the number of theoretical plates. For example, the following table gives values for N for a solute eluting with a retention time of 10.0 min and a peak width of 1.00 min.

b	a	T	N
0.5	0.5	1.00	1850
0.6	0.4	1.50	1520
0.7	0.3	2.33	1160
0.8	0.2	4.00	790

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9.3: Optimizing Chromatographic Separations

Now that we have defined the solute retention factor, selectivity, and column efficiency we are able to consider how they affect the resolution of two closely eluting peaks. Because the two peaks have similar retention times, it is reasonable to assume that their peak widths are nearly identical. If the number of theoretical plates is the same for all solutes—not strictly true, but not a bad assumption—then from equation 12.2.15, the ratio t_r/w is a constant. If two solutes have similar retention times, then their peak widths must be similar. Equation 12.2.1, therefore, becomes

$$R_{AB} = \frac{t_{r,B} - t_{r,A}}{0.5(w_B + w_A)} \approx \frac{t_{r,B} - t_{r,A}}{0.5(2w_B)} = \frac{t_{r,B} - t_{r,A}}{w_B} \quad (9.3.1)$$

where B is the later eluting of the two solutes. Solving equation 12.2.15 for w_B and substituting into Equation 9.3.1 leaves us with the following result.

$$R_{AB} = \frac{\sqrt{N_B}}{4} \times \frac{t_{r,B} - t_{r,A}}{t_{r,B}} \quad (9.3.2)$$

Rearranging equation 12.2.8 provides us with the following equations for the retention times of solutes A and B .

$$t_{r,A} = k_A t_m + t_m \quad \text{and} \quad t_{r,B} = k_B t_m + t_m$$

After substituting these equations into Equation 9.3.2 and simplifying, we have

$$R_{AB} = \frac{\sqrt{N_B}}{4} \times \frac{k_B - k_A}{1 + k_B}$$

Finally, we can eliminate solute A 's retention factor by substituting in equation 12.2.9. After rearranging, we end up with the following equation for the resolution between the chromatographic peaks for solutes A and B .

$$R_{AB} = \frac{\sqrt{N_B}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_B}{1 + k_B} \quad (9.3.3)$$

In addition to resolution, another important factor in chromatography is the amount of time needed to elute a pair of solutes, which we can approximate using the retention time for solute B .

$$t_{r,s} = \frac{16R_{AB}^2 H}{u} \times \left(\frac{\alpha}{\alpha - 1} \right)^2 \times \frac{(1 + k_B)^3}{k_B^2} \quad (9.3.4)$$

where u is the mobile phase's velocity.

Although Equation 9.3.3 is useful for considering how a change in N , α , or k qualitatively affects resolution—which suits our purpose here—it is less useful for making accurate quantitative predictions of resolution, particularly for smaller values of N and for larger values of R . For more accurate predictions use the equation

$$R_{AB} = \frac{\sqrt{N}}{4} \times (\alpha - 1) \times \frac{k_B}{1 + k_{\text{avg}}}$$

where k_{avg} is $(k_A + k_B)/2$. For a derivation of this equation and for a deeper discussion of resolution in column chromatography, see Foley, J. P. "Resolution Equations for Column Chromatography," *Analyst*, **1991**, 116, 1275-1279.

Equation 9.3.3 and Equation 9.3.4 contain terms that correspond to column efficiency, selectivity, and the solute retention factor. We can vary these terms, more or less independently, to improve resolution and analysis time. The first term, which is a function of the number of theoretical plates (for Equation 9.3.3) or the height of a theoretical plate (for Equation 9.3.4), accounts for the effect of column efficiency. The second term is a function of α and accounts for the influence of column selectivity. Finally, the third term in both equations is a function of k_B and accounts for the effect of solute B 's retention factor. A discussion of how we can use these parameters to improve resolution is the subject of the remainder of this section.

Using the Retention Factor to Optimize Resolution

One of the simplest ways to improve resolution is to adjust the retention factor for solute B . If all other terms in Equation 9.3.3 remain constant, an increase in k_B will improve resolution. As shown by the green curve in Figure 12.3.1, however, the improvement is greatest if the initial value of k_B is small. Once k_B exceeds a value of approximately 10, a further increase produces only a marginal improvement in resolution. For example, if the original value of k_B is 1, increasing its value to 10 gives an 82% improvement in resolution; a further increase to 15 provides a net improvement in resolution of only 87.5%.

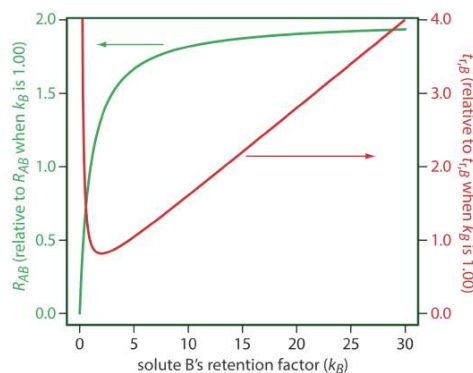


Figure 12.3.1. Effect of k_B on the resolution for a pair of solutes, R_{AB} , and the retention time for the later eluting solute, $t_{r,B}$. The y-axes display the resolution and retention time relative to their respective values when k_B is 1.00.

Any improvement in resolution from increasing the value of k_B generally comes at the cost of a longer analysis time. The red curve in Figure 12.3.1 shows the relative change in the retention time for solute B as a function of its retention factor. Note that the minimum retention time is for $k_B = 2$. Increasing k_B from 2 to 10, for example, approximately doubles solute B 's retention time.

The relationship between retention factor and analysis time in Figure 12.3.1 works to our advantage if a separation produces an acceptable resolution with a large k_B . In this case we may be able to decrease k_B with little loss in resolution and with a significantly shorter analysis time.

To increase k_B without changing selectivity, α , any change to the chromatographic conditions must result in a general, nonselective increase in the retention factor for both solutes. In gas chromatography, we can accomplish this by decreasing the column's temperature. Because a solute's vapor pressure is smaller at lower temperatures, it spends more time in the stationary phase and takes longer to elute. In liquid chromatography, the easiest way to increase a solute's retention factor is to use a mobile phase that is a weaker solvent. When the mobile phase has a lower solvent strength, solutes spend proportionally more time in the stationary phase and take longer to elute.

Adjusting the retention factor to improve the resolution between one pair of solutes may lead to unacceptably long retention times for other solutes. For example, suppose we need to analyze a four-component mixture with baseline resolution and with a run-time of less than 20 min. Our initial choice of conditions gives the chromatogram in Figure 12.3.2 a. Although we successfully separate components 3 and 4 within 15 min, we fail to separate components 1 and 2. Adjusting conditions to improve the resolution for the first two components by increasing k_2 provides a good separation of all four components, but the run-time is too long (Figure 12.3.2 b). This problem of finding a single set of acceptable operating conditions is known as the **general elution problem**.

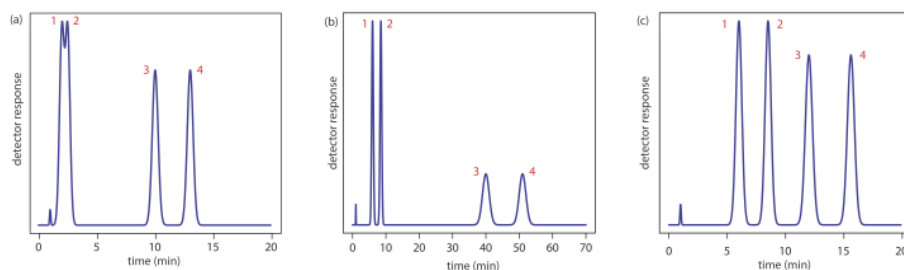


Figure 12.3.2. Example showing the general elution problem in chromatography. See text for details.

One solution to the general elution problem is to make incremental adjustments to the retention factor as the separation takes place. At the beginning of the separation we set the initial chromatographic conditions to optimize the resolution for early eluting solutes. As the separation progresses, we adjust the chromatographic conditions to decrease the retention factor—and, therefore, to decrease the retention time—for each of the later eluting solutes (Figure 12.3.2 c). In gas chromatography this is accomplished by temperature programming. The column's initial temperature is selected such that the first solutes to elute are resolved fully. The temperature is then increased, either continuously or in steps, to bring off later eluting components with both an acceptable resolution and a reasonable analysis time. In liquid chromatography the same effect is obtained by increasing the solvent's eluting strength. This is known as a gradient elution. We will have more to say about each of these in later sections of this chapter.

Using Selectivity to Optimize Resolution

A second approach to improving resolution is to adjust the selectivity, α . In fact, for $\alpha \approx 1$ usually it is not possible to improve resolution by adjusting the solute retention factor, k_B , or the column efficiency, N . A change in α often has a more dramatic effect on resolution than a change in k_B . For example, changing α from 1.1 to 1.5, while holding constant all other terms, improves resolution by 267%. In gas chromatography, we adjust α by changing the stationary phase; in liquid chromatography, we change the composition of the mobile phase to adjust α .

To change α we need to selectively adjust individual solute retention factors. Figure 12.3.3 shows one possible approach for the liquid chromatographic separation of a mixture of substituted benzoic acids. Because the retention time of a compound's weak acid form and its weak base form are different, its retention time will vary with the pH of the mobile phase, as shown in Figure 12.3.3 a. The intersections of the curves in Figure 12.3.3 a show pH values where two solutes co-elute. For example, at a pH of 3.8 terephthalic acid and *p*-hydroxybenzoic acid elute as a single chromatographic peak.

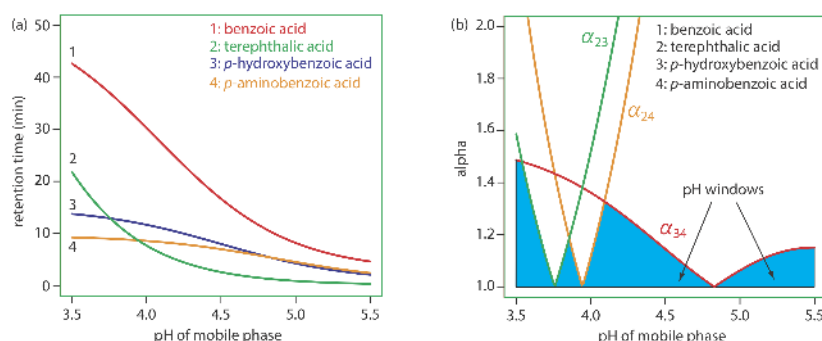


Figure 12.3.3 . Example showing how the mobile phase pH in liquid chromatography affects selectivity: (a) retention times for four substituted benzoic acids as a function of the mobile phase's pH; (b) alpha values for three pairs of solutes that are difficult to separate. See text for details. The mobile phase is an acetic acid/sodium acetate buffer and the stationary phase is a nonpolar hydrocarbon. Data from Harvey, D. T.; Byerly, S.; Bowman, A.; Tomlin, J. "Optimization of HPLC and GC Separations Using Response Surfaces," *J. Chem. Educ.* **1991**, 68, 162–168.

Figure 12.3.3 a shows that there are many pH values where some separation is possible. To find the optimum separation, we plot α for each pair of solutes. The red, green, and orange curves in Figure 12.3.3 b show the variation in α with pH for the three pairs of solutes that are hardest to separate (for all other pairs of solutes, $\alpha > 2$ at all pH levels). The blue shading shows windows of pH values in which at least a partial separation is possible—this figure is sometimes called a window diagram—and the highest point in each window gives the optimum pH within that range. The best overall separation is the highest point in any window, which, for this example, is a pH of 3.5. Because the analysis time at this pH is more than 40 min (Figure 12.3.3 a), choosing a pH between 4.1–4.4 might produce an acceptable separation with a much shorter analysis time.

Let's use benzoic acid, $\text{C}_6\text{H}_5\text{COOH}$, to explain why pH can affect a solute's retention time. The separation uses an aqueous mobile phase and a nonpolar stationary phase. At lower pHs, benzoic acid predominately is in its weak acid form, $\text{C}_6\text{H}_5\text{COOH}$, and partitions easily into the nonpolar stationary phase. At more basic pHs, however, benzoic acid is in its weak base form, $\text{C}_6\text{H}_5\text{COO}^-$. Because it now carries a charge, its solubility in the mobile phase increases and its solubility in the nonpolar stationary phase decreases. As a result, it spends more time in the mobile phase and has a shorter retention time.

Although the usual way to adjust pH is to change the concentration of buffering agents, it also is possible to adjust pH by changing the column's temperature because a solute's pK_a value is pH-dependent; for a review, see Gagliardi, L. G.; Tascon,

M.; Castells, C. B. "Effect of Temperature on Acid–Base Equilibria in Separation Techniques: A Review," *Anal. Chim. Acta*, **2015**, 889, 35–57.

Using Column Efficiency to Optimize Resolution

A third approach to improving resolution is to adjust the column's efficiency by increasing the number of theoretical plates, N . If we have values for k_B and α , then we can use Equation 9.3.3 to calculate the number of theoretical plates for any resolution. Table 12.3.1 provides some representative values. For example, if $\alpha = 1.05$ and $k_B = 2.0$, a resolution of 1.25 requires approximately 24 800 theoretical plates. If our column provides only 12 400 plates, half of what is needed, then a separation is not possible. How can we double the number of theoretical plates? The easiest way is to double the length of the column, although this also doubles the analysis time. A better approach is to cut the height of a theoretical plate, H , in half, providing the desired resolution without changing the analysis time. Even better, if we can decrease H by more than 50%, it may be possible to achieve the desired resolution with an even shorter analysis time by also decreasing k_B or α .

Table 12.3.1 . Minimum Number of Theoretical Plates to Achieve Desired Resolution for Selected Values of k_B and α

k_B	$R_{AB} = 1.00$		$R_{AB} = 1.25$		$R_{AB} = 1.50$	
	$\alpha = 1.05$	$\alpha = 1.10$	$\alpha = 1.05$	$\alpha = 1.10$	$\alpha = 1.05$	$\alpha = 1.10$
0.5	63500	17400	99200	27200	143000	39200
1.0	28200	7740	44100	12100	63500	17400
1.5	19600	5380	30600	8400	44100	12100
2.0	15900	4360	24800	6810	35700	9800
3.0	12500	3440	19600	5380	28200	7740
5.0	10200	2790	15900	4360	22900	6270
10.0	8540	2340	13300	3660	19200	5270

To decrease the height of a theoretical plate we need to understand the experimental factors that affect band broadening. There are several theoretical treatments of band broadening. We will consider one approach that considers four contributions: variations in path lengths, longitudinal diffusion, mass transfer in the stationary phase, and mass transfer in the mobile phase.

Multiple Paths: Variations in Path Length

As solute molecules pass through the column they travel paths that differ in length. Because of this difference in path length, two solute molecules that enter the column at the same time will exit the column at different times. The result, as shown in Figure 12.3.4 , is a broadening of the solute's profile on the column. The contribution of **multiple paths** to the height of a theoretical plate, H_p , is

$$H_p = 2\lambda d_p \quad (9.3.5)$$

where d_p is the average diameter of the particulate packing material and λ is a constant that accounts for the consistency of the packing. A smaller range of particle sizes and a more consistent packing produce a smaller value for λ . For a column without packing material, H_p is zero and there is no contribution to band broadening from multiple paths.

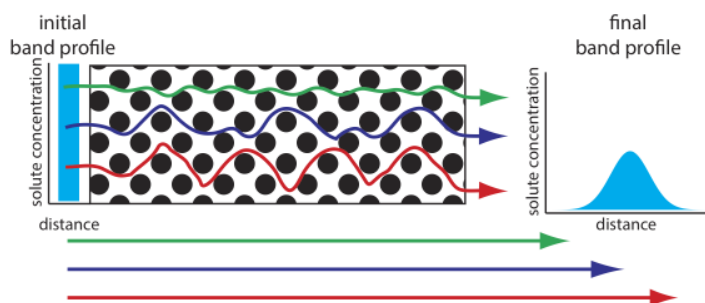


Figure 12.3.4 . The effect of multiple paths on a solute's band broadening. The solute's initial band profile is rectangular. As this band travels through the column, individual solute molecules travel different paths, three of which are shown by the meandering colored paths (the actual lengths of these paths are shown by the straight arrows at the bottom of the figure). Most solute molecules travel paths with lengths similar to that shown in blue, with a few traveling much shorter paths (green) or much longer paths (red). As a result, the solute's band profile at the end of the column is broader and Gaussian in shape.

An inconsistent packing creates channels that allow some solute molecules to travel quickly through the column. It also can create pockets that temporarily trap some solute molecules, slowing their progress through the column. A more uniform packing minimizes these problems.

Longitudinal Diffusion

The second contribution to band broadening is the result of the solute's **longitudinal diffusion** in the mobile phase. Solute molecules are in constant motion, diffusing from regions of higher solute concentration to regions where the concentration of solute is smaller. The result is an increase in the solute's band width (Figure 12.3.5). The contribution of longitudinal diffusion to the height of a theoretical plate, H_d , is

$$H_d = \frac{2\gamma D_m}{u} \quad (9.3.6)$$

where D_m is the solute's diffusion coefficient in the mobile phase, u is the mobile phase's velocity, and γ is a constant related to the efficiency of column packing. Note that the effect of H_d on band broadening is inversely proportional to the mobile phase velocity: a higher velocity provides less time for longitudinal diffusion. Because a solute's diffusion coefficient is larger in the gas phase than in a liquid phase, longitudinal diffusion is a more serious problem in gas chromatography.

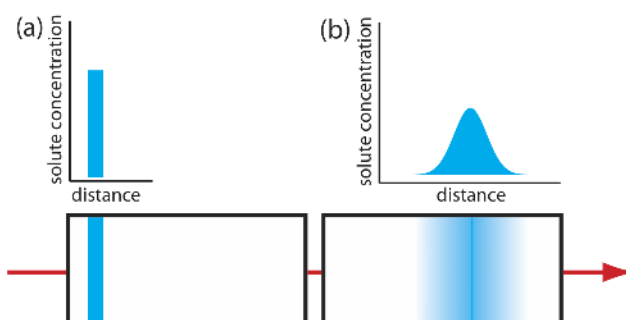


Figure 12.3.5 . The effect of longitudinal diffusion on a solute's band broadening. Two horizontal cross-sections through the column and the corresponding concentration versus distance profiles are shown, with (a) being earlier in time. The red arrow shows the direction in which the mobile phase is moving.

Mass Transfer

As the solute passes through the column it moves between the mobile phase and the stationary phase. We call this movement between phases **mass transfer**. As shown in Figure 12.3.6 , band broadening occurs if the solute's movement within the mobile phase or within the stationary phase is not fast enough to maintain an equilibrium in its concentration between the two phases. On average, a solute molecule in the mobile phase moves down the column before it passes into the stationary phase. A solute molecule in the stationary phase, on the other hand, takes longer than expected to move back into the mobile phase. The contributions of mass transfer in the stationary phase, H_s , and mass transfer in the mobile phase, H_m , are given by the following equations

$$H_s = \frac{qkd_f^2}{(1+k)^2 D_s} u \quad (9.3.7)$$

$$H_m = \frac{fn(d_p^2, d_c^2)}{D_m} u \quad (9.3.8)$$

where d_f is the thickness of the stationary phase, d_c is the diameter of the column, D_s and D_m are the diffusion coefficients for the solute in the stationary phase and the mobile phase, k is the solute's retention factor, and q is a constant related to the column packing material. Although the exact form of H_m is not known, it is a function of particle size and column diameter. Note that the effect of H_s and H_m on band broadening is directly proportional to the mobile phase velocity because a smaller velocity provides more time for mass transfer.

The abbreviation fn in Equation 9.3.7 means “is a function of.”

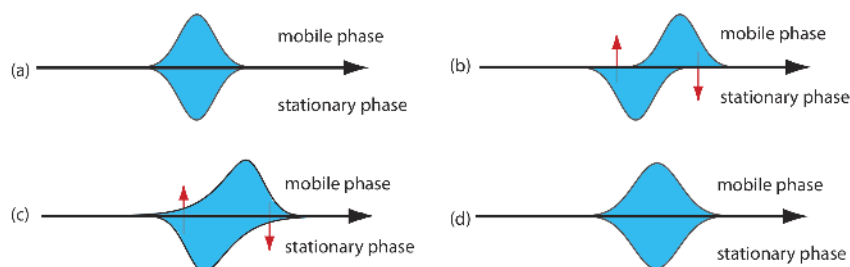


Figure 12.3.6 . Effect of mass transfer on band broadening: (a) Ideal equilibrium Gaussian profiles for the solute in the mobile phase and in the stationary phase. (b, c) If we allow the solute's band to move a small distance down the column, an equilibrium between the two phases no longer exists. The red arrows show the movement of solute—what we call the mass transfer of solute—from the stationary phase to the mobile phase, and from the mobile phase to the stationary phase. (d) Once equilibrium is reestablished, the solute's band is now broader.

Putting It All Together

The height of a theoretical plate is a summation of the contributions from each of the terms affecting band broadening.

$$H = H_p + H_d + H_s + H_m \quad (9.3.9)$$

An alternative form of this equation is the **van Deemter equation**

$$H = A + \frac{B}{u} + Cu \quad (9.3.10)$$

which emphasizes the importance of the mobile phase's velocity. In the van Deemter equation, A accounts for the contribution of multiple paths (H_p), B/u accounts for the contribution of longitudinal diffusion (H_d), and Cu accounts for the combined contribution of mass transfer in the stationary phase and in the mobile phase (H_s and H_m).

There is some disagreement on the best equation for describing the relationship between plate height and mobile phase velocity [Hawkes, S. J. *J. Chem. Educ.* **1983**, 60, 393–398]. In addition to the van Deemter equation, other equations include

$$H = \frac{B}{u} + (C_s + C_m) u$$

where C_s and C_m are the mass transfer terms for the stationary phase and the mobile phase and

$$H = Au^{1/3} + \frac{B}{u} + Cu$$

All three equations, and others, have been used to characterize chromatographic systems, with no single equation providing the best explanation in every case [Kennedy, R. T.; Jorgenson, J. W. *Anal. Chem.* **1989**, 61, 1128–1135].

To increase the number of theoretical plates without increasing the length of the column, we need to decrease one or more of the terms in Equation 9.3.9. The easiest way to decrease H is to adjust the velocity of the mobile phase. For smaller mobile phase velocities, column efficiency is limited by longitudinal diffusion, and for higher mobile phase velocities efficiency is limited by the

two mass transfer terms. As shown in Figure 12.3.7—which uses the van Deemter equation—the optimum mobile phase velocity is the minimum in a plot of H as a function of u .

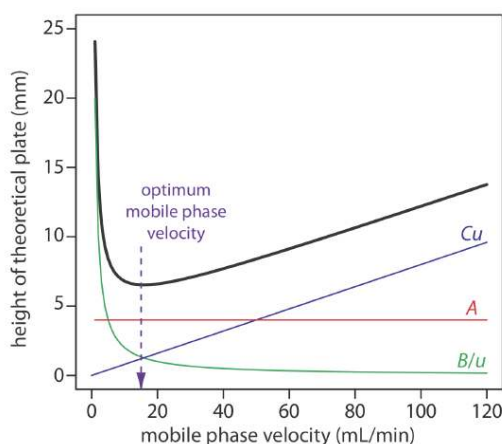


Figure 12.3.7 . Plot showing the relationship between the height of a theoretical plate, H , and the mobile phase's velocity, u , based on the van Deemter equation.

The remaining parameters that affect the terms in Equation 9.3.9 are functions of the column's properties and suggest other possible approaches to improving column efficiency. For example, both H_p and H_m are a function of the size of the particles used to pack the column. Decreasing particle size, therefore, is another useful method for improving efficiency.

For a more detailed discussion of ways to assess the quality of a column, see Desmet, G.; Caooter, D.; Broeckhaven, K. "Graphical Data Representation Methods to Assess the Quality of LC Columns," *Anal. Chem.* **2015**, 87, 8593–8602.

Perhaps the most important advancement in chromatography columns is the development of open-tubular, or **capillary columns**. These columns have very small diameters ($d_c \approx 50\text{--}500\ \mu\text{m}$) and contain no packing material ($d_p = 0$). Instead, the capillary column's interior wall is coated with a thin film of the stationary phase. Plate height is reduced because the contribution to H from H_p (Equation 9.3.5) disappears and the contribution from H_m (Equation 9.3.8) becomes smaller. Because the column does not contain any solid packing material, it takes less pressure to move the mobile phase through the column, which allows for longer columns. The combination of a longer column and a smaller height for a theoretical plate increases the number of theoretical plates by approximately $100\times$. Capillary columns are not without disadvantages. Because they are much narrower than packed columns, they require a significantly smaller amount of sample, which may be difficult to inject reproducibly. Another approach to improving resolution is to use thin films of stationary phase, which decreases the contribution to H from H_s (Equation 9.3.7).

The smaller the particles, the more pressure is needed to push the mobile phase through the column. As a result, for any form of chromatography there is a practical limit to particle size.

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9.4: High-Performance Liquid Chromatography

In **high-performance liquid chromatography** (HPLC) we inject the sample, which is in solution form, into a liquid mobile phase. The mobile phase carries the sample through a packed or capillary column that separates the sample's components based on their ability to partition between the mobile phase and the stationary phase. Figure 12.5.1 shows an example of a typical HPLC instrument, which has several key components: reservoirs that store the mobile phase; a pump for pushing the mobile phase through the system; an injector for introducing the sample; a column for separating the sample into its component parts; and a detector for monitoring the eluent as it comes off the column. Let's consider each of these components.

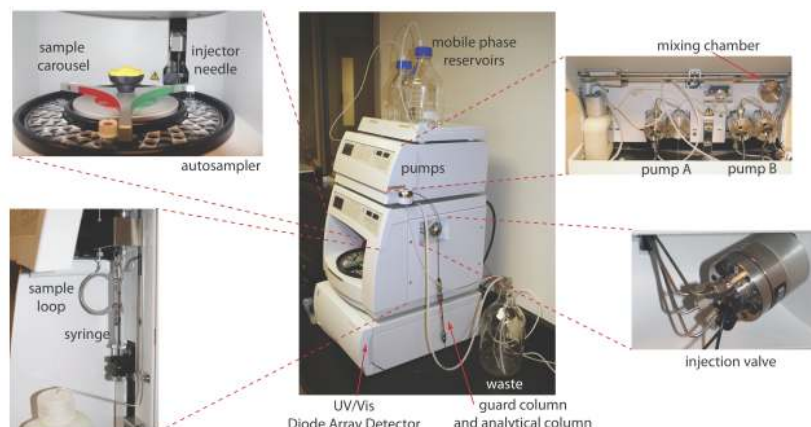


Figure 12.5.1 . Example of a typical high-performance liquid chromatograph with insets showing the pumps that move the mobile phase through the system and the plumbing used to inject the sample into the mobile phase. This particular instrument includes an autosampler. An instrument in which samples are injected manually does not include the features shown in the two left-most insets, and has a different style of loop injection valve.

A solute's retention time in HPLC is determined by its interaction with the stationary phase and the mobile phase. There are several different types of solute/stationary phase interactions, including liquid–solid adsorption, liquid–liquid partitioning, ion-exchange, and size-exclusion. This chapter deals exclusively with HPLC separations based on liquid–liquid partitioning. Other forms of liquid chromatography receive consideration in [Chapter 12.6](#).

HPLC Columns

An HPLC typically includes two columns: an analytical column, which is responsible for the separation, and a guard column that is placed before the analytical column to protect it from contamination.

Analytical Columns

The most common type of HPLC column is a stainless steel tube with an internal diameter between 2.1 mm and 4.6 mm and a length between 30 mm and 300 mm (Figure 12.5.2). The column is packed with 3–10 μm porous silica particles with either an irregular or a spherical shape. Typical column efficiencies are 40000–60000 theoretical plates/m. Assuming a $V_{\text{max}}/V_{\text{min}}$ of approximately 50, a 25-cm column with 50 000 plates/m has 12 500 theoretical plates and a peak capacity of 110.



Figure 12.5.2 . Typical packed column for HPLC. This particular column has an internal diameter of 4.6 mm and a length of 150 mm, and is packed with 5 μm particles coated with stationary phase.

Capillary columns use less solvent and, because the sample is diluted to a lesser extent, produce larger signals at the detector. These columns are made from fused silica capillaries with internal diameters from 44–200 μm and lengths of 50–250 mm. Capillary columns packed with 3–5 μm particles have been prepared with column efficiencies of up to 250 000 theoretical plates [Novotony, *M. Science*, **1989**, 246, 51–57].

One limitation to a packed capillary column is the back pressure that develops when pumping the mobile phase through the small interstitial spaces between the particulate micron-sized packing material (Figure 12.5.3). Because the tubing and fittings that carry the mobile phase have pressure limits, a higher back pressure requires a lower flow rate and a longer analysis time. Monolithic columns, in which the solid support is a single, porous rod, offer column efficiencies equivalent to a packed capillary column while allowing for faster flow rates. A monolithic column—which usually is similar in size to a conventional packed column, although smaller, capillary columns also are available—is prepared by forming the monolithic rod in a mold and covering it with PTFE tubing or a polymer resin. Monolithic rods made of a silica-gel polymer typically have macropores with diameters of approximately 2 μm and mesopores—pores within the macropores—with diameters of approximately 13 nm [Cabrera, K. Chromatography Online, April 1, 2008].

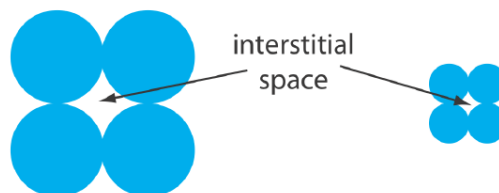


Figure 12.5.3 . The packing of smaller particles creates smaller interstitial spaces than the packing of larger particles. Although reducing particle size by 2 $\times$ increases efficiency by a factor of 1.4, it also produces a 4-fold increase in back pressure.

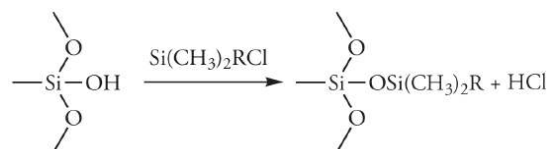
Guard Columns

Two problems tend to shorten the lifetime of an analytical column. First, solutes that bind irreversibly to the stationary phase degrade the column's performance by decreasing the amount of stationary phase available for effecting a separation. Second, particulate material injected with the sample may clog the analytical column. To minimize these problems we place a guard column before the analytical column. A Guard column usually contains the same particulate packing material and stationary phase as the analytical column, but is significantly shorter and less expensive—a length of 7.5 mm and a cost one-tenth of that for the corresponding analytical column is typical. Because they are intended to be sacrificial, guard columns are replaced regularly.

If you look closely at Figure 12.5.1 , you will see the small guard column just above the analytical column.

Stationary Phases for Liquid-Liquid Chromatography

In liquid-liquid chromatography the stationary phase is a liquid film coated on a packing material, typically 3–10 μm porous silica particles. Because the stationary phase may be partially soluble in the mobile phase, it may elute, or bleed from the column over time. To prevent the loss of stationary phase, which shortens the column's lifetime, it is bound covalently to the silica particles. **Bonded stationary phases** are created by reacting the silica particles with an organochlorosilane of the general form $\text{Si}(\text{CH}_3)_2\text{RCl}$, where R is an alkyl or substituted alkyl group.



To prevent unwanted interactions between the solutes and any remaining —SiOH groups, $\text{Si}(\text{CH}_3)_3\text{Cl}$ is used to convert unreacted sites to $\text{—SiOSi}(\text{CH}_3)_3$; such columns are designated as end-capped.

The properties of a stationary phase depend on the organosilane's alkyl group. If R is a polar functional group, then the stationary phase is polar. Examples of polar stationary phases include those where R contains a cyano ($\text{—C}_2\text{H}_4\text{CN}$), a diol ($\text{—C}_3\text{H}_6\text{OCH}_2\text{CHOHCH}_2\text{OH}$), or an amino ($\text{—C}_3\text{H}_6\text{NH}_2$) functional group. Because the stationary phase is polar, the mobile phase is a nonpolar or a moderately polar solvent. The combination of a polar stationary phase and a nonpolar mobile phase is called **normal-phase chromatography**.

In **reversed-phase chromatography**, which is the more common form of HPLC, the stationary phase is nonpolar and the mobile phase is polar. The most common nonpolar stationary phases use an organochlorosilane where the R group is an *n*-octyl (C_8) or *n*-octyldecyl (C_{18}) hydrocarbon chain. Most reversed-phase separations are carried out using a buffered aqueous solution as a polar

mobile phase, or using other polar solvents, such as methanol and acetonitrile. Because the silica substrate may undergo hydrolysis in basic solutions, the pH of the mobile phase must be less than 7.5.

It seems odd that the more common form of liquid chromatography is identified as reverse-phase instead of normal phase. You might recall that one of the earliest examples of chromatography was Mikhail Tswett's separation of plant pigments using a polar column of calcium carbonate and a nonpolar mobile phase of petroleum ether. The assignment of normal and reversed, therefore, is all about precedence.

Mobile Phases

The elution order of solutes in HPLC is governed by polarity. For a normal-phase separation, a solute of lower polarity spends proportionally less time in the polar stationary phase and elutes before a solute that is more polar. Given a particular stationary phase, retention times in normal-phase HPLC are controlled by adjusting the mobile phase's properties. For example, if the resolution between two solutes is poor, switching to a less polar mobile phase keeps the solutes on the column for a longer time and provides more opportunity for their separation. In reversed-phase HPLC the order of elution is the opposite that in a normal-phase separation, with more polar solutes eluting first. Increasing the polarity of the mobile phase leads to longer retention times. Shorter retention times require a mobile phase of lower polarity.

Choosing a Mobile Phase: Using the Polarity Index

There are several indices that help in selecting a mobile phase, one of which is the polarity index [Snyder, L. R.; Glajch, J. L.; Kirkland, J. J. *Practical HPLC Method Development*, Wiley-Interscience: New York, 1988]. Table 12.5.1 provides values of the polarity index, P' , for several common mobile phases, where larger values of P' correspond to more polar solvents. Mixing together two or more mobile phases—assuming they are miscible—creates a mobile phase of intermediate polarity. For example, a binary mobile phase made by combining solvent A and solvent B has a polarity index, P'_{AB} , of

$$P'_{AB} = \Phi_A P'_A + \Phi_B P'_B \quad (9.4.1)$$

where P'_A and P'_B are the polarity indices for solvents A and B, and Φ_A and Φ_B are the volume fractions for the two solvents.

Table 12.5.1 . Properties of HPLC Mobile Phases

mobile phase	polarity index (P')	UV cutoff (nm)
cyclohexane	0.04	210
<i>n</i> -hexane	0.1	210
carbon tetrachloride	1.6	265
<i>i</i> -propyl ether	2.4	220
toluene	2.4	286
diethyl ether	2.8	218
tetrahydrofuran	4.0	220
ethanol	4.3	210
ethyl acetate	4.4	255
dioxane	4.8	215
methanol	5.1	210
acetonitrile	5.8	190
water	10.2	—

✓ Example 12.5.1

A reversed-phase HPLC separation is carried out using a mobile phase of 60% v/v water and 40% v/v methanol. What is the mobile phase's polarity index?

Solution

Using Equation 9.4.1 and the values in Table 12.5.1, the polarity index for a 60:40 water–methanol mixture is

$$P'_{AB} = \Phi_{\text{water}}P'_{\text{water}} + \Phi_{\text{methanol}}P'_{\text{methanol}}$$

$$P'_{AB} = 0.60 \times 10.2 + 0.40 \times 5.1 = 8.2$$

? Exercise 12.5.1

Suppose you need a mobile phase with a polarity index of 7.5. Explain how you can prepare this mobile phase using methanol and water.

Answer

If we let x be the fraction of water in the mobile phase, then $1 - x$ is the fraction of methanol. Substituting these values into Equation 9.4.1 and solving for x

$$7.5 = 10.2x + 5.1(1 - x)$$

$$7.5 = 10.2x + 5.1 - 5.1x$$

$$2.4 = 5.1x$$

gives x as 0.47. The mobile phase is 47% v/v water and 53% v/v methanol.

As a general rule, a two unit change in the polarity index corresponds to an approximately 10-fold change in a solute's retention factor. Here is a simple example. If a solute's retention factor, k , is 22 when using water as a mobile phase ($P' = 10.2$), then switching to a mobile phase of 60:40 water–methanol ($P' = 8.2$) decreases k to approximately 2.2. Note that the retention factor becomes smaller because we are switching from a more polar mobile phase to a less polar mobile phase in a reversed-phase separation.

Choosing a Mobile Phase: Adjusting Selectivity

Changing the mobile phase's polarity index changes a solute's retention factor. As we learned in Chapter 12.3, however, a change in k is not an effective way to improve resolution when the initial value of k is greater than 10. To effect a better separation between two solutes we must improve the selectivity factor, α . There are two common methods for increasing α : adding a reagent to the mobile phase that reacts with the solutes in a secondary equilibrium reaction or switching to a different mobile phase.

Taking advantage of a secondary equilibrium reaction is a useful strategy for improving a separation [(a) Foley, J. P. *Chromatography*, **1987**, 7, 118–128; (b) Foley, J. P.; May, W. E. *Anal. Chem.* 1987, 59, 102–109; (c) Foley, J. P.; May, W. E. *Anal. Chem.* 1987, 59, 110–115]. Figure 12.3.3, which we considered earlier in this chapter, shows the reversed-phase separation of four weak acids—benzoic acid, terephthalic acid, *p*-aminobenzoic acid, and *p*-hydroxybenzoic acid—on a nonpolar C_{18} column using an aqueous buffer of acetic acid and sodium acetate as the mobile phase. The retention times for these weak acids are shorter when using a less acidic mobile phase because each solute is present in an anionic, weak base form that is less soluble in the nonpolar stationary phase. If the mobile phase's pH is sufficiently acidic, the solutes are present as neutral weak acids that are more soluble in the stationary phase and take longer to elute. Because the weak acid solutes do not have identical pK_a values, the pH of the mobile phase has a different effect on each solute's retention time, allowing us to find the optimum pH for effecting a complete separation of the four solutes.

Acid–base chemistry is not the only example of a secondary equilibrium reaction. Other examples include ion-pairing, complexation, and the interaction of solutes with micelles. We will consider the last of these in Chapter 12.7 when we discuss micellar electrokinetic capillary chromatography.

In [Example 12.5.1](#) we learned how to adjust the mobile phase's polarity by blending together two solvents. A polarity index, however, is just a guide, and binary mobile phase mixtures with identical polarity indices may not resolve equally a pair of solutes. Table 12.5.2, for example, shows retention times for four weak acids in two mobile phases with nearly identical values for P' . Although the order of elution is the same for both mobile phases, each solute's retention time is affected differently by the choice of organic solvent. If we switch from using acetonitrile to tetrahydrofuran, for example, we find that benzoic acid elutes more quickly and that *p*-hydroxybenzoic acid elutes more slowly. Although we can resolve fully these two solutes using mobile phase that is 16% v/v acetonitrile, we cannot resolve them if the mobile phase is 10% tetrahydrofuran.

Table 12.5.2 . Retention Times for Four Weak Acids in Mobile Phases With Similar Polarity Indexes

retention time (min)	16% acetonitrile (CH ₃ CN) 84% pH 4.11 aqueous buffer ($P' = 9.5$)	10% tetrahydrofuran (THF) 90% pH 4.11 aqueous buffer ($P' = 9.6$)
$t_{r,BA}$	5.18	4.01
$t_{r,PH}$	1.67	2.91
$t_{r,PA}$	1.21	1.05
$t_{r,TP}$	0.23	0.54

Key: BA is benzoic acid; PH is *p*-hydroxybenzoic acid; PA is *p*-aminobenzoic acid; TP is terephthalic acid

Source: Harvey, D. T.; Byerly, S.; Bowman, A.; Tomlin, J. "Optimization of HPLC and GC Separations Using Response Surfaces," *J. Chem. Educ.* **1991**, 68, 162–168.

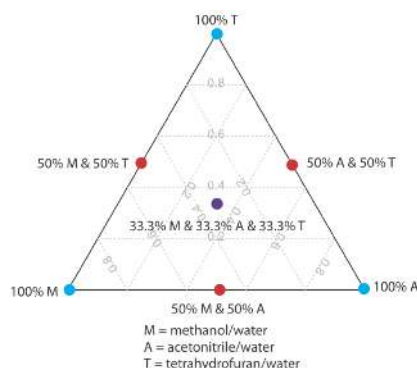


Figure 12.5.4 . Solvent triangle for optimizing a reversed-phase HPLC separation. The three blue circles show mobile phases consisting of an organic solvent and water. The three red circles are binary mobile phases created by combining equal volumes of the pure mobile phases. The ternary mobile phase shown by the purple circle contains all three of the pure mobile phases.

One strategy for finding the best mobile phase is to use the solvent triangle shown in Figure 12.5.4, which allows us to explore a broad range of mobile phases with only seven experiments. We begin by adjusting the amount of acetonitrile in the mobile phase to produce the best possible separation within the desired analysis time. Next, we use Table 12.5.3 to estimate the composition of methanol/H₂O and tetrahydrofuran/H₂O mobile phases that will produce similar analysis times. Four additional mobile phases are prepared using the binary and ternary mobile phases shown in Figure 12.5.4. When we examine the chromatograms from these seven mobile phases we may find that one or more provides an adequate separation, or we may identify a region within the solvent triangle where a separation is feasible. Figure 12.5.5 shows a resolution map for the reversed-phase separation of benzoic acid, terephthalic acid, *p*-aminobenzoic acid, and *p*-hydroxybenzoic acid on a nonpolar C₁₈ column in which the maximum desired analysis time is set to 6 min [Harvey, D. T.; Byerly, S.; Bowman, A.; Tomlin, J. *J. Chem. Educ.* **1991**, 68, 162–168]. The areas in blue, green, and red show mobile phase compositions that do not provide baseline resolution. The unshaded area represents mobile phase compositions where a separation is possible.

The choice to start with acetonitrile is arbitrary—we can just as easily choose to begin with methanol or with tetrahydrofuran.

Table 12.5.3 . Composition of Mobile Phases With Approximately Equal Solvent Strengths

%v/v CH ₃ OH	% v/v CH ₃ CN	%v/v THF

%v/v CH ₃ OH	% v/v CH ₃ CN	%v/v THF
0	0	0
10	6	4
20	14	10
30	22	16
40	32	24
50	40	30
6	50	36
70	60	44
80	72	52
90	87	62
100	99	71

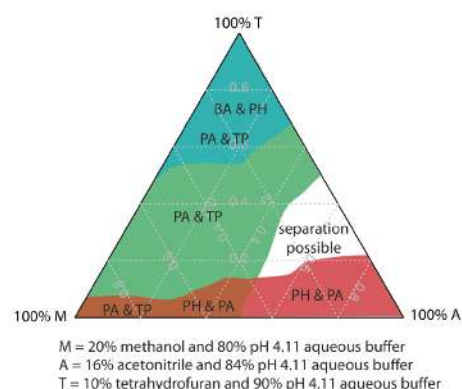


Figure 12.5.5 . Resolution map for the separation of benzoic acid (BA), terephthalic acid (TP), *p*-aminobenzoic acid (PA), and *p*-hydroxybenzoic acid (PH) on a nonpolar C₁₈ column subject to a maximum analysis time of 6 min. The shaded areas represent regions where a separation is not possible, with the unresolved solutes identified. A separation is possible in the unshaded area. See Harvey, D. T.; Byerly, S.; Bowman, A.; Tomlin, J. "Optimization of HPLC and GC Separations Using Response Surfaces," *J. Chem. Educ.* **1991**, 68, 162–168 for details on the mathematical model used to generate the resolution map.

Choosing a Mobile Phase: Isocratic and Gradient Elutions

A separation using a mobile phase that has a fixed composition is an **isocratic elution**. One difficulty with an isocratic elution is that an appropriate mobile phase strength for resolving early-eluting solutes may lead to unacceptably long retention times for late-eluting solutes. Optimizing the mobile phase for late-eluting solutes, on the other hand, may provide an inadequate separation of early-eluting solutes. Changing the mobile phase's composition as the separation progresses is one solution to this problem. For a reversed-phase separation we use an initial mobile phase that is more polar. As the separation progresses, we adjust the composition of mobile phase so that it becomes less polar (see Figure 12.5.6). Such separations are called **gradient elutions**.

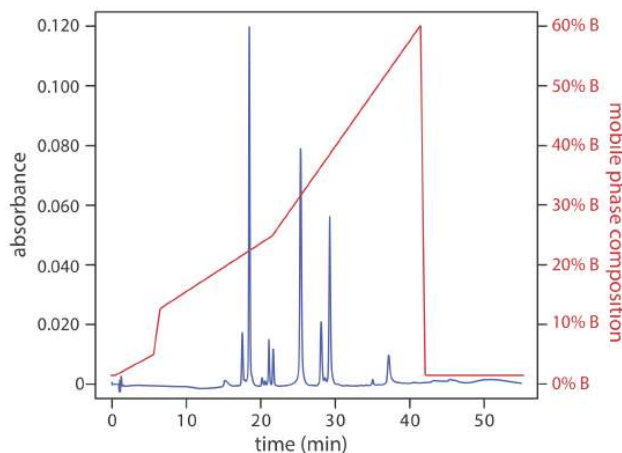


Figure 12.5.6 . Gradient elution separation of a mixture of flavonoids. Mobile phase A is an aqueous solution of 0.1% formic acid and mobile phase B is 0.1% formic acid in acetonitrile. The initial mobile phase is 98% A and 2% B. The percentage of mobile phase B increases in four steps: from 2% to 5% over 5 min, beginning at 0.5 min; from 5% to 12% over 1 min, beginning at 5.5 min; from 12% to 25% over 15 min, beginning at 6.5 min; and from 25% to 60% over 20 min, beginning at 21.5 min. Data provided by Christopher Schardon, Kyle Meinhardt, and Michelle Bushey, Department of Chemistry, Trinity University.

HPLC Plumbing

In a gas chromatograph the pressure from a compressed gas cylinder is sufficient to push the mobile phase through the column. Pushing a liquid mobile phase through a column, however, takes a great deal more effort, generating pressures in excess of several hundred atmospheres. In this section we consider the basic plumbing needed to move the mobile phase through the column and to inject the sample into the mobile phase.

Moving the Mobile Phase

A typical HPLC includes between 1–4 reservoirs for storing mobile phase solvents. The instrument in [Figure 12.5.1](#) , for example, has two mobile phase reservoirs that are used for an isocratic elution or a gradient elution by drawing solvents from one or both reservoirs.

Before using a mobile phase solvent we must remove dissolved gases, such as N_2 and O_2 , and small particulate matter, such as dust. Because there is a large drop in pressure across the column—the pressure at the column’s entrance is as much as several hundred atmospheres, but it is atmospheric pressure at the column’s exit—gases dissolved in the mobile phase are released as gas bubbles that may interfere with the detector’s response. Degassing is accomplished in several ways, but the most common are the use of a vacuum pump or sparging with an inert gas, such as He, which has a low solubility in the mobile phase. Particulate materials, which may clog the HPLC tubing or column, are removed by filtering the solvents.

Bubbling an inert gas through the mobile phase releases volatile dissolved gases. This process is called sparging.

The mobile phase solvents are pulled from their reservoirs by the action of one or more pumps. [Figure 12.5.7](#) shows a close-up view of the pumps for the instrument in [Figure 12.5.1](#) . The working pump and the equilibrating pump each have a piston whose back and forth movement maintains a constant flow rate of up to several mL/min and provides the high output pressure needed to push the mobile phase through the chromatographic column. In this particular instrument, each pump sends its mobile phase to a mixing chamber where they combine to form the final mobile phase. The relative speed of the two pumps determines the mobile phase’s final composition.

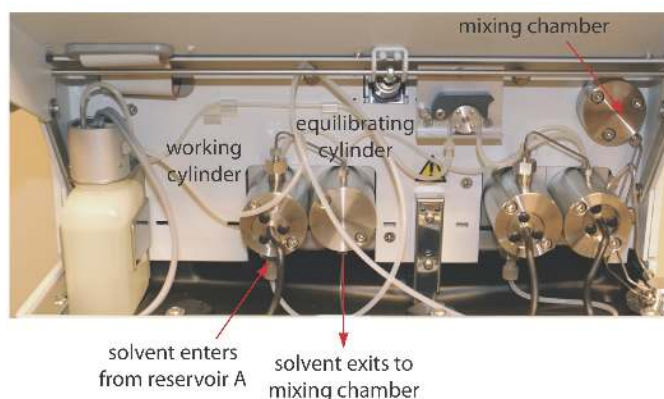


Figure 12.5.7 . Close-up view of the pumps for the instrument shown in [Figure 12.5.1](#) . The working cylinder and the equilibrating cylinder for the pump on the left take solvent from reservoir A and send it to the mixing chamber. The pump on the right moves solvent from reservoir B to the mixing chamber. The mobile phase's flow rate is determined by the combined speeds of the two pumps. By changing the relative speeds of the two pumps, different binary mobile phases can be prepared.

The back and forth movement of a reciprocating pump creates a pulsed flow that contributes noise to the chromatogram. To minimize these pulses, each pump in [Figure 12.5.7](#) has two cylinders. During the working cylinder's forward stroke it fills the equilibrating cylinder and establishes flow through the column. When the working cylinder is on its reverse stroke, the flow is maintained by the piston in the equilibrating cylinder. The result is a pulse-free flow.

There are other possible ways to control the mobile phase's composition and flow rate. For example, instead of the two pumps in [Figure 12.5.7](#) , we can place a solvent proportioning valve before a single pump. The solvent proportioning valve connects two or more solvent reservoirs to the pump and determines how much of each solvent is pulled during each of the pump's cycles. Another approach for eliminating a pulsed flow is to include a pulse damper between the pump and the column. A pulse damper is a chamber filled with an easily compressed fluid and a flexible diaphragm. During the piston's forward stroke the fluid in the pulse damper is compressed. When the piston withdraws to refill the pump, pressure from the expanding fluid in the pulse damper maintains the flow rate.

Injecting the Sample

The operating pressure within an HPLC is sufficiently high that we cannot inject the sample into the mobile phase by inserting a syringe through a septum, as is possible in gas chromatography. Instead, we inject the sample using a **loop injector**, a diagram of which is shown in [Figure 12.5.8](#) . In the load position a sample loop—which is available in a variety of sizes ranging from 0.5 μL to 5 mL—is isolated from the mobile phase and open to the atmosphere. The sample loop is filled using a syringe with a capacity several times that of the sample loop, with excess sample exiting through the waste line. After loading the sample, the injector is turned to the inject position, which redirects the mobile phase through the sample loop and onto the column.

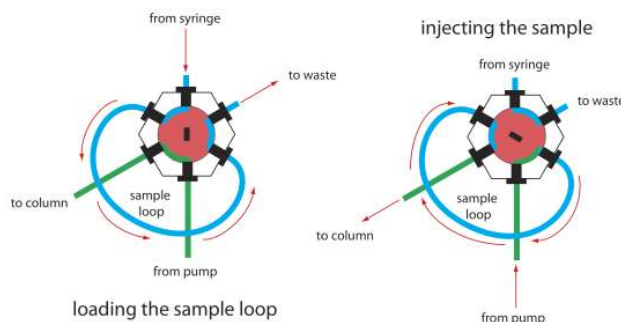


Figure 12.5.8 . Schematic diagram of a manual loop injector. In the load position the flow of mobile phase from the pump to the column (shown in green) is isolated from the sample loop, which is filled using a syringe (shown in blue). Rotating the inner valve (shown in red) to the inject position directs the mobile phase through the sample loop and onto the column.

The instrument in [Figure 12.5.1](#) uses an autosampler to inject samples. Instead of using a syringe to push the sample into the sample loop, the syringe draws sample into the sample loop.

Detectors for HPLC

Many different types of detectors have been used to monitor HPLC separations, most of which use the spectroscopic techniques from [Chapter 10](#) or the electrochemical techniques from [Chapter 11](#).

Spectroscopic Detectors

The most popular HPLC detectors take advantage of an analyte's UV/Vis absorption spectrum. These detectors range from simple designs, in which the analytical wavelength is selected using appropriate filters, to a modified spectrophotometer in which the sample compartment includes a flow cell. Figure 12.5.9 shows the design of a typical flow cell when using a diode array spectrometer as the detector. The flow cell has a volume of 1–10 μL and a path length of 0.2–1 cm.

To review the details of how we measure absorbance, see [Chapter 10.2](#). More information about different types of instruments, including the diode array spectrometer, is in [Chapter 10.3](#).

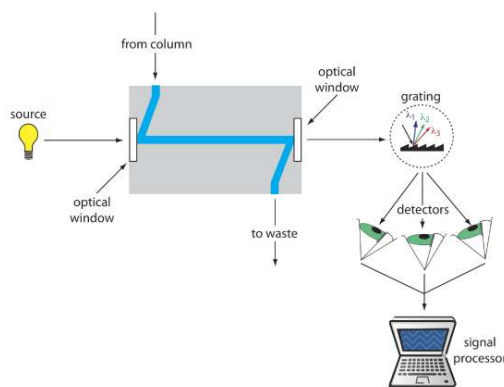


Figure 12.5.9 . Schematic diagram of a flow cell for a detector equipped with a diode array spectrometer.

When using a UV/Vis detector the resulting chromatogram is a plot of absorbance as a function of elution time (see Figure 12.5.10). If the detector is a diode array spectrometer, then we also can display the result as a three-dimensional chromatogram that shows absorbance as a function of wavelength and elution time. One limitation to using absorbance is that the mobile phase cannot absorb at the wavelengths we wish to monitor. [Table 12.5.1](#) lists the minimum useful UV wavelength for several common HPLC solvents. Absorbance detectors provide detection limits of as little as 100 pg–1 ng of injected analyte.

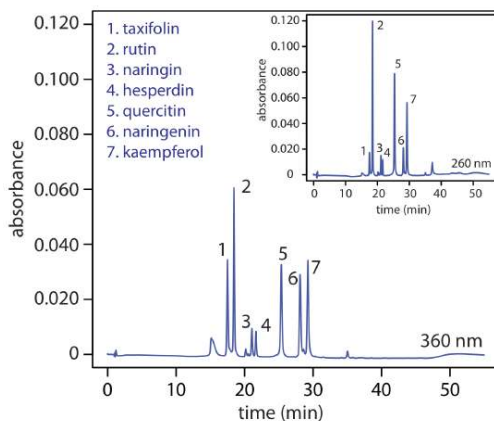


Figure 12.5.10 . HPLC separation of a mixture of flavonoids with UV/Vis detection at 360 nm and, in the inset, at 260 nm. The choice of wavelength affects each analyte's signal. By carefully choosing the wavelength, we can enhance the signal for the analytes of greatest interest. Data provided by Christopher Schardon, Kyle Meinhardt, and Michelle Bushey, Department of Chemistry, Trinity University.

If an analyte is fluorescent, we can place the flow cell in a spectrofluorimeter. As shown in Figure 12.5.11 , a fluorescence detector provides additional selectivity because only a few of a sample's components are fluorescent. Detection limits are as little as 1–10 pg of injected analyte.

See [Chapter 10.6](#) for a review of fluorescence spectroscopy and spectrofluorimeters.

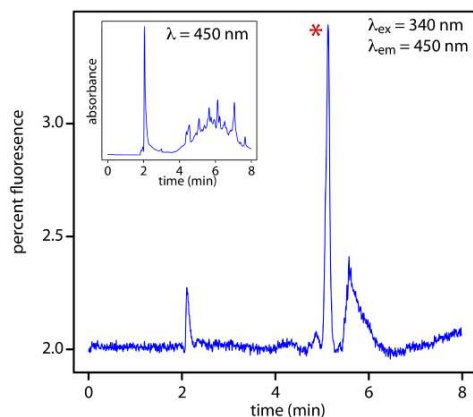


Figure 12.5.11 . HPLC chromatogram for the determination of riboflavin in urine using fluorescence detection with excitation at a wavelength of 340 nm and detection at 450 nm. The peak corresponding to riboflavin is marked with a red asterisk (*). The inset shows the same chromatogram when using a less-selective UV/Vis detector at a wavelength of 450 nm. Data provided by Jason Schultz, Jonna Berry, Kaelene Lundstrom, and Dwight Stoll, Department of Chemistry, Gustavus Adolphus College.

Electrochemical Detectors

Another common group of HPLC detectors are those based on electrochemical measurements such as amperometry, voltammetry, coulometry, and conductivity. Figure 12.5.12, for example, shows an amperometric flow cell. Effluent from the column passes over the working electrode—held at a constant potential relative to a downstream reference electrode—that completely oxidizes or reduces the analytes. The current flowing between the working electrode and the auxiliary electrode serves as the analytical signal. Detection limits for amperometric electrochemical detection are from 10 pg–1 ng of injected analyte.

See [Chapter 11.4](#) for a review of amperometry.

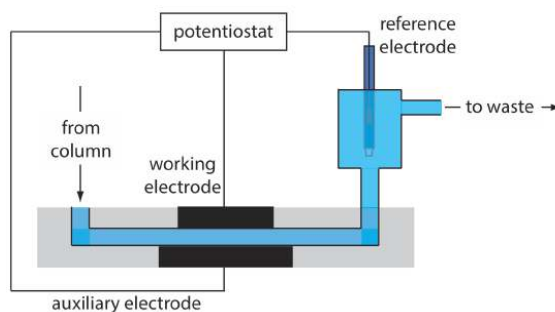


Figure 12.5.12 . Schematic diagram showing a flow cell for an amperometric electrochemical detector.

Other Detectors

Several other detectors have been used in HPLC. Measuring a change in the mobile phase's refractive index is analogous to monitoring the mobile phase's thermal conductivity in gas chromatography. A refractive index detector is nearly universal, responding to almost all compounds, but has a relatively poor detection limit of 0.1–1 μg of injected analyte. An additional limitation of a refractive index detector is that it cannot be used for a gradient elution unless the mobile phase components have identical refractive indexes.

Another useful detector is a mass spectrometer. Figure 12.5.13 shows a block diagram of a typical HPLC–MS instrument. The effluent from the column enters the mass spectrometer's ion source using an interface that removes most of the mobile phase, an essential need because of the incompatibility between the liquid mobile phase and the mass spectrometer's high vacuum environment. In the ionization chamber the remaining molecules—a mixture of the mobile phase components and solutes—undergo ionization and fragmentation. The mass spectrometer's mass analyzer separates the ions by their mass-to-charge ratio (m/z). A detector counts the ions and displays the mass spectrum.

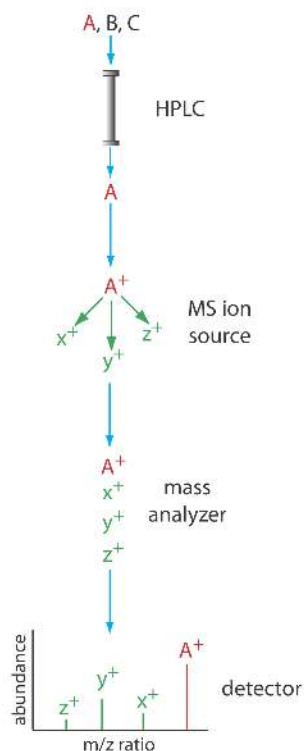


Figure 12.5.13 . Block diagram of an HPLC–MS. A three component mixture enters the HPLC. When component A elutes from the column, it enters the MS ion source and ionizes to form the **parent ion** and several **fragment ions**. The ions enter the mass analyzer, which separates them by their mass-to-charge ratio, providing the mass spectrum shown at the detector.

There are several options for monitoring the chromatogram when using a mass spectrometer as the detector. The most common method is to continuously scan the entire mass spectrum and report the total signal for all ions reaching the detector during each scan. This total ion scan provides universal detection for all analytes. As seen in Figure 12.5.14 , we can achieve some degree of selectivity by monitoring only specific mass-to-charge ratios, a process called selective-ion monitoring.

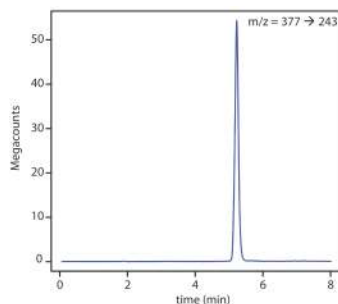


Figure 12.5.14 . HPLC–MS/MS chromatogram for the determination of riboflavin in urine. An initial parent ion with an m/z ratio of 377 enters a second mass spectrometer where it undergoes additional 20 ionization; the fragment ion with an m/z ratio of 243 provides the signal. The selectivity of this detector is evident when you compare this chromatogram to the one in Figure 12.5.11 , which uses fluorescence detection. Data provided by Jason Schultz, Jonna Berry, Kaelene Lundstrom, and Dwight Stoll, Department of Chemistry, Gustavus Adolphus College.

The advantages of using a mass spectrometer in HPLC are the same as for gas chromatography. Detection limits are very good, typically 0.1–1 ng of injected analyte, with values as low as 1–10 pg for some samples. In addition, a mass spectrometer provides qualitative, structural information that can help to identify the analytes. The interface between the HPLC and the mass spectrometer is technically more difficult than that in a GC–MS because of the incompatibility of a liquid mobile phase with the mass spectrometer's high vacuum requirement.

For more details on mass spectrometry see Introduction to Mass Spectrometry by Michael Samide and Olujide Akinbo, a resource that is part of the Analytical Sciences Digital Library.

Quantitative Applications

High-performance liquid chromatography is used routinely for both qualitative and quantitative analyses of environmental, pharmaceutical, industrial, forensic, clinical, and consumer product samples.

Preparing Samples for Analysis

Samples in liquid form are injected into the HPLC after a suitable clean-up to remove any particulate materials, or after a suitable extraction to remove matrix interferents. In determining polycyclic aromatic hydrocarbons (PAH) in wastewater, for example, an extraction with CH_2Cl_2 serves the dual purpose of concentrating the analytes and isolating them from matrix interferents. Solid samples are first dissolved in a suitable solvent or the analytes of interest brought into solution by extraction. For example, an HPLC analysis for the active ingredients and the degradation products in a pharmaceutical tablet often begins by extracting the powdered tablet with a portion of mobile phase. Gas samples are collected by bubbling them through a trap that contains a suitable solvent. Organic isocyanates in industrial atmospheres are collected by bubbling the air through a solution of 1-(2-methoxyphenyl)piperazine in toluene. The reaction between the isocyanates and 1-(2-methoxyphenyl)piperazine both stabilizes them against degradation before the HPLC analysis and converts them to a chemical form that can be monitored by UV absorption.

Quantitative Calculations

A quantitative HPLC analysis is often easier than a quantitative GC analysis because a fixed volume sample loop provides a more precise and accurate injection. As a result, most quantitative HPLC methods do not need an internal standard and, instead, use external standards and a normal calibration curve.

An internal standard is necessary when using HPLC–MS because the interface between the HPLC and the mass spectrometer does not allow for a reproducible transfer of the column's eluent into the MS's ionization chamber.

✓ Example 12.5.2

The concentration of polynuclear aromatic hydrocarbons (PAH) in soil is determined by first extracting the PAHs with methylene chloride. The extract is diluted, if necessary, and the PAHs separated by HPLC using a UV/Vis or fluorescence detector. Calibration is achieved using one or more external standards. In a typical analysis a 2.013-g sample of dried soil is extracted with 20.00 mL of methylene chloride. After filtering to remove the soil, a 1.00-mL portion of the extract is removed and diluted to 10.00 mL with acetonitrile. Injecting 5 μL of the diluted extract into an HPLC gives a signal of 0.217 (arbitrary units) for the PAH fluoranthene. When 5 μL of a 20.0-ppm fluoranthene standard is analyzed using the same conditions, a signal of 0.258 is measured. Report the parts per million of fluoranthene in the soil.

Solution

For a single-point external standard, the relationship between the signal, S , and the concentration, C , of fluoranthene is

$$S = kC$$

Substituting in values for the standard's signal and concentration gives the value of k as

$$k = \frac{S}{C} = \frac{0.258}{20.0 \text{ ppm}} = 0.0129 \text{ ppm}^{-1}$$

Using this value for k and the sample's HPLC signal gives a fluoranthene concentration of

$$C = \frac{S}{k} = \frac{0.217}{0.0129 \text{ ppm}^{-1}} = 16.8 \text{ ppm}$$

for the extracted and diluted soil sample. The concentration of fluoranthene in the soil is

$$\frac{16.8 \text{ g/mL} \times \frac{10.00 \text{ mL}}{1.00 \text{ mL}} \times 20.00 \text{ mL}}{2.013 \text{ g sample}} = 1670 \text{ ppm fluoranthene}$$

? Exercise 12.5.2

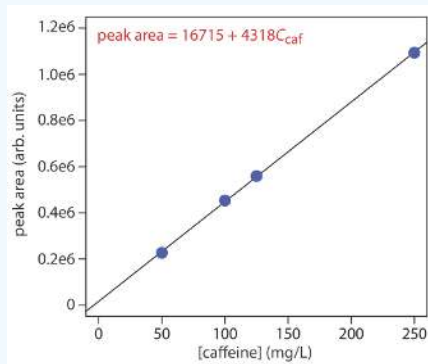
The concentration of caffeine in beverages is determined by a reversed-phase HPLC separation using a mobile phase of 20% acetonitrile and 80% water, and using a nonpolar C₈ column. Results for a series of 10-μL injections of caffeine standards are in the following table.

[caffeine] (mg/L)	peak area (arb. units)
50.0	226724
100.0	453762
125.0	559443
250.0	1093637

What is the concentration of caffeine in a sample if a 10-μL injection gives a peak area of 424195? The data in this problem comes from Kusch, P.; Knupp, G. "Simultaneous Determination of Caffeine in Cola Drinks and Other Beverages by Reversed-Phase HPTLC and Reversed-Phase HPLC," *Chem. Educator*, **2003**, 8, 201–205.

Answer

The figure below shows the calibration curve and calibration equation for the set of external standards. Substituting the sample's peak area into the calibration equation gives the concentration of caffeine in the sample as 94.4 mg/L.



The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of fluoxetine in serum provides an instructive example of a typical procedure. The description here is based on Smyth, W. F. *Analytical Chemistry of Complex Matrices*, Wiley Teubner: Chichester, England, 1996, pp. 187–189.

Representative Method 12.5.1: Determination of Fluoxetine in Serum

Description of Method

Fluoxetine is another name for the antidepressant drug Prozac. The determination of fluoxetine in serum is an important part of monitoring its therapeutic use. The analysis is complicated by the complex matrix of serum samples. A solid-phase extraction followed by an HPLC analysis using a fluorescence detector provides the necessary selectivity and detection limits.

Procedure

Add a known amount of the antidepressant protriptyline, which serves as an internal standard, to each serum sample and to each external standard. To remove matrix interferences, pass a 0.5-mL aliquot of each serum sample or standard through a C₁₈ solid-phase extraction cartridge. After washing the cartridge to remove the interferences, elute the remaining constituents, including the analyte and the internal standard, by washing the cartridge with 0.25 mL of a 25:75 v/v mixture of 0.1 M HClO₄ and acetonitrile. Inject a 20-μL aliquot onto a 15-cm × 4.6-mm column packed with a 5 μm C₈-bonded stationary phase. The isocratic mobile phase is 37.5:62.5 v/v acetonitrile and water (that contains 1.5 g of tetramethylammonium perchlorate and 0.1 mL of 70% v/v HClO₄).

Monitor the chromatogram using a fluorescence detector set to an excitation wave- length of 235 nm and an emission wavelength of 310 nm.

Questions

1. The solid-phase extraction is important because it removes constituents in the serum that might interfere with the analysis. What types of interferences are possible?

Blood serum, which is a complex mixture of compounds, is approximately 92% water, 6–8% soluble proteins, and less than 1% each of various salts, lipids, and glucose. A direct injection of serum is not advisable for three reasons. First, any particulate materials in the serum will clog the column and restrict the flow of mobile phase. Second, some of the compounds in the serum may absorb too strongly to the stationary phase, degrading the column's performance. Finally, although an HPLC can separate and analyze complex mixtures, an analysis is difficult if the number of constituents exceeds the column's peak capacity.

2. One advantage of an HPLC analysis is that a loop injector often eliminates the need for an internal standard. Why is an internal standard used in this analysis? What assumption(s) must we make when using the internal standard?

An internal standard is necessary because of uncertainties introduced during the solid-phase extraction. For example, the volume of serum transferred to the solid-phase extraction cartridge, 0.5 mL, and the volume of solvent used to remove the analyte and internal standard, 0.25 mL, are very small. The precision and accuracy with which we can measure these volumes is not as good as when we use larger volumes. For example, if we extract the analyte into a volume of 0.24 mL instead of a volume of 0.25 mL, then the analyte's concentration increases by slightly more than 4%. In addition, the concentration of eluted analytes may vary from trial-to-trial due to variations in the amount of solution held up by the cartridge. Using an internal standard compensates for these variation. To be useful we must assume that the analyte and the internal standard are retained completely during the initial loading, that they are not lost when the cartridge is washed, and that they are extracted completely during the final elution.

3. Why does the procedure monitor fluorescence instead of monitoring UV absorption?

Fluorescence is a more selective technique for detecting analytes. Many other commonly prescribed antidepressants (and their metabolites) elute with retention times similar to that of fluoxetine. These compounds, however, either do not fluoresce or are only weakly fluorescent.

4. If the peaks for fluoxetine and protriptyline are resolved insufficiently, how might you alter the mobile phase to improve their separation?

Decreasing the amount of acetonitrile and increasing the amount of water in the mobile will increase retention times, providing more time to effect a separation.

Evaluation

With a few exceptions, the scale of operation, accuracy, precision, sensitivity, selectivity, analysis time, and cost for an HPLC method are similar to GC methods. Injection volumes for an HPLC method usually are larger than for a GC method because HPLC columns have a greater capacity. Because it uses a loop injection, the precision of an HPLC method often is better than a GC method. HPLC is not limited to volatile analytes, which means we can analyze a broader range of compounds. Capillary GC columns, on the other hand, have more theoretical plates, and can separate more complex mixtures.

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