Chapter 13

Kinetic Methods

Chapter Overview

There are many ways to categorize analytical techniques, several of which we introduced in earlier chapters. In Chapter 3 we classified techniques by whether the signal is proportional to the absolute amount of analyte or the relative amount of analyte. For example, precipitation gravimetry is a total analysis technique because the precipitate's mass is proportional to the absolute amount, or moles, of analyte. UV/Vis absorption spectroscopy, on the other hand, is a concentration technique because absorbance is proportional to the relative amount, or concentration, of analyte.

A second way to classify analytical techniques is to consider the source of the analytical signal. For example, gravimetry encompasses all techniques in which the analytical signal is a measurement of mass or a change in mass. Spectroscopy, on the other hand, includes those techniques in which we probe a sample with an energetic particle, such as the absorption of a photon. This is the classification scheme used in organizing Chapters 8–11.

An additional way to classify analytical techniques is by whether the analyte's concentration is determined under a state of equilibrium or by the kinetics of a chemical reaction or a physical process. The analytical methods described in Chapter 8–11 mostly involve measurements made on systems in which the analyte is at equilibrium. In this chapter we turn our attention to measurements made under nonequilibrium conditions.

13A Kinetic Methods Versus Equilibrium Methods

In an EQUILIBRIUM METHOD the analytical signal is determined by an equilibrium reaction that involves the analyte or by a steady-state process that maintains the analyte's concentration. When we determine the concentration of iron in water by measuring the absorbance of the orange-red $Fe(phen)_3^{2+}$ complex (see Representative Method 10.1), the signal depends upon the concentration of $Fe(phen)_3^{2+}$, which, in turn, is determined by the complex's formation constant. In the flame atomic absorption determination of Cu and Zn in tissue samples (see Representative Method 10.2), the concentration of each metal in the flame remains constant because each step in the process of atomizing the sample is in a steady-state. In a KINETIC METHOD the analytical signal is determined by the rate of a reaction that involves the analyte or by a nonsteady-state process. As a result, the analyte's concentration changes during the time in which we monitor the signal.

In many cases we can choose to complete an analysis using either an equilibrium method or a kinetic method by changing when we measure the analytical signal. For example, one method for determining the concentration of nitrite, NO_2^-, in groundwater utilizes the two-step diazotization reaction shown in Figure 13.1.[1] The final product, which is a reddish-purple azo dye, absorbs visible light at a wavelength of 543 nm. Because neither reaction in Figure 13.1 is rapid, the absorbance—which is directly proportional to the concentration of nitrite—is measured 10 min after we add the last reagent, a lapse of time that ensures that the concentration of the azo dyes reaches the steady-state value required of an equilibrium method.

We can use the same set of reactions as the basis for a kinetic method if we measure the solution's absorbance during this 10-min development period, obtaining information about the reaction's rate. If the measured rate is a function of the concentration of NO_2^-, then we can use the rate to determine its concentration in the sample.[2]

1 Method 4500-NO_2^- B in *Standard Methods for the Analysis of Waters and Wastewaters*, American Public Health Association: Washington, DC, 20th Ed., 1998.

2 Karayannis, M. I.; Piperaki, E. A.; Maniadaki, M. M. *Anal. Lett.* **1986**, *19*, 13–23.

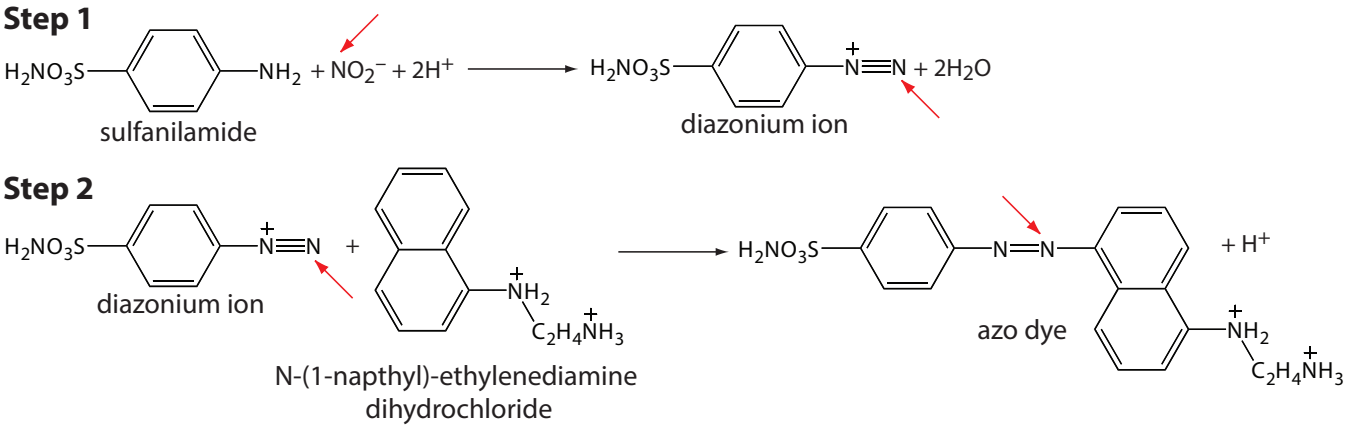


Figure 13.1 Analytical scheme for the analysis of NO_2^- in groundwater. The **red** arrows highlights the nitrogen in NO_2^- that becomes part of the azo dye.

There are many potential advantages to a kinetic method of analysis, perhaps the most important of which is the ability to use chemical reactions and systems that are slow to reach equilibrium. In this chapter we examine three techniques that rely on measurements made while the analytical system is under kinetic control: chemical kinetic techniques, in which we measure the rate of a chemical reaction; radiochemical techniques, in which we measure the decay of a radioactive element; and flow injection analysis, in which we inject the analyte into a continuously flowing carrier stream, where its mixes with and reacts with reagents in the stream under conditions controlled by the kinetic processes of convection and diffusion.

13B Chemical Kinetics

The earliest analytical methods based on chemical kinetics—which first appear in the late nineteenth century—took advantage of the catalytic activity of enzymes. In a typical method of that era, an enzyme was added to a solution that contained a suitable substrate and their reaction was monitored for a fixed time. The enzyme's activity was determined by the change in the substrate's concentration. Enzymes also were used for the quantitative analysis of hydrogen peroxide and carbohydrates. The development of chemical kinetic methods continued in the first half of the twentieth century with the introduction of nonenzymatic catalysts and noncatalytic reactions.

Despite the diversity of chemical kinetic methods, by 1960 they no longer were in common use. The principal limitation to their broader acceptance was a susceptibility to significant errors from uncontrolled or poorly controlled variables—temperature and pH are two such examples—and the presence of interferents that activate or inhibit catalytic reactions. By the 1980s, improvements in instrumentation and data analysis methods compensated for these limitations, ensuring the further development of chemical kinetic methods of analysis.[3]

13B.1 Theory and Practice

Every chemical reaction occurs at a finite rate, which makes it a potential candidate for a chemical kinetic method of analysis. To be effective, however, the chemical reaction must meet three necessary conditions: the reaction must not occur too quickly or too slowly; we must know the reaction's rate law; and we must be able to monitor the change in concentration for at least one species. Let's take a closer look at each of these requirements.

The material in this section assumes some familiarity with chemical kinetics, which is part of most courses in general chemistry. For a review of reaction rates, rate laws, and integrated rate laws, see the material in Appendix 17.

Reaction Rate

The RATE of the chemical reaction—how quickly the concentrations of reactants and products change during the reaction—must be fast enough that we can complete the analysis in a reasonable time, but also slow enough that the reaction does not reach equilibrium while the reagents are mixing. As

3 Pardue, H. L. *Anal. Chim. Acta* **1989**, *216*, 69–107.

We will consider two examples of instrumentation for studying reactions with fast kinetics in Section 13B.3.

a practical limit, it is not easy to study a reaction that reaches equilibrium within several seconds without the aid of special equipment for rapidly mixing the reactants.

Rate Law

The second requirement is that we must know the reaction's RATE LAW—the mathematical equation that describes how the concentrations of reagents affect the rate—for the period in which we are making measurements. For example, the rate law for a reaction that is first order in the concentration of an analyte, A, is

Because the concentration of A decreases during the reactions, $d[A]$ is negative. The minus sign in equation 13.1 makes the rate positive. If we choose to follow a product, P, then $d[P]$ is positive because the product's concentration increases throughout the reaction. In this case we omit the minus sign; see equation 13.21 for an example.

$$\text{rate} = -\frac{d[A]}{dt} = k[A] \qquad 13.1$$

where k is the reaction's RATE CONSTANT. An INTEGRATED RATE LAW often is a more useful form of the rate law because it is a function of the analyte's initial concentration. For example, the integrated rate law for equation 13.1 is

$$\ln [A]_t = \ln [A]_0 - kt \qquad 13.2$$

or

$$[A]_t = [A]_0 e^{-kt} \qquad 13.3$$

where $[A]_0$ is the analyte's initial concentration and $[A]_t$ is the analyte's concentration at time t.

Unfortunately, most reactions of analytical interest do not follow a simple rate law. Consider, for example, the following reaction between an analyte, A, and a reagent, R, to form a single product, P

$$A + R \underset{k_r}{\overset{k_f}{\rightleftharpoons}} P$$

where k_f is the rate constant for the forward reaction, and k_r is the rate constant for the reverse reaction. If the forward and the reverse reactions occur as single steps, then the rate law is

The first term, $k_f[A][R]$ accounts for the loss of A as it reacts with R to make P, and the second term, $k_r[P]$ accounts for the formation of A as P converts back to A and to R.

$$\text{rate} = -\frac{d[A]}{dt} = k_f[A][R] - k_r[P] \qquad 13.4$$

Although we know the reaction's rate law, there is no simple integrated form that we can use to determine the analyte's initial concentration. We can simplify equation 13.4 by restricting our measurements to the beginning of the reaction when the concentration of product is negligible. Under these conditions we can ignore the second term in equation 13.4, which simplifies to

$$\text{rate} = -\frac{d[A]}{dt} = k_f[A][R] \qquad 13.5$$

The integrated rate law for equation 13.5, however, is still too complicated to be analytically useful. We can further simplify the kinetics by making further adjustments to the reaction conditions.[4] For example, we can ensure

4 Mottola, H. A. *Anal. Chim. Acta* **1993**, *280*, 279–287.

pseudo-first-order kinetics by using a large excess of R so that its concentration remains essentially constant during the time we monitor the reaction. Under these conditions equation 13.5 simplifies to

$$\text{rate} = -\frac{d[A]}{dt} = k_f[A][R]_0 = k'[A] \qquad 13.6$$

where $k' = k_f[R]_0$. The integrated rate law for equation 13.6 then is

$$\ln[A]_t = \ln[A]_0 - k't \qquad 13.7$$

or

$$[A]_t = [A]_0 e^{-k't} \qquad 13.8$$

To say that the reaction is pseudo-first-order in A means the reaction behaves as if it is first order in A and zero order in R even though the underlying kinetics are more complicated. We call k' the pseudo-first-order rate constant.

It may even be possible to adjust the conditions so that we use the reaction under pseudo-zero-order conditions.

$$\text{rate} = -\frac{d[A]}{dt} = k_f[A]_0[R]_0 = k''t \qquad 13.9$$

$$[A]_t = [A]_0 - k''t \qquad 13.10$$

To say that a reaction is pseudo-zero-order means the reaction behaves as if it is zero order in A and zero order in R even though the underlying kinetics are more complicated. We call k'' the pseudo-zero-order rate constant.

Equation 13.10 is the integrated rate law for equation 13.9.

where $k'' = k_f[A]_0[R]_0$.

Monitoring the Reaction

The final requirement is that we must be able to monitor the reaction's progress by following the change in concentration for at least one of its species. Which species we choose to monitor is not important: it can be the analyte, a reagent that reacts with the analyte, or a product. For example, we can determine the concentration of phosphate by first reacting it with Mo(VI) to form 12-molybdophosphoric acid (12-MPA).

$$H_3PO_4(aq) + 6\text{Mo(VI)}(aq) \longrightarrow \text{12–MPA}(aq) + 9H^+(aq) \qquad 13.11$$

Reaction 13.11 is, of course, unbalanced; the additional hydrogens on the reaction's right side come from the six Mo(VI) that appear on the reaction's left side where Mo(VI) is thought to be present as the molybdate dimer $HMo_2O_6^+$.

Next, we reduce 12-MPA to heteropolyphosphomolybdenum blue, PMB. The rate of formation of PMB is measured spectrophotometrically, and is proportional to the concentration of 12-MPA. The concentration of 12-MPA, in turn, is proportional to the concentration of phosphate.[5] We also can follow reaction 13.11 spectrophotometrically by monitoring the formation of the yellow-colored 12-MPA.[6]

13B.2 Classifying Chemical Kinetic Methods

Figure 13.2 provides one useful scheme for classifying chemical kinetic methods of analysis. Methods are divided into two broad categories: direct-computation methods and curve-fitting methods. In a direct-computation method we calculate the analyte's initial concentration, $[A]_0$, using the appropriate rate law. For example, if the reaction is first-order in analyte, we can use equation 13.2 to determine $[A]_0$ given values for k, t, and $[A]_t$. With a curve-fitting method, we use regression to find the best fit between the

5 (a) Crouch, S. R.; Malmstadt, H. V. *Anal. Chem.* **1967**, *39*, 1084–1089; (b) Crouch, S. R.; Malmstadt, H. V. *Anal. Chem.* **1967**, *39*, 1090–1093; (c) Malmstadt, H. V.; Cordos, E. A.; Delaney, C. J. *Anal. Chem.* **1972**, *44(12)*, 26A–41A.

6 Javier, A. C.; Crouch, S. R.; Malmstadt, H. V. *Anal. Chem.* **1969**, *41*, 239–243.

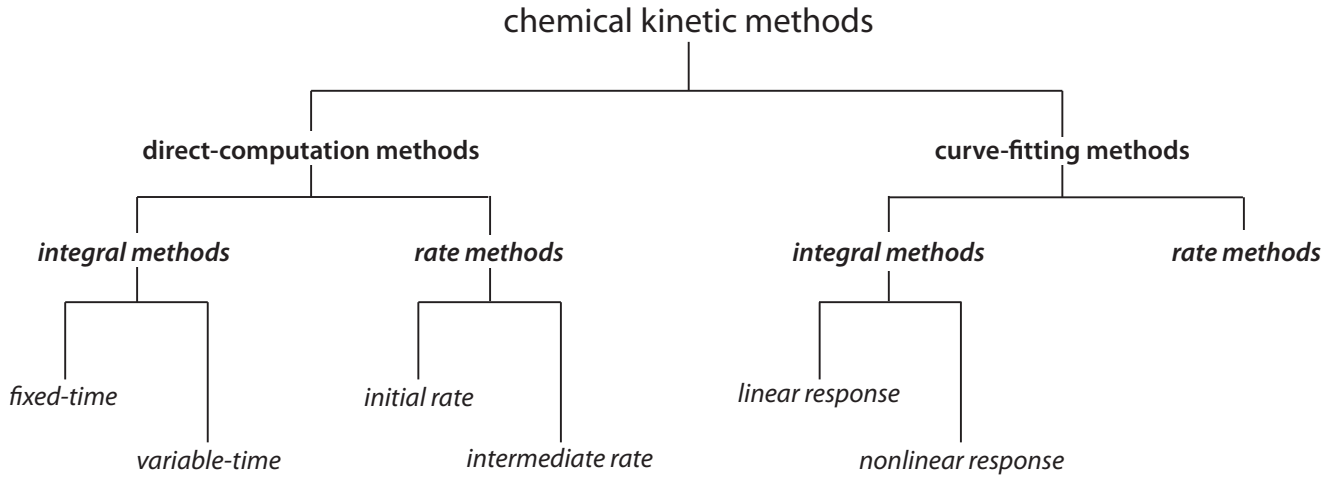


Figure 13.2 Classification of chemical kinetic methods of analysis adapted from Pardue, H. L. "Kinetic Aspects of Analytical Chemistry," *Anal. Chim. Acta* **1989**, *216*, 69–107.

data—for example, $[A]_t$ as a function of time—and the known mathematical model for the rate law. If the reaction is first-order in analyte, then we fit equation 13.2 to the data using k and $[A]_0$ as adjustable parameters.

Direct-Computation Fixed-Time Integral Methods

A direct-computation integral method uses the integrated form of the rate law. In a ONE-POINT FIXED-TIME INTEGRAL METHOD, for example, we determine the analyte's concentration at a single time and calculate the analyte's initial concentration, $[A]_0$, using the appropriate integrated rate law. To determine the reaction's rate constant, k, we run a separate experiment using a standard solution of analyte. Alternatively, we can determine the analyte's initial concentration by measuring $[A]_t$ for several standards that contain known concentrations of analyte and construct a calibration curve.

Example 13.1

The concentration of nitromethane, CH_3NO_2, is determined from the kinetics of its decomposition reaction. In the presence of excess base the reaction is pseudo-first-order in nitromethane. For a standard solution of 0.0100 M nitromethane, the concentration of nitromethane after 2.00 s is 4.24×10^{-4} M. When a sample that contains an unknown amount of nitromethane is analyzed, the concentration of nitromethane remaining after 2.00 s is 5.35×10^{-4} M. What is the initial concentration of nitromethane in the sample?

Solution

First, we determine the value for the pseudo-first-order rate constant, k'. Using equation 13.7 and the result for the standard, we find its value is

$$k' = \frac{\ln [A]_0 - \ln [A]_t}{t} = \frac{\ln (0.0100) - \ln (4.24 \times 10^{-4})}{2.00\ \text{s}} = 1.58\ \text{s}^{-1}$$

Next we use equation 13.8 to calculate the initial concentration of nitromethane in the sample.

$$[A]_0 = \frac{[A]_t}{e^{-k't}} = \frac{5.35 \times 10^{-4}\ \text{M}}{e^{-(1.58\ \text{s}^{-1})(2.00\ \text{s})}} = 0.0126\ \text{M}$$

Equation 13.7 and equation 13.8 are equally appropriate integrated rate laws for a pseudo-first-order reaction. The decision to use equation 13.7 to calculate k' and equation 13.8 to calculate $[A]_0$ is a matter of convenience.

Practice Exercise 13.1

In a separate determination for nitromethane, a series of external standards gives the following concentrations of nitromethane after a 2.00 s decomposition under pseudo-first-order conditions.

$[CH_3NO_2]_0$ (M)	$[CH_3NO_2]$ (M) at $t = 2.00$ s
0.0100	3.82×10^{-4}
0.0200	8.19×10^{-4}
0.0300	1.15×10^{-3}
0.0400	1.65×10^{-3}
0.0500	2.14×10^{-3}
0.0600	2.53×10^{-3}
0.0700	3.21×10^{-3}
0.0800	3.35×10^{-3}
0.0900	3.99×10^{-3}
0.100	4.15×10^{-3}

Analysis of a sample under the same conditions gives a nitromethane concentration of 2.21×10^{-3} M after 2 s. What is the initial concentration of nitromethane in the sample?

Click here to review your answer to this exercise.

In Example 13.1 we determine the analyte's initial concentration by measuring the amount of analyte that has not reacted. Sometimes it is more convenient to measure the concentration of a reagent that reacts with the analyte, or to measure the concentration of one of the reaction's products. We can use a one-point fixed-time integral method if we know the reaction's stoichiometry. For example, if we measure the concentration of the product, P, in the reaction

The kinetic method for phosphate described earlier is an example of a method in which we monitor the product. Because the phosphate ion does not absorb visible light, we incorporate it into a reaction that produces a colored product. That product, in turn, is converted into a different product that is even more strongly absorbing.

$$A + R \longrightarrow P$$

then the concentration of the analyte at time t is

$$[A]_t = [A]_0 - [P]_t \qquad 13.12$$

because the stoichiometry between the analyte and product is 1:1. If the reaction is pseudo-first-order in A, then substituting equation 13.12 into equation 13.7 gives

$$\ln([A]_0 - [P]_t) = \ln[A]_0 - k't \qquad 13.13$$

which we simplify by writing in exponential form.

$$[A]_0 - [P]_t = [A]_0 e^{-k't} \qquad 13.14$$

Finally, solving equation 13.14 for $[A]_0$ gives the following equation.

$$[A]_0 = \frac{[P]_t}{1 - e^{-k't}} \qquad 13.15$$

Example 13.2

The concentration of thiocyanate, SCN^-, is determined from the pseudo-first-order kinetics of its reaction with excess Fe^{3+} to form a reddish-colored complex of $Fe(SCN)^{2+}$. The reaction's progress is monitored by measuring the absorbance of $Fe(SCN)^{2+}$ at a wavelength of 480 nm. When using a standard solution of 0.100 M SCN^-, the concentration of $Fe(SCN)^{2+}$ after 10 s is 0.0516 M. The concentration of $Fe(SCN)^{2+}$ in a sample that contains an unknown amount of SCN^- is 0.0420 M after 10 s. What is the initial concentration of SCN^- in the sample?

Solution

First, we must determine a value for the pseudo-first-order rate constant, k'. Using equation 13.13, we find that its value is

$$k' = \frac{\ln[A]_0 - \ln([A]_0 - [P]_t)}{t} = \frac{\ln(0.100) - \ln(0.100 - 0.0516)}{10.0\ \text{s}} = 0.0726\ \text{s}^{-1}$$

Next, we use equation 13.15 to determine the initial concentration of SCN^- in the sample.

$$[A]_0 = \frac{[P]_t}{1 - e^{-k't}} = \frac{0.0420\ \text{M}}{1 - e^{-(0.0726\ \text{s}^{-1})(10.0\ \text{s})}} = 0.0868\ \text{M}$$

Figure 6.15 shows the color of a solution containing the $Fe(SCN)^{2+}$ complex.

A one-point fixed-time integral method has the advantage of simplicity because we need only a single measurement to determine the analyte's initial concentration. As with any method that relies on a single determination, a one-point fixed-time integral method can not compensate for a constant determinate error. In a TWO-POINT FIXED-TIME INTEGRAL METHOD we correct for constant determinate errors by making measurements at two points in time and use the difference between the measurements to determine the analyte's initial concentration. Because it affects both measurements equally, the difference between the measurements is independent of a constant determinate error. For a pseudo-first-order reaction in which we measure the

See Chapter 4 for a review of constant determinate errors and how they differ from proportional determinate errors.

Practice Exercise 13.2

In a separate determination for SCN^-, a series of external standards gives the following concentrations of $Fe(SCN)^{2+}$ after a 10.0 s reaction with excess Fe^{3+} under pseudo-first-order conditions.

$[SCN^-]_0$ (M)	$[Fe(SCN)^{2+}]$ (M) at $t = 10.0$ s
5.00×10^{-3}	1.79×10^{-3}
1.50×10^{-2}	8.24×10^{-3}
2.50×10^{-2}	1.28×10^{-2}
3.50×10^{-2}	1.85×10^{-2}
4.50×10^{-2}	2.21×10^{-2}
5.50×10^{-2}	2.81×10^{-2}
6.50×10^{-2}	3.27×10^{-2}
7.50×10^{-2}	3.91×10^{-2}
8.50×10^{-2}	4.34×10^{-2}
9.50×10^{-2}	4.89×10^{-2}

Analysis of a sample under the same conditions gives an $Fe(SCN)^{2+}$ concentration of 3.52×10^{-2} M after 10 s. What is the initial concentration of SCN^- in the sample?

Click here to review your answer to this exercise.

analyte's concentration at times t_1 and t_2, we can write the following two equations.

$$[A]_{t_1} = [A]_0 e^{-k' t_1} \qquad 13.16$$

$$[A]_{t_2} = [A]_0 e^{-k' t_2} \qquad 13.17$$

Subtracting equation 13.17 from equation 13.16 and solving for $[A]_0$ leaves us with

$$[A]_0 = \frac{[A]_{t_1} - [A]_{t_2}}{e^{-k' t_1} - e^{-k' t_2}} \qquad 13.18$$

To determine the rate constant, k', we measure $[A]_{t_1}$ and $[A]_{t_2}$ for a standard solution of analyte. Having obtained a value for k', we can determine $[A]_0$ by measuring the analyte's concentration at t_1 and t_2. We also can determine the analyte's initial concentration using a calibration curve consisting of a plot of $([A]_{t_1} - [A]_{t_2})$ versus $[A]_0$.

A fixed-time integral method is particularly useful when the signal is a linear function of concentration because we can replace the reactant's concentration with the corresponding signal. For example, if we follow a

reaction spectrophotometrically under conditions where the analyte's concentration obeys Beer's law

$$(Abs)_t = \varepsilon b [A]_t$$

then we can rewrite equation 13.8 and equation 13.18 as

$$c = e^{-k't}\varepsilon b$$

$$(Abs)_t = [A]_0 e^{-k't}\varepsilon b = c[A]_0$$

$$c' = \frac{1}{(\varepsilon b)(e^{-k't_1} - e^{-k't_2})}$$

$$[A]_0 = \frac{(Abs)_{t_1} - (Abs)_{t_2}}{e^{-k't_1} - e^{-k't_2}} \times (\varepsilon b)^{-1} = c'[(Abs)_{t_1} - (Abs)_{t_2}]$$

where $(Abs)_t$ is the absorbance at time t, and c and c' are constants.

Direct-Computation Variable-Time Integral Methods

In a VARIABLE-TIME INTEGRAL METHOD we measure the total time, Δt, needed to effect a specific change in concentration for one species in the chemical reaction. One important application is the quantitative analysis of catalysts, which takes advantage of the catalyst's ability to increase the rate of reaction. As the concentration of catalyst increased, Δt decreases. For many catalytic systems the relationship between Δt and the catalyst's concentration is

$$\frac{1}{\Delta t} = F_{cat}[A]_0 + F_{uncat} \qquad 13.19$$

where $[A]_0$ is the catalyst's concentration, and F_{cat} and F_{uncat} are constants that account for the rate of the catalyzed and uncatalyzed reactions.[7]

Example 13.3

Sandell and Kolthoff developed a quantitative method for iodide based on its ability to catalyze the following redox reaction.[8]

$$As^{3+}(aq) + 2Ce^{4+}(aq) \longrightarrow As^{5+}(aq) + 2Ce^{3+}(aq)$$

An external standards calibration curve was prepared by adding 1 mL of a KI standard to a mixture of 2 mL of 0.05 M As^{3+}, 1 mL of 0.1 M Ce^{4+}, and 1 mL of 3 M H_2SO_4, and measuring the time for the yellow color of Ce^{4+} to disappear. The following table summarizes the results for one analysis.

$[I^-]$ (μg/mL)	Δt (min)
5.0	0.9
2.5	1.8
1.0	4.5

What is the concentration of I^- in a sample if Δt is 3.2 min?

7 Mark, H. B.; Rechnitz, G. A. *Kinetics in Analytical Chemistry*, Interscience: New York, 1968.
8 Sandell, E. B.; Kolthoff, I. M. *J. Am. Chem. Soc.* **1934**, *56*, 1426.

SOLUTION

Figure 13.3 shows the calibration curve and the calibration equation for the external standards based on equation 13.19. Substituting 3.2 min for Δt gives the concentration of I^- in the sample as 1.4 μg/mL.

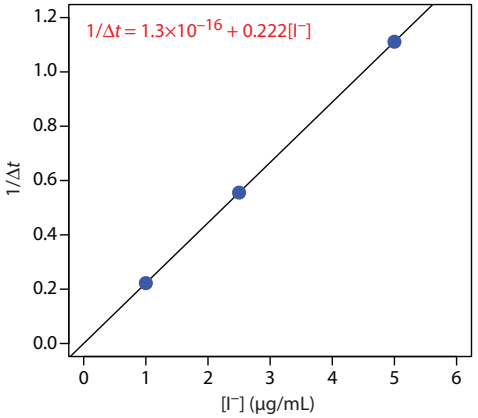


Figure 13.3 Calibration curve and calibration equation for Example 13.3.

DIRECT-COMPUTATION RATE METHODS

In a RATE METHOD we use the differential form of the rate law—equation 13.1 is one example of a differential rate law—to determine the analyte's concentration. As shown in Figure 13.4, the rate of a reaction at time t, $(rate)_t$, is the slope of a line tangent to a curve that shows the change in concentration as a function of time. For a reaction that is first-order in analyte, the rate at time t is

$$(rate)_t = k[A]_t$$

Substituting in equation 13.3 leaves us with the following equation relating the rate at time t to the analyte's initial concentration.

$$(rate)_t = k[A]_0 e^{-kt}$$

If we measure the rate at a fixed time, then both k and e^{-kt} are constant and we can use a calibration curve of $(rate)_t$ versus $[A]_0$ for the quantitative analysis of the analyte.

There are several advantages to using the reaction's INITIAL RATE ($t = 0$). First, because the reaction's rate decreases over time, the initial rate provides the greatest sensitivity. Second, because the initial rate is measured under nearly pseudo-zero-order conditions, in which the change in concentration with time effectively is linear, it is easier to determine the slope. Finally, as the reaction of interest progresses competing reactions may develop, which complicating the kinetics: using the initial rate eliminates these complications. One disadvantage of the initial rate method is that there may be insufficient time to completely mix the reactants. This problem is avoided by using an INTERMEDIATE RATE measured at a later time ($t > 0$).

As a general rule (see Mottola, H. A. "Kinetic Determinations of Reactants Utilizing Uncatalyzed Reactions," *Anal. Chim. Acta* **1993**, *280*, 279–287), the time for measuring a reaction's initial rate should result in the consumption of no more than 2% of the reactants. The smaller this percentage, the more linear the change in concentration as a function of time.

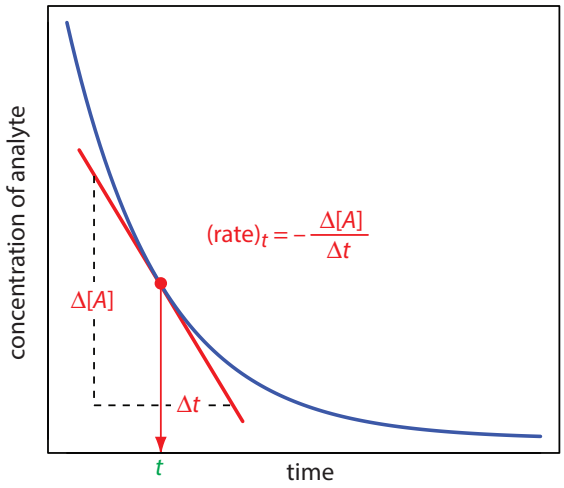
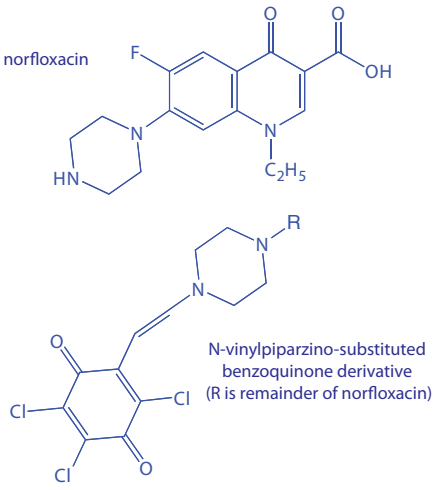


Figure 13.4 Determination of a reaction's instantaneous rate at time t from the slope of a **line** tangent to a **curve** that shows the change in the analyte's concentration as a function of time.



AU stands for absorbance unit.

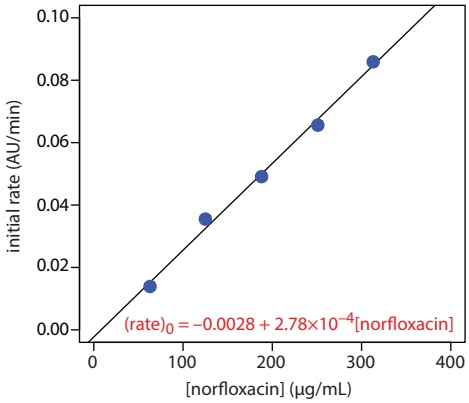


Figure 13.5 Calibration curve and calibration equation for Example 13.4.

Example 13.4

The concentration of norfloxacin, a commonly prescribed antibacterial agent, is determined using the initial rate method. Norfloxacin is converted to an N-vinylpiperazine derivative and reacted with 2,3,5,6-tetrachloro-1,4-benzoquinone to form an N-vinylpiperazino-substituted benzoquinone derivative that absorbs strongly at 625 nm.[9] The initial rate of the reaction—as measured by the change in absorbance as a function of time (AU/min)—is pseudo-first order in norfloxacin. The following data were obtained for a series of external norfloxacin standards.

[norfloxacin] (μg/mL)	initial rate (AU/min)
63	0.0139
125	0.0355
188	0.0491
251	0.0656
313	0.0859

To analyze a sample of prescription eye drops, a 10.00-mL portion is extracted with dichloromethane. The extract is dried and the norfloxacin reconstituted in methanol and diluted to 10 mL in a volumetric flask. A 5.00-mL portion of this solution is diluted to volume in a 100-mL volumetric flask. Analysis of this sample gives an initial rate of 0.0394 AU/min. What is the concentration of norfloxacin in the eye drops in mg/mL?

Solution

Figure 13.5 shows the calibration curve and the calibration equation for the external standards. Substituting 0.0394 AU/min for the initial rate and solving for the concentration of norfloxacin gives a result of 152 μg/mL. This is the concentration in a diluted sample of the extract. The concentration in the extract before dilution is

$$\frac{152\ \mu g}{mL} \times \frac{100.0\ mL}{5.00\ mL} \times \frac{1\ mg}{1000\ \mu g} = 3.04\ mg/mL$$

Because the dried extract was reconstituted using a volume identical to that of the original sample, the concentration of norfloxacin in the eye drops is 3.04 mg/mL.

Curve-Fitting Methods

In a direct-computation method we determine the analyte's concentration by solving the appropriate rate equation at one or two discrete times. The relationship between the analyte's concentration and the measured response is a function of the rate constant, which we determine in a separate experi-

9 Darwish, I. A.; Sultan, M. A.; Al-Arfaj, H. A. *Talanta* **2009**, *78*, 1383–1388.

ment using a single external standard (see Example 13.1 or Example 13.2), or a calibration curve (see Example 13.3 or Example 13.4).

In a CURVE-FITTING METHOD we continuously monitor the concentration of a reactant or a product as a function of time and use a regression analysis to fit the data to an appropriate differential rate law or integrated rate law. For example, if we are monitoring the concentration of a product for a reaction that is pseudo-first-order in the analyte, then we can fit the data to the following rearranged form of equation 13.15

$$[P]_t = [A]_0(1 - e^{-k't})$$

using $[A]_0$ and k' as adjustable parameters. Because we use data from more than one or two discrete times, a curve-fitting method is capable of producing more reliable results.

Example 13.5

The data shown in the following table were collected for a reaction that is known to be pseudo-zero-order in analyte. What is the initial concentration of analyte in the sample and the rate constant for the reaction?

time (s)	$[A]_t$ (mM)	time (s)	$[A]_t$ (mM)
3	0.0731	8	0.0448
4	0.0728	9	0.0404
5	0.0681	10	0.0339
6	0.0582	11	0.0217
7	0.0511	12	0.0143

Solution

From equation 13.10 we know that for a pseudo-zero-order reaction a plot of $[A]_t$ versus time is linear with a slope of $-k'$, and a y-intercept of $[A]_0$. Figure 13.6 shows a plot of the kinetic data and the result of a linear regression analysis. The initial concentration of analyte is 0.0986 mM and the rate constant is 0.00677 M^{-1} s^{-1}.

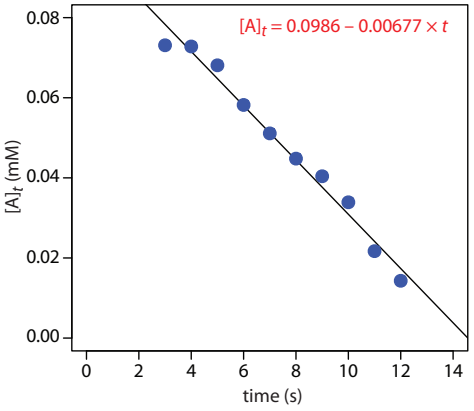


Figure 13.6 Result of fitting equation 13.10 to the data in Example 13.5.

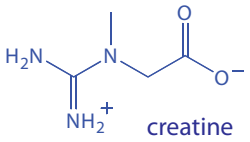
Representative Method 13.1

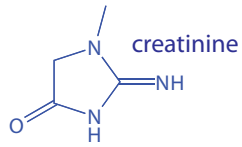
Determination of Creatinine in Urine

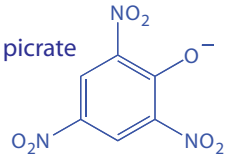
Description of Method

Creatine is an organic acid in muscle tissue that supplies energy for muscle contractions. One of its metabolic products is creatinine, which is excreted in urine. Because the concentration of creatinine in urine and serum is an important indication of renal function, a rapid method for its analysis is clinically important. In this method the rate of reaction between creatinine and picrate in an alkaline medium is used to determine the con-

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 creatinine in urine provides an instructive example of a typical procedure. The description here is based on Diamandis, E. P.; Koupparis, M. A.; Hadjiioannou, T. P. "Kinetic Studies with Ion Selective Electrodes: Determination of Creatinine in Urine with a Picrate Ion Selective Electrode," *J. Chem. Educ.* **1983**, *60*, 74–76.







centration of creatinine in urine. Under the conditions of the analysis the reaction is first order in picrate, creatinine, and hydroxide.

$$\text{rate} = k[\text{picrate}][\text{creatinine}][\text{OH}^-]$$

The reaction is monitored using a picrate ion selective electrode.

Procedure

Prepare a set of external standards that contain 0.5–3.0 g/L creatinine using a stock solution of 10.00 g/L creatinine in 5 mM H_2SO_4, diluting each standard to volume using 5 mM H_2SO_4. Prepare a solution of 1.00×10^{-2} M sodium picrate. Pipet 25.00 mL of 0.20 M NaOH, adjusted to an ionic strength of 1.00 M using Na_2SO_4, into a thermostated reaction cell at 25 °C. Add 0.500 mL of the 1.00×10^{-2} M picrate solution to the reaction cell. Suspend a picrate ion selective in the solution and monitor the potential until it stabilizes. When the potential is stable, add 2.00 mL of a creatinine external standard and record the potential as a function of time. Repeat this procedure using the remaining external standards. Construct a calibration curve of $\Delta E/\Delta t$ versus the initial concentration of creatinine. Use the same procedure to analyze samples, using 2.00 mL of urine in place of the external standard. Determine the concentration of creatinine in the sample using the calibration curve.

Questions

1. The analysis is carried out under conditions that are pseudo-first order in picrate. Show that under these conditions the change in potential as a function of time is linear.

   The potential, E, of the picrate ion selective electrode is given by the Nernst equation

   $$E = K - \frac{RT}{F}\ln[\text{picrate}]$$

   where K is a constant that accounts for the reference electrodes, the junction potentials, and the ion selective electrode's asymmetry potential, R is the gas constant, T is the temperature, and F is Faraday's constant. We know from equation 13.7 that for a pseudo-first-order reaction, the concentration of picrate at time t is

   $$\ln[\text{picrate}]_t = \ln[\text{picrate}]_0 - k't$$

   where k' is the pseudo-first-order rate constant. Substituting this integrated rate law into the ion selective electrode's Nernst equation leaves us with the following result.

   $$E_t = K - \frac{RT}{F}(\ln[\text{picrate}]_0 - k't)$$

   $$E_t = K - \frac{RT}{F}\ln[\text{picrate}]_0 + \frac{RT}{F}k't$$

   Because K and $(RT/F)\ln[\text{picrate}]_0$ are constants, a plot of E_t versus t is a straight line with a slope of RTk'/F.

For a review of the Nernst equation for ion selective electrodes, see Section 11B.3 in Chapter 11.

2. Under the conditions of the analysis, the rate of the reaction is pseudo-first-order in picrate and pseudo-zero-order in creatinine and OH^-. Explain why it is possible to prepare a calibration curve of $\Delta E/\Delta t$ versus the concentration of creatinine.

   The slope of a plot of E_t versus t is $\Delta E/\Delta t = RTk'/F$ (see the previous question). Because the reaction is carried out under conditions where it is pseudo-zero-order in creatinine and OH^-, the rate law is

   $$\text{rate} = k[\text{picrate}][\text{creatinine}]_0[OH^-]_0 = k'[\text{picrate}]$$

   The pseudo-first-order rate constant, k', is

   $$k' = k[\text{creatinine}]_0[OH^-]_0 = c[\text{creatinine}]_0$$

   where c is a constant equivalent to $k[OH^-]_0$. The slope of a plot of E_t versus t, therefore, is linear function of creatinine's initial concentration

   $$\frac{\Delta E}{\Delta t} = \frac{RTk'}{F} = \frac{RTc}{F}[\text{creatinine}]_0$$

   and a plot of $\Delta E/\Delta t$ versus the concentration of creatinine can serve as a calibration curve.

3. Why is it necessary to thermostat the reaction cell?

   The rate of a reaction is temperature-dependent. The reaction cell is thermostated to maintain a constant temperature to prevent a determinate error from a systematic change in temperature, and to minimize indeterminate errors from random fluctuations in temperature.

4. Why is it necessary to prepare the NaOH solution so that it has an ionic strength of 1.00 M?

   The potential of the picrate ion selective electrode actually responds to the activity of the picrate anion in solution. By adjusting the NaOH solution to a high ionic strength we maintain a constant ionic strength in all standards and samples. Because the relationship between activity and concentration is a function of ionic strength, the use of a constant ionic strength allows us to write the Nernst equation in terms of picrate's concentration instead of its activity.

For a review of the relationship between concentration, activity, and ionic strength, see Chapter 6I.

13B.3 Making Kinetic Measurements

When using Representative Method 13.1 to determine the concentration of creatinine in urine, we follow the reactions kinetics using an ion selective electrode. In principle, we can use any of the analytical techniques in Chapters 8–12 to follow a reaction's kinetics provided that the reaction does not proceed to an appreciable extent during the time it takes to make a measurement. As you might expect, this requirement places a serious limitation on kinetic methods of analysis. If the reaction's kinetics are slow relative to the analysis time, then we can make a measurement without the analyte undergoing a significant change in concentration. If the reaction's

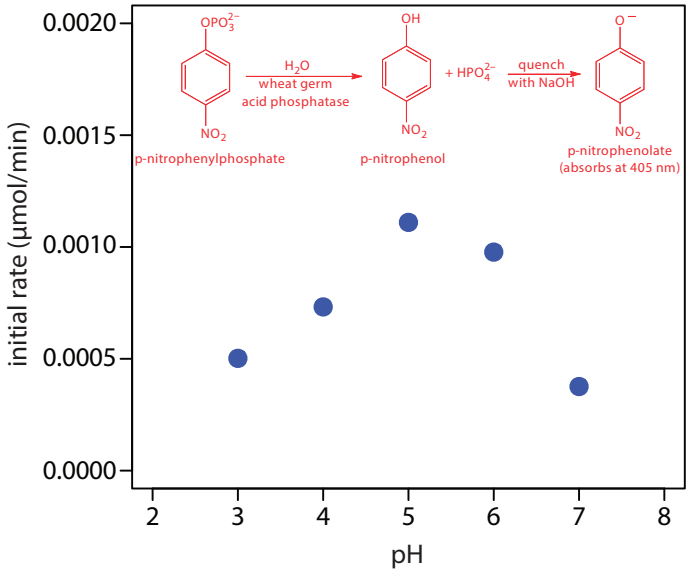


Figure 13.7 Initial rate for the enzymatic hydrolysis of *p*-nitrophenylphosphate using wheat germ acid phosphatase. Increasing the pH quenches the reaction and coverts colorless *p*-nitrophenol to the yellow-colored *p*-nitrophenolate, which absorbs at 405 nm.

rate is too fast—which often is the case—then we introduce a significant error if our analysis time is too long.

One solution to this problem is to stop, or QUENCH the reaction by adjusting experimental conditions. For example, many reactions show a strong dependence on pH and are quenched by adding a strong acid or a strong base. Figure 13.7 shows a typical example for the enzymatic analysis of *p*-nitrophenylphosphate, which uses the enzyme wheat germ acid phosphatase to hydrolyze the analyte to *p*-nitrophenol. The reaction has a maximum rate at a pH of 5. Increasing the pH by adding NaOH quenches the reaction and converts the colorless *p*-nitrophenol to the yellow-colored *p*-nitrophenolate, which absorbs at 405 nm.

An additional problem when the reaction's kinetics are fast is ensuring that we rapidly and reproducibly mix the sample and the reagents. For a fast reaction, we need to make our measurements within a few seconds—or even a few milliseconds—of combining the sample and reagents. This presents us with a problem and an advantage. The problem is that rapidly and reproducibly mixing the sample and the reagent requires a dedicated instrument, which adds an additional expense to the analysis. The advantage is that a rapid, automated analysis allows for a high throughput of samples. Instruments for the automated kinetic analysis of phosphate using reaction 13.11, for example, have sampling rates of approximately 3000 determinations per hour.

A variety of instruments have been developed to automate the kinetic analysis of fast reactions. One example, which is shown in Figure 13.8, is the STOPPED-FLOW ANALYZER. The sample and the reagents are loaded into separate syringes and precisely measured volumes are dispensed into a mixing chamber by the action of a syringe drive. The continued action of the syringe drive pushes the mixture through an observation cell and into a stopping syringe. The back pressure generated when the stopping

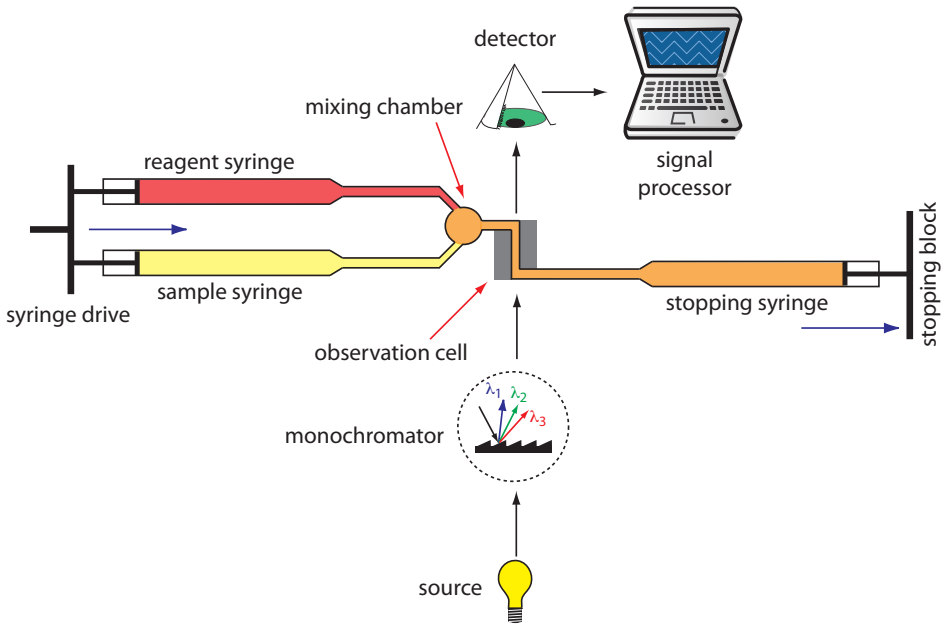


Figure 13.8 Schematic diagram of a stopped-flow analyzer. The **blue** arrows show the direction in which the syringes are moving.

syringe hits the stopping block completes the mixing, after which the reaction's progress is monitored spectrophotometrically. With a stopped-flow analyzer it is possible to complete the mixing of sample and reagent, and initiate the kinetic measurements in approximately 0.5 ms. By attaching an autosampler to the sample syringe it is possible to analyze up to several hundred samples per hour.

Another instrument for kinetic measurements is the CENTRIFUGAL ANALYZER, a partial cross section of which is shown in Figure 13.9. The sample and the reagents are placed in separate wells, which are oriented radially around a circular transfer disk. As the centrifuge spins, the centrifugal force pulls the sample and the reagents into the cuvette where mixing occurs. A single optical source and detector, located below and above the transfer disk's outer edge, measures the absorbance each time the cuvette passes through the optical beam. When using a transfer disk with 30 cuvettes and rotating at 600 rpm, we can collect 10 data points per second for each sample.

The ability to collect lots of data and to collect it quickly requires appropriate hardware and software. Not surprisingly, automated kinetic analyzers developed in parallel with advances in analog and digital circuitry—the hardware—and computer software for smoothing, integrating, and differentiating the analytical signal. For an early discussion of the importance of hardware and software, see Malmstadt, H. V.; Delaney, C. J.; Cordos, E. A. "Instruments for Rate Determinations," *Anal. Chem.* **1972**, *44(12)*, 79A–89A.

13B.4 Quantitative Applications

Chemical kinetic methods of analysis continue to find use for the analysis of a variety of analytes, most notably in clinical laboratories where automated methods aid in handling the large volume of samples. In this section we consider several general quantitative applications.

Enzyme-Catalyzed Reactions

ENZYMES are highly specific catalysts for biochemical reactions, with each enzyme showing a selectivity for a single reactant, or SUBSTRATE. For example, the enzyme acetylcholinesterase catalyzes the decomposition of the neurotransmitter acetylcholine to choline and acetic acid. Many enzyme–

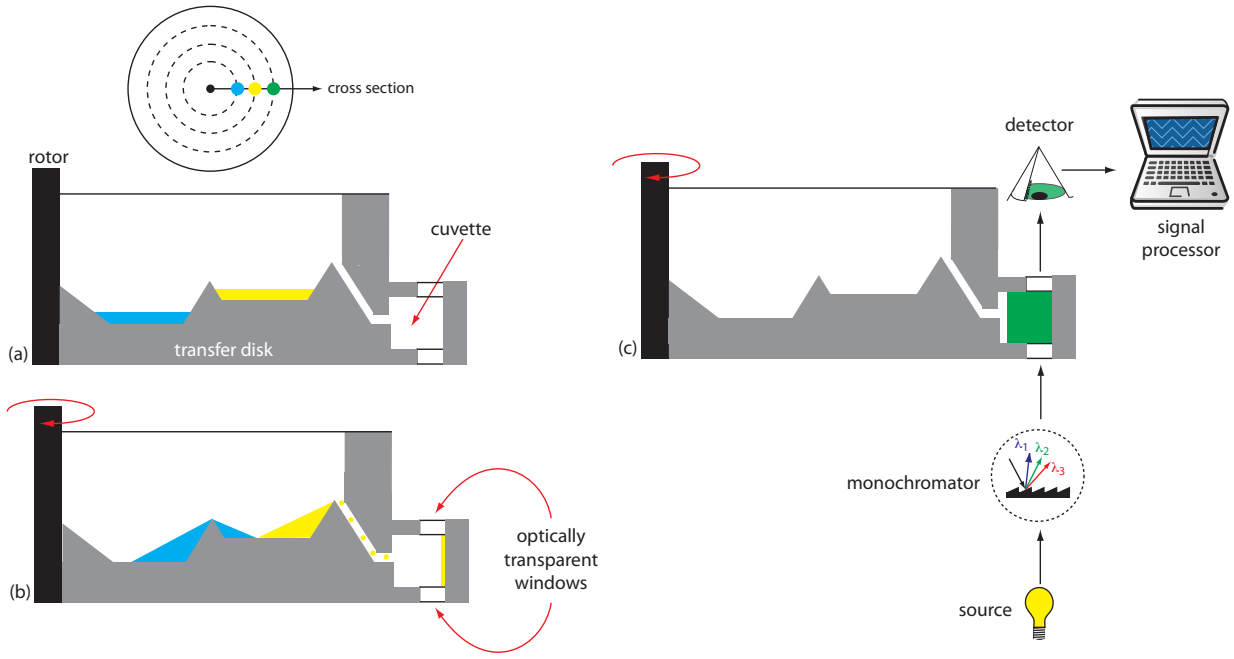


Figure 13.9 Cross sections through a centrifugal analyzer showing (a) the wells that hold the sample and the reagents, (b) the mixing of the sample and the reagents, and (c) the configuration of the spectrophotometric detector.

substrate reactions follow a simple mechanism that consists of the initial formation of an enzyme–substrate complex, *ES*, which subsequently decomposes to form product, releasing the enzyme to react again.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \qquad 13.20$$

where k_1, k_{-1}, k_2, and k_{-2} are rate constants. If we make measurement early in the reaction, the concentration of products is negligible and we can ignore the step described by the rate constant k_{-2}. Under these conditions the reaction's rate is

$$\text{rate} = \frac{d[P]}{dt} = k_2[ES] \qquad 13.21$$

To be analytically useful we need to write equation 13.21 in terms of the concentrations of the enzyme, *E*, and the substrate, *S*. To do this we use the STEADY-STATE APPROXIMATION, in which we assume the concentration of *ES* remains essentially constant. Following an initial period, during which the enzyme–substrate complex first forms, the rate at which *ES* forms

$$\frac{d[ES]}{dt} = k_1[E][S] = k_1([E]_0 - [ES])[S] \qquad 13.22$$

is equal to the rate at which it disappears

$$-\frac{d[ES]}{dt} = k_{-1}[ES] + k_2[ES] \qquad 13.23$$

where $[E]_0$ is the enzyme's original concentration. Combining equation 13.22 and equation 13.23 gives

Note the use of a conservation of mass on the enzyme, which requires that

$$[E]_0 = [E] + [ES]$$

as the enzyme is present either in its uncomplexed form or in the enzyme-substrate complex.

$$k_1([E]_0 - [ES])[S] = k_{-1}[ES] + k_2[ES]$$

which we solve for the concentration of the enzyme–substrate complex

$$[ES] = \frac{[E]_0[S]}{\frac{k_{-1} + k_2}{k_1} + [S]} = \frac{[E]_0[S]}{K_m + [S]} \qquad 13.24$$

where K_m is the MICHAELIS CONSTANT. Substituting equation 13.24 into equation 13.21 leaves us with our final rate equation.

$$\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_m + [S]} \qquad 13.25$$

A plot of equation 13.25, as shown in Figure 13.10, helps us define conditions where we can use the rate of an enzymatic reaction for the quantitative analysis of an enzyme or a substrate. For high substrate concentrations, where $[S] >> K_m$, equation 13.25 simplifies to

$$\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_m + [S]} \approx \frac{k_2[E]_0[S]}{[S]} = k_2[E]_0 = V_{max} \qquad 13.26$$

where V_{max} is the maximum rate for the catalyzed reaction. Under these conditions the reaction is pseudo-zero-order in substrate, and we can use V_{max} to calculate the enzyme's concentration, typically using a variable-time method. At lower substrate concentrations, where $[S] << K_m$, equation 13.25 becomes

$$\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_m + [S]} \approx \frac{k_2[E]_0[S]}{K_m} = \frac{V_{max}[S]}{K_m} \qquad 13.27$$

Because reaction is first-order in substrate we can use the reaction's rate to determine the substrate's concentration using a fixed-time method.

Chemical kinetic methods have been applied to the quantitative analysis of a number of enzymes and substrates.[10] One example, is the determination of glucose based on its oxidation by the enzyme glucose oxidase

$$\text{glucose}(aq) + H_2O(l) + O_2(g) \xrightarrow{\text{glucose oxidase}} \text{gluconolactone}(aq) + H_2O_2(aq)$$

under conditions where equation 13.20 is valid. The reaction is monitored by following the rate of change in the concentration of dissolved O_2 using an appropriate voltammetric technique.

One method for measuring the concentration of dissolved O_2 is the Clark amperometric sensor described in Chapter 11.

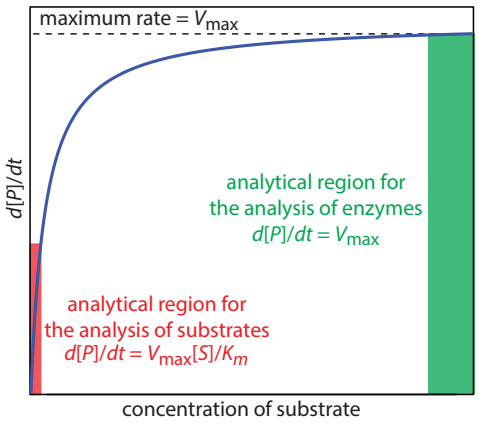


Figure 13.10 Plot of equation 13.25 showing limits for the analysis of substrates and enzymes in an enzyme-catalyzed chemical kinetic method of analysis. The curve in the region highlighted in **red** obeys equation 13.27 and the curve in the area highlighted in **green** follows equation 13.26.

Nonenzyme-Catalyzed Reactions

The variable-time method also is used to determine the concentration of nonenzymatic catalysts. One example uses the reduction of H_2O_2 by thiosulfate, iodide, or hydroquinone, a reaction catalyzed by trace amounts of selected metal ions. For example the reduction of H_2O_2 by I^-

$$2I^-(aq) + H_2O_2(aq) + 2H_3O^+(aq) \longrightarrow 4H_2O(l) + I_2(aq)$$

10 Guilbault, G. G. Handbook of Enzymatic Methods of Analysis, Marcel Dekker: New York, 1976.

is catalyzed by Mo(VI), W(VI), and Zr(IV). A variable-time analysis is conducted by adding a small, fixed amount of ascorbic acid to each solution. As I_2 is produced it rapidly oxidizes the ascorbic acid and is reduced back to I^-. Once all the ascorbic acid is consumed, the presence of excess I_2 provides a visual endpoint.

Noncatalytic Reactions

Chemical kinetic methods are not as common for the quantitative analysis of analytes in noncatalytic reactions. Because they lack the enhancement of reaction rate that a catalyst affords, a noncatalytic method generally is not useful for determining small concentrations of analyte. Noncatalytic methods for inorganic analytes usually are based on a complexation reaction. One example is the determination of aluminum in serum by measuring the initial rate for the formation of its complex with 2-hydroxy-1-napthaldehyde *p*-methoxybenzoyl-hydrazone.[11] The greatest number of noncatalytic methods, however, are for the quantitative analysis of organic analytes. For example, the insecticide methyl parathion has been determined by measuring its rate of hydrolysis in alkaline solutions.[12]

13B.5 Characterization Applications

Chemical kinetic methods also find use in determining rate constants and in elucidating reaction mechanisms. Two examples from the kinetic analysis of enzymes illustrate these applications.

Determining V_{MAX} and K_M for Enzyme-Catalyzed Reactions

The value of V_{max} and K_m for an enzymatic reaction are of significant interest in the study of cellular chemistry. For an enzyme that follows the mechanism in reaction 13.20, V_{max} is equivalent to $k_2 \times [E]_0$, where $[E]_0$ is the enzyme's concentration and k_2 is the enzyme's turnover number. An enzyme's turnover number is the maximum number of substrate molecules converted to product by a single active site on the enzyme, per unit time. A turnover number, therefore, provides a direct indication of the active site's catalytic efficiency. The Michaelis constant, K_m, is significant because it provides an estimate of the substrate's intracellular concentration.[13]

An enzyme's turnover number also is know as k_{cat} and is equal to $V_{max}/[E]_0$. For the mechanism in reaction 13.20, k_{cat} is equivalent to k_2. For more complicated mechanisms, k_{cat} is a function of additional rate constants.

As shown in Figure 13.10, we can find values for V_{max} and K_m by measuring the reaction's rate for small and for large concentrations of the substrate. Unfortunately, this is not always practical as the substrate's limited solubility may prevent us from using the large substrate concentrations needed to determine V_{max}. Another approach is to rewrite equation 13.25 by taking its reciprocal

11 Ioannou. P. C.; Piperaki, E. A. *Clin. Chem.* **1986**, *32*, 1481–1483.
12 Cruces Blanco, C.; Garcia Sanchez, F. *Int. J. Environ. Anal. Chem.* **1990**, *38*, 513–523.
13 (a) Northup, D. B. *J. Chem. Educ.* **1998**, *75*, 1153–1157; (b) Zubay, G. *Biochemistry*, Macmillan Publishing Co.: New York, 2nd Ed., p 269.

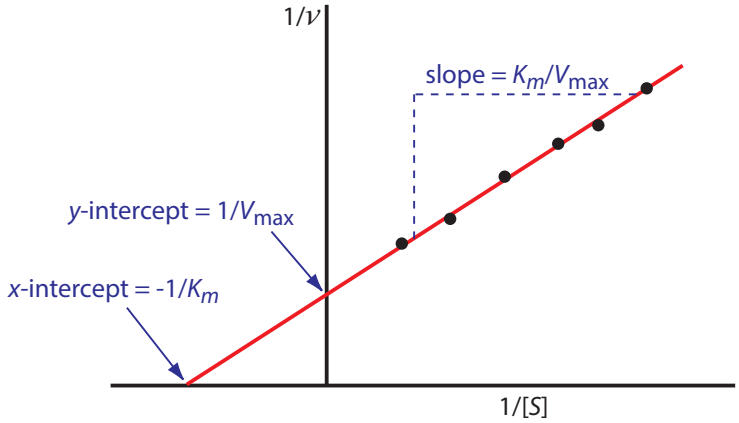


Figure 13.11 Lineweaver–Burk plot of equation 13.25 using equation 13.28.

$$\frac{1}{d[P]/dt} = \frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \qquad 13.28$$

where v is the reaction's rate. As shown in Figure 13.11, a plot of $1/v$ versus 1/[S], which is called a double reciprocal or LINEWEAVER–BURK PLOT, is a straight line with a slope of K_m/V_{max}, a y-intercept of $1/V_{max}$, and an x-intercept of $-1/K_m$.

Example 13.6

The reaction between nicotineamide mononucleotide and ATP to form nicotineamide–adenine dinucleotide and pyrophosphate is catalyzed by the enzyme nicotinamide mononucleotide adenylyltransferase.[14] The following table provides typical data obtained at a pH of 4.95. The substrate, S, is nicotinamide mononucleotide and the initial rate, v, is the μmol of nicotinamide–adenine dinucleotide formed in a 3-min reaction period.

[S] (mM)	v (μmol)	[**S**] (mM)	v (μmol)
0.138	0.148	0.560	0.324
0.220	0.171	0.766	0.390
0.291	0.234	1.460	0.493

Determine values for V_{max} and K_m.

SOLUTION

Figure 13.12 shows the Lineweaver–Burk plot for this data and the resulting regression equation. Using the y-intercept, we calculate V_{max} as

$$V_{max} = \frac{1}{y - \text{intercept}} = \frac{1}{1.708\ \mu\text{mol}^{-1}} = 0.585\ \mu\text{mol}$$

and using the slope we find that K_m is

$$K_m = \text{slope} \times V_{max} = 0.7528\ \mu\text{mol}^{-1}\,\text{mM} \times 0.585\ \mu\text{mol} = 0.440\ \text{mM}$$

14 (a) Atkinson, M. R.; Jackson, J. F.; Morton, R. K. *Biochem. J.* **1961**, *80*, 318–323; (b) Wilkinson, G. N. *Biochem. J.* **1961**, *80*, 324–332.

In Chapter 5 we noted that when faced with a nonlinear model—and equation 13.25 is one example of a nonlinear model—it may be possible to rewrite the equation in a linear form. This is the strategy used here. Linearizing a nonlinear model is not without limitations, two of which deserve a brief mention. First, because we are unlikely to have data for large substrate concentrations, we will not have many data points for small values of 1/[S]. As a result, our determination of the y-intercept's value relies on a significant extrapolation. Second, taking the reciprocal of the rate distorts the experimental error in a way that may invalidate the assumptions of a linear regression. Nonlinear regression provides a more rigorous method for fitting equation 13.25 to experimental data. The details are beyond the level of this textbook, but you may consult Massart, D. L.; Vandeginste, B. G. M.; Buydens, L. M. C. De Jong, S.; Lewi, P. J.; Smeyers-Verbeke, J. "Nonlinear Regression," which is Chapter 11 in *Handbook of Chemometrics and Qualimetrics: Part A*, Elsevier: Amsterdam, 1997, for additional details. The simplex algorithm described in Chapter 14 of this text also can be used to fit a nonlinear equation to experimental data.

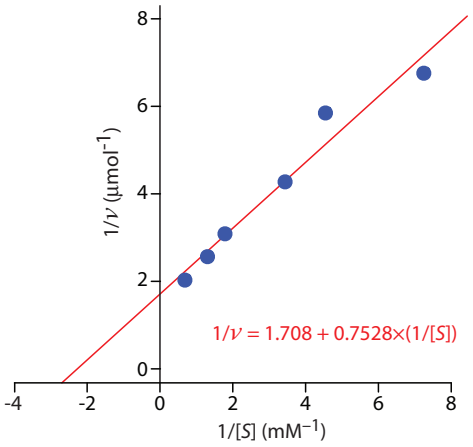
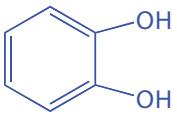
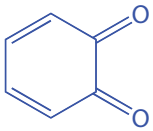


Figure 13.12 Lineweaver–Burk plot and regression equation for the data in Example 13.6.



catechol



o-quinone

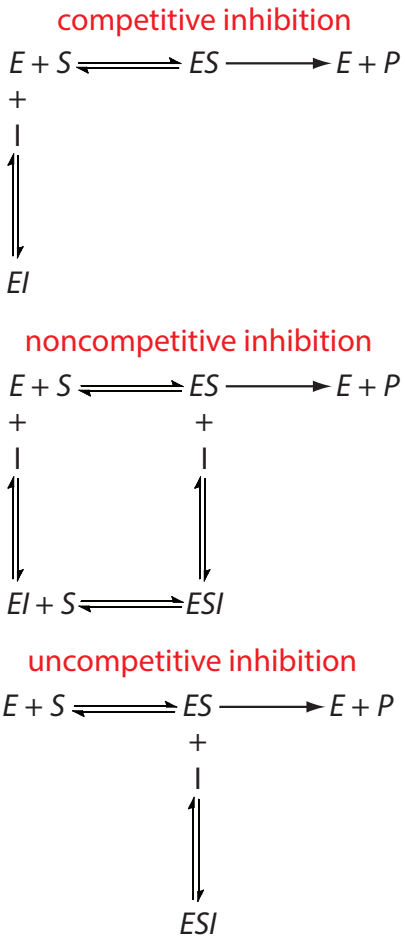


Figure 13.13 Mechanisms for the reversible inhibition of enzyme catalysis. *E*: enzyme, *S*: substrate, *P*: product, *I*: inhibitor, *ES*: enzyme–substrate complex, *EI*: enzyme–inhibitor complex, *ESI*: enzyme–substrate–inhibitor complex.

Practice Exercise 13.3

The following data were collected during the oxidation of catechol (the substrate) to *o*-quinone by the enzyme *o*-diphenyl oxidase. The reaction was followed by monitoring the change in absorbance at 540 nm. The data in this exercise is adapted from jkimball.

[catechol] (mM)	0.3	0.6	1.2	4.8
rate (ΔAU/min)	0.020	0.035	0.048	0.081

Determine values for V_{max} and K_m.

Click here to review your answer to this exercise.

Elucidating Mechanisms for the Inhibition of Enzyme Catalysis

When an INHIBITOR interacts with an enzyme it decreases the enzyme's catalytic efficiency. An irreversible inhibitor binds covalently to the enzyme's active site, producing a permanent loss in catalytic efficiency even if we decrease the inhibitor's concentration. A reversible inhibitor forms a noncovalent complex with the enzyme, resulting in a temporary decrease in catalytic efficiency. If we remove the inhibitor, the enzyme's catalytic efficiency returns to its normal level.

There are several pathways for the reversible binding of an inhibitor and an enzyme, as shown in Figure 13.13. In COMPETITIVE INHIBITION the substrate and the inhibitor compete for the same active site on the enzyme. Because the substrate cannot bind to an enzyme–inhibitor complex, *EI*, the enzyme's catalytic efficiency for the substrate decreases. With NONCOMPETITIVE INHIBITION the substrate and the inhibitor bind to different active sites on the enzyme, forming an enzyme–substrate–inhibitor, or *ESI* complex. The formation of an *ESI* complex decreases catalytic efficiency because only the enzyme–substrate complex reacts to form the product. Finally, in UNCOMPETITIVE INHIBITION the inhibitor binds to the enzyme–substrate complex, forming an inactive *ESI* complex.

We can identify the type of reversible inhibition by observing how a change in the inhibitor's concentration affects the relationship between the rate of reaction and the substrate's concentration. As shown in Figure 13.14, when we display kinetic data using as a Lineweaver-Burk plot it is easy to determine which mechanism is in effect. For example, an increase in slope, a decrease in the *x*-intercept, and no change in the *y*-intercept indicates competitive inhibition. Because the inhibitor's binding is reversible, we can still obtain the same maximum velocity—thus the constant value for the *y*-intercept—by adding enough substrate to completely displace the inhibitor. Because it takes more substrate, the value of K_m increases, which explains the increase in the slope and the decrease in the *x*-intercept's value.

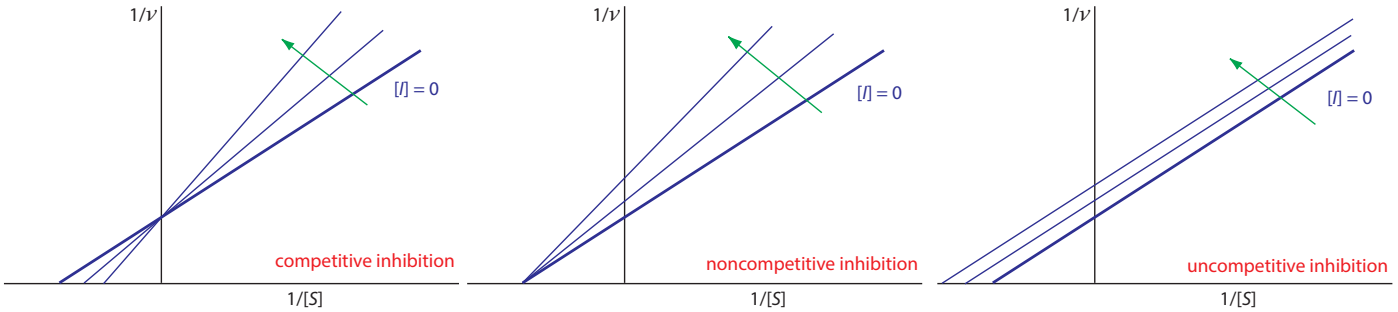


Figure 13.14 Lineweaver–Burk plots for competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition. The **thick blue** line in each plot shows the kinetic behavior in the absence of inhibitor, and the **thin blue** lines in each plot show the change in behavior for increasing concentrations of the inhibitor. In each plot, the inhibitor's concentration increases in the direction of the **green** arrow.

Example 13.7

Practice Exercise 13.3 provides kinetic data for the oxidation of catechol (the substrate) to *o*-quinone by the enzyme *o*-diphenyl oxidase in the absence of an inhibitor. The following additional data are available when the reaction is run in the presence of *p*-hydroxybenzoic acid, PBHA. Is PBHA an inhibitor for this reaction and, if so, what type of inhibitor is it? The data in this exercise are adapted from jkimball.

[catechol] (mM)	0.3	0.6	1.2	4.8
rate (ΔAU/min)	0.011	0.019	0.022	0.060

Solution

Figure 13.15 shows the resulting Lineweaver–Burk plot for the data in Practice Exercise 13.3 and Example 13.7. Although the two *y*-intercepts are not identical in value—the result of uncertainty in measuring the rates—the plot suggests that PBHA is a competitive inhibitor for the enzyme's reaction with catechol.

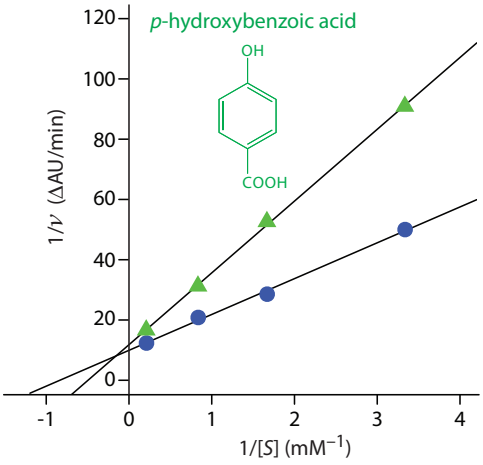


Figure 13.15 Lineweaver–Burk plots for the data in Practice Exercise 13.3 and Example 13.7.

Practice Exercise 13.4

Practice Exercise 13.3 provides kinetic data for the oxidation of catechol (the substrate) to *o*-quinone by the enzyme *o*-diphenyl oxidase in the absence of an inhibitor. The following additional data are available when the reaction is run in the presence of phenylthiourea. Is phenylthiourea an inhibitor for this reaction and, if so, what type of inhibitor is it? The data in this exercise are adapted from jkimball.

[catechol] (mM)	0.3	0.6	1.2	4.8
rate (ΔAU/min)	0.010	0.016	0.024	0.040

Click here to review your answer to this exercise.

13B.6 Evaluation of Chemical Kinetic Methods

Scale of Operation

The detection limit for a chemical kinetic method ranges from minor components to ultratrace components, and is determined by two factors: the rate of the reaction and the instrumental technique used to monitor the rate. Because the signal is directly proportional to the reaction's rate, a faster reaction generally results in a lower detection limit. All other factors being equal, detection limits are smaller for catalytic reactions than for noncatalytic reactions. Not surprisingly, some of the earliest chemical kinetic methods took advantage of catalytic reactions. For example, ultratrace levels of Cu (<1 ppb) are determined by measuring its catalytic effect on the redox reaction between hydroquinone and H_2O_2.

See Figure 3.5 to review the meaning of minor, trace, and ultratrace components.

In the absence of a catalyst, most chemical kinetic methods for organic compounds use reactions with relatively slow rates, which limits the analysis to minor and to higher concentration trace analytes. Noncatalytic chemical kinetic methods for inorganic compounds that use metal–ligand complexation reactions may be fast or slow, with detection limits ranging from trace to minor analyte.

The second factor that influences a method's detection limit is the instrumentation used to monitor the reaction's progress. Most reactions are monitored spectrophotometrically or electrochemically. The scale of operation for these techniques are discussed in Chapter 10 and Chapter 11.

Accuracy

As noted earlier, a chemical kinetic method potentially is subject to larger errors than an equilibrium method due to the effect of uncontrolled or poorly controlled variables, such as temperature or pH. Although a direct-computation chemical kinetic method can achieve moderately accurate results (a relative error of 1–5%), the accuracy often is much worse. Curve-fitting methods provide significant improvements in accuracy because they use more data. In one study, for example, accuracy was improved by two orders of magnitude—from errors of 500% to 5%—by replacing a direct-computation analysis with a curve-fitting analysis.[15] Although not discussed in this chapter, data analysis methods that include the ability to compensate for experimental errors can lead to a significant improvement in accuracy.[16]

Precision

The precision of a chemical kinetic method is limited by the signal-to-noise ratio of the instrumentation used to monitor the reaction's progress. When

15 Pauch, J. B.; Margerum, D. W. *Anal. Chem.* **1969**, *41*, 226–232.

16 (a) Holler, F. J.; Calhoun, R. K.; MClanahan, S. F. *Anal. Chem.* **1982**, *54*, 755–761; (b) Wentzel, P. D.; Crouch, S. R. *Anal. Chem.* **1986**, *58*, 2851–2855; (c) Wentzel, P. D.; Crouch, S. R. *Anal. Chem.* **1986**, *58*, 2855–2858.

using an integral method, a precision of 1–2% is routinely possible. The precision for a differential method may be somewhat poorer, particularly if the signal is noisy.

Sensitivity

We can improve the sensitivity of a one-point fixed-time integral method by making measurements under conditions where the concentration of the monitored species is as large as possible. When monitoring the analyte's concentration—or the concentration of any other reactant—we want to take measurements early in the reaction before its concentration decreases. On the other hand, if we choose to monitor one of the reaction's products, then it is better to take measurements at longer times. For a two-point fixed-time integral method, we can improve sensitivity by increasing the difference between times t_1 and t_2. As discussed earlier, the sensitivity of a rate method improves when we choose to measure the initial rate.

Selectivity

The analysis of closely related compounds, as discussed in earlier chapters, often is complicated by their tendency to interfere with each other. To overcome this problem we usually need to separate the analyte and the interferent before completing the analysis. One advantage of a chemical kinetic method is that it often is possible adjust the reaction conditions so that the analyte and the interferent have different reaction rates. If the difference in their respective rates is large enough, then one species will react completely before the other species has a chance to react.

The need to analyze multiple analytes in complex mixtures is, of course, one of the advantages of the separation techniques covered in Chapter 12. Kinetic techniques provide an alternative approach for simple mixtures.

We can use the appropriate integrated rate laws to find the conditions necessary to separate a faster reacting species from a more slowly reacting species. Let's consider a system that consists of an analyte, A, and an interferent, B, both of which show first-order kinetics with a common reagent. To avoid an interference, the relative magnitudes of their rate constants must be sufficiently different. The fractions, f, of A and B that remain at any point in time, t, are defined by the following equations

$$(f_A)_t = \frac{[A]_t}{[A]_0} \qquad 13.29$$

$$(f_B)_t = \frac{[B]_t}{[B]_0} \qquad 13.30$$

where $[A]_0$ and $[B]_0$ are the initial concentrations of A and B, respectively. Rearranging equation 13.2 and substituting in equation 13.29 or equation 13.30 leaves use with the following two equations.

$$\ln \frac{[A]_t}{[A]_0} = \ln (f_A)_t = -k_A t \qquad 13.31$$

$$\ln \frac{[B]_t}{[B]_0} = \ln (f_B)_t = -k_B t \qquad 13.32$$

where k_A and k_B are the rate constants for A and for B. Dividing equation 13.31 by equation 13.32 leave us with

$$\frac{k_A}{k_B} = \frac{\ln (f_A)_t}{\ln (f_B)_t}$$

Suppose we want 99% of A to react before 1% of B reacts. The fraction of A that remains is 0.01 and the fraction of B that remains is 0.99, which requires that

$$\frac{k_A}{k_B} = \frac{\ln (f_A)_t}{\ln (f_B)_t} = \frac{\ln (0.01)}{\ln (0.99)} = 460$$

the rate constant for A must be at least 460 times larger than that for B. When this condition is met we can determine the analyte's concentration before the interferent begins to react. If the analyte has the slower reaction, then we can determine its concentration after we allow the interferent to react to completion.

This method of adjusting reaction rates is useful if we need to analyze an analyte in the presence of an interferent, but is impractical if both A and B are analytes because the condition that favors the analysis of A will not favor the analysis of B. For example, if we adjust conditions so that 99% of A reacts in 5 s, then 99% of B must react within 0.01 s if it has the faster kinetics, or in 2300 s if it has the slower kinetics. The reaction of B is too fast or too slow to make this a useful analytical method.

What do we do if the difference in the rate constants for A and B are not significantly different? We still can complete an analysis if we can simultaneously monitor both species. Because both A and B react at the same time, the integrated form of the first-order rate law becomes

$$C_t = [A]_t + [B]_t = [A]_0 e^{-k_A t} + [B]_0 e^{-k_B t} \qquad 13.33$$

where C_t is the total concentration of A and B at time, t. If we measure C_t at times t_1 and t_2, we can solve the resulting pair of simultaneous equations to determine values $[A]_0$ and $[B]_0$. The rate constants k_A and k_B are determined in separate experiments using standard solutions of A and B.

Equation 13.33 can also serve as the basis for a curve-fitting method. As shown in Figure 13.16, a plot of $\ln(C_t)$ as a function of time consists of two regions. At shorter times the plot is curved because A and B react simultaneously. At later times, however, the concentration of the faster reacting component, A, decreases to zero, and equation 13.33 simplifies to

$$C_t \approx [B]_t = [B]_0 e^{-k_B t}$$

Under these conditions a plot of $\ln(C_t)$ versus time is linear. Extrapolating the linear portion to $t = 0$ gives $[B]_0$, with $[A]_0$ determined by difference.

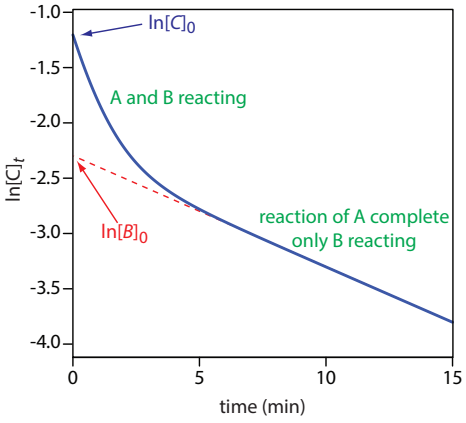


Figure 13.16 Kinetic determination of a slower reacting analyte, B, in the presence of a faster reacting analyte, A. The rate constants for the two analytes are: $k_A = 1$ min^{-1} and $k_B = 0.1$ min^{-1}. Example 13.8 asks you to use this data to determine the concentrations of A and B in the original sample.

Example 13.8

Use the data in Figure 13.16 to determine the concentrations of A and B in the original sample.

Solution

Extrapolating the linear part of the curve back to $t = 0$ gives $\ln[B]_0$ as –2.3, or a $[B]_0$ of 0.10 M. At $t = 0$, $\ln[C]_0$ is –1.2, which corresponds to a $[C]_0$ of 0.30 M. Because $[C]_0 = [A]_0 + [B]_0$, the concentration of A in the original sample is 0.20 M.

Time, Cost, and Equipment

An automated chemical kinetic method of analysis provides a rapid means for analyzing samples, with throughputs ranging from several hundred to several thousand determinations per hour. The initial start-up costs may be fairly high because an automated analysis requires a dedicated instrument designed to meet the specific needs of the analysis. When measurements are handled manually, a chemical kinetic method requires routinely available equipment and instrumentation, although the sample throughput is much lower than with an automated method.

13C Radiochemistry

Atoms that have the same number of protons but a different number of neutrons are ISOTOPES. To identify an isotope we use the notation ${}^{A}_{Z}E$, where E is the element's atomic symbol, Z is the element's atomic number, and A is the element's atomic mass number. Although an element's different isotopes have the same chemical properties, their nuclear properties are not identical. The most important difference between isotopes is their stability. The nuclear configuration of a stable isotope remains constant with time. Unstable isotopes, however, disintegrate spontaneously, emitting radioactive particles as they transform into a more stable form.

An element's atomic number, Z, is equal to the number of protons and its atomic mass, A, is equal to the sum of the number of protons and neutrons. We represent an isotope of carbon-13 as

$${}^{13}_{6}\text{C}$$

because carbon has six protons and seven neutrons. Sometimes we omit Z from this notation—identifying the element and the atomic number is repetitive because all isotopes of carbon have six protons and any atom that has six protons is an isotope of carbon. Thus, ^{13}C and C–13 are alternative notations for this isotope of carbon.

The most important types of radioactive particles are alpha particles, beta particles, gamma rays, and X-rays. An ALPHA PARTICLE, α, is equivalent to a helium nucleus, ${}^{4}_{2}\text{He}$. When an atom emits an alpha particle, the product in a new atom whose atomic number and atomic mass number are, respectively, 2 and 4 less than its unstable parent. The decay of uranium to thorium is one example of alpha emission.

$${}^{238}_{92}\text{U} \longrightarrow {}^{234}_{90}\text{Th} + \alpha$$

A BETA PARTICLE, β, comes in one of two forms. A NEGATRON, ${}^{0}_{-1}\beta$, is produced when a neutron changes into a proton, increasing the atomic number by one, as shown here for lead.

$${}^{214}_{82}\text{Pb} \longrightarrow {}^{214}_{83}\text{Bi} + {}^{0}_{-1}\beta$$

A negatron, which is the more common type of beta particle, is equivalent to an electron. You may recall from Section 12D.5 that the electron capture detector for gas chromatography uses ^{63}Ni as a beta emitter.

The conversion of a proton to a neutron results in the emission of a POSITRON, ${}^{0}_{1}\beta$.

$$ {}^{30}_{15}\mathrm{P} \longrightarrow {}^{30}_{14}\mathrm{Si} + {}^{0}_{1}\beta $$

The emission of an alpha or a beta particle often produces an isotope in an unstable, high energy state. This excess energy is released as a GAMMA RAY, γ, or as an X-ray. Gamma ray and X-ray emission may also occur without the release of an alpha particle or a beta particle.

13C.1 Theory and Practice

A radioactive isotope's rate of decay, or activity, follows first-order kinetics

$$ A = -\frac{dN}{dt} = \lambda N \qquad 13.34 $$

where A is the isotope's activity, N is the number of radioactive atoms present in the sample at time t, and λ is the isotope's decay constant. Activity is expressed as the number of disintegrations per unit time.

Note the similarity between equation 13.34 and equation 13.1

$$ \text{rate} = -\frac{d[A]}{dt} = k[A] $$

where activity is equivalent to rate, N is equivalent to $[A]$, and λ is equivalent to k.

As with any first-order process, we can rewrite equation 13.34 in an integrated form.

$$ N_t = N_0 e^{-\lambda t} \qquad 13.35 $$

Substituting equation 13.35 into equation 13.34 gives

$$ A = \lambda N_0 e^{-\lambda t} = A_0 e^{-\lambda t} \qquad 13.36 $$

If we measure a sample's activity at time t we can determine the sample's initial activity, A_0, or the number of radioactive atoms originally present in the sample, N_0.

An important characteristic property of a radioactive isotope is its HALF-LIFE, $t_{1/2}$, which is the amount of time required for half of the radioactive atoms to disintegrate. For first-order kinetics the half-life is

$$ t_{1/2} = \frac{0.693}{\lambda} \qquad 13.37 $$

Suppose we begin with an N_0 of 1200 atoms During the first half-life, 600 atoms disintegrate and 600 remain. During the second half-life, 300 of the 600 remaining atoms disintegrate, leaving 300 atoms or 25% of the original 1200 atoms. Of the 300 remaining atoms, only 150 remain after the third half-life, or 12.5% of the original 1200 atoms.

Because the half-life is independent of the number of radioactive atoms, it remains constant throughout the decay process. For example, if 50% of the radioactive atoms remain after one half-life, then 25% remain after two half-lives, and 12.5% remain after three half-lives.

Kinetic information about a radioactive isotope usually is given in terms of its half-life because it provides a more intuitive sense of the isotope's stability. Knowing, for example, that the decay constant for ${}^{90}_{38}\mathrm{Sr}$ is 0.0247 yr^{-1} does not give an immediate sense of how fast it disintegrates. On the other hand, knowing that its half-life is 28.1 yr makes it clear that the concentration of ${}^{90}_{38}\mathrm{Sr}$ in a sample remains essentially constant over a short period of time.

13C.2 Instrumentation

Alpha particles, beta particles, gamma rays, and X-rays are measured by using the particle's energy to produce an amplified pulse of electrical current in a detector. These pulses are counted to give the rate of disintegration. There are three common types of detectors: gas-filled detectors, scintillation counters, and semiconductor detectors. A gas-filled detector consists of a tube that contains an inert gas, such as Ar. When a radioactive particle enters the tube it ionizes the inert gas, producing an Ar^+/e^- ion-pair. Movement of the electron toward the anode and of the Ar^+ toward the cathode generates a measurable electrical current. A GEIGER COUNTER is one example of a gas-filled detector. A SCINTILLATION COUNTER uses a fluorescent material to convert radioactive particles into easy to measure photons. For example, one solid-state scintillation counter consists of a NaI crystal that contains 0.2% TlI, which produces several thousand photons for each radioactive particle. Finally, in a semiconductor detector, adsorption of a single radioactive particle promotes thousands of electrons to the semiconductor's conduction band, increasing conductivity.

You can learn more about these radiation detectors and the signal processors used to count particles by consulting this chapter's additional resources.

13C.3 Quantitative Applications

In this section we consider three common quantitative radiochemical methods of analysis: the direct analysis of a radioactive isotope by measuring its rate of disintegration, neutron activation, and isotope dilution.

Direct Analysis of Radioactive Analytes

The concentration of a long-lived radioactive isotope remains essentially constant during the period of analysis. As shown in Example 13.9, we can use the sample's activity to calculate the number of radioactive particles in the sample.

Example 13.9

The activity in a 10.00-mL sample of wastewater that contains $^{90}_{38}Sr$ is 9.07×10^6 disintegrations/s. What is the molar concentration of $^{90}_{38}Sr$ in the sample? The half-life for $^{90}_{38}Sr$ is 28.1 yr.

Solution

Solving equation 13.37 for λ, substituting into equation 13.34, and solving for N gives

$$N = \frac{A \times t_{1/2}}{0.693}$$

Before we can determine the number of atoms of $^{90}_{38}Sr$ in the sample we must express its activity and its half-life using the same units. Converting the half-life to seconds gives $t_{1/2}$ as 8.86×10^8 s; thus, there are

$$\frac{(9.07 \times 10^{6}\ \text{disintegrations/s})\,(8.86 \times 10^{8}\ \text{s})}{0.693} = 1.16 \times 10^{16}\ \text{atoms } {}^{90}_{38}\text{Sr}$$

The concentration of ${}^{90}_{38}\text{Sr}$ in the sample is

$$\frac{1.16 \times 10^{16}\ \text{atoms } {}^{90}_{38}\text{Sr}}{(6.022 \times 10^{23}\ \text{atoms/mol})\,(0.01000\ \text{L})} = 1.93 \times 10^{-6}\ \text{M } {}^{90}_{38}\text{Sr}$$

The direct analysis of a short-lived radioactive isotope using the method outlined in Example 13.9 is less useful because it provides only a transient measure of the isotope's concentration. Instead, we can measure its activity after an elapsed time, t, and use equation 13.36 to calculate N_0.

Neutron Activation Analysis

Few analytes are naturally radioactive. For many analytes, however, we can induce radioactivity by irradiating the sample with neutrons in a process called neutron activation analysis (NAA). The radioactive element formed by NEUTRON ACTIVATION decays to a stable isotope by emitting a gamma ray, and, possibly, other nuclear particles. The rate of gamma-ray emission is proportional to the analyte's initial concentration in the sample. For example, if we place a sample containing non-radioactive ${}^{27}_{13}\text{Al}$ in a nuclear reactor and irradiate it with neutrons, the following nuclear reaction takes place.

$${}^{27}_{13}\text{Al} + {}^{1}_{0}\text{n} \longrightarrow {}^{28}_{13}\text{Al}$$

The radioactive isotope of ${}^{28}_{13}\text{Al}$ has a characteristic decay process that includes the release of a beta particle and a gamma ray.

$${}^{28}_{13}\text{Al} \longrightarrow {}^{28}_{14}\text{Al} + {}^{0}_{-1}\beta + \gamma$$

When irradiation is complete, we remove the sample from the nuclear reactor, allow any short-lived radioactive interferences to decay into the background, and measure the rate of gamma-ray emission.

The initial activity at the end of irradiation depends on the number of atoms that are present. This, in turn, is a equal to the difference between the rate of formation for ${}^{28}_{13}\text{Al}$ and its rate of disintegration

$$\frac{dN_{{}^{28}_{13}\text{Al}}}{dt} = \Phi\sigma N_{{}^{27}_{13}\text{Al}} - \lambda N_{{}^{28}_{13}\text{Al}} \qquad 13.38$$

where Φ is the neutron flux and σ is the reaction cross-section, or probability that a ${}^{27}_{13}\text{Al}$ nucleus captures a neutron. Integrating equation 13.38 over the time of irradiation, t_i, and multiplying by λ gives the initial activity, A_0, at the end of irradiation as

$$A_0 = \lambda N_{{}^{28}_{13}\text{Al}} = \Phi\sigma N_{{}^{28}_{13}\text{Al}}(1 - e^{-\lambda t})$$

If we know the values for A_0, Φ, σ, λ, and t_i, then we can calculate the number of atoms of ${}^{27}_{13}\text{Al}$ initially present in the sample.

A simpler approach is to use one or more external standards. Letting $(A_0)_x$ and $(A_0)_s$ represent the analyte's initial activity in an unknown and in an external standard, and letting w_x and w_s represent the analyte's weight in the unknown and in the external standard, we obtain the following pair of equations

$$(A_0)_x = kw_x \qquad 13.39$$

$$(A_0)_s = kw_s \qquad 13.40$$

that we can solve to determine the analyte's mass in the sample.

As noted earlier, gamma ray emission is measured following a period during which we allow short-lived interferents to decay into the background. As shown in Figure 13.17, we determine the sample's or the standard's initial activity by extrapolating a curve of activity versus time back to $t = 0$. Alternatively, if we irradiate the sample and the standard at the same time, and if we measure their activities at the same time, then we can substitute these activities for $(A_0)_x$ and $(A_0)_s$. This is the strategy used in the following example.

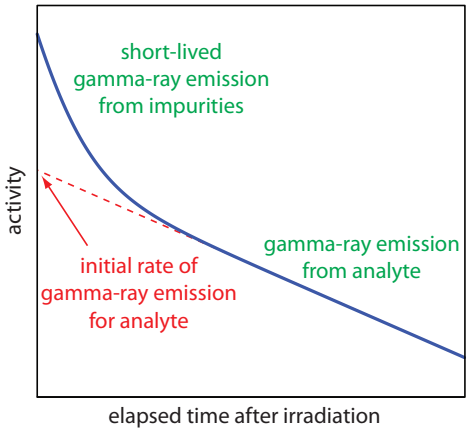


Figure 13.17 Plot of gamma-ray emission as a function of time showing how the analyte's initial activity is determined.

Example 13.10

The concentration of Mn in steel is determined by a neutron activation analysis using the method of external standards. A 1.000-g sample of an unknown steel sample and a 0.950-g sample of a standard steel known to contain 0.463% w/w Mn are irradiated with neutrons for 10 h in a nuclear reactor. After a 40-min delay the gamma ray emission is 2542 cpm (counts per minute) for the unknown and 1984 cpm for the external standard. What is the %w/w Mn in the unknown steel sample?

Solution

Combining equation 13.39 and equation 13.40 gives

$$w_x = \frac{A_x}{A_s} \times w_s$$

The weight of Mn in the external standard is

$$w_s = \frac{0.00463 \text{ g Mn}}{\text{g steel}} \times 0.950 \text{ g steel} = 0.00440 \text{ g Mn}$$

Substituting into the above equation gives

$$w_x = \frac{2542 \text{ cpm}}{1984 \text{ cpm}} \times 0.00440 \text{ g Mn} = 0.00564 \text{ g Mn}$$

Because the original mass of steel is 1.000 g, the %w/w Mn is 0.564%.

Among the advantages of neutron activation are its applicability to almost all elements in the periodic table and that it is nondestructive to the sample. Consequently, NAA is an important technique for analyzing archeological and forensic samples, as well as works of art.

How we process the sample depends on the analyte and the sample's matrix. We might, for example, digest the sample to bring the analyte into solution. After filtering the sample to remove the residual solids, we might precipitate the analyte, isolate it by filtration, dry it in an oven, and obtain its weight.

Given that the goal of an analysis is to determine the amount of nonradioactive analyte in our sample, the realization that we might not recover all the analyte might strike you as unsettling. Recall from Chapter 7G, that a single liquid–liquid extraction rarely has an extraction efficiency of 100%. One advantage of isotope dilution is that the extraction efficiency for the nonradioactive analyte and for the tracer are the same. If we recover 50% of the tracer, then we also recover 50% of the nonradioactive analyte. Because we know how much tracer we added to the sample, we can determine how much of the nonradioactive analyte is in the sample.

Isotope Dilution

Another important radiochemical method for the analysis of nonradioactive analytes is ISOTOPE DILUTION. An external source of analyte is prepared in a radioactive form with a known activity, A_T, for its radioactive decay—we call this form of the analyte a TRACER. To prepare a sample for analysis we add a known mass of the tracer, w_T, to a portion of sample that contains an unknown mass, w_x, of analyte. After homogenizing the sample and tracer, we isolate w_A grams of analyte by using a series of appropriate chemical and physical treatments. Because these chemical and physical treatments cannot distinguish between radioactive and nonradioactive forms of the analyte, the isolated material contains both. Finally, we measure the activity of the isolated sample, A_A. If we recover all the analyte—both the radioactive tracer and the nonradioactive analyte—then A_A and A_T are equal and $w_x = w_A - w_T$. Normally, we fail to recover all the analyte. In this case A_A is less than A_T, and

$$A_A = A_T \times \frac{w_A}{w_x + w_T} \qquad 13.41$$

The ratio of weights in equation 13.41 accounts for any loss of activity that results from our failure to recover all the analyte. Solving equation 13.41 for w_x gives

$$w_x = \frac{A_T}{A_A} w_A - w_T \qquad 13.42$$

Example 13.11

The concentration of insulin in a production vat is determined by isotope dilution. A 1.00-mg sample of insulin labeled with ^{14}C having an activity of 549 cpm is added to a 10.0-mL sample taken from the production vat. After homogenizing the sample, a portion of the insulin is separated and purified, yielding 18.3 mg of pure insulin. The activity for the isolated insulin is measured at 148 cpm. How many mg of insulin are in the original sample?

Solution

Substituting known values into equation 13.42 gives

$$w_x = \frac{549 \text{ cpm}}{148 \text{ cpm}} \times 18.3 \text{ mg} - 1.00 \text{ mg} = 66.9 \text{ mg insulin}$$

Equation 13.41 and equation 13.42 are valid only if the tracer's half-life is considerably longer than the time it takes to conduct the analysis. If this is not the case, then the decrease in activity is due both to the incomplete recovery and the natural decrease in the tracer's activity. Table 13.1 provides a list of several common tracers for isotope dilution.

An important feature of isotope dilution is that it is not necessary to recover all the analyte to determine the amount of analyte present in the

Table 13.1 Common Tracers for Isotope Dilution

isotope	half-life
^{3}H	12.5 years
^{14}C	5730 years
^{32}P	14.3 days
^{35}S	87.1 days
^{45}Ca	152 days
^{55}Fe	2.91 years
^{60}Co	5.3 years
^{131}I	8 days

original sample. Isotope dilution, therefore, is useful for the analysis of samples with complex matrices, where a complete recovery of the analyte is difficult.

13C.4 Characterization Applications

One example of a characterization application is the determination of a sample's age based on the decay of a radioactive isotope naturally present in the sample. The most common example is carbon-14 dating, which is used to determine the age of natural organic materials.

As cosmic rays pass through the upper atmosphere, some $^{14}_{7}N$ atoms in the atmosphere capture high energy neutrons, converting them into $^{14}_{6}C$. The $^{14}_{6}C$ then migrates into the lower atmosphere where it oxidizes to form C-14 labeled CO_2. Animals and plants subsequently incorporate this labeled CO_2 into their tissues. Because this is a steady-state process, all plants and animals have the same ratio of $^{14}_{6}C$ to $^{12}_{6}C$ in their tissues. When an organism dies, the radioactive decay of $^{14}_{6}C$ to $^{14}_{7}N$ by $^{0}_{-1}\beta$ emission ($t_{1/2}$=5730 years) leads to predictable reduction in the $^{14}_{6}C$ to $^{12}_{6}C$ ratio. We can use the change in this ratio to date samples that are as much as 30 000 years old, although the precision of the analysis is best when the sample's age is less than 7000 years. The accuracy of carbon-14 dating depends upon our assumption that the natural $^{14}_{6}C$ to $^{12}_{6}C$ ratio in the atmosphere is constant over time. Some variation in the ratio has occurred as the result of the increased consumption of fossil fuels and the production of $^{14}_{6}C$ during the testing of nuclear weapons. A calibration curve prepared using samples of known age—examples of samples include tree rings, deep ocean sediments, coral samples, and cave deposits—limits this source of uncertainty.

There is no need to prepare a calibration curve for each analysis. Instead, there is a universal calibration curve known as IntCal. The most recent such curve, IntCal13 is described in the following paper: Reimer, P. J., et. al. "IntCal13 and Marine 13 Radiocarbon Age Calibration Curve 0–50,000 Years Cal BP," *Radiocarbon* **2013**, *55*, 1869–1887. This calibration spans 50 000 years before the present (BP).

Example 13.12

To determine the age of a fabric sample, the relative ratio of $^{14}_{6}C$ to $^{12}_{6}C$ was measured yielding a result of 80.9% of that found in modern fibers. How old is the fabric?

Solution

Equation 13.36 and equation 13.37 provide us with a method to convert a change in the ratio of $^{14}_{6}C$ to $^{12}_{6}C$ to the fabric's age. Letting A_0 be the ratio of $^{14}_{6}C$ to $^{12}_{6}C$ in modern fibers, we assign it a value of 1.00. The ratio of $^{14}_{6}C$ to $^{12}_{6}C$ in the sample, A, is 0.809. Solving gives

$$t = \ln\frac{A_0}{A} \times \frac{t_{1/2}}{0.693} = \ln\frac{1.00}{0.809} \times \frac{5730\text{ yr}}{0.693} = 1750\text{ yr}$$

Other isotopes can be used to determine a sample's age. The age of rocks, for example, has been determined from the ratio of the number of radioactive atoms $^{238}_{92}U$ to the number of stable $^{206}_{82}Pb$ atoms produced by radioactive decay. For rocks that do not contain uranium, dating is accomplished by comparing the ratio of radioactive $^{40}_{19}K$ to the stable $^{40}_{18}Ar$. Another example is the dating of sediments collected from lakes by measuring the amount of $^{210}_{82}Pb$ that is present.

13C.5 Evaluation

Radiochemical methods routinely are used for the analysis of trace analytes in macro and meso samples. The accuracy and precision of radiochemical methods generally are within the range of 1–5%. We can improve the precision—which is limited by the random nature of radioactive decay—by counting the emission of radioactive particles for as long a time as is practical. If the number of counts, M, is reasonably large ($M \geq 100$), and the counting period is significantly less than the isotope's half-life, then the percent relative standard deviation for the activity, $(\sigma_A)_{rel}$, is approximately

See Figure 3.5 to review the meaning of macro and meso samples.

$$(\sigma_A)_{rel} = \frac{1}{\sqrt{M}} \times 100$$

For example, if we determine the activity by counting 10 000 radioactive particles, then the relative standard deviation is 1%. A radiochemical method's sensitivity is inversely proportional to $(\sigma_A)_{rel}$, which means we can improve the sensitivity by counting more particles.

Selectivity rarely is of concern when using a radiochemical method because most samples have only a single radioactive isotope. When several radioactive isotopes are present, we can determine each isotope's activity by taking advantage of differences in the energies of their respective radioactive particles or differences in their respective decay rates.

In comparison to most other analytical techniques, radiochemical methods usually are more expensive and require more time to complete an analysis. Radiochemical methods also are subject to significant safety concerns due to the analyst's potential exposure to high energy radiation and the need to safely dispose of radioactive waste.

13D Flow Injection Analysis

The focus of this chapter is on methods in which we measure a time-dependent signal. Chemical kinetic methods and radiochemical methods are two examples. In this section we consider the technique of flow injection analysis in which we inject the sample into a flowing carrier stream that gives rise to a transient signal at the detector. Because the shape of this transient signal depends on the physical and chemical kinetic processes that take place in the carrier stream during the time between injection and detection, we include flow injection analysis in this chapter.

13D.1 Theory and Practice

Flow injection analysis (FIA) was developed in the mid-1970s as a highly efficient technique for the automated analyses of samples.[17] Unlike the centrifugal analyzer described earlier in this chapter (see Figure 13.9), in which the number of samples is limited by the transfer disk's size, FIA allows for the rapid, sequential analysis of an unlimited number of samples. FIA is one example of a continuous-flow analyzer, in which we sequentially introduce samples at regular intervals into a liquid carrier stream that transports them to the detector.

A schematic diagram detailing the basic components of a flow injection analyzer is shown in Figure 13.18. The reagent that serves as the carrier is stored in a reservoir, and a propelling unit maintains a constant flow of the carrier through a system of tubing that comprises the transport system. We inject the sample directly into the flowing carrier stream, where it travels through one or more mixing and reaction zones before it reaches the detector's flow-cell. Figure 13.18 is the simplest design for a flow injection analyzer, which consists of a single channel and a single reagent reservoir. Multiple channel instruments that merge together separate channels, each

17 (a) Ruzicka, J.; Hansen, E. H. *Anal. Chim. Acta* **1975**, *78*, 145–157; (b) Stewart, K. K.; Beecher, G. R.; Hare, P. E. *Anal. Biochem.* **1976**, *70*, 167–173; (c) Valcárcel, M.; Luque de Castro, M. D. *Flow Injection Analysis: Principles and Applications*, Ellis Horwood: Chichester, England, 1987.

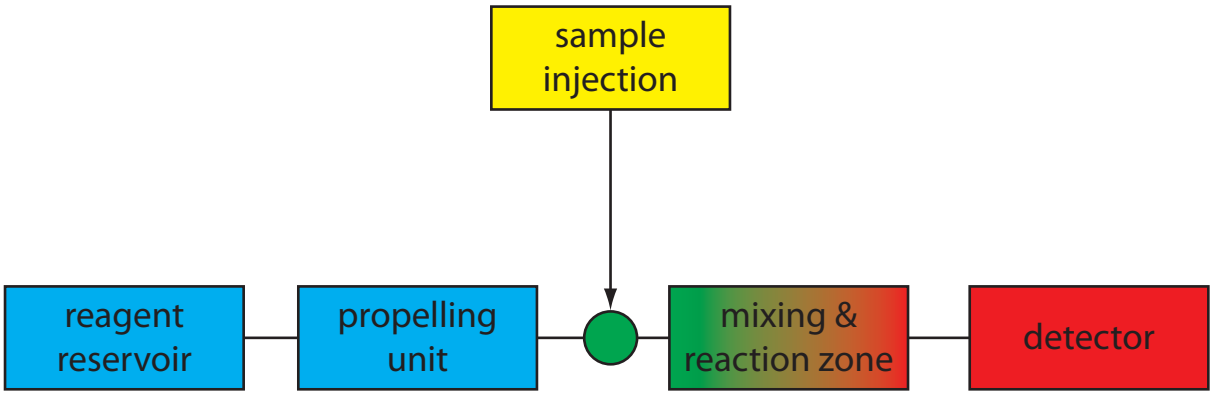


Figure 13.18 Schematic diagram of a simple flow injection analyzer showing its basic components. After its injection into the carrier stream the samples mixes and reacts with the carrier stream's reagents before reaching the detector.

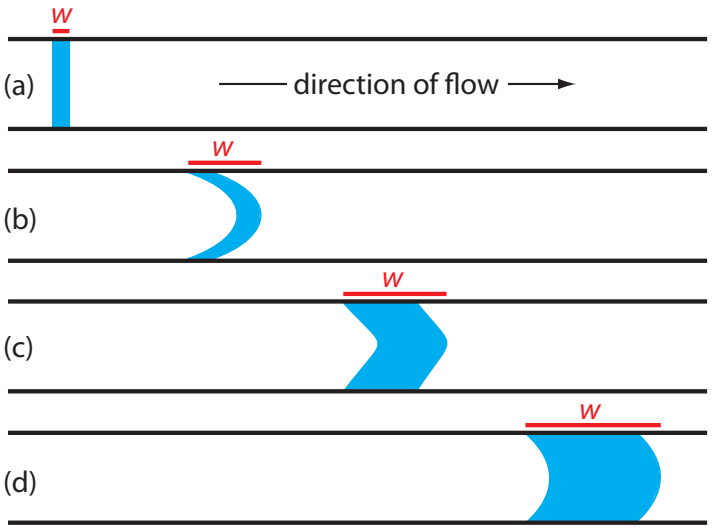


Figure 13.19 Effect of dispersion on the shape of a sample's flow profile, shown in **blue**, at different times during a flow injection analysis: (a) at injection; (b) when convection dominates dispersion; (c) when convection and diffusion contribute to dispersion; and (d) when diffusion dominates dispersion. The **red** line shows the width, w, of the samples flow profile.

of which introduces a new reagent into the carrier stream, also are possible. A more detailed discussion of FIA instrumentation is found in the next section.

When we first inject a sample into the carrier stream it has the rectangular flow profile of width w shown in Figure 13.19a. As the sample moves through the mixing zone and t he reaction zone, the width of its flow profile increases as the sample disperses into the carrier stream. Dispersion results from two processes: convection due to the flow of the carrier stream and diffusion due to the concentration gradient between the sample and the carrier stream. Convection occurs by laminar flow. The linear velocity of the sample at the tube's walls is zero, but the sample at the center of the tube moves with a linear velocity twice that of the carrier stream. The result is the parabolic flow profile shown in Figure 13.19b. Convection is the primary means of dispersion in the first 100 ms following the sample's injection.

The second contribution to the sample's dispersion is diffusion due to the concentration gradient that exists between the sample and the carrier stream. As shown in Figure 13.20, diffusion occurs parallel (axially) and perpendicular (radially) to the direction in which the carrier stream is moving. Only radial diffusion is important in a flow injection analysis. Radial diffusion decreases the sample's linear velocity at the center of the tubing, while the sample at the edge of the tubing experiences an increase in its linear velocity. Diffusion helps to maintain the integrity of the sample's flow profile (Figure 13.19c) and prevents adjacent samples in the carrier stream from dispersing into one another. Both convection and diffusion make significant contributions to dispersion from approximately 3–20 s after the sample's injection. This is the normal time scale for a flow injection analysis. After approximately 25 s, diffusion is the only significant contributor to dispersion, resulting in a flow profile similar to that shown in Figure 13.19d.

An FIA curve, or FIAGRAM, is a plot of the detector's signal as a function of time. Figure 13.21 shows a typical fiagram for conditions in which both

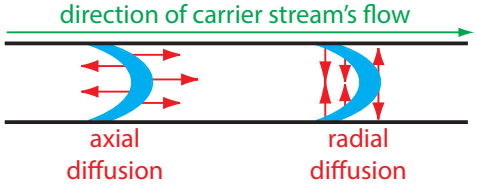


Figure 13.20 Illustration showing axial and radial diffusion. The **blue** band is the sample's flow profile and the **red** arrows indicate the direction of diffusion.

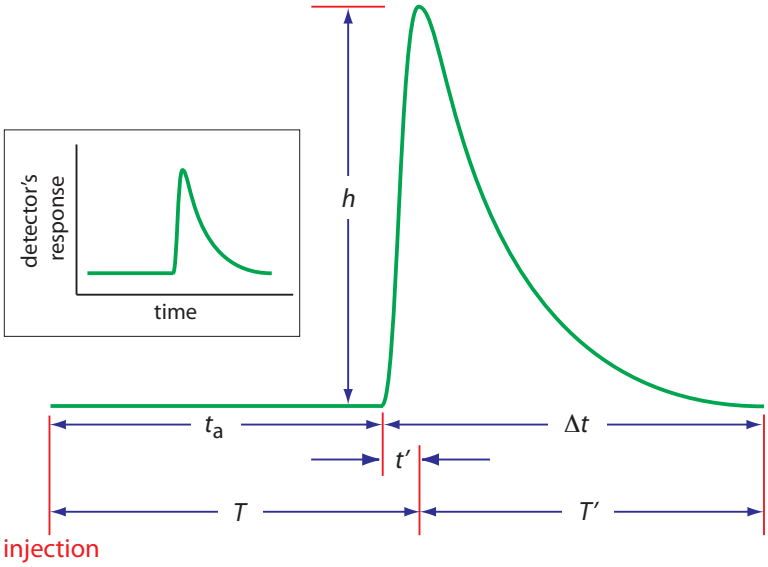


Figure 13.21 Typical fiagram for flow injection analysis showing the detector's response as a function of time. See the text for an explanation of the parameters t_a, t', Δt, T, T', and h.

convection and diffusion contribute to the sample's dispersion. Also shown on the figure are several parameters that characterize a sample's fiagram. Two parameters define the time for a sample to move from the injector to the detector. Travel time, t_a, is the time between the sample's injection and the arrival of its leading edge at the detector. Residence time, T, on the other hand, is the time required to obtain the maximum signal. The difference between the residence time and the travel time is t', which approaches zero when convection is the primary means of dispersion, and increases in value as the contribution from diffusion becomes more important.

The time required for the sample to pass through the detector's flow cell—and for the signal to return to the baseline—is also described by two parameters. The baseline-to-baseline time, Δt, is the time between the arrival of the sample's leading edge to the departure of its trailing edge. The elapsed time between the maximum signal and its return to the baseline is the return time, T'. The final characteristic parameter of a fiagram is the sample's peak height, h.

Of the six parameters shown in Figure 13.21, the most important are peak height and the return time. Peak height is important because it is directly or indirectly related to the analyte's concentration. The sensitivity of an FIA method, therefore, is determined by the peak height. The return time is important because it determines the frequency with which we may inject samples. Figure 13.22 shows that if we inject a second sample at a time T' after we inject the first sample, there is little overlap of the two FIA curves. By injecting samples at intervals of T', we obtain the maximum possible sampling rate.

Peak heights and return times are influenced by the dispersion of the sample's flow profile and by the physical and chemical properties of the flow injection system. Physical parameters that affect h and T' include the volume of sample we inject, the flow rate, the length, diameter and geometry

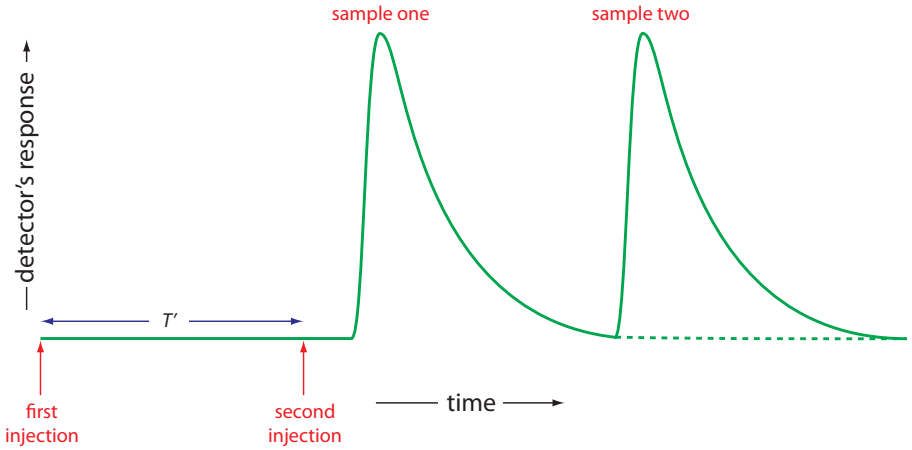


Figure 13.22 Effect of return time, T', on sampling frequency.

of the mixing zone and the reaction zone, and the presence of junctions where separate channels merge together. The kinetics of any chemical reactions between the sample and the reagents in the carrier stream also influence the peak height and return time.

Unfortunately, there is no good theory that we can use to consistently predict the peak height and the return time for a given set of physical and chemical parameters. The design of a flow injection analyzer for a particular analytical problem still occurs largely by a process of experimentation. Nevertheless, we can make some general observations about the effects of physical and chemical parameters. In the absence of chemical effects, we can improve sensitivity—that is, obtain larger peak heights—by injecting larger samples, by increasing the flow rate, by decreasing the length and diameter of the tubing in the mixing zone and the reaction zone, and by merging separate channels before the point where the sample is injected. With the exception of sample volume, we can increase the sampling rate—that is, decrease the return time—by using the same combination of physical parameters. Larger sample volumes, however, lead to longer return times and a decrease in sample throughput. The effect of chemical reactivity depends on whether the species we are monitoring is a reactant or a product. For example, if we are monitoring a reactant, we can improve sensitivity by choosing conditions that decrease the residence time, T, or by adjusting the carrier stream's composition so that the reaction occurs more slowly.

13D.2 Instrumentation

The basic components of a flow injection analyzer are shown in Figure 13.23 and include a pump to propel the carrier stream and the reagent streams, a means to inject the sample into the carrier stream, and a detector to monitor the composition of the carrier stream. Connecting these units is a transport system that brings together separate channels and provides time for the sample to mix with the carrier stream and to react with the reagent

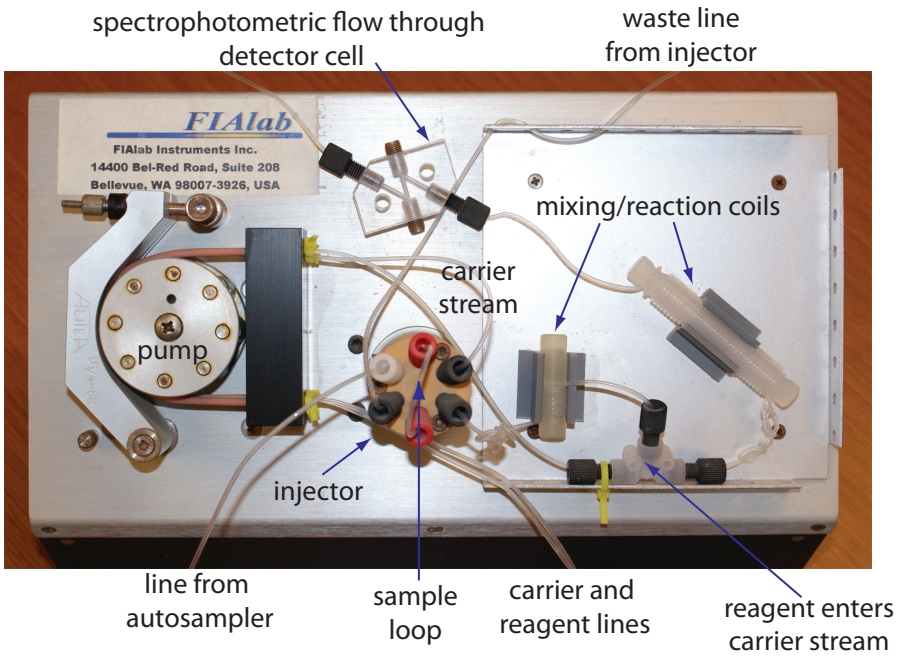


Figure 13.23 Example of a typical flow injection analyzer that shows the pump, the injector, the transport system, which consists of mixing/reaction coils and junctions, and the detector (minus the spectrophotometer). This particular configuration has two channels: the carrier stream and a reagent line.

streams. We also can incorporate separation modules into the transport system. Each of these components is considered in greater detail in this section.

Propelling Unit

The propelling unit moves the carrier stream through the flow injection analyzer. Although several different propelling units have been used, the most common is a PERISTALTIC PUMP, which, as shown in Figure 13.24, consists of a set of rollers attached to the outside of a rotating drum. Tubing from the reagent reservoirs fits between the rollers and a fixed plate. As the drum rotates the rollers squeeze the tubing, forcing the contents of the tubing to move in the direction of the rotation. Peristaltic pumps provide a constant flow rate, which is controlled by the drum's speed of rotation and the inner diameter of the tubing. Flow rates from 0.0005–40 mL/min are possible, which is more than adequate to meet the needs of FIA where flow rates of 0.5–2.5 mL/min are common. One limitation to a peristaltic pump is that it produces a pulsed flow—particularly at higher flow rates—that may lead to oscillations in the signal.

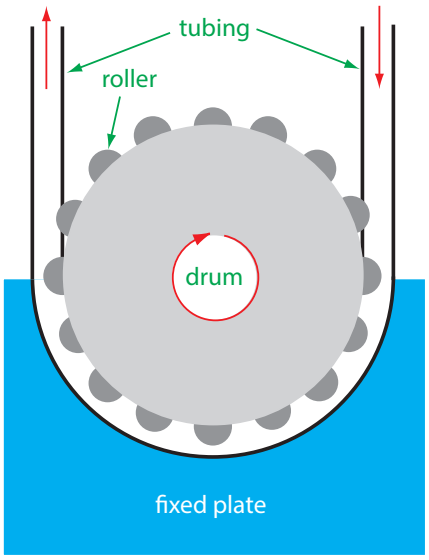


Figure 13.24 Schematic diagram of a peristaltic pump.

Injector

The sample, typically 5–200 μL, is injected into the carrier stream. Although syringe injections through a rubber septum are possible, the more common method—as seen in Figure 13.23—is to use a rotary, or loop injector similar to that used in an HPLC. This type of injector provides for

Figure 12.39 and Figure 12.45 show examples of an HPLC loop injector.

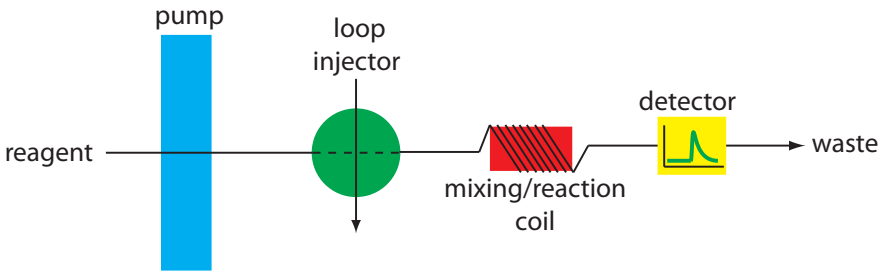


Figure 13.25 Example of a single-channel manifold in which the reagent serves as the carrier stream and as a species that reacts with the sample. The mixing/reaction coil is wrapped around a plastic cylinder.

a reproducible sample volume and is easily adaptable to automation, an important feature when high sampling rates are needed.

Detector

The most common detectors for flow injection analysis are the electrochemical and optical detectors used in HPLC. These detectors are discussed in Chapter 12 and are not considered further in this section. FIA detectors also have been designed around the use of ion selective electrodes and atomic absorption spectroscopy.

Transport System

The heart of a flow injection analyzer is the transport system that brings together the carrier stream, the sample, and any reagents that react with the sample. Each reagent stream is considered a separate channel, and all channels must merge before the carrier stream reaches the detector. The complete transport system is called a MANIFOLD.

The simplest manifold has a single channel, the basic outline of which is shown in Figure 13.25. This type of manifold is used for direct analysis of analyte that does not require a chemical reaction. In this case the carrier stream serves only as a means for rapidly and reproducibly transporting the sample to the detector. For example, this manifold design has been used for sample introduction in atomic absorption spectroscopy, achieving sampling rates as high as 700 samples/h. A single-channel manifold also is used for determining a sample's pH or determining the concentration of metal ions using an ion selective electrode.

We can also use the single-channel manifold in Figure 13.25 for an analysis in which we monitor the product of a chemical reaction between the sample and a reactant. In this case the carrier stream both transports the sample to the detector and reacts with the sample. Because the sample must mix with the carrier stream, a lower flow rate is used. One example is the determination of chloride in water, which is based on the following sequence of reactions.

$$Hg(SCN)_2(aq) + 2Cl^-(aq) \rightleftharpoons HgCl_2(aq) + 2SCN^-(aq)$$

$$Fe^{3+}(aq) + SCN^-(aq) \rightleftharpoons Fe(SCN)^{2+}(aq)$$

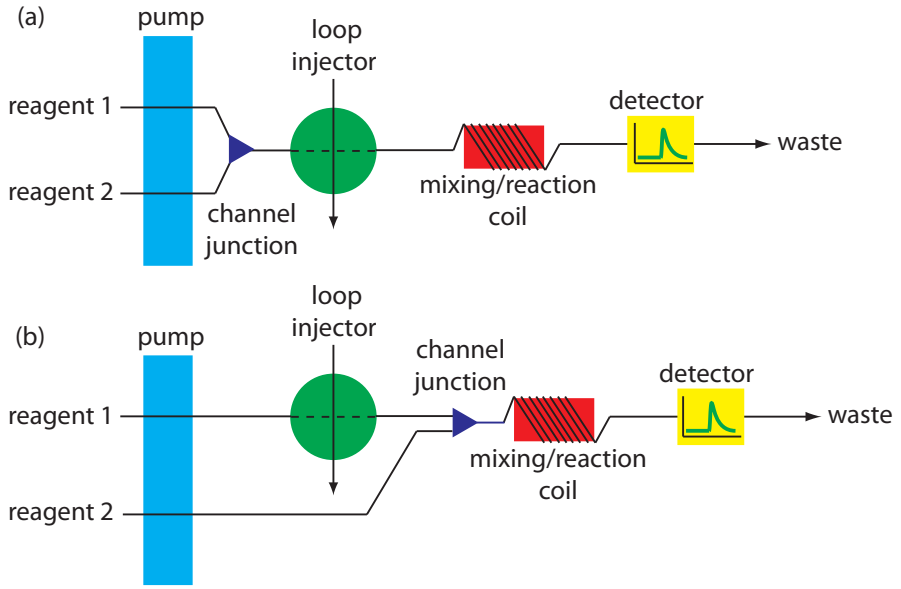


Figure 13.26 Two examples of a dual-channel manifold for flow injection analysis. In (a) the two channels merge before the loop injector, and in (b) the two channels merge after the loop injector.

The carrier stream consists of an acidic solution of $Hg(SCN)_2$ and Fe^{3+}. Injecting a sample that contains chloride into the carrier stream displaces thiocyanate from $Hg(SCN)_2$. The displaced thiocyanate then reacts with Fe^{3+} to form the red-colored $Fe(SCN)^{2+}$ complex, the absorbance of which is monitored at a wavelength of 480 nm. Sampling rates of approximately 120 samples per hour have been achieved with this system.[18]

Most flow injection analyses that include a chemical reaction use a manifold with two or more channels. Including additional channels provides more control over the mixing of reagents and the interaction between the reagents and the sample. Two configurations are possible for a dual-channel system. A dual-channel manifold, such as the one shown in Figure 13.26a, is used when the reagents cannot be premixed because of their reactivity. For example, in acidic solutions phosphate reacts with molybdate to form the heteropoly acid $H_3P(Mo_{12}O_{40})$. In the presence of ascorbic acid the molybdenum in the heteropoly acid is reduced from Mo(VI) to Mo(V), forming a blue-colored complex that is monitored spectrophotometrically at 660 nm.[18] Because ascorbic acid reduces molybdate, the two reagents are placed in separate channels that merge just before the loop injector.

A dual-channel manifold also is used to add a second reagent after injecting the sample into a carrier stream, as shown in Figure 13.26b. This style of manifold is used for the quantitative analysis of many analytes, including the determination of a wastewater's chemical oxygen demand (COD).[19] Chemical oxygen demand is a measure of the amount organic matter in the wastewater sample. In the conventional method of analysis, COD is determined by refluxing the sample for 2 h in the presence of acid and a strong oxidizing agent, such as $K_2Cr_2O_7$ or $KMnO_4$. When refluxing is complete, the amount of oxidant consumed in the reaction is determined

You will find a more detailed description of the redox titrimetric method for COD in Chapter 9.

18 Hansen, E. H.; Ruzicka, J. *J. Chem. Educ.* **1979**, *56*, 677–680.
19 Korenaga, T.; Ikatsu, H. *Anal. Chim. Acta* **1982**, *141*, 301–309.

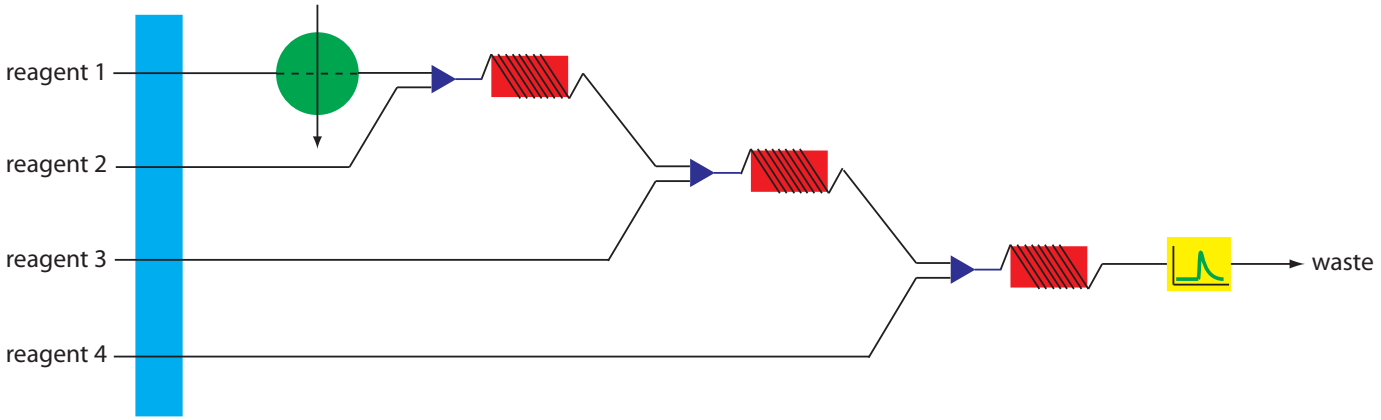


Figure 13.27 Example of a four-channel manifold for a flow injection analysis.

by a redox titration. In the flow injection version of this analysis, the sample is injected into a carrier stream of aqueous H_2SO_4, which merges with a solution of the oxidant from a secondary channel. The oxidation reaction is kinetically slow and, as a result, the mixing coil and the reaction coil are very long—typically 40 m—and submerged in a thermostated bath. The sampling rate is lower than that for most flow injection analyses, but at 10–30 samples/h it is substantially greater than the redox titrimetric method.

More complex manifolds involving three or more channels are common, but the possible combination of designs is too numerous to discuss. One example of a four-channel manifold is shown in Figure 13.27.

Separation Modules

By incorporating a separation module into the flow injection manifold we can include a separation—dialysis, gaseous diffusion and liquid-liquid extractions are examples—in a flow injection analysis. Although these separations are never complete, they are reproducible if we carefully control the experimental conditions.

Dialysis and gaseous diffusion are accomplished by placing a semipermeable membrane between the carrier stream containing the sample and an acceptor stream, as shown in Figure 13.28. As the sample stream passes through the separation module, a portion of those species that can cross the semipermeable membrane do so, entering the acceptor stream. This type of separation module is common for the analysis of clinical samples, such as serum and urine, where a dialysis membrane separates the analyte from its complex matrix. Semipermeable gaseous diffusion membranes are used for the determination of ammonia and carbon dioxide in blood. For example, ammonia is determined by injecting the sample into a carrier stream of aqueous NaOH. Ammonia diffuses across the semipermeable membrane into an acceptor stream that contains an acid–base indicator. The resulting acid–base reaction between ammonia and the indicator is monitored spectrophotometrically.

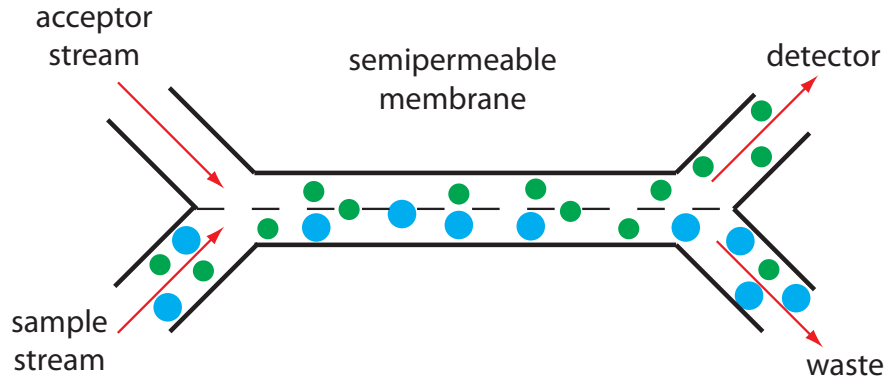


Figure 13.28 Separation module for a flow injection analysis using a semipermeable membrane. The smaller **green** solutes can pass through the semipermeable membrane and enter the acceptor stream, but the larger **blue** solutes cannot. Although the separation is not complete—note that some of the **green** solute remains in the sample stream and exits as waste—it is reproducible if we do not change the experimental conditions.

Liquid–liquid extractions are accomplished by merging together two immiscible fluids, each carried in a separate channel. The result is a segmented flow through the separation module, consisting of alternating portions of the two phases. At the outlet of the separation module the two fluids are separated by taking advantage of the difference in their densities. Figure 13.29 shows a typical configuration for a separation module in which the sample is injected into an aqueous phase and extracted into a less dense organic phase that passes through the detector.

13D.3 Quantitative Applications

In a quantitative flow injection method a calibration curve is determined by injecting a series of external standards that contain known concentrations of analyte. The calibration curve's format—examples include plots of absorbance versus concentration and of potential versus concentration—depends on the method of detection. Calibration curves for standard spectroscopic and electrochemical methods are discussed in Chapter 10 and in Chapter 11, respectively and are not considered further in this chapter.

Example 13.13 shows a typical example of a calibration curve for a flow injection analysis.

Flow injection analysis has been used to analyze a wide variety of samples, including environmental, clinical, agricultural, industrial, and phar-

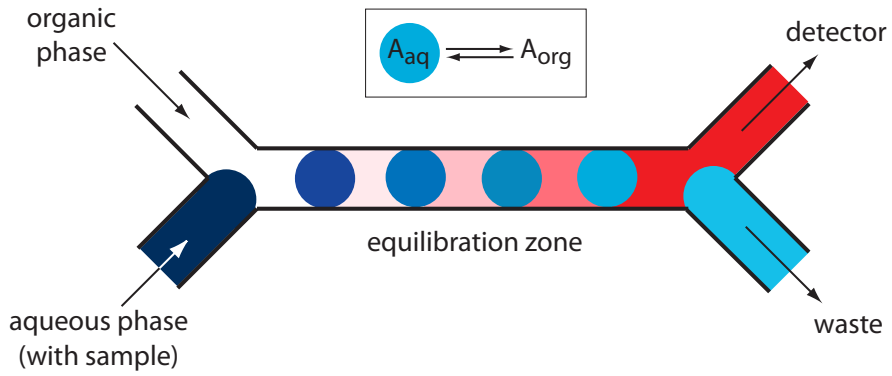


Figure 13.29 Separation module for flow injection analysis using a liquid–liquid extraction. The inset shows the equilibrium reaction. As the sample moves through the equilibration zone, the **analyte** extracts into the organic phase.

Table 13.2 Selected Flow Injection Analysis Methods for Environmental Samples

analyte	sample	sample volume (μL)	concentration range	sampling frequency (h^{-1})
Ca^{2+}	freshwater	20	0.8–7.2 ppm	80
Cu^{2+}	groundwater	70–700	100–400 ppb	20
Pb^{2+}	groundwater	70–700	0–40 ppb	20
Zn^{2+}	sea water	1000	1–100 ppb	30-60
NH_4^+	sea water	60	0.18–18.1 ppb	288
NO_3^-	rain water	1000	1–10 ppm	40
SO_4^{2-}	fresh water	400	4–140 ppm	180
CN^-	industrial	10	0.3–100 ppm	40

Source: Adapted from Valcárcel, M.; Luque de Castro, M. D. *Flow-Injection Analysis: Principles and Practice*, Ellis Horwood: Chichester, England, 1987.

maceutical samples. The majority of analyses involve environmental and clinical samples, which is the focus of this section.

The three examples are: the determination of chloride, the determination of phosphate, and the determination of chemical oxygen demand.

Quantitative flow injection methods have been developed for cationic, anionic, and molecular pollutants in wastewater, freshwaters, groundwaters, and marine waters, three examples of which were described in the previous section. Table 13.2 provides a partial listing of other analytes that have been determined using FIA, many of which are modifications of standard spectrophotometric and potentiometric methods. An additional advantage of FIA for environmental analysis is the ability to provide for the continuous, in situ monitoring of pollutants in the field.[20]

As noted in Chapter 9, several standard methods for the analysis of water involve an acid–base, complexation, or redox titration. It is easy to adapt these titrations to FIA using a single-channel manifold similar to that shown in Figure 13.25.[21] The titrant—whose concentration must be stoichiometrically less than that of the analyte—and a visual indicator are placed in the reagent reservoir and pumped continuously through the manifold. When we inject the sample it mixes thoroughly with the titrant in the carrier stream. The reaction between the analyte, which is in excess, and the titrant produces a relatively broad rectangular flow profile for the sample. As the sample moves toward the detector, additional mixing occurs and the width of the sample's flow profile decreases. When the sample passes through the detector, we determine the width of its flow profile, Δt, by monitoring the indicator's absorbance. A calibration curve of Δt versus log[analyte] is prepared using standard solutions of analyte.

Flow injection analysis has also found numerous applications in the analysis of clinical samples, using both enzymatic and nonenzymatic methods. Table 13.3 summarizes several examples.

20 Andrew, K. N.; Blundell, N. J.; Price, D.; Worsfold, P. J. *Anal. Chem.* **1994**, *66*, 916A–922A.
21 Ramsing, A. U.; Ruzicka, J.; Hansen, E. H. *Anal. Chim. Acta* **1981**, *129*, 1–17.

Table 13.3 Selected Flow Injection Analysis Methods for Clinical Samples

analyte	sample	sample volume (μL)	concentration range	sampling frequency (h^{-1})
nonenzymatic methods				
Cu^{2+}	serum	20	0.7–1.5 ppm	70
Cl^-	serum	60	50–150 meq/L	125
PO_4^{3-}	serum	200	10–60 ppm	130
total CO_2	serum	50	10–50 mM	70
chloropromazine	blood plasma	200	1.5–9 μM	24
enzymatic methods				
glucose	blood serum	26.5	0.5–15 mM	60
urea	blood serum	30	4–20 mM	60
ethanol	blood	30	5–30 ppm	50

Source: Adapted from Valcárcel, M.; Luque de Castro, M. D. *Flow-Injection Analysis: Principles and Practice*, Ellis Horwood: Chichester, England, 1987.

Representative Method 13.2

Determination of Phosphate by FIA

Description of Method

The FIA determination of phosphate is an adaptation of a standard spectrophotometric analysis for phosphate. In the presence of acid, phosphate reacts with ammonium molybdate to form a yellow-colored complex in which molybdenum is present as Mo(VI).

$$H_3PO_4(aq) + 12H_2MoO_4(aq) \rightleftharpoons H_3P(Mo_{12}O_{40})(aq) + 12H_2O(l)$$

In the presence of a reducing agent, such as ascorbic acid, the yellow-colored complex is reduced to a blue-colored complex of Mo(V).

Procedure

Prepare the following three solutions: (a) 5.0 mM ammonium molybdate in 0.40 M HNO_3; (b) 0.7% w/v ascorbic acid in 1% v/v glycerin; and a (c) 100.0 ppm phosphate standard using KH_2PO_4. Using the phosphate standard, prepare a set of external standards with phosphate concentrations of 10, 20, 30, 40, 50 and 60 ppm. Use a manifold similar to that shown in Figure 13.26a, placing a 50-cm mixing coil between the pump and the loop injector and a 50-cm reaction coil between the loop injector and the detector. For both coils, use PTFE tubing with an internal diameter of 0.8 mm. Set the flow rate to 0.5 mL/min. Prepare a calibration curve by injecting 50 μL of each standard, measuring the absorbance at 650 nm. Samples are analyzed in the same manner.

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 phosphate provides an instructive example of a typical procedure. The description here is based on Guy, R. D.; Ramaley, L.; Wentzell, P. D. "An Experiment in the Sampling of Solids for Chemical Analysis," *J. Chem. Educ.* **1998**, *75*, 1028–1033. As the title suggests, the primary focus of this chapter is on sampling. A flow injection analysis, however, is used to analyze samples.

Questions

1. How long does it take a sample to move from the loop injector to the detector?

   The reaction coil is 50-cm long with an internal diameter of 0.8 mm. The volume of this tubing is

   $$V = l\pi r^2 = 50\text{ cm} \times 3.14 \times \left(\frac{0.08\text{ cm}}{2}\right)^2 = 0.25\text{ cm}^3 = 0.25\text{ mL}$$

   With a flow rate of 0.5 mL/min, it takes about 30 s for a sample to pass through the system.

2. The instructions for the standard spectrophotometric method indicate that the absorbance is measured 5–10 min after adding the ascorbic acid. Why is this waiting period necessary in the spectrophotometric method, but not necessary in the FIA method?

   The reduction of the yellow-colored Mo(VI) complex to the blue-colored Mo(V) complex is a slow reaction. In the standard spectrophotometric method it is difficult to control reproducibly the time between adding the reagents to the sample and measuring the sample's absorbance. To achieve good precision we allow the reaction to proceed to completion before we measure the absorbance. As seen by the answer to the previous question, in the FIA method the flow rate and the dimensions of the reaction coil determine the reaction time. Because this time is controlled precisely, the reaction occurs to the same extent for all standards and samples. A shorter reaction time has the advantage of allowing for a higher throughput of samples.

3. The spectrophotometric method recommends using phosphate standards of 2–10 ppm. Explain why the FIA method uses a different range of standards.

   In the FIA method we measure the absorbance before the formation of the blue-colored Mo(V) complex is complete. Because the absorbance for any standard solution of phosphate is always smaller when using the FIA method, the FIA method is less sensitive and higher concentrations of phosphate are necessary.

4. How would you incorporate a reagent blank into the FIA analysis?

   A reagent blank is obtained by injecting a sample of distilled water in place of the external standard or the sample. The reagent blank's absorbance is subtracted from the absorbances obtained for the standards and samples.

Example 13.13

The following data were obtained for a set of external standards when using Representative Method 13.2 to analyze phosphate in a wastewater sample.

$[PO_4^{3-}]$ (ppm)	absorbance
10.00	0.079
20.00	0.160
30.00	0.233
40.00	0.316
60.00	0.482

What is the concentration of phosphate in a sample if it gives an absorbance of 0.287?

Solution

Figure 13.30 shows the external standards calibration curve and the calibration equation. Substituting in the sample's absorbance gives the concentration of phosphate in the sample as 36.1 ppm.

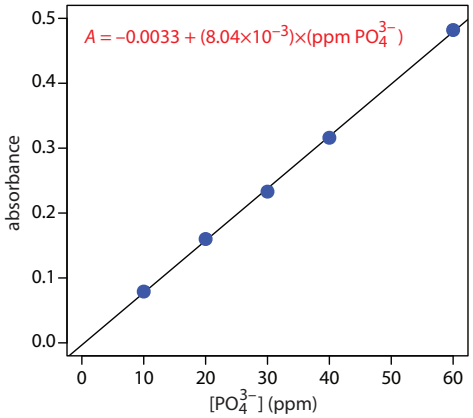


Figure 13.30 Calibration curve and equation for the data in Example 13.13.

13D.4 Evaluation

The majority of flow injection analysis applications are modifications of conventional titrimetric, spectrophotometric, and electrochemical methods of analysis; thus, it is appropriate to compare FIA methods to these conventional methods. The scale of operations for FIA allows for the routine analysis of minor and trace analytes, and for macro, meso, and micro samples. The ability to work with microliter injection volumes is useful when the sample is scarce. Conventional methods of analysis usually have smaller detection limits.

See Figure 3.5 to review the meaning minor and trace analytes, and the meaning of macro, meso and micro samples.

The accuracy and precision of FIA methods are comparable to conventional methods of analysis; however, the precision of FIA is influenced by several variables that do not affect conventional methods, including the stability of the flow rate and the reproducibility of the sample's injection. In addition, results from FIA are more susceptible to temperature variations.

In general, the sensitivity of FIA is less than that for conventional methods of analysis for at least two reasons. First, as with chemical kinetic methods, measurements in FIA are made under nonequilibrium conditions when the signal has yet to reach its maximum value. Second, dispersion dilutes the sample as it moves through the manifold. Because the variables that affect sensitivity are known, we can design the FIA manifold to optimize the method's sensitivity.

Selectivity for an FIA method often is better than that for the corresponding conventional method of analysis. In many cases this is due to

the kinetic nature of the measurement process, in which potential interferents may react more slowly than the analyte. Contamination from external sources also is less of a problem because reagents are stored in closed reservoirs and are pumped through a system of transport tubing that is closed to the environment.

Finally, FIA is an attractive technique when considering time, cost, and equipment. When using an autosampler, a flow injection method can achieve very high sampling rates. A sampling rate of 20–120 samples/h is not unusual and sampling rates as high as 1700 samples/h are possible. Because the volume of the flow injection manifold is small, typically less than 2 mL, the consumption of reagents is substantially smaller than that for a conventional method. This can lead to a significant decrease in the cost per analysis. Flow injection analysis does require the need for additional equipment—a pump, a loop injector, and a manifold—which adds to the cost of an analysis.

For a review of the importance of flow-injection analysis, see Hansen, E. H.; Miró, M. "How Flow-Injection Analysis (FIA) Over the Past 25 Years has Changed Our Way of Performing Chemical Analyses," *TRAC, Trends Anal. Chem.* **2007**, *26*, 18–26.

13E Key Terms

alpha particle	beta particle	centrifugal analyzer
competitive inhibitor	curve-fitting method	enzyme
equilibrium method	fiagram	flow injection analysis
gamma ray	Geiger counter	half-life
inhibitor	initial rate	integrated rate law
intermediate rate	isotope	isotope dilution
kinetic method	Lineweaver-Burk plot	manifold
Michaelis constant	negatron	neutron activation
noncompetitive inhibitor	one-point fixed-time integral method	peristaltic pump
positron	quench	rate
rate constant	rate law	rate method
scintillation counter	steady-state approximation	stopped-flow analyzer
substrate	tracer	two-point fixed-time integral method
uncompetitive inhibitor	variable time integral method	

13F Summary

Kinetic methods of analysis use the rate of a chemical or s physical process to determine an analyte's concentration. Three types of kinetic methods are discussed in this chapter: chemical kinetic methods, radiochemical methods, and flow injection methods.

Chemical kinetic methods use the rate of a chemical reaction and either its integrated or its differential rate law. For an integral method, we determine the concentration of analyte—or the concentration of a reactant or

product that is related stoichiometrically to the analyte—at one or more points in time following the reaction's initiation. The initial concentration of analyte is then determined using the integrated form of the reaction's rate law. Alternatively, we can measure the time required to effect a given change in concentration. In a differential kinetic method we measure the rate of the reaction at a time t, and use the differential form of the rate law to determine the analyte's concentration.

Chemical kinetic methods are particularly useful for reactions that are too slow for other analytical methods. For reactions with fast kinetics, automation allows for sampling rates of more than 100 samples/h. Another important application of chemical kinetic methods is the quantitative analysis of enzymes and their substrates, and the characterization of enzyme catalysis.

Radiochemical methods of analysis take advantage of the decay of radioactive isotopes. A direct measurement of the rate at which a radioactive isotope decays is used to determine its concentration. For an analyte that is not naturally radioactive, neutron activation can be used to induce radioactivity. Isotope dilution, in which we spike a radioactively-labeled form of analyte into the sample, is used as an internal standard for quantitative work.

In flow injection analysis we inject the sample into a flowing carrier stream that usually merges with additional streams of reagents. As the sample moves with the carrier stream it both reacts with the contents of the carrier stream and with any additional reagent streams, and undergoes dispersion. The resulting fiagram of signal versus time bears some resemblance to a chromatogram. Unlike chromatography, however, flow injection analysis is not a separation technique. Because all components in a sample move with the carrier stream's flow rate, it is possible to introduce a second sample before the first sample reaches the detector. As a result, flow injection analysis is ideally suited for the rapid throughput of samples.

13G Problems

1. Equation 13.18 shows how $[A]_0$ is determined using a two-point fixed-time integral method in which the concentration of A for the pseudo-first-order reaction

$$A + R \longrightarrow P$$

is measured at times t_1 and t_2. Derive a similar equation for the case where the product is monitored under pseudo-first order conditions.

2. The concentration of phenylacetate is determined from the kinetics of its pseudo-first order hydrolysis reaction in an ethylamine buffer. When a standard solution of 0.55 mM phenylacetate is analyzed, the concentration of phenylacetate after 60 s is 0.17 mM. When a sample

is analyzed the concentration of phenylacetate that remains after 60 s is 0.23 mM. What is the concentration of phenylacetate in the sample?

3. In the presence of acid, iodide is oxidized by hydrogen peroxide

$$2I^-(aq) + H_2O_2(aq) + 2H_3O^+(aq) \longrightarrow 4H_2O(l) + I_2(aq)$$

When I^- and H_3O^+ are present in excess, we can use the reaction's kinetics of the reaction, which is pseudo-first order in H_2O_2, to determine the concentration of H_2O_2 by following the production of I_2 with time. In one analysis the solution's absorbance at 348 nm was measured after 240 s. Analysis of a set of standard gives the results shown below.

$[H_2O_2]$ (μM)	absorbance
100.0	0.236
200.0	0.471
400.0	0.933
800.0	1.872

What is the concentration of H_2O_2 in a sample if its absorbance is 0.669 after 240 s?

4. The concentration of chromic acid is determined by reducing it under conditions that are pseudo-first order in analyte. One approach is to monitor the reaction absorbance at a wavelength of 355 nm. A standard of 5.1×10^{-4} M chromic acid yields absorbances of 0.855 and 0.709 at 100 s and 300 s after the reaction's initiation. When a sample is analyzed under identical conditions, the absorbances are 0.883 and 0.706. What is the concentration of chromic acid in the sample?

5. Malmstadt and Pardue developed a variable time method for the determination of glucose based on its oxidation by the enzyme glucose oxidase.[22] To monitor the reaction's progress, iodide is added to the samples and standards. The H_2O_2 produced by the oxidation of glucose reacts with I^-, forming I_2 as a product. The time required to produce a fixed amount of I_2 is determined spectrophotometrically. The following data was reported for a set of calibration standards

[glucose] (ppm)	time (s)		
5.0	146.5	150.0	149.6
10.0	69.2	67.1	66.0
20.0	34.8	35.0	34.0
30.0	22.3	22.7	22.6
40.0	16.7	16.5	17.0
50.0	13.3	13.3	13.8

22 Malmstadt, H. V.; Pardue, H. L. *Anal. Chem.* **1961** *33*, 1040–1047.

To verify the method a standard solution of 20.0 ppm glucose was analyzed in the same way as the standards, requiring 34.6 s to produce the same extent of reaction. Determine the concentration of glucose in the standard and the percent error for the analysis.

6. Deming and Pardue studied the kinetics for the hydrolysis of *p*-nitrophenyl phosphate by the enzyme alkaline phosphatase.[23] The reaction's progress was monitored by measuring the absorbance of *p*-nitrophenol, which is one of the reaction's products. A plot of the reaction's rate (with units of μmol mL^{-1} sec^{-1}) versus the volume, *V*, in milliliters of a serum calibration standard that contained the enzyme, yielded a straight line with the following equation.

$$\text{rate} = 2.7 \times 10^{-7}\,\mu\text{mol mL}^{-1}\text{s}^{-1} + (3.485 \times 10^{-5}\,\mu\text{mol mL}^{-2}\text{s}^{-1})\,V$$

A 10.00-mL sample of serum is analyzed, yielding a rate of 6.84×10^{-5} μmol mL^{-1} sec^{-1}. How much more dilute is the enzyme in the serum sample than in the serum calibration standard?

7. The following data were collected for a reaction known to be pseudo-first order in analyte, *A*, during the time in which the reaction is monitored.

time (s)	$[A]_t$ (mM)
2	1.36
4	1.24
6	1.12
8	1.02
10	0.924
12	0.838
14	0.760
16	0.690
18	0.626
20	0.568

What is the rate constant and the initial concentration of analyte in the sample?

8. The enzyme acetylcholinesterase catalyzes the decomposition of acetylcholine to choline and acetic acid. Under a given set of conditions the enzyme has a K_m of 9×10^{-5} M and a k_2 of 1.4×10^4 s^{-1}. What is the concentration of acetylcholine in a sample if the reaction's rate is 12.33 μM s^{-1} in the presence of 6.61×10^{-7} M enzyme? You may assume the concentration of acetylcholine is significantly smaller than K_m.

23 Deming, S. N.; Pardue, H. L. *Anal. Chem.* **1971**, *43*, 192–200.

9. The enzyme fumarase catalyzes the stereospecific addition of water to fumarate to form L-malate. A standard 0.150 μM solution of fumarase has a rate of reaction of 2.00 μM min^{-1} under conditions in which the substrate's concentration is significantly greater than K_m. The rate of reaction for a sample under identical condition is 1.15 μM min^{-1}. What is the concentration of fumarase in the sample?

10. The enzyme urease catalyzes the hydrolysis of urea. The rate of this reaction is determined for a series of solutions in which the concentration of urea is changed while maintaining a fixed urease concentration of 5.0 μM. The following data are obtained.

[urea] (μM)	rate (μM s^{-1})
0.100	6.25
0.200	12.5
0.300	18.8
0.400	25.0
0.500	31.2
0.600	37.5
0.700	43.7
0.800	50.0
0.900	56.2
1.00	62.5

Determine the values of V_{max}, k_2, and K_m for urease.

11. To study the effect of an enzyme inhibitor V_{max} and K_m are measured for several concentrations of inhibitor. As the concentration of the inhibitor increases V_{max} remains essentially constant, but the value of K_m increases. Which mechanism for enzyme inhibition is in effect?

12. In the case of competitive inhibition, the equilibrium between the enzyme, E, the inhibitor, I, and the enzyme–inhibitor complex, EI, is described by the equilibrium constant K_{EI}. Show that for competitive inhibition the equation for the rate of reaction is

$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m\{1 + ([I]/K_{EI})\} + [S]}$$

where K_I is the formation constant for the EI complex

$$E + I \rightleftharpoons EI$$

You may assume that $k_2 << k_{-1}$.

13. Analytes A and B react with a common reagent R with first-order kinetics. If 99.9% of A must react before 0.1% of B has reacted, what is the minimum acceptable ratio for their respective rate constants?

14. A mixture of two analytes, A and B, is analyzed simultaneously by monitoring their combined concentration, $C = [A] + [B]$, as a function of time when they react with a common reagent. Both A and B are known to follow first-order kinetics with the reagent, and A is known to react faster than B. Given the data in the following table, determine the initial concentrations of A and B, and the first-order rate constants, k_A and k_B.

time (min)	$[C]$ (mM)
1	0.313
6	0.200
11	0.136
16	0.098
21	0.074
26	0.058
31	0.047
36	0.038
41	0.032
46	0.027
51	0.023
56	0.019
61	0.016
66	0.014
71	0.012

15. Table 13.1 provides a list of several isotopes used as tracers. The half-lives for these isotopes also are listed. What is the rate constant for the radioactive decay of each isotope?

16. ^{60}Co is a long-lived isotope ($t_{1/2} = 5.3$ yr) frequently used as a radiotracer. The activity in a 5.00-mL sample of a solution of ^{60}Co is 2.1×10^7 disintegrations/sec. What is the molar concentration of ^{60}Co in the sample?

17. The concentration of Ni in a new alloy is determined by a neutron activation analysis. A 0.500-g sample of the alloy and a 1.000-g sample of a standard alloy that is 5.93% w/w Ni are irradiated with neutrons in a nuclear reactor. When irradiation is complete, the sample and the standard are allowed to cool and their gamma ray activities measured. Given that the activity is 1020 cpm for the sample and 3540 cpm for the standard, determine the %w/w Ni in the alloy.

18. The vitamin B_{12} content of a multivitamin tablet is determined by the following procedure. A sample of 10 tablets is dissolved in water and

diluted to volume in a 100-mL volumetric flask. A 50.00-mL portion is removed and 0.500 mg of radioactive vitamin B_{12} having an activity of 572 cpm is added as a tracer. The sample and tracer are homogenized and the vitamin B_{12} isolated and purified, producing 18.6 mg with an activity of 361 cpm. Calculate the milligrams of vitamin B_{12} in a multivitamin tablet.

19. The oldest sample that can be dated by ^{14}C is approximately 30 000 yr. What percentage of the ^{14}C remains after this time span?

20. Potassium–argon dating is based on the nuclear decay of ^{40}K to ^{40}Ar ($t_{1/2} = 1.3 \times$ yr). If no ^{40}Ar is originally present in the rock, and if ^{40}Ar can not escape to the atmosphere, then the relative amounts of ^{40}K and ^{40}Ar can be used to determine the age of the rock. When a 100.0-mg rock sample is analyzed it is found to contain 4.63×10^{-6} mol of ^{40}K and 2.09×10^{-6} mol ^{40}Ar. How old is the rock sample?

21. The steady state activity for ^{14}C in a sample is 13 cpm per gram of carbon. If counting is limited to 1 hr, what mass of carbon is needed to give a percent relative standard deviation of 1% for the sample's activity? How long must we monitor the radioactive decay from a 0.50-g sample of carbon to give a percent relative standard deviation of 1.0% for the activity?

22. To improve the sensitivity of a FIA analysis you might do any of the following: inject a larger volume of sample, increase the flow rate, decrease the length and the diameter of the manifold's tubing, or merge separate channels before injecting the sample. For each action, explain why it leads to an improvement in sensitivity.

23. Figure 13.31 shows a fiagram for a solution of 50.0-ppm PO_4^{3-} using the method in Representative Method 13.2. Determine values for h, t_a, T, t', Δt, and T'. What is the sensitivity of this FIA method, assuming a linear relationship between absorbance and concentration? How many samples can be analyzed per hour?

24. A sensitive method for the flow injection analysis of Cu^{2+} is based on its ability to catalyze the oxidation of di-2-pyridyl ketone hydrazone (DPKH).[24] The product of the reaction is fluorescent and is used to generate a signal when using a fluorimeter as a detector. The yield of the reaction is at a maximum when the solution is made basic with NaOH. The fluorescence, however, is greatest in the presence of HCl. Sketch an appropriate FIA manifold for this analysis.

24 Lazaro, F.; Luque de Castro, M. D.; Valcárcel, M. Analyst, 1984, 109, 333–337.

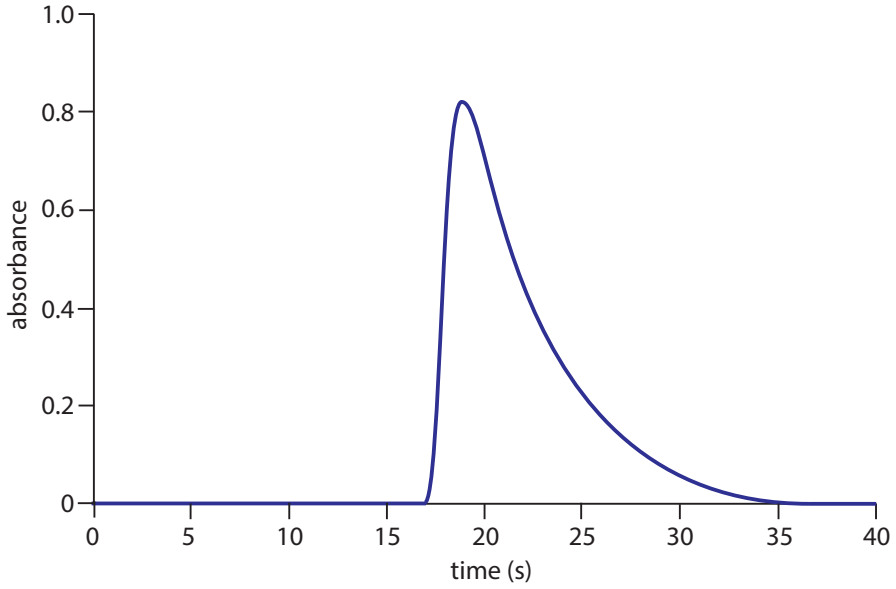


Figure 13.31 Fiagram for Problem 13.23.

25. The concentration of chloride in seawater is determined by a flow injection analysis. The analysis of a set of calibration standards gives the following results.

$[Cl^-]$ (ppm)	absorbance	$[Cl^-]$ (ppm)	absorbance
5.00	0.057	40.00	0.478
10.00	0.099	50.00	0.594
20.00	0.230	75.00	0.840
30.00	0.354		

A 1.00-mL sample of seawater is placed in a 500-mL volumetric flask and diluted to volume with distilled water. When injected into the flow injection analyzer an absorbance of 0.317 is measured. What is the concentration of Cl^- in the sample?

26. Ramsing and co-workers developed an FIA method for acid–base titrations using a carrier stream that is 2.0×10^{-3} M NaOH and that contains the acid–base indicator bromothymol blue.[25] Standard solutions of HCl were injected, and the following values of Δt were measured from the resulting fiagrams.

[HCl] (M)	Δt (s)	[HCl] (M)	Δt (s)
0.008	3.13	0.080	7.71
0.010	3.59	0.100	8.13
0.020	5.11	0.200	9.27
0.040	6.39	0.400	10.45
0.060	7.06	0.600	11.40

25 Ramsing, A. U.; Ruzicka, J.; Hansen, E. H. *Anal. Chim. Acta* **1981**, *129*, 1–17.

A sample with an unknown concentration of HCl is analyzed five times, giving values of 7.43, 7.28, 7.41, 7.37, and 7.33 s for Δt. Determine the concentration of HCl in the sample.

27. Milardovíc and colleagues used a flow injection analysis method with an amperometric biosensor to determine the concentration of glucose in blood.[26] Given that a blood sample that is 6.93 mM in glucose has a signal of 7.13 nA, what is the concentration of glucose in a sample of blood if its signal is 11.50 nA?

28. Fernández-Abedul and Costa-García developed an FIA method to determine cocaine in samples using an amperometric detector.[27] The following signals (arbitrary units) were collected for 12 replicate injections of a 6.2×10^{-6} M sample of cocaine, $C_{17}H_{21}NO_4$.

24.5	24.1	24.1
23.8	23.9	25.1
23.9	24.8	23.7
23.3	23.2	23.2

(a) What is the relative standard deviation for this sample?

(b) The following calibration data are available

[cocaine] (μM)	signal (arb. units)
0.18	0.8
0.36	2.1
0.60	2.4
0.81	3.2
1.0	4.5
2.0	8.1
4.0	14.4
6.0	21.6
8.0	27.1
10.0	32.9

In a typical analysis a 10.0-mg sample is dissolved in water and diluted to volume in a 25-mL volumetric flask. A 125-μL aliquot is transferred to a 25-mL volumetric flask and diluted to volume with a pH 9 buffer. When injected into the flow injection apparatus a signal of 21.4 (arb. units) is obtained. What is the %w/w cocaine in the sample?

26 Milardović, S.; Kruhak, I.; Iveković, D.; Rumenjak, V.; Tkalčec, M.; Grabarić, B. S. *Anal. Chim. Acta* **1997**, *350*, 91–96.

27 Fernández-Abedul, M; Costa-García, A. *Anal. Chim. Acta* **1996**, *328*, 67–71.

29. Holman, Christian, and Ruzicka described an FIA method to determine the concentration of H_2SO_4 in nonaqueous solvents.[28] Agarose beads (22–45 µm diameter) with a bonded acid–base indicator are soaked in NaOH and immobilized in the detector's flow cell. Samples of H_2SO_4 in *n*-butanol are injected into the carrier stream. As a sample passes through the flow cell, an acid–base reaction takes place between H_2SO_4 and NaOH. The endpoint of the neutralization reaction is signaled by a change in the bound indicator's color and is detected spectrophotometrically. The elution volume needed to reach the titration's endpoint is inversely proportional to the concentration of H_2SO_4; thus, a plot of endpoint volume versus $[H_2SO_4]^{-1}$ is linear. The following data is typical of that obtained using a set of external standards.

$[H_2SO_4]$ (mM)	end point volume (mL)
0.358	0.266
0.436	0.227
0.560	0.176
0.752	0.136
1.38	0.075
2.98	0.037
5.62	0.017

What is the concentration of H_2SO_4 in a sample if its endpoint volume is 0.157 mL?

13H Solutions to Practice Exercises

Practice Exercise 13.1

Figure 13.32 shows the calibration curve and the calibration equation for the external standards. Substituting 2.21×10^{-3} M for $[CH_3NO_2]_{t=2\,s}$ gives $[CH_3NO_2]_0$ as 5.21×10^{-2} M.

Click here to return to the chapter.

Practice Exercise 13.2

Figure 13.33 shows the calibration curve and the calibration equation for the external standards. Substituting 3.52×10^{-2} M for $[Fe(SCN)^{2+}]_{t=10\,s}$ gives $[SCN^-]_0$ as 6.87×10^{-2} M.

Click here to return to the chapter.

Practice Exercise 13.3

Figure 13.34 shows the Lineweaver–Burk plot and the equation for the data in Practice Exercise 13.3. The *y*-intercept of 9.974 min/ΔAU is

28 Holman, D. A.; Christian, G. D.; Ruzicka, J. *Anal. Chem.* **1997**, *69*, 1763–1765.

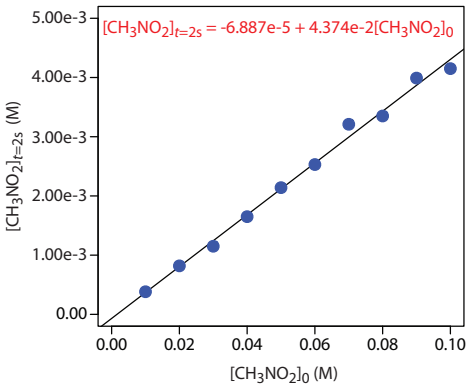


Figure 13.32 Calibration curve and calibration equation for Practice Exercise 13.1.

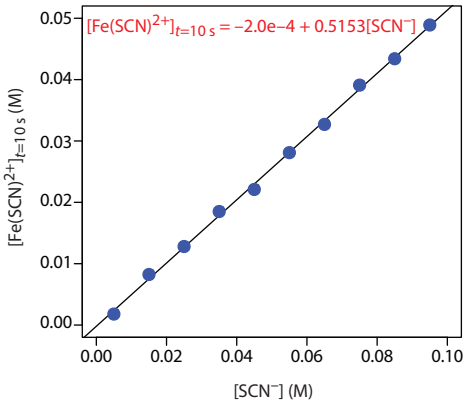


Figure 13.33 Calibration curve and calibration equation for Practice Exercise 13.2.

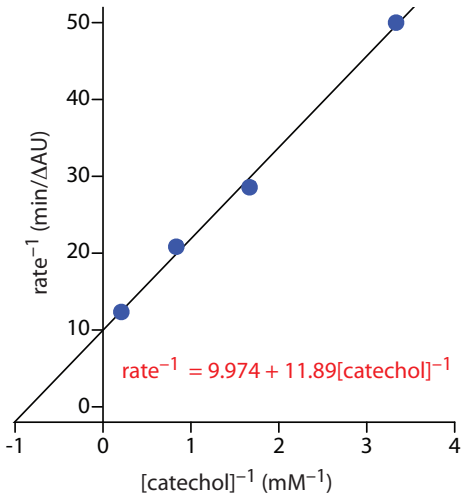


Figure 13.34 Lineweaver–Burk plot and equation for Practice Exercise 13.3.

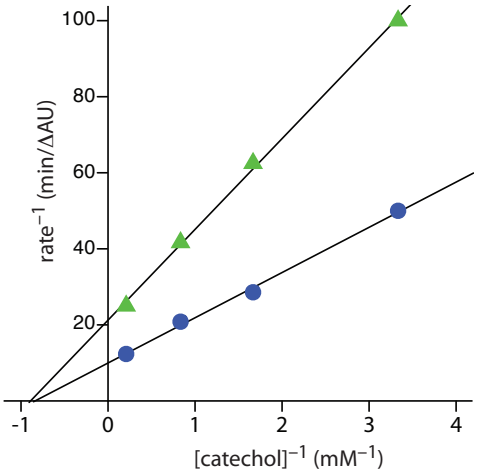


Figure 13.35 Lineweaver–Burk plots for Practice Exercise 13.4.

equivalent to $1/V_{max}$; thus, V_{max} is 0.10 ΔAU/min. The slope of 11.89 min/ΔAU•mM is equivalent to K_m/V_{max}; thus, K_m is 1.2 mM.

Click here to return to the chapter.

Practice Exercise 13.4

Figure 13.35 shows the Lineweaver–Burk plots for the two sets of data in Practice Exercise 13.4. The nearly identical *x*-intercepts suggests that phenylthiourea is a noncompetitive inhibitor.

Click here to return to the chapter.