Chapter 7

Collecting and Preparing Samples

Chapter Overview

When we use an analytical method to solve a problem, there is no guarantee that our results will be accurate or precise. In designing an analytical method we consider potential sources of determinate error and indeterminate error, and take appropriate steps to minimize their effect, such as including reagent blanks and calibrating instruments. Why might a carefully designed analytical method give poor results? One possibility is that we may have failed to account for errors associated with the sample. If we collect the wrong sample, or if we lose analyte while preparing the sample for analysis, then we introduce a determinate source of error. If we fail to collect enough samples, or if we collect samples of the wrong size, then our precision may suffer. In this chapter we consider how collecting samples and preparing them for analysis affects the accuracy and precision of our results.

NaBr   **F.W.102.90**

Certificate of Actual Lot Analysis

Barium (Ba) ----------------- 0.002%
Bromate (BrO₃) --------------- P.T.
Calcium, Magnesium,
 and R₂O₃ Precipitate --------- 0.001%
Chloride (Cl) ---------------- 0.1%
Heavy Metals (as Pb) --------- 4ppm
Insoluble Matter ------------- 0.002%
Iron (Fe) -------------------- 1ppm
Nitrogen Compounds (as N) ---- 3ppm
pH of a 5% Solution ---------- 5.5
Sulfate (SO₄) ---------------- 0.002%
*Potassium (K) --------------- 0.01%

*
ACS LIMIT ...0.005%

Figure 7.1 Certificate of analysis for a production lot of NaBr. The result for iron meets the ACS specifications, but the result for potassium does not.

Although equation 7.1 is written in terms of a standard deviation, s, a propagation of uncertainty is written in terms of variances, s^2. In this section, and those that follow, we will use both standard deviations and variances to discuss sampling uncertainty.

7A   The Importance of Sampling

When a manufacturer lists a chemical as ACS Reagent Grade, they must demonstrate that it conforms to specifications set by the American Chemical Society (ACS). For example, the ACS specifications for NaBr require that the concentration of iron be ≤ 5 ppm. To verify that a production lot meets this standard, the manufacturer collects and analyzes several samples, reporting the average result on the product's label (Figure 7.1).

If the individual samples do not accurately represent the population from which they are drawn—what we call the TARGET POPULATION—then even a careful analysis must yield an inaccurate result. Extrapolating this result from a sample to its target population introduces a determinate sampling error. To minimize this determinate sampling error, we must collect the right sample.

Even if we collect the right sample, indeterminate sampling errors may limit the usefulness of our analysis. Equation 7.1 shows that a confidence interval about the mean, $\bar{X}$, is proportional to the standard deviation, s, of the analysis

$$\mu = \bar{X} \pm \frac{ts}{\sqrt{n}} \qquad 7.1$$

where n is the number of samples and t is a statistical factor that accounts for the probability that the confidence interval contains the true value, μ.

Each step of an analysis contributes random error that affects the overall standard deviation. For convenience, let's divide an analysis into two steps—collecting the samples and analyzing the samples—each characterized by a standard deviation. Using a propagation of uncertainty, the relationship between the overall variance, s^2, and the variances due to sampling, s^2_{samp}, and the analytical method, s^2_{meth}, is

$$s^2 = s^2_{samp} + s^2_{meth} \qquad 7.2$$

Equation 7.2 shows that the overall variance for an analysis may be limited by either the analytical method or the collecting of samples. Unfortunately, analysts often try to minimize the overall variance by improving only the method's precision. This is a futile effort, however, if the standard deviation for sampling is more than three times greater than that for the method.[1] Figure 7.2 shows how the ratio s_{samp}/s_{meth} affects the method's contribution to the overall variance. As shown by the dashed line, if the sample's standard deviation is $3\times$ the method's standard deviation, then indeterminate method errors explain only 10% of the overall variance. If indeterminate sampling errors are significant, decreasing s_{meth} provides only a nominal change in the overall precision.

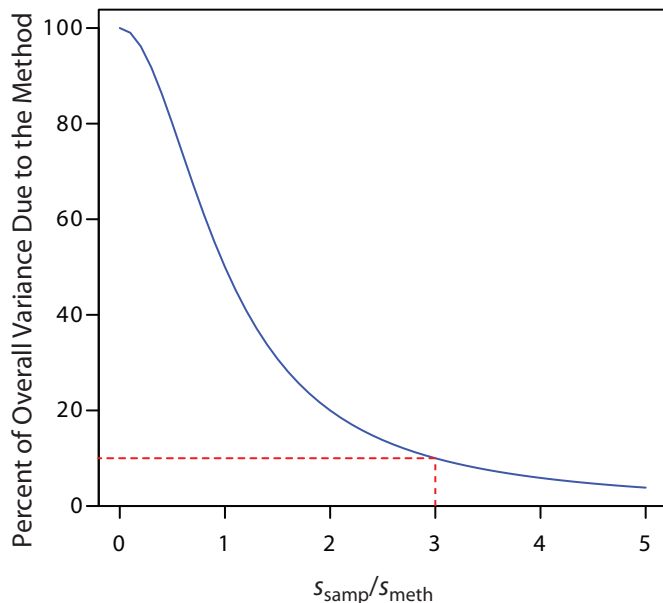1   Youden, Y. J. *J. Assoc. Off. Anal. Chem.* **1981**, *50*, 1007–1013.

Figure 7.2 The blue curve shows the method's contribution to the overall variance, s^2, as a function of the relative magnitude of the standard deviation in sampling, s_{samp}, and the method's standard deviation, s_{meth}. The dashed red line shows that the method accounts for only 10% of the overall variance when $s_{samp} = 3 \times s_{meth}$. Understanding the relative importance of potential sources of indeterminate error is important when considering how to improve the overall precision of the analysis.

Example 7.1

A quantitative analysis gives a mean concentration of 12.6 ppm for an analyte. The method's standard deviation is 1.1 ppm and the standard deviation for sampling is 2.1 ppm. (a) What is the overall variance for the analysis? (b) By how much does the overall variance change if we improve s_{meth} by 10% to 0.99 ppm? (c) By how much does the overall variance change if we improve s_{samp} by 10% to 1.89 ppm?

Solution

(a) The overall variance is

$$s^2 = s_{samp}^2 + s_{meth}^2 = (2.1 \text{ ppm})^2 + (1.1 \text{ ppm})^2 = 5.6 \text{ ppm}^2$$

(b) Improving the method's standard deviation changes the overall variance to

$$s^2 = (2.1 \text{ ppm})^2 + (0.99 \text{ ppm})^2 = 5.4 \text{ ppm}^2$$

Improving the method's standard deviation by 10% improves the overall variance by approximately 4%.

(c) Changing the standard deviation for sampling

$$s^2 = (1.9 \text{ ppm})^2 + (1.1 \text{ ppm})^2 = 4.8 \text{ ppm}^2$$

improves the overall variance by almost 15%. As expected, because s_{samp} is larger than s_{meth}, we obtain a bigger improvement in the overall variance when we focus our attention on sampling problems.

Practice Exercise 7.1

Suppose you wish to reduce the overall variance in Example 7.1 to 5.0 ppm^2. If you focus on the method, by what percentage do you need to reduce s_{meth}? If you focus on the sampling, by what percentage do you need to reduce s_{samp}?

Click here to review your answer to this exercise

To determine which step has the greatest effect on the overall variance, we need to measure both s_{samp} and s_{meth}. The analysis of replicate samples

There are several ways to minimize the standard deviation for sampling. Here are two examples. One approach is to use a standard reference material (SRM) that has been carefully prepared to minimize indeterminate sampling errors. When the sample is homogeneous—as is the case, for example, with aqueous samples—a useful approach is to conduct replicate analyses on a single sample.

provides an estimate of the overall variance. To determine the method's variance we analyze samples under conditions where we may assume that the sampling variance is negligible. The sampling variance is determined by difference.

Example 7.2

The following data were collected as part of a study to determine the effect of sampling variance on the analysis of drug-animal feed formulations.[2]

% Drug (w/w)			% Drug (w/w)		
0.0114	0.0099	0.0105	0.0105	0.0109	0.0107
0.0102	0.0106	0.0087	0.0103	0.0103	0.0104
0.0100	0.0095	0.0098	0.0101	0.0101	0.0103
0.0105	0.0095	0.0097			

The data on the left were obtained under conditions where both s_{samp} and s_{meth} contribute to the overall variance. The data on the right were obtained under conditions where s_{samp} is known to be insignificant. Determine the overall variance, and the standard deviations due to sampling and the analytical method. To which factor—sampling or the method—should you turn your attention if you want to improve the precision of the analysis?

SOLUTION

See Chapter 4 for a review of how to calculate the variance.

Using the data on the left, the overall variance, s^2, is 4.71×10^{-7}. To find the method's contribution to the overall variance, s^2_{meth}, we use the data on the right, obtaining a value of 7.00×10^{-8}. The variance due to sampling, s^2_{samp}, is

$$s^2_{samp} = s^2 - s^2_{meth} = 4.71 \times 10^{-7} - 7.00 \times 10^{-8} = 4.01 \times 10^{-7}$$

Converting variances to standard deviations gives s_{samp} as 6.32×10^{-4} and s_{meth} as 2.65×10^{-4}. Because s_{samp} is more than twice as large as s_{meth}, improving the precision of the sampling process has the greatest impact on the overall precision.

2   Fricke, G. H.; Mischler, P. G.; Staffieri, F. P.; Houmyer, C. L. *Anal. Chem.* **1987**, *59*, 1213–1217.

Practice Exercise 7.2

A polymer's density provides a measure of its crystallinity. The standard deviation for the determination of density using a single sample of a polymer is 1.96×10^{-3} g/cm^3. The standard deviation when using different samples of the polymer is 3.65×10^{-2} g/cm^3. Determine the standard deviations due to sampling and the analytical method.

Click here to review your answer to this exercise.

7B   Designing A Sampling Plan

A SAMPLING PLAN must support the goals of an analysis. For example, a material scientist interested in characterizing a metal's surface chemistry is more likely to choose a freshly exposed surface, created by cleaving the sample under vacuum, than a surface previously exposed to the atmosphere. In a qualitative analysis, a sample does not need to be identical to the original substance, provided that there is sufficient analyte to ensure its detection. In fact, if the goal of an analysis is to identify a trace-level component, it may be desirable to discriminate against major components when collecting samples.

For a quantitative analysis, the sample's composition must accurately represent the target population, a requirement that necessitates a careful sampling plan. Among the issues to consider are these five questions.

1. From where within the target population should we collect samples?
2. What type of samples should we collect?
3. What is the minimum amount of sample for each analysis?
4. How many samples should we analyze?
5. How can we minimize the overall variance for the analysis?

For an interesting discussion of the importance of a sampling plan, see Buger, J. et al. "Do Scientists and Fishermen Collect the Same Size Fish? Possible Implications for Exposure Assessment," *Environ. Res.* **2006**, *101*, 34–41.

7B.1   Where to Sample the Target Population

A sampling error occurs whenever a sample's composition is not identical to its target population. If the target population is HOMOGENEOUS, then we can collect individual samples without giving consideration to where to sample. Unfortunately, in most situations the target population is HETEROGENEOUS. Due to settling, a medication available as an oral suspension may have a higher concentration of its active ingredients at the bottom of the container. The composition of a clinical sample, such as blood or urine, may depend on when it is collected. A patient's blood glucose level, for instance, changes in response to eating and exercise. Other target populations show both a spatial and a temporal heterogeneity. The concentration of dissolved O_2 in a lake is heterogeneous due both to the changing seasons and to point sources of pollution.

If the analyte's distribution within the target population is a concern, then our sampling plan must take this into account. When feasible, homogenizing the target population is a simple solution—in most cases, however, this is impracticable. Additionally, homogenization destroys information about the analyte's spatial or temporal distribution within the target population, information that may be of importance.

The composition of a homogeneous target population is the same regardless of where we sample, when we sample, or the size of our sample. For a heterogeneous target population, the composition is not the same at different locations, at different times, or for different sample sizes.

RANDOM SAMPLING

The ideal sampling plan provides an unbiased estimate of the target population's properties. A RANDOM SAMPLING is the easiest way to satisfy this

requirement.[3] Despite its apparent simplicity, a truly random sample is difficult to collect. Haphazard sampling, in which samples are collected without a sampling plan, is not random and may reflect an analyst's unintentional biases.

Here is a simple method for ensuring that we collect random samples. First, we divide the target population into equal units and assign a unique number to each unit. Then, we use a random number table to select the units to sample. Example 7.3 provides an illustrative example.

Example 7.3

To analyze a polymer's tensile strength, individual samples of the polymer are held between two clamps and stretched. In evaluating a production lot, the manufacturer's sampling plan calls for collecting ten 1 cm × 1 cm samples from a 100 cm × 100 cm polymer sheet. Explain how we can use a random number table to ensure that our samples are random.

SOLUTION

As shown by the grid, we divide the polymer sheet into 10 000 1 cm × 1 cm squares, each identified by its row number and its column number, with numbers running from 0 to 99. For example, the blue square is in row 98 and column 1. To select ten squares at random, we enter the random number table in Appendix 14 at an arbitrary point, and let the entry's last four digits represent the row and the column for the first sample. We then move through the table in a predetermined fashion, selecting random numbers until we have 10 samples. For our first sample, let's use the second entry in the third column, which is 76831. The first sample, therefore, is row 68 and column 31. If we proceed by moving down the third column, then the 10 samples are as follows:

Sample	Number	Row	Column	Sample	Number	Row	Column
1	76831	68	31	6	41701	17	01
2	66558	65	58	7	38605	86	05
3	33266	32	66	8	64516	45	16
4	12032	20	32	9	13015	30	15
5	14063	40	63	10	12138	21	38

In collecting a random sample we make no assumptions about the target population, making this the least biased approach to sampling. On the

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3   Cohen, R. D. *J. Chem. Educ.* **1991**, *68*, 902–903.

other hand, a random sample often requires more time and expense than other sampling strategies because we need a greater number of samples to ensure that we adequately sample the target population.[4]

JUDGMENTAL SAMPLING

The opposite of random sampling is selective, or JUDGMENTAL SAMPLING in which we use prior information about the target population to help guide our selection of samples. Judgmental sampling is more biased than random sampling, bur requires fewer samples. Judgmental sampling is useful if we wish to limit the number of independent variables influencing our results. For example, if we are studying the bioaccumulation of PCB's in fish, we may choose to exclude fish that are too small or that appear diseased.

SYSTEMATIC SAMPLING

Random sampling and judgmental sampling represent extremes in bias and in the number of samples needed to characterize the target population. SYSTEMATIC SAMPLING falls in between these extremes. In systematic sampling we sample the target population at regular intervals in space or time. Figure 7.3 shows an aerial photo of the Great Salt Lake in Utah. A railroad line divides the lake into two sections with different chemical compositions. To compare the lake's two sections—and to evaluation spatial variations within each section—we use a two-dimensional grid to define sampling locations.

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4   Borgman, L. E.; Quimby, W. F. in Keith, L. H., ed. *Principles of Environmental Sampling*, American Chemical Society: Washington, D. C., 1988, 25–43.
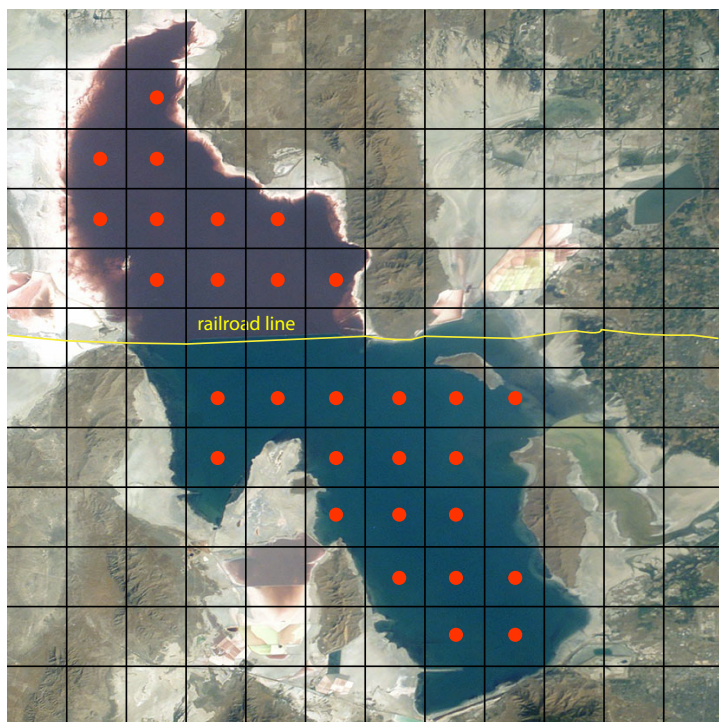

Figure 7.3 Aerial photo of the Great Salt Lake in Utah, taken from the International Space Station at a distance of approximately 380 km. The railroad line divides the lake into two sections that differ in chemical composition. Superimposing a two-dimensional grid divides each section of the lake into sampling units. The red dots at the center of each unit represent sampling sites. Photo courtesy of the Image Science and Analysis Laboratory, NASA Johnson Space Center, Photo Number ISS007-E-13002 (eol.jsc.nasa.gov).
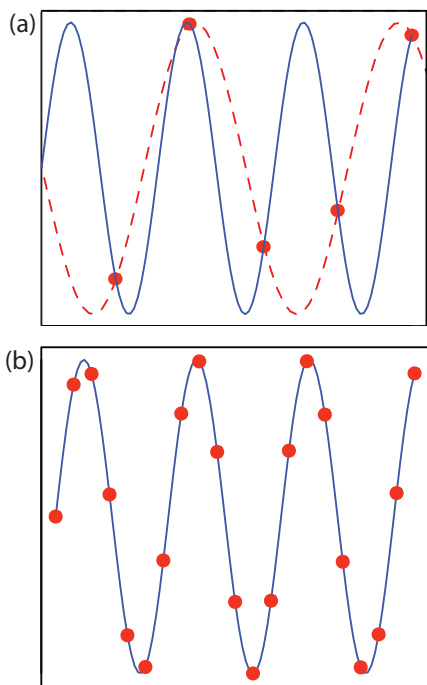
Figure 7.4 Effect of sampling frequency when monitoring a periodic signal. Individual samples are shown by the red dots (•). In (a) the sampling frequency is approximately 1.5 samples per period. The dashed red line shows the apparent signal based on five samples and the solid blue line shows the true signal. In (b) a sampling frequency of approximately 5 samples per period accurately reproduces the true signal.

When a population's heterogeneity is time-dependent, as is common in clinical studies, samples are drawn at regular intervals in time.

If a target population's properties have a periodic trend, a systematic sampling will lead to a significant bias if our sampling frequency is too small. This is a common problem when sampling electronic signals where the problem is known as aliasing. Consider, for example, a signal consisting of a simple sign wave. Figure 7.4a shows how an insufficient sampling frequency underestimates the signal's true frequency. The apparent signal, shown by the dashed red line passing through the five data points, is significantly different from the true signal shown by the solid blue line.

According to the NYQUIST THEOREM, to accurately determine a periodic signal's true frequency, we must sample the signal at least twice during each cycle or period. If we collect samples at an interval of Δt, the highest frequency we can accurately monitor is $(2\Delta t)^{-1}$. For example, if our sampling rate is 1 sample/hr, the highest frequency we can monitor is $(2 \times 1 \text{ hr})^{-1}$ or 0.5 hr^{-1}, corresponding to a period of less than 2 hr. If our signal's period is less than 2 hours (a frequency of more than 0.5 hr^{-1}), then we must use a faster sampling rate. Ideally, the sampling rate should be at least 3-4 times greater than the highest frequency signal of interest. If our signal has a period of one hour, we should collect a new sample every 15-20 minutes.

SYSTEMATIC–JUDGMENTAL SAMPLING

Combinations of the three primary approaches to sampling are also possible.[5] One such combination is SYSTEMATIC–JUDGMENTAL SAMPLING, in which we use prior knowledge about a system to guide a systematic sampling plan. For example, when monitoring waste leaching from a landfill, we expect the plume to move in the same direction as the flow of groundwater—this helps focus our sampling, saving money and time. The systematic–judgmental sampling plan in Figure 7.5 includes a rectangular grid for most of the samples and linear transects to explore the plume's limits.[6]

5   Keith, L. H. *Environ. Sci. Technol.* **1990**, *24*, 610–617.
6   Flatman, G. T.; Englund, E. J.; Yfantis, A. A. in Keith, L. H., ed. *Principles of Environmental Sampling*, American Chemical Society: Washington, D. C., 1988, 73–84.

Figure 7.5 Systematic–judgmental sampling plan for monitoring the leaching of pollutants from a landfill. The sampling sites, shown as red dots (•), are on a systematic grid straddling the direction of the groundwater's flow. Sampling along linear transects helps establish the plume's limits.
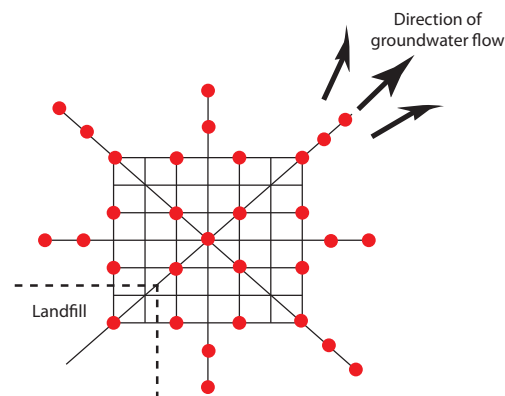
STRATIFIED SAMPLING

Another combination of the three primary approaches to sampling is judgmental–random, or STRATIFIED SAMPLING. Many target populations consist of distinct units, or strata. For example, suppose we are studying particulate Pb in urban air. Because particulates come in a range of sizes—some visible and some microscopic—and from many sources—road dust, diesel soot, and fly ash to name a few—we can subdivide the target population by size or source. If we choose a random sampling plan, then we collect samples without considering the different strata. For a stratified sampling, we divide the target population into strata and collect random samples from within each stratum. After analyzing the samples from each stratum, we pool their respective means to give an overall mean for the target population. The advantage of stratified sampling is that individual strata usually are more homogeneous than the target population. The overall sampling variance for stratified sampling is always at least as good, and often better than that obtained by simple random sampling.

CONVENIENCE SAMPLING

One additional method of sampling deserves brief mention. In CONVENIENCE SAMPLING we select sample sites using criteria other than minimizing sampling error and sampling variance. In a survey of rural groundwater quality, for example, we can choose to drill wells at randomly selected sites or we can make use of existing wells, which is usually the preferred choice. In this case cost, expedience, and accessibility are more important than ensuring a random sample.

7B.2   What Type of Sample to Collect

After determining where to collect samples, the next step in designing a sampling plan is to decide what type of sample to collect. There are three common methods for obtaining samples: grab sampling, composite sampling, and in situ sampling.

The most common type of sample is a GRAB SAMPLE, in which we collect a portion of the target population at a specific time and/or location, providing a "snapshot" of the target population. If our target population is homogeneous, a series of random grab samples allows us to establish its properties. For a heterogeneous target population, systematic grab sampling allows us to characterize how its properties change over time and/or space.

A COMPOSITE SAMPLE is a set of grab samples that we combine into a single sample before analysis. Because information is lost when we combine individual samples, we normally analyze grab sample separately. In some situations, however, there are advantages to working with a composite sample.

One situation where composite sampling is appropriate is when our interest is in the target population's average composition over time or space.

For example, wastewater treatment plants must monitor and report the average daily composition of the treated water they release to the environment. The analyst can collect and analyze individual grab samples using a systematic sampling plan, reporting the average result, or she can combine the grab samples into a single composite sample. Analyzing a single composite sample instead of many individual grab samples, saves time and money.

Composite sampling is also useful when a single sample can not supply sufficient material for the analysis. For example, analytical methods for determining PCB's in fish often require as much as 50 g of tissue, an amount that may be difficult to obtain from a single fish. By combining and homogenizing tissue samples from several fish, it is easy to obtain the necessary 50-g sample.

A significant disadvantage of grab samples and composite samples is that we cannot use them to continuously monitor a time-dependent change in the target population. In situ sampling, in which we insert an analytical sensor into the target population, allows us to continuously monitor the target population without removing individual grab samples. For example, we can monitor the pH of a solution moving through an industrial production line by immersing a pH electrode in the solution's flow.

Example 7.4

A study of the possible relationship between traffic density and the concentrations of lead, cadmium, and zinc in roadside soils, made use of the following sampling plan.[7] Samples of surface soil (0–10 cm) were collected at perpendicular distances of 1, 5, 10, 20, and 30 m from the roadway. At each distance, 10 samples were taken from different locations and mixed to form a single sample. What type of sampling plan is this? Explain why this is an appropriate sampling plan.

SOLUTION

This is an example of a systematic–judgemental sampling plan using composite samples. These are good choices given the goals of the study. Automobile emissions release particulates containing elevated concentrations of lead, cadmium, and zinc—this study was conducted in Uganda where leaded gasoline is still in use—which settle out on the surrounding roadside soils as "dry rain." Sampling in areas near roadways, and sampling at fixed distances from the roadway provides sufficient data for the study, while limiting the total number of samples. Combining samples from the same distance into a single, composite sample has the advantage of decreasing sampling uncertainty. Because variations in metal concentrations perpendicular to the roadway is not of interest, the composite samples do not result in a loss of information.

7   Nabulo, G.; Oryem-Origa, H.; Diamond, M. *Environ. Res.* **2006**, *101*, 42–52.

7B.3   How Much Sample to Collect

To minimize sampling errors, samples must be of an appropriate size. If a sample is too small, its composition may differ substantially from that of the target population, introducing a sampling error. Samples that are too large, however, require more time and money to collect and analyze, without providing a significant improvement in the sampling error.

Let's assume that our target population is a homogeneous mixture of two types of particles. Particles of type A contain a fixed concentration of analyte, and particles without analyte are of type B. Samples from this target population follow a binomial distribution. If we collect a sample containing n particles, the expected number of particles containing analyte, n_A, is

For a review of the binomial distribution, see Chapter 4.

$$n_A = np$$

where p is the probability of selecting a particle of type A. The standard deviation for sampling is

$$s_{samp} = \sqrt{np(1-p)} \qquad 7.3$$

To calculate the relative standard deviation for sampling, s_{samp}^{rel}, we divide equation 7.3 by n_A, obtaining

$$s_{samp}^{rel} = \frac{\sqrt{np(1-p)}}{np}$$

Solving for n allows us to calculate the number of particles providing the desired relative sampling variance.

$$n = \frac{1-p}{p} \times \frac{1}{(s_{samp}^{rel})^2} \qquad 7.4$$

Example 7.5

Suppose we are analyzing a soil where the particles containing analyte represent only $1 \times 10^{-7}\%$ of the population. How many particles must we collect to give a percent relative standard deviation for sampling of 1%?

SOLUTION

Since the particles of interest account for $1 \times 10^{-7}\%$ of all particles, the probability, p, of selecting one of these particles is only 1×10^{-9}. Substituting into equation 7.4 gives

$$n = \frac{1-(1\times10^{-9})}{1\times10^{-9}} \times \frac{1}{(0.01)^2} = 1\times10^{13}$$

To obtain a relative standard deviation for sampling of 1%, we need to collect 1×10^{13} particles.

A sample containing 10^{13} particles may be fairly large. Suppose this is equivalent to a mass of 80 g. Working with a sample this large clearly is not practical. Does this mean we must work with a smaller sample and accept a larger relative standard deviation for sampling? Fortunately the answer is no. An important feature of equation 7.4 is that the relative standard deviation for sampling is a function of the number of particles, not the combined mass of the particles. If we crush and grind the particles to make them smaller, then a sample containing 10^{13} particles will have a smaller mass. If we assume that a particle is spherical, then its mass is proportional to the cube of its radius.

$$\text{mass} \propto r^3$$

Decreasing a particle's radius by a factor of 2, for example, decreases its mass by a factor of 2^3, or 8.

Example 7.6

Assume that a sample of 10^{13} particles from Example 7.5 weighs 80 g and that the particles are spherical. By how much must we reduce a particle's radius if we wish to work with 0.6-g samples?

SOLUTION

To reduce the sample's mass from 80 g to 0.6 g, we must change its mass by a factor of

$$\frac{80}{0.6} = 133 \times$$

To accomplish this we must decrease a particle's radius by a factor of

$$r^3 = 133 \times$$
$$r = 5.1 \times$$

Decreasing the radius by a factor of approximately 5 allows us to decrease the sample's mass from 80 g to 0.6 g.

Treating a population as though it contains only two types of particles is a useful exercise because it shows us that we can improve the relative standard deviation for sampling by collecting more particles. Of course, a real population contains more than two types of particles, with the analyte present at several levels of concentration. Nevertheless, many well-mixed populations, in which the population's composition is homogeneous on the scale at which we sample, approximate binomial sampling statistics. Under these conditions the following relationship between the mass of a random grab sample, m, and the percent relative standard deviation for sampling, R, is often valid

$$mR^2 = K_s \qquad\qquad 7.5$$

where K_s is a sampling constant equal to the mass of a sample producing a percent relative standard deviation for sampling of $\pm1\%$.[8]

Example 7.7

The following data were obtained in a preliminary determination of the amount of inorganic ash in a breakfast cereal.

Mass of Cereal (g)	0.9956	0.9981	1.0036	0.9994	1.0067
% w/w Ash	1.34	1.29	1.32	1.26	1.28

What is the value of K_s and what size samples are needed to give a percent relative standard deviation for sampling of $\pm2.0\%$. Predict the percent relative standard deviation and the absolute standard deviation if we collect 5.00-g samples.

SOLUTION

To determine the sampling constant, K_s, we need to know the average mass of the cereal samples and the relative standard deviation for the amount of ash. The average mass of the cereal samples is 1.0007 g. The average % w/w ash and its absolute standard deviation are, respectively, 1.298% w/w and 0.03194% w/w. The percent relative standard deviation, R, therefore, is

$$R = \frac{s_{samp}}{\overline{X}} \times 100 = \frac{0.03194\% \text{ w/w}}{1.298\% \text{ w/w}} \times 100 = 2.46\%$$

Solving for K_s gives its value as

$$K_s = mR^2 = (1.0007 \text{ g})(2.46)^2 = 6.06 \text{ g}$$

To obtain a percent relative standard deviation of $\pm2\%$, samples need to have a mass of at least

$$m = \frac{K_s}{R^2} = \frac{6.06 \text{ g}}{(2.0)^2} = 1.5 \text{ g}$$

If we use 5.00-g samples, then the expected percent relative standard deviation is

$$R = \sqrt{\frac{K_s}{m}} = \sqrt{\frac{6.06 \text{ g}}{5.00 \text{ g}}} = 1.10\%$$

and the expected absolute standard deviation is

$$s_{samp} = \frac{R\overline{X}}{100} = \frac{(1.10)(1.298\% \text{ w/w})}{100} = 0.0143\% \text{ w/w}$$

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8   Ingamells, C. O.; Switzer, P. *Talanta* **1973**, *20*, 547–568.

Practice Exercise 7.3

Olaquindox is a synthetic growth promoter in medicated feeds for pigs. In an analysis of a production lot of feed, five samples with nominal masses of 0.95 g were collected and analyzed, with the results shown in the following table.

mass (g)	0.9530	0.9728	0.9660	0.9402	0.9576
mg olaquindox/kg feed	23.0	23.8	21.0	26.5	21.4

What is the value of K_s and what size samples are needed to obtain a percent relative deviation for sampling of 5.0%? By how much do you need to reduce the average particle size if samples must weigh no more than 1 g?

Click here to review your answer to this exercise.

7B.4   How Many Samples to Collect

In the previous section we considered how much sample we need to minimize the standard deviation due to sampling. Another important consideration is the number of samples to collect. If our samples are normally distributed, then the confidence interval for the sampling error is

$$\mu = \bar{X} \pm \frac{ts_{samp}}{\sqrt{n_{samp}}}$$

7.6

where n_{samp} is the number of samples and s_{samp} is the standard deviation for sampling. Rearranging equation 7.6 and substituting e for the quantity $\bar{X} - \mu$, gives the number of samples as

$$n_{samp} = \frac{t^2 s_{samp}^2}{e^2}$$

7.7

Because the value of t depends on n_{samp}, the solution to equation 7.7 is found iteratively.

When we use equation 7.7, the standard deviation for sampling, s_{samp}, and the error, e, must be expressed in the same way. Because s_{samp} is given as a percent relative standard deviation, the error, e, is given as a percent relative error. When you use equation 7.7, be sure to check that you are expressing s_{samp} and e in the same way.

Example 7.8

In Example 7.7 we determined that we need 1.5-g samples to establish an s_{samp} of ±2.0% for the amount of inorganic ash in cereal. How many 1.5-g samples do we need to obtain a percent relative sampling error of ±0.80% at the 95% confidence level?

SOLUTION

Because the value of t depends on the number of samples—a result we have yet to calculate—we begin by letting $n_{samp} = \infty$ and using $t(0.05, \infty)$ for t. From Appendix 4, the value for $t(0.05, \infty)$ is 1.960. Substituting known values into equation 7.7 gives the number of samples as

$$n_{samp} = \frac{(1.960)^2(2.0)^2}{(0.80)^2} = 24.0 \approx 24$$

Letting $n_{samp} = 24$, the value of $t(0.05, 23)$ from Appendix 4 is 2.073. Recalculating n_{samp} gives

$$n_{samp} = \frac{(2.073)^2(2.0)^2}{(0.80)^2} = 26.9 \approx 27$$

When $n_{samp} = 27$, the value of $t(0.05, 26)$ from Appendix 4 is 2.060. Recalculating n_{samp} gives

$$n_{samp} = \frac{(2.060)^2(2.0)^2}{(0.80)^2} = 26.52 \approx 27$$

Because two successive calculations give the same value for n_{samp}, we have an iterative solution to the problem. We need 27 samples to achieve a percent relative sampling error of $\pm 0.80\%$ at the 95% confidence level.

With 24 samples, the degrees of freedom for t is 23.

Practice Exercise 7.4

Assuming that the percent relative standard deviation for sampling in the determination of olaquindox in medicated feed is 5.0% (see Practice Exercise 7.3), how many samples do we need to analyze to obtain a percent relative sampling error of $\pm 2.5\%$ at $\alpha = 0.05$?

Click here to review your answer to this exercise.

Equation 7.7 provides an estimate for the smallest number of samples that will produce the desired sampling error. The actual sampling error may be substantially larger if s_{samp} for the samples we collect during the subsequent analysis is greater than s_{samp} used to calculate n_{samp}. This is not an uncommon problem. For a target population with a relative sampling variance of 50 and a desired relative sampling error of $\pm 5\%$, equation 7.7 predicts that 10 samples are sufficient. In a simulation using 1000 samples of size 10, however, only 57% of the trials resulted in a sampling error of less than $\pm 5\%$.[9] Increasing the number of samples to 17 was sufficient to ensure that the desired sampling error was achieved 95% of the time.

7B.5 Minimizing the Overall Variance

A final consideration when developing a sampling plan is to minimize the overall variance for the analysis. Equation 7.2 shows that the overall variance is a function of the variance due to the method, s_{meth}^2, and the variance due to sampling, s_{samp}^2. As we have seen, we can improve the sampling variance by collecting more samples of the proper size. Increasing the number

For an interesting discussion of why the number of samples is important, see Kaplan, D.; Lacetera, N.; Kaplan, C. "Sample Size and Precision in NIH Peer Review," Plos One, 2008, 3(7), 1–3. When reviewing grants, individual reviewers report a score between 1.0 and 5.0 (two significant figure). NIH reports the average score to three significant figures, implying that differences of 0.01 are significant. If the individual scores have a standard deviation of 0.1, then a difference of 0.01 is significant at $\alpha = 0.05$ only if there are 384 reviews. The authors conclude that NIH review panels are too small to provide a statistically meaningful separation between proposals receiving similar scores.

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9   Blackwood, L. G. *Environ. Sci. Technol.* **1991**, *25*, 1366–1367.

of times we analyze each sample improves the method's variance. If s^2_{samp} is significantly greater than s^2_{meth}, we can ignore the method's contribution to the overall variance and use equation 7.7 to estimate the number of samples to analyze. Analyzing any sample more than once will not improve the overall variance, because the method's variance is insignificant.

If s^2_{meth} is significantly greater than s^2_{samp}, then we need to collect and analyze only one sample. The number of replicate analyses, n_{rep}, needed to minimize the error due to the method is given by an equation similar to equation 7.7.

$$n_{rep} = \frac{t^2 s^2_{meth}}{e^2}$$

Unfortunately, the simple situations described above are often the exception. For many analyses, both the sampling variance and the method variance are significant, and both multiple samples and replicate analyses of each sample are necessary. The overall error in this case is

$$e = t\sqrt{\frac{s^2_{samp}}{n_{samp}} + \frac{s^2_{meth}}{n_{samp} n_{rep}}} \qquad 7.8$$

Equation 7.8 does not have a unique solution as different combinations of n_{samp} and n_{rep} give the same overall error. How many samples we collect and how many times we analyze each sample is determined by other concerns, such as the cost of collecting and analyzing samples, and the amount of available sample.

Example 7.9

An analytical method has a percent relative sampling variance of 0.40% and a percent relative method variance of 0.070%. Evaluate the percent relative error ($\alpha = 0.05$) if you collect 5 samples, analyzing each twice, and if you collect 2 samples, analyzing each 5 times.

SOLUTION

Both sampling strategies require a total of 10 analyses. From Appendix 4 we find that the value of $t(0.05, 9)$ is 2.262. Using equation 7.8, the relative error for the first sampling strategy is

$$e = 2.262\sqrt{\frac{0.40}{5} + \frac{0.070}{5 \times 2}} = 0.67\%$$

and that for the second sampling strategy is

$$e = 2.262\sqrt{\frac{0.40}{2} + \frac{0.070}{2 \times 5}} = 1.0\%$$

Because the method variance is smaller than the sampling variance, we obtain a smaller relative error if we collect more samples, analyzing each fewer times.

Practice Exercise 7.5

An analytical method has a percent relative sampling variance of 0.10% and a percent relative method variance of 0.20%. The cost of collecting a sample is $20 and the cost of analyzing a sample is $50. Propose a sampling strategy that provides a maximum percent relative error of ±0.50% ($\alpha = 0.05$) and a maximum cost of $700.

Click here to review your answer to this exercise.

7C   Implementing the Sampling Plan

Implementing a sampling plan normally involves three steps: physically removing the sample from its target population, preserving the sample, and preparing the sample for analysis. Except for in situ sampling, we analyze a sample after removing it from its target population. Because sampling exposes the target population to potential contamination, the sampling device must be inert and clean.

After removing a sample from its target population, there is a danger that it will undergo a chemical or physical change before we can complete its analysis. This is a serious problem because the sample's properties no longer are representative of the target population. To prevent this problem, we often preserve samples before transporting them to the laboratory for analysis. Even when analyzing samples in the field, preservation may still be necessary.

The initial sample is called the primary or GROSS SAMPLE, and may be a single increment drawn from the target population, or a composite of several increments. In many cases we cannot analyze the gross sample without first reducing the sample's particle size, converting the sample into a more readily analyzable form, or improving its homogeneity.

7C.1   Solutions

Typical examples of solution samples include those drawn from containers of commercial solvents; beverages, such as milk or fruit juice; natural waters, including lakes, streams, seawater and rain; bodily fluids, such as blood and urine; and, suspensions; such as those found in many oral medications. Let's use the sampling of natural waters and wastewaters as a case study in how to sample solutions.

Although you may never work with the specific samples highlighted in this section, the case studies presented here may help you in envisioning potential problems associated with your samples.
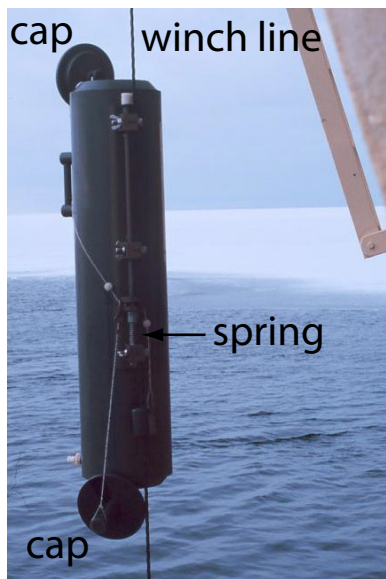
SAMPLE COLLECTION

The chemical composition of a surface water—such as a stream, river, lake, estuary, or ocean—is influenced by flow rate and depth. Rapidly flow-

Figure 7.6 A Niskin sampling bottle for collecting water samples from lakes and oceans. After lowering the bottle to the desired depth, a weight is sent down the winch line, tripping a spring that closes the bottle. Source: NOAA (photolib.noaa.gov).

ing shallow streams and rivers, and shallow (<5 m) lakes are usually well mixed, and show little stratification with depth. To collect a grab sample we submerge a capped bottle below the surface, remove the cap and allow the bottle to fill completely, and replace the cap. Collecting a sample this way avoids the air–water interface, which may be enriched with heavy metals or contaminated with oil.[10]

Slowly moving streams and rivers, lakes deeper than five meters, estuaries, and oceans may show substantial stratification. Grab samples from near the surface are collected as described earlier, and samples at greater depths are collected using a sample bottle lowered to the desired depth (Figure 7.6).

Wells for sampling groundwater are purged before collecting samples because the chemical composition of water in the well-casing may be significantly different from that of the groundwater. These differences may result from contaminants introduced while drilling the well, or by a change in the groundwater's redox potential following its exposure to atmospheric oxygen. In general, a well is purged by pumping out a volume of water equivalent to several well-casing volumes, or until the water's temperature, pH, or specific conductance is constant. A municipal water supply, such as a residence or a business, is purged before sampling because the chemical composition of water standing in a pipe may differ significantly from the treated water supply. Samples are collected at faucets after flushing the pipes for 2-3 minutes.

Samples from municipal wastewater treatment plants and industrial discharges often are collected as a 24-hour composite. An automatic sampler periodically removes an individual grab sample, adding it to those collected previously. The volume of each sample and the frequency of sampling may be constant, or may vary in response to changes in flow rate.

Sample containers for collecting natural waters and wastewaters are made from glass or plastic. Kimax and Pyrex brand borosilicate glass have the advantage of being easy to sterilize, easy to clean, and inert to all solutions except those that are strongly alkaline. The disadvantages of glass containers are cost, weight, and the ease of breakage. Plastic containers are made from a variety of polymers, including polyethylene, polypropylene, polycarbonate, polyvinyl chloride, and Teflon. Plastic containers are lightweight, durable, and, except for those manufactured from Teflon, inexpensive. In most cases glass or plastic bottles may be used interchangeably, although polyethylene bottles are generally preferred because of their lower cost. Glass containers are always used when collecting samples for the analysis of pesticides, oil and grease, and organics because these species often interact with plastic surfaces. Because glass surfaces easily adsorb metal ions, plastic bottles are preferred when collecting samples for the analysis of trace metals.

10 Duce, R. A.; Quinn, J. G. Olney, C. E.; Piotrowicz, S. R.; Ray, S. J.; Wade, T. L. *Science* **1972**, *176*, 161–163.

Table 7.1	Preservation Methods and Maximum Holding Times for Selected Analytes in Natural Waters and Wastewaters	
Analyte	Preservation Method	Maximum Holding Time
ammonia	cool to $4\,^{\circ}C$; add H_2SO_4 to $pH < 2$	28 days
chloride	none required	28 days
metals—Cr(VI)	cool to $4\,^{\circ}C$	24 hours
metals—Hg	HNO_3 to $pH < 2$	28 days
metals—all others	HNO_3 to $pH < 2$	6 months
nitrate	none required	48 hours
organochlorine pesticides	1 mL of 10 mg/mL $HgCl_2$ or immediate extraction with a suitable non-aqueous solvent	7 days without extraction 40 days with extraction
pH	none required	analyze immediately

In most cases the sample bottle has a wide mouth, making it easy to fill and remove the sample. A narrow-mouth sample bottle is used if exposing the sample to the container's cap or to the outside environment is a problem. Unless exposure to plastic is a problem, caps for sample bottles are manufactured from polyethylene. When polyethylene must be avoided, the container cap includes an inert interior liner of neoprene or Teflon.

SAMPLE PRESERVATION AND PREPARATION

After removing a sample from its target population, its chemical composition may change as a result of chemical, biological, or physical processes. To prevent a change in composition, samples are preserved by controlling the solution's pH and temperature, by limiting its exposure to light or to the atmosphere, or by adding a chemical preservative. After preserving a sample, it may be safely stored for later analysis. The maximum holding time between preservation and analysis depends on the analyte's stability and the effectiveness of sample preservation. Table 7.1 provides a list of representative methods for preserving samples and maximum holding times for several analytes of importance in the analysis of natural waters and wastewaters.

Most solution samples do not need additional preparation before analysis. This is the case for samples of natural waters and wastewaters. Solution samples with particularly complex matrices—blood and milk are two examples—may need additional processing to separate the analytes from interferents, a topic covered later in this chapter.

Here our concern is only with the need to prepare the gross sample by converting it into a form suitable for analysis. Analytical methods may include additional sample preparation steps, such as concentrating or diluting the analyte, or adjusting the analyte's chemical form. We will consider these forms of sample preparation in later chapters focusing on specific analytical methods.

7C.2 Gases

Typical examples of gaseous samples include automobile exhaust, emissions from industrial smokestacks, atmospheric gases, and compressed gases. Also

included in this category are aerosol particulates—the fine solid particles and liquid droplets that form smoke and smog. Let's use the sampling of urban air as a case study in how to sample gases.

SAMPLE COLLECTION

One approach for collecting a sample of urban air is to fill a stainless steel canister or a Tedlar/Teflon bag. A pump pulls the air into the container and, after purging, the container is sealed. This method has the advantage of simplicity and of collecting a representative sample. Disadvantages include the tendency for some analytes to adsorb to the container's walls, the presence of analytes at concentrations too low to detect with accuracy and precision, and the presence of reactive analytes, such as ozone and nitrogen oxides, that may react with the container or that may otherwise alter the sample's chemical composition during storage. When using a stainless steel canister, cryogenic cooling, which changes the sample from a gaseous state to a liquid state, may limit some of these disadvantages.

Most urban air samples are collected using a trap containing a solid sorbent or by filtering. Solid sorbents are used for volatile gases (vapor pressures more than 10^{-6} atm) and semi-volatile gases (vapor pressures between 10^{-6} atm and 10^{-12} atm). Filtration is used to collect aerosol particulates. Trapping and filtering allows for sampling larger volumes of gas—an important concern for an analyte with a small concentration—and stabilizes the sample between its collection and its analysis.

In solid sorbent sampling, a pump pulls the urban air through a canister packed with sorbent particles. Typically 2–100 L of air are sampled when collecting volatile compounds, and 2–500 m^3 when collecting semi-volatile gases. A variety of inorganic, organic polymer, and carbon sorbents have been used. Inorganic sorbents, such as silica gel, alumina, magnesium aluminum silicate, and molecular sieves, are efficient collectors for polar compounds. Their efficiency for collecting water, however, limits their sorption capacity for many organic compounds.

Organic polymeric sorbents include polymeric resins of 2,4-diphenyl-p-phenylene oxide or styrene-divinylbenzene for volatile compounds, and polyurethane foam for semi-volatile compounds. These materials have a low affinity for water, and are efficient collectors for all but the most highly volatile organic compounds, and some lower molecular weight alcohols and ketones. The adsorbing ability of carbon sorbents is superior to that of organic polymer resins, which makes them useful for highly volatile organic compounds that can not be collected by polymeric resins. The adsorbing ability of carbon sorbents may be a disadvantage, however, since the sorbed compounds may be difficult to desorb.

Non-volatile compounds are normally present either as solid particulates, or are bound to solid particulates. Samples are collected by pulling large volumes of urban air through a filtering unit, collecting the particulates on glass fiber filters.

1 m^3 is equivalent to 10^3 L.

The short term exposure of humans, animals, and plants to atmospheric pollutants is more severe than that for pollutants in other matrices. Because the composition of atmospheric gases can vary significantly over a time, the continuous monitoring of atmospheric gases such as O_3, CO, SO_2, NH_3, H_2O_2, and NO_2 by in situ sampling is important.[11]

SAMPLE PRESERVATION AND PREPARATION

After collecting a gross sample of urban air, there is generally little need for sample preservation or preparation. The chemical composition of a gas sample is usually stable when it is collected using a solid sorbent, a filter, or by cryogenic cooling. When using a solid sorbent, gaseous compounds are released for analysis by thermal desorption or by extracting with a suitable solvent. If the sorbent is selective for a single analyte, the increase in the sorbent's mass can be used to determine the amount of analyte in the sample.

7C.3 Solids

Typical examples of solid samples include large particulates, such as those found in ores; smaller particulates, such as soils and sediments; tablets, pellets, and capsules used for dispensing pharmaceutical products and animal feeds; sheet materials, such as polymers and rolled metals; and tissue samples from biological specimens. Solids are usually heterogeneous and samples must be collected carefully if they are to be representative of the target population. Let's use the sampling of sediments, soils, and ores as a case study in how to sample solids.

SAMPLE COLLECTION

Sediments from the bottom of streams, rivers, lakes, estuaries, and oceans are collected with a bottom grab sampler, or with a corer. A bottom grab sampler (Figure 7.7) is equipped with a pair of jaws that close when they contact the sediment, scooping up sediment in the process. Its principal advantages are ease of use and the ability to collect a large sample. Disadvantages include the tendency to lose finer grain sediment particles as water flows out of the sampler, and the loss of spatial information—both laterally and with depth—due to mixing of the sample.

An alternative method for collecting sediments is a cylindrical coring device (Figure 7.8). The corer is dropped into the sediment, collecting a column of sediment and the water in contact with the sediment. With the possible exception of sediment at the surface, which may experience mixing, samples collected with a corer maintain their vertical profile, preserving information about how the sediment's composition changes with depth.

Collecting soil samples at depths of up to 30 cm is easily accomplished with a scoop or shovel, although the sampling variance is generally high. A

Figure 7.7 Bottom grab sampler being prepared for deployment. Source: NOAA (photolib.noaa.gov).

11 Tanner, R. L. in Keith, L. H., ed. *Principles of Environmental Sampling*, American Chemical Society: Washington, D. C., 1988, 275–286.
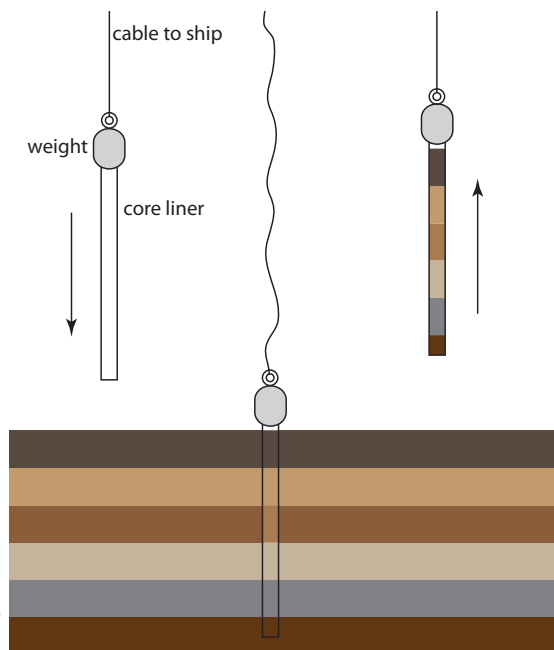
Figure 7.8 Schematic diagram of a gravity corer in operation. The corer's weight is sufficient to penetrate into the sediment to a depth of approximately 2 m. Flexible metal leaves on the bottom of the corer are pushed aside by the sediment, allowing it to enter the corer. The leaves bend back and hold the core sample in place as it is hauled back to the surface.

better tool for collecting soil samples near the surface is a soil punch, which is a thin-walled steel tube that retains a core sample after it is pushed into the soil and removed. Soil samples from depths greater than 30 cm are collected by digging a trench and collecting lateral samples with a soil punch. Alternatively, an auger may be used to drill a hole to the desired depth and the sample collected with a soil punch.

For particulate materials, particle size often determines the sampling method. Larger particulate solids, such as ores, are sampled using a riffle (Figure 7.9), which is a trough containing an even number of compartments. Because adjoining compartments empty onto opposite sides of the
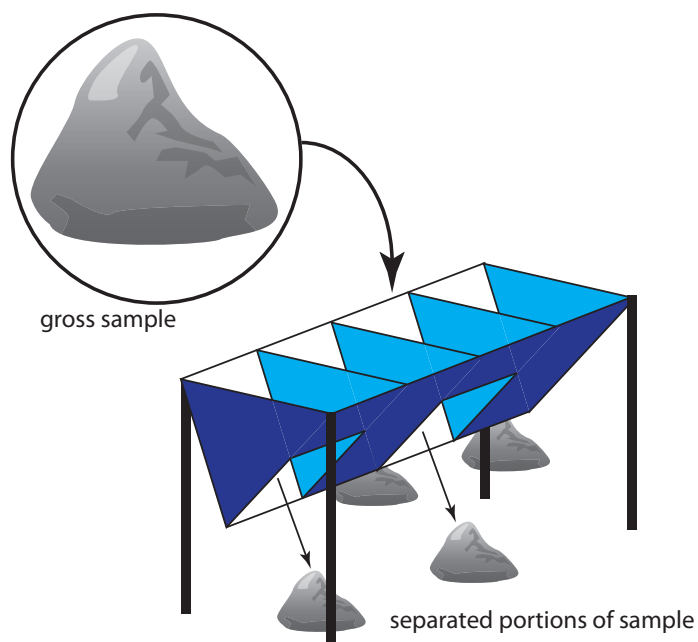

Figure 7.9 Example of a four-unit riffle. Passing the gross sample, shown within the circle, through the riffle divides it into four piles, two on each side. Combining the piles from one side of the riffle provides a new sample, which may be passed through the riffle again or kept as the final sample. The piles from the other side of the riffle are discarded.

riffle, dumping a gross sample into the riffle divides it in half. By repeatedly passing half of the separated material back through the riffle, a sample of any desired size may be collected.

A sample thief (Figure 7.10) is used for sampling smaller particulate materials, such as powders. A typical sample thief consists of two tubes that are nestled together. Each tube has one or more slots aligned down the sample thief's length. Before inserting the sample thief into the material being sampled, the slots are closed by rotating the inner tube. When the sample thief is in place, rotating the inner tube opens the slots, which fill with individual samples. The inner tube is then rotated to the closed position and the sample thief withdrawn.

Figure 7.10 Sample thief showing its open and closed positions.

SAMPLE PRESERVATION

Without preservation, a solid sample may undergo a change in composition due to the loss of volatile material, biodegradation, and chemical reactivity (particularly redox reactions). Storing samples at lower temperatures makes them less prone to biodegradation and to the loss of volatile material, but fracturing of solids and phase separations may present problems. To minimize the loss of volatiles, the sample container is filled completely, eliminating a headspace where gases collect. Samples that have not been exposed to O_2 are particularly susceptible to oxidation reactions. For example, the contact of air with anaerobic sediments must be prevented.

SAMPLE PREPARATION

Unlike gases and liquids, which generally require little sample preparation, a solid sample usually needs some processing before analysis. There are two reasons for this. First, as discussed in section 7B.3, the standard deviation for sampling, s_{samp}, is a function of the number of particles in the sample, not the combined mass of the particles. For a heterogeneous material consisting of large particulates, the gross sample may be too large to analyze. For example, a Ni-bearing ore with an average particle size of 5 mm may require a sample weighing one ton to obtain a reasonable s_{samp}. Reducing the sample's average particle size allows us to collect the same number of particles with a smaller, more manageable mass. Second, many analytical techniques require that the analyte be in solution.

REDUCING PARTICLE SIZE

A reduction in particle size is accomplished by a combination of crushing and grinding the gross sample. The resulting particulates are then thoroughly mixed and divided into SUBSAMPLES of smaller mass. This process seldom occurs in a single step. Instead, subsamples are cycled through the process several times until a final LABORATORY SAMPLE is obtained.

Crushing and grinding uses mechanical force to break larger particles into smaller particles. A variety of tools are used depending on the particle's
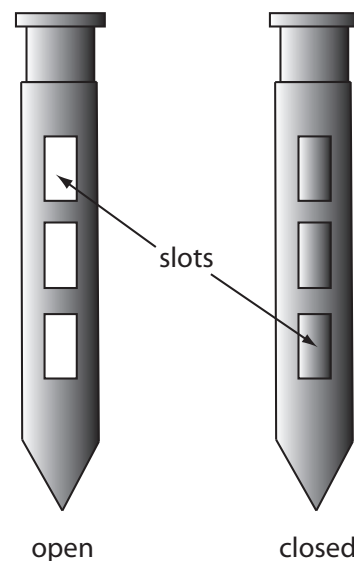
size and hardness. Large particles are crushed using jaw crushers capable of reducing particles to diameters of a few millimeters. Ball mills, disk mills, and mortars and pestles are used to further reduce particle size.

A significant change in the gross sample's composition may occur during crushing and grinding. Decreasing particle size increases the available surface area, which increases the risk of losing volatile components. This problem is made worse by the frictional heat that accompanies crushing and grinding. Increasing the surface area also exposes interior portions of the sample to the atmosphere where oxidation may alter the gross sample's composition. Other problems include contamination from the materials used to crush and grind the sample, and differences in the ease with which particles are reduced in size. For example, softer particles are easier to reduce in size and may be lost as dust before the remaining sample is processed. This is a particular problem if the analyte's distribution between different types of particles is not uniform.

The gross sample is reduced to a uniform particle size by intermittently passing it through a sieve. Those particles not passing through the sieve receive additional processing until the entire sample is of uniform size. The resulting material is mixed thoroughly to ensure homogeneity and a subsample obtained with a riffle, or by CONING AND QUARTERING. As shown in Figure 7.11, the gross sample is piled into a cone, flattened, and divided into four quarters. After discarding two diagonally opposed quarters, the remaining material is cycled through the process of coning and quartering until a suitable laboratory sample remains.
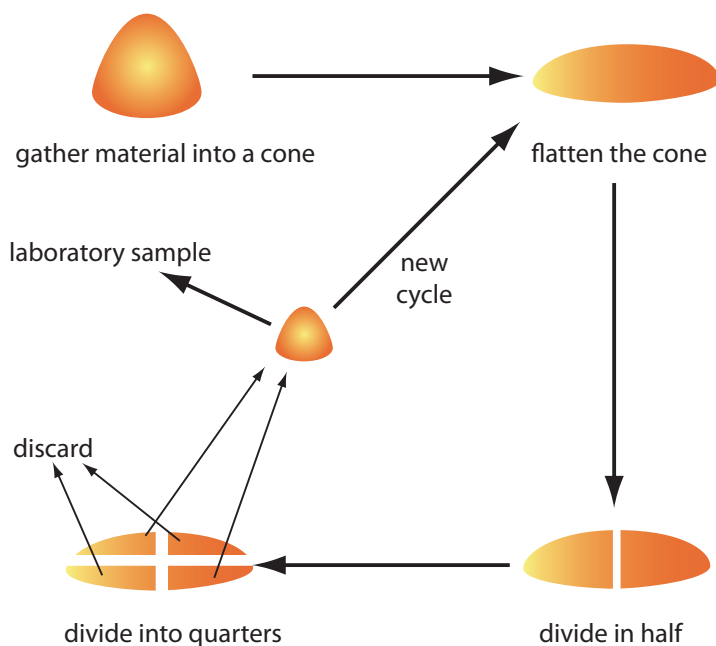

gather material into a cone

flatten the cone

laboratory sample

new cycle

discard

divide into quarters

divide in half

Figure 7.11 Illustration showing the method of coning and quartering for reducing sample size. After gathering the gross sample into a cone, the cone is flattened, divided in half, and then divided into quarters. Two opposing quarters are combined to form the laboratory sample, or the subsample is sent back through another cycle. The two remaining quarters are discarded.

BRINGING SOLID SAMPLES INTO SOLUTION

If you are fortunate, your sample will easily dissolve in a suitable solvent, requiring no more effort than gently swirling and heating. Distilled water is usually the solvent of choice for inorganic salts, but organic solvents, such as methanol, chloroform, and toluene are useful for organic materials.

When a sample is difficult to dissolve, the next step is to try digesting it with an acid or a base. Table 7.2 lists several common acids and bases, and summarizes their use. Digestions are carried out in an open container, usually a beaker, using a hot-plate as a source of heat. The main advantage of an open-vessel digestion is cost because it requires no special equipment. Volatile reaction products, however, are lost, resulting in a determinate error if they include the analyte.

Many digestions are now carried out in a closed container using microwave radiation as the source of energy. Vessels for microwave digestion are manufactured using Teflon (or some other fluoropolymer) or fused silica. Both materials are thermally stable, chemically resistant, transparent to microwave radiation, and capable of withstanding elevated pressures. A typical microwave digestion vessel, as shown in Figure 7.12, consists of an insulated vessel body and a cap with a pressure relief valve. The vessels are placed in a microwave oven (typically 6–14 vessels can be accommodated) and microwave energy is controlled by monitoring the temperature or pressure within one of the vessels.

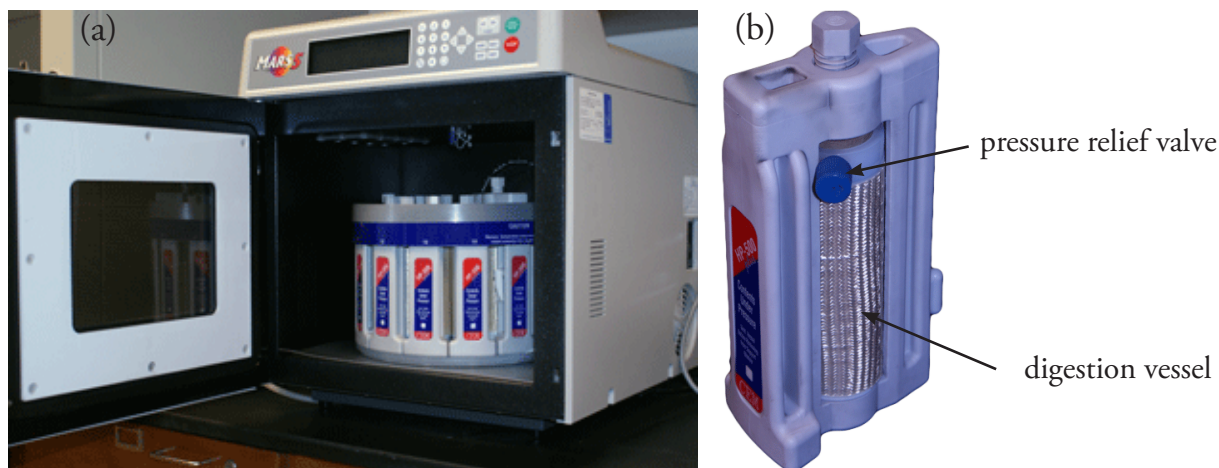| Table 7.2 Acids and Bases Used for Digesting Samples	
Solution	Uses and Properties
HCl (37% w/w)	• dissolves metals more easily reduced than H_2 ($E° < 0$) • dissolves insoluble carbonate, sulfides, phosphates, fluorides, sulfates, and many oxides
HNO_3 (70% w/w)	• strong oxidizing agent • dissolves most common metals except Al, Au, Pt, and Cr • decomposes organics and biological samples (wet ashing)
H_2SO_4 (98% w/w)	• dissolves many metals and alloys • decomposes organics by oxidation and dehydration
HF (50% w/w)	• dissolves silicates by forming volatile SiF_4
$HClO_4$ (70% w/w)	• hot, concentrated solutions are strong oxidizing agents • dissolves many metals and alloys • decomposes organics (*Caution: reactions with organics often are explosive; use only in a specially equipped hood with a blast shield and after prior decomposition with HNO_3*)
HCl:HNO_3 (3:1 v/v)	• also known as aqua regia • dissolves Au and Pt
NaOH	• dissolves Al and amphoteric oxides of Sn, Pb, Zn, and Cr

Figure 7.12 Microwave digestion unit. (a) View of the unit's interior showing the carousel holding the digestion vessels. (b) Close-up of a Teflon digestion vessel, which is encased in a thermal sleeve. The pressure relief value, which is part of the vessel's cap, contains a membrane that ruptures if the internal pressure becomes too high.

A microwave digestion has several important advantages over an open-vessel digestion, including higher temperatures (200–300 °C) and pressures (40–100 bar). As a result, digestions requiring several hours in an open-vessel may need no more than 30 minutes when using a microwave digestion. In addition, a closed container prevents the loss of volatile gases. Disadvantages include the inability to add reagents during the digestion, limitations on the sample's size (typically < 1 g), and safety concerns due to the high pressures and corrosive reagents.

Inorganic samples that resist decomposition by digesting with acids or bases often can be brought into solution by fusing with a large excess of an alkali metal salt, called a flux. After mixing the sample and the flux in a crucible, they are heated to a molten state and allowed to cool slowly to room temperature. The melt usually dissolves readily in distilled water or dilute acid. Table 7.3 summarizes several common fluxes and their uses. Fusion works when other methods of decomposition do not because of the high temperature and the flux's high concentration in the molten liquid.

Table 7.3	Common Fluxes for Decomposing Inorganic Samples		
Flux	Melting Temperature (°C)	Crucible	Typical Samples
Na_2CO_3	851	Pt	silicates, oxides, phosphates, sulfides
$Li_2B_4O_7$	930	Pt, graphite	aluminosilicates, carbonates
$LiBO_2$	845	Pt, graphite	aluminosilicates, carbonates
NaOH	318	Au, Ag	silicates, silicon carbide
KOH	380	Au, Ag	silicates, silicon carbide
Na_2O_2	—	Ni	silicates, chromium steels, Pt alloys
$K_2S_2O_7$	300	Ni, porcelain	oxides
B_2O_3	577	Pt	silicates, oxides

Disadvantages include contamination from the flux and the crucible, and the loss of volatile materials.

Finally, we can decompose organic materials by dry ashing. In this method the sample is placed in a suitable crucible and heated over a flame or in a furnace. The carbon present in the sample oxidizes to CO_2, and hydrogen, sulfur, and nitrogen leave as H_2O, SO_2, and N_2. These gases can be trapped and weighed to determine their concentration in the organic material. Often the goal of dry ashing is to remove the organic material, leaving behind an inorganic residue, or ash, that can be further analyzed.

7D   Separating the Analyte from Interferents

When an analytical method is selective for the analyte, analyzing samples is a relatively simple task. For example, a quantitative analysis for glucose in honey is relatively easy to accomplish if the method is selective for glucose, even in the presence of other reducing sugars, such as fructose. Unfortunately, few analytical methods are selective toward a single species.

In the absence of interferents, the relationship between the sample's signal, S_{samp}, and the concentration of analyte, C_A, is

$$S_{samp} = k_A C_A \qquad 7.9$$

where k_A is the analyte's sensitivity. If an interferent, is present, then equation 7.9 becomes

$$S_{samp} = k_A C_A + k_I C_I \qquad 7.10$$

where k_I and C_I are, respectively, the interferent's sensitivity and concentration. A method's selectivity for the analyte is determined by the relative difference in its sensitivity toward the analyte and the interferent. If k_A is greater than k_I, then the method is more selective for the analyte. The method is more selective for the interferent if k_I is greater than k_A.

Even if a method is more selective for an interferent, we can use it to determine C_A if the interferent's contribution to S_{samp} is insignificant. The SELECTIVITY COEFFICIENT, $K_{A,I}$, which we introduced in Chapter 3, provides a way to characterize a method's selectivity.

$$K_{A,I} = \frac{k_I}{k_A} \qquad 7.11$$

Solving equation 7.11 for k_I, substituting into equation 7.10, and simplifying, gives

$$S_{samp} = k_A (C_A + K_{A,I} \times C_I) \qquad 7.12$$

An interferent, therefore, does not pose a problem as long as the product of its concentration and its selectivity coefficient is significantly smaller than the analyte's concentration.

In equation 7.9, and the equations that follow, you can replace the analyte's concentration, C_A, with the moles of analyte, n_A when working with methods, such as gravimetry, that respond to the absolute amount of analyte in a sample. In this case the interferent also is expressed in terms of moles.

$$K_{A,I} \times C_I << C_A$$

If we cannot ignore an interferent's contribution to the signal, then we must begin our analysis by separating the analyte and the interferent.

7E   General Theory of Separation Efficiency

The goal of an analytical separation is to remove either the analyte or the interferent from the sample's matrix. To achieve this separation there must be at least one significant difference between the analyte's and the interferent's chemical or physical properties. A significant difference in properties, however, may not be enough. A separation that completely removes the interferent may also remove a small amount of analyte. Altering the separation to minimize the analyte's loss may prevent us from completely removing the interferent.

Two factors limit a separation's efficiency—failing to recover all the analyte and failing to remove all the interferent. We define the analyte's RECOVERY, R_A, as

$$R_A = \frac{C_A}{(C_A)_o} \qquad 7.13$$

where C_A is the concentration of analyte remaining after the separation, and $(C_A)_o$ is the analyte's initial concentration. A recovery of 1.00 means that no analyte is lost during the separation. The interferent's recovery, R_I, is defined in the same manner

$$R_I = \frac{C_I}{(C_I)_o} \qquad 7.14$$

where C_I is the concentration of interferent remaining after the separation, and $(C_I)_o$ is the interferent's initial concentration. We define the extent of the separation using a separation factor, $S_{I,A}$.[12]

$$S_{I,A} = \frac{R_I}{R_A} \qquad 7.15$$

The meaning of trace and macro, as well as other terms for describing the concentrations of analytes and interferents, is presented in Chapter 2.

In general, $S_{I,A}$ should be approximately 10^{-7} for the quantitative analysis of a trace analyte in the presence of a macro interferent, and 10^{-3} when the analyte and interferent are present in approximately equal amounts.

Example 7.10

An analytical method for determining Cu in industrial plating baths gives poor results in the presence of Zn. To evaluate a method for separating the analyte from the interferent, samples containing known concentra-

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12   (a) Sandell, E. B. *Colorimetric Determination of Trace Metals*, Interscience Publishers: New York, 1950, pp. 19–20; (b) Sandell, E. B. *Anal. Chem.* **1968**, *40*, 834–835.

tions of Cu or Zn are prepared and analyzed. When a sample containing 128.6 ppm Cu is taken through the separation, the concentration of Cu remaining is 127.2 ppm. Taking a 134.9 ppm solution of Zn through the separation leaves behind a concentration of 4.3 ppm Zn. Calculate the recoveries for Cu and Zn, and the separation factor.

SOLUTION

Using equation 7.13 and equation 7.14, the recoveries for the analyte and interferent are

$$R_{Cu} = \frac{127.2 \text{ ppm}}{128.6 \text{ ppm}} = 0.9891, \text{ or } 98.91\%$$

$$R_{Zn} = \frac{4.3 \text{ ppm}}{134.9 \text{ ppm}} = 0.032, \text{ or } 3.2\%$$

and the SEPARATION FACTOR is

$$S_{Zn,Cu} = \frac{R_{Zn}}{R_{Cu}} = \frac{0.032}{0.9891} = 0.032$$

Recoveries and separation factors are useful for evaluating a separation's potential effectiveness. They do not, however, provide a direct indication of the error that results from failing to remove all the interferent or from failing to completely recover the analyte. The relative error due to the separation, E, is

$$E = \frac{S_{samp} - S^*_{samp}}{S^*_{samp}} \qquad 7.16$$

where S^*_{samp} is the sample's signal for an ideal separation in which we completely recover the analyte.

$$S^*_{samp} = k_A (C_A)_o \qquad 7.17$$

Substituting equation 7.12 and equation 7.17 into equation 7.16, and rearranging

$$E = \frac{k_A (C_A + K_{A,I} \times C_I) - k_A (C_A)_o}{k_A (C_A)_o}$$

$$E = \frac{C_A + K_{A,I} \times C_I - (C_A)_o}{(C_A)_o}$$

$$E = \frac{C_A}{(C_A)_o} - \frac{(C_A)_o}{(C_A)_o} + \frac{K_{A,I} \times C_I}{(C_A)_o}$$

leaves us with

$$E = (R_A - 1) + \frac{K_{A,I} \times C_I}{(C_A)_o} \qquad 7.18$$

A more useful equation is obtained by solving equation 7.14 for C_I and substituting into equation 7.18.

$$E = (R_A - 1) + \frac{K_{A,I} \times (C_I)_o}{(C_A)_o} \times R_I \qquad 7.19$$

The first term of equation 7.19 accounts for the analyte's incomplete recovery, and the second term accounts for failing to remove all the interferent.

Example 7.11

Following the separation outlined in Example 7.10, an analysis is carried out to determine the concentration of Cu in an industrial plating bath. Analysis of standard solutions containing either Cu or Zn give the following linear calibrations.

$$S_{Cu} = 1250 \text{ ppm}^{-1} \times C_{Cu}$$

$$S_{Zn} = 2310 \text{ ppm}^{-1} \times C_{Zn}$$

(a) What is the relative error if we analyze samples without removing the Zn? Assume that the initial concentration ratio, Cu:Zn, is 7:1. (b) What is the relative error if we first complete the separation, obtaining the recoveries from Example 7.10? (c) What is the maximum acceptable recovery for Zn if the recovery for Cu is 1.00 and the error due to the separation must be no greater than 0.10%?

SOLUTION

(a) If we complete the analysis without separating Cu and Zn, then R_{Cu} and R_{Zn} are exactly 1 and equation 7.19 simplifies to

$$E = \frac{K_{Cu,Zn} \times (C_{Zn})_o}{(C_{Cu})_o}$$

Using equation 7.11, we find that the selectivity coefficient is

$$K_{Cu,Zn} = \frac{k_{Zn}}{k_{Cu}} = \frac{2310 \text{ ppm}^{-1}}{1250 \text{ ppm}^{-1}} = 1.85$$

Given the initial concentration ratio, Cu:Zn, of 7:1, the relative error if we do not attempt a separation of Cu and Zn is

$$E = \frac{1.85 \times 1}{7} = 0.264, \text{ or } 26.4\%$$

(b) To calculate the relative error we substitute the recoveries from [Example 7.10](#) into [equation 7.19](#), obtaining

$$E = (0.9891 - 1) + \frac{1.85 \times 1}{7} \times 0.032$$

$$E = -0.0109 + 0.0085 = -0.0024$$

or –0.24%. Note that the negative determinate error due to the analyte's incomplete recovery is partially offset by a positive determinate error from failing to remove all the interferent.

(c) To determine the maximum recovery for Zn, we make appropriate substitutions into [equation 7.19](#)

$$E = 0.0010 = (1 - 1) + \frac{1.85 \times 1}{7} \times R_{Zn}$$

and solve for R_{Zn}, obtaining a recovery of 0.0038, or 0.38%. Thus, we must remove at least

$$100.00\% - 0.38\% = 99.62\%$$

of the Zn to obtain an error of 0.10% when R_{Cu} is exactly 1.

7F   Classifying Separation Techniques

We can separate an analyte and an interferent if there is a significant difference in at least one of their chemical or physical properties. Table 7.4 provides a partial list of separation techniques, classified by the chemical or physical property being exploited.

| Table 7.4 Classification of Separation Techniques	
Basis of Separation	Separation Technique
size	filtration
	dialysis
	size-exclusion chromatography
mass or density	centrifugation
complex formation	masking
change in physical state	distillation
	sublimation
	recrystallization
change in chemical state	precipitation
	electrodeposition
	volatilization
partitioning between phases	extraction
	chromatography
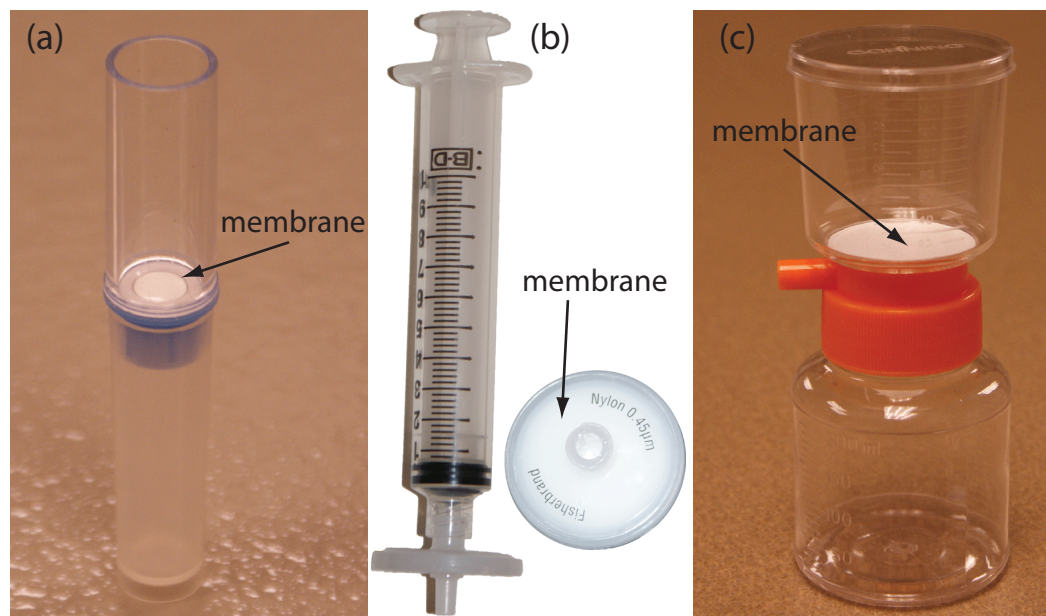
Figure 7.13 Three types of membrane filters for separating analytes from interferents. (a) A centrifugal filter for concentrating and desalting macromolecular solutions. The membrane has a nominal molecular weight cut-off of 1×10^6 g/mol. The sample is placed in the upper reservoir and the unit is placed in a centrifuge. Upon spinning the unit at $2000 \times g$–$5000 \times g$, the filtrate collects in the bottom reservoir and the retentate remains in the upper reservoir. (b) A 0.45 μm membrane syringe filter. The photo on the right shows the membrane filter in its casing. In the photo on the left, the filter is attached to a syringe. Samples are placed in the syringe and pushed through the filter. The filtrate is collected in a test tube or other suitable container. (c) A disposable filter system with a 0.22 μm cellulose acetate membrane filter. The sample is added to the upper unit and vacuum suction is used to draw the filtrate through the membrane and into the lower unit. To store the filtrate, the top half of the unit is removed and a cap placed on the lower unit. The filter unit shown here has a capacity of 150 mL.

7F.1   Separations Based on Size

Size is the simplest physical property we can exploit in a separation. To accomplish the separation we use a porous medium through which only the analyte or the interferent can pass. Examples of size-based separations include filtration, dialysis, and size-exclusion.

In a FILTRATION we separate a particulate interferent from dissolved analytes using a filter whose pore size retains the interferent. The solution passing through the filter is called the FILTRATE, and the material retained by the filter is the RETENTATE. Gravity filtration and suction filtration using filter paper are techniques with which you should already be familiar. Membrane filters, available in a variety of micrometer pores sizes, are the method of choice for particulates that are too small to be retained by filter paper. Figure 7.13 provides information about three types of membrane filters.

DIALYSIS is another example of a separation technique that uses size to separate the analyte and the interferent. A dialysis membrane is usually constructed from cellulose and fashioned into tubing, bags, or cassettes. Figure 7.14 shows an example of a commercially available dialysis cassette. The sample is injected into the dialysis membrane, which is sealed tightly

For applications of gravity filtration and suction filtration in gravimetric methods of analysis, see Chapter 8.

Figure 7.14 Example of a dialysis cassette. The dialysis membrane in this unit has a molecular weight cut-off of 10 000 g/mol. Two sheets of the membrane are separated by a gasket and held in place by the plastic frame. Four ports, one of which is labeled, provide a means for injecting the sample between the dialysis membranes. The cassette is inverted and submerged in a beaker containing the external solution, which is stirred using a stir bar. A foam buoy, used as a stand in the photo, serves as a float so that the unit remains suspended above the stir bar. The external solution is usually replaced several time during dialysis. When dialysis is complete, the solution remaining in the cassette is removed through an injection port.

by a gasket, and the unit is placed in a container filled with a solution whose composition is different from the sample. If the concentration of a particular species is different on the membrane's two sides, the resulting concentration gradient provides a driving force for its diffusion across the membrane. While small species freely pass through the membrane, larger species are unable to pass. Dialysis is frequently used to purify proteins, hormones, and enzymes. During kidney dialysis, metabolic waste products, such as urea, uric acid, and creatinine, are removed from blood by passing it over a dialysis membrane.

Size-exclusion chromatography is a third example of a separation technique that uses size as a means for effecting a separation. In this technique a column is packed with small, approximately 10-μm, porous polymer beads of cross-linked dextrin or polyacrylamide. The pore size of the particles is controlled by the degree of cross-linking, with more cross-linking producing smaller pore sizes. The sample is placed into a stream of solvent that is pumped through the column at a fixed flow rate. Those species too large to enter the pores pass through the column at the same rate as the solvent. Species entering into the pores take longer to pass through the column, with smaller species needing more time to pass through the column. Size-exclusion chromatography is widely used in the analysis of polymers, and in biochemistry, where it is used for the separation of proteins.

A more detailed treatment of size-exclusion chromatography, which also is called gel permeation chromatography, is in Chapter 12.

7F.2   Separations Based on Mass or Density

If the analyte and the interferent differ in mass or density, then a separation using centrifugation may be possible. The sample is placed in a centrifuge tube and spun at a high angular velocity, measured in revolutions per minute (rpm). The sample's constituents experience a centrifugal force that pulls them toward the bottom of the centrifuge tube. The species experiencing the greatest centrifugal force has the fastest sedimentation rate, and

Table 7.5 Conditions for Separating Selected Cellular Components by Centrifugation		
Components	Centrifugal Force ($\times g$)	Time (min)
eukaryotic cells	1000	5
cell membranes, nuclei	4000	10
mitochondria, bacterial cells	15 000	20
lysosomes, bacterial membranes	30 000	30
ribosomes	100 000	180

Source: Adapted from Zubay, G. *Biochemistry*, 2nd ed. Macmillan: New York, 1988, p.120.

is the first to reach the bottom of the centrifuge tube. If two species have equal density, their separation is based on mass, with the heavier species having the greater sedimentation rate. If the species are of equal mass, then the species with the largest density has the greatest sedimentation rate.

Centrifugation is an important separation technique in biochemistry. Table 7.5, for example, lists conditions for separating selected cellular components. We can separate lysosomes from other cellular components by several differential centrifugations, in which we divide the sample into a solid residue and a supernatant solution. After destroying the cells, the solution is centrifuged at 15 000 $\times g$ (a centrifugal force that is 15 000 times that of the earth's gravitational force) for 20 minutes, leaving a solid residue of cell membranes and mitochondria. The supernatant, which contains the lysosomes, is isolated by decanting it from the residue and centrifuged at 30 000 $\times g$ for 30 minutes, leaving a solid residue of lysosomes. Figure 7.15 shows a typical centrifuge capable of produce the centrifugal forces needed for biochemical separations.

An alternative approach to differential centrifugation is DENSITY GRADI-ENT CENTRIFUGATION. To prepare a sucrose density gradient, for example, a solution with a smaller concentration of sucrose—and, thus, of lower density—is gently layered upon a solution with a higher concentration of

Figure 7.15 Bench-top centrifuge capable of reaching speeds up to 14 000 rpm and centrifugal forces of 20 800 $\times g$. This particular centrifuge is refrigerated, allowing samples to be cooled to temperatures as low as –4 °C.
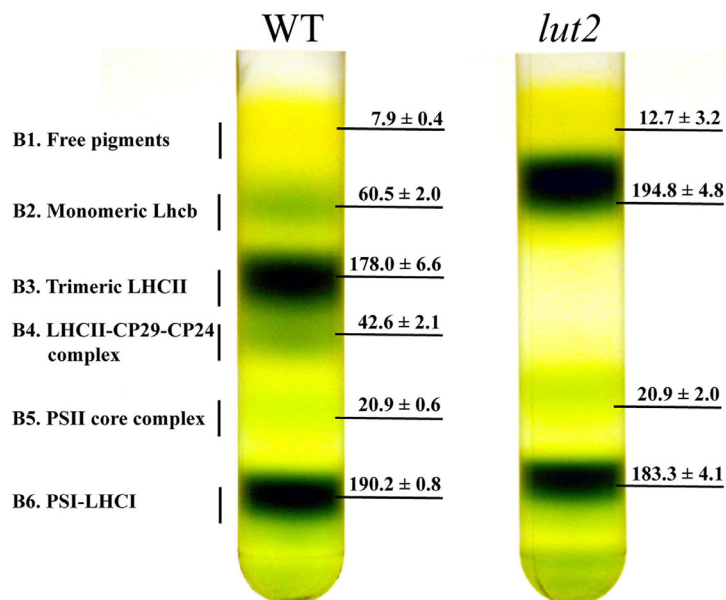
Figure 7.16 Example of a sucrose density gradient centrifugation of thylakoid membranes from wild type (WT) and *lut2* plants. The thylakoid membranes were extracted from the plant's leaves and separated by centrifuging in a 0.1–1 M sucrose gradient for 22 h at $280\,000 \times g$ at $4\,^{\circ}$C. Six bands and their chlorophyll contents are shown. Adapted from Dall'Osto, L.; Lico, C.; Alric, J.; Giuliano, G.; Havaux, M.; Bassi, R. *BMC Plant Biology* **2006**, *6:32.*

sucrose. Repeating this process several times, fills the centrifuge tube with a multi-layer density gradient. The sample is placed on top of the density gradient and centrifuged using a force greater than $150\,000 \times g$. During centrifugation, each of the sample's components moves through the gradient until it reaches a position where its density matches the surrounding sucrose solution. Each component is isolated as a separate band positioned where its density is equal to that of the local density within the gradient. Figure 7.16 provides an example of a typical sucrose density centrifugation for separating plant thylakoid membranes.

7F.3 Separations Based on Complexation Reactions (Masking)

One widely used technique for preventing an interference is to bind the interferent in a strong, soluble complex that prevents it from interfering in the analyte's determination. This process is known as MASKING. As shown in Table 7.6, a wide variety of ions and molecules are useful MASKING AGENTS, and, as a result, selectivity is usually not a problem.

Technically, masking is not a separation technique because we do not physically separate the analyte and the interferent. We do, however, chemically isolate the interferent from the analyte, resulting in a pseudo-separation.

Example 7.12

Using Table 7.6, suggest a masking agent for an analysis of aluminum in the presence of iron.

SOLUTION

A suitable masking agent must form a complex with the interferent, but not with the analyte. Oxalate, for example, is not a suitable masking agent

Table 7.6 Selected Inorganic and Organic Masking Agents for Metal Ions	
Masking Agent	Elements Whose Ions Can Be Masked
CN^-	Ag, Au, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pd, Pt, Zn
SCN^-	Ag, Cd, Co, Cu, Fe, Ni, Pd, Pt, Zn
NH_3	Ag, Co, Ni, Cu, Zn
F^-	Al, Co, Cr, Mg, Mn, Sn, Zn
$S_2O_3^{2-}$	Au, Ce, Co, Cu, Fe, Hg, Mn, Pb, Pd, Pt, Sb, Sn, Zn
tartrate	Al, Ba, Bi, Ca, Ce, Co, Cr, Cu, Fe, Hg, Mn, Pb, Pd, Pt, Sb, Sn, Zn
oxalate	Al, Fe, Mg, Mn
thioglycolic acid	Cu, Fe, Sn

Source: Meites, L. *Handbook of Analytical Chemistry*, McGraw-Hill: New York, 1963.

because it binds both Al and Fe. Thioglycolic acid, on the other hand, is a selective masking agent for Fe in the presence of Al. Other acceptable masking agents are cyanide (CN^-) thiocyanate (SCN^-), and thiosulfate ($S_2O_3^{2-}$).

Practice Exercise 7.6

Using Table 7.6, suggest a masking agent for the analysis of Fe in the presence of Al.

Click here to review your answer to this exercise.

As shown in Example 7.13, we can judge a masking agent's effectiveness by considering the relevant equilibrium constants.

Example 7.13

Show that CN^- is an appropriate masking agent for Ni^{2+} in a method where nickel's complexation with EDTA is an interference.

SOLUTION

The relevant reactions and formation constants are

$$Ni^{2+}(aq) + Y^{4-}(aq) \rightleftharpoons NiY^{2-}(aq) \quad K_1 = 4.2\times10^{18}$$

$$Ni^{2+}(aq) + 4CN^-(aq) \rightleftharpoons Ni(CN)_4^{2-}(aq) \quad \beta_4 = 1.7\times10^{30}$$
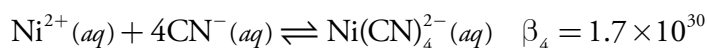
where Y^{4-} is an abbreviation for EDTA. Cyanide is an appropriate masking agent because the formation constant for the $Ni(CN)_4^{2-}$ is greater than that for the Ni–EDTA complex. In fact, the equilibrium constant for the reaction in which EDTA displaces the masking agent

$$Ni(CN)_4^{2-}(aq) + Y^{4-}(aq) \rightleftharpoons NiY^{2+}(aq) + 4CN^-(aq)$$
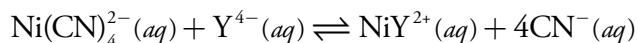
You will find the formation constants for these reactions in Appendix 12.

$$K = \frac{K_1}{\beta_4} = \frac{4.2 \times 10^{18}}{1.7 \times 10^{30}} = 2.5 \times 10^{-12}$$

is very small, indicating that $Ni(CN)_4^{2-}$ is relatively inert in the presence of EDTA.

Practice Exercise 7.7

Use the formation constants in Appendix 12 to show that 1,10-phenanthroline is a suitable masking agent for Fe^{2+} in the presence of Fe^{3+}. Use a ladder diagram to define any limitations on using 1,10-phenanthroline as a masking agent. See Chapter 6 for a review of ladder diagrams.

Click here to review your answer to this exercise.

7F.4 Separations Based on a Change of State

Because an analyte and its interferent are usually in the same phase, we can achieve a separation if one of them undergoes a change in its physical state or its chemical state.

CHANGES IN PHYSICAL STATE

When the analyte and the interferent are miscible liquids, a DISTILLATION may be possible if their boiling points are significantly different. Figure 7.17 shows the progress of a distillation as a plot of temperature versus the mixture's vapor-phase and liquid-phase composition. The initial mixture (point A), contains more interferent than analyte. When this solution is brought to its boiling point, the vapor phase in equilibrium with the liquid phase is enriched in analyte (point B). The horizontal line connecting points A and B represents this vaporization equilibrium. Condensing the vapor phase at point B, by lowering the temperature, creates a new liquid phase whose composition is identical to that of the vapor phase (point C). The vertical line connecting points B and C represents this condensation
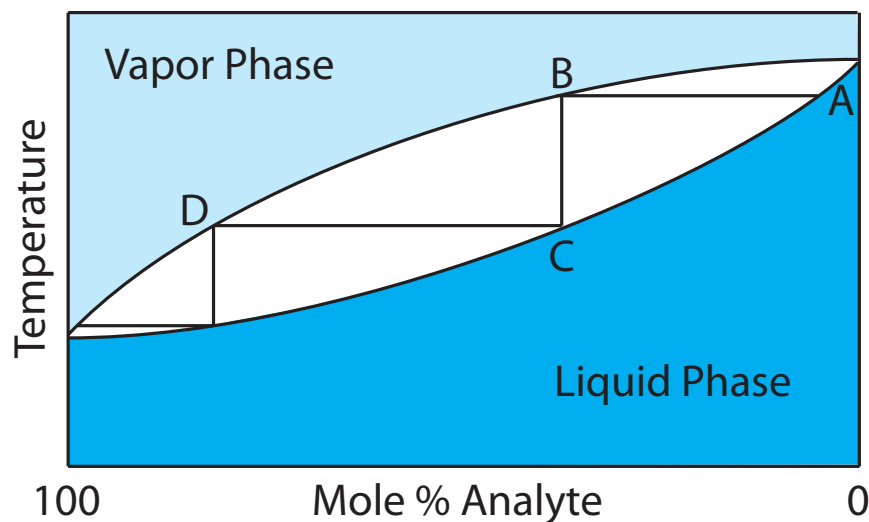

Figure 7.17 Boiling point versus composition diagram for a near-ideal solution consisting of a low-boiling analyte and a high-boiling interferent. The horizontal lines represent vaporization equilibria and the vertical lines represent condensation equilibria. See the text for additional details.
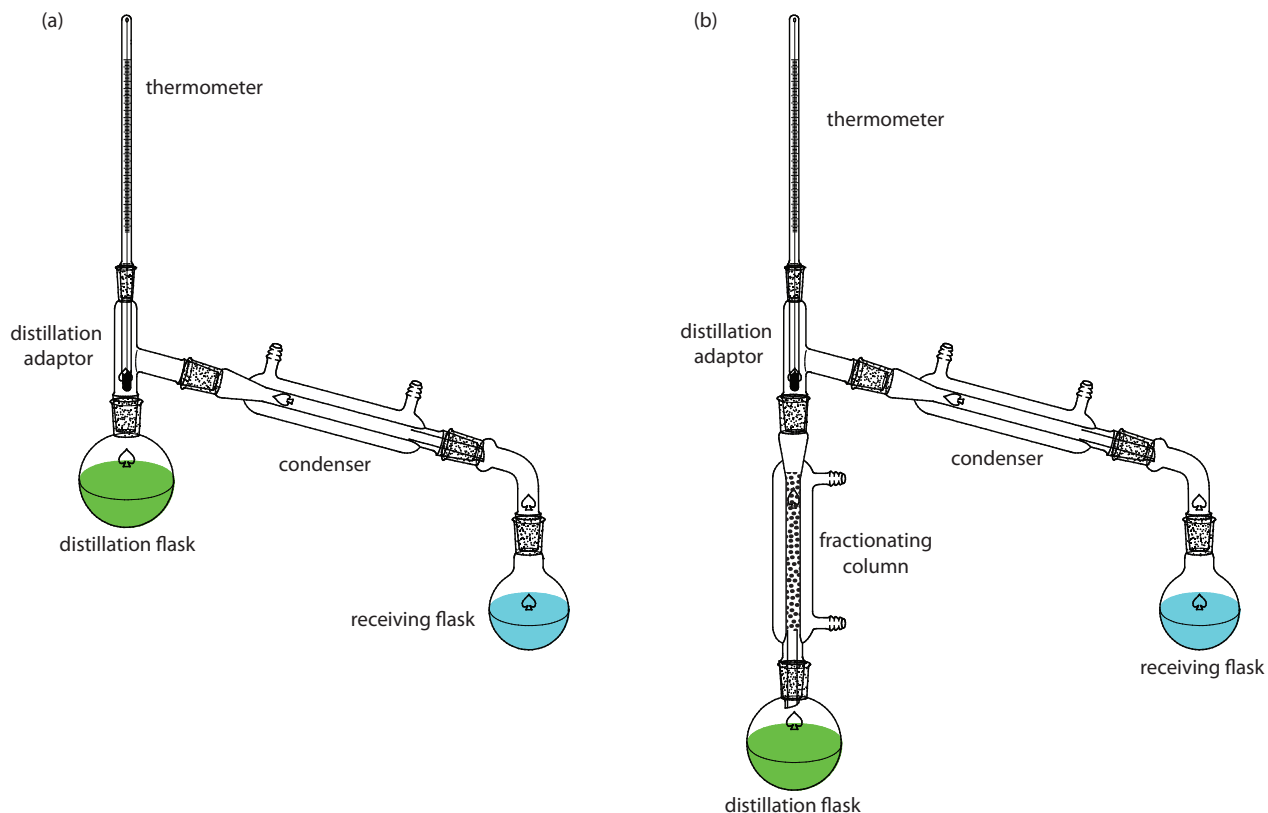
Figure 7.18 Typical experimental set-up for (a) a simple distillation, and (b) a fractional distillation.
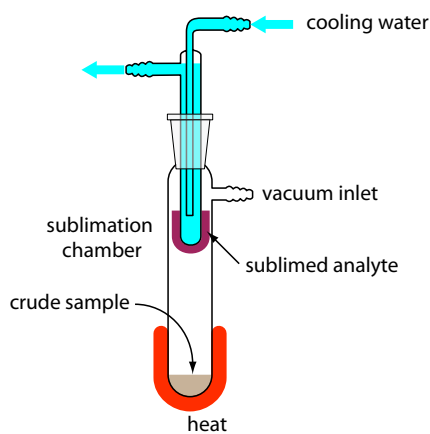

Figure 7.19 Typical experimental set-up for a sublimation. The sample is placed in the sublimation chamber, which may be evacuated. Heating the sample causes the analyte to vaporize and sublime onto a cooled probe. Modified from Slashme (commons. wikipedia.org).

equilibrium. The liquid phase at point C has a lower boiling point than the original mixture, and is in equilibrium with the vapor phase at point D. This process of repeated vaporization and condensation gradually separates the analyte and interferent.

Two experimental set-ups for distillations are shown in Figure 7.18. The simple distillation apparatus shown in Figure 7.18a does not produce a very efficient separation, and is useful only for separating a volatile analyte (or interferent) from a non-volatile interferent (or analyte), or for an analyte and interferent whose boiling points differ by more than $150\,^\circ$C. A more efficient separation is achieved by a fractional distillation (Figure 7.18b). Packing the distillation column with a high surface area material, such as a steel sponge or glass beads, provides more opportunity for the repeated process of vaporization and condensation necessary to effect a complete separation.

When the sample is a solid, SUBLIMATION may provide a useful separation of the analyte and the interferent. The sample is heated at a temperature and pressure below the analyte's triple point, allowing it to vaporize without passing through the liquid state. Condensing the vapor recovers the purified analyte (Figure 7.19). A useful analytical example of sublimation is the isolation of amino acids from fossil mollusk shells and deep-sea sediments.[13]

13   Glavin, D. P.; Bada, J. L. *Anal. Chem.* **1998**, *70*, 3119–3122.

RECRYSTALLIZATION is another method for purifying a solid. A solvent is chosen in which the analyte's solubility is significant when the solvent is hot, and minimal when the solvent is cold. The interferents must be less soluble in the hot solvent than the analyte, or present in much smaller amounts. After heating a portion of the solvent in an Erlenmeyer flask, small amounts of the sample are added until undissolved sample is visible. Additional hot solvent is added until the sample redissolves, or until only insoluble impurities remain. This process of adding sample and solvent is repeated until the entire sample has been added to the Erlenmeyer flask. Any insoluble impurities are removed by filtering the hot solution. The solution is allowed to cool slowly, promoting the growth of large, pure crystals, and then cooled in an ice bath to minimize solubility losses. The purified sample is isolated by filtration and rinsed to remove any soluble impurities. Finally, the sample is dried to remove any remaining traces of the solvent. Further purification, if necessary, can be accomplished by additional recrystallizations.

CHANGES IN CHEMICAL STATE

Distillation, sublimation, and recrystallization use a change in physical state as a means of separation. Chemical reactivity also can be a useful tool for separating analytes and interferents. For example, we can separate SiO_2 from a sample by reacting it with HF to form SiF_4. Because SiF_4 is volatile, it is easy to remove by evaporation. If we wish to collect the reaction's volatile product, then a distillation is possible. For example, we can isolate the NH_4^+ in a sample by making the solution basic and converting it to NH_3. The ammonia is then removed by distillation. Table 7.7 provides additional examples of this approach for isolating inorganic ions.

Another reaction for separating analytes and interferents is precipitation. Two important examples of using a precipitation reaction in a separation are the pH-dependent solubility of metal oxides and hydroxides, and the solubility of metal sulfides.

Table 7.7	Examples of Using a Chemical Reaction and a Distillation to Separate an Inorganic Analyte From Interferents	
Analyte	Treatment	Isolated Species
CO_3^{2-}	$CO_3^{2-}(aq) + 2H_3O^+(aq) \rightarrow CO_2(g) + 3H_2O(l)$	CO_2
NH_4^+	$NH_4^+(aq) + OH^-(aq) \rightarrow NH_3(g) + H_2O(l)$	NH_3
SO_3^{2-}	$SO_3^{2-}(aq) + 2H_3O^+(aq) \rightarrow SO_2(g) + 3H_2O(l)$	SO_2
S^{2-}	$S^{2-}(aq) + 2H_3O^+(aq) \rightarrow H_2S(g) + 2H_2O(l)$	H_2S

Separations based on the pH-dependent solubility of oxides and hydroxides usually use strong acids, strong bases, or an NH_3/NH_4Cl buffer. Most metal oxides and hydroxides are soluble in hot concentrated HNO_3, although a few oxides, such as WO_3, SiO_2, and SnO_2 remain insoluble even under these harsh conditions. In determining the amount of Cu in brass, for example, we can avoid an interference from Sn by dissolving the sample with a strong acid and filtering to remove the solid residue of SnO_2.

Most metals form a hydroxide precipitate in the presence of concentrated NaOH. Those metals forming amphoteric hydroxides, however, do not precipitate because they react to form higher-order hydroxo-complexes. For example, Zn^{2+} and Al^{3+} do not precipitate in concentrated NaOH because the form the soluble complexes $Zn(OH)_3^-$ and $Al(OH)_4^-$. The solubility of Al^{3+} in concentrated NaOH allows us to isolate aluminum from impure samples of bauxite, an ore of Al_2O_3. After crushing the ore, we place it in a solution of concentrated NaOH, dissolving the Al_2O_3 and forming $Al(OH)_4^-$. Other oxides in the ore, such as Fe_2O_3 and SiO_2, remain insoluble. After filtering, we recover the aluminum as a precipitate of $Al(OH)_3$ by neutralizing some of the OH^- with acid.

The pH of an NH_3/NH_4Cl buffer ($pK_a = 9.26$) is sufficient to precipitate most metals as the hydroxide. The alkaline earths and alkaline metals, however, do not precipitate at this pH. In addition, metal ions that form soluble complexes with NH_3, such as Cu^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+} also do not precipitate under these conditions.

The use of S^{2-} as a precipitating reagent is one of the earliest examples of a separation technique. In Fresenius's 1881 text *A System of Instruction in Quantitative Chemical Analysis*, sulfide is frequently used to separate metal ions from the remainder of the sample's matrix.[14] Sulfide is a useful reagent for separating metal ions for two reasons: (1) most metal ions, except for the alkaline earths and alkaline metals, form insoluble sulfides; and (2) these metal sulfides show a substantial variation in solubility. Because the concentration of S^{2-} is pH-dependent, we can control which metal precipitates by adjusting the pH. For example, in Fresenius's gravimetric procedure for determining Ni in ore samples (see Figure 1.1 in Chapter 1 for a schematic diagram of this procedure), sulfide is used three times as a means of separating Co^{2+} and Ni^{2+} from Cu^{2+} and, to a lesser extent, from Pb^{2+}.

7F.5   Separations Based on a Partitioning Between Phases

The most important group of separation techniques uses a selective partitioning of the analyte or interferent between two immiscible phases. If we bring a phase containing a solute, S, into contact with a second phase, the solute partitions itself between the two phases, as shown by the following equilibrium reaction.

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14   Fresenius. C. R. *A System of Instruction in Quantitative Chemical Analysis*, John Wiley and Sons: New York, 1881.

$$S_{phase\ 1} \rightleftharpoons S_{phase\ 2} \qquad\qquad 7.20$$

The equilibrium constant for reaction 7.20

$$K_D = \frac{[S_{phase\ 2}]}{[S_{phase\ 1}]}$$

is called the distribution constant or PARTITION COEFFICIENT. If K_D is sufficiently large, then the solute moves from phase 1 to phase 2. The solute remains in phase 1 if the partition coefficient is sufficiently small. When we bring a phase containing two solutes into contact with a second phase, if K_D is favorable for only one of the solutes a separation of the solutes is possible. The physical states of the phases are identified when describing the separation process, with the phase containing the sample listed first. For example, if the sample is in a liquid phase and the second phase is a solid, then the separation involves liquid–solid partitioning.

EXTRACTION BETWEEN TWO PHASES

We call the process of moving a species from one phase to another phase an EXTRACTION. Simple extractions are particularly useful for separations where only one component has a favorable partition coefficient. Several important separation techniques are based on a simple extraction, including liquid–liquid, liquid–solid, solid–liquid, and gas–solid extractions.

LIQUID–LIQUID EXTRACTIONS

A liquid–liquid extraction is usually accomplished with a separatory funnel (Figure 7.20). After placing the two liquids in the separatory funnel, we shake the funnel to increase the surface area between the phases. When the
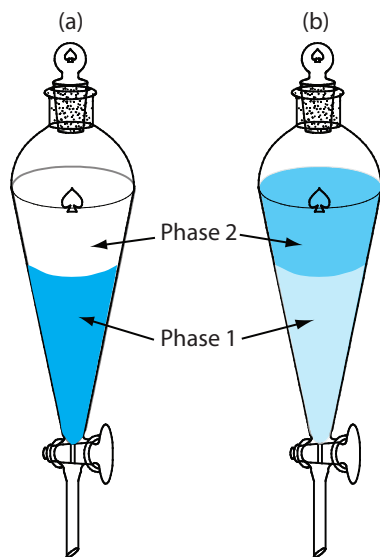

Figure 7.20 Example of a liquid–liquid extraction using a separatory funnel. (a) Before the extraction, 100% of the analyte is in phase 1. (b) After the extraction, most of the analyte is in phase 2, although some analyte remains in phase 1. Although one liquid–liquid extraction can result in the complete transfer of analyte, a single extraction usually is not sufficient. See Section 7G for a discussion of extraction efficiency and multiple extractions.
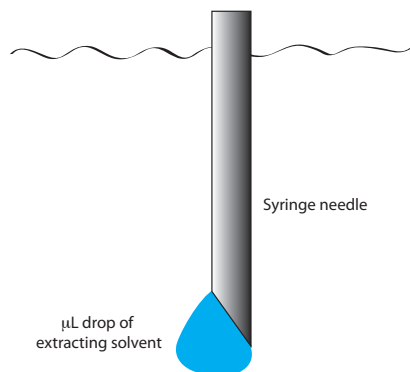
Figure 7.21 Schematic of a liquid–liquid microextraction showing a syringe needle with a µL drop of the extracting solvent.

extraction is complete, we allow the liquids to separate. The stopcock at the bottom of the separatory funnel allows us to remove the two phases.

We also can carry out a liquid–liquid extraction without a separatory funnel by adding the extracting solvent to the sample container. Pesticides in water, for example, are preserved in the field by extracting them into a small volume of hexane. Liquid–liquid microextractions, in which the extracting phase is a 1-µL drop suspended from a microsyringe (Figure 7.21), also have been described.[15] Because of its importance, a more thorough discussion of liquid–liquid extraction is in section 7G.

SOLID PHASE EXTRACTIONS

In a solid phase extraction of a liquid sample, we pass the sample through a cartridge containing a solid adsorbent, several examples of which are shown in Figure 7.22. The choice of adsorbent is determined by the species we wish to separate. Table 7.8 provides several representative examples of solid adsorbents and their applications.

As an example, let's examine a procedure for isolating the sedatives secobarbital and phenobarbital from serum samples using a C-18 solid adsorbent.[16] Before adding the sample, the solid phase cartridge is rinsed with 6 mL each of methanol and water. Next, a 500-µL sample of serum is pulled through the cartridge, with the sedatives and matrix interferents retained by a liquid–solid extraction (Figure 7.23a). Washing the cartridge with distilled water removes any interferents (Figure 7.23b). Finally, we elute the sedatives using 500 µL of acetone (Figure 7.23c). In comparison to a liquid–liquid extraction, a solid phase extraction has the advantage of being easier, faster, and requiring less solvent.

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15 Jeannot, M. A.; Cantwell, F. F. *Anal. Chem.* **1997**, *69*, 235–239.
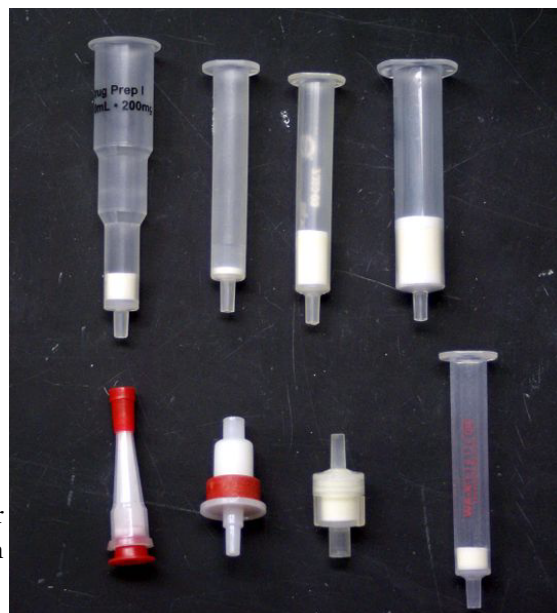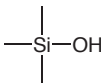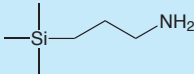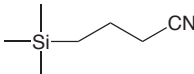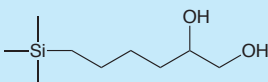16 Alltech Associates *Extract-Clean SPE Sample Preparation Guide*, Bulletin 83.

Figure 7.22 Selection of solid phase extraction cartridges for liquid samples. The solid adsorbent is the white material in each cartridge. Source: Jeff Dahl (commons.wikipedia.org).

Table 7.8	Representative Adsorbents for the Solid Phase Extraction of Liquid Samples	
Adsorbent	Structure	Properties and Uses
silica	—Si—OH	• retains low to moderate polarity species from organic matricies • fat soluble vitamins, steroids
aminopropyl	—Si~~~NH₂	• retains polar compounds • carbohydrates, organic acids
cyanopropyl	—Si~~~CN	• retains wide variety of species from aqueous and organic matricies • pesticides, hydrophobic peptides
diol	—Si~~~OH OH	• retains wide variety of species from aqueous and organic matricies • proteins, peptides, fungicides
octadecyl (C-18)	—$C_{18}H_{37}$	• retains hydrophobic species from aqueous matricies • caffeine, sedatives, polyaromatic hydrocarbons, carbohydrates, pesticides
octyl (C-8)	—C_8H_{17}	• similar to C-18

CONTINUOUS EXTRACTIONS

An extraction is possible even if the analyte has an unfavorable partition coefficient, provided that the sample's other components have significantly smaller partition coefficients. Because the analyte's partition coefficient is unfavorable, a single extraction will not recover all the analyte. Instead we continuously pass the extracting phase through the sample until a quantitative extraction is achieved.
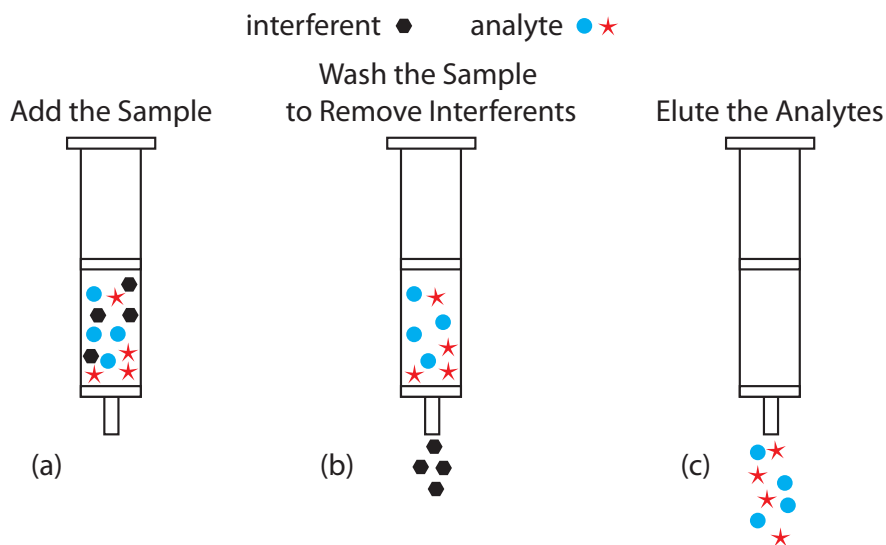

Figure 7.23 Steps in a typical solid phase extraction. After preconditioning the solid phase cartridge with solvent, (a) the sample is added to the cartridge, (b) the sample is washed to remove interferents, and (c) the analytes eluted.
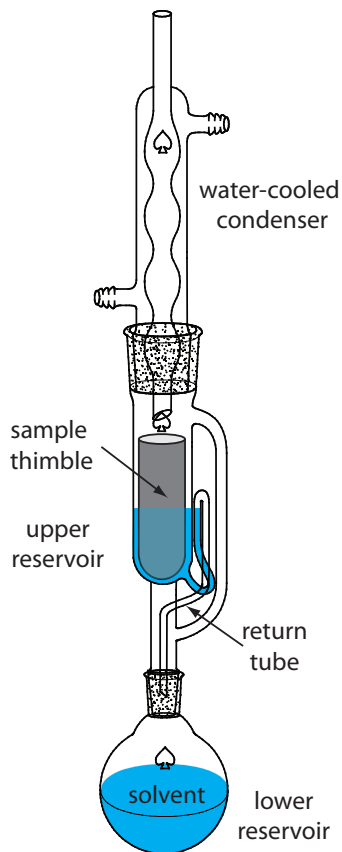
A continuous extraction of a solid sample is carried out using a SOXH-LET EXTRACTOR (Figure 7.24). The extracting solvent is placed in the lower reservoir and heated to its boiling point. Solvent in the vapor phase moves upwards through the tube on the far right side of the apparatus, reaching the condenser where it condenses back to the liquid state. The solvent then passes through the sample, which is held in a porous cellulose filter thimble, collecting in the upper reservoir. When the solvent in the upper reservoir reaches the return tube's upper bend, the solvent and extracted analyte are siphoned back to the lower reservoir. Over time the analyte's concentration in the lower reservoir increases.

Microwave-assisted extractions have replaced Soxhlet extractions in some applications.[17] The process is the same as that described underlined earlier for a microwave digestion. After placing the sample and the solvent in a sealed digestion vessel, a microwave oven is used to heat the mixture. Using a sealed digestion vessel allows the extraction to take place at a higher temperature and pressure, reducing the amount of time needed for a quantitative extraction. In a Soxhlet extraction the temperature is limited by the solvent's boiling point at atmospheric pressure. When acetone is the solvent, for example, a Soxhlet extraction is limited to 56 °C, but a microwave extraction can reach 150 °C.

Two other continuous extractions deserve mention. Volatile organic compounds (VOCs) can be quantitatively removed from liquid samples by a liquid–gas extraction. As shown in Figure 7.25, an inert purging gas, such as He, is passed through the sample. The purge gas removes the VOCs, which are swept to a primary trap where they collect on a solid absorbent. A second trap provides a means for checking to see if the primary trap's capacity is exceeded. When the extraction is complete, the VOCs are re-

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17   Renoe, B. W. *Am. Lab* August **1994**, 34–40.

Figure 7.24 Soxhlet extractor. See text for details.
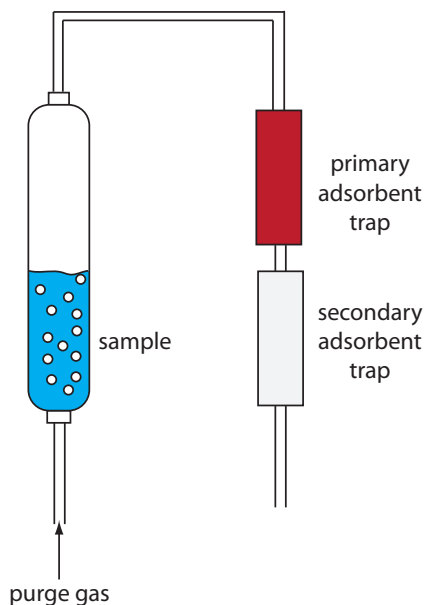

Figure 7.25 Schematic diagram of a purge-and-trap system for extracting volatile analytes. The purge gas releases the analytes, which subsequently collect in the primary adsorbent trap. The secondary adsorption trap is monitored for evidence of the analyte's breakthrough.

moved from the primary trap by rapidly heating the tube while flushing with He. This technique is known as a PURGE-AND-TRAP. Because the analyte's recovery may not be reproducible, an internal standard is necessary for quantitative work.

Continuous extractions also can be accomplished using supercritical fluids.[18] If we heat a substance above its critical temperature and pressure, it forms a SUPERCRITICAL FLUID whose properties are between those of a gas and a liquid. A supercritical fluid is a better solvent than a gas, which makes it a better reagent for extractions. In addition, a supercritical fluid's viscosity is significantly less than that of a liquid, making it easier to push it through a particulate sample. One example of a supercritical fluid extraction is the determination of total petroleum hydrocarbons (TPHs) in soils, sediments, and sludges using supercritical CO_2.[19] An approximately 3-g sample is placed in a 10-mL stainless steel cartridge and supercritical CO_2 at a pressure of 340 atm and a temperature of 80 °C is passed through the cartridge for 30 minutes at flow rate of 1–2 mL/min. To collect the TPHs, the effluent from the cartridge is passed through 3 mL of tetrachloroethylene at room temperature. At this temperature the CO_2 reverts to the gas phase and is released to the atmosphere.

CHROMATOGRAPHIC SEPARATIONS

In an extraction, the sample is one phase and we extract the analyte or the interferent into a second phase. We also can separate the analyte and interferents by continuously passing one sample-free phase, called the mobile phase, over a second sample-free phase that remains fixed or stationary. The sample is injected into the mobile phase and the sample's components partition themselves between the mobile phase and the stationary phase. Those components with larger partition coefficients are more likely to move into the stationary phase, taking a longer time to pass through the system. This is the basis of all chromatographic separations. Chromatography provides both a separation of analytes and interferents, and a means for performing a qualitative or quantitative analysis for the analyte. For this reason a more thorough treatment of chromatography is found in Chapter 12.

7G Liquid–Liquid Extractions

A liquid–liquid extraction is an important separation technique for environmental, clinical, and industrial laboratories. A standard environmental analytical method illustrates the importance of liquid–liquid extractions. Municipal water departments routinely monitor public water supplies for trihalomethanes ($CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$) because they are known or suspected carcinogens. Before their analysis by gas chroma-

The Environmental Protection Agency (EPA) also publishes two additional methods for trihalomethanes. Method 501.1 and Method 501.3 use a purge-and-trap to collect the trihalomethanes prior to a gas chromatographic analysis with a halide-specific detector (Method 501.1) or a mass spectrometer as the detector (Method 501.3). You will find more details about gas chromatography, including detectors, in Chapter 12.

18 McNally, M. E. *Anal. Chem.* **1995**, *67*, 308A–315A.
19 "TPH Extraction by SFE," ISCO, Inc. Lincoln, NE, Revised Nov. 1992.

tography, trihalomethanes are separated from their aqueous matrix by extracting with pentane.[20]

In a simple liquid–liquid extraction the solute partitions between two immiscible phases. One phase usually is aqueous and the other phase is an organic solvent, such as the pentane used in extracting trihalomethanes from water. Because the phases are immiscible they form two layers, with the denser phase on the bottom. The solute is initially present in one of the two phases; after the extraction it is present in both phases. EXTRACTION EFFICIENCY—that is, the percentage of solute moving from one phase to the other—is determined by the equilibrium constant for the solute's partitioning between the phases and any other reactions involving the solute. Examples of other reactions affecting extraction efficiency include acid–base reactions and complexation reactions.

7G.1 Partition Coefficients and Distribution Ratios

As we learned earlier in this chapter, a solute's partitioning between two phases is described by a partition coefficient, K_D. If we extract a solute from an aqueous phase into an organic phase

$$S_{aq} \rightleftharpoons S_{org}$$
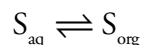
then the partition coefficient is

$$K_D = \frac{[S_{org}]}{[S_{aq}]}$$

A large value for K_D. indicates that the solute's extraction into the organic phase is favorable.

To evaluate an extraction's efficiency we must consider the solute's total concentration in each phase. We define the DISTRIBUTION RATIO, D, as the ratio of the solute's total concentration in each phase.

$$D = \frac{[S_{org}]_{total}}{[S_{aq}]_{total}}$$

The partition coefficient and the distribution ratio are identical if the solute has only one chemical form in each phase. If the solute exists in more than one chemical form in either phase, then K_D and D usually have different values. For example, if the solute exists in two forms in the aqueous phase, A and B, only one of which, A, partitions between the two phases, then

$$D = \frac{[S_{org}]_A}{[S_{aq}]_A + [S_{aq}]_B} \leq K_D = \frac{[S_{org}]_A}{[S_{aq}]_A}$$

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20 "The Analysis of Trihalomethanes in Drinking Water by Liquid Extraction," EPA Method 501.2 (EPA 500-Series, November 1979).

This distinction between K_D and D is important. The partition coefficient is an equilibrium constant and has a fixed value for the solute's partitioning between the two phases. The distribution ratio's value, however, changes with solution conditions if the relative amounts of A and B change. If we know the equilibrium reactions taking place within each phase and between the phases, we can derive an algebraic relationship between K_D and D.

7G.2 Liquid–Liquid Extraction With No Secondary Reactions

In a simple liquid–liquid extraction, the only reaction affecting extraction efficiency is the solute's partitioning between the two phases (Figure 7.26). In this case the distribution ratio and the partition coefficient are equal.

$$D = \frac{[S_{org}]_{total}}{[S_{aq}]_{total}} = K_D = \frac{[S_{org}]}{[S_{aq}]} \qquad 7.21$$

Let's assume that the solute is initially present in the aqueous phase and that we are extracting it into the organic phase. A conservation of mass requires that the moles of solute initially present in the aqueous phase equal the combined moles of solute in the aqueous phase and the organic phase after the extraction.

$$(\text{moles } S_{aq})_0 = (\text{moles } S_{aq})_1 + (\text{moles } S_{org})_1 \qquad 7.22$$

where the subscripts indicate the extraction number. After the extraction, the solute's concentration in the aqueous phase is

$$[S_{aq}]_1 = \frac{(\text{moles } S_{aq})_1}{V_{aq}} \qquad 7.23$$

and its concentration in the organic phase is

$$[S_{org}]_1 = \frac{(\text{moles } S_{org})_1}{V_{org}} \qquad 7.24$$

where V_{aq} and V_{org} are the volumes of the aqueous phase and the organic phase. Solving equation 7.22 for $(\text{moles } S_{org})_1$ and substituting into equation 7.24 leave us with

$$[S_{org}]_1 = \frac{(\text{moles } S_{aq})_0 - (\text{moles } S_{aq})_1}{V_{org}} \qquad 7.25$$

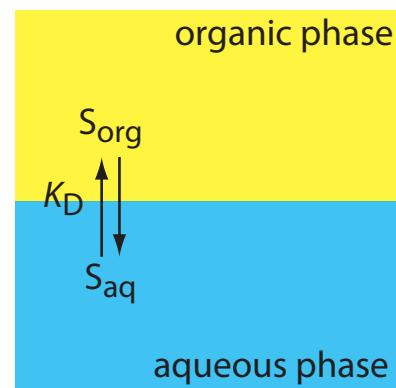Substituting equation 7.23 and equation 7.25 into equation 7.21 gives

Figure 7.26 Scheme for a simple liquid–liquid extraction in which the solute's partitioning depends only on the K_D equilibrium.

The subscript 0 represents the system before the extraction and the subscript 1 represents the system after the first extraction.

$$D = \frac{\dfrac{(\text{moles } S_{aq})_0 - (\text{moles } S_{aq})_1}{V_{org}}}{\dfrac{(\text{moles } S_{aq})_1}{V_{aq}}} = \frac{(\text{moles } S_{aq})_0 \times V_{aq} - (\text{moles } S_{aq})_1 \times V_{aq}}{(\text{moles } S_{aq})_1 \times V_{org}}$$

Rearranging, and solving for the fraction of solute remaining in the aqueous phase after one extraction, $(q_{aq})_1$, gives

$$(q_{aq})_1 = \frac{(\text{moles } S_{aq})_1}{(\text{moles } S_{aq})_0} = \frac{V_{aq}}{DV_{org} + V_{aq}} \qquad 7.26$$

The fraction present in the organic phase after one extraction, $(q_{org})_1$, is

$$(q_{org})_1 = \frac{(\text{moles } S_{org})_1}{(\text{moles } S_{org})_0} = 1 - (q_{aq})_1 = \frac{DV_{aq}}{DV_{org} + V_{aq}}$$

Example 7.14 shows how we can use equation 7.26 to calculate the efficiency of a simple liquid-liquid extraction.

Example 7.14

A solute has a K_D between water and chloroform of 5.00. Suppose we extract a 50.00-mL sample of a 0.050 M aqueous solution of the solute with 15.00 mL of chloroform. (a) What is the separation's extraction efficiency? (b) What volume of chloroform must we use to extract 99.9% of the solute?

SOLUTION

For a simple liquid–liquid extraction the distribution ratio, D, and the partition coefficient, K_D, are identical.

(a) The fraction of solute remaining in the aqueous phase after the extraction is given by equation 7.26.

$$(q_{aq})_1 = \frac{V_{aq}}{DV_{org} + V_{aq}} = \frac{50.00 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}} = 0.400$$

The fraction of solute in the organic phase is 1– 0.400, or 0.600. Extraction efficiency is the percentage of solute moving into the extracting phase. The extraction efficiency, therefore, is 60.0%.

(b) To extract 99.9% of the solute $(q_{aq})_1$ must be 0.001. Solving equation 7.26 for V_{org}, and making appropriate substitutions for $(q_{aq})_1$ and V_{aq} gives

$$V_{org} = \frac{V_{aq} - (q_{aq})_1 V_{aq}}{(q_{aq})_1 D} = \frac{50.00 \text{ mL} - (0.001)(50.00 \text{ mL})}{(0.001)(5.00)} = 9990 \text{ mL}$$

This is large volume of chloroform. Clearly, a single extraction is not reasonable under these conditions.

In Example 7.14, a single extraction provides an extraction efficiency of only 60%. If we carry out a second extraction, the fraction of solute remaining in the aqueous phase, $(q_{aq})_2$, is

$$(q_{aq})_2 = \frac{(\text{moles } S_{aq})_2}{(\text{moles } S_{aq})_1} = \frac{V_{aq}}{DV_{org} + V_{aq}}$$

If V_{aq} and V_{org} are the same for both extractions, then the cumulative fraction of solute remaining in the aqueous layer after two extractions, $(Q_{aq})_2$, is

$$(Q_{aq})_2 = \frac{(\text{moles } S_{aq})_2}{(\text{moles } S_{aq})_0} = (q_{aq})_1 \times (q_{aq})_2 = \left(\frac{V_{aq}}{DV_{org} + V_{aq}}\right)^2$$

In general, for a series of n identical extractions, the fraction of analyte remaining in the aqueous phase after the last extraction is

$$(Q_{aq})_n = \left(\frac{V_{aq}}{DV_{org} + V_{aq}}\right)^n \qquad\qquad 7.27$$

Example 7.15

For the extraction described in Example 7.14, determine (a) the extraction efficiency for two extractions and for three extractions; and (b) the number of extractions required to ensure that we extract 99.9% of the solute.

SOLUTION

(a) The fraction of solute remaining in the aqueous phase after two extractions and three extractions is

$$(Q_{aq})_2 = \left(\frac{50.00 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}}\right)^2 = 0.160$$

$$(Q_{aq})_3 = \left(\frac{50.00 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}}\right)^3 = 0.0640$$

The extraction efficiencies are 84.0% with two extractions and 93.6% with three extractions.

(b) To determine the minimum number of extractions for an efficiency of 99.9%, we set $(Q_{aq})_n$ to 0.001 and solve for n in equation 7.27.

$$0.001 = \left(\frac{50.00 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}}\right)^n = (0.400)^n$$

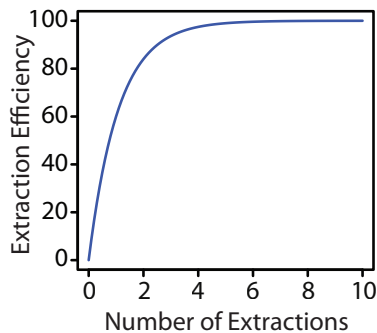Taking the log of both sides and solving for n

$$\log(0.001) = n\log(0.400)$$

$$n = 7.54$$

we find that a minimum of 8 extractions is necessary.

Figure 7.27 Plot of extraction efficiency versus the number of extractions for the liquid–liquid extraction in Example 7.15.

The last two examples provide us with an important observation—for any extraction efficiency, we need less solvent if we complete several extractions using smaller portions of solvent instead of one extraction using a larger volume of solvent. For the conditions in Example 7.14 and Example 7.15, an extraction efficiency of 99.9% requires one extraction with 9990 mL of chloroform, or 120 mL when using eight 15-mL portions of chloroform. Although extraction efficiency increases dramatically with the first few multiple extractions, the effect quickly diminishes as we increase the number of extractions (Figure 7.27). In most cases there is little improvement in extraction efficiency after five or six extractions. For the conditions in Example 7.15, we reach an extraction efficiency of 99% after five extractions and need three additional extractions to obtain the extra 0.9% increase in extraction efficiency.

Practice Exercise 7.8

To plan a liquid–liquid extraction we need to know the solute's distribution ratio between the two phases. One approach is to carry out the extraction on a solution containing a known amount of solute. After extracting the solution, we isolate the organic phase and allow it to evaporate, leaving behind the solute. In one such experiment, 1.235 g of a solute with a molar mass of 117.3 g/mol is dissolved in 10.00 mL of water. After extracting with 5.00 mL of toluene, 0.889 g of the solute is recovered in the organic phase. (a) What is the solute's distribution ratio between water and toluene? (b) If we extract 20.00 mL of an aqueous solution containing the solute with 10.00 mL of toluene, what is the extraction efficiency? (c) How many extractions will we need to recover 99.9% of the solute?

Click here to review your answer to this exercise.

7G.3 Liquid–Liquid Extractions Involving Acid–Base Equilibria

As shown by equation 7.21, in a simple liquid–liquid extraction the distribution ratio and the partition coefficient are identical. As a result, the distribution ratio does not depend on the composition of the aqueous phase or the organic phase. Changing the pH of the aqueous phase, for example, will not affect the solute's extraction efficiency.

If the solute participates in an additional equilibrium reaction within a phase, then the distribution ratio and the partition coefficient may not be the same. For example, Figure 7.28 shows the equilibrium reactions affecting the extraction of the weak acid, HA, by an organic phase in which
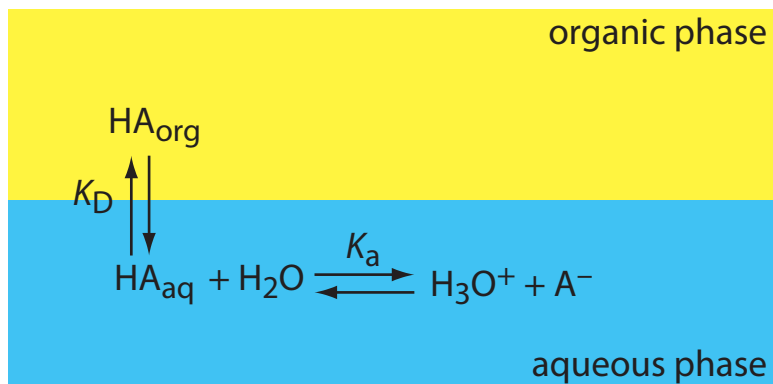
Figure 7.28 Scheme for a liquid–liquid extraction of the weak acid, HA. Although the weak acid is soluble in both phases, its conjugate weak base, A⁻, is soluble only in the aqueous phase. The K_a reaction, which is called a SECONDARY EQUILIBRIUM REACTION, affects the extraction efficiency because it controls the relative abundance of HA in solution.

ionic species are not soluble. In this case the partition coefficient and the distribution ratio are

$$K_D = \frac{[HA_{org}]}{[HA_{aq}]} \qquad 7.28$$

$$D = \frac{[HA_{org}]_{total}}{[HA_{aq}]_{total}} = \frac{[HA_{org}]}{[HA_{aq}] + [A^-_{aq}]} \qquad 7.29$$

Because the position of an acid–base equilibrium depends on the pH, the distribution ratio is pH-dependent. To derive an equation for D that shows this dependency, we begin with the acid dissociation constant for HA.

$$K_a = \frac{[H_3O^+_{aq}][A^-_{aq}]}{[HA_{aq}]} \qquad 7.30$$

Solving equation 7.30 for the concentration of A⁻

$$[A^-_{aq}] = \frac{K_a \times [HA_{aq}]}{[H_3O^+_{aq}]}$$

and substituting into equation 7.29 gives

$$D = \frac{[HA_{org}]}{[HA_{aq}] + \dfrac{K_a \times [HA_{aq}]}{[H_3O^+_{aq}]}}$$

Factoring [HA_{aq}] from the denominator, replacing [HA_{org}]/[HA_{aq}] with K_D (equation 7.28), and simplifying leaves us with a relationship between the distribution ratio, D, and the pH of the aqueous solution.

$$D = \frac{K_D[H_3O^+_{aq}]}{[H_3O^+_{aq}] + K_a} \qquad 7.31$$

Example 7.16

An acidic solute, HA, has a K_a of 1.00×10^{-5} and a K_D between water and hexane of 3.00. Calculate the extraction efficiency if we extract a 50.00 mL sample of a 0.025 M aqueous solution of HA, buffered to a pH of 3.00, with 50.00 mL of hexane. Repeat for pH levels of 5.00 and 7.00.

SOLUTION

When the pH is 3.00, $[H_3O^+_{aq}]$ is 1.0×10^{-3} and the distribution ratio is

$$D = \frac{(3.00)(1.0 \times 10^{-3})}{1.0 \times 10^{-3} + 1.0 \times 10^{-5}} = 2.97$$

The fraction of solute remaining in the aqueous phase is

$$(Q_{aq})_1 = \frac{50.00 \text{ mL}}{(2.97)(50.00 \text{ mL}) + 50.00 \text{ mL}} = 0.252$$

The extraction efficiency, therefore, is almost 75%. The same calculation at a pH of 5.00 gives the extraction efficiency as 60%. At a pH of 7.00 the extraction efficiency is only 3% .
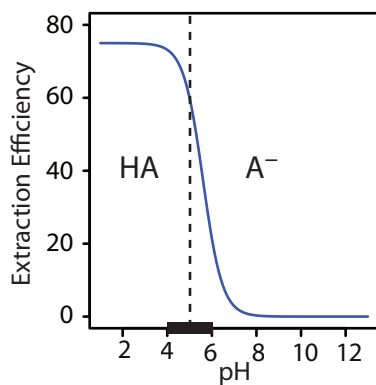

Figure 7.29 Plot of extraction efficiency versus pH of the aqueous phase for the extraction of the weak acid HA in Example 7.16. A ladder diagram for HA is superimposed along the x-axis, dividing the pH scale into regions where HA and A^- are the predominate aqueous phase species. The greatest change in extraction efficiency occurs as the pH moves through HA's buffer region.

The extraction efficiency in Example 7.16 is greater at more acidic pH levels because HA is the solute's predominate form in the aqueous phase. At a more basic pH, where A^- is the solute's predominate form, the extraction efficiency is smaller. A graph of extraction efficiency versus pH is shown in Figure 7.29. Note that extraction efficiency is essentially independent of pH for pHs more acidic than the weak acid's pK_a, and that it is essentially zero for pHs more basic than the pK_a. The greatest change in extraction efficiency occurs at pHs where both HA and A^- are predominate species. The ladder diagram for HA along the graph's x-axis helps illustrate this effect.

Practice Exercise 7.9

The liquid–liquid extraction of the weak base B is governed by the following equilibrium reactions:

$$B(aq) \rightleftharpoons B(org) \; K_D = 5.00$$

$$B(aq) + H_2O(l) \rightleftharpoons OH^-(aq) + HB^+(aq) \; K_b = 1.0 \times 10^{-4}$$
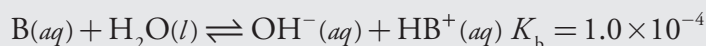
Derive an equation for the distribution ratio, D, and calculate the extraction efficiency if 25.0 mL of a 0.025 M solution of B, buffered to a pH of 9.00, is extracted with 50.0 mL of the organic solvent.
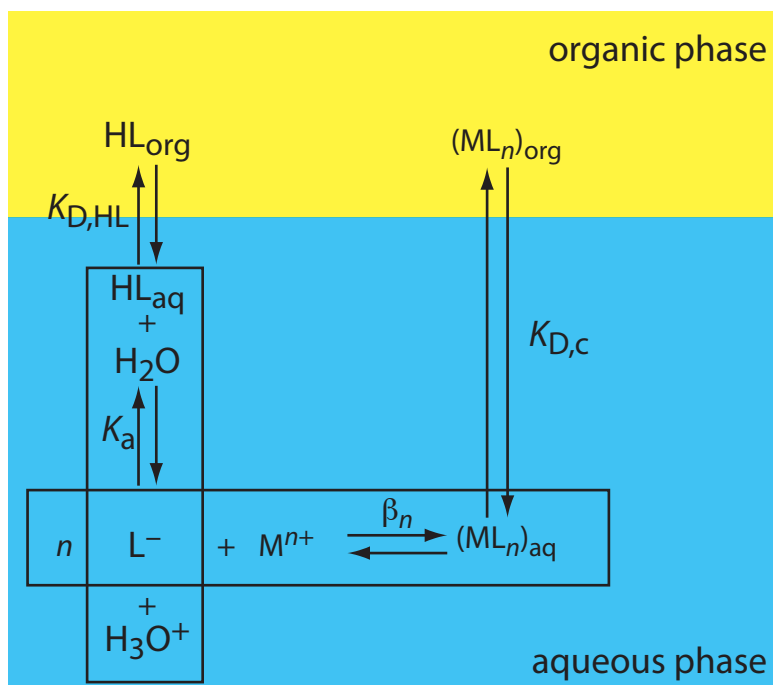
Click here to review your answer to this exercise.

Figure 7.30 Scheme for the liquid–liquid extraction of a metal ion, M^{n+}, by the ligand L^-. The ligand initially is present in the organic phase as HL. Four equilibrium reactions are needed to explain the extraction efficiency.

7G.4 Liquid–Liquid Extraction of a Metal–Ligand Complex

One important application of liquid–liquid extractions is the selective extraction of metal ions using a ligand. Unfortunately, many ligands are not very soluble in water or undergo hydrolysis or oxidation in aqueous solutions. For these reasons the ligand is added to the organic solvent instead of the aqueous phase. Figure 7.30 shows the relevant equilibria (and equilibrium constants) for the extraction of M^{n+} by the ligand HL, including the ligand's extraction into the aqueous phase ($K_{D,HL}$), the ligand's acid dissociation reaction (K_a), the formation of the metal–ligand complex (β_n), and the complex's extraction into the organic phase ($K_{D,c}$).

   If the ligand's concentration is much greater than the metal ion's concentration, then the distribution ratio is

$$D = \frac{\beta_n K_{D,c}(K_a)^n (C_{HL})^n}{(K_{D,HL})^n [H_3O^+]^n + \beta_n (K_a)^n (C_{HL})^n} \qquad 7.32$$

where C_{HL} is the ligand's initial concentration in the organic phase. As shown in Example 7.17, the extraction efficiency for metal ions shows a marked pH dependency.

Example 7.17

A liquid–liquid extraction of the divalent metal ion, M^{2+}, uses the scheme outlined in Figure 7.30. The partition coefficients for the ligand, $K_{D,HL}$, and for the metal–ligand complex, $K_{D,c}$, are 1.0×10^4 and 7.0×10^4, respectively. The ligand's acid dissociation constant, K_a, is 5.0×10^{-5}, and the formation constant for the metal–ligand complex, β_2, is 2.5×10^{16}.
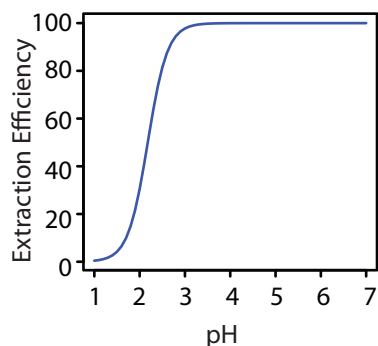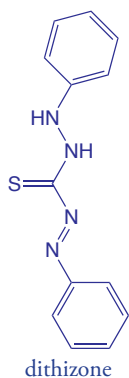
Figure 7.31 Plot of extraction efficiency versus pH for the extraction of the metal ion, M^{2+}, in Example 7.17.

dithizone

Calculate the extraction efficiency if we extract 100.0 mL of a 1.0×10^{-6} M aqueous solution of M^{2+}, buffered to a pH of 1.00, with 10.00 mL of an organic solvent that is 0.1 mM in the chelating agent. Repeat the calculation at a pH of 3.00.

SOLUTION

When the pH is 1.00 the distribution ratio is

$$D = \frac{(2.5 \times 10^6)(7.0 \times 10^{4})(5.0 \times 10^{-5})^2(1.0 \times 10^{-4})^2}{(1.0 \times 10^4)^2(0.10)^2 + (2.5 \times 10^6)(5.0 \times 10^{-5})^2(1.0 \times 10^{-4})^2}$$

$$D = 0.0438$$

and the fraction of metal ion remaining in the aqueous phase is

$$(Q_{aq})_1 = \frac{100.0 \text{ mL}}{(0.0438)(10.00 \text{ mL}) + 100.0 \text{ mL}} = 0.996$$

At a pH of 1.00, we extract only 0.40% of the metal into the organic phase. Changing the pH to 3.00, however, increases the extraction efficiency to 97.8%. Figure 7.31 shows how the pH of the aqueous phase affects the extraction efficiency for M^{2+}.

One advantage of using a ligand to extract a metal ion is the high degree of selectivity that it brings to a liquid–liquid extraction. As seen in Figure 7.31, a divalent metal ion's extraction efficiency increases from approximately 0% to 100% over a range of 2 pH units. Because a ligand's ability to form a metal–ligand complex varies substantially from metal ion to metal ion, significant selectivity is possible by carefully controlling pH. Table 7.9 shows the minimum pH for extracting 99% of a metal ion from an aqueous solution using an equal volume of 4 mM dithizone in CCl_4.

Example 7.18

Using Table 7.9, explain how you can separate the metal ions in an aqueous mixture of Cu^{2+}, Cd^{2+} and Ni^{2+} by extracting with an equal volume of dithizone in CCl_4.

SOLUTION

From Table 7.9, a quantitative separation of Cu^{2+} from Cd^{2+} and Ni^{2+} is possible if we acidify the aqueous phase to a pH of less than 1. This pH is greater than the minimum pH for extracting Cu^{2+} and significantly smaller than the minimum pH for extracting either Cd^{2+} or Ni^{2+} After the extraction is complete, buffering the aqueous phase to 4.0 allows us to extract the Cd^{2+} leaving the Ni^{2+} in the aqueous phase.

Table 7.9	Minimum pH for Extracting 99% of an Aqueous Metal Ion Using 4.0 mM Dithizone in CCl_4 ($V_{aq} = V_{org}$)	
Metal Ion	**Minimum pH**	
Hg^{2+}	-8.7	
Ag^+	-1.7	
Cu^{2+}	-0.8	
Bi^{3+}	0.9	
Zn^{2+}	2.3	
Cd^{2+}	3.6	
Co^{2+}	3.6	
Pb^{2+}	4.1	
Ni^{2+}	6.0	
Tl^+	8.7	

Source: Kolthoff, I. M.; Sandell, E. B.; Meehan, E. J.; Bruckenstein, S. *Quantitative Chemical Analysis*, Macmillan: New York, 1969, p. 353.

7H   Separation Versus Preconcentration

Two common analytical problems are: (1) matrix components that interfere with an analyte's analysis; and (2) an analyte with a concentration that is too small to analyze accurately. We have seen that we can use a separation to solve the first problem. Interestingly, we often can use a separation to solve the second problem as well. For a separation in which we recover the analyte in a new phase, it may be possible to increase the analyte's concentration. This step in an analytical procedure is known as a PRECONCENTRATION.

An example from the analysis of water samples illustrate how we can simultaneously accomplish a separation and a preconcentration. In the gas chromatographic analysis for organophosphorous pesticides in environmental waters, the analytes in a 1000-mL sample may be separated from their aqueous matrix by a solid-phase extraction using 15 mL of ethyl acetate.[21] After the extraction, the analytes in the ethyl acetate have a concentration that is 67 times greater than that in the original sample (assuming the extraction is 100% efficient).

$$\frac{1000 \text{ mL}}{15 \text{ mL}} \approx 67 \times$$

7I   Key Terms

centrifugation	composite sample	coning and quartering
convenience sampling	density gradient centrifugation	dialysis
distillation	distribution ratio	extraction
extraction efficiency	filtrate	filtration

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

21 Aguilar, C.; Borrul, F.; Marcé, R. M. *LC•GC* **1996**, *14*, 1048–1054.

grab sample	gross sample	heterogeneous
homogeneous	in situ sampling	judgmental sampling
laboratory sample	masking	masking agents
Nyquist theorem	partition coefficient	preconcentration
purge-and-trap	random sampling	recovery
recrystallization	retentate	sampling plan
secondary equilibrium reaction	selectivity coefficient	separation factor
size exclusion chromatography	Soxhlet extractor	stratified sampling
sublimation	subsamples	supercritical fluid
systematic–judgmental sampling	systematic sampling	target population

7J   Chapter Summary

An analysis requires a sample. How we acquire that sample is critical. The samples we collect must accurately represent their target population, and our sampling plan must provide a sufficient number of samples of appropriate size so that the uncertainty in sampling does not limit the precision of our analysis.

A complete sampling plan requires several considerations, including: the type of sample (random, judgmental, systematic, systematic–judgmental, stratified, or convenience); whether to collect grab samples, composite samples, or in situ samples; whether the population is homogeneous or heterogeneous; the appropriate size for each sample; and, the number of samples to collect.

Removing a sample from its population may induce a change in its composition due to a chemical or physical process. For this reason, we collect samples in inert containers and we often preserve them at the time of collection.

When an analytical method's selectivity is insufficient, we may need to separate the analyte from potential interferents. Such separations can take advantage of physical properties—such as size, mass or density—or chemical properties. Important examples of chemical separations include masking, distillation, and extractions.

7K   Problems

1. Because of the risk of lead poisoning, the exposure of children to lead-based paint is a significant public health concern. The first step in the quantitative analysis of lead in dried paint chips is to dissolve the sample. Corl has evaluated several dissolution techniques.[22] In this study, samples of paint were collected and pulverized with a Pyrex mortar and pestle. Replicate portions of the powdered paint were then taken for

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22 Corl, W. E. *Spectroscopy* **1991**, *6(8)*, 40–43.

analysis. The following table shows results for a paint sample and for a standard reference material. Both samples and standards were digested with HNO_3 on a hot plate.

Replicate	% w/w Pb in Sample	% w/w Pb in Standard
1	5.09	11.48
2	6.29	11.62
3	6.64	11.47
4	4.63	11.86

(a) Determine the overall variance, the variance due to the method and the variance due to sampling. (b) What percentage of the overall variance is due to sampling? (c) How might you decrease the variance due to sampling?

2. To analyze a shipment of 100 barrels of an organic solvent, you plan to collect and analyze single samples from ten barrels using a random sampling. From which barrels should you collect samples if the first barrel is given by the 12th entry in the random number table in Appendix 14, with subsequent barrels given by every third entry? Assume that entries in the random number table are arranged by rows.

3. The concentration of dissolved O_2 in a lake shows a daily cycle from the effect of photosynthesis, and a yearly cycle due to seasonal changes in temperature. Suggest an appropriate systematic sampling plan for monitoring the daily change in dissolved O_2. Suggest an appropriate systematic sampling plan for monitoring the yearly change in dissolved O_2.

4. The data in the following table were collected during a preliminary study of the pH of an industrial wastewater stream.

Time (h)	pH	Time (h)	pH
0.5	4.4	9.0	5.7
1.0	4.8	9.5	5.5
1.5	5.2	10.0	6.5
2.0	5.2	10.5	6.0
2.5	5.6	11.0	5.8
3.0	5.4	11.5	6.0
3.5	5.4	12.0	5.6
4.0	4.4	12.5	5.6
4.5	4.8	13.0	5.4
5.0	4.8	13.5	4.9

Time (h)	pH	Time (h)	pH
5.5	4.2	14.0	5.2
6.0	4.2	14.5	4.4
6.5	3.8	15.0	4.0
7.0	4.0	15.5	4.5
7.5	4.0	16.0	4.0
8.0	3.9	16.5	5.0
8.5	4.7	17.0	5.0

Construct a graph of pH as a function of time and suggest an appropriate sampling frequency for a long-term monitoring program.

5. You have been asked to monitor the daily fluctuations in atmospheric ozone in the downtown area of a city to determine if there is relationship between daily traffic patterns and ozone levels. (a) Which of the following sampling plan will you use: random, systematic, judgmental, systematic–judgmental, or stratified? (b) Do you plan to collect and analyze a series of grab samples, or will you form a single composite sample? (c) Will your answers to these questions change if your goal is to determine if the average daily ozone level exceeds a threshold value? If your answer is yes, then what is your new sampling strategy?

6. The distinction between a homogeneous population and a heterogeneous population is important when you are developing a sampling plan. (a) Define homogeneous and heterogeneous. (b) If you collect and analyze a single sample, can you determine if the population is homogeneous or heterogeneous?

7. The sampling of a heterogeneous population is particularly challenging. Explain why you can minimize the sampling variance for a heterogeneous population by increasing the number of samples, but not by increasing the mass of individual samples.

8. Beginning with equation 7.4, derive equation 7.5. Assume that the particles are spherical with a radius of r and a density of d.

9. The sampling constant for the radioisotope ^{24}Na in homogenized human liver has been reported as approximately 35 g.[23] (a) What is the expected relative standard deviation for sampling if you analyze 1.0-g samples? (b) How many 1.0-g samples must you analyze to obtain a maximum sampling error of $\pm 5\%$ at the 95% confidence level?

23 Kratochvil, B.; Taylor, J. K. *Anal. Chem.* **1981**, *53*, 924A–938A.

10. Engels and Ingamells reported the following results for the % w/w K_2O in a mixture of amphibolite and orthoclase.[24]

0.247	0.300	0.236	0.258	0.304	0.330
0.247	0.275	0.212	0.311	0.258	0.187

Each of the 12 samples had a nominal weight of 0.1 g. Determine the approximate value for K_s and the mass of sample needed to achieve a percent relative standard deviation of 2%.

11. The following data has been reported for the determination of KH_2PO_4 in a mixture of KH_2PO_4 and NaCl.[25]

Nominal Mass (g)	Actual Mass (g)	% w/w KH_2PO_4
0.10	0.1039	0.085
	0.1015	1.078
	0.1012	0.413
	0.1010	1.248
	0.1060	0.654
	0.0997	0.507
0.25	0.2515	0.847
	0.2465	0.598
	0.2770	0.431
	0.2460	0.842
	0.2485	0.964
	0.2590	1.178
0.50	0.5084	1.009
	0.4954	0.947
	0.5286	0.618
	0.5232	0.744
	0.4965	0.572
	0.4995	0.709
1.00	1.027	0.696
	0.987	0.843
	0.991	0.535
	0.998	0.750
	0.997	0.711
	1.001	0.639

24 Engels, J. C.; Ingamells, C. O. *Geochim. Cosmochim. Acta* **1970**, *34*, 1007–1017.
25 Guy, R. D.; Ramaley, L.; Wentzell, P. D. *J. Chem. Educ.* **1998**, *75*, 1028–1033.

Nominal Mass (g)	Actual Mass (g)	% w/w KH_2PO_4
2.50	2.496	0.766
	2.504	0.769
	2.496	0.682
	2.496	0.609
	2.557	0.589
	2.509	0.617

(a) Prepare a graph of % w/w KH_2PO_4 versus the actual sample mass and discuss how this graph is consistent with your understanding of the factors affecting sampling variance. (b) For each nominal mass, calculate the percent relative standard deviation for the analysis. The value of K_s for this analysis has been estimated as 350. For each nominal mass, use this K_s to determine the percent relative standard deviation due to sampling. Considering these calculations, what is your conclusion about the importance of indeterminate sampling errors for this analysis? (c) For each nominal mass, convert the percent relative standard deviation to an absolute standard deviation. Plot points on your graph corresponding to ± 1 absolute standard deviations about the overall average % w/w KH_2PO_4. Draw smooth curves through these two sets of points. Considering these curves, does the sample appear to be homogeneous on the scale at which it is sampled?

12. In this problem you will collect and analyze data in a simulation of the sampling process. Obtain a pack of M&M's (or other similar candy). Collect a sample of five candies and count the number that are red (or any other color of your choice). Report the result of your analysis as % red. Return the candies to the bag, mix thoroughly, and repeat the analysis for a total of 20 determinations. Calculate the mean and the standard deviation for your data. Remove all candies from the bag and determine the true % red for the population. Sampling in this exercise should follow binomial statistics. Calculate the expected mean value and the expected standard deviation, and compare to your experimental results.

13. Determine the error ($\alpha = 0.05$) for the following situations. In each case assume that the variance for a single determination is 0.0025 and that the variance for collecting a single sample is 0.050. (a) Nine samples are collected, each analyzed once. (b) One sample is collected and analyzed nine times. (c) Three samples are collected, each analyzed three times.

14. Which of the sampling schemes in problem 13 is best if you wish to limit the overall error to less than ± 0.25, and the cost of collecting a single sample is 1 (arbitrary units), and the cost of analyzing a single

sample is 10? Which is the best sampling scheme if the cost of collecting a single sample is 7, and the cost of analyzing a single sample is 3?

15. Maw, Witry, and Emond evaluated a microwave digestion method for Hg against the standard open-vessel digestion method.[26] The standard method requires a 2-h digestion and is operator-intensive. The microwave digestion is complete in approximately 0.5 h and requires little monitoring by the operator. Samples of baghouse dust from air-pollution-control equipment were collected from a hazardous waste incinerator and digested in triplicate before determining the concentration of Hg in ppm. Results are summarized in the following tables.

ppm Hg Following Microwave Digestion

Sample	Replicate 1	Replicate 2	Replicate 3
1	7.12	7.66	7.17
2	16.1	15.7	15.6
3	4.89	4.62	4.28
4	9.64	9.03	8.44
5	6.76	7.22	7.50
6	6.19	6.61	7.61
7	9.44	9.56	10.7
8	30.8	29.0	26.2

ppm Hg Following Standard Digestion

Sample	Replicate 1	Replicate 2	Replicate 3
1	5.50	5.54	5.40
2	13.1	12.8	13.0
3	5.39	5.12	5.36
4	6.59	6.52	7.20
5	6.20	6.03	5.77
6	6.25	5.65	5.61
7	15.0	13.9	14.0
8	20.4	16.1	20.0

Does the microwave digestion method yields acceptable results in comparison to the standard digestion method?

16. Simpson, Apte, and Batley investigated methods for preserving water samples collected from anoxic (O_2-poor) environments that contain high concentrations of dissolved sulfide.[27] They found that preserving water samples with HNO_3 (a common method for preserving aerobic samples) gave significant negative determinate errors when analyzing

26 Maw, R.; Witry, L.; Emond, T. *Spectroscopy* **1994**, *9*, 39–41.
27 Simpson, S. L.: Apte, S. C.; Batley, G. E. *Anal. Chem.* **1998**, *70*, 4202–4205.

for Cu^{2+}. Preserving samples by first adding H_2O_2 and then adding HNO_3, eliminated the determinate error. Explain their observations.

17. In a particular analysis the selectivity coefficient, $K_{A,I}$, is 0.816. When a standard sample with an analyte-to-interferent ratio of 5:1 is carried through the analysis, the error in determining the analyte is +6.3%. (a) Determine the apparent recovery for the analyte if $R_I = 0$. (b) Determine the apparent recovery for the interferent if $R_A = 0$.

18. The amount of Co in an ore is to be determined using a procedure for which Fe in an interferent. To evaluate the procedure's accuracy, a standard sample of ore known to have a Co/Fe ratio of 10.2:1 is analyzed. When pure samples of Co and Fe are taken through the procedure the following calibration relationships are obtained

$$S_{Co} = 0.786 \times m_{Co}$$
$$S_{Fe} = 0.669 \times m_{Fe}$$

where S is the signal and m is the mass of Co or Fe. When 278.3 mg of Co are taken through the separation step, 275.9 mg are recovered. Only 3.6 mg of Fe are recovered when a 184.9 mg sample of Fe is carried through the separation step. Calculate (a) the recoveries for Co and Fe; (b) the separation factor; (c) the error if no attempt is made to separate the Co and Fe before the analysis; (d) the error if the separation step is carried out; and (e) the maximum recovery for Fe if the recovery for Co is 1.00, and the maximum allowed error is 0.05%.

19. The amount of calcium in a sample of urine was determined by a method for which magnesium is an interferent. The selectivity coefficient, $K_{Ca,Mg}$, for the method is 0.843. When a sample with a Mg/Ca ratio of 0.50 was carried through the procedure, an error of –3.7% was obtained. The error was +5.5% when using a sample with a Mg/Ca ratio of 2.0. (a) Determine the recoveries for Ca and Mg. (b) What is the expected error for a urine sample in which the Mg/Ca ratio is 10.0?

20. Using the formation constants in Appendix 12, show that F^- is an effective masking agent for preventing a reaction between Al^{3+} and EDTA. Assume that the only significant forms of fluoride and EDTA are F^- and Y^{4-}.

21. Cyanide is frequently used as a masking agent for metal ions. Its effectiveness as a masking agent is better in more basic solutions. Explain the reason for this pH dependency.

22. Explain how an aqueous sample consisting of Cu^{2+}, Sn^{4+}, Pb^{2+}, and Zn^{2+} can be separated into its component parts by adjusting the pH of the solution.

23. A solute, S, has a distribution ratio between water and ether of 7.5. Calculate the extraction efficiency if you extract a 50.0-mL aqueous sample of S using 50.0 mL of ether as: (a) a single portion of 50.0 mL; (b) two portions, each of 25.0 mL; (c) four portions, each of 12.5 mL; and (d) five portions, each of 10.0 mL. Assume that the solute is not involved in any secondary equilibria.

24. What volume of ether is needed to extract 99.9% of the solute in problem 23 when using: (a) 1 extraction; (b) 2 extractions; (c) four extractions; and (d) five extractions.

25. What is the minimum distribution ratio if 99% of the solute in a 50.0-mL sample is to be extracted with a single 50.0-mL portion of an organic solvent? Repeat for the case where two 25.0-mL portions of the organic solvent are used.

26. A weak acid, HA, with a K_a of 1.0×10^{-5} has a partition coefficient, K_D, of 1.2×10^3 between water and an organic solvent. What restriction on the sample's pH is necessary to ensure that 99.9% of the weak acid in a 50.0-mL sample is extracted with a single 50.0-mL portion of the organic solvent?

27. For problem 26, how many extractions are needed if the sample's pH cannot be decreased below 7.0?

28. A weak base, B, with a K_b of 1.0×10^{-3} has a partition coefficient, K_D, of 5.0×10^2 between water and an organic solvent. What restriction on the sample's pH is necessary to ensure that 99.9% of the weak base is in a 50.0-mL sample is extracted with two 25.0-mL portions of the organic solvent?

29. A sample contains a weak acid analyte, HA, and a weak acid interferent, HB. The acid dissociation constants and the partition coefficients for the weak acids are as follows: $K_{a,HA} = 1.0 \times 10^{-3}$, $K_{a,HB} = 1.0 \times 10^{-7}$, $K_{D,HA} = K_{D,HB} = 5.0 \times 10^2$. (a) Calculate the extraction efficiency for HA and HB when a 50.0-mL sample, buffered to a pH of 7.0, is extracted with 50.0 mL of the organic solvent. (b) Which phase is enriched in the analyte? (c) What are the recoveries for the analyte and the interferent in this phase? (d) What is the separation factor? (e) A quantitative analysis is conducted on the phase enriched in analyte. What is the expected relative error if the selectivity coefficient, $K_{HA,HB}$, is 0.500 and the initial ratio of HB/HA is 10.0?
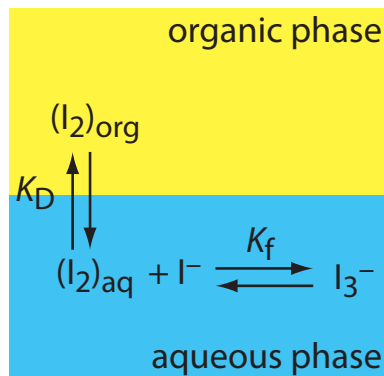
Figure 7.32 Extraction scheme for Problem 7.30.
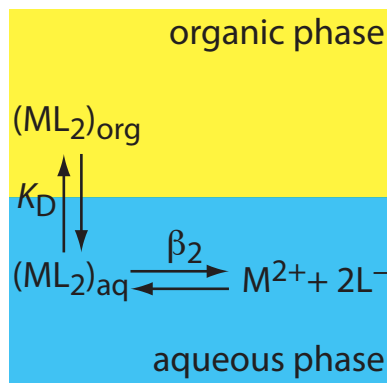

Figure 7.33 Extraction scheme for Problem 7.31.

30. The relevant equilibria for the extraction of I_2 from an aqueous solution of KI into an organic phase are shown in Figure 7.32. (a) Is the extraction efficiency for I_2 better at higher or at lower concentrations of I^-? (b) Derive an expression for the distribution ratio for this extraction.

31. The relevant equilibria for extracting the metal-ligand complex ML_2 from an aqueous solution into an organic phase are shown in Figure 7.31. (a) Derive an expression for the distribution ratio for this extraction. (b) Calculate the extraction efficiency when a 50.0-mL aqueous sample that is 0.15 mM in M^{2+} and 0.12 M in L^- is extracted with 25.0 mL of the organic phase. Assume that K_D is 10.3 and that β_2 is 560.

32. Derive equation 7.32.

33. The following information is available for the extraction of Cu^{2+} by CCl_4 and dithizone: $K_{D,c} = 7 \times 10^4$; $\beta_2 = 5 \times 10^{22}$; $K_{a,HL} = 2 \times 10^{-5}$; $K_{D,HL} = 1.01 \times 10^4$; and $n = 2$. What is the extraction efficiency if a 100-mL sample of an aqueous solution that is 1.0×10^{-7} M Cu^{2+} and 1 M in HCl is extracted with 10 mL of CCl_4 containing 4.0×10^{-4} M dithizone (HL)?

34. Cupferron is a ligand whose strong affinity for metal ions makes it useful as a chelating agent in liquid–liquid extractions. The following table provides pH-dependent distribution ratios for the extraction of Hg^{2+}, Pb^{2+}, and Zn^{2+} from an aqueous solution to an organic solvent.

| | Distribution Ratio for | | |
pH	Hg^{2+}	Pb^{2+}	Zn^{2+}
1	3.3	0.0	0.0
2	10.0	0.43	0.0
3	32.3	999	0.0
4	32.3	9999	0.0
5	19.0	9999	0.18
6	4.0	9999	0.33
7	1.0	9999	0.82
8	0.54	9999	1.50
9	0.15	9999	2.57
10	0.05	9999	2.57

(a) Suppose you have a 50.0-mL sample of an aqueous solution containing Hg^{2+}, Pb^{2+}, and Zn^{2+}. Describe how you can effect a separation of these metal ions. (b) Under the conditions of your extraction for Hg^{2+}, what percent of the Hg^{2+} remains in the aqueous phase after three 50.0-mL extractions with the organic solvent? (c) Under the conditions of your extraction for Pb^{2+}, what is the minimum volume of

organic solvent needed to extract 99.5% of the Pb^{2+} in a single extraction? (d) Under the conditions of your extraction for Zn^{2+}, how many extractions are needed to remove 99.5% of the Zn^{2+} if each extraction uses 25.0 mL of organic solvent?

7L   Solutions to Practice Exercises

Practice Exercise 7.1

To reduce the overall variance by improving the method's standard deviation requires that

$$s^2 = 5.00 \text{ ppm}^2 = s_{samp}^2 + s_{meth}^2 = (2.1 \text{ ppm})^2 + s_{meth}^2$$

Solving for s_{meth} gives its value as 0.768 ppm. Relative to its original value of 1.1 ppm, this is reduction of $3.0 \times 10^1\%$. To reduce the overall variance by improving the standard deviation for sampling requires that

$$s^2 = 5.00 \text{ ppm}^2 = s_{samp}^2 + s_{meth}^2 = s_{samp}^2 + (1.1 \text{ ppm})^2$$

Solving for s_{samp} gives its value as 1.95 ppm. Relative to its original value of 2.1 ppm, this is reduction of 7.1%.

Click here to return to the chapter.

Practice Exercise 7.2

The analytical method's standard deviation is 1.96×10^{-3} g/cm^3 as this is the standard deviation for the analysis of a single sample of the polymer. The sampling variance is

$$s_{samp}^2 = s^2 - s_{meth}^2 = (3.65 \times 10^{-2})^2 - (1.96 \times 10^{-3})^2 = 1.33 \times 10^{-3}$$

Converting the variance to a standard deviation gives s_{meth} as 3.64×10^{-2} g/cm^3.

Click here to return to the chapter.

Practice Exercise 7.3

To determine the sampling constant, K_s, we need to know the average mass of the samples and the percent relative standard deviation for the concentration of olaquindox in the feed. The average mass for the five samples is 0.95792 g. The average concentration of olaquindox in the samples is 23.14 mg/kg with a standard deviation of 2.200 mg/kg. The percent relative standard deviation, R, is

$$R = \frac{s_{samp}}{\overline{X}} \times 100 = \frac{2.200 \text{ mg/kg}}{23.14 \text{ mg/kg}} \times 100 = 9.507 \approx 9.51$$

Solving for K_s gives its value as

$$K_s = mR^2 = (0.95792 \text{ g})(9.507)^2 = 86.58 \text{ g} \approx 86.6 \text{ g}$$

To obtain a percent relative standard deviation of 5.0%, individual samples need to have a mass of at least

$$m = \frac{K_s}{R^2} = \frac{86.58 \text{ g}}{(5.0)^2} = 3.5 \text{ g}$$

To reduce the sample's mass from 3.5 g to 1 g, we must change the mass by a factor of

$$\frac{3.5 \text{ g}}{1 \text{ g}} = 3.5\times$$

If we assume that the sample's particles are spherical, then we must reduce a particle's radius by a factor of

$$r^3 = 3.5\times$$
$$r = 1.5\times$$

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Practice Exercise 7.4

Because the value of t depends on the number of samples—a result we have yet to calculate—we begin by letting $n_{samp} = \infty$ and using $t(0.05, \infty)$ for the value of t. From Appendix 4, the value for $t(0.05, \infty)$ is 1.960. Our first estimate for n_{samp} is

$$n_{samp} = \frac{t^2 s_{samp}^2}{e^2} = \frac{(1.960)^2 (5.0)^2}{(2.5)^2} = 15.4 \approx 15$$

Letting $n_{samp} = 15$, the value of $t(0.05, 14)$ from Appendix 4 is 2.145. Recalculating n_{samp} gives

$$n_{samp} = \frac{t^2 s_{samp}^2}{e^2} = \frac{(2.145)^2 (5.0)^2}{(2.5)^2} = 18.4 \approx 18$$

Letting $n_{samp} = 18$, the value of $t(0.05, 17)$ from Appendix 4 is 2.103. Recalculating n_{samp} gives

$$n_{samp} = \frac{t^2 s_{samp}^2}{e^2} = \frac{(2.103)^2 (5.0)^2}{(2.5)^2} = 17.7 \approx 18$$

Because two successive calculations give the same value for n_{samp}, we need 18 samples to achieve a sampling error of $\pm 2.5\%$ at the 95% confidence interval.

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Practice Exercise 7.5

If we collect a single sample (cost $20), then we can analyze that sample 13 times (cost $650) and stay within our budget. For this scenario, the percent relative error is

$$e = t\sqrt{\frac{s^2_{samp}}{n_{samp}} + \frac{s^2_{meth}}{n_{samp}n_{rep}}} = 2.179\sqrt{\frac{0.10}{1} + \frac{0.20}{1 \times 13}} = 0.74$$

where $t(0.05, 12)$ is 2.179. Because this percent relative error is larger than $\pm 0.50\%$, this is not a suitable sampling strategy.

Next, we try two samples (cost $40), analyzing each six times (cost $600). For this scenario, the percent relative error is

$$e = t\sqrt{\frac{s^2_{samp}}{n_{samp}} + \frac{s^2_{meth}}{n_{samp}n_{rep}}} = 2.2035\sqrt{\frac{0.10}{2} + \frac{0.20}{2 \times 6}} = 0.57$$

where $t(0.05, 11)$ is 2.2035. Because this percent relative error is larger than $\pm 0.50\%$, this also is not a suitable sampling strategy.

Next we try three samples (cost $60), analyzing each four times (cost $600). For this scenario, the percent relative error is

$$e = t\sqrt{\frac{s^2_{samp}}{n_{samp}} + \frac{s^2_{meth}}{n_{samp}n_{rep}}} = 2.2035\sqrt{\frac{0.10}{3} + \frac{0.20}{3 \times 4}} = 0.49$$

where $t(0.05, 11)$ is 2.2035. Because both the total cost ($660) and the percent relative error meet our requirements, this is a suitable sampling strategy.

There are other suitable sampling strategies that meet both goals. The strategy requiring the least expense is to collect eight samples, analyzing each once for a total cost of $560 and a percent relative error of $\pm 0.46\%$. Collecting 10 samples and analyzing each one time, gives a percent relative error of $\pm 0.39\%$ at a cost of $700.
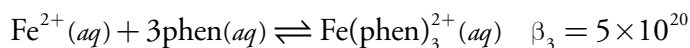
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Practice Exercise 7.6

The fluoride ion, F^-, is a suitable masking agent as it binds with Al^{3+} to form the stable AlF_6^{3-} complex, leaving iron in solution.

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Practice Exercise 7.7
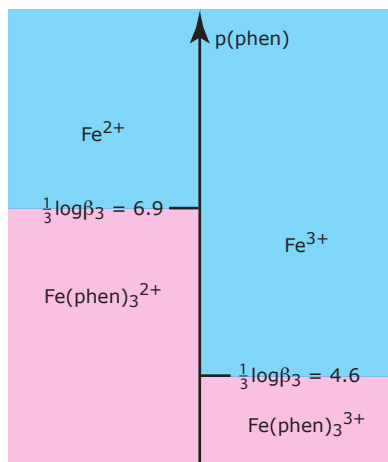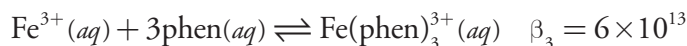
The relevant reactions and equilibrium constants are

$$Fe^{2+}(aq) + 3phen(aq) \rightleftharpoons Fe(phen)_3^{2+}(aq) \quad \beta_3 = 5 \times 10^{20}$$

Figure 7.34 Ladder diagram for Practice Exercise 7.7.

$$Fe^{3+}(aq) + 3phen(aq) \rightleftharpoons Fe(phen)_3^{3+}(aq) \quad \beta_3 = 6 \times 10^{13}$$

where phen is an abbreviation for 1,10-phenanthroline. Because β_3 is larger for the complex with Fe^{2+} than it is for the complex with Fe^{3+}, 1,10-phenanthroline will bind Fe^{2+} before it binds Fe^{3+}. A ladder diagram for this system (Figure 6.34) suggests that an equilibrium p(phen) between 5.6 and 5.9 will fully complex Fe^{2+} without any significant formation of the $Fe(phen)_3^{3+}$ complex. Adding a stoichiometrically equivalent amount of 1,10-phenanthroline to a solution of Fe^{2+} will be sufficient to mask Fe^{2+} in the presence of Fe^{3+}. Adding a large excess of 1,10-phenanthroline, however, will decrease p(phen) and allow the formation of both metal–ligand complexes.

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Practice Exercise 7.8

(a) The solute's distribution ratio between water and toluene is

$$D = \frac{[S_{org}]}{[S_{aq}]} = \frac{0.889 \text{ g} \times \dfrac{1 \text{ mol}}{117.3 \text{ g}} \times \dfrac{1}{0.00500 \text{ L}}}{(1.235 \text{ g} - 0.889 \text{ g}) \times \dfrac{1 \text{ mol}}{117.3 \text{ g}} \times \dfrac{1}{0.01000 \text{ L}}} = 5.14$$

(b) The fraction of solute remaining in the aqueous phase after one extraction is

$$(q_{aq})_1 = \frac{V_{aq}}{DV_{org} + V_{aq}} = \frac{20.00 \text{ mL}}{(5.14)(10.00 \text{ mL}) + 20.00 \text{ mL}} = 0.280$$

The extraction efficiency, therefore, is 72.0%.

(c) To extract 99.9% of the solute requires

$$(Q_{aq})_n = 0.001 = \left[\frac{20.00 \text{ mL}}{(5.14)(10.00 \text{ mL}) + 20.00 \text{ mL}} \right]^n = 0.280^n$$

$$\log(0.001) = n\log(0.280)$$

$$n = 5.4$$

a minimum of six extractions.

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Practice Exercise 7.9

Because the weak base exists in two forms, only one of which extracts into the organic phase, the partition coefficient, K_D, and the distribution ratio, D, are not identical.

$$K_D = \frac{[B_{org}]}{[B_{aq}]}$$

$$D = \frac{[B_{org}]_{total}}{[B_{aq}]_{total}} = \frac{[B_{org}]}{[B_{aq}] + [HB^+_{aq}]}$$

Using the K_b expression for the weak base

$$K_b = \frac{[OH^-_{aq}][HB^+_{aq}]}{[B_{aq}]}$$

we solve for the concentration of HB^+ and substitute back into the equation for D, obtaining

$$D = \frac{[B_{org}]}{[B_{aq}] + \dfrac{K_b \times [B_{aq}]}{[OH^-_{aq}]}} = \frac{[B_{org}]}{[B_{aq}]\left(1 + \dfrac{K_b}{[OH^-_{aq}]}\right)} = \frac{K_D[OH^-_{aq}]}{[OH^-_{aq}] + K_b}$$

At a pH of 9.0, the $[OH^-]$ is 1×10^{-5} M and the distribution ratio has a value of

$$D = \frac{K_D[OH^-_{aq}]}{[OH^-_{aq}] + K_b} = \frac{(5.00)(1.0 \times 10^{-5})}{1.0 \times 10^{-5} + 1.0 \times 10^{-4}} = 0.455$$

After one extraction, the fraction of B remaining in the aqueous phase is

$$(q_{aq})_1 = \frac{25.00 \text{ mL}}{(0.455)(50.00 \text{ mL}) + 25.00 \text{ mL}} = 0.524$$

The extraction efficiency, therefore, is 47.6%. At a pH of 9, most of the weak base is present as HB^+, which explains why the overall extraction efficiency is so poor.

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