3 chapter

Dilutions and Concentrations

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3.1 INTRODUCTION

This chapter builds upon the information presented in the previous Chap. 1 (Laboratory Standard Operating Procedures). This chapter covers the following topics:

1. Reasons for performing dilutions and concentrations in food analysis
2. Basic calculations and strategies for calculating the final concentration, given the initial concentration and a known dilution/concentration scheme (and vice versa)
3. Strategies for designing and performing dilutions to obtain standard curves
4. Example and practice problems

This information will be used repeatedly in a food analysis course (e.g., homework, laboratories, and exams). More importantly, the principles described in this chapter are essential for virtually all laboratory or bench work in food science, including quality assurance/quality control, analysis for food labeling, and product formulation.

3.2 REASONS FOR DILUTIONS AND CONCENTRATIONS

There are various reasons why dilutions and concentrations are performed, including:

1. Dilution:
   (a) To reduce high analyte concentrations in the sample down to levels within the operating range or optimal range of a method/instrument
   (b) To reduce high analyte concentrations in the sample down to levels within the linear region of a method/instrument or to levels within a defined standard curve (see *Practice Problems 5 and 7*)
   (c) To dilute the background matrix to levels that do not interfere with the analysis
   (d) Reagent addition, which dilutes the sample by increasing the volume
   (e) Solvent extraction, which dilutes the sample by using large volumes of solvent to favor transfer of the analyte from sample to solvent (see *Practice Problem 1*)
2. Concentration:
   (a) To increase low analyte concentrations in the sample up to levels within the operating range or optimal range of a method/instrument
   (b) To increase low analyte concentrations in the sample up to levels in the linear region of a method or to within a standard curve (see *Practice Problem 1*)
   (c) Post-extraction evaporation of solvent (see *Practice Problem 1*)

3.3 USING VOLUMETRIC GLASSWARE TO PERFORM DILUTIONS AND CONCENTRATIONS

The use of laboratory glassware was covered in Chap. 1. However, a few key points are sufficiently important for calculations regarding dilutions and concentrations to mention again:

1. Class A glass volumetric flasks are used for bringing samples or solutions up to a known "**to contain**" (*TC*) volume.
2. Class A glass volumetric pipettes are used for transferring (delivering) a known "**to deliver**" (*TD*) volume of sample.
3. Other types of pipettes (non-class A volumetric glass pipettes, adjustable pipettors, automatic pipettors, reed pipettors, graduated measuring pipettes) and other glassware (graduated cylinders, beakers, etc.) are less accurate and less precise and should not be used for quantitative volume measurements.
4. Dilutions can be performed as "**diluted to**" or "**diluted to a final volume of**" or as "**diluted with.**" These are very different terms (see Chap. 1 for definitions). It is critical to understand the differences between these types of dilutions to perform lab procedures and calculations correctly.

3.4 CALCULATIONS FOR DILUTIONS AND CONCENTRATIONS

3.4.1 Introduction

Calculations involving dilutions or concentrations are critical for quantitative measurements. Many analytical methods (such as spectrophotometric assays, chromatography, titrations, protein determinations, etc.) are performed on diluted or concentrated samples, and the analytical result must be converted to the concentration in the undiluted or unconcentrated sample for labeling or research purposes. Instances for which these calculations are used include, but are not limited to:

1. Preparation of standard curves (see *Practice Problems 3 and 4*).
2. Determining the necessary dilution required to obtain a sample concentration within a specified range (see *Practice Problems 5 and 7*)
3. Determining the necessary range of dilutions for preparing a range of concentrations for a standard curve from a stock standard solution (see *Practice Problems 5 and 7*)

4. For converting an analytical result obtained from a diluted or concentrated sample to the undiluted or concentrated food (see *Practice Problems 1 and 6*)

3.4.2 Expressing Concentration

Recall that the concentration of an analyte in a sample or solution is defined as follows:

$$C = \frac{X}{m} \quad \text{or} \quad C = \frac{X}{V} \tag{3.1}$$

C = concentration

X = amount of analyte (g, mol, etc.)

V = sample volume

m = mass

Note that concentrations also can be expressed in terms of **percentages** (%), parts per million (ppm), etc. (see Chap. 2, Table 2.1). Percentage is a particular problem as it can refer to ratios expressed as weight per weight, weight per volume, or volume per volume. Simply saying 5% ethanol is unclear as it could represent 5% w/w, w/v, or v/v. Therefore, % are typically expressed with the accompanying notation (w/w, w/v, etc.) to clarify the meaning of the % value. Rearranging Eq. 3.1, the amount of analyte can be expressed as:

$$X = Cm \quad \text{or} \quad X = CV \tag{3.2}$$

For each step in a dilution or concentration, a portion (mass or volume) of a sample or solution is either diluted with additional liquid or reduced in volume. The mass, volume, and concentration of the sample change, but the amount (mol, g, etc.) of the analyte present in the amount of the sample that is diluted or concentrated does not change. Therefore, the following is true:

$$X_1 = X_2 \tag{3.3}$$

X_1 = the amount of analyte in the sample before the dilution / concentration step

X_2 = the amount of analyte in the sample after the dilution / concentration step

Example D1 Suppose that a stock solution of thiamine is made by dissolving 168 mg thiamine to a volume of 150 mL, and then 0.25 mL of that stock solution is added to a volumetric flask and water is added so that the final volume is 200 mL. The concentration of thiamine in the stock solution is:

$$C = \frac{X}{V} = \frac{168\,\text{mg thiamine}}{150\,\text{mL solution}} = \frac{1.12\,\text{mg thiamine}}{\text{mL solution}}$$

The amount of thiamine in the 0.25 mL that is diluted is:

$$X = CV = (0.25\,\text{mL solution})\left(\frac{1.12\,\text{mg thiamine}}{\text{mL solution}}\right)$$
$$= 0.28\,\text{mg thiamine}$$

Note that we are only concerned with the amount of analyte in the portion of the sample that is diluted/concentrated (0.25 mL in this example) and *not* the amount of analyte in the whole initial sample (150 mL in this case). When the 0.25 mL of the stock solution (containing a total of 0.28 mg thiamine) is diluted to a final volume of 200 mL, the total amount of thiamine (0.28 mg) does not change because the added water does not contain any thiamine. Therefore:

$$X_1 = X_2 = 0.28\,\text{mg thiamine}$$

However, the concentration of thiamine changes, because now the 0.28 mg thiamine is present in 200 mL instead of 0.25 mL:

$$C = \frac{X}{V} = \frac{0.28\,\text{mg thiamine}}{200\,\text{mL solution}} = \frac{0.0014\,\text{mg thiamine}}{\text{mL solution}}$$

Therefore, the thiamine solution has been diluted, as the final concentration (0.0014 mg/mL) is less than the initial concentration (1.68 mg/mL).

"Concentration" and "amount" are very distinct concepts. Concentration is a ratio of the amount of analyte to the amount of sample. The concentration (mg/mL, M, N, %, ppm, etc.) of an analyte present in a sample is not dependent on the amount (g, mL, etc.) of sample.

Example D2 If a pesticide is present at 2.8 parts per billion (ppb) (note that 1 ppb = 1 µg/kg) in a sample of applesauce, the pesticide is uniformly distributed throughout the applesauce, and therefore the concentration is 2.8 ppb regardless of whether 1 µg, 1 mg, 1 g, 1 kg, 1 mL, or 1 L of applesauce is analyzed. However, the total amount of the analyte present does depend on the size of the sample. We can easily see that

0.063 and 2.2 kg of applesauce contain the same concentration, but vastly different total amounts, of the pesticide:

$$X = \mathrm{Cm} \tag{3.4}$$

$$0.063\,\mathrm{kg\ applesauce}\left(\frac{2.8\,\mu\mathrm{g\ pesticide}}{\mathrm{kg\ applesauce}}\right) = 0.176\,\mu\mathrm{g\ pesticide}$$

$$2.2\,\mathrm{kg\ applesauce}\left(\frac{2.8\,\mu\mathrm{g\ pesticide}}{\mathrm{kg\ applesauce}}\right) = 6.16\,\mu\mathrm{g\ pesticide}$$

Once we know the concentration of a sample or solution, we can take any part, or all, of the sample. This changes the amount of the analyte but not the concentration. Then, diluting or concentration the aliquot chosen changes the concentration, but not the amount, of the analyte. These are two critical concepts that must be implicitly understood to master the concepts of dilution and concentrations. Since the total amount of analyte does not change during each step, the following equations can be derived:

$$X_i = X_f \tag{3.5}$$

$$\text{and } X = \mathrm{Cm} \quad \text{or} \quad X = \mathrm{CV}, \text{therefore:}$$

$$C_i V_i = C_f V_f \quad \text{or} \quad C_i m_i = C_f m_f \tag{3.6}$$

$$i = \text{initial}\left(\text{before dilution or concentration}\right)$$

$$f = \text{final}\left(\text{after dilution or concentration}\right)$$

3.4.3 Forward Calculations

The relationship between the initial and final concentration can be used to determine the starting and/or final concentration if one concentration and the masses or volumes used in the dilution are known. If the starting concentration is known, the final concentration can be calculated (a **"forward" calculation**):

$$C_f = \frac{C_i V_i}{V_f} \quad \text{or} \quad C_f = \frac{C_i m_i}{m_f} \tag{3.7}$$

Similarly, if the final concentration is known, the starting concentration can be calculated (a **"back" calculation**). From this relationship, it becomes evident that the final concentration can be expressed as the initial concentration multiplied by the ratio of the initial and final concentrations (or vice versa for the initial concentration):

$$C_f = \frac{C_i V_i}{V_f} = C_i\left(\frac{V_i}{V_f}\right) \quad \text{or} \quad C_f = \frac{C_i m_i}{m_f} = C_i\left(\frac{m_i}{m_f}\right) \tag{3.8}$$

This is intuitive, as the ratio of the final to the initial volumes, masses, or concentrations is referred to as the "fold" of the dilution. The ratio of the starting and final masses or volumes for each step is referred to as the **dilution factor** *(DF)* for that step (see *Practice Problem 2*):

$$\mathrm{DF} = \frac{V_i}{V_f} \tag{3.9}$$

The final concentration is the product of the initial concentration and the DF (the DF is a "multiplier" that can be used to convert the initial concentration to the final concentration):

$$C_f = \frac{C_i V_i}{V_f} = C_i\left(\frac{V_i}{V_f}\right) = C_i\left(\mathrm{DF}\right) \tag{3.10}$$

Another way to think of the DF is the ratio of the final and initial concentrations for each step:

$$\mathrm{DF} = \frac{C_f}{C_i} \tag{3.11}$$

Some conventions that apply to the term "dilution factor" are as follows:

1. DF always refers to the forward direction (initial mass or volume divided by final mass or volume for each step).
2. The term DF is usually used even if the step is a concentration; less commonly, the term "concentration factor" may be used.
3. Since DF is a multiplier of the initial concentration:
   (a) If the step is a dilution, DF < 1.
   (b) If the step is a concentration, DF > 1.

The "fold" or "X" of a dilution is defined as follows:

$$\text{dilution "fold" or "X"} = \frac{1}{\mathrm{DF}} \tag{3.12}$$

Example D3 For the thiamine solution described earlier, if 1 mL of the solution is added to a 10 mL volumetric flask and diluted to volume, the ratio of the final to initial volumes is 10:1 or a "tenfold" or "10X" dilution, and therefore the final concentration is tenfold (10X) lower than, or 1/10th of, the initial concentration:

$$C_f = \frac{C_i V_i}{V_f} = C_i\left(\frac{V_i}{V_f}\right) = \frac{1.12\,\text{mg thiamine}}{\text{mL solution}}\left(\frac{1\,\text{mL}}{10\,\text{mL}}\right) = \frac{0.112\,\text{mg thiamine}}{\text{mL solution}}$$

For a tenfold dilution:

$$\text{dilution "fold" or "X"} = \frac{1}{\text{DF}}$$

$$\text{Therefore, DF} = \frac{1}{\text{dilution "fold" or "X"}}$$

$$\text{DF} = \frac{1}{10} = 0.1$$

$$C_f = C_i(\text{DF}) = \frac{1.12\,\text{mg thiamine}}{\text{mL solution}}(0.1) = \frac{0.112\,\text{mg thiamine}}{\text{mL solution}}$$

As discussed above, note that this is different than adding 10 mL water to 1 mL of the thiamine solution, which makes the final volume 11 mL for an 11-fold or 11X dilution:

$$C_f = C_i\left(\frac{V_i}{V_f}\right) = \frac{1.12\,\text{mg thiamine}}{\text{mL solution}}\left(\frac{1\,\text{mL}}{1\,\text{mL} + 10\,\text{mL}}\right) = \frac{1.12\,\text{mg thiamine}}{\text{mL solution}}\left(\frac{1\,\text{mL}}{11\,\text{mL}}\right) = \frac{0.102\,\text{mg thiamine}}{\text{mL solution}}$$

For concentration processes, the calculation is the same except the analyte concentration increases from initial to final. For example, suppose that 12.8 mL of the thiamine solution is reduced (by boiling, freeze drying, rotary evaporation, etc.) to 3.9 mL. The final concentration is:

$$C_f = C_i\left(\frac{V_i}{V_f}\right) = \frac{1.12\,\text{mg thiamine}}{\text{mL solution}}\left(\frac{12.8\,\text{mL}}{3.9\,\text{mL}}\right) = \frac{3.68\,\text{mg thiamine}}{\text{mL solution}}$$

Note that masses can be used instead of volumes:

$$C_i m_i = C_f m_f \qquad (3.13)$$

Furthermore, both masses and volumes can be used:

$$C_i m_i = C_f V_f \quad \text{or} \quad C_i V_i = C_f m_f \qquad (3.14 \text{ and } 3.15)$$

Example D4 Suppose that soybean oil contains 175 mg oleic acid per g, and 2.5 g of the oil is diluted with hexane to a final volume of 75 mL. The concentration of oleic acid in the final solution can be determined as follows:

$$C_i m_i = C_f V_f$$

$$C_f = C_i\left(\frac{m_i}{V_f}\right) = \frac{175\,\text{mg oleic acid}}{\text{g oil}}\left(\frac{2.5\,\text{g oil}}{75\,\text{mL}}\right) = \frac{5.83\,\text{mg oleic acid}}{\text{mL}}$$

3.4.4 Back Calculations

The examples worked up to this point involve "forward" calculations (calculating the final concentration from a known initial concentration, see *Practice Problems 3 and 4*). However, many food analysis calculations will involve "back" calculations (calculating the initial sample concentration from a final concentration obtained by analysis of the diluted or concentrated sample, see *Practice Problems 1 and 6*). This calculation is simply the reverse of the "forward" calculation, for which the initial concentration is solved for as opposed to the final concentration:

$$C_i V_i = C_f V_f \quad \text{or} \quad C_i m_i = C_f m_f, \text{ therefore:}$$

$$C_i = \frac{C_f V_f}{V_i} = C_f\left(\frac{V_f}{V_i}\right) \quad \text{or}$$

$$C_i = \frac{C_f m_f}{m_i} = C_f\left(\frac{m_f}{m_i}\right) \qquad (3.16)$$

Note that for back calculations, we flip the DF:

$$C_i = \frac{C_f V_f}{V_i} = C_f\left(\frac{V_f}{V_i}\right) = C_f\left(\frac{1}{\text{DF}}\right) \quad \text{or}$$

$$C_i = \frac{C_f m_f}{m_i} = C_f\left(\frac{1}{\text{DF}}\right) \qquad (3.17)$$

Example D5 Suppose that 50 mL of water is added to a 35 mL sample of apple juice (therefore, initial volume = 35 mL and final volume = 85 mL), and titration indicates that the concentration of malic acid in the diluted sample is 0.24 % w/v. What is the concentration of malic acid in the undiluted juice? This can be determined using Eq. 3.17 above:

$$C_i = C_f\left(\frac{V_f}{V_i}\right) = 0.24\%\ \text{w/v}\left(\frac{85\,\text{mL}}{35\,\text{mL}}\right) = 0.583\%\ \text{w/v}$$

A special case of concentration is when a solution is completely evaporated or dried (so that there is no liquid left, only nonvolatile solutes) and then reconstituted with a volume of liquid smaller than the initial volume. This is technically composed of two distinct steps: a concentration step (drying the sample or solution until only solutes, usually a very small mass, are left) followed by a dilution step (diluting the residual dry mass to a define volume).

Example D6 Suppose that 10 mL apple juice, containing 0.025 g malic acid/mL, is freeze dried, leaving a residue of 0.09 g. This residue is then reconstituted to a final volume of 3 mL with water. What is the concentration of malic acid in the final solution? For the first step, the problem is set up as:

$$C_f = C_i\left(\frac{V_i}{V_f}\right) = \frac{0.025\,\text{g malic acid}}{\text{mL juice}}\left(\frac{10\,\text{mL juice}}{0.09\,\text{g residue}}\right) = \frac{2.78\,\text{g malic acid}}{\text{g residue}}$$

For the second step, the problem is set up as:

$$C_f = C_i\left(\frac{V_i}{V_f}\right) = \frac{2.78\,\text{g malic acid}}{\text{g residue}}\left(\frac{0.09\,\text{g residue}}{3\,\text{mL}}\right) = \frac{0.0833\,\text{g malic acid}}{\text{mL}}$$

However, in actual laboratory practice, this is typically treated as a single step (starting with the initial volume and ending with the final diluted volume) for two reasons. First, the whole dried sample is typically reconstituted, so the final mass for the first step and the initial mass of the second step are the same and thus cancel each other out in the calculation. Second, for most concentrations involving solutions, the mass of the solutes remaining after complete drying is much too small to be measured accurately with balances commonly found in most food analysis laboratories, and it is difficult to get all of the residue out accurately for weighing. Therefore, these problems are typically solved in a single step as follows:

$$C_f = C_i\left(\frac{V_i}{V_f}\right) = \frac{0.025\,\text{g malic acid}}{\text{mL juice}}\left(\frac{10\,\text{mL juice}}{3\,\text{mL}}\right) = \frac{0.0833\,\text{g malic acid}}{\text{mL}}$$

As long as the entire residue is reconstituted, the calculation can be treated as a single step.

3.4.5 Multiple-Step Dilutions and Concentrations

Up to this point, we have dealt exclusively with single-step dilutions and concentrations. However, real-world analytical practice often requires that several dilutions and/or concentrations be performed (see *Practice Problems 1, 2, and 6*). There are two ways to approach this problem. Suppose that three dilutions are used as follows:

$$\text{Step 1}: C_1 \rightarrow C_2 \text{ by performing } V_1 \rightarrow V_2$$

$$\text{Step 2}: C_2 \rightarrow C_3 \text{ by performing } V_3 \rightarrow V_4$$

$$\text{Step 3}: C_3 \rightarrow C_4 \text{ by performing } V_5 \rightarrow V_6$$

The obvious approach is to solve for the final (or initial) concentration of the first step, use the final (or initial) concentration of the first step as the initial (or final) concentration of the second step, and so forth:

$$C_2 = C_1\left(\frac{V_1}{V_2}\right) \text{ then } C_3 = C_2\left(\frac{V_3}{V_4}\right) \text{ then } C_4 = C_3\left(\frac{V_5}{V_6}\right)$$

This can be time-consuming. Furthermore, performing multiple calculations can introduce rounding errors and increases the probability of making other errors. Notice that we can substitute the formula used to calculate a concentration in one step for that value in the next step:

$$C_2 = C_1\left(\frac{V_1}{V_2}\right)$$

$$C_3 = C_2\left(\frac{V_3}{V_4}\right) = \left[C_1\left(\frac{V_1}{V_2}\right)\right]\left(\frac{V_3}{V_4}\right)$$

$$C_4 = C_3\left(\frac{V_5}{V_6}\right) = \left[C_1\left(\frac{V_1}{V_2}\right)\left(\frac{V_3}{V_4}\right)\right]\left(\frac{V_5}{V_6}\right)$$

Therefore, as long as we have the volumes (or masses) from each step, we can reduce this multistep calculation to a single-step calculation that goes directly from the initial to the final concentration (or vice versa), keeping in mind that when going forward (from initial sample to final solution), the initial concentration is multiplied by a series of dilution factors for each step, with the starting mass/volume divided by the final mass/volume for each step. If we have a dilution or concentration scheme with n steps, we can set up the calculations so that:

$$\text{Step 1}: C_i \rightarrow C_2 \text{ by performing } V_1 \rightarrow V_2$$

$$\text{Step 2}: C_2 \rightarrow C_3 \text{ by performing } V_3 \rightarrow V_4$$

and so forth until the last step, where:

$$\text{Step } n: C_3 \rightarrow C_f \text{ by performing } V_{k-1} \rightarrow V_k$$

Then, a general formula for multistep forward calculations is:

$$C_f = C_i\left(\frac{m \text{ or } V_1}{m \text{ or } V_2}\right)\left(\frac{m \text{ or } V_3}{m \text{ or } V_4}\right)\cdots\left(\frac{m \text{ or } V_{k-1}}{m \text{ or } V_k}\right) = C_i(\mathrm{DF}_1)(\mathrm{DF}_2)\ldots(\mathrm{DF}_n) \quad (3.18)$$

We can simplify this by calculating the "overall DF" (DF_Σ), the product of DFs from each step:

$$\mathrm{DF}_\Sigma = (\mathrm{DF}_1)(\mathrm{DF}_2)\ldots(\mathrm{DF}_n) \quad (3.19)$$

$$C_f = C_i(\mathrm{DF}_\Sigma) \quad (3.19)$$

When going from final solution to initial sample, the calculation is reversed: the initial concentration is multiplied by dilution factors for each step, with the final mass/volume divided by the initial mass/volume for each step. A general formula for multistep back calculations is:

$$C_i = C_f\left(\frac{m \text{ or } V_2}{m \text{ or } V_1}\right)\left(\frac{m \text{ or } V_4}{m \text{ or } V_3}\right)\cdots\left(\frac{m \text{ or } V_k}{m \text{ or } V_{k-1}}\right) = C_f\left(\frac{1}{\mathrm{DF}_1}\right)\left(\frac{1}{\mathrm{DF}_2}\right)\cdots\left(\frac{1}{\mathrm{DF}_n}\right) \quad (3.20)$$

$$C_f = C_i\left(\frac{1}{\mathrm{DF}_\Sigma}\right) \quad (3.21)$$

$$\mathrm{DF}_\Sigma = \frac{C_f}{C_i} \quad (3.22)$$

This approach of solving dilution or concentration problems in a single step can be used for dilutions, concentrations, and mixed procedures involving both dilutions and concentrations.

Example D7 Suppose that 0.56 g of green tea extract is diluted in boiling water to a volume of 500 mL, 25 mL of the resulting solution is mixed with 100 mL phosphate buffer, 75 mL of this solution is freeze dried, and the residue is dissolved to a final volume of 50 mL in methanol, and all of this solution is then combined with 50 mL ether. The final concentration of caffeine is analyzed by HPLC and is 12.9 μg/mL. What is the concentration of caffeine in the green tea extract (in μg/g)?

We can set the problem up like this:

$$\text{Step 1: } C_i = ?\ 0.56\,\mathrm{g}(m_1) \text{ diluted to } 500\,\mathrm{mL}(V_2)$$

$$\text{Step 2: } 25\,\mathrm{mL}(V_3) \text{ diluted to } 125\,\mathrm{mL}\ (V_4, 25\,\mathrm{mL} + 100\,\mathrm{mL})$$

$$\text{Step 3: } 75\,\mathrm{mL}\ (V_5) \text{ concentrated to } 50\,\mathrm{mL}(V_6)$$

$$\text{Step 4: } 50\,\mathrm{mL}(V_7) \text{diluted to } 100\,\mathrm{mL}\ (V_8, 50\,\mathrm{mL} + 50\,\mathrm{mL}),\quad C_f = \frac{12.9\,\mu\mathrm{g}}{\mathrm{mL}}$$

The calculation is a back calculation of the initial concentration from the final calculation:

$$C_i = C_f\left(\frac{m \text{ or } V_2}{m \text{ or } V_1}\right)\left(\frac{m \text{ or } V_4}{m \text{ or } V_3}\right)\cdots\left(\frac{m \text{ or } V_k}{m \text{ or } V_{k-1}}\right) = C_f\left(\frac{1}{\mathrm{DF}_1}\right)\left(\frac{1}{\mathrm{DF}_2}\right)\cdots\left(\frac{1}{\mathrm{DF}_n}\right)$$

There are four steps, so there must be four individual DFs (this is a good way to make sure that you are doing the problem correctly; does the number of DFs you used equal the number of steps?):

$$C_i = C_f\left(\frac{m \text{ or } V_2}{m \text{ or } V_1}\right)\left(\frac{m \text{ or } V_4}{m \text{ or } V_3}\right)\left(\frac{m \text{ or } V_6}{m \text{ or } V_5}\right)\left(\frac{m \text{ or } V_8}{m \text{ or } V_7}\right)$$

$$C_i = \frac{12.9\,\mu\mathrm{g\,caffeine}}{\mathrm{mL}}\left(\frac{500\,\mathrm{mL}}{0.56\,\mathrm{g\,green\,tea\,extract}}\right)\left(\frac{125\,\mathrm{mL}}{25\,\mathrm{mL}}\right)\left(\frac{50\,\mathrm{mL}}{75\,\mathrm{mL}}\right)\left(\frac{100\,\mathrm{mL}}{50\,\mathrm{mL}}\right) = \frac{76{,}800\,\mu\mathrm{g\,caffeine}}{\mathrm{g\,green\,tea\,extract}}$$

Another way to make sure that this calculation has been done correctly is to assign each solution a letter or number, and those should "cancel each other out" along with the units. If the units and/or solution identities don't cancel out properly, one or more concentrations, volumes, or masses have been used incorrectly. For this problem, the solutions could be identified as follows:

$$\text{Step 1: } C_i = ?\ m\,0.56\,\mathrm{g}(m_1) \text{ diluted to } 500\,\mathrm{mL}\ (V_2, \text{solution } A)$$

$$\text{Step 2: } 25\,\mathrm{mL}(V_3, \text{solution } A) \text{ diluted to } 125\,\mathrm{mL}\ (V_4, \text{solution } B, 25\,\mathrm{mL} + 100\,\mathrm{mL})$$

$$\text{Step 3: } 75\,\mathrm{mL}\ (V_5, \text{solution } B) \text{ concentrated to } 50\,\mathrm{mL}\ (V_6, \text{solution } C)$$

Step 4: 50 mL (V_7, solution C) diluted to 100 mL (V_8, solution D, 50 mL + 50 mL)

$$C_i = \frac{12.9\,\mu g}{mL}$$

The problem would then be solved the same way with the solutions identified:

$$C_i = \frac{12.9\,\mu g\ caffeine}{mL\ solution\ D}\left(\frac{500\,mL\ solution\ A}{0.56\,g\ green\ tea\ extract}\right)\left(\frac{125\,mL\ solution\ B}{25\,mL\ solution\ A}\right)\left(\frac{50\,mL\ solution\ C}{75\,mL\ solution\ B}\right)\left(\frac{100\,mL\ solution\ D}{50\,mL\ solution\ C}\right) = \frac{76{,}785.7\,\mu g\ caffeine}{g\ green\ tea\ extract}$$

3.5 SPECIAL CASES

3.5.1 Extraction

There are several special cases regarding dilution that merit specific mention: extraction and homogenization/mixing. Regarding **extraction**, often, we use a solvent for which an analyte has a high affinity (e.g., lipids are more soluble in nonpolar solvents such as hexane or chloroform than the foods they are in, so these solvents "pull" the lipid out of the food) to extract that analyte from a sample. The solvent is typically immiscible with the sample as a whole. This means that the sample and the solvent do not mix and can be easily separated by centrifugation or by using a separatory funnel. Most of the analyte is transferred to the solvent, while most of the sample remains behind. For dilution and concentration purposes, we assume that 100 % of the desired analyte is quantitatively transferred from the sample into the solvent (see *Practice Problem 1*). While this assumption is never actually true, it is necessary for our purposes. Further dilutions and/or concentrations may then be performed on the extraction solvent.

In some cases, we use a single extraction step. In this situation, the sample is mixed with the solvent to achieve transfer (extraction) of the analyte from the solvent. The sample and solvent are then separated. This is different than the simple dilution problems we have done previously, in that the sample remains behind while only the analyte is transferred to the solvent. However, the principle is still the same: 100 % of the analyte that was in the sample is assumed to now reside in the solvent, and we can use the equations previously discussed (Eqs. 3.16 and 3.17).

Example E1 Suppose that peanut butter (PB) contains 0.37 g fat/g, and 1.4 g peanut butter is extracted with 100 mL hexane. What is the concentration of fat in the hexane (the assumption here is that the volume of the hexane doesn't change)?

This problem can be solved using the standard procedure:

$$C_f = C_i\left(\frac{m_i}{V_f}\right) = \frac{0.37\,g\ fat}{g\ PB}\left(\frac{1.4\,g\ PB}{100\,mL}\right) = \frac{0.00518\,g\ fat}{mL}$$

Note that this is the same as if the entire sample was diluted into the solvent, but for an extraction only a part of the sample (but presumably all of the analyte) is actually transferred.

In reality, only a fraction (generally unknown) of the analyte is transferred from the sample to the solvent. In order to overcome this, repeated extractions are often performed. These are the same as single extractions, except the extraction is repeated with fresh solvent each time and the solvent volumes from each extraction are combined into a single extract. In this case, we can treat this as a single extraction where the combined solvent volume is the final volume, and again we assume that 100 % of the analyte is transferred to the solvent.

Example E2 Suppose that the peanut butter example above is adjusted so that the peanut butter is extracted three times with 75 mL of fresh hexane, and the three extracts are combined. What is the concentration of fat in the hexane?

In this case, the final volume would be 3×75 mL = 225 mL:

$$C_f = C_i\left(\frac{m_i}{V_f}\right) = \frac{0.37\,g\ fat}{g\ PB}\left(\frac{1.4\,g\ PB}{225\,mL}\right) = \frac{0.00230\,g\ fat}{mL}$$

Extraction procedures often specify that the extraction solvent be brought up to a known final volume after extraction. This accounts for potential loss of solvent, or significant changes in volume due to extraction of sample components, during extraction and also increases the precision and accuracy of the extraction. This is handled by simply making this final volume the final volume for the dilution calculation.

Example E3 Suppose that the peanut butter example above is adjusted so that the peanut butter is extracted 2X with 100 mL hexane, and the pooled hexane extract is diluted to a final volume of 250 mL. What is the concentration of fat in the hexane?

In this case, the final volume would simply be 250 mL, as the pooled extracts (~200 mL) are further diluted with ~50 mL to an exact total volume of 250 mL. The problem would then be solved as shown above, using 250 mL as the final volume.

In summary, extraction can be thought of as a dilution (or concentration) in which the final volume is the total volume of solvent. Regardless of the number of extractions, it can be viewed as a single step as long as all the solvent extracted from the sample is kept and the final volume into which the sample is extracted is known. After extraction, dilutions or concentrations of the extract may be performed; these are handled as any other dilution or concentration.

3.5.2 Homogenization/Blending/Mixing

The other scenario that warrants consideration is **homogenization**, **blending**, and **mixing**. Often, samples need to be dispersed or blended into a solvent for sample preparation. This is typically done using a lab blender, food processor, Polytron homogenizer, stomacher, sonicator, etc. These processes cannot typically be done in volumetric glassware. Furthermore, the problem with these processes, from a calculation standpoint, is that homogenization, blending, and mixing of solids with liquids typically change the volume of a sample.

Example E4 Suppose you homogenize 2.5 g cheese (of an unknown volume) in 100 mL phosphate buffer. What is the final volume?

The final volume of the liquid will be >100 mL and is not easily measured by volumetric glassware. Therefore, in this example, the final volume is unknown. This poses a challenge for accurate dilution calculations. This process is usually overcome similarly to the extractions described above: the sample is homogenized, quantitatively transferred to a volumetric flask, and then diluted to a known final volume with the same solvent used for homogenization.

Example E5 Suppose that 22.7 g swordfish fillet is homogenized in a lab blender with 150 mL denaturing buffer. Following homogenization, the liquid is decanted into a 500 mL flask. Fresh denaturing buffer (~25 mL) is used to rinse the blender, and the rinse liquid is also decanted into the flask. The flask is brought to volume with denaturing buffer. The concentration of methylamines in the diluted homogenate is 0.92 µmol/mL. What is the concentration of methylamines in the fish?

In this case, the sum of the homogenization process is that 22.7 g fish is diluted into 500 mL final volume. Therefore, the calculation is performed as follows:

$$C_i = C_f\left(\frac{V_f}{V_i}\right) = \frac{0.92\,\mu\text{mol methylamines}}{\text{mL}}\left(\frac{500\,\text{mL}}{22.7\,\text{g fish}}\right)$$

$$= \frac{20.3\,\mu\text{mol methylamines}}{\text{g fish}}$$

In summary, mixing processes can be thought of as a dilution (or concentration) step for which the final volume is the total volume of solvent. Regardless of the number of steps involved, it can be viewed as a single step as long as all the homogenate from whole sample is kept and the final volume into which the sample is diluted is known. Keep in mind that, after mixing and quantitative dilution, subsequent dilutions or concentrations of the homogenate may be performed, and these are handled as any other dilution or concentration.

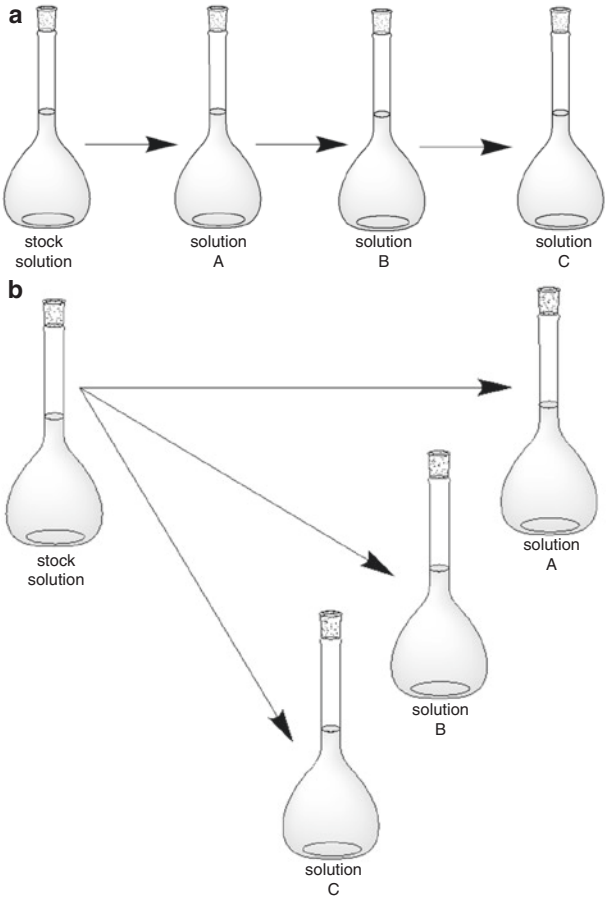
3.6 STANDARD CURVES

It is very common for analytical procedures to require a **standard curve**, which is a set of solutions containing different concentrations of the analyte. Typically, a **stock solution** containing a high concentration of the analyte is prepared, and various dilutions are performed to get the desired standard curve solutions (*see Practice Problems 3, 4, 5, and 7*). These dilutions can be done with either sequential dilutions (Fig. 3.1a) or parallel dilutions (Fig. 3.1b).

3.6.1 Sequential Versus Parallel Dilutions

In the case of **sequential dilutions**, each dilution is used to prepare the next dilution in the series:

$$\text{stock solution} \rightarrow \text{solution 1} \rightarrow \text{solution 2}$$
$$\rightarrow \text{solution 3, etc.}$$



3.1 figure Example standard curve dilution schemes for sequential dilutions (**a**) and parallel dilutions (**b**)

Example F1 Suppose a 1 *M* solution of citric acid is serially diluted as follows: 1 mL is diluted with 2 mL water, and the resulting solution is diluted the same way (1 mL+2 mL water) until four diluted solutions are obtained. What are the concentrations in each solution?

We can solve this problem by assigning each solution a name (Solutions A–D) and then calculating the concentration in each. For each step, the initial volume is 1 mL and the final volume is 3 mL, since 2 mL water is combined with each solution, and the initial concentration is the concentration of the previous solution (stock when making Solution A, Solution A when making Solution B, etc.):

$$\text{Solution } A: C_f = C_i\left(\frac{V_i}{V_f}\right) = 1M\left(\frac{1\text{mL}}{3\text{mL}}\right) = 0.333M$$

$$\text{Solution } B: C_f = C_i\left(\frac{V_i}{V_f}\right) = 0.333M\left(\frac{1\text{mL}}{3\text{mL}}\right) = 0.111M$$

$$\text{Solution } C: C_f = C_i\left(\frac{V_i}{V_f}\right) = 0.111M\left(\frac{1\text{mL}}{3\text{mL}}\right) = 0.0370M$$

$$\text{Solution } D: C_f = C_i\left(\frac{V_i}{V_f}\right) = 0.0370M\left(\frac{1\text{mL}}{3\text{mL}}\right) = 0.0123M$$

For **parallel dilutions**, different DFs are applied to the stock to obtain the desired solutions:

$$\text{stock solution} \xrightarrow{\text{DF1}} \text{solution 1}$$

$$\text{stock solution} \xrightarrow{\text{DF2}} \text{solution 2}$$

$$\text{stock solution} \xrightarrow{\text{DF3}} \text{solution 3}$$

Example F2 Suppose the 1 *M* stock solution of citric acid is diluted as follows: 1 mL of stock solution is diluted to volume in a 5 mL, 10 mL, 25 mL, and 50 mL volumetric flask. What are the citric acid concentrations in each solution? We can solve this problem by assigning each solution a name (Solutions A–D) and then calculating the concentration in each. For each dilution, the starting concentration is the stock concentration (since they were made in parallel), and the final volume is the volume of each volumetric flask:

$$\text{Solution } A: C_f = C_i\left(\frac{V_i}{V_f}\right) = 1M\left(\frac{1\text{mL}}{5\text{mL}}\right) = 0.200M$$

$$\text{Solution } B: C_f = C_i\left(\frac{V_i}{V_f}\right) = 1M\left(\frac{1\text{mL}}{10\text{mL}}\right) = 0.100M$$

$$\text{Solution } C: C_f = C_i\left(\frac{V_i}{V_f}\right) = 1M\left(\frac{1\text{mL}}{25\text{mL}}\right) = 0.040M$$

$$\text{Solution } D: C_f = C_i\left(\frac{V_i}{V_f}\right) = 1M\left(\frac{1\text{mL}}{50\text{mL}}\right) = 0.020M$$

Preparing standards in parallel is typically more accurate than in series, as each dilution is performed in a single step from the stock. This reduces error associated with each solution.

Example F3 If preparing a dilution involves an error of ~1% (a factor of 0.01), what is the error in the third solution if the solutions are prepared in parallel?
Performing three dilutions in parallel will result in each solution being off by ~1%:

$$\text{stock solution} \xrightarrow{\text{DF1}} \text{solution 1}\left(X \pm 1\% \text{ or } 1.01X\right)$$

$$\text{stock solution} \xrightarrow{\text{DF2}} \text{solution 2}\left(X \pm 1\% \text{ or } 1.01X\right)$$

$$\text{stock solution} \xrightarrow{\text{DF3}} \text{solution 3}\left(X \pm 1\% \text{ or } 1.01X\right)$$

If dilutions are performed in series, the error compounds because each solution is used to prepare the next solution.

Example F4 For the same three dilutions, what is the error in the third solution if the dilutions are prepared in series?

When prepared in series, the error increases with each subsequent dilution:

$$\text{stock solution} \rightarrow \text{solution 1}\left(\pm 1\% \text{ or } 1.01X\right) \rightarrow \text{solution 2}\left(\pm 1\% \text{ or } 1.01X\right) \rightarrow \text{solution 3}\left(\pm 1\% \text{ or } 1.01X\right)$$

$$\text{error in solution } 1 = \pm 1.01X = \pm 1\%$$

$$\text{error in solution } 2 = \pm(1.01)(1.01)X = \pm 1.0201X = \pm 2.01\%$$

$$\text{error in solution } 3 = \pm(1.01)(1.01)(1.01)X = \pm 1.030301X = \pm 3.0301\%$$

Parallel dilutions are often preferred over series dilutions for accuracy and improved quantification for standard curves. Regardless of how they are performed, the principles of dilution calculations apply. As with any dilution, these dilutions can be performed as "dilute to" or "dilute with" procedures, and the calculations should be performed accordingly.

3.6.2 Designing Dilution Schemes

Occasionally, you may be given the stock solution concentration and the needed concentrations of standard curve solutions and asked to design a dilution scheme. The way to do this is to calculate the needed DFs for each solution and then design a scheme to achieve the DFs.

Example F5 Suppose you have a 0.2% w/v NaCl solution and you need standard curve solutions of 0.1, 0.04, 0.02, 0.01, and 0.002% w/v. How would you make these?

First, start by calculating each DF from the stock solution:

$$\text{DF} = \frac{C_f}{C_i}$$

$$\text{DF}_\text{A} = \frac{C_f}{C_i} = \frac{0.1\%}{0.2\%} = 0.5\left(\text{i.e.}, \frac{1}{2}\right)$$

$$\text{DF}_\text{B} = \frac{C_f}{C_i} = \frac{0.04\%}{0.2\%} = 0.2\left(\text{i.e.}, \frac{1}{5}\right)$$

$$\text{DF}_\text{C} = \frac{C_f}{C_i} = \frac{0.02\%}{0.2\%} = 0.1\left(\text{i.e.}, \frac{1}{10}\right)$$

$$\text{DF}_\text{D} = \frac{C_f}{C_i} = \frac{0.01\%}{0.2\%} = 0.05\left(\text{i.e.}, \frac{1}{20}\right)$$

$$\text{DF}_\text{E} = \frac{C_f}{C_i} = \frac{0.002\%}{0.2\%} = 0.01\left(\text{i.e.}, \frac{1}{100}\right)$$

From this point, there are several options. You can dilute in series or in parallel. And, you can "dilute to" or "dilute with" to achieve the desired concentrations. For simplicity, the parallel "dilute to" example is shown in Table 3.1.

3.1 table — Dilution example for a standard curve

Solution	Ci (% w/v)	Dilute (mL)	To final volume (mL)	DF	Cf (% w/v)
A	0.2	1	2	0.5	1
B	0.2	1	5	0.2	0.04
C	0.2	1	10	0.1	0.02
D	0.2	1	20	0.05	0.01
E	0.2	1	100	0.002	0.002

You should be able to design a scheme to dilute a stock solution to a wide array of diluted solutions using both parallel and series dilutions and perform

either with "dilute to" or "dilute with" procedures. One consideration to keep in mind is that class A volumetric glassware comes in discreet volumes, and only those volumes can be used for dilutions where maximum accuracy and precision are desired. The most common volumes available are:

1. Class A volumetric flasks: 5, 10, 25, 50, 100, 200, 250, 500, and 1000 mL
2. Class A volumetric transfer pipettes: 1, 2, 3, 4, 5, 10, 20, 25, 50, and 100 mL

Therefore, your designed dilution scheme should use only those volumes for transferring volumes (pipettes) and bringing to volume (flasks), if possible, to optimize accuracy and precision. Some schemes may require the use of an adjustable pipettor (to transfer or add volumes such as 0.1 mL, 950 µL, etc.), particularly when small volumes are used. This is often the case when preparing standard curves (see *Practice Problems 3 and 4*).

Example F6 Suppose that you have a stock solution of 2000 ppm Fe(II) in 0.1 N HCl. You need to create 1 mL of 1000, 750, 500, 250, and 100 ppm Fe(II) by combining different volumes of the stock solution and 0.1 N HCl. What volumes should be used?

We know the starting (2000 ppm) and final concentration (1000–100 ppm) and final volume (1 mL). Therefore, we must calculate the initial volume of stock solution for each solution:

$$C_iV_i = C_fV_f \; and \; V_i = \frac{C_fV_f}{C_i}$$

$$\text{for } 1000\,\text{ppm},\; V_i = \frac{C_fV_f}{C_i} = \frac{(1000\,\text{ppm})(1\,\text{mL})}{2000\,\text{ppm}} = 0.5\,\text{mL}$$

$$\text{for } 750\,\text{ppm},\; V_i = \frac{C_fV_f}{C_i} = \frac{(750\,\text{ppm})(1\,\text{mL})}{2000\,\text{ppm}} = 0.375\,\text{mL}$$

and so forth. Once the volume of stock solution is known for each dilution, we then calculate the amount of 0.1 N HCl needed to dilute to 1 mL:

$$\text{for 1000 ppm, } 0.5\,\text{mL} \rightarrow 1\,\text{mL} - 0.5\,\text{mL} = 0.5\,\text{mL 0.1 N HCl}$$

$$\text{for 750 ppm, } 0.375\,\text{mL} \rightarrow 1\,\text{mL} - 0.375\,\text{mL} = 0.625\,\text{mL 0.1 N HCl}$$

and so forth. The dilution scheme would thus be as shown in Table 3.2.

3.2 table Dilution example for a standard curve

Fe(II) (ppm)	*Stock (mL)*	*0.1 N HCL (mL)*	*Total volume (mL)*
1000	0.5	0.5	1.0
750	0.375	0.625	1.0
500	0.25	0.75	1.0
250	0.125	0.875	1.0
100	0.05	0.95	1.0

In cases when an adjustable pipettor is used, accuracy and precisions should be optimized by frequently calibrating and maintaining the pipettors. Furthermore, using proper (and consistent) manual pipetting technique is critical in these scenarios.

3.7 UNIT CONVERSIONS

Up to this point, we have examined calculations for which the units of the dilutions and the units of the concentrations that you have been given "match up" so that no conversions are required to get the correct answer. However, this will not always be the case in actual lab practice. This will require you to perform unit conversions to get an answer that is correct and makes sense.

Example G1 Suppose that you dilute 14.4 g of yogurt to a final volume of 250 mL with water. HPLC analysis of the diluted sample indicates a riboflavin concentration of 0.0725 ng/µL. What is the riboflavin content in the yogurt?

This is a straightforward dilution problem that can be solved as follows:

$$C_i = C_f\left(\frac{V_f}{V_i}\right)$$

However, if the calculation were performed without unit conversions, the answer obtained would be as follows:

$$C_i = C_f\left(\frac{V_f}{V_i}\right) = \frac{0.0725\,\text{ng riboflavin}}{\mu\text{L diluted sample}}$$

$$\left(\frac{250\,\text{mL diluted sample}}{14.4\,\text{mL yogurt}}\right) = \frac{1.26\,\text{ng riboflavin}\times\text{mL}}{\text{g yogurt}\times\mu\text{L}}$$

Obviously, (ng × mL)/(g × µL) is not an acceptable unit for expressing the riboflavin concentration in a product. Therefore, we must change

either µL to mL or mL to µL for the units to be expressed correctly in the final answer. The calculation is performed correctly as follows:

$$C_i = C_f\left(\frac{V_f}{V_i}\right) = \frac{0.0725\,\text{ng riboflavin}}{\mu\text{L diluted sample}}\left(\frac{1000\,\mu\text{L}}{\text{mL}}\right)$$
$$\left(\frac{250\,\text{mL diluted sample}}{14.4\,\text{mL yogurt}}\right) = \frac{1,260\,\text{ng riboflavin}}{\text{g yogurt}}$$

If the units are not converted, the magnitude (number only) of the final answer is off by a factor of 1000 in this case, in addition to having the wrong units.

By keeping track of the units, you easily catch an incorrect value and adjust the calculation.

3.8 AVOIDING COMMON ERRORS

The most common errors associated with dilutions and corrections are:

1. Setting up the calculation incorrectly (incorrectly using the information provided)
2. Lack of needed unit conversion or incorrect unit conversion

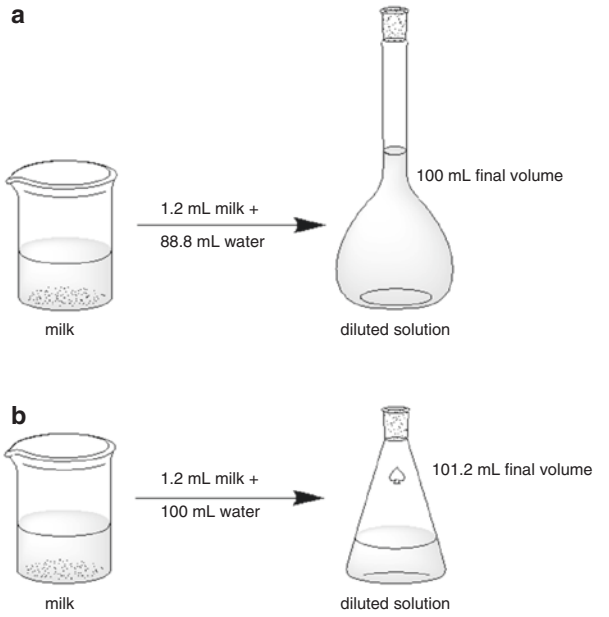
Three strategies can be employed to avoid these mistakes or catch them if they have been made:

1. Draw a picture of the dilution or concentration scheme.
2. Perform **unit analysis** (also referred to as **dimensional analysis** or the **factor-label method**) and assign "names" to each solution in a scheme.
3. Perform the "sniff test."

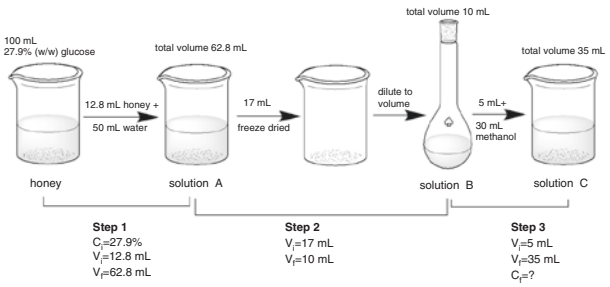
3.8.1 Draw a Picture

Drawing a picture can be very useful for making sure the problem has been set up correctly. This allows you to visualize the dilution or concentration scheme, which is often helpful. Drawing a picture or diagram can help clarify the meaning of the information provided and assist in setting up the calculation.

Example H1 Suppose you are told that 1.2 mL skim milk is diluted to 100 mL with water. The picture that would be drawn might look something like Fig. 3.2a. By comparison, if you are told that 1.2 mL skim milk is diluted with 100 mL water, the picture that would be drawn might look something like Fig. 3.2b.



3.2 figure Dilution schemes for a "dilute to" (**a**) and "dilute with" (**b**) scenario



3.3 figure Diagram of a complex multistep dilution and concentration scheme

Drawing a picture can be particularly useful for visualizing complex multistep processes.

Example H2 You have 100 mL honey (27.9% glucose by weight). 12.8 mL honey is dissolved by adding 50 mL water, 17 mL of this solution is freeze dried and reconstituted to a total volume of 10 mL with water, and 5 mL of this solution is combined with 30 mL methanol. What is the % glucose by weight in the final solution?

This information can be diagramed as shown in Fig. 3.3.

Figure 3.3 shows a fairly complex scheme, and organizing the information in your head can be challenging. Drawing this scheme allows you to assign names to various solutions and organize the information so that it can easily be plugged into the calculation.

3.8.2 Unit Analysis

We have already discussed unit analysis, a key step in verifying that the calculation has been set up correctly. Along with this, assigning names to each solution in a scheme and incorporating those into the calculation can assure that each step has been set up correctly in the calculation. If the problem has been set up correctly, each solution name for the intermediate steps should be "canceled out," leaving only the final solution or sample when the calculation is complete.

Example H3 For the example in Fig. 3.3, the concentration of the final solution would be calculated as follows:

$$C_f = 27.9\%\ \text{glucose}\left(\frac{12.8\,\text{mL sample}}{62.8\,\text{mL solution}\,A}\right)\left(\frac{17\,\text{mL solution}\,A}{10\,\text{mL solution}\,B}\right)\left(\frac{5\,\text{mL solution}\,B}{35\,\text{mL solution}\,C}\right) = 1.38\%\ \text{glucose}$$

In this example, we see that both the units *and* the intermediate solution names cancel out. However, it would be very easy to accidentally reverse one of the dilution factors. For example, if we accidentally reversed the DF for step A → B:

$$C_f = 27.9\%\ \text{glucose}\left(\frac{12.8\,\text{mL sample}}{62.8\,\text{mL solution}\,A}\right)\left(\frac{10\,\text{mL solution}\,B}{17\,\text{mL solution}\,A}\right)\left(\frac{5\,\text{mL solution}\,B}{35\,\text{mL solution}\,C}\right) = 0.478\%\ \text{glucose}$$

The units still cancel out, but you would get an incorrect answer. However, by keeping track of the assigned names of each solution, you would quickly see that this step's DF is reversed because the intermediate solution names do not cancel out. Therefore, this is an easy way to quickly catch and correct errors in dilution calculations.

3.8.3 "Sniff Test"

Finally, use the "sniff test" on your calculations to detect any obvious errors. For example:

1. If the procedure is an overall dilution, then the final concentration should be lower than the initial concentration.
2. If the procedure is an overall concentration, then the final concentration should be higher than the initial concentration.
3. With very few exceptions (such as % moisture expressed on a dry weight basis for some samples), the % of an analyte in a sample will never be ≥100 %.
4. Does the calculated value make sense? For example, one would not expect the following calculated values to be true:
   (a) % moisture > a few % in very dry products
   (b) % solids > a few % in dilute solutions
   (c) % fat, protein, ash, or carbohydrates that don't make sense given what is known about the product (such as vegetable oil containing 35 % moisture or 12 % fat)
   (d) Analyte levels that do not make sense given what is known about typical product composition (particularly ≥1 order of magnitude greater than expected)

For the sniff test, it is important that you know a little about the product you are analyzing, if possible. If the "sniff test" suggests an error, carefully reexamine how you set up the problem and how the calculation was performed.

3.9 PRACTICE PROBLEMS

(Note: Answers to problems are in the last section of the laboratory manual.)

The following example problems demonstrate real-world calculations involving dilutions and concentrations:

1. You randomly select a cup of applesauce (containing 113 g applesauce) from the processing line. You extract 10.3 g of the applesauce 3X with 50 mL ethyl acetate, pool the extracts, and dilute to 250 mL with ethyl acetate. You evaporate 25 mL of the extract to dryness under a stream of N_2 and redissolve the residue to 5 mL with methanol. GC analysis of the methanol indicates a methoxyfenozide concentration of 0.00334 µg/mL. What is the concentration of methoxyfenozide in the applesauce (µg/g)? What is the total amount of methoxyfenozide in the applesauce cup (µg)?
2. You are given a stock solution of (-)-epicatechin (EC, MW = 290.26 g/mol) in water (0.94 mg/mL). You prepare a series of solutions by serial dilution as follows: (1) diluting 0.5 mL of the stock solution to 10 mL with water (solution A), (2) diluting 1.5 mL of solution A with 4 mL water (solution B), and (3) diluting 3 mL solution B with 9 mL water

(solution C). What is the concentration of solution C (mg/mL and μM)? What is the overall DF? How many "fold" has the stock solution been diluted to solution C?

3. You are performing a spectrophotometric assay for riboflavin using a commercial kit. The kit comes with 2 mL of a solution of riboflavin (1.45 mg/mL). The instructions tell you to make a stock solution by diluting the riboflavin with 25 mL water. Then, the instructions tell you to make a set of standard solutions by diluting 100 μL of the stock solution with 0.5 mL (A), 0.75 mL (B), 1 mL (C), 2 mL (D), and 5 mL (E) water. What is the riboflavin concentration (mg/mL) in the stock solution and the five standards?
4. You are using a colorimetric method to measure anthocyanin pigments in raspberry juice. The method requires a total sample volume of 2 mL. You have a stock solution of 160 g/L anthocyanins, and you need solutions of 0, 0.1, 0.2, 0.3, and 0.5 mg/mL. Design a dilution scheme employing commonly available volumetric flasks, volumetric pipettes, and/or an adjustable pipettor (but do *not* use any volumes less than 0.2 mL, and the final volume of each solution must be at least 2 mL) to obtain the desired concentrations.
5. As discussed previously, samples often need to be diluted or concentrated in order to obtain analyte concentrations in the range of the standard curve. Ideally, the sample concentration would be near the middle of the standard curve values. This can be challenging, because you often do not know the approximate sample concentration prior to the analysis. For the standard curve described in *Problem 4*, the standards cover the range of 0–0.5 mg/mL. Suppose you get a sample of a new type of juice into the QA lab and you have no idea how to dilute the juice for analysis. Scientific literature suggests that this juice can have anthocyanin concentrations of anywhere from 750 to 3000 μg/mL. Based on this information, design a dilution scheme that will likely yield diluted juice samples within the range of the standard curve.
6. You are analyzing Ca content of milk using atomic absorption spectroscopy (AAS). This analysis requires dry ashing to isolate the minerals from a sample (dry ashing is essentially a concentration and extraction step: the sample is incinerated to remove all organic mineral and leave only the minerals). You dry ash a 2.8 mL sample of milk, dissolve the ash in 12 mL 1 N HCl, and dilute the solution to 50 mL with 1 N HCl. You further dilute 7 mL of this solution by adding 13 mL 1 N HCl. You then analyze 0.5 mL sample and 0.5 mL of standards (10–100 ppm Ca) by AAS. You find that the diluted sample contains 28.2 ppm Ca. What is the Ca content of the undiluted milk in ppm?
7. You are measuring caffeine (194.2 g/mol) in drinks by high-performance liquid chromatography (HPLC). You prepare standard solutions of 0–100 μM caffeine. Your method calls for dilution of the sample to 250 mL with water prior to analysis. You know that a particular drink contains 170 mg caffeine per 400 mL. How many mL should be diluted to 250 mL total to obtain a concentration in the middle of your standard curve?