LESSON ASSIGNMENT

LESSON 6 Dilutions.

TEXT ASSIGNMENT Paragraphs 6-1 through 6-5.

LESSON OBJECTIVE After completing this lesson, you should be able to:

6-1 Calculate simple dilutions, serial dilutions, working solutions made from stock solutions, and new standards by mixing known standard concentrations.

SUGGESTION After completing the assignment, complete the exercises of this lesson. These exercises will help you to achieve the lesson objective.
LESSON 6

DILUTIONS

6-1. DISCUSSION

A dilution is a laboratory procedure in which the concentration of a sample or solution is reduced by the addition of solvent (diluent). In the laboratory, a dilution is commonly performed when the concentration of an unknown is greater than the limits of linearity of a given quantitative procedure or when a working solution must be prepared from a stock.

6-2. SIMPLE DILUTIONS

Dilutions are expressed as a ratio between the volume of the original solution to the volume of final solution. The conventional form is to express the dilution as the ratio of one unit of the original volume to the final volume. Thus, when 2 mL of sample is added to 8 mL of diluent, the result is a 2:10 dilution, but this is preferably expressed as a 1:5 dilution. The reciprocal of the dilution is the dilution factor. This is the factor by which quantitative results are multiplied to give the final concentration for the original, undiluted sample. For a serum sample diluted 1:10 for a glucose determination, the test result must be multiplied by the dilution factor, 10, to determine the actual concentration of the undiluted serum sample. To calculate the concentration of a dilute solution, multiply the concentration of the original solution by the dilution, expressed as a fraction.

NOTE: A one to ten dilution may be expressed as 1:10 or equivalently as 1/10. This, of course, applies to all dilutions.

a. Example 1. A specimen is diluted by combining 3 mL of serum with 21 mL of saline. What is the dilution of the serum?

Solution. Read the problem carefully and calculate the dilution.

NOTE: Volume units must be the same when calculating dilutions.

\[
\begin{align*}
3 \text{ mL (parts serum)} & + 21 \text{ mL (parts saline)} \\
& = 24 \text{ mL (total parts)} \\
\text{Dilution} & = 3 \text{ mL} ÷ 24 \text{ mL}
\end{align*}
\]
Put the ratio in standard form by dividing each side of the ratio by the sample volume (3).

\[
\frac{3 \text{ mL}}{24 \text{ mL}} = \frac{1}{8}
\]

The dilution of this specimen is 1:8 and if this information were to be used in calculating the concentration of the specimen, the concentration of the diluted sample would be multiplied by 8 in order to determine the concentration of the original, undiluted sample.

**NOTE:** Dilutions and dilution factors have no unit of report, because the units divide out.

b. **Example 2.** A 5 mol/L standard solution has been diluted 1:5, what is the concentration of the dilute solution?

**Solution.** Read the problem carefully, and determine the unknown quantity.

The unknown quantity is the concentration of the dilute solution. In order to solve this type of problem you must express the dilution as a fraction. If you have a fraction of a number, as is the case with this problem, you are in effect multiplying the fraction times the original number.

\[
\frac{1}{5} \text{ mol/L} \times \frac{1}{5} = \frac{1 \text{ mol/L}}{5}
\]

c. **Example 3.** A glucose determination was performed on a serum specimen that was diluted 1:10. The concentration of the diluted specimen was determined to be 73 mg/dL. What is the concentration of the undiluted serum?

**Solution.** Read the problem carefully and determine the unknown quantity.

In this particular instance you need to determine the concentration of a serum specimen, based upon a glucose determination that was performed on a diluted sample.

The dilution factor is the reciprocal of the dilution. This is the factor by which the quantitative results are multiplied to determine the concentration of the undiluted serum sample.

\[
(73 \text{ mg/dL})(10) = 730 \text{ mg/dL}
\]
d. **Example 4.** Determine the amount of serum in 40 mL of a 1:5 dilution of serum with saline.

Solution. Read the problem carefully and use ratio and proportion to determine the amount of serum in the given volume of solution.

\[
\frac{1 \text{ part serum}}{5 \text{ total parts}} \times \frac{x \text{ mL serum}}{40 \text{ mL total}}
\]

\[
(5 \text{ total parts})(x \text{ mL serum}) = (40 \text{ mL total})(1 \text{ part serum})
\]

\[
(40 \text{ mL total})(1 \text{ part serum}) \quad \frac{5 \text{ total parts}}{x \text{ mL serum}} = x \text{ mL serum}
\]

\[
x \text{ mL serum} = 8 \text{ mL serum}
\]

**6-3. SERIAL DILUTIONS**

Serial dilution techniques are used when it is necessary to test several successive dilutions on the same sample. The procedure involves the preparation of an initial dilution and subsequent redilution of aliquots (portions) to give progressively more dilute solutions. In order to calculate the final or intermediate dilutions, use each dilution of interest as a multiplicative factor, of which the resulting product is the dilution.

a. **Example 1.** A serum specimen was successively diluted 1:2, 1:2, and 1:2 with saline. What is the final dilution of the specimen?

Solution. When determining the final dilution of a series of dilutions use each dilution as a multiplicative factor. The resulting product is the final dilution.

\[
\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8}
\]

or equivalently, 1:8
b. **Example 2.** A 1:10 dilution of a substance is rediluted 3:5, 2:15, and 1:2. What is the final dilution?

Solution. When determining the final dilution of a series of dilutions, use each dilution as a multiplicative factor. The resulting product is the final dilution.

\[
\frac{1}{10} \times \frac{3}{5} \times \frac{2}{15} \times \frac{1}{2} = \frac{6}{1500}
\]

Express the dilution in lowest terms.

1:250

c. **Example 3.** A 5 mol/L solution of HCl is diluted 1:5. The resulting solution is diluted 1:10. Determine the concentration of the final dilution.

Solution. When determining the final dilution of a series of dilutions use each dilution as a multiplicative factor. The resulting product is the final dilution.

\[
\frac{1}{5} \times \frac{1}{10} = \frac{1}{50}
\]

Once the final dilution has been determined, the original concentration is multiplied by the dilution to yield the concentration of the dilute HCl solution.

\[
\frac{1}{50} \times 5 \text{ mol/L} = \frac{5}{50} \text{ mol/L} \quad \text{or, equivalently,} \quad 0.1 \text{ mol/L}
\]

6-4. **PREPARATION OF A WORKING SOLUTION FROM A STOCK SOLUTION**

In some instances, a laboratory may utilize a stock solution that will be diluted in order to be used as a working solution. In changing the concentration of the solution, a basic relationship is employed. This relationship is based on the fact that the amount of solute remains constant in both solutions and can be expressed as follows: The volume of the given solution times the concentration of that solution equals the volume of the resulting solution times the concentration of the second solution. An expression that describes this relationship is as follows.
\[ C_1V_1 = C_2V_2 \]

Where:

- \( C_1 \) = Concentration of the stock solution
- \( V_1 \) = Volume of the stock solution
- \( C_2 \) = Concentration of the working solution
- \( V_2 \) = Volume of the working solution

**NOTE:** The relationship between the volumes and concentrations involved is that of an inverse proportion; that is, when the volume of one solution is greater, the concentration of the solution of greater volume is less.

a. **Basic Rules for Solving \( C_1V_1 = C_2V_2 \) Problems.**

1. Three of the 4 values must be known.
2. The units of volume and concentration must be the same respectively.
3. It is crucial that the volume and concentration that relate to one another are identified.
4. Any unit of volume or concentration may be used.

b. **Example 1.** How much 30.0% alcohol is required to make 100 mL of a 3.0% alcohol solution?

Solution. Read the problem carefully and determine the relationship that will allow you to determine the unknown quantity.

\[ C_1V_1 = C_2V_2 \]

Ensure that the concentration and volume units are the same respectively.

Using the appropriate expression, substitute the given values.

\[ (30.0\%)V_1 = (3.0\%)(100\mL) \]
Solve for the unknown quantity.

\[
V_1 = \frac{(3.0\%) (100 \text{ mL})}{30.0} = 10 \text{ mL}
\]

10 mL of 30.0 % alcohol must be added to a 100 mL volumetric flask and q.s. to the mark with water in order to attain a concentration of 3.0 %.

Solution via dimensional analysis:

Calculate the amount of solute in the final (diluted) solution.

\[
\frac{1 \text{ dL}}{100 \text{ mL}} \times \frac{3.0 \text{ mL}}{100 \text{ mL}} = 3.0 \text{ mL alcohol}
\]

Multiply the amount of solute needed in the final solution times the percent concentration of the original solution, used appropriately as a factor.

\[
\frac{3.0 \text{ mL alcohol}}{30.0 \text{ mL alcohol}} = 0.10 \text{ dL}
\]

Expressing the volume of original solution in milliliters yields the same results as before.

\[
\frac{100 \text{ mL}}{0.10 \text{ dL}} = 10 \text{ mL}
\]

**NOTE:** A key for this particular type of problem is that in most instances the problem refers to the preparation of a dilute solution from a concentrated stock. Another key is in the fact that this type of problem will have either two concentrations and one volume or two volumes and one concentration.
c. **Example 2.** How much 0.100 Eq/L Ca(OH)\(_2\) can be prepared from 10.0 mL of 6.00 mol/L Ca(OH)\(_2\)?

Solution. Read the problem carefully and determine the relationship that will allow you to solve the problem for the unknown quantity.

\[ C_1V_1 = C_2V_2 \]

Initial inspection of the problem reveals that the concentration units are different. Either concentration may be converted, the requirement is that both units must be the same.

Express 0.100 Eq/L Ca(OH)\(_2\) in terms of mol/L

\[
\frac{0.100 \text{ Eq}}{\text{L}} \times \frac{\text{mol}}{2 \text{ Eq}} = 0.0500 \text{ mol/L}
\]

Using the appropriate expression substitute the values.

\[ C_1V_1 = C_2V_2 \]

\[
(6.00 \text{ mol/L})(10.0 \text{ mL}) = (0.0500 \text{ mol/L})V_2
\]

Solve for the unknown quantity.

\[
V_2 = \frac{6.00 \text{ mol/L} \times (10.0 \text{ mL})}{0.0500 \text{ mol/L}} = 1200 \text{ mL}
\]

### 6-5. PREPARATION OF STANDARDS BY MIXING

When preparing a new standard by mixing two or more standards of known concentration, the concentration of the new standard times its volume is equal to the sum of the concentration of the first standard times its volume plus the concentration of the second standard times its volume and so on depending on the number of standards being mixed. Keep in mind that the concentration of the new standard will never be greater than the highest standard nor less than the lowest standard. All concentrations and volumes must be in the same units respectively. An expression that describes the relationship between the standards being mixed is as follows.
\[ C_1V_1 = C_2V_2 + C_3V_3 \ldots + C_nV_n \]

Where:

- \( C_1 \) = Concentration of the new standard
- \( V_1 \) = Volume of the new standard (total volume)
- \( C_2, C_3, C_n \) = Concentration of the standards used in preparation
- \( V_2, V_3, V_n \) = Volume of the standards used in preparation

### Example 1

You are preparing a series of standards for an automated glucose procedure. You have made a 50.0 mg/dL and a 300 mg/dL standard and are making intermediate concentrations by mixing various quantities of the two. If you mix 3.00 mL of the 50.0 mg/dL standard with 2.00 mL of the 300 mg/dL standard, what is the concentration of the new standard?

**Solution.** Read the problem carefully and select the formula that will allow you to solve the problem for the unknown quantity.

\[ C_1V_1 = C_2V_2 + C_3V_3 \ldots + C_nV_n \]

Ensure that all concentrations and volumes are expressed in the same units, respectively. (This problem does not require any concentration or volume unit conversion.)

To determine \( V_1 \), add the volumes of each of the standards being mixed. In this problem, \( V_1 \) equals 5.00 mL since 3.00 mL of the 50.0 mg/dL standard and 2.00 mL of the 300 mg/dL standard are being mixed.

Substitute the given information and solve for the unknown quantity.

\[
\begin{align*}
C_1 &= \frac{(50.0 \text{ mg/dL})(3.00 \text{ mL}) + (300 \text{ mg/dL})(2.00 \text{ mL})}{5.00 \text{ mL}} \\
C_1 &= \frac{150 \text{ mg/dL}}{5.00 \text{ mL}} \\
C_1 &= 150 \text{ mg/dL}
\end{align*}
\]
b. Example 2. What is the concentration of a standard solution that was prepared by mixing 5.0 mL of a 6.0 Eq/L Ca₃(PO₄)₂ standard, 8.0 mL of a 2.0 mol/L Ca₃(PO₄)₂ standard, and 3.0 mL of a 31 g/dL Ca₃(PO₄)₂ standard?

Solution. Read the problem carefully and determine the relationship that will allow you to solve the problem for the unknown quantity.

\[ C_1V_1 = C_2V_2 + C_3V_3 \ldots + C_nV_n \]

Ensure that all concentrations and volumes are expressed in the same units, respectively.

**NOTE:** In this example, convert the concentrations to mol/L. Consistency of the units is the only requirement. One unit is not preferred over another in this type of calculation.

Convert 6.0 Eq/L Ca₃(PO₄)₂ to mol/L.

\[
\frac{6.0 \text{ Eq}}{1 \text{ mol}} \times \frac{1 \text{ mol}}{6 \text{ Eq}} = 1.0 \text{ mol/L}
\]

Convert 31 g/dL Ca₃(PO₄)₂ to mol/L.

Calculate the GMW of Ca₃(PO₄)₂.

\[
\text{Ca₃(PO₄)₂} \\
\text{Ca} \times 40.1 \times 3 = 120.3 \\
\text{P} \times 31.0 \times 2 = 62.0 \\
\text{O} \times 16.0 \times 8 = 128.0 \\
\text{GMW} = \frac{310.3 \text{ g/mol}}{}
\]

Use the appropriate factors to determine the mol/L concentration.

\[
\frac{31 \text{ g}}{1 \text{ mol}} \times \frac{1 \text{ mol}}{310.3 \text{ g}} \times \frac{10 \text{ dL}}{1 \text{ L}} = 1.0 \text{ mol/L}
\]

Determine the total volume.

\[
\begin{align*}
1.0 \text{ mol/L} & \times 5.0 \text{ mL} \\
2.0 \text{ mol/L} & \times 8.0 \text{ mL} \\
1.0 \text{ mol/L} & \times 3.0 \text{ mL} \\
& = 16.0 \text{ mL}
\end{align*}
\]
Substitute the given information and solve for the unknown quantity.

\[ C_1 V_1 = C_2 V_2 + C_3 V_3 \ldots + C_n V_n \]

\[ (C_1)(16.0 \text{ mL}) = (1.0 \text{ mol/L})(5.0 \text{ mL}) + (2.0 \text{ mol/L})(8.0 \text{ mL}) + (1.0 \text{ mol/L})(3.0 \text{ mL}) \]

\[ C_1 = \frac{(1.0 \text{ mol/L})(5.0 \text{ mL}) + (2.0 \text{ mol/L})(8.0 \text{ mL}) + (1.0 \text{ mol/L})(3.0 \text{ mL})}{6 \text{ mL}} \]

\[ C_1 = 1.5 \text{ mol/L} \]

Continue with Exercises
EXERCISES, LESSON 6

INSTRUCTIONS: Answer the following exercises by writing the answer in the space provided at the end of the question.

After you have completed all the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. A 10.0 Eq/L NaOH solution was diluted 1:10, rediluted 1:5 and again to 3:15.
   a. Calculate the final dilution factor.
   b. Calculate the final concentration of the diluted NaOH.

2. How much 20 percent NaOH can be prepared from 1.5 mL of 70% NaOH?

3. How many milliliters of 6.0 mol/L HCl is needed to make 6.0 liters of 0.010 mol/L HCl?

4. How many milliliters of 3.0 Eq/L LiCO₃ must be diluted to prepare 5.0 L of a 20 mEq/L LiCO₃ solution?
5. A serum specimen was diluted by adding 100 uL of serum to 0.50 mL of saline and 0.10 mL of deionized water. A glucose determination was performed and the concentration of the diluted sample was found to be 123 mg/dL. What is the concentration of the undiluted patient specimen?

6. A urea standard was prepared by mixing 2.0 mL of a 0.050 g/dL solution and 2.0 mL of a 10 mg/dL standard. What is the mg/dL concentration of the prepared standard urea standard?

7. How much 12.0 Eq/L H$_2$SO$_4$ is required to prepare 100 mL of a 60 mEq/L solution?

8. A 10 L solution of 0.50 mol/L concentration was prepared by adding 250 mL of stock solution and adjusting the volume to 10 L. What is the concentration of the stock solution?

9. How much water must be added to 500 mL of a 10 g/dL NaOH to dilute the concentration to 0.50 Eq/L?

10. What is the Eq/L concentration of a sodium standard that was prepared by mixing 2.0 mL of a 5.0 Eq/L Na$_2$HPO$_4$ with 4.0 mL of a 1.0 mol/L Na$_2$HPO$_4$?
SOLUTIONS TO EXERCISES, LESSON 6

1. a 1:250 (para 6-3)
   b 0.040 Eq/L (para 6-3)

2. 5.2 mL (para 6-4)

3. 10.0 mL (para 6-4)

4. 33.33 mL (para 6-4)

5. 861.0 mg/dL (para 6-2)

6. 30.0 mg/dL (para 6-5)

7. 0.5 mL (para 6-4)

8. 20.0 mol/L (para 6-4)

9. 2000.0 mL (para 6-4) The problem asks “how much water must be added.”

10. 3.0 Eq/L (para 6-5)

End of Lesson 6