Calculations for Molecular Biology and Biotechnology

A Guide to Mathematics in the Laboratory

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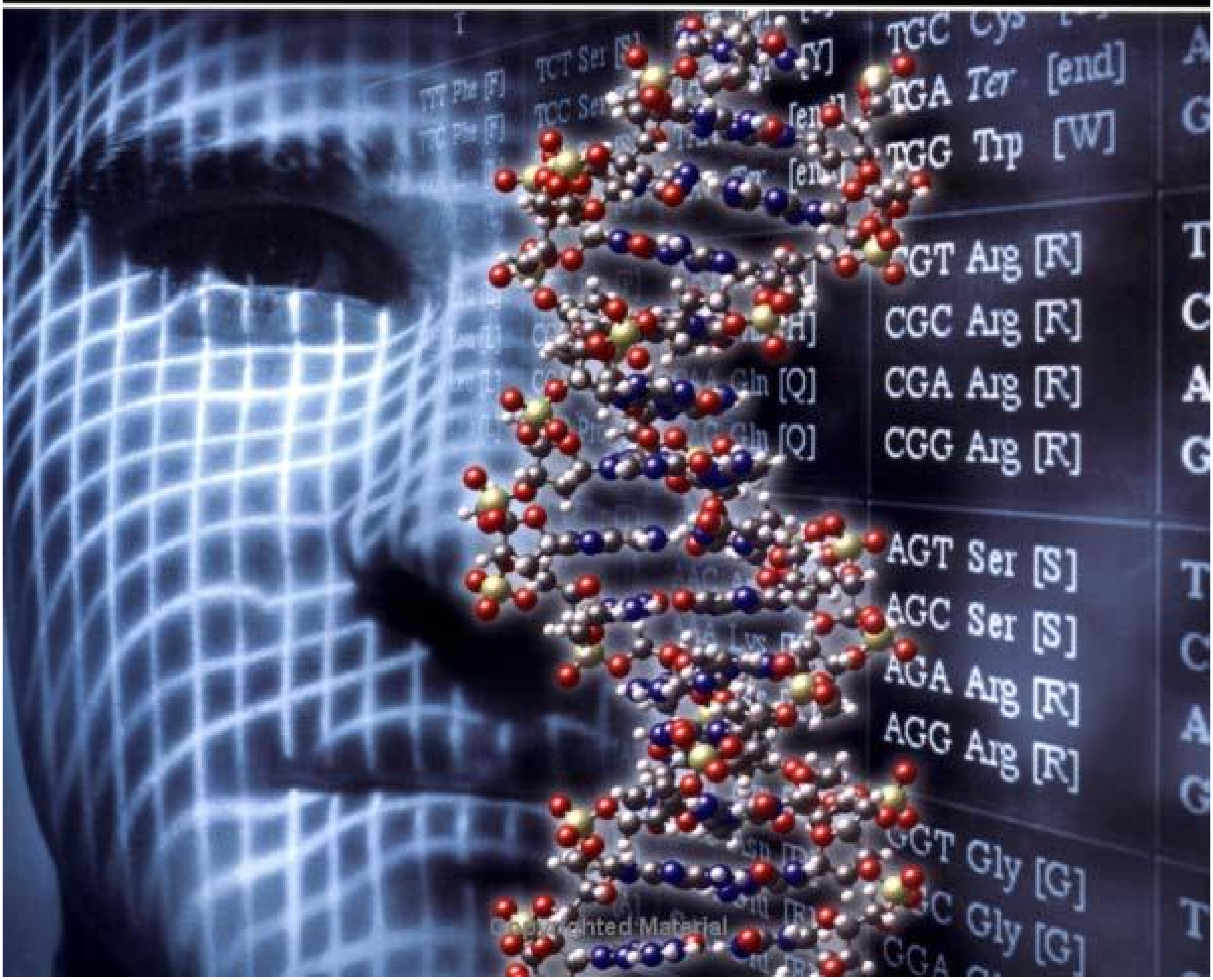

Solutions, Mixtures, and Media

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Introduction

Whether it is an organism or an enzyme, most biological activities function at their optimum only within a narrow range of environmental conditions. From growing cells in culture to sequencing of a cloned DNA fragment or assaying an enzyme's activity, the success or failure of an experiment can hinge on paying careful attention to a reaction's components. This section outlines the mathematics involved in making solutions.

Calculating Dilutions: A General Approach

Concentration is defined as an amount of some substance per a set volume:

$$\text{concentration} = \frac{\text{amount}}{\text{volume}}$$

Most laboratories have found it convenient to prepare concentrated stock solutions of commonly used reagents, those found as components in a large variety of buffers or reaction mixes. Such stock solutions may include 1 *M* Tris, pH 8.0, 500 m*M* EDTA, 20% sodium dodecylsulfate (SDS), 1 *M* $MgCl_2$, and any number of others. A specific volume of a stock solution at a particular concentration can be added to a buffer or reagent mixture so that it contains that component at some concentration less than that in the stock. For example, a stock solution of 95% ethanol can be used to prepare a solution of 70% ethanol. Since a higher percent solution (more concentrated) is being used to prepare a lower percent (less concentrated) solution, a **dilution** of the stock solution is being performed.

There are several methods that can be used to calculate the concentration of a diluted reagent. No one approach is necessarily more valid than another. Typically, the method chosen by an individual has more to do with how his or her brain approaches mathematical problems than with the legitimacy of the procedure. One approach is to use the equation $C_1V_1 = C_2V_2$ where

C_1 is the initial concentration of the stock solution,
V_1 is the amount of stock solution taken to perform the dilution,
C_2 is the concentration of the diluted sample, and
V_2 is the final, total volume of the diluted sample.

For example, if you were asked how many µL of 20% sugar should be used to make 2 mL of 5% sucrose, the $C_1V_1 = C_2V_2$ equation could be used. However, to use this approach, all units must be the same. Therefore, you first need to convert 2 mL into a microliter amount. This can be done as follows:

$$2 \text{ mL} \times \frac{1000 \ \mu\text{L}}{1 \text{ mL}} = 2000 \ \mu\text{L}$$

C_1, then, is equal to 20%, V_1 is the volume you wish to calculate, C_2 is 5%, and V_2 is 2000 µL. The calculation is then performed as follows:

$$C_1V_1 = C_2V_2$$

$$(20\%)V_1 = (5\%)(2000 \ \text{mL})$$

Solving for V_1 gives the following result.

$$V_1 = \frac{(5\%)(2000 \ \mu\text{L})}{20\%} = 500 \ \mu\text{L}$$

The % units cancel since they are in both the numerator and the denominator of the equation, leaving µL as the remaining unit. Therefore, you would need 500 µL of 20% sucrose plus 1500 µL (2000 µL – 500 µL = 1500 µL) of water to make a 5% sucrose solution from a 20% sucrose solution.

Dimensional analysis is another general approach to solving problems of concentration. In this method, an equation is set up such that the known concentration of the stock and all volume relationships appear on the left side of the equation and the final desired concentration is placed on the right side. Conversion factors are actually part of the equation. Terms are set up as numerator or denominator values such that all terms cancel except for that describing concentration. A dimensional analysis equation is set up in the following manner.

$$\text{starting concentration} \times \text{conversion factor} \times \frac{\text{unknown volume}}{\text{final volume}} = \text{desired concentration}$$

Using the dimensional analysis approach, the problem of discovering how many microliters of 20% sucrose are needed to make 2 mL of 5% sucrose is written as follows:

$$20\% \times \frac{1 \text{ mL}}{1000 \ \mu\text{L}} \times \frac{x \ \mu\text{L}}{2 \text{ mL}} = 5\%$$

Notice that all terms on the left side of the equation will cancel except for %. Solving for x µL gives the following result:

$$\frac{(20\%)x}{2000} = 5\%$$

$$x = \frac{(5\%)(2000)}{20\%} = 500$$

Since x is a µL amount, you need 500 µL of 20% sucrose in a final volume of 2 mL to make 5% sucrose. Notice how similar the last step of the solution to this equation is to the last step of the equation using the $C_1V_1 = C_2V_2$ approach.

Making a conversion factor part of the equation obviates the need for performing two separate calculations, as is required when using the $C_1V_1 = C_2V_2$ approach. For this reason, dimensional analysis is the method used for solving problems of concentration throughout this book.

Concentrations by a Factor of X

The concentration of a solution can be expressed as a multiple of its standard working concentration. For example, many buffers used for agarose or acrylamide gel electrophoresis are prepared as solutions 10-fold (10X) more concentrated than their standard running concentration (1X). In a 10X buffer, each component of that buffer is 10-fold more concentrated than in the 1X solution. To prepare a 1X working buffer, a dilution of the more concentrated 10X stock is performed in water to achieve the desired volume. To prepare 1000 mL (1 L) of 1X Tris-borate-EDTA (TBE) gel running buffer from a 10X TBE concentrate, for example, add 100 mL of 10X solution to 900 mL of distilled water. This can be calculated as follows:

$$10\text{X Buffer} \times \frac{n\ \text{mL}}{1000\ \text{mL}} = 1\text{X Buffer}$$

n mL of 10X buffer is diluted into a total volume of 1000 mL to give a final concentration of 1X. Solve for n.

$$\frac{10\text{X}n}{1000} = 1\text{X}$$

Multiply numerator values.

$$(1000) \times \frac{10\text{X}n}{1000} = 1\text{X}\ (1000)$$

Use the Multiplication Property of Equality (see the following box) to multiply each side of the equation by 1000. This cancels out the 1000 in the denominator on the left side of the equals sign.

$$10Xn = 1000X$$

$$\frac{10Xn}{10X} = \frac{1000X}{10X}$$

Divide each side of the equation by 10X. (Again, this uses the Multiplication Property of Equality.)

$$n = 100$$

The X terms cancel since they appear in both the numerator and the denominator. This leaves n equal to 100.

Therefore, to make1000 mL of 1X buffer, add 100 mL of 10X buffer stock to 900 mL of distilled water (1000 mL – 100 mL contributed by the 10X buffer stock = 900 mL).

Multiplication Property of Equality

Both sides of an equation may be multiplied by the same nonzero quantity to produce equivalent equations. This property also applies to division: both sides of an equation can be divided by the same nonzero quantity to produce equivalent equations.

Problem 2.1 How are 640 mL of 0.5X buffer prepared from an 8X stock?

Solution 2.1 We start with a stock of 8X buffer. We want to know how many milliliters of the 8X buffer should be in a final volume of 640 mL to give us a buffer having a concentration of 0.5X. This relationship can be expressed mathematically as follows:

$$8\text{X buffer} \times \frac{n \text{ mL}}{640 \text{ mL}} = 0.5\text{X buffer}$$

Solve for n.

$$\frac{8Xn}{640} = 0.5X$$

Multiply numerator values on the left side of the equation. Since the mL terms appear in both the numerator and the denominator, they cancel out.

$8Xn = 320X$	Multiply each side of the equation by 640.
$n = \frac{320X}{8X} = 40$	Divide each side of the equation by 8X. The X terms, since they appear in both the numerator and the denominator, cancel.

Therefore, add 40 mL of 8X stock to 600 mL of distilled water to prepare a total of 640 mL of 0.5X buffer (640 mL final volume – 40 mL 8X stock = 600 mL volume to be taken by water).

Preparing Percent Solutions

Many reagents are prepared as a percent of solute (such as salt, cesium chloride, or sodium hydroxide) dissolved in solution. Percent, by definition, means "per 100." 12%, therefore, means 12 per 100, or 12 out of every 100. 12% may also be written as the decimal 0.12 (derived from the fraction 12/100 = 0.12).

Depending on the solute's initial physical state, its concentration can be expressed as a weight per volume percent (% w/v) or a volume per volume percent (% v/v). A percentage in weight per volume refers to the weight of solute (in grams) in a total of 100 mL of solution. A percentage in volume per volume refers to the amount of liquid solute (in mL) in a final volume of 100 mL of solution.

Most microbiology laboratories will stock a solution of 20% (w/v) glucose for use as a carbon source in bacterial growth media. To prepare 100 mL of 20% (w/v) glucose, 20 grams of glucose are dissolved in enough distilled water so that the final volume of the solution, with the glucose completely dissolved, is 100 mL.

Problem 2.2 How can the following solutions be prepared?

a) 100 mL of 40% (w/v) polyethylene glycol (PEG) 8000
b) 47 mL of a 7% (w/v) solution of sodium chloride
c) 200 mL of a 95% (v/v) solution of ethanol

Solution 2.2

a) Weigh out 40 grams of PEG 8000 and dissolve in distilled water so that the final volume of the solution, with the PEG 8000 completely dissolved, is 100 mL. This is most conveniently done by initially dissolving the PEG 8000 in approximately 60 mL of distilled water. When the granules are dissolved, pour the solution into a 100-mL graduated cylinder and bring the volume up to the 100-mL mark with distilled water.

b) First, 7% of 47 must be calculated. This is done by multiplying 47 by 0.07 (the decimal form of 7%; $\frac{7}{100} = 0.07$):

$$0.07 \times 47 = 3.29$$

Therefore, to prepare 47 mL of 7% sodium chloride, weigh out 3.29 grams of sodium chloride and dissolve the crystals in some volume of distilled water less than 47 mL, a volume measured so that, when the 3.29 grams of NaCl are added, it does not exceed 47 mL. When the sodium chloride is completely dissolved, dispense the solution into a 50-mL graduated cylinder and bring the final volume up to 47 mL with distilled water.

c) 95% of 200 mL is calculated by multiplying 0.95 (the decimal form of 95%) by 200:

$$0.95 \times 200 = 190$$

Therefore, to prepare 200 mL of 95% ethanol, measure 190 mL of 100% (200 proof) ethanol and add 10 mL of distilled water to bring the final volume to 200 mL.

Diluting Percent Solutions

When approaching a dilution problem involving percentages, express the percent solutions as fractions of 100. The problem can be written as an equation in which the concentration of the stock solution ("what you have") is positioned on the left side of the equation and the desired final concentration ("what you want") is on the right side of the equation. The unknown volume (x) of the stock solution to add to the volume of the final mixture should also be expressed as a fraction (with x as a numerator and the final desired volume as a denominator). This part of the equation should also be positioned on the left side of the equals sign. For example, if 30 mL of 70% ethanol is to be prepared from a 95% ethanol stock solution, the following equation can be written:

$$\frac{95}{100} \times \frac{x \text{ mL}}{30 \text{ mL}} = \frac{70}{100}$$

You then solve for x.

$$\frac{95x}{3000} = \frac{70}{100}$$

Multiply numerators together and multiply denominators together. The mL terms, since they are present in both the numerator and the denominator, cancel.

$$\frac{3000}{1} \times \frac{95x}{3000} = \frac{70}{100} \times \frac{3000}{1}$$

Multiply both sides of the equation by 3000.

$$95x = \frac{210,000}{100}$$

Simplify the equation.

$$95x = 2100$$

$$\frac{95x}{95} = \frac{2100}{95}$$

Divide each side of the equation by 95.

$$x = 22$$

Round off to two significant figures.

Therefore, to prepare 30 mL of 70% ethanol using a 95% ethanol stock solution, combine 22 mL of 95% ethanol stock with 8 mL of distilled water.

Problem 2.3 If 25 grams of NaCl are dissolved into a final volume of 500 mL, what is the percent (w/v) concentration of NaCl in the solution?

Solution 2.3 The concentration of NaCl is 25 g/500 mL (w/v). To determine the % (w/v) of the solution, we need to know how many grams of NaCl are in 100 mL. We can set up an equation of two ratios in which x represents the unknown number of grams. This relationship is read "x grams is to 100 mL as 25 grams is to 500 mL":

$$\frac{x \text{ g}}{100 \text{ mL}} = \frac{25 \text{ g}}{500 \text{ mL}}$$

Solving for x gives the following result:

$$x \text{ g} = \frac{(25 \text{ g})(100 \text{ mL})}{500 \text{ mL}} = \frac{2500 \text{ g}}{500} = 5 \text{ g}$$

Therefore, there are 5 grams of NaCl in 100 mL (5 g/100 mL), which is equivalent to a 5% solution.

Problem 2.4 If 8 mL of distilled water are added to 2 mL of 95% ethanol, what is the concentration of the diluted ethanol solution?

Solution 2.4 The total volume of the solution is 8 mL + 2 mL = 10 mL. This volume should appear as a denominator on the left side of the equation. This dilution is the same as if 2 mL of 95% ethanol were added to 8 mL of water. Either way, it is a quantity of the 95% ethanol stock that is used to make the dilution. The "2 mL," therefore, should appear as the numerator in the volume expression on the left side of the equation:

$$\frac{95}{100} \times \frac{2 \text{ mL}}{10 \text{ mL}} = \frac{x}{100}$$

$$\frac{190}{1000} = \frac{x}{100}$$

The mL terms cancel. Multiply numerator values and denominator values on the left side of the equation.

$$0.19 = \frac{x}{100}$$

Simplify the equation.

$$19 = x$$

Multiply both sides of the equation by 100.

If x in the original equation is replaced by19, it is seen that the new concentration of ethanol in this diluted sample is 19/100, or 19%.

Problem 2.5 How many microliters of 20% SDS are required to bring 1.5 mL of solution to 0.5%?

Solution 2.5 In previous examples, there was control over how much water we could add in preparing the dilution to bring the sample to the desired concentration. In this example, however, a fixed volume (1.5 mL) is used as a starting sample and must be brought to the desired concentration. Solving this problem will require the use of the Addition Property of Equality (see the following box).

Addition Property of Equality

You may add (or subtract) the same quantity to (from) both sides of an equation to produce equivalent equations. For any real numbers a, b, and c, if $a = b$, then $a + c = b + c$, and $a - c = b - c$.

Since concentration, by definition, is the amount of a particular component in a specified volume, by adding a quantity of a stock solution to a fixed volume, the final volume is changed by that amount and the concentration is changed accordingly. The amount of stock solution (x mL) added in the process of the dilution must also be figured into the final volume, as follows.

$$\frac{20}{100} \times \frac{x \text{ mL}}{1.5 \text{ mL} + x \text{ mL}} = \frac{0.5}{100}$$

$$\frac{20x}{150 + 100x} = \frac{0.5}{100}$$

Multiply numerators and denominators. The mL terms cancel out.

$$\frac{150 + 100x}{1} \times \frac{20x}{150 + 100x} = \frac{0.5}{100} \times \frac{150 + 100x}{1}$$

Multiply both sides of the equation by 150 + 100x.

$$20x = \frac{75 + 50x}{100}$$

Simplify the equation.

$$\frac{100}{1} \times 20x = \frac{75 + 50x}{100} \times \frac{100}{1}$$

Multiply both sides of the equation by 100.

$$2000x = 75 + 50x$$

Simplify.

$$1950x = 75$$

Subtract 50x from both sides of the equation (Addition Property of Equality).

$$x = \frac{75}{1950} = 0.03846 \text{ mL}$$

Divide both sides of the equation by 1950.

$$0.03846 \text{ mL} \times \frac{1000 \ \mu\text{L}}{\text{mL}} = 38.5 \ \mu\text{L}$$

Convert mL to µL and round off to one significant figure to the right of the decimal point.

Therefore, if 38.5 µL of 20% SDS are added to 1.5 mL, the SDS concentration of that sample will be 0.5% in a final volume of 1.5385 mL. If there were some other component in that initial 1.5 mL, the concentration of that component would change by the addition of the SDS. For example, if NaCl were present at a concentration of 0.2%, its concentration would be altered by the addition of more liquid. The initial solution of 1.5 mL would contain the following amount of sodium chloride:

$$\frac{0.2\ \text{g}}{100\ \text{mL}} \times 1.5\ \text{mL} = 0.003\ \text{g}$$

Therefore, 1.5 mL of 0.2% NaCl contains 0.003 grams of NaCl.

In a volume of 1.5385 mL (the volume after the SDS solution has been added), 0.003 grams of NaCl is equivalent to a 0.195% NaCl solution, as shown here:

$$\frac{0.003}{1.5385} \times 100 = 0.195\%$$

Moles and Molecular Weight: Definitions

A **mole** is equivalent to 6.023×10^{23} molecules. That molecule may be a pure elemental atom or a molecule consisting of a bound collection of atoms. For example, a mole of hydrogen is equivalent to 6.023×10^{23} molecules of hydrogen. A mole of glucose ($C_6H_{12}O_6$) is equivalent to 6.023×10^{23} molecules of glucose. The value 6.023×10^{23} is also known as **Avogadro's number**.

The **molecular weight** (**MW**, or **gram molecular weight**) of a substance is equivalent to the sum of its atomic weights. For example, the gram molecular weight of sodium chloride (NaCl) is 58.44, the atomic weight of Na (22.99 g) plus the atomic weight of chlorine (35.45 g). Atomic weights can be found in the periodic table of the elements. The molecular weight of a compound, as obtained commercially, is usually provided by the manufacturer and is printed on the container's label. On many reagent labels, a **formula weight (FW)** is given. For almost all applications in molecular biology, this value is used interchangeably with molecular weight.

Problem 2.6 What is the molecular weight of sodium hydroxide (NaOH)?

Solution 2.6

Atomic weight of Na	22.99
Atomic weight of O	16.00
Atomic weight of H	+ 1.01
Molecular weight of NaOH	40.00

Problem 2.7 What is the molecular weight of glucose ($C_6H_{12}O_6$)?

Solution 2.7 The atomic weight of each element in this compound must be multiplied by the number of times it is represented in the molecule:

Atomic weight of C = 12.01		
$12.01 \times 6 =$		72.06
Atomic weight of H = 1.01		
$1.01 \times 12 =$		12.12
Atomic weight of O = 16.00		
$16 \times 6 =$	+	96.00
Molecular weight of $C_6H_{12}O_6$		180.18

Therefore, the molecular weight of glucose is 180.18.

Molarity

A 1 molar (1 *M*) solution contains the molecular weight of a substance (in grams) in 1 liter of solution. For example, the molecular weight of NaCl is 58.44. A 1 *M* solution of sodium chloride (NaCl), therefore, contains 58.44 grams of NaCl dissolved in a final volume of 1000 mL (1 L) water. A 2 *M* solution of NaCl contains twice that amount (116.88 grams) of NaCl dissolved in a final volume of 1000 mL water.

Problem 2.8 How are 200 mL of 0.3 *M* NaCl prepared?

Solution 2.8 The molecular weight of NaCl is 58.44. The first step in solving this problem is to calculate how many grams are needed for 1 L of a 0.3 *M* solution. This can be done by setting up a ratio stating, "58.44 grams is to 1 *M* as *x* grams is to 0.3 *M*." This relationship, expressed mathematically, can be written as follows. We then solve for *x*.

$$\frac{58.44 \text{ g}}{1\ M} = \frac{x \text{ g}}{0.3\ M}$$

Because units on both sides of the equation are equivalent (if we were to multiply one side by the other, all terms would cancel), we will disregard them. Multiplying both sides of the equation by 0.3 gives

$$\frac{0.3}{1} \times \frac{58.44}{1} = \frac{x}{0.3} \times \frac{0.3}{1}$$

$$17.53 = x$$

Therefore, to prepare 1 L of 0.3 *M* NaCl, 17.53 grams of NaCl are required.

Another ratio can now be written to calculate how many grams of NaCl are needed if 200 mL of a 0.3 *M* NaCl solution are being prepared. It can be expressed verbally as "17.53 grams is to1000 mL as *x* grams is to 200 mL," or written in mathematical terms:

$$\frac{17.53 \text{ g}}{1000 \text{ mL}} = \frac{x \text{ g}}{200 \text{ mL}}$$

$$\frac{17.53 \times 200}{1000} = x$$

Multiply both sides of the equation by 200 to cancel out the denominator on the right side of the equation and to isolate *x*.

$$\frac{3506}{1000} = x = 3.51$$

Simplify the equation.

Therefore, to prepare 200 mL of 0.3 *M* sodium chloride solution, 3.51 grams of NaCl are dissolved in distilled water to a final volume of 200 mL.

Problem 2.9 How are 50 mL of 20 millimolar (m*M*) sodium hydroxide (NaOH) prepared?

Solution 2.9 First, because it is somewhat more convenient to deal with terms expressed as molarity (*M*), convert the 20 m*M* value to an *M* value:

$$20 \text{ m}M \times \frac{1 \ M}{1000 \text{ m}M} = 0.02 \ M$$

Next, set up a ratio to calculate the amount of NaOH (40.0 gram molecular weight) needed to prepare 1 L of 0.02 *M* NaOH . Use the expression "40.0 grams is to 1 *M* as *x* grams is to 0.02 *M*." Solve for *x*.

$$\frac{40.0 \text{ g}}{1 \ M} = \frac{x \text{ g}}{0.02 \ M}$$

$$\frac{(40.0)(0.02)}{1} = x$$

Multiply both sides of the equation by 0.02.

$$0.8 = x$$

Therefore, if 1 L of 0.02 *M* NaOH is to be prepared, add 0.8 grams of NaOH to water to a final volume of 1000 mL.

Now, set up a ratio to determine how much is required to prepare 50 mL and solve for *x*. (The relationship of ratios should read as follows: 0.8 grams is to 1000 mL as *x* grams is to 50 mL.)

$$\frac{0.8 \text{ g}}{1000 \text{ mL}} = \frac{x \text{ g}}{50 \text{ mL}}$$

$$\frac{(0.8)(50)}{1000} = \frac{(x)(50)}{50}$$

Multiply both sides of the equation by 50. This cancels the 50 in the denominator on the right side of the equation.

$$\frac{40.0}{1000} = x = 0.04$$

Simplify the equation.

Therefore, to prepare 50 mL of 20 m*M* NaOH, 0.04 grams of NaOH is dissolved in a final volume of 50 mL of distilled water.

Problem 2.10 How many moles of NaCl are present in 50 mL of a 0.15 *M* solution?

Solution 2.10 A 0.15 *M* solution of NaCl contains 0.15 moles of NaCl per liter. Since we want to know how many moles are in 50 mL, we need to use a conversion factor to convert liters to milliliters. The equation can be written as follows:

$$x \text{ mol} = 50 \text{ mL} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times \frac{0.15 \text{ mol}}{\text{L}}$$

Notice that terms on the right side of the equation cancel except for moles. Multiplying numerator and denominator values gives

$$x \text{ mol} = \frac{(50) \times (1) \times (0.15) \text{ mol}}{1000} = \frac{7.5 \text{ mol}}{1000} = 0.0075 \text{ mol}$$

Therefore, 50 mL of 0.15 *M* NaCl contains 0.0075 moles of NaCl.

Diluting Molar Solutions

Diluting stock solutions prepared in molar concentration into volumes of lesser molarity is performed as previously described (see page 18).

Problem 2.11 From a 1 *M* Tris solution, how is a 400 mL of 0.2 *M* Tris prepared?

Solution 2.11 The following equation is used to solve for *x*, the amount of 1 *M* Tris added to 400 mL to yield a 0.2 *M* solution.

$$1\ M \times \frac{x\ \text{mL}}{400\ \text{mL}} = 0.2\ M$$

$$\frac{1x\ M}{400} = 0.2\ M$$

The mL units cancel since they appear in both the numerator and the denominator on the left side of the equation. Multiply numerator values.

$$x = (0.2)(400)$$

Multiply both sides of the equation by 400.

$$x = 80$$

Therefore, to 320 mL (400 mL – 80 mL = 320 mL) of distilled water, add 80 mL of 1 *M* Tris, pH 8.0, to bring the solution to 0.2 *M* and a final volume of 400 mL.

Problem 2.12 How are 4 mL of 50 m*M* NaCl solution prepared from a 2 *M* NaCl stock?

Solution 2.12 In this example, a conversion factor must by included in the equation so that molarity (*M*) can be converted to millimolarity.

$$2\ M \times \frac{1000\ \text{m}M}{M} \times \frac{x\ \text{mL}}{4\ \text{mL}} = 50\ \text{m}M$$

$$\frac{2000x\ \text{m}M}{4} = 50\ \text{m}M$$

Multiply numerators. (On the left side of the equation, the *M* and mL units both cancel since these terms appear in both
the numerator and the denominator.)

$$2000x\ \text{mM} = 200\ \text{m}M$$

Multiply both sides of the equation by 4.

$$x = \frac{200\ \text{m}M}{2000\ \text{m}M} = 0.1$$ Divide each side of the equation by 2000 m*M*.

Therefore, to 3.9 mL of distilled water, add 0.1 mL of 2 *M* NaCl stock solution to produce 4 mL final volume of 50 m*M* NaCl.

Converting Molarity to Percent

Since molarity is a concentration of grams per 1000 mL, it is a simple matter to convert it to a percent value, an expression of a gram amount in 100 mL. The method is demonstrated in the following problem.

Problem 2.13 Express 2.5 *M* NaCl as a percent solution.

Solution 2.13 The gram molecular weight of NaCl is 58.44. The first step in solving this problem is to determine how many grams of NaCl are in a 2.5 *M* NaCl solution. This can be accomplished by using an equation of ratios: "58.44 grams is to 1 *M* as *x* grams is to 2.5 *M*." This relationship is expressed mathematically as follows:

$$\frac{58.44\ \text{g}}{1\ M} = \frac{x\ \text{g}}{2.5\ M}$$

Solve for *x*.

$$\frac{(58.44)(2.5)}{1} = x$$ Multiply both sides of the equation by 2.5.

$$146.1 = x$$ Simplify the equation.

Therefore, to prepare a 2.5 *M* solution of NaCl, 146.1 grams of NaCl are dissolved in a total volume of 1 liter.

Percent is an expression of concentration in parts per 100. To determine the relationship between the number of grams of NaCl present in a 2.5 *M* NaCl solution and the equivalent percent concentration, ratios can be set up that state, "146.1 g is to 1000 mL as *x* g is to 100 mL."

$$\frac{146.1\ \text{g}}{1000\ \text{mL}} = \frac{x\ \text{g}}{100\ \text{mL}}$$

Solve for *x*.

$$\frac{(146.1)(100)}{1000} = x$$

Multiply both sides of the equation by 100 (the denominator on the right side of the equation).

$$\frac{14610}{1000} = x = 14.6$$

Simplify the equation.

Therefore, a 2.5 *M* NaCl solution contains 14.6 grams of NaCl in 100 mL, which is equivalent to a 14.6% NaCl solution.

Converting Percent to Molarity

Converting a solution expressed as percent to one expressed as a molar concentration is a matter of changing an amount per 100 mL to an equivalent amount per liter (1000 mL) as demonstrated in the following problem.

Problem 2.14 What is the molar concentration of a 10% NaCl solution?

Solution 2.14 A 10% solution of NaCl, by definition, contains 10 grams of NaCl in 100 mL of solution. The first step to solving this problem is to calculate the amount of NaCl in 1000 mL of a 10% solution. This is accomplished by setting up a relationship of ratios as follows:

$$\frac{10 \text{ g}}{100 \text{ mL}} = \frac{x \text{ g}}{1000 \text{ mL}}$$

Solve for x.

$$\frac{(10)(1000)}{100} = x$$

Multiply both sides of the equation by 1000 (the denominator on the right side of the equation).

$$\frac{10,000}{100} = x = 100$$

Simplify the equation.

Therefore, a 1000-mL solution of 10% NaCl contains 100 grams of NaCl.

Using the gram molecular weight of NaCl (58.44), an equation of ratios can be written to determine molarity. In the following equation, we determine the molarity (*M*) equivalent to 100 grams:

$$\frac{x\ M}{100\ \text{g}} = \frac{1\ M}{58.44\ \text{g}}$$

Solve for x.

$$x = \frac{100}{58.44} = 1.71$$

Multiply both sides of the equation by 100 and divide by 58.44.

Therefore, a 10% NaCl solution is equivalent to 1.71 M NaCl.

Normality

A 1 normal (1 N) solution is equivalent to the gram molecular weight of a compound divided by the number of hydrogen ions present in solution (i.e., dissolved in one liter of water). For example, the gram molecular weight of hydrochloric acid (HCl) is 36.46. Since, in a solution of HCl, one H^+ ion can combine with Cl^- to form HCl, a 1 N HCl solution contains 36.46/1 = 36.46 grams HCl in 1 liter. A 1 N HCl solution, therefore, is equivalent to a 1 M HCl solution. As another example, the gram molecular weight of sulfuric acid (H_2SO_4) is 98.0. Since, in a H_2SO_4 solution, two H^+ ions can combine with SO_4^{2-} to form H_2SO_4, a 1 N H_2SO_4 solution contains 98.0/2 = 49.0 grams of H_2SO_4 in 1 liter. Since half the gram molecular weight of H_2SO_4 is used to prepare a 1 N H_2SO_4 solution, a 1 N H_2SO_4 solution is equivalent to a 0.5 M H_2SO_4 solution.

Normality and molarity are related by the equation

$$N = nM$$

where n is equal to the number of replaceable H^+ (or Na^+) or OH^- ions per molecule.

Problem 2.15 What is the molarity of a 1 N sodium carbonate (Na_2CO_3) solution?

Solution 2.15 Sodium carbonate has two replaceable Na^+ ions. The relationship between normality and molarity is

$$N = nM$$

Solving for M gives the following result.

$\frac{N}{n} = M$	Divide both sides of the equation by n (the number of replaceable Na^+ ions).
$\frac{1}{2} = M$	In this problem, the number of replaceable + ions, n, is 2. The normality, N, is 1.
$0.5 = M$	

Therefore, a 1 N sodium carbonate solution is equivalent to a 0.5 M sodium carbonate solution.

pH

The first chemical formula most of us learn, usually during childhood, is that for water, H_2O. A water molecule is composed of two atoms of hydrogen and one atom of oxygen. The atoms of water, however, are only transiently associated in this form. At any particular moment, a certain number of water molecules will be dissociated into hydrogen (H^+) and hydroxyl (OH^-) ions These ions will reassociate within a very short time to form the H_2O water molecule again. The dissociation and reassociation of the atoms of water can be depicted by the following relationship:

$$H_2O \rightleftharpoons H^+ + OH^-$$

In actuality, the hydrogen ion is donated to another molecule of water to form a hydronium ion (H_3O^+). A more accurate representation of the dissociation of water, therefore, is as follows:

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$$

For most calculations in chemistry, however, it is simpler and more convenient to think of the H^+ hydrogen ion as being a dissociation product of H_2O rather than the H_3O^+ hydronium ion.

A measure of the hydrogen ion (H^+) concentration in a solution is given by a **pH** value. A solution's pH is defined as the negative logarithm to the base 10 of its hydrogen ion concentration:

$$pH = -\log[H^+]$$

In this nomenclature, the brackets signify concentration. A **logarithm (log)** is an exponent, a number written above, smaller, and to the right of another number, called the **base**, to which the base should be raised. For example, for 10^2, the 2 is the exponent and the 10 is the base. In 10^2, the base, 10, should be raised to the second power, which means that 10 should be multiplied by itself a total of two times. This will give a value of 100, as shown here:

$$10^2 = 10 \times 10 = 100$$

The log of 100 is 2 because that is the exponent of 10 that yields 100. The log of 1000 is 3 since $10^3 = 1000$:

$$10^3 = 10 \times 10 \times 10 = 1000$$

The log of a number can be found on most calculators by entering the number and then pressing the **log** key.

In pure water, the H^+ concentration ($[H^+]$) is equal to 10^{-7} *M*. In other words, in 1 L of water, 0.0000001 moles of hydrogen ion will be present. The pH of water, therefore, is calculated as follows:

$$pH = -\log(10^{-7}) = -(-7) = 7$$

Pure water, therefore, has a pH of 7.0.

pH values range from 0 to 14. Solutions having pH values less than 7 are acidic. Solutions having pH values greater than 7 are alkaline, or basic. Water, with a pH of 7, is considered a neutral solution; it is neither acidic nor basic.

When an acid, such as hydrochloric acid (HCl), is added to pure water, the hydrogen ion concentration increases above 10^{-7} *M*. When a base, such as sodium hydroxide (NaOH), is added to pure water, the OH^- ion is dissociated from the base. This hydroxyl ion can associate with the H^+ ions already in the water to form H_2O molecules, reducing the solution's hydrogen ion concentration and increasing the solution's pH.

Problem 2.16 The concentration of hydrogen ion in a solution is 10^{-5} *M*. What is the solution's pH?

Solution 2.16 pH is the negative logarithm of 10^{-5}.

$$pH = -\log[H^+] = -\log(10^{-5}) = -(-5) = 5$$

Therefore, the pH of the solution is 5. It is acidic.

Problem 2.17 The concentration of hydrogen ion in a solution is 2.5×10^{-4} M. What is the solution's pH?

Solution 2.17 The **product rule for logarithms** states that for any positive numbers M, N, and a (where a is not equal to 1), the logarithm of a product is the sum of the logarithms of the factors:

$$\log_a MN = \log_a M + \log_a N$$

Since we are working in base 10, a is 10.

The product rule of logarithms will be used to solve this problem.

$$\begin{aligned} \text{pH} &= -\ \log\left(2.5 \times 10^{-8}\right) \\ &= -\ \left(\log 2.5 + \log 10^{-5}\right) \\ &= -[0.40 + (-5)] \\ &= -(0.40 - 5) = -(-4.6) = 4.6 \end{aligned}$$

Therefore, the solution has a pH of 4.6.

Note: *In Problem 2.16, the hydrogen ion concentration was stated to be 10^{-5}. This value can also be written as 1×10^{-5}. The log of 1 is 0. If the product rule for logarithms is used to calculate the pH for this problem, it would be equal to $-[0 + (-5)]$, which is equal to 5.*

Problem 2.18 The pH of a solution is 3.75. What is the concentration of hydrogen ion in the solution?

Solution 2.18 To calculate the hydrogen ion concentration in this problem will require that we determine the **antilog** of the pH. An antilog is found by doing the reverse process of that used to find a logarithm. The log of 100 is 2. The antilog of 2 is 100. The log of 1000 is 3. The antilog of 3 is 1000. For those calculators that do not have an antilog key, this can usually be obtained by entering the value, pressing the **10^x^** key, and then pressing the = sign. (Depending on the type of calculator you are using, you may need to press the **SHIFT** key to gain access to the **10^x^** function.)

$\text{pH} = -\log[\text{H}^+]$	Equation for calculating pH.
$-\log[\text{H}^+] = 3.75$	The pH is equal to 3.75.
$\log[\text{H}^+] = -3.75$	Multiply each side of the equation by –1.

$[H^+] = 1.8 \times 10^{-4}$

Take the antilog of each side of the equation.

***Note**: Taking the antilog of the log of a number, since they are opposite and canceling operations, is equivalent to doing nothing to that number. For example, the antilog of the log of 100 is 100.*

Therefore, the hydrogen ion concentration is 1.8×10^{-4} *M*.

Since water, H_2O, dissociates into both H^+ and OH^- ions, the H^+ concentration must equal the OH^- concentration. Just as water has a pH, so does it have a **pOH**, which is defined as the negative logarithm of the OH^- (hydroxyl ion) concentration.

$$pOH = -\log[OH]$$

and

$$pOH = 14 - pH$$

Problem 2.19 A solution has a pH of 4.5. What is the solution's pOH?

Solution 2.19 The pOH is obtained by subtracting the pH from 14.

$$pOH = 14 - pH$$

$$pOH = 14 - 4.5 = 9.5$$

Therefore, the pOH of the solution is 9.5.

Problem 2.20 What is the pH of a 0.02 *M* solution of sodium hydroxide (NaOH).

Solution 2.20 Sodium hydroxide is a strong base and, as such, is essentially ionized completely to Na^+ and OH^- in dilute solution. The OH^- concentration, therefore, is 0.02 *M*, the same as the concentration of NaOH. For a strong base, the H^+ ion contribution from water is negligible and so will be ignored. The first step to solving this problem is to determine the pOH. The pOH value will then be subtracted from 14 to obtain the pH.

$$\begin{aligned} \text{pOH} &= -\log(0.02) \\ &= -(-1.7) = 1.7 \\ \text{pH} &= 14 - 1.7 = 12.3 \end{aligned}$$

Therefore, the pH of the 0.02 *M* NaOH solution is 12.3.

pK_a and the Henderson–Hasselbalch Equation

In the Bronsted concept of acids and bases, an **acid** is defined as a substance that donates a proton (a hydrogen ion). A **base** is a substance that accepts a proton. When a Bronsted acid loses a hydrogen ion, it becomes a Bronsted base. The original acid is called a **conjugate acid**. The base created from the acid by loss of a hydrogen ion is called a **conjugate base**.

Dissociation of an acid in water follows the general formula

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

Where HA is a conjugate acid, H_2O is a conjugate base, H_3O^+ is a conjugate acid, and A^- is a conjugate base.

The acid's ionization can be written as a simple dissociation, as follows:

$$HA \rightleftharpoons H^+ + A^-$$

The dissociation of the HA acid will occur at a certain rate characteristic of the particular acid. Notice, however, that the arrows go in both directions. The acid dissociates into its component ions, but the ions come back together again to form the original acid. When the rate of dissociation into ions is equal to the rate of ion reassociation, the system is said to be in **equilibrium**. A strong acid will reach equilibrium at the point where it is completely dissociated. A weak acid will have a lower percentage of molecules in a dissociated state and will reach equilibrium at a point less than 100% ionization. The concentration of acid at which equilibrium occurs is called the **acid dissociation constant**, designated by the symbol $\boldsymbol{K_a}$. It is represented by the following equation:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

A measure of K_a for a weak acid is given by its pK_a, which is equivalent to the negative logarithm of K_a:

$$pK_a = -\log K_a$$

pH is related to pK_a by the **Henderson–Hasselbalch equation**:

$$pH = pK_a + \log\frac{[\text{conjugate base}]}{[\text{acid}]}$$

$$= pK_a + \log\frac{[A^-]}{[HA]}$$

The Henderson–Hasselbalch equation can be used to calculate the amount of acid and conjugate base to be combined for the preparation of a buffer solution having a particular pH, as demonstrated in the following problem.

Problem 2.21 You wish to prepare 2 liters of 1 *M* sodium phosphate buffer, pH 8.0. You have stocks of 1 *M* monobasic sodium phosphate (NaH_2PO_4) and 1 *M* dibasic sodium phosphate (Na_2HPO_4). How much of each stock solution should be combined to make the desired buffer?

Solution 2.21 Monobasic sodium phosphate (NaH_2PO_4) in water exists as Na^+ and $H_2PO_4^-$ ions. $H_2PO_4^-$ (phosphoric acid, the conjugate acid) dissociates further to HPO_4^{2-} (the conjugate base) + H^+ and has a pK_a of 6.82 at 25°C. [pK_a values can be found in the Sigma chemical catalogue (Sigma, St. Louis, MO) or in *The CRC Handbook of Chemistry and Physics*.] The pH and pK_a values will be inserted into the Henderson–Hasselbalch equation to derive a ratio of the conjugate base and acid to combine to give a pH of 8.0. Note that the stock solutions are both at a concentration of 1 *M*. No matter in what ratio the two solutions are combined, there will always be 1 mole of phosphate molecules per liter.

$$\text{pH} = \text{p}K_a + \log\frac{[\text{A}^-]}{[\text{HA}]}$$

Insert pH and $\text{p}K_a$ values into Henderson–Hasselbalch equation.

$$8.0 = 6.82 + \log\frac{[\text{HPO}_4^{\ 2-}]}{[\text{H}_2\text{PO}_4^{\ -}]}$$

$$1.18 = \log\frac{[\text{HPO}_4^{\ 2-}]}{[\text{H}_2\text{PO}_4^{\ -}]}$$

Subtract 6.82 from both sides of the equation.

$$\text{antilog } 1.18 = \frac{[\text{HPO}_4^{\ 2-}]}{[\text{H}_2\text{PO}_4^{\ -}]} = 15.14$$

Take the antilog of each side of the equation.

Therefore, the ratio of $HPO_4^{\ 2-}$ to $H_2PO_4^{\ -}$ is equal to 15.14. To make 1 *M* sodium phosphate buffer, 15.14 parts Na_2HPO_4 should be combined with 1 part NaH_2PO_4. 15.14 parts Na_2HPO_4 plus 1 part NaH_2PO_4 is equal to a total of 16.14 parts. The amounts of each stock to combine to make 2 liters of the desired buffer is then calculated as follows:

For Na_2HPO_4, the amount is equal to

$$\frac{15.14}{16.14} \times 2\ \text{L} = 1.876\ \text{L}$$

For NaH_2PO_4, the amount is equal to

$$\frac{1}{16.14} \times 2\ \text{L} = 0.124\ \text{L}$$

When these two volumes are combined, you will have 1 *M* sodium phosphate buffer having a pH of 8.0.