

PREPARING SOLUTIONS FOR BIOLOGY EVENTS

Solute - The substance which dissolves in a solution

Solvent - The substance which dissolves another to form a solution. For example, in a sugar and water solution, water is the solvent; sugar is the solute.

Solution - A mixture of two or more pure substances. In a solution one pure substance is dissolved in another pure substance homogeneously. For example, in a sugar and water solution, the solution has the same concentration throughout, i.e. it is homogeneous.

Mole - A fundamental unit of mass (like a "dozen" to a baker) used by chemists. This term refers to a large number of elementary particles (atoms, molecules, ions, electrons, etc) of any substance. 1 mole is 6.02×10^{23} molecules of that substance. (Avogadro's number).M

1. Simple Dilution (Dilution Factor Method based on ratios)

A *simple dilution* is one in which a *unit volume* of a liquid material of interest is combined with an appropriate volume of a *solvent* liquid to achieve the desired concentration. The *dilution factor* is the total number of unit volumes in which your material will be dissolved. The diluted material must then be thoroughly mixed to achieve the true dilution. For example, a 1:5 dilution (verbalize as "1 to 5" dilution) entails combining 1 unit volume of *solute* (the material to be diluted) + 4 unit volumes of the *solvent* medium (hence, $1 + 4 = 5 =$ dilution factor).

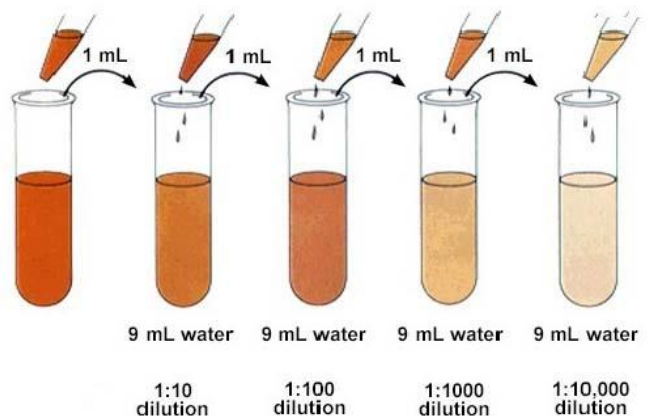
Example 1: Frozen orange juice concentrate is usually diluted with 4 additional cans of cold water (the dilution solvent) giving a dilution factor of 5, i.e., the orange concentrate represents one unit volume to which you have added 4 more cans (same unit volumes) of water. So the orange concentrate is now distributed through 5 unit volumes. This would be called a 1:5 dilution, and the OJ is now 1/5 as concentrated as it was originally. So, in a simple dilution, *add one less unit volume of solvent* than the desired dilution factor value.

Example 2: Suppose you must prepare 400 ml of a disinfectant that requires 1:8 dilution from a concentrated stock solution with water. Divide the volume needed by the dilution factor ($400 \text{ ml} / 8 = 50 \text{ ml}$) to determine the unit volume. The dilution is then done as 50 ml concentrated disinfectant + 350 ml water.

2. Serial Dilution

A *serial dilution* is simply a series of simple dilutions which amplifies the dilution factor quickly beginning with a small initial quantity of material (i.e., bacterial culture, a chemical, orange juice, etc.).

It is the repeated dilution of a solution to achieve a geometric dilution of the original solution. It's commonly performed in experiments requiring highly dilute solutions with great accuracy. The source of dilution material (solute) for each step comes from the diluted material of the previous dilution step. In a serial dilution the *total dilution factor* at any point is the *product* of the individual dilution factors in each step leading up to it.



3. Making fixed volumes of specific concentrations from liquid reagents:

V1C1=V2C2 Method

Very often you will need to make a specific volume of known concentration from stock solutions, or perhaps due to limited availability of liquid materials (some chemicals are very expensive and are only sold and used in small quantities, e.g., micrograms), or to limit the amount of chemical waste. The formula below is a quick approach to calculating such dilutions where:

V = volume, C = concentration; in whatever units you are working.

(stock solution attributes) **V1C1=V2C2** (new solution attributes)

Example: Suppose you have 3 ml of a stock solution of 100 mg/ml ampicillin (= **C1**) and you want to make 200 ul (= **V2**) of solution having 25 mg/ml (= **C2**). You need to know what volume (**V1**) of the stock to use as part of the 200 ul total volume needed.

V1 = the volume of stock you will start with. **This is your unknown.**
C1 = 100 mg/ml in the stock solution
V2 = total volume needed at the new concentration = 200 ul = 0.2 ml
C2 = the new concentration = 25 mg/ml

By algebraic rearrangement:

$$\mathbf{V1} = (\mathbf{V2} \times \mathbf{C2}) / \mathbf{C1}$$

$$\mathbf{V1} = (0.2 \text{ ml} \times 25 \text{ mg/ml}) / 100 \text{ mg/ml}$$

and after cancelling the units,

$$\mathbf{V1} = 0.05 \text{ ml, or } 50 \text{ ul}$$

So, you would take 0.05 ml = **50 ul of stock solution** and dilute it with 150 ul of solvent to get the 200 ul of 25 mg/ml solution needed. Remember that the amount of solvent used is based upon the final volume needed, so you have to subtract the starting volume from the final to calculate it.

4. Percent Solutions (% = parts per hundred or grams/100 ml)

Many reagents are mixed as *percent concentrations* as weight per volume for dry reagent OR volume per volume for solutions. When working with a dry reagent it is mixed as *dry mass (g) per volume* and can be simply calculated as the *% concentration (expressed as a proportion or ratio) x volume needed = mass of reagent to use*.

by weight (w/v) - The formula for weight percent (w/v) is: [Mass of solute (g) / Volume of solution (ml)] x 100

and by volume (v/v). The formula for volume percent (v/v) is: [Volume of solute (ml) / Volume of solution (ml)] x 100

Example 1: If you want to make 200 ml of 3 % NaCl you would dissolve 0.03 g/ml x 200 ml = 6.0 g NaCl in 200 ml water.

When using **liquid reagents** the percent concentration is based upon *volume per volume*, and is similarly calculated as *% concentration x volume needed = volume of reagent to use*.

Example 2: If you want to make 2 L of 70% acetone you would mix 0.70 ml/ml x 2000 ml = 1400 ml acetone with 600 ml water.

5. Moles and Molar solutions (unit = M = moles/L)

Sometimes it may be more efficient to use **molarity** when calculating concentrations. A **mole** is defined as one gram molecular weight of an element or compound, and comprised of exactly 6.023×10^{23} atoms or molecules (this is called Avagadro's number). The mole is therefore a unit expressing the *amount of a chemical*. The *mass* (g) of one mole of an element is called its **molecular weight (MW)**. When working with compounds, the mass of one mole of the compound is called the **formula weight (FW)**. The distinction between MW and FW is not always simple, however, and the terms are routinely used interchangeably in practice. Formula (or molecular) weight is always given as part of the information on the label of a chemical bottle.

The number of moles in an arbitrary mass of a dry reagent can be calculated as:

$$\# \text{ of moles} = \text{weight (g)} / \text{molecular weight (g)}$$

Molarity is the unit used to describe the number of moles of a chemical or compounds in one liter (L) of solution and is thus a unit of *concentration*. By this definition, a 1.0 Molar (1.0 M) solution is equivalent to one *formula weight* (FW = g/mole) of a compound dissolved in 1 liter (1.0 L) of solvent (usually water).

Example 1: To prepare a liter of a simple molar solution from a dry reagent

Multiply the *formula weight* (or MW) by the desired molarity to determine how many grams of reagent to use:

Chemical FW = 194.3 g/mole; to make 0.15 M solution use

$$194.3 \text{ g/mole} * 0.15 \text{ moles/L} = 29.145 \text{ g/L}$$

Example 2: To prepare a specific volume of a specific molar solution from a dry reagent

A chemical has a FW of 180 g/mole and you need 25 ml (0.025 L) of 0.15 M (M = moles/L) solution. How many grams of the chemical must be dissolved in 25 ml water to make this solution?

$$\# \text{grams} / \text{desired volume (L)} = \text{desired molarity (mole/L)} * \text{FW (g/mole)}$$

by algebraic rearrangement,

$$\# \text{grams} = \text{desired volume (L)} * \text{desired molarity (mole/L)} * \text{FW (g/mole)}$$

$$\# \text{grams} = 0.025 \text{ L} * 0.15 \text{ mole/L} * 180 \text{ g/mole}$$

after cancelling the units,

$$\# \text{grams} = 0.675 \text{ g}$$

So, you need **0.675 g/25 ml**

To convert from % solution to molarity, multiply the % solution by 10 to express the percent solution grams/L, then divide by the formula weight.

$$\text{Molarity} = \frac{(\text{grams reagent}/100 \text{ ml}) * 10}{\text{FW}}$$

Example 1: Convert a 6.5 % solution of a chemical with FW = 325.6 to molarity,

$$[(6.5 \text{ g}/100 \text{ ml}) * 10] / 325.6 \text{ g/mole} = [65 \text{ g/L}] / 325.6 \text{ g/mole} = 0.1996 \text{ M}$$

To convert from molarity to percent solution, multiply the molarity by the FW and divide by 10:

$$\% \text{ solution} = \frac{\text{molarity} * \text{FW}}{10}$$

Example 2: Convert a 0.0045 M solution of a chemical having FW 178.7 to percent solution:

$$[0.0045 \text{ moles/L} * 178.7 \text{ g/mole}] / 10 = 0.08 \text{ \% solution}$$

6. Concentrated stock solutions - using "X" units

Stock solutions of stable compounds are routinely maintained in labs as more concentrated solutions that can be diluted to working strength when used in typical applications. The usual working concentration is denoted as 1x. A solution 20 times more concentrated would be denoted as 20x and would require a 1:20 dilution to restore the typical working concentration.

Example: A 1x solution of a compound has a molar concentration of 0.05 M for its typical use in a lab procedure. A 20x stock would be prepared at a concentration of $20 \times 0.05 \text{ M} = 1.0 \text{ M}$. A 30X stock would be $30 \times 0.05 \text{ M} = 1.5 \text{ M}$.

7. Normality (N): Conversion to Molarity

Normality = $n \times M$ where n = number of protons (H^+) in a molecule of the acid.

Example: In the formula for concentrated sulfuric (36 N H_2SO_4), there are two protons, so, its molarity = $N/2$. So, $36\text{N } \text{H}_2\text{SO}_4 = 36/2 = 18 \text{ M}$.