PREPARING SOLUTIONS AND MAKING DILUTIONS

NOTE: Much of this beginner's guide to solutions has been taken verbatim from a webpage prepared by Greg Anderson of Bates College, Lewiston, ME (click here to see Mr. Anderson's *How to Make Simple Solutions and Dilutions* webpage). Mr. Anderson's text and examples are used with his permission.

Unit Definitions

milligram = mg = 1/1000 of a g or 10^{-3} g
gram = g
kilogram = kg = 1000 g or 10^{3} g
mole = 6.023 x 10^{23} molecules
molarity = moles per liter
molar = M = term used to discuss the molarity of solutions (*e.g.*, 2.5 M means 2.5 moles of solute dissolved in one liter)
millimole = 1/1000 of a mole
millimolar = mM = term used to discuss molarity in thousandths of a mole (*e.g.*, a 20 mM solution contains 20/1000 = 0.02 moles per liter)
w/v = weight (of solute) per final solution volume
v/v = volume (of reagent) per final solution volume

1. Simple Dilution (Dilution Factor Method)

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 diluent (the material to be diluted) + 4 unit volumes of the solvent medium (hence, 1 + 4 = 5 = dilution factor).

Example 1: To dilute a streptavidin solution 1:300

Mix 1 unit volume of streptavidin solution with 299 volumes of solvent medium.

In some instances, solutions are expressed in terms of "X," an indicator of relative solute concentration. For example, the buffer saline sodium citrate (commonly referred to as SSC) is usually made up as a 20X stock solution. However, it is typically diluted before use. The same rules discussed above apply for making dilutions from "X" stock solutions.

Example 2: A 1X solution of SSC can be prepared from a 20X SSC stock solution by mixing one unit volume of 20X SSC with 19 volumes of water.

2. Mixing parts or volumes

Mixing of parts or volumes is often confused with simple dilution. For example, if you are instructed to, "...add 1 part glacial acetic acid with 3 parts water," you are literally mixing 1 unit volume of glacial acetic acid and 3 unit volumes of water. You would do the same if asked to mix 1 volume of glacial acetic acid with 3 volumes of water. Confusion can arise due to the use of a colon in some mixing recipes (see example below).

Example: If you are instructed to make up a 1:3 acetic ethanol solution, you are probably supposed to mix one unit volume of acetic acid and three unit volumes of ethanol. However, if you are asked to make a 1:3 dilution of acetic acid in ethanol, you would mix one unit volume of acetic acid with two unit volumes of ethanol. Confused? If you see the word "dilution" or "dilute" in your instructions, you are doing a simple dilution as discussed in section 1. If you are not sure, ask advice from someone who will probably know.

3. 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 (e.g., DNA, restriction enzyme, *etc.*). The source of dilution material for each step comes from the diluted material of the previous. In a serial dilution the *total dilution factor* at any point is the *product* of the individual dilution factors in each step up to it.

Final dilution factor (DF) = DF1 * DF2 * DF3, *etc*.

Example: In the microbiology lab BIOLOGY 201 the students perform a *three step* 1:100 serial dilution of a bacterial culture. The initial step combines 1 unit volume culture (10 µl) with 99 unit volumes of broth (990 µl) = 1:100 dilution. In the next step, one unit volume *of the 1:100 dilution* is combined with 99 unit volumes of broth now yielding a total dilution of 1:100 * 100 = 1:10,000. Repeated again (the third step) the total dilution would be 1:100 * 10,000 = 1:1,000,000 total dilution. The concentration of bacteria is now one million times *less* than in the original sample.

4. Making fixed volumes of specific concentrations from liquid reagents: *V1C1=V2C2*

Very often you'll need to make a specific volume of known concentration 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 waste. The formula below is a quick approach to calculating such dilutions where:

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

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

Example 1: Suppose you have 3 ml of a stock solution of 100 mg/ml ampicillin (= **C1**) and you want to make 200 µl (= **C2**) of solution having 25 mg/ml (= **V2**). You need to know what volume (**V1**) of the stock to use as part of the 200 µl total volume needed.

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

By algebraic rearrangement:
V_1 = (V2 x C2) / C1
V_1 = (0.2 ml x 25 mg/ml) / 100 mg/ml

After canceling the units,

= 0.05 ml = 50 µl

So, you would take 50 µl of stock solution and dilute it with 150 µl of solvent to get the 200 µl 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.)

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

Sometimes it may be more efficient to use ***molarity*** when calculating concentrations. A 1.0 Molar (1.0 M) solution is equivalent to 1 *formula weight* (FW) (g/mole) of chemical dissolved in 1 liter (1.0 L) of solvent (usually water). Formula weight is always given on the label of a chemical bottle (use *molecular weight* if it is not given).

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 g/mole * 0.15 moles/L = 29.145 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 to make up 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?

#grams/desired volume (L) = desired molarity (mole/L) * FW (g/mole)

By algebraic rearrangement,
#grams = desired volume (L) * desired molarity (mole/L) * FW (g/mole)
#grams = 0.025 L * 0.15 mole/L * 180 g/mole

After canceling the units,
#grams = 0.675 g

So to make 25 ml of a 0.15 M solution, you need to place 0.675 g in a container and add water until the final volume is 25 ml.

6. Percent Solutions (= parts per hundred)

Many reagents are mixed as *percent concentrations.* When working with a dry chemical it is mixed as *dry mass (g) per volume* where #g/100 ml = percent concentration. A 10% NaCl solution is equal to 10 g dissolved in 100 ml of solvent. Because the solid is measured based upon its weight (w) while the solvent is measured based upon its volume, the NaCl solution discussed above should be labeled as 10% w/v NaCl.

Example 1: If you want to make 3% w/v NaCl you would dissolve 3.0 g NaCl in 100 ml water (or the equivalent for whatever volume you needed).

However, if you want to make up less or more than 100 ml of solution, the proper amount of solute can be determined by multiplying the number of grams in a 1% w/v solution by the desired final volume divided by 100.

Example 2: To make up 350 ml of 12% w/v NaCl in water,

12 g * (350 ml/100 ml) = 12 g x 3.5 = 42 g

So, place 42 g of NaCl in a beaker and add water up to 350 ml

Example 3: Make up 15 ml of 0.05% w/v $AgNO_3$

0.05 * (15 ml/100 ml) = 0.0075 g of 7.5 mg

So, place 0.0075 g $AgNO_3$ in a beaker and add water up to 15 ml.

When using **liquid reagents** the percent concentration is based upon *volume per volume*, *i.e.*, # ml/100 ml.

Example 4: If you want to make 70% v/v ethanol you would mix 70 ml of 100% ethanol with 30 ml water

Making up volumes less than 100 ml is handled the same way as for solutions prepared from solids.

Example 5: To make up 912 ml of 21.5% v/v Triton X-100,

21.5 ml * (912 ml/100 ml) = 196.1 ml

So, mix 196.1 ml of Triton X-100 with 715.9 ml of water and you'll have a 21.5% v/v solution (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.)

7. Conversions from % to molarity and from molarity to %

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

$$\text{Molarity} = \frac{(\%\ \text{solution}) * 10}{\text{FW}}$$

Example 6: To convert a 6.5% solution of a chemical with FW = 325.6 to a molarity value,

[(6.5 g/100 ml) * 10] / 325.6 g/L = 0.1996 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 7: Convert a 0.0045 M solution of a chemical having FW 178.7 to a percent solution:

[0.0045 moles/L * 178.7 g/mole] / 10 = 0.08 % solution