APPENDIX II. Concentration and Dilution

The most common expressions of concentration in biochemistry are *mass per unit volume molarity* and *percent-of-solute*.

Mass per unit volume is defined as the number of grams of solid (solute) dissolved in a given volume of liquid (solvent). In biochemistry we encounter a wide range of concentrations dependent on the solubility and abundance of the given solute. You may encounter a typical range of 1 microgram/milliliter (mcg/mL) to 100 milligrams/milliliter (mg/mL).

Molarity is defined as the number of moles of solute per liter of solution (denoted M). Molarity usually describes solutions of accurate concentration, where the molecular weight of the solute is known. Solutes are weighed on an analytical balance, and volumes are measured in volumetric flasks. In biochemistry, most concentrations are in the millimolar (mM), micromolar (µM), or nanomolar (nM) range.

Percent-of-solute is frequently used for liquids and solids of undetermined molecular weight. Three designations are used: volume per volume (v/v), weight per volume (w/v), and weight per weight (w/w). A 5% (v/v) solution contains 5 ml solute in 100 ml total solution. A 5% (w/v) solution contains 5 g solute per 100 ml total solution. A 5% (w/w) solution contains 5 g solute per 100 g total solution. Of these three designations, weight per volume expressions are used most commonly in biochemistry. Sometimes these concentrations are not expressed as percentages, but rather simply as the number of mg (or µg) per ml solution. Often times stock solutions contain a mixture of components. In these instances they are often prepared at five or ten times (5X or 10X) the final working concentration. A simple 1:5 or 1:10 dilution of the stock solution provides the correct working concentration.

Biochemical experiments frequently require the dilution of a particular sample or **stock solution**. If the concentration of stock solution and that needed in the experiment are known, the *dilution factor* can be calculated. For example, if you want to dilute a 1 M stock solution to 5 mM in your experiment, the dilution factor is 0.005/1 or 1/200 (also indicated as a 1:200 dilution). This means you must dilute 1 part stock solution with 199 parts solvent. Another way of stating this is that 1 part stock solution must be diluted into 200 parts total solution. This is commonly described by biochemists as a one ***in*** 200 (or two hundred-fold) dilution. Generally, if you want 200 ml of the diluted solution, you would mix 1 ml stock solution with 199 ml solvent. This is a commonly accepted practice in biochemistry.

You can determine the concentration of a diluted solution (C_{final}) or calculate what volume of stock solution ($V_{initial}$) must be added to solvent to make a diluted solution of known concentration.

The following formula may be useful:

$$C_{initial}V_{initial} = C_{final}V_{final} \qquad \text{Eq. 1}$$

For example, if you have a stock solution of 5 mg/mL protein, and you want to make 250 mcL of a 50 mcg/mL working solution, you would add 25 mcL of the stock solution to 225 mcL of solvent. This is a 1:100 dilution. It is a good practice to always add smaller volumes to the larger volume of water or solvent.

The reverse calculation is often employed when calculating the concentration of an unknown stock solution (the initial concentration). If we measure the final concentration and the amount of dilution (the dilution factor) we can rearrange Equation 1 to the form:

$$C_{initial} = \frac{C_{final}V_{final}}{V_{initial}} \qquad \text{Eq. 2}$$

Note that this equation can be written $M_{initial}V_{initial} = M_{final}V_{final}$, where M represents the molar concentration.

From the previous example suppose we measured the final concentration to be 50 mcg/mL. Intuitively we know that our stock solution must be more concentrated because we diluted 25 mcL ($V_{initial}$) into a final volume of 250 mcL. In this case the dilution factor is 100, the stock solution is 100 times more concentrated than the measured solution.

Keep in mind the limitations of the equipment and amounts of material present. In biochemistry most dilutions will be made in a V_{final} of less than 1 mL. This mean a typical 1:200 dilution will involve adding 1 mcL of stock solution to 199 mcL of diluent. Unfortunately the limitations of the pipettors does not accurately allow the measurement of 1 mcL. Therefore a typical 1:200 dilution would most likely be done by adding 5 mcL of stock solution to 995 mcL of diluent.

A special type of dilution is the *serial dilution*. In this method, a stock solution is diluted systematically in fixed steps of the same dilution factor (see Fig. 1). A high degree of accuracy and precision is required, because errors made in an early dilution are carried to all subsequent dilutions. Serial dilutions are used frequently in immunological and microbiological experiments.

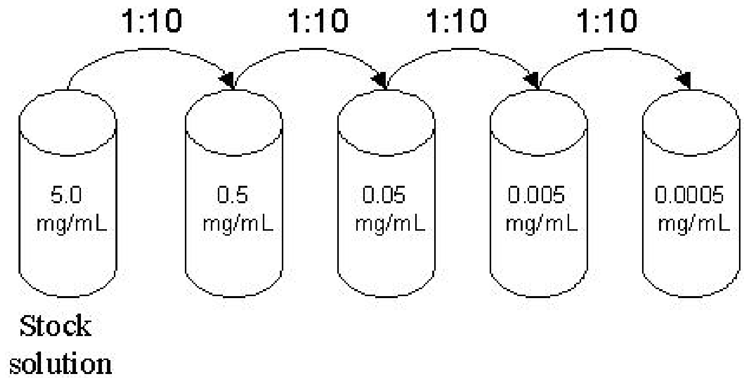

Fig. 1. A serial dilution scheme.

A more accurate method for preparing dilutions is the systematic dilution of a single stock standard. Note: it is not always reasonable to prepare all dilutions from a single standard.

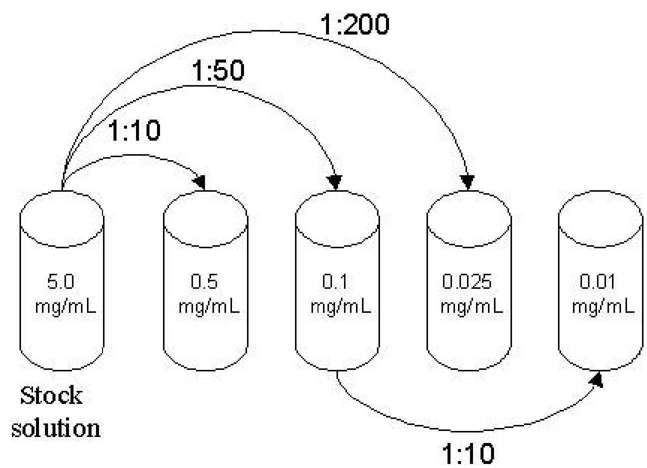

Fig. 2. Systematic dilution scheme