BISC 367 Plant Physiology

Preparations of solutions

(Adapted from Plant Physiology Manual. D.J. Armstrong, Oregon State University)

Good laboratory practice requires the preparation of solutions that contain particular concentrations or amounts of various chemical reagents. Thus, you must be familiar with calculations related to the preparation of solutions and with the various means of measuring and dispensing solutions. The following material is designed to provide you with the basic information needed to prepare and handle solutions in the laboratory.

A. The concept of a Mole:

The concept of a mole is fundamental to many laboratory operations and is defined as a gram molecular weight, meaning that a mole of a compound is an amount of that substance equal to its formula weight expressed in grams. One mole of any compound contains 6.02×10^{23} molecules/atoms (Avogadro's number) of that compound.

B. Molar solutions:

The concentration of a solute in a solution can be expressed as **molarity**. A 1M solution contains one mole of *solute* per liter of solution. To prepare a 1 M solution of sucrose in water, one mole of sucrose is placed in a volumetric flask, dissolved in water and the solutions is made to a final volume of 1 liter in the flask.

It is important to distinguish between a **mole** (which is an *amount* of material) and a **molar concentration** (which is a measure of *concentration*). Molar concentrations are abbreviated as **M** whereas there is no abbreviation for mole.

In working with molar solutions, it is useful to build in the following relationships:

1M = I mole in a liter = 1 millimole in one mL of solution

0.001M = 1 mM = 1 mmole in a liter = 1 micromole (μmol) in one mL of solution

0.001 mM = 1 μmole in a liter = 1 nanomole in one mL of solution

The expression of concentration in units of molarity is useful in work involving chemical reactions between solutes because the use of molar concentrations makes it easy to adjust the relative number of reacting molecules. Thus, 1 mL of a 1M solution of compound A will contain the same number of molecules of A as 1 mL of a 1 M solution of compound B.

C. Molal solutions

The concentration of a solute in a solution may also be expressed in units of molality. A one molal solution contains 1 mole of solute per 1000 g (1 kg) of solvent. To prepare a one molal solution of sucrose you would weigh one mole of sucrose into a container and add 1000 g water (1 liter). Note that volume of solvent rather than the volume of solution is measured. Molal concentrations are abbreviated as **m**.

Molal concentrations are not used in work involving chemical reactions because molal solutions of different solutes will contain a different number of solute molecules per given volume of solution. Molal solutions are used in work involving the thermodynamic properties of solutions because these properties depend on the ratio of solute and solvent molecules in the system. Examples of properties of solutions that are directly proportional to molal concentration include such things as freezing point depression, boiling point elevation and other **colligative** properties (a colligative property is one that is dependent on the number of solute molecules rather than the nature of the solute molecules). A significant colligative property of dissolved solutes to plant physiologists is **osmotic potential**.

D. Normal solutions

It is common to see the concentration of acids or bases in solution expressed in units of Normality, which is a measure of the number of reactive species in a solution. A one normal (1N) solution of an acid or base contains one gram equivalent weight of the acid or base. Gram equivalent weights for acids or bases are calculated by dividing the molecular weight by the number of ionizable hydrogen or hydroxyl groups contained in a molecule of acid or base. Thus, a gram equivalent weight of sulphuric acid is the same as a 2N solution of sulphuric acid. Similarly, a 1 M solution of HCl or NaOH is the same as the 1N solutions of these compounds. Another way to calculate the normality of an acid or base solution is to multiply the molarity by the number of ionizable hydrogen or hydroxyl groups. The concept of normality can also be extended to include compounds that undergo oxidation/reduction reactions.

It is useful to know the approximate normalities of the concentrated commercial solutions of commons acids and bases although precise work is necessary to measure the exact acidity and alkalinity of these solutions.

E. Percent solution (or mass percentage)

It is quite common for many laboratory reagents to be expressed as percent values. This denotes the mass of a substance in a solution as a percentage of the mass of the entire solution. There are three different ways to expression concentration as a mass percentage.

Percent by weight per volume (% w/v) = grams of solute per 100 mL solution. To prepare a 1% (w/v) solution of sucrose, 1 g of sucrose is dissolved in water after which the volume is adjusted to 100 mL by the addition of water.

Percent per volume (% v/v) = milliliter of solute per 100 mL solution. To prepare a 1% (v/v) solution of acetone, 1 mL acetone is dissolved in water and the volume adjusted to 100 mL by the addition of water.

Percent by weight (% w/w) = grams of solute per 100 grams of solution. To prepare a 1% (w/w) solution of sucrose, 1 g sucrose is dissolved in 99g (99 mL) of water.

When preparing a % (v/v) solution it is worth noting that when you mix two volumes of two solvents (such as ethanol and water) the volumes are not necessarily additive and attention must be paid to the final volume of the mixture because this will affect the final concentration.

F. Dilution of stock solutions

Solutions prepared using concentration units that are based on a final volume of solution (e.g. M, N, % (w/v) or (v/v)) may be used as a stock solution to prepare a more dilute solution of the same compound. The amount of a stock solution required to prepare a more dilute solution is calculated according to the following formula:

$$\mathbf{C_1 \times V_1 = C_2 \times V_2}$$ where:

C_1 is the concentration of the stock solution
V_1 is the volume of stock solution required to make the diluted solution
C_2 is the concentration of the dilute solution
V_2 is the volume of the dilute solution

For example, to prepare 1000 mL of a 30% (v/v) solution of ethanol from the usual 95% commercial laboratory ethanol stock you would use the following based on the above formula.

$$95 \times V_1 = 30 \times 1000$$

Solving for V1 gives you 316 mL. Therefore, to prepare the desired volume of 30% (v/v) ethanol, you need to measure 316 mL 95% ethanol into a 1000 mL volumetric flask and make the volume up to 1000 mL.

G. Measurement of solution volumes

Measurement of a large volume of solution is normally made using a volumetric flask or a graduated cylinder. Volumetric flasks are more accurate than graduated cylinders. Beakers and erlenmyer flasks should not be used to measure the volume of a solution because the volume makings are not accurate. Measurement of a small volume of solution requires the use of pipettes. Please note the following:

1) Never ever mouth pipette! Always use the pipette adapter or bulb provided
2) When you use a pipette it is important to remember that anytime you adjust the meniscus of the liquid in the pipette to a graduation on the pipette the tip of the pipette should be in contact with the side of a container. Failing to do so will introduce a small error in the volume measured.

The following pipettes are commonly found in laboratories:

1) Graduated Mohr pipette: on these pipettes the graduation marks do not extent to the tip of the pipette and volume in the tip is undefined. Therefore, you must stop expelling the measured liquid when it reaches the bottom graduation mark (this type of pipette never has all the liquid expelled).
2) Graduated serological pipette: on these pipettes the graduations extend to the end of the pipette. Therefore, for these pipettes you must expel all the liquid in the pipette with the tip of the pipette held in contact with the side of the container that is receiving the liquid.

Make up the following solutions:

1) 200 mL of 250 mM NaCl. The molecular weight of NaCl is 58.4

2) 200 mL of 0.25 molal NaCl.

3) 200 mL 0.5 N NaOH. The molecular weight of NaOH is 40. Be careful, NaOH is very caustic!

4) 200 mL of 15% (w/v) NaCl

5) 200 mL 70% (v/v) ethanol (the lab stock is 95%)

6) Prepare 200 mL of a 20 mM NaCl solution from your 250 mM NaCl stock solution.

Problem set

The following problems are designed to provide you with experience in some common types of laboratory calculation encountered in the preparation of solutions:

1) How would you prepare 2 liters of 0.05 M KCl? (the molecular weight of KCl is 74.6)

2) How would you prepare 500 mL of 0.2 molal sucrose? (the molecular weight of sucrose is 342.3)
3) How would you prepare 250 mL of 0.2 M NaOH (the molecular weight of NaOH is 40)
    a. What is the normality of this solution?
    b. What is the concentration of this solution expressed as % (w/v)
4) Express each of the following in molar, millimolar, and micromolar units of concentration:
    a. 0.3 moles/L
    b. 0.3 mmoles/L
    c. 0.3 mmoles/mL
    d. 0.3 μmoles/mL
5) How would you prepare 250 mL of 30% (v/v) ethanol solution from a 95% (v/v) ethanol stock?
6) How many micromoles acetic acid are present in 1 mL of a 20 mM acetic acid solution?
7) How would you prepare 250 mL of 0.2 M solution of KCl from a 10% (w/v) KCl stock solution? (molecular weight of KCl is 74.6).