1

GENERAL BIOCHEMISTRY

1.1 INTRODUCTION

Solutions of chemical reagents are a big part of biochemistry, biological and chemical based work. For a beginner of experimental procedure making solutions can also be the most frustrating part. Preparation and handling solutions are essential part of experimental biochemistry. Thus any of new science graduates should be clear in preparing reagents, buffers, and accuracy in pipeting.

The concentration of a dissolved salt in water refers to the amount of salt (solute) that is dissolved in water (solvent). Solutes are the substance of interest to be dissolved and the term solvent denotes the material in which the solute is dissolved.

Solution is a mixture that contains solute and a solvent. Solute can be denoted as the component of a solution present in the lesser amount and the solvent is the component of a solution present in the greater amount. Concentration can be written as the amount of a solute present in a solution per amount of solvent.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the importance of solution preparation in biochemistry
- explain different concentration units
- describe different terms of percent solutions

1.2 UNITS OF CONCENTRATION

- There are many ways to express concentrations. Concentration may be expressed several different ways and some of the more common concentration units are:
  1. Equivalent weight
  2. Molarity
  3. Molality
  4. Normality
  5. Percent solution (weight/weight)
  6. Percent solution (weight/volume)
  7. Percent solution (volume/volume)

1.2.1 Equivalent Weight

The equivalent weight is determined by dividing the atomic or molecular weight by the valence. A major use of the concept of equivalents is that one equivalent of an ion or molecule is chemically equivalent to one equivalent of a different ion or molecule. The mass of a substance especially in grams is chemically equivalent to eight grams of oxygen or one gram of hydrogen **:** the atomic or molecular weight divided by the valence

Valance could be determined as

1. The absolute value of ion charge
2. The number of H^+ or OH^- that a species can react with
3. The absolute value of change in charge on a species when undergoing a chemical reaction.

1.2.1.1 Preparation of NaOH

Solutions of NaOH can be prepared by either dissolving solid NaOH pellets in water or by diluting a concentrated solution of NaOH. However, the exact concentration of the solution prepared by these methods cannot be calculated from the weighed mass or using the dilution equation for two reasons:

1. Solid sodium hydroxide is hygroscopic (“water-loving”). Pellets of NaOH exposed to air will increase in mass as they become hydrated so the actual mass of pure NaOH is not accurately known.
2. Sodium hydroxide in solution reacts with carbonic acid and its concentration decreases over time. The acid is formed when small amounts of CO_2 gas (which is always present in air) dissolves in solution.

$$H_2CO_3(aq) + NaOH(aq) \rightleftharpoons H_2O + Na^+(aq) + HCO_3^-(aq)$$

The water used to make the NaOH solution can be boiled to expel the dissolved CO_2 gas but this time-consuming procedure is often not possible in a short laboratory period. A stock solution of NaOH can be made in advance with boiled water but will re-absorb CO_2 over a period of time unless stored in airtight containers. Therefore, if we want to know the exact concentration of a freshly made NaOH solution, we need to "standardize" it. That is, determine its exact concentration by titrating it with a known mass of a primary standard acid.

A "primary standard" is a substance that is used to determine the concentration of a solution. A primary standard should have the following properties: It should be available in very pure form at reasonable cost and should have a high equivalent weight to minimize weighing errors. It should be stable at room temperature, easy to dry, and should not easily absorb water when exposed to air (hygrophobic).

Potassium hydrogen phthalate ("KHP") is the primary standard reagent commonly used to standardize NaOH. It is a monoprotic acid whose formula is $KHC_8H_4O_4$ and molecular weight is 204.22 g/mol.

$$KHC_8H_4O_4(aq) + NaOH(aq) \longrightarrow H_2O + Na^+(aq) + K^+(aq) + C_8H_4O_4(aq)$$

The white powdery acid is normally heated at 110°C for one hour to remove any loosely bound waters of hydration and then cooled in a desiccator before use. The exact mass (and number of moles of acid) is determined by weighing the dried acid on an analytical balance. The acid is then dissolved in water and NaOH is added until an endpoint (the point at which an indicator changes color) is reached. The phenophthalein indicator used in this experiment is colorless in acid and pink in base. Therefore, the solution containing KHP will remain colorless as long as some KHP is still present. Once the last of the KHP has reacted, the solution will turn pink with one excess drop of base.

The exact concentration of NaOH is calculated by using the stoichiometry from reaction to convert the number of moles of KHP used to moles of NaOH and then dividing by the volume of NaOH used to reach the endpoint of the reaction.

1.2.1.2 Equivalent Weight of an Acid

In an acid base titration, the equivalence point is the volume of added base where the moles of –OH added (from the base) equal the moles of H+ initially present (from the acid). (i.e) moles of H^+ initially present = moles –OH added *(at equivalence point)*

To approximate the equivalence point, an indicator with an endpoint close to the equivalence point is added to the analyte solution. A balanced equation can be written describing the chemical reaction occurring between the titrant (the base in this experiment) and analyte (the acid in this experiment) if the identity of both is known.

For example, the titration of hydrochloric acid with potassium hydroxide can be written:

$$HCl(aq) + KOH(aq) \longrightarrow H_2O + KCl(aq)$$

In the case if the identity of the acid is unknown, but the number of acidic hydrogens (H^+) carried by the acid is known, a balanced equation can still be written.

For example, the titration of a triprotic acid (an acid with 3 H+) with sodium hydroxide can be written:

$$H_3X(aq) + 3KOH(aq) \longrightarrow 3H_2O(l) + K_3X(aq)$$

(X : the unknown anion of the acid)

The formula weight of this unknown acid can be calculated by using dimensional analysis. First, the base's concentration is used to convert the base's volume at the endpoint to moles. Then, multiplying by the mole ratio between acid and base from the balanced chemical equation allows for the calculation of the moles of the acid. Now the mass of acid titrated must be divided by the moles of acid calculated giving a result with the units of g/mol.

1.2.1.3 Equivalent Weight of an Oxidizing Agent

The concept of equivalents and equivalent mass is not restricted to acid-base reactions alone. Unlike acid-base reactions in redox reactions, the electrons are the active units (the equivalents) and the equivalent weights are the masses of oxidizing or reducing agent that deliver or accept 1 mole of electrons. But in case of acid and base the hydrogen or hydroxide ions plays key role in determination of equivalent weight.

INTEXT QUESTIONS 1.1

1 The equivalent weight is determined by dividing the atomic or molecular weight by the ....

2 The mass of a substance especially in grams is chemically equivalent to .... grams of oxygen

(a) 2 (b) 5 (c) 8 (d) 10

3 .... is the primary standard reagent commonly used to standardize NaOH

1.2.2 Molarity

Molarity is based on the volume of solution containing the solute. Since density is a temperature dependent property a solution's volume, and thus its molar concentration, changes with temperature. By using the solvent's mass in place of the solution's volume, the resulting concentration becomes independent of temperature.

Molarity is the common way of referring to concentrations of solutions. The goal of most basic molarity problems shall be to get the moles from grams by dividing the molecular weight and then dividing by the total number of liters or by given the molarity find the number of grams of the solution by multiplying the volume then the molecular weight. Molarity might give you the density of the solution, from which you can obtain the mass by multiplying the density by the volume.

Although there are several ways in which the concentration of a solution can be quantified, molarity is one of the most basic and widely used. Molarity (M) is defined as the number of moles of solute dissolved in one liter of solution. The higher the molarity, the more concentrated or strong the solution is. For example, a 12 M (which is said "twelve molar") solution of HCl (ie. hydrochloric acid) is much more concentrated than a 0.10 M solution! The basic formula for calculating molarity is:

Molarity (M) = moles of solute (mol) per liters of solution (L)

To solve for moles of solvent, we can use algebra to manipulate the above equation producing the following derived formulas:

moles of solute (mol) = Molarity (M) × liters of solution (L)

In simple terms, the following formula could be used for preparation of molar solutions for lab solutions preparation

For preparation of molar solution

$$\frac{\text{Molecular weight of the compound (A)}}{1000} \times \text{Required morality (B)} \times \text{Required volume of solution (C)} = \text{D gram}$$

In the above equation for preparation of solution of 'B' molarity, 'D' grams of the solute could be dissolved in 'C' ml of solvent.

INTEXT QUESTIONS 1.2

1. .... is based on the volume of solution containing the solute

   (a) Normality (b) Molarity (c) Molality (d) Percent

2. Molarity is the common way of referring to .... of solutions

3. .... is defined as the number of moles of solute dissolved in one liter of solution
4. The higher the ...., the more concentration of the solution

1.2.3 Molality

The molal unit is not used nearly as frequently as the molar unit and is used in thermodynamic calculations where a temperature independent unit of concentration is needed. A molality is the number of moles of solute dissolved in one kilogram of solvent. The term molality and molarity should not be confused. While expressing the Molality it is represented by a small "m," whereas molarity is represented by an upper case "M."

In case of preparation of molar solution except water all other solvent must be weighed. The water is exempted from weighing because; one liter of water has a specific gravity of 1.0 and weighs one kilogram. So one can measure out one liter of water and the solute could be directly added to it. But other solvents might have a specific gravity greater than or less than one. Therefore, one liter of any solvent other than water is not likely to occupy a liter of space.

For example to make a one molal aqueous (water) solution of sodium chloride (NaCl), measure out one kilogram of water and add one mole of the solute, NaCl to it. The atomic weight of sodium is 23 and the atomic weight of chlorine is 35. Therefore the formula weight for NaCl is 58. So 58 grams of NaCl could be dissolved in 1kg water for preparation of 1 molal solution of NaCl.

INTEXT QUESTIONS 1.3

1. .... is the unit used in thermodynamic calculations where a temperature independent unit of concentration is needed.
2. A molality is the number of moles of solute dissolved in .... kilogram of solvent

   (a) 3 (b) 2 (c) 1 (d) 6
3. Molality it is represented by a ....

   (a) Small 'm' (b) Capital 'M' (c) Small 'n' (d) Capital 'N'

1.2.4 Normality

The concentration of a solution could also be expressed in terms of Normality. It is based on an alternate chemical unit of mass called the equivalent weight. The normality of a solution is the concentration expressed as the number of equivalent weights (equivalents) of solute per liter of solution. In a chemical mixture 1 normal (1 N) solution contains 1 equivalent weight of solute per liter of solution. Since normality simplifies the calculations required for chemical concentration, it is widely used in analytical chemistry.

Every substance may be assigned an equivalent weight. The equivalent weight may be equal to the formula weight (molecular weight, mole weight) of the substance or equal to an integral fraction of the formula weight (i.e., molecular weight divided by 2, 3, 4, and so on).

The above phenomenon could be better explained with the following example to gain an understanding of the meaning of equivalent weight:

$$\begin{array}{ccccc} HCl(aq) & + & NaOH(aq) & \longrightarrow & NaCl(aq) + H_2O \\ \text{1 mole} & & \text{1 mole} & & \\ \text{(36.5 grams)} & & \text{(40.0 grams)} & & \\ H_2SO_4(aq) & + & 2NaOH(aq) & \longrightarrow & Na_2SO_4(aq) + 2H_2O \\ \text{1 mole} & & \text{1 mole} & & \\ \text{(98.1 grams)} & & \text{(80.0 grams)} & & \end{array}$$

In the above chemical reaction 1 mole of hydrochloric acid (HCl) reacts with 1 mole of sodium hydroxide (NaOH) and 1 mole of sulfuric acid (H_2SO_4) reacts with 2 moles of NaOH. If you made 1 molar solutions of these substances, 1 liter of 1 M HCl will react with 1 liter of 1 M NaOH and 1 liter of 1 M H_2SO_4 will react with 2 liters of 1 M NaOH. Therefore, H_2SO_4 has twice the chemical capacity of HCl when reacting with NaOH. The equivalent weight of HCl is equal to its molecular weight, but that of H_2SO_4 is ½ its molecular weight.

Expressions for normality are shown below. Notice the similarity to molar solution definition.

$$\text{Normality (N)} = \frac{\text{Number of equivalents of solute}}{\text{1 liter of solution}} = \frac{\text{Equivalents}}{\text{liter}}$$

where $\quad \text{Number of equivalents of solute} = \dfrac{\text{grams of solute}}{\text{equivalent weight of solute}}$

$$\text{finally N} = \frac{\text{grams of solute}}{\text{eq wt solute} \times \text{L solution}} = \frac{\text{grams}}{\text{eq wt} \times \text{L}}$$

So, 1 liter of solution containing 36.5 grams of HCl would be 1 N, and 1 liter of solution containing 49.0 grams of H_2SO_4 would also be 1 N. A solution containing 98.1 grams of H_2SO_4 (1 mole) per liter would be 2 N.

INTEXT QUESTIONS 1.4

1. .... based on an alternate chemical unit of mass called the equivalent weight

   (a) Normality (b) Molarity (c) Percent (d) Molality

2. One mole of .... reacts with 1 mole of sodium hydroxide (NaOH)

3. One mole of sulfuric acid (H_2SO_4) reacts with .... moles of NaOH

4. The equivalent weight of HCl is equal to its ....

1.2.5 Percentage Solutions

The percentage solution could be expressed in terms of weight percent (% w/w), volume percent (% v/v) and weight-to-volume percent (% w/v) units of solute present in 100 units of solution. For example a solution of 1.5% w/v NH_4NO_3, contains 1.5 gram of NH_4NO_3 in 100 mL of solution.

1.2.5.1 Percent by weight (% w/w)

In case of preparing a solution based on percentage by weight, one would simply determine what percentage was required (for example, a 20% by weight aqueous solution of sodium chloride) and the total quantity to be prepared.

If the total quantity needed is 1 kg, then it would simply be a matter of calculating 20% of 1 kg which, of course is:

20 /100 * 1000 g/kg = 200 g NaCl/kg.

Thus finally to bring the total quantity to 1 kg, it would be necessary to add 800g water.

1.2.5.2 Percent by volume (% w/v)

Preparation of solutions based on percent by volume it requires the calculation same as for percent by weight, except that calculations are based on volume. In simple terms one should plan that what percentage was desired (for example, a 20% by volume aqueous solution of sodium chloride) and the total quantity to be prepared in terms of volume.

For example if 20 % is to be prepared for a total quantity of 1 liter, then it would simply be a matter of calculating 20% of NaCl in 1 liter, the formula can be written as:

20/100 * 1000 ml/l = 200 g NaCl/l.

1.2.5.3 Percent by volume (% v/v)

Volume percent or volume/volume percent most often is used when preparing solutions of liquids. This is typically only used for mixtures of liquids. Volume percent is relative to volume of solution, not volume of solvent. The advantage of volume/volume units is that gaseous concentrations reported in these units do not change as a gas is compressed or expanded.

For example, 70% v/v rubbing alcohol may be prepared by taking 700 ml of isopropyl alcohol and adding sufficient water to obtain 1000 ml of solution (which will not be 300 ml).

Table 1.1 Common Units for Reporting Concentration

Sl.No	Name	Units	Symbol
1.	Molarity	moles solute liters solution	M
2.	Normality	equivalents solute liters solution	N
3.	Molality	moles solute kilograms solvent	m
4.	Weight percent	grams solute 100 grams solution	% w/w
5.	Volume percent	mL solute 100 mL solution	% v/v
6.	weight-to-volume percent	grams solute 100 mL solution	% w/v

INTEXT QUESTIONS 1.5

1. Percent by weight could be expressed as ....
2. For preparation of 20 % NaCl by (w/v) .... grams of NaCl is to be dissolved in 1 L of water.

   (a) 200 (b) 100 (c) 400 (d) 50
3. volume/volume percent most often is used when preparing solutions of ....
4. Match the following:

(i)	Molarity	(a)	N
(ii)	Normality	(b)	m
(iii)	Molality	(c)	M

Notes

WHAT HAVE YOU LEARNT

- Preparation solutions of chemical reagents are very important task for the beginners of biochemistry.
- Concentration of a biological solution could be expressed in terms of equivalent weight, normality, molarity, molality, percent solution (weight/ weight, weight/ volume, volume /volume).
- The equivalent weight is determined by dividing the atomic or molecular weight by the valence.
- Molarity could be simply termed as "M" and are units of moles of solute per liter of solution.
- Molality is units of moles of solute per kilogram of solution and is termed as "m". While the normality is termed as "N" and are units of equivalent of solute per liter of solution.
- The percentage solution could be expressed in terms of weight percent (% w/w), volume percent (% v/v) and weight-to-volume percent (% w/v) units of solute present in 100 units of solution.

TERMINAL QUESTIONS

1. Write a brief note about the importance of solution preparation in biochemistry.
2. Write a note on Equivalent weight.
3. Describe about molarity and molality.
4. Write a short note on normality.
5. Write about different terms of percent solutions.
6. Tabulate the units and symbols of different modes of concentrations.

ANSWERS TO INTEXT QUESTIONS

1.1

1. Valence
2. 8
3. Potassium hydrogen phthalate

1.2

1. Molarity
2. Concentrations
3. Molarity
4. Molarity

1.3

1. Molality
2. 1
3. Small 'n'

1.4

1. Normality
2. Hydrochloric acid
3. Two
4. Molecular weight

1.5

1. (% w/w)
2. 200
3. Liquids
4. Match the following:
   (i) (b)
   (ii) (a)
   (iii) (c)