

**Course : PG-Pathshala**

**Paper 1: Fundamental of Biophysics**

**Module 31: Aqueous solutions, Acids & Bases, measurements of pH, Henderson Haselbatch equation, Titration curve & pK values, Buffers**

**Content Writer: Dr. M. R. Rajeswari, AIIMS, New Delhi**

**Introduction:** In our day to day life we rarely come across pure substances. Most of these are mixtures of two or more pure substances. The importance of such substances in life depends on their composition. For example, the properties of German silver (mixture of copper, zinc and nickel) are quite different from those of bronze (mixture of copper and tin) or brass (mixture of copper and zinc); 1 part per million of fluoride ions in H<sub>2</sub>O prevents tooth decay whereas increasing concentration to 1.5 ppm causes the tooth to become mottled and can be poisonous (used in rat poison). The intravenous injections are always dissolved in water containing salts at particular ionic concentrations that match with blood plasma concentrations and so on.

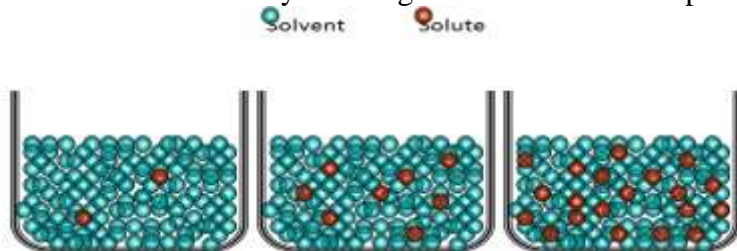
In this Unit, we will discuss aqueous solutions and their properties. This will be followed by studying the quantitative properties of the solutions, like concentration and pH. We will begin with brief introduction on solutions and their property.

**Objectives:**

- ❖ Express concentration of solution in different units
- ❖ Describe properties of solutions
- ❖ Distinguish between acidic and basic solutions
- ❖ Application of Henderson Haselbatch equation
- ❖ Explain the functions of buffers
- ❖ Explain titration curve & pK values

**Aqueous solution**

**Solution** is a homogeneous mixture of two or more chemically non-reacting substances whose composition can be varied within certain limits. In a homogeneous mixture, all the particles are of molecular size i.e. up to  $10^{-9}$  m. Every solution is made up of a solvent and one or more solutes. Solvent is that component of the solution which is present in large amount by the weight than the other component, termed as solvent. **Solute** is that component of the solution which is present in small amount by the weight than the other component, termed as solute.



**Units of Concentration:**

## 1 Ppm and ppb

The concentration of a solution is the amount of the solute dissolved in a known amount of the solvent or solution. **Parts per million parts (ppm)** is defined as the mass of the solute present in one million ( $10^6$ ) parts by mass of the solution. Thus, for a solute A, ppm is  $(\text{mass of A} \times 10^6) / (\text{mass of solution})$ . Often biologists express the concentration of solution in **percentage**. 'The percentage by weight' means the weight of the solute in grams present in 100g of the solution. 'The percentage by volume' means the weight of the solute in grams dissolved in 100  $\text{cm}^3$  of the solution, in case of a solid dissolved in a liquid. But in case of a liquid dissolved in another liquid, 'percentage by volume' means the volume of the liquid solute in  $\text{cm}^3$  present in 100  $\text{cm}^3$  of the solution.

The 'strength' of a solution is defined as the amount of the solute in grams present in one litre or  $\text{dm}^3$  of the solution. It is expressed in g/litre or  $\text{g}/\text{dm}^3$ .

**2 Molarity** is defined as the number of moles of the solute dissolved per litre of solution. It is represented by 'M' and is given by:  $(\text{Number of moles of solute}) / (\text{Volume of the solution in litre})$ .

12.6 g of NaCl are dissolved in water making 344mL of solution. Calculate the molar concentration

$$= \frac{12.6 \text{ g NaCl} \left( \frac{1 \text{ mol NaCl}}{58.44 \text{ g NaCl}} \right)}{344 \text{ mL} \left( \frac{1 \text{ L}}{1000 \text{ mL}} \right) \text{ solution}}$$

Ans = 0.627M

**3 Molality** : Molality (m) is defined as the number of moles of solute per kilogram of solvent.  
molality = moles of solute/kilograms of solvent

Note : **Molarity** is a measurement of the moles in the **total volume of the solution**, whereas **molality** is a measurement of the **moles in relationship to the mass of the solvent**.

### Example:

Compare the molar and molal volumes of 1 mol of a solute dissolved in  $\text{CCl}_4$  ( $d = 1.59/\text{mL}$ ).

For a **1 molal** solution, 1 mol of solute is dissolved in 1 kg of  $\text{CCl}_4$ .

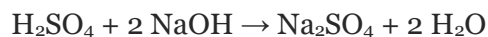
$1 \text{ kg of CCl}_4 \times (1,000 \text{ g}/1 \text{ kg}) \times (\text{mL}/1.59 \text{ g}) = 629 \text{ mL CCl}_4$

**4 Normality** is defined as the number of gram equivalents of the solute dissolved per litre of given solution. It is denoted by 'N'.  $(\text{number of gram equivalents of solute}) / (\text{Volume of the solution in litre})$ . Therefore, Normality (N) equal to the gram equivalent weight divided by liters of solution:

$N = \text{gram equivalent weight} / \text{liters of solution}$  (often expressed in g/L)

or it may be the molarity multiplied by the number of equivalents:

$N = \text{molarity} \times \text{equivalent}$  Ex: normality of 0.1 M  $\text{H}_2\text{SO}_4$  (sulfuric acid) for the reaction:




2 moles of H<sup>+</sup> ions (2 equivalents) from sulfuric acid react with sodium hydroxide (NaOH) to form sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) and water. Using the equation:

$$N = \text{molarity} \times \text{equivalents} \quad , \quad N = 0.1 \times 2, \quad N = 0.2 \text{ N}$$

**5 Mole Fraction:** Mole fraction of a constituent is the fraction obtained by dividing number of moles of that constituent (n<sub>1</sub>) by the total number (n<sub>1</sub>+n<sub>2</sub>+n<sub>3</sub> ...) of moles of all the constituents present in the solution. It is denoted by 'x'. Example is given below:

**Mole Fraction**



**Mole Fraction ( $\chi$ )**

$$\chi_A = \frac{\text{moles of A } \textcircled{A}}{\text{sum of moles of all components } \textcircled{A} + \textcircled{B}}$$

$$\chi_B = \frac{\text{moles of B } \textcircled{B}}{\text{sum of moles of all components } \textcircled{A} + \textcircled{B}}$$

Since A + B make up the entire mixture, their mole fractions will add up to one.  $\chi_A + \chi_B = 1.00$

The following Table summarises the units

Unit	Definition
ppm (Parts per million)	The mass of the solute present in one million (10 <sup>6</sup> ) parts by mass of the solution. Thus, for a solute A, ppm is (mass of A x 10 <sup>6</sup> ) / (mass of solution)
Percentage by Weight, % wt	The weight of the solute in grams present in 100g of the solution.
Percentage by volume, % vol	The weight of the solute in grams dissolved in 100 cm <sup>3</sup> of the solution.
Molarity, M	The number of moles of the solute dissolved per litre of solution.
Normality, N	The number of gram equivalents of the solute dissolved per litre of given solution..
Mole Fraction, X	The fraction obtained by dividing number of moles of that constituent (n <sub>1</sub> ) by the total number (n <sub>1</sub> +n <sub>2</sub> +n <sub>3</sub> ...) of moles of all the constituents present in the solution.

## Acid and Bases

Acid is a substance which is capable of furnishing  $H^+$  ions in aqueous solution or which is capable of donating proton or which can accept a pair of electrons. Base is a substance which is capable of furnishing  $OH^-$  ions in aqueous solution or which is capable of accepting proton or which can donate a pair of electrons.

**Strength of Acids and Bases:** It is usually expressed in terms of dissociation constant of acid ( $K_a$ ) and base ( $K_b$ ).



Or,

$$\frac{[H^+] \times [A^-]}{[HA]} = K_a$$

If concentration of acid  $HA = C$  mole/litre and also since  $[H^+] = [A^-]$  therefore, for monobasic acids,

$$\frac{[H^+]^2}{C} = K_a$$

Similarly, for monobasic base,

$$\frac{[OH^-]^2}{C} = K_b$$

It is noted that greater the value of  $[H^+]$  or  $[OH^-]$  greater is the strength of the acid or base respectively.

## Measurements of pH

The ability of water to dissociate into  $H^+$  and  $OH^-$  is a major controller of cellular pH.

$$[H^+] = [OH^-] = 1 \times 10^{-7}; \text{pH} = 7$$

The term pH defined as the negative logarithm of the hydrogen ion concentration in a given solution i.e.

$$\text{pH} = \log ( 1/ [H^+] )$$

The term pH is used to represent the scale of acidity. The concept of pH may be better understood by studying the ionization of pure water.

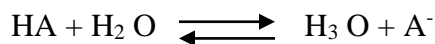
In an acidic solution,  $[H^+] > 10^{-7}$ , pH is less than 7

In an alkaline solution,  $[H^+] < 10^{-7}$ , pH is more greater than 7

## Buffers:

A buffer is a substance or mixture of substances that permit solutions to resist large changes in pH upon the addition of small amount of  $H^+$  or  $OH^-$  ions i.e. it helps to maintain a near constant pH upon the addition of small amount of  $H^+$  or  $OH^-$  ions. A buffer is a solution which resists any change in its pH value on dilution or on addition of an acid or alkali.

Common buffer mixtures contain two substances, a conjugate acid and a conjugate base. An 'acidic' buffer contains a weak acid and a salt of the weak acid (conjugate base). A 'basic' buffer contains a weak base and a salt of the weak base (conjugate acid).



According to the law of mass action;

$$k_1 [\text{HA}] [\text{H}_2\text{O}] = K_2 [\text{H}_3\text{O}^+] [\text{A}^-]$$

or, 
$$\frac{[\text{H}_3\text{O}^+] [\text{A}^-]}{[\text{HA}] [\text{H}_2\text{O}]} = \frac{k_1}{k_2} = K$$

or 
$$\frac{[\text{H}^+] \times [\text{A}^-]}{[\text{HA}]} = K'$$

The  $K'$  is the apparent equilibrium constant of the acid, depends on temperature and the ionic strength.

So, 
$$[\text{H}^+] = K' \frac{[\text{HA}]}{[\text{A}^-]}$$

Then take the logarithm of both sides;

$$\log [\text{H}^+] = \log K' + \log \frac{[\text{HA}]}{[\text{A}^-]}$$

$$-\log [\text{H}^+] = -\log K' + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

Replacing  $-\log [\text{H}^+]$  by pH and  $-\log K'$  by  $\text{p}K'$

$$\text{pH} = \text{p}K_a + \log \left( \frac{[\text{A}^-]}{[\text{HA}]} \right)$$

For Example, buffer solution made from 0.20 M  $\text{HC}_2\text{H}_3\text{O}_2$  and 0.50 M  $\text{C}_2\text{H}_3\text{O}_2^-$  that has an acid dissociation constant for  $\text{HC}_2\text{H}_3\text{O}_2$  of  $1.8 \times 10^{-5}$ .

$$\text{pH} = \text{p}K_a + \log \left( \frac{[\text{C}_2\text{H}_3\text{O}_2^-]}{[\text{HC}_2\text{H}_3\text{O}_2]} \right)$$

$$\text{pH} = -\log (1.8 \times 10^{-5}) + \log (0.50 \text{ M} / 0.20 \text{ M})$$

$$\text{pH} = -\log (1.8 \times 10^{-5}) + \log (2.5)$$

$$\text{pH} = 4.7 + 0.40$$

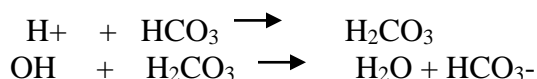
**Ans : pH = 5.1**

For a base, BH<sup>+</sup>,

$$pOH = pK_b + \log \frac{[BH^+]}{[B]}$$

This is known as **Henderson-Hasselbach** equation. This equation provides a method for the determination of pH of a buffer system when concentration of the acid and its salt is known. “Buffer capacity can be defined as the number of moles per litre of H<sup>+</sup> or OH<sup>-</sup> required causing a given change in pH”. In other words, the ability of a buffer to resist changes in pH is called ‘**Buffer capacity**’. It is denoted by  $\beta$ - Buffer value.

The process by which added H<sup>+</sup> and OH<sup>-</sup> ions are removed so that pH remains constant is known as buffer action. A mixture of carbonic acid (H<sub>2</sub>CO<sub>3</sub>) + sodium hydrogen carbonate (NaHCO<sub>3</sub>) buffer system is found in blood and helps in maintaining pH of the blood close to 7.4. Any excess of H<sup>+</sup> ion entering the blood stream is removed by H<sub>2</sub>CO<sub>3</sub> as in other buffer solutions.



Moreover, the pH value of gastric juice is maintained between 1.6 and 1.7 due to buffer system. The major buffer systems found in living cells are those of phosphate, bicarbonate and histidine residues in protein complexes. Buffer systems are important practically in all physiological system as these help in maintaining the optimum H<sup>+</sup> concentration (i.e. pH) which is necessary for the optimal activity of enzymes. By far the most important buffer for maintaining acid-base balance in the blood is the carbonic-acid-bicarbonate buffer.

Other buffers perform a more minor role than the carbonic-acid-bicarbonate buffer in regulating the pH of the blood. The phosphate buffer consists of phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) in equilibrium with dihydrogen phosphate ion (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) and H<sup>+</sup>. The pK for the phosphate buffer is 6.8, which allows this buffer to function within its optimal buffering range at physiological pH.

**Some commonly used buffers in biological systems and their range are given below :**

Buffer	pH range
1. Hydrochloric Acid-Potassium Chloride (HCl-KCl)	1.0 to 2.2
2. Glycine-HCl	2.2 to 3.6
3. Citrate	3.0 to 6.2
4. Sodium Acetate - Acetic Acid	3.6 to 5.6
5. Citrate-Phosphate	2.6 to 7.0
6. Phosphate Buffer (Sodium phosphate monobasic -	5.8 to 8.0

Sodium phosphate dibasic)	
7. Tris-HCl	7.2 to 9.0
8. Glycine-Sodium Hydroxide	8.6 to 10.6
9. Carbonate-Bicarbonate	9.2 to 10.6

## Titration Curve

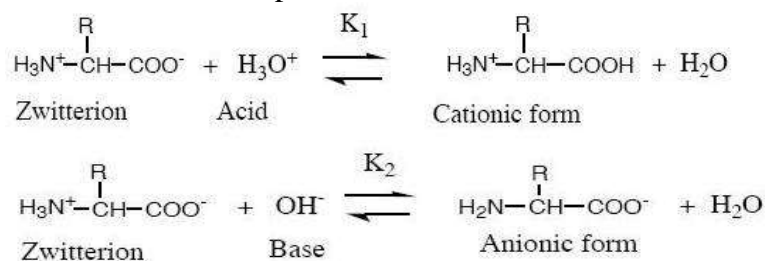
A titration curve is a plot of pH vs. the amount of titrant added. Typically the titrant is a strong (completely) dissociated acid or base. Such curves are useful for determining endpoints and dissociation constants of weak acids or bases.

### Features of the Strong Acid-Strong Base Titration Curve:

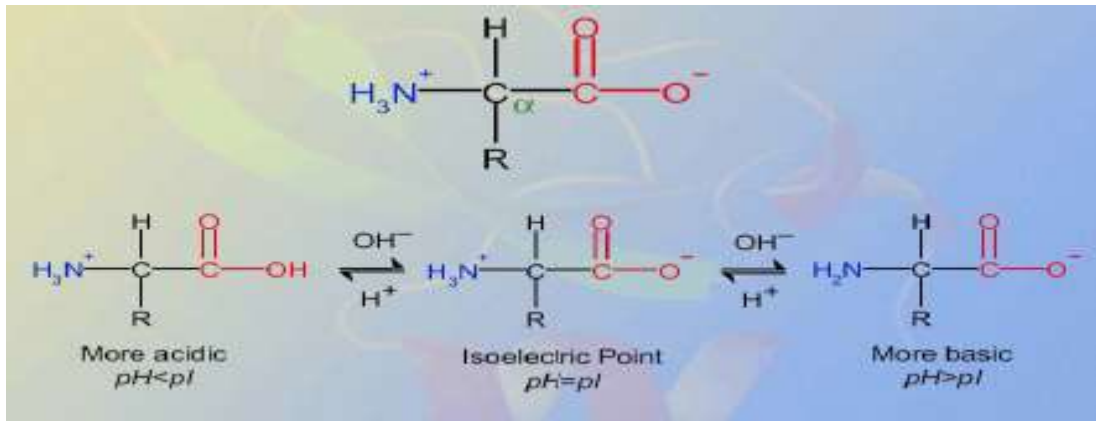
1. The pH starts out low, reflecting the high  $[H_3O^+]$  of the strong acid and increases gradually as acid is neutralized by the added base.
2. Suddenly the pH rises steeply. This occurs in the immediate vicinity of the equivalence point. For this type of titration the pH is 7.0 at the equivalence point.
3. Beyond this steep portion, the pH increases slowly as more base is added.

## Titration of Amino Acids

Amino acids play an integral role in the structure and function of proteins and participate in nearly every physiological event within a cell. The acid-base properties of protein depends on its constituent amino acids. Each amino acid contain at least one amino and carboxylic acid group, which make them amphoteric substances that react with both acids and bases as follows:



The molecules which contain an equal number of ionizable groups of opposite charges, therefore bears a **net charge 'zero'** are called "**zwitterions or dipolar ion**". The amino acids with net charge zero are also called **ampholytes**. Zwitterionic form of any amino acid is-



Amino acids behave as ampholytes at any pH depending on the pK of the side chain group. **Isoelectric point is that pH at which there is no net charge** on the amino acid and exists in the Zwitterionic form, and is represented by pI. At this pH (pI) the amino acid does not migrate to any electrode under the influence of electric field.

The pI of an amino acid depends on the nature of side chain and its ionization. The order of **proton dissociation depends on the acidity of the proton** i.e. group which is most acidic (lower pKa) will dissociate first. Therefore hydrogen on the  $\alpha$ -COOH group ( $pK_1$ ) will dissociate before that of  $\alpha$ -NH<sub>3</sub> group ( $pK_2$ ). Amino acids which have additional acidic or basic side chain, have different pI because of the influence of the extra ionizable group. **The net charge on an amino acid is the algebraic sum of the positively and negatively charged groups present** and it depends upon the pKa value of the each functional group and on the pH of surrounding medium. For polyfunctional acids, pI is also approximately the **midway between the pKa values on either side of the isoionic species**. That means acidic amino acid have the pI between the two acid group while basic amino acids have the pI in the mid way between two NH<sub>2</sub> group.

The pI for lysine is simply the average of the  $pK_a$ 's of the two -NH<sub>3</sub><sup>+</sup> groups.

$$pI = (pk_2 + pk_3) / 2 = (9.18 + 10.79) / 2 = 9.99.$$

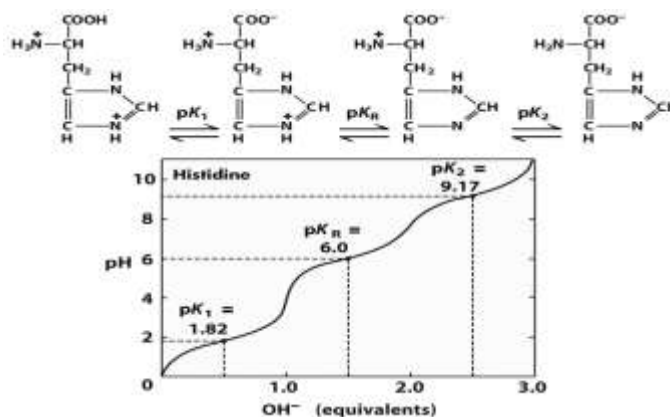


Fig: Titration curve of histidine showing the three ionizable states.



The pKa value varies with the environment. In a protein, **polar environment amino acid residue favors charge separation**, while **nonpolar environment favors the uncharged species**. A nonpolar solution determines the pKa of a COOH group making it weak acid and lowers pKa of the amino group, making it as strong acid.

## Summary:

- **Solutions** are defined as homogeneous mixtures of two or more pure substances. Every solution is made up of a solvent and one or more solutes. Solvent is that component of the solution which is present in large amount by the weight than the other component, termed as solvent.
- Concentration and pH are two important quantitative properties of aqueous solutions. The concentration of a solution is the amount of the solute dissolved in a known amount of the solvent or solution. Concentration is expressed in terms of **molarity, molality, mole fraction, parts ratio, percentage weight etc.**
- Acid is a substance which is capable of furnishing H<sup>+</sup> ions in aqueous solution. Base is a substance which is capable of furnishing OH<sup>-</sup> ions in aqueous solution. pH is used to represent the scale of acidity. A buffer is a solution which resists any change in its pH value on dilution or on addition of an acid or alkali.
- **The Henderson–Hasselbalch Equation** describes the derivation of pH as a measure of acidity in biological and chemical systems. The equation is also useful for estimating the pH of a buffer solution. It is widely used to calculate the isoelectric point of proteins (point at which protein neither accept nor yield proton) in titration curve.
- Titration curves are obtained when the pH of given volume of a sample solution varies after successive addition of acid or alkali. It is used in estimating the acid base behaviour and pKa values of the ionizable groups of the amino acid.
- Amino acids are Zwitterions and their pI depends on the pKs of side chain group. Glycine has only hydrogen as side chain and has pI of 6.06