Sub- Physical Pharmacy, B. Pharm, 3rd semester

Unit- V: pH, Buffers and Isotonic solutions.

SYLLABUS TOPIC- SORENSON'S pH SCALE, pH DETERMINATIONS

Sorenson's pH scale:

pH refers to potential of hydrogen ions concentration. Sorenson's has defined pH of a solution as the logarithm of the reciprocal of the hydrogen ions or hydronium ions concentration [H_3O^+].

Mathematically

pH= log 1/ [H_3O^+] ---------- (1)

The above equation can be rearranged as

pH= log 1- log [H_3O^+] ---------- (2)

=> pH= - log [H_3O^+] or pH= - log [H^+] {as the value of log 1 is Zero} ---------- (3)

Hence pH can also defined as the negative logarithm of hydrogen ion or hydronium ions concentration. The concentration of [H_3O^+] is expressed in molarity, mol/L, etc.

In pure water [H^+] = 1.0 X 10^{-7}

So pH of neutral (pure) water is –log (10^{-7}) = 7

Acidic solution: The solutions having [H^+] value greater than 10^{-7} are called acidic solution and the solutions having [H^+] value less than 10^{-7} are called basic solution. Hence pH value of all acidic solutions are less than 7 and pH value of all basic solutions are greater than 7.

Sorenson developed a scale based on the pH value and different concentration of H_3O^+ in a solution which is called Sorenson's pH scale (Table-1).

The magnitude of the hydrogen ion is represented by means of the normality factor with regard to hydrogen ion, and this factor is written in the form of a negative power of 10. Sorenson employ the name 'hydrogen ion exponent' and the symbol pH for the numerical value of this power.

Sorenson's scale (Table-1) assigns a pH of 0 to 14, with 0 being the most acidic, 14 being the most basic, and 7 being neutral (neither acidic nor basic). The pH scale works in powers of ten, so each jump in number is a multiple of ten in concentration. For example a pH of 1 is 10 times more acidic than a pH 2. The value 7 at which the hydrogen and hydroxyl ion concentrations are about equal at room temperature is referred to as the neutral point, or neutrality. The neutral pH at 0°C is 7.47, and at 100°C it is 6.15.

The generalisations reported above regarding the acidity, neutrality and basicity hold good only when

1. Solvent is water
2. Temperature is 25°C
3. No other factors present that cause deviations.

Table-1: The pH scale and corresponding hydrogen and hydroxyl ion concentrations

pH	$[H_3O^+]$ (moles/liter)	$[OH^{-1}]$ (moles/liter)	
0	10^0	10^{-14}	
1	10^{-1}	10^{-13}	
2	10^{-2}	10^{-12}	
3	10^{-3}	10^{-11}	Acidic
4	10^{-4}	10^{-10}	
5	10^{-5}	10^{-9}	
6	10^{-6}	10^{-8}	
7	10^{-7}	10^{-7}	Neutral
8	10^{-8}	10^{-6}	
9	10^{-9}	10^{-5}	
10	10^{-10}	10^{-4}	
11	10^{-11}	10^{-3}	Basic
12	10^{-12}	10^{-2}	
13	10^{-13}	10^{-1}	
14	10^{-14}	10^0	

Applications:

The pH of the solutions must be controlled in pharmacy particularly in formulations of eye drops, ear drops, injections and liquid orals for the following reasons

1. ***Enhancing solubility and stability:*** The pH of the pharmaceutical preparations should be adjusted so as to make the API soluble and remain physically stable in the formulation.
2. ***Improving purity:*** The purity of the protein can be determined as the amphoteric compounds are least soluble at their isoelectric points.
3. ***Absorption of drugs:*** The drug molecules are absorbed differently from various parts of the GIT as the later differs in their pH.
4. ***Optimising biological activity:*** Enzymes have maximum activity at a definite pH value.
5. ***Comforting the body:*** The pH of the formulations that are administered to different tissues of the body should be optimum to avoid irritation (eyes), haemolysis (blood) or burning sensation (abraded surface).
6. ***Storage of products:*** Special type of glass is used in case the glass container imparts alkalinity and alters the pH of the contents.

The pH indicators:

The pH indicator is a weak acid or weak base that exists in tautomeric form that readily interconvert. It is a solution when added to test solution produces a colour change, which helps in determining the pH of the test solution. The colour of any indicator depends on the pH of the solution (Table-2).

Ex- Phenolpthalein, methyl red, Thymol blue etc.

Universal indicator

Universal indicator is defined as a mixture of several indicators, which gives different color shades as the pH of the solution varies, in a particular pH range.

Table-2: Some indicators and change of colours with pH.

Name of the indicator	pH range	Colour change	Universal indicator
Methyl yellow	3.1-4.4	Blue-yellow	Mixture of all indicator range of pH is 1 to 11.
Methyl red	4.2-6.2	Red-yellow	
Bromothymol Blue	6.0-7.6	Yellow-blue	
Thymol blue	8.0-9.6	Yellow-blue	
Phenolpthalein	8.3-10.0	Colourless-pink	

Measurement of pH:

The are two widely accepted methods for the determination of the pH of a solution

(a) Colorimetric method
(b) Electrometric method

Colorimetric method:

This method based on the principle of colour comparison of the test solution to that of the standard both treated with universal indicator. This method is used to determine the pH of the solution in the pH range of 3 to 11 ± 0.2 units. Commercially available indicator strips of filter papers are used for identifying the pH. Otherwise several standard solutions can be prepared or procured which are mixed solution of buffer and indicator. Also **Capillators** and **Comparators** are commercially available for this purpose.

Capillators: Standard solutions (mixture of buffer solution and universal indicator) of small volume placed in capillary tubes are called capillators.

Comparators: Standard solutions (mixture of buffer solution and universal indicator) of large volume placed in capillary tubes are called comparators. This is useful in examining turbid and coloured solutions.

Method:

Step-1: Standard buffer solutions of known pH ranging from 3.0 to 11.0 are prepared with 1.0 pH interval.
Step-2: A few drops of universal indicator solutions are added to the above solution that produce different colours. Similarly few drops of universal indicator are also added to the solution to be tested. It produce a colour depending upon its pH.
Step-3: The colour of the test solution is compared with the colour of the standard solutions. The pH of the standard solution that has nearly same colour as that of test is consider as the approximate pH of test solution.
Step-4: In a similar way the test solution is again compared with the colour of the indicator treated standard solution of narrow pH range with 0.2 pH interval.
Step-5: Step 2 and 3 are again repeated and the pH of the solution is reported.

Precautions:

Standard solutions must be protected from light to avoid colour fading
All tubes must have same dimension. i.e. tube diameter and thickness of glass.

Advantages:

- ✓ Less expensive
- ✓ Acid-base reaction of non-aqueous solution can be studied.
- ✓ Easy estimation of pH unless the drug shows buffer action.

Disadvantage:

- ✓ This method is less accurate and less convenient
- ✓ It is not useful for coloured or turbid solution.
- ✓ The indicators used may impart a deviation in pH to buffered solution.
- ✓ This is not useful in presence of salts, proteins etc.

Electrometric method

Principle:

The magnitude in the potential difference between glass and a solution containing hydrogen ion varies with concentration of H^+ concentration. Hence the pH of the solutions are determined by means of the electrodes. Hydrogen electrode and glass electrodes are used for this purpose. However glass electrodes are commonly used. The instrument used to determine the pH of unknown solution by this method is called pH meter.

Method:

A pH metre with its control knobs are presented in fig.1. The glass electrode is attached to the instrument.

Figure 1: The pH meter with glass membrane electrode.

Step-1: At first the instrument temperature is set to that of the solution temperature.
Step-2: The electrode is immersed into a standard buffer solution of pH 7.0. The potential control knob is adjusted till the pH reading in digital meter becomes 7.0.
Step-3: Then the instrument is calibrated using standard buffers of pH 4.0 (M/20 potassium hydrogen phthalate) or/and pH 9.14.
Step-4: The electrode is now rinsed with distilled water properly and re-immersed into the test solution. The pH value is obtained from the digital meter.

The pH of the test solution can be changed by the addition of slight amount acid or base solution (depending upon the desired direction of change) and the procedure is followed till the desired pH is obtained.

Advantages:

- ✓ It gives an accurate measurement of pH.
- ✓ Glass electrode is not affected by oxidation-reduction system.
- ✓ The electrode establishes equilibrium rapidly.
- ✓ The indicator need not required.
- ✓ The pH range of measurement is large.

Disadvantages:

- ✓ The cost of pH meter is high compared to colorimetric method.
- ✓ This method is not suitable for viscous solutions and gels because of poor ionic mobility.

SYLLABUS TOPIC: APPLICATION OF BUFFERS, BUFFER EQUATION, BUFFER CAPACITY, BUFFERS IN PHARMACEUTICAL AND BIOLOGICAL SYSTEMS.

Buffers: Buffers are defined as a compound or a mixture of compounds that resists the pH upon the addition of small quantities of acid or alkali. Buffer have definite pH value. The pH will not change after keeping it for a long period of time. The pH value altered negligibly by the addition of small quantities of acid /base.

Buffer action: The resistance to a change in pH is known as buffer action. So buffers can be added to show buffer action.

Buffer capacity: The amount of acid/base required to produce a unit change in pH in a solution is called buffer capacity.

Applications of Buffers:

- **Solubility enhancement:** The pH of the pharmaceutical formulations are adjusted to an optimum value so that the drug remain solubilised though out its shelf-life and not precipitated out.
- **Increasing stability:** To prevent hydrolysis and for maximum stability**,** the pH of the medium should be adjusted suitably.
- **Improving purity:** The purity of proteins can be identified from its solubility at their isoelectric point as they are least soluble at this point. The isoelectric pH can be maintained using suitable buffers.
- **Optimising biological activity:** Enzymes have maximum activity at definite pH values. Hence buffer of desired pH is added to the preparation.
- **Comforting the body:** The pH of the formulations that are administered to different tissues of the body should be optimum to avoid irritation (eyes), haemolysis (blood) or burning sensation (abraded surface). The pH of the preparation must be added with suitable amount of buffers to match with the pH of the physiological fluid

Buffer systems:

The buffer systems are classified as followings

(a) Weak acid and its conjugate base, i.e. salt of week acid with a strong base. Ex- acetic acid and sodium acetate.
(b) Weak base and its conjugate acid, i.e. salt of week base with a strong acid. Ex- ammonium hydroxide and ammonium chloride.
(c) Two salts acts as acid-base pair. Ex- Potassium hydrogen phosphate and potassium dihydrogen phosphate.
(d) Amphoteric electrolyte. Ex- Solution of glycine.
(e) Solution of strong acid and solution of strong base. Ex- Strong HCl with KCl.

Some important buffer system and their pH is given below in table-3

Table-3: Some important buffer system and their pH.

System	pH
HCl and KCl	1.2 to 2.2
HCl and potassium hydrogen phthalate	2.2 to 4.0
Sodium hydroxide and potassium hydrogen phthalate	4.2 to 5.8
Boric acid and sodium carbonate monohydrate	5.0 to 9.0
Potassium dihydrogen phosphate and sodium hydroxide	5.8 to 8.0
Boric acid, sodium hydroxide and potassium chloride	8.0-10.0

Mechanism of Buffer action

In a buffer solution, the components interact with each other and produce a dynamic equilibrium. When a small quantity of acid or base is added, the dynamic equilibrium shifts and nullifies the effect of the addition.

Buffer action of acidic buffer:

Consider an acid buffer, i.e. acetic acid and sodium acetate. The ionization equation are written as:

Strong electrolyte:

$CH_3COONa \xrightarrow{H_2O} Na^+ + CH^3COO^-$ completely ionized

Weak acid:

$CH_3COOH + H_2O \Longleftrightarrow H_3O^+ + CH_3COO^-$ slightly ionized

Therefore, the solution contains very few H_3O^+ ions, but has an excess sodium ions and acetate ions. When a small amount of acid is added, the H_3O^+ ions present in the solution react with CH_3COO^- as

$H_3O^+ + CH_3COO^- \rightarrow CH_3COOH + H_2O$

Since added free H_3O^+ ions are not available, pH does not change. When a small amount of base is added, the hydroxyl ions furnished by the base are neutralised by acetic acid as:

$OH^- + CH_3COOH \rightarrow CH_3COO^- + H_2O$

Since added free OH^- ions are not available, pH does not change. Thus buffer action is maintained when a small amount of acid or base is added. This process continues until entire acetate ions or acetic acid is consumed, action is not unlimited.

The mechanism of buffer action of acid-base pair (example is phosphate buffer) is similar to that mentioned above. In phosphate buffer, weak acid conjugate base are involved, i.e. ion $H_2PO_4^-$ serves as weak acid and HPO_4^- acts as its conjugate base.

Buffer Action of Alkaline Buffer

Buffer action of a mixture of a weak base and its salt, for example ammonium hydroxide and ammonium chloride, is considered. The ionization equation is written as:

Strong electrolyte:

$$NH_4Cl \xrightarrow{H_2O} NH_4^+ + Cl^- \quad \text{- completely ionised}$$

Weak base:

$$NH_4OH \xrightarrow{H_2O} NH_4^+ + OH^- \quad \text{- slightly ionized}$$

Therefore, the solution contains very few OH^- ions, but has an excess of ammonium ions and chloride ions.

When a small amount of acid is added, the H_3O^+ ions obtained from acid react with NH_4OH as

$$H_3O^+ + NH_4OH <=> NH_4^+ + 2\ H_2O$$

Since added free H_3O^+ ions are not available, pH does not change.

When a strong base is added, the hydroxyl ions furnished by the base are neutralised by NH_4^+ as:

$$OH^- + NH_4^+ <=> NH_4OH$$

Since added free OH^- ions are not available, pH does not change. Thus buffer action is maintained when a small amount of acid or base is added. This process continues until entire ammonium hydroxide or ammonium ions are consumed. Hence buffer action is not unlimited.

Ampholytic Substances:

Ampholytes and amphoteric electrolytes are the substances that capable of acting both as an acid and a base. For example, glycine, like an acid as shown below.

$$NH_2CH_2COOH + H_2O <=> NH_2CH_2COO^- + H_3O^+$$

Glycine also behaves as a base as shown below.

$$NH_2CH_2COO^- + H_3O^+ <=> {}^+NH_3CH_2COO^- + H_2O$$

These doubly charged ions are known as zwitter ions or dipolar ions he above system reacts with H_3O^+ ions or OH^- ions and nullify the influence of the added substances.

Buffer equation-Henderson-Hasselbalch equation:

The buffer equation is also known as Henderson-Hasselbalch equation. Two separate equations are obtained for each type of buffer, acidic and basic. Buffer equation is developed based on the effect of salt on the ionization of a weak acid, when the salt and acid have a common ion.

An acid buffer, acetic acid and sodium acetate, is considered for deriving the buffer equation. The ionization equilibrium equation for weak acid (acetic acid) may be shown as:

Weak acid:

$CH_3COOH + H_2O <=> H_3O^+ + CH_3COO^-$ -slightly ionized

Applying the Law of Mass Action, the acid dissociation constant (K_a) is written as:

$K_a = [H_3O^+] [CH^3COO^-]/ [CH_3COOH] = 1.75 \times 10^{-5}$ ----------(1)

When sodium acetate is added to acetic acid, equation (1) is momentarily disturbed. Since, salt also supplies the acetate ion, the term $[CH_3COOH]$ in the numerator increases. In order to re-establish the constant K_a at 1.75×10^{-5}, the hydronium ion $[H_3O^+]$ in the numerator instantaneously decreases. In other words, the equilibrium is shifted in the direction shown below.

$CH^3COO^- + H_3O^+ \rightarrow H_2O + CH_3COOH$

In other words, common ion, $[CH^3COO^-]$ repressed ionization of acetic acid. This is an example of common ion effect.

The pH of the final solution may be obtained by rearranging equation (1).

$[H_3O^+] = K_a [CH_3COOH] / [CH^3COO^-]$ ----------(2)

Since, the acid is weak and ionizes slightly, $[CH_3COOH]$ may remain unaltered. Hence, $[CH_3COOH]$ = [acid].

Since salt is completely ionized, the entire $[CH^3COO^-]$ may be obtained directly from the salt and be written as [salt]. Hence, $[CH^3COO^-]$ = [salt).

Substituting them in equation (2) gives:

$[H_3O^+] = K_a$ [acid] / [salt] ----------(3)

Taking logarithm of equation (3) and reversing the signs give:

$- \log [H_3O^+] = - \log K_a - \log$ [acid] / [salt]----------(4)

But $pH = -\log [H_3O^+]$ and $pK_a = -\log K_a$. By substituting these values in equation (4) gives:

$pH = pK_a + \log$ [acid] / [salt]----------(5)

Equation (5) is known as buffer equation or Henderson-Hasselbalch equation for acid buffer. Similarly buffer equation for a solution containing weak base and the corresponding salt may be derived in a similar manner. Equation for the calculation of $[OH^-]$ may be written as:

$[OH^-] = K_b$ [base]/[salt] ----------(6)

Henderson-Hasselbalch's equation for basic buffers is:

$pH = pK_w + pK_b + \log$[salt]/[acid] ----------(7)

Applications:

- ✓ For a definite pH solution, it is essential to add salt and acid (or base) to water in a desired ratio. This ratio is determined by Henderson-Hasselbalch equation.
- ✓ Since salt and acid are added in the preparation of a buffer solution, their concentrations are known. Using this data, the resultant pH of a solution can be calculated using buffer equation.
- ✓ Equations (5) and (7) permit the calculation of the percent of drug ionized (or ionized) in the solution. This knowledge is important in predicting the drug absorption, because only unionized molecules can penetrate cell membranes (lipid in nature) more readily than ionized molecules.
- ✓ The pK_a of various drugs can be determined from pH of solutions
- ✓ The solubility of a substance at any pH can be predicted provided intrinsic solubility and pK_a are known.
- ✓ A suitable sält forming substance can be selected based on Henderson Hasselbalch equation.

Buffer Capacity:

Buffer efficiency or buffer capacity is defined as the ratio of the increment of strong base (or acid) to the small change in pH brought about by this addition.

Buffers resist the change in pH. However, the pH of the solution does change when a large quantity of acid or base is added. The magnitude of the resistance of a buffer to pH change is referred to as the buffer capacity, buffer index and buffer value.

Buffer capacity, β, is mathematically expressed as:

$\beta = \Delta B / \Delta pH$ ----------(8)

where B = concentration of base (or acid) added, gram-Eq/L

According to equation (8), the buffer capacity has a value of 1 when I gram equivalent of strong base (or acid) is added to 1 litre of buffer solution, if the change in pH is I unit.

The buffer has its greatest capacity, when [salt]/[acid] is equal to 1. Therefore, Henderson-Hasselbalch equation may be written as $pH = pK_a$. Buffer capacity decreases appreciably as the pH deviates more than 1 unit on each side of the pK_a value (fig-2). Buffer capacity is not a fixed value for a given buffer system, but depends on the amount of base added. Buffer capacity changes as the ratio of log [salt]/[acid] increases with added base. Buffer equation can be used to calculate the pH of the solution after the addition of base.

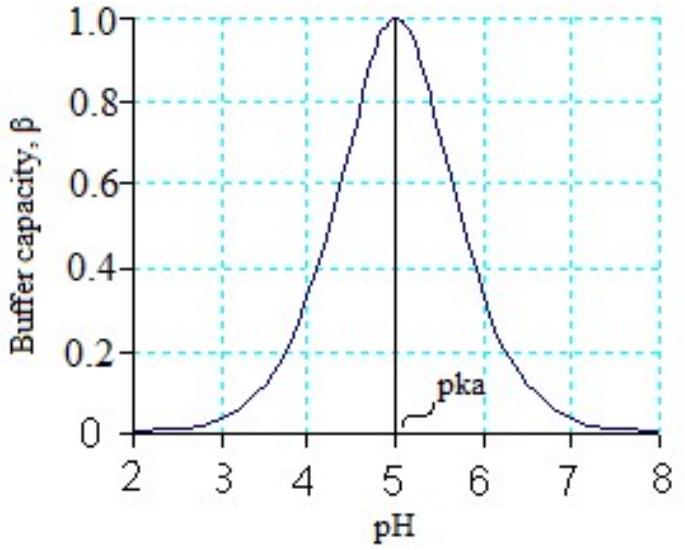

Figure 2- A typical Buffer capacity diagram of a buffer solution.

Buffer capacity is also influenced by the total concentration of the ier constituents. The greater the concentration of salt and acid, the greater is the buffer capacity. For this reason, buffers are expressed in terms of molar concentrations namely 0.2 M, 0.02 M, etc.

Van Slyke's equation can be used for calculating the buffer capacity of a buffer.

$$\beta = 2.303\ C\ \left\{K_a\,[H_3O^+]/\ (K_a + [H_3O^+])^2\right\} \quad \text{----------(9)}$$

where C= concentration of total buffer (sum of acid and salt)

Equation (9) permits the calculation of Bat any hydrogen concentrate At maximum capacity, pH=pK_a or the term $[H_3O^+] = K_a$

Hence, equation (9) changes to $\beta_{max} = 0.576\ C$ ----------(10)

BUFFERS IN BIOLOGICAL SYSTEMS:

Blood: Blood consists of primary (plasma) and secondary buffer Erythrocytes) systems contributing the pH 7.4. When the pH of the blood s below 7.0 or above 7.8, life is in danger. The pH of the blood in diabetic coma is reported to drop as low as 6.8.

Primary buffers present in plasma are carbonic acid-bicarbonate system and acid/alkali salts of phosphoric acid system.
Secondary buffers that are present in erythrocytes are Haemoglobin/oxyhaemoglobin system and acid/alkali salts of phosphoric acid system.
The buffer capacity is 0.0318+ 0.0035 for the whole blood, in which 0.031 is contributed by the cells and 0.008 is contributed by the plasma.

Lacrimal fluids: Lacrimal fluids (or tears) have been found to have a great degree of buffer capacity, allowing dilution of 1:15 with neutral distilled water. The pH of tears is about 7.4, with a range of 7.0 to 8.0. Normally, pure conjunctival fluid is more acidic than the tear fluids

commonly employed in pharmacy. The pH increases rapidly when the sample is removed for analysis because of loss of carbon dioxide from the tear fluid.

Urine: The pH of urine is 6.0 for normal subjects (adults), when 24 h urine was collected. The pH may be as low as 4.5 or as high as 7.8. The pH of urine is maintained in the following manner.

If urine pH is low (4.5), hydronium ions are excreted into it urine by the kidneys.
If urine pH is high (7.4), hydronium ions are retained by the action of kidneys.

SYLLABUS TOPIC: BUFFERED ISOTONIC SOLUTION

Buffered isotonic solution:

Isotonic buffered solution is defined as a solution which maintains the iso-tonicity and the pH as that of the body fluids.

Isotonic buffer solution should be compatible with the body fluids for the following reasons.

- ✓ Blood and lacrimal fluids are in vivo buffer systems. Any solution that comes in contact with these fluids should be buffered to a desired pH, so that these are compatible with the body fluids.
- ✓ Some solutions are meant for the application on delicate membranes of the body. Such solutions may cause haemolysis, tissue irritation, necrosis and tissue toxicity. In such cases, solutions must be just to the same osmotic pressure and tonicity as that of the body fluids.

Applications:

Isotonicity should be adjusted for several dosage forms.

1. Parenteral preparations should be isotonic with blood plasma. There can be some flexibility depending on the route of administration and Quantity of solution to be injected.
   (a) Intravenous infusions, irrigating solutions, lotions for open wound
   (b) Subcutaneous injection.
   (c) Parenteral preparations meant for diagnostic purposes, in order to avoid false reaction,
   (d) Solutions meant for intrathecal injection, because the volume of CSF (Cerebro Spinal Fluid) is only 60 to 80 mL. Hence, hence or hypotonic solutions though in small volumes, will disturb the osmotic pressure and may cause vomiting and other effects.
2. Aqueous solutions used as nasal drops.
3. Ophthalmic drops

Preparation of Isotonic Buffer Solution:

1. The drug and other ingredients are dissolved in water.
2. The pH of the solution is determined and adjusted to the desired value.
3. The tonicity value of the solution is calculated procedures using standard procedures.
4. The amount of sodium chloride required to adjust the tonicity is calculated.
5. The required amount of sodium chloride is added to the solution, so that the final solution becomes isotonic.
6. Isotonic diluting solution is added for maintaining the drug concentration to the desired level (dose).
7. If the pH is also needs to be maintained, then buffered isotonic diluting solution is added to make up the desired volume (dose).

Since the drug solution is already isotonic, any isotonic diluting solution (electrolytes) can be used to dilute the solution. Some of the isotonic diluting solutions are:
Isotonic sodium chloride solution, Dextrose solution, Ringer solution etc

The isotonic buffered diluting solutions are available in acidic, neutral and alkaline range. Isotonic buffered diluting solutions are:

Osmosis - Osmotic Pressure:

Osmosis is defined as a process in which the solvent molecules pass through a semipermeable membrane from a pure solvent to a solution or from a dilute solution to a concentrated solution.

Consider a case in which a solution is confined in a membrane that is permeable to solvent. When it is placed in a solvent, diffusion of solvent molecule is observed. This phenomenon is termed as osmosis. The semipermeable membranes (animal and vegetable) regulate the passage of solvent molecules by electrostatic and chemical interactions. The process of osmosis proceeds to equalize the concentration in contact with each other. Thus equilibrium state is achieved. A semipermeable membrane is a barrier which selectively permits the passage of solvent molecules, but not the solute molecules.

The experimental setup for demonstrating the osmosis experiment is shown in Fig-3. Thistle tube has a wide opening at one end. A piece of untreated cellophane is stretched and tied. The tube is partly filled with a concentrated solution of sucrose (a non-volatile substance). The thistle tube is immersed in a beaker of water (Fig-3).

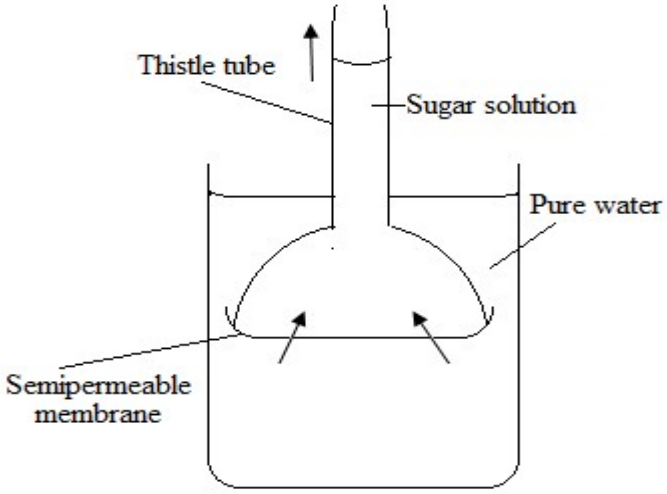

Figure 3: Demonstration of osmotic experiment

The diffusion of solvent molecules takes place in both directions. But more number of water molecules passes from the beaker (escaping tendencies) through the semipermeable membrane into the tube. This is an attempt to dilute the solute and rise the vapour pressure to its original value. This process creates enough pressure to drive the sugar solution rise up in the tube. At one point, the rise of solution in the tube stops, i.e. equilibrium is achieved.

At equilibrium: Hydrostatic pressure of the column of liquid is equal to Flow of water (osmotic pressure) causing the water to pass through the membrane and enters the tube.

Since vapour pressure of the solvent is higher than that of the solute, water molecules diffuse.

Osmotic pressure may be defined as the hydrostatic pressure build up on the solution, which just stops the osmosis of pure water into the solution through a semipermeable membrane.

The external pressure may be adjusted so as to prevent the osmosis of pure water into solution this provides another definition of osmotic pressure. Osmotic pressure may be defined as the external pressure applied to the solution in order to stop the osmosis of solvent into solution separated by a semipermeable membrane,

Applications:

Osmotic pressure principles are used in the preparation of isotonic intravenous and isotonic lacrimal fluids. Such solutions are compatible to body fluids and prevent the damage of delicate biological membranes. In experimental physiology, the tissue is immersed in salt solutions, which are isotonic. Otherwise, tissue gets damaged due to osmosis. A similar behaviour is observed with red blood cells (RBS).

When the solutions are in contact with the cells or membranes, tonicity of the cells may be altered, i.e. functions are altered. Based on this behaviour, solutions are designated in different ways.

Iso-osmotic Solutions:

Iso-osmotic solutions are those osmotic pressure as that of the cell contents in question, but the solute is solutions which produce the same permeable through the cell membrane thereby altering the tone of the cell. Example of iso-osmotic solution is 1.8 % solution of urea. Iso-osmocity does not necessarily mean isotonic. For example, 1.8 % solution of urea has the same osmotic pressure as that of 0.9% solution of sodium chloride, but former solution produces haemolysis due to permeability of water. Therefore, 1.8% solution of urea is not isotonic, though iso-osmotic.

Isotonic Solutions:

Isotonic solutions are those solutions which produce the same osmotic pressure as that of the cell contents in question, without net gain or loss of both solutions, provided the cell membrane is impermeable to the solutes.

Isotonic solutions are iso-osmotic as well as isotonic with the cells and membranes. Some of the standard isotonic solutions are:

- ✓ 0.9% w/v Normal saline (sodium chloride) solution
- ✓ 5.0% w/v Dextrose solution
- ✓ 2.0% w/v Boric acid solution

These solutions do not cause swelling or shrinking of tissues when applied. Therefore, discomfort would not be caused when instilled into the eyes, nasal tract and when injected into blood or other body fluids.

In the human body, different types of cell membranes are available. All are not having same-level of permeability to a single substance. For example, red blood cell membrane and mucous

lining of the eye are not the same. Therefore, isotonic solutions of 0.9% w/v sodium chloride also need not necessarily be isotonic with respect to all the living membranes, but many of them are roughly isotonic.

Hypertonic Solutions:

Hypertonic solutions are defined as those solutions containing the solute in higher concentration than that is required for isotonic solutions.

Some hypertonic solutions are:

- ✓ 2.0% w/v Normal saline (sodium chloride) solution (concentration >0.9 % w/v).
- ✓ 10.0 % w/v Dextrose solution (concentration > 5.0% w/v).
- ✓ 3.0 % w/v Boric acid solution (concentration > 2.0% w/v).

When red blood cells are suspended in a 2.0 % w/v solution of sodium chloride, the water within the cells passes out through the cell membranes in an attempt to dilute the surrounding salt solution. This process continues until the salt concentrations on both sides of the erythrocyte membrane are equal. Thus outward passage of water causes the cells to shrink and becomes wrinkled or crenated. Such a salt solution is said to be hypertonic with respect to blood.

Hypotonic Solution:

Hypotonic solutions are defined as those solutions containing the Solute in lower concentration than that is required for isotonic solutions

Some hypotonic solutions are:

- ✓ 0.2% w/v Normal saline (sodium chloride) solution (concentration <0.9 % w/v).
- ✓ 3.0% w/v Dextrose solution (concentration <5.0% w/v).
- ✓ 1.0% w/v Boric acid solution (concentration < 2.0 % w/v).

When blood cells are suspended in a 0.2 % w/v solution of sodium chloride (or in distilled water), water enters the blood cells causing them to swell and finally burst with the liberation of haemoglobin. This process is known as haemolysis. Such a weak salt solution is said to be hypotonic with respect to blood.

Nature of Living Membranes-Isotonicity

The term 'isotonicity' is restricted to a particular membrane. In other words, all the membranes do not behave in a similar manner. The following examples illustrate the differences.

- ✓ The red blood cell (RBC) membrane is not a perfect impermeable membrane. It allows some drugs to permeate. RBC membrane is also not a perfect semipermeable membrane. It allows water, urea, ammonium chloride and boric acid to permeate. A2% w/w boric acid solution is iso-osmotic. The boric acid pass through the membrane and 2% w/w boric acid solution is hypotonic and bring about haemolysis.

- ✓ The mucous membrane of the eye is a true semipermeable membrane and boric acid (2 % w/w) solution serve as isotonic.

In a similar manner, 0.9 g sodium chloride per 100 mL solution need not necessarily be isotonic with respect to the living membrane.

Measurement of Tonicity

Isotonicity value is defined as the concentration of an aqueous sodium chloride solution having same colligative properties as the solution in question

Apart from sodium chloride, a number of chemicals and drugs are also included in the formulations. These ingredients also contribute to the tonicity of the solution. Therefore, methods are needed for verifying the tonicity and adjusting the tonicity. Two methods are mentioned below.

Hemolytic method: Red blood cells (RBCs) are suspended in various drug solutions and the swelling of RBCs is observed bursting, shrinking and wrinkling of the blood cells. In hypotonic solutions, oxyhemoglobin is released, which is in direct proportion to the number of cells hemolyzed.

- ✓ In hypertonic solutions, the cells shrink and become wrinkled or crenated
- ✓ In isotonic solutions, the cells do not change their morphology.

This method is used for the determination of isotonicity value.

Cryoscopic method or depression of freezing point:

Colligative properties of solutions are helpful in determining the isotonicity values. Among them, freezing point depression is extensively applied. Water has the freezing point of 0 °C. When substances such as sodium chloride are added to water, the freezing point of water decreases. The depression of the freezing point (ΔT_f) of blood and tears is 0.52 °C. Therefore, the value of 0.9 % w/w NaCl solution should also be -0.52 °C. Such a solution shows same osmotic pressure as that of the blood. Hence, the functions of RBC and tissues do not alter.

Methods of adjusting the tonicity:

Normally, solution dosage forms contain drugs of desired dose ana several excipients needed for formulation. In order to render such solutions isotonic, sodium chloride, dextrose, etc. are added. Several methods are available for adjusting the tonicity. Osmotic pressure is not a readily measurable quantity, but freezing point depression (another colligative property) is more easily measured.

Class I methods: In this type, sodium chloride or other substances are added to the solution in sufficient quantity to make it isotonic. Then the preparation is brought to its final volume with an isotonic or a buffered isotonic diluting solution.

These methods are of two types: Cryoscopic method and Sodium chloride equivalent method.

Class II methods: In this type, water is added in sufficient quantity make the preparation isotonic. Then the preparation is brought to its volume with an isotonic or a buffered isotonic diluting solution.

These methods are of two types: White-Vincent method and Sprowls method.

Cryoscopic Method of Adjusting the Tonicity:

Principle: Water has the freezing point of 0 °C. Blood contains a number of substances such as carbonic acid, carbonates, salts of phosphoric acid and hemoglobin. As a result, the depression in the freezing point of the blood is -0.52 °C.

When substances such as sodium chloride are added to water, the freezing point of water decreases. The extent of depression in the freezing point depends on the concentration of the added substance. For example, sodium chloride at 1 % w v solution decreases the freezing point of water to - 0.58°. In order to make the drug solution isotonic, the freezing point depression of the solution must be maintained at-0.52°.

Initially the drug solution is prepared whose depression in the freezing point (ΔT_f) is known. The remaining (ΔT_f) value is adjusted by adding additional substances such as sodium chloride.

For the purpose of calculate, the freezing point depression of a number of drugs are determined experimentally or theoretically a concentration of 10 % w/v (or sometimes 0.5 % w/v). Similarly the freezing point depression values of 1 w/v solution of sodium chloride and other general ingredients are also determined.

Derivation: Freezing point depression (ΔT_f) of blood 0.52°C. Since the drug solution must be isotonic, it must have ΔT_f, same as that of the blood, i.e. ΔT_f =0.52°C.

Total drug solution ΔT_f = ΔT_f of drug + ΔT_f adjusting substance ----------(1)

Freezing point depression (ΔT_f) of the total drug solution = 0.52°C

ΔT_f, value of the drug = x X ΔT_f of 1 % drug solution = d

where x = drug concentration in the preparation, g/100 mL

ΔT_f for adjusting solution = w X a

where w=weight of the adjusting substance, g/100 mL

a = ΔT_f of the adjusting substance (sodium chloride), 1% (=0.58)

For an isotonic solution, equation (1) is substituted by the terms mentioned above.

0.52° = d + wa

The % w/v of adjusting substance needed is:

W= (0.52-d)/a = (0.52-d)/0.58 ----------(2)

Equation (2) is valid, if 1 % drug solution is specified. For any given percentage strength of medicament (PSM), equation (2) may be modified as:

$W = [0.52 - (PSM \times d)] / 0.58$ ----------(3)

Thus, the desired concentration of adjusting substance is calculated and added in order to make the drug preparation isotonic with blood. Each solute exerts its effect on the freezing point, although others are present.

Hence, if two or more substances are present, a sum of their freezing point depression should be considered.

Advantage: Determination of depression in the freezing point is much simpler and more convenient.

Sodium Chloride Equivalent Method:

Tonicic equivalent or sodium chloride equivalent method is used to adjust the tonicity of pharmaceutical solutions,

Sodium chloride equivalent (D) of a drug is the amount of sodium chloride that is equivalent to 1 g of the drug. In this definition, equivalent refers to sodium chloride concentration having the same osmotic effect as that of the drug. In the absence of available data, the E value of a new drug can be calculated from equation (4).

$E = [17 \times L_{iso}] / M$ ----------(4)

where M = molecular mass, AMU

L_{iso} = freezing point depression of the drug solution for showing isotonicity

Method: The percent of sodium chloride required for adjusting isotonicity can be calculated using equation (5).

PSA = 0.9 - (PSM E of medicament) ----------(5)

where PSM= Percent strength of medicament

PSA = Percent of sodium chloride for adjustment of isotonicity Equation (5) is used to calculate the amount of adjusting substance (sodium chloride) required for making the solution isotonic. It is valid for 100 mL solution.

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