CHAPTER III

SOME PHYSICO-CHEMICAL LAWS APPLIED TO PHYSIOLOGY

(BIOPHYSICS)

Physiological processes are dependent upon certain physio-chemical principles which are described in this chapter.

Units of concentration of solutions

1. **Percentage.** Weight in gms of solute per 100 gms of solution **is per cent. by weight** and the same amount dissolved in 100 ml is **per cent. by volume.**

2. **Molar solution.** Molecular weight in gms of a solute dissolved in 1000 ml is a *molar solution*. EXAMPLE—mol. wt. of H_2SO_4 is 98·016, so a molar solution is 98·016 gms per 1000 ml.

Molal solution. Molecular weight in gram of a solute dissolved in 1000 gms of solvent.

A *mol* (M) or gram-molecular weight is the amount of substance in grams equal to the molecular weight of the substance.

One **millimol** (mM) is equal to one-thousandth of a mol (M) and **1 micromol** (μM), to one-thousandth of a millimol.

3. **Normal solution.** A normal solution is one which contains per litre, the amount equal to gram-molecular weight of the substance divided by equivalent weight or number of replaceable hydrogen atoms. EXAMPLE—mol. wt. of H_2SO_4 is 98·016 where $H^+ + H^+$ and SO_4^{--} ions are present. As number of replaceable hydrogen atoms is 2, then normal solution of H_2SO_4 will contain 98·016 divided by 2, i.e., 49·008 gms in a litre.

4. **Gram equivalent** is the molecular weight in grams divided by the number of valence of ions present. One **milliequivalent** (mEq) is one-thousandth of such gram equivalent.

Ions, Electrolytes and Non-electrolytes

1. **Ions.** On an electric current being passed through solution of a compound in water, some elements migrate at the positive pole or anode and some at the negative pole or cathode. The charged particles are called **ions.** Negative ions **(anions)** move towards the anode and positive ions **(cations)** to cathode due to attraction of unlike charges.

2. **Electrolytes.** Electrolytes are compounds which can be dissociated into anions and cations on an electric current being passed. EXAMPLE—acid, bases, salts, etc.

3. **Non-electrolytes.** Compounds which cannot be dissociated are called *non-electrolytes.*

FILTRATION

Filtration *is the process by which undissolved particles are separated from a liquid through a membrane as a result of a mechanical force* (filtering force). **It is** done through a porous substance, such as a piece of linen or filter paper. This filtering force is either gravity or hydrostatic pressure—positive or negative.

Physiological importance. Whenever there is a difference of hydrostatic pressure between the two sides of a membrane, filtration will occur.

The important examples are—(1) Absorption from the small intestine, (2) Passage of water, salts, foodstuffs, etc., from the blood stream to the tissue fluid—hydrostatic pressure in the capillaries being higher than in the latter.

DIFFUSION

Molecules of a substance are continuously in motion. This motion is least in the solids, maximum in the gases and intermediate in the liquids. When two such substances, (for instance—a solid and a liquid, or two miscible liquids, or a liquid and a gas or two gases) are kept in contact, the molecules of the two substances will pass into each other, until an uniform admixture is obtained. If a layer of water be carefully added upon a layer of concentrated sugar solution, the two liquids will gradually run into each other and ultimately the sugar concentration will be same everywhere. This *spontaneous admixture of the molecules of the two substances in contact* (*due to inherent molecular movement*) *is called* **diffusion** (FIG. 84).

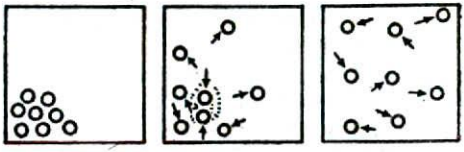


FIG. 84. In the schematic representation of diffusion the molecules become randomly distributed. In the left-sided diagram the molecules are introduced into one corner of the container. In the middle diagram the molecules are in random movement due to their kinetic energy. They can collide with one another and with the walls of the container. In the right-sided diagram the molecules are commencing to be evenly distributed throughout.

Anything that alters the molecular movement also alters the rate of diffusion proportionally, viz., heat increases and cold depresses. The diffusibility of different substances is not same. Other factors remaining constant, *it depends upon the weight and size of the molecules.*

Physiological importance. When two substances, as mentioned above, come in contact, directly or through a permeable membrane—diffusion will take place, when the molecular concentration of a substance in solution is higher in one part of a liquid than in another and when any absolute barrier does not intervene (FIG. 85). Some of the important examples are—(1) Admixture of foodstuffs with digestive juices, (2) Absorption from the intestine, (3) Exchange between plasma and red cells, (4) Exchange in the capillary bed; e.g., foodstuffs, oxygen, etc., pass out from

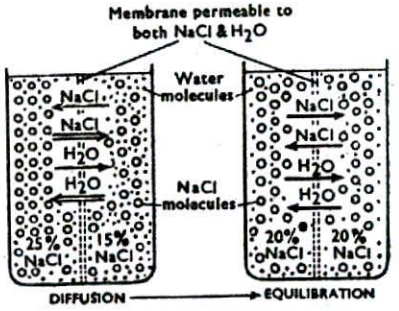


FIG. 85. Figure shows the mechanism of diffusion of two solutions of unequal strength keeping separated by a membrane having permeability to both the substances. Beaker on the left side contains 25% and 15% NaCl solutions, keeping separated by a membrane. 25% of NaCl solution diffuses rapidly to the compartment containing 15% NaCl. H_2O from the same compartment diffuses slowly to the compartment containing 15% NaCl. But H_2O from compartment containing 15% NaCl diffuses rapidly to the compartment containing 25% NaCl. But NaCl of the same compartment diffuses very slowly. Beaker on the right side shows the equilibration of two solutions after diffusion through the membrane.

the blood stream (highest concentration) to the tissue fluid (lower concentration) and then to the tissue cells (lowest concentration), where they are used up. The metabolites including CO_2, on the other hand, come out of the cells (highest concentration) where they are produced, to the tissue fluid (lower concentration), and then to the blood stream (lowest concentration). (5) Exchange in the lung capillaries, e.g., O_2 from air (higher concentration) enters the venous blood (lower concentration), while CO_2 from venous blood (higher concentration) diffuses out into the air (lower concentration). (6) Admixture of gases in the lungs. There are many other examples.

OSMOSIS

The diffusion of water through a semipermeable membrane is called **osmosis.** If a layer of water be separated from a sugar solution by a semipermeable membrane (which will allow only the water molecules to pass but not the sugar molecules) it will be seen that the sugar solution gradually increases in volume for some time and then there will be no further change (Fig. 86). What happens is that, the sugar molecules being impermeable, more water molecules will pass from the water layer into the sugar solution than will pass from the latter to the former (Fig. 86–A & B). Due to this, the volume and therefore the level of sugar solution will rise. This will raise the hydrostatic pressure of the sugar solution and this increased pressure will force more and more water molecules to pass out of the sugar solution. Thus a time will come when the movement of water molecules on either side will be same, so that no further alteration of volume will take place. At this stage (Fig. 86–C), the hydrostatic pressure of sugar solution S exactly neutralises the attractive force of the solution for water molecules. This attractive force is the osmotic pressure. The amount of pressure which has to be applied on solution S to prevent the movement of water molecules is defined as the osmotic pressure of the particular concentration of the substance. If the semipermeable membrane is placed between two sugar solutions of different strengths, water molecules will continuously pass from one side to the other till the concentrations on two sides are equal. Stronger solution will always draw more water from the weaker solution. Therefore, *the force under which a solvent moves from a solution of lower solute concentration to a solution of higher solute concentration when a selectively permeable membrane separates these solutions, is called* **osmotic (oncotic) pressure** (Fig. 86). In true sense, it is the pressure which must be put upon a solution to keep it in an equilibrium with the pure solvent when the two are separated by a semipermeable membrane. For this reason osmotic pressure does not depend upon the *size* of the molecules but upon the

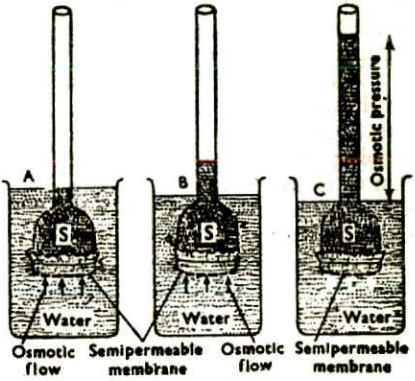


Fig. 86. Osmosis. S=Strong solution.

total number of discrete particles per unit volume. If the solute be ionisable, the osmotic pressure (O. P.) will be proportionally more. If more pressure (weight) is applied on piston (Fig. 87) water will be forced out from the stronger solution S to the weaker solution W. This passage of water against concentration gradient is known as **ultrafiltration.** If two solutions, separated by a membrane, have the same O. P., they are called **isotonic.** One having lesser O. P. is called **hypotonic,** while that having higher O. P., **hypertonic.** 0·9 per cent. sodium chloride solution is isotonic with blood plasma, commonly known as *normal saline* or *physiological saline.* A 5 per cent. solution of glucose has also similar osmotic pressure. Either of them can be injected intravenously in human patient if properly sterilised. Two solutions having same number of particles per unit volume are called **isosmotic.** But since, their permeability through a membrane may vary, they are not necessarily isotonic.

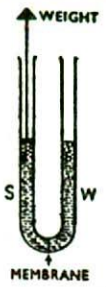


Fig. 87. Measuring O. P. by weight.

Physiological importance. Osmotic pressure plays a great role in physiology. A few examples are as follows: (1) Absorption from the intestine, (2) Exchange in the capillary bed. Continuous osmotic exchange is going on between blood, tissue fluid, tissue cells and lymph. (3) Regulation of urine formation. (4) Reabsorption of cerebrospinal fluid. (5) Continuous osmotic exchange between plasma and red cells. (6) *Clinical use*—Injections of isotonic, hypotonic and hypertonic saline and other solutions are given in suitable cases by way of treatment. Saline purgatives (magnesium sulphate, etc.) and saline diuretics also work on osmotic principle. (7) *Laboratory use*—Sodium chloride and other solutions of different osmotic pressures are used in the laboratory for various experiments.

Methods of measurement. O.P. can be measured in various ways: (A) **Mechanical methods.** (1) *By putting weights*—The simplest way is to apply adequate pressure (i.e., weight) upon the stronger solution to prevent any rise of volume. That pressure which is just needed to stop the increase of volume of a particular solution is the measure of its O. P. (Fig. 87). (2) *By a manometer*—The same thing can be done by connecting the apparatus with a suitable manometer in which the pressure will gradually rise till it equalises with the O. P. of the solution, at which point further rise will stop (Peffer's method, Fig. 88). (B) **Biological methods.** (1) *Hamburger's Red Corpuscle Method*—Red cells are kept in the unknown solution for some time after which the cell volume is noted. If the cell volume be reduced, the solution is hypertonic than plasma (hence, water has been drawn out), if the cells swell up, the solution is hypotonic (so that water has entered), if no change—the solution is isotonic. If sufficiently hypotonic, the red cells will gradually swell up and ultimately burst (*Haemolysis*). (2) *De Vris' Plant Cell Method*—The same principle is followed as above, only plant cells are used instead of red cells and the comparison is made with the cell sap inside. In hypertonic solutions, the cells will shrink, in isotonic—there will be no change, while in hypotonic solutions the cells will swell up and may burst (*Plasmolysis*). (C) **Physical**

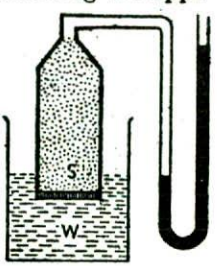


Fig. 88. Measuring O. P. with manometer.

methods. (1) By noting the *depression of freezing* point (symbol Δ)—Higher the concentration, lower will be the freezing point, and therefore higher will be the O.P. (2) By noting the *vapour tension*—higher the concentration, lower will be the rate of evaporation from the solution and higher the O. P. (*i*) **Hill's method**—using Thermopile. Higher the rate of evaporation, more will be the fall of temperature and less will be the O. P. Comparison is made with a solution of known O. P. (*ii*) **Barger's Capillary Glass Tube Method**—Alternate drops of known and unknown solutions separated by air bubbles are drawn in a standard capillary glass tube and after some time the edges of the solutions noted. The edges will shift according to the rate of evaporation. From these data O. P. can be calculated.

ULTRAFILTRATION

Ultrafiltration is a kind of filtration through a jelly filter or any *ultrafilter* which serves to separate colloid solutions from crystalloids and to separate particles of different size in a colloid mixture. Due to the opposite of osmosis (*vide* page 107) ultrafiltration results from the exertion of a pressure on a solution. This pressure forces the solution through a membrane impermeable to one or more of the solutes.

Physiological importance. The blood plasma is placed in a vessel of which one end is a collodion membrane. Now, if a pressure is exerted on the blood plasma an ultrafiltrate will be separated. This separation will take out from the solution all the constituents of the plasma except the protein which has been contained in the gross plasma. This occurrence results in due to small pores of the collodion membrane. The extracellular fluid (ECF) is also an ultrafiltrate into the plasma through the porous capillary membrane. If the counter pressure is not exerted on the plasma, the ECF shall pass back into the plasma.

DIALYSIS

Dialysis *is a process by which the more diffusible materials can be separated from non-diffusible materials.* In FIG. 89-A, water solution of egg albumin and sugar has taken in the upper smaller container whose open bottom is covered with a semipermeable membrane. The semipermeable membrane has got selective permeability to water and sugar molecules but not to macromolecules—the egg albumin. This container is suspended (partially) in the water of a large container. Due to selective permeability, the sugar molecules will ultimately go into the water leaving behind only the albumin and little water. As the albumin is impermeable to this membrane, this will rebound from the membrane during the process of dialysis (Fig. 89).

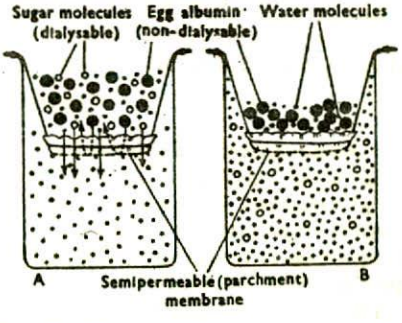


FIG. 89. Showing the process of dialysis. A=onset of the process, B=after completion of the process.

Physiological importance. Whenever the bigger particles are held back and only the smaller particles are allowed to pass through a membrane—depending upon osmotic pressure

(i.e., diffusion), dialysis comes into action. For instance, (1) during absorption from the intestine bigger food particles are held back, (2) in the capillary area the bigger albumin, globulin, etc., particles are not ordinarily allowed to pass into the tissue fluid. It is to be noted that diffusion, osmosis and dialysis are the manifestations of the same principle (inherent molecular movement) and usually go on simultaneously.

SURFACE TENSION

Surface tension *is the manifestation of attracting forces in between atoms or molecules.* As elsewhere, so also in a liquid, the molecules attract each other. Within the depth of the liquid, each molecule is attracted equally from all directions (FIG. 90). Hence, the molecule can move freely in all directions. But a molecule at the liquid-air surface, is attracted only by the molecules within the depth of the liquid and there are relatively few molecules in the gas above the water surface to exert any upward force. Consequently, it tends to be pulled inwards and its freedom of movement is restricted. Thus at the surface of a liquid a layer is formed in which the molecules are arranged more densely. For the same reason the surface of the liquid tries to pull itself together and shrinks in order to occupy the least possible area. This energy with which the surface molecules closely adhere together is called *surface tension.*

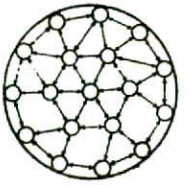


FIG. 90. Showing the surface tension of water molecules in a beaker as seen from above.

Solutes alter surface tension. Inorganic salts generally raise while organic substances reduce surface tension of water. Of the latter—bile salts, proteins, phospholipids, oils, soaps, etc., are important examples (Hay's sulphur test, for the detection of bile salts in urine, depends upon this principle.)

Physiological importance. (1) The globular shape of an oil drop in water, of the fat particles in milk, etc., is due to surface tension. (2) Bile salts reduce the surface tension of fat converting it into an emulsion in the intestine. This helps digestion and absorption of fat. (3) The formation of cell membrane is at least partly due to surface tension of the cell cytoplasm. There are innumerable such examples.

ADSORPTION

Adsorption *is a peculiar form of combination in which substances adhere together on their surfaces. It is a sort of union by surface contact.* It is not a true chemical reaction, because no definite quantitative relation is found. The possible mechanisms of adsorption are as follows: (1) *Surface tension*—Adsorption is probably a manifestation of surface tension developed from the attraction of dissimilar molecules of two substances. (2) *Residual valency*—The chemical nature of the particles and their residual valencies may be such as to make such a loose combination possible. (3) *Electrical state*—The electrical charge of the particles may be of opposite nature; so that they attract each other and thus make a sort of contact combination. Failure of adsorption means that the electrical charges are similar, so that they repel each other. During the process of adsorption much kinetic energy is lost and this kinetic energy appears as heat of adsorption.

Physiological importance. In physiology adsorption plays a great role. Some of them are as follows: (1) Enzyme action—both the enzyme and the substrate are colloidal in nature. By process of adsorption they come in closer contact and the interaction is hastened. (2) The combination between toxin and antitoxin, by which they neutralise each other, is another adsorption phenomenon. (3) Various adsorption compounds are formed in the body, such as lecithin with protein (found in the brain) and such others. The blue compound formed by adding iodine with starch is an adsorption compound.

HYDROTROPY

Certain substances have the property of making water-insoluble substances soluble in water. This is called **hydrotropy** or **hydrotropic action.** How this action is brought about is not definitely known. It is said that the hydrotropic substances form loose compounds with the insoluble substances and thereby make them soluble and diffusible through membranes. This view is supported by the fact that a quantitative relation is often found between them. For instance, when glycocholic acid forms hydrotropic compound with oleic acid they have a molecular ratio of 3 : 1. *Hydrotropic substances* of physiological importance are: (1) Bile salts and sueh other compounds of cholic acid (*vide* under 'Bile Salts'). This is possibly the chief member. (2) Lecithin, soaps of higher fatty acids, phenylacetic acid, benzoic acid, hippuric acid, etc. (3) Hydrotropic substances of unknown nature are also found in the intestinal juice, in the intestinal mucosa, in the blood plasma and possibly in other tissues and body fluids.

The *insoluble substances* which are made soluble in this way are—fats, certain phospholipids, sterols—specially cholesterol, insoluble soaps, uric acid and inorganic salts of Ca, Mg, and possibly of Fe, Cu, Mn, etc.

Physiological importance. This is of immense physiological value. There are many chemicals in the body which are kept in solution with the help of hydrotropic action. Some examples are given below: (1) In the bile (in the liver, gall-bladder, common bile duct) cholesterol and certain other compounds are kept in solution with the help of bile salts. (2) In the intestine the insoluble soaps, sterols, fatty acids, inorganic salts, etc., make hydrotropic compounds with bile salts and other hydrotropic substances present in the intestinal juice and mucosa and are thus easily absorbed. (3) In the blood plasma and other body fluids (and possibly in the tissues) there are a number of hydrotropic substances which keep many otherwise insoluble substances in solution, for instance, sterol, Ca and other inorganic salts, uric acid, etc.

DONNAN EQUILIBRIUM

When a saline solution and distilled water are separated by a permeable membrane final equilibrium will be reached when the concentration of salt on both sides will be same. But if there be any non-diffusible ion on one side, a different phenomenon will be seen. Suppose **NaP** is an ionisable compound of which **P** is non-diffusible. If this compound is kept on one side and NaCl on the other, **Na** and **Cl** ions will freely pass but not **P.** When the final equilibrium will be reached, it will be seen that the

product of **Na** and **Cl** on one side is equal to the product of the same two ions on the other side. For example—

INITIAL STATE		FINAL STATE
Na : Na	⟶	**Na : Na**
P : Cl		**Cl : Cl**
:		**P :**

In the final state **Na** × **Cl** (left) = **Na** × **Cl** (right).

It is obvious that total **Na** of the left side is greater than total **Na** of the right side and the total **chloride** of the right side is greater than total chloride of the left side.

Similarly, if **NaP** be on the left side and H_2O on the right side, in the final state the reaction on the right side will be alkaline due to **Na** and **OH** ions. If a compound **Cl-P** be on the left and H_2O on the right, in the final state the reaction on the right side will be acid due to **H** and **Cl** ions. This type of equilibrium in which *the products of the same pair of ions on two sides of the permeable membrane become same, is called* **Donnan equilibrium.** It is obvious that in the final state there will be a great difference in the nature and quantity of diffusible ions on two sides of the membrane which will lead to a difference of electric potential and chemical reaction on the two sides. Since, in our body there are many compounds of the **NaP** type, Donnan equilibrium is of great **physiological importance.** It explains how difference of electric potential can be established on two sides of a membrane, how stomach can secrete a strongly acid juice and pancreas can secrete an alkaline juice. The phenomenon of chloride shift can also be explained from this standpoint.

COLLOID

When sugar, urea, NaCl, etc., be dissolved in water the result is a clear permanent *true solution.* But if proteins, starch, glycogen, etc., be placed in water, they will make a thick opalescent *unstable solution.* Graham (1861) called the first type as the **crystalloidal solution,** because solute particles form crystals and pass through parchment membrane, and called the second group—**colloidal solution,** for the solute particles in this case show reverse properties. *Modern idea* indicates that the difference between true solution and colloidal solution depends on the size of the solute (the dispersed phase) molecules in the solvent (the dispersion medium). If the size is greater than 200 mμ they remain as suspensions and if less than 1 mμ as true solution. The size of the molecules in colloidal solutions varies from 1 mμ to 200 mμ.

To understand the difference between a crystalloidal and a colloidal solution, one should remember that the forces which keep the solute particles in solution, are roughly as follows: (1) Inherent movement of the solute particles, i.e., their diffusibility. (2) Inherent movement of the solvent molecules which continuously dash against the solute particles and thus help to keep them in solution. (3) Electric charge—positive or negative—carried by the particles, which by constant attraction and repulsion also help uniform solution. (4) Hydration (carrying water molecules) of the solute particles, and so on. If the solute particles be very small (below 1 mμ—*amicrons*) all these forces will be acting to their maximum and the result will be a permanent true solution. Now, if the solute particles be gradually made larger and larger, (1–200 mμ—*submicrons*) their own movement (diffusibility) will gradually be reduced and ultimately will be almost

nil. The first factor, mentioned above, will, therefore, be completely out of the field. The other forces, such as the dashing by the solvent molecules, electric charge, etc., will then try to keep the large solute particles somehow in solution and will be able to do so up to a certain extent. Such a solution will be unstable and *heterogeneous*—a sort of *pseudosolution.* This is a colloid. If then, the solute particles be made still larger (over 200 mμ—*microns*) all the forces will completely fail and the solute particles will not go into solution at all. Hence, if the solute-solvent relations be studied as a series of phenomena, it will be found that, at one extreme there is complete solubility (true solution), at the other extreme—complete insolubility, while in the intermediate stages there will be a phenomenon of semi-solubility. This is **colloidal solution.**

Thus, other factors remaining constant, the real difference between a true solution and colloidal solution lies in the size of the solute particles, and not upon their chemical nature.

Thus, a **colloid** may be defined *as a substance,* (e.g., gelatin or cell cytoplasm), *which, by reason of the size of its molecules, is slowly diffusible rather than soluble in water (its hydrates being gelatinous in consistence), and is incapable of passing through an animal membrane (a semipermeable membrane).* In this substance *the solute particles are proportionally larger than the solvent molecules.*

Emulsoid and Suspensoid. Colloids fall into two classes—**emulsoid** (emulsion) or **lyophilic colloids** and **suspensoid** (suspension) or **lyophobic colloids.** In emulsoids the solutes have a great affinity for the solvent so they are called *lyophilic* and when the solvents are water, they are **hydrophilic colloids.** In suspensoids, there is no affinity between the solute and the solvent. A large quantity of electrolyte is required to precipitate an emulsoid which is *reversible.* In case of suspensoids a very small amount is required for such precipitation which is *irreversible.* Examples of suspensoids are colloidal solution of inorganic salts, metals, etc., and those of emulsoids are polysaccharides, proteins, etc., and are of greater physiological importance.

Emulsoids being more stable, can impart their own stability to suspensoids. In other words, when suspensions of solid particles are made in a solvent which is already an emulsion, the resulting suspensoid will be relatively more stable.* This is called the *protective action* of emulsoids. For instance, blood is a suspension of red cells (7·2 μ) in plasma, which in itself is an emulsion of proteins in water. This makes blood, a relatively stable suspensoid.

Sol and Gel

A colloid may remain in two states—as a liquid or as a solid. The former is called **sol,** the latter, **gel.** These two states are reversible. Sol can be transformed into gel by altering temperature, H-ion concentration, salt concentration, concentration of the solute, etc. During this process, a relatively small amount of solute particles runs together and sets into a semi-solid mass, entangling in its meshes a fairly large amount of the solvent in the form of isolated droplets. The explanation is as follows: Being heterogeneous, colloidal solution has two distinct components—called *phases.* The solvent, being continuous, is called the *continuous phase.* The solute, being

* Many pharmacopoeial emulsions, such as of bismuth salts are made upon this principle, by adding to the mixture—gum acacia and such other substances which with water easily makes an emulsion.

discontinuous or dispersed, is known as *dispersed phase*. The two phases are interchangeable. When the solute is more or less solid and the solvent (continuous phase) is liquid—it becomes a sol (if the liquid is water—a hydrosol). If, on the other hand, the solvent (continuous phase) is more or less solid and the solute (dispersed phase) be liquid—it makes a gel (if the latter is water—a hydrogel). For instance, a hot solution of agar agar is a liquid—a sol. On cooling, it sets into a semisolid jelly—a gel. Milk is a sol, butter is a gel (FIG. 91).

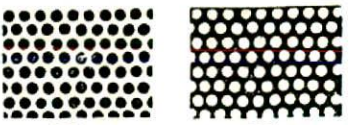


FIG. 91. Showing reversal of phases of a colloid. SOL=solid solute(*black*), liquid solvent (*white*). GEL=phases reversed. Now *black* is solvent and solid, *white* is solute and liquid.

Properties of Colloids. (1) **Brownian movement**—Due to the impact of the solvent molecules, the colloid particles are continuously moving about. (2) **Faraday-Tyndall phenomenon** (Optical phenomenon)—When a beam of light is passed through a colloidal solution and observed at right angles, the track of light, which is invisible in a true solution, may be visible here as a white line. This is caused by the dispersal of light rays by the colloid particles. If the particles be sufficiently small, the light, when viewed through a Nicol's prism, will be found to be polarised at right angles to the beam. (3) **Electrical phenomenon**—(*a*) Colloid particles carry electric charge—which may be positive or negative (*colloidal ions*). (*b*) *Isoelectric* pH—By addition of salts, weak acids, weak alkalies, etc., the hydrogen-ion concentration of the medium can be adjusted to an isoelectric pH where the electric charge of the colloidal ions will be completely neutralised. At this pH the colloid particles become less soluble and may be precipitated. Almost every colloid, particularly proteins, has its characteristic isoelectric pH (*vide* page 80). (*c*) *Electrophoresis* (*vide* below & page 80). (4) **Precipitation**—The colloid particles can be precipitated in various ways. Isoelectric precipitation has been mentioned above. In precipitation, there is no intramolecular change and it can be made to go back into solution again. (5) **Coagulation**—This also is a property of the colloid. Here, the intramolecular change takes place. The coagulum cannot be redissolved. (6) **Osmotic pressure (Oncotic pressure)**—Colloids exert some osmotic pressure. But the particles being large, it is proportionally much smaller. However, it is of great physiological importance (*vide* page 118). (7) **Imbibition of water**—Emulsoid particles can imbibe a good amount of water. (8) **Adsorption**—This also is a colloidal phenomenon (*vide* page 110).

Separation of Colloids. May be done by: (1) **Dialysis** (*vide* page 109). (2) **Isoelectric precipitation** (*vide* above). (3) **Salting out**—By adding suitable amounts of various salts. It is a kind of precipitation of a protein from its solution by saturation or partial saturation with such neutral salts as sodium, chloride, magnesium sulphate, or ammonium sulphate. (4) **Electrophoresis**—If an electric current be passed through a colloidal solution, the positively charged colloid ions will accumulate around the negative pole, while the negatively charged particles will accumulate around the positive pole. In this way they can be separated. Even their rate of movement can be measured by noting the concentration of a particular colloid at different points in the electric field (*vide*

page 80). (5) **Adsorption**—By suitable methods, colloids can be isolated by adsorption. (6) **Ultracentrifugalisation**—If centrifugalised at high speed colloid particles collect at the bottom of the centrifuge tubes at different rates, the heavier ones settle out faster. (7) **Ultrafiltration**—By this process the colloidal (larger) and crystalloid (smaller) dissolved molecules can be separated from each other (*vide* page 109). Colloid of different particle sizes can also be separated from one another by ultrafiltration through membranes of different pore sizes.

Physiological importance. Colloids are of immense physiological value. Some examples are as follows: (1) **Cell protoplasm**—in every cell —exists in a colloidal state—mostly as emulsoid. 90% of organic matter of the body remains as colloid. (2) **Milk, plasma** and **lymph**—are all emulsoids. Blood is a suspension of red cells in plasma. (3) **Interfacial reaction** —Being dispersed in the form of minute particles, colloids afford a very large surface area for various reactions to occur, such as adsorption, surface tension, enzyme action, etc. For instance, (*a*) the emulsification of fats during digestion, provide a large surface of contact for the enzymes to act and thus quickens the process of digestion. (*b*) The total surface area of red cells being very large, facilitates rapid exchange of gases, ions, etc., in the blood stream. (4) **Imbibition of water**—Since emulsoids readily imbibe water, a good deal of water remains stored in the body in this way. (5) **Adsorption**—which is of great physiological importance—is a colloidal phenomenon. (6) **Blood clotting**—is essentially a colloidal process—in which a sol (plasma) is converted into a gel (Clot). (7) **Colloid osmotic pressure**—When human red blood corpuscles are suspended in 0·5% sodium chloride solution (hypotonic), water and sodium chloride pass into the cells due to lower osmotic pressure inside the cells. The red cells are swollen and burst. If the red cells are suspended in more than 1·0% sodium chloride solution (hypertonic) opposite change occurs, i.e., water and salt pass out of the red cells. The red cells are shrunken and become crenated. If the red cells are suspended in normal saline solution (0·9%) there is practically no change in the cells.

Plasma contains both *diffusible* and *non-diffusible* large colloidal particles. The *total* osmotic pressure of plasma is about 6·5 atmospheres. This is due to crystalloids and colloids. The osmotic pressure of the plasma proteins is 25 mm of Hg. The osmotic pressure of the plasma proteins depends on *serum albumin* and *serum globulin*. Serum albumin exerts a greater osmotic pressure than serum globulin. By virtue of the osmotic pressure of the plasma proteins, the fluid in the blood capillaries is retained and the plasma volume is maintained. The capillary blood pressure is a filtering force tending to drive protein-free fluid into the interstitial spaces, whereas the osmotic pressure of the plasma protein exerts an opposite effect. The osmotic pressure of plasma proteins also play a great role in glomerular filtration.

THE ATOMIC STRUCTURE

The atom of all elements (103 known) is composed of the smallest and elementary particles. *Neutrons*, *protons* and *electrons* are the elementary particles. The neutron and proton occur in the centre of an atom where they form an *atomic nucleus*. The electron orbits the atomic nucleus at some distance from its centre where it is virtually weightless and carries *negative* electrical charge. Each neutron and proton (nucleons) has one unit of *atomic weight* or *mass* and is about 1800 times heavier than the electron. A proton's electrical charge is positive, whereas a neutron is electrically neutral. Hence, the number of

positive and negative charges are exactly balanced and so an atom is electrically neutral. The structure of atoms of some elements as shown (FIG. 92) indicate each element has a distinctive number of protons and electrons.

The chemical properties of the elements however depend upon the number and configuration of electrons. The number of such planetary electrons in the neutral atom of any element is equal to the number of protons in the nucleus of that atom. An element is characterised by a fixed and definite atomic number of protons in the nuclei of its atom balanced by an equal number of planetary electrons. With the *exception of the nucleus of the ordinary hydrogen* all atomic nuclei contain one or more neutrons in addition to protons.

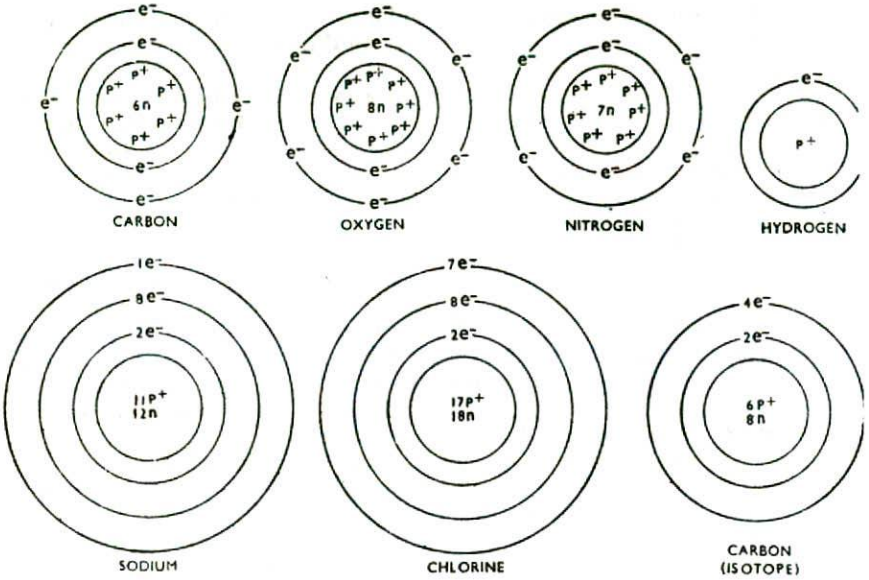


FIG. 92. The electron shells of some atoms. The circle represents the atomic nucleus of the atom containing protons (p^+) and neutron (n) and electrons (e^-). The distance of electron from the atomic nucleus denotes various energy levels of atoms. The *isotope of carbon* varies only in number of neutrons contained in the atomic nucleus.

The number of neutrons in the nuclei of the atoms of an element are not fixed, but vary within certain limits. The atoms of most elements contain nuclei with different numbers of neutrons and consequently of different mass numbers. Each such atomic variety with a specific mass of an element constitutes isotopes. Therefore, **isotopes** *are atoms of the same elements whose nuclei contain different numbers of neutrons but the same number of electrons and protons.* Isotopic atoms (nuclides) can be detected and distinguished physically but not chemically.

Natural Isotopes. They belong to the first class and remain as mixtures in all natural sources and can be isolated by careful fractionation. The isotopes of physiological importance are those of **carbon, hydrogen, nitrogen, oxygen, sulphur, chlorine,** etc. For instance,—carbon having the familiar atomic wt. 12 has isotopes with atomic wt. 11, 12, 13 and 14 (^{11}C, ^{12}C, ^{13}C, ^{14}C or written as C^{11}, C^{12}, C^{13}, C^{14}). Hydrogen has the usual atomic wt. 1, but isotopic *heavy* hydrogen has an atomic wt. 2 (^2H). Oxygen with atomic wt. 16, has isotopes with atomic wt. 17 and 18 (^{17}O, ^{18}O). Similarly sulphur (atomic wt. 32) has isotopes with atomic wt. 33 and 34 (^{33}S, ^{34}S) and chlorine with the usual atomic wt. of 35 may have an isotopic variety with 37 (^{37}Cl).

Of special interest are the *hydrogen isotopes* (*radiohydrogen*) (^2H or H^δ) and ^3H which have been given special names—**deuterium** and **tritium** respectively. The deuterium is symbolised by **D** (Urey, 1932). $\mathbf{D_2O}$ (*heavy water*) is present to some extent in all natural

samples of water (tap water contains 1 part in 9,000 parts) and traces of D_2O (deuterium oxide) and THO (radioactive tritium oxide) are present throughout the body fluids. By determining D_2O and THO spaces, the total body fluid would be obtained. Electrolysis of water preferentially dissociates H_2O but leaves D_2O untouched. Consequently after electrolysis of water fairly concentrated solution of D_2O can be obtained and can be subsequently isolated by fractionation. Although in chemical properties it is identical with H_2O, D_2O has different mass, recognised by *mass spectrometer*.

Radioactive Isotopes. Some isotopes are stable, but some are unstable due to their relative number of protons and neutrons in the nuclei. These are *radioactive isotopes* which break down constantly into more stable atoms. Elements like *uranium* and *radium* are radioactive, they emit α-, β- and γ-rays. Although majority of the naturally occurring elements are not radioactive, *radioactive isotopes of all of them can be prepared artificially by bombardment with a* **cyclotron** or by **uranium pile.** The emitting α-radiation from the disintegrating atom is not easily detected. β-radiation and γ-radiation are usually measured with *Geiger-Müller* counter. In some special cases either *Windowless* or *Scintillation counter* is used. The stability of a radioactive isotope is measured by its *half-life*. The half-life of a radioactive isotope is the time taken by the radiation to come down to half the original strength. The half-life of radioactive elements varies from minutes to years. Those applied for physiological studies must obviously have sufficiently long life to enable an elaborate study of their life-history in the body.

HALF-LIFE OF SOME RADIOACTIVE ISOTOPES: ^{42}K, 12·4 h.; ^{24}Na, 15 h.; ^{131}I, 8 d.; ^{59}Fe, 45 d.; ^{32}P, 14·3 d.; ^{35}S, 87·1 d.; ^{45}Ca, 152 d.; ^{60}Co, 5·3 y.; ^{14}C, 5700 y.; ^{3}H, 12·5 y.; etc. (h denotes hours, d, days and y, years.)

Physiological application of isotopes. The isotopes, either natural or radioactive, have identical chemical properties with the parent element and so they are treated similarly in the living system as their normal homologue. A very low concentration of them can however be detected by physical method and so they can be used to 'label' molecules and are used extensively in different physiological and clinical studies in recent times.

The latest advancement in the study of metabolic processes is the application of natural and radioactive isotopes. Compounds labelled with such isotopes are administered. The changes undergone in such 'labelled' compounds, their migration from place to place and finally their excretion from the body, are traced and observed with the help of suitable instruments and of other physico-chemical properties. Hence, such elements are referred to as *tracer elements*.

Some physiological studies based on application of radioactive isotopes are listed below:

A. By dilution of radioactivity added: (*a*) Measurement of red cell volume with ^{51}Cr labelled red blood corpuscles. (*b*) Determination of plasma volume with ^{131}I labelled serum albumin. (*c*) Determination of sodium space with either ^{22}Na or ^{24}Na. (*d*) Estimation of total body water with iodo-antipyrine labelled with ^{131}I.

B. Membrane-transfer measurement: (*a*) Absorption studies of iron (^{59}Fe), vitamin B_{12} (^{60}Co), fatty acids (^{14}C oleic acid), etc. (*b*) Transport of Na and K across the intestinal wall. (*c*) Exchange of ions in the kidney tubules.

C. Studies based on distribution of a particular isotope: (*a*) Uptake of ^{131}I by thyroid gland in thyroid function test. (*b*) Uptake of ^{32}P by malignant tissue in its tracing in the body. (*c*) Distribution of different drugs.

D. Metabolic studies. This is one of the widest application of radioactive isotope. Studies on synthesis, degradation and isolation of intermediate products in almost all metabolic pathways in normal physiological conditions and its variations in different diseases have been attempted.

They include carbohydrate, protein and nucleic acids, fatty acids and steroids and mineral metabolism. Synthesis and degradation of various hormones have also been studied.

E. As a radiation source in medicine: (*a*) ^{131}I in the treatment of hyperthyroidism and thyroid cancer. (*b*) ^{32}P in the treatment of leukaemia and polycythemia vera. (*c*) Clinically for destruction of tumour.

Autoradiography. The capacity of radioactive isotope to blacken photographic emulsion is termed the technique of autoradiography. A mixture of substances are first of all separated by paper chromatography and then the paper is kept in contact with X-ray film or similar photographic emulsion. The grade of black hue gives the amount of radioactivity of the particular fraction. If applied on histological section this technique can detect the presence of radioactivity inside the different cellular compartments (nuclei, mitochondria, etc.).

ACID, BASE, HYDROGEN-ION CONCENTRATION AND pH

An *acid* may be defined as a substance which, when dissolved in water, produces hydrogen ions $[H^+]$ or in other words, it is the compound of electronegative element with *ionisable hydrogen* $[H^+]$. Similarly a *base* or *alkali* is a substance which produces hydroxyl ions $[OH^-]$ when dissolved in water. That is electropositive elements with *ionisable hydroxyl groups* $[OH^-]$ are the bases. A normal solution of any acid will exactly neutralise an equal volume of a normal solution of any base, no matter, how strong or weak, may be characteristics of two reactants. During neutralisation equal numbers of hydrogen ions of acid and hydroxyl ions of base unite to form water.

Free acidity. Amount of acidity present in a solution in free state and not combined with any other substance.

Actual acidity. Amount of $[H^+]$ ion concentration in a solution.

Total acidity or Titratable acidity. Amount of free acid *plus* that present in combination. This can be titrated against a base.

The equilibrium governing the ionisation of water is as follows:

$$H_2O \rightleftharpoons [H^+]+[OH^-].$$

At a particular temperature the product of a number of hydrogen ions multiplied by a number of hydroxyl ions, is a constant. Thus at 23°C.,

$$[H^+]\times[OH^-]=10^{-14}.$$

Concentration of $[H^+]$ and $[OH^-]$ ions in aqueous solution is such that their product is always equal to 10^{-14} gram-molecular weight per litre. This concentration product or dissociation constant of water can be denoted as Kw*. So the equation will be

$$[H^+]\times[OH^-]=Kw=0{\cdot}00000000000001=1\times10^{-14}.$$

In pure water the concentration of $[H^+]$ ions is equal to the concentration of $[OH^-]$.

So $[H^+]\times[OH^-]=1\times10^{-14}$ and

$[H^+]=1\times10^{-7}$, $[OH^-]=1\times10^{-7}$.

Therefore pH of the pure water will be $pH=\log(1/[H^+])=\log(1/[1\times10^{-7}])=$**7·0.** So the pure water having equal number $[H^+]$ and $[OH^-]$ ions is neutral at pH 7·0.

Hydrogen-ion concentration (cH) is a measure of extent of acidity or alkalinity of a solution. As the product of hydrogen- and hydroxyl-ion concentrations in a solution is constant, if the former is known, the latter can be calculated. Thus the hydrogen-ion concentration of neutral water is 10^{-7} and of normal sodium hydroxide is 10^{-14}.

The **pH** of a solution is defined as the negative logarithm (base 10) of the hydrogen-ion concentration, i.e., $pH=-\log[H^+]$ or $pH=\log(1/[H^+])$.

Calculation of pH. For calculation of pH, $[H^+]$ ion concentration must be ascertained. If the $[H^+]$ ion concentration of a solution is known then the pH of a solution can be calculated. When the $[H^+]$ ion concentration of a solution is $2{\cdot}86\times10^{-4}$ then the pH can be calculated as follows:

$$[H^+]=2{\cdot}86\times10^{-4}$$
$$pH=\log(1/[H^+])$$
$$=\log(1/[2{\cdot}86\times10^{-4}])$$

*By Law of Mass Action $\dfrac{[H^+][OH^-]}{[H_2O]}=K$, where K is constant. So $[H^+][OH^-]=Kw$.

$$= \log (1/[10^{0.456} \times 10^{-4}])$$
$$= \log (1/10^{-3.544}) = 3{\cdot}544$$
$$\text{or, } pH = 3{\cdot}544.$$

For calculation of $[H^+]$ ion concentration from a known pH value, the calculation will be reversed. Supposing the pH value of a solution is 3·544 then the $[H^+]$ ion concentration will be

$$pH\ 3{\cdot}544 = \log 10^{3.544}$$
$$= \log (1/10^{-3.544})$$
$$= \log (1/[10^{0.456} \times 10^{-4}])$$
$$= \log (1/[2{\cdot}86 \times 10^{-4}]) \text{ (Antilog of } 0{\cdot}456 = 2{\cdot}86)$$

$[H^+]$ ion concentration will be $2{\cdot}86 \times 10^{-4}$.

Greater the value of pH, lower will be the value of acidity and conversely the lower value of pH will indicate the higher value of acidity.

Hydrogen-ion concentration (cH) can be expressed in three ways :

	pH (−log)	Power of Ten	Decimal	
Acid	pH 0	$1\,\mathcal{N}$	1·0 $\mathcal{N}$	Acid
	pH 1	$10^{-1}\,\mathcal{N}$	0·1 $\mathcal{N}$	
	pH 2	$10^{-2}\,\mathcal{N}$	0·01 $\mathcal{N}$	
	pH 3	$10^{-3}\,\mathcal{N}$	0·001 $\mathcal{N}$	
	pH 4	$10^{-4}\,\mathcal{N}$	0·0001 $\mathcal{N}$	
	pH 5	$10^{-5}\,\mathcal{N}$	0·00001 $\mathcal{N}$	
	pH 6	$10^{-6}\,\mathcal{N}$	0.000001 $\mathcal{N}$	
Neutral	pH 7	$10^{-7}\,\mathcal{N}$	0·0000001 $\mathcal{N}$	Neutral
Alkaline	pH 8	$10^{-8}\,\mathcal{N}$	0·00000001 $\mathcal{N}$	Alkaline
	pH 9	$10^{-9}\,\mathcal{N}$	0·000000001 $\mathcal{N}$	
	pH 10	$10^{-10}\,\mathcal{N}$	0·0000000001 $\mathcal{N}$	
	pH 11	$10^{-11}\,\mathcal{N}$	0·00000000001 $\mathcal{N}$	
	pH 12	$10^{-12}\,\mathcal{N}$	0·000000000001 $\mathcal{N}$	
	pH 13	$10^{-13}\,\mathcal{N}$	0·0000000000001 $\mathcal{N}$	
	pH 14	$10^{-14}\,\mathcal{N}$	0·00000000000001 $\mathcal{N}$	

The pH scale. Sorensen introduced the simple pH scale. The degree of acidity can be measured on a scale of pH units. Pure water is neutral, i.e., neither acid nor

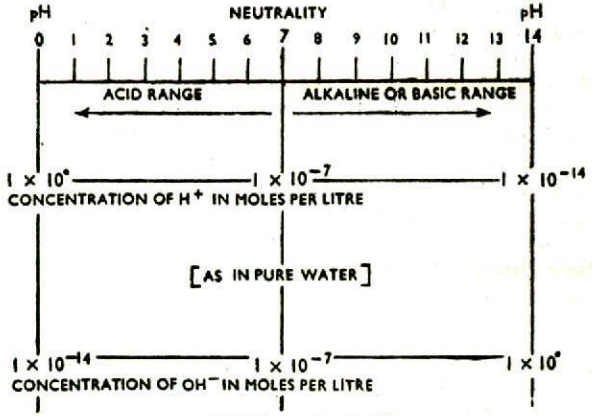


FIG. 93. The scale of pH.

alkaline, has a pH of 7·0. Acid substances produce more $[H^+]$ ions than those are found *in* pure water. They possess pH values of *less* than 7·0. Alkaline (basic) substances

combine with $[H^+]$ ions leaving fewer than those are found in pure water. They have pH values of *more* than 7·0 (Fig. 93). A change of one unit on the pH scale means a tenfold change in hydrogen-ion concentration. A twofold change in hydrogen-ion concentration is expressed by a movement of 0·3 on the pH scale.

Indicators. For standardisation of solution, an indicator is generally used to determine the end point. Indicator can be defined as the very weak organic acid or base that undergoes change of structure with consequent change of colour in the presence of certain concentration of hydrogen and hydroxyl ions. Some common indicators along with their range of colour over a range of concentration have been presented in the following table:

Some common indicators	Ranges of pH	Changes in colour
Alizarin red	5·0 to 6·8	yellow to red
Bromcresol green ..	4·0 to 5·6	yellow to blue
Bromcresol purple ..	5·4 to 7·0	yellow to purple
Congo red	3·0 to 5·0	blue to red
Litmus	4·5 to 8·3	red to blue
Methyl orange	3·1 to 4·4	orange-red to yellow
Methyl red	4·2 to 6·3	red to yellow
Phenolphthalein ..	8·3 to 10·0	colourless to red
Phenol red	6·6 to 8·2	yellow to red
Töpfer's reagent	2·9 to 4·0	red to yellow

Physiological importance of maintenance of pH. A large number of living cells are extremely sensitive to changes in the pH of their extracellular fluids. For the extracellular fluid (ECF) an useful requirement is that it would not be either too acid or too alkaline (basic). The pH of human blood plasma is usually maintained between 7·36 and 7·45. If these limits change, i.e., above pH 7·8 or below 6·8, fatal illness and perhaps death would ensue. Luckily our blood plasma equalises this limit with substances called **buffers** which act to prevent any sudden shift in the pH.

Buffers. Buffers are usually *a mixture of either weak acids with their salts of strong bases or strong acids with their salts of weak bases.* The buffer system of the body has the capability of resisting the change in pH by accepting either the $[H^+]$ or $[OH^-]$ ions. Buffering action is very important in the biological system in maintenance of normal acid-base balance of the body. It is most important in the action of enzymes, both *in vivo* and *in vitro*.

Henderson-Hasselbalch equation can be used for determination of pH of a buffer solution or for determination of relative concentration of the salt and acid required for achieving the normal pH.

The pH of the buffer solution prepared by mixing 35 ml of (N/10) acetic acid with 15 ml of (N/10) NaOH can be determined by the Henderson-Hasselbalch equation:

$pH = pKa + \log$ (salt/acid), where pKa is $\log (1/Ka)$. Ka* is the **dissociation constant of the acid.**

*Consider the equilibrium holding in a pure solution of a weak acid is

$$HA \rightleftharpoons [H^+]+[A^-]$$

By Law of Mass Action $\dfrac{[H^+]\,[A^-]}{[HA]} = Ka$ or $[H^+] = \dfrac{Ka\,[HA]}{[A^-]}$.

So $\log [H^+] = \log Ka + \log [HA] - \log [A^-]$

or, $-\log [H^+] = -\log Ka - \log [HA] + \log [A^-]$.

According to Sorensen $-\log [H^+] = pH$, by similar reasoning $-\log Ka = pKa$

Therefore $pH = pKa + \log \dfrac{[A^-]}{[HA]}$.

When these two solutions are mixed up 15 ml of (N/10) NaOH will neutralise 15 ml of (N/10) acetic acid so as to form 15 ml of the salt, Na-acetate. So 20 ml of acetic acid will remain unneutralised.

So in the buffer solution, the salt and unneutralised acid ratio will be (15/20). The dissociation constant (*Ka*) of acetic acid is ($1{\cdot}86 \times 10^{-5}$) and so the p*Ka* will be log (1/*Ka*), that is 4·73.

So in Henderson-Hasselbalch equation,

$$\begin{aligned} \text{pH} &= 4{\cdot}73 + \log\ (15/20) \\ &= 4{\cdot}73 + \log\ 0{\cdot}75 \\ &= 4{\cdot}73 + \bar{1}{\cdot}8751 \\ &= \mathbf{4{\cdot}6.} \end{aligned}$$

If the ratio of salt and unneutralised acid ratio becomes 1 : 1, then the pH value of the buffer mixture will be equal to p*Ka* of the acid.

As for example, if 30 ml of (N/10) acetic acid and 15 ml of (N/10) NaOH are mixed up then 15 ml of salt—Na-acetate will be formed and 15 ml of acetic acid will remain as unneutralised acid. So the salt and acid ratio will be (15/15) = 1. So interpolating the value in the Henderson-Hasselbalch equation the result will be

$$\begin{aligned} \text{pH} &= \text{p}Ka + \log\ (\text{salt/acid}) \\ &= 4{\cdot}73 + \log\ (15/15) \\ &= 4{\cdot}73 + \log\ 1 \\ &= 4{\cdot}73 + 0 \\ &= \mathbf{4{\cdot}73.} \end{aligned}$$

So the buffering action in this case is at its maximum and can react either as acid or as base.