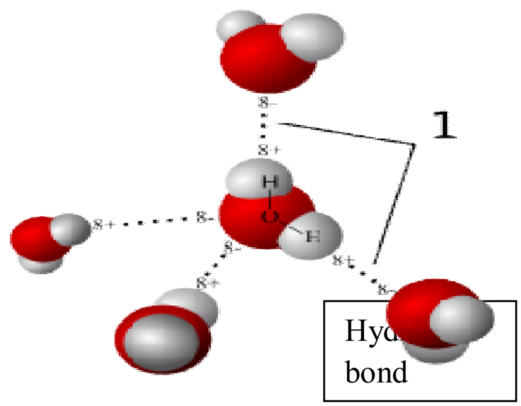
UNIT III. Biological Chemistry Part I

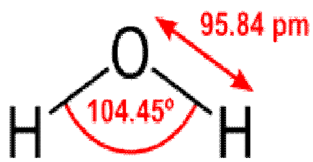
WATER

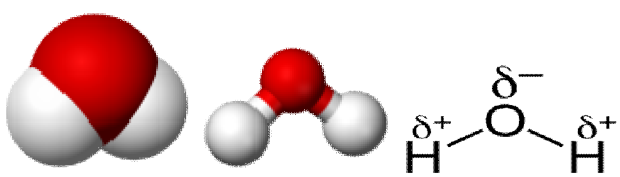
Water (H_2O- *dihydrogen monoxide*) is the most abundant compound on Earth's surface, covering about 70 percent of the planet. In nature, water exists in liquid, solid, and gaseous states. Life on earth is totally dependant on water. Many substances dissolve in water and it is commonly referred to as *the universal solvent*. Water usually makes up 55% to 78% of the human body. Water forms 70-90% of protoplasm, 55% of water is confined to the cells as intracellular fluids e.g. blood, lymph and tissue fluids. In living systems water occurs in two states:

i) Bound water or intramolecular water

ii) Free water or as a solvent







Water is asymmetric molecule where, one molecule of water has two hydrogen atoms covalently bonded to a single oxygen atom. Oxygen is more electronegative and attracts electrons much more strongly than hydrogen, resulting in a net positive charge on the hydrogen atoms, and a net negative charge on the oxygen atom. Hence, water molecule is said to be polarized which contains hydrogen atom with partial positive charge (+) and oxygen with partial negative charge (-). Electrical attraction between O atom of one water molecule to the H atom of another water results in formation of hydrogen bond. One water molecule forms four hydrogen bonds with four neighboring water molecules to form tetrahedral structure.

Properties of Water

1. Water molecule has neutral charge and its pH is 7.0.
2. At room temperature, it is a tasteless, odorless and colorless liquid. It exists in three forms (liquid, vapor and solid form).

3. It has high heat of evaporation i.e. it requires a large amount of heat to change from a liquid to gaseous state.
4. Water has a very high specific heat capacity and hence can absorb or release a relatively large amount of heat with a little change in temperature. This properties allow water to moderate Earth's climate by resisting large fluctuations in temperature.
5. Water has a high surface tension. Polar structure of water allow it to adhere to any other charged molecules or surfaces, this gives "wetting" capacity of water. This property keeps the body of animals moist.
6. High viscosity of water allow the organisms to float on it. It works like lubricant and protect the aquatic organisms from mechanical disturbances.
7. The density of water increases with decrease in temperature and water molecules come closer. In the course of bonding on further decrease in temperature molecules move apart forming molecular lattice work (ice crystal). Hence ice becomes less dense than liquid water and therefore it floats on water.
8. Pure water is poor conductor of electricity.
9. Water is also a good solvent, due to its polarity. Substances that will mix well and dissolve in water (e.g. salts) are polar and known as hydrophilic ("water-loving") substances, while those that do not mix well with water (e.g. fats and oils), are non-polar and known as hydrophobic ("water-fearing") substances.
10. Salinity of water is because of various salts present in it. E.g. Na, K, Ca, Mg, Cl, S, NO_3, $NaHCO_3$ etc.
11. Chemically, water is amphoteric: it can act as either an acid or a base in chemical reactions.

Dissociation of Water

Almost all reactions in the living body occurs in aqueous medium where H+ ion concentration changes slightly. But, to ensure homeostasis, extracellular and intracellular fluids must contain balanced quantities of acids and base. Chemically, water is amphoteric: it can act as either an acid or a base in chemical reactions. As an acid it donates a proton (a H^+ ion) and as a base it receives a proton. Water can be described as a polar liquid that slightly dissociates disproportionately into the hydronium ion (H_3O^+) and an associated hydroxide ion (OH^-).

Water dissociates into hydroxyl ion (OH^-) and proton (H^+) as follows:

$H_2O \rightarrow H^+ + OH^-$ OR

$2H_2O \rightarrow H_3O^+ + OH^-$

$H_3O^+ \rightleftharpoons H^+ + H_2O \rightleftharpoons H^+ + OH^-$

Hydronium ion	Amphoteric water	Hydroxyl ion
(Acid)		(Base)

Proton combines with variety of molecules as follows

1. $H^+ + H_2O \rightarrow H_3O^+$ (Hydronium ion)

2. $H^+ + OH^- \rightarrow H_2O$ (Water)

3. $H^+ + {}^-NH_2 \rightarrow {}^-NH^+_3$

   Proton + Amino group → Charged amine

For dissociation of water dissociation constant K can be given as

$$K = \frac{[H^+][OH^-]}{[H_2O]} = \frac{\text{Molar concentration of reactant}}{\text{Molar concentration of product}}$$

$$1.8 \text{ X } 10^{-16} = \frac{[H^+][OH^-]}{55.5}$$

Where, Electrical conductivity of water = $1.8 \text{ X } 10^{-16}$M

Molar concentration of water = 55.56 mol/L

Now

Ionic product of water,

$$K_w = [H^+][OH^-]$$

Therefore,

$$K_w = (1.8 \text{ X } 10^{-16}) \text{ X } (55.56)$$

$$K_w = 10^{-14} \text{ M}^2$$

At equilibrium $[H^+] = [OH^-]$

$$[H^+][OH^-] = [H^+]^2$$

Therefore, $[H^+] = \sqrt{Kw}$

In pure water

$[H^+] = 10^{-7}$ M and pH of pure water is 7.0.

Significance of water to living organisms

1. Water is also a good solvent as it interacts with both inorganic and organic substances. Hydrophilic compounds mixes well and dissolve in water (e.g. salts, sugars) are known as ("water-loving") substances, while hydrophobic compounds do not mix well with water (e.g. fats and oils), are known as ("water-fearing") substances.

2. It forms major component of cell protoplasm as well as body fluids (intercellular matrix).
3. Water dissolves or suspend life-supporting substances e.g. starch, glycogen, protein and transport them to intercellular and intracellular fluids.
4. As it dissolves or suspend many substances it acts as an ideal medium in which reactions take place which are carried out by enzymes. Reactions provide energy (non-matter) for living.
5. Water stabilizes structural organization of living matter e.g. folded or three dimensional structure of protein and phospholipid bilayer to form plasma membrane.
6. Water serves as a medium for distribution of organisms as aquatic plants and animals. Aquatic living organisms extract nutrients from water, yet maintaining a balance of electrolyte and nourishment concentration in their cells.
7. Due to solvent action and amphoteric nature, water helps to maintain a constant pH of cell.
8. Because of high heat of evaporation, water causes elimination of excess of body heat through evaporation of sweat and maintains body temperature.
9. Water dissolves nitrogenous waste products which are eliminated out in the form of urine.
10. Because of high viscosity it acts as a lubricant. Water is a major part of mucous and other lubricating fluids.
11. Water contains many inorganic substances or minerals are essential to life.

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pH

Water dissociates into the hydronium ion (H_3O^+) and an associated hydroxide ion (OH^-) and again combines to form water molecule. These protons are released in surrounding medium and accepted by other molecules. Acids are proton donors and bases are proton acceptors. Some of the examples of acids are as follows.

i) $H_3O^+ \rightleftharpoons H^+ + H_2O \rightleftharpoons H^+ + OH^-$

Hydronium ion (Acid) — Amphoteric water — Hydroxyl ion (Base)

ii) $CH_3COOH \rightarrow CH3COO^- + H^+$

Acetic acid — Acetate ion (Base) — Proton

iii) $H^+ + {}^-NH_2 \rightarrow {}^-NH^+_3$

Proton — Amino group — Charged amine

From above example acids and bases exists in pairs, thus a base is called conjugated base. In these conjugates acids are in liquilibrium with its conjugate base (Acid + Base + H^+).

Hydrogen ions (H^+) are responsible for acidity and hydroxyl ions (OH^-) are responsible for alkalinity of solution. Chemical reactions in the body are sensitive to slight change in hydroxyl or hydrogen ion concentration. Thus, to maintain homeostasis intracellular and ectracellular fluids must contains balanced quantities of acids and bases.

Acidity of solution is measured as concentration of hydrogen ion i.e. pH.

Definition: According to Sörensen (1909) pH is negative log of the hydrogen ion concentration (in moles per liter). (p- Negative $\log_{10}$)

$$pH = -\log [H+]$$

A pH scale in terms of acidity and alkalinity of solution ranges from 1 to 14. Solution with less than 7 are acidic and solutions with pH greater than 7 are alkaline. pH 7 is neutral pH.

e.g. Solution with pH 4 is acidic and H^+ ion concentration is 1×10^{-4}.

e.g. Solution with pH 9 is alkaline and H^+ ion concentration is 1×10^{-9}.

Henderson-Hasselbalch Equation- Consider the dissociation of a weak acid (HA) into a proton (H^+) and its conjugate base (A^-) in aqueous solution.

$$HA = H^+ + A^-$$

$$pH = -\log [H+]$$

The dissociation constant for the acid, Ka is given as

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

Simplifying this we get

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

OR

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

Taking log to the both side,

$$\log [H^+] = \log Ka + \log \frac{[HA]}{[A^-]}$$

Change sign of both side multiplying by -1

$$-\log [H^+] = -\log Ka - \log \frac{[HA]}{[A^-]}$$

Now $-\log[H^+] = pH$

$-\log Ka = pKa$

Substituting we get,

$$pH = pKa - \log \frac{[HA]}{[A^-]}$$

Invert equation by changing sign we get,

$$pH = pKa + \log \frac{[A^-]}{[HA]}$$

Above equation is known as the ***Henderson—Hasselbalch equation,*** and it provides a convenient means of calculating the pH of mixture of weak acid and their salts. E.g. pH of blood can be determined if concentration of salt bicarbonate (0.025 M) and carbonic acid (0.00125 M) is known. It is also used to determine pH of unknown buffer. $(pKa = 6.1)$

$$pH = 6.1 + \log \frac{0.025}{0.00125}$$

Therefore pH of blood is $pH = 7.4$

BUFFERS

Most biological processes are sensitive to pH because it affects ionic state of biological molecules. In order to resist the fluctuations in pH, body is protected by chemical compounds called Buffers.

"Buffer is a solution that resists a change in pH on addition of alkali or acid." Buffers are solution containing weak acid and its conjugate base.

e.g. Acetic acid : Sodium acetate

Ammonium hydroxide : Ammonium chloride

- Buffering action is the process by which added H^+ or OH^- ions are removed.
- Buffering capacity is extent of resistance of change in pH by buffer.

Hydrogen ions are produced in the body during metabolism of various substances as follows:

1. Carbonic acid (H_2CO_3)- It is the chief acid in the body. Oxidation of C- compounds produces about 10-20 moles of CO_2 which combines with water to give carbonic acid.
2. Sulphuric acid (H_2SO_4)- It is produced during oxidation of S- containing amino acids e.g. Cystein and methionine.
3. Phosphoric acid (H_3PO_4)- It is produced during metabolism of phosphoproteins, nucleoprotein, Phosphatids and hydrolysis of phosphoesters.
4. Organic acids are produced as metabolic intermediates e.g Pyruvic acid, lactic acid, ace to-acetic acid etc.

There are three primary systems that regulate the H^+ concentration in the body fluids to prevent acidosis or alkalosis:

First-line defense-

(1) The *chemical acid-base buffer systems of the body fluids,* which immediately combine with acid or base to prevent excessive changes in H^+ concentration.

(2) The *respiratory center,* which regulates the removal of CO_2 (and, therefore, H_2CO_3) from the extracellular fluid.

Second line defense-

(3) The *kidneys,* which can excrete either acid or alkaline urine, thereby readjusting the extracellular fluid H^+ concentration toward normal during acidosis or alkalosis.

Buffers in biological systems

1. Carbonic acid – bicarbonate buffer
2. Phosphate buffer
3. Protein buffer

When there is a change in H^+ concentration, the *buffer systems* of the body fluids react within a fraction of a second to minimize these changes. Buffer systems do not eliminate H^+ from or add them to the body but only keep them tied up until balance can be reestablished.

1. Carbonic acid – bicarbonate buffer-

The bicarbonate buffer system consists of a water solution that contains two ingredients: a weak acid, Carbonic acid (H_2CO_3), and a bicarbonate ion (HCO_3^-). It is found in both extracellular and intracellular fluids and H_2CO_3 is formed in the body by the reaction of CO_2 with H_2O.

- Bicarbonate ion acts as a weak base and removes H^+ as follows:

$$H^+ + HCO_3^- \rightarrow H_2CO_3$$

Hydrogen ion — Bicarbonate ion (Weak base) — Carbonic acid

Subsequently, H_2CO_3 dissociates in to water and CO2 which exhaled by lungs.

- Carbonic acid acts as a weak acid and provides H^+ whenever there is shortage of H^+ as follows:

$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

Carbonic acid (Weak acid) — Hydrogen ion — Bicarbonate ion

At pH 7.4 HCO_3^-concentration is about 24 m mol./L while H_2CO_3 is about 1.2 m mol./L.

2. Phosphate buffer

Although the phosphate buffer system is not important as an extracellular fluid buffer, it plays a major role in buffering renal tubular fluid and intracellular fluids. Components of the phosphate buffer system are dihydrogen phosphate ($H_2PO_4^-$) and Monohydrogen phosphate ion (HPO_4^-).

- Dihydrogen phosphate ($H_2PO_4^-$) acts as a weak acid and buffers strong bases such as OH^- as follows:

$$OH^- + H_2PO_4^- \rightarrow H_2O + HPO_4^{2-}$$

Hydroxyl ion (Strong base) — Dihydrogen phosphate (Weak acid) — Water — Monohydrogen phosphate (Weak base)

- Monohydrogen phosphate (HPO_4^{2-}) acts as a weak base and buffers H^+ released by strong acid such as HCl as follows:

$$H^+ + HPO_4^{2-} \rightarrow H_2PO_4^-$$

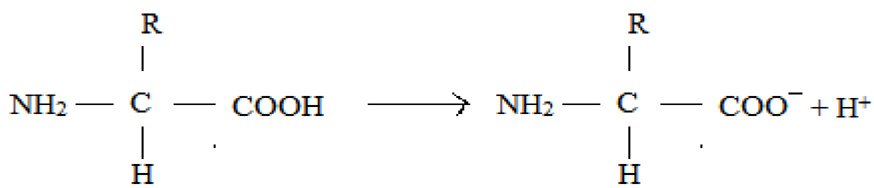
Hydrogen ion (Strong acid) — Monohydrogen phosphate (Weak base) — Dihydrogen phosphate (Weak acid)

Concentration of phosphate buffer is highest in intracellular fluids, therefore it regulates pH in cytoplasm. It also acts at extracellular fluids of kidney such as urine. When H^+ combines with HPO_4^{2-} it gives $H_2PO_4^-$ which then passes in to urine and blood pH is maintained.

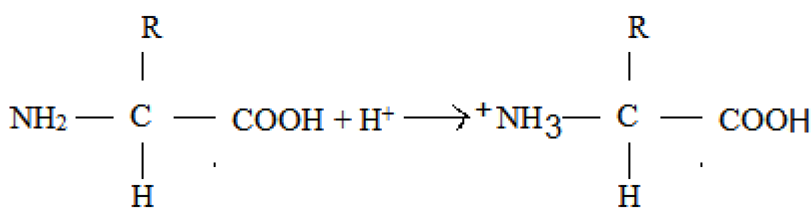
3. Protein buffer

It is important buffer in intracellular fluid and plasma. In the red blood cell, protein hemoglobin (Hb) and in plasma, albumin are important buffers. The carboxyl group (-COOH) and amino group ($-NH_2$) are functional components of protein buffer system.

- When pH rises i.e. becomes alkaline, carboxyl group acts like acid and release H^+ which reacts with excess OH^- and forms water as follows:



- When pH falls i.e. becomes acidic, free amino group at the other end of protein acts as a base and combines with H^+ as follows:



Thus, proteins can buffer both acids and bases.

- In red blood cells haemoglobin effectively buffers H^+ in the following way:

  In blood capillaries CO_2 from tissues enters in to red blood cells and combines with H_2O to form carbonic acid H_2CO_3 which again dissociates in to H^+ and HCO_3^-. At the same time oxyhaemoglobin ($Hb.O_2$) gives oxygen to tissues. Reduced haemoglobin (de-oxyhaemoglobin) is excellent buffer of H^+. As it picks H^+ reduced haemoglobin is written as Hb.H.

H_2O (Water) + CO_2 (Entering cells) → H_2CO_3 (Carbonic acid)

H_2CO_3 → H^+ (Hydrogen ion) + HCO_3^- (Bicarbonate ion)

$Hb.O_2$ (Oxyhaemoglobin) + H^+ (Hydrogen ion From carbonic acid) → Hb.H (Reduced haemoglobin) + O_2 (Released to cells)