MICROBIOLOGY COURSE MATERIAL

Semester – II (CC 3)

CC 3 : Biochemistry
Unit 1: Bioenergetics

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CC-3: Biochemistry (THEORY)
SEMESTER – II

❖ Properties of Water & Concept of pH and Buffers

Biological systems need water because it provides many functions. Those functions can be explained as follows -

- **As a medium of transport**

Water is acts as a transport medium for different molecules, cells, and other materials. The plasma, which makes up about 55 % of the fluid part of blood, contains 90% of water. This water transports various materials like blood cells and hormones throughout the human body. Water is also essential in intra- and intercellular transport.

- **As a solvent**

Water in the body is a universal solvent. It dissolves many compounds such as sodium chloride and other salts. Water dissolves urea and other nitrogenous compounds like uric acid. This is essential for the excretion of these compounds.

- **As a metabolite**

Water in the body is a metabolite and takes part in many reactions.

- **Mobility of Spermatozoa**

Spermatozoa can move only by their flagella in water. If there is no water, they cannot move at all. The process of fertilization cannot take place.

- **As a Structure Determinant**

Water determines the characteristic structure and biological properties of biomolecules. Proteins, nucleic acids, and polar lipids have both hydrophobic and hydrophilic parts. They tend to form structures in which the non-polar hydrophobic parts can hide from water.

- **As a Coolant**

The high specific heat of water allows it to act as a coolant and regulate the body temperature in hot conditions. The temperature of the organism can remain constant as the air temperature fluctuates. The high heat of evaporation of water also helps maintain body temperature. When we sweat the water from the skin evaporates and produces a cooling effect.

- **As a lubricant**

Water acts as a lubricant in the body during digestion. The water present in saliva lubricates the food and makes the passage to lower digestive tract easy. Also, water around our eyeballs, muscles, and joints ensures that they can move without friction. These roles can be explained once we have understood the structure and bonding in a water molecule, and between water molecules.

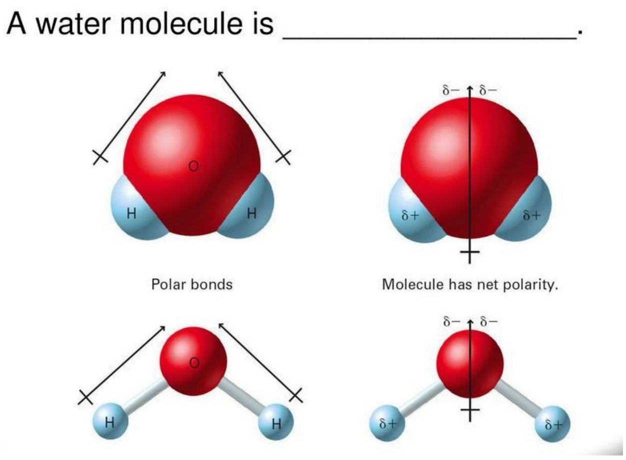
- **A molecular compound**

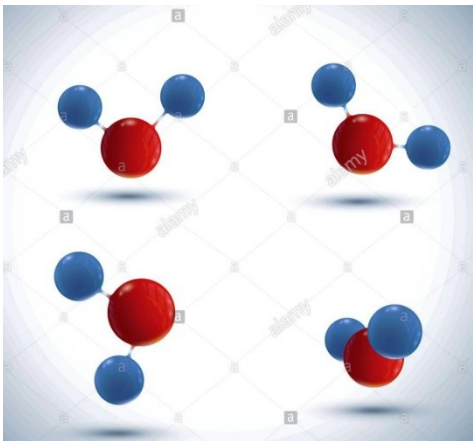
Water is a molecular compound, with molecular formula H_2O. The atoms in a water molecule are held together by strong covalent bonds. These are very difficult to break.

Diagram of a Water Compound

The dot-and-cross diagram for a water molecule shows there are two **bonding pairs of electrons** and two **non-bonding pairs of electrons**. The four pairs repel one another, forming a tetrahedral pattern. In this way they are as far from one another as possible. The molecule itself (the spatial distribution of atoms) is described as 'bent', 'angular' or 'non-linear'. The two electrons in each oxygen-hydrogen bond are not shared equally. They are more strongly attracted to the oxygen atom. The bond is **polar**, it has a 'negative end' (the oxygen atom) and a 'positive end' (the hydrogen atom).

A **hydrogen bond** forms between a non-bonding pair of electrons on the oxygen atom of one water molecule and the hydrogen atom ('positive end') of another water molecule. The hydrogen bond is about ten times weaker than a single covalent bond.





Water molecule in liquid state **Water molecules in Ice**

- **Water as Solvent**

Most compounds with **ionic bonding**, e.g. metal salts, dissolve in water. The oxygen atoms of water molecules are attracted to cations (ions with a positive charge) and water molecules surround it. These water molecules attract more water molecules and hydrogen-bonds form between them. The result is a cluster of water molecules around the ion. We say the ion is hydrated.

Similarly anions (ions with a negative charge) become surrounded by clusters of water molecules. This time it is the positive ends of the water molecule, the hydrogen atoms that are attracted to the anion.

A wide range of molecular compounds also dissolve in water, including sugars, amino acids, small nucleic acids and proteins. All these molecules are **polar**. This means they have a positive end and a negative end as the result of polar covalent bonds within them. Of the important biological molecules only the non-polar lipids (fats and oils) and large polymers (e.g. polysaccharides, large proteins and DNA) do not dissolve.

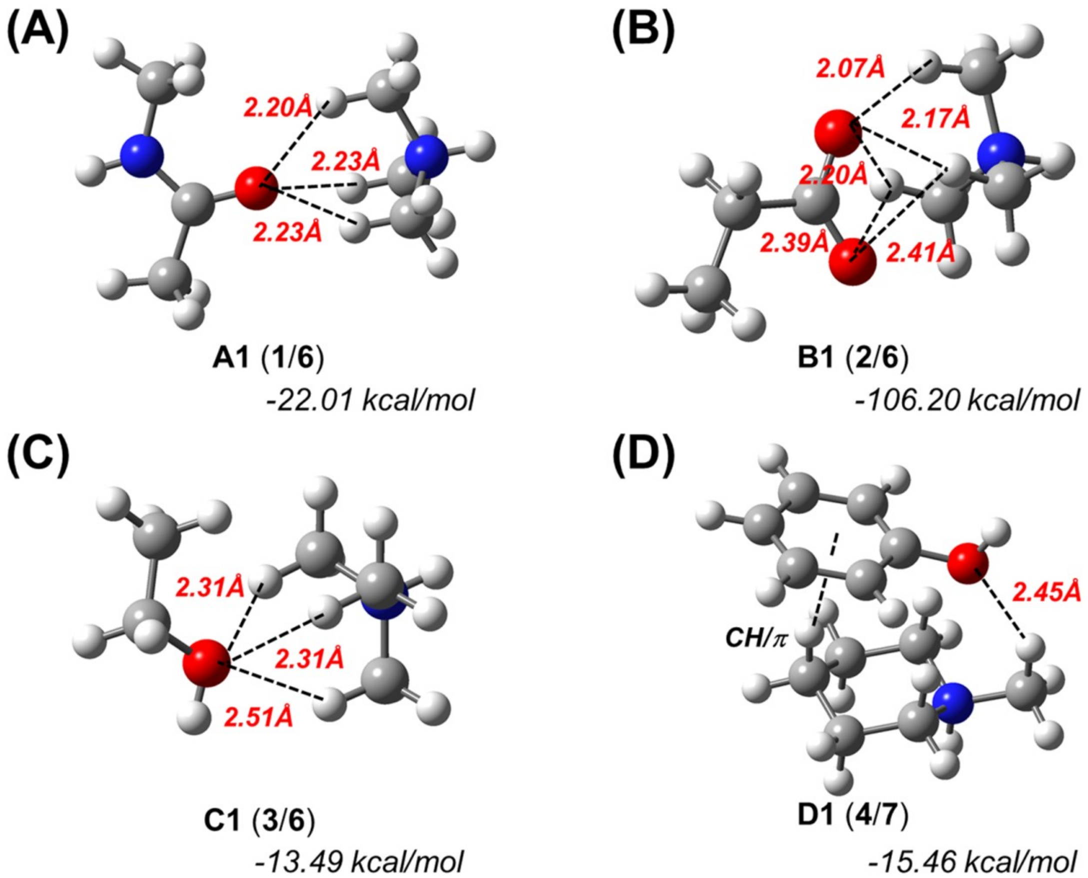


Diagram 3: Hydrogen Bonds in Protein Ligand Complexes

The water acts as a solvent for chemical reactions and also helps transport dissolved compounds into and out of cells. As a salt such as NaCl dissolves, the Na^+ and Cl^- ions leaving the crystal lattice acquire far greater freedom of motion (Fig. 4-6). The resulting increase in the entropy (randomness) of the system is largely responsible for the ease of dissolving salts such as NaCI in water. In thermodynamic terms, formation of the solution occurs with a favourable change in free energy: $\Delta G = \Delta H - T\Delta S$, where ΔH has a small positive value and $T\Delta S$ a large positive value; thus ΔG is negative.

The biologically important gases like CO_2, O_2, and N_2 are non-polar. These gases are consequently very poorly soluble in water.

Another important property is that many compounds dissolve and transfer a proton (a hydrogen nucleus) to a water molecule. The result is an acidic solution with pH < 7. Compounds that release a proton in this way are called **acids**. For example,

$$\mathbf{CH_3COOH\ (aq) + H_2O\ (aq) \rightleftharpoons CH_3COO^-\ (aq) + H_3O^+\ (aq)}$$

H_3O^+(aq) is called a **hydroxonium ion** and is responsible for the acidic properties of the solution.

$pH = -\log_{10}[H_3O^+]$ and provides a scale for acidity and basicity.

Some molecules receive a proton from a water molecule. The result is an alkaline solution with pH > 7. Compounds that accept a proton in this way are called **bases**. For example, OH^-(aq) is called a **hydroxide ion** and is responsible for the alkaline properties of the solution.

❖ Temperature management

Cells host a huge range of chemical reactions. Many of these are catalysed by Enzymes. Enzyme activity is sensitive to temperature and reactions only occur in a narrow range of temperatures. Water helps to manage temperature changes because of its relatively high **specific heat capacity** (the heat required to raise 1 kg of water by 1 $^{\circ}$C). It also has relatively large **enthalpy of vaporisation** (heat energy required to convert a liquid to a gas) and **enthalpy of fusion** (heat energy required to convert a solid to a liquid). This is reflected in the unusually high boiling and melting points of water and these are due to H bonds in it. These properties are a consequence of **hydrogen bonding.**

- **Metabolite**

Chemical reactions take place in cells. Collectively these reactions together are called metabolism, i.e. all the chemical and physical processes within a cell. The chemicals produced are called metabolites. Water is a metabolite in many reactions. It's involved in photosynthesis, digestion, metabolism and energy production.

When water reacts with a chemical to break it into smaller molecules the reaction is described as **hydrolysis**. When water is formed as one of the products when two molecules join together the reaction is described as **condensation**.

- **Living environment**

Many organisms, such as fish, live in water and cannot survive out of it. They have adapted to living in it. Ice floats on water. This is because ice is less dense than water. The reason is that ice has a giant structure with every oxygen atom at the centre of a tetrahedral arrangement of hydrogen atoms (two are covalently bonded and two are hydrogen-bonded).

In freezing weather, ice forms on the surface of ponds and lakes forming an insulating layer above the water below. This provides a living environment for some organisms until the ice melts. Organisms can also live under the ice. The surfaces of ponds and lakes (and other forms of water) are covered in a 'skin' of water molecules. While most objects break through this skin, it is strong enough to support small insects such as pond skaters. The skin forms because of the increased attraction between water molecules (**cohesive forces**) at the surface.

Carbohydrates, lipids, proteins and nucleic acid are 4 major biomolecules which forms the living system in terms of their solubility, which depends on their composition of basic elements which are made up of likes of carbon, hydrogen, nitrogen, Phosphate, oxygen and sulphur.

Carbohydrates: Simple carbohydrates are generally water soluble example Sucrose (table sugar), Glucose (rice), fructose (fruit sugar), maltose (malt) etc. in their monomer and dimer forms all sugars are water soluble if sugar units in their ring structure (Howerth ring structure), are attached to lipids they are insoluble mainly because they are attached to lipids.

Lipids: they are known water insoluble biomolecules.

Proteins: Proteins are of two major classes one is globular plasma soluble proteins which are made up of polar, polar charged, basic and acidic amino acids, hence they are water soluble and these proteins have physiological functions. Examples are Albumin, Immunoglobulin, Bovine Serum Albumin, Casein etc. Another class is the tough, elastic fibers which are water insoluble mainly because they are made up of non polar amino acids which do not soluble in water.

Nucleic acids: Nucleic acids are hereditary materials which are DNA (Deoxyribonucleic acids) and RNA (Ribose-sugar nucleic acids), the structural building block for both DNA and RNA are nucleotides which are made up of 3 components: one is a purine or pyrimidine ring, a 5 carbon sugar and a phosphate group. Since Both DNA and RNA has phosphate groups on the backbone which is extended outward from the main chain and since they are ionized having charges they contribute their water solubility. Nucleic acids are completely water soluble.

❖ Acids, Bases and pH

There are millions of chemical substances in the world. Some of them have acidic properties, others, basic properties. ***Acids* are substances which free hydrogen ions (H^+) or can accept pair of electrons , when they are mixed with water. *Bases* are substances which free hydroxide ions (OH^-) or accept proton when they are mixed with water**. (This freeing of ions is called dissociation in both cases). Free hydroxide ions react with the hydrogen ions producing water molecules: $H^+ + OH^- = H_2O$. in this way, bases diminish the concentration of hydrogen ions. A solution rich in hydrogen ions is acidic, a solution poor in hydrogen ions is basic. Some acids dissociate only in part and they are called *weak acids*; others dissociate completely, freeing large amounts of hydrogen ions, and they are called *strong acids*. In the same way, the bases can be stronger or weaker. Diluted acids and bases are less concentrated and less aggressive in their actions. The acidic or basic degree of substances is measured in *pH* units. The scale used spans from 0 to 14. Substances with pH lower than 7 are considered acids, those with pH equal to 7 are considered neutral, and those with pH higher than 7 are considered bases. Substances with low pH are very acidic, while those with high pH are highly basic.

Concentrated acidic and basic substances are very corrosive and dangerous.

pH is the measure of the concentration of hydrogen ions in a solution. As this concentration can extend over several orders of magnitude, it is convenient to express it by means of logarithms of base ten. As this concentration is always less than one, its logarithm always has the minus sign. To avoid having to always write the minus sign, it has been agreed to write this value with the positive sign. (This is the same as using the logarithm of the reciprocal of the hydrogen ion concentration). So, the pH is the logarithm of the concentration of hydrogen ions, with the sign changed**: pH = - log $[H^+]$.** Thus, when pH has low values, the concentration of hydrogen ions is high.

Distilled water has pH = 7. So how it is possible that distilled water has free hydrogen ions? Their presence is due to the casual dissociation of some water molecules because of the thermal agitation ($H_2O \leftrightarrow H^+ + OH^-$). Immediately after, these ions recombine themselves, but other molecules dissociate themselves, thus keeping a constant equilibrium of a certain concentration of dissociated molecules.

The degree of ionization of water at equilibrium is small; at 25°C, only about one of every 10^7 molecules in pure water is ionized at any instant. The equilibrium constant for the reversible ionization of water is: $K_{eq} = [H^+][OH^-]/[H_2O]$. In pure water at 25°C, the concentration of water is 55.5 M (i.e., grams of H_2O in 1 litre divided by gram molecular weight or 1000/18 M = 55.5 M). This value is essentially constant in relation to the very low concentrations of H+ and OH– , namely 1 × 10^7 M. Accordingly, on substituting 55.5 M in the equilibrium constant expression, we get : Keq = [H+][OH–] /55.5 M which, on rearranging, becomes : (55.5 M) (Keq) = [H+] [OH–] = Kw where Kw designates the product (55.5 M) (Keq), the ion product of water at 25°C. The value for Keq is 1.8 × 10^{-16} M at 25°C as calculated from electrical conductivity measurements. Substituting this value for Keq in above equation:

$$\mathbf{(55.5\ M)\ (1.8 \times 10^{-16}\ M)}$$

$$\mathbf{= [H+\ ]\ [OH-\ ]\ 99.9 \times 10^{-16}\ M^2 = [H+\ ]\ [OH-]1.0 \times 10^{-14}\ M^2}$$

$$\mathbf{= [H+\ ]\ [OH-\ ]}$$

$$\mathbf{= Kw}$$

Thus, the product [H+] [OH–] in aqueous solutions at 25°C always equals 1 × 10^{-14} M^2 . When there are exactly equal concentrations of both H+ and OH–, as in pure water, the solution is said to be at neutral pH. At this pH, the concentration of H+ and OH– can be calculated from the ion product of water as follows:

$$\mathbf{[H+\ ] = [OH-\ ] = 10^{-7}\ M.}$$

As the ion product of water is constant, whenever the concentration of H+ ions is greater than 1 × 10^{-7} M, the concentration of OH– must become less than 1 × 10^{-7} M, and vice versa. When the concentration of H+ is very high, as in a solution of hydrochloric acid, the OH– concentration must be very low. Living system produce acids like lactic acid, pyruvic acid, acetic acid hydrochloric acid etc. Living system produces bases like adenine, guanine, cytosine, thymine and so on.

pH is very important for biochemical reactions because almost all biochemical reactions are catalyzed by enzymes and enzymes show their optimum activity at an optimum pH . If pH of the reaction is not maintained the optimum activity will not be available. Again to maintain the particular pH particular buffer is required.