

Water & pH



BIOCHEMISTRY





- Water is essential for life. It covers $\frac{2}{3}$ of the earth's surface and every living thing is dependent upon it.
- The human body is comprised of over 70% water, and it is a major component of many bodily fluids including blood, urine, and saliva.



- Water (H_2O) is made up of 2 hydrogen atoms and one oxygen atom,
- With a total atomic weight of 18 daltons.
- The structure of the electrons surrounding water is tetrahedral, resembling a pyramid.





- Water has important effects on all biological systems.
- Water has two very important properties:
 1. Physical Properties
 2. Chemical Properties



1. Physical Properties:

- Polar molecule
- Hydrophilic substances dissolve
- Hydrophobic substances aggregate
- Osmosis
- Diffusion



2. Chemical Properties :

- Ionizes to O and OH
- Acids and bases alter pH
- Buffers resists changes in pH

Physical properties



1. Water is a polar molecule

A water molecule is formed when two atoms of hydrogen bond covalently with an atom of oxygen.



- In a covalent bond electrons are shared between atoms. In water the sharing is not equal. The oxygen atom attracts the electrons more strongly than the hydrogen.



- This gives water an asymmetrical distribution of charge.
- Molecules that have ends with partial negative and positive charges are known as polar molecules.
- It is this polar property that allows water to separate polar solute molecules and explains why water can dissolve so many substances.



- The HOH bond angle is 104.5
- The oxygen nucleus attracts electrons more strongly than does the hydrogen nucleus (a proton);
- that is, oxygen is more electronegative.

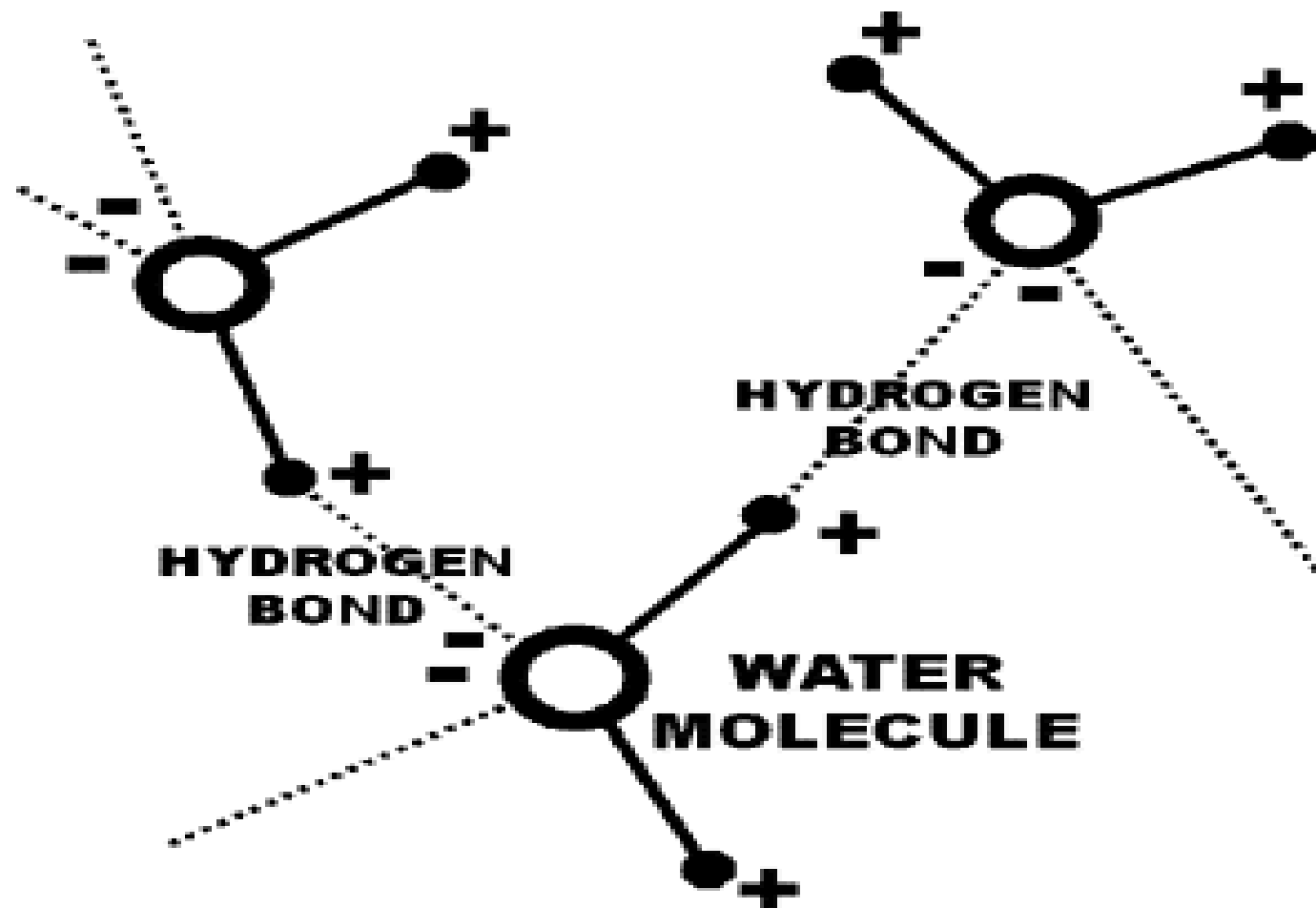
- The sharing of electrons between H and O is therefore unequal;



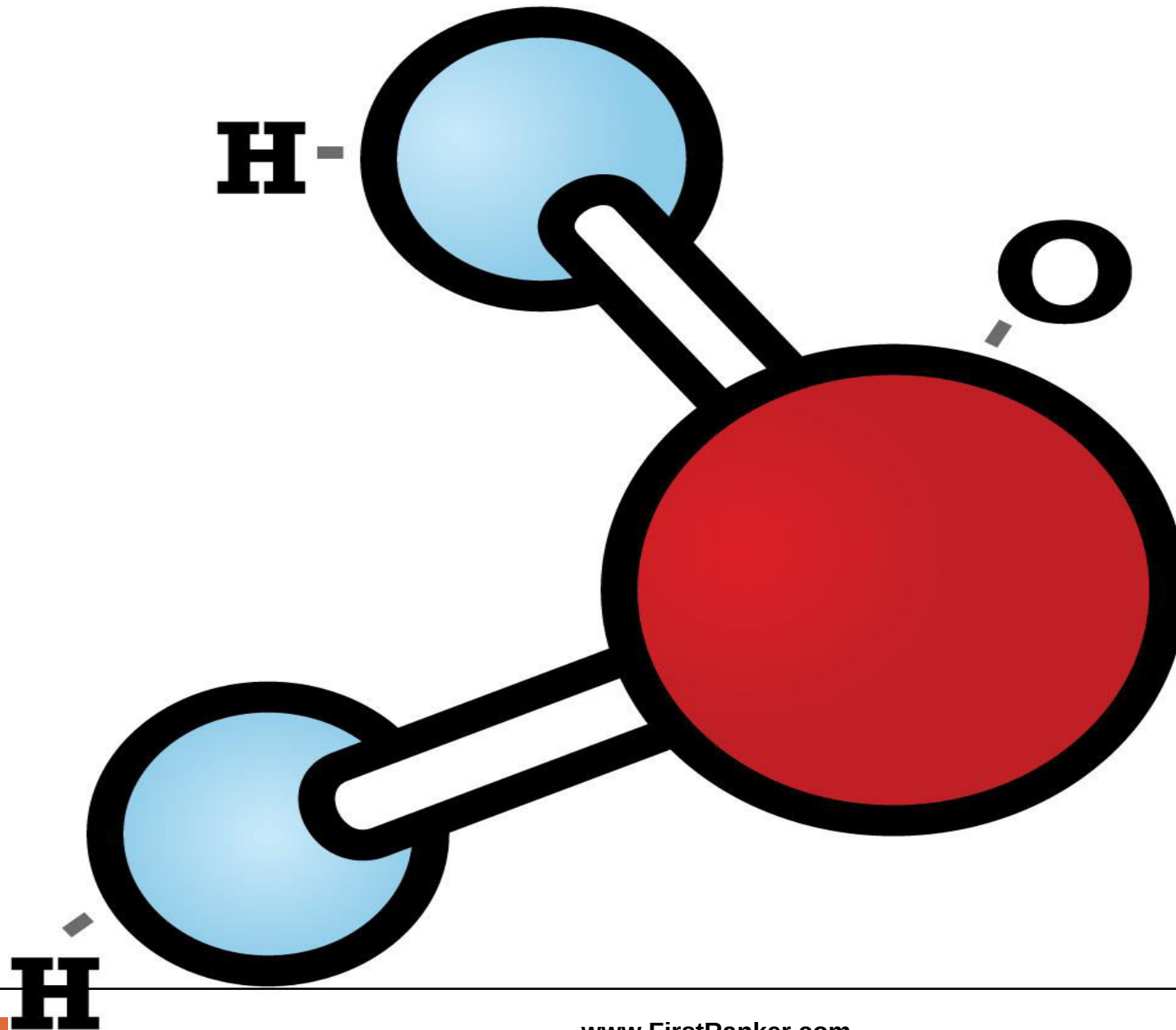
- The result of this unequal electron sharing is two electric dipoles in the water molecule:
- one along each of the H O bonds:
- each hydrogen bears a partial positive charge
- And the oxygen atom bears a partial negative charge equal to the sum of the two partial positives



- As a result, there is an electrostatic attraction between the oxygen atom of one water molecule and the hydrogen of another called a **hydrogen bond**.



– WATER MOLECULES ARE POLAR DUE TO PARTIAL CHARGES. THEY WILL LINE-UP DUE TO ATTRACTION AND REPULSION OF THESE PARTIAL CHARGES.



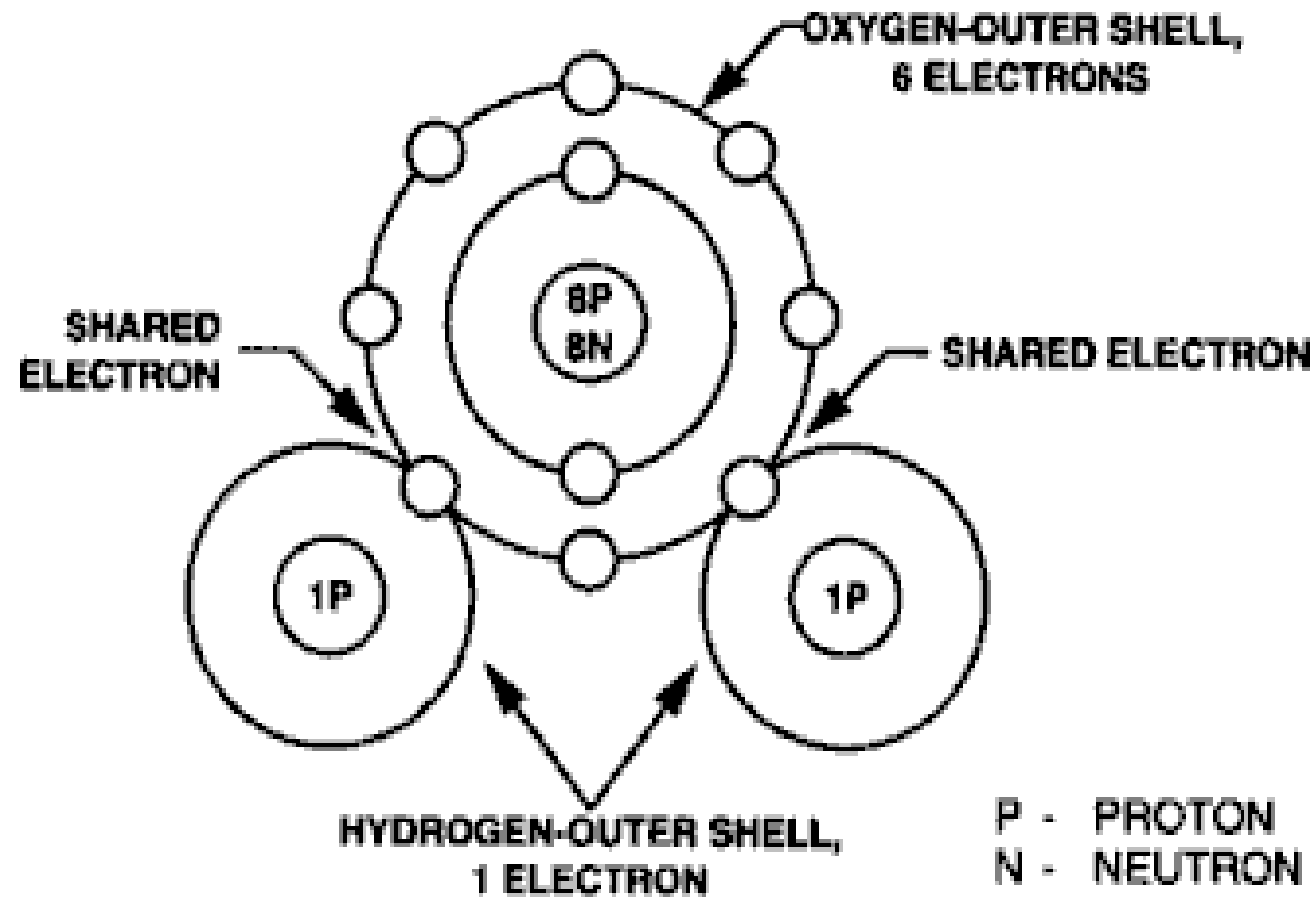
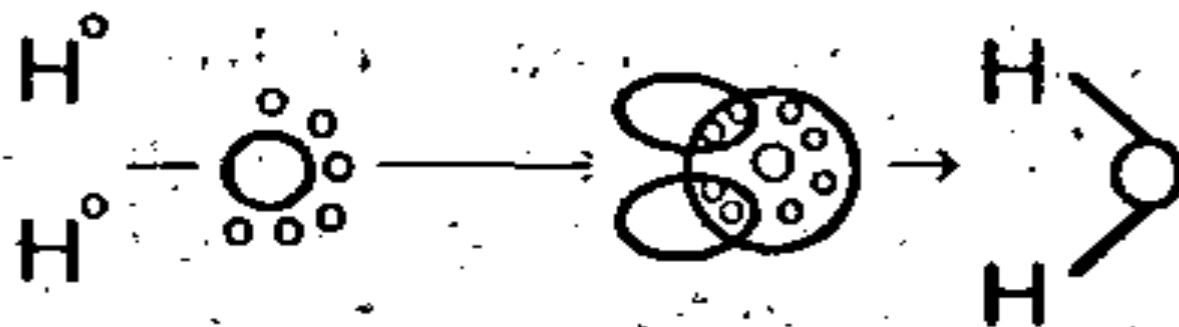
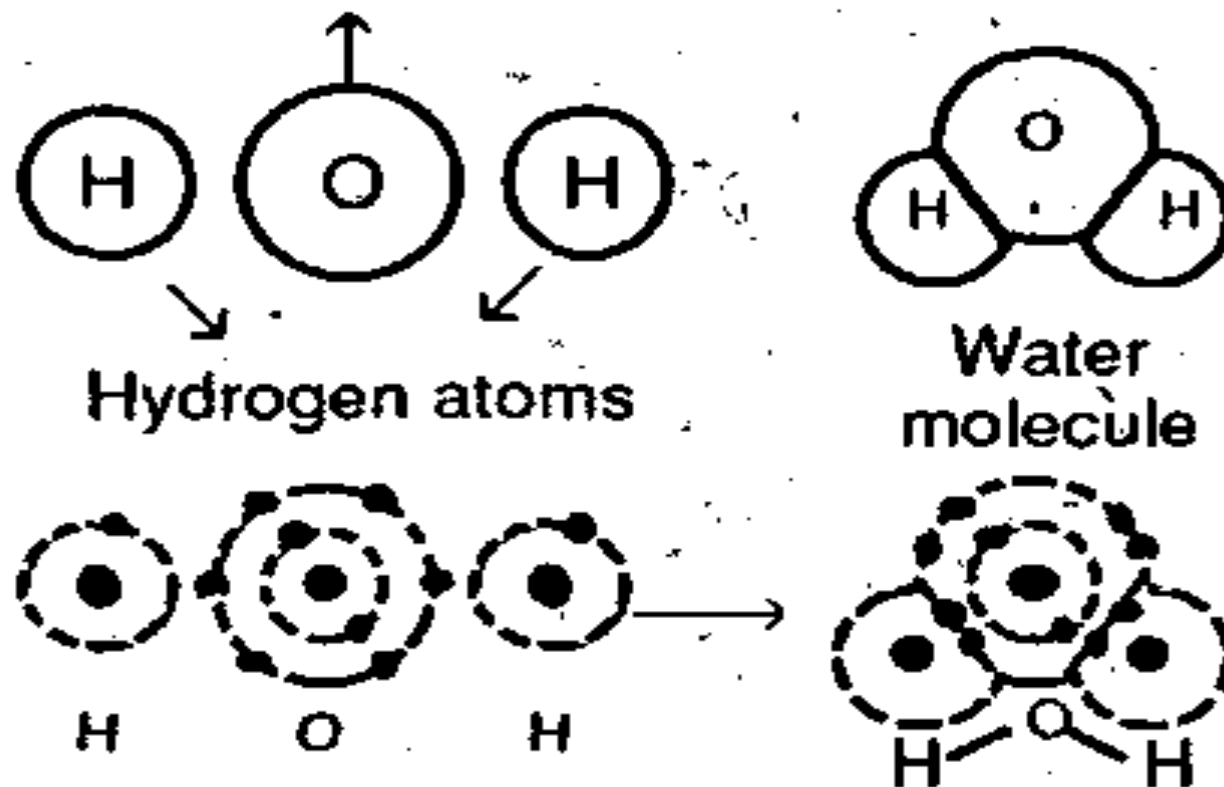


Figure 2-II. Illustration of a Molecule (H_2O)



Molecular structure of water



2. Hydrophilic & Hydrophobic

- Water is a polar solvent. It readily dissolves most biomolecules, which are generally charged or polar compounds.



- Compounds that dissolve easily in water are **hydrophilic (Greek, “water-loving”)**.

In contrast,

- Nonpolar solvents such as chloroform and benzene are poor solvents for polar biomolecules but easily dissolve those that are **hydrophobic—nonpolar molecules** such as lipids and waxes.



- Water dissolves salts such as NaCl by hydrating and stabilizing the Na and Cl ions, weakening the electrostatic interactions between them.
- The same factors apply to charged biomolecules,
- Water readily dissolves such compounds by replacing solute- solute hydrogen bonds with solute-water hydrogen bonds, thus screening the electrostatic interactions between solute molecules.



Amphipathic compounds contain regions that are:

- **Polar** (or charged) and regions that are **Nonpolar**.



- When an amphipathic compound is mixed with water, the polar hydrophilic region interacts favorably with the solvent and tends to dissolve.
- but the nonpolar, hydrophobic region tends to avoid contact with the water.



- These stable structures of amphipathic compounds in water are called **micelles**, May contain hundreds or thousands of molecules.
- Many biomolecules are amphipathic; e.g
Proteins, pigments, certain vitamins, and the sterols and phospholipids of membranes all have polar and nonpolar surface regions

Hydrophobic interactions



- The forces that hold the non polar regions of the molecules together are called **hydrophobic interactions**.
- (nonpolar compounds self associate in an aqueous environment)



- Hydrophobic interactions among lipids, and between lipids and proteins, are the most important determinants of structure in biological membranes.



- Hydrophobic interactions between non polar amino acids also stabilize the three-dimensional structures of proteins.

Covalent Bonds



- ❑ A covalent bond is a bond that is formed when two atoms share electrons.
- ❑ A single bond is formed when 1 pair of electrons is shared; a double bond is formed when 2 pairs of electrons are shared; and a triple bond is formed when 3 pairs of electrons are shared.

Ionic Bonds



- Sodium and chlorine combine during a reaction that will transfer one valence electron from sodium to chlorine.
- After the transfer sodium and chlorine are no longer neutral. The sodium and chlorine have become ions (atoms with unequal number of protons and electrons)..



- The chlorine now has a net charge of negative 1 (-1) and the sodium ion has a charge of +1.
- Since objects of opposite charge attract each other, the two ions will be held tightly together. This type of attraction is called an **ionic bond**.

Hydrogen Bonds



- Hydrogen bonds are formed when a hydrogen atom is shared between two molecules
- Hydrogen bonds have polarity. The hydrogen atom in water is covalently attached to a very electronegative oxygen atom .
- It also shares its partial positive charge with a second electronegative oxygen atom.

Van der Waals interactions

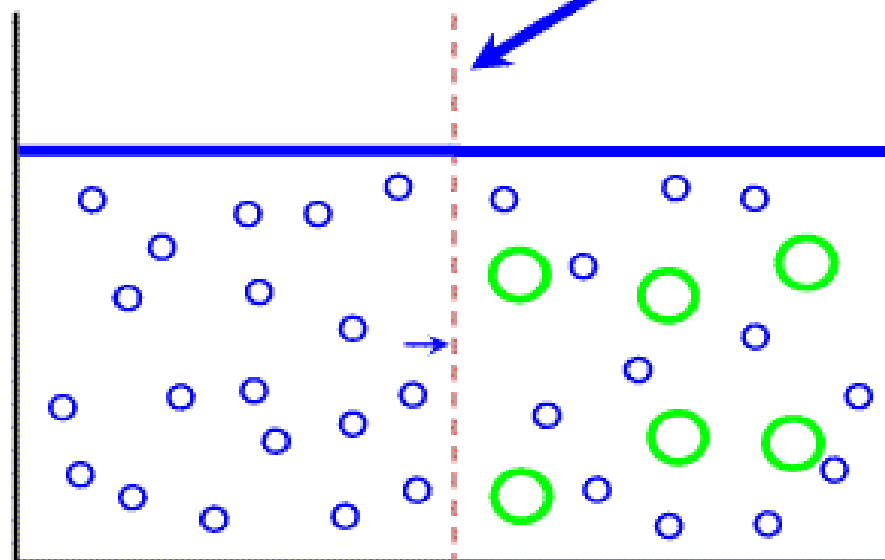


- Rapid movement of the electrons around one nucleus may create a transient electric dipole, which induces a transient, opposite electric dipole in the nearby atom.
- The two dipoles weakly attract each other, bringing the two nuclei closer. These weak attractions are called **van der Waals interactions**.

Osmosis

○ - Water
○ - Sugar

Selectively Permeable Membrane



Low Sugar Concentration High Sugar Concentration
High Water Concentration Low Water Concentration

Osmosis



- Osmosis is the passage of water from a region of high water concentration through a semi-permeable membrane to a region of low water concentration.



- **osmosis** , transfer of a liquid solvent through a semipermeable membrane that does not allow dissolved solids (solutes) to pass.



- The definition contains three important statements:
- Osmosis is **the passage of water** from a region of high water concentration **through a semi-permeable membrane** to a region of low water concentration.



- Osmosis is **the passage of water from a region of high water concentration** through a semi-permeable membrane to a region of low water concentration.



- Osmosis is **the passage of water** from a region of high water concentration through a semi-permeable membrane **to a region of low water concentration.**



- *Semi-permeable membranes* are very thin layers of material (cell membranes are semi-permeable) which allow some things to pass through them but prevent other things from passing through.



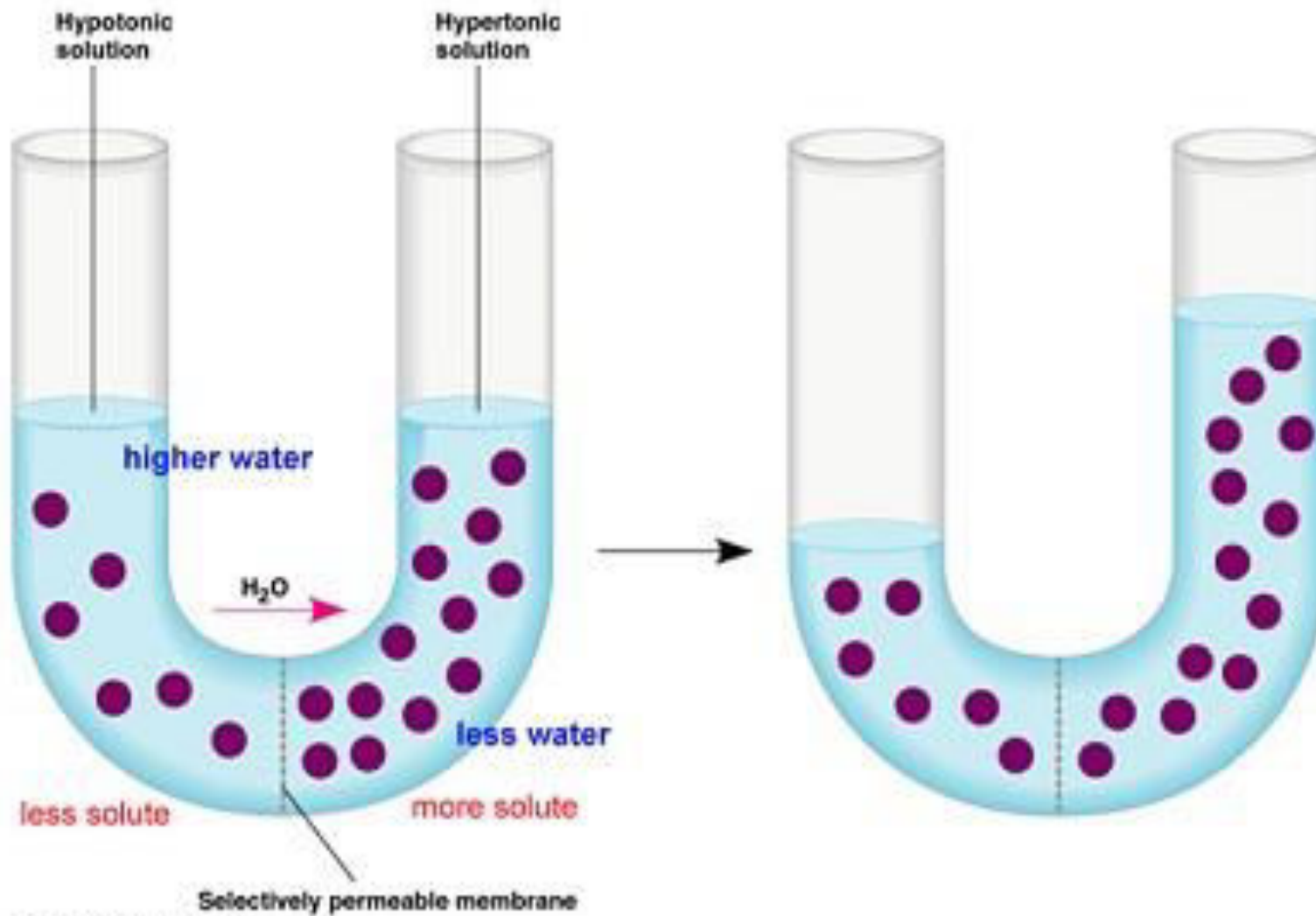
- Cell membranes will allow small molecules like Oxygen, water, Carbon Dioxide, Ammonia, Glucose, amino-acids, etc. to pass through.
- Cell membranes will not allow larger molecules like Sucrose, Starch, protein, etc. to pass through.



- *A region of high concentration of water* is either a very dilute solution of something like sucrose or pure water. In each case there is a lot of water: there is a high concentration of water.



- *A region of low concentration of water* is a concentrated solution of something like sucrose. In this case there is much less water.

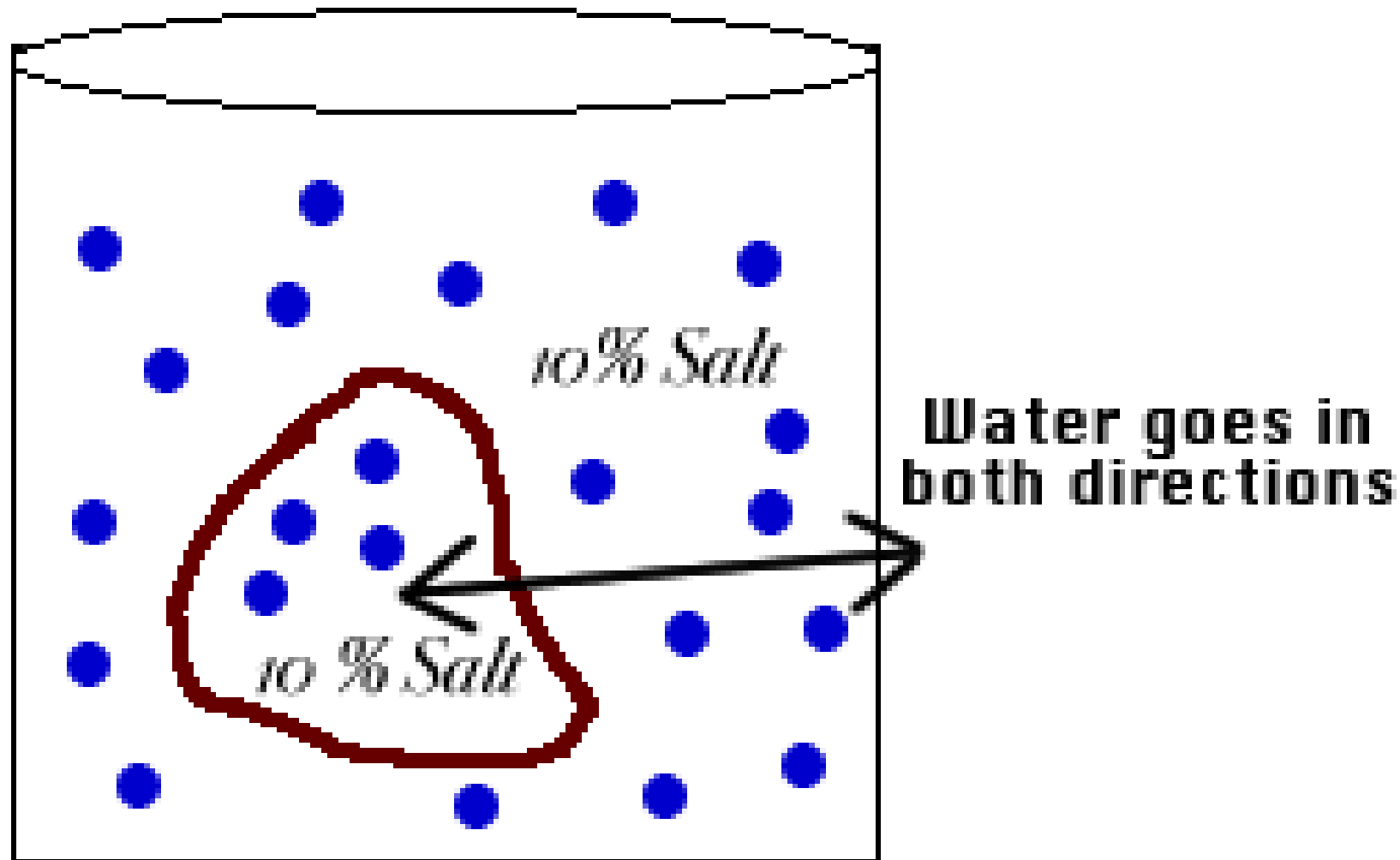


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- Salt is a solute, when it is concentrated inside or outside the cell, it will draw the water in its direction.

Solution is Isotonic

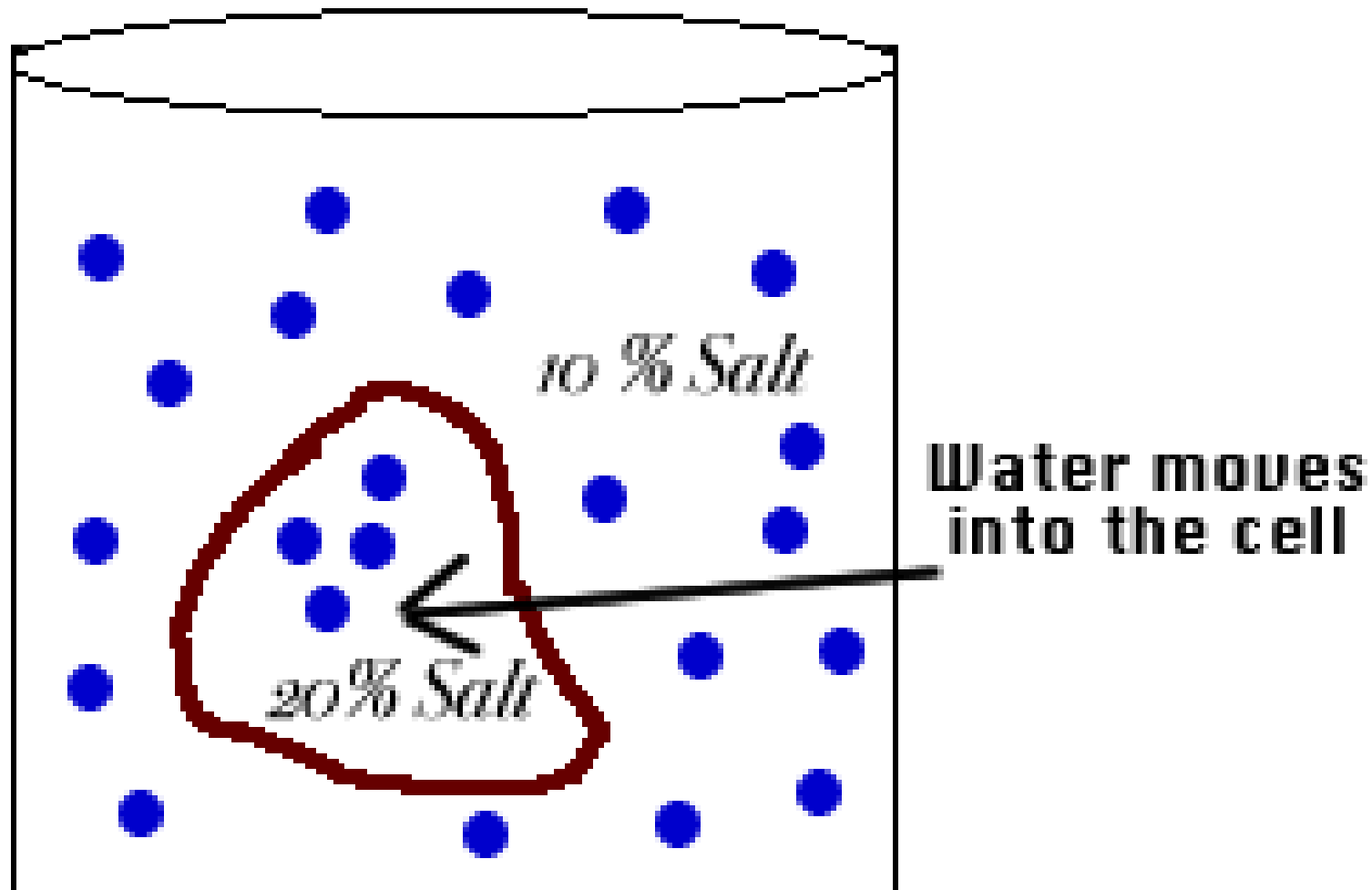


● Water Molecules



- If the concentration of solute (salt) is equal on both sides, the water will move back in forth but it won't have any result on the overall amount of water on either side.
- **"ISO"** means the same

Solution is Hypotonic

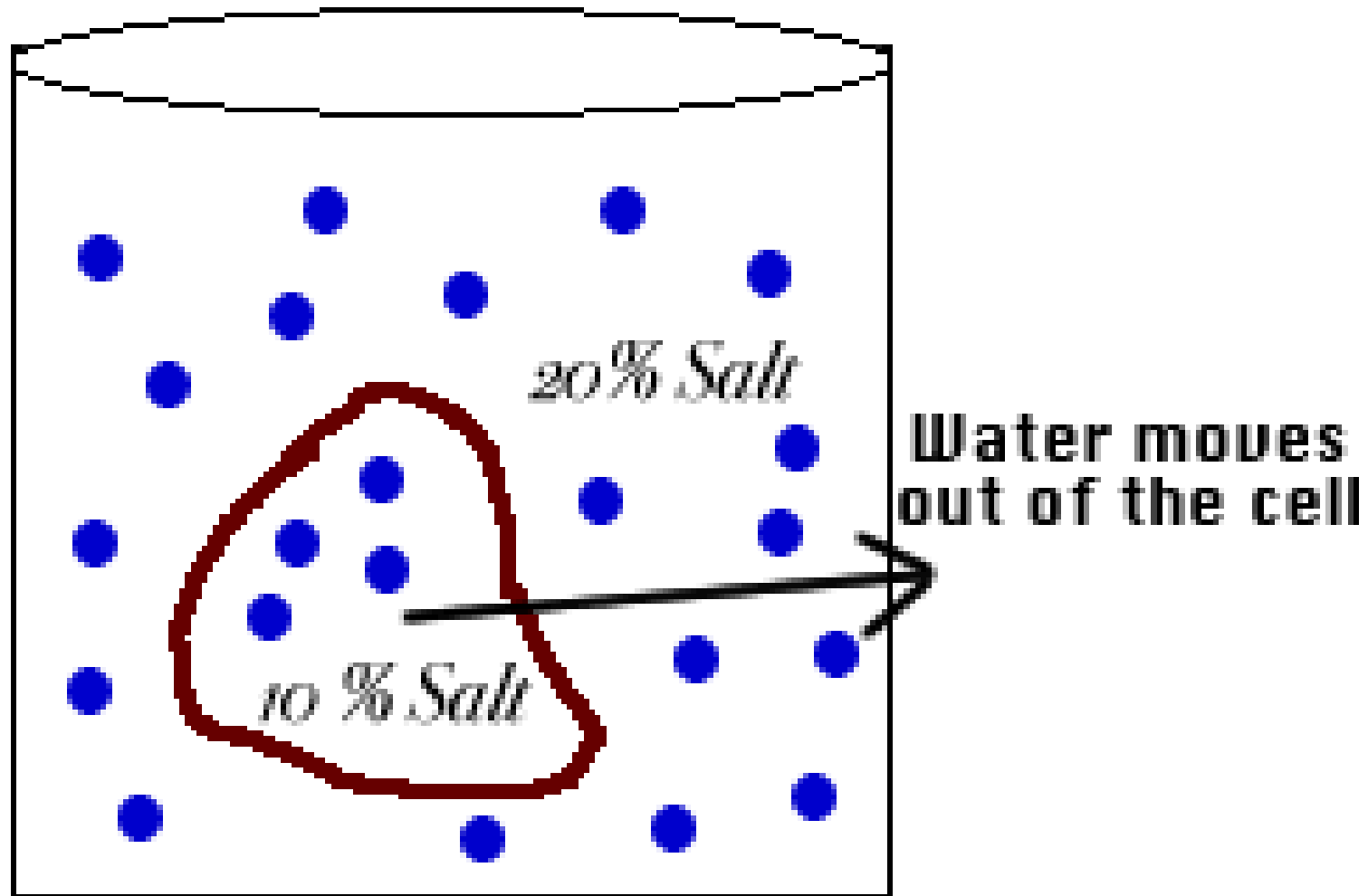


● Water Molecules



- The word "**HYP**O" means less, in this case there are less solute (salt) molecules outside the cell, since salt sucks, water will move into the cell.

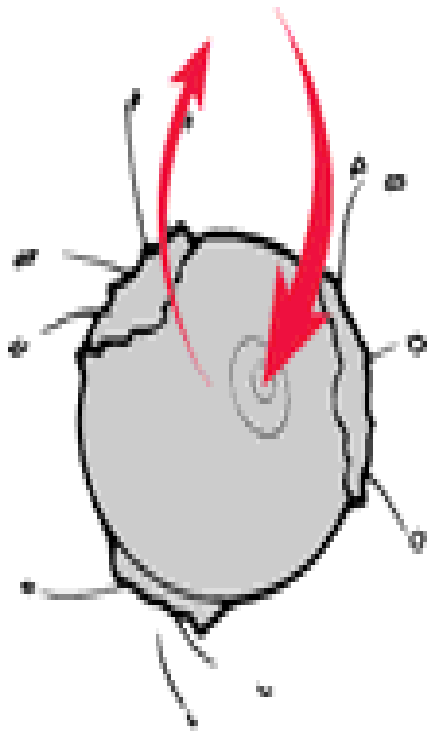
Solution is Hypertonic



● Water Molecules



- The word "**HYPER**" means more, in this case there are more solute (salt) molecules outside the cell, since salt sucks, water will move out of the cell.



Hypotonic
(Swollen cell)



Isotonic
(Normal
looking cell)



Hypertonic
(Shriveled cell)



- The process of osmosis and the factors that influence it are important clinically in the maintenance of adequate body fluids and in the proper balance between volumes of extracellular and intracellular fluids.

osmotic pressure



- The minimum pressure necessary to stop solvent transfer is called the ***osmotic pressure***.



- The pressure exerted by the molecules of the solvent on the membrane they pass through is called **osmotic pressure**.



- The term osmotic pressure refers to the amount of pressure necessary to stop the flow of water across the membrane..



- The hydrostatic pressure of the water exerts an opposite effect; that is, it exerts pressure in favor of the flow of water across the membrane.



- The osmotic pressure of the particles in a solute depends on the relative concentrations of the solutions on either side of the membrane, and on the area of the membrane.



- The osmotic pressure exerted by the non diffusible particles in a solution is determined by the:

numbers of particles in a unit of fluid and
not by the mass of the particles.



- Osmotic pressure is the energy driving osmosis and is important for living organisms because it allows water and nutrients dissolved in water to pass through cell membranes.

Biological Importance of Osmosis



1. Osmosis and dialysis are of prime importance in living organisms, where they influence the **distribution of nutrients** and the **release of metabolic waste products**.



2. The process of osmosis and the factors that influence it are **important clinically in the maintenance of adequate body fluids** and in the proper balance between volumes of extracellular and intracellular fluids.



3. Living cells of both plants and animals are enclosed by a semi permeable membrane called the cell membrane, which regulates the flow of liquids and of dissolved solids and gases into and out of the cell.



- The membrane forms a selective barrier between the cell and its environment; not all substances can pass through the membrane with equal facility.



- Without this selectivity, the substances necessary to the life of the cell would diffuse uniformly into the cell's surroundings, and toxic materials from the surroundings would enter the cell.



4. If blood cells (or other cells) are placed in contact with an isotonic solution, they will neither shrink nor swell. If the solution is hypertonic, the cells will lose water and shrink.



- If the solution is hypotonic (or if pure solvent is used) the cells will swell; the osmotic pressure that is developed may even be great enough to rupture the cell membrane.



- In plants osmosis is at least partially responsible for the absorption of soil water by root hairs and for the elevation of the liquid to the leaves of the plant.



- Osmosis refers only to transfer of solvent; transfer of solute is called **dialysis**.



- In either case the direction of transfer is from the area of higher concentration of the material transferred to the area of lower concentration.



- This spontaneous migration of a **material** from a region of higher concentration to a region of lower concentration is called **diffusion**.

Diffusion



- Diffusion refers to the movement of a SOLUTE across a plasma membrane..



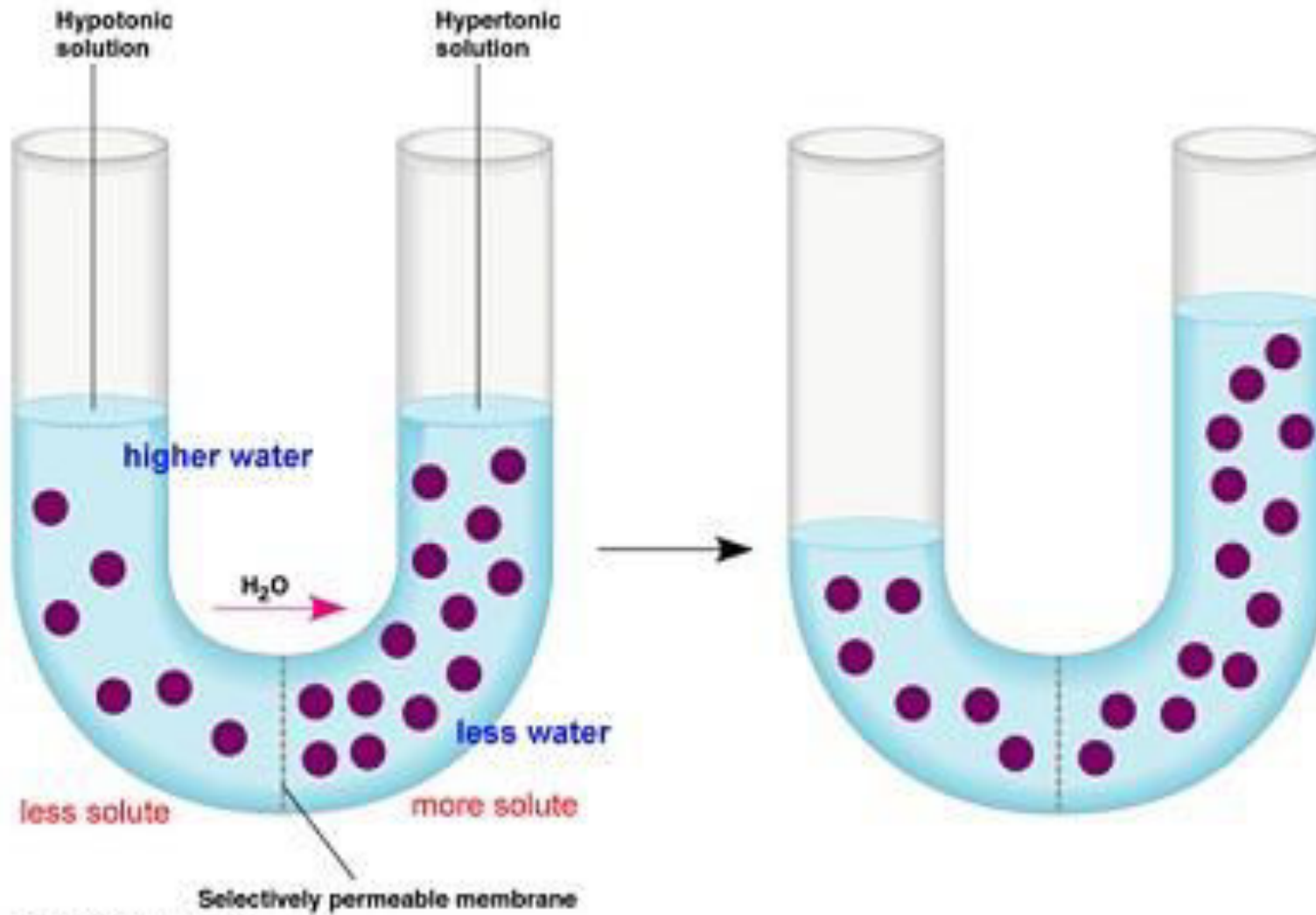
- Solutes move from the area of higher concentration to the area of lower concentration (from the hypertonic compartment to the hypotonic compartment) until an isotonic solution is reached.



- Osmosis refers to the movement of WATER across a plasma membrane. Water does the opposite of what a solute does in diffusion:



- Water will move from the area of lower solute concentration to the area of higher solute concentration (from the hypotonic compartment to the hypertonic compartment.)



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pH and pKa



- Water has a slight propensity to dissociate into hydroxide ions and Protons.
- The acidity of the aqueous solution is usually reported using the logarithmic pH scale.
- Buffers usually maintain the pH of the extracellular fluid between 7.35-7.45.

pH values in the organism



pH values in the cell and in the extracellular fluid are kept constant within narrow limits.

- In the blood, the pH value normally ranges only between 7.35 and 7.45.
- The pH value of cytoplasm is slightly lower than that of blood at 7.0–7.3.



- In lysosomes pH 4.5–5.5
- Extreme values are found in the stomach pH 2 and in the small bowel > 8 .
- Since the kidney can excrete either acids or bases, depending on the state of the metabolism, the pH of urine has a particularly wide range of variation 4.8–7.5.



- Acidic and basic are two extremes that describe a chemical property of chemicals. Mixing acids and bases can cancel out or neutralize their extreme effects. A substance that is neither acidic nor basic is neutral.



- pH term was introduced in 1909 by Sorensen.
- **pH** is defined as the negative log of the hydrogen ion concentration.

$$\text{pH} = -\log (\text{H}^+)$$



- The pH scale measures how acidic or basic a substance is.
- The pH scale ranges from 0 to 14.
- A pH of 7 is neutral.
- A pH less than 7 is acidic.
- A pH greater than 7 is basic.



- The pH scale is logarithmic and as a result, each whole pH value below 7 is ten times more acidic than the next higher value.
- For example, pH 4 is ten times more acidic than pH 5 and 100 times (10 times 10) more acidic than pH 6.



- The same holds true for pH values above 7, each of which is ten times more alkaline (another way to say basic) than the next lower whole value.
- For example, pH 10 is ten times more alkaline than pH 9 and 100 times (10 times 10) more alkaline than pH 8.



- **pKa** - the negative log of the dissociation constant, which is a measure of strength of an acid/base .
- when $pK_a = pH$, there is equal concentration of acid and its conjugate base.



- **pKa** of an acid group is defined as the pH at which the protonated and unprotonated species are present in equal concentrations.

OR

- **pka** - is defined as the pH where a drug exists as 50% ionized and 50% unionized



- The exact probability that a molecule will be protonated or deprotonated depends on the pK_a of the molecule and the pH of the solution.



- **Protonation** is the addition of a proton (H^+) to an atom, molecule, or ion.



- **Deprotonation** is the removal of a proton (H^+) from a molecule, forming the conjugate base.



- **The relative ability of a molecule to give up a proton is measured by its pK_a value.**
- A low pK_a value indicates that the compound is acidic and will easily give up its proton to a base.



- At pH values less(acidic) than pK_a , protonated acid form is more (it will give out its H^+ ions)
- At pH values(basic) greater than the pK_a , deprotonated base form is more in the solution (it will take H^+ ions to neutralize the pH and bring it down).

Ionization of Water:



- Pure water is neutral. But when chemicals are mixed with water, the mixture can become either acidic or basic
- Water molecules exist in equilibrium with hydrogen ions and hydroxide ions.





- The product $[H^+][OH^-]$ —the **ion product of water**—is constant even when additional acid–base pairs are dissolved in the water.
- At 25 °C, pure water contains H^+ and OH^- at concentrations of $1 \times 10^{-7} \text{ mol L}^{-1}$ each;
- it is **neutral and has a pH value of exactly 7.**



- The **water equilibrium constant** is written as:

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

Experimentally, it has been found that the concentration of:

$$H^+ = OH^- = 10^{-7}$$

$$\text{Therefore: } K_w = [10^{-7}][10^{-7}] = [10^{-14}]$$

Acids and bases



- **Acids are defined as substances** that can donate hydrogen ions (protons),
- while **Bases are compounds that accept protons.**



- Acid–base reactions always involve *pairs of acids and the associated conjugated bases*
- The stronger the acid or base, the *weaker the conjugate base or acid, respectively*.
For example, the very strongly acidic **hydrogen chloride** belongs to the very weakly **basic chloride ion**



- **The weakly acidic ammonium ion is conjugated with the moderately strong base ammonia.**



- Because both the H^+ and OH^- concentrations of pure water are 10^{-7} M , pH scale runs from a ***maximum of 14*** (strongly alkaline) to a minimum of (strongly acidic).
- As it is a log scale, one unit reflects a *10-fold change* in proton concentration.

Henderson -Hasselbalch equation



- The quantitative relationship between the pH of the solution and the concentration of a weak acid and its conjugate base is described by :

Henderson -Hasselbalch equation

$$\text{pH} = \text{pK}_a + \log \frac{(\text{A}^-)}{(\text{HA})}$$



HA is the weak acid. It is disassociated into:

H^+ proton

A^- its conjugate base

Applications of Henderson Hasselbalch equation



1. How the pH of the physiologic solution will respond to changes in the concentration of a weak acid and its base.
 - e.g. Changes in the HCO_3 buffer system.
 - increase in HCO_3 will increase the pH
 - In turn CO_2 will be retained to decrease the pH.



2. It is used to calculate the ionized and unionized form of the drugs.

Uncharged drug will cross the membrane more readily than the charged form



- Many biomolecules are **amphoteric in aqueous solution** that is they can accept or donate protons.



- At physiologic pH all the amino acids have a positive group and a negative group. Thus they can act as an acid or a base.
- Such substances are called **Ampholytes**.



- Since protonation-deprotonation effects are responsible for the charges on biomacromolecules which maintain their solubility in water, their solubility is often **lowest at their isoelectric point pI, the pH value at which the molecule has no net charge.**



Importance:

- Separation of plasma proteins by charge is done in this manner.
- pH is kept higher than the isoelectric pH (pI). The proteins will have negative charge and so the proteins will move towards the positive electrode. Variations in the mobility pattern are suggestive of diseases.

Titration Curves



- Titration is used to determine the amount of an acid in a given solution. A measured volume of the acid is titrated with a solution of a strong base, usually sodium hydroxide (NaOH), of known concentration.



- The NaOH is added in small increments until the acid is consumed (neutralized), as determined with an indicator dye or a pH meter. The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added.





- As NaOH is gradually introduced, the added OH combines with the free H in the solution to form H₂O
- As free H is removed, HAc dissociates further to satisfy its own equilibrium constant
- The net result as the titration proceeds is that more and more HAc ionizes, forming Ac, as the NaOH is added.



- At the midpoint of the titration, at which exactly 0.5 equivalent of NaOH has been added, one-half of the original acetic acid has undergone dissociation, so that the concentration of the proton donor, [HAc], now equals that of the proton acceptor, [Ac].



- At this stage the pH of the equimolar solution of acetic acid and acetate is exactly equal to the pK_a of *acetic acid.i.e.*
- $pH = pka$



- The most important point about the titration curve of a weak acid is that it shows graphically that a weak acid and its anion—a conjugate acid-base pair—can act as a buffer.

Importance



- Measurement of pH is one of the most important and frequently used procedures in biochemistry. The pH affects the structure and activity of biological macromolecules;
 1. for example, the catalytic activity of enzymes is strongly dependent on pH
 2. Measurements of the pH of blood and urine are commonly used in medical diagnoses.



3. pKa helps to understand the nature of acid and base like pH:

- $\text{pK}_a < 2$ --strong acid
- $\text{pK}_a > 2$ but < 7 -- weak acid
- $\text{pK}_a > 7$ but < 10 -- weak base
- $\text{pK}_a > 10$ --strong base



4. Pka of amino acid side chains play an important role in defining the pH-dependent characteristics of a protein.

e.g. The pH-dependent activity of enzymes and the pH-dependence of protein stability are determined by the pKa values of amino acid side chain .



5. Most drugs are either weak acids or weak bases. At the stomach pH (1.5) aspirin a weak acid which has got a pK of 3.5 will be protonated (COOH) and uncharged. Uncharged drugs easily cross the membranes.