UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
BIOCHEMISTRY AND MOLECULAR BIOLOGY
LECTURE HANDOUTS: YEAR 2: B. Pham, BMLS, BDS
WATER, pH, ACIDS & BUFFERS

Structure of Water (H_2O)

Brief describe the structure of water.

- Molecule of water is written as H_2O
- Oxygen atom is more electronegative than the Hydrogen atom
- The bond angle in a molecule H_2O is about 104.5^o
- Distribution of electrons within the molecule of water is such that the portion of the molecule near the Oxygen atom is slightly negative, and the portion near the Hydrogen atom is slightly positive
- Such a molecule is called a Dipole and is said to have a Dipole moment.
- Water molecules interact with each other because the partial positively charged Hydrogen atom on one H_2O molecule is attracted to the negatively charged Oxygen atom on another water molecule
- Forming a weak bond (called Hydrogen bond) between the two water molecules
- The interaction results in the Tetrahedral structure of H_2O molecules
- Five H_2O molecules form the tetrahedral structure of water (i.e., association of a central water molecule with four other water molecules by hydrogen bonding)
- The tetrahedral structure is typical of Ice and to a lesser extent of Liquid water
- Each hydrogen bond is relatively weak compared to a covalent bond, but the large number of hydrogen bonds between water molecules in liquid water is the reason for the stability of water

{Take note: Why is water liquid at room temperature, but Ammonia is gas at room temperature? What is Hydrogen bond?}

<u>Water as a solvent</u>:

Why is water a good solvent for most compounds?

- The ability of water to serve as a solvent for Ions and some Organic and Bioorganic molecules is because of its dipolar nature and its ability to form Hydrogen bonds
- Molecules that are capable of forming Hydrogen bonds can do so with H_2O
- These molecules contain charges or dipoles
- Their charges or Dipoles interact with positive or negative ions in H_2O
- In aqueous solution these molecules are surrounded by several molecules of water, thus they become soluble (or dissolve) in water
- Many Inorganic Ionic compounds dissolve in water and exist as separate ions surrounded by water; e.g. NaCl dissolves in water as Na^+ and Cl^- ions
- Biomolecules containing non-ionic but weakly Polar groups dissolve in H_2O because of the attraction of the polar groups to water molecules
- Sugars and Alcohols are readily soluble in water for this reason
- Non-polar (or Apolar) groups such as those present in Hydrocarbons do not form Hydrogen bonds therefore they are not soluble in water (i.e., they do not dissolve in water). They are said to be Hydrophobic!!!

{Take note: Explain why non-polar (or Hydrophobic) molecules added to water usually form spherical droplets with minimum water-exposed surface (e.g., oil drops in water)?}

Dissociation of water:

Water molecules have a limited tendency to reversibly dissociate (i.e., ionize) into a **Hydrogen ion** or P**roton (H^+)** and a **Hydroxyl ion** (**OH^-**).

$$H_2O \quad \leftarrow===\rightarrow \quad H^+ \quad + \quad OH^-$$

An alternative description of this dissociation process is:

$$2H_2O \quad \leftarrow===\rightarrow \quad H_3O^+ \quad + \quad OH^-$$

(Where H_3O^+ is referred to as Hydronium ion).

The tendency of water to dissociate is given by the expression:

$$\mathbf{K = [H^+][OH^-] / [H_2O]} \qquad \text{Expression 1.}$$

Where the terms in **square brackets** [ ] represent **Molar concentrations** of Hydrogen ions, Hydroxyl ions and undissociated water molecules at equilibrium.
K represents the equilibrium constant or the dissociation constant of water.

(It is important at this point to make the assumption that at the Temperature of 25^oC, the value of K is about 1.8×10^{-16} mol/L.)

- We can however calculate the Molar concentration (mol/L. or M) of Pure water.
- Thus, One liter (1000g) of pure water (molecular weight = 18) contains 1000/18 = 55.56M.
- This high Molar concentration (55.56M) of water in not significantly affected by dissociation, therefore it can be assumed to be constant.

Thus we can rewrite Expression 1 above as:

$$\mathbf{K\,[H_2O] = [H^+][OH^-]} \qquad \text{Expression 2.}$$

As stated above K is a constant and $[H_2O]$ is also a constant, therefore $K\,[H_2O]$ represents a new constant, which is called the ionic product of water, K_w.

$$\text{Thus:} \quad \mathbf{K_w = K\,[H_2O] = [H^+][OH^-]} \qquad \text{Expression 3}$$

At 25^{oC} the value of $K = 1.8 \times 10^{-16}$ mol/L, and

By calculation the value of $[H_2O] = 55.56M$. Since $K_w = K\,[H_2O]$
We can calculate the value of K_w as follows:

$$\mathbf{K_w = 1.8 \times 10^{-16}\ mol/L \times 55.56\ mol/L = 1.00 \times 10^{-14}\ (mol/L)^2}$$

By calculation the value of $K_w = 1.00 \times 10^{-14}\ (mol/L)^2$

This can be represented simply as $K_w = 10^{-14}\ M^2$

We can now represent Expression 3 as follows:

$$\mathbf{K_w = [H^+][OH^-] = 10^{-14}\ M^2}$$

- **It must be realized that the Ion Product of Water, namely $[H^+][OH^-]$, is constant for all aqueous solutions, even those that contain dissolved acids or dissolved bases.**
- **If a large amount of H^+ ions are added to pure water, the concentration of OH^- ions will decrease in order that the Ion Product $[H^+][OH^-]$ remain constant at 10^{-14} M^2 at 25^oC.**

What is the significance of the Ion Product of Water?
The expression $[H^+][OH^-] = 10^{-14}$ means that:

- In pure water as well as in all aqueous solutions the product of $[H^+][OH^-]$ is constant and it is equal to 10^{-14}.
- This simply means that none of these two concentrations can be changed alone.
- If, for example an acid is added and $[H^+]$ increased, then $[OH^-]$ must decrease to maintain the Ion Product constant
- It also means that in pure water or neutral solution, where $[H^+]$ and $[OH^-]$ are equal, each of them can be calculated as: $[H^+] = [OH^-] = \sqrt{10^{-14}} = 10^{-7}$ mol/L.
- The calculation of $[H^+]$ can be calculated if $[OH^-]$ is known and vice versa.

How can the pH scale be related to the property of H_2O?
The expression $[H^+][OH^-] = 10^{-14}$ can be converted to the p-scale of numbers, by first taking the logarithm of both sides. Thus:

$$\textbf{Log } \{[H^+][OH^-]\} = \textbf{Log } 10^{-14}$$

This expression can be written as:

$$\textbf{Log } [H^+] + \textbf{Log } [OH^-] = -14$$

By multiplying both sides by –1 the expression becomes:

$$-\textbf{Log } [H^+] - \textbf{Log } [OH^-] = 14 \qquad \textbf{Expression 4.}$$

It must be noted at this point that by definition:

$$-\textbf{Log } [H^+] = pH. ; \qquad -\textbf{Log } [OH^-] = pOH$$

Thus expression 4 can be written as follows:

$$pH + pOH = 14$$

Using this equation it is very easy to find the pH, if the pOH is known and vice versa:
Note that: $pH = 14 - pOH$

The pH-scale is normally used from 0 to 14. In a Neutral solution the pH = pOH
Therefore the pH = 7, and pOH = 7.
In an acidic solution pH values are below 7, in basic solution pH values are over 7.
The value 0 and 14 at the end of the pH-scale implies the following:

pH = 0 implies a 1.0 molar solution of H^+ ions that is a 1.0 M strong acid.
pH=14 implies, that pOH = 14 – pH = 0, it is a 1.0 M solution of a strong univalent base.

ACIDS and BASES:

Is the Strength of an acid (base) the same as the Concentration of the acid (base)?

- The strength of an acid or base is not the same as the concentration of the acid or base.
  - For example HCl is a strong acid because it dissociates completely in solution.
  - When HCl reacts with water the equilibrium of the reaction is shifted well to the right regardless of the concentration of HCl.

$$HCl + H_2O \leftarrow\!=\!=\!=\!=\!=\!=\!=\rightarrow H_3O^+ + Cl^-$$

  - The same goes for NaOH, which is a strong base:

$$NaOH + H_2O \leftarrow\!=\!=\!=\!=\!=\!=\!=\!=\!=\rightarrow Na^+ + OH^- + H_2O$$

- Acetic Acid is a weak acid because it is only slightly dissociated in aqueous solution.
- When acetic acid reacts with water the equilibrium lies well to the left regardless of the concentration of acetic acid.

$$CH_3COOH + H_2O \leftarrow\!=\!=\!=\!=\!=\!=\!=\!=\rightarrow CH_3COO^- + H_3O^+$$

The Concentration of an acid or base depends on the amount (in grams) of the acid or base in 1000 ml of solution.

Thus, a 0.5 M HCl is less concentrated than a 2.0 M HCl solution.

Similarly a 0.5 M Acetic acid solution is less concentrated than a 2.0 M Acetic acid solution.

However, 0.5 M HCl solution and 0.5 M Acetic acid solution are of the same concentration but the HCl adds more H^+ ions to the solution than the acetic acid, therefore the HCl is a stronger acid

Briefly explain the terms acid and base in biochemical systems?

- The definition of Acids and Bases proposed by **Bronsted** and **Lowry** is the most convenient for the biochemical systems.
  - According to them:
    - **An acid is a proton donor**
    - **A base is a proton acceptor**

- **According to this concept a Conjugate Base always accompanies an Acid, and Conjugate Acid accompanies a Base**.
  - Thus for a weak acid: $\mathbf{HA \leftarrow\!=\!=\!=\!=\rightarrow H^+ + A^-}$

- Where **HA** is the undissociated acid and $\mathbf{A^-}$ is its Conjugate base, since it is a proton acceptor. Water for example can act as a base and as an acid thus:

$$\mathbf{H_2O + H_2O \leftarrow\!=\!=\!=\!=\!=\rightarrow H_3O^+ + OH^-}$$

The dissociation of a weak acid can be described by the following expression:

$$\mathbf{Ka = [A^-][H^+] / [HA] = Keq}$$

(Where **Ka** is the dissociation constant for the weak acid, for acid ionization it is called Acidity constant.)

- In **Biochemical and Medical applications**, it is usual to designate the concentration in terms of moles per liter in the expression for **Ka**.
- In such a case, the constant **Ka** becomes the "**Apparent dissociation constant**" and is designated as **Ka`**
- The Real or True (i.e., Thermodynamic) Dissociation Constant, Ka requires the use of ion activities in place of molar concentration for its calculation.
- Thus, in Biochemical calculations we have to use the expression for the Apparent Dissociation Constant: The expression is: **$Ka` = [A^-][H^+] / [HA]$**

- Note that the relative strengths of weak acids and of weak bases are expressed quantitatively as their Apparent Dissociation Constants, which express their tendency to ionize.
- Thus the **pKa`** value of an Acid group is that pH at which the Protonated Un-protonated species are present at equal concentrations.

Examples of some physiologically relevant weak acids and their conjugate bases:

Acid	Conjugate base	PKa`
Ammonium ion (NH_4^+)	Ammonia (NH_3)	9.25
Carbonic acid (H_2CO_3)	Bicarbonate ion (HCO_3^-)	6.37
Dihydrogen phosphate ion ($H_2PO_4^-$)	Monohydrogen phosphate ion (HPO_3^{2-})	6.86
Lactic acid ($CH_3CHOHCOOH$)	Lactate ion ($CH_3CHOHCOO^-$)	3.86

- The tendency of a Conjugate Acid to dissociate could be evaluated from the Ka` or pKa` value.
- The smaller the Ka` the lower the tendency to dissociate and the weaker the acid.
- The larger the Ka` values the higher the tendency to dissociate and the stronger is the acid.
- Note also that the smaller the Ka`, the larger the pKa`. Thus, the larger the pKa` value the weaker the acid the smaller the pKa` the stronger the acid.

What is the HANDERSON-HASSELBALCH EQUATION?

- The relationship between the pH of a solution containing a weak acid and its acid dissociation constant can be stated using the Handerson-Hasselbalch equation.
- This equation for a weak acid can be expressed as follows:

Briefly show how to derive the Handerson-Hasselbalch equation:
Consider a weak acid HA, that dissociates thus:

$$HA \leftarrow\!=\!=\!=\!=\!=\!=\!\rightarrow H^+ + A^-$$

$$Kd = [H^+]\,[A^-] / [HA]$$

This can be arranged thus:

$$[H^+] = Kd\,([HA] / [A^-])$$

Taking the Log of both sides gives:

$$\text{Log}\,[H^+] = \text{Log}\,Kd + \text{Log}\,([HA] / [A^-])$$

Multiply both sides of the equation by – 1 gives:

$- \text{Log } [H^+] = -\text{Log Kd} - \text{Log } ([HA] / [A^-])$

By definition: **$-\text{Log } [H^+] = \text{pH}$, in addition $-\text{Log Kd} = \text{pKa}$**

Thus substituting in the equation above gives the expression:

Thus: $\text{pH} = \text{pKa} - \text{Log } ([HA] / [A^-])$

This expression can be written as: $\text{pH} = \text{pKa} + \text{Log } ([A^-] / [HA])$

This expression is called the **Handerson-Hasselbalch** equation.

What are the major uses of the Handerson-Hasselbalch equation?

- One major use of the Henderson-Hasselbalch equation is for the preparation of buffer solutions of known pH.
- It is also important in calculating the pH of biochemical solutions.

What do you understand by the term buffer solution?

- By definition a buffer solution is a solution that can resist change in pH when small amounts of acid or base are added to the solution.
- **The Acidic buffer solution is made up of a weak acid and the salt of the weak acid**.
- **The Basic buffer solution is made up of a weak base and the salt the weak base.**

List some of the factors that can affect the pH value of a buffer solution are:

The Handerson-Hasselbalch equation is used to prepare buffer solutions:

$\text{pH} = \text{pKa} + \text{Log } ([A^-] / [HA])$

- The strength of the weak acid (Ka` or pKa` is the measure of acid strength and it is constant for a given weak acid or weak base.
- The $[A^-]/[HA]$ ratio. Note that when the $[A^-] = [HA]$ then $\text{pH} = \text{pKa}`$
- The greater the [HA], the lower the pH of the buffer solution. Remember that lower pH means more acidity
- An increase in temperature causes decrease in the value of pKa` and thus shifts the pH value lower.

What is the pH range of a buffer solution?

The **pH range of the buffer solution** can be estimated by using the limit over which the ratio of $[A^-]/[HA]$ can be varied.

That is when:

The ratio of $[A^-]/[HA] = 10:1$. At this ratio the $\text{pH} = \text{pKa}` + 1$
The ratio of $[A^-]/[HA] = 1:10$. At this ratio the $\text{pH} = \text{pKa}` - 1$.

Thus, the expression $\text{pH} = \text{pKa}` \pm 1$ is the range of the buffer solution.

What do you understand by the term Buffer Capacity?

The ability of a buffer solution to resist change in pH is called the buffer capacity.

By definition: **the Buffer capacity is the number of moles of protons or hydrogen ions required to change the pH of one liter of buffer solution by 1.0 pH unit.**

What are the factors that can influence the buffer capacity of a solution?

The buffer capacity can be influence by the following:

- **The final concentration of the buffer solution** – thus, the higher the concentration of the buffer solution the stronger the buffer capacity.
- **The ratio $[A^-]/[HA]$**.
  When the **ratio is greater than one** the buffer capacity in the acid direction is greater than in the basic direction.
  When the **ratio is less than one** then the buffer capacity in the base direction is higher than in the acid direction.
  When the **ratio is equal to one**, then the buffer capacity in one direction is equal to the other. The buffer capacity is maximal at this point, i.e., when pH = pKa`.

Why does the pH of a buffer solution change when diluted?

- Since the pH of a buffer solution depends upon the ratio $[A^-]/[HA]$, change is not expected in the pH of the buffer solution upon dilution of the buffer.
- However, the pH does change.
- This is because the ion activities of the conjugate base A^- and the conjugate acid HA change to different degrees upon dilution.

What are the BLOOD BUFFERS?

The important buffers in the metabolic system are as follows:

- Bicarbonate buffer system H_2CO_3/HCO_3^-.
  - Note that the actual value of $[H_2CO_3] = \{[H_2CO_3] + [CO_{2\ dissolved}]\}$.
- The Hemoglobin buffer system HHb/Hb^-
- The Oxyhemoglobin buffer system $HHbO_2/HbO_2^-$
- The Phosphate buffer system $H_2PO_4^-/HPO_4^{2-}$
- The Protein buffer system $RCOOH\ (NH_3^+)\ /\ RNH_2\ (COO^-)$

In the Red Blood Cells the main buffer systems are the Hemoglobin buffer, Oxyhemoglobin buffer and the Bicarbonate buffer.

In the Blood Plasma the main buffer systems are the Bicarbonate buffer, Protein buffer and Phosphate buffer.

An expression for the Bicarbonate buffer system in the blood is:

$$\mathbf{CO_2 + H_2O \leftarrow\!=\!=\!=\rightarrow H_2CO_3 \leftarrow\!=\!=\rightarrow H^+ + HCO_3^-}$$

The Bicarbonate buffer system is effective as a blood buffer because:

- Equilibrium exists between gaseous CO_2 in the lungs and dissolved CO_2 in the plasma. This is an effective mechanism for the removal of CO_2 from the metabolic system and thus controls the H^+ ions in the blood.
- The enzyme Carbonic Anhydrase catalyzes the interconversion of dissolved CO_2 to Carbonic Acid, thus regulating the concentration of dissolved CO_2 and consequently gaseous CO_2.
- The equilibrium associated with the dissociation of Carbonic Acid is also under the control of Carbonic Anhydrase. The enzyme ensures that the Bicarbonate buffer functions as an Open System.

STUDY QUESTIONS:

- Describe the structure of water.
- Why is water liquid at room temperature, but Ammonia is gas at room temperature?
- Define Hydrogen bond
- Why is water a good solvent for most compounds?
- Explain why non-polar (or Hydrophobic) molecules added to water usually form spherical droplets with minimum water-exposed surface (e.g., oil drops in water)?
- What is the significance of the Ion Product of Water?
- How can the pH scale be related to the property of H_2O?
- Explain why the Strength of an acid is different from the Concentration of the acid.
- Briefly explain the terms acid and base in biochemical systems?
- Write the HANDERSON-HASSELBALCH EQUATION
- Briefly show how to derive the Handerson-Hasselbalch equation
- What are the major uses of the Handerson-Hasselbalch equation?
- What do you understand by the term buffer solution?
- List some of the factors that can affect the pH value of a buffer solutions
- What is the pH range of a buffer solution?
- What do you understand by the term Buffer Capacity?
- What are the factors that can influence the buffer capacity of a solution?
- Why does the pH of a buffer solution change when diluted?
- List the main buffer systems in red blood cells
- List the main buffer systems in blood plasma.

UNIVERSITY OF PNG
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DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
CARBOHYDRATES CHEMISTRY
MONOSACCHARIDES:

What are carbohydrates?

- Carbohydrates as a group of compounds that are:
  - Hydrated Carbons with general formula $\mathbf{C_xH_{2x}O_x}$ (where x is an integer)
  - **Polyhydroxy Aldehyde** or **Polyhydroxy Ketones** or compounds that can be hydrolyzed into those compounds

What are the major groups of carbohydrates?

- Four major groups of Carbohydrates:
  Monosaccharides (**Simple Sugars**): Carbohydrates that cannot be hydrolyzed any further (e.g., Glucose, Galactose, and Fructose)
  Disaccharides: Carbohydrates that can be hydrolyzed into two Monosaccharides (e.g., sucrose, maltose)
  Oligosaccharides: Carbohydrates that produce between two and ten Monosaccharides on complete hydrolysis (e.g., Raffinose a tri-saccharide)
  Polysaccharides: Carbohydrates that yield more than Ten Monosaccharides on complete hydrolysis (e.g., Glycogen, Amylose, Amylopectin, Cellulose)

What are the functions of Carbohydrates?

- Serve as substrate for energy in most organisms
- Give structure to cell membranes and cell walls (in plants)
- Serve as metabolic intermediates
- Components of Nucleotides and Nucleic Acids (DNA and RNA)
- Involve in Lubrication, Cellular Inter-communication, and Immunity

What are the groups of Monosaccharides?
Classification and Nomenclature of Monosaccharides:

- Monosaccharides separated into two major groups based on Type of Carbonyl Functional Group (Reactive Group) on the molecule
  - **Aldose:** Monosaccharides that contain **Aldehyde** (-CHO) functional group (e.g., Glucose)
  - **Ketose:** Monosaccharides that contain **Ketone** (=C=O) functional group (e.g., Fructose)

What nomenclature is used for Monosaccharides?

- Systematic Nomenclature of Monosaccharides:
- Formed by adding Suffix **– ose** to Prefix that indicates the Number of Carbon Atoms in the Monosaccharide
  - Carbon atoms in Monosaccharide are numbered sequentially, with the Carbon containing the Functional (Carbonyl) group having the lowest possible number
  - **Examples**: Monosaccharide containing **3 Carbon atoms** is called a **Tri-ose; 4 Carbon atoms** is a **Tetr-ose**; **5 Carbon atoms** is **Pent-ose**; **6 carbon atoms** is **Hex-ose**

- To indicate the Carbonyl Functional Group the Prefix **Aldo-** or **Keto-** is added as appropriate
- Examples: **Figs. 1 & 2**:
    - **Aldo-triose**; **Aldo-tetrose**; **Aldo-pentose**; **Aldo-hexose** to denote the presence of **Aldehyde functional group** as the first carbon atom in these Monosaccharides
    - **Keto-triose; Keto-tetrose; Keto-pentose; Keto-hexose** to denote the presence of the Ketone functional group as the second carbon atom in these Monosaccharides
    - Three-carbon sugars (Triose sugars) are the smallest carbohydrates
        - Glyceraldehyde (an Aldotriose) and Dihydroxyacetone (a Ketotriose)

What is Chiral Carbon?

- Chiral Carbon is an Asymmetric Carbon
    - A carbon bonded to four different atoms or groups
- Most Monosaccharides contain at least one asymmetric (Chiral) carbon atom, thus they are Optically Active Enantiomers

What are Enantiomers?

- Compounds are called Enantiomers of one another if their Structural Formulas are Non-super imposable mirror images
- Enantiomers are Stereoisomers because they differ from each other only in the way their atoms or groups are oriented in space
    - That is, they have different spatial arrangement of atoms or groups in their molecules

- Enantiomers have similar Physical properties except for the Direction of Rotation of the Plane of Plane Polarized Light
    - Enantiomers rotate the Plane of Plane Polarized Light to Equal Extent but in Opposite Direction

What do you understand by Optical Activity molecule or compound?

- **Optically Active molecule is one whose Aqueous Solution can Rotate the Plane of Plane-Polarized Light**
- **Optically Active molecule contains one or more Chiral centers**
- Example**:**
    - **Glucose is optically active because in solution it can rotate the plane of plane polarized light, a property that is due to the Chiral centers in the molecule of glucose**

How is the optical activity of a compound expressed?

- Quantitatively optical activity of molecules in a compound is expressed as its Specific Rotation
- Specific Rotation is represented as:

$$[\alpha]^{25}_{D} = \text{Observed Degree of Rotation} / lc$$

{Where **l** is Optical Path Length in **dm**; **c** is concentration in **gcm^{-3}; D** is the **D-line** in the Spectrum of **Sodium 589.3nm}**

How is observed degree of rotation and sign {(+) or (–)} of a compound determined?

- Polarimeter is used to determine Observed Degree of Rotation of the Plane of Plane-Polarized Light passing through an Aqueous Solution containing know amount of the Optically Active Compound
- Direction and degree of rotation of the Plane of the Polarized Light is observed
    - If the rotation is in Clockwise direction (i.e., to the Right), then the compound is said to be Dextrorotatory, and is given the (+) sign
    - If the rotation is in Anti-clockwise direction (i.e., to the Left), then the compound is said to be Laevorotatory and is given the (–) sign

- Dextrorotatory compounds are assigned Positive (+) values of Specific Rotation,
- Laevorotatory compounds are assigned Negative (–) values of Specific Rotation
- Optically Active Enantiomers are designated by:
- Prefix (+) if they are Dextrorotatory; e.g., Glucose
- Prefix (–) if they are Laevorotatory; e.g., Fructose

What is the meaning of Prefix D- and L- in the names of compounds?

- Prefix D- or L- designate the Configuration of compounds (i.e., Enantiomers)
- **Emil Fischer** proposed this convention to indicate Enantiomers
- According to Emil Fischer's Convention:
    - **Absolute Configuration** of Enantiomers of Glyceraldehyde can be indicated by the Prefix D- or L-

- Prefix D- or L- denotes the Spatial Arrangements of the –H and –OH atoms about the Chiral Carbon (C-2) atom in Glyceraldehyde (Aldo-triose)
    - In D-Glyceraldehyde the position of –OH group is on the Right and the –H atom is on the Left of the Chiral Carbon
    - Position of –OH and –H are interchanged in the L-Glyceraldehyde (**See diagram below**)

D-Glyceraldehyde **L-Glyceraldehyde**.

- L-Glyceraldehyde is the Mirror Image (Enantiomer) of D-Glyceraldehyde
- All compounds structurally related to D-stereoisomer of Glyceraldehyde are said to have D-structure
- All compounds structurally related to L-stereoisomer of Glyceraldehyde are said to have L-structure respectively

TAKE NOTE:

- Prefix D- or L- designates Spatial (Three-dimensional) Arrangements of atoms about the Chiral Carbon in Enantiomers **NOT** direction of rotation of plane of plane-polarized light
- Property of light rotation is designated by the sign (+) for Dextrorotatory or (–) for Laevorotatory
- D-isomer can be either Dextrorotatory (+) or Laevorotatory (–) compound
- Keto-triose (Dihydroxyacetone) is not Optically Active because its molecules do not contain Chiral Carbon

What are the Stereo-chemical isomers of Monosaccharides?

- Stereo-chemically, Monosaccharides are related to either D- or L- stereoisomer of Glyceraldehyde

- Monosaccharides that are D-stereoisomers:
  - Orientation of –OH group and –H atom around the Chiral carbon atom that is **farthest** from the Aldehyde or Ketone functional group should be similar to orientation of–OH and –H on the Chiral carbon atom in D-Glyceraldehyde
- Monosaccharides that are L-stereoisomers:
  - Orientation of –OH group and –H atom around the Chiral carbon atom that is **farthest** from the Aldehyde or Ketone functional group should be similar to orientation of–OH and –H on the Chiral carbon atom in L-Glyceraldehyde

- In other words, for monosaccharides having more than one Chiral Carbon in their molecules, the Prefix D- or L- always refers to the Configuration of the Chiral Carbon atom most distal to the Carbonyl Carbon

What nomenclature is used to indicate Ketose sugars?

- Systematic names of some Ketose sugars are derived from the names of the corresponding Aldose sugars by inserting –**ul** before the suffix – **ose**
- Example: D-Xylose; D-Ribose; D-Erythrose are Aldose sugars
- Corresponding Ketose sugars are: **D-Xylulose; D-Ribulose; D-Erythrulose**

How is the closed-ring structure of Monosaccharides formed?

- Monosaccharides with 5 or more Carbon atoms have closed-ring structures not linear structures
- In Aldohexose sugars (e.g., Glucose) the alcohol group (-OH) on C-5 reacts intra-molecularly with the Aldehyde group on C-1 to form **Cyclic Hemiacetal**
  - Newly formed –OH group on C-1 is called **Hemiacetal OH**
  - Six-member ring structure of Glucose formed is called a **Pyranose**, because it is similar to the ring structure of Pyran, which is the simplest compound containing such a ring (**Fig. 3**)

- In Ketohexose sugars (e.g., Fructose) the –OH group on C-5 reacts intra-molecularly with the Ketone group on C-2 to form **Cyclic Hemiketal**
  - Newly formed OH group on C-2 is called **Hemiketal OH**
  - Five-member ring structure of Fructose formed is called a **Furanose**, because of its resemblance to the ring structure of Furan, which is the simplest compound containing such a ring

- Ring structure of Aldose sugar is an Internal Hemiacetal,
- Ring structure of Ketose sugar is an Internal Hemiketal
- **In Aldose sugars Hemiacetal OH is as reactive as an Aldehyde group**
- **In Ketose sugars Hemiketal OH behaves like a Ketone group**

What are Anomers?

- Formation of ring structure in Monosaccharide creates an additional Chiral Carbon in the Cyclic molecule that gives rise to TWO Additional Isomers
- Newly formed Chiral Carbon atom (C-1 in an Aldose sugar, and C-2 in a Ketose sugar) is called **Anomeric Carbon** in both cases
- Newly formed Isomer can be either an **α-Anomer** or **β-Anomer**
  - **α-Anomer** and **β-Anomer** are called **Diastereomers**, because they are Not mirror images of each other

Use diagram to illustrate structure difference between α-Anomer and β-Anomer.

- Haworth structures of both Glucose and Fructose:
  - For the α-Anomer the –OH group on the Anomeric Carbon is below the Plane of the Ring structure;
  - For the β-Anomer the –OH group is above the Plane of the Ring structure (diagram below)

α-D-Glucopyranose **β-D-Glucopyranose**

- **Figure 4** illustrates the ring structures of some common Monosaccharides

What is Mutarotation?

- The α-Anomeric form and the β-Anomeric form of sugars are inter-convertible
- Mutarotation is conversion of α-Anomeric form to the β-Anomeric form via the Acyclic form of the sugar in solution to give an equilibrated mixture of both Anomers
  - Equilibrium mixture of Glucose in solution contains about 63.6% of β-D-Glucopyranose, 36.4% α-D-Glucopyranose and 0.03% of Acyclic Form of Glucose (**Fig. 3**)

What are Epimers?

- Epimers are Monosaccharides that differ in the configuration of only one Carbon atom
- Examples:
  - D-Galactose is a C-4 epimer of D-Glucose
  - D-Mannose is a C-2 epimer of D-Glucose

What are Reducing Sugars?

- Reducing Sugars are those that can:
  - Reduce Cu^{2+} ions to Cu^{+} ions (Fehling's solution) or
  - Give Positive Silver Mirror Test by reducing Ammonical Silver (1) Nitrate to Metallic Silver (Tollen's reagent)
- Reducing property is due mainly to the presence in the sugar of Aldehyde group that is oxidized to a Carboxylic Acid group
- Aldose sugars have greater reducing powers than Ketose sugars
- Anomeric carbon is usually free in reducing sugar,
- In Non-reducing sugar the Anomeric carbon is not available because it participates in the formation of Glycosidic bond

Give explanation for the reducing property of Ketose sugars? (**Figs. 5 & 6**)

- Ketose sugars like Fructose have reducing property of an Aldehyde because they are capable of reducing Fehling's solution and Tollen's reagent, which are not normally affected by Ketone

- This property in Ketose sugars can be explained by **Keto-enol Tautomerism** that occurs when **Ketose sugars is in Alkaline medium**
- In alkaline medium, Ketose sugar (such as Fructose) undergoes Keto-enol Tautomerism forming equilibrium with an Aldose sugar (such as Glucose) via an ene-diol (such as Fructenediol) intermediate

Ketose ⟵⟶ enediol ⟵⟶ Aldose

D-fructose ⟵⟶ Fructenediol ⟵⟶ D-glucose

- **In both reactions the forward reaction occurs in an alkaline medium, while the reverse reaction occurs in an acidic medium**
- **In an alkaline medium the equilibrium is shifted to the right resulting in the conversion of the Ketose sugar to an Aldose sugar which then reacts positively with Fehling's solution or Tollen's reagent**

What are sugar derivatives? **Fig. 7**

- Sugar derivatives are Monosaccharides with modified chemical structures
  - **Deoxy sugars**: Monosaccharides in which an OH group is replaced with an H are called Deoxy sugars
    - Examples include: β-D-2-Deoxyribose sugar (also called 2-deoxy-β-D-ribofuranose)
  - **Amino sugars**: Monosaccharides in which one or more OH groups are replaced by $–NH_2$ group
  - In most cases the OH group in C-2 of the sugar is replaced by $–NH_2$
  - If the amino group is Acetylated then it becomes an N-acetyl sugar
    - Examples include:
      - α-D-Glucosamine (also called 2-amino-2-deoxy-α-D-Glucopyranose);
      - α-D-Acetyl-Glucosamine (also called 2-acetylamino-2-deoxy-α-D-Glucopyranose)

What are sugar acids?

- **Monosaccharides in which either the Anomeric carbon or a hydroxyl carbon or both are oxidized to Carboxylic acid**
- **Sugar acids can be either an** Aldonic acid **or a** Uronic acid
- Aldonic acids**:**
  - **Compounds formed when the Carbonyl carbon (Anomeric carbon) in an Aldose sugar is oxidized**
    - **It is the oxidation of the Aldehyde group in an Aldose sugar to a Carboxylic group**
  - **Aldonic acids are named by adding the suffix –**onic acid **to the root name of the Aldose sugar**
  - **Examples include: D-Gluconic Acid; D-Galactonic Acid**

- Uronic acids**:**
- **Compounds formed when the Primary Alcohol group (C-6 in an Aldohexose) of an Aldose sugar is oxidized to a Carboxylic acid group**

- Individual sugar acids formed are named by replacing the ending –ose with –uronic acid
- **Examples include: D-Glucuronic acid; D-Galacturonic acid; D-Mannuronic acid**

What are sugar alcohols?

- Monosaccharides in which the Carbonyl Carbon is reduced to an Alcohol, producing the corresponding Acyclic Poly-hydroxyl Alcohol
- Sugar alcohols are generally called **ALDITOLS**
- Alditol formed from a given sugar is named by replacing the ending **–ose** with –**itol**
- Examples of Alditols formed from Aldose sugars are: Glucitol (Sorbitol); Galactitol (Dulcitol); Mannitol; Ribitol
- Each Ketose sugar usually produces Two sugar alcohol isomers because of the additional Chiral carbon atom formed in the process
- Example: D-fructose gives D-Mannitol and D-Sorbitol.

What is the structure of L-ASCORBIC ACID (VITAMIN C)? (**Figs. 7 & 8**)

- L-Ascorbic acid is a Gamma-Lactone of a sugar derivative of Glucose
- It is a strong reducing agent that is readily oxidized to give L-Dehydroascorbic acid, which also possesses Vitamin C activity
- Hydrolysis of the Lactone ring gives the inactive L-Diketogulonate

What are Glycosides and Glycosidic Bond?

- Glycosides are compounds formed in condensation reactions between:
  - OH group on the Anomeric Carbon of Monosaccharide and the OH group on a Second compound, which may or may not be a Carbohydrate
- Type of bond formed is called **O-Glycosidic bond**
- O-Glycosidic bond is by definition either an **Acetal** bond or a **Ketal** bond
- Names of Glycosides depend on the Monosaccharide involved when the second compound is also a Carbohydrate: Examples:
  - Glucose forms **Glucosides**
  - Galactose forms **Galactosides**
  - Fructose forms **Fructosides**

- When the second compound is not a Carbohydrate then product is called **Aglycone**
- Some common Aglycones contain either Methanol, or Glycerol, or Sterol, or Phenol as the second compound
  - Example: Cardiac Glycosides contain Steroids as the Aglycone components

DISACCHARIDES:

What are disaccharides?

- Sugars made up of Two Monosaccharide residues linked via a Glycosidic bond
  Give names and structures of common disaccharides of Physiological importance
- Disaccharides of Physiological importance: Maltose, Sucrose, Lactose
- Structures of these disaccharides: **Fig. 9**

Briefly explain the systematic nomenclature in Disaccharides

- Disaccharide has Glycosidic linkage

- Glycosidic linkages are by convention, named by reading from left to right
- Example: Sucrose (Common Sugar),
  - Systematic name is **O-α-Glucopyranosyl- (1,2)-β-D-Fructofuranoside**
  - This imply two monosaccharides linked by an O-α-(1,2)-glycosidic bond
  - Oxygen bridge between **α-D-glucopyranose and β-D-fructofuranose** is linking the Anomeric carbon C-1 of Glucose and Anomeric carbon C-2 of Fructose

- Ending **–ide** indicates that the Anomeric carbon of the Second Monosaccharide residue (which is Fructose in this case) takes part in the formation of the O-glycosidic bond
  - When the –OH on the Anomeric carbon of the Second Monosaccharide residue takes part in the formation of the Glycosidic bond, the residue also becomes a Glycoside, thus forming a **Pyranoside** or **Furanoside**

- When the –OH on Anomeric carbon of the Second Monosaccharide residue is free (i.e. it does not take part in the formation of the Glycosidic bond) then the ending **–ose** is used (See nomenclature for Maltose: **Fig. 9**)

Why is sucrose a non-reducing sugar but maltose is a reducing sugar?

- Sucrose is a non-reducing sugar because both Anomeric carbon atoms are involved in the formation of the Glycosidic bond, therefore they cannot react with Fehling's solution and Tollen's reagent

- Maltose is a reducing sugar because the –OH on the Anomeric carbon on the Second Glucose residue is Free and is therefore able to give Positive Test with Fehling's solution and Tollen's reagent

What is invert sugar?

- Invert sugar is the name given to the solution obtained when Sucrose is completely hydrolyzed
- Solution of sucrose is Dextrorotatory
- Sucrose is a disaccharide made up of Glucose which is Dextrorotatory and Fructose which is Laevorotatory
- Complete hydrolysis of sucrose gives Equal amount of Glucose and Fructose
- Laevorotatory power of Fructose is greater than the Dextrorotatory power of Glucose, therefore the resulting solution is Laevorotatory
- Invert sugar refers to the Inversion in the Polarization of Plane-Polarized Light caused by the hydrolyzed solution

POLYSACCHARIDES:

What are the different classes of polysaccharides?

- Two classes of Polysaccharides:
  - Homo-polysaccharides **are Polysaccharides that consist of only one type of Monosaccharide linked by Glycosidic bonds**
  - **Depending on the repeating Monosaccharide unit a Homo-polysaccharide can be called:**
    - **Glucan** or **Glucosan** if the repeating unit is Glucose;
    - **Galactan** if the repeating unit is Galactose;
    - **Fructosan** if the repeating unit is Fructose
  - Examples: Starch, Glycogen, Cellulose

- **Hetero-polysaccharides** are Polysaccharides that are made up of more than one type of Monosaccharides

Brief description the structures of Starch, Glycogen and Cellulose?

- **STARCHES**:
  - Major storage form of Glucose in most plants
  - Two main constituents of starch are **Amylopectin** and **Amylose**
  - Amylopectin **constitute about 80 to 85% of most natural starch**
  - **Structurally, Amylopectin is Branched Chain Polysaccharide containing two types of linkages:**
    - **α-D-Glucopyranose units linked by α-(1,4)-Glucosidic bonds** along the chain
    - **α-(1,6)-Glucosidic bonds at the** Branched points
    - Branches occur at intervals of between 25 to 30 Glucose residues
  - Amylose **constitutes about 15 to 20% of most natural starch**
    - **Amylose is a Linear (Un-branched) Polysaccharide made up of:**
      - **α-D-Glucopyranose units linked by α-(1,4)-Glucosidic bonds**
  - **Repeating disaccharide unit of Amylose is Maltose**

- **GLYCOGEN (Animal starch):**
  - Major storage form of carbohydrate in animals
  - Structurally it is a more highly branched form of Amylopectin, with branch points after every 12 to 14 Glucose residues
  - Glucose residues are linked along the chain by α-(1,4)-Glucosidic bonds and by α-(1,6)-Glucosidic bonds at the branched points

- **CELLULOSE:**
  - Major structural polysaccharide of plant cells
  - Un-branched Polysaccharide made up of **β-D-Glucopyranose** units, linked by **β-(1,4)-Glucosidic** bonds to form a long straight chain
  - Several of these chains are held together by Cross-linked Hydrogen bonds
  - Repeating disaccharide unit in Cellulose is called **Cellobiose**

Briefly explain Cellulose is un-digestible carbohydrate but Glycogen, Amylose and Amylopectin are digestible carbohydrates

- Cellulose is an insoluble carbohydrate
  - Monosaccharide units in cellulose is **β-D-Glucopyranose**
  - Cellulose is not digestible because the Amylase enzymes in humans cannot hydrolyze the **β-(1,4)-Glucosidic** linkages in cellulose

- Glycogen, Amylose and Amylopectin are soluble carbohydrates
- Amylase enzymes in humans can hydrolyze the:
  - α-(1,4)-Glucosidic bonds along the chain and α-(1,6)-Glucosidic bonds at the branched points in both Glycogen and Amylopectin
  - α-(1,4)-Glucosidic bonds in Amylose

What is Dextrin?

- **Dextrin** (Starch Gum):
  - Products obtained during partial break down of starch either by heating in an autoclave or by enzymatic or chemical reactions
  - Dextrin varies in size and amount of branching
  - For medical purposes dextrin serve as Excipients (Inert fillers) of Dry Extracts and Pills and in preparation of Emulsions and Dry Bandages
  - Other uses of dextrin include: Modifiers to milk and milk products in infant formulas and as a source of carbohydrate in animal feeds

What are Dextrans?

- Dextrans:
- Polysaccharides produced by certain Bacteria, grown on Sucrose
- Structurally, Dextrans are Polymers of D-Glucopyranosyl residues linked by α-(1,6)-Glycosidic bonds along the chain and by α-(1,3)-Glycosidic bonds at Branch Points
- Various types of Dextrans differ in Chain Length and Degree of Branching
- Specially prepared Dextrans are used clinically and in research laboratories
- Examples:
  - Clinical use of Dextran includes:
    - Plasma Volume Extender to hold water in the blood stream in order to avoid Fatal Drop in Blood Volume and Pressure of Patients in Shock
    - Dextran-sulfate complex is used therapeutically as an anticoagulant
  - Dextrans are used as Chromatographic Matrix in research laboratories

What are Glycosaminoglycans (Mucopolysaccharides)?

- Glycosaminoglycans (GAG) are also called Mucopolysaccharides
- Un-branched Hetero-polysaccharides made up of repeating disaccharide units in which **one component** is always an **Amino Sugar** (D-Glucosamine or D-Galactosamine) the other component is usually a Uronic Acid

- Seven types of Glycosaminoglycans of Physiological importance:
  - Hyaluronic acid;
  - Chondroitin Sulfate (Chondroitin 4-Sulfate and Chondroitin 6-Sulfate);
  - Keratan Sulfate I & II
  - Heparin;
  - Heparan Sulfate;
  - Dermatan Sulfate

- Names of the Monosaccharide components in the Disaccharide units of Glycosaminoglycans are listed in the Table as **Fig. 10**

What are Proteoglycans?

- Glycosaminoglycans covalently linked to Proteins form complex structures called PROTEOGLYCANS
- Polysaccharide portions of Proteoglycans are called Glycosaminoglycans

What are the different types of Glycopeptide linkages? (Fig. 11)

- Covalent linkage of Sugars to Peptide chain is a central part of Glycoprotein structure
- There are three major types of Glycopeptide linkages, these are:
  - N-Glycosyl to Asparagine,
  - O-Glycosyl to Serine or Threonine;

- O-Glycosyl to 5-hydroxylysine

STUDY QUESTIONS:

- Name the major groups of carbohydrates
- What nomenclature is used for Monosaccharides?
- What is a chiral carbon?
- What are Enantiomers
- Why are some compounds optically active
- What is the meaning of the Prefix D- and L- in the names of Carbohydrates?
- What is indicated by the Sign (+) or (–) in front of a Monosaccharide?
- How is the closed-ring structure of Monosaccharides formed?
- Use diagrams to explain the meaning of Anomers
- What is Mutarotation?
- Why are some sugars called Reducing sugars?
- Why is sucrose a non-reducing sugar but maltose is a reducing sugar?
- What is invert sugar?
- Briefly describe the structures of Glycogen, Amylose and Amylopectin

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Definitions of Lipids, Fatty acids, Saponifiable and Non-Saponifiable lipids, Saponification number and Iodine number:

What are Lipids?
Lipids are biomolecules that are:

- Hydrophobic in nature because of the high amount of Hydrocarbons in their structure
- Relatively insoluble in water but readily soluble in non-polar solvents such as Chloroform, Benzene and Ether
- Easily separated from other biological materials by extraction into organic solvents because of their hydrophobic properties

Examples of lipids include such compounds as:

- Fats, Oils, Steroids, Waxes and the Fat-soluble Vitamins (Vitamins A, D, E and K)

What are Fatty Acids (give some examples)?
Fatty Acids:

- These are Aliphatic Carboxylic Acids containing Long Hydrocarbon chains that may be Saturated or Unsaturated
- A molecule of fatty acid has both Hydrophobic and Hydrophilic properties, thus fatty acids are Amphipathic in nature
- A molecule of fatty acid can be separated into two distinct parts:
  - Non-polar hydrophobic hydrocarbon chain (the tail) and
  - A polar (-COOH) group (the hydrophilic head)
- Most of the naturally occurring fatty acids, obtained from the hydrolysis of natural fats and oils, contain an even number of carbon atoms because they are usually synthesized from Two-carbon units.

Examples of fatty acids include:

- Palmitic Acid, Oleic Acid, Arachidonic Acid, Linoleic Acid, Linolenic Acid, etc.
- (The general structure of a fatty acid is presented in **Page 1a**.)

What are Saponifiable lipids?

- These are lipids that can be hydrolyzed either by Heat, Alkaline or Acid solutions
- The hydrolyzed products usually include, Fatty Acids (salts of fatty acids), Glycerol, and in some cases other molecular components contained in the lipid
  - Examples of Saponifiable lipids include: Neutral fats and Phospholipids.

What are Non-Saponifiable Lipids?

- Non-saponifiable lipids are those lipids that cannot be hydrolyzed
  - Examples: Terpenes, Steroids and the Fat-soluble Vitamins.

What is the saponification number of a lipid?

- The saponification number of a lipid is the number of milligrams of KOH that is needed to saponify one gram of fat.
    - Since each molecule of fat regardless of its size requires 3 molecules of KOH to saponify it, the Saponification number also indicates the number of molecules of fat in one gram of fat

What is the iodine number of fat?

- The iodine number of fat is the number of grams of iodine that is absorbed by 100 grams of fat.
- It is a measure of the degree of un-saturation of the fatty acids in the structure of the fat

NOMENCLATURE OF SATURATED FATTY ACIDS

Briefly describe the Systematic nomenclature of saturated fatty acids?

- In the nomenclature of saturated fatty acids:
    - The IUPAC system (Systematic name or Genevan system) and
    - The Common names

- The **IUPAC or Systematic name** of a fatty acid is formed by replacing the **ending -e** with the **suffix -oic acid** in the name of the **Alkane** with the same number of carbon atoms.
- In this system the Carboxyl Carbon is carbon number one (**page 1a)**

Examples:

- **16C fatty acid** is called **Hexadecanoic acid**;
- **18C fatty acid** is **Octadecanoic acid**.

- **The Common names** of fatty acids are generally derived from either the Latin or Greek name of their source of origin
    - Greek letters or symbols **(α, β, γ, δ, ε,** etc.) can be used to denote or to number carbon atoms in fatty acid molecules (**see examples in page 1a**).
    - Examples of Common names:
        - Palmitic acid from the Latin - Palma (palm tree);
        - Arachidic acid from Greek - Arachne (spider), etc.

Take Note:

- In the Nomenclature of fatty acids one should not mix Greek letters or symbols with the Systematic names of fatty acids, nor should numerals be mixed with Common names of fatty acids.
- The Carboxyl Carbon in a fatty acid molecule is always considered as the First carbon (C-1) in the Systematic name, it has no corresponding Greek letter or symbol in the Common name.
- In the Systematic name:
    - The Second carbon atom (C-2) in the fatty acid molecule corresponds to the α-carbon in the Common name,
    - The Third carbon atom (C-3) in fatty acid molecule corresponds to the β-carbon atom in the Common name and so on.
    - **The last or terminal carbon atom in a fatty acid molecule is considered as the ω-carbon or the n-carbon atom** (see page 1a).

The Common names, Systematic names and Short Hand Formula of some Saturated Fatty Acids are presented in the table below:

Common name	**Systematic name**	**Short-hand structural formula**
Valeric acid	Pentanoic acid	$CH_3(CH_2)_3COOH$
Caproic acid	Hexanoic acid	$CH_3(CH_2)_4COOH$
Caprylic acid	Octanoic acid	$CH_3(CH_2)_6COOH$
Capric acid	Decanoic acid	$CH_3(CH_2)_8COOH$
Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$
Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$
Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$
Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$
Arachidic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$
Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$
Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$

NOMENCLATURE OF UNSATURATED FATTY ACIDS
(Including the OMEGA-numbering and the n-numbering systems):

- In all naturally occurring Unsaturated fatty acids the double bond is always in the **cis-configuration**
- **In the Nomenclature of Unsaturated fatty acids:**
  - **Both Systematic names and**
  - **Common names can be used.**
- **Most of the Common names of Unsaturated fatty acids are derived from the Latin or Greek names of their source of origin.**

Briefly describe the Systematic nomenclature of Unsaturated Fatty Acids?
SYSTEMATIC NAMES:

- The Carboxyl carbon is always the first carbon atom (C-1)
- **Systematic names** for unsaturated fatty acids indicate:
  - **Number of Carbon atoms in the fatty acid,**
  - **Number of Double bonds (unless it has only one double bond),**
  - **Position of the Double bonds and**
  - **Always contain the suffix enoic**
  - The **delta (Δ) numbering system** is used to indicate the position of the double bond in fatty acids.
  - Example:
    - Oleic acid an 18C unsaturated fatty acid with a double bond between carbon atoms 9 and 10, thus the systematic name is cis-Δ^9-Octadecenoic acid;
    - Linoleic acid an 18C fatty acid with two double bonds (the first between carbon 9 and 10, the second between carbon 12 and 13) the systematic name is cis-$\Delta^{9,12}$-Octadecadienoic acid

- The Systematic nomenclature can also be used without the delta sign.
  - Oleic acid is then written as cis-9-octadecenoic acid, and
  - Linoleic acid as cis-9, 12-octadecadienoic acid

- **A shortened form of nomenclature can be used**.
  - For Oleic acid it is 18:1; 9. This implies 18 carbon atoms, one double bond between carbon 9 and 10.
  - For Linoleic acid it is 18:2;9,12, (see page 2b for examples)

Explain the Omega numbering system for Unsaturated fatty acids

- In unsaturated fatty acid molecule the last carbon atom (the terminal methyl carbon) is called the ω-carbon or the n-carbon.
- In unsaturated fatty acids the **ω-numbering system or the n-numbering system** is used to indicate the position of a double bond by counting from the ω-carbon or the n-carbon.
- Examples, using Omega numbering system:
  - **Oleic acid is ω 9, C18:1 or n-9,18:1**.
    - The ω 9 or n-9 implies that Oleic acid contains a double bond on the ninth carbon atom counting from the ω-carbon atom or the n-carbon atom (that is, from the last carbon atom in the fatty acid molecule).
    - C18:1, means 18 carbon atom and one double bond.

  - **Linoleic acid is ω 6, C18:2 or n-6, 18:2**.
    - The ω 6 or n-6 implies that Linoleic acid contains a double bond on the sixth carbon atom counting from the ω-carbon atom or the n-carbon atom (that is from the last carbon atom in the fatty acid molecule),
    - C182 means 18 carbon atoms, two double bonds and that the first double bond is between the sixth and seventh carbon atoms counting from the ω-carbon or the n-carbon atom (see example in page 2b).

TAKE NOTE:

- In the Omega numbering system and the n-numbering system the position of the second double bond is not indicated.
- The general principle is that in Polyunsaturated fatty acids, the double bonds tend to occur at every third carbon atom towards the methyl end of the molecule:

  (**- CH = CH - CH_2 - CH = CH - CH_2 - CH = CH -**)
- In other words the double bonds in Polyunsaturated fatty acids are NOT in the Conjugated form (such as - CH = CH - CH = CH -)
- The double bonds in Polyunsaturated fatty acids are usually separated by at least two single bonds, not by just one single bond
  - Thus, in the case of Linoleic acid (ω 6, C18:2) the second double bond will be between the ninth and tenth carbon atoms from the ω-carbon or the n-carbon atom.

What are the groups or families of polyunsaturated fatty acids?

- Polyunsaturated fatty acids can be grouped into three series or families based on the Omega-numbering system of nomenclature.
- **The groups are**:
  - **ω 9 or n-9 series**:
    - These are fatty acids in which the first double bond is between the ninth and tenth carbon atoms from the ω-carbon atom.
    - Examples: Oleic acid and Gondoic acid.

- **ω 6 or n-6 series**:
  - These are fatty acids in which the first double bond is between the sixth and seventh carbon atoms from the ω-carbon atom.
  - Examples: Linoleic acid and Arachidonic acid
- **ω 3 or n-3 series**:
  - These are fatty acids in which the first double bond is between the third and fourth carbon atoms from the ω-carbon atom.
  - Examples: α-Linolenic acid and Timnodonic acid are examples.

Can mammalians tissues biosynthesize polyunsaturated fatty acids?

- **Most animals can biosynthesize the ω 9 series of polyunsaturated fatty acids because of the presence of the Δ^9-Desaturase enzyme system in their liver and other tissues**.
- This enzyme system is capable of introducing double bonds only between C-9 and the Carboxyl group.
- The ω 6 series and ω 3 series of polyunsaturated fatty acids cannot be biosynthesized by most animals including humans, because of **lack of the Desaturase enzyme system** capable of introducing double bonds beyond C-9 (carbon atom number 9 counting from the Carboxyl carbon).

The Systematic names and Shortened names of some unsaturated fatty acids are listed in the Table below:

Common names	Systematic names (all-cis-)	Shortened names
Palmitoleic acid	9-Hexadecenoic acid	16:1;9
Oleic acid	9-Octadecenoic acid	18:1;9
Vaccenic acid	11-Octadecenoic acid	18:1;11
Linoleic acid	9,12-Octadecadienoic acid	18:2;9,12
γ-Linolenic acid	6,9,12-Octadecatrienoic acid	18:3;6,9,12
α-Linolenic acid	9,12,15-Octadecatrienoic acid	18:3;9,12,15
Gondoic acid	11-Eicosenic acid	20:1;11
Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	20:4;5,8,11,14
Timnodonic acid	5,8,11,14,17-Eicosapentaenoic acid (EPA)	20:5;5,8,11,14,17
Erucic acid	13-Docosenoic acid	22:1;13
Clupanodonic acid	7,10,13,16,19-Docosapentaenoic acid	22:5;7,10,13,16,19
Cervonic acid	4,7,10,13,16,19-Docosahexaenoic acid (DHA)	22:6;4,7,10,13,16,19
Nervonic acid	15-Tetracosenoic acid	24:1;15

THE NONESSENTIAL AND ESSENTIAL FATTY ACIDS:

What are the non-essential fatty acids (give examples)?

- The non-essential unsaturated fatty acids are those that can be biosynthesized in the body therefore, they do not need to be specifically included in the diet.
- For example, Palmitoleic acid (16:1;9) and Oleic acid are non-essential monounsaturated fatty acids.
- **These unsaturated fatty acids and others derived from them can be biosynthesized from saturated fatty acids in animal tissues that contain the Δ^9-desaturase enzyme system.**
- This enzyme system is capable of introducing double bonds between C-9 and the carboxyl group in fatty acids.
- These fatty acids are members of the ω 9 series of fatty acids.

What are the essential fatty acids (give examples)?

- The ESSENTIAL FATTY ACIDS are those **unsaturated** fatty acids that **cannot** be biosynthesized in the tissues of some animals including humans, thus they must be obtained in the diet.
- For example **Linoleic acid (18:2;9,12) and α-Linolenic acid (18:3;9,12,15)** cannot be synthesize in the tissues of most animals including humans,
- This is **because of lack of the enzyme system capable of introducing a double bond beyond C-9 (counting from the carboxyl group) in a fatty acid chain**.
- **Most of the essential fatty acids are members of the ω 6 and ω 3 series of fatty acids**.

Take note:
Some animals including humans can biosynthesize **Arachidonic acid** from Linoleic acid obtained in the diet.

- Thus, **Linoleic acid** is usually said to be the **True Essential Fatty Acid** (**See pages 2c and 2d**).

SOME PHYSICAL PROPERTIES OF FATTY ACIDS:

State some of the physical properties of fatty acids:

- Fatty acids are Amphipathic, this is because the molecule of fatty acid consist of hydrophobic (the hydrocarbon) part and hydrophilic (the –COOH) group.
- The longer the hydrocarbon chain in a fatty acid molecule the higher the melting point of the fatty acid.
- The greater the number of double bonds in the molecule of a fatty acid the lower the melting point of the fatty acid.
- Thus, unsaturated fatty acids have substantially lower melting points than saturated fatty acids.
- For example the melting point of:
  - Stearic acid (18:0) is 70^{o} C
  - Oleic acid (18:1;9) is 13^{o} C,
  - Linoleic acid (18:2;9,12) is -11^{o} C.

CLASSIFICATION OF LIPIDS:

What are the major classes of lipids?
Lipids can be separated into three major classes:

- **Simple lipids**:
  - These are esters that fatty acids form with various alcohols.
  - This class can be further divided into fats, oils and waxes.
  - Fats and oils are esters of fatty acids and glycerol.
  - Waxes are esters of fatty acids and higher molecular weight monohydric alcohols.

- **Complex lipids**:
  - These are esters made up of fatty acids, alcohol and other chemical compounds.
  - This class can be divided into the following**:**
    - **Phospholipids, Glycolipids, Glycosphingolipids, Sulfolipids, Aminolipids and Lipolipids**.

- **Precursor and derived lipids**:
  - These include the following:
    - Fatty acids, Glycerol, Steroids,
    - Other alcohols in including: Sterols, Fatty Aldehydes, Ketone bodies, Hydrocarbons, Lipid-soluble vitamins and Hormones.

SIMPLE LIPIDS
(Stereospecific numbering system of Glycerol):

What is the stereospecific (sn-) numbering system?

- The Triacylglycerols (also called Triglycerides or neutral fats) are Triesters of Glycerol and three fatty acids.
- The general structure of a Triacylglycerol (**see page 4a**) contains three fatty acyl groups linked by ester bonds to a molecule of glycerol (Propane-1, 2, 3-triol).
- If the fatty acyl groups that are esterified to C-1 and C-3 of the glycerol molecule are different, then the C-2 of the Glycerol molecule is asymmetric (a Chiral center).
- The fatty acyl group esterified to C-2 is written to the left of C-2 in a Fisher projection formula to designate the L-configuration of naturally occurring Triacylglycerols.
- The spatial arrangements of the -OH groups in C-1 and C-2 of a glycerol molecule are not identical.
  - Therefore, the three carbon atoms in a glycerol molecule are usually designated either by:
    - The **Stereospecific numbering** system (**sn-,** 1, 2, 3) or by
    - An older numbering system that uses the symbols α, β and α '.

Why is the stereospecific (sn-) numbering system of Glycerol important?

- The -sn- numbering system of Glycerol is significant because some enzymes can readily distinguish the sn-3 carbon atom from the sn-1 carbon atom in glycerol molecule.
  - For example, the enzyme Glycerol kinase catalyzes the addition of a phosphate group to the -OH on the sn-3 carbon of Glycerol to produce the compound Glycerol-3-phosphate and not Glycerol-1-phosphate (see page 4a).

Why are Simple Triacylglycerols different from Mixed Triacylglycerols (Give examples in each case)**?**

- The Triacylglycerol that contains identical fatty acyl groups that are esterified to the three-ester positions of Glycerol is called a **Simple Triacylglycerol**.
  - For example,
    - Triolein (Trioleoylglycerol) contains three molecules of Oleic acid residues esterified to a molecule of Glycerol.
    - Tristearin (Tristearoylglycerol) contains three Stearic acid residues esterified to a molecule of glycerol.

- The **Mixed Triacylglycerols** usually contain two or three different types of fatty acid residues Esterified to a molecule of Glycerol.
  - Such compounds are named according to the placement of the fatty acid residues on the glycerol molecule.
    - Examples:
      - 1-palmitoleoyl-2-linoleoyl-3-stearoyl-glycerol;
      - 1,3-dipalmitoleoylstearoyl-glycerol.

How are fats different from Oils?

- **Fats and Oils** are sometimes called **Neutral fats**.
  - They are usually complex mixtures of simple and mixed Triacylglycerols, whose composition of fatty acid residues varies with the organism that produced them.
  - One major difference between fats and oils is that fats are solid or semi-solid at room temperature, while oils are liquid at room temperature.
  - This is because in oils the Triacylglycerols are made up mainly of unsaturated fatty acid residues, while those in fats are made up mainly of saturated fatty acid residues.
  - The melting points of unsaturated fatty acids are significantly lower than those for saturated fatty acids.

COMPLEX LIPIDS:

- **PHOSPHOLIPIDS**:

What are Phospholipids (give examples)?

- Phospholipids are lipids that contain Phosphorous and have a backbone of Glycerol
- Examples:
  - Phosphoglycerides, Sphingosine (e.g., Sphingomyelin).

- These are the major lipid constituents of cellular membranes and occur in high concentrations in lipids of Glandular organs, Blood plasma, Egg yolk, and in the Seeds of legumes.
- **Phosphoglycerides** (also called Glycerophospholipids or Phosphoacylglycerols) are major components of the Phospholipids that are important constituents of biological membranes.

Give the general structure of Phosphoglyceride.

- The general structure (**see page 4a**) of a Phosphoglyceride is usually made up of an sn-3-phosphorylated Glycerol (sn-Glycerol-3-phosphate) esterified at C-1 and C-2 positions with two fatty acids.
- Furthermore, a third ester linkage is formed between the Phosphate group at sn-C-3 and a polar alcohol ("X").
- Phosphoglycerides are Amphiphilic (Amphipathic) molecules because of the two non-polar aliphatic hydrophobic chains (the "Tails") and the polar hydrophilic Phosphoryl-X group (the "Head").
- Phosphoglyceride is Amphoteric because the molecule contains negatively charged and positively charged groups. The charged polar heads make Phosphoglycerides the most hydrophilic lipids (**see page 4b**).
- Saturated fatty acid residues with either 16 Carbon atoms or 18 Carbon atoms are usually esterified to the sn- C-1 position of the Glycerol in some Phosphoglycerides; while to the sn-C-2 position in the Glycerol is usually esterified an unsaturated fatty acid that contains between 16 to 20 Carbon atoms per molecule.

The structures (**see page 5b**) and names of some Phosphoglycerides and their corresponding polar alcohol groups are presented in the Table:

(Phosphatidic acid is the simplest Phosphoglyceride in which "X" = -H)

Phosphoglyceride (Glycerophospholipid)	**Polar alcohol group ("X")**
Phosphatidic acid	-H
Phosphatidylethanolamine (Cephaline)	Ethanolamine
Phosphatidylcholine (Lecithin)	Choline
Phosphatidylserine	Serine
Phosphatidylinositol	Myo-inositol
Phosphatidylglycerol	Glycerol

What are Lysophospholipids (Give one example)?

Lysophospholipids are Glycerophospholipids that contain only one fatty acyl residue in their molecule.

- For example **Lecithin** (**Phosphatidylcholine**) contains two fatty acyl residues in its molecule, while Lysolecithin contains only one fatty acyl residue.

What are Plasmalogens?

- **Plasmalogens** are Glycerophospholipids (Phosphoglycerides).
- They are structurally different from other Glycerophospholipids.
- The sn-1 carbon of Glycerol is linked by an ether bond to a **cis-α, β -unsaturated alcohol** instead of the normal ester linkage to a saturated fatty acid as in other Glycerophospholipids.
- In Plasmalogen the polar alcohol (head) group "X" can either be Ethanolamine, Choline or Serine (**see page 6a**).

SPHINGOLIPIDS:

What are Sphingolipids?

- All Sphingolipids are derived from **Sphingosine**.
- The different types of Sphingolipids include:
  - Sphingomyelin, (which are the only Sphingolipid that contain phosphate and have no sugar moiety),
  - Glycosphingolipids.

- **Sphingosine** (**also called 4-Sphingenine**) is an 18 carbon unsaturated amino alcohol (a diol).
- It has two asymmetric carbon atoms (C-2 and C-3) and a double bond between C-4 and C-5 in trans- configuration.
- Trans-1, 3-dihydroxo-2-amino-octadec-4-ene is the systematic name for Sphingosine.
- The saturated form of Sphingosine is Dihydrosphingosine (1, 3-dihydroxo-2-amino-octadecane).

What are Ceramides and how are they related toe Sphingomyelins?

- **Ceramide** is the compound formed when a fatty acid molecule is linked to the $-NH_2$ group in Sphingosine via an amide bond.
- Ceramides are the N-fatty acyl derivatives of Sphingosine.
- Ceramides form the core structure of naturally occurring Sphingolipids.
  - Example, the systematic name of a Ceramide is trans-1, 3-dihydroxo-2-N-acyl-octadec-4-ene.

- **Sphingomyelins** are the most common Sphingolipids in the brain and nerve tissue.
- Structurally, Sphingomyelins are Ceramides that contain either Phosphocholine or Phosphoethanolamine head group, thus they are usually classified as Sphingophospholipids.
- The Sphingomyelins are the only Sphingosine-derived Phospholipids in membranes.

Hydrolysis of a molecule of Sphingomyelin yields:

- **One molecule of a fatty acid,**
- **A phosphoric acid,**
- **Amino-alcohol - Sphingosine, and either**
- **Choline or Ethanol-amine**
- No glycerol is present in a Sphingomyelin.

- The conformation of Sphingomyelins resemble that of Glycerophospholipids because of:
  - The polar head group (which is either Phosphocholine or Phosphoethanolamine esterified to C-1 of Sphingosine) and
  - Two non-polar "tails", one of which is the long unsaturated hydrocarbon portion of Sphingosine and the other a fatty acid attached to the -NH on the C-2 atom of Sphingosine.
- Sphingomyelins are Amphoteric and Amphipathic in nature (**see appropriate diagrams in page 6b**).
- **Glycosphingolipids (Sphingoglycolipids**) are Sphingolipids that contain Carbohydrate as part of their molecular structure.

What are the different classes of Glycosphingolipids?
There are four possible classes of Glycosphingolipids:

- **Cerebrosides**:
  - These are sometimes called Ceramide Monohexosides, because they are Ceramides that contain a single sugar residue as the head group.
- **Sulfatides**:
  - These are Ceramides that contain sulfated galactose residue as the head group.
- **Globosides** (also called **Ceramide Oligosaccharides)**.
  - They are Ceramides that contain between two to four sugar molecules as head groups.
- **Gangliosides**:
  - They are usually derived from the Cerebroside called Glucocerebrosides or Glucosylceramides.
  - Gangliosides are Ceramide Oligosaccharides that contain one or more Sialic acid residue (N-acetylneuraminic acid and its derivatives) see diagram on page 6c.

<u>The EICOSANOIDS</u>:

- The Eicosanoids are a group of compounds derived from the metabolism of the Eicosapolyenoic fatty acids (that is the polyunsaturated fatty acids that contain 20 carbon atoms).
- Some of the compounds included in the group are:
  - **Prostanoids, Leukotrienes (LTs) and Lipoxins (LXs)**

- The Prostanoids, Leukotrienes and Lipoxins are collectively called Eicosanoids because they are all 20C unsaturated fatty acid derivatives of Eicosapolyenoic fatty acids.
- **Prostanoids** are a group of compounds that include:
  - **Prostaglandins (PGs), Prostacyclins (PGIs) and Thromboxanes (TXs)**

What are the Prostaglandins?
Prostaglandins:

- The carbon skeleton of the Prostaglandins is similar to the structure of Prostanoic acid a 20C – saturated fatty acid in which carbon atoms 8 to12 forms a Cyclopentane ring (see page 8a).
- Prostaglandins are divided into **three series** based on the **number of double** bonds in the side chains attached to the Cyclopentane ring, **not** the double bonds inside the Cyclopentane ring.
- There are three series of Prostaglandins, these are:
  - **The Series – 1**: **Prostaglandins (PG_1)** are those that have **only one double bond in their side chain**
  - **The Series –2: Prostaglandins (PG_2)** are those that have **two bonds in their side chains**.
  - **The Series – 3 Prostaglandins (PG_3)** have **three double bonds in their side chains**

In the nomenclature of the Prostaglandins the following should be noted:

- **PG** - is the abbreviation for Prostaglandin.
- The letters A, B... indicate the type of Prostaglandin, which is based on the substituent groups attached to the Cyclopentane ring.
- The numerical Subscript refers to the Series of Srostaglandin, which is based on the number of double bonds in the side chains attached to the cyclopentane ring structure.
  - **Thus, PGE_2 means Prostaglandin, Type E, Series 2**

What are the precursor molecules for the synthesis of Prostaglandins?

The **Three series of Prostaglandins** can be related to three Eicosapolyenoic acids as precursors.

- **Series – 1 Prostaglandins** are derived from all cis-8,11,14-Eicosatrienoic acid. This fatty acid is also called Homo-γ - Linolenic acid (ω 6; 20:3;8,11,14).
- **Series - 2 Prostaglandins** are derived from all cis-5,8,11,14-Eicosatetraenoic acid, also called Arachidonic acid (ω 6; 20:4;5,8,11,14).
- **Series - 3 Prostaglandins** are derived from all cis-5,8,11,14,17-Eicosapentaenoic acid (EPA) also called Timnodonic acid (ω 3; 20:5;5,8,11,14,17).

STEROIDS:

- **The steroids are often found in association with fats, usually in the un-saponifiable fraction.**
- **Steroids are lipids that contain four fused carbon rings that form the so-called Steroid nucleus.**
- **The steroid nucleus is a Cyclo-Pentano-Perhydro-Phenanthrene.**

What are Sterols (give examples)?

- The **Sterols** are a group of Steroids that contain one or more -OH groups (but no carbonyl or carboxyl groups) attached to the second steroid nucleus.
- The names of Sterols usually have the Suffix -ol.

Example of Sterol is Cholesterol:

- The steroid nucleus of Cholesterol contains an -OH group on C-3, a double bond between C-5 and C-6 and a branched aliphatic side chain (with 8 carbon atoms) attached to C-17.
- Cholesterol contains a total of 27 carbon atoms. The Systematic name of Cholesterol is:
  - **3-Hydroxy-5, 6-Cholestene** (see page 9a).
- Cholesterol is the major Sterol in the human body, it is a structural component of cell membranes and plasma lipoproteins;
- Cholesterol is the precursor for the biosynthesis of steroid hormones and Bile acids (Cholic acid, Chenodeoxycholic acid, Deoxycholic acid and Lithocholic acid)

Study Questions on Lipids:

- What are Saponifiable lipids?
- What are Non-Saponifiable Lipids?
- What is the saponification number of a lipid?
- What is the iodine number of fat?
- Briefly describe the Systematic nomenclature of saturated fatty acids?
- Briefly describe the Systematic nomenclature of Unsaturated Fatty Acids?
- Explain the Omega numbering system for Unsaturated fatty acids
- What are the groups or families of polyunsaturated fatty acids?
- What are the non-essential fatty acids (give examples)?
- What are the essential fatty acids (give examples)?
- State some of the physical properties of fatty acids
- What are the major classes of lipids?
- Why is the stereospecific (sn-) numbering system of Glycerol important?
- Why are Simple Triacylglycerols different from Mixed Triacylglycerols
- How are fats different from Oils?
- What are Phospholipids (give examples)?
- Give the general structure of Phosphoglyceride.
- Draw the structure of Cholesterol

University of Papua New Guinea
School of Medicine and Health Sciences
Discipline of Biochemistry and Molecular Biology
BIOMEMBRANE: FUNCTIONS, STRUCTURE & TRANSPORT

What are some of the functions of the biological membrane?

- Boundaries around cells (Plasma Membrane)
- Boundaries round distinct subcellular compartments (e.g. Nucleus, Mitochondria, Lysosomes, Golgi bodies, etc.).
    - Compartmentalize and Segregate intracellular events, and Separate cells from one another
- Membranes mediate regulation of cellular functions by:
    - Acting as selective barriers,
    - Allowing inside environment of cells or organelles to differ from outside
- Membranes are involved in signalling processes:
    - Contain specific receptors for external stimuli
    - Involved in both chemical and electrical signal generation
- Specific enzyme systems are localized on membranes
- Plasma membrane is a selectively permeable outer boundary of cell
- Plasma membrane contains:
    - Specific systems; Pumps, Channels, Transporters used for exchange of nutrients and other materials with the environment

NB:

- Normal cellular function starts with normal cell membrane:
    - Damage to membrane structure can affect water balance and ion influx among others, and therefore grossly alter most processes within the cell

What are the basic components in biological membrane?

- Two basic components are Lipids and Proteins
        - Some membranes also contain carbohydrate
- Composition of lipid, protein and carbohydrate varies from one membrane to another
- Ratio of Lipid to Protein is not fixed in most membranes
- Lipid to Protein ratio in membranes varies widely from **4:1 to 1:4**, depending on some specific functions of the membrane

What are the major lipids in the membrane?

- Major lipids in biological membranes are:
    - Phospholipids, Glycolipids, and Cholesterol
- Lipids in membranes are Amphipathic (Amphiphilic) molecules,
    - Have both Hydrophobic (dislike for water) and Hydrophilic (water loving) ends

- Orientation of Amphipathic compounds (Lipids) in aqueous solution is to prevent Hydrophobic region coming into contact with water molecules

What is the structure of a micelle?

- Interaction of fatty acid and alkaline forms fatty acid salt (eg. Sodium Palmitate, a constituent of soap)
- Molecules of Sodium Palmitate in water forms a spherical micelle structure
    - Orientation of molecule is such that the hydrophobic fatty acid chains are hidden inside the micelle and the hydrophilic head-groups interact with the surrounding water molecules (**Fig 1**)

How is the lipid bilayer formed?

- Molecule of Phospholipid is Amphipatic made up of:
    - Two Non-polar (Hydrophobic) groups: Tails
    - One polar (Hydrophilic) group: Head
- When in contact with aqueous solution, the two non-polar (fatty acyl chains) are too bulky to fit into the interior of a micelle, thus,
- Stable structure for most phospholipids in aqueous solution is a two-dimensional bimolecular sheet or lipid bilayer (**Fig 2**)
- Lipid bilayer, the Phospholipid molecules are orientated with
    - Hydrophobic chains in the interior of the structure and
    - Hydrophilic head groups on the surface
- Each layer in the lipid bilayer is referred to as Inner and outer leaflets

What are the types of Phospholipids in the biological membrane?

- Biological membranes contain different types of individual lipids asymmetrically distributed between the two leaflets
- Example:
    - Plasma membrane of Erythrocytes:
    - Sphingomyelin and Phosphatidyl-Choline are preferentially located in the **Outer leaflet**,
    - Phosphatidyl-Ethanolamine and Phosphatidyl-Serine are mainly in the **Inner leaflet**.

What forces are used to maintain the structural integrity of bio-membranes?

- Lipid bilayer will spontaneously self-assemble in aqueous solution
- Major driving force for this to occur is the hydrophobic effect:
    - Hydrophobic fatty acid chains avoid contact with water molecules
- Once formed, the bilayer structure is maintained by:
- Multiple non-covalent interactions including:
    - Hydrophobic interactions and
    - Van Der Waals forces between the hydrocarbon chains
- Charge interactions and Hydrogen bonding between Polar Head-groups, and
- Hydrogen bonding between Head-groups and surrounding water molecules

What are the different types of proteins in the bio-membrane?

- Two basic types of membrane proteins **(Fig 3)**:
    - Integral (Intrinsic) membrane proteins, and
    - Peripheral membrane proteins

How is the integral membrane different from the peripheral membrane protein?

- **Integral (Intrinsic) membrane proteins**:
    - Tightly associated with hydrophobic core of the lipid bilayer
    - Interact extensively with the hydrocarbon tails of lipids, and often span the lipid bilayer
    - Can be removed from the membrane only with organic solvents or detergents that disrupt the membrane structure
    - **Transmembrane proteins** are Integral proteins that are exposed on both the Outer and Inner sides of the membrane; thus, they extend across the width of the membrane

Peripheral membrane proteins:

- Peripheral membrane proteins are usually loosely bound by hydrogen bonding or electrostatic interactions to the exposed surface of integral proteins
- Peripheral membrane proteins can readily be removed by washing the membranes with solutions of high ionic strength
- High ionic strength disrupts the non-covalent ionic and hydrogen bonds holding the proteins on the surface of the membrane

What is the Extracellular matrix?

- Surface of animal cells is usually covered with a flexible and sticky layer of complex carbohydrates, proteins and lipids called Extracellular matrix
- Complex coating of cell (Extracellular matrix):
    - Is cell-specific, Serves in cell-cell recognition and communication, Creates cell adhesion, and Provides a protective outer layer

Outline the Fluid Mosaic model of membrane structure: (Fig 4)

- Fluid Mosaic model for structure of biological membranes was proposed by Jonathan Singer and Garth Nicholson in 1972
    - Biological membrane can be viewed as Two-dimensional solutions of oriented lipids and globular proteins
    - Integral membrane proteins can be considered as "Icebags" floating in a two-dimensional lipid "Sea"

They proposed the following:

- Bilayer organization of lipids act both as a solvent for the Amphipathic integral membrane proteins and as a permeability barrier
- Some lipids may interact with certain membrane proteins, and that these interactions would be essential for the normal functioning of the protein
- Free lateral movement of proteins in plane of bilayer
- Proteins cannot flip from one side of bilayer to the other side

MEMBRANE TRANSPORT

- Plasma membrane is a selectively permeable barrier
- Movement across bilayer can be:
    - Unmediated or Carrier-free transport: (eg., Water, Gases and Urea)
    - Carrier mediated transport, may require presence of integral membrane transport proteins (eg. sugars, amino acids, ions etc.)
- Passive transport of molecules across a membrane does not require an input of metabolic energy
- Rate of transport (diffusion) is proportional to Concentration Gradient of the molecules across the membrane

What are the two types of passive transport?

- Two types of passive transport:
    - Simple Diffusion and Facilitated Diffusion

What is simple diffusion?

- **Simple Diffusion (unmediated, Carrier-free)**:
    - Small uncharged or hydrophobic molecules (H_2O, O_2, CO_2, other gases, urea, ethanol, esters, ethers, etc.) cross the lipid bilayer by simple diffusion
    - No membrane proteins are involved, so there is no specificity
    - Unmediated (Carrier-free) transport proceeds always in the direction of the concentration gradient
    - Rate of diffusion is directly proportional to:
        - Concentration gradient of the molecule across the membrane,
        - Diffusion constant, Temperature and/or the Magnitude of the Partition Coefficient

- Simple diffusion cannot be saturated

What is Facilitated (Mediated) Diffusion?

- Unlike simple diffusion, the facilitated (or carrier-mediated) diffusion of a molecule across a biological membrane is dependent on specific integral membrane proteins called **Uniporters**

- Uniporter facilitates translocation of molecules across the membrane in the direction of the concentration gradient without any supply of energy
- Molecule binds to protein on one side of the membrane the protein then undergoes a conformational change, transports the molecule across the membrane and then releases it on the other side
    - Molecules transported across membranes in this way include hydrophilic molecules such as Glucose, other sugars and amino acids (**Fig. 5**)
- Transport proteins (Uniporters) are specific for one particular molecule or a group of structurally similar molecules
- Transport proteins can be saturated
- Transport protein can be affected by temperature, pH, and inhibitor molecules.
- When the transport protein is used for the translocation of one molecule in one direction and another molecule in the other direction without energy supply, the process is called **Exchange diffusion**.

What is active Transport?

- Active transport process makes it possible to transport molecules from a site of lower concentration to that of a higher concentration
- Active transport requires carrier-protein and supply of metabolic energy, as ATP
- Energy for active transport can be derived either from direct coupling to the hydrolysis of ATP or by coupling to the movement of an ion down its concentration gradient

Active transport may serve for:

- Translocation of a single molecule in one direction (**Uni-port**), or
- Translocation of two molecules in opposite directions (**Anti-port**) or
- Translocation of two molecules in the same directions (**Symport)**

These processes are also known as **COUPLED ACTIVE TRANSPORT**

Study Questions:

1. What are the functions of the biological membrane?
2. Use a simple diagram to show the components parts of the lipid bilayer.
3. List some of the lipids in the outer and inner leaflets of the lipid bilayer.
4. List the different types of non-covalent interactions in the lipid bilayer.
5. How do membrane proteins interact with the membranes?
6. With the help of a simple labelled diagram describe the “Fluid mosaic model” of membrane structure.
7. Briefly describe the two types of passive transport across membranes.
8. Briefly describe active transport across the membranes

University of Papua New Guinea
School of Medicine and Health Sciences
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Discipline of Biochemistry and Molecular Biology

ENZYMES

What is an Enzyme?

- Enzyme is an organic catalyst capable of increasing rate of chemical reaction without any change to the enzyme during the process
- Enzyme can increase rate of chemical reaction up to a million-fold
- Enzyme-catalyzed reactions occur under mild conditions, such as:
  - Body temperature, Atmospheric pressure, Neural pH
- Most enzymes are **highly substrate specific**
- Activity of enzyme can regulated
- Most enzymes are proteins, except a few catalytically active **RNA molecules** (Ribozymes)

How is Activation Energy related to Transition state?

- For chemical reaction to proceed E**nergy barrier** must be overcome (**Fig. 1**)
  - Energy needed to transform substrate into **"Transition state"**
  - Transition state has the **highest "Free energy"** of any component in the reaction pathway
- "**Gibbs" Free Energy of Activation (ΔG*) is the difference in Free energy between Transition state and Substrate (Fig. 1)**

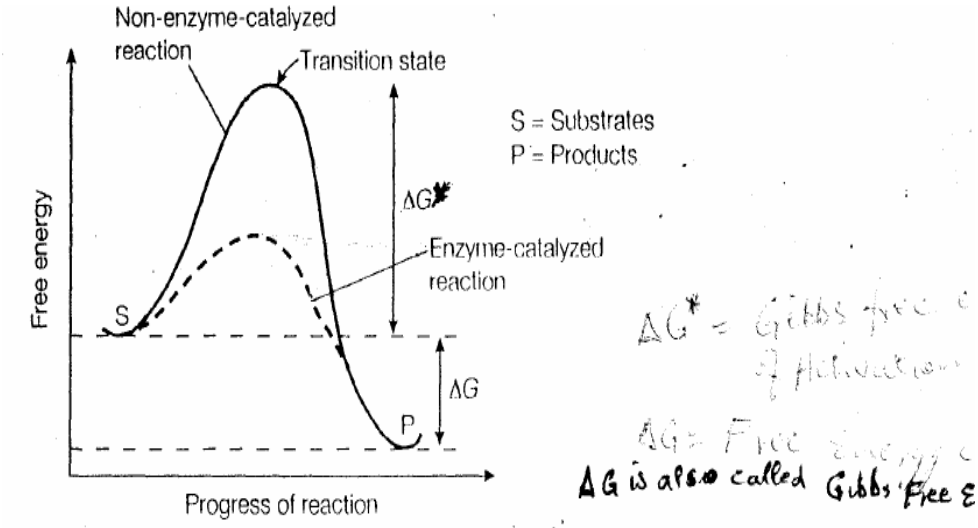


Fig. 1. The energy changes taking place during the course of a biochemical reaction.

Is the Gibbs free energy of activation affected by an enzyme?

- Enzyme decreases the Gibbs free energy of activation (ΔG*)
- Enzyme stabilizes the Transition state of a chemical reaction
- Enzyme does not alter the energy levels of substrates and products
- Enzyme increases the rate at which the reaction occurs, but has no effect on the overall change in energy of the reaction
- Enzyme increases the rate of chemical reaction by decreasing the Gibbs free energy of activation **(ΔG*) {**Fig. 1}

Is Gibbs free energy of Activation (ΔG*), the same as Gibbs free energy change (ΔG)?

- Gibbs Free Energy change (**ΔG**) is different from Gibbs Free Energy of Activation (**ΔG***) {**Fig. 1**}
    - Gibbs Free Energy change (**ΔG**) is the free energy change between SUBSTRATE [S], AND PRODUCT [P]

        - **ΔG = Free energy of [S] – Free energy of [P]**

- **ΔG** indicates if a reaction is energetically favorable or not
- **ΔG** is independent of the path of the chemical reaction,
- **ΔG** provides no information about the rate of a chemical reaction since the rate of the chemical reaction is governed by **ΔG***
- Negative **ΔG** indicates that the reaction is thermodynamically favorable in the direction indicated (i.e., it is likely to occur spontaneously), {**Fig. 1**}
- Positive **ΔG** indicates that the reaction is not thermodynamically favorable and requires an input of energy to proceed in the direction indicated
    - Input of energy can be achieved coupled reactions
- Standard Free Energy change (**ΔG°**), indicates defined [S] and [P] under specified biochemical conditions

ENZYME REACTIONS:

- General expression for an enzyme catalyzed reaction:

$$E + S \leftarrow\!=\!=\!=\!=\!=\rightarrow ES =\!=\!=\!=\!=\!=\rightarrow E + P$$

(***Where E = Enzyme; S= Substrate; ES = Enzyme-Substrate Complex; P = Product***)

- Concept of “Active site” or “Catalytic site” or “Substrate-binding” site is needed to understand formation of **ES-complex**

What is the Active site or Catalytic site of an enzyme?

- Active site or Catalytic site of an enzyme:
    - Region that binds Substrate(s) and converts it into Product(s)
    - Relatively small part of the whole enzyme molecule
    - Three-dimensional entity formed by amino acid residues that can lie far, apart in the linear polypeptide chain
- Substrate(s) binds in the active site by multiple weak forces:
    - Electrostatic interactions, Hydrogen bonds, Van der Waals bonds, or Hydrophobic interactions, Reversible covalent bonds
- Binding of Substrate to Active site gives: Enzyme-Substrate complex (ES)

- Catalytically active residues within the active site acts on Substrate, forming "Transition state" and then Product(s), which is released

Briefly explain the Lock-and-Key Model for Enzyme-Substrate binding

- **Lock-and-Key Model**: (**Fig. 2a**)
  - Proposed by **Emil Fischer** in 1894
  - According to this model the shape of the Substrate and the Active site on the enzyme fit together like a Key into its Lock
  - Both shapes are considered Rigid and Fixed, and perfectly complement each other when brought together in the right alignment

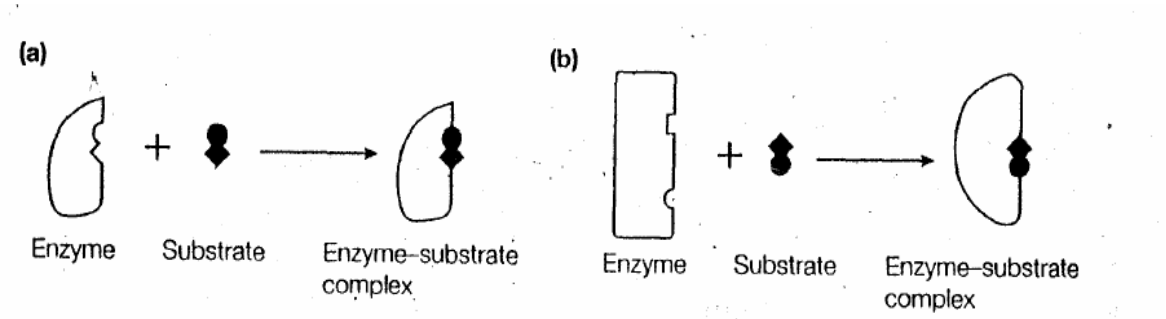


Fig. 2. Binding of a substrate to an enzyme. (a) Lock-and-key model; (b) induced-fit model.

Briefly explain the Induced-Fit Model for Enzyme-Substrate binding

- **Induced-Fit Model**: (**Fig. 2b**)
  - Proposed in 1958 by Daniel Koshland, Jr
  - Binding of Substrate Induces a conformational change in the Active site of the enzyme
  - Enzyme may distort the Substrate, forcing it into a conformation similar to that of the "Transition state"

How are enzymes classified?

Common names:

- Many enzymes are named by adding the suffix **"-ase"** to the name of their substrate
  - **Urease** is the enzyme that catalyzes hydrolysis of Urea,
  - **Maltase** is the enzyme that catalyzes hydrolysis of Maltose
- Some enzymes, such as **Trypsin** and **Chymotrypsin**, have names that do not denote their substrate
- Some other enzymes have several alternative names

International Classification of Enzymes:

- International System of Enzyme Nomenclature was formulated to Standardized and Rationalize Names of Enzymes
- Enzymes are place into one of Six Major classes based on the type of reaction catalyzed
    - Enzyme is identified uniquely by using a Four-Digit Classification number

- Six Major classes are: Oxido-reductases, Transferases, Hydrolases, Lyases, Isomerases, Ligases or Synthases

What is the velocity or rate of an enzyme reaction?

- Velocity or Rate of an Enzyme-catalyzed reaction is the change in the amount of Substrate or Product per unit time
- Velocity of Enzyme is measured under "**Steady-State"** conditions, when the amount of Substrate is very large compared to amount of enzyme
- Velocity is usually reported as the value at time zero (**Vo**)
    - **Initial velocity (Vo)** and is expressed as **μmol/min**
- Velocity is fastest at time zero, the point when no product has been formed (**Fig. 3**)
- Fig. 3 shows Typical graph of Product formed against Time for an Enzyme-catalyzed reaction
- Initial period of rapid product formation gives the linear portion of the graph
- Slowing-down of the enzyme velocity as Substrate is used up and/or as the enzyme loses activity
- Initial Velocity (Vo) is obtained by drawing a straight line through the linear part of the curve, starting at the zero time-point
- Slope of this straight line is equal to **Vo**

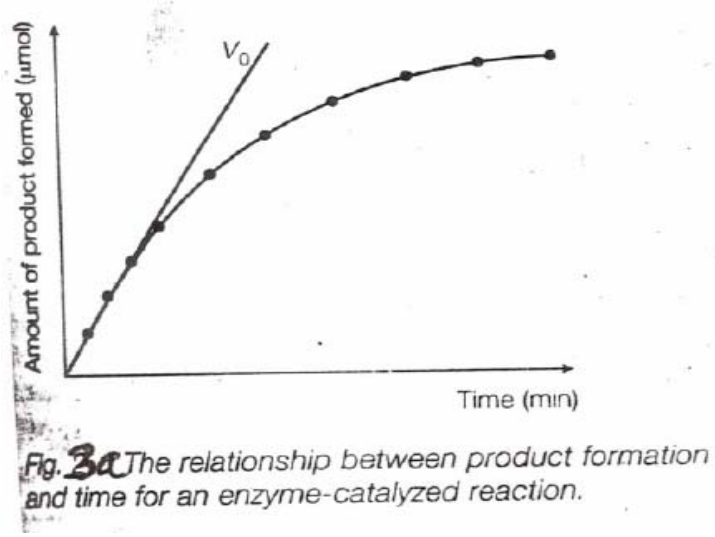


Fig. 3a The relationship between product formation and time for an enzyme-catalyzed reaction.

How do you express the activity of an enzyme (Enzyme units)?

- An enzyme unit (U) is the amount of enzyme that catalyzes the transformation of 1.0μmol of Substrate per minute at 25°C under optimal conditions for that enzyme
- Activity of an Enzyme, is the Total Units of Enzyme in the sample,
- Specific Activity is the Number of Units per milligram of Protein (**units/mg**)
- Specific Activity is a measure of the purity of an enzyme;
    - During the purification of an enzyme, the Specific Activity increases and becomes maximal and constant when the enzyme is pure

How does Enzyme concentration affect reaction velocity?

- When the Substrate concentration is constant, the Vo is directly proportional to the concentration of the Enzyme
- Increasing the amount of Enzyme also increases Vo
- Straight-line graph is obtained when Vo is plotted against Enzyme concentration

What is zero-order reaction?

- For a given amount of Enzyme the Velocity of the reaction is Constant and Independent of Substrate concentration
- Increasing the Substrate concentration **[S]** has no effect on reaction velocity
    - Addition of more substrate will not speed up the reaction

What is First-order reaction?

- For a given amount of enzyme, Velocity of reaction is directly proportional to [S]
- Increasing the [S] also cause increase in Velocity of reaction
- Relationship between Velocity and [S] is Linear

How does [S] affect velocity of enzyme reaction?

- At low [S]:
    - Doubling of [S] will lead to a doubling of the Vo
        - **First-order reaction**
- At higher [S]:
    - Enzyme becomes saturated,
    - Further increases in [S] lead to very small changes in Vo
        - **Zero-order reaction**
- Shape of graph when Vo is plotted against [S] is called Hyperbolic curve (**Fig. 3b**)
- Graph can be separated into three sections:
    - When [S] is low, reaction is First-order with respect to the substrate
    - Vo is directly proportional the [S]

- At mid [S], reaction is mixed-order, i.e., proportionality is changing
- At high [S], reaction is zero-order,
- V is independent of [S]
- **At this point the enzyme is said to have its maximum velocity (Vmax)**
- Increasing [S] further will not have any effect on the Velocity of reaction, because the enzyme is saturated
    - Enzymes that support this type of kinetics are called **Michaelis-Menten** enzymes

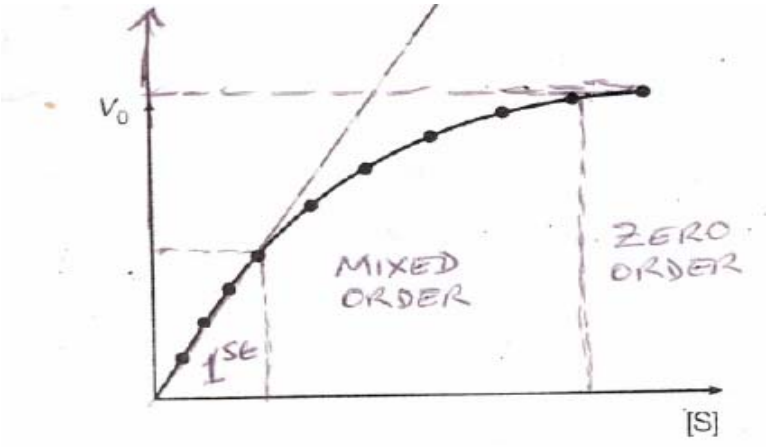
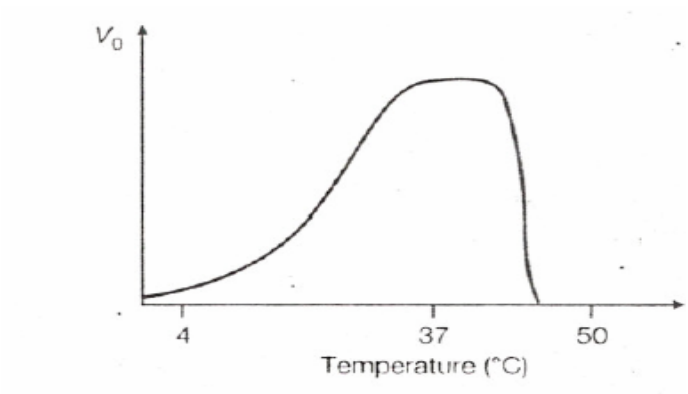


Fig. 3b The relationship between substrate concentration [S] and initial reaction velocity (V_0).

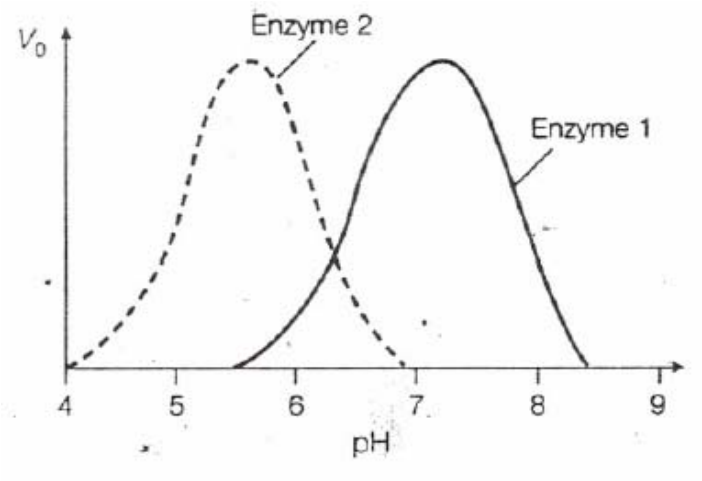
How does Temperature affect velocity of enzyme reaction?

- Temperature affects velocity of enzyme reactions in two ways
  - **First**: Rise in temperature increases Velocity of reaction
  - **Second**: Increasing temperature above a certain value causes inactivation of the enzyme (denature or unfolding of the enzyme protein) and thus reduction in Velocity of reaction. Most enzymes are Thermo-labile
  - Under conditions of Hypothermia (i.e., abnormal low body temperature) most enzyme reactions are depressed, which accounts for the decreased oxygen demand of living organisms at low temperature
- Graph of Temperature plotted against Vo gives a curve with well-defined Temperature optimum (**Fig. 4a**)
- Temperature optimum is around 37°C



How does pH affect enzyme reaction?

- Each enzyme has an Optimum pH at which Velocity of reaction is maximum
  - Small deviations in pH from optimum value lead to reduced velocity, caused by changes in ionization of groups at the active site of enzyme
  - Larger deviations in pH lead to denature of the enzyme protein itself, due to interference with the many weak non-covalent bonds maintaining its three-dimensional structure
    - Graph of Vo plotted against pH gives bell-shaped curve (**Fig. 4b**)
  - Optimum pH of most enzymes is around 6.8, but there is diversity in the pH optima, due to different environments of enzyme functions
    - Digestive enzyme Pepsin works in acidic pH of stomach (pH is around 2.0)



ENZYME KINETICS:

What is the Michaelis-Menten equation?

- Michaelis-Menten equation is:

$$V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

  - *Vo = Initial velocity;*
  - *Vmax = Maximum velocity*
  - *[S] = Substrate concentration*
  - *Km = Michaelis constant*

What does the Michaelis-Menten equation represent?

- Relationship between Initial velocity (Vo) and substrate concentration [S]

What are the three basic assumptions of the Michaelis-Menten equation?

- [S] is very large compared to [E], (i.e., no free enzyme is available)
- [ES] complex is in a "steady-state", (i.e., Rate of formation of ES complex is equal to its rate of breakdown)
- Initial velocity (Vo) of reaction must be used

What is Km?

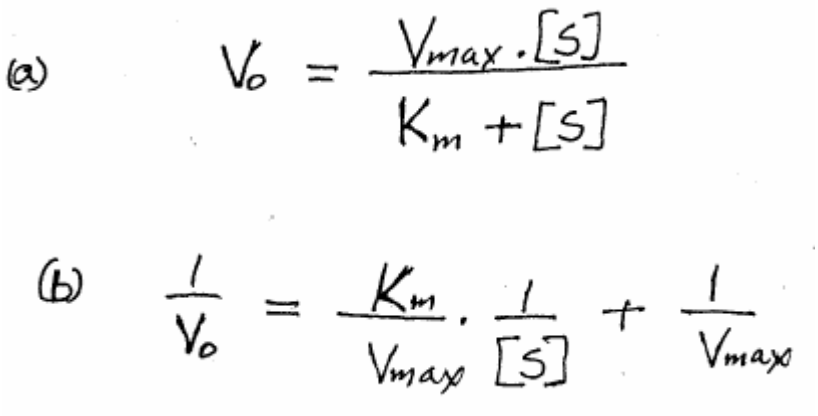
- **Km** is equal to the [S] at which the Initial velocity (Vo) is equal to half the maximal velocity,
  - That is: **Km = [S], when Vo = 0.5 Vmax**
- Km is a measure of the stability of the ES complex

How significance is Km?

- **Small value of Km**, means **high affinity** of the enzyme for the Substrate
- **Large value of Km**, means **low affinity** of enzyme for the Substrate
- Km is characteristic for a particular enzyme with a given substrate
- Km is an important parameter in relation to metabolic control in cell
- Km values are near to the concentration of substrates in cells

What is the Lineweaver-Burk Plot (Double-Reciprocal Plot)?

- Lineweaver-Burk Plot (Double-Reciprocal plot) is used to determine Km and Vmax for an enzyme
- Michaelis-Menten equation is rearranged to give the following expression (Double-reciprocal equation):



- Equation is similar to **Y = MX + C**, which is straight-line equation
- Equation is used to plat a graph called Double-reciprocal plot
- Following information can be obtained from the graph

Y = MX + C

- **Slope of graph: M = Km/Vmax**
- **Intercept on Y-axis: C = 1/Vmax**
- **Intercept on X-axis: when 1/Vo = 0, gives – 1/Km**

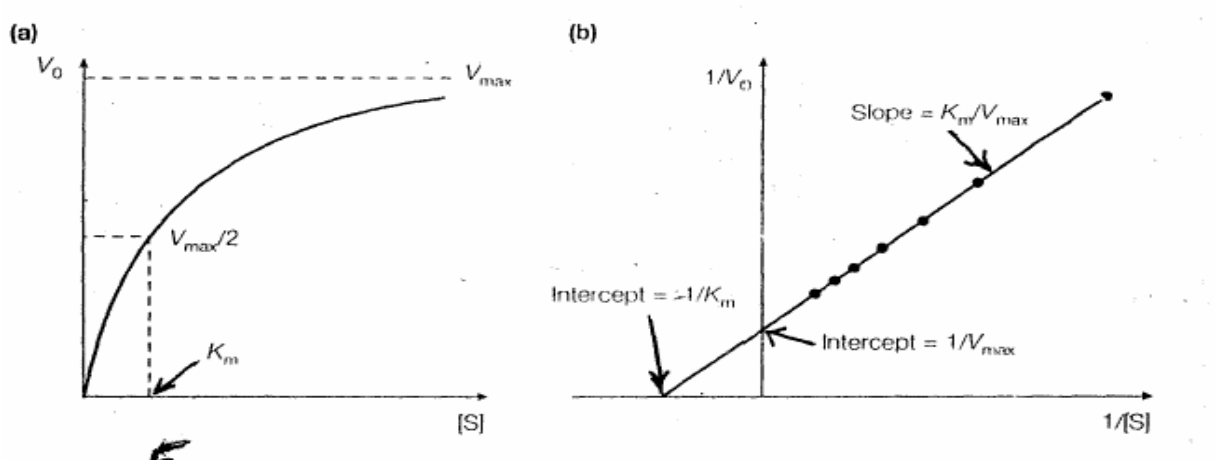


Fig. 5 The relationship between substrate concentration [S] and initial reaction velocity (V_0). (a) A direct plot, (b) a Lineweaver–Burk double-reciprocal plot.

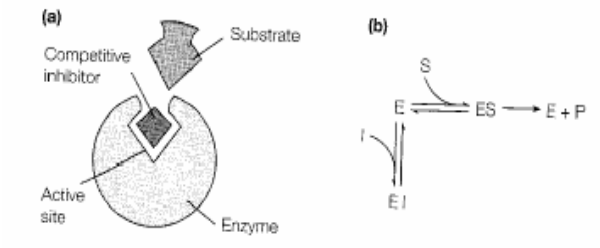
ENZYME INHIBITION:

- Some compounds can inhibit velocity of enzyme reaction
- Two main types of Enzyme inhibition:
  - **Reversible Inhibition** and **Irreversible Inhibition**

- Reversible inhibition can be subdivided into:
  - **Competitive inhibition and Non-competitive inhibition**

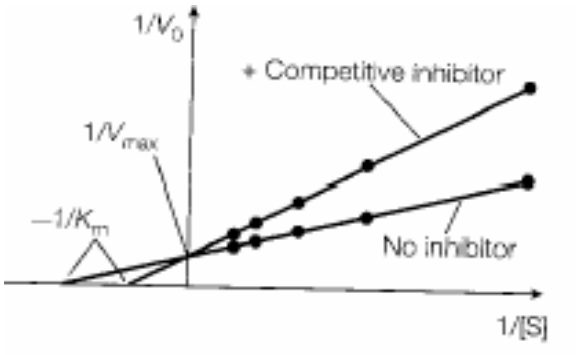
What is Competitive inhibitor (Give examples)?

- Competitive inhibitor has some structural similarities to normal substrate
- Competitive inhibitor competes with substrate for enzyme active site
- Enzyme may bind either Substrate or Inhibitor, but not both at the same time
- Competitive inhibitor binds reversibly to the active site
- High [S] can displace competitive inhibitor from active site of enzyme
- **Examples** of competitive inhibitors:
  - Sulfanilamide competes with Para-Aminobenzoic Acid (PABA) in reaction catalyzed by Dihydropteroate Synthetase in biosynthesis of Folate
  - Methotrexate competes with Dihydrofolate in reaction catalyzed by Dihydrofolate Reductase (Methotrexate is structural analogue of Folate)



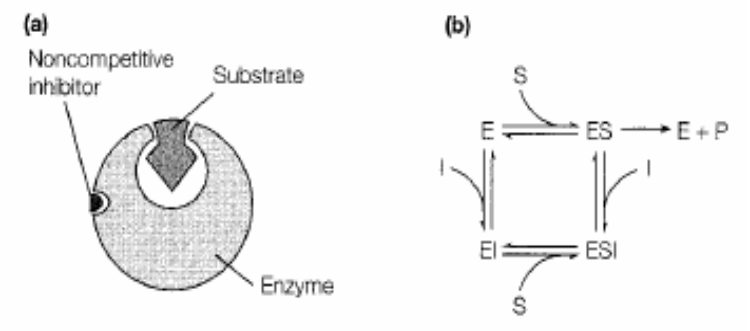
How does competitive inhibitor affect Michaelis-Menten equation?

- Measure Vo of the enzyme at different [S] in the absence and presence of a fixed concentration of competitive inhibitor
- **Draw Lineweaver-Burk plot** for Inhibited and Non-inhibited enzyme (**See Diagram below**)
- No change in Vmax of enzyme,
- Change in Km of enzyme for substrate
    - Competitive inhibitor increases the Km of an enzyme for its substrate



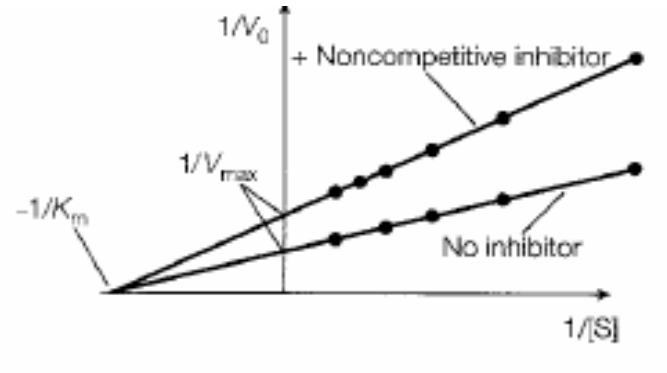
What is non-competitive inhibitor (Give example)?

- Non-competitive inhibitor binds reversibly to a site other than the active site of the enzyme (see diagram)
- **Enzyme can bind the inhibitor, the substrate or both the inhibitor and substrate together**
- Effects of non-competitive inhibitor cannot be overcome by increasing [S]
- Example of Non-competitive inhibitor:
    - Inhibition of Renin by Pepstatin



How does non-competitive inhibitor affect Michaelis-Menten equation?

- Measure Vo of the enzyme at different [S] in the absence and presence of a fixed concentration of non-competitive inhibitor
- **Draw Lineweaver-Burk plot** for Inhibited and Non-inhibited enzyme (**See Diagram below**)
- Km is not affected, thus affinity of the enzyme for Substrate in unchanged
- Vmax of inhibited enzyme is decreased



What is Irreversible inhibition (Give examples)?

- Irreversible competitive inhibitors irreversible inhibit enzymes by binding very tightly to the active sites of the enzymes
- Some highly toxic, naturally occurring, and manufactured compounds are irreversible enzyme inhibitors
- Examples:
  - Penicillin inhibits Trans-peptidase that is important in development of bacterial membrane, thus destroying normal growth of bacteria
  - Aspirin inhibits Cyclooxygenase in biosynthesis of Eicosanoids
  - Allopurinol inhibits Xanthine Oxidase in degradation of Purines

Give examples of enzymes that are used in diagnosis:

- Acid Phosphatase: tumor marker in cancer of Prostate
- Alanine Aminotransferase (ALT): Indicator of hepatocellular damage
- Alkaline Phosphatase (ALP): increases in Cholestatic liver disease, and a marker of Osteoblast activity in bone disease
- Amylase: Indicator of cell damage in acute Pancreatitis
- Aspartate Aminotransferase (AST): hepatocellular and myocardial damage
- Creatine Kinase (CK-MB): Myocardial damage
- Gamma-glutamyl transpeptidase (GGT): Hepato-Biliary damage
- Lactate Dehydrogenase (LDH): Muscle damage
- Cholinesterase involved in impulse transmission at neuromuscular and synaptic junctions, and in hydrolysis of Succinylcholine, a muscle-relaxing drug used in Anesthesia
- Plasma Cholinesterase measurements are useful in diagnosis of poisoning with pesticides and insecticides, which are inhibitors of Cholinesterase

STUDY QUESTIONS TO LECTURE IN ENZYMES

- Briefly state how enzymes affect (a) Gibbs free energy of activation; (b) Gibbs free energy.
- Name two models proposed to explain how an enzyme binds substrate to the active site.
- Name the six major classes of enzymes.
- Briefly explain the term "Linked or Coupled enzyme assays".
- Define the following (a) Enzyme units; (b) Specific activity of an enzyme
- Explain how substrate concentration affects the rate of enzyme-catalyzed reaction.
- State the significance of the Michaelis-Menten constant (Km).
- Explain how the Double-reciprocal plot (Lineweaver-Burk Plot) is used to determine Km and Vmax.
- Use graph to explain competitive and noncompetitive inhibition of an enzyme.
- How is a competitive inhibition different from non-competitive inhibition, in the way they affect the Km and Vmax of an enzyme?
- List five irreversible inhibitors and the enzyme or enzymes affected by each.
- List five enzymes with diagnostic value.

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ELECTRON TRANSPORT CHAIN, OXIDATIVE PHOSPHORYLATION, SUPEROXIDE

What is metabolism?

- It is sum total of all chemical reactions involved in maintaining the living state of all cells
- Categories of Metabolism:
  - Catabolism or Break down of molecules to obtain energy
    - Examples: break down of Glucose to obtain energy
  - Anabolism or Synthesis of all compounds needed by the cells
    - Examples: synthesis of DNA, RNA, or Proteins
- Amphibolism:
  - It is the link between Anabolism and Catabolism
  - TCA (Krebs Cycle) is the major Amphibolic pathway because it links anabolic and catabolic pathways
- Bioenergetics is a term which describes the biochemical or metabolic pathways by which the cell ultimately obtains energy

How is energy stored in cells?

- Catabolism provides energy needed for performing useful work
- Energy is stored in cells mainly as Adenosine Tri-phosphate (ATP)
- ATP links Exothermic and Endothermic Reactions
- ATP is made of Adenosine and Ribose bonded to 3-Phosphate groups via Phosphate Ester bonds
  - Two bonds in ATP are High-energy bonds (bond energy = 7 kcal/mole)
- ADP contains 2-Phosphate groups:
  - One of the two bonds is high energy bond
- AMP contains 1-Phosphate group,

- Hydrolysis of ATP:
  - $ATP + H_2O$ =====➔ $ADP + P + Energy$

- Under certain conditions ATP may be hydrolyzed directly to AMP
  - $ATP + H_2O$ ====➔ $AMP + PP + Energy$

- ATP Formation:
  - $ADP + P + Energy$ =====➔ $ATP + H_2O$

- Other High energy Phosphates molecules are:
- GTP, Creatine Phosphate ($CrPO_3$), Phosphoenolpyruvate (PEP), 1,3-Bisphosphoglycerate (1,3BPG), Succinyl-CoA, etc.

What are Coupled reactions, give examples?

- Some biochemical reactions produce energy (Exothermic),
- Other biochemical reactions require energy (Endothermic)
- Both processes are carried out efficiently when they are "Coupled"
- Couple reaction means:
  - Two reactions occurring simultaneously to support each other
  - Fists reaction must be Exothermic
  - Second reaction which is Endothermic, picks up the energy produce by Exothermic reaction
- Couple reaction requires ATP or other high-energy compound
  - Example of a coupled reactions:
    - Hydrolysis of ATP and Contraction of muscle tissue
    - Hydrolysis of ATP releases energy used by muscles to contract
  - Coupled reaction:
    - $ATP + H_2O$ ====➔ $ADP + P$ + Energy
    - Relaxed muscle + Energy ====➔ Contracted muscle
- Another example of a coupled reaction
  - Hydrolysis of $CrPO_3$ to release energy used for formation of ATP
  - Coupled reaction:
    - $CrPO_3 + H_2O$ =====➔ Creatine + HPO_4^{-3} + Energy
    - $ADP + HPO_4^{-3}$ + Energy =====➔ $ATP + H_2O$
- During periods of resting muscular activity is low, therefore the reactions are reversed to replenish the supplies of ATP and $CrPO_3$
  - ATP + Creatine ======➔ $CrPO_3 + ADP$
- Energy for the formation of ATP is supplied by other metabolic reactions

ATP Synthesis in mitochondria

- Cells use Proton-Pumping System made up of proteins inside the Mitochondria to generate ATP
- Synthesis of ATP is coupled with Oxidation of NADH and the reduction of O_2 in the Electron Transport Chain (ETC) also known as Oxidative Phosphorylation
- Process involved 3 key steps:
  - Transfer of electrons from NADH, via a series of Electron carriers, to O_2
  - Transfer of electrons by these carriers generates a Proton (H^+) Gradient across the Inner Mitochondrial membrane
  - ATP is synthesized when H^+ spontaneously diffuses back across the Inner Mitochondrial membrane
- ATP Synthetase converts the Free Energy of the Proton Gradient to Chemical Energy in the form of ATP

What is the Electron Transport Chain (ETC)?

- Electron Transport (Respiration) Chain is the Final Common Pathway in Aerobic cells by which Electrons derived from various substrates are transferred to Oxygen
- ETC is composed of a series of highly organized Oxidation-Reduction Enzymes whose reactions can be represented by:
    - **Reduced A + Oxidized B ←=======→ Oxidized A + Reduced B**

Where is the Electron Transport Chain located?

- ETC is located in the inner membrane in the Mitochondria
- Enzymes of the ETC are embedded in the inner membrane in association with the enzymes of Oxidative Phosphorylation

What are the major components of ETC?

- ETC is made up of Four Major Complexes
    - Complex I:
        - NADH, Coenzyme Q Reductase
        - Point of entry into ETC for electrons from NADH
    - Complex II:
        - Succinate, Coenzyme Q Reductase
        - Point of entry into ETC for electrons from Succinate
    - Complex III:
        - Coenzyme Q, Cytochrome C Reductase
        - Electron acceptor for Coenzyme Q
    - Complex IV:
        - Cytochrome C Oxidase
        - Electron acceptor for Cytochrome C

What do you understand by Oxidative Phosphorylation?

- It is the main source of energy in Aerobic metabolism
- It is a process by which Free Energy released when electrons are transferred along the Electron Transport Chair is coupled to the formation of ATP from ADP and Pi
- Two possibilities must be considered:
    - Intact Mitochondria:
        - Transport of Electrons and Oxidative Phosphorylation of ADP are tightly Coupled reactions
        - Free Energy released is stored as ATP
    - Damaged Mitochondria:
        - Electron transport may occur without Oxidative Phosphorylation
        - Free Energy that is released as Electrons are transported will not be stored as ATP but will instead be lost as heat

What are reducing equivalents?

- Reducing equivalents are molecules that serve as sources of electrons for the ETC
- Two major reducing equivalents:
    - Reduced Nicotinaminde-Adenine Dinucleotide ($NADH + H^+$)
    - Reduced Flavin-Adinine Dinucleotide ($FADH_2$)

How many ATP molecules is produce by each Reducing Equivalent?

- Electrons that enter the ETC from NADH produces 3 molecules of ATP
- Electrons that enter the ETC from $FADH_2$, by pass Complex I and therefore produces only 2 molecules of ATP

What is Superoxide?

- Partial reduction of molecular Oxygen gives a highly reactive, highly unstable molecule called Superoxide (O_2^-)
- Superoxide is an anion free radical that can react with and damage DNA, Proteins and Cell membranes in general

Where is Superoxide formed?

- In the mitochondria by reactions of O_2 with $FADH_2$ and reduces Cytochrome Q
- In reactions involving molecular Oxygen
- In Red Blood Cells, because they contain Hb that forms HbO_4

How can Superoxide be removed from cells?

- Enzymatic reactions for removal of Superoxide: Two step reactions;
- First Step:
  - Superoxide Dismutase:
  - Metallo-enzyme that catalyzes the removal of Superoxide from cells
    - **$2\ O_2^- + 2\ H^+ \text{=====} \rightarrow H_2O_2 + O_2$**

- Second Step:
  - Hydrogen Peroxidase catalyzed break down of Hydrogen Peroxide formed
    - **$2\ H_2O_2 \text{======} \rightarrow 2H_2O + O_2$**

STUDY QUESTIONS:

1. What are Superoxides and how can they be removed from cells?
2. What is the Electron Transport Chain and were is it located in the cell?
3. How is energy stored in the cell?
4. What are coupled reactions (give examples)?
5. What are the major components of the Electron Transport Chain?
6. How many molecules of ATP are produced by one molecule of NADH, $FADH_2$?

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COLORIMETRY and SPECTROPHOTOMETRY - Overview

- Several methods of analysis of chemical compounds are based on ability of molecules to absorb light (Electromagnetic Radiation)
- Analytical methods using electromagnetic radiation are based on two general principles:
    - A given chemical compound cannot absorb light of all wavelengths (**Why?**)
        - Each compound has a specific characteristic wavelength (**λ**)
            - Thus, a compound can absorb light containing its characteristic wavelength
    - Intensity of light absorption is Proportional to the Concentration of the compound in solution

Why do chemical compounds absorb light of certain wavelength? (Fig. 1)

- Chemical compounds are made up of molecules, which are made up of atoms
- Atoms contain electrons located in orbitals whose energy levels are determined by quantum mechanics
    - Each orbital has a precisely determined Energy level
- Number of energy levels in any given atom is grater than the number of electron pairs in the atom
- Electrons occupy the lower energy levels while the higher levels are empty
- Electrons in Lower energy levels can be transferred to Higher empty energy levels
    - This occurs when electron in the lower energy level acquires energy that precisely equals the energy difference between the upper and lower energy levels (ΔE)

- Energy difference between 2 neighboring electron energy levels in a chemical compound is equal to either the energy of:
    - Visible light (λ = 380nm – 1000nm), or UV light (λ = 200nm – 380nm)

- Rule for the possibility of light absorption is as follows:
    - Electromagnetic radiation (Light) in the visible or UV range can be absorbed by a molecule, if the energy of the electromagnetic radiation is exactly equals to the energy difference between two electron energy levels in the molecule (**Fig. 1**)

Energy of radiation (Light energy) is determined as:

$$E = h\nu$$

{Note that $\nu = c/\lambda$} Thus substitute ν in the equation, gives

$$E = hc/\lambda$$

- Where **E =** Light energy; **h** = Planks constant; **ν** = Frequency of light energy; **λ** = Wavelength of Light energy; **c =** Speed of Light

Rules for light absorption can be expressed mathematically as:

$$\Delta E = hc/\lambda.$$

- Where **ΔE** = difference in energy between a filled and an empty electron energy level (or orbital)

Briefly explain why some chemical compounds can absorb more than one wavelength of light (Fig. 2)

- Some times transfer of electrons are possible between one filled electron energy level to several free energy levels, or from several filled electron energy levels to the same empty energy level
- This is the reason why a given chemical compound can absorb not only one but several different wavelengths of light
    - Thus a compound can absorb and therefore show several characteristic wavelengths of light (**Fig. 2**)

What do you understand by Absorption Spectrum of a chemical compound?

- In order to use light absorption for analysis of a chemical compound, the wavelength characteristics of the compound must be establish
    - Thus the need to determine the Absorption Spectrum of the chemical compound
- Absorption Spectrum is the dependence of light absorption by the given chemical compound on the wavelength of light
- Absorption Spectrum of a chemical compound is a Curve, which shows the Dependence of light absorption by the molecules in the chemical compound versus the Wavelength of visible light and UV light (See **Fig. 3**)

TAKE NOTE:

- To record Absorption Spectrum of a compound the following is needed:
    - A device for select appropriate wavelengths
    - A device to measure light absorption at the selected wavelengths
        - **Colorimeter**, **Spectrophotometer**

Fig. 3a: Shows the Electromagnetic Spectrum scales

Why such broad absorption bands in the absorption spectrum?

- If it was possible to record Absorption Spectrum at Absolute Zero Temperature then, the spectrum would have absorption at the exactly determined wavelengths,
    - That is, there would be just Absorption Lines instead of Absorption Bands in the spectrum
- At temperature greater than absolute zero, Thermal Expansion of Spectral Lines occurs, and Absorption Bands appear instead of Sharp Absorption Lines, **WHY?**
- Because at temperatures higher than absolute zero:
    - Molecules are vibrating and a given electron energy level is split into several vibrating sub-levels, thus resulting in changing the Line Spectrum into Bands
    - The λ_{max} is the one that more precisely corresponds to the energy difference between the two electron energy levels

What is Spectrophotometry? Figs. 4 & 5

- It is the measurement of light absorption by a sample
- Spectrophotometer and Colorimeter are instruments used to measure light absorption
- A beam of light is passed through the sample to be measured
- Light produced by the spectrophotometer is called Incident light (**Io**)
- Light that passed through the sample is called Transmitted light (**I**)
- Difference between the Intensities of Incidence light (Io) and Transmitted light (I) is the amount of light absorbed by the compound in the sample

What are some of the uses of spectrophotometer in Biochemistry?

- Measurement of concentration of a compound in solution
- Identification of compounds in solution using their characteristic absorption spectrum
- Measurement of rates of enzyme catalyzed reactions

What is Transmittance? (Fig. 6)

- Difference in Intensities **Io** and **I** represents amount of light Absorbed by sample
- Energy Ratio **I/Io** are called the Transmittance (T) of the solution.
- Express as a Percentage (**T%**):
    - Thus:

$$\mathbf{T = I/Io \times 100}$$

- Example, if only 20% of the light is Transmitted it means that the solution absorbs 80% of the light

What is Absorbance? (Fig. 6):

- Absorption of light can be defined as the Logarithm of the Ratio between the **Intensity** of Light falling on the sample of a compound (**Io**) and the **Intensity** of Light Transmitted through the sample of compound (**I**)

$$\mathbf{A = \log Io/I} \quad \text{(where A is Absorption)}$$

- Biochemical analysis more common use the Absorbance of a sample
    - Absorbance can be defined as:

$$\mathbf{A = \log_{10} 1/T} \qquad \text{Where } \mathbf{T = I/Io} \text{ (Transmittance)}$$

$$\text{Thus:} \qquad \mathbf{A = \log_{10} Io / I}$$

What is Lambert's Law?

Lambert's Law states that:

- For a Monochromatic light passing through a solution of absorbing molecules at Concentration (c), the fraction of light absorbed by the solution is Proportional to the Path Length (l) through which the light passes but is independent of the actual light intensity
- Lambert's law can be represented simple as:

$$\mathbf{A \propto l}\text{; which can be rewritten as } \mathbf{A = kl}$$

{**k** represent proportionality constant}

What is Beer's Law?

- Beer's Law indicates the relationship between the fraction of monochromatic light being absorbed and the concentration (c) of absorbing molecules
- Beer's law can be represented simple as:

$$\mathbf{A} \propto \mathbf{c}\text{; which can be rewritten as } \mathbf{A = kc}$$

{**k** represent proportionality constant}

What is Beer-Lambert's equation?

- Beer-Lambert equation expresses the relationship between the Light Absorbed (A) by a solution and the Concentration (c) of the Absorbing Compound in that solution
- It is the combination of Lambert's law and Beer's law:
- Beer – Lambert's equation:

$$A = \in cl$$

{**Extinction Coefficient** ($\in$) represents proportionality constants in both laws}

What is Molar Extension Coefficient?

- Absorbance (**A**) is a ratio of Intensities and therefore has no unit
- Path-Length (**l**) is expressed in cm
- When concentration (**c**) is expressed in moles per liter (i.e., Molar or M) the constant $\in$ has the unit of $M^{-1}cm^{-1}$ and is called the Molar Extinction Coefficient
- Extinction Coefficient ($\in$) is equal to the Absorbance of a 1.0 M solution of a compound in a 1.0 cm path-length cuvette at a given wavelength
- Since the absorbance of a solution varies with wavelength, the Molar Extinction Coefficient will also change for different wavelengths
- Thus, when $\in$ is written the wavelength must be written as a subscript:
  - Molar Extinction Coefficient of NADH: $\mathbf{\in_{340nm} = 6220\ M^{-1}cm^{-1}}$
- Unknown compounds can be identified by calculation their Molar Extension Coefficient