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CHAPTER-1

CONCEPTS OF BIOCHEMISTRY



Concept 1: Structure of Atom

- Living cells are composed of a variety of different elements, but the four most abundant elements in living cells are carbon (C), hydrogen (H), nitrogen (N), and oxygen (O).
- The elemental composition of biological system is different from earth crust but similar to aquatic system, suggest aquatic ancestry.
- Life on Earth is carbon-based because carbon is a highly versatile element that can form many different types of chemical bonds with other elements, including hydrogen, oxygen, nitrogen, and sulphur.

Element by	Symbol	Percentage in
weight		human body
Oxygen	0	65
Carbon	С	18.5
Hydrogen	Н	9.5
Nitrogen	N	3.2
Calcium	Ca	1.5
Phosphorus	Р	1.0
Potassium	K	0.4
Sulfur	S	0.3
Sodium	Na	0.2
Chlorine	Cl	0.2
Magnesium	Mg	0.1
Trace	B, Cr, Co, F, I, Fe, Mn,	less than 0.1
elements	Mo, Se, Si, Sn, V, Zn	

- Atoms are composed of three types of subatomic particles: protons, neutrons, and electrons.
- Protons are positively charged particles found in the nucleus of an atom.
- The number of protons in an atom's nucleus determines its atomic number and thus its identity as a specific element.
- Neutrons are uncharged particles found in the nucleus of an atom.
- The number of neutrons in an atom's nucleus can vary, leading to different isotopes of an element with different atomic weights.
- Electrons are negatively charged particles that orbit the nucleus in shells or energy levels.
- The number of electrons in an atom's outermost shell determines its chemical properties and how it interacts with other atoms.

	Mass	Charge		
Proton	1.6726 x 10 ⁻²⁷	(+) 1.6022 x 10 ⁻¹⁹		
	kg.	Coulomb		
Neutron	1.6749 x 10 ⁻²⁷	No charge		
	kg.			
Electron	9.1 x 10 ⁻³¹ kg.	(-) 1.6022 x 10 ⁻¹⁹ Coulomb		

• Distribution of electrons in shells and sub shells is follows:

Shell number			2	3			4			
Shell name	К		L	M			N			
Subshell name	0	0	1	0	1	2	0	1	2	3
Subshell Number	S	S	р	S	р	d	S	р	d	f
Number of electron in subshells	2	2	6	2	6	10	2	6	10	14
Total number of electrons in subshell			8		18			;	32	

Isotope

• Elements having the same numbers of protons (atomic number), but different numbers of neutrons (Atomic Mass). Example: ¹²C, ¹³C and ¹⁴C

Isobar

• Elements that have similar mass numbers and different atomic numbers. Example: 14C and 14N

Isotone

- Elements which have the same number of neutrons.
- Example: 12C (6 neturons) and 13N (6 neutrons)
- Radioisotopes are isotopes of an element that are unstable and emit radiation as they decay into more stable forms.

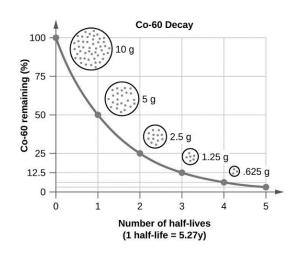
Туре	Nuc	clear equation		Representation		Change in mass/atomic numbers
Alpha decay	ξx	⁴ ₂ He + ^{A-4} _{Z-2} Y		***************************************		A: decrease by 4 Z: decrease by 2
Beta decay	AX	$_{-1}^{0}e + _{Z+1}^{A}Y$		V >	8,33	A: unchanged Z: increase by 1
Gamma decay	ĝχ	⁰ γ + ^Δ γ	Excited nuclea	γ > γ		A: unchanged Z: unchanged
Positron emission	Δ×	0 +1e + Y-1 Y		V >		A: unchanged Z: decrease by 1
Electron capture	AX	0 -1e + Y-1 Y		X-ray VVV>		A: unchanged Z: decrease by 1

Examples:

- Alpha Decay: ²³⁸Uranium
- Beta Decay (Negatron): ³H, ¹⁴C, ³²P (strong), ³⁵S
- Beta Decay (Positron): ¹¹C, ¹⁸F
- Gamma Decay: ⁶⁰Co, ¹³¹I

Half Life of Radio Isotopes

- Time in which original radioactive material reduces to half.
- It is constant for radioisotope, donot change with concentration.
- $T_{1/2} = \frac{0.693}{k}$, where 'k' is first order rate constant



Isotope Remaining			
$= \left(\frac{1}{2}\right)^n x$ Starting Material			
Where n = the number of half-lives			

Isotope	Half-life
³ H	12.3 years
¹⁴ C	5730 years
³² P	14.3 days
²³⁵ U	7.1 x 10 ⁸ years
²³⁸ U	4.51 x 10 ⁹ years
²³⁹ Pu	24,400 years

Concept 2: Molecular Composition of Cell

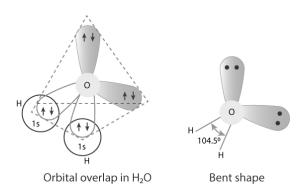
- Water is most abundant molecule, followed by proteins (carbohydrates in plants)
- Amount of RNA is 5-6 times more as compare to DNA

	E. coli	Animal	Green Plant
	(%)	(Rat liver)%	(Spinach) %
Water	70	69	93
Protein	15	21	2.3
DNA	1	0.2	0.2
RNA	6	1.0	1.0
Carbohydrates	3	3.8	3.8
Lipids	2	6	0.3

Important properties of water

- Water has a higher melting point, boiling point, and heat of vaporization compared to most other solvents.
- These properties arise due to strong intermolecular attractions between adjacent water molecules, leading to high internal cohesion in liquid water.
- Water is densest at 4°C, which is why ice floats on liquid water.
- The electron structure of water molecules explains these intermolecular attractions.
- Each hydrogen atom in water shares an electron pair with the central oxygen atom.
- The shape of
- The water molecule is influenced by the outer electron orbitals of oxygen, forming a rough tetrahedral structure, which are similar to the sp³ bonding orbitals of carbon.
- The bond angle in water (104.5°) is slightly less than a perfect tetrahedron due to the influence of the nonbonding orbitals.

Properties	Values
Molarity of Pure Water	55.56 M
Heat of vaporization, cal/g	540
Heat capacity, cal/g	1,000
Heat of fusion, cal/g	79.7
Surface tension	72.8
Dielectric constant	78.5 (= 80)
Hybridization	sp ³
Bond angle	104.5 ⁰



Concept 3: Covalent and Ionic Bonding

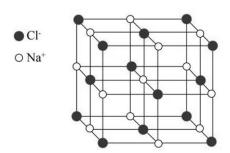
Covalent Bonds

- Covalent bonds involve the sharing of electron pairs between atoms, typically between nonmetals like carbon, oxygen, nitrogen, and hydrogen.
- They are formed when there is no difference or low difference in electronegativity of bonded atoms
- Strength: Covalent bonds range from 150-1000 kJ/mol, with bond dissociation energies typically in the range of 200-500 kJ/mol.
- Factors Affecting Strength:
- Size: Smaller atoms like oxygen and carbon have stronger orbital overlap, creating stronger bonds.
- Polarity: A greater difference in electronegativity (like in C-O bonds) creates highly polar and stronger covalent bonds.
- Bond Type: Single, double, and triple covalent bonds vary in strength, with single bonds usually being weaker than multiple bonds.
- **Example**: The C-O bond is strong (400 kJ/mol) due to small atomic size and high polarity.
- Pi-pi Interactions: Weaker than covalent bonds (1-50 kJ/mol), commonly found in aromatic systems, contributing to molecular stability and restricted rotation.

Ionic Bonds

- Ionic bonds form when one atom donates electrons to another, typically between a metal and a nonmetal.
 This leads to electrostatic attraction between positively and negatively charged ions.
- When there is large difference in electronegativity of bonded atoms there is transfer of electrons.
- Strength: Ionic bonds depend on the charge magnitude of the ions and the distance between them and dielectric constant of medium in which ions has been placed.

$$E = \frac{q_1 q_2}{r.D}$$

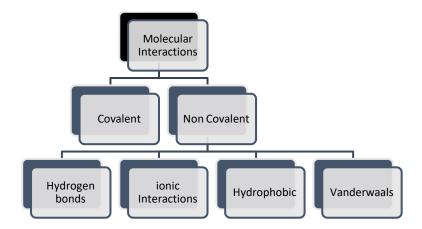


FCC Structure of NaCl

- Factors Affecting Strength:
- **Dielectric Constant**: Higher dielectric constant of the solvent (e.g., water = 80) reduces electrostatic forces and stabilizes ions, promoting dissolution of ionic compounds. Such solvent with high D, better dissolve ionic compounds by reducing the electrostatic forces between ions.
- They are 80 times weaker in water as compare to air or vacuum.
- Crystal Structure: NaCl (salt) forms a strong ionic lattice due to the alternating arrangement of Na⁺ and Cl⁻ ions in a cubic unit. Each chloride ions are surrounded by six sodium ions in a face-centered cubic structure, influencing bond strength and stability.

	Covalent Bonding	Ionic Bonding
Bond Energy	200-450 KJ/mol	600-4000 KJ/mol
Electrons	Shared	Transfer
Interactions	Balance of attractive	Electrostatic forces
	and repulsive forces	keep ions bonded
	between atoms	together
	Can be polar, non-	
	polar	
Examples	Water (H₂O)	NaCl

Concept 4: Molecular Interactions



- Molecular interactions are the forces that act between molecules and play a critical role in maintaining the structure and function of biological systems.
- These interactions include covalent bonds and noncovalent bonds such as hydrogen bonds, electrostatic (ionic) interactions, hydrophobic interactions, van der Waals forces.
- Covalent bonds are strongest in aqueous environment (in presence of water), almost 10 times or more stronger than other interactions.
- Hydrogen bonds are strongest non-covalent interactions in polar environment (in presence of water).
- Salt bridge of ionic interactions are strongest noncovalent interactions in non-polar environment.

- Ionic Van der Waals interactions are weakest, nonspecific and most sensitive to distance.
- Primary structure of biomolecules is stabilized by covalent bonds.
- Secondary structure of biomolecules is mainly stabilized by hydrogen bonding.
- Tertiary structure of biomolecules (protein/DNA) is mainly stabilized by hydrophobic interactions

Interaction	Strength (KJ/mol)
Covalent bonds	200-450
Hydrogen Bonds	12-30
Ionic interactions	6-40
Hydrophobic interactions	4-12
Van der Waals interactions	2-4

Solubility of Salt in Water

- As a salt such as NaCl dissolves, the and ions leaving the crystal lattice acquire far greater freedom of motion.
- The resulting increase in entropy (randomness) of the system is largely responsible for the ease of dissolving salts such as NaCl in water.

The **solubility of gases in water** depends on their interaction with water molecules:

- Ammonia (NH₃) is highly soluble due to its ability to form hydrogen bonds with water.
- Sulfur dioxide (SO₂) is also quite soluble, forming dipole interactions and ionizing partially.
- Carbon dioxide (CO₂) is moderately soluble as it reacts with water to form carbonic acid.
- Oxygen (O₂) is the least soluble due to its nonpolar nature.

Electrostatic Interactions (Salt Bridge or Ionic bonds)

- Formed between charged molecules where transfer of electrons are possible.
- Energy = $\frac{Kq_1q_1}{r.D}$ and Force = $\frac{Kq_1q_1}{r^2.D}$
- Factors affecting strength:
- Distance: The energy of electrostatic interactions is inversely proportion to distance and force is inversely proportion to square of distance (r²)
- Dielectric Constant: In polar environments like water, the dielectric constant (around 80) reduces the strength of ionic interactions by screening the charges.
 In nonpolar environments, ionic interactions are strongest due to the lack of screening.

- Electrostatic interactions can be attractive or repulsive in nature depending on charges involved
- Attractive: Histone and DNA, Peripheral protein and plasma membrane
- Repulsive: Two strands of DNA, folded protein
- Addition of salt to solution decrease electrostatic interactions, whether attractive or repulsive.

Hydrogen Bonds

- Weak bond that forms between a hydrogen atom bonded to electronegative atom and an electronegative atom having lone pair of electron such as nitrogen or oxygen.
- They are 10% covalent due to overlaps in the bonding orbitals, and about 90% electrostatic.
- Factors affecting strength:
- Electronegativity: More strength when donor is more electronegative than acceptor (e.g., O-H...O bonds are stronger than N-H...N bonds).
- Geometry: Strongest when the angle between the donor-hydrogen-acceptor is 180°, ensuring optimal orbital overlap.
- HA Distance: Distance between H atom and donor is lesser than vanderwall contact distance
- Environment: Hydrogen bonds are stronger in nonpolar solvents (low D) where there is less competition from solvent molecules.
- Examples: Double stranded DNA, secondary structures of protein, water in liquid or ice state
- Maximum number of hydrogen bond can be formed by a molecule depends on hydrogen which can be donated and lone pair of electrons. Water can maximally form four hydrogen bonds.

Hydrophobic Interactions

- Hydrophobic interactions occur when nonpolar molecules or parts of molecules aggregate to avoid contact with water.
- These interactions are driven by the tendency of water molecules to exclude nonpolar substances.
- Addition of salt to water increases hydrophobic interactions (leads to salting out of proteins).
- With temperature there is bell shaped curve for free energy or strength.
- Example: Folding of globular proteins, membrane lipid bilayer structure

Vander wall Interactions

- Weakest, non-specific, arise from temporary dipoles created by electron movement
- Strength increases with size of atoms, highly distancedependent, maximum strength when two atoms are contact with each other (at Vander wall contact distance)
- Free energy change during Van der Waals interaction
- $\Delta G_{Van} = \frac{A}{r^{12}} \frac{B}{r^6} + \frac{q_1q_2}{r}$, where constant 'A' represents electron shell repulsion and constant 'B' respresent dipole-dipole attraction
- Attraction is due to induced dipole-dipole attraction when two atoms comes in contact

- Repulsion is due to electron shell overlapping when two non-bonded atoms are closer than Vander wall contact distance. This is responsible for steric hindrance during conformational changes.
- Interaction decreases rapidly as distance is greater than Vander wall contact distance (inversely proportional to the sixth power of the distance)
- Interaction decreases more rapidly if distance between two non-bonded atom is lesser than Vander wall contact distance (inversely proportional to the twelfth power of the distance)

Concept 5: Solutions and Colligative Properties

Parts Per Million (PPM)

• 1 PPM: 1 mg of solute in 1000 mL of solution

Moles Concept

• A mole is equivalent to 6.023 x 10²³ molecules (Avogadro's number).

Molar Solution

• Molarity(M) = $\frac{\text{gram molecular wieght of solute}}{1 \text{ L volume of solution}}$

Molarity of Mixture of two Molar Solutions

 $\bullet \ M_1V_1 + M_2V_2 = M_3(V_1 + V_2)$

Normal Solution

• Normality(N) = $\frac{\text{gram equivalent wieght of solute}}{1 \text{ L volume of solution}}$

Stock Solution and Dilutions

• $C_1V_1 = C_2V_2$

Ionic strength of salt

$$\bullet I = \frac{1}{2} \sum C_i Z_i^2$$

 The free energy (ΔG) of a dissolved solute increases with solute concentration. This is because as the concentration increases, the chemical potential of the system rises.

Formula: $\Delta G = \Delta G^0 + 2.303 \text{ RT log [solute]}$

- Molarity (M) is temperature-dependent because solution volume can change with temperature.
- Normality (N) can vary depending on the reaction type (acid-base, redox).
- ppm is used for very dilute solutions.

 Percent solutions are practical and widely used in different forms (w/v, w/w, v/v).

Conversion Relationships:

- From Normality to Molarity: Normality = Molarity × nfactor
- From ppm to Molarity (for dilute solutions):

$$M = \frac{ppm}{molar\ mass} \times 10^{-3}$$

Colligative Properties

- The effect of solute concentration on the colligative properties of water is independent of the chemical properties of the solute
- It depends only on the *number* of solute particles (molecules or ions) in a given amount of water.

Examples

- Decrease in Vapor pressure
- Increase in Boiling point elevation
- Decrease in Freezing point
- Increase in Osmotic pressure
- When two aqueous compartments are separated by a semipermeable membrane, water moves across that membrane to equalize the osmolarity in the two compartments. This tendency for water to move across a semipermeable membrane produces the osmotic pressure (π= i. C. R. T).
- The van't Hoff factor accounts for the degree of dissociation or association of solute particles in the solution.
- For non-electrolytes like glucose, sucrose, starch the value of i=1
- For electrolytes, 'i' reflects the number of ions produced (e.g., NaCl dissociates into Na⁺ and Cl⁻, so i=2).
- Salts (electrolytes) will have more effect on colligative property as compare to non-electrolyte if kept is same concentration.
- For example, a compound such as NaCl, which dissociates in solution, has an effect on osmotic pressure that is twice that of an equal number of moles of a non-dissociating solute such as glucose.

Concept 6: pH and Buffer of aqueous solutions

Kw and pH

- Ionic product of water (Kw) =[H⁺] x [OH⁻] = 10⁻¹⁴ (at 25°C).
- If [H⁺] is given [OH⁻] can be calculated and vice versa.
- pH= -log[H⁺] and pOH= -log[OH⁻]
- pH + pOH = 14 (at 25°C). pH + pOH can vary with temperature
- The pH of biological systems can vary, and understanding the relationship between pH and [H⁺] is crucial:

 Lysosome (pH 4.2) vs. Cytosol (pH 7.2): The hydrogen ion concentration [H⁺] in lysosome is approximately 1000 times more than in cytosol.

pH of Strong Acids:

- Strong acids completely dissociate in solution, so [H⁺]
 can be directly calculated from the molarity of the
 acid.
- pH of aqueous solutions ranges from 0-14 at 25⁰ C
- At lower temperature pH + pOH can be higher than 14
- At higher temperature pH + pOH can be lower than 14
- Theoretically pH of strong acids can be lesser than 0 (negative)
- When two solutions with different pH are mixed, the resulting pH is affected by the H⁺ and OH⁻ concentrations.
- We cannot take average of two pH values to get pH of two solutions
- For determining the average pH over multiple measurements, calculate the [H⁺] concentration for each pH value, then average the concentrations and convert back to pH
- pH of mixture of two acid added in equal volume will be lower than average and closer to low pH
- pH of mixture of two bases added in equal volume will be Higer than average and closer to higher pH
- pH of mixture of acid and base added in equal volume will be closer to 7
- For example, mixing equal volumes of pH 4.0 and pH 10.0 solutions results in a neutral solution with an approximate pH of 7.0.
- Dilute solutions of strong acids: In very dilute acidic solutions, the contribution of H⁺ from water must be considered, as seen in extremely dilute HCl solutions.
- pH of very dilute (10⁻⁸ M) acidic solution will be slightly lesser than 7 (6.96).

Ka and pKa for Weak Acids

- Weak acid do not dissociate completely into [H⁺]
- The acid dissociation constant (Ka) is a measure of the strength of an acid in solution. It quantifies the degree to which an acid dissociates into its ions (H⁺ and its conjugate base) in water.
- $K_a = \frac{[H^+][A^-]}{[HA]}$
- A high Ka value indicates a strong acid (more dissociation) and low Ka value indicates a weak acid (less dissociation).

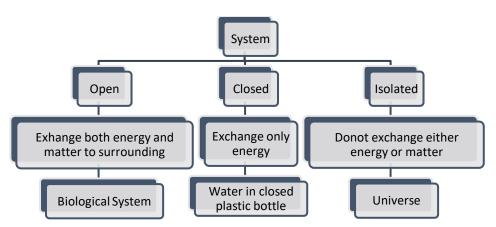
- **pKa** is the negative logarithm of the acid dissociation constant $(pK_a = -log K_a)$
- The larger the Ka, the stronger the acid, and hence the smaller the pKa.
- Strong acid has low pKa and weak acid has high pKa
- pKa is pH where acid get 50 % dissociated. Means where 50% is acid form and rest 50 % is conjugate base form.
- Polyprotonic acids have multiple pKa (equal to number of ionizable groups)

Buffer

- It is mixture of weak acid and its conjugate base
- Buffer retard the change in the pH of a solution on addition of strong acids or strong bases.
- $pH = pK_a + log \frac{[A^-]}{[HA]}$
- When there is more than one pKa choose pKa closest to desired pH

- If you know pH and pKa for any buffer, you can calculate [base]/[acid] ratio using this equation.
- $\frac{[Base]}{[Acid]} = \frac{[A^-]}{[HA]} = Antilog (pH pK_a)$
- The bicarbonate buffer system maintain pH in the blood
- Generally pH of blood is around 7.4, thus HCO₃-/CO₂ ratio is 20
- [base]>[acid] means pH>pKa,
 [base]=[acid] means pH=pKa and
 [base]<[acid] means pH<pKa
- Buffering zone is pKa + 1 pH
- pH of a buffer is theoretically independent of dilution (to limited extent 10 or 100 times)
- When dilution is very large (10⁶ or more), buffer fails to maintain pH and it approaches near 7.
- The molarity of the buffer is the number of moles of acid form + base form in 1 liter solution

Concept 7: Thermodynamics and Bioenergetics



- **First Law of Thermodynamics**: Energy cannot be created or destroyed, only transformed.
 - $\Delta U = q + w$ (change in internal energy = heat + work).
- **Second Law of Thermodynamics**: The entropy (S) of an isolated system always increases over time.
- Entropy (ΔS): Represents the disorder of a system. An increase in entropy favors the spontaneity of a reaction.
- Enthalpy (ΔH): The total heat content of a system operating at constant pressure. Negative ΔH (exothermic) contributes to a negative ΔG, favoring spontaneity.

Gibb's Free Energy

- Free energy is energy available to do work.
- It measures the maximum reversible work a system can perform at constant temperature and pressure.
- ΔG predicts the direction of a reaction
- Negative ΔG: Reaction is spontaneous in forward direction.
- Positive ΔG: Reaction is non-spontaneous in forward direction but spontaneous in backward direction.
- ΔG is zero: $\Delta G = 0$, the reactants are in equilibrium. Forward rate is equal to backward rate.
- Gibbs Free Energy (ΔG) determines the spontaneity (direction) but not the rate of a reaction. The rate is determined by the activation energy (Ea).
- The change in free energy, $\Delta G = \Delta H T \Delta S$
- At the thermal melting point (Tm) of DNA, lipids or proteins ΔG is zero. Thus, ΔH = TΔS or ΔS= ΔH/T

ΔΗ	ΔS	T less than ΔH/ΔS	T higher than ΔH/ΔS	
+	+	ΔG positive; not	ΔG negative; favored	
		favored		
+	-	ΔG positive; not	ΔG positive; not	
		favored	favored	
-	+	ΔG negative;	ΔG negative; favored	
		favored		
-	-	ΔG negative;	ΔG positive; not	
		favored	favored	

Gibb's Free Energy change under physiological conditions

- The free energy change under physiological condition (ΔG) of the reaction A ⇒ B depends on the concentration of the reactant and product under given conditions.
- At constant temperature and pressure, the following relationship can be derived
- $\Delta G = \Delta G^0 + 2.303 RT \log \frac{[B]}{[A]}$
- Where, ΔG^0 is standard energy change and [B] and [A] are cellular concentrations of products and reactants respectively.

Standard Gibb's Free Energy

- ΔG°: Free energy change under standard conditions
- Reactants and products are kept at 1 mol/L concentrations, temperature 25°C, pH=0 and Pressure = 1 atmospheric pressure
- ΔG°: Free energy change under standard conditions for biological system.
- All standard condition same except pH = 7
- $\Delta G^o RT \ln K_{eq}$
- $\bullet \quad \Delta G^o = -2.303 \, RT \log K_{eq}$
- $\Delta G^o = -1.36 \, kcal/mol \log K$ (To calculate answer in Kcal/mole)
- $\Delta G^o = -5.7 \, kJ/mol \log K$ (To calculate answer in KJ/mole)

Equilibrium Constant (K'eq):

- The ratio of product to substrate concentration at equilibrium.
 - K'eq = [products]/[reactants]
- If $K_{eq}>1$, then $\Delta G^0 = -ve$: It suggest reaction is favoured forward and at equilibrium have mainly products
- If K_{eq}<1, then ΔG⁰ = + ve: It suggest reaction is favoured backward and at equilibrium contains mainly substrate
- If K_{eq} =1, then ΔG⁰ = 0: It suggest that substrate and products are in equal concentration, thus, reaction is already at equilibrium.

- If Keq=100 for a reaction at 300 K, the free energy change is: ΔG^0 =-2.303×1.987×300×log (100) =-2746 cal/mol
- Variation of K_{eq} with ΔG_o at 25° C

K _{eq}	log K _{eq}	ΔG_o	ΔG_o
		(kcal.mol ⁻¹)	(kJ.mol ⁻¹)
10000 (10 ⁴)	4	-5.45	-22.8
1000 (10³)	3	-4.08	-17.1
100 (10 ²)	2	-2.72	-11.4
10(10 ¹)	1	-1.36	-5.7
1(10°)	0	0	0
0.1 (10 ⁻¹)	-1	+1.36	+5.7
0.01 (10 ⁻²)	-2	+2.72	+11.4
0.001 (10 ⁻³)	-3	+4.08	+17.1
0.0001 (10 ⁻⁴)	-4	+5.45	+22.8

 Standard free energy of coupled and sequential reactions are additive and Keq are multiplicative.

Energy Rich Compounds

 The term high-energy compounds or energy rich compounds are usually applied to substances which possess sufficient free energy to liberate at least 7.3 Kcal/mol at pH 7.0

Compound with High	ΔG°′	ΔG°′
Energy Bonds	(Kcal/mol)	(KJ/mol)
1. Phosphoenolpyruvate	- 14.8	- 61.2
2. Carbamoyl phosphate	- 12.3	- 51.6
3. 1,3-Bisphosphoglycerate	- 11.8	- 49.6
4. ATP (→ AMP + PPi)	- 10.9	- 45.8
5. Phosphate creatine	- 10.3	- 43.3
6. Acetyl phosphate	- 10.1	- 42.4
7. Arginine phosphate	- 8.0	- 33.6
8. Acetyl CoA	- 7.5	- 31.5
9. ATP (→ ADP + Pi)	- 7.3	- 30.6

- Chemical basis for large free energy change with ATP hydrolysis (ΔG°' ≈ -61.9 kJ/mol)
- Charge repulsion in substrate
- Resonance stabilization of product
- Hydration stability of products
- Ionization favors forward reaction
- Chemical basis for large free energy change with PEP hydrolysis (ΔG°' ≈ -61.9 kJ/mol)
- Enol to keto tautomerization of pyruvate
- Resonance stabilization in resulting pyruvate
- · Charge separation upon hydrolysis

Concept 8: Carbohydrates

Monosaccharides

- Carbohydrates are composed of carbon (C), hydrogen (H), and oxygen (O).
- They are simplest sugars having general formula $C_nH_{2n}O_n$, where n ≥ 3
- The simplest carbohydrates are monosaccharides (simple sugars), which can be classified as either aldoses (containing an aldehyde group, -CHO) or ketoses (containing a ketone group, -C=O).
- Seliwanoff's Test: Distinguish between Aldehyde and Keto sugar. It is given positive by Keto sugars.

		Aldoses	Ketoses
3C	Triose	Glyceraldehyde	Dihydroxy
			acetone
4C	Tetrose	Erythrose, Threose	Erythrulose
5C	Pentose	Ribose, Xylose,	Ribulose
		Arabinose, Lyxose	
6C	Hexose	Glucose, Galactose,	Fructose
		Mannose	

- D-Lyxose is a Pentose sugar and is a constituent of 'Lyxoflavin' isolated from human heart muscle.
- Bial's test: is specific for Pentoses (bluish color).
 Hexoses generally react to give green, red or brown products.
- Reducing Sugar: Sugars with a free aldehyde or ketone group in their open-chain form can reduce Cu²⁺ to Cu⁺.
- All monosaccharides are reducing sugars
- Tests for Reducing Sugars: Fehling's Test, Benedict's Test where color of solution changes from blue to red if sugar is reducing.
- Reducing sugars with phenyl hydrazine reducing forms osazone and with Tollen's reagent (AgNO₃) form silver mirror.

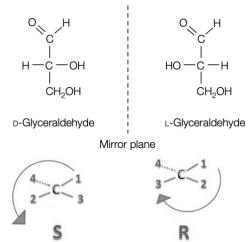
Stereoisomers

- Stereoisomers are molecules with the same molecular formula and bond structure but differ in the arrangement of atoms in space.
- Chirality: A carbon atom bonded to four different groups is a chiral center, and this property is responsible for the optical activity of carbohydrates.
- Isomers having same molecular formula but different orientation of functional group and bonds in 3D space
- Number of stereoisomers = 2ⁿ, where 'n' is number of chiral carbon

- Aldehydes has one extra chiral carbon as compare to ketone sugars
- Enantiomers are non-super imposable mirror images

DL Nomenclature system

- D-L system is based on the configuration of the hydroxyl group on the chiral carbon farthest from the carbonyl group in a sugar.
- **D**: Hydroxyl group on the right in a Fischer projection.
- L: Hydroxyl group on the left in a Fischer projection.
- D and L forms are enantiomers.
- The D-L system does not correlate directly with the optical rotation (dextrorotatory or laevorotatory) of the sugar.
- Enantiomers rotates plane polarized light in opposite direction
- Most of sugar are in D-form and amino acids are in Lform in biological system.



The RS Nomenclature system

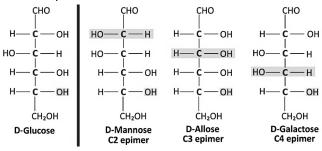
- It is a method of naming molecules with multiple chiral centres to describe their stereochemistry unambiguously.
- D-glyceraldehyde is in the R form and L-glyceraldehyde is in the S form.
- Assigning Priority: Each group attached to a chiral carbon is assigned a priority based on atomic number and other rules.
- Common substituent priorities: -SH>-OH>-NH₂>-COOH>-CHO>-CH₂OH>-H.
- View the molecule: Look at the chiral carbon with the group of lowest priority (usually hydrogen) pointing away from you.
- Assign priorities (1, 2, 3, 4): Based on the atomic number of the atoms directly attached to the chiral carbon.

 Clockwise or counterclockwise: If the priority of the groups decreases in clockwise order, the configuration is R (Latin rectus, meaning "right"). If the priority of the groups decreases in counterclockwise order, the configuration is S (Latin sinister, meaning "left").

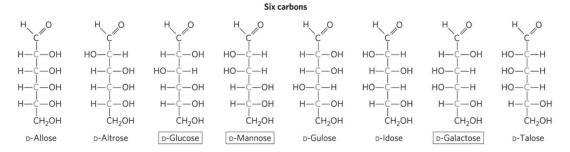
Diastereomers

- Diastereomers are stereoisomers that are not mirror images of each other.
- Diastereomers differ at 1 or more chiral carbon but not at all chiral carbons.
- Epimers: A specific type of diastereomers that differ at only one chiral carbon.

- D-glucose and D-mannose: Epimers differing at the C-2 position (C-2 epimer).
- D-glucose and D-galactose: Epimers differing at the C-4 position (C-4 epimer).
- D-glucose and D-allose: Epimer of D-glucose differing at the C-3 position.



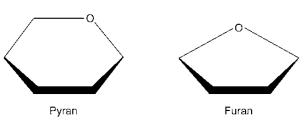
• D-talose: It is not an epimer of glucose; it differs at both C-2 and C-4 positions, making it a diastereomer, not an epimer.



Ring Structure

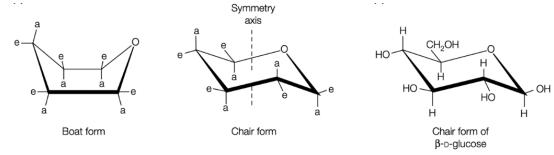
- The aldehyde or ketone group can react with a hydroxyl group to form a covalent bond.
- The reaction between an aldehyde and the hydroxyl group of a sugar (an alcohol) creates a hemiacetal (Equation 1) whereas a ketone reacts with a hydroxyl group (alcohol) to form a hemiketal (Equation 2).

- For making Ring/Cyclic structure, minimum number of carbons present should be 4.
- Pyranose a 6 membered ring with one Oxygen and 5 Carbons.
- Furanose a 5 membered ring with one Oxygen and 4 Carbons.

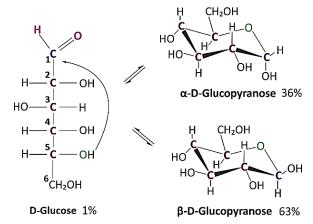


- Glucose is mainly Pyranose, i.e. 99% Pyranose but 1% Furanose also exist
- Fructose is mainly Furanose, i.e. 99% Furanose but 1% Pyranose also exist.
- The fructose in honey is mainly in the β -D-pyranose form
- The sweetness of honey gradually decreases at a high temperature.
- Increasing the temperature shifts the equilibrium in the direction of the β -D-furanose (stable) form, reducing the sweetness of the solution.
- The pyranose ring of a six-carbon sugar can exist in either a boat or a chair configuration.
- In the boat form, there is considerable steric hindrance between the various groups attached to the carbon atoms of the ring and hence this form is less favorable energetically.

Thus the chair form predominates, as shown for β-D-glucose, where all the axial positions are occupied by hydrogen atoms.



Anomers



- Anomers are diastereoisomers of cyclic forms of sugars
- Anomers are a type of epimer that differ at the anomeric carbon (the carbon derived from the carbonyl group during ring closure in sugars).
- Anomeric carbon is -1 atom of an aldose or the C-2 atom of a ketose.
- α-D-glucose and β-D-glucose are anomers that differ at the hemiacetal (or hemiketal) carbon in the cyclic form.
- At equilibrium, the β-anomer of D-glucose predominates, because the -OH group of the anomeric carbon is in the more stable equatorial position of the more stable chair structure
- Mutarotation: In aqueous solution, the α and β forms of glucose can interconvert, leading to an equilibrium mixture of both anomers. The equilibrium mixture has 63.6% of the β -anomer and 36.4% of the α -anomer

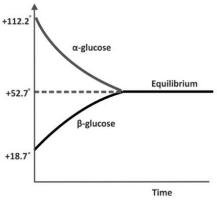
Optical Activity

- The ability of a compound to rotate plane-polarized light.
- Chiral molecules with non-superimposable mirror images exhibit optical activity.

- If rotate clockwise are termed as dextrorotatory (+) and if rotate counter clockwise are termed as levorotatory (-)
- The specific rotation $[\propto_D^{25^0C}]$ of an optically active compound is

D - Glucose	+ 52.70	L- Glucose	+ 52.70
D - Fructose	- 92.7 ⁰	L - Fructose	-+ 92.7 ⁰
Sucrose	+ 66 ⁰		

 Racemic Mixtures: A 1:1 mixture of enantiomers (e.g., D- and L-glucose) that is optically inactive because the optical rotations cancel each other out.



- Anomeric Mixtures: Mixtures of α- and β-anomers of D-glucose exhibit a net optical rotation because both forms rotate light in the same direction (both are positive).
- A freshly-prepared aqueous solution of α -D-glucose has a specific rotation, $\alpha_D^{25^0C}$ of +112.2°. And when this solution is allowed to stand, the rotation falls to +52.7° suggesting α -form converting to β -anomers
- A fresh solution of β-D-glucose has a rotation value of +18.7°; on standing, it also changes to the same value, +52.7°, suggesting β -form converting to α -D-glucose.

Derived Sugars

1. Glycosides:

- Formed by condensation between the hydroxyl group of the anomeric carbon of a monosaccharide and another compound (may be a monosaccharide or nonsugar like an aglycone).
- O-glycosidic bonds form when the second group is hydroxyl, e.g., glucoside (from glucose) or galactoside (from galactose).
- N-glycosidic bonds form when the second group is an amine, e.g., cytidine in nucleosides.
- Important in medicine: Cardiac glycosides (e.g., ouabain) inhibit Na⁺-K⁺ ATPase and are used for heart conditions. Other glycosides include antibiotics like streptomycin.

2. Sugar Acids:

- Gluconic acid: Formed by oxidation of C-1 of glucose.
- Glucuronic acid: Formed by oxidation at C-6 of glucose, important in polysaccharides and for detoxification by coupling with drugs, hormones, and pollutants.
- Vitamin C (Ascorbic acid): A biologically important sugar acid.

3. Amino Sugars:

- Formed when an amino group (NH₂) is introduced into hexoses.
- Glucosamine: Found in complex polysaccharides, usually as N-acetyl glucosamine.
- N-acetyl galactosamine: Found in glycoproteins and glycolipids.
- Sialic acids: Derived from neuraminic acid, important in glycoproteins and gangliosides, widely distributed in animal tissues.

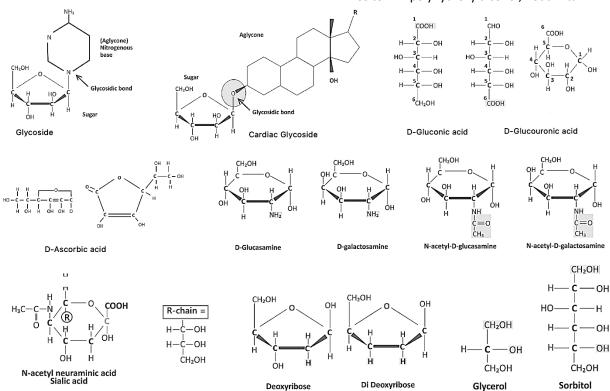
4. Deoxy Sugars:

- Deoxyribose: Formed by the loss of oxygen from ribose, a key component of DNA.
- 2-Deoxyglucose: Used experimentally to inhibit glucose metabolism, also used in cancer and COVID-19 treatments.
- Dideoxyribose: Used in nucleotides as chain terminators during DNA replication.

5. Sugar Alcohols:

- Formed by hydrogenation of sugar, reducing the carbonyl group to a hydroxyl group.
- Examples include glycerol, sorbitol, and mannitol (found in brown algae), commonly used as sweeteners.

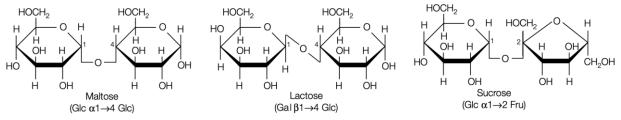
Inositol – A polyhydroxy alcohol, not a Vitamin



Disaccharides

- They are simplest oligo-saccharides having only two sugars
- They are reducing if there is free anomeric carbon or non-reducing if anomeric carbons is involved in glycosidic bond formation from both sides Molecular weight of disaccharides given below is 342 Dalton

Example	Unit 1	Unit 2	Bond	Reducing
				Sugar
Maltose	Glucose	Glucose	α(1→4)	Yes
Lactose	Galactose	Glucose	β(1→4)	Yes
Lactulose	Galactose	Fructose	β(1→4)	Yes
Cellobiose	Glucose	Glucose	β(1→4)	Yes
Sucrose	Glucose	Fructose	α(1→2)β	No
Trehalose	Glucose	Glucose	α(1→1)α	No



• The name 'invert sugar' is given to mixture of D-glucose and D-fructose because sucrose (which is dextrorotatory with a specific rotation, $\alpha_D^{25^0C}$ of + 66.7°), upon hydrolysis, gives a mixture of equimolar quantities of D(+) glucose (dextrorotatory; $\alpha_D^{25^0C}$ of + 52.5°) and D(-) fructose (levorotatory; $\alpha_D^{25^0C}$ of - 92.0°).

 $= -92.0^{\circ}$

• Mixture of glucose and fructose is levorotatory with $\alpha_D^{25^0C}$ value of (92.0 - 52.5)= - 39.5°. This reaction which is called "inversion of sucrose" is catalyzed by the enzyme sucrose (invertase) and also by H⁺ ions.

Polysaccharides

- Polysaccharides, also known as glycans. They vary in types of recurring monosaccharide units, the length of their chains, the types of bonds that connect these units, and the extent of branching.
- Homopolysaccharides consist of only one type of monomeric sugar, while heteropolysaccharides are composed of two or more different types of monomers.

Homo-polysaccharides

 It includes- Starch (amylose and amylopectin), glycogen, cellulose, chitin and dextran.

- Amylose has linear chains of (α1→4)Glucose (Glc), composed of 50–5,000 glucose residues. It is a component of starch, used for energy storage in plants mainly in amyloplast (plastid).
- Amylopectin has linear (α1→4)Glucose (Glc) backbone with (α1→6)Glc branches every 24–30 residues. It is branched starch and contains up to 10⁶ glucose units. Another component of starch, stores energy in plants.
- Starch (amylose and amylopectin) are helical structure, stabilized by intra-chain hydrogen bonding
- Starch synthesis occurs in chloroplast of leaf mesophyll cells or amyloplast of tuber cells using ADP-glucose.
- Glycogen has (α1→4)Glucose (Glc) backbone with (α1→6)Glc branches every 8–12 residues, having upto to 50,000 glucose units. It is energy storage in bacteria and animal cells.
- In animals glycogen is mainly stored in cytosol of liver and muscle cells
- Cellulose is linear chains of (β1→4)Glucose (Glc). It is composed upto to 15,000 glucose units.
- Cellulose is structural polysaccharide in plants, providing rigidity and strength to cell walls. It has sheet like structure and stabilized by inter-chain hydrogen bonding.
- Chitin has linear chains of (β1→4)N-acetylglucosamine (GlcNAc), mainly forming cell wall of fungi.
- Chitin provides structural rigidity and strength to the exoskeletons of insects, spiders, and crustaceans.
- Cellulose is most abundant polysaccharide in nature.
 Chitin is second most abundant.
- Dextran has (α1→6)Glucose (Glc) backbone with (α1→3) branches. They have structural role, acts as an extracellular adhesive in bacteria.

Туре	Repeating	Linkages	
	sugar		
Amylose	D-Glucose	α1 → 4	
Amylopectin	D-Glucose	$\alpha 1 \rightarrow 4$ $\alpha 1 \rightarrow 6$	
Glucagon	D-Glucose	$\alpha 1 \rightarrow 4$ $\alpha 1 \rightarrow 6$	
Cellulose	D-Glucose	β1 → 4	
Chitin	N-Acetyl	β1 → 4	
	D-Glucosamine		
Dextran	D-Glucose	α1 → 6	α1 → 3

Hetero-polysaccharides

- They have more than one type of repeating sugars
- Most of them play structural role (bacterial cell wall, matrix of animals)
- Pectin is a heteropolysaccharide made up of D-Galacturonic acid in $\alpha(1 \rightarrow 4)$ linkages. It is a soluble dietary fiber in fruits. Softening of ripe tomato occurs by enzyme Poly Galacturonase. Gene knock down of enzyme's gene helps to preserve tomatoes (Flavr-savr tomato).
- The rigid component of bacterial cell walls (peptidoglycan) is a heteropolymer of alternating (β1→4) -linked N-acetylglucosamine and N-acetylmuramic acid residues
- The enzyme lysozyme kills bacteria by hydrolyzing the β1→4 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid.
- Heteropolysaccharides are referred as GAGs (Glycosaminoglycans) and often has repeated acidic sugar and amino sugar
- One of the two monosaccharides is always either N-acetylglucosamine or N-acetylgalactosamine; the other is in most cases a uronic acid, usually D-glucuronic or L-iduronic acid.
- Keratan Sulfate does not contain acidic sugar

Туре	Component of	Linkages	,
	disaccharide repeats		
Hyaluronic	D-glucouronic acid+ N-	β1 → 3	β1 → 4
acid	acetyl-D-glucasamine		
Chondroitin	D-glucouronic acid+ N-	β1 → 3	β1 → 4
sulfate	acetyl-D-galactosamine-		
	4-Sulfate		
Dermatan	D-iduronic acid+ N-	β1 → 3	β1 → 4
sulfate	acetyl-D-galactosamine-		
	4-Sulfate		
Kerato	D-galactose + N-acetyl-	β1 → 4	β1 → 3
sulfate	D-glucasamine-6-Sulfate		

- Most of heteropolysacharides attach to small molecular weight protein to form glycoproteins to form proteoglycans.
- Hyaluronic Acid is not attached to Proteins (donot form proteoglycans)
- Dermatan Sulfate in excess is responsible for Atherosclerosis
- GAGs are slimy and slippery because of negative charge given by Carboxy, Sulfate and Acetate.
- Hyaluronic Acid is not sulfated
- Heparan has highest negative charge
- In the presence of heparan sulfate or heparin, the binding affinity of thrombin for antithrombin increases 2,000-fold, and thrombin is strongly inhibited. Thus, heparin act as anti-coagulant.
- Heparan Sulfate in excess is responsible for mental retardation
- Glycolipids are plasma membrane components in which the hydrophilic head groups are oligosaccharides.
- Glycoprotein are carbohydrate-protein conjugates in which the glycans are branched and are much smaller and more structurally diverse than the huge glycosaminoglycans of proteoglycans.
- The carbohydrate is attached at its anomeric carbon through a glycosidic link to the —OH of a Ser or Thr residue (O-linked), or through an N-glycosyl link to the amide nitrogen of an Asn residue (N-linked).
- Blood Group Antigens (A,B,O) are glycosphingolipids in RBCs and glycoproteins in secretions, where carbohydrate is O-linked oligosaccharide.

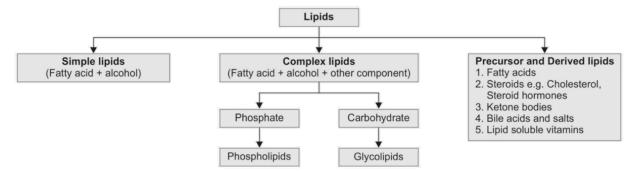
Concept 9: Lipids

- Lipids are a class of organic compounds that are insoluble in water but soluble in non-polar solvents (e.g., chloroform, ether).
- They are hydrophobic (water-repelling) due to their long hydrocarbon chains but can be amphipathic (containing both hydrophobic and hydrophilic regions), especially in membrane-forming lipids
- They are preferred energy storage molecule in animals as they are more reduced and occupy lesser space (Example Tri Acyl Glycerol)

Functions

 Energy Storage: Lipids, particularly triacylglycerols, store more energy per gram (9 kcal/g) than carbohydrates due to their highly reduced structure.

- Structural Role: Lipids are major components of biological membranes, such as the phospholipid bilayer in cell membranes.
- Signalling: Lipids like steroids (e.g., cholesterol) and eicosanoids function as hormones (primary
- messenger) or lipid like diacyl glycerol and phosphatidyl inositol 3,4,5 tris phosphate act as second messengers in signalling pathways.
- Insulation and Protection: Lipids act as insulators (thermal insulation) and provide cushioning to organs.



Simple Lipids

- Lipids can be broadly classified into two categories: simple Lipids and complex Lipids
- Simple lipids are esters of fatty acids and polyhydroxy alcohol
- Fatty Acids has long-chain carboxylic acids (typically 12-24 carbon atoms).
- Saturated Fatty Acids: Contain no double bonds

Saturated fatty acids					
Formula		Common	Melting		
		Name	Point		
CH ₃ (CH ₂) ₁₀ COOH	12C	Lauric acid	45 °C		
CH ₃ (CH ₂) ₁₂ COOH	14C	Myristic acid	55 °C		
CH ₃ (CH ₂) ₁₄ COOH	16C	Palmitic acid	63 °C		
CH ₃ (CH ₂) ₁₆ COOH	18C	Stearic acid	69 ºC		
CH ₃ (CH ₂) ₁₈ COOH	20C	Arachidic acid	76 °C		

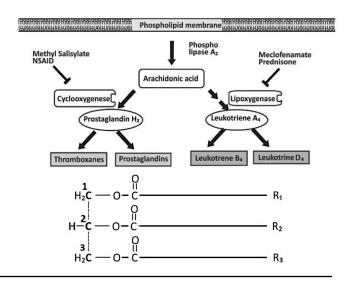
- Unsaturated Fatty Acids: Contain one or more double bonds (e.g., oleic acid, linoleic acid). They can be cis (natural configuration) or trans (from industrial hydrogenation).
- Numbering of Double Bond: Delta system—when counting starts from carboxy end and Omega system when counting starts from the methyl group

 Essential Fatty Acids: Fatty acids that cannot be synthesized by the body, such as linoleic acid (omega-6) and alpha-linolenic acid (omega-3), thus has to be taken essentially from diet. They are also termed as

- polyunsaturated fatty acids (PUFA). They belong to ω^3 and ω^6 Family
- Tm of fatty acids (thus lipids) increases with chain length and decreases with degree of unsaturation.

16:1 Cis-Δ ⁹	ω^7	Palmitoleic acid	0 °C
18:1 Cis-Δ ⁹	ω9	Oleic acid	13 °C
18:2 Cis-Δ ^{9, 12}	ω6	Linoleic acid	- 5 °C
18:3 Cis-Δ ^{6,9, 12}	ω^6	γ- Linolenic acid	- 11 °C
18:3 Cis-Δ ^{9, 12,15}	ω^3	α- Linolenic acid	- 17ºC
20:4 Cis-Δ ^{5,8,11,14}	ω^6	Arachidonic acid	- 49 °C
20:5 Cis-Δ ^{5,8,11,14,17}	ω^3	Timnodonic acid	- 69 °C
22:6 Cis-Δ ^{4,7,10,13,16, 19}	ω^3	Cervonic acid	- 92ºC

- Arachidonic acids often released from membrane lipids by action of phospholipase A₂ is a precursor for eicanosoid
- Eicanosoids Prostaglandin/Prostacyclin (Inflammatory response), thromboxanes (Platelet Aggregation) and leukotrienes (Bronchio-constriction) are important physiological mediators.
- The receptors for eicanosoids belongs to GPCR family



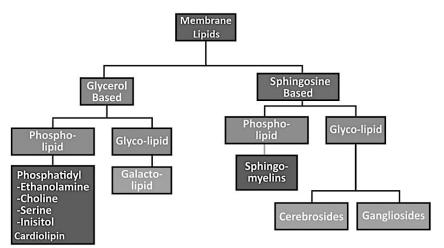
Simple lipids: Glycerol backbone esterified with one, two or three fatty acids.

- Monoacyl glycerol has one fatty acid attached to glycerol. It behaves like detergent and forms micelles when suspended in water
- Diacyl glycerol has two fatty acids forming ester bond with glycerol. It is important secondary messanger.
- Triacylglycerol: Non-polar (also known as Neutral Fat/Triglycerides/TAGs). It donot form membrane structure.

- TAGs are the main lipids present in diet and also the main storage form of lipids in the body. They are present in the adipose tissues. They typically have saturated fatty acid at C1, unsaturated fatty acid at C2 and fatty acid at C3 can be saturated or unsaturated.
- The enzyme which breaks down TAG in adipose tissue is Hormone Sensitive Lipase (HSL) present in adipose tissues. But HSL cannot break fatty acid at position 2.
- Phospholipase act as specific site on phospholipids

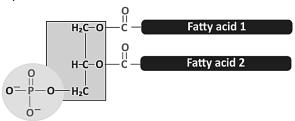
Complex Lipids (Membrane Lipids)

- Phospholipids are complex lipids made up of 3 components i.e Alcohol, FA and Phosphate. They are also known as phosphoglycerides.
- Phospholipids are of two types: Glycerophospholipids— Parent polyhydroxy alcohol is glycerol (3C) and sphingo-phospholipids— Parent alcohol is sphingosine (18C) which is polyhydroxy amino alcohol.



Glycerol based Phospholipids

 Phosphatidic acid, the parent compound for the glycerol-based phospholipids, consists of sn-glycerol-3-phosphate, with fatty acids esterified at the 1- and 2-positions



- Glycerophospholipids are derivatives of phosphatidic acid. In eukaryotes they are synthesised in smooth ER.
- Phosphatides with choline or ethanolamine are referred to as phosphatidylcholine (known commonly

- as lecithin) or phosphatidylethanolamine ((known commonly as cephalin), respectively
- Phosphatidylcholine (Lecithin) = Glycerol + 2 FA + Phosphate + Choline
- Phosphatidylethanolamine (Cephalin) = Glycerol + 2 FA
 + Phosphate + Ethanolamine
- Phosphatidyl Serine = Serine + Phosphatidic Acid (PA)
- Phosphatidyl Inositol = Inositol + Phosphatidic Acid
 (PA)
- Phosphatidyl Glycerol = Glycerol + PA
- Lecithin and Cephalin are the most abundant phospholipids in most eukaryotic cells.
- Dipalmitoyl lecithin is a very effective surface active agent and a major constituent of the surfactant secreted by alveolar type II cells