BIO-MOLECULES & ANALYTICAL TECHNIQUES

UNIT I: Carbohydrates, Proteins, and Lipids

Carbohydrates:

1. Classification:
2. Monosaccharides: Glucose, Fructose, Galactose
3. Disaccharides: Sucrose, Lactose, Maltose
4. Polysaccharides: Starch, Glycogen, Cellulose
5. Structure and Properties:
6. Monosaccharides: Structure (open chain and cyclic forms), isomerism
7. Disaccharides: Glycosidic bond formation
8. Polysaccharides: Structural differences and functional roles

1. Classification of Carbohydrates

Carbohydrates are classified based on their complexity and the number of sugar units they contain:

A. Monosaccharides

- **Definition:** Simple sugars with a single sugar unit.
- **Examples:** Glucose, Fructose, Galactose.
- **Structure:**
  - **Glucose:** An aldohexose with the formula $C_6H_{12}O_6$.
  - **Fructose:** A ketohexose with the formula $C_6H_{12}O_6$.
  - **Galactose:** An aldohexose similar to glucose but with a different arrangement of hydroxyl groups.

B. Disaccharides

- **Definition:** Composed of two monosaccharide units linked by a glycosidic bond.
- **Examples:**
  - **Sucrose (table sugar):** Glucose + Fructose.
  - **Lactose (milk sugar):** Glucose + Galactose.
  - **Maltose (malt sugar):** Two glucose units.
- **Structure:**
  - **Glycosidic Bond:** A covalent bond formed between the hydroxyl group of one monosaccharide and the anomeric carbon of another.

C. Polysaccharides

- **Definition:** Long chains of monosaccharide units (more than ten).
- **Examples:**
  - **Starch:** A storage form of glucose in plants, consisting of amylose (linear) and amylopectin (branched).
  - **Glycogen:** A storage form of glucose in animals, highly branched.
  - **Cellulose:** A structural component in plant cell walls, composed of β-glucose units linked by β (1→4) glycosidic bonds.
- **Structure:**
  - **Amylose:** Linear chains of α(1→4) linked glucose units.
  - **Amylopectin:** Branched chains with α (1→4) linked glucose and α (1→6) branches.
  - **Glycogen:** Similar to amylopectin but with more frequent branching.

2. Structure of Carbohydrates

A. Monosaccharides

- **General Formula:** (CH2O)n, where n ranges from 3 to 7.
- **Isomerism:**
  - **Structural Isomers:** Differ in the arrangement of atoms (e.g., glucose and fructose).
  - **Stereoisomers:** Differ in the spatial arrangement of atoms (e.g., D- and L-forms).
  - **Anomers:** Differ at the anomeric carbon (e.g., α and β forms of glucose).

B. Disaccharides

- **Glycosidic Linkage:** Formed through a dehydration reaction.
- **Example:** In sucrose, the glycosidic bond is between the anomeric carbon of glucose and the fructose carbon.

C. Polysaccharides

- **Branching:** Affects the solubility and functionality of the polysaccharide.
  - **Starch and Glycogen:** Have α-linkages which make them digestible by humans.
  - **Cellulose:** Has β-linkages which cannot be broken down by human digestive enzymes.

3. Properties of Carbohydrates

A. Physical Properties

- **Solubility:**
  - Monosaccharides and disaccharides are generally soluble in water due to their hydroxyl groups forming hydrogen bonds with water.
  - Polysaccharides have varying solubility depending on their structure (e.g., cellulose is insoluble).
- **Taste:** Many monosaccharides and disaccharides taste sweet (e.g., glucose, sucrose).

B. Chemical Properties

- **Reducing and Non-reducing Sugars:**
  - **Reducing Sugars:** Contain a free anomeric carbon capable of acting as a reducing agent (e.g., glucose, lactose).
  - **Non-reducing Sugars:** Do not have a free anomeric carbon (e.g., sucrose).
- **Benedict's Test:** Used to detect reducing sugars. A positive test results in a color change due to the reduction of copper (II) sulfate to copper(I) oxide.
- **Hydrolysis:** Polysaccharides and disaccharides can be broken down into monosaccharides by enzymes or acids.
  - **Enzymatic Hydrolysis:** Specific enzymes (e.g., amylase, lactase) catalyze the breakdown of complex carbohydrates.

Proteins:

1. Amino Acids:
2. Classification: Essential, non-essential, based on side chain properties (hydrophobic, hydrophilic, acidic, basic)
3. Structure and Properties: General structure, zwitterion formation, isoelectric point
4. Peptide Bond and Peptides:
5. Formation: Condensation reaction, peptide bond characteristics
6. Peptide Structure: Dipeptides, tripeptides, oligopeptides
7. Protein Structure:
8. Primary: Amino acid sequence
9. Secondary: α-Helix, β-Sheet, hydrogen bonding

10. Tertiary: 3D folding, disulfide bridges, hydrophobic interactions
11. Quaternary: Subunit assembly, functional complexes
12. Functions of Proteins: Enzymes, structural proteins, transport proteins, antibodies
13. Denaturation and Renaturation: Factors causing denaturation (pH, temperature), reversibility

Classification of Proteins

1. **Based on Composition:**
   - **Simple Proteins:** Yield only amino acids upon hydrolysis (e.g., albumins, globulins).
   - **Conjugated Proteins:** Contain a prosthetic group (non-protein part) (e.g., glycoproteins, lipoproteins, metalloproteins).
   - **Derived Proteins:** Result from the partial hydrolysis of simple or conjugated proteins.
2. **Based on Function:**
   - **Structural Proteins:** Provide support and shape (e.g., collagen in connective tissues).
   - **Enzymes:** Catalyse biochemical reactions (e.g., amylase, protease).
   - **Transport Proteins:** Carry substances (e.g., haemoglobin transports oxygen).
   - **Defensive Proteins:** Protect the body (e.g., antibodies).
   - **Signal Proteins:** Coordinate cellular activities (e.g., insulin).
   - **Contractile Proteins:** Involved in muscle contraction (e.g., actin, myosin).

Structure of Proteins

1. **Primary Structure:**
   - **Definition:** Linear sequence of amino acids in a polypeptide chain.
   - **Importance:** Determines the protein's unique characteristics and function.
   - **Peptide Bonds:** Link amino acids together in a specific order.
2. **Secondary Structure:**
   - **α-Helix:**
     - **Description:** Right-handed coil stabilized by hydrogen bonds between every fourth amino acid.
     - **Example:** α-keratin in hair.
   - **β-Sheet:**
     - **Description:** Sheet-like structure formed by hydrogen bonds between parallel or anti-parallel polypeptide chains.
     - **Example:** Silk fibroin.
3. **Tertiary Structure:**
   - **Description:** Three-dimensional folding pattern of a protein due to interactions among R groups (side chains).
   - **Interactions:** Hydrogen bonds, ionic bonds, hydrophobic interactions, disulfide bridges.
   - **Example:** Globular proteins like enzymes.
4. **Quaternary Structure:**
   - **Description:** Assembly of multiple polypeptide chains (subunits) into a functional protein complex.
   - **Importance:** Adds functionality and regulation.
   - **Example:** Haemoglobin, composed of four subunits.

Properties of Proteins

1. **Solubility:**
   - Depends on the protein and the environment (pH, ionic strength, temperature).
   - Globular proteins are generally soluble in water, whereas fibrous proteins are not.
2. **Charge:**
   - Determined by the ionizable side chains of amino acids.
   - Proteins can act as buffers.
3. **Denaturation:**
   - Loss of native structure due to disruption of non-covalent interactions.
   - Caused by factors such as heat, pH changes, solvents, or detergents.
   - Denatured proteins lose their biological function.
4. **Renaturation:**
   - Some proteins can regain their native structure if the denaturing agent is removed.
   - Depends on the primary structure's ability to guide proper folding.

Functions of Proteins

1. **Enzymatic Catalysis:**
   - Speed up biochemical reactions by lowering activation energy.
   - Highly specific for their substrates.
2. **Transport:**
   - Carry molecules across cell membranes or through the bloodstream.
   - Example: Haemoglobin transports oxygen; albumin transports fatty acids.
3. **Structural Support:**
   - Provide rigidity and elasticity.
   - Example: Collagen in connective tissue, keratin in hair and nails.
4. **Movement:**
   - Enable muscle contraction and cellular motility.
   - Example: Actin and myosin in muscle fibres.
5. **Regulation:**
   - Act as hormones or receptors to regulate biological processes.
   - Example: Insulin regulates glucose levels.
6. **Défense:**
   - Protect the body against pathogens.
   - Example: Antibodies (immunoglobulins) neutralize foreign invaders.

Denaturation and Renaturation of Proteins

1. **Denaturation:**
   - **Definition:** Process by which a protein loses its native conformation and function.
   - **Causes:**
     - **Heat:** Breaks hydrogen bonds and hydrophobic interactions.
     - **pH Extremes:** Alters ionization states of amino acids, disrupting ionic bonds.
     - **Chemicals:** Urea, guanidine hydrochloride, and detergents interfere with non-covalent interactions.
     - **Mechanical Agitation:** Disrupts weak interactions through shear forces.
   - **Consequences:** Loss of biological activity, often irreversible in severe conditions.
2. **Renaturation:**

- **Definition:** Refolding of a denatured protein into its native structure.
- **Factors Influencing Renaturation:**
  - **Removal of Denaturing Agent:** Gradual return to physiological conditions.
  - **Presence of Chaperones:** Proteins that assist in proper folding.
  - **Primary Structure Integrity:** The ability of the amino acid sequence to guide refolding.

Lipids:

1. Fatty Acids:
2. Classification: Saturated, unsaturated (mono- and polyunsaturated)
3. Structure and Properties: Chain length, degree of unsaturation, cis/trans configuration
4. Glycolipids and Phospholipids:
5. Structure: Glycerol backbone, fatty acid chains, phosphate group (for phospholipids)
6. Functions: Membrane structure, cell signalling
7. Cholesterol:
8. Structure: Steroid nucleus, hydroxyl group
9. Functions: Membrane fluidity, precursor to steroids and bile acids

Introduction to Lipids

- Definition: Lipids are a diverse group of hydrophobic molecules including fats, oils, waxes, phospholipids, and steroids.
- Functions: Energy storage, structural components of cell membranes, signalling molecules.

Classification of Lipids

1. Simple Lipids:
   - Fats and Oils: Esters of fatty acids with glycerol.
   - Waxes: Esters of fatty acids with long-chain alcohols.
2. Complex Lipids:
   - Phospholipids: Contain a phosphate group.
     - Glycerophospholipids: Glycerol backbone, two fatty acids, phosphate group.
     - Sphingophospholipids: Sphingosine backbone, one fatty acid, phosphate group.
   - Glycolipids: Contain a carbohydrate group.
3. Derived Lipids:
   - Steroids: Cholesterol and its derivatives.
   - Eicosanoids: Prostaglandins, thromboxane's, leukotrienes.

Fatty Acids

- Structure:
  - Saturated Fatty Acids: No double bonds, straight chains (e.g., palmitic acid).
  - Unsaturated Fatty Acids: One or more double bonds, kinked chains (e.g., oleic acid, linoleic acid).
- Properties:
  - Melting Point: Increases with chain length, decreases with unsaturation.
  - Solubility: Insoluble in water, soluble in organic solvents.
  - Reactivity: Prone to oxidation, hydrogenation.

Phospholipids

- Structure:
    - Glycerophospholipids: Glycerol backbone, two fatty acid tails, phosphate group with an alcohol (e.g., phosphatidylcholine).
    - Sphingophospholipids: Sphingosine backbone, one fatty acid, phosphate group with an alcohol (e.g., sphingomyelin).
- Properties:
    - Amphipathic Nature: Hydrophilic head and hydrophobic tail.
    - Role in Membranes: Form bilayers, basis of cell membranes.

Glycolipids

- Structure: Sphingosine backbone, one fatty acid, carbohydrate group.
- Types: Cerebrosides (single sugar), gangliosides (complex sugars).
- Functions: Cell recognition, signalling, stability of membrane structure.

Cholesterol

- Structure: Steroid nucleus, hydroxyl group at position 3, hydrocarbon tail.
- Functions:
    - Membrane Fluidity: Modulates membrane fluidity and stability.
    - Precursor Molecule: Bile acids, steroid hormones, vitamin D.

Functions of Lipids

- Energy Storage: High energy content, stored as triglycerides in adipose tissue.
- Structural Components: Major components of cell membranes (phospholipids, cholesterol).
- Insulation and Protection: Thermal insulation and cushioning of vital organs.
- Signalling Molecules: Hormones (steroids), intracellular signalling (phosphatidylinositol derivatives).

Properties of Saturated and Unsaturated Fatty Acids

- Saturated Fatty Acids:
    - Solid at Room Temperature: Due to tight packing of molecules.
    - Sources: Animal fats, butter, lard.
    - Health Impact: High intake associated with cardiovascular diseases.
- Unsaturated Fatty Acids:
    - Liquid at Room Temperature: Due to kinks in the hydrocarbon chain preventing tight packing.
    - Sources: Plant oils (olive oil, canola oil), fish.
    - Health Impact: Beneficial for cardiovascular health, essential fatty acids (omega-3 and omega-6).

Synthesis and Metabolism of Lipids

- Biosynthesis: Fatty acid synthesis, triglyceride formation, phospholipid synthesis.
- Catabolism: β-oxidation of fatty acids, ketogenesis in liver.

Visualization of Lipid Structures

- Chemical Structures: Diagrams of glycerol, fatty acids, triglycerides, phospholipids, cholesterol.
- Membrane Models: Phospholipid bilayer, role of cholesterol in membrane fluidity.

UNIT II: Nucleic Acids, Vitamins, and Bioenergetics

Nucleic Acids:

1. Structure and Functions of DNA and RNA:
2. DNA: Double helix, base pairing, replication
3. RNA: Types (mRNA, tRNA, rRNA), transcription, translation

Nucleic acids are essential biomolecules that store and transmit genetic information. The two main types of nucleic acids are DNA (Deoxyribonucleic Acid) and RNA (Ribonucleic Acid).

Structure of Nucleic Acids

1. Basic Components:

- **Nucleotides:** The monomers of nucleic acids. Each nucleotide consists of three components:
  - **Nitrogenous Base:** There are two types of nitrogenous bases:
    - **Purines:** Adenine (A) and Guanine (G)
    - **Pyrimidines:** Cytosine (C), Thymine (T) in DNA, and Uracil (U) in RNA
  - **Pentose Sugar:** A five-carbon sugar
    - **Deoxyribose** in DNA
    - **Ribose** in RNA
  - **Phosphate Group:** Links the 3' carbon of one sugar to the 5' carbon of the next sugar, creating a sugar-phosphate backbone

2. DNA Structure:

- **Double Helix:** DNA is a double-stranded helix formed by two complementary strands running in opposite directions (antiparallel).
- **Base Pairing:**
  - Adenine (A) pairs with Thymine (T) via two hydrogen bonds.
  - Guanine (G) pairs with Cytosine (C) via three hydrogen bonds.
- **Major and Minor Grooves:** The double helix has alternating major and minor grooves which are important for protein binding.

3. RNA Structure:

- **Single-Stranded:** RNA is typically single-stranded but can fold into complex structures.
- **Base Pairing:**
  - Adenine (A) pairs with Uracil (U).
  - Guanine (G) pairs with Cytosine (C).

Functions of Nucleic Acids

1. DNA Functions:

- **Genetic Information Storage:** DNA stores genetic information in the sequence of its bases.
- **Replication:** DNA can replicate itself during cell division, ensuring genetic continuity.
- **Transcription:** DNA serves as a template for RNA synthesis.

2. RNA Functions:

- **mRNA (Messenger RNA):** Carries the genetic information from DNA to the ribosome, where proteins are synthesized.
- **tRNA (Transfer RNA):** Brings amino acids to the ribosome during protein synthesis.
- **rRNA (Ribosomal RNA):** Combines with proteins to form ribosomes, the site of protein synthesis.
- **Other RNAs:** Include snRNA, siRNA, and miRNA, which play roles in gene regulation and mRNA processing.

DNA Replication

1. Semi-Conservative Process:

- Each new DNA molecule consists of one old (parental) strand and one new (daughter) strand.

2. Steps of DNA Replication:

- **Initiation:** Replication begins at specific locations called origins of replication.
- **Unwinding:** Helicase unwinds the double helix, creating a replication fork.
- **Primer Synthesis:** Primase synthesizes RNA primers to provide a starting point for DNA synthesis.
- **Elongation:** DNA polymerase adds nucleotides to the growing DNA strand in a 5' to 3' direction.
- **Leading and Lagging Strands:**
  - **Leading Strand:** Synthesized continuously.
  - **Lagging Strand:** Synthesized discontinuously as Okazaki fragments.
- **Primer Removal and Ligation:** RNA primers are removed and replaced with DNA, and DNA ligase seals the gaps.

Transcription (RNA Synthesis)

1. Process of Transcription:

- **Initiation:** RNA polymerase binds to the promoter region of DNA.
- **Elongation:** RNA polymerase synthesizes RNA by adding nucleotides complementary to the DNA template strand.
- **Termination:** RNA polymerase reaches a terminator sequence and releases the newly synthesized RNA.

Translation (Protein Synthesis)

1. Process of Translation:

- **Initiation:** mRNA binds to the ribosome, and the first tRNA brings an amino acid to the start codon.
- **Elongation:** tRNAs bring amino acids to the ribosome, and peptide bonds form between amino acids.
- **Termination:** When a stop codon is reached, the newly synthesized protein is released.

Types of RNA and Their Functions

1. mRNA (Messenger RNA):

- Carries genetic code from DNA to the ribosome for protein synthesis.

2. tRNA (Transfer RNA):

- Brings amino acids to the ribosome during translation.

3. rRNA (Ribosomal RNA):

- Forms the core of the ribosome's structure and catalyzes protein synthesis.

4. Other RNAs:

- **snRNA (Small Nuclear RNA):** Involved in RNA splicing.
- **miRNA (MicroRNA) and siRNA (Small Interfering RNA):** Involved in gene regulation.

Vitamins:

Source, Structure, Biological Role, and Deficiency:

1. Vitamin A: Vision, skin health
2. Vitamin B: Energy metabolism (B1, B2, B3, B6, B12, Folate)
3. Vitamin C: Antioxidant, collagen synthesis
4. Vitamin D: Calcium metabolism, bone health
5. Vitamin E: Antioxidant, protects cell membranes
6. Vitamin K: Blood clotting

Vitamins are organic compounds that are essential for normal growth and nutrition. They are required in small quantities in the diet because they cannot be synthesized by the body. Vitamins are classified into two categories: fat-soluble and water-soluble.

Fat-Soluble Vitamins

1. **Vitamin A (Retinol)**
    - **Source:**
        - Animal sources: Liver, fish oils, milk, eggs
        - Plant sources (as provitamin A carotenoids): Carrots, sweet potatoes, spinach
    - **Structure:**
        - Retinol, retinal, and retinoic acid are the active forms.
    - **Biological Role:**
        - Vision: Retinal is a component of rhodopsin, a protein in the eyes.
        - Immune function: Maintains epithelial tissues.
        - Cell growth: Retinoic acid regulates gene expression.
    - **Deficiency:**
        - Night blindness
        - Xerophthalmia (dry eyes)
        - Increased susceptibility to infections
2. **Vitamin D (Calciferol)**
    - **Source:**
        - Sunlight exposure (synthesis in the skin)
        - Foods: Fish liver oils, fatty fish, fortified milk
    - **Structure:**
        - D2 (Ergocalciferol) and D3 (Cholecalciferol)
    - **Biological Role:**
        - Calcium and phosphorus metabolism: Enhances absorption in the gut.
        - Bone health: Prevents rickets and osteomalacia.
    - **Deficiency:**
        - Rickets in children
        - Osteomalacia in adults
        - Osteoporosis
3. **Vitamin E (Tocopherol)**
    - **Source:**
        - Vegetable oils, nuts, seeds, green leafy vegetables
    - **Structure:**
        - Tocopherols and tocotrienols, with α-tocopherol being the most active.
    - **Biological Role:**
        - Antioxidant: Protects cell membranes from oxidative damage.
    - **Deficiency:**
        - Haemolytic anemia
        - Neuromuscular problems
4. **Vitamin K**
    - **Source:**
        - Green leafy vegetables, fermented foods, synthesized by gut bacteria

- **Structure:**
  - Phylloquinone (K1) and menaquinones (K2)
- **Biological Role:**
  - Blood clotting: Essential for the synthesis of clotting factors.
  - Bone health: Regulates bone mineralization.
- **Deficiency:**
  - Increased bleeding and bruising
  - Haemorrhagic disease in newborns

Water-Soluble Vitamins

1. **Vitamin B Complex**
   - **Thiamine (B1)**
     - **Source:** Whole grains, pork, legumes
     - **Structure:** Thiamine pyrophosphate (TPP) is the active form.
     - **Biological Role:** Carbohydrate metabolism, nerve function
     - **Deficiency:** Beriberi, Wernicke-Korsakoff syndrome
   - **Riboflavin (B2)**
     - **Source:** Dairy products, eggs, green leafy vegetables
     - **Structure:** Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)
     - **Biological Role:** Energy production, cellular function
     - **Deficiency:** Ariboflavinosis, mouth sores
   - **Niacin (B3)**
     - **Source:** Meat, fish, whole grains
     - **Structure:** Nicotinic acid and nicotinamide
     - **Biological Role:** DNA repair, metabolism
     - **Deficiency:** Pellagra (dermatitis, diarrhea, dementia)
   - **Pantothenic Acid (B5)**
     - **Source:** Meat, whole grains, avocados
     - **Structure:** Component of coenzyme A
     - **Biological Role:** Synthesis of coenzyme A, fatty acid metabolism
     - **Deficiency:** Rare, but may cause fatigue, irritability
   - **Pyridoxine (B6)**
     - **Source:** Poultry, fish, potatoes
     - **Structure:** Pyridoxal phosphate (PLP) is the active form.
     - **Biological Role:** Amino acid metabolism, neurotransmitter synthesis
     - **Deficiency:** Anemia, peripheral neuropathy
   - **Biotin (B7)**
     - **Source:** Egg yolk, nuts, legumes
     - **Structure:** Biotin is a coenzyme.
     - **Biological Role:** Carboxylation reactions, metabolism of fats and carbohydrates
     - **Deficiency:** Dermatitis, alopecia
   - **Folate (B9)**
     - **Source:** Leafy greens, citrus fruits, beans
     - **Structure:** Tetrahydrofolate (THF) is the active form.
     - **Biological Role:** DNA synthesis, cell division

- **Deficiency:** Megaloblastic anemia, neural tube defects

- **Cobalamin (B12)**
  - **Source:** Animal products (meat, dairy, eggs)
  - **Structure:** Contains cobalt; cyanocobalamin and methyl cobalamin
  - **Biological Role:** Red blood cell formation, nerve function
  - **Deficiency:** Pernicious anemia, neurological issues

2. **Vitamin C (Ascorbic Acid)**
   - **Source:**
     - Citrus fruits, strawberries, bell peppers, broccoli
   - **Structure:**
     - Ascorbic acid is the active form.
   - **Biological Role:**
     - Collagen synthesis, antioxidant, immune function
   - **Deficiency:**
     - Scurvy (bleeding gums, joint pain, anemia)

Bioenergetics:

Thermodynamics: Free energy, entropy, enthalpy

Redox Potential: Oxidation-reduction reactions

High-Energy Compounds: ATP, GTP

Metabolic Pathways:

Glycolysis: Glucose breakdown, ATP production

TCA Cycle: Acetyl-CoA oxidation, energy extraction

Electron Transport System and Oxidative Phosphorylation: ATP synthesis, proton gradient

Definition: Bioenergetics is the study of the transformation of energy in living organisms. It involves understanding how cells harness and utilize energy to perform various functions.

2. Thermodynamics in Biological Systems

Free Energy (G):

- Free energy is the portion of a system's energy that can perform work when temperature and pressure are uniform throughout the system.
- Gibbs Free Energy Change (ΔG): Indicates the spontaneity of a reaction.
  - $\Delta G < 0$: Spontaneous reaction (exergonic)
  - $\Delta G > 0$: Non-spontaneous reaction (endergonic)
  - $\Delta G = 0$: Equilibrium

Entropy (S):

- Entropy is a measure of disorder or randomness in a system.
- Biological systems tend to move towards increased entropy.

Enthalpy (H):

- Enthalpy is the total heat content of a system.
- Change in enthalpy (ΔH) can be used to determine if a reaction is exothermic (releases heat) or endothermic (absorbs heat).

Relationship: $\Delta G = \Delta H - T\Delta S$

3. Redox Potential

Oxidation-Reduction (Redox) Reactions:

- Involves the transfer of electrons between molecules.

- Oxidation: Loss of electrons
- Reduction: Gain of electrons

Redox Potential (E_0):

- The tendency of a molecule to gain or lose electrons.
- Standard redox potential is measured under standard conditions.

4. High-Energy Compounds

ATP (Adenosine Triphosphate):

- ATP is the primary energy carrier in cells.
- Structure: Adenine base, ribose sugar, and three phosphate groups.
- Hydrolysis of ATP releases energy: $ATP + H2O \rightarrow ADP + Pi + Energy$

Other High-Energy Compounds:

- GTP (Guanosine Triphosphate)
- Creatine phosphate

5. Metabolic Pathways

Glycolysis:

- Breakdown of glucose into pyruvate, yielding ATP and NADH.
- Occurs in the cytoplasm.
- Key steps:
    1. Glucose is phosphorylated to glucose-6-phosphate.
    2. Fructose-1,6-bisphosphate splits into two 3-carbon molecules.
    3. ATP is generated through substrate-level phosphorylation.
    4. Pyruvate is the end product.

TCA Cycle (Krebs Cycle/Citric Acid Cycle):

- Oxidation of acetyl-CoA to CO_2, generating NADH, $FADH_2$, and ATP.
- Occurs in the mitochondrial matrix.
- Key steps:
    1. Acetyl-CoA combines with oxaloacetate to form citrate.
    2. Citrate undergoes a series of transformations, releasing CO_2 and generating high-energy electron carriers.

Electron Transport System (ETS) and Oxidative Phosphorylation:

- ETS: Series of protein complexes in the inner mitochondrial membrane that transfer electrons from NADH and $FADH_2$ to oxygen.
- Proton gradient is established across the inner membrane.
- ATP synthase uses the proton gradient to produce ATP from ADP and inorganic phosphate. $ADP + Pi + Energy \rightarrow ATP$

Oxidative Phosphorylation:

- The process of ATP generation coupled with electron transfer to oxygen.

6. Integration of Metabolism

Interconnections:

- Glycolysis, TCA cycle, and ETS are interconnected.
- Metabolic pathways ensure the efficient utilization of energy resources.
- Regulation: Enzymes and hormones regulate these pathways to meet the energy demands of the cell.

UNIT III: Centrifugation, Chromatography, and Electrophoresis

Centrifugation:

1. Basic Principles: Sedimentation, centrifugal force
2. Types: Differential, density gradient

Centrifugation is a technique used to separate particles from a solution according to their size, shape, density, viscosity of the medium, and rotor speed. It uses the centrifugal force generated by spinning the sample at high speeds.

Basic Principles of Sedimentation

Sedimentation:

- The process by which particles settle to the bottom of a liquid and form a sediment.
- Sedimentation rate depends on the force acting on the particles, their size, shape, and density, as well as the viscosity and density of the medium.

Centrifugal Force:

- Formula: $Fc = m \cdot \omega 2 \cdot r$
  - Fc: Centrifugal force
  - mmm: Mass of the particle
  - ω: Angular velocity
  - r: Radius of rotation

Relative Centrifugal Force (RCF):

- Expressed in multiples of gravity (g).
- Formula: $RCF=1.118\times10^{-5}r(RPM)$
  - r: Radius in cm
  - RPM: Revolutions per minute

Types of Centrifugations

1. Differential Centrifugation:

- Principle: Separates based on size and density by progressively increasing centrifugal force.
- Application: Commonly used for cell fractionation, separating cellular components like nuclei, mitochondria, and ribosomes.

2. Density Gradient Centrifugation:

- Principle: Particles are separated based on their density by spinning in a gradient medium (e.g., sucrose, caesium chloride).
- Types:
  - **Rate-Zonal Centrifugation:** Separates particles by size and shape; particles move through a pre-formed gradient at different rates.
  - **Isopycnic (Equilibrium) Centrifugation:** Separates particles solely by density; particles migrate to the point in the gradient where their density matches the gradient density.
- Application: Purification of nucleic acids, viruses, and subcellular organelles.

Instrumentation of Centrifuges

Components of a Centrifuge:

- **Rotor:** The rotating unit that holds the tubes containing the samples. Types include fixed-angle, swinging-bucket, and vertical rotors.
- **Drive Shaft:** Connects the rotor to the motor.
- **Chamber:** Encloses the rotor and samples, often refrigerated to prevent sample heating.
- **Control Panel:** Used to set the speed, time, and temperature.

Types of Centrifuges:

- **Microcentrifuges:** Used for small volumes (0.5-2 mL), typically for DNA/RNA extraction and protein purification.
- **High-Speed Centrifuges:** Handle larger volumes and have higher speeds (up to 30,000 RPM).
- **Ultracentrifuges:** Reach very high speeds (up to 100,000 RPM) for detailed analysis of macromolecules, such as protein and DNA.

Applications of Centrifugation

Biological Applications:

- **Cell Fractionation:** Isolating different cellular components for biochemical analysis.
- **DNA/RNA Purification:** Separating nucleic acids from other cellular debris.
- **Protein Purification:** Isolating proteins based on size and density.

Industrial Applications:

- **Pharmaceuticals:** Purifying drugs and vaccines.
- **Food Industry:** Clarifying fruit juices, separating cream from milk.
- **Environmental Science:** Testing water and soil samples for contaminants.

Chromatography:

1. Principles, Instrumentation, Application:
2. Partition and Absorption Chromatography
3. Paper and Thin Layer Chromatography (TLC)
4. Ion Exchange, Gel Permeation, Affinity Chromatography
5. Advanced Techniques: HPLC, GCMS, LCMS

Chromatography is a laboratory technique for the separation of a mixture into its components. It involves passing a mixture dissolved in a "mobile phase" through a "stationary phase," which separates the analyte to be measured from other molecules in the mixture based on differences in their movement through the stationary phase.

Basic Principles:

- **Stationary Phase:** The phase that does not move and interacts with the components of the mixture.
- **Mobile Phase:** The phase that moves and carries the mixture through the stationary phase.
- **Elution:** The process of washing out a compound through the stationary phase using the mobile phase.
- **Retention Factor (Rf):** A measure of the time a compound spends in the stationary phase relative to the mobile phase.

Types of Chromatography:

1. **Partition Chromatography:**
    - **Principle:** Separation based on differential partitioning between two liquid phases.
    - **Applications:** Separation of amino acids, peptides, and small organic molecules.
2. **Adsorption Chromatography:**
    - **Principle:** Separation based on adsorption onto a solid stationary phase.
    - **Applications:** Separation of dyes, lipids, and vitamins.
3. **Paper Chromatography:**
    - **Principle:** Separation based on differential migration on paper.
    - **Instrumentation:** Chromatography paper, solvent system.
    - **Applications:** Identification of pigments, amino acids, and sugars.

4. **Thin Layer Chromatography (TLC):**
    - **Principle:** Similar to paper chromatography, but uses a thin layer of silica or alumina on a glass plate.
    - **Instrumentation:** TLC plates, developing chamber, UV light for visualization.
    - **Applications:** Monitoring reaction progress, analysing plant extracts, and drug testing.
5. **Ion Exchange Chromatography:**
    - **Principle:** Separation based on charge interactions between analytes and the stationary phase.
    - **Instrumentation:** Ion exchange resins, elution buffers.
    - **Applications:** Purification of proteins, nucleotides, and water treatment.
6. **Gel Permeation Chromatography (Size Exclusion Chromatography):**
    - **Principle:** Separation based on molecular size; larger molecules elute first.
    - **Instrumentation:** Gel filtration columns, appropriate buffer systems.
    - **Applications:** Molecular weight determination, purification of macromolecules like proteins and polysaccharides.
7. **Affinity Chromatography:**
    - **Principle:** Separation based on specific interactions between an immobilized ligand and its target molecule.
    - **Instrumentation:** Affinity columns, ligand-bound stationary phase.
    - **Applications:** Purification of enzymes, antibodies, and receptor proteins.

Advanced Chromatographic Techniques:

1. **High-Performance Liquid Chromatography (HPLC):**
    - **Principle:** High-resolution separation using high-pressure pumps to pass the solvent through a column filled with a finely divided stationary phase.
    - **Instrumentation:** HPLC system (pump, injector, column, detector, data system).
    - **Applications:** Drug analysis, clinical research, food industry quality control.
2. **Gas Chromatography-Mass Spectrometry (GC-MS):**
    - **Principle:** Separation of volatile compounds using gas chromatography followed by identification using mass spectrometry.
    - **Instrumentation:** GC system (injector, column, detector) coupled with a mass spectrometer.
    - **Applications:** Environmental analysis, forensic science, flavor, and fragrance analysis.
3. **Liquid Chromatography-Mass Spectrometry (LC-MS):**
    - **Principle:** Separation of non-volatile and thermally labile compounds using liquid chromatography followed by mass spectrometric detection.
    - **Instrumentation:** LC system (pump, column, detector) coupled with a mass spectrometer.
    - **Applications:** Proteomics, metabolomics, pharmaceutical research.

Electrophoresis:

1. Principles: Electric field, charge-to-mass ratio
2. Types: Agarose gel, polyacrylamide gel (PAGE)
3. Factors Affecting Migration: Voltage, buffer composition
4. Advanced Techniques: 2D Electrophoresis, Isoelectric Focusing

Electrophoresis is a laboratory technique used to separate charged molecules, such as DNA, RNA, or proteins, based on their size and charge. The process involves applying an electric field to move the molecules through a gel or other medium.

Basic Principles of Electrophoresis

1. Electric Field: An electric current is applied across a gel matrix.
2. Migration: Charged molecules move towards the opposite charge (anions to anode, cations to cathode).
3. Separation: Molecules are separated based on size and charge; smaller molecules move faster than larger ones.

Types of Electrophoresis

Agarose Gel Electrophoresis:

- Use: Primarily for DNA and RNA separation.
- Gel: Made of agarose, a natural polysaccharide.
- Resolution: Suitable for larger fragments of nucleic acids.

Polyacrylamide Gel Electrophoresis (PAGE):

- Use: Commonly for protein and small nucleic acids.
- Gel: Made of acrylamide, allows higher resolution.
- Types:
    - Native PAGE: Separates proteins in their native state.
    - SDS-PAGE: Uses sodium dodecyl sulfate to denature proteins, separating them by molecular weight.

Factors Affecting Electrophoretic Migration

1. Molecular Size: Smaller molecules migrate faster.
2. Charge: Higher charge increases migration speed.
3. Gel Concentration: Higher gel concentration slows down migration.
4. Buffer Composition: pH and ionic strength affect molecule charge and gel conductance.
5. Voltage: Higher voltage increases migration speed but can generate heat, affecting results.

Advanced Electrophoresis Techniques

Two-Dimensional Electrophoresis (2D):

- Process: Combines isoelectric focusing (IEF) and SDS-PAGE.
- Use: Separates proteins first by isoelectric point, then by molecular weight.
- Advantage: High-resolution separation of complex protein mixtures.

Isoelectric Focusing (IEF):

- Principle: Proteins are separated based on their isoelectric point (pI).
- Process: A pH gradient is established, and proteins migrate to the pH where their net charge is zero.

Applications of Electrophoresis

1. DNA Analysis:
    - Example: Restriction fragment length polymorphism (RFLP), PCR product analysis.
2. Protein Analysis:
    - Example: Protein purity checks, molecular weight determination, identification of protein isoforms.
3. RNA Analysis:
    - Example: mRNA quality assessment, RNA integrity checks.

UNIT IV: Spectroscopy, Microscopy, and Laser Techniques

Spectroscopy:

Beer-Lambert Law: Absorbance, concentration

Instrumentation:

Photoelectric Calorimeter

UV-Visible Spectrophotometer

Beer-Lambert Law: The Beer-Lambert Law, also known as Beer's Law, describes the relationship between the absorption of light by a substance and its concentration in solution. It is expressed as:

$$A = \epsilon \cdot b \cdot c$$

Where:

- A is the absorbance of the sample.
- ϵ (epsilon) is the molar absorptivity or molar extinction coefficient, which is specific to each substance at a particular wavelength.
- b is the path length of the light through the sample (typically in cm).
- ccc is the concentration of the substance in solution (in mol/L or Molarity).

Key Concepts:

- **Absorbance (A):** The measure of how much light a substance absorbs at a particular wavelength. It is directly proportional to the concentration and path length according to the Beer-Lambert Law.
- **Molar Absorptivity (ϵ\epsilonϵ):** This is a constant for a given substance at a specific wavelength and indicates how strongly the substance absorbs light. Higher ϵ\epsilonϵ values indicate stronger absorption.
- **Path Length (b):** The distance the light travels through the sample. Standard path length in spectrophotometers is typically 1 cm.
- **Concentration (c):** The amount of substance in solution, usually expressed in molarity.

Instrumentation: Photoelectric Calorimeter

Photoelectric Calorimeter: A photoelectric calorimeter is an instrument used to measure the amount of radiant energy absorbed or emitted by a substance. It operates based on the principle of converting light energy into heat energy, which is then measured.

Working Principle:

1. **Absorption of Light:** The substance absorbs incident light, which leads to the promotion of electrons to higher energy levels (excitation).
2. **Heat Generation:** Excited electrons release excess energy as heat when they return to lower energy levels (relaxation).
3. **Calorimetric Measurement:** The heat generated is proportional to the amount of light absorbed. This heat is quantified using a calorimeter, which measures the temperature change induced by absorbed light.

Applications:

- **Chemical Analysis:** Determining concentrations of solutions by measuring light absorption.
- **Environmental Monitoring:** Assessing pollutant levels in air or water samples.
- **Biological Research:** Quantifying biomolecules such as proteins and nucleic acids.

Instrumentation: UV-Visible Spectrophotometer

UV-Visible Spectrophotometer: A UV-Visible spectrophotometer measures the absorption of ultraviolet (UV) and visible light by substances. It is widely used in chemical analysis, biochemistry, and various other fields.

Principle:

1. **Light Source:** UV-Vis spectrophotometers use a light source that emits UV and visible light (usually a deuterium lamp for UV and a tungsten lamp for visible light).
2. **Sample Interaction:** Light passes through a sample, and the amount of light absorbed at specific wavelengths is measured.
3. **Detector:** The detector records the intensity of transmitted light, which is then converted into absorbance values.
4. **Data Analysis:** The absorbance values obtained are used to determine the concentration of the substance in the sample using the Beer-Lambert Law.

Applications:

- **Quantitative Analysis:** Determining concentrations of substances based on their absorbance.
- **Qualitative Analysis:** Identifying substances by their absorption spectra.
- **Biochemical Studies:** Analysing biomolecules such as proteins, nucleic acids, and vitamins.

Microscopy:

1. Types and Design:
2. Compound Microscope
3. Phase Contrast Microscope
4. Fluorescent Microscope
5. Electron Microscopy: TEM, SEM

Definition: Microscopy refers to the technique of using microscopes to observe objects that are too small to be seen with the naked eye.

Importance: Microscopy allows scientists to:

- Study cellular structures and organelles.
- Investigate microorganisms, tissues, and molecular structures.
- Perform detailed analysis in fields like medicine, biology, materials science, and nanotechnology.

Types of Microscopes

Compound Microscope

Principle: A compound microscope uses multiple lenses to magnify the image of a sample. It typically employs visible light to illuminate the specimen.

Components:

- **Objective Lens:** Primary magnifying lens close to the specimen.
- **Eyepiece (Ocular Lens):** Further magnifies the image for the viewer.

Applications: Used in biological sciences for observing cells, tissues, and small organisms.

Phase Contrast Microscope

Principle: Enhances the contrast of transparent and colourless specimens by exploiting differences in refractive index.

Components:

- **Phase Plate:** Introduces phase shifts in light passing through the specimen.
- **Phase Annulus:** Adjusts phase contrast for optimal imaging.

Applications: Useful for viewing live cells and observing intracellular structures without staining.

Fluorescence Microscope

Principle: Excites fluorescent dyes in the specimen with specific wavelengths of light, producing fluorescence.

Components:

- **Excitation Filter:** Selects light wavelength for excitation.
- **Dichroic Mirror:** Reflects excitation light to the specimen and transmits emitted fluorescence.

Applications: Enables visualization of specific molecules (e.g., proteins, DNA) tagged with fluorescent markers.

Electron Microscopy (EM)

Principle: Uses electron beams rather than light to image specimens, achieving much higher resolution.

Types:

- **Transmission Electron Microscope (TEM):** Transmits electrons through thin sections of a specimen to create detailed images.
- **Scanning Electron Microscope (SEM):** Scans a focused electron beam across the specimen's surface, generating 3D images.

Applications: Provides ultra-high resolution for studying subcellular structures, nanoparticles, and materials.

Applications of Microscopy

Biological Applications

- **Cell Biology:** Studying cellular structures, organelles, and processes.
- **Microbiology:** Identifying microorganisms, observing microbial interactions.

Medical Applications

- **Histopathology:** Examining tissue samples for diagnostic purposes.
- **Clinical Microscopy:** Analysing bodily fluids for pathogens or abnormalities.

Materials Science and Nanotechnology

- **Material Characterization:** Analysing surface features, crystal structures, and defects.
- **Nanotechnology:** Imaging nanoparticles, nanostructures, and thin films

Radioisotopes:

Introduction, Measurement: Scintillation counter, autoradiography

Definition: Radioisotopes are unstable forms of elements that decay into more stable forms, emitting radiation in the process.

Types of Radiation:

- **Alpha (α) Radiation:** Consists of helium nuclei (2 protons and 2 neutrons), low penetration power.
- **Beta (β) Radiation:** Electrons or positrons emitted during nuclear decay, moderate penetration power.
- **Gamma (γ) Radiation:** High-energy electromagnetic radiation emitted from the nucleus, very high penetration power.

Applications:

- **Medicine:** Diagnosis (e.g., PET scans) and treatment (e.g., radiotherapy).
- **Research:** Tracing biological processes (e.g., protein synthesis), studying molecular structures.
- **Industry:** Analysing materials (e.g., thickness measurement in manufacturing).

Measurement Techniques

Scintillation Counter

Principle: A scintillation counter detects radiation by converting the energy of incoming particles into flashes of light (scintillations) using a scintillator material.

Components:

- **Scintillator:** Converts radiation energy into light photons.
- **Photomultiplier Tube (PMT):** Amplifies and detects light pulses produced by the scintillator.
- **Analyzer:** Counts and measures the intensity of detected radiation.

Applications:

- **Quantitative Analysis:** Measuring radioactivity levels in samples (e.g., environmental monitoring, nuclear medicine).
- **Particle Detection:** Identifying specific types of radiation (alpha, beta, gamma).

Autoradiography

Principle: Autoradiography visualizes the distribution of radioactive substances within a sample by exposing a photographic emulsion or film to radiation emitted by the sample.

Procedure:

- **Sample Preparation:** Incorporate or apply radioactive material to the sample (e.g., tissue section).
- **Exposure:** Place the sample in contact with a photographic emulsion or film for an appropriate duration.
- **Development:** Process the emulsion or film to reveal areas where radiation was emitted (dark spots).

Applications:

- **Biological Research:** Studying cellular processes (e.g., DNA replication, protein synthesis) by tracking labelled molecules.
- **Medical Imaging:** Using radiolabelled compounds to visualize metabolic activity or receptor binding in tissues.

UNIT V: Biostatistics

Statistical Measures:

Mean, Median, Mode: Central tendency

Standard Deviation: Variability

Statistical Tests:

One-Way ANOVA: Comparing means of three or more groups

Two-Way ANOVA: Interaction between two factors

t-Test: Comparing means between two groups

F-Test: Variance comparison

Chi-Square Test: Independence test

Mean, Median, Mode

Mean:

- **Definition:** The arithmetic average of a set of values.
- **Formula:** $Mean = \sum i = 1nxin$ are individual data points and n is the number of data points.
- **Example:** Calculate the mean height of 5 individuals: 160 cm, 165 cm, 170 cm, 175 cm, 180 cm. $Mean = 160 + 165 + 170 + 175 + 1805 = 170\ cm$

Median:

- **Definition:** The middle value in a sorted list of numbers.
- **Example:** Find the median income from the following dataset: $30,000, $35,000, $40,000, $45,000, $200,000.
    - Arrange in ascending order: $30,000, $35,000, $40,000, $45,000, $200,000.
    - Median = $40,000

Mode:

- **Definition:** The most frequently occurring value in a dataset.
- **Example:** Identify the mode of blood types in a sample of 20 individuals: A, B, AB, O, O, A, B, A, AB, O, O, A, A, B, O, A, B, A, O, O.
    - Mode = O (appears most frequently).

Standard Deviation

Definition: Measures the amount of variation or dispersion of a set of values around the mean.

Formula:

Standard Deviation $(\sigma) = \sum i = 1n(xi - x^{-})2n - 1$

Example: Calculate the standard deviation of ages in a sample: 25, 30, 35, 40, 45 years.

- **Step 1:** Calculate the mean age: $Mean = 25 + 30 + 35 + 40 + 455 = 35\ years$
- **Step 2:** Calculate deviations from the mean: −10, −5,0,5,10-10, -5, 0, 5, 10−10, −5,0,5,10.
- **Step 3:** Square deviations: 100,25,0,25,100100, 25, 0, 25, 100100,25,0,25,100.
- **Step 4:** Sum of squared deviations: 250250250.
- **Step 5:** Divide by n−1n-1n−1 (5-1 = 4) and take the square root
- **Statistical Tests**

One-Way ANOVA (Analysis of Variance)

Definition: Compares means of three or more groups to determine if there are statistically significant differences.

Example: Compare the effectiveness of three different diets (A, B, C) on weight loss in obese individuals.

- **Null Hypothesis (H0):** There is no significant difference in mean weight loss among diets A, B, and C.

- **Alternative Hypothesis (Ha):** There is a significant difference in mean weight loss among diets A, B, and C.

Two-Way ANOVA

Definition: Examines the interaction between two independent categorical variables on a continuous dependent variable.

Example: Investigate the effects of two factors (gender and exercise intensity) on blood pressure reduction in hypertensive patients.

- **Null Hypothesis (H0):** There is no interaction effect between gender and exercise intensity on blood pressure reduction.
- **Alternative Hypothesis (Ha):** There is a significant interaction effect between gender and exercise intensity on blood pressure reduction.

t-Test (Independent Samples)

Definition: Compares means of two independent groups to determine if there is a significant difference between them.

Example: Compare the mean cholesterol levels between patients treated with Drug X and patients treated with Drug Y.

- **Null Hypothesis (H0):** There is no significant difference in mean cholesterol levels between patients treated with Drug X and Drug Y.
- **Alternative Hypothesis (Ha):** There is a significant difference in mean cholesterol levels between patients treated with Drug X and Drug Y.

F-Test (Variance Comparison)

Definition: Tests the equality of variances between two or more groups.

Example: Compare the variance in blood pressure readings between male and female patients.

- **Null Hypothesis (H0):** There is no significant difference in variance of blood pressure readings between male and female patients.
- **Alternative Hypothesis (Ha):** There is a significant difference in variance of blood pressure readings between male and female patients.

Chi-Square Test (Independence Test)

Definition: Tests the independence of two categorical variables.

Example: Assess the association between smoking status (smoker, non-smoker) and lung cancer diagnosis (yes, no) in a sample population.

- **Null Hypothesis (H0):** Smoking status and lung cancer diagnosis are independent.
- **Alternative Hypothesis (Ha):** Smoking status and lung cancer diagnosis are not independent.