

M.SC., BIOCHEMISTRY

I SEMESTER

BC: 1.1: Chemistry of Biomolecules

Unit – 1

Amino acids – classification, structure and physicochemical properties, chemical synthesis of peptides – solid phase peptide synthesis. Proteins – classification, purification, and criteria of homogeneity. Structural organization, sequence determination and characterization of proteins. Conformation of proteins – Ramachandran plots. Denaturation of proteins.

Unit – 2

Classification, chemical properties of carbohydrates, Chemistry and biological roles of homo and heteropolysaccharides, peptidoglycan, glycosaminoglycans, glycoconjugates, glycoproteins, Structural elucidation of polysaccharides; Oligosaccharides – lectin interaction in biochemical processes.

Unit – 3

Classification of Lipids, Fatty acids and their physicochemical properties. Structure and properties of Prostaglandins. Fats and waxes, physicochemical properties and characterization of fats and oil. Structure, properties and biological roles of phospholipids and Sphingolipids. Chemistry and properties of Sterols and Steroids. Salient features of bacterial and plant lipids.

Unit – 4

Nucleic acids – bases, nucleosides, nucleotides, physicochemical properties of nucleic acids, cleavage of nucleic acids by enzymatic methods, non – enzymatic transformation of nucleotides and nucleic acids, methylation, Sequencing, chemical synthesis of DNA. Three dimensional structure of DNA. Different forms of DNA – circular DNA and Supercoiling. Types of RNA. Structure of t-RNA. Nucleotides as regulatory molecules, enzyme cofactors and mediators of chemical energy in cells. Porphyrins – Structure and properties of porphyrins – heme, Chlorophyll and Cytochromes.

BC 1.2: Biochemical Techniques

Unit – 1:

Separation Techniques: Principles, methods and applications of chromatography – Paper, thin layer, ion exchange, gel filtration and affinity chromatography, GLC, HPLC and chromatofocussing.

Unit – 2:

Tissue homogenization. Disruption of tissues and cells, Centrifuges – Principle, applications and types. Differential and density gradient centrifugation. Preparative and analytical ultracentrifuge. Principles and applications of manometry and oxygen electrode, Principle and applications of microscopy, types of microscopes, phase contrast, fluorescent and electron microscopes.

Unit – 3:

Basic Principles of spectroscopy, basic laws of light absorption; instrumentation and applications of UV-visible, IR, ESR, NMR, atomic absorption and Mass spectroscopy, fluorimetry, flame photometry, nephelometry, ORD, CD, X-ray diffraction.

Unit – 4:

Nuclear techniques – nature of radioactivity, detection and measurements of radioactivity, Radio isotopic techniques, Biochemical uses of isotopes. Radiation hazards and methods of radioactive disposal. Principles, methods and applications of electrophoresis, moving boundary electrophoresis, zone electrophoresis, paper, starch, agarose, PAGE, High voltage and Capillary electrophoresis, Isoelectric focusing, two-dimensional electrophoresis, PFGE.

BC 1.3: Physiology and Bioenergetics

Unit – 1:

Composition of blood, erythrocytes, leucocytes, thrombocytes, Coagulation of blood and fibrinolysis. Respiratory organs and mechanism of respiration. Hemoglobin and transport of gases, Physiology of heart, Digestion and absorption of foods. Structure of kidney and nephron. Physiology of kidney. Regulation of electrolyte, water and acid base balance in the body.

Unit – 2:

Structure and organization of muscle cell, types of muscles. Molecular organization of contractile systems and molecular mechanisms of contraction and relaxation of muscle. Biochemical changes associated with muscle contraction and relaxation. Structure of nerve cell, origin of membrane potential, mechanism of propagation of nerve impulse in unmyelinated and myelinated nerve fibers. Synapse – types of synapses, transmission at adrenergic and cholinergic nerve endings. Blood brain barrier, Neurotransmitters. Physiology of vision.

Unit – 3:

Composition and structure of cell membranes, Molecular constituents of membranes, asymmetric organization of lipids and proteins, fluidity of membranes, different membrane models, Membrane channels and pumps, ligand gated ion channels, Ionic channels. Molecular models of transport mechanism, Membrane biogenesis, cell- cell interactions, ionophores, gap junctions, artificial membranes and liposomes.

Unit – 4:

Principles of thermodynamics, free energy, enthalpy and entropy, Free energy changes in biological transformations in living systems. Redox potential, phosphate group transfer potential and ATP, High-energy compounds, oxidation and reduction reactions. Oxidation and reduction enzymes, utilization of oxygen by oxygenases, superoxide dismutase and catalase. Mitochondrial electron transport system – organization of components and importance. Substrate level phosphorylation, oxidative phosphorylation, respiratory control, Mechanism and theories of oxidative

phosphorylation. Respiratory chain inhibitors and uncouplers of oxidative phosphorylation. Mitochondrial electron transport system. Bioluminescence.

BC 1.4: Enzymology

Unit: 1

Classification of enzymes, Remarkable properties of enzymes – catalytic power, specificity. Transformation of different forms of energy. Enzyme localization and assay of enzymes, Units of enzyme activity, Active site – Fisher and Koshland models, formation of enzyme – substrate complex and experimental evidences. Nature of active site, mapping of enzyme active site through chemical procedures and site directed mutagenesis, Factors affecting enzyme activity, Modern concepts of evolution of catalysis, ribozymes, abzyme and synzymes.

Unit – 2:

Kinetics of single substrate enzyme catalyzed reactions, Michaelis – Menten equation, Lineweaver - Burk, Eadie – Hofstee and Hanes plots. Significance of V_{max} , K_m , K_{cat} , specificity constant (K_{cat}/K_m)

Kinetics of multisubstrate reaction – Classification with examples. Rate expression for non-sequential (ping-pong) and sequential (ordered and random) mechanisms. Use of initial velocity, Inhibition

and exchange studies to differentiate between multi substrate reaction mechanisms. Flexibility and conformational mobility of enzymes.

Enzyme inhibition – reversible inhibition – competitive, non-competitive, uncompetitive inhibition; irreversible inhibition, Determination of K_i values

Unit – 3;

Types of reaction catalysis – General acid – base, electrostatic, covalent, intermolecular, metal – ion catalysis, Proximity and orientation.

Mechanism of reaction catalyzed by serine proteases – trypsin and chymotrypsin, carboxypeptidase, lysozyme, triose phosphate isomerase, ribonuclease
Rotational catalysis – ATPase.

Mechanism of catalysis with coenzymes – pyridoxal phosphate, flavin nucleotides, thiamine pyrophosphate, biotin, tetrahydrofolate, lipoic acid.

Unit – 4:

Enzyme regulation – general mechanisms of enzyme regulation. Allosteric enzymes (ATCase). Cooperativity phenomenon. Hill and Scatchard plots. Sigmoidal kinetics and their physiological significance, Symmetric and sequential models of action of allosteric enzymes and their significance. Feedback inhibition and feed forward stimulation., Control of enzymatic activity by products and substrates. Reversible and irreversible activation Isoenzymes, Multifunctional enzymes, Multi – enzyme systems – properties, mechanism of action and regulation of Pyruvate dehydrogenase and Fatty acid synthase complex

PRACTICAL – I

BC 1.5: Biochemical Techniques

Paper chromatography – ascending and descending – separation of amino acids, sugars, purines and pyrimidines. Qualitative tests for their identification.

Thin – layer chromatography of amino acids and lipids.

Column chromatographic separation of plant pigments.

Separation of amino acids by paper electrophoresis.

Polyacrylamide Gel Electrophoresis of serum proteins.

Ion Exchange chromatography of amino acids.

Absorption spectrum of chlorophyll extracted from green leaves.

Absorption spectrum of aromatic amino acids, purines, pyrimidines and heme.

Determination of Molar absorption coefficient of tyrosine.

Optical rotation of glucose and fructose using polarimeter.

Sub – cellular fraction of organelles of liver cells and identification by the marker enzymes.

Affinity Chromatography.

N and C terminal analysis of proteins. (End group analysis of proteins).

Peptide mapping.

Molecular weight of protein by SDS-PAGE

Estimation of proteins by Spectrophotometric method

Density gradient centrifugation – Isolation of rat liver mitochondria.

2- Dimensional electrophoresis of lproteins

Isoelectric focusing

PRACTICAL – II

BC 1.6: Enzymology

Assay of Amylase from saliva

Assay of Acid phosphatase from potato

Assay of Trypsin

Assay of urease from Horse – gram

Assay of Succinate dehydrogenase from the liver

Isoenzymes of LDH – electrophoretic separation and specific staining technique

Time course of enzyme activity

Effect of PH on enzyme activity and determination of optimum PH

Effect of temperature on enzyme activity and calculation of energy of activation.

Effect of substrate concentration on enzyme activity and determination of Michealis constant.

Enzyme inhibition – irreversible inhibition of Papain Or Serine proteases by appropriate inhibitors

Effect of substrate and regulators on allosteric enzyme – Phosphorylase Or ATCase

Enzyme purification by 3 or 4 steps

- a) Acetone precipitation
- b) Ammonium sulphate fractionation
- c) Ion – exchanging chromatography
- d) Gel filtration
- e) Electrophoresis

Effect of metal ions on enzyme ions on enzyme – Alcohol dehydrogenase