

Credits: 4

Class Test -12 Marks
Teachers Assessment
Attendance - 12 Marks
End Semester Exam

Prerequisite: - Biochemistry, Molecular Biology, Microbiology &

Course Objectives:

The objective of this course is to make the students familiar with concepts of Enzyme Kinetics, Inhibition, regulation and specificity.

Detailed syllabus

UNIT I

Enzymology: Introduction, General characteristics of enzymes, Activation of enzymes, active site and its importance, Factors influencing catalytic efficiency, Michaelis-Menten Equilibrium, Henry-Nucgaekkus-Menten's equations, Steady State approach, Michaelis-Menten equation, Velocity vital Substrate concentration curves. **Methods of plotting** Lineweaver-Burk, Hanes-Woolf, Woolf-Augustinsson-Hofstee, Eadie-Scatchard, Advantages and disadvantages of the methods, Comparisons and applications; Integrated form of Michaelis-Menten equation.

UNIT II

Equilibrium dialysis, Scatchard plot for equilibrium binding, Effect of pH on enzyme activity, Effect of temperature on enzyme stability, Arrhenius equation. Formation of reaction intermediates, transient kinetics, flow techniques (continuous, stopped, quenched flow). **mechanistic principles:** Role of proximity effect, bound distortion, multistep catalysis and solvent effects.

UNIT III

Enzyme Inhibition: Models and types of inhibition. **Regulation of enzyme activity:** reversible covalent modification, irreversible covalent modification, allosteric regulation, transcarbamylase, ligand-protein interaction, scatchard plot, Hill plot, cooperative binding, allosteric (MWC, KNF), Half site reactivity.

UNIT IV

Applied enzymology: Application of enzymes in analytical labs (clinical and industrial catalysts, Immobilized enzymes, enzyme electrodes, assay of enzyme activity for various purposes, abzymes, recent developments.

UNIT V

Techniques: X-ray Crystallography. Chemiluminescence & Phosphorescence. Hydrophobic interaction. Centrifugation Sedimentation, partial specific volume and diffusion co-efficient, Purification & Chromatography: Gel filtration, ion-exchange, hydrophobic interaction, hydroxyapatite and affinity chromatography, FPLC HPLC. Molecular spectroscopy, Biomolecular fluorescence complementation assay. Mass spectrometry. Radioisotope labeling, biology, autoradiography, radioactive labeling of biological macromolecules.

MMB201: ENZYME AND TECHNIQUES IN BIOCHEMISTRY

Teaching Scheme Lectures: 4 hrs/Week Credits: 4	Examination Scheme Class Test -12Marks Teachers Assessment - 6Marks Attendance – 12 Marks End Semester Exam – 70 marks
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Prerequisite: - Biochemistry, Molecular Biology, Microbiology & Industrial Applications.

Course Objectives:

The objective of this course is to make the students familiar with concepts of enzyme, Enzyme kinetics, Inhibition, regulation and specificity.

Detailed syllabus

UNIT I Enzymology: Introduction, General characteristics of enzymes, Activation energy, Coupled reactions, active site and its importance, Factors influencing catalytic efficiency. Enzyme kinetics: Rapid Equilibrium, Henry-Nucgaekkus-Menten's equations, Steady State approach, significance of K_m , Haldane equation, Velocity vital Substrate concentration curves. Methods of plotting enzyme kinetics data: Lineweaver-Burk, Hanes-Woolf, Woolf-Augustinsson-Hofstee, $E_{0.5}$ -Scatchard; Advantages and disadvantages of the methods, Comparisons and applications; Integrated form of the Henry-Michaelis-Menten equation.
UNIT II Equilibrium dialysis, Scatchard plot for equilibrium binding, Effect of pH on enzyme stability and activity, Effect of temperature on enzyme stability, Arrhenius equation. Formation of E.S covalent intermediates, transient kinetics, flow techniques (continuous, stopped, quenched), Temp-Jump. General mechanistic principles: Role of proximity effect, bound distortion, multistep catalysis, bi-functional catalysis and solvent effects.
UNIT III Enzyme Inhibition: Models and types of inhibition. Regulation of enzyme activity: Feedback inhibition, reversible covalent modification, irreversible covalent modification, allosteric concept, Aspartate transcarbamylase, ligand-protein interaction, scatchard plot, Hill plot, cooperativity index, Models for allostery (MWC, KNF), Half site reactivity.
UNITIV Applied enzymology: Application of enzymes in analytical labs (clinical and industrial), enzymes as industrial catalysts, Immobilized enzymes, enzyme electrodes, assay of enzyme activities for diagnostic purposes, abzymes, recent developments.
UNIT V Techniques: X-ray Crystallography. Chemiluminescence & Phosphorescence. Hydrodynamic methods, Centrifugation Sedimentation, partial specific volume and diffusion co-efficient, Viscosity. Protein purification & Chromatography: Gel filtration, ion-exchange, hydrophobic interaction chromatography, hydroxyapatite and affinity chromatography, FPLC HPLC. Molecular spectroscopy, IR, ESR, FRET, Biomolecular fluorescence complementation assay. Mass spectrometry. Radioisotope and their use in biology, autoradiography, radioactive labeling of biological macromolecules.