



MANGALORE UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY

MSc Biochemistry

HARDCOREBCH403: ANALYTICAL BIOCHEMISTRY

Total Number of LectureHours: 56
Number of Credits:04

Total

Course objectives

- To understand the use of animal models in various experiments
- To learn different techniques in cell fractionation
- To know various chromatographic techniques
- To study the principle and applications of different electrophoretic and spectroscopic techniques
- To have knowledge of use of isotopes in biochemistry.

Course outcome:

- Students get to learn how to measure errors in estimations
- Various techniques such as cell fractionation, centrifugation, chromatography, electrophoresis, spectroscopy would be known by the student
- Knowledge of isotopes and their applications in biochemistry would be clear.

Unit I

Preliminary techniques in Biochemistry: Animal and plant models, Investigation with isolated organs and tissues, Introduction to animal and plant cell culture. Investigation with microorganism and their mutant (auxotroph), yeast, *Ceanorhabditis elegans*, *Arabidopsis thaliana* and *Drosophila melanogaster* as model specimen for biochemical investigations.

Cell fractionation techniques: Cell lysis, homogenization, extraction, salting in, salting out, dialysis and ultra -filtration. **Centrifugation:** Basic principles of sedimentation, types of centrifuges and rotors. Preparative Centrifugation – Differential and Density gradient, Sub-cellular fractionation, Marker enzyme analysis, Analytical Centrifugation - application and design.

Unit II

Chromatography: Introduction, partition coefficient, Modes of chromatography, liquid and solid phases, paper chromatography and Thin-layer Chromatography

(TLC): Principle, procedure and application, Column chromatography: Basic components, selection of stationary and mobile phase, matrices. Adsorption chromatography (hydroxyapatite and Hydrophobic interaction), Partition (normal phase and reverse phase) Ion exchange (Cation and anion exchange), Gel filtration, affinity chromatography, High performance liquid chromatography (HPLC), Fast protein liquid chromatography (FPLC), Gas liquid chromatography (GLC).

Unit III

14

hrs.

Electrophoretic techniques: Principle, Non-denaturing, denaturing electrophoresis, agarose gel electrophoresis, isoelectric focusing, pulsed field electrophoresis, capillary electrophoresis, Visualizing separated components - staining for proteins and nucleic acids, fluorescence, PAS staining, zymogram.

Spectroscopic techniques: Beer-Lambert's Law and its limitations, Extinction coefficient, Principles & Applications: Colorimeter, UV-Vis Absorption spectroscopy, Fluorescence Spectroscopy, Mass spectrometry, Infrared and Raman Spectroscopy, Nuclear Magnetic Resonance, Electron Spin Resonance, Circular dichroism spectroscopy, X-ray crystallography.

Unit IV

1

4hrs.

Isotopes in Biochemistry: Isotopes, Types of radioactive decay, Units of radioactivity, Interaction of radioactivity with matter, Detection and measurement of radioactivity: Methods based on gas ionization (Geiger-Muller counter), Excitation (Scintillation counting) and Photographic methods. Specific activity, commonly used isotopes (Tritium, Carbon-14, Phosphorus-32, Sulfur-35, Iodine-131), Advantages and restriction of radiotracer experiments, safety aspects, Applications of radioisotopes in biological sciences.

REFERENCES:

1. Freifelder D. M. Physical Biochemistry- Application to Biochemistry and Molecular Biology, 2nd ed., W.H. Freeman, 1982.
2. Principles and Techniques of Biochemistry and Molecular Biology, ed., Keith Wilson & John Walker, March 2010, Cambridge Univ. Press.
3. West & Todd. Biochemistry. 4th ed., Oxford and IBH.
4. Upadhyay and Upadhyay. Biophysical Chemistry
5. USA

