

## MST201: ANALYTICAL TECHNIQUES

<p><b>Teaching Scheme</b> Lectures: 4 hrs/Week</p> <p>Credits: 4</p>	<p><b>Examination Scheme</b> Class Test -12Marks Teachers Assessment - 6Marks Attendance – 12 Marks End Semester Exam – 70 marks</p>
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**Course Objectives:** The objective of this laboratory course is to introduce students to experiments in biochemistry. The course is designed to teach students the utility of set of experimental methods in biochemistry in a problem oriented manner.

### Unit I

**Basic Techniques** Buffers; Methods of cell disintegration; Enzyme assays and controls; Detergents and membrane proteins; Dialysis, Ultrafiltration and other membrane techniques

**Spectroscopy Techniques** UV, Visible and Raman Spectroscopy; Theory and application of Circular Dichroism; Fluorescence; MS, NMR, PMR, ESR and Plasma Emission spectroscopy

### Unit II

**Chromatography Techniques** TLC and Paper chromatography; Chromatographic methods for macromolecule separation - Gel permeation, Ion exchange, Hydrophobic, Reverse-phase and Affinity chromatography; HPLC and FPLC; Criteria of protein purity

**Electrophoretic techniques** Theory and application of Polyacrylamide and Agarose gel electrophoresis; Capillary electrophoresis; 2D Electrophoresis; Disc gel electrophoresis; Gradient electrophoresis; Pulsed field gel electrophoresis

### Unit III

**Centrifugation** Basic principles; Mathematics & theory (RCF, Sedimentation coefficient etc); Types of centrifuge - Microcentrifuge, High speed & Ultracentrifuges; Preparative centrifugation; Differential & density gradient centrifugation; Applications (Isolation of cell components); Analytical centrifugation; Determination of molecular weight by sedimentation velocity & sedimentation equilibrium methods.

### Unit IV

**Microscopic Techniques:** History, basic types of light microscopy and their applications in brief; Simple, compound, inverted, stereo, fluorescence, dark field and bright field microscope. Phase contrast microscopy: Amplitude and phase objects, wave terminology, positive or dark phase contrast and negative or bright phase contrast microscopy. Electron microscopy: Transmission Electron Microscope and Scanning Electron Microscope, sample preparation for EM, basic concept of confocal microscope.

### Unit V

**Advanced Techniques:** Protein crystallization- X-ray diffraction and X-ray crystallography and their application. **Mass Spectrometry:** Theory and methods; Different components of a mass spectrometer, types of ionization techniques and types of mass analyzers. MALDI-TOF. Mass precision, mass measurement accuracy, mass resolution, ionization energy and appearance energy.

### Texts/References

1. Freifelder D., Physical Biochemistry, Application to Biochemistry and Molecular Biology, 2nd Edition, W.H. Freeman & Company, San Francisco, 1982.
2. Keith Wilson and John Walker, Principles and Techniques of Practical Biochemistry, 5th Edition, Cambridge University Press, 2000.
3. D. Holme & H. Peck, Analytical Biochemistry, 3rd Edition, Longman, 1998.
4. R. Scopes, Protein Purification - Principles & Practices, 3rd Edition, Springer Verlag, 1994.
5. Selected readings from Methods in Enzymology, Academic Press.