Programme: M. Sc. (Biochemistry)

Course Code: **BCC 402** **Title of the Course:** Analytical Biochemistry-I

Number of Credits: 3

Effective from AY: 2020-21

Prerequisites for the course:	Students should have studied the theory/ instrumentation and application of some of the basic analytical techniques. It is assumed that students have a basic knowledge of fundamentals in biochemistry.	
Course Objectives:	1. Introduction of various bioanalytical techniques for analysis. 2. Evaluate the utility of various analytical techniques as a qualitative and quantitative tool. 3. This course develops concepts in techniques used for routine biochemical work such as chromatography, spectrophotometry, centrifugation, microscopy, electrophoresis.	
Course Outcomes:	1. Students should be in a position to differentiate between various analytical techniques based on their theory and sensitivity achieved. 2. Explain the principles of various techniques and apply the knowledge of the techniques for designing various experiments in research and development.	
Content:	1. **Acid, bases and buffers:** concept of ph, eh, acid-base associations, buffers, buffering capacity, mechanism of dissociation of macromolecules, dissociation constants, pka, pi, solvents (eluotropic series), peroxide values, solubility and affinity constants. 2. **Centrifugation:** Principle of centrifugation, concepts of RCF, different types of instruments and rotors, preparative, differential and density gradient centrifugation, analytical ultra-	**6 h** **5 h**

	centrifugation, determination of molecular weights and other applications, subcellular fractionation.	
	3. **Electrophoretic techniques:** Principles of electrophoretic separation. Types of electrophoresis including paper, cellulose, acetate/nitrate and gel. Slab gel, tube, Continuous and discontinuous, etc; Gel electrophoresis - types of gel, Agarose GE, Polyacrylamide gel electrophoresis PAGE, SDS- PAGE, Isoelectric Focusing and ampholytes, 2-D, native, gradient gels, PFGE, DGGE, TGGE. Capillary electrophoresis-instrumentation, sample introduction in CE, types of CE methodology, electrophoretic mobility and electroosmatic mobility, total mobility, efficiency and resolution in CE column. Separation of neutral molecule by MEKC. Staining strategies and procedures: Coomassie Brilliant blue R/G 250, Silver, Fluorescent stains Flamingo, Oriole, SYPRO-Ruby; Stain-free gels.	**8 h**
	4. Separation techniques: Solvent extraction: Basic principle, types of extractions and application. Separations based on a partitioning between phases based on chemical nature and polarity of analyte. Dialysis: Principles, and applications of equilibrium dialysis and ultrafiltration. Artificial membranes, semi-permeable membranes, Donnan membrane equilibrium, and biological significance of osmosis and micelles.	**7 h**
	5. **Chromatographic techniques:** Basic principles and application of thin-layer, paper chromatography, column chromatography, HPLC, GC, separation matrixes - Ion-exchange, Affinity, Molecular exclusion and Adsorbtion (hydrophobic interaction chromatography, DNA cellulose chromatography, MAK hydroxyl-apatite chromatography). Concept of mobile phases;	**10h**

	gradient elution (concave, convex and linear) and stationary phases	
Pedagogy:	Lectures (online or physical)/ tutorials/ seminars/ term papers/assignments/ presentations/ self-study or a combination of some of these. Sessions shall be interactive in nature to enable peer group learning.	
Text Books/ References / Readings	1. Wilson K, Walker J; Principles and Techniques of Practical Biochemistry; Cambridge University Press; 2010/ 7th Edition 2. Christian G. D., Dasgupta P. K , Schug K. A; Analytical Chemistry; John Wiley & Sons; 2013/ 7th Edition 3. Norris J. R., Ribbons D.W.; In Methods in Microbiology; Academic Press; 1971/1st Edition. 4. Parakhia M. V., Tomar, R. S., Patel S., Golakiya B. A.: Molecular Biology and Biotechnology: Microbial Methods; New India, 2010 5. Homes D. J., Peck H; Analytical Biochemistry; Pearson education Limited; 1998. 6. Douglas A. Skoog, F. James Holler, Stanley R. Crouch, Principles of Instrumental Analysis; Cengage Learning 2016/ 7th Edition.	