Name of the Programme: M.Sc. Part-I (Biochemistry)

Course Code: CHB-501 **Title of the Course:** Analytical Biochemistry-I

Number of Credits: 4

Effective from AY: 2022-23

Pre-requisites for the Course:	Students should have graduate level knowledge either in chemical or life sciences or should have qualified change of discipline test.	
Course Objectives:	1. To introduce various bioanalytical techniques for separation and purification of biomolecules. 2. To develop concepts in techniques used for routine biochemical work such as chromatography, spectrophotometry, centrifugation, microscopy, electrophoresis. 3. To evaluate the utility of various analytical techniques as a qualitative and quantitative tool.	
Content:		**No of hours**
	1. General principles of analytical biochemistry a. Selection of valid methods for analysis, Instrumental methods, physiological methods, assessment of analytical methods. b. Quality assurance in analytical biochemistry: quality control and quality assessment, c. Accreditation of laboratories: standard operating procedure and good laboratory practice, sampling for analysis, calibration and graphical representation of data.	4
	2. Acid, bases and buffers a. Units used in quantitative biochemical measurements: molarity, normality, parts per million and percentage by weight/ volume, concept of pH using pH electrode and other ion selective electrodes., Eh, acid-base associations. b. Buffers, buffering capacity, measurement of pH, mechanism of dissociation of macromolecules, dissociation constants, pKa, pI, solvents (eluotropic series), peroxide values, solubility and affinity constants.	10
	3. Colligative Properties a. Definitions, Factors affecting and Physiological Applications of Osmosis. b. Measurement of osmotic pressure, Osmoregulation, Adsorption, Colloids, Surface Tension and Viscosity. c. Numerical Problems based on above concepts.	4
	4. **Centrifugation:** a. Principle of centrifugation, concepts of RCF, different types of instruments and rotors. b. Preparative, differential and density gradient centrifugation, analytical ultra-centrifugation. c. Determination of molecular weights and other applications, subcellular fractionation.	8
	5. Electrophoretic techniques:	10

	a. Principles of electrophoretic separation, Types of electrophoresis including paper, cellulose, acetate/nitrate and gel (introduction to concepts of slab gel, tube, continuous and discontinuous, etc). b. 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. c. Capillary electrophoresis - instrumentation, sample introduction in CE, types of CE, electrophoretic mobility and electroosmotic mobility, total mobility, efficiency and resolution in CE column. d. Separation of neutral molecules by MEKC. e. Staining strategies and procedures: Coomassie Brilliant blue R/G 250, Silver, Fluorescent stains Flamingo, Oriole, SYPRO-Ruby; Stain-free gels. f. Examples of separation of biomolecules by electrophoresis.	
	6. Solvent extraction a. Basic principle, types of extractions and application. b. Separations based on a partitioning between phases based on chemical nature and polarity of analyte. c. Introduction to Soxhlet apparatus, solid phase extraction, microwave assisted extraction, ultrasound assisted extraction, counter current extraction.	5
	7. Dialysis a. Principles and applications of equilibrium dialysis and ultrafiltration. b. Dialysis and Concentration, reverse dialysis. c. Artificial membranes, semi-permeable membranes, Donnan membrane equilibrium. d. Biological significance of osmosis and micelles.	5
	8. Chromatographic techniques: a. Introduction to chromatography: definitions, theories, principle of chromatographic technique, terms and parameters used in chromatography, classification of chromatographic methods, concept of mobile phases; gradient elution (concave, convex and linear) and stationary phases. b. Basic principles, instrumentation and application of thin-layer, paper chromatography, column chromatography, HPLC, GC, ion-exchange chromatography, affinity chromatography, molecular exclusion chromatography and adsorption chromatography. c. Special chromatographic techniques for nucleic acids: DNA cellulose chromatography, MAK hydroxyl-apatite chromatography. d. Introduction to Supercritical-Fluid Chromatography and hyphenated techniques like LCMS, GCMS.	14
Pedagogy:	Mainly lectures and tutorials. Seminars / term papers /assignments / presentations / self-study or a combination of some of these can also be used.	

	ICT mode should be preferred. Sessions should be interactive in nature to enable peer group learning.
References/ Readings:	1. K. Wilson, J. Walker, Principles and Techniques of Practical Biochemistry; Cambridge University Press, 7th Edition, 2010. 2. G. D. Christian, P. K .Dasgupta, K. A. Schug, Analytical Chemistry, John Wiley & Sons, 7th Edition, 2013. 3. M. V. Parakhia, R. S. Tomar, S. Patel, B. A. Golakiya, Molecular Biology and Biotechnology, Microbial Methods, New India, 2010. 4. D. J. Homes, H. Peck, Analytical Biochemistry,Pearson education Limited,1998. 5. A. Skoog Douglas, F. James Holler, Stanley R. Crouch, Principles of Instrumental Analysis, 7th Edition, Cengage Learning, 2016. 6. D. J. Holme., H. Peck, Analytical Biochemistry, 3rd Edition, Prentice Hall 1998.
Course Outcomes:	1. Students will be able to explain the principles of various separation techniques 2. Students will be in a position to differentiate between various analytical techniques for separation and purification of biomolecules based on their principles 3. Students will be able to choose appropriate separation technique and isolate and purify biomolecules. 4. Students will be able to apply the knowledge of these techniques for designing various experiments in research and development