Name of the Programme: M. Sc -I (Analytical Chemistry)

Course Code: CHA-503 **Title of the course:** Separation Techniques

Number of Credits: 04

Effective from AY: 2022-23

Prerequisites for the course:	Students should have studied analytical chemistry courses at M.Sc. Chemistry in semester I	
Course Objective:	1. Introduction of various separation techniques. 2. Evaluate the use of chromatographic techniques for chemical analysis.	
Content:	**1. Basic Separation Technique:** General aspects of separation techniques-role of separation technique in analysis; separating the analyte from interferents, general theory of separation efficiency: separation factor. Classifying separation techniques: Separations based on size; separations based on mass or density, separations based on complexation reactions (Masking); separations based on a change of state; separations based on partitioning between phases. (Note: Following techniques shall be discussed as representative example) Basic principles of distillation; theory of vacuum, steam, azeotropic and fractional distillation. Fractionation by solvent extraction: based on chemical nature and based on polarity of analyte. Membrane techniques: dialysis, reverse osmosis, ultrafiltration. Centrifugation techniques: Sedimentation velocity, Sedimentation equilibrium, analytical and preparative centrifugation, differential centrifugation, density gradient centrifugation; applications in separation.	No of hours 10
	2. Chromatographic Methods: Introduction to chromatography: Principle of chromatographic technique, terms and parameters used in chromatography, classification of chromatographic methods, partition versus adsorption chromatography, qualitative and quantitative analysis by chromatography; Planar Chromatography (Paper and thin layer): Paper Chromatography: Principle, types (ascending, descending, circular, two dimensional paper chromatography), choice of solvent, adsorbents, multiple development, qualitative and quantitative measurement applications.	30

	Thin Layer Chromatography (TLC): Principle; efficiency of thin layer plates, methodology (technique), criteria for selection of stationary and mobile phases (numerical to calculate elution strength of mixed solvents used as mobile phase), choice of adsorbents, preparation of plates, spotting (spot capacity), development of chromatogram, identification and detection using physical and chemical methods, reproducibility of Rf values and improving resolution, Two-dimensional TLC, comparison of TLC with paper chromatography and column chromatography, thin layer ionophoresis and electrophoresis, qualitative, quantitative evaluation and applications. High-performance TLC (HPTLC): Introduction, theory, classification (classical, high performance, ultra, preparative HPTLC), difference between TLC and HPTLC with respects to the parameters, scanning densitometer, quantitative analysis and applications. Column Chromatography: Introduction, types (conventional, flash, LPLC, Dry column vacuum chromatography), principle, packing, loading, eluting and collecting eluent in the column chromatography and experimental requirements, theory of development, migration rates of solutes, band broadening, resolution and column efficiency, variables that affect column efficiency, van Deemter equation, qualitative and quantitative analysis, numericals and applications. Gas Chromatography (GC): Instrumentation, selection of operating condition, carrier gases, stationary phases, choices of GC column, temperature selection, sampling techniques, methods to prepare derivatives of samples (silylation, acylation, alkylation), factors affecting separation, working principle of GC detectors such as TCD, ECD, FID, quantification methods such as normalizing peak area, internal std., external std, standard addition, advances in GC, hyphenated techniques; GC-FTIR, GC-MS. Analysis of data obtained using GC chromatogram, GC-MS. Liquid-Liquid Partition Chromatography: HPLC Introduction, selection of stationary and mobile phase, types of bonded phase chromatography-NPC and RPC and stationary phases used, reversed phase partition chromatography, steps in HPLC method development in partition chromatography, elution techniques (isocratic and gradient), ion pairing agents, buffer agents, organic modifiers, optimization of capacity factor, gradient selectivity factor and column plate numbers, numericals on method development using Snyder's polarity index, advances in LC, Preparative vs analytical HPLC, Chiral	

	chromatography- Pirkle stationary phases, examples of enantiomer separation such as ibuprofen, calculation of enantiomeric excess. Choosing detectors- working principle of RI, UV-Vis, conductivity and ELSD, hyphenated techniques; LC-MS. Analysis of chemical data obtained using HPLC chromatogram, LC-MS. application of HPLC method development in food analysis/drugs, etc.	
	3. Other Chromatographic Methods: Size Exclusion Chromatography: Principle, types, stationary phases in gel chromatography, physical and chemical characteristics of gel, mechanism of gel permeation chromatography (GPC), instrumentation of GPC, applications of GPC- determination of molecular weight of polymer with numericals. Supercritical-Fluid Chromatography: Introduction, important properties of supercritical-fluids, instrumentation and variables, SFC column vs other column, applications and data analysis. Affinity Chromatography: Principle, affinity matrix, ligands, mobile phase, separation mechanism, application in the separation of proteins, etc. Ion Exchange Chromatography: Introduction, mechanism of separation, types of stationary phases, factor affecting separation; Ion exclusion chromatography; separation mechanism- Donnan theory, application in the separation of alkaloids, carboxylic acids etc.	10
	4. Electrophoresis: Theory of electrophoresis, Types- Free solution and supporting medium electrophoresis, paper electrophoresis, capillary electrophoresis and gel electrophoresis. Capillary electrophoresis- Instrumentation, sample introduction in CE, types of CE methodology, electrophoretic mobility and electroosmotic mobility, total mobility, efficiency and resolution in CE column, numericals. Gel electrophoresis - types of gel, Polyacrylamide gel electrophoresis PAGE, Agarose GE, SDS-PAGE, 2D Gel electrophoresis, factors affecting separation; Capillary Electrochromatography. Separation of neutral molecule by MEKC; Separation and determination of Vitamin B-complex by using CZE and MEKC. Staining and detecting electrophoresis band.	10
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. G. D. Christian, Analytical Chemistry, 6th Ed.; John Wiley, 2004. 2. D. A. Skoog, D. M. West, F. J. Holler, S. R. Crouch, Fundamentals of Analytical Chemistry, 9th Ed.; Cengage Learning, 2014. 3. David. Harvey, Modern Analytical Chemistry, 1st Ed.; The McGraw-Hill, 2000. 4. L. R. Snyder, J. J. Kirkland, J. W. Dolan, Introduction to modern liquid chromatography, 3rd Ed.; John Wiley & Sons, 2009. 5. H. H. Willard, L. L. Merritt, J. A. Dean, F. A. Settle, Instrumental methods of Analysis, 7th Ed.; CBS Publishing, 1986. 6. G. H. Jeffery, J. Bassett, J. Mendham, R. C. Denney, Vogel's Text Book of Quantitative Chemical Analysis, 5th Ed.; John Wiley, 1989. 7. H. Gunzler, A. Williams, Handbook of analytical techniques, 1st Ed.; Wiley, 2002. 8. F. W. Fifield, D. Kealey, Principles and Practice of Analytical Chemistry, 5th Ed.; Blackwell Science Ltd., 2000. 9. A. Braithwaite, F. J. Smith, Chromatographic methods, 5th Ed.; Kluwer academic publishers, 1999. 10. J. Inczedy, Analytical Applications of Ion Exchangers, 1st Ed.; Oxford Pergamon Press, 1966.
Course outcomes:	1. Students will be able to select the separation techniques for purification of analytes from interferents. 2. Students will be able to analyse data and interpret chromatogram. 3. Students will be able to perform qualitative and quantitative estimation using HPLC data. 4. Students will understand and will be able to apply various chromatographic techniques.

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