

CA S 515: SEPARATION TECHNIQUES

Course Outcomes

- Enable the students demonstrate the fundamentals of different solvent extraction techniques and their applications in separation.
- Illustrate the principles, separation mechanisms and applications of affinity chromatography of chromatography.
- Explain the basic concepts, Instrumentation, methodology, separation and identification of compounds by electrophoresis technique.
- Understand the theory, instrumentation and applications of GC and HPLC.
- Demonstrate the principle of GFC and GPC techniques used in isolation, purification, identification and analysis of macromolecules.
- Learn applications of ultracentrifugation in purification and characterization of biomolecules.
- Acquire a critical knowledge of capillary electrophoresis and Capillary electro chromatography for the separation of organic, inorganic and natural products.

UNIT-I

[12 Hours]

Solvent Extraction: Definition, Types, Principle and Efficiency of extraction. Sequence of extraction process. Factors effecting extraction-pH, Oxidation state, Modifiers, Synergistic, Masking and salting out agents. Techniques-Batch and continuous extraction; Applications.

Affinity Chromatography: Definitions. Separation mechanism - Matrices, Matrix activation, Role of spacer arms and applications.

Electrophoresis: Theory and classification; Factors influencing the mobility-Macromolecular size and Charge, Interaction with supporting electrolyte, pH and Concentration discontinuities. Factors affecting electrophoretic phenomena-Electrolysis, Electroosmosis, Temperature and Supporting media, Instrumentation; Methodology-Preparation of gels-Staining and Destaining, Applications.

UNIT-II

[12 Hours]

High Pressure Liquid Chromatography (HPLC): Apparatus, pumps, column packing, characteristics of liquid chromatographic detectors-UV, IR, refractometer & fluorescence detectors, Advantages & Applications.

Exclusion Chromatography: Theory & principle of size exclusion chromatography. Experimental techniques for gel filtration chromatography (GFC) and gel-permeation Chromatography (GPC), Materials for packing-Factors governing column efficiency, Methodology and Applications.

Gas Chromatography (GC): Principle, comparison of GSC and GLC; Instrumentation Columns-Packed and tubular; Study of detectors-Thermal conductivity, Flameionization, Electron capture and Mass spectrometry, Factors affecting separation, Applications of GC/MS technique.

UNIT-III

[12 Hours]

Ultra centrifugation: Principle, Sedimentation constant, Sedimentation equilibrium, Sedimentation velocity, Methodology and Applications.

Capillary electrophoresis: Overview, Types, The basis for electrophoretic separations, Migration rates and Plate heights, Electro-osmotic flow, Instrumentation, Capillary zone electrophoresis, Capillary gel electrophoresis, Capillary isoelectrophoresis, Capillary isoelectric focusing.

Capillary electro-chromatography: Packed column electro-chromatography, micellar electrokinetic capillary chromatography and applications.

References

1. Chromatography, Part A and Part B, 5th edn., E. Heftman, Elsevier (1992).
2. Chromatography Today, D. F. Poole and S. K. Poole, Elsevier (1991).
3. Principles of Instrumental Analysis, 7th ed., D. A. Skoog, F. J. Holler and S. R. Crouch, Cengage India Pvt. Ltd., (2020).
4. Principles of Instrumental Analysis, 4th ed., D. A. Skoog and D. M. West, Saunders College Publications (1992).
5. Quantitative Analysis, 5th edn., R. A. Day and A. L. Underwood, Prentice-Hall (1998).
6. Analytical Chemistry, 5th edn., G. D. Christian, John Wiley and Sons (2001).
7. Instrumental Methods of Analysis, 7th edn., H. H. Willard, L. L. Merritt and J. A. Dean, CBS Publishers, New Delhi (1988).
8. Instrumental Methods of Chemical Analysis, 19th edn., B. K. Sharma, Goel, 2000.