

SEMESTER IV

11.1.10. Course Code: DSC 10: ANALYTICAL CHEMISTRY4 (AC4)

Course Title: SEPARATION METHODS-II

Total Credits: 04 (Credits: Theory-02, Practical-02)

(Total Lectures: Theory- 30, Practical-60)

Objectives: Objective of this course is to learn the separation techniques and its application.

Learning Outcomes:

By the end of this course, students will be able to learn:

- Various types of separation techniques and their applications
- Electrophoretic techniques

Unit 1: Column Chromatography

A. General: columns, matrix materials, stationary phase, column packing, application of sample, column development and sample elution, detectors and fraction collectors, applications.

B. High performance liquid chromatography: Principle, column, matrices and stationary phases, column packing, mobile phase and pumps, application of sample, detectors, applications. **C. Adsorption chromatography:** Principle, adsorbents, solvents, nature of solute, operating parameters, retention volumes and times, applications.

D. Liquid-liquid partition, chromatography: Principle, normal phase chromatography, reversed phase liquid chromatography, applications.

E. Ion-exchange chromatography: Principle, ion exchangers, ion-exchange equilibria, ion-exchange resin selectivity, column operations (column development, detection of solute bands), factors affecting retention volumes, applications.

F. Gel chromatography: Principle, types of gels, separation by gel chromatography, applications.

(Lectures: 20)

Unit 2: Electrophoretic Techniques:

A. Principle, apparatus, support media (paper, cellulose acetate membranes, gels)

B. SDS-PAGE, native gels, gradient gels, isoelectric focusing, 2D-PAGE, continuous flow electrophoresis, detection, estimation and recovery of proteins in gels.

C. Western Blotting, Electrophoresis of Nucleic Acids, Capillary Electrophoresis.

D. Isoelectric Focusing.

(Lectures: 10)

PRACTICALS (Credits: 02, Laboratory Periods: 60)

1. Determination of the residual chlorine in city water supply using colorimetry (*Take at least two samples*).
2. Determination of adsorption isotherm of acetic acid on activated charcoal and determination of the adsorption constant (k).
3. Determination of the capacity of at least two anion exchange resins *e.g.* Amberlite type II (Dimethyl-2-hydroxyethylbenzyl ammonium-based and Amberlite type I trialkyl ammonium-based, DOWEX type II, etc).
4. Determination of the capacity of at least two cation exchange resins *e.g.* DOWEX-50 (sulphonic acid based), sodium polystyrene sulfonate, Amberlite-sulphonic acid based, etc).
5. To remove the hardness of the water by using ion exchange resins.
6. To separate Ni (II) and Zn (II) by ion exchange resins and quantify it by complexometric titration.
7. Determination of the solubility of CaSO₄ by ion exchange and complexometric titrations.

8. Separation of compounds using adsorption column chromatography.
 - (a) Separation of the mixture of *o*-nitro phenol and *p*-nitro phenol.
 - (b) Separation of the mixture of dyes (methylene blue and methyl orange).
 - (c) Separation of the mixture of *o*-nitro aniline and *p*-nitro aniline.
9. Determination of the void volume of a gel column.
10. Visit to Water Purification Plants.

REFERENCES:

- Mikes, O. (2000), Laboratory Handbook of Chromatographic methods, D. Van Nostrand Company Inc.
- Fifield, F.W.; Kealey, D. (2000), Principles and Practice of Analytical Chemistry, Wiley.
- Mendham, J.; Denney, R.C.; Barnes, J.D.; Thomas, M.J.K.; (2000), Vogel's Quantitative Chemical Analysis, Prentice Hall.
- Wilson, K.; Walker, J. (2000), Principles and Techniques of Practical Biochemistry, Cambridge University Press. Additional Resources:
- Holme, D.J.; Peck, H. (1998), Analytical Biochemistry, Prentice Hall.
- Freifelder, D. (1983), Physical Biochemistry, W.H. Freeman & Company.
- Plummer, D.T. (2001), Introduction to Practical Biochemistry, McGraw-Hill.

Additional Resources:

- Holme, D.J.; Peck, H. (1998), Analytical Biochemistry, Prentice Hall.
- Freifelder, D. (1983), Physical Biochemistry, W.H. Freeman & Company.

Teaching Learning Process:

- Conventional chalk and board teaching.
- Class interactions and discussions

Assessment Methods:

- Class Tests at Periodic Intervals.
- Written assignment (s) / Presentation by individual students
- End semester University Theory and Practical Examination

Keywords: Column Chromatography. Electrophoretic Techniques