Department of Chemistry
Dumkal College
Basantapur, Dumkal
Murshidabad

STUDY MATERIALS

CHROMATOGRAPHY

YASIN NUREE
Department of Chemistry
Dumkal College

Chromatography

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1. Definition of Chromatography

Chromatography is a physical method of separation in which the components of a mixture are distributed between two phases:

- **Stationary phase –** fixed in place
- **Mobile phase –** moves over the stationary phase

The separation occurs because different components travel at different rates due to differences in adsorption, partition, solubility, or affinity.

2. General Principles of Chromatography

Basic Principle

When a mixture is placed in contact with two phases:

- One phase remains **stationary**
- The other phase **moves**

Components separate because of:

- Differences in **adsorption**
- Differences in **partition coefficient**
- Differences in **molecular size**
- Differences in **affinity**

Important Terms

1. Stationary Phase

Solid or liquid fixed on a solid support.

2. Mobile Phase

Liquid or gas that moves through the stationary phase.

3. Adsorption

Adhesion of molecules onto the surface of solid.

4. Partition Coefficient (K)

$$K = \frac{\text{Concentration in stationary phase}}{\text{Concentration in mobile phase}}$$

5. Retention Factor (Rf value)

In planar chromatography:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Rf value ranges from **0 to 1.**

3. Classification of Chromatography

1. **Based on Principle**
    - Adsorption chromatography

    - Partition chromatography
    - Ion exchange chromatography
    - Gel filtration chromatography
2. **Based on Mobile Phase**
    - Gas chromatography (GC)
    - Liquid chromatography (LC)
3. **Based on Technique**
    - Column chromatography
    - Paper chromatography
    - Thin Layer Chromatography (TLC)

4. Paper Chromatography

Definition

Paper chromatography is a type of **partition chromatography** in which cellulose paper acts as the stationary phase and a suitable solvent acts as the mobile phase.

Principle

Separation is based on **partition of solutes between water (held in paper fibers) and moving solvent**.

Types of Paper Chromatography

1. Ascending
2. Descending
3. Radial (Circular)
4. Two-dimensional

Procedure (General)

1. Draw pencil line near bottom of paper.
2. Apply sample using capillary tube.
3. Place paper in solvent chamber.
4. Allow solvent to rise.
5. Remove and dry.
6. Visualize spots (using reagents if required).
7. Calculate Rf value.

Applications

- Separation of amino acids
- Separation of metal ions
- Separation of plant pigments
- Forensic analysis

5. Thin Layer Chromatography (TLC)

Definition

TLC is a type of **adsorption chromatography** where a thin layer of adsorbent (silica gel or alumina) is coated on a glass/aluminum plate.

Principle

Based on **differential adsorption** of components between:

- Stationary phase (silica/alumina)
- Mobile phase (solvent)

Procedure

1. Prepare TLC plate.
2. Apply sample spot.
3. Place in developing chamber.
4. Allow solvent to rise.
5. Remove and dry.
6. Visualize under UV or spray reagent.
7. Calculate Rf value.

Advantages of TLC

- Faster than paper chromatography
- Better separation
- Requires small sample
- High sensitivity

Applications

- Checking purity
- Monitoring reaction
- Drug analysis
- Separation of metal ions

6. Paper Chromatographic Separation of Fe^{3+} and Al^{3+}

Aim

To separate Fe^{3+} and Al^{3+} ions using paper chromatography.

Principle

Fe^{3+} and Al^{3+} form complexes with suitable reagents and move differently due to differences in partition coefficient.

Materials Required

- Whatman filter paper
- $FeCl_3$ solution
- $AlCl_3$ solution
- Solvent: Butanol : Acetic acid : Water (4:1:5)
- Spray reagents:
  - Potassium ferrocyanide (for Fe^{3+})
  - Aluminon reagent (for Al^{3+})

Procedure

1. Draw baseline with pencil.
2. Apply mixture of Fe^{3+} and Al^{3+}.
3. Place paper in solvent chamber.
4. Allow solvent to rise about 3/4 height.
5. Remove and dry.
6. Spray with detecting reagents:
   - $Fe^{3+} \rightarrow$ Blue colour with potassium ferrocyanide.
   - $Al^{3+} \rightarrow$ Red lake with aluminon.

Observation

Two separate spots appear at different heights.

Calculate Rf:

$$Rf = \frac{\text{Distance travelled by metal ion}}{\text{Distance travelled by solvent}}$$

Result

Fe^{3+} and Al^{3+} are separated due to difference in their partition behaviour.

7. Comparison: Paper Chromatography vs TLC

Feature	Paper Chromatography	TLC
Principle	Partition	Adsorption
Stationary Phase	Cellulose paper	Silica gel / Alumina
Speed	Slow	Fast
Resolution	Moderate	High
Sensitivity	Less	More

8. Advantages of Chromatography

- Simple
- Accurate

- Small sample required
- High resolving power
- Widely applicable

Types of Chromatography

1. Column Chromatography

Definition

Column chromatography is a type of **adsorption chromatography** in which the stationary phase (silica gel/alumina) is packed in a glass column and the mobile phase (solvent) flows through it.

Principle

Separation is based on **differential adsorption** of components of a mixture on the stationary phase.

- More strongly adsorbed → moves slowly
- Less strongly adsorbed → moves faster

Components of Column Chromatography

1. **Column** – Glass tube
2. **Stationary Phase** – Silica gel / Alumina
3. **Mobile Phase** – Suitable solvent or solvent mixture
4. **Eluent** – Solvent used for separation

Procedure

1. **Preparation of Column**
    - Plug cotton at bottom.
    - Add sand layer.
    - Pack silica/alumina (wet or dry method).
    - Add thin layer of sand on top.
2. **Loading Sample**
    - Dissolve mixture in minimum solvent.
    - Pour carefully over stationary phase.
3. **Elution**
    - Add solvent.
    - Collect fractions separately.
4. **Detection**
    - Use TLC to check separated fractions.

Applications

- Separation of organic compounds
- Purification of natural products
- Separation of dyes
- Pharmaceutical analysis

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2. Ion-Exchange Chromatography

Definition

Ion-exchange chromatography is a method of separation based on **exchange of ions between solution and an insoluble ion-exchange resin**.

Principle

Ion exchange resin contains:

- Fixed charged groups
- Mobile counter ions

When solution passes through resin:

$$\text{Resin–X} + \text{Y}^+ \rightarrow \text{Resin–Y} + \text{X}^+$$

Types of Ion Exchange Resins

1. Cation Exchange Resin

- Contains acidic groups ($–SO_3H$, $–COOH$)
- Exchanges H^+ or other cations
- Example: Dowex-50

2. Anion Exchange Resin

- Contains basic groups ($–NH_3^+$)
- Exchanges OH^- or other anions
- Example: Dowex-1

Classification

Type	Functional Group	Exchange Ion
Strong Acid Cation	$–SO_3H$	H^+
Weak Acid Cation	$–COOH$	H^+
Strong Base Anion	$–NR_3^+$	OH^-
Weak Base Anion	$–NH_2$	OH^-

Applications

- Water softening
- Separation of rare earth elements
- Purification of proteins
- Removal of heavy metals
- Pharmaceutical industry

3. Determination of Ion Exchange Capacity (Batch Method)

(Used when column method is not feasible)

Definition

Ion Exchange Capacity (IEC) is the number of milliequivalents (meq) of ions exchanged per gram of dry resin.

$$\text{IEC} = \frac{\text{milliequivalents of ions exchanged}}{\text{weight of dry resin (g)}}$$

Unit: meq/g

A. Determination of Cation Exchange Capacity (Batch Method)

Principle

Cation exchange resin in H^+ form exchanges H^+ with Na^+ ions from NaCl solution. The released H^+ is titrated with NaOH.

Chemicals Required

- Cation exchange resin (H^+ form)
- Standard NaCl solution
- Standard NaOH solution
- Phenolphthalein indicator
- Distilled water

Procedure

1. Take known weight (~1 g) of dry resin.
2. Add 100 mL of standard NaCl solution.
3. Shake for 1–2 hours.
4. Filter solution.
5. Titrate liberated H^+ with standard NaOH.

Reaction

$$\text{Resin–H} + \text{NaCl} \rightarrow \text{Resin–Na} + \text{HCl}$$

HCl formed is titrated:

$$\text{HCl} + \text{NaOH} \rightarrow \text{NaCl} + \text{H}_2\text{O}$$

Calculation

$$\text{IEC} = \frac{N \times V}{W}$$

Where:

- N = Normality of NaOH
- V = Volume of NaOH used (mL)
- W = Weight of dry resin (g)

B. Determination of Anion Exchange Capacity (Batch Method)

Principle

Anion exchange resin in OH^- form exchanges OH^- with Cl^- from NaCl. The liberated OH^- is titrated with standard HCl.

Chemicals Required

- Anion exchange resin (OH^- form)
- NaCl solution
- Standard HCl
- Methyl orange indicator

Reaction

$$\text{Resin–OH} + \text{NaCl} \rightarrow \text{Resin–Cl} + \text{NaOH}$$

NaOH formed is titrated:

$$\text{NaOH} + \text{HCl} \rightarrow \text{NaCl} + \text{H}_2\text{O}$$

Calculation

$$\text{IEC} = \frac{N \times V}{W}$$

Unit: meq/g

4. Advantages of Batch Method

- Simple
- No column required
- Suitable for undergraduate practical
- Requires less apparatus

5. Viva Questions (Important for Practical Exam)

1. Define ion exchange capacity.
2. Why is resin converted to H^+/OH^- form before experiment?
3. What is the unit of IEC?
4. Why is shaking necessary?
5. Difference between strong and weak ion exchange resin?
6. Why is NaCl used?
7. What happens if resin is not washed properly?

6. Important Points for Exam

✓ Resin must be dried before weighing

✓ Remove excess electrolyte by washing

✓ Use standard solutions

✓ Avoid air bubbles

7. Recommended Books (B.Sc. Honours Level)

1. Vogel's Textbook of Quantitative Chemical Analysis
2. B.K. Sharma – Instrumental Methods of Chemical Analysis

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3. Skoog, Holler & Crouch – Principles of Instrumental Analysis
4. Willard, Merritt, Dean & Settle – Instrumental Methods of Analysis