Unit-V
Separation techniques-II

Paper Chromatography:

1. Principle:

Paper chromatography is a form of partition chromatography in which the stationary phase is the thin layer of water bound to cellulose fibers in filter paper, and the mobile phase is a suitable solvent or solvent mixture. When a mixture is applied, its components separate because those more soluble in the mobile phase travel faster, while those with greater affinity for the stationary phase move more slowly.

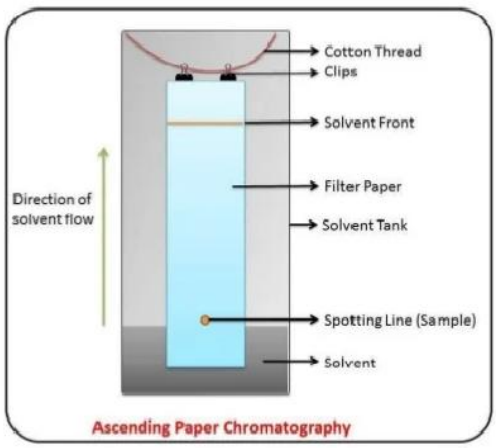
2. Experimental Procedure:

1) Take a strip/sheet of chromatographic paper.
2) Draw a light pencil line (~2 cm from the bottom) → baseline.
3) Apply small drops of the sample solution on the baseline using a capillary tube.
4) Place the paper in a chromatography chamber containing a shallow layer of solvent (ensuring spots are above the solvent Take level).
5) Allow the solvent to rise by capillary action (or flow down in descending type).
6) When solvent reaches near the end, remove the paper and mark the solvent front.
7) Dry the paper and visualize spots by:
   - UV light
   - Iodine vapors
   - Spraying with detecting reagents (ninhydrin, etc.)
8) Calculate R_f values for identification.

$$R_f = \frac{Distance\,traveled\,by\,solute}{Distance\,traveled\,by\,solvent\,front}$$

Types of Paper Chromatography:

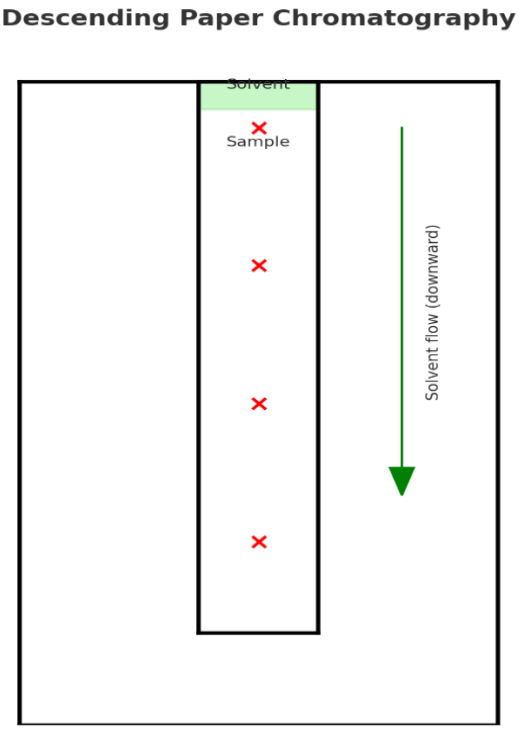
(a) Ascending Chromatography



1. The filter paper is clipped at the top and suspended vertically inside a closed chamber.
2. A spotting line is marked near the bottom, above the level of the solvent, where the sample is applied.

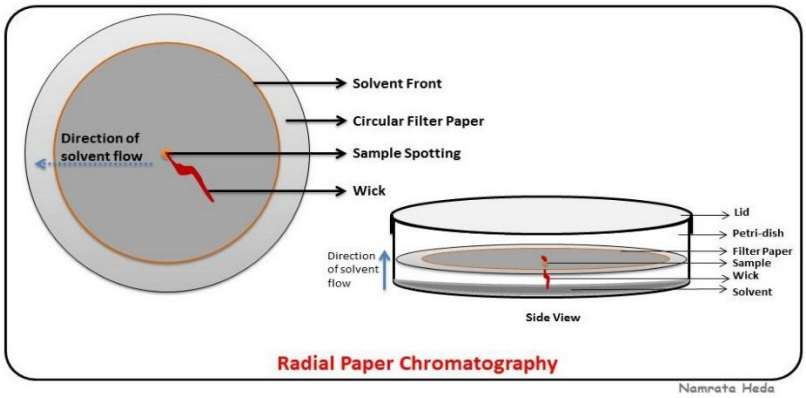
3. The solvent (mobile phase) is placed at the bottom of the chamber in the solvent tank.
4. As the experiment proceeds, the solvent rises upward through the paper by capillary action.
5. The sample components dissolve in the solvent and travel at different speeds depending on their affinity for the stationary phase (water bound in paper fibers) and their solubility in the mobile phase.
6. The solvent front (the farthest point reached by the solvent) is marked, and the separated spots can later be visualized.

(b) Descending Chromatography



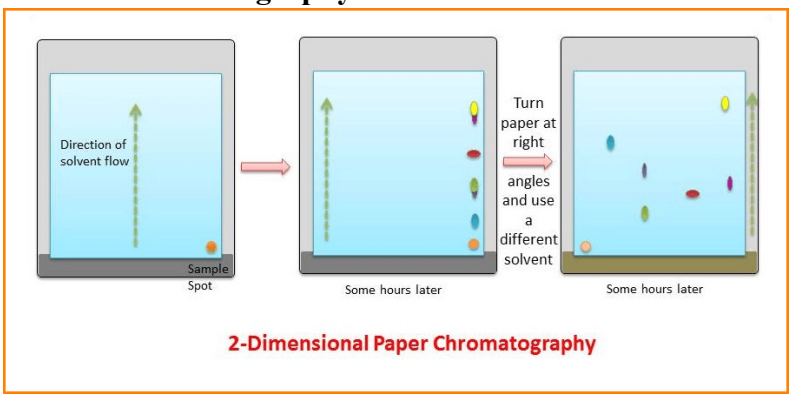
1. The filter paper is clipped at the top with a solvent trough above it.
2. The sample is spotted near the top of the paper.
3. The solvent flows downward due to gravity and capillary action, carrying the sample components at different rates.
4. The separated spots appear along the length of the paper as the solvent moves down.

(c) Radial (Circular) Chromatography



1. Circular filter paper is placed inside a Petri dish.
2. A sample spot is applied at the center of the paper.
3. A wick (small strip of paper) connects the solvent in the dish to the center of the filter paper.
4. The solvent rises through the wick and spreads radially outward across the paper.
5. As the solvent front moves outward in all directions, it carries the components of the sample, which separate into circular zones around the center.
6. The side view shows the arrangement inside the Petri dish: solvent at the bottom, wick dipping into it, and circular filter paper on top.

(d) Two-Dimensional Chromatography



1. The sample is first spotted at one corner of square paper.
2. In the first run, the solvent moves upward, separating components in the vertical direction.
3. The paper is dried, then rotated 90°, and developed again in a different solvent, separating components in the horizontal direction.
4. Final result: a two-dimensional pattern of spots, giving better resolution for complex mixtures (e.g., amino acids).

Applications of Paper Chromatography

1. **Biochemistry and Biology**
   a. Separation and identification of amino acids, sugars, nucleotides, and plant pigments (chlorophylls, carotenoids, anthocyanins).
   b. Useful in studying metabolic pathways and photosynthesis.
2. **Pharmaceutical Industry**
   a. Quality control of drugs and formulations.
   b. Detection of impurities, degradation products, and adulterants in medicines.
3. **Food Industry**

a. Identification of preservatives, sweeteners, artificial colors, and contaminants in food and beverages.
b. Ensures food quality and safety standards.

4. **Forensic Science**
   a. Examination of inks, dyes, poisons, drugs, and explosives in criminal investigations.
   b. Used for document verification and toxicological analysis.
5. **Clinical and Medical Applications**
   a. Detection of abnormal metabolites in body fluids (e.g., aminoaciduria, phenylketonuria).
   b. Helps in diagnosing metabolic disorders and monitoring treatment**.**

Column Chromatography

1. Principle

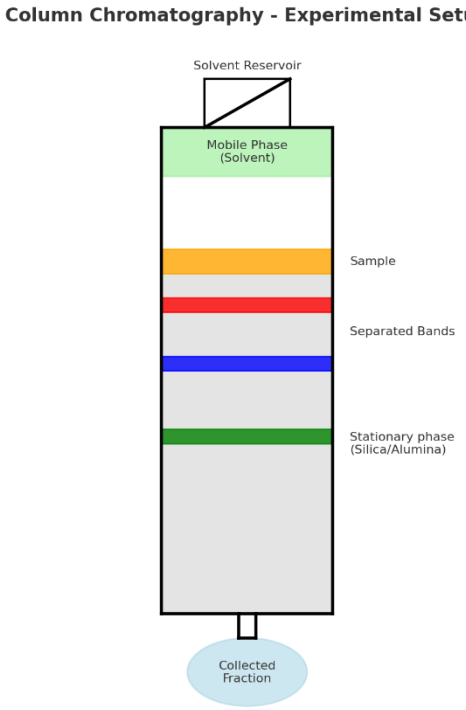
Column chromatography is based on the principle of differential adsorption. The stationary phase is a solid adsorbent (commonly silica gel or alumina) packed into a vertical glass column, and the mobile phase is a liquid solvent or mixture of solvents that flows through it. When a mixture is introduced, its components interact differently with the stationary phase: those with stronger adsorption move slowly, while those more soluble in the mobile phase move faster, leading to separation into distinct bands as they travel down the column.

2. Classification

Column chromatography can be classified based on the separation mechanism:

1. **Adsorption Column Chromatography:** separation based on differences in adsorption of solutes on the solid adsorbent.
2. **Partition Column Chromatography:** separation based on distribution between stationary liquid phase and mobile liquid phase.
3. **Ion-Exchange Column Chromatography:** uses charged stationary groups to separate cations or anions.
4. **Gel Filtration (Size-Exclusion) Column Chromatography:** separation based on molecular size; larger molecules elute first.
5. **Affinity Column Chromatography:** based on specific binding interactions (e.g., antigen–antibody, enzyme–substrate).

3. Experimental Procedure



1. Preparation of Column

A clean glass column is filled with a slurry of adsorbent (silica gel/alumina) in a suitable solvent. The column is packed uniformly without air bubbles.

2. Application of Sample

The mixture to be separated is dissolved in a minimum quantity of solvent and applied carefully on top of the column.

3. Elution

The mobile phase (eluent) is allowed to flow through the column. Components move at different rates depending on their interaction with stationary and mobile phases.

Elution can be:

- Isocratic elution – single solvent throughout.
- Gradient elution – solvent polarity gradually increased.

4. Collection and Detection

Eluted fractions are collected in test tubes or flasks. The separated compounds are detected by color, UV absorption, or chemical tests.

4. Applications

1. Separation and purification of natural products such as alkaloids, glycosides, and plant pigments.

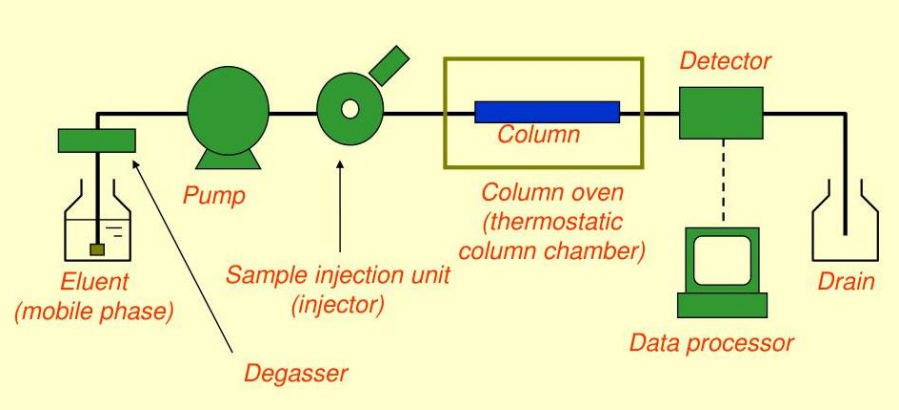
2. Isolation of pure compounds from synthetic reaction mixtures.
3. Pharmaceutical industry: purification of drugs, vitamins, and antibiotics.
4. Biochemical analysis: separation of proteins, amino acids, nucleotides, and enzymes.
5. Environmental studies: detection of pollutants, pesticides, and toxins.
6. Industrial use: purification of chemicals, dyes, and essential oils.

High Performance Liquid Chromatography (HPLC)

1. Principle

High Performance Liquid Chromatography (HPLC) separates components of a mixture based on their differential partitioning between a stationary phase (packed column) and a mobile phase (liquid under high pressure). Compounds with stronger interaction with the stationary phase are retained longer, while those with weaker interaction elute faster. The use of high-pressure pumps ensures rapid, high-resolution separation even for complex mixtures.

2. Instrumentation (Block Diagram & Components)



Main Components

1. **Eluent & Degasser**: Stores the mobile phase and removes dissolved gases to prevent bubbles.
2. **Pump**: Delivers the solvent at high pressure and constant flow.
3. **Injector**: Introduces the sample into the mobile phase stream.
4. **Column (with Oven)**: The core part where separation occurs as compounds interact differently with stationary and mobile phases.
5. **Detector**: Senses separated compounds as they elute and converts response to an electronic signal.
6. **Data Processor**: Produces a chromatogram showing peaks for each compound.

7. **Drain**: Eluent and separated compounds are directed to drain or collected in fractions.

3. Applications of HPLC

1. **Pharmaceutical Industry**
    - Quality control of drugs and formulations.
    - Determination of drug purity, stability, and degradation products.
    - Bioavailability and pharmacokinetic studies.
2. **Biochemistry and Biotechnology**
    - Separation of amino acids, peptides, proteins, nucleotides, and vitamins.
    - Analysis of enzymes and metabolites.
3. **Food Industry**
    - Detection of preservatives, colorants, sweeteners, and contaminants.
    - Analysis of natural products like caffeine, sugars, and organic acids.
4. **Environmental Analysis**
    - Detection of pesticides, herbicides, and pollutants in water and soil.
    - Monitoring toxic compounds in effluents.
5. **Forensic Science and Clinical Research**
    - Analysis of drugs of abuse, toxins, and poisons.
    - Blood and urine analysis for therapeutic drug monitoring.