CHAPTER 3: CHROMATOGRAPHY

1. Principles of Chromatography:

- Chromatography is a separation technique based on the differential distribution of components between two phases: a stationary phase and a mobile phase.
- It relies on differences in adsorption, partition, and solubility of the components.
- Key principles include:
  - Adsorption Chromatography: Separation based on the ability of a substance to adhere to the surface of the stationary phase.
  - Partition Chromatography: Separation based on the differential solubility of compounds between two immiscible phases.

2. Mobile and Stationary Phases

- Mobile Phase: The solvent or gas that carries the mixture through the stationary phase (e.g., water, ethanol, helium).
- Stationary Phase: The fixed phase that selectively retains the components (e.g., silica gel, cellulose, alumina).
- The choice of phases affects the separation efficiency and resolution.

3. Types of Chromatographic Techniques

- Paper Chromatography (PC): Uses cellulose paper as the stationary phase, ideal for separating pigments and amino acids.
- Thin Layer Chromatography (TLC): Uses a thin layer of adsorbent material (e.g., silica) on a glass or plastic plate.
- Column Chromatography (CC): Involves a vertical glass column filled with a stationary phase for separating bulk mixtures.
- High-Performance Liquid Chromatography (HPLC): A highly efficient liquid chromatography method using high pressure.
- Gas Chromatography (GC): Uses an inert gas as the mobile phase, suitable for volatile and thermally stable compounds.

1. Paper Chromatography:

Principle:

Separation is based on the differential partitioning of compounds between the mobile and stationary phases.

More soluble compounds travel farther, while less soluble ones stay closer to the origin.

Materials:

1)Chromatography paper (e.g., Whatman filter paper)

2)Solvent (e.g., water, ethanol)

3)Sample mixture (e.g., plant pigments, ink)

4)Capillary tube or micropipette

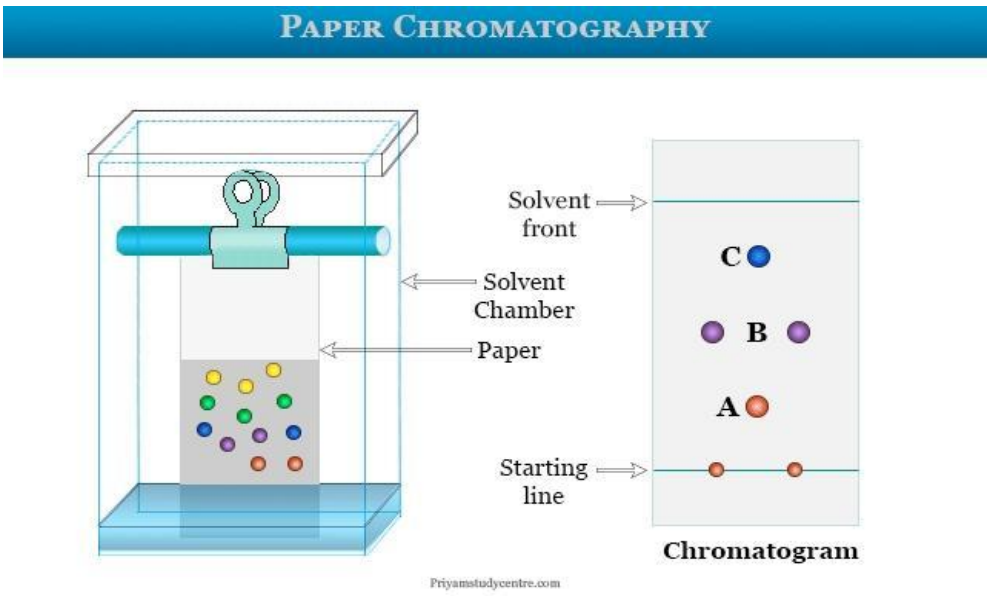
5)Beaker or chromatography chamber

6)Pencil, ruler, and forceps

Procedure:

1. **Sample Preparation:** Apply a small drop of the sample on the chromatography paper near the bottom.
2. **Marking the Baseline:**Draw a pencil line 1-2 cm from the bottom where the sample is applied.
3. **Solvent Preparation:** Add the mobile phase (solvent) to the chamber, ensuring it is below the baseline.
4. **Paper Placement:** Hang or roll the paper into the chamber without touching the sides.
5. **Development:** Allow the solvent to move up the paper, separating the components.
6. **Stopping the Run:** Remove the paper when the solvent front is near the top and mark it.
7. **Drying and Observation:** Let the paper dry completely and observe the separated spots.
8. **Calculating Rf Values:** Measure distances and calculate Rf values.

Diagram:



Applications:

1.Separation of plant pigments – To identify different pigments like chlorophyll, carotenoids, and xanthophylls.

2. Amino acid analysis – To separate and identify amino acids in a mixture.

3. Detection of drugs and poisons – Used in forensic science to detect substances in biological samples.

4. Analysis of food products – To detect adulterants or components like dyes and additives.

5. Separation of mixtures in pharmaceuticals – To check the purity of compounds

2.Thin layer chromatography:

Principle:

TLC is based on the separation of components in a mixture due to their different affinities between a stationary phase (usually a solid adsorbent) and a mobile phase (a solvent). Components travel at different rates on the stationary phase because of differences in adsorption and solubility, leading to their separation.

Materials:

1. TLC plate

2. Developing chamber

3. Solvent or mobile phase

4. Capillary tubes or micropipettes

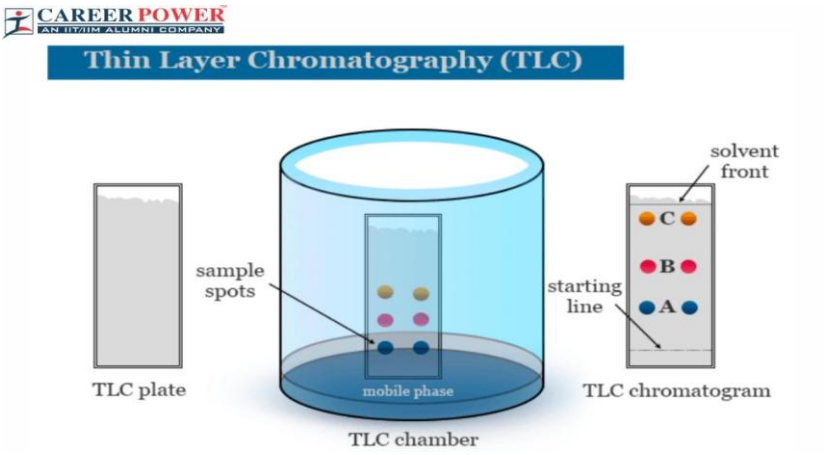
5. UV lamp or iodine chamber

Procedure:

1. Draw a light pencil line about 1 cm from the bottom of the TLC plate.
2. Use a capillary tube to put a small drop of your sample on the pencil line.
3. Let the spot dry completely.

4. Pour a small amount of solvent into a developing chamber (make sure it's below the pencil line).
5. Place the TLC plate upright inside the chamber without touching the solvent with the spot.
6. Close the chamber and let the solvent move up the plate by capillary action.
7. When the solvent front reaches near the top, remove the plate.
8. Mark the solvent front with a pencil immediately.
9. Allow the plate to dry.
10. Observe the separated spots under UV light or use a chemical spray if needed.
11. Measure the distances to calculate the Rf values.

Diagram:



Applications:

1.Identification of compounds in mixtures.

2. Checking purity of a substance.

3. Separation of plant pigments like chlorophyll and carotenoids.

3.Column chromatography:

Principle:
Column chromatography separates components of a mixture based on their different adsorption affinities between a stationary phase (usually a solid like silica gel or alumina packed in a column) and a mobile phase (a liquid solvent). Components move through the column at different speeds, leading to their separation.

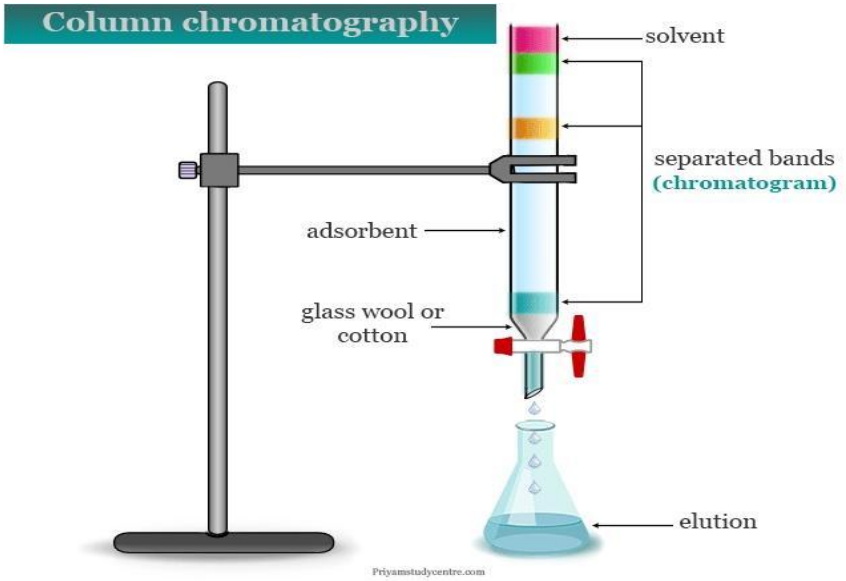
Materials:

1. **Glass column** – for packing the stationary phase.
2. **Stationary phase** – usually silica gel or alumina powder.
3. **Mobile phase (eluent)** – suitable solvent or solvent mixture.
4. **Sample solution** – mixture to be separated.
5. **Beaker or flask** – to collect the eluted fractions.
6. **Cotton or glass wool** – to plug the bottom of the column and hold the stationary phase.
7. **Funnel** – for pouring solvents and sample.

Procedure:

1. Plug the bottom of the glass column with cotton or glass wool to prevent the stationary phase from leaking out.
2. Fill the column with the stationary phase (silica gel or alumina) using a slurry method with the mobile phase solvent. Make sure no air bubbles remain.
3. Allow the stationary phase to settle and pack tightly.
4. Add a small layer of sand on top to protect the stationary phase surface.
5. Carefully apply the sample solution on top of the packed column without disturbing the layer.
6. Pour the mobile phase solvent slowly into the column to start elution.
7. Collect the eluted fractions in separate containers as they come out.
8. Monitor the fractions (using TLC or color changes) to identify separated components.
9. Continue elution until all components are separated.

Diagram:



Applications:

1. Isolation of antibiotics and other pharmaceuticals.

2. Separation of proteins, enzymes, and biomolecules in biochemistry.

3. Purification of organic compounds in chemical synthesis.

4.. Analysis of complex mixtures in forensic and environmental samples.

4.Gas Chromatography:

Principle:
Gas chromatography separates volatile components of a mixture based on their distribution between a mobile gas phase (carrier gas) and a stationary liquid or solid phase inside a column. Components with different boiling points and affinities travel at different speeds, leading to their separation.

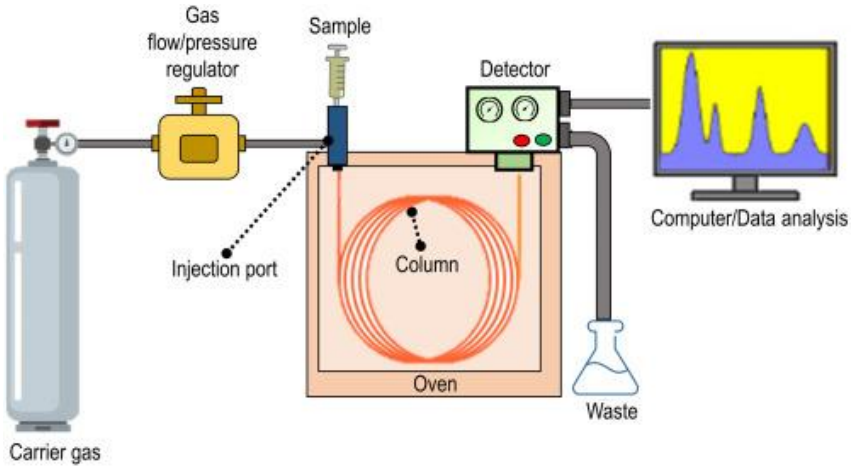
Materials:

1. **Carrier gas** (mobile phase) – usually helium, nitrogen, or hydrogen.
2. **Injection port** – to introduce the sample into the system.
3. **Column** – coated with stationary phase, placed inside an oven.
4. **Oven** – to control the temperature of the column.
5. **Detector** – to identify and measure the separated components (e.g., Flame Ionization Detector, Thermal Conductivity Detector).
6. **Recorder or computer** – to record the chromatogram output.

Procedure:

1. Prepare the sample (usually liquid or gas).
2. Inject a small amount of sample into the heated injection port.
3. Carrier gas (e.g., helium) carries the vaporized sample through the column.
4. Components separate inside the column based on their interaction with the stationary phase and boiling points.
5. Separated components reach the detector one by one.
6. The detector sends signals to the recorder/computer to produce a chromatogram.
7. Analyze the chromatogram to identify and quantify the components.

Diagram:



Applications:

1.Analysis of volatile organic compounds in air, water, and soil samples.

2. Quality control in pharmaceuticals to check purity and composition.

3. Detection of drugs and toxins in forensic science.

4. Testing food and beverages for flavor compounds, preservatives, and contaminants

5.High performance liquid chromatography:

Principle:
HPLC separates components of a mixture based on their different interactions with a stationary phase (packed inside a column) and a liquid mobile phase that is pumped through under high pressure. Components move at different rates, leading to separation.

Materials:

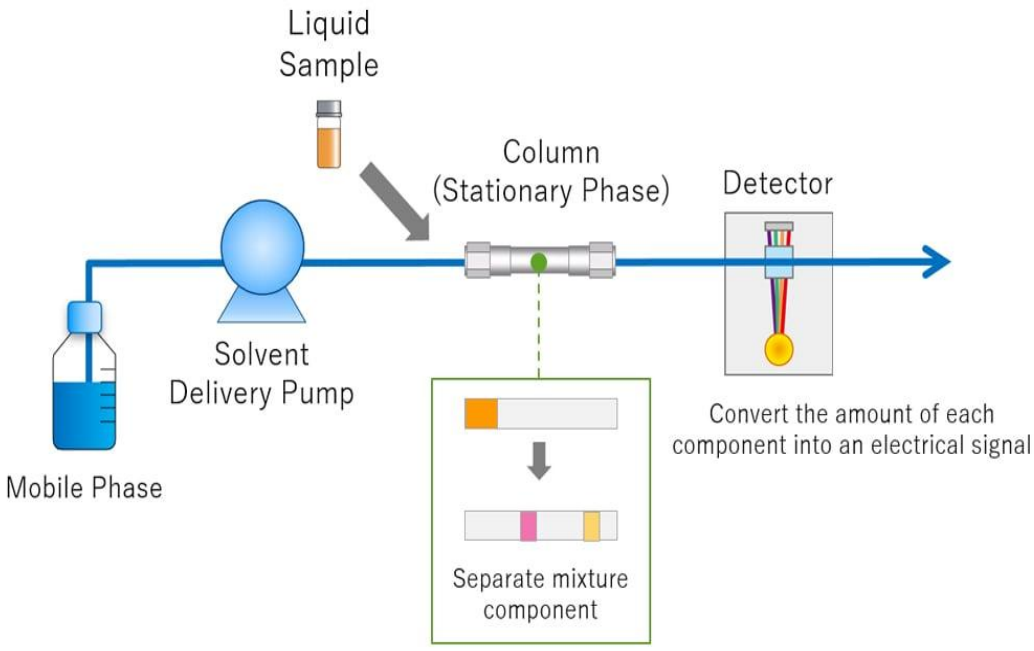
1. **Solvent reservoir** – contains the mobile phase (liquid solvent).

2. **Pump** – to push the mobile phase through the column at high pressure.
3. **Injector** – to introduce the sample into the mobile phase flow.
4. **Column** – packed with stationary phase particles (like silica).
5. **Detector** – to detect separated components (e.g., UV detector).
6. **Data processor/recorder** – to record and analyze the output chromatogram.

. Procedure:

1. Prepare the mobile phase (solvent) and degas it to remove air bubbles.
2. Fill the solvent reservoir with the mobile phase.
3. Turn on the pump to deliver the mobile phase through the column at high pressure.
4. Inject a small volume of the sample into the injector.
5. The sample passes through the column where components separate based on their interaction with the stationary phase.
6. The separated components reach the detector, producing signals.
7. The signals are recorded and displayed as a chromatogram.
8. Analyze the chromatogram to identify and quantify components.

Diagram:



Applications:

1. Environmental analysis – detecting pollutants in water and soil.

2. Biochemistry – separating and analyzing proteins, nucleotides, and amino acids.

3. Forensic science – detecting drugs and toxic substances.

4. Clinical diagnostics – measuring blood or urine components.