UNIT-5

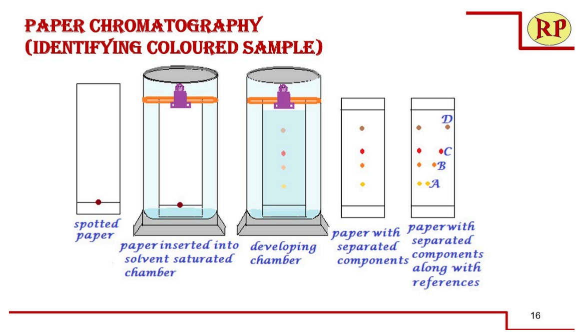
Separation techniques-2

Syllabus - 9 hours

Paper chromatography- Principle, experimental procedure, ascending, descending, radial and Two-dimensional applications. Column chromatography - Principle, classification, experimental procedure and applications.HPLC- Principle, Instrumentation – block diagram and applications.

Paper Chromatography:

Paper chromatography is a type of partition chromatography where the separation of components in a mixture is based on their differential solubility and mobility between two phases: a stationary phase (the paper) and a mobile phase (the solvent). The paper used in this method is typically made of cellulose, which contains water molecules bound in its structure, making it both a stationary and a support medium.



Principle:

The fundamental principle of paper chromatography is partitioning between the mobile and stationary phases. When a solvent (mobile phase) moves through the paper, it carries the dissolved substances with it. Different components of the mixture travel at different rates depending on:

- *Their solubility in the mobile phase.*
- *Their affinity for the stationary phase (which contains the water in the cellulose fibers).*

Compounds that have a higher affinity for the mobile phase will move faster, while those that are more attracted to the stationary phase will move more slowly, resulting in the separation of components.

Experimental Procedure of Paper Chromatography

1. *Preparation of Paper:*
   - *Use chromatography paper (usually made of cellulose) as the stationary phase.*

- A pencil line is drawn near the bottom of the paper to mark the starting point for the sample application (about 1-2 cm from the bottom).

2. Sample Application:
    - A small amount of the sample mixture is applied as a spot on the pencil line using a capillary tube or micropipette. It is important to ensure that the spot is small to avoid overlapping during the separation process.
3. Selection of Solvent (Mobile Phase):
    - Choose an appropriate solvent (or mixture of solvents) based on the solubility of the compounds being separated. Common solvents include water, ethanol, acetone, or mixtures of organic solvents.
    - The solvent must be immiscible with the paper (stationary phase) and should not dissolve it.
4. Development of Chromatogram:
    - The chromatography paper is placed in a development chamber (such as a beaker or jar) with a small amount of the solvent at the bottom. The solvent level should be below the sample spot to prevent it from dissolving the sample directly.
    - The chamber is covered to maintain a saturated atmosphere, allowing the solvent to rise up the paper through capillary action.
    - As the solvent moves up, it carries the different components of the sample along with it at varying speeds.
5. Detection and Visualization:
    - Once the solvent front has moved a desired distance, the paper is removed from the chamber, and the solvent front is marked immediately with a pencil.
    - Allow the paper to dry. If the separated components are colorless, chemical reagents, such as ninhydrin (for amino acids) or iodine (for lipids), may be used to visualize the spots.
    - The Rf values (retention factors) for each spot are calculated using the formula:
6. Rf=Distance traveled by the soluteDistance traveled by the solvent front\text{Rf} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front}}Rf=Distance traveled by the solvent frontDistance traveled by the solute

Types of Paper Chromatography

1. Ascending Paper Chromatography:
    - In ascending paper chromatography, the paper is suspended vertically, with the lower edge dipping into the solvent in a developing chamber.
    - The solvent travels upward by capillary action, carrying the sample components with it.
    - This is the most common type of paper chromatography used in laboratories.
2. Descending Paper Chromatography:
    - In descending chromatography, the solvent moves downward due to gravity.
    - The paper is suspended from the top, with the solvent reservoir placed at the top of the chamber. The solvent flows down the paper by both capillary action and gravity.
    - This method is faster than ascending chromatography and is often used when a quicker separation is needed.

3. Radial or Circular Paper Chromatography:

- *In radial chromatography, a circular piece of paper is used. The sample is applied at the center of the paper.*
- *The paper is then placed horizontally on a flat surface, and the solvent is allowed to move outward from the center, forming a circular pattern of separated components.*
- *This technique is useful for quick, small-scale separations.*

4. Two-Dimensional Paper Chromatography:

- *In two-dimensional chromatography, the sample is applied at one corner of a rectangular or square piece of chromatography paper.*
- *First, the paper is developed in one solvent (as in ascending chromatography) to separate the components.*
- *After drying, the paper is rotated 90 degrees, and it is developed again in a second solvent with a different polarity.*
- *This technique allows for the separation of complex mixtures with overlapping spots in the first solvent system, making it highly effective for analyzing mixtures of amino acids and peptides.*

Applications of Paper Chromatography

1. Qualitative Analysis of Organic Compounds:

- *Paper chromatography is used to identify organic compounds such as amino acids, carbohydrates, nucleic acids, and lipids in various biological samples.*
- *It is frequently employed in the analysis of plant extracts, fermentation products, and other natural materials.*

2. Separation of Complex Mixtures:

- *It is widely used to separate mixtures of organic compounds that cannot be easily separated by other methods.*
- *Examples include the separation of plant pigments (like chlorophyll and carotenoids), dyes, or inks.*

3. Analysis of Pharmaceuticals:

- *In pharmaceutical industries, paper chromatography is employed to test the purity of drugs and identify any impurities.*
- *It is also used in the separation and identification of medicinal compounds in formulations.*

4. Forensic Science:

- *Paper chromatography is an essential tool in forensic science for analyzing substances found at crime scenes, such as ink in questioned documents or toxic substances in biological samples.*

5. Food and Beverage Industry:

- *Paper chromatography is used to detect adulterants and additives in food products. It can also be employed to separate and identify food dyes, preservatives, and other food additives.*

6. Environmental Monitoring:

- Paper chromatography is useful for analyzing environmental pollutants like pesticides, herbicides, and heavy metals in water and soil samples.
- It can also be employed to detect toxic substances in air and soil samples.

7. Educational Purposes:

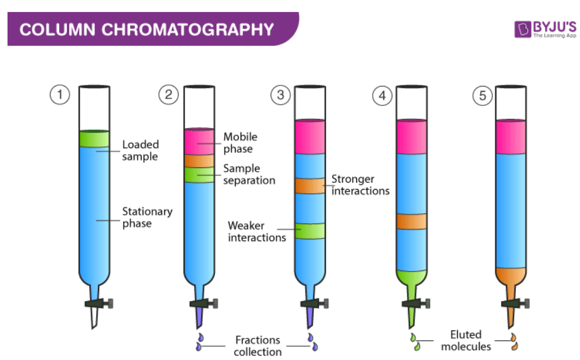
- It is a common technique used in schools and universities to demonstrate basic concepts of chromatography, including separation of mixtures, solubility, and molecular interactions.

Summary

Paper chromatography is a simple, cost-effective, and widely applicable separation technique used to analyze mixtures of organic and inorganic compounds. Its versatility allows for various modes of operation (ascending, descending, radial, and two-dimensional), making it suitable for a wide range of applications in fields like pharmaceuticals, forensic science, environmental monitoring, and biological research. By using this method, scientists and researchers can easily identify, separate, and analyze components in complex mixtures based on their physical and chemical properties.

Column Chromatography

Column Chromatography is a widely used technique in analytical chemistry for separating and purifying individual compounds from mixtures. It operates on the principle of selective adsorption, where components of a mixture are separated based on their different affinities toward the stationary and mobile phases. The sample mixture is passed through a column packed with a stationary phase, and components move at different rates due to their interaction with the stationary and mobile phases, leading to separation.



Principle of Column Chromatography

The principle of column chromatography is adsorption and partitioning. In this technique:

- Stationary Phase: A solid material (usually silica gel, alumina, or other adsorbents) is packed into a column. The stationary phase adsorbs the compounds from the sample mixture based on their polarity or other chemical properties.
- Mobile Phase: A solvent (or mixture of solvents) is passed through the column. The mobile phase carries the compounds of the mixture through the stationary phase.

Separation Mechanism:

- *The components of the sample mixture have varying degrees of interaction with the stationary phase based on their polarity, size, or affinity.*
- *Components that interact more strongly with the stationary phase (strongly adsorbed) move more slowly through the column, while those that have a greater affinity for the mobile phase (less strongly adsorbed) move faster.*
- *This differential migration of components through the column allows for the separation of the individual substances.*

The separation can occur through adsorption chromatography (where the stationary phase adsorbs the sample) or partition chromatography (where separation is based on the solubility differences between the stationary and mobile phases).

Classification of Column Chromatography

Column chromatography can be classified based on different criteria:

1. Based on the Type of Adsorption/Interaction:

- *Adsorption Chromatography: This technique is based on the adsorption of sample components onto a stationary phase (usually a solid like silica gel or alumina). Components are separated based on their adsorption affinity for the solid adsorbent.*
- *Partition Chromatography: In this type, the stationary phase is often a liquid or gel-like material. Components separate based on their partitioning between the stationary liquid phase and the mobile phase.*

2. Based on the Type of Mobile Phase:

- *Liquid Column Chromatography: A liquid mobile phase is passed through a solid stationary phase, leading to separation.*
- *Gas Column Chromatography: This involves a gaseous mobile phase and is often referred to as Gas Chromatography (GC), although this is not the focus here since liquid mobile phases are common in column chromatography.*

3. Based on the Flow of the Mobile Phase:

- *Gravity Column Chromatography: The solvent flows through the column by gravity. This is the simplest form of column chromatography.*
- *Flash Chromatography: A variant of gravity column chromatography, where the solvent is pushed through the column using pressure, resulting in faster separation and analysis.*
- *High-Performance Liquid Chromatography (HPLC): A more advanced and sophisticated form of column chromatography where the solvent is pumped through the column under high pressure. HPLC is often used for separating very small or complex samples with high precision.*

Experimental Procedure of Column Chromatography

1. *Preparation of the Column:*
   - *Selection of Stationary Phase: The stationary phase (usually silica gel or alumina) is chosen based on the nature of the compounds being separated. Silica gel is commonly used for separating polar compounds, while alumina is preferred for less polar substances.*
   - *Packing the Column: The column is packed with the stationary phase. This can be done by either a dry packing method (adding dry stationary phase and then adding solvent) or a wet packing method (making a slurry of the stationary phase with the solvent and then pouring it into the column).*
   - *Tapping the Column: Once the stationary phase is packed, the column is tapped to remove any air bubbles or gaps, ensuring even flow during the separation.*
2. *Sample Loading:*
   - *The sample mixture to be separated is dissolved in a small amount of the solvent (mobile phase) and applied to the top of the packed column. It is important to apply the sample carefully to avoid disturbing the top layer of the stationary phase.*
3. *Elution of Components:*
   - *Selection of Solvent (Mobile Phase): A suitable solvent or a mixture of solvents is chosen as the mobile phase. The polarity of the solvent is selected to optimize the separation of compounds. For example, non-polar solvents (e.g., hexane) are used to separate non-polar compounds, while polar solvents (e.g., ethanol, methanol) are used for polar compounds.*
   - *Elution Process: The mobile phase is poured into the column, and it moves through the stationary phase by gravity or external pressure (in flash chromatography). As the mobile phase moves down the column, it carries the different components of the mixture at different rates based on their affinities toward the stationary and mobile phases.*
   - *Gradient Elution (Optional): A mixture of solvents with varying polarities can be used in gradient elution to improve separation efficiency, starting with a non-polar solvent and gradually increasing the polarity.*
4. *Collection of Fractions:*
   - *As the components of the mixture move through the column, they form separate bands or zones. These bands can be seen if the compounds are colored; otherwise, UV light or chemical reagents can be used for detection.*
   - *The different components (fractions) are collected separately at the bottom of the column, using test tubes or vials. The collection is based on the elution of different zones.*
5. *Analysis of Fractions:*
   - *The separated fractions are analyzed using various techniques, such as thin-layer chromatography (TLC), UV-visible spectroscopy, or mass spectrometry, to identify and quantify the components.*

Advantages of Column Chromatography

- *Versatile Separation Technique: Column chromatography can be used to separate a wide range of compounds, including polar, non-polar, small, large, organic, and inorganic molecules.*
- *Large-Scale Purification: This method is suitable for both small-scale analytical separations and large-scale purification of substances, making it highly useful in pharmaceutical and chemical industries.*
- *Choice of Solvents: A variety of solvents can be used as mobile phases, providing flexibility in optimizing separations for different types of samples.*
- *Simple and Inexpensive: Basic column chromatography does not require expensive equipment and can be performed with easily available materials like glass columns, silica gel, and solvents.*
- *Effective for Purification: Column chromatography is effective for the purification of individual compounds from mixtures, making it a critical tool in chemical synthesis and drug production.*

Applications of Column Chromatography

1. ***Purification of Compounds:***
   - ***Column chromatography is widely used to purify organic compounds after synthesis. It is commonly employed in laboratories to purify drugs, chemicals, and natural products.***
   - ***This method is effective in isolating pure compounds from reaction mixtures or plant extracts.***
2. ***Separation of Mixtures:***
   - ***Column chromatography is used to separate complex mixtures of compounds. It is particularly useful for separating components that have similar boiling points or are difficult to separate by other techniques like distillation.***
   - ***Examples include the separation of proteins, amino acids, lipids, and other biomolecules.***
3. ***Pharmaceutical Industry:***
   - ***In the pharmaceutical industry, column chromatography is used to purify active pharmaceutical ingredients (APIs), identify impurities, and optimize drug formulations.***
   - ***It is also utilized for the quality control and purification of intermediates during drug production.***
4. ***Natural Product Research:***
   - ***Column chromatography plays a vital role in isolating and purifying bioactive compounds from natural sources, such as plant extracts and marine organisms.***
   - ***It is used to separate alkaloids, flavonoids, terpenoids, and other secondary metabolites from natural products.***
5. ***Biochemistry and Biotechnology:***
   - ***In biochemistry, column chromatography is frequently used to purify proteins, nucleic acids (DNA, RNA), and enzymes.***
   - ***It is an essential technique in the study of biomolecules and the development of biotechnology products.***
6. ***Environmental Analysis:***
   - ***Column chromatography is applied to analyze environmental samples for pollutants, pesticides, and toxic chemicals in water, air, and soil.***

- It is used for monitoring environmental contaminants and assessing pollution levels.

7. Food and Beverage Industry:
    - This technique is used in the food industry to analyze and purify food additives, preservatives, and flavors.
    - It is also used to detect contaminants or residues in food products.
8. Forensic Science:
    - In forensic analysis, column chromatography is employed to separate and identify compounds such as drugs, toxins, and other substances found in biological samples or at crime scenes.

Summary

Column chromatography is a versatile and powerful separation technique used extensively in laboratories and industries for the separation, purification, and analysis of compounds. Its flexibility, scalability, and efficiency make it ideal for a wide range of applications, including organic synthesis, drug production, biochemical research, and environmental monitoring. The technique offers the advantage of being simple and customizable, allowing for the separation of complex mixtures of compounds with high precision.