Chapter 19:

Chromatography

Short Questions (Exercise)

(i) Define chromatography.

Chromatography is a separation technique that separates components of a mixture based on their different interactions with a stationary phase and a mobile phase.

Example: Separating pigments in ink using paper chromatography.

(ii) Define locating agent.

A locating agent is a chemical that reacts with colorless compounds on a chromatogram to produce visible spots, making the components identifiable.

Example: Ninhydrin is used as a locating agent for amino acids.

(iii) What is Rf value?

The retention factor (Rf) is the ratio of the distance traveled by a compound to the distance traveled by the solvent front in chromatography.

Formula:

Rf = Distance traveled by the compound / Distance traveled by the solvent.

Example: If the compound moves 2 cm and the solvent moves 5 cm, Rf = 0.4.

(iv) Define paper chromatography.

Paper chromatography is a technique where a mixture is separated by allowing the solvent (mobile phase) to carry the components up a piece of paper (stationary phase) through capillary action.

Example: Separating plant pigments.

(v) What do you mean by paper chromatogram?

A paper chromatogram is the result of paper chromatography, where separated components appear as distinct spots on the paper at different distances from the origin.
Example: Pigments separated from a plant extract can be seen as colored spots.

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2. Explain in detail paper chromatography.

Definition

Paper chromatography is a method used to separate and identify components in a mixture using a piece of chromatography paper as the stationary phase and a liquid solvent as the mobile phase.

Steps

1. **Sample Application**: A small spot of the sample is placed near the base of the paper.
2. **Mobile Phase Setup**: The paper is suspended in a solvent so that the solvent travels up the paper by capillary action.
3. **Separation**: Components of the mixture travel at different speeds depending on their affinity to the paper and the solvent.
4. **Result**: Different components appear as distinct spots on the chromatogram.

Applications

- **Food Industry**: Testing for food color dyes.
- **Botany**: Analyzing plant pigments.
- **Forensics**: Identifying ink or dye compositions.

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3. How can you identify an unknown substance by chromatography?

1. **Perform Chromatography**: Run the unknown sample alongside known reference substances under identical conditions.
2. **Compare Rf Values**: Calculate the Rf value of the unknown and compare it with the Rf values of known substances.
3. **Identify the Substance**: If the Rf value matches that of a reference compound, the unknown is identified.

Example: Comparing an unknown pigment's Rf value to known plant pigments to identify it.

Differentiate between stationary and mobile phase.

Property	Stationary Phase	Mobile Phase

Definition	The phase that remains fixed in chromatography.	The phase that moves and carries the sample.
Nature	Solid or liquid.	Liquid or gas.
Function	Separates components based on affinity.	Transports the sample through the system.
Example in Paper Chromatography	Paper.	Solvent (e.g., water, ethanol).

Extera Short Questions (Topic Wise)

19.1: Principle of Chromatography

1. What is the principle of chromatography?

Chromatography separates components of a mixture based on their distribution between a stationary phase and a mobile phase. Components move at different rates due to differences in solubility and affinity.
Example: In paper chromatography, pigments in ink are separated as they travel with the solvent.

2. What are the phases involved in chromatography?

- **Stationary Phase**: A solid or liquid surface on which the separation occurs.
- **Mobile Phase**: A liquid or gas that carries the mixture through the stationary phase.
  Example: In paper chromatography, the stationary phase is the paper, and the mobile phase is the solvent.

3. Why do different substances travel at different speeds in chromatography?

Substances differ in their solubility in the mobile phase and their interaction with the stationary phase. Those with higher affinity to the stationary phase move slower.
Example: Polar compounds interact more with polar stationary phases, moving slower.

4. How is retention factor (Rf) calculated in chromatography?

The Rf value is the ratio of the distance traveled by the substance to the distance traveled by the solvent.
Formula: Rf = Distance traveled by the compound / Distance traveled by the solvent.
Example: If a compound moves 2 cm and the solvent moves 5 cm, Rf = 2/5 = 0.4.

19.2: Paper Chromatography

1. What is paper chromatography?

Paper chromatography is a simple technique where the stationary phase is a piece of paper, and the mobile phase is a solvent that travels up the paper by capillary action.
Example: It is used to separate pigments in ink.

2. What types of mixtures can be separated using paper chromatography?

Mixtures of dyes, pigments, or amino acids can be separated using this technique.
Example: Separating food color dyes in a sample.

3. How does the solvent choice affect paper chromatography?

The solvent must dissolve the mixture components effectively. Polar solvents are used for polar compounds, and nonpolar solvents are used for nonpolar compounds.
Example: Ethanol is used to separate polar compounds.

4. What are the advantages of paper chromatography?

- Simple and inexpensive.
- Requires minimal equipment.
- Can separate small quantities of substances.
  Example: Analyzing the pigments in leaf extracts.

19.3: Thin-Layer Chromatography (TLC)

1. What is thin-layer chromatography (TLC)?

TLC is a chromatography technique where a thin layer of silica gel or alumina is used as the stationary phase on a glass or plastic plate.
Example: TLC is used in drug analysis to check the purity of pharmaceuticals.

2. How does TLC differ from paper chromatography?

- TLC uses silica or alumina as the stationary phase, whereas paper chromatography uses paper.
- TLC is faster and more efficient for separating small quantities.
  Example: TLC can separate more closely related compounds than paper chromatography.

3. What is the role of a UV lamp in TLC?

A UV lamp helps visualize compounds that are not visible under normal light. Many compounds fluoresce under UV light, revealing their positions on the TLC plate.
Example: Detecting drug metabolites in forensic science.

4. What are the advantages of TLC?

- Faster and more sensitive than paper chromatography.
- Provides better separation and allows for quantitative analysis.
  Example: Used in the cosmetic industry to test product purity.

19.4: Applications of Chromatography

1. How is chromatography used in pharmaceuticals?

Chromatography is used to test the purity of drugs, identify compounds, and separate active ingredients.
Example: Gas chromatography is used to analyze anesthetic gases.

2. What is the role of chromatography in environmental science?

Chromatography helps detect pollutants and monitor water and air quality.
Example: Detecting pesticides in groundwater using liquid chromatography.

3. How is chromatography applied in forensic science?

Chromatography is used to identify drugs, toxins, and other chemical evidence in criminal investigations.
Example: Gas chromatography identifies alcohol levels in blood samples.

4. What industries rely on chromatography?

Chromatography is widely used in food testing, cosmetics, biochemistry, and petrochemicals.
Example: High-performance liquid chromatography (HPLC) tests for preservatives in food.

Extera Long Questions (Topic Wise)

1. Principle of Chromatography

Definition of Chromatography

Chromatography is a separation technique used to isolate components of a mixture based on their different affinities toward the stationary phase and the mobile phase. It is widely employed in analytical chemistry for identifying, quantifying, and purifying compounds.

Working Principle

Chromatography operates on the principle of differential partitioning between two phases:

1. **Stationary Phase**: A solid or liquid phase that remains fixed in place.
2. **Mobile Phase**: A liquid or gas that flows over the stationary phase, carrying the components of the mixture.

Key Concept: Retention Factor (Rf)

The distance a compound travels relative to the solvent front in the mobile phase is expressed as the retention factor (Rf):
Formula: Rf = Distance traveled by the compound / Distance traveled by the solvent.
Example: If the compound moves 2 cm and the solvent moves 4 cm, Rf = 2/4 = 0.5.

Mechanism

- Components of the mixture interact differently with the stationary and mobile phases.
- Those with higher solubility in the mobile phase move faster, while those with greater affinity to the stationary phase move slower.

Example

In paper chromatography, pigments in ink separate based on their solubility in the solvent and their interaction with the cellulose fibers in the paper.

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2. Paper Chromatography

Definition of Paper Chromatography

Paper chromatography is a simple and inexpensive method used to separate and identify components in a mixture. It involves a stationary phase (paper) and a mobile phase (solvent) that moves through the paper by capillary action.

Steps in Paper Chromatography

1. A small spot of the sample mixture is placed near the bottom of a piece of chromatography paper.
2. The paper is suspended in a container with the mobile phase (e.g., water, ethanol).

3. The solvent travels up the paper, carrying the components of the mixture.
4. Different components travel at different speeds, creating distinct spots.

Advantages

1. Inexpensive and simple setup.
2. Suitable for small quantities.
3. Effective for separating non-volatile substances.

Applications

1. **Food Testing**: Detecting artificial colorings in food samples.
2. **Plant Pigment Analysis**: Separating chlorophyll and carotenoids from leaves.
3. **Forensic Science**: Analyzing dyes in inks or fibers.

Example

Separating the pigments in a leaf extract reveals different colors such as green (chlorophyll) and orange (carotenoids).

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3. Thin Layer Chromatography (TLC)

Definition of Thin Layer Chromatography

TLC is a chromatographic technique where the stationary phase is a thin layer of silica gel or alumina coated on a plate, and the mobile phase is a solvent. It is widely used for rapid and efficient separations.

Procedure for TLC

1. A small spot of the sample is applied to the base of a TLC plate.
2. The plate is placed in a container with the mobile phase.
3. The solvent rises through capillary action, carrying the sample components.
4. The separated spots are visualized under UV light or by spraying with a detecting agent.

Advantages of TLC

1. Faster and more efficient than paper chromatography.
2. High sensitivity for small quantities.
3. Can be used for both qualitative and quantitative analysis.

Applications of TLC

1. **Pharmaceuticals**: Testing the purity of drugs and detecting impurities.
2. **Forensic Science**: Identifying drug residues in criminal investigations.
3. **Food Chemistry**: Analyzing preservatives and contaminants.

Example

In drug analysis, TLC can separate different analgesic compounds like aspirin and paracetamol, showing distinct spots on the TLC plate.

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4. Applications of Chromatography

1. Pharmaceutical Industry

Chromatography is used to:

- Test drug purity.
- Separate and identify active ingredients.
- Detect impurities and degradation products.
  Example: High-performance liquid chromatography (HPLC) is used to test the purity of antibiotics.

2. Environmental Analysis

Chromatography helps detect pollutants in air, water, and soil.
Example: Gas chromatography-mass spectrometry (GC-MS) identifies pesticide residues in drinking water.

3. Forensic Science

Chromatography is crucial for analyzing samples in criminal investigations, such as:

- Detecting drugs in blood or urine.
- Analyzing dyes in fibers and inks.
  Example: GC-MS identifies alcohol levels in blood samples.

4. Food and Beverage Industry

Chromatography ensures food safety by detecting contaminants, preservatives, and artificial colorings.
Example: HPLC analyzes the caffeine content in coffee and tea.

5. Biotechnology and Research

Chromatography is used to purify proteins, enzymes, and DNA in research and production.
Example: Affinity chromatography isolates specific proteins for research.

Conclusion

Chromatography is an indispensable tool in science and industry due to its versatility, sensitivity, and accuracy. From analyzing pharmaceuticals to monitoring environmental pollutants, chromatography provides reliable methods for separating, identifying, and quantifying compounds in complex mixtures. Its wide range of applications and techniques like paper chromatography, TLC, and gas chromatography make it one of the most important analytical techniques in modern science.

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19.1: Principle of Chromatography

1. **What is the principle of chromatography?**
   a) Boiling point differences
   b) **Separation based on distribution between stationary and mobile phases** ✓
   c) Separation by density
   d) Absorption of light

2. **What are the two phases in chromatography?**
   a) Active and inactive phases
   b) **Stationary and mobile phases** ✓
   c) Solid and liquid phases
   d) Static and dynamic phases

3. **What is the stationary phase in chromatography?**
   a) The phase that moves with the solvent
   b) **The phase that remains fixed during the process** ✓
   c) The phase that evaporates
   d) The gaseous phase

4. **What is the mobile phase in chromatography?**
   a) **The phase that carries the components through the stationary phase** ✓
   b) The phase that remains fixed
   c) The phase that separates ions
   d) The solid phase

5. **What is the formula for calculating the retention factor (Rf)?**
   a) Rf = Distance traveled by solvent / Distance traveled by compound
   b) **Rf = Distance traveled by compound / Distance traveled by solvent** ✓
   c) Rf = Time taken by compound / Total time
   d) Rf = Distance by stationary phase

6. **What does a higher Rf value indicate?**
   a) Stronger interaction with stationary phase
   b) **Stronger interaction with mobile phase** ✓
   c) Heavier molecular weight
   d) Poor solubility

19.2: Paper Chromatography

7. **What is paper chromatography used for?**
   a) Separating metals
   b) **Separating pigments or small molecules** ✓

c) Separating gases
d) Purifying water

8. **What acts as the stationary phase in paper chromatography?**
   a) Silica gel
   b) Alumina
   c) **Paper** ✔
   d) Plastic sheet

9. **What is the role of the solvent in paper chromatography?**
   a) To dry the sample
   b) To make the sample heavier
   c) **To carry the components of the mixture up the paper** ✔
   d) To react with the paper

10. **What is an advantage of paper chromatography?**
    a) It requires expensive equipment
    b) It is only useful for large-scale separations
    c) **It is simple and inexpensive** ✔
    d) It requires a long time to separate components

11. **Which mixtures are commonly separated using paper chromatography?**
    a) Solid metal mixtures
    b) **Pigments, dyes, or amino acids** ✔
    c) Gases
    d) Heavy oils

12. **What is a practical application of paper chromatography?**
    a) Separating proteins
    b) **Analyzing plant pigments** ✔
    c) Refining crude oil
    d) Separating metals

19.3: Thin-Layer Chromatography (TLC)

13. **What is used as the stationary phase in TLC?**
    a) Paper
    b) **A thin layer of silica gel or alumina** ✔
    c) Liquid solvent
    d) Air

14. **What is the mobile phase in TLC?**
    a) Solids
    b) **A solvent or liquid** ✔
    c) Gases
    d) Plastic sheets

15. **How is TLC better than paper chromatography?**
    a) It is slower but more accurate

b) **It is faster and provides better separation** ✔
c) It requires less equipment
d) It uses less solvent

16. **What is the role of UV light in TLC?**
a) To increase reaction speed
b) To dry the plate
c) **To visualize the separated compounds** ✔
d) To identify metals

17. **What is an application of TLC?**
a) Detecting gases in the atmosphere
b) **Testing drug purity** ✔
c) Refining petroleum
d) Analyzing water hardness

18. **What is the main limitation of TLC?**
a) It cannot separate small molecules
b) It requires expensive equipment
c) **It cannot separate very similar compounds** ✔
d) It is too slow

19.4: Applications of Chromatography

19. **What is gas chromatography primarily used for?**
a) Separating solids
b) **Analyzing volatile substances** ✔
c) Testing solubility
d) Separating dyes

20. **Which industry relies heavily on chromatography for quality control?**
a) Automobile
b) **Pharmaceutical** ✔
c) Construction
d) Textile

21. **What is the main use of chromatography in environmental science?**
a) Analyzing gases
b) **Detecting pollutants in water and air** ✔
c) Testing pH levels
d) Calculating density

22. **What does HPLC stand for?**
a) High Precision Liquid Chromatography
b) **High-Performance Liquid Chromatography** ✔
c) Heat-Powered Liquid Chromatography
d) High-Pressure Liquid Chromatography

23. **What is the stationary phase in gas chromatography?**
    a) A liquid or solid ✔
    b) A gas
    c) A paper sheet
    d) A solvent

24. **What is the main application of chromatography in food science?**
    a) Calculating calories
    b) Measuring mass
    c) **Detecting food additives and contaminants** ✔
    d) Measuring solubility

25. **Which chromatography technique is best for analyzing large biomolecules?**
    a) Paper chromatography
    b) **Affinity chromatography** ✔
    c) Gas chromatography
    d) TLC

26. **What is the use of chromatography in forensics?**
    a) Testing bullet residues
    b) **Identifying drugs and toxins in biological samples** ✔
    c) Measuring DNA concentration
    d) Separating proteins

27. **What property is used to separate components in gas chromatography?**
    a) Solubility
    b) **Boiling points** ✔
    c) Density
    d) Magnetic properties

28. **Why is chromatography useful in biochemistry?**
    a) **For purifying and isolating proteins, enzymes, and DNA** ✔
    b) For heating chemicals
    c) For testing solvents
    d) For measuring pH

29. **Which chromatography is used for essential oil extraction?**
    a) TLC
    b) Paper chromatography
    c) **Steam distillation chromatography** ✔
    d) HPLC

30. **What is the primary advantage of chromatography?**
    a) It is inexpensive
    b) It is time-consuming
    c) **It separates and identifies complex mixtures efficiently** ✔
    d) It requires minimal training