

Paper Chromatography

Paper Chromatography (PC)

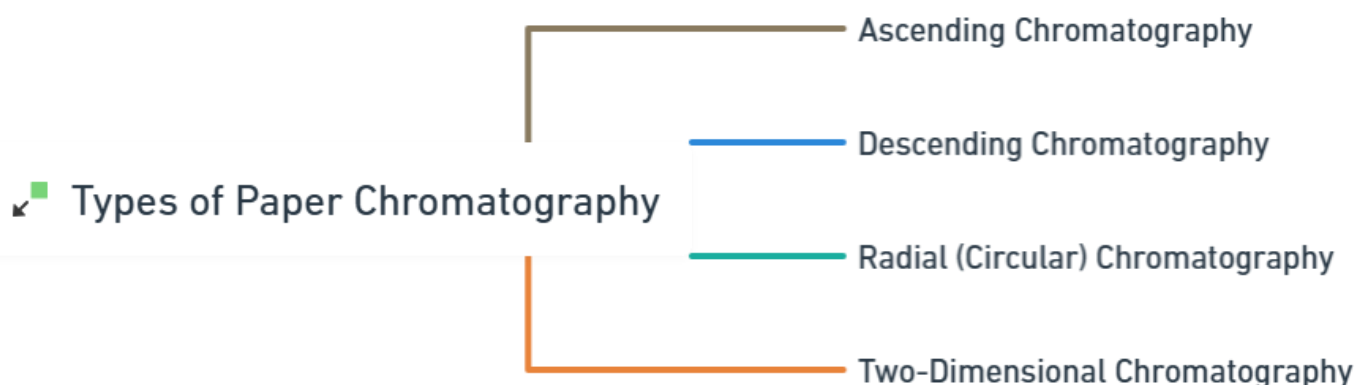
Introduction

- Paper Chromatography is one of the oldest and simplest forms of planar chromatography.
- It involves the use of special filter paper as the stationary phase, and a suitable liquid solvent or solvent mixture as the mobile phase.
- Though now less commonly used in routine pharmaceutical laboratories (being largely replaced by TLC and HPLC), it remains highly valuable in educational labs, natural product analysis, and preliminary qualitative drug testing, especially for polar compounds like amino acids, alkaloids, and sugars.

Principle

- **Paper Chromatography operates primarily on the principle of partition chromatography.**
 - The stationary phase is water molecules adsorbed onto the cellulose fibers of the paper. Even though the paper appears dry, it holds water due to hydrogen bonding.
 - The mobile phase is an organic solvent or solvent mixture that travels by capillary action along the paper.
- **As the mobile phase moves:**
 - Each component of the sample partitions itself between the stationary (water) and mobile (organic solvent) phases.
 - The extent of partitioning depends on the solubility and polarity of the compound:
 - Hydrophilic (polar) drugs remain in the stationary phase longer.
 - Lipophilic (non-polar) drugs migrate more with the mobile phase.
- As a result, components in the drug mixture separate as distinct spots or zones on the paper.

Types of Paper Chromatography



1. Ascending Chromatography

- The mobile phase moves upward on the paper.

- The lower edge of the paper (with sample spots) is dipped in solvent, and capillary action pulls the solvent upward.
- Commonly used due to simplicity.

2. Descending Chromatography

- Solvent moves downward, aided by gravity as well as capillary action.
- Provides faster and better separation, especially for longer paper strips or larger molecules.

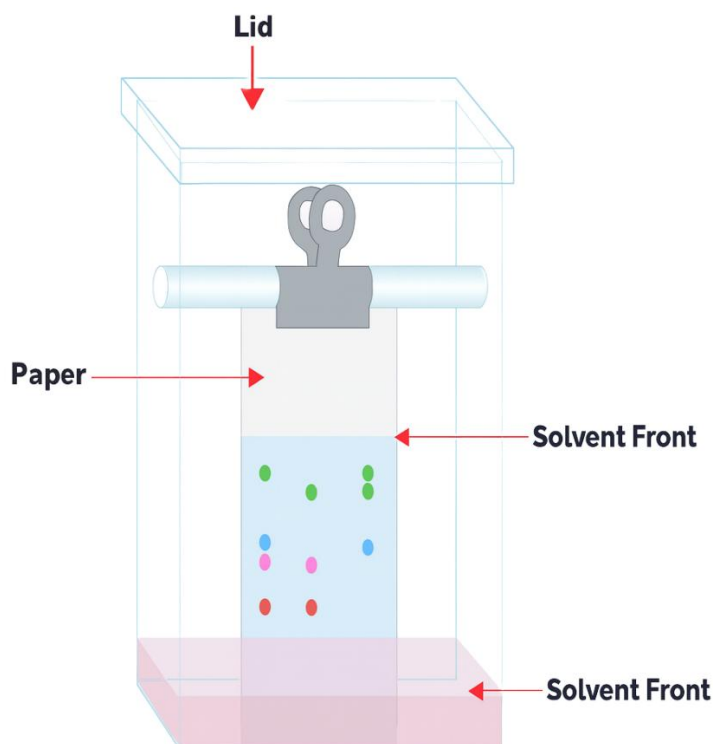
3. Radial (Circular) Chromatography

- A circular filter paper is used.
- Sample is applied at the center, and solvent is allowed to travel from the center outward in all directions.
- Often used in teaching labs.

4. Two-Dimensional Chromatography

- Used when the sample contains many components that are not well separated by single development.
- The paper is first developed in one direction, then dried and rotated 90°, and developed again with a different solvent system.
- Produces a two-dimensional map of separated components.

Preparation Techniques



A. Choice of Paper

- Use Whatman filter paper (No. 1 or 3) or other high-quality chromatography paper.

- The paper should be uniform in thickness, free from impurities, and have good adsorptive and partitioning properties.

B. Sample Preparation

- Pharmaceutical samples are dissolved in volatile solvents like methanol, ethanol, or water.
- Sample should be filtered to remove insoluble excipients.
- Volume applied is small (1–10 μL), using a micropipette or capillary.

C. Spotting the Sample

- A small concentrated spot is placed 1.5–2.0 cm from the edge of the paper.
- Allow spot to air dry before development.
- For complex mixtures, multiple samples and standards can be spotted on the same line.

Development Techniques

1. Solvent Saturation

- The chromatography chamber must be pre-saturated with solvent vapor using filter paper soaked in mobile phase to prevent solvent evaporation during development.

2. Solvent Systems

- The choice depends on the nature of the drug:
 - For **polar drugs**: Butanol : Acetic acid : Water (4:1:5) – upper layer used
 - For **non-polar drugs**: Benzene : Ethanol or Chloroform : Methanol
 - For **alkaloids**: Ethyl acetate : Methanol : Ammonia

3. Development

- Paper is suspended in the chamber so that the sample spots do not touch the solvent.
- Solvent travels up (or down) the paper and separates the sample components.

4. Drying and Visualization

- After the solvent has traveled an appropriate distance (~10–15 cm), the paper is removed and air-dried.
- Visualization depends on drug type:
 - UV light for aromatic or conjugated drugs.
 - Iodine vapors for non-UV-active compounds.
 - **Chemical reagents**:
 - **Ninhydrin**: detects amino acids and amines (purple/blue spots).
 - **Dragendorff's reagent**: detects alkaloids.

- **Sulfuric acid + heating:** for general detection by charring.

Rf Value (Retention Factor)

- As with TLC, the Rf value in paper chromatography is calculated using:

$$R_f = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent front}}$$

- Rf values are compared with standard compounds to identify unknowns.
- Each compound has a characteristic Rf value under fixed conditions.

Applications in Pharmaceutical Analysis

A. Identification of Drugs

- PC is used to confirm the identity of drugs in formulations by comparing Rf values and color reactions with reference standards.
- **Example:** Differentiating caffeine, aspirin, and acetaminophen in multi-ingredient tablets.

B. Detection of Impurities

- Additional spots besides the main drug indicate impurities or degradation products.
- **Example:** In vitamin C tablets, oxidative degradation can be detected by additional spots.

C. Separation of Drug from Excipients

- Excipients like sugars and starches are either non-migratory or do not react with common visualizing agents, so they don't interfere with drug detection.
- PC is useful to isolate active drugs from inactive matrix.

D. Stability Testing

- PC can show breakdown products formed during storage (heat, light, moisture).
- **Example:** Hydrolysis of penicillin to penicilloic acid shows distinct spots.

E. Analysis of Natural Products and Herbal Drugs

- PC is used extensively in alkaloid, flavonoid, and glycoside analysis.
- Helps in standardization of herbal formulations.

F. Monitoring Reaction Progress

- In chemical synthesis of drug molecules, PC tracks intermediate formation and completion of reaction.

Advantages:

- Simple, inexpensive, and easy to perform.



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