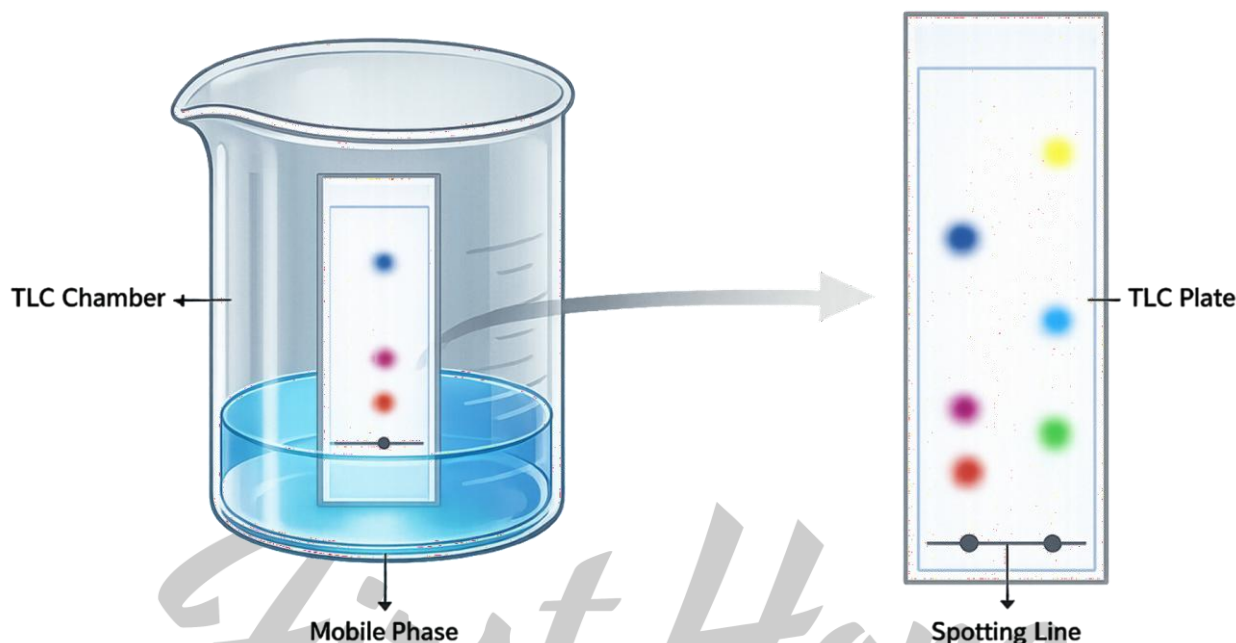


Chromatography

Thin Layer Chromatography (TLC)

Principle

- Thin **Layer Chromatography** is based mainly on the adsorption principle, although sometimes partition also plays a role.



Phases involved:

- **Stationary phase:** Thin layer of adsorbent (like silica gel or alumina) coated on a plate
- **Mobile phase:** Solvent or mixture of solvents

What happens:

- Sample is applied as a small spot on the plate
- Solvent moves upward by capillary action
- **Components interact differently with:**
 - Stationary phase (adsorption)
 - Mobile phase (solubility)

Separation occurs because:

- Strong adsorption → slow movement
- Weak adsorption → fast movement

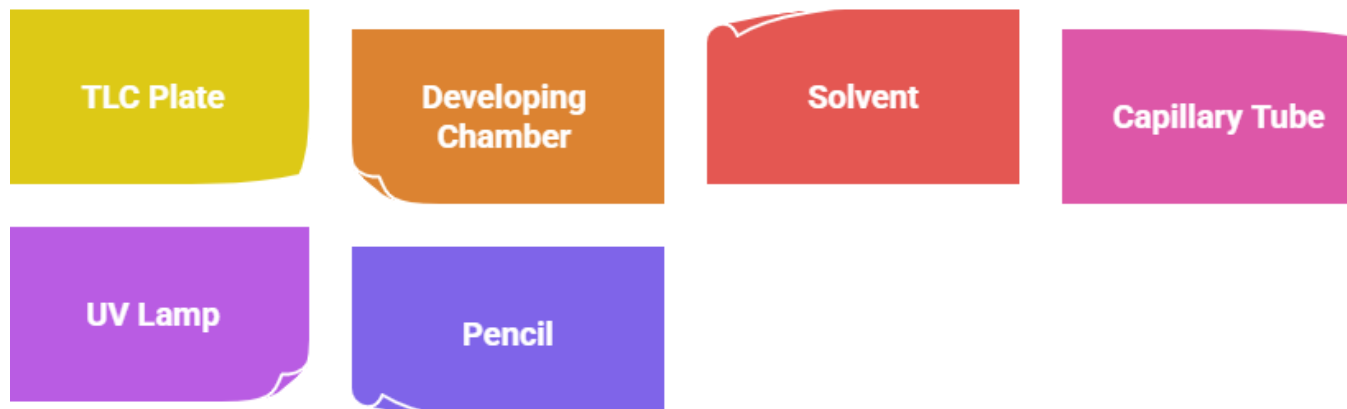
Polarity concept:

- Polar compounds → stick strongly → low movement
- Nonpolar compounds → move with solvent → high movement

Apparatus

TLC setup is simple but more advanced than paper chromatography.

TLC Apparatus



1. TLC plate

- Glass, aluminum, or plastic plate
- **Coated with:**
 - Silica gel (most common)
 - Alumina

2. Developing chamber

- Closed container with solvent

3. Solvent (mobile phase)

- Selected based on polarity

4. Capillary tube or micropipette

- For applying for sample

5. UV lamp or detecting reagents

- For visualization

6. Pencil

- For marking baseline

Instrumentation (Setup and Working)

1. Preparation of plate

- Draw baseline about 1 cm from bottom

2. Sample application

- Apply small, concentrated spot
- Let it dry

3. Development

- Place plate in chamber with solvent
- Solvent level below spot

4. Solvent movement

- Solvent rises by capillary action

5. Separation

- Components separate based on adsorption strength

6. Stopping

- Remove plate when solvent nears top

7. Detection

- **Methods:**
 - UV light
 - Iodine chamber
 - Spraying reagents

Chromatographic Parameters

R_f value (Retention factor)

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

Characteristics:

- Value between 0 and 1
- Unique for a compound under fixed conditions

Other parameters:

- **Resolution (R):** Degree of separation between two spots
- **Retention behavior:** Depends on polarity and adsorption strength

Factors Affecting Resolution

1. Nature of adsorbent

- Silica gel (polar) gives different separation than alumina

2. Solvent system

- Most critical factor
- Polar solvent → increases movement of polar compounds

3. Thickness of layer

- Thick layer → poor resolution
- Thin, uniform layer → better separation

4. Sample size

- Large spot → tailing and overlapping

5. Temperature

- Affects solvent flow and interactions

6. Chamber saturation

- Saturated chamber gives uniform solvent movement

7. Particle size of adsorbent

- Smaller particles → better separation

Applications

TLC is widely used because it is fast, sensitive, and simple.

Uses:

- Identification of compounds
- Checking purity of substances
- Monitoring chemical reactions
- Separation of plant components
- Drug analysis in pharmaceuticals
- Detection of adulterants in food
- Forensic analysis (drugs, poisons)

High Performance Thin Layer Chromatography (HPTLC)

Introduction

- High Performance Thin Layer Chromatography (HPTLC) is an advanced form of TLC used for separation, identification, and quantification of compounds.
- The sample is applied on a precoated plate and separated using a mobile phase.
- HPTLC provides better accuracy, sensitivity, and reproducibility than conventional TLC.
- It is widely used in pharmaceutical analysis, herbal drug analysis, food testing, and forensic science.

Principle

- HPTLC works on the principle of differential migration of compounds over a stationary phase under the influence of a mobile phase.
- Compounds move at different speeds depending on polarity, solubility, and affinity toward stationary and mobile phases.
- Compounds with greater attraction toward the stationary phase move slowly.
- Compounds with greater attraction toward the mobile phase move faster.
- Separation occurs because each compound travels a different distance on the plate.

Apparatus

- HPTLC plate coated with silica gel or alumina
- Sample applicator
- Developing chamber
- UV chamber
- Densitometer or scanner
- Micropipettes and capillary tubes

Instrumentation

1. Sample Applicator

- Automatic applicators are used to apply small and accurate volumes of sample on the plate.
- Application is usually done in the form of bands rather than spots for better separation.

2. HPTLC Plates

- Plates are precoated with fine particle size silica gel.
- Fine particles provide better resolution and faster separation.

3. Development Chamber



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