Thin layer chromatography (TLC)

Thin layer chromatography – TLC, is one of the simplest, fastest, easiest and less expensive chromatographic technique used in qualitative and quantitative analysis to separate organic compounds and test the purity of compounds.

Principle:

TLC is a method of separation or identification of mixture of components into individual components by using finely divided solid adsorbent coated on a glass plate and liquid as a mobile phase.

Thin Layer Chromatography is based on the principle of **adsorption**. It works through the differential affinity (attraction) of the compounds towards:

- The **stationary phase** (typically a thin layer of adsorbent like silica gel or alumina coated on a plate), and
- The **mobile phase** (a suitable solvent or mixture of solvents).

Compounds in a mixture travel with the solvent up the plate by capillary action. Depending on their polarity and solubility:

- Some compounds interact more with the **stationary phase** and travel **less**.
- Others interact more with the **mobile phase** and travel **farther**.

This difference in travel distance results in **separation** of components on the TLC plate.

Theory of Thin Layer Chromatography:

TLC relies on a dynamic **equilibrium** between the **adsorption** of a compound on the stationary phase and its **desorption** into the mobile phase.

1. Stationary Phase:

- Usually **polar** (silica gel SiO_2 or alumina Al_2O_3).
- It provides a surface for adsorption of analytes.

2. Mobile Phase (Eluent):

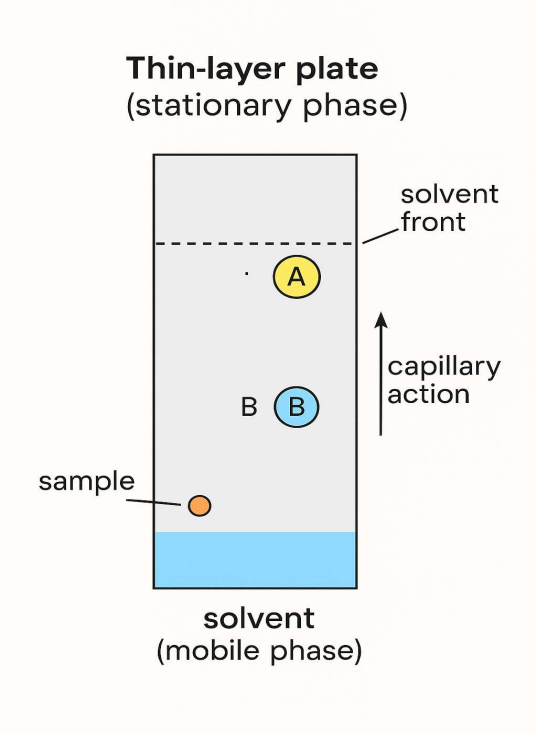
- Can be polar or nonpolar depending on the sample's nature.
- Moves upward by **capillary action**.
- Carries analytes to varying degrees depending on their **polarity and affinity**.

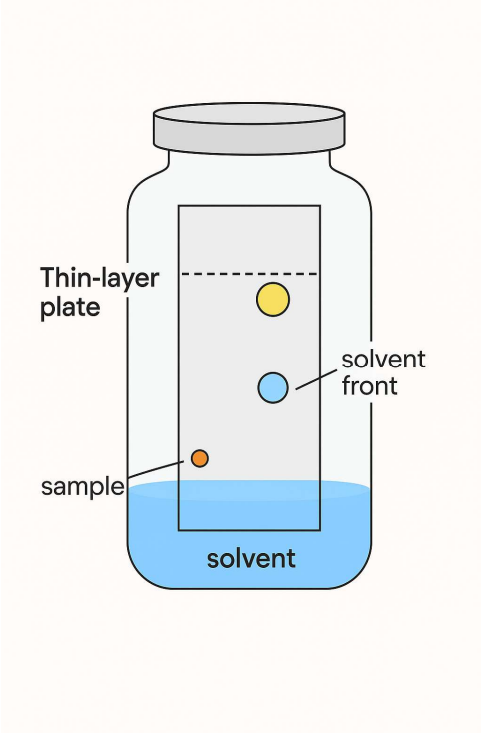
3. Retention Factor (Rf):

A measure of how far a compound travels:

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

- Values range from 0 to 1.
- More polar compounds → lower Rf (stronger interaction with the stationary phase).
- Less polar compounds → higher Rf (move faster with the solvent front).





Apparatus Required:

- **TLC plate:** A glass, plastic, or aluminium sheet coated with a thin layer of adsorbent (usually silica gel or alumina).
- **Sample mixture:** A solution containing the analytes to be separated.
- **Capillary tubes or micropipette:** For applying the sample.
- **Developing chamber:** A jar or beaker with a lid or cover to hold the solvent.
- **Mobile phase (eluent):** A suitable solvent or solvent mixture.
- **Pencil and ruler:** For marking the baseline and solvent front.
- **UV lamp or iodine chamber:** For visualizing the spots (if colorless).

Step-by-Step Setup and Procedure - TLC:

Step 1: Preparing the TLC Plate

TLC plates can either be purchased pre-coated or prepared manually in the laboratory using a suitable adsorbent like silica gel or alumina. Below is the manual preparation process, which is useful for academic and experimental learning purposes.

- **Cleaning the Glass Plates**

- Thoroughly clean glass plates with water and ethanol or acetone.
- Dry them completely; any grease or dust will interfere with adhesion of the adsorbent layer.

- **Preparing the Adsorbent Slurry**

- Mix Silica gel G (with binder) with distilled water in a 1:2 ratio (e.g., 25 g silica gel + 50 mL water).
- Stir to form a smooth, lump-free slurry.

- **Coating the Plates**

- Pour the slurry into a TLC spreader or coat manually using a glass rod.
- Spread a uniform thin layer (0.25 mm to 0.5 mm) over the surface of the glass plate.
- Let the plates rest vertically to ensure an even coating and settle air bubbles.

- **Drying the Coated Plates**

- Air-dry the plates for 30 minutes or until visibly dry.
- Then place them in a hot air oven at 100–120 °C for about 30–60 minutes to remove all moisture and activate the adsorbent.

- **Storage**

- Store dried plates in a dry and dust-free container.
- Preferably use them immediately or keep them in a desiccator if not used soon.

- **Usage of TLC Plate**

- Cut the TLC plate to a manageable size (e.g., 5–10 cm).
- Draw a light pencil line about 1 cm from the bottom – this is the **baseline** where the sample is spotted.
- Label spots for different samples.

Step 2: Applying the Sample

- Use a capillary tube to apply a small amount of the sample solution onto the baseline.
- Allow the spots to dry and repeat if necessary for better visualization.

Step 3: Preparing the Developing Chamber

- Pour the mobile phase into the chamber to a depth of about 0.5–1 cm.
- Line the chamber with filter paper to saturate the vapor (improves development).
- Close the lid and allow the solvent to equilibrate.

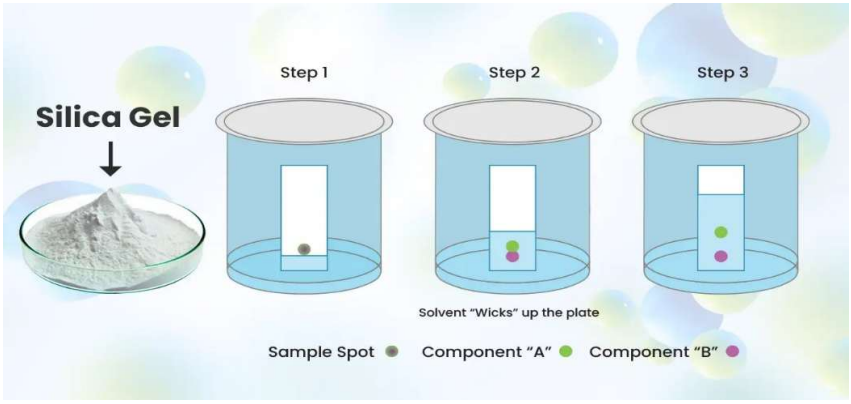
Step 4: Selection of Solvent in Thin Layer Chromatography (TLC)

- The solvent (mobile phase) plays a critical role in TLC, as it is responsible for carrying the analytes (components of a mixture) up the plate by capillary action.
- The choice of solvent directly affects the Rf values and the quality of separation.

Solvent	Polarity	Common Use
Hexane	Non-polar	For non-polar compounds
Toluene	Slightly polar	Aromatic compounds
Diethyl ether	Moderate polarity	Esters, ethers
Ethyl acetate	Polar	Broad range; used in mixtures
Acetone	Polar	Ketones, alcohols
Methanol / Ethanol	Highly polar	Polar compounds, amines
Water	Very polar	Rare in TLC due to high surface tension

Step 4: Developing the Plate

- Place the TLC plate carefully into the chamber, ensuring the sample spots are above the solvent level.
- Seal the chamber and let the solvent rise up the plate by capillary action.



Step 5: Removing and Drying

- Once the solvent front has moved an adequate distance (~3/4 of the plate), remove the plate.
- Immediately mark the solvent front with a pencil.
- Allow the plate to dry.

Step 6: Detection of Spots in Thin Layer Chromatography (TLC)

After developing a TLC plate, the separated compounds appear as invisible spots (unless colored), so detection techniques are used to visualize and identify the analytes.

a. Visualizing the Spots

- Applicable when: The compounds are colored.
- Example: Organic dyes, some metal complexes.
- Observation: Colored spots appear directly on the plate.
- Use a UV lamp or iodine vapor chamber to detect colorless compounds.
- Circle the spots with a pencil for documentation.

b. UV Light (Ultraviolet Illumination)

- **Principle:** Many compounds **absorb UV light** or **quench the fluorescence** of a fluorescent TLC plate.
- **TLC Plate Type:** Silica gel coated with a **fluorescent indicator (e.g., F254)**.
- **Wavelengths Used:**
    - **Shortwave UV:** 254 nm
    - **Longwave UV:** 365 nm
- **Observation:** Spots appear as **dark areas** on a bright fluorescent background.
- **Advantage: Non-destructive**, can be used before further derivatization.

c. Chemical Staining (Post-spray or Dip Methods)

- After development, TLC plates can be sprayed or dipped in detecting reagents that react with analytes to form colored compounds.

Common Stains:

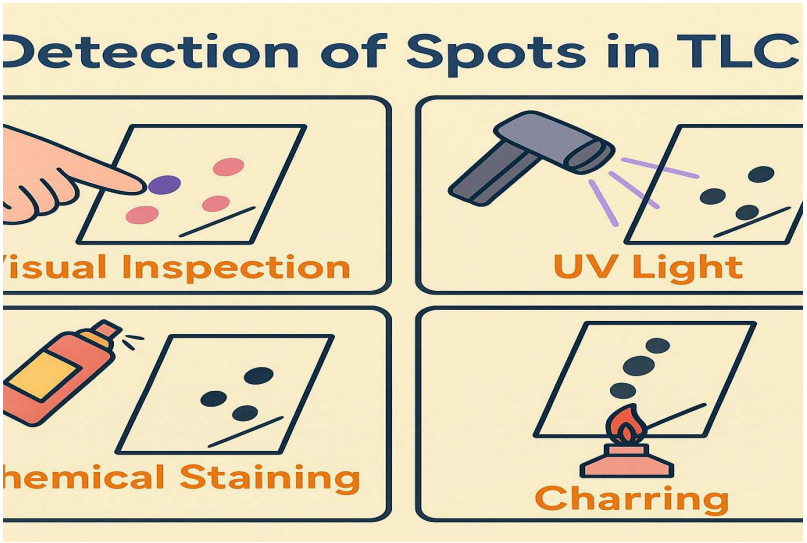
Reagent	Detects	Appearance
Iodine vapor	Unsaturated compounds, alkaloids	Brown spots
Ninhydrin	Amino acids	Purple/violet spots
Dragendorff's reagent	Alkaloids	Orange/red spots
Anisaldehyde–sulfuric acid	Carbohydrates, steroids	Purple/blue/green spots
$KMnO_4$ (Potassium permanganate)	Alkenes, alcohols	Yellow-brown background, white/yellow spots

d. Charring

- Plate is sprayed with a reagent (e.g., sulfuric acid) and **heated** to char the compounds.
- **Used for:** Carbohydrates, lipids, etc.
- **Observation:** Black or brown charred spots.

e. Fluorescence Detection

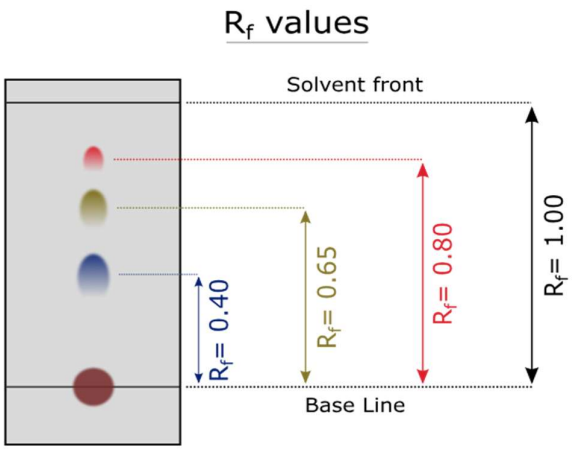
- Some compounds fluoresce naturally under UV light.
- Used in pharmaceutical and biochemical TLC.



Observations and Calculations: recording the results

- Measure the distance traveled by each spot (from baseline to center of spot).
- Measure the distance traveled by the solvent front.
- Calculate **Rf value** for each component:

$$Rf = \frac{\text{Distance moved by compound}}{\text{Distance moved by solvent front}}$$



Applications:

1. **Qualitative and Quantitative Analysis by Thin Layer Chromatography (TLC)**

TLC is a powerful analytical tool used for both **qualitative** (what is present?) and **quantitative** (how much is present?) analysis of compounds in a mixture.

Qualitative Analysis (Identification of Compounds):

- Run the sample alongside **known reference standards** on the same TLC plate.
- Develop the plate in a suitable **solvent system**.
- Visualize the spots using UV light or staining reagents.
- Compare **Rf values** and spot characteristics (e.g., shape, color) of unknown with known compounds.
- **Rf Value Calculation:** $$Rf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}$$

2. Quantitative Analysis:

a. Spot Intensity Comparison

- Compare the **intensity (color/density)** of sample spots with **standard solutions** of known concentrations.
- Use **visual inspection** or **image analysis software**.

b. Densitometry (TLC Scanner)

- A **densitometer** scans the developed TLC plate.
- Measures **light absorption/reflection** from each spot.
- Generates **peak area** or **intensity vs. concentration** plots.

c. Elution and Spectrophotometry

- Scrape off the spot from the TLC plate.
- Dissolve in suitable solvent.
- Measure **absorbance** using a **UV-Vis spectrophotometer**.
- Apply Beer-Lambert law to calculate concentration.

3.

Field	Application
Organic Chemistry	Reaction monitoring, compound purity
Pharmaceuticals	Drug ID, stability, and impurity testing
Natural Products	Plant metabolite profiling
Forensics	Drug & ink analysis
Environment	Pesticide/toxin detection
Food Industry	Additive and preservative analysis