

# THIN LAYER CHROMATOGRAPHY (TLC)

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# KEY CONCEPTS

- Introduction
- General Principle
- TLC Technique
- Applications

# INTRODUCTION

- Thin Layer Chromatography (TLC) is an important technique used for identification and separation of mixture of chemical compounds into its individual components.
- TLC is a form of liquid chromatography consisting of two phases: **A mobile phase (liquid)** and **A stationary phase (solid)**.
- Differences in the interactions between the solutes and stationary and mobile phases enable separation.

# PRINCIPLE

- TLC technique involves the distribution of components of a mixture to be separated between two phases.
- The components of the **mixture are partitioned between an adsorbent** (stationary phase), and a **solvent** (mobile phase).
- Different compounds will have different solubility and adsorption to the two phases between which they are to be partitioned.
- In TLC separation of the individual substances is based on their relative affinities towards stationary and mobile phases.

# PRINCIPLE

- **The stationary phase:** is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.
- **The mobile phase:** is a developing liquid which flows through the stationary phase, carrying the samples with it.
- Components with more affinity towards stationary phase travels slower.
- Components with less affinity towards stationary phase travels faster.

# METHOD

- **Adsorbents used as Stationary Phase:**
  - **Inorganic:** Silica Gel, Kieselguhr, Aluminium Silicate, Bentonite.
  - **Organic:** Cellulose & its acetylates, Charcoal & activated Charcoal, Dextran Gel, Polyamides.
- **Solvents used as Mobile Phase:**
  - Petroleum ether, Benzene, Carbon tetrachloride.
- **Selection of Adsorbents and Solvents:**
  - ✓ Adsorbent should not adhere to glass plate.
  - ✓ Solvents should be of high purity.
  - ✓ Selected based on the nature of the compound to be separated (polar or non polar.)

# R<sub>f</sub> VALUE

- R<sub>f</sub> value indicates the position of migrated spots on chromatogram.
- In TLC the results are represented by R<sub>f</sub> value which represents the migration of solute relative to the solvent front.
- The R<sub>f</sub> value is calculated as:-

$$\text{R}_f \text{ Value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

# TLC - TECHNIQUE

## **STEP 1: Preparation of Slurry**

- A plastic, glass or aluminum sheet is coated with a thin layer of silica gel (adsorbent).
- Plates must be dried, activated and stored in desiccator until used.

## **STEP 2: Preparation of Tank**

- Solvent mixtures should be freshly prepared for analysis.
- Solvent is poured down side of the tank (1.5cm depth).
- Tank is covered with the glass lid and kept for saturation.

## **STEP 3: Application of Sample (Spot)**

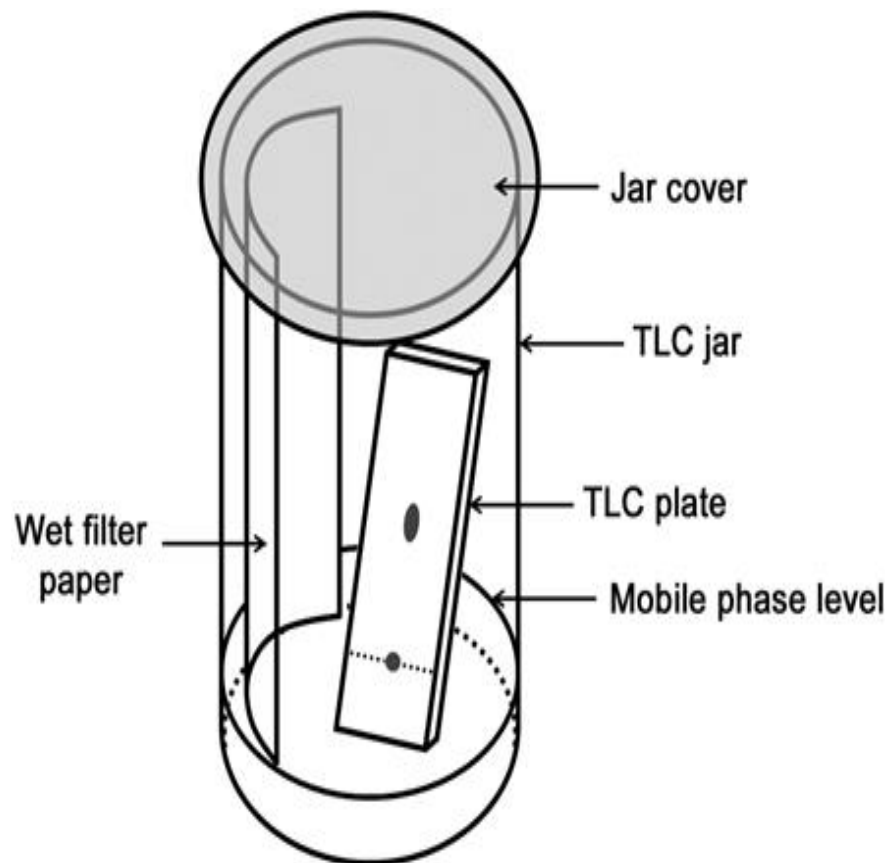
- A very small amount of sample (solution) to be analyzed is applied in a small spot with a capillary tube, ~1cm from the bottom of the TLC plate.



# TLC - TECHNIQUE

- The TLC is developed in a chamber which contains the mobile phase (solvent).
- When the mobile phase rises up the plate up by capillary action, the components dissolve in the solvent and move.

A TLC experimental set-up



# TLC - TECHNIQUE

- Individual components in the sample move up at different rates.
- More **polar analytes interact** more **strongly** with the **stationary phase** **move very slowly up.**
- More **nonpolar analytes interact** less **strongly** with the **polar silica gel** and **more strongly** with the less polar **mobile phase** **move higher up.**
- Once the solvent reaches the top (below ~1-2 cm) of the TLC sheet the plate is removed from the developing chamber and position of solvent front is marked.

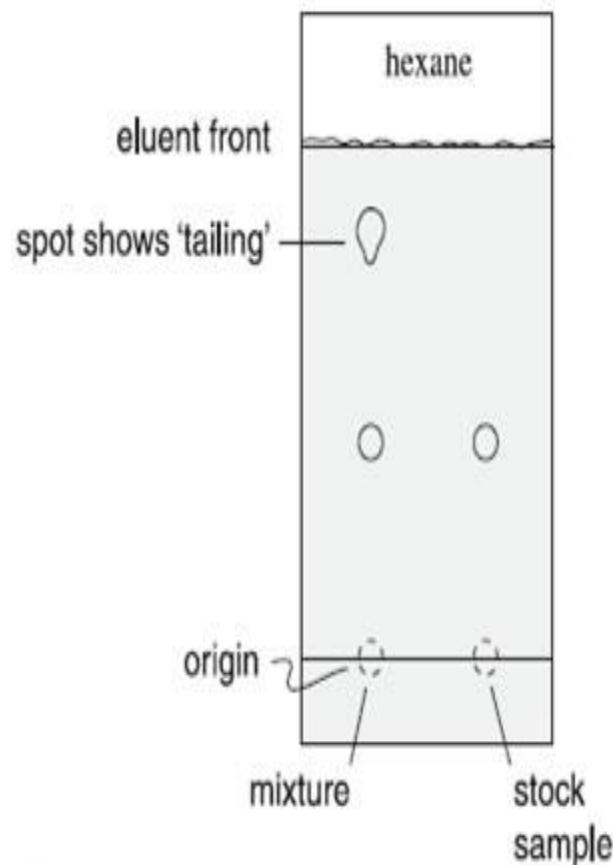
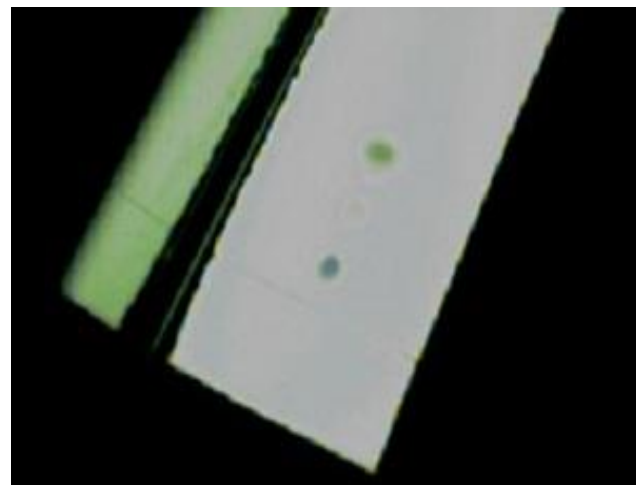
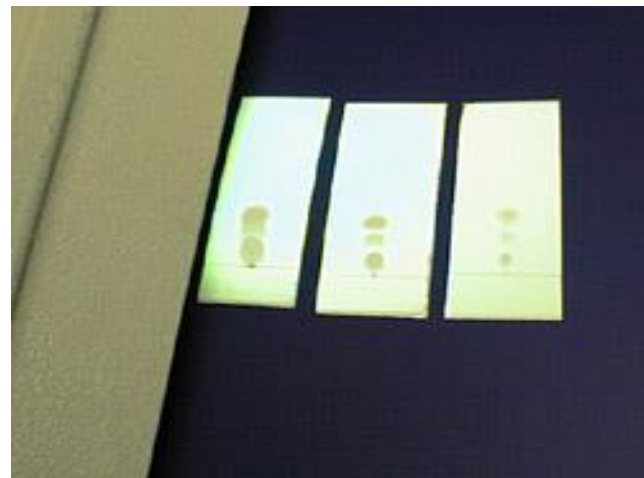


Figure 3 A completed chromatogram

# TLC - TECHNIQUE

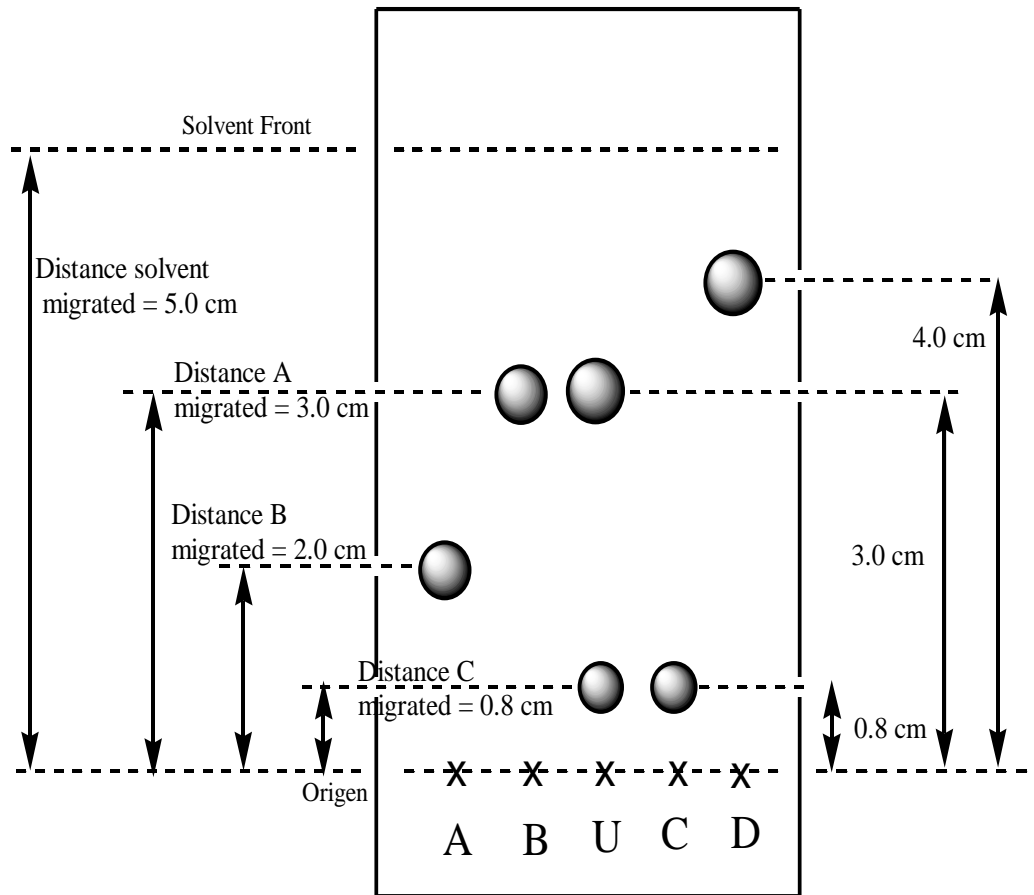
- The solvent is allowed to evaporate from the TLC sheet.
- As the compound is colorless, it can be visualized by suitable methods.
  - Lipids - Iodine vapors
  - Amino acids - Ninhydrin reagent.
- Also, manganese-activated zinc silicate (**fluorescent compound**), is added to the adsorbent that allows the visualization of spots under a black light (**UV<sub>254</sub> lamp**).
- Once visible, the  $R_f$  value of each spot can be determined.



<http://orgchemboulder.com/Technique/Procedures/TLC/TLC.shtml>

# TLC – CALCULATION OF R<sub>f</sub> VALUE

$$R_f = \frac{\text{Distance from centre of solute spot (cm) to the baseline}}{\text{Distance from solvent front to baseline (cm)}}$$



$$R_f(A) = \frac{2.0 \text{ cm}}{5.0 \text{ cm}} = 0.40$$

$$R_f(B) = \frac{3.0 \text{ cm}}{5.0 \text{ cm}} = 0.60$$

$$R_f(C) = \frac{0.8 \text{ cm}}{5.0 \text{ cm}} = 0.16$$

$$R_f(D) = \frac{4.0 \text{ cm}}{5.0 \text{ cm}} = 0.80$$

$$R_f(U_1) = \frac{3.0 \text{ cm}}{5.0 \text{ cm}} = 0.60$$

$$R_f(U_2) = \frac{0.8 \text{ cm}}{5.0 \text{ cm}} = 0.16$$

# RESULTS OF TLC - $R_f$ VALUE

- **Qualitative results of TLC**
  - expressed as fractions of 1.0
  - can be expressed from  $R_f$  values (Ex:  $R_f \times 100$ )
  - no more than two decimal places
- **$R_f$  values can be used to aid in the identification of a substance by comparison to standards.**
- **Comparison should be made only between spots on the same sheet, run at the same time.**
- Identical substances will have the same  $R_f$  value, whereas non- identical compounds will differ in their  $R_f$  values.

# APPLICATIONS

- TLC is used in qualitative and quantitative analysis to separate organic compounds and to test the purity of compounds.
- This technique is useful for separation of lipids, amino acids and sugars etc.
- It is useful in:
  - Identification of components of a mixture.
  - Following the course of a reaction,
  - Analyzing fractions collected during purification,
  - Analyzing the purity of a compound.