

Chromatographic Separations.

TLC & PC

BSc (Hons) Chemistry

Practical

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by

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Thin layer Chromatography (TLC)

It is a chromatographic technique in which an adsorbent layer is developed on glass plates. Elution is carried out with solvents as in the case of paper chromatography.

Steps involved in carrying out TLC

(i) Preparation of chromatoplates :

- (i) Plates (2.5 cm × 10 cm) are washed with chromic acid and then with water and dried in an oven.
- (ii) (a) A slurry of adsorbent is prepared; usually by shaking 10 g of adsorbent in 30 ml water in a conical flask and poured into the *applicator* and drawn immediately the applicator. After 15 mins. the plate is dried in an oven at 120° for 1-2 hrs. The plate may be preserved in a desiccator [See fig. 3(a) and 3(b)].

(b) The washed and dried glass slides are dipped into a slurry of adsorbent silical gel or alumina and taken out. The paste is wiped out from the edges with the thumb and forefinger and then dried.

(ii) Application of the sample :

The sample is applied in solution form with a suitable solvent by a fine capillary on a pencilled line near one edge of the chromatoplate [See fig. 3(c)]. The spot is allowed to dry.

(iii) Developing :

In case of TLC, ascending technique is always applied. The chromatoplate after spotting is made to stand in a solvent or mixture of solvents in a jar preferably vertically fitted with a lid. when the solvent reaches almost the top of the plate the plate is taken out [See fig. 3(e)].

(iv) Location :

After developing, the spot is located at its new position, easily if the sample is coloured or by placing the chromatoplate in a chamber containing a few crystals of iodine. Almost all organic compounds develop a brown colour in contact with iodine vapour. These spots are known as *chromatograms*. Then R_f value is calculated in the usual procedure.

Separation of leaf pigments of spinach leaves by TLC

Chemicals required :

- (i) Fresh spinach leaves
- (ii) Silical gel G for TLC
- (iii) Chloroform
- (iv) Petroleum ether (60°-80°)
- (v) Acetone

Apparatus required :

- (i) Mortar and pestle
- (ii) glass funnel
- (iii) cotton
- (iv) glass plate (12 cm × 4 cm)
- (v) Measuring cylinder
- (vi) Capillary tubes
- (vii) Beaker (50 ml)
- (viii) Solvent chamber

Procedure :

- (i) **Preparation of chromatoplate :**
- (ii) **Application of the sample :** Extract of the spirach leaves with petroleum ether : acetone - 80:20 is spotted with capillary tube after filtering through cotton.
- (iii) **Developing :** Developing solvent is petroleum ether : acetone (80:20) and dried the chromatogram.
- (iv) **Determination of R_f values :**
 - A grey spot ($R_f \Rightarrow 0.6$)
 - A yellow spot ($R_f \Rightarrow 0.53$)
 - Two green spots ($R_f \Rightarrow 0.49$ and 0.45)
 - A greenish yellow spot ($R_f \Rightarrow 0.4$)

Separation of a mixture of dyes by TLC

Chemicals required :

- (i) Methylene blue
 - (ii) Fluorescein
 - (iii) Silica gel G
 - (iv) Chloroform
 - (v) Methanol
 - (vi) Ethanol
- } solvents

Apparatus required :

- (i) TLC plate (12 cm × 4 cm)
- (ii) Solvent chamber
- (iii) Capillary tube or micropipette for spotting

Procedure :

- (i) Preparation of chromatoplate
- (ii) Solvent chamber is made ready with chloroform : methanol = 90:10
- (iii) 10 ml of each dye is dissolved in 50% ethanol (10 ml) for spotting.
- (iv) The chromatoplate is placed in the solvent chamber and developed.
- (v) The plate is taken out and dried. R_f values of the two dyes are determined.
Fluorescein ($R_f \Rightarrow 0.56$)
Methyferre blue ($R_f \Rightarrow 0.16$)

Seperation and identification of sugars present in a given mixture by paper chromatography

Apparatus required :

- (i) Chromatographic chamber
- (ii) Oven
- (iii) Chromotographic filter paper
- (iv) Dropper
- (v) Reagent bottles etc.

Solutions :

- (i) Glucose $\Rightarrow 1 \text{ mg ml}^{-1}$
- (ii) Fructose $\Rightarrow 1 \text{ mg ml}^{-1}$
- (iii) Sucrose $\Rightarrow 1 \text{ mg ml}^{-1}$
- (iv) Mixture of unknown sugars

Solvents (Developer) :

Normal butanol : pyridine : Water = 6:4:3 (by volume)

Spraying reagent :

1.66 g of Phthalic acid and 0.91 ml of aniline are dissolved in 48 ml of n-butanol. This mixture is diluted with 48 ml of ether containing 4 ml of water.

Procedure :

A strip of whatman No. 1 chromatographic paper (40 cm × 5 cm) is taken. A line is drawn about two inch from the top of the paper with a pencil. Four small circles are drawn with a pencil and marked G (for glucose), F (for fructose), S (for sucrose) and M (for mixture). Two drops of each solution are given as a spot in the respective positions with the help the capillary tubes. These spots are dried in air for half an hour. Meanwhile the chromatographic chamber is kept undisturbed with the proper lid for equilibration with the solvent vapour. The spotted paper is placed in the chromatographic chamber. Paper is allowed to elute till the wet surface reaches the other end of the paper. The paper is taken out and allowed to dry. The spraying reagent is spread and paper is heated at 110° in an air oven, so that brownish spots are marked with pencils and R_f values are calculated.

Experimental results :

No. of Observations	Sugars	Distance travelled by the component from origin	Distance travelled by the solvent front
1.	Glucose		
2.	Fructose		
3.	Sucrose	—	—
4.	Mixture		

The sugars are identified from their respective R_f values -

No. of Observations	Sugars	Distance travelled by the component from origin	Distance travelled by the solvent front
1.	Glucose		0.17
2.	Fructose		0.25
3.	Sucrose		0.08

Paper chromatographic separation of inorganic ions :

Chemicals required :

- Solutions (a) $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, (b) $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, (c) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. (5 mg each) in distilled water. Metal ion $\Rightarrow 1 \text{ mg ml}^{-1}$ in solution.
- Spraying agent : 10 mg rubeanic acid in 10 ml rectified spirit.
- Unknown solution : Any of the above solutions.