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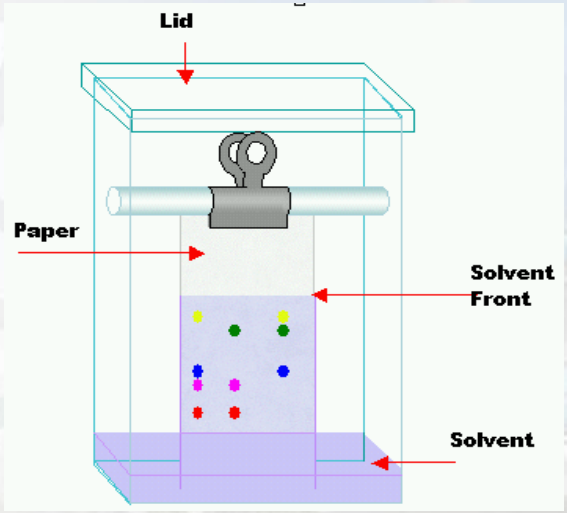
الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

THIN LAYER CHROMATOGRAPHY (TLC) METHOD



By
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INTRODUCTION

- TLC is universal analytical technique in chemical analysis for organic and inorganic matter

- HISTORY-
- In 1938 Izmailov and Shraiber describe basic principle used for seperation of plant extract.
- In 1958 Stahl worked on preparing plates and separation of wide variety of compound.

- TLC is simple and rapid method using thin layer of adsorbent on plates.

ADVANTAGES

- Low cost
- Short analysis time
- All spots can be visualized
- Adaptable to most pharmaceuticals
- Uses small quantities of solvents
- Requires minimal training
- Reliable and quick
- Minimal amount of equipment is needed
- Densitometers can be used to increase accuracy of spot concentration

- It requires little equipment
- Requires a short time for separation
- It is more sensitive
- Very small quantity of sample required for analysis
- The method used for adsorption, partition, ion exchange chromatography
- Components which are saparated can be recovered easily
- Quantitative separations of spot and zone are possible.

PRINCIPLE OF TLC

- TLC is categorised under both adsorption and partition chromatography.
- Separation of component may result due to adsorption or partition or both phenomenon depend upon nature of adsorbent used on plate and solvent system used for development.

TECHNIQUE

- TLC is carried out on glass, aluminum or plastic plate which is coated with thin uniform layer of adsorbent such as silica gel
- Plate is activated
- Sample applied by capillary or microsyringe at bottom of plate
- After drying the plate is placed in a suitable tank
- Solvent moves by capillary action
- Resolving sample mixture into discrete spot
- Separated spots are located and identified by various physical and chemical methods

CHROMATOGRAPHY

- STATIONARY PHASE
- MOBILE PHASE

ADSORBENT

Adsorbent used such as silica gel, alumina, cellulose

Factors for choosing the right adsorbent

1. **Characteristic of compound to be separated**
2. **Solubility of compound**
3. **Nature of substance to be separated**
4. **The compound does not react chemically with adsorbent.**
5. **Adsorbents do not adhere to TLC plate.**

COMMON ADSORBENTS - INORGANIC

Silica gel
Alumina
Calcium phosphate
Glass powder
Magnesium silicate
Calcium silicate
Ferric oxides
Zinc carbonate

COMMON ADSORBENTS - ORGANIC

Celluose
Charcoal & activated carbon
Starch
Sucrose
Manitol
Dextran gel

MOBILE PHASE – SOLVENT SYSTEM

- Choice of mobile phase depends on nature of substance to be separated.
- And also depends on adsorbent material to be used.
- Polarity of solvent and substance to be separated plays important role in selection.
- Purity of solvent is also important.

FACTOR AFFECTING MOBILE PHASE

- Nature of the substance to be separated.
- Nature of the stationary phase used.
- Mode of chromatography.
- Nature of separation.
- Suitable eluents are usually selected by trial and error method, literature review
- The solvent used should be of high purity.
- Other factors include polarity, solubility etc.
- Combination of two solvents gives better separation than with a single solvent

COMMON SOLVENTS

1. Petroleum ether
2. Benzene
3. Carbon tetrachloride
4. Chloroform
5. Diethyl ether
6. Ethanol
7. Methanol
8. Acetone
9. Dichloromethane
10. Diethyl form amide

SAMPLE ANALYSIS

The area of application is kept as small as possible for sharper and greater resolution of sample.

For preparative work, the sample is applied in a narrow band

The pipette or syringe is used for applying sample.

The spot should be within 2-5 mm diameter.

For preparative work, sample is applied up to 4 mg at the starting line

DEVELOPMENT

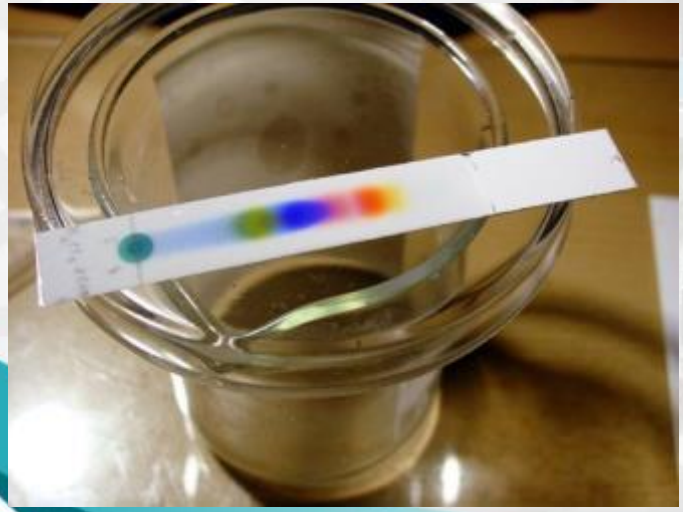
TLC plate is placed vertically in a rectangular chromatography tank or chamber .

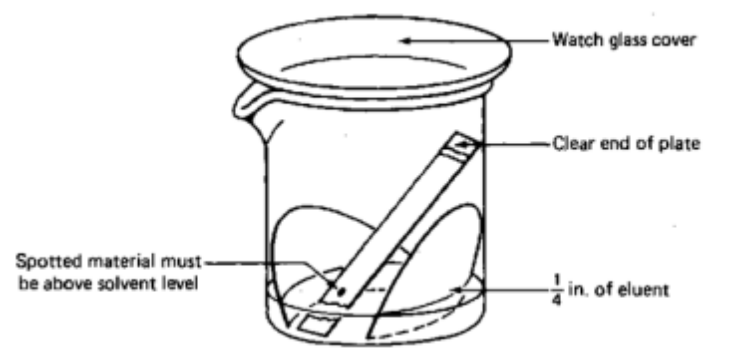
Glass and stainless steel are suitable chamber.

If tank is not saturated, solvent will evaporate and affect the Rf value.

Development should be carried out at room temp by covering chamber with glass plate.


CAMAG
CAMAG

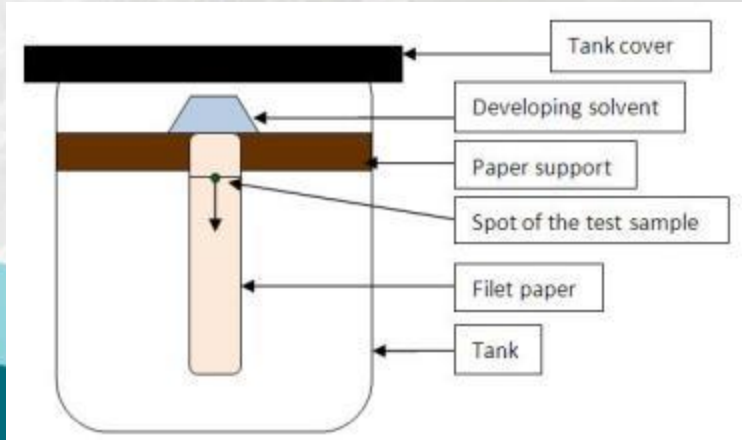




Ascending development-

plate after spotting placed in chamber .
and flow of solvent from bottom to top.

Descending –
in this flow of solvent from reservoir to plate by means of filter paper strip.
solvent move from top to bottom.

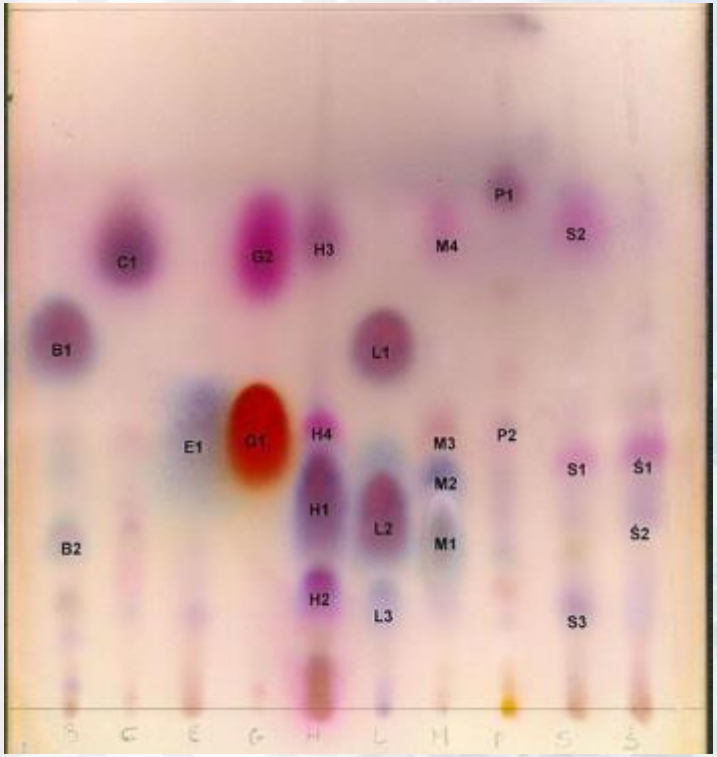


DETECTION

Physical method includes ultravoilet, fluoroscence or radioactive counting.

In chemical method by spraying
Conc. Sulphuric acid is used as locating agent to produce coloured spot which visible in dayligjt
Iodine vapoure use for organic substance

DETECTION


C1
G2
H3
M4
P1
S2
B1
L1
E1
Q1
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M2
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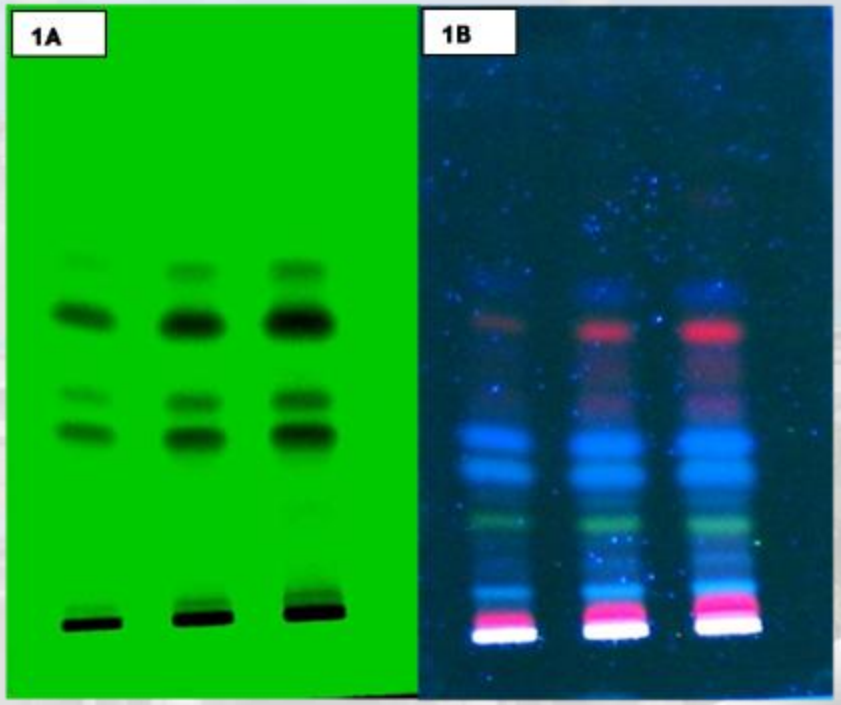
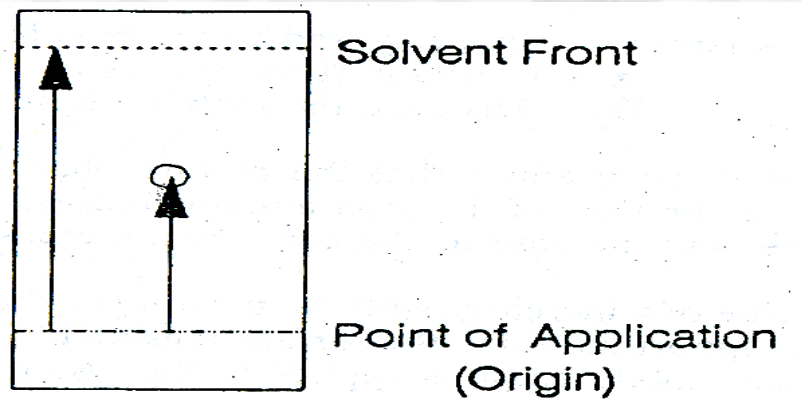

1A
1B

R_f value (Retardation factor)

$$R_f = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}$$



R_f value is constant for each component only under identical experimental condition.

It depends on following factors-

- Nature of adsorbent
- Mobile phase
- Thickness of layer
- The temperature
- Equilibration
- Dipping zone
- Chromatographic technique

APPLICATIONS

1. Purity of sample
2. Examination of reaction
3. Identification of compound
4. Biochemical analysis
5. In chemistry
6. In pharmaceutical industry
7. In seperation of multicomponent pharmaceutical formulation
8. In food and cosmetic industry

THANK YOU