# THIN LAYER CHROMATOGRAPHY( TLC)

## (BY:RANJEET RANJAN)

The technique of thin layer chromatography closely resemble to those of column and paper chromatography. In thin layer chromatography, partition however occurs on a layer of finely divided adsorbent, which is supported on a glass plate. This chromatography using thin layers of an adsorbent held on a glass plate or other supporting medium is known or thin layer chromatography.

## ADVANTAGES OF TLC:

1) It is an elegantly simple procedure for chromatography in all kinds of solid-liquid & liquid system.
2) It is performed in analytical & operative in large preparation scale.
3) Applicable to almost all chemical compounds.
4) Because of its rapid speed it can be employed for checking the course of chemical reaction; in laboratory as wall as industrial scale.
5) It has great resolving power, and so can be used for uncovering adulteration of foods and drugs caused by improper storage or incorrect use.
6) TLC can readily detect compounds, which are encountered in trace amount, due to high sensitivity. (e.g. Narcotics; Air pollutant; pesticides etc.)

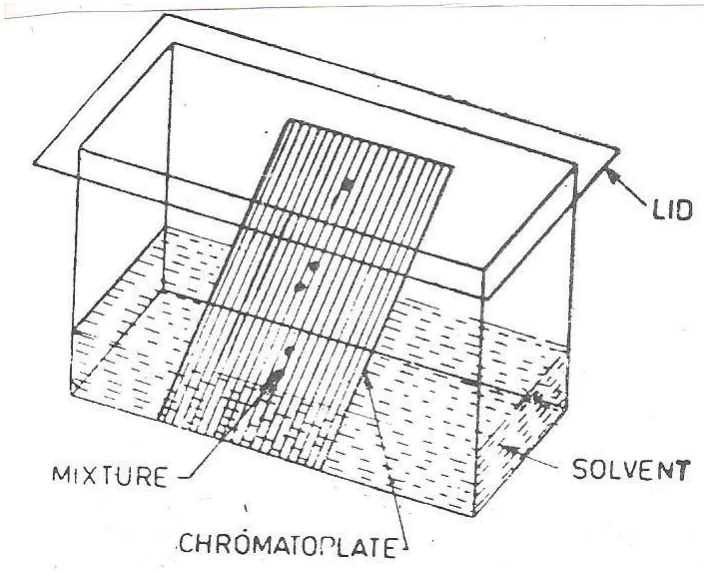


Fig.6- Apparatus for Thin layer chromatography

## Basic operations involved in TLC:

### 1) Methods for production of thin layer plates:

Spreading, pouring, spraying or dipping can achieve coating of glass plates with adsorbent layer. Most uniform layers are obtained by spreading.

Layers are classified in to two types:

a) Solid layers.

b) Loose layer.

i. For solid layers a uniform layer of the adsorbent material is applied to a clean glass plate with help of applicator.

ii. For loose layer may be prepared by.

I. Pouring of suspension on plate.

II. Dipping of plates in suspension.

III. Spraying with thin suspension.

### 2) Application of sample on chromatoplates:

In analytical TLC, 0.1% solution of sample is applied to the plates with the help of capillaries, micropipettes. The solvent in which the substances are dissolved is allowed to evaporate. Solutions are applied as single spots in a row along one side of the plate about 2 cm from edge. It is desirable to chromatograph a sample to be analysed in different

amounts e.g. 1,5,10 and 60 Ng on one plate. The amount of sample that can be applied in one spot depends upon:

- Thickness of layer:

  In absorption TLC, whore 25 mm thick layer is used 70-500 Ng of mixture of lipophilic substances can be fractioned.

- Principle of chromatography employed.

## 3) Choice of adsorbent:

The common adsorbents used in TLC are silicagel alumnae, Kieselguhr and powdered cellulose, coating materials used in TLC, depends upon their acidity or basicity, Activity and separating mechanism. So it will depend and change according to nature of compound.

Normally a 0.25 mm thick layer can be prepared by spreading aqueous slurry of adsorbent with applicator on glass plates. Thick layers (1-2 mm) of silica gel can be prepared by slurring silica gel G with water in ratio 25:40. The layers are air dried for about leman and then activated by heating in an over at about 11 for 2 hrs.

A binding a gent usually plasters of Paris is after incorporated to hold adsorbent firmly.

## Preparation of thin layers in plates:

A large number of applicators are commercially available which are used for coating the glass plates with different adsorbent layers of uniform thickness. The various methods of preparing layers are:

I. Pouring:

   A measured amount of slurry is put on a given size of plate, which is kept on a level surface. The plate is then tipped back and forth to spread the slurry uniformly over the surface.

II. Dipping:

   In this method plates are prepared by dipping then two at a time back to back in $CHCl_3$ slurries of adsorbent.

III. Spraying:

   A small point sprayer for distribution of the slurry on glass plate. This is not used because it is difficult to obtain uniform layers on a single plate and also there may be a variation from plate to plate.

IV. Spreading:

The slurry is an applicator. This is either moved over the stationary plate or it is tied stationary and the plate is posted or pulled through. The apparatus developed by state for getting actsorpont layers insists of aligning tray in which the plates are sot in a line and spreader, which takes up the spreading mixture and applies it uniformly on thin layer.

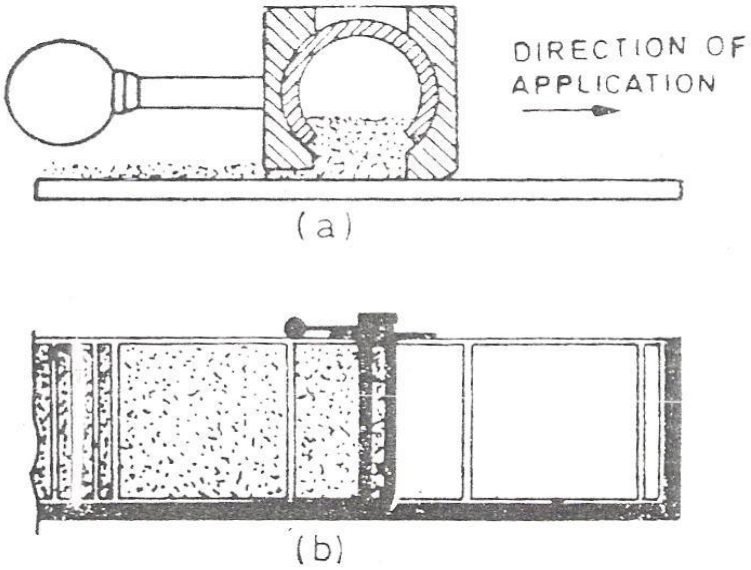


Fig.7 (a) Operation of thin-layer spreader.

V. Precoated plates:

Ready to use thin layers of the common aolsortmts are now available pirouetted on glass or plastic stets. These plates are quite expensive. The thickness of precoated plastic sheets usually varies from 0.1 to 0.2 mm.

4) Choice of solvent:

If one does not know about the nature of communists of the mixture to be separated the best lucent is found by trial and error using small very rapid running TLC plates. If the nature of component is known then it is possible to know a suitable solvent by using original stain's triangle, which has inter-relating adsorbent activity, nature of solute and nature of solvent. If the triangle is rotated so that the corner M points to the type of mixture to be separated, this specifies at corners S and E respectively, the necessary activity of the adsorbent and the optimum polarity of the event with mixtures of solvents, it is possible to obtain, intermediate elution behaviour. Mixtures of two or more solvent of different polarity often give better operations than chemically homogenous solvents.

Sine polar solvents produce the greater migration, a better operations is effected in their presence.

## 5) Detecting reagents:

Compounds separated by thin layer chromatography are colourless their positions are thus located or detected with help of some reagents known as locating or detecting reagents. Iodine vapour and sulphuric acid (mixed with aromatic aldehydes or oxidising agents like KMNO4; 1 + NO 3; chromic acid etc.) are common locating agents. Iodine forms a number of coloured loose complexes with a variety of compounds. Sulphuric acid also forms coloured complexes, which are visible in daylight and ultraviolet light.

The process of detecting the sports on the sheet after completion of the development is called visualisation. Where over there is a sport, the fluorescence of the sport is quenched with respect to the background upon scanning with a U.V lamp. Amino acids are detected by spraying ninhydrin on the sheep, to get fluorescent derivatives.

## 6) Developing chamber:

In the TLC plates are usually developed by placing them on edge in jar containing a 0.5 – 10 cm layer of solvent. It is a type of chromate jar or a common tank, is used which is closed form all sides. According to requirements the jar is jacketed and connected to a thermostat in order to develop a chromatogram at constant temperature.

Usually it is possible to develop micro chromatoplatos in body food jars or in glass beaker covered with aluminium foil. The jar is saturated with solvent vapour. by lining the inside of jar with filter paper, as it has following advantage

- It yields straight solvent fronts
- Developing time is reduced to one third.
- RF values are must less than in unsaturated tanks.

## 7) Development & Detection:

Chromatoplates are usually developed once with a single solvent by either horizontal, ascending or descending elution. Some techniques are as:

### 1. Ascending or vertical development:

The sample is spotted at one end of the plate and then developed by the ascending technique used in paper chromatography. The plates are placed vertically in a container saturated with developer vapour and the solvents as ends from bottom to top.

## 2. Horizontal development:

It is useful with adhering thin layers and loss layers. The sample is placed in the centre of the plate and developed either by slowly dripping solvent on it from micropipette. This procedure is also called circular TLC.

## 3. Multiple developments:

In this the development is carried repeatedly with same solvent in same direction each time after drying.

## 4. Stepwise development:

It is carried out consecutively with two different solvents but is same direction. One of the solvents is run to a height of 15 – 18 cm and the other to 10 – 12 cm.

## 5. Gradient development:

Sometimes it is advantageous especially when fractionating compounds of widely different properties change the composition of the solvent continuously during chromatography. This technique is called gradient elution. In this technique the chrumatoplate is lowered into a jar containing a solvent and then a second more polar solvent is added in chamber with help of burette. The elute is continuously stirred. As a result polarity of the former solvent polarity of the former solvent is modified by second solvent.

## 6. Continuous development:

When there are small differences in RF values, the development distance is increased in order to achieve complete separation. This is done by continuous development in which a solvent is forced to run over the edges of the chromatoplates. Where it can be collected instead of being left to evaporate.

## 7. Two dimensional development:

It is also possible to develop the square plates in two dimensions. A sample is spotted in a corner of the plate and then developed consecutively, in two directions either with two same solvent or different solvents.

The location of compound after development is dire by spraying different developing reagents. If substance shows dark spots against a florescent background they can be made visible under a U.V. lamp

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## Applications:

- For checking purity and progress of reaction.
- For purification as well as identifying compounds. Such as amino acids; protein; peptides & Antibiotics.