

## Paper and Thin Layer Chromatography

Paper and Thin layer chromatography Method for testing the purity of compounds and identifying substances.

Useful technique because it is relatively quick and requires small quantities of material.

# PAPER CHROMATOGRAPHY

Paper chromatography is an analytical technique used for separating and qualitatively analyzing the components of a mixture this method also used for testing the purity of substances and also identifying them .

The main principle of this method is that the components of the mixture will get separated between a stationary phase and a Mobile phase .

Most commonly used stationary phase is a high quality filter paper and the mobile phase will be a solution that will travel up the stationary phase , carrying the samples along with it .

The separation of the components depend on the strength with which they get adsorbed to the stationary phase versus how readily they dissolve in the mobile phase .

Due to the different molecular structures of each component , each will have a different solubility in the mobile phase causing them to separate .

More soluble a component is , more the distance travelled by it .

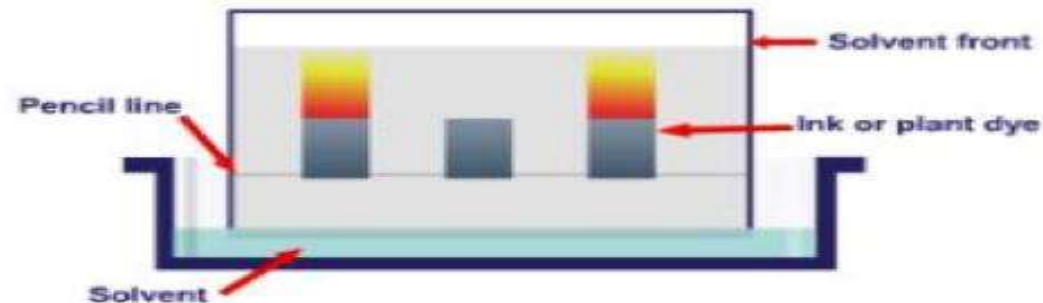
Paper chromatography - is one of the types of chromatography • procedures which runs on a piece of specialized paper.

Phases in which substance will be separated

The stationary phase is a water molecules bound to an (inert) cellulose matrix

Mobile phase is the solvent (in our lab butanol with acetic acid)

[solutes are separated according to their solubility in mobile phase, or their attraction to the stationary phase] -(Depend in the polarity).



Two types of paper chromatography:

## Paper Chromatography •



Descending

-In this method, the solvent is kept in a trough at the top of the chamber and is allowed to flow down the paper. -The liquid moves down by capillary action as well as by the gravitational force. -In this case, the flow is more rapid as compared to the ascending method.



Ascending

In this method, the solvent moves upward against gravitational force. The only force that cause the motion of solvent and the compounds is capillary force. So the speed of the process is slow.

# Thin Layer Chromatography

is a technique used to isolate non-volatile mixtures. The experiment is •  
conducted on a sheet of aluminum foil, plastic, or glass which is coated  
with a thin layer of adsorbent material. The material usually used  
is [aluminium oxide](#), cellulose, or silica gel.

On completion of the separation, each component appears as spots •  
separated vertically. Each spot has a retention factor ( $R_f$ ) expressed as:

$$R_f = \text{dist. travelled by sample} / \text{dist. travelled by solvent} \bullet$$

The factors affecting retardation factor are the solvent system, amount of •  
material spotted, adsorbent and temperature. TLC is one of the fastest,  
least expensive, simplest and easiest chromatography technique. The  
method is rapid and separations can be completed in less than one hour. •  
TLC is a widely used.

Phases in which substance will be separated

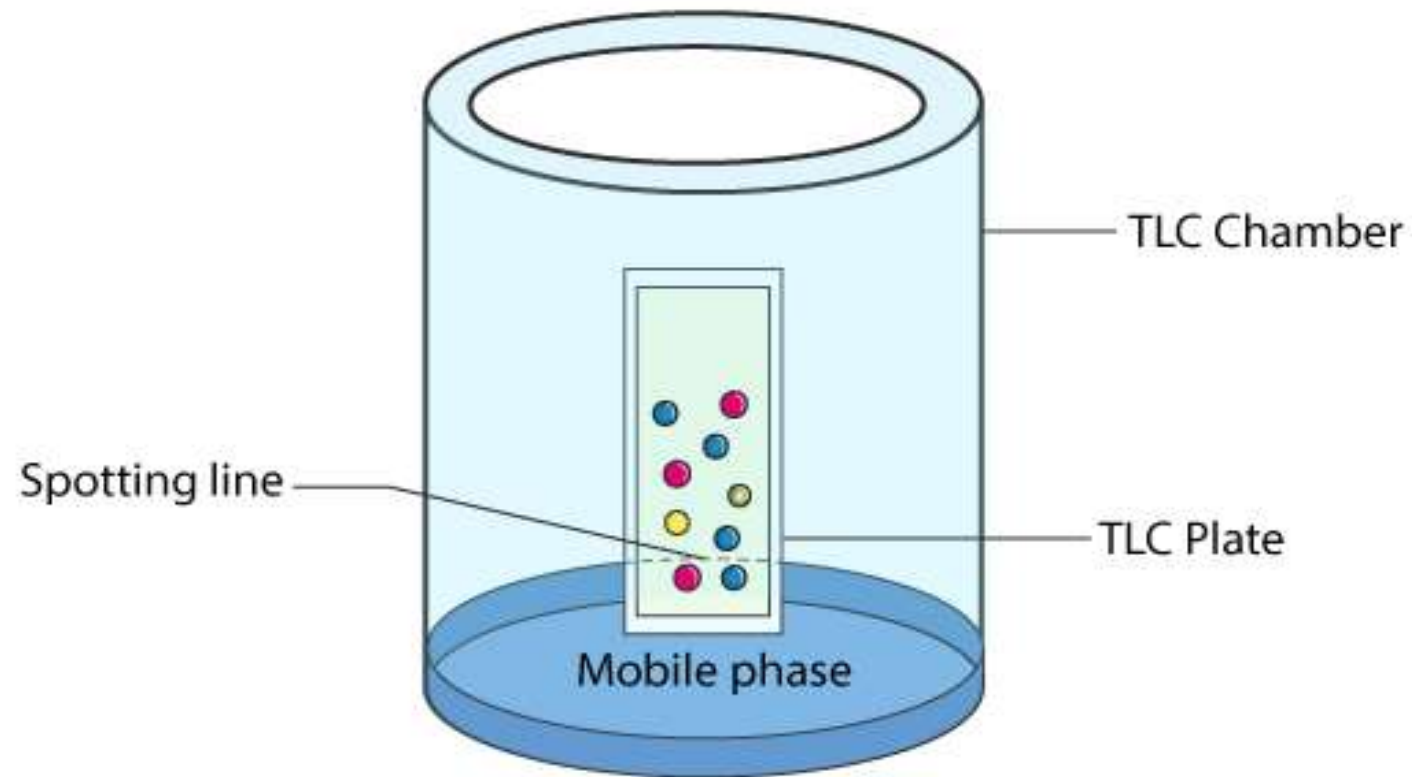
The stationary phase  
[Stationary phase: adsorbent]

Mobile phase  
is the solvent (in our lab butanol  
with acetic acid)

Is a thin layer (0.25 – 0.5 mm) of adsorbent . like silica gel - a polar substance- , [aluminum oxide or magnesium silicate] spread uniformly over the surface of a flat, inert surface of the glass plastic plate. [The stationary phase+ support medium should be inert].

Mixture of solvents

# THIN LAYER CHROMATOGRAPHY



# Thin Layer Chromatography Principle

Like other chromatographic techniques, thin layer chromatography (TLC) • depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the [separation of the mixture](#) is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.



# Thin Layer Chromatography Experiment

- The stationary phase that is applied to the plate is made to dry and stabilize.
- To apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil.
- Apply sample solutions to the marked spots.
- Pour the mobile phase into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase.
- Place the plate in the TLC chamber and close it with a lid. It is kept in such a way that the sample faces the mobile phase.
- Immerse the plate for development. Remember to keep the sample spots well above the level of the mobile phase. Do not immerse it in the solvent.
- Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber.



# Thin Layer Chromatography Applications

The qualitative testing of Various medicines such as sedatives, local anaesthetics, anticonvulsant tranquilisers, analgesics, antihistamines, steroids, hypnotics is done by TLC.

TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.

Thin layer chromatography can be used to identify natural products like essential oils or volatile oil, fixed oil, glycosides, waxes, alkaloids, etc

It is widely used in separating multicomponent pharmaceutical formulations.

It is used to purify of any sample and direct comparison is done between the sample and the authentic sample

It is used in the food industry, to separate and identify colours, [sweetening agent](#), and preservatives

It is used in the cosmetic industry.

It is used to study if a reaction is complete.

# Disadvantages Of Thin Layer Chromatography

- Thin Layer Chromatography plates do not have longer stationary phase.
- When compared to other chromatographic techniques the length of separation is limited.
- The results generated from TLC are difficult to reproduce.
- Since TLC operates as an open system, some factors such as humidity and temperature can be consequences to the final outcome of the chromatogram.
- The detection limit is high and therefore if you want a lower detection limit, you cannot use TLC.
- It is only a qualitative analysis technique and not quantitative.

# The major difference between Thin layer chromatography and paper chromatography?

(TLC).	paper chromatography
Wide choice between different adsorbents[stationary phase].	cellulose
It has better resolution and to allow for quantitation.	Low resolution but also allow for quantitation.
compact zonal spread[concentrated for quantitation analysis in need ].	Expanded zonal spread[ not concentrated for quantitation analysis in need ].