Thin-Layer Chromatography -TLC

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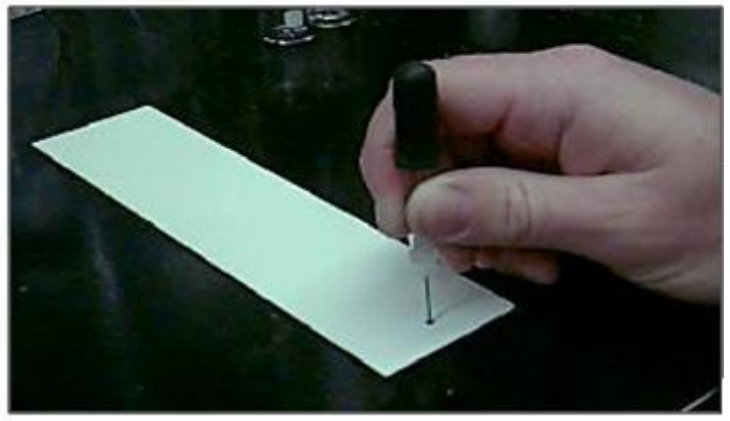


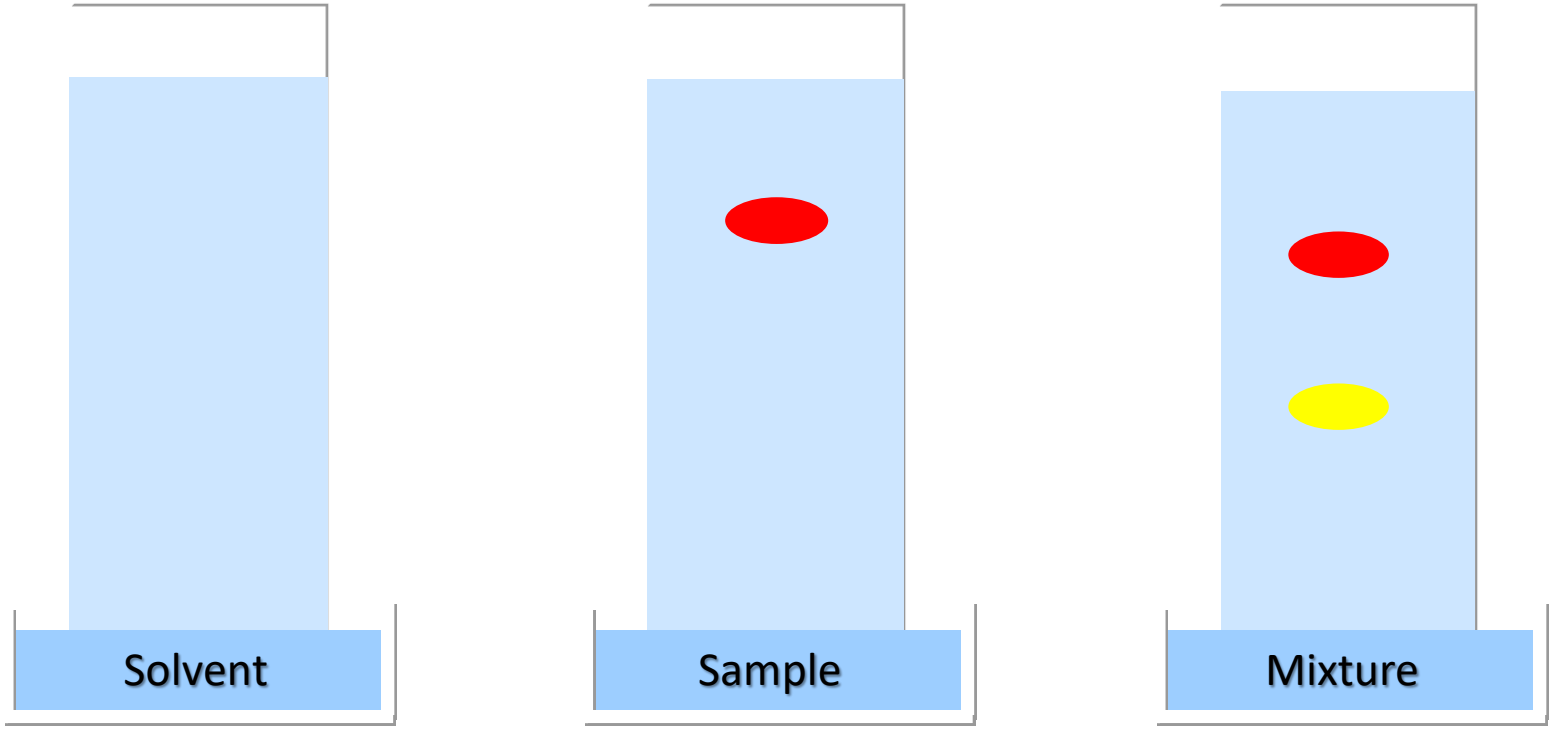
❑ System Components

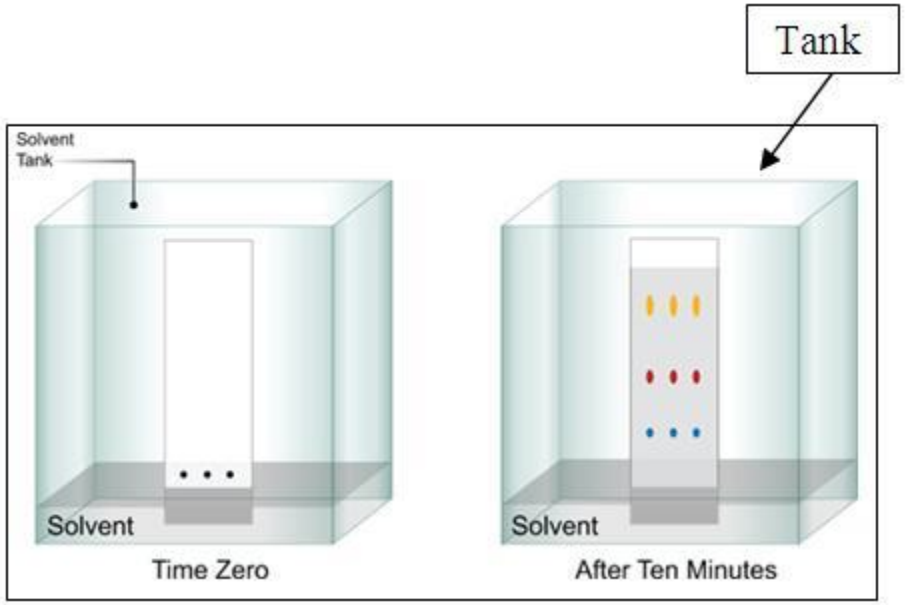
TLC system components consists of

- **TLC plates**, preferably ready made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.
- **TLC chamber**. This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.
- **Mobile phase**. This comprises of a solvent or solvent mixture The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.

Principles of (TLC)

Chromatography is carried out on active particulate material (silica gel or alumina) dispersed on an Inert support (flat glass plates or flat polymeric films)




Tank
Solvent
Tank
Solvent
Time Zero
Solvent
After Ten Minutes

Principle of separation

Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. The separation depends 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 move faster. A competition therefore, is set up between the silica gel plate and the development solvent for the spotted compound. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate.

The outcome depends upon a balance among three polarities - that of the plate, the development solvent and the spot material. If the development solvent is polar enough, the spot will move some distance from its original location. Different components in the original spot, having different polarities, will move different distances from the original spot location and show up as separate spots. When the solvent has traveled almost to the top of the plate, the plate is removed, the solvent front marked with a pencil, and the solvent allowed to evaporate

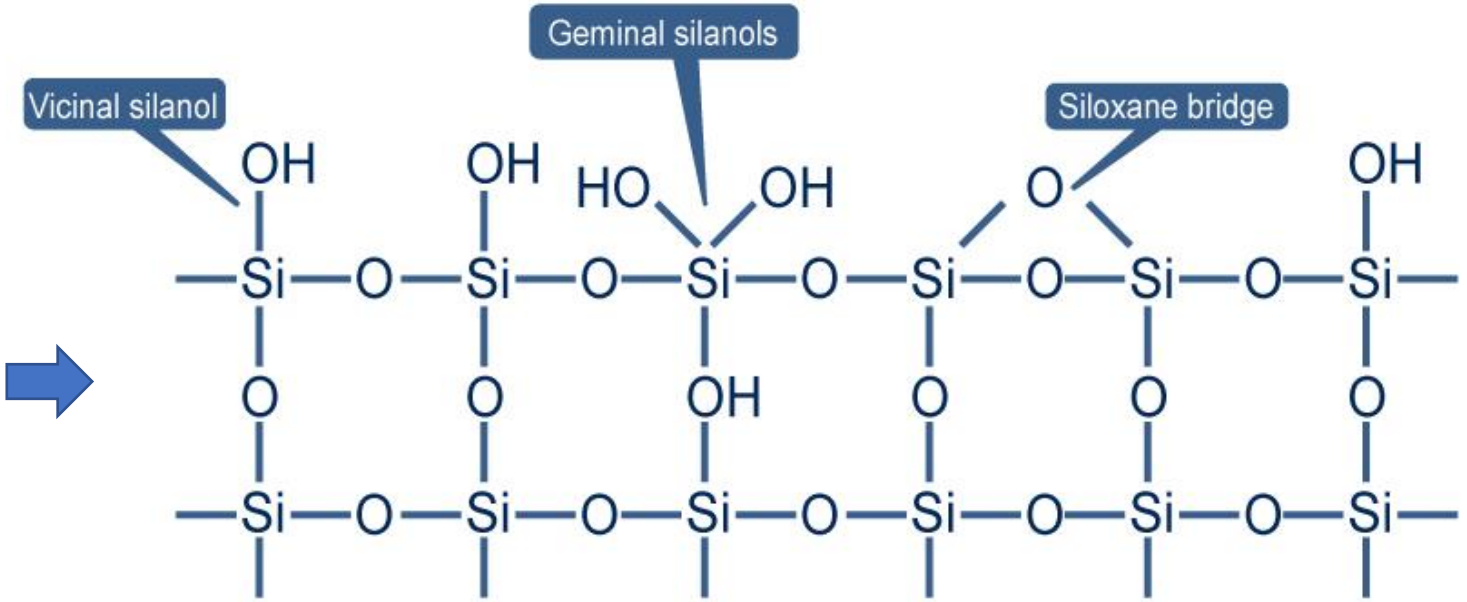
A deep insight

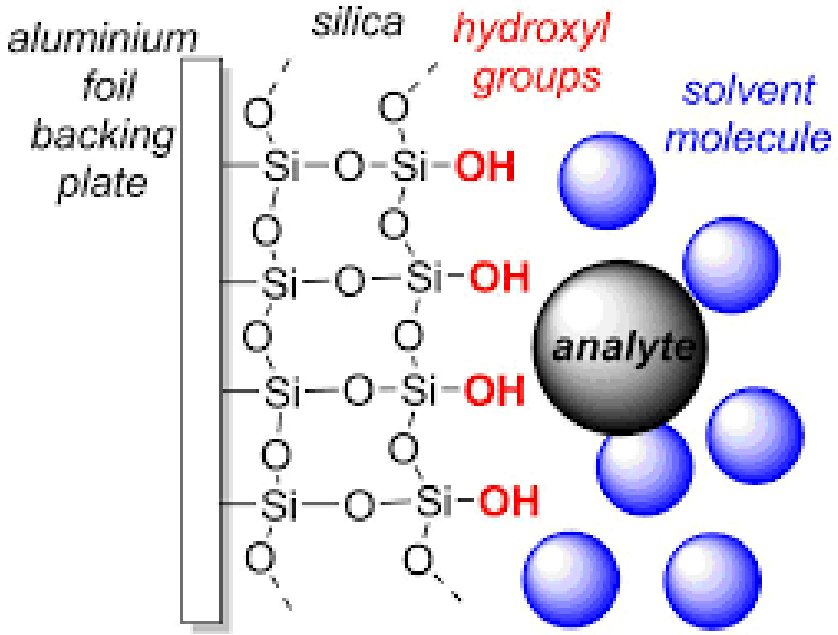
Silica gel consists of a three-dimensional network of thousands of alternating silicon and oxygen bonds, with O-H groups on the outside surface. Silica gel is simply very finely ground very pure sand. It should be noted that silica gel is highly polar and is capable of hydrogen bonding. As the solvent travels up the plate, over the spot, an equilibrium is set up, as development solvent competes with the TLC plate for the solute. The silica gel binds to the solute and the development solvent tries to dissolve it away, carrying the solute(s) along as the solvent travels up the plate. A balance of intermolecular forces determines the position of equilibrium and thus the ability of the solvent to move the solute up the plate.

The balance depends upon (1) the polarity of the TLC plate (constant and high), (2) the polarity of the development solvent (can be varied by using different solvents), and (3) the polarity of the compounds in the spot (this varies depending upon what compounds are in the spot).

For example, if a sample consists of two components, one more polar than the other, the more polar will tend to stick more tightly to the plate and the less polar will tend to move along more freely with the solvent. Using a more polar development solvent would cause both to move along further. If the approximate structures of the solutes are known, it is possible to make an educated guess as to what solvent or mixture of solvents to use. In practice though, for a given mixture of compounds to be analyzed, a solvent or mixture of solvents is chosen by trial and error to give the best separation. For compounds having very low polarity however, a lower-polarity solvent may be more effective in moving the solute up the plate.)

Silica Gel structure



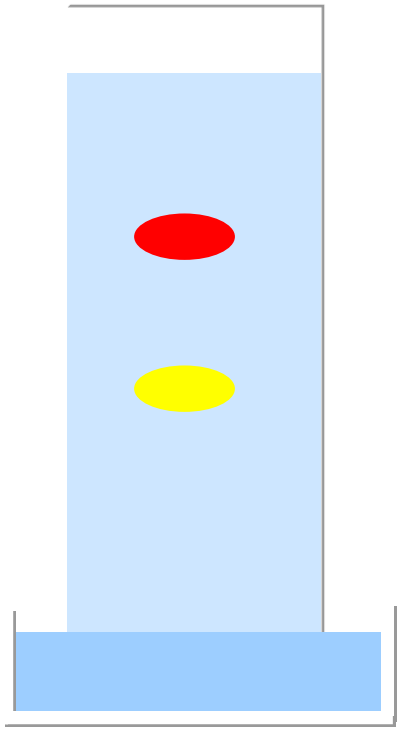


- n-Hexane
- Cyclohexene
- Toluene
- Benzene
- Diethyl ether
- Chloroform
- Dichloromethane
- 1,2 dichloroethane
- Acetone
- Ethyl acetate
- Acetonitrile
- Propanol
- Methanol
- Acetic acid
- Water.

Increasing polarity

Basic Steps of TLC Technique

- **Preparation of the Plate**
- **Sample Application**
- **Chromatogram Development**
- **Locating of the Spots**



The Procedure

TLC Plates - ready-made plates are used which are chemically inert and stable. The stationary phase is applied on its surface in the form of a thin layer. The stationary phase on the plate has a fine particle size and also has a uniform thickness. Slurry of the active material in appropriate solvent is uniformly spread over the plate. Air-drying overnight, or oven-drying (Depending upon the nature of the solvent)

TLC Chamber - Chamber is used to develop plates. It is responsible to keep a steady environment inside which will help in developing spots. Also, it prevents the solvent evaporation and keeps the entire process dust-free.

TLC Mobile phase - Mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate-free. The higher the quality of purity the development of spots is better.

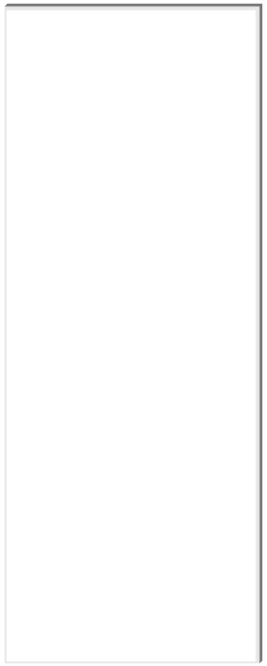
TLC Filter Paper – It has to be placed inside the chamber. It is moistened in the mobile phase.

Preparation of the Plate

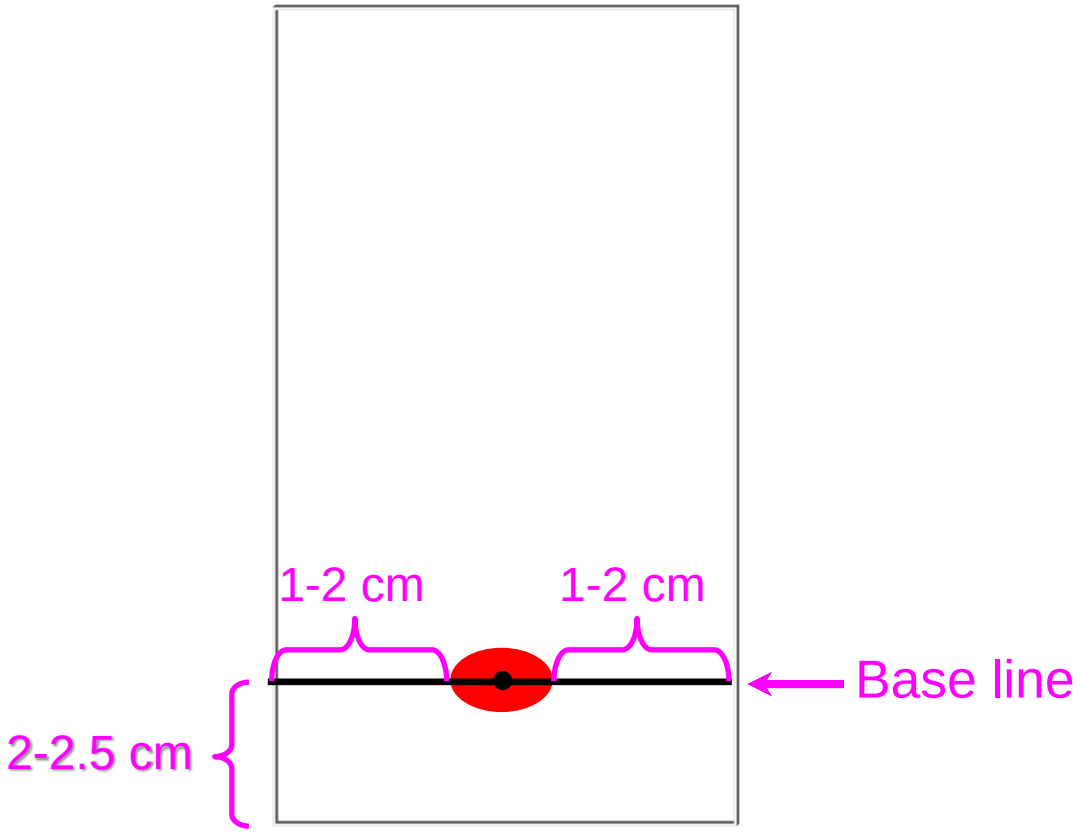
- **Slurry of the active material in appropriate solvent is uniformly spread over the plate.**

- **Air-drying overnight, or oven-drying at 80-90 °C (Depending upon the nature of the solvent) for about 30 minutes.**

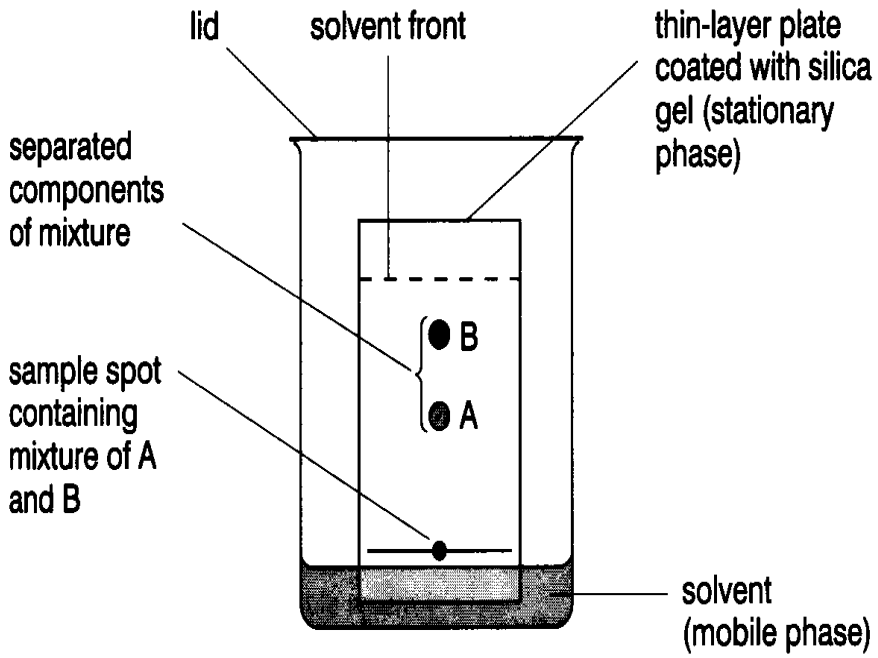
- **Ready to use thin layers (pre-coated plates) are also commercially available.**



Sample Application



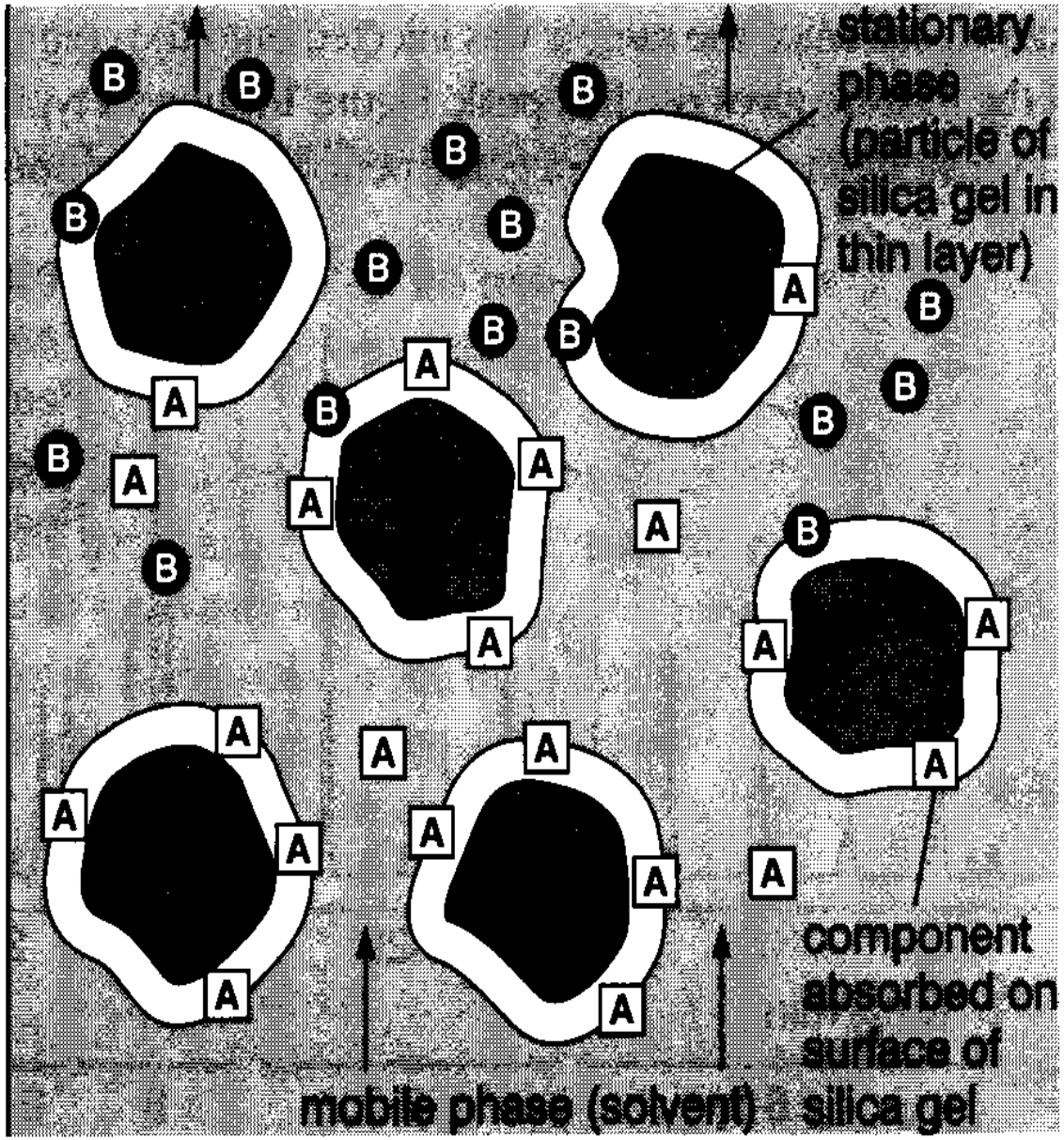
TLC



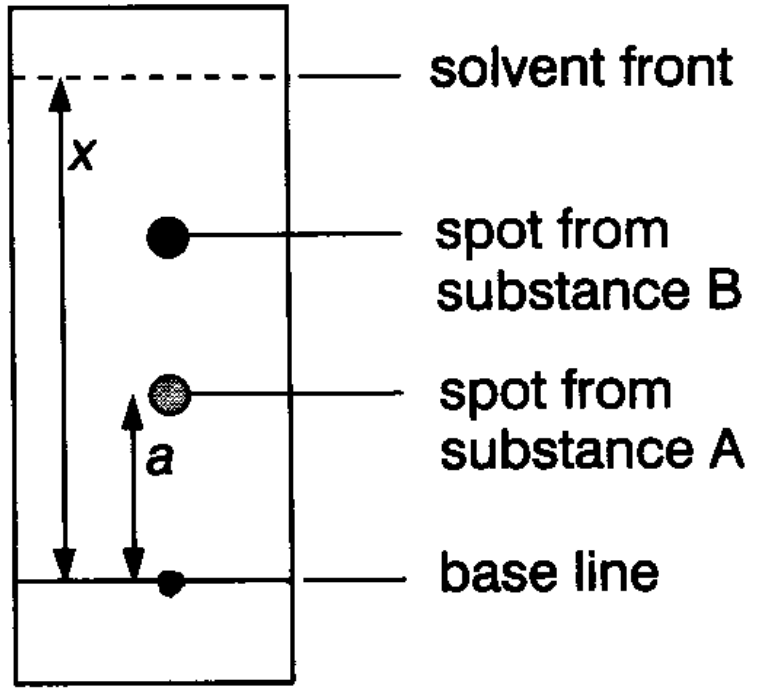
- Series of spots forms
- Compare samples in mixture with known substances.
- Measure R_f values.
- Coloured compounds & colourless compounds.

Separation and identification.

Component B has greater affinity for mobile phase. Gets carried further.

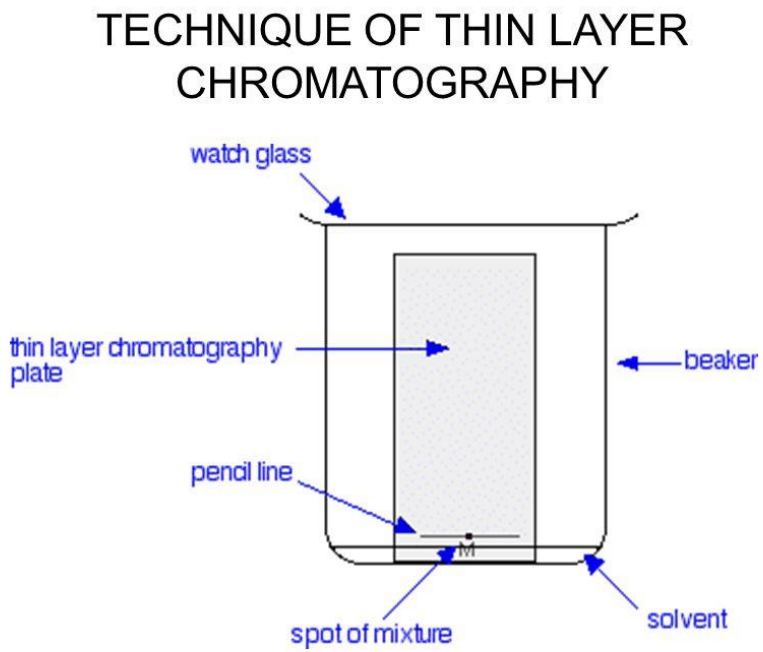


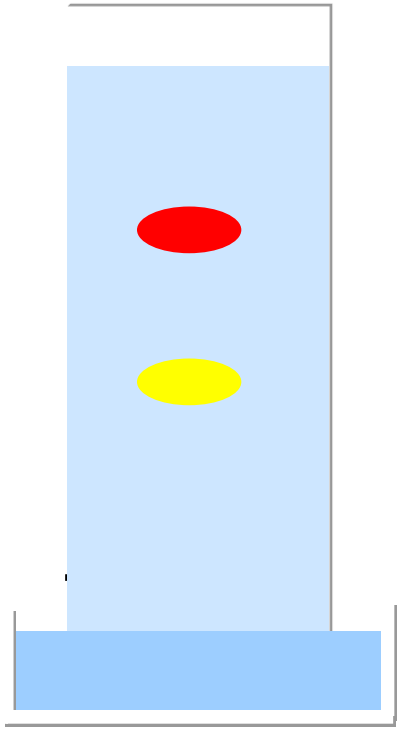
Component A has greater affinity for stationary phase. Does not get carried so far by mobile phase.



R_f value for substance A = $\frac{a}{x}$

Chromatogram Development

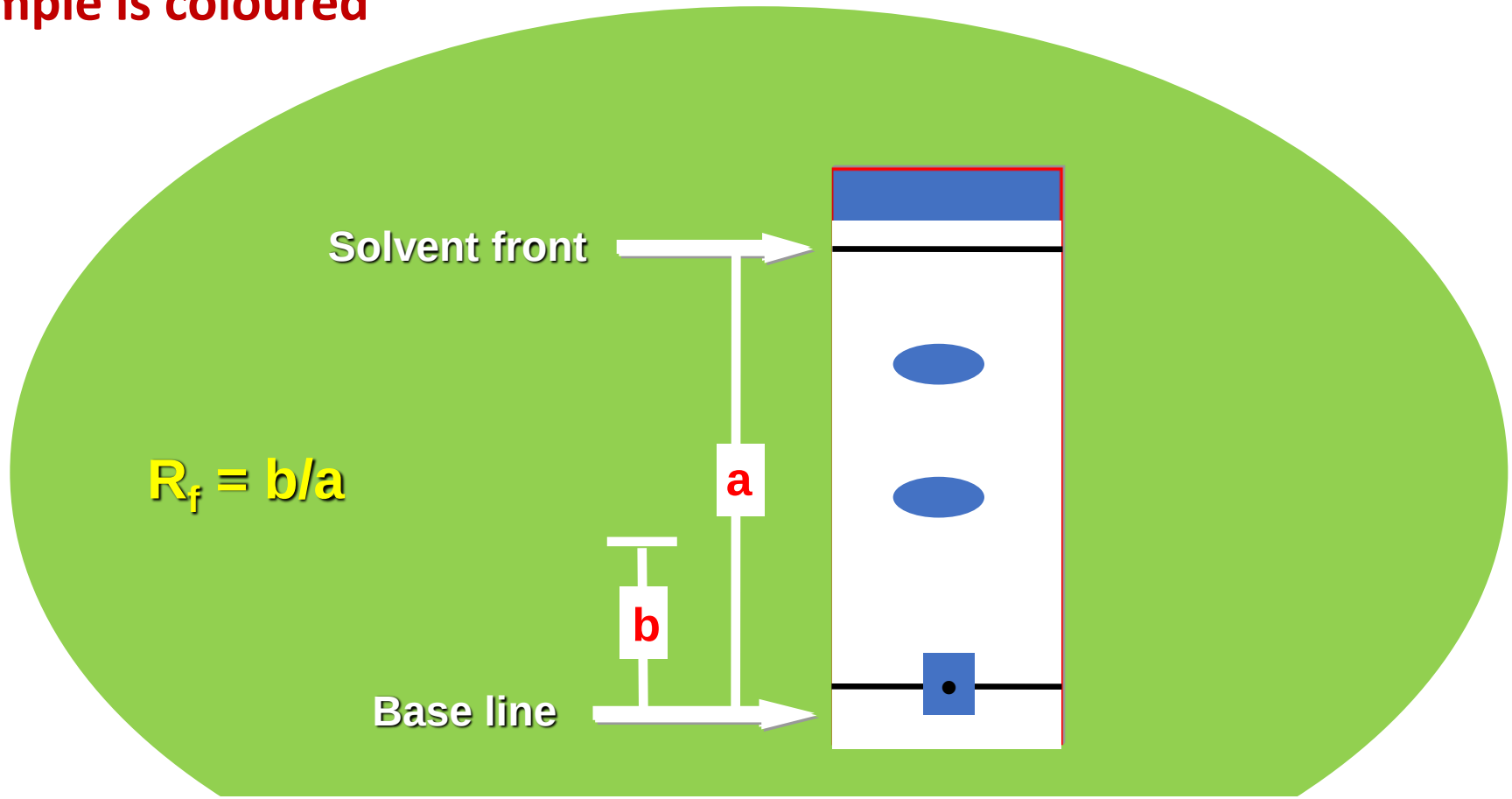




- **Direct contact between the sample and the solvent system is avoided**
- **As the developing solvent travels through the plate in upward direction, it dissolves the sample and carries it along.**
- **The sample gets distributed between the mobile phase and the stationary phase.**

How to visualize the spots ?

When the sample is coloured

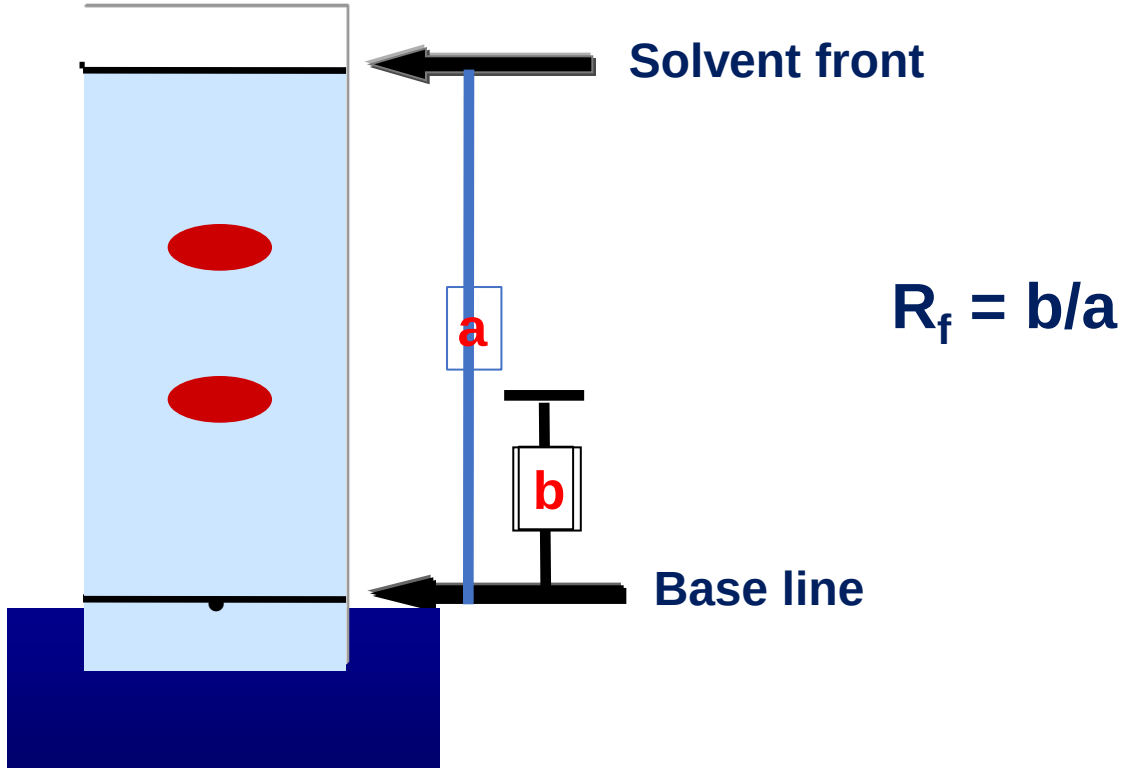


On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (R_f) expressed as: R_f = dist. travelled by sample / dist. travelled by solvent

The sample is colourless

The spots are not visible

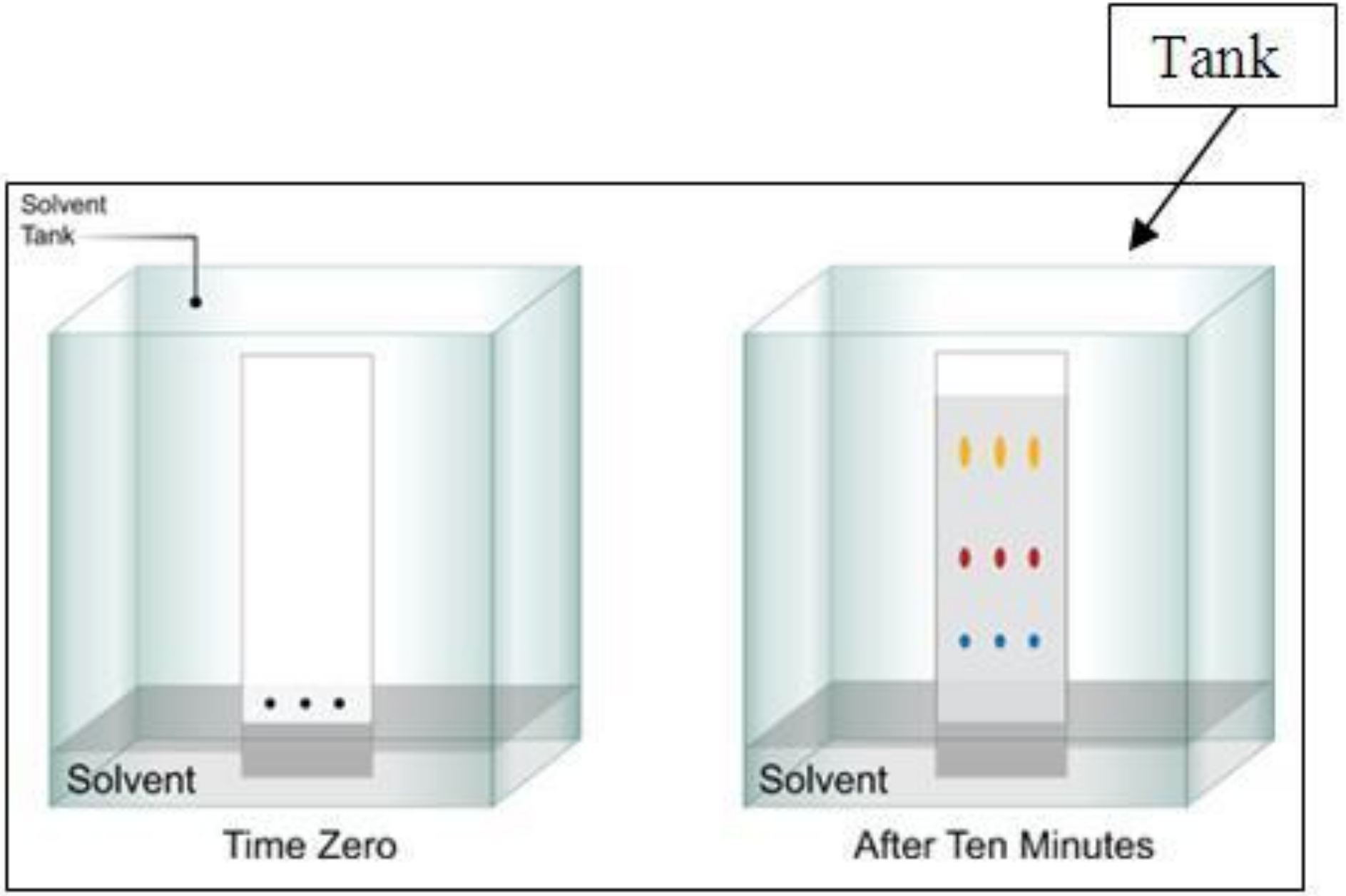
Developing agent such as Iodine, 2,4-Dinitrophenylhydrazine or sulphuric acid are used for many organic mixtures.
Ninhydrin is used for amino acids.
Silver nitrate is used for carbohydrates



$R_f = b/a$

Visualization Techniques

Visualization of colored compounds is simple : the spots can be directly observed after development. Because most compounds are colorless however, a visualization method is needed. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light. A spot will interfere with the fluorescence and appear as a dark spot on a glowing background. While under the UV light, the spots can be outlined with a pencil to mark their locations. A second method of visualization is accomplished by placing the plate into iodine vapors for a few minutes. Most organic compounds will form a dark-colored complex with iodine. Ninhydrin is used for amino acids. Silver nitrate is used for carbohydrates.


Tank
Solvent
Tank
Solvent
Time Zero
Solvent
After Ten Minutes

R_f & Its significance

The R_f value is used to quantify the movement of the materials along the plate. R_f is equal to the distance traveled by the substance divided by the distance traveled by the solvent. Its value is always between zero and one. If a development solvent of very high a polarity is used, all components in the mixture will move along with the solvent and no separation will be observed (R_f will be too large). If the solvent is of too low a polarity the components will not move enough, and again separation will not occur (R_f will be too small). In practice, different solvents or mixtures of solvents are tried until a good separation is observed. Typically an effective solvent is one that gives R_f s in the range of 0.3 - 0.7.

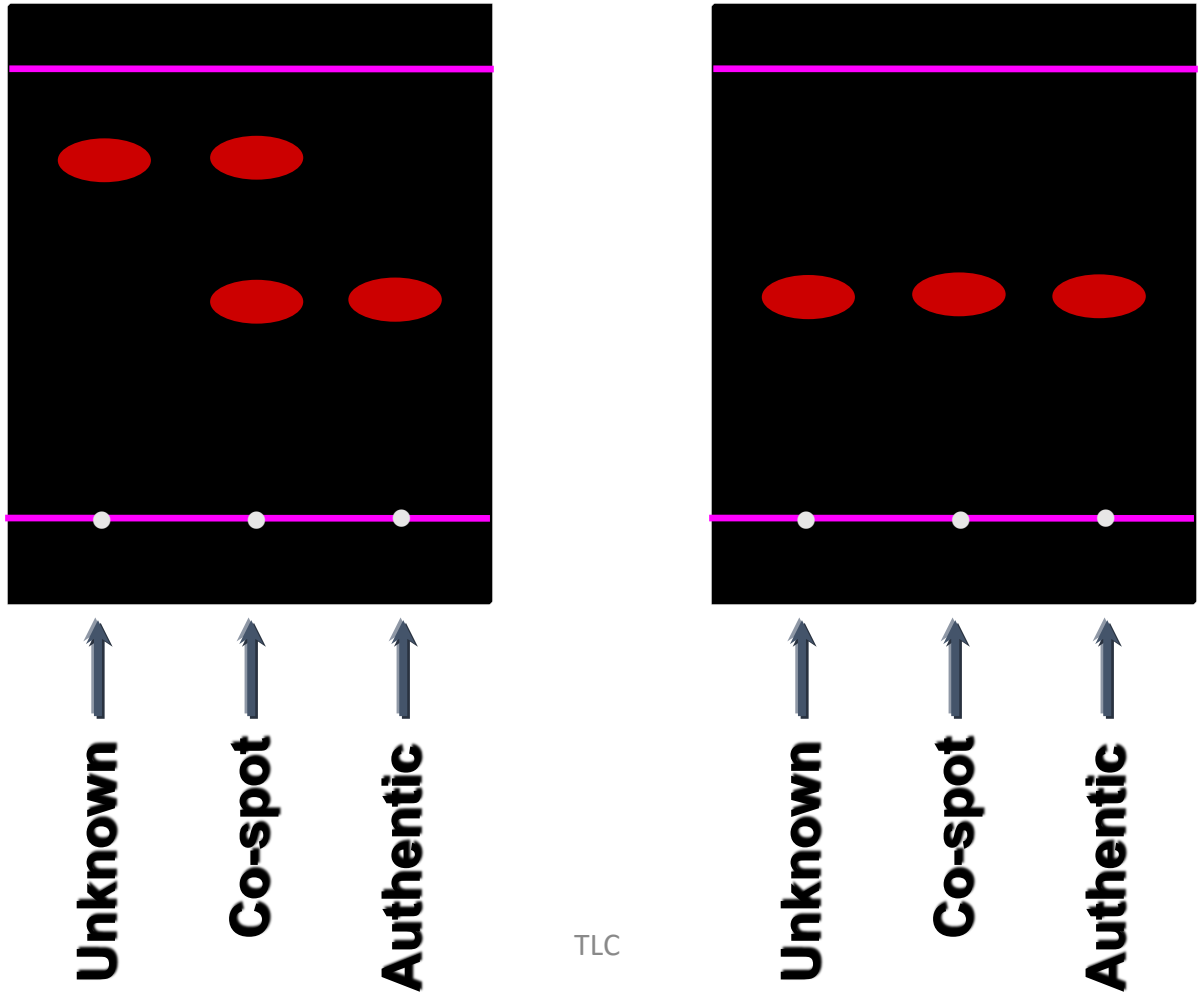
Spotting solvent & Developing solvent

Remember that the spotting solvent is simply used as a vehicle to transfer the material to be analyzed to the TLC plate. Once the transfer is made the spotting solvent evaporates. It has no effect on the separation. It is the development solvent that effects the separation.

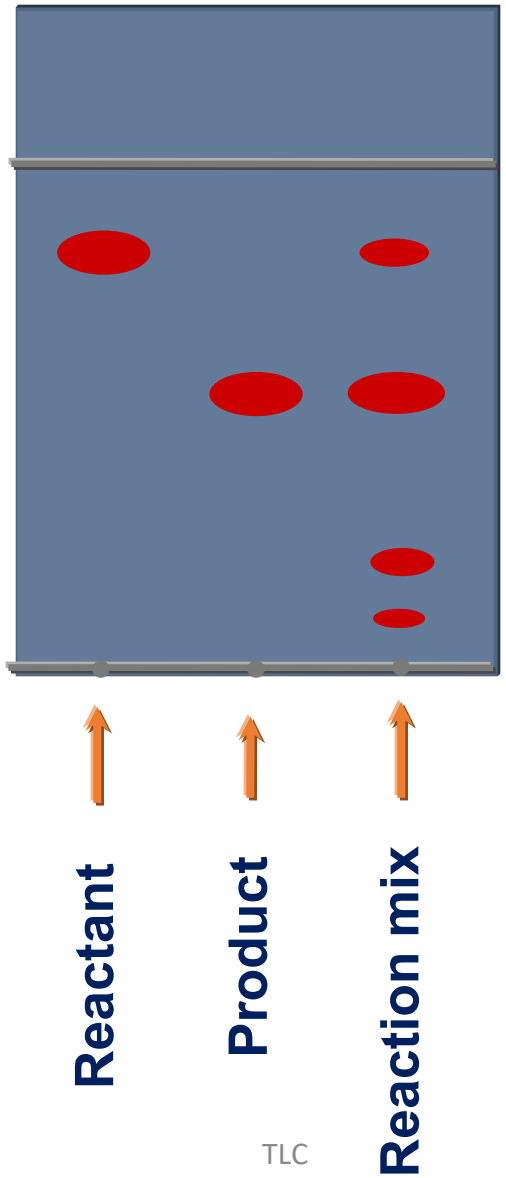
Thin Layer Chromatography Applications

- 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 agents and preservatives
- It is used in the cosmetic industry.
- It is used to study if a reaction is complete.

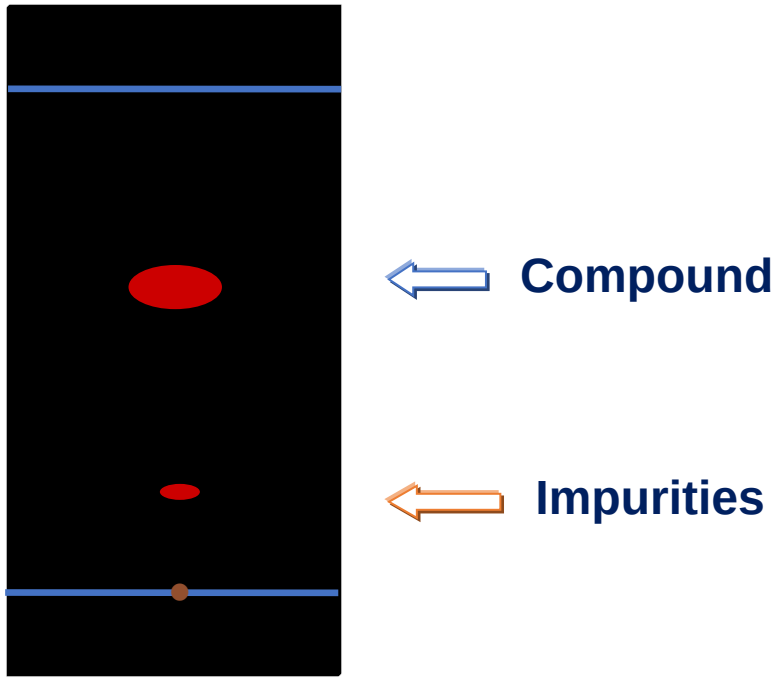
Identification of Unknown Compounds



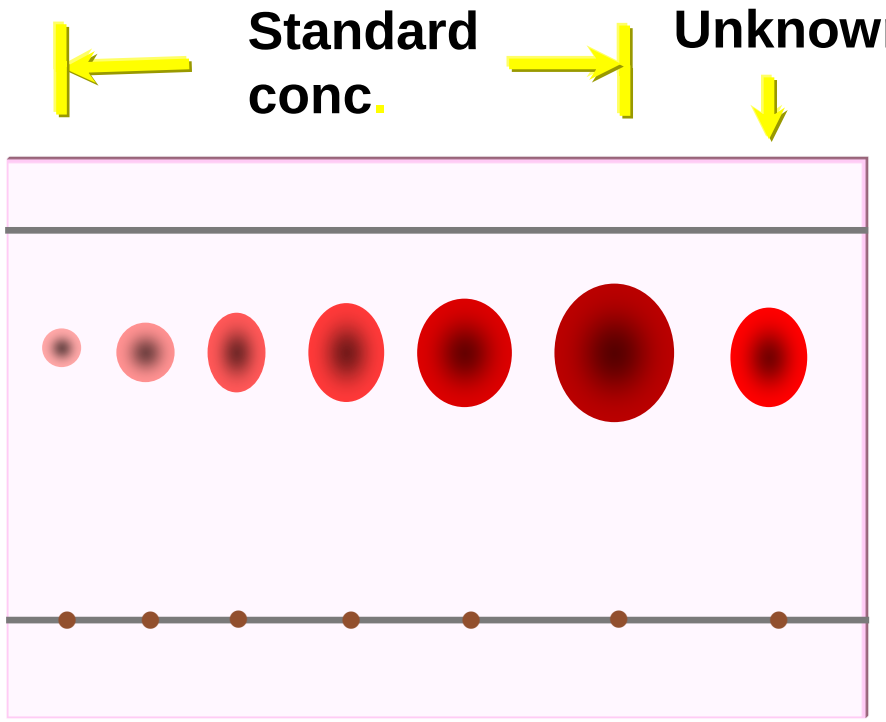
Analysis of reaction mixture

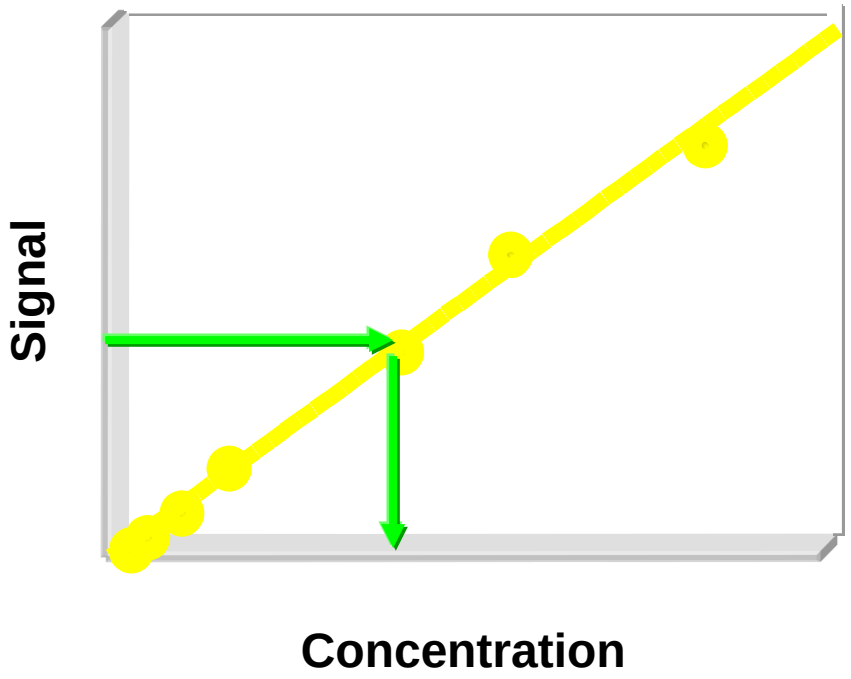


Determination of the Purity of compound



Quantitative estimation of an unknown concentration

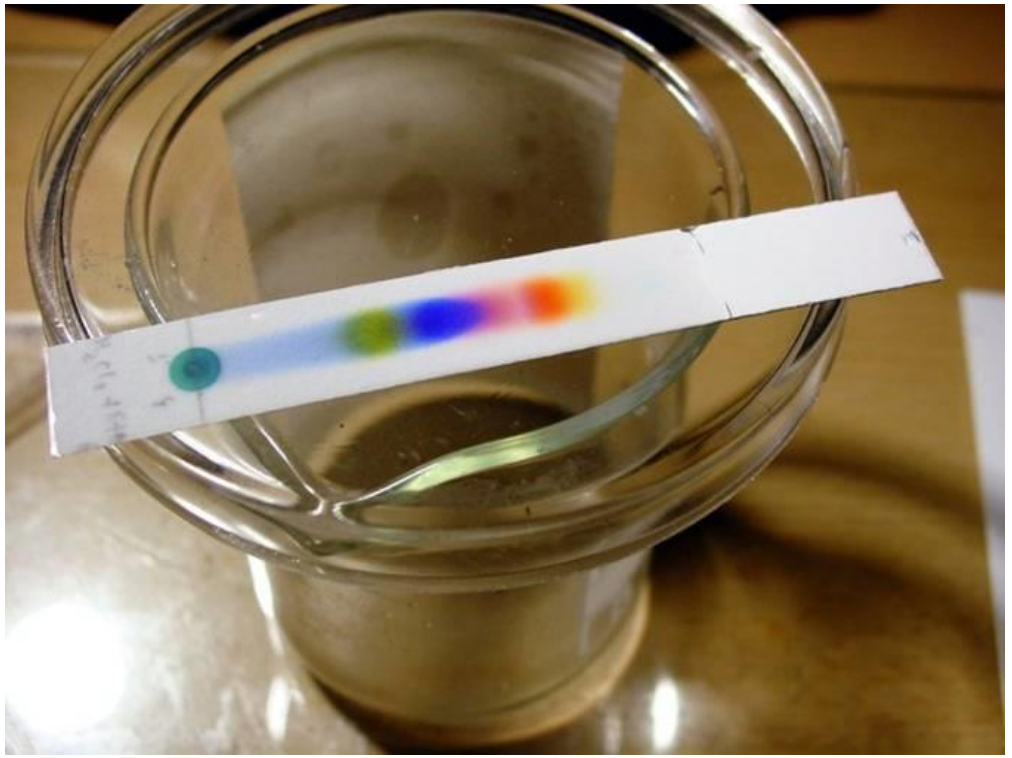




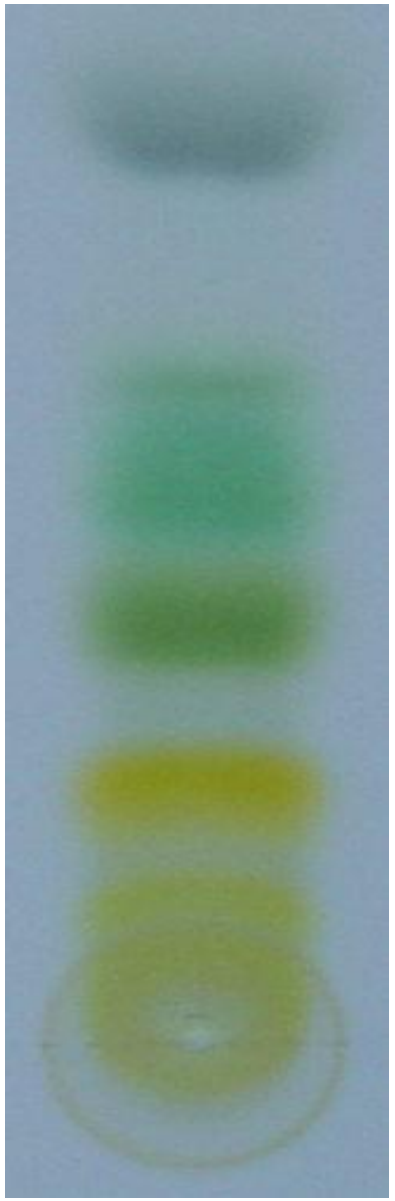
Calibration curve

Examples on TLC separations

Separation of black ink on a TLC

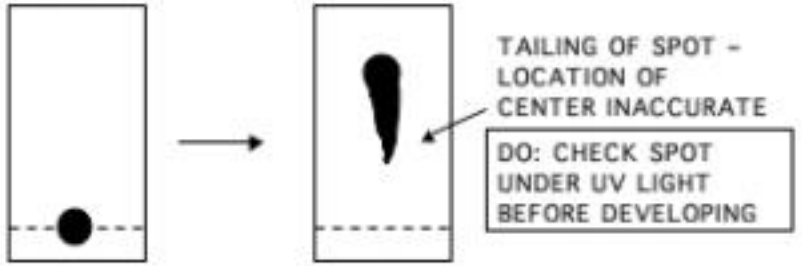


The chromatography of an extract of green leaves (for example spinach)



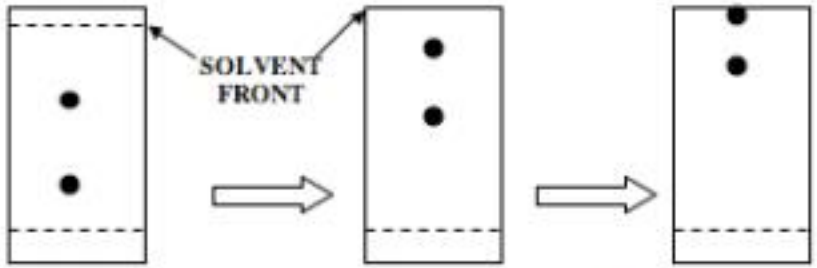
THINGS TO WATCH OUT FOR.

* OVERLOADING THE SPOT (SPOT TOO LARGE)

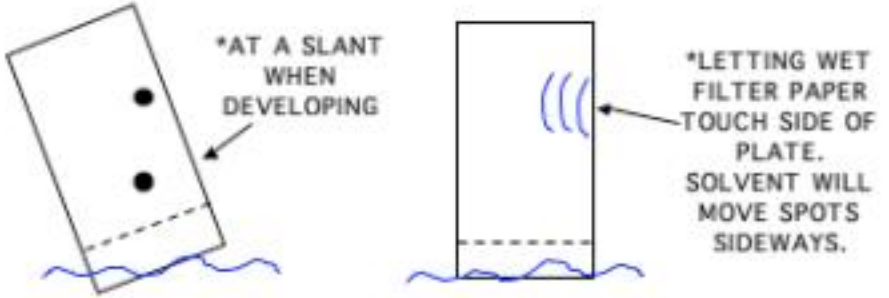


* SPOT TOO SMALL – DIFFICULT TO SEE

* ALLOWING SOLVENT FRONT TO REACH TOP OF PLATE



IF THE SOLVENT FRONT CANNOT BE SEEN, THE R_f CANNOT BE CALCULATED. STOP BEFORE FRONT REACHES TOP OF PLATE.



Preparative TLC

- TLC can also be used on a small semi-preparative scale to separate mixtures of up to a few hundred milligrams. The mixture is not "spotted" on the TLC plate as dots, but rather is applied to the plate as a thin even layer horizontally to and just above the solvent level. When developed with solvent the compounds separate in horizontal bands

Limitations of Thin Layer Chromatography

1.Thin Layer Chromatography plates do not have longer stationary phase.

2.When compared to other chromatographic techniques the length of separation is limited.

3.The results generated from TLC are difficult to reproduce.

4.Since TLC operates as an open system, some factors such as humidity and temperature can be consequences to the final outcome of the chromatogram.

5. Detection limit is high and therefore if you want a lower detection limit, you can't use TLC.

6.It is only a qualitative analysis technique and has very limited use in quantitative analysis.

What does the RF value tell you?

The retention factor, or **Rf**, is defined as the distance traveled by the compound divided by the distance traveled by the solvent. ... Conversely, if **you** know the structures of the compounds in a mixture, **you** can predict that a compound of low polarity will have a larger **Rf value** than a polar compound run on the same plate.

What factors affect RF values?

factors which affect Rf value are:-•

The **solvent system** and its **composition**. **Temperature**. The quality of the paper. **Distance** through which the **solvent** runs.