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An account on thin layer chromatography

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ABSTRACT

In this study, we address the basic expects such as idea, principle, mechanism and working of thin layer chromatography in analytical as well as preparation methods. From most of journals we found that TLC is a simple, cost effective, less time consuming technique. This technique is used from last few decades for identification and analysis of different biological compounds and detection of impurities. Study highlights the review on TLC and its application of qualitative and quantitative analysis.

INTRODUCTION:

Chromatography is a technique used for separation of mixture into components. The technique is based on the difference in the rates at which the components of a mixture are absorbed on a suitable absorbent. The material on which various components are absorbed is called stationary phase (alumina, silica gel, calcium carbonate, activated charcoal). The mixture to be separated is dissolved in a suitable medium; moving phase. The moving phase is run on stationary phase and the separation is based on the principle that components of a mixture present in moving phase move at different rate through the stationary phase.

There are different types of chromatography like paper chromatography, thin layer chromatography, column chromatography, high performance liquid chromatography (HPLC). Thin layer chromatography is a special type of chromatography in which a thin layer of adsorbent such as silica or alumina is spread over a sheet of glass, metal or plastic. A small amount of mixture to be analyzed is spotted at a distance of about 2 cm from the bottom with the help of fine capillary tube. The plate is now dried and places in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid (mobile phase) and its slowly rises up the TLC plate by capillary action as the solvent moves past the spot, an equilibrium is established for each component of the mixture between the molecules of that component which are absorbed on the solid and the molecules which are in solution. When the solvent has reached top of the plate the plate is removed from chamber, dried and the separated components are visualized. If the components are colored visualization is straight forward and for colorless compound UV lamp is used for visualization.

Rf value: Once visible, the Rf value or" retardation factor" or "ratio to front" can be calculated by dividing the distance the product travelled by the distance the solvent front travelled using the initial spotting site as reference. These values depend on the solvent used and the type of TLC plate.

$$\text{Rf Value} = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by Solvent}}$$

PROCEDURE TO RUN A TLC PLATE

1. PREPARATION OF TLC PLATE

TLC plates are commercially available. They are prepared by dissolving silica powder in a appropriate volume of water with some binders like plaster of paris, gypsum etc and is stirred using a glass rod to form a slurry. It is allowed to dry in oven at 110˚C for one hour or 60˚C for overnight. The standard size of the plate should be 6.5cm length by 2.5-5cm width is enough.

2. PREPARATION OF MOBILE PHASE

The choice of suitable depend upon nature of substance and adsorbent used on plate and I should be inert. The mobile phase is an organic solvent or a mixture of organic solvent. The solvent is poured into a designed chamber, a jar with a lid or a beaker with a watch glass on the top to a depth of just less than 0.5 cm.

3. SPOTTING THE TLC PLATE

Measure 0.5cm from the bottom of the TLC plate and draw a line at this mark. Now take a micro-capillary and deep it in the solution and gently touch it onto the proper location on TLC plate. The plate is dipped in solvent placed in a sealed container. The solvent moves up the plate by capillary action and needs the sample mixture which is dissolved and carried up the plate by the solvent. Different compounds in the sample mixture travel at different rates due to the difference in their attraction to

the stationary phase because of difference in solubility in the solvent. Allow the solvent to travel up the plate until 1cm from the top. Take the plate out and ark the solvent front immediately. Do not allow the solvent to run over the edge of the plate. Allow the plate to dry.

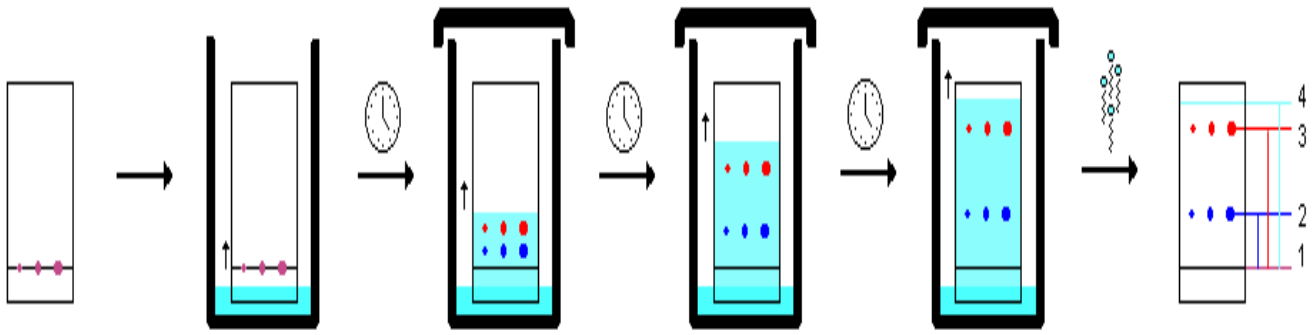


Figure 1: Sequence of TLC

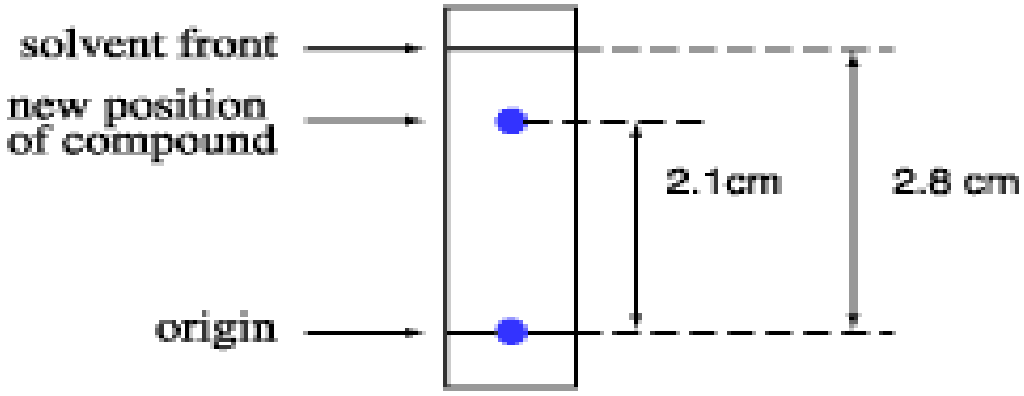
4. VISUALISATION OF THE SPOT

After taking out the plate and after evaporation has complete the spots are being detected. If the component of the sample is colored circle them lightly with a pencil and observe directly. If the spots are colorless they are visualized by standing the plate in a Iodine vapour atmosphere. Sometimes the spots can also be visualized by spraying the plate with reagents like Ninhydrin, Sulphuric acid etc.

5. ANALYSIS

The components are visible as separated spots are identified by the comparing the distance they have travelled with those of the unknown reference.

The retardation factor (or Rf) is defined as the distance travelled by component divided by the distance travelled by the solvent. Measure the distance of the start line to the solvent front then measure the distance of the centre of the spot to the start line divide the distance the individual spot moved the resulting ratio is called the Rf value. For example



$$R_f = \frac{2.1}{2.8} = 0.75$$

Graph 1:

APPLICATIONS

Tlc is a very common and very useful technique to affect the separation and purification of a given sample. The important applications are as follows:

1. This technique is particularly used for characterizing and isolating organic compounds such as amino acids, alcohols, proteins, acids, amines and antibiotics.
2. It has a very high efficiency for separation and it can also be performed very quickly.

3. It is used in identification of dye raw materials and products, preservatives, fatty acids and constitutes of so it has wide applications in cosmetology.
4. This technique has wide applications in food analysis as it is used for determination of fungicides and pesticides in drinking water, residues in vegetables, salad and meat, vitamins in soft drink, aflatoxins in milk and milk products.
5. It is used generally for analysis of petroleum and coal products.
6. It is used to check the purity of a compound which has been purified by some other suitable method.
7. It requires a very small sample of a substance to be analyzed.
8. TLC can be used for improving organic farming for better crop results.
9. Secondary metabolites, naturally producing compounds like alkaloids, esters, flavonoids, isoflavinoids, tannins, salicylic acid and lignin are identified from plant explants by using tlc.
10. TLC is used for determination of active substance and their metabolites in biological matrix, diagnose of metabolic disorders like phenolketolunaria , cystinuria, and maple syrup disease in babies.

CONCLUSION

TLC is a simple, easy, convenient, cost-effective and easy to operate technique. It gives reproducible results. It has wide applications in drugs and biochemical industries of drugs, in preparative analysis and has general applications in clinical, pharmaceutical, forensic, biological industries for identification and purification of compounds.

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