



AN OVERVIEW ON THIN LAYER CHROMATOGRAPHY

Name : Khandagale Jyoti Kadubal

Loknete Marutrao Ghule Patil Mahavidyalaya

Dahigaon- Ne Tal Shevgaon Dist Ahmednagar 414502

Abstract : This attempt has been made to review the basic principles and significance of thin Layer chromatography (TLC) in different analytical methods, research in general and in phytochemistry. TLC can be performed with less complicated technique, takes minimum time, low cost, simple so easy to operate. It has a wide range of application in general chemistry laboratories for several decades to routinely separate chemical and biomolecule products. Also it has wide application in pharmaceutical analysis and in identifying impurities in a compound. TLC can be used as a preliminary analytical method prior to HPLC. It is also useful to monitor the progress of a reaction and identification of compound present in a given substance. Study highlights the review on TLC and its application in various fields like environmental toxicology, food and in herbal analysis.

Keywords: Thin Layer Chromatography, Principle of TLC Rf values, Advantages of TLC, Application of TLC.

Introduction-

Thin layer chromatography (TLC) is a chemical analysis procedure which is used for the separation of mixture into their components. This chromatographic technique was discovered in 1906 by M. Tswett. This technique involves

spotting a sample solution onto a TLC plate, stationary phase is a solid or a liquid supported on a solid.

TLC plate is coated with a thin layer of adsorbent material, like silica gel, aluminum oxide, or cellulose. This thin layer of adsorbent is known as the stationary phase.

After the sample spotted near the bottom of this plate placing the plate into a vessel containing the mobile phase. Capillary force drawn the mobile phase through the plate, which drive the separation of the sample mixture in chromatography.

After drying the TLC plate, we may observed the individual bands via absorbance or fluorescence.

Thin layer chromatography can be used to identify compound present in a given substance, for monitoring the progress of reaction. The separation is based on differential partitioning between the mobile phase and stationary phase. There are different types of chromatographic techniques such as column chromatography, paper chromatography etc. Among them Thin layer chromatography (TLC) is a widely used laboratory technique. This technique is similar to paper chromatography. In paper chromatography we use paper acts as a stationary phase.

However, In thin layer chromatography involves a stationary phase of a thin layer of adsorbent as compare to paper chromatography, it means faster, gives better separation.

In Thin layer chromatography separation of compound is based on the competition of the solute and the mobile phase for binding places on the stationary phase.

For example, If silica gel is used as the stationary, considered it is polar in nature and two compounds with different polarity spotted near the bottom of TLC plate, the more compound has a stronger interaction with the silica gel and therefore more tendency to dispel the mobile phase from the binding places. However, the less polar compound moves higher up the plate.

If the mobile phase is changed to a more polar solvent, it is more capable of dispelling solute from the silica binding places and all compounds on the plate will move higher up the plate. For example Ethyl acetate results in higher R_f values. And that will not result in reversed order of running of the compound on the TLC plate.

Principle of TLC: TLC is normally done on a small glass or plastic plate coated with a thin layer of a solid- the most common are silica (SiO_2) or alumina (Al_2O_3). This is the stationary phase. The mobile phase is an organic solvent or solvent mixture. The sample mixture is applied near the bottom of the plate as a small spot, then placed in a jar containing a few ml of solvent. The solvent climbs up the plate by capillary action, carrying the sample mixture along with it. Each compound in the mixture moves at a different rate, depending upon its solubility in the mobile phase and strength of its absorption to the stationary phase. When the solvents get's near the top of the plate, it is allowed to evaporate, leaving behind the components of the mixture at various distances from the point of origin. The ratio of distance a compound moves to the distance the solvent moves is the R_f values (retention factor). This value is characteristics of the compound, the solvent and the stationary phase.

Rf Values:

The behavior of an individual compound in TLC is characterized by a quantity known as R_f and is expressed as a decimal fraction. The term R_f is associated with the migration of the solute relative to the solvent front as:

$$R_f = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

Measuring R_f values

Measurement are often taken from the plate in order to help identify the compound present. These measurements are the distance travelled by the solvent, and the distance travelled by individual spots, when the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.

The measurements are taken as:

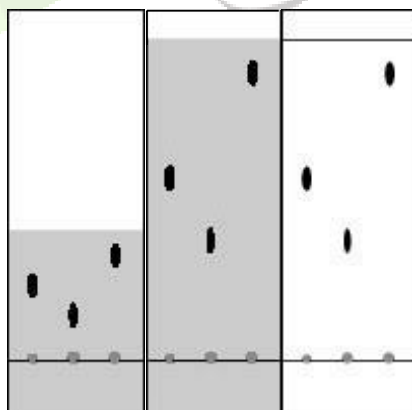


Fig: Spots as compounds.

For example, if the red component travelled 1.7 cm from the base line while the solvent had travelled 5.0 cm, then the R_f values for the red component is

$$R_f = \frac{1.7}{5.0}$$

$$= 0.34$$

If we could repeat this experiment under exactly the same condition, then the R_f values for each component could always be the same. For example, the R_f values for the red dye would always be 0.34. However, if anything changes (the temperature, the exact composition of the solvent, & so on), that is no longer true.

The R_f for a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant.

1. **Solvent system:** The purity of solvents and quantity of solvent mixed should be strictly controlled. It should be made freshly for each run if one of the solvents is very volatile or hygroscopic. Example- acetone.
2. **Adsorbent:** Different adsorbents will give different R_f value for same solvent. Reproducibility is only possible for given adsorbent of constant particle size and binder. Plates should be stored over silica gel in desiccators before use and the sample should be applied quickly so that the water vapor in the atmosphere is not adsorbed by the plate. Because of the difficulties associated with activation procedures, it is far better to use plates stored at room temperature and to activate them.
3. **Thickness of the adsorbent:** Standard plates approximately 250 micrometer is the preferable thickness of layer. Below 200, the R_f value vary considerably. The layer may be of higher or lower thickness in individual compounds.
4. **Amount of material spotted:** Increasing the mass of sample on the plate will often increase the R_f of drug, especially if it normally tails in the system. However, if a plate is grossly overloaded, this too will give a tailing spot and will have the effect of apparently decreasing the R_f value. The two situations are normally easy to distinguish by the intensity of the spot.

Plates (Stationary phase): The plates also known as chromatoplates can be prepared in the lab, but are most commonly purchased. Silica gel and alumina are among the most common stationary phases, but others are available as well. Many plates incorporate a compound which fluoresces under short-wave UV (254nm). The backing of TLC plates is often composed of glass, aluminum, or plastic. Glass plates are chemically inert and best withstand reactive stains and heat, but are brittle and can be difficult to cut. Aluminum and plastic plates can be cut with scissors. The thickness of the adsorbent layer is typically around 0.1-0.25mm for analytical purposes and around 0.5-2.0 mm for preparative TLC.

Spotting of plates: The thin end of the spotter is placed in the dilute solution; the solution will rise up in the capillary (capillary forces). Touch the plate briefly at start line. Allow the solvent to evaporate and spot at the same place again. This way you will get a concentrated and small spot. Try to avoid spotting too much material, because this will deteriorate the quality of the separation considerably ('tailing'). The spots should be far enough away from the edges and from each other as well. If possible, you should spot the compound or mixture together with the starting materials and possible intermediates on the plate.

Location of spot: The position of various solute separated by TLC can be located by various methods. Colored substances can be seen directly when viewed against stationary phase, while colorless substances can be detected only by making them visible by using of some spraying agent, which produces colored areas in the region which they occupy.

Specifically in TLC following can be used for spraying the visible spot:

1. Being purely inorganic in nature, corrosive agents may also be used for spraying on the invisible spots.
2. Dilute solution of potassium dichromate in concentrated sulfuric acid. In the process, potassium dichromate (yellow) is reduced to chromic sulfate (green) by most of the organic compounds, particularly used for sugars.
3. Vapors of sulfur trioxide, produced on warming fuming sulfuric acid, chars organic compound and makes them visible as dark spots.
4. Solution of potassium permanganate.
5. Iodine vapors.

Other common reagents include saturated solution of hydrogen sulfide, 0.2N aqueous ammonium sulfide, 0.1% alcoholic quercetin, 0.2% methanolic oxine, and 0.5% aqueous sodium rhodizonate. If the adsorbent used for TLC plate contains a fluorescing material, the solutes can be viewed under ultraviolet light.

Development solvents : The choice of a suitable solvent depends upon: Nature of substance, and adsorbent used on the plate. A development solvent should be such that, does not react chemically with the substance in the mixture under examination. Carcinogenic solvents (benzene etc) or environmentally dangerous solvents should always be avoided. Solvent system range from non-polar to polar solvents. Non-polar solvents are generally used, as highly polar solvents cause the adsorption of any component of the solvent mixture. Commonly used development solvents are petroleum ether, carbon tetrachloride, pyridine, glycerol, diethyl ether, formamide, methanol, ethanol, acetone, and n-propanol.

Mobile Phase: For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvent. As the mobile phase moves pass the surface of silica gel it transports the analyte pass the particles of the stationary phase. However, the analyte molecules are only free to move with the solvent if they are not bound to the surface of the silica gel. Thus the fraction of the time that the analyte is bound to the surface of the silica gel relative to the time it spends in solution determines the retention factor of the analyte. The ability of an analyte to bind to the surface of the silica gel in the presence of a particular solvent or mixture of solvents can be viewed as the sum of two competitive interactions. First, polar groups in the solvent can compete with the analyte for binding sites on the surface of the silica gel. Therefore, if a highly polar solvent is used, it will interact strongly with the surface of the silica gel and will leave few sites on the stationary phase free to bind with the analyte. The analyte will, therefore move quickly pass the stationary phase. Similarly, polar groups in the solvent can interact strongly with polar functionality in the analyte and prevent interaction of the analyte with the surface of the silica gel.

This effect also leads to rapid movement of the analyte pass the stationary phase. The polarity of a solvent to be used for chromatography can be evaluated by examining the dielectric constant (**symbol**) and dipole moment (**symbol**) of the solvent. The larger these two numbers, the more polar is the solvent. In addition, the hydrogen bonding ability of the solvent must also be considered. For example methanol is a strongly hydrogen bond donor and will severely inhibit the ability of all but the most polar analytes to bind the surface of the silica gel.

Developing a Plate : A TLC can be developed in a beaker or closer jar. Place a small amount of solvent (mobile phase) in a container. A small spot of solution containing the sample is applied to a plate, about one centimeter from the base. The plate is then dipped into a suitable solvent, such as hexane or ethyl acetate, and placed in a sealed container. The solvent moves up the plate by capillary action and meets the sample mixture, which is dissolved and is carried up the plate by the solvent.

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in their solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_f value) can be adjusted. The solvent level has to be below the line of the TLC, otherwise the spots will dissolve away. The lower edge of the plate is then dipped in a solvent. The solvent (eluent) travels up the matrix by capillarity, moving the components of the samples at various rates because of their different degree of interaction with the matrix (stationary phase) and solubility in the developing solvent. Non-polar solvents will force non-polar compounds to be top of the plate, because the compounds dissolve well and do not interact with the polar stationary phase. Allow the solvent to travel up the plate until ~1 cm from the top. Take the plate out and mark the solvent front immediately. Do not allow the solvent to run over the edge of the plate. Next, let the solvent evaporate completely.

Precaution during sample application:

1. Sample should be dissolved in a nonpolar solvent as polar solvent has a tendency to spread out the starting spot.
2. Solvent used for dissolving sample should be volatile.
3. While applying sample, the surface of the adsorbent should not be disturbed as this distorts the shapes of the spots on subsequent developed chromatogram, hindering the accuracy of quantitative measurements.
4. The sample spot should be within 2-5 mm in diameter.

The TLC Experiment:



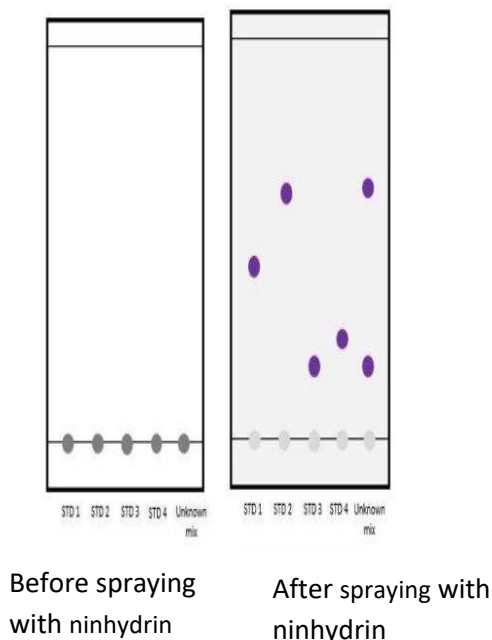
Visualization: When the solvent front has moved to within about 1 cm of the top end of the adsorbent (after 15 to 45 minutes), the plate should be removed from the developing chamber, the position of the solvent front marked, and the solvent allowed to evaporate. If the components of the sample are colored, they can be observed directly. If not, they can sometimes be visualized by shining ultraviolet light on the plate or by allowing the plate to stand for a few minutes in a closed container in which the atmosphere is saturated with iodine vapor. Sometimes the spots can be visualized by spraying the plate with a reagent that will react with one or more of the components of the sample.

Analysis: The components, visible as separated spots, are identified by comparing the distances they have travelled with those of the known reference materials. Measure the distance of the start line to the solvent front. Then measure the distance of center of the spot to the start line. Divide the distance the solvent moved by the distance the individual spot moved. The resulting ratio is called R_f -value. As the chemicals being separated may be colorless, several methods exist to visualize the spot. Often a small amount of a fluorescent compound, usually manganese activated zinc silicate, is added to the adsorbent that allows the visualization of spot under a backlight (UV_{254}). The adsorbent layer will thus fluoresce light green by itself, but spot of analyte quench this fluorescence, iodine vapour are a general unspecific color reagent, specific color reagent exist into which the TLC plate is dipped or which are sprayed onto the plate. Once visible, the R_f value, or retention factor, of each spot can be determined by dividing the distance travelled by the product by the total distance traveled by the solvent (the solvent front). These values depend on the solvent used, and the type of TLC plate, and are not physical constants.

Using thin layer chromatography to identify compounds

Suppose you had a mixture of amino acids and wanted to find out which particular amino acids the mixture contained. For simplicity we'll assume that you know the mixture can only possibly contain five of the common amino acids. A small drop of the mixture is placed on the base line of the thin layer plate, and similar small spots of the known amino acids are placed alongside it. The plate is then stood in a suitable solvent and left to develop as before. In the diagram, the mixture is M, and the known amino acids are labeled 1 to 5. The left-hand diagram shows the plate after the solvent front has almost reached the top. The spots are still invisible. The second diagram shows what it might look like after spraying with ninhydrin.

There is no need to measure the R_f values because you can easily compare the spots in the mixture with those of the known amino acids- both from their positions and their colours.



In this example, the mixture contains the amino acids labeled as 1,4 and 5. And what if the mixture contained amino acids other than the ones we have used for comparison

Applications

Thin layer chromatography has been a useful tool in numerous applications of pharmaceutical importance.

Amino acids

TLC of amino acids is more difficult than TLC of inks, because amino acids are colorless. Therefore, one cannot see the spots with the naked eye once the plate fully developed and dried. To see the spots, it is necessary to use either the ninhydrin or the black-light visualization techniques. E.g., Amino acids, proteins and peptides 8: A mixture of 34 amino acids, proteins and peptides has been successfully separated and isolated from urine using silica gel plates. All these substances were found to be ninhydrin positive. The development was carried out first with chloroform-methanol-20% ammonium hydroxide (2:2:1) and then with phenol-water.

Pharmaceuticals and drugs

TLC is used in the identification, purity testing and determination of the concentration of active ingredients, auxiliary substances and preservatives in drugs and drug preparations, process control in synthetic manufacturing processes. Various pharmacopoeias have accepted TLC technique for the detection of impurity in a drug or chemical. E.g., Antibiotics: Penicillin's have been separated on silica gel 'G' by using the two solvents, acetone-methanol (1:1) and iso-propanol-methanol(3:7). As the detecting agent, the iodine- azide reaction was employed by spraying the dried plates with a 0.1% iodine solution containing 3.5% of sodium azide.

Separation of multicomponent pharmaceutical formulations

It is also used in separation of multicomponent pharmaceutical formulations.

Qualitative analysis of alkaloids

It is used in qualitative analysis of alkaloids in control phase of both pharmaceutical formulations and vegetable drugs. TLC has been used for the isolation and determination of alkaloids in toxicology where the 30-60 minute runs give a great advantage in comparison to the 12-24 hours required for paper chromatography. Purine alkaloids have been separated by TLC on silicic acid, silica gel and aluminium oxide. The spots are visualized by spraying first with an alcoholic iodine potassium iodine solution followed by 25% HCl-96% ethanol (1:1).

Clinical chemistry and Biochemistry

For the determination of active substances and their metabolites in biological matrices, diagnosis of metabolic disorders such as phenylketonuria, cystinuria and maple syrup disease in babies. It serve as an useful tool in analysis of urinary constituent derived from lipids in analysis of many urinary constituents such as steroids, amino acids, porphyrins and bile acids. Urinary analysis by TLC is most effective when done in conjunction with other chromatographic processes, so that minor metabolites can be detected and resolved completely free of other components.

Cosmetology

In the identification of dye raw materials and end products, preservatives, surfactants, fatty acids and constituents of perfumes.

Food Analysis

For the determination of pesticides and fungicides in drinking water, residues, salad and meat, vitamins in soft drinks, banned additives in Germany (e.g. sandalwood extract in fish and meat products), compliance with limit values (e.g. polycyclic compounds in drinking water, aflatoxins in milk and milk products).

Analysis of Heavy Petroleum product

Thin-Layer Chromatography (TLC), which is commonly used in the analysis of complex mixtures, is seldom used in the investigation of petroleum products, may be the most complex objects. In particular, with respect to heavy petroleum products, no such information has been found in the literature. At the same time, the simplicity, economy, and efficiency of this technique in comparison with column chromatography are advantages that are widely known. TLC technique used (in the preparative variant) for a rapid determination of the group composition of petroleum products (asphalts, pitches, residues), and in connection with spectroscopic studies of the chemical composition of the fractions obtained.

Separation Of Aromatic Amines

Cationic and non-ionic surfactant-mediated systems have been used as mobile phases in thin-layer chromatographic separation of aromatic amines on silica gel layers. The effect of surfactant concentration below and above its critical micellar concentration on mobility of amines was examined. The influences of organic and inorganic additives such as alcohols, urea, NaCl and NaBr in micellar solutions on the mobility and separation efficiency of amines is also assessed

Application Related To Organic Chemistry

- a) It has been widely used for checking number of other separation processes. TLC has also been applied successfully in various purification processes, checking of distillation fractions and for checking the progress of purification by molecular distillation.
- b) TLC has been used as an analytical tool in organic chemistry due to its high speed of separation and its applicability in a large number of chemical compounds. Its important use is in the separation and isolation of individual components of a mixture, but in organic chemistry it has been used for: checking the purity of samples, as purification process, for identification of organic compounds, for studying various organic reactions, in characterizing and isolating a number of compounds such as acids, alcohols, glycols, amides, alkaloids, vitamins, amino acids, antibiotics, food stuffs and examination of reaction. The reaction mixture is examined by TLC to assess whether the reaction is complete or otherwise. The method is also used in checking other separation processes and purification processes like distillation, molecular distillation etc.

REFEERENCES:

1. Singhal S., Singhal N., Agarwal S., Pharmaceutical Analysis II, Thin layer chromatography, Pragati prakashan, First edition, 2009, 98-111.
2. Kasture A. V., Mahadik K. R., Wadodkar S.G., More H.N., A textbook of pharmaceutical analysis, Instrumental methods, Nirali Prakashan, 9th edition, 2005, vol II, 18-30.
3. Quach H. T., Steeper R. L., Griffin G.W., Separation of plant pigments by thin layer chromatography . Journal of chemical education. 2004, 81, 385-7.
4. Skoog D. A., Holler F. J., and Nieman T. A., ' Principles of instrumental analysis', Saunders college publishing, 5th edition, 2006,761-766.
5. Chatwal G. R., Anand S. K., Instrumental method of chemical analysis, Himalaya publishing house, 5th edition, 2008, 2.599-2.616.
6. Beckett A. h., Stenlake j. b., Practical pharmaceutical chemistry, Thin layer chromatography, CBS publishers, 4th edition, 2005, 115-128.
7. Ali M. and Agrawal V., Thin layer chromatography of aromatic amines, Separation Science and technology, 2002, 37, 363-377.
8. Sharma b. k., Instrumental methods of chemical analysis, Goal publishing house, Meerut, 5th edition, 2007, 241-264.
9. Vidya sagar, Instrumental methods of drug analysis, Pharma Med Press, First edition 2009,263.
10. Justus G. Kirchner, Thin-layer chromatographic quantitative analysis, Journal of chromatography A , 1 August 1973, Volume 82, Issue1, 101-115.

