Review On Comprehensive Working, Principles And Applications Of Thin Layer Chromatography

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Abstract- *In this present article, we address the basic aspects such as idea, mechanism and working of Thin layer Chromatography (TLC) in analytical as well as preparative preparation methods with its application. We have gone through diverse journals for gathering complete package of TLC and found that TLC is very simple, easy, less time consuming, costeffective and multiple samples could be run in one go hence, is constantly the first choice for varied application in qualitative analysis of pharmaceutical products. TLC holds good promise for identification and analysis of different bioactive compounds, secondary metabolites, Vitamins and amino acids. It can be used for separating compounds from crude extracts and separating impurities from a compound. Identical compounds from the mixture can be easily separated by analytical and further by preparative TLC. Many standard methods in industrial chemistry, environmental toxicology, steroids, food chemistry, water, inorganic pesticide, dye purity, cosmetics, plant materials, and herbal analysis rely upon TLC as the preferred approach.*

Keywords- TLC, Principle of TLC, Applications of TLC, Separation, silica gel, staining, Preparative TLC, solvent system, Organic solvents, capillary action.

I. INTRODUCTION

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures into their components. They all have a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The mobile phase flows through the stationary phase and carries the components of the mixture with it. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. There are different types of chromatography such as Column chromatography, Paper chromatography etc. among them Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. .Thin layer chromatography (TLC) is among the most useful tools for following the progress of organic chemical reactions and for assaying the purity of organic compounds in phytochemistry and Biotechnology. Like all chromatographic methods, TLC takes advantage of the different affinity of the analyze with the mobile and stationary phases to achieve the separation of complex mixtures of organic molecules. A TLC plate is a sheet of glass, metal or plastic which is coated with a thin layer of a solid adsorbent. A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the solvent, is the mobile phase, and it slowly rises up the TLC plate by capillary action. To determine the best solvent or mixture of solvents (a "solvent system") to develop a TLC plate or chromatography column loaded with an unknown mixture, vary the polarity of the solvent in several trial runs: a process of trial and error. Carefully observe and record the results of the chromatography in each solvent system. You will find that as you increase the polarity of the solvent system, all the components of the mixture move faster (and vice versa with lowering the polarity). The ideal solvent system is simply the system that gives the best separation. TLC elution patterns usually carry over to column chromatography elution patterns. Since TLC is a much faster procedure than column chromatography, TLC is often used to determine the best solvent system for column chromatography. Therefore a mixture is analyzed by TLC to determine the ideal solvent(s) for a flash chromatography procedure.Thin layer chromatography can be used to Monitor the progress of a reaction, identify compounds present in a given substance, and determine the purity of a substance. Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal phase silica gel is used as the stationary phase it can be

considered polar. Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places. Consequently, the less polar compound moves higher up the plate. If the mobile phase is changed to a more polar solvent or mixture of solvents, it is more capable of dispelling solutes from the silica binding places and all compounds on the TLC plate will move higher up the plate. Practically this means that if you use a mixture of ethyl acetate and heptane as the mobile phase, adding more ethyl acetate results in higher R_f values for all compounds on the TLC plate.

II. PRINCIPLE OF TLC

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate and a liquid mobile phase, which is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate. The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied. The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups. The solubility rule "Like Dissolves Like" is followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind

- **R_f 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 R_f is calculated by dividing the distance the compound traveled from the original position by the distance the solvent travelled from the original position (the solvent front). Nature of 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 not to activate them.
- **Measuring R_f values-** Measurements are often taken from the plate in order to help identify the compounds 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.

An R_f value is "retardation factor" or "ratio to front" is calculated by using the formula.

R_f = Distance traveled by compound / Distance traveled by solvent front

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

- Solvent system
- Adsorbent
- Thickness of the adsorbent
- Amount of material spotted

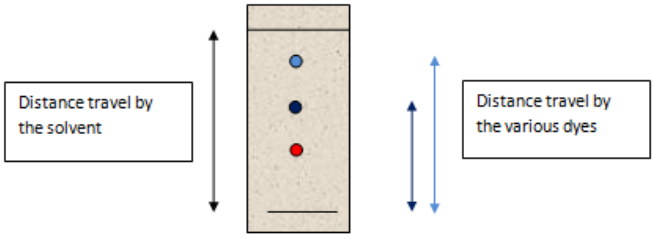

Figure 1: Spots as compounds- Identification of compounds

- **Plate preparation-** TLC plates are usually commercially available, with standard particle size ranges to improve reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water. This mixture is spread as thick slurry on an unreactive carrier sheet, usually glass, thick aluminum foil, or plastic. The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the adsorbent layer is typically around 0.1- 0.25 mm for analytical purposes and around 0.5- 2.0 mm for preparative TLC
- **Spotting the plate-** 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 the 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 spots-** The position of various solutes 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 making use of some spraying agent, which produces colored areas in the region which they occupy. Specifically in TLC following can be used for spraying the invisible spots:

  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.

- **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 substances in the mixture under examination. Carcinogenic solvents (benzene etc) or environmentally dangerous solvents (dichloromethane etc) should always be avoided. Solvent systems 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, glycol, glycerol, diethyl ether, formamide, methanol, ethanol, acetone, and n-propranol
- **Mobile Phase-** For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvents. As the mobile phase moves pass the surface of the 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 a 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 and dipole moment of the solvent. The larger these two numbers, the more polar is the solvent.
- **Developing a Plate-** A TLC plate can be developed in a beaker or closed jar. Place a small amount of solvent (mobile phase) in the 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 in to 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 solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components (measured by the Rf value) can be adjusted. The solvent level has to be below the starting line of the TLC, otherwise the spots will dissolve away. The lower edge of the plate is then dipped in a solvent. The solvent travels up the matrix by capillarity, moving the components of the samples at various rates because of their different degrees of interaction with the matrix (stationary phase) and solubility in the developing solvent. 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.
- **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.

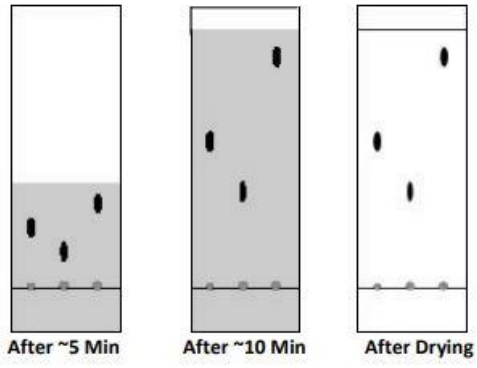

Figure 2: Spot visualization

- **Interactions between the Compound and the Adsorbent-** The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces. With silica gel, the dominant interactive forces between the adsorbent and the materials to be separated are of the dipole-dipole type.

III. APPLICATIONS

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

- TLC of 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 is 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
- 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.
- 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.
- 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.
- Cosmetology: In the identification of dye raw materials and end products, preservatives, surfactants, fatty acids, constituents of perfumes.
- Food Analysis: For the determination of pesticides and fungicides in drinking water, residues in vegetables, salads and meat, vitamins in soft drinks.
- Analysis of Heavy Petroleum Product: Thinlayer chromatography (TLC), which is commonly used in the analysis of complex mixtures, is seldom used in the investigation of petroleum products, maybe the most complex objects.
- 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.

IV. CONCLUSION

Thin layer chromatography is simple, cost-effective, and easy-to-operate technique in phytochemistry and biochemistry with numerous applications which use in the development of new drugs and various types of formulations from medicinal plants. Further needed detailed documentation for the sustainable development in education and research.

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