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Dimensional Chromatography

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HUMAN

Masarrat Mukadam[1]*, Saeed Chowdhry[2], Ayesha Karimji[3], Atafaraz Ansari[4]

Anjuman-I-Islam's Kalsekar Technical Campus, School of Pharmacy, New Panvel, Khandagaon near Thana Naka District Raigad, Maharashtra, India.

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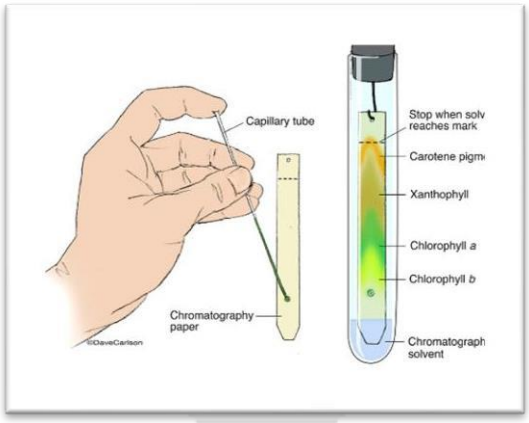
ABSTRACT

Chromatography is a well-known technique used for the separation of compounds. Among all, chromatographic techniques, paper chromatography is a type of analytical tool which is used for the separation of coloured components. The principle involved may be separation and partition of components based on their affinity towards the stationary phase Further, the investigation should be made to make new advancements in the field of chromatography separation involving the identification of types of paper used and gel permeation process.

INTRODUCTION

The technique of paper chromatography was first discovered by Synge and Martin in 1943. Paper chromatography is a specific type of technique that operates on a specific piece of paper. It is a type of planar chromatography, in which separation of compounds is performed using a filter paper made up of cellulose which acts as a stationary phase. Themethod is comparatively cheap and helps to separate dissolved chemical substances by their different rates of migration through paper sheets. The method requires a very minute quantity of samples for analysis.

PRINCIPLE



The basic principle involved in paper chromatography is partition in which the various components get distributed or partitioned between liquid phases. It involves the use of aqueous solvent held in pores of filter paper which acts as stationary phase whereas mobile phase travels over the paper [3, 4]. Dueto differences in their affinity towards water (instationary phase) and mobile phase solvents, the compounds in the mixture get separated through capillary action of the pores in the paper. The components may also be separated based on the principle of adsorption between solid and liquid phases, where the solid surface of paper serves as stationary phase and the mobile phase is a liquid solvent. Although the main working principle of paper chromatography is partitioning this is employed in many pharmaceutical applications.

Stationary and mobile phase

Paper chromatography is essentially partition chromatography and there is a wide variety of useful combinations of stationary and mobile phases. The two systems don't need to be immiscible. The types of stationary phases that are used can be classified as aqueous, hydrophilic, and hydrophobic systems. Stationary phases.

(1) Aqueous stationary phase

Water is readily held by paper. Therefore, water-equilibrated paper is attached by suspending paper in a closed chamber whose atmosphere is saturated with water. If an aqueous buffer or salt phase is required, the paper is drawn through the respective solution and then exposed to a water-saturated atmosphere in a chamber. This type of system is particularly suited for the separation of moderate polarto extremely polar mixtures.

(2) Hydrophilic stationary phase

An organic solvent can be used for the hydrophilic stationary phase. If the solvent is volatile enough, the paper can be equilibrated in a chamber whose atmosphere is saturated with solvent. Alternatively, the stationary phase solvent is dissolved in a very volatile diluent evaporates leavingthe stationary phase liquid uniformly distributed throughout the paper. Commonly used hydrophilic solvents include formamide, methanol, glycerol, and glycols.

(3) Hydrophobic stationary phase

The paper must be modified previously before it will tend to retain the hydrophobic stationary phase. Equilibration in the vapors of the solvent is the dipping technique in a solution of the solvent and a volatile diluent is used for introducing the hydrophobic solvent into the modified paper. Solvents such as dimethylformamide, aromatic and aliphatic hydrocarbons, and kerosene are commonly used.

Mobile phase

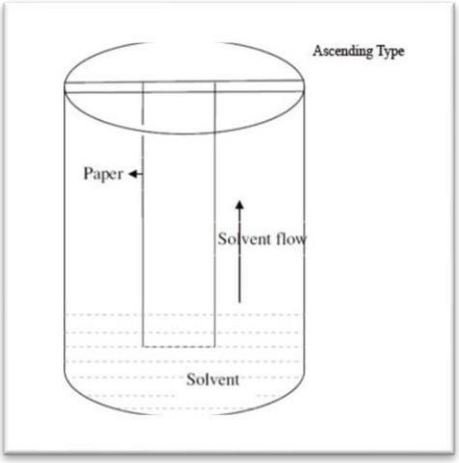
The mobile phase in various combinations can be used in paper chromatography. Choosing the optimum eluting condition is a trial and error process. However, certain guidelines can beused to predict eluting conditions. For example,the characteristics of the components in themixture and the type of stationary phase being employed should be considered. The solvent system used in paper chromatography is usuallya mixture of organic solvent with water. The ionization of analytes can be controlled by

MODES OF PAPER CHROMATOGRAPHY

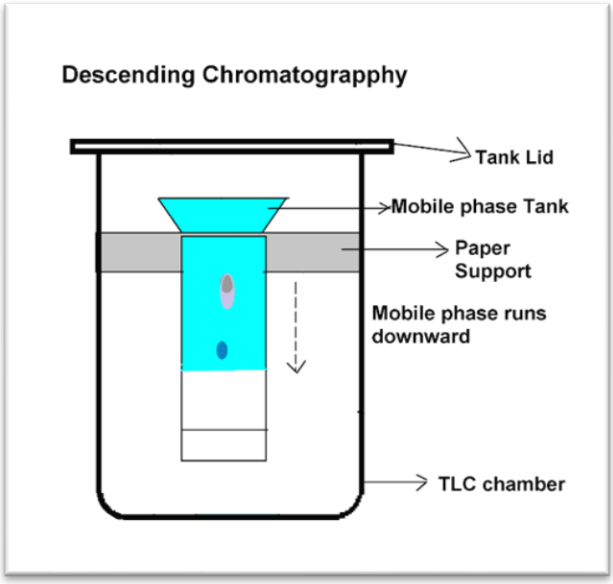
- Ascending chromatography

addition of acids (HCl, HNO_3, acetic acid) and bases (NH_3). Different combinations of the

solvent system can be used for the identification ofcompounds based on their chemical nature for example; for amino acids, the solvent system used is acetic acid: water: n-butanol in the ratio of 1:5: 4. For separation of sugar; solvent system composed of ethyl acetate: pyridine: water; water: ethyl acetate is suitable. For inorganic ions, solvents like pyridine: water or HCl: water is more popular.



In ascending technique, the chromatogram is attached in a way that the spot is touched with the solvent where the solvent is atthe bottom. The development of the chromatogram or the separation of the spot is against gravity. This is why this is termed as ascending technique. There is paper support on thetop of this tank. The mobile phase (solvent) is at the bottom of the tank. The filter paper is attached to the tank by the paper support and filter paper will touch the solvent. But the spot should not touch the solvent. The mobile phase will gradually rise upwards and carry the spot substances. The most polar substance will be at the bottom concerning the tank whereas the least polar will be on the top end of the tank. Ascending technique is relatively a slow process.



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Descending chromatography

In this chromatography, the development of the chromatogram is done by allowing the solvent to travel down the paper. The solvent reservoir is kept at the top and the process of movement of the solvent is assisted against gravity. This method is preferred over simple ascending chromatography due to

(i) Constant flow rate if solvent,

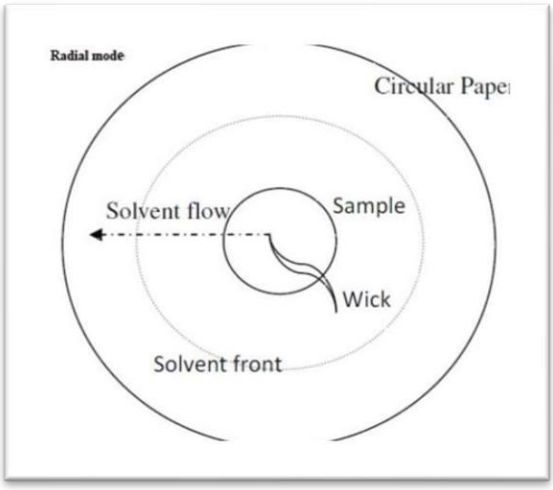
(ii) Less time consuming

(iii) The ease of separation of solutes with low Rf value. It only drawback of this technique compared with ascending chromatography is a requirement of extensive apparatus.

➢ Ascending- descending chromatography

This describes a modified form of paper chromatography that involves ascending and descending flow of solvent on the same piece of paper. The advantages of this method over other methods are (i) The run time is reduced, i.e. needs short period, (ii) Components with RF value > 0.50 can be detected individually as they will have their channel, (iii) Longer flow distance available which gives better resolution. The RF value obtained by ascending descending chromatography is not significantly different from those of ordinary techniques.

- Radial chromatography



The term radial chromatography was described by Rutter and involves the use of circular filter paper in whichcomponents get separated in the form of consent ringsrather than a single spot. A list of advantages was alsogiven which includes

(i) Sharpness and resolution of separation

(ii) Speedy separation

(iii) Simplicity and compactness of the employed apparatus

(iv) Control on the rate of flow of solvent

(v) Reproducibility

(vi) Ease of removal of test samples during andafter development.

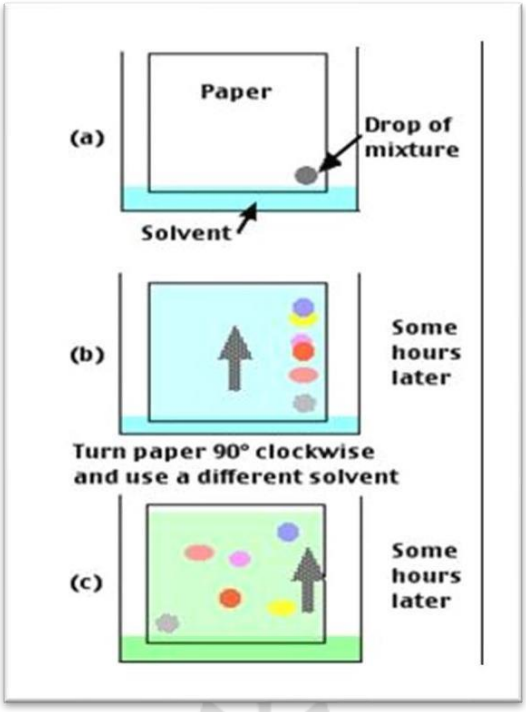
The technique employs the use of filter paper immersed in eluant and placed horizontally in a petri dish. The eluant flows from the center towards the periphery of the paper and is kept in a covered Petri plate for the development of the chromatogram. The wick of the paper is dipped in the mobile phase and solvent flows over the paper and the spots appear as concentric rings.

- Horizontal or Circular Paper Chromatography

This allows the separation of sample components in the form of concentric circular zones through the radial movement of the liquid phase.

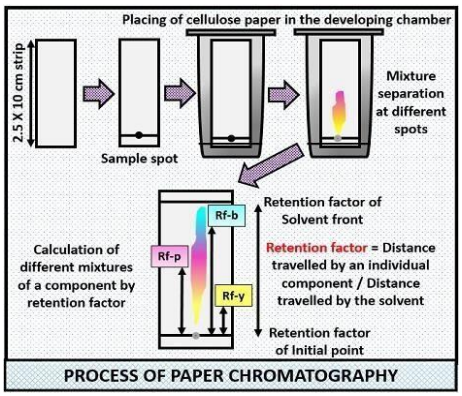
- Two-Dimensional Chromatography

This helps in resolving substances that have similar Rf values.



Where, Retardation factor (Rf) = The distance traveled by the solute/ distance traveled bythe solvent front

PROCEDURE OF PAPER CHROMATOGRAPHY



1. Selection of the ideal type of development: Based on factors such as the complexity of the solvent, mixture, paper, etc. the development type is chosen. Mostly either Radial or Ascending type of paper chromatography is employed because of the easiness they offer while handling and performing which ultimately leads to obtaining the chromatogram faster

within a shorter duration of time.

2. Selection of Filter paper: As per the pores' size andthe sample quality.

3. Sample preparation: This involves the dissolutionof the sample in an ideal solvent that is being utilized in developing the mobile phase.

4. Sample loading or spot on the paper: With the help of a capillary tube, micropipette, the sample is spotted on the paper at an accurate position. This promotes the interpretation of the chromatogram more quickly and easily.

5. Chromatogram development: This is carried by the paper immersion in the mobile phase. The mobile phase crosses over the sample on the paper because of the capillary action of the paper.

6. Drying of paper and detection of the compound: With the aid of an air drier, the paper is dried as soon as the chromatogram is developed. On the chromatogram developed paper, the detecting solution is sprayed and dried thoroughly for the identification of the sample chromatogram spots.

TYPES OF CHROMATOGRAPHY PAPERS

A wide variety of papers, which are very uniform from lot to let are commercially available in different sizes, porosities, shapes, thicknesses, and chemical treatments. In general, cellulose fiber is the main component of filter paper. The cellulose fiber is a linear polymeric carbohydrate chain owning hydrophilic character and is further cross-linked with a stable hydrogen-bonded system. Water or other very popular types of solvents are tightly held within the hydrophilic cellulose system and can beconsidered to be different from bulk water or polar system.

The cellulose papers can be altered in different ways to modify their chromatographic comportment. For example, paper can beimpregnated with diatomaceous earth, alumina,silica gel, ion exchange resins. These kinds of papers will exhibit properties of theseadsorbents and consequently, influence the retention of the stationary liquid and the adsorption or partition sequence of a mixture. The ion exchange resin impregnated paper will have either cation or anion exchange properties. If the paper is acetylated, the paper takes on a hydrophobic property. That is, it tends to retaina hydrophobic type solvent rather than a hydrophilic type

solvent as a stationary phase. This type of application is referred to as reverse phase chromatography. This paper can be made hydrophobic by silicone treatment or by impregnating it with inert non-polar typeorganic polymers.

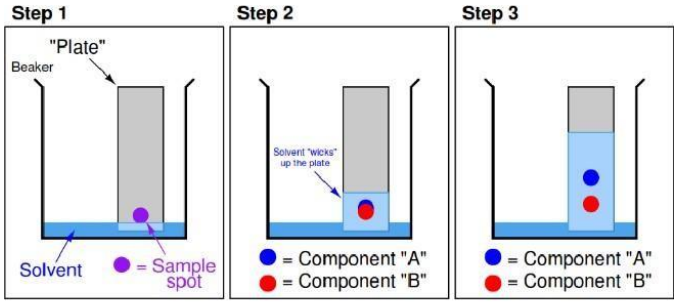
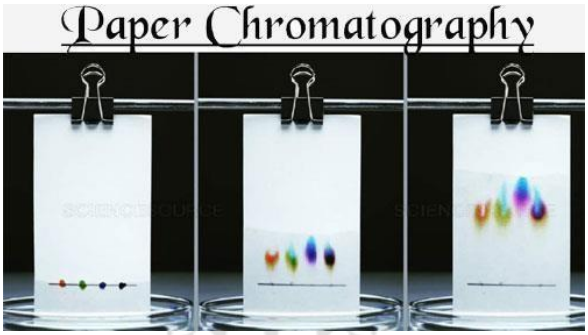


Figure 2



APPLICATIONS

1. Paper chromatography is a useful method to identify several constituents present in a sample,with a correctly chosen mobile phase.

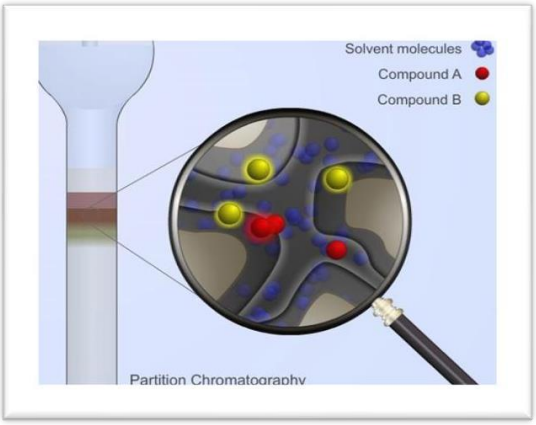
2. This method requires a small-scale setup, involves the very minute quantity of sample, and is also cost-effective.

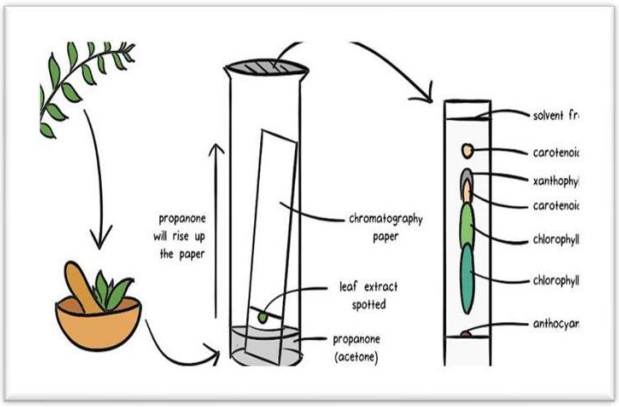
3. Paper chromatography is an effective tool for the separation of free amino acids present in humanserum.

4. It also offers a rapid method of separating and estimating sugars quantitatively; however, the identification depends upon the determination of their physical constants and the formation of characteristicderivatives.

5. Paper chromatographic technique is also used for carrying out the assay of pharmaceutical compounds such as a mixture of phenylephrine hydrochloride, chlorpheniramine hydrochloride, and dextromethorphan hydrochloride.

6. The technique is also useful in the isolation of pair of components having sample RF values using two-dimensional paper chromatography.





7. To analyze or separate the different constituents of a mixture, paper chromatography is used. It is one of the methods of qualitative analysis. We can say it is a useful tool for separating polar as well as nonpolar solutes. To analyze the different compounds in drugs, most pharmaceutical companies use this technique.

It is used in determining the pollutants in water and testing antibiotics.

FOR ISOLATION AND PURIFICATION

For components of the mixture, paper chromatography has been put to use as a purification and isolation technique. Using spectrophotometric methods, the separated components on the paper are cut, dissolved in suitable solvents and their absorption is characterized at specific wavelengths.

In food industry

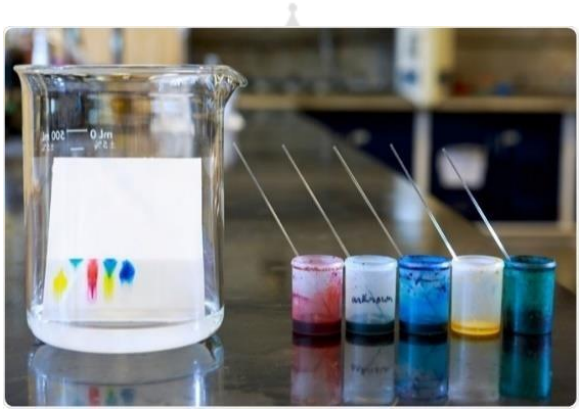
Both natural and synthetic food colours are added to foods to improve their acceptability and to make them more popular. Paper chromatography has been primarily used for the analysis of food colours in ice creams, sweets, drinks and beverages, jams, and jellies. To ensure that no non-permitted colouring agents are added to the foods, only edible colours are permitted for use. That's how quantification and identification become more important.

CONCLUSION

Initially, chromatographic techniques were used to separate substances based on their colour as was the case with herbal pigments. With time its application area was extended considerably. Nowadays, chromatography is accepted as an extremely sensitive, and effective separation method. Column chromatography is one of the useful separation and determination methods. Column chromatography is a protein purification method realized especially based on one of the characteristic features of proteins. Besides, these methods are used to control the purity of a protein. HPLC technique which has many superior features including especially its higher sensitivity, rapid turnover rate, its use as a quantitative method, can purify amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, antibiotics, and steroids.

II. TLC

INTRODUCTION

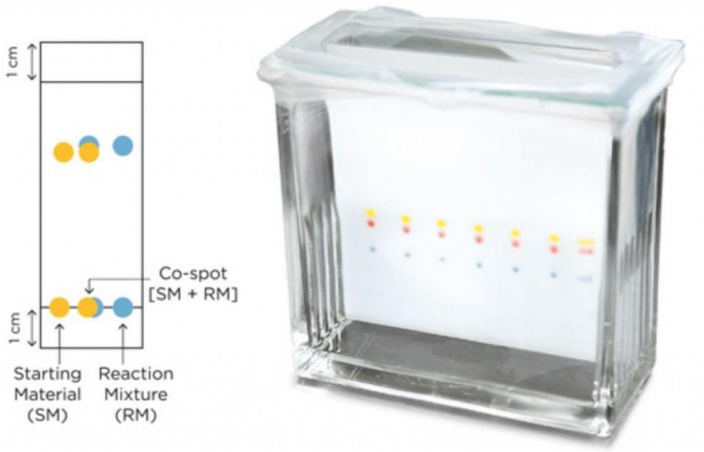


Thin Layer Chromatography (TLC) is an analytical technique in forensic science. It is a chromatography technique used to separate mixtures. Chromatography was discovered by M. Tswett in 1906. Thin-layer chromatography (TLC) is a type of liquid chromatography in which the stationary phase is in the form of a layer on glass, and aluminum, or plastic support. The term “planar chromatography” is often used for both TLC and paper chromatography (PC) because each avail a planar stationary phase rather than a packed column. PC, which utilizes plain, modified, or saturated paper (cellulose) as the stationary phase, involves many of the same basic techniques as TLC, but it has not evolved into an efficient, sensitive, quantitative, instrument-based analytical method and has many disadvantages as compared to TLC. TLC is highly selective and flexible because of the great variety of layers of stationary phase. It

has proven to be as sensitive as HPLC in many analyses as the solvent usage per sample is very low.

PRINCIPLE

Thin-layer chromatography is a method of separation or identification of a mixture of components by using finely divided adsorbent Solid/ liquid over a glass plate and liquid as a mobile phase.



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❖ Adsorption of substances on thestationary phase

❖ Separation of adsorbed substances bythe mobile phase.

❖ Separated substances are recoveredby the mobile phase through elution.

❖ Qualitative and quantitative analysisof eluted substances.

1. The distinction depends on the relative affinity between the stationary and mobile phases of the compounds. The compounds move over the surface of the stationary phase under the influence of the mobile phase (driven by capillary action). The compounds with greater affinity for the stationary phase travel slowly during this movement, while the others travel faster. The isolation of components in the mixture is thus accomplished. The individual components are visualized as spots at a different stage of travel on the plate once separation happens.

2. In a solid phase, the adsorbent is coated onto a solid support such as a sheet of glass, aluminum,and plastic as a thin layer of about 0.25mm thick.

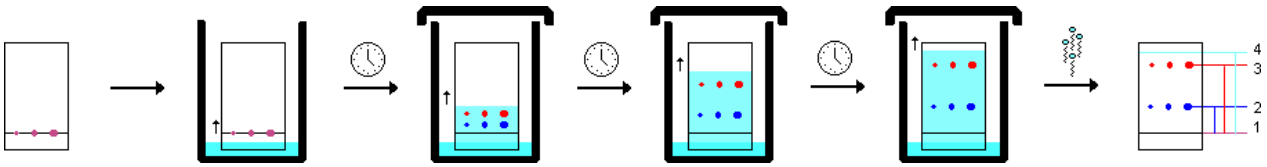
3. The mixture that needs to be separated is dissolved in a solvent to form a solution. Thus this solution is applied at the base of the thin-layer plate. The solution is allowed to move in an upward direction under the influence of capillary action. The solid will absorb some fraction of each component of the mixture and the remaining will be left in the solution. Anyone molecule will remain attached to the solid surface while the other components continue moving up the plate with solvent.

4. Separation of mixtures on a flat surface by the movement of solvent due to differences in solubility, migrating rates, size, and charge.

5. Elution is stopped when the solvent reaches the opposite side of the silica-coated glass.

INSTRUMENTATION

Most chromatography methods work because of a difference in polarity. The stationary phase keeps the components of a mixture of similar polarity combined with it causing them to move more slowly than the mobile phase. Because of differences in solubility of compounds in the mobile phase, and because of the strength of attraction to the stationary phase, some components move faster than others. This results in the separation of the compounds. As the solvent rises, the compounds in the samples move to various heights on the plate. Each component of the compound appears as spots that are visualized either by UV light or an iodine chamber. The relative intensity of the spots is not an accurate indication of the amount of compound present. The distance the spot travels on the plate is expressed as "ratio to front" or the Rf value given in the equation below. Rf = Distance of center of the spot from starting point / Distance of solvent front from starting point. The Rf value can be affected by many experimental conditions. The only way to get the identity of an unknown compound through TLC that is identical to a known compound is to spot a solution of the known or standard compound on the same TLC plate.



APPLICATION OF TLC

1. Pharmaceuticals and Drugs

Identification, purity testing, and determination of active substances and preservatives in drugsand drug preparations.

2. Clinical, Forensic, and Biochemistry

Determination of active substances and their metabolites in biological matrices, diagnosis of metabolic disorders like phenylketonuria, cystinuria, and maple syrup disease in babies.

3. Cosmetology

Dye raw materials and end products, preservatives, surfactants, fatty acids, constituents of perfumes.

4. Food Analysis

Determination of pesticides and insecticides in drinking water, residues in vegetables, salads,and meat, and vitamins in soft drinks.

5. Environmental Analysis

Groundwater analysis, determination of pollutants from soils and surface water.

6. Identification of Drugs, Poisons, and explosives:

Thin-layer chromatography (TLC) is one of the most widely used techniques for the separation and identification of drugs, whether they come from proprietary preparations, illicitly prepared material, or biological samples. It is a supreme technique because of its simplicity, low cost, and the selectivity of detection reagents. However, an analyst challenged with a new or unknowncompound has the problem of choosing a suitable system or systems from the hundreds that have been proposed, sometimes without any knowledge of their effectiveness. During the last few years, much effort has been put into choosing the best TLC systems for general screening and identification purposes to create several standardized systems. The main advantages gained by such standardization are that (a) analyses are performed more efficiently since only the moreeffective system are used, (b) chromatographic data built up by one laboratory are easily transferable to another laboratory, and (c) any

disagreements between the findings of two or more laboratories analyzing the same sample should be minimized.

7. Identification of dyes and ink

Forensic document examination, especially the analysis of inks, can be divided into two approaches including non-destructive document and destructive document. Non- destructive analytical methods will choose specific characteristics of ink to serve as parameters, such as its colors,luminescence, and radiationabsorption. Questionable documents may be differentiated by properties of transmission, reflection, and fluorescence spectra obtained for inks deposited on the paper surface. However, the methods of physic-chemical analysis can determine the type and composition of the ink, leading to ink identification Destructive document analysis starts by removing a small section from the ink line with an extraction solvent to open up more avenues of analysis. In particular, the chromatographic separation of colored pigments from component dyes can be useful. Even though a blue ballpoint pen can only write in one color, the ink is made from a mixture of different-colored pigments. This method hasproven highly productive for thecomparison and matching of inkwith the database of chromatograms.

8. Identification of Pesticides

TLC and HPTLC complement gas chromatography(GC) and high-performance column liquid chromatography (HPLC) for pesticide separation, detection, identification, and quantification becauseof their following unique advantages over column chromatography: single use of the layer simplifies sample preparation procedures; simplicity of development by dipping the plate into a mobile phase in a chamber; high sample throughput with low operating cost because multiple samples can be run simultaneously with standards on a single plate using a very low volume of solvent; high resolution through multiple developments or two-dimensional (2D) development on a plate with a singleadsorbent or dual adsorbents; selective and sensitive post chromatographic detection and identification with a very wide variety of chromogenic, fluorogenic, and biological reagents and coupled spectrometric techniques; highresolution and accurate and precise quantification achieved on HPTLC plates, especially with the automated sample application, development, and densitometric scanning methods; visual observation and direct recording of the entire chromatogram including all sample

components, the origin, and the mobile phase front; and the ability to repeat detection and quantification steps under different conditions.

✓ **ADVANTAGES**

1. A simple method of component separation.

2. Fewer types of equipment are used in this technique. As the components elute rapidly,the separation is achieved in a very short time.

3. It is possible to visualize all elements of UV light.

4. By this process, the non-volatile compounds can be isolated.

5. It is also possible to separate the microlitre volume of the sample through TLC.

6. Easy isolation and recovery of the components of complex mixtures.

✓ **DISADVANTAGES**

1. It is difficult to reproduce the findings obtained from the experiment.

2. Applicable for components of soluble mixtures only.

3. Qualitative analysis, not the analysis in quantitative terms.

4. Not a mechanism that's automatic.

5. A thin layer of chromatography operates in an open system, humidity and temperaturecan influence the outcomes.

6. As plate length is limited, the separation process takes place up to a certain length.

CONCLUSION

As old and simple as TLC is, it has been found its way still in modern science as it can be used as a first indicator of the components, before more advanced techniques can be used such as HPLC, GC etc. It provides fast, low cost qualitative analyses and screening in order to obtain information such assample stability, purity, and uniformity and to follow the course of a reaction. Samples that are difficult to prepare can be analyzed readily, and detection is

especially flexible in the absence of the mobile phase and with a variety of parameters. TLC has been applied virtually in all areas of analysis, including chemistry, biochemistry, biology, industrial, agricultural, environmental, food, pharmaceutical, clinical, natural products, toxicology, forensics, plant science, bacteriology, parasitology, and entomology.

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10. Jump up to:a b Reich, E.; Schibli A. (2007). High-performance thin-layer chromatography for the analysis of medicinal plants (Illustrated ed.). New York: Thieme. ISBN 978-3-13-141601-8.
11. Tables showing the thickness value of commercial regular and preparative Thin Layer Chromatography plates