A REVIEW ON THIN LAYER CHROMATOGRAPHY: PRINCIPLES, APPLICATIONS AND ADVANCES

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ABSTRACT

Thin Layer Chromatography (TLC) is a versatile analytical technique that has been widely used for separation, identification, and quantification of compounds in various fields. This review provides a comprehensive overview of the principles, applications and advances including its use in pharmaceutical, food, environmental, and biomedical analysis. Recent advances in TLC, including High-Performance TLC (HPTLC) and TLC-Mass Spectrometry (TLC-MS), are discussed. The challenges and limitations of TLC, including resolution and sensitivity, are highlighted, and potential solutions are proposed. The review concludes that TLC is a powerful analytical technique with a wide range of applications. Its advantages, including simplicity, cost-effectiveness, and versatility, make it an attractive option for various analytical tasks. The recent advances in TLC, including HPTLC and TLC-MS, have enhanced its potential and expanded its applications.

KEYWORDS: *Thin Layer Chromatography; Rf value; stationary phase; mobile phase*

INTRODUCTION

Chromatography is a technique used to separate components of a mixture based on their distribution between a moving fluid (mobile phase) and a stationary phase. Mobile Phase is typically volatile, it moves over the stationary phase through capillary action between process where a liquid moves along a solid's surface due to stronger attraction between the liquid molecules and the solid than among the liquid molecules themselves. Stationary Phase is often a highly polar substance, such as aluminum oxide or silica, that interacts with the solutes in the mixture. This process allows for the effective separation and analysis of various substances.

Thin layer Chromatography

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures and identify the compounds. Chromatography was discovered by M. Tswett in 1906. It is a simple, inexpensive separation technique that is used in organic chemistry to monitor the progress of chemical reaction at every step. It is commonly used as a diagnostic tool to determine whether an unknown sample contains a single compound or multiple compounds, to check if the product has started forming in an organic reaction, and to assess if the reaction has been completed. It is generally used as a qualitative tool. TLC can also be used to identify the compounds in a mixture and can also check the purity of synthesized compounds. This is done by comparison of TLC of an unknown sample with known materials.

Thin layer chromatography (TLC) is conducted on a sheet of glass, plastic, or aluminum foil coated with a thin layer of adsorbent material, typically silica gel, aluminum oxide, or cellulose (blotter paper). This coating, known as the stationary phase, holds the sample in place. Once the sample is applied, a solvent or solvent mixture, referred to as the mobile phase, moves up the plate by capillary action. As different analytes ascend the plate at varying speeds, separation of the components is achieved.

Principle of TLC

TLC operates on the principle of "like dissolves like," utilizing the separation of polar, non-polar, and mid-polar compounds from a mixture. This is achieved through interaction with both the stationary phase (a static adsorbent layer) and the mobile phase (a solvent or solvent mixture that moves across the stationary phase). When a sample is applied, the compounds in the mixture interact differently with these phases based on their polarity. Compounds that are less soluble in the mobile phase exhibit a stronger affinity for the stationary phase and thus travel shorter distances, while more soluble compounds move further along the plate.

For colorless compounds, techniques such as fluorescence, radioactivity, or chemical reagents can be used to produce visible colored reactions, allowing for identification of the compounds' positions on the chromatogram. These color changes can be observed under normal light or UV light. The position of each compound is quantified by calculating the ratio between the distance traveled by the compound and the distance traveled by the solvent. This value, known as the retention factor (Rf), is used for qualitative analysis of the compounds.

Calculating the Rf value of a compound

The distance a compound travels relative to the solvent front is influenced by the molecule's structure, making thin layer chromatography (TLC) useful for both identifying and separating compounds. This relationship is quantified by the retention factor (Rf), not "retardation factor." The Rf value is calculated as the ratio between the distance traveled by the compound and the distance traveled by the solvent front, providing a standard measure for comparison. Rf value is usually expressed as

Rf value = Distance traveled by compound / Distance traveled by solvent front

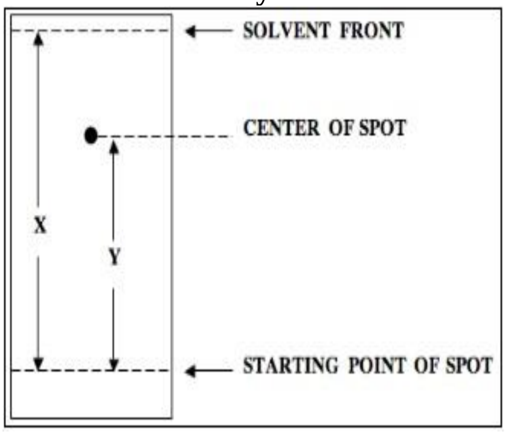

Fig.1 Spotting of TLC plate

Rf values are highly dependent on the nature of the adsorbent and the solvent system used, which is why experimental Rf values may not always match those found in the literature. Generally, low-polarity compounds have higher Rf values compared to more polar compounds. Rf values are determined by observing the spots on TLC plates, often under a UV transilluminator at 365 nm to detect compounds that are otherwise invisible to the naked eye.

The Rf values of separated compounds can be compared to standard or literature values to help identify the components of a mixture. With proper calibration, thin layer chromatography (TLC) can be used for both qualitative and quantitative analysis, making it a fast and versatile technique. TLC is widely used in fields such as chemistry, biochemistry, and pharmacology for purposes like determining compound purity, monitoring chemical reactions, and identifying the components of mixtures.

Rf values are always less than 1 because they represent the ratio of the distance traveled by the solute (analyte) to the distance traveled by the solvent front. This ratio can never exceed 1 since the solvent front always moves faster than the solute due to the solute's interaction with the stationary phase. The Rf value is calculated by dividing the distance the solute travels by the distance the solvent front moves. However, Rf values remain consistent only if all experimental conditions, such as solvent system and adsorbent type, are kept constant for each analysis.

Factors influencing Rf values include the following:

Nature of Adsorbent

In thin layer chromatography (TLC), the "adsorbent" refers to the solid substance coated as a thin layer on a flat surface, such as a glass or plastic plate. Common adsorbents used in TLC include materials like silica gel or alumina. The separation of ßsample components on the TLC plate is influenced by the sample's properties, particularly particle size and chemical composition. As sample molecules move across the TLC plate, they interact with the adsorbent, which causes them to separate based on their affinity for the adsorbent and the mobile phase.

Different adsorbents can be used depending on the specific separation requirements of the experiment. For instance, cellulose, a natural polysaccharide, can serve as an adsorbent in TLC and is particularly useful for separating chiral compounds when modified to create chiral stationary phases. Reverse-phase (RP) silica, a non-polar adsorbent, is commonly used to separate non-polar or hydrophobic compounds, typically in combination with non-polar solvents.

Some TLC plates are also impregnated with fluorescent materials, making it easier to visualize the separated compounds under UV light. When selecting the appropriate adsorbent, the nature of the compounds to be separated and the optimal conditions for separation must be considered. Polar adsorbents like silica gel tend to interact more effectively with polar compounds, while non-polar adsorbents such as RP silica are better suited for separating non-polar substances. The success of TLC separation is also influenced by factors such as the solvent system and the thickness of the adsorbent layer.

Mobile Phase

The mobile phase in TLC refers to the solvent used to separate compounds based on their chemical composition and polarity. It is crucial to choose a solvent that matches the sample's properties to ensure proper separation. Volatile or hygroscopic solvents, like acetone, require fresh batches for each run. The solvent's composition and polarity significantly influence the movement and separation of compounds on the TLC plate, causing them to travel at different rates. This, in turn, determines how the molecules can be visualized and analyzed.

Temperature

Temperature affects the migration and separation of compounds in thin layer chromatography (TLC) by influencing the interactions between the compounds and the mobile phase. The characteristics of the mobile phase, such as its polarity and composition, directly impact how compounds move across the TLC plate. Different compounds interact with the mobile phase in varying ways, leading to different migration speeds and facilitating separation. Changes in temperature can alter these interactions, affecting the rates of migration and separation and, consequently, how compounds are visualized and analyzed.

Apparatus and working

TLC consists of glass plates, stationary phase, solvent system, aluminum foil, chromatographic chamber.

1. TLC Plate: Coated with a stationary phase (e.g. silica gel, alumina)

2. Solvent System: A mixture of solvents (e.g. hexane, ethyl acetate)

3. Spotting device: For applying samples to the TLC plate

4. Detection methods: UV lamp, fluorescent lamp or chemical staining.

Techniques for Thin Layer Chromatography

Spotting of the sample

To begin, we can acquire a pre-coated TLC plate from the laboratory instructor, which is already coated with silica gel as the adsorbent. The first step involves drawing a light pencil line, leaving a distance of 0.5 to 1 cm from the edge of the plate. It's crucial to exercise caution when drawing this line; otherwise, the pre-coated silica gel may be dislodged. This line serves as the baseline for our sample spotting.

A minimal quantity of the compound is then used for spotting. The sample is dissolved in the appropriate solvent, which will be specified by the laboratory instructor, either in a sodium fusion tube or an Eppendorf vial. A thin capillary is employed to dispense a small amount of the sample solution onto the baseline. Typically, a single sample spot is placed in the center of the baseline.
If multiple samples are to be spotted, it's important to ensure an appropriate distance is maintained between each spot to prevent them from merging during the elution process.

Preparation of container for developing TLC

To begin, select a dry beaker with a capacity ranging from 100 to 150 mL or a TLC chamber. If the beaker is not already dry, it should be rinsed with the solvent intended for use. It is crucial to exercise caution and avoid rinsing with any other solvent, particularly water. Next, place a clean, dry piece of filter paper standing upright against the wall of the beaker. This paper will absorb some of the solvent, thereby saturating the surrounding air with solvent vapor. This process will expedite the separation of the components on the TLC plate by reducing the rate of solvent evaporation from the plate.

Pouring the Solvent

Carefully pour a small amount of solvent into the beaker, ensuring it is only a few millimeters above the base. To minimize solvent evaporation, cover the beaker with a watch glass or Petri dish.

Adjusting the Solvent Level

If too much solvent is used, the level will exceed the baseline on the TLC plate, which could result in the spot being drained. It is essential that the height of the solvent in the beaker does not surpass the baseline on the TLC plate.

Running the TLC Plate

Gently place the TLC plate into the beaker, using forceps if necessary. Cover the beaker with a watch glass. Ensure that the baseline of the solvent system is not submerged. Monitor the solvent front as it travels up the plate, which may take some time, especially with less volatile solvents. Once the solvent front is approximately 0.5 to 1.0 cm from the top edge of the plate, carefully remove the plate with forceps. Immediately mark the position of the solvent front with a pencil. This marking is essential for calculating the Retention Factor (Rf) values.

Visualization

If the component(s) present in the sample are colored, they will be visible. If the substances are not colored, they can be visualized using UV light. The aromatic rings in organic compounds absorb UV light, resulting in dark spots where the compound is present. The plate is observed under a UV lamp, and any spots visible under UV light are circled to note their positions after the plate is removed from the UV chamber.

Compounds that are not UV active can be identified through appropriate staining of the TLC plate. This can be achieved by using a chamber containing iodine, or by spraying the plate with a suitable reagent. Reactions that cause a color change occur with the compound on the plate, making the spots visible.

For any TLC experiments, it is recommended to sketch the diagram of each plate in the notebook as part of the experiment's record, followed by reporting the Rf values of the spots. Additionally, a photo can be taken using a mobile phone and included in the report.

Preparation

Depending on the compounds to be resolved, choose the TLC sorbent, developing solvent system, method of detection and visualization, development chamber and method of development.

Step 1: Prepare the TLC Plate.

Step 2: Apply the sample on the plate

Step 3: Develop the TLC plate

Step 4: Detect and Visualise the compounds on the plate

Advantages

Thin-layer chromatography (TLC) is simple, inexpensive, and easy to teach. It requires minimal materials and can be applied to other chromatographic techniques like HPLC once the best solvent is identified. Multiple compounds can be separated on one plate, and the mobile phase can be easily adjusted with different solvents. TLC is useful for checking compound purity with UV light and identifying compounds using Rf values. Chromatography conditions can also be modified for better resolution.

Disadvantages

Thin-layer chromatography (TLC) has several disadvantages: it has limited separation due to short stationary phases, a higher detection limit compared to other chromatographic methods, and is sensitive to environmental factors like humidity and temperature because it operates as an open system.

Advancements in TLC

1. Instrumental Advancements

Thin Layer Chromatography (TLC) has seen notable instrumental enhancements, particularly with the emergence of High-Performance TLC (HPTLC), which boosts resolution and sensitivity. Automated TLC systems have increased precision and throughput, making TLC more appealing for industrial use. Additionally, the integration of TLC with Mass Spectrometry (TLC-MS) has significantly improved detection and identification capabilities.

2. Methodological Advancements

Recent methodological improvements have broadened the scope of TLC applications. Two-Dimensional TLC (2D-TLC) enhances separation and resolution, while Micellar TLC facilitates the separation of hydrophobic compounds. Innovations such as Ionic Liquid-based TLC and Chiral TLC allow for better separation of polar and enantiomeric compounds, respectively. Bioaffinity TLC also enables the selective detection of biomolecules.

3. Stationary Phase Advancements

Innovations in stationary phases have further elevated TLC performance. Nano-porous TLC plates offer better resolution and sensitivity, while functionalized plates provide selective detection of specific compounds. Monolithic TLC plates enhance separation efficiency, and bio-based plates present sustainable, eco-friendly options.

4. Detection Advancements

Advancements in detection methods have markedly improved TLC's sensitivity and selectivity. Techniques such as Hyperspectral Imaging and Chemiluminescence Detection enhance sensitivity, whereas Fluorescence Detection and Mass Spectrometry Detection offer improved selectivity.

Future Directions

The combination of TLC with Mass Spectrometry has emerged as a powerful tool, particularly in pharmaceutical and biomedical research. Looking ahead, TLC is expected to integrate Artificial Intelligence (AI) and Machine Learning (ML) to enhance data analysis. There are anticipated developments in new stationary phases and the further expansion of TLC-MS applications, alongside the potential for portable and handheld TLC devices for field-based analysis.

Recent Research

Current research efforts are focused on the TLC-MS analysis of pharmaceuticals, detection of food contaminants, and assessment of environmental pollutants. Additionally, there is ongoing work in TLC-based disease diagnosis and the analysis of biological samples using TLC-MS.

Applications of TLC

Thin Layer Chromatography (TLC) is a widely employed analytical technique known for its versatility and efficiency in separating and identifying compounds in various industries, including pharmaceuticals, food, cosmetics, and biochemistry. One of its primary applications is in the pharmaceutical sector, where it is crucial for assessing the purity of drug samples and determining the concentration of active ingredients, auxiliary substances, and preservatives. By allowing direct comparisons between a sample and an authentic reference, TLC facilitates qualitative testing of various medications, such as sedatives, analgesics, and steroids. This capability is vital for adhering to pharmacopoeial standards, which mandate rigorous impurity detection and process control.

In the realm of compound identification, TLC proves invaluable for analyzing a range of natural products, including essential oils, glycosides, and secondary metabolites like polyphenols and flavonoids. Its application extends to identifying illegal substances, particularly in sports, where it is used to detect banned anabolic steroids. This aspect highlights the technique's importance in both regulatory and safety contexts.

TLC also plays a significant role in biochemical analysis, where it is used to separate and isolate metabolites from biological matrices such as blood plasma, urine, and serum. This capability is essential for diagnosing metabolic disorders, including phenylketonuria and cystinuria, particularly in newborns. When combined with other chromatographic techniques, TLC enhances the detection of minor metabolites, providing a comprehensive understanding of urinary constituents derived from lipids, amino acids, and other compounds.

In the food industry, TLC serves as a vital tool for ensuring the safety and quality of food products. It is employed to identify colors, sweeteners, and preservatives, as well as to detect pesticide residues in vegetables, fruits, and meats. This application is critical for compliance with safety regulations, ensuring that food products meet legal standards for contaminants and additives.

The cosmetic industry also benefits from TLC, which is used to identify raw materials and constituents in dyes, preservatives, and fragrances, helping to ensure the safety and efficacy of cosmetic products. Moreover, in general chemistry, TLC is utilized for the separation and identification of closely related compounds and for analyzing cationic and non-ionic surfactants.

Overall, Thin Layer Chromatography is an essential analytical tool that not only facilitates quality control and compliance across various industries but also plays a pivotal role in scientific research and development, making it indispensable in modern analytical chemistry. Its ability to provide rapid and reliable results underscores its significance in ensuring product safety and efficacy.

CONCLUSION

TLC is a versatile and straightforward technique for separating and analyzing compounds. It is an unique combination of simplicity, speed, and sensitivity makes it an indispensable tool in analytical chemistry. The future of TLC holds much promise, with ongoing research and development poised to unlock new possibilities and applications, further cementing its status as a vital analytical tool.

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