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THIN LAYER CHROMATOGRAPHY: A COMPREHENSIVE REVIEW

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ABSTRACT

Thin Layer Chromatography (TLC) is a popular analytical method for separation and identification of components in mixture. This review provides a wide overview by covering the basic principles, methodology, retention factor calculations, and their applications in various scientific fields. Its simplicity, low cost, and versatility have made this technique a necessary instrument in pharmaceutical analysis, natural product chemistry, forensic science, and quality control laboratories. The review paper discusses the importance of TLC in modern analytical chemistry as well as its advantages and limitations.

KEYWORDS: Thin layer chromatography, retention factor, stationary phase, mobile phase, analytical technique, Visualization techniques.

1. INTRODUCTION

Chromatography is considered one of the most significant separation methods in analytical chemistry, and it is used in the pharmaceutical, environmental, food, and forensic sciences. Among the existing chromatographic techniques, thin-layer chromatography deserves to be listed among the most basic techniques that are equally effective at the same time. TLC, initially invented in the early 1950s, is now a competent technique to analyze chemical compounds qualitatively and semi-quantitatively.

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The name chromatography is a combination of the Greek terms “chroma” (colour) and “graphein” (to write), as it is based upon the separation of coloured pigments of plants. The separation of compounds on a thin layer of adsorbent material coated on a flat support is the specific process that is involved in TLC. Unlike column chromatography, where the compounds are separated in a vertical direction using the packed column, TLC separations are done in a horizontal or at an angle on a plane surface, with the ability to analyze numerous samples at once.

The popularity of TLC in the laboratories worldwide is due to a combination of several factors: low equipment needs, fast analysis time, low costs of operating, and the possibility of simultaneous analysis of multiple samples. These properties render TLC especially useful in the initial screening, monitoring reaction progress, and in the determination of optimal conditions for other separation methods [1].

2. Principle of Thin Layer Chromatography

The principle that is the basis of TLC is the differential distribution of compounds between two phases, one of which is a stationary phase and another, a mobile phase. The stationary phase includes a thin film of the adsorbent substance attached to a solid structure, whereas the mobile phase is a solvent/mixture of solvents that moves through the stationary phase by capillary action.

2.1 Adsorption and Partition Mechanisms

TLC uses two main mechanisms, namely adsorption and partition. In adsorption chromatography, which is the most typical in TLC, the separation is achieved through the differences in the adsorption of the sample components on the stationary phase surface. Polar compounds interact more strongly with polar adsorbents such as silica gel, and less polar compounds interact with the adsorbents weaklier and move easily with the mobile phase.

The partition mechanism, conversely, is the distribution of the analyses across two liquid phases. This is achieved by modifying the stationary phase with a liquid coating to get a liquid-liquid partitioning system instead of solid-liquid adsorption [2].

2.2 Polarity and Separation

The process of separating compounds in TLC is very much dependent on the relative polarities of the compounds. One of the most important rule of chromatography is the

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statement that “like dissolves like”. The polar compounds are held longer and travel shorter distances in normal-phase TLC, where the mobile phase is less polar than the stationary one. On the other hand, in reversed-phase TLC, the stationary phase is a non-polar phase, and non-polar compounds are more strongly retained.

Separation efficiency is directly dependent upon the mobile phase polarity. A more polar mobile phase will bring compounds higher up the plate, and a less polar solvent system will lead to lesser migration. This relationship enables the chromatographers to maximize separations by modifying the solvent composition [3].

3. METHODOLOGY

3.1 Components and Materials

A common TLC system has a number of important components:

Stationary Phase: The silica gel (SiO_2) is the most widely adopted adsorbent, which is characterized by the large surface area, the uniform size of particles, and the high affinity to adsorption. Alumina (Al_2O_3), cellulose, and modified silica phases have also been used as other adsorbents. These materials are coated onto the glass, aluminum or plastic backing in layers usually between 0.1 and 0.25 millimeters thick.

Mobile Phase: The mobile phase is pure solvents or mixtures of solvents that are chosen in terms of polarity and the type of compounds that will be separated. The typical solvents are hexane, chloroform, ethyl acetate, methanol, and mixtures of these solvents. The solvent system should be optimized in such a way that good separation is attained without too long migration times.

Sample Application: The samples are dissolved in volatile solvents and placed in small spots or bands towards the bottom of the TLC plate. The sample should be properly applied because large or diffuse spots will result in inefficient separation and ambiguous outcomes [4].

3.2 Experimental Procedure

Systemic approach of TLC procedure is followed by:

1. **Plates preparation:** Commercially pre-coated plates are most widely used, although laboratory-prepared plates can also be used. Before using, plates may be activated to remove the adsorbed moisture.

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2. **Sample Application:** Small portions of sample solution are to be spotted on the plate using a capillary tube or micropipette, usually 1-2 centimeters from the bottom edge. Several samples may be placed in a horizontal line with sufficient spacing between the spots.

3. **Development:** The spotted plate is to be put in an upright position inside a closed chamber containing the mobile phase solvent. The level of the solvent should not exceed the spots of the samples. The solvent is pulled by capillary action up the plate, taking with it the components of the sample. To maintain the solvent vapor saturation and ensure reproducible results the chamber ought to be kept covered.

4. **Detection:** Once the solvent front has moved a certain distance (usually 10-15 centimeters), the plate is taken out, and the solvent front position is marked immediately. When compounds are coloured, they can be seen directly. In the case of colourless compounds, the visualization methods are used [5].
   Source: https://www.chemistrylearner.com/chromatography/thin-layer-chromatography

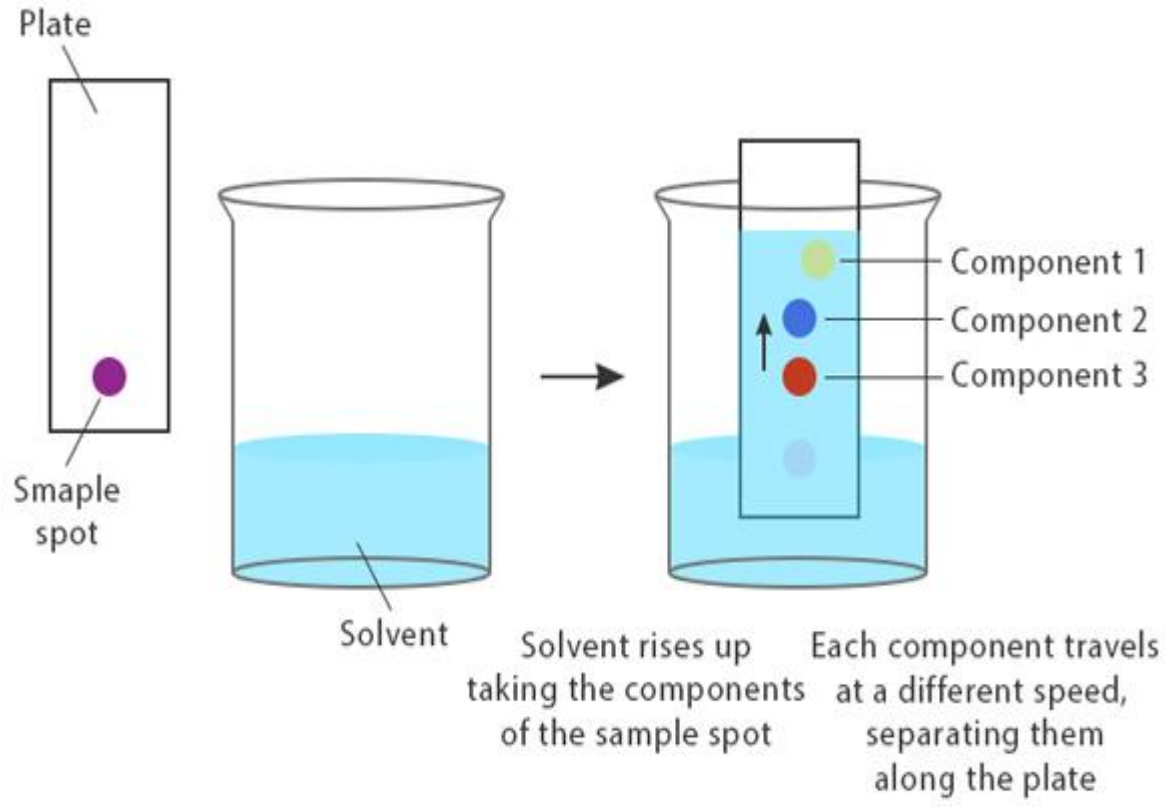

Fig. 1: Procedure for Thin Layer Chromatography.

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3.3 Visualization Techniques

There are a number of ways to identify the separated compounds:

1. **UV Illumination:** The UV light at 254 or 366 nanometers is absorbed by many organic compounds. Plates with fluorescent indicators appears bright under UV light, with compounds appearing as dark spots by quenching fluorescence [6].

2. **Chemical Reagents:** Ninhydrin used in amino acids, and sulfuric acid used for general visualization through charring [7].

3. **Physical Methods:** Iodine vapor chambers offer a temporary visualization because iodine adsorbs onto organic compounds to form brown spots that disappear with time [8].

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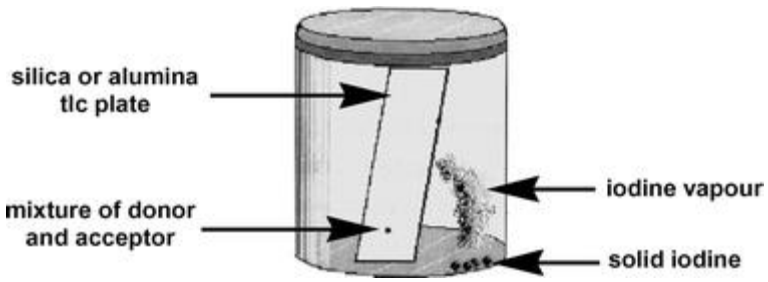

Fig. 2: A Demonstration of Iodine-Based Visualization in Thin-Layer Chromatography

4. Retention Factor (Rf) Values

The retention factor, often referred to as Rf, gives quantitative measure of the migration of compounds in TLC. This dimensionless parameter is calculated by the following formula:

Rf = distance travelled by solute (compound) / distance travelled by solvent.

The values of Rf are always between 0 and 1; 0 means no movement, and 1 means movement same to the solvent front. Practically, useful Rf values are generally known to be between 0.2 and 0.8 because extreme values do not give much information on the identification of the compound [9].

4.1 Factors Affecting Rf Values

Rf values depend on a number of experimental parameters:

Stationary Phase: Retention is altered by various adsorbents and coating thickness. The moisture content influences the activity of silica gel, which greatly affects the Rf values.

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Mobile Phase Composition: The polarity of solvents has a direct relationship with the migration of compounds. Small changes in solvent ratios can have significant effects on Rf values.

Temperature: Rf values are generally elevated by raising temperature, thus increasing the mobility of the molecules and decreasing the viscosity of the solvent.

Saturation: Chamber saturation with solvent vapor ensures reproducible conditions. Unsaturated chambers tend to yield less reproducible high Rf values.

Sample Load: Overloading generates distorted spots of Rf values with altered true values because of non-linear adsorption effects [10].

4.2 Applications of Rf Values

The uses of Rf values in TLC analysis include:

Compound Identification: Rf values are used to determine unknown compounds under standardized conditions by comparing them with reference standards that are also analyzed at the same time.

Purity Assessment: The occurrence of a single spot with consistent Rf values between different solvent systems would indicate that the compound is pure, whereas the presence of many spots would indicate impurities.

Method Development: Rf values are used to optimize separation conditions for preparative or analytical applications.

It should be noted that the Rf values are not absolute constants but system-dependent parameters that need to be carefully standardized in order to make valid comparisons [11].

5. Advantages of Thin Layer Chromatography

TLC also has many practical advantages that contribute to its long-term popularity:

Simplicity: It is a simple technique that needs only minimal training and can be done using simple laboratory equipments. Routine applications do not require any complicated instrumentation or a large amount of theoretical knowledge.

Cost-Effectiveness: TLC is much cheaper to capitalize in and run than other instrumental methods such as HPLC or GC. Pre-coated plates are cheap, and only a small amount of solvent is needed.

Speed: This method can be used to analyze several samples at the same time, and the average analysis duration is between 30 minutes and an hour. This parallel processing feature enables high sample throughput.

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Versatility: TLC is compatible with a variety of sample types and classes of compounds. The method is equally applicable to both organic and inorganic compounds, small molecules as well as moderately sized biomolecules.

Visual Detection: The visualization of all components in a single plate will give instant qualitative information of sample composition and purity.

Flexibility: The stationary phase and mobile phase can be adjusted for the specific application with ease by chromatographers. And the detection methods can also be changed in order to optimize separations.

Sample Recovery: Unlike the destructive methods the compounds can be scraped off the plate and extracted for further use or analysis.

Minimal sample requirement: TLC needs a sample in microliter amounts, which conserves the precious or scarce materials [12].

6. Disadvantages and Limitations

Despite of its many advantages, TLC has some limitations:

Limited Resolution: TLC has lower resolution and peak capacity as compared to high-performance liquid chromatography. Complex mixtures with numerous closely related compounds might not be separated sufficiently.

Qualitative Nature: TLC is a qualitative method, although densitometry can also be used semi-quantitatively. Specialized equipment and careful calibration is required for precise quantification.

Reproducibility Problems: Rf values may differ in laboratories or even between runs due to environmental factors such as temperature and humidity. To have reliable results, standardization is necessary.

Manual Operation: TLC uses manual sample application, development, and interpretation which have operator-dependent variability unlike automated instrumental analysis methods.

Limitations on Sensitivity: Detection limits have been found to be generally larger than instrumental methods, typically in the microgram range rather than nanogram or picogram level.

Destructive Visualization: In many visualization techniques, especially those that use chemical reagents and charring, the separated compounds are irreversibly altered or destroyed, preventing recovery.

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Time-Intensive Quantification: When quantitative analysis is needed, densitometric scanning increases time and equipment costs, negating some of TLC's simplicity advantages [13,14].

7. Applications

TLC is used in several different scientific and industrial fields:

7.1 Pharmaceutical Analysis

TCL has wide applications in pharmaceutical industry in quality control, formulation development and regulatory compliance. It is used in identity testing of raw materials, testing purity of active pharmaceutical ingredients, testing stability of degradation products and detection of drug counterfeits. Under proper validation, TLC is a quick and cost-efficient way of performing routine pharmaceutical testing which is compliant with regulatory requirements [15].

7.2 Natural Product Chemistry

Researchers involved in the study of plant extracts, essential oils and marine natural products use TLC in preliminary screening and monitoring fraction during an isolation process. The method assists in the identification of the classes of compounds, guide optimization of extraction process, and tracking of compounds through purification schemes. The fact that crude extracts can be handled in TLC without the need of extensive sample preparation makes it useful in the study of natural products [16].

7.3 Forensic Science

Forensic laboratories use TLC to analyze drugs of abuse, traces of explosives, inks, dyes, and other evidence materials. This method is simple and inexpensive thus it can be used in field testing and preliminary screening with confirmatory analysis by more advanced methods after positive results [17].

7.4 Food Chemistry

TLC is used by food analysts to identify pesticide residues, food additives, vitamins, antioxidants and contaminants. They are used in food analysis involving edible oils, monitoring of food processing reactions, and verifying food authenticity. TLC offers quick screening for quality control in food production and regulatory monitoring [18].

7.5 Environmental Analysis

In environmental laboratories, TLC is used to analyse pesticides, polycyclic aromatic hydrocarbons and other contaminants in water, soil and air samples. Although instrumental

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techniques are better in terms of sensitivity, TLC is still capable of preliminary screening and educational purposes [19].

7.6 Clinical and Biochemical analysis

TLC is used in clinical labs as a method of analyzing lipids, amino acids, steroids, and drugs in biological fluids. TLC has mostly been replaced by automated instrumental techniques in the routine clinical practice, but it still has its place in specialized uses and limited resources settings [20].

7.7 Research and Education

TLC also serves as a good pedagogic tool in introducing students to the principles of chromatography. Its visual quality enables the learners to comprehend the separation mechanisms, polarity theory, and methodology of analysis. TLC is commonly used in the research laboratory to monitor progress of reaction, fraction checking of columns, and to develop other methods [21].

8. Modern Developments

New developments have improved the TLC capacities and increased its applications. High-performance thin layer chromatography (HPTLC) uses smaller and more uniform particles on its plates, and provides better sensitivity and resolution. Automated sample application and development systems reduce operator variabilities and increase throughput. Through the use of densitometric scanning and image analysis software, the TLC technique can be transformed from a strict qualitative technique to a quantitate analytical method [22].

Two-dimensional TLC greatly improves separation power for complex mixtures by employing distinct solvent systems to develop the mixtures successively in perpendicular directions. This method can be especially useful when it comes to the analysis of botanical extracts and biological samples that have many components [23].

The combination of TLC and mass spectrometry with other spectroscopies gives strong hyphenated procedures that combine the separation potential of TLC with advanced identification instrumentation. These advances make TLC still up-to-date in modern analytical laboratories despite competition from advanced instrumental techniques [24].

9. CONCLUSION

Thin layer chromatography is a basic analytical method which is simple yet effective. Nevertheless, TLC continues to be useful in analytical labs all over the globe despite the advanced instrumental techniques are available. Its low cost, minimal equipment

requirement, fast analysis, and multiple samples processing ensure continued relevance in various applications.

The knowledge of the TLC principles, methodology, and limitations gives analysts a flexible tool that they can be used in many analytical challenges. TLC, however, provides practical answers that can supplement more advance methods, whether it is in the routine quality control, preliminary research screening, educational demonstrations or development of methods.

As analytical chemistry continues evolving, TLC adapts through advancements in technology, but it remains same in its fundamental nature as a convenient and efficient way of separation.

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