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Review Article

Thin Layer Chromatography (TLC) VS. Paper Chromatography: A Review

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Abstract

In the field of analytical chemistry and various pharmaceutical fields the separation techniques are widely used to study specific compounds from a mixture or a complex material. One such separation technique is called Chromatography. It takes place on the basis of the relative volume of each solute present in the moving fluid stream. The discovery of chromatography was done by Mikhail S. Tsvet, who was a Russian botanist in the year 1901. The separation is done through stationary phase and a mobile phase. Chromatography is used for separation, analysis, and purification of various components which can include food, pesticides, pharmaceuticals, tissue extracts etc. In this paper 2 major types of chromatography are discussed; these include Paper Chromatography and Thin Layer Chromatography. The principles of both the types will be discussed and the contrast will be established between the two in a tabular format.

Keywords: Analytic; Pharmaceutical; Chromatography; Mobile Phase; Stationary Phase

Introduction

Chromatography is a technique used for separating the solutes or components present in a mixture. This separation takes place on the basis of the relative volume of each solute present in the moving fluid stream, which is known as mobile phase, and also present in the stationary phase. The mobile phase can comprise of a liquid or a gas and the stationary phase comprises of a solid or a liquid. The discovery of chromatography was done by Mikhail S. Tsvet, who was a Russian botanist. In the year 1901, Tsvet discovered the physicochemical basis of separation and applied it in a scientific way to separate plant pigments [1]. He specifically focused on the carotenoids and the chlorophylls of the plants. Since he did his research majorly on the coloured components of the plants, hence he named this method as chromatography which also derives meaning from Greek words. Chromatography can be effectively used for the purpose of separation, analysis, and purification of various components which can include food, pesticides, pharmaceuticals, tissue extracts and also air and water samples.

This technique is widely used in many pharmaceutical and chemistry domains because of its precision and applicability in these areas. Some of the important uses of Chromatography are as follows:

- **Chromatography and manufacturing of drugs:** In the pharmaceutical industry the drugs are produced primarily using high-performance liquid chromatography. This technique is considered apt for compound separation before characterization while making the drug. This technique defines the quantitative composition of various compounds

in a drug. Hence, it is widely used in quality control testing of the drug. This type of chromatography is also used in drug characterization [2]. It is also used in purification of products during various stages of synthesis, using modern automated processes in a timely manner. This exercise results in a more refined final product.

- **Chromatography and testing of drugs:** Chromatography is widely used in drug testing by various law bodies like police and forensic. In the testing the bodily fluids such as blood or urine are tested using chromatography to separate the naturally occurring compounds that result from metabolic breakdown of ingested material. In drug testing urine samples are preferred over blood samples because most of the drug compounds have relatively shorter half life in blood rather than that in urine. In urine many drug compounds can be detected even after several months of consumption. These compounds can include cannabinoids, opiates and cocaine. While testing chromatography is paired with mass spectrometry of the compounds for authentic results. For the quantitative analysis of drugs in a urine sample usually gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry is used. Gas chromatography-mass spectrometry is more time and sample consuming however it detects the wider range of compounds present in the sample.
- **Chromatography in vaccine production:** During the making or in the final stage of production, many vaccines are purified using the technique of chromatography. Chromatography can also be used to isolate the antigen of interest of the vaccine which can be then amplified for mass production. For instance, the recent SARS coronavirus spike protein was isolated using liquid chromatography technique which allowed it to be produced in bulk quantities. This isolation enables the researchers to perform many tests on the isolated protein which help them understand the structural and biochemical characteristics of the protein. This understanding helps in making vaccines that can counter the virus effectively. Chromatography is potent in separating the component of interest from the by-products present in the growth medium which enables the researchers to isolate the inactivated or attenuated virus that can be used as an antigen to make an effective vaccine.
- **Chromatography in analysis of food items:** Due to the manufacturing of harvesting the food products generally contain complex mixtures of compounds which are in bulk quantities. These compounds can occur either naturally or they are imparted while they are processed. For the purpose of analyzing these complex combinations of compounds present in the food, chromatography is used. Chromatography can help the researchers to separate these complex mixtures to have a deeper knowledge of their composition. This becomes necessary in the type of foods where there is high concentration of some particular compounds in food. For example, any product which is plant-based can have some parts of harmful pesticides which can be separated with the help of chromatography. Moreover, in any non-vegetarian food there can be a presence of a veterinary drug in the animal flesh which can be harmful for human consumption. Hence, chromatography is widely used for such analysis of food products. Moreover, chromatography helps in quality control of the food products by detecting any potential toxic impurities in them. This technique is also used for nutritional profiling of food items by allowing them to be standardized and authenticated.

Two major types of chromatography which are Paper chromatography and Thin Layer Chromatography will be discussed as follows.

Paper chromatography

Chromatography works on the separation of compounds based on their polarity towards either the mobile phase or the stationary phase. Due to this reason this technique is analytical in nature. For the purpose of separating solid and liquid, paper chromatography is used in which cellulose filter paper is the stationary phase and the liquid is the mobile phase. The paper chromatography technique was discovered in the year 1943 by Synge and Martin [3]. This technique functions on a particular type of paper, hence it is called a planar chromatography technique. A cellulose paper is used in this technique which acts as a stationary phase and helps in the separation of compounds.

Principle of paper chromatography [4-6]

The paper chromatography involves the principle of partition, which distributes various components between the liquid phases.

In this, the stationary phase which is the filter paper, holds the aqueous solvent. The mobile phase moves over the paper. The separation process is possible due to capillary action of the pores. The friendliness of these components with water decides the amount of separation. In a different method, separation may occur on the basis of adsorption. In adsorption, the surface of the paper behaves like a stationary phase and the liquid solvent behaves like a mobile phase. Hence, the adsorption actually happens between the solid and the liquid phases. Paper chromatography finds its application in various pharmaceutical industries.

Its applicability is wide because it is cost effective as compared to other methods. It separates the dissolved components by the degree of migration through the cellulose paper. However, for the results to be most accurate, the sample should be precise and minute.

Stationary phase

Paper chromatography is also known as partition chromatography. In this type of chromatography the usefulness of the mobile and stationary phase has huge applicability. It also focuses on the fact that there does not exist any particular rule for phases to be immiscible. The classification of the types of stationary phase are aqueous, hydrophilic and hydrophobic systems.

Aqueous stationary phase

The paper has the property of holding water. Hence, in this technique a paper which is already water-equilibrated is attached to a suspending paper inside a closed chamber whose atmosphere is completely water saturated. When there is a requirement of a salt phase or an aqueous buffer then this paper is moved through the solution and thereafter it is exposed to that part of the chamber which has a water saturated atmosphere. This type of stationary phase is best when the separation is to be done for a polar mixture (moderate to high).

Hydrophilic stationary phase

Hydrophilic stationary phase is made up of an organic solvent. Here, the key indicator of separation of any compound is the volatility of organic solvent. However, if the organic solvent is found to be sufficiently volatile then the paper is equilibrated in the solvent saturated atmosphere of the chamber. In a different method, the solvent of the stationary phase is dissolved in a highly volatile diluent that evaporates which distributes the liquid of the stationary phase uniformly throughout the paper. Some of the most common hydrophilic solvents are methanol, formamide, glycerol and glycols.

Hydrophobic stationary phase

For the paper to be hydrophobic it needs to be processed in advance so that it can depict the characteristic of retaining the hydrophobic stationary phase. This technique uses the dripping method in which the equilibration of solvent is done by vapors. Moreover the mixture of solvent and a volatile diluent are chosen to make the hydrophobic solvent react with the paper. Some of the commonly used solvents are, kerosene, dimethylformamide, aromatic and aliphatic hydrocarbons.

Mobile phase

In the process of paper chromatography, mobile phase can be used in multiple combinations. However, the process of selecting the required optimum eluting is only possible through trial and error as there is no specific way of selecting it. Yet, there are particular guidelines that can be referred while making such selection and also for estimating the conditions of elution. For example, in order to make the optimum selection, the component characteristics as well as the stationary phase used in the mixture should be studied well. In this type of chromatography, an organic solvent mixed with water acts like the solvent system. However, in order to control the ionization of analytes, there can be an addition of some acids or bases. Some of the acids used can be HCl, HNO3 and acetic acid, whereas bases like NH3 can also be used to control the ionization. For the purpose of better identification of the compounds, various combinations of solvent systems are used. This is based on the chemical nature of those compounds.

Thin layer chromatography

This method of chromatography uses a solid-liquid adsorption for the purpose of isolating non-volatile mixtures. In this method, the mobile phase is a liquid but the stationary phase is a silica gel coated glass plate. The polarity of the particles towards these phases (mobile and stationary) helps in their separation from one another. The technique of chromatography was discovered in the year 1906 by M. Tswettin. The procedure of thin layer chromatography for the most part utilizes a sheet of plastic, glass or aluminum foil, which is

covered with a thin layer of adsorbent material, typically cellulose, silica gel, aluminum oxide. This coated layer is known as the fixed (stationary) phase. This process follows the following steps:

- First of all, the sample is applied on the glass plate which is the stationary phase.
- A solvent or a mixture of these solvents which comprise the mobile phase is drawn up on that glass plate through capillary action.
- Due to this, numerous analytes begin moving onto the glass plate (stationary phase) at differing rates.
- This movement of analytes results in the separation of the compounds. happens due to the coming down of various analytes onto the stationary phase plate at different rates in the thin layer chromatography process.

TLC has been used extensively by the researchers for analyzing the progress of a reaction, finding multiple components that constitute any mixture and also for checking the purity of any compound. In this process there is a strife between the solute and the mobile phase in order to bind with the immobile (stationary) phase. The strife of these leads to the separation of the desired components. For instance, if in the stationary phase, silica gel is used then it is polar in nature. Hence, if two compounds are used that possess different polarity from each other, then the compound which is more polar will react more with silica. Due to this reaction the more polar compound will be able to remove the mobile phase from the binding places easily.

Subsequently, the compound which is less polar tends to move high on to the glass plate which leads to the inference that the value of Rf has enhanced. Nonetheless, in the event that the mobile stage is adjusted and is made with a more polar combination of solvents then it will disperse the solutes from the silica restriction. In this manner, all mixtures on the thin layer chromatography plate will climb high. For instance, in the event that a combination of ethyl acetate and heptane is utilized as the mobile stage, then adding more ethyl acetic acid will bring about high Rf values for every one of the mixtures. However, any adjustment of the polarity of the mobile stage by and large doesn't bring about the opposite movement of the compounds on the TLC plate.

Principle of TLC

In this type of chromatography, for the purpose of stationary phase a glass plate is used which is usually covered with silica gel or aluminum oxide. whereas for the mobile phase, a solvent is used which is chosen based on the mixture properties. Thin layer chromatography works on the principle of distribution of a compound between a solid stationary phase and a liquid mobile phase.

Comparison between Paper chromatography and Thin layer chromatography (TLC) [7-10]

BASIS	Paper Chromatography	Thin Layer Chromatography
Postulate	It is postulated on the principle of partition chromatography	It is postulated on the principle of adsorption chromatography
Stationary Phase	The stationary phase is made up of cellulose paper.	The stationary phase is made up of a silica coated glass plate.
Mobile Phase	There can be two types of mobile phases- Hydrophilic: These include compounds like ammonia, methanol, water and isopropanol (Hydrophobic: These include compounds like dimethyl ether, kerosene, cyclohexane and isopropanol	The mobile phase includes compounds like glycerol, carbon tetrachloride, pyridine and acetone.
Preparation time	Preparation time needed is less	Comparatively more preparation time is needed
Heat requirement	High heat is not required, therefore there is no need for prolonged heating of the paper inside the oven.	There is a high heat requirement therefore the TLC plate is heated for a longer period of time inside the oven.
Sample required	Sample amount needed is less.	Sample amount needed is more.
Silica Gel	Silica gel is not needed	Silica gel is used

Physical Separation	It can be done using mostly ascending technique	Physical separation is not done. Separation is done using descending technique.
Separation efficiency	Suitable for polar water soluble compounds	Suitable for less polar compounds
Time	Particle separation happens quickly.	Particle separation takes more time.
UV Analysis	Ultraviolet light analysis is not possible.	Ultraviolet light analysis is possible.
Cost	Less cost required	More cost required

Table 1

Conclusion

There are many separation techniques used to separate and study specific compounds from a complex mixture or material. In this paper two major chromatographic techniques are discussed which are: (1) Paper Chromatography (2) Thin Layer Chromatography. It is a scientific method used to isolate particles on the basis of their compatibility with the mobile as well as the stationary phase. Out of the two types of chromatography, paper chromatography is used when there is a need for a solid-liquid partition. In this technique the stationary phase and the mobile phase are actually cellulose filter paper and fluid respectively. This method is a adsorption of solid and liquid. This technique is highly useful for the separation and analysis of those compounds that possess non-volatile combinations. In thin layer chromatography, there exists a fixed phase (stationary) which is made up of silica gel covered glass plate and another phase is mobile which is a liquid. In this technique also just like the paper chromatography, the isolation of the components is based on their compatibility with the mobile and stationary phase. Both the techniques are used widely in analytical chemistry and other sciences as they both have their potent advantages.

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Conflict of Interest

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