

Paper Chromatography: A Review

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Abstract

Chromatography is a well known technique used for separation of compounds. Among all, chromatographic techniques, paper chromatography is a type of analytical tool which is used for separation of colored components. The principle involved may be separation and partition of components based on their affinity towards stationary phase. Literature survey reveals that paper chromatography has been used from ancient times, especially in countries like United State for separation of organic and inorganic components. Further, investigation should be made to make new advancement in the field of chromatography separation involving identification of types of paper used and gel permeation process.

Keywords: Paper chromatography; technique; principle; separation.

Introduction

The technique of paper chromatography was first discovered by Synge and Martin in 1943. Paper chromatography is specific type of technique that operates on a specific piece of paper [1]. 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. The method is comparatively cheap and helps to separate dissolved chemical substance by their different rates of migration through paper sheets. The method requires very minute quantity of sample for analysis [2].

Principle

The basic principle involved in paper chromatography is partition in which the various components get distributed or partitioned between liquid phases. It involves use of aqueous solvent held in pores of filter paper which acts as stationary phase whereas mobile phase travels over the paper [3, 4]. Due to differences in their affinity towards water (in stationary 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 on the basis of principle of adsorption between solid and liquid phases, where solid surface of paper serves as stationary phase and mobile phase is a liquid solvent. Although the main working principle of paper chromatography is partitioning this is employed in many pharmaceutical applications [5].

Migration parameters

The positions of migrated spots on the chromatography are indicated by different terms such as R_F , R_I , R_M and R_C . These parameters are also qualitative and quantitative parameters of substance.

R_F = The term R_F is associated with the migration of the solute relative to the solvent front as:

$$R_f = \frac{\text{Distance traveled by the solute from origin line}}{\text{Distance traveled by the solvent from origin line}}$$

The R_F is the function of the partition coefficient, it is constant for the given substance, provided the condition of chromatographic systems are kept constant with respect to temperature, type of paper, duration, direction of development, nature and the shape and size of the wick. The R_F values of a substance depends on no of factors such as

- Solvent system
- Medium of separation i.e. quality of paper

- c) Nature of mixture
- d) Temperature
- e) Dimensions of vessel in which operation is to be carried out.

Experimental details for qualitative analysis

- a) **Choice of proper chromatographic technique:** The first and prime job is to identify the mode of paper chromatographic technique required which mainly depends upon nature of the sample to be analyzed [6].
- b) **Choice of the filter paper:** The success of paper chromatography depends upon appropriate use of filter paper. The choice of paper is dependent upon the type of problem i.e. sample under investigation. The prime factors, that governs the choice are as follows:
 - (1) Type of analysis is qualitative or quantitative in nature or it is analytical or preparative technique
 - (2) Nature of the substance i.e. hydrophilic or lipophilic neutral or charged species [2, 7].

Types of papers

A wide variety of papers, which are very uniform from lot to lot are commercially available in different sizes, porosities, shapes, thickness and chemical treatments. In general, cellulose fiber is the main component of filter paper. The cellulose fiber is a linear polymeric carbohydrate chains owning hydrophilic character and is further cross linked with stable hydrogen bonded system. Water or other very popular types solvents are tightly held within the hydrophilic cellulose system and can be considered to be different from bulk water or polar system [8].

The cellulose papers can be altered in different ways to modify its chromatographic compartment. For example, paper can be impregnated with diatomaceous earth, alumina, silica gel, ion exchange resins. These kinds of papers will exhibit properties of these adsorbents 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 retain a 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 nonpolar-type organic polymers [9].

A glass fiber type paper can be used if very corrosive eluting conditions are needed. Adsorption symptoms due to the glass can be reduced by special treatment. Different types of Whatman chromatography papers are available and the choice of paper relies on type of separation. The most commonly used Whatman filter papers have a content of 90% of α -cellulose. Cellulose paper is also used as a support for various adsorbents like alumina, silica, zirconium oxide etc. which get precipitated in the pores of the filter paper to produce a thin sheet of adsorbent with the flexibility of the paper but having adsorbing characteristics of the precipitation [10].

Stationary and mobile phase

Paper chromatography is essentially partition chromatography and there is wide variety of useful combinations of stationary and mobile phases. It is not necessary that the two systems be immiscible. The types of stationary phase 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 water saturated atmosphere in a chamber. This type of system is particularly suited for separation of moderate polar to extremely polar mixtures [11].
- (2) **Hydrophilic stationary phase:** An organic solvent can be used for 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 leaving the stationary phase liquid uniformly distributed throughout the paper. Commonly used hydrophilic solvents include, formamide, methanol, glycerol and glycols [12, 13].

- (3) **Hydrophobic stationary phase:** The paper must be modified previously, before it will exhibit a tendency to retain hydrophobic stationary phase. Equilibration in the vapours of the solvent is the dipping technique in a solution of the solvent and a volatile diluent are used for introducing the hydrophobic solvent into the modified paper. Solvents such as dimethylformamide, aromatic and aliphatic hydrocarbons and kerosene are commonly used [13].

Mobile phase

Mobile phase in various combinations can be used in paper chromatography. Choosing optimum eluting condition is a trial and error process. However, certain guidelines can be used to predict eluting conditions. For example, the characteristics of the components in the mixture and the type of stationary phase being employed should be considered [14]. The solvent system used in paper chromatography is usually a mixture of organic solvent with water. The ionization of analytes can be controlled by addition of acids (HCl, HNO₃, acetic acid) and bases (NH₃). Different combination of solvent system can be used for identification of compounds based on their chemical nature for example; for amino acids the solvent system used is acetic acid: water: n-butanol in ratio of 1:5: 4. For separation of sugar; solvent system composed of ethylacetate: pyridine: water; water: ethyl acetate is suitable. For inorganic ions, solvents like pyridine: water or HCl: water more popular [15].

Modes of Paper Chromatography

Paper chromatography can be performed on the basis of method of development of chromatogram. Broadly; it can be classified as:

1. Ascending chromatography

The technique of ascending chromatography was introduced by Consden, Gordan and Martin and was later modified by Williams and Kirby [16]. As the name indicates, the chromatogram is allowed to ascend and development of chromatogram takes place due to movement of solvent in upward direction on the paper. In this technique, solvent reservoir is kept at the bottom of the beaker and piece of paper with loaded sample is dipped in solvent. It is always recommended to take care that the spot should remain above the solvent system. Furthermore, it is also important to take care about size of paper to avoid bending and crumpling.

2. Descending chromatography

In this chromatography, the development of chromatogram is done by allowing the solvent to travel down the paper. The solvent reservoir is kept at the top and process of movement of solvent is assisted against the 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 R_F value. Its only drawback of this technique compared with ascending chromatography is requirement of extensive apparatus [17].

3. Ascending- descending chromatography

This describes modified form of paper chromatography which 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 span of time, (ii) Components with R_F value > 0.50 can be detected individually as they will have own individual channel, (iii) Longer flow distance available which gives better resolution. The R_F value obtained by ascending descending chromatography is not significantly different those from ordinary techniques [18].

4. Radial chromatography

The term radial chromatography was described by Rutter and involves use of circular filter paper in which components get separated in the form of concentric rings rather than a single spot. A list of advantages was also given which includes (i) Sharpness and resolution of separation, (ii) speedy separation, (iii) Simplicity and compactness of the employed apparatus, (iv) Control on rate of flow of solvent, (v) Reproducibility, (vi) Ease of removal of test samples during and after development. The technique employs use of filter paper immersed in elutant and placed horizontally in a petri dish. The elutant flows from the center towards periphery of the paper and kept in covered petri plate for development of the chromatogram. The wick of the paper is dipped in mobile phase and solvent flows over the paper and the spots appear as concentric rings [19, 20].

5. Two-dimensional chromatography

Two-dimensional chromatography (Circular chromatography); is one of best method for separation of organic and inorganic compounds. Development of chromatogram is carried out in two different directions at right angle to each other. The sample is spotted on one of the corner of Circular paper and is allowed to develop in one direction followed by immersing of paper again in same solvent at right angle to the first one. The diagrammatic represents of various types of paper chromatography is depicted in **Figure 1**.

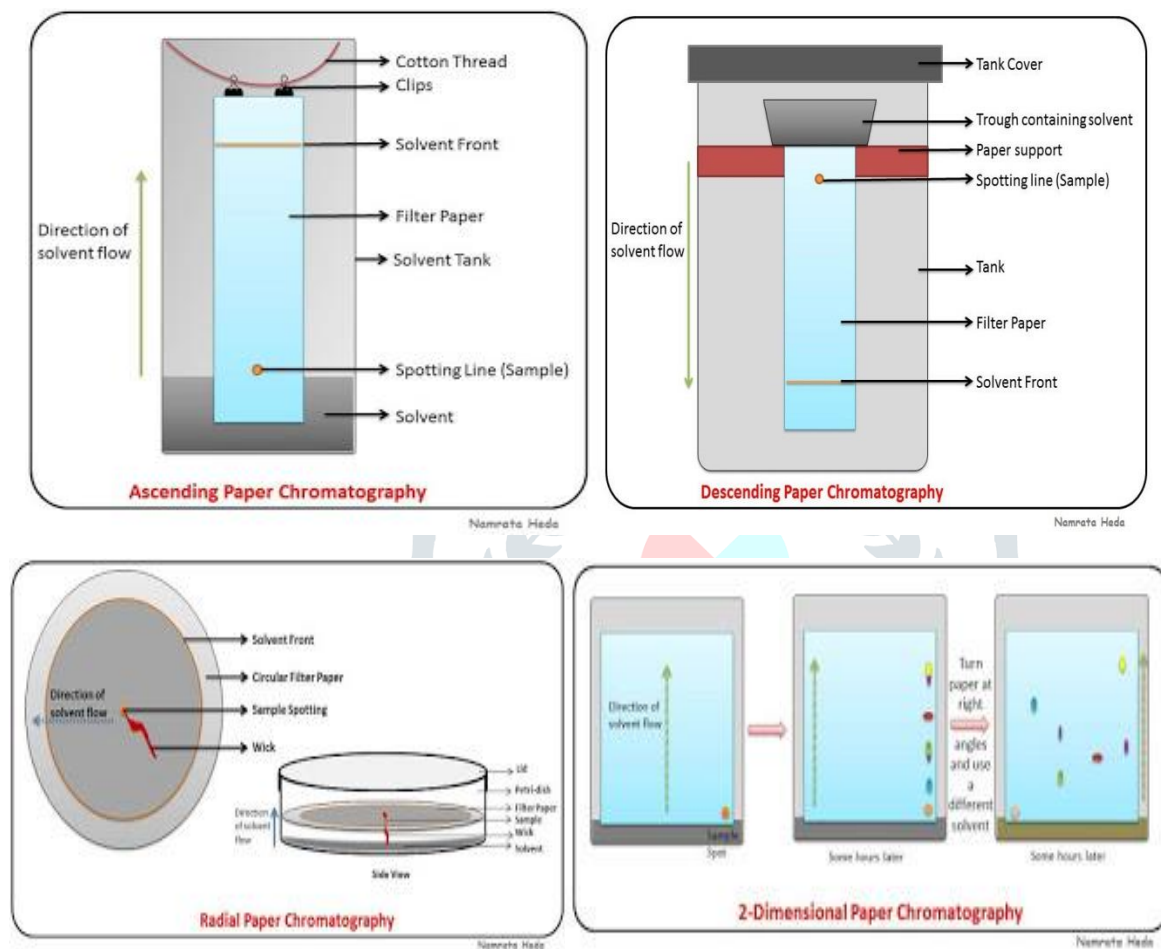


Figure 1: Different types of paper chromatography techniques

Procedure

The basic procedure for performing paper chromatography is explained below:

1. Selecting a suitable type of development

The type of development is selected on the basis of complexity of the solvent, type of paper used and nature of sample etc. Most of the time, radial chromatography is preferred due to its ease of operation and high resolution. Moreover, it is easy to perform, less time consuming and results are reproducible.

2. Selecting a suitable filter paper

Sample quality and pore size of filter paper helps to identify the appropriate type of filter paper to be used. Usually, a thin layer of filter paper (Whatman No. 1) is commonly used.

3. Prepare the sample

Sample is prepared by dissolving it in a suitable solvent and should be inert with sample under analysis and most of the times mobile phase is used for the purpose.

4. Spot the sample on the paper

Using a capillary tube, the sample should be appropriately spotted in the center on the paper and should be at a proper position.

5. Chromatogram development

The chromatogram is developed by immersing paper in the mobile phase. As soon as the filter paper gets the mobile phase through capillary action and along with the samples components also move based on their affinity towards mobile phase.

6. Paper drying and compound detection

After the development of the chromatogram, the paper is dried at room temperature followed by using an air drier. The components are identified by use of detecting agents and are characteristic for different chemical compounds.

Applications of paper chromatography

1. Paper chromatography is a useful method to identify number of constituents present in a sample, with a correctly chosen mobile phase.
2. This method requires small scale setup, involves very minute quantity of sample and is also cost effective [21].
3. Paper chromatography is an effective tool for separation of free amino acids present in human serum [22, 23].
4. It also offers a rapid method of separating and estimating sugars quantitatively; however the identification depends upon determination of their physical constants and formation of characteristic derivatives [24].
5. Paper chromatographic technique is also used for carrying out assay of pharmaceutical compounds such as mixture of phenylephrine hydrochloride, chlorpheniramine hydrochloride and dextromethorphan hydrochloride [25, 26].
6. The technique is also useful in isolation of pair of components having sample R_F values using two-dimensional paper chromatography [27].
7. Paper chromatography also involves inorganic applications such as separation of cations like cadmium, zinc, mercury, beryllium and calcium [28].
8. It technique is also helpful in identification of accelerator and anti-oxidant in rubber and is useful for determining its quality [29].
9. Paper chromatography is widely used in detection of various plant constituents such as opium, quinine alkaloids [30, 31].
10. Paper chromatography is also used to determine rate of ongoing reaction. Therefore, is a valuable tool in synthetic chemistry [32].

Conclusion

Future outlook of paper chromatography indicated that the technique may be continuously used for identification and separation of various pharmaceutical compounds because of its low cost and convenience factors. Further new advancements should be made to advance applications of this technique.

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