International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 276-279

Received: 22-01-2018
Accepted: 26-02-2018

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Paper chromatography analysis: A vital tool for chemistry

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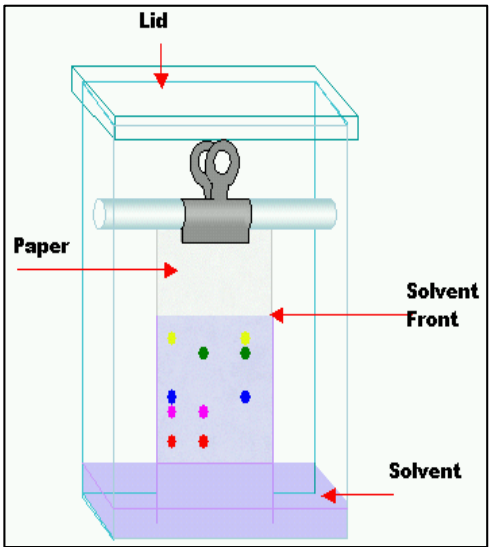
Abstract
A separation process is a method that converts a mixture or solution of chemical substances into two or more distinct products. Now-a-days, a large number of techniques are used to separate the components of a mixture like separating funnel, centrifugation, simple distillation, fractional distillation, chromatography etc. Chromatography is a one of the best technique for separation. Paper chromatography is a simplest and faster technique compare to other chromatography techniques. Paper chromatography as the name indicates it carried out on paper. An simple filter paper can be used for it. Different type of paper can also be used in it, depending on solvent. The main advantage of paper chromatography is its simplicity, low cost and hassle free operation. It can be run in various modes such as ascending, descending, 2-D mode etc. Its detection is analyzed by visually, fluoresce of substances using UV, Enzymatic and microbiological methods.

Keywords: Separation methods, chromatography, paper chromatography

Introduction
It is found that most materials in our surroundings are mixtures of two or more components. Mixtures are either homogeneous or heterogeneous. Homogeneous mixtures are uniform in composition, but heterogeneous mixtures are not uniform in composition**.** These mixtures can be separated into their components by several methods**.** A separation process is a method that converts a mixture or solution of chemical substances into two or more distinct product mixtures. There are a large number of techniques to separate the components of a mixture, such as separating funnel, centrifugation, simple distillation, fractional distillation, chromatography, magnetic separation, crystallization, sublimation, evaporation, precipitation, leaching, electrophoresis etc.
Chromatography is a technique for the separation of components from a mixture. It consists mainly two phases, mobile phase and stationary phase. As the name indicate mobile phase is a moving fluid stream, it may be either a gas or a liquid and Stationary phase is motionless, it may be either a solid or a liquid.



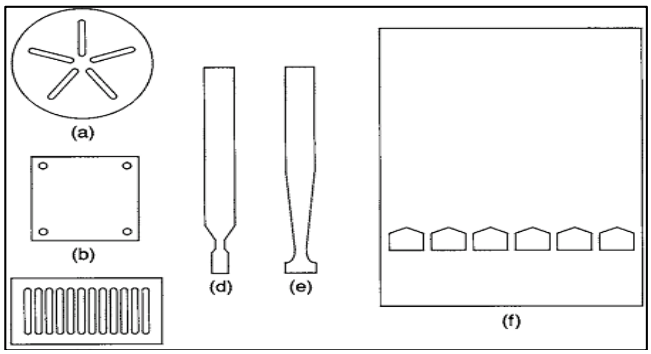
Chromatography was first employed in Russia by the scientist Mikhail S. Tsweet in 1900. He continued to work with chromatography in the first decade of the 20th century, mainly for the separation of plant pigments such as chlorophyll and xanthophylls. Tsweet developed a technique that is used today in the similar form. He took a vertical glass column with an adsorptive material, such as alumina, silica, or powdered sugar and added a solution of the plant pigments (chlorophyll and xanthophylls) to the top of the column, and washed the pigments through the column with a solvent. The pigments separated into a series of discrete coloured bands on the column. In Greek words colour meaning is chromatography, so M.S. Tsweet called the method chromatography. German chemist Richard Kuhn and his student Edgar Lederer reported the use of chromatography in the resolution of a number of biologically important materials. Novel types of chromatography developed during the 1930 year to 1940 year. In 1941 two British chemists, Archer J.P. Martin and Richard L.M. Synge, used a technique for the study of the amino acid composition of wool. Their technique was similar as that of M.S. Tsweet. There are different types of chromatographic techniques such as column chromatography, thin layer chromatography (TLC), paper chromatography and gas chromatography.

In this paper, we are providing a systematic investigation of paper chromatography with different technology and different filter papers. The advantage of paper chromatography over other different chromatography are also discussed. This work is expected to provide fundamental insights into the paper chromatography.

Result and Discussion

Paper Chromatography is a technique that is used to separate and to identify components of a mixture. Paper chromatography is one of the most important and simple chromatographic methods. Paper chromatography has proved to be very successful in the analysis of chemical compound and lipid sample in particular. In this chromatography, it uses paper as the stationary phase and a liquid solvent as the mobile phase the sample mixture is placed on a piece of paper, the edge of the paper is carefully immersed in a solvent, after that the solvent moves up the paper due to capillary action. Components of the mixture are carried along with the solvent up the paper to varying degrees, in other words the components of the mixture rise up at different degrees and thus are separated from one another depending on the compound's preference to be adsorbed onto the paper versus being carried along with the solvent. In order to obtain the extent of movement of a component in a paper chromatography, we can calculate retention factor "R_f value" for each separated component in the developed chromatogram. The R_f value is a number that is defined as the ratio of the distance traveled by the solute to the distance traveled by the solvent.

Nature of paper in paper chromatography is very important. The paper generally is of highly purified cellulose. The cellulose is a polysaccharide consisting of chains of glucose monomers. The paper generally is of highly purified cellulose. The paper shows weak ion exchange with adsorptive properties. Modified forms of paper have been produced in which the paper has been infused with alumina, silica gel, and ion-exchange resin etc. These papers are consists mainly two types of shape, either rectangular or circular sheets. There is easy commercial availability of these papers. The standard width of paper is very crucial because a slight changes in the width of paper at the point of entrance of solvent may affect separation. Hence, availability of standardized papers is important. Some specially shaped types of papers are also available as shown in figure below.



There is a different variety of papers used especially for paper chromatography are; 1.Less dense papers for more rapid flow rates (e.g., Whatman 54 and 4) 2.Smooth papers 3. Acid-washed papers 4. Preparative papers.

Modified cellulose paper: It is found that, situations become different if polar substances have to use from mixture. Then, for separations of polar substances, papers with increased capacity have been introduced. Papers with high carboxyl content (Schleicher and Schuell I or II) are appropriate for the separation of polar compounds (amines, amino acids, and cations etc.) from mixture. There become more possibilities of ion-exchange if the Papers which we are using in chromatography contains ion-exchange resins. If the substances which have to separate are hydrophobic substances, then the filter paper which have to use should be prepared from cellulose esters. kieselguhr filter paper (e.g., Schleicher and Schuell 287) is used in hydrophobic compounds separation from mixture. For example, acetylated papers (Schleicher and Schuell 2043b/6 or Machery-Nagel 214 AC) or silicone-treated papers (e.g., silicone oil-impregnated Whatman 1, Machery-Nagel 212, or Schleicher and Schuell 2043b) are used.

Glass filter paper: These papers have been used, in some suitable applications, because of their following character; It is found that some of Reagents are too corrosive for cellulose papers, at that place, this paper is used, Analysis time is reduced significantly compare to cellulose paper.

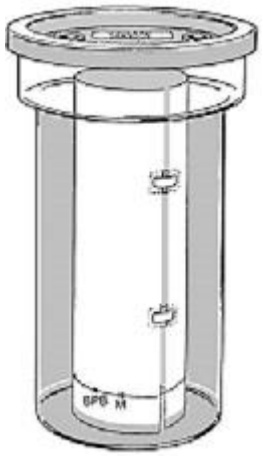
Membrane Filters: In case of macromolecules, Nitrocellulose membranes is used as carrier materials in the separation of macromolecules from mixture. This technique utilizes the fact that high molecular weight materials form a homogeneous immobilized film on the surface of the microporous carrier that is capable of specific interactions with various substances. One of the main problem which arises using membrane filters is sorption. The problem of the sorption can be diminished by utilizing neutral detergents. To apply a sample on the chromatography paper for development, it is necessary to solubilize solid samples in a solvent that can be easily volatilized.

Techniques

There are some apparatus, which are required for paper chromatography, these are support for paper, Solvent trough, Airtight chamber, Whattmann filter paper number 1, Capillary

tubes, Samples – Amino acids (or) Pigments, solvent, Platinum loop. There are two main technique used for paper chromatography, these are Ascending and Descending.

Ascending paper chromatography; In the ascending mode of chromatography the paper is suspended so that the lower edge of paper is below the level of the solvent, and the solvent moves up with the help of capillary action. The most widely appropriate solvent mixture in ascending paper chromatography is of n-Butanol, Acetic acid, Water and the ratio used in making solvent should be (4:1:5) respectively. The solvent of Butanol, acetic acid and water is abbreviated as BAW. The sheet of paper is supported on a frame with the button edge in contact with solvent. The arrangement is contained in an airtight tank lined with paper saturated with the solvent to prove a constant atmosphere and separations are carried out in a constant temperature room. The solvent will ascend into the paper via capillary action this process is called "Ascending Chromatography".



Descending Paper Chromatography: In descending paper chromatography the upper end of the paper is immersed in a solvent contained in a suspended trough so that the flow, initiated as in the ascending mode by capillary action, is sustained by gravity and will continue so long as there is solvent to feed it. In this chromatography, the solvent will descend into the paper and this process is then termed "Descending Chromatography". It is reported that the results obtained for a particular sample/solvent system combination run in either ascending or descending mode were generally similar. The descending paper chromatography is usually faster than ascending paper chromatography.

Applications: The importance of paper chromatography is well known in a large number of field. A list of its applications covers all types of analytes, including proteins, peptides, amino acids, poly-, oligo-, di- and monosaccharides, natural products, sterols, steroids, bile acids, pigment, dyes and inorganic species etc. It is helpful to check the control of purity of pharmaceuticals, for the detection of alloys/pollutants/ingredients, to detect the contaminants in foods and drinks, for the detection of drugs and dopes in animals & humans, analysis of cosmetics, also for the analysis of the reaction mixtures in biochemical labs. Portugeuse in 1937 begin the work on electrophoresis. It is possible to trace the development of paper electrophoresis. Paper electrophoresis is broadly divided into three main techniques: low voltage, high voltage and continuous. Out of these three techniques, the low voltage (up to 1000 V) technique is probably the most widely used. As with paper chromatography, the applications of paper electrophoresis also included amino acids, organic acids, natural products such as alkaloids, polysaccharides, nucleotides, proteins, peptides, pigments and inorganic species etc.

Detection and Quantification of Substances on Paper Chromatograms: The papers are removed from the developing chamber and dried. Depending on the solvents that were employed and the stability of the resolved samples, the chromatogram is dried at room temperature, or higher temperature may be used, such as forced hot air. As discussed above that paper chromatography consists two phase; mobile phase and stationary phase. The mobility of components in mobile phase plays an important for the separation of components from mixture. This mobility is expressed by R_f value. Sometimes the mobility values are expressed as Rf values relative to a known reference material's R_f value. The R_f values are generally constant under strictly controlled conditions. Temperature and humidity play a very momentous role in changing the R_f value, so it is necessary to control temperature and humidity during paper chromatography experiment. The colored spots can be detected visually; however, there is some difficulty in detection for colorless materials, it is necessary to use alternate methods. UV light plays a curial role in case of colorless material detection. UV light between 250 and 260 nm can be used for a large number of compounds. On irradiation with UV light from a high-pressure mercury vapor lamp, many organic substances show fluoresce. Enzymatic and microbiological methods also found to be useful for detection of some substances. For example, enzymatic methods can be used for detection of enzymes rather than the substrate. Amylase may be detected by spraying the paper with starch solution, incubating for a suitable period, then spraying with iodine vapors. After that Amylases appear as white spots on a blue background. For monitoring radioactive samples radioscanner can be used.

Conclusion

Paper chromatography is one of technique from all chromatography techniques of considerable importance. According to solvent different type of papers are used in it. The main thing is that it is a simple technique and can be performed faster. Their descending mode is found faster than ascending mode of paper chromatography. Its detection is analyzed by visually, fluoresce of substances using UV, Enzymatic and microbiological methods. Hence its detection and quantitation can be done without any expensive instrumentation. The temperature and humidity must be controlled for constant R_f value. It covers all types of analytes, including proteins, peptides, amino acids, poly-, oligo-, di- and monosaccharides, natural products, sterols, steroids, bile acids, pigment, dyes and inorganic species etc.

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