IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
November 2018 Vol.:13, Issue:4

Review Article: Chromatography Principle and Applications

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

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Submission: 29 October 2018
Accepted: 4 November 2018
Published: 30 November 2018

www.ijppr.humanjournals.com

Keywords: Chromatography, TLC, HPTLC, Column, Mobile phase, Stationary phase

ABSTRACT

Chromatography is a separation technique. The mixture is dissolved in a liquid called mobile phase, which carries it through a structure holding another material called stationary phase. The separation of components depends on differential partitioning between mobile and stationary phase. Mobile Phase is Solvent. Stationary Phase is Column Packing Material. Analytical purpose of Chromatography is to determine the chemical composition of a sample and Preparative purpose is to purify and collect one or more components of a sample. This article discusses the classification and type of Chromatographic techniques with its principle & instrumentation for understanding the fundamental principle of Chromatography.

INTRODUCTION

Chromatography technique developed substantially because of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s, for which they won the 1952 Nobel Prize in Chemistry[1]. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several chromatographic methods: paper chromatography, gas chromatography and what would become known as high-performance liquid chromatography. Since then, technology has advanced rapidly. Researchers found that the main principles of Tsvet's chromatography could be applied in many different ways, resulting in the different varieties of chromatography described below. Advances are continually improving the technical performance of chromatography, allowing the separation of increasingly similar molecules.

Definition

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation[2].

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction[3].

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive[4].

Principle of Chromatography[5]

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster.

Three components thus form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- Mobile phase: This phase is always composed of "liquid" or a "gaseous component".
- Separated molecules.

The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other.

The Classification of Chromatography[6]

Chromatographic methods can be differentiated based on the physical means of bringing the stationary and mobile phases into contact it means the basis of classification is how the stationary base and mobile phase come into contact.

Column Chromatography

The stationary phase is held in a narrow tube through which the mobile phase is forced either by pressure or by gravity. Examples include:

- Simple column chromatography
- High-pressure liquid chromatography (HPLC)
- Gas chromatography (GC)

Planar Chromatography

The stationary phase is supported on a flat plate or in the fibers of a paper. Here the mobile phase moves through the stationary phase by capillary action or by gravity. Examples include:

- Paper chromatography
- Thin layer chromatography (TLC)

Column Chromatography

It can be further differentiated based on the types of stationary and mobile phases and the kinds of equilibria involved in solute transfer between the phases.

There are two broad categories in this classification scheme:

- Liquid chromatography (for instance simple column or HPLC)
- Gas Chromatography (GC)

Based on the polarity of phases classification can be:

- Normal Phase Chromatography
- Reversed Phase Chromatography

Based on experimental parameter variation over separation period classification may be:

- Isothermal in which Temperature is kept constant.
- Temperature Programming in which column temperature changes systematically.
- Isocratic in which solvent composition is held constant.
- Solvent Programmed in which solvent composition and concentration changes systematically.

Uses of Chromatography in Chemistry[7]

Chromatography has gained immense importance in the field of chemistry from detecting the optical isomer to determining the amount of mixture present in a sample. Following are some of the uses of chromatography in chemistry.

- Chromatography is used to figure out the relation of different mixtures with one another.
- It is a very effective technique to test the purity of the sample.
- The amount of mixture present in a sample can be calculated by using chromatography.
- Chiral compounds, which are very similar in molecular weight, elemental composition, and physical properties and differ only in optical isomers, can be separated using chromatography.
- It is used for the separation of a mixture of compounds. Paper chromatography is particularly very effective in detection and separation of a mixture of compounds.

Uses of Chromatography in Medicine[7]

- In pharmaceutical companies, a large amount of pure chemicals for making further medicines is prepared by using chromatography.
- Paper chromatography is used to separate the various inks or dyes from the mixture.
- Presence of alcohol or some other drugs in blood or urine is detected by using gas chromatography.
- Chiral compounds resemble each other greatly in terms of molecular weight, physical composition, and elemental weight. However, they have different optical isomers due to which they have different biological activities. Chromatography is a very effective technique to separate the isomers. For example, thalidomide is compound with two isomers one of them causes birth defects; chromatography is used to separate the isomer from its harmful counterpart.
- In pharmacy, chromatography is very important to analyze whether correct medicine is manufactured or not.

- In forensic science, it helps in solving many cases by detecting residual burnt particles and flammable chemicals present in the body parts in case of fire or explosions.

- Paper chromatography and Gas chromatography are employed in the fingerprint, DNA RNA analysis.

SUMMARY

Chromatographic techniques have become very important in industry for purification and separation of synthesis. Chromatography techniques increase productivity in chemistry and instrumentation providing more information as it gives an increased resolution, speed and sensitivity. The time spent optimizing new methodologies can be reduced significantly.

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