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REVIEW ARTICLE ON EXPLORING CHROMATOGRAPHY: PRINCIPLES, TECHNIQUES & APPLICATION

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ABSTRACT
A separation method called chromatography separates a mixture into discrete molecules according to their solubility, affinity, and interaction with a solid phase. In a moving phase, constituents travel across a stationary phase at varying speeds. Thin layer chromatography method(TLC) and column chromatography method are two examples of the several kinds of chromatography. TLC uses adsorption and partition to separate materials on a plate; ninhydrin is frequently used for visualization. By passing a mobile phase through a solid stationary phase, column chromatography—including HPLC (High Performance Liquid Chromatography)—separates mixtures and enables component identification, measurement, or purification.

KEYWORDS: Chromatography, Static phase, Moving Phase, Paper Chromatography, TLC, HPLC.

INTRODUCTION

Chromatography is the Method of sepration mixture of component into individual component through equilibrium distribution between two phases. which translates to "color-writing," is a physical separation technique that allows a mixture of substances to be segregated, isolated, and refinied into distinct bits that varying velocity of dispersion based on 1. Solubility 2. Affinity (Based on polarity and non polarity molecules). A element of mixture are distributed between the mobile phase, which moves in a predetermined direction at different speeds, and the stationary phase which solid or viscous liquid/ / solvent coates on surface.[1] [2] The partition is useful to seprate and identify small bits like sacchrides, fatty acids, amino alkanoic acid; while ion exchange is useful for supermolecules like DNA, RNA.[4] These days, the food business relies heavily on liquid chromatography, which uses adsorption in order to eleminate an enormous non-flavor-and flavour active ffood constituents.[5]

It is well known that in 1901, Russian botanist Michael Tswett noticed that a column filled with calcium carbonate and allow its mixture to run through it, chlorophyll pigments divided into several colored components. Thus, he is referred to as the father and founder of chromatography.[2][3]

PRINCIPLES

1) Adsorption
2) Partition
3) Affinity
4) Ion exchange
5) Size Exclusion

1. Adsorption: It is the process in which due to intermolecular forces the components of mixture adhere to surface of stationary phase is called Adsorption
2. Partition: The Process where sepration of component mixture is depend on their differential portioning between two immisible phases.
3. Affinity: Affinity means attraction. Affinity is based on specific interaction between molecule (protein) and ligand immobilized on chromatography column. Molecule of interest will selectively binds to ligand while other molecule pass through the column.
4. Ion exchange: sepration of charged molecules based on their interaction with stationary phase with charged groups.
5. Size Exclusion: It is the process sepration of molecules based on their size.[1]

Classification

1. The classification based on stationary phase's shape:

Example. Planner chromatography

2. The classification based on static and moving phase:

Sr. No.	Static phase	Moving phase	Name
1.	Solid	Liquid	-Plane chromatography -Paper chromatography -TLC -Adsorption column chromatography -HPLC
2.	Solid (ion exchange resin)	Liquid	-Ion exchange chromatography
3.	solid	Gas	-Gas-solid chromatography (GSC)
4.	Solid matrix	Liquid	-Gel permeation chromatography (GPC)
5.	Liquid	Gas	-Gas-liquid chromatography (GLC)
6.	Liquid	Liquid	-liquid-liquid chromatography (LLC)

[1]

1. Planner chromatography:

In this case, the moving phase that, via capillary action or gravity, run through the static phase, which may be liquid or cellulose (paper chromatography) or solid with silica gel or alumina (thin layer chromatography).[2]

2. Paper chromatography

Principle: This method is a kind of partition chromatography where the material is split between two liquids: the stationary phase, which is a stationary liquid that is contained in the paper's cellulose fibers, and the mobile phase, which is a moving liquid or developing solvent. The mixture's components that need to be separated move at varying speeds and show up as spots at various locations on the paper.

Paper chromatography was first introduced as a way to separate mixtures of organic chemicals, such dyes and amino acids, but it is today preferred for separating cations and anions as well as inorganic substances.[2][9]

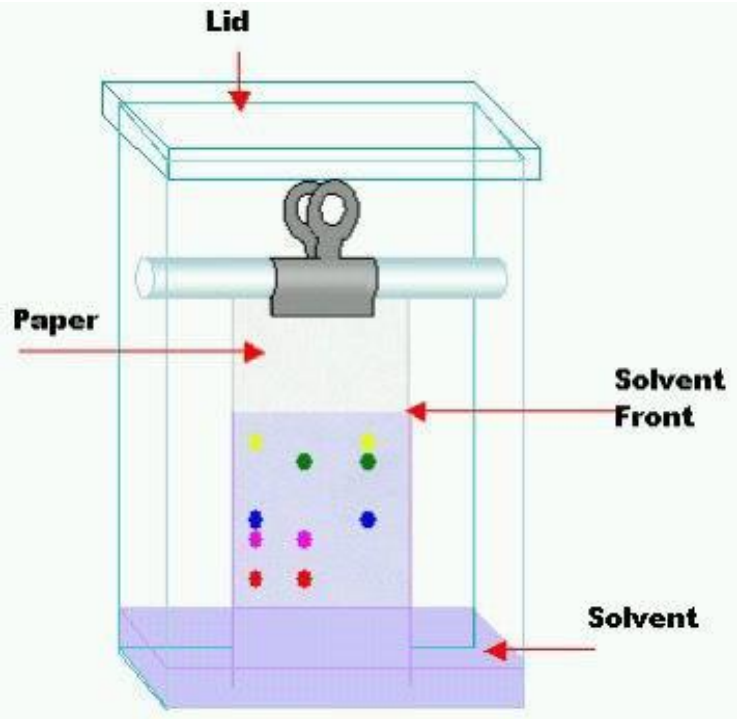

Figure 1: Paper Chromatography.

3. Thin liquid chromatography

Principle: In the classification of chromatographic method TLC has been included under both absorption and partition principle of chromatography. The various materials of different adsorptive power are used in TLC. The separation of component is based of partion and adsorption or both phenomenon.

Solid-liquid adsorption is created in TLC when the static phase interacts with a large surface area and the moving phase is liquid. The moving phase is propelled upwelling the static phase via capillary action. The polarity of the material, solid phase, and solvent all have an impact on this upward motion rate. The most popular ingredient is ninhydrin, which can be visualized using blacklight. This allows us to get segreted compounds.[2][6]

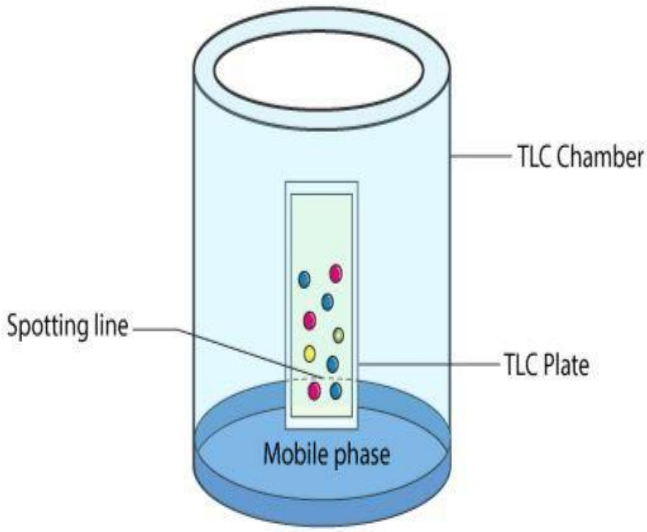

Figure 2: TLC

4. Column chromatography

The experiment depicted in the diagram involves applying column chromatography to a mixture consisting of two components.

The static phase is a solid substance that is loaded into the column. The solvent is poured through column also known as an eluting solution. When the mixture is placed on top of the wet column, more eluent is added. Gravity pulls the mobile phase down through the stationary phase, causing the constituents to start flowing through the column at different rates. In the fig.3, component 2 is held on the column for a longer amount of period than component 1 because component 1 moves more quickly than component 2. The two compounds' different solubility in the solvent and/or attraction to the solid packing material are usually the causes of this. A large amount of moving phase is run through the column, the components will exit the column on their own. For every component, the retention time the amount of time it takes to leave the column will be repeatable under the specified parameters of temperature, column width, and mobile and stationary phase identities.[2][9]

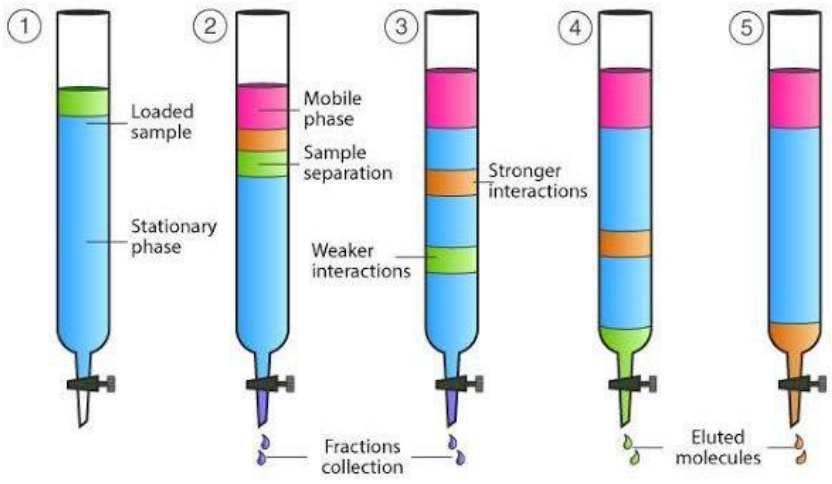

Figure 3: Column Chromatography.

5. HPLC technique

High Performance Liquid Chromatography technique is mentioned as HPLC. before the development of HPLC was accomplished by flow of the cluent due to gravity. The analysis time was able to be somewhat reduced even with the latter enhancements. "Column chromatography" or "low pressure chromatography" are the names given to those traditional/beginning LC systems.[2][12][13]

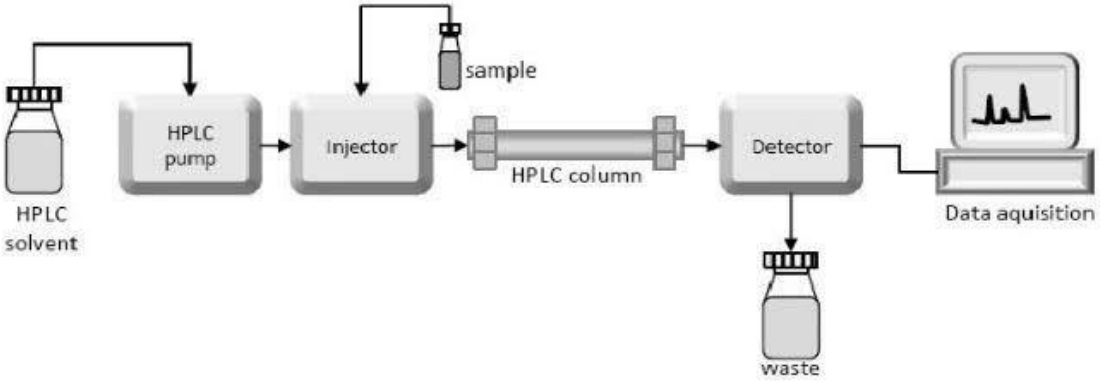

Figure 5: HPLC.

Application

1. Applications of Paper Chromatography techniques

A summary of some of the several disciplines in which paper chromatography finds use is provided below:

• To diversify drug brews
• To separate proteins, vitamins, antibiotics, and carbohydrates
• To identify drugs
• To identify impurities
• To confirm the clarity of medications
• To inspect mixture of reaction in biochemical labs
• The detection of drugs and narcotics in mans and animals.[13]

2. Thin layer technique Application

TLC used for testing of drugs, like calming medications, regional anesthetics, epileptic tranquilizers, analgesics, allergy medications, steroids, and hypnotic drugs.

• TLC is extremely beneficial in biochemical analysis, which includes isolating or separating biochemical metabolites from bodily fluids such as serum, urine, blood plasma, etc.
• The thin-layer chromatography technique can be used to identify natural chemical like volatile oils, fixed oils, essential oils, alkaloids, etc.
• The sample and the original sample are immediately compared after it has been cleaned. to identify and distinguish between preservatives, sweeting agents, flavors in the food industry.
• It is used to assess the completeness of a response.[13]

3. Column Chromatography techniques Applications

Column chromatography is efficient technique to extracting active agents from phytoconstituents.

• To clear the vital components of impurities.
• Distilling the metabolites of important components.
• Used to quantify the amount of phytomenadione present in injections and tablets.
• The amounts of betamethasone, acetonide, and flucinolone in formulations.
• For separating inorganic ions, including those derived from nickel, cobalt, and copper.
• Quantification of quinine in an ethanolic solution.
• Beneficial for isolating carbohydrates from their byproducts.
• To separate natural compound combinations, including alkaloids and glycosides.
• Phenothiazine detection in the presence of carbazole and diphenylamine.[13]

REFERENCE

1. Gurudeep R. Chatwal, Instrumental method chemical Analysis. Mumbai; Himalayan publication House, 2019; 2: 567.
2. Mohamed Ahmed Sayed, "A Review of chromatography: Principles, Classification, Application" 2021; 3-4.
3. Lodha Laxminarayan, Sharma Neha, Vishwas Amit, Dr. Khinchi M.P "A Review of Chromatographic Techiques", Asian Journal of Pharmaceutical Reasearch and Devlopment, 2017; 1.
4. Ebre EC, Obinna IB and Winkor VA, "Application of Column, Paper Thin layer and Ion exchange Chromatography in purifying samples: Mini Review", SF Journal of Pharmaceutical and Analytical Chemistry, 2019; 2(2): 1018.
5. Shima S. Saffarionpour, Tessa F.de Jong, Luuk A.M. Van der wielen, Eric Brouwer, Marcel Ottens, "column chromatography for sepration and fractanation for flavor active estres on hydrophobic resins and Stimulation of breakthrough Behaviour", sepration and purification technology, 2018; 304.
6. Mimansha Patel, "Chromatography Principle and Application- A Review", International journal of Pharmacy and Pharmaceutical Research, 2018; 13(4): 291.
7. Dr. Dinesh Mishra, Mr. Gaurav Sarsodia, Dr. Gurmeet S. Chhabba, Practical Book of Instrumental Analysis, Pune; Nirali Prakashan, 2020: 20-23.
8. Subarna Ganguli, Practical Book Instrumental Analysis, Pune; Nirali Prakashan, 2020; 14-25.
9. Shashank Tiwari and Shreya Talreja,"Thin Layer Chromatography (TLC) VS. Paper Chromatography- A Review", ACTA SCINTIFIC PHARMACEUTICAL SCINECES, 2022; 6(9): 5-9.
10. Ranjith Reddy Kondeti, Kranti shri Mulpuri, Bharathi Meruga, "Advancements in Column Chromatography- A Review", World Journal of Pharmaceutical Sciences, 2014; 1-2.

11. Santosh Kumar Bhardwaj, K.Dwivedi, D.D.Agrwal "A-Review, HPLC Method Devlopment and validation" , International Journal of Analytical and Bioanalytical Chemistry, 2015; 5(4): 76-81.
12. Mr.Gorhe S G, Miss. Pawar G R "A – Review on High Performance Liquid Chromatography (HPLC), INTERNATIONAL JOURNAL OF ADVANCE SCIENTIFIC RESEARCH AND ENGINEERINF TRENDS, 2018; 3(1): 2456.
13. Rushikesh B, Mayur B, Sandip C, Piyush B, Jayesh G, Vijay Raj S and Ganesh S,"A Brief Review On Different Chromatographic technique", Open Access Journal Of Pharmaceutical Research, 2024; 8(1): 4-15.