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REVIEW ARTICLE

A Brief Review on Different Chromatography Techniques

Samyakt Jain*[1], Ankit Jain[2]

[1] *Student, IPS Academy College of Pharmacy, Rajendra Nagar, Indore, India*
[2] *Associate professor, Department of Pharmacy, IPS Academy College of Pharmacy, Rajendra, Indore, India*

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Abstract:
This review discusses about chromatography techniques employed for the separation of compounds, elucidating the intricate dynamics involved in this pivotal analytical process. The main focus is about categorization of materials constituting the mobile and stationary phases, forming the backbone of chromatographic methodologies. An in-depth analysis of solute-stationary phase interactions is presented, encompassing pivotal factors such as cavity formation, dispersion forces, lone-pair electron interactions, dipole-type interactions, and hydrogen-bonding interactions. Various chromatography systems, including but not limited to column chromatography, gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), ion exchange chromatography, and affinity chromatography are explored in this work. Each system is scrutinized for its unique contribution to retention mechanisms and separation efficiency. Method development in chromatography is further investigated, emphasizing the significance of assessing the solvation characteristics inherent in novel stationary phases. The role of interfacial adsorption in the context of gas chromatography technique development is expounded upon, shedding light on its impact on retention determination. This work serves as a guide for the evaluation of diverse material properties, aligning with the operational characteristics inherent in chromatography. The relevance of this analytical approach to material characterization is emphasized, providing a holistic perspective on the diverse applications and implications of chromatography in contemporary scientific research and analysis.

Keywords: Chromatography; HPLC; Stationary Phase; Mobile Phase; Retention time; Adsorption

1. Introduction

Chromatography serves as a physical method of separation, involving the division of elements into two phases – a mobile phase flowing in a specific direction and a stationary phase. The efficacy of this separation process relies on molecular properties associated with adsorption (liquid-solid), partition (liquid-solid), affinity, or variations in molecular weight. The nature of the mixture plays a pivotal role in achieving effective separation between molecules. Partition-based chromatography techniques are adept at successfully separating and identifying minute molecules like amino acids, carbohydrates, and fatty acids. Conversely, larger entities such as proteins and macromolecules like nucleic acids find more successful separation through affinity chromatography, also known as ion-exchange chromatography. [1,2]

Various chromatographic methods cater to diverse separation needs. Paper chromatography proves useful in protein separation and protein synthesis investigations, while gas-liquid chromatography is employed for separating alcohol, ether, lipids, and amino groups, as well as monitoring enzymatic interactions. Agarose gel in chromatography serves to purify viral, DNA, and RNA particles. In chromatography, the stationary phase, either solid or liquid, coats a solid phase, with a liquid or gaseous mobile phase flowing over it. The nomenclature shifts to gas chromatography (GC) when the mobile phase is gas and liquid chromatography (LC) when it is liquid. GC analyzes gases and combinations of solid and volatile liquid materials, while LC is particularly suitable for thermally unstable and non-volatile materials. Beyond separation, chromatography finds application in quantitative analysis, aiming for a balanced separation within a reasonable timeframe. [3]

A spectrum of chromatographic techniques, including gel permeation chromatography, column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, high-pressure liquid chromatography, and affinity chromatography, has been devised to meet these objectives. The foundation of these techniques lies in non-covalent connections between the molecules to be separated and the adsorbent, incorporating ion exchange and hydrophobic interactions in chromatography for the extraction of proteins and other biological macromolecules. The objective of this review is to briefly explore the principles, techniques, and applications of chromatography as a versatile method for the separation, identification, and quantitative analysis of diverse compounds. [4]

* Corresponding author: Samyakt Jain and Ankit Jain

2. Types of Chromatography Techniques

The various types of chromatography techniques used in the pharmaceutical industry are illustrated in Figure 1.

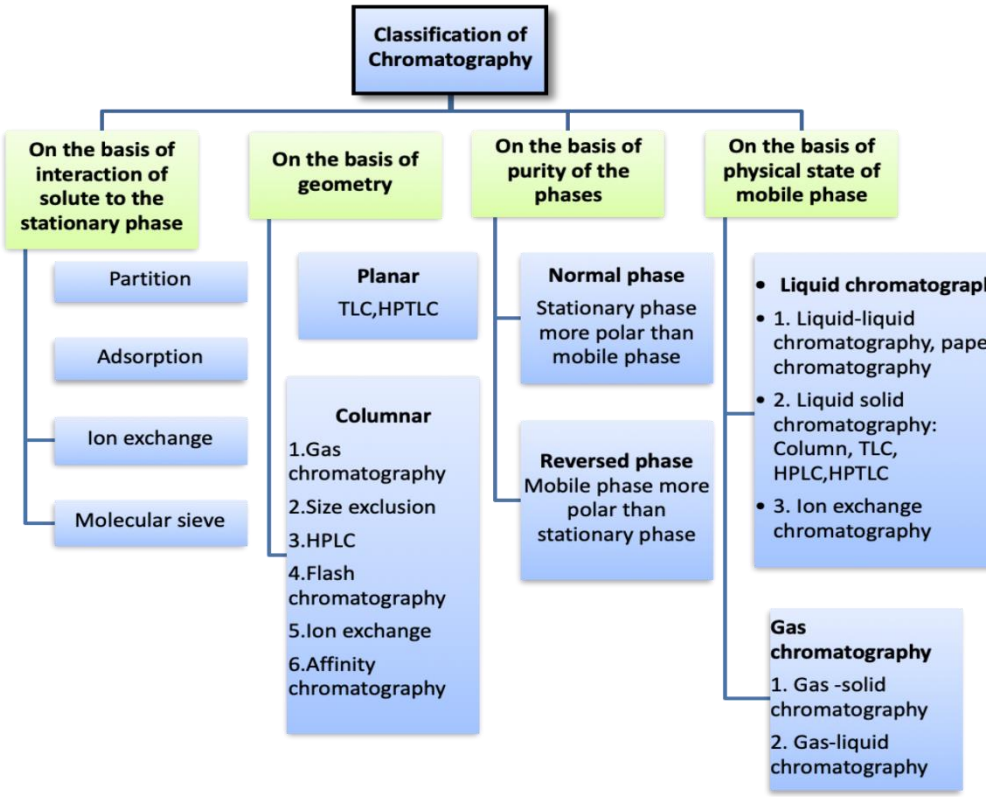

Figure 1. Classification of Chromatography Techniques

2.1. Column Chromatography

Chromatography, a physical separation method, involves the partitioning of components into mobile and stationary phases based on the principle of differential adsorption. This principle asserts that varying affinities for the adsorbent in the stationary phase result in differential movement rates through the column. Molecules with higher affinity exhibit prolonged adsorption, leading to slower movement, while those with weaker affinity travel faster, enabling their separation into distinct fractions. In column chromatography, the mobile phase, a liquid facilitating smooth column flow, interacts reversibly with the solid stationary phase (usually silica). [5, 6] The adsorbents and solvents used in column chromatography are shown in Table 1.

Table 1. Adsorbent & Solvent used in column chromatography

Polarity	Adsorbents	Solvent
Weak	Sucrose	Petroleum ether
	Starch	Carbon tetrachloride
	Inulin	Cyclohexane
	talc	Carbon di-sulphide
	Sodium carbonate	Ether (ethanol free)
Medium	Calcium carbonate	Acetone
	Calcium phosphate	Benzene

	Magnesium carbonate	Toluene
	Magnesium oxide	Esters
	Calcium hydroxide	Chloroform
	Activated magnesium silicate	Acetonitrile
Strong	Activated alumina	Alcohols
	Activated charcoal	Water
	Activated magnesia	Pyridine
	Activated silica	Organic acids
	Fuller's earth	Mixtures of acids or bases with ethanol or pyridine

2.2. Thin Layer Chromatography

hin Layer Chromatography (TLC) is a method that involves the application of a finely divided adsorbent on a chromatographic plate to separate or identify elements within a mixture. The mobile phase solvent moves through the plate via capillary action, counteracting gravitational force. Components migrate based on their affinity for the adsorbent, with the slower-traveling ones having a stronger attraction to the stationary phase and the faster ones having less affinity. This results in the division of components on the thin-layer chromatographic plate according to their interaction with the stationary phase. The stationary phase in this process is solid silica (SiO2), composed of tetrahedral silica atoms connected by bent oxygen atoms, forming an extended structure. The highly polar silanol (Si-O-H) groups terminate the solid's surface, causing it to adhere to the glass plate and remain stationary during chromatography. Various detecting agents are employed to determine the nature of compounds or for identification purposes in TLC.[7] Examples include ferric chloride for phenolic compounds and tannins, ninhydrin in acetone for amino acids, Dragendroff reagent for alkaloids, and 2,4-dinitrophenyl hydrazine for aldehydes and ketones. Stationary phases used in TLC are listed out in Table 2.

Table 2 Some examples of stationary phase used in TLC

Name	**Composition**	**Adsorbent: Water ratio**
Silicagel H	Silicagel without binder	1:1.5
Silicagel GF	Silicagel + Binder + Fluorescent indicator	1:2
Silicagel G	Silicagel + $CaSO_4$(gypsum)	1:2
Alumina	Al_2O_3 without binder	1:1.1
Al_2O_3 G	Al_2O_3 + binder	1:2

2.3. Paper Chromatography

Paper chromatography (PC) is a form of planar chromatography where specific paper, such as Whatman filter paper, is employed for chromatographic procedures. Regarded as the simplest and most widely utilized among chromatographic techniques, it finds application in the isolation, identification, and quantitative determination of both organic and inorganic compounds. [8] The fundamental concept behind separation in paper chromatography is partition, distinguishing it from adsorption. The process involves two substance distribution phases: the stationary phase, where moisture in the cellulose layers of filter paper acts as the stationary component, and the mobile phase, utilizing organic solvents or buffers. As the developing solution moves up the stationary phase, carrying the sample, the ease of separation for sample constituents is determined by their adsorption onto the stationary phase relative to their solubility in the mobile phase.

2.4. Gas Chromatography

Modern gas chromatography (GC) offers significant advantages, including high resolution, speed, sensitivity, precision, and accuracy. The GC system comprises four key components: a carrier gas source, sample introduction system, column, and detector. This

versatile technique has evolved from its initial application for analyzing gases and vapors of highly volatile substances. Gas chromatography serves as a multipurpose analytical method, particularly useful for product identification under controlled conditions, requiring a direct coupling to a mass spectrometer when more detailed information, such as positive peak identification on the chromatogram, is essential. [9]

2.5. High Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC), initially termed high-pressure liquid chromatography, is employed for the separation, identification, and quantification of individual components within solutions. This technique utilizes pumps to propel a pressurized liquid solvent, containing the sample mixture, through a column filled with solid adsorbent material. As the components exit the column, they undergo separation due to their unique interactions with the adsorbent material, leading to varying flow rates for each component. Analyzing steroids presents challenges due to their low levels and complex sample matrices. High-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) proves highly effective for routine steroid measurement and detection, benefiting from fluorescence's sensitivity, selectivity, user-friendliness, and cost-effectiveness. Various types of HPLC further diversify its applications, including Normal Phase HPLC, Reverse Phase HPLC, Size-exclusion HPLC, and Ion-Exchange HPLC [10, 11]

2.6. Affinity Chromatography

Affinity chromatography, a form of liquid chromatography, involves the isolation or separation of a target analyte from a sample or complex mixture using a biologically related agent. This method relies on precise and reversible interactions commonly observed in biological systems, such as antibody-antigen or enzyme-substrate interactions, to retain the target. In this approach, the affinity ligand serves as the stationary phase, acting as the immobilized binding agent. The support for this binding agent can vary, ranging from silica particles and glass beads in high-performance affinity techniques to carbohydrate- or polymeric-based beads and gels in low-performance affinity separations. [12] In commercial monoliths for liquid chromatography, flow-through pores typically have diameters between 1.5 and 1.7 μm, while diffusion pores boast diameters below 100 nm

2.7. Electrophoresis Chromatography

In electrophoresis, the separation process relies on variations in k' and/or effective mobility, which are determined by the velocities of the distinct separands. Unlike chromatography, where eluent flow is fundamental, electrophoretic procedures typically suppress electroosmotic flow to prevent disrupting sample zone borders, as these zones migrate at a maximum velocity of 10^{-3} m s^{-1}. This is particularly crucial for isotachophoresis separations, where separands traverse successive zones with nearly imperceptible lengths of about 0.1 mm. Linear liquid flow velocities can reach $2 \times 10-3$ m s^{-1} when electroosmotic flow is used as a transport mechanism through capillaries as small as approximately 50 μm. In the separation compartment, this velocity may exceed the electrophoretic migration velocity, especially for ionic species with limited effective mobility. Consequently, cationic, non-ionic, and anionic separands can move in the same direction, influencing the electrophoretic separation power based on the interplay of repulsive or attractive forces. [13] Electrophoresis chromatography is categorized into three types: Paper Electrophoresis, Gel Electrophoresis, and Capillary Electrophoresis

2.8. Gel Chromatography

Gel-filtration chromatography, a form of partition chromatography, is employed for the separation of molecules based on their varying molecular sizes. This method is known by several names, including gel-permeation, gel-exclusion, size-exclusion, and molecular-sieve chromatography. The underlying principle of gel filtration is straightforward—the relative sizes of molecules cause them to be distributed between a stationary phase, consisting of a matrix with defined porosity, and a mobile phase. Columns constructed with this matrix, often in bead form, possess measurable liquid volumes: the external volume, found between the beads, and the internal volume, located within the beads. [14] A key advantage of gel-filtration chromatography lies in its ability to achieve separation under conditions tailored to preserve the stability and activity of the target molecule without compromising resolution. Additionally, the absence of a molecule-matrix binding stage protects critical molecules from unnecessary damage, resulting in excellent activity recoveries in gel-filtration separations

3. Applications

Applications of chromatography span various sectors, notably in the pharmaceutical industry. It serves to identify and examine samples for trace elements, categorize compounds based on molecular weight and element quantity, and determine the purity of unidentified substances. Additionally, chromatography plays a crucial role in drug manufacturing, employing gas chromatography for the separation of volatile mixtures and high-pH anion-exchange chromatography for carbohydrates and oligosaccharides. Its utility extends to detecting impurities in pharmaceutical industries. [14]

In the food industry, chromatography aids in identifying signs of food decay, assessing additives, and determining nutritional value. The chemical industry benefits from chromatography in testing water samples and assessing air quality, with gas chromatography and high-performance liquid chromatography commonly used to identify pollutants. [15]

Molecular biology studies find extensive applications, notably in protein separation techniques such as enzyme purification, plasma fractionation, and insulin purification using high-performance liquid chromatography (HPLC). HPLC is also instrumental in biochemical processes, biotechnology, and the fuel business. Additionally, chromatography proves valuable in forensic pathology and crime scene testing, particularly in examining bloodstreams and hair samples. [15]

4. Conclusion

Chromatography techniques, including Gas Chromatography (GC), Liquid Chromatography (LC), High-Performance Liquid Chromatography (HPLC), Thin-Layer Chromatography (TLC), Ion Exchange Chromatography, and Affinity Chromatography, are widely employed as effective methods for separation and analysis across various scientific disciplines. The utilization of a diverse array of instruments enables the separation and analysis of numerous chemicals through chromatography. The choice of technique is influenced by factors such as the type of sample, the target analytes, and the required level of specificity and resolution. Ongoing technological advancements continually enhance the capabilities of chromatography, solidifying its status as an indispensable tool in industry, research, and various scientific applications.

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Author's short biography

Samyakt Jain

Mr. Samyakt Jain is a final-year student at IPS Academy College of Pharmacy, Indore. After completing graduation moved to the master's programs and always worked in the pharmaceutical research field. He always feels proud of his family, teachers, friends, and the nation.

Ankit Jain

Mr. Ankit Jain is an esteemed associate professor within the Department of Pharmacy at the IPS Academy College of Pharmacy, Indore. He has completed his post-graduation in Pharmaceutical medicinal Chemistry at Narsee Monjee Institute of Management Studies (NIMIMS), Mumbai. He has vast experience in Research and Development in various industries and Organizations. Her present interest in research in Computer Aided Drug Design with a strong background in various pharmaceutical fields and fifteen years of experience in Academic Fields. He has published many research articles in peer-reviewed national and international.