WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 4, 121-133. Review Article ISSN 2277–7105

CHROMATOGRAPHY TECHNIQUES AND THEIR DIVERSE APPLICATIONS: A COMPREHENSIVE REVIEW

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Article Received on 23 December 2023,
Revised on 13 Jan. 2024,
Accepted on 03 Feb. 2024
DOI: 10.20959/wjpr20244-31232

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ABSTRACT

Chromatography stands as a crucial biophysical technique that facilitates the separation, identification, and purification of components within a mixture, catering to both qualitative and quantitative analyses. For proteins, purification is achieved by exploiting various characteristics such as size and shape, total charge, surface hydrophobicity, and binding capacity with the stationary phase. The separation of proteins is accomplished through four distinct techniques, each grounded in molecular characteristics and interaction types: ion exchange, surface adsorption, partition, and size exclusion chromatography. Techniques relying on the stationary bed further enhance chromatography methodologies and include column, thin layer, and paper chromatography. Among these, column chromatography stands out as one of the most prevalent methods employed for protein purification. By leveraging the diverse properties of proteins, chromatography emerges as a versatile tool for biophysical analyses, contributing significantly to advancements in biochemical research and aiding in the purification of proteins for various applications.

KEYWORDS: Chromatography, protein purification, biophysical technique, separation, identification, qualitative analysis, quantitative analysis, molecular characteristics, ion exchange chromatography, surface adsorption chromatography, partition chromatography,

size exclusion chromatography, stationary phase, column chromatography, thin layer chromatography, paper chromatography, biochemical research, protein applications.

INTRODUCTION

Mikhail Tswett, a Russian botanist, is credited with coining the term chromatography in 1906. However, the first analytical application of chromatography was detailed by James and Martin in 1952. They explored the use of gas chromatography for analyzing fatty acid mixtures.

Chromatography, in its various forms, relies on differences in size, binding affinities, charge, and other properties to affect the separation of materials. This powerful separation tool finds extensive application across all branches of science and is frequently the sole method available for isolating components from intricate mixtures. The versatility of chromatography has made it an indispensable technique in analytical chemistry and various scientific disciplines.

Classification of chromatography

1. Based on the nature of stationary Phase and Mobile phase

- Gas-Solid Chromatography
- Gas-Liquid Chromatography
- Solid-Liquid Chromatography (Column Chromatography, TLC, HPLC)
- Liquid-Liquid Chromatography (Paper Partition Chromatography, Column Partition Chromatography)

2. Based on the principle of separation

- **Adsorption chromatography:** In this type, the stationary phase is solid, and the mobile phase can be liquid or gas.

Examples: include Thin Layer Chromatography, Column Chromatography, Gas-Solid Chromatography, and High-Performance Liquid Chromatography (HPLC).

- **Partition chromatography:** This method relies on the principle of partition for separation. The stationary phase is liquid over a solid or gas surface, while the mobile phase can be liquid or gas.

Examples: include Gas-Liquid Chromatography, Paper Partition Chromatography, and Column Partition Chromatography.

3. Based on the modes of chromatography

- Normal phase chromatography: In this type, the stationary phase is polar, and the mobile phase is non-polar. This mode is not widely used in pharmacy.
- The diverse classification schemes highlight the versatility of chromatography in addressing different separation needs based on the properties of the stationary and mobile phases, the principles of separation, and the specific mode employed.
- Reverse Phase Chromatography: In this type of chromatography, the stationary phase is non-polar, and the mobile phase is polar. It is widely employed in pharmaceutical analysis**.**

4. Other types of chromatography

- Ion-Exchange Chromatography
- Gel Permeation Chromatography (Gel Filtration, Size Exclusion Chromatography)
- Chiral Chromatography

Types of chromatography

I. Column Chromatography
II. Thin-Layer Chromatography
III. Paper Chromatography
IV. Gas Chromatography
V. Ion-Exchange Chromatography
VI. Gel Permeation Chromatography
VII. Ultra-Performance Liquid Chromatography (UPLC)
VIII. High-Performance Liquid Chromatography (HPLC)

I. Column chromatography

Column chromatography is a widely used method for purifying biomolecules, particularly proteins, due to their distinct characteristics such as size, shape, net charge, stationary phase affinity, and binding capacity. This technique facilitates the separation of different components based on these specific characteristics.

In column chromatography, the sample to be separated is initially applied to a column (Stationary phase), followed by the introduction of a wash buffer (Mobile phase). The flow of these components through the column material, typically supported by fiberglass, is carefully controlled. As the samples traverse through the column, they interact with the stationary phase, leading to the separation of biomolecules based on their unique properties. The separated components are collected at the bottom of the column in a time- and volume-dependent manner.[1]

This method allows for the efficient purification of biomolecules, including proteins, making it a valuable tool in various biochemical and biotechnological applications.

Applications of column chromatography

1. **Separation of mixture of compounds**

Column chromatography is widely utilized for the separation of complex mixtures into individual components. It exploits the distinct properties of different compounds to achieve a high level of purification.

2. **Isolation of active constituents**

In pharmaceutical and natural product research, column chromatography is employed to isolate the active constituents from crude extracts. This is crucial for obtaining pure compounds for further analysis and testing.

3. **Isolation of metabolites from biological fluids**

Column chromatography plays a pivotal role in isolating metabolites from biological fluids, aiding in the study of biochemical pathways and understanding the composition of complex biological samples.

4. **Removal of impurities or purification process**

The technique is valuable for removing impurities from a sample or purifying a target compound. It is particularly useful in the pharmaceutical industry for obtaining highly pure drugs.

5. **Estimation of drugs in formulations or crude extracts**

Column chromatography is employed for the quantitative estimation of drugs in pharmaceutical formulations or crude extracts. It provides a reliable method for determining the concentration of specific compounds in a given sample.

Thin-layer chromatography (TLC) is a technique utilized for the separation of non-volatile mixtures.[2] In this method, a sheet of an inert substrate, such as glass, plastic, or aluminum foil, is employed. This substrate is coated with a thin layer of adsorbent material, typically silica gel, aluminum oxide (Alumina), or cellulose. The coated layer, known as the stationary phase, interacts with a liquid mobile phase.

Principle: The underlying principle of separation in thin-layer chromatography is adsorption chromatography. In this process, one or more compounds are applied as spots onto a thin layer of adsorbent material on a chromatographic plate. The mobile phase, or solvent, then moves through the plate via capillary action. As the mobile phase progresses, the components of the mixture move according to their affinity for the adsorbent.

Components with a higher affinity for the stationary phase travel more slowly, while those with lesser affinity move faster. This differential affinity results in the separation of the mixture into distinct bands or spots on the chromatographic plate.[3] The visualization of these separated components allows for qualitative and quantitative analysis of the original mixture.

TLC is a versatile and widely used chromatographic technique, particularly in analytical chemistry and biochemistry, for its simplicity, quick analysis, and effectiveness in separating a variety of compounds.

Applications of Thin-Layer Chromatography (TLC)

- **Separation of mixtures of drugs**

TLC is widely employed for separating mixtures of drugs, whether of chemical or biological origin, as well as plant extracts. This is particularly useful in pharmaceutical research and quality control.

- **Separation of various compounds**

TLC is versatile and can be applied to separate a diverse range of compounds, including carbohydrates, vitamins, antibiotics, proteins, alkaloids, glycosides, and more. This makes it a valuable tool in various fields such as biochemistry and pharmaceuticals.

- **Identification of related compounds in drugs**

TLC is utilized for identifying related compounds in drugs, aiding in the analysis of the composition and purity of pharmaceutical formulations. This is crucial for ensuring the quality and safety of medications.

- **Detection of foreign substances in drugs**

One of the essential applications of TLC is to detect the presence of foreign substances in drugs. This is imperative for quality control purposes, helping to identify and eliminate any contaminants that may compromise the efficacy and safety of pharmaceutical products.[4]

Paper chromatography is a technique where the separation of unknown substances is achieved primarily through the flow of solvents on specially designed filter paper.

Principle: The principle underlying paper chromatography is primarily partition, as opposed to adsorption. A cellulose layer in the filter paper contains moisture, serving as the stationary phase. Organic solvents or buffers are employed as the mobile phase.[8,43]

Paper used: The choice of filter paper depends on factors such as thickness, flow rate, purity, and technique. Different grades of Whatman filter papers, including No.1, No.2, No.3MM, and No.17, are commonly used.

Modified paper: Various modifications can be applied to filter paper to enhance its performance. Acid- or base-washed filter paper and glass fiber-type paper are examples of modified paper.

Hydrophilic papers: Hydrophilic papers, modified with substances like methanol, formamide, glycol, and glycerol, are also utilized. These modifications help optimize the chromatographic separation on the filter paper.

Paper chromatography, with its simplicity and versatility, finds application in various fields, particularly in qualitative and quantitative analysis of complex mixtures. The choice of paper and its modifications play a critical role in tailoring the chromatographic process for specific analytical needs.

Applications of paper chromatography

- **Identification of Drugs and Impurities**

Paper chromatography is extensively utilized for the identification of drugs and impurities in pharmaceutical formulations. It aids in assessing the composition and purity of pharmaceutical products.

- **Separation of Mixtures with Polar and Non-Polar Compounds**

This technique is well-suited for separating mixtures that consist of both polar and non-polar compounds. Paper chromatography's ability to handle a wide range of compounds makes it valuable in the analysis of diverse samples.

- **Control of purity in pharmaceuticals**

Paper chromatography plays a crucial role in quality control by monitoring the purities of pharmaceuticals. It ensures that pharmaceutical products meet the required standards and regulatory specifications.[5]

Gas Chromatography (GC) is a chromatographic technique where the mobile phase is in the gaseous state. It stands as one of the most widely used methods for the separation and analysis of compounds.

In gas chromatography, the sample is vaporized and injected into a chromatograph, which contains a stationary phase (Typically a coated capillary column) and a carrier gas as the mobile phase. As the sample travels through the column, it interacts with the stationary phase, leading to the separation of its components based on their physical and chemical properties.

Gas chromatography is highly versatile and applicable to a wide range of compounds. Its popularity stems from its efficiency, speed, and sensitivity, making it an indispensable tool in various scientific and industrial fields for tasks such as quantitative analysis, qualitative analysis, and the identification of different chemical substances.

Principle: Gas chromatography operates on the principle of partitioning the analyte between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid. This partitioning behavior is dependent on the differences in the interaction of organic compounds with the mobile gas phase and the stationary phase.[6,7]

Components within the sample are separated based on their partition coefficients. Those with higher solubility in the liquid stationary phase will elute later, while those with lower solubility will elute first. The partitioning behavior between the mobile and stationary phases allows for the effective separation of the individual components present in the sample.

Carrier gas: Carrier gases, such as hydrogen, helium, nitrogen, or argon, serve as the mobile phase in gas chromatography.[4,5] These gases facilitate the transportation of the sample through the chromatographic column and contribute to the efficiency of the separation process. The choice of carrier gas depends on factors such as the type of analysis and the specific requirements of the separation.

Applications of gas chromatography

- **Purification of compounds**

Gas chromatography is employed for the purification and determination of compounds in various drugs, such as clove oil, atropine sulfate, and stearic acid. It aids in assessing the quality and purity of pharmaceutical substances.

- **Quality Control and Analysis of drug products**

Gas chromatography plays a crucial role in quality control and the analysis of drug products, including antibiotics, general anesthetics, antivirals, and more. It ensures that pharmaceutical formulations meet regulatory standards and specifications.

- **Determination of metabolite levels in body fluids**

Gas chromatography is utilized to determine the levels of metabolites in body fluids like blood plasma, serum, and urine. This application is vital for clinical and diagnostic purposes, providing valuable information about the metabolic profile of an individual.[4]

Gas chromatography's versatility and precision make it a valuable tool in various scientific and medical fields, particularly in pharmaceuticals, where it is applied to ensure the quality, purity, and efficacy of drugs. Additionally, its applications extend to clinical and diagnostic settings for the analysis of biological samples.

High-performance liquid chromatography (HPLC) is a chromatographic technique that enables structural and functional analysis as well as the purification of various molecules in a short time. This method is highly effective in the separation and identification of a wide range of biomolecules, including amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules.

In HPLC, the mobile phase passes through columns under high pressure, typically ranging from 10 to 400 atmospheric pressure, with a high flow rate of 0.1 to 5 cm/s. This technique employs small particles, and the application of high pressure enhances the solvent flow rate, thereby increasing the separation power of HPLC. The use of small particles and high pressure allows for a rapid and efficient analysis.

The essential components of an HPLC device include a solvent depot, high-pressure pump, commercially prepared column, detector, and recorder. The duration of separation is controlled by a computerized system, ensuring precise and automated control over the process. The analysis is completed in a short time, making HPLC a highly efficient and widely used technique in various scientific and industrial applications.[8]

Applications of High-Performance Liquid Chromatography (HPLC)

- **Pharmaceutical application**

Identify active constituents in dosage forms: HPLC is used in the pharmaceutical industry to identify the active constituents in various dosage forms, ensuring the quality and consistency of pharmaceutical products.

Evaluate Pharmaceutical Product Shelf-Life: HPLC is employed to assess the shelf-life of pharmaceutical products, ensuring their stability and efficacy over time.

Dopamine in Levodopa: HPLC is used for the analysis of dopamine in levodopa, a crucial application in the field of neuropharmacology.[9,10]

Environmental application

Identify diphenhydramine in deposited sample: HPLC is utilized in environmental applications for the identification of compounds such as diphenhydramine in deposited samples.

Pollutant biomonitoring: HPLC is employed in environmental monitoring for the identification and quantification of pollutants, contributing to environmental assessment and management.[10,11]

- **Clinical application**

Analysis of antibiotics: In clinical settings, HPLC is used for the analysis of antibiotics, ensuring accurate quantification and characterization of these essential pharmaceutical agents.[12]

High-Performance Liquid Chromatography proves to be a versatile analytical technique with applications spanning pharmaceuticals, environmental monitoring, and clinical analysis. Its precision, sensitivity, and efficiency make it a valuable tool in diverse scientific and industrial contexts.[13]

Application of chromatography

- **Pharmaceutical industry**

Drug development: Chromatography is crucial in drug development for the separation and analysis of various drug components. It ensures the purity and quality of pharmaceutical products.[14]

Quality control: Chromatography is used to verify the composition and quality of pharmaceutical formulations, ensuring they meet regulatory standards.[15]

- **Clinical and Medical research**

Diagnostics: Chromatography is applied in clinical laboratories for diagnostic purposes, including the analysis of blood, urine, and other bodily fluids.

Biochemical research: In biochemistry, chromatography is used to study biomolecules such as proteins, nucleic acids, and enzymes.[16]

- **Environmental monitoring**

Pollutant analysis: Chromatography aids in the identification and quantification of pollutants in air, water, and soil samples, contributing to environmental monitoring and assessment.[17]

Waste management: It is used to analyze and characterize waste products for proper disposal and environmental impact assessment.[18]

- **Food and Beverage industry**

Food Quality control: Chromatography ensures the quality and safety of food products by analyzing additives, preservatives, and contaminants.[19]

Flavor and Fragrance analysis: It is employed to identify and quantify components in flavors and fragrances.

- **Chemical industry**

Chemical analysis: Chromatography is widely used in chemical laboratories for analyzing and separating different chemical compounds.

Product quality control: It ensures the quality of chemical products by verifying the composition and purity of raw materials and final products.[20]

- **Forensic science**

Drug testing: Chromatography is used in forensic laboratories for drug testing and the analysis of substances found at crime scenes.

Toxicology: It helps identify and quantify toxic substances in forensic investigations.[21]

- **Biotechnology**

Protein purification: Chromatography is an integral part of protein purification processes in biotechnology, facilitating the isolation of specific proteins.

Genomic analysis: It is used in DNA sequencing and genomics research for separating and analyzing DNA fragments.[22]

- **Petroleum industry**

Fuel analysis: Chromatography is employed to analyze and characterize components in fuels, ensuring compliance with industry standards.

Oil refining: It aids in the analysis of crude oil and its fractions during the refining process.[24]

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