Study of Chromatography Techniques and Its Application

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ABSTRACT: ***Chromatography is a vital biophysical method for qualitative and quantitative analysis that allows for the identification, separation as well as purification of the components of a mixture. Size or shape, total charge, hydrophobic groups on the surface, and binding ability with the stationary phase are all factors that may be used to purify proteins. Ion exchange, surface adsorption, partition, or size exclusion are four separation strategies based on molecule properties and interaction type. Other chromatography techniques, such as column, thin layer, and paper chromatography, are based on the stationary bed. One of the most popular methods of protein purification is column chromatography. The primary goal of this article is to get a better understanding of chromatography techniques, kinds, and applications. Chromatographic methods will give increasingly precise findings in the future. Affinity chromatography is increasingly being used to explore PTM protein and protein interactions, particularly with the objective of creating innovative universal tag systems and chemical derivatization methods for peptide affinity selection.***

KEYWORDS: ***Affinity Chromatography, Column Chromatography, Gas Chromatography, Mobile Phase, Stationary Phase.***

1. INTRODUCTION

Chromatography is based on the concept of molecules in a mixture being applied to a surface or a solid, as well as the fluid stationary phase separating from one another while moving with the assistance of a mobile phase. On this separation procedure, molecular characteristics related to adsorption (liquid-solid), partition, or affinity or differences among their molecular weights are beneficial. As a result of these differences, certain components of the mixture stay longer in the stationary phase and travel slowly through the chromatographic system, while other flow swiftly into the mobile phase and exit the system[1].

1.1.The chromatographic techniques is built on two component based on this approaches:

- Stationary Phase: A "solid" phase or a layer of a liquid adsorbed on the surface of a solid support is always present in this phase.
- Mobile Phase: A “liquid” or a “gaseous components” is always present in this phase.

The kind of interaction between the stationary phase, mobile phase, and chemicals present in the mixture is the major component effective in separating molecules from each other. Partition chromatography methods may be used to separate and identify small molecules such as amino acids, carbohydrates, and fatty acids. However, affinity chromatography (ion-exchange chromatography) is more effective in the isolation of biomolecules such as nucleic acids or proteins. On the other hand, gas-liquid chromatography may be used to separate alcohol or Esther group, as well as monitor enzymatic interactions molecular sieve chromatography determines the molecular weight of proteins. Agarose-gel chromatography is used to purify RNA, DNA, and viruses. There are two types of stationary phases: solid and liquid. The stationary phase is traversed by a gaseous or a Whenever the mobile phase is liquid, liquid chromatography (LC) is employed, while gas chromatography is utilised when the mobile phase is gas chromatography is used to analyse gases, volatile liquid mixes, and solid materials. Particularly suitable for compounds that are non-volatile and thermally unstable[2] is liquid chromatography. Molecules can be separated using a variety of chromatographic methods, shows in Figure 1.

1.2.Types of Chromatographic Techniques:

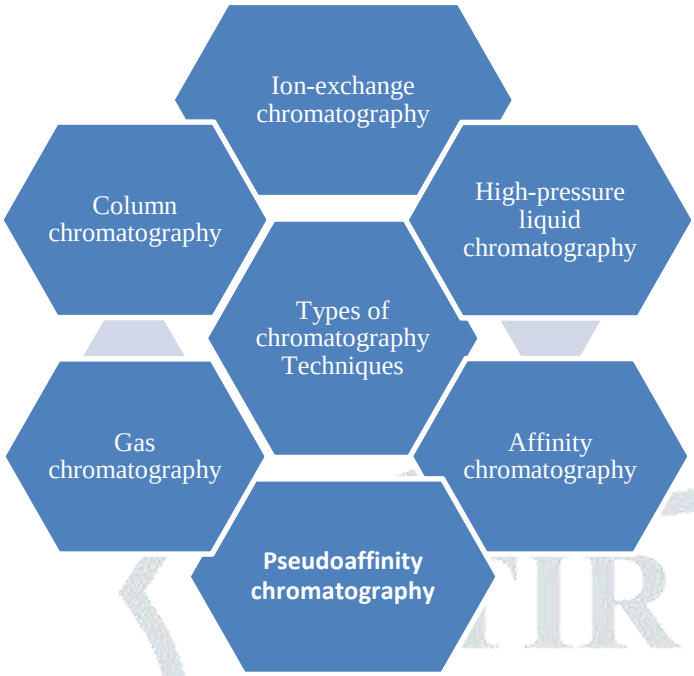

Figure 1: Illustrates some major types of chromatography techniques.

1.2.1. Techniques of Ion-Exchange Chromatography:

In Ion-Exchange Chromatography, electrostatic interactions between charged protein groups and solid support materials are the driving. This is because the protein to be separated has a different ion load than the matrix. The protein's affinity for the column is determined by ionic interactions. pH, ion salt concentration and/or ionic strength of the buffer solution are all used to separate the proteins. They are ion-exchange matrices that have a positive charge and are used to adsorb proteins with a negatively charged. As an alternative, negatively charged cation-exchange matrices absorb positively charged protein[3].

1.2.2. Column chromatography :

Using chromatographic methods, proteins may be separated based on their structure, net charge and the size of the stationary phase they are bound to. Most of them employ column chromatography. This technique is used to purify biomolecules. Prior to applying the sample to be separated, wash buffer is added to a column (mobile phase). Its flow is ensured by the internal column materials, which is mounted on a fiber glass support structure that They are collected in a volume- & time-dependent manner at the bottom of the device, shows in Figure 2.

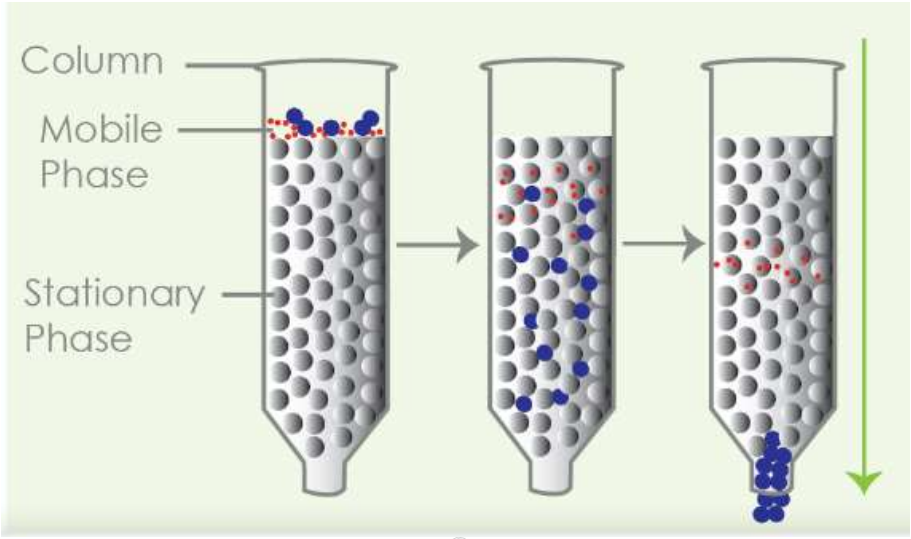

Figure 2: Agarose beads serve as the stationary phase in column chromatography, remaining immobile within the column[4].

1.2.3. Gas chromatography :

Alternatively, a liquid stationary phase is adsorbed on the surface of an inert solid. There are two types of gas chromatography gas and liquid chromatography. During the carrier phase, He and N2 is used as a carrier gas The mobile phase, an inert gas, is forced through a column under high pressure. Materials are vaporized and transported into a gaseous mobile phase for further analysis and analysis There are two phases in a sample: a mobile phase, as well as a stationary phase, on a solid support It differs from because the mobile phase is a gas, as well as the component are separated as vapors,

There are two types of samples that may be used: liquids and gases. Because of its small molecules & chemical inertness, helium is employed as a carrier gas in gas chromatography. When pressure is applied, the sample is pushed through the column by the mobile phase. Component are separated with the use of a stationary phase-coated, show in figure 3.

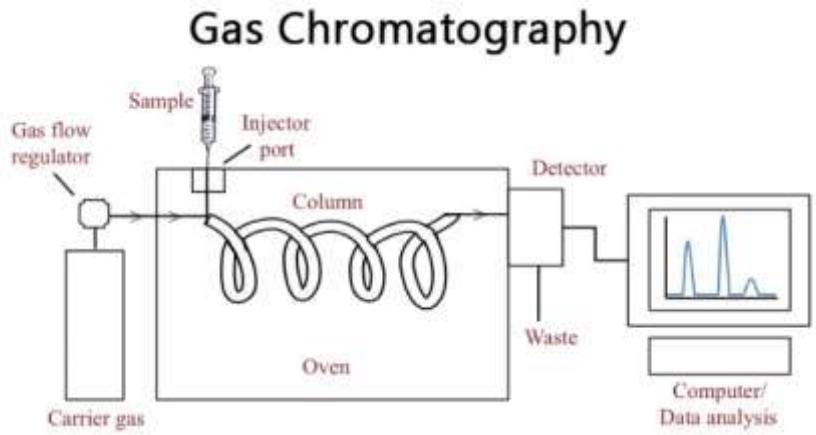

Figure 3: Illustrate the diagram showing working of gas chromatography[6].

1.2.4. High Performance Liquid Chromatography:

It is feasible to undertake structural and functional analysis as well as purification of numerous compounds using this chromatography technology in a short amount of time. Amino acids, lipids, carbohydrates, nucleic acids, proteins, steroids, or other physiologically active substances may all be separated and identified using this approach. High-performance liquid chromatography (HPLC) involves moving the mobile phase over column at a high flow rate (0.1 to 5 cm/sec) while HPLC's separation power is enhanced by using small particles as well as high pressure on the solvents flow rate in this method, which allows the analysis to be completed in a short amount of time. Each element of an HPLC system is crucial, including the solvent storage,

high-pressure pump, column, detector, and recorder (R-O). An automated system controls the length of the separations, as well as the accumulation of materials[7].

1.2.5. Pseudo Affinity Chromatography:

Since anthraquinone dyes including azo-dyes have affinity for dehydrogenases & reductases, they can be used as ligands. Affinity chromatography with immobilized metals is the most well-known version of this type of chromatography

1.2.6. Affinity Chromatography:

In addition to purifying enzymes and hormones, chromatography may also filter nucleic acid, antibodies, and other biological molecules. A chemical compound that can be produced with specific proteins binds the column's filament (dextran, cellulose ,polyacrylamide etc). Un complexed ligand-protein complexes, however, are linked to matrix and remain in column, whereas un complexed ligand For the protein-bound ions to be removed from the columns, the osmolarity of the solution must (pH or adding salts)[8].

1.2.6.1. Principle of affinity chromatography:

While there is some specificity to the interactions between adsorbent and material to be separated, there is still an attraction force between the atoms that draws them together and keeps them together. A reversible binding to a ligand linked to an insoluble matrix is required for the separation material.

Covalently linked substrates (ligands) expose reactive groups necessary for targets molecule interaction in the stationary phase. When the crude mix of compound passes through the chromatography column, substances with binding sites for the immobilized substrate bind to the stationary phase, while all other substances elute into the column empty volume. Other compounds can be removed from mobile phase by introducing competing ligands or by changing pH, ionic strength, and/or polarity conditions after the bound target molecules have been removed. A macromolecular complex interacts with the ligand, as shown in figure 4.

Enzymes were the original target for the invention of affinity chromatography, however it has subsequently been broadened to accommodate other molecules such as DNA, nucleic acids immune cells.

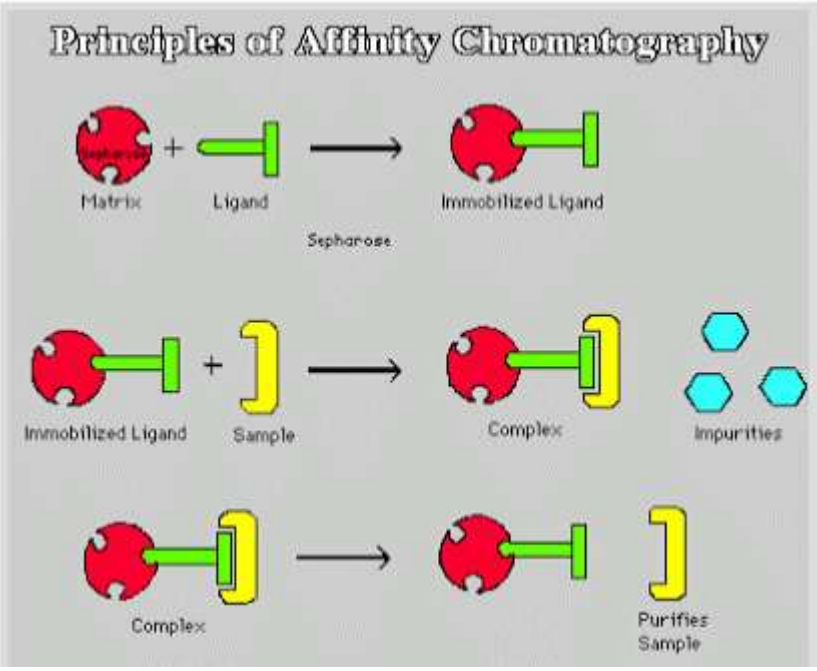

Figure 4: Illustrate the interaction between the ligand and the macromolecular complex[9]**.**

1.3.Chromatography's Applications Include:

1.3.1. Pharmaceutical Sector:

- Determine the existence of trace elements or compounds in samples by identifying and analyzing them.
- Compound segregation based on molecular weight and element content.
- Detects unknown chemicals and mixture purity.

- In the pharmaceutical industry.

1.3.2. Chemical industry:

- HPLC and GC are widely used for identifying different pollutants including such polychlorinated biphenyl in pesticides and oils, as well as checking air quality.
- In a variety of applications in the life sciences.
- In testing water sample or also checks air quality.

1.3.3. Food Industry:

- Distinguishing food deterioration or additives.
- Analyzing the nutritional value of food.

1.3.4. Molecular biology Studies:

- A variety of approaches that combine hyphenated chromatographic techniques, such as ECLC-MS, are utilised in research on metabolomics or proteomics, as well as nucleic acid research.
- There are a variety of applications for HPLC including protein separation procedures such as Insulin purification (plasma fractionation) and enzyme purifying (enzyme purification).

2. LITERATURE REVIEW

Justyna Płotka et al. studied about green chromatography. The analysis of organic molecules in samples with varying matrix compositions is critical in a variety of fields. Gas or liquid chromatographic techniques are used to determine the vast majority of chemical compounds. As a result, it's critical that these approaches have little environmental effect. Because helium is a non-renewable resource, it is best to avoid utilizing it as a carrier gas in gas chromatography. At all stages of the analysis, from sample collection and preparation through separation and final determination, chromatographic methods have the potential to be more environmentally friendly. The method's environmental effect is frequently influenced by the instrument's placement in relation to the sample collecting point[10].

Detlev Helmig described about gas chromatography's air analysis A special attention is placed on capillary gas chromatography. A database of chemical groups of the substances examined and a table of stationary phases are used to list these numbers. In this chapter, the features of Capillary Columns and important terminology such as Carrier Gas (CGA), Detection, Film Thickness, Liquid Stationary Phase (LSP), Injection, Multi - dimensional GC, Oven Programming, PLOT Column, Packed Columns, as well as Sampling Techniques A brief overview of emerging alternate approaches to classic GC techniques is also provided[11].

Harald Pasch studied about the molecular heterogeneity of larger molecules is spread in several directions. They are commonly distributed in terms of chemical composition, functionality, or molecular architecture, in additional to molar mass. It is important to utilize a variety of analytical methods to characterize the various forms of molecular heterogeneity. These approaches should ideally be targeted at a certain sort of heterogeneity. Two dimensional information on molecular heterogeneity is expected to be obtained by combining two selective analytical methods. The most promising hyphenated approaches protocols involve combining two separate chromatographic methods as well as chromatography as well as spectroscopy. The basic concepts of two-dimensional chromatography including the hyphenation of liquid chromatography with selective detectors will be discussed in this review[12].

3. DISCUSSION

It is common to use chromatographic techniques as part of analytic operations in order to separate, quantify, or identify many different types of analytes in samples with complex and variable matrix composition There are several ways to separate or determine substances, and column chromatography is one Using one of a protein's unique characteristics, column chromatography is a technique of proteins purification. Amino acid-binding affinity chromatography is an effective protein separation technique that employs immobilized ligands to interact with Similar to how pigments were separated using natural pigments, the earliest application of chromatographic methods was to separate compounds based on Its range of applications has grown significantly over time. On the other hand, chromatography is now universally recognized as a sensitive and efficient Separation and determination using column chromatography is one of the most useful techniques As an important tool for biochemists, chromatography is also relatively easy to utilize in clinical laboratory

research. A paper chromatography technique is used, for example, to detect sugars and amino acids in bodily fluids that may be connected to hereditary metabolic disorders Using gas chromatography, steroid, fat and barbiturates are tested in laboratories. The chromatographic technique is used to separate vitamins and proteins.

4. CONCLUSION

First, chromatographic techniques were used to separate chemicals depending on their colour. Throughout the years, its application range has expanded substantially. It is now well accepted that chromatography is a highly sensitive or effective separation method. Column chromatography is a proteins purification technology based on one of proteins' distinctive properties. A protein's purity can also be maintained using these approaches. As a result of its increased sensitivity and rapid turnover rate or as a quantitative method, HPLC is capable of purifying a variety of substances including protein, RNA, hydrocarbons, amino acids, carbohydrate, medications, antibiotics, Similar to how pigments were separated using natural pigments, the earliest application of chromatographic methods was to separate compounds based on Its range of applications has grown significantly over time. On the other hand, chromatography is now universally recognized as a sensitive and efficient There are several ways to separate and determine substances, and column chromatography is one Using a protein's unique characteristic as a guide, column chromatography purifies the protein. It is the major objective of this page to provide a better understanding of chromatography techniques, types, & applications To understand how these parameters affect the entire cycle duration, further research should examine resin lifespan problems and the frequency with which each process cycle's cleaning phase should be done.

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