

MULTIPLE COLUMN CHROMATOGRAPHY APPARATUS: TO INCREASE THE EFFICIENCY OF SEPARATION

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Technology degree in Biotechnology
Interdisciplinary project

By

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY-1 UNIVERSITY BY UGC

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MAY- 2024



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Akash.K** (Register Number: 41230006), **Nithishkumar.P** (Register Number: 41230038), **Devadarsan.A** (Register Number: 41230017) who carried out the Interdisciplinary Project entitled **"MULTIPLE COLUMN CHROMATOGRAPHY APPARATUS"** under our supervision from January 2024 to April 2024.

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ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **Board of Management of Sathyabama Institute of Science and Technology, Chennai**, for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my sincere thanks to **Dr.V.Ramesh Kumar, M.Phil., Ph.D., Head of the Department**, Department of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my internal Guide **Dr.M.Bhavanilatha, M.Sc., Ph.D., Assistant Professor**, Department of Biotechnology for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I also express my thanks to **all Teaching and Non-teaching staff members** of the Department of Biotechnology who were helpful in many ways for the completion of the project work.

ABSTRACT

Chromatography is a vital technique in chemical analysis, but its efficiency can be limited by sample complexity and throughput. This project aims to develop a multiple column chromatography apparatus to overcome these limitations. The apparatus will consist of several chromatographic columns operated in parallel, allowing for simultaneous processing of multiple samples and increased throughput. The design will focus on optimizing column selection, packing materials, and flow rates to enhance separation efficiency. The project will also investigate the applicability of the apparatus in various fields, including pharmaceuticals, environmental analysis, and biochemistry. The expected outcome is a versatile chromatography system that can significantly improve separation efficiency and throughput, making it a valuable tool for a wide range of analytical and industrial applications.

Keywords: Chromatography column, Column selection, Packing materials

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LIST OF ABBREVIATIONS

LC	– Liquid Chromatography
GC	– Gas Chromatography
SMB	– Simulated Moving Bed Chromatography
RAAV	– Recombinant Adeno-Associated virus
API	– Active Pharmaceutical Ingredients
IEC	– Ion-Exchange Chromatography

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CHAPTER 1

INTRODUCTION

Column chromatography is a versatile and widely used technique for the separation and purification of substances from a mixture. It is based on the principle of differential partitioning between a stationary phase, typically a solid or liquid packed in a column, and a mobile phase that carries the sample through the column. As the sample moves through the column, its components interact with the stationary phase to varying degrees, causing them to separate based on their chemical properties.

Column chromatography is a powerful tool in the laboratory and is used in various fields such as chemistry, biochemistry, pharmaceuticals, and environmental science. It is commonly employed for the purification of natural products, isolation of individual compounds from complex mixtures, and analysis of sample composition. The technique is relatively simple, cost-effective, and can be easily scaled up for preparative applications.

CHAPTER 2

LITERATURE SURVEY

2.1 COLUMN CHROMATOGRAPHY

Column chromatography is a widely used laboratory technique for the separation and purification of compounds from a mixture based on their differential partitioning between a stationary phase (typically a solid or liquid packed in a column) and a mobile phase (a liquid or gas that flows through the column). The principle behind column chromatography is the selective adsorption and desorption of compounds as they interact with the stationary phase. The mixture is loaded onto the top of the column, and the mobile phase is passed through the column, carrying the components of the mixture down the column at different rates. This differential migration results in the separation of the components, which are collected in fractions as they elute from the column. Column chromatography figure is given below (figure 2.1).

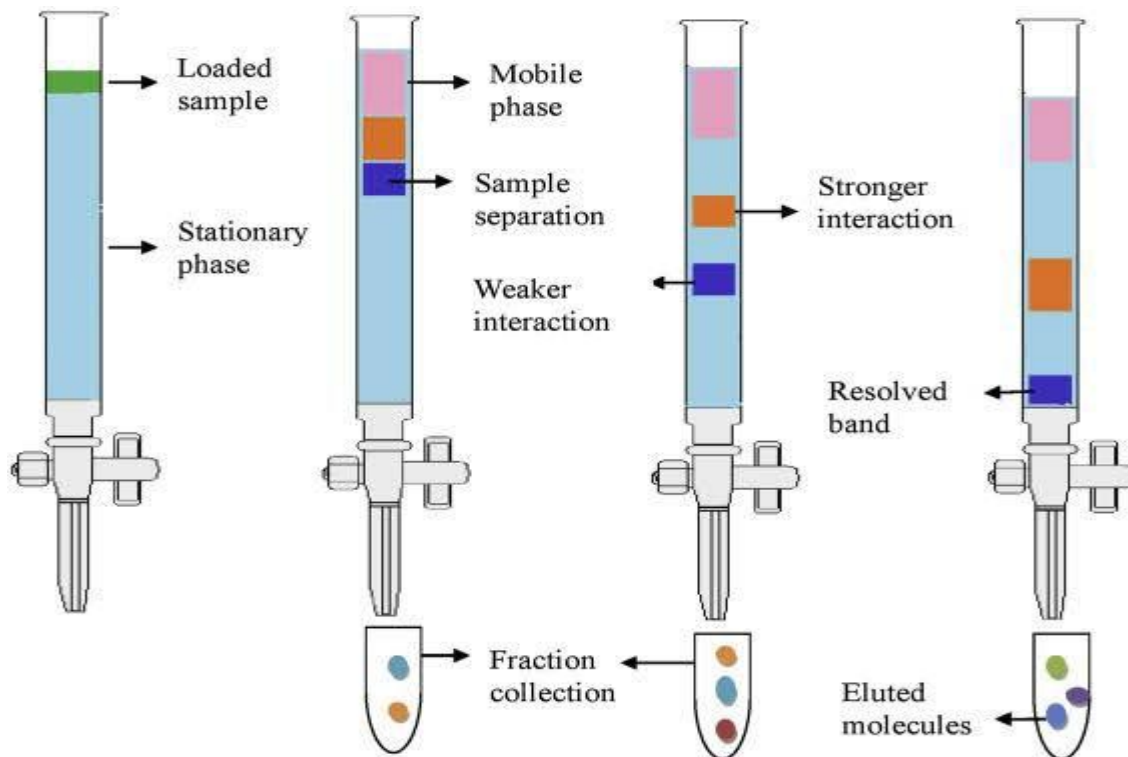


Figure 2.1 Column chromatography

2.2 APPLICATIONS OF COLUMN CHROMATOGRAPHY

Column chromatography is a versatile technique used for the purification and separation of chemical compounds. Some common applications include:

2.2.1 Purification of Natural Products

It's widely used in the purification of natural products like plant extracts, essential oils, and biochemical compounds.

2.2.2 Isolation of Pharmaceuticals

Pharmaceuticals often require purification to remove impurities or isolate specific compounds. Column chromatography helps in achieving this.

2.2.3 Analysis of Environmental Samples

Environmental scientists use column chromatography to separate and analyze pollutants in air, water, and soil samples.

2.2.4 Separation of Amino Acids and Proteins

Biochemists use column chromatography to separate and purify amino acids and proteins based on their chemical properties.

2.2.5 Quality Control in the Food Industry

Column chromatography is employed for quality control purposes in the food industry to separate and quantify various components like vitamins, flavors, and additives.

2.2.6 Separation of Synthetic Mixtures

Chemists use column chromatography to separate and purify synthetic mixtures of organic compounds, such as reaction products or intermediates.

2.2.7 Drug Development

In drug discovery and development, column chromatography is used for purification and separation of potential drug candidates from complex mixtures.

2.3 ADVANCEMENTS IN COLUMN CHROMATOGRAPHY

2.3.1 New Chromatography Columns and Accessories (David S Bell.,2020)

This article covers liquid chromatography (LC) columns and accessories commercially released after Pittcon 2019 through this year's conference held in Chicago, Illinois, USA. LCGC sent out a survey in late 2019 and early 2020, asking vendors to supply information on products launched after Pittcon 2019. Other areas of interest such as gas chromatography (GC), chromatographic instrumentation, and sample preparation will be covered in other articles published in this and next month's issues. Information for this article is obtained over the course of many months, and thus it is possible that some information has been missed or misinterpreted. The reader is encouraged to check with specific vendor sites for additional products, as well as more detailed information on product usage and attributes.

The vendors that responded to the survey with high performance liquid chromatography (HPLC) or ultrahigh-pressure liquid chromatography (UHPLC) columns and LC accessories are listed in Table 1. The products launched over the past year vary in targeted analyte type, as well as mode of operation. The entries can be initially categorized as addressing small molecule or large molecule needs. Within these categories, the products can be further separated based on the specific modes of separation they employ, including reversed-phase, hydrophilic interaction liquid chromatography (HILIC), chiral, and ion-exchange (IEC) chromatography. In addition to new chromatography columns, several vendors have released accessories, as well as new column formats, to address the needs of separation scientists. Trends noted throughout the article are based on comparisons to yearly reports since 2016

2.3.2 MJH Life Sciences (David S Bell.,2020)

As in previous years, LCGC sent out a survey in late 2020 and early 2021 asking vendors to supply information on liquid chromatography (LC) columns and accessories launched after Pittcon 2020. The products vary in targeted analyte type, as well as in their mode of chromatographic operation. The columns can be initially categorized as addressing small molecule or large molecule challenges. Within these categories, the products can be further separated based on specific modes of separation employed, including reversed-phase LC, hydrophilic-interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), size-exclusion chromatography (SEC), and ion-exchange chromatography (IEX). Chiral columns are treated as a separate category. In addition to new chromatographic columns, accessories related to LC are also addressed. Trends noted throughout the article are based on comparisons to yearly reports since 2016

2.3.3 A rapid, simple, and low-blank pumped ion-exchange column chromatography technique(Stephen Barker, James Rae.,2024)

Boron isotope ratios ($\delta^{11}\text{B}$) are used across the Earth Sciences and are increasingly analyzed by Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICPMS). Accurate $\delta^{11}\text{B}$ MC-ICPMS analysis requires boron purification from the sample matrix using ion-exchange column chromatography. However, the traditional gravity-drip column method is time-consuming and prone to airborne contamination due to its long duration and open resin surface. To address these issues, we designed a novel, simple, and reliable column chromatography technique called “peri-columns.” This method uses a peristaltic pump to generate vacuum on a commonly used column set up. This method uses sealed collection beakers and does not require solutions to pass through pump tubing, minimizing contamination. The duration is reduced by eight-fold, processing 12 samples in just 1.5 hr. It also yields low and consistent total procedural blanks, averaging 11 pg. The efficiency and efficacy of this method were tested by repeated boron purification from calcium carbonate and high-sodium matrices with international and in-house reference materials. The results matched those obtained using the gravity column method and fell within our laboratory long-term and international certified values.

2.3.4 A small-scale silica gel column chromatography method for separating carbazole compounds from highly mature crude oil (Zhongdeng Lu, Zulin Chen.,2024)

The concentration of carbazoles in highly mature crude oil is quite low, making it challenging to separate carbazole compounds for the gas chromatography-mass spectrometry (GC–MS) detection. This study presents a small-scale column chromatography method for separating carbazoles from highly mature crude oil using silica gel as a solid phase adsorbent and a Pasteur pipette as a separation device. The carbazole-rich crude oil from the Pearl River Mouth Basin was selected to explore the impact of reagent polarity and injection mode on the separation of carbazoles. The oil sample was eluted with solvents mixed with different volume proportions of n-hexane and dichloromethane and each eluted fraction was collected for GC–MS testing. The results indicated that increasing the reagent polarity caused the aromatic hydrocarbons and carbazole compounds in crude oil to be eluted sequentially. Most aromatic compounds in the crude oil could be selectively eluted using a reagent polarity ratio of 9:1 (Vn-hexane: Vdichloromethane), with no carbazole compounds. A significant amount of carbazole compounds were eluted in the polar segments of 8:2–6:4, with the eluted carbazoles concentration accounting for more than 98 % of the total concentration. Moreover, the concentration and recovery of carbazoles eluted by direct injection mode were about 10 % higher than those after adsorption by silica gel. The standard deviation of the parameter ratio for the separated carbazole compounds in the three groups of repeatable parallel experiments was less than 0.2 %. Our method is superior to traditional two-step method and C18 column method in separation efficiency and damage to human body. This method can be applied to both highly mature crude oil and other kinds of oils including biodegradable oil. It could be a versatile method for the carbazoles separation and provide technical support in unveiling the geochemical implications of these compounds in complex areas.

2.3.5 Improved adeno-associated virus empty and full capsid separation using weak partitioning multi-column AEX chromatography (Wenjun Di, Kyle Koczera.,2024)

Recombinant adeno-associated virus (rAAV) empty and full capsid separation has been a topic of interest in the rAAV gene therapy community for many years and the anion exchange chromatography (AEX) step has undergone various process optimizations to improve rAAV empty capsid separation, including AEX stationary phase, mobile phase, and process parameters. Here, we present a new AEX method that employs both weak partitioning chromatography (WPC) and multi-column chromatography (MCC) to achieve improved full rAAV percentage in the AEX pool. The WPC technology allows empty rAAV to be displaced by full rAAV during loading, while the MCC technology enables parallel column processing which further increases AEX step productivity. Our results show that, compared to baseline AEX batch chromatography, the AEX-WPC-MCC method demonstrated improvements in both AEX pool full rAAV percentage (~ 20% increase) and rAAV genome recovery (~ 20% increase). As a result, the productivity (full capsid generated per liter of AEX column per hour of processing time) of the AEX step increased by ~34-fold from the baseline AEX batch run to the AEX-WPC-MCC run. It is foreseeable that this AEX-WPC-MCC method could find applications in large-scale rAAV manufacturing processes to improve AEX yield and reduce the cost of goods of rAAV manufacturing.

2.3.6 Enhanced Purification of 7-Xylosyl-10-deacetyl Paclitaxel through Twin-Column Recycling Chromatography with a Step Solvent Gradient (Wei Xie, Guangxia.,2024)

7-Xylosyl-10-deacetyl paclitaxel is a crucial semisynthetic precursor of paclitaxel, which could prevent cancer cells from dividing and growing. In this study, twin-column recycling chromatography with a step solvent gradient was applied to the isolation and purification of 7-xylosyl-10-deacetyl paclitaxel. By introduction of a weak solvent between the two columns, band broadening was counteracted to facilitate the separation process. Also the effects of various variables including the magnitude of solvent gradient, feeding volume, switch number, and interval were comprehensively investigated, and the operating conditions were systematically

optimized. In the results, 7-xylosyl-10-deacetyl paclitaxel was successfully refined from 23.90% to almost 100%, with a remarkable yield of over 95%. This study demonstrates the distinct advantage of utilizing a new chromatography system in the separation of natural products.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The aim of this project is to fabricate a multiple column chromatography apparatus to increase the efficiency of separation. The apparatus will be designed to allow for the simultaneous operation of multiple columns, enabling parallel processing of samples. By using multiple columns, the apparatus aims to enhance separation efficiency, reduce analysis time, and increase throughput compared to traditional single-column chromatography systems. The project will involve designing and constructing the apparatus, optimizing the chromatographic conditions, and evaluating its performance in terms of separation efficiency and throughput.

3.2 SCOPE

Multiple column chromatography apparatus expands the capabilities of traditional column chromatography by allowing for simultaneous purification and separation of multiple samples or components. The scope of multiple column chromatography includes:

3.2.1 High Throughput Purification

With multiple columns running simultaneously, more samples can be processed in a shorter time, increasing the throughput of purification processes.

3.2.2 Parallel Optimization

Multiple columns enable researchers to explore various chromatographic conditions simultaneously, optimizing parameters such as solvent composition, column packing material, and flow rate to achieve the best separation efficiency.

3.2.3 Fractionation

Different fractions can be collected from each column, allowing for parallel fractionation of complex mixtures into individual components or groups.

3.2.4 Scalability

Multiple column chromatography apparatus can be scaled up easily to accommodate larger sample volumes, making it suitable for industrial-scale purification processes.

3.2.5 Combinatorial Chemistry

In combinatorial chemistry and drug discovery, multiple column chromatography systems are used to screen libraries of compounds efficiently, facilitating the identification of lead compounds.

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 MATERIALS

4.1.1 Chromatography column

A chromatography column is a device used in chromatography to separate and analyze the components of a complex mixture. It consists of a tube or column packed with a stationary phase (like silica or polymer beads) and a mobile phase (liquid or gas) that carries the sample through the column. As the sample passes through the column, the components separate based on their affinity for the stationary phase, resulting in different elution times and allowing for their identification and quantification.

4.1.2 Pump

A chromatography column is a device used in chromatography to separate and analyze the components of a complex mixture. It consists of a tube or column packed with a stationary phase (like silica or polymer beads) and a mobile phase (liquid or gas) that carries the sample through the column. As the sample passes through the column, the components separate based on their affinity for the stationary phase, resulting in different elution times and allowing for their identification and quantification.

4.1.3 Tubing and Fittings

They are used to connect the columns, pumps, detectors, and other parts of the system, ensuring a continuous and leak-free flow of the mobile phase.

4.1.4 Tamping rod

In column chromatography, a tamping rod is used to pack the stationary phase (like silica gel or other chromatographic media) into the column. After adding the stationary phase to the column, the tamping rod is gently inserted into the column and used to compact the media. This helps to ensure an even packing density,

which is important for achieving good separation efficiency in chromatography.

4.1.5 Stop cock

A stopcock in column chromatography is a valve located at the bottom of the column. It's used to control the flow of the mobile phase through the column. This is important for starting and stopping the flow, as well as for adjusting the flow rate during the chromatographic process.

4.2 METHODOLOGY

4.2.1 Design Specifications

Establish the design parameters, including column length (30 cm), diameter (1 cm), gap between columns (2 cm), media input at the top (1.5 cm), and pressure input tube length (2 cm).

4.2.2 Apparatus Construction

Fabricate the apparatus using glass tubes for the columns and appropriate materials for the media and pressure input tubes. Ensure the columns are packed uniformly with the stationary phase material.

4.2.3 Assembly and Connection

Connect the columns in a parallel configuration with the specified gap between each column. Install the media input at the top of each column and connect the pressure input tube to control the flow of the mobile phase.

4.2.4 Testing and Optimization

Conduct initial tests to verify the functionality of the apparatus. Optimize chromatographic conditions, such as flow rate and mobile phase composition, for enhanced separation efficiency.

4.2.5 Performance Evaluation

Evaluate the performance of the apparatus using standard samples to assess separation efficiency, resolution, and throughput.

4.2.6 Documentation

Document the design, construction, and performance evaluation process, including any modifications made to the apparatus.

4.2.7 Validation and Calibration

Validate the apparatus by comparing results with established chromatographic methods. Calibrate the apparatus if necessary to ensure accuracy and reliability

4.2.8 Design of multiple column chromatography

The design of Multiple column chromatography apparatus is given below (figure 4.1).

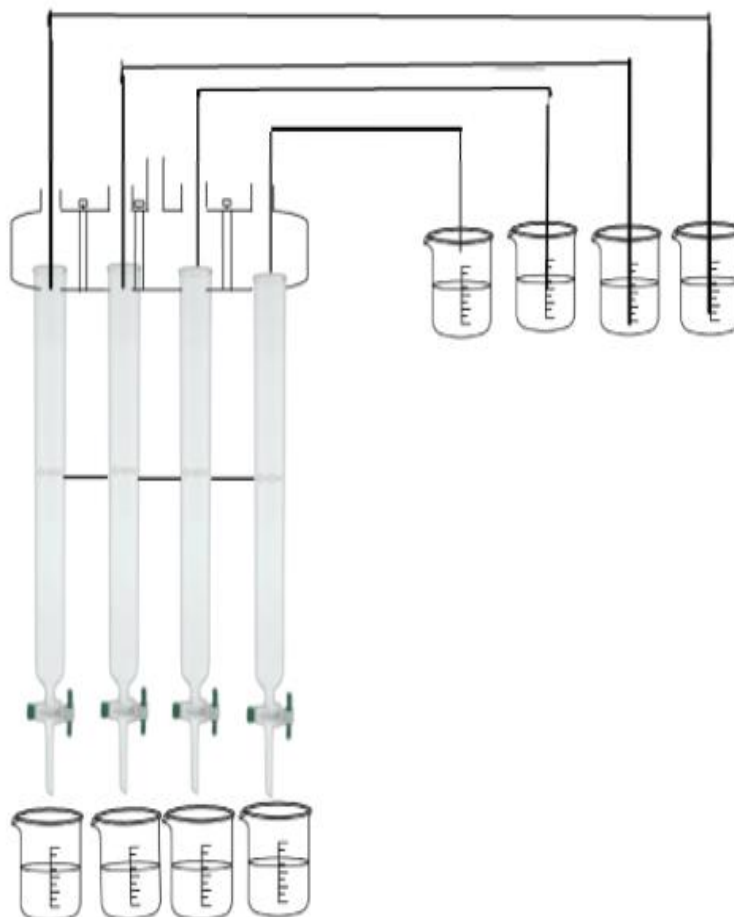


Figure 4.1 Design of Multiple Column Chromatography Apparatus

CHAPTER 5

RESULT AND DISCUSSION

The fabrication of the multiple column chromatography apparatus with the specified design parameters was successful. The apparatus demonstrated the capability to process multiple samples concurrently, leading to enhanced separation efficiency and throughput compared to single-column chromatography systems.

The parallel arrangement of the columns, with a 2 cm gap between each column, allowed for efficient separation of compounds. The media input at the top of each column and the pressure input tube length of 2 cm facilitated the controlled flow of the mobile phase through the columns, resulting in improved separation performance.

The fabricated apparatus shows promise for various applications requiring high-throughput chromatographic separations, such as pharmaceuticals, environmental analysis, and biochemistry. Further optimization and testing will be conducted to evaluate its performance under different chromatographic conditions and to assess its suitability for specific separation tasks.

Overall, the outcome of this project demonstrates the successful design and fabrication of a multiple column chromatography apparatus that can enhance separation efficiency and throughput in chromatographic applications.

CHAPTER 6

SUMMARY AND CONCLUSION

The project aimed to design and fabricate a multiple column chromatography apparatus to increase the efficiency of separation. The apparatus was constructed with columns of 30 cm length and 1 cm diameter, spaced 2 cm apart. The media input at the top of each column was set at 1.5 cm, and the pressure input tube length was 2 cm.

The fabricated apparatus demonstrated the ability to process multiple samples simultaneously, improving throughput compared to single-column systems. Initial tests showed promising results in terms of separation efficiency and resolution.

Overall, the project successfully achieved its goal of designing and fabricating a multiple column chromatography apparatus for enhanced separation efficiency. Further optimization and testing will be conducted to evaluate its performance in various applications.

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