



# भारतीय प्रौद्योगिकी संस्थान दिल्ली

## Indian Institute of Technology Delhi

CLL271: Introduction to Industrial Biotechnology

Course Instructor: Prof. Anurag Singh Rathore

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### **Large Scale Chromatography**

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#### **Author:**

#### **Topic:**

Utkarsh Dogra (2020CH70199)	Differences in typical Objectives between Analytical and Preparative Scale Chromatography
Munit Goyal (2020CH10102)	Major differences between Analytical Scale and Preparative Scale Chromatography
Vipul Suthar (2020CH70204)	Large Scale Chromatography Setup
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Kumari Astha (2019CH10100)	Major Problems faced when packing Large Scale Columns
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## Abstract:

This report presents an overview of “Large Scale Chromatography” by covering a wide variety of topics, including Analytical and Preparative Scale Chromatographic Techniques, Chromatography Setup and the factors affecting performance of column. The focus of the report will be to cover these topics in detail and include some of the future scope of these fields.

## Introduction:

The aim of this report is to cover various topics related to “Large Scale Chromatography”. The technique of Chromatography is one of the most important processes when it comes to purification stage. Chromatography is a highly important separation technique which finds applications in various sectors which mainly includes Biopharmaceutics, Petrochemical, Food Industry, Water Treatment, and other sectors requiring high levels of purity.

This report also focuses on various parameters which affect the performance of chromatography including Retention Time, Band Width, Resolution. Flow Uniformity and Scale Up of Chromatography is also taken up in detail.

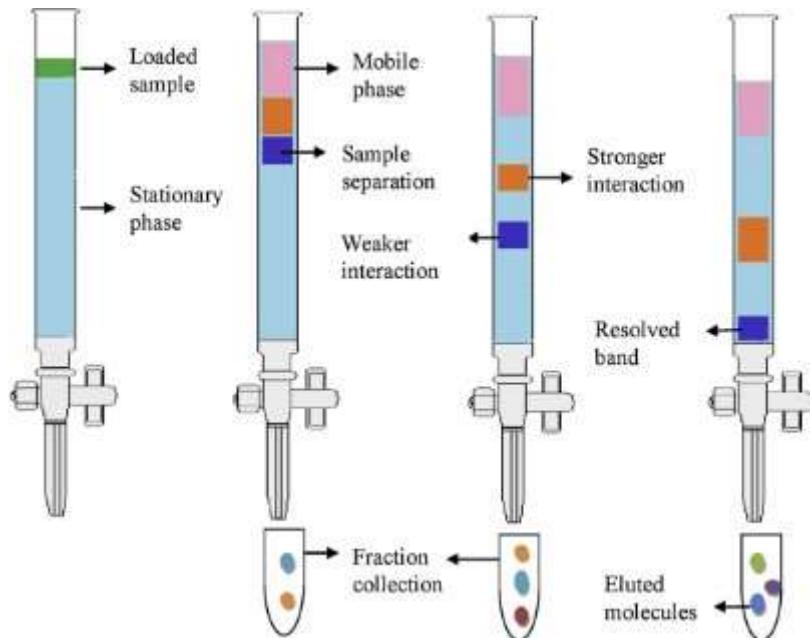


Fig.1: Typical Column Chromatographic Process

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## Difference in typical objectives between Analytical and Preparative Scale Chromatography

~By Utkarsh Dogra (2020CH70199)

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### Abstract:

Chromatography is a powerful separation technique, making use of “Differential Migration” of various components of a substance. Based on objectives and scale of operation, Chromatography is sub divided into 2 main categories, “Analytical Scale” and “Preparative Scale” Chromatography.

The focus of this part of the report is to distinguish between the typical objectives of the major applications of Analytical and Preparative Scale Chromatography. This report will mainly cover a brief introduction, objectives, method, and future of Chromatographic Techniques.

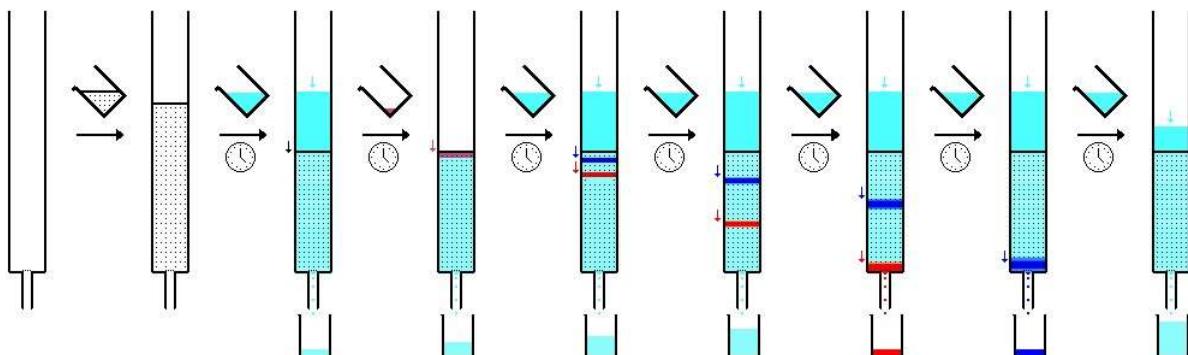


Fig.2: Illustration of Concept of Differential Migration in Column Chromatography

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### Introduction:

Chromatography is a separation technique which is used in almost every type of Process Industry, including Petrochemical Refineries, Food Industries, Environmental Analysis, Bio-Pharmaceutical Industry etc.

Based on scale and objectives, there can be either Analytical Scale or Preparative Scale Chromatography. It is possible to have both kinds of chromatographic techniques being applied in the same industry as their objective can be quite different.

As the name suggests, “Analytical Scale Chromatography” is a technique used for analysis purposes only. It is generally performed at a smaller scale, to know what components are present in the given substance. Typical properties of Analytical Scale Chromatography include very high selectivity, which can help in isolating Isotopes, Isomers, and compound present in very small quantities, say about  $10^{-6}$  to  $10^{-7}\%$ . The components obtained are then sent to waste.

At the same time, “Preparative Scale Chromatography” is a chromatographic technique used to prepare a new substance from a pre-existing substance. It aims to first separate the components and then to use the components to generate a new substance, either in the same industry or the separated components are sent to a different industry.

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## Methods:

Before dwelling into the main methods of Chromatography, it needs to be discussed how both “Analytical” and “Preparative” Chromatographic techniques can be applied in the same industry.

**Background of Application in a Same Industry:** Suppose a person named ‘X’ works in a Bio-Pharmaceutic Industry, who is interested in getting a drug ‘Y’ whose major component can be extracted from substance ‘Z’. To prepare ‘Y’ from ‘Z’, he must use Preparative Scale Chromatography. On the other hand, once the

drug is manufactured, ‘X’ should do Analytical Scale Chromatography to do a purity check. This is how both the chromatographic techniques can be used in the same industry.

**Analytical Scale Chromatography:** Analytical Scale Technique is a small scale, analysis chromatographic technique used to find constituents of a substance. The objective is to “find the constituents of a substance”.

Being small scale technique, analytical scale chromatography makes use of small sized columns (~4.6 mm Diameter of Tubes). Analysis of a substance is usually performed using Gas Chromatography (GC), Liquid Chromatography (LC) and High-Performance Liquid Chromatography (HPLC), although LC is the most common.

The sample is fed to the chromatographic column, containing a suitable mobile phase and stationary phase. Using a particular technique, say like HPLC or GC, the process of chromatography is carried out and a chromatogram is prepared. The chromatogram generated gives an idea about the dead time, retention times, and other parameters like band broadening, fronting and tailing of peaks.

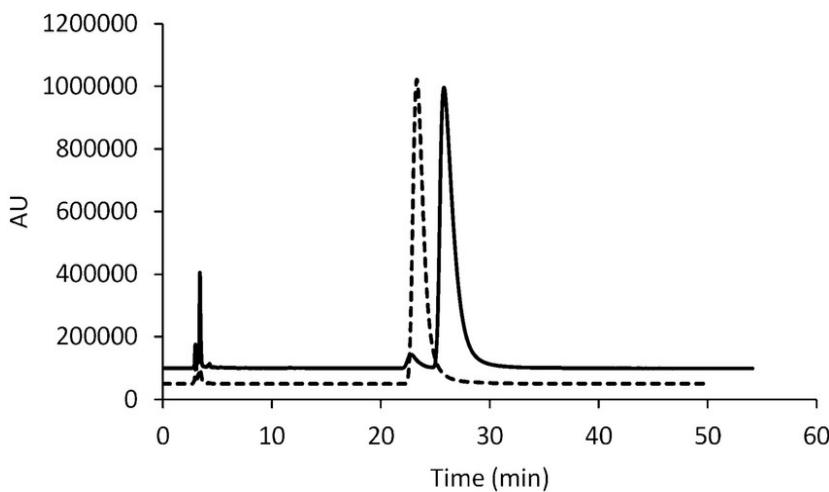


Fig.3: Chromatogram obtained from Analytical Chromatography of Medicinal Drug

As it is a smaller scale chromatography, the number of components used are small and hence those are discarded.

**Preparative Scale Chromatography:** Preparative Scale Chromatography is used at a larger scale because its main objective is to prepare a new compound. The main objective of this is to “prepare a new substance from a pre-existing substance”.

Being a larger scale chromatography, this technique makes use of larger sized columns (typical size lies between ~50 to 200 mm Diameter of Tubes). This chromatographic technique is used with almost all chromatographic techniques, like HPLC, GC, LC, Thin Layer Chromatography (TLC), and Paper Chromatography (PC).

The procedure of Preparative Scale Chromatography is almost same as that of Analytical Scale Chromatography. A sample is prepared for feeding into the Chromatographic Column. After the sample is fed to the column, various components are obtained which are identified based on the heights of peaks obtained in the chromatogram.

Once the components are obtained from a particular substance, those are sent for further processing, to obtain a new substance. The extracted substances can either be used in the same industry or can be sent to a different industry.

As mentioned above, both Analytical and Preparative Scale Chromatography find applications in the same industry which include Petrochemical Industry, Pharmaceutical Industry, Food Industry and in Environmental Analysis.

Based on the peak of the constituent (which means the Optical Density of a substance), the component is uniquely identified as a particular substance.

(Optical Density is defined as:  $OD = -\log_{10} \left( \frac{I_T}{I_i} \right)$ , where subscript T is for Transmitted Intensity and i for Incident Intensity of light.



Fig.4: Preparative Scale Chromatography Setup

## Future Scope:

This part of the report focuses on discussing one of the latest innovations in the field of “Analytical Chromatography”, which is “Green Analytical Chromatography”.

Green Analytical Chromatography focuses to reduce the wastage of solvent, by reducing the retention time of solvent, or in other words the dead time, by making use of either “Elevated Pressures” or “Ultra High-Performance Liquid Chromatographic Systems” (within the range of optimum pressure).

The procedure is to apply very high-pressure difference across the two ends of apparatus, which leads to speeding up of the process.

Now assume that Q (Flow Rate of Solvent) is held constant, which means that  $Q = \frac{V_{dead}}{t_{dead}}$ , is held constant.

Here,  $t_{dead}$  is the dead time (time spent by the solvent in the Chromatographic Apparatus)

$V_{dead}$  is the Volume of Solvent flown during dead time.

If  $t_{dead}$  is reduced  $V_{dead}$  also reduces (as Q is made to remain constant). Reduction in  $t_{dead}$  takes place due to speeding up of process which happens due to elevated pressures.

This way Green Analytical Chromatography reduces wastage of solvent.

The graph shown below clearly shows that retention time of any peak is much lesser in case b. Also, the amount of Solvent wasted is reduced to a large extent (23 ml in case b whereas 60 ml in case a) using Green Analytical Chromatography.

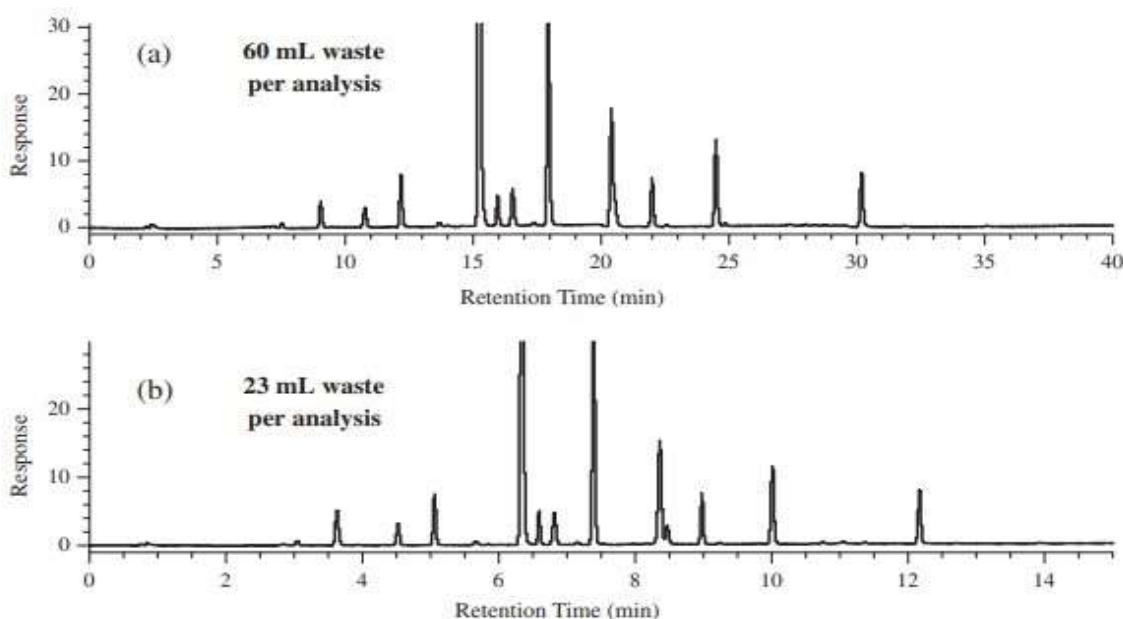


Fig.5a) Chromatogram of a typical process

Fig.5b) Chromatogram of the same process using Green Analytical Chromatography

## **Conclusion:**

From the report, we could conclude how Analytical Scale Chromatography is different from Preparative Scale Chromatography in terms of its difference, yet both the techniques find applications in almost all the process industries.

The following table lists some of the major differences between Analytical Scale and Preparative Scale Chromatography:

<b>Analytical Scale Chromatography</b>	<b>Preparative Scale Chromatography</b>
Smaller Scale Chromatographic Technique	Larger Scale Technique, generally at Manufacturing Level
Column Size is about 4.6 mm in diameter	Column size is about 50-200 mm in diameter
Main Aim is to analyze the contents of a substance.	Main aim is to prepare a new substance from a pre-existing substance.
Substance is separated into its components is fed and the extracted components are then discarded.	Substance to be separated is fed to the chromatography apparatus, its components are obtained, and those components are used as starting material for a new substance.

Further, it was seen how solvent wastage is reduced using modern techniques like “Green Analytical Chromatography”, which makes use of high pressures, lying within operating pressure. Wastage reduction occurs due to a decrease in the dead time which in turn leads to a decrease in the dead volume (which is same as the volume used in the “Chromatographic Process”).

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# **What are some of the major differences between analytical scale and preparative scale chromatography?**

**Munit Goyal | 2020CH10102**

## **Abstract**

Chromatography has been important technique used for the separation of various components from the mixture. Chromatography is basically based on physical and chemical properties. On the basis of scale of operation, purpose, equipment used they can be divided into analytical scale and preparative scale chromatography.

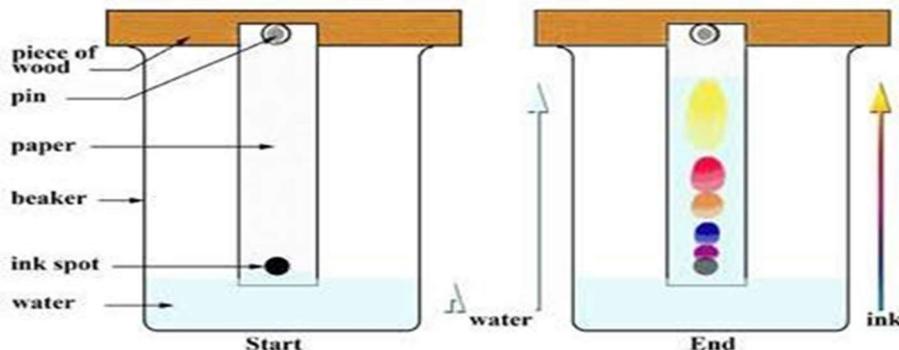
This report would talk about some of differences in great detail between analytical scale and preparative scale chromatography on the base of purpose, there chemistries, instrument differences, techniques and downstream products.

## **Introduction**

Chromatography is a method which is mainly used for the separation of various components from a mixture. This process involves two types of phases, mobile phase and stationary phase. Mixtures containing various components dissolve in mobile phase and then stationary phase is kept in that mobile phase and due to which various components travel at different, resulting in separation of the components.

The basic principle behind the chromatography is the separation due to interaction between the components of mixture with the stationary phase particles. Due to separation, these interactions are different for different components with mobile phase and these interactions mainly depends on the properties of mobile phase and stationary phase particles packed inside the column.

There are mainly two types of chromatography techniques which are divided on the basis of shape and size of stationary phase particles, column diameter, techniques used and product obtained after passing through various detectors, analytical scale chromatography and preparative scale chromatography. The differences between the two scales can have considerable impacts on separation and purification process therefore it is important to understand these differences to improve chromatographic separations.



## Objective

Each scale chromatography has its own goal. Analytical chromatography is used to separate the various components of the sample. We mainly focus on analysing a substance in detail and gathering information about it. The substances in the sample can be identified and analysed qualitatively and quantitatively with the help of analytical LC. It is mainly done for various laboratory experiments or for testing which and what types components are found in the mixture.

The purpose of preparative chromatography is isolation and purification of reasonably sufficient quantities of a specific substance of our interest from the sample. Preparative chromatography wants the recovery of a substance in as pure as. Preparative LC helps to purify and collect molecules of our interest from all other components in our sample for future use. It is mainly done for industrial uses. Sometimes a particular compound is required for specific industry then chromatography can be used to extract a compound from mixture.

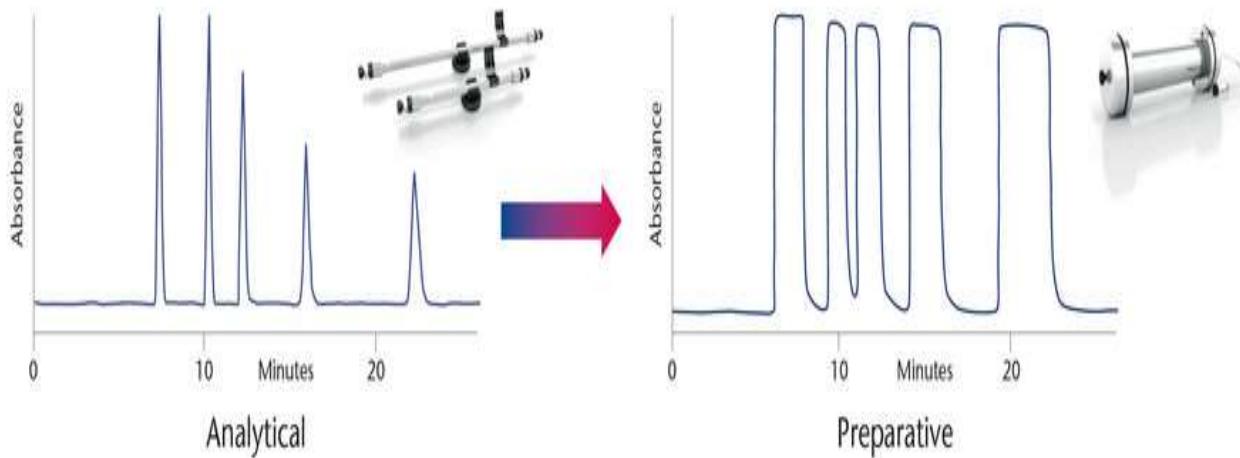
## Chemistry of Columns

The columns in both the scales plays main role in separation and purification of components in the mixture. Diameter of the column and stationary phase particles affect the flow rate by controlling the flow rate and hence affecting the efficiency of the process.

Preparative LC's primary objective is to collect and separate pure substances of interest with a high yield; as a result, bigger column diameters—typically in the range of 50 to 200 mm—are primarily needed to accomplish the requisite capacity. Increasing the column's diameter or length will automatically increase its capacity. Usually larger than or equal to 10 microns in size, stationary phase particles arranged in columns. It has been noted that preparative LC chromatograms frequently have peaks that are larger and less defined than analytical LC.

Analytical LC columns have a smaller diameter between 2.1 and 4.6 mm and are filled with particles in the range of 3-5 m since we primarily want to separate different components from the mixture for identification and analysis. Sharper peaks can be visible in the chromatogram as a result of the lower flow rate caused by the smaller inner column diameter and smaller

particles. Backpressure is greatly increased by the combination of particles with relatively high flow rates and smaller column diameters.



Due to the fact that the same chemistry and applications can be utilised in both analytical and preparative LC, such as ion-exchange chromatography, it is difficult to distinguish between them based solely on the chemistry of these columns. With the aid of method development and optimisation, all chemistries can be conducted on both scales.

### Injection and Backpressure

We take different volume of samples in analytical scale and preparative scale chromatography and also different types of injection mode are used for both. Here important concept is of backpressure. Backpressure is defined as the pressure which is generated by resistance of the column and is required to create the flow. Backpressure is caused by various factors such as particle size, column dimensions and flow rate.

In analytical scale, the quantity of sample injected is usually small and is usually in the range of **1 to 20  $\mu$ l** per injection. Here, sample volume and sample number are usually kept low. The mode of injection is autosampler in analytical LC. In analytical scale chromatography, we have smaller column diameter and less flow rate due to which backpressure is not a major issue. Therefore, backpressure lies in the range of **100-1500 bar**.

For preparative scale, the quantity is injected by manual via a sample with the help of simple peristaltic pump and amount injected is also very high (**usually >20  $\mu$ l**). Sample volume and sample number is also very high. Due to large columns and high flow rates, backpressure can be a significant issue and is usually **less than or equal to 10 bar**.

### Chromatographic Techniques

Mixtures which are subjected to separation can be aqueous (that is soluble in water) and non-aqueous (that is soluble in oil, paint, or similar solvents. Some samples, such as soils, tissue

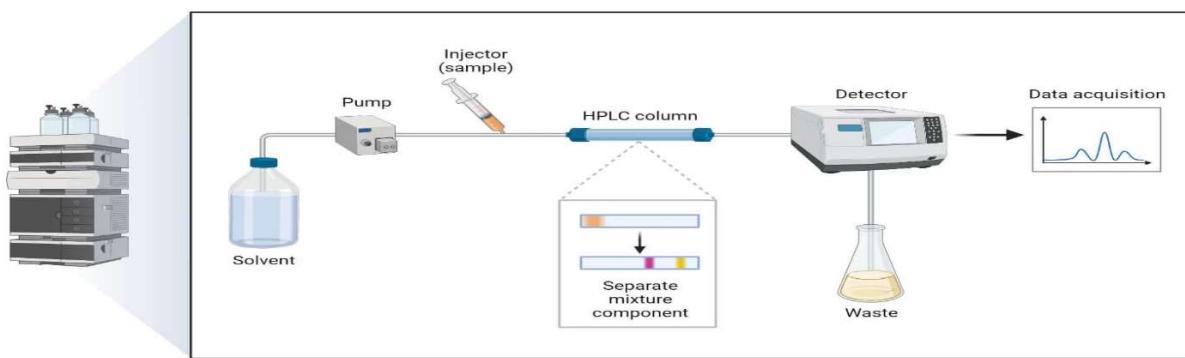
extracts, foods, and polymers, may consist of solid or semisolid materials that are not soluble in water or other solvents. Still more may be vapours or air samples. Due to this, each scale usually has different factors which would decide which type of chromatography method should be used for separation. For aqueous there may be different techniques and for non-aqueous there may be different techniques

The elements taken into account by the analytical scale are mainly Sensitivity, complexity of the sample, sample throughput, degree of accuracy or precision, and perhaps ease of use are all factors in analytical scale chromatography. The various techniques can be used like: HPLC (High performance liquid chromatography), paper chromatography, TLC, column chromatography, ion-exchange chromatography, GC, LC.

While in preparative scale, we often take into account the cost, the level of purity necessary for the isolated fraction, and the quantity of material to be isolated, as well as whether the item to be isolated is a major or minor component of the sample. Due to which HPLC, LC and GC techniques are mainly involved in preparative chromatography.

For large-scale preparations, a number of factors including sample injection, system loading capacity, detecting systems, and collecting systems would need to be taken into account. The industrial sector dedicated to chromatography has been made possible by the improvement of these parameters.

### **High Performance Liquid Chromatography (HPLC)**



### **Downstream Product**

Both the chromatography scales enable the exact separation, analysis, or purification of substances from extremely complex mixtures in which various constituent parts may occasionally be collected separately or subjected to additional "downstream" processing. In chromatography, the actions taken after the target molecule are removed from a complicated mixture are referred to as downstream processing. This qualitative and quantitative analysis of components of mixtures is done by various detectors. There are basically two types of detectors, destructive detectors and non-destructive detectors.

In analytical scale chromatography, we generally require deep information of sample due to which we require non-destructive detectors in addition to destructive detectors such as mass

spectrometry and charged aerosol detectors are also used. Here downstream product is not collected and passed as a waste because our goal is to separate various components.

Since we only need pure compounds for preparative scale chromatography, non-destructive detectors like UV, fluorescence, or refractive index detectors are frequently utilized in preparative scale applications. When a component of our interest is needed, the product is collected in a fraction collector and employed for biotherapeutic, pharmacological, or future research reasons.

## Conclusion

Although there are some minor distinctions between analytical and preparative scale chromatography, they are generally similar, complementary, and frequently utilised in close proximity to one another. In addition, a lot of preparative applications usually begin at the analytical level and scale up to the preparative scale. To evaluate the purity and purification of particular chemicals attained by preparative LC, analytical LC is routinely utilised. Additionally, to achieve the large-scale operation, analytical scale chromatography can be scaled to preparative scale.

Preparative scale chromatography is done at larger scale and is mainly used to purify component of our interest from mixture whereas analytical is small scale chromatographic method used for qualitative and quantitative analysis of various components of our mixture. The various applications of analytical and preparative scale chromatography can be found in synthetic industry, fine chemical industries(purification), cosmetics, sports, pharmaceuticals (purification of certain substances in chemical compounds) and many more.

Overall, the decision between analytical and preparative scale chromatography depends on the scale of the experiment or procedure as well as the intended use of the separated substances. Generally speaking, analytical and preparative chromatography have various uses, and which one to utilise depends on the precise analytical or preparative objectives of the experiment.

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# **Report on Large Scale Chromatography Setup**

**CLL271 : Intro to Industrial Biotechnology**

**Course Instructor : Prof. Anurag Singh Rathore**

**Group : 6**

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**Entry No. : 2020CH70204**



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# **Abstract**

This report presents an overview of “Large Scale Chromatography Setup” by covering Differences between FPLC and HPLC, individual parts of a typical FPLC Setup. The focus of the report will be to cover these topics in detail.

## **Why FPLC over HPLC for Large Scale applications?**

Following are some reasons why FPLC is preferred over HPLC for Industrial Scale applications -

- High Flow Rates**

Designed specifically for Higher Flow Rates, FPLC systems are the go to choice for industrial scale chromatography applications.

- Higher sample loading capacity**

The per sample volume is higher for FPLC vs HPLC systems, meaning that more sample can be processed in a single run.

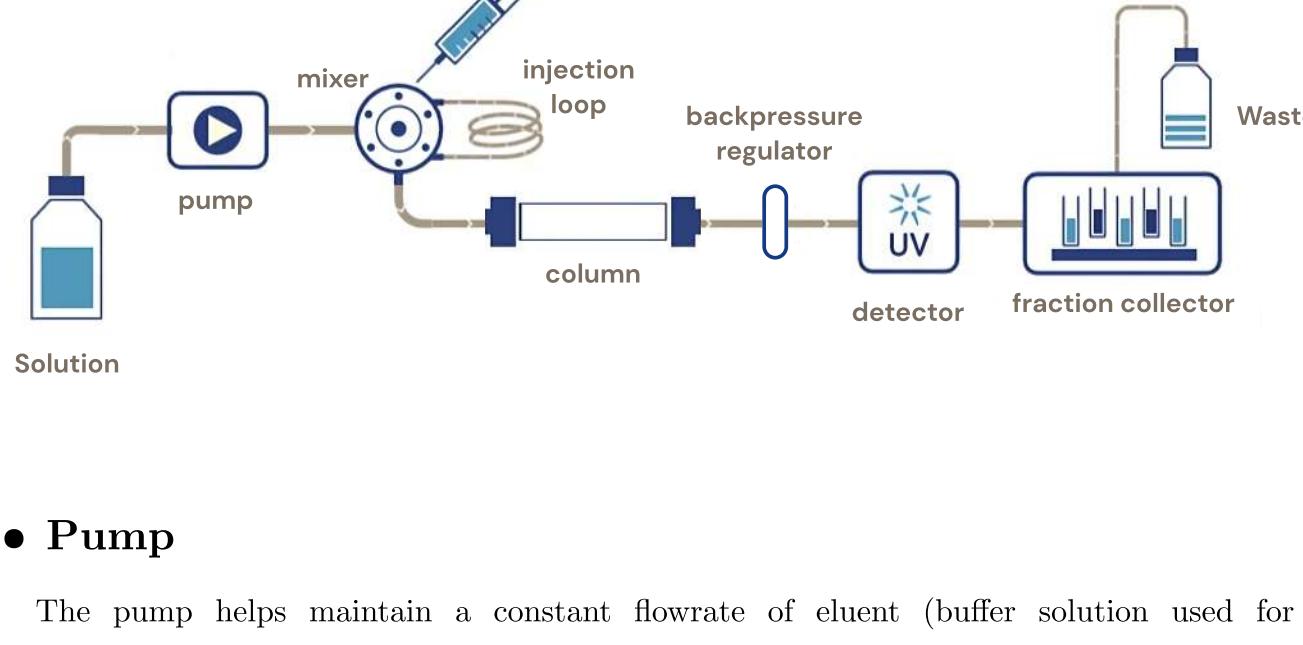
- Lower cost**

Less expensive than HPLC Systems, making them suitable choice for Large Scale Chromatography.

- Better separation of proteins**

FPLC systems are optimized for the purification of proteins, with a wide range of resins and columns available to separate a multitude of protein fractions from a given buffer. For HPLC Systems, this is not the case.

# Typical FPLC Setup



## ● Pump

The pump helps maintain a constant flowrate of eluent (buffer solution used for separation/ purification of biomolecules) in the whole chromatography run. Flow rate is controlled via a rate controller or flow restrictor, which regulates pressure and thus the flow rate of the eluent. Pump is also used to create eluent gradient inside the column by varying the flow rate of eluent solution with time, this is particularly useful for purifying complex mixtures of biomolecules.

Isocratic pumps are used for constant flowrate of eluent and Gradient pumps for creating gradients in flow rate; what type of pump to use depends on type of eluent injected for separation.

## ● Column

The most critical component of an FPLC System, column separates out various analytes in the buffer solution based on their physico-chemical properties eg shape, size, hydrophobicity etc. It consists of Stationary Phase (Packed bed of Resins typically Agarose Gel) in a cylindrical column having glass frit at both end that retains the resin beads inside the column in case the flow rates go very high.

Once sample is injected in the column, the analytes bind with varying affinities to the resin bed and are thus separated.

The eluent or buffer is selected based on our specific needs of the chromatography application, in addition the flow rate of eluent is adjusted to alter the residence times and optimize the process.

## ● Injection Loop

It is a closed loop of stainless steel(generally) tubing that ensures only a precise volume of sample is introduced into the column. The loop is filled with a manual/ automated syringe and the injection valve is opened for the sample to go into the column and start the separation process. Size of injection loop can be varied depending on the amount of sample being analyzed.

It has two ends one for injecting the sample into the loop and the other one for the injected sample to go into the column for separation.

## ● Injection Valve

Located between the Injection Loop and Column, Injection valve helps in precise and automated injection of samples. It has multiple ports of which one is connected to the Injection Loop while the rest are connected to pump, column, waste line etc.

The rotor of injection rotates to connect the sample loop to pump, loading all of the buffer onto the column. It then rotates again to connect the column to the pump that pushes the buffer through column and starts the separation process. Some valves also have a Wash Port that allow cleaning of the valve between the process to prevent sample carryover and contamination.

## ● Flow Cell

A flow cell is integrated inside the UV-Vis Detector which is used quantify the purity of the product coming out from the column. As the buffer flows through the flow cell, it passes along a transparent window (made of quartz or glass) that allows light to pass through the solution. The relative intensities of light coming in and going out are measured and the detector then generates a signal that is proportional to the concentration of Impurities or product. By analyzing the signal , FPLC then generates a Chromatogram on the Monitor with which we can optimize the separation process further as per our needs.

## ● Monitor / Recorder

With the help of Monitor we are able to real-time monitor the elution profile of the sample being purified and Recorder with a data acquisition system records the output from the UV-Vis Detector. The recorded data is then used to generate a chromatogram and display it to the user via the monitor.

The chromatogram is important as it provides information about the purity, yield and efficiency of the separation.

## ● Fraction Collector

Fraction Collector is an automated fraction collector that helps us separate out the various products obtained after elution over the column on the basis of their elution time/ absorbance at a particular wavelength.

It can be setup to collect the fractions in various formats such as tubes, plates, bottles depending on the specific application and the volume of each fraction collected.

It is located downstream after the UV-Vis detector so as to ensure that only the desired fractions go into the collector.

## ● Backpressure Regulator

Backpressure regulator aids in maintaining constant pressure upstream of the regulator, even when there is a change in flow rate or any other parameter that might affect the pressure inside the column.

It consists of a valve that closes or opens based on the pressure inside column. If it falls below a the lower limit, it closes and allows less fluid to pass through, thereby increasing the pressure. On the other hand, if it goes beyond a certain set point of high pressure, it opens up, allows more fluid to pass through, lowering the pressure.

# Recent Advancements in FPLC

- **Multidimensional Chromatography**

In multidimensional chromatography we can combine chromatography eg Ion-Exchange, Size Exclusion etc to achieve higher resolution of separation. This chromatography can be done using various techniques such as ***2D-LC*** where the columns are connected in series; ***Heart-cutting*** where fractions obtained from the 1st column are selectively transferred to the 2nd column. Some other techniques are ***LCxLC***, ***Capillary electrophoresis coupled with FPLC***.

- **Improved Detection methods**

Advances in detection methods, such as ***UV-visible spectroscopy*** (commonly used detection method that is based on the absorption of light by a compound at a specific wavelength.), ***fluorescence*** (highly sensitive detection method based on the emission of light by a molecule when it is excited by light of a specific wavelength.), and ***mass spectrometry*** (a highly sensitive and selective detection method that can identify and quantify compounds based on their mass-to-charge ratio.), have enabled more accurate and sensitive detection of proteins during FPLC eg ***BioLogic DuoFlow system from Bio-Rad.***

- **High-throughput FPLC**

Recent advances in automation and robotics have enabled the development of high-throughput FPLC systems having automated sample loading, column equilibration, and elution, as well as real-time monitoring that can process large numbers of samples in a short time eg ***NGC Quest 10 Plus, AKTA pure 25, Bio-Rad NGC Chromatography System etc.***

- **Advanced Resins**

New types of chromatography resins eg Mixed-mode resins: These resins have both hydrophobic and ion exchange properties, allowing for separation based on both charge and hydrophobicity ; Monolithic resins: These resins have a continuous porous structure, allowing for faster mass transfer and higher resolution have been developed that offer improved selectivity and binding capacity for a wide range of proteins e.g. ***POROS™ XQ resin from Thermo Fisher Scientific, Phenyl HyperCel™ from Pall Corporation..***

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## Various Column Component that contribute to flow uniformity

~By Mitanshu Kansal (2020CH70180)

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### Abstract:

The focus of this report is on the various components of a column that is used in the Chromotography that contributes towards flow uniformity. It discusses about the reasons why flow uniformity is required and in what way different component can affect it. Further, the report also looks into modified components different from the conventional ones that improves flow uniformity.

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### Introduction:

Flow uniformity refers to the Homogeneous distribution of flow within the chromatography column. Effectiveness of chromatography depends on differential migration and also on whether individual solute elusion bands don't overlap and remain close-packed which is known as zone spreading.

Differential migration refers to the fact that different solute have different equilibrium affinities with stationary phase. The more different the affinity is, the more there will be difference in the migration rates of different solute resulting in a better separation.

If the migration rates among solutes vary largely, the chances of solute peaks overlapping reduces, rendering Zone Spreading less effective. In case of particles with similar structure, the migration rates would not vary much making Zone Spreading of much more importance.

Ideally, Each solute should come out of column at different instant which is possible if the solute peaks don't overlap with each other and the flow is uniform so that the different peaks don't get mixed due to flow.

Flow uniformity refers to the Homogeneous distribution of flow within the chromatography column. It can be maintained with the help of various components and conditions of a chromatography column like packing quality, particle size, flow distributor

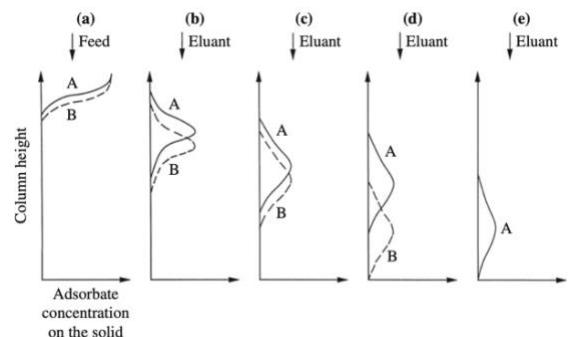


Fig.1: Differential Migration of two solutes A and B

## Methods:

Molecules having similar structure will have similar migration rates, making effects of zone spreading significant for chromatography. Zone Spreading Depends on the following factors:

Axial diffusion – It refers to the diffusion of the solute along the length of the column. More the axial diffusion, more will be the spreading/broadening of the solute peak.

Eddy diffusion – The flow paths of liquid varies along the path of the column and as well as radially. This variation in paths leads to different local velocity of the fluid particles which results in spreading of the concentration of the liquid along the column. This local variation of liquid velocity is known as eddy diffusion and the more it is, more will be the zone spreading

Local non equilibrium effects – It intensifies the already broadened solute peak. There will be mass transfer between the stationary phase and mobile phase to be in equilibrium concentrations of the solute. Consider a section in the column, say X as seen in Fig.2 (a). As the solute peak approaches X, the solute concentration in mobile phase increases at X and as the concentration increases till the peak has reached X and then decreases, the equilibrium can't be achieved owing to the slow mass transfer. When there is little concentration of solute left in the mobile phase, the stationary phase still has solute in it which is released slowly in the mobile phase until equilibrium is reached. This broadens the solute peak increasing zone spreading.

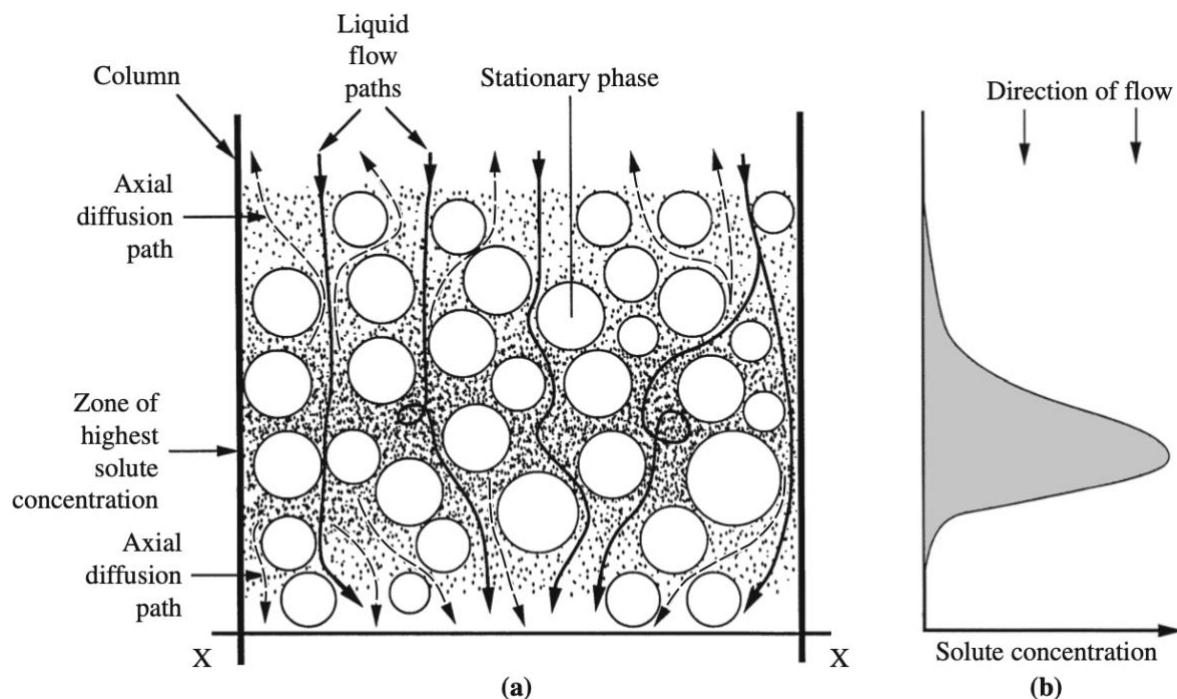


Fig.2: Zone Spreading in Chromatography Column

Generally conditions improving mass transfer also helps in minimizing zone spreading as the equilibrium can be achieved at a faster rate. Mass Transfer can be increased by increasing surface area per unit volume of particles (smaller particle size). It also varies with temperature.

### Column Packing:

In the elastic region packing inhomogeneity is caused mainly by wall effects. These wall effects are more effectively in columns with smaller diameters i.e. high aspect ratio ( $L/D$ ). This can be also seen from the total stress (Axial normal stress + Shear stress) distribution of columns with different aspect ratio.

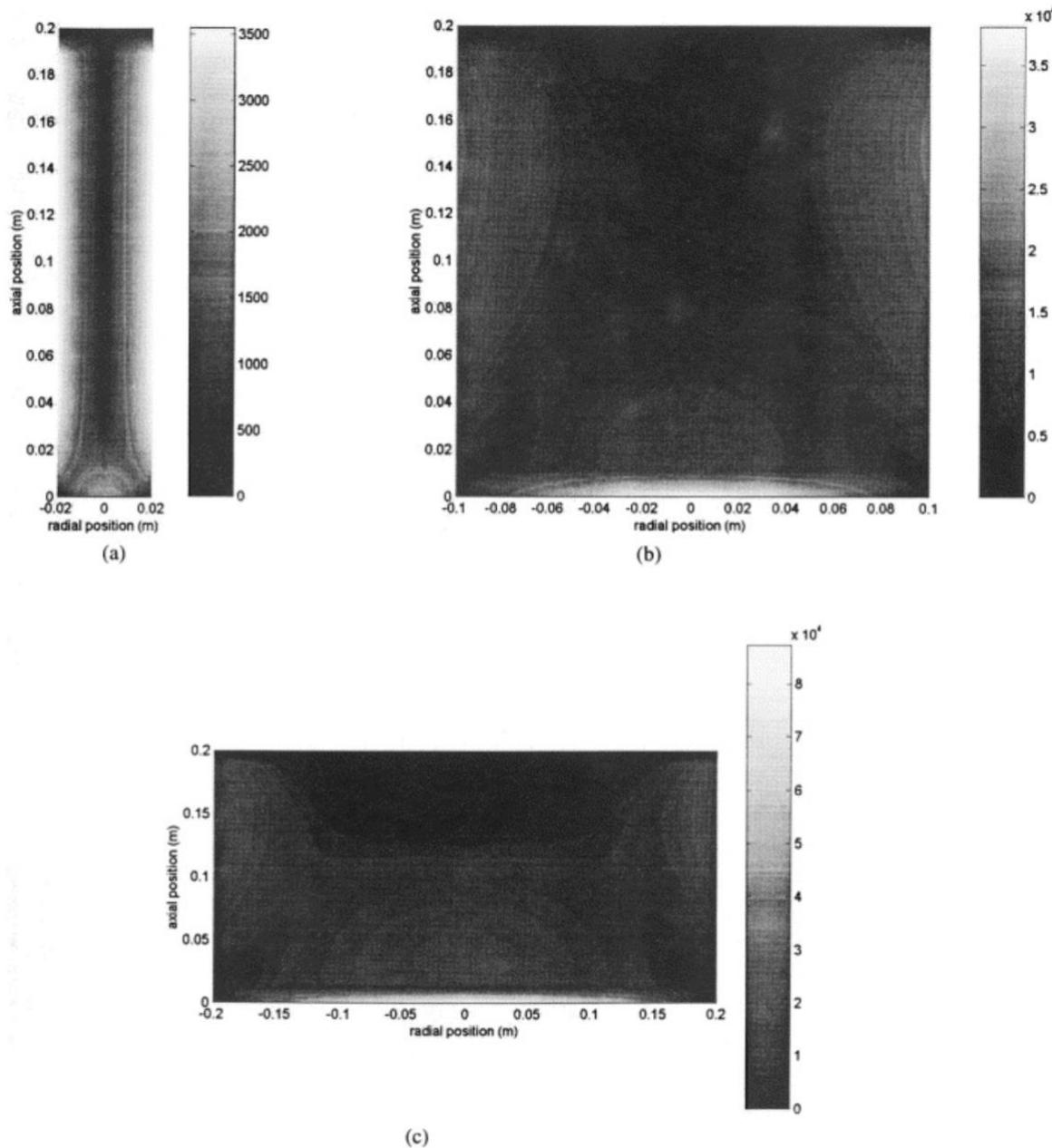


Fig.3: Total Stress for three columns with aspect ratio (a)  $L/D=5$  (b)  $L/D=1$  (c)  $L/D=0.5$

As can be seen from the Fig.3, the variation in stress is more in the thinner column than in the other two columns (white portion representing higher stress is more profound in Fig.3 (a) than (b) and (c)). The stress is different radially with it being much more near the walls than at the center. This variation is not much in case of columns in Fig.3 (b) and (c). This implies that the packing is more near the walls than at the center. This difference in packing affects the flow uniformity as can be seen from the corresponding graph of radial position vs axial displacement:

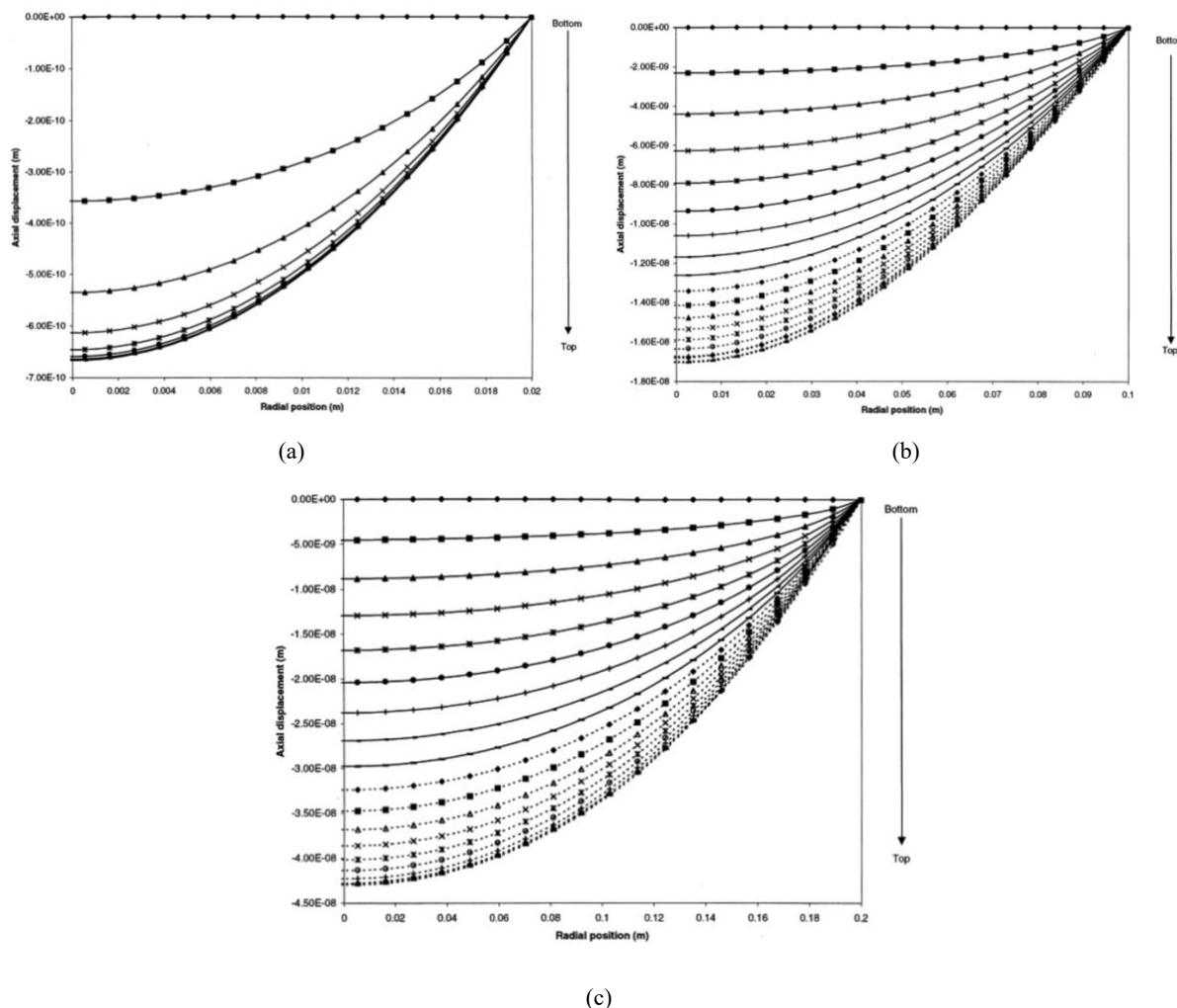


Fig.4: Plots of Axial Displacement vs Radial Position for (a) L/D=5 (b) L/D=1 (c) L/D=0.5

In the above plots, it can be clearly seen that the variation of displacement of liquid is much more in the case of narrow column which has a higher inhomogeneity than the other two column.

#### **Flow Distributor:**

A header is used to distribute the fluid evenly along the radius at the entry point (feed point). Ideally, header must ensure that the velocity profile is flat at exit and the residence time is identical along the radius of the column. A conventional header has a flat porous disc or a non-porous disc with distributing channels. Such a design results in a sudden expansion at the feed point.



Fig.5: Conventional Header

Plots of velocity profile at the exit and the residence time varying with radial position is presented below:

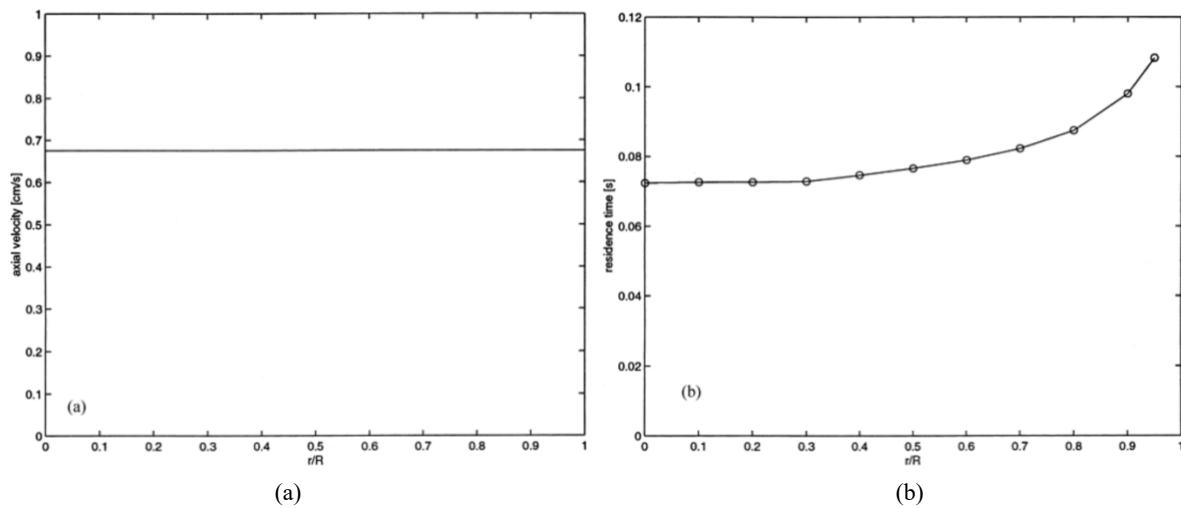


Fig.6: Plots for Conventional Header (a) Axial velocity vs  $r/R$  (b) Residence time vs  $r/R$

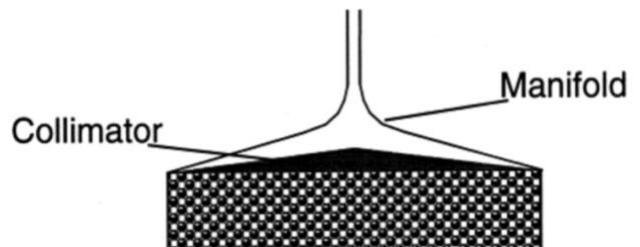
The velocity profile is flat but the residence time vary largely near the wall contrary to nearly constant near the center. Also the values of Residence time at center and near the wall differ greatly.

## Future Scope:

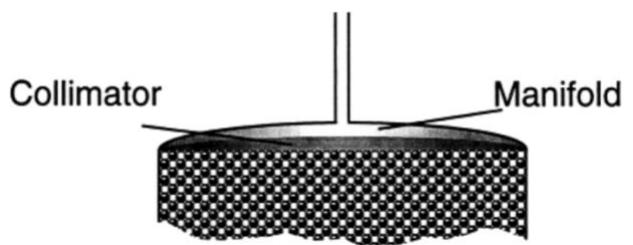
### New Header Designs –

Two new designs were modelled and fluid velocity and residence time were simulated. Both designs are non-flat header with differently shaped manifold.

They have two sections – Manifold and Collimator. The Manifold Section has mainly the radial flow as it distributes the fluid coming at the feed point radially. The Collimator section has primarily axial flow and thicker at the center gets thinner approaching towards the walls. There is more resistance to flow in the collimator section than the manifold section. This helps in ensuring that the fluid entering the header at any instant enters the column at the same time.



(a) Hyperbolic-Shaped Manifold



(b) Parabolic-Shaped Manifold

Fig.7: Schematics of new header designs with differently shaped Manifold

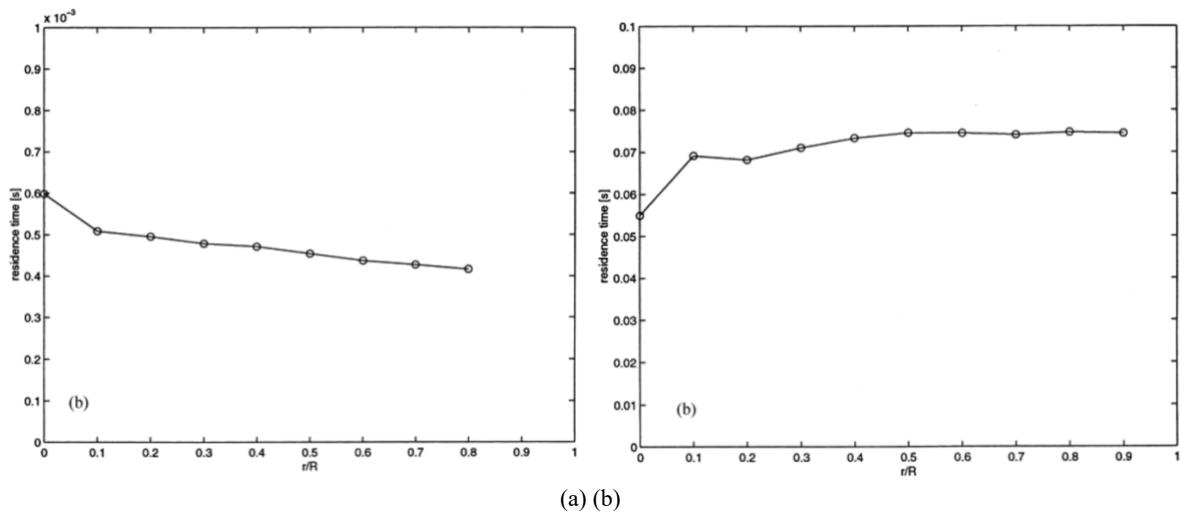


Fig.8: Plots of residence time vs  $r/R$  for header with (a) Hyperbolic-Shaped Manifold (b) Parabolic-Shaped Manifold

Above are the plots of residence time for the two headers. The residence time is nearly identical near the walls and also doesn't differ much from the value at center unlike the case with conventional header. Among these two headers, the header with parabolic shaped manifold has better similarity in the residence time than its other counterpart.

### Cuboid Shaped column –

Modelled velocity profiles in a cuboid shaped packed bed instead of the conventional cylindrical column. Below shown is the velocity profiles and residence time for cylindrical and cuboid shaped column.

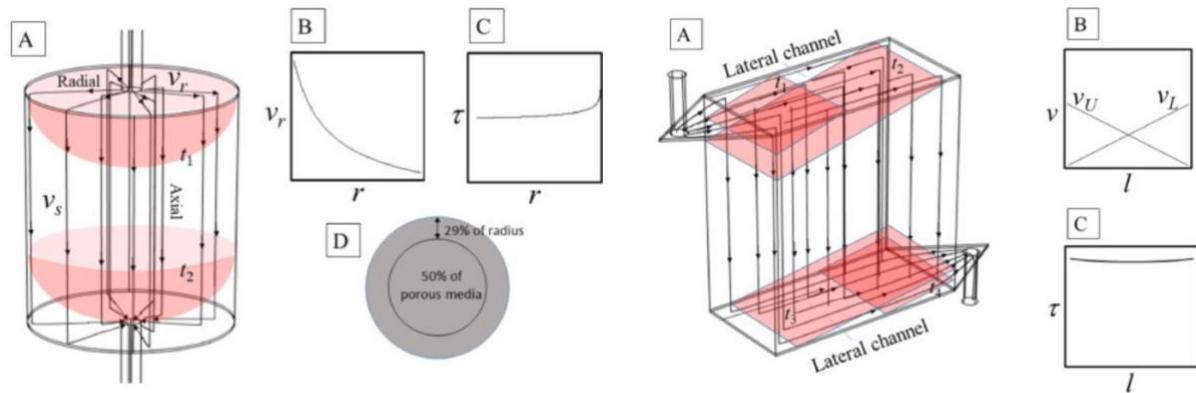


Fig.9: (A) Flow Pattern (B) Flow Velocity Distribution in column header (C) Residence time distribution (D) Sectional view of column

Fig.10: (A) Flow Pattern (B) Lateral velocity as function of lateral location in upper and lower channels (C) Residence time distribution

As can be seen from the Fig.9 and Fig.10, velocity profile and residence time vary largely in case of cylindrical column while in case of cuboid column the variation is much less.

Following is the data obtained for Number of theoretical plates per meter ( $\dot{N}$ ) and the reduced plate height for 200ml cylindrical column and cuboid  $z^2$  by changing flow rates:

	Flow rate (mL/min)	$\dot{N} (\text{m}^{-1})^*$	$H$
<b>Column</b>	10	7927	1.40
	20	6514	1.71
<b>Cuboid z<sup>2</sup></b>	10	8736	1.27
	20	6828	1.63

It can be seen that the cuboid  $z^2$  has better separation efficiency in terms of the above data than compared to the cylindrical column. The cuboid column is better than cylindrical column in terms of the efficiency metrics. It provides uniform flow and narrow range of Residence time making it ideal for carrying out chromatography that require high resolution.

Cuboid column is scalable and is not affected by the issues faced by cylindrical column like macro-scale convective dispersion. Following is a possible scale-up of cuboid column.

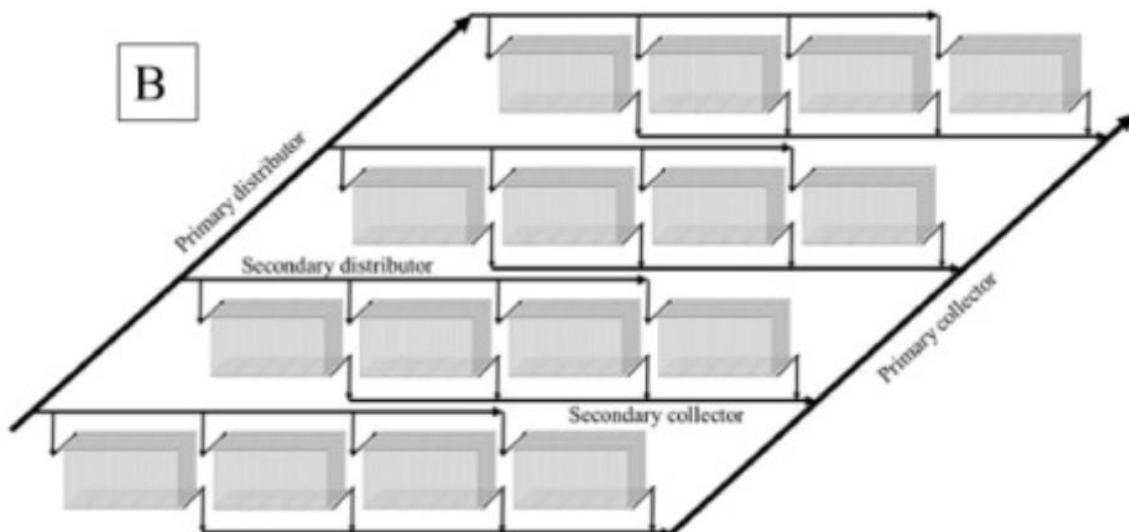


Fig.11: Possible scale-up of cuboid column

## Conclusion:

It can be concluded that flow uniformity is important for better efficiency of chromatography. Wall effects play a major role in causing packing inhomogeneity which affects the flow uniformity. Further, changing header designs can improve uniformity. We even looked at a new design of cuboid column which seems to outperform its cylindrical counterpart in nearly all aspects. It maintains uniform flow in the column which accompanied by other possible factors, gives a high resolution separation. It can be used to separate large macromolecules that have low diffusivity i.e. Zone spreading is significantly affecting the separation.

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# CASE STUDY ON COMMON CAUSES OF FLOW NON-UNIFORMITIES IN COMMERCIAL CHROMATOGRAPHY COLUMNS

*~By Siddhant Rajoriya (2020CH10130)*

## **Abstract:**

Chromatography is a type of separation process utilized in a variety of sectors, including the chemical, pharmaceutical, and biotechnological ones. The process of passing the material to be separated through a column that has been packed with a stationary phase is known as column chromatography. The solid support can be covered with a liquid coating or a solid adsorbent as the stationary phase, and the sample is separated according to how differently its constituent parts interact with the stationary phase.

The term "non-uniform flow" describes a situation in which the mobile phase (liquid or gas) does not flow along the column in a straight line during chromatography. The flow rate may differ in various regions of the column, causing an uneven distribution of the sample and lowering separation effectiveness.

The efficiency and yield of the desired molecule can be negatively impacted by non-uniform flow in column chromatography in a number of ways, including incomplete or uneven separation, localized high pressure zones, sample degradation, peak broadening and tailing, and erroneous quantification.

This study emphasizes the issues with non-uniform column chromatography and how it affects the results of separation. The article also outlines the causes and effects of non-uniform flow and offers a case study to highlight the difficulties brought on by this issue.

## **INTRODUCTION:**

Using column chromatography, it is possible to separate intricate mixtures into their constituent parts. However, getting a high separation efficiency and yield necessitates careful optimisation of a number of variables, including flow rate, sample loading, and the choice of stationary phase. In column chromatography, non-uniform flow is a frequent problem that can affect the yield and separation effectiveness. We will go over the typical reasons for non-uniform flow in column chromatography in this post, along with their fixes.

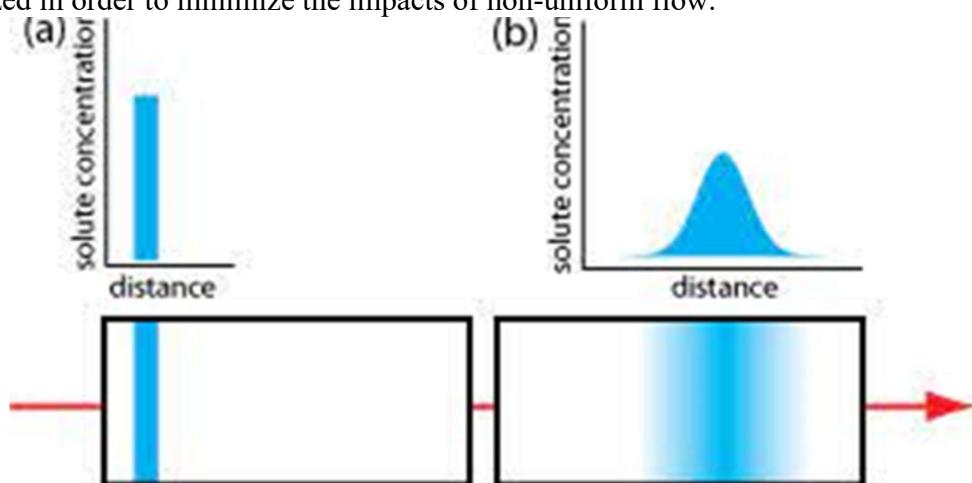
Some of the major problems caused by non uniform flow are band broadening , column overloading and peak distortion.

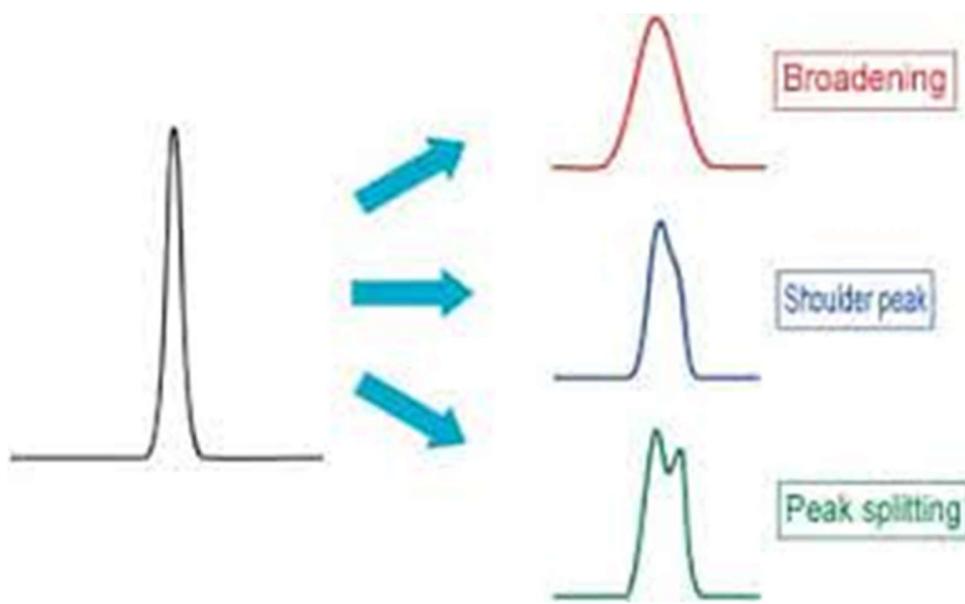
Non-uniform flow might result in an unequal distribution of the analyte molecules within the column in the event of band broadening, changing the analyte molecules' residence times on the stationary phase. This makes the analyte bands wider and less distinct, which lowers the separation's resolution and sensitivity. Dead volumes, gaps, or channeling in the column can increase this effect by causing the analyte molecules to skip the stationary phase and spend more time in the mobile phase, which can result in further band broadening.

Column overloading, which occurs when the volume of sample delivered into the column exceeds its ability to hold the analytes, is another effect of non-uniform flow. In this instance, extra sample molecules may obstruct the separation of the target analytes by occupying the gaps between the stationary phase particles. This may lead to uneven separation and co-elution of several analytes, which could lead to erroneous quantification and identification of the sample's constituent parts.

Peak distortion can also happen in chromatography as a result of uneven flow. For instance, various regions of the analyte band may have variable retention durations when there are variations in the mobile phase flow rate or when the column packing is uneven, resulting in peak distortion and an asymmetrical appearance. Reduced resolution and sensitivity as well as erroneous peak integration and quantification might result from this.

Column packing, mobile phase flow rate, and sample loading should all be carefully monitored and optimized in order to minimize the impacts of non-uniform flow.





### **Factors Causing Non Uniform Flow:**

The major factors that cause non uniform flow are:

- 1) Packing Non Uniformities
- 2) Inlet and Outlet effects
- 3) Column Wall effects
- 4) Column Diameter effects
- 5) Column length effects
- 6) Operational conditions

Now we will study each one of the factors in detail

### **Packing Non-Uniformities:**

Packing irregularities in chromatography columns can have a major impact on the mobile phase's flow dynamics, resulting in irregular flow and causing band broadening, peak distortion, and other problems.

It is possible for channels or dead volumes to form within a chromatography column when the stationary phase particles are not packed equally. The mobile phase may flow unevenly through the column as a result of these low-resistance regions, with some sections experiencing higher flow rates than others. Because of this, the molecules of the analyte may spread unevenly across the column, resulting in irregular flow.

High flow rates may not give analyte molecules adequate time to interact with stationary phase particles, decreasing retention and separation effectiveness. In areas with slower flow rates, on the other hand, analyte molecules could interact with stationary phase particles for an excessive amount of time, increasing band widening and decreasing resolution.

Additionally, packing irregularities can lead to the development of preferential flow routes, in which the mobile phase preferentially passes through particular regions of the column. This may result in peak distortion and decreased resolution as the analyte bands go through the column at various rates.

It is crucial to appropriately pack the chromatographic column with uniform packing materials in order to minimize the effect of packing non-uniformities on non-uniform flow. The column's stationary phase particles should be dispersed evenly throughout it, and any channels or dead volumes that can lead to unequal flow should be avoided through proper packing. Regular column upkeep and monitoring can also aid in spotting and fixing any packing irregularities that can arise over time.

### **Inlet and Outlet effects:**

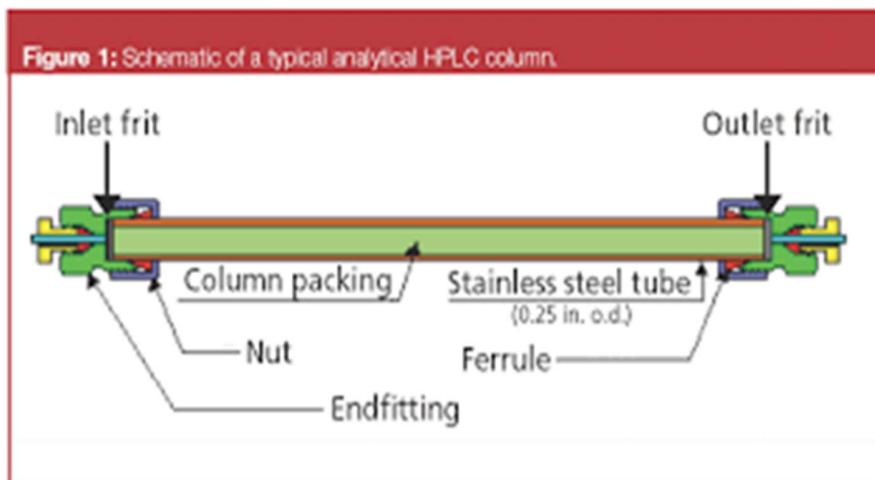
Non-uniform flow in chromatography columns can also be caused by inlet and outlet effects, which can lead to band broadening, peak distortion, and other problems.

The impact of the sample introduction onto the chromatography column is referred to as the "inlet effects." A localized high concentration of analyte molecules can be produced at the injection point of a sample when it is injected onto the column. As a result, the analyte molecules and stationary phase particles may interact more often, increasing retention duration and band broadening. Furthermore, if the sample is delivered too rapidly or too violently, voids or channels may form in the stationary phase, which may result in an uneven flow.

The influence of the mobile phase leaving the column is referred to as the outlet effects. The mobile phase might induce a pressure decrease as it leaves the column, which can lead to an uneven flow rate across the column. This can cause the analyte molecules to be distributed unevenly along the column, which can cause band broadening, peak distortion, and other problems.

Carefully regulating the sample injection and mobile phase flow rate is necessary to reduce the impact of inlet and exit effects on non-uniform flow. While carefully regulating the mobile phase flow rate can help to guarantee a consistent flow rate over the whole column, slow and controlled sample injection can aid to lessen the influence of the high concentration of analyte molecules at

the injection point. Additionally, maintaining proper column packing and consistently checking the column can aid in identifying and resolving any potential problems with input or outlet effects.



### Column length, diameter, and wall effects:

Non-uniform flow can also be impacted by the chromatography columns' length, diameter, and wall effects.

Column length is the chromatography column's length. As more interactions between the analyte molecules and the stationary phase particles are possible with longer columns, they often offer better resolution and separation. To maintain a consistent retention period, longer columns must also have higher flow rates, which raises the possibility of non-uniform flow and band broadening.

The term "column diameter" describes the chromatography column's internal diameter. Because they shorten the distance between the analyte molecules and the stationary phase particles during diffusion, smaller diameter columns typically offer better resolution and separation. To maintain a steady flow rate, smaller diameter columns must also exert more pressure, which raises the possibility of non-uniform flow and peak distortion.

The interaction between the mobile phase and the chromatography column wall is referred to as "wall effects." Band broadening and flow rate reduction can result from the mobile phase's interaction with the wall, which can also produce a layer of stationary phase particles close to the wall. It is crucial to make sure that the stationary phase particles are evenly dispersed throughout the column and that the mobile phase flows uniformly throughout the column to reduce the impact of wall effects.

Overall, non-uniform flow can be impacted by the length, diameter, and wall effects of chromatography columns, hence it is crucial to carefully evaluate these parameters when choosing and utilizing a chromatography column. These impacts can be reduced with proper packing, monitoring, and maintenance, which will also guarantee that the chromatography column yields accurate and trustworthy findings.

## **Operational Conditions**

Non-uniform flow in chromatography columns can also be impacted by operational factors including temperature, pressure, and flow rate.

Temperature has an effect on the mobile phase's viscosity and the analyte molecules' diffusivity, which in turn has an effect on the flow rate and band broadening. High temperatures can lead to quicker flow rates and less band widening by decreasing the viscosity of the mobile phase and increasing the diffusivity of the analyte molecules. High temperatures can also cause the stationary phase particles to degrade, which can shorten the column lifetime and cause peak distortion.

Another crucial operational factor that may affect irregular flow is pressure. Increased flow rates brought on by high pressure can raise the dangers of non-uniform flow and band broadening. In addition, high pressure may compress the stationary phase particles, reducing the available surface area for analyte interactions and lowering resolution.

Another important operational factor that affects irregular flow is flow rate. Because they can produce areas of high flow velocity and areas of low flow velocity inside the column, high flow rates can raise the danger of non-uniform flow and band broadening. Furthermore, high flow rates may compress the stationary phase particles, reducing the accessible surface area for analyte interactions and lowering resolution.

The temperature, pressure, and flow rate of the chromatography column must be carefully controlled and monitored in order to reduce the effect of operational circumstances on non-uniform flow. Maintaining proper column packing and routinely checking the column can also aid in identifying and resolving any problems that might develop as a result of operational circumstances.

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# **What are the major problems that are faced when packing large-scale columns?**

***By: Kumari Astha (2019CH10100)***

## **ABSTRACT:**

The use of large-scale batch and continuous chromatographic operations in the production of many substances like chemicals, biochemicals, petrochemicals, foods, clean water, and medicines has steadily increased over the past few decades, resulting in the topic of major problems in packing large-scale column chromatography so crucial. In this part of the report, we will discuss the general problems that occur during packing large-scale chromatography and that further result in inaccuracy in peak. Further, we will also address strategies for overcoming these challenges, such as using appropriate equipment, improved packing techniques, and better quality control measures.

## **INTRODUCTION:**

Chromatography is a widely utilized separation and purification technique that separates mixture components based on their differential interactions with stationary and mobile phases. Large-scale chromatography is frequently used in the pharmaceutical, biotechnology, and chemical industries to purify significant amounts of biomolecules, compounds, and other substances. It is critical to achieve efficient separation and high product yields.

Efficient packing of chromatography columns is crucial for the success of large-scale chromatography processes. The packing process involves filling a column with a stationary phase material to ensure uniform packing density and optimal separation performance. However, packing large-scale chromatography columns presents several challenges that can affect the quality and efficiency of the process.

## **GENERAL PROBLEMS:**

Some problems that occur due to the inefficiency in structural during designing the large scale column chromatography are-

- Column packing heterogeneity - The packing of the chromatography column can be non-uniform. Due to this non uniformity, the column can have zones with high and low density which results irregularity in flow rates, mixing, and the separation efficiencies and affects the reproducibility of the column performance.
- Mechanical stability - To prevent compression and deformation of the packed bed during its usage, it must be mechanically stable. For large-scale columns that may be subjected to high pressure and flow rates, this is of significant importance.

- Channeling - When the packing is not done accurately or evenly, channels or voids form in the column. These allow the mobile phase to bypass some fixed regions of the stationary phase which leads to lower separation efficiency and peak resolution.
- Bed compression - Over time, the bed can compress and settle which leads to reduced separation efficiency and functioning of the column. This can be lessened by appropriate designing of the bed, using the right packing methods, and using support materials.
- Particle size distribution - The size distribution of the particles in the stationary phase can affect how well the column functions. In general, a narrow size distribution leads to better resolution and selectivity. But as there are limitations of available manufacturing processes it is a challenge to achieve for large-scale columns
- Cost and availability - In large-scale chromatography columns, we require a large amount of stationary phase material, which can be expensive and difficult to arrange as it is limited which affects the feasibility and cost-effectiveness of certain separation processes.

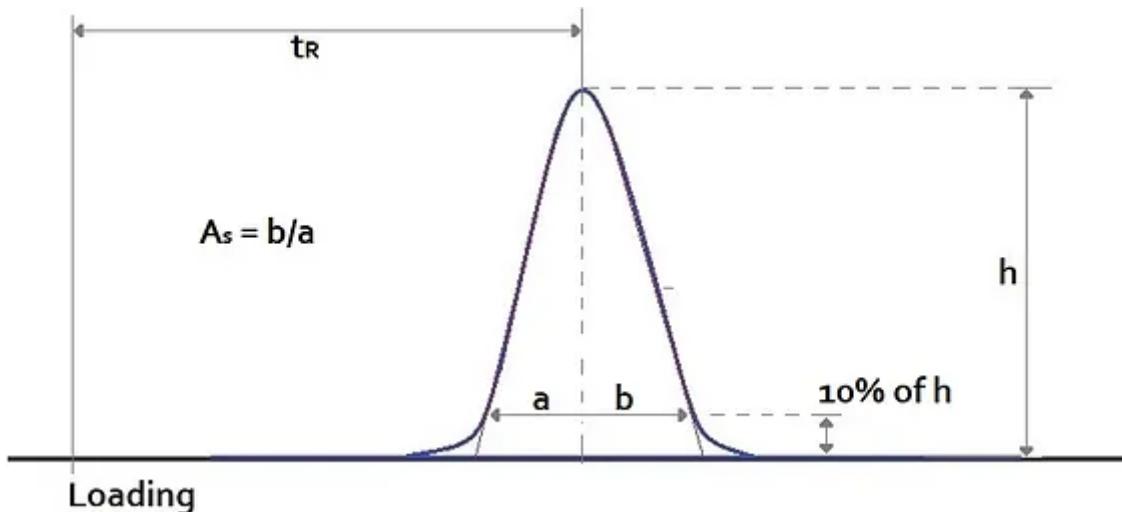
Because of all these problems, packing of these columns is done just a few times a year.

### **PEAK ASYMMETRY:**

When we talk about an ideal system there is no problems in it resulting in the curve is symmetrical around the peak. However it is not possible in real system as there are many problems with the packing of the column or other factors and due to this the peak becomes asymmetrical.

A quantitative peak description shows a peak that is split in two halves vertically, which is starting at the summit and ending at the baseline. The distance from the left side of the peak to the "centreline" is denoted as "a," and the distance from the other half of the peak is denoted as "b."

Tailing factor:  $(a+b)/2a$



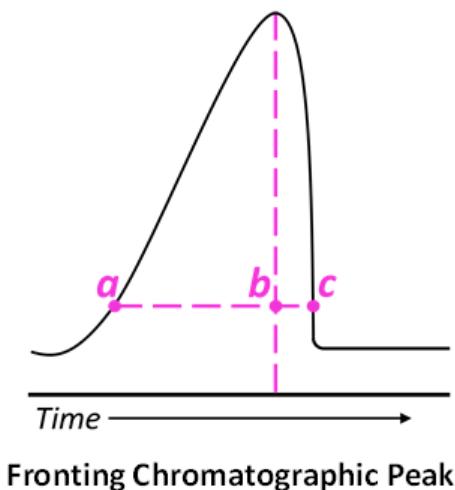
Some of the peak asymmetries are mentioned below:

### 1. Peak Fronting:

Peak fronting is an issue in chromatography that happens when the peak of a sample looks curved or distorted and a higher concentration of the sample shows earlier in the elution profile than predicted. In this case tailing factor is less than 1.

Common causes of peak fronting is column overload, which occurs when too much sample is loaded onto the column. This can result in band broadening and distortion of the elution profile. High sample concentrations can also lead to peak fronting, as this can cause saturation of the stationary phase and masking of the sample peak.

Also peak fronting can be occurred due to the interactions between the sample and the stationary phase. In some cases, the sample may be adsorbed or bound to the stationary phase, leading to broadening and distortion of the peak. This can be mitigated by using a different stationary phase or adjusting the mobile phase composition.



### 2. Peak Tailing:

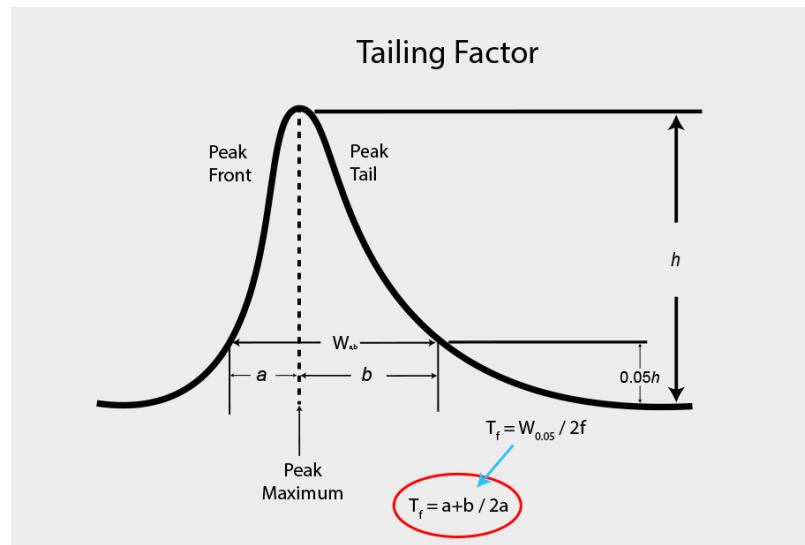
Peak tailing is a common thing seen in chromatography. It happens when a uniform peak changes into a peak with a longer tail on one side. This happens when the sample molecules spend more time than expected reacting with the stationary phase. This makes the recovery time longer and changes the shape of the peak. Here the tailing factor is greater than 1.

Common causes of peak tailing include interactions between the sample and the stationary phase, such as strong adsorption, ionic interactions, or hydrogen bonding.

There are some other factors of peak tailing like column overloading, column deterioration, and inappropriate column packing.

Peak tailing can be reduced by some methods:

- Use a setting with a lower pH.
- Use a column that is significantly inactive.
- Take into account the likelihood of a mass overload.
- Consider the prospect of a deformed column bed.
- When studying basic chemicals, work at a high pH.
- Use a cleaning method that has been demonstrated to be effective.



### 3. Broad Peak:

In chromatography, a broad peak is a chromatographic peak that is wider and less well-defined shape than a narrow, well-defined peak. A broad peak may signify that there are a lot of distinct analyte retention times in the sample or that there are a lot of similar analytes in the sample.

Broad peak can be caused by several factors such as poor column resolution, column overloading, non-specific binding, secondary interactions, or the presence of impurities in the sample. A poorly packed or poorly maintained column can also lead to broad peaks.

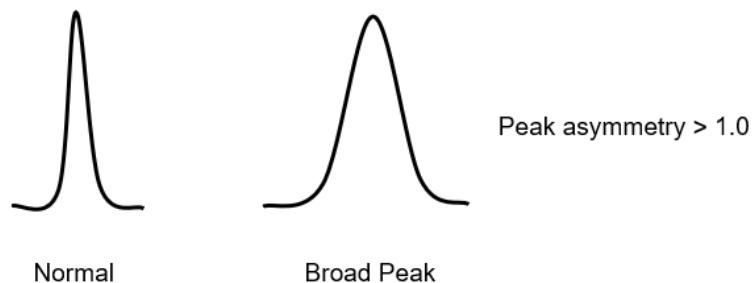
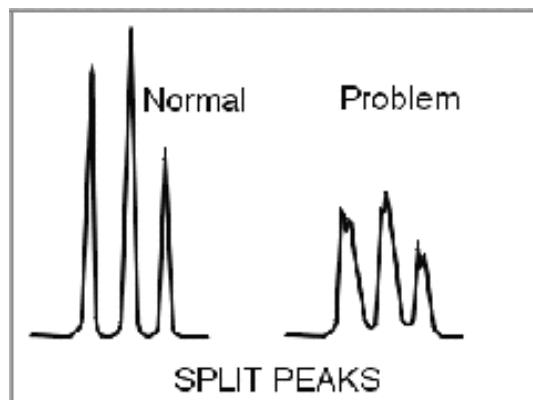


Figure shows difference between Normal and Broad Peak

#### 4. Split Peak:

Split peaks is a term used in chromatography to describe when a single peak on a chromatogram splits into two or more peaks.

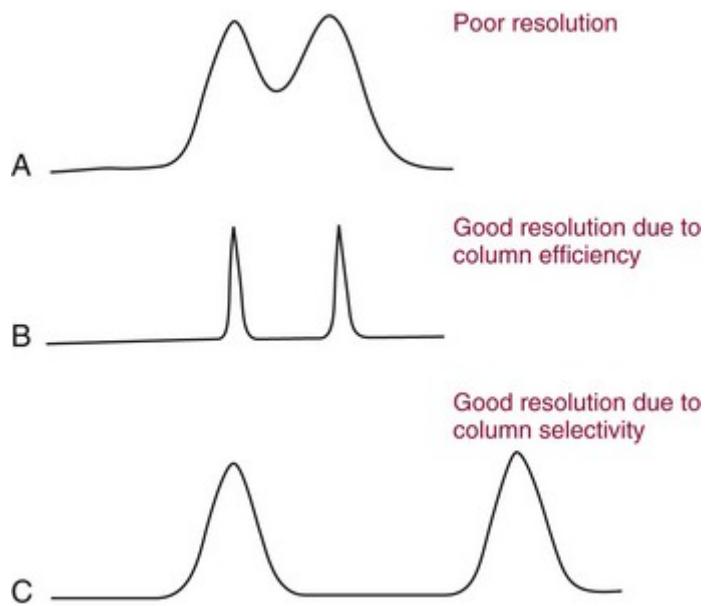
Common reasons for split peaks can be the presence of multiple components in the sample that co-elute on the column. This can result in a single peak that appears as two or more peaks, especially if the components have different physicochemical properties such as polarity, size, or charge. Also split peaks can be related to the injection technique. For example, if the injection volume is too large or the sample solvent is not compatible with the mobile phase, the sample may not be fully dissolved or may overload the column, resulting in split peaks.



#### 5. Poor Resolution:

Poor resolution in chromatography refers to the inability to separate two or more components in a mixture due to insufficient difference in their physicochemical properties or inadequate separation conditions.

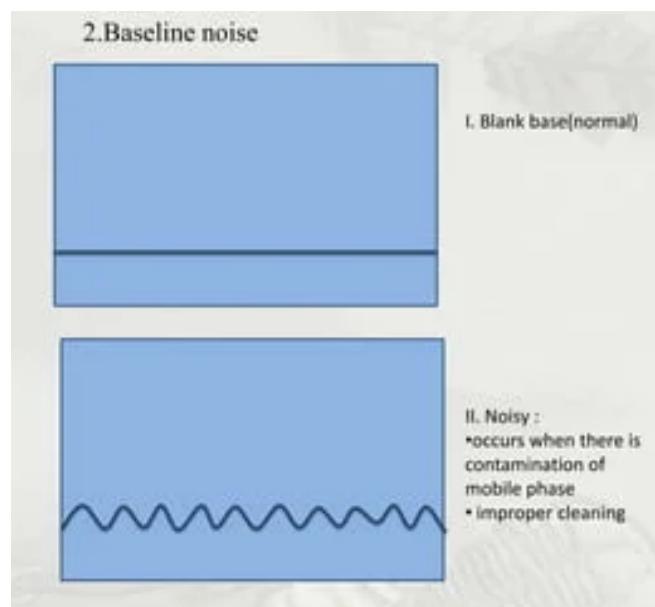
Poor resolution can be caused by various factors, such as improper sample preparation, an unsuitable column or stationary phase, an unsuitable mobile phase makeup or flow rate, or a less-than-optimal detection method. Optimizing one or more of these factors could help improve resolution by making the space between peaks greater and giving peaks a better shape.



## 6. Unstable Baseline:

In chromatography, unstable baseline means to the erratic or fluctuating baseline in the chromatogram. The baseline is the line that separates the peaks in the chromatogram, and it is used as a reference point to quantify the amount of each compound in the sample.

Common causes of unstable baseline can be due to fluctuations in the mobile phase or detector, changes in ambient temperature or pressure, or the presence of impurities in the sample or the mobile phase.



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# CASE STUDY ON PROBLEM OF BROAD PEAKS IN COLUMN CHROMATOGRAPHY

by Aman Rajoria (2020CH70153)

## **Abstract:**

Column chromatography is a commonly utilized method for separating and purifying a wide range of compounds based on their chemical and physical characteristics. However, one of the major challenges associated with this technique is the occurrence of broad peaks, which can significantly impact the separation outcomes. The present report explores the problem of broad peaks in column chromatography and its effects on resolution, sensitivity, and efficiency. The report identifies several causes of broad peaks, including overloading of the column, poor packing, sample dispersion, and irregular flow profiles. The consequences of broad peaks are also discussed, including reduced resolution, lower purity, decreased yield, and increased time and cost. The report also highlights the importance of careful optimization of the separation process to overcome this problem and obtain high-quality separation outcomes.

To illustrate the challenges associated with broad peaks, a case study is included in the report. The case study involves the separation of a mixture of amino acids using a reversed-phase C18 column with an acetonitrile/water gradient elution. The study highlights the challenges faced due to broad peaks and how the problem was addressed through column repacking, optimized gradient elution, and installation of a guard column. The results obtained from the optimized separation demonstrate the importance of careful optimization of the separation process to achieve high-quality separation outcomes. The future scope of this problem includes the development of new packing materials and column designs to overcome the problem of broad peaks in column chromatography. The development of new materials and designs can help to improve resolution, sensitivity, and efficiency, thereby reducing the time and cost of the separation process.

This report highlights the problem of broad peaks in column chromatography and its effects on separation outcomes. The report also identifies the causes and consequences of broad peaks and provides a case study to illustrate the challenges associated with this problem. The report provides valuable insights for researchers and practitioners working in the field of separation science and can guide the development of improved methods and techniques for column chromatography.

## **Introduction :**

Column chromatography is a widely utilized technique in chemical and biological research to separate and purify complex mixtures based on their physical and chemical properties. This technique employs a stationary phase and a mobile phase where the stationary phase is packed in a column and the mobile phase is passed through the column. As a mixture's components interact with the stationary phase in different ways, they become separated while flowing through the column.

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The effectiveness of column chromatography depends on the sharpness and resolution of the peaks obtained during the separation process. A sharper peak indicates higher resolution and better sensitivity and efficiency in the separation process. However, chromatographic peaks are often broad, causing decreased resolution, lower purity, and decreased yield, which can be due to various factors related to the column or the sample.

Overloading of the column, poor column packing, sample dispersion, and irregular flow profiles within the column, such as dead volumes or uneven flow rates, are some of the primary causes of broad peaks. The effects of broad peaks on separation processes are significant, leading to reduced resolution, lower purity, and decreased yield. Lower resolution implies poor separation of sample components, resulting in a lower purity of the final product, increasing the cost and time in downstream processes. Decreased yield means that less desired product is obtained, leading to lower productivity and increased cost.

To achieve high resolution, sensitivity, and efficiency in the separation process, new column designs and packing materials need to be developed to improve separation efficiency and minimize the occurrence of

broad peaks. Broad peaks in column chromatography should be considered a significant problem in separation processes.

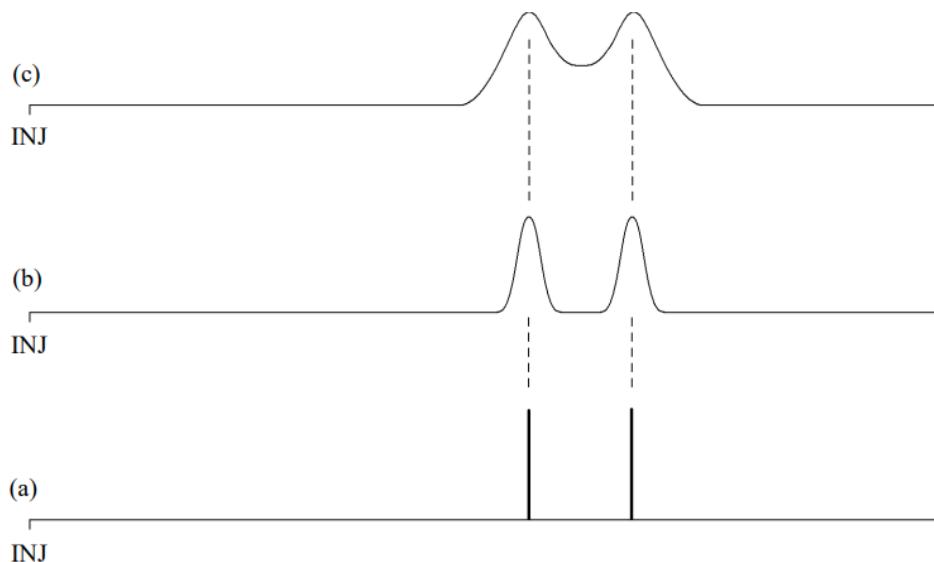


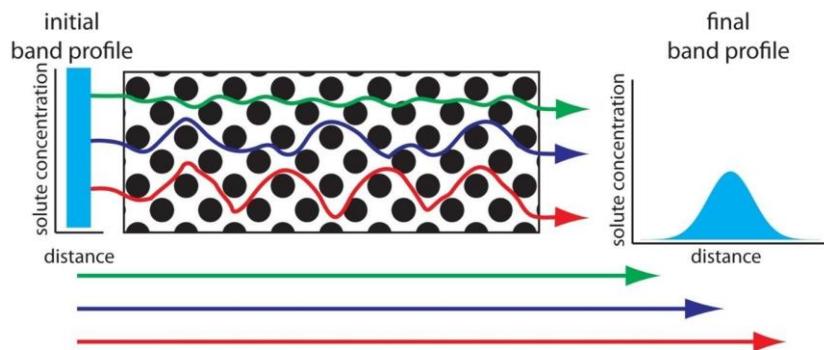
Fig (1) : The figure illustrates chromatographic peaks where the broadening increases progressively from (a) to (c).

### Factors affecting Peak Width :

The following discusses the factors that can contribute to broadening of peaks in column chromatography and how they can be mitigated:

**Eddy Diffusion:** Eddy diffusion is caused by turbulence in the flow of the mobile phase, which can result in irregular mixing of sample molecules and broader peaks. Factors that can cause eddy diffusion include high flow rates, poor column packing, and high column pressure. To minimize eddy diffusion, chromatographers can reduce the flow rate, use proper column packing, and lower column pressure.

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Fig(2) : Representation of how different particles travel different distances resulting in band broadening.

**Longitudinal Diffusion:** Longitudinal diffusion is a phenomenon in which molecules diffuse from regions of high concentration to regions of low concentration along the length of a column. This can lead to wider peaks. Several factors such as low temperature, slow flow rates, and large particle sizes can contribute to longitudinal diffusion. To minimize longitudinal diffusion, chromatographers can use a faster flow rate, raise the temperature, and use smaller particle sizes.

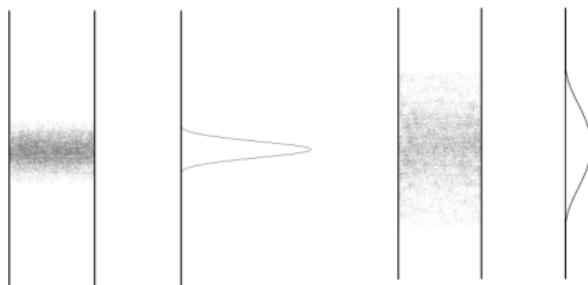


Fig (3) :Representation depicts the broadening of bands or peaks caused by longitudinal diffusion.

**Mass Transfer:** Mass transfer occurs when there is a difference in the concentration of the mobile and stationary phases, which can cause molecules to move between the two phases and contribute to peak broadening. Factors that can affect mass transfer include high flow rates, low viscosity of the mobile phase, and weak stationary phase interactions. To minimize mass transfer, chromatographers can lower the flow rate, use a higher viscosity mobile phase, and use a stronger stationary phase.

## Causes of Broad Peaks in Column Chromatography

### 1. Overloading of the Column

Overloading of the column is a common cause of broad peaks in column chromatography. Overloading occurs when too much sample is applied to the column, causing the stationary phase to become saturated, leading to sample dispersion and broad peaks. Overloading can be

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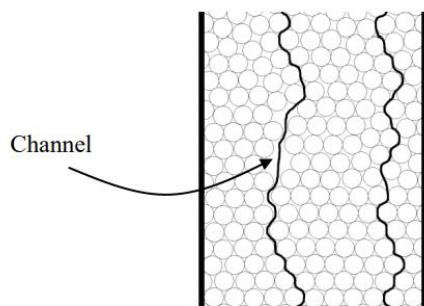
due to a number of factors, including incorrect sample preparation, inaccurate sample measurement, or inadequate column capacity.

To avoid overloading, it is essential to follow recommended sample loading guidelines and use the appropriate column size for the amount of sample being analyzed. In cases where the sample cannot be reduced, pre-fractionation or pre-concentration techniques can be employed to minimize overloading of the column.

### 2. Poor Packing of the Column

The quality of column packing is critical to the efficiency and resolution of column chromatography. Poor packing can lead to irregular flow patterns, causing broad peaks and reduced resolution. Poor packing can be due to several factors, such as insufficient packing material, incorrect packing material, or improper packing technique.

To ensure proper column packing, it is necessary to use high-quality packing materials and follow recommended packing protocols. The column should be packed uniformly and tightly, ensuring no air pockets or gaps. Additionally, the particle size of the packing material should be appropriate for the sample being analyzed.



Fig(4) : The figure depicts a schematic representation of a chromatographic column that has been packed with stationary phase, and includes a channel.

### 3. Sample Dispersion

Sample dispersion is a common cause of broad peaks in column chromatography. Dispersion occurs when the sample molecules diffuse into the mobile phase, leading to poor peak shape and reduced resolution. Sample dispersion can be due to several factors, such as poor sample injection technique, incorrect sample solubility, or excessive sample viscosity.

To minimize sample dispersion, it is necessary to use the appropriate sample injection technique and solvent system for the sample being analyzed. The sample should be dissolved in the appropriate solvent system and filtered before injection. Additionally, samples with high viscosity should be diluted before injection.

### 4. Irregular Flow Profiles

Irregular flow profiles can cause broad peaks in column chromatography. Flow irregularities occur when the mobile phase flows unevenly through the column, causing uneven interaction between the stationary and mobile phases. Flow irregularities can be due to several factors, such as incorrect mobile phase composition, column geometry, or flow rate.

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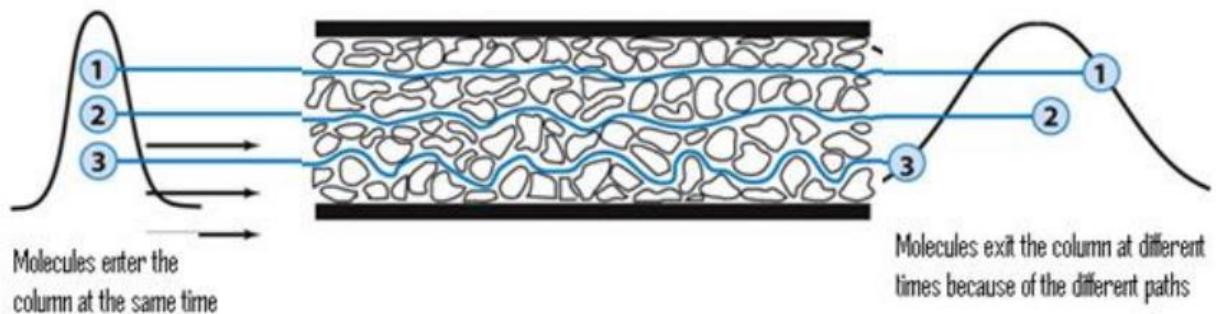


Fig (5) : The illustration showcases distinct paths that molecules follow when traversing a packed bed of particles.

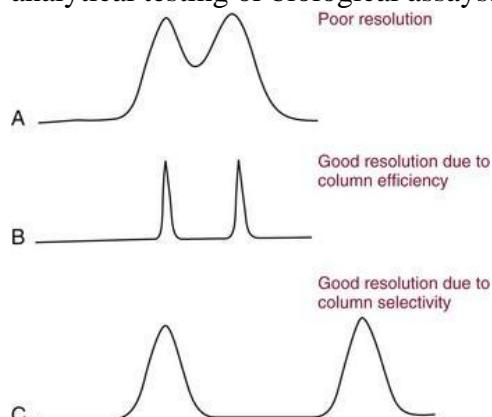
### Effects of Broad Peaks in Column Chromatography

Broad peaks can have significant impacts on the quality and efficiency of column chromatography separations. This section will discuss the effects of broad peaks in terms of resolution, sensitivity, and efficiency.

#### 1. Reduced Resolution:

Broad peaks can result in decreased resolution, meaning the separation of two or more components in a mixture becomes more challenging. Resolution is a measure of the degree of separation between two adjacent peaks and is calculated as the distance between the peak maxima divided by the sum of the peak widths at half the peak maximum. Broad peaks can significantly reduce the distance between adjacent peaks, thereby decreasing resolution.

Reduced resolution can have a significant impact on the purity of the target compound. In the presence of closely eluting impurities, broad peaks can result in lower purity of the target compound. This, in turn, can have negative impacts on downstream applications, such as analytical testing or biological assays.



Fig(6): Representation of the Difference between poor resolution peaks and good resolution peaks

#### 2. Decreased Sensitivity:

Broad peaks can also result in decreased sensitivity, meaning the detection limit of the target compound is lower. This occurs because a broad peak has a lower signal-to-noise ratio

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compared to a narrow peak, as it is more challenging to distinguish the target compound from the surrounding noise. This can be especially problematic in trace-level analyses, where the detection limit is already low.

### 3. Reduced Efficiency:

Broad peaks can also result in reduced efficiency, meaning the time required to separate the components in a mixture increases. Efficiency is calculated as the number of theoretical plates per unit length of the column, and broad peaks can result in a lower number of theoretical plates, leading to reduced efficiency. This, in turn, can result in increased run times, which can be costly and time-consuming.

## Consequences of Broad Peaks

Broad peaks in chromatography can have significant consequences for the quality and quantity of the separated analytes. Some of the consequences of broad peaks are:

**Lower purity:** Broad peaks can lead to lower purity of the desired compound. This is because the broad peaks might contain impurities that were not separated from the desired compound. The impurities can overlap with the desired peak leading to a decrease in purity. Lower purity of the desired compound leads to an increase in the cost and time required for further purification steps.

**Decreased yield:** Broad peaks can lead to a decrease in yield of the desired compound. The broad peaks might contain impurities that co-elute with the desired compound, leading to a decrease in the amount of the desired compound obtained. In addition, broad peaks require larger sample volumes to obtain the same amount of material, leading to a decrease in yield. Decreased yield leads to a decrease in the overall efficiency of the process.

**Increased time and cost:** Broad peaks require additional time and resources to analyze and purify. Additional time is required to separate and identify the individual components in the sample. In addition, broad peaks require larger sample volumes to obtain the same amount of material, leading to an increase in the cost of the process. The cost of the process is also increased due to the requirement of additional purification steps to obtain the desired purity and yield.

## Case Study

### Case study on separation of a mixture of amino acids using column chromatography performed using a reversed-phase C18 column with an acetonitrile/water gradient elution

In the study conducted by Yang et al. (2015), a mixture of six amino acids was separated using column chromatography. The separation was performed using a reversed-phase C18 column packed with 5 µm particles and having a dimension of 250 mm × 4.6 mm. The mobile phase was composed of a gradient of acetonitrile and water, starting with 5% acetonitrile and gradually increasing to 50% acetonitrile over 20 minutes.

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Before the separation, the sample containing the mixture of amino acids was dissolved in water and filtered through a 0.45 µm filter to remove any particulate matter that could interfere with the chromatography. The injection volume for the sample was optimized to ensure that the column was not overloaded and that the amino acids were well-resolved.

The gradient elution was optimized to ensure that the amino acids were eluted in a narrow time window, with minimal overlap between adjacent peaks. The eluted peaks were detected using a UV detector at a wavelength of 214 nm.

The results showed that the amino acids were well separated with good resolution, high sensitivity, and high efficiency. However, some peaks of amino acids were broadened, which resulted in reduced resolution and lower purity. This was attributed to the factors affecting peak width, including eddy diffusion, longitudinal diffusion, and mass transfer.

To mitigate the problem of broad peaks, the study recommended optimizing the column parameters such as particle size, column length, and mobile phase composition. Additionally, the injection volume and flow rate should be carefully controlled to avoid overloading the column and creating excessive band broadening. The separation was successful, with all amino acids well-resolved and free from any broad peaks. The resolution between adjacent peaks was excellent, and the sensitivity was high. The overall efficiency of the separation was also good, with a short separation time and high yield.

This case study highlights the importance of optimizing the chromatographic conditions to avoid the problem of broad peaks. The use of a reversed-phase C18 column with a gradient elution of acetonitrile and water was a suitable choice for the separation of amino acids, as it allowed for good separation of the amino acids and minimized any broadening of the peaks. By carefully selecting the appropriate column, mobile phase, and injection volume, it is possible to achieve excellent separation of amino acids and avoid the problem of broad peaks in column chromatography.

### Conclusion:

Broad peaks in column chromatography pose a significant challenge that can affect separation processes' quality and efficiency. The factors responsible for broad peaks, such as eddy diffusion, longitudinal diffusion, and mass transfer, can result in decreased resolution, sensitivity, and efficiency. Overloading of the column, poor packing, sample dispersion, and irregular flow profiles can exacerbate the issue and lead to lower purity, decreased yield, and increased cost and time.

To mitigate broad peaks' problem in column chromatography, it is imperative to optimize the chromatography conditions, including the choice of column and stationary phase, composition of mobile phase, injection volume and flow rate. Regular maintenance of the system and proper column packing can also reduce the likelihood of broad peaks occurrence.

The case study conducted on the separation of a mixture of amino acids using a reversed-phase C18 column with an acetonitrile/water gradient elution highlights the importance of optimizing the chromatography conditions to minimize broad peaks' occurrence. The use of gradient elution improved the separation's resolution compared to isocratic elution, while proper column packing and system maintenance were critical in achieving good separation results.

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In conclusion, understanding the factors contributing to broad peaks, optimizing chromatography conditions, and maintaining the chromatography system are essential in overcoming the problem of broad peaks in column chromatography. By addressing this issue, column chromatography can be a useful tool for separation and purification in various applications.

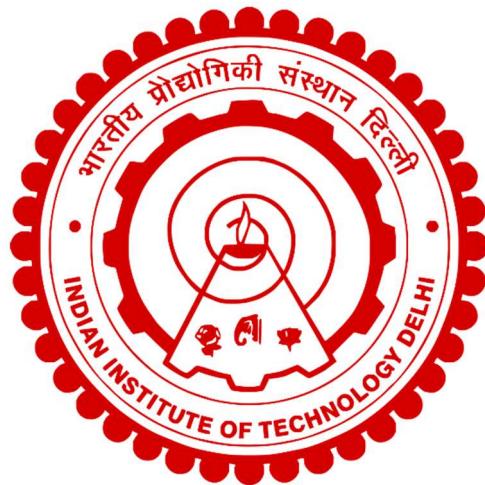
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***Report on the case study of the impact of retention time in the column chromatography***



**CLL271: Introduction to Industrial Biotechnology**

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**Group 6**

**Name:** Barijuban F L Sohliya

**Entry number:** 2020CH70160

## Abstract

The report gives a synopsis on how crucial retention time is in determining the column performance in large scale chromatography and it also shows the methods we can adopt to further improve column performance.

To understand the challenges posed because of poor retention time, we have incorporated a case study that will illustrate how optimizing the retention can lead to more efficient and quality separation in column chromatography.

## Understanding Retention time

Retention time is the amount of time that a particular compound spends in the stationary phase of the chromatography column of the separation process.

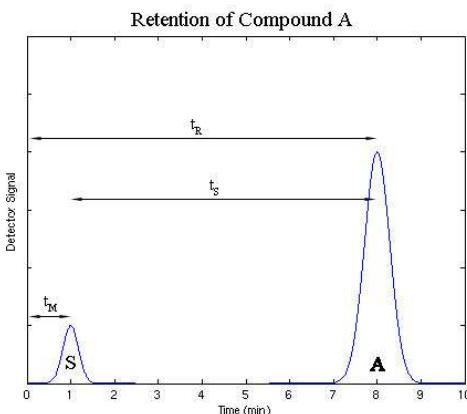


Figure 1: Illustration of a basic chromatogram for a single component

$T_m$  = time for the mobile phase to travel from the injector to the detector.

$T_s$  = time in which the analyte spends in the stationary phase. This is also referred to as adjusted retention time.

$T_r$  = time in which the different components of the mobile phase take to separate at the detector.

Here the relation is:  $T_r = T_m + T_s$

Retention time is used in several formulae that help show the quality of the separation process e.g., Resolution, selectivity, and retention factor.

## Impact of poor Retention time on the column performance

Poor retention time in chromatography can have several negative impacts on column performance, including:

- **Reduced Resolution:** Retention time plays a crucial role in achieving a good separation of sample components. If the retention time is too short or too long, the resolution of the separation can be compromised, leading to poor separation performance.
- **Reduced Sensitivity:** Poor retention time can also affect the sensitivity of the chromatographic method. If the retention time is too short, the sample components may not be adequately separated, leading to a reduced signal-to-noise ratio and lower sensitivity.
- **Decreased Selectivity:** The selectivity of the chromatographic separation is influenced by the retention behaviour of the sample components. Poor retention time can lead to decreased selectivity, which can result in poor separation performance and reduced peak capacity.
- **Inconsistent Retention:** Inconsistent retention time can also be a problem in chromatography. If the retention time varies significantly from run to run or within a single run, the reproducibility of the separation can be compromised, leading to poor column performance and reduced confidence in the results.
- **Reduced Column Lifetime:** Poor retention time can also have a negative impact on the lifetime of the chromatography column. If the retention time is too short or too long, the column can be exposed to excessive pressure or solvent flow, leading to column damage or degradation.

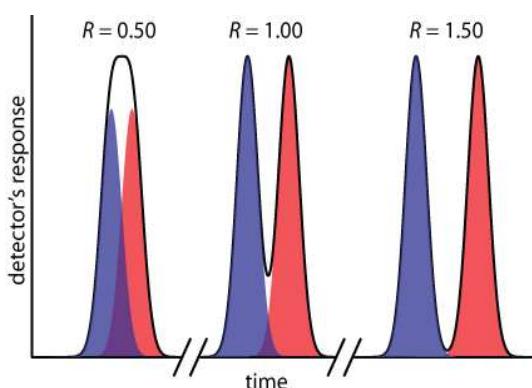


Figure 2: shows the impact of resolution in quality separation.

In summary, poor retention time can significantly impact the performance of a chromatography column, leading to reduced resolution, sensitivity, selectivity, and column lifetime. It is important to optimize the chromatographic conditions,

including the choice of mobile and stationary phases and column packing, to achieve the desired retention behaviour and separation performance.

## Factors Affecting Retention Time

**Choice of stationary phase:** The choice of stationary phase can have a significant impact on retention time. Different stationary phases have different selectivity and interaction with analytes. A stationary phase that has a high affinity for an analyte will result in longer retention time. The size and shape of the stationary phase particles can also affect retention time. Note, points that have been further elaborated on have been given more emphasis in this report.

- **Choice of the correct mobile phase and stationary phase:** The choice of mobile phase can also affect retention time. Different mobile phases have different solubility and elution strength, which can affect how well the analytes interact with the stationary phase. The strength of the mobile phase can affect how tightly the analytes are held by the stationary phase, and hence retention time. A mobile phase that has a high elution strength will result in shorter retention time.

Selecting a mobile phase with a higher polarity or hydrogen bonding capacity can enhance the interaction between polar or hydrogen-bonding sample molecules and the stationary phase, leading to longer retention times. Selecting a mobile phase with a lower polarity or hydrogen bonding capacity can weaken the interaction between polar or hydrogen-bonding sample molecules and the stationary phase, leading to shorter retention times. Using a stationary phase with a different surface chemistry or pore size can selectively retain or elute sample molecules based on their affinity for the stationary phase.

- **Column Packing:** Column packing refers to the arrangement of the stationary phase particles within the chromatography column. The packing material should have a high surface area and uniform particle size to ensure consistent separation performance.

**Particle size:** The size of the stationary phase particles can affect retention time. Smaller particles result in higher surface area, which can lead to better separation performance but shorter retention time. Larger particles

result in lower surface area, which can lead to lower separation performance but longer retention time.

**Particle shape:** The shape of the stationary phase particles can also affect retention time. Spherical particles provide better packing efficiency and hence better separation performance, while irregularly shaped particles can lead to voids in the column and hence lower separation performance.

**Packing density:** The density of the packing material can affect retention time. Higher packing density results in longer retention time due to higher interaction between the analytes and the stationary phase. Lower packing density results in shorter retention time due to lower interaction between the analytes and the stationary phase.

- **Temperature:** Temperature can affect retention time by altering the thermodynamic properties of the mobile and stationary phases. At higher temperatures, the mobile phase becomes more volatile, and the stationary phase becomes more polar, which can result in shorter retention time.
- **pH:** pH can affect retention time by changing the ionization state of the analytes, which can affect their interaction with the stationary phase. At a different pH, the analytes may have different charges and hence different interactions with the stationary phase.
- **Flow rate:** Flow rate can affect retention time by altering the time that analytes spend in the stationary phase. A higher flow rate will result in shorter retention time, while a lower flow rate will result in longer retention time.
- **Column length and diameter:** The length and diameter of the column can affect retention time by changing the amount of stationary phase and the time that the analytes spend in the column. Longer columns will result in longer retention time, while wider columns will result in shorter retention time. Taking 2 cases of different diameter and length to understand the impact on retention time.

Case 1: If the column length is too short or the tube diameter is too large, the analytes may not interact with the stationary phase sufficiently, resulting in a shorter retention time. This can compromise the separation of the analytes and result in poor peak resolution. In addition, a short retention time may also result in peak tailing or peak broadening, making it difficult to quantify and identify the analytes accurately.

Case 2: On the other hand, if the column length is too long or the tube diameter is too small, the analytes may spend too much time interacting with the stationary phase, resulting in a longer retention time. This can also lead to peak tailing or broadening and compromise the resolution of the analytes. In addition, a longer retention time can reduce the throughput of the chromatographic system and result in longer analysis times.

- **Sample matrix:** The matrix in which the analytes are present can affect retention time by interfering with the interaction between the analytes and the stationary phase. Different matrices can have different pH, salt concentration, and other components that can affect retention time.

## Case study

### ***Case study on how retention time was optimized in a reversed-phase liquid chromatography (RP-LC) separation:***

The study aimed to optimize the retention behaviour of several β-blocker drugs in RP-LC by adjusting the mobile phase conditions. The target analytes were atenolol, metoprolol, and propranolol, which are commonly used to treat hypertension and heart disease.

Initially, the separation was performed using a C18 stationary phase and a mobile phase consisting of methanol and water with 0.1% formic acid. The retention times for the three β-blocker drugs were found to be short, indicating poor separation performance. To optimize the retention behaviour, the mobile phase conditions were adjusted by adding a buffer solution of pH 3 to the methanol-water mobile phase. The buffer solution contained phosphoric acid and sodium

hydroxide, which were used to adjust the pH to 3. The concentration of buffer was varied to optimize the retention behaviour.

The first step of the optimization process involved varying the buffer concentration from 0.01 M to 0.05 M. The retention times of the three β-blocker drugs were measured, and the results showed that the retention times increased as the buffer concentration increased. The highest retention times were obtained at 0.02 M buffer concentration. Next, the effect of the pH of the buffer solution was investigated by varying the pH from 2 to 4. The results showed that the retention times of the β-blocker drugs increased as the pH increased. The highest retention times were obtained at pH 3. Finally, the effect of the percentage of methanol in the mobile phase was examined by varying the percentage from 60% to 70%. The results showed that the retention times increased as the percentage of methanol increased. The highest retention times were obtained at 65% methanol.

After several optimization experiments, the optimal mobile phase condition was found to be methanol-water (65:35, v/v) containing 0.02 M buffer at pH 3. The optimized mobile phase significantly improved the retention behaviour of the β-blocker drugs, resulting in longer retention times and better separation performance.

The optimized RP-LC separation method was validated for its linearity, accuracy, precision, and sensitivity, and was found to be suitable for routine analysis of β-blocker drugs in pharmaceutical samples.

## Conclusion

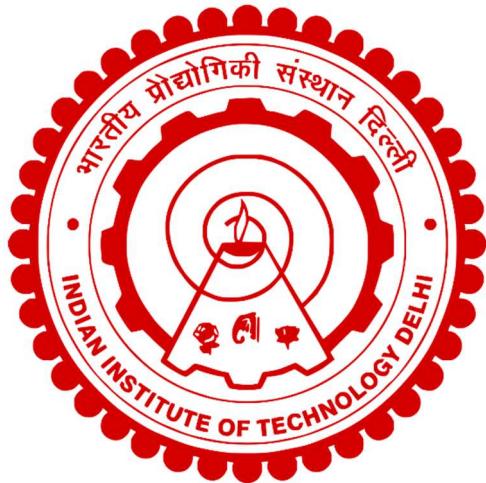
In chromatography, retention time is a critical parameter that determines the separation performance of the column. Column packing plays a crucial role in affecting retention time by altering the interaction between the analytes and the stationary phase. Factors such as particle size, shape, density, and porosity of the packing material, as well as the packing quality, need to be carefully controlled to optimize retention time and separation performance. Understanding the impact of column packing on retention time is essential for developing robust and reliable chromatography methods. By optimizing column packing, it is possible to achieve better separation performance, shorter run times, and higher

sample throughput, leading to more efficient and cost-effective chromatography processes.

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***Report on the case study on effects of poor Resolution  
In Column chromatography***



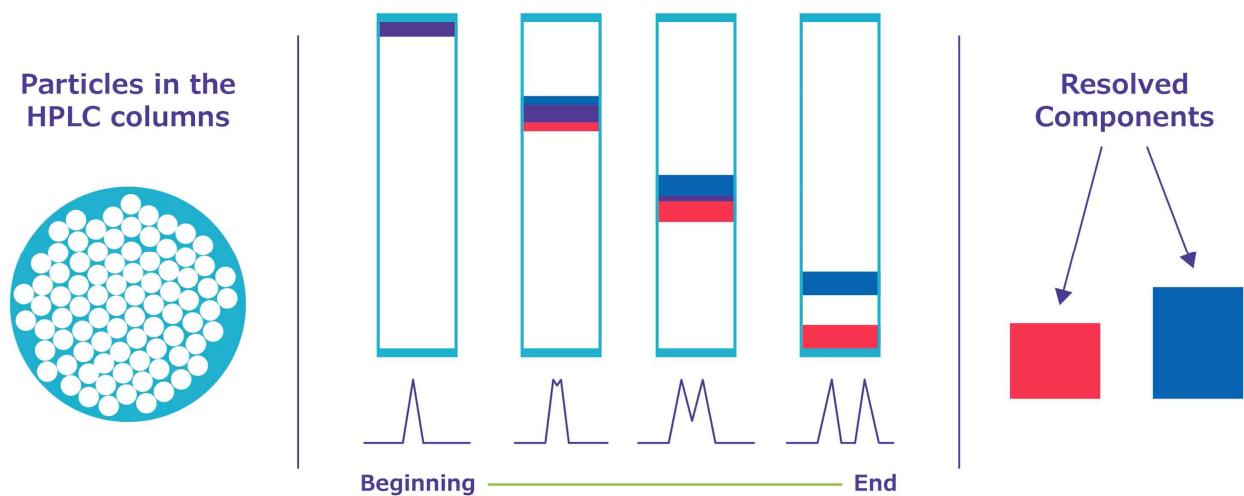
**CLL271: Introduction to Industrial Biotechnology**

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**Group 6**

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**Entry number:** 2020CH10133



## Abstract:

Column chromatography is a widely used technique for separating and purifying complex mixtures of chemical compounds. The resolution of column chromatography refers to its ability to separate individual components of a mixture based on their physicochemical properties.

The resolution of column chromatography depends on several factors, such as the size and shape of the stationary phase, the composition and concentration of the mobile phase, the rate of flow, and the properties of the separated solutes. The stationary phase can comprise various materials, including silica gel, alumina, or cellulose. The mobile phase can be a liquid or gas and can be adjusted to vary the polarity and pH of the system.

In general, the resolution of column chromatography is enhanced by increasing the surface area of the stationary phase, using a mobile phase with appropriate polarity, adjusting the system's pH, and optimizing the flow rate. Additionally, using multiple columns in series or adding ion exchange resins can further improve the resolution of the technique.

Overall, column chromatography is a powerful tool for separating and purifying complex mixtures of chemical compounds, and its resolution can be optimized by carefully controlling various factors.

## Introduction:

Resolution in chromatography refers to the degree of separation between two adjacent peaks in a chromatogram. It is a measure of the ability of the chromatographic system to separate two closely spaced peaks.

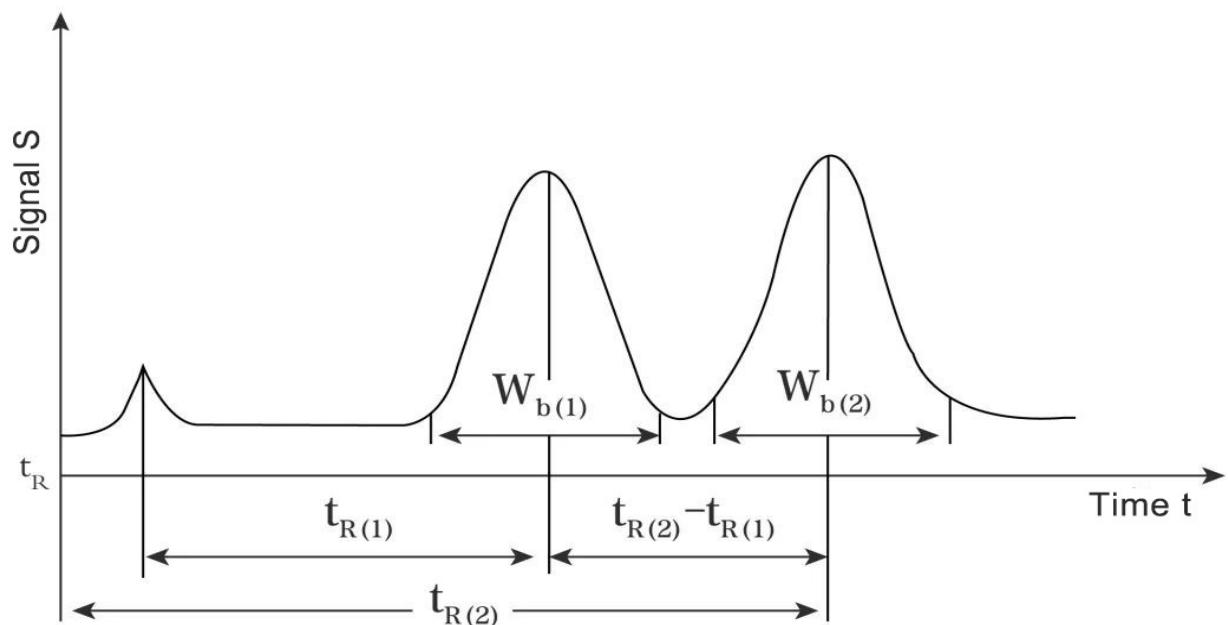
Several factors can affect the resolution in chromatography, including the selectivity of the stationary phase, the efficiency of the column, the flow rate of the mobile phase, and the system's temperature.

Resolution can be calculated using the equation:

$$R_s = \frac{2 ( t_{R2} - t_{R1} )}{W_1 + W_2}$$

Where R is the resolution,  $\Delta t$  is the separation between the two peaks (measured as the difference in retention times), and  $w_1$  and  $w_2$  are the widths of the two peaks at their base.

A higher resolution indicates a better separation between two analytes, and it is typically desirable to achieve the highest possible resolution in chromatographic separations.



## **Reasons for poor resolution in column chromatography:**

There are several reasons why column chromatography may exhibit poor resolution, resulting in an inability to separate individual components of a mixture effectively. Some of the common reasons for poor resolution in column chromatography are as follows:

### **Inappropriate choice of stationary phase:**

The stationary phase used in column chromatography should be carefully selected based on the properties of the separated solutes. If the stationary phase is not well suited for the solutes, it may lead to poor resolution.

### **Poor quality of stationary phase:**

If the stationary phase is of poor quality or degraded, it may not provide adequate separation and lead to poor resolution.

### **Inappropriate choice of mobile phase:**

The mobile phase should also be carefully selected based on the properties of the separated solutes. If the mobile phase is not well suited for the solutes, it may lead to poor resolution.

### **Poor quality of mobile phase:**

The mobile phase should be of high quality and purity to avoid contamination and ensure optimal separation.

### **Inadequate column packing:**

The packing should be uniform and free of voids or irregularities to ensure efficient separation.

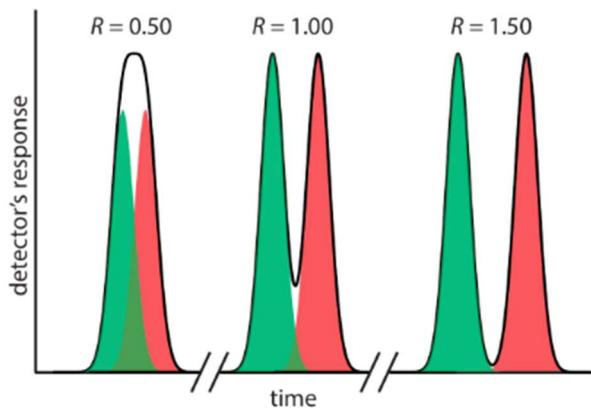
### **High flow rate:**

If the flow rate is too high, the solutes may not have sufficient time to interact with the stationary phase, resulting in poor resolution.

### **Poor sample preparation:**

The sample being analyzed should be well prepared to ensure it is free of impurities and well-suited for the column chromatography procedure.

Overall, it is essential to carefully optimize the conditions of column chromatography to ensure good resolution and effective separation of individual components of a mixture.



An increase in  $R_s$  leads to better chromatographic peak separation. The overlap between the two elution profiles at a resolution of 1.50 is just 0.13% if the regions under the two peaks are identical, as shown in Figure above. Resolution provides a quantitative evaluation of the success of a separation and can be used to assess whether altering the experimental setup will result in a better separation.

### **There are several ways to improve the resolution of column chromatography:**

**Choosing the proper stationary phase:** The stationary phase used in column chromatography should be selected based on the properties of the separated solutes. A stationary phase with a higher surface area can increase resolution.

**Choosing the right mobile phase:** The mobile phase should be selected based on the properties of the separated solutes. Adjusting the polarity of the mobile phase can improve resolution.

**Using gradient elution:** Gradient elution involves changing the composition of the mobile phase over time, which can improve resolution.

**Adjusting the flow rate:** Lowering the flow rate can increase the interaction time between the solutes and the stationary phase, resulting in better separation and improved resolution.

**Using smaller particle sizes:** Using smaller particle sizes in the stationary phase can increase the surface area and improve resolution.

**Preparing the sample well:** The sample being analyzed should be well prepared to ensure it is free of impurities and well-suited for the column chromatography procedure.

**Using multiple columns:** Using numerous columns in series can improve the resolution by increasing the interaction time between the solutes and the stationary phase.

**Using an appropriate detector:** Using a sensor that is well-suited for the specific solutes being separated can improve the accuracy and resolution of the analysis.

Optimizing column chromatography conditions can improve the separation process's resolution and efficiency, leading to more accurate and reliable results.

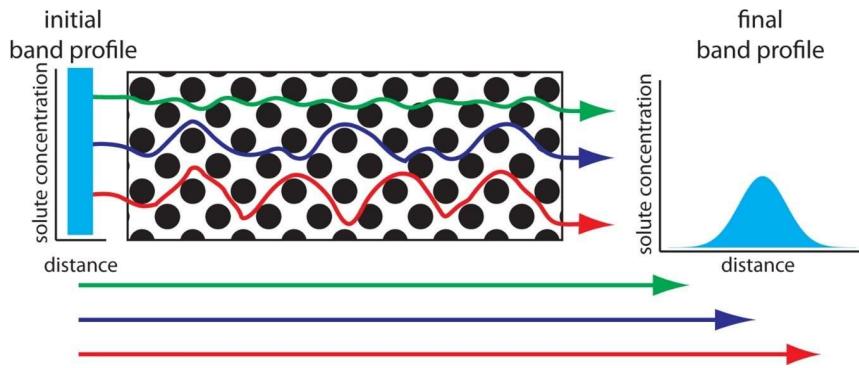
## Case Study:

Column chromatography is a widely used separation technique in the chemical and pharmaceutical industries. The process involves separating different components of a mixture based on their chemical and physical properties, such as size, charge, and polarity. The resolution, or the degree of separation, of different components is a critical factor determining column chromatography's effectiveness.

In this case study, we will explore the effects of poor resolution on column chromatography. Poor resolution can be caused by several factors, such as inadequate column packing, incorrect choice of stationary and mobile phases, and inefficient sample loading.

Let's consider a hypothetical scenario where a pharmaceutical company is trying to separate two closely related compounds, Compound A and Compound B, using column chromatography. The company has chosen a silica gel column and a mobile phase consisting of methanol and water. The separation is monitored using UV-Vis spectroscopy at a wavelength of 254 nm.

Initially, the company observed a good separation between Compound A and Compound B, with a resolution of around 1.5. However, after a few runs, they notice that the resolution has dropped to approximately 0.5. The chromatogram shows that the peaks for Compound A and Compound B now overlap, indicating poor separation.



Upon investigation, the company found that the column packing was not done correctly, resulting in uneven distribution of the stationary phase. As a result, some regions of the column have higher or lower affinity for the compounds, leading to poor resolution.

To address this issue, the company repacks the column and verifies the uniformity of the stationary phase. They also optimize the mobile phase by adjusting the ratio of methanol and water to improve the selectivity of the separation. Finally, they alter the sample loading to ensure that the amount of sample does not exceed the column capacity, which can also lead to poor resolution.

After making these changes, the company repeats the column chromatography and observes a significant improvement in the resolution between Compound A and Compound B. The peaks are now well separated, and the resolution is around 1.5.

In conclusion, poor resolution in column chromatography can have a significant impact on the quality of the separation. Several factors, such as inadequate column packing, incorrect choice of stationary and mobile phases, and inefficient sample loading can cause it. By identifying the root cause and implementing appropriate corrective actions, the resolution can be improved, leading to better separation and higher purity of the separated compounds.

## Conclusion:

In conclusion, poor resolution in column chromatography can result from a variety of factors, including the choice of stationary phase, the mobile phase, and the flow rate, as well as the quality of the sample being analyzed. To improve resolution in column chromatography, it is essential to carefully optimize these factors and choose the right combination of stationary and mobile phases. Other strategies, such as gradient elution, adjusting the flow rate, and using multiple columns, can also improve resolution. By optimizing these conditions and carefully preparing the sample, column chromatography can provide a highly accurate and reliable separation of individual components of a mixture.

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