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Investigation of Column Packing & Flow Profiles in Packed Chromatographic Columns

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ABSTRACT

Liquid chromatography is an important downstream operation in the health care, biotechnology, biopharmaceutical, and bioprocessing industries. Its high resolving power is utilized to capture valuable materials such as therapeutic proteins, antibodies, peptides, and nucleic acids. Column chromatography relates to a separation and/or purification technique in which a stationary "bed" of a packing medium or resin is contained within a rigid tube. The column efficiency is measured in terms of Number of plates and Asymmetry. Column Efficiency loss can occur during packing, during storage and during transportation. The detailed study has been done for column sizes 2.5 cm and 5 cm inner diameter to improve column efficiency to the customer by studying the impact of Frit thickness, number of adaptor vanes and the effect of column internal smoothness. Column efficiency improved (19-38%) with reducing frit thickness, (15-25%) with increasing number of vanes and 10% with reducing column internal roughness. With the improvements achieved we can deliver more stable prepacked columns to the end user.

Keywords: Column packing, Flow profiles, Column diameter, Asymmetry, Number of plates, Column efficiency,

Summary for Lay Audience

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid (gas or solvent) called the mobile phase, which carries it through a system (a column, a capillary tube, a plate, or a sheet) on which a material called the stationary phase (like Resin) is fixed. The different constituents of the mixture have different affinities for the stationary phase. The different molecules stay longer or shorter on the stationary phase, depending on their interactions with its surface sites. So, they travel at different apparent velocities in the mobile fluid, causing them to separate.

In the present study we are dealing with Column packing for various types of resins used in pharmaceutical applications for the separation of proteins and other components. Better the quality of the columns packed, better will be the results of separation.

Quality of the column is determined by the parameters such as Asymmetry and number of plates. Asymmetry in layman terms is the column should have been packed fully tight without any air bubbles or any other impurities and suppose if we pass a liquid through the column bed, it will be distributed evenly and ultimately it will give better separation.

We study various options for improving the efficiency of the columns packed so that when the columns are used for applications or for any separation of components, it should give good results.

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DEDICATION

This Thesis is dedicated to my Family members and my Team members at PolyAnalytik Inc.

LIST OF ABBREVATIONS

AKTA – Trade mark of GE/ Amersham FPLC

As - Asymmetry

EMG – Exponentially modified Gaussian

FPLC – Fast protein Liquid chromatography

GMP – Good manufacturing practices

HETP – Height equivalent to theoretical plate

HIC – Hydrophobic interaction chromatography

N –number of plates

PP – Polypropylene

PPV – Pressurised packing vessel

PE – Polyethylene

SEC – Size exclusion chromatography

SP – Sulfopropyl

Ve – Elution volume

W – Width of peak

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CHAPTER 1

INTRODUCTION

1.1 Overview

Liquid chromatography is an important downstream operation in the health care, biotechnology, biopharmaceutical, and bioprocessing industries. Its high resolving power is utilized to capture valuable materials such as therapeutic proteins, antibodies, peptides, and nucleic acids. Good Manufacturing Practice (GMP) dictates that the chromatographic operation is carried out in a consistent and reproducible fashion and provides products of desired purity and quality. Column chromatography is the workhorse for both analytical and preparative scale in these industries. Column chromatography relates to a separation and/or purification technique in which a stationary "bed" of a packing medium or resin is contained within a rigid tube. The packing medium ("stationary phase"), usually resin particles, is often suspended to form a slurry, which is then pumped, poured, or sucked into the column to form a packed bed.

The liquid sample is passed through the column to achieve differential association with the stationary phase which in turn separates the compound of interest. This interaction could be ionic charge (ion-exchange chromatography), hydrophobicity (hydrophobic interaction chromatography), porosity (size exclusion chromatography) or affinity chromatography, where the packing medium includes binding agents, such as antigens, antibodies, or ligands, that specifically bind to one or more desired compounds or molecules in the liquid sample. Thus, as the liquid sample flows through the packing

medium, only the desired compounds or molecules remain in the column. A subsequent flow through the packing medium of an eluting liquid separates the captured molecules of interest from the binding agents attached to the packing medium or separates the binding agents from the packing medium(Giddings, 1965).

The packing efficiency of the chromatography resin can greatly impact chromatography performance. A poorly packed column can result in product dilution or insufficient resolving ability to meet the purification objective.

The objective of this thesis is to improve the performance efficiency of packed columns by studying the effect of packing density, radial fluid flow profile and solid phase (resin) properties. PolyAnalytik will develop these parameters and will help commercialize the designs for larger diameter columns. Secondly, the study will be used to improve the performance and efficiency of the current size columns which are 2.5 cm and 5 cm diameter columns.

1.2 Limitations & Challenges

Despite all developments and works carried out by various authors for column packing, there are below limitations with respect to column performance:

- Limitation of packing the column bed at certain pressure, keeping the particles compressed without breaking resin and without any voids in the column
- Having stable bed over a certain period ensuring consistent Asymmetry and number of plates. Trend is now for prepacked columns which involves shipping

(vibrations) and keeping column on shelf. Asymmetry and plates tend to shift due to vibrations, possible voids, and higher flow rates.

- Some aspects of Hardware design like Frit thickness, Number of vanes in adaptor & column internal smoothness are still unexplored in terms of column performance.

1.3 Organisation of Thesis

Chapter 2 has been organized for Literature review. It comprises of background of the project, the reason for choosing this topic, how it would benefit the company and finally the analysis of the impact on the society for future generations. It has been devoted to a detailed literature review of the proposed study. Also, would like to mention what studies have been done so far and how it has been presented successfully.

Chapter 3 encompasses Materials & Methods and has been done in three sections. Section 3.1 Includes Materials used for the study and in detail properties of the materials and what impact it will have on the column performance and the studies being carried out. Section 3.2 details out the equipment used for the column packing and analysis of these columns. Details about the Pressurized packing vessel (PPV) has been included along with the operation and performance. Equipment used is specific to the PolyAnalytik Inc.

Section 3.3 includes Experimental procedure used and how to ensure smooth packing of the columns by following required operating procedure

Chapter 4 comprises in detail all the results and discussion

Chapter 5 includes conclusions and recommendations from the data collected, observations and it's applications for future column packing.

1.4 Novelty & Research Contribution

The main purpose of the study is to improve the performance efficiency of packed columns after studying the flow distribution characteristics inside the column with respect to frit thickness, Adaptor liquid distribution by changing number of vanes, and column internal smoothness. This study standardizes these parameters to help PolyAnalytik get better performing columns and further support to commercialize the designs for larger diameter columns.

The important factor affecting the column efficiency is the fluid profile in the bed and it depends on frits thickness, Adaptor distribution vanes (baffles), and column internal smoothness. Based on these factors below is the list of studies done at PolyAnalytik lab:

- Study the effect of Frit thickness on the column efficiency in terms of number of plates in the column.
- Study the effect of number of vanes(baffles) in the adaptor on the column efficiency and Asymmetry and flow distribution performance in the column
- Columns of the size 2.5 and 5 cm internal diameter are studied for three types of resins.

- Three types of resins selected are of different particle size and different properties with different applications of each resin.
- Establish a relationship between Fluid profile and parameters- frits thickness, Adaptor distribution number of vanes, and column internal smoothness. This is done for 2.5 cm and 5 cm diameter columns.

CHAPTER 2

Literature Review

2.1 Background

Chromatography is the workhorse for the purification of biotherapeutics because of its excellent resolving power. To meet the stringent purification specifications required by regulatory authorities, it is rare to find a bioprocess design that does not incorporate at least one chromatography step and more often at least two. Such requirements place an increasing demand to design more efficient, reliable, and scalable chromatographic steps (Goyon et. al, 2017). To achieve high product purity, the selection of chromatography resin and process development on the selected resin plays a significant role. The packing efficiency of the chromatography resin can greatly impact chromatography performance. Packing heterogeneity has long been known to cause band broadening and reduced separation efficiencies. A poorly packed column can result in product dilution or insufficient resolving ability to meet the purification objective. End users look to bio manufacturers to demonstrate packing consistency and packed bed integrity (Siu et al, 2014).

A packed chromatographic column is a maze of channels through which flows the mobile phase. Along the way, it carries all the analyte molecules that happen to be dissolved into it at any moment. There are countless pathways along which a molecule of either the solvent or the analyte may migrate from one end of the column to the other (Figure 2.1). At any given time, the local velocity of molecules following different pathways, or

moving at different locations along the same pathway, may be very different in magnitude and/or in direction. The flow pattern (i.e., the detailed relative distribution of the mobile phase velocity in magnitude and direction) is essentially the sum of all the local velocity biases or differentials which are so important to the analyte zone spreading [Giddings, 1965; Peters et al, 1974]. Substantial amount of work has been done in the field of fluid dynamics in porous media. This field encompasses a wide range of solid porous structures combined with fluids both different in nature and moving in different flow regimes. The properties of the migrating liquids to be considered are viscosity, diffusivity, and capacity to wet the solid surface of the porous medium they are flowing through. This variability in porous structures and fluids in motion makes the spectrum of practical situations to be investigated very large, since systems can be very different from each other (Farcas, 1997).

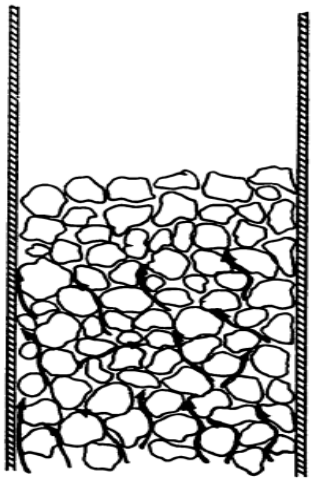


Figure 2.1: Flow through a packed bed depicting the countless pathways by which molecules in the mobile phase may travel from one end of the column to the other, Karger et al. 1973

Different packing methods and conditions can result in beds that have different structural characteristics. Best practices of different packing methods can be found in the respective vendor application notes and industry guidelines. The packing technique applied depends on several critical factors such as the mechanical and physical properties of the resin (including rigidity, particle shape, shear resistance, density, and size) as well as hardware capabilities.

For example, soft resins are compressible and can tolerate moderate pressures, which will reduce the porosity of the bed or can even disrupt the bed integrity. Therefore, as much as 30% additional resin is needed to achieve the required column volume

Scale-up of a chromatography process might appear as simply increasing the column diameter to accommodate for the higher quantity of material to be processed, while maintaining the same bed height and flow velocity (i.e., residence time). In practice, however, additional aspects such as method of column packing, hydrodynamic pressure drop, and efficiency of liquid flow distribution need to be considered. A typical problem to be addressed during scale up is the loss of wall support for the chromatography resin because of the large change in aspect ratio, causing larger compression of the resin and an additional hydrodynamic pressure drop (Hagel et al, 2008).

2.2 Column Packing Approach

There are two common ways to fill a chromatography column. The first a “dry packing” method where dry solid phase packing medium is introduced into the column and buffer (or the starting solvent) is then used to saturate the medium. The second method a “wet packing” method, where the buffer or starting solvent is mixed with dry medium to form slurry, which is then introduced into the column. It is crucial that the medium is evenly packed and that there are no bubbles or irregularities in the packed resin bed. Several approaches are available in the case of the more common “wet packing” technique, the “tap- packing” method is applied as a common approach at the laboratory scale involving physical tapping to the column to disrupt bubbles and uniformly distribute the resin and bed consolidation. Another approach used for column packing is to flow the resin into the column slowly (“stop and flow”). At larger scales, mechanical vibrations and/or pressure is applied during packing. These approaches have been shown to successfully apply resin in larger scale chromatographic systems (Poole and Poole, 1993).

Various sizes of chromatography packed columns are used in industry. Small lab scale columns (1 ml and 5 ml) may be used for process development, scale down or design. At the preparatory scale, ready-to-use pre-packed columns or columns packed by the end user are utilized. Once the column is packed, the sample to be separated is introduced into the flowing mobile phase upstream of the column. The uniformity of sample distribution at the column inlet, particularly as the cross section of the chromatographic column increases, is a critical feature in the separation of substances of interest. This relies on the liquid distribution and collection system at the fluid inlet and outlet of the

packed bed. Ideally, the carrier liquid is uniformly introduced throughout the surface at the top of the packing medium, and it flows through the packing at the same velocity throughout the packing cross section and is uniformly removed at the outlet of the packed bed. Wall effects may however, become prominent as the column diameter increases (Ameriga et al, 2010).

Various studies and patents have appeared on column packing design to overcome the problems outlined above. Koh and Guiochon (2006) investigated the packing density, the external porosity, the permeability, and the column efficiency for two types of particles. They found that packing methods affected the efficiency of the columns. (Farkas et al, 1997) applied fluorescence-detection for the determination of the radial distribution of the column velocity and the column packing efficiency. They established that the lack of homogeneity of the column packing led to band broadening of the eluent peaks and unsymmetrical band profiles with tailing. Various studies on column packing investigated axial and radial inhomogeneity as a function of the packing method applied. The local fluid velocity and local HETP (Height Equivalent to Theoretical Plate) values vary across the column and over time for axial and radial as well as for slurry compressed columns. Patent US20080217248 (GE Healthcare) discloses a method and system for improving the packing of chromatography columns which utilizes an external drive means to compress a bed of particulate medium to a target bed height. US20130193052 (Repligen) discloses the manufacture of chromatography column tubes from plastic/thermoplastic or composite materials (such as polypropylene (PP), polyethylene (PE), polyamides, acetals, or glass-filled plastics, such as glass fiber plastics). Securing at least one of two

flow distributors within the column tube with a tight interference or press fit, results in chromatography columns with reduced or no dead zones around the press fit flow distributor and they have an adjustable packing medium volume. US8702983 (GE Healthcare) discloses a column comprising a first port for mobile phase and a transverse fluid distribution channel for distributing fluid uniformly throughout the packed bed.

However, despite all the above developments in column chromatography, certain crucial challenges remain relating to packing of the resins and uniform liquid distribution in the column. In particular, the main challenges in packed columns are (i) packing the bed at a certain pressure; (ii) keeping the particles compressed without breaking them, achieving a uniform bed with no void space, and (iii) achieving good distribution of the analyte throughout the column (i.e., having the analyte divide up and flow through the whole bed, rather than just the center of the column). PolyAnalytik has developed several proprietary approaches to overcome these issues listed above. At the present time, a fundamental analysis of the hydrodynamic column behaviour on the particle scale remains problematic. Furthermore, the accessibility of relevant parameters which influence column hydrodynamics and determine the degree of packing homogeneity is still limited.

There are three major techniques that have traditionally been employed for qualifying chromatographic column performance. First, pulsed-input method to experimentally measure the height equivalent of a theoretical plate (HETP) is commonly used to measure column packing efficiency and peak broadening. The pulse response approach using small non-interacting solutes allows for an excellent evaluation of column packing.

Alternatively, a step response (frontal analysis) can be applied to analyze the breakthrough curve. Thirdly sophisticated modelling approaches are available to account for fundamental factors controlling column performance. Pulse response experiments and the chromatographic peak statistical moment analysis are a function of the experimental setup, the peak analysis method and the amount and type of injected solute. Peak analysis is done using either direct numerical integration of the data or peak fitting to functions such as Gaussian or the exponentially modified Gaussian (EMG) function. Noise compensation and baseline drift corrections are needed as well for accurate peak analysis.(Guiochon et al. 1997). Gritti and Guiochon have provided an overview of all mathematical models available to-date. In their view, the empirical van Deemter equation continues to be most appropriate for analysing column performance.

The van Deemter equation relates height equivalent to theoretical plate (HETP), a parameter used to measure performance of a packed bed, to the diffusive and adsorptive processes and is given as equation 2.1

$$HETP = A + \frac{B}{u_0} + Cu_0 \qquad (2.1)$$

where A, B, and C terms represent axial dispersion; molecular diffusion; and combined triple effects, viz., solid-liquid film mass transfer, effective intraparticle diffusion, and adsorption effects, respectively. HETP is directly related to the breakthrough profile.

2.3 Modelling of Flow through Chromatographic Bed

The size and relative location of the pores, channels, and cavities of a chromatographic bed determine the rate of the fluid flow and the nature of the various dispersion and mass transfer phenomena. All these interrelationships have an important role in the effectiveness of any separation. It would be most desirable to be able to define a geometrical quantity that would characterize the pore system in any porous medium. Unfortunately, the pore system of a porous body forms a very complicated surface, one that is difficult to describe geometrically. Intuitively, one would like to talk about the 'size' of pores, a convenient measure of the 'size' being the 'diameter. However, the term diameter makes sense geometrically only if the pores are of spherical shape, unless some further specifications are made. If we consider the flow of fluids through interparticle space, the pores must be visualized instead as rather tube-shaped voids. One would then call the diameter of such a tube the 'pore diameter'. Unfortunately, this visualization of the term diameter is again geometrically quite meaningless unless the tubes are cylindrical. In general, they will not be so, and they will not even possess a pattern for their cross-section since the walls will be irregularly diverging and converging. (Scheidegger, 1974). With randomly arranged channels, a small element of fluid in laminar flow will zig-zag erratically as it strives to avoid the solid particles in its path. While moving along such a channel, the volume elements of the fluid would do so at a constantly changing local velocity, depending upon the local size of the 'pore diameter they are crossing at a given moment. These elements of fluid would also constantly change their relative positions, when filling a larger void, or immediately next, while squeezing into

the narrow space situated between two adjacent packing particles. Thus, in reality, the fluid is not moving in a capillary as in Poiseuille flow since the interconnecting voids generate a tortuous stream path which does not have continuous walls. It's the local 'diameter* which determines the local flow rate (i.e., local linear velocity), and which changes constantly and erratically. The moving fluid can fill this nondescript space only by rearranging its elements constantly. The resulting constant mixing inside the interparticle channels is resembling to some extent to that encountered in turbulent flow, with the capital difference that it does not involve the whole volume of fluid, but it is limited to the volume of fluid moving in a particular interparticle channel, and it's not the energy state of the fluid elements responsible for this mixing, but the chaotic change in flow space characteristics (such as volume and direction of flow) throughout the bed.

2.4 Chromatographic Column Packing Techniques

The procedures used for the packing of columns for high performance liquid chromatography are dry packing, slurry packing and packing under stress (axial, radial or annular). Dry and conventional slurry packing is used for most analytical and some preparative columns [Poole and Poole, 1993]. Dynamic mechanical compression, either in the axial or annular direction, is used for packing wide bore columns meant to be used for preparative applications (radial compression has also been used for analytical columns (Little et al., 1976; McDonald etal., 1980; Rausch etal., 1980). All these packing techniques involve the application of a certain level of mechanical stress to the particles,

either individually (in the slurry) or as a group (in the growing bed). The magnitude of this mechanical stress is widely different depending on the axial and radial position inside the column. The local values of the packing density, the external porosity and the permeability are a function of the history of the local stress applied during packing and during the life of the column [Guiochon etal., 1995]. This could provide for a general mechanism explaining all the factors considered above.

Slurry Packed Columns - In the first procedure columns are packed by forcing a more or less dilute slurry (ca 5-30% packing material suspended in a solvent such as methanol, isopropanol, etc., which wets and disperses well the material [Bristow, 1978; Unger, 1979; Poole and Poole, 1993]) into the column, and which has its exit end closed with a frit. A pushing solvent is pumped at high flow rate, under a head pressure of up to 800 atm into the slurry container and then through the column pointing either upward or downward [Kaminski et al., 1982]. Bed consolidation is achieved by running the pushing solvent for 15-30 minutes through the packing.

The interaction between the particles and the high velocity fluid stream has two origins. First, the particles in the slurry are earned rapidly by the fluid stream and impinge vigorously onto the rising surface of the bed. They get imbedded in it and find a metastable position. Second, the viscous shear of the fast-moving stream of solvent pushes the particles forward and consolidates the bed. The extent of this consolidation is shown by finding that the external porosity of the packed bed decreases linearly from 0.434 to 0.399 (a 10% decrease), with increasing pressure of the packing solvent, for

columns packed with Zorbax 10 µm particles, under head pressures ranging from 72 to 770 atm.

Axial Compression Columns- These columns are similar to huge syringes which have their barrels filled with packing material. Their piston is moved by a hydraulic jack which can apply a mechanical stress adjustable up to ca 100 kg/cm^2 (Godbille et al., 1976; Colin et al., 1990; Sarker et al., 1995). The column barrel is filled with slurry (ca 30% of packing material homogeneously suspended in the packing fluid) and closed with a frit held by a bolted flange. When the piston is moved in order to compress the slurry, the excess liquid is expelled at the end opposed to the piston and a consolidated bed builds up progressively. The force of the jack moving the piston causes a stress applied against one end of the packed bed. However, stress does not convey homogeneously in solids, whether divided or not, as it does in liquids and no mechanism guarantees that the local stress be constant throughout the bed volume. It has been shown by Train [1956; 1957; 1960] that the distribution of stress in a powder mass during its compression inside a cylindrical die is far from uniform in the entire volume of the resulting tablet. Under a compression stress equivalent to 45 kg/cm^2 applied to the piston located at the top of a 5.3 x 14.5 cm die, the local stress at the end of the bed opposite to the piston was approximately 15 kg/cm^2. At the same time, this stress was 20 kg/cm^2 in the geometrical center of the bed, and against the piston wall, at its center. The stress increased with increasing distance from piston center toward the comer formed by the piston and the die wall. Three annular regions of increasing radii experienced a stress of 30, 40 and 50

kg/cm^2, respectively, with the highest stress measured in the very corner, in compassing the annular region situated between the limits.

Radial Compression Columns- These columns use a plastic cartridge closed at both ends by frits and have a stream distributor at inlet. The cartridge is filled with the packing material and is placed inside a steel cylinder. The designs of the plastic cartridge and of the steel cylinder allow a leak-proof seal at both ends. A hydraulic fluid is introduced under pressure to fill the space between the plastic cartridge and the steel cylinder and in this manner compress the bed radially [Little etal., 1976; McDonald etal., 1980; Rausch et al., 1980]. Implementations of this method of bed compression are available for both analytical and preparative columns. Columns prepared with this procedure exhibit good performance [Eon, 1978; Sarker et al., 1994, 1995, 1996; Carta et al., 1995]. Measurement of the porosity and the permeability of these columns have demonstrated the strong influence of the intensity of the radial compression stress on these properties [Sarker etal., 1994; 1996]. The external porosity of the bed decreases with increasing compression stress while the head pressure required achieving a certain flow rate increases accordingly. This result shows that the permeability follows the Blake - Kozeny correlation [Bird et al., 1960]. Nevertheless, the effects observed here are smaller than in axial compression columns due to the fact that the design of the instrument allows only a much lower compression stress (100 psi versus 1500 psi in axial compression). Although the geometry of the stress distribution in a radially compressed bed is more complex than in an axially compressed bed, a model has been proposed to relate the compression stress with the mobile phase flow rate, and the head pressure [Carta et al.,

1994]. This model is in agreement with experimental measurements obtained by Sarker et al. [1994; 1996]. It shows that the radial compression stress applied should correspond to a hydraulic pressure in the compression chamber in excess of half the head pressure. Insufficient compression levels result in dramatic failure in column performance, which is a result of cracks being formed in the bed. This model could be used, along with the independent data on the Young modulus acquired with axial compression columns, to relate the intensity of the radial compression stress to the distribution of the packing density throughout the column bed.

Flow Profiles in chromatographic Columns –

The flow profile is the image of the redistribution of the mobile phase volume elements during their travel through the column packing. It is impossible to monitor the movement of these elements with a detection system commonly used in chromatography. In fact, these detectors are designed so that their response to the mobile phase be as small as possible. At the same time, these detectors monitor promptly the passage of analyte molecules earned along by any mobile phase volume element. If these molecules travel along the column all the time with the liquid element, they entered the column with, they can function as markers of the mobile phase propagation through the column. The three-dimensional redistribution of the concentration maximum within the band of an unretained analyte exiting the column should reflect the flow profile inside the chromatographic column.

In all previous work single channel detection has been considered for mapping out the three-dimensional shape of an analyte band. This means that during one run, one series of data points was collected at one point of the column exit and compared later to data points collected in other points during subsequent runs. In this manner any variations in experimental conditions between runs could influence the differences resulted from data comparison. For this reason, it was decided to map out the flow profile inside the column by performing local, multiple channel detection, with several parallel detection channels increasing with increasing column diameter. This approach can compensate for inevitable experimental variations in the peak height, area, shape, and retention time which are usually encountered even when experimental conditions are maintained constant with special care.

2.5 Calculation Method for Asymmetry & HETP

2.5.1 Asymmetry

The asymmetry factor is a measure of peak tailing. It is defined as **the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope**, with all measurements made at 10% of the maximum peak height.

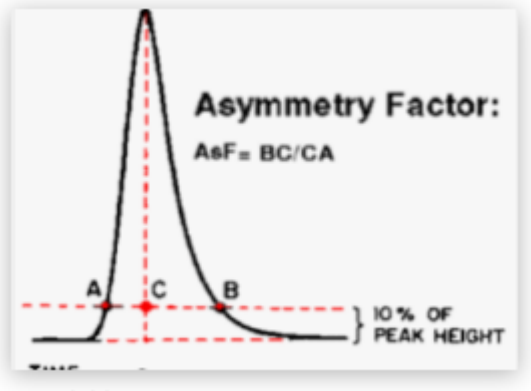


Figure 2.2: Asymmetry calculation Curve (Giddings, 1965)

The peak asymmetry is defined as the tail width / front width. A chromatogram is a representation of the separation that has chemically [chromatographically] occurred in the HPLC column. A series of peaks rising from a baseline is drawn on a time axis. Each peak represents the detector response for a different compound (Tosoh Bio science, 2018).

Peak width is the distance between points where lines tangent to the peak's left and right inflection points intersects the baseline and is calculated using equation (1). The USP (United States Pharmacopeia) uses this method. This results in small N (number of plates) values when peak overlap is large.

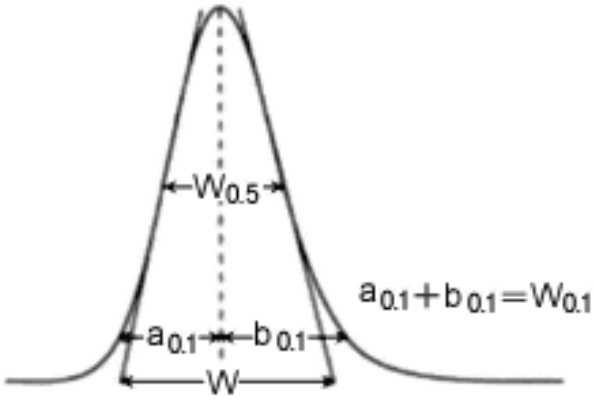


Figure 2.3: Peak width calculation (Giddings, 1965)

2.5.2 HETP (Height Equivalent to Theoretical plates)

Theoretical plates represent a hypothetical division of chromatographic columns, and each plate represents an equilibrated partitioning of the solute between the stationary and mobile phases. Any chromatography does not have any physical plates, but it is result of mathematical calculation. The columns having higher number of theoretical plates are considered more efficient in HPLC separation. A more efficient column has a narrower peak than the less efficient column with same retention time (Giddings, 1965).

Theoretical plates should be determined under specific set conditions. Temperature plays an important role and can alter the no of plates. All the columns don't have same number of plates, but it depends on flow rate, viscosity of mobile phase and the molecular weight of the compound to be analysed.

Theoretical plate number (N) is an index that indicates column efficiency. It describes the number of plates as defined according to plate theory and can be used to determine

column efficiency based on calculation in which the larger the theoretical plate number the sharper the peaks.

Theoretical plates in column chromatography are directly related to the efficiency of its column. Therefore, it can assist in improving the resolution, which is directly proportional to the square root of theoretical plates' number when all other variables are kept constant.

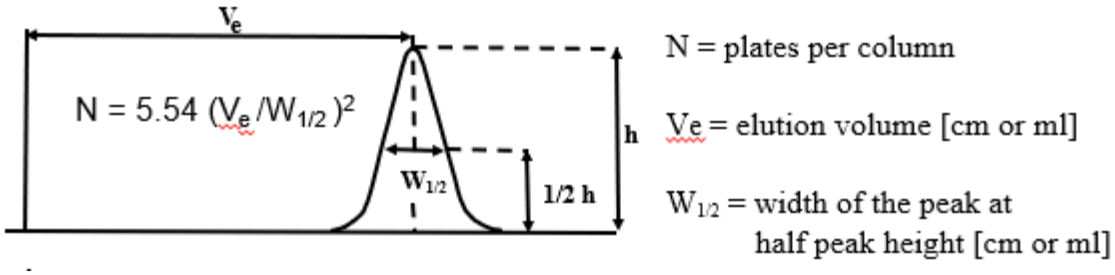


Figure 2.4: HETP calculation (Giddings, 1965)

2.5.3 Peak Area

The area under a peak [peak area count] is a measure of the concentration of the compound it represents. This area value is integrated and calculated automatically by the computer data station.

In a LC chromatogram, the size and area of the component peak are proportional to the amount of the component reaching the detector. The peak area is proportional to the amount of the component.

2.5.4 Peak Height

It is the distance from the bottom or baseline of the peak to its apex. The bottom of the peak is defined by either a zero-absorbance value or a calculated baseline for increased accuracy.

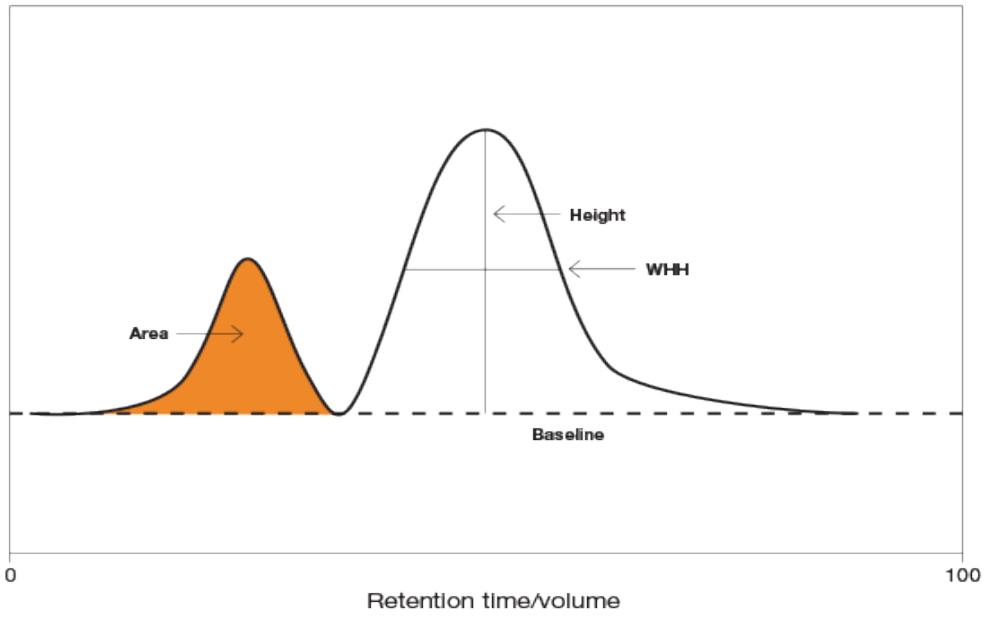


Figure 2.5: Peak height calculation (Giddings, 1965)

CHAPTER 3

Materials & Methods

3.1 Materials

This section describes in detail various materials used for the study. Materials used are Resin (three types of resins are used), Hardware (which includes column tube, adaptors, frits, hexagonal caps, and O rings. Also, used are various chemicals. Chemicals used for making resin slurry and chemicals used for analysing the columns when installed on AKTA^{TM} (FPLC).

Resin - Chromatography resins encompass ion exchange, affinity and mixed mode chemistries coupled with resin beads that offer high flow and superior purification capabilities. Use of salt-tolerant or mixed-mode resins can lead to significant time and cost savings by eliminating dilution and adjustment steps. Resins are industry proven to perform packing and purification from benchtop to commercial scale, with several formats to accommodate screening through commercial production needs. Chromatography resins deliver high purity within a simplified process design for small and medium-sized biomolecules. Resins offer flexible process integration without the need for feed stream manipulation. Broad portfolio covers end users' process requirements and addresses their purification challenges. Unique selectivity with mixed-mode ligands for both capture and purification will increase the productivity of the existing standard purification platforms.

3.1.1 Chromatography Resins from Tosoh Corporation

TOYOPEARL® chromatography resins are hydrophilic, macroporous, bulk processing media designed especially for large-scale chromatography applications. TOYOPEARL® resins are available for the most common modes of liquid chromatography: size exclusion (SEC), hydrophobic interaction (HIC), ion exchange and Affinity chromatography. Based on their semi-rigid backbone structure TOYOPEARL® resins assure excellent pressure/flow characteristics. TOYOPEARL® is stable over the pH 2-13 range. Particle sizes are 20-40 µm superfine grade (S) for the highest performance, 40-90 µm for economical purification (M), and 90-120 µm coarse grade (C) and 100 - 300 µm extra coarse grade (EC) for the largest scale chromatography.

Following Resins were used for current study and their properties are listed below:

3.1.1.1 TOYOPEARL® Phenyl-650M

TOYOPEARL® Phenyl-650M is a hydrophobic interaction chromatography (HIC) resin for biomolecule purification. The resin is composed of a base material of hydroxylated methacrylic polymer beads that have been functionalized with a Phenyl ligand group.

TOYOPEARL® Phenyl-650M is based on a 100 nm (1000 Å) pore size polymethacrylate base material bonded with phenyl groups. This resin has an intermediate hydrophobicity. Primary applications are for the separation of proteins, their isoforms, and aggregate removal. It is typically used in intermediate purification and polishing steps. For high resolution separations, the use of TSKgel®SP-5PW (20) media is suggested. Both products have the same selectivity.

TOYOPEARL® Phenyl-650M is available in three particle sizes, S-, M-, and C-grade.

Table 3.1: Properties of TOYOPEARL® Phenyl-650M resin

Product Attributes

Pore size (mean):	100 nm
Particle size (mean):	100 µm (C-grade) 65 µm (M-grade) 35 µm (S-grade)
Pressure rating:	0.3 Mpa
Shipping buffer:	20% ethanol
pH stability:	>1-13
Shelf life:	10 years (estimated)

TOYOPEARL® Phenyl-650M resin can be used for high throughput capture, intermediate purification, and polishing process steps. The primary applications of this resin are for the separation of proteins, their isoforms, and aggregate removal.

3.1.1.2 TOYOPEARL® Hexyl-650C

TOYOPEARL® Hexyl-650C is based on a 100 nm (1000 Å) pore size polymethacrylate base material bonded with C6 groups. This resin has the highest hydrophobicity of the TOYOPEARL HIC resins. It is used for very hydrophilic proteins or where a low salt elution environment is required. It is useful as an early capture step or for intermediate purification.

TOYOPEARL chromatographic resins are based on a rigid methacrylic polymer, resulting in high mechanical and chemical stability. Resins are available as non-functionalized "HW" series resins for size exclusion separations, and derivative with surface chemistries for alternative modes of chromatography such as ion exchange, hydrophobic interaction, or affinity separations. TOYOPEARL® Hexyl-650C chromatographic resins are designed for hydrophobic interaction chromatography. This chromatographic mode separates molecules on the basis of hydrophobic interactions between the sample and the ligand. The separation is usually accomplished in buffered aqueous solution with a gradient of decreasing ionic strength.

Table 3.2: Properties of TOYOPEARL® Hexyl-650C resin

Product Attributes

Pore size (mean):	100 nm
Particle size (mean):	100 µm (C-grade)
Pressure rating:	0.3 Mpa
Shipping buffer:	20% ethanol
pH stability:	>1-13
Shelf life:	10 years (estimated)

TOYOPEARL® Hexyl-650C resin has the highest hydrophobicity of the HIC ligands offered by TBL. It is used for very hydrophilic proteins or where a low salt elution environment is required. It is useful as an early capture step or for inter resin purification.

3.1.1.3 TSKgel ® SP-5PW (20)

TSKgel ® SP-5PW (20) is a strong cation exchange resin for biomolecule purification. It is composed of highly crosslinked polymethacrylate beads that have been functionalized with sulfopropyl (SP) strong cation exchange groups. TSKgel ® SP-5PW (20) resins are available in two particle sizes: 30 µm and 20 µm, and ideally suited for high resolution polishing steps. TSKgel ® SP-5PW (20) chromatographic resin is designed for ion exchange chromatography. This chromatographic mode separates molecules based on ionic interactions between the sample and the resin. The separation is usually accomplished in buffered aqueous solution with a gradient of increasing ionic strength. Alternatively, pH adjustment may be used for control of elution.

Table 3.3: Properties of TSKgel ® SP-5PW (20) resin

Product Attributes

Pore size (mean):	100 nm
Particle size (mean):	30 µm 20 µm
Pressure rating:	0.2 Mpa
Shipping buffer:	20% ethanol
pH stability:	2-13
Shelf life:	10 years (estimated)

3.1.2 Column Hardware

Column Hardware is one of the most important items for packed bed design. In present study it is made of Polymethylmethacrylate as we want to have see through columns and has below properties which makes it favorable for use in this application –

Polymethylmethacrylate is a crystalline material noted for its high strength to weight ratio, excellent chemical resistance, and high performance in thermoforming and corrosive environments.

Various components used in the hardware are as shown in the Figures 3.1 and 3.2 below:

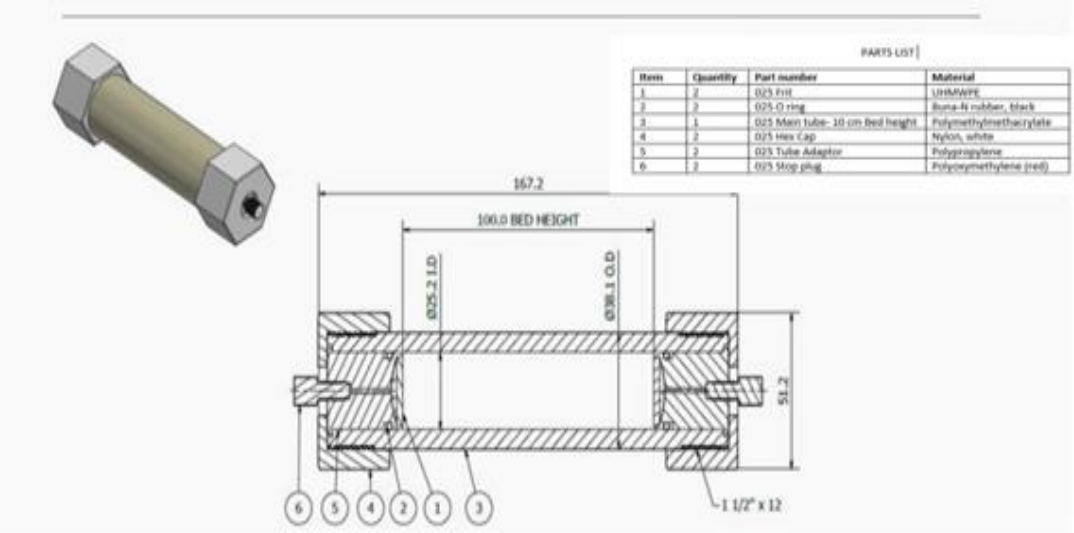


Figure 3.1: Detailed description of Hardware for 2.5 cm column (PolyAnalytik, 2019)

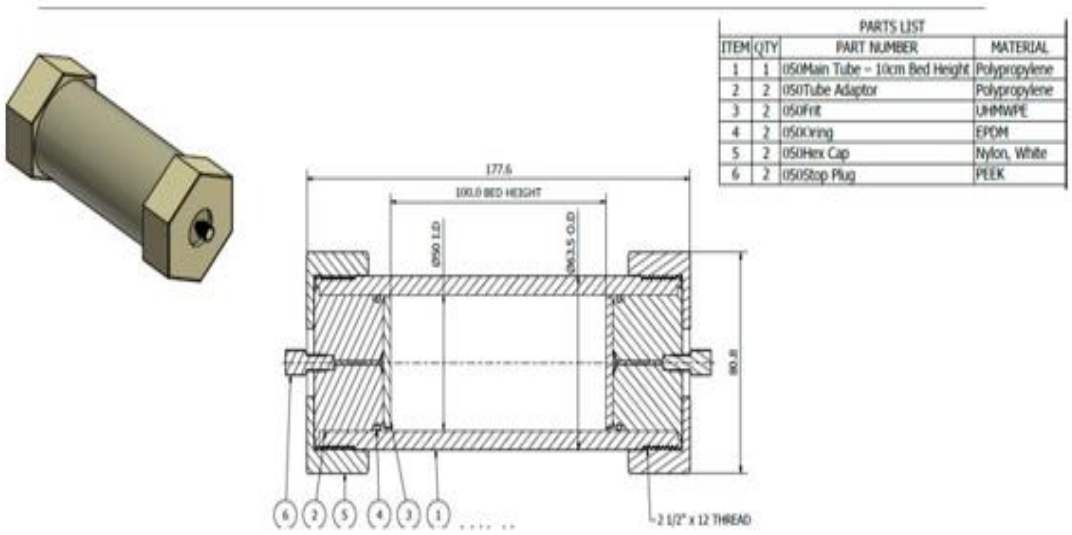


Figure 3.2: Detailed description of Hardware for 5 cm column PolyAnalytik, 2019)

Details of individual components for 2.5 cm Column are as below:

Frits – Frits are used at top and bottom of the column to ensure support for the resin and no passage of resin along with buffer.

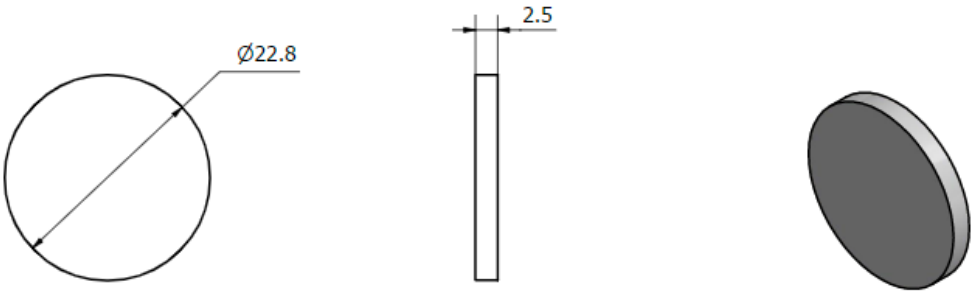


Figure 3.3: Frit design used in adaptor for 2.5 cm column (PolyAnalytik, 2019)

Hexagonal Cap- This is used for tightening the adaptors after the column is packed, so that the packed bed is secured tightly avoiding any displacement of resin and the column contents.

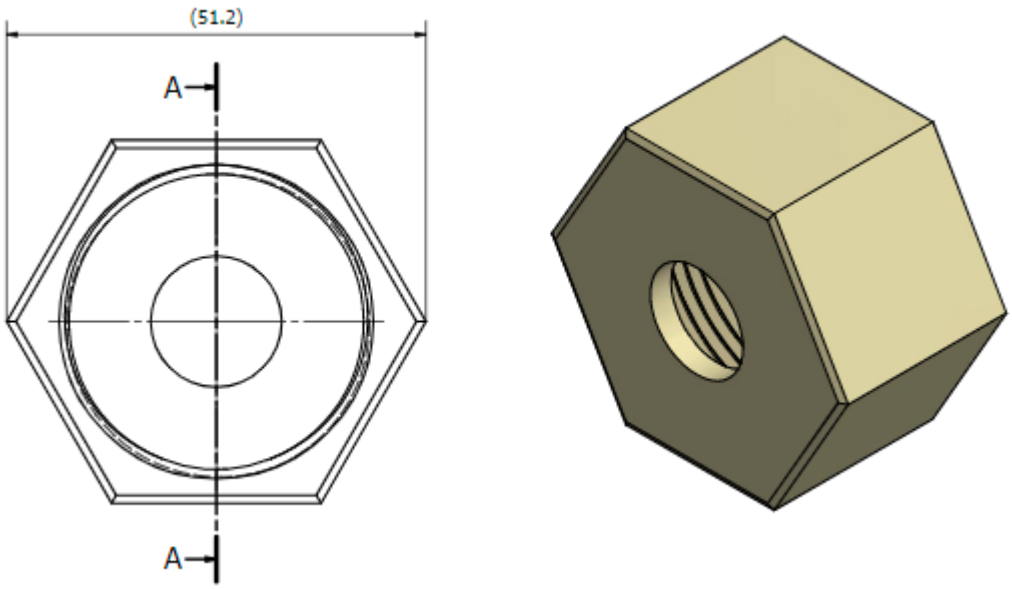


Figure 3.4: Hexagonal cap for column ends (PolyAnalytik, 2019)

Main Column Tube- Tube is used for packing the resin and acts as packed column area and avoiding any voids in the resin bed.

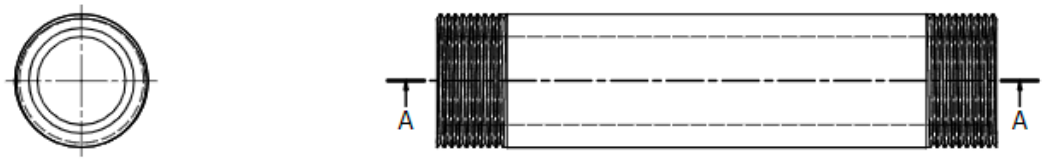


Figure 3.5: Main column tube for resin bed (PolyAnalytik, 2019)

Tube Adaptor – Used on both sides of the column tube to hold resin in place in the bed and to ensure proper distribution of buffer (liquid) with the help of vanes (baffles) inside each adaptor.

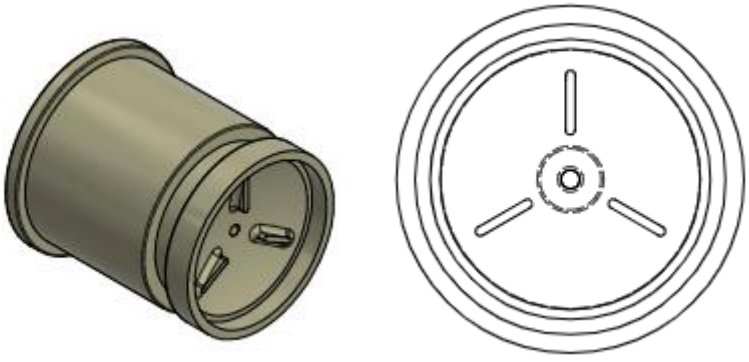


Figure 3.6: Adaptor for 2.5 cm column (PolyAnalytik,2019)

O ring – This is used in the Tube adaptor to secure the adaptor firmly to the main column tube ensuring no leakage of resin out of the column

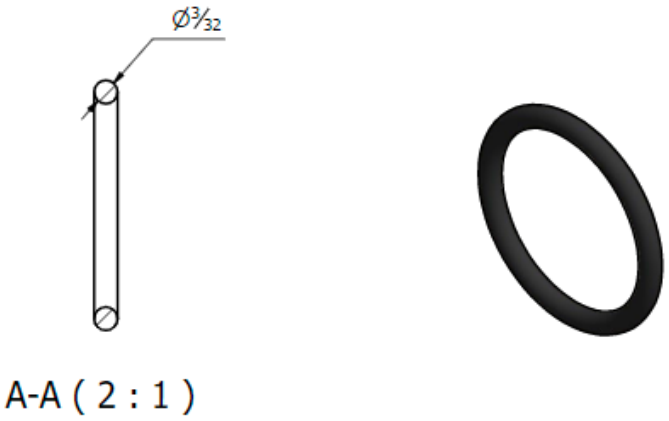


Figure 3.7: O ring for adaptor (PolyAnalytik, 2019)

Stop Plug – This is used to plug the inlet of the Tube adaptor, after the column has been packed fully and tightening the adaptor with hexagonal cap. This will prevent buffer leakage out of the column and ensure column does not dry up on storage.

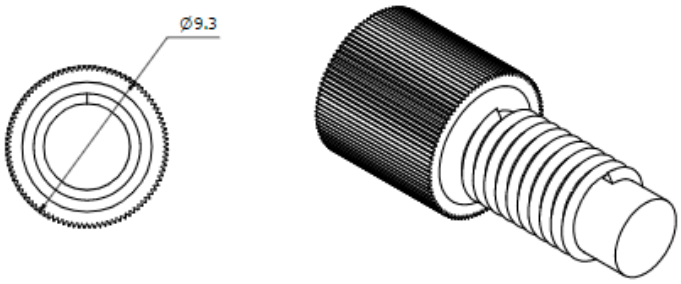


Figure 3.8: Stop plug to seal column ends (PolyAnalytik,2019)

3.1.3 Chemicals

Chemicals were used during the process of treatment of resin (slurry preparation) and during packing of the column and then finally were required for analyzing the columns on FPLC.

For all the three resins used for the study need 2M Sodium chloride which is used for making slurry and for doing solvent exchange for all the resins. Also, all the three resins use 2M Sodium chloride as packing buffer.

For analysis of the columns 1M Sodium chloride is used along with 3M Sodium chloride as tracer.

Various concentration of solutions were prepared from solid Sodium chloride crystals and then filtered before using them for making slurry or using it for FPLC for column analysis.

Sodium chloride is easily soluble in water and partially soluble or insoluble in other liquids. In its aqueous state NaCl acts as a good conductor of electricity due to the free movement of the ions and hence is commonly used as a conductor.

3.2 Equipment

Various packing methods and conditions can result in beds that have different structural characteristics. Best practices of different packing methods can be found in the respective vendor application notes and industry guidelines. The packing technique applied depends on several critical factors such as the mechanical and physical properties of the resin (including rigidity, particle shape, shear resistance, density, and size) as well as hardware capabilities and integrity. Therefore, as much as 30% additional resin is needed to achieve the required column volume.

PolyAnalytik has developed a procedure where Pressurised packing vessel (PPV) is used for packing the columns of diameters like 2.5 cm and 5 cm. Using PPV for packing columns has below advantages over other packing equipment's used in an industry:

- Lighter design
- Fast resin chamber refill capability
- Fine pressure control
- Gentle stirring mechanism
- More robust packing adapter
- Larger vessel capacity
- Sanitary fittings throughout
- Easy to clean and maintain while changing from one resin to another

Below Figure shows the basic design of Pressurized packing vessel (PPV) –

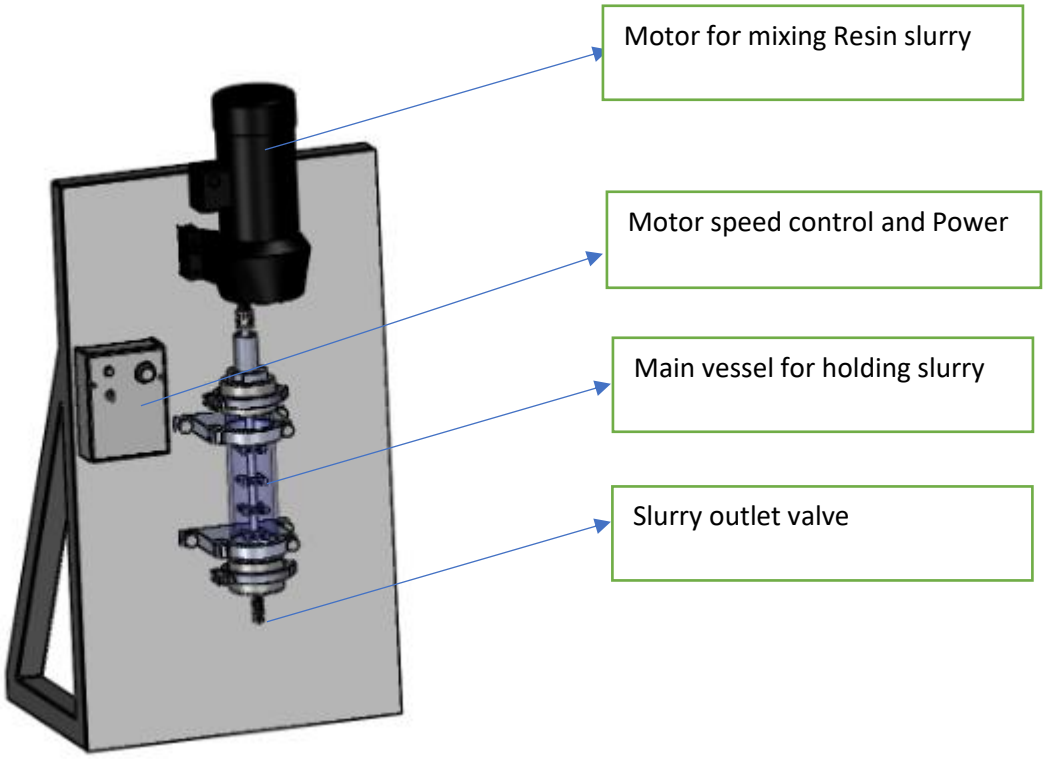


Figure 3.9: Pressurized packing vessel (PPV) (PolyAnalytik, 2019)

3.3 Experimental Set up

3.3.1 Packing apparatus Preparation

1. Pressurised packing vessel (PPV) is cleaned (i.e., no residual resin, no build-up, no dust, or dirt. Vessel is taken out from the assembly and cleaned fully with DI water and soap and flushed well with DI water till clean and then fixed to the assembly. Cleaning is done every time we want change resin type.
2. 1250 mL Vessel is positioned to the support in the drawer. Bottom valve is kept closed.
3. Slurry previously prepared is mixed until it is a homogenous suspension by mixing the resin with paddle in jar. Resin is added quickly and carefully to the vessel. Top of the vessel is returned to the assembly. Ensure the black rubber seal is seated properly on the vessel. Vessel is sealed with sanitary fitting and tightened with the bolt.
4. Charged vessel is taken and aligned with the motor and spider (black hexagon rubber) pushed up into place while also ensuring it is positioned in the support system. Supports are fastened on the top and bottom portion of the vessel making sure it is still making a good connection with the motor.
5. N_2 line is attached by connecting the N_2 line to the push-to-connect fitting on the vessel lid. N_2 tank is opened and ensuring the regulator on the N_2 tank is no higher than 8 bar (116 psi). Valve connecting the N_2 tank regulator is opened, and the regulator connected to PPV.
6. Pressure is set to the correct setting for the resin being packed.

Figure 3.10: Picture of a clean vessel for PPV from the top (left) and side (right).

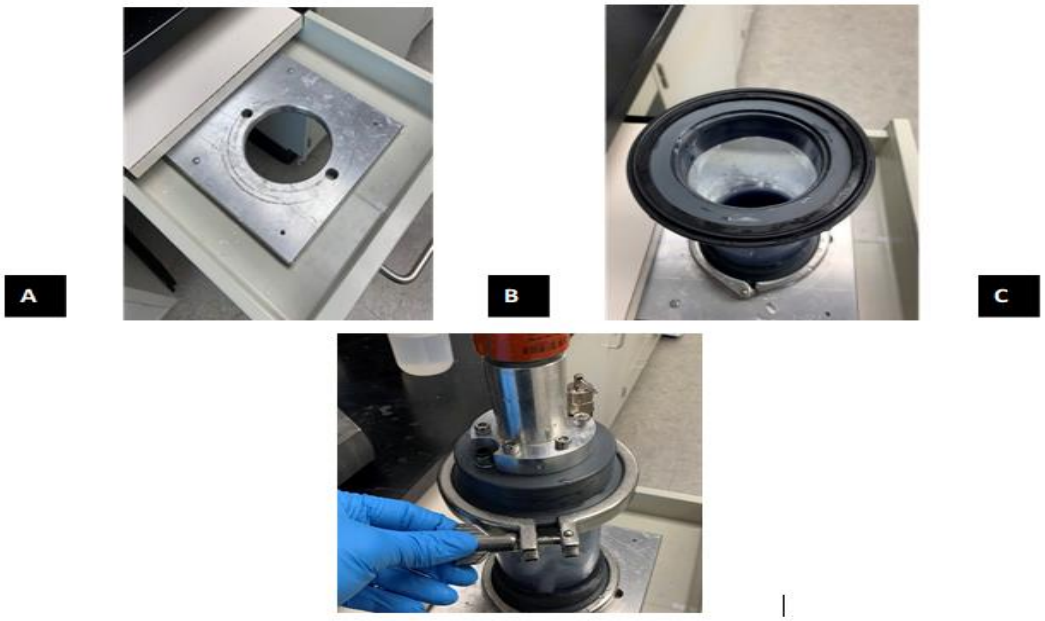


Figure 3.11. PPV assembly before adding resin

A) The support for PPV **B)** The top of the vessel with the black rubber ring installed **C)** Tightening the sanitary fitting on PPV after adding the resin slurry to the vessel.

3.3.2 Operating Procedure

Steps followed for packing the columns:

1. Three resins, TOYOPEARL® Phenyl-650M, TOYOPEARL® Hexyl 650 C and TSKgel ® SP-5PW (20) from Tosoh Bioscience are used for packing 2.5 cm & 5 cm columns
2. Each resin and each parameter be packing 3 columns each for the study to have an average of 3 columns for the readings and observations

All the resins are solvent exchanged with respective buffers as per list below:

Table 3.4: Packing conditions of Resins used for study

Resin Name	Slurry: Buffer ratio	Packing Buffer	Packing pressure(bar)	Packing adaptor	Vibration
TOYOPEARL Phenyl 650 M	50:50	2M NaCl	0.25	Regular	No
TOYOPEARL Hexyl 650C	50:50	2M NaCl	1.25 for 2.5 cm 0.75 for 5 cm	Regular	No
TSKgel SP-5PW(20)	26:74	2M NaCl	1.0	Regular	Yes(1bar)

3. Packing conditions of the columns are as per Table 3.4 above and Pressurized packing vessel (PPV), of bigger size is used.
4. Once the columns are packed, they are put on AKTA™ Explorer 100 for analysing Asymmetry (AS), Number of plates and observe peak details, in terms of height and Area.

5. Once AS is within required range (0.8-1.4), the column is considered pass.
6. All the columns are transparent (opaque) and are observed from outside with light for bed packing structure or for any voids and relate it to the AS values obtained.
7. Columns are installed again on AKTA^{TM} and a Tracer Dye injected in the same direction as normal flow of buffer till we get dye at the bottom of the column and then stop injecting the dye.
8. The above step is done on one column, out of the three columns packed.
9. Dye is injected to determine the flow distribution of the buffer in the column and to observe the packing of the resin. This is done visually, and observations noted.
10. Column is taken where dye has been injected and cut into pieces, to observe the dye distribution along the length of the column.
11. Pictures of the cut column are taken for dye distribution and observations noted.
12. These observations help in predicting the column bed structure and the performance.
13. All the results and observations are tabulated in the observation sheet.

3.3.3 Experimental Parameters Selection

Research study requires proper planning of the parameters to be studied depending on the objective of the study. PolyAnalytik have been packing the columns and were aware of the parameters followed for the packing of 2.5 cm and 5 cm diameter columns. Based on those parameters, below were planned for study which are crucial for the performance of the column:

Table 3.5: Experimental Parameters for the study

Experimental Parameters	Current	Changed to	Significance	To see impact on
Frit Thickness(mm) 2.5 cm column	2.5	1.5, 3.2	Placed in adaptor & determines liquid flow while packing and separation of resin from buffer	Packing time, Asymmetry, Number of Plates
Frit Thickness(mm) 5 cm column	3.2	1.5, 6.3	Placed in the adaptor & determines liquid flow while packing and separation of resin from buffer	Packing time, Asymmetry, Number of Plates
Adaptor number of vanes (2.5 cm)	3	6,9	Stationary baffles in adaptor to have better liquid distribution in the bed	Asymmetry, Number of plates, packing time
Adaptor number of vanes(5 cm)	8	12,16	Stationary baffles in adaptor to have better liquid distribution in the bed	Asymmetry, Number of plates, packing time
Column Surface roughness (micron RA)	0.8	0.4	Help improve liquid flow along the walls	Time for Buffer to flow

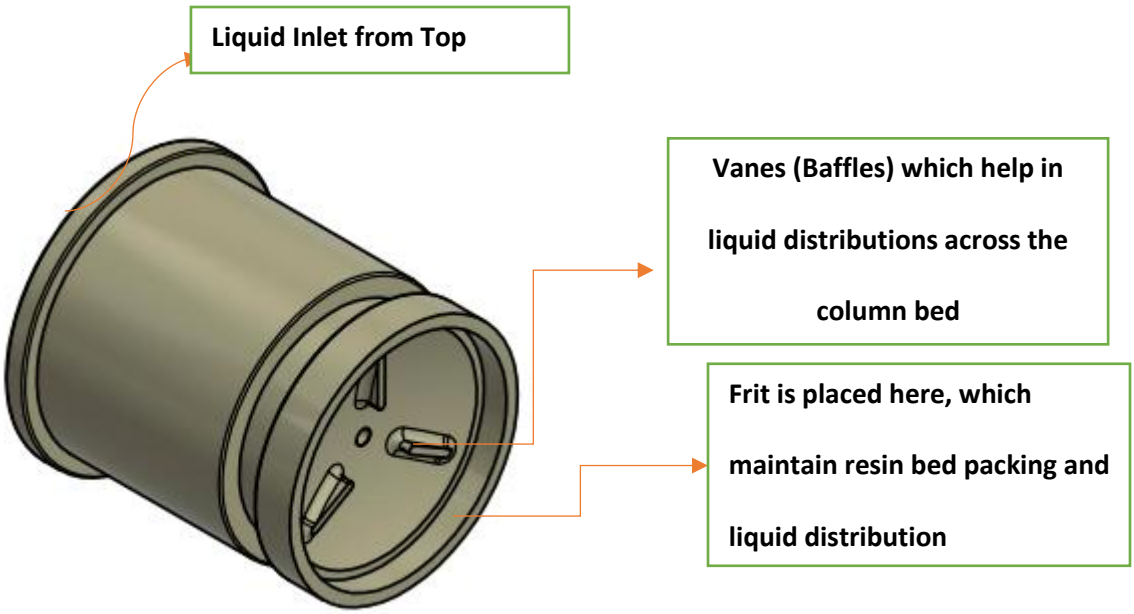


Figure 3.12: Parameters to study Schematic (PolyAnalytik, 2019)

All the parameters planned carefully ensuring the maximum impact on the column performance based on our experience and based on the data available in the literature.

3.3.4 Experimental Run

a. All the columns are packed as per steps detailed out in section 3.3.2. Resin is prepared per procedure and as per Table 3.4 in respective buffer and ratio indicated.

b. Refer to the figures below for the steps followed.

Detailed description and photos of each stage of the process are depicted as below for easy understanding:

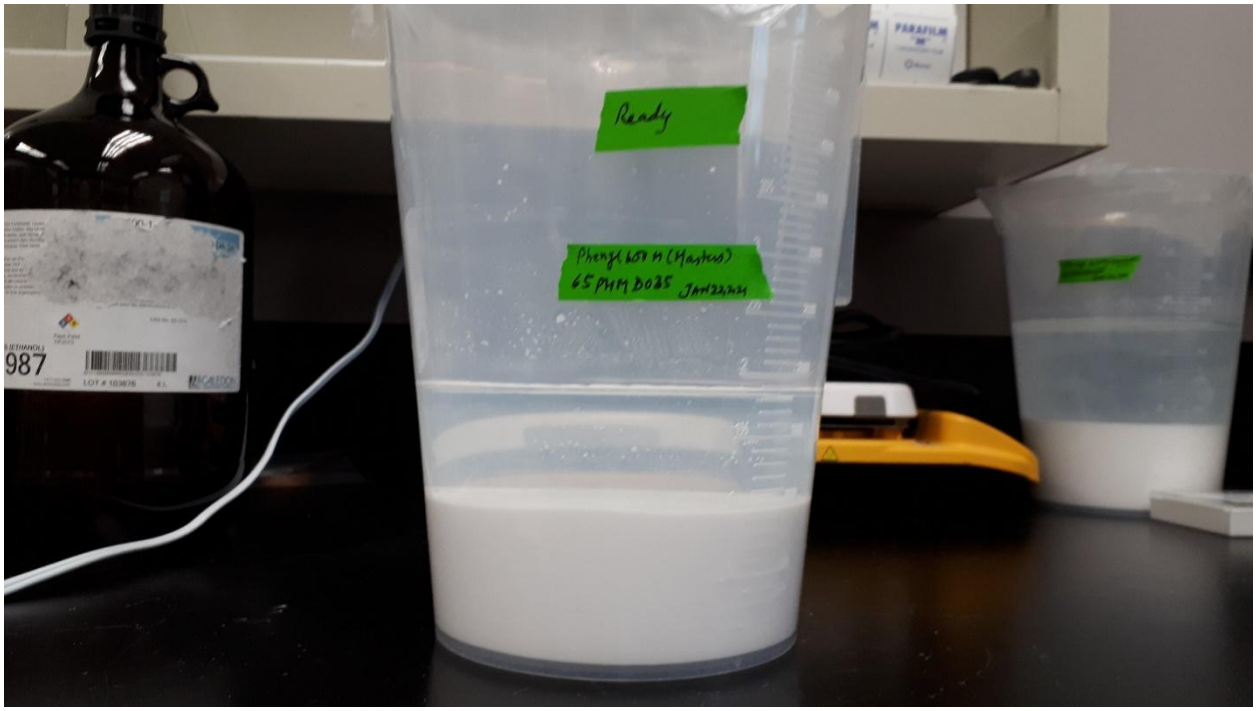


Fig 3.13: Resin Ready in Buffer (Resin preparation for packing column)

Fig 3.14: Hardware Ready (Hardware inspected and made ready with Frits/O rings

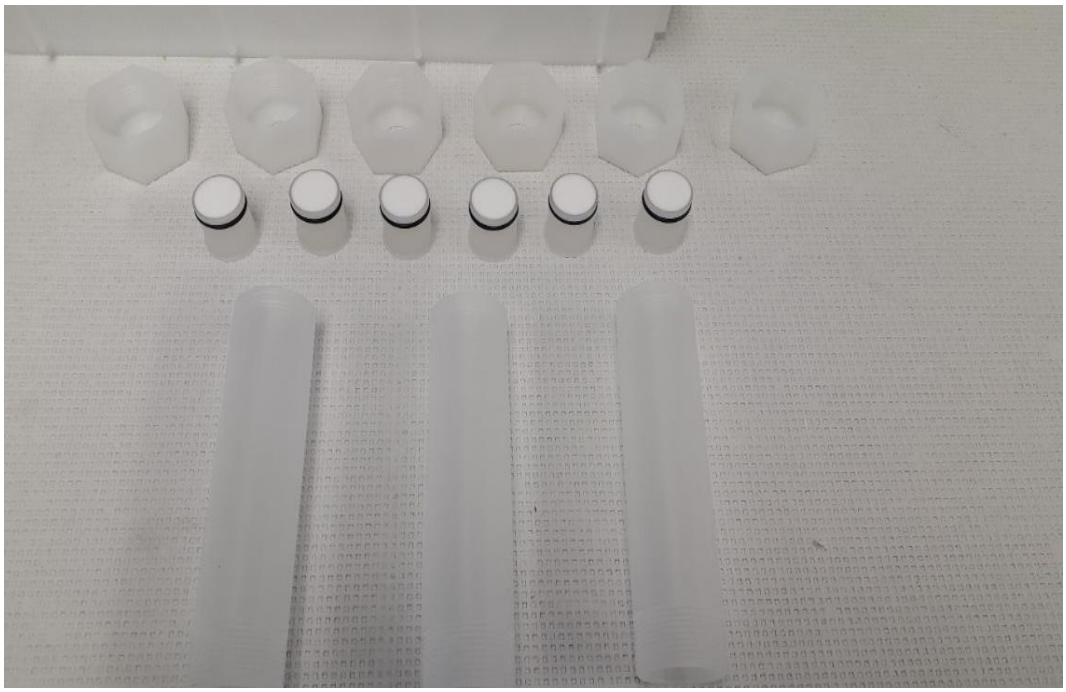


Fig 3.15: Hardware ready (Hardware inspected and made ready with O rings& frits)

Fig 3.16: PPV with Column attached (as the column being packed)

Fig 3.17: Columns Packed (Columns packed and ready for Analysis)

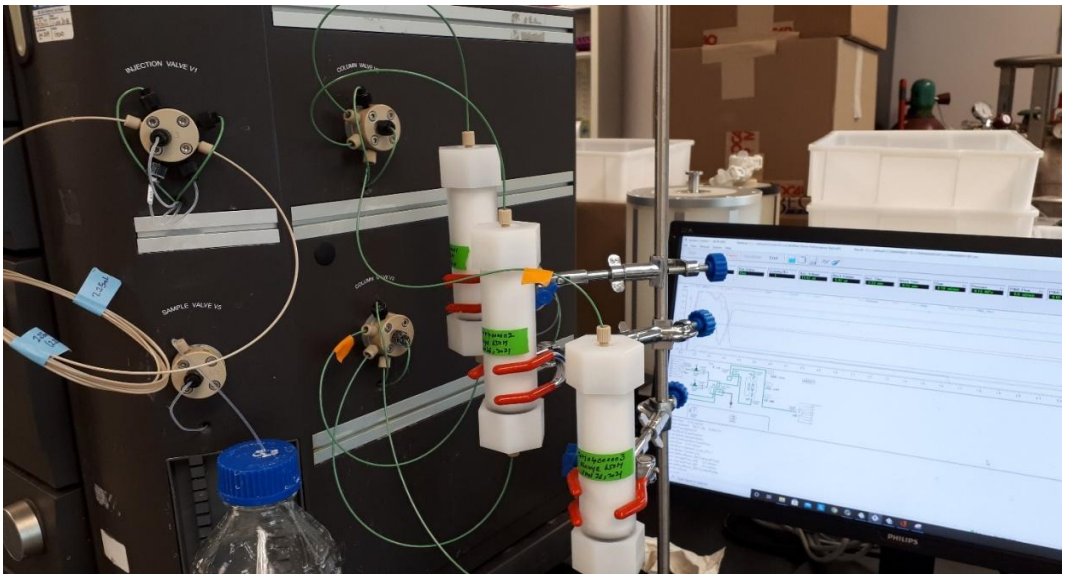


Fig 3.18: Columns on AKTA (for analysis)

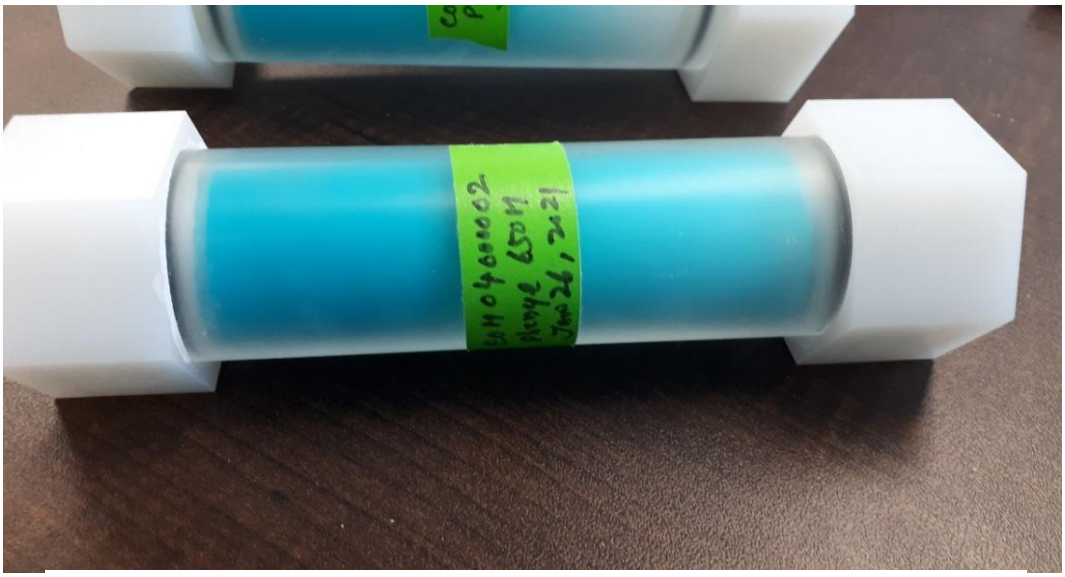
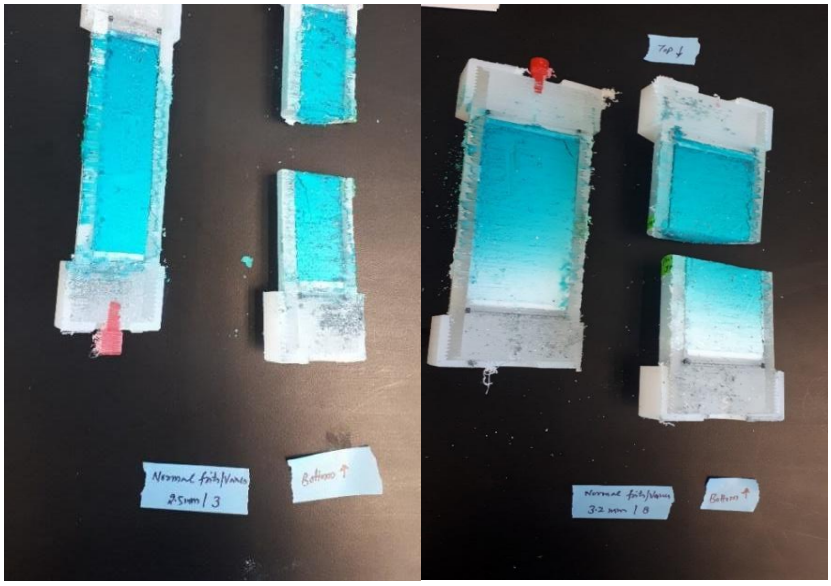


Fig 3.19: 2.5 cm Column after dye study (to study liquid distribution)



2.5 cm Column Cut	5.0 cm Column Cut

Fig: 3.20: Liquid Flow distribution Cross section

CHAPTER 4

RESULTS & DISCUSSION

This Chapter discusses various results obtained through the Study performed. Three different types of resins were used for the study. Method and materials used for the study are covered in Chapter 3. Three resins were selected in a way that they have different particle size to see if that has any impact on the column's performance ensuring similar packing procedures.

Column diameters selected were 2.5 cm and 5 cm. Different columns were packed with different set of variables as indicated in Table 3.4 and packing conditions followed are indicated in Table 3.5. Three columns were packed of each resin with each variable and then average of readings done. Each column was run on AKTA^{TM} explorer (FPLC) and obtained 3 chromatograms for each column.

Main Goal of the study performed is to understand the ways to improve packed column efficiency and to improve the performance of the packed columns in terms of liquid distribution, which will help the column usage for various applications.

Below is the list of various studies conducted:

1. To study effect of frit thickness on the column efficiency
2. To study the effect of no of vanes(baffles) on column efficiency
3. To study effect of Internal Surface roughness of column on liquid distribution
4. To study effect of Frit thickness on Peak area

5. To study the effect of frit thickness on Asymmetry of the column

4.1 TOYOPEARL® Phenyl-650M

4.1.1 Column Diameter- 2.5 cm

- **Effect of frit thickness on Column efficiency**: Efficiency is ultimately related to the plates in a packed column. The higher the number of plates, more efficient the column is. The figure below clearly shows as we increase frit thickness the column efficiency drops. We need to optimize for frit thickness to get best efficiency and satisfy other requirements for the column.

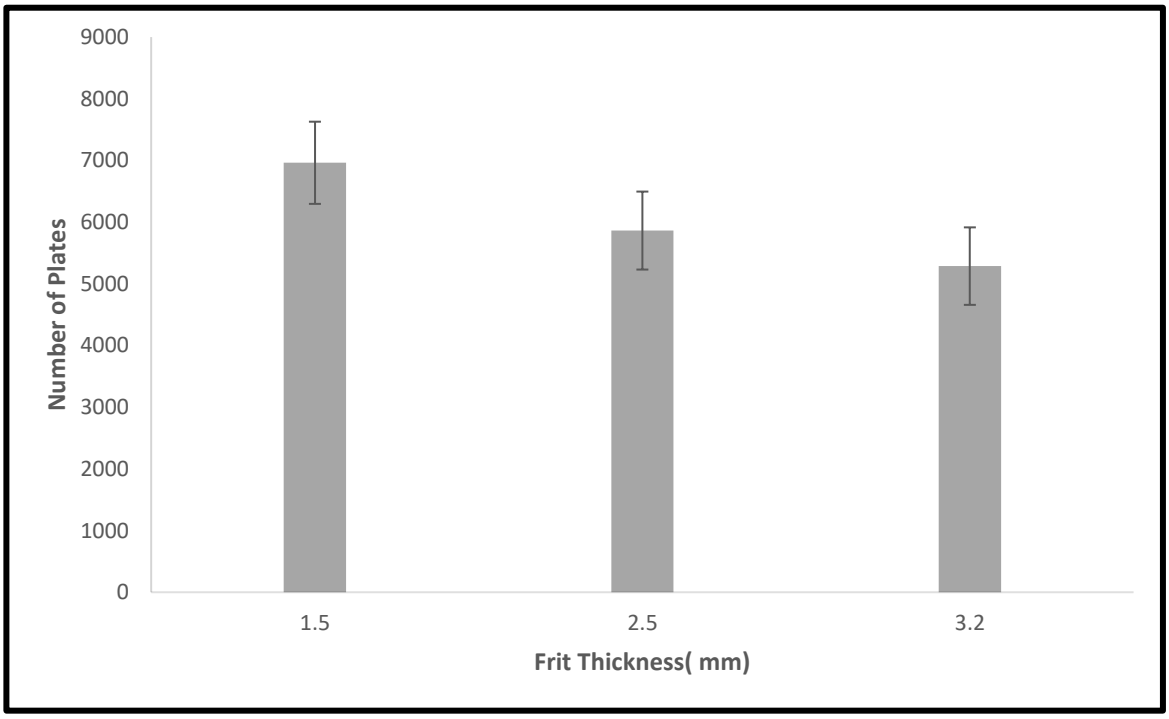


Figure 4.1: Effect of frit thickness on Plates for 2.5 cm column- Phenyl 650M resin

-Effect of frit thickness on Peak Area: Peak area is the direct measure of column efficiency. Broader the peak means poorer the column efficiency. Peak shape can indicate about the column structure. The figure below shows the effect of frit thickness on Peak area. It is clear from the figure below, as frit thickness is increased peak area increases and hence the column efficiency drops

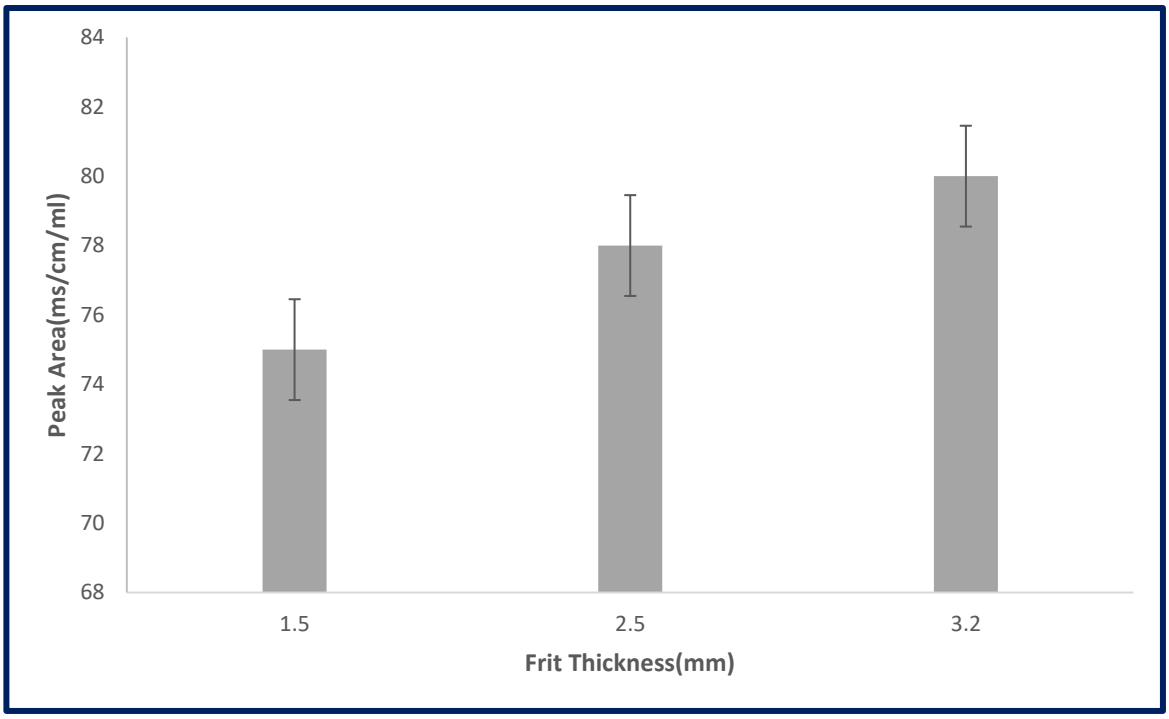


Figure 4.2: Effect of frit thickness on Peak area for 2.5 cm column – Phenyl 650M resin

-Effect of No of vanes on Liquid Flow distribution: Adaptor is used at the top of the column for keeping the column bed in position and this adaptor have vanes (baffles) which help in better liquid distribution inside the packed column. While using the column for certain applications, interaction between liquid and the resin bed is very important and determines the recovery desired.

The figure below shows the effect of changing no of vanes on the flow distribution efficiency. As the no of vanes are increased in the adaptor, flow distribution increases.

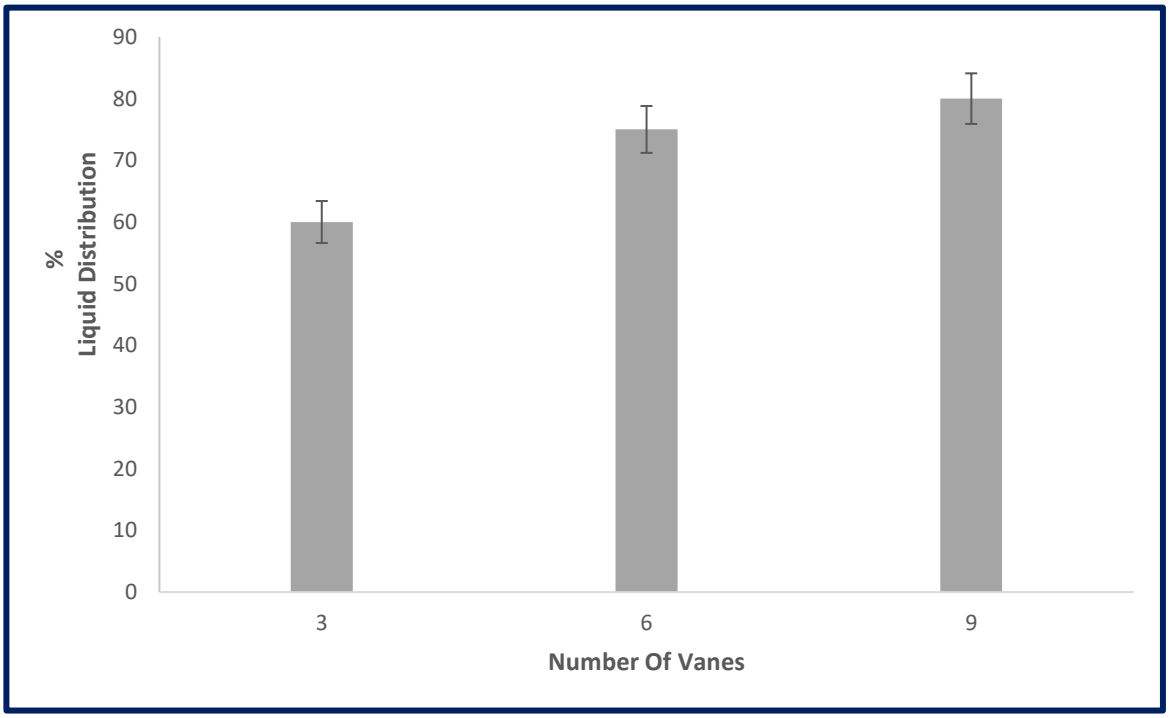


Figure 4.3: Effect of number of vanes on Liquid flow distribution for 2.5 cm column – Phenyl 650M resin

-Effect of Column Internal surface Roughness on flow distribution: Columns used are made of polypropylene and the smoothness of the column bed can play a major role in the bed integrity and flow distribution of the liquid which can impact the column effectiveness. Column roughness has tendencies to cause voids in the column and can impact column efficiency.

The figure below shows how reducing the roughness improves flow distribution inside the column.

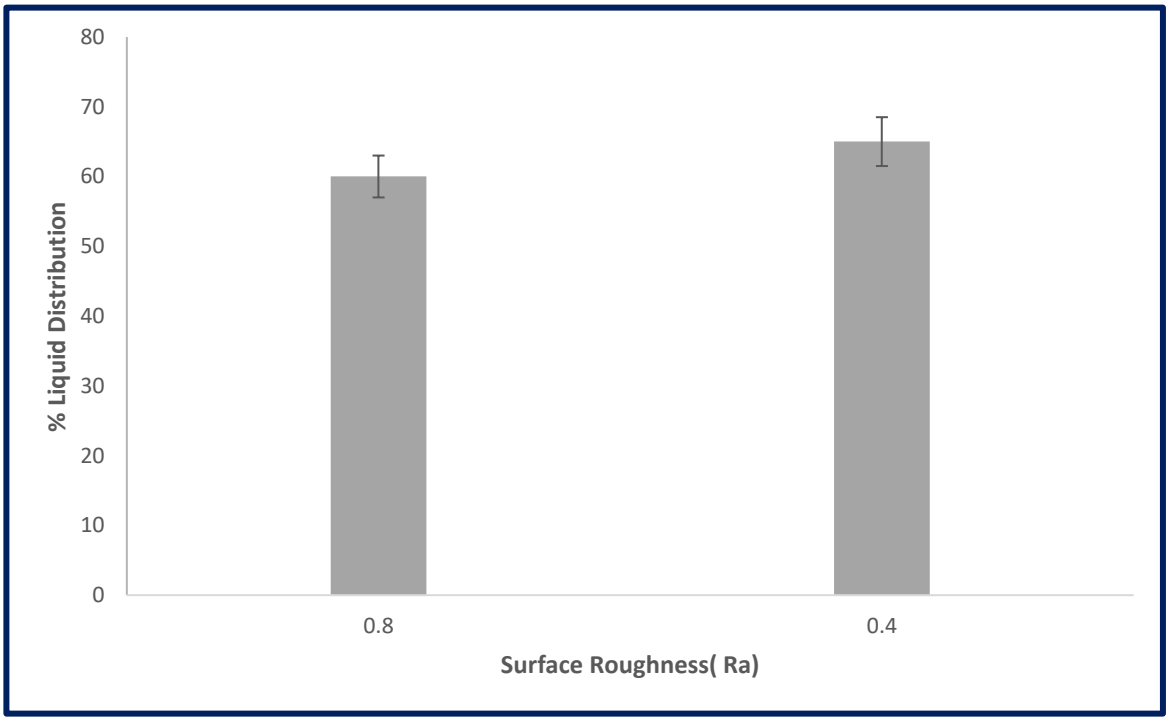


Figure 4.4: Effect of surface roughness n Liquid flow distribution for 2.5 cm column –

4.1.2 Column Diameter- 5 cm

- Effect of frit thickness on Column efficiency: It is ultimately related to the plates in a packed column. Higher the number of plates, more efficient the column is. The figure below clearly shows as we increase frit thickness the column efficiency drops. We need to optimize for frit thickness to get best efficiency and satisfy other requirements for the column.

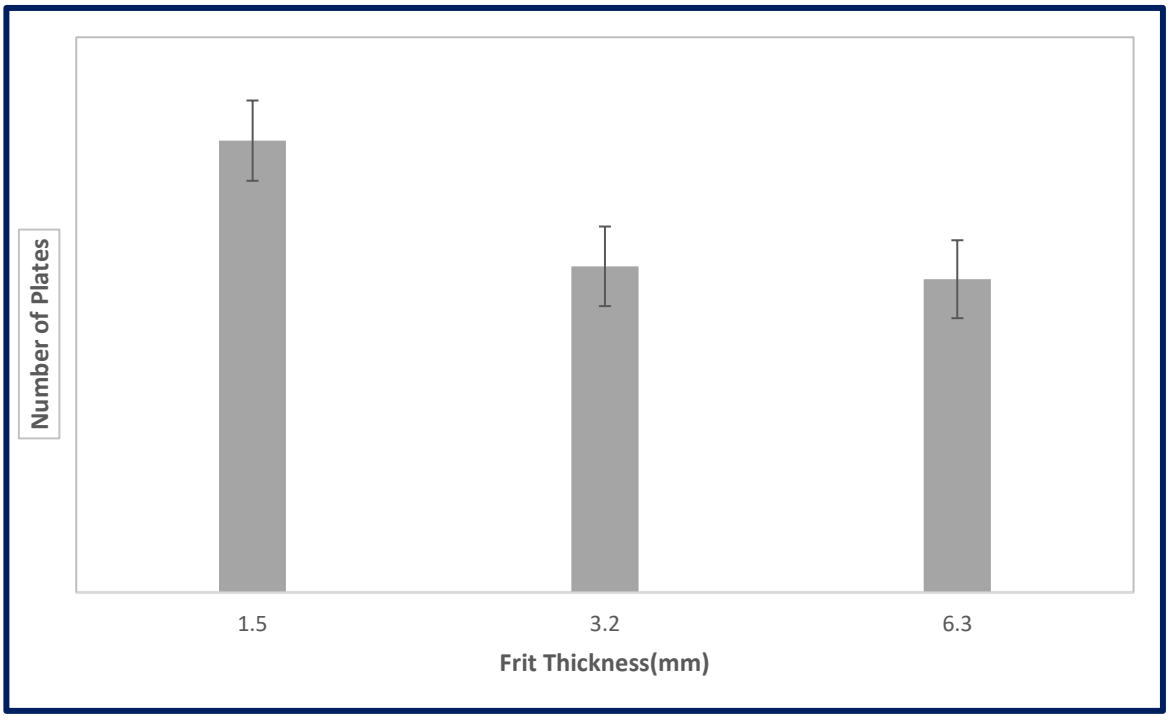


Figure 4.5: Effect of frit thickness on Plates for 5 cm column- Phenyl 650M resin

-Effect of frit thickness on Peak Area: Peak area is the direct measure of column efficiency. Broader the peak means poorer the column efficiency. Peak shape can indicate about the column structure. The figure below shows the effect of frit thickness on Peak area. It is clear from the figure below, as frit thickness is increased peak area increases and hence the column efficiency drops

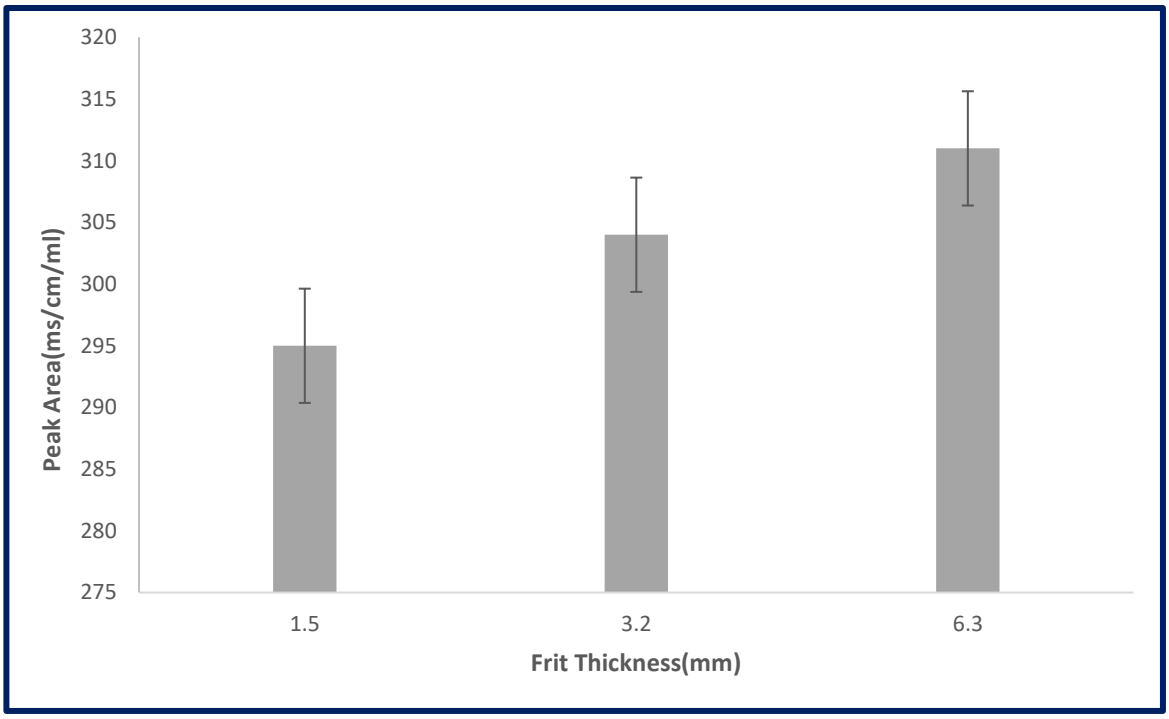


Figure 4.6: Effect of frit thickness on Peak area for 5 cm Column – Phenyl 650M resin

-Effect of No of vanes on Liquid Flow distribution: Adaptor is used at the top of the column for keeping the column bed in position and this adaptor have vanes (baffles) which help in better liquid distribution inside the packed column. While using the column for certain applications, interaction between liquid and the resin bed is very important and determines the recovery desired.

The figure below shows the effect of changing no of vanes on the flow distribution efficiency. As the no of vanes are increased in the adaptor, flow distribution increases.

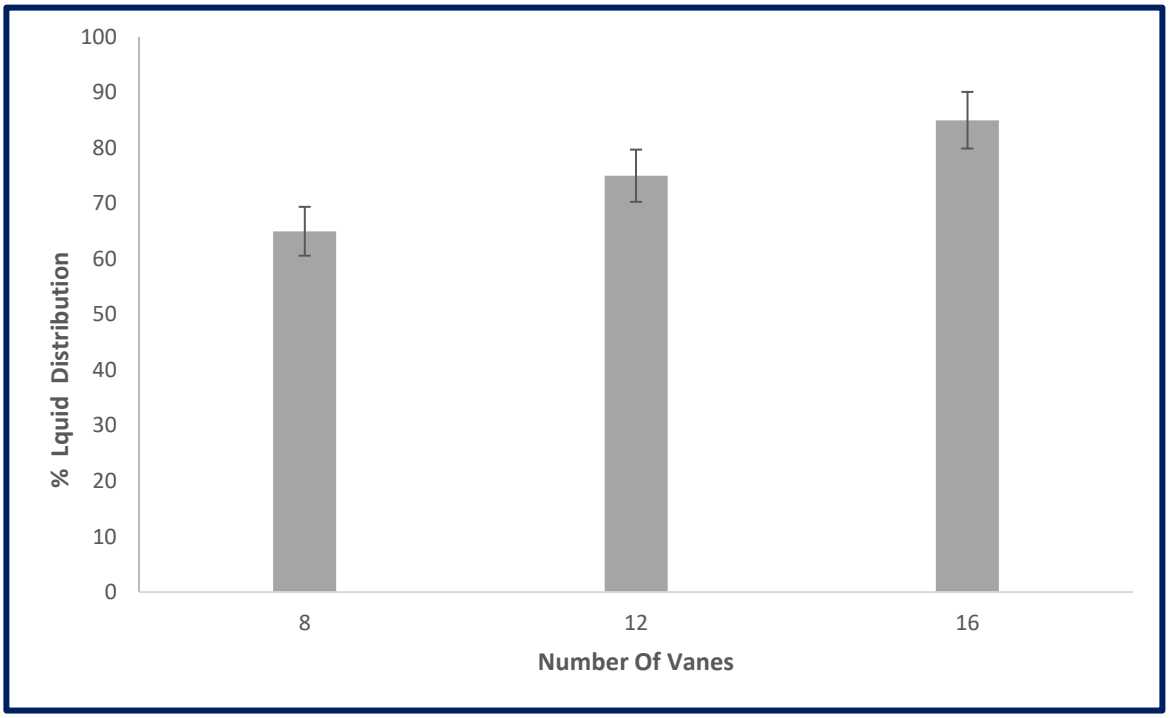


Figure 4.7: Effect of number of Vanes on liquid flow distribution for 5 cm column – Phenyl 650M resin

Summary: Phenyl-650M resin is used for high throughput capture, intermediate purification, and polishing process steps. The primary applications of this resin are for the separation of proteins, their isoforms, and aggregate removal.

Based on the results, it is seen that if we increase the frit thickness the No of theoretical plates are less, hence the column will be less efficient in removal of proteins and other desired components. On the other hand, if we increase the no of vanes (baffles) in the adaptor the distribution improves, means will help in better interaction between the resin and solute being used.

The reasons behind the plates increase as we reduce frit thickness are:

- Width of the peak decreases with frit thickness decrease
- Sample does not get diluted when we decrease frit thickness
- More band broadening

Still we cannot go to infinity small frit thickness as we still need required liquid distribution in the column.

Peak area should not change with any parameter change but in our experiments peak area change is observed due to error with sample pump or if may be tracer get trapped in the column. Similar results seen for Polyanalytik columns also. This need further study.

Better surface roughness of column internal helps in better liquid distribution, means less liquid accumulation on the walls.

To get the best results of separation, we need optimise with Frit thickness and no of vanes.

4.2 TOYOPEARL Hexyl 650 C

4.2.1 Column Diameter- 2.5 cm

- **Effect of frit thickness on Column efficiency**: It is ultimately related to the plates in a packed column. Higher the number of plates, more efficient the column is. The figure below clearly shows as we increase frit thickness the column efficiency increases. We need to optimize for frit thickness to get best efficiency and satisfy other requirements for the column.

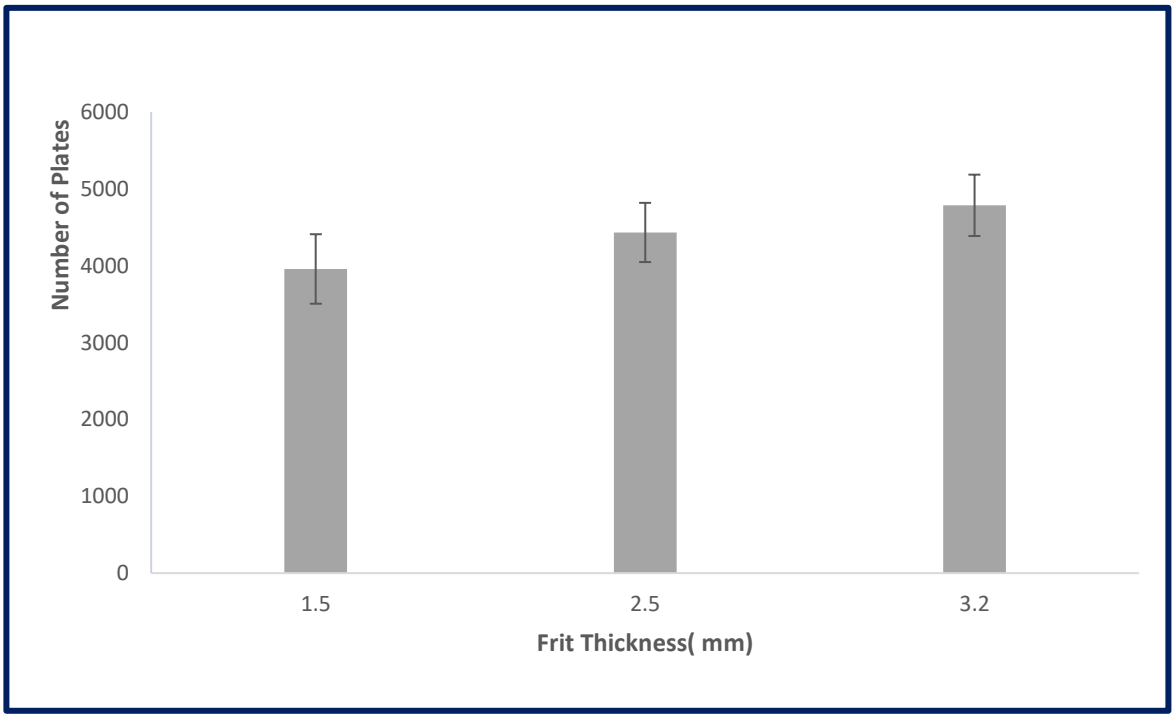


Figure 4.8: Effect of frit thickness on plates for 2.5 cm Column – Hexyl 650C resin

-Effect of frit thickness on Peak Area: Peak area is the direct measure of column efficiency. Broader the peak means poorer the column efficiency. Peak shape can indicate about the column structure. The figure below shows the effect of frit thickness on Peak area. It is clear from the figure below, as frit thickness is increased peak area decreases and hence the column efficiency increases.

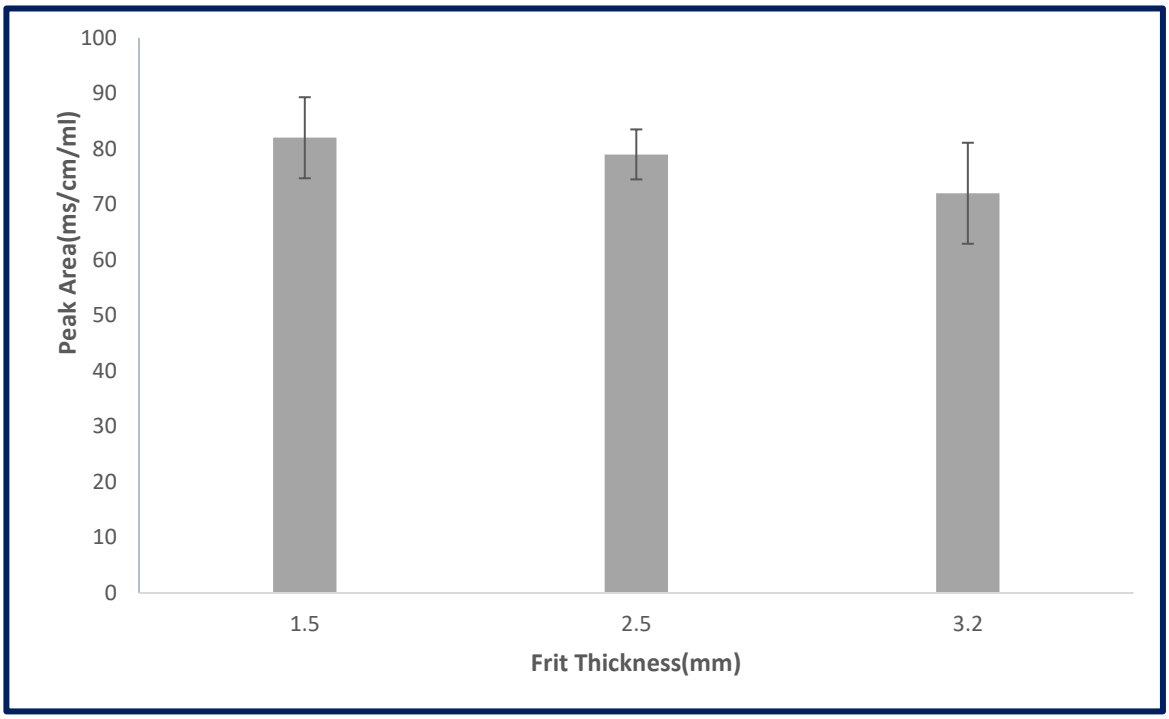


Figure 4.9: Effect of frit thickness on Peak area for 2.5 cm column – Hexyl 650C resin

-Effect of No of vanes on Flow distribution: Adaptor is used at the top of the column for keeping the column bed in position and this adaptor have vanes (baffles) which help in better liquid distribution inside the packed column. While using the column for certain applications, interaction between liquid and the resin bed is very important and determines the recovery desired.

The figure below shows the effect of changing no of vanes on the flow distribution efficiency. As the no of vanes are increased in the adaptor, flow distribution increases.

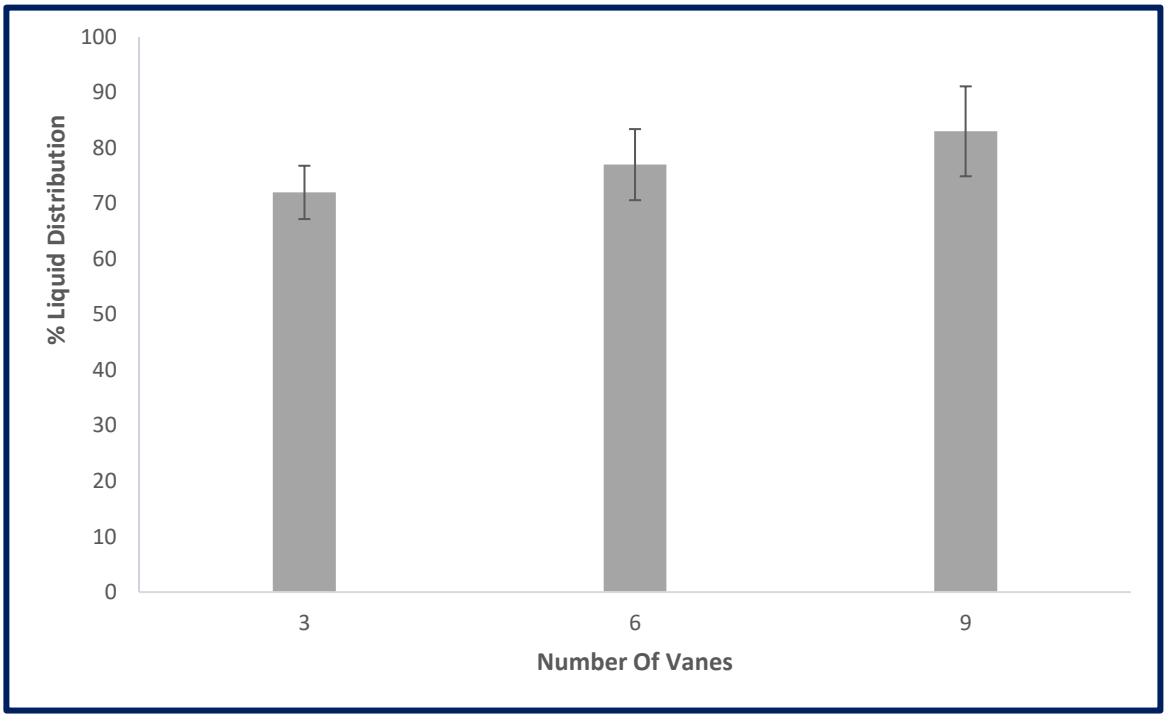


Figure 4.10: Effect of number of vanes on liquid Flow distribution for 2.5 cm column – Hexyl 650C resin

4.2.2 Column Diameter- 5 cm

- **Effect of frit thickness on Column efficiency**: It is ultimately related to the plates in a packed column. Higher the number of plates, more efficient the column is. The figure below clearly shows as we increase frit thickness the column efficiency increases. We need to optimize for frit thickness to get best efficiency and satisfy other requirements for the column.

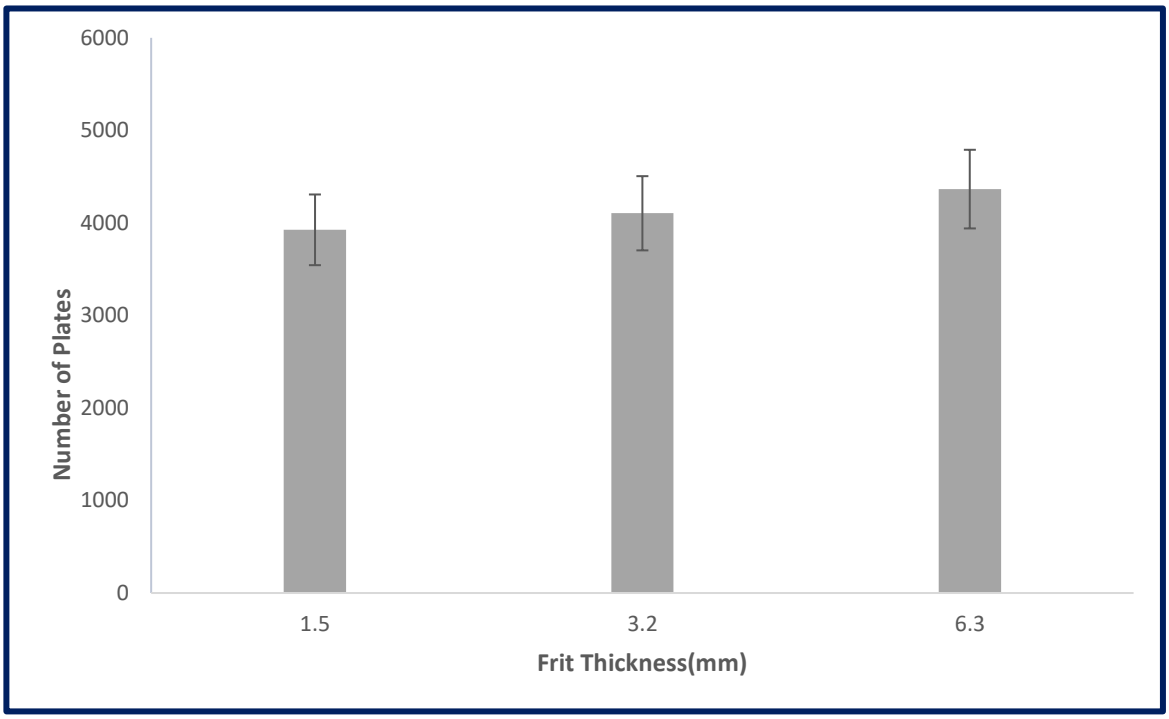


Figure 4.11: Hexyl 650 C Effect of Frit thickness on plates 5 cm Column – Hexyl 650C

-Effect of frit thickness on Peak Area: Peak area is the direct measure of column efficiency. Broader the peak means poorer the column efficiency. Peak shape can indicate about the column structure. The figure below shows the effect of frit thickness on Peak area. It is clear from the figure below, as frit thickness is increased peak area decreases and hence the column efficiency increases.

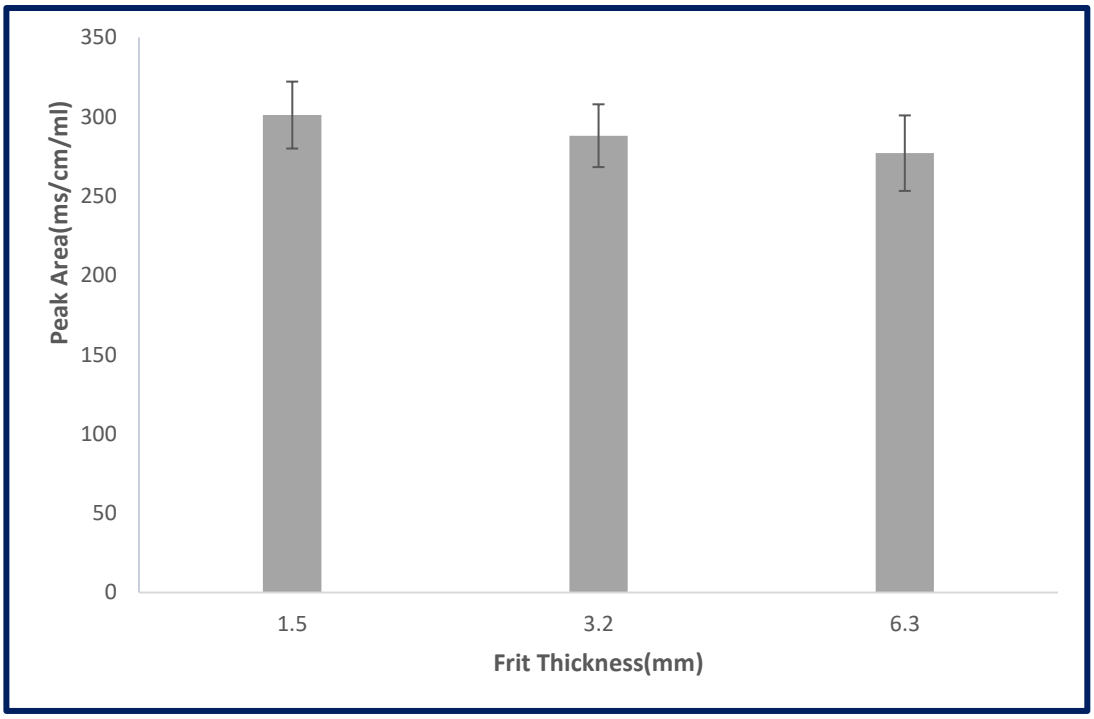


Figure 4.12: Effect of frit thickness on peak area for 5 cm column – Hexyl 650C

-Effect of No of vanes on Flow distribution: Adaptor is used at the top of the column for keeping the column bed in position and this adaptor have vanes (baffles) which help in better liquid distribution inside the packed column. While using the column for certain applications, interaction between liquid and the resin bed is very important and determines the recovery desired.

The figure below shows the effect of changing no of vanes on the flow distribution efficiency. As the no of vanes are increased in the adaptor, flow distribution increases.

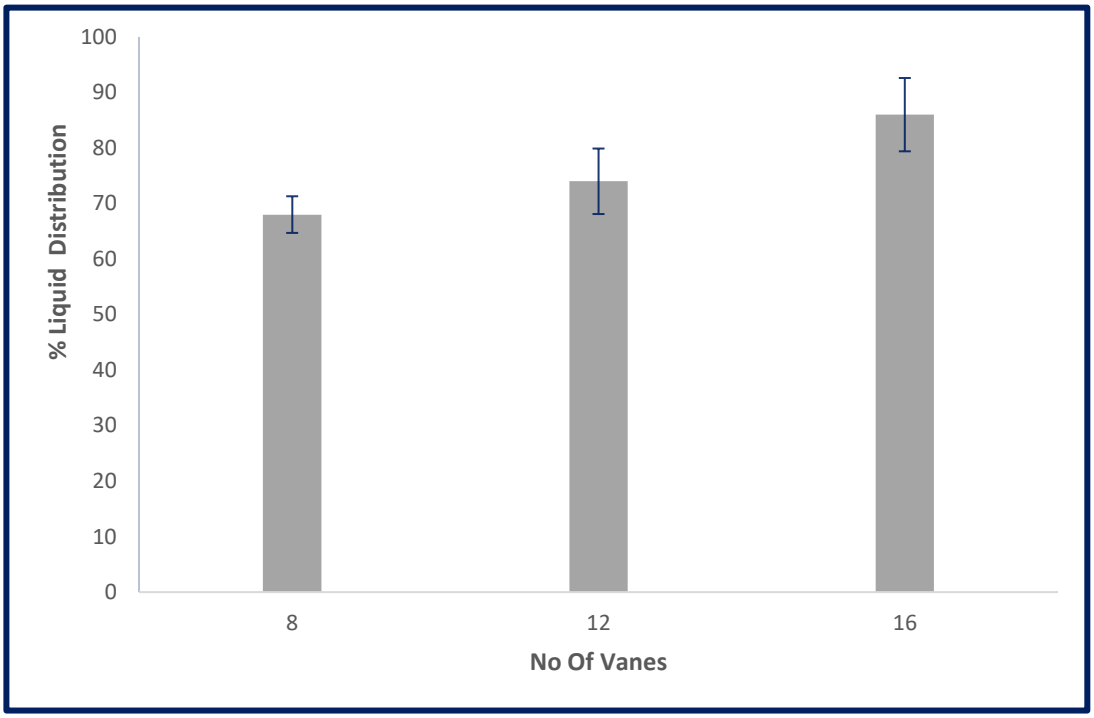


Figure 4.13: Effect of number of vanes on liquid flow distribution for 5 cm column- Hexyl 650C

Summary: Hexyl 650 C resin has the highest hydrophobicity of the TOYOPEARL HIC resins. It is used for very hydrophilic proteins or where a low salt elution environment is required. It is useful as an early capture step or for intermediate purification.

Based on the results, it is seen that if we increase the frit thickness the Number of theoretical plates are more, hence the column will be more efficient in removal of proteins and other desired components. On the other hand, if we increase the number of vanes (baffles) in the adaptor the distribution improves, means will help in better interaction between the resin and elute.

For Hexyl 650 C the results are opposite to that of Phenyl 650 M as Hexyl 650 C has the highest hydrophobicity which makes it unique.

To get the best results of separation, we need optimise with Frit thickness and no of vanes.

Based on the results above we can have further detailed studies with different combinations of variables and implement those results in the production.

4.3 TSKgel SP-5PW (20)

4.3.1 Column Diameter- 2.5 cm

- **Effect of frit thickness on Column efficiency:** It is ultimately related to the plates in a packed column. Higher the number of plates, more efficient the column is. The figure below clearly shows as we increase frit thickness the column efficiency drops. We need to optimize for frit thickness to get best efficiency and satisfy other requirements for the column.

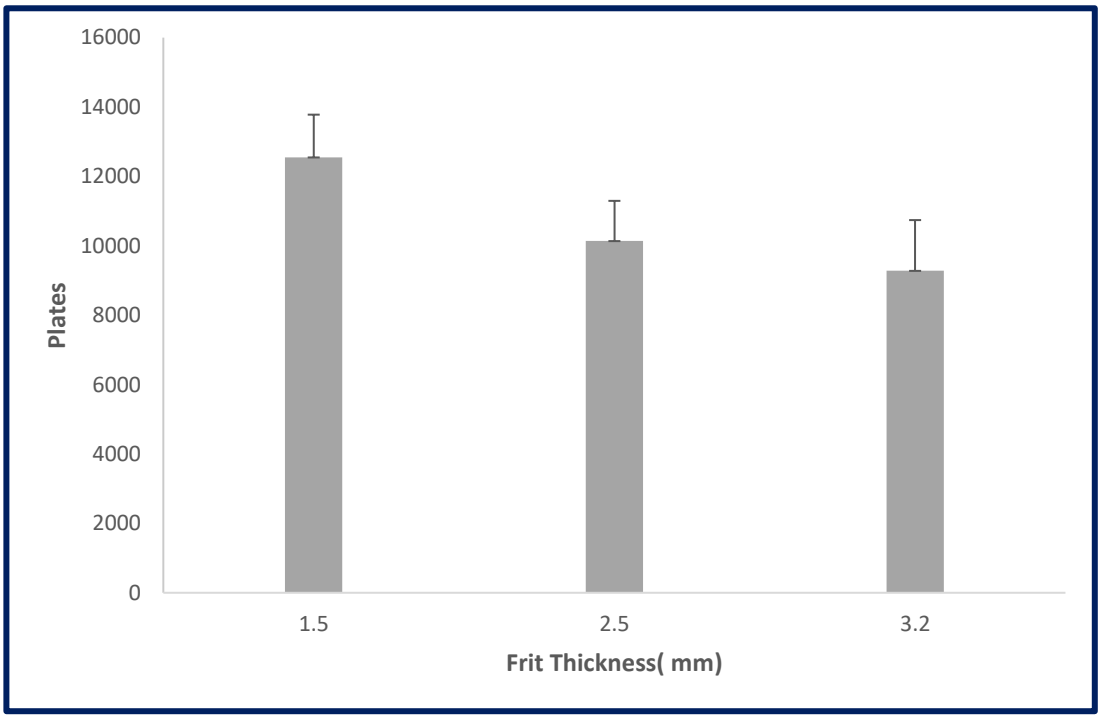


Figure 4.14: Effect of frit thickness on plates for 2.5 cm column – SP -5PW (20) resin

-Effect of frit thickness on Peak Area: Peak area is the direct measure of column efficiency. Broader the peak means poorer the column efficiency. Peak shape can indicate about the column structure. The figure below shows the effect of frit thickness on Peak area. It is clear from the figure below, as frit thickness is increased peak area increases and hence the column efficiency drops

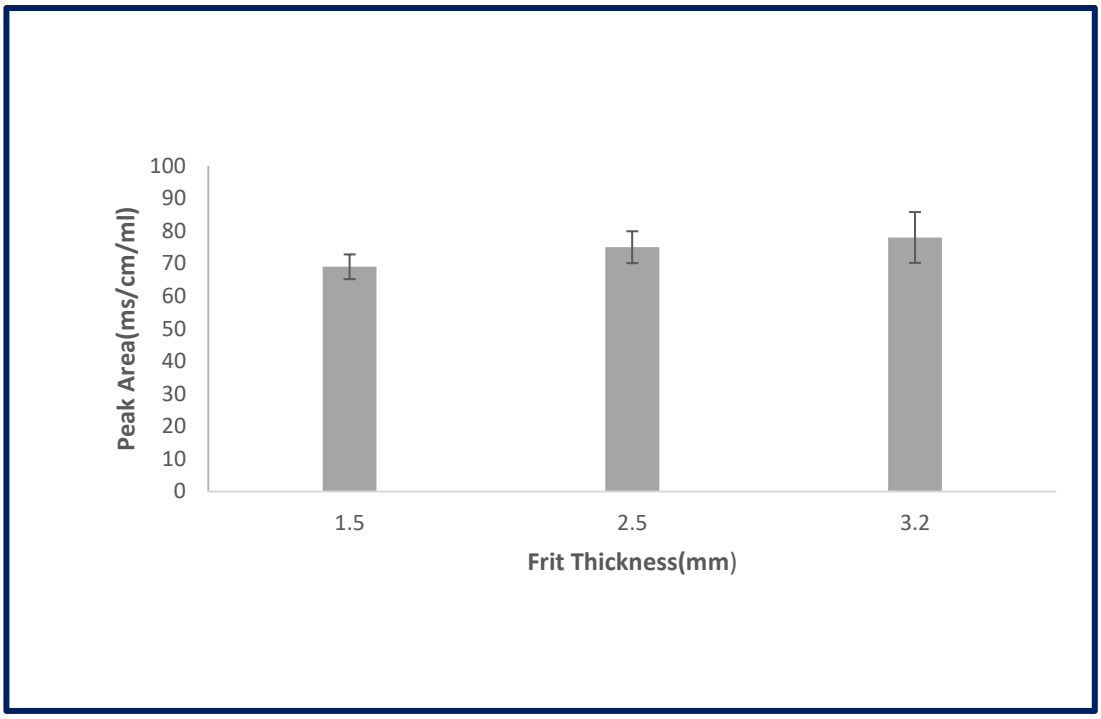


Figure 4.15: Effect of frit thickness on Peak area for 2.5 cm column – SP 5PW (20) resin

-Effect of No of vanes on Flow distribution: Adaptor is used at the top of the column for keeping the column bed in position and this adaptor have vanes (baffles) which help in better liquid distribution inside the packed column. While using the column for certain applications, interaction between liquid and the resin bed is very important and determines the recovery desired.

The figure below shows the effect of changing no of vanes on the flow distribution efficiency. As the no of vanes are increased in the adaptor, flow distribution increases.

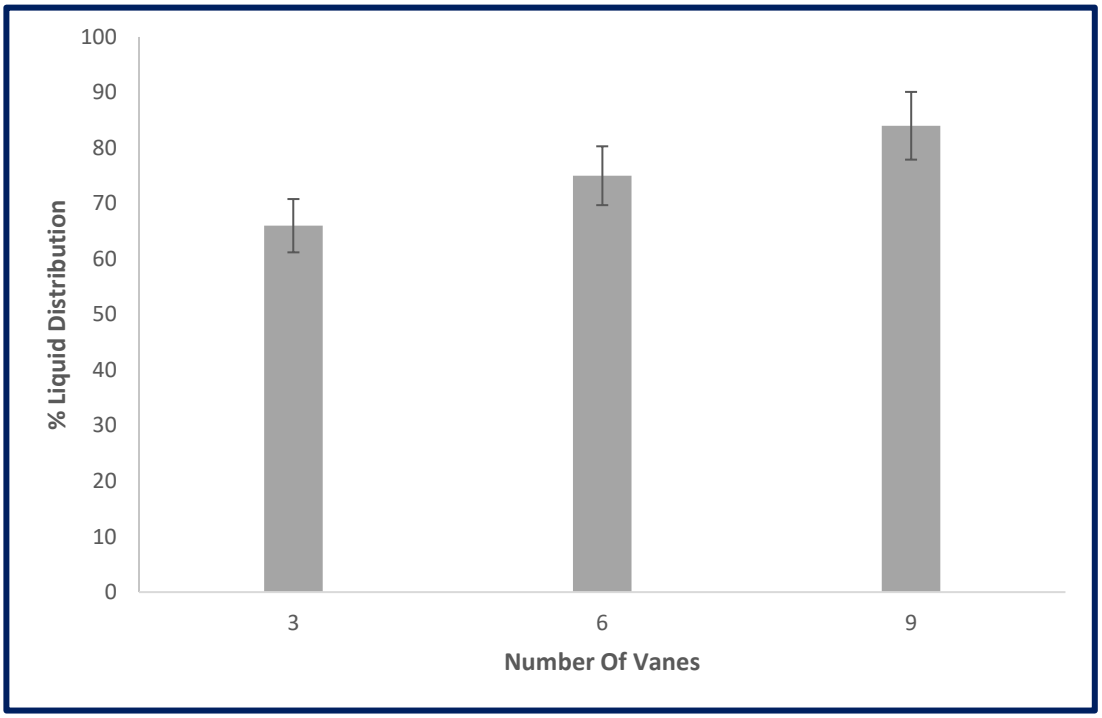


Figure 4.16: Effect of no of vanes on liquid flow distribution 2.5 cm column – SP 5PW (20) resin

4.3.2 Column Diameter- 5 cm SP- 5PW (20) resin

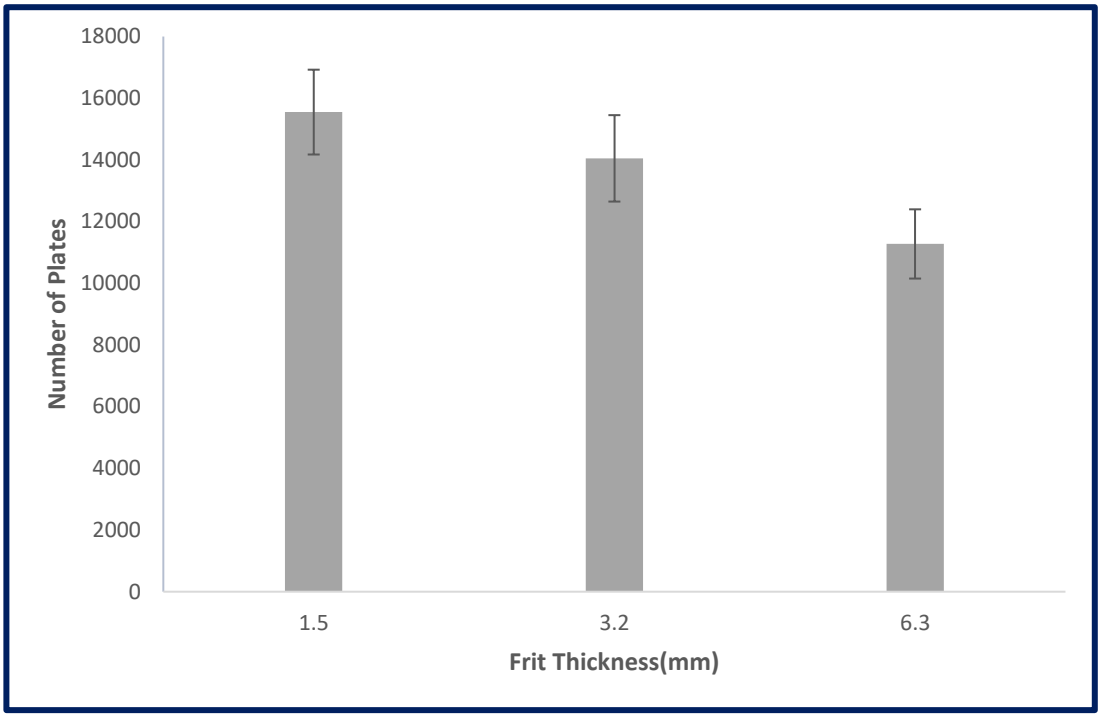


Figure 4.17: Effect of frit thickness on plates for 5 cm column – SP 5PW (20) resin

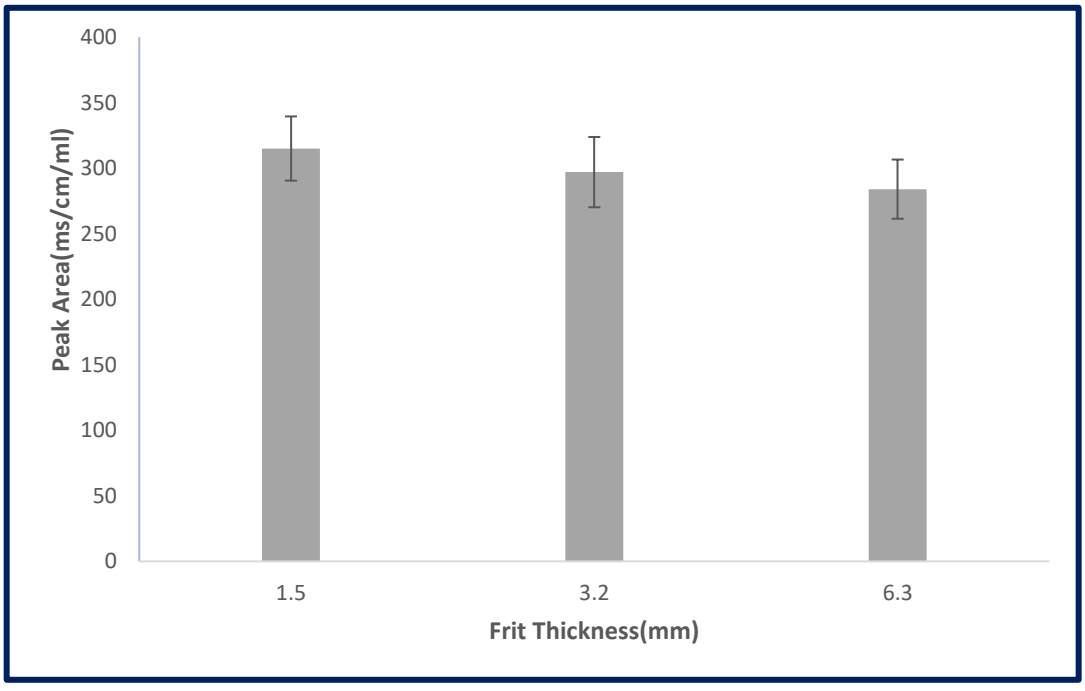


Figure 4.18: Effect of frit thickness on peak area for 5 cm column – SP 5PW (20) resin

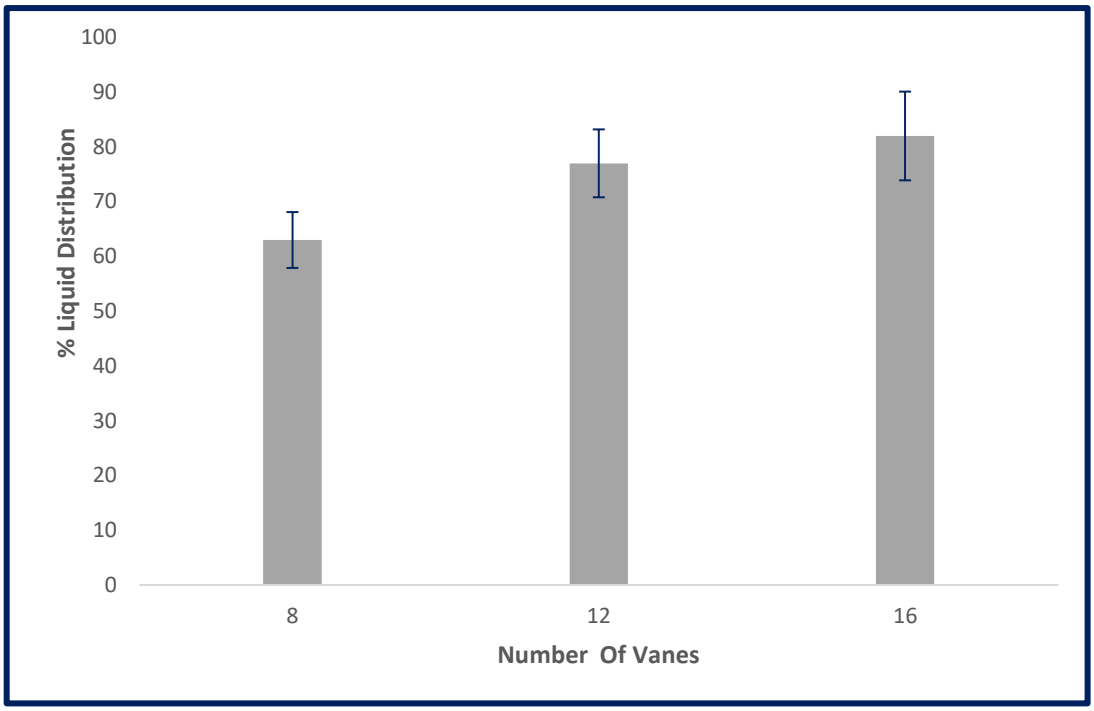


Figure 4.19: Effect of no of vanes on liquid flow distribution for 5 cm column – SP 5PW(20) resin

Summary: TSKgel SP-5PW (20) resin is a strong cation exchange resin for biomolecule purification. TSKgel SP-5PW (20) resin is best used for high resolution process polishing steps.

Based on the results, it is seen that if we increase the frit thickness the Number of theoretical plates are less, hence the column will be more efficient if we reduce the frit thickness. On the other hand, if we increase the number of vanes (baffles) in the adaptor the distribution improves, means will help in better interaction between the resin and elute. The reasons behind the plates increase as we reduce frit thickness are:

- Width of the peak decreases with frit thickness decrease

- Sample does not get diluted when we decrease frit thickness
- More band broadening

Still we cannot go to infinity small frit thickness as we still need required liquid distribution in the column.

To get the best results of separation, we need optimise with Frit thickness and no of vanes.

Based on the results above further detailed studies should be conducted with different combinations of variables and implement those results in the production.

4.4 Asymmetry Results

Below are the results for Asymmetry for columns of 2.5 cm and 5 cm diameter. Results indicate as we decrease the frit thickness, we have better and Asymmetrical columns, which are what is, required in the industry applications.

Earlier we have presented and discussed plates and peak area for various frit thickness and these results are indicative of the column performance improvement when we go down on frit thickness for 2.5 cm and 5 cm columns

4.4.1 Column Diameter (Phenyl 650 M) - 2.5 cm

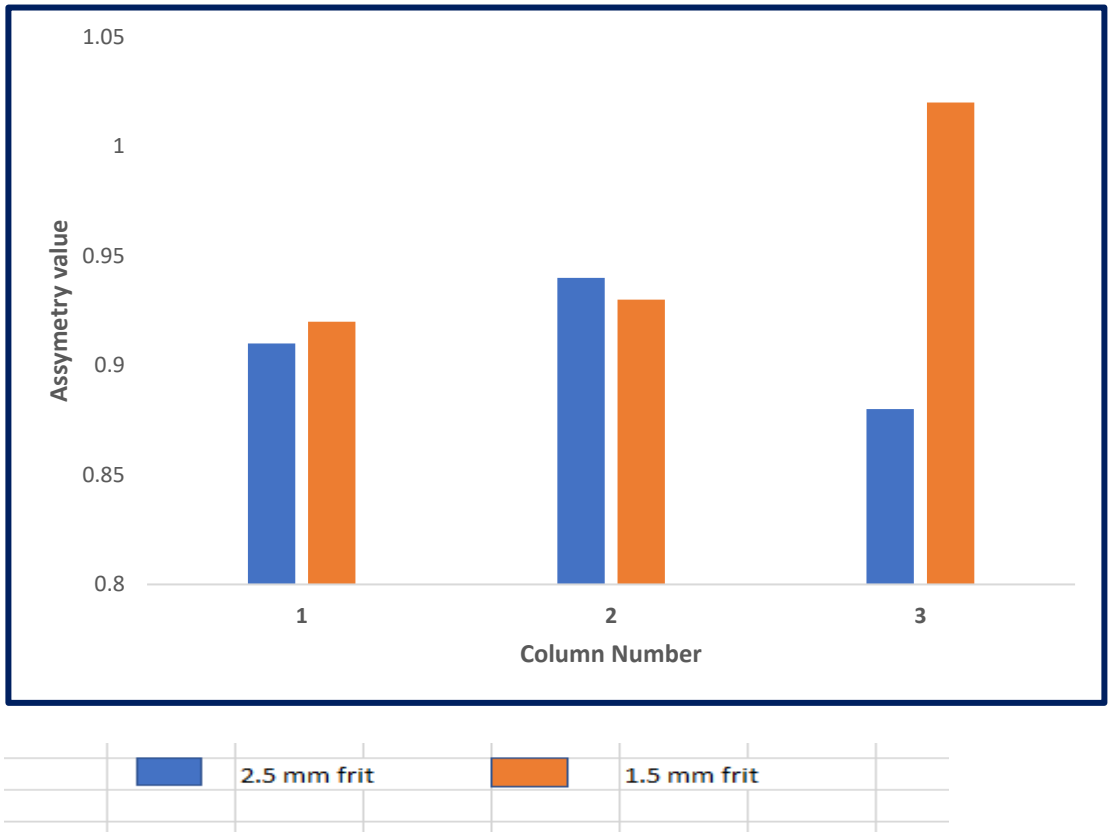


Figure 4.20: Effect of Frit thickness on Asymmetry for 2.5 cm column – Phenyl 650 M resin

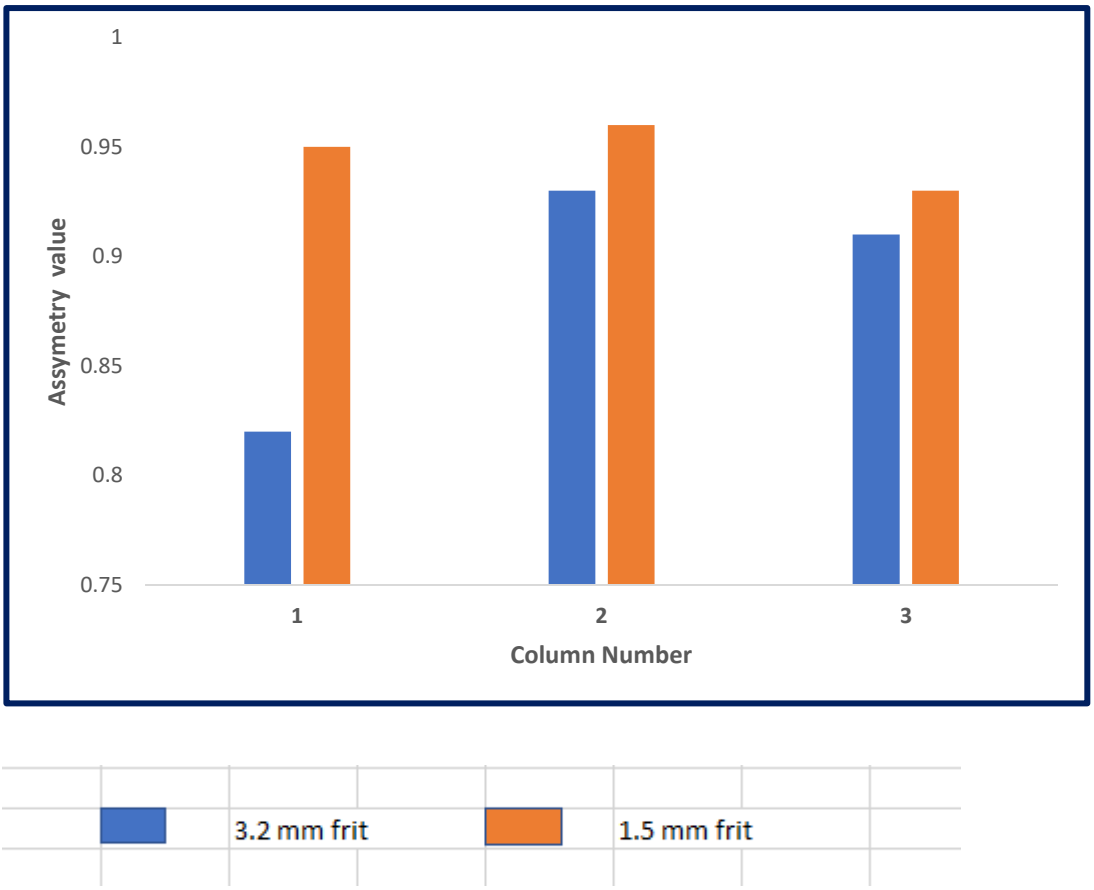


Figure 4.21: Effect of Frit thickness on Asymmetry for 5 cm column – Phenyl 650 M

CHAPTER 5

CONCLUSIONS & RECOMMENDATIONS

The research topic (Investigation of Column Packing & Flow Profiles in Packed Chromatographic Columns) undertaken in this thesis is relatively new and it is still a largely unstudied area, especially in Column sizes of 2.5 cm and 5 cm diameter and greater than these diameters. In this chapter of the thesis, the results obtained in this study are concisely summarized and few recommendations are outlined.

5.1 Conclusions

Flow studies carried out for 2.5 cm and 5 cm packed columns for various resins and different variables were thoroughly investigated. Each column size and resin used has been followed with detailed AKTA^{TM} analysis for each column including flow studies for the columns to see liquid distribution in the column. The most important conclusions are as follows:

1. The topic studied in this thesis is considered a novel research investigation since no similar study has been previously done on 2.5 cm or 5 cm columns. Most if not all studies performed so far has been in smaller columns of size 0.5 cm and smaller. So far there is no guideline to perform successful experiments for columns of diameter 2.5 cm and 5 cm. The first part of this thesis was to establish a reliable experimental procedure and how to study various parameters in column diameters 2.5 cm and 5 cm which has been done successfully.

2. A set of experiments were performed according to the procedure developed at PolyAnalytik and different studies were performed as stated in the objectives and ensuring we get stable columns with different types of resins for various pharmaceutical applications. The aim of the current study was to investigate the performance of the columns packed for the applications and suggest an improvement if possible.
3. Preliminary study suggests opting for a lower thickness of the frit being used for the column adaptors for separation of Resin and buffer as it improves column efficiency in the range of 10% to 38% for resins TOYOPEARL® Phenyl-650M and TSKgel ® SP-5PW (20) for 2.5 cm and 5 cm diameter columns.
4. Second study was done by varying the number of vanes for the column adaptor to see the impact on liquid flow distribution. Increasing the number of vanes from 3 to 6 and to 9, improved liquid flow distribution by 14 to 25% for all the resins studied and for column diameters 2.5 cm and 5 cm.
5. Third study was done to see the impact of column internal roughness on liquid flow distribution. Column internal roughness was changed from 0.8 Ra to 0.4 Ra and this has given improvement of 9-10% in liquid flow distribution for packed columns for 2.5 cm and 5 cm diameters.

6. The results of experimental study are summarized as below:

Table 5.1: Summary of Results Obtained- TOYOPEARL® Phenyl-650M

Column Diameter (cm)	Parameter Studied	Parameter Change	Results Obtained	Measurement Variable
2.5	Frit thickness	2.5 mm to 1.5 mm	19% higher	Plates
5.0	Frit thickness	3.2 mm to 1.5 mm	38.5% higher	Plates
2.5	Number of Vanes	3 to 6	25% higher	Liquid distribution
5.0	Number of Vanes	8 to 12	15.4% higher	Liquid distribution
2.5	Internal roughness	0.8 Ra to 0.4 Ra	9% higher	Liquid distribution
5.0	Internal roughness	0.8 Ra to 0.4 Ra	10% higher	Liquid distribution
2.5	Frit thickness	2.5 mm to 1.5 mm	5% higher	Asymmetry
5.0	Frit thickness	3.2 mm to 1.5 mm	7% higher	Asymmetry

Table 5.2: Summary of Results Obtained- TSKgel ® SP-5PW (20)

Column Diameter (cm)	Parameter Studied	Parameter Change	Results Obtained	Measurement Variable
2.5	Frit thickness	2.5 mm to 1.5 mm	23.7% higher	Plates
5.0	Frit thickness	3.2 mm to 1.5 mm	10.7% higher	Plates
2.5	Number of Vanes	3 to 6	14% higher	Liquid distribution
5.0	Number of Vanes	8 to 12	22% higher	Liquid distribution

5.2 Recommendations

Based on the results obtained in this study, the following recommendations are done for future studies.

1. To investigate the combination of Frit thickness and number of vanes impact on the column efficiency and fine tune the study to get the best performance in the industry for various applications.
2. To implement the results obtained in the study above for columns of larger diameter of 8 cm and 20 cm which are the requirements for the industrial applications.

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KARAMJIT SINGH, P.Eng.

Plant Manager

SUMMARY OF QUALIFICATIONS

- Two years working at PolyAnalytik Inc as a Product Development Engineer
- Extensive Canadian & International industrial experience as a Production Manager, Process Engineer and Plant Manager with experience in Packaging equipment's and processes
- Extensive knowledge of specialized technologies used in Asia, Europe, and the United States related to the Oleo Chemical and Biodiesel industries related to Quality and Quality management
- Proven ability to manage and conduct studies of plant production installations and maintenance systems. Well versed with methodologies like TPM and TQM.
- Certified Lead Auditor for ISO 9001 and ISO 14001; conducted over 100 audits for QMS and EMS
- Demonstrated skills in implementation and auditing of QMS and EMS, and implementation of HACCP and GMP systems
- Well versed with Lean manufacturing, Six sigma and problem-solving methodologies
- Experience in production & planning process
- Good experience with project management
- Well versed with MS office, word and Excel & Email use and well versed with SAP.
- Extensive experience in Health and Safety as a Safety Chair
- Expertise in Six Sigma; certified Green Belt & well versed with problem solving tools like GSTD, 5 why, root cause analysis.
- Excellent organizational, analytical, and problem-solving skills combined with staff training and supervisory skills
- Self-starter, persuasive, trainer and a leader.
- Ability to coach and mentor members of team.

PROFESSIONAL EXPERIENCE

Product Development Engineer
PolyAnalytik Inc. London, Ontario May 2019 – Till Date

- Managing the commercialization and production of Packed Columns for Pharmaceutical Industry in collaboration with Japanese Company.
- Managing Quality Management system for the company.

Industrial Engineer (IP Manager)
Nestle Inc. London, Ontario Oct 2015 – Sept 2017

- Managed the Factory operational line, labor and material usage standards and ensure accuracy for the annual operational plan as part of the budget process.
- Facilitated teams in implementing sustained improvements to reduce factory costs.
- Key driver in implementing continuous improvements across factory operations leading to Nestle Continuous excellence (NCE)

- Participated in new product cost estimates & feasibility for Innovation/ Renovation activities driven by product development
- Budget preparation, discussion and proposal
- Plan, organize, direct & control daily operations

- Member of Lean value stream pillar
  - Initiating DMAIC & SMED projects and follow up to meet targets for the year
  - Initiating, suggesting & implementing Opex projects to achieve savings target of 3.2 million for the factory for 2016 and achieved 4.2 million against these targets
  - Fully supported Autobahn project to ensure timely approval for funding by submitting required Technical data
  - Interacting with various departments in the factory to ensure desired results for the factory
  - Call to Arms – supported and ensured all data submitted in time to Vevey with required discussions
  - Ensured all the standards/ Targets for 2017 are prepared / discussed and submitted in time

Process Engineer
Renix Inc. London, Ontario, Jan 2012 – May 2015

- Responsible for managing all the Scale up and Design activities for the commercialization of CFIX technology for various applications
- Work on Lab scale set up to prove proof of concept and ensuring process conditions, chemical usage and efficiency of the process.
- Scale up activities includes design, costing, purchasing, procurement, installation and commissioning.
- Worked on a project independently and successfully completed the project, starting from design to successful commissioning and delivering to the client.

Plant Manager
Markmore Biofuels Ltd Kuala Lumpur, Malaysia 2009 – 2010

- Managed projects and operations for a Biodiesel plant and refinery with a capacity of 250,000TPA
- Organized commissioning of plant and installation of equipment and systems leading to cost savings for the company
- Recruited, trained, and supervised a team of engineers and managers, enabling successful plant operations

Plant Manager
Mission Biotechnologies Sdn Bhd Kuantan, Malaysia 2008 – 2009

- Handled complete operations of pre-treatment plant (330TPD), Biodiesel plant (300 TPD), and Glycerine Refining plant as Senior Production Manager
- Organized and managed logistics, quality control, purchasing and maintenance of equipment and systems leading to reduction in labour for the company
- Saved company labour costs by fulfilling responsibility of Management Representative for ISO implementation and as Chair of the Safety Committee
- Trained and supervised 65 staff members in company operations, standards and procedures
- Planned and managed operations for an additional Biodiesel plant project with a capacity of 250,000 TPA

Production Manager
IFFCO Malaysia SDN BHD Johor, Malaysia 2002 – 2008

- Managed troubleshooting, “de-bottle-necking”, operations, and maintenance of Oleo Chemical complex, including saponification and effluent treatment plants
- Maintained “Just in Time” Production systems, Quality Control systems, and plant and production improvements, resulting in improved productivity and market
- Achieved significant cost reductions in utilities and chemical consumption costs, leading to increased profits for the company
- Managed processes, including oil and fat hydrogenation and splitting; distillation and fractionation of fatty acids; glycerine evaporation/distillation; effluent treatment; boilers/utilities; and tank farm
- Implemented TQM and HACCP throughout the plant, resulting in sales improvements
- Supervised 50 staff members and served as Chair of the Safety Committee, increasing efficiency, and ensuring no workplace accidents during operations

General Manager (Research and Development)
SRAAC Ltd Kurnool, India 2001 – 2002

- Managed Oleo Chemical and Soap Plant operations, increasing productivity
- Revamped the bar soap making line, increasing production from 10 TPD to 40 TPD
- Trained and supervised 50 staff members in operation and process control, resulting in increased production capacity

Production Manager
WIPRO Ltd Bangalore, India 1989 – 2001

- Managed Oleo Chemical and Soap Plant operations, implementing high quality standards to ensure optimal production
- Maintained ISO 9001 Quality System, volume of production, Profit before Tax (PBT) targets, and manufacturing efficiencies including yields, usage variances, and working capital management
- Supervised 265 staff members and served as the Safety Coordinator

PROFESSIONAL ASSOCIATIONS

P. Eng., Professional Engineers Ontario (Member in good standing since Oct 2013)

EDUCATION

Post Graduate Diploma in Management
International Institute of Advanced Marketing — Mumbai, India — 2001

Master of Technology in Chemical Engineering Degree (Gold Medalist)
Nagpur University — Nagpur, India — 1989

- Assessed by International Credential Assessment Service of Canada (ICAS) to represent a level of education in Ontario comparable to: Master's Degree

Bachelor of Technology Degree in Chemical Engineering
Nagpur University — Nagpur, India — 1987

- Assessed by ICAS to represent a level of education in Ontario comparable to: Bachelor's Degree (Four Years)

ADDITIONAL COURSES

ISO 9001 and ISO 14001 Lead Auditor Certifications
International Registrar of Certified Auditors (IRCA) — 2000/2001