Biotechnology Ion Exchange Chromatography Improvement

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Abstract *— This research investigates the influence of different stop collection percentages on ion exchange chromatography in laboratory-scale separations. A direct association was seen between reduced stop collection (percentages of peak max) and increased chromatographic area under the curves, resulting in enhanced substance elution. Four (4) experimental runs showed linear behavior (97%, 95%, 93%, 90%, and 88% peak max). The presence of variability among runs serves to emphasize the susceptibility of outcomes, hence it is important to consider the control of key parameters such as chromatography process conditions and sample stability. The suggestions for future research include (1) adjusting column conditions and (2) investigating causes of variability to enhance reproducibility. This study offers significant contributions to the productivity of a blockbuster drug, by enhancing the uses of ion exchange chromatography. The supporting data was generated using a scaled-down model of the large-scale process operations.*

Key Terms *—Chromatography, Ion Exchange, Scale-Down Model, Stop Collection*

INTRODUCTION

The Biotechnology Industry provides innovative protein-based therapeutics for diseases with limited to no treatment, receiving FDA support through a Fast Track program. These programs focus on regulatory tactics, faster approval processes, and innovative research and development methods to expedite the availability of medical goods to the public.

Several projects aim to accelerate the translation of scientific findings into medicinal goods, often overseeing the optimization of the manufacturing process. As a result, several opportunities and improvement projects arise once the commercialization of the medical product is established. The project presented in this document aims to improve an Ion Exchange Chromatography Process for a peptibody with microbial origin. This involves evaluating historical data, manufacturing process inputs and outputs, adverse chemical conditions, and equipment capabilities for process optimization. The project will be conducted in a process development laboratory using a scale-down model, providing a controlled environment for systematic experimentation, optimization, and quality assessment. The scale-down model is crucial in bridging the gap between conceptual optimizations and their practical implementation at commercial scale manufacturing processes. The main goal is to contribute to increased efficiency and productivity while maintaining product quality.

LITERATURE REVISION

Biotechnology is a multidisciplinary science that applies biological principles to develop new technologies for a variety of purposes. It combines genetic engineering, molecular biology, bioinformatics, and medicines. Upstream processing is the initial cell/bacteria cultures to provide an ideal environment for the production of desired biomolecules. Downstream processing is the separation, purification, and formulation of collected biomolecules into a final product. To isolate and purify the target biomolecule, this method uses a variety of unit operations such as filtrations and several chromatograph styles.

The development of this project considers the biopharmaceutical downstream purification process of drug substances with the technical knowledge of process development laboratories. The manufacture of a biopharmaceutical drug substance involves three stages: (1) acquiring/producing the initial ingredients, (2) conducting the upstream production

of the biopharmaceutical, and (3) finalizing the downstream purification of the biopharmaceutical [1]. The downstream purification procedure eliminates or reduces impurities that are relevant to the product or the manufacturing process, as well as any product-related impurities that may be present in the biopharmaceutical-produced material.

The purification process downstream requires strict adherence to current Good Manufacturing Practices (cGMPs) and precise regulation of the process parameters. The stringent regulation of the initial manufacturing stages, known as critical process parameters (CPPs), is essential and demanding to ensure the reduction of different impurities in the refined biopharmaceutical drug substance. The downstream purification process for protein-based products (recombinant proteins and monoclonal antibodies) and gene therapy-based biopharmaceuticals (viral vectors and genetically modified patient cells, mRNA non-viral vector) must meet strict expectations and significant requirements set by regulatory authorities [1].

Typically, the purification process for recombinant proteins and monoclonal antibodies involves taking the harvested material from the bioreactor, which has been clarified, and passing the solution containing the biopharmaceutical through a specific sequence of chromatographic and filtration steps. The order in which these steps are performed is crucial for achieving the desired level of purification. In our project, we will be improving an Ion Exchange Chromatography (IEC) process. IEC employs a method of segregating proteins based on their charged residues. Charged residues on protein surfaces include the side chains of amino acids, the α-amino and α-carboxyl termini of the chains, and the sialic acid residues on glycoproteins [1]. The residues possess amphoteric properties, hence causing the sign and overall charge of proteins to be dependent on the pH level and electric potential of the product media. The isoelectric point (pI) of a protein is the pH at which its positive charge is equal to its negative charge. A protein forms a bond with an anion exchanger, which is a resin that carries a negative charge when the pH is higher than its isoelectric point (pI). A protein forms a complex with a cation exchanger, which is a resin that carries a positive charge when the pH is lower than its isoelectric point (pI).

To create a purification process that is both reliable and consistent in producing a product of satisfactory quality, the fundamental aspects of each chromatography phase in the overall plan are evaluated via small-scale trials [2]. When developing a purification process at a scale-down model, it is crucial to consider the anticipated end size of the process. This allows for a comprehensive evaluation of particular challenges related to scaling up the process. This is especially crucial if the protein has to be purified following the regulations of current good manufacturing practices, as established by regulatory agencies. In a standard downstream process, chromatography is often divided into three separate phases: capture, intermediate purification, and final polishing [2]. Each step needs a distinct emphasis, and as the purification process is expanded, new obstacles will arise.

After conducting preliminary small-scale scouting tests, the purification process will usually have the ability to generate protein amounts in the range of milligrams. The primary outcome of these process optimization initiatives is the thorough consideration and, where feasible, the definition of product information and process parameters. The process of scaling up from laboratory to pilot plant often requires multiplying the size by a factor of 50 to 100 [2]. When scaling up chromatography, it is important to carefully evaluate many elements relating to chromatography, non-chromatography, and equipment. These factors together contribute to the effective process scale-up.

To obtain a larger scale of chromatographic purification, it is common to raise the diameter of the column and the rate at which the liquid flows through it. However, it is important to keep the height of the media bed and the speed at which the liquid flows through the column constant. Successful scale-up may be achieved by increasing the column diameter while maintaining a

proportional increase in the column cross-sectional area with the process volume while keeping the bed height constant. This is because the total duration that the target molecule spends in the process, from small-scale development to large-scale columns, stays unchanged. Theoretically, the separations should be almost the same for both column diameters, thus serving as the foundation for a successful scale-up. Nevertheless, when the size of the column grows, additional considerations arise, including the selection and preparation of buffers, the arrangement of the media, the design of the column, and the maintenance of cleanliness in the manufacturing process.

Most of the recombinant proteins are synthesized by utilizing mammalian (Chinese Hamster Ovary cells) and microbial expression methods (Escherichia coli and Saccharomyces cerevisiae). Mammalian post-translational modifications are often necessary for precise interactions with target molecules in systems, whereas microbial expression methods are favored for achieving larger quantities of expression. Utilizing E. coli as biological platforms for the regulated manufacture of polypeptides via recombinant technology is a cost-effective approach [3]. The whole procedure might be executed by using microbial cell factories with comparatively uncomplicated approaches and instruments. Furthermore, the creation of proteins outside the cell facilitates the process of purification that follows.

Therapeutic proteins and peptides provide several benefits, with the most significant being their ability to give effectiveness, selectivity, and specificity compared to tiny chemical compounds [3]. Protein and peptide treatments are considered to be largely safe due to their composition, whether modified or artificially produced. Proteins and peptides are considered safe for use in living organisms and are not considered alien to their systems. Peptides operate as hormones, chemokines, and cytokines, playing a crucial role in transmitting signals throughout living organisms. Owing to the aforementioned factors, the potential for unexpected adverse reactions is unpredictable when it comes to peptide medications. Indeed, it has been noted that the adverse effects associated with peptide medicines are often caused by either incorrect doses or local responses at the injection sites [3].

Therapeutic proteins and peptides have a strong affinity for certain targets. This refers to the minimum or complete lack of non-specific binding to unwanted targets, resulting in a high therapeutic index. For instance, therapeutic proteins with low molecular weight, such as monoclonal antibodies and hormones, exhibit a high degree of specificity in binding to their target receptors, even at very low concentrations of picomolar magnitude [3].

Ion-exchange chromatography (IEX) is a well-established method often used to thoroughly analyze therapeutic proteins. Before release or process improvements, it is necessary to conduct a comprehensive investigation on the identity, heterogeneity, impurity content, and activity of every new batch of therapeutic proteins. This examination employs a diverse array of analytical techniques, such as reversed-phase liquid chromatography (RPLC), hydrophobic interaction chromatography (HIC), and/or size exclusion chromatography (SEC). Cation-exchange chromatography (CEX) is the predominant form of IEX employed for protein purification and characterization. CEX is acknowledged as the gold standard for charge-sensitive analysis, although technique parameters, such as column type, mobile phase pH, and salt concentration gradient, typically need to be tuned for each specific protein [4]. Presently, IEX employs two methodologies, namely salt-gradient and pH-gradient, each with its own merits and limitations. Currently, the primary constraint of the pH-gradient ion exchange (IEX) technique is the exorbitant expense associated with commercial buffers, hence impeding the broader use of this method. A recommended approach for developing methods including therapeutic proteins is to use a polymeric non-porous stationary phase that is linked with a strong cation exchanger containing sulfonic acid groups. To determine the

optimal mobile phase parameters, such as pH, salt type, and concentration, it is crucial to take into account the isoelectric point (pI) of the proteins. So far, IEX has effectively been used to analyze many types of modifications on peptide chains, such as deamidation, C-terminal lysine truncation, N-terminal pyroglutamate, methionine oxidation, or glycosylation variations, in various therapeutic proteins, including monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) [4].

Hanke and Ottens investigated the vital significance of liquid chromatography in biopharmaceutical purification, recognizing it as an essential step in most purification methods. The research underlines the need for novel, logical, methodical, and generally applicable process development methodologies to solve the bottlenecks often associated with biopharmaceutical production [5]. The authors emphasize that, despite the widespread use of liquid chromatography, its knowledge is not properly used in process development. The study provides an overview of recent achievements in detecting and quantifying the features and interactions involved in downstream process evolution. The emphasis is on the mixture's composition, solute molecular characteristics, and interactions with resins. The Quality by Design (QbD) and Process Analytical Technology (PAT) efforts emphasize the need to improve process and product knowledge.

The purification difficulty in biopharmaceuticals is described by the complicated cascade of unit activities necessary to reach high levels of product purity. The downstream process developer confronts issues due to slight differences in the upstream process and the reliance on single-unit operations. High-throughput process development (HTPD) and the transition from trial-and-error methodologies to more mechanistic models. Mechanistic models based on basic principles provide a greater degree of process knowledge, bridging the gap between early-stage design decisions and advanced-stage optimization [5]. All these factors are required to have a great representation of a scaled-down model.

PROBLEM STATEMENT

This research consists of optimizing the chromatography process step for a microbial-derived peptide, with the primary goals of boosting step yield, with a potential recovering an additional 2 to 4 grams of product, keeping excellent quality, and guaranteeing regulatory compliance. This will be achieved using engineering knowledge and a scientific approach by conducting scale-down model experiments of Ion Exchange Chromatography to provide consistent evidence of the optimization. Scale-down models serve as a manufacturing representation of the process in which various scenarios can be tested as part of continuous improvements of manufacturing processes.

This work is significant because it has the potential to transform biotechnological manufacturing systems. The research intends to boost overall production productivity, minimize waste, and strengthen the supply chain by improving chromatography efficiency and yield. This not only improves the plant's capacity to produce additional goods, but it also corresponds with sustainability goals by limiting environmental effects. Furthermore, the emphasis on product quality and regulatory compliance emphasizes the company's dedication to providing safe and effective remedies. In essence, this study addresses major issues in biopharmaceutical production, resulting in significant breakthroughs that can lead to improved patient outcomes, a more robust supply chain, and a more sustainable biotechnology business.

Research Objectives

The key goals of this research project are to improve the chromatography process for producing a microbial-derived peptide, to achieve a higher step yield, and to recover an extra 2 to 4 grams of the target product. The initiative aims to preserve and even improve product quality while complying with tight regulatory criteria through thorough design and refining. The main purpose of

improving these essential factors is to contribute to a more efficient and sustainable manufacturing process. Finally, the project aims to provide a significant advancement in biotechnological production, ensuring increased product output, improved quality, and regulatory compliance, thereby providing a valuable contribution to both the scientific community and the biopharmaceutical industry.

RESEARCH CONTRIBUTIONS

The main contribution to the development of this research is to create value for patients ensuring more products are manufactured. This will be achieved by optimizing an ion exchange chromatography process, resulting in improved efficiency and yield during the separation and purification process. This will directly contribute to higher step yields and greater grams of the target product recovered. The contribution will be developed in a Process Development lab conducting Scientific Experiments scale-down model contributions that will set the reference for the posterior laboratory runs of the process. This laboratory scale optimization will be traduced to the production process, ensuring that excellent product quality is maintained. The optimization of the process and increased plant capabilities to produce additional goods, as well as the decrease in lots manufactured every year, indicate a commitment to a more sustainable and flexible manufacturing environment. Furthermore, the enhanced supply chain guarantees that patients have constant and timely product availability. This research project blends biotechnology developments with a focus on sustainability and productivity, providing a comprehensive solution that benefits patients, the manufacturing process, and the industry as a whole.

METHODOLOGY

This research project investigates the variation of the stop-collect parameter in chromatography processes using an experimental approach. It simulates large-scale outcomes using industry laboratories and bench-scale columns. The study aims to examine the effects of decreasing the stop-collect percentage on grams recovered. The results will provide insights for larger-scale process improvements, ensuring safety and adherence to protocols.

Research Design

This Research will have an experimental approach to conduct a prospective study. This project will be done using Industry Laboratories in which a scale-down model the Chromatography will be used to simulate Large-scale results of the variation of the stop-collect parameter of the process. To experiment bench-scale columns with the required resin will be packed, buffers for the process will be prepared, the Chromatograph System method will be created, and four experimental runs will be conducted to evaluate various scenarios. To run the experiments the equipment that will be used is a Modular Chromatography System. The variation of the Stop Collect will be conducted at the values of 95%, 93%, 90%, and 88% peak max (PM). Data analysis will be conducted to evaluate the difference in terms of yield and grams of collecting beyond 97% PM. Since the chromatograph provides the whole peak, for each run the area in 97%, 95%, 93%, 90%, and 88% PM will be evaluated. This experiment will have Controlled Variables in which the temperatures, pressure, experimental buffers, and columns will remain the same to reduce variability.

Hypothesis

As the Stop Collect Percentage of Maximum Peak is lowered, more grams will be retrieved.

Experimental Variables

For this experiment, the independent variables will be the Start Collect, the Stop Collect, the Peak Max, and process Flow Rates. The dependent variables of this experiment will be the Volume, Concentration of the Pool, Grams of Product, and Step Yield.

Data Collection

Every experimental run data will be collected in the Modular Chromatography System and the data will be exported to an Excel.CSV file that will be used for data processing. Data acquired from each chromatography run will be the volume, UV signal, Conductivity, and pressure of each phase of the run. We will be evaluating the Elution phase of the run in which the chromatograph peak appears. The concentration of the pool will be determined using lab equipment and that data will be used to determine the grams acquired at each standpoint. All the data will be stored in an Excel file and will be processed in statistical tools such as Minitab. Statistical P-value using a Confidence interval of 95% will be used to determine statistical significance. Also, a graph of each chromatograph will be presented to analyze the difference between each run.

Safety Measures

Experiments will be conducted in an industry-qualified laboratory, using Personal Protection Equipment such as safety glasses, globes, and Lab Coats. Also, the Safety Data Sheet will be evaluated before any experimentation.

Limitations of this Research Design

An experimental run will be conducted with the available product pool for the corresponding chromatography. This research will provide data at a laboratory scale for the feasibility of an improvement on a Manufacturing Scale, but the process and requirements of a manufacturing implementation are out of the scope of this research. All the experiments will be equivalent to the expected inputs and outputs of the manufacturing process and will serve as a guide for the modifications required before implementation.

RESULTS DISCUSSION

A summary of the results of the experimental runs is provided in Table 1. The complex nature of ion exchange chromatography is highlighted by the variation in grams and volume across various stop collection percentages within each run. Subtle oscillations can be seen when analyzing the means of grams and volume collected at different stop-collection percentages. This suggests that column qualities, among other things, have a big impact on the reproducibility of data. These discrepancies could be the result of things like inconsistent packing, possible contaminants in the sample, or deterioration of the column over time. Because of how susceptible the chromatographic process is to these changes, it is crucial to keep column conditions constant to guarantee accurate and repeatable results. Any change in column characteristics might affect elution patterns, which in turn affects mass yield and volume because the separation process is largely dependent on the interactions between the analyte and stationary phase. Therefore, attaining dependable and repeatable ion exchange chromatography findings requires close attention to column maintenance and quality control.

Table 1
Experimental Run Results Summary

Run	Volume Collected (mL)	Concentration (mg/mL)	Grams (mg)
Run 1 95% PM	26.68	1.2789	34.12
Run 2 93% PM	25.27	1.2408	31.35
Run 3 90% PM	25.89	1.2242	31.69
Run 4 88% PM	25.57	1.2590	32.20

In Figure 1, we can see the elution profiles for each run. The observed variability is reflective of the dynamic nature of chromatographic separations, where factors like column packing consistency, sample impurities, or minor variations in operating conditions can contribute to fluctuations in the elution profiles. Comprehending and measuring this fluctuation is essential for interpreting outcomes and refining chromatographic procedures. Extensive research on the particular variables causing variability, such as thorough evaluations of sample purity and column conditions, can provide valuable information for improving the

repeatability of ion exchange chromatography results. Through the identification and mitigation of these causes of variability, scientists can enhance their methodology and attain chromatographic analysis results that are more consistent and dependable.

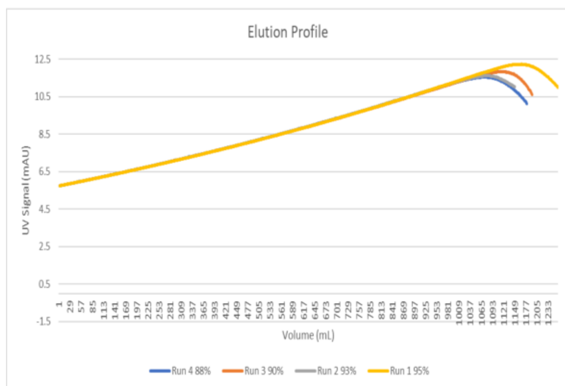

Figure 1
Runs Elution Profile

An examination of the chromatographic data reveals a distinct impact of reducing the stop collection percentage on the area under the chromatographic peaks, as depicted in Table 2. The area, represented in mL*mAU, serves as a quantitative measure of the eluted substance, reflecting the extent of the chromatographic separation. Notably, as the stop collection percentage decreases, there is a corresponding increase in the area under the peaks. This observation is particularly evident in Run 4, where the area at 88% PM stop collection is 10,299, whereas if the collection was halted at 97% PM, only 9,731 mL*mAU would have been collected. The direct correlation between stop collection percentage and area signifies that a reduction in the percentage of stop collection results in a larger area, indicating a more extensive elution of the target substance. Consequently, this expanded area directly contributes to a greater mass yield. In essence, on average, diminishing the stop collection percentage manifests as an effective strategy for enhancing the collection of grams, affirming the crucial role of stop collection optimization in ion exchange chromatography.

Table 2
Experimental Area Comparison for Each Run

Stop Collect (%) PM	Run 1 95% Areas (mL*mAU)	Run 2 93% Areas (mL*mAU)	Run 3 90% Areas (mL*mAU)	Run 4 88% Areas (mL*mAU)
97	10842	9782	10163	9731
95	11005	9982	10276	9908
93	11143	10157	10376	10027
90	11322	10401	10495	10207
88	11441	10566	10569	10299

We can see this trend in Figure 2, in which the area is at its highest at 88% PM stop collect and lowest at 97% PM:

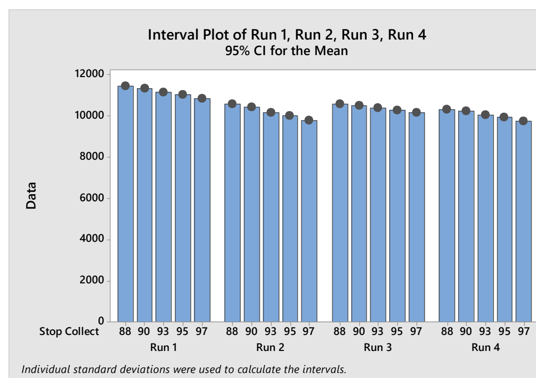

Figure 2
Interval Plot for Area (mL*mAU) per Run

One-way ANOVA was used with a 95% Confidence Interval to determine if there was a statistically significant difference between the areas of each run. This information gives information on the variability present from each run. The P-value was 0 and the F-value was 21.04. With this information, we can reject the null hypothesis, and conclude that at least one mean is different from the rest. In Figure 3, we can see the variation from the means of all runs.

Overall, the product quality review appears to have gone well, as all runs passed most of the tests, including the CEX-HPLC, SE-HPLC, RE-HPLC, and ECP Elisa tests, refer to Table 3. This continuous success using several analytical techniques points to the eluted materials from Runs 1, 2, and 3 having a high level of purity and conformance. The dependability of these findings supports both the general caliber of the samples that

were gathered and the strength of the chromatographic separation procedure.

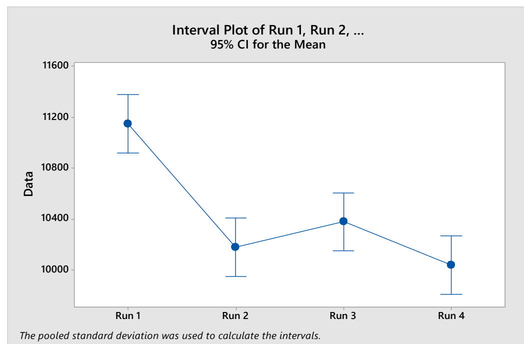

Figure 3
Interval Plot for Mean per Run

Nonetheless, it is important to pay attention to the fact that Run 4 failed the CEX- and RE-HPLC tests. The failures in these particular tests, about reverse-phase and cation-exchange chromatography, may point to the existence of contaminants or fluctuations in the composition of the substance that was eluted in Run 4. Comprehending the causes of these failures is essential to identify possible problems with the chromatographic procedure or sample preparation related to Run 4.

Variations in column conditions, sample deterioration, or problems with the elution procedure could all be contributing factors to these failures. The differences between the RE-HPLC and CEX-HPLC tests may be explained by more research into the chromatographic factors unique to Run 4, such as the makeup of the mobile phase and column integrity.

Table 3
Product Quality Results Summary

Run	RE-HPLC	CEX-HPLC	SE-HPLC	ECP Elisa
Run 1 95%	Pass	Pass	Pass	Pass
Run 2 93%	Pass	Pass	Pass	Pass
Run 3 90%	Pass	Pass	Pass	Pass
Run 4 88%	Fail	Fail	Pass	Pass

CONCLUSION

The study on ion exchange chromatography experiments has revealed the complex mechanisms involved in the separation procedure. The optimization of the stop collection percentage is crucial for maximizing volume and mass yield, with a 95% rate being the most effective. The efficiency of this method in boosting drug elution is attributed to the direct association between lower stop-collection percentages and the increased area beneath chromatographic peaks. The heterogeneity in the region of collection across different percentages of stop collection within each run highlights the susceptibility of ion exchange chromatography to subtle alterations in experimental parameters. The presence of variability in chromatographic procedures necessitates careful consideration of essential parameters, including column conditions and sample purity, to improve reproducibility.

Positive findings were obtained for Runs 1, 2, and 3 of the product quality assessments, including RE-HPLC, CEX-HPLC, SE-HPLC, and ECP Elisa testing. However, the lack of success in Run 4 during the RE-HPLC and CEX-HPLC tests suggests potential problems unique to this particular run. Further research on column conditions and sample integrity is necessary.

The research enhances understanding of the chromatographic procedure and optimization ideas but also emphasizes the importance of careful consideration of experimental parameters for reproducibility and dependability. Future studies should focus on expanding stop collection percentages and systematically investigating sources of variability to improve methodologies and promote consistent and reliable ion exchange chromatography outcomes.

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