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 and increased area under chromatographic peaks, resulting in enhanced substance elution, as evidenced by four runs conducted at varying percentages (97%, 95%, 93%, 90%, and 88%). The presence of variability among runs serves to emphasize the susceptibility of outcomes to differences in experimental conditions, hence emphasizing the importance of careful consideration of parameters such as column conditions and sample integrity. Suggestions for further research including adjusting column conditions and investigating causes of variability to enhance reproducibility. This study offers significant contributions to the optimization of stop collection percentages, hence enhancing the uses of ion exchange chromatography in laboratory-scale separations as a tool for large scale process improvement.

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.

Background

Biotechnology is a multidisciplinary field that uses biological principles to create new technologies. It involves genetic engineering, molecular biology, bioinformatics, and medicines. Upstream processing involves the initial culture of cells or microorganisms to produce desired biomolecules. Downstream processing involves the separation, purification, and formulation of collected biomolecules into final products. This project focuses on the biopharmaceutical downstream purification process of drug substances, using technical knowledge from process development laboratories. The manufacturing process involves three stages: acquiring initial ingredients, conducting upstream production, and finalizing downstream purification. The downstream purification procedure eliminates or reduces impurities and accidental contaminants from the biopharmaceutical produced upstream.

Problem

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.

Methodology

This study consist on bench-scale columns with resin will be packed, buffers prepared, and four experimental runs will be conducted using a Modular Chromatography System. The experiment will be conducted at 95%, 93%, 90%, and 88%, with data analysis to evaluate differences in yield and collecting grams beyond 97%. Controlled variables will ensure consistent temperatures, pressure, experimental buffers, and columns to reduce variability. The hypothesis is that as the Stop Collect Percentage of Maximum Peak is lowered, more grams will be retrieved.

Experimental variables include Start Collect, Stop Collect, Peak Max, and process Flow Rates, with dependent variables including Volume, Concentration of the Pool, Grams of Product, and Step Yield. Data collected from each experimental run will be stored in an Excel file and processed using statistical tools like Minitab. A statistical P-value with a confidence interval of 95% will be used to determine statistical significance.

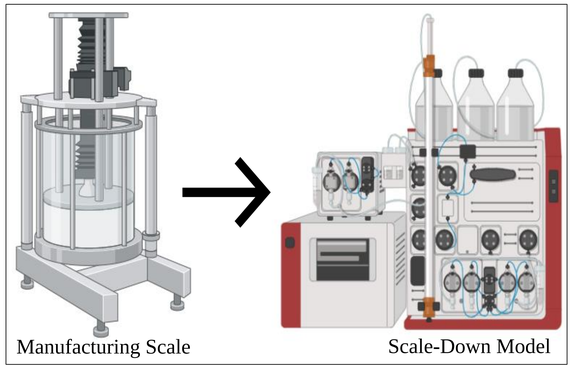

Figure 1
Manufacturing Scale to Scale-Down Model

Results and Discussion

Figure 2 demonstrates chromatographic separations' dynamic variability, influenced by factors like column packing consistency, sample impurities, and operating conditions, requiring understanding and measurement for improved repeatability and consistency.

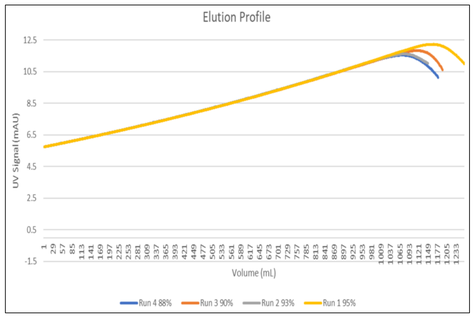

Figure 2
Runs Elution Profile

The study reveals that decreasing stop collection percentage increases the area under chromatographic peaks, indicating greater chromatographic separation, particularly in Run 4, leading to greater mass yield.

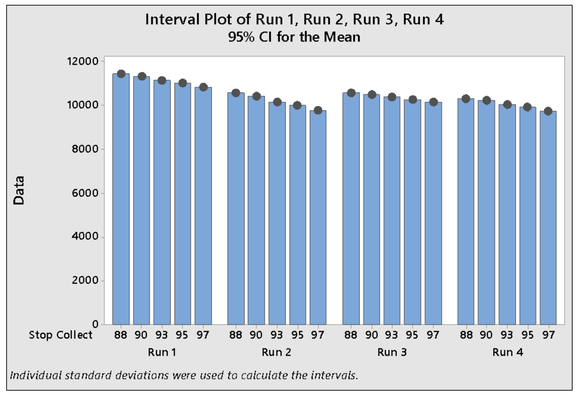

Figure 3
Interval Plot for Area (mL*mAU) per run

The study used one-way ANOVA to examine the variability in each run, finding a statistically significant difference with a P-value of 0 and F-value of 21.04, rejecting the null hypothesis. In Table 1, show results of product quality.

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

Table 1
Product Quality Results Summary

Conclusions

The study on ion exchange chromatography experiments reveals the complex mechanisms involved in separation procedures, with a 95% rate being most effective. The efficiency of this method is attributed to the association between lower stop collection percentages and increased area beneath chromatographic peaks. The research emphasizes the importance of careful consideration of experimental parameters for reproducibility and dependability.

Future Work

Future research in ion exchange chromatography should focus on exploring a wider range of stop collection percentages to maximize volume and mass yield, improving efficiency and reproducibility. Exploring factors affecting sample integrity, such as impurities or degradation, can address challenges in product quality tests. Collaboration between chromatographers, analytical chemists, and biotechnologists can facilitate a multidisciplinary approach to optimization, using advanced analytical techniques and computational modeling.

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