

# **OPTIMIZATION OF INTEGRATED OPERATIONS IN BIOLOGICS MANUFACTURING AND ADOPTING PROCESS MONITORING TOOLS**

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by

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Submitted

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to the



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*Dedicated to my family*

## **CERTIFICATE**

This is to certify that the thesis entitled “OPTIMIZATION OF INTEGRATED OPERATIONS IN BIOLOGICS MANUFACTURING AND ADOPT ONLINE PROCESS MONITORING TOOLS” being submitted by ANJALI RAMAKRISHNA to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy is a record of the original bonafide research work carried out by her under my guidance and supervision. The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

I certify that she has pursued the prescribed course of research.

Prof. Anurag S. Rathore

Department of Chemical Engineering

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## ABSTRACT

The inspiration for this work is the growing focus on process intensification and continuous manufacturing of biopharmaceuticals, to lower production costs. Most biopharma manufacturers today operate in a batch or semi-continuous mode. In batch mode, sampling and analysis are performed offline, inherently causing a halt in the process. The sequential scheduling leads to suboptimal equipment utilisation with long standby periods. By overcoming these shortcomings, continuous operation can enable manufacturers to cope up with increasing market demand due to higher throughputs. It also provides advantages such as reduction in the scale and footprint of the plants, lower process costs, better resin utilisation in chromatography steps, among others. However, there are certain challenges in continuous manufacturing such as the need for in-process monitoring and control to ensure safety, efficacy and the quality standards expected by global regulatory authorities. This has been achieved through process analytical technology (PAT) based tools, in recent times. A typical process consists of several upstream and downstream unit operations, starting from cell culture followed by chromatographic purification, viral clearance, and formulation. Downstream purification, consisting of clarification, capture, polishing, and formulation, can contribute up to 80% of total Mab production costs. Up to 60% of which are due to chromatography steps with the Affinity step contributing to 50-55% of the total COGs making the intensification of this step an area of interest. The aim of this work is to develop process intensification and process analytical technology (PAT) solutions for monitoring and control of downstream unit operations for mAb production using mechanistic, spectroscopic, or data-driven models for improved efficiency, reduced cost of goods sold (COGS), and automation.

The first work is process intensification of protein A chromatography by proposing MFR (multi flow rate) based loading strategies to maximize utilization of binding sites on the resin. This is achieved by adjusting flow trajectory based on simulated breakthrough curves using estimated diffusion constants at specific residence times. The strategy involves utilization of variable flow rate during the loading phase or negative step gradient of residence time. A switch time is defined to transition from one flow rate to the next. It was observed that by operating a process at multiple flow rates, a near maxima of both adsorbent capacity and the productivity could be obtained. In comparison to the reported methods using flow-trajectory based loading, the

proposed method follows a simpler approach and displays increased resin utilization (~15% higher than batch) and improved productivity.

The next work is aimed at enabling a data-based monitoring tool to predict and prolong resin aging in Affinity chromatography. The high cost of protein A resins makes it one of the most expensive raw materials used in biopharmaceutical manufacturing. The resin is often used for multiple cycles (as much as two hundred times). There are situations where concurrent validation at the manufacturing scale may be more appropriate due to increased variability contributed by factors such as multiple lots of feed, scale related differences with regards to packing/unpacking, frequency of repacking, variations in contaminant levels in feed, and impact of proteases. In this work, two case studies were considered to understand DBC decline during resin aging and a model was designed to predict it. Based on the prediction, a control strategy to improve resin performance is also demonstrated aimed at increasing the resin lifetime.

The next work is to develop a UV based PAT tool for online dilution-free concentration determination at in-process stages of downstream manufacturing. A way to circumvent the challenges that arise in concentration estimation due to saturation and non-linearity of signal/UV at higher concentrations is proposed. Chemometrics has been used for visualisation and prediction. The developed tool used method of partial least squares (PLS) to predict concentrations up to 100g/L, validated with differing proteins and buffer systems. A few tools exist in literature that enable online concentration measurements such as Flow VPE (variable path length), Raman, NIR (near infra-red) or fluorescence-based spectroscopy. The proposed tool is simpler and involves little or no capital investment as compared to current industry considerations.

The final work was to develop a PAT tool using Raman spectroscopy, which can predict multiple critical quality attributes such as fragments, acidic variants, in addition to protein concentration. A series of machine learning tools were applied and tested before arriving at the best model in terms of R<sup>2</sup> and least RMSE for each attribute. To enable continuous manufacturing, having tools in place that can determine the quality of feed entering the next unit operation, without pausing the unit operation is the need of the hour. Other spectroscopic methods have been proposed in literature that serve as PAT tools; however, Raman spectroscopy is highly specific and displays the least impact from water interference. The tool would serve in online monitoring



of product quality and enable quality by design. The main objective is reduction of the number of in-process testing at manufacturing scale to monitor critical quality attributes (CQAs), which are both expensive and time consuming. The models were aimed to be successfully validated at all downstream in-process stages, with multiple IgG1 molecules and a few applications and industrial case studies have been illustrated.

## सारांश

इस काम के लिए प्रेरणा उत्पादन लागत को कम करने के लिए बायोफार्मास्यूटिकल्स की प्रक्रिया गहनता और निरंतर विनिर्माण पर बढ़ता ध्यान है। अधिकांश बायोफार्मा निर्माता आज एक बैच या अर्ध-निरंतर मोड में काम करते हैं। बैच मोड में, नमूनाकरण और विश्लेषण ऑफ़लाइन किया जाता है, स्वाभाविक रूप से प्रक्रिया में ठहराव पैदा करता है। अनुक्रमिक शेड्यूलिंग लंबी स्टैंडबाय अवधि के साथ उप-इष्टतम उपकरण उपयोग की ओर जाता है। इन कमियों पर काबू पाने से, निरंतर संचालन निर्माताओं को उच्च थ्रूपुट के कारण बढ़ती बाजार मांग का सामना करने में सक्षम बना सकता है। यह पौधों के पैमाने और पदचिह्न में कमी, कम प्रक्रिया लागत, क्रोमैटोग्राफी चरणों में बेहतर राल उपयोग जैसे लाभ भी प्रदान करता है। हालांकि, निरंतर विनिर्माण में कुछ चुनौतियां हैं जैसे कि वैश्विक नियामक प्राधिकरणों द्वारा अपेक्षित सुरक्षा, प्रभावकारिता और गुणवत्ता मानकों को सुनिश्चित करने के लिए इन-प्रोसेस निगरानी और नियंत्रण की आवश्यकता। यह हाल के दिनों में प्रक्रिया विश्लेषणात्मक प्रौद्योगिकी (पीएटी) आधारित उपकरणों के माध्यम से हासिल किया गया है। एक विशिष्ट प्रक्रिया में कई अपस्ट्रीम और डाउनस्ट्रीम यूनिट ऑपरेशन होते हैं, जो सेल कल्चर से शुरू होते हैं जिसके बाद क्रोमैटोग्राफिक शुद्धिकरण, वायरल क्लीयरेंस और फॉर्मूलेशन होता है। डाउनस्ट्रीम शुद्धिकरण, जिसमें स्पष्टीकरण, कैप्चर, पॉलिशिंग और फॉर्मूलेशन शामिल हैं, कुल एमएबी उत्पादन लागत का 80% तक योगदान कर सकते हैं। जिनमें से 60% तक क्रोमैटोग्राफी चरणों के कारण होते हैं, जिसमें एफिनिटी चरण कुल सीओजी के 50-55% में योगदान देता है, जिससे इस चरण की गहनता रुचि का क्षेत्र बन जाती है। इस कार्य का उद्देश्य बेहतर दक्षता, बेची गई वस्तुओं की कम लागत (सीओजीएस), और स्वचालन के लिए यंत्रवत, स्पेक्ट्रोस्कोपिक, या डेटा-संचालित मॉडल का उपयोग करके एमएबी उत्पादन के लिए डाउनस्ट्रीम यूनिट संचालन की निगरानी और नियंत्रण के लिए प्रक्रिया गहनता और प्रक्रिया विश्लेषणात्मक प्रौद्योगिकी (पीएटी) समाधान विकसित करना है।

पहला काम राल पर बाध्यकारी साइटों के उपयोग को अधिकतम करने के लिए एमएफआर (बहु प्रवाह दर) आधारित लोडिंग रणनीतियों का प्रस्ताव करके प्रोटीन ए क्रोमैटोग्राफी की प्रक्रिया गहनता है। यह विशिष्ट निवास समय पर अनुमानित प्रसार स्थिरांक का उपयोग करके नकली सफलता घटना के आधार पर प्रवाह प्रक्षेपवक्र को समायोजित करके प्राप्त किया जाता है। रणनीति में लोडिंग चरण के दौरान परिवर्तनीय प्रवाह दर या निवास समय के नकारात्मक चरण ढाल का उपयोग शामिल है। एक स्विच समय को एक प्रवाह दर से अगले में संक्रमण के लिए परिभाषित किया गया है। यह देखा गया कि बहुविध

प्रवाह दरों पर एक प्रक्रिया का प्रचालन करके अधिशोषक क्षमता और उत्पादकता दोनों का लगभग अधिकतम प्राप्त किया जा सकता है। प्रवाह-प्रक्षेपक आधारित लोडिंग का उपयोग करके रिपोर्ट किए गए तरीकों की तुलना में, प्रस्तावित विधि एक सरल दृष्टिकोण का अनुसरण करती है और बढ़ी हुई राल उपयोग (बैच से ~ 15% अधिक) और बेहतर उत्पादकता प्रदर्शित करती है।

अगला काम एफ़िनिटी क्रोमैटोग्राफी में राल उम्र बढ़ने की भविष्यवाणी और लम्बा करने के लिए डेटा-आधारित निगरानी उपकरण को सक्षम करने के उद्देश्य से है। प्रोटीन ए रेजिन की उच्च लागत इसे बायोफार्मासिटिकल निर्माण में उपयोग किए जाने वाले सबसे महंगे कच्चे माल में से एक बनाती है। राल का उपयोग अक्सर कई चक्रों (दो सौ बार) के लिए किया जाता है। ऐसी परिस्थितियां हैं जहां विनिर्माण पैमाने पर समवर्ती सत्यापन अधिक उपयुक्त हो सकता है क्योंकि कई लॉट फीड, पैकिंग/अनपैकिंग के संबंध में पैमाने से संबंधित अंतर, रीपैकिंग की आवृत्ति, फ़ीड में दूषित स्तरों में भिन्नता, और प्रोटीज के प्रभाव जैसे कारकों द्वारा योगदान की गई परिवर्तनशीलता में वृद्धि। इस काम में, राल उम्र बढ़ने के दौरान डीबीसी गिरावट को समझने के लिए दो केस स्टडीज पर विचार किया गया था और इसकी भविष्यवाणी करने के लिए एक मॉडल तैयार किया गया था। भविष्यवाणी के आधार पर, राल के प्रदर्शन में सुधार के लिए एक नियंत्रण रणनीति भी राल जीवनकाल को बढ़ाने के उद्देश्य से प्रदर्शित की जाती है।

अगला काम डाउनस्ट्रीम विनिर्माण के इन-प्रोसेस चरणों में ऑनलाइन कमजोर पड़ने से मुक्त एकाग्रता निर्धारण के लिए यूवी आधारित पीएटी उपकरण विकसित करना है। उच्च सांद्रता पर संतृप्ति और सिग्नल / यूवी की गैर-रैखिकता के कारण एकाग्रता अनुमान में उत्पन्न होने वाली चुनौतियों को दरकिनार करने का एक तरीका प्रस्तावित है। केमोमेट्रिक्स का उपयोग विजुअलाइज़ेशन और भविष्यवाणी के लिए किया गया है। विकसित उपकरण ने 100g/L तक सांद्रता की भविष्यवाणी करने के लिए आंशिक कम से कम वर्गों (PLS) की विधि का उपयोग किया, जो अलग-अलग प्रोटीन और बफर सिस्टम के साथ मान्य है। साहित्य में कुछ उपकरण मौजूद हैं जो फ्लो वीपीई (चर पथ लंबाई), रमन, एनआईआर (इन्फ्रा-रेड के पास) या प्रतिदीप्ति-आधारित स्पेक्ट्रोस्कोपी जैसे ऑनलाइन एकाग्रता माप को सक्षम करते हैं। प्रस्तावित उपकरण सरल है और इसमें वर्तमान उद्योग विचारों की तुलना में बहुत कम या कोई पूंजी निवेश शामिल नहीं है।

अंतिम काम रमन स्पेक्ट्रोस्कोपी का उपयोग करके एक पीएटी उपकरण विकसित करना था, जो प्रोटीन एकाग्रता के अलावा टुकड़े, अम्लीय वेरिएंट जैसे कई महत्वपूर्ण गुणवत्ता विशेषताओं की भविष्यवाणी कर सकता है। मशीन लर्निंग टूल्स की एक श्रृंखला को लागू किया गया और प्रत्येक विशेषता के लिए R<sup>2</sup> और कम से कम RMSE के संदर्भ में सर्वश्रेष्ठ मॉडल पर पहुंचने से पहले परीक्षण किया गया। निरंतर विनिर्माण को सक्षम करने के लिए, ऐसे उपकरण होना जो यूनिट ऑपरेशन को रोके बिना, अगली यूनिट ऑपरेशन

में प्रवेश करने वाले फ़ीड की गुणवत्ता निर्धारित कर सकें, समय की आवश्यकता है। साहित्य में अन्य स्पेक्ट्रोस्कोपिक तरीकों का प्रस्ताव किया गया है जो पीएटी उपकरण के रूप में काम करते हैं; हालांकि, रमन स्पेक्ट्रोस्कोपी अत्यधिक विशिष्ट है और पानी के हस्तक्षेप से कम से कम प्रभाव प्रदर्शित करता है। यह उपकरण उत्पाद की गुणवत्ता की ऑनलाइन निगरानी में काम करेगा और डिजाइन द्वारा गुणवत्ता को सक्षम करेगा। मुख्य उद्देश्य महत्वपूर्ण गुणवत्ता विशेषताओं (सीक्यूए) की निगरानी के लिए विनिर्माण पैमाने पर इन-प्रोसेस परीक्षण की संख्या में कमी करना है, जो महंगा और समय लेने वाला दोनों हैं। मॉडल को सभी डाउनस्ट्रीम इन-प्रोसेस चरणों में सफलतापूर्वक मान्य करने का लक्ष्य रखा गया था, जिसमें कई आईजीजी 1 अणु और कुछ अनुप्रयोग और औद्योगिक मामले के अध्ययन शामिल किए गए हैं।

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