DEVELOPMENT OF MONITORING AND CONTROL STRATEGIES FOR DOWNSTREAM PROCESSES

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by

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Dedicated to my family

Certificate

This is to certify that the thesis entitled "DEVELOPMENT OF MONITORING AND CONTROL STRATEGIES FOR DOWNSTREAM PROCESSES" being submitted by VISHWANATH S HEBBI 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 him 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 he has pursued the prescribed course of research.

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VISHWANATH S HEBBI

Abstract

Manufacturing biopharmaceuticals is a complex process. Monitoring and controlling of process variables is necessary to ensure consistent quality of the product. However, in-process quality control is traditionally limited to off line analysis. This results in delay in taking critical decisions, batch failures, and at the times loss of valuable product. In view of this, regulatory authorities have proposed process analytical technology (PAT) guidance for the biotech industries with an aim of achieving consistent product quality. PAT offers an approach to facilitate application of process knowledge for improvement of control, delivering consistent product quality, and delivering superior operational efficiency.

Implementation of PAT requires establishment of process models, mathematical relationships between critical quality attributes, critical process parameters and feed material attributes. While mechanistic models are desirable, most biotech unit operations are too complex to create mechanistic models that are simple enough for use in commercial manufacturing. Use of empirical models or hybrid models is more of a norm in industry. The two major platforms used in biopharmaceutical manufacturing are the bacterial *Escherichia coli* system, and the mammalian Chinese Hamster Ovary system. In *E. Coli* based process, refolding is one of the important unit operations which decides the yield, impurity profile of the products.

Protein refolding is known to be significantly impacted by critical process parameters and feed material attributes including composition and pH of the solubilisation and refolding buffers. Hence, to achieve robust process control and product quality, these attributes and parameters need to be monitored. A PAT tool has been developed using online oxidation reduction potential, pH, and temperature probes to create a fingerprint of a typical refolding batch within the pre-defined design and operating spaces. A database of successful runs about a set-point is then created and used to develop statistical process control charts to monitor the evolution of the batch with respect to the addition of each subsequent component. These charts have been used for real-time monitoring of process deviations. This prevents wastage of valuable inclusion body or solubilized inclusion bodies material and saves time by allowing early detection of the otherwise unsalvageable batches. The tool also allows a history of batch information to be saved in the form of easily interpretable profiles for future reference. The probes are robust, cost-effective, and can be easily integrated with data collection systems. Using these three online indicators in real time has been demonstrated to ensure that the process is being operated within the established design space, thereby potentially eliminating or at least

minimizing the need for testing of the product critical quality attributes using offline methods such as reverse phase-high performance liquid chromatography.

Many times biotherapeutic faces challenges such as lower half life and stability inside the body. PEGylation of the product is a well-known method to improve the pharmacological properties and stability of biotherapeutic proteins. Proper control of the PEGylation reaction step is critical as the molecule's size and nature changes significantly during the reaction, as does the impurity profile of the process stream. A near infrared spectroscopy-based PAT approach has been developed for monitoring and control of the PEGylation reaction of G-CSF. NIRS spectra have been used to build calibrated and validated PLS models for the three species involved in the reaction, namely G-CSF and its monoPEGylated and diPEGylated variants. A NIRS immersion probe was deployed in the reaction vessel to provide real time information about the progress of the reaction. A pH probe integrated with a peristaltic pump facilitated automated quenching of the reaction at the deserved time. The NIRS spectra have also been used to build a Batch Evolution Model for the reaction from end-to-end, including the addition of the reactants to the reaction vessel, the progress of the reaction for 70 minutes, and the final quenching with Tris base. Comparing real-time data with the expected trajectory of statistical process control charts from the Batch Evolution Model allowed for the deviations to be identified as and when they occur, and aid in root cause analysis.

Snakebite has been a neglected disease for decades until the World Health Organization included it in the list of tropical neglected diseases in 2009. A process for manufacturing the recombinant Lethal Toxin Neutralizing factor (rLTNF) has been developed with overall recovery of 78–80%. A manufacturing plant capable of producing 10 Kg of rLTNF per batch was simulated and techno economic analysis performed. The production cost of rLTNF was estimated to be $5–6 per g (or $0.09 per 15 mg dose), significantly lower in cost to that of currently available antivenom in the market (approximately $6 per dose).Further, a novel three-step process for manufacturing of rLTNF monomer to reduce the manufacturing cost by 30%. The tolerance of alpha chymotrypsin enzyme to denaturing conditions was used to develop a simultaneous solubilization and cleavage reaction in single step, in which the denaturant urea not only aids inclusion body solubilization but also opens the peptide chains for efficient cleavage. The cleavage reaction was identified to be critical as it determines product yield and purity, and thus all further downstream processes and was optimized using Design of Experiments techniques. The proposed process offers reduced manufacturing cost by up to 30% from the previously published process and can be easily adapted to any peptide manufacturing process. Finally, a PAT implementation for monitoring and control of the rLTNF enzymatic

cleavage reaction was developed using ATR-FTIR spectroscopy which provides distinctive spectra for rLTNF concatemer and urea concentration in the reaction mixture. PLS calibration models were built to correlate the at-line spectra with concentration of rLTNF concatemer, allowing the progress of the reaction and percentage conversion to be tracked in real time. PAT implementation ensured that the product formed meets critical quality attribute targets of purity, yield and impurity profile. The tool also enabled identification of deviations, such as deviations in the reaction buffer, concentration of concatemer in the IBs, enzyme loading relative to protein concentration, and reaction time with late quenching, enabling robust monitoring and control.

In protein therapeutics, it is critical that the excipient concentrations in the drug product are maintained within the prescribed specifications. This task is quite straightforward when the protein concentration in drug product is low. However, as the protein concentrations rise in the drug product, especially in the case of subcutaneous formulations, maintaining precise levels of excipients becomes challenging. At higher concentration (in excess of 100 mg/ml), protein molecules tend to attract/repel the charged excipients when processed through tangential flow filtration membrane, resulting in a significant drift of excipient concentration in the final formulated drug substance. This in turn alters the pH, conductivity, and osmolality of the solution. Limited literature exists on methods for analysis of excipients in protein drug products and this is so due to incompatibility of protein with organic solvents and wide diversity with respect to the nature of excipients used in protein therapeutic formulations. Two different chromatographic modes, ion exclusion chromatography and reversed phase chromatography, were deployed to analyse a total of seventeen excipients that are widely present in protein formulations ranging from peptides to monoclonal antibodies. Both the methods were validated as per ICH guidelines. The present methods did not require sample pre-treatment. The application of the methods has been demonstrated by analysing excipients concentration in various innovator products and their biosimilars. A mechanistic model incorporating coupling of the charge fluxed through the membrane was developed to explain the drift in the excipient concentration observed in DF and UF operations in the processing of mAbs. It was found that the drift of the excipients in both the DF and UF operations is most strongly influenced by their diffusivity, followed by the charge on the mAb and its concentration. The initial/ exchange buffer concentration also had a significant influence on the drift of the excipients. The model was validated with experimental results, and the match between the experimental and the model-simulated values were within 8%. It has been demonstrated that the model can be used to predict the initial/ exchange buffer concentration for a target final concentration after the

UF/DF operation. The effect of system parameters on the drift of the excipients was determined from the model, and the diffusivities of the excipients and mAb charge were found to have the strongest influence.

We believe that the studies presented here as a part of this thesis will promote development of highly efficient PAT tools for manufacturing of biotherapeutics.

सार

विनिर्माण बायोफार्मास्युटिकल्स एक जटिल प्रक्रिया है। उत्पाद की निरंतर गुणवत्ता सुनिश्चित करने के लिए प्रक्रिया चर की निगरानी और नियंत्रण आवश्यक है। हालांकि, इन-प्रोसेस गुणवत्ता नियंत्रण पारंपरिक रूप से ऑफ लाइन विश्लेषण तक सीमित है। यह महत्वपूर्ण निर्णयों, बैच विफलताओं, और मूल्यवान उत्पाद के नुकसान के समय में देरी को रोकता है। इसे देखते हुए, नियामक अधिकारियों ने लगातार उत्पाद गुणवत्ता प्राप्त करने के उद्देश्य से बायोटेक उद्योगों के लिए प्रक्रिया विश्लेषणात्मक प्रौद्योगिकी (प्याट) मार्गदर्शन का प्रस्ताव किया है। पीएटी नियंत्रण के सुधार, निरंतर उत्पाद की गुणवत्ता प्रदान करने और बेहतर परिचालन दक्षता प्रदान करने के लिए प्रक्रिया ज्ञान के अनुप्रयोग की सुविधा के लिए एक दृष्टिकोण प्रदान करता है।

पीएटी के कार्यान्वयन के लिए प्रक्रिया मॉडल की स्थापना, महत्वपूर्ण गुणवत्ता विशेषताओं, महत्वपूर्ण प्रक्रिया मापदंडों और फ़ीड सामग्री विशेषताओं के बीच गणितीय संबंध की आवश्यकता होती है। जबकि मैकेनिस्टिक मॉडल वांछनीय हैं, अधिकांश बायोटेक यूनिट संचालन यांत्रिकी मॉडल बनाने के लिए बहुत जटिल हैं जो वाणिज्यिक निर्माण में उपयोग के लिए पर्याप्त सरल हैं। अनुभवजन्य मॉडल या हाइब्रिड मॉडल का उपयोग उद्योग में एक आदर्श है। बायोफर्मासिटिकल मैन्युफैक्चरिंग में इस्तेमाल किए जाने वाले दो प्रमुख प्लेटफॉर्म हैं बैक्टीरियल एस्चेरिचिया कोलाई सिस्टम और स्तनधारी चीनी हम्सटर ओवेरियन सिस्टम। ई। कोलाई आधारित प्रक्रिया में, रेफोल्डिंग उन महत्वपूर्ण इकाइयों के संचालन में से एक है जो उत्पादों की उपज, अशुद्धता प्रोफ़ाइल का फैसला करता है।

प्रोटीन रिफ़ॉल्डिंग को महत्वपूर्ण प्रक्रिया मापदंडों और फ़ीड सामग्री विशेषताओं से प्रभावित होने के लिए जाना जाता है, जिसमें सॉल्यूबिलेशन और पीएच की संरचना और बफर को शामिल किया जाता है। इसलिए, मजबूत प्रक्रिया नियंत्रण और उत्पाद की गुणवत्ता प्राप्त करने के लिए, इन विशेषताओं और मापदंडों पर नजर रखने की आवश्यकता है। पूर्व-निर्धारित डिज़ाइन और ऑपरेटिंग रिक्त स्थान के भीतर एक विशिष्ट रीफ़ॉल्डिंग बैच का एक फिंगरप्रिंट बनाने के लिए ऑनलाइन ऑक्सीकरण कमी क्षमता, पीएच और तापमान जांच का उपयोग करके एक पैट टूल विकसित किया गया है। सेट-पॉइंट के बारे में सफल रन का एक डेटाबेस तब बनाया जाता है और प्रत्येक बाद के घटक के अलावा के साथ बैच के विकास की निगरानी के लिए सांख्यिकीय प्रक्रिया नियंत्रण चार्ट विकसित करने के लिए उपयोग किया जाता है। इन चार्टों का उपयोग प्रक्रिया विचलन की वास्तविक समय की निगरानी के लिए किया गया है। यह मूल्यवान समावेशन के अपव्यय को रोकता है या समावेशी निकाय सामग्री को घुलित करता है और अन्यथा अप्राप्य बैचों का शीघ्र पता लगाने की अनुमति देकर समय बचाता है। उपकरण भविष्य के संदर्भ के लिए आसानी से व्याख्या करने योग्य प्रोफाइल के रूप में बैच जानकारी के इतिहास को सहेजने की अनुमति देता है। जांच मजबूत, लागत प्रभावी है, और आसानी से डेटा संग्रह प्रणालियों के साथ एकीकृत किया जा सकता है। वास्तविक समय में इन तीन ऑनलाइन संकेतकों का उपयोग करके यह सुनिश्चित करने के लिए प्रदर्शन किया गया है कि यह प्रक्रिया स्थापित डिज़ाइन स्थान के भीतर संचालित हो रही है, जिससे ऑफ़लाइन चरण का उपयोग करते हुए उत्पाद महत्वपूर्ण गुणवत्ता के परीक्षण की आवश्यकता को कम से कम या कम से कम किया जा सके, जैसे कि रिवर्स चरण- उच्च उत्पादन द्रव्य वर्णलेखन। कई बार जैव-चिकित्सीय चुनौतियों का सामना करना पड़ता है, जैसे निचले आधे जीवन और शरीर के अंदर स्थिरता। औषधीय गुणों को बेहतर बनाने के लिए और जैव-चिकित्सीय प्रोटीन की स्थिरता को बेहतर बनाने के लिए उत्पाद का पेगीलेशन एक प्रसिद्ध विधि है। अणु प्रतिक्रिया के चरण का उचित नियंत्रण महत्वपूर्ण है क्योंकि प्रतिक्रिया के दौरान अणु का आकार और प्रकृति में महत्वपूर्ण परिवर्तन होता है, जैसा कि प्रक्रिया स्ट्रीम की अशुद्धता प्रोफ़ाइल करता है। जी-सीएसएफ के पेगीलेशन प्रतिक्रिया की निगरानी और नियंत्रण के लिए एक निकट अवरक्त स्पेक्ट्रोस्कोपी-आधारित पैट दृष्टिकोण विकसित किया गया है। एनआईआरयस स्पेक्ट्रा का उपयोग प्रतिक्रिया में शामिल तीन प्रजातियों के लिए कैलिब्रेटेड और वैलिडेटेड पीएलएस मॉडल के निर्माण के लिए किया गया है, अर्थात् जी-सीएसएफ और इसके मोनोपेगलेटेड और डायपेगलेटेड वेरिएंट। प्रतिक्रिया की प्रगति

के बारे में वास्तविक समय की जानकारी प्रदान करने के लिए प्रतिक्रिया पोत में एक एनआईआरएस विसर्जन जांच तैनात की गई थी। एक पीएच जांच एक क्रमिक वृत्तों में सिकुड़नेवाला पंप के साथ एकीकृत, योग्य समय पर प्रतिक्रिया की स्वचालित शमन की सुविधा। येनआईरयस स्पेक्ट्रा का उपयोग एंड-टू-एंड से प्रतिक्रिया के लिए एक बैच इवोल्यूशन मॉडल बनाने के लिए भी किया गया है, जिसमें रिएक्टर पोत पर रिएक्टर्स को जोड़ना, 70 मिनट के लिए प्रतिक्रिया की प्रगति और ट्रिस बेस के साथ अंतिम शमन शामिल है। । बैच विकास मॉडल से सांख्यिकीय प्रक्रिया नियंत्रण चार्ट की अपेक्षित प्रक्षेपवक्र के साथ वास्तविक समय के आंकड़ों की तुलना विचलन के लिए अनुमति दी जाती है जब वे होते हैं और मूल कारण विश्लेषण में सहायता करते हैं।

सर्पदंश दशकों से एक उपेक्षित बीमारी है, जब तक कि २००९ में विश्व स्वास्थ्य संगठन ने इसे उष्णकटिबंधीय उपेक्षित बीमारियों की सूची में शामिल नहीं किया था। पुनः संयोजक घातक टॉक्सिन न्यूट्रालाइजिंग फैक्टर (आरएलटियेनऍफ़) के निर्माण की एक प्रक्रिया को ७०-८० % की समग्र वसूली के साथ विकसित किया गया है। प्रति बैच १० टन आरएलटियेनऍफ़ के उत्पादन में सक्षम एक विनिर्माण संयंत्र का अनुकरण किया गया और तकनीकी आर्थिक विश्लेषण किया गया। आरएलटीएनएफ की उत्पादन लागत $ ५-६ प्रति ग्राम (या $ ०.०९ प्रति १५ मिलीग्राम खुराक) होने का अनुमान था, बाजार में वर्तमान में उपलब्ध एंटीवेनम (लगभग $ ६ प्रति खुराक) की लागत से काफी कम है। एक उपन्यास तीन-चरण विनिर्माण लागत को 30% तक कम करने के लिए आरएलटियेनऍफ़ मोनोमर के निर्माण की प्रक्रिया। शर्तों को निरूपित करने के लिए अल्फा काइमोट्रिप्सिन एंजाइम की सहिष्णुता का उपयोग एकल चरण में एक साथ घुलनशीलता और दरार की प्रतिक्रिया को विकसित करने के लिए किया गया था, जिसमें डिनैब्यूटेंट यूरिया न केवल शरीर को जोड़ने में सहायक होता है, बल्कि कुशल दरार के लिए पेप्टाइड श्रृंखला भी खोलता है। दरार की प्रतिक्रिया को महत्वपूर्ण माना गया क्योंकि यह उत्पाद की उपज और शुद्धता को निर्धारित करता है, और इस प्रकार आगे की सभी डाउनस्ट्रीम प्रक्रियाओं और डिजाइन ऑफ एक्सपेरिमेंट तकनीकों का उपयोग करके अनुकूलित किया गया था। प्रस्तावित प्रक्रिया पहले से प्रकाशित प्रक्रिया से विनिर्माण लागत को ३०% तक कम कर देती है और इसे आसानी से किसी भी पेप्टाइड निर्माण प्रक्रिया के लिए अनुकूलित किया जा सकता है। अंत में, आरटीटीएनएफ एंजाइमैटिक क्लीवेज रिएक्शन की निगरानी और नियंत्रण के लिए एक पैट कार्यान्वयन एटीआर-एफटीआईआर स्पेक्ट्रोस्कोपी का उपयोग करके विकसित किया गया था जो प्रतिक्रिया मिश्रण में आरएलएनटीएन एफटीएटर और यूरिया एकाग्रता के लिए विशिष्ट स्पेक्ट्रा प्रदान करता है। पियलयस कैलिब्रेशन मॉडल को आरएलटियेनऍफ़ कंसट्रेटर की एकाग्रता के साथ ऑन-लाइन स्पेक्ट्रा को सहसंबंधित करने के लिए बनाया गया था, जिससे प्रतिक्रिया और प्रतिशत रूपांतरण की प्रगति को वास्तविक समय में ट्रैक किया जा सके। पीएटी कार्यान्वयन ने सुनिश्चित किया कि गठित उत्पाद शुद्धता, उपज और अशुद्धता प्रोफ़ाइल के महत्वपूर्ण गुणवत्ता विशेषता लक्ष्यों को पूरा करता है। उपकरण ने विचलन की पहचान को भी सक्षम किया, जैसे कि प्रतिक्रिया बफर में विचलन, आईबी में कंसट्रेटर की एकाग्रता, प्रोटीन एकाग्रता के सापेक्ष एंजाइम लोड करना, और देर से शमन के साथ प्रतिक्रिया समय, मजबूत निगरानी और नियंत्रण को सक्षम करना।

प्रोटीन चिकित्सा विज्ञान में, यह महत्वपूर्ण है कि दवा उत्पाद में निर्धारित सांद्रता निर्धारित विनिर्देशों के भीतर बनाए रखी जाती है। ड्रग उत्पाद में प्रोटीन की मात्रा कम होने पर यह कार्य काफी सीधा है। हालांकि, दवा उत्पाद में प्रोटीन सांद्रता में वृद्धि के रूप में, विशेष रूप से चमड़े के नीचे योगों के मामले में, एक्ससपिएंट्स के सटीक स्तर को बनाए रखने चुनौतीपूर्ण है। उच्च सांद्रता में (१०० मिली ग्राम / मिली लीटर से अधिक), प्रोटीन अणु स्पर्शरेखा प्रवाह निस्पंदन झिल्ली के माध्यम से संसाधित होने पर आवेशित वाहकों को आकर्षित / प्रतिकर्षित करते हैं, जिसके परिणामस्वरूप अंतिम तैयार दवा पदार्थ में प्रवाहक एकाग्रता का एक महत्वपूर्ण बहाव होता है। यह बदले में पीएच, चालकता, और समाधान की परासरणता को बदल देता है। प्रोटीन दवा उत्पादों में एक्ससपिएंट्स के विश्लेषण के लिए सीमित साहित्य मौजूद है और ऐसा कार्बनिक सॉल्वैंट्स के साथ प्रोटीन की असंगति और प्रोटीन चिकित्सीय योगों में उपयोग किए गए एक्ससपिएंट्स की प्रकृति के संबंध में व्यापक विविधता के कारण होता है। दो अलग-अलग

क्रोमैटोग्राफिक मोड, आयन अपवर्जन क्रोमैटोग्राफी और उल्टे चरण क्रोमैटोग्राफी, कुल सत्रह एक्सक्सपिएंट्स का विश्लेषण करने के लिए तैनात किए गए थे जो कि व्यापक रूप से पेप्टाइड्स से मोनोक्लोनल एंटीबॉडी तक के प्रोटीन योगों में मौजूद हैं। दोनों विधियों को आईसीएच दिशानिर्देशों के अनुसार मान्य किया गया था। वर्तमान विधियों में नमूना पूर्व उपचार की आवश्यकता नहीं थी। विभिन्न इनोवेटर उत्पादों और उनके बायोसिमिलर में एक्सक्सपिएंट्स एकाग्रता का विश्लेषण करके विधियों के आवेदन का प्रदर्शन किया गया है। झिल्ली के माध्यम से प्रवाहित आवेश के युग्मन को सम्मिलित करने वाला एक यंत्रवत मॉडल विकसित किया गया था ताकि मयाबस के प्रसंस्करण में डिएफ और यूएफ क्रियाओं में मनाया जाने वाले उत्कृष्ट सांद्रता में बहाव को समझाया जा सके। यह पाया गया कि डिएफ और यूएफ संचालन दोनों में एक्सफ़िलिएंट्स का बहाव उनके डिफ्यूसिटी से सबसे अधिक प्रभावित होता है, इसके बाद मयाबस और उसके सांद्रता पर चार्ज होता है। प्रारंभिक / विनिमय बफर एकाग्रता का भी एक्सक्सपिएंट्स के बहाव पर एक महत्वपूर्ण प्रभाव था। मॉडल को प्रयोगात्मक परिणामों के साथ मान्य किया गया था, और प्रयोगात्मक और मॉडल-सिम्युलेटेड मूल्यों के बीच मैच ८% के भीतर थे। यह प्रदर्शित किया गया है कि मॉडल का उपयोग डिएफ और यूएफ ऑपरेशन के बाद लक्ष्य अंतिम एकाग्रता के लिए प्रारंभिक / विनिमय बफर एकाग्रता की भविष्यवाणी करने के लिए किया जा सकता है। एक्सक्सपिएंट्स के बहाव पर सिस्टम मापदंडों का प्रभाव मॉडल से निर्धारित किया गया था, और एक्सक्सपिएंट्स और मयाबस चार्ज के विचलन को सबसे मजबूत प्रभाव पाया गया था।

हमारा मानना है कि इस थीसिस के एक भाग के रूप में यहां प्रस्तुत अध्ययन जैव-तंत्र के विनिर्माण के लिए अत्यधिक कुशल पैट उपकरणों के विकास को बढ़ावा देगा।

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List of Abbreviations

CQA	*Critical Quality Attribute*
CPP	*Critical Process Parameters*
PAT	*Process Analytical Technology*
DOE	*Design of Experiments*
MVDA	*Multivariate Data Analysis*
MSPC	*Multivariate Statistical Process Control*
QbD	*Quality by Design*
DOT	*Dissolve Oxygen Tension*
FTIR	*Fourier Transformed Infrared Spectroscopy*
DLS	*Dynamic Light Scattering*
LTNF	*Lethal Toxin Neutralizing Factor*
IFPAC	*International Foundation Process Analytical Chemistry*
FMEA	*Failure Mode Effect Analysis*
rhGCSF	*Recombinant Human Granulocyte Colony Stimulating factor*
DTT	*Dithiothreitol*
PEG	*Polyethylene Glycol*
HPLC	*High Performance Liquid chromatography*
IB	*Inclusion Body*
SIB	*Solubilized Inclusion Bodies*
RP-HPLC	*Reverse Phase- High Performance Liquid chromatography*
BSPC	*Batch Statistical Process Control*
ORP	*Oxidation reduction Potential*
MPLS	*Multivariate Statistical Process Control*
GAA	*Glacial Acetic Acid*
TFA	*Trifluoroacetic acid*
NIPALS	*Nonlinear Iterative Partial Least Squares*
BEM	*Batch Evolution Modelling*
SPC	*statistical process control*
WHO	*World Health Organization*
GMP	*good manufacturing practice*
COGs	*cost of goods*
HCDNA	*Host Cell Deoxyribonucleic Acid*
HCP	*Host Cell Protein*
V	*Volume of the reservoir (m^3)*
$F_{i,P}$	*Rate of outflow of species 'i' through the permeate stream ($m^3\ s^{-1}$)*
F_P	*Permeate flow rate($m^3\ s^{-1}$)*
$C_{i,r}$	*Concentrations of species 'i' in retentate(mol m^{-3})*
$C_{i,b}$	*Concentrations of species 'i' in exchange buffer(mol m^{-3})*
A	*Membrane area(m^2)*
J_v	*Permeate flux(m s^{-1})*
J_i	*Flux of the species(mol s^{-1})*
F	*Faraday's constant(C mol^{-1})*
T	*Temperature(K)*
D_i	*Diffusivity of species 'i' ($m^2\ s^{-1}$)*

z_i	*Valency (charge in electronic unit) of species i*
ϕ	*Electrostatic potential(J C^{-1})*
x	*Space variable in the direction of the length of the pore of the membrane(m)*
l	*Length of the pore(m)*
$C_{i,m0}$	*Concentration of species i at the pore mouth(mol m^{-3})*
$C_{i,ml}$	*Concentration of species i at the pore end(mol m^{-3})*
ϕ_0	*Electric potential at the pore mouth(J C^{-1})*
ϕ_l	*Electric potential at the pore end(J C^{-1})*
mAb	*Monoclonal antibody*
UF/DF	*Ultrafiltration/Diafiltration*
v_p	*Partial volume of the protein in the reservoir(m^3)*
C_p	*Concentration of the protein in the reservoir(mol m^{-3})*
n	*Number of diafiltration volumes*
D_{Fi}	*Fractional drift in the concentration of i^{th} excipient*
C_i	*Concentration of i^{th} excipient in the mAb solution(mol m^{-3})*
C_{ib}	*Concentration of i^{th} excipient in the exchange buffer(mol m^{-3})*
his^+	*Histidine ion*
Ac^-	*Acetate ion*
Na^+	*Sodium ion*
Cl^-	*Chloride ion*
his^0	*Neutral histidine*
$C_{i,f}$	*Final concentration of the 'i' excipient after the UF/DF operation(mol $^{-3}$)*