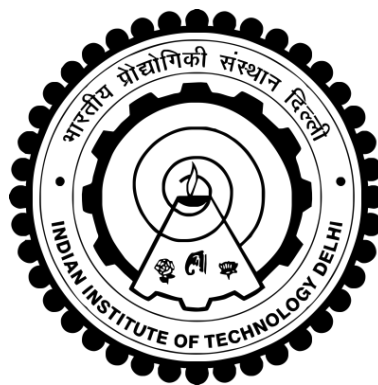


**CONTINUOUS PROCESSING FOR
PURIFICATION OF
BIOTECH THERAPEUTICS**

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INDIAN INSTITUTE OF TECHNOLOGY DELHI
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PURIFICATION OF
BIOTECH THERAPEUTICS**

by

NIKHIL KATEJA

Department of Chemical Engineering

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

FEBRUARY 2020

Dedicated to my family

Certificate

This is to certify that the thesis entitled “**CONTINUOUS PROCESSING FOR PURIFICATION OF BIOTECH THERAPEUTICS**” being submitted by **NIKHIL KATEJA** 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.

Prof. Anurag S. Rathore

Department of Chemical Engineering

Indian Institute of Technology Delhi

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NIKHIL KATEJA

Abstract

Despite the extraordinary success, the biopharma industry is presently facing multiple economic, political and regulatory challenges. As a result, there is a significant drive to boost the overall productivity of biotherapeutic manufacturing by utilization of continuous processing. However, the actual implementation of continuous manufacturing is highly contingent on development of novel continuous processing technologies and their efficient integration. In this thesis, we attempt to alleviate a number of challenges associated with continuous bioprocessing by following a two-fold approach: (a) development of novel continuous manufacturing technologies; (b) utilization of these technologies for development of cost effective alternatives to the current batch manufacturing .

While the technology for continuous upstream processing has been explored for more than a decade (perfusion), options available for continuous downstream processing are limited and subject to ongoing development. Such enabling technologies are especially lacking for processes which require reaction and mixing like refolding and precipitation. In our attempt to solve this problem, we report (chapter 3) on development of a novel coiled flow inversion reactor (CFIR). The CFIR has been proposed as a configuration consisting of helical coils bent at equidistant right angles to cause flow inversion for improved cross sectional mixing in the tube. The configuration can be appropriately utilized to provide a sharper residence time distribution along with good cross-sectional mixing and a better emulation of plug flow than a simple straight tube or a helix. The novel reactor has been explored for continuous protein refolding and continuous precipitation. The performance of the continuous protocol has been compared with an optimized batch protocol. It has been demonstrated that operation in CFIR allows for refolding at higher protein concentrations (2 folds) along with a decrease in the time required to reach equilibrium conversion (1.5 folds). In case of continuous precipitation, improved clearance of host cell proteins (HCP) and host cell deoxyribonucleic acid (DNA) was obtained compared to batch. In both the case studies, continuous configuration offered a 6-16X increase in productivity as non-productive steps like cleaning and filling steps were eliminated and buffer consumption was significantly reduced without any adverse effect on product quality. Effectively, the configuration can significantly contribute towards creation of an integrated continuous bioprocessing platform.

Actual implementation of continuous manufacturing is highly contingent on integration between different unit operations. Recent times has therefore seen an impetus towards development of solutions for efficient integration of continuous unit operations. Integrated

chromatographic platform processes have the potential to simplify the downstream processing train without sacrificing the product quality requirements. Thus, the primary motivation (chapter 5) was to develop an integrated chromatographic platform for purification of monoclonal antibody (mAb) therapeutics that can deliver the desired separation of both charge variants and aggregates, in addition to the process related impurities like HCP and host cell DNA. To achieve the same, an integrated two-stage chromatographic process platform consisting of cation exchange chromatography and multimodal chromatography has been proposed. The versatility of the proposed platform has been successfully demonstrated for three different mAbs. It has been shown that in each case charge variant separation was achieved with the required clearance of aggregates ($< 1\%$), HCP (< 10 ppm), and DNA (< 5 ppb). Moreover, the proposed platform is conducive to use for development of a continuous process and offers 50% reduction in process time, absence of any hold steps, lower buffer utilization, lower risk of external contamination and decreased operational costs when compared to the conventional purification platforms.

Based on the success of creation of technology enablers in the form of the CFIR and integrated chromatographic platforms, the later part of thesis focusses on utilization of these developed enablers for creation of end-to-end continuous platforms. Creation of two platforms was targeted- one for proteins expressed in bacterial hosts as inclusion bodies and the other for monoclonal antibody therapeutics expressed in mammalian hosts. In chapter 4, we propose an end-to-end integrated continuous downstream process (from inclusion bodies to unformulated drug substance) for a therapeutic protein expressed in *Escherichia coli* as inclusion bodies, with granulocyte colony stimulating factor (GCSF) as our model molecule. The final process for GCSF consisted of continuous refolding in a coiled flow inverter reactor directly coupled to a three-column periodic counter-current chromatography for capture of the product followed by a three-column con-current chromatography for polishing. The continuous bioprocessing train was run uninterrupted for 26 h to demonstrate its capability and the resulting output was analyzed for the various critical quality attributes, namely product purity ($>99\%$), high molecular weight impurities ($<0.5\%$), host cell proteins (<100 ppm), and host cell DNA (<10 ppb). All attributes were found to be consistent over the period of operation. The developed assembly offers smaller facility footprint, higher productivity, fewer hold steps, and significantly higher equipment and resin utilization. The complexities of process integration in the context of continuous processing have also been highlighted.

For the case of mAb therapeutics (chapter 6), Protein A capture chromatography, the core of a mAb purification platform, is known to account for more than 50% of downstream processing

costs along with other limitations including lack of complete stability to alkaline cleaning solutions, relatively lower binding capacity, and ligand leaching. Researchers have explored alternatives to protein A chromatography, both chromatographic and non-chromatographic, but with limited success. In this work, we have proposed a non-protein A purification platform for continuous processing of monoclonal antibodies (mAbs). The proposed platform consists of precipitation in coiled flow inverter reactor, cation exchange chromatography for capture, multimodal chromatography and a salt tolerant anion exchange membrane as polishing steps. The versatility of the proposed platform has been successfully demonstrated for three different mAbs. In all cases, acceptable process yield was obtained (70 to 80 %) and the product quality attributes of the final unformulated drug substance were consistent and well within accepted limits (HCP <100 ppm, DNA < 10 ppb, % aggregate content < 1%) along with desired charge variant composition.

We believe that the studies presented here as a part of this thesis will promote development of highly efficient, universal, end-to-end, fully continuous platforms for manufacturing of biotherapeutics.

सार

असाधारण सफलता के बावजूद, बायोफार्मा उद्योग वर्तमान में कई आर्थिक, राजनीतिक और नियामक चुनौतियों का सामना कर रहा है। नतीजतन, निरंतर प्रसंस्करण के उपयोग द्वारा जैव-चिकित्सीय विनिर्माण की समग्र उत्पादकता को बढ़ावा देने के लिए एक महत्वपूर्ण ड्राइव है। हालांकि, निरंतर निर्माण का वास्तविक कार्यान्वयन उपन्यास निरंतर प्रसंस्करण प्रौद्योगिकियों के विकास और उनके कुशल एकीकरण पर अत्यधिक आकस्मिक है। इस थीसिस में, हम एक दोहरी दृष्टिकोण का पालन करते हुए निरंतर बायोप्रोसेसिंग से जुड़ी कई चुनौतियों को दूर करने का प्रयास करते हैं: (ए) उपन्यास निरंतर निर्माण प्रौद्योगिकियों का विकास; (बी) वर्तमान बैच विनिर्माण के लिए लागत प्रभावी विकल्पों के विकास के लिए इन प्रौद्योगिकियों का उपयोग।

जबकि सतत अपस्ट्रीम प्रसंस्करण के लिए प्रौद्योगिकी को एक दशक से अधिक (छिड़काव) के लिए खोजा गया है, निरंतर बहाव प्रसंस्करण के लिए उपलब्ध विकल्प सीमित हैं और चल रहे विकास के अधीन हैं। ऐसी सक्षम प्रौद्योगिकियों में विशेष रूप से ऐसी प्रक्रियाओं की कमी होती है जिनके लिए प्रतिक्रिया और मिश्रण की आवश्यकता होती है जैसे कि रीफोल्डिंग और प्रेसिपीटेशन। इस समस्या को हल करने के हमारे प्रयास में, हम एक उपन्यास कोइलेड फ्लो इन्वर्टर रिएक्टर (सीएफआईआर) के विकास पर रिपोर्ट (अध्याय 3) करते हैं। सीएफआईआर को एक विन्यास के रूप में प्रस्तावित किया गया है, जो नली में बेहतर पार अनुभागीय मिश्रण के लिए प्रवाह उलटा पैदा करने के लिए विषुवत समकोण पर सहायक कुंडली से बना है। विन्यास का उपयोग उचित क्रॉस-अनुभागीय मिश्रण और एक सरल स्ट्रेट ट्यूब या हेलिक्स की तुलना में प्लग प्रवाह के बेहतर अनुकरण के साथ एक तेज निवास समय वितरण प्रदान करने के लिए किया जा सकता है। उपन्यास रिएक्टर को निरंतर प्रोटीन रीफॉल्डिंग और निरंतर प्रेसिपीटेशन के लिए खोजा गया है। निरंतर प्रोटोकॉल के प्रदर्शन की तुलना एक अनुकूलित बैच प्रोटोकॉल के साथ की गई है। यह प्रदर्शित किया गया है कि सीएफआईआर में ऑपरेशन उच्च प्रोटीन सांद्रता (दो गुना) के साथ-साथ संतुलन रूपांतरण (डेढ़ गुना) तक पहुंचने के लिए आवश्यक समय में कमी के लिए

अनुमति देता है। निरंतर प्रेसिपीटशन के मामले में, मेजबान सेल प्रोटीन (एचसीपी) और मेजबान सेल डीऑक्सीराइबोन्यूक्लिक एसिड (डीएनए) की बेहतर निकासी बैच की तुलना में प्राप्त की गई थी। दोनों मामले के अध्ययन में, निरंतर कॉन्फिगरेशन ने उत्पादकता में 6-16X वृद्धि की पेशकश की क्योंकि सफाई और भरने वाले कदम जैसे गैर-उत्पादक कदम समाप्त हो गए और उत्पाद की गुणवत्ता पर कोई प्रतिकूल प्रभाव डाले बिना बफर खपत को काफी कम कर दिया गया। प्रभावी रूप से, कॉन्फिगरेशन एक एकीकृत सतत बायोप्रोसेसिंग प्लेटफॉर्म के निर्माण में महत्वपूर्ण योगदान दे सकता है।

विभिन्न इकाइयों के संचालन के बीच एकीकरण पर निरंतर विनिर्माण का वास्तविक कार्यान्वयन अत्यधिक आकस्मिक है। इसलिए हाल के दिनों में निरंतर यूनिट संचालन के कुशल एकीकरण के लिए समाधानों के विकास की दिशा में गति देखी गई है। एकीकृत क्रोमैटोग्राफिक प्लेटफॉर्म प्रक्रियाओं में उत्पाद की गुणवत्ता की आवश्यकताओं का त्याग किए बिना डाउनस्ट्रीम प्रसंस्करण ट्रेन को सरल बनाने की क्षमता है। इस प्रकार, प्राथमिक प्रेरणा (अध्याय 5) को मोनोक्लोनल एंटीबॉडी (मैब) चिकित्सीय की शुद्धि के लिए एक एकीकृत क्रोमैटोग्राफिक प्लेटफॉर्म विकसित करना था जो एचसीपी और डीएनए के अलावा चार्ज वेरिएंट और एग्रीगेट दोनों के वांछित पृथक्करण को वितरित कर सकता है। उसी को प्राप्त करने के लिए, एक एकीकृत दो-चरण क्रोमैटोग्राफिक प्रक्रिया मंच जिसमें कटियन एक्सचेंज क्रोमैटोग्राफी और मल्टीमॉडल क्रोमैटोग्राफी शामिल है, प्रस्तावित किया गया है। प्रस्तावित मंच की बहुमुखी प्रतिभा को तीन अलग-अलग मैबस के लिए सफलतापूर्वक प्रदर्शित किया गया है। यह दिखाया गया है कि प्रत्येक मामले में चार्ज वेरिएंट पृथक्करण समुच्चय (<1%), एचसीपी (<10 पीपीएम), और डीएनए (<5 पीपीबी) की आवश्यक मंजूरी के साथ हासिल किया गया था। इसके अलावा, प्रस्तावित मंच एक सतत प्रक्रिया के विकास के लिए उपयोग करने के लिए अनुकूल है और पारंपरिक शुद्धि प्लेटफार्मों की तुलना में प्रक्रिया समय में 50% की कमी, किसी भी होल्ड स्टेप्स की अनुपस्थिति, कम बफर उपयोग, बाहरी संदूषण के कम जोखिम और परिचालन लागत में कमी की पेशकश करता है।

सीएफआईआर और एकीकृत क्रोमैटोग्राफिक प्लेटफॉर्म के रूप में प्रौद्योगिकी एनबलर्स के निर्माण की सफलता के आधार पर, एंड-टू-एंड निरंतर प्लेटफार्मों के निर्माण के लिए इन विकसित एनबलर्स के

उपयोग पर थीसिस का बाद का हिस्सा फोकस्सड है। दो प्लेटफार्मों के निर्माण को लक्षित किया गया था - एक बैक्टीरिया होस्ट में शामिल किए गए प्रोटीन के लिए और दूसरा माममालियन होस्ट में मोनोक्लोनल एंटीबॉडी चिकित्सा विज्ञान के लिए व्यक्त किया गया। अध्याय 4 में, हम अपने मॉडल अणु के रूप में ग्रेनुलोसाइट कॉलोनी स्टिमुलेटिंग फैक्टर (जी सी एस ऍफ) के साथ एस्चेरीचिअ कोली में शामिल चिकित्सीय प्रोटीन के लिए एक एंड-टू-एंड एकीकृत निरंतर बहाव प्रक्रिया (असंरचित दवा पदार्थ से) का प्रस्ताव करते हैं। जीसीएसएफ के लिए अंतिम प्रक्रिया में एक कुंडलित प्रवाह पलटनेवाला रिएक्टर में निरंतर रीफॉल्डिंग शामिल थी जो सीधे उत्पाद के कैचर और पॉलिशिंग के लिए एक आवधिक काउंटर-करंट क्रोमैटोग्राफी के लिए युग्मित था। निरंतर बायोप्रोसेसिंग ट्रेन को 26 घंटों के लिए अपनी क्षमता का प्रदर्शन करने के लिए निर्बाध रूप से चलाया गया था और परिणामी आउटपुट का विश्लेषण विभिन्न महत्वपूर्ण गुणवत्ता विशेषताओं, अर्थात् उत्पाद शुद्धता (> 99%), उच्च आणविक भार अशुद्धियों (<0.5%), मेजबान सेल प्रोटीन (<100 पीपीएम), और मेजबान सेल डीएनए (<10 पीपीबी) के लिए किया गया था। सभी विशेषताओं को ऑपरेशन की अवधि के अनुरूप पाया गया। विकसित असंबली छोटी सुविधा के पदचिह्न, उच्च उत्पादकता, कम होल्ड स्टेप्स और काफी उच्च उपकरण और रेसिन उपयोग प्रदान करती है। निरंतर प्रसंस्करण के संदर्भ में प्रक्रिया एकीकरण की जटिलताओं को भी उजागर किया गया है।

मैब थैरेप्यूटिक्स (अध्याय 6) के मामले में, प्रोटीन ए कैचर क्रोमैटोग्राफी, मैब शुद्धि प्लेटफॉर्म का मुख्य भाग, 50% से अधिक डाउनस्ट्रीम प्रोसेसिंग लागत के साथ-साथ अन्य सीमाओं के साथ खाते में जाना जाता है, जिसमें सफाई समाधानों के लिए पूर्ण स्थिरता की कमी, अपेक्षाकृत कम बाध्यकारी क्षमता, और लिगेंड लीचिंग शामिल है। शोधकर्ताओं ने प्रोटीन ए क्रोमैटोग्राफी, क्रोमैटोग्राफिक और गैर-क्रोमैटोग्राफिक दोनों के विकल्पों का पता लगाया है, लेकिन सीमित सफलता के साथ। इस काम में, हमने मोनोक्लोनल एंटीबॉडी (मैबस) के निरंतर प्रसंस्करण के लिए एक गैर-प्रोटीन ए शुद्धि मंच का प्रस्ताव दिया है। प्रस्तावित मंच में कुंडलित प्रवाह इन्वर्टर रिएक्टर में प्रेसिपीटशन, कैचरिंग के लिए कटियन एक्सचेंज क्रोमैटोग्राफी, पॉलिशिंग चरणों के रूप में मल्टीमॉडल क्रोमैटोग्राफी और एक नमक सहिष्णु

आयनों विनिमय झिल्ली है। प्रस्तावित मंच की बहुमुखी प्रतिभा को तीन अलग-अलग मैबस के लिए सफलतापूर्वक प्रदर्शित किया गया है। सभी मामलों में, स्वीकार्य प्रक्रिया उपज (70 से 80%) प्राप्त की गई थी और अंतिम असंबंधित दवा पदार्थ की उत्पाद गुणवत्ता विशेषताएँ सुसंगत और अच्छी तरह से स्वीकार्य सीमा के भीतर थीं (एचसीपी <100 पीपीएम, डीएनए <10 पीपीबी, % एग्रीगेट <1%) वांछित चार्ज संस्करण रचना के साथ।

हमारा मानना है कि इस थीसिस के एक हिस्से के रूप में यहां प्रस्तुत अध्ययन जैव-तंत्र के निर्माण के लिए अत्यधिक कुशल, सार्वभौमिक, एंड-टू-एंड, पूरी तरह से निरंतर प्लेटफार्मों के विकास को बढ़ावा देगा।

Contents

Certificate	i
Acknowledgements	ii
Abstract	iv
List of Figures	xiv
List of Tables	xvii
1 Introduction	1
1.1 Background.....	2
1.2 Problem definition, scope and objectives of research	3
1.2.1 Development of technology enablers to facilitate continuous processing.....	3
1.2.2 Integrated continuous downstream processing of proteins expressed as inclusion bodies.....	4
1.2.3 Integrated chromatographic platform development for purification of monoclonal antibody therapeutic products	4
1.2.4 Non-protein A purification platform for continuous processing of monoclonal antibody therapeutics	5
2 Literature Survey	6
2.1 Continuous processing for manufacturing of biotherapeutics.....	7
2.2 Continuous manufacturing technologies for processing of biotherapeutics	9
2.3 Process integration in continuous processing	12
2.3.1 Modular Approach	13
2.3.2 Adaptation Approach	16
2.3.3 Merger Approach	18
2.4 Continuous downstream process development	19
2.5 Integrated continuous biomanufacturing platform	22
3 Development of technology enablers to facilitate continuous processing	25
3.1 Introduction.....	26
3.1.1 Coiled flow inversion reactor (CFIR).....	26
3.2 Continuous refolding of a recombinant protein in a coiled flow inversion reactor (CFIR).28	
3.2.1 Background	28
3.2.2 Materials and methods.....	29
3.2.3 Results and discussion.....	33

3.2.4	Summary on use of CFIR for refolding.....	36
3.3	Continuous precipitation of process related impurities from clarified cell culture supernatant using the coiled flow inversion reactor (CFIR).....	36
3.3.1	Background	36
3.3.2	Materials and methods.....	38
3.3.3	Results and Discussion.....	42
3.3.4	Summary on use of CFIR for precipitation	50
4	Integrated continuous downstream processing of proteins expressed as inclusion bodies	51
4.1	Introduction.....	52
4.2	Materials and methods.....	53
4.2.1	Materials.....	53
4.2.2	Methods.....	54
4.3	Results and discussion.....	59
4.3.1	Continuous refolding in CFIR.....	59
4.3.2	Purification process development.....	59
4.3.3	Integrated continuous process development.....	63
4.3.4	Comparison of batch process and continuous process	68
4.4	Conclusions.....	69
5	Integrated chromatographic platform development for purification of monoclonal antibody therapeutic products.....	70
5.1	Introduction.....	71
5.2	Materials and methods.....	72
5.2.1	Materials.....	72
5.2.2	Methods.....	73
5.3	Results and discussion.....	80
5.3.1	Selection of chromatography steps.....	80
5.3.2	Optimization of cation exchange chromatography.....	81
5.3.3	Optimization of multimodal chromatography	83
5.3.4	Integrated two-stage chromatographic platform.....	86
5.3.5	Adaptation of platform for different mAbs	87
5.3.6	Merits of the integrated platform.....	87
5.4	Conclusions.....	90

6	Non-protein A purification platform for continuous processing of monoclonal antibody therapeutics.....	91
6.1	Introduction.....	92
6.2	Materials and Methods	93
6.2.1	Materials.....	93
6.2.2	Methods.....	95
6.3	Theory.....	100
6.3.1	Principles of Scheduling of CMC Operations.....	101
6.4	Results and Discussions.....	103
6.4.1	Preliminary Batch Studies.....	104
6.4.2	Integrated DOE-based optimization.....	107
6.4.3	Integrated Continuous Operation	111
6.5	Adaption of Platform for other mAbs.....	114
6.6	Continuous Purification Schemes- Benefits and Concerns.....	116
6.7	Conclusions.....	119
7	Conclusions and scope of future work.....	120
7.1	Conclusions.....	121
7.2	Scope of future work	122
	References.....	124
	Bio-Data.....	139

List of Figures

Figure 2.1 Schematic of a new integrated continuous manufacturing platform. Adapted from (Godawat et al., 2015).	8
Figure 2.2 List of continuous manufacturing technologies available for different stages of biopharmaceutical manufacturing.....	9
Figure 2.3 Technology modules that facilitate integration: (A,B) Alternating tangential flow filtration (ATF) and acoustic wave separator (AWS) for continuous cell retention, (C) Inline concentrator for continuous ultrafiltration, (D,E) Co-current and counter-current diafiltration units for continuous buffer exchange.....	14
Figure 2.4 Flowchart for integration of unit operations highlighting the steps to be followed and considerations to be taken into account for efficient integration.	18
Figure 2.5 Illustration of the various considerations that need to be kept in mind during development of a continuous downstream process: stepwise approach and strategies.....	21
Figure 2.6 Process flow diagram of the end-to-end continuous bioprocessing platform for production of a monoclonal antibody therapeutic proposed by Godawat et al. The platform includes a perfusion bioreactor with ATF as cell retention device for upstream processing. The downstream process included two 3-Column PCC systems.	23
Figure 3.1 Design comparison between (A) Straight helical reactor and (B) Coiled flow inversion reactor with 90° bend.....	28
Figure 3.2 (A) Process flow diagram of continuous refolding in CFIR, (B) RTD curve (dimensionless concentration, $F(\theta)$, vs. dimensionless time, θ) for the designed CFIR configuration compared to the calculated RTD curve of a straight tube reactor.....	31
Figure 3.3 Methodology followed for optimization of refolding condition in the coiled flow inversion reactor.....	32
Figure 3.4 Actual versus predicted plot for % purity and productivity models obtained from the DOE study.....	34
Figure 3.5 Comparison of batch versus continuous refolding performance over the entire duration of operation.	35
Figure 3.6 Schematic illustration of the experimental setup used for performing precipitation in the CFIR (top view) along with the tabulated details of the flow rate(s), tank(s), pump(s), and injection valves used in the different precipitation protocols.	40
Figure 3.7 Results of DOE study showing (A, B) Actual vs. predicted plot for DNA and HCP concentration, (C, D) Interaction plots for DNA and HCP concentration, (E, F) Prediction	

profilers for DNA and HCP concentration, (G, H, I) Effect of hold time on DNA concentration, recovery (%), and HCP concentration for the optimized batch condition obtained from DOE.	44
Figure 3.8 (A) Comparison of the pH change pattern in the batch precipitation with the pH change slices in continuous pH precipitation experiments (B, C, D) Comparison of recovery (%), DNA concentration and HCP concentration for the continuous pH precipitation with batch precipitation, (E) Results of DOE study for the continuous pH precipitation in CFIR showing actual vs. predicted plot for recovery %, DNA and HCP concentration (F) Prediction profiles for recovery %, DNA and HCP concentration.	46
Figure 3.9 (A, B) Overlay of isoform distribution after protein A purification as determined by CE-HPLC analytical chromatography with sigmoidal elution gradient for mAb A and mAb C respectively, (C, D) Pictures of neutralized protein A elute samples for non-precipitated and pH precipitated harvest respectively taken after centrifugation. A clear white pellet was obtained for the case of non-precipitated harvest (Figure C) but no pellet was obtained for the pH precipitated harvest (Figure D).	49
Figure 4.1 Results of DOE study showing (A) Actual vs. predicted plot for purity % and recovery %, (B) Prediction profiles for purity % and recovery %, (C) Contour profiles for purity % and recovery %.	62
Figure 4.2 Architecture of the developed integrated continuous downstream process. The assembly includes a CFIR reactor for continuous refolding, two surge vessels, and a 3 column CEX PCC for capture step followed by a 3 column MMC CCC for polishing step.	65
Figure 4.3 UV profiles for the PCC capture and the CCC polishing step (A) UV profile for loading of the 3 column CEX PCC step (B) Zoomed-in overlays of all CEX elution peaks for the 12 column operations (C) Zoomed-in overlays of all MMC elution peaks for the 60 column operations to show process consistency.	65
Figure 4.4 In-process performance indicators and critical quality attributes over the entire duration of continuous operation (A) Refolding output (B) Unformulated drug substance. ...	66
Figure 4.5 Intact mass analysis of the (A) Refolding output and (B) Purified GCSF (five replicate injections).	67
Figure 5.1 Schematic of the experimental setup used for performing two stage chromatography along with tabulated details of the flow rate(s), tank(s) and pump(s) used for different mAb samples.	76
Figure 5.2 Visual representation for the calculation of ϕ	80

Figure 5.3 (A, B) Cumulative acidic and basic species plots for Eshmuno CPX resin at different salt concentrations, (C, D) Cumulative acidic and basic species plots for Poros HS resin at different salt concentrations, (E, F) Cumulative HMWI % vs. cumulative monomer % plots for Eshmuno CPX and Poros HS resin at different salt concentrations, (G) Cumulative acidic and basic species profiles for Eshmuno CPX at pH gradients, (H) Cumulative acidic and basic species profiles for Eshmuno CPX for different salts.	85
Figure 5.4 (A, B, C) Chromatogram overlays for single CEX and integrated CEX-MMC for mAb C, mAb B and mAb A. Volume and absorbance have been normalized in the chromatograms for overlay purpose. In combined run, the HMWI were observed to elute in the regeneration step. Regeneration peak has been encircled in the chromatogram (D, E, F) Cumulative acidic and basic vs. cumulative main profile for single CEX and integrated CEX-MMC platform for mAb C, mAb B, and mAb A.	89
Figure 6.1 Architecture of the integrated continuous non-protein A platform.	96
Figure 6.2 Comparison of the PCC with CMC mode of continuous chromatography.	103
Figure 6.3 Analytical cation exchange and size exclusion chromatograms for different process steps (A, B) Precipitation (C, D) CEX chromatography, and (E, F) MMC chromatography respectively.	106
Figure 6.4 (A) Design of the central composite DOE study, (B) DOE results showing Actual versus model predicted plots, (C) Prediction profiler for output parameters with respect to input parameters, (D) Design space for operation under defined set of constraints.	110
Figure 6.5 Decision of peak cutting criteria for acidic species based on (A) Plot between Elution CV and % clearance of acidic species, (B) Plot between % recovery loss of main species and % clearance of acidic species.	110
Figure 6.6 Chromatographic profile for the (A, B) 3 column continuous CEX loading and 4 column continuous MMC loading in CMC setup, (C, D) zoomed in clubbed profiles of load and elution/washes of CEX and MMC and (E) overlays of all CEX elution profiles.	114

List of Tables

Table 2.1 Summary of recent advancements in various continuous upstream and downstream unit operations.....	10
Table 3.1 Effect of process parameters on refolding performance.	35
Table 3.2 Comparison between recoveries (%), fold reduction in DNA, HCP concentrations and % HMWI for batch and the continuous precipitation experiments.	48
Table 4.1 Detailed description of the CEX chromatography process.	56
Table 4.2 Detailed description of the MMC chromatography process.....	56
Table 4.3 Comparison of the model prediction and the actual experimental results.	63
Table 4.4 Comparison of batch and continuous processing for individual unit operations as well as the entire process. Data has been normalized to batch processing for the ease of direct comparison.....	68
Table 5.1 Detailed description of the CEX chromatographic process.	74
Table 5.2 Detailed description of the MMC chromatographic process.....	74
Table 5.3 Comparison of ϕ values for the resins considered at different salt concentration..	82
Table 5.4 Effect of gradient on ϕ values of cumulative acidic and basic profiles.....	83
Table 5.5 Effect of salts on ϕ values of cumulative acidic and basic profiles.....	83
Table 5.6 Aggregate levels and recovery in the flow through pool for different MMC resins at varying condition of pH and salt concentration.....	84
Table 5.7 Details of the single vs. integrated system in terms of HMWI %, recovery % and Φ values for the three mAbs.....	88
Table 6.1 Details of chromatographic steps.....	97
Table 6.2 Details of continuous chromatography operations for unit cycle.....	98
Table 6.3 Performance of individual unit operations in batch purification.	106
Table 6.4 Process and quality attributes for the integrated continuous platform for different mAbs.	115
Table 6.5 Comparison of continuous and batch operations for mAb C.	116