APPLICATION OF PAT TOWARDS THE DOWNSTREAM PROCESSING OF BIOPHARMACEUTICALS

GAUTAM KAPOOR

DEPARTMENT OF CHEMICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY DELHI

AUGUST 2019

APPLICATION OF PAT TOWARDS THE DOWNSTREAM PROCESSING OF BIOPHARMACEUTICALS

by

Gautam Kapoor

Department Of Chemical Engineering

Submitted

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

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

AUGUST 2019

CERTIFICATE

This is to certify that the thesis entitled **"Application of PAT towards the Downstream Processing of Biopharmaceuticals**" being submitted by **Gautam Kapoor** to the Indian Institute of Technology, Delhi, for the award of the degree of Doctor of Philosophy, is a record of bonafide research work carried out by him. **Gautam Kapoor** has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis.

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.

Prof. **Anurag S. Rathore**
Department of Chemical Engineering
Indian Institute of Technology,
Delhi-110016

This thesis is dedicated to my parents.

For their endless love, support and encouragement.

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Prof. Anurag S. Rathore for the continuous support of my Ph.D. study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study. I will always remain indebted for his kind words not only on the academic issue but also on personal front so that I remained focused on my work.

Besides my advisor, I would like to thank the rest of my research committee: Prof. S. Basu, Prof. M. Shaikh and Prof. J. Gomes, for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives.

I thank my fellow lab mates for the stimulating discussions, for the sleepless nights we were working tirelessly. I would like to thank my fellow doctoral, undergraduate and postgraduate students for their feedback, cooperation and of course friendship. In addition, I would like to express my gratitude to the staff of Bioprocessing and Bioseparations Lab, IIT-D for the last-minute favors.

I would like to thank my friends for accepting nothing less than excellence from me. Last but not the least, I would like to thank my family: my parents for supporting me spiritually throughout writing this thesis and my life in general.

Gautam Kapoor

ABSTRACT

Biopharmaceutical production processes involve a series of unit operations. Downstream processing involves multiple purification steps to capture the target protein and remove endogenous and adventitious contaminants. The protein is then formulated with appropriate excipients to make it fit for use.

Process Analytical Technology (PAT) is defined as a system for designing, analysing and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. Biotech processes and products present unique challenges with respect to Process Analytical Technology implementation.

The first part of the thesis deals with implementation of PAT in process chromatography. Fairly robust and accurate predictive models were developed from the information derived from different analytical tools to predict the percentage of aggregation and charge variants in an unknown sample and thus aid in pooling decisions during the elution stage of process chromatography.

The second part of the thesis deals with implementation of PAT in UF/DF. The feasibility of developing a PAT based controller for industrial application was established through extensive experimentation and planned alteration of controlled and manipulated process variables of the mechanistic model developed by a separate group.

The last part of the thesis deals with implementation of PAT in protein refolding. It was established that intrinsic fluorescence could not be used as a tool to monitor the refolding of two different classes of biomolecules for different reasons.

सार

बायोफर्मासिटिकल उत्पादन प्रक्रियाओं में यूनिट संचालन की एक श्रृंखला शामिल है। डाउनस्ट्रीम प्रसंस्करण में लक्ष्य प्रोटीन को पकड़ने और अंतर्जात और साहसी संदूकों को हटाने के लिए कई शुद्धि चरण शामिल हैं। प्रोटीन को तब उपयुक्त रसायन के साथ तैयार किया जाता है ताकि उपयोग के लायक बनाया जा सके।

प्रक्रिया विश्लेषणात्मक प्रौद्योगिकी को अंतिम उत्पाद की गुणवत्ता सुनिश्चित करने के लक्ष्य के साथ कच्चे और इन-प्रोसेस सामग्री और प्रक्रियाओं की महत्वपूर्ण गुणवत्ता और प्रदर्शन विशेषताओं के समय पर माप (अर्थात प्रसंस्करण के दौरान) के माध्यम से विनिर्माण को डिजाइन, विश्लेषण और नियंत्रित करने के लिए एक प्रणाली के रूप में परिभाषित किया गया है। । बायोटेक प्रक्रियाएं और उत्पाद प्रक्रिया विश्लेषणात्मक प्रौद्योगिकी कार्यान्वयन के संबंध में अद्वितीय चुनौतियां पेश करते हैं।

थीसिस का पहला हिस्सा प्रक्रिया क्रोमैटोग्राफी में पीएटी के कार्यान्वयन से संबंधित है। एक अनजान नमूने में एकत्रीकरण और चार्ज वेरिएंट के प्रतिशत की भविष्यवाणी करने के लिए विभिन्न प्रकार के विश्लेषणात्मक उपकरणों से प्राप्त जानकारी से काफी मजबूत और सटीक पूर्वानुमान मॉडल विकसित किए गए थे और इस प्रकार प्रक्रिया क्रोमैटोग्राफी के क्षणिक चरण के दौरान निर्णय लेने में सहायता करते हैं।

थीसिस का दूसरा भाग यूएफ / डीएफ में पैट के कार्यान्वयन से संबंधित है। औद्योगिक अनुप्रयोग के लिए एक पीएटी आधारित नियंत्रक विकसित करने की व्यवहार्यता एक अलग समूह द्वारा विकसित यांत्रिकीय मॉडल के नियंत्रित और हेरफेर प्रक्रिया चर के व्यापक प्रयोग और योजनाबद्ध परिवर्तन के माध्यम से स्थापित की गई थी।

थीसिस का अंतिम भाग प्रोटीन रीफिलिंग में प्रक्रिया विश्लेषणात्मक प्रौद्योगिकी के कार्यान्वयन से संबंधित है। यह स्थापित किया गया था कि आंतरिक प्रतिदीप्ति को अलग-अलग कारणों से बायोमोलेक्युलस के दो अलग-अलग वर्गों की तह की निगरानी के लिए एक उपकरण के रूप में इस्तेमाल नहीं किया जा सकता है।

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES