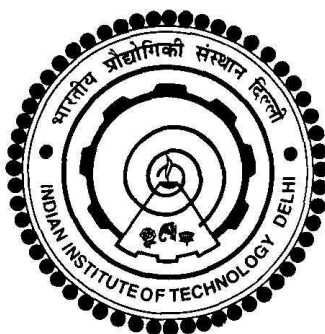


**THEORETICAL AND EXPERIMENTAL STUDIES FOR
DEVELOPMENT AND OPTIMIZATION OF MEMBRANE
SEPARATIONS**

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AUGUST 2016**

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by

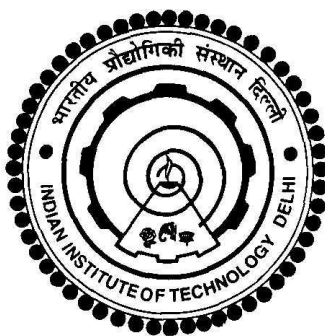
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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

AUGUST 2016

Dedicated to my Parents, Sister and Brother

CERTIFICATE

This is to certify that the thesis entitled “**THEORETICAL AND EXPERIMENTAL STUDIES FOR DEVELOPMENT AND OPTIMIZATION OF MEMBRANE SEPARATIONS**” being submitted by **S. MUTHU KUMAR** 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.

Anurag S. Rathore

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ACKNOWLEDGEMENTS

This thesis would not have been possible without the support of many people in my tough times. Without them it would be a dream. I placed my immense gratitude for their kindness, support, contribution and ideas.

I owe my deepest gratitude to my Professor, Dr. Anurag S Rathore for his encouragement and support in each phase of my research endeavor. As a supervisor, he acts as a driving force of my research work from the beginning of the doctoral program. Personally as a well-wisher he helped me to overcome numerous obstacles. He supported me not only guiding for my research but, also emotionally, giving ample of moral support and freedom.

With his insightful discussions and constructive feedback, he channelized my research work in proper direction. Being my inspiration he taught how to make things in a perfect manner. Without his guidance and continuous optimism this dissertation would not have been possible. I owe my profound thanks to him for his constant support and his effort to shape my dissertation till the last moment. He is an unrivalled mentor who accompanied throughout the gestation of my work. I am very grateful to his trust in me and his positive stimulation.

I am pleased to acknowledge my PhD research committee members, Prof. S.K. Gupta, Prof. Anupam Shukla and Prof. Biswajit Kundu for their significant influence in formulating the ideas. Their insights helped me move forward.

I would like to specially thank some of my best friends Rahul Bhambure, Varsha Joshi, Vijesh Kumar, Viki Chopda, Mili Pathak, Gautham Kapoor, Rohit Bansal, Kathiresan, Sumit Singh, Lalita Kanwar, Neh Nupur, Nikil Kateja, Gunjan and Jyoti Batra for their scintillating cooperation during the time when I felt exhausted.

I would also like to gratefully acknowledge the support of Rakesh Mendhe and Sandip Hadpe for helpful discussions and for their help rendered in carrying out the experiments.

Special thanks to my parents and my relatives who have put in their efforts and prayers for me to attain success in life. I am falling short of words to express my feelings towards them. Their blessings and encouragement were essential for my successful completion of this work.

S. Muthu Kumar

ABSTRACT

In bioseparation, applications of membrane technologies include clarification, concentration, buffer exchange, purification and sterilization. Clarification processes are critical steps during production of biological products because they directly affect yield, product quality, and reproducibility. Traditional fed-batch process design includes both centrifugation and filtration for the clarification of the harvest. Clarification techniques are evolving to incorporate feed pretreatment, flocculation, and different filtration technologies such as normal-flow, tangential-flow, and depth filtration. The objective is to increase process capacities and filtrate quality, ultimately reducing biomanufacturing costs. In case of purification, membrane chromatography has emerged as a potential alternative to conventional packed bed chromatography with advantages including reduced hardware requirement, operational ease, and shorter processing time. While development of such a step is relatively simpler when compared to packed bed chromatography, it still involves optimization of the various parameters experimentally as a first step towards commercialization. This is often a time and resource intensive exercise. Use of miniaturization and automation in the form of high throughput process development (HTPD) offers a potential solution.

In this thesis we have examined the feasibility of using the various combined models for the use of microfiltration and depth filtration of a therapeutic product expressed in *Pichia pastoris* at constant pressure. Next we addressed development of a HTPD platform for membrane chromatography. The proposed platform has been successfully applied towards process development for purification of a biotech therapeutic, Granulocyte Colony Stimulating Factor (GCSF). Further, we have also validated the platform by comparing the results obtained with the HTPD platform against those obtained at the traditional laboratory scale. Third, we have examined different membrane adsorbers for purification of Granulocyte Colony Stimulating Factor (GCSF), an *E coli* based biotherapeutic. Membranes that have been

explored include those based on cation exchange, anion exchange, hydrophobic interaction and salt tolerance exchange interactions. Performance of the various membrane adsorbers has been characterized with respect to step recovery, product quality as well as non-specific binding of the product to the membrane. Fourth and final, we have compared the performance of the traditional column chromatography with membrane chromatography for the two major classes of biotherapeutics, a microbial product and a mAb.

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ABBREVIATIONS

HTPD- High throughput process development

DOE- Design of Experiments

GCSF- Granulocyte Colony Stimulating Factor

IEX- Ion exchange chromatography

mAb- Monoclonal antibody

CEX- Cation exchange chromatography

AEX- Anion exchange chromatography

HIC- Hydrophobic interaction chromatography

HCP- Host cell proteins

SSR- Sum of squared residuals

STIC AEX- Salt tolerant anion exchange chromatography

SDS PAGE- Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SEM- Scanning electron microscope

CD Spectroscopy- Circular dichroism spectroscopy