THEORETICAL AND EXPERIMENTAL STUDIES FOR DEVELOPMENT AND OPTIMIZATION OF MEMBRANE SEPARATIONS

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THEORETICAL AND EXPERIMENTAL STUDIES FOR DEVELOPMENT AND OPTIMIZATION OF MEMBRANE SEPARATIONS

by

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Submitted

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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.

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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