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# High throughput process development (HTPD) platform for membrane chromatography

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ABSTRACT

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. Transport of molecules in such membranes is primarily driven by convection with very limited pore diffusion as compared to packed bed column chromatography resulting in increased mass transfer and quicker capture of the product and also allowing us to work at very high flow rates. 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. It is expected that companies have better understanding and control of their manufacturing processes. Biotech manufacturers, however, are under ever-increasing pressure to reduce the cost of development and commercialization of a biotech therapeutic. Use of miniaturization and automation in the form of high throughput process development (HTPD) offers a potential solution. In this paper, we address development of such a platform for membrane chromatography. The platform proposed in this paper 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 (7 μl membrane volume) against those obtained at the traditional laboratory scale (0.18 mL membrane volume). Statistical analysis of the data has been performed to assess the strengths and limitations of the platform.



## 1. Introduction

Chromatography is by far the most widely used technique for purification of therapeutic proteins [1]. Conventionally, chromatography is carried out using packed beds. However, some key limitations that are associated to packed bed chromatography include high pressure drop across the bed and dependence on intra-particle diffusion for the transport of solute molecules to their binding sites within the pores of such media. Membrane chromatography offers a viable and efficient alternative to traditional packed bed chromatography and is fast gaining acceptance in the biotech industry [2–5].

Membrane chromatography can be performed in several different modes, including ion-exchange, affinity, hydrophobic interaction and reversed-phase. Affinity and ion exchange chromatography are widely used to purify therapeutic proteins. Attachment of the various charged groups like sulfonic acid, sulfopropyl, diethylaminoethyl and quaternarium ammonium on the ion exchange membrane enhances the retention of charged molecules on its surface. The convective flow across the membrane module facilitates binding of the solute molecules to the ligand, thus overcoming the diffusion related limitations that are typically associated with the traditional resin beads [6–11]. Several applications involving membrane chromatography of proteins have been published in the last decade. Zeng and Ruckenstein [12] have described the various methods employed for preparation of adsorptive membranes and has reported the use of macro porous chitin and chitosan membranes for protein purification. Knudsen et al. [13] have demonstrated cost effectiveness of implementing anion exchange membrane chromatography for removal of low levels of host cell impurities such as DNA, host cell protein, and viruses. Boi et al. [14] have used protein A affinity membrane chromatography for adsorption of human IgG. The authors also developed a mathematical model for affinity membrane chromatography and validated the model with the experimental data. The model was found to satisfactorily describe all the process stages and could serve as an useful tool for process optimization. Bhut et al. [15] have purified anthrax protein antigen PA protein from E. coli lysates using weak anion exchange membranes. Yang et al. [16] have evaluated the capture of both a

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small protein (α lactalbumin) and a large protein (thyroglobulin) using anion exchange membranes. The authors concluded that membranes with a high capacity for large biomolecules (20–300 nm) and a low capacity for small molecules (such as host-cell proteins and endotoxins) are preferable. Grein et al. [17] have used ion exchange membrane chromatography to improve the clearance of the host cell proteins and DNA during production of a recombinant baculovirus. Shiosaki et al. [18] have compared the membrane chromatography with the packed bed adsorber for the adsorption of two different proteins (myoglobin and ovalbumin). Kokpinar et al. [19] have developed two different membrane chromatography devices with different functionalities. The devices made it possible to load the membrane directly with preclarified fermentation broth or cell lysate and separate the protein of interest often in a single step.

High throughput process development (HTPD) has also emerged as a tool of interest in bioprocessing. The primary motivation for creating such a platform is that is allows us to examine the effects of and interactions amongst the numerous process parameters that can impact step performance (such as pH, buffer molarity and protein concentration in ion exchange membrane chromatography) by performing larger number of experiments with relatively limited time and resources [20–24]. A few authors have explored the topic of HTPD for membrane chromatography. Chandler and Zydney [25] have developed a high throughput screening process for microfiltration of yeast suspensions. They found that the data obtained in 96 well plates was consistent with that from large scale micro filters.

In this paper we report creation and validation of a HTPD platform for membrane chromatography *via* a micro-scale platform containing 7 μl membrane volume in each of the 96 wells. We have performed statistical analysis to demonstrate comparability of the results obtained with the HTPD platform to the traditional laboratory scale membrane chromatography.

## 2. Material and methods

### 2.1. Materials

AcroPrep™ advance 96-well filter plate with Mustang S and Mustang Q were obtained from pall life sciences (Bangalore, India). Lab scale devices Acrodisc® Units with Mustang® Q and S membranes were also purchased from pall life Sciences. Glacial acetic acid, sodium acetate (anhydrous), sodium chloride, sodium hydroxide, glycerol, acetonitrile (HPLC grade), and trifluoroacetic acid (TFA) were purchased from Merck Chemicals India. Acrylamide, ammonium persulfate, bisacrylamide, beta-mercaptoethanol, glycine, sodium dodecyl sulfate, sodium salt of ethylenediaminetetraacetic acid (EDTA), sodium thiosulphate, and N, N, N′, N′-tetramethylethylenediamine (TEMED) were purchased from Sigma Aldrich Co, India.

### 2.2. Instrumentation

AcroPrep™Advance 96-well filter plate with Mustang S and Mustang Q with 7 μl membrane volume per well were used for the HTPD platform to study the binding behavior of Granulocyte Colony Stimulating Factor (GCSF). Automation in liquid dispensing was achieved using multichannel Eppendorf Research® pro electronic pipette. Ika MTS 2/4 digital shaker was used to fully suspend the sample/buffer in the medium during incubation at various stages of the experiment. Whatman® vacuum manifold was used for vacuum filtration of sample and buffer from the 96-well plates. Traditional lab scale ion exchange membrane chromatography experiments were done using an ÄKTA Purifier chromatography system (GE Healthcare Bio-Sciences, Uppsala, Sweden). GCSF protein solution was injected into the Mustang membrane chromatography unit using a sample loop of capacities from 10–2000 μl (GE Healthcare Bio-Sciences). The output from the membrane chromatography was monitored using pH, conductivity and UV detection at 280, 260 and 215 nm detectors. The pressure drop across the membrane chromatography was monitored by pressure transducers installed in the ÄKTA Purifier system. All data were recorded and viewed in Unicorn 5.2 software (GE Healthcare Bio-Sciences). The purified protein samples were collected in 15 mL polypropylene BD® centrifuge tubes using a Frac-920 fraction collector (GE Healthcare Bio-Sciences) connected to the ÄKTA Purifier 10 system. Measurement of absorbance at 280 nm (*A*280) for determining protein concentration was performed with an Epoch micro plate spectrophotometer (BioTek® Instruments Inc., Winooski, VT, USA). Reversed phase high performance liquid chromatography (RP-HPLC) analysis for protein concentration measurement was carried out using a 4.6 mm × 150 mm Zorbax Eclipse XDB C18 column (Agilent Technologies, Palo Alto, CA) on a Dionex Ultimate 3000 LC system. RP-HPLC data was recorded and analyzed using Chromeleon®6.80 software (Dionex Corporation, USA).

## 3. Experimental methods

### 3.1. Refolding and sample preparation

Refolded GCSF expressed in *Escherichia coli* cells was used in this investigation. Inclusion bodies were initially solubilized using urea as a denaturant followed by the refolding using the dilution method at 7 ± 2 °C [26]. The refolded product was analyzed by RP-HPLC for measuring the unfolded and refolded product forms. Refolded GCSF protein was concentrated by ultrafiltration using 3 kDa Minimate™ tangential flow filtration capsule. The pH of the concentrated protein sample was then adjusted to pH 4.0. The protein sample was then centrifuged at 8000 rpm (4 °C). Supernatant was collected and then buffer exchanged into the respective buffers as per the DOE. The buffer exchanged samples were used as an input for the cation and anion exchange membrane chromatography. Approximately 90% of the total protein in the feed material is GCSF. The level of DNA in the feed material is approximately 10 ng/ml. Finally, product purity is approximately 70% in the feed material.

### 3.2. HTPD platform

AcroPrep™ Advance 96-well filter plate with Mustang S and Mustang Q were used in this investigation. Operation involved equilibration, sample loading, washing and elution as shown in Fig. 1. The ion exchange membranes were equilibrated using 200 μl of the respective equilibration buffer (3 cycles) and incubated for 5 min at room temperature. Vacuum was applied at −300 mbar gauge to remove the filter plate using the vacuum manifold. After equilibration, pre-treated protein solution was added into the wells and incubated for 20 min. Next, three cycles of wash were performed and incubated with each cycle of 5 min with the equilibration buffer to remove any unbound protein. Salt based elution was performed using acetate buffer containing 1 M NaCl. Three cycles, each of 200 μl elution buffer and incubation time of 10 min were performed to completely elute the protein from the ion exchange membranes as shown in Table 1. Liquid fractions from the AcroPrep™ plate were collected by vacuum filtration at −300 mbar gauge for 10 s using Whatman® vacuum manifold. All filtrate fractions, except the initial buffer washes, were collected in Costar UV readable microtiter plates (Corning Incorporated, NY) and absorbance was read at 280 nm using an