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A novel primary amine-based anion exchange membrane adsorber☆

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ABSTRACT

A novel anion exchange membrane adsorber is presented which shows excellent impurity removal under different buffer conductivities ranging from 2 to 27 mS/cm. The membrane utilizes a primary amine ligand (polyallylamine) and was designed specifically to bind impurities at high salt concentrations. Studies with DNA, endotoxin, and virus spiked into buffer at varying salt conditions were done, resulting in clearance of >3, 4, and 4 LRV, respectively, with negligible change on increasing salt up to 27 mS/cm conductivities. Verification of virus removal in mAb feedstocks is also shown. The data are compared with other membrane adsorbers and a conventional resin which utilize traditional chemistries to demonstrate improved purification performance with the primary amine ligand. Additional data on scale-up of the membrane adsorber device is discussed. A stacked flat-sheet design was implemented to ensure linear scale-up of performance using bovine serum albumin (BSA) as a model. The linearly scalable device, coupled with the highly effective membrane for virus, DNA, and endotoxin removal, represents a step forward in polishing technology for the purification of monoclonal antibodies and recombinant proteins.

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1. Introduction

Membrane adsorbers have become a viable technology for the removal of trace impurities in the purification of monoclonal antibodies [1–3]. In the past, a few of the most striking challenges included low binding capacities (as compared to conventional chromatography resins), poor scale-down models [4], and high cost [5,6]. However, many of these challenges have been overcome with new membrane adsorber technology, allowing for excellent purification performance in an easy-to-use, disposable format, overcoming some of the challenges associated with packing traditional chromatography resin columns [7,8].

Typically, the purification of a monoclonal antibody follows a three-step chromatography column platform [9]. In the first step, the protein of interest is captured on a Protein A column; impurities flow through the column, and the product is eluted under low pH conditions. Following a low pH hold to inactivate viruses, the pH of the protein A pool is adjusted, and the solution is processed through a series of polishing steps (typically ion exchange). For example, the Protein A pool can be further purified using a cation exchange (CEX) purification column in a second bind-elute step. The product binds to the column along with closely related impurities (such as product aggregates) while a population of additional impurities flows through. The product is then eluted using a high salt buffer, separating the closely related impurities such as aggregates from the product and the CEX pool is diluted 3–4 times to reduce the salt concentration and pH-adjusted before loading onto the next step, a flow-through anion exchange (AEX) polishing column where impurities are bound and the product is allowed to pass through the column.

While chromatography columns have been used extensively as described, there are a number of limitations associated with such bead-based technologies. For instance, the binding of species within a chromatography resin is generally flow-rate dependent and diffusion limited [10,11]. In addition, the permeability of the packed bed of resin is strongly related and inversely proportional to the resin's particle diameter. Increasing the resin's diameter reduces the resin bed's external surface area per volume. The external surface area of the bead is important because only the external surface is exposed to the convective flow of the column and is used for binding species too large to enter the diffusional pore structure (such as DNA and viruses) [12]. Therefore, in order to improve volumetric throughput, resin diameters are increased at the expense of external surface area, potentially limiting the bead column's capacity for large species such as DNA and viruses. Furthermore, for a typical flow-through AEX column, the limited bed permeability also requires the column diameters (resin volume) to be oversized to increase the normal flow area, thus increasing the volumetric throughput in order to process large volumes of feed in a reasonable amount of time [1,9,13,14]. This

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underutilization of column capacity can result in unfavorable process economics.

Membranes have several fundamental advantages over traditional resin approaches, especially for flow-through applications. Surface-modified microporous membranes have ligands that are immediately accessible to the binding species and consequently, minimal diffusion resistance exists. The flow rate through multiple layers of membrane can be many times higher than through a column, while binding the same amount or more of impurities [8].

Membrane chromatography has actually been under investigation for almost two decades. A number of reviews and the references therein give a comprehensive summary of membrane chromatography, commonly used chemistries, mass transport and scalability in membrane adsorbers, and their application in the purification of proteins [5,10,12,15–17]. Several AEX membrane adsorbers are now commercially available [18–21].

One of the most common chemistries used for an AEX membrane adsorber is quaternary amine (Q) chemistry, which is also a common ligand in AEX resins. However, Q chemistry provides high binding capacity only at low conductivity. The CEX pools, which are typically ~15 mS/cm, need to be diluted to reduce the conductivity before loading onto the AEX column in order to achieve acceptable dynamic binding capacities [9]. Feed dilution results in increased volumes for mixers, holding tanks, and buffers along with added process time. Membrane adsorbers with Q chemistry also suffer from these drawbacks; they can usually only operate at low conductivities [4]. In order to overcome this particular issue, Riordan et al. have screened a number of alternate ligands to traditional Q chemistry in order to extend the salt-tolerance of anion-exchange membranes to sustain requisite viral retention at elevated conductivities. However, bovine serum albumin (BSA) was used as a model for HCP and the capacity values reported were very low [22].

Another challenge to the widespread adoption of membrane adsorbers in monoclonal antibody processes has been scalability from lab to pilot scale [5]. At the bench-scale, flat sheet geometries dominate, but this geometry has not been practical at the large scale. As such, an alternative spiral wound format is used for the large scale, but performance from bench- to process-scale can be difficult to predict or requires specialized devices for scale-down work. It has been shown that flat sheet device designs can provide excellent scalability from lab to pilot scale [3].

In this paper, the use of a primary amine ligand membrane adsorber is highlighted. The chemistry chosen provides good binding capacity even at high conductivity. Studies were done to test for virus, DNA, and endotoxin retention. A comparison with Q-based AEX membrane adsorbers and a conventional AEX resin show the benefit of using a primary amine ligand. Additionally, the development of a stacked-disc format is detailed, which reliably scales up the flat sheet geometry used on the bench-scale.

2. Materials and methods

2.1. Devices

ChromaSorb™ 0.08 mL, 50 mL and 500 mL devices were obtained from Millipore Corporation (Billerica, MA, USA). The ChromaSorb membrane has a pore size of 0.65 μm and is coated with a primary amine-containing hydrogel. Sartobind® Q SingleSep nano 1 mL devices were obtained from Sartorius (Weender Landstrasse, Goettingen, Germany). Mustang® Q 0.35 mL coin devices were obtained from Pall Life Sciences (Northborough, MA, USA). HiTrap™ Q Sepharose® Fast Flow (QSFF) agarose-based, 1 mL columns were obtained from GE Life Sciences (Piscataway, NJ, USA).

2.2. Reagents

Trizma® hydrochloride, Trizma base, sodium chloride, and sodium hydroxide were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used to prepare all buffers described. All solutions were sterile-filtered with a 0.22 μm membrane prior to use. Herring sperm DNA, bulk endotoxin *Escherichia coli* lipopolysaccharide (LPS), and bovine serum albumin (BSA) were all purchased from Sigma (St. Louis, MO, USA).

2.3. Protein feed stocks

Two proprietary mAb feed stocks were obtained for virus spiking experiments. mAb A was prepared in mammalian cells, clarified to remove insoluble impurities, processed through a Protein A chromatography step and then processed through a CEX column. mAb A was formulated at a protein concentration of 5.88 g/L in a proprietary buffer with a conductivity of 12 mS/cm and pH 7.5. An aliquot of this mAb was diluted using deionized water to generate another feed at a concentration of 2.96 g/L and conductivity of 6 mS/cm. mAb B was prepared in mammalian cells, clarified to remove insoluble impurities, processed through a Protein A chromatography column and formulated at a concentration of 15.8 g/L in 20 mM Tris buffer with 25 mM sodium chloride at pH 7.2. Conductivity was adjusted either by dilution using deionized water or by addition of 5 M sodium chloride to generate three feeds at conductivities of 5, 8.6 and 13 mS/cm. Bovine serum albumin (BSA) feed was formulated at a concentration of 0.05 mg/mL in 25 mM Tris buffer with 100 mM sodium chloride at pH 8.0, resulting in a conductivity of ~10 mS/cm.

2.4. Virus preparation

Crude and high titer Minute viruses of mice (MVM) stocks were prepared as previously described [23]. Briefly, crude MVM stocks were produced by infecting 324K.PT cells (P. Tattersall, Yale University Medical Center, New Haven, CT, USA) growing in high glucose Dulbecco's modified eagle medium (HG-DMEM) with 1% fetal bovine serum (FBS). Virus was harvested from culture 5–7 days post infection by three freeze-thaw cycles, and cell lysates were clarified by centrifugation (300 × *g* for 5 min) to remove cell debris. The supernatant was then 0.22 μm pre-filtered and stored at −80 °C until ready for use. High titer stocks were subjected to additional purification by ultracentrifugation to concentrate the virus which was then resuspended in protein-free storage buffer.

Crude bacteriophage PP7 stock was prepared as previously described [24]. Briefly, crude PP7 stocks were produced by infecting *Pseudomonas aeruginosa* (A.T.C.C.15692-B2) growing in tryptic soy broth (TSB) medium. Phage was harvested from culture 24 h post-infection by clarifying cell lysate by centrifugation (4200 rpm for 20 min) to remove cell debris. The supernatant was then 0.45 μm and 0.22 μm pre-filtered and centrifuged for 2 h at 90,000 × *g*. The phage pellet was resuspended in protein-free PBS buffer and stored at −80 °C until ready for use.

2.5. Device preparation

For all tests, all ChromaSorb devices were sanitized with 0.5 N NaOH for 30 min at 2.5 membrane volumes (MV)/min prior to use and equilibrated for 10 min at 12.5 MV/min with the appropriate buffer. The load was also run at 12.5 MV/min. Mustang Q, Sartobind Q and QSFF were prepared and run according to the manufacturers' recommendations unless otherwise noted. Briefly, Mustang Q, Sartobind Q, and QSFF were equilibrated with the appropriate buffer at the same flow as the load for a minimum of 10 membrane or column volumes until the pH of the permeate was the same as the pH

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