Journal of Chromatography A, 1218 (2011) 5386–5392

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A novel primary amine-based anion exchange membrane adsorber☆

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ARTICLE INFO

Article history:
Received 2 December 2010
Received in revised form 23 March 2011
Accepted 25 March 2011
Available online 4 April 2011

Keywords:
Anion exchange
Membrane adsorber
Polyallylamine
Salt tolerant
Impurity removal

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.

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

☆ Presented at the 13th Symposium on Preparative and Industrial Chromatography and Allied Techniques, Stockholm, Sweden, 12–15 September 2010.

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doi:10.1016/j.chroma.2011.03.068

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

of the equilibration buffer; specific load flow rates for each device and experiment are detailed in the sections below.

2.6. DNA retention experiments

A 0.2 mg/mL DNA solution was made in 25 mM Tris buffer, pH 8.0 with 0 (~2 mS/cm) or 250 mM NaCl (~27 mS/cm). Membrane devices were loaded to 20 mg/mL of membrane at a flow rate of 12.5, 10 and 30 MV/min for ChromaSorb, Mustang Q and Sartobind Q, respectively. The effluent was collected and sampled for assaying. For QSFF, the feed was loaded at 1 CV/min to a total load of 1.5 mg/mL of media. Effluent samples were collected, and DNA assays were done with a PicoGreen® kit from Cygnus (Southport, NC, USA).

2.7. Endotoxin retention experiments

A 60,000 endotoxin units (EU)/mL solution was made in 25 mM Tris buffer, pH 8.0 with 0 or 250 mM NaCl. Membrane adsorbers were loaded to 25 million EU/mL of membrane at a flow rate of 12.5, 10 and 30 MV/min for ChromaSorb, Mustang Q and Sartobind Q, respectively, and effluent samples were collected at loadings of 15 and 25 million EU/mL of membrane. The QSFF column was loaded at 1 CV/min to 2.0 million EU/mL of media, and effluent samples were taken at 1.2 and 2.0 million EU/mL.

Endotoxin assays were performed using the Endochrome-K kit and Kinetic software, EndoScan V, version 4 from Charles River Laboratories (Wilmington, MA, USA). Limulus Amebocyte Lysate (LAL) reagent water and control standard endotoxin (CSE) stock solution were also supplied by Charles River Laboratories. All materials in direct contact with the analyte solutions in the assay procedure (i.e. plastic dilution tubes, pipettes, microplates, and reservoirs) were endotoxin-free and disposable.

2.8. Virus retention experiments

Crude MVM stock was added to the pools of mAb A at conductivities of 6 and 12 mS/cm to a target spike titer of 1×10^6 tissue culture infectious dose 50% per mL ($TCID_{50}$/mL). High titer MVM stock was added to the pools of mAb B at conductivities of 5.0, 8.3 and 12.0 mS/cm to a final target titer of 1×10^6 $TCID_{50}$/mL. For all devices tested, feed samples were collected for titer determination post-0.22 μm filtration. Samples were kept at room temperature during the course of the experiment and assayed concurrently with the filtrate samples at the end of the experiment.

High titer MVM stock was spiked into 25 mM Tris 150 mM NaCl, pH 8.0, resulting in a conductivity of 11.4 mS/cm, to a target spike titer of 2.0×10^6 $TCID_{50}$/mL. Bacteriophage PP7 was spiked into 25 mM Tris 100 mM NaCl, pH 8.0, resulting in a conductivity of 11.4 mS/cm to a target spike titer of 2.0×10^8 plaque forming units (pfu)/mL.

All MVM or PP7 spiked feeds were filtered with a 0.22 μm filter prior to loading. ChromaSorb 0.08 mL and Sartobind Q devices were run at 12.5 MV/min. QSFF columns were run at 0.2 column volumes (CV)/min. Effluent samples were collected directly from devices during processing at selected mass loadings and assayed for virus infectivity.

MVM titers were determined using a tissue culture infectious dose 50% ($TCID_{50}$) assay as described in [25]. Test samples were diluted to mitigate cytotoxicity and viral interference, then serially diluted 10-fold for infectivity assays. Sub-confluent 324K.PT cells were inoculated with sample dilutions and then incubated at 37 °C in 5% CO_2 for 10–12 days. After incubation, infected wells were visually assessed for cytopathic effect (CPE) and virus titers were calculated using the Spearman–Kärber equation for calculation of $TCID_{50}$ [26,27].

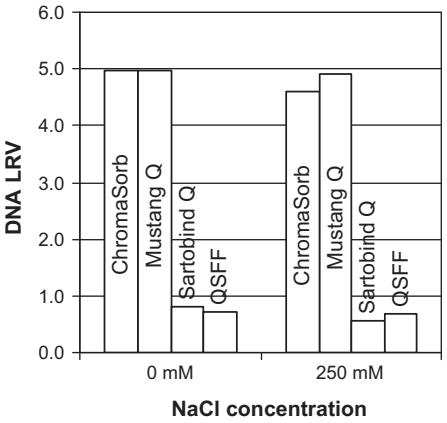

Fig. 1. DNA log reduction value (LRV) of ChromaSorb, Mustang Q, Sartobind Q devices, and Q Sepharose Fast Flow column at 0 and 250 mM NaCl. The load was 20 mg/mL membrane and 1.6 mg/mL resin at 0 mM NaCl and 18 mg/mL membrane and 1.4 mg/mL resin at 250 mM NaCl.

The infectious titer of PP7 in samples was determined using a plaque assay method. Samples were serially diluted (10-fold), mixed with *P. aeruginosa* in liquid overlay agar, and the mixture was poured onto solid agar plates. The plates were then incubated overnight at 37 °C, and the plaques were counted on plates that contained between 10 and 300 plaques. Titers were determined by multiplying the number of plaques by the dilution factor and then dividing by the plated sample volume.

2.9. Scalability experiments

ChromaSorb 0.08 mL, 50 mL and 500 mL devices were tested for BSA dynamic binding capacity. Solutions of 0.5 mg/mL BSA in 25 mM Tris, pH 8.0 were loaded onto each device at 12.5 MV/min using a peristaltic pump. Effluent fractions were collected every minute to 50% breakthrough based on UV absorbance at 280 nm relative to the feed. The dynamic capacity at 10 and 50% breakthrough were calculated.

Bacteriophage PP7 was spiked into 25 mM Tris 100 mM NaCl, pH 8.0 to a target titer of 2.0×10^8 pfu/mL. ChromaSorb 0.08 mL and 50 mL devices were loaded at 12.5 MV/min to 5.39×10^{10} pfu/mL of membrane. A 2 mL final pool sample, corresponding to 30 mL of spiked feed processed across ChromaSorb 0.08 mL devices and 19.5 L of spiked feed processed across ChromaSorb 50 mL devices was collected for assaying.

2.10. Log reduction value (LRV) determination

DNA, endotoxin and virus reduction were expressed in terms of LRV which was calculated as shown in equation below:

$$\mathrm{LRV} = \mathrm{Log}_{10}\left(\frac{C_{Feed}V_{Feed}}{C_{final}V_{final}}\right)$$

where LRV, Log reduction value; C_{Feed}, concentration of DNA, endotoxin or virus in the feed; V_{Feed}, starting volume of feed; C_{final}, concentration of DNA, endotoxin or virus in the effluent sample; V_{final}, final volume (post processing).

3. Results and discussion

3.1. DNA removal

DNA removal in ChromaSorb, Mustang Q, Sartobind Q, and QSFF at varying salt concentrations is shown in Fig. 1. At 0 mM NaCl, ChromaSorb and Mustang Q had >4.5 LRV, while Sartobind Q and

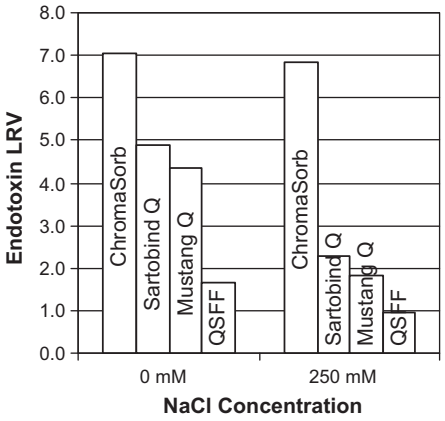

Fig. 2. Endotoxin LRV of ChromaSorb, Mustang Q, Sartobind Q devices, and Q Sepharose Fast Flow (QSFF) column at 0 and 250 mM NaCl. The load was 25 and 15 million EU endotoxin/mL membrane and 2.0 and 1.5 million EU endotoxin/mL resin at 0 and 250 mM NaCl, respectively.

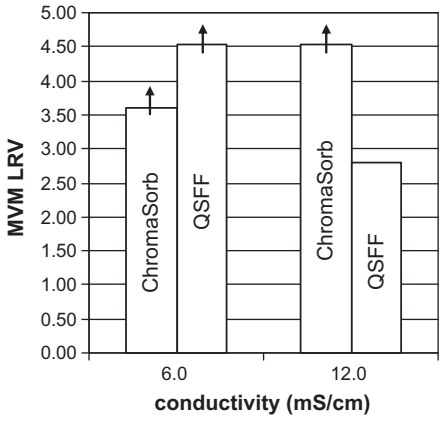

Fig. 3. Minute virus of mice (MVM) retention in ChromaSorb devices and QSFF columns of virus-spiked mAb A feed at varying conductivities. ChromaSorb devices were loaded to 6 kg/L while QSFF columns were loaded to 0.07 kg/L. Chromasorb devices were run in triplicate; no error bars are shown because virus was not detected in any of the samples. QSFF columns were run in singlet. Arrows indicate all reported LRVs are greater than or equal to values shown.

the QSFF column exhibited early breakthrough of DNA, resulting in less than 1.0 LRV DNA. The membrane adsorbers are expected to perform better than the column due to better mass transfer, but only two of the three adsorbers tested resulted in high levels of DNA clearance. Both Mustang and Sartobind use a quaternary ammonium ligand (Q); therefore, the ligand chemistry cannot account for the difference in DNA removal. However, Mustang has a base matrix of 0.65 μm pore-size hydrophilic polyethersulfone [20] while Sartobind is a 3 μm pore-size base-stable cellulose [18]. The smaller surface area associated with a larger pore size and possible differences in the amount of ligand available for binding may explain the lower DNA capacity of Sartobind Q devices. DNA loading onto the column was notably lower relative to membrane adsorbers, likely due to the size of the resin column tested (1 mL). This observation is consistent with the common practice of using an over-sized resin column for flow-through AEX trace impurity removal.

At 250 mM NaCl, the DNA removal was similar to that of the low salt condition for all three membrane adsorbers, with all media tested seeing an insignificant decrease in LRV. The equivalent binding at both low and high salt is likely because DNA has a very high density of negative charge. The resulting high affinity binds DNA strongly to the positively charged anion exchangers and is less affected by competition of buffer-based electrostatic interactions.

3.2. Endotoxin removal

In Fig. 2, the removal of endotoxin at low and high salt is shown. Again, the three membrane adsorbers perform better than the resin column. However, the effect of conductivity can be seen clearly: all of the samples except the ChromaSorb device show a decline in endotoxin removal at 250 mM NaCl. In fact, for the ChromaSorb device, the amount of endotoxin in the effluent for both salt conditions was below the limit of detection of the assay (0.005 EU/mL), resulting in endotoxin retention of ~7 LRV. Because of its unique ligand, the ChromaSorb device is insensitive to increasing salt concentrations, and the performance is maintained even at high conductivities.

The mechanism of the salt-tolerant interaction between the target molecule and the primary-amine containing ChromaSorb membrane is a combination of charge and hydrogen bonding [28]. The ChromaSorb membrane possess a higher charge density than other membrane adsorbers. Experiments using urea as a hydrogen-bonding modifier showed reduced binding strength for BSA in the presence of salt and urea compared to salt alone. In fact, due to competition between the product mAb and the ChromaSorb membrane for impurities, it has been suggested that impurity removal could be better at higher conductivities (10–30 mS/cm) [28]. Johansson et al. have also shown that anion-exchange ligands based on primary amines are optimal for the capture of proteins at higher conductivities because they take advantage of both electrostatic and hydrogen bond interactions [29].

The reduction in endotoxin removal by the two Q-membrane adsorbers was good (~5 LRV) at low conductivity. However, at higher conductivity, the endotoxin removal dropped below 3 LRV. This is likely an effect of the ligand affinity being reduced by the higher conductivity. These results are consistent with Sartobind Q's operating manual which recommends operation only up to 20 mS/cm.

Endotoxin removal by QSFF was poor (LRV<2) at low salt and declined further (LRV~1.0) at high salt. The poor performance at low salt could be attributable to slower mass transfer due to diffusion into the resin and the reduced external surface area that is exposed to the convective flow for resins as compared to the membrane adsorbers. In addition, the small column size (1 mL, 2.5 cm bed height) could contribute to the reduced endotoxin binding. Nor surprisingly, the reduction in endotoxin binding with increasing salt is consistent with the Q-membrane adsorbers as both media possess similar ligand chemistries.

3.3. Virus removal

MVM retention by ChromaSorb devices and QSFF columns was compared at conductivities of 6 and 12 mS/cm using a representative monoclonal antibody feed (mAb A). ChromaSorb devices fully retained virus at mAb mass loadings of up to 6 kg/L of membrane at both conductivities, Fig. 3. In contrast, QSFF gave good viral clearance at the lower conductivity condition while virus breakthrough was seen for the high conductivity condition. Although the QSFF resin showed good performance at low conductivities, the overall loading of the resin to 0.07 kg/L is much lower than that for the ChromaSorb 0.08 mL devices which were loaded to ~6 kg/L.

In a separate experiment, mAb B feed adjusted to 5.0, 8.3 and 12 mS/cm was spiked with MVM and loaded onto ChromaSorb and Sartobind Q devices. Both ChromaSorb and Sartobind Q devices were run to the same loading (5 kg/L). The virus removal in the two membrane adsorbers is shown in Fig. 4. As in the previous experiment, virus retention by the ChromaSorb device was not sensitive to feed conductivity and MVM LRV>4 were seen in all cases. In contrast, Sartobind devices retained virus best under low

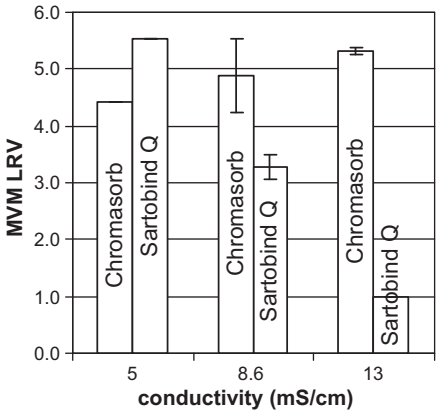

Fig. 4. Minute virus of mice (MVM) retention in ChromaSorb and Sartobind Q devices of virus-spiked mAb B feed at varying conductivities. Both membrane adsorbers were loaded to 5 kg/L in each case.

Table 1
Virus LRV in ChromaSorb and Mustang Q devices at two conductivities. MVM was spiked to a target titer of ~2.0×10^6 $TCID_{50}$/mL in 25 mM Tris, pH 8.0; NaCl was added to control conductivity. Each device was run in quadruplicate and the flowthrough pools for each were collected for assaying.

Conductivity (mS/cm)	Average MVM LRV	
	ChromaSorb	Mustang Q
4	≥4.25	≥4.25
14	≥4.31	1.90

conductivity conditions and exhibited a marked reduction in virus retention with increasing conductivity.

In an earlier study, MVM retention by ChromaSorb and Mustang Q devices was also compared at low and high conductivities, 4 and 14 mS/cm, respectively, as shown in Table 1 [30]. Virus retention by the ChromaSorb device was not sensitive to feed conductivity, giving MVM LRV>4 at both low and high conductivities. The Mustang Q device retained virus best under low conductivity conditions with a reduction in virus retention (LRV<2) at higher conductivity.

The results seen in the virus retention experiments are consistent with the endotoxin experiments and further highlight the performance disadvantage of using Q-chemistry based AEX chromatography in either conventional bead-based or membrane formats. The primary amine-based ChromaSorb chemistry demonstrated robust DNA, endotoxin and virus clearance at high salt concentrations. This is likely an effect of the unique ligand which possess both a high level of positive charge and hydrogen bonding interactions with target biomolecules. The higher affinity interactions provided by the primary amine potentially allow for the AEX membrane flow-through polishing step to be run without dilution and provides a more robust and wider conductivity range in the process step. This has favorable process implications, as described earlier, making it a suitable candidate to replace conventional AEX beads.

3.4. Scalability

The performance of the ChromaSorb device is dependent on the volume of membrane used. In order to make the 0.08 mL, 50 mL and 500 mL devices linearly scalable, the 8-layer flat-sheet membrane geometry needed to be preserved. To make the larger devices, this was accomplished by using a stacked-disc approach where one disc is composed of 2 sets of 8 layers of membrane – one set bonded to each side of the disc – with these discs stacked in parallel. A schematic of the three different device sizes is shown in Fig. 5. The feed stock is introduced through the inlet and travels through the stack upstream of the membrane layers. Once the fluid penetrates the membrane, impurities are adsorbed, and the purified fluid collects along small channels on the permeate side and exits through the outlet. For the 50 mL device, two stacks are used to form the required volume of membrane, while for the 500 mL device, 20 stacks are used. This design enables the preservation of the 8 layers of membrane and the associated flow rate for scaling up without having to increase substantially the planar area of the device.

Typical BSA dynamic breakthrough curves for ChromaSorb 0.08 mL, 50 mL and 500 mL devices built with identical membrane are shown in Fig. 6. The curves are similar, showing relatively sharp breakthroughs, consistent with good mass transport and efficient utilization of the ligand. Table 2 shows the average BSA dynamic binding capacities and standard deviations at 10 and 50% breakthrough for multiple 0.08 mL and 50 mL devices. Smaller populations for the 50 mL devices were used due to the larger volumes of load required. However, several different membrane lots were included to capture the variability that could be seen in the manu-

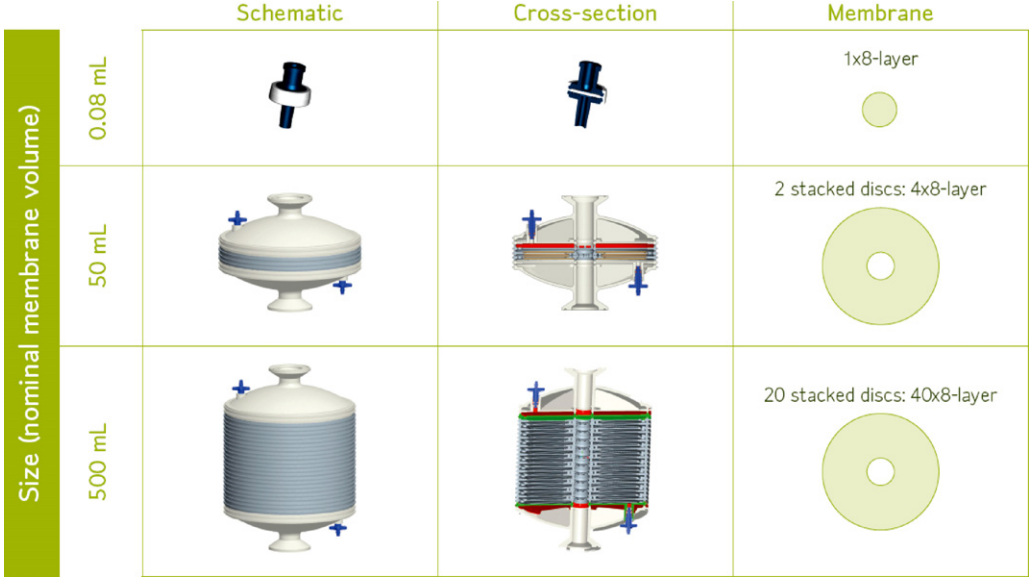

Fig. 5. Schematic of the three different size scales of ChromaSorb devices.

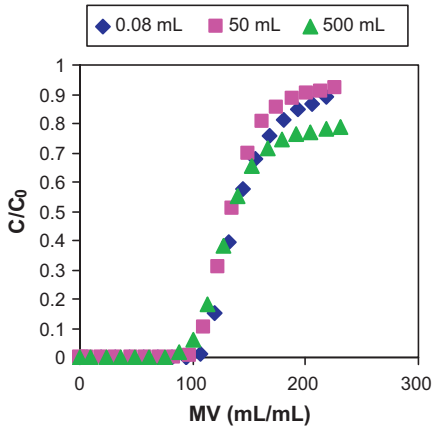

Fig. 6. BSA breakthrough curves for each ChromaSorb device size. The membrane for each device was taken from the same lot. Devices were loaded with 0.5 mg/mL BSA in 25 mM Tris, pH 8.0 at 12.5 membrane volumes (MV)/min until at least 80% breakthrough was detected.

Table 2
Average BSA capacity for the 0.08 mL and 50 mL devices. Sample size (N) and standard deviations (σ) are shown.

Device size (mL)	N	Average BSA capacity (mg/mL)			
		10% breakthrough	σ	50% breakthrough	σ
0.08	54	73.5	8.5	83.5	10.2
50	30	67.4	9.8	82.3	10.4

facturing process: 8 lots were used for the 0.08 mL device and 7 lots were used for the 50 mL device, with an overlap of 5 lots in both the device sizes. The average BSA binding capacity at 10% breakthrough for the 50 mL is within 10% of that for the 0.08 mL device format. At 50% breakthrough, the difference in binding capacity is insignificant.

Fig. 7 shows the 10% breakthrough average (μ) and standard deviation (σ) for the 0.08 and 50 mL devices plotted assuming the data follow a Gaussian distribution. The standard deviation of the two devices is similar as seen in the spread of the x-axis. The capacity for 95% (2σ) of the 50 mL devices falls within $\mu \pm 3\sigma$ of that for the 0.08 mL devices.

To minimize this standard deviation, a strategy of using blended membrane lots was introduced to the large scale devices. The variability in performance can be caused by variability in different membrane lots. For comparison, 50 mL devices were built by blending membrane from four lots. Each 8-layer stack consisted of 2 layers from each of the 4 lots; each stack being identical. Fig. 8

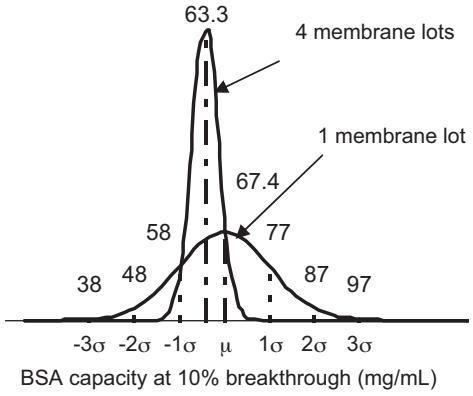

Fig. 8. Comparison of the Gaussian distribution of the average BSA capacity at 10% breakthrough for 50 mL devices built from 1 and 4 membrane lots.

illustrates the resulting Gaussian curve. The single membrane lot devices show 15% variability in capacity, as compared to ~5%, for the 4-lot devices. Lot-to-lot differences are minimized by incorporating 4 lots of membrane in one device. Comparing this with Fig. 7, we see that now, 95% of these devices fall within $\mu \pm 2\sigma$ of the 0.08 mL devices. This demonstrates that performance at the bench scale using 0.08 mL devices is a reasonable predictor of performance at the pilot scale with 50 mL devices having blended membrane from 4 lots.

BSA capacity testing would not be practical using a sufficiently large sample size for the 500 mL devices so this testing was not performed. However, based on similarities of the BSA breakthrough curves for 50 mL and 500 mL devices observed in Fig. 6 we would not expect to see a capacity difference between these device scales. Moreover, the construction and flow characteristics for both 50 and 500 mL devices are identical making capacity differences such as those seen between 0.08 mL and 50 mL devices highly unlikely.

BSA binding capacity is one metric for the evaluation of device scalability. ChromaSorb scalability was also evaluated for virus retention. However, spiking studies using MVM at this scale presented some challenges, both in terms of safety and cost. As an alternative, bacteriophage PP7 was evaluated as a potential model for MVM. PP7 is approximately the same size as MVM and has a single stranded RNA genome with medium physiochemical resistance [24]. MVM and PP7 had similar breakthrough profiles for ChromaSorb 0.08 mL devices: full retention for both MVM and PP7 was observed up to a loading of 15.6 g/L, with breakthrough thereafter, as shown in Table 3. The LRV values for PP7 were slightly higher because of higher PP7 spike titers. However, similar ChromaSorb 0.08 mL device breakthrough curves for PP7 and MVM (not shown)

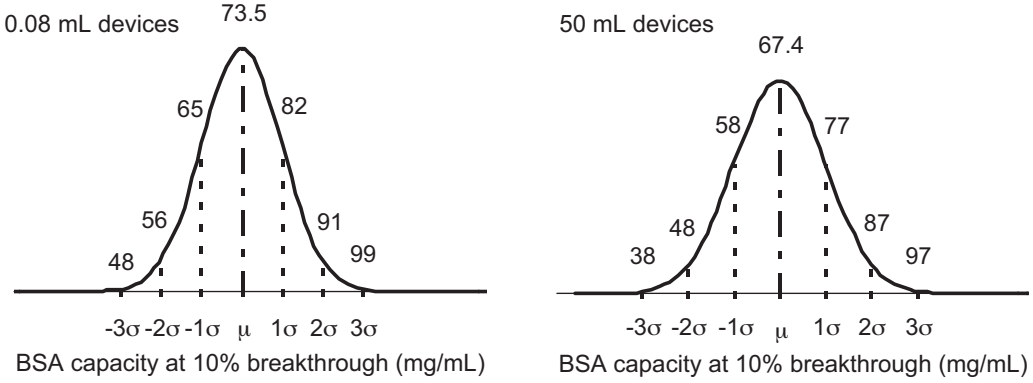

Fig. 7. Gaussian distribution of the average BSA capacity at 10% breakthrough for 0.08 mL devices (left) and 50 mL devices (right). The sample size (N) for the 0.08 mL and 50 mL devices was 54 and 30, respectively.

Table 3
LRV at different BSA mass loadings. MVM was spiked to a titer of 2.0×10^6 $TCID_{50}$/mL and PP7 was spiked to a titer of 2.0×10^8 pfu/mL in 0.5 mg/mL BSA in 25 mM Tris 100 mM NaCl, pH 8.0. Differences in LRV of MVM and PP7 are due to a higher starting titer for PP7.

BSA mass loading (g/L)	MVM LRV	Bacteriophage PP7 LRV
15.6	≥5.5	≥7.6
25.0	2.2	<4.7

support the use of PP7 as a model for MVM in ChromaSorb devices where testing with MVM presents challenges. PP7 retention by 0.08 mL and 50 mL devices was identical with both device scales achieving LRV ≥7.6. The virus retention data further demonstrate the scalability of the ChromaSorb devices.

4. Conclusions

Impurity binding performance of the ChromaSorb device, a polyallylamine-based membrane adsorber, was studied using DNA, endotoxin and mammalian virus as model streams. Robust performance at low and high conductivities was verified up to 250 mM NaCl. Device scalability from bench to pilot scale was demonstrated via a comprehensive BSA binding study using multiple lots of membrane. It can be concluded that by preserving the stacked disc format at the various sizes, BSA capacities for the 50 mL device within 10% of the 0.08 mL device can be achieved. The ChromaSorb device represents a step forward in polishing technology for the purification of monoclonal antibodies and recombinant proteins by providing a scalable, highly effective membrane adsorber for DNA, endotoxin and virus removal. Additional impurity clearance work with antibody feed stocks is in progress and will be reported in the future.

Acknowledgments

The authors thank the Analytical services and Virology groups at Millipore for assay support.

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