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Membrane ion-exchange chromatography for process-scale antibody purification

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Abstract

The large-scale production of recombinant monoclonal antibodies demands economical purification processes with high throughputs. The potential for ion-exchange membrane adsorbers to replace traditional ion-exchange columns was evaluated. Breakthrough capacities of commercially available cation-exchange membranes were determined as a function of flow-rate and layer number. Due to economic and process restrictions, cation-exchange membranes may not currently be advantageous for process-scale antibody purification in a bind and elute mode. However, anion-exchange membranes in a flow-through mode may provide a reasonable alternative to columns for the removal of low levels of impurities such as DNA, host cell protein, and virus.

Keywords: Preparative chromatography; Membranes; Breakthrough volumes; Antibodies

1. Introduction

Recombinant monoclonal antibodies have recently seen increasing importance as therapeutics in treating human disease such as cancer [1–5]. Downstream purification processes for therapeutic monoclonal antibody production typically involve multiple chromatography steps, including one or more ion-exchange columns. Conventional ion-exchange column chromatography steps are effective and reliable, but generally have low product throughput (kg processed/h). As monoclonal antibodies become more widely used, more efficient process-scale production is necessary. Membrane chromatography has increasingly been reported as a potentially advantageous tool to purify proteins [6–13]. One advantage of membranes over conventional preparative beads or gels is the elimination of pores with long diffusive path lengths. For the membranes, binding sites are located along the through-pores rather than nestled within long diffusive pores. Accordingly, mass transport of the monoclonal antibody to the binding site relies on convection rather than diffusion. Consistent with convective rather than diffusive mass transport, binding capacities of ion-exchange membranes have been found to be independent of flow-rate [14–17]. In addition, breakthrough capacities of ion-exchange membranes are often comparable in magnitude to commonly used ion-exchange resins [14,18,19], further supporting the need to investigate the potential higher throughputs membranes offer. Furthermore, improved large-scale ion-exchange membrane adsorber devices have recently become available [20], making membrane chromatography a promising al-

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ternative for use in the commercial purification of therapeutic monoclonal antibodies.

To understand the basic chromatographic properties of ion-exchange membranes, the effect of flow-rate on the breakthrough capacity of cation-exchange membranes and columns was compared. This enabled the characterization of membranes as chromatographic systems, allowing direct comparison to commonly used bead-type sorbents as well as the selection of an appropriate membrane scale-down system for use in process development. Large-scale cation-exchange membrane adsorbers were found to have comparable breakthrough capacities to those of process-scale cation-exchange columns, but were not likely to be clearly advantageous in the bind and elute mode at this time.

Recombinant monoclonal antibody processes often employ anion-exchange chromatography to bind trace levels of impurities and potential contaminants such as DNA, host cell protein, and virus, while allowing the antibody to flow through. In concept, such a purification step requires only a small amount of anion-exchange sorbent relative to the large amount of antibody being processed since only small quantities of impurities and potential contaminants are being adsorbed. However, current commercial purification schemes often use large anion-exchange columns, primarily due to the flow-rate limitations of the resins and the process throughput requirements. Thus a small membrane, whose breakthrough capacity is independent of flow-rate, may be well suited for this type of purification step. Anion-exchange membranes were therefore evaluated for their ability to remove trace levels of DNA, host cell protein, and virus while processing relatively large amounts of antibody. Use of a membrane would be advantageous for this application if current process throughputs were maintained or improved and the unit operation was significantly simplified due to the use of a small, prefabricated, single-use device with minimal buffer requirements.

2. Experimental

2.1. Materials

Ion-exchange membranes were from Sartorius (Edgewood, NY, USA and Goettingen, Germany) and Pall BioPharmaceuticals (Membrane Technology Center, Pensacola, FL, USA; some of the prototype membrane material was generously provided). Swinny stainless steel 13 mm O.D. (10 mm I.D.) filter holders were from Millipore (Bedford, MA, USA). The prototype 25 mm stainless steel membrane holder was from Pall BioPharmaceuticals. A prototype PTFE housing with stainless steel distributing adapters was provided by Sartorius for the 15-, 30- and 60-layer process modules. For Sartorius membranes, approximately 50 cm^2 of membrane area=1 ml membrane volume, and the average membrane thickness was assumed to be 0.0275 cm. For Pall membranes, approximately 72 cm^2 of membrane area=1 ml membrane volume, and the average membrane thickness was 0.014 cm.

Poros 20HS and Poros 50HS cation-exchange resins and 100×4.6 mm column hardware were from PerSeptive Biosystems (Framingham, MA, USA). Q-Sepharose Fast Flow (FF) anion-exchange resin was from Amersham Pharmacia Biotech (Piscataway, NJ, USA). The 19×0.66 cm column hardware was from Omnifit (Cambridge, UK). Salmon sperm DNA was obtained from Gibco BRL (Life Technologies, Gaithersburg, MD, USA). Xenotropic murine leukemia virus was from BioReliance (Rockville, MD, USA). Load material was recombinant humanized monoclonal antibody (IgG_1) produced in Chinese hamster ovary cells and purified by alternative methods. Type I load material was purified by tangential flow filtration [21] followed by protein A chromatography, whereas Type II load material was purified by expanded bed cation-exchange chromatography followed by protein A chromatography. This load material was obtained from Genentech (South San Francisco, CA, USA).

2.2. Instruments

Chromatography was run on either an AKTA Explorer 100 or a BioSkid chromatography system, both from Amersham Pharmacia Biotech.

2.3. Methods

Chromatographic methods are described in the figure captions. Host cell protein (Chinese hamster

ovary cell protein, or CHOP) levels were measured by an enzyme-linked immunosorbent assay (ELISA) developed at Genentech. Briefly, affinity-purified goat anti-CHOP antibodies were immobilized on microtiter plate wells. Dilutions of samples were incubated in the wells, followed by an incubation with goat anti-CHOP conjugated to horseradish peroxidase. Horseradish peroxidase enzymatic activity was quantified with *o*-phenylenediamine. The product was quantified by reading absorbance at 490 nm.

3. Results and discussion

3.1. Cation-exchange chromatography

Breakthrough curves were used to compare the effect of flow-rate on the binding capacity of cation-exchange membranes and columns. Poros 20HS and Poros 50HS cation-exchange columns, respectively, and a Sartorius strong cation-exchange membrane unit were loaded with antibody in 10 m*M* acetate buffer, pH 5.0 (1 g/l) to complete breakthrough at three flow-rates. To allow direct comparison between membranes and columns, flow-rates which result in equal residence times in each sorbent were used and the amount of antibody loaded was normalized relative to the volume of sorbent, or g antibody per l sorbent. The term sorbent therefore denotes either ion-exchange resin or membrane, and the volume of sorbent is either the volume of resin or the volume of membrane. Flow-rate significantly affected the breakthrough curves for both columns, but only slightly affected the breakthrough curves of the membrane (Fig. 1). For the columns, as the flow-rate increased, breakthrough occurred sooner and the shape of the breakthrough curve changed dramatically (Fig. 1A and B). This effect was more pronounced with the Poros 50HS column, as would be expected due to the larger bead diameter and the therefore increased diffusion path length. For the membrane, however, the shape of the breakthrough curve did not change with flow-rate and the breakthrough curve changed only slightly (Fig. 1C). These breakthrough curves are summarized in Fig. 2, which compares the effect of flow-rate on the breakthrough capacity of each sorbent. The breakthrough capacity of the columns declined approximately logarithmically with increasing flow-rate, while for the membrane it was quite constant over a wide range of flow-rates.

When developing chromatography processes for large-scale purification, it is critical to use a representative scale-down system. A linearly scaleable system which both minimizes feedstock requirements and accurately predicts the behavior at large-scale is necessary. To test the linear scaleability of commercially available Sartorius modules, breakthrough curves were generated on cation-exchange membrane units ranging from 1 to 60 layers over the operable range of linear flow-rate. Breakthrough curves for the 15-layer radial flow process module were consistent in shape with those seen above for the five-layer disk unit, and did not deteriorate at high flow-rates (Fig. 3). Breakthrough curve shapes were consistent for the one-, five-, 30- and 60-layer units as well (data not shown).

The breakthrough capacity increased logarithmically with layer number, rising sharply from 7 g antibody/l sorbent for one layer to nearly 30 g/l for the 15-layer unit and up to 40 g/l on the 60-layer unit (Fig. 4). The comparatively low breakthrough capacity on the one-layer unit is not surprising since flow will be preferentially directed through the largest pores. This behavior is expected to be minimized with the addition of subsequent layers. The continued rise in breakthrough capacity even beyond 15 and 30 layers of membrane may be due to inefficient flow distribution within the process modules or the housing and associated apparatus. To adequately assess the question of flow distribution, residence time distributions should be measured and standard analyses performed according to the methods described by Gebauer et al. [18]. Therefore, although the breakthrough capacity was independent of flow-rate, it did depend on layer number, making the selection of a representative scale-down system less straightforward.

In a bind and elute mode, the flow-independent binding capacities are not easily exploited so as to result in a clearly advantageous manufacturing process for the purification of kilograms of antibody. Although the binding capacities of ion-exchange membranes are comparable to those of resins, the cost of membrane on a per volume basis is currently much higher. A significant improvement (five- to

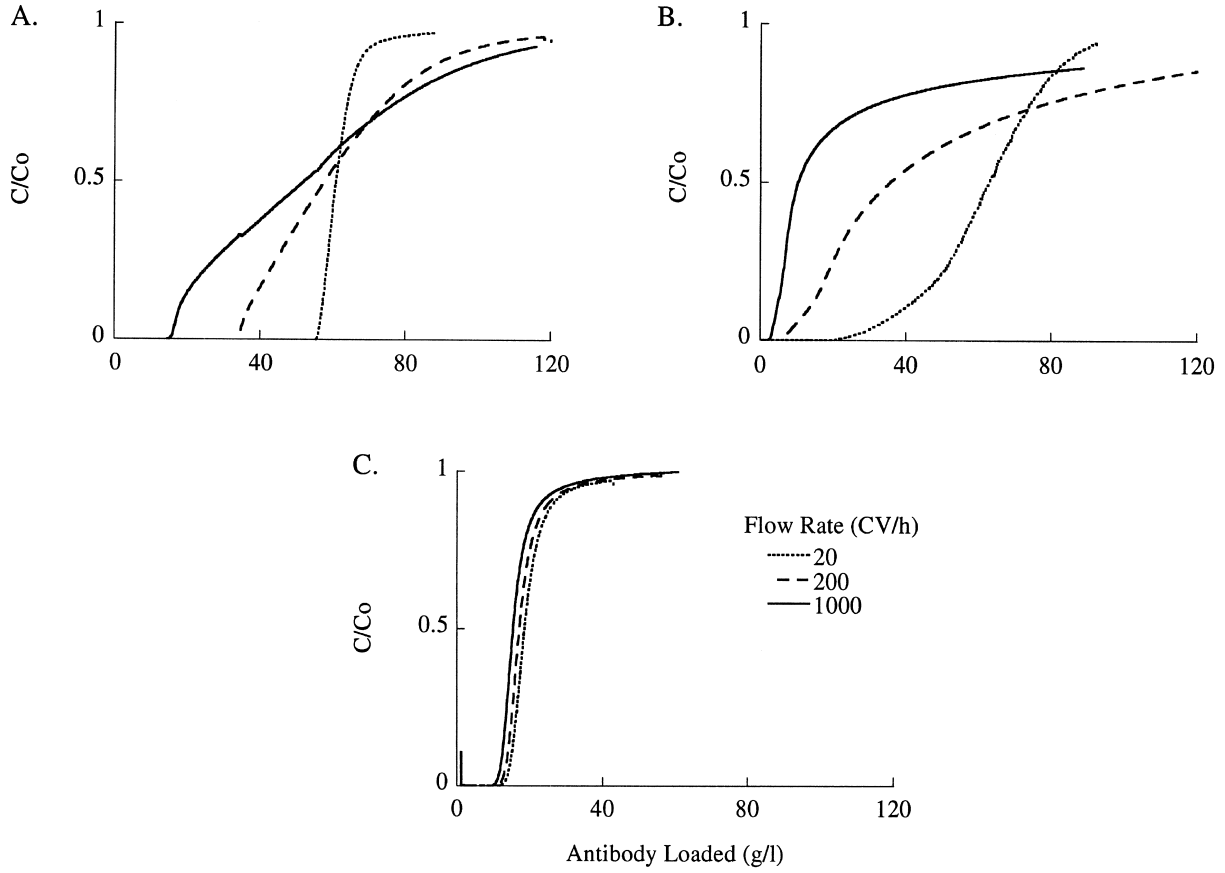

Fig. 1. Effect of flow-rate on the breakthrough curves of cation-exchange columns and membranes. C/Co is the ratio of antibody concentration in the effluent to that in the load. Antibody loaded is g antibody/l sorbent. Breakthrough at 5% was monitored by absorbance at 280 nm. Chromatography was run at flow-rates of 20, 200 and 1000 column volumes per hour on 100 mm×4.6 mm I.D. columns containing (A) Poros 20HS and (B) Poros 50HS cation-exchange resins and (C) a five-layer, 100 cm^2 Sartorius S100 strong cation-exchange membrane unit. Load material Type I was prepared by diafiltration into 10 m*M* acetate, pH 5 (buffer A) followed by dilution to 1 g/l in buffer A. Each system was equilibrated with 10 column volumes of buffer A, loaded with 1 g/l monoclonal antibody to complete breakthrough, washed with 10 column volumes of buffer A, then regenerated with 10 column volumes of 10 m*M* acetate, 1 *M* NaCl, pH 5.

10-fold) in throughput may be achieved by maintaining the cost while modestly increasing the number of cycles performed on a given set of membrane modules per batch of antibody to be purified. Alternatively, a significant cost savings (five- to 10-fold) may be achieved by maintaining the throughput while dramatically increasing the number of cycles. While it does seem possible to realize both increased product throughput and decreased sorbent cost, this may require 10 or even 100 cycles per batch. Performing multiple cycles is likely to require sanitization and re-equilibration between cycles, as well as increased validation studies. Therefore, as the number of cycles increases, the benefits of using membranes may diminish, possibly making the implementation of ion-exchange membranes in a bind and elute mode for the purification of kilogram quantities of monoclonal antibody less desirable.

3.2. Anion-exchange chromatography

Ion-exchange membranes may provide a good alternative for processing steps in which the purpose of the step is to bind trace impurities while the antibody is unretained, or flows through. For the purification of monoclonal antibodies with a high

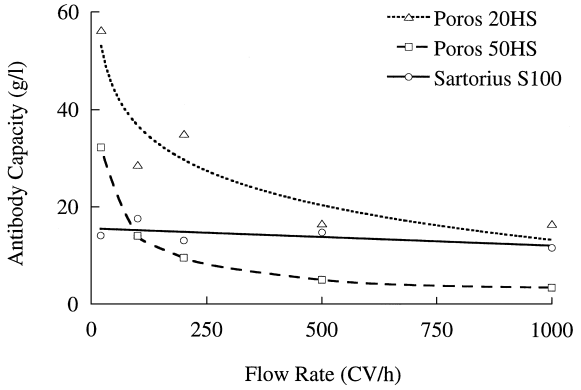

Fig. 2. Effect of flow-rate on the breakthrough capacity of cation-exchange columns and membranes at equivalent residence times. Antibody capacity is g antibody/l sorbent. Breakthrough capacity was measured at 5% on the curves shown in Fig. 1. CV=Column volume.

isoelectric point, anion-exchange chromatography is often used in a flow-through mode to remove trace levels of impurities and potential contaminants such as DNA, host cell protein, and virus. Chromatography is typically run on a Q-Sepharose column which has been sized to overcome the flow-rate limitations of the resin, thus allowing adequate throughput of antibody. The outcome is a process step which is binding only mg of impurity per liter of sorbent, or far less than the expected total binding

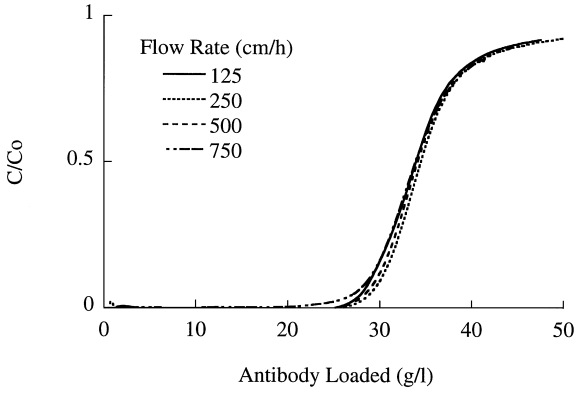

Fig. 3. Effect of flow-rate on the breakthrough curves of a representative Sartobind Factor-Two cylindrical, radial flow cation-exchange membrane adsorber module. C/Co is the ratio of antibody concentration in the effluent to that in the load. Antibody loaded is g antibody/l sorbent. Breakthrough at 5% was monitored by absorbance at 280 nm. Chromatography was run on a 15-layer module according to the procedure described in Fig. 4.

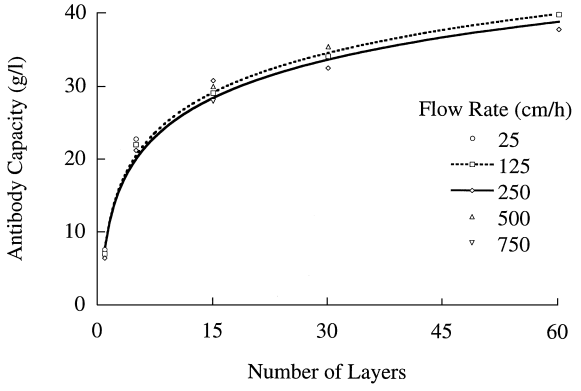

Fig. 4. Effect of flow-rate and number of layers on the binding capacity of Sartorius cation-exchange membranes. Antibody capacity is g antibody/l sorbent. Breakthrough capacity, monitored by absorbance at 280 nm, was measured at 10% (breakthrough curves not shown). Chromatography was run on two configurations of Sartorius strong cation-exchange membranes. Sartobind disk units had one and five layers of membrane (5 and 100 cm^2 of membrane area, respectively). Sartobind Factor-Two cylindrical, radial flow modules with a height of 3 cm had 15, 30 and 60 layers of membrane (1250, 2500 and 5000 cm^2 of membrane area, respectively). Flow-rates above 500 cm/h and 250 cm/h were not obtainable on the 30- and 60-layer modules, respectively, due to pressure limitations (3 bar) on the Bioskid chromatography instrument. Load material Type I was prepared by an approximately 30-fold dilution into 50 m*M* acetate, 25 m*M* NaCl, pH 5.6 (buffer A) to a final concentration of 0.2 g/l antibody. Disk units were washed with 10 ml buffer A, 10 ml 500 m*M* acetate, pH 5.6 (buffer B), and 20 ml buffer A, loaded to at least 75% breakthrough, then washed with 20 ml buffer A, 30 ml 1 *M* NaOH, and finally 30 ml 0.1 *M* NaOH for storage. Cylindrical modules were washed with 1 l buffer B and 2 l buffer A, loaded to at least 20% breakthrough, then washed with 2 l buffer A, 1 l 1 *M* NaOH, and finally 1 l 0.1 *M* NaOH for storage. Prior to loading each membrane, chromatography systems were flushed with load material up to the inlet of the membrane to minimize the delay of breakthrough due to the physical set-up and/or mixing in the chromatography system. Volumetric flow-rate was calculated by (membrane volume×linear flow-rate)/(number of layers×average membrane thickness).

capacity (on the order of 10 g protein/l sorbent). In such a scenario, a comparatively small anion-exchange membrane, which can maintain the same throughput as a large column due to the flow-independent breakthrough capacity, is a good candidate for implementation into a purification process.

As previously mentioned, one of the key functions of the anion-exchange chromatography step in monoclonal antibody purification is the complete removal

of trace levels of DNA. Using a model load material of a dilute solution of purified salmon sperm DNA, breakthrough curves on anion-exchange membranes were generated by monitoring absorbance at 260 nm over a wide range of pH and conductivity (data not shown). The breakthrough capacity for DNA was constant over a pH range of 4 to 9, and at conductivities ≤40 mS/cm (Fig. 5). These ranges of pH and conductivity extend well beyond typical operating parameters for anion-exchange chromatography. Using one-layer Sartobind Q membranes, Charlton et al. [22] observed immediate breakthrough of calf thymus DNA under similar buffer conditions. They speculated that binding capacity may be improved by increasing the number of layers, supporting our findings that binding capacity is a function of the number of layers. Therefore, due to the constant breakthrough capacity over a wide range of both pH and conductivity, as well as the acidity of DNA, the complete removal of trace levels of DNA in real process streams is expected. This does remain to be confirmed, however, either by direct measurement or by spike clearance studies in the presence of appropriate concentrations of monoclonal antibody.

The anion-exchange chromatography step is also relied upon for removal of trace levels of host cell proteins. The ability of an anion-exchange membrane to compete with a more traditional sorbent was tested. A single-use membrane was sized according to pressure limitations and cost equivalency to a multi-cycle use column. That is, a single-use membrane was required to maintain the same throughput (kg antibody processed/h) for approximately the same cost as an ion-exchange resin, whose price has been minimized by bulk purchase and multiple cycle use. Due to these imposed economic and process restrictions, the ratio of the load volume to the sorbent volume is two orders of magnitude greater for membranes than for columns. That is, for membranes, the load volume is approximately 1000-times the membrane volume, whereas for columns, the load volume is approximately 10-times the resin volume. Although a 15-layer, 50 cm height Sartorius process module met these preliminary requirements, the unavailability of a sufficiently small 15-layer device as well as the extremely large amount of antibody processed per volume of membrane required the use of a very small three-layer disk unit.

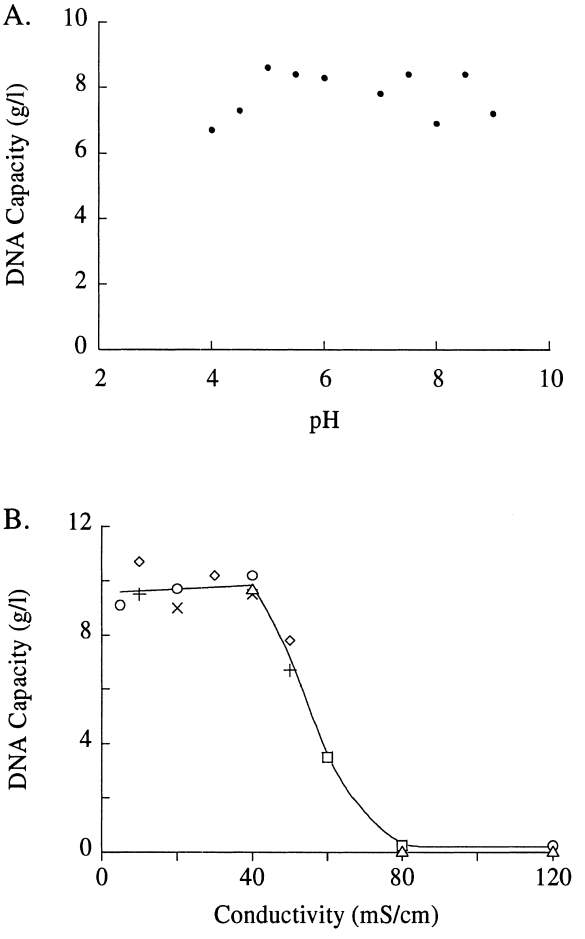

Fig. 5. Effect of conductivity and pH on the breakthrough capacity of Sartorius Q15 membranes for salmon sperm DNA. DNA capacity is g DNA/l sorbent. Breakthrough at 5% was monitored by absorbance at 260 nm. Load was salmon sperm DNA diluted to 0.05 mg/ml with the appropriate buffer A (see below). Chromatography, run at 500 CV/h (2.5 ml/min), was 20 ml buffer A, 70–75 ml load, 20 ml buffer A, 30 ml buffer B. (x, pH 3.5; diamond, pH 4; square, pH 5; circle, pH 6; +, pH 7; triangle, pH 8). (A) For pH 3.5–6, buffer A was 25 m*M* citrate (conductivity 5–5.5 mS/cm) and buffer B was 25 m*M* citrate, 1.0 *M* NaCl, pH 5.0. For pH 7–10, buffer A was 25 m*M* Tris (conductivity 2–2.5 mS/cm) and buffer B was 25 m*M* Tris, 1.0 *M* NaCl, pH 8.0. Chromatography was followed by 20 ml 1 *M* NaOH, 20 ml buffer A, and 30 ml 1 *M* KCl, 20% EtOH. (B) Buffer A was either 25 m*M* citrate (pH 3.5–6) or 25 m*M* Tris (pH 7–8), with NaCl added as needed for conductivity. Buffer B was 0.5 *M* NaOH, 2 *M* NaCl. Chromatography was followed by 20 ml buffer A and 20 ml 2 *M* KCl, 20% EtOH.

Knowing that layer number affects the breakthrough capacity, the use of three layers to predict the behavior of 15 layers introduces the problem of whether to maintain the linear flow-rate through each membrane layer regardless of total layer number, or maintain the total residence time in the membrane. As a first pass, this comparison was carried out under worst-case conditions by maintaining the linear flow-rate rather than the residence time, resulting in a rigorous testing of membrane performance.

Using load material typical of a full-scale manufacturing process, the removal of very low levels of host cell proteins (CHOP) by a Q-Sepharose FF column and a Sartorius Q membrane was compared (Table 1). The Q-Sepharose FF column removed all detectable host cell protein when loaded to 50 g antibody/l resin at 76 cm/h. The Sartorius Q membrane likewise removed all detectable host cell protein, even out to a total load of 15 000 g antibody/l membrane at 620 cm/h. The ability of both systems to remove host cell protein was further examined by using less purified load material. When loaded to 100 g antibody/l sorbent, both the Q-Sepharose FF column and the Sartorius Q membrane reduced the host cell protein approximately fourfold, from 389 ng CHOP/mg antibody to 140 ng/mg and 93 ng/mg, respectively (Fig. 6). The concentration of host cell protein present in fractions taken across the load of the membrane increased logarithmically out to the total load of 15 000 g antibody/l membrane, by which time the concentration of host cell protein was relatively constant at approximately 270 ng/mg. Therefore, a three-layer Sartorius Q membrane is capable of reducing the level of host cell protein from approximately 10 ng/mg to less than 2.3 ng/mg, making it a viable alternative to traditional Q-Sepharose chromatography for the removal of very low levels of host cell proteins.

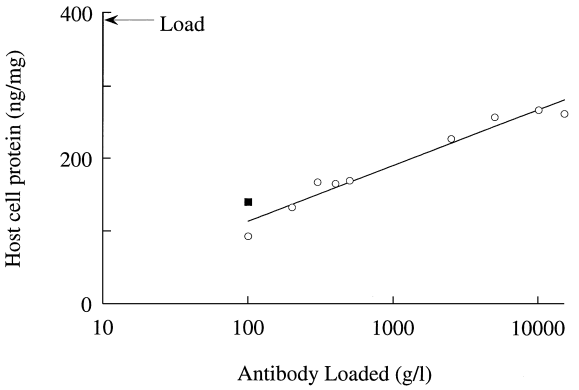

Fig. 6. Removal of moderate levels of host cell protein by a Q-Sepharose FF column and a Sartorius Q15 membrane. Host cell protein is ng CHOP/mg antibody. Antibody loaded is g antibody/l sorbent. The data forms a conventional breakthrough curve, but is shown here in a semi-log format in order to distinguish the comparative data point on the anion-exchange resin (at 100 g antibody/l sorbent). A 19×0.66 cm Q-Sepharose FF column (filled square) was loaded to 50 g antibody/l resin at 76 cm/h (0.43 ml/min) whereas a Q15 membrane (open circles) was loaded to 15 000 g antibody/l membrane at 620 cm/h (37.6 ml/min). The effluent was collected in fractions across the load. Load material Type I was adjusted to pH 8, conductivity 7 mS/cm with 1.5 *M* Tris base and purified water to a final concentration of 2.5 g/l. Chromatography consisted of a 45 min exposure to 0.5 *M* NaOH, 10–15 ml 250 m*M* Tris, 0.5 *M* NaCl, pH 8 (buffer B), 30–35 ml 25 m*M* Tris, 50 m*M* NaCl, pH 8 (buffer A), load, 20 ml buffer A, 10–15 ml buffer B, 45 min exposure to 0.5 *M* NaOH, and 20 ml 0.1 *M* NaOH.

To further test the ability of anion-exchange

Table 1
Removal of very low levels of host cell protein by a Q-Sepharose FF column and a Sartorius Q15 membrane[a]

	Host cell protein (ng/mg antibody)	Antibody loaded (g antibody/l sorbent)	Flow-rate (cm/h)
Load	10.6	–	–
Q-Sepharose FF	<2	50	76
Sartorius Q15	<2	15 000	620

[a] A 19×0.66 cm Q-Sepharose FF column was loaded to 50 g antibody/l resin at 76 cm/h (0.43 ml/min) whereas a Q15 membrane was loaded to 15 000 g antibody/l membrane at 620 cm/h (37.6 ml/min). The effluent was collected in fractions across the load. No host cell protein was detected in any of the fractions. Load material was Type I further purified by cation-exchange chromatography then adjusted to pH 8, conductivity 7 mS/cm with 1.5 *M* Tris base and purified water to a final concentration of 2.5 g/l. Chromatography consisted of a 45 min exposure to 0.5 *M* NaOH, 10–15 ml 250 m*M* Tris, 0.5 *M* NaCl, pH 8 (buffer B), 30–35 ml 25 m*M* Tris, 50 m*M* NaCl, pH 8 (buffer A), load, 20 ml buffer A, 10–15 ml buffer B, 45 min exposure to 0.5 *M* NaOH, and 20 ml 0.1 *M* NaOH.

membranes to remove host cell protein, ion-exchange membranes from two manufacturers were compared. Strong anion-exchange membranes from Sartorius and Pall, when loaded to 1000 g antibody/l membrane at 62 and 620 cm/h, each reduced the level of host cell protein approximately twofold (Fig. 7). Consistent with the flow-rate independent breakthrough capacities observed for antibody, there was also no flow-rate dependence for binding trace levels of these impurities. Unlike the host cell protein breakthrough observed previously, no change in the amount of host cell protein in the membrane effluent was observed. Therefore, a similar experiment was repeated using membranes loaded only to 200 g antibody/l membrane. The level of host cell protein was again reduced approximately twofold, regardless of flow-rate, starting at loads of 50 g antibody/l membrane and continuing to 200 g antibody/l membrane (Fig. 8). However, breakthrough was observed between 10 and 50 g antibody loaded/l membrane, and the rate of host cell protein breakthrough was slower at 76 cm/h than at 620 cm/h.

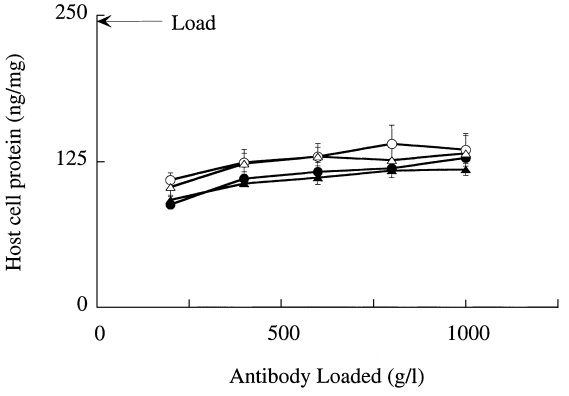

Fig. 7. Effect of flow-rate on host cell protein removal by Sartorius and Pall strong anion-exchange membranes. Host cell protein is ng CHOP/mg antibody. Antibody loaded is g antibody/l sorbent. Ten layers of Sartorius Q (open symbols) or Pall Q_p (filled symbols) membrane were stacked into a Swinny 13 mm filter holder and loaded to 1000 g antibody/l membrane at flow-rates of 62 cm/h (diamonds) and 620 cm/h (circles), or 0.81 and 8.1 ml/min, respectively. The effluent was collected in five equal volume fractions. Load material Type II was adjusted to pH 8, conductivity 4 mS/cm with 1.5 *M* Tris base and purified water to a final concentration of 4.4 g/l. Chromatography was 25 ml 250 m*M* Tris, 0.5 *M* Na_2SO_4, pH 8 (buffer B), 50 ml 25 m*M* Tris, 12 m*M* Na_2SO_4, pH 8 (buffer A), load, 25 ml buffer A, 25 ml buffer B, 25 ml 0.5 *M* NaOH, and 25 ml 0.1 *M* NaOH.

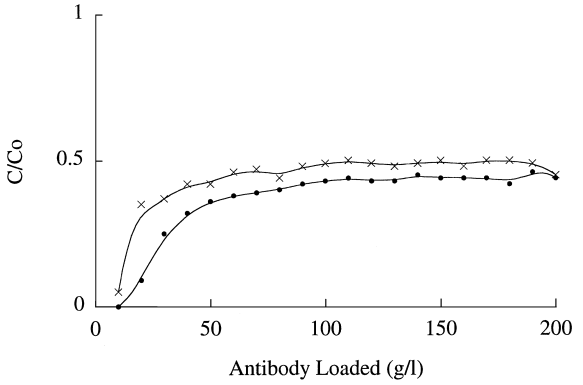

Fig. 8. Effect of flow-rate on the breakthrough of host cell protein on Pall strong anion-exchange membranes. C/Co is the ratio of host cell protein concentration in the effluent to that in the load. Antibody loaded is g antibody/l sorbent. Breakthrough at 5% was monitored by absorbance at 280 nm. Ten layers of Q_p membrane were stacked into a Pall 25 mm prototype holder and loaded to 200 g antibody/l membrane at flow-rates of 76 cm/h (filled circles) and 620 cm/h (x). The effluent was collected in fractions across the load. Load material Type II was adjusted to pH 8, conductivity 4 mS/cm with 1.5 *M* Tris base and purified water to a final concentration of 4.2 g/l. Chromatography was 25 ml 250 m*M* Tris, 0.5 *M* Na_2SO_4, pH 8 (buffer B), 50 ml 25 m*M* Tris, 12 m*M* Na_2SO_4, pH 8 (buffer A), load, 25 ml buffer A, 25 ml buffer B, 25 ml 0.5 *M* NaOH, and 25 ml 0.1 *M* NaOH.

Therefore, although complete removal of these impurities is achieved only at very low loads, strong anion-exchange membranes are capable of reducing host cell protein levels at least twofold, essentially independently of flow-rate and total amount of antibody loaded.

One possible explanation for the incomplete host cell protein removal observed is described by Petsch et al. [23], who studied endotoxin removal on anion-exchange membranes. Basic proteins were found to act as endotoxin carriers by competing with the anion-exchange ligand for endotoxin binding. In a similar manner, it is possible for antibody to be acting as host cell protein carriers under the conditions we studied. This behavior could be elucidated by determining the equilibrium binding isotherms for host cell proteins and other very low concentration protein impurities, both as single components as well as in the presence of high concentrations of antibody. Certainly, anion-exchange membranes have the potential to be useful for processing large volumes of feedstreams in which only partial removal of host

cell protein is needed. Furthermore, anion-exchange steps used in series would be interesting to investigate, especially if each unit is able to independently provide the same fold-reduction.

Lastly, anion-exchange chromatography is often a crucial processing step for the removal of potentially contaminating virus during the large-scale production of monoclonal antibodies. Generally, the anion-exchange chromatography step must demonstrate at least four to five logs of removal of virus when used in current processing schemes. The ability of Sartorius Q membranes to remove xenotropic murine leukemia virus was tested and compared to a Q-Sepharose FF column (Table 2). The Q-Sepharose FF column, loaded to 50 g antibody/l resin at 76 cm/h, provided greater than a $10^{5.1}$-fold reduction of virus infectivity (that is, 5.1 log removal of virus). The Sartorius membrane system, when loaded to 2000 g antibody/l membrane at 620 cm/h, provided a $10^{2.3}$-fold reduction of virus infectivity. The removal of virus decreased with increasing load, resulting in less than or equal to $10^{1.0}$ particles removed after 4000 g antibody/l membrane. Due to the nature of the testing procedure, in which a slightly turbid solution of high titer virus-spiked load is applied onto the membranes, it is possible that the virus may have fouled the membrane and/or exceeded the number of available binding sites. The pressure drop across the membrane doubled by the end of the load, indicating some fouling of the membrane, likely with virus since the virus-spiked load solution was slightly turbid and the pressure drop for the corresponding control (no virus) run only slightly increased. Therefore, to more accurately assess the ability of anion-exchange membranes to remove virus, the membranes could be tested using lower titer virus loads coupled with a more sensitive assay, as well as possibly using a smaller virus that would not have as much potential of fouling the membrane pores.

Acknowledgements

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Table 2
Xenotropic murine leukemia virus removal by a Q-Sepharose FF column and Sartorius Q membranes[a]

	Log removal of virus	Antibody loaded (g antibody/l sorbent)	Flow-rate (cm/h)
Q-Sepharose FF	>5.1	50	76
Sartorius Q	2.3	2000	620
(10 layers)	1.0	4000	
	0.5	6000	
	0.7	8000	
	0.3	10 000	

[a] A 19×0.66 cm Q-Sepharose FF column was loaded to 50 g antibody/l resin at 76 cm/h (0.43 ml/min), whereas the membrane was loaded to 10 000 g antibody/l membrane at 620 cm/h (8.1 ml/min). Ten layers of Sartorius Q membrane were stacked into a Swinny 13 mm filter holder. Membrane volume was calculated as the product of the exposed surface area, the membrane thickness, and the number of layers. Load material was Type II protein A pool adjusted to pH 8, conductivity 7 mS/cm with 1.5 *M* Tris base and purified water to a final concentration of 8.0 g/l, followed by addition of virus. Xenotropic murine leukemia virus, at a starting concentration of approximately 10^7–10^8 tissue culture infectious dose 50 ($TCID_{50}$)/ml, was spiked into the load material at a 1:50 dilution. The chromatography was 20–25 ml buffer B, 30–50 ml buffer A, load, 25 ml buffer A, 10–25 ml buffer B, 30 ml 0.5 *M* NaOH, and 20–25 ml 0.1 *M* NaOH. Buffer A was 25 m*M* Tris, 50 m*M* NaCl, pH 8 and buffer B was 250 m*M* Tris, 0.5 *M* NaCl, pH 8. A $TCID_{50}$ assay was used to determine the amount of infectious virus presenting in each pool sample. The PG-4 cells (Moloney sarcoma virus-transformed brain, cat, *Felis catus*, ATCC CRL 2032; ATCC, Manassas, VA, USA) were seeded into 96-well plates to a density of about $5{\cdot}10^4$ cells/ml. The plates were incubated at 36–38°C for 7 days before being examined for viral introduced cytopathic effect. The virus titer was calculated using the Karber method [24]. Log removal of virus (LRV) was calculated by subtracting the total virus (log $TCID_{50}$) recovered from the total virus (log $TCID_{50}$) added.

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