Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1078 (2005) 74–82
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Performance of a membrane adsorber for trace impurity removal in biotechnology manufacturing

Michael Phillips [a], Jason Cormier [a,*], Jennifer Ferrence [b], Chris Dowd [b], Robert Kiss [c], Herbert Lutz [a], Jeffrey Carter [a]

[a] *Biopharmaceutical R&D Division, Millipore Corp., 32 Wiggins Ave, Bedford, MA 01730, USA*
[b] *Department of Recovery Sciences, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA*
[c] *Department of Manufacturing Sciences and Technology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA*

Received 23 February 2005; received in revised form 26 April 2005; accepted 2 May 2005

Abstract

Membrane adsorbers provide an attractive alternative to traditional bead-based chromatography columns used to remove trace impurities in downstream applications. A linearly scalable novel membrane adsorber family designed for the efficient removal of trace impurities from biotherapeutics, are capable of reproducibly achieving greater than 4 log removal of mammalian viruses, 3 log removal of endotoxin and DNA, and greater than 1 log removal of host cell protein. Single use, disposable membrane adsorbers eliminate the need for costly and time consuming column packing and cleaning validation associated with bead-based chromatography systems, and minimize the required number and volume of buffers. A membrane adsorber step reduces process time, floor space, buffer usage, labor cost, and improves manufacturing flexibility. This "process compression" effect is commonly associated with reducing the number of processing steps. The rigid microporous structure of the membrane layers allows for high process flux operation and uniform bed consistency at all processing scales.

Keywords: Membrane adsorber; Membrane chromatography; Preparative chromatography; Antibody

1. Introduction

Biotechnology manufacturing processes are intended to produce therapeutics that meet regulatory and company standards for safety, purity and efficacy. Recombinant expression systems present a challenging array of biological impurities that must be removed during purification and prior to final fill. In addition to several upstream purification steps, it is common for manufacturers to employ an anion exchange chromatography column as a polishing step toward the end of the manufacturing process solely to adsorb trace levels of DNA, host cell protein (HCP) and, for bacterial fermentation, endotoxin impurities. Endotoxin and DNA are negatively charged molecules (p*I* values of less than 3) that are amenable to removal by AEX. Conversely, HCP represents a range of diverse cellular proteins, each with its particular p*I* and affinity for the quaternary amine ligand. In addition, anion exchange chromatography has been validated to provide significant removal of adventitious viruses. In this context, trace impurities are defined as $\leq 10^7$ viruses per total volume of feedstock, $\leq 1\,\mu g/mL$ of DNA, ≤ 100 EU/mL of endotoxin, and ≤ 100 ng/mL of HCP.

Membrane adsorbers provide an attractive alternative technology to traditional bead-based chromatographic separations. The membrane adsorber utilizes the same strong anion exchange ligand, quaternary amine, as standard anion exchange polishing chromatography columns. Although the removal mechanisms are the same, membrane adsorber devices provide several potential performance advantages over column chromatography. The main performance advantage of membrane-based chromatographic separations derives from the extremely fast mass transfer rates. Unlike bead-based chromatography, where most of the adsorption

* Corresponding author. Tel.: +1 781 533 2828; fax: +1 781 533 3134.
E-mail address: Jason_Cormier@millipore.com (J. Cormier).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.05.007

sites are internal to the bead and the rate of mass transfer is controlled by pore diffusion, the adsorption sites of membrane adsorbers are within the convective flow path of the fluid [1,2]. Additionally, a tight membrane pore size distribution coupled with an effective flow distributor at the device inlet and multiple membrane layers in series could be optimized such that "plug flow" uniformity is maximized and the dispersion is minimized through the entire device, thus providing effective utilization of all active sites [3,4]. For these reasons, mass transfer rates of membrane adsorber media may be an order of magnitude greater than that of standard bead-based chromatography media [5], allowing for both high efficiency and high-flux separations. In addition to the performance advantage, membrane adsorbers are disposable, thus they can provide several cost and ease-of-use benefits including a reduction in the number and volume of buffers due to the elimination of resin storing, cleaning, sanitization, and flushing validation, as well as the elimination of the need for column hardware and packing [4].

Membrane-based chromatography has been successfully employed for preparative separations with much work predominantly for protein separations [6–13]. However, universal adoption of this technology has been slow because membrane chromatography has been limited by the lower binding capacity than that of bead-based columns, even though the high flux advantages provided by membrane adsorbers would lead to higher productivity [8]. Although bead-based chromatography is still predominant and effective for product bind-elute operations, it has several inherent disadvantages for trace-impurity removal or polishing applications. For trace-impurity applications, adsorptive capacity is not the limitation. Furthermore, the adsorptive binding capacity of bead-based columns used in this application is typically 3–4 orders of magnitude larger than required because columns are normally sized to achieve a desired flow rate rather than capacity. Since membrane-based systems have a distinct flow rate advantage and sufficient capacity for binding trace levels of impurities and contaminants, membrane adsorbers are ideally suited for this application. Work has been done recently using membrane chromatography to remove DNA, HCP, endotoxin, and virus from antibody manufacturing solutions with reasonable success [14–17].

To meet this application need, high throughput anion exchange membrane adsorbers were developed for trace impurity removal. A well-designed membrane adsorber provides excellent flow distribution to ensure that essentially all binding sites are used before impurity breakthrough occurs. Reproducibility in the manufacture of the derivatized membrane complements good device design, leading to consistency in device performance and providing confidence that the device will reliably perform its intended function. Lastly, performance of a family of devices must be scalable if one is to effectively use a scale-down device as an indicator of process-scale capsule performance.

In this paper, data are provided demonstrating the feed stream chemistries and process windows within which this novel membrane adsorber may be used successfully for trace DNA, endotoxin, HCP, and model bacteriophage removal. Specific performance targets for the designed membrane adsorber were 3 log reduction of virus and DNA, 2 log reduction of endotoxin, and 1 log reduction of HCP. Data on removal of three model mammalian viruses, mouse minute virus (MMV), xenotropic murine leukemia virus (MLV) and simian virus 40 (SV40) are provided. In addition to trace impurity/contaminant removal, this paper presents data on device scaling and manufacturing reproducibility. Lastly, we present a study in which HCP removal is evaluated under conditions at pilot plant scale.

2. Experimental

2.1. Devices, chemicals, reagents, viruses, and scaling

2.1.1. Devices

The membrane and adsorber devices were fabricated at Millipore Corp. (Billerica, MA, USA). The membrane adsorber contains eight layers (0.1 cm bed height) of 0.65 μm hydrophilic polyvinylidene fluoride (PVDF) base membrane which is then derivatized with a quaternary amine ligand that provides anion exchange capacity to the filter matrix. This is the same membrane process which is used to make 0.2 μm PVDF sterile absolute membranes. Thus, the pore size distribution on this membrane is very tight and well controlled. Three scales of membrane adsorbers were used in this study. A small-scale device intended for the use in process development, feasibility and validation studies, has been engineered to be scalable to the process scale 2- and 6-stack capsules. In the stacked disk capsule configuration, the eight layers of membrane are immobilized onto a perforated plastic disk designed to allow flow through the membrane and the disk. To increase the amount of membrane, multiple disks are added to a capsule. Thus, the 6-stack capsule contains three times the membrane of the 2-stack capsule. In the stacked disk configuration, flow occurs in parallel through each disk; bed height remains constant. A physical description of the three devices is provided in Table 1.

2.1.2. DNA preparation

DNA removal studies were performed using herring sperm DNA (HS-DNA, stock concentration of 10 mg/mL) obtained from Promega (Madison, WI, USA). A working stock of 50 μg/mL was prepared from the concentrated DNA stock

Table 1
Physical characteristics of membrane adsorbers used in this study

Device	Frontal area (cm^2)	Membrane bed volume (mL)[a]
Small scale	3.5	0.35
2-Stack	150	15
6-Stack	450	45

[a] Membrane bed volume represents the frontal area multiplied the total thickness of the eight membrane layers in the device.

solution. The working stock concentration was verified spectrophotometrically by using the relationship that one A_{260} unit corresponds to 50 μg/mL dsDNA. PicoGreen dsDNA quantitation reagent was obtained from Molecular Probes (Eugene, OR, USA). Reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA) or Fisher Scientific (Fair Lawn, NJ, USA).

2.1.3. Endotoxin preparation

Endotoxin assays were performed using the Limulus Amebocyte Lysate (LAL) Kinetic-QCL Endotoxin Assay system and WinKQCL software from BioWhittaker (Walkersville, MD, USA). Lyophilized LAL reagent was supplied by BioWhittaker. LAL reagent water was generated using a Milli-Q Ultrapure water system from Millipore Corp. The endotoxin levels of this water were below the limit of detection of the endotoxin assay. The endotoxin bulk source used for spiking the feedstock was *Escherichia coli* (055:B5-#L2880) lipopolysaccharide (LPS), supplied by Sigma-Aldrich. Control standard endotoxin (CSE) used to generate standard curves was supplied by BioWhittaker. Trizma pre-set crystals and NaCl were supplied by Sigma–Aldrich. All materials that contacted the analyte solutions in the assay procedure (i.e. plastic dilution tubes, pipettes, microplates, and reservoirs) were endotoxin-free and disposable.

2.1.4. HCP preparation

Load material for the HCP removal studies contained a recombinant humanized monoclonal antibody (MAb) produced in Chinese hamster ovary (CHO) cells. The material was purified by Protein-A chromatography, cation exchange chromatography, and virus filtration.

2.1.5. Bacteriophage/mammalian virus preparation

Bacteriophages ϕX174 and ϕ6 and their host cells, *E. coli* and *Pseudomonas pseudoalcaligenes* S4, respectively, were obtained and propagated as described elsewhere [18].

MMV [ATCC VR-1346] was propagated on human embryonic cells (324 K, Yale University, New Haven, CT, USA) grown in HG DMEM containing 10% fetal bovine serum (FBS). Subconfluent cell monolayers were infected with virus and replenished with HG DMEM containing 1% FBS. The cultures were examined regularly for cytopathic effect (CPE). When viral CPE was evident, the culture supernatant and cell debris was harvested by using a freeze thaw method. The cell debris was removed by centrifugation and aliquots of the supernatant were frozen at or below −70 °C. All virus stocks were sonicated and filtered through a 0.22 μm sterilizing grade filter prior to use.

Xenotropic MuLV from the Institute for Cancer Research (London, UK) was propagated on *Mus dunni* cells (ATCC CRL-2017) grown in McCoys buffer containing 10% FBS. Subconfluent cell monolayers were infected with virus and the cells were passaged every 3–4 days for four passages. Three to four days after the fourth passage, the culture supernatant was harvested. The cell debris was removed by centrifugation and aliquots of the supernatant were frozen at or below −70 °C. All virus stocks were sonicated and filtered through a 0.22 μm sterilizing grade filter prior to use.

SV40 (ATCC VR-305) was propagated on African green monkey kidney C1008 cells (Vero cells, ATCC 1586) grown in HG DMEM medium containing 10% FBS. Subconfluent cell monolayers were infected with virus and the culture was examined regularly for CPE. When viral CPE was evident, the culture supernatant and cell debris from each virus infection was harvested by using a freeze thaw method. The cell debris was removed by centrifugation and aliquots of the supernatant were frozen at or below −70 °C. All virus stocks were sonicated and filtered through a 0.22 μm sterilizing grade filter prior to use.

2.1.6. Scaling/reproducibility studies

Dynamic binding capacity studies on small scale devices (0.35 mL bed volume) were conducted by using a BioCAD Sprint chromatographic workstation from Applied Biosystems (Foster City, CA, USA). For the dynamic binding capacity studies on 2-stack (150 mL bed volume) and 6-stack (450 mL bed volume) capsules, the fluid flow rate was maintained by using a Model 7225 Micropump from Barnant Co. (Barrington, IL, USA). The effluent from the large-scale capsules was monitored by using a Model 500 UV detector from Lab Alliance (State College, PA, USA) equipped with a semi-prep flow cell. The protein and salts used were *N*-tosyl-L-glutamic acid, bovine albumin, Tris-base, Tris–HCl, and NaCl and were purchased from Sigma–Aldrich. All solutions were filtered prior to use through a 0.22 μm sterilizing grade filter.

2.2. Biological assays

2.2.1. DNA removal studies

To quantify DNA with the PicoGreen system, samples were processed following the manufacturer recommendations. Five standard solutions, ranging from 0.5 ng/mL to 2 μg/mL, were prepared by dilution of the HS-DNA working stock solution. Triplicate 100 μL aliquots of each standard DNA solution and of test samples were added to a 96-well plate. The PicoGreen reagent was diluted 200-fold in TE (10 mM Tris buffer, 1 mM EDTA, pH 8.0) and 100 μL were added to each sample well. Fluorescence of the samples and of the standards was measured using a SpectraFLUOR Plus plate reader from Tecan U.S. (Research Triangle Park, NC, USA). The excitation and emission wavelengths were set at 485 and 535 nm, respectively. The fluorescence value of a blank sample (0 ng/mL HS-DNA) was subtracted from that of the samples and standards. The mean and standard deviation was calculated for the standard curve solutions and for each sample. A five-point standard curve of fluorescence versus DNA concentration was generated and used to determine the DNA concentration of the test samples.

2.2.2. Endotoxin removal studies

To quantify endotoxin, the LAL Kinetic-QCL Endotoxin Assay system was used according to manufacturer's instructions. Briefly, 100 μL aliquots of CSE standards and test samples were placed into 96-well microplates, and 100 μL of the LAL reagent was added to each sample well. Continuous monitoring of the absorbance at 405 nm was used to provide real-time quantitation of the endotoxin-dependent enzymatic reaction. The reaction rate was determined automatically by the LAL plate reader. In this assay, reaction time is inversely proportional to the amount of endotoxin present.

A standard curve relating CSE concentration (EU/mL) to LAL assay reaction time was created for each solution used in the endotoxin removal studies. Each standard curve spanned 0.05–50 EU/mL and had a linear regression coefficient of at least 0.98. The LPS concentrations for feed and permeate samples were calculated using the standard curve.

2.2.3. HCP removal studies

CHO cell protein concentration was determined by an enzyme-linked immunosorbent assay (ELISA) developed at Genentech, Inc. Affinity-purified goat whole anti-CHO protein antibodies (i.e. anti-HCP antibodies) were immobilized on microtiter plate wells. Dilutions of samples containing HCP were added to the wells and incubated to allow binding of HCP to the immobilized antibodies. This was followed by incubation with conjugated-peroxidase whole anti-CHO-protein antibody. The wells were washed to remove unbound conjugated antibody, and horseradish peroxidase activity was quantified with the substrate *o*-phenylenediamine by reading the absorbance at 492 nm. In this assay, horseradish peroxidase activity is correlated to HCP concentration. The dynamic range for the ELISA was typically 5–320 ng/mL. Because samples were typically diluted by two-fold, the detection limit was 10 ng/mL HCP.

2.2.4. Protein product yield

MAb product yield was determined by UV spectrophotometry using a Shimadzu UV-1601 UV Spectrophotometer (Columbia, MD, USA). Sample absorbance at 280 nm was used to calculate the MAb concentration. Yields were determined by comparing the total pool volume and concentration to the total load volume and concentration.

2.2.5. Bacteriophage/mammalian virus removal studies

Bacteriophage ϕX174 and ϕ6 quantitation was performed by plaque assays that have been described elsewhere [18]. In brief, serial dilutions of phage-containing samples were added to host bacteria (*E. coli* for ϕX174 and *P. pseudoalcaligenes* for ϕ6). These virus–host suspensions were added to solidified growth medium and allowed to incubate until phage plaques became visible. The number of plaques and the dilution factor were multiplied to calculate the concentration of phage in the original sample.

Mammalian virus assays were performed using a standard tissue culture infectious dose ($TCID_{50}$) quantal infectivity assay. In brief, five-fold serial dilutions of virus-containing fluid were prepared and at least eight 0.25 mL aliquots of each dilution was added to monolayers of PG4 cells (for X-MuLV), Vero cells (for SV40), or 324 K cells (for MMV) grown in 24-well plates. Negative controls of virus-free buffer and positive controls containing known titers of virus were prepared in parallel. The plates were incubated at 35–39 °C, 4–6% CO_2 for 1–2 h, and the wells were fed with approximately 1.5 mL of tissue culture medium. Assays were viewed for CPE, using light microscopy, according to the following schedules: X-MuLV cultures were viewed every two days for 1 week or until CPE was observed; SV40 cultures were viewed every week for 3 weeks or until CPE was observed; MMV cultures were viewed every three days for 12 days or until CPE was observed. $TCID_{50}$ values were calculated by using the Kärber statistical method [19]. If the minimum detection limit of the assay was reached, the Poisson distribution at 98% confidence was applied.

2.3. Purification experiments

With the exception of the scaling studies, all purification experiments were performed using the small scale devices. All challenge solutions were pre-filtered using a 0.22 μm Sterivex prefilter (Millipore) placed in-line upstream of the device. To monitor pressure, pressure gauges were placed upstream of both the 0.22 μm prefilter and the adsorber device. Prior to use, all the adsorber devices were tested for integrity by wetting the device with DI water and then measuring the air-diffusion rate at a test pressure of 103.4 mPa. Air pressure was measured with a 0–206.8 mPa digital pressure gauge from DCT Instruments (Columbus, OH, USA) with a 0.25% full-scale accuracy. The permeate air flow was measured by visually inspecting the water droplet movement in a 1 mL pipette (accuracy down to 0.01 mL/min) in a 5–10 min timeframe. All devices exhibited less than 0.01 mL/min air flow after equilibration indicating a fully wetted and integral device. Water permeability measurements were made using DI water at a test pressure of 137.9 mPa. The resulting water flow rate was measured with a stop watch and graduated cylinder. All devices had a measured permeability greater than 58 L/(m^2 h mPa).

Unless otherwise specified, the challenge solutions were as follows: HS-DNA challenge solution at 1 μg/mL in 25 mM Tris buffer, pH 8.0; 2000 EU/mL endotoxin in 25 mM Tris buffer, pH 8.1; approximately 1.5×10^7 pfu/mL bacteriophage in 25 mM Tris buffer, pH 8.1; between 700 and 1000 ng/mL HCP in 25 mM Tris buffer, pH 8.0 at a conductivity between 4 and 7.5 mS/cm. Challenge flow rates were 20 mL/min (340 cm/h) for the small scale devices, and 260 mL/min for the 2- and 6-stack capsules. Challenge volumes for the small scale devices were 1000 mL of HS-DNA, 300 mL of endotoxin, 300 mL of bacteriophage suspension, and 700 ml of HCP challenge. HCP was challenged with a volume up to 200 L for the 6-stack. Between 10 and 20 L

of challenge solution was used for the 2- and 6-stack tosyl glutamic acid dynamic capacity testing.

During the DNA removal studies, instantaneous 1 mL samples were collected at 5 min, 25 min and every 10 min thereafter. For the endotoxin and bacteriophage removal studies, feed samples and pooled filtrate samples were collected. Each sample was assayed for its model impurity and the results were used to calculate log removal values by using the following equation:

$$\mathrm{LRV} = \log_{10}\left(\frac{C_{\mathrm{feed}}}{C_{\mathrm{permeate}}}\right)$$

For the mammalian virus challenge experiments, the challenge suspension consisted of virus spiked into 100 or 150 mL of either 50 mM NaCl (6.9 mS/cm) or 150 mM NaCl (16.6 mS/cm) in 25 mM Tris, pH 8.1. The challenge titers were approximately 5.0×10^5 TCID_{50}/mL, 4.7×10^5 TCID_{50}/mL, and 1.8×10^6 TCID_{50}/mL for MuLV, MMV, and SV40, respectively. The challenge flow rate was 20 mL/min (340 cm/h). Mammalian virus LRV was calculated as above.

2.4. HCP clearance experiments—laboratory scale

The laboratory scale HCP clearance experiments were conducted using small scale devices. The load solution was typically 3 g/L MAb and 700–900 ng/mL HCP at a conductivity of 7.5 mS/cm and pH 8.0. Load solution pH was adjusted with 1.5 M Tris-base to pH 8.0 and load conductivity was adjusted with purified water. The load solution was pre-filtered using a 0.22 μm Millipak 20 filter (Millipore) and pre-filtered in-line using a 0.22 μm, 10 cm^2 Sterivex filter (Millipore). Pressure transducers from Becton Dickinson (Franklin Lakes, NJ, USA) were located on the inlet of both the Sterivex pre-filter and the small scale device. The pressure profiles were monitored by a Netdaq Data Acquisition system from Fluke (Everett, WA, USA).

Before use, the small scale membrane adsorber device was flushed with purified water at 3000 L/(m^2 h) for a minimum of 5 min. The device was then equilibrated with buffer, 25 mM Tris-base, 50 mM NaCl, adjusted to the appropriate pH and conductivity per load solution conditions, at a flux rate of 3000 L/(m^2 h) for a minimum of 10 min. The MAb solution was loaded at a rate of 340 cm/h or 20 mL/min (3400 L/(m^2 h)) for a throughput of 6000 mg MAb/mL membrane or 2.1 g MAb. The filtrate was collected in 14 mL fractions, which were analyzed for HCP removal and MAb product yield.

2.5. HCP clearance experiments—pilot plant scale

The pilot plant scale HCP clearance experiments on 6-stack capsules were performed using a 1/2 in. chromatography skid from Millipore. The load solution was approximately 3 g/L MAb and 700–1000 ng/mL HCP. Load solution pH was adjusted with 1.5 M Tris-base to pH 8.0 and the conductivity was adjusted with purified water to 7.5 or 4 mS/cm. The load solution was pre-filtered in-line using a 0.22 μm, 10 in. sterilizing grade Durapore cartridge filter (Millipore). Typically, the 6-stack capsule was loaded to 8–13 g MAb/mL membrane or 400–600 g MAb.

Before use, the 6-stack capsule was wetted with 10 L of purified water at 34.5 mPa, tested for integrity and equilibrated with 30 L of 25 mM Tris-base, 50 mM NaCl, pH 8.0 at 3 L/min (340 cm/h). The MAb load solution flow rate was maintained at 340 cm/h by using a Viking S1L pump (Cedar Falls, IA, USA). Filtrate split stream samples were collected in 30 mL fractions every minute using a Foxy 200 fraction collector from ISCO, Inc. (Lincoln, NE, USA) and these fractions were analyzed for HCP removal and MAb product yield.

3. Results and discussion

3.1. Bacteriophage and mammalian virus removal

As is common when characterizing virus clearance technologies, bacteriophages were used in this study as indicator models to evaluate the full range of device performance. Mammalian virus removal provided support to the principle that mammalian virus removal is accurately or conservatively modeled by that of the bacteriophages.

The membrane adsorber devices were challenged with ϕX174 and ϕ6 suspended in fluids of varying pH or NaCl concentration. As seen in Fig. 1, ϕ6 retention was unaffected by pH changes from 6 to 8 (because of stability issues with

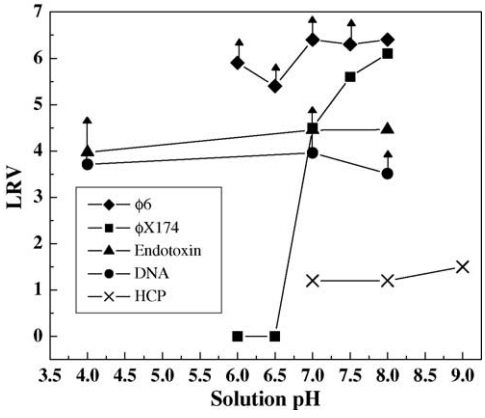

Fig. 1. Effect of solution pH on bacteriophage, DNA, endotoxin, and HCP retention. Upward arrows indicate that impurity was not detectable in the filtrate. The following experimental conditions were used: HS-DNA challenge solution at 1 μg/mL in 25 mM buffer; 2000 EU/mL endotoxin in 25 mM buffer; approximately 1.5×10^7 pfu/mL ϕX174 and ϕ6 bacteriophage in 25 mM buffer; between 700 and 1000 ng/mL HCP in 3 g/L MAb/25 mM Tris buffer. Challenge flow rates were 20 mL/min (340 cm/h) for the small scale devices. Challenge volumes for the small scale devices were 1000 mL of HS-DNA, 300 mL of endotoxin, 300 mL of ϕX174 bacteriophage suspension, and 700 ml of HCP challenge. For pH of 4.0, 6.0–7.0, and 7.5–9.0 and acetate, phosphate, and Tris buffer solution was used.

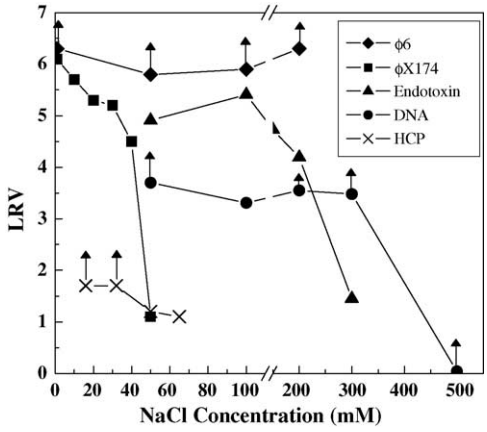

Fig. 2. Effect of NaCl concentration on bacteriophage, DNA, endotoxin, and HCP retention. Upward arrows indicate that impurity was not detectable in the filtrate. The following experimental conditions were used: HS-DNA challenge solution at 1 μg/mL in 25 mM Tris buffer, pH 8.0; 2000 EU/mL endotoxin in 25 mM Tris buffer, pH 8.1; approximately 1.5×10^7 pfu/mL ϕX174 and ϕ6 bacteriophage in 25 mM Tris buffer, pH 8.1; between 700 and 1000 ng/mL HCP in 3 g/L MAb/25 mM Tris buffer, pH 8.0. Challenge flow rates were 20 mL/min (340 cm/h) for the small scale devices. Challenge volumes for the small scale devices were 1000 mL of HS-DNA, 300 mL of endotoxin, 300 mL of ϕX174 bacteriophage suspension, and 700 ml of HCP challenge.

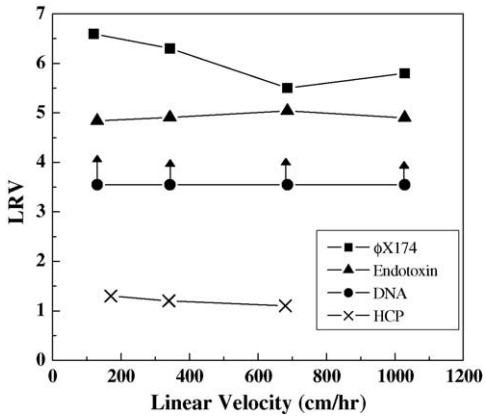

Fig. 3. Effect of linear velocity on ϕX174, DNA, endotoxin, and HCP retention. Upward arrows indicate that DNA was not detectable in the filtrate. The following experimental conditions were used: HS-DNA challenge solution at 1 μg/mL in 25 mM Tris buffer, pH 8.0; 2000 EU/mL endotoxin in 25 mM Tris buffer, pH 8.1; approximately 1.5×10^7 pfu/mL ϕX174 bacteriophage in 25 mM Tris buffer, pH 8.1; between 700 and 1000 ng/mL HCP in 3 g/L MAb/25 mM Tris buffer, pH 8.0 at a conductivity between 4 and 7.5 mS/cm. Challenge volumes for the small scale devices were 1000 mL of HS-DNA, 300 mL of endotoxin, 300 mL of ϕX174 bacteriophage suspension, and 700 ml of HCP challenge.

ϕ6, we could not test outside of the range tested). Conversely, for ϕX174, greater than 4 LRV was observed at a pH greater than the p*I* of the virus (pH 6.7), and minimal retention was observed at pH values below the p*I*. Fig. 2 shows the effect of NaCl concentration on bacteriophage removal. Again, ϕ6 removal was unaffected by change of NaCl concentration between 0 and 200 mM, and ϕX174 removal falls from 6 LRV to less than 1 LRV between 0 and 50 mM NaCl. The sensitivity of ϕX174 retention to high salt and to pH values below the p*I* is consistent with an ion exchange mechanism and is similar to that observed of ion-exchange chromatography of proteins.

The devices were also challenged with three mammalian viruses at two different salt concentrations (Table 2). At 50 mM NaCl, the LRVs of all three mammalian viruses and ϕ6 were greater than 5.0. At this salt concentration, the removal of ϕX174 was less than 1 LRV, indicating that ϕX174 was not as strongly bound to the adsorber medium as opposed to the mammalian viruses. Therefore, of the five viruses evaluated, ϕX174 was the model virus chosen in the detailed characterization of membrane adsorber performance since it was the most sensitive to the changes in pH and conductivity and, therefore, most weakly bound.

Table 2
Log removal values (LRV) for bacteriophage and mammalian virus

Virus	LRV		
	0 mM NaCl	50 mM NaCl	150 mM NaCl
MuLV	N/D	>5.5	>4.36
MMV	N/D	>5.2	1.05
SV40	N/D	>5.8	4.55
ϕ6	6.5	5.8	6.3
ϕX-174	6.1	1.1	N/D

Bacteriophage and mammalian virus tests were performed in 25 mM Tris, pH 7.5 and the indicated concentration of NaCl. Feed samples were taken at the beginning. Each separate experiment consisted of processing 150 mL of virus solution through a small scale device and taking a permeate pool sample. N/D represents conditions that were not done.

Three additional process or solution characteristics relevant to the robustness of anion exchange (AEX) removal are linear velocity, volume processed per unit of AEX medium and protein concentration. These performance characteristics were tested with ϕX174. Fig. 3 shows that the membrane adsorber consistently provides a ϕX174 LRV of at least 5 at linear velocities ranging from 10 to 60 mL/min or 170 to 1030 cm/h (flux rates of 1700–10,000 L/(m^2 h)) through a small scale device. Fig. 4 shows that ϕX174 LRV is independent of process volume, up to 4000 mL through the small scale device (1143 mL/cm^2). Lastly, Fig. 5 shows that performance is unaffected by the presence of MAb (p*I* 9.1, 150 kD) at concentrations of 13 g/L or lower. In order to achieve these levels of removal, it is imperative that the solution conditions are selected such that the MAb does not competitively bind to the matrix and the virus does not bind to the MAb through ionic interactions. This can be prevented by setting the solution conditions to a pH that is slightly below the MAb p*I* (ca. <0.5 pH unit below the p*I* of the antibody).

3.2. Biomolecular clearance

DNA, endotoxin and HCP removal were characterized for sensitivity to pH (Fig. 1), ionic strength (Fig. 2), and linear velocity (Fig. 3). DNA and endotoxin removal was also characterized for sensitivity to MAb concentration

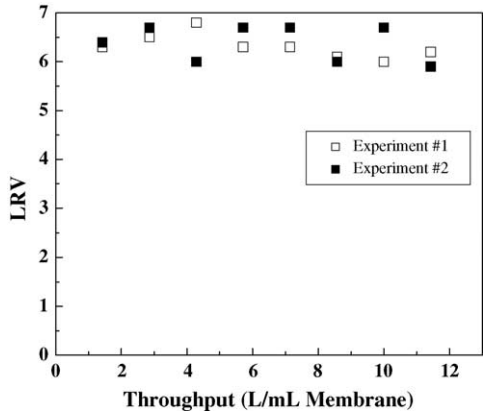

Fig. 4. Effect of throughput on ϕX174 retention. Approximately 1.5×10^7 pfu/mL of ϕX174 bacteriophage was spiked into 25 mM Tris buffer, pH 8.1, conductivity = 1.5 mS/cm. The challenge flow rate for the small scale was set at 20 mL/min (340 cm/h), and the challenge volume was 4 L.

(Fig. 5). Removal of DNA and of endotoxin was insensitive to pH over a range of 4–8. Removal of HCP was somewhat sensitive to pH, showing a 0.5 LRV increase at pH 9 versus pH 7–8. Removal of DNA and endotoxin was insensitive to ionic strengths up to 300 mM NaCl and 150 mM NaCl, respectively. Removal of HCP was sensitive to ionic strength. HCP was reduced to undetectable levels (below 10 ng/mL) at NaCl concentrations of 32 mM and lower, and was reduced by 1 LRV at 50 mM NaCl or greater. Removal of all three impurities was insensitive to changes in linear velocity. Lastly, removal of endotoxin and DNA was insensitive to the presence of MAb at concentrations less than 13 g/L.

With the exception of the flow rate sensitivity data, these results are consistent with performance of standard AEX chromatography. In standard bead-based AEX chromatography, removal efficiency typically decreases as the flow rate is increased.

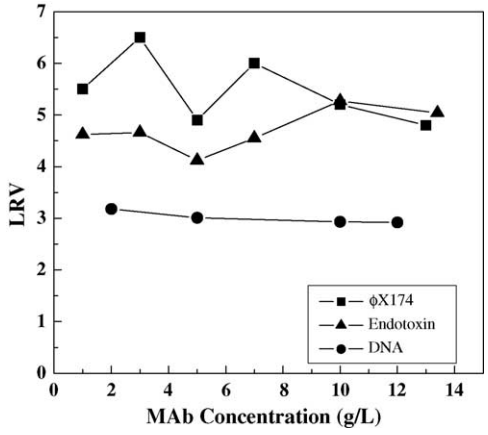

Fig. 5. Effect of MAb concentration on ϕX174, DNA, and endotoxin retention. The following experimental conditions were used: HS-DNA challenge solution at 1 μg/mL in 25 mM Tris buffer, pH 8.0; 2000 EU/mL endotoxin in 25 mM Tris Buffer, pH 8.1; approximately 1.5×10^7 pfu/mL ϕX174 bacteriophage in 25 mM Tris buffer, pH 8.1. Challenge flow rates were 20 mL/min (340 cm/h) for the small scale devices, challenge volumes for the small scale devices were 1000 mL of HS-DNA, 300 mL of endotoxin, and 300 mL of bacteriophage suspension.

3.3. Reproducibility and scalability

Permeability and dynamic binding capacity, standard membrane characterization tests, were performed on devices of three different scales: small scale, 2- and 6-stack. Each device lot contained the same lot of derivatized membrane. Permeability is a measure of membrane porosity and provides an indication of the expected range of process flow rates. The data in Fig. 6 show that the permeability is independent of device configuration or scale.

Dynamic binding capacity provides an indication of the consistency with which membrane is derivatized with the quaternary amine ligand and the uniformity of flow distribution within the device. A small indicator molecule, *N*-tosyl-L-glutamic acid, was used in the dynamic binding capacity studies (Fig. 7). Due to the much higher diffusion coefficient associated with small molecules, the resulting breakthrough curves with *N*-tosyl-L-glutamic acid are much sharper than those of larger molecules, such as proteins. Thus, the sensitivity for detecting device defects is significantly enhanced (i.e. the noise for the signal/noise ratio has been dramatically reduced). The breakthrough curves are characterized by a sharp transition from zero to complete breakthrough, indicating uniform utilization of the available binding sites. The very sharp breakthrough curves are an indication of a highly efficient device. The efficiency is a function of both the media (tight pore size distribution) and device (uniform flow distribution). This performance characteristic occurs independently of membrane lot and device configuration. The location of the curves indicates that binding capacity is likewise independent of device size and configuration or scale.

Removal of four trace impurity models, DNA, endotoxin, ϕX174, and ϕ6 was evaluated on three lots of small scale

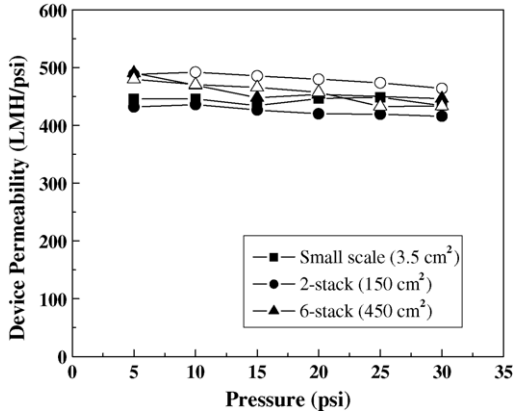

Fig. 6. Relationship between pressure and device water permeability for three sizes of membrane adsorber devices. The coefficient of variation for these data is 10%. The open circle and triangle represent repeats of the 2- and 6-stack experiments. DI water (at 23 °C) was used.

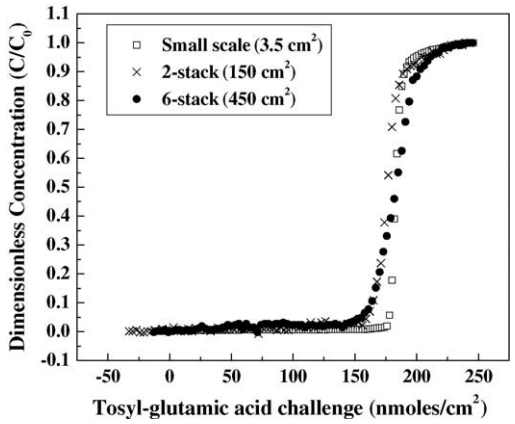

Fig. 7. Effect of membrane adsorber device size on the dynamic breakthrough curves of tosyl glutamic acid. Tosyl glutamic acid binding capacity had inter-lot variability of less than 5% and intra-lot variability of less than 2% (data not shown). The solution was made up of 50 μg/mL of tosyl glutamic acid in 2.5 mM Tris buffer, pH 8.0. The challenge flow rate was at 10 mL/min (171 cm/h) for the small scale devices and 260 mL/min (104 and 35 cm/h) for the 2- and 6-stacks.

devices, each of which contained a different lot of membrane. The data from these tests (Fig. 8) indicate that trace impurity removal is consistent among the three membrane lots. This is further evidence of the consistency of device performance and manufacturability.

Lastly, HCP removal capabilities of two small scale devices and 6-stack capsules were evaluated. The small scale and 6-stack devices performed comparably at two conductivities (Fig. 9), one of which led to full retention (4.0 mS/cm) and one of which led to partial HCP retention (7.5 mS/cm).

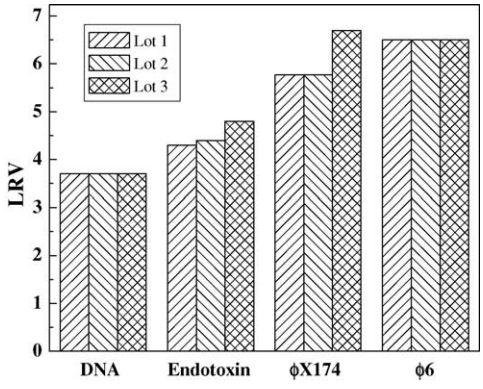

Fig. 8. Reproducibility of model impurity removal by three different lots of device membrane. Coefficients of variation for the endotoxin and ϕX174 data are 9% and 8%, respectively. Coefficients of variation for the DNA and ϕ6 data are not presented because neither DNA nor ϕ6 was detected in the filtrates. The following experimental conditions were used: HS-DNA challenge solution at 1 μg/mL in 25 mM Tris buffer, pH 8.0; 2000 EU/mL endotoxin in 25 mM Tris buffer, pH 8.1; approximately 1.5×10^7 pfu/mL ϕX174 bacteriophage in 25 mM Tris buffer, pH 8.1. Challenge flow rates were 20 mL/min (340 cm/h) for the small scale devices, challenge volumes for the small scale devices were 1000 mL of HS-DNA, 300 mL of endotoxin, and 300 mL of ϕX174 bacteriophage suspension.

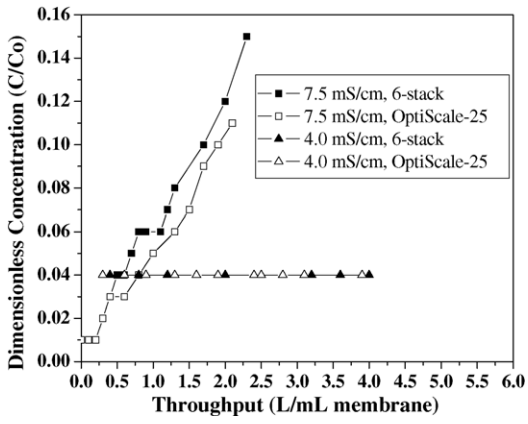

Fig. 9. Effect of scale on HCP removal. Dimensionless concentration (C/C_0) is a ratio of the HCP concentration in the filtrate to that in the load. Throughput on the device is litres of MAb load solution per millilitres of device membrane. The MAb load solution was conditioned to pH 8.0 and contained 3 g/L MAb and 1000 ng/mL HCP for the 7.5 mS/cm case (square symbols) and contained 1.5 g/L MAb and 230 ng/mL HCP for the 4 mS/cm case (triangular symbols). The solution was loaded at 3 L/min (340 cm/h) for both device scales. Both plots reflect equivalent MAb loading on a mass basis. The detection limit of the assay is 10 ng/mL HCP ($C/C_0 = 0.04$ for 4 mS/cm data and $C/C_0 = 0.01$ for 7.5 mS/cm data).

4. Conclusion

Under solution conditions common in antibody manufacturing, the membrane adsorber devices provided log retention values of >3 for DNA, >4 for endotoxin, >4 for virus, and >1 for host cell protein. To achieve >1 log removal of host cell protein removal, the load to the membrane adsorber was conditioned to pH 8.0 and a conductivity of <4.0 mS/cm. However, at pH 8.0 and a conductivity of 7.5 mS/cm, the membrane adsorber did not achieve >1 log removal of host cell protein clearance. Host cell protein represents a range of proteins each with a different specific binding affinity and equilibrium constant for the derivatized membrane. Thus, to utilize membrane adsorber devices effectively for host cell protein removal in a similar fashion that optimizes standard chromatography processes, one must either lower the conductivity of the protein solution (i.e. increase equilibrium binding constant) or increase the volume of membrane used in the process (i.e. increase the number or size of devices). By measurement of permeability, dynamic ion exchange capacity and impurity retention, the small scale device performance is fully scalable to that of the larger format capsules.

5. Proprietary names

WinKQCL is a registered trademark of Biowhittaker Technologies, Inc.
ATCC is a registered trademark of American Type Culture Collection.
BioCAD is a registered trademark of PerSeptive Biosystems, Inc.
SpectraFLUOR is a trademark of Tecan Group AG.
Foxy is a registered trademark of Isco, Inc.

Acknowledgements

The authors wish to acknowledge the individuals who participated in the bacteriophage (especially Jessica Shaw for her contributions) and mammalian virus assays, the DNA (especially Marta Portoles for her contributions) and endotoxin assays, and Analytical Operations of Genentech, Inc. for the HCP assays.

References

[1] J. Thommes, M.R. Kula, Biotechnol. Prog. 11 (1995) 357.
[2] P. Sridhar, Chem. Eng. Technol. 19 (1996) 398.
[3] D.K. Roper, E.N. Lightfoot, J. Chromatogr. A 702 (1995) 3.
[4] R. Ghosh, J. Chromatogr. A 952 (2002) 13.
[5] R.R. Deshmukh, T.N. Warner, F. Hutchison, M. Murphy, W.E. Leitch, P. De Leon, G.S. Srivatsa, D.L. Cole, Y.S. Sanghvi, J. Chromatogr. A 890 (2000) 179.
[6] C.S. Rao, Process Biochem. 37 (2001) 247.
[7] J.A. Gerstner, R. Hamilton, S.M. Cramer, J. Chromatogr. 596 (1992) 173.
[8] T.B. Tennikova, B.G. Belenkii, F. Svec, J. Liq. Chromatogr. 13 (1990) 63.
[9] M.B. Tennikov, N.V. Gazdina, T.B. Tennikova, F. Svec, J. Chromatogr. A 798 (1998) 55.
[10] T.B. Tennikova, F. Svec, J. Liq. Chromatogr. 646 (1993) 279.
[11] F.T. Sarfert, M.R. Etzel, J. Chromatogr. A 764 (1997) 3.
[12] S.Y. Suen, M.R. Etzel, J. Chromatogr. A 686 (1994) 179.
[13] M.A. Teeters, T.W. Root, E.N. Lightfoot, J. Chromatogr. A 944 (2002) 129.
[14] H.L. Knudsen, R.L. Fahrner, Y. Xu, L.A. Norling, G.S. Blank, J. Chromatogr. A 907 (2001) 145.
[15] R. Van Reis, A. Zydney, Curr. Opin. Biotechnol. 12 (2001) 208.
[16] H.R. Charlton, J.M. Relton, K.H.N. Slater, Bioseparation 8 (1999) 281.
[17] F.B. Anspach, D. Petsch, Process Biochem. 35 (2000) 1005.
[18] H. Brough, C. Antoniou, J. Carter, J. Jacubik, Y. Xu, H. Lutz, Biotechnol. Prog. 18 (2002) 782.
[19] P. Payment, M. Trudel (Eds.), Methods and Techniques in Virology, Marcel Dekker, New York, 1993.