ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Development of a Polishing Step Using a Hydrophobic Interaction Membrane Adsorber With a PER.C6® -Derived Recombinant Antibody

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Received 12 June 2009; revision received 28 August 2009; accepted 2 September 2009

Published online 8 September 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/bit.22538

ABSTRACT: Membrane chromatography has already proven to be a powerful alternative to polishing columns in flow-through mode for contaminant removal. As flow-through utilization has expanded, membrane chromatography applications have included the capturing of large molecules, including proteins such as IgGs. Such bind-and-elute applications imply the demand for high binding capacity and larger membrane surface areas as compared to flow-through applications. Given these considerations, a new Sartobind Phenyl™ membrane adsorber was developed for large-scale purification of biomolecules based on hydrophobic interaction chromatography (HIC) principles. The new hydrophobic membrane adsorber combines the advantages of membrane chromatography—virtually no diffusion limitation and shorter processing time—with high binding capacity for proteins comparable to that of conventional HIC resins as well as excellent resolution. Results from these studies confirmed the capability of HIC membrane adsorber to purify therapeutic proteins with high dynamic binding capacities in the range of 20 mg-MAb/cm^3-membrane and excellent impurity reduction. In addition the HIC phenyl membrane adsorber can operate at five- to ten-fold lower residence time when compared to column chromatography. A bind/elute purification step using the HIC membrane adsorber was developed for a recombinant monoclonal antibody produced using the PER.C6® cell line. Loading and elution conditions were optimized using statistical design of experiments. Scale-up is further discussed, and the performance of the membrane adsorber is compared to a traditional HIC resin used in column chromatography.

Biotechnol. Bioeng. 2009;xxx: xxx–xxx.

KEYWORDS: membrane adsorber; hydrophobic interaction chromatography (HIC); purification; downstream processing; PER.C6 cells

Introduction

The traditional downstream processing of therapeutic proteins is built on packed bed column chromatography as the main purification agent due to its simplicity and high resolution (Curling and Gottschalk, 2007). The most commonly used chromatography techniques are ion exchange chromatography, affinity chromatography, and hydrophobic interaction chromatography (HIC). The resin chemistries and the sequence of the steps are selected to meet the objectives of the purification process in terms of selectivity, speed, recovery, and capacity. HIC is used to separate molecules based on differences in their hydrophobicity. The sample is loaded at high concentration of a lyotropic salt, promoting the interaction between the hydrophobic surface of the protein and the hydrophobic surface of the adsorbent (Tiselius, 1948). With increasing ionic strength, an increasing number of hydrophobic pockets are exposed on proteins due to the release of the ordered layer of water molecules surrounding the hydrophobic ligand and pockets of the protein, resulting in stronger binding. Most of the proteins adsorbed are effectively eluted by simply washing the HIC adsorbent with a low ionic strength buffer solution (Hjerten et al., 1974; Porath et al., 1973).

HIC is a useful technique for large-scale purification of recombinant proteins (Evans et al., 2008). It has been described as a powerful and indispensable tool for the purification of therapeutic proteins (Gagnon et al., 1995a,b; Gagnon and Grund, 1996a,b). HIC has also been presented as an efficient mode of removing dimers and higher molecular weight aggregates when used as a polishing step in a monoclonal antibody purification process (Li et al., 2005). Nevertheless, developing preparative HIC methods can be challenging. In particular, the flow rate and diffusion limitation associated with packed-bed HIC can increase the risk of protein denaturation due to long contact time on the

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hydrophobic surface and the high concentration of lyotropic salt around the protein, both of which can cause severe product losses (Jungbauer et al., 2005). The use of a convective chromatography technique, such as membrane chromatography, reduces these problems by allowing much faster processing time. Because the transport of the molecules to the binding sites occurs mainly by convection (while pore diffusion is minimal), the mass transfer resistance is reduced so that capture is rapid and largely independent of flow rate. Due to the hydrodynamic benefits, membrane chromatography technology involves much smaller devices than columns with a similar throughput. This can significantly reduce buffer consumption, processing time, and space requirements; flow-through membrane chromatography in particular can save up to 95% of buffer and 66% of process time as compared with traditional column chromatography (Zhou and Tressel, 2006). Furthermore, membrane adsorbers are available in a ready-to-use disposable capsule format that eliminates the need for packing and qualification or re-use validation at large scale (Gottschalk et al., 2004; Zhou et al., 2007). These features translate into reduced process time and complexity while adding flexibility to the manufacturing facility.

Advantages and performances of membrane chromatography have been well described in the literature (Becerra-Arteaga et al., 2008; Fraud, 2008; Ghosh, 2002; Lim et al., 2007; Mora et al., 2006). Several groups have reported on membranes for HIC (Kawai et al., 2003; Kim et al., 1991; Kubota et al., 1995, 1996). Ghosh and Wang had previously examined the feasibility of the purification of a humanized monoclonal antibody by hydrophobic interaction membrane chromatography using commercially available microporous membranes (Ghosh and Wang, 2006; Wang et al., 2006). The novel hydrophobic phenyl membrane adsorber used in the present study is the first designed specifically for this purpose and was recently introduced in articles from Liu et al. (2009) and Fraud et al. (2009).

Here we describe some basic properties of the new Sartobind Phenyl^{TM} membrane adsorber using model protein systems and a case study for the purification of a monoclonal IgG_1 expressed using the PER.C6® human cell line. Process development data generated through the use of statistical design of experiments was used to identify critical parameters for the phenyl membrane and optimize loading and elution conditions for delivering high capacity, yield, and resolution. The binding capacity and selectivity of the hydrophobic phenyl membrane adsorber were found to be comparable to those of a conventional HIC resin. Scale-up is further discussed.

Materials and Methods

The model proteins cytochrome *c*, ribonuclease A, lysozyme, α-chymotrypsinogen A, β-lactoglobulin, ovalbumin, and γ-globulin were all purchased from Sigma–Aldrich (St. Louis, MO). All proteins were prepared as stock solutions (25 mg/cm^3) in 0.9% sodium chloride, diluted into the appropriate buffer system, and filtered through a 0.2 μm membrane before use.

For the industrial case study, a human monoclonal antibody (IgG_1, p$I = 8.3$, 150 kDa) was produced at PERCIVIA, LLC using the PER.C6 cell line in a fed-batch process with chemically defined growth medium. PER.C6 cells are human embryonic retinal cells immortalized by the adenovirus E1 gene as described in US Patent 5,994,128 (Fallaux et al., 1999). The crude media was clarified by centrifugation at 15,000*g* followed by depth filtration and sterile filtration. The clarified media was partially purified by column chromatography. The partially purified material contained about 1.0–1.5% aggregate and about 11 μg-HCP/mg-MAb.

The Sartobind Phenyl hydrophobic interaction membrane adsorber was provided by Sartorius Stedim Biotech (Goettingen, Germany). The membrane adsorber is based on hydrophilic regenerated stabilized cellulose with the hydrophobic phenyl groups covalently attached to the cellulose matrix. This membrane is assembled into a 30-layer radial flow capsule. Scale-down "Nano" devices had 3 cm^3 of membrane volume and the pilot scale device had 150 cm^3 of membrane volume.

The Toyopearl PPG-600M and Phenyl-650M HIC resins used in this work were purchased from Tosoh Bioscience (Montgomeryville, PA). For the model protein work, both resins were used in the pre-packed 1 cm^3 Toyoscreen format. For the industrial case study, the PPG-600M was packed in an XK16 column (GE Healthcare Life Sciences, Piscataway, NJ) with a bed height of 4.8 cm (bed volume of 9.65 cm^3). High-Sub and Low-Sub Phenyl Sepharose FF were purchased from GE Healthcare Life Sciences in the pre-packed 1 cm^3 HiTrap format.

All small-scale chromatography experiments were carried out with an ÄKTA Explorer 100 (GE Healthcare Life Sciences), and the scale-up experiment was performed with a BioProcess chromatography system (GE Healthcare Life Sciences).

USP grade dibasic and monobasic sodium phosphate, ammonium sulfate, acetic acid, and sodium hydroxide were purchased from JT Baker (Phillipsburg, NJ). ACS grade ethanol was also purchased from JT Baker. Reagent grade ethylene glycol was purchased from BDH Chemicals (Dorset, UK). All buffers were prepared using Milli-Q-grade water (Millipore, Billerica, MA) and were filtered by 0.22 μm filtration before use. The equilibration buffers contained 50 mM sodium phosphate at pH 6.5–7.5 with 0.7–0.9 M ammonium sulfate. The elution buffers were 50 mM sodium phosphate, pH 6.5–7.5 with 0.10–0.25 M ammonium sulfate. The strip buffers were 50 mM sodium phosphate, pH 6.5–7.5, and the regeneration buffer was 50% ethylene glycol. Cleaning in place (CIP) was performed with 0.5 M sodium hydroxide. Load material was prepared by diluting the partially purified MAb with Milli-Q water and then adding a 2× concentrate of the equilibration buffer gradually while mixing until the conductivity of the load

material matched that of the equilibration buffer. The pH was adjusted, if needed, by addition of 10% acetic acid.

Minitab software (Minitab, Inc., State College, PA) was used to plan and analyze the full-factorial loading and elution studies. In both studies, two factors—ammonium sulfate concentration and pH of the loading and elution buffers—were tested at three levels each, and the run order was randomized.

Model Protein Separations

Two model protein mixtures were used. Protein mixture A was composed of 3 mg/cm^3 cytochrome *c*, 6 mg/cm^3 ribonuclease A, 3 mg/cm^3 lysozyme, and 6 mg/cm^3 α-chymotrypsinogen. Protein mixture B was composed of 3 mg/cm^3 cytochrome *c*, 6 mg/cm^3 ribonuclease A, 3 mg/cm^3 lysozyme and 12 mg/cm^3 β-lactoglobulin. Both mixtures were formulated in 1.7 M ammonium sulfate and 50 mM potassium phosphate buffer, pH 7.0 (equilibration buffer). All steps were performed at flow rate of 1 cm^3/min at ambient temperature. The membrane adsorber and the resins were equilibrated with 10 cm^3 equilibration buffer. After equilibration, a 0.25 cm^3 pulse of one of the protein mixtures was injected via a sample loop, and the units were washed with 2 cm^3 of equilibration buffer. A linear gradient from equilibration buffer to 50 mM potassium phosphate buffer, pH 7.0 was performed in 10 cm^3 followed by 10 cm^3 of 50 mM potassium phosphate buffer, pH 7.0.

Protein solutions containing 1 mg/cm^3 lysozyme in 1.5 M ammonium sulfate in 50 mM potassium phosphate buffer, pH 7.0, 1 mg/cm^3 ovalbumin in 1.5 M ammonium sulfate in 50 mM potassium phosphate buffer, pH 7.0, and 1 mg/cm^3 γ-globulin in 0.9 M ammonium sulfate in 50 mM potassium phosphate buffer, pH 7.0 were used to determine the dynamic binding capacities of various HIC adsorbents. All steps were performed at flow rate of 10 cm^3/min with the membrane adsorber and 1 cm^3/min with the resins at ambient temperature. The membrane adsorber and the resins were equilibrated with 30 cm^3 of equilibration buffer. Loading was performed with 150 cm^3 of the respective protein solution. Washing was performed with 30 cm^3 equilibration buffer. Elution was performed by applying a linear gradient from equilibration buffer to 50 mM potassium phosphate buffer, pH 7.0 in 60 cm^3.

Loading Study

Chromatography experiments in the loading study were carried out as follows: The adsorber was first flushed with equilibration for 10 membrane volumes at a residence time of 18 s (10 cm^3/min). The load material was then applied at the same residence time until 10% breakthrough was observed by monitoring the UV absorbance at 280 nm. The adsorber was then washed with 30 membrane volumes of equilibration buffer. Elution was carried out in a single step at 0.17 M ammonium sulfate in sodium phosphate, pH 7.0 for 20 membrane volumes. After stripping and regeneration, the membrane was sanitized with 10 membrane volumes of sodium hydroxide at a residence time of 60 s (3 cm^3/min), neutralized with equilibration buffer, and stored in 20% ethanol.

Elution Study

In the elution study equilibration was performed with 50 mM sodium phosphate pH 7.0 containing 0.75 M ammonium sulfate for 10 membrane volumes at a residence time of 18 s. The load material was then applied at the same residence time to a loading of 15 mg-MAb/cm^3-membrane. The adsorber was then washed with 20 membrane volumes of equilibration buffer. Elution was carried out in a single step with 20 membrane volumes. The adsorber was then stripped with 50 mM sodium phosphate for 10 membrane volumes and regenerated with 50% ethylene glycol for 10 membrane volumes at a residence time of 36 s. Sanitization and storage were performed as previously described.

Cycling Study

For the cycling study, the adsorber was first flushed with equilibration buffer—50 mM sodium phosphate pH 7.0 containing 0.75 M ammonium sulfate—for 10 membrane volumes at a residence time of 18 s. The load material was then applied at the same residence time until 10% breakthrough was observed by monitoring the UV absorbance at 280 nm. The adsorber was then washed with 30 membrane volumes of equilibration buffer. Elution was carried out in a single step using 50 mM sodium phosphate pH 7.0 with 0.17 M ammonium sulfate for 20 membrane volumes. The adsorber was then stripped with 50 mM sodium phosphate pH 7.0 for 10 membrane volumes and regenerated with ethylene glycol for 10 membrane volumes at a residence time of 36 s. Finally, the membrane was sanitized with 10 membrane volumes of sodium hydroxide at a residence time of 180 s, neutralized with stripping buffer, and stored in 20% ethanol.

Scale-Up

The Sartobind Phenyl process was scaled up from a 3 cm^3 Sartobind Nano to a 150 cm^3 pilot scale device manufactured with the same lot of membrane. The devices were operated under identical conditions with respect to buffers, load material, and residence time. In the first experiment, both devices were equilibrated with 10 membrane volumes of buffer, loaded to 10% breakthrough, washed for 20 membrane volumes with equilibration buffer, and eluted for 20 membrane volumes, all at a residence time of 18 s. Next the adsorbers were regenerated with 10 membrane volumes of 20% ethanol at a residence time of 36 s, sanitized with 10 volumes of 0.5 M NaOH at a residence time of 60 s,

and neutralized with five membrane volumes of 50 mM sodium phosphate, pH 7.0. In the second experiment, the method was similar except that the devices were loaded to 16 mg-MAb/cm^3-membrane and the wash was shortened to 10 membrane volumes.

The current column chromatography resin used in the purification of this antibody was run side-by-side with the membrane adsorber. Optimized conditions were used for each operation.

Analytical Techniques

The concentration of the MAb was determined spectrophotometrically. Aggregate levels were measured by size exclusion chromatography (SEC) using a TSKgel G3000SWXL column from Tosoh Bioscience and a Waters 2695 separations module (Milford, MA) with peak detection by UV absorbance at 280 nm. Host cell proteins were quantified by a proprietary ELISA at the DSM Groningen QC laboratory.

Product recovery was determined by Equation (1)

$$\text{Recovery} = \frac{\text{mass of MAb in elution}}{\text{mass of MAb applied to column}} \quad (1)$$

For experiments in which the column or membrane was loaded to breakthrough, yield was determined by Equation (2)

$$\text{Yield} = \frac{\text{mass of MAb in elution}}{\text{mass of MAb applied to column} - \text{mass of MAb not bound to the column}} \quad (2)$$

The reduction of HCPs was calculated as follows:

$$\text{Reduction of HCP} = 1 - \frac{(\mu\text{g of HCP/mg of MAb}) \text{ in elution}}{(\mu\text{g of HCP/mg of MAb}) \text{ in load}} \quad (3)$$

Similarly, the reduction of aggregates was calculated according to the formula below:

$$\text{Reduction of aggregate} = 1 - \frac{\%\text{ aggregate in elution}}{\%\text{ aggregate in load}} \quad (4)$$

Results and Discussion

Model Protein Separations

To compare the separation properties of the selected resins with the membrane adsorber, two model protein mixtures were used. Small pulses of these mixtures were injected onto the Sartobind Phenyl membrane adsorber and four commercially available HIC resins and eluted by a linear gradient. The elution chromatograms are shown in Figure 1. For both mixtures, the Sartobind Phenyl demonstrated better resolution than the Sepharose and Toyopearl resins. Interestingly, the order in which the proteins eluted in the linear gradient differs between the membrane adsorber and the resins. The cytochrome *c* and ribonuclease A eluted in the same order for the membrane and resins, but the lysozyme eluted last from the membrane adsorber whereas it eluted from the columns before the α-chymotrypsinogen (mixture A) or the β-lactoglobulin (mixture B). This behavior might be explained by the differences in mass transport between membrane adsorbers and resins, differences in the way in which the HIC ligands are attached, or by different unfolding of the proteins on the adsorbents (Hahn et al., 2003; Lienqueo and Mahn, 2005). It is possible that better resolution could be obtained for the resins if longer columns were employed.

The dynamic binding capacity at 10% breakthrough was determined for the phenyl membrane adsorber and the resin columns for three different proteins. The results are summarized in Table I. In all cases, the binding capacity at 10% breakthrough of the Sartobind Phenyl was at least as good as the resins. It is typically expected that membranes will have lower binding capacities than resins due to the lower number of available binding sites; it is likely that given longer residence times, the capacities of the resins would in fact be higher.

The influence of the length of the gradient and the flow rate on the performance of a 3 cm^3 Nano unit was investigated using the model protein mixture B. The linear gradient volume was increased from 10 to 50 cm^3 as shown in Figure 2. Only for the shortest gradient was there a negative impact on resolution; when the gradient lengths were 30 and 50 cm^3, the resolutions were qualitatively equivalent. Using a constant gradient length of 30 cm^3, the flow rate was varied from 1 to 5 cm^3/min (Fig. 3). The minimal diffusion limitation is evident by the similarities of the chromatograms at all three flow rates tested.

Industrial Case Study for a Monoclonal IgG_1

Loading Study

Previous scouting studies (data not shown) determined appropriate binding conditions in the range of 0.7–0.9 M ammonium sulfate in neutral sodium phosphate buffer (data not shown). To further characterize the performance of the Sartobind Phenyl membrane, a statistical design of experiments was undertaken to explore the effect of the salt concentration and pH of the loading buffer on the capacity,

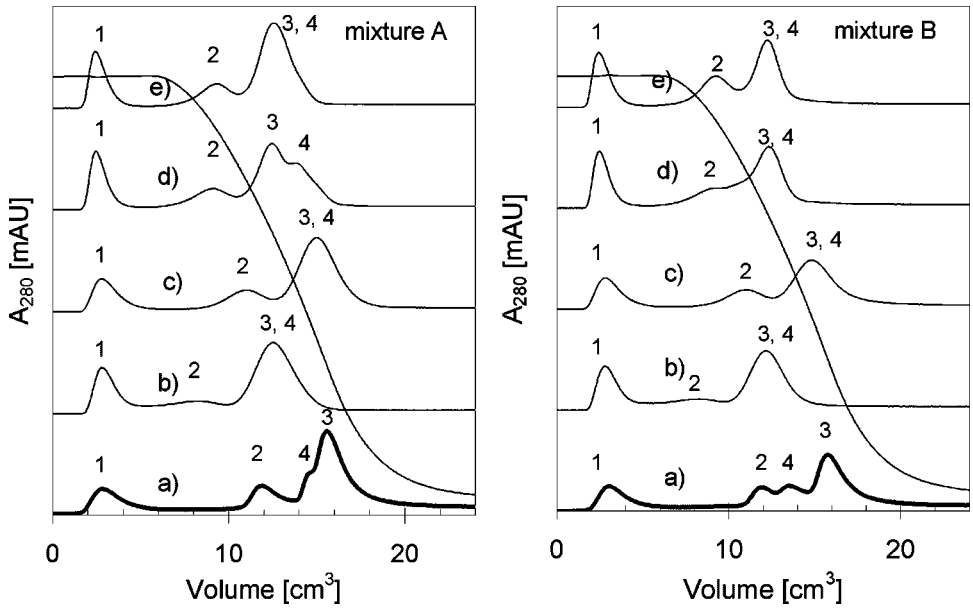

Figure 1. Separation of two mixtures of model proteins using different HIC media. Mixture A (**left**): 1, cytochrome *c*; 2, ribonuclease A; 3, lysozyme; 4, α-chymotrypsinogen. Mixture B (**right**): 1, cytochrome *c*; 2, ribonuclease A; 3, lysozyme; 4, β-lactoglobulin. The media used were Sartobind Phenyl (**a**), Phenyl FF low sub (**b**), Phenyl FF high sub (**c**), Toyopearl Phenyl-650M (**d**), and Toyopearl PPG-600M (**e**). The retention volumes are normalized to the non-binding cytochrome *c* tracer.

yield, and selectivity of the adsorber. Figure 4 shows the performance of the adsorber over the design space. The incipient breakthrough capacity for this MAb is higher than the 10% breakthrough capacity measured using polyclonal IgG (Table I), but the binding capacity of any adsorbent can vary significantly from one molecule to the next, even within the same class. Not surprisingly, the capacity increases with ammonium sulfate concentration. The capacity, however, must be balanced against the yield of the product, and the strongest binding conditions also represented the lowest yields. For all conditions tested, aggregate level in the eluate was $\leq$0.5%, corresponding to a reduction of $\geq$50% from the starting material. As observed with the antibody, higher salt conditions promoted stronger binding of HCPs reducing product purity in the eluate. The highest HCP clearance was observed in the range of 0.7–0.8 M ammonium sulfate at pH 6.5–7.0; under these process conditions, HCP clearance was $\geq$60%, from 11 μg-HCP/mg-MAb in the starting material to 4 μg-HCP/mg-MAb in the elution pool. Based on the results of the loading study, 0.75 M ammonium sulfate in 50 mM sodium phosphate at a pH of 7.0 was selected as the optimal loading condition to maximize capacity (~21 mg-MAb/cm^3-membrane), yield (~85%), and impurity levels in the eluate (0.25% aggregate and 4 μg-HCP/mg-MAb).

Table I. Dynamic binding capacities at 10% breakthrough for different HIC media challenged with three different model proteins.

Adsorbent	τ (s)	Protein	$DBC_{10\%}$ (mg/cm^3)
Sartobind Phenyl	18	γ-Globulin	17
		Ovalbumin	28
		Lysozyme	31
Phenyl Sepharose 6 Fast Flow (high sub)	60	γ-Globulin	15
		Ovalbumin	25
		Lysozyme	18
Phenyl Sepharose 6 Fast Flow (low sub)	60	γ-Globulin	5
		Ovalbumin	12
		Lysozyme	4
Toyopearl Phenyl-650M	60	γ-Globulin	7
		Ovalbumin	10
		Lysozyme	11
Toyopearl PPG-600M	60	γ-Globulin	12
		Ovalbumin	8
		Lysozyme	3

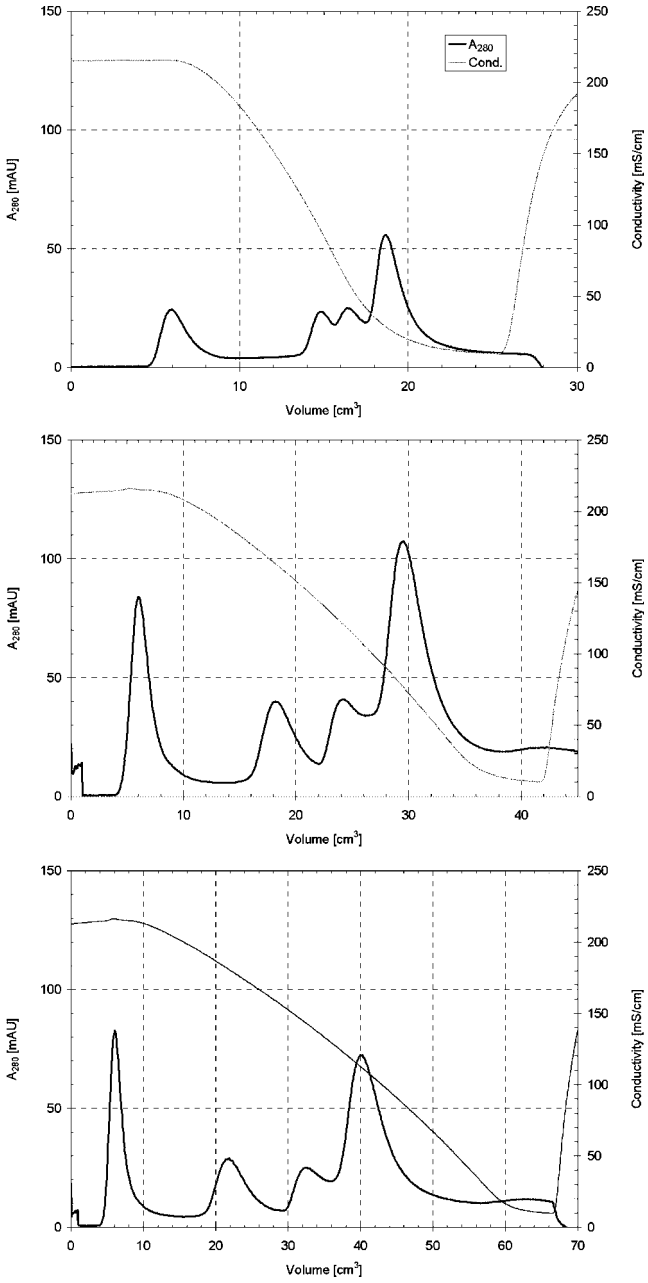

Figure 2. Separation of "mixture B" on Sartobind Phenyl using linear gradients of 10 (**top**), 30 (**middle**), and 50 cm^3 (**bottom**). In order of elution, the peaks are cytochrome *c*, ribonuclease A, lysozyme, and β-lactoglobulin.

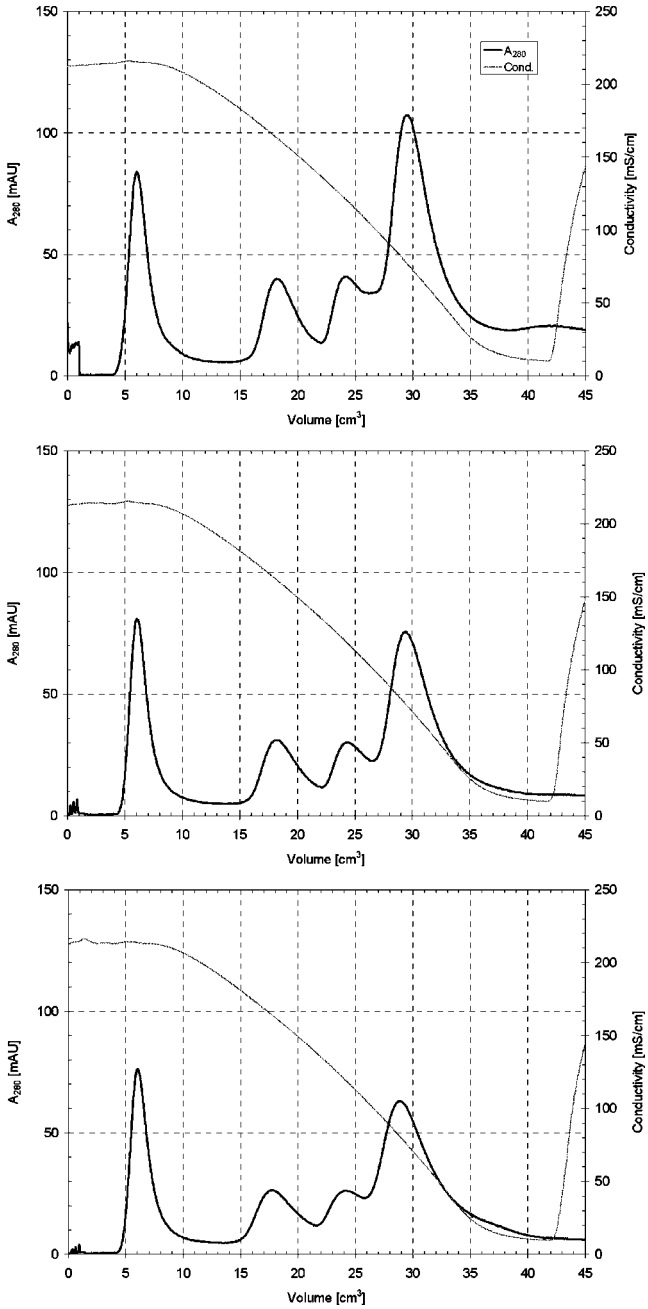

Figure 3. Separation of "mixture B" on Sartobind Phenyl using linear gradients at 1 (**top**), 2 (**middle**), and 5 cm^3/min (**bottom**). In order of elution, the peaks are cytochrome *c*, ribonuclease A, lysozyme, and β-lactoglobulin.

Elution Study

Using the optimized loading conditions, a second statistical design of experiments was carried out to determine the optimal elution conditions. For these experiments, the membrane was loaded to approximately 75% of the incipient breakthrough capacity, that is, 15 mg-MAb/cm^3-membrane. As shown in Figure 5, yield improved when lower salt levels were used for elution, from 80–84% at the highest salt level to 87–93% at the lowest salt level. It appears also that yield increased when the elution buffer was at a higher pH. As expected, decreasing the salt concentration in the elution buffer promoted the elution of aggregates, but the trend was not very strong. There was no clear trend with the amount of HCPs in the eluate. Based on yield and aggregate reduction, the center point of the DOE was selected as the target elution condition.

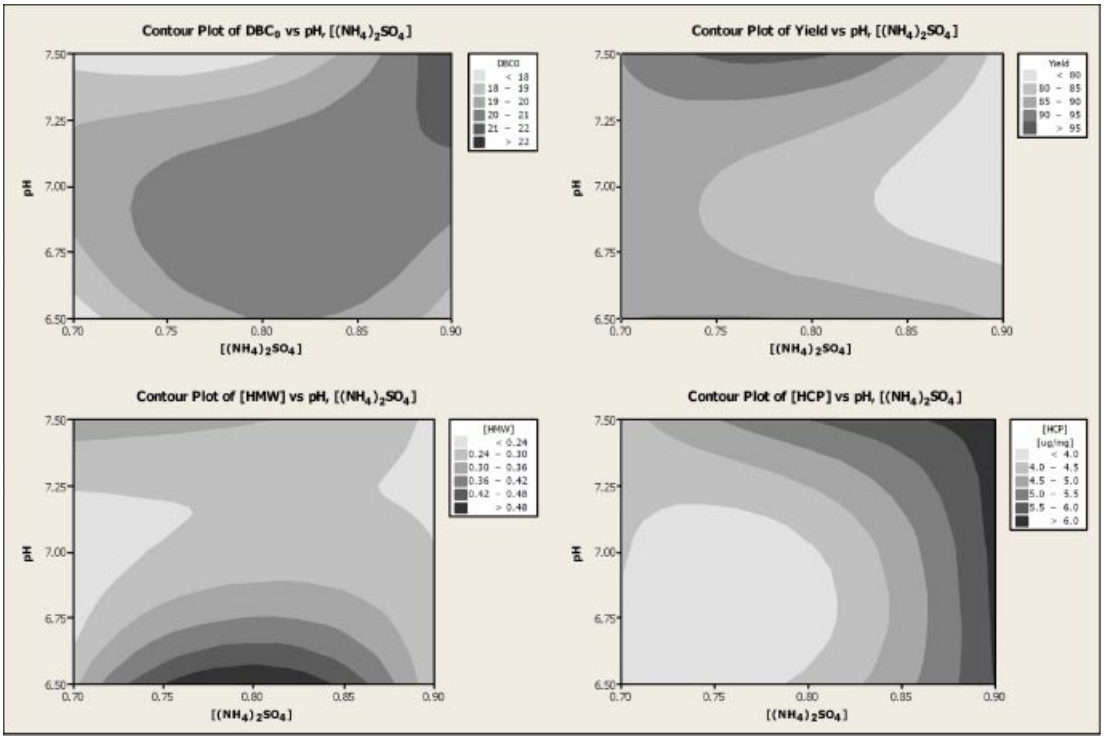

Figure 4. Performance of Sartobind Phenyl loaded under different conditions. **Top-left**: Dynamic binding capacity (in mg of MAb per cm^3 of membrane) at incipient breakthrough. **Top-right**: Yield (in per cent). **Bottom-left**: Aggregate ("HMW") content (in per cent) of the elution pool (starting material: 1.0%). **Bottom-right**: Host cell protein content (in μg of HCP per mg of MAb) of the elution pool (starting material: 11 μg-HCP/mg-MAb).

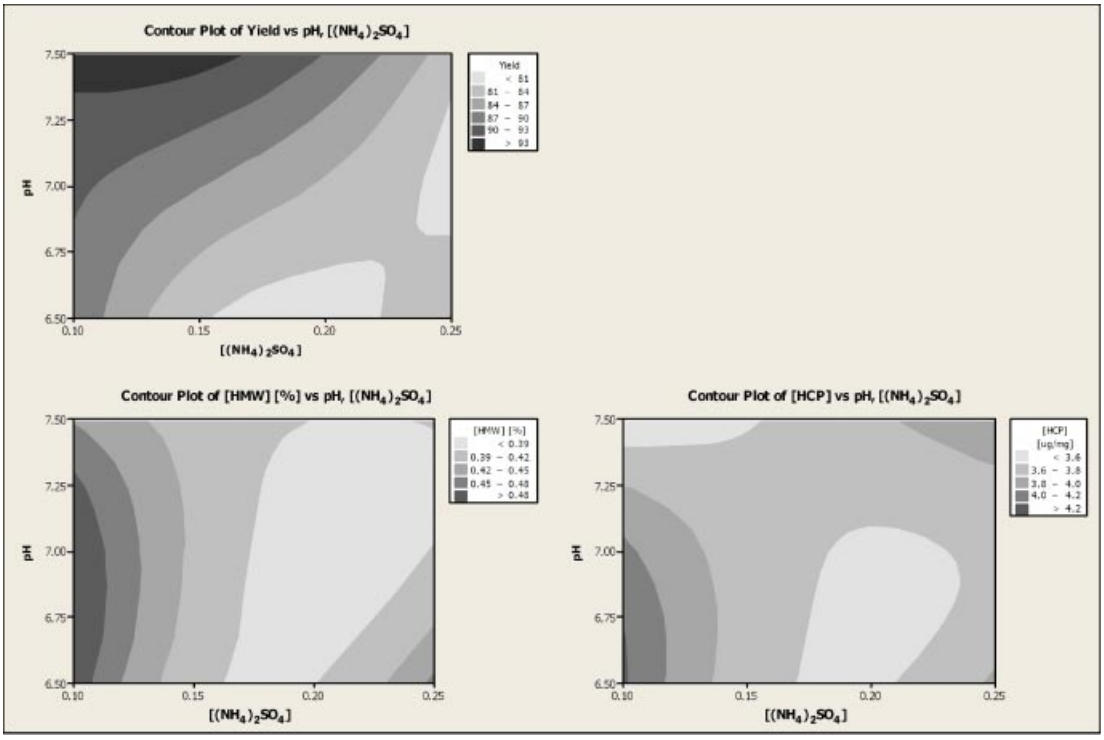

Figure 5. Performance of Sartobind Phenyl eluted under different conditions. **Top**: Yield (in per cent). **Bottom-left**: Aggregate ("HMW") content (in per cent) of the elution pool (starting material: 1.0%). **Bottom-right**: Host cell protein content (in μg of HCP per mg of MAb) of the elution pool (starting material: 11 μg-HCP/mg-MAb).

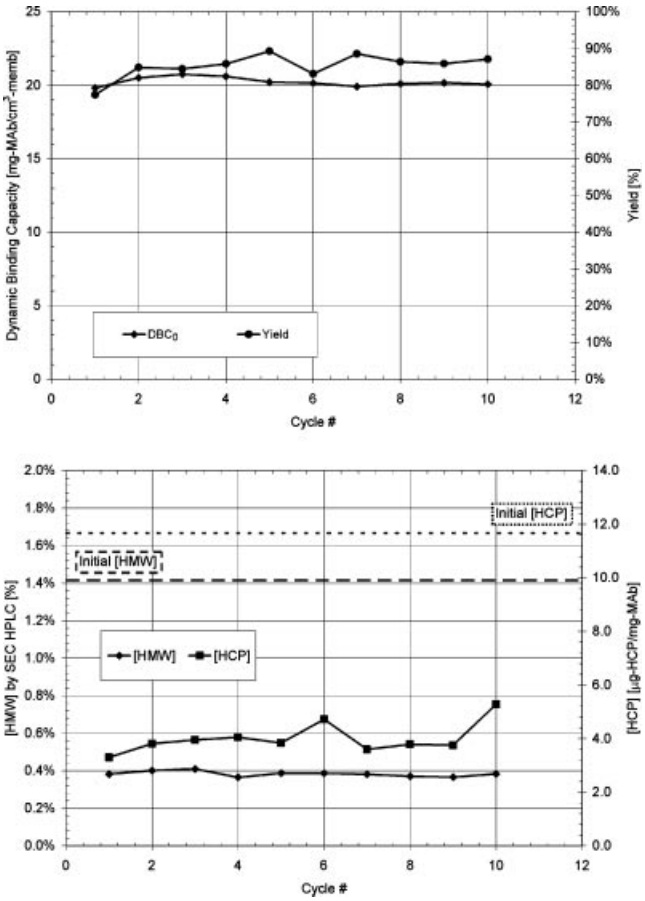

Figure 6. Performance of Sartobind Phenyl over 10 cycles. **Top**: Dynamic binding capacity at incipient breakthrough (DBC_0) and yield. **Bottom**: Aggregate (HMW) and HCP clearance.

Cycling Study

In order to best exploit the fast mass transfer characteristic of the Sartobind Phenyl membrane adsorber and maximize the throughput of this step, it is desirable to design the polishing step to run in multiple cycles rather than a single cycle. This would allow for smaller system and device sizing while maintaining a rapid processing time. The capacity data in Figure 6 demonstrate that incipient breakthrough occurred consistently around 20–21 mg-MAb/cm^3-membrane, suggesting that the membrane can be used for at least ten cycles. The breakthrough data is further supported by the yield and impurity data; the yield and elution impurity profiles were consistent across all ten cycles.

Scale-Up

The dynamic binding capacities for the two devices were within about 5% of each other (Table II). Furthermore, both devices showed similar performance in terms of recovery and removal of aggregates and HCP. These findings represent a successful 50-fold scale-up of the HIC membrane adsorber step.

Table II. Dynamic binding capacity, recovery, and elution impurity burden (aggregate and HCP) for Sartobind Phenyl at two scales.

	5″ Capsule MV = 150 cm^3	Nano- MV = 3 cm^3
DBC_0 (mg-MAb/cm^3-membrane)	19	18
DBC_{10} (mg-MAb/cm^3-membrane)	21	20
Recovery (%)	90	92
Elution [HMW] (%) (load = 0.9%)	0.4	0.4
Elution [HCP] (µg-HCP/mg-MAb) (load = 11 µg-HCP/mg-MAb)	2.3	1.6

Comparison of Membrane- and Resin-Based Processes

The Sartobind Phenyl membrane and Toyopearl PPG-600M resin showed comparable performance in terms of yield and removal of aggregates and HCPs (Table III). While the resin had twofold higher capacity, the membrane adsorber had a sixfold shorter cycle time.

Conclusions

Recent advances in vector technology, host cell lines, and cell culture conditions have resulted in extremely high titers and cell densities (Coco-Martin and Harmsen, 2008). The PER.C6 human cell line in particular has reported expression levels of over 10 g/L in fed-batch culture and 27 g/L in the XD process for a monoclonal antibody (Chon, 2009). These advances in titers have lead to new opportunities for the manufacturing of biologics including single-use technology bioreactors since smaller vessels can produce enough material not only for clinical studies but even for commercial batches. Downstream processing has lagged behind in incorporating these new single-use technologies primarily because of current membrane chromatography capacity limitations. While membranes have been accepted in flow-through applications, low

Table III. Comparison of the capacity, recovery, and impurity reduction of Sartobind Phenyl and Toyopearl PPG-600M.

	Sartobind Phenyl Nano 3 cm^3	Toyopearl PPG-600M XK16 9.65 cm^3
Usable capacity (mg-MAb/cm^3-membrane)	16	33
Recovery (%)	92	97
Elution [HMW] (%) (load = 0.9%)	0.4	0.7
Elution [HCP] (µg-HCP/mg-MAb) (load = 11 µg-HCP/mg-MAb)	1.6	2.1

binding capacities have prevented their use in bind/elute applications.

The Sartobind Phenyl membrane adsorber presents a new opportunity in downstream processing: the ability to purify a recombinant therapeutic protein in a bind/elute mode using a membrane adsorber at industrially relevant scales. The HIC membrane has a dynamic binding capacity comparable to currently available HIC resins used in many processes and shows excellent resolution, both with model proteins and a recombinant monoclonal antibody produced using the PER.C6 human cell line. In the present work, a usable dynamic binding capacity of 16 mg/cm^3 was achieved with yields of 90%, HCP reduction of approximately 80% and final aggregate levels below 1% for a monoclonal IgG_1. The antibody used in this work is quite stable and therefore the aggregate challenge to the adsorber was not particularly aggressive. The HCP burden of the load material, however, was very high due to the fact that it was purified through an un-optimized capture step, and other preceding purification steps were omitted.

The step was shown to be scalable over a 50-fold range, and it was also demonstrated that the membrane could be cycled at least 10 times if needed without any significant change in performance. These performance studies confirm that the phenyl membrane adsorber is capable of impurity removal comparable to that of a packed-bed column but in a significantly shorter processing time.

The high flow rate and minimal diffusion limitations allow for the rapid processing of batches while eliminating the need for the packing, qualification, and cleaning validation studies associated with packed bed chromatography. This results in reduced process complexity, labor, and time while still maintaining the required product purity. Among the alternative formats for purification unit operations, membrane chromatography technology is beginning to make a real impact in the biopharmaceutical industry. In addition to the advantages clearly demonstrated in the present study, membrane adsorber provides enhanced process flexibility.

Nomenclature

CIP	cleaning in place
CV	column volume
DBC_0	dynamic binding capacity at incipient breakthrough
DBC_{10}	dynamic binding capacity at 10% breakthrough
DOE	design of experiments
ELISA	enzyme-linked immunosorbent assay
HCP	host cell protein
HIC	hydrophobic interaction chromatography
HMW	high molecular weight (aggregate)
HPLC	high performance liquid chromatography
MAb	monoclonal antibody
MV	membrane volume
PPG	polypropylene glycol
SEC	size exclusion chromatography
t	time
τ	residence time

The authors would like to thank the PERCIVIA Protein and Analytical Sciences group and the QC department at DSM Biologics in Groningen, The Netherlands, for assay support in this work. Additionally, special thanks to Blanca Lain and Emily Schirmer for feedback and helpful discussions.

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