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Sartobind® Rapid A: Robust mAb Capture at High Productivity

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Abstract

The mAb capture step represents the current bottleneck in downstream processing. Protein A resins are diffusion-limited chromatography materials that require low flow rates to achieve a binding capacity above 30 g/L, which results in low productivity. Here, we present a novel chromatography membrane that combines high binding capacities with high flow rates for increased productivity. Four different monoclonal antibodies were tested on the membrane with a standard purification protocol. Resulting in robust performance over 200 cycles without any adaptation of the purification protocol. Further, product quality attributes were analyzed showing comparable product quality as state-of-the-art protein A resins.

This novel technology, with its high productivity and short cycle times, purifies monoclonal antibodies with 10× less chromatography material used per batch and allows for full utilization of the membrane within one batch. Plus, as a disposable consumable, it provides the opportunity to remove column handling in bioprocesses and resin reuse over multiple batches.

Introduction

Processes to purify recombinant monoclonal antibodies (mAb) for therapeutic treatment are well established in the market. However, manufacturer's are continuously improving their processes to lower patient risks and treatment costs. Over the past decade, efforts to improve efficiency have focused on upstream processes, resulting in a shift of the bottleneck towards downstream processes [1, 2]. Downstream purification typically relies on three chromatographic steps. Of these, the mAb capture step still depends on protein A resin in a packed bed column format, leading to obvious shortcomings. For example, packed bed columns have high diffusional resistance and long process times. They must also be reused over multiple batches to make them economical, which in turn requires extensive cleaning and validation efforts, as well as tedious column packing [3].

So far, available chromatographic matrices fall into two categories based on their dominant mass transport capabilities. The first category, porous chromatographic resins (i.e., diffusive materials), has a mass transport dependent on diffusion into the porous structure. Inside of resins, the effective pore diffusion is slow and the distances to be covered are comparatively large (~30 – 50 µm). This leads to process operation at low flow rates and high residence times, resulting in low productivities (10 – 30 g/L×h) [4, 5].

The second category contains purely convective materials such as membranes, fiber beds, or monoliths. In these materials, the binding sites reside at the surface of the convective pores, so the predominant transport mechanism is based on convection. This structure supports good accessibility of the ligand, but a trade-off between binding capacity and pressure drop occurs because both values are linked to the pore size but in an inverse manner. Pore sizes that ensure acceptable binding capacities for a protein-A-functionalized material (> 30 g/L) are in the sub-micron range (e.g., 0.3 µm). This forces the user to either accept high pressure drop, increased fouling propensity, or low binding capacity [5].

Researchers are making considerable efforts to identify alternative chromatographic materials that overcome these challenges while providing comparable product quality and support therapeutics that are affordable to more people [6]. A new generation of chromatographic materials is emerging that combines the structural and performance aspects of resins with the benefits of purely convective materials. This new convecdiff membrane contains a high binding gel phase with a short diffusional path length (2 – 3 µm) and large convective pores for fast transport to the gel phase. This combination offers robust and scalable high binding capacities at short residence times. In addition, the large convective pore sizes ensure low fouling propensity, easy cleanability, and high permeability, allowing for bed heights of about 4 mm with low pressure drops [5].

The data presented in this application note demonstrate that the novel convecdiff membrane with a protein A ligand provides typical binding capacities > 30 g/L for a set of tested Fc-containing molecules at residence times of 12 seconds and robust performance and product quality over 200 cycles, illustrating how the novel convecdiff membrane is a viable alternative to protein A resins used for mAb capture.

Materials and Methods

Buffers, Reagents and Monoclonal Antibodies

Chemicals used for buffer preparation were purchased from Carl Roth (Karlsruhe, Germany), with buffer constitutions listed in Table 1. Buffers and recipes used in this study are subject of internal platform approach.

Table 1: *Buffers Utilized for the Chromatographic Experiments.*

Buffer	Phase	Ingredients	pH
PBS	(Re-)Equilibration, Wash, HPLC Mobile Phase	1 × PBS	7.4 ± 0.2
Elution-buffer	Elution	0.1 M acetic acid, 150 mM NaCl	2.9 ± 0.1[1] | 3.2 ± 0.1[2]
Reg-buffer	Regeneration | Cleaning	0.2 M NaOH	> 12.5

[1] Elution pH mAbs 1–3 [2] Elution pH mAbs 4

All recombinant human monoclonal antibodies were expressed in Chinese hamster ovary (CHO) cells using standard cell culture techniques (stirred bioreactor). The cultivations were done in Sartorius 5 L Biostat® reactors in batch mode for 14 days. Cell clarification was performed in a two-step depth filtration using Sartorius Sartoclear® DL20 and DL60 with subsequent sterile filtration using Sartorius Sartopore® 2 XLG. Table 2 summarizes the antibodies used in this work.

Table 2: *Monoclonal Antibodies and Their Properties.*

Molecule	Class	pI	MW [kDa]
mAb1	Antibody IgG1	8.36	145.41
mAb2	Antibody IgE	7.33	146.50
mAb3	Antibody IgG1	8.09	146.53
mAb4	Antibody IgG1	8.68	145.23

Protein A Chromatography Devices

Protein A chromatographic devices used were novel Sartorius Sartobind® Rapid A with membrane volumes (MV) of 1.2 mL.

Protein Concentration and Monomer Determination by Size Exclusion HPLC

Protein concentrations and monomer | aggregate levels of HCCF and purified samples were determined by analytical high-performance size exclusion chromatography (SEC-HPLC) using a TSKgel® G3000SWXL-column (30 mm ID × 7.8 cm) from Tosoh (Griesheim, Germany) with an UltiMate™ 3000 HPLC System from Thermo Fisher Scientific (Dreieich, Germany). The HPLC system was operated at 1 mL/min with PBS as mobile phase applying 10 µL of sample. The elution profile was monitored at λ = 280 nm using the system's spectrophotometer. Elution peak area was converted to protein concentration using a standard curve generated with purified material. Aggregate levels were determined as a ratio of peak areas of the early-eluting aggregate peak(s), late-eluting fragment peak(s), and the monomer peak.

Dynamic Binding Capacity Measurements

DBC is defined as maximum amount of target protein that can be loaded onto a stationary phase without causing unnecessary loss, measured under realistic experimental conditions. Dynamic binding capacity (g of mAb per L of membrane | resin) was determined for chromatographic devices (see chapter Protein A Chromatography Devices) using an ÄKTA™ Avant 150.

For the cycling studies, the DBC was determined with HCCF (harvest cell culture fluid). The device was equilibrated and then loaded with the HCCF containing the mAbs until visible overloading appeared (at 12 seconds residence time). The flowthrough was fractionated in 1 mL portions to identify the volume when the stationary phase is fully saturated and mAb breakthrough occurs. A DBC (total bound mAb) was calculated for the amount of HCCF loaded where no monomer was measurable in the breakthrough. The exact protein concentration of the feed and the breakthrough-fractions were determined with SE-HPLC.

$$(1)\ DBC = \frac{V_{0\%} \times C_0}{V_{membrane}}$$

Where $V_{0\%}$= volume at which no mAb can be measured in the flowthrough fraction (L), C_0 is the mAb concentration (g/L) and $V_{membrane}$ is the volume of the membrane in the chromatographic devices. Determination of DBC with HCCF reflects the real process due to physicochemical mAb interactions with impurities, as well as competition and hindering of accessibility to the ligand.

Determination of Productivity

The productivity of the utilized chromatography devices, as well as of the purification of the different mAbs, was calculated according to:

$$(2)\ PR = \frac{m_{mAb}}{V_{membrane} \times t_c}$$

Where PR [g/L_{MV}×h] is the productivity, m_{mAb} [g] is the average eluted mass of monoclonal antibody, $V_{membrane}$ [L] is the volume of the membrane in the chromatographic devices and t_c [h] is the average cycle time over the whole process.

Protein A Capture Chromatography From Harvested Cell Culture Fluid

Capture of monoclonal antibodies from harvested cell culture fluid (HCCF) was conducted with Sartobind® Rapid A membrane with a membrane volume of 1.2 mL for cycling studies with different mAbs. Chromatography was performed with buffers and chromatography recipes mentioned in Table 1 and Table 3.

Table 3: *Chromatography Recipes for One Cycle of mAb Capture With Protein A Membrane Adsorber Versus Resin.*

	Sartobind® Rapid A	
Phase	**V [MV]**	**Flow rate [MV/min]**
Equilibration	5	10
Load [g/L]	34.4	5
Wash	12	10
Elution	12[1]	5
Regeneration	9 – 10[2]	5
Re-Equilibration	15 – 16[3]	10
Avg. Cycle Time [min]	—	9.6

[1] Fractionation of elution peak from 100 – 100 mAU at λ = 280 nm
[2] Hold until pH ≥ 12.3, then 4 MV
[3] Hold until pH ≤ 7.5, then cycle ends

The load was calculated as 80% of the $DBC_{10\%}$ measured at a residence time of 12 seconds for the membrane. The load density was chosen conservatively to achieve the desired number of cycles without product loss. Elution pools were collected from 100 – 100 mAU (using ÄKTA™ spectrophotometer with a 2 mm path length at λ = 280 nm). Step yield was determined using mass of product in the load and pool (both determined by SE-HPLC).

Host Cell Protein, hcDNA and Leached Protein A Measurements

Host cell protein (HCP) concentrations were measured using the CHO HCP ELISA Kit3G F550-1 Kit from Cygnus Technologies (Southport, USA). Host cell DNA concentrations have been measured using the Quant-iT PicoGreen dsDNA Assay Kit from Thermo Fisher Scientific (Dreieich, Germany). The log-reduction-value (LRV) of both impurities has been determined by means of the decadic logarithm of the quotient of impurity concentration in the feed and the impurity concentration in the elution fraction. Leached protein A has been quantified using the Protein A ELISA Kit (9000-1) from Repligen (Waltham, USA). The values listed refer to ng protein A per mg mAb. All assays have been performed according to the manufacturer's instructions and analyzed in an Infinite M Nano+ plate reader from Tecan (Maennedorf, Switzerland). For each HCCF used in this study, every 10th elution fraction was collected and analyzed regarding different CQAs (critical quality attributes) and CPPs.

Results

Robustness of New Convecdiff Sartobind® Rapid A Membrane — Evaluation of Chromatographic Performance and Product Quality Attributes Over 200 Cycles

The convecdiff Sartobind® Rapid A membrane was evaluated for its capability to run 200 cycles robust and reproducible. The cycling study included detection of UV traces, pressure drops, elution peak symmetry and critical quality attributes of the product, such as yield, monomer content, HCP and hcDNA removal, as well as protein A ligand leaching. Further, to demonstrate robustness of the convecdiff membrane, the same chromatography protocol (see Table 3) was used for all four mAbs.

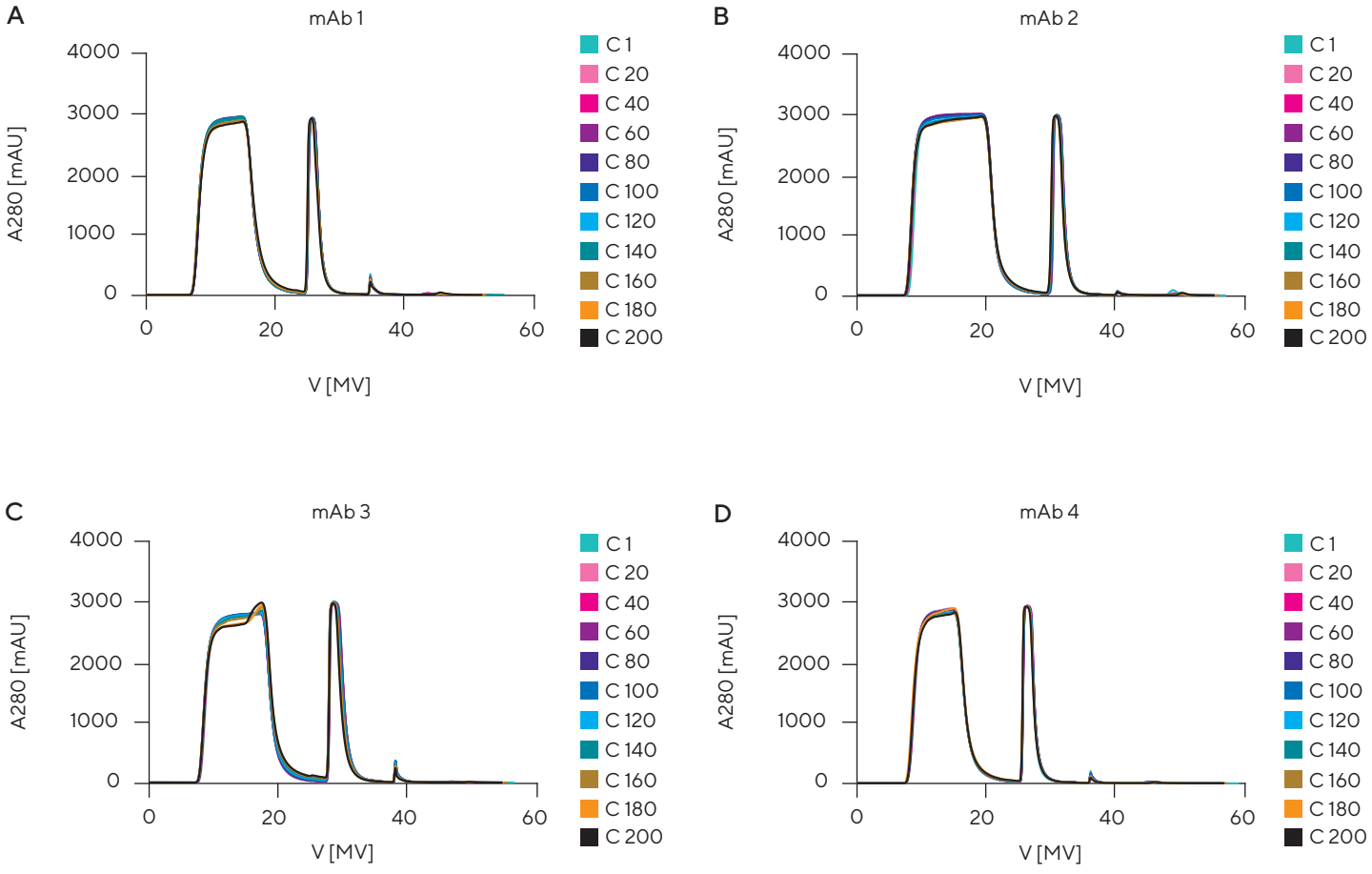
Figure 1 shows the overlays of UV adsorption at 280 nm for all four mAbs over the 200 cycles. During the load phase of the feed, unbound components flow through the convecdiff membrane, resulting in high UV absorbance after approx. 8 MV. In the following wash step, unbound components are flushed out of the membrane, leading to a decrease of the UV signal to zero (at ~27 MV). For elution of the bound mAb, the pH is decreased, resulting in release of the target molecule from the membrane, which again results in an increase of the UV signal at approx. 30 MV. After the elution block, the regeneration solution is applied on the membrane, resulting

in release of non-eluted mAbs and sticky impurities. A small peak during regeneration appeared during each experiment. Subsequently, the membrane was re-equilibrated to flush out the regeneration buffer and to restore optimal conditions for the next bind and elute cycle of mAb.

For each of the four mAbs, the intended number of mAb capture cycles was achieved with very high reproducibility. The peak shapes of the UV signals are very consistent. No significant peak broadening or shifts were observed over the whole process time. Only mAb 3 showed a slight change of the UV signal during the loading. This was caused by degradation of the HCCF during the process time and resulted in a decrease in yield. The overall process time for 200 cycles was around 34 hours.

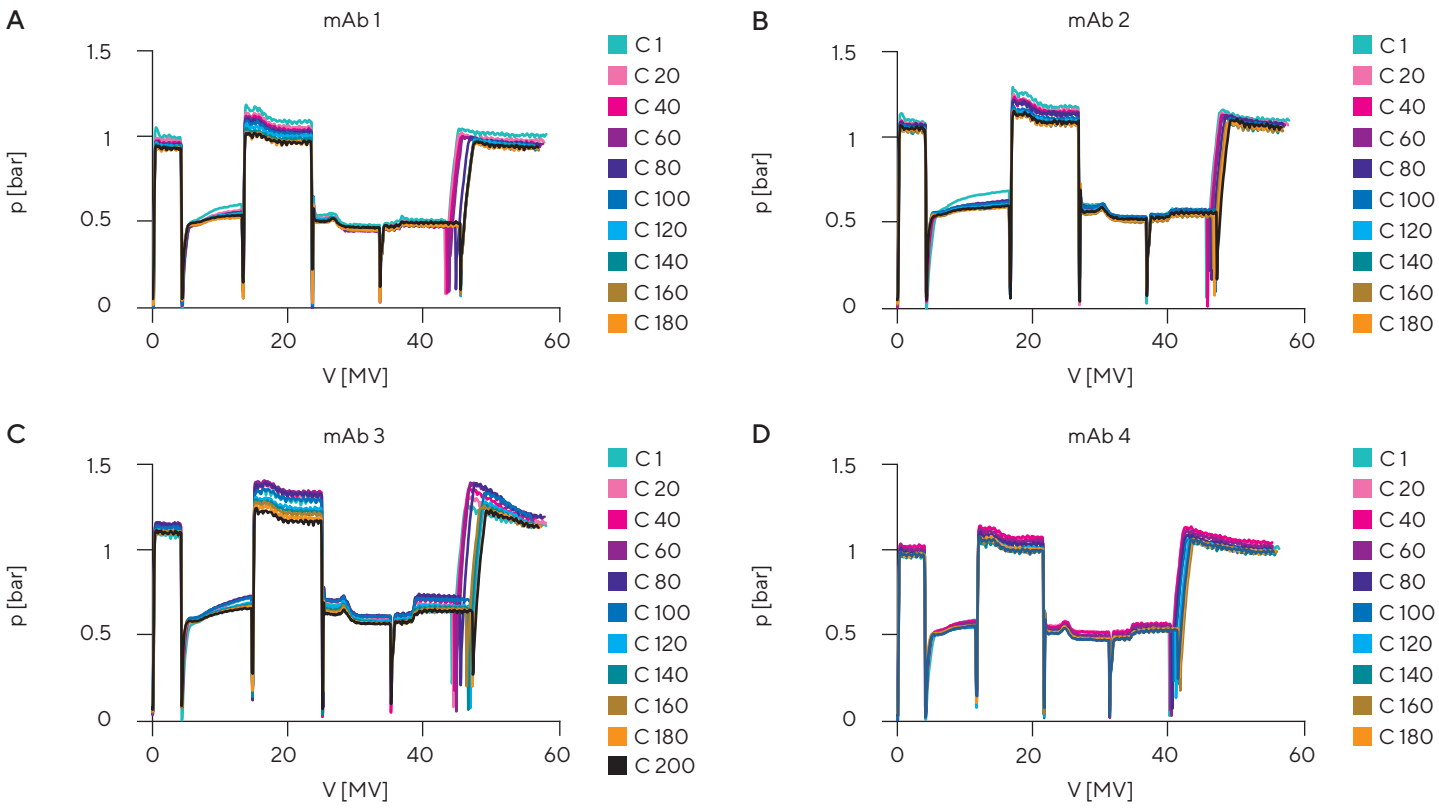
In addition, the pressure behavior over 200 cycles was detected. The highest observed pressure drops were detected during the wash step, for which a flowrate of 10 MV/min was applied, and when the stationary phase was saturated. Here, a maximum pressure between 1.1 – 1.4 bar (0.11 – 0.14 MPa; see Figure 2) was detected. Overall, the pressure behavior of the convecdiff Sartobind® Rapid A membrane showed only small variations within 0.2 bar indicating that the membrane was not affected by considerable fouling under the applied conditions over 200 cycles.

Figure 1: *Overlays of UVTraces of 200 Bind and Elute Cycles of mAbs 1 – 4 (A – D) Using Novel Convecdiff Membrane.*



Note. Shown is an overlay of every 20th cycle for each experiment.

Figure 2: *Overlays of Pressure Traces of 200 Bind and Elute Cycles of mAbs 1 – 4 (A – D).*

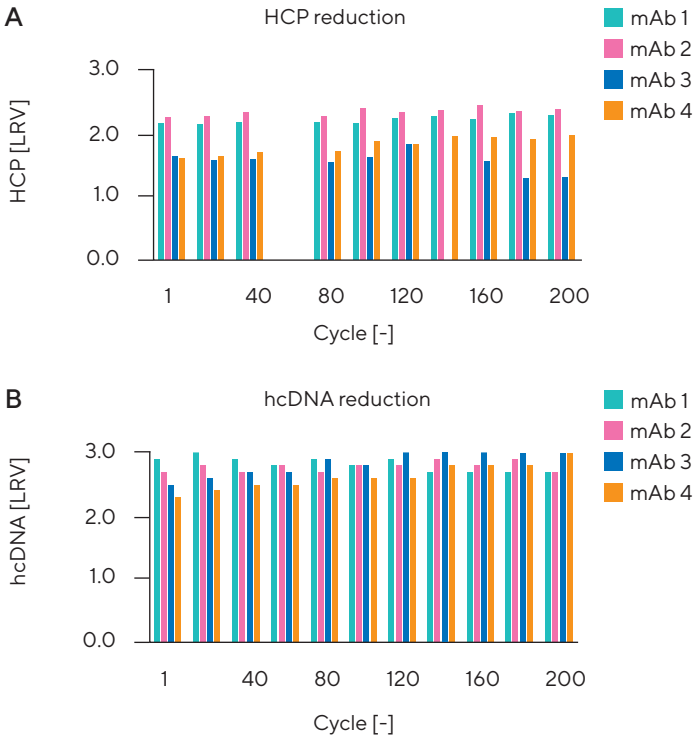


Note. Shown is an overlay of every 20th cycle for each experiment.

Additionally, the CQAs of the product were analyzed, including HCP removal, hcDNA removal, protein A ligand leaching, and high and low molecular weight (HMW and LMW) species. As shown in Figure 3, consistent results were achieved for the 200 cycles for HCP and hcDNA removal for each HCCF. mAb 3 and 4 show lower log reduction values (LRV) due to lower HCP and hcDNA content in the HCCF. As described earlier, mAb 3 HCCF was not stable over the processing time, which also results in a decrease of the LRV at cycles above 130. However, for hcDNA depletion, no effect could be observed. With a consistent removal of both, HCP and hcDNA were confirmed over 200 cycles for four different mAbs, demonstrating the cycling capability and stability of Sartobind® Rapid A without optimization of the purification protocol.

Further process- and product-related parameters are summarized in Table 4. For three of the four mAbs, all analyzed CPP and CQA values showed a very high consistency over the 200 cycles. mAb 3 showed consistent values until cycle 130. After this, the HCCF started to degrade and form aggregates. Therefore, two values are given in Table 4 for this mAb to demonstrate that, until cycle 130, same good CPP and CQA values were achieved with Sartobind® Rapid A.

Figure 3: *Capability of HCP and hcDNA Removal (Log Reduction Value) of Sartobind® Rapid A at Certain Intervals Capturing mAbs 1 – 4.*



Discussion

Purification with the new convecdiff Sartobind® Rapid A membrane resulted in very high yields for three of the four mAbs. mAb 4 showed lower yields. An improvement of the yield for this mAb can be achieved by further optimization of the protocol. Further, the membrane showed a low propensity for aggregation and fragment formation resulting in a remarkably high monomer concentration in the elution fractions. Also, the elution volumes and mAb concentration in the elution showed no significant change over the 200 cycles (5 – 6 MV; 5 – 6.5 g/L, depending on HCCF). Another important parameter is leaching of the protein A ligand from the membrane during purification. The analysis of this value in the product showed very low levels with an average of 3.4 ppm. Compared to usual protein A resins, the leached ligand is at a comparable level, but there was no noticeable decrease in binding capacity despite cleaning with NaOH after every single cycle, as is typically seen with resins [7].

The underlying calculated productivities were exceptionally high, which is a result of very short cycle times (average cycle times of 10 – 11 minutes) and very low stationary phase of the chromatography material (1.2 mL MV). Variations between the four experiments are due to different feed concentrations and binding capacities achieved, which directly influence the cycle time.

Table 4: *Overview of Process-Related Parameters and Product Quality Attributes.*

	mAb 1	mAb 2	mAb 3	mAb 4
Titer [g/L]	3.12	2.25	3.50	4.30
DBC [g/L]	42.2	41.8	54.3	49.9
Load[1] [g/L]	32.8	32.6	42.0	38.7
Yield [%]	95.7 ± 1.3	96.3 ± 2.6	92.1% ± 1.6[2] 88.3 ± 7.7[3]	87.3 ± 1.7
Monomer [%]	> 99.5	> 99.5	99.0[1] 97.5[2]	> 99.5
HCP reduction [LRV]	2.2 ± 0.1	2.4 ± 0.1	1.5 ± 0.2[2]	1.8 ± 0.1
hcDNA reduction [LRV]	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.2[2]	2.6 ± 0.2
Protein A leached [ppm]	2.7 ± 0.7	–	–	2.8 ± 0.3
Avg. Productivity [g/L × h]	167.7	160.3	204.7[1] 196.2[2]	206.9

[1] Load 77.5% of DBC [2] For cycles 1 – 130 [3] For cycles 1 – 200

This study investigated the effectiveness of the novel convecdiff Sartobind® Rapid A membrane in capturing mAbs from HCCF . It analyzed CCPs and CQAs, proving that there is no membrane-related decrease of performance over 200 cycles. As the protein A membrane consists of a novel structure in which mass transport is neither primarily diffusive nor purely convective, evaluation of the robustness of the membrane in a housing cannot be tested in the same manner as packed beds in columns with HETP or plate number [8]. For this reason, investigators assessed the preservation of binding capacity (measured with yield), the pressure behavior, impurity removal, leached ligand and elution volume over the membrane lifetime. Those parameters are indicators of robustness and fouling propensity of the protein A stationary phases [9, 10].

The parameters analyzed for the four different mAbs varied within a very narrow range over the entire number of cycles. The stationary phase (membrane) maintained its integrity over 200 cycles, revealing that the fouling propensity of the membrane adsorber is very low. Adding a short regeneration | cleaning step after each bind and elute cycle minimized the fouling process. This also indicated that the ligand was stable after repeated exposure to caustic reagents. Protein A ligands tend to undergo hydrolysis during caustic exposure, which typically leads to loss of binding capacity and yield [7, 11]. The lowest yields in this study occurred with the highest load density besides, though this yield was constant over 200 cycles. Slight decreases in yield for mAb 3 were related to changes in the feed constitution. Between cycle 130 and 140, the feed showed an increase in aggregate formation, indicating that the applied conditions led to a longer holding time of the HCCF. A lower yield for mAb 4 compared to the other mAbs seemed to result from the chosen elution conditions (elution mAbs 1 – 3: pH 2.9 ± 0.1; mAb 4: pH 3.2 ± 0.1). Since pH reduction is the main driver for the release of a mAb from protein A, the lower pH elution buffer resulted in better elution performance and thus higher yields. Some mAbs aggregate at lower pH (not seen in this study, see Table 4), leading investigators to test a higher pH.

Conclusion

This new technology can eliminate two main pain points of the industry: packed bed chromatography, which can fail, and column reuse, which is not economically viable. In addition, this new ready-to-use and disposable alternative will provide cost benefits in certain processes, such as clinical-scale processes and low-demand molecules, where resins are typically underutilized. Human resources can focus on higher-value tasks because they won't be occupied with column packing and cleaning validation. Further, from a regulatory perspective, this technology will mitigate several common problems, such as bed failure events, bioburden issues, and cross-contamination of batches .

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