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Intensifying mAb Purification With Sartobind® Membrane Chromatography

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Abstract

The growing use of monoclonal antibodies (mAbs) across diverse therapeutic applications requires continued improvement in purification methods, with enhanced productivity and reduced development timelines as central objectives.

Replacing conventional resin-based chromatography with membrane-based purification technologies provides a practical solution for increasing efficiency. The unique properties of membrane chromatography make it particularly well-suited for the purification of high-quality mAbs, and these methods can be seamlessly integrated into an established downstream purification process.

This application note describes a collaboration with Enzene Biosciences Ltd, through which we developed and implemented a three-step mAb purification process. The workflow consisted of capture and polishing steps and relied exclusively on ready-for-use Sartobind® membrane chromatography consumables. Efficient mAb capture was accomplished using Sartobind® Rapid A, followed by ion-exchange polishing steps. Anion-exchange chromatography with Sartobind® Q achieved substantial host cell DNA reduction and excellent clearance of host cell proteins. Subsequent cation exchange with Sartobind® S demonstrated high performance in aggregate removal, achieving a significant (50%) reduction in high molecular weight species.

ENZENE Biosciences Ltd.

Introduction

Continuous efficiency improvements in the cost-intensive, GMP-compliant downstream processing of mAbs are essential to meet the growing demand for these powerful therapeutics. Key drivers of this progress include accelerated production timelines, increased yields, reduced handling time and effort, and a minimized equipment footprint.

In this context, membrane chromatography has emerged as a promising alternative to traditional resin-based systems, offering distinct advantages that align with the evolving demands of modern biomanufacturing.

With the Sartobind® membrane chromatography portfolio, Sartorius provides a high-performance solution for both capture and polishing steps in GMP-compliant biopharmaceutical downstream processing. Unlike porous resins that rely on slow diffusive mass transfer, Sartobind® membranes operate primarily through convective mass transfer. This mechanism enables rapid binding of target molecules to the binding sites in bind-elute mode, supports high flow rates, and results in significantly shorter processing times. Their linear scalability simplifies process development, while the compact, ready-for-use design contributes to a reduced equipment footprint, minimized manual handling, shorter setup times, lower overall process complexity, and reduced capital expenditure requirements. Additionally, in flow-through mode, membrane chromatography typically achieves higher contaminant removal capacities than resins.

Methods and Results

Study design

This application note presents the development and implementation of a membrane-based mAb purification process employing exclusively Sartobind® chromatography consumables, developed in collaboration with Enzene Biosciences Ltd. Designed as a proof-of-concept study, mAb purification was performed at a small scale to showcase the efficacy and feasibility of this fully membrane-based approach. mAb capture was achieved using Sartobind® Rapid A, followed by Sartobind® Q and S for subsequent anion- and cation-exchange polishing steps, respectively. Both polishing steps were performed in flow-through mode, simplifying operation, enabling high flow rates, and further reducing processing time while improving overall efficiency.

The results are organized into two parts. The first part presents findings from initial test runs using the three Sartobind® membrane devices: Sartobind® Rapid A for capture and Sartobind® Q and S for polishing. The aim of this application note is not to describe each optimization step in detail, but rather to highlight parameters that can be adjusted to develop an efficient and tailored membrane-based mAb purification process, and to show how a connected operation can be enabled with minimal changes to the matrix after neutralization of the capture step eluate following low-pH virus inactivation (VI). The second part focuses on the three-step chromatography purification process using all Sartobind® devices in series.

Optimization of membrane-based mAb capture and polishing using Sartobind® membrane chromatography

Capture with Sartobind® Rapid A

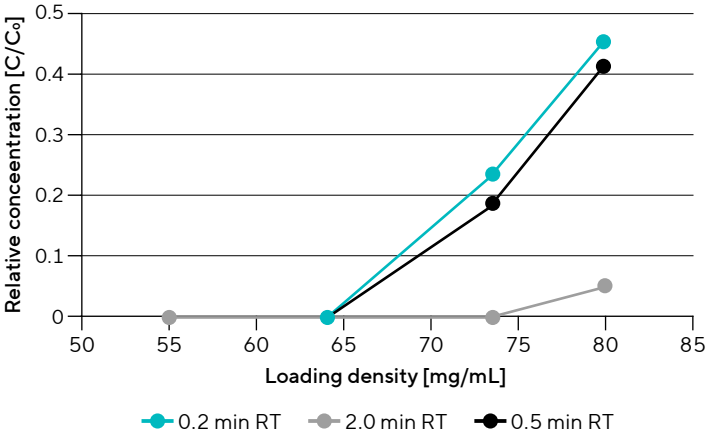
Determination of dynamic binding capacity at different residence times

Optimization of mAb capture began with the assessment of the dynamic binding capacity (DBC) of Sartobind® Rapid A for the target mAb. Clarified cell culture harvest (CCCH) was loaded onto a 1.2 mL membrane at residence times (RTs) of 0.2, 0.5, and 2 minutes until breakthrough of the mAb was observed.

Plotting the relative mAb concentration in the flow-through against the loading density (mg/mL of column volume [CV]) revealed a comparable DBC across all three RTs. At RTs of 0.2 and 0.5 minutes, breakthrough was observed at ~65 mg mAb/mL CV, while at an RT of 2 minutes, breakthrough started slightly later at 75 mg/mL CV (Figure 1).

Considering the commonly accepted practice of loading at ~80% of the 10% DBC, the selected parameters of 40 mg/mL CV at 0.2 min RT were highly conservative, corresponding to only ~60% of the 10% DBC. The selected binding capacity applies two safety margins: (1) operating at 80% of the measured DBC and (2) accounting for cycle-dependent capacity loss with an additional 80% factor. The findings support the feasibility of shorter residence times, which can accelerate processing and enhance overall downstream efficiency.

Figure 1: DBC determination of the Sartobind® Rapid A membrane device (1.2 mL CV) for the target mAb at different RTs: 0.2 min, 0.5 min, and 2 min. The relative mAb concentration in the flow-through is plotted against the loading density (mg/mL CV)



Optimization of the chromatography protocol

To enhance the efficiency of the Sartobind® Rapid A mAb capture step, the chromatography protocol used for determining the DBC was further refined.

- High-salt wash (wash 2): NaCl concentration was reduced to as low as 0.25 M to identify the minimal NaCl concentration required for efficient yield and purification.
- Buffer volume: Equilibration, wash, and elution volumes were optimized to reduce overall buffer consumption, decrease waste volumes, shorten process times, and ensure yield and purity.
- Process duration: RTs for buffer solutions (excluding loading and elution) were reduced from 0.2 min to 0.1 min without significantly impacting mAb yield, purity, and leached Protein A.
- Elution window: Collection of the fixed elution volume was adjusted to 50 – 100 mAU to ensure optimal mAb recovery and purity and to enable a seamless transition to subsequent polishing steps.
- Eluate pH: The pH of wash buffer 3 and the concentration of acetic acid in the elution buffer were systematically adjusted to obtain an eluate at pH 4 with minimal conductivity. An eluate at pH 4 supports mAb stability, enabling pooling of fractions from consecutive runs prior toVI, while low conductivity (< 4 mS/cm) after neutralization is essential for the subsequent Sartobind® Q anion exchange polishing step.

The resulting chromatography protocol applied for mAb capture with Sartobind® Rapid A is summarized in Table 1.

Table 1: Optimized chromatography protocol for mAb capture using Sartobind® Rapid A (1.2 mL CV). Buffer volumes for each process step correspond to 20 CV, except initial equilibration, which is executed using 15 CV

Process parameters	Specification
mAb load density [mg/mL CV]	40
Residence time for loading and elution phase [min]	0.2
Residence time for other process steps [min]	0.1
Elution collection criteria (for 2 mm UV flow cell path length) [mAU]	50 – 100

Process step	Buffer composition
Equilibration	50 mM sodium phosphate, pH 7.0
Loading	CCCH (at 40 mg/mL loading density)
Post-load wash | wash 1	50 mM sodium phosphate, pH 7.0,
Wash 2	50 mM sodium phosphate + 0.25 M NaCl, pH 7.0
Wash 3	10 mM sodium acetate, pH 7.3
Elution	50 mM acetic acid, pH 3.0
Clean-in-place (CIP)	0.1 N NaOH
Equilibration	50 mM sodium phosphate, pH 7.0
Storage	20% ethanol | 2% benzyl alcohol

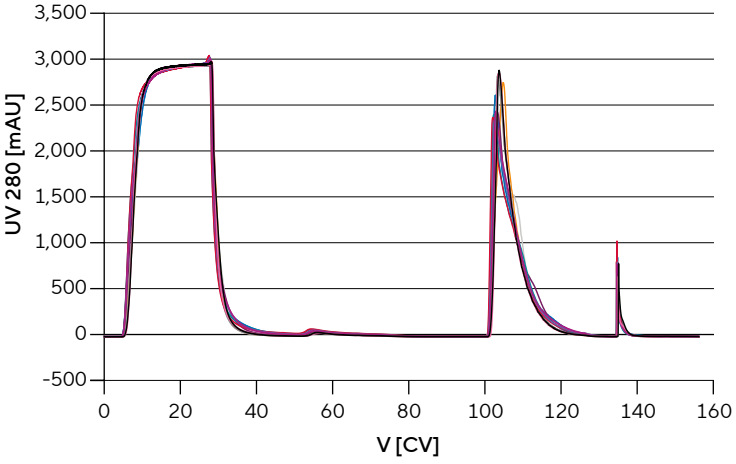
Re-use of Sartobind® Rapid A

The reusability of the Sartobind® Rapid A membrane (1.2 mL CV) was evaluated across 75 consecutive cycles, applying the optimized chromatography protocol for mAb capture (Table 1) with the following modifications: the equilibration buffer volume at the end of each cycle and the beginning of the next was reduced to 10 CVs. After 25 cycles, the membrane was held in 0.2 M NaOH for 15 min; after 50 cycles, it was regenerated with 0.5 N NaOH for 15 min.

Monitored performance indicators included pH, conductivity, UV-280 nm adsorption profile, pressure drop, recovery, and quality of the purified mAb. Across 75 cycles, no significant differences were observed in pH and conductivity profiles. The UV-280 nm profiles were highly reproducible, showing only minor variations in the elution peak profile, which correlated with a slight decrease in mAb recovery over time (Figure 2). Nonetheless, mAb yield obtained from Sartobind® Rapid A capture remained consistently high at 100% during the first 25 cycles and above 90% through cycle 75, while purity remained unaffected throughout all cycles. A gradual increase in transmembrane pressure (TMP) was observed with increasing cycle number, which may be related to the impurity level of the feed. However, further optimization of the washing steps could minimize this effect.

Depending on feedstream properties and the target molecule, Sartobind® Rapid A exhibits a lifetime of up to 200 cycles, as shown in a separate application note.[1]

Figure 2: Chromatographic profiles from every fifth run (cycles 1, 5, 10, etc.) of the 75-cycle re-use study using Sartobind® Rapid A (1.2 mL CV)



Polishing step 1 with Sartobind® Q

Sartobind® Q (1 mL CV) was used in flow-through mode for polishing step 1 to remove host cell proteins (HCP) and host cell DNA (hcDNA) by anion-exchange (AEX) chromatography (Table 2).

The eluate obtained from the Sartobind® Rapid A capture step was adjusted to pH 8.2 using 2 M Tris buffer and diluted with WFI to a conductivity of 3.59 mS/cm. At pH ~ 8.0 and conductivity ≤ 4 mS/cm, the target mAb does not bind to the membrane, whereas negatively charged hcDNA and HCP exhibit strong binding affinity. The neutralized Protein A eluate was then prefiltered through a Sartopore® 2 XLG filter and loaded onto Sartobind® Q, pre-equilibrated with 20 mM sodium phosphate buffer (pH 8.0), at a loading density of 2.5 g/mL CV.

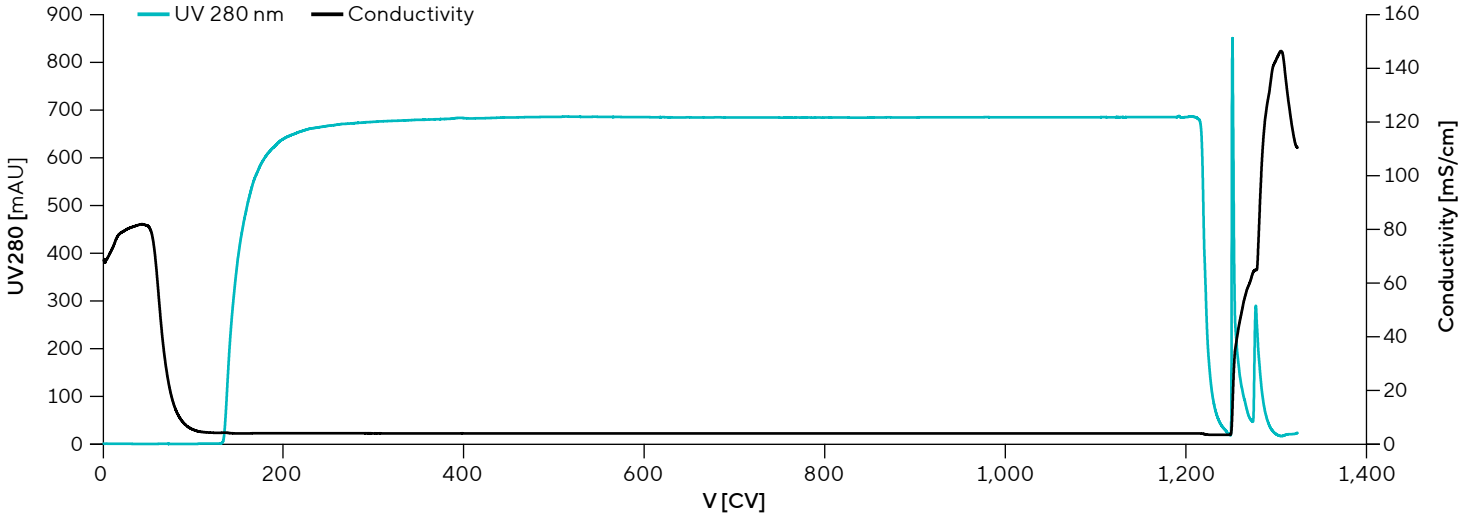
Table 2: Chromatography protocol for polishing step 1 carried out with Sartobind® Q for AEX in flow-through mode

Process parameters	Specification
Load pH	8.2
Load conductivity [mS/cm]	3.59
Protein concentration [mg/mL]	2.34
Load volume [mL]	1,070
Loading density [g/mL CV]	2.5
Residence time for loading and post-load wash | wash 1 phase [min]	0.2
Residence time for remaining phases [min]	0.1

Process step	Buffer composition
High-salt wash (30 CV)	20 mM sodium phosphate, 1 M NaCl, pH 8.0
Equilibration buffer (75 CV)	20 mM sodium phosphate, pH 8.0
Loading	Neutralized Protein A eluate
Post-load wash | wash 1 (30 CV)	20 mM sodium phosphate, pH 8.0
High-salt wash | stripping (30 CV)	20 mM sodium phosphate, 1 M NaCl, pH 8.0
CIP (30 CV)	1 M NaOH

The chromatographic profile of the AEX run with Sartobind® Q is shown in Figure 3. No increase in delta or pre-column pressure was observed during either the loading or the non-loading phases.

Figure 3: Chromatographic profile of mAb polishing step 1 using Sartobind® Q for the removal of process-related impurities by AEX in flow-through mode

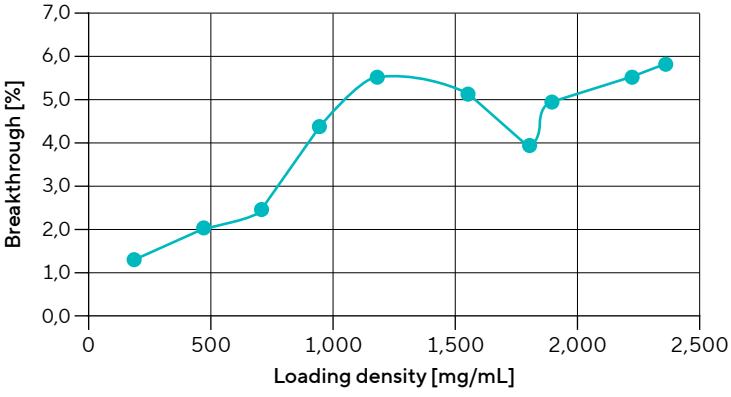


The flow-through was collected in fractions of 250 mg (107 mL each) and analyzed for purity by size-exclusion high-performance liquid chromatography (SE-HPLC), hcDNA, leached Protein A, and HCP, and compared to the feed. hcDNA and Protein A were reduced from 1 ng/mL and 1 ppm to below the quantification limits of < 30 pg/mL and < 310 pg/mL, respectively.

The efficiency of HCP removal is illustrated in Figure 4. At a loading density of ~ 940 mg/mL CV, 4% HCP breakthrough was observed, increasing slightly to 6% at ~ 2.2 g/mL CV. Overall, Sartobind® Q achieved a significant HCP reduction from 689 ppm to 28.2 ppm, corresponding to a log reduction value (LRV) of 1.39.

These findings demonstrate the excellent clearance capacity of Sartobind® Q for process-related impurities, supporting its use in the production of clinical-grade mAbs. In view of these results, no further optimization of the AEX protocol was considered necessary.

Figure 4: HCP breakthrough relative to the membrane loading density (mg/mL CV) applied to the Sartobind® Q membrane device (1 mL CV). The relative HCP concentration in the flow-through is plotted against the loading density (mg/mL CV).



Polishing step 2 with Sartobind® S

Sartobind® S (1 mL CV) was employed in polishing step 2 to remove aggregates via cation-exchange chromatography (CEX). To optimize aggregate clearance conditions, several test runs were conducted using Sartobind® Q flow-through that had been prefiltered by Sartopore® 2 XLG, evaluating two approaches:

- Displacement flow-through mode
- Overload bind-elute (OBE) mode

In both modes, the antibody initially binds to the stationary phase but is displaced into the flow-through due to the stronger binding affinity of aggregates. This occurs when the membrane is loaded beyond its actual binding capacity for the target mAb.

The flow-through of each run was collected in multiple fractions, which were analyzed by SE-HPLC to assess the removal of high-molecular-weight species (HMWS) and low-molecular-weight species (LMWS).

Three test runs in displacement flow-through mode were conducted, employing feed solutions with varying pH and conductivity conditions:

- pH 4.5 and 4.2 mS/cm
- pH 4.0 and 4.1 mS/cm
- pH 8.1 and 3.5 mS/cm

The loading density was kept constant at around 1 g/mL CV, and the RT was 0.3 minutes for both the loading and the non-loading phases. Analysis of the flow-through revealed minimal to no HMWS clearance, likely due to the already low aggregate content (0.9%) in the Sartobind® Q eluate.

To further challenge the method, HMWS were artificially enriched by adjusting the sample to pH 3.5 and incubating it at 40 °C for 15 hours. The resulting material, containing 5.6% HMWS, was then adjusted to pH 4.5 and a conductivity of 4.1 mS/cm before being loaded onto the membrane at 0.47 g/mL CV. RT was maintained as in the previous runs.

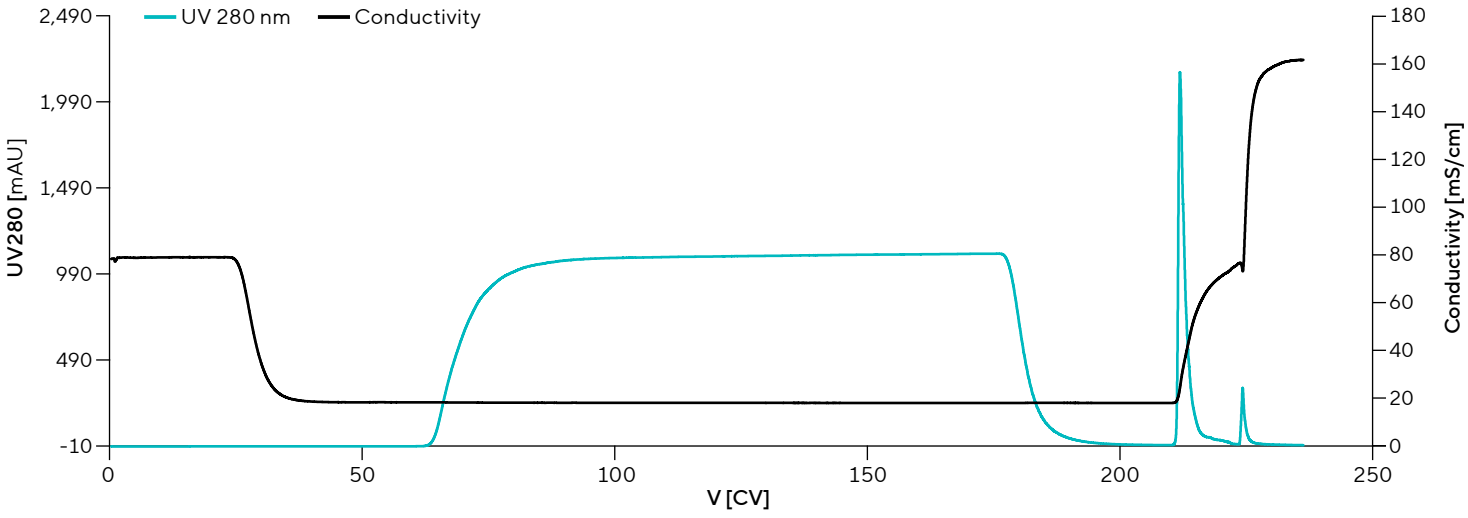
Since the flow-through analysis of the enriched HMWS showed no significant clearance, a subsequent test run was conducted in OBE mode using Sartobind® Rapid A eluate enriched with HMWS as described above. The membrane was equilibrated with 20 mM sodium acetate, pH 5.5, adjusted to a conductivity of 18 mS/cm. The feed was similarly adjusted to pH 5.5 and 18.2 mS/cm. Further process details of the OBE run are summarized in Table 3.

Table 3: Chromatography protocol for polishing step 2 employing Sartobind® S for CEX in OBE mode

Process parameters	Specification
Load pH	5.5
Load conductivity [mS/cm]	18.2
Protein concentration [mg/mL]	4.1
Load volume [mL]	80
Loading density [g/mL CV]	0.33
Residence time for loading and post-load wash phase [min]	0.6
Residence time for non-loading phase [min]	0.3
HMWS load [%]	2.7
Process step	**Buffer composition**
Pre-loading	
High salt wash (30 CV)	20 mM sodium acetate, 1 M NaCl, pH 5.5
Equilibration buffer (30 CV)	20 mM sodium acetate, pH 5.5, conductivity adjusted to 18 mS/cm using 2 M NaCl
Loading	
Post-load wash | wash 1 (30 CV)	20 mM sodium acetate, pH 5.5, conductivity adjusted to 18 mS/cm using 2 M NaCl
High salt wash | stripping (30 CV)	20 mM sodium acetate, 1 M NaCl, pH 5.5
CIP (30 CV)	1 M NaOH

The chromatographic profile of the OBE run is shown in Figure 5. No increase in delta column or pre-column pressure was observed during either the loading or the non-loading phases. Initially, the mAb bound to the membrane; however, under overload conditions, it started to elute at ~ 60 mL.

Figure 5: Chromatographic profile of mAb polishing step 2 using Sartobind® S for aggregate removal via CEX operated in OBE mode



Ten flow-through fractions, collected during the loading and post-load wash phases, were analyzed for their mAb and aggregate content using OD 280 nm and SE-HPLC, respectively (Tables 4 and 5, Figure 6).

The observed low mAb content in fraction 2 and its increase in the subsequent fractions (Table 4) correspond well with the initial binding of the mAb to the membrane and its subsequent elution under overload conditions (Figure 6).

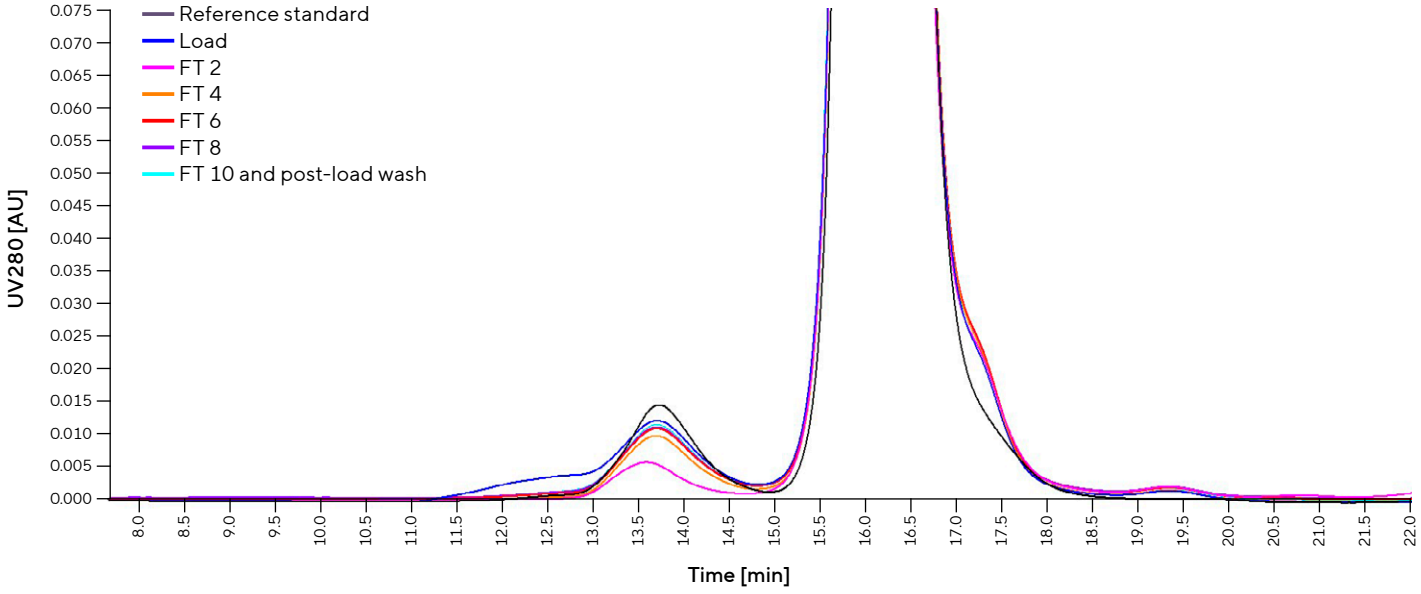
Table 4: Results of the OD 280 nm analysis for mAb yield in flow-through (FT) fractions obtained from Sartobind® S CEX operated in OBE mode

Sample	Concentration by OD 280 nm [mg/mL]
Load	4.1
FT 2	1.6
FT 4	3.8
FT 6	3.9
FT 8	3.9
FT 10 and post-load wash	2.0

Table 5: Results of the SE-HPLC analysis for aggregate removal in flow-through (FT) fractions obtained from Sartobind® S CEX operated in OBE mode

Sample	% HMWS	% Monomer peak	% LMWS
Load	2.7	95.4	1.9
FT 2	0.8	97.2	2.0
FT 4	1.5	96.5	2.0
FT 6	1.7	96.4	2.0
FT 8	1.7	96.3	2.0
FT 10 and post-load wash	1.9	96.0	2.2

Figure 6: SE-HPLC profiles of flow-through fractions collected during polishing step 2 using Sartobind® S for aggregate removal via CEX operated in OBE mode



Fraction 2 also exhibited the lowest level of HMWS, indicating efficient early retention of aggregates. In the following fractions, HMWS continued to be effectively removed (Table 5, Figure 6, while only a minor proportion of mAb was retained. These findings highlight the higher binding affinity of aggregates to the membrane under the applied mAb overload conditions.

Overall, HMWS levels were significantly reduced by ~50%, as quantified by SE-HPLC, demonstrating the strong performance of the Sartobind® S membrane device and its suitability for efficient aggregate clearance. LMWS were not retained under the conditions tested, as shown by overlapping SEC profiles of the load and flow-through fractions (Table 5, Figure 5).

These findings support the effectiveness of the aggregate removal strategy utilizing Sartobind® S in OBE mode, while also indicating potential for improving LMWS clearance in future optimization efforts.

Implementation of a three-step, fully membrane-based mAb purification process using Sartobind® membranes

Once the process conditions for mAb capture and polishing had reached a well-optimized state, all three chromatography steps were carried out in series. To enhance process efficiency, the capture step was performed in a continuous mode using parallel batch multi-column chromatography (B-MCC).

Two Sartobind® Rapid A devices were operated in an alternating loading | non-loading mode: one membrane was loaded, while the other simultaneously underwent washing, elution, regeneration, and (re-)equilibration. The eluate was then subjected to low-pH VI, followed by neutralization and intermediate filtration using a Sartopore® 2 XLG filter (0.8/0.2 µm pore size). Subsequently, the polishing steps were carried out using Sartobind® Q and Sartobind® S (Figure 7).

mAb capture using two Sartobind® Rapid A membranes in parallel B-MCC

The capture step was performed by applying the optimized buffer recipes listed in Table 1. Buffer volumes and RTs were adjusted to enable alternating operation of the two Sartobind® Rapid devices (Table 6). Due to flow rate limitations of the chromatography system, RTs during non-loading phases were increased to 0.2 min. Additionally, the duration of the loading phase was intentionally set longer than the non-loading phase to ensure continuous loading. Temporary interruptions during elution (if the loading phase is still ongoing) did not affect overall process continuity, as eluates were pooled for subsequent VI.

A total of 10 capture cycles (5 per membrane) were performed using two 10 mL Sartobind® Rapid A devices, each operated at a loading density of 40 mg/mL, to capture 4 g of mAb from 2.22 L CCCH feed solution. The device sizes were chosen to enable a feasible dimensioning for the subsequent polishing steps.

Figure 7: Overview of the small-scale membrane-based mAb purification process, depicting the sequential use of Sartobind® chromatography devices for capture and polishing

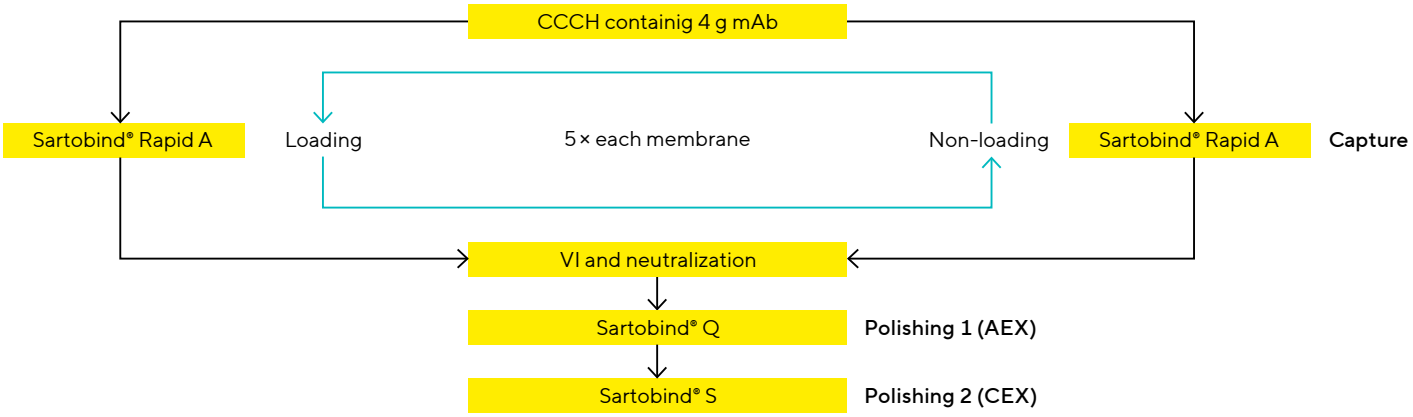
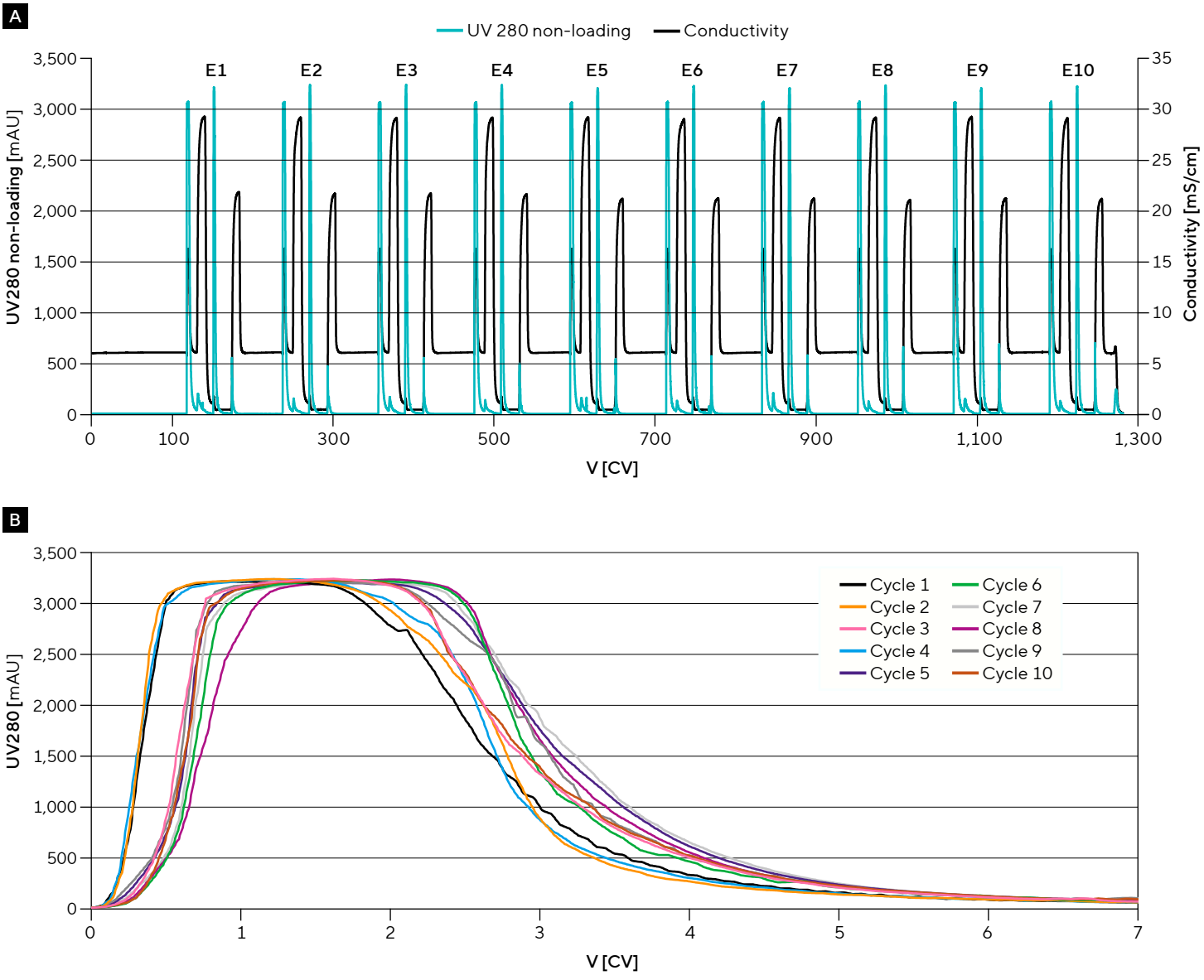


Table 6: Chromatography protocol for continuous mAb capture with two Sartobind® Rapid A devices (10 mL CV each), operated in an alternating loading | non-loading mode

Process step	CV	RT [min]	Flow rate [mL/min]	Time [min]
Equilibration	10	0.20	50.0	2.0
Loading	2.22 L CCCH pH 7.26 16.87 mS/cm pre-filtered through 0.2 µm PES filter	1.05	9.50	23.3
Post-load wash | wash 1	10	0.20	50.0	2.0
Wash 2	10	0.20	50.0	2.0
Wash 3	10	0.20	50.0	2.0
Elution	20	0.20	50.0	4.0
Regeneration	10	0.20	50.0	2.0
Equilibration	10	0.20	50.0	2.0
Total duration per run				41.3

Figure 8: Chromatographic profile of the mAb capture step performed with two 10 mL Sartobind® Rapid A membrane devices operated in an alternating parallel loading | non-loading mode **(A)** and overlay of all elution peaks **(B)**



The chromatographic profile of the capture step is presented in Figure 8. The elution peaks (E1–E10) were consistent across all 10 cycles. Throughout the process, both pre-column and delta-column pressures remained low, indicating stable performance of the two Sartobind® Rapid A devices.

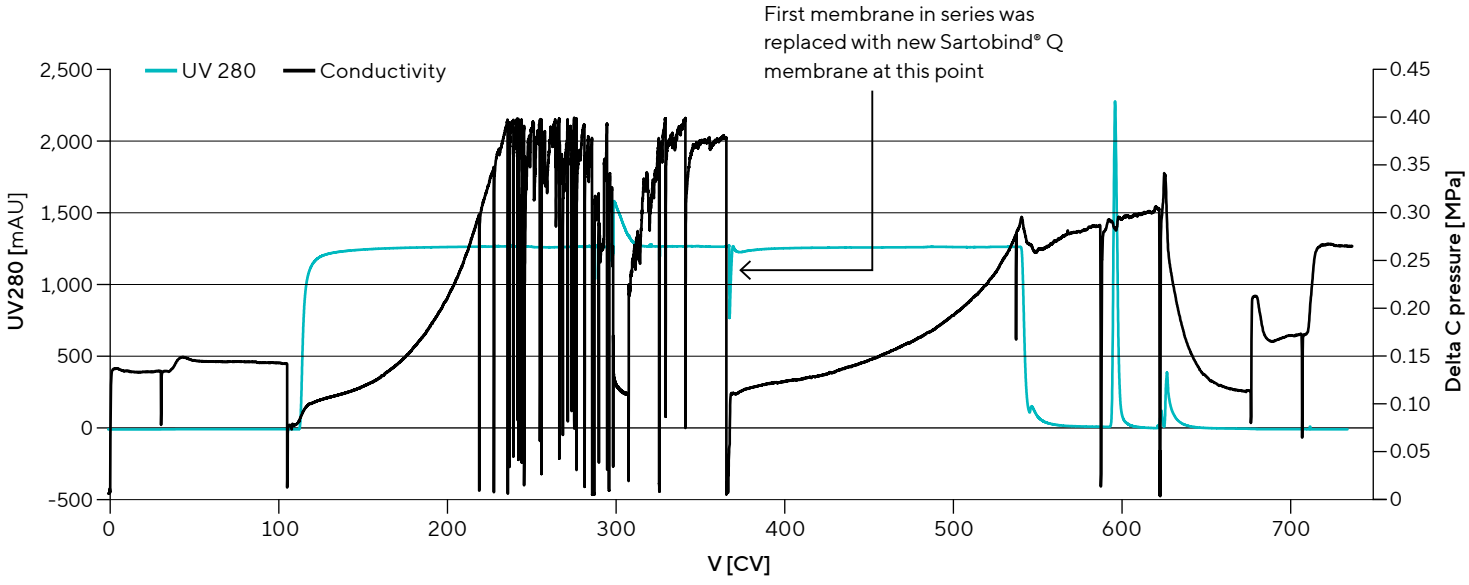
The Protein A eluates (pH 4.0, 0.65 mS/cm) were pooled and adjusted to pH 3.5 by the addition of glacial acetic acid. The solution was incubated for 60 minutes, in batch mode, at low pH to inactivate potential enveloped viruses. After incubation, neutralization to pH 8.0 was performed by adding 2 M Tris, resulting in a final conductivity of 5.36 mS/cm.

Polishing step 1 using Sartobind® Q in AEX

The neutralized Protein A eluate was subjected to AEX chromatography in flow-through mode using two 1 mL Sartobind® Q devices connected in series.

Prior to loading, the neutralized eluate was diluted with WFI to reduce the conductivity to 3.77 mS/cm. The prepared eluate (864 mL, 5 mg/mL mAb) was filtered in-line through a Sartopore® 2 XLG filter and loaded onto the Sartobind® Q membrane. The membranes were operated at a loading density of around 2 g/mL CV. Further process parameters were consistent with those outlined in Table 2.

Figure 9: Chromatographic profile of polishing step 1 utilizing two 1 mL Sartobind® Q membrane devices connected in series for AEX in flow-through mode



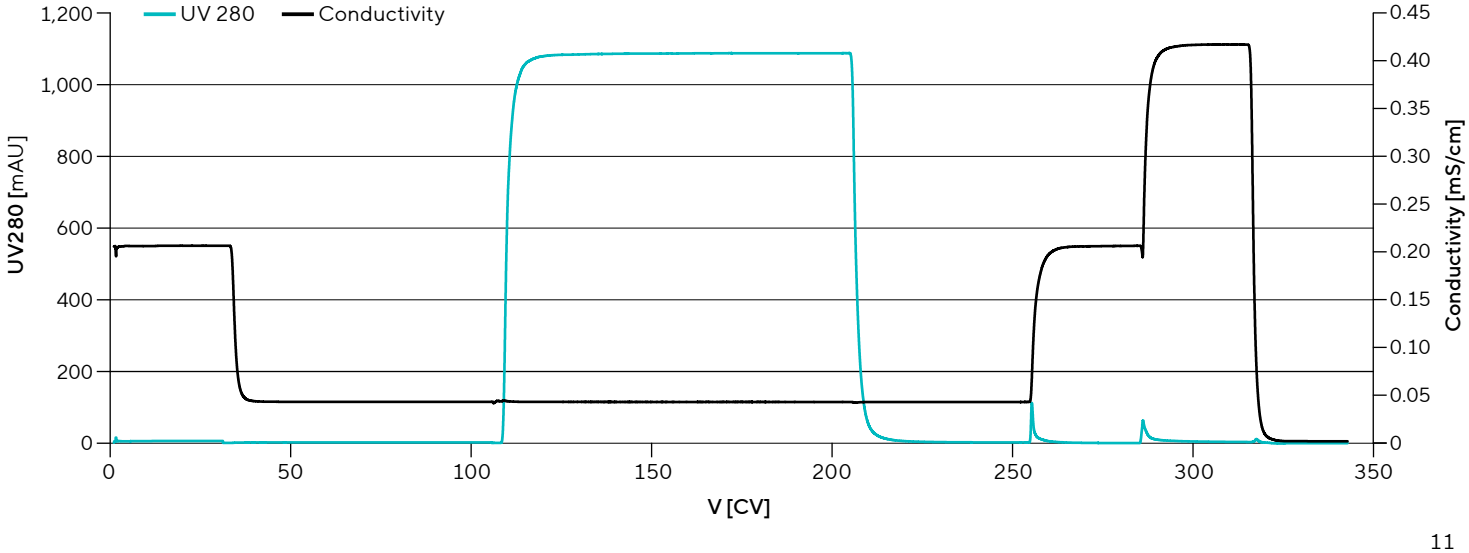
Since the differential pressure increased gradually during the loading phase (Figure 9), the residence time was extended from 0.1 to 0.67 minutes. Following a continued rise in pressure, the first Sartobind® Q device connected in series was replaced. The sustained, albeit slower, increase in pressure was likely due to the high hcDNA content of the load (Table 8).

Polishing step 2 using Sartobind® S in CEX

The flow-through and post-load wash fractions obtained from AEX polishing step 1 were pooled and adjusted to a pH of 5.4 and a conductivity of 18 mS/cm to enable aggregate removal in OBE mode, as established during initial optimization runs.

As in polishing step 1, the feed (973 mL, 3.9 mg/mL mAb) was filtered in-line through a Sartopore® 2 XLG filter before loading onto a 10 mL Sartobind® S membrane at a loading density of 0.38 g/mL CV. Buffer compositions, volumes, and residence times were applied as described in Table 3.
The chromatographic profile of polishing step 2, conducted with Sartobind® S in OBE mode, is shown in Figure 10.

Figure 10: Chromatographic profile of polishing step 2 using a 10 mL Sartobind® S CEX membrane device operated in OBE mode



Analysis of mAb purification performance and impurity clearance in the fully membrane-based process

Samples (load and output) from the mAb capture step employing Sartobind® Rapid A, as well as from subsequent polishing steps conducted with Sartobind® Q and S, were analyzed to assess process efficiency in terms of mAb recovery, purity, and impurity clearance. The results are presented in Tables 7 and 8.

The consistently high step yields (>90%) and overall recovery of 91.4% confirm the efficiency of mAb purification using Sartobind® chromatography devices for capture and polishing (Table 7). Notably, the mAb concentration nearly doubled during purification, increasing from 1.9 mg/mL in the CCCH to 3.5 mg/mL in the final output of the Sartobind® S polishing step (Table 7).

The 114.4% recovery observed for the Sartobind® Rapid A capture step is likely due to analytical method differences: the load (CCCH) was quantified by Protein A HPLC, whereas the eluate was measured at UV 280 nm.

SEC-HPLC analysis of the monomer peak, along with HMWS and LMWS species, confirmed high molecular purity of the mAb (Table 7). The monomer peak remained consistently above 97% throughout all three purification steps, while HMWS remained < 1% and LMWS did not exceed 1.5%.

Consistent with previous findings, these low levels of product-related impurities were not further reduced during AEX polishing, indicating that aggregate removal had reached its efficiency limit.

In addition to high mAb recovery rates and substantial purity, the membrane-based purification process demonstrated excellent clearance of process-related impurities. HCP, hcDNA, and Protein A were reduced to levels well below commonly accepted clinical thresholds (Table 8).

HCP levels were significantly reduced by 2.6 LRV during mAb capture with Sartobind® Rapid A, followed by a further reduction of 1.66 LRV during AEX polishing with Sartobind® Q. Finally, after CEX polishing with Sartobind® S, HCP levels were reduced to 14 ppm.

hcDNA was reduced by 3.0 LRV during mAb capture and to below the detection limit of 0.3 pg/10 µL in the subsequent polishing steps. Leached Protein A, initially present at a low concentration of 3 ppm in the Sartobind® Rapid A eluate, was also reduced to below detectable levels.

In summary, the high recovery rates and effective impurity clearance demonstrate the efficiency of the purification process, based exclusively on Sartobind® devices, and meet key requirements for clinical-grade mAb production.

Table 7: Summary of mAb recovery and purity analysis of the fully membrane-based purification process using exclusively Sartobind® chromatography devices

Analytical results	Sartobind® Rapid A		Sartobind® Q (AEX)		Sartobind® S (CEX)	
	Load	Output	Load	Output	Load	Output
mAb [g]	4.22	4.82	4.32	3.92	3.8	3.9
mAb [mg/mL]	1.9	7.8	5.0	4.2	3.9	3.5
mAb step recovery [%]	114.4		90.8		101.6	
mAb total process recovery [%]			91.4			
Monomer peak [%]	n.a.	97.6	97.9	97.8	98.0	97.9
HMWS [%]	n.a.	0.9	0.9	0.9	0.7	0.7
LMWS [%]	n.a.	1.5	1.2	1.3	1.3	1.4

Table 8: Summary of impurity removal analysis of the fully membrane-based purification process using exclusively Sartobind® chromatography devices

Analytical results	Sartobind® Rapid A		Sartobind® Q (AEX)		Sartobind® S (CEX)
	C1 load	C1 output*	C2 load	C2 output	C3 output
HCP [ppm]	3.35×10^5	9.01×10^2	6.93×10^2	1.5×10^1	1.4×10^1
HCP LRV	2.6		1.66		n.a.
hcDNA [ng/mL]	1.9×10^5	171	37	1	BDL
hcDNA LRV	3.0		1.6		n.a.
rPA [ppm]	n.a.	3	2	1	BDL

Note. Below detection limit (BDL) values are as follow—hcDNA: 0.3 pg/10 µL, rPA: 0.16 ng/mL.

*pH of the eluate adjusted to pH 8.0 using 2 M Tris solution

Discussion

Traditional resin-based chromatography, while effective, presents inherent limitations in processing speed, scalability, and operational flexibility. In this study, we explored an intensified fully membrane-based purification process, employing exclusively Sartobind® chromatography devices for mAb capture and both AEX and CEX polishing steps.

Given the unique characteristics of both the mAb-producing cell culture and the mAb itself, the initial phase of the study focused on optimizing the capture and polishing steps under these specific conditions.

mAb capture using Sartobind® Rapid A was optimized by evaluating the DBC to assess loading capacity and RT dependency. A conservative loading density of 40 mg/mL CV at 0.2 min RT was chosen, significantly below the commonly accepted 80% of the 10% DBC. Further process optimization focused on several key parameters:

- Minimizing NaCl concentration during high-salt washing to enhance mAb recovery and impurity removal while reducing salt consumption
- Reducing RT to 0.1 min for all steps beyond loading and elution to accelerate processing
- Adjusting the pH of the final wash buffer and the acetic acid concentration in the elution buffer to achieve an eluate with pH 4 and low conductivity, ensuring mAb stability prior to VI and optimal conditions for subsequent CEX
- Defining the appropriate elution window to maximize product yield and purity

The refined chromatographic protocol was successfully used for mAb capture in the three-step mAb purification process, in which all three Sartobind® chromatography steps were carried out in series. Although buffer volumes were not included in the optimization studies, they were reduced in this process to further enhance efficiency.

Using two 10 mL Sartobind® Rapid A devices in parallel B-MCC, 4 g mAb from 10 L HCCF were purified in 10 capture cycles (5 in parallel). Each cycle was completed in ~40 minutes, which is significantly faster than the ~2 hours typically required with Protein A resin, allowing full processing of 10 L feed within 3.3 h.

Beyond rapid processing, the Sartobind® Rapid A eluate contained high-quality mAb, as reflected in a monomer peak content of 97.6%, and low aggregate levels of 0.9% HMWS and 1.5% LMWS. Furthermore, HCP was substantially reduced by 2.6 LRV, while leached Protein A was low (3 ppm), highlighting the successful refinement of buffer solutions.

The initial strategy for optimizing Sartobind® Q AEX polishing involved adjusting the Protein A eluate to pH 8.0 and a conductivity below 4 mS/cm, allowing flow-through of the mAb while effectively retaining process-related impurities. This approach already demonstrated excellent clearance efficiency, achieving a ~ 1.4 LRV for HCP, while hcDNA and leached Protein A were reduced to below the detection limits.

Therefore, no further optimization was performed when Sartobind® Q was used in the three-step mAb purification process. Although a pressure increase necessitated the replacement of the first of two serially connected membranes, AEX polishing achieved a remarkable mAb recovery of ~91%. Process-related impurities were reduced to clinical-grade levels, with HCP clearance of 1.66 LRV even exceeding the results of the test run.

CEX polishing with Sartobind® S followed the initially tested OBE approach showed an efficient HMWS reduction of ~50%. However, in this test run, aggregate levels had been artificially increased, as the natural content was already at an accepted clinical-grade level and below the membrane's clearance capability. Since no artificial aggregate increase was performed in the three-step purification process, the low aggregate levels remained unchanged. Notably, in this specific process, a single AEX polishing step using Sartobind® Q would have been sufficient. However, CEX utilizing Sartobind® S was included to demonstrate the feasibility of a complete three-step mAb purification process using Sartobind® chromatographic devices as a smart alternative to traditional resin-based chromatography, and to highlight the flexibility in applying the CEX step to feed material with elevated HMW content.

Conclusion

Replacing traditional resin-based chromatography with membrane chromatography consumables presents a compelling strategy for streamlining downstream processing of mAbs. Compared to resins, membrane devices offer faster processing, linear scalability, easy handling, and a smaller footprint without compromising product quality.

Analytical results from the purification process, which relied exclusively on Sartobind® membrane devices for both mAb capture and polishing, demonstrate clinical-grade mAb purity, highlighting the strong potential of membrane-based approaches as a rapid, robust, and efficient alternative to resin chromatography for fast development and process validation of mAb-based therapies.

References

1. Gruenberg M, Kuchemueller KB, Toeppner K, Busse RA. (2022) Sartobind® Rapid A: Robust mAb Capture at High Productivity. [Application Note] Sartorius. www.sartorius.com/download/1692296/sartobind-rapid-a-robust-capture-high-productivity-applicati-1-data.pdf

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