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Application Note

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Optimizing and Intensifying ADC Aggregate Removal: A Design of Experiments (DoE) Approach to Membrane Chromatography and Rapid Cycling

Geoffrey Pressac[1,*], Timo Schmidberger[2]

1. Sartorius Stedim FMT S.A.S., Zi des Paluds, Avenue de Jouques – CS 91051, 13781 Aubagne Cedex
2. Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen

*Correspondence
Email: geoffrey.pressac@sartorius.com

Abstract

Antibody-drug conjugates (ADCs) combine monoclonal antibodies with potent small-molecule payloads for targeted cancer treatment. The conjugation process usually promotes the generation of aggregates and undesired drug-to-antibody ratio (DAR) species which must be removed by following purification steps. This study explores the feasibility of using single-use chromatography membrane technology as an alternative to resin-based chromatography in an ADC process.

Introduction

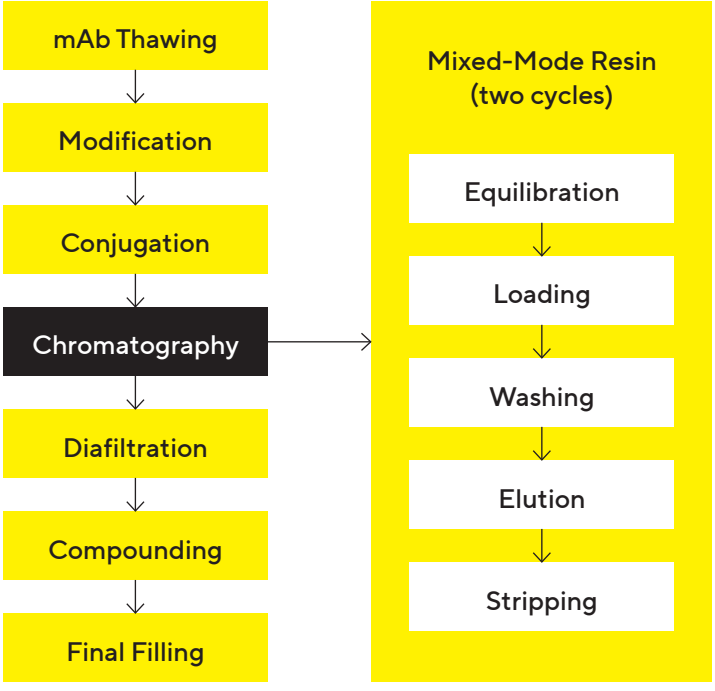
Chromatography resins have historically been the mode of choice for downstream purification in bioprocesses. While they are widely established in the industry, they are limited by low flow rates, meaning they suffer from low productivity. Additionally, resins are expensive and require non-value-added activities such as column packing and cleaning. As an alternative, chromatography membranes have large pores which support convective mass transport, enabling a 10-fold increase in process flow rates compared to resin-packed columns. The resulting high productivity can enable a rapid-cycling chromatography (RCC) approach that further reduces footprint. Single-use membrane adsorbers also reduce the risk of cross-contamination while freeing up facility resources.

ADCs represent a growing therapeutic segment of the oncology field. They combine highly potent small-molecule payloads with monoclonal antibodies (mAbs) to improve their specificity as a cancer treatment. ADC manufacturing requires fully purified antibodies, which are grafted chemically with cytotoxic agents by linker molecules. Aggregates, free payloads, and both low- and high-DAR species are the main impurities present in ADC process streams.

Our goal in this study was to assess a single-use chromatography membrane technology as a potential replacement for resin currently used in an established ADC process (figure 1) in which a mixed-mode resin is loaded at <30 g/L sorbent, achieving a yield of 98%, 30% clearance of high–molecular-weight (HMW) species, and overall productivity of 4 $g_{ADC}/L_{resin}/h$.

Sartorius' Sartobind® membranes were able to intensify the established ADC process (figure 1) and enhance aggregate clearance without compromising yield. These results were originally published in BioProcess International in October 2022[1].

Figure 1: *Current Downstream Process for the Studied Antibody–Drug Conjugate (ADC).*



Materials and Methods

Full materials and methods are available in Bendelac et al. (2022)[1] and are briefly outlined below.

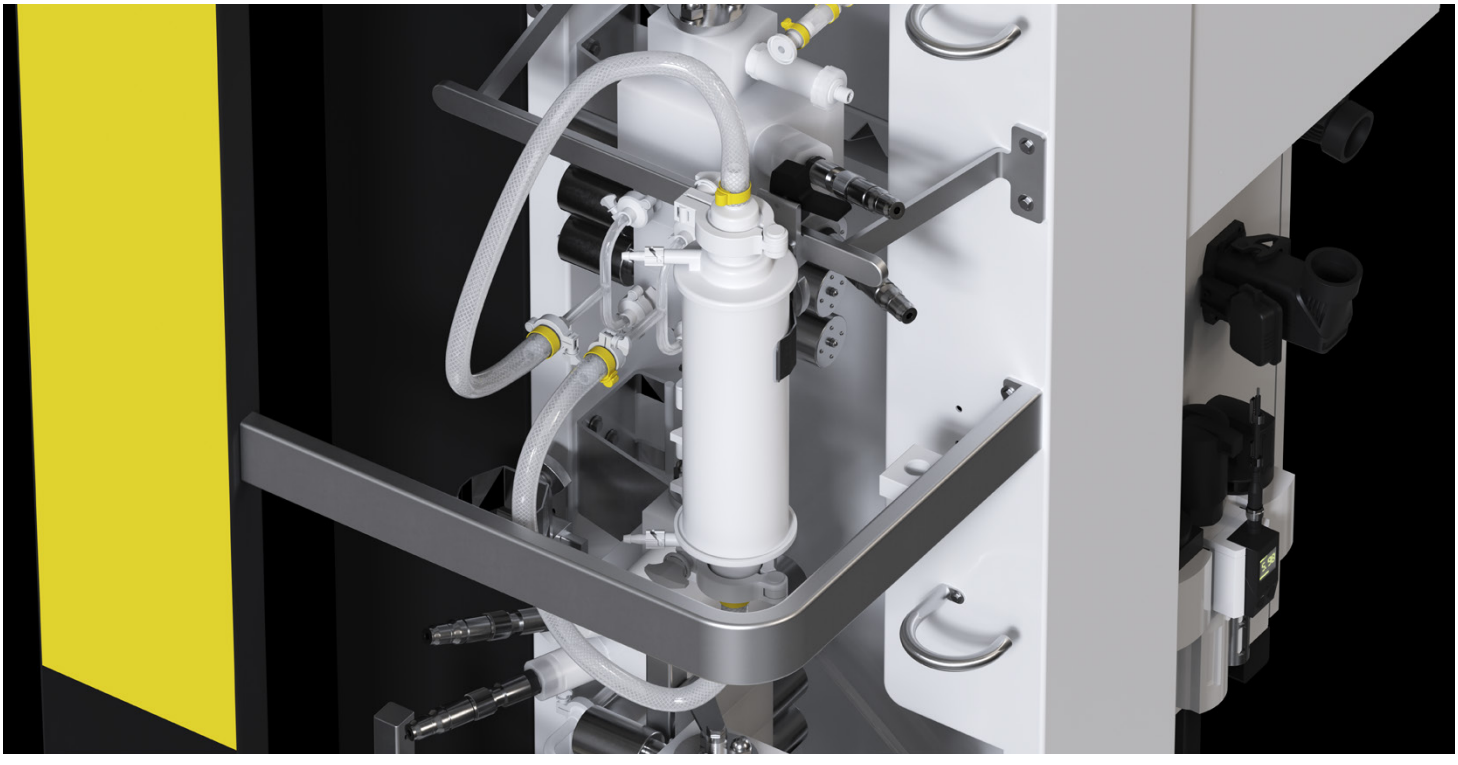
All tests used an ADC produced from stochastic conjugation on IgG_1. Small-scale experiments were conducted with Sartobind® Q Nano capsules (1 mL of membrane and 4 mm bed height for initial screening and 3 mL of membrane and 8 mm bed height for optimization).

A design of experiments (DoE) approach was applied to optimize the step and evaluate more precisely the influence of both pH and conductivity on membrane performance. This informed the selection of working conditions for a second DoE study to define the design space in a quality by design (QbD) approach. A Sartobind® Q Nano 3 mL capsule and 180 mg of ADC were used to study the impact of pH, conductivities, and ADC concentration on yield, DAR, and HMW clearance. The selected working conditions were used to study 10 cycles on the same Sartobind® Q Nano membrane.

Analytics
DAR was determined using three techniques: UV spectroscopy, size exclusion chromatography (SEC), high-performance liquid chromatography (HPLC), and high-resolution mass spectrometry (HRMS). Recovery was calculated by comparing the total amount of ADC loaded with that recovered in the flow-through and buffer flush solutions. HMW clearance was assessed using the same approach.

Data Analysis
Sartorius' MODDE® software (version 13) was used for experimental design and analysis at each phase of screening and optimization. Based on the nature of the factors tested (multilevel) for the screening phase, a D-optimal design was chosen. For the optimization, a face-centered central composite design was chosen. Both could support models to account for primary effects, interactions, and quadratic effects.

For model fitting, we used the software's analysis wizard and removed nonsignificant parameters to optimize the model's predictive power, indicated by Q^2 values. We could then use the optimized models to decide which process condition was superior based on a contour plot. R^2 shows how well a generated model describes the data; Q^2 shows how well the model can be applied to future data that were not used for the model. The latter value is determined by a leave-one-experiment-out methodology. High values in R^2 and Q^2 were targeted, with the maximum value being 1 in each case.

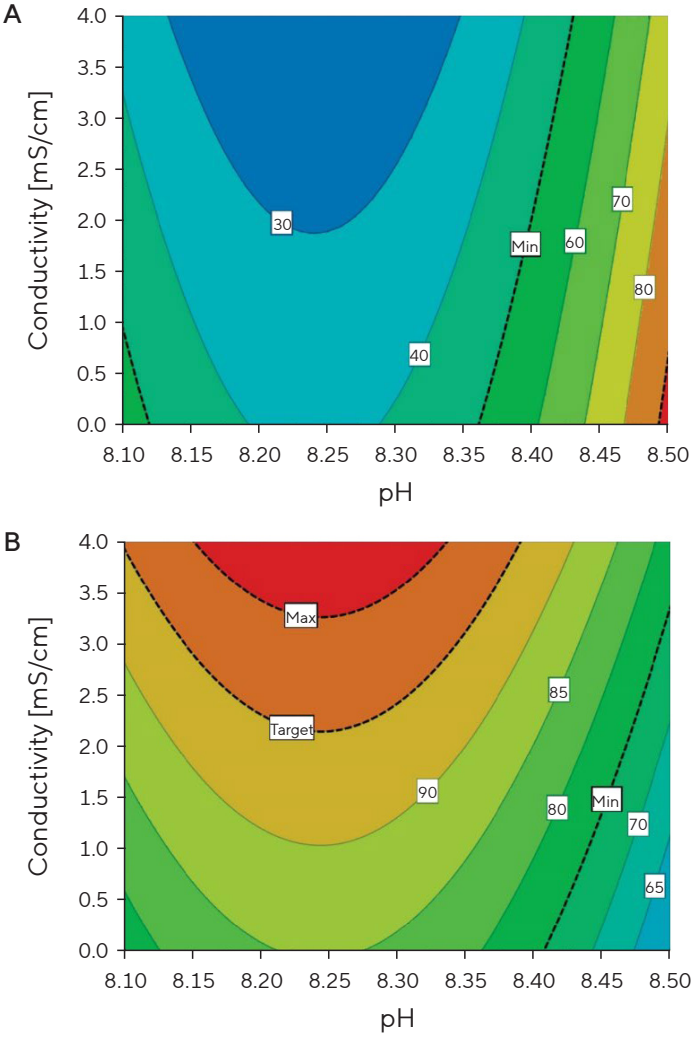


Results

Screening DoE

The influence of pH and conductivity on HMW retention, yield, and DAR were examined closely. Using a DoE approach, we investigated three levels of pH (all above pI) and three levels of conductivity with a D-optimal factorial plan, including three repetitions of a center point (figure 3).

Figure 3: *Effects of Buffer Conditions on HMW Clearance (A) and Yield (B) Using a DoE Approach*



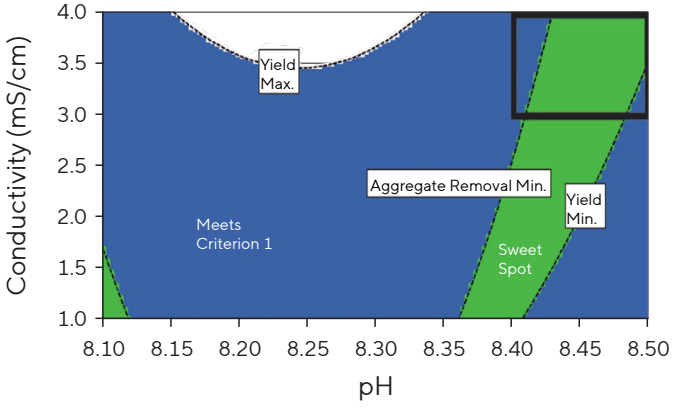
Note. ADC starting solution was adjusted in nine phosphate buffers (pH 8.1, 8.3, and 8.5; conductivity 1, 2, and 4 mS/cm). At a starting concentration of 3.6 g/L, the suspensions were filtered through a Sartobind® Q Nano 1 mL capsule (4 mm bed height) at 10 MV/min. Yield (B) and aggregate removal (A) were evaluated with UV spectroscopy and size-exclusion (SEC) high-performance liquid chromatography (HPLC), respectively.

For both HMW removal and yield, we identified a significant nonlinear effect. A plateau is observed (e.g., for pH between 8.2 and 8.3) when small changes in pH and conductivity do not change HMW removal or yield, indicating process robustness. The resulting model quality metric showed acceptable validity, with acceptable descriptive power for yield ($R^2 = 0.86$) and HMW ($R^2 = 0.73$). But for HMW, the model was not strongly predictive ($Q^2 = 0.23$). The best aggregate clearance was obtained at higher pH (>8.4). The influence of conductivity was limited once pH was >8.35 and was stronger between pH 8.15 and 8.35.

We obtained the highest yield between pH 8.15 and 8.35 with conductivities in the upper range (> 2 mS/cm). DAR remained in the targeted range throughout; nevertheless, we observed that high-DAR species tended to be retained more at low conductivities. Also, lower DAR in the flow-through correlated with stronger HMW removal.

Comparing these two plots shows an inverse correlation of both parameters: High yield mostly correlates with low aggregate removal. Consequently, optimal yield and HMW clearance did not overlap. The software's sweet-spot functionality was used to find the best compromise between these two factors (figure 4). The acceptable compromise was at pH >8.4 and conductivities >3 mS/cm, which also met our DAR target. This information was used to perform a new DoE study centered on the optimal point to assess process performance using a QbD approach (black box in figure 4).

Figure 4: *Sweet-Spot Plot Balancing HMW Clearance and Yield*



Optimization DoE and Test of the Design Space

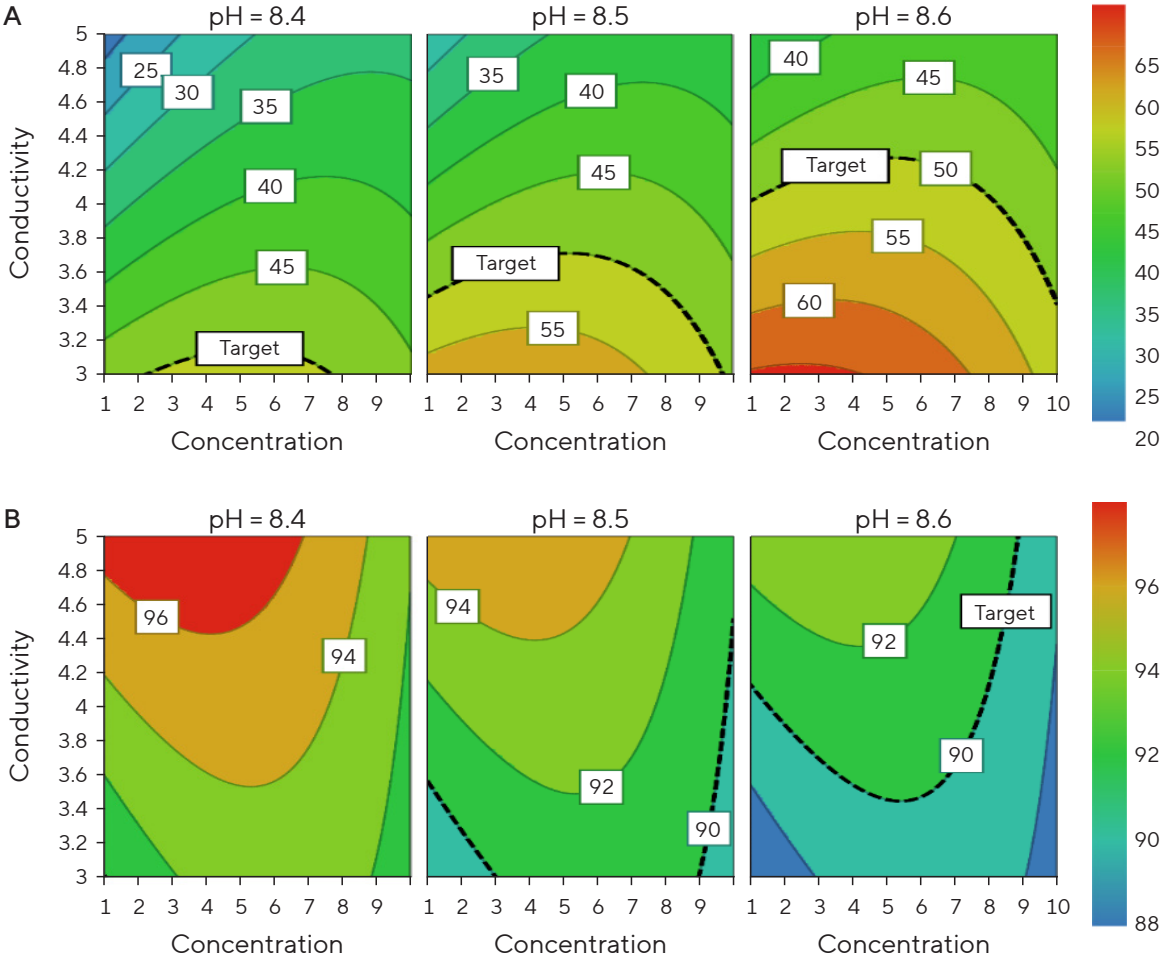
Selected pH levels for this DoE study were 8.3–8.5; selected conductivities were 3–5 mS/cm. ADC concentrations at 1, 5.5, and 10 g/L were also tested. Figure 5 shows the resulting contour plots, with responses for HMW clearance and product yield. The HMW clearance model gave an R^2 of 0.88, Q^2 of 0.67, and a strong validity (0.66). For the yield model, R^2 was 0.77, Q^2 was 0.31, and model validity was lower (0.33) but still >0.25 (below 0.25 would indicate a significant lack of fit).

Aggregate removal was higher than in the established column chromatography process (30%) in most conditions (figure 5A) and negatively affected by high conductivities, high concentrations (>8 g/L), and lower pH levels (8.4). The yield was also >90% target in most conditions (figure 5B). It was maximum at lower pH levels and higher conductivities, confirming an opposite trend for HMW removal. However, yield was also limited by high ADC concentrations (>8 g/L).

Working conditions had less of an influence on DAR. It was nearest to the starting value when the yield was high and aggregate removal was low. DAR in this study was at most 0.3 units below that observed with the established purification process.

In all cases, the model allowed us to determine that the parameter with the strongest influence overall was conductivity in this design space. Aggregate removal was efficient with the membrane adsorber because the key attributes almost always met the target specifications. However, this DoE also highlights the need for tight control over both pH and conductivity.

Figure 5: D*esign Space for Aggregate Removal on Sartobind® Q*



Note. The impact of working conditions on aggregate removal (A) and yield (B). ADC starting solution was adjusted in different phosphate buffers (pH of 8.4, 8.5 or 8.6 and conductivity of 3, 4 or 5 mS/cm); and concentrations of 1; 5.5 or 10 g/L. The suspensions were filtered through Sartobind® Q Nano 3 mL (8 mm bed height) at 5 MV/min. Yield, aggregate removal and DAR were evaluated using SEC HPLC and HRMS. Target aggregate clearance was set at 50 % and target yield was set at 90 %.

Process Intensification Through Membrane Cycling

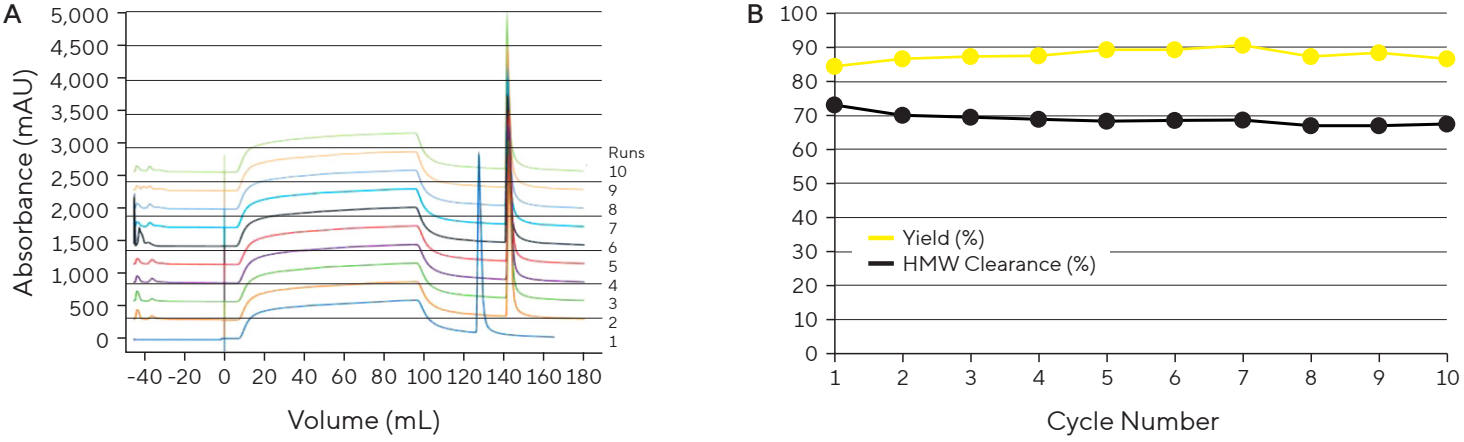
Membrane loading was identified as a key parameter from the beginning of our study. Therefore, we expected cycling to greatly benefit footprint and costs. Ten cycles were run on a Sartobind® Q Nano 3 mL capsule as proof of concept for an RCC approach. According to our model, the influence of pH was low within the working range. Therefore, we chose loading conditions that achieve a central pH from the DoE and maximize HMW clearance while targeting a yield of 90%: phosphate buffer, pH 8.5, conductivity 3.5 mS/cm, and 2 g/L ADC concentration.

The cycling study achieved stable pressure and overlapping UV280 peaks for 10 cycles, suggesting no membrane fouling and stable performance (figure 6A). Yield was comparable to the model's predicted value (88–90%), ranging from 85–91%, and the total pool value was 87% (figure 6B). We do not consider the observed variations to be significant.

However, HMW removal was above expectations: 67–73% (a 69% average value from the filtrate pool), with 55–60% predicted by the model. This remained stable over 10 cycles, with a minor trend toward reduction in the clearance, which dropped from 73% for the first cycle to 68% for the final cycle. This could be within the assay variability but should be confirmed to determine whether the regeneration procedure should be optimized. Nevertheless, HMW clearance results exceeded our initial target of 50% and the resin benchmark of 30%.

Finally, the DAR was slightly below (by 0.3 units) what was observed with the established process but remained within specifications.

Figure 6: *Membrane Cycling Study*



Note. A) Overlay of chromatograms from 10 cycles; B) Evolution of key quality attributes over 10 cycles. The cycling study used 1 mM phosphate buffer at pH 8.5 and 3.2 mS/cm conductivity. ADC concentration was adjusted to 2 g/L. Suspensions were filtered through a Sartobind® Q Nano 3 mL capsule (8 mm bed height) at 5 MV/min. UV280 absorptions were overlapped from the 10 cycles to detect performance issues over reuse (left). Yield and aggregate removal were evaluated using SEC-HPLC, then plotted over the 10 cycles (right).

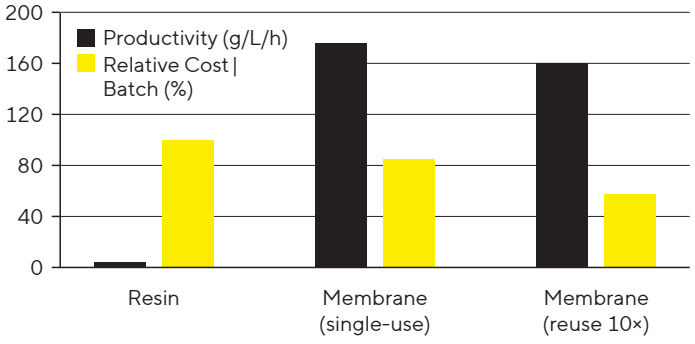
Discussion

Two conclusions can be drawn from these experiments. First, the MODDE® model predicted the performance of our membrane-adsorber-based process well in terms of yield (for which predictability was the lowest among the three parameters). The real results of HMW removal were higher than the model predicted. Thus, the DoE model can be considered a worst-case scenario.

Second, a rapid-cycling approach maximizes the use of the membrane adsorber without significantly compromising the quality or yield of our ADC end product compared to a resin-based process. Further studies should use a higher number of cycles and possible refinement of the regeneration procedure (only 1 M NaCl was used herein).

Using process projections, we evaluated the effect of switching from our established column-chromatography process to one based on this membrane-adsorber technology (figure 7). Based on our cost model tool, the switch could enable us to reduce our process footprint significantly by replacing 31.8 L resin with a 1.2 L membrane. The associated increase in productivity (from 4 to 176 g/L of sorbent per hour), with reductions in both time (from 12 to 6.5 hours) and buffer requirements (saving >40%), provides a significant cost reduction of 15%. In addition, switching from a resin to a membrane process would make it possible to perform this process step in a single work shift, facilitating production planning.

Figure 7: *Comparison of Cost and Productivity Across Different Chromatography Methods*



Note. A process modeling tool was used to assess the costs of using the current chromatography resin (31.8 L volume, reused for 50 batches) or switching to a single-use Sartobind® Q 1.2 L membrane for 20 cycles per batch. The possibility of reusing the membrane for 10 batches also was evaluated. All costs were considered, including working time, column packing once per year, column maintenance, reuse validation, resin lifetime studies, buffers, and hardware investments.

Figure 7 also highlights that, if needed, the membrane could be reused for 10 batches and thus decrease the process costs further (~43% compared with the resin-based process). Further studies would be required to demonstrate the stability of membrane performance over time. However, Sartorius has demonstrated reusability of the membrane up to 1,000 cycles.

Conclusion

Our study demonstrated that Sartobind® membrane used in RCC mode can:

- Remove HMW species from the ADC drug product more efficiently than the current resin (70% reduction) without significantly impacting the DAR
- Maintain a high yield (90%)
- Multiply productivity by 44-fold (from 4 to 176 g/L of sorbent per hour)
- Reduce consumable volume by 26x (from 31.8 to 1.2 L)
- Reduce process time from 12 to 6.5 hours, manageable within a shift
- Reduce buffer consumption by over 40% (in single use)
- Reduce costs by 15% (single use) to 43% (if reused),
- Enable faster time to market by alleviating cleaning validation and shelf life studies (if single-batch used)
- Enable more agile manufacturing (no column storage, smaller footprint, smaller consumable, and faster processing)

Sartorius' Field Application Team is ready to support all customers to improve their processes and build flexible, highly productive facilities. Contact a representative today at www.sartorius.com.

References

[1] Bendelac, A., Schmidberger, T., Fabre, B., Lacoste, E., & Pressac, G. (2022). Optimizing and Intensifying ADC Aggregate Removal. BioProcess International, 20(10). https://bioprocessintl.com/analytical/downstream-development/optimizing-and-intensifying-adc-aggregate-removal-a-doe-approach-to-membrane-chromatography-and-rapid-cycling/

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further information, visit
www.sartorius.com