Antibody therapeutics

Leveraging 3 distinct resins to provide effective impurity removal for mAbs and novel antibody derivatives

Authors
Michelle Nolasco Rivera, Andrew Siemers, Kristina Pleitt, Alejandro Becerra, Thermo Fisher Scientific

The use of monoclonal antibodies (mAbs) and novel antibody derivatives continues to grow thanks to the need for more efficacious drugs for patients and increasingly innovative and scalable platforms. However, drug sponsors and CDMOs looking to manufacture these molecules must adeptly navigate high levels of product-related impurities. More complex antibody molecules — such as bispecifics, Fc-fusion proteins, and Fab fragments — often have additional product-related impurities to remove, posing added purification challenges. These molecules may lack traditional protein A binding sites, be pH sensitive, present higher impurity levels, and/or have a higher propensity for aggregation.

Due to these challenges, biomanufacturers need a robust toolkit of purification techniques to manufacture these molecules and achieve target purity levels to make them safe and efficacious.

Background

Protein aggregation can be caused by a number of manufacturing steps, including higher expression in cell culture systems, operating conditions (pH/conductivity), mechanical stresses (e.g., shear), freeze/thaw cycles, or storage conditions. Higher aggregate levels can lead to process related problems like filter fouling or reduced column loadings, increasing overall costs of production. These impurities can also impact the efficacy and safety of the drug product.

Ideally, it is best to prevent product aggregation from the outset of a project through optimization of the upstream, including selecting clones and molecule constructs which produce low aggregate levels. Regardless, the downstream process needs to remove process- and product-related impurities, including aggregates, to meet target levels.

Among chromatographic approaches for aggregate removal, cation exchange (CEX) and hydrophobic interaction chromatography (HIC) are the most widely used; significant industry-wide knowledge is available for these techniques. Anion exchange (AEX) and mixed mode anion exchange (MMAEX) chromatography are sometimes utilized, though they may be less effective or require more optimization. Mixed-mode cation exchange chromatography (MMCEX) can also be effective to remove aggregates and other product and process related impurities.

The downstream process for mAbs typically begins with affinity capture with Protein A, followed by two polishing steps. The aggregate removal resins can be positioned as the first or second step after affinity capture, depending on the needs of a specific process.

The other polishing step, most commonly AEX or MMAEX, is part of most processes to ensure robust virus clearance and to remove any residual impurities, particularly DNA and host cell proteins (HCP).

Case study: Evaluation of different tools

This case study examines the aggregate removal capabilities of three Thermo Scientific POROS™ resins. Thermo Scientific™ POROS™ XS Cation Exchange Resin (CEX), Thermo Scientific™ POROS™ Benzyl Ultra Hyrdophobic Interaction Chromatography Resin (HIC), and POROS™ Caprylate Mixed-Mode Cation Exchange Resin (MMCEX) were evaluated using the same load sample. An IgG1 monoclonal antibody (pI ~8) was produced in CHO cell culture and initially purified with Thermo Scientific™ MabCaptureC Protein A resin. The aggregate level of this pool was ~ 7% and the aim of the study was to reduce these high molecular weight species (HMWS) to ≤ 2%. As mentioned above, CEX and flowthrough HIC are commonly used in the field, thus, the screening and optimization of POROS XS and POROS Benzyl Ultra relied on prior knowledge with those resins. On the other hand mixed mode chromatography resins typically require more optimization of operating conditions. Thus, for POROS Caprylate, an initial partition coefficient (Kp) screening followed by a Design of Experiments (DoE) approach were taken to find the optimal window of operation of this resin in isocratic mode.

I. Aggregate removal with POROS XS Cation Exchange Resin

POROS XS was equilibrated in 50 mM acetate buffer at pH 5.3 + 20 mM NaCl, and post-Protein A IgG1 mAb material — adjusted to match the equilibration pH and conductivity — was loaded to 80 g/L_{resin}. After loading, the column was washed with 5 column volumes (CV) of 50 mM acetate + 50 mM NaCl. Next, the mAb was eluted using a linear gradient from wash buffer to 50 mM acetate pH 5.3 + 500 mM NaCl over 20 CV. All steps were operated at a residence time of 3 minutes. The chromatogram for this run is shown in Figure 1.

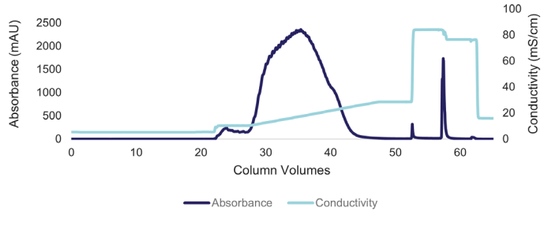

Figure 1. A chromatogram for mAb purification with POROS XS; UV 280 nm is shown in dark blue and conductivity is shown in light blue.

During the elution, fractions were collected and analyzed by analytical size exclusion chromatography (aSEC) to determine the monomer and aggregate content. As illustrated in Figure 2, the monomer eluted first at about 10-12 mS/cm and the aggregate co-eluted toward the end of the monomer peak with measurable levels only in fractions above ~20 mS/cm.

A closer look at the aSEC chromatograms in Figure 3 reveals the initial sample had both dimers and trimers. Both of those HMWS were removed completely in the initial fractions with no detectable peaks in the fraction collected at 16 mS/cm.

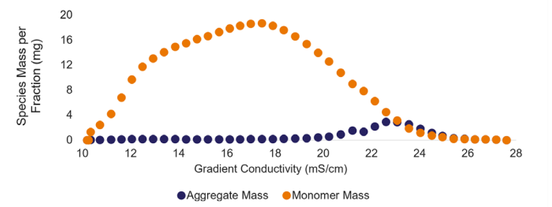

Figure 2. Analytical aSEC chromatograms showing monomer and aggregate levels during elution with POROS XS; aggregate shown in dark blue and monomer shown in orange. Relative composition of monomer and aggregate during gradient elution with POROS XS

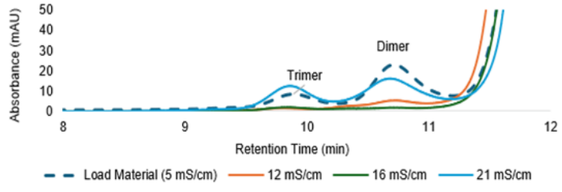

Figure 3. Overlay of aSEC chromatograms for the load sample and selected fractions from the gradient elution with POROS XS.

A theoretical elution pool was calculated taking the fractions collected from 10 mS/cm to 21 mS/cm. This product pool achieved a reduction of 82% and 58% of dimer and trimer, respectively, with a monomer recovery of 88%.

II. Aggregate removal with POROS Benzyl Ultra Hydrophobic Interaction Chromatography Resin

Next, POROS Benzyl Ultra was operated in flow-through mode with the post-Protein A mAb sample adjusted to 10 mM citrate at pH 6. The resin was loaded to 250 g/L_{resin} at a 3-minute residence time. As shown in the chromatogram in Figure 4, the UV absorbance does not increase during the load phase until approximately 5 CV, suggesting that both the monomer and aggregate are binding.

As the loading continues, the monomer species are displaced by aggregates. This is illustrated in Figure 5, where, at 25 g/L_{resin} loading, there is no breakthrough of monomer whereas, at loadings ≥75 g/L_{resin} , the monomer breakthrough is ~100% (C/Co = 1). In the case of the aggregates, the breakthrough curve is shallow and remained relatively flat throughout the 250 g/L_{resin} loading.

The quantitative analysis of the fractions taken during the loading indicated a monomer recovery of ~92% and a reduction in aggregate levels from 7% to 1.6% at 250 g/L_{resin} loading. The aSEC profile shown in Figure 6 indicates trimers were present on the 125 and 250 g/L_{resin} fraction, which is the likely reason for the shallow and relatively flat aggregate breakthrough curve. A closer look at the individual HMWS species indicated a reduction of ~87% dimer and ~44% trimer.

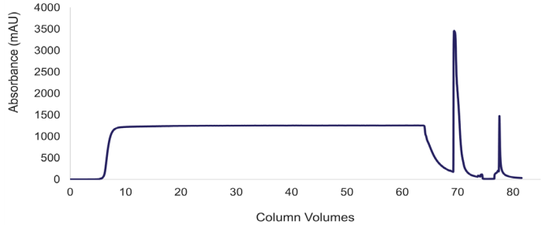

Figure 4. UV 280 nm Chromatogram for POROS Benzyl Ultra operated in flowthrough mode. The equilibration step is not shown and the load phase starts at 0 CV.

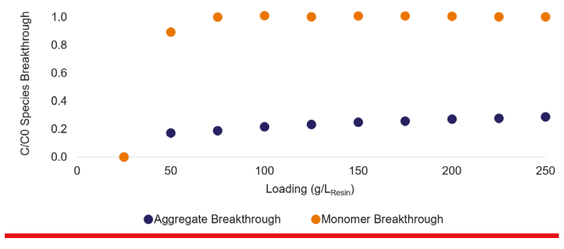

Figure 5. Breakthrough curves of monomer and aggregate as a function of loading onto POROS Benzyl Ultra..

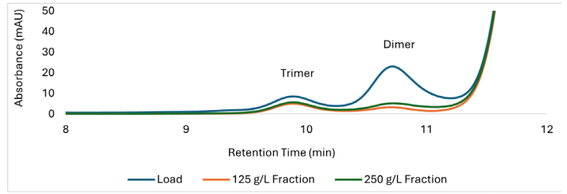

Figure 6. aSEC chromatogram for load sample and selected fractions for POROS Benzyl Ultra.

III. Aggregate removal with POROS Caprylate Mixed-Mode Cation Exchange Resin

POROS Caprylate is a recently launched mixed-mode cation exchange resin and thus the prior knowledge with this product is limited. Taking that into consideration, a more comprehensive experimental plan was undertaken to find optimal conditions for aggregate removal with the mAb tested. First, a Kp screen was conducted to determine the relative strength of the binding to the resin for both the monomer and the aggregate over a broad range of pH (4.5 to 7.5) and NaCl concentrations (0-400 mM). Briefly, a series of batch binding experiments at low loading (10 g/L_{resin}) were performed in this operating space. The goal was to identify the pH and conductivity ranges where the Kp of the monomer and the Kp of the aggregate are relatively low and high, respectively. The Kp values for monomer and aggregate are shown in Figure 7. Based on these data, the pH and NaCl concentration range for column experiments was narrowed down to 5.0-5.5 and 320-480 mM, respectively.

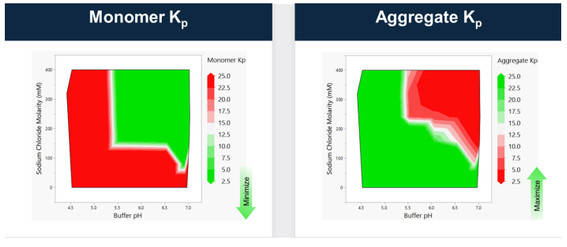

Figure 7. K_p contour plots for monomer and aggregate with POROS Caprylate.

These operating ranges were then explored in a DoE using 1 mL columns and target mAb loadings of 100 g/L_{resin} at a 3 min residence time. The results of these experiments are shown in Figure 8. The aggregate levels were reduced by >75% in the experimental space tested with increasing clearance at lower pH levels. On the other hand, the monomer recovery ranged from 82-96% with higher recoveries at higher pH and NaCl concentrations. These data were further analyzed using a statistical software and the optimal operating range was calculated to be pH ~5.3 and a NaCl concentration of ~375-400 mM.

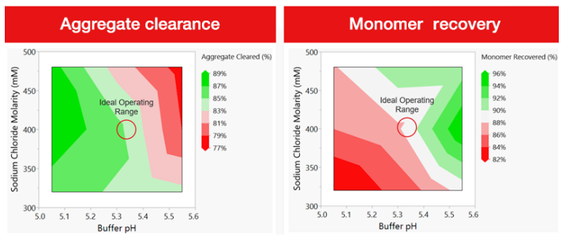

Figure 8. DoE results for POROS Caprylate optimization. Left, % aggregate cleared. Right, % monomer recovery. The loading was fixed at 100 g/L_{resin} in this set of experiments.

Using these conditions, a 1 mL POROS Caprylate column was loaded to 250 g/L_{resin} at a 3-minute residence time. The chromatogram shown in Figure 9 shows a similar UV trace to that observed with POROS Benzyl Ultra. Specifically, the increase in absorbance during the load is not immediate but occurs after a few CVs, suggesting partial binding where both species are binding and aggregates are displaced in the monomer.

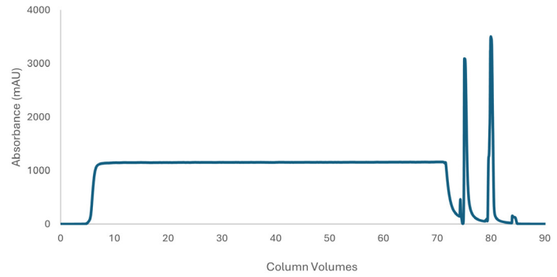

Figure 9. UV 280 nm chromatogram for POROS Caprylate operated in flowthrough mode. The equilibration step is not shown and the load phase starts at 0 CV.

The breakthrough curves of monomer and aggregate are shown in Figure 10. At a loading of 50 g/L_{resin} the monomer has broken through completely whereas the aggregate shows a shallow breakthrough curve.

In Figure 11, the aSEC chromatogram shows removal of HMWS species. Specifically, a reduction of 84% and 38% of dimer and trimer species, respectively, was achieved. The final pool at 250 g/L_{resin} had aggregate levels < 2% and a monomer recovery of ~97%.

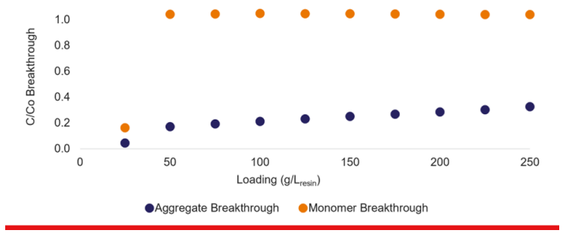

Figure 10. Breakthrough curves of monomer and aggregate for the POROS Caprylate run.

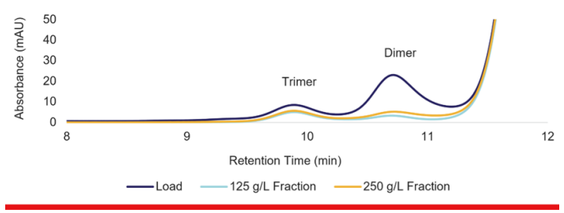

Figure 11. Analytical SEC chromatogram for load and selected fractions for POROS Caprylate run.

In summary, for each resin a set of operating conditions (i.e., pH, conductivity, loading) which resulted in an aggregate level of ≤ 2% were identified. The recoveries were also relatively high ranging from 88% with POROS XS to 97% with POROS Caprylate. These findings are summarized in Table 1.

Table 1. Summary of optimized conditions for POROS resins to achieve ≤ 2% aggregate levels.

POROS Resin	Mode of operation	Operating conductivity (mS/cm)	pH	Loading (g/L_{resin})	Recovery (%)
POROS XS	Bind/elute	21	5.3	80	88
Benzyl Ultra	Flowthrough	2	6.0	250	92
Caprylate	Flowthrough	38	5.3	250	97

Considerations for process development and scale up

Several factors need to be considered when selecting a resin. Process development timelines tend to be short so leveraging prior knowledge whenever possible can shorten optimization requirements. For example, there is significant knowledge in the field about CEX and particularly with POROS XS in mAb processes. The use of HIC in flowthrough mode has grown in the last decade so the optimization efforts for resins like POROS Benzyl Ultra are also relatively low.

Conversely, finding the specific operating window for mixed-mode chromatography resins require more resources, particularly for newer products like POROS Caprylate where prior knowledge is limited. The reuse and cleanability of the resins are also important factors to consider as these could impact the overall process economics. IEX and HIC resins are generally easier to clean because they can withstand high concentrations of NaOH. However, there are instances where highly hydrophobic mAbs, impurities, and/or resins may require additional efforts to optimize the cleaning regime.

There are also scale up factors to be considered. The position of a step in a process can impact the dilution requirements and facility fit , e.g., tank limitations, needs to be assessed. The column sizes , which depend on column loading, also affect the buffer and resin needs as well as associated costs.

To further assess these considerations, a typical clinical manufacturing mAb process case scenario was modeled using the results from the aggregate removal studies. Specifically, a 2,000 L bioreactor with a mAb titer of 4 g/L_{resin} using MabCapture C Protein A resin as a capture step was used for the calculations. A few process constraints were included in the modeling, including a maximum loading per cycle of 80 g/L_{resin} for POROS XS and 200 g/L_{resin} for POROS Benzyl Ultra and POROS Caprylate. For column sizing, a 20 cm bed height was used for all chromatography steps, and the position of the aggregate removal step was set as variable, i.e., before or after AEX chromatography. The output parameters of the model were CV, number of cycles per step, and intermediate product pool volumes.

In this scenario the column size required for POROS XS would be about 2x higher than that of POROS Benzyl Ultra or POROS Caprylate (57 vs 32 L). The lower CV requirement for POROS Benzyl Ultra or POROS Caprylate will also result in a concomitant decrease in buffer consumption (~70% less). In addition, the bind-and-elute modality with POROS XS usually required more washes than flowthrough operations. POROS XS would also need 2 cycles to process the same mass of antibody compared to 1 cycle for flowthrough operation with POROS Benzyl Ultra or POROS Caprylate.

The intermediate product pool volumes depend on the position of the aggregate removal step because the corresponding pH/conductivity adjustments need to be made, particularly dilution to operate the AEX step at low conductivity. As shown in Figure 12, when the aggregate removal step is positioned after Protein A, i.e., Polish 1, the pool volume after AEX is the lowest for HIC (~600 L) and the highest for MMCEX (~2000 L). Depending on the tank sizes available, this relatively large volume could be a limitation to implement MMCEX in this position.

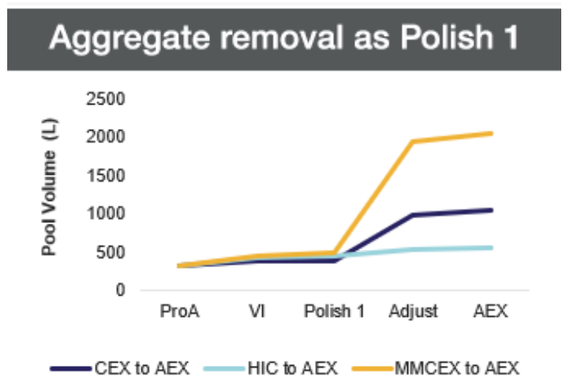

Figure 12. Intermediate product pool volumes for aggregate removal step positioned as Polish 1 (post Protein A).

Conversely, when the aggregate removal step is placed after AEX (Polish 2), the intermediate pool volumes are lower because dilution requirements are reduced. In this scenario, the largest intermediate corresponds to the adjusted pool when using CEX after AEX. The final pools are in the range of 430 L to 660 L for all cases as shown in Figure 13. It should be noted that placing the aggregate removal step after AEX will result in a higher impurity burden on that unit operation. While the risk is low, these additional impurities could negatively affect the HCP or virus clearance achieved with this step. These scale-up and process considerations are important when selecting resinsto develop a process.

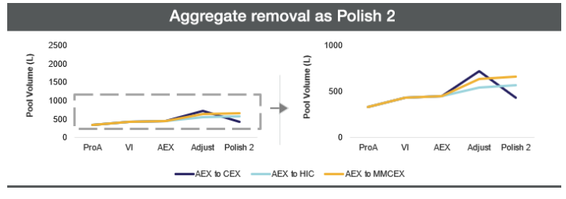

Figure 13. Intermediate product pool volumes for aggregate removal step positioned as Polish 2 (post AEX).

Conclusion

In this work, several chromatography options for effective aggregate removal in a mAb purification process were demonstrated. These products can also be used for complex antibodies where challenges with aggregates are more common. Tools like POROS XS (CEX) have been widely implemented in purification processes and therefore may be easier to develop. To address higher aggregate levels or scenarios where a higher loading is required due to facility fit constraints, POROS Benzyl Ultra (flow through HIC) or POROS Caprylate (MMCEX) may be better suited. The choice of resin also depends on the development timelines and experience with each option. Although there is no one-size-fits-all approach, Thermo Fisher Scientific solutions are available to develop a robust purification process for novel and traditional antibody formats.

There are three features that distinguish POROS™ technology from other chromatography resins:

- **A poly(styrene-divinylbenzene) backbone:** this highly rigid material enables a linear relationship between pressure and flow; it is robust, chemically stable, and easily scalable.
- **Particle size of the base bead:** the 50 µm material provides a good balance between resolution and pressure flow characteristics.

Large through pores: these allow for reduced mass transfer resistance, which in practice means sharper elution peaks and steeper breakthrough curves Thermo Fisher Scientific has multiple resin types for affinity capture and polishing:

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- **Thermo Scientific™ POROS™ ion exchange resins**
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- **POROS Caprylate Mixed-Mode Cation Exchange Chromatography Resin**

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