Aggregate Clearance in Flow-Through Mode Using Mixed-Mode Chromatography

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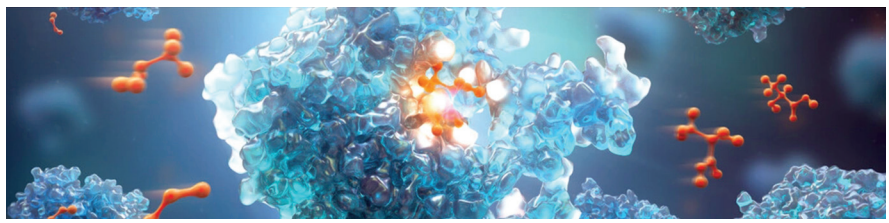

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Symmetric bispecific antibodies (bsAbs) contain two different binding domains per epitope and can be structured in many ways. For example, single-chain variable fragment (scFv) regions can be appended to different locations on a parent antibody, such as at the end of the crystallizable fragment (Fc) region or at the tops of the antigen-binding fragment (Fab) arms.

Symmetric molecules present purification challenges that differ from those of asymmetric bsAbs (including higher rates of aggregate formation), including higher-order aggregates, and fragments, as well as instability in a low-pH environment. A number of options are available for controlling aggregates during antibody production, each of which has limitations.

For example, bind-and-elute chromatography methods using cation-exchange (CEX) resins often do not deliver sufficient aggregate separation. Ceramic hydroxyapatite (CHT) and other mixed-mode resins offer better aggregate control. Flow-through methods typically allow for high loading and simple integration into continuous processes. Such options include mixed-mode anion-exchange (AEX) resins or chromatography media based on AEX, CEX, or hydrophobic-interaction chromatography (HIC) for frontal or weak partitioning. However, the latter technology typically comes with robustness issues and narrow loading windows.

Low product titers resulting from the high aggregate content in symmetric bsAb feeds have driven the need for process improvements to meet the commercial demand for such antibodies. Here, I describe my team's application of a mixed-mode CEX resin that was developed by Thermo Fisher in collaboration with AstraZeneca (**1**). It enables improved aggregate control for both bsAb and monoclonal antibody (mAb) feeds.

Case Study

The POROS Caprylate mixed-mode CEX resin was designed to enable flow-through operation with selective binding of aggregates, offering a new option for purification of feeds that have extremely high aggregate levels. Key features of this resin include a novel caprylic-acid ligand and a POROS backbone that enables linear pressure-flow characteristics in manufacturing at scale.

Figure 1: pH effects on aggregate selectivity

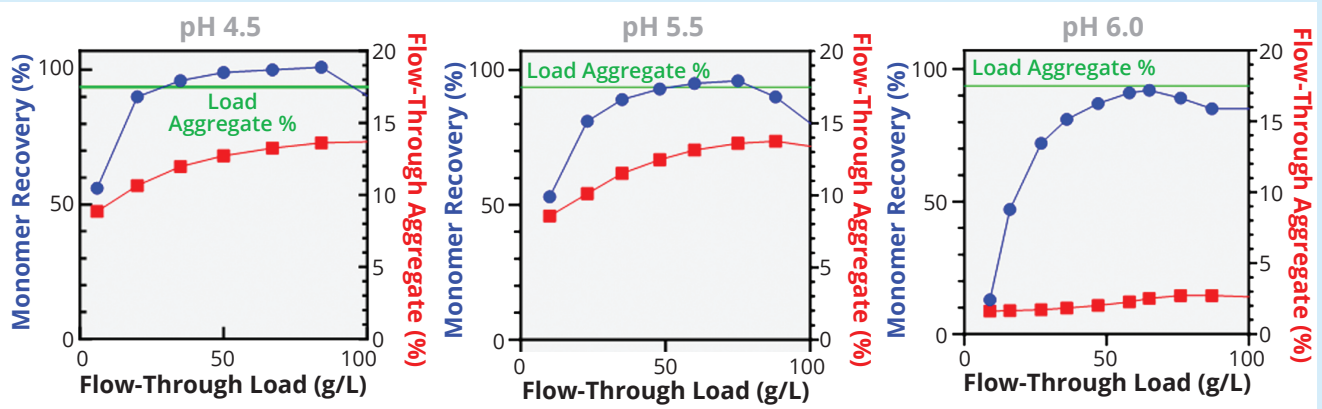
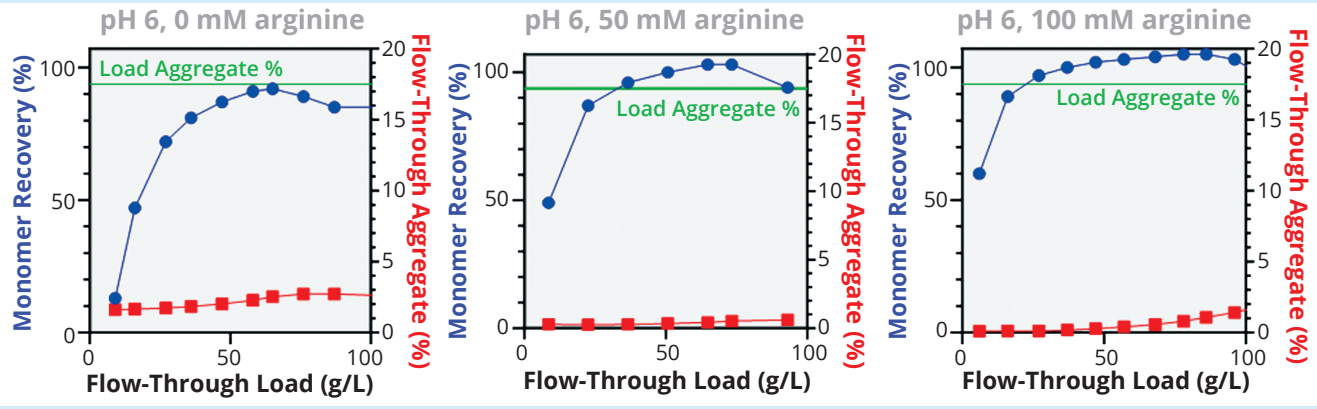


Figure 2: Arginine effects on aggregate selectivity



To evaluate the resin for aggregate control capabilities, we used a model symmetric bispecific antibody (BisA) with 17.5% aggregates and an isoelectric point (pI) of 9.14. Initial work demonstrated strong binding at neutral pH levels rather than the pH of 5 or 6 typically used for AEX chromatography. Those studies also revealed that adding arginine as a mobile-phase modifier significantly augmented selective product binding. Results of a design of experiments (DoE) study showed that the separation was sensitive to both pH and arginine concentration.

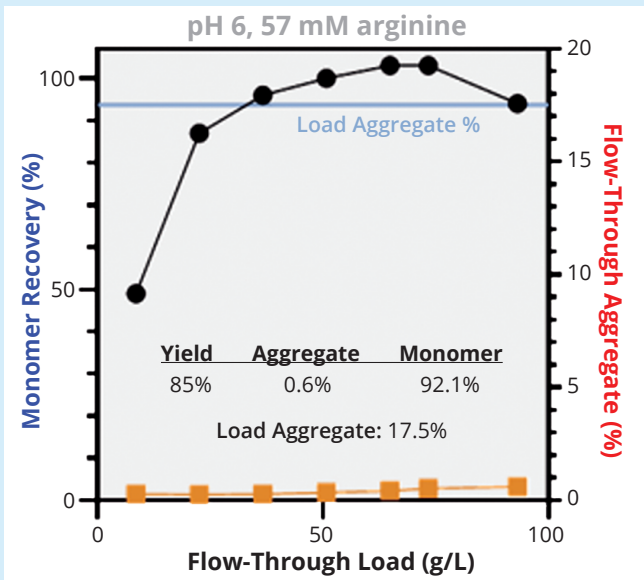
Strong aggregate binding was observed at mildly acidic pH values, but elution was insufficient. Increasing the pH enabled stronger binding and improved selectivity for aggregates with delayed product breakthrough, reducing yields while increasing sensitivity to load challenge levels (Figure 1). Operation at pH 6 provided good selectivity between the aggregate and the main-product peaks. A loading of 100 g/L allowed very little breakthrough of aggregate with a monomer yield in the 80% range.

Although selectivity was excellent at higher pH, we also explored adding arginine to improve product yield (Figure 2). Increasing concentrations of arginine augmented aggregate binding to the column, which increased the apparent capacity for aggregates. With higher levels of arginine, almost all of the monomer fraction flowed through the column, showing a robust separation of aggregates that remained bound to the column after loadings of up to 100 g/L.

We also evaluated different POROS Caprylate prototype resins with distinct hydrophobicity levels to determine which one gave the best separation of aggregates and monomer recovery (data not shown). The selected POROS Caprylate resin achieved high purity (85%) and monomer yield (92.1%) across a range of conditions for BisA, with the best results achieved using high pH and moderate arginine on a medium-hydrophobicity resin (Figure 3). The aggregate level was reduced from an initial load of 17.5% to 0.6%.

POROS Caprylate chromatography resin also can be used to clear aggregates from traditional mAb feeds. As shown in Figure 4, high yield and aggregate clearance were achieved across a range of conditions. In this study, the resin did not reach full aggregate breakthrough at 100 g/L loading for any condition.

Figure 3: Optimized conditions and results for purification of BisA with POROS Caprylate mixed-mode cation-exchange chromatography resin

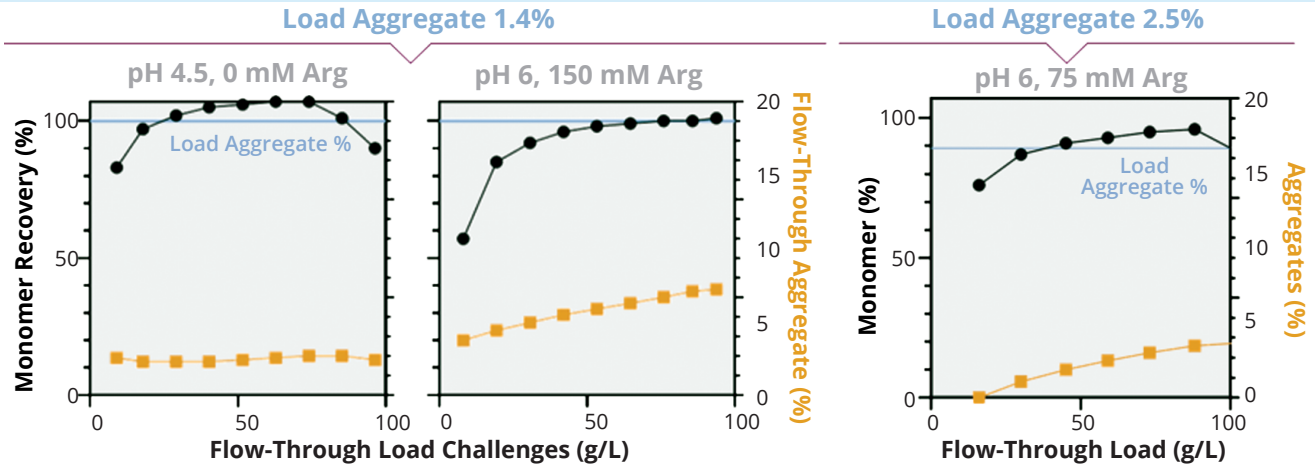


A Selective Alternative

Using the POROS Caprylate mixed-mode CEX chromatography resin provides a significant advantage for clearing impurities in flow-through mode and achieves robust clearance of high levels of aggregates. The unique selectivity of the caprylate ligand contributes to a high yield of targeted monomers while using established CEX buffer systems. Robust aggregate selectivity has been demonstrated for multiple molecular formats, including different bispecific formats and traditional mAbs under a broad range of operating conditions.

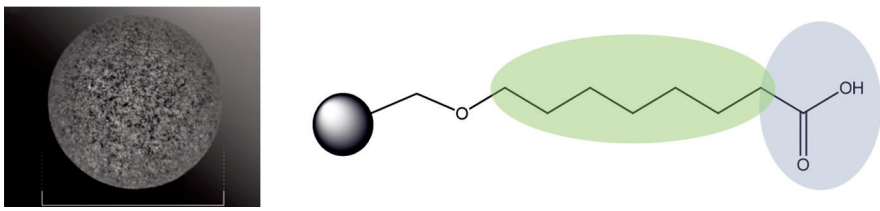
POROS caprylate resin potentially allows users to purify feeds with extremely high aggregate levels through two flow-through chromatography steps with robust control of aggregates. That can reduce costs and accelerate processing times, especially for users working with engineered mAb derivatives. In addition, the resin offers a manufacturing-compatible alternative to other resins such as CHT for high-aggregate products.

Figure 4: Removal of aggregates from mAb feeds using POROS Caprylate mixed-mode cation-exchange chromatography resin



Reference

1 Motobar L, Aspelund S, Aspelund M. Manufacturing-Friendly Aggregate Clearance in Downstream Processing of Bispecific and Traditional Antibodies Using a Novel POROS Mixed-Mode Chromatography Medium. *BioProcess Int.* webinar, 6 June 2024; https://www.bioprocessintl.com/chromatography/manufacturing-friendly-aggregate-clearance-in-downstream-processing-of-bispecific-and-trafitional-antibodies-using-a-novel-poros-mixed-mode-chromatography-media.



POROS Caprylate bead (50 µm) and ligand structure

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Learn more at https://www.thermofisher.com/mixed-mode-chromatography.

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