Antibody therapeutics

Advances in flow-through technology to enhance mAb polishing

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Summary

The rapidly evolving landscape of monoclonal antibody (mAb) development has led to increasing demand for innovative purification approaches, particularly during the polishing stage. Advances in flow-through technology have enhanced mAb polishing, with a growing emphasis on hydrophobic interaction chromatography (HIC) and mixed-mode chromatography. These approaches can remove challenging species not easily removed by other methods, enhancing product quality and manufacturability. This makes flow-through technology an essential tool to address the increasing complexity and variety of mAbs in clinical development.

Increasingly complex antibody molecules

In recent years, the variety of mAbs has expanded beyond traditional IgG formats. The emergence of alternative antibody derivatives such as antibody-drug conjugates (ADCs), bispecific mAbs, Fab fragments and Fc-fusion proteins presents unique challenges (Figure 1). For example, some of these formats display absent or altered protein A binding sites, overexpression of free light chains or increased propensity for aggregation. These challenges place considerable pressure on downstream development teams to continuously evolve and maintain a robust purification approach.

Figure 1. Different mAb modalities that demand additional tools for efficient purification.

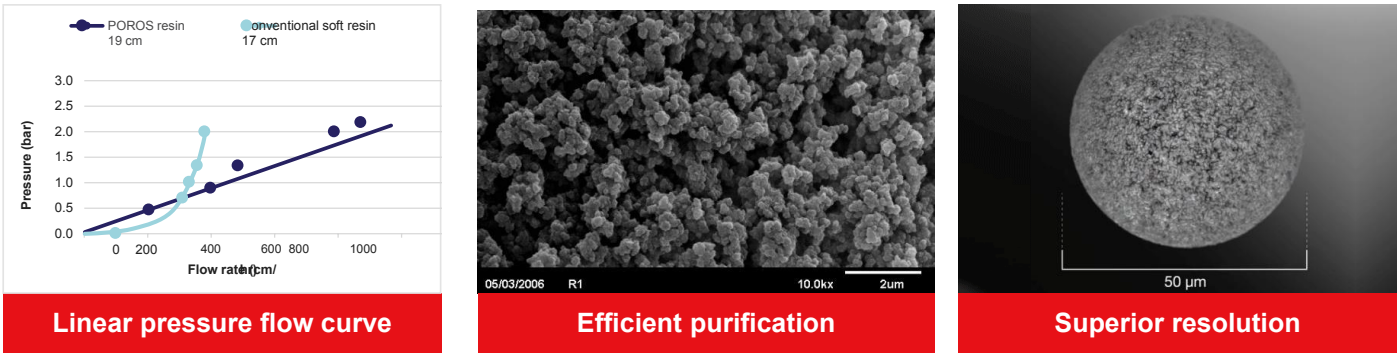


Figure 2. Key features of the POROS bead.

Key considerations in downstream processing

Developing effective downstream antibody processing involves balancing multiple factors. First and foremost, product quality is of paramount concern. Chromatography resins must provide high resolution to effectively separate the product of interest from impurities, such as aggregates and host cell proteins (HCPs). Ideally, resins should offer high capacity and throughput, allowing researchers to minimize costs, reduce processing times and manage intermediate pool volumes. POROS chromatography resins are designed with these factors in mind and allow for simple downstream processing.

The POROS base beads are comprised of polystyrene-divinylbenzene, a rigid polymer that results in a stable packed bed and a linear relationship between flow rate and pressure (Figure 2). This makes scale-up, as well as optimization of flow rate and process efficiency, more straightforward with respect to column pressure-drop. The POROS base bead also has large through-pores, which reduces resistance to mass transfer. This translates to more robust binding capacity and resolution with respect to flow rate. Moreover, the bead itself has an average diameter of 50 µm. This size allows for a proper balance between resolving power and the ability to maintain scalability and sufficient pressure flow characteristics.

Chromatography can be operated in either bind-and-elute mode or flow-through mode. In bind-and-elute mode, the resin binds both the product of interest and impurities (such as aggregates), and then the product of interest is selectively eluted from the column. This mode is advantageous for separating closely related species,

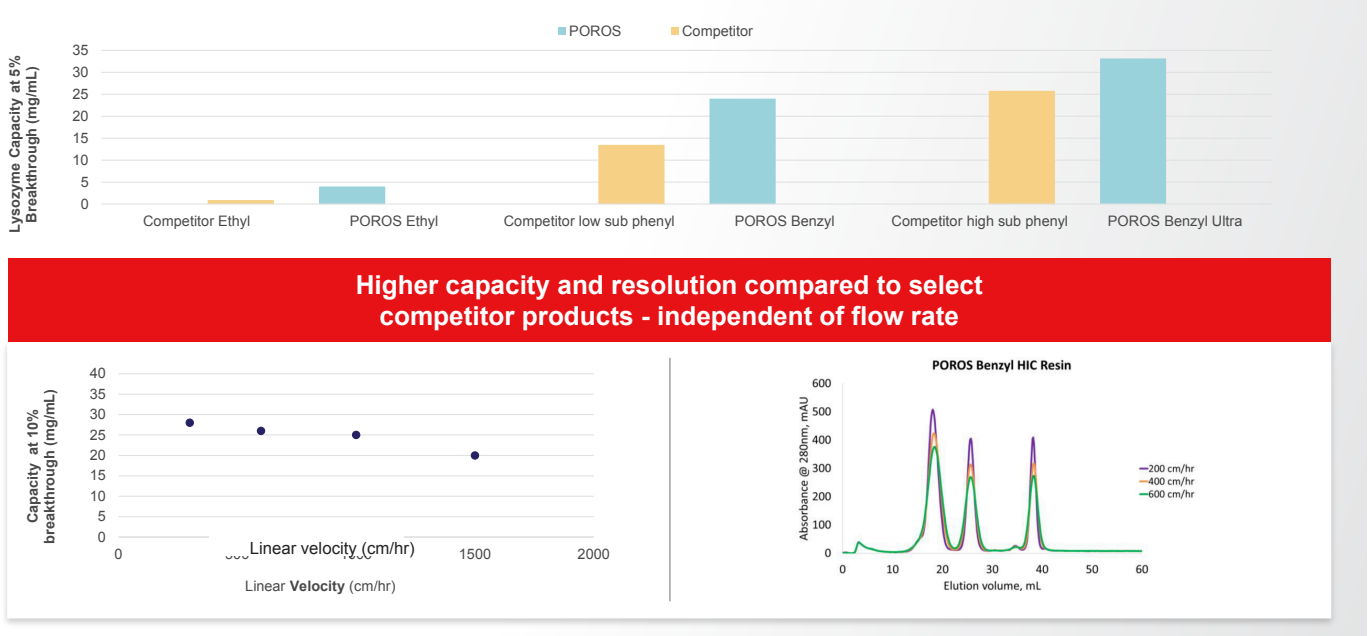


Figure 3. Comparison of key characteristics of POROS beads compared to select competitor products, highlighting higher capacity and resolution, independent of flow rate.

such as charge variants. However, for aggregate and host cell protein removal, comparable product quality can be achieved using flow-through chromatography. In flow-through mode, only impurities bind to the stationary phase, allowing for higher mass loading, which results in reduced resin usage, fewer processing steps and lower buffer consumption. All these benefits contribute to shorter processing times and a smaller equipment footprint.

POROS hydrophobic interaction chromatography resins

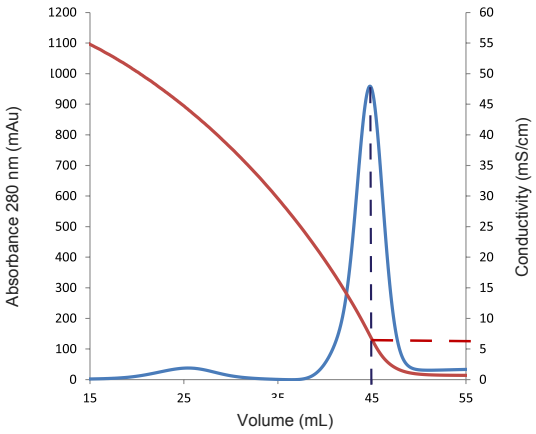
The POROS HIC family of resins includes POROS Ethyl, POROS Benzyl and POROS Benzyl Ultra resins. POROS Ethyl is the least hydrophobic while POROS Benzyl and POROS Benzyl Ultra offer higher hydrophobicity. POROS Benzyl Ultra is designed specifically for flow-through mode under low-salt conditions. These POROS HIC resins display higher capacity and resolution independent of flow rate compared to competitors' products (Figure 3). Moreover, they display consistent lot-to-lot performance as well as the linear pressure-flow drop, which is characteristic of the POROS base bead, making them ideal for large-scale bioprocessing.

HIC can be used in a range of applications across different therapeutic areas. For example, it can also be used for enzyme, recombinant protein and virus purification. It is also useful for reducing aggregates and other product and process-related impurities during mAb purification, including ADC purification for the resolution of individual drug–antibody ratio (DAR) species and Fc fusion type molecules. The following case studies highlight the effective use of flow-through applications for HIC.

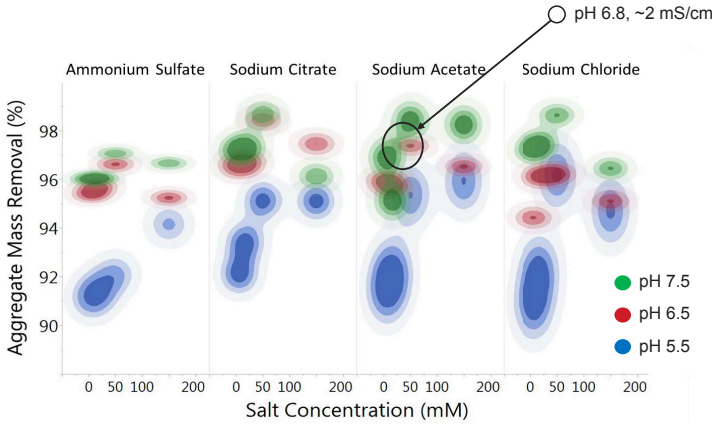
Case Study 1: Optimizing a mAb purification polishing step in flow-through mode using POROS HIC chromatography in flow-through mode

The first case study focuses on mAb A, a clinical-stage antibody with an existing process involving affinity capture, depth filtration, low pH hold, anion exchange in flow-through mode and a mixed-mode bind-and-elute step to reduce high aggregate levels (>12%) (Figure 4). Despite achieving 99% monomer purity and 90% recovery with the mixed-mode step, the throughput was limited to 25 g/L of resin at a 6-minute residence time.

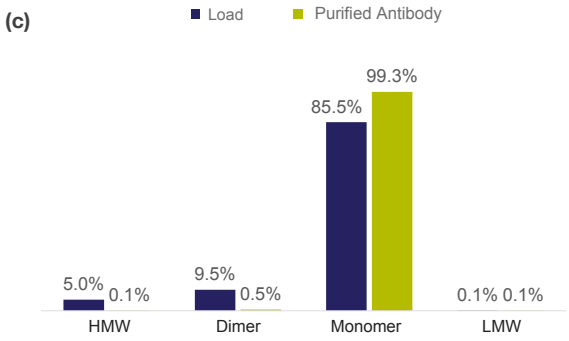
(a) mAB A: Bind-Elute POROS Benzyl Ultra



(b) Conditions: 0–150 mM Salt, pH 5.5–7.5



(c)



(d)

mAb-A Process	Mixed-Mode BE	POROS Benzyl Ultra-FT
Load density (g/L resin)	25	**80**
Monomer purity Pool (%)	99	**>99**
Monomer recovery (%)	90	**98**
Residence time (min)	6	**1.2**
HCP (ppm)	<LLOQ	**<LLOQ**

Figure 4. Bind and elute experiment (a) performed with POROS Benzyl Ultra to determine the starting point for flow through conductivity, with peak elution at ~7 mS/cm. (b) Heat map highlighting aggregate mass removal from high throughput screening performed using POROS Benzyl Ultra and four salts (ammonium sulfate, sodium citrate, sodium acetate, sodium chloride) from 0–150 mM, pH 5.5–7.5. (c) Verification run to show effective reduction of aggregates in no-salt FT process with high recovery, carried out at flow rate 500 cm/hr, 1.2 min residence time, load density 80 g/L. (d) Comparison of product quality, with FT showing an 8% increase in monomer recovery and ~threefold increase in load density.

To optimize this process, the mixed-mode step was replaced with a flow-through POROS HIC step. The process development work involved three stages:

1. Determining optimal flow-through conductivity: A low-loading bind-and-elute experiment was conducted with a decreasing conductivity gradient to establish a starting point for flow-through conductivity optimization.
2. High throughput screening (HTS): During this step, various salt types, concentrations and pH levels were evaluated to optimize conditions, focusing on POROS Benzyl Ultra.
3. Scale down model: Column loading studies were performed to assess residence time effects.

Results showed that the POROS Benzyl Ultra resin operated in flow-through mode provided comparable aggregate removal to the mixed-mode separation operated in bind-and-elute mode. Although product quality in terms of aggregate removal was equivalent in both modes, the flow-through HIC step noticeably improved recovery, with a boost of 8% (Figure 4). Similarly, column loading capacity was almost three-fold higher with the flow-through HIC step, with column loading increased to 80 g/L. Furthermore, the residence time, or flow rate, was five times faster with the flow-through HIC step. Thus, the flow-through HIC step matches the product quality of the mixed-mode bind-and-elute step and is more efficient with respect to binding capacity and flow rate, resulting in productivity gains.

Case Study 2: POROS Benzyl Ultra viral clearance and impurity removal in an ADC manufacturing process

The next case study involved the evaluation of the POROS Benzyl Ultra resin for viral clearance and impurity removal during an ADC manufacturing process. The company producing this ADC utilizes synthetic amino acids that allow for site-specific conjugation of the drug linker, creating a highly homogenous DAR. However, this process can result in high levels of aggregates (7–11%).

The POROS Benzyl Ultra resin was used to reduce high molecular weight (HMW) species as well as host cell proteins for pre-conjugated mAbs in three different processes. The results showed good reduction of host cell proteins and HMW impurities using high loading densities (Figure 5). In a viral clearance study for mAb A, yield was comparable across qualification, XMuLV-spiked and MVM-spiked runs, averaging 85%. A log reduction value (LRV) of >5.97 was achieved for XMuLV and a LRV of 4.56 was achieved for MVM, demonstrating effective viral clearance of a model parvovirus and retrovirus for this process.

Flow-through high aggregate mAb polishing using POROS Caprylate Mixed-Mode Cation Exchange Chromatography Resin

Thermo Scientific POROS Caprylate Mixed-Mode Cation Exchange resin is a unique mixed-mode chromatography tool designed for high aggregate selectivity in flow-through mode that became commercially available in 2024. The ligand, caprylic acid, imparts both hydrophobic and weak cation exchange characteristics.

POROS Caprylate Mixed-Mode Cation Exchange resin is suitable for moderate to high aggregate levels (up to 20%) and operates over a broad pH (4.5–7.0) and conductivity range (10–30 mS/cm). To demonstrate the aggregate removal capability of the

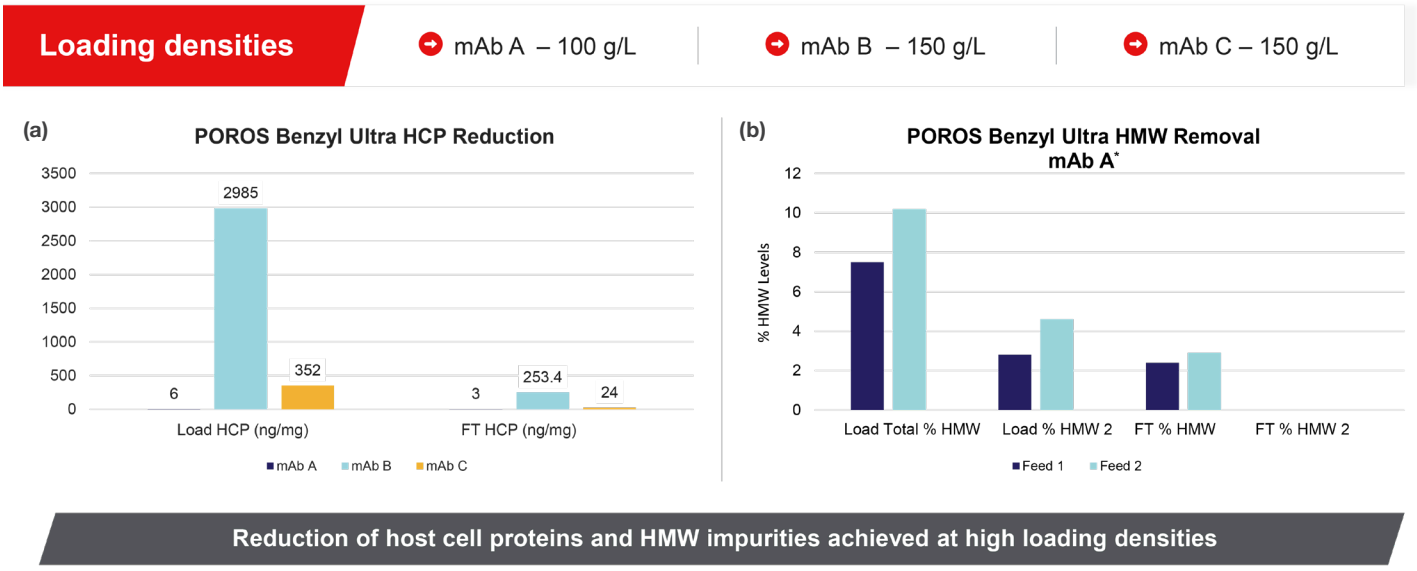


Figure 5. (a) Host cell protein reduction for three different mAbs using POROS Benzyl Ultra and (b) a summary of aggregate removal for mAb A using two feed streams. *Post POROS Benzyl Ultra HMW levels for mAb B and C were <1%.

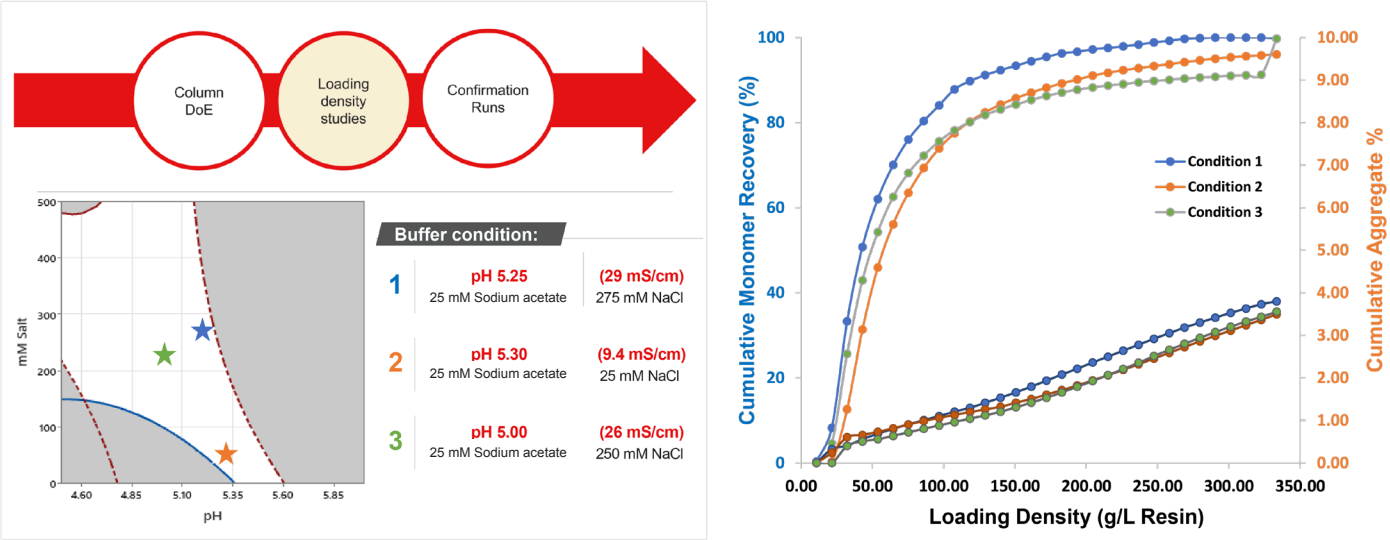


Figure 6. Loading density studies, performed across three buffer conditions, to confirm that POROS caprylate can facilitate effective polishing with high monomer yield and purity.

resin an IgG1 mAb was purified via Protein A capture and subjected to high and low pH adjustments to generate up to 10% aggregate in the feed stream. A bench-scale design-of-experiment (DOE) was performed to evaluate the impact of pH (4.5–6.0) and sodium chloride concentration (0–500 mM) on the responses of yield and high molecular weight (HMW) reduction using POROS Caprylate Mixed-Mode Cation Exchange resin in flow-through mode. Column loading was kept constant at 100 g/L resin in the DOE. The results showed >75% monomer recovery and robust aggregate removal (<2% aggregate) across a wide range of conditions, with monomer recovery expected to increase with higher column loading.

Next, loading density studies were performed at three different conditions within the DOE space (Figure 6). For all three conditions, <2.0% aggregate in the product pool was achievable with ≥90% monomer recovery at 160–180 g/L loading density. Additionally, HCP and leached Protein A were reduced by approximately 95% for all three operating conditions. Further characterization of HCPs by HPLC-MS/MS showed that POROS Caprylate Mixed-Mode Cation Exchange resin was able to reduce the number of individual HCP species from 380 to 79, with complete removal of many HCPs considered to be high risk or challenging to remove in mAb processes.

Conclusion

Advancements in flow-through chromatography technology, particularly with POROS chromatography resins, offers significant enhancements for polishing in mAb manufacturing processes.

The case studies highlighted here demonstrate the applications and benefits of these advanced resins, paving the way for more efficient and effective bioprocessing strategies.

Watch the complete webinar with Robert Stairs here.

Learn more at **thermofisher.com/mixed-mode-chromatography**

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