# High Aggregate Levels with Engineered Monoclonal Antibodies

## An Innovative Approach to Addressing the Challenge

**Ying Chen and Al de Leon**

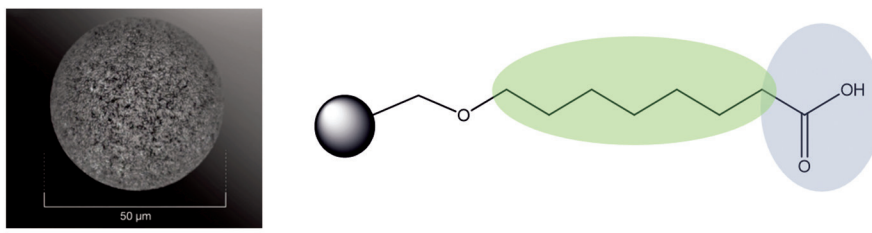
The new generation of engineered antibodies has an increased tendency for aggregation, creating new challenges for downstream processing. Aggregates are large, tangled clusters of antibody molecules that can form irreversibly during upstream production, downstream processing, and storage (**1**). Extreme levels of pH, ionic strength, temperature, concentration, shear forces, and other processing conditions exacerbate aggregation. The resulting particles can expose different epitopes, which decreases overall product efficacy, increases immunogenicity, and (depending on particle size) even introduces the potential to block blood vessels in recipients (**2**).

Given the importance of aggregate removal, shortcomings among standard techniques create the need for economical and effective purification solutions. A new chromatography resin offers unique selectivity with favorable process economics for robust removal of aggregates, host-cell proteins (HCPs), and leached protein A from antibody manufacturing processes.

### An Opportunity To Expand Operating Ranges

Several methods are used for aggregate removal based on different separation principles including size, charge, and hydrophobicity (**3**). Although such approaches are effective, they also present a number of challenges. Ion-exchange chromatography (IEC) has a relatively low binding capacity, hydrophobic-interaction chromatography (HIC) resins are difficult to clean, and both size-exclusion and hydroxyapatite media present obstacles for packing columns and scaling up processes.

On the next page, the left panel of Figure 1 summarizes typical operating conditions for chromatographic aggregate removal, with shaded areas representing conditions that provide for high yield and antibody purity with each approach. HIC in flow-through mode can process feed with a 5–15% aggregate levels at a mass loading of 65–200 g/L resin. Cation-exchange chromatography (CEX) in bind–elute mode can process feed with ≤5% aggregate at a maximum loading of 100 g/L resin; overloaded CEX resins can process similar aggregate levels at much higher mass loading. However, CEX gives low monomer recovery at low mass loading, so process-development operations require substantial amounts of feed material.




**POROS Caprylate bead (50 µm) and ligand structure**

The right panel of Figure 1 shows corresponding operating conditions. Blue boxes define the opportunity to expand typical operating ranges using the POROS Caprylate mixed-mode CEX resin, which offers unique selectivity.

### Resin Features

POROS Caprylate resin balances the removal of aggregates and other impurities, such as HCPs and leached protein A, with robust monoclonal antibody (mAb) recovery — a combination that is challenging for other mixed-mode resins operated in flow-through mode. The ability to operate in flow-through mode increases mass loading capability, requires lower volumes of buffer and resin, uses a smaller equipment footprint, and can be accomplished in shorter processing times than bind–elute mode takes. Additional process benefits are enabled by these product attributes:

- Caprylic acid functionality enables high aggregate selectivity, reducing the number of chromatography steps needed while increasing process yield.

**Figure 1:** Typical operating ranges for intermediate polishing steps reveal the opportunity (blue boxes) to expand these ranges using a chromatographic technique with unique selectivity; B–E = bind–elute, CEX = cation-exchange chromatography, FT = flow-through, HIC = hydrophobic-interaction chromatography.

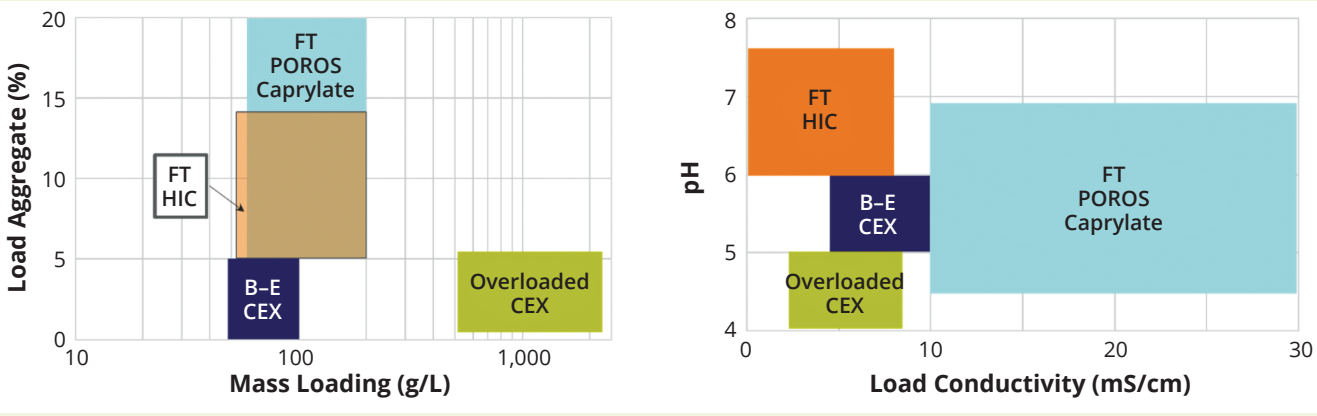



**Figure 2:** White area of contour plot represents operating conditions that resulted in an aggregate level of ≤1% and monomer recovery ≥75%; HCP = host-cell protein, LPrA = lipoprotein A

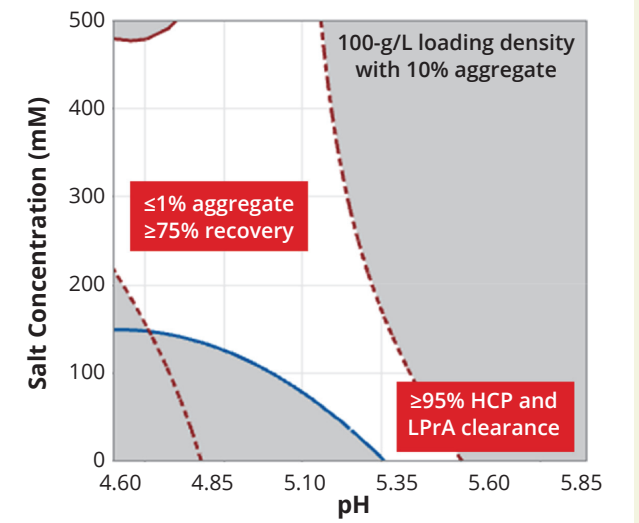
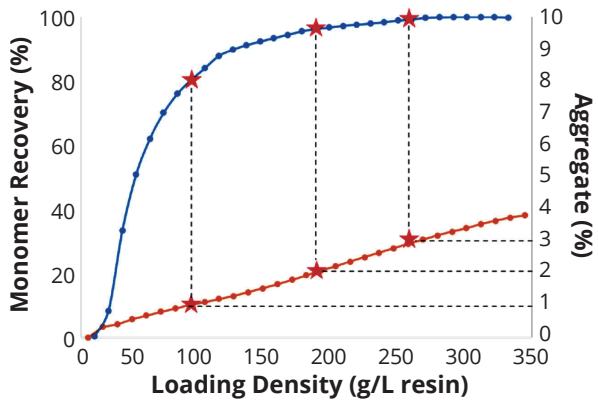



**Figure 3:** Monomer recovery and aggregate percentage in flow-through fraction as a function of loading density




| Aggregate | Loading Density | Monomer Recovery |
|---|---|---|
| 1% | 85.6 g/L resin | 80.4% |
| 2% | 181.9 g/L resin | 96.3% |
| 3% | 256.8 g/L resin | 99.2% |

• The 50-µm bead size and large through-pores provide high aggregate capacity for increased productivity and reduced cost.

• The poly(styrene-*co*-divinylbenzene) backbone offers predictable scalability with a linear pressure-flow curve.

## Optimizing Aggregate Removal and mAb Recovery

To demonstrate the ability of POROS Caprylate resin to remove aggregates with high monomer recovery, we exposed a purified immunoglobulin G (IgG1) mAb to high and low pH multiple times to generate aggregates at levels up to 10%. Batch and column experiments started with high-throughput screening (HTS) to define and provide directionality on the effects of pH, salt concentration, and loading density on both aggregate removal and monomer recovery.

HTS experiments included 200-g/L and 100-g/L loading densities with initial aggregate levels of 5% and 10%, respectively, at different pH levels and with salt concentrations. Results showed that aggregate removal was favored at low pH, and high monomer recovery was achievable with higher salt concentrations. Use of those conditions could improve efficiency by requiring fewer adjustments to the process stream following protein A affinity capture.

We used a column-based design of experiments (DoE) study centered on conditions of pH 5.25 and 28.6-mS/cm conductivity to confirm aggregate removal and monomer recovery at 100-g/L loading and 10% aggregates. As Figure 2 shows, the operating condition for POROS Caprylate resin can be broadened depending on aggregate-level acceptability. Note that ≥95% reduction in HCP and leached protein A also were demonstrated.

A loading-density study showed that POROS Caprylate resin facilitated effective polishing with high monomer yield and purity. The feed had 89.4% monomer purity and 10.6% aggregates, and residence time was three minutes. Figure 3 shows cumulative monomer recovery and aggregate percentage in the flow-through as a function of loading density. A 1% aggregate level corresponds to an 85.6 g/L loading density and 80.4% monomer recovery. Both the loading density and monomer

**Table 1:** Host-cell protein (HCP) classification and relative signal intensity (total ion count) after the protein A step and following POROS Caprylate purification

| | | Detection in IgG1 Material After Purification with . . . | |
|---|---|---|---|
| **HCP Classification** | **Identified HCP** | **Protein A Affinity Resin** | **POROS Caprylate Resin** |
| High risk | 8-kDa glucose-regulated protein (GRP78, BiP) | $6.64 \times 10^5$ | Not detected |
| | Alpha-enolase (2-phospho-D-glycerate hydrolysase | $2.43 \times 10^4$ | Not detected |
| | Cathepsin B (CatB) | $1.00 \times 10^6$ | Not detected |
| | Cathepsin L (CatL) | $4.16 \times 10^4$ | Not detected |
| | Cathepsin Z (CatZ) | $7.52 \times 10^4$ | Not detected |
| | Glutathione S-transferase P1 (GSTP1) | $4.06 \times 10^5$ | Not detected |
| | Lysosomal acid lipase (LAL) | $2.67 \times 10^5$ | Not detected |
| | Matrix metalloproteinase 19 (MMP-19) | $2.08 \times 10^5$ | Not detected |
| | Phospholipase B-like 2 (PLBL2) | $1.67 \times 10^5$ | Not detected |
| | Monocyte chemoattractant protein 1 (MCP-1) | $1.72 \times 10^6$ | $1.02 \times 10^5$ |
| | Peroxiredoxin 1 (PRDX1) | $4.20 \times 10^5$ | $1.12 \times 10^5$ |
| Difficult to remove | Cathepsin D (CatD) | $8.43 \times 10^4$ | Not detected |
| | Insulin-like growth-factor binding protein 4 | $7.46 \times 10^4$ | Not detected |
| | Metalloproteinase inhibitor | $2.08 \times 10^5$ | Not detected |
| | Galectin-3 binding protein | $2.15 \times 10^5$ | $3.31 \times 10^4$ |
| | Lipoprotein lipase | $2.72 \times 10^6$ | $7.42 \times 10^5$ |
| High risk and difficult to remove | Clusterin (Clu) | $2.24 \times 10^7$ | $1.56 \times 10^6$ |

recovery increased with higher aggregate levels.

A range of different buffer conditions also can be used, including lower pH and salt concentrations. The ability to use a lower salt concentration would eliminate the need for sample manipulation before the next chromatographic step.

Comparing POROS Caprylate resin with a commercially available alternative that also is designed to remove aggregates in flow-through mode revealed that the POROS resin had 75% higher monomer recovery and 230% higher HCP reduction at a mass loading of 100 g/L using optimized buffer conditions. POROS Caprylate resin also enabled removal of many high-risk and challenging HCPs. Table 1 classifies those proteins according to their risk and difficulty to remove. Most identified HCPs were undetectable; others were reduced by at least an order magnitude.

## A Flow-Through Polish Step

Aggregates will remain a challenge for the next generation of antibody products. POROS Caprylate resin offers a new tool to polish mAb products with aggregate levels of up to 20% while operating at a broad range of pH and conductivity and reducing both HCPs and leached protein A. Because the resin was designed to operate in flow-through mode, using it reduces processing times and buffer use — and thus overall costs — compared with IEC in bind–elute mode.

A range of different buffer conditions also can be used, including lower pH and salt concentrations. The ability to use a lower salt concentration would **ELIMINATE** the need for sample manipulation before the next step.

## References

**1** Joshi V, Yadav N, Rathore AS. Aggregation of Monoclonal Antibody Products: Formation and Removal. *BioPharm Int.* 26(3) 2013; https://www.biopharminternational.com/view/aggregation-monoclonal-antibody-products-formation-and-removal.

**2** Lundahl MLE, et al. Aggregation of Protein Therapeutics Enhances Their Immunogenicity: Causes and Mitigation Strategies. *RSC Chem. Biol.* 2(4) 2021: 1004–1020; https://doi.org/10.1039/D1CB00067E.

**3** Vázquez-Rey M, Lang DA. Aggregates in Monoclonal Antibody Manufacturing Processes. *Biotechnol. Bioeng.* 108(7) 2011: 1494–1508; https://doi.org/10.1002/bit.23155.

***Al de Leon*** *is a research and development manager (al.deleon@thermofisher.com), and* ***Ying Chen*** *is a research and development staff scientist (ying.chen@thermofisher.com), both at Thermo Fisher Scientific in Bedford, MA.*

*POROS resins are pharmaceutical-grade reagents for manufacturing and laboratory use only.*