Enhanced aggregate and HCP removal with mixed-mode chromatography

Abstract

Monoclonal antibodies (mAbs) play a vital role in the treatment of various diseases, including cancer, but their therapeutic efficacy can be compromised by the presence of aggregates and impurities. This study explores the use of Thermo Scientific™ POROS™ Caprylate Mixed-Mode Cation Exchange Chromatography Resin for the purification of mAbs, leveraging the hydrophobic and weak cation exchange properties of the ligand to effectively separate aggregates from monomers, and optimize monomer recovery.

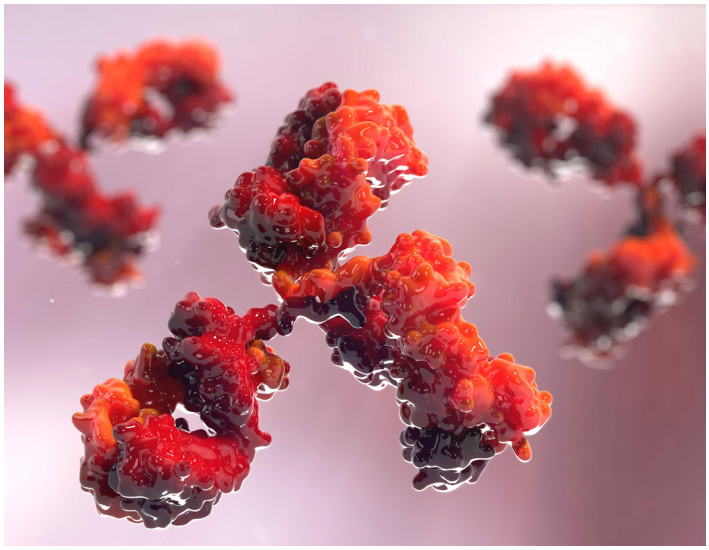
Through design of experiments (DOE) studies, we have identified that salt concentration and pH are critical factors influencing purification outcomes. The use of POROS Caprylate resin has been confirmed to effectively remove high molecular weight (HMW) aggregates, as demonstrated by size-exclusion chromatography (SEC). Additionally, the results of enzyme-linked immunosorbent assays (ELISAs) for total host cell protein (HCP) quantification and liquid chromatography tandem mass spectrometry (LC/MS/MS) for individual HCP identification showed significant reductions in HCP levels. Protein A leachate levels were also significantly reduced as determined by ELISA.

Overall, POROS Caprylate resin proves to be a robust purification tool, enabling the production of safe and efficacious mAbs by maximizing monomer recovery and effectively removing HMW species and HCPs.

Introduction

The development of a new generation of engineered antibodies has posed challenges for downstream processing, as impurities produced during upstream production can affect the efficacy of therapeutic mAb-based drugs.

In traditional mAb processes, aggregates have been typically removed using either cation exchange (CEX) chromatography in bind-and-elute mode or hydrophobic interaction chromatography (HIC) in flow-through mode. CEX chromatography in bind-and-elute mode can process mAb feed with up to 5% aggregate content at a maximum loading of 100 g per liter (L) of resin. In contrast, HIC in flow-through mode can handle mAb feed with 5–15% aggregate levels at mass loadings of 65–200 g per liter (L) of resin. Notably, CEX chromatography in flow-through mode can utilize nearly its full binding capacity for aggregates, allowing



for significantly higher loading and substantial reduction in HCPs and other impurities [1,2]. Yet, in modern processes higher aggregate levels and more complex HMW species can challenge traditional polish approaches, leading to lower purity and yield. Newer purification tools are needed.

In 2012, Brodsky et al. [3] suggested using caprylic acid (octanoic acid) as an additive to remove aggregates and HMW species via flocculation. Building on this idea, a chromatography resin was developed by chemically attaching caprylic acid to porous poly(styrene-co-divinyl benzene) beads. This product, POROS Caprylate resin, is used in flow-through mode and demonstrates excellent capabilities in aggregate removal and monomer recovery percentages while overcoming the limitation of binding capacity, thereby achieving a high yield of monomers from traditional bind-and-elute resins.

This article describes the POROS Caprylate resin, which possesses unique selectivity and robustly removes aggregates, HCPs, and leached protein A.

Summary of the studies

POROS Caprylate resin is a mixed-mode hydrophobic weak CEX resin that offers a robust solution for the efficient purification of mAbs and related antibody products in flow-through format.

The following study demonstrates that POROS Caprylate resin can facilitate efficient removal of impurities while achieving high monomer recovery for a traditional mAb in a flow-through format. This approach helps ensure high recovery of monomers and effective removal of aggregates and HCPs, both factors which are critical for the safety and efficacy of the final therapeutic product while enabling lower manufacturing costs. Additionally, POROS Caprylate resin has shown versatility by being compatible with various buffer combinations and pH levels, enabling it to fit into diverse purification processes.

A biosimilar version of HERCEPTIN® (trastuzumab) mAb was initially purified using a Thermo Scientific™ POROS™ MabCapture™ A resin before being subjected to pH cycling to generate aggregates. A DOE study was performed to determine the optimal operating range. The final conditions were confirmed using column chromatography. Monomer recovery and aggregate removal were measured. Impurities including HCPs and leached protein A were quantified using typical methods, and specific HCPs were identified and quantified by LC/MS/MS.

Materials

Product	Manufacturer	Cat. No.
Trastuzumab	Thermo Fisher Scientific	NA (internally produced)
POROS MabCapture A Affinity Chromatography Resin	Thermo Fisher Scientific	4374735
POROS Caprylate Mixed-Mode Cation Exchange Chromatography Resin	Thermo Fisher Scientific	A51051
Tris(hydroxymethyl)aminomethane	Thermo Fisher Scientific	T393-212
Acetic Acid, Glacial	Thermo Fisher Scientific	A38-212
Sodium Phosphate, Monobasic	Thermo Fisher Scientific	446222500
Sodium Phosphate, Dibasic	Thermo Fisher Scientific	S375-500
Sodium Chloride	Thermo Fisher Scientific	S640-10
Sodium Hydroxide (5 N)	Thermo Fisher Scientific	SS256-500
Hydrochloric Acid (6 M)	Thermo Fisher Scientific	S25857
Sodium Acetate	Thermo Fisher Scientific	BP333-500
Sodium Citrate	Thermo Fisher Scientific	BP327-1
CHO HCP ELISA Kit	Cygnus	F550-1
Protein A ELISA kit	Repligen	9000-1
MAbPac SEC-1 Size Exclusion Chromatography HPLC Columns	Thermo Fisher Scientific	074696
Acclaim VANQUISH C18 UHPLC Columns	Thermo Fisher Scientific	074812-V
Disposable PES filter units, 0.2 µm filter	Thermo Fisher Scientific	FB12566504
Amicon Ultra Centrifugal Filter	MilliporeSigma	UFC9010

Instruments

Product	Manufacturer	Cat. No.
UltiMate 3000 HPLC System	Thermo Fisher Scientific	Contact our sales support team for purchase
Varioskan LUX Multimode Microplate Reader	Thermo Fisher Scientific	VLBLATD1
NanoDrop 2000C Spectrophotometer	Thermo Fisher Scientific	ND-2000C
Orbitrap Ascend BioPharma Tribrid Mass Spectrometer	Thermo Fisher Scientific	B51003849
Vanquish Flex UHPLC system	Thermo Fisher Scientific	Contact our sales support team for purchase
ÄKTA pure Chromatography System	Cytiva	cytivalifesciences.com
Orion Versa Star Pro pH/Conductivity Multiparameter Benchtop Meter	Thermo Fisher Scientific	13645584
Sorvall Legend XTR Centrifuge	Thermo Fisher Scientific	E311966

Methods

The general purification scheme is shown in Figure 1. Briefly, trastuzumab was purified from clarified cell culture using POROS MabCapture A affinity capture resin with 100 mM Tris, pH 7.4 buffer at a target concentration of 30 mg per mL of resin. The flow rate was 300 cm/hr. The resin was washed with (1) 100 mM Tris, pH 7.0, (2) 100 mM Tris, 0.5 M NaCl, pH 7.0, and (3) 25 mM sodium acetate, pH 5.5. The mAb was eluted using 25 mM sodium acetate, pH 3.2. The eluted mAb was held at low pH for 1 hour, then the pH was adjusted to the desired level and filtered through a 0.2 µm filter. The purified mAb was kept in a 4°C refrigerator until the further polishing step with POROS Caprylate resin.

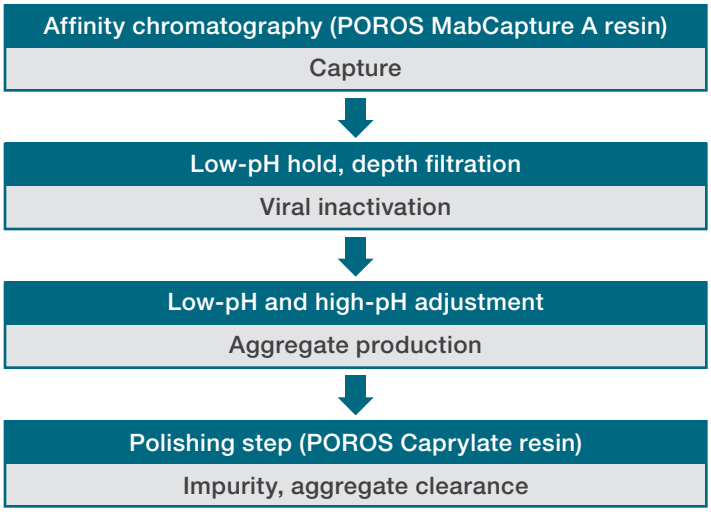


Figure 1. General experimental overview.

Generation of 5% or 10% mAb aggregates

After purification using POROS MabCapture A resin, the level of trastuzumab aggregates was increased from <2% to approximately 5% or 10% HMW by subjecting the antibody to four cycles of high and low pH adjustments (pH 3.5, pH 12.5, pH 8.0, pH 4.5) [4–6]. The levels of aggregates were measured by high-performance liquid chromatography (HPLC) size-exclusion chromatography (SEC) analysis (Thermo Scientific™ UltiMate™ 3000 HPLC System; Thermo Scientific™ MAbPac™ SEC-1 column; mobile phase: 50 mM sodium phosphate, 300 mM NaCl, pH 6.5; flow rate: 0.2 mL/min).

Figure 2 shows a typical HPLC-SEC chromatogram used to quantify mAb monomers and aggregates. Peaks 1 and 2 represent the aggregates, peak 3 is the monomeric mAb, and peak 4 is the low molecular weight species.

Finding optimal conditions by high-throughput screening

To determine the optimal pH and salt concentration that maximize aggregate removal at high monomer recovery, each well of a 96-well filter plate was filled with 10 µL of POROS Caprylate resin. The resin in each well was equilibrated with 190 µL of buffer of

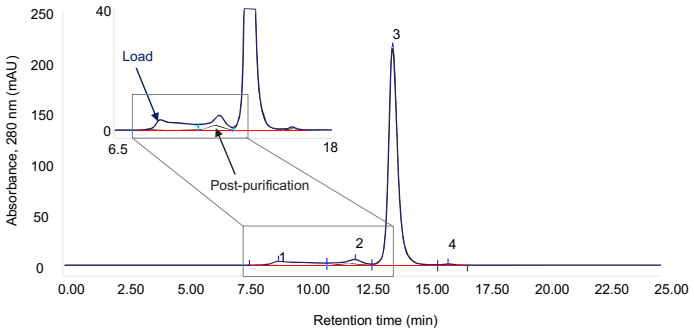


Figure 2. SEC chromatogram of mAb feed prior to purification using POROS Caprylate resin (blue) and after purification (black). HPLC-SEC was used to determine aggregate levels on a mockup feed solution. Inset is an expanded section showing the reduction of HMW species post-purification.

various salt concentrations and pH, as detailed in Figure 3. The plate was then centrifuged at 1,200 rpm for 3 min. This process was repeated three times. Following resin equilibration, 40 µL of 50 mg/mL mAb with ~5% aggregate mAb or 20 µL of 50 mg/mL mAb with ~10% aggregate mAb after POROS MabCapture A resin purification were added into each well, targeting the load densities of 200 mg or 100 mg per mL of resin for ~5% HMW or ~10% HMW aggregates, respectively. The 96-well plate was shaken for 30 min at 400 rpm, and flow-through was collected in a 1 mL 96 deep-well plate. Then 100 µL of each collected sample was transferred to a UV-transparent plate, in which protein concentration was determined by A_{280} measurement on a Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader, while monomer purity and aggregate levels were analyzed by HPLC-SEC on the UltiMate 3000 system with a MAbPac SEC-1 column.

Experimental details:

- Base buffer: sodium acetate or sodium citrate
- Load mAb: 5% aggregate mAb at 200 mg per mL of resin, 10% aggregate mAb at 100 mg per mL of resin
- Salt type: sodium chloride
- Salt concentrations: 0–300 mM for the 5% aggregates, 0–500 mM for the 10% aggregates
- pH: 4.5–7.5

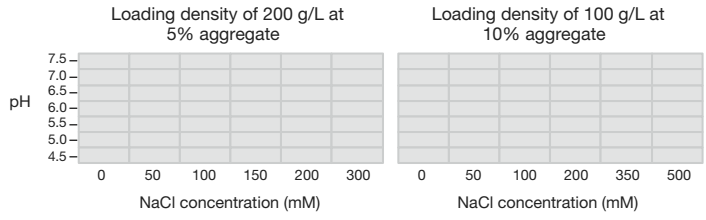


Figure 3. Plate layout for screening experiment to find the optimum buffer composition for maximizing aggregate removal and high monomer recovery with POROS Caprylate resin.

Finding optimal conditions with column chromatography

The pH and salt concentration were further optimized for the POROS Caprylate resin in column format. POROS Caprylate resin was packed into a 0.66 cm (D) x 3 cm (L) (1 mL) column. Experiments were conducted using an ÄKTA pure™ Chromatography System. Each column was equilibrated with 25 mM sodium acetate buffer containing varying concentrations of NaCl and adjusted to specific pH levels. Trastuzumab with ~10% aggregates in various salt concentrations and pH levels was loaded onto the column at a load density of 100 mAb per mL of resin. The flow-through pool was collected and analyzed for monomer and aggregate content by HPLC-SEC on the UltiMate 3000 system with a MAbPac SEC-1 column.

Experimental details:

- Buffer: sodium acetate
- Salt type: sodium chloride
- Salt concentrations: 0–500 mM
- pH: 4.5–6.0
- Load mAb: 10% aggregates at 100 mg mAb per mL of resin

Breakthrough analysis

To determine the effect of loading density on aggregate and impurity removal across three buffer conditions, POROS Caprylate resin was packed into a 0.66 cm (D) x 3 cm (L) (1 mL) column. The column was equilibrated with 25 mM sodium acetate, 275 mM NaCl, pH 5.25, at 3 min residence time. The column was loaded with trastuzumab (~10% aggregates, up to 325 mg of mAb per mL of resin) in 25 mM sodium acetate, 275 mM NaCl, pH 5.25, at 3 min residence time. Flow-through and wash fractions were collected in 96 deep-well plates at a volume of 1 mL per well over 5 column volumes (CVs). The concentration of mAb was determined by A_{280} measurement on the Varioskan LUX Multimode Microplate Reader. The monomer recovery, aggregate level, and monomer purity were determined by HPLC-SEC on the UltiMate 3000 system with a MAbPac SEC-1 column. The same experiments were performed for the other two buffers (25 mM sodium acetate, 75 mM NaCl, pH 5.3, and 25 mM sodium acetate, 250 mM NaCl, pH 5.0).

Experimental details:

Condition	Buffer
1	25 mM sodium acetate, 275 mM NaCl, pH 5.25
2	25 mM sodium acetate, 75 mM NaCl, pH 5.3
3	25 mM sodium acetate, 250 mM NaCl, pH 5.0

- Column: 0.66 cm (D) x 3 cm (L) (1 mL)
- Max. load density: 325 mg of mAb per mL of resin
- Initial aggregate content: ~10%
- Concentration of mAb: ~10 mg/mL
- Residence time: 3 min (20 cm/hr linear flow rate)
- Flow-through pool: load plus wash
- Collection fraction: 1 mL

Confirmation studies

Confirmation studies were conducted to verify the observed trends in aggregate removal and monomer recovery from the flow-through breakthrough analysis of POROS Caprylate resin across three buffer conditions.

POROS Caprylate resin was packed into a 0.66 cm (D) x 3 cm (L) 1 mL column. The mAb purification experiments were carried out in flow-through mode, based on the previously tested three buffer conditions and corresponding loading densities.

Experimental details:

Condition	Buffer	Max. load density (mg per mL of resin)
1	25 mM sodium acetate, 275 mM NaCl, pH 5.25	160
2	25 mM sodium acetate, 75 mM NaCl, pH 5.3	175
3	25 mM sodium acetate, 250 mM NaCl, pH 5.0	170

- Column: 0.66 cm (D) x 3 cm (L) (1 mL)
- Initial aggregate content: ~10%
- Concentration of mAb: ~10 mg/mL
- Residence time: 3 min (20 cm/hr linear flow rate)
- Flow-through pool: load plus wash

Quantification of protein A leachate

To assess the ability of POROS Caprylate resin to remove the leached protein A ligand, the Protein A ELISA Kit was used to quantify any contaminating protein A in the antibody purification process. This was done by comparing the mAb load to the flow-through fractions.

Serially diluted samples from load and flow-through fractions were incubated in a microtiter plate with immobilized antibodies. A biotinylated anti–protein A probe and streptavidin peroxidase conjugate were added, followed by a colorimetric reaction with tetramethylbenzidine (TMB). Absorbance was measured at 450 nm, and protein A concentration was determined using a standard curve.

Quantification of total CHO HCP content

To evaluate the effectiveness of POROS Caprylate resin in reducing HCP levels from the mAb load during purification, the CHO HCP ELISA Kit was used to measure Chinese hamster ovary (CHO) HCPs. This kit helped monitor total HCP clearance throughout the mAb purification process.

The CHO HCP ELISA Kit employs HRP-labeled CHO HCP antibodies and capture CHO HCP antibodies on microtiter strips. Serially diluted samples were incubated with these antibodies, followed by the addition of TMB substrate. The reaction was stopped, and absorbance was measured at 450 nm/600 nm. CHO HCP concentrations were calculated in ng/mL using a standard curve and a 4-parameter model.

Identification of individual CHO HCP

To address the limitations of the ELISA method in analyzing potentially thousands of HCPs in a mAb sample, LC/MS/MS was employed as a complementary method.

Both load and flow-through fraction samples were digested with trypsin. The resulting peptides were separated using a Thermo Scientific™ Acclaim™ VANQUISH™ C18 UHPLC Column and analyzed with a Thermo Scientific™ Orbitrap™ Ascend BioPharma Tribrid™ Mass Spectrometer. HCP identification and quantification were performed using Thermo Scientific™ Proteome Discoverer™ 3.1 Software [7].

Results

High-throughput screening for flow-through mode studies

The buffer conditions (salt concentration and pH) for POROS Caprylate resin were optimized through high-throughput screening in flow-through mode to maximize aggregate removal and monomer recovery. Key parameters for optimization included salt concentrations in the ranges of 0–300 mM or 0–500 mM, and pH values from 4.5 to 7.5. Two loading scenarios were evaluated: 5% aggregates at 200 mg per mL of resin and 10% aggregates at 100 mg per mL of resin.

Figure 4 shows that both loading density scenarios exhibited a consistent purification trend—aggregate removal was more effective at low pH, while higher salt concentrations enhanced monomer recovery. These insights informed the selection of a buffer system for subsequent separation studies. Furthermore, the findings suggested that minimal adjustment is needed after protein A purification before proceeding to the next polishing step, which typically involves reducing conductivity for ion exchange binding.

This study elucidated critical relationships of salt concentration and pH with aggregate removal and monomer recovery, thereby guiding buffer selection to enhance purification efficiency and cost-effectiveness.

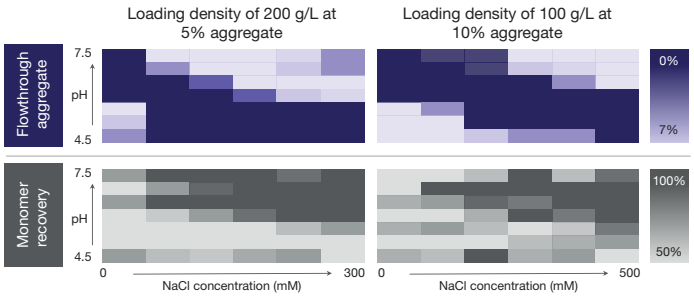


Figure 4. pH and conductivity screening at two different loading scenarios corresponding to aggregates and monomer recovery.

Chromatography optimization in scale-down mode

DOE statistical analysis guided buffer selection and predicted monomer recovery with aggregate removal for nine buffer combinations with varying pH (4.5–6.0) and NaCl concentrations (50–500 mM) (Figure 5).

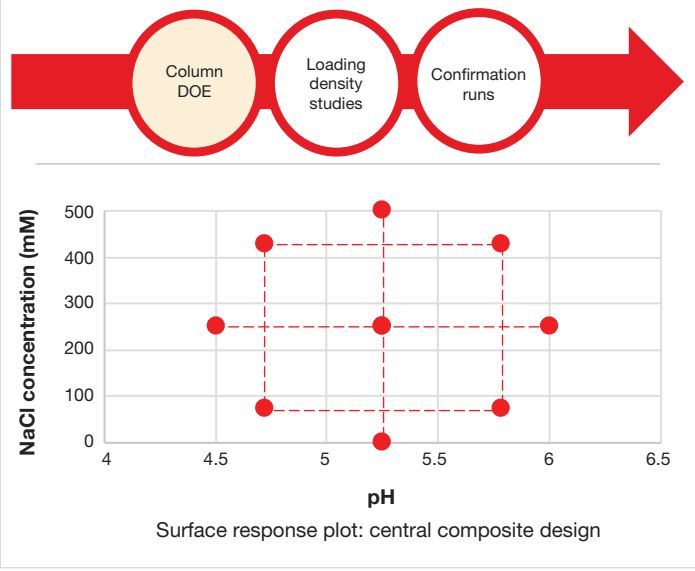


Figure 5. Central composite design for different pH values and NaCl concentrations.

The contour plots generated using Minitab™ software illustrate the relationships among monomer recovery, aggregate removal, and the selectivity factors of pH and NaCl concentration (Figure 6). The plots indicate that low pH (4.50–5.35) and NaCl concentrations below 250 mM are effective in removing high molecular weight species. Conversely, higher pH and NaCl concentrations improve monomer recovery but reduce the removal of high molecular weight species. These findings are consistent with high-throughput screening trends, highlighting the significant impact of pH and salt conditions on the efficiency of purification using the hydrophobic and weak cation exchange properties of POROS Caprylate resin.

The contour plots clearly demonstrate buffer selectivity based on pH and salt requirements, providing valuable insights for optimizing buffer combinations. Monomer recovery and aggregate removal values were calculated using a mass balance equation based on total concentration and SEC-HPLC purity data.

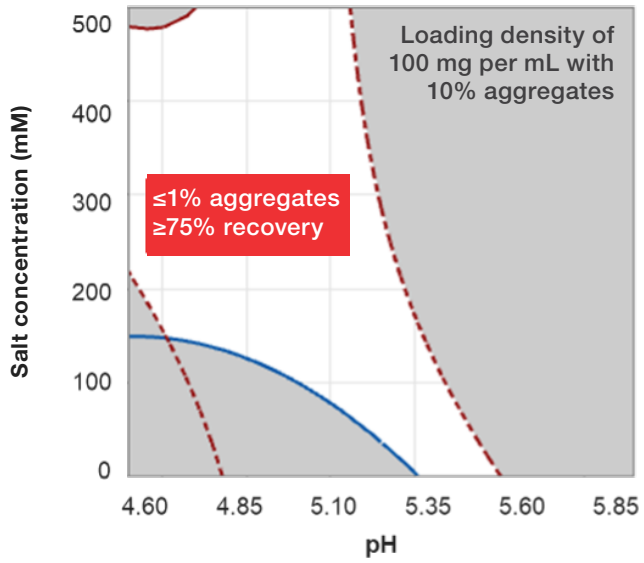


Figure 6. Contour plots for relationships among monomer recovery, aggregate removal, and the selectivity factors.

Chromatography optimization in scale-down model: breakthrough analysis

Chromatography optimization in the scale-down model demonstrated significant advancements in the loading density study conducted in overload mode. Three buffer conditions with varying pH and salt conductivity were evaluated, revealing breakthrough points for monomer purity, recovery, and aggregate percentage. A representative breakthrough curve, as shown in Figure 7, highlights the relationship between loading density and purification outcomes under one of the buffer conditions.

Understanding this relationship aids in selecting optimal loading densities, thereby improving monomer recovery and minimizing aggregate levels in the POROS Caprylate resin flow-through mode.

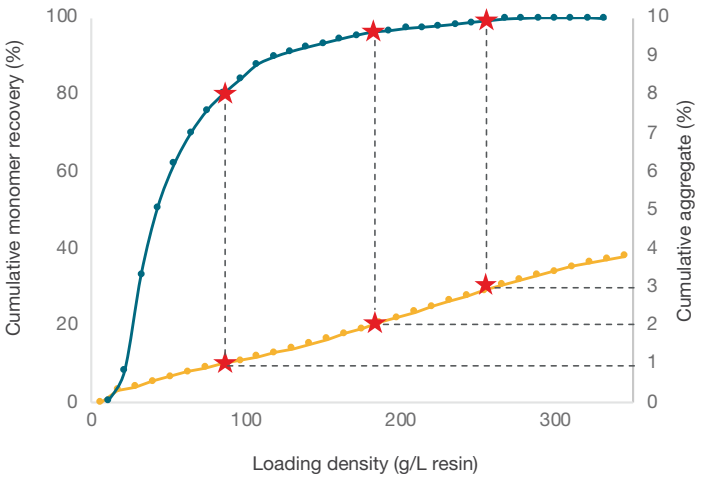


Figure 7. POROS Caprylate resin can facilitate a highly effective polishing step with high monomer yield and purity.

Additional buffer conditions with different pH and salt conductivity (µS/cm) were selected for loading density studies as well. Table 1 summarizes the monomer recovery corresponding to aggregate levels in at three different buffer conditions. These results highlight the effects of loading density on monomer recovery and aggregate levels, providing valuable insights into their relationship across varying buffer conditions.

Table 1. mAb monomer recovery at three different loading densities and aggregate percentages across three different buffer conditions.

Aggregate level	Loading density (mg per mL of resin)	Monomer recovery (%)
Buffer 1: 25 mM sodium acetate, 275 mM NaCl, pH 5.25		
1%	85.6	80.4
2%	181.9	96.3
3%	256.8	99.2
Buffer 2: 25 mM sodium acetate, 75 mM NaCl, pH 5.3		
1%	86.1	69.3
2%	215.2	81.7
3%	290.5	90.8
Buffer 3: 25 mM sodium acetate, 250 mM NaCl, pH 5.0		
1%	118.4	80.1
2%	215.2	88.7
3%	290.5	90.8

Table 2. Confirmation tests for the loading density studies.

Test	pH	NaCl (mM)	Conductivity (µS/cm)	Load (mg)	Monomer recovery (%)	Aggregates (%)
Confirmation test 1	5.25	275	28.6	160	93.88	1.76
Confirmation test 2	5.3	75	9.4	175	90.92	1.55
Confirmation test 3	5.0	250	25.9	170	89.64	1.70

Confirmation tests

To verify the balance between aggregate removal and monomer recovery observed in the loading density studies across three buffer conditions, column studies were conducted. Each confirmation test involved loading the column with 160, 175, or 170 mg per mL of resin to facilitate effective aggregate removal. The final flow-through pools exhibited monomer recovery of ≥89% and aggregate levels below 2%, improved from the initial aggregate level of ~10% (Table 2). These results confirm the observed trends and demonstrate successful balancing of aggregate removal and monomer recovery. Overall, these findings support the use of POROS Caprylate resin in the purification process, highlighting its effectiveness in removing aggregates and enhancing monomer purity.

Impurity: leached protein A

To help ensure final drug efficacy without leached protein A contamination, the Protein A ELISA Kit was employed to detect and quantify this impurity. Figure 8 shows that POROS Caprylate resin reduced protein A leachate by over 90% across three buffer conditions, while preserving the antibody integrity.

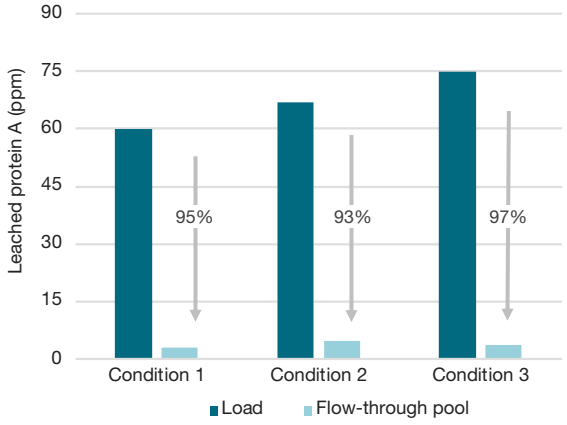


Figure 8. Clearance of leached protein A using POROS Caprylate resin.

Impurity: total HCP content

HCPs are process-related impurities originating from a host organism that can affect the stability, quality, and efficacy of biotherapeutics. Levels of residual HCPs are monitored, and clearance is required by regulatory agencies.

The ELISA method was employed to determine the clearance of HCPs by POROS Caprylate resin in this study. As shown in Figure 9, POROS Caprylate resin removed over 95% of total HCPs from the initial load, in flow-through mode, helping to ensure effective purification.

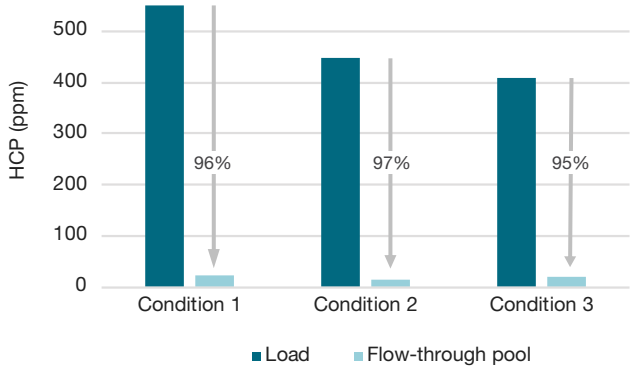


Figure 9. HCP clearance using POROS Caprylate resin.

CHO HCPs: LC/MS/MS method to quantify individual HCPs

While ELISA is the standard analytical assay for total HCP quantification, it cannot identify the nature of the individual HCPs. Particular attention should be paid to the clearance of two specific categories of HCPs—high-risk and difficult-to-remove. High-risk HCPs need to be reduced to very low levels, as they have been determined to be immunogenic, biologically active, or enzymatically active with the potential to degrade either product molecules or excipients used in formulation. Difficult-to-remove HCPs can cause trouble in downstream purification schemes, as they exhibit characteristics in common with the product or are sometimes co-eluted, making it difficult to meet HCP clearance requirements.

LC/MS/MS analysis was performed to identify and relatively quantify individual HCPs, to better understand the composition of the initial HCP load and POROS Caprylate resin's ability to reduce or remove specific HCPs.

There were 380 individual HCPs identified in the protein A pool and 78 HCPs identified in the polish pool. Table 3 lists 21 of the notable HCPs identified in the protein A pool and polish pool. After polishing with POROS Caprylate resin, 11 of these HCPs were completely removed to below detection levels, and those found in the polish pool showed very high reduction levels (Figure 10).

Table 3. List of individual HCPs identified by LC/MS/MS in pre- and post-polish pools.

Identified HCP	UniProt accession No.	Classification	Sample 1, protein A pool	Sample 2, polish pool
8 kDa glucose-regulated protein (GRP78, BiP)	G3I8R9	High-risk	•	•
Alpha-enolase (2-phospho-D-glycerate hydrolase)	G3I0W1	High-risk	•	
Cathepsin B (CatB)	G3H0L9	High-risk	•	•
Cathepsin Z (CatZ)	Q9EPP7	High-risk	•	
Clusterin (CLU)	G3HNJ3	High-risk	•	•
Glutathione S-transferase P1 (GSTP1)	G3I3Y6	High-risk	•	•
Lipoprotein lipase (LPL)	G3H6V7	High-risk	•	•
Lysosomal acid lipase (LAL)	G3HQY6	High-risk	•	
Matrix metalloproteinase-19 (MMP-19)	G3HRK9	High-risk	•	
Monocyte chemoattractant protein 1 (MPC-1)	G3GTT2	High-risk	•	•
Peroxiredoxin-1 (PRDX1)	Q9JKY1	High-risk	•	•
Phospholipase B–like 2	G3I6T1	High-risk	•	
Procollagen-lysine 2-oxoglutarate 5-deoxygenase 1 (PLOD1)	G3IIE7	High-risk	•	
Protein S100-A6 (S100A6)	G3HC31	High-risk	•	•
Pyruvate kinase (PK)	G3H3Q1	High-risk	•	•
Cathepsin D	G3I4W7	Difficult-to-remove	•	
Lipoprotein lipase	A0A3L7IKX6	Difficult-to-remove	•	•
Galectin-3–binding protein	G3H3E4	Difficult-to-remove	•	
G-protein–coupled receptor 56	A0A9J7FJA0	Difficult-to-remove	•	
Metalloproteinase inhibitor 1	G3IBH0	Difficult-to-remove	•	
Nidogen-1	A0A8C2LYQ4	Difficult-to-remove	•	

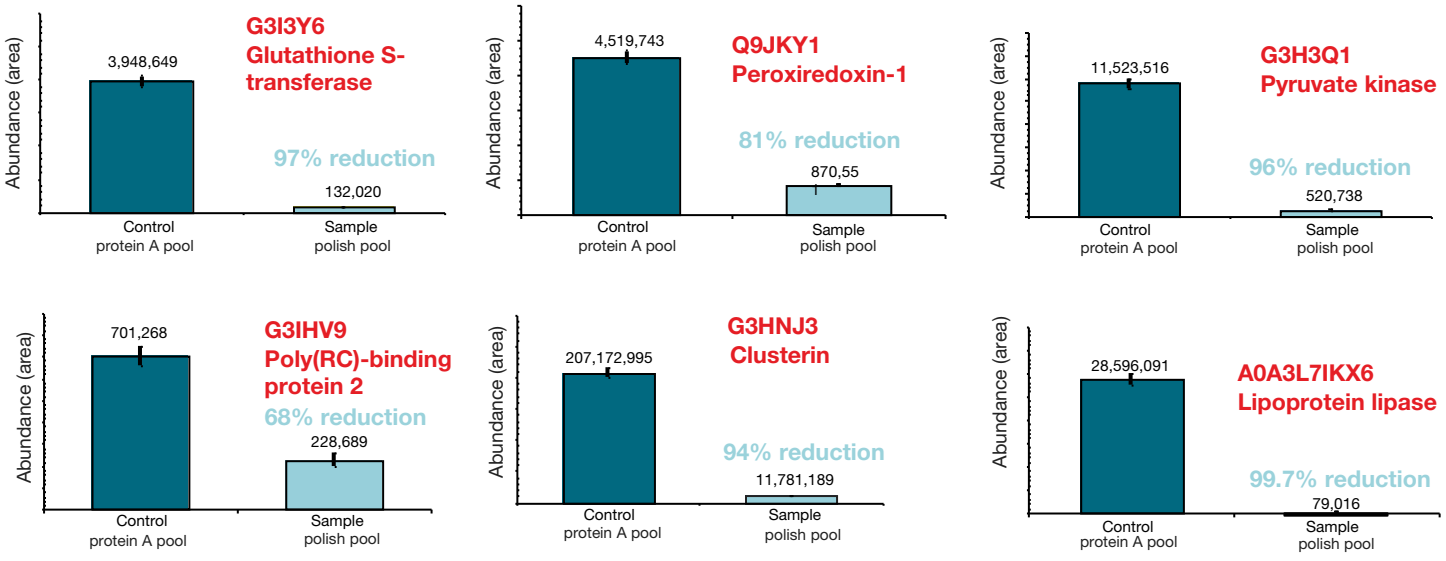


Figure 10. Relative quantities of 6 high-risk or difficult-to-remove HCPs found in the protein A pool and polish pool, showing significant reduction by POROS Caprylate resin.

Conclusions

These studies on POROS Caprylate resin, from high-throughput screening to process scale-up using column verification, have successfully demonstrated process optimization. The studies provided valuable insights into the purification process, particularly in separating monomers from impurities. Utilizing flow-through mode has resulted in a more efficient and cost-effective purification process. Optimal buffer conditions, loading densities, and pH levels were identified to achieve high monomer recovery while effectively removing impurities, including aggregates, HCPs, and protein A.

The successful optimization of the POROS Caprylate resin process offers multiple benefits, including improved overall efficiency, reduced production costs, and the production of high-quality monoclonal antibodies with high monomer purity. Additionally, the flow-through mode streamlines operations by eliminating the need for extra sample manipulation or buffer conditioning steps.

Overall, these studies mark a significant advancement in monoclonal antibody purification, providing a robust and optimized process for separating monomers from impurities. This can help lead to more efficient and cost-effective production of therapeutic antibodies.

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