Simultaneous aggregate and high-risk HCP clearance using mixed-mode cation exchange chromatography in flow-through mode

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Introduction

Engineered monoclonal antibodies (mAbs) can present downstream processing challenges due to product- and process-related impurities, including aggregates and host cell proteins (HCPs). Traditional aggregate removal in the polishing step relies on cation exchange (CEX) or hydrophobic interaction chromatography (HIC). But these approaches can be limited by binding capacity, load material, and development time — particularly as modern mAb and multispecific antibodies production often contain elevated levels of high molecular weight (HMW) aggregate and host cell proteins (HCPs).

Caprylic acid–based flocculation has been evaluated as an alternative impurity removal strategy [1]. However, this introduces its own challenges such as scalability and product recovery. Instead, caprylic acid was immobilized onto a porous cross-linked poly(styrene-divinylbenzene) beads to create Thermo Scientific™ POROS™ Caprylate Mixed-Mode Cation Exchange Resin to employ the chemical properties of caprylic acid via standard chromatography procedures. This offers a new tool for flow-through polishing for simultaneous HMW and HCP removal while maintaining high monomer recovery at increased capacities over traditional bind-and-elute methods.

This study evaluates the performance of Thermo Scientific™ POROS™ Caprylate resin for flow-through mode purification of a traditional mAb feed containing high levels of HMW aggregates.

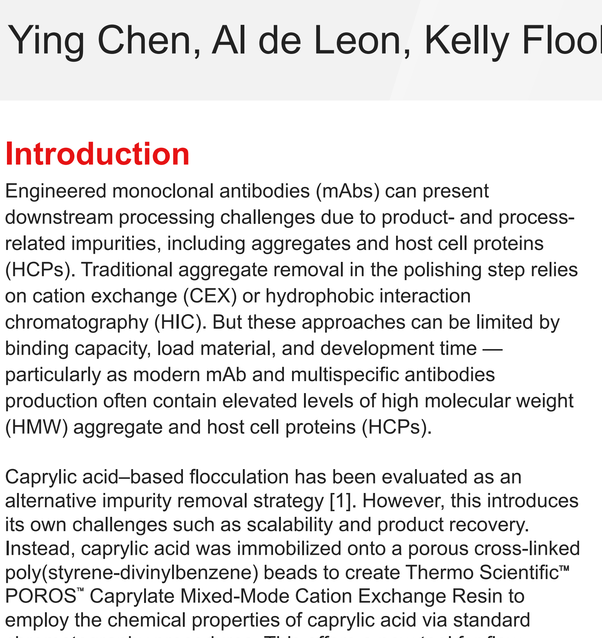


Figure 1: POROS™ beads and caprylic acid form a mixed-mode, hydrophobic weak cation exchange resin—POROS Caprylate Mixed-Mode Cation Exchange Resin

Materials and methods

Sample preparation of mAb aggregates

- IgG1 mAb was produced in-house using a Thermo Scientific™ Protein A affinity resin
- Aggregated mAb was generated by multiple exposure to pH stress [2]
- Aggregated mAb was titrated back to the stock pool to generate a ~10% aggregate starting material

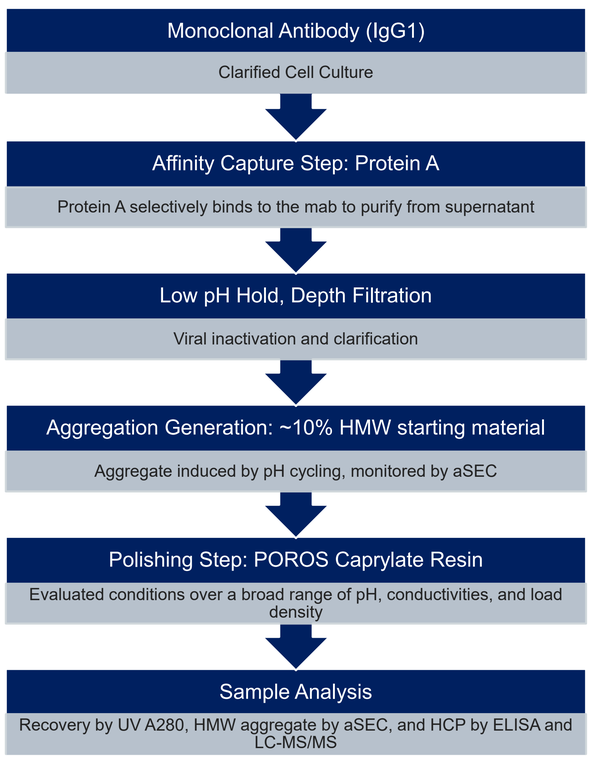


Figure 2: Schematic of sample generation, aggregate generation, resin evaluation, and sample analysis

Methods (continued)

Quantification of HMW aggregate species

- Analytical SEC HPLC (aSEC) was performed with Thermo Scientific™ MabPac™ SEC-1 on Thermo Scientific™ UltiMate™ 3000
  - **Mobile Phase**: 50 mM Sodium Phosphate, 300 mM NaCl, pH 6.5
  - **Detection:** UV at 280nm

aSEC-HPLC used for aggregate level determination

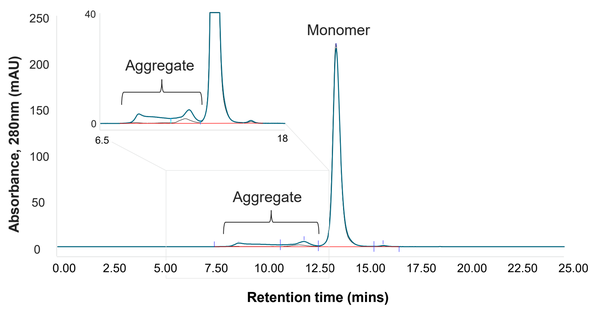


Figure 3: aSEC chromatogram of load material for POROS Caprylate resin (blue) and post Caprylate purification (black). Inset magnifies image of HMW section showing the reduction of HMW species post-purification.

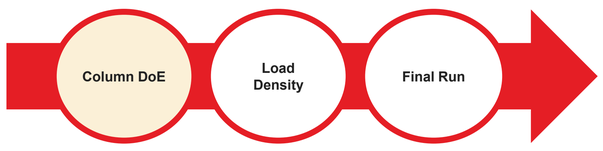
Quantification of total CHO HCP Content

- HCP quantification analysis was performed with Cygnus CHO Host Cell Protein ELISA-kit

Identification of individual CHO HCP [3]

- HCP identification was performed using LC-MS/MS Thermo Scientific™ Acclaim™ Vanquish™ C18 UHPLC column and analyzed using an Thermo Scientific™ Orbitrap™ Ascend BioPharma Tribrid™ mass spectrometer
- Analysis and quantification of HCP using Thermo Scientific™ Proteome Discoverer™ 3.1 software

Results—DoE study



Design of Experiment (DoE)

DoE study used to determine buffer composition for the highest monomer recovery and aggregate removal. Nine buffer combinations with varying pH and sodium chloride (NaCl) concentrations were evaluated in flowthrough mode.

Design parameters:

- Load density: 100 mg/mL of resin
- Loading conditions: 10% aggregate levels
- pH range: pH 4.5 to pH 6.0
- NaCl concentration: 0 to 500 mM

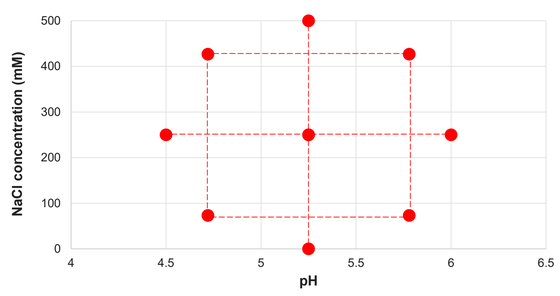


Figure 4: Design Space, [NaCl] and pH vs. monomer and aggregate response

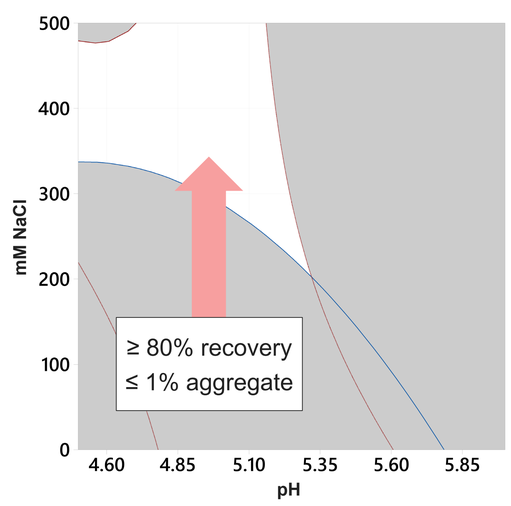


Figure 5: Design space for ≥ 80% monomer recovery with <1% aggregate

Results—DoE study (continued)

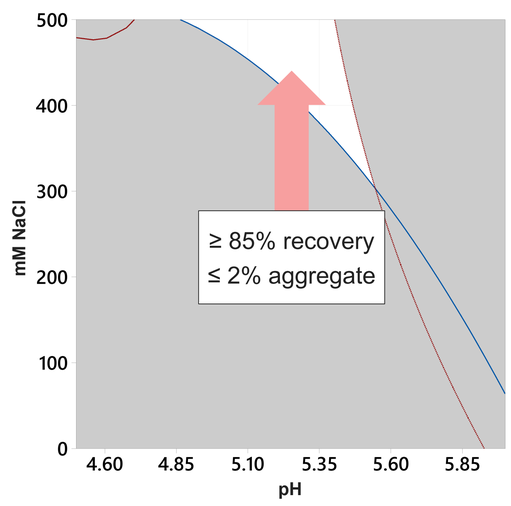
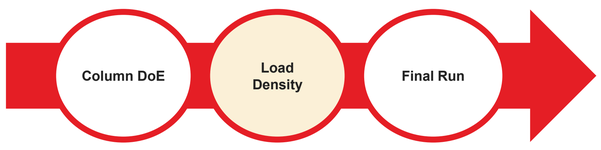


Figure 6: Narrower design space that resulted in higher recovery (>85% monomer) but a slight increase aggregate

- Low conductivity conditions, POROS Caprylate Mixed-Mode Resin was able to reduce aggregate levels down to 1%-2% in flowthrough mode
- Aggregate removal was more effective at lower pH
- High salt concentration enhanced monomer recovery
- The 2-D representation of the design space showed relatively large ranges for high yield and purity

Results—Load density study



Load density study was conducted to determine breakthrough points for the monomer purity, recovery, and aggregate percentage.

Loading conditions:

- Max loading: 325 g/L resin
- Monomer Purity: 89.4%
- % Aggregate: 10.6%

Buffer & residence time:

- 25 mM Sodium Acetate, 275 mM NaCl, pH 5.25
- Residence Time: 3 mins

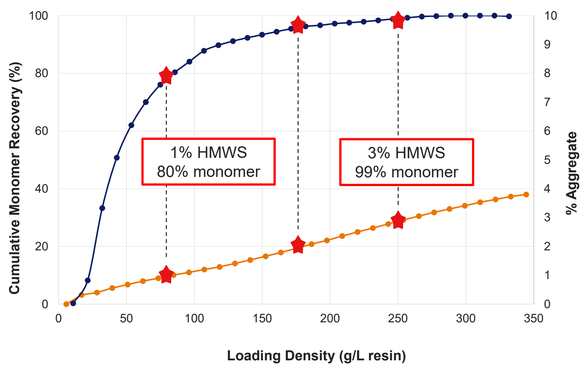


Figure 7: Monomer recovery (dark blue) vs aggregate accumulation (orange), with aggregate levels marked for 1%, 2% and 3%

- < 5% Total accumulated HMW aggregate at the highest load density tested at 325 mg/mL of resin
- As the purity increases, the monomer recovery decrease

Table 1: Loading density and monomer recovery at target aggregate impurity levels

Target % Aggregate	Loading density (g/L resin)	Monomer recovery (%)
1%	85.6	80.4
2%	181.9	96.3
3%	256.8	99.2

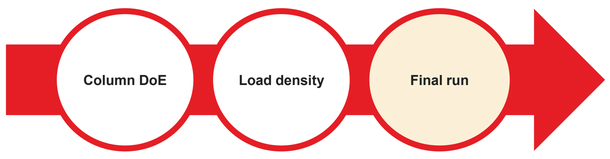
Additional analysis was performed to evaluate bulk HCP clearance. A large reduction of HCP was shown, from > 400 ppm in the protein A pool down to < 25 ppm in the final pool post POROS Caprylate resin purification.

Table 2: Load density experiment HCP analysis*

Parameter	Quantity
Total load	160 mg
HCP Pre-column	555 ppm
HCP Post-Column	24 ppm

***sample buffer conditions:** 25mM sodium acetate, 275mM NaCl, pH 5.25

Results—Final run



Completed a final run with a goal to achieve 80% recovery and <2% aggregate using the observations and parameters from DoE and load density study.

Conditions:

- Column Size: 1mL POROS Caprylate mixed-mode column
- Loading density: 160 mg/mL of resin
- Loading buffer: 25 mM Sodium Acetate, 275 mM NaCl, pH 5.25
- Residence time: 3 minutes
- Mode: Flow-through

Table 3: final Run

Run	Monomer recovery	HMW aggregates
1	94%	1.8%

- The final flowthrough pools had > 90% recovery and aggregate levels below 2%an improvement from the initial aggregate level of ~ 10%.

Results—HCP identification and quantification via LC-MS/MS

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

- 380 HCPs were identified in the protein A pool
- 78 HCPs were identified in the Caprylate pool

Identification of 21 HPCs that were classified as high risk or difficult to remove in the protein A and/or Caprylate pool. After polishing step with POROS Caprylate resin, eleven of these HCPs were below detectable levels by LC-MS/MS. In figure 7, lists some of those HPCs. Additional data available in application note.

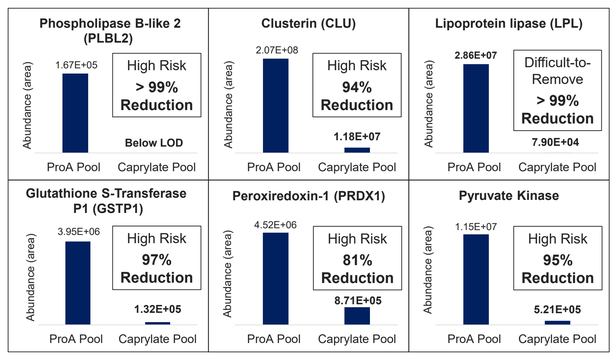


Figure 8: Relative quantities of 6 high risk or difficult to remove HCPs found in the protein A and Caprylate pool, showing significant reduction by POROS Caprylate resin

Conclusions

The purification of a high aggregate (10%) mAb feedstock using POROS Caprylate Mixed-Mode CEX Resin was able to:

- Exceed target monomer recovery (94% > 80%) and aggregate reduction (1.8% < 2% HMWs)
- Simultaneously reduce high risk or difficult-to-remove HCPs
- Operate at high binding capacity in flow-through mode

Operating in flow-through mode, this method can offer intensified process economics when implemented in place of bind-and-elute CEX polishing.

References

1. Brodsky Y, Zhang C, Yigzaw Y, Vedantham G. Caprylic acid precipitation for purification of monoclonal antibodies. *Biotechnol Bioeng.* 2012;109(10):2589–2598.
2. Potty SR, Xenopoulos A. Stress-induced antibody aggregates. *BioProcess Int.* 2013;11(3):44–49.
3. Kiyonami R, Melani R, Chen Y, et al. Applying UHPLC-HRAM MS/MS method to assess host cell protein clearance during the purification process development of therapeutic mAbs. *Int J Mol Sci.* 2024;25(17):9687.

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