An innovative approach to addressing high aggregate challenges in engineered monoclonal antibodies

Ying Chen, Al de Leon, Kelly Flook, Thermo Fisher Scientific, Bedford, MA 01730 USA

Abstract

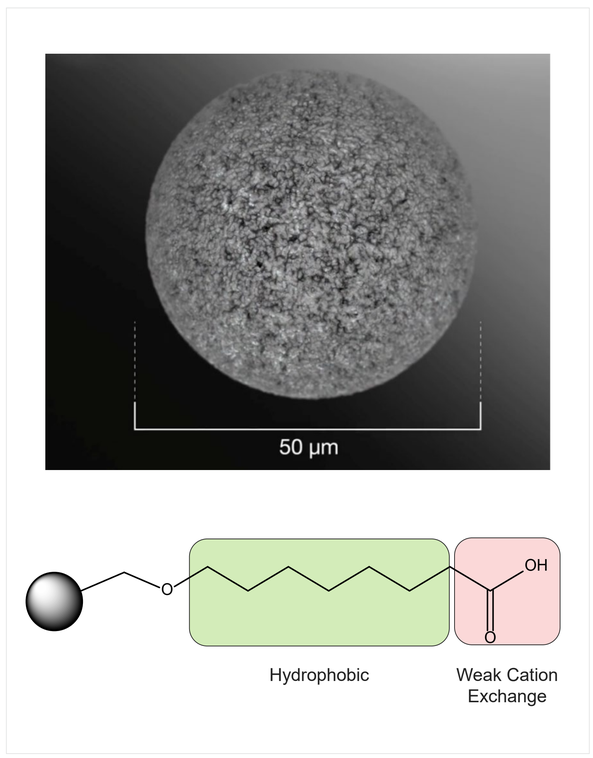
With advances in engineered antibody designs, treatment performance improves, but higher aggregate levels are often produced in the cell culture creating new purification challenges. Current solutions for aggregate removal include bind and elute strategies with cation exchange or hydrophobic interaction chromatography resins which, whilst effective, often result in poor process economics and low recoveries. Alternatively, caprylic acid has been successfully used as a flocculant for antibody aggregates but requires a filtration step resulting in a more labor intensive and complicated process. The work in this poster describes the performance of a resin-based approach using immobilized Caprylic acid. It effectively removes high levels of aggregates as well as leached ligand from protein A affinity resin and 301 out of 380 HCPs identified using HPLC-MS/MS2 including those that are either high-risk and/or challenging to remove.

Introduction

With the need of designing therapeutics with higher efficacy, more engineered monoclonal antibody derivatives are actively pursued for the next generation of mAb-based drugs. With the more complex structures, like symmetric, asymmetric or fragment-based bispecifics, the downstream process developer is challenged by mis-paired products, undesired fragments and higher levels of aggregates. Alternative new mAb designs are equally challenging. The use of caprylic acid as a flocculant for aggregate removal and high molecular weight species has been earlier suggested by Brodsky et al.[1] in 2012. The precipitation step though requires to introduce additional filtration and sedimentation steps.

By chemically attaching caprylic acid (octanoic acid) to large pore POROS™ divinylbenzene polymeric beads, a chromatography resin with excellent aggregate removal capabilities was developed. The work described here tests the final design of the resin on loading, aggregate elimination and also best operational conditions (for our simulated mAb high aggregate test solution).

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

A IgG1 type mAb was produced in-house and purified using Thermo Scientific™ MabCaptureA™ affinity resin. In order to mimic high aggregate levels, the mAb was then stressed through multiple exposures to high and low pH adjustments, until the aggregate level reached approximately 10%. [2]

- Purified mAb was then applied to 1mL POROS Caprylate Mixed-Mode resin packed into OmniFit glass column (6.6mmID x 30 mmL).
- HPLC-SEC was performed with a Thermo Scientific MabPac™ SEC-1 on Thermo Scientific UltiMate™ 3000. Buffer: 50mM Sodium Phosphate, 300 mM NaCl, pH 6.5; flow rate: 0.2 mL/min; detection: UV at 280nm.
- HCP and Protein A ligand leach was performed with Cygnus CHO Host Cell Protein ELISA-kit and Repligen Protein A ELISA-Kit, respectively.

Materials and methods (continued)

To simulate an antibody with high levels of aggregate, a biosimilar version of Herceptin was produced and subjected to pH cycling to induce aggregate formation. Aggregate levels and monomer purity were determined using size exclusion chromatography (SEC)

Figure 2: Schematic of sample generation, aggregate induction and resin performance test.

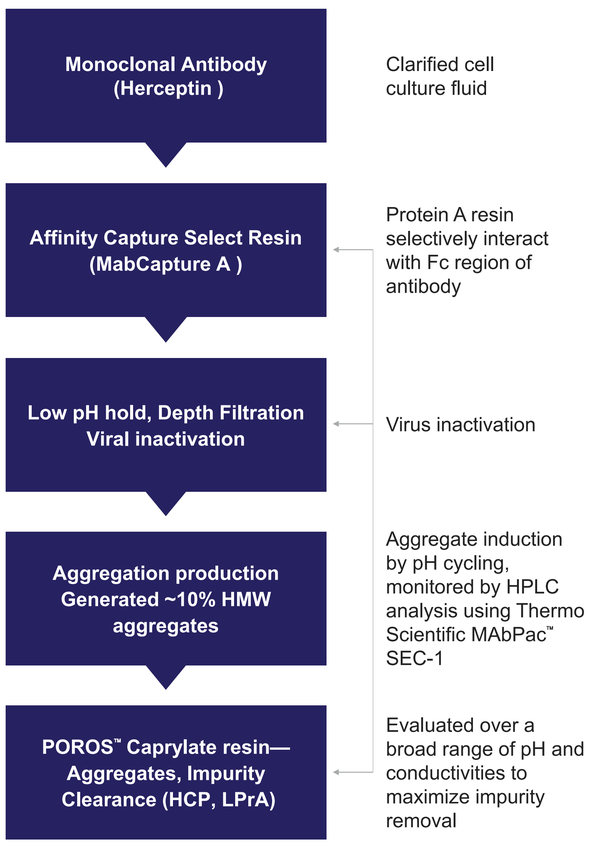


Figure 3: HCP characterization and relative quantification using HPLC MS-MS/MS method

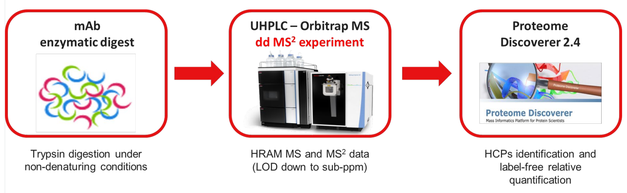
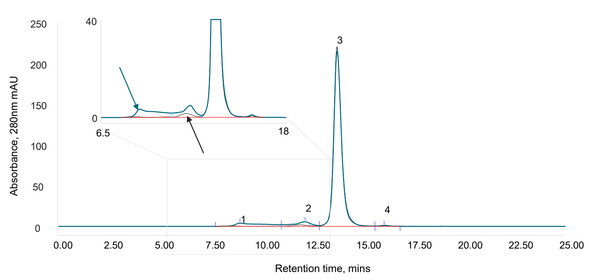


Figure 4: SEC chromatograph of mAb feed prior to purification using POROS Caprylate resin (blue) and after (black). Inset is an expanded section of high molecular weight species.

HPLC – SEC used for aggregate level determination on mock-up feed solution



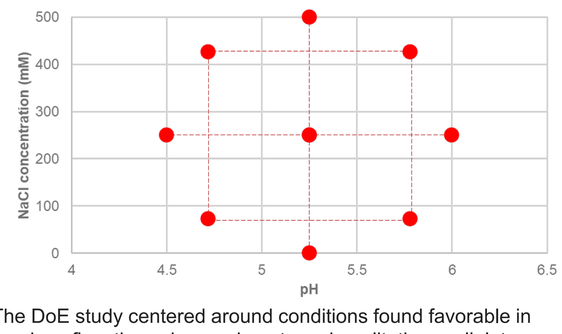
Results—DoE study

Finding optimal conditions

A Design of Experiment (DoE) study was used to evaluate the optimum mobile phase pH and conductivity to achieve monomer yield > 80% and reduction of aggregate levels to < 2%.

The design space: pH range 4.5–6.0, [NaCl] from 0–500mM. Load density was kept constant at 100mg / mL resin.

Figure 5: Design Space, [NaCl] and pH vs. monomer and aggregate response

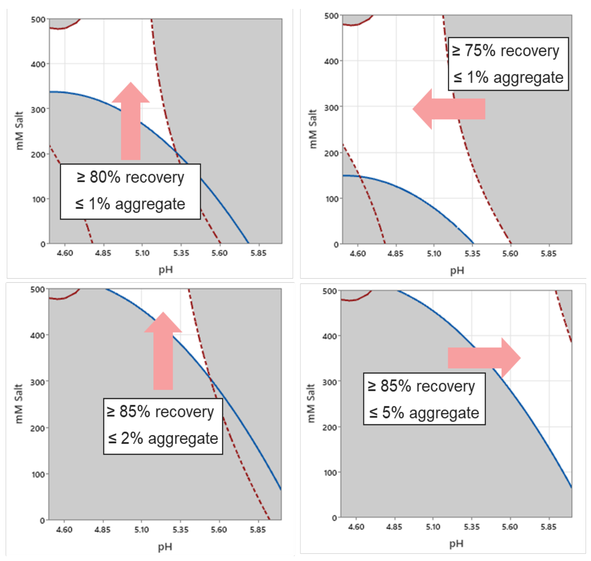


The DoE study centered around conditions found favorable in previous flow-through experiments and qualitative, wellplate based HTS screening. The pH range was chosen from 4.5 to 6.0, the NaCl concentration from 0 to 500 mM.

Results—DoE study (continued)

The 2-dimensional representation of the design space below show relatively large design conditions for high yield and purity expectations..

Figure 6: Design space for monomer vs. aggregate percentage



Even with lower conductivity conditions, POROS Caprylate Mixed-Mode resin is able to reduce aggregate levels down to 1–2%

This option is favorable for a directly following low salt anion exchange process step in the overall polishing process. As the AEX polishing is also often run in flow-through mode, the suggested savings pull through then at that step as well (lower buffer consumption, lower COGS, smaller column sizes, fast high yield break through).

Results—Load density study

Conditions used for load density study

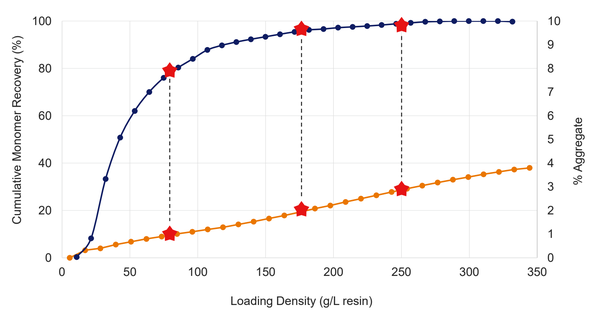
Feed:

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

Buffer & Residence Time:

- Sodium Acetate pH 5.25
- 275mM NaCl (28.62 mS/cm)
- Residence Time: 3 min

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



Result show very favorable monomer yield for the given aggregate impurity levels.

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

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

Results—Reduction of other HWMS

POROS™ Caprylate Mixed-Mode resin is also effective in removing other high molecular weight species (HMWS), like host cell proteins (HCP) or leached Protein A resin ligand.

Parameter	Unit	Loading density experiment R&D Batch A	Loading density Experiment R&D Batch B	Production Validation Batch MMCEX-001
Total load	[mg]	160	175	100
Buffer conditions		25mM sodium acetate 275mM NaCl, pH 5.25	25mM sodium acetate, 75mM NaCl, pH 5.30	25mM sodium acetate, 12mM NaCl, pH 4.5
Host cell protein in load	[ppm]	555	450	648
Host cell protein after column	[ppm]	24	14	36
Leached protein A in load	[ppm]	60.3	67.5	78.5
Leached protein A after column	[ppm]	3.1	4.7	1.3
Text System		1mL CV Omnifit column	6.6mm ID x 300mmL	residence time 3 minutes

Table 2: HCP & Leached Protein A ligand reduction, 3 different experiments/conditions

HCP Classification	Identified HCP	IgG-1 after Protein A purification	IgG-1 after POROS Caprylate Purification
High Risk	8 kDa glucose regulated protein(GRP78, BiP)	6.64E+05	n.d.
	Alpha-enolase (2-phospho-D-glycerate hydro-lyase)	2.43E+04	n.d.
	Cathepsin B (CatB)	1.00E+06	n.d.
	Cathepsin L (CatL)	4.16E+04	n.d.
	Cathepsin Z (CatZ)	7.52E+04	n.d.
	Glutathione S-transferase P 1 (GSTP1)	4.06E+05	n.d.
	Lysosomal Acid Lipase (LAL)	2.67E+05	n.d.
	Matrix metalloproteinase-19 (MMP-19)	2.08E+05	n.d.
	Phospholipase B-like 2 (PLBL2)	1.67E+05	n.d.
	Monocyte Chemoattractant Protein-1 (MCP-1)	1.72E+06	1.02E+05
	Peroxiredoxin-1 (PRDX1)	4.20E+05	1.12E+05
Challenging to Remove	Cathepsin D	8.43E+04	n.d.
	Insulin-like growth factor-binding protein 4	7.46E+04	n.d.
	metalloproteinase inhibitor	2.08E+05	n.d.
	galectin-3-binding protein	2.15E+05	3.31E+04
	lipoprotein lipase	2.72E+06	7.42E+05
High Risk and Challenging	Clusterin (CLU)	2.24E+07	1.56E+06

Table 3: Identification of HCP and relative quantification (total ion count) before and after POROS Caprylate purification

Conclusions

Simulated high aggregate levels in our mAb test solution has shown that POROS Caprylate Mixed-Mode resin operated in flow-through mode, is very promising for

- Effective removal of high (10%) aggregate levels in mAbs using flow-through mode
- Delivering high monomer yields (> 80%) with low aggregate impurity levels (< 2%)
- Improved mAb purification process designs, were flow through can be used for the cation exchange step and the anion exchange-based final polishing step
- The economics of a such intensified process design can be highly advantageously for existing and new modalities

References

1. Brodsky Y, Zhang C, Yigzaw Y, Vedantham G. CaprylicVan, Biotechnol Bioeng. 2012 Oct
2. Stress-Induced Antibody Aggregates, Ajish SR Potty and Alex Xenopoulos, p44 ff, BioProcess International 11(3) March 2013, BioProcess

Intended use statement

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