Overcoming aggregate removal challenges in the therapeutic antibody purification workflow – three downstream strategies

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Abstract

High aggregate content is often linked to bispecific antibodies (bsAb) but can also be problematic in monoclonal antibodies (mAbs) and Fc-fusion proteins, with aggregate levels sometimes exceeding 10%.

Resin manufacturers are tasked with providing better tools for process developers. This poster presents three case studies on the aggregate removal of challenging antibody feeds, using resins with the potential to advance the field.

Introduction

Regulatory guidelines mandate that therapeutic antibodies achieve purity levels exceeding 99%, and aggregate levels must be kept at minimal levels. However, purifying antibody feeds with high aggregate levels poses significant manufacturing challenges, such as reduced process yields and overall efficiency of the downstream process.

Process optimization becomes critical, as capture and polish steps must be meticulously adjusted to separate aggregates efficiently. High aggregate levels can also shorten resin lifespan, increasing operational costs. Overcoming these challenges is essential for ensuring the production of high-quality, safe antibody therapeutics.

Case study 1

Aggregate removal during the initial capture step using affinity purification

This case study is published by Wuxi Biologics in a short communication (ref 1).

Introduction: Protein A affinity purification is commonly used as a capture step in antibody manufacturing. Affinity chromatography can significantly reduce impurity levels however, Protein A has generally poor efficiency in removing aggregates. Aggregate removal often relies on subsequent polishing steps in the downstream process.

Goal of the study: Researchers observed increased aggregate removal in the capture step using Thermo Scientific ™ CaptureSelect™ FcXp affinity matrix. The resin was further evaluated for this feature.

Aggregate separation potential in bsAb-1

- bsAb-1 aggregate content is 16.6%
- Comparison between Protein A and CaptureSelect FcXP affinity matrix using linear pH gradient elution

Study format: Loading at 5 mg/mL, 5 min residence time. **Elution (Fig 1A):** 50 mM NaAc-HAc pH 5.5 to pH 3.5 over 20 CV. **Elution (Fig. 1B):** 50 mM NaAc-HAc pH 5.5 to pH 3.5 in 20 CV

Protein A separation

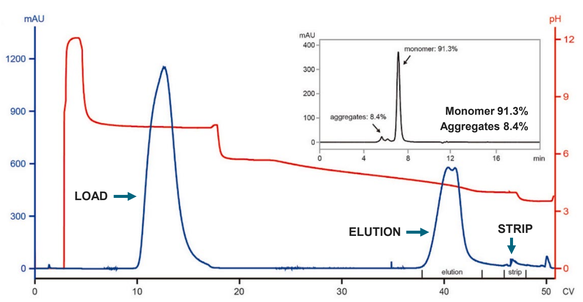


Fig. 1A. Chromatogram showing Protein A separation of bsAb-1

FcXP separation

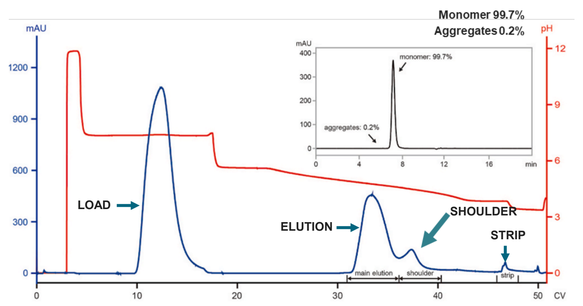


Fig. 1B. Chromatogram showing separation of bsAb-1 using the FcXp resin. The elution peak shows a distinct shoulder.

Results comparison Protein A and FcXP resin:

- The Protein A resin is not able to efficiently separate the monomer from the aggregates.
- The small shoulder peak in the FcXP-chromatogram contains a high percentage of aggregates.
- SEC-HPLC data demonstrates that the FcXP resin probably possesses a stronger aggregate separation potential than Protein A.

Aggregate separation potential in bsAb-2

- bsAB-2 aggregate content is 22.6%
- Investigating FcXP affinity matrix separation potential using various elution strategies

Study format: Loading at 15mg/mL, 5 min residence time. **Elution (Fig 2A):** 30 mM NaAc-HAc, pH 5.5, to 30 mM NaAc-HAc, 15 mm NaCl, pH 3.5 over 20 CV. **Elution (Fig. 2B):** 50mM NaAC-HAc, 2.7 mM NaCl, pH 4.3 to 50mM NaAC-Hac, pH 4.3 over 20CV

Linear pH elution

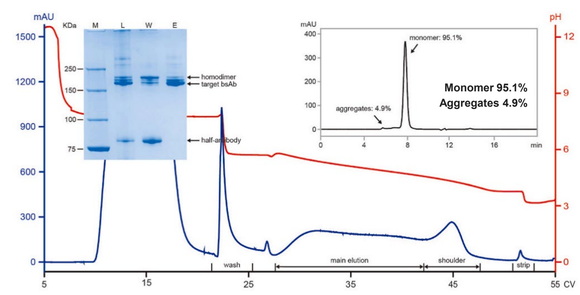


Fig. 2A. Chromatogram showing separation of bsAb-2 with a linear pH gradient elution at a defined conductivity, showing promising aggregate separation. Final monomer recovery was 86,3%.

Linear conductivity elution

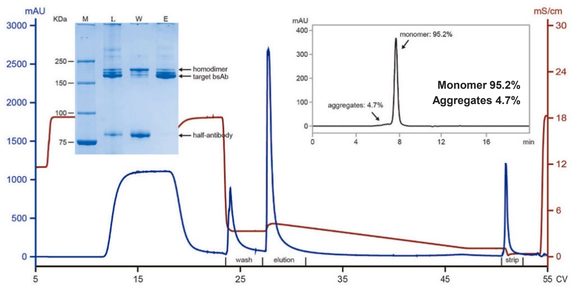


Fig. 2B. Chromatogram showing separation of bsAb-2 with a linear conductivity gradient elution. The sharp single elution peak has a monomer purity of 95.2%. Final monomer recovery was 84,3%.

Results elution study:

- The FcXP resin provides effective separation under pH and conductivity elution conditions
- Having most aggregates removed at the capture step could be a significant advantage in improving the overall robustness of the process

Conclusion: This study suggests that the FcXP affinity resin has a higher avidity to aggregates due to multiple ligand contact points and could potentially be an alternative for capture when aggregate content is high.

Case study 2

Innovative resin design to address high aggregate challenges

Introduction: Caprylic acid can be used as a flocculant for aggregate removal, but precipitation methods are not optimal in large-scale manufacturing. Hence, a unique resin was developed, attaching caprylic acid to POROS™ high-performance resin beads. This resin is designed to be used in **flowthrough mode**.

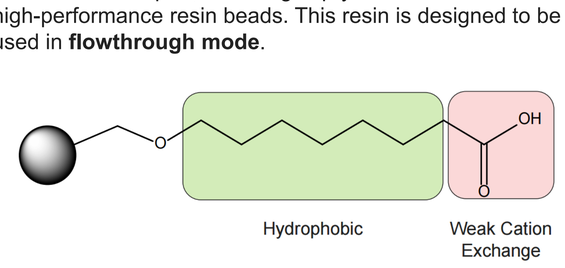
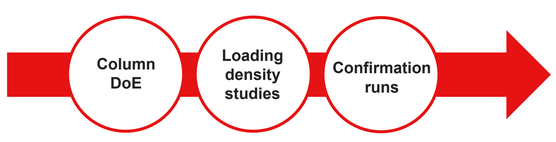


Fig. 3. Thermo Scientific™ POROS™ Caprylate Mixed-Mode Cation Exchange Chromatography resin contains a ligand with both hydrophobic and weak cation exchange features.

This study describes the experimental work performed, testing the resin on loading, aggregate removal and optimal process conditions. For the complete overview, check reference 2.



Column DoE & loading density studies

- Aggregate induced Herceptin mAb containing 10.6% aggregates after Protein A purification
- Acceptable monomer yield = > 80% with a minimum reduction of aggregate levels to < 2%
- Column DoE study conducted to find the optimal mobile phase conditions demonstrates a large design space*

* Further details and additional study results can be found in ref. 2.

Study format: maximum load density 322 g/L, 3 min residence time. Three buffer conditions were tested using 25mM Sodium acetate at different pH and conductivity conditions: (1) **pH 5.25 (29mS/cm / 275 mM NaCl),** (2) **pH 5.30 (9.4 mS/cm / 25 mM NaCl),** (3) **pH 5.00 (26mS/cm / 250mM NaCl)**

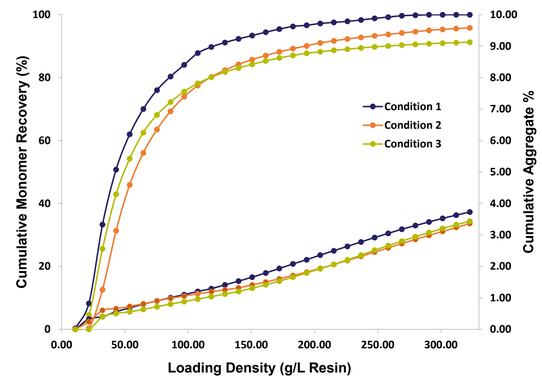


Fig. 4. Results loading density study, using 3 conditions varying pH and conductivity.

Results of the loading density study:

- Higher monomer recovery is achieved at higher loading densities
- Minimal differences are seen between the 3 conditions tested

Confirmation runs

- 3 confirmation runs using conditions selected from the load density study (see table 1)
- Determination of monomer recovery, aggregate, HCP, and leached Protein A ligand removal

Confirmation Run	pH	NaCl concentration (mM)	Conductivity (mS/cm)	Loading density (mg/mL)
1	5.25	275	28.6	160
2	5.30	75	9.4	175
3	5.00	250	25.9	170

Table 1. Overview of conditions used in the confirmation runs

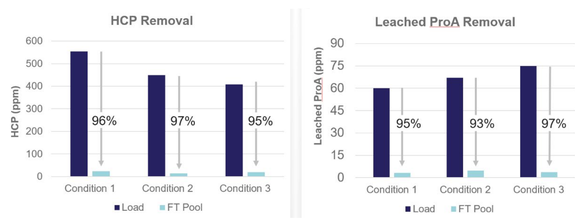


Fig. 5. High HCP and leached Protein A ligand removal in all confirmation runs.

Results confirmation runs:

- High HCP and leached Protein A removal can be achieved at high loading density
- Identification of HCPs shows successful removal of many high risk and challenging HCPs [ref.2.]

Conclusion: The POROS Caprylate resin is highly effective in removing aggregates and ensuring high monomer yields. Additionally, its use in flowthrough mode offers several advantages. The resin's large design space also plays a crucial role in enhancing process design.

Case study 3

Aggregate removal using hydrophobic interaction chromatography (HIC) in flowthrough (FT) mode

This study was performed to further optimize a downstream process of a clinical mAb feed containing a high level of aggregates (>12%).

The goal was to develop a more efficient, robust, cost-effective polish step using HIC (Thermo Scientific™ POROS™ Benzyl Ultra HIC resin) in FT mode.

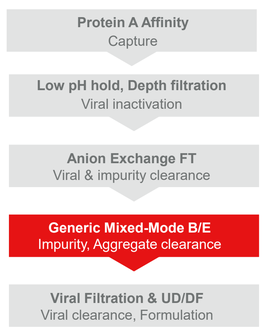


Fig. 6. Original purification process with a generic Mixed-Mode resin in bind/elute mode as the final polishing step.

The study workflow consisted of 3 steps: determining salt tolerability, high throughput screening, and performing scale-down model process development. For the complete study[ref.3.]

Breakthrough analysis

Study format: Flow rate 300 cm/hr, 2 min residence time, max load density 350 g/L, column 0.66 cm x 10cm (DxL)

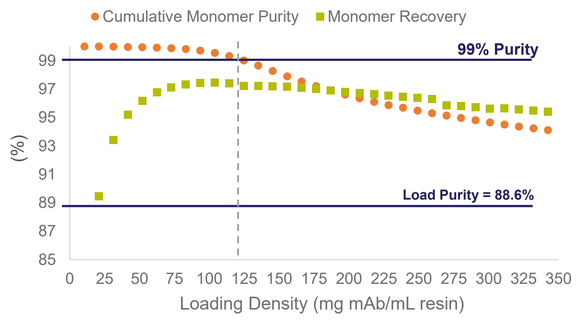


Fig. 10. Breakthrough analysis showing monomer purity (blue dotted line) and recovery (green dotted line).

Results breakthrough analysis:

- < 1% breakthrough of aggregates up\to 125 g/L resin loading
- 97% Monomer recovery over a wide range of load densities
- HIC flow-through process uses **no additional buffer manipulation post AEX pool**

Flow through verification run (500cm/hr)

Study format: Flow rate 500 cm/hr, residence time 1.2 min, max load density 80 g/L, column 0.66 cm x 10cm (DxL), loading buffer Tris-Acetate pH6.8 (same as AEX step)

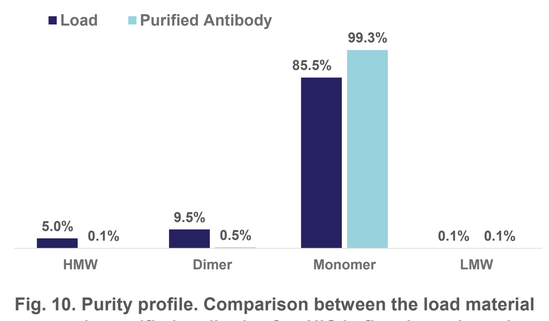


Fig. 10. Purity profile. Comparison between the load material versus the purified antibody after HIC in flowthrough mode.

mAb-A Process	Generic Mixed-Mode BE	POROS Benzyl Ultra-FT
Load Density (g/L resin)	25	**80–100**
Monomer Purity Pool	99%	**>99%**
Monomer Recovery	90%	**98%**
Residence time (min)	6	**1.2**

Table 4. Overall results compared between the original polishing step (Generic Mixed-Mode in bind elute) versus the new step.

Results verification run:

- Final run shows effective reduction of aggregates in a low conductivity FT process, with high recovery

Conclusion: The new process allows increased load density and delivers higher purity and improved aggregate clearance.

In summary

Antibody aggregation remains a challenge, especially for complex molecules and downstream processes need to be optimized for efficient and effective removal. With several solutions available for capture and polishing steps, process developers can select the suitable method for their process.

References

1. Dong, W., Zhang, D., & Li, Y. (2024). *CaptureSelect FcXP affinity medium exhibits strong aggregate separation capability. Protein Expression and Purification*, 220, 106503. https://doi.org/10.1016/j.pep.2024.106503
2. Chen, Y., de Leon, A., Flook, K., (poster 2024) *An innovative approach to addressing high aggregate challenges in engineered monoclonal antibodies.* Thermo Fisher Scientific, Bedford, MA.
3. Li, J. J., Terova, O., & De Rooij, J. (2018). *Improving aggregate removal to enhance clinical manufacturing of mAbs.* BioPharm International.

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