thermo scientific

Efficient monoclonal antibody aggregate removal by Hydrophobic Interaction Chromatography (HIC)

John J. Li, Jessica De Rooij, Alejandro Becerra, and Orjana Terova,
Thermo Fisher Scientific, 35 Wiggins Ave, Bedford MA, 01730 USA

INTRODUCTION

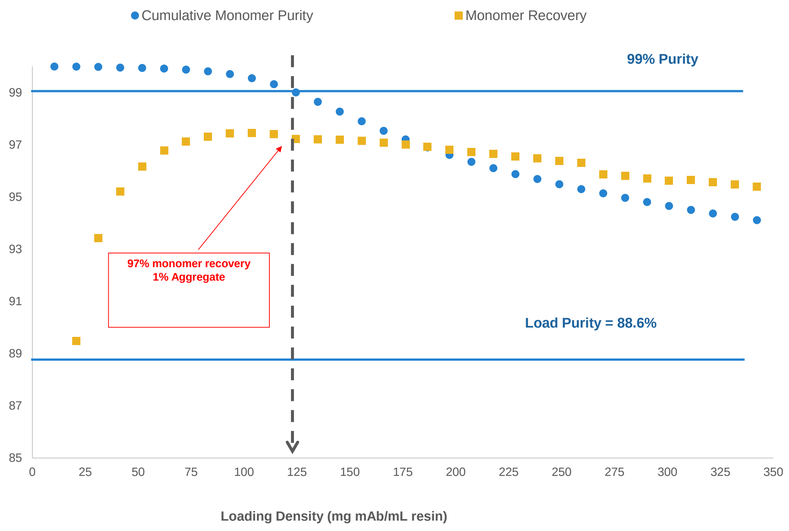
This case study shows the optimization of POROS™ HIC resin-use in high-throughput screening (HTS) and subsequently upscaling in both Bind-Elute (B/E) and Flow-Through (FT) mode. Our study shows that a well-designed process together with a robust resin are key to a successful and efficient Mab polishing process.

GOAL OF THE STUDY

Design a more efficient, robust and cost-effective polish step utilizing POROS™ HIC resins as an alternative to the mixed-mode step in the original purification process of a clinical Mab containing >10% aggregates.

Salt Tolerability (Data not Shown) → High Throughput Screening → Scale Down Model

POROS Benzyl Ultra in Flow-through mode

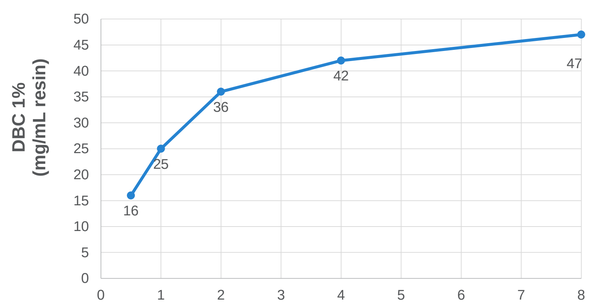


Study Format

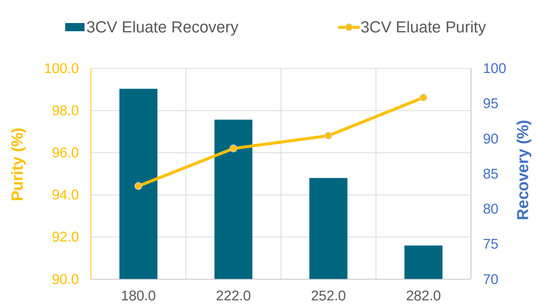
- 0.8cmx10cmL (CV 5mL)
- **Load 350 mg/mL resin**
- 2 min residence time
- Load monomer 89%
- 2 mS/cm conductivity
- pH6.8 chosen based on optimized pH of previous AEX polish step

✓ **1% Aggregate breakthrough after 125mg/mL resin loading**
✓ **Complete Aggregate clearance achieved with POROS Benzyl Ultra at low salt and flexible pH**

POROS Benzyl in Bind/elute mode



Dynamic Binding Capacity 1% vs. Residence time



Elution Conductivity Optimization Elution 180-282mM Sodium Citrate

✓ **High DBC at short residence time (2min, 32mg/L resin)**
✓ **High recovery and purity in Elution (265mM Sodium Citrate)**

HTS: Resin Selection for Scale-Down

Screening variables used to predict conditions for scale down model: resin type, salt type and salt concentration. Partition selectivity ratio is used to determine to level of separation.

Resin type	POROS Ethyl				POROS Benzyl				POROS Benzyl ultra			
Salt type	Sodium Chloride	Sodium Acetate	Amm. Sulfate	Sodium Citrate	Sodium Chloride	Sodium Acetate	Amm. Sulfate	Sodium Citrate	Sodium Chloride	Sodium Acetate	Amm. Sulfate	Sodium Citrate
Salt concentration ↑				2.43		1.84						
				3.12	2.74	2.18						
			2.48	4.19	2.59	2.41		2.35	2.38	2.07		
					2.84	2.52	2.70	2.63	2.20	2.27	2.03	
					2.43		2.66	3.53	2.45	3.12	2.02	2.19
							2.54	3.79	2.39	3.73	2.55	2.68
Non-Binding Region →									3.12	3.46	4.34	3.76
									5.36	4.23	7.17	8.16

Screen Constants:

- pH constant at 7.0
- mAb Load, 66mg/mL resin
- Resin volume 10 µL resin

Higher the number = greater separation

✓ **POROS Benzyl selected for polish in bind/elute**
✓ **POROS Benzyl Ultra selected for polish in flow-through**

POROS HIC RESINS

POROS Ethyl → Non-POROS HIC resins → POROS Benzyl → POROS Benzyl Ultra

Increasing hydrophobicity

✓ **Designed for use with lower salt concentrations**
✓ **Differentiated selectivity and ligand chemistry**
✓ **Novel 50 µm base bead**
✓ **Improved recovery, resolution and capacity**
✓ **Superior pressure-flow characteristics**
✓ **Consistent lot-to-lot performance**
✓ **Robust chemical and base stability**

RESULTS SUMMARY AND CONCLUSIONS

Process Summary	Mixed-Mode (Clinical Process)	POROS Benzyl Bind-Elute Mode	POROS Benzyl Ultra Flow-through Mode
Load Monomer Purity (%)	90	**89**	**85.5**
Load Density (g/L resin)	25	**32**	**100**
Monomer Purity Pool (%)	99	**99**	**>99**
Monomer Recovery (%)	90	**>99**	**98**
HCP (ppm)	NA	**120 to 12ppm**	**100 to 35 ppm**
Residence time (min)	6	**2**	**1.2**
Pool Volume (50-50mAu)	5CV	**4CV**	**NA**
MMV Clearance	NA	**2**	**1**
XmuLV Clearance	NA	**>5**	**>5**
Productivity (g/L/hr)	**7**	**27**	**89**

POROS HIC resins drastically improve Mab polish step:

✓ **Increased load density**
✓ **Improved monomer recovery**
✓ **Shorter residence time**
✓ **4-12 times higher process step efficiency**

contact: John.Li3@thermofisher.com

ThermoFisher SCIENTIFIC