EFFICIENT MONOCLONAL ANTIBODY AGGREGATE REMOVAL BY HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC)

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Bioprocessing

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 >12% aggregates.

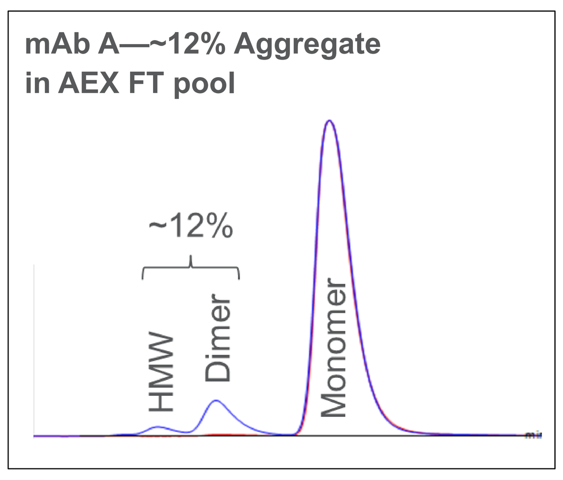


Fig. 1A

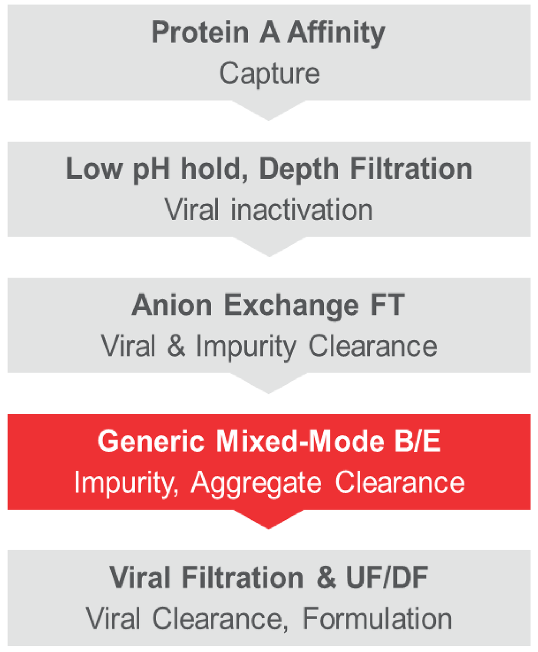


Fig. 1B

Fig. 1 A. Size Exclusion Chromatogram of mAb A, a clinical mAb containing >12% aggregates.

Fig. 1 B. Original purification process of mAb A

Fig. 2 Study design for the optimization of POROS HIC resin use in clinical mAb purification process.

Salt Tolerability Window → High Throughput Screening → Scale Down Model

HIGH TRHOUGHPUT SCREENING – RESIN SELECTION FOR SCALE DOWN

Screening variables used to predict conditions for scale down model (FT & B/E):

- resin type
- salt type
- salt concentration

Partition selectivity ratio is used to determine to level of separation.

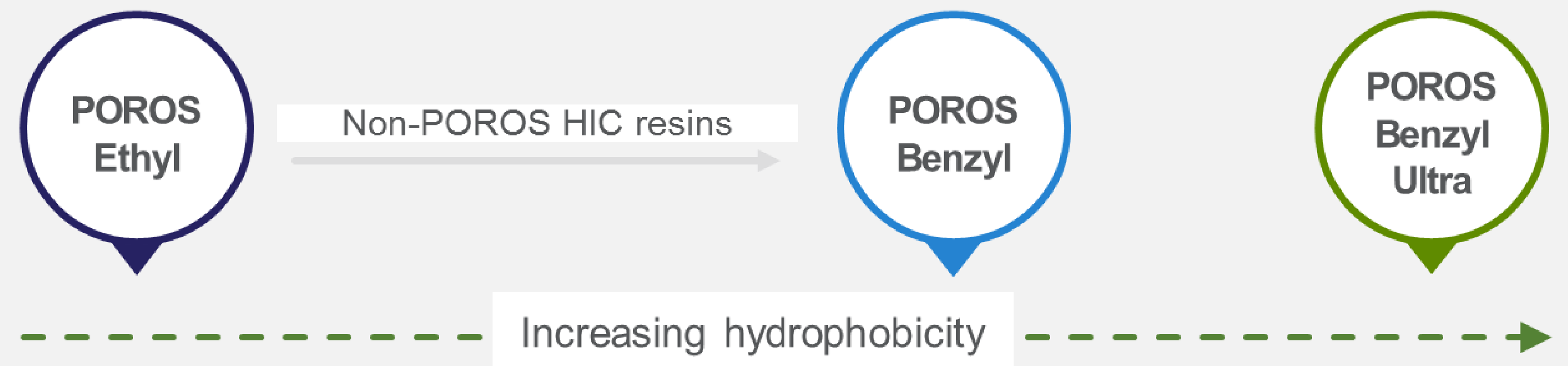
Resin type	POROS Ethyl				POROS Benzyl				POROS Benzyl Ultra			
Salt type	Sodium Chloride	Sodium Acetate	Ammonium Sulfate	Sodium Citrate	Sodium Chloride	Sodium Acetate	Ammonium Sulfate	Sodium Citrate	Sodium Chloride	Sodium Acetate	Ammonium Sulfate	Sodium Citrate
Partition Selectivity (Salt ↑)				2.43		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
									3.12	3.46	4.34	3.76
									5.36	4.23	7.17	8.16

Fig. 3 Overview HTS results; aggregate partitioning as a function of resin type, salt and salt concentration. Highly selective monomer/aggregates partitioning observed at low to no salt conditions (red boxes)

Higher the number = greater separation

- ✓ **POROS Benzyl chosen for Bind-Elute optimization**
- ✓ **POROS Benzyl Ultra chosen for Flow-Through optimization**

POROS HIC RESINS



- ✓ **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**

OPTIMIZATION SCALE DOWN MODEL – POROS BENZYL IN BIND / ELUTE MODE

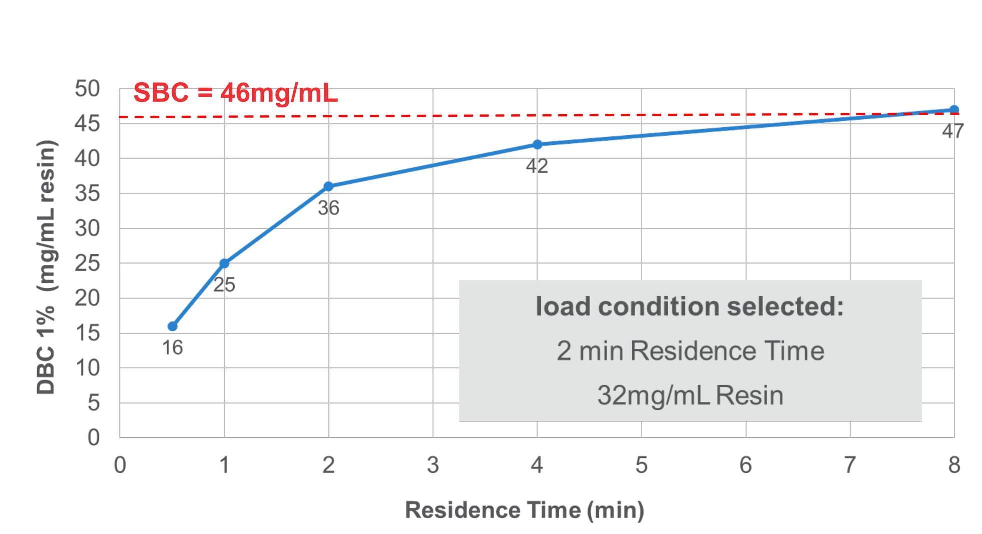


Fig. 4 DBC at 1% breakthrough as function of residence time. Maximum static capacity is reached at ~5min. In order to obtain a faster loading process than the original process, the final load conditions selected were: 32 mg/mL at 2 min residence time.

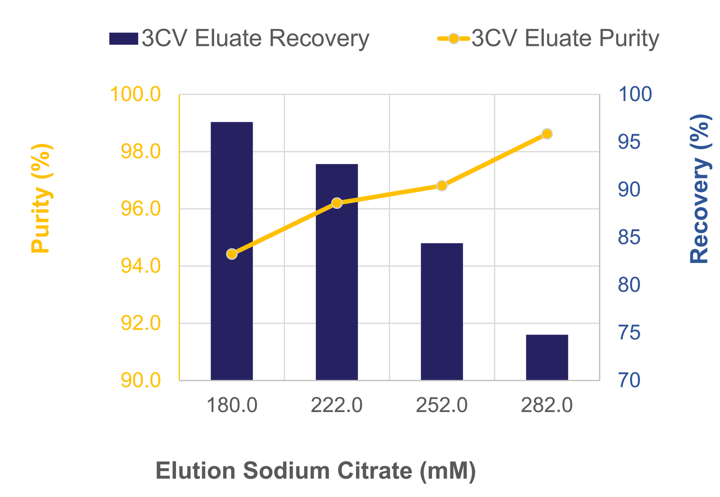
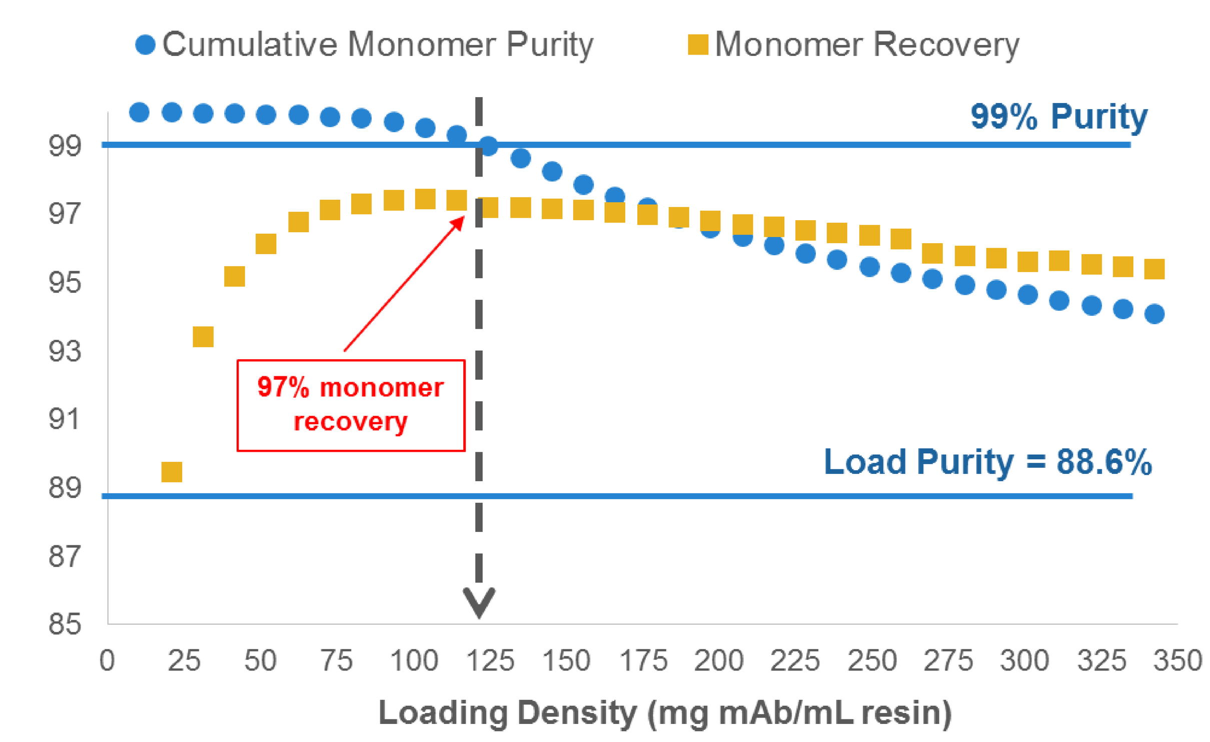


Fig. 5 Elution optimization results. Optimal recovery and purity as function of salt concentration and column volumes.

- ✓ **High Dynamic Binding Capacity at short residence time**
- ✓ **High recovery and purity from a fast elution profile**

OPTIMIZATION SCALE DOWN MODEL – POROS BENZYL ULTRA IN FLOWTHROUGH MODE



Study Format

- 0.8cmx10cmL (CV 5mL)
- **Load 350 mg/mL resin**
- Flow rate: 300 cm/hr
- 2 min residence time
- Load monomer 89%
- 2 mS/cm conductivity
- pH6.8 chosen based on optimized pH of previous AEX polish step

Fig. 6 Flow through purification using Benzyl Ultra. Along the loading step fractions were collected and analyzed for monomer recovery and purity. No additional buffer manipulation was needed after the AEX step.

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

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
Productivity (g/L/hr)	**7**	**27**	**89**

POROS HIC resins drastically improve mAb A polishing step:

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

TRADEMARKS/LICENSING