POROS Benzyl Ultra Viral Clearance and Impurity Removal for a Novel Antibody Format Used in an Enhanced Antibody Drug Conjugate (ADC)

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INTRODUCTION

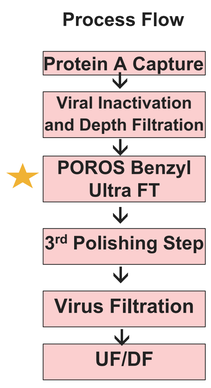
Ambrx Inc develops novel ADCs for oncological targets. The technology utilizes synthetic amino acids, which lends to a few advantages: site-specific and stable conjugation of drug-linker, highly homogenous drug-to-antibody ratios (DAR), and extended half-life.

Novel antibody formats may require unique purification solutions to address challenges such as aggregate removal and host cell protein (HCP) clearance. In this study, the performance of POROS™ Benzyl Ultra, a hydrophobic interaction chromatography resin, was evaluated for the reduction of HMW species that are challenging to remove through standard bind and elute cation exchange chromatography. This is due to both the properties of HMW species formed in the platform in addition to the overall high starting levels. In addition to impurity reduction, viral clearance capabilities were demonstrated. The use of POROS Benzyl Ultra also showed an improvement in scalability and overall performance as the process can be operated in flow through at high loading densities and while maintaining excellent recovery.

METHODS

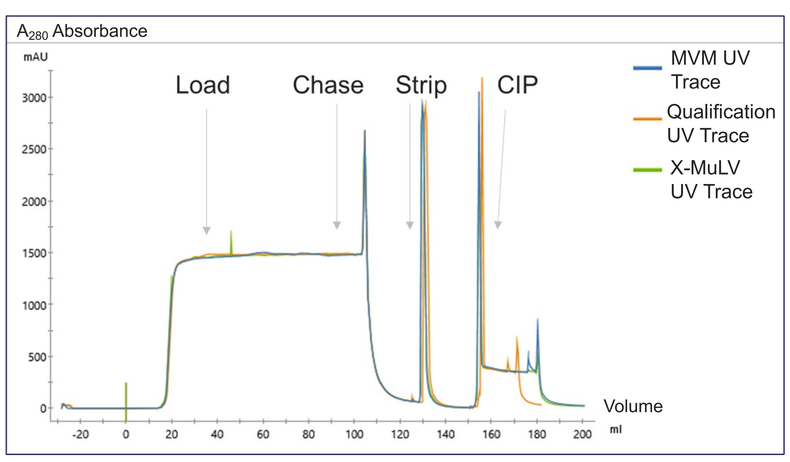
The viral clearance study was evaluated for a monoclonal antibody utilizing synthetic amino acids (prior to conjugation), denoted as mAb A. The viral clearance study was performed using a 0.8 cm i.d. x 10 cm bed height column, with all steps performed at a 4 minute residence time per the buffer sequence shown below. mAb A was produced in recombinant CHO cell culture and purified using the process flow diagram shown below. The load material for POROS™ Benzyl Ultra had 7.5 – 10.2% overall HMW levels, with 2.8 – 4.6% of a particularly challenging aggregate denoted as HMW 2. The recoveries, log reduction of viruses, and impurity reductions were evaluated. Impurity reduction for two additional mAbs utilizing a similar platform but different therapeutic target were further evaluated for impurity reduction with POROS Benzyl Ultra:

Step	Buffer/Solution	Length
Equilibration	50 mM sodium acetate, pH 5.5	5 CV
Load	**IDF (Intermediate depth Filter) pool, pH 5.5**	**100 g/L**
Wash 1	50 mM sodium acetate, pH 5.5	5 CV
Strip	WFI (water for injection)	5 CV
Post-use CIP/Sanitization	1.0 N Sodium Hydroxide	5 CV
Storage	0.1 N Sodium Hydroxide	5 CV



POROS™ HIC resin	Particle Size	Base Bead Mean Pore Size	Base Bead Material
POROS™ Benzyl Ultra	50 µm	40 nm	Poly(styrene-divinylbenzene)

Viral Clearance Study Results



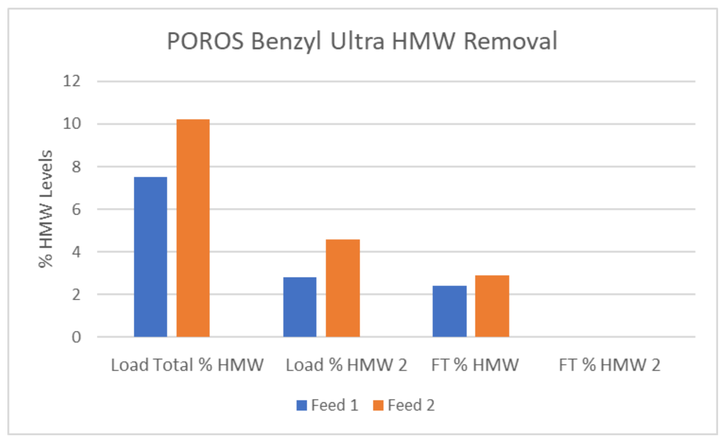
Chromatogram Overlay – Consistent performance with XMuLV, MVM and no virus/qualification runs

Run	Yield (%)	LRV
Qualification	85%	-
X-MuLV	84%	>5.97
MVM	85%	4.56

- ✓ Complete removal of X-MuLV and robust clearance of MVM was achieved
- ✓ Yields were comparable for all runs at approximately 85%
- ✓ POROS HIC demonstrated to be an effective tool for clearance of retro and parvoviruses for this molecule and process

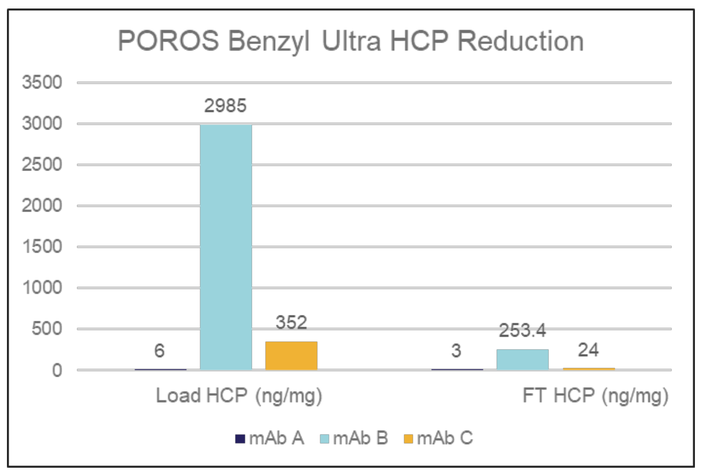
Aggregate Removal for mAb A

Load and flow through pools for mAb A on Benzyl Ultra were evaluated using SE-HPLC. In addition to high HMW levels (> 10%), HMW 2 was a challenging species to separate using traditional B/E methods



- ✓ High load % HMW (>10%) was reduced to acceptable levels with Benzyl Ultra
- ✓ Challenging HMW 2 species was removed completely in both feeds

Additional Impurity Clearance Results for mAb A, B and C



Loading Densities

mAb A: 100 g/L

mAb B: 150 g/L

mAb C: 150 g/L

- ✓ Reduction of host cell proteins and HMW impurities was achieved at high loading densities
- ✓ mAb B and mAb C pools reduced HMW impurities to < 1.0%

Process Performance Improvements

Secondary polishing after Protein A capture from 2000 L clarified cell culture

Resin	Residence Time (min)	Loading Density (g/L)	Cycles	CV (L)	Process Time (h)	Yield %	Buffer Vol (L)
POROS Benzyl Ultra	4	100 - 200	2	16	4.25	85%	720
IEX Bind and Elute	4	50 - 100	4	16	8.25	60 - 70%	1680
IEX Bind and Elute	4	50 - 100	2	32	4.29	60 - 70%	1760

Column volumes and calculations for process time, yield, and buffer volume based on predicted titer and expected parameters for the data demonstrated. For standard bind and elute processes calculations based on expected parameters from literature and field

- ✓ Potential 40 – 60% reduction in process time, buffer volume, and resin volume compared to standard bind/elute methods
- ✓ Significant improvement in productivity

CONCLUSIONS

- POROS Benzyl Ultra reduced total HMW levels to acceptable levels and showed complete removal of a challenging HMW 2 species
- Effective viral clearance for this novel antibody in flow through mode
- Reduction of host cell proteins and HMW impurities for additional mAbs
- Improved process performance leads to 40 – 60% reduction in overall process time, buffer volume, and resin volume, resulting in better process economics, reduced costs, and improved facility fit at 2000 L GMP scale

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