thermo scientific

Innovative Hydrophobic Interaction Chromatography (HIC) Resins for Next Generation Purification Challenges

John J. Li, Moira Lynch, Jinsong Liu, James Molinari, Rich LaPolla, Dave Thomas, Kelly Flook, Jessica de Rooij, Andy Tomlinson and Shelly Parra, Thermo Fisher Scientific, 35 Wiggins Ave, Bedford MA, 01730 USA

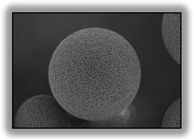
INTRODUCTION

Advances in biotherapeutics are generating more diversified classes of biomolecules that are presenting unique and often difficult purification challenges. To meet industry demand for more differentiated purification tools, a series of HIC resins were developed using extensive user input (Figure 1). Design goals focused on introducing resins addressing typical pain points for current HIC separations such as resolution, capacity and poor product recovery. Since there is a growing trend in downstream purification to use HIC in the flow through mode for product polishing, design also focused on maintaining superior resin performance at lower salt concentrations or with weaker lyotropic salts.

Here we present the superior performance of one of our HIC resins in a typical monoclonal antibody aggregate removal process.

RESIN DESIGN

A new 50µm POROS™ polystyrene divinylbenzene base bead was created to meet design goals. Novel coating and functionalization procedures were used to graft unique alkly or aromatic ligands onto the bead structure to provide a differentiated range of hydrophobicity compared to current market offerings. Design features include:



- ✓ Differentiated selectivity & improved resolution
- ✓ High performance with lower salt concentrations
- ✓ Improved recovery and higher capacity for a range of molecules
- ✓ Consistent performance, robust chemical stability and alternate storage solutions

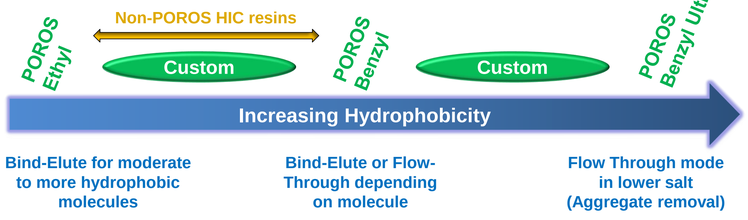


Figure 1. Hydrophobicity of POROS-HIC Resins. POROS Ethyl and POROS Benzyl bracket the commercially available range of HIC resins, while POROS Benzyl Ultra is considerably more hydrophobic than anything currently on the market, designed specifically for flow-through applications in lower salt. Moreover, a deep understanding of POROS resin chemistry allows for build-to-suit custom hydrophobicity resins to meet unique purification challenges.

HIGHER DYNAMIC BINDING CAPACITY

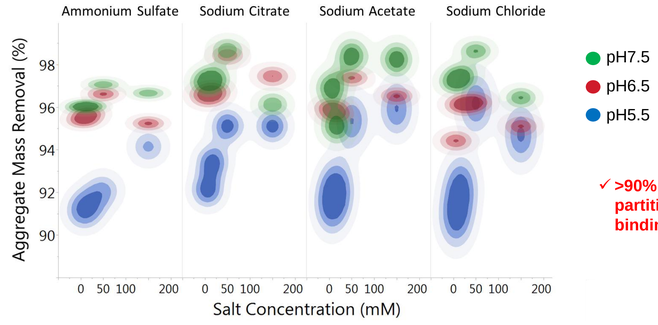
Stationary Phase	Lysozyme DBC C5 (mg/mL)
Non-POROS ethyl	<1
POROS Ethyl	4
Non-POROS low sub phenyl	13
POROS Benzyl	24
Non-POROS high sub phenyl	25
POROS Benzyl Ultra	33

Figure 2. Dynamic Binding Capacity Comparison with Competitors. Buffer: 1.5M ammonium sulfate, 50 mM sodium phosphate pH 7.0; Residence time: 4 minutes; Column format: 0.66cmD x 20cmL; Protein loaded until 5% breakthrough. Detection: UV at 280nm

MONOCLONAL ANTIBODY AGGREGATE REMOVAL

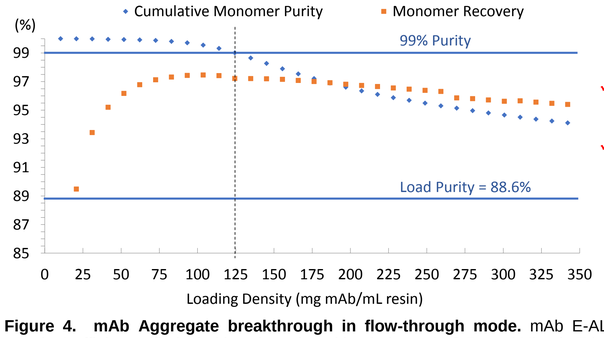
A critical requirement in the final formulation of therapeutic monoclonal antibodies is the lowest possible concentration of aggregates, typically less than 1%. HIC is uniquely positioned as an ideal tool for aggregate removal , since aggregates tend to be more hydrophobic than their monomeric counterparts. Both bind-elute and flow-through mode HIC applications have been successfully used to remove mAb aggregates in commercial processes.

In the following example, a highly efficient flow-through process for mAb aggregate removal was developed. Using POROS Benzyl Ultra, high-throughput screening (HTS) was performed to gage the effect of pH, salt concentration and salt type on aggregate partitioning (Figure 3). Aggregate breakthrough analysis in scale-down model column format (0.66cmDx10cm) was performed (Figure 4, 5, 6). Neutral pH and low conductivity was chosen based on the seamless integration of HIC Benzyl Ultra into an existing in-process condition post protein-A elution.



✓ **>90% Aggregate partitioning in static binding HTS format**

Figure 3. mAb aggregate removal by flow-through HTS screening. HTS screening was performed using a 96-well filter plate format (phase ratio 1:6, 5mg/mL resin loading). Percent aggregate removal was measured using a combination of UV280 concentration and HPLC-SEC purity data, and plotted as a function of salt type, salt concentration and pH. Monomer recovery in flow through was acceptable (Data not shown)



✓ **125g/L resin load at 1% aggregate breakthrough**

✓ **97% Monomer recovery yield**

Figure 4. mAb Aggregate breakthrough in flow-through mode. mAb E-AL-02 (ImmunoGen Inc.) was protein-A affinity purified, viral inactivated, and loaded onto HIC Benzyl Ultra in Tris-Acetate buffer at neutral pH, 1.7mS/cm. No additional buffer manipulation or dilution was performed. Linear velocity: 300 cm/hr; Column format: 0.66cmD x 10cmL; Residence time 2.0 min. Max resin loading: 350g/L. Breakthrough fractions were analyzed every 15 mL of mAb load. Loading density at 1% aggregate breakthrough is marked by a dashed line.

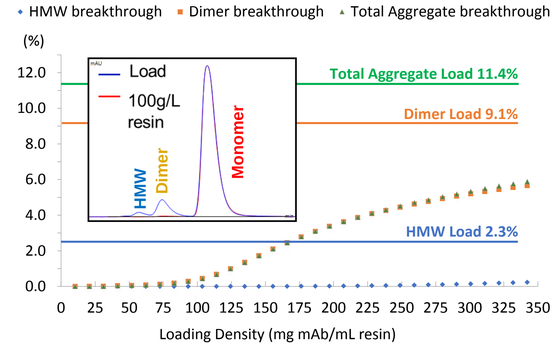
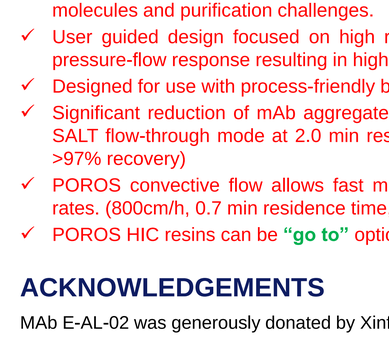


Figure 5 mAb Aggregate breakthrough by species. mAb aggregate analysis was carried out using analytical size exclusion chromatography with the Thermo Fisher MAbPac SEC1 column on the Ultimate 3000 HPLC system. The SEC separation was performed at ambient temperature isocratically using a mobile phase of 50mM Sodium Phosphate pH 6.8 and filtered 0.2µm prior to use.



Sample	HCP (ppm)
HIC Load	1275
HIC Eluate Pool	923

✓ **75g/L resin load at 1% aggregate breakthrough (800cm/hr or 0.7 min residence time)**

Figure 6 mAb Aggregate breakthrough at 800 cm/hr. Column format: 0.66cmD x 10cmL; Max resin loading: 145g/L resin. For HCP, HIC Load was spiked with 0.1%v/v CHO culture fluid. Pooled HIC Eluate (100g/L resin) was measured for HCP using qPCR (ProteinSEQ™ CHO HCP Quantitation Kit)

CONCLUSIONS

- ✓ A family of HIC resins with a wide range of hydrophobicity accommodates a variety of molecules and purification challenges.
- ✓ User guided design focused on high resolution, capacity and recovery with improved pressure-flow response resulting in high performance products.
- ✓ Designed for use with process-friendly buffers, salts and conductivities.
- ✓ Significant reduction of mAb aggregate on POROS Benzyl Ultra was achieved in LOW SALT flow-through mode at 2.0 min residence time. (125g/L resin loading, >99% purity, >97% recovery)
- ✓ POROS convective flow allows fast mass transfer and high performance at high flow rates. (800cm/h, 0.7 min residence time, 80g/L resin loading, 99% purity, >98% recovery)
- ✓ POROS HIC resins can be **"go to"** options in the purification tool box.

ACKNOWLEDGEMENTS

MAb E-AL-02 was generously donated by Xinfang Li and Thomas Ryll, ImmunoGen Inc.