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

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INTRODUCTION

Advances in biomolecules are generating a wider range of molecules that are presenting unique and often difficult purification challenges. Based on these complex purification needs, a series of HIC resins were developed with a range of hydrophobicity. Utilizing extensive user input, design goals for the resins were established that focused on high resolution, high capacity, and mass transfer characteristics for high throughput with increased product recovery. An additional goal was the maintenance of resin performance at lower salt concentrations or with weaker kosmotropic salts.

To meet the design criteria, a new 50µm POROS™ polystyrene divinylbenze base bead was designed that is suitable for both bind/elute and flow-through HIC applications for a wide variety of biomolecules and sizes. A novel coating procedure was developed and functionalization with unique ligands to provide three new resin products that span a wide range of hydrophobicity.

Better Separations Through Chemistry

Protein purification can be achieved through a variety of mechanisms. Selection of the right purification resin along with method optimization is critical for achieving good purities, acceptable yields and with the greatest productivity. When the target molecule is challenging and typical approaches are unsuccessful, having a wider range of selectivity available greatly increases the chance of success. In cases where the molecule is highly hydrophobic, or contains a very hydrophobic conjugate, it can be difficult to elute even in low salt. By altering the hydrophobicity of the resin surface, the retention strength of the resin can be increased or decreased to produce a range of resins to cover a wide variety of molecule hydrophobicity.

DESIGN PRIORITIES

Purification Challenges

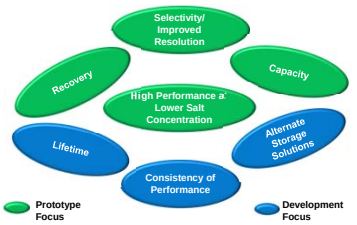


Figure 1. POROS HIC Resin Design and Development Priorities

User desired features were ranked to guide resin design and the most important features (Figure 1) were incorporated into prototypes. These concentrated on the performance characteristics: resolution, capacity, recovery and high performance in low salt. Three products have been developed. Figure 2 plots the relative hydrophobicity of the three products against several commercial products based on lysozyme elution conductivity under the same conditions.

✓ POROS Ethyl and POROS Benzyl bracket the commercially available range but designed with higher performance

✓ POROS Benzyl Ultra is considerably more hydrophobic than anything currently on the market, designed specifically for flow-through applications in **lower salt**

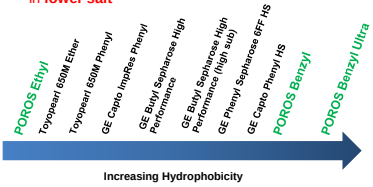


Figure 2. Hydrophobicity Range of Resins
Based on lysozyme gradient elution. Column size: 0.66cmD X 20cmL; 1.7M ammonium sulfate, 50mM sodium phosphate pH 7.0; Gradient Elution: 1.7M ammonium sulfate/50 mM sodium phosphate pH 7.0 to 50 mM sodium phosphate pH 7.0 over 10 column volumes; Flow rate: 100 cm/hr.

COMPARISON OF PRODUCTS

Figure 3 shows the increase in hydrophobicity from POROS Ethyl the least hydrophobic to POROS Benzyl Ultra the most hydrophobic.

✓ Product range accommodates a wide range of molecules and purification challenges

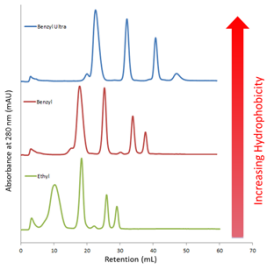


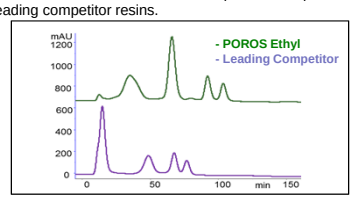
Figure 3. Separation Comparison of POROS HIC Resins
Separation of a mixture of standard proteins using POROS Ethyl, POROS Benzyl and POROS Benzyl Ultra resins. Column size: 0.46cmD X 20cmL; Protein Mixture: Ribonuclease A, Lysozyme, Chymotrypsin and Chymotrypsinogen in 1.7M ammonium sulfate, 50mM sodium phosphate pH 7.0; Gradient Elution: 1.7M ammonium sulfate/50 mM sodium phosphate pH 7.0 to 50 mM sodium phosphate pH 7.0 over 10 column volumes (CV); Flow rate: 100 cm/hr.

NOTE: These conditions were chosen to demonstrate the differences in resin hydrophobicity. POROS Benzyl and Benzyl Ultra are considerably hydrophobic and have been designed for lower salt applications.

BETTER RESOLUTION

High Performance

Due to the improved resolving power of the optimized POROS base bead, each POROS HIC resin provides differentiated resolution and elution profiles compared to leading competitor resins.



Resin	Resolution		
	Ribonuclease A - Lysozyme	Lysozyme - Chymotrypsin	Chymotrypsin - Chymotrypsinogen
POROS Ethyl	2.2	3.2	1.6
Leading Competitor	-	1.9	1

Figure 4. Example Separation Comparison
Separation of a mixture of standard proteins using POROS Ethyl Column size: 0.66cmD X 20cmL; Protein Mixture: Ribonuclease A, Lysozyme, Chymotrypsin and Chymotrypsinogen in 1.7M ammonium sulfate, 50mM sodium phosphate pH 7.0; Gradient Elution: 1.7M ammonium sulfate/50 mM sodium phosphate pH 7.0 to 50 mM sodium phosphate pH 7.0 over 10 column volumes (CV); Flow rate: 100 cm/hr.

Resolution Maintained in Lower Salt

POROS HIC resins were designed for high performance in a variety of buffered salts and salt concentrations. Loading salt conditions can be optimized based on purification needs while maintaining the resolving characteristics of the resin. Figure 5 shows POROS Benzyl Ultra separation of four proteins loaded in three different salt concentrations (gradient slope constant).

✓ Flexibility to optimize with lower conductivity buffers while still maintaining resolution

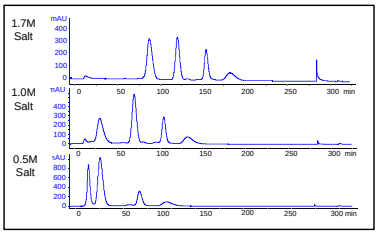


Figure 5. Separation Comparison in Different Salt Concentrations Separation of a mixture of standard proteins using POROS Benzyl Ultra Column size: 0.66cmD X 20cmL; Protein Mixture: Ribonuclease A, Lysozyme, Chymotrypsin and Chymotrypsinogen ; Eluent A: 1.7M, 1.0M or 0.5M ammonium sulfate/50 mM sodium phosphate pH 7.0; Eluent B: 50 mM sodium phosphate pH 7.0; Gradient: 0 to 100% B at 0.17M ammonium sulfate change per CV to keep slope the same; Flow rate: 100 cm/hr; Detection: UV at 280nm.

HIGH DYNAMIC BINDING CAPACITY

Comparisons were done with competitors.

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 6. Capacity Comparison with Leading Competitors
Protein standard: 1.5 mg/mL lysozyme; Buffer: 1.5M ammonium sulfate, 50 mM sodium phosphate pH 7.0; Residence time: 4 minutes; Detection: UV at 280nm; Column format: 0.66cmD x 20cmL; Protein loaded until breakthrough, measurement taken at 5% breakthrough.

NOTE: These conditions were chosen to demonstrate the differences in resin capacity. POROS Benzyl and Benzyl Ultra are considerably hydrophobic and have been designed for lower salt applications.

WIDE LINEAR VELOCITY RANGE

Predictable and Linear Pressure Flow Response

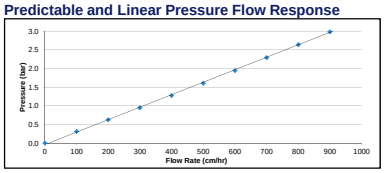


Figure 7. Pressure-Flow Response
Buffer: 0.1M NaCl; Linear velocity increased in increments of 100 cm/hr. until 3 bar is reached; Column format: 1.6cmD x 20cmL.

Performance Maintained over a Wide Range of Flow Rates

✓ Resolution is independent of linear flow rates

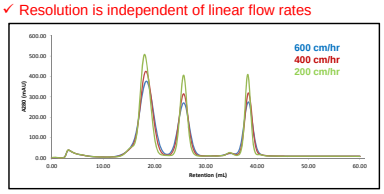


Figure 8. Separation Comparison at Different Flow Rates
Protein mixture: ribonuclease A, lysozyme, and chymotrypsinogen A ; Eluent A: 1.7M ammonium sulfate/50 mM sodium phosphate pH 7.0; Eluent B: 50 mM sodium phosphate pH 7.0; Gradient: 0 to 100% B in 10 CV; Linear velocity: 200, 400 or 600 cm/hr; Detection: UV at 280nm; Column format: 0.46cmD x 20cmL; Resin: POROS Benzyl.

BIND/ELUTE MODE

POROS HIC resins offer solutions to challenging bind/elute purifications. In the example below, the goal was to separate the product molecule from a dominant process-related impurity in addition to removing DNA. Commercially available HIC resins did not provide a satisfactory solution for the customer. Product fractionation was achieved but required a low flow rate due to poor flow properties the 34 µm agarose bead. POROS Ethyl resin allowed fractionation of the product from the impurity at a high flow rate (4.5 min residence time) while maintaining satisfactory operating pressures. DNA was also removed in the flow-through. POROS Ethyl resin demonstrated an advantage over a commercially available resin due to intrinsic flow properties.

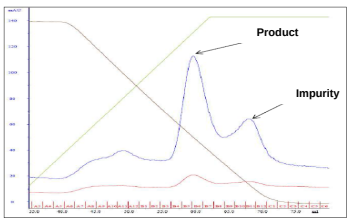


Figure 9. Customer Bind/elute Example
Load: ~7 mg total protein; Eluent A: Buffered 1.5M ammonium sulfate; Eluent B: Buffer; Gradient: 0 to 100% B over 5CV; Residence time: 4.5 minutes; Detection: UV at 280 and 254 nm; Column format: 1.0cmD x 7.5cmL POROS Ethyl.

FLOW-THROUGH MODE

Different target molecules will exhibit varying levels of hydrophobicity as well as biophysical characteristics. In the example below conditions have been optimized for a monoclonal antibody (mAb) to be run in flow-through mode in low salt to remove dimers and high molecular weight material. Initial optimization was performed with a small load (~1 mg protein/mL of resin) at varying low salt conditions to determine the optimal conductivity allowing the mAb monomer to flow through. Analysis of fractions determined the purity of the target molecule for each condition. The fraction at which the desired purity is observed can be used as a starting condition for higher load flow-through mode experiments.

✓ Effective reduction of dimer and high molecular weight aggregates in low conductivity solutions

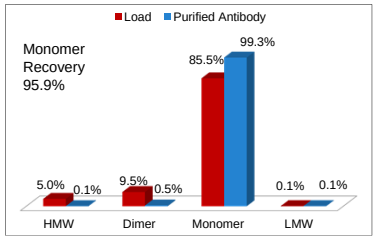


Figure 10. Customer Flow -Through Example for mAb Polish
Resin: POROS Benzyl Ultra , Buffer: sodium citrate with a conductivity of 1.7 mS/cm. Flow rate: 500 cm/h; Product residence time: 1.2 minutes; mAb load: 80 g/L.

CONCLUSIONS

Increasing numbers of challenging target molecules have scientists looking for new purification tools. HIC is being turned to in some cases as a last resort based on traditional HIC purification conditions (high molarity kosmotropic salts).

POROS HIC resins offer scientists a new set of tools for purification that are capable of using the properties of HIC in process preferable conditions such as lower salt concentrations or with weaker kosmotropic salts. With this performance capability, HIC resins no longer need to be a last resort option and instead can be a "go to" option in the purification tool box.

New therapeutic molecules such as multi-target antibodies lead to higher product specific impurities and new purification challenges. HIC resins have increasing utility for purification of such molecules. POROS HIC resins span a wider range of hydrophobicity than all commercially available resins providing more purifications options. Benefit of the most hydrophobic resins is the use of lower concentrations of weaker salts.

Extensive user input guided the design and development of high performing POROS HIC resins. These resins are suitable for both bind/elute as well as flow-through applications and have excellent resolution, high capacity, and fast flow rate capability when compared to other HIC products.

✓ User guided design and development focus on high performance resolution, capacity, recovery, and pressure flow response

✓ A family of HIC resins with a wide range of hydrophobicity - accommodates a variety of molecules and purification challenges

✓ Fractionation of product from impurity at a high flow rate in bind/elute mode on POROS Ethyl

✓ Significant reduction of mAb impurities on POROS Benzyl Ultra in flow-through mode in low salt and with a 1.2 minute residence time

TRADEMARKS

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