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POROS® HQ 50 and PI 50 resins in bind/elute chromatography applications

Why and where to start?

Introduction

Anion exchange (AEX) chromatography is commonly used in downstream purification processes in bind/elute mode to bind the target molecule and remove impurities. It can be a powerful tool to capture the target molecule by selectivity, or to help with polishing the final product, where impurity separation and resolution are key factors. POROS® HQ has both weak and strong AEX capabilities since it has a mixture of primary, secondary, tertiary, and quaternary amines on the bead. The unique chemistry differences of both POROS® PI and HQ mean capacity and selectivity may be different from those of other AEX resins, thereby affecting binding, purity, and recovery. This has proved beneficial in a number of applications using POROS® HQ and PI over other anion exchangers.

Table 1. POROS® HQ and PI comparison.

	Functional group	Type of AEX
POROS® PI	Polyethyleneimine	Weak
POROS® HQ	Quaternized polyethyleneimine	Strong, with some weak functionality

Table 2. POROS® chromatography resin attributes and process benefits.

POROS® resin attribute	Process benefit
Unique bead morphology and convective flow properties of the base bead	Allows for high mass transfer rates and more efficient binding and eluting, leading to increased yield and smaller pool volumes
Flow rate–independent performance	Increases volumetric throughput and allows for smaller column sizes and less buffer usage
50 µm particle size	Provides superior resolution for better impurity clearance, higher product yield, and step vs. gradient resolution
pH and salt tolerance	Provides process robustness and flexibility when designing a purification scheme
Rigid and inert polymer and robust chemical stability of functional group	Allows for the use of aggressive cleaning agents that can extend column life/reuse

Materials and methods

Bovine serum albumin (BSA) dynamic binding capacity

Each column was precharged with 20 mM Tris, 1 M NaCl, pH 8.0, followed by an equilibration with 20 mM Tris, pH 8.0 (1.1 mS/cm). The column format was 0.46 cm (D) x 20 cm (L), 3.3 mL. Each column was loaded with 10 mg/mL BSA (Sigma Cat. No. A7906, pI 4.7–5.3, 66 kDa) in equilibration buffer with a final conductivity of <2 mS/cm at flow rates of 100–900 cm/hr. Binding capacities at 5% breakthrough (C5) were determined based on UV absorbance.

BSA capacity as function of pH and salt concentration

Each column was precharged with 20 mM Bis-Tris propane, 1 M NaCl, pH 7.5, followed by equilibration with 20 mM Bis-Tris propane at pH 6.0, 7.5, and 9.0 and salt concentrations at 0, 50, 100, and 150 mM NaCl. The column format was 0.46 cm (D) x 20 cm (L), 3.3 mL. Each column was loaded with 10 mg/mL BSA (Sigma Cat. No. A7906, pI 4.7–5.3, 66 kDa) in equilibration buffer at 300 cm/hr. Binding capacities at 5% breakthrough (C5) were determined based on UV absorbance.

Protein separation

Each column was equilibrated with 20 mM Tris, pH 8.0 (1.1 mS/cm). The column format was 0.46 cm (D) x 20 cm (L), 3.3 mL. Each column was loaded with a sample protein mixture of 5 mg/mL transferrin (pI 5.6), 10 mg/mL chicken ovalbumin (pI 4.6), and 4 mg/mL soybean trypsin inhibitor (pI 4.5). The total protein load was 4.4 mg on each column, or 1.3 mg per mL of resin. A gradient was run from 20 mM Tris, pH 8.0, to 20 mM Tris/500 mM NaCl, pH 8.0, over 10 column volumes (CVs) at flow rates from 300 to 1,000 cm/hr.

Results and discussion

Both POROS® HQ and PI resins show good BSA capacity over a wide flow rate range (100–900 cm/hr) compared to a conventional agarose resin (Figure 1). POROS® resin provides high dynamic binding capacities and excellent capture efficiencies that are independent of linear velocities. This attribute can be exploited to allow the use of smaller column volumes, reduced buffer consumption, and decreased process time, all of which promote higher process throughput.

The data from the BSA capacity study over a range of salt and pH conditions are summarized in Figure 2. POROS® HQ has the highest capacity, but

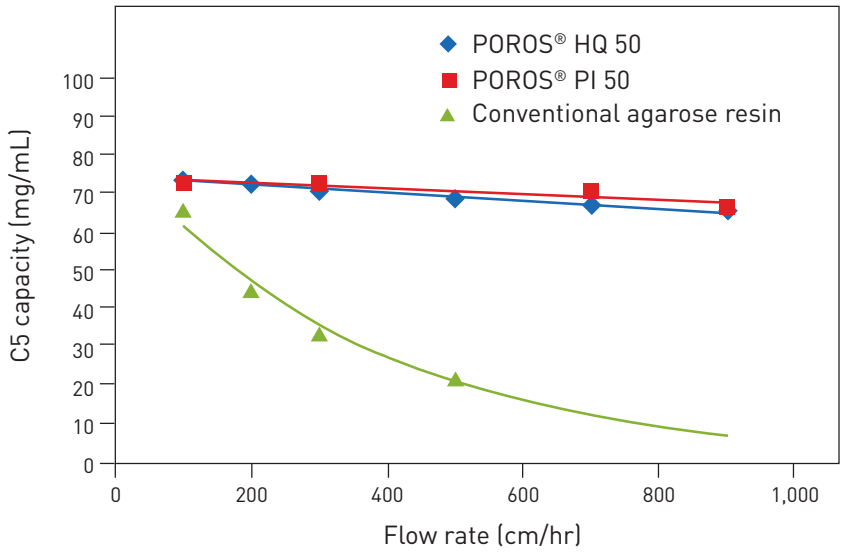

Figure 1. BSA-binding capacity of POROS® HQ and PI at 5% breakthrough as a function of linear flow rate, compared to a conventional agarose-based resin.

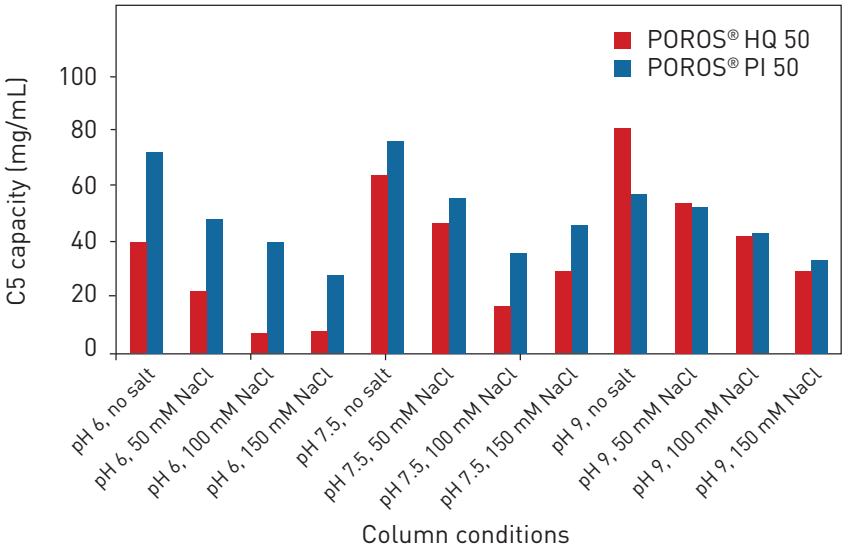

Figure 2. BSA capacity on POROS® HQ and PI at 5% breakthrough over the range of pH 6–9 and 0–150 mM NaCl.

POROS® PI has good BSA capacity even at salt concentrations up to 150 mM NaCl over a pH range from 6 to 9. POROS® HQ performs as expected for a strong anion exchange resin, exhibiting increased capacity at higher pH values. POROS® PI resin retains good capacity over the range of pH 6–9, with capacities greater than 30 mg/mL at salt concentrations up to 150 mM NaCl.

Both POROS® HQ and PI resins deliver excellent separation using a very steep gradient over a wide flow rate range (300–1,000 cm/hr), even with a challenging protein mix (Figure 3). Chicken ovalbumin (pI 4.6) and soybean trypsin inhibitor (pI 4.5) (Figure 3, middle and right peak, respectively) are only 0.1 pI units apart, making them difficult to resolve with most resins under these conditions. Resolution is maintained as linear flow rate increases on both resins, which is typical for POROS® resin performance. Additionally, POROS® PI and HQ have different selectivity for this particular protein mixture, allowing for increased retention and better resolution (Figure 4).

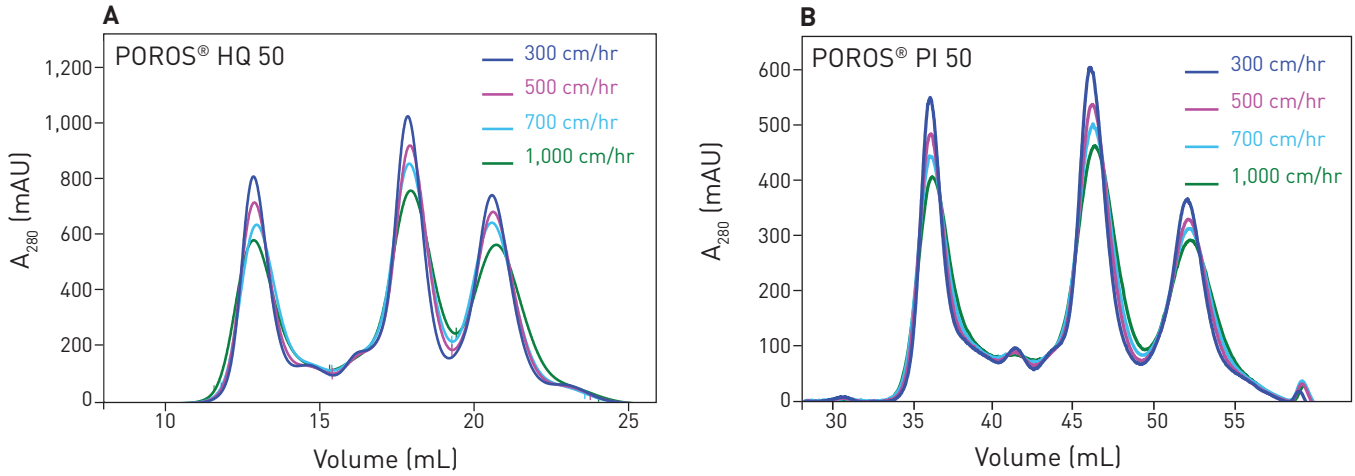

Figure 3. Separation on (A) POROS® HQ and (B) POROS® PI, as a function of linear flow rate. Protein peaks, from left to right: transferrin (pI 5.6), chicken ovalbumin (pI 4.6), soybean trypsin inhibitor (pI 4.5).

Where to start?

Background

Even when comparing commercially available weak-to-weak or strong-to-strong AEX resins, the optimal binding and elution conditions can vary significantly due to a number of resin characteristics. Different AEX resins operated with the same process conditions will yield variable results; therefore, standardized conditions or platform-type evaluations are not recommended. For this reason, it is important to test different loading and elution conditions to optimize capacity, separation, and yield based on the target molecule characteristics and process challenges. It is important to optimize the chromatography step such that the eluting peaks will become more separated during the process by moving some bound impurities into the flow through/wash and allowing others to remain bound and eluting during the strip. This can be done by optimizing the pH and conductivity of the equilibration buffer and loading pool as well as the elution scheme. The keys are to use the highest–ionic strength buffer that still allows the target material to bind, and to elute with the lowest–ionic strength buffer that achieves good elution of the material but retains the DNA, viruses, endotoxins, etc.

Always filter the load through a 0.22 µm or 0.45 µm filter before loading to reduce fouling of the column screens.

Optimize impurity-binding conditions

- **pH**—The load solution and the column equilibration buffer should be identical. The binding pH should be 1 to 3 units above the pI of the target molecule. The dynamic binding capacity (DBC) will increase as pH increases (away from the pI). If the pI is not known, try pH 8.5.
- **Buffer system**—Although no buffer system has proven advantageous over another on POROS® AEX resins, citrate, MES, Tris, and sodium phosphate are capable of buffering in this pH range and are often used. Other biological buffer systems that function well in this pH range, but which are typically more expensive, are Bis-Tris propane, succinate, and HEPES. When choosing buffer systems, consider molecule stability, binding optimization, and the ability of the buffer to control pH in the desired operating range.
- **Conductivity**—In general, DBC decreases as buffer conductivity increases. The load conductivity should be between 1 and 8 mS/cm or about 0–75 mM NaCl; however, the optimum buffer condition depends on the target molecule and buffer pH. Most proteins require some salt for stability. These conditions are driven by the physical characteristics and stability of the protein.
- **Flow rate**—The target operating flow rate is flexible, since there is minimal change with increased residence time on POROS® HQ and PI. Typical flow rates are 100–500 cm/hr in a bind/elute application.

Optimize elution conditions

Elution optimization should begin with a gradient elution. Most often, once elution performance is understood, a step elution can be implemented.

- **Salt gradient**—To determine where the target molecule and contaminants/impurities are eluting, start with a 20 CV gradient from low salt, typically matched to the wash buffer, to approximately 500 mM–1 M NaCl. This can be accomplished by assaying fractions across the peaks (~1/10 CV). Based on this information, the process can be further optimized.

- **pH**—Initially, the elution pH should be matched to the binding pH. However, the pH of the elution buffer should be optimized, as the optimum binding and eluting pH can often be different.
- **DBC**—Separation as a function of DBC should be assessed. The maximum DBC at which a given separation can be obtained depends on a number of factors, including sample solubility, column selectivity, buffer pH, and buffer conductivity.
- **Bed height**—Initial screening can be run with shorter bed heights, but development should be conducted at the final desired bed height, typically 15–25 cm.

Optimize column cleaning conditions

Contaminants such as nucleic acids, endotoxins, viruses, lipids, metal ions, etc., bind very tightly to AEX resins even in bind/elute mode, so it is important to clean the column sufficiently to increase column lifetime. One of the benefits of POROS® resins is that harsh cleaning conditions can be used, if needed, to optimize column cleaning and reuse.

POROS® HQ and PI resins can typically be cleaned with 3 to 5 CVs of 1–2 M NaCl followed by 3 to 5 CVs of 0.5–1 M NaOH. Different solutions may be required for column cleaning if resin is used for capture chromatography.

Points to consider for optimizing resin cleaning protocols

- Monitor A_{214}, A_{260}, and A_{280} to determine if different impurities are eluting.
- Run all strip/cleaning/regeneration steps in upflow, if possible.
- During the optimization of cleaning procedures, equilibrate the column with equilibration buffer or water in between each proposed cleaning solution. This allows the effect of the cleaning solution to be studied.
- Run enough column volumes to ensure the desired buffer/solution effect is reached and the entire cleaning peak has been eluted before starting the next solution, i.e., 3 to 5 CVs.
- Incorporate a static hold so as to increase the residence time of cleaning solution while minimizing solution volume requirements.
- Clean/sanitize with NaOH solutions last, to minimize the risk of irreversibly binding impurities to the resin.
- Perform a gradient or sequential washes to slowly strip the column and reduce the chance of precipitation on the column (i.e., gradient with increased conductivity and decreased pH). For example, 50 mM NaCl, pH 7, to 1 M NaCl, pH 5 on AEX, or with increased pH on CEX (pH 8–9).
- Perform a mixing/precipitation study in a glass tube to determine if precipitation could be occurring on the column.

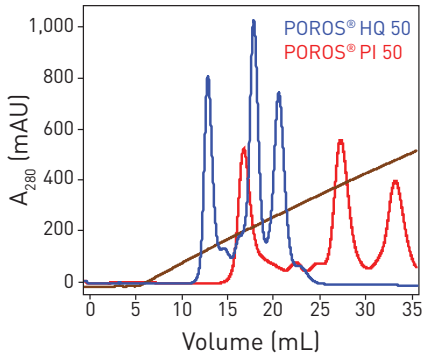

Figure 4. Chromatography overlay of protein separation on POROS® HQ and PI showing selectivity difference using the same gradient and protein sample mixture (300 cm/hr).

Recommended solution series for AEX resin

Test the following (in this order) and then optimize cleaning based on your results:

- 1 M NaCl
- Static hold (30 min)
- 1 M NaCl
- 3 M NaCl
- Water or equilibration buffer
- 1 M acetic acid and/or citric acid
- Water or equilibration buffer
- 0.1 M HCl
- 1 M HCl
- Water or equilibration buffer
- 30% ethanol
- Water or equilibration buffer
- 0.1 M NaOH
- 1 M NaOH
- 1 M NaOH/1M NaCl
- 1 M NaOH/30% ethanol

Other solutions to try if you observe very dirty feed in capture mode: guanidine hydrochloride, urea, EDTA, Tween® in NaCl, IPA.

Conclusion

POROS® HQ and PI have been shown to have high binding capacity and excellent resolution over a range of process conditions. The unique functional groups of POROS® HQ and PI and the 50 µm pore size and bead morphology deliver different selectivity and separation than those observed with other commercially available AEX resins. If disposability is a factor, Life Technologies now offers Go-Pure™ Pre-Packed Chromatography Columns for maximum convenience, to promote faster processing times and reduced changeover/setup time between processes. Packed-bed chromatography, and POROS® resin specifically, allow for reusability at commercial scales, ease of packing at different scales in traditional column formats, and the ability to design for commercial scale from the beginning to reduce redevelopment costs.

References

PerSeptive BioSystems, "The Busy Researcher's Guide to BioMolecule Chromatography" (1996) Publication PL 128.

Scientific contributors

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Speak to a technical specialist about how these resins can improve your current process at **bp@lifetech.com**.

Product	Quantity	Cat. No.
POROS® HQ 50 – Strong anion exchange resins		
POROS® HQ 50 µM Quaternized Polyethyleneimine	50 mL	1-2559-06
POROS® HQ 50 µM Quaternized Polyethyleneimine	250 mL	1-2559-11
POROS® HQ 50 µM Quaternized Polyethyleneimine	1 L	1-2559-07
POROS® HQ 50 µM Quaternized Polyethyleneimine	5 L	1-2559-09
POROS® HQ 50 µM Quaternized Polyethyleneimine	10 L	1-2559-08
POROS® PI 50 – Weak anion exchange resins		
POROS® PI 50 µM Polyethyleneimine	50 mL	1-2459-06
POROS® PI 50 µM Polyethyleneimine	250 mL	1-2459-11
POROS® PI 50 µM Polyethyleneimine	1 L	1-2459-07
POROS® PI 50 µM Polyethyleneimine	5 L	1-2459-09
POROS® PI 50 µM Polyethyleneimine	10 L	1-2459-08

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