Characterization of a Novel High Capacity Weak Anion Exchange Resin

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Abstract

- Ion exchange resins with increased selectivity and capacity are now in great demand. A new high capacity weak anion exchange resin was recently developed and this poster will focus on this resin and its ability to purify proteins and other biomolecules.
- A high capacity weak anion exchange resin was developed by Tosoh Corporation that utilizes the well-known TOYOPEARL® HW-65 size exclusion resin as its base bead. The base resin is chemically modified with diethylaminoethanol groups to provide a high level of anionic binding sites, creating the TOYOPEARL GigaCap® DEAE-650M resin.
- The chemical attachment of the cationic groups on the 100 nm pore size of the base resin results in greater than 100 g/L dynamic binding capacity for BSA-type molecules. In addition, the resin is stable at high linear velocities with good pressure-flow characteristics due to its 75 µm particle size. The resin has a pressure rating of 0.3 MPa and is stable in the pH range of 2-13. The higher capacity and stability of this resin allows for increased throughput in downstream purification steps while still maintaining excellent binding and elution kinetics.
- The new DEAE-type resin effectively separates proteins even at very high loads. When loaded to 96 g/L protein, the resin was able to separate ovalbumin from trypsin inhibitor.
- Also as part of this study, solutions of up to 50 g/L of β-lactoglobulins were loaded onto the column. The new resin was compared to other commercially available anion exchange resins and demonstrated excellent selectivity under increasing load conditions. The separation of β-lactoglobulins is particularly impressive since β-lactoglobulins A and B differ by only one charge. An aspartic acid in variant A is substituted for a glycine in variant B.

Introduction

- TOYOPEARL DEAE-650M chromatography media is a weak anion exchanger often used for blood fractionation and other biomolecule purification. It can be used for high throughput capture, intermediate purification, and polishing process steps. The mean particle size is 65 µm, and the average pore size is 100 nm. The TOYOPEARL GigaCap DEAE-650M chromatography media is a high capacity version of the TOYOPEARL DEAE-650M.
- TOYOPEARL HW-65 resin is the polymeric base bead for both of these resins, and they each have a pressure rating of 0.3 MPa. Both resins are stable in the pH range of 2-13 and are intended to be used for capture and/or intermediate purification.
- β-lactoglobulins were used to demonstrate how well the resins can separate proteins and to compare their selectivities. The two forms of β-lactoglobulin, a 162 residue protein, differ by only one charge. In β-lactoglobulin A, residue 64 is aspartic acid, whereas in β-lactoglobulin B, it is glycine. Studies for ovalbumin and trypsin inhibitor were also performed.

Table 1: DEAE Anion Exchange Resin Binding Capacity Comparisons

Resin	Particle Size (µm)	Ion-Exchange Capacity (meq/L)	Binding Capacity (g/L)		Recovery (%) (DBC)
			Static	Dynamic*	
TOYOPEARL GigaCap DEAE-650M	**50 - 100**	**0.23**	**179**	**165**	**100**
TOYOPEARL DEAE-650M	40 - 90	0.11	30	25	97
Capto™ DEAE	90 (median)	0.29 - 0.35	143	140	96
Fractogel® DEAE (M)	40 - 90	N/A	52	N/A	N/A

DBC Conditions:

Resin:	as indicated
Column size:	6 mm ID × 4 cm
Mobile phase:	A: 50 mmol/L Tris, pH 8.5
	B: mobile phase A + 1.0 mol/L NaCl
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	BSA (1.0 g/L)

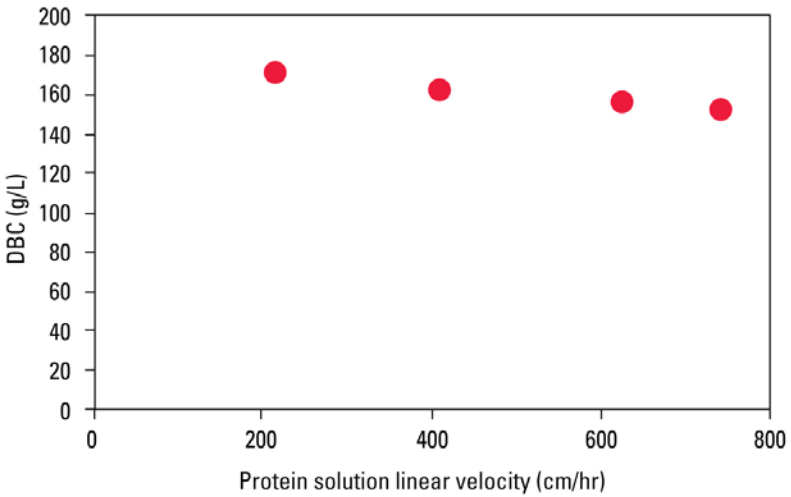
SBC Conditions:

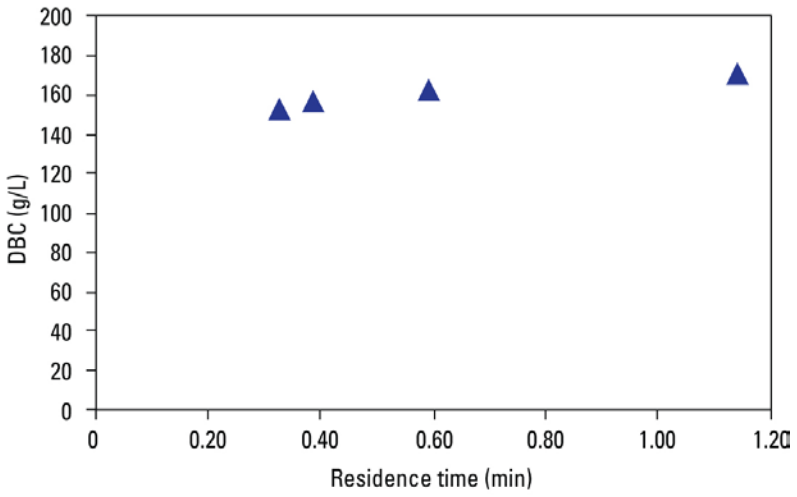
Resin:	as indicated
Adsorption buffer:	50 mmol/L Tris, pH 8.5
Sample:	BSA (10.0 g/L)

TOYOPEARL GigaCap DEAE-650M has a higher binding capacity than TOYOPEARL DEAE-650M and competing DEAE products.

*Dynamic binding capacities were determined at 10% breakthrough

Figure 1: DBC vs. Flow Rate and Residence Time





Resin:	TOYOPEARL GigaCap DEAE-650M
Column size:	6 mm ID × 4 cm
Mobile phase:	A: 0.05 mol/L Tris, pH 8.5
	B: mobile phase A + 1.0 mol/L NaCl
Flow rate:	212, 407, 623, and 739 cm/hr
Detection:	UV @ 280 nm
Sample:	BSA (1.0 g/L)

Efficient mass transfer kinetics allow this resin to maintain a high DBC even with increased linear velocities and decreased residence time.

*Dynamic binding capacities were determined at 10% breakthrough

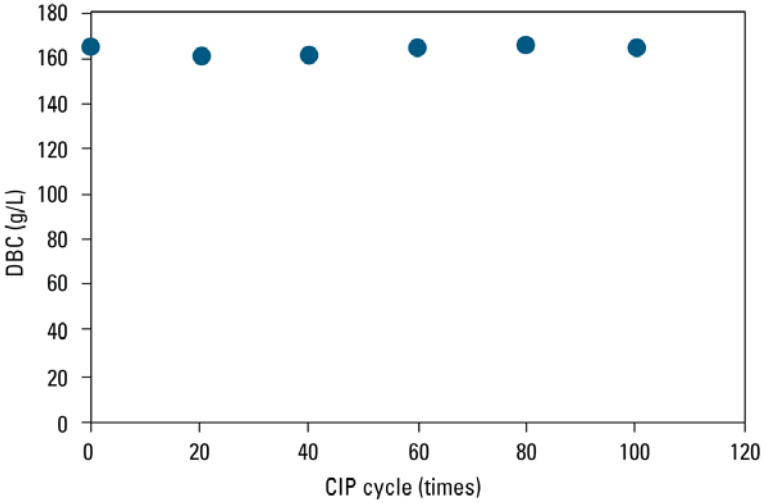
Table 2: Effect of Protein Size on DBC

Resin	Dynamic Binding Capacity (g/L)	
	BSA[*1] 66 kDa	Human IgG[*2] 160 kDa
TOYOPEARL GigaCap DEAE-650M	170	103 (113[*3])
TOYOPEARL GigaCap Q-650S	181	92 (113[*3])
TOYOPEARL GigaCap Q-650M	173	104 (117[*3])
TOYOPEARL Q-600C AR	108	90
TOYOPEARL Super Q-650M	145	13
TOYOPEARL QAE-550C	29	32
TOYOPEARL DEAE-650M	25	31

Column size: 6 mm ID × 4 cm
Mobile phase: [*1] 0.05 mol/L Tris-HCl, pH 8.5
[*2] 0.015 mol/L Tris-HCl, pH 8.7
[*3] 0.020 mol/L Piperazine-HCl, pH 9.5
Flow rate: 212 cm/hr
Detection: UV @ 280 nm
Temperature: ambient
Injection vol.: until 10% breakthrough
Sample: 1.0 g/L protein

Like other products in the TOYOPEARL GigaCap series, the TOYOPEARL GigaCap DEAE-650M exhibits high binding capacities for proteins of various sizes.

Figure 2: Stability of Resin After CIP



CIP conditions:

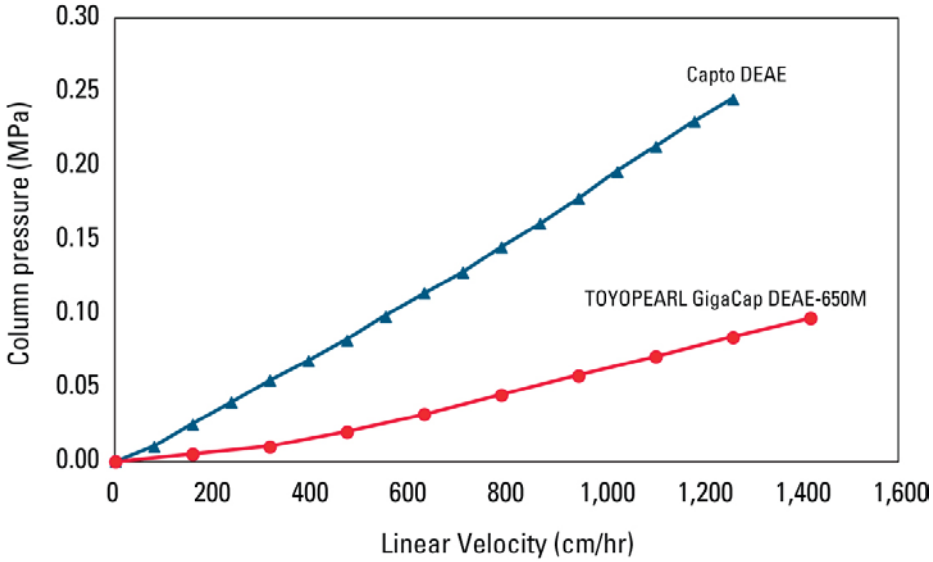
Alkaline washing solution:	0.5 mol/L NaOH
Buffer washing solution:	50 mmol/L Tris, 0.5 mol/L NaCl, pH 8.5
Flow rate:	106 cm/hr (0.5 mL/min)
Alkaline wash volume:	27 CV/cycle
Alkaline contact time:	1 hr
Buffer wash volume:	10 CV/cycle

DBC Conditions:

Resin:	TOYOPEARL GigaCap DEAE-650M
Column size:	6 mm ID × 4 cm
Mobile phase:	A: 50 mmol/L Tris, pH 8.5
	B: mobile phase A + 1.0 mol/L NaCl
Flow rate:	212 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	BSA (1.0 g/L)

- The DBC was measured after every set of 20 clean in place (CIP) cycles. Each CIP cycle consisted of a 27 CV (1 hour) wash with 0.5 mol/L NaOH followed by 10 CV of a pH 8.5 buffer.
- The DBC is retained through at least 100 CIP cycles, showing good stability characteristics.

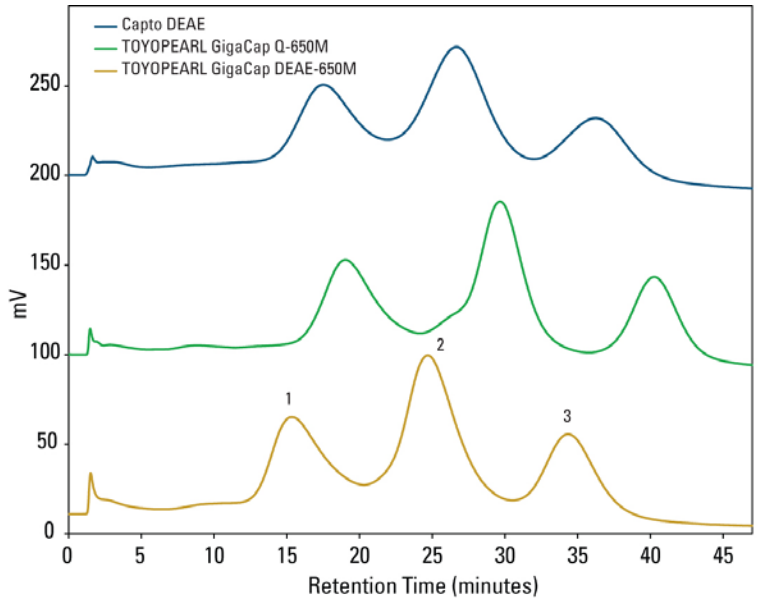
Figure 3: Pressure-Flow Curves



Column size: 2.2 cm ID × 20 cm
Mobile phase: 0.1 mol/L NaCl

- TOYOPEARL GigaCap DEAE-650M and Capto DEAE were packed into 2.2 cm ID × 20 cm columns to determine pressure-flow characteristics.
- Even though Capto DEAE has a larger particle size of 90 µm, compared to the 75 µm of TOYOPEARL GigaCap DEAE-650M, at all flow rates tested, TOYOPEARL GigaCap DEAE-650M showed a lower pressure drop than Capto DEAE.
- At 1,400 cm/hr, the TOYOPEARL GigaCap DEAE-650M column pressure was only ~0.10 MPa, whereas Capto DEAE had a pressure of approximately 0.25 MPa.

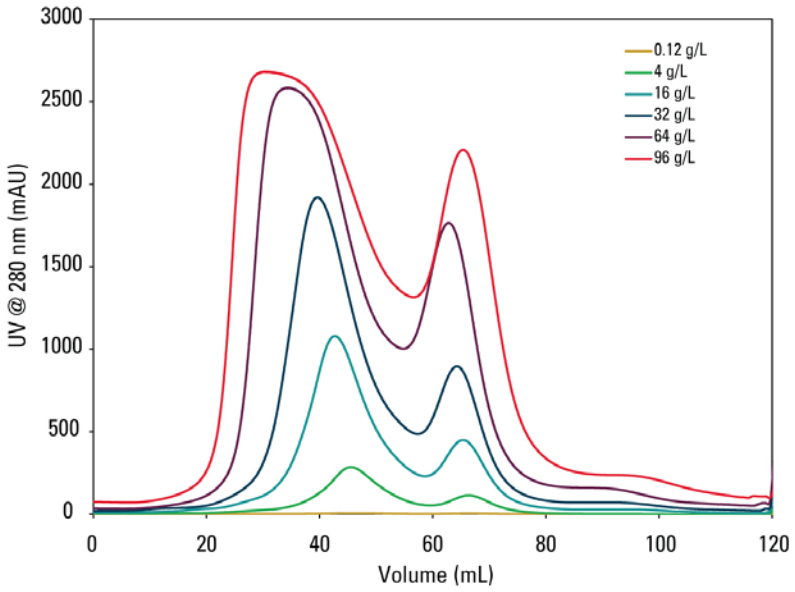
Figure 4: Selectivity Comparisons



Resin:	as indicated
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	A: 50 mmol/L Tris-HCl, pH 8.5
	B: mobile phase A + 1.0 mol/L NaCl, pH 8.5
Gradient:	120 minutes, 0 - 100% B
Flow rate:	136 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	100 µL
Samples:	1. transferrin, 2.9 g/L
	2. ovalbumin, 6.5 g/L
	3. trypsin inhibitor, 10.0 g/L
Sample load:	1.94 mg total protein

- The selectivity of TOYOPEARL GigaCap DEAE-650M differs from other chromatography resins, such as TOYOPEARL GigaCap Q-650M and Capto DEAE.
- The TOYOPEARL GigaCap DEAE-650M tends to retain proteins to a lesser extent compared to the two other resins, allowing for lower elution conductivities while still maintaining high binding capacities.

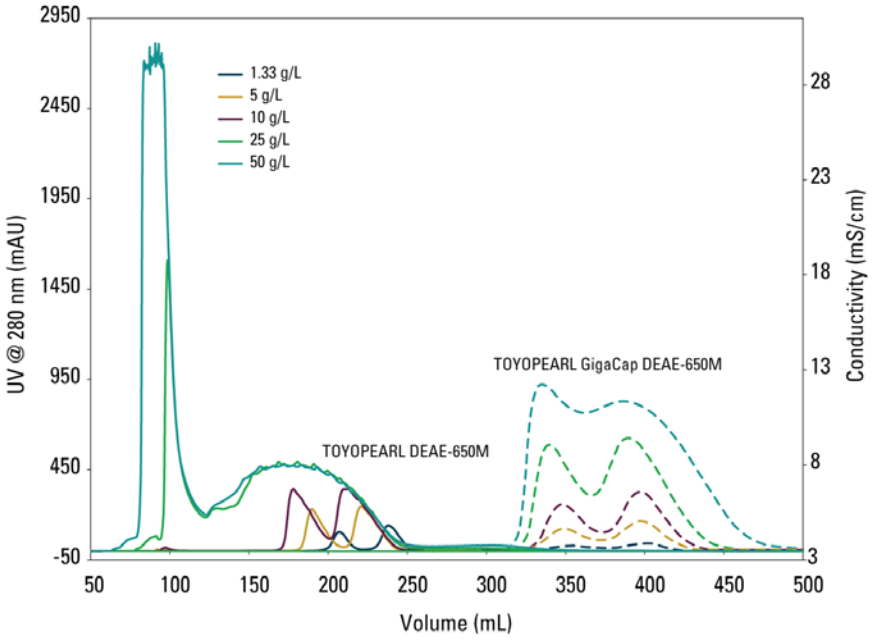
Figure 5: Standard Protein Loading



Resin:	TOYOPEARL GigaCap DEAE-650M
Column size:	10 mm ID × 14.7 cm
Mobile phase:	A: 50 mmol/L Tris-HCl, pH 8.5
	B: mobile phase A + 0.5 mol/L NaCl
Gradient:	50 CV, 0 - 100% B
Flow rate:	136 cm/hr (1.78 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	0.01 - 480% CV
Samples:	1. ovalbumin, 10 g/L
	2. trypsin inhibitor, 10 g/L
Sample load:	2.35 - 1132.98 mg total protein
Method:	sanitize column with 2.5 CV of 1.0 mol/L NaOH
	equilibrate with mobile phase A for 5 CV
	inject sample
	wash with mobile phase A for 2 CV
	elute with gradient
	sanitize column with 2.5 CV of 1.0 mol/L NaOH

- The separation of ovalbumin and trypsin inhibitor was compared under increasing load conditions of a standard protein solution, ranging from 0.12 - 96 g total protein per L of resin.
- At 96 g/L, excellent resolution between the two peaks is still achieved.

Figure 6: β-lactoglobulin Loading

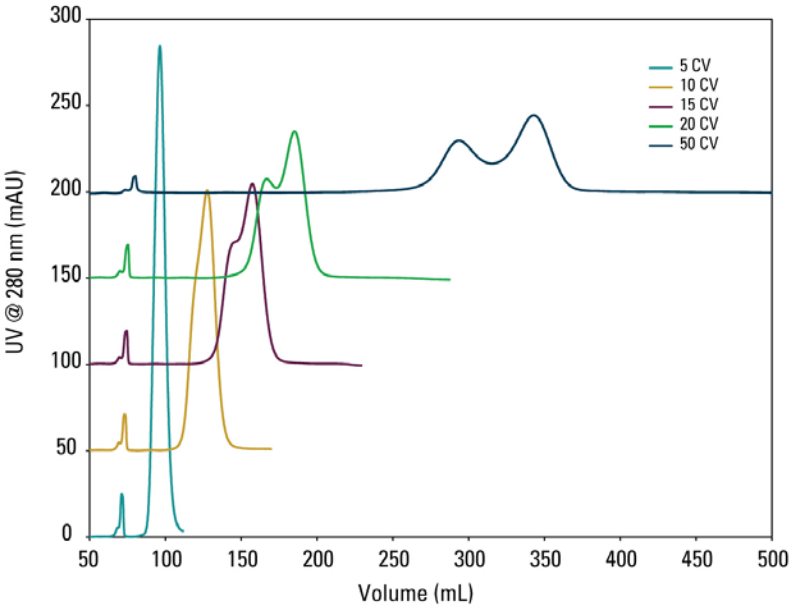


Resins:	TOYOPEARL GigaCap DEAE-650M TOYOPEARL DEAE-650M
Column size:	10 mm ID x 14.7 cm
Mobile phase:	A: 20 mmol/L Na_2HPO_4, pH 6.0 B: mobile phase A + 0.5 mol/L NaCl, pH 6.0
Gradient:	120 minutes, 0 - 100% B
Flow rate:	136 cm/hr (1.78 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	7 - 250% CV
Samples:	20 g/L β-lactoglobulins
Method:	sanitize column with 2.5 CV of 1.0 mol/L NaOH equilibrate with mobile phase A for 5 CV inject sample wash with mobile phase A for 2 CV elute with gradient sanitize column with 2.5 CV of 1.0 mol/L NaOH

Figure 6: β-lactoglobulin Loading, continued

- The influence of protein load was studied. 1.33 - 50 g β-lactoglobulins/L resin were loaded onto either a TOYOPEARL DEAE-650M (solid lines) or TOYOPEARL GigaCap DEAE-650M (dotted lines) column and eluted under the same conditions.
- For the TOYOPEARL DEAE-650M resin, the 25 g/L (green) and 50 g/L (teal) runs appeared similar. This similarity may be due to the column being saturated. In addition, at 25 g/L and 50 g/L, the TOYOPEARL DEAE-650M resin did not appear to resolve the β-lactoglobulins.
- On the other hand, all loading concentrations up to 50 g/L showed two peaks with the TOYOPEARL GigaCap DEAE-650M.
- Baseline or near baseline separation was achieved for both resins up to at least 5 g/L β-lactoglobulins loading.
- The TOYOPEARL DEAE-650M runs eluted at a lower conductivity compared to the TOYOPEARL GigaCap DEAE-650M, potentially indicating a higher salt tolerance in the TOYOPEARL GigaCap DEAE-650M resin.

Figure 7: β-lactoglobulin Gradients



Resin:	TOYOPEARL GigaCap DEAE-650M
Column size:	10 mm ID × 14.7 cm
Mobile phase:	A: 20 mmol/L sodium phosphate, pH 6.0
	B: mobile phase A + 0.5 mol/L NaCl, pH 6.0
Gradient:	0 - 100%
Flow rate:	136 cm/hr (1.78 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	7% CV
Samples:	20 g/L β-lactoglobulins in mobile phase A
Method:	sanitize column with 2.5 CV of 1.0 mol/L NaOH
	equilibrate with mobile phase A for 5 CV
	inject sample
	wash with mobile phase A for 2 CV
	elute with gradient
	sanitize column with 2.5 CV of 1.0 mol/L NaOH

The influence of gradient slope on the elution of 1.33 g β-lactoglobulins/L are as follows:

- The β-lactoglobulins did not visibly show two peaks with a 5 CV gradient.
- With a 10 CV gradient, a shoulder was visible.
- With at least a 15 CV gradient, two distinct peaks were seen, and the resolution increased with decreasing gradient slope.
- The effect of column length on protein resolution was not studied.

Conclusions

- TOYOPEARL GigaCap DEAE-650M is a high capacity weak anion exchange resin useful for both capture and intermediate purifications. For example, it successfully separated two proteins at up to 96 g/L total protein as well as β-lactoglobulins at up to 50 g/L.
- TOYOPEARL GigaCap DEAE-650M retains its high binding capacity even with decreased residence time and increased linear flow rate as well as repeated CIP cycles.
- TOYOPEARL GigaCap DEAE-650M exhibits good pressure-flow characteristics, allowing for decreased pool volumes while maintaining excellent binding and elution kinetics.
- At the recommended linear flow rate for performance testing, the reduced plate height is approximately 4-5 bead widths. However, the minimum HETP was achieved at a much lower flow rate. (Data not shown).
- The high DBC of TOYOPEARL GigaCap DEAE-650M minimizes the need for larger columns and decreases buffer consumption, especially for higher titer proteins, allowing increased throughput while maintaining excellent binding and elution kinetics.