



Characterization of Two Novel High Capacity, High Resolution Strong Ion Exchange Resins

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Introduction

- TOYOPEARL GigaCap[®] Q-650S media is a high capacity, strong quaternary ammonium anion exchange resin optimized for the purification and polishing of both small and large proteins.
- TOYOPEARL GigaCap S-650S media is a high capacity, strong sulfonic cation exchange resin optimized specifically for the purification and polishing of IgG and smaller proteins.
- TOYOPEARL[®] HW-65 resin, the polymeric base bead for both resins, is chemically modified to provide higher capacity. Both resins have a pressure rating of 0.3 MPa, and they are stable in the pH range 3-13.
- Comparisons were made between the TOYOPEARL GigaCap Q-650S and the TOYOPEARL GigaCap S-650S to their M-grade counterparts showing increased selectivity as well as to other commercially available high resolution resins.



Anion Exchange Resin Binding Capacity Comparisons

Resin	Particle Size (µm)	pH Stability	Base Bead	Ion Exchange Capacity (meq/L)	Binding Capacity (g/L)		DBC Recovery (%)	DBC Elution Volume (CV)
					Static	Dynamic ¹		
TOYOPEARL GigaCap Q-650S	20 - 50	3 - 13	Polymethacrylic	0.20	200	191	99	1.7
TOYOPEARL GigaCap Q-650M	50 - 100	3 - 13	Polymethacrylic	0.17	191	172	97	15.8
Capto™ Q ImpRes	36 - 44	2 - 12	Agarose	0.12	92	40	100	ND ²
Q Sepharose™ HP	24 - 44	2 - 12	Agarose	0.15	114	81	99	ND ²

¹Dynamic binding capacities were determined at 10% breakthrough

²Values not determined

Dynamic Binding Capacity (DBC) Conditions:

Column size: 6 mm ID × 4 cm
 Mobile phase A: 50 mmol/L Tris-HCl buffer, pH 8.5
 B: mobile phase A + 0.5 mol/L NaCl
 Linear velocity: 212 cm/hr
 Detection: UV @ 280 nm
 Sample: 1.0 g/L BSA

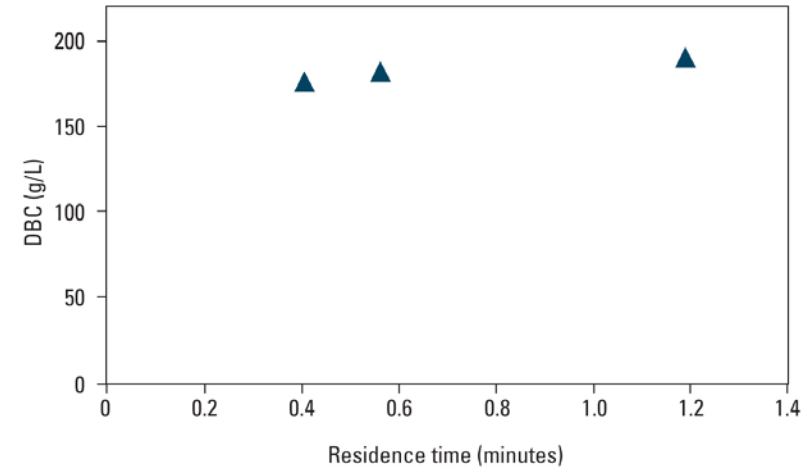
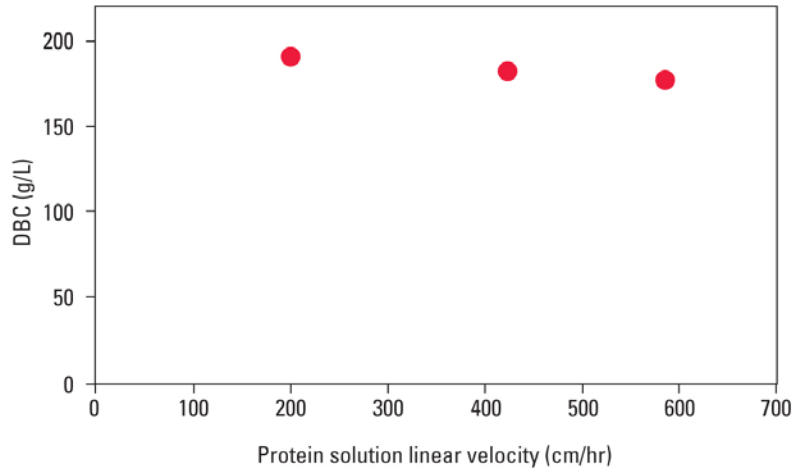
Static Binding Capacity (SBC) Conditions:

Adsorption buffer: 50 mmol/L Tris-HCl, pH 8.5
 Protein concentration: 10.0 g/L

- The TOYOPEARL GigaCap series has a significantly higher binding capacity compared to GE Healthcare's Capto Q ImpRes and Q Sepharose HP, while maintaining nearly 100% recovery.
- In addition, the TOYOPEARL GigaCap Q-650S elutes in a very narrow band compared to the TOYOPEARL GigaCap Q-650M, allowing more efficient handling in subsequent downstream processing steps.



DBC vs. Flow Rate: Residence Time



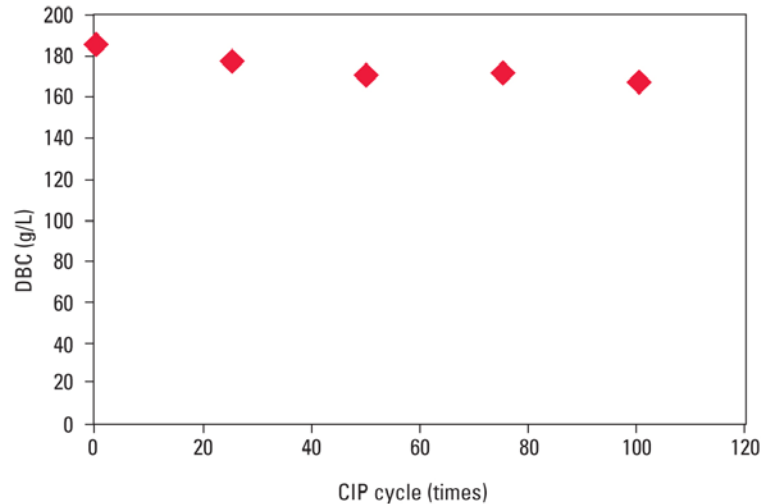
DBC Conditions:

Resin: TOYOPEARL GigaCap Q-650S
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L Tris-HCl buffer, pH 8.5
B: mobile phase A + 0.5 mol/L NaCl
Linear velocity: 201, 424, or 585 cm/hr
Residence time: 1.2 min, 0.6 min, or 0.4 min
Detection: UV @ 280 nm
Sample: 1.0 g/L BSA

Efficient mass transfer kinetics allow the TOYOPEARL GigaCap Q-650S to maintain a high DBC even with increased linear velocities and decreased residence time.



Stability of Resin After CIP



DBC conditions:

Resin: TOYOPEARL GigaCap Q-650S
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L Tris-HCl, 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: 1.0 g/L BSA

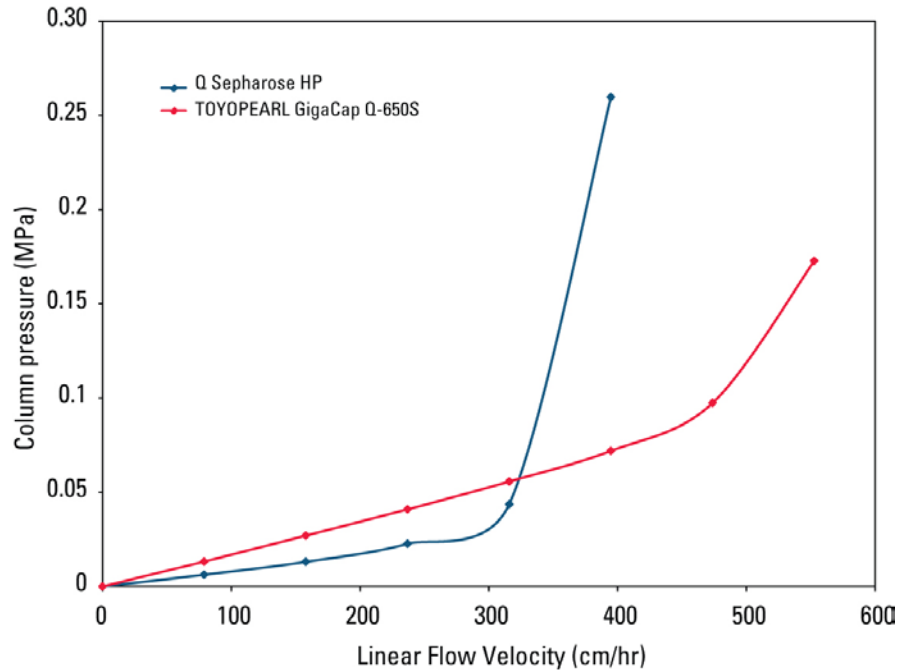
CIP conditions:

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

- The DBC of TOYOPEARL GigaCap Q-650S was measured after every set of 20 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 maintained through at least 100 CIP cycles, showing excellent stability.



Pressure-Flow Curves

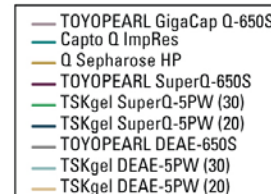
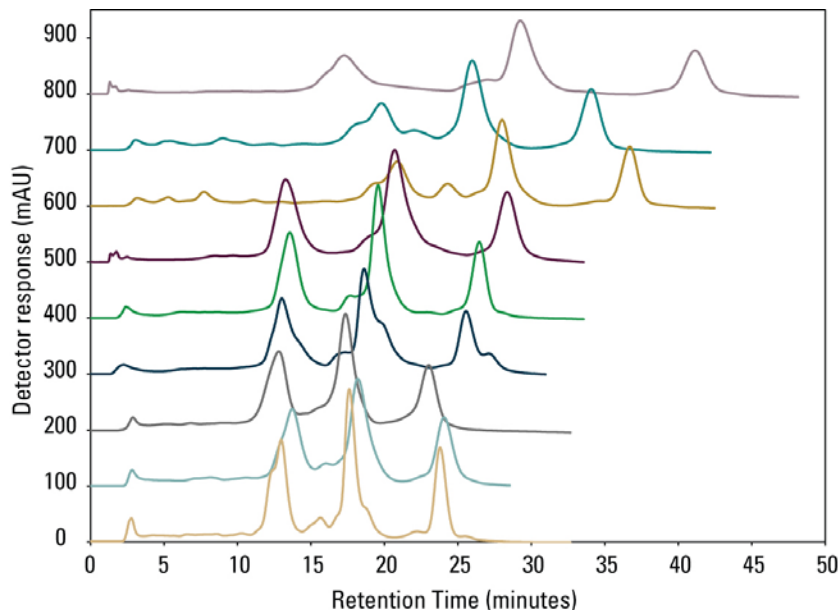


Resins: as indicated
Column size: 2.2 cm ID × 20 cm
Mobile phase: 0.1 mol/L NaCl

- TOYOPEARL GigaCap Q-650S and Q Sepharose HP exhibited similar pressure-flow response for flow rates up to 300 cm/hr. Above 300 cm/hr, the Q Sepharose HP resin reached its critical velocity.¹ The particle sizes are 35 μm and 34 μm , respectively.
- The GigaCap Q-650S resin had a much higher critical velocity and can be operated at increased flow rates.



Selectivity Comparisons

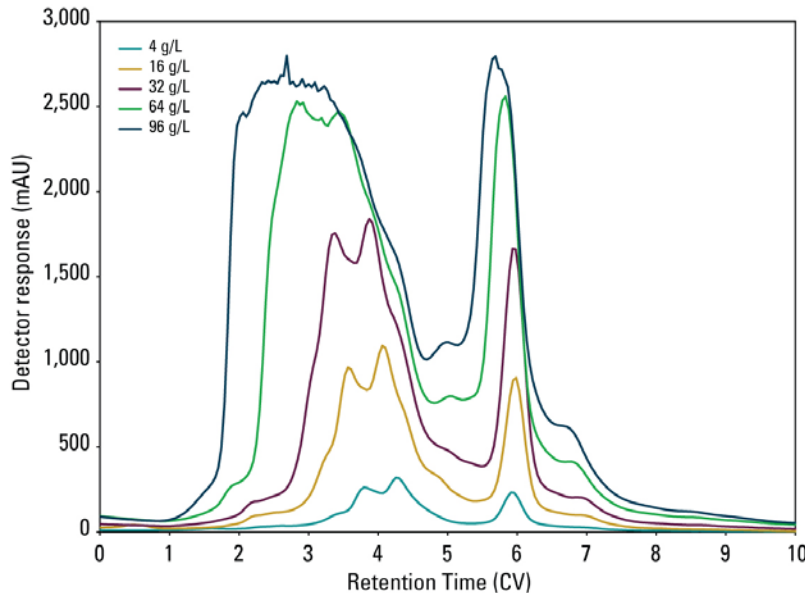


Column size: 7.5 mm ID × 7.5 cm
Mobile phase: A: 50 mmol/L Tris-HCl, pH 8.5
B: mobile phase A + 1.0 mol/L NaCl
Gradient: 0-100% mobile phase B (120 min)
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Injection vol.: 100 µL
Sample: 2.9 g/L transferrin, 6.5 g/L ovalbumin,
10.0 g/L trypsin inhibitor

- The TOYOPEARL GigaCap Q-650S retains proteins to a higher conductivity than most of the resins tested.
- This data may indicate the TOYOPEARL GigaCap Q-650S resin is more salt tolerant than the other TOYOPEARL and TSKgel® Q-type resins.
- The retention is similar to Q Sepharose HP and Capto Q ImpRes. However, TOYOPEARL GigaCap Q-650S has improved selectivity compared to those two resins.



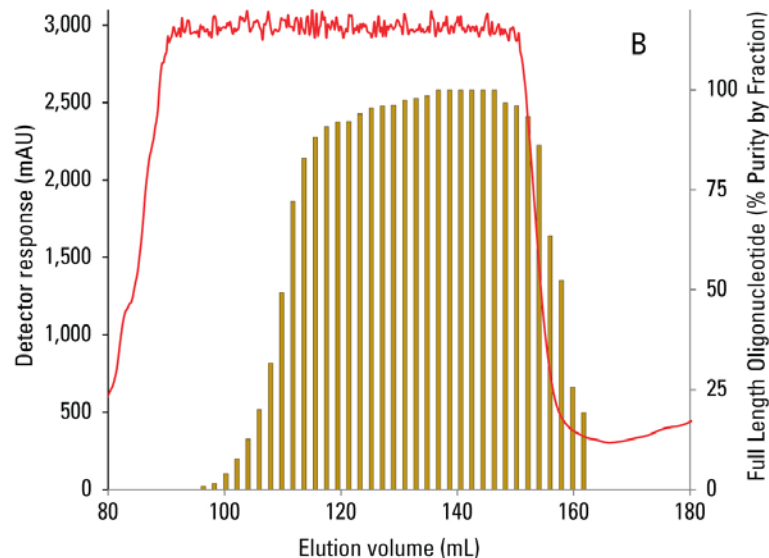
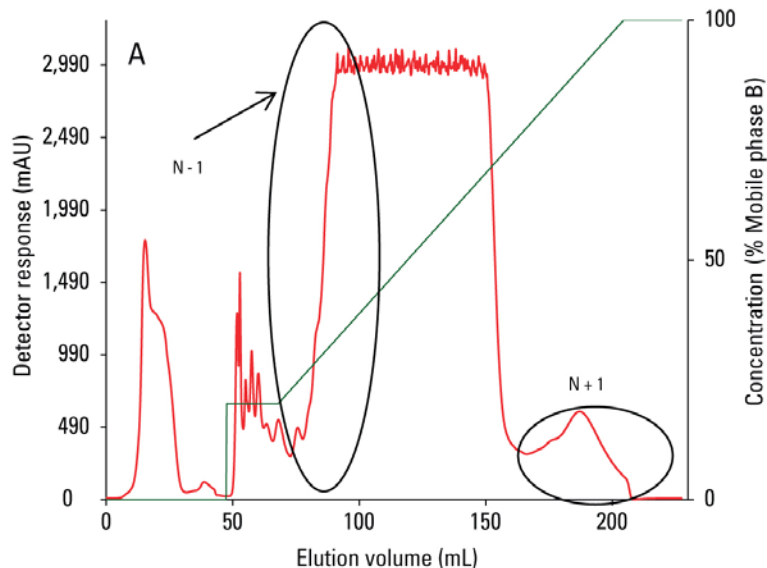
Standard Protein Loading



Resin: TOYOPEARL GigaCap Q-650S
Column size: 1.0 cm ID × 15.5 cm
Mobile phase: A: 50 mmol/L Tris-HCl, pH 8.7
B: mobile phase A + 0.5 mol/L NaCl
Gradient: 0-100% mobile phase B (10 CV)
Flow rate: 136 cm/hr (1.78 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Injection vol.: 0.4-9.6 CV
Sample: 4.5 g/L ovalbumin, 5.4 g/L trypsin inhibitor
Sample load: 4-96 g protein/L resin
Method: Column sanitized with 1.0 mol/L NaOH for 2.5 CV. Equilibrate column with mobile phase A for 2 CV. Load proteins. Wash column with mobile phase A for 3 CV. Run 0-100% mobile phase B gradient over 10 CV. Clean column with 5 CV using 1.0 mol/L NaOH.

- Shown are chromatograms of TOYOPEARL GigaCap Q-650S resin with increasing protein load, ranging from 4 g protein/L resin to 96 g protein/L resin.
- At lower protein loads, the resin was able to separate various components in the ovalbumin.
- Even at 96 g/L protein loading, this resin has sufficient capacity and selectivity to separate the ovalbumin and its impurities from the trypsin inhibitor.

Oligonucleotide Purification



Resin: TOYOPEARL GigaCap Q-650S
 Column size: 6.6 mm ID × 18 cm (6.16 mL)
 Mobile phase: A: 20 mmol/L NaOH
 B: mobile phase A + 3.0 mol/L NaCl
 Gradient: 20% - 100% B (2 CV)
 100% B (2 CV)
 Flow rate: 200 cm/hr (1.14 mL/min)
 Detection: UV @ 254 nm
 Injection vol.: 4.63 mL (181.4 mg)
 Sample: crude phosphorothioate deoxyribonucleotide

- TOYOPEARL GigaCap Q-650S effectively purified a crude phosphorothioate deoxyribonucleotide at 80% DBC loading.
- Although the UV trace at 254 nm went off scale, an enlarged image of the main oligonucleotide peak, when overlaid with a histogram showing HPLC results for fraction purity, shows the full length oligonucleotide completely contained in the main peak at 80% DBC loading.
- In addition, the TOYOPEARL GigaCap Q-650S is able to elute in a smaller pool volume compared to other resins (data not shown).



Cation Exchange Resin Binding Capacity Comparisons

Resin	Particle Size (µm)	pH Stability	Base Bead	Ion Exchange Capacity (meq/L)	Binding Capacity (g/L)		DBC Recovery (%)	DBC Elution Volume* (CV)
					Static	Dynamic*		
TOYOPEARL GigaCap S-650S	20 - 50	3 - 13	Polymethacrylic	0.24	177	164	99	4.0
TOYOPEARL GigaCap S-650M	50 - 100	3 - 13	Polymethacrylic	0.16	156	145	98	13.5
Capto SP ImpRes	36 - 44	2 - 12	Agarose	0.12	89	27	100	ND**
SP Sepharose HP	24 - 44	2 - 12	Agarose	0.15	105	65	100	ND**

* Elution volumes calculated using lysozyme at 10% breakthrough.

** Values not determined.

DBC Conditions:

Column size: 6 mm ID × 4 cm
 Mobile phase: A: 50 mmol/L acetate buffer, pH 4.7
 B: mobile phase A + 0.5 mol/L NaCl
 Linear velocity: 212 cm/hr
 Detection: UV @ 280 nm
 Sample: 1.0 g/L γ-globulin

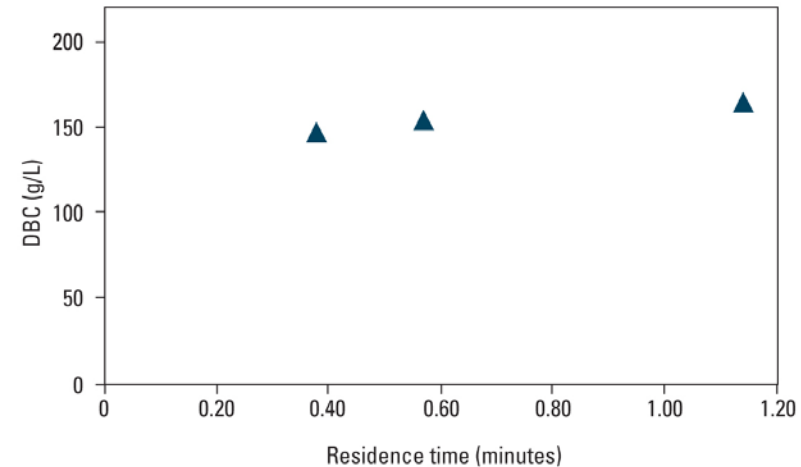
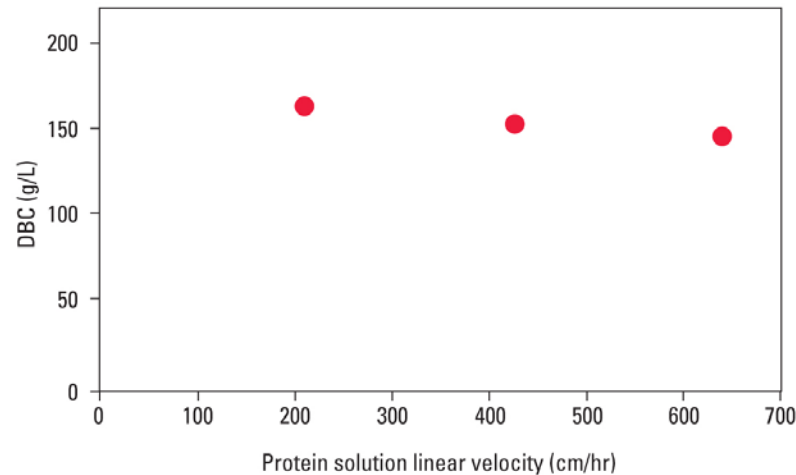
SBC Conditions:

Adsorption buffer: 50 mmol/L acetate buffer, pH 4.7
 Sample: 10.0 g/L γ-globulin

- Both TOYOPEARL GigaCap resins have a significantly higher binding capacity compared to GE Healthcare's Capto SP ImpRes and SP Sepharose HP while maintaining nearly 100% recovery.
- In addition, the TOYOPEARL GigaCap S-650S elutes in a very narrow band compared to TOYOPEARL GigaCap S-650M, allowing for more efficient handling in subsequent downstream processing steps.



DBC vs. Flow Rate: Residence Time



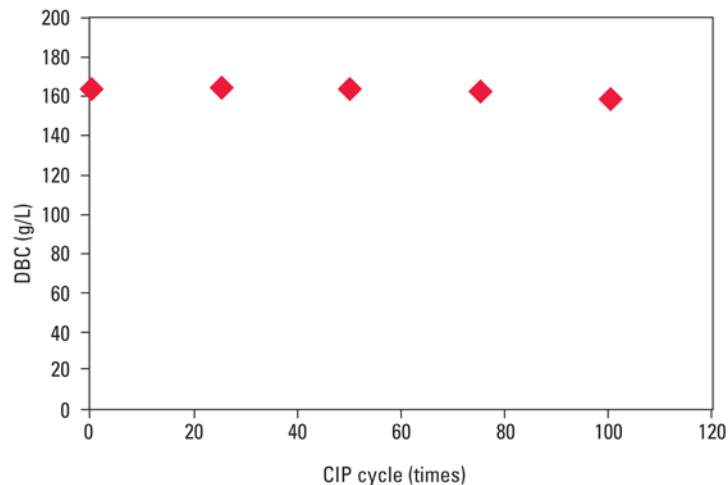
DBC Conditions:

Resin: TOYOPEARL GigaCap S-650S
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L acetate pH 4.7
B: mobile phase A + 0.5 mol/L NaCl
Linear velocity: 211, 425, or 637 cm/hr
Residence time: 1.1 min, 0.6 min, or 0.4 min
Detection: UV @ 280 nm
Sample: 1.0 g/L γ -globulin

Efficient mass transfer kinetics allow the TOYOPEARL GigaCap S-650S to maintain a high DBC even with increased linear velocities and decreased residence time.



Stability of Resin After CIP



DBC conditions:

Resin: TOYOPEARL GigaCap S-650S
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L acetate, pH 4.7
B: mobile phase A + 1.0 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: 1.0 g/L γ -globulin

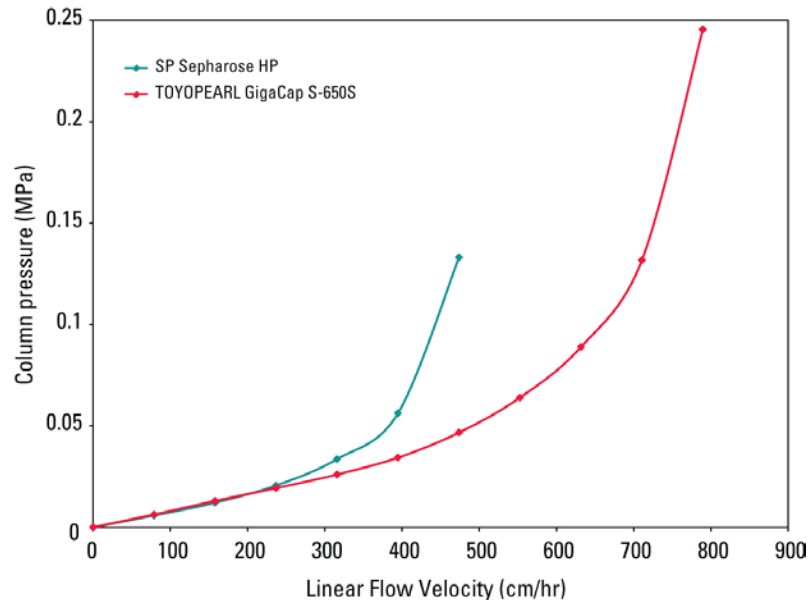
CIP conditions:

Alkaline wash: 0.5 mol/L NaOH
Buffer wash: 50 mmol/L acetate + 0.5 mol/L NaCl, pH 4.7
Flow rate: 106 cm/hr (0.5 mL/min)
CIP wash volume: 27 CV/cycle
Contact time: 1 hr
Buffer wash volume: 10 CV/cycle

- The DBC of TOYOPEARL GigaCap S-650S was measured after every set of 20 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 maintained through at least 100 CIP cycles, showing excellent stability.



Pressure-Flow Curves

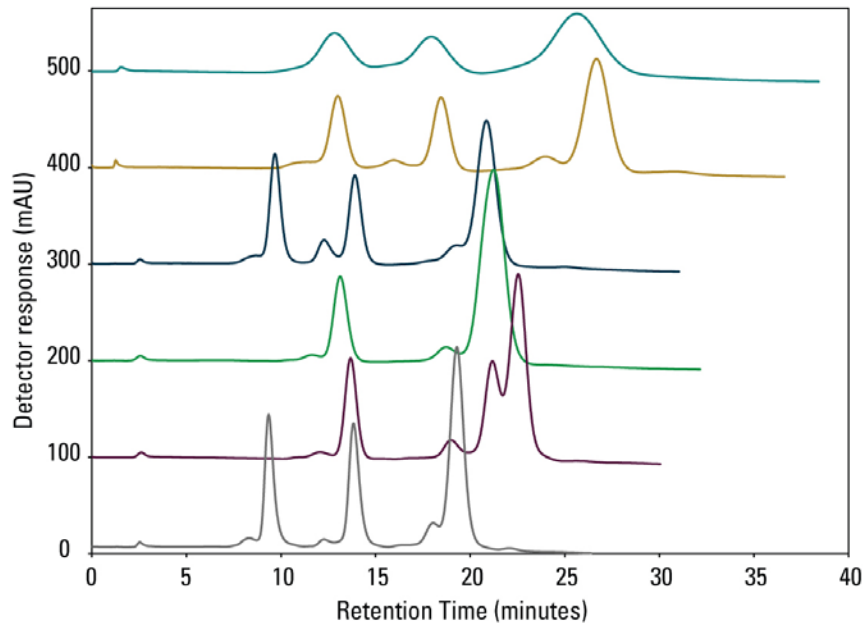


Resins: as indicated
Column size: 2.2 cm ID × 20 cm
Mobile phase: 0.1 mol/L NaCl

- TOYOPEARL GigaCap S-650S and SP Sepharose HP exhibited similar pressure-flow responses for flow rates up to 225 cm/hr. Above 450 cm/hr, the SP Sepharose HP resin reached its critical velocity.¹ The particle sizes are 35 μm and 34 μm , respectively.
- The GigaCap S-650S resin had a much higher critical velocity and can be operated at increased flow rates.



Selectivity Comparisons



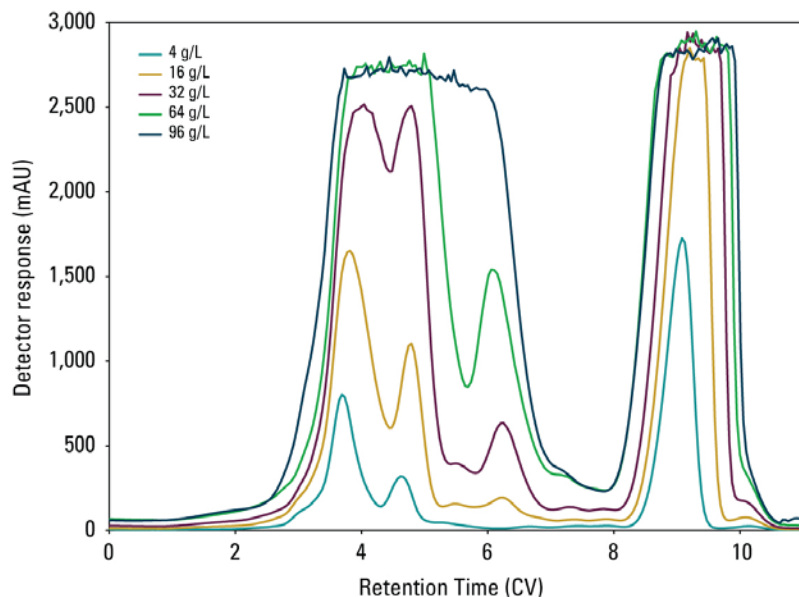
— TOYOPEARL GigaCap S-650M
— TOYOPEARL GigaCap S-650S
— TOYOPEARL SP-650S
— Capto SP ImpRes
— SP Sepharose HP
— TSKgel SP-5PW (20)

Column size: 7.5 mm ID × 7.5 cm
Mobile phase: A: 20 mmol/L phosphate, pH 7.0
B: mobile phase A + 1.0 mol/L NaCl
Gradient: 0-100% mobile phase B (60 min)
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Injection vol.: 20 µL
Samples: ribonuclease A, 9.8 g/L
cytochrome C, 3.6 g/L
lysozyme, 6.4 g/L

- The TOYOPEARL GigaCap S-650 resins retain proteins at higher conductivities than the TSKgel SP-5PW (20) and TOYOPEARL SP-650S.
- Although the TOYOPEARL GigaCap S-650S shows similar retentivity to Capto SP ImpRes, SP Sepharose HP, and TOYOPEARL GigaCap S-650M, the TOYOPEARL GigaCap S-650S elutes with improved selectivity and lower pool volumes.



Standard Protein Loading



Resin: TOYOPEARL GigaCap S-650S
Column size: 1.0 cm ID × 15.8 cm
Mobile phase: A: 20 mmol/L sodium phosphate, pH 4.7
B: mobile phase A + 0.5 mol/L NaCl
Gradient: 0-100% mobile phase B (10 CV)
Flow rate: 300 cm/hr
Detection: UV @ 280 nm
Temperature: ambient
Injection vol.: 0.4-9.6 CV
Sample: α -chymotrypsin, 2.0 g/L
lysozyme, 2.0 g/L
Sample load: 4-96 g protein/L resin
Method: Column sanitized with 1.0 mol/L NaOH for 2.5 CV. Equilibrate column with mobile phase A for 2 CV. Load proteins. Wash column with mobile phase A for 3 CV. Run 0-100% mobile phase B gradient over 10 CV. Clean column with 5 CV using 1.0 mol/L NaOH.

- Shown are chromatograms of TOYOPEARL GigaCap Q-650S resin with increasing protein load, ranging from 4 g protein/L resin to 96 g protein/L resin.
- At lower protein loads, the resin was able to separate various components in the α -chymotrypsin.
- Even at 96 g/L loading, the resin still had sufficient capacity and selectivity to separate the α -chymotrypsin and its impurities from lysozyme.



Conclusions

- TOYOPEARL GigaCap Q-650S and TOYOPEARL GigaCap S-650S are high capacity and high resolution strong ion exchange resins useful in both purification and polishing steps.
- Like their M-grade counterparts, the TOYOPEARL GigaCap Q-650S and TOYOPEARL GigaCap S-650S resins have high binding capacities and improved elution kinetics, resulting in a decreased elution pool volume.
- Dynamic binding capacity of the TOYOPEARL GigaCap Q-650S was higher than the TOYOPEARL GigaCap Q-650M at around 191 g BSA/L resin, compared to 172 g BSA/L resin.
- Dynamic binding capacity of the TOYOPEARL GigaCap S-650S resin was also higher than its M-grade counterpart, coming in at around 164 g γ -globulin/L resin, compared to 145 g γ -globulin/L resin.
- The elution kinetics for the TOYOPEARL GigaCap Q-650S resin are significantly improved over the TOYOPEARL GigaCap Q-650M. The BSA eluted in approximately 1.7 CV, almost an order of magnitude less than the TOYOPEARL GigaCap Q-650M, which eluted in 15.8 CV.
- The TOYOPEARL GigaCap S-650S eluted lysozyme in approximately 4.0 CV, whereas the TOYOPEARL GigaCap S-650M eluted lysozyme in 13.5 CV.
- Both resins have a high capacity, even at increased flow rates.
- Both resins also exhibit reasonable selectivity, allowing for some separation of the test proteins, even at a protein load of 96 g total protein/L resin.



Reference

¹ Stickel JJ; Fotopoulos A. Pressure-flow relationships for packed beds of compressible chromatography media at laboratory and production scale. *Biotechnol Prog* **2001**, *17*, 744-751.