



TOYOPEARL GigaCap[®] Series

INTRODUCTION

Ion Exchange Chromatography (IEC) is one of the most frequently used chromatographic modes for the separation and purification of biomolecules. Compared with other chromatographic modes, modern ion exchange media offer high dynamic binding capacities and a straightforward method development. IEC is used at all stages and scales of purification of therapeutic proteins: from laboratory scale purification to industrial scale downstream processing (DSP).

The development of cell lines with high expression levels has triggered the demand for high throughput DSP. This requires rigid resins that offer high binding capacities and recoveries at high flow rates and withstand harsh purification procedures. TOYOPEARL GigaCap ion exchange resins were developed to meet the actual needs in high throughput purification of monoclonal antibodies and other proteins by packed bed chromatography.

The TOYOPEARL GigaCap family consists of two cation exchange resins - GigaCap S-650 and GigaCap CM-650 - and the GigaCap Q-650 and DEAE-650 anion exchange resins. While all GigaCap media are available in M-grade particle size (75 µm) for capture and intermediate process steps, GigaCap S and Q are also available in smaller particle size (S-grade; 35 µm) for high resolution intermediate and polishing purification.

STATIC VS DYNAMIC BINDING CAPACITIES FOR HUMAN IgG

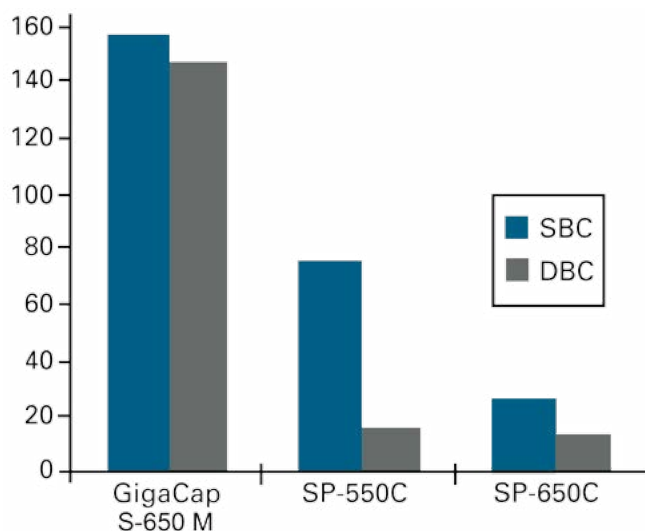


Figure 1
 DBC for GigaCap S-650M calculated at 10% breakthrough
 Column size: 6 mm ID x 4 cm L; Sample: polyclonal human IgG;
 Linear velocity: 212 cm/h

HIGHLIGHTS

- High binding capacities at short residence times
- Fast elution kinetics generating low elution pool volumes
- Excellent mechanical and chemical stability
- Superior pressure flow properties

TOYOPEARL GigaCap S-650

TOYOPEARL GigaCap S-650 is a strong cation exchange resin designed for high throughput process chromatography. Based on the well proven methacrylic polymer backbone of TOYOPEARL and TSKgel media TOYOPEARL GigaCap S combines excellent pressure flow characteristics (Figure 5) with unmatched dynamic binding capacities (DBC) and high recoveries for a wide range of biomolecules. Latest surface modification technology was applied to increase the content of functional groups at the particle surface thus reaching binding capacities up to 150 mg IgG per mL resin (Figure 1, 2). Similar binding capacities can be achieved for smaller proteins such as insulin (Figure 3).

TOYOPEARL GigaCap S-650M BREAKTHROUGH CURVES AT DIFFERENT LINEAR VELOCITIES

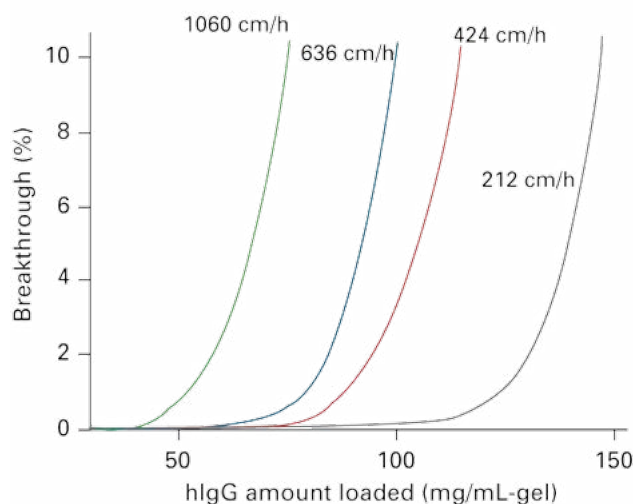


Figure 2
 DBC for GigaCap S-650M calculated at 10% breakthrough
 Column size: 6 mm ID x 4 cm L bed height;
 Sample: Polyclonal human IgG (1 mg/mL); Buffer: 0.1 mol/L acetate buffer (pH 4.7); Linear velocity: 212, 424, 636, 1060 cm/h; Detection: UV@280nm

TOYOPEARL DYNAMIC BINDING CAPACITIES FOR INSULIN

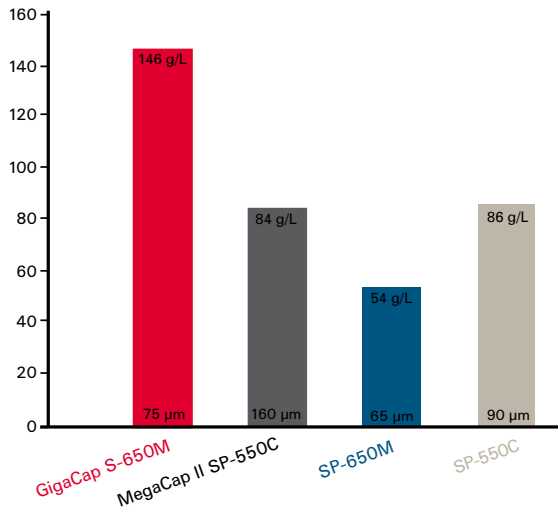


Figure 3
Column size: 6.6 mm ID x 22 mm L; 100 mM citrate buffer (ph 3.0)

The applied ligand chemistry enables a better access of proteins to the charged groups. Besides binding capacity this improves also mass transfer and thereby reduces the target molecule elution volume.

Figure 4 shows the breakthrough curve for TOYOPEARL GigaCap S compared with the most equivalent competitive resin. Each trace shows the dynamic binding capacity of the resin up to 10% breakthrough plus the elution profile. Elution pool volume of TOYOPEARL GigaCap S is remarkably reduced when compared to the competitive resin. The concentration of the eluted protein is proportionally increased as well. It is possible to achieve reductions in elution pool volumes of over 75% (Jackewitz A. BioProcess Int. 6 (7) 2008: 108-110). This has potentially very significant impact on reducing the cost of further downstream process steps. Similar reductions of elution pool volumes can be reached with all GigaCap resins.

TOYOPEARL GigaCap S shows an excellent chemical stability resulting in a minimal loss of IgG binding capacity

REDUCED POOL VOLUME

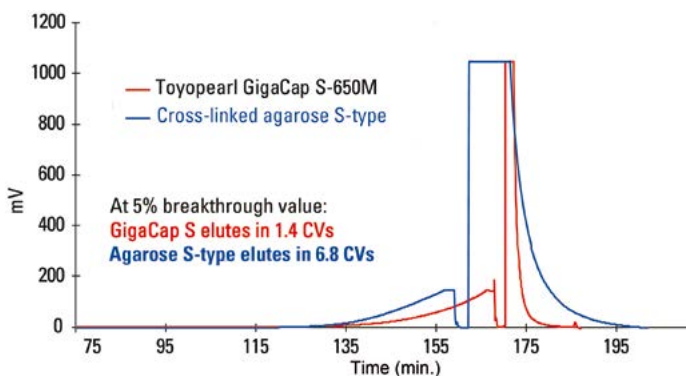


Figure 4
Column size: TOYOPEARL GigaCap S-650M, 6 mm ID x 4 cm L; Sample: polyclonal human IgG (1 mg/mL); Loading buffer: 0.1 mol/L acetate buffer pH 4.7; Elution buffer: 0.1 mol/L acetate buffer pH 4.7 + 1.0 mol/L NaCl; Linear velocity: 212 cm/h; Detection: UV@280 nm

PRESSURE FLOW CURVES OF CATION EXCHANGE RESINS

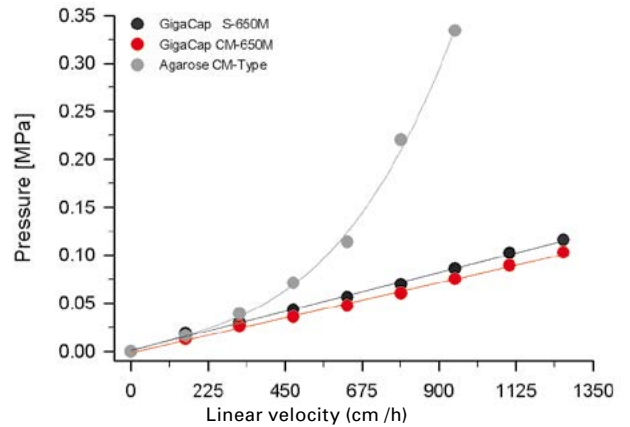


Figure 5
Column size: 22 mm ID x 20 cm L; Mobile phase: Water; Temperature: 25° C

after exposure to 1 M NaOH (Table 1) for 5 weeks. It is therefore ideally suited for process scale protein purification in large industrial process columns.

The small particle size TOYOPEARL GigaCap S-650S offers a higher binding capacity and resolution than the M grade. It is ideally suited for high purity separations needed in final polishing steps. Figure 6 shows the high resolution achieved with TOYOPEARL GigaCap S-650S and its advantageous selectivity, when compared with agarose based cation exchange media with similar particle size.

TOYOPEARL GigaCap S ALKALINE STABILITY

Time (week)		0	1	3	5
IEC capacity	meq/mL	0.159	0.157	0.158	0.156
hIgG-DBC (10% breakthr.)	mg/mL-gel	143	144	140	135
hIgG-recovery	%	99	101	100	99

Table 1
TOYOPEARL GigaCap S-650M stored in 1 M NaOH solution at RT

SELECTIVITY OF SMALL PARTICLE SIZE MEDIA

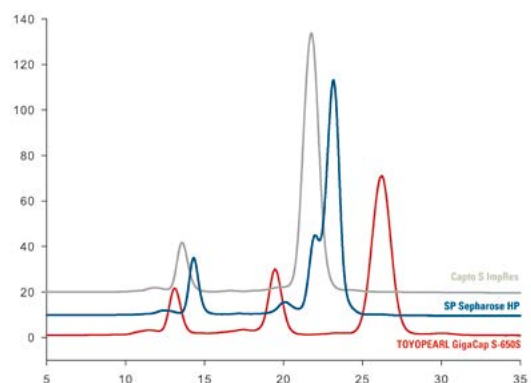


Figure 6
Column: 7.5 mm ID x 7.5 cm; Buffer A: 0.02 mol/L phosphate buffer (pH 7.0), Buffer B: 0.02 mol/L phosphate buffer + 1.0 mol/L NaCl (pH 7.0); Flow rate: 1.0 mL/min; Gradient: 60 min linear from 100% buffer A to 100% buffer B; Detection: UV @ 280nm; Sample: 1. ribonuclease A (9.2 g/L), 2. cytochrome C (4.7 g/L), 3. lysozyme (9.5 g/L); Injection volume: 20 µL

TOYOPEARL GigaCap CM-650M

TOYOPEARL GigaCap CM-650M is a weak cation exchange resin that combines excellent pressure flow characteristics (Figure 5) and high alkaline stability (Table 2) with high dynamic binding capacities and recoveries for both, IgGs and smaller proteins. Latest surface modification technology was applied to increase the content of functional groups at the particle surface thus reaching binding capacities up to 100 mg IgG per mL resin (Figure 7).

Depending on the specific characteristics of the target molecule, GigaCap S-650M and CM-650M exhibit different selectivity as shown in Figure 8 for some standard proteins. Therefore it is strongly recommended to evaluate both, GigaCap CM-650M and S-650M when developing a cation exchange purification step. For fast resin screening all GigaCap resins are available in 1 or 5 mL pre-packed ToyoScreen columns.

TOYOPEARL GigaCap CM ALKALINE STABILITY

Time (week)		0	1	3	5
IEX capacity	meq/mL	0.24	0.23	0.24	0.23
hIgG-DBC (10% breakthr.)	mg/mL-gel	99	101	100	99

Table 2

TOYOPEARL GigaCap CM-650M stored in 0.5 M NaOH solution at RT

BREAKTHROUGH CURVES FOR IgG ON CES RESINS

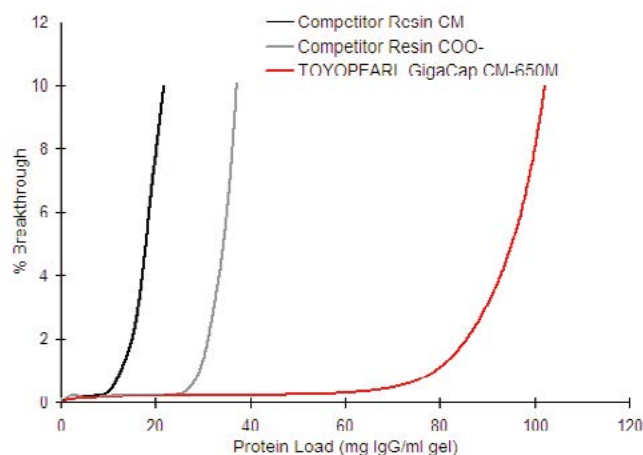


Figure 7

Column size: 6 mm ID x 4 cm L; Sample: polyclonal human IgG (1 mg/mL); Loading buffer: 50 mM sodium acetate buffer (pH 4.7); Elution buffer: 50 mM sodium acetate buffer (pH 4.7) + 0.5 mM NaCl; Linear velocity: 212 cm/h; Detection: UV@280 nm

SELECTIVITY OF TOYOPEARL GigaCap S-650M AND TOYOPEARL GigaCap CM-650M

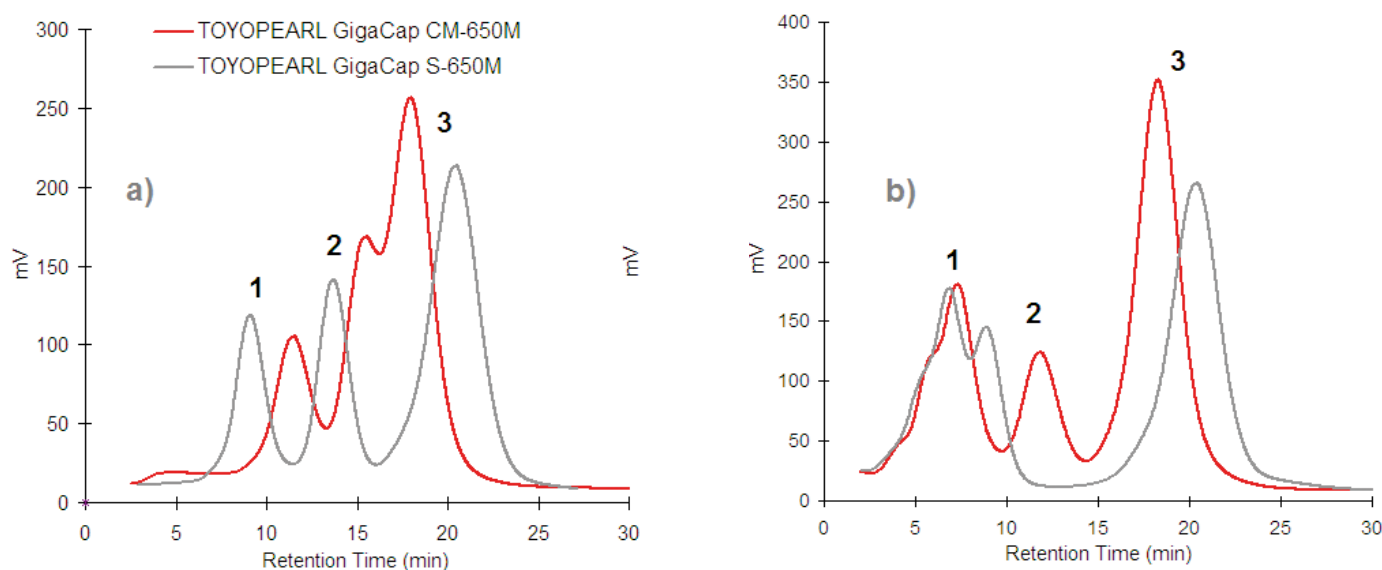


Figure 8

Column size: 6 mm ID x 4 cm L; Flow rate: 1.0 ml/min; Buffer A: 20 mM Phosphate (pH 7.0); Buffer B: 20 mM Phosphate + 1.0 M NaCl (pH 7.0); Gradient: 60 min linear gradient from 100% A to 100% B; Injection Volume: 25 µL; Detection: UV@280nm;

Sample: (a) 1. ribonuclease A (5.0 mg/mL), 2. cytochrome C (1.9 mg/mL), 3. lysozyme (3.8 mg/mL); (b) 1. trypsinogen (3.8 mg/mL), 2. ribonuclease A (5.0 mg/mL), 3. lysozyme (3.8 mg/mL)

TOYOPEARL GigaCap Q-650

TOYOPEARL GigaCap Q-650 is a strong anion exchange resin. It combines excellent pressure flow characteristics (Figure 9) with high dynamic binding capacities and high recoveries for a broad range of proteins. It binds up to 175 mg BSA per mL resin at 212 cm/h and still 164 mg BSA per mL resin at a linear flow of 800 cm/h (Figure 10).

High dynamic binding capacities can be achieved for much larger proteins as well. Even for a very large protein like thyroglobulin a dynamic binding capacity of 49 mg/mL gel can be achieved. As usual the difference between static (SBC) and dynamic binding capacity (DBC) is increasing with the increase in molecular weight (Table 3).

TOYOPEARL GigaCap Q-650M is the ideal anion exchange resin for large scale DSP unit operations. Examples range from flow-through steps in antibody purification to bind-elute steps in plasma protein purification. In flow through steps, TOYOPEARL GigaCap Q-650M can provide a viral clearance of 4 logs for MVM virus and X-MuLV virus.

The small particle size TOYOPEARL GigaCap Q-650S (35 μm) offers a higher binding capacity (190g BSA/L) and higher resolution than the respective M grade. The purification of oligonucleotides using anion exchange chromatography has traditionally fallen to resins such as TSKgel® SuperQ-5PW (20) that offer high resolution and selectivity in conjunction with excellent mechanical stability at very high column pressures. TOYOPEARL GigaCap Q-650S resin offers a low pressure alternative to oligonucleotide purification while preserving the selectivity, resolution and yields of those higher pressure processes. Figure 11 shows that the N-1 peak was slightly better resolved with the TSKgel SuperQ-5PW (20) than with the TOYOPEARL GigaCap Q-650S, perhaps due to the smaller particle size of the TSKgel resin. HPLC analysis of fractions taken across the peaks (data not shown) revealed that both resins were able to adequately resolve the full length oligonucleotide.

PRESSURE FLOW CURVES FOR ANION EXCHANGE RESINS

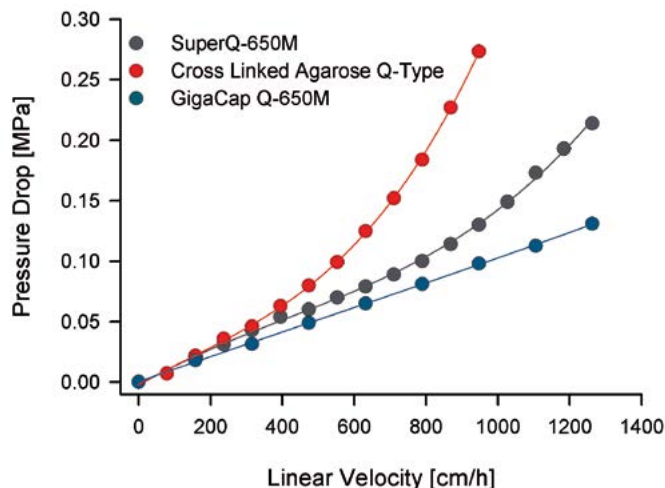


Figure 9

GigaCap Q BSA BINDING CAPACITY AT DIFFERENT FLOW RATES

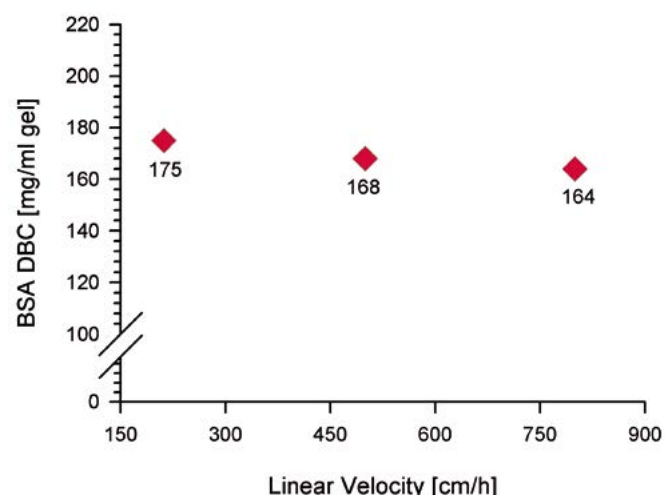


Figure 10

DBC measured at 10% breakthrough; Column size: 6 mm ID x 4 cm L; Sample: Bovine serum albumin (BSA 1 mg/mL) in Tris-HCL pH 8.5

PURIFICATION OF OLIGONUCLEOTIDES

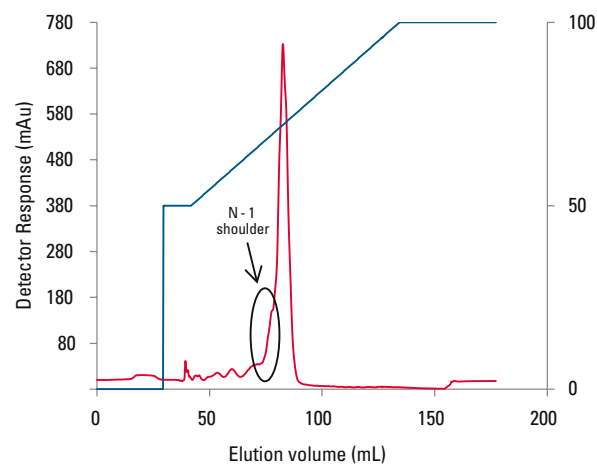
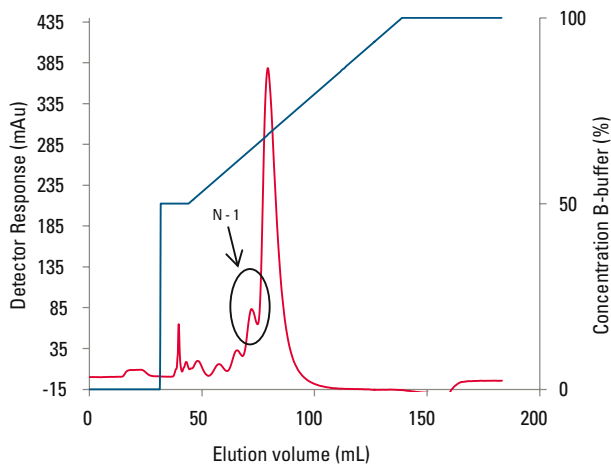


Figure 11

Resin: TSKgel SuperQ-5PW (20); Column size: 6.6 mm ID x 18.5 cm (6.3 mL); Mobile phase: A: 20 mmol/L NaOH; B: 20 mmol/L NaOH, 3.0 mol/L NaCl; Gradient: 50% B (2 CV) 50-100% B (15 CV), 100% B (2 CV); Flow rate: 200 cm/hr (1.14 mL/min); Detection: UV @ 254 nm; Sample load: 1.0 mg; Sample: crude phosphorothioate deoxyoligonucleotide

Resin: TOYOPEARL GigaCap Q-650S; Column size: 6.6 mm ID x 18.5 cm (6.3 mL); Mobile phase: A: 20 mmol/L NaOH; B: 20 mmol/L NaOH, 3.0 mol/L NaCl 50% B (2 CV); Gradient: 50-100% B (15 CV), 100% B (2 CV); Flow rate: 200 cm/hr (1.14 mL/min); Detection: UV @ 254 nm; Sample load: 1.0 mg; Sample: crude phosphorothioate deoxyoligonucleotide

TOYOPEARL GigaCap DEAE-650

TOYOPEARL GigaCap DEAE-650M is a weak anion exchange resin for process scale applications. The polymeric base bead was chemically modified to provide a greater number of anionic binding sites, resulting in increased binding capacity. TOYOPEARL GigaCap DEAE-650M exhibits typical dynamic binding capacities (DBC) approaching 170 g/L for bovine serum albumin (BSA) and 100 g/L for IgG. It maintains high capacity across a range of linear velocities and exhibits excellent pressure-flow characteristics. The high capacity and low back pressure creates opportunities for increased throughput in various anion exchange purification steps and is ideally suited for efficient plasma protein purification.

Good mass transfer kinetics enable the resin to maintain DBC at faster linear velocities (Figure 12). This fast uptake ability, when coupled with the narrow elution peak typical of TOYOPEARL GigaCap resins, results in smaller and more concentrated in-process pool volumes, thus reducing the amount of water for injection needed and increasing process throughput downstream.

TOYOPEARL GigaCap DEAE-650M is base stable for at least 100 cleaning-in-place (CIP) cycles with 0.5 mol/L NaOH, making multiple uses of the resin possible (Figure 13).

DBC OF TOYOPEARL Gigacap DEAE-650M AT DIFFERENT FLOW-RATES

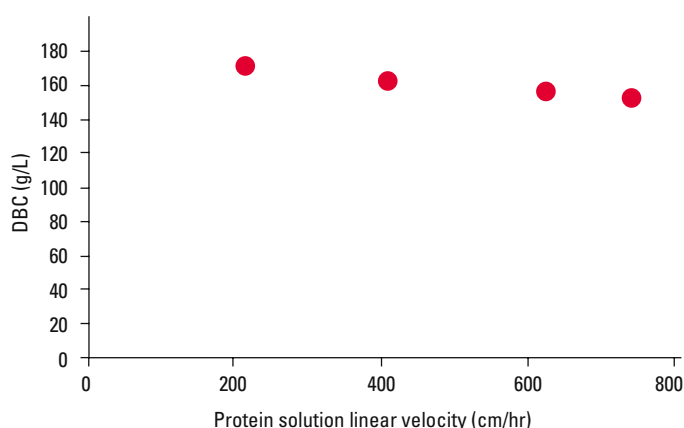


Figure 12
 DBC was determined at 10% breakthrough ; Column size: 6.0 mm ID x 4.0 cm; Sample: BSA (1.0 g/L) in 0.05 mol/L Tris, pH 8.5; Flow rate: 212 cm/h; Detection: UV @ 280 nm

CIP STABILITY OF TOYOPEARL GigaCap DEAE-650M

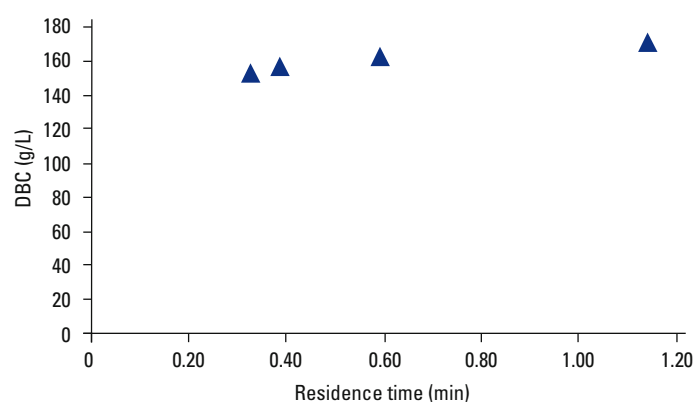


Figure 13
 Alkaline washing solution: 0.5 mol/L NaOH;
 Buffer washing solution: 0.5 mol/L Tris, 0.5 mol/L NaCl, pH 8.5; Flow rate: 106 cm/hr (0.5 mL/min); Alkaline wash volume: 27 CV/cycle; Alkaline contact time: 1 hr; Buffer wash volume: 10 CV/cycle

COMPARISON OF TOYOPEARL GigaCap RESINS







TOYOPEARL	Structure	Particle Size	DBC	IEX Capacity
CATION EXCHANGE:				
GigaCap S-650M	 $\text{O-R}'\text{-CH}_2\text{-SO}_3$	75 μm	150g hIgG/L	0.15 eq/L
GigaCap S-650S	 $\text{O-R}'\text{-CH}_2\text{-SO}_3$	35 μm	145g hIgG/L	0.24 eq/L
GigaCap CM-650M	 $\text{O-R}'\text{-CH}_2\text{-COO}^-$	75 μm	100g hIgG/L	0.225 eq/L
ANION EXCHANGE				
GigaCap Q-650M	 $\text{O-R}'\text{-CH}_2\text{-N}^+(\text{CH}_3)_3$	75 μm	175 g BSA/L	0.15 eq/L
GigaCap Q-650S	 $\text{O-R}'\text{-CH}_2\text{-N}^+(\text{CH}_3)_3$	35 μm	190 g BSA/L	0.20 eq/L
GigaCap DEAE-650M	 $\text{O-CH}_2\text{-CH}_2\text{-NH}^+(\text{C}_2\text{H}_5)_2$	75 μm	170 g BSA/L	0.23 eq/L

Table 3

ORDERING INFORMATION

TOYOPEARL GigaCap®

Cation Exchange Resins

Part-No.	Description	Resin volume
0021833	TOYOPEARL GigaCap® S-650M	100 mL
0021834	TOYOPEARL GigaCap® S-650M	250 mL
0021835	TOYOPEARL GigaCap® S-650M	1 L
0021836	TOYOPEARL GigaCap® S-650M	5 L
0021837	TOYOPEARL GigaCap® S-650M	50 L
0021868	ToyoScreen GigaCap S-650M	1 mL x 6 ea.
0021869	ToyoScreen GigaCap S-650M	5 mL x 6 ea.
0045001	ToyoScreen RoboColumn S-650M	200µl x 8 ea.
0045002	ToyoScreen RoboColumn S-650M	600µl x 8 ea.

0022875	TOYOPEARL GigaCap® S-650S	25 mL
0022876	TOYOPEARL GigaCap® S-650S	250mL
0022877	TOYOPEARL GigaCap® S-650S	1 L
0022878	TOYOPEARL GigaCap® S-650S	5 L
0022879	TOYOPEARL GigaCap® S-650S	50 L

0021946	TOYOPEARL GigaCap® CM-650M	100 mL
0021947	TOYOPEARL GigaCap® CM-650M	250 mL
0021948	TOYOPEARL GigaCap® CM-650M	1 L
0021949	TOYOPEARL GigaCap® CM-650M	5 L
0021950	TOYOPEARL GigaCap® CM-650M	50 L
0021951	ToyoScreen GigaCap CM-650M	1 mL x 6 ea.
0021952	ToyoScreen GigaCap CM-650M	5 mL x 6 ea.
0045005	ToyoScreen RoboColumn CM-650M	200µl x 8 ea.
0045006	ToyoScreen RoboColumn CM-650M	600µl x 8 ea.

Anion Exchange Resins

Part-No.	Description	Resin volume
0021854	TOYOPEARL GigaCap® Q-650M	100 mL
0021855	TOYOPEARL GigaCap® Q-650M	250 mL
0021856	TOYOPEARL GigaCap® Q-650M	1 L
0021857	TOYOPEARL GigaCap® Q-650M	5 L
0021858	TOYOPEARL GigaCap® Q-650M	50 L
0021859	ToyoScreen GigaCap Q-650M	1 mL x 6 ea.
0021860	ToyoScreen GigaCap Q-650M	5 mL x 6 ea.
0045003	ToyoScreen RoboColumn Q-650M	200µl x 8 ea.
0045004	ToyoScreen RoboColumn Q-650M	600µl x 8 ea.

0022881	TOYOPEARL GigaCap® Q-650S	25 mL
0022882	TOYOPEARL GigaCap® Q-650S	250 mL
0022883	TOYOPEARL GigaCap® Q-650S	1 L
0022884	TOYOPEARL GigaCap® Q-650S	5 L
0022885	TOYOPEARL GigaCap® Q-650S	50 L

0022865	TOYOPEARL GigaCap® DEAE-650M	100 mL
0022866	TOYOPEARL GigaCap® DEAE-650M	250 mL
0022867	TOYOPEARL GigaCap® DEAE-650M	1 L
0022868	TOYOPEARL GigaCap® DEAE-650M	5 L
0022869	TOYOPEARL GigaCap® DEAE-650M	50 L
0022872	ToyoScreen GigaCap® DEAE-650M	1 mL x 6 ea.
0022873	ToyoScreen GigaCap® DEAE-650M	5 mL x 6 ea.
0045007	ToyoScreen RoboColumn DEAE-650M	200µl x 8 ea.
0045008	ToyoScreen RoboColumn DEAE-650M	600µl x 8 ea.