Novel High Binding Capacity Ion Exchange Resins for Biochromatography

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Introduction

Ion exchange chromatography (IEC) is often used as a capture or intermediate step in the purification of biopharmaceutical proteins, including monoclonal antibodies. Method development and production groups at biopharmaceutical and contract manufacturing organizations are constantly faced with the task of reducing unit operation costs. Increased throughput using novel IEC resins with increased binding capacity and better mechanical stability are an ideal strategy to reduce these production costs.

Recently we developed two novel high binding capacity IEC resins; TOYOPEARL GigaCap® S-650M and TOYOPEARL GigaCap® Q-650M. The TOYOPEARL GigaCap® family of resins are based on methacrylic polymers designed for protein purification from bench-top to manufacturing scale. These resins are designed to exhibit high binding capacity and superior rigidity characteristic of all TOYOPEARL® resins.

In this poster, we report the physical properties of these two new IEC resins, including their pore characteristics, protein binding capacity, protein selectivity, pressure-flow trends and chemical stability in comparison to several commercially available IEC resins.

Experimental

SP Sepharose® Fast Flow, SP Sepharose® XL, Capto® S, Q Sepharose® XL and Capto® Q were purchased from GE Healthcare (Sweden). Fractogel EMD SE Hicap and Fractogel EMD TMAE Hicap was from Merck KGaA (Germany). TOYOPEARL SP-650C, TOYOPEARL SP-550C TOYOPEARL QAE-550C, TOYOPEARL SuperQ-650M and TOYOPEARL SuperQ-650C were from Tosoh Corporation (Japan).

Polyclonal human IgG was purchased from Baxter Chemicals (USA). Bovine serum albumin (BSA) was purchased from Wako chemicals (Japan). Human IgG (Product name; "KAKETSUKEN" Gamma-Globulin) was from Kaketsuken (Japan). Lysozyme, ribonuclease A, cytocrome C, thyroglobulin, trypsin inhibitor from hen egg, transferrin and ovalbumin, were from Sigma chemicals (USA).

Instrumentation

Pump: DP-8020 (Tosoh)
Detector: UV-8020 (Tosoh)
Sample injector: Model 7520 (Rheodyne)
Data processing: LC-8020 or SC-8020 (Tosoh)

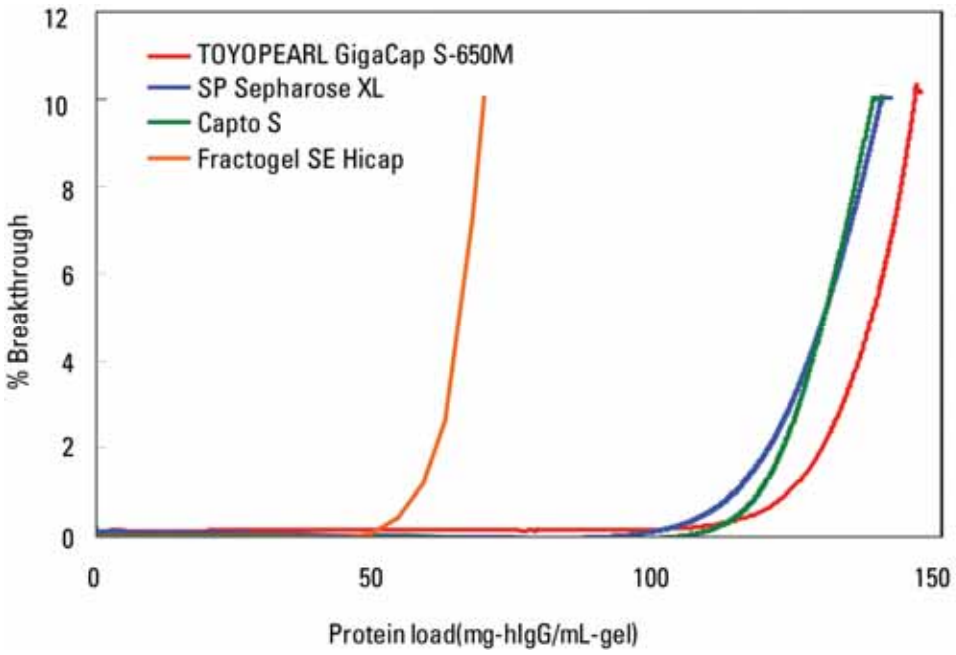
Table 1 Comparison of dynamic binding capacity for polyclonal human IgG

Ion exchanger	Particle size (µm)	Ion exchange capacity (meq/mL resin)	Dynamic Binding capacity (mg/mL -gel)	Recovery (%)
TOYOPEARL GigaCap® S-650M	50 - 100	0.16	145	98
TOYOPEARL SP650C	50 - 150	0.12	12	98
TOYOPEARL SP550C	50 - 150	0.13	14	98
SP Sepharose XL	50 - 150	0.17	140	98
Capto S	90(median)	0.13	138	96
Fractogel SE HiCap	40 - 90	0.08	68	97

Conditions;
Dynamic binding capacities were determined at 10% breakthrough. Column size: 6 mm I.D. x 40mm height
Sample: polyclonal human IgG (1 mg/mL), Loading buffer 0.1mol/L acetate buffer (pH 4.7)
Elution buffer: 0.1mol/L acetate buffer (pH 4.7) + 1.0mol/L NaCl, Linear velocity: 212 cm/hr. Detection: UV @ 280 nm

The DBC and recovery of polyclonal hIgG samples as well as the physical properties of various cation exchange resins are summarized in Table I.

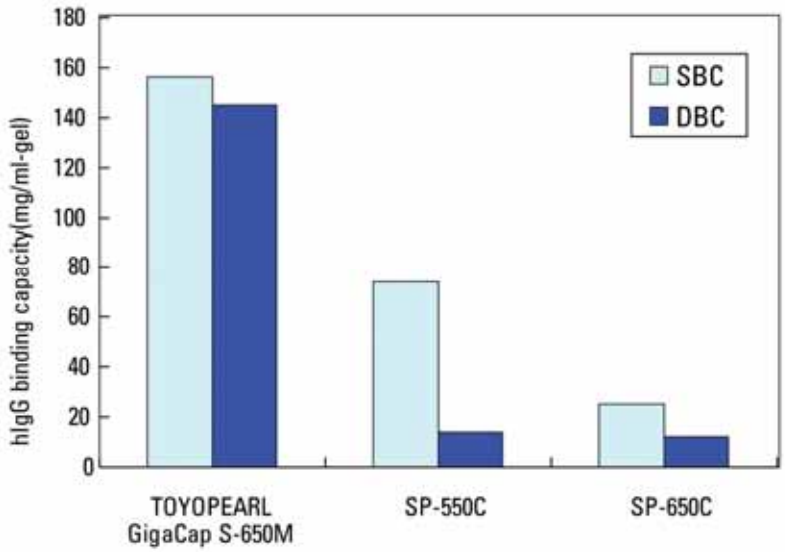
Figure 1. Breakthrough curves of polyclonal human IgG



Column size:	6 mm I.D. x 40mm height
Sample:	polyclonal human IgG (1 mg/mL)
Loading buffer:	0.1mol/L acetate buffer (pH 4.7)
Elution buffer:	0.1mol/L acetatebuffer (pH 4.7) +1.0mol/L NaCl, Linear velocity: 212 cm/hr,
Detection:	UV @ 280 nm

TOYOPEARL GigaCap S-650M has the highest dynamic binding capacity at more than 140 mg/mL-gel and also exhibits a superior shape to the breakthrough curve, as shown in Figure 1.

Figure 2. Comparison between static and dynamic binding capacities for polyclonal human IgG



Column size:	6 mm I.D. x 40mm height
Sample:	polyclonal human IgG (1 mg/mL)
Loading buffer:	0.1mol/L acetate buffer (pH 4.7)
Elution buffer:	0.1mol/L acetatebuffer (pH 4.7) +1.0mol/L NaCl, Linear velocity: 212 cm/hr,
Detection:	UV @ 280 nm
SBC conditions;	
Adsorption buffer:	54mmol/L acetate buffer (pH4.7)
Protein sample:	polyclonal human IgG
Protein concentration:	5mg/mL
Total protein:	270mg/mL-gel
Adsorption time:	3hr, Adsorption temperature : 25°C

In Figure 2, the DBCs of our commercially available SP resins are significantly reduced in comparison to the measured static binding capacities (SBCs). Conversely, there is only a small difference between the DBC and SBC using TOYOPEARL GigaCap S-650M.

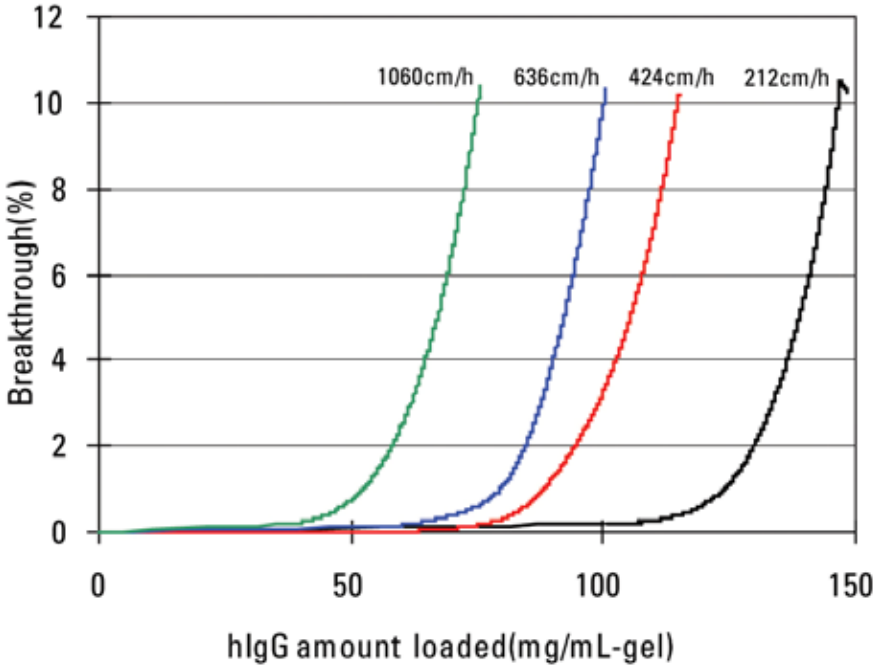
Table 2 DBCs for polyclonal human IgG at various linear velocity

	DBC(mg-hIgG/mL-gel)		
Linear velocity(cm/h)	212	636	1060
TOYOPEARL GigaCap® S-650M	145	99	75
TOYOPEARL SP-650C	12	5	3
TOYOPEARL SP-550C	14	5	3
Capto S	138	101	N.D.
SP Sepharose FF	21	8	5
Fractogel SE Hicap	69	39	27

Column size:	6mm I.D. x 40mm bed height
Sample:	Polyclonal human IgG(1mg/mL)
Buffer:	0.1mol/L acetate buffer (pH 4.7)
Linear velocity:	212 , 424, 636, 1060 cm/hr
Detection:	UV@280 nm

The DBCs of polyclonal hIgG on various commercially available SP resins at various linear velocities are shown in Table 2.

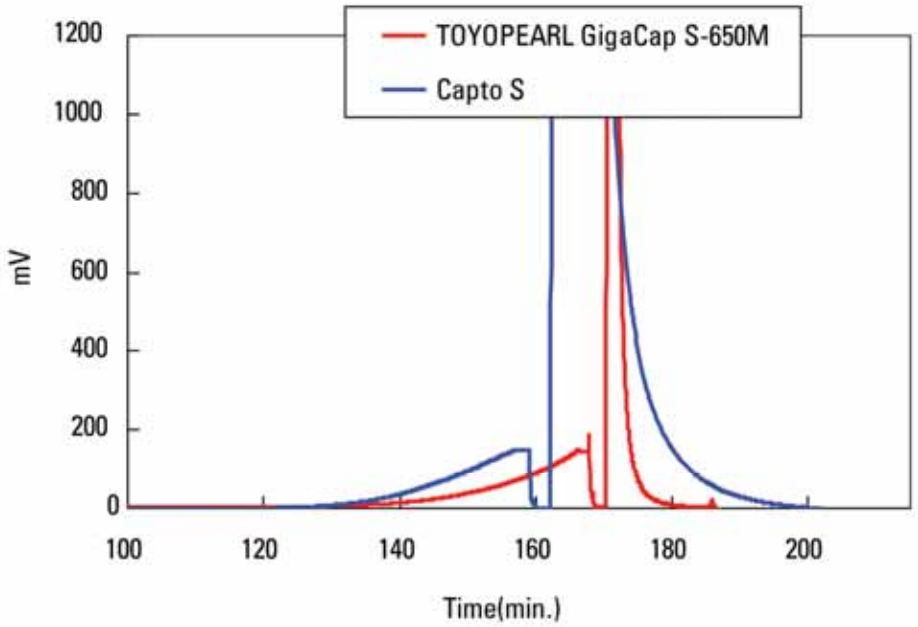
Figure 3. IgG breakthrough curves at various linear velocity



Column size:	6mm ID × 4cm bed height.
Sample:	polyclonal human IgG (1mg/mL)
Buffer:	0.1mol/L acetate buffer (pH 4.7)
Linear velocity:	212, 424, 636, 1060 cm/hr
Detection:	UV @ 280nm

The breakthrough curves are shown in Figure 3.

Figure 4. Desorption profiles of polyclonal human IgG



Column size:	6 mm I.D. x 40mm height
Sample:	polyclonal human IgG (1mg/mL)
Loading buffer:	0.1mol/L acetate buffer (pH 4.7)
Loading linear velocity:	212 cm/hr
Elution buffer:	0.1mol/L acetate buffer (pH 4.7) +1.0mol/L NaCl
Elution linear velocity:	424 cm/hr
Detection:	UV @ 280 nm

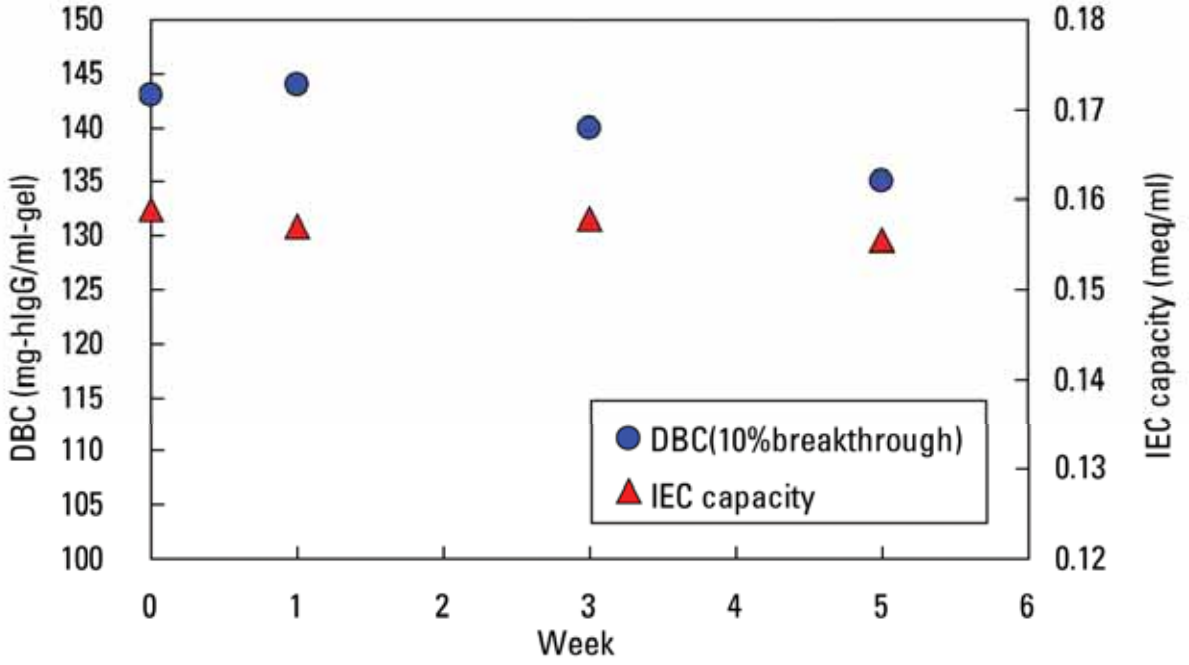
The desorption characteristics are illustrated in Figure 4.

Table 3 Elution volumes of desorption fractions

	Elution Volume (mL)	Recovery (%)
TOYOPEARL GigaCap® S-650M	8	96
Capto S	38	96

The elution of the polyclonal hIgG fraction from TOYOPEARL GigaCap S-650M was collected approximately 5 times faster than that of other commercially available resin (Table 3).

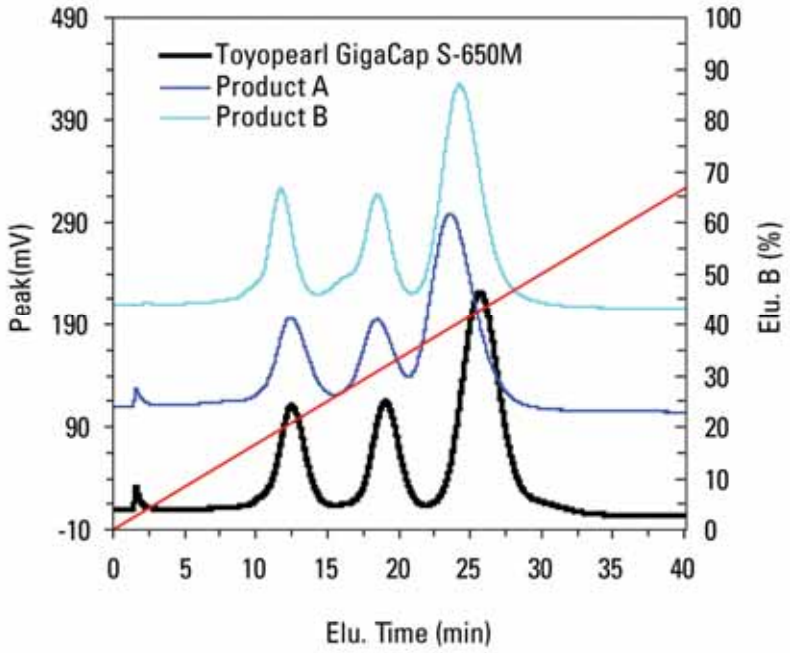
Figure 5. Alkaline stability of TOYOPEARL GigaCap S-650M



TOYOPEARL GigaCap S-650M was stored in 1.0mol/L NaOH solution at room temperature, DBC was measured at 10% height breakthrough of polyclonal human IgG.

Figure 5 shows the alkaline stability of TOYOPEARL GigaCap S-650M. The DBC and ion exchange capacity decreased only 5% and <1%, respectively, after a 5 week exposure to 1.0mol/L NaOH.

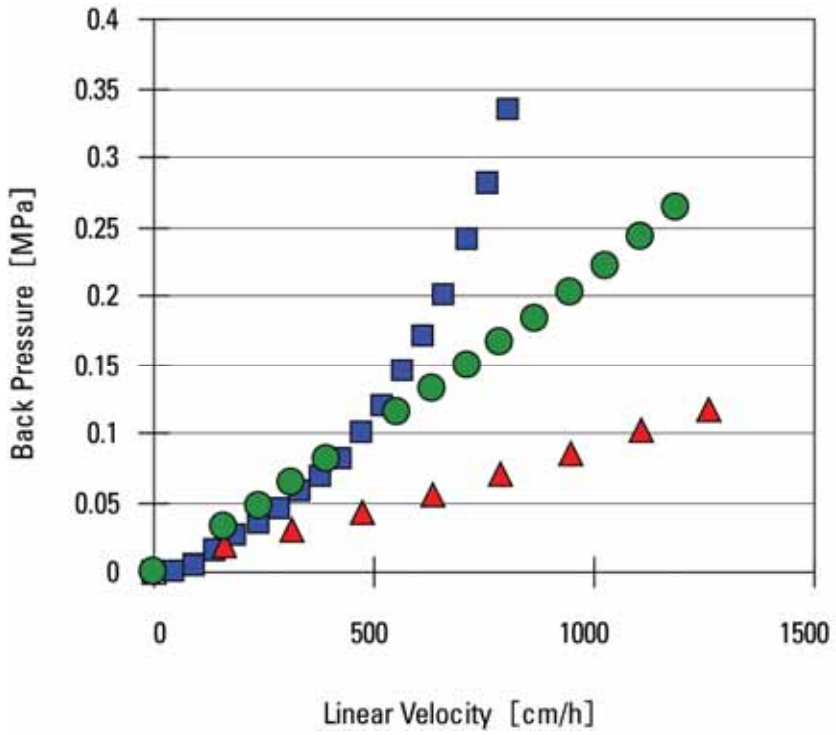
Figure 6. Comparison of protein selectivity



Column:	7.5mm I.D. × 7.5cm
Flow rate:	1.0mL/min
Sample:	ribonuclease A (9.9mg/mL), cytochrome C (3.5mg/mL) and lysozyme (6.6mg/mL)
Inj. Vol.:	25mL
Eluent:	A : 20mmol/L phosphate (pH 7.0)
	B : 20mmol/L phosphate + 1.0mol/L NaCl (pH 7.0)
Gradient:	60-min linear gradient from buffer A to buffer B
Detection:	UV @ (280nm)

The elution profiles of a standard protein mixture on various SP resins are illustrated in Figure 6.

Figure 7. Pressure-flow curves



Column size; 22 mm ID × 20 cm
Mobile phase; distilled water
Temp.; 25°C
▲: Toyopearl GigaCap S-650M
●: Toyopearl SP-650M
■: SP Sepharose XL

The pressure-flow characteristics of the tested resins are shown in Figure 7. TOYOPEARL GigaCap S-650M has excellent pressure-flow durability over a wide range of linear velocities when compared to the other resins.

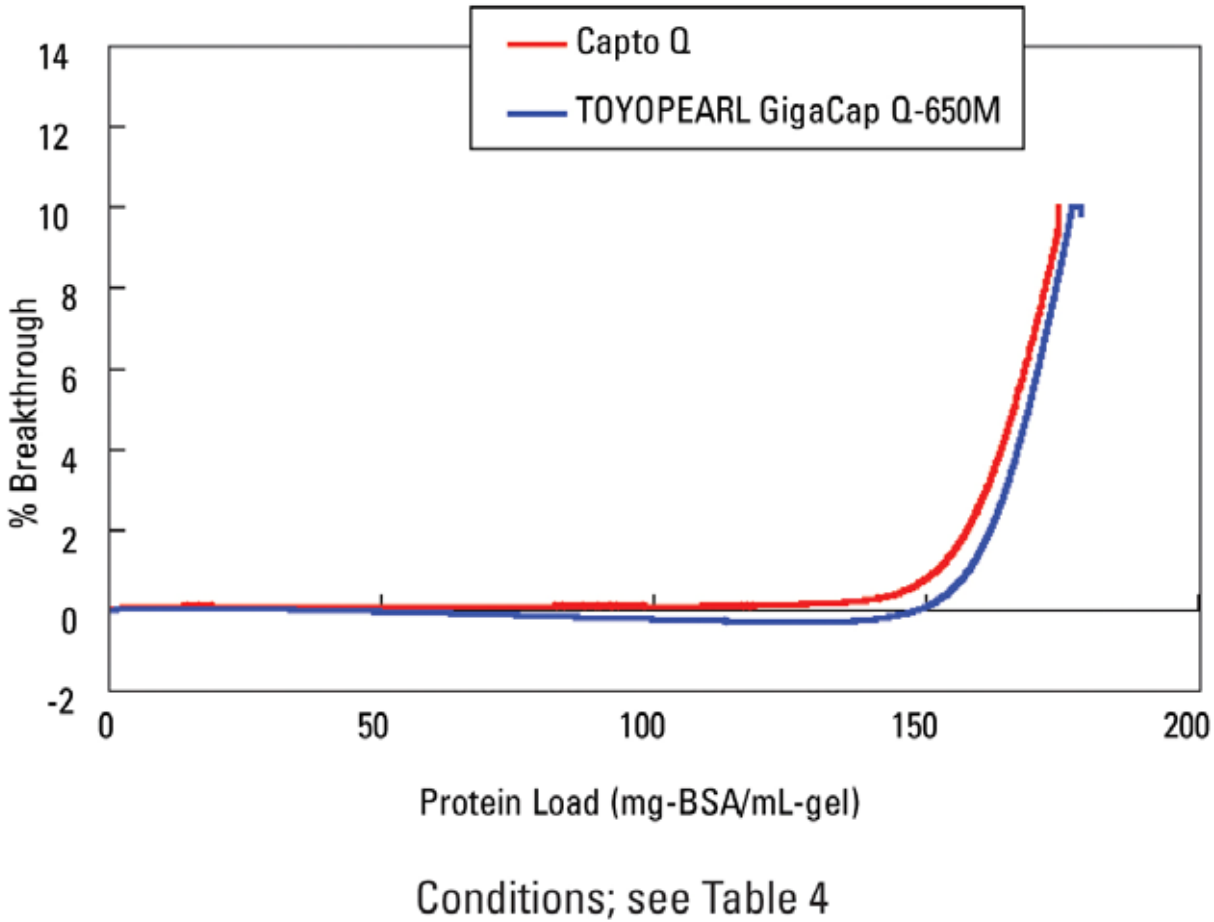
Table 4 Comparison of dynamic binding capacity for bovine serum albumin

Ion exchanger	Particle size (μm)	Ion exchange capacity (meq/mL resin)	Dynamic Binding capacity (mg/mL -gel)	Recovery (%)
TOYOPEARL GigaCap® Q-650M	50 - 100	0.14	175	97
TOYOPEARL® QAE-550C	50 - 150	0.32	18	96
TOYOPEARL® SuperQ-650M	40 - 90	0.24	145	98
TOYOPEARL® SuperQ-650C	50 - 150	0.23	123	97
Capto Q	90(median)	0.18	166	98
Fractogel EMD TMAE HiCap	40 - 90	0.08	138	98

Conditions;
Dynamic binding capacities were determined at 10% breakthrough. Column size: 6 mm I.D. x 40mm height
Sample: polyclonal human IgG (1 mg/mL), Loading buffer 0.1mol/L acetate buffer (pH 4.7)
Elution buffer: 0.1mol/L acetate buffer (pH 4.7) + 1.0mol/L NaCl, Linear velocity: 212 cm/hr. Detection: UV @ 280 nm

The DBC and recovery of BSA samples as well as the physical properties of various anion exchange resins are summarized in Table 4.

Figure 8. Breakthrough curves of bovine serum albumin on anion exchange resins



Conditions; see Table 4

TOYOPEARL GigaCap Q-650M has the highest dynamic binding capacity at more than 170 mg/mL-gel and also exhibits a superior shape to the breakthrough curve, as shown in Figure 8.

Table 5 Effect of protein size on binding capacity

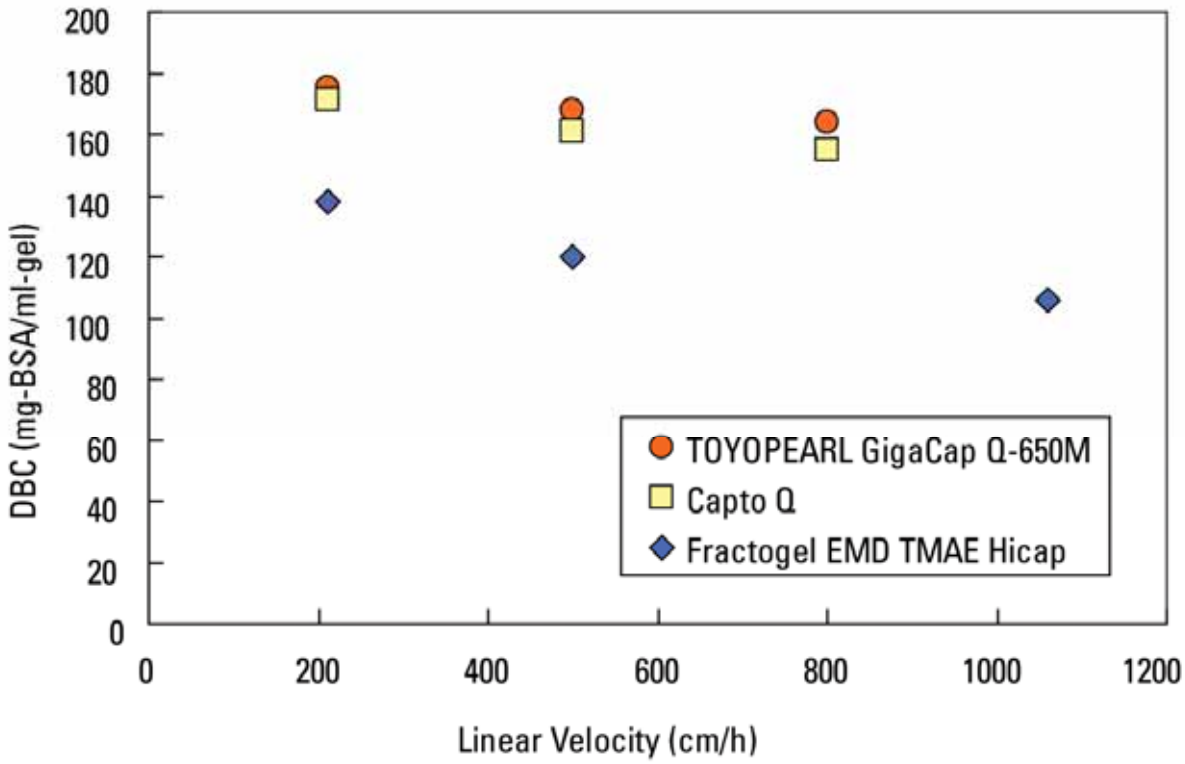
Resin		Binding capacity (mg/mL-gel)		
		BSA 66kDa	human IgG 160kDa	Thyroglobulin 660kDa
TOYOPEARL GigaCap Q-650M	SBC	187	139	94
	DBC	173	108	46
Capto Q	SBC	183	128	71
	DBC	168	95	7

SBC conditions ;
(1) BSA : adsorption buffer 50mmol/L Tris-HCl buffer (pH8.5)
Protein concentration : 10mg/ml, Total protein : 250 mg
(2) human IgG : adsorption buffer 15mmol/L Tris-HCl buffer (pH8.7), Protein concentration : 4.7mg/ml
Total protein : 240 mg
(3) thyroglobulin : adsorption buffer 15mmol/L Tris-HCl buffer (pH8.7) +0.1mol/L NaCl, Protein concentration : 4.7mg/ml
Total protein : 240 mg
Adsorption time : 3hr, Temperature : 25°C

DBC conditions ;
Column size: 6 mm I.D. x 40mm height,
Protein sample 1 mg/mL, Linear velocity: 212 cm/hr
Detection: UV @ 280 nm
(1) BSA : see Table 4
(2) human IgG : Loading buffer 15mmol/L Tris-HCl (pH8.7)
(3) thyroglobulin : Loading buffer 15mmol/L Tris-HCl (pH8.7) + 0.1mol/L NaCl

The effect of protein size on the binding capacity is summarized in Table 5. As the size of the protein increases, the binding capacity on both commercially available resins were gradually reduced. However, the newly developed TOYOPEARL GigaCap Q-650M had very high dynamic binding capacity for all proteins including the very large thyroglobulin.

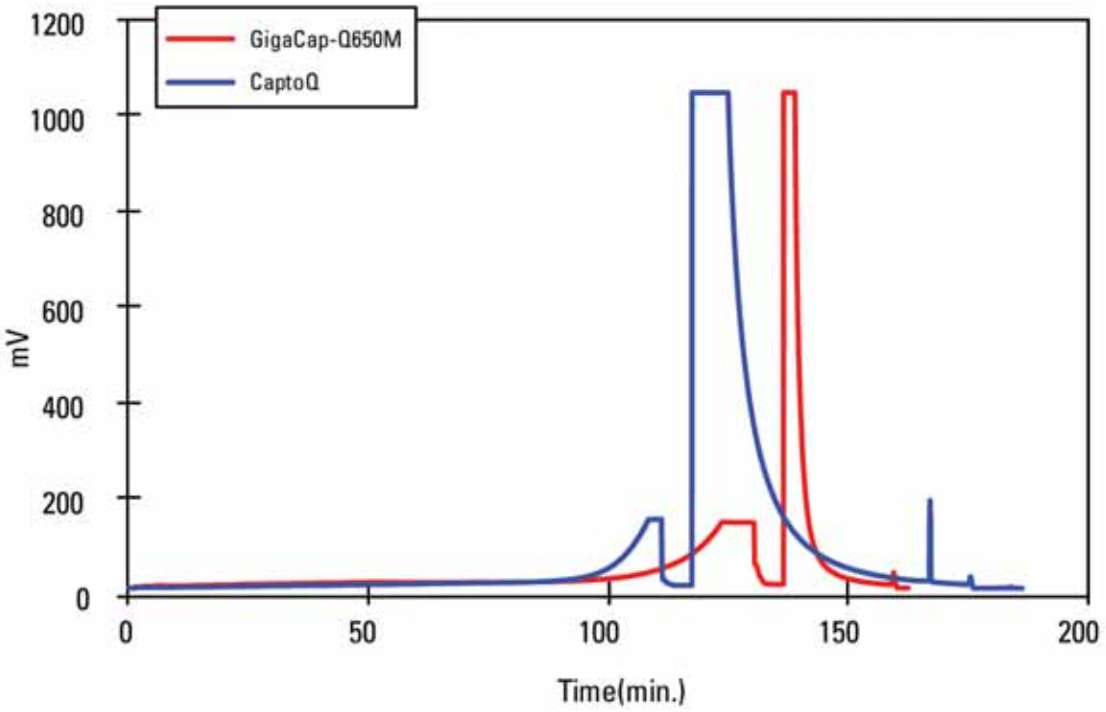
Figure 9. Dynamic binding capacities for BSA at various linear velocities



Conditions; see Table 4
Linear velocity: 212 , 424, 636, 1060 cm/hr

The DBCs of BSA on various commercially available Q resins at various linear velocities are shown in Figure 9.

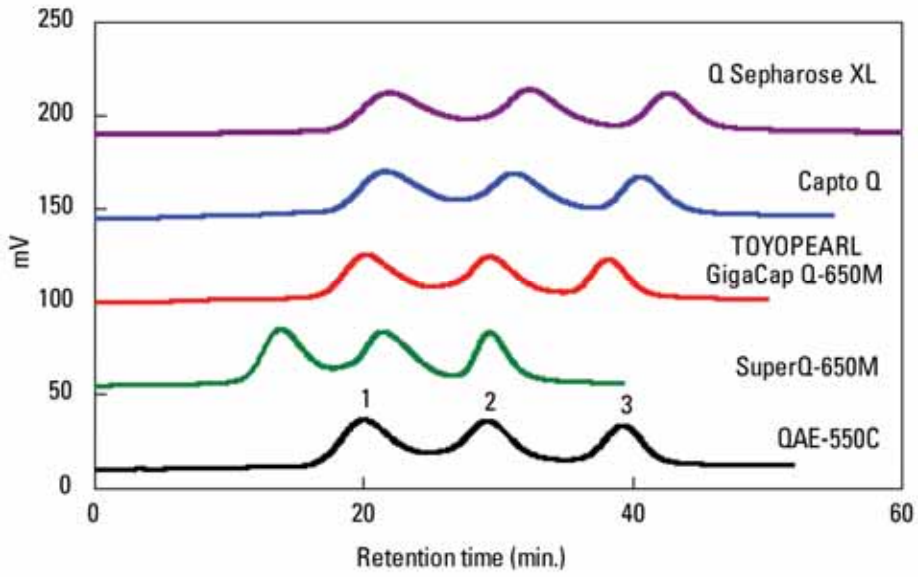
Figure 10. Desorption profiles of human IgG



Loading :	see Table 5
Elution buffer :	15mmol/L Tris-HCl buffer (pH8.7) + 1.0mol/L NaCl
Elution linear velocity :	424 cm/hr

The desorption characteristics are illustrated in Figure 10. The elution of human IgG fractions from TOYOPEARL GigaCap Q-650M was collected approximately 4 times faster than that of other commercially available resin.

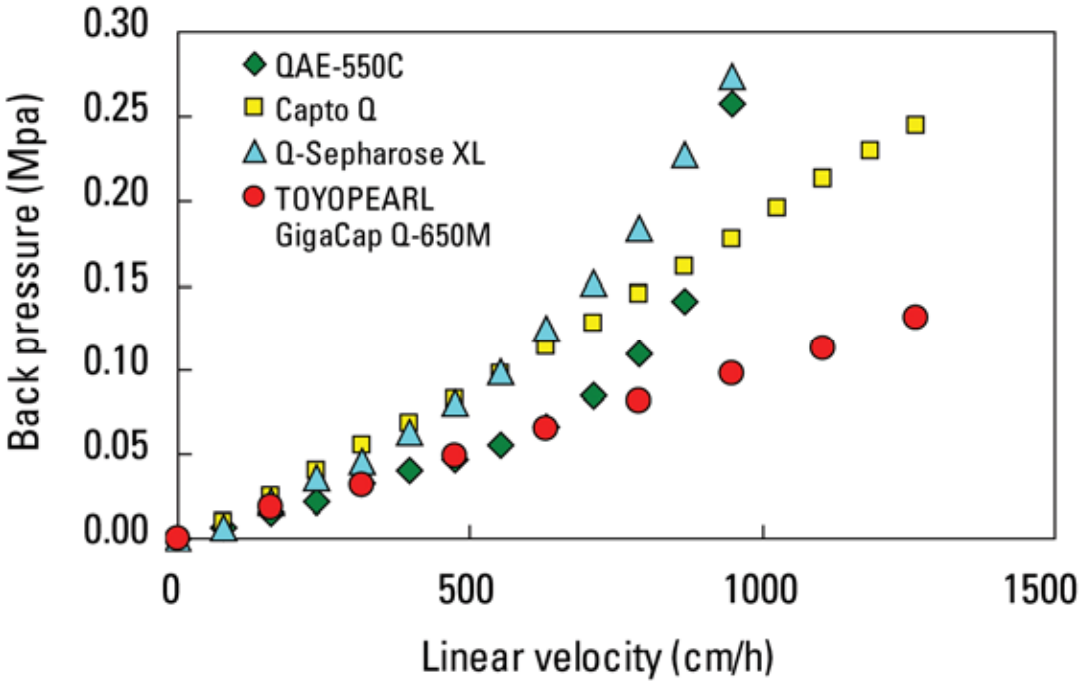
Figure 11. Comparison of protein selectivity



Column size:	7.5mm I.D. x 75mm
Flow rate:	1.0mL/min
Sample:	1. transferrin (1.0mg/mL) 2. ovalbumin (1.5mg/mL) 3. trypsin inhibitor (1.8mg/mL)
Injection volume:	25µL
Buffer A:	50mmol/L Tris-HCl (pH 8.7)
Buffer B:	50mmol/L Tris-HCl + 1.0mol/L NaCl (pH 8.7)
Gradient:	60-min linear gradient from buffer A to buffer B
Detection:	UV @280nm

The elution profiles of a standard protein mixture on various Q resins are illustrated in Figure 11.

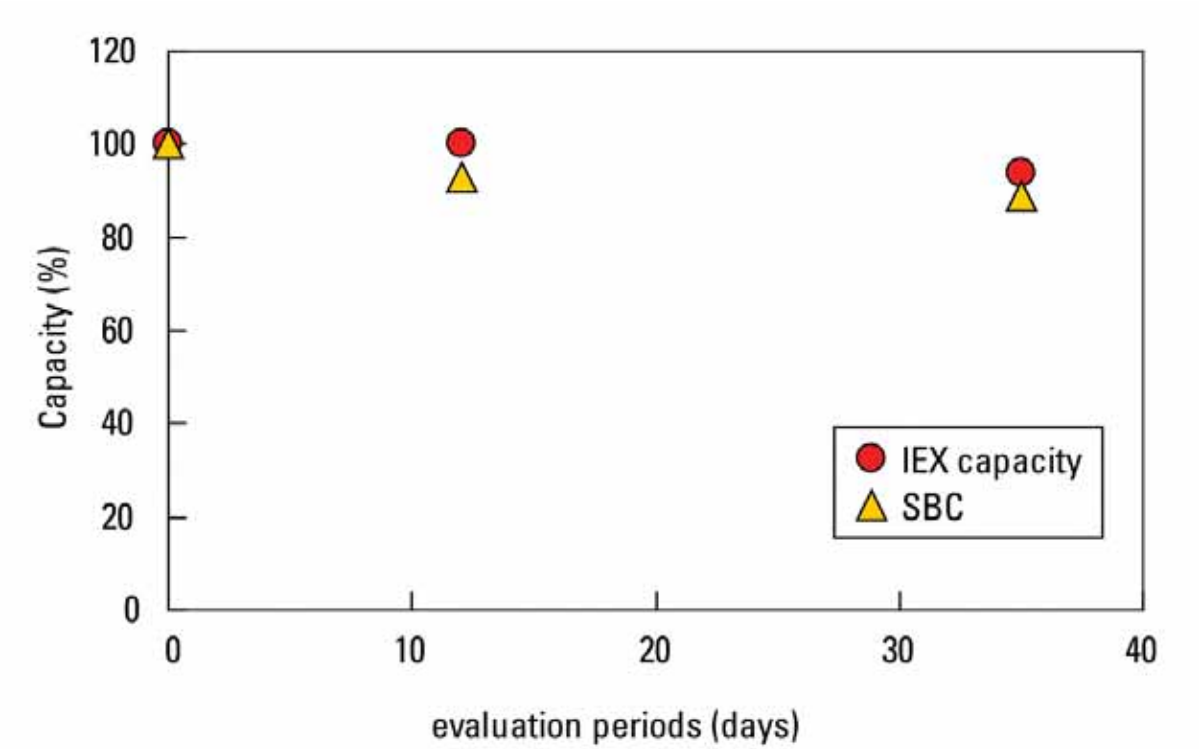
Figure 12. Pressure-flow curves



Column size: 22 mm I.D. x 20 cm,
Mobile phase: Distilled water,
Temperature: 25 °C

The pressure-flow characteristics of the tested resins are shown in Figure 12. TOYOPEARL GigaCap Q-650M has excellent pressure-flow durability over a wide range of linear velocities when compared to the other resins.

Figure 13. Alkaline stability of TOYOPEARL GigaCap Q-650M



TOYOPEARL GigaCap Q-650M was stored in 0.1mol/L NaOH solution at 25 °C, SBC condition: see Table 5, Protein: BSA

Figure 13 shows the alkaline stability of TOYOPEARL GigaCap Q-650M. The SBC decreased only 10% after a 35 day exposure to 0.1mol/L NaOH.

Conclusions

We developed two new IEC resins with high binding capacity and Excellent mechanical stability; TOYOPEARL GigaCap S-650M and TOYOPEARL GigaCap Q-650M. These resins were designed for capture or intermediate purification steps.

1: TOYOPEARL GigaCap resins have high dynamic binding capacities even at high linear velocities.

2: TOYOPEARL GigaCap resins have excellent pressure-flow characteristics.

3: TOYOPEARL GigaCap resins have high recovery of proteins in smaller elution volumes.

TOYOPEARL GigaCap resins can significantly reduce production costs by utilizing their high binding capacity and shortened purification time (better throughput).