A High Capacity Strong Cation Exchange Resin* for the Chromatographic Purification of Monoclonal Antibodies and Other Proteins

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*Specifically designed for packed bed use.

Abstract

Recent technological advances in cell culture production will result in the expression of 5 - 10 g/L of potential therapeutic protein in the near future. Downstream processing groups are challenged to keep up with the added demands. These downstream steps include chromatographic purification generally comprised of three steps; capture, intermediate purification and polishing. A typical capture step for an MAb includes the use of Protein A which results in excellent throughput (i.e. capacity and speed) and is very important in concentrating the target molecule. The second step in MAb purifications is generally ion exchange or HIC. We have developed a strong cation exchange resin in the Toyopearl® family that meets the demands of enhanced MAb production. This new resin exhibits very high binding capacities at fast flow rates. The new cation exchange resin has an impressive >140 mg/mL dynamic binding capacity for human IgG molecules at a flow rate typically achieved in normal production processes. The pKa and small ion capacities were comparable to both Toyopearl SP-650M and Toyopearl SP-550C. The pressure flow curves were similar to the existing Toyopearl SP resin of similar particle size indicating that the packing of this resin will be predictable at large scale. Additional physical and chromatographic characterization will also be included.

Introduction

Recent advances in the ability of upstream processes to manufacture biotherapeutic proteins at levels up to 10 mg/ml in the cell culture fluid have placed increasing demands on downstream unit operations. One of the potential bottlenecks in the downstream processes is the binding capacity of the chromatographic resin. If more capacity can be engineered into the resin, smaller columns, improved throughput and less buffer consumption can be utilized. Tosoh Corporation designed a new cation exchange resin that has very high binding capacity for the purification of biotherapeutic molecules to alleviate this potential bottleneck. This new resin, Toyopearl GigaCap® S-650M, has a dynamic binding capacity close to 145 mg/mL for IgG and is designed to have a long lifetime, withstand high flow rates and tolerate aggressive cleaning regimens.

Experimental

Measurement of dynamic binding capacity for proteins:

Dynamic binding capacity was measured by breakthrough experiments with chicken egg white lysozyme, α-chymotrypsin and polyclonal human IgG. The column size was either 6mmI.D. x 4cm or 3mmI.D. x 15cm (10cm for α-chymotrypsin). Buffered solutions (lysozyme: 50mmol/L glycine buffer, pH 9.0, IgG; 100mmol/L sodium acetate buffer, pH 4.7 and 50mmol/L sodium acetate for α-chymotrypsin), each at 1 mg/mL were fed to the column at various flow velocities. Breakthrough curves were monitored using UV detection at 280 nm. The dynamic binding capacity was determined at 10% breakthrough.

Chromatographic apparatus:

Most experiments were carried out an ÄKTAprime® or ÄKTAexplorer® (GE Healthcare) liquid chromatography system at ambient temperature.

Alkaline stability test.

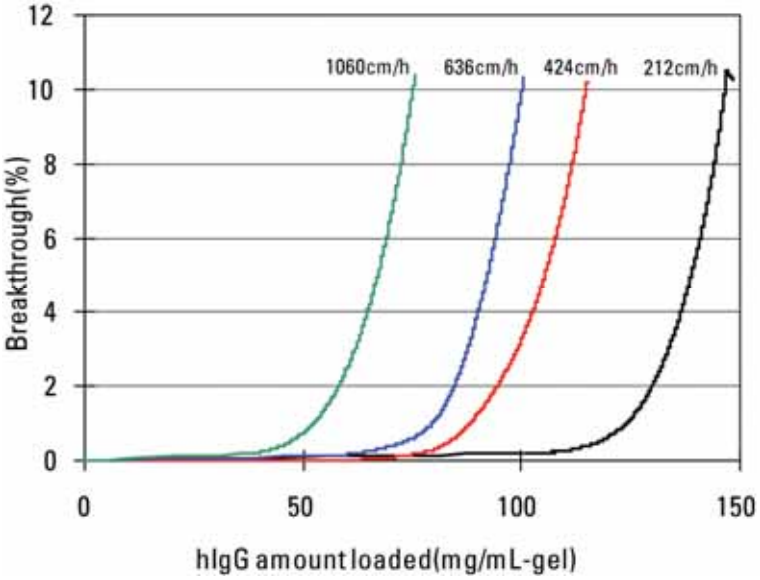
The stability of the experimental resin was tested in either 1.0mol/L of sodium hydroxide at 25ºC. After exposure to sodium hydroxide, dynamic binding capacity for lysozyme was measured.

Table 1: Typical Properties of Toyopearl GigaCap S-650M

	Toyopearl SP-650M	Toyopearl SP-550C	Toyopearl GigaCap S-650M
Particle size (µm)	40 - 90	50 - 150	50 - 100
Ion exchange capacity (meq/mL resin)	0.13 - 0.17	0.14 - 0.18	0.1 - 0.2
Binding capacity (mg/mL -gel)			
lysozyme @ 212cm/hr	48	81	167 (280cm/hr)
IgG @ 212cm/hr	43	14	145

Typical Properties of Toyopearl GigaCap S-650M were compared to existing strong cation exchange resins provided through Tosoh Bioscience. Toyopearl GigaCap S-650M is synthesized on a 1000 Angstrom polymethacryate base with nominal particle size of 75µm. The resin is designed to have high capacities for both large (IgG) and small (lysozyme) proteins.

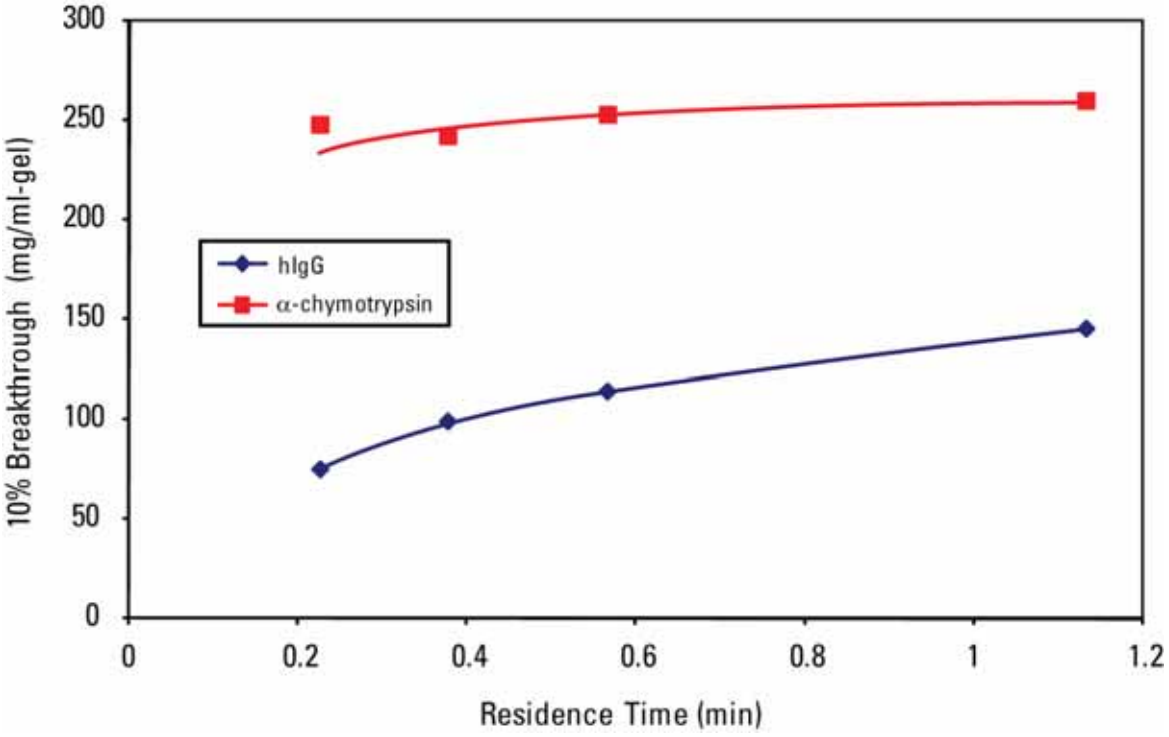
Figure 1: Human IgG DBC at Various Flow Rates



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

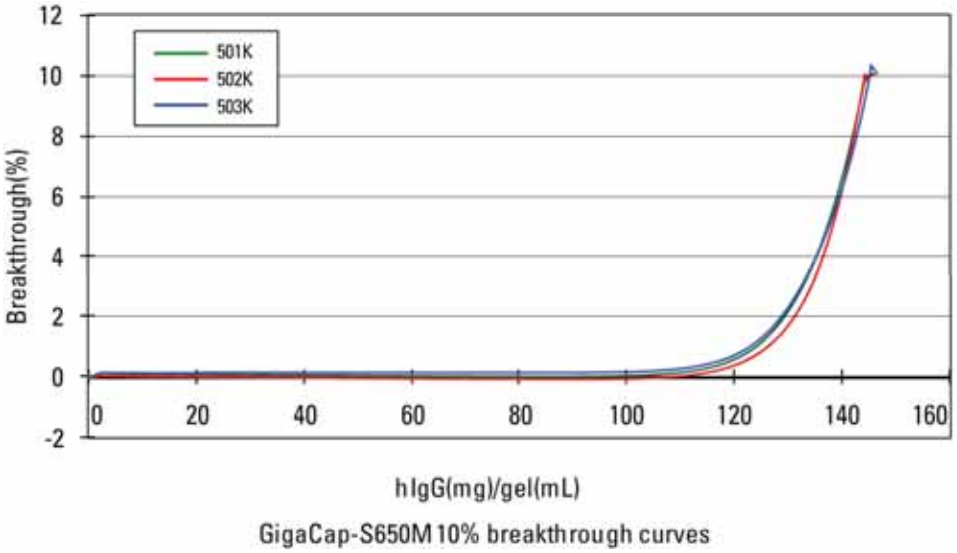
The dynamic binding capacity at 10% breakthrough of Toyopearl GigaCap S-650M was determined using a human polyclonal IgG sample at 1mg/mL. At extremely fast flow rates of 1060 cm/hr the new resin had a 75 mg/mL-resin binding capacity whereas at a more reasonable 212 cm/hr the binding capacity was an impressive 145 mg/mL-resin. Recoveries were essentially quantitative (data not shown).

Figure 2: Binding Capacity at Different Residence Times



Using the data obtained from Figure 1 as well as additional data for α-chymotrypsin, the dynamic binding capacity at 10% breakthrough was plotted vs. the residence time of the load. The larger IgG protein had excellent binding kinetics even at very short residence times. The smaller α-chymotrypsin had almost no change in capacity except at extremely short residence times.

Figure 3: Toyopearl GigaCap S-650M Breakthrough Curves



GigaCap-S650M 10% breakthrough curves

Column size:	6mm I.D.×4cm bed height.
Sample:	polyclonal human IgG (1mg/mL).
Buffer:	0.1mol/L acetate buffer (pH 4.7).
Flow velocity:	212 cm/hr.
Detection:	UV 280 nm

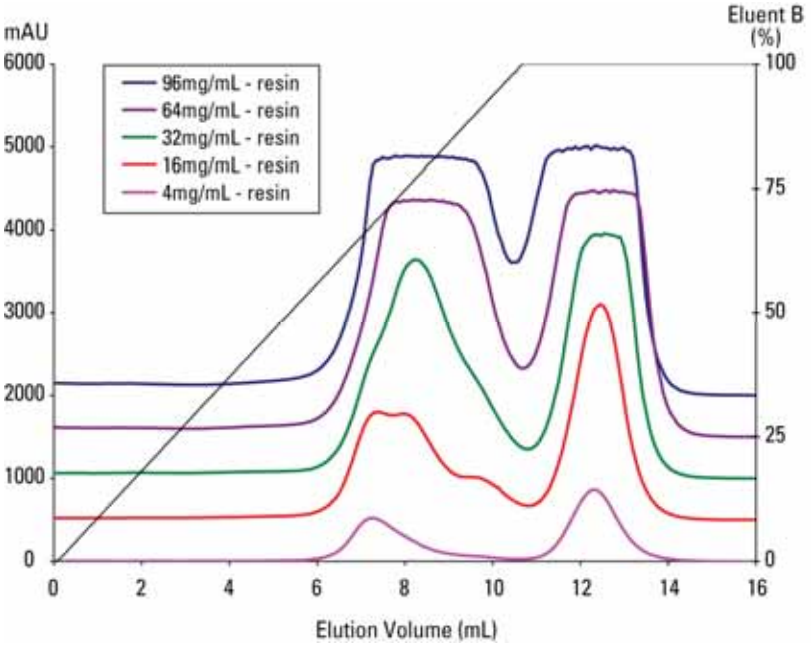
Three lots of Toyopearl GigaCap S-650M were tested for binding capacityZ reproducibility at 212 cm/hr as described in Figure 1. There was essentially no change in capacity between lots under these experimental conditions.

Table 2: Lot Reproducibility

	unit	501K	502K	503K
Ion exchange capacity	meq/mL	0.161	0.172	0.160
DBC	mg/mL-gel	145	144	145
Recovery	%	97	96	98

The results from figure 3 are summarized in this table. The ion exchange capacities of the three lots were very similar. There was no change in binding capacity nor in amount of protein recovered in each of the experiments indicating excellent lot reproducibility for the new Toyopearl GigaCap S-650M.

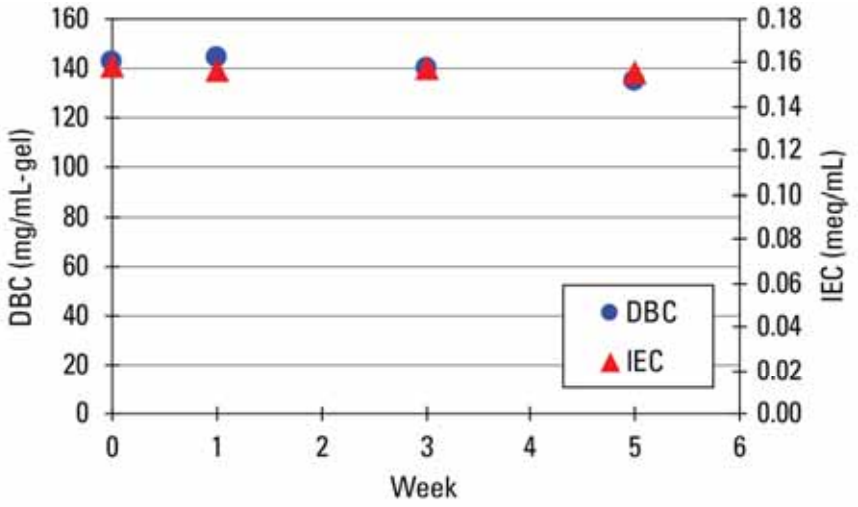
Figure 4: Good Resolution at High Loading Capacities



Columns:	3mmID x 15cm
Linear velocity:	300cm/h
Sample:	α-chymotrypsin (2mg/mL) lysozyme (2mg/mL) (total of 4mg proteins/mL)
Eluent:	A: 20mmol/L phosphate buffer (pH 6.0) B: 20mmol/L phosphate buffer + 500mmol/L NaCl (pH 6.0)
Gradient:	10 CV linear gradient from 0 to 100%B (0-500mmol/L NaCl)
Detection:	UV (280nm)

Increasing amounts of α-chymotrypsin and lysozyme were loaded onto a Toyopearl GigaCap S-650M column. Despite the large increase in binding capacities the selectivity, as shown by the resolution between the two peaks, did not suffer significantly under near saturating conditions.

Figure 5: Alkaline Stability

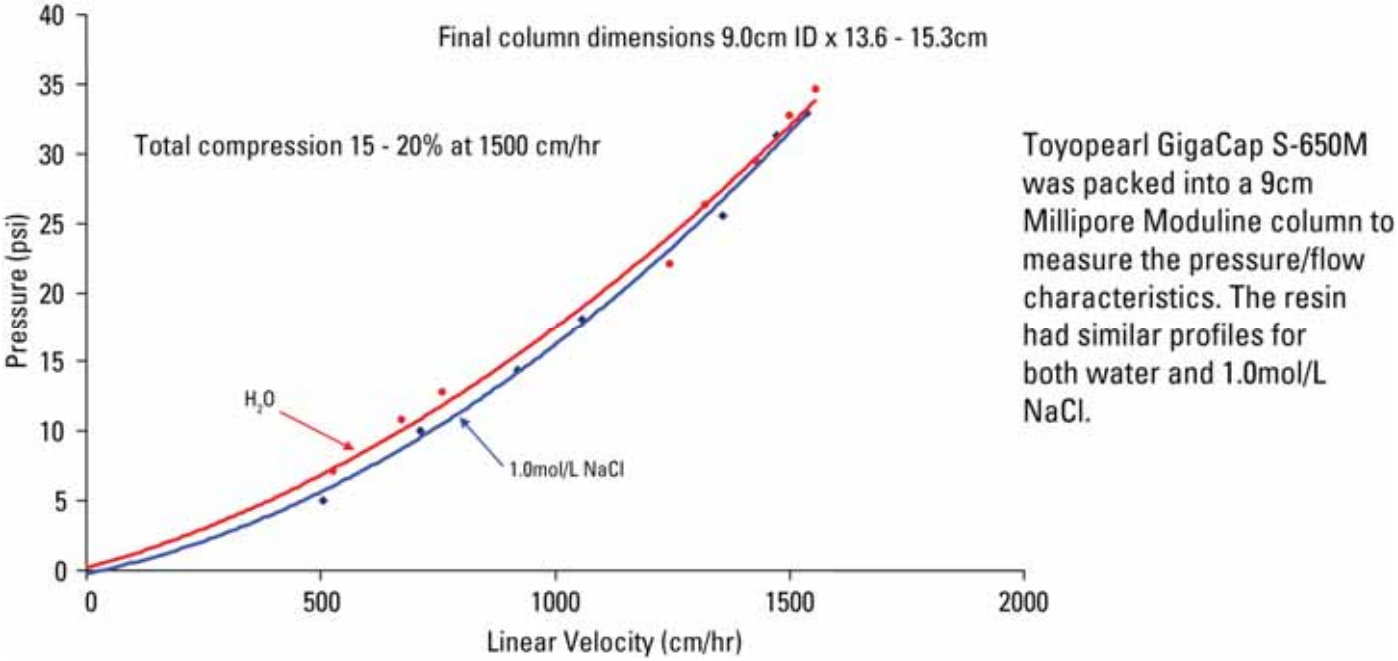


Conditions: Toyopearl GigaCap S-650M is stored in 1mol/L NaOH solution at room temperature.

Evaluation period (soaking)	week	0	1	3	5
Ion exchange capacity	meq/mL	0.15	0.15	0 .158	0.156
hIgG-DBC (10 reakthrough)	mg/mL-gel	143	144	140	135
hIgG-recovery	%	99	101	100	99

Toyopearl GigaCap S-650M was subjected to continuous 1.0mol/L NaOH exposure at room temperature. After at least five weeks time, the ion exchange capacity had little to no change. The dynamic binding capacity was essentially unchanged and the recoveries remained quantitative.

Figure 6: Pressure-Flow Characteristics of Toyopearl GigaCap S-650M



Toyopearl GigaCap S-650M was packed into a preparative column in either water or 1.0mol/L NaCl. At a typical process-scale bed height of approximately 15 cm and a 1000cm/hr linear flow rate the pressure observed over the column was less than 15psi. The compression of the resin over the course of the experiment was linear.

Conclusions

1. Through a proprietary optimization process that allows us to bind more protein onto the resin, a new Toyopearl was introduced that has very high capacities and quantitative recoveries for both large (IgG) and small (lysozyme and α-chymotrypsin) proteins.
2. The kinetics of protein binding are exceptional allowing high level loading (near saturation) at increased flow rates. Selectivity remains constant.
3. The resin is based on a 75µm particle size which provides superior pressure flow characteristics.
4. The resin is stable when subjected to 1.0mol/L NaOH at room temperature for at least 5 weeks.
5. Manufactured lots of the new resin exhibit excellent reproducibility.
6. Toyopearl GigaCap® S-650M is now commercially available through Tosoh Bioscience.