A Very High Capacity Cation Exchange Resin for Purification of Monoclonal Antibodies

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Abstract

Downstream processing, including chromatographic purification of proteins in the biopharmaceutical industry is generally comprised of three steps; capture, intermediate purification and polishing. A typical capture step 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 cation exchange resin in the Toyopearl® family that meets the demands of an MAb produced in large quantities. This new resin exhibits very high binding capacities at fast flow rates. The new cation exchange resin has an impressive >125mg/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 should be straight forward at large scale. Additional physical and chromatographic characterization will also be included.

Rationale

- The need for higher capacity resins in response to the development of more efficient and more productive upstream processes
- Cation is used most frequently after a Protein A capture step in many MAb platform technologies
- Previously we developed an SP resin that was further improved. This new experimental S resin is a further improvement of the previous version.

Experimental

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

Chromatographic apparatus: Most experiments were carried out on an ÄKTA Prime or ÄKTA Explorer (GE Healthcare) liquid chromatography system at ambient temperature.

Ion exchange chromatography: The Toyopearl SP-650M, SP-550C or experimental resin was packed in a column (6mmID x 4 cm, 3mmID x 15 cm, or 10mm ID x 17.9 cm) using standard techniques. The columns were equilibrated with starting buffer (buffer A: 20mmol/L sodium phosphate, pH 6.0). After protein loading, elution was carried out with elution buffer (buffer B: 20mmol/L sodium phosphate containing 0.5mol/L NaCl, pH 6.0). The typical elution method was a 60 min linear gradient from buffer A to buffer B.

Alkaline stability test: The stability of the experimental resin was tested in either 0.5mol/L or 1.0mol/L of sodium hydroxide at 25ºC. After exposure to sodium hydroxide, dynamic binding capacity for lysozyme was measured.

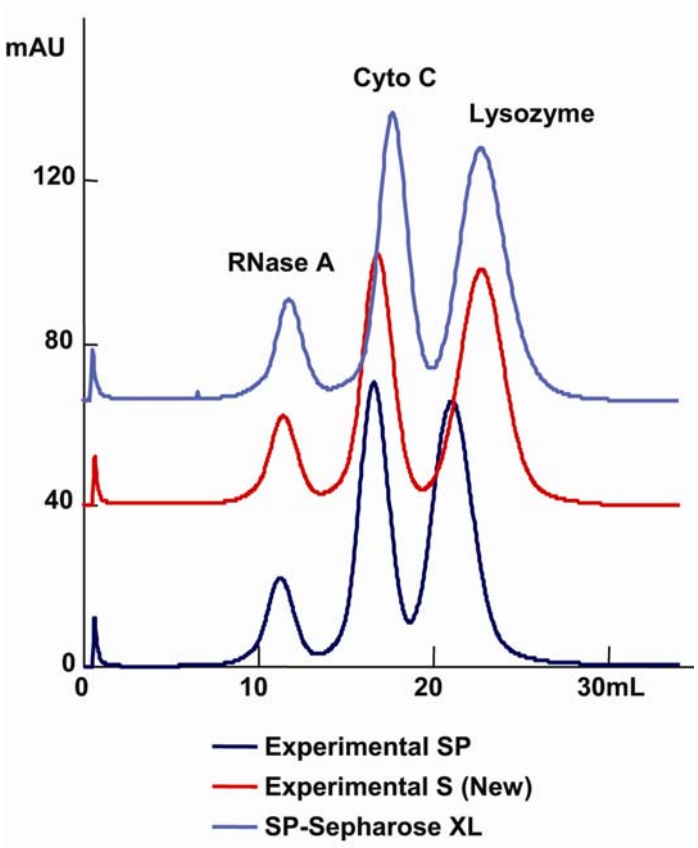
Table 1: Comparison of basic properties

	Toyopearl SP-650M	Toyopearl SP-550C	Experimental Toyopearl S (No.1)	Experimental Toyopearl S (No.2)
Particle size (µm)	40 - 90	50 - 150	50 - 100	50 - 100
Ion exchange capacity (eq/L resin)	0.13 - 0.17	0.14 - 0.18	0.16	0.17
Binding capacity (g/L -gel)				
lysozyme @ 106cm/hr*	50	111	ND	ND
lysozyme @ 212cm/hr*	48	81	167* (at 280cm/hr)	ND
lysozyme @ 424cm/hr*	40	49	ND	ND
IgG @ 212cm/hr	43	14	145	144

* different size column

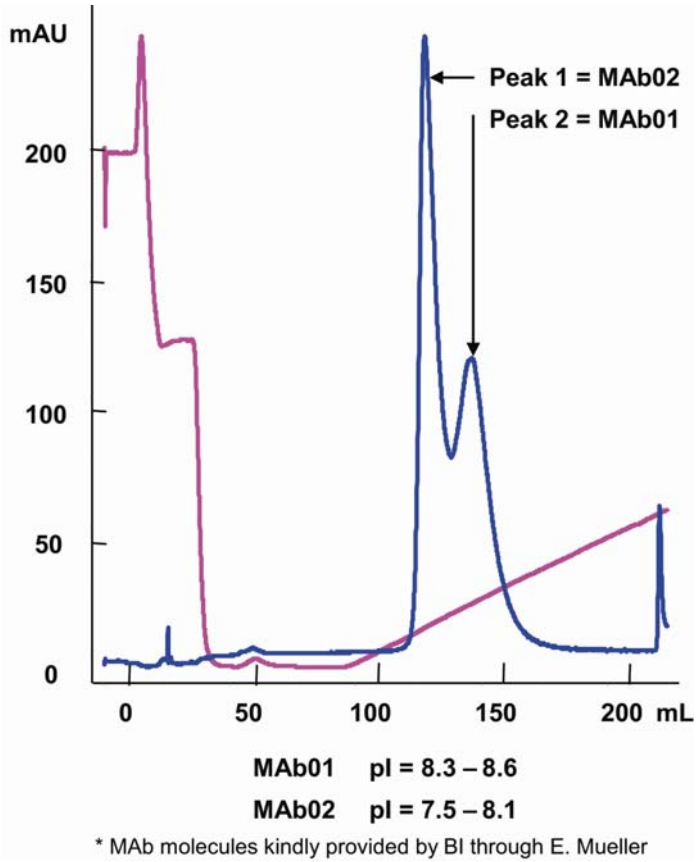
Comparison of basic properties of three different cation exchange resins. Although each resin has strong cation exchange groups (either sulphopropyl or sulphonyl) and the ion exchange capacity was similar, the dynamic binding capacity was different. The experimental resin had a much higher binding capacity for both small proteins (lysozyme) and large proteins (human IgG).

Figure 1a: Selectivity



Column size:	6mmID x 4cm bed height
Flow rate:	1.0mL/min
Sample:	ribonuclease A (10 mg/mL), cytochrome C (3.5 mg/ml) and lysozyme (6.5mg/mL)
Sample load:	100mL
Buffer A:	20mmol/L phosphate (pH 7.0)
Buffer B:	20mmol/L phosphate + 1.0mol/L NaCl (pH 7.0)
Gradient:	60min linear gradient from buffer A to buffer B
Detection:	UV (280 nm)

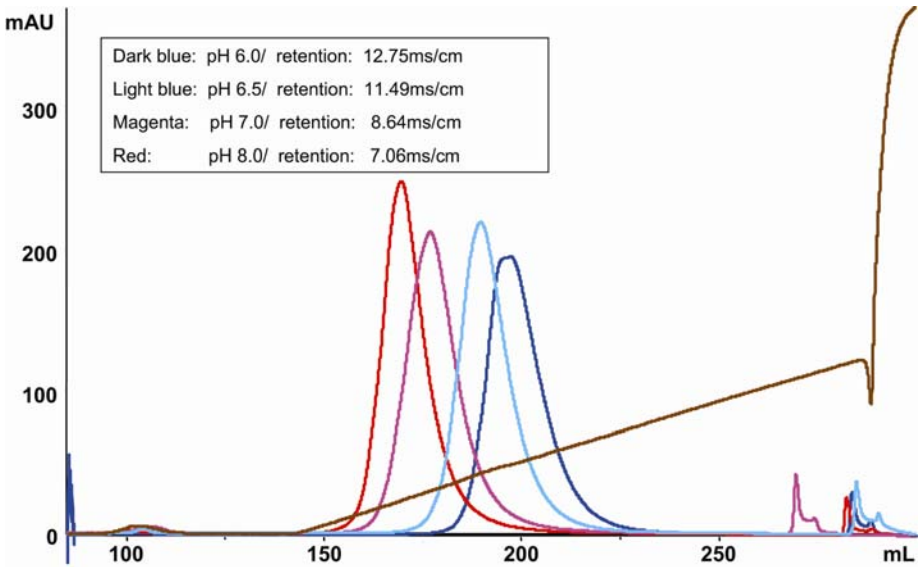
Figure 1b: Selectivity



Column Size:	10mmID x 17.9cm bed height
Flow Rate:	4mL/min (300cm/hr)
Sample:	MAb01 (3.6mg/ml) and MAb02 (4.5mg/mL) diluted 1:4 each in buffer A.
Sample load:	8mL (4mL of MAb01 and 4mL of MAb02) were loaded (8 - 11 mg total for each protein)
Buffer A:	20mmol/L phosphate (pH 6.0)
Buffer B:	20mmol/L phosphate + 0.5mol/L NaCl (pH 6.0)
Gradient:	0 - 50% B (0 - 500mmol/L NaCl) , 5CV length. Hold for 2CV at 50%B (500mmol/L NaCl).
Detection:	UV (280nm)

Selectivity on the experimental resin was tested using two methods. In the first, ribonuclease A, cytochrome C and lysozyme were separated on the experimental S resin, an experimental SP resin (PREP 2005 poster) and SP-Sepharose XL (GE Healthcare) resin. In the second, two humanized monoclonal antibodies (MAb01 and MAb02, kindly provided by Boehringer Ingelheim (through Dr. Egbert Mueller, Tosoh Bioscience GmbH) were separated on the experimental S resin at pH 6.0.

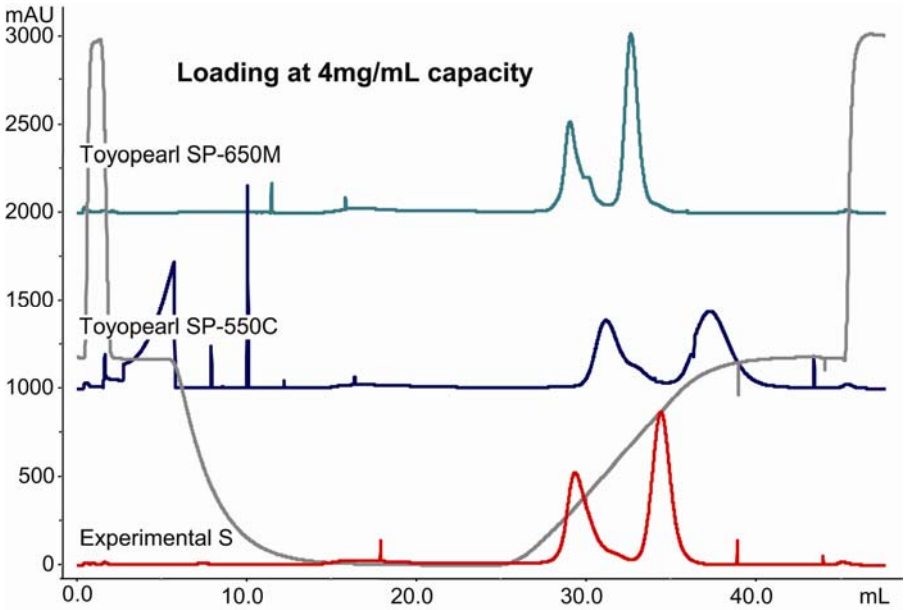
Figure 2: pH Scouting of MAb 01



Column Size:	10mmID x 17.9cm bed height
Flow Rate:	4mL/min (300cm/hr)
Sample:	MAb01
Sample Load:	5.0mL of 1.8mg/mL in buffer A
Buffer A:	20mmol/L phosphate (pH 6.0)
Buffer B:	20mmol/L phosphate + 0.5mol/L NaCl (pH 6.0)
Gradient:	0 - 50% B (0 - 250mmol/L NaCl) , 5CV length. Hold for 2CV at 50%B (250mmol/L NaCl).
Detection:	UV (280nm)

The retention of MAb01 (pI 8.3 - 8.6) was performed at various pHs. The conductivity at peak elution is listed in the figure. Not surprisingly, the monoclonal antibody exhibited more retentivity at lower pH.

Figure 3a: Capacity vs Resolution for Tosoh cation exchangers: SP-550C, SP-650M, Experimental S.



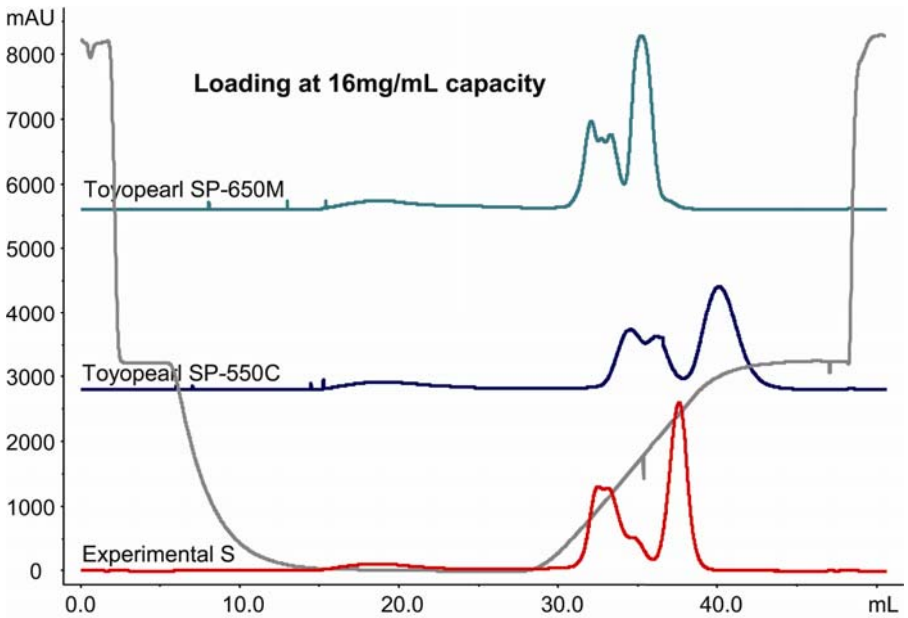
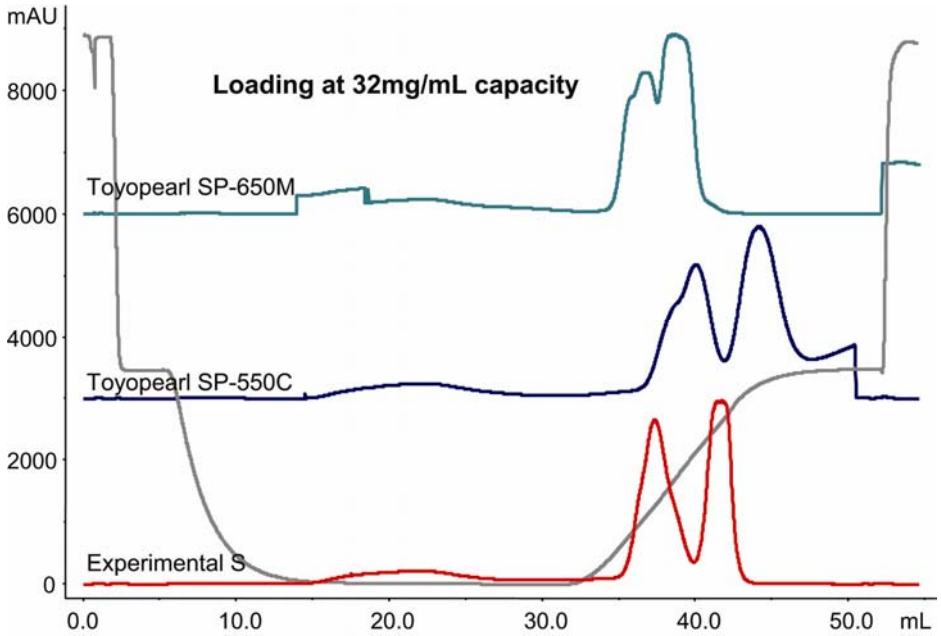


Figure 3b: Capacity vs Resolution for Tosoh cation exchangers: SP-550C, SP-650M, Experimental S.



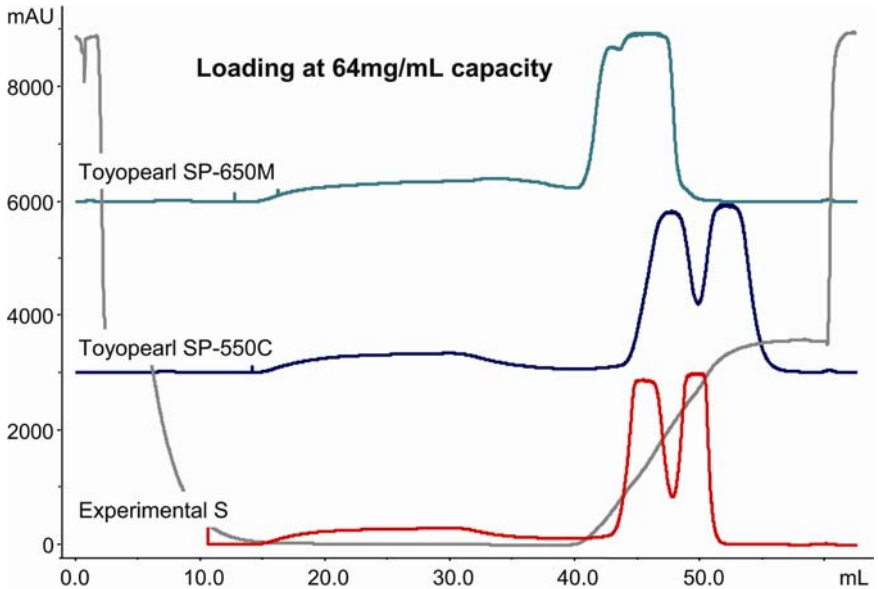
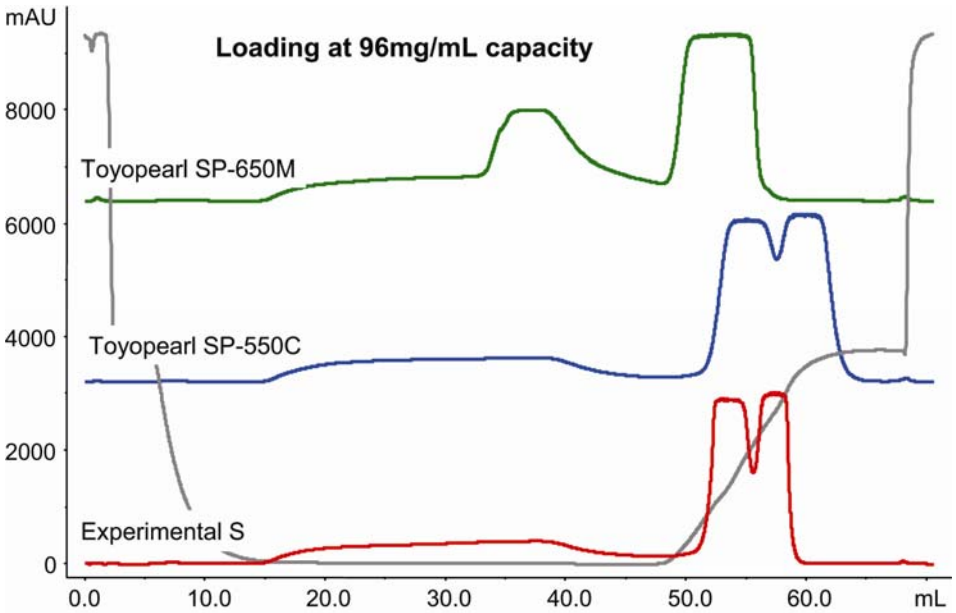


Figure 3c: Capacity vs Resolution for Tosoh cation exchangers: SP-550C, SP-650M, Experimental S.

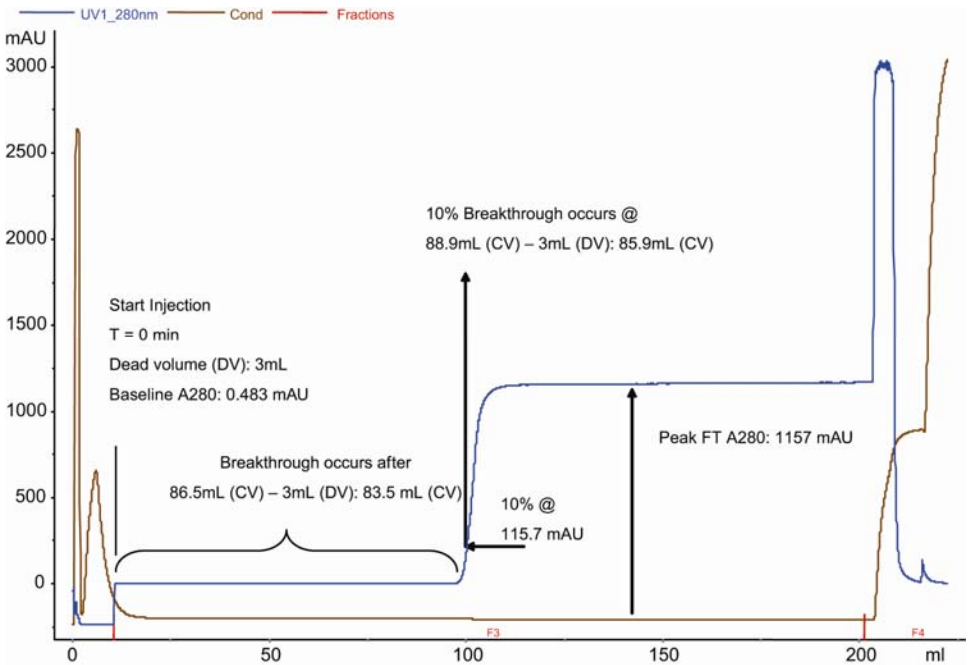


Columns:	3mmID x 15cm bed height for each
Flowrates:	300cm/h (0.35mL/min) during load and elution; 850cm/h (1mL/min) during equilibration, wash, & regeneration. Maximum pressure @ 1.6 bar for 1mL/min.
Load:	a-Chymotrypsin/ Lysozyme: at 2mg/mL each for a total of 4mg/mL concentration. dissolved 400mg of each lyopholized powder into 200mL of low salt buffer (20mmol/L phosphate, pH 6.0). A280 @ 1:10 dilution: 0.842; 8.42AU/mL total
Buffers:	20mmol/L phosphate (pH 6.0) (low salt for equilibration) 20mmol/L phosphate + 500mmol/L NaCl (pH 6.0) (High salt for elution)
Gradient:	0 - 100%B (0-500mmol/L NaCl), 10CV length. Held for 10CV past 100% B
Detection:	UV (280nm)

The resolution between lysozyme and a-chymotrypsin were tested at various saturating conditions. A total of 4, 16, 32, 64 and 96 total mg of protein were loaded. At the highest concentrations there was essentially no resolution on the Toyopearl SP-650M resin but very good resolution on the new experimental S resin.

Figure 4a: Capacity studies

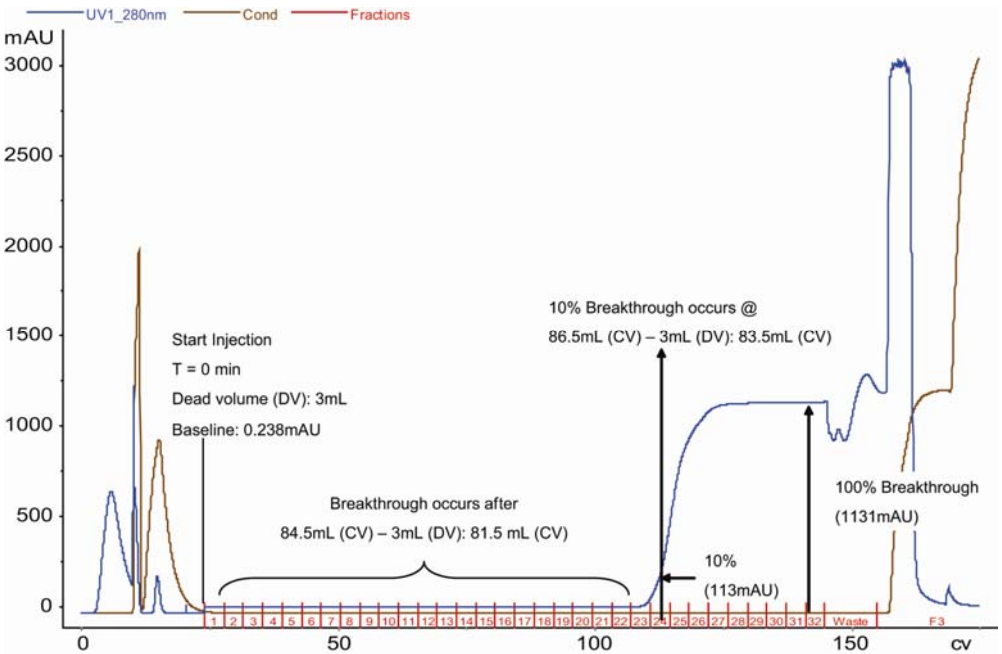
Experimental S. Fresh resin: Loading Lysozyme: 1mL x 15cm



Sample load:	Lysozyme : (Sigma/ lot 093K1455/ Cat L-6876) diluted 413mg into 200mL for ~ 2mg/ml solution. A280 of 1:10 diluted load = 0.468; 4.68 AU total. loading 180mL for a total of 360mg loading.
Column:	Experimental S. 1mL x 5cm
Flow rate:	0.4mL/min; 280cm/hr
Buffers:	20mmol/L phosphate (pH 6.0) 20mmol/L phosphate + 500mmol/L NaCl (pH 6.0)
Regen:	4CV in 1mol/L NaOH collecting FT/Wash to measure absorbance.

Figure 4b: Capacity studies

Experimental S. after 50 cycles: Loading Lysozyme: 1mL x 15cm



Sample load:	Lysozyme : (Sigma/ lot 093K1455/ Cat L-6876) diluted 500mg into 250mL for ~ 2mg/mL solution. A280 of 1:10 diluted load = 0.459; 4.59 AU total. loading 200mL for a total of 400mg loading.
Column:	Experimental S. 1mL x 5cm
Flow rate:	0.4mL/min; 280cm/hr
Buffers:	20mmol/L phosphate (pH 6.0) 20mmol/L phosphate + 500mmol/L NaCl (pH 6.0) Fractionated flow through every 4mL (4CV)
Regen:	4CV in 1mol/L NaOH

Based on simple overlays of both chromatography runs at 50 cycles and fresh resin, breakthrough occurs approximately at the same volume loading at 2mg/mL. Exposure time was 1h in 1mol/L NaOH for each cycle. Caustic stability data shows that the Experimental S resin stands up to multiple cleanings with no measurable loss in binding capacity.

Table 2: The dynamic binding capacity after exposure to NaOH

	Prior to NaOH	After 50 cycles
Experimental Toyopearl S	167g/L - resin	163g/L - resin

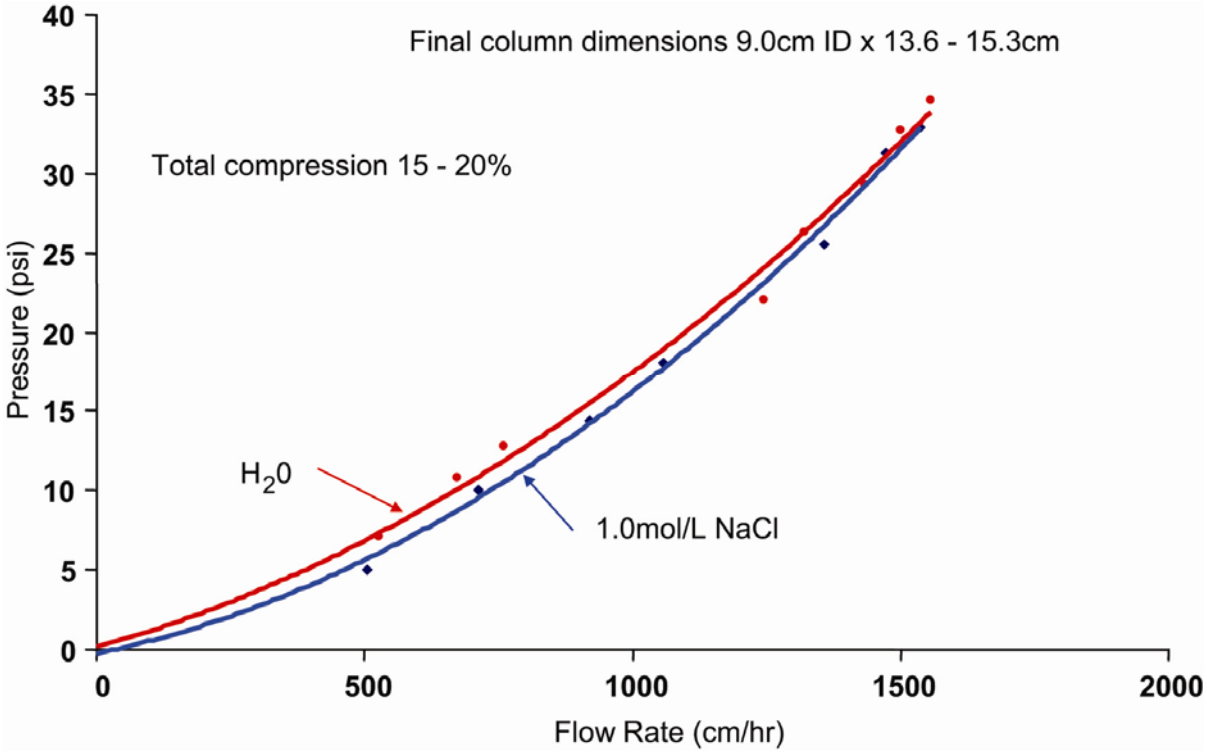
CIP Study: Column cycled 50 times including a 1.0mol/L NaOH cleaning (exposure time at least 1h per cycle)

Table 3: Prolonged exposure to NaOH

	New Resin	1 month exposure to 0.5mol/L NaOH	1 month exposure to 1.0mol/L NaOH
Ion Exchange Capacity	0.17eq/L	0.18eq/L	0.18eq/L
Dynamic Binding Capacity	167g/L	142g/L	142g/L

The small ion exchange capacity was determined after 1 month exposure to 0.5 or 1.0mol/L NaOH. The QC method for Toyopearl SP-650 was used (Evaluation Methods and Specifications for Toyopearl SP-650 Resins).

Figure 5: Pressure Flow Characteristics



The experimental S resin was packed into a 9cm Millipore column to measure the pressure/flow characteristics. The resin had similar profiles for both water and 1.0mol/L NaCl.

Table 4: Pore Size Characterization

	Toyopearl SP-650M	Experimental Toyopearl S
Avg. PEO size	Approx. 4,500	Approx. 1,000
Calc. Avg. PEO size	20Å	8Å
Max. PEO size	Approx. 400,000	Approx. 10,000
Calc. Max. PEO size	264Å	31Å
Avg. Dextran size	Approx. 7,500	Approx. 1,000
Calc. Avg. Dextran size	25Å	10Å
Max. Dextran size	Approx. 2,000,000	Approx. 20,000
Calc. Max. Dextran size	292Å	42Å

Preliminary inverse size exclusion results for the pore size characterization of the experimental S resin compared to commercially available Toyopearl SP-650. The column sizes used in this study were 10mmID x 46cm.
The flow rates used were either 75 or 11cm/h and the solvent was distilled water.

Discussion

Tosoh Corporation has developed a new cation exchange resin with very high binding capacity for IgG molecules. The resin exhibits comparable selectivity when compared to previous experimental resins as well as a common high capacity cation exchanger currently on the market. The resin was also tested for its selectivity using two closely related MAbs and found to be able to separate the proteins (this separation was not optimized). The true test of the resin is the selectivity at very high loading conditions and the new resin did an excellent job of separating lysozyme and α-chymotrypsin when loading up to 98 mg/mL total protein load.

The new resin also exhibited excellent stability when exposed to 0.5 and 1.0N NaOH. The binding capacity remained very high and the small ion exchange capacity was unchanged. The pressure flow characteristics are excellent. Initial pore size characterization using inverse size exclusion chromatography revealed some interesting data. The pore of the new resin appears exclude the uncharged polymer standards when compared to the Toyopearl SP-650M. However, the capacity values for lysozyme and MAbs indicate charged molecules may be able to enter the pores under appropriate binding conditions.

Conclusions

A new cation exchange resin with very high capacity was developed. This resin has excellent selectivity when using small and large sized proteins. It is very stable to high concentrations of NaOH with minimal changes in binding capacity and ion exchange capacity. The pressure flow characteristics are excellent and should be quite amenable to large scale applications.

Interestingly, despite the high capacity, the elution of MAbs from the resin still occurred at "normal" conductivities. What may be a potential issue though, is the concentration of the protein as it elutes from the column. At very high concentrations at elution, the protein solubility may become an issue. Also, although it was not seen at small scale, there may be an increase in system backpressure when eluting at such high protein concentrations. Tosoh plans to commercialize this resin in the next few months.

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