Characterization of a Novel High-Capacity Weak Cation Exchange Resin

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Abstract

Ion exchange resins with increased selectivity and binding capacity are now in demand and it is imperative for chromatographers to have such resins in their repertoire. The Toyopearl GigaCap® family of resins was created to meet these demands. This poster will focus on Toyopearl GigaCap CM-650M resin as a novel high-capacity weak cation exchange media for the purification of biomolecules.

A polymethacrylic base bead, Toyopearl HW-65 resin, was chemically modified with carboxymethyl groups in the 1000Å pores of the bead which result in greater than 100 mg/mL dynamic binding capacity for IgG-type molecules. The binding capacity for other proteins such as lysozyme and chymotrypsinogen are also significantly increased.

Toyopearl GigaCap CM-650M resin binds mAbs under a variety of conditions. Even at pH 3.6, Toyopearl GigaCap CM-650M resin bound a humanized mAb in a buffer containing greater than 300mmol/L NaCl. Experiments were performed to determine the upper and lower pH boundaries for adsorption, the optimal pH for binding of specific proteins and the maximal conductivity for mAb binding. Resin selectivity is demonstrated by resolving two proteins with pI values less than two units apart. In addition, experiments were performed to determine the impact of increasing protein loads on selectivity.

Introduction

Toyopearl GigaCap CM-650M resin binds mAbs under a variety of conditions. For example, even at pH 3.6, Toyopearl GigaCap CM-650M resin bound a humanized mAb in greater than 300mmol/L NaCl. Thus, we wanted to further explore the operating range of this resin. We experimentally determined the practical upper and lower pH boundaries for adsorption and the optimal pH for a specific humanized mAb. From there, we determined the maximal conductivity for this mAb's binding.

Additional experiments described in this poster focus on the operational capabilities and limits of the Toyopearl GigaCap CM-650M resin. Dynamic binding capacity was determined by 10% breakthrough of Human IgG at pH 4.7 (data not shown). Selectivity for this resin was demonstrated using two mixtures of two compounds with pI values that were less than two units apart. Using pH scouting at low protein loading conditions, the optimal pH for resolution between the two proteins was determined. The optimal pH for total elution volume was also determined. The effects of increased protein loading on resin selectivity were demonstrated at pH 3.5, 5.5, and 7.0.

Experimental Information

For the for initial selectivity comparisons, Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M (Tosoh Bioscience LLC) resins were packed in 0.60cm ID x 4.0cm columns as described in the *Instruction Manual for Toyopearl and TSKgel PW type Resins*. The columns were performance tested and found to be acceptable for use in these experiments.

Toyopearl GigaCap CM-650M resin was packed in 1.0cm ID x 16cm columns for pH scouting and conductivity scouting experiments.

Lysozyme, trypsinogen and α-chymotrypsinogen were purchased from Sigma-Aldrich.

Monoclonal antibody (BI mAb 02) was generously supplied by Boehringer-Ingelheim through Dr. Egbert Mueller of Tosoh Bioscience GmbH. The estimated pI of this antibody is 8.2.

All experiments were carried out using an AKTA® Explorer at ambient temperature.

Separation and scouting conditions are listed with their respective chromatograms.

Properties of the Cation Exchange Resins Used in this Study

Resin	Toyopearl GigaCap CM-650M	Toyopearl GigaCap S-650M
Particle Size (µm)	75	75
Pore Size (Å)	1000	1000
Ion Exchange Capacity (meq/mL resin)	0.17- 0.28	0.14 - 0.18
Binding Capacity (mg/mL resin)	>110 Static (γ-globulin) >100 Dynamic (γ-globulin)	80-120 Dynamic (BSA)
Estimated pKa of Functional Group	3.6	1.2

Selectivity of Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins
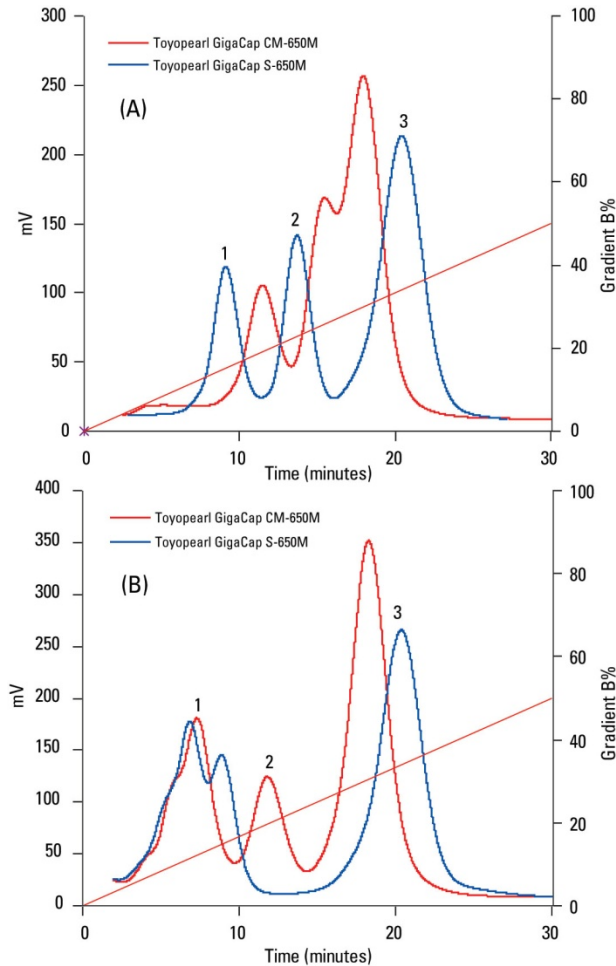
Resins: Toyopearl GigaCap CM-650M
                 Toyopearl GigaCap S-650M
Column Size: 0.6cm ID x 4.0cm (1.13mL)
Buffer A: 20mmol/L phosphate, pH 7.0
Buffer B: Buffer A + 1.0mol/L NaCl
Gradient: 60 minutes 0%B – 100%B
Flow Rate: 212cm/ hr
Detection: UV@280nm
Temperature: Ambient
Sample A: 1. ribonuclease A (5.0mg/mL)
                 2. cytochrome C (1.9mg/mL)
                 3. lysozyme (3.8mg/mL)
Sample B: 1. trypsinogen (3.8mg/mL)
                 2. ribonuclease A (5.0mg/mL)
                 3. lysozyme (3.8mg/mL)
Sample Loaded: 25µL

Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins have different selectivities. Depending on the particular set of proteins being separated the optimal selectivity could be either of these cation exchange resins.

Effect of pH on the Selectivity of Toyopearl GigaCap CM-650M Resin (trypsinogen/lysozyme mixture)
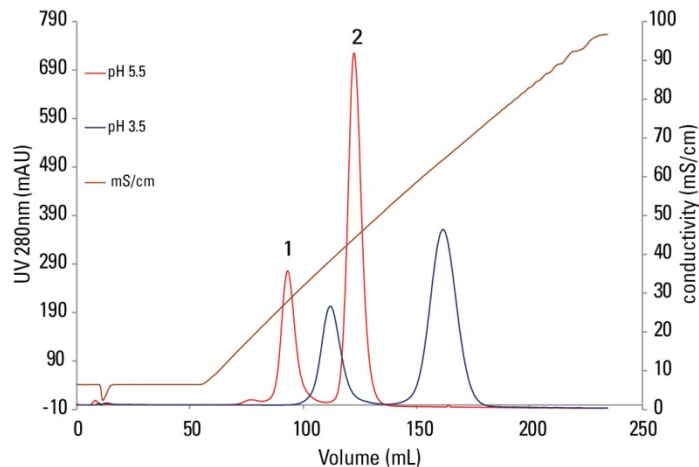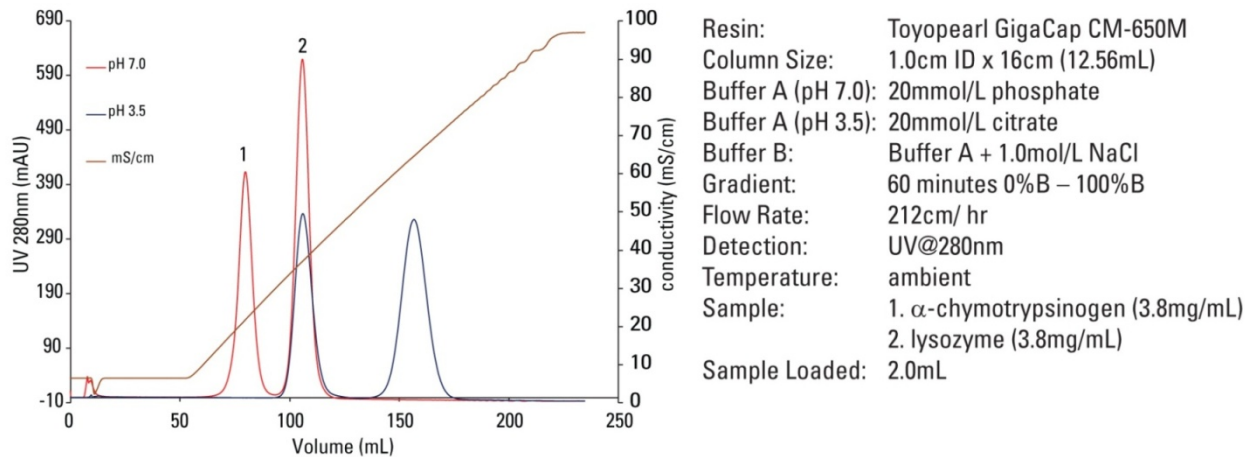

Resin: Toyopearl GigaCap CM-650M
Column Size: 1.0cm ID x 16cm (12.56mL)
Buffer A (pH 5.5): 20mmol/L citrate
Buffer A (pH 3.5): 20mmol/L citrate
Buffer B: Buffer A + 1.0mol/L NaCl
Gradient: 60 minutes 0%B − 100%B
Flow Rate: 212cm/ hr
Detection: UV@280nm
Temperature: ambient
Sample: 1. trypsinogen (3.8mg/mL)
2. lysozyme (3.8mg/mL)
Sample Loaded: 2.0mL

Toyopearl GigaCap CM-650M resin showed good selectivity between trypsinogen (pI = 9.3) and lysozyme (pI = 10.7) at the pH values shown. Selecting for the elution of the sharpest peak and the peak with the greatest height, pH 5.5 was most suitable. The best resolution between peaks was observed at pH = 3.5 and was used for additional studies. The study included conditions performed at pH 7.5, 7.0, 6.5, 6.0, 5.0, 4.5 and 4.0. (Data not shown).

Effect of pH on Selectivity of Toyopearl GigaCap CM-650M Resin

Resin: Toyopearl GigaCap CM-650M
Column Size: 1.0cm ID x 16cm (12.56mL)
Buffer A (pH 7.0): 20mmol/L phosphate
Buffer A (pH 3.5): 20mmol/L citrate
Buffer B: Buffer A + 1.0mol/L NaCl
Gradient: 60 minutes 0%B – 100%B
Flow Rate: 212cm/ hr
Detection: UV@280nm
Temperature: ambient
Sample: 1. α-chymotrypsinogen (3.8mg/mL)
2. lysozyme (3.8mg/mL)
Sample Loaded: 2.0mL

Toyopearl GigaCap CM-650M resin showed good selectivity between α-chymotrypsinogen (pI = 9.5) and lysozyme (pI = 10.7). As the pH decreases, the resolution between the two proteins increased slightly. The elution volume of each peak also increased as the pH was lowered. At pH 3.5, the two proteins were more strongly bound to the resin and required a higher salt concentration to desorb relative to the pH 7.0 purification. The study included conditions performed at pH 7.5, 6.5, 6.0, 5.5, 5.0, 4.5, and 4.0 (data not shown). The effect of increased protein loads will demonstrate if the selectivity can be maintained. (Slide 10.)

# pH Scouting for Separation of a Two Protein Mixture

	pH	Retention (mL)	Width (mL)	Height (mAU)	Resolution
Trypsinogen	7.0	71.3	30.0	274.3	-
Lysozyme	7.0	106.2	29.6	689.5	1.05
Trypsinogen	5.5	93.2	27.8	273.2	-
Lysozyme	5.5	122.6	27.9	724.5	1.06
Trypsinogen	3.5	108.5	39.2	203.2	-
Lysozyme	3.5	156.9	44.2	362.9	1.16

	pH	Retention (mL)	Width (mL)	Height (mAU)	Resolution
α-chymotrypsinogen	7.0	79.8	30.3	410.5	-
Lysozyme	7.0	105.9	27.3	618.8	0.9
α-chymotrypsinogen	5.5	92.5	30.9	373.7	-
Lysozyme	5.5	121.2	32.2	581.3	0.98
α-chymotrypsinogen	3.5	106.0	34.1	337.9	-
Lysozyme	3.5	156.7	43.9	328.8	1.30

Toyopearl GigaCap CM-650M resin showed good selectivity between the chosen molecules. This partial list of pH scouting results highlights the three pH values that were selected for further study. Additional runs were performed at pH 7.5, 6.5, 6.0, 5.0, 4.5, and 4.0 (data not shown). Width of the peaks were measured using baseline extrapolation.

# Effect of Column Load on Selectivity at Various pH Values
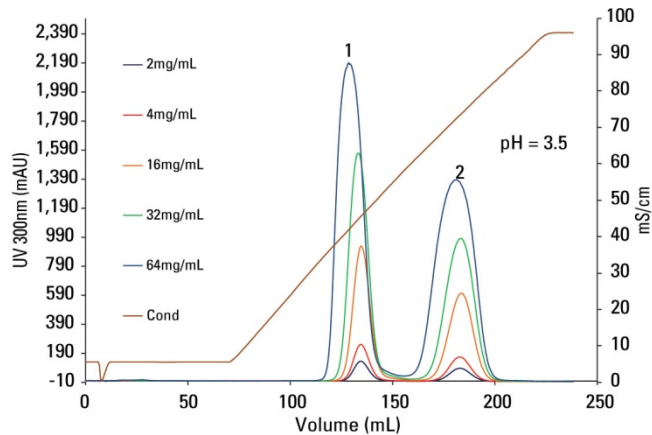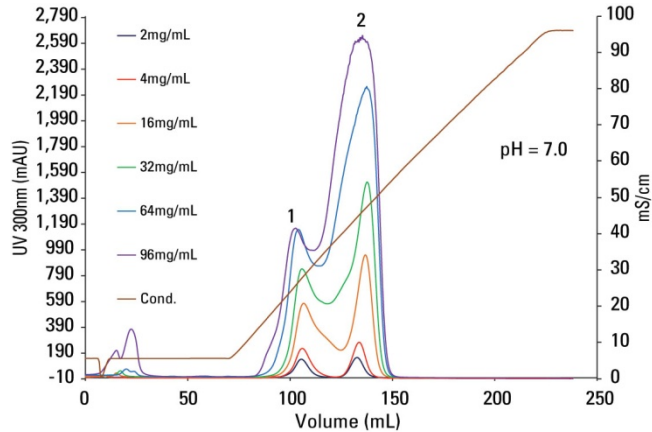
Resin: Toyopearl GigaCap CM-650M
Column Size: 1.0cm ID x 16cm (12.56mL)
Buffer A (pH 7.0): 20mmol/L phosphate
Buffer A (pH 3.5): 20mmol/L citrate
Buffer B: Buffer A + 1.0mol/L NaCl
Gradient: 60 minutes 0%B – 100%B
Flow Rate: 212cm/hr
Detection: UV@300nm
Temperature: Ambient
Sample loaded: 1. α-chymotrypsinogen
2. lysozyme (quantity as indicated in figure)

Selectivity was maintained over the pH range selected. The pH 5.5 (data not shown) and 3.5 maintained greater selectivity for higher loads than at pH 7.0. Even at very high total protein loads (96.0mg/mL resin) selectivity was maintained.

Please Note: The absorbance was recorded at 300nm (de-tuned) to prevent the higher column loads from going off scale at 280nm.

pH Scouting for a humanized monoclonal antibody on Toyopearl GigaCap CM-650M resin
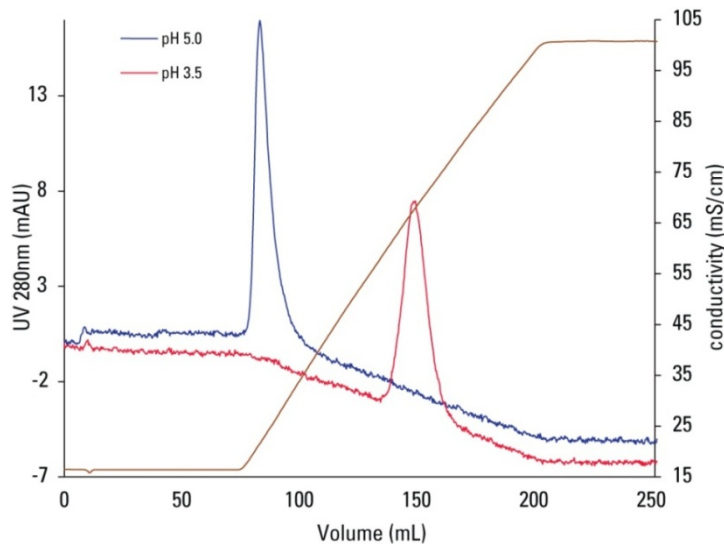
Resin:	Toyopearl GigaCap CM-650M
Column Size:	10mm ID x 16.0cm (12.56mL)
Mobile Phase:	Buffer A: 20mmol/L citrate, pH 3.5 (15mS/cm) 20mmol/L acetate, pH 5.0, (15mS/cm) Buffer B: Buffer A with 1.0mol/L NaCl (95mS/cm)
Gradient:	60 minutes 0%B - 100%B
Flow Rate:	212cm/hr
Detection:	UV@280nm
Temperature:	ambient
Sample:	4.5g/L mAb (diluted 1:5 mAb:running buffer)
Sample Load	0.628mL diluted mAb

In the pH range 6.0-3.0 (not all data shown), the monoclonal antibody peak shifted to the right and peak shape broadened. Based on peak shape and gradient position, it was determined that the pH where this specific humanized monoclonal antibody had the best peak shape was 4.5. However, at pH 4.0, better binding characteristics were achieved.


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pH Scouting to Determine Optimal pH for a mAb Elution

pH	Retention (mL)	Width (mL)	Height (mAU)
3.5	147.15	30.90	11.095
4.0	135.71	30.28	13.164
4.5	106.49	23.26	17.572
5.0	82.81	32.43	16.870

This partial list of pH scouting results details the trends shown in the previous slide. Peak widths were wider and peak height decreased when pH decreased for this particular mAb. Width of the peaks were measured using baseline extrapolation. The optimal peak shape occurs at pH 4.5 for this monoclonal antibody when loaded at a conductivity of 15mS/cm. However, better binding characteristics were achieved at a pH value of 4.0, as shown in the following slides.

Conductivity Scouting for Humanized Monoclonal Antibody on Toyopearl GigaCap CM-650M Resin
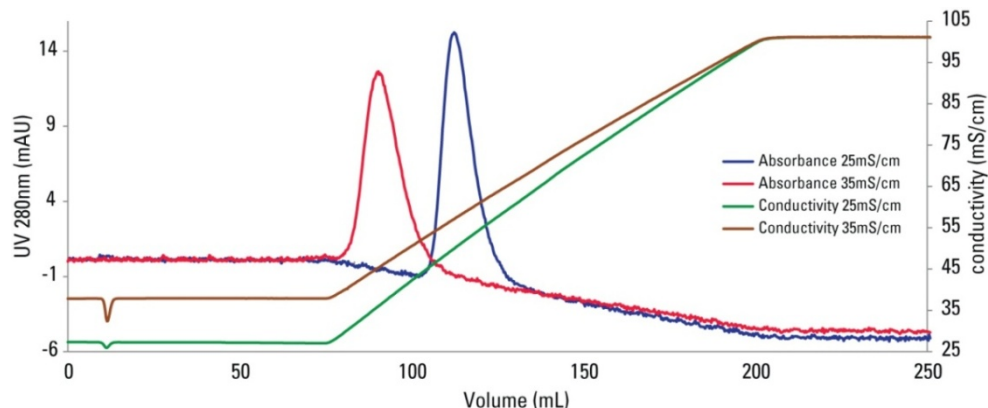
Resin:	Toyopearl GigaCap CM-650M
Column Size:	10mm ID x 16.0cm (12.56mL)
Mobile Phase:	Buffer A: 20mmol/L acetate, pH 4.0 (25mS/cm, 35mS/cm)
	Buffer B: Buffer A with 1.0mol/L NaCl (95mS/cm)
Flow Rate:	212cm/hr
Gradient:	60 minutes 0%B – 100%B
Detection:	UV@280nm
Temperature:	ambient
Sample:	4.5g/L mAb (diluted 1:5 mAb:running buffer)
Sample Load	0.628mL diluted mAb

Not surprisingly, as conductivity increased, the monoclonal antibody peak shifted to the left, indicating earlier elution. Based on these results, it was determined that Toyopearl GigaCap CM-650M resin could still bind this humanized monoclonal antibody at a feed conductivity of 35mS/cm and at pH 4.0. For comparison, 300mmol/L NaCl has a conductivity of approximately 28mS/cm at ambient temperature.

Conductivity Scouting to Determine Maximal Conductivity for mAb Binding

pH	Conductivity (mS/cm)	Retention (mL)	Width (mL)	Height (mAU)
4.0	15	82.83	28.77	16.870
	25	112.31	26.01	16.664
	35	90.36	20.71	12.931
	45	Monoclonal antibody eluted in void volume.		
	55			
4.5	15	106.49	23.26	17.572
	25	Monoclonal antibody eluted in void volume.		
	35			
	45			
	55			
5.0	15	135.71	30.28	13.164
	25	Monoclonal antibody eluted in void volume.		
	35			
	45			
	55			

This table of conductivity scouting results details the trends shown in the previous slide. Peak widths decreased with conductivity increases, resulting in a smaller elution volume. Width of the peaks were measured using baseline extrapolation.

Conclusions

- Toyopearl GigaCap CM-650M resin has demonstrated a high capacity for protein adsorption, unlike any other weak cation exchange resin commercially available.

- Toyopearl GigaCap CM-650M resin demonstrated good selectivity over a wide pH range.   This flexibility allows chromatographers versatility in selecting an optimal pH for their protein separations.

- Toyopearl GigaCap CM-650M resin maintained selectivity even at very high protein loading. Thus, making the use of the full binding capacity of the resin possible.

- Toyopearl GigaCap CM-650M resin bound a humanized monoclonal antibody over a wide range of pH values and high salt concentrations, again maximizing the flexibility of this resin in a variety of applications.