TOYOPEARL GigaCap® DEAE-650M Performance Comparison to TOYOPEARL® DEAE-650M

TOYOPEARL GigaCap DEAE-650M, a very high capacity anion exchange resin for process scale applications, was recently introduced by Tosoh Corporation. This resin, with dynamic binding capacities of over 150 g/L for bovine serum albumin (BSA), is the newest member of the TOYOPEARL product line.

Introduction

TOYOPEARL GigaCap DEAE-650M and TOYOPEARL DEAE-650M anion exchange chromatography resins share more than just the same ligand. They also share the same polymethacrylic backbone and the same 100 nm pore size. It is there that the physical similarity between these two resins ends.

TOYOPEARL GigaCap DEAE-650M resin has a larger mean particle size (75 µm vs. 65 µm), larger ion exchange capacity, and more than a six-fold dynamic binding capacity compared to the TOYOPEARL DEAE-650M resin. The proprietary TOYOPEARL GigaCap ligand attachment chemistry results in the preferential placement of functional groups into the larger, more protein accessible pores in the polymer bead. This promotes both higher protein dynamic binding capacities and improved resin binding and desorption. *Table 1* shows the increased capacities of TOYOPEARL GigaCap DEAE-650M compared to the TOYOPEARL DEAE-650M resin. The experiments detailed below were carried out to see if the difference in performance between these two resins was limited to just binding capacity.

Table 1. Typical Properties of TOYOPEARL GigaCap DEAE-650M

	TOYOPEARL DEAE-650M	TOYOPEARL GigaCap DEAE-650M
Particle Size (µm)	40-90	50-100
Ion Exchange Capacity (eq/L resin)	0.11	0.23
SBC (g/L resin)	30	179
DBC (g/L resin)	25	165

Experimental Conditions/Results

β-lactoglobulin is a glycoprotein that is one of the major whey proteins found in cow's milk. It consists of two monomeric 18 kD subunits that make a 36 kD dimer at neutral to slightly acidic pH values. Several genetic variants of β-lactoglobulin have been identified, of which the A and B phenotypes are most prevalent. The A variant differs from the B variant by one charge difference caused by a single amino acid change from glycine to aspartic acid. The two variants are easily separated using anion exchange chromatography.

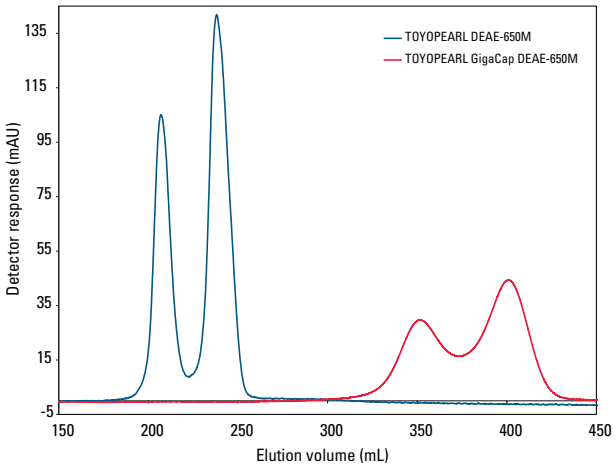
The data presented here compare the separations of β-lactoglobulins A and B using TOYOPEARL DEAE-650M and TOYOPEARL GigaCap DEAE-650M.

In order to compare the performance of the two resins, separation experiments were carried out on 1.0 cm ID × 15.0 ± 0.5 cm columns packed with TOYOPEARL DEAE-650M and TOYOPEARL GigaCap DEAE-650M.

Each column was first loaded with a small quantity of β-lactoglobulin and the A and B variants were separated. The columns were under loaded to better visualize any differences in performance, as seen in *Figure 1*. The performance of the resins was then tested at increasing amounts *(Figure 2)* of β-lactoglobulin.

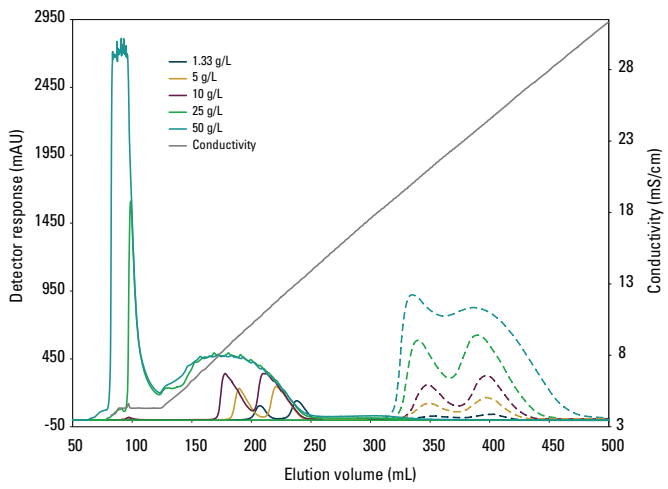
For all experiments, the columns were first sanitized with 2.5 column volumes (CV) of 1.0 mol/L NaOH. The columns were then equilibrated with 5 CV of 20 mmol/L Na_2HPO_4 (mobile phase A). An injection of 20 g/L β-lactoglobulin was then loaded onto the column, and the column was washed with 2 CV of mobile phase A. A 50 CV gradient going from 0-100% mobile phase B, comprised of mobile phase A with 0.5 mol/L NaCl, was then completed. After the gradient, the column was again sanitized with 2.5 CV of 1.0 mol/L NaOH.

Figure 1. TOYOPEARL DEAE-650M vs. TOYOPEARL GigaCap DEAE-650M at low loading conditions



Resins:	TOYOPEARL GigaCap DEAE-650M TOYOPEARL DEAE-650M
Column size:	1.0 cm ID × 15.0 ± 0.5 cm
Mobile phase:	A: 20 mmol/L Na_2HPO_4, pH 6.0 B: mobile phase A + 0.5 mol/L NaCl
Gradient:	0 – 100% (50 CV)
Flow rate:	135.9 cm/hr (1.78 mL/min)
Detection:	UV @ 280 nm, conductivity
Temperature:	ambient
Injection vol.:	7% CV
Sample:	20 g/L β-lactoglobulin in mobile phase A
Sample load:	1.33 g/L total protein

Figure 2. Influence of protein load on TOYOPEARL DEAE-650M and TOYOPEARL GigaCap DEAE-650M



Resins:	TOYOPEARL GigaCap DEAE-650M TOYOPEARL DEAE-650M
Column size:	1.0 cm ID × 15.0 ± 0.5 cm
Mobile phase:	A: 20 mmol/L Na_2HPO_4, pH 6.0 B: mobile phase A + 0.5 mol/L NaCl
Gradient:	0 – 100% (50 CV)
Flow rate:	135.9 cm/hr (1.78 mL/min)
Detection:	UV @ 280 nm, conductivity
Temperature:	ambient
Injection vol.:	7% - 250% CV
Sample:	20 g/L β-lactoglobulin in mobile phase A
Sample load:	1.33 – 50 g/L total protein

Conclusions

Both TOYOPEARL DEAE-650M and TOYOPEARL GigaCap DEAE-650M can separate β-lactoglobulin at loads of up to 10 g/L with near baseline resolution. For loads of 25 g/L and greater, TOYOPEARL GigaCap DEAE-650M was able to separate β-lactoglobulins A and B, though without baseline resolution as was seen at lower loading levels. Elution of β-lactoglobulin also occurs at significantly higher conductivities on the TOYOPEARL GigaCap DEAE-650M compared to the TOYOPEARL DEAE-650M indicating that the TOYOPEARL GigaCap DEAE-650M may have a higher salt tolerance than the TOYOPEARL DEAE-650M resin. This salt tolerance can be beneficial when using TOYOPEARL GigaCap DEAE-650M to capture proteins in crude feedstock at physiological conditions.

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