



## COMPARISON OF TOYOPEARL AF-rPROTEIN A HC-650F BINDING CAPACITY AT VARIOUS BED HEIGHTS AND CONSTANT LINEAR VELOCITY

Protein A chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest.

Protein A chromatography is a very robust purification procedure and is used as a capture step due to its specificity. In protein A chromatography, crude feedstock is passed through a column under conditions that promote binding. After loading is complete, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any nonspecific interactions between process impurities (host cell proteins, etc.) and the stationary phase.

The bound protein is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding. Low pH elution can have a negative effect on protein stability and it is advised that the eluted protein solution be neutralized to minimize aggregation and denaturation.

### INTRODUCTION

TOYOPEARL AF-rProtein A HC-650F is a high capacity protein A resin for the purification of monoclonal antibodies (mAbs). This resin exhibits dynamic binding capacities (DBC) of 70 g/L at 5 minutes residence time. It is based on a hydroxylated methacrylic polymer particle. Table 1 lists the properties and dynamic binding capacities of this resin.

TOYOPEARL AF-rProtein A HC-650F resin remains dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL particles do not distort under flow rates that generate up to 0.3 MPa pressure. These resin properties, combined with a narrow particle size distribution, result in excellent pressure-flow characteristics for the packed TOYOPEARL bed.

### PROPERTIES OF TOYOPEARL AF-rPROTEIN A HC-650F

Particle size	45 $\mu$ m
Pore diameter	100 nm
DBC (5 min)	70 g/L
DBC (2 min)	50 g/L
Caustic stability	> 200 CIP cycles (0.1 mol/L NaOH)

### Table 1

Pressure is an important factor to consider throughout the separation process. Longer, thinner columns are subjected to higher pressure loads than wider columns with shorter bed heights. If it can be shown that an identical volume of resin packed in a shorter, wider column performs as well as a taller, thinner column, scientists using TOYOPEARL AF-rProtein A HC-650F can expect to see a decrease in column pressure while increasing productivity by reducing column residence time.

The data presented here demonstrates the capabilities of TOYOPEARL AF-rProtein A HC-650F to purify a human IgG1 monoclonal antibody from crude feedstock with a fixed column volume at multiple bed heights and constant linear velocity of 400 cm/hr.

### EXPERIMENTAL CONDITIONS/RESULTS

Experiments were carried out on 6.6 mm ID  $\times$  19.5 cm (6.67 mL), 1.0 cm  $\times$  8.4 cm (6.60 mL), and 1.5 cm  $\times$  3.5 cm (6.20 mL) columns packed with TOYOPEARL AF-rProtein A HC-650F resin. The columns successfully passed performance tests for use in these experiments.

The columns were equilibrated with 10 mmol/L Na<sub>2</sub>HPO<sub>4</sub>, 150 mmol/L NaCl, pH 7.40, and loaded with clarified feedstock at approximately 90% of the resin DBC (63 g/L-resin) at a constant linear velocity of 400 cm/h. This linear velocity corresponds to residence times of 2.9 minutes, 1.2 minutes, and 0.5 minutes for the 19.5, 8.4, and 3.5 cm bed heights respectively.

A linear velocity of 400 cm/hr was selected for this series of experiments as it is representative of the fastest flow rates used in manufacturing scale operations. A more typical linear velocity would be in the 300 to 350 cm/hr range.

6.6 MM ID × 19.5 CM COLUMN

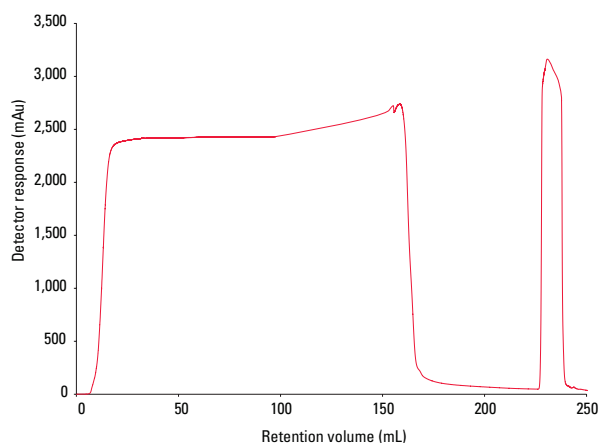


Figure 1

Resin: TOYOPEARL AF-rProtein A HC-650F  
 Column size: 6.6 mm ID × 19.5 cm (6.67 mL)  
 Mobile phase: A: 10 mmol/L Na<sub>2</sub>HPO<sub>4</sub>, 150 mmol/L NaCl, pH 7.40  
 B: 100 mmol/L Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, pH 3.0  
 C: 0.1 mol/L NaOH  
 Flow rate: 400 cm/h (2.28 mL/min)  
 Residence time: 2.9 min  
 Detection: UV @ 280 nm  
 Sample: crude feedstock (titer = 3.0 g/L)  
 Sample load: 155 mL

1.5 CM ID × 3.5 CM COLUMN

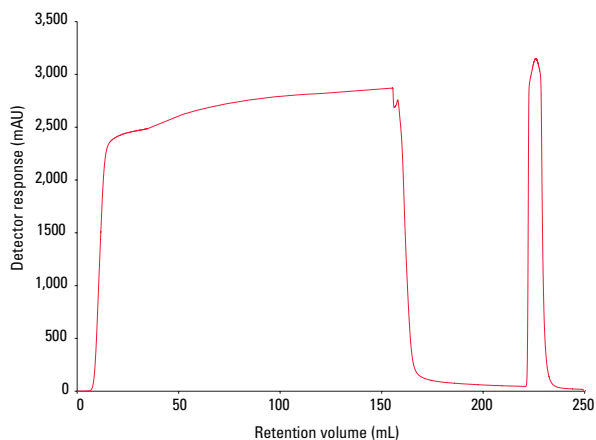


Figure 3

Resin: TOYOPEARL AF-rProtein A HC-650F  
 Column size: 1.0 cm ID × 8.4 cm (6.60 mL)  
 Mobile phase: A: 10 mmol/L Na<sub>2</sub>HPO<sub>4</sub>, 150 mmol/L NaCl, pH 7.40  
 B: 100 mmol/L Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, pH 3.0  
 C: 0.1 mol/L NaOH  
 Flow rate: 400 cm/h (5.23 mL/min)  
 Residence time: 0.5 min  
 Detection: UV @ 280 nm  
 Sample: crude feedstock (titer = 3.0 g/L)  
 Sample load: 155 mL

After loading, the column was washed with 5 CV of equilibration buffer to remove any unbound impurities and then eluted with 100 mmol/L citrate buffer, pH 3.0.

Post-elution, the column was washed with 3 CV of equilibration buffer and sanitized with 3 CV of 0.1 mol/L NaOH (15 minutes contact time).

As can be seen in Figure 1 (6.6 mm ID × 19.5 cm column size), Figure 2 (1.0 cm × 8.4 cm column size) and Figure 3 (1.5 cm × 3.5 cm column size), the elution peaks are sharply defined and exhibit minimal tailing.

1.0 CM ID × 8.4 CM COLUMN

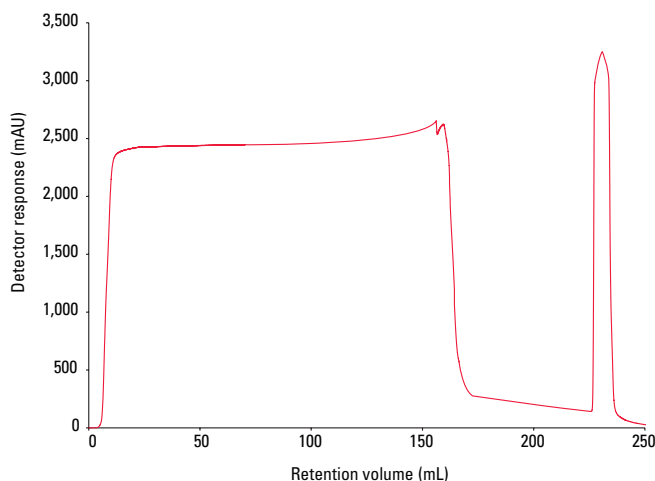


Figure 2

Resin: TOYOPEARL AF-rProtein A HC-650F  
 Column size: 1.0 cm ID × 8.4 cm (6.60 mL)  
 Mobile phase: A: 10 mmol/L Na<sub>2</sub>HPO<sub>4</sub>, 150 mmol/L NaCl, pH 7.40  
 B: 100 mmol/L Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, pH 3.0  
 C: 0.1 mol/L NaOH  
 Flow rate: 400 cm/h (5.23 mL/min)  
 Residence time: 1.2 min  
 Detection: UV @ 280 nm  
 Sample: crude feedstock (titer = 3.0 g/L)  
 Sample load: 155 mL

REPRESENTATIVE SEC HPLC ANALYSIS OF ELUTED MAB

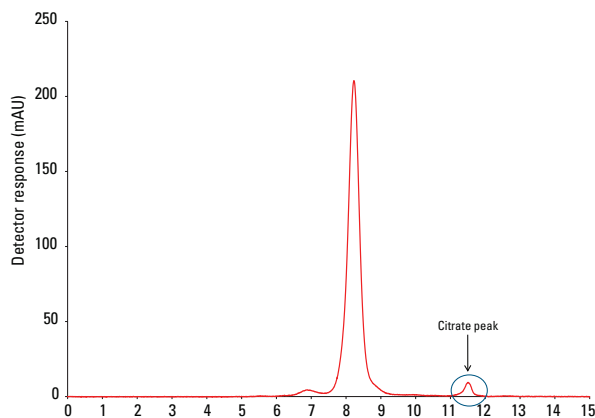


Figure 4

Column: TSKgel G3000SW<sub>XL</sub>, 5 μm, 7.8 mm ID x30 cm  
 Mobile phase: 0.1 mol/L Na<sub>2</sub>PO<sub>4</sub>, 0.1 mmol/L Na<sub>2</sub>SO<sub>4</sub>  
 0.05% azide, pH 6.7  
 Flow rate: 1 mL/min  
 Detection: UV @ 280 nm  
 Inj. Vol.: 1.0 μL  
 Sample: protein A purified mAb

MAB PURITY AND YIELD

Column dimension (cm ID x cm)	Residence time (min)	Sample loaded (mg)	mAb recovered (mg)	Elution volume (mL)	Yield (%)	Purity (%)	Resin capacity 400 cm/hr	Column pressure (MPa)
0.66 x 19.5	2.9	464.7	391.6	37	84.3	96.2	58.7 g/L	0.21
1.0 x 8.4	1.2	464.7	343.2	37	74.1	96.9	52.2 g/L	0.11
1.5 x 3.5	0.5	464.7	224.2	26	48.2	94.7	36.2 g/L	0.07

➤ **Table 2**

Table 2 shows the capacity, yield, pressure, and purity for each of the purifications performed.

A representative HPLC analysis of the elution pool by SEC (Figure 4) indicates that column bed height has a minimal effect on the amount of aggregates present in the purified product.

Recovery was determined by comparing the amount of mAb present in the crude sample loaded onto the column to the amount of mAb present in the elution pool.

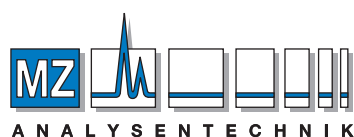
**CONCLUSIONS**

TOYOPEARL AF-rProtein A HC-650F is capable of delivering high purity monoclonal antibodies with excellent recovery at loading levels approaching the resin capacity in columns with bed heights as short as 3.5 cm with a small increase in aggregate levels at the shortest bed height.

While this study clearly shows that capacity and yield will decrease for a fixed resin volume in columns of increasing ID, the reduction in performance is relatively minor. At a bed height less than half of the 6.6 mm ID column, the difference in capacity for the 1.0 cm ID column was only 6.5 g/L and was still greater than 50 g/L overall.

Most notable was the pressure difference of 0.1 MPa (1 bar) between the 6.6 mm and 1.0 cm ID columns. These values suggest that a bed height of 20 cm is not necessary when using TOYOPEARL AF-rProtein A HC-650F in order to maximize resin performance and productivity. Columns packed to a bed height of approximately 10 cm to 15 cm will perform similarly to a 20 cm bed height column, but at reduced pressures and increased product throughput.

Further optimization of the protein A column by adjusting linear velocity and bed height may eliminate the need for chromatographers to use a 20 cm bed height for protein A process steps without any loss in column capacity.



**AUTHORIZED DISTRIBUTOR**

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