



# **Purification of Monoclonal Antibodies Using Cation Exchange Toyopearl GigaCap<sup>®</sup> Resins**

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# Abstract

In the biopharmaceutical industry, downstream processing typically includes three chromatographic steps; capture, intermediate purification and polishing. Protein A is most frequently used as a capture step which results in excellent throughput (i.e., capacity and speed) and is very important in concentrating the target molecule. The second step in mAb production is generally ion exchange or HIC. Our experiments focused on the use of two new cation exchange resins, Toyopearl GigaCap S-650M and Toyopearl GigaCap CM-650M, for further purification of mAb after capture by Protein A. These resins exhibit excellent base stability in 1.0mol/L NaOH and showed robust clean-in-place properties with minimal loss of capacity after 50 x 1 hour cycles of caustic exposure to 1.0mol/L NaOH. Both resins had very high binding capacities at fast flow rates. Toyopearl GigaCap S-650M resin had an impressive >150mg/mL dynamic binding capacity and Toyopearl GigaCap CM-650M resin had >100mg/mL dynamic binding capacity for human IgG molecules at a flow rate typically utilized in production processes. These resins can tolerate high salt concentration and their best selectivity for mAb adsorption at a wide range of low pH values. A mAb isolated from Protein A was further purified to greater than 98% with 90% recovery using Toyopearl GigaCap CM-650M resin. Both resins are stable at high linear velocities, have good pressure flow characteristics and will easily withstand back pressures up to 3bar. The high capacity and stable back pressure stability of the Toyopearl GigaCap cation exchange series of resins create opportunities for increased throughput in downstream purification steps.

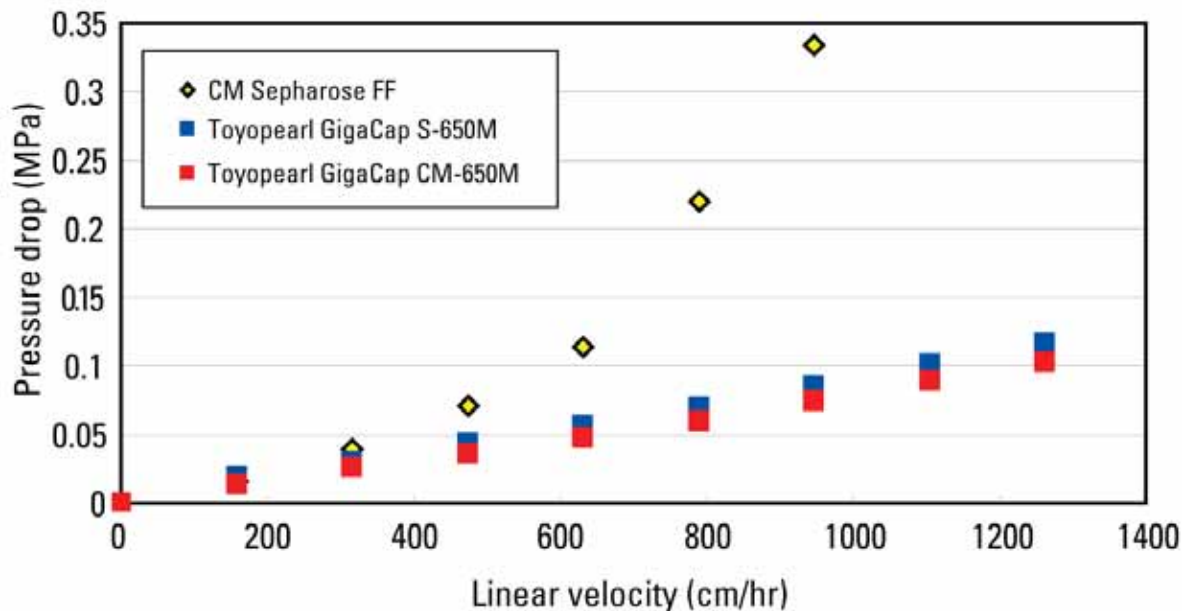


# Rationale

Therapeutic antibodies are a major product class in the biopharmaceutical industry. Protein A chromatography is typically used as a capture step for purifying antibodies. Impurities are removed after Protein A capture utilizing one or two additional chromatographic steps (cation exchange, anion exchange, HIC or some combination of these). In this poster, we demonstrate the usage of either Toyopearl GigaCap CM-650M resin or Toyopearl GigaCap S-650M resin in removing impurities and improving antibody yield after Protein A chromatography.



# Figure 1: Relationship between Flow Rate and Pressure Drop on a Cation-Exchange Resins

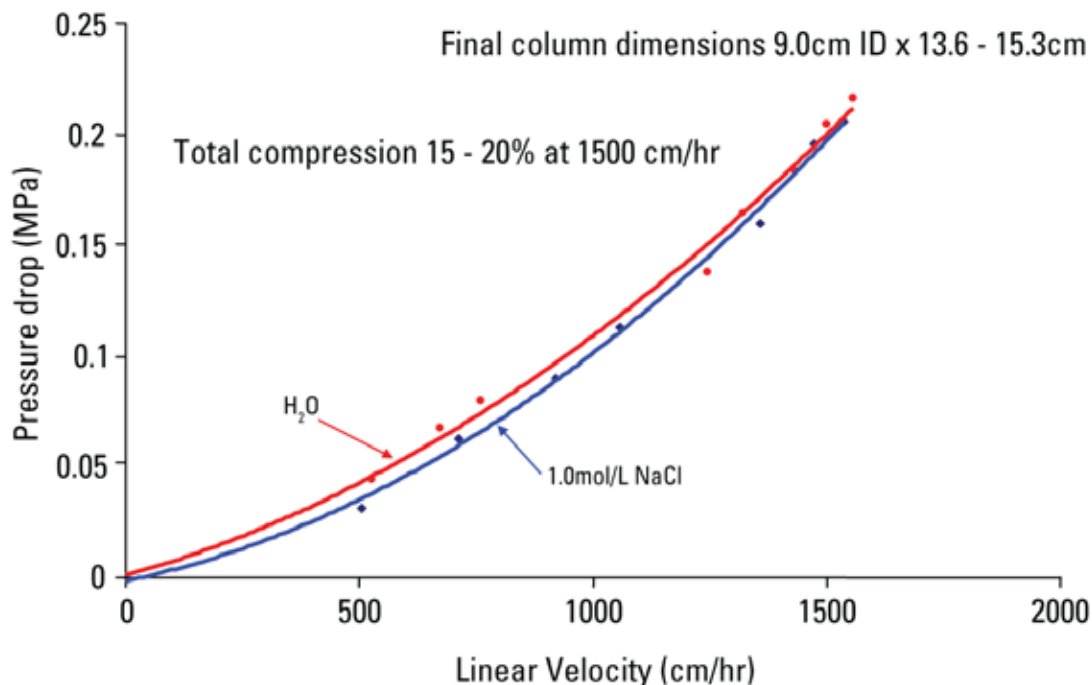


Column: 22mm ID x 20cm L  
Mobile phase: Water

Toyopearl GigaCap S-650M and Toyopearl GigaCap CM-650M resins had essentially the same pressure response to increasing flow rates. The pressure-flow characteristics of both Toyopearl GigaCap resins were significantly lower than for CM Sepharose™ FF, a commercial cation exchange resin.



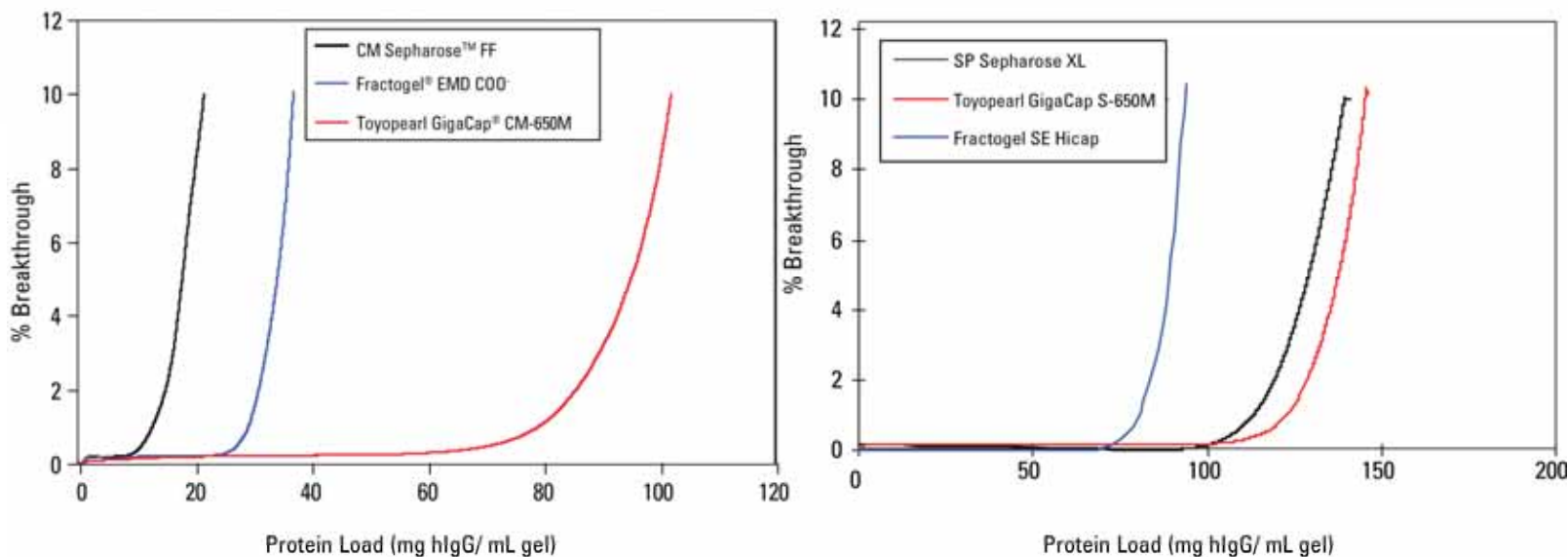
## Figure 2: Pressure-Flow Properties of Toyopearl GigaCap S-650M Resin using Water or 1.0mol/L NaCl



Toyopearl GigaCap S-650M resin was packed into a 9.0cm ID x 15cm bed height column to measure the pressure-flow characteristics. Data showed that Toyopearl GigaCap S-650M resin had similar profiles when packed and run in both water and 1.0mol/L NaCl. Similar data was observed for Toyopearl GigaCap CM-650M resin (data not shown).



# Figure 3: IgG Breakthrough Curves on Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M Resins

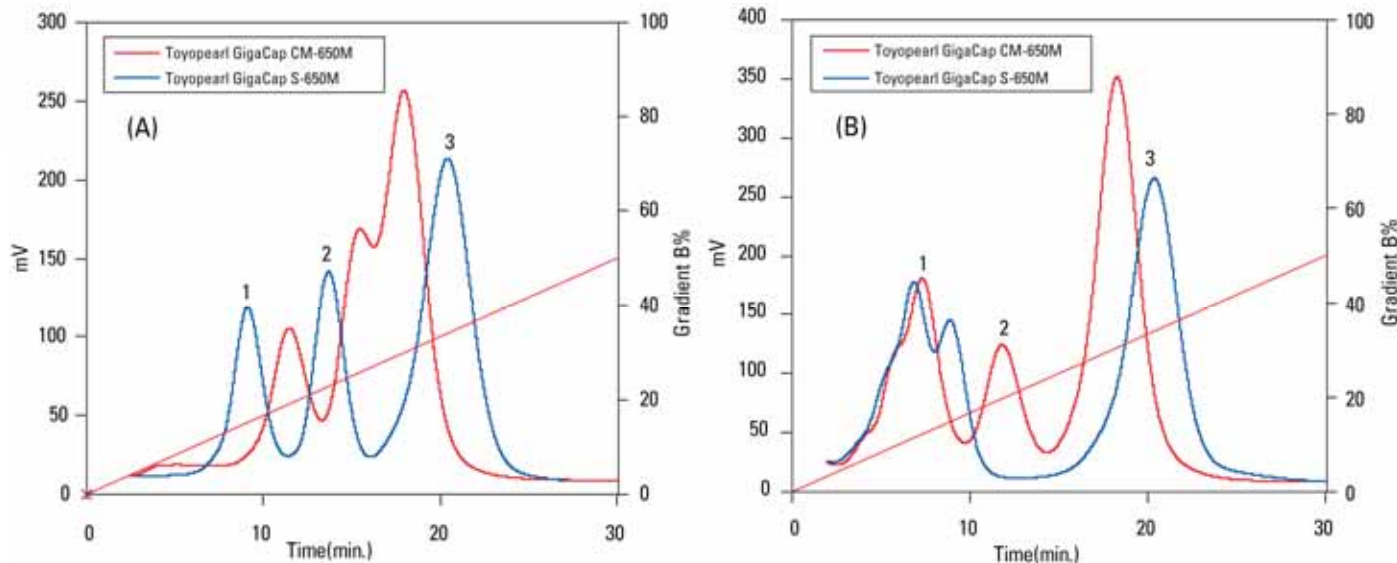


Column size: 6mm ID x 4cm L  
Buffer A: 0.1mol/L acetate buffer, pH 4.6  
B: 0.1mol/L acetate buffer + 1.0mol/L, pH 4.6  
Injection: loading until break through curve observed  
Flow rate: 212cm/hr  
Detection: UV @ 280nm  
Sample: human polyclonal IgG (1mg/mL)

Both Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins have increased capacity for IgG compared to other commercially available cation exchange resins.



# Figure 4: Selectivity of Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M Resins

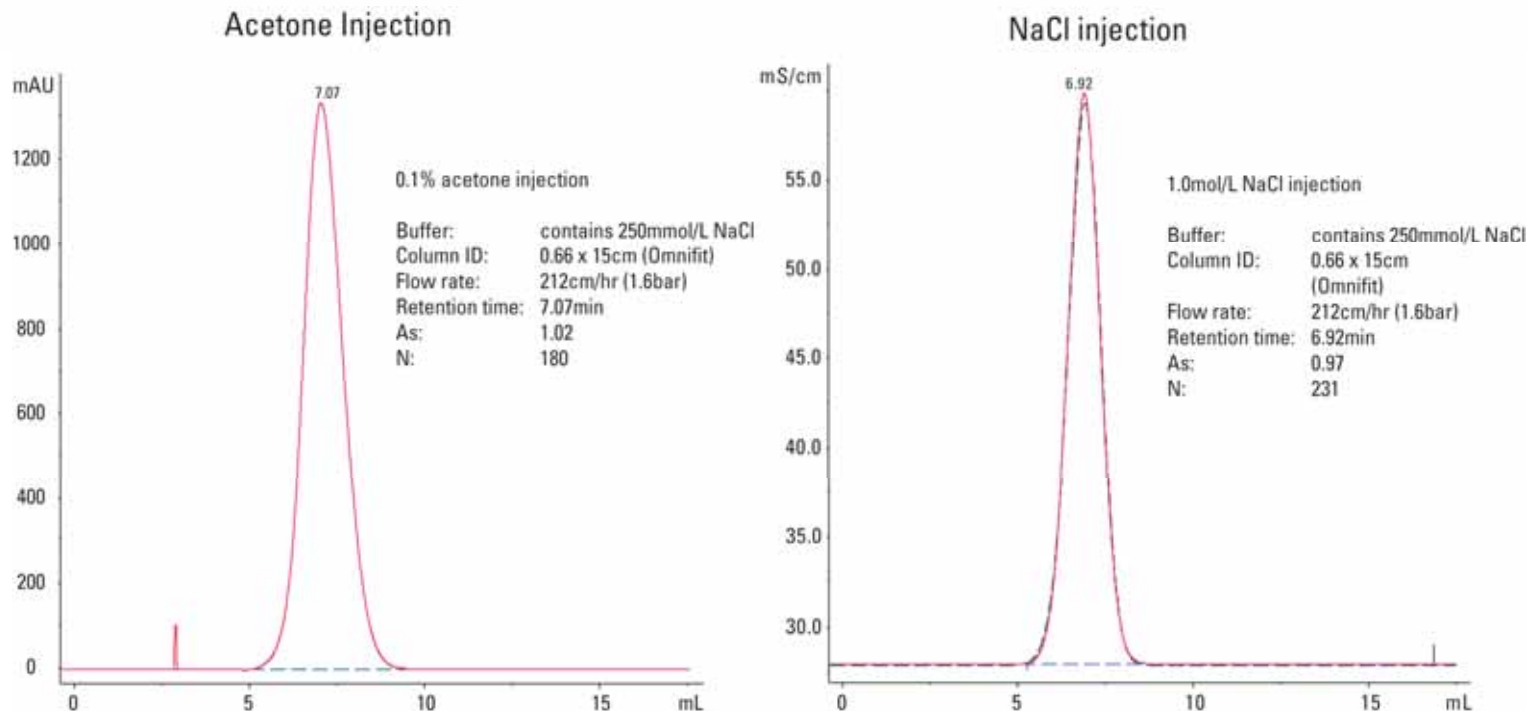


Column size: 6mm ID x 4cm,  
Buffer A: 20mmol/L Phosphate pH 7.0  
B: 20mmol/L Phosphate + 1.0mol/L NaCl pH 7.0  
Gradient: 60min linear gradient from buffer A to buffer B  
Flow rate: 1.0mL/min  
Injection volume: 25µL  
Detection: UV @ 280nm  
Sample: (A) 1. ribonuclease A (5.0mg/mL),  
2. cytochrome C (1.9mg/mL)  
3. lysozyme (3.8mg/mL)  
(B) 1. trypsinogen (3.8mg/mL),  
2. ribonuclease A (5.0mg/mL)  
3. lysozyme (3.8mg/mL)

Toyopearl GigaCap S-650M and Toyopearl GigaCap CM-650M resins have different selectivities which can be very useful for impurity removal.



# Figure 5: Toyopearl GigaCap CM-650M Resin - Testing Column Performance

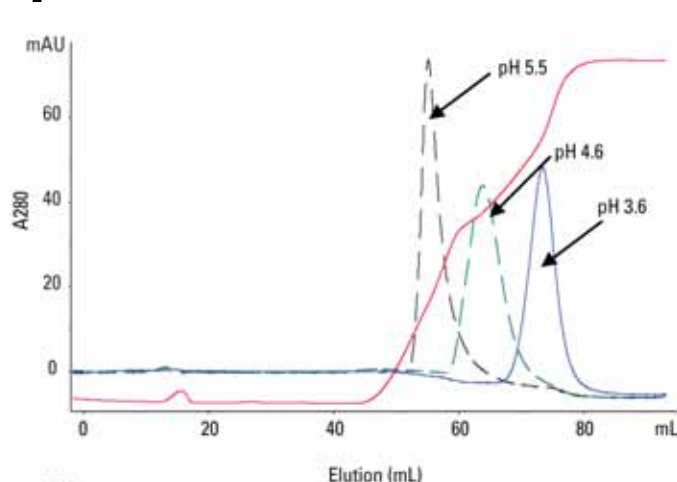


Both 0.1% acetone and 1.0mol/L NaCl can be used to evaluate column packing performance for Toyopearl GigaCap S-650M (not shown) and Toyopearl GigaCap CM-650M resins as long as there is at least 250mmol/L of NaCl in the running buffer.





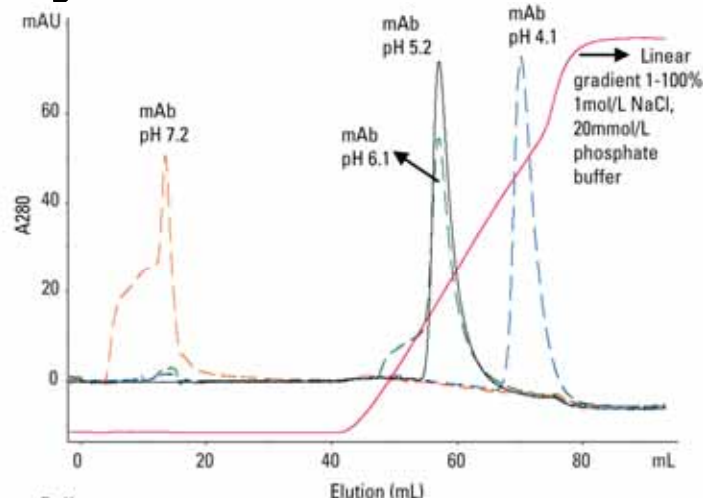
## Figure 6: Toyopearl GigaCap CM-650 Resin – pH and Buffer Selectivity



Buffer:  
A: binding buffer 20mmol/L acetate buffer pH 3.6, 4.6 and 5.5  
B: eluting buffer 20mmol/L acetate buffer + 1.0mol/L NaCl

Sample loaded: 1mg/column  
Separation conditions: washed with 5CV 100% buffer A followed by (1mg) 11mL injection. Then washed with 3CV 100% buffer A followed by 6CV of linear gradient 1-100% buffer B. Finally, column is washed with 5CV 100% buffer B.

Detection: Abs @ 280nm  
Column ID: 0.66cm ID x 15cm (Omnifit)  
Flow rate: 212cm/hr (1.6bar)



Buffer:  
A: binding buffer, 20mmol/L phosphate (no NaCl)  
B: eluting buffer A + 1.0mol/L NaCl

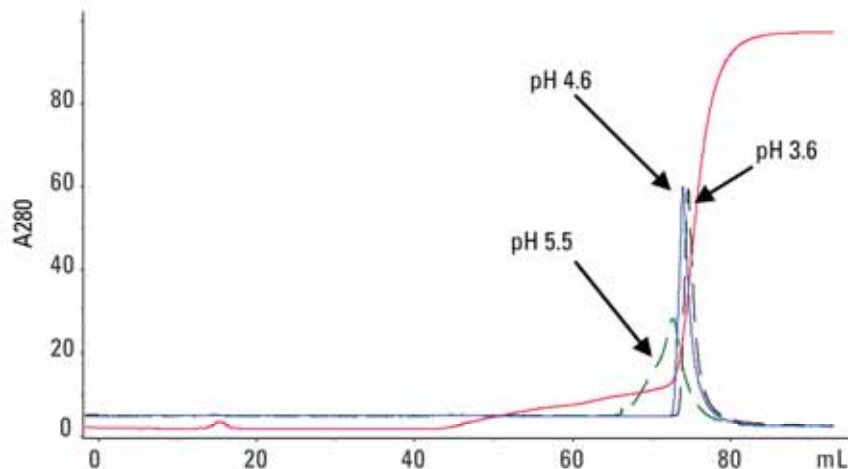
Sample loaded: 1mg/column  
Separation conditions: Column is washed with 5CV 100% buffer A followed by (1mg) 11mL injection. Column is then washed with 3CV 100% buffer A followed by 6CV of linear gradient 1-100 buffer B. Finally, column is washed with 5CV 100% buffer B.

Detection: A280  
Column ID: 0.66 x 15cm (Omnifit)  
Flow rate: 212cm/hr (1.6bar)

Phosphate buffer and acetate buffer (20mmol/L) adjusted to different pH values (pH 3.6, 4.6 and 5.5) were tested. On the binding strength of Toyopearl GigaCap CM-650M resin, data indicated that pH 3.6 and 4.1 provided the most symmetrical mAb peaks and allowed mAb to be adsorbed tightly onto the resins as shown by the higher salt concentration necessary to elute mAb.



## Figure 7: Toyopearl GigaCap S-650M Resin – pH Scouting



**Buffer:**

A: binding buffer, 20mmol/L acetate buffer pH 3.6, 4.6 and 5.5

B: eluting buffer, 20mmol/L acetate buffer + 1.0mol/L NaCl

Sample loaded: 1mg/column

Separation conditions: washed with 5CV 100% buffer A followed by (1mg) 11mL injection. Then washed with 3CV 100% buffer A followed by 6CV of linear gradient 1-12% buffer B. Finally, column is washed with 5CV 100% buffer B.

Detection: Abs @ 280nm

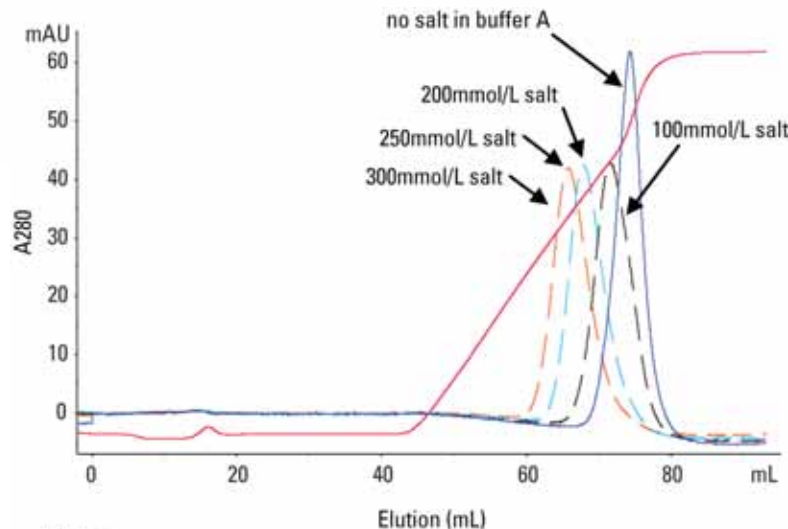
Column ID: 0.66 x 15cm (Omnifit)

Flow rate: 212cm/hr (1.6bar)

Acetate buffer (20mmol/L) adjusted to different pH values (pH 3.6, 4.6 and 5.5) was tested. On the binding strength of Toyopearl GigaCap S-650M resin, binding of mAb was optimal in the pH range of 3.6 - 4.6.



## Figure 8: Toyopearl GigaCap CM-650M Resin – Salt Tolerance at pH 3.6



### Buffer:

A: Binding buffer, 20mmol/L acetate buffer pH 3.6 + 0, 100, 200, 250 and 300mmol/L NaCl

B: Eluting buffer, 20mmol/L acetate buffer + 1.0mol/L NaCl

Sample loaded: 1mg/column

Separation conditions: Column is washed with 5CV 100% buffer A followed by (1mg) 11mL injection. Column is then washed with 3CV 100% buffer A followed by 6CV of linear gradient 1-100 buffer B. Finally, column is washed with 5CV 100% buffer B.

Detection: Abs @ 280nm

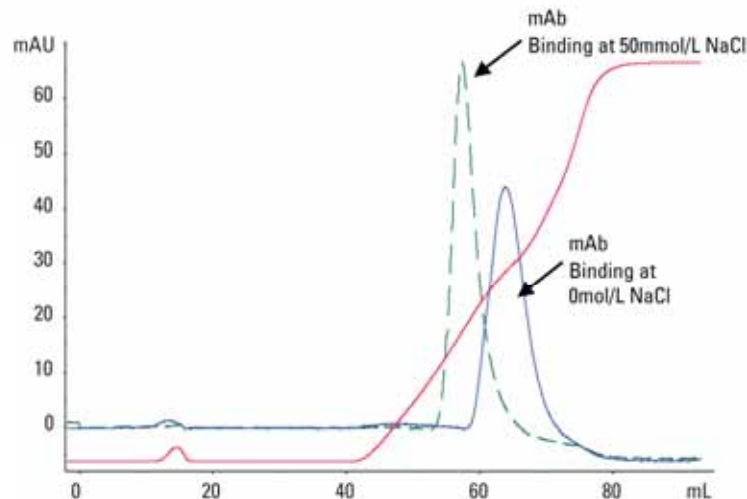
Column ID: 0.66 x 15cm (Omnifit)

Flow rate: 212cm/hr (1.6bar)

A salt tolerance study indicated that at pH 3.6, Toyopearl GigaCapCM-650M resin adsorbed and retained mAb even at high salt concentrations.



## Figure 9: Toyopearl GigaCap CM-650M Resin - Salt Tolerance at pH 4.6



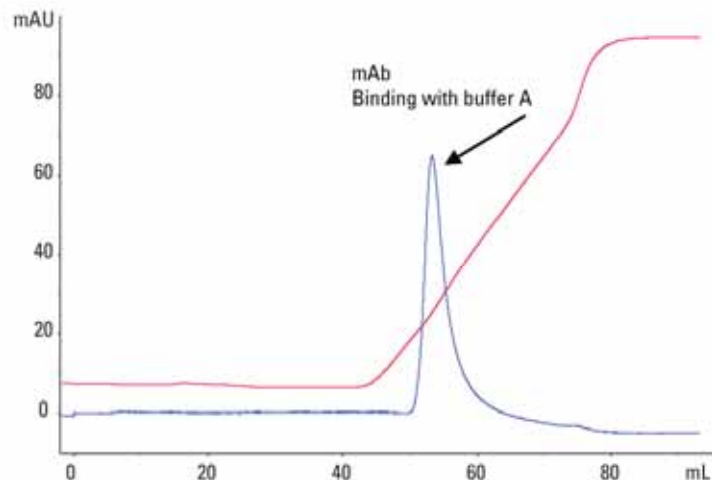
Buffer:

A: Binding buffer, 20mmol/L acetate buffer pH 4.6 + 0 and 50mmol/L NaCl  
B: Eluting buffer, 20mmol/L acetate buffer + 1.0mol/L NaCl

Sample loaded: 1mg/column

Separation conditions: Column is washed with 5CV 100% buffer A followed by (1mg) 11mL injection. Column is then washed with 3CV 100% buffer A followed by 6CV of linear gradient 1-100 buffer B. Finally, column is washed with 5CV 100% buffer B.

Detection: Abs @ 280nm  
Column ID: 0.66 x 15cm (Omnifit)  
Flow rate: 212cm/hr (1.6bar)



Buffer:

A: Binding buffer, 20mmol/L acetate buffer pH 4.6 + 100mmol/L NaCl  
B: Eluting buffer, 20mmol/L acetate buffer + 1.0mol/L NaCl

Sample loaded: 1mg/column

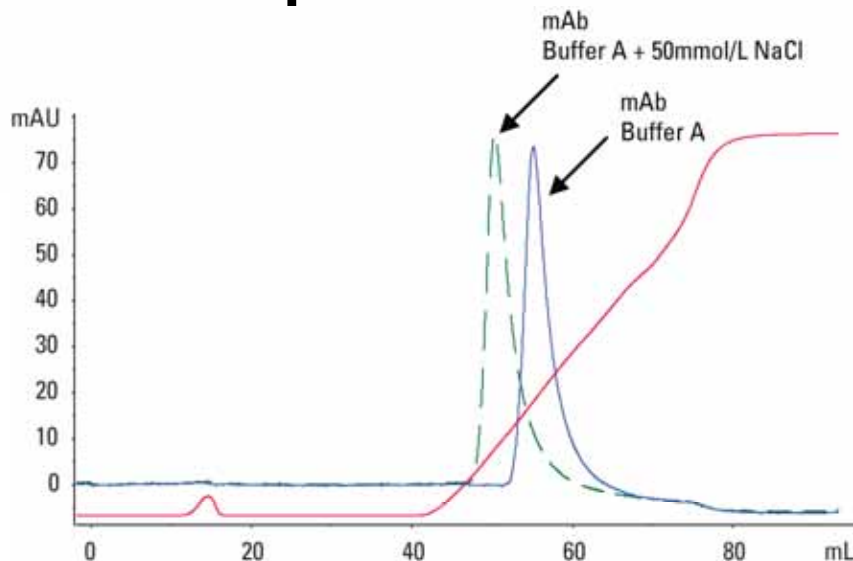
Separation conditions: Column is washed with 5CV 100% buffer A followed by (1mg) 11mL injection. Column is then washed with 3CV 100% buffer A followed by 6CV of linear gradient 1-100 buffer B. Finally, column is washed with 5CV 100% buffer B.

Detection: Abs @ 280nm  
Column ID: 0.66 x 15cm (Omnifit)  
Flow rate: 212cm/hr (1.6bar)

At pH 4.6, Toyopearl GigaCap CM-650M resin adsorbs mAb in the presence of 50mmol/L NaCl. mAb was still bound when 100mmol/L NaCl was added to the starting buffer at pH 4.6. When the salt concentration exceeded 100mmol/L, no mAb adsorption was observed.



## Figure 10: Toyopearl GigaCap CM-650M Resin - Salt Tolerance at pH 5.5



### Buffer:

A: Binding buffer, 20mmol/L acetate buffer pH 5.5 + 50mmol/L NaCl

B: Eluting buffer, 20mmol/L acetate buffer + 1.0mol/L NaCl

Sample loaded: 1mg/column

Separation conditions: Column is washed with 5CV 100% buffer A followed by (1mg) 11mL injection. Column is then washed with 3CV 100% buffer A followed by 6CV of linear gradient 1-100 buffer B. Finally, column is washed with 5CV 100% buffer B.

Detection: Abs @ 280nm

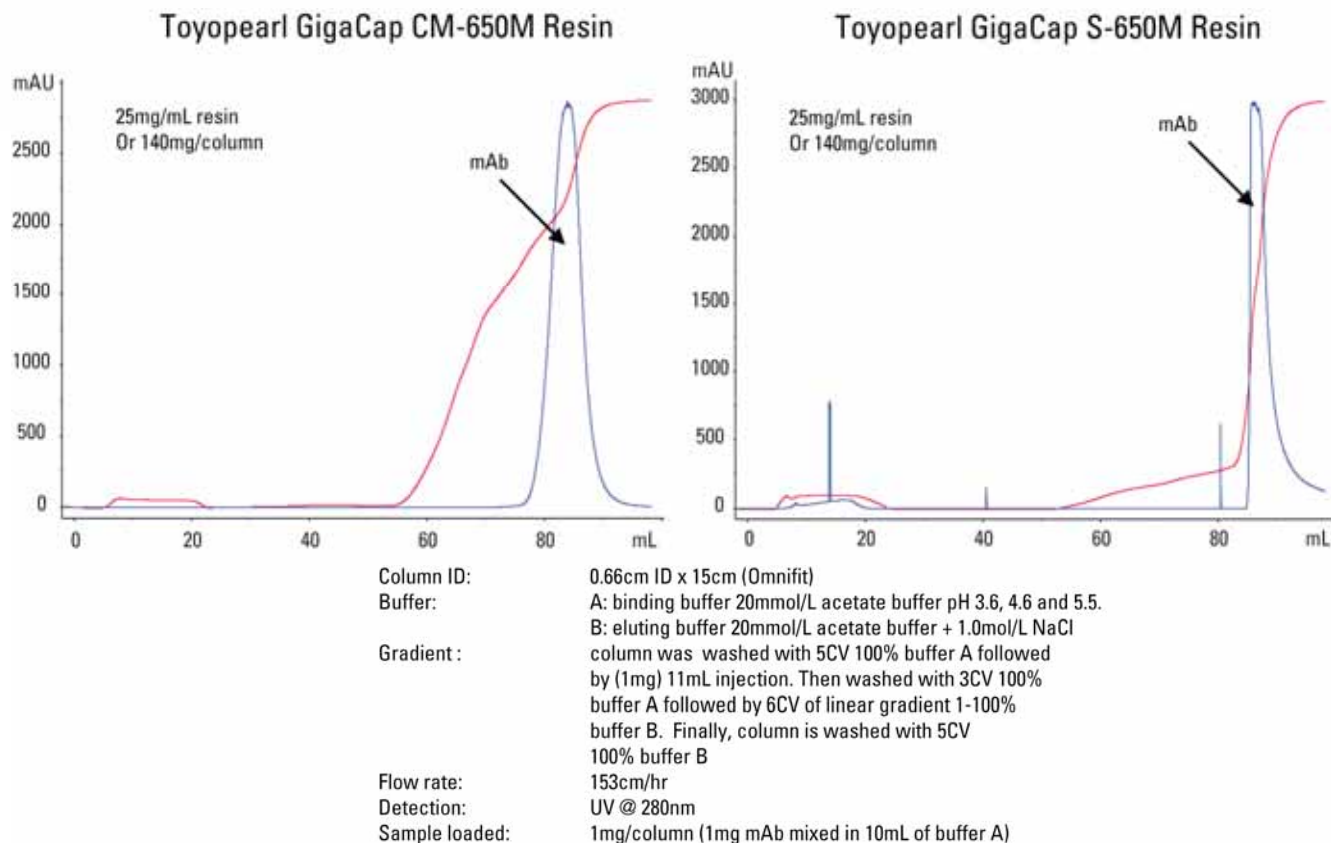
Column ID: 0.66 x 15cm (Omnifit)

Flow rate: 212cm/hr (1.6bar)

At pH 5.5, Toyopearl GigaCap CM-650M resin bound mAb at 50mmol/L NaCl or less.



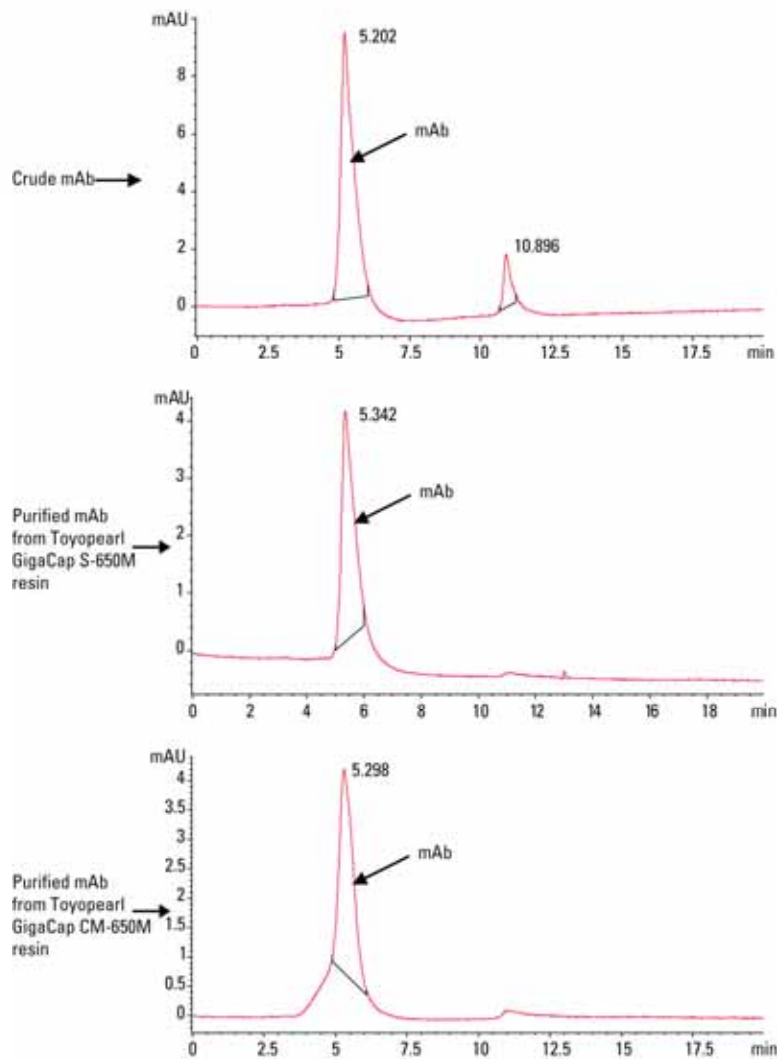
# Figure 11: Loading Study for Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M Resins Using mAb



At 25mg mAb/mL resin loading capacity indicated that both resins still generated very nice peaks. This suggested that both resins can separate a higher loading concentration of mAb material.



## Figure 12: mAb Purity Analysis



Crude mAb and purified mAb after Toyopearl GigaCap S-650M and Toyopearl GigaCap CM-650M purification were injected onto a TSKgel G3000SW<sub>XL</sub> column 7.8mm ID x 30cm. Data indicated that the purity of the mAb after purification on Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins was >98%.



# Conclusions

- Toyopearl GigaCap CM-650M resin can adsorb mAb at a very high salt concentrations up to 300mmol/L at pH  $\leq$  3.6
- Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins can adsorb mAb under a variety of experimental conditions
- Both Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins have excellent binding capacity for proteins, particularly monoclonal antibodies
- Both Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins were able to purify a mAb to >98% purity after a Protein A capture step
- The recovery of mAb from both cation exchange resins was >93%
- Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins have different selectivities at different pH and salt concentrations

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