# New cation exchanger for separation of IgG monomer from dimers and aggregates

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# Abstract

In this poster, we describe the characterization of a new experimental cation exchange (carboxyl type) resin in the Toyopearl family. This new resin was developed to improve the separation of IgG monomer from dimers and aggregates in therapeutic antibody purification. This was accomplished by optimizing the pore size and surface modification of the resin.

The new experimental resin has different selectivity for dimers and aggregates when compared to Toyopearl SP-650M or CM-650M and exhibited better resolution between monomer and aggregates. We could purify IgG monomer by using the new resin with a bind-and-elute mode (linear or stepwise gradient) or in a flow through mode. The recovery of IgG monomer was greater than 90% and the purity of monomer was estimated to be more than 99.5% by size exclusion chromatography.

# Introduction

A major product in the biopharmaceutical industry is therapeutic antibodies. Most antibodies are purified using Protein A affinity chromatography as the capture step followed by two or three additional chromatography steps. Impurities that must be removed include high molecular weight aggregates, host cell proteins, nucleic acids, leached Protein A, and viral contaminants. Among these impurities, high molecular weight aggregates can not be removed by Protein A affinity chromatography and thus require additional chromatographic steps. Although cation exchange, hydrophobic interaction, or ceramic hydroxylapatite chromatography is used to remove dimers and aggregates, each of these techniques alone is not sufficient to remove aggregates. To address these issues, we have developed a novel chromatographic support.

In this poster, we present the characteristics and applications for the purification of IgG monomer.

# Experimental

**Measurement of dynamic binding capacity for Lysozyme**

Dynamic binding capacity was measured by breakthrough experiments with chicken egg white Lysozyme. The column dimensions were 6mmID x 4cm. Buffered solution (50mmol/L glycine buffer, pH 9.0) containing 5mg/mL of lysozyme were loaded onto the column at various flow velocities. Breakthrough curves were monitored by a UV detector at 280nm. The dynamic binding capacity was determined at 10% breakthrough.

**Antibody**

A humanized polyclonal IgG1 with a pI of 8.2 and purified on Protein A affinity chromatography was used in each of the antibody experiments. The IgG fraction was heated (60° C, 1hr) to increase the contents of dimers and aggregates.

**Chromatographic apparatus**

A majority of the experiments were carried out on a liquid chromatography system, ÄKTA Prime (GE Healthcare) or HPLC system (Tosoh) at 25° C.

**Ion exchange chromatography**

Toyopearl SP-650M, CM-650M or the experimental resin was packed in a 6mmID x 4cm column. The column was equilibrated with a starting buffer (buffer A: 50mmol/L acetate, pH 5.0, 50mmol/L acetate, pH 5.5, or sodium phosphate, pH 6.0). After protein loading, elution was carried out with the elution buffer (buffer B) containing 1.0mol/L NaCl in buffer A. The typical elution method was a linear or stepwise gradient from buffer A to buffer B over the length of the gradient in each of the figures.

**Purity check of IgG**

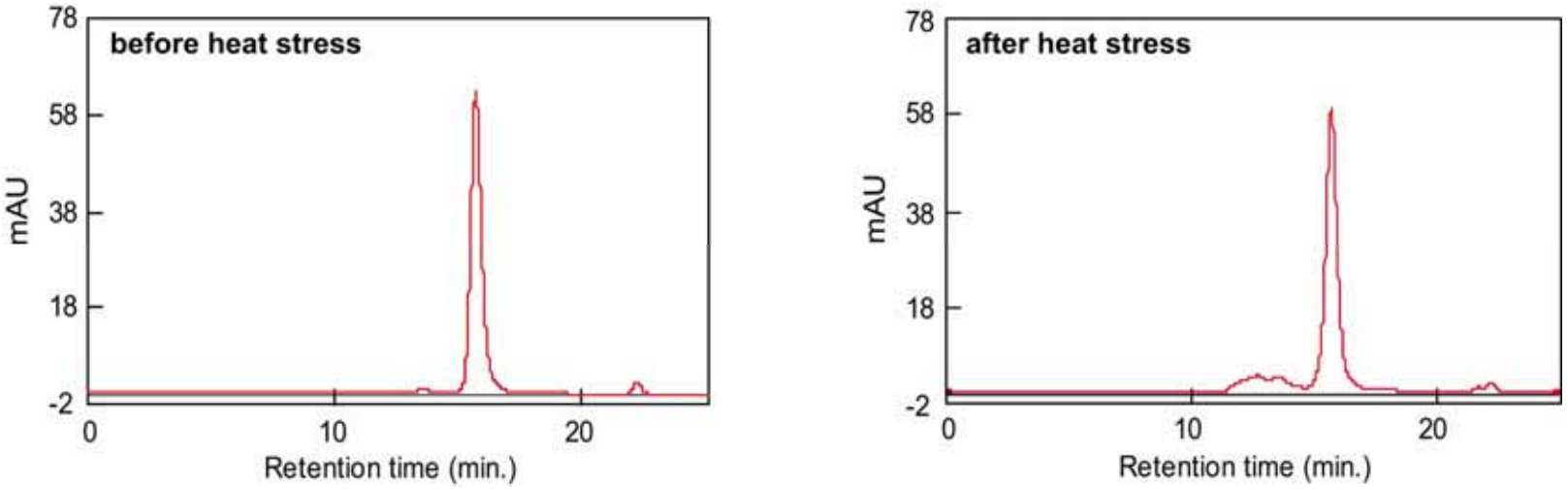
IgG monomer, dimer or aggregate was measured by size exclusion chromatography (SEC). A TSKgel G3000SW$_{XL}$ (7.8mmID x 30cm, Tosoh) was run at 0.5mL/min and a mobile phase containing 50mmol/L sodium phosphate with 200mmol/L arginine, pH 6.8.

# Table 1: Basic properties

| | Toyopearl SP-650M | Toyopearl CM-650M | Experimental resin |
|---|---|---|---|
| **Particle size** | 40 - 90µm | 40 - 90µm | 40 - 90µm |
| **Ion exchange capacity** | 0.13 - 0.17 meq/mL resin | 0.08 - 0.12 meq/mL resin | 0.10 meq/mL resin |
| **Functional group** | sulfonic acid | carboxylic acid | carboxylic acid |
| **Binding capacity** | | | |
| **lysozyme @ 106cm/hr*** | 50 | 62 | 84 |
| **lysozyme @ 212cm/hr*** | 48 | 60 | 80 |
| **lysozyme @ 424cm/hr*** | 40 | 53 | 53 |
| **IgG**** | 43 | 40 | 50 |

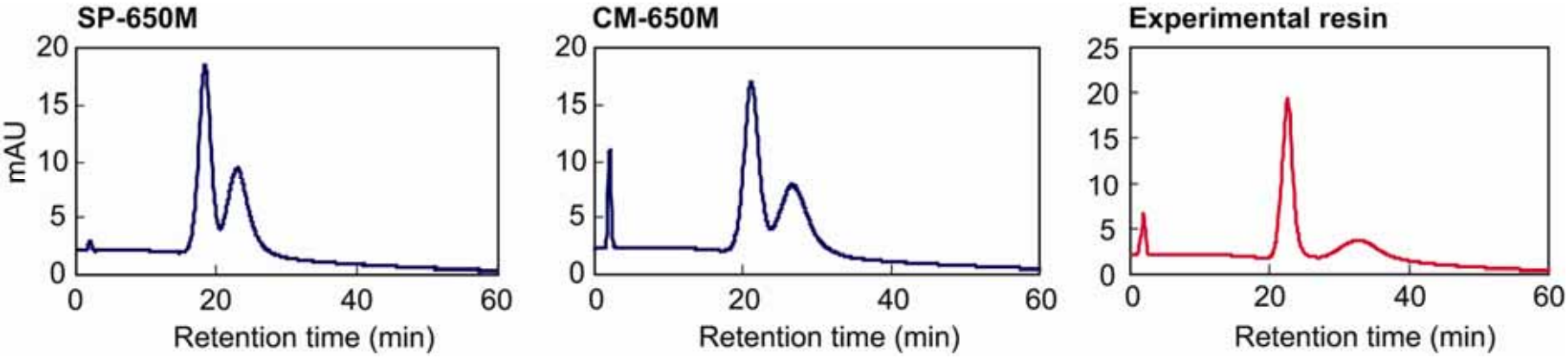
* binding capacity was measured by breakthrough curves.
** binding capacity was measured by static method at pH 4.5

# Figure 1: Effect of heat on the formation of aggregates of humanized IgG



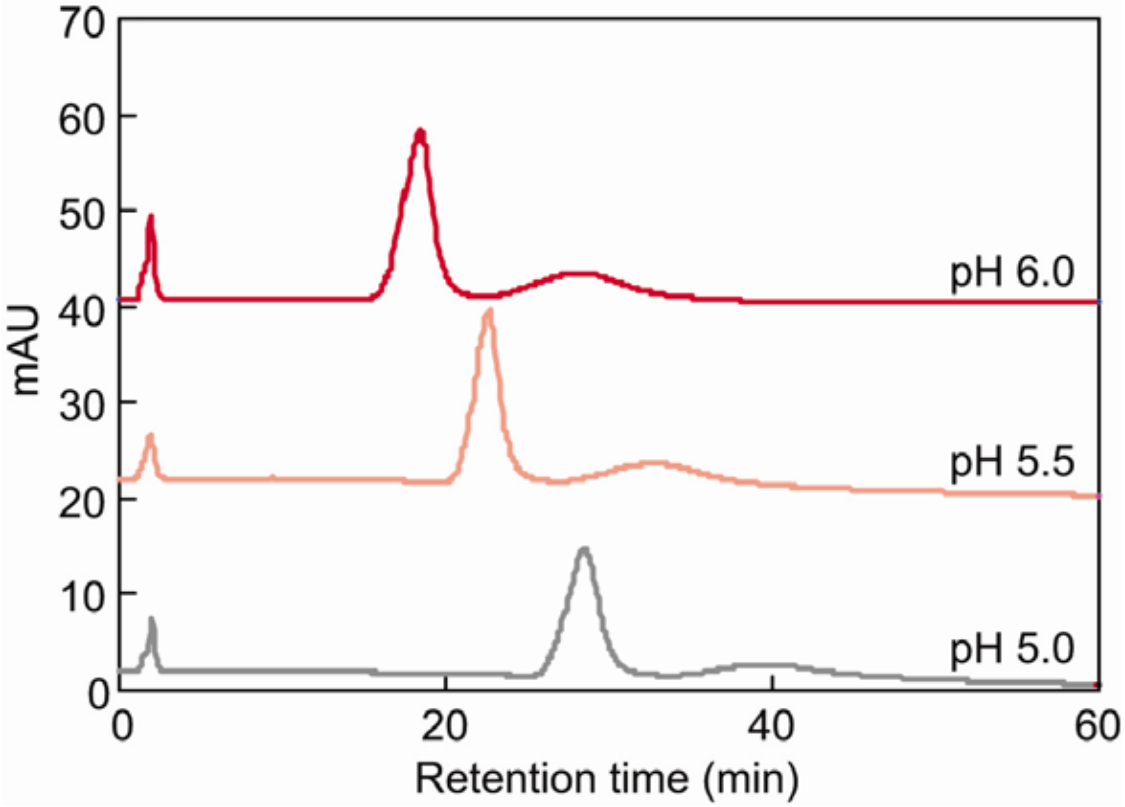

IgG samples before and after heat stress (15µg) were loaded onto a TSKgel G3000SW$_{XL}$
7.8 x 300mm column at a flow-rate of 0.5mL/min in 50mmol/L sodium phosphate containing 200mmol/L of arginine, pH 6.8.

# Figure 2: Separation of heat stressed IgG antibody by cation exchangers

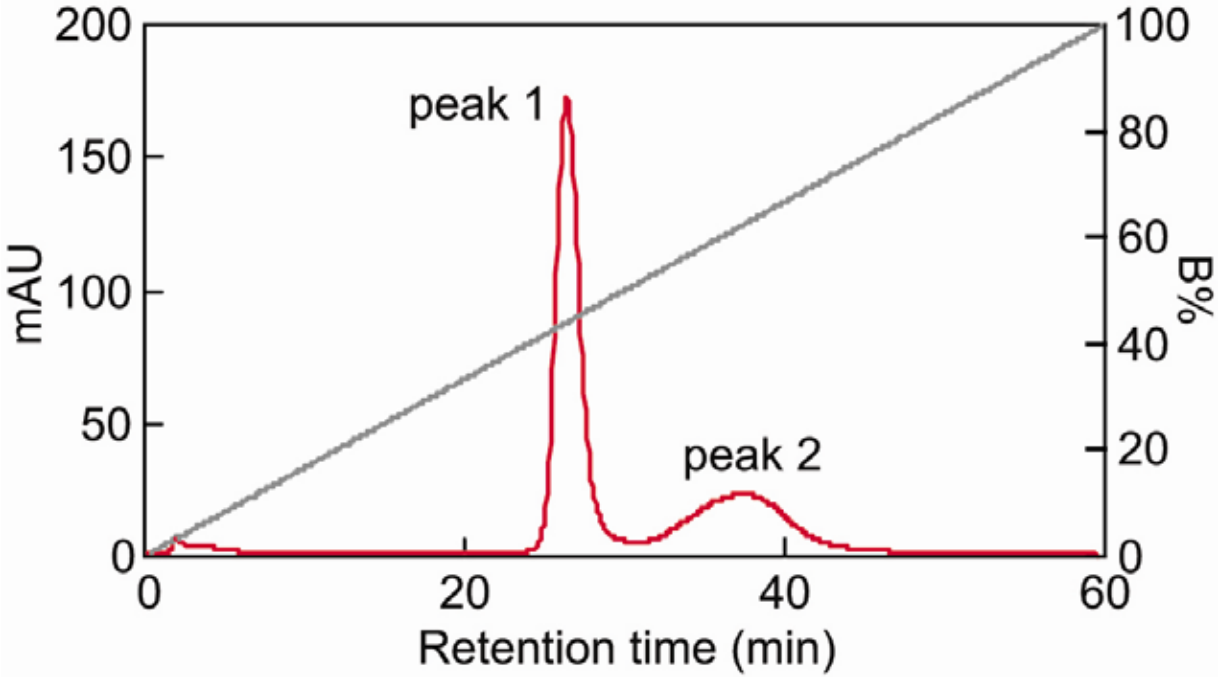



Each column was equilibrated with 50mmol/L acetate, pH 5.5 (buffer A). The heat stressed IgG (100µg) was loaded onto each column at a flow-rate of 0.5mL/min. The IgG was then eluted with a 60-min linear gradient from 0 to1.0mol/L NaCl in buffer A.

# Figure 3: Effect of pH on IgG separation using the experimental resin

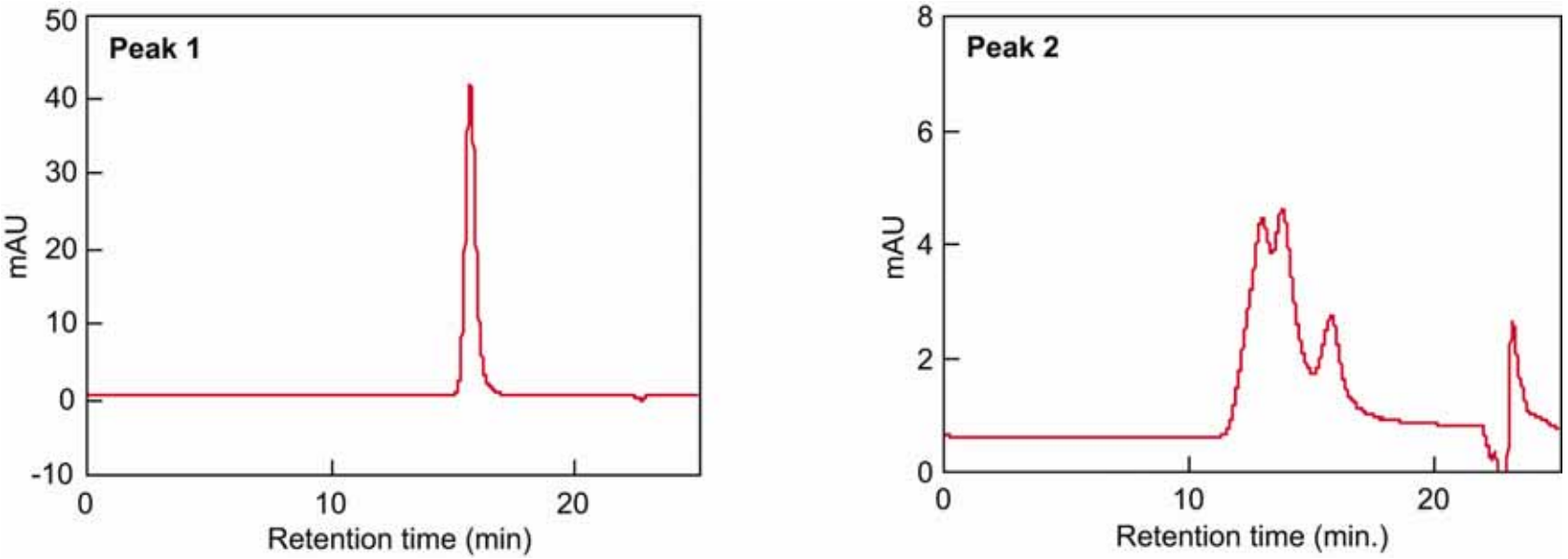


# Figure 4: Purification of IgG monomer with linear gradient using the experimental resin



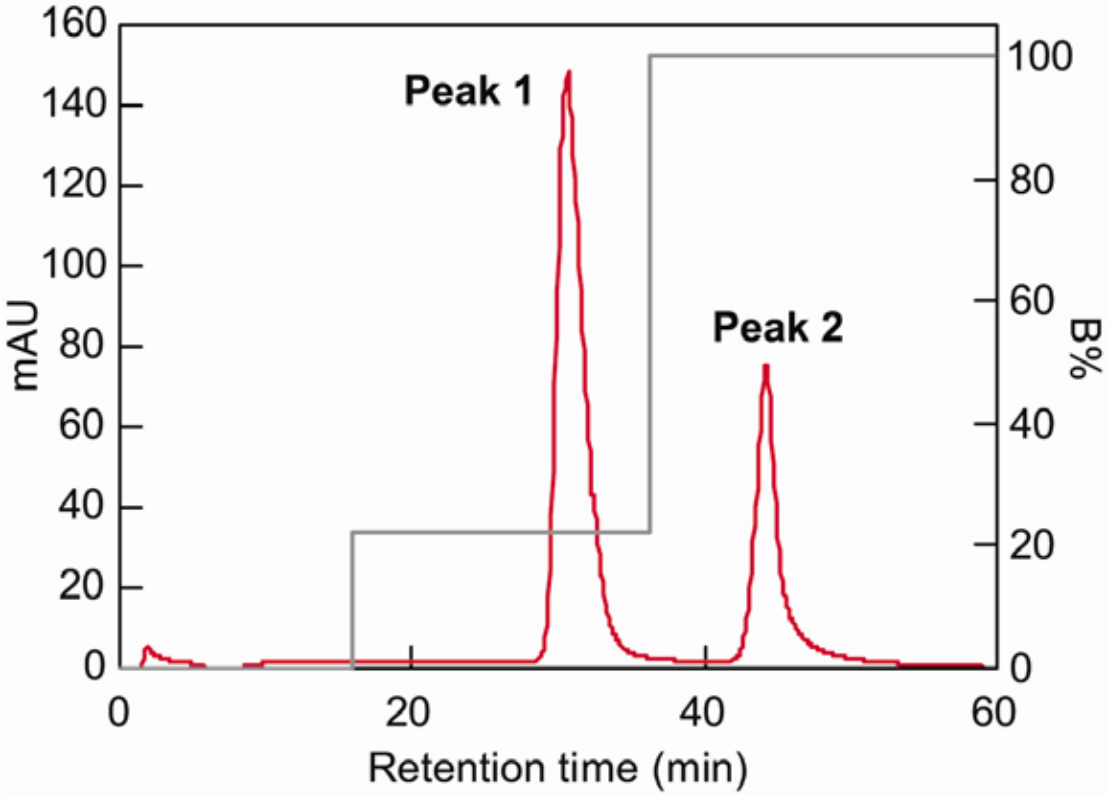

The column was equilibrated with 50mmol/L acetate, pH 5.5 (buffer A). The heat stressed IgG (4mg) was loaded onto the column at a flow-rate of 0.5mL/min. The IgG was then eluted with a 60-min linear gradient from 0 to1.0mol/L NaCl in buffer A.

# Figure 5: SEC chromatograms of fractions from figure 4



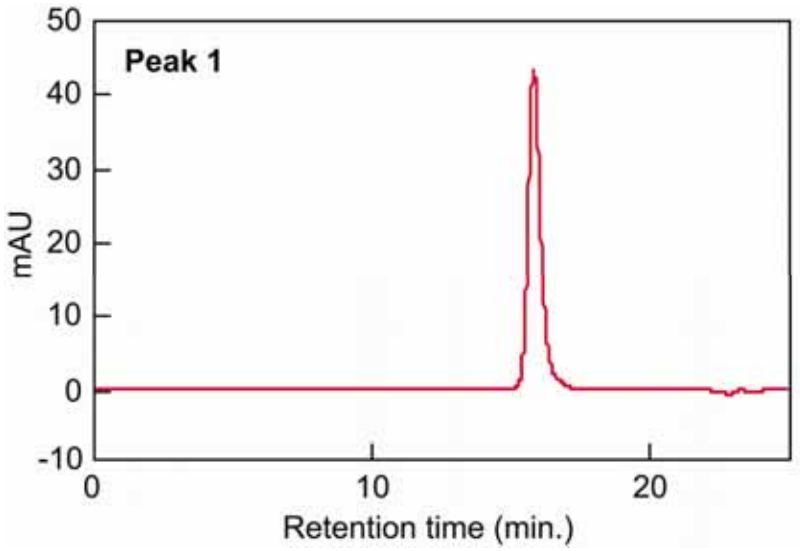

The fractions of peak 1 and 2 in figure 4 were loaded onto a TSKgel G3000SW$_{XL}$ at a flow-rate of 0.5mL/min.

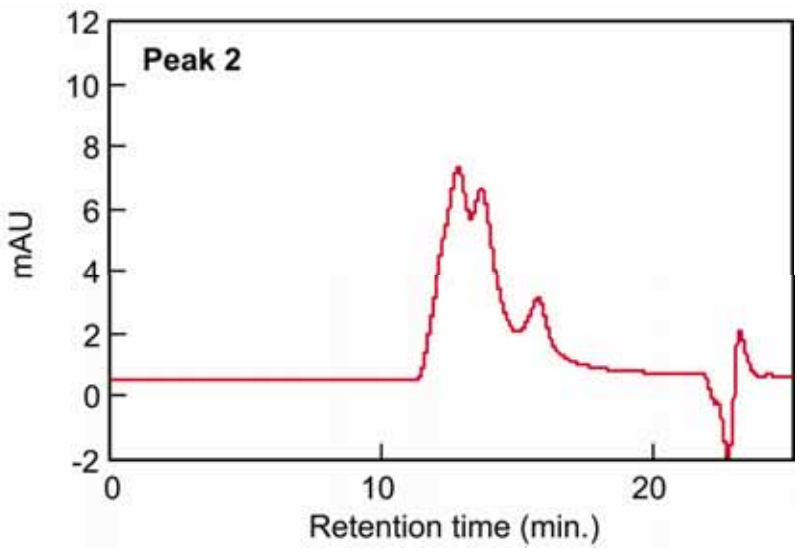
# Figure 6: Purification of IgG monomer by stepwise salt gradient on the experimental resin




The column was equilibrated with 50mmol/L acetate, pH 5.5 (buffer A). The heat stressed IgG (4mg) was loaded onto the column at a flow-rate of 0.5mL/min. The IgG was then eluted by a stepwise gradient of 0.22 and 1.0 mol/L NaCl in buffer A.

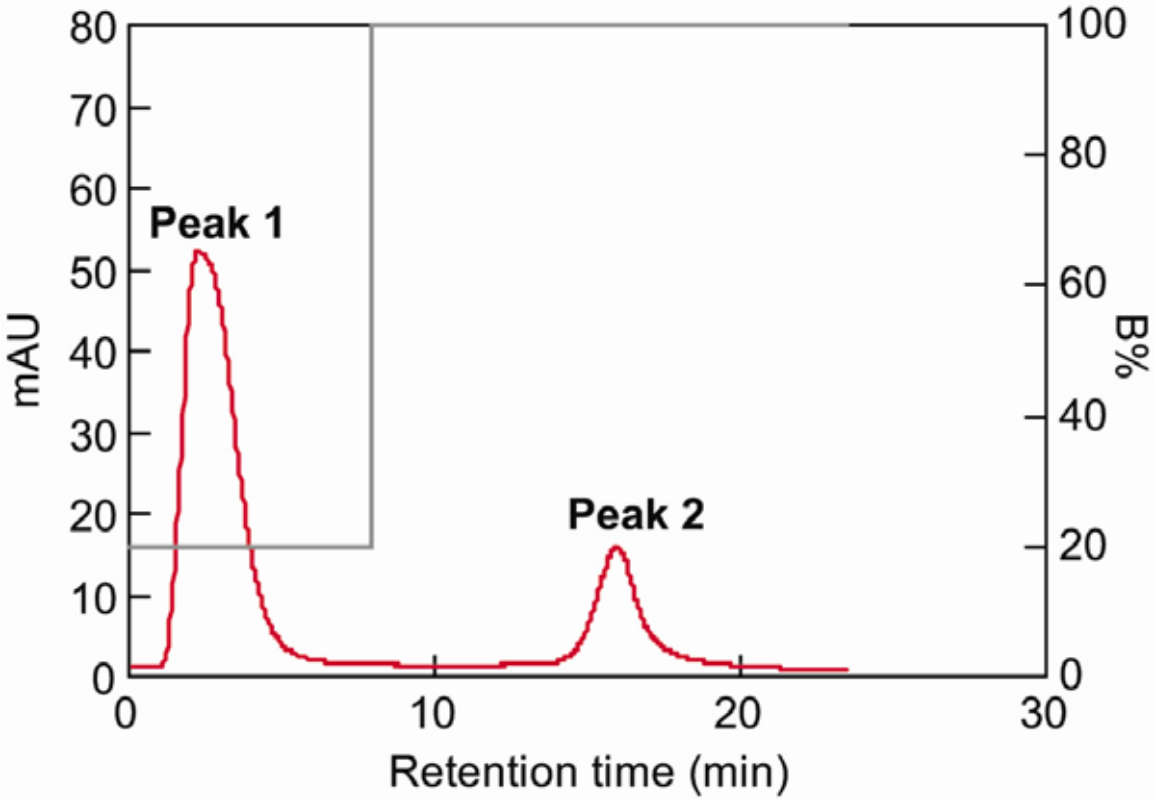
# Figure 7: SEC chromatograms of fractions from figure 6



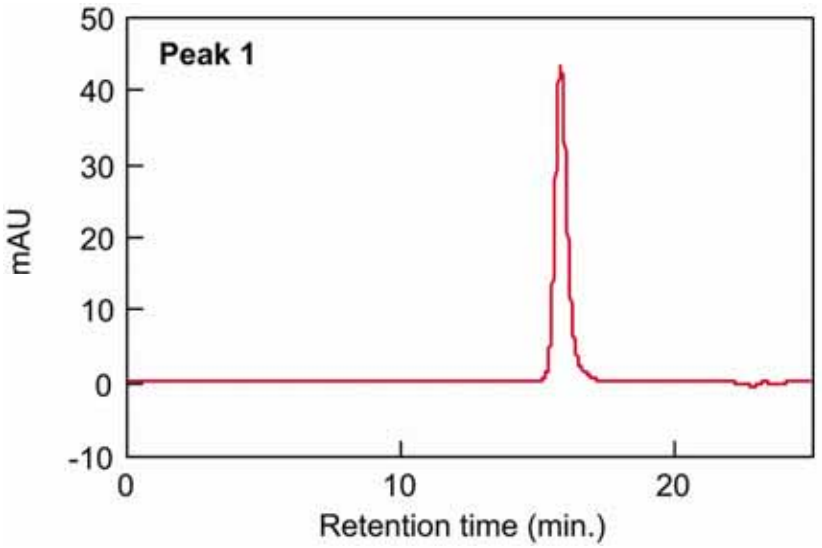

The fractions of peak 1 and 2 in figure 6 were loaded onto a TSKgel G3000SW$_{XL}$ at a flow-rate of 0.5mL/min.

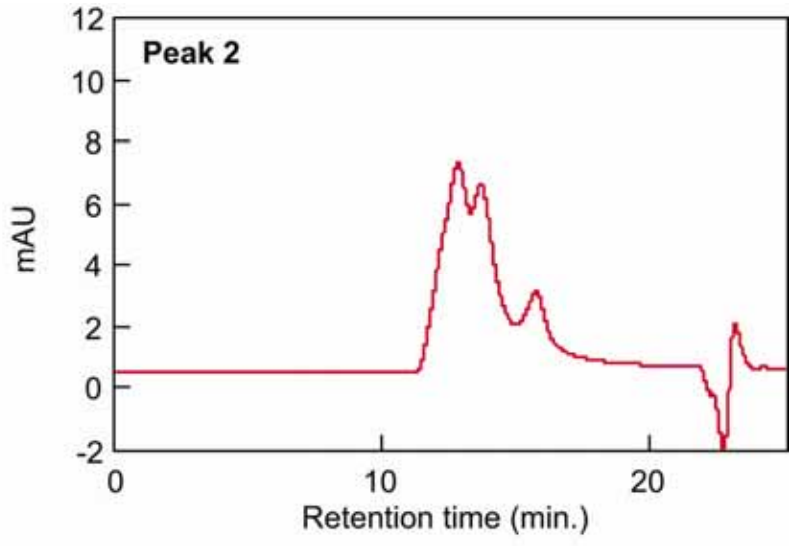
# Figure 8: Purification of IgG monomer by flow-through mode on the experimental resin




The column was equilibrated with 50mmol/L acetate containing of 200mmol/L NaCl, pH 5.5 (buffer A). The heat stressed IgG (1mg) was loaded onto the column at a flow-rate of 0.5mL/min. The IgG was then eluted by a stepwise gradient of 1.0mol/L NaCl in buffer A.

# Figure 9: SEC chromatograms of fractions from figure 8




The fractions of peak 1 and 2 in figure 8 were loaded onto a TSKgel G3000SW$_{XL}$ at a flow-rate of 0.5mL/min.

# Discussion

Table 1 lists the basic characteristics of the three different cation exchange resins used in this study. The experimental resin had higher binding capacity for lysozyme and human polyclonal IgG than SP-650M and CM-650M.

Figure 1 shows SEC chromatograms of humanized IgG before and after heat stress. We have artificially increased the concentration of dimers and aggregates because this humanized IgG purified by Protein A affinity chromatography was fairly low in these isoforms. Using this technique, the concentration of dimers and aggregates was increased from 3 to 15% in IgG according to the peak areas in the chromatograms.

Figure 2 describes the separation of heat stressed IgG by cation exchange chromatography. The peak with greater absorbancy corresponds to IgG monomer and the peak with lower absorbancy corresponds to dimers and aggregates. The carboxylic type resins were more retentive than sulfonic type under the conditions employed here and the experimental resin had better resolution between monomer and aggregates.

Figure 3 exhibits the effect of pH on IgG separation utilizing the experimental resin. As expected, the retention time decreased with increasing pH. However, no significant effect on the selectivity was observed.

# Discussion Cont.

Figures 4-7 demonstrates the purification of IgG monomer using bind-and-elute modes on the experimental resin. In ion-exchange chromatography, elution can be performed by linear or stepwise gradient. Linear gradient elution provides better resolution. In contrast, stepwise elution is more amenable to large scale processes. Figures 4-7 show that IgG monomer can be purified effectively by both linear and stepwise gradient elutions on the experimental resin. The recoveries of the IgG monomer (Peak 1) in the linear and stepwise gradient elutions were 92% and 94%, respectively. The purities of the IgG monomer in both elutions were estimated to be greater than 99.5% according to the SEC on G3000SW$_{XL}$.

Figures 8 and 9 illustrate the purification of IgG monomer using flow through mode. In some cases it may be more advantageous to actually select conditions at which the IgG monomer will flow through the column while dimers and aggregates will bind. This mode of separation is often referred to as "flow through mode" or "negative chromatography". The experimental resin could also be utilized in this mode. By adjusting the pH and ionic strength of both the sample solution and the loading buffer (buffer A), IgG monomer was purified effectively in this mode. The recovery of the IgG monomer (Peak 1) was 95% and the purity was estimated to be greater than 99.5% based on the SEC on G3000SW$_{XL}$.

# Conclusions

We have demonstrated that the experimental resin has:

- improved selectivity for IgG monomer and aggregates separation
- better resolution between the monomer and aggregates

*and*

- IgG monomer was purified with high recoveries (greater than 90%) and high purity (greater than 99.5%)
- IgG monomer was purified by not only stepwise or linear gradient elution but also in a flow though mode