Purification of MAb by cation exchange chromatography in a flow-through mode

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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 aggregates in therapeutic antibody purification. This was accomplished by optimizing the pore size and surface modifications of the resin.

The new experimental resin had different selectivity for 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 in a flow through mode. The recovery of IgG monomer was greater than 90% and the purity of monomer was estimated greater than 99.5 by size exclusion chromatography.

We also present results for separation of protein A from IgG monomer.

Introduction

Therapeutic antibodies have become the major product in the biopharmaceutical industry. Most of the antibodies are purified by protein A affinity chromatography as a capture step followed by two or three 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 require additional chromatography steps. Although cation exchange, hydrophobic interaction, or ceramic hydroxyapatite chromatography has been used to remove aggregates, several technical issues need to be addressed in each chromatography step. To overcome these problems, we have developed a novel chromatographic support.

In this poster, we present 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 6 mmI.D. ×4 cm. Buffered solution (50 mmol/L glycine, pH 9.0) containing 5 mg/mL of lysozyme were loaded onto the column at various flow velocities. Breakthrough curves were monitored by a UV detector at 280 nm. The dynamic binding capacity was determined at 10% breakthrough.

Antibody

The antibody used was humanized IgG_1 with pI of 8.2 and purified using protein A affinity chromatography.

Chromatographic apparatus

Most experiments were carried out on a liquid chromatography system ÄKTAprime (GE Healthcare) or HPLC system (Tosoh) at 25°C.

Ion exchange chromatography (pH screening)

The experimental resin was packed in a column (6 mm I.D. X 1 cm). The column was equilibrated with a starting eluent (Eluent A: 20mmol/L sodium acetate, pH 5.0 and 5.5, sodium phosphate, pH 6.0 and 6.5). After loading of proteins, elution was carried out with the final elution eluent (Eluent B) containing 0.5 mol/L NaCl in eluent A.

Purity check of IgG

Fractions were collected and fractions were examined with size exclusion chromatography. A TSKgel G3000SW_{XL} (7.8 mmI.D. ×30 cm, Tosoh) was run at 0.5 mL/min using an eluent containing 50 mmol/L sodium phosphate and 300mmol/L NaCl, pH 6.8.

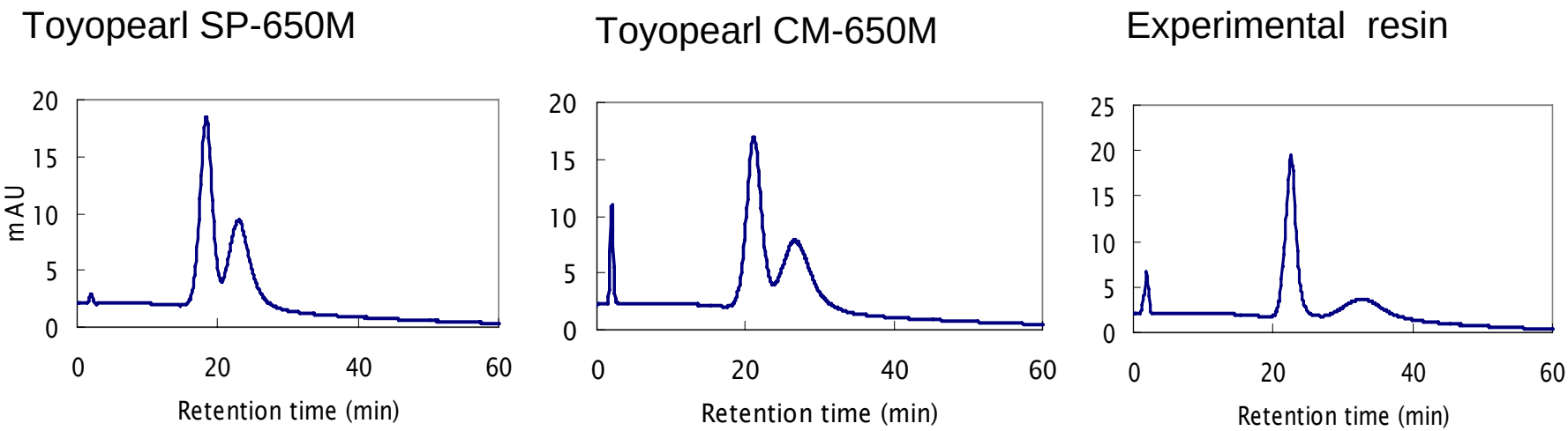
Table 1. Basic properties

	SP-650M	CM-650M	Experimental resin
Particle size (μm)	40-90	40-90	40-90
Ion exchange capacity	0.13-0.17 (meq/ml resin)	0.08-0.12 (meq/ml resin)	0.1 (meq/ml resin)
functional group	sulfonic acid	carboxylic acid	carboxylic acid
Binding capacity:			
lysozyme[a)] at 106 cm/hr	50	62	84
at 212 cm/hr	48	60	80
at 424 cm/hr	40	53	53
IgG[b)]	43	40	50

a)Binding capacity was mesured by breakthrough curves.
b)IgG binding capacity was measured by static method at pH 4.5.

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

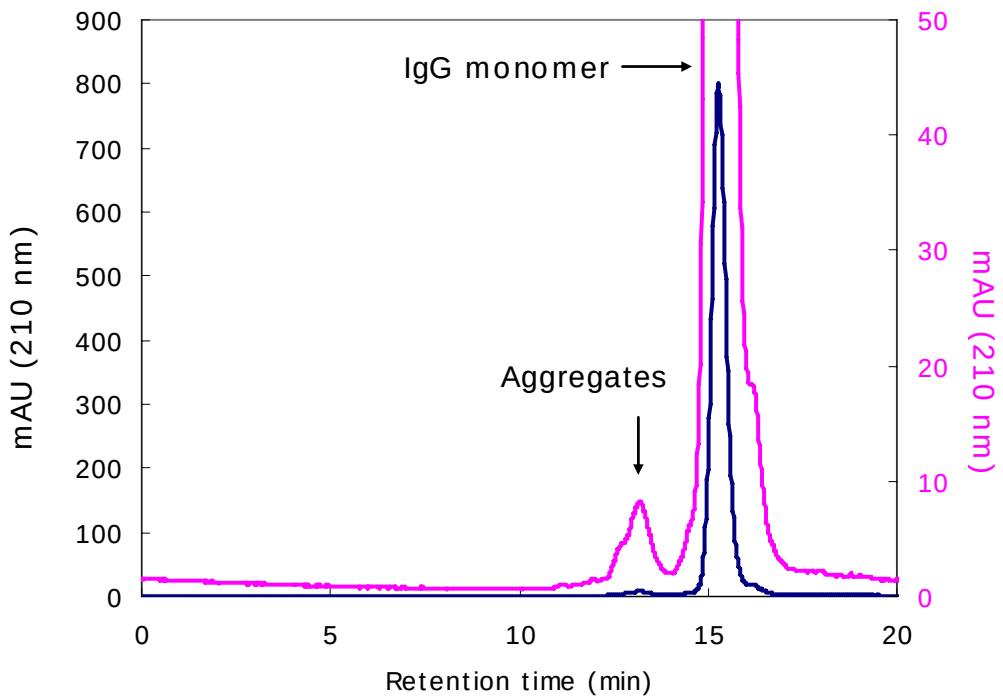
Fig. 1 Separation of heat stressed antibody by cation exchangers



Each column was equilibrated with 50 mmol/L sodium acetate, pH 5.5 (buffer A). The heat stressed antibody (100mg) was loaded onto each column (6 X 40 mm) at a flow-rate of 0.5 mL/min. The sample was then eluted with a 60-min linear gradient from 0 to 1.0 mol/L NaCl in buffer A.

Fig.1 shows the separation of heat stressed IgG (50°C, 1hr) by cation exchange chromatography. The higher peak corresponds to IgG monomer and the lower peak corresponds to aggregates. We can see that carboxylic type resins were more retentive than sulfonic type and the experimental resin had the best resolution between monomer and aggregates.

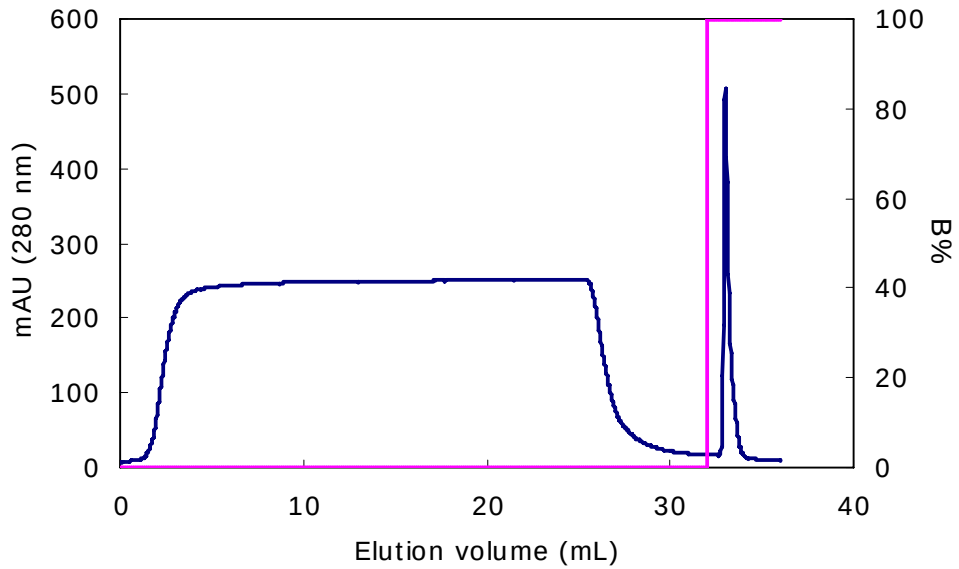
Fig. 2 Chromatogram of antibody purified by protein A affinity chromatography on TSKgel G3000SW$_{XL}$



IgG sample (5 mg) was loaded onto a TSKgel G3000SW$_{XL}$ 7.8 mmI.D. X 30 cm column at a flow-rate of 0.5mL/min in 50mmol/L sodium phosphate containing 300 mmol/L of NaCl, pH 6.8.

Fig. 2 shows SEC chromatogram of IgG purified by protein A affinity chromatography. IgG aggregates were not be able to remove by protein A affinity chromatography. We estimated aggregates contents at 1.3% judging from peak areas.

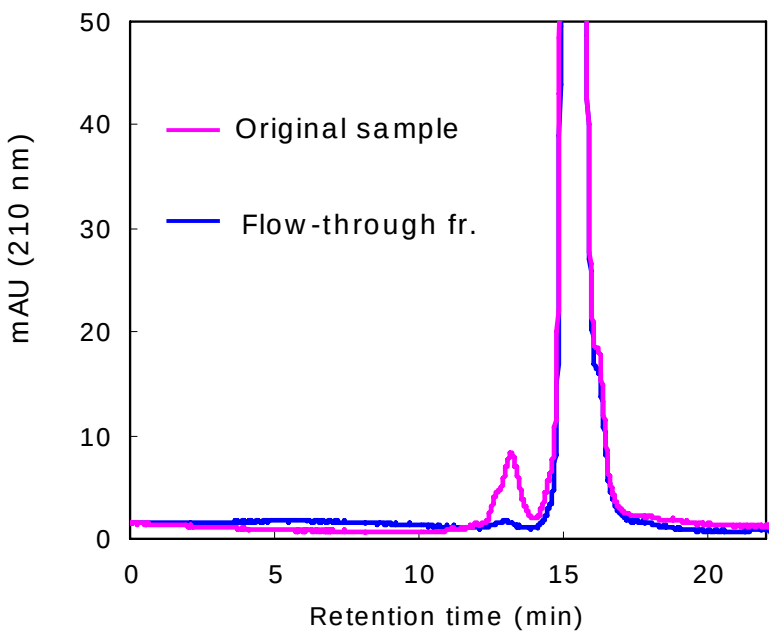
Fig. 3 Purification of IgG monomer in a flow through mode by the experimental resin

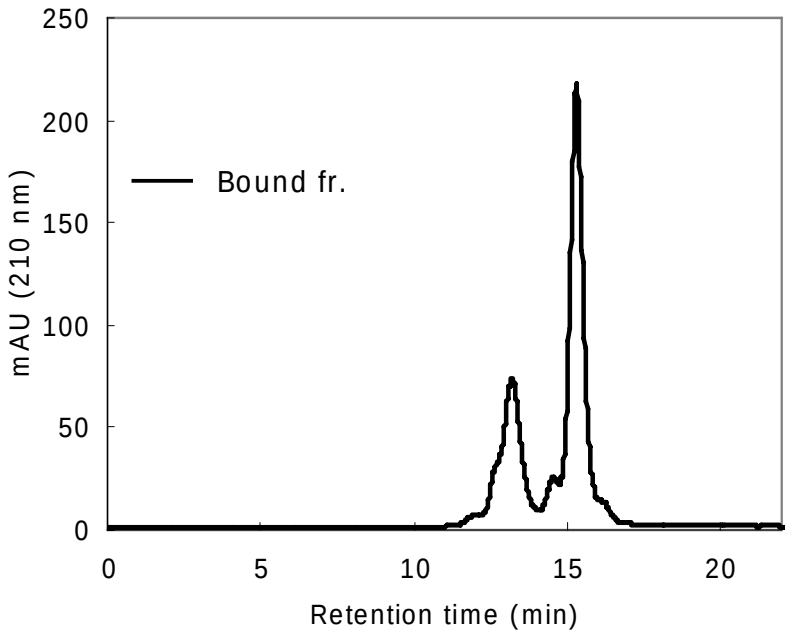


The column (6 mmI.D. X 1 cm) was equilibrated with 50mmol/L sodium acetate containing of 110 mmol/L NaCl, pH 5.5 (Eluent A). The antibody (2 mg/mL, 25 mL) was loaded onto the column at a flow-rate of 0.2 mL/min. The sample was then eluted with step wise gradient of 1.0 mol/L NaCl in buffer A.

Fig. 3 and 4 show the purification of IgG monomer with flow through mode. It may be more advantageous to actually select conditions at which IgG monomer will flow through while dimers and aggregates will bind. This mode of binding is often referred to as "flow through mode". We have tried this mode on IgG separation by using the experimental resin. Total protein recovery was 99%, and IgG contents in flow-through fractions were 91%. We can see that IgG monomer was purified effectively in this mode. We estimated the purity of IgG monomer in flow-through fractions at 99.8%.

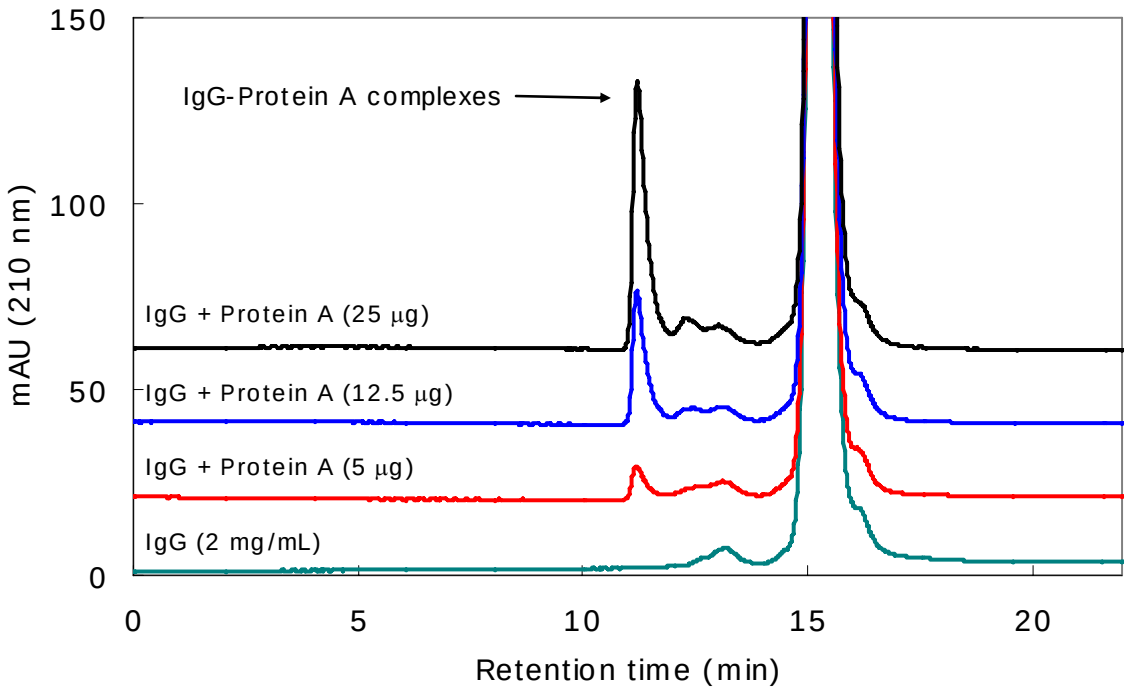
Fig.4 Chromatograms of the original sample, flow through and bound fractions





The original sample, flow-through and bound fractions in fig.3 were loaded onto a TSKgel G3000SW$_{XL}$ at a flow-rate of 0.5 mL/min.

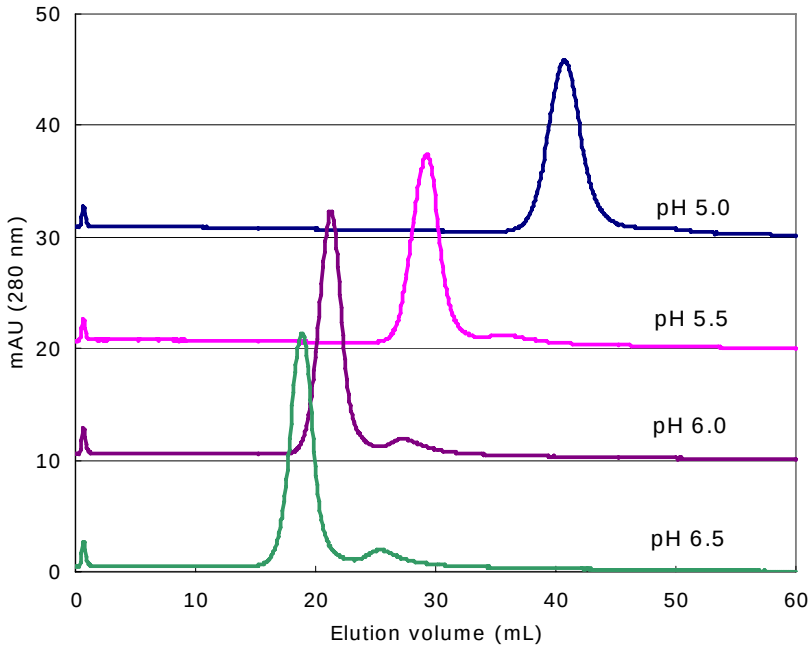
Fig. 5 Chromatograms of humanized antibody and spiked with increasing amounts of recombinant protein A on TSKgel G3000SW$_{XL}$



Each sample (2.5 mL) was loaded onto a TSKgel G3000SW$_{XL}$ 7.8 mm I.D. X 30 cm column at a flow-rate of 0.5mL/min in 50 mmol/L sodium phosphate containing 300 mmol/L of NaCl, pH 6.8.

Fig. 5 shows chromatograms of IgG and spiked with increasing amounts of recombinant protein A. IgG-protein A complexes were eluted at 11.3 min and the amounts of complexes were increased with amounts of protein A.

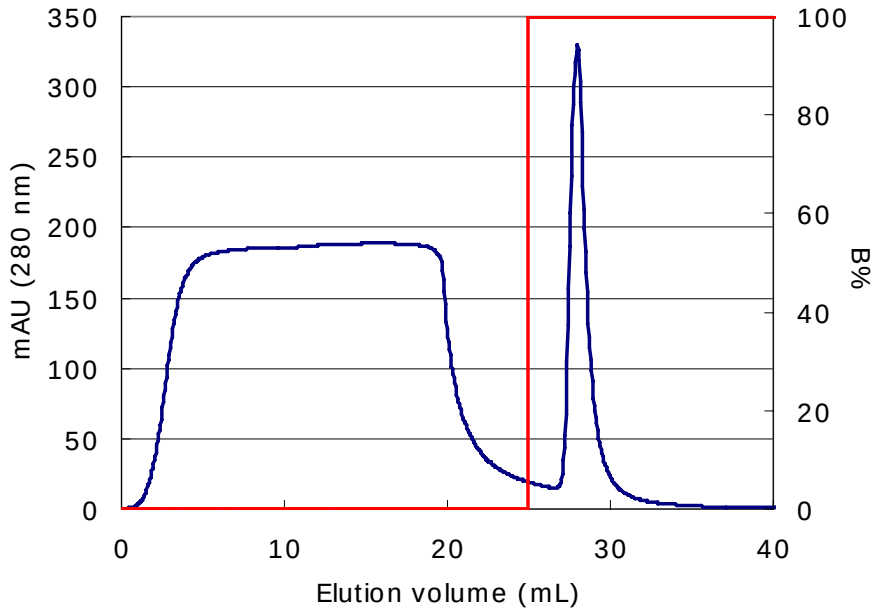
Fig. 6 Chromatograms of pH screening on IgG and spiked with protein A by the experimental cation exchanger



The column (6 mmI.D. X 1 cm) was equilibrated with 50mmol/L sodium acetate (pH 5.0 and 5.5) or 50 mmol/L sodium phosphate (pH 6.0 and 6.5) (Eluent A). A 100 mL sample containing 200 mg IgG and 2.5 mg protein A was loaded onto the column at a flow-rate of 0.5 mL/min. The sample was then eluted with a 120-min linear gradient from 0 to 0.5 mol/L NaCl in eluent A.

Fig. 6 shows the results of pH screening of protein A spiked IgG sample on cation exchange chromatography. IgG-protein A complexes were more retentive than IgG monomer.

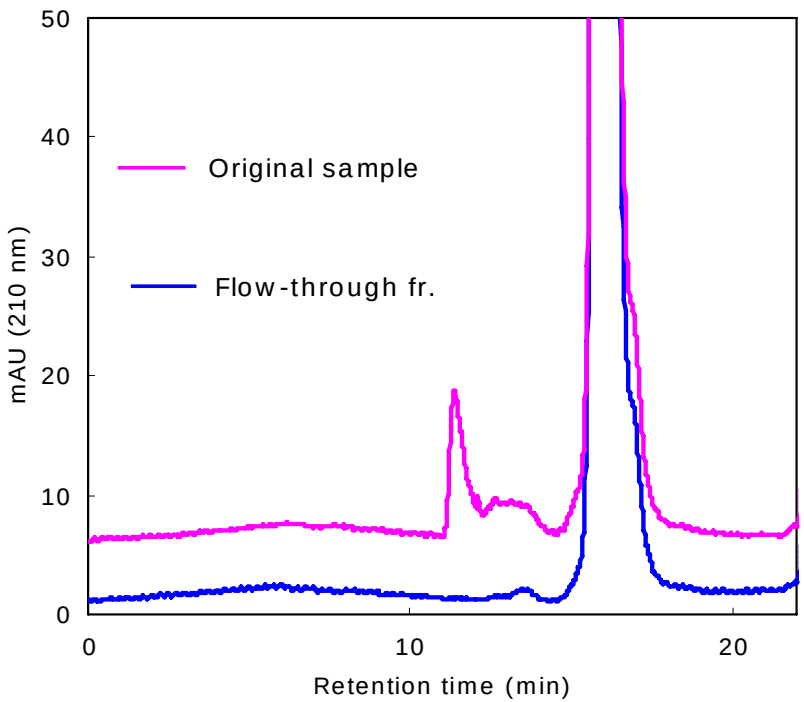
Fig. 7 Purification of IgG monomer in a flow through mode by the experimental resin

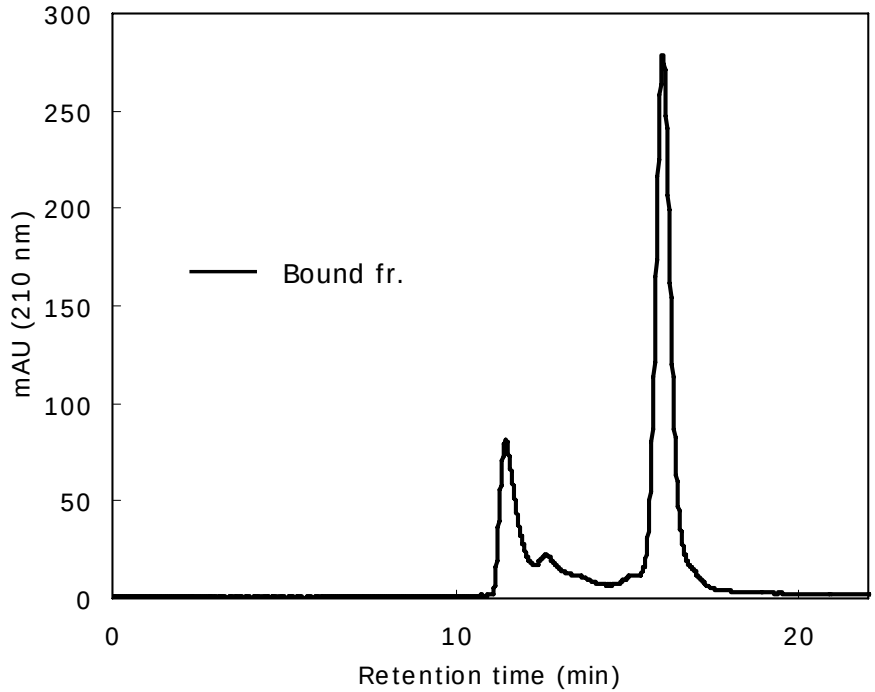


The column (6 mmI.D. X 1 cm) was equilibrated with 50mmol/L sodium acetate containing of 90mmol/L NaCl, pH 5.5 (Eluent A). Antibody (2.1 mg/mL, 20 mL containing 250 mg of protein A) was loaded onto the column at a flow-rate of 0.2 mL/min. The sample was then eluted with step wise gradient of 1.0 mol/L NaCl in eluent A.

Fig. 7 and 8 show the purification of IgG monomer from protein A spiked sample in a flow through mode. A 250 mg of protein A was spiked to 42 mg of IgG. Sample load was 150 mg/mL-gel. Flow-through fractions contained small amounts of aggregates but IgG-protein A complexes were not detected by SEC. Bound fractions contained large amounts of IgG-protein A complexes. We are going to determine the protein A amounts by ELISA.

Fig.8 Chromatograms of the original sample, flow-through and bound fractions





The original sample, flow-through and bound fractions in fig.7 were loaded onto a TSKgel G3000SW$_{XL}$ at a flow-rate of 0.5 mL/min.

Conclusions

- The experimental cation exchange resin had improved selectivity for the IgG separation
- The experimental cation exchange resin showed better resolution between IgG monomer and aggregate
- IgG monomer was purified in the flow through mode with high recoveries (greater than 90%) and high purity (greater than 99.5%)
- Leached protein A would be removed from IgG monomer on the experimental cation exchange resin in the flow through (further study is on-going)