Purification of Immunoglobulin M Using a Novel Ion-exchanger

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Abstract

The use of monoclonal antibodies (Mab) is becoming more important in diagnosis and treatment of disease. IgM in particular has shown to possess unique and beneficial characteristics relative to other immunoglobulin classes.

IgM is a large molecule comprised of five IgG subunits resulting in a relatively unstable and difficult to purify molecule. Unlike single chain antibodies, IgM cannot be purified by Protein A (an affinity material commonly used for it's high binding capacity and excellent selectivity for antibodies) due to steric hindrance. Alternative affinity methods have been developed with thiophillic absorbents but result in low binding capacity.

In this study, we examine an alternative purification method of IgM by ion exchange chromatographyusing a novel high capacity stationary phase (TSKgel BioAssist).

Experimental

HPLC

HPLC system consisted of two pumps(DP-8020), a column oven(CO-8020C), an UV detector(UV-8020) and an autosampler(AS-8020,all from TOSOH Co.,Japan).

Reagents

All proteins were purchased from Sigma(Japan). IgG and IgM was gifted by TOSOH Research Laboratories. All chemical reagents employed were from Kishida Chemicals(Oosaka,Japan).

Binding capacity

The resins were packed into 10mm x 4.6mmI.D. column. The protein solution was purged into the column and base line was monitored by UV detector to obtain a break through curve. The binding capacity was calculated by purged volume at the 10% height of the break through curve.

Purification conditions of IgM from mouse ascites fluid

Sample: 9.5mg/mL IgM in mouse ascites fluid

Experimental

Chromatography conditions:

Cation-exchange chromatography

Column:	TSKgel BioAssist S 5cm x 4.6mmI.D.
Eluent :	20mmol/L Sodium phosphate buffer pH6.0
Gradient :	NaCl
Flow rate :	1.0mL/min

Size exclusion chromatography

Column :	TSKgel BioAssist G4SWXL 30cm x 7.8mmI.D.
Eluent :	0.3mol/L NaCl in 50mmol/L Sodium phosphate buffer pH6.5
Flow rate :	1.0mL/min

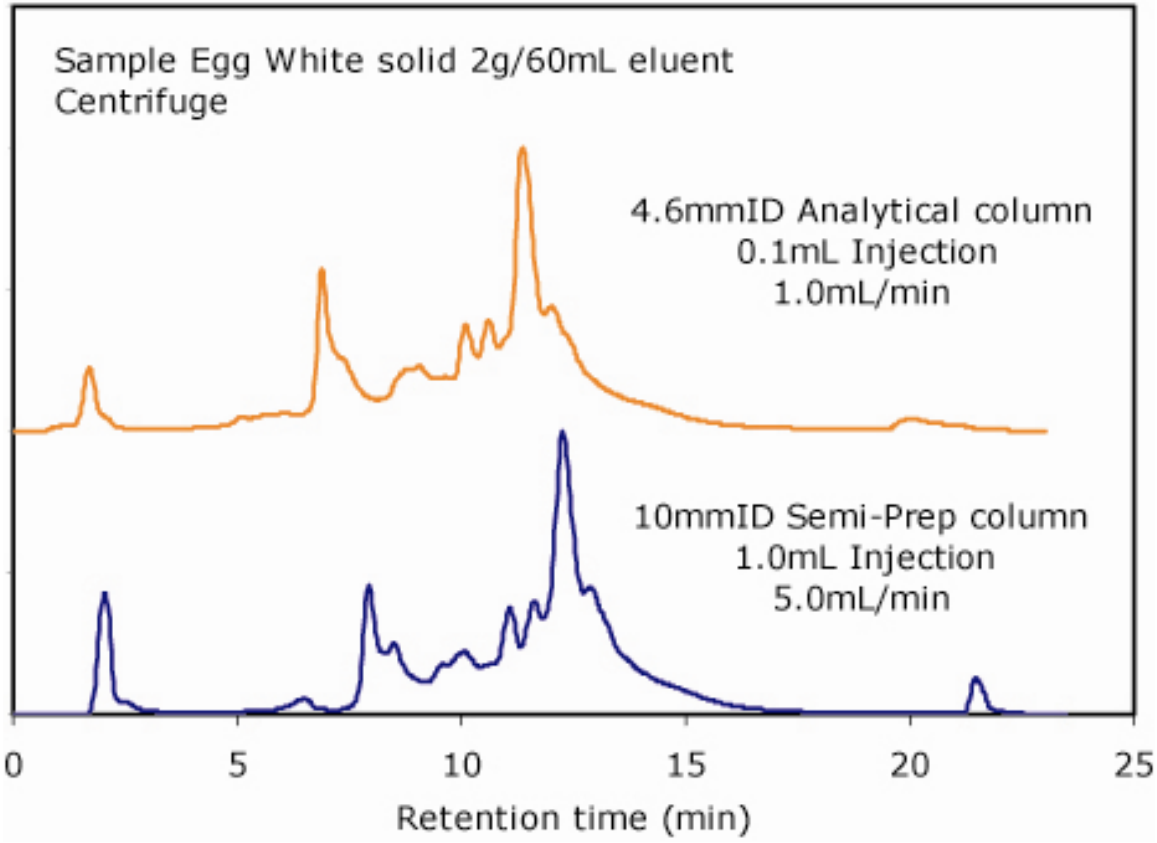
Column

Characteristics of IEC Columns evaluated

	TSKgel BioAssist Q	TSKgel BioAssist S
Base Matrix	hydrophilic resin porous	hydrophilic resin porous
Particle size		
4.6mmID column	10 μm	7 μm
10mmID column	13 μm	13 μm
Pore size (Å)	ca. 4000	ca.1300
Ion exchange	ca. 0.1meq/L	ca. 0.1 meq/L
Ion site	Polyamine	Sulfapropyl
Column size	50mm x 4.6mmID	50mm x 4.6mmID
	10cm x 10mmID	10cm x 10mmID
	PEEK	PEEK
Optimal flow rate		
4.6mmID column	10.mL/min	0.8mL/min
10mmID column	5.0mL/min (10)	5.0mL/min (10)
Loading volume	<10mg	<100mg

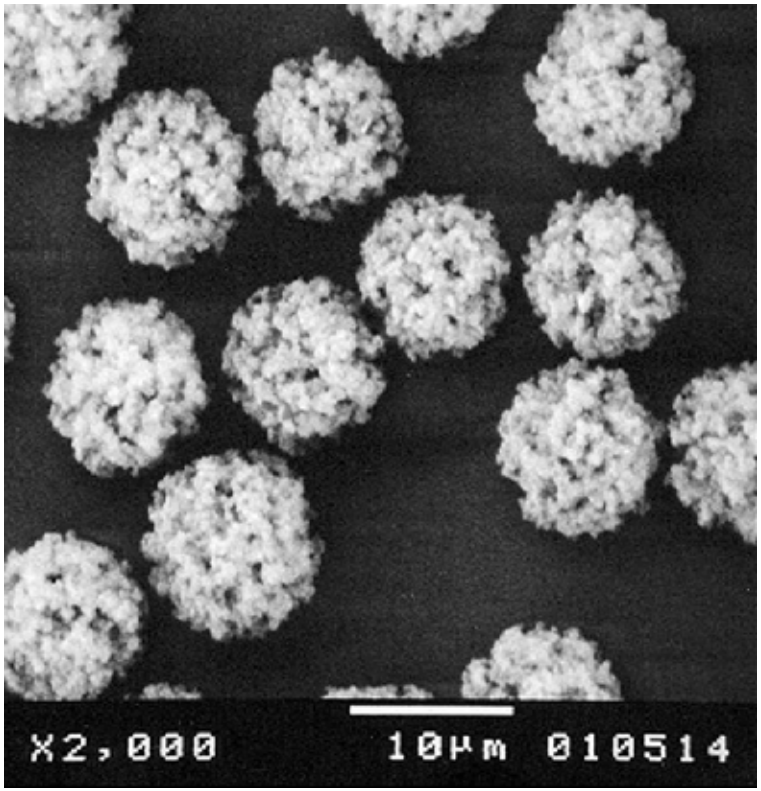
Column

Scalability comparison

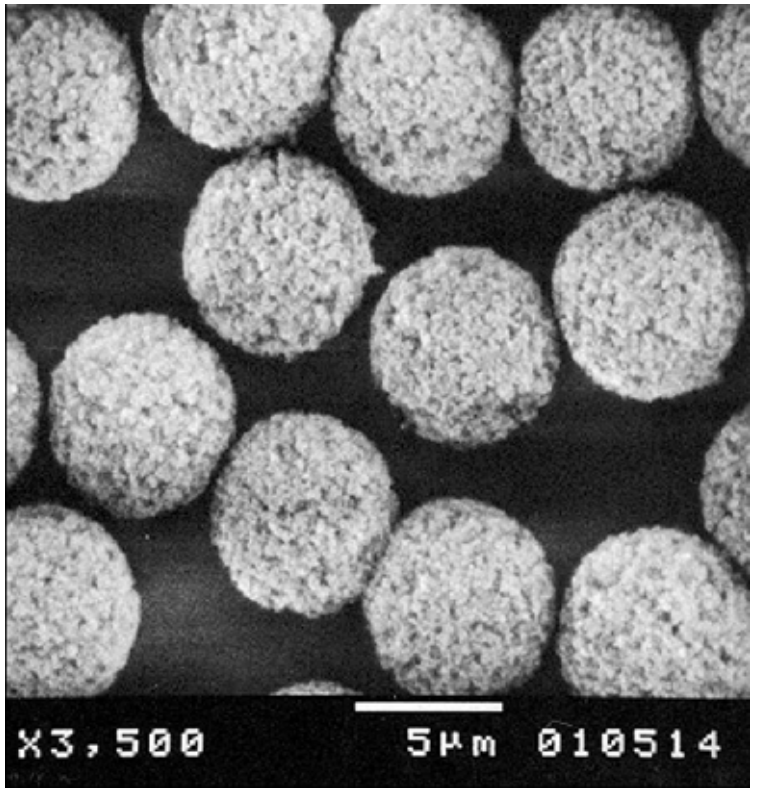


Column

SEM Photographs of BioAssist Resins



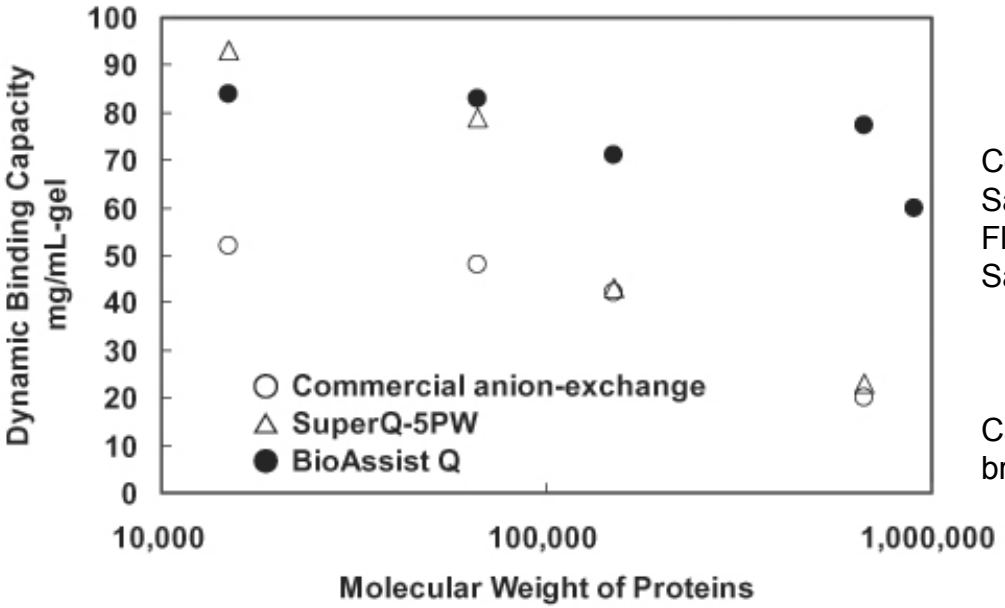
BioAssist Q Approx. 4000Å



BioAssist S Approx. 1300Å

Dynamic Binding Capacity

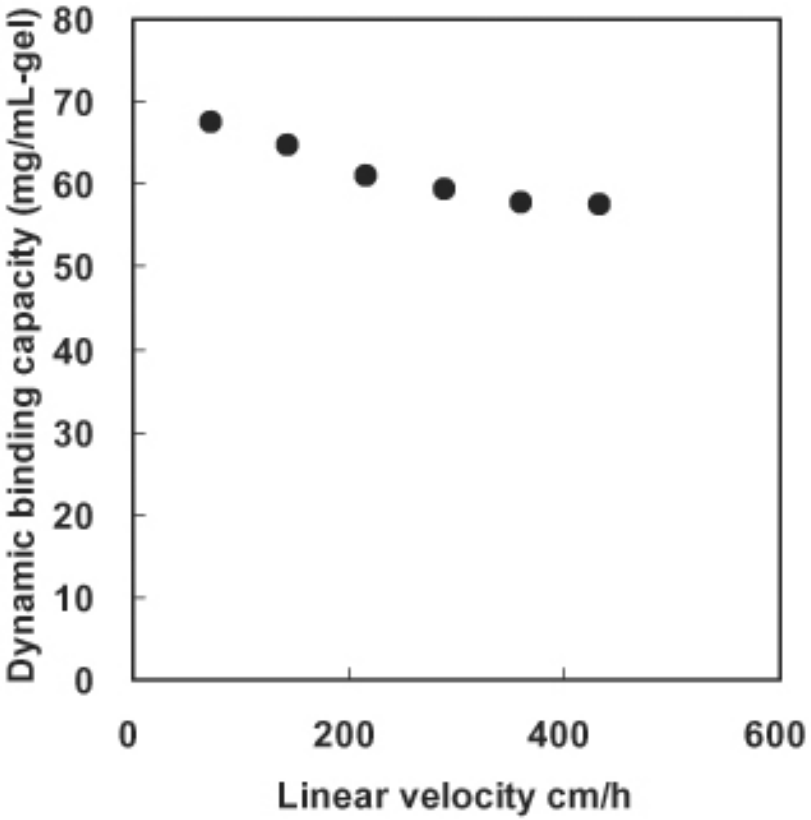
Binding Capacity vs. MW



Column size: 10mm x 4.6mmID
Sample: 20mmol/L Tris-HCl buffer (pH8.0)
Flow rate: 0.38mL/min Temperature: ambient
Sample: Trypsin inhibitor (15KDa, 10mg/mL), HAS (66.5KDa, 10mg/mL), IgG1 (150KDa, 2.3mg/mL), Thyroglobulin (66.9KDa, 5mg/mL), IgM (900KDa, 5mg/mL)
Capacities were calculated by 10% height of the breakthrough curve

Dynamic Binding Capacity

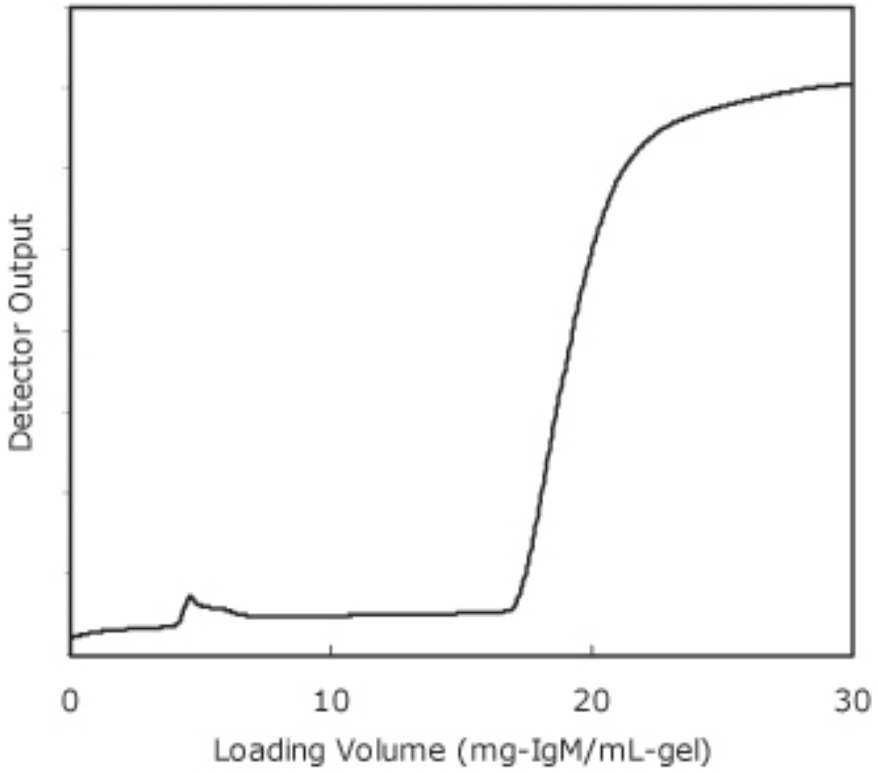
Binding Capacity vs. Velocity



Column: BioAssist Q (10 μ m), 4.6mm ID x 1cm
Sample: 5mg/mL IgM in 20mM Tris-HCl buffer pH8.0
Flow rate: 0.2mL/min (72cm/h) to 1.2mL/min (433cm/h)

Dynamic Binding Capacity

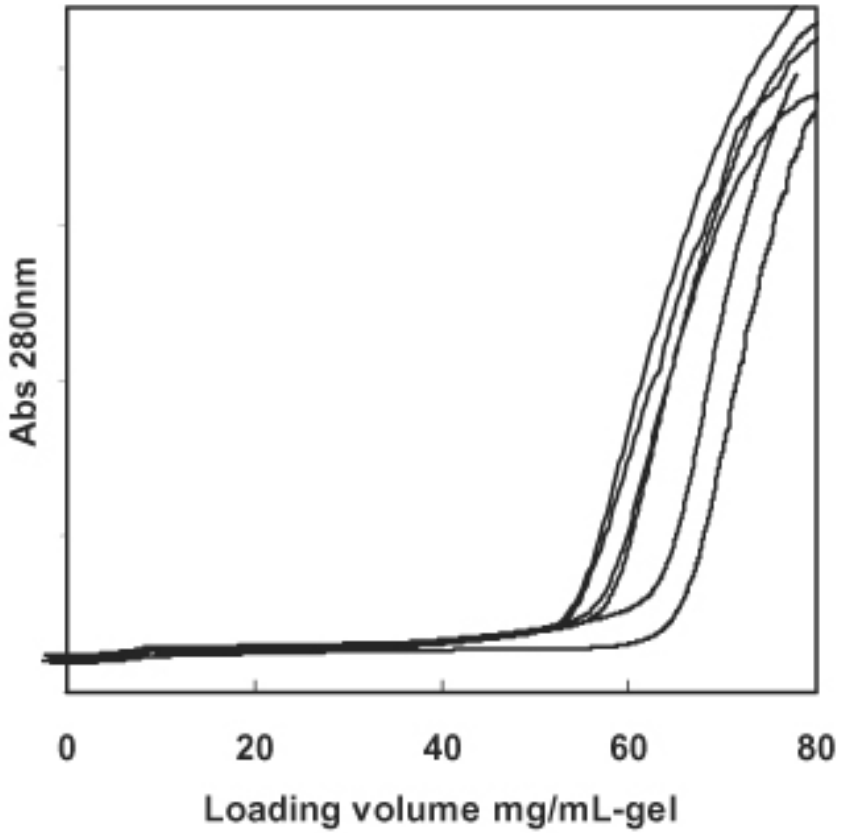
Breakthrough Curves BioAssist S



Column:	BioAssist S (7 μ m), 4.6mm ID x 1cm
Sample:	2mg/mL IgM in 20mM sodium phosphate buffer pH6.0
Flow rate:	0.38mL/min

Dynamic Binding Capacity

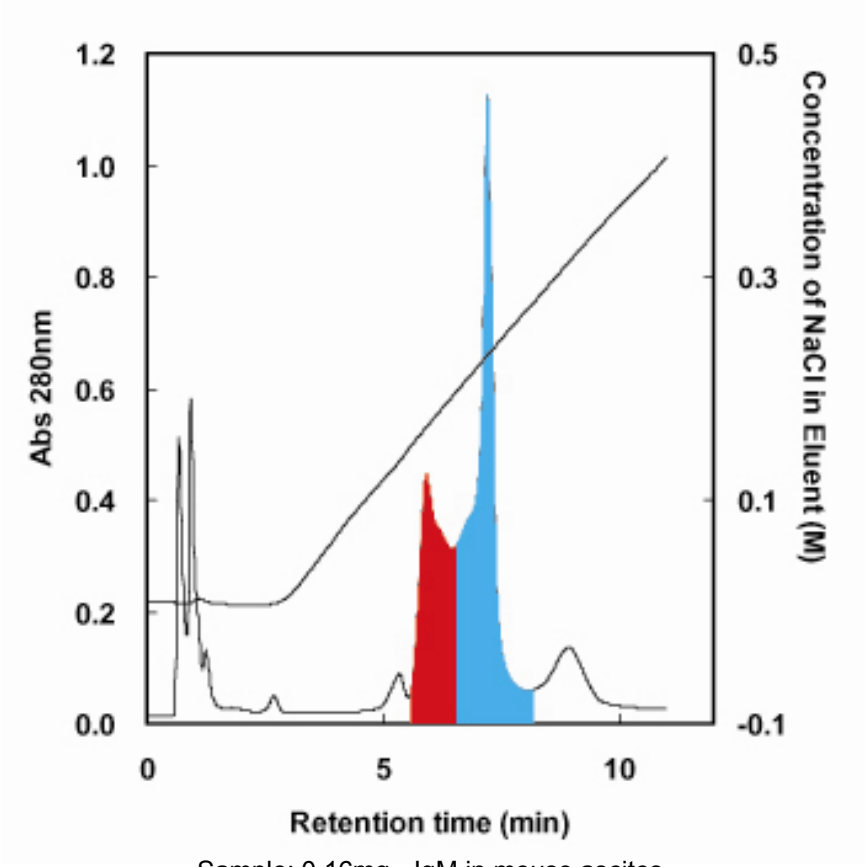
Breakthrough Curves BioAssist Q



Column: BioAssist Q (10 μ m), 4.6mm ID x 1cm
Sample: 5mg/mL IgM in 20mM Tris-HCl buffer pH8.0
Flow rate: 0.2mL/min (72cm/h) to 1.2mL/min (433cm/h)

Separation of IgMs

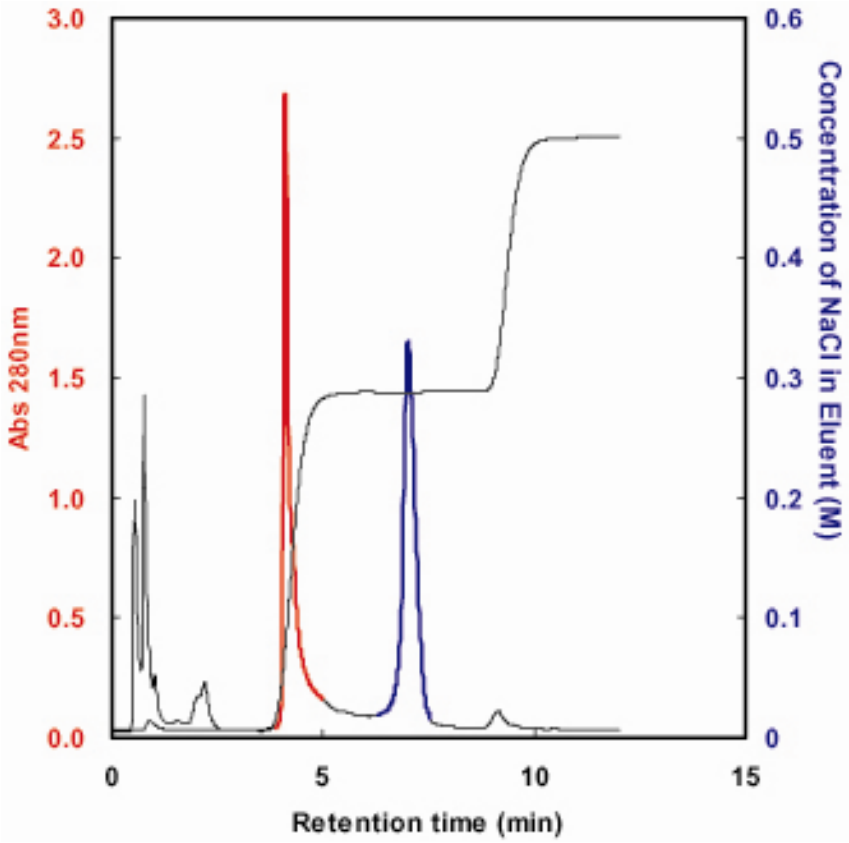
Separation of IgM in mouse ascites on BioAssist S by linear gradient of NaCl



Sample: 0.16mg - IgM in mouse ascites

Separation of IgMs

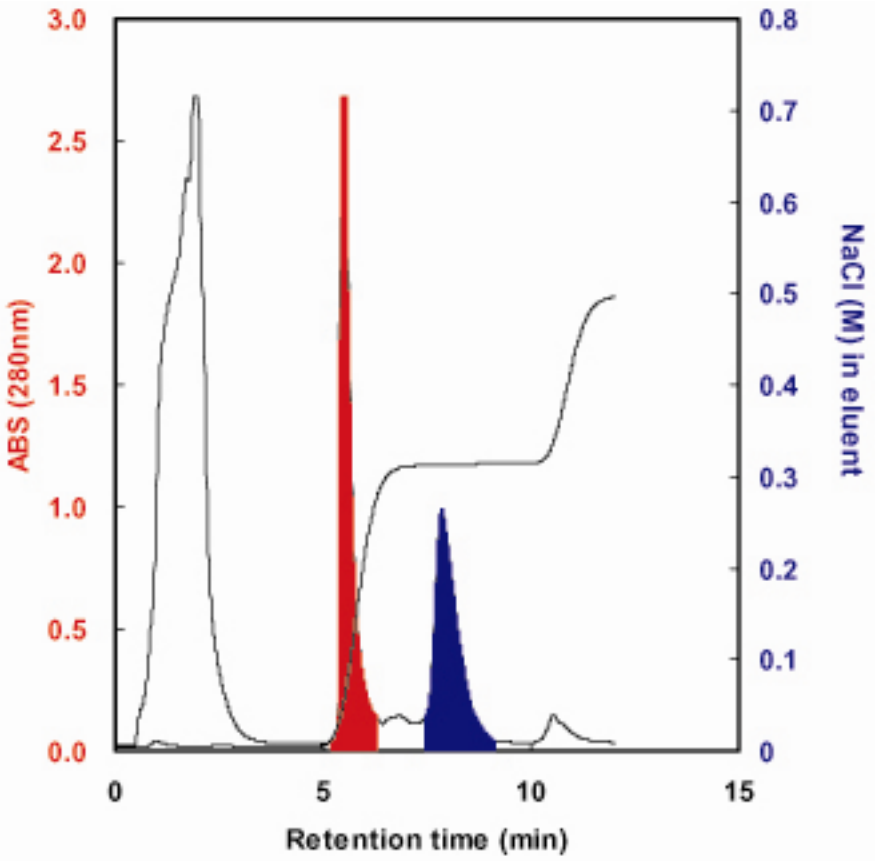
Separation of IgM in mouse ascites on BioAssist S by step gradient



Sample: 0.16mg - IgM in mouse ascites

Separation of IgMs

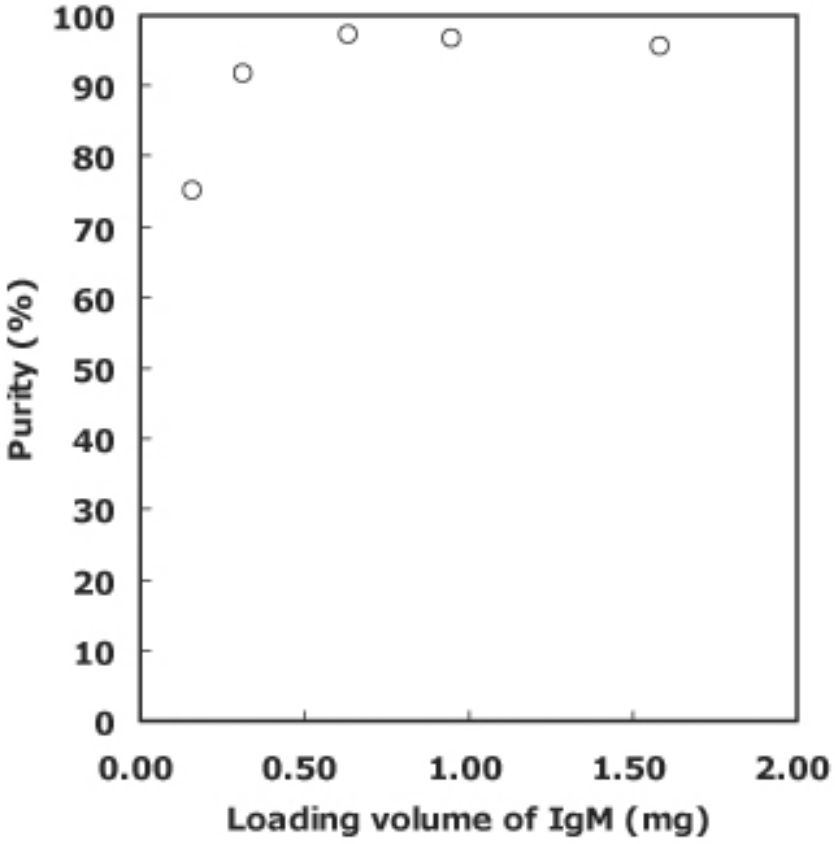
Separation of IgM in mouse ascites on BioAssist S



Sample: 4.75mg - IgM in mouse ascites

Separation of IgMs

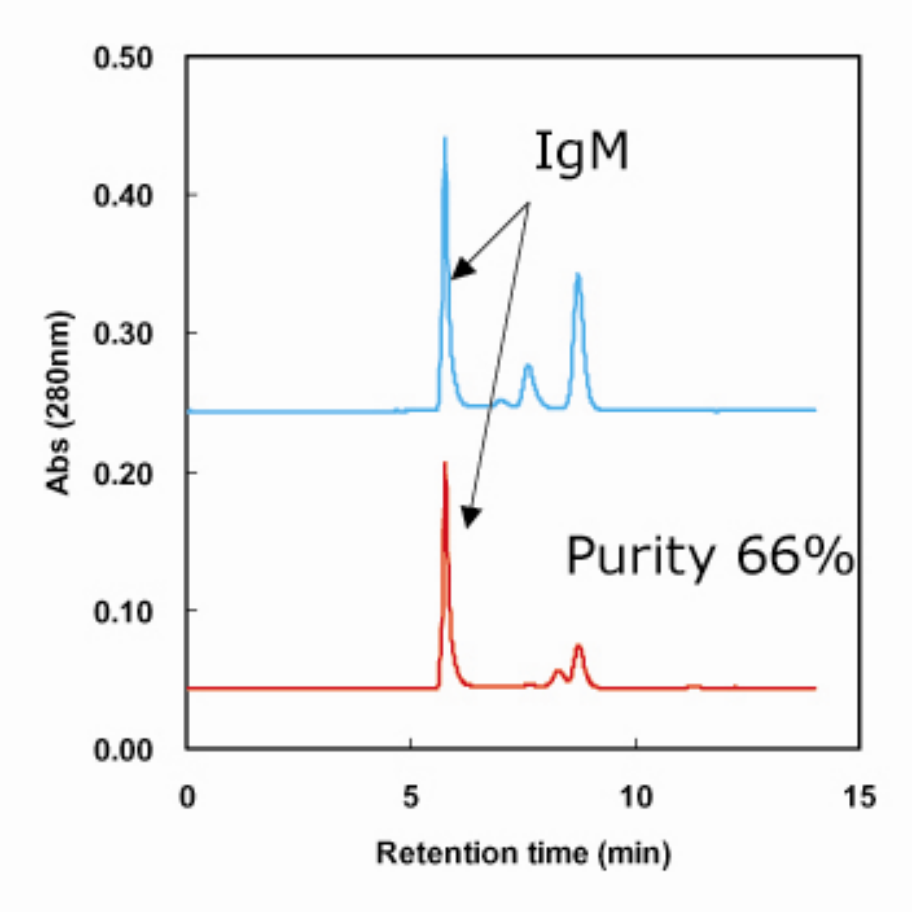
Influence of loading volume



Conditions: IgM eluted with step gradient of 0.3M NaCl

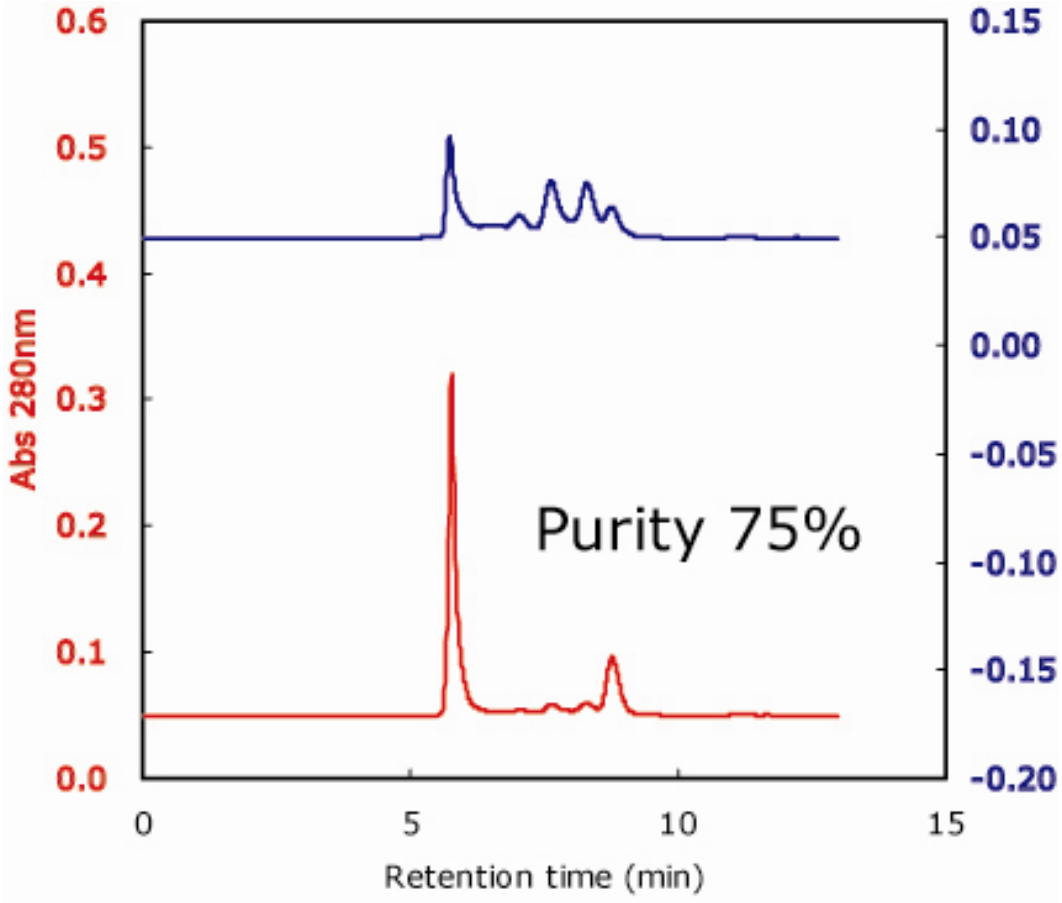
Separation of IgMs

Purity check by SEC on BioAssist G4SW$_{XL}$



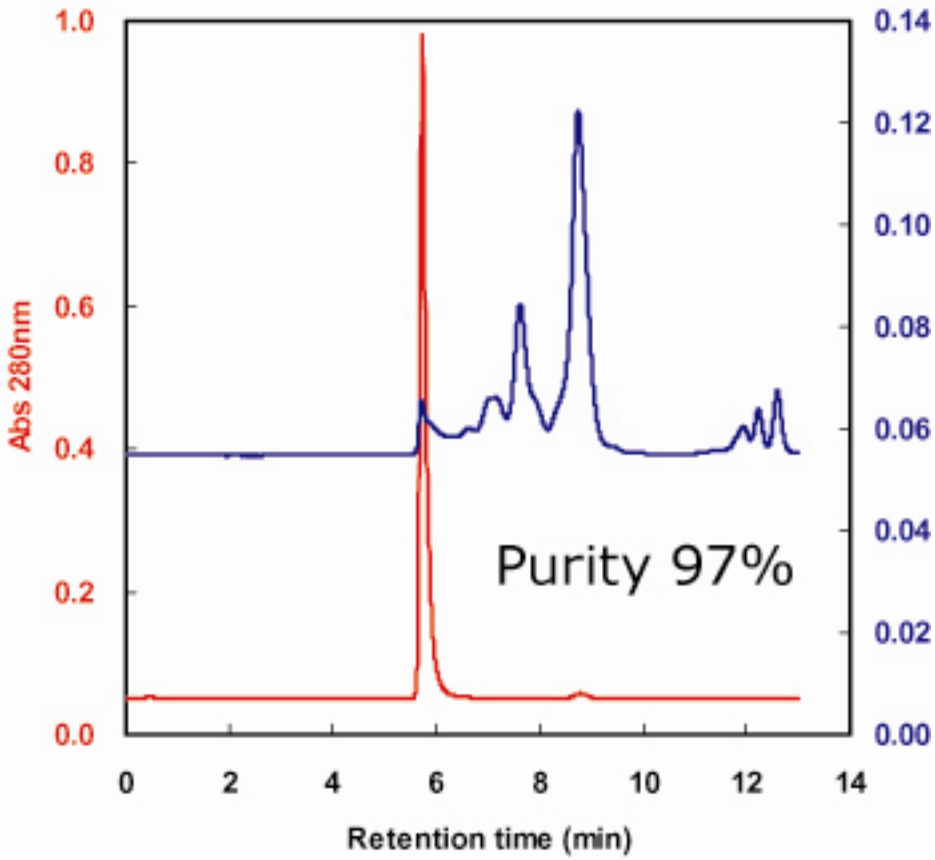
Separation of IgMs

Purity check by SEC on BioAssist G4SW$_{XL}$



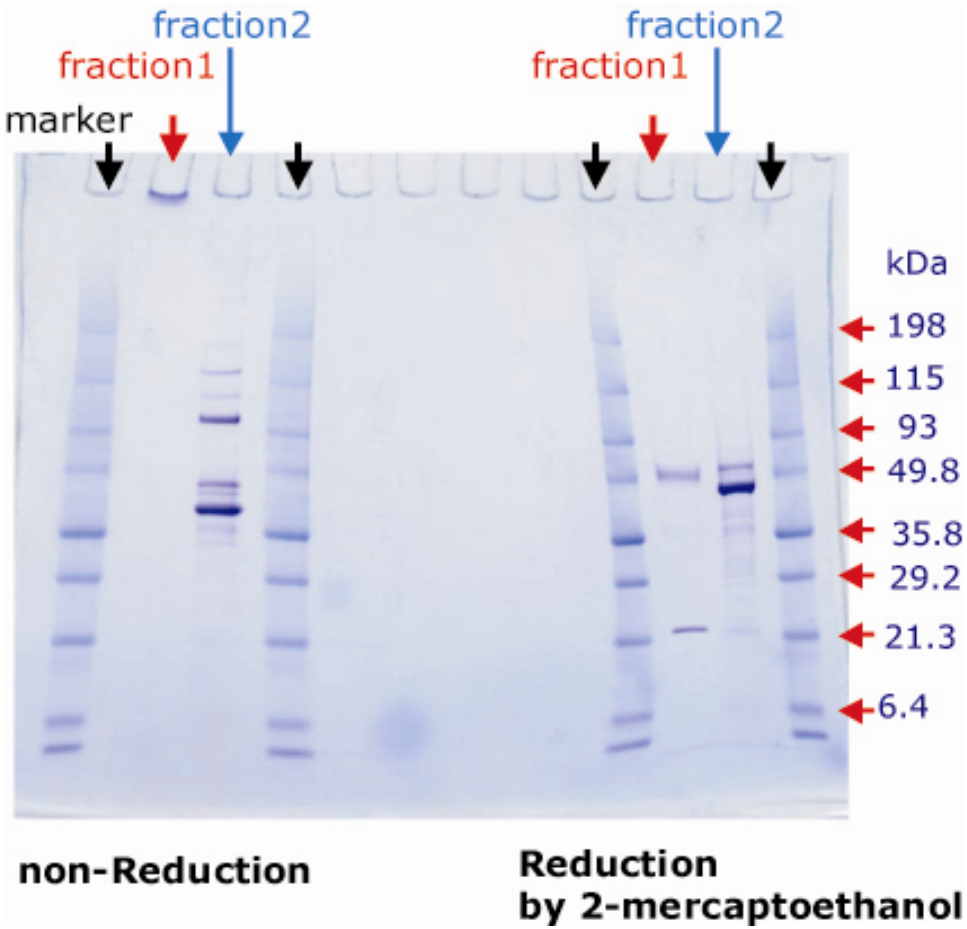
Separation of IgMs

Purity check by SEC on BioAssist G4SW$_{XL}$



Separation of IgMs

SDS-Page



Conclusions

- Binding capacity of the TSKgel BioAssist materials remains high (ca. 60mg/mL) over a wide molecular weight and flow rate range relative popular conventional porous materials.

- In the separation of IgM purified from mouse ascites fluid, purity was shown to improve with increased sample load. A purity of 97% was obtained when 4.75mg IgM was loaded onto the column. This is postulated this phenomenon may be the result of competitive displacement of albumin at the ion exchange sites as IgM load increases.