Separation of IgM using TSKgel BioAssist S

TSKgel
APPLICATION NOTE

Abstract

A novel macroporous ion exchange material has been developed to provide high retention and capacity of large biomolecules such as IgM. TSKgel BioAssist columns are offered in an analytical format and most recently in a new 13micron particle size for semi-preparative purifications.

Introduction

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; it 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 often result in low binding capacity.

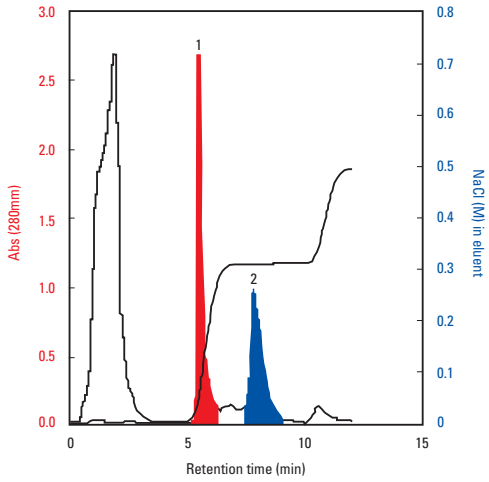
In this note we examine an alternative purification method of IgM by ion exchange chromatography using a novel high capacity stationary phase.

Results

As shown in *Figure 1*, baseline separation of IgM from other contaminants is achieved using a 0.3M NaCl step gradient after elution of albumin. Linear gradient separations, although possible, did not yield equivalent baseline separations (data not shown). Purity analysis by size exclusion of both fractions taken during anion exchange is shown in *Figure 2*. The IgM fraction was determined to be 97% pure. Interestingly, our work showed that increasing the sample load increased the purity yield of the IgM fraction. *Figure 3* shows that below 0.75mg loading, the purity decreases rapidly. It is postulated that the IgM competitively displaces the albumin from the sulfpropyl binding sites, accounting for the increase in purity as the IgM concentration increases.

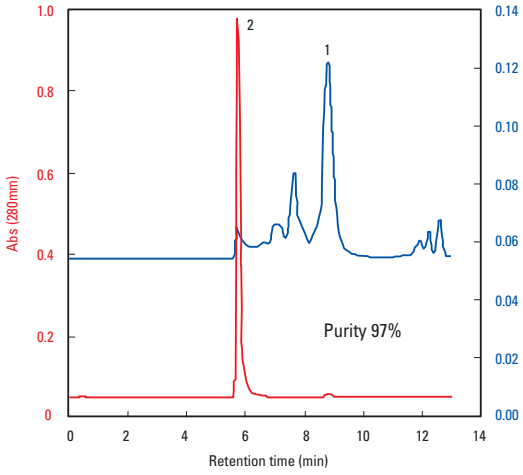
The separation performance is maintained up to capacities of over 60mg/mL of resin (data not shown). Researchers with a need to purify amounts greater than the 20mg/column possible with the analytical format should use the recently commercialized 13micron semi-preparative column. To compensate for the loss in resolution attributed to the increase in particle size, the semi-preparative column was lengthened relative to the analytical format.

Figure 1. Separation of IgM by Cation Exchange



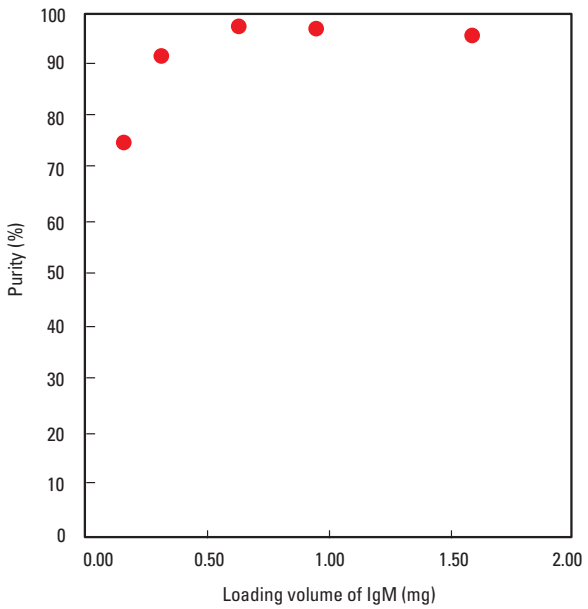
Columns:	TSKgel BioAssist S, 7µm, 4.6mmID x 5cm, PEEK Housing
Eluent:	20mmol/L sodium phosphate buffer, pH 6.0
Gradient	0mol/L - 0.3mol/L NaCl @ t = 5 min., 0.3mol/L - 0.5mol/L NaCl @ t = 10 min.
Flow Rate:	1mL/min.
Sample:	500µL of 9.5mg/mL IgM in mouse ascites fluid; shaded peaks represent albumin and IgM respectively
Detection:	UV @ 280nm
Instrument:	Tosoh HPLC

Figure 2. Purity Determination by Size Exclusion Chromatography



Columns:	TSKgel BioAssist G4SW$_{XL}$, 8µm, 7.8mmID x 30cm, PEEK Housing
Eluent:	0.3mol/L NaCl in 50mmol/L sodium phosphate buffer, pH 6.5
Flow Rate:	1mL/min
Sample:	Fractions 1 and 2 from Cation Exchange Purification
Detection:	UV @ 280nm
Instrument:	Tosoh HPLC

Figure 3. Influence of Loading Volume



IgM eluted with step gradient of 0.3M NaCl

Conclusions

The macroporous TSKgel BioAssist S offers high binding capacity and excellent resolution for purification of antibodies. More information about column attributes or the IgM purification can be found on the TSKgel BioAssist S product page of the website, www.tosohbioscience.com/seperation/us.

TOSOH

TOSOH BIOSCIENCE

TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com