APPLICATIONS

Selectivity Differences in Size-Exclusion Chromatography for Monoclonal Antibodies; How Pore Size Effects Retention

Helen Whitby, M. Christina Malinao, Brian Rivera and Chad Eichman
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501

Introduction

The control of chronic diseases is an ongoing challenge in public health systems. In recent years, the trend has switched and, instead of traditional small molecule drug targets, pharmaceutical companies are designing biotherapeutics as the next generation therapies to address the shortcomings of small molecule therapies. Biotherapeutics, however, are not chemically synthesized like small molecules and instead are produced recombinantly by engineered host cells. These biotherapeutics have a successful record in treating many life-threatening and chronic diseases, however, the potential immunogenicity of active substances produced under differing manufacturing conditions must also be considered.

Size exclusion chromatography (SEC) is widely used to characterize monoclonal antibodies (mAbs) and to quantify levels of aggregation, a critical quality attribute required for all therapeutic mAbs. The identification of fragments is also of prime importance during analysis as these fragments can significantly affect the efficacy of the protein and can have safety implications.

Monoclonal antibody aggregates are often found at very low levels (<0.1 % by peak area compared to monomer) and to address this need for low level detection a robust set of bioZen™ SEC columns were developed which combine UHPLC efficiency and high sensitivity to drive resolution and identification of even lower level targets.

The scope of this study is to demonstrate the selectivity differences that can be observed when working with column packing media of different pore sizes. The bioZen SEC-2 media has a 150 Å pore and is typically recommended for the separation of smaller mAbs and antibody fragments. The bioZen SEC-3 contains a 300 Å pore size that is better for separation of mAb aggregates from monomer. Below we present the results from this study using two well-characterized mAbs, rituximab and trastuzumab.

Materials and Methods:

Trastuzumab and rituximab were obtained from Pall (MA, USA). All chemicals were purchased from Sigma Chemical (St. Louis, MO). All applications were performed on an Agilent® 1260 Infinity II LC system equipped with a UV-Vis detector.

Conditions for analysis

Columns:	bioZen 1.8 µm SEC-2 bioZen 1.8 µm SEC-3
Dimensions:	300 x 4.6 mm
Part Nos.:	00H-4769-E0 (SEC-2) 00H-4772-E0 (SEC-3)
Mobile Phase:	50 mM KH_2PO_4, 250 mM KCl, pH 6.8
Flow Rate:	0.4 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Sample:	As indicated

Results and Discussion:

The scope of this study was to show the different selectivity exhibited by size exclusion columns with varying pore sizes. We looked at both rituximab and trastuzumab within the scope of the investigation and the results are presented below.

In all cases a distinct and sharp peak for each mAb was observed regardless of the pore size of the column used. Both bioZen SEC-2 and bioZen SEC-3 gave good separation of the main peak from high molecular weight (HMW) aggregates and its variable region fragments.

For both mAbs, separation of the aggregate peaks from the main peak are more defined when using a larger pore size SEC column (bioZen SEC-3) confirmed by greater peak to valley ratio's between the aggregate and the main peak in each example (Fig 1 rituximab & Fig 2 trastuzumab). We can conclude, particularly in the case of trastuzumab where HMW aggregates may be in

excess of 400 kDa in size, resolution of these different aggregates is lower when working with a smaller pore size material such as the bioZen SEC-2 column. Greater separation of aggregate components is also apparent in figure 1b for rituximab when using the 300 Å equivalent. Using bioZen SEC-2 may hold an advantage in some environments where there is a benefit to a lower overall resolution of the different aggregate peaks allowing a total area for aggregates to be easily measured. Some QC environments do not need to record individual forms of aggregation and simply look for a value summed over the area of all peaks.

In the case of the rituximab fragment peaks, which elute after the main peak, little or no separation was observed with either column showing no distinction regardless of the pore size of the media used. Trastuzumab, however, offered better resolution in the fragment region with the bioZen SEC-2 highlighting some of the benefits of using a smaller pore size column for mAbs.

Fig 1a
Rituximab – bioZen 1.8 µm SEC-2 (150 Å)

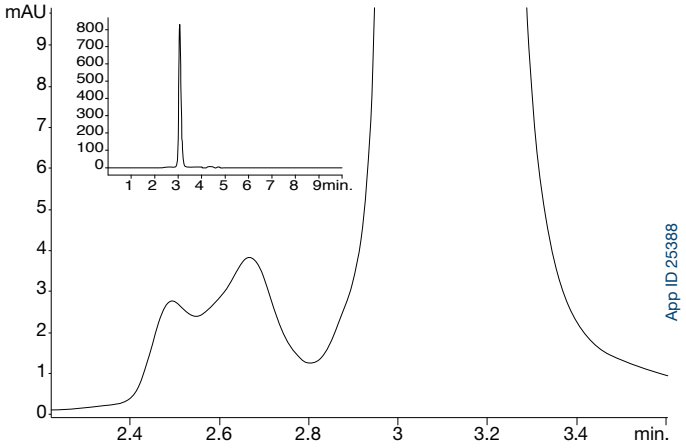


Fig 1b
Rituximab – bioZen 1.8 µm SEC-3 (300 Å)

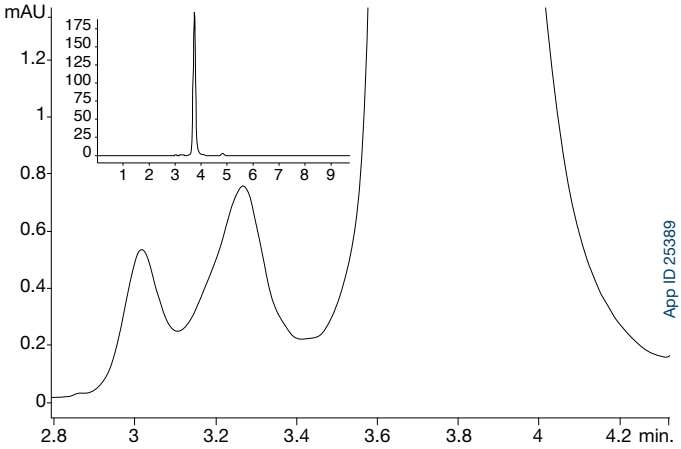


Fig 2a
Trastuzumab - bioZen 1.8 µm SEC-2 (150 Å)

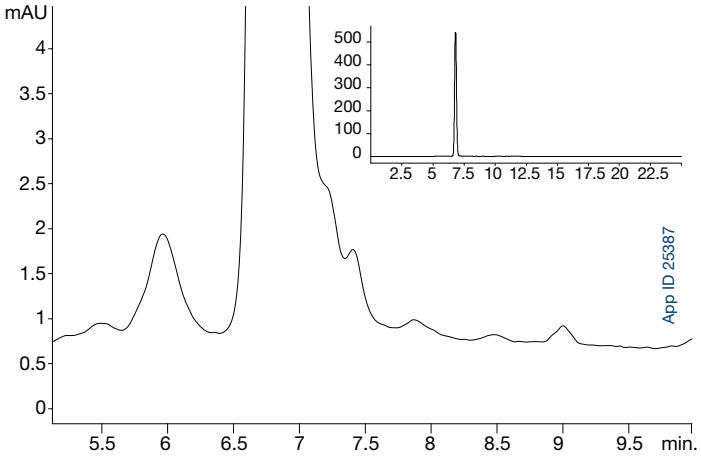
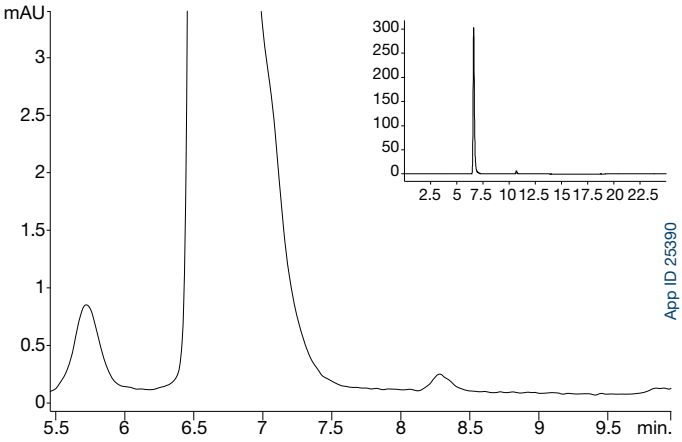


Fig 2b
Trastuzumab – bioZen 1.8 µm SEC-3 (300 Å)



Conclusion:

Although smaller porosity medias such as the bioZen SEC-2 are regarded as preferred for low molecular weight (LMW) separation ranges (such as fragment analysis) we have seen, in the case of both trastuzumab and rituximab, the bioZen SEC-2 column also offers good peak shape and separation of HMW aggregates. If a more defined separation of the component HMW aggregates is required or separation from the main peak is not adequate, improved resolution is typically observed with a larger pore size media such as the 300 Å bioZen SEC-3. Depending on the properties of the analyte of interest or the goal of the analysis, either the bioZen SEC-2 or SEC-3 may be more appropriate.

Ordering Information bioZen™

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	50 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
						/3pk	—	ea
bioZen 2.6 µm Glycan	50 x 2.1	00D-4773-AN	00F-4773-AN	—	—	AJ0-9800	—	AJ0-9000
						/3pk	—	ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	AJ0-9803	—	AJ0-9000
						/10pk	/10pk	ea
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	00F-4771-E0	AJ0-7605	AJ0-7606	KJ0-4282
						/3pk	—	ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	AJ0-9806	—	AJ0-9000
						/3pk	/3pk	ea
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00B-4768-E0	00F-4768-E0	AJ0-9806	AJ0-9808	AJ0-9000
						/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00D-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0	AJ0-9809	AJ0-9811	AJ0-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0	AJ0-9812	AJ0-9814	AJ0-9000

	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	300 x 4.6		for 4.6 mm	Holder
							/3pk	ea
bioZen 1.8 µm SEC-2	—	—	00F-4769-E0	—	00H-4769-E0	—	AJ0-9850	AJ0-9000
bioZen 1.8 µm SEC-3	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJ0-9851	AJ0-9000
							/10pk	ea
bioZen 6 µm WCX	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	—	AJ0-9400	KJ0-4282

Sample Preparation

bioZen Solid Phase Extraction	Format	Sorbent Mass	Part Number	Unit
bioZen N-Glycan Clean-Up	Microelution 96-Well Plate	5 mg/well	8M-S009-NGA	1/box

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

BE-HAPPY™ guarantee

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

www.phenomenex.com/behappy

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN67200319_W

Having trouble reproducing this method? We would love to help!
*Visit **www.phenomenex.com/LiveChat** to get in touch with one of our Technical Specialists*