Effect of Particle Size, Pore Size and Column Configuration for the Analysis of Antibody Aggregates by SEC

Jeffrey A. Kakaley[1], Takashi Sato[2], Ernest J. Sobkow[1], Jeff Wilson[1]

1. YMC America, Inc., Allentown, PA USA 2. YMC Co., Ltd., Kyoto, Japan

Introduction

Size Exclusion Chromatography (SEC) is a pervasive technique for monitoring aggregate formation for antibody and other protein solutions in the pharmaceutical and biotechnology industries. The increased use of various HPLC and uHPLC systems designed for narrow dispersion and rapid throughput has led to increased interest in faster SEC analyses using smaller stationary phase particle sizes. This investigation looks at the effects on resolution and speed of separation for various combinations of particle physical attributes coupled with variations in column geometries (length and inner diameter).

Experimental

Sample Preparation

Samples were made by diluting a 25mg/mL stock solution of Avastin (Genentech Corp.) to 1mg/mL using a 1X solution of phosphate buffered saline (PBS) as the diluent. Samples were mixed well and kept refrigerated (4°C) when not in use.

Mobile Phase

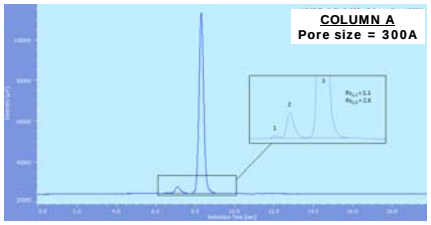
Analyses were run isocratically using 100mM sodium phosphate buffer at pH=7.0 with 200mM sodium chloride. All columns were equilibrated with a minimum of 10 column volumes of mobile phase prior to 1st injection.

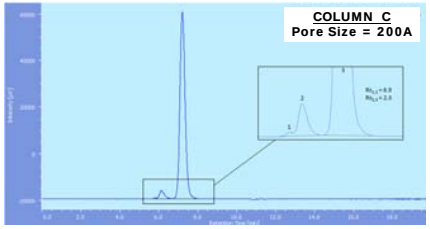
Instrument Parameters

HPLC System:	Jasco X-LC
Flow rate:	1 mL/min, unless it is noted in chromatogram
Column Temperature:	25°C
Detection λ:	215 nm
Injection Volume:	5µL for columns with 8.0mm I.D. 1.5µL for columns with 4.6mm I.D.

Effect of Pore Size (5um particle)

300A versus 200A in 8x300 mm configuration

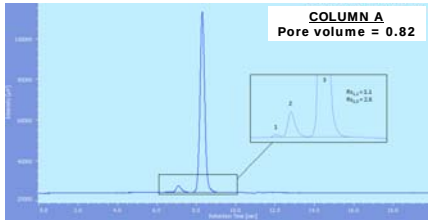


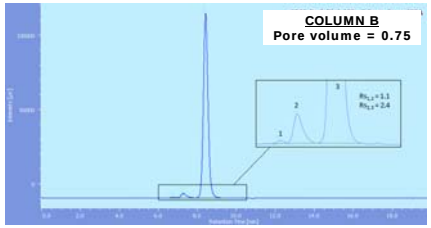


For Avastin, 300A slightly outperformed 200A at normal flow rates (1ml/min) for aggregate and higher order aggregate separation from monomer.

Effect of Pore Volume on Resolution

5um, 300A Particle





Increasing Pore Volume can lead to small improvements in resolution of aggregate from monomer.

SEC COLUMN TABLE

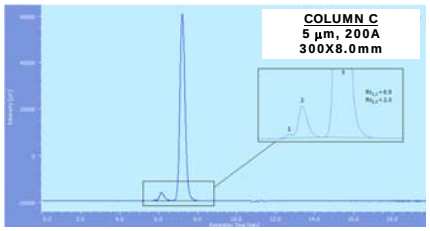
Column	YMC Part Num.	Dimension(mm)	Column Number	Particle Size (um)	Pore Size (A)	Pore Vol. (ml/g)	Surface Area (m2/g)	% C	Rs (1-2)	Rs (2-3)	Approx. Run Time(min)
A	DL30505-3000WT	8.0x300	0830001720	4.8	287	0.82	113	2.8	1.1	2.6	11
B	DL30505-3000WT	8.0x300	0830001294	4.8	310	0.75	97	2.5	1.1	2.4	11
C	DL30505-3000WT	8.0x300	0830001621	4.6	229	1.05	108	4.7	0.9	2.3	11
D	DL30503-3046WT	4.6x300	YT-466	2.7	222	0.94	169	4.3	NM	1.7	6
E	DL30503-1546WT	4.6x150	YT-465	2.8	326	0.86	106	2.6	NM	1.3	3
F	DL30503-1546WT	4.6x150	YT-464	2.7	222	0.94	169	4.3	NM	1.2	3
G	DL20502-1546WT	4.6x150	YT-458	2.1	204	0.92	180	4.4	NM	1.2	2

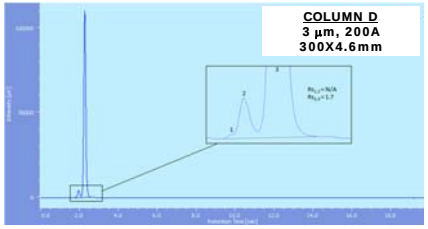
YT-XXX designates prototype
NM = not meaningful

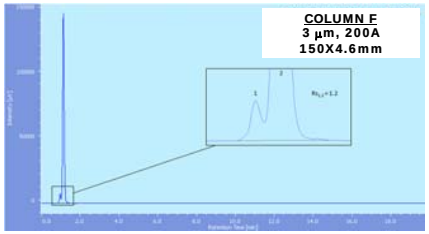
NOTES ON COLUMNS:

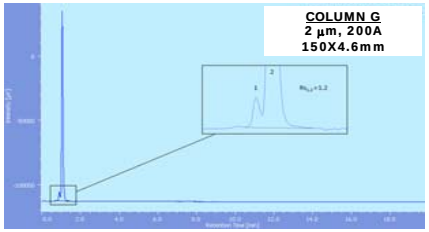
1. All columns manufactured by YMC Co., Ltd., Kyoto, Japan
2. All columns are silica based bonded with 1,2-dihydroxypropane.
3. For additional information on non-prototype columns visit www.ymcamerica.com

Effect of Decreasing Particle Size on Resolution



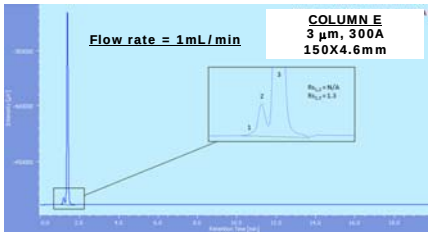


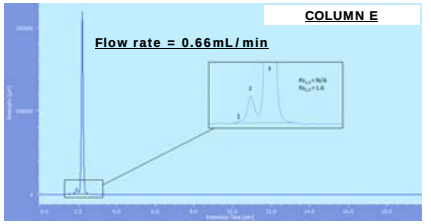


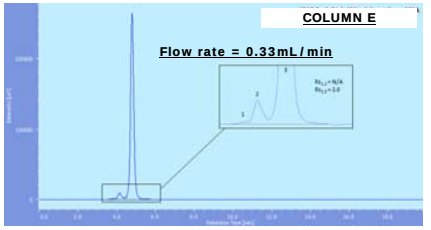


Decreasing particle size and column length at constant flow (increased linear velocity) results in loss of resolution between aggregate and higher order aggregate from mAb. Unlike reverse phase chromatography, in size exclusion chromatography, when particle size is decreased and linear velocity are increased in order to gain speed of analysis, resolution is decreased.

Effect of Decreasing Linear Velocity on Speed of Analysis on Resolution







Decreasing flow rate increases resolution but speed of analysis is longer.

Conclusions

Larger pore size improved resolution for aggregate and mAb.

Increasing Pore Volume can lead to small improvements in resolution of aggregate from monomer. In some methods where resolution is poor, choosing a column with increased pore volume can lead to improved resolution and method ruggedness.

Unlike reverse phase chromatography (RPC), decreasing particle size and column length results in a loss of resolution between aggregate and higher order aggregate from mAb. Speed does increase at the expense of a loss of resolution.

Unlike small molecules in RPC, SEC mode shows improved resolution with decreasing flow rate and accompanying increase in analysis time for mAb and aggregate.