Investigation of Pore Size, Particle Size, and Column Geometry Effects on SEC Analysis of Monoclonal Antibodies

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Introduction

Smaller particle size stationary phases have seen increased use in the modern analytical laboratory due to their ability to exhibit greater resolution while enabling the use of smaller column geometries. Shorter column lengths and narrower bores allow for faster runtimes and lower flowrates, decreasing laboratory costs in terms of solvent consumption and man-hours needed to run the analyses. This poster examines the effects of smaller particle sizes in various column geometries on size-exclusion chromatography (SEC) methods and the method variables affecting the characterization of antibody products.

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 & Instrument Parameters

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 (CV) of mobile phase prior to 1st injection. All runs were made on an Agilent 1260 with flowrates of 1.0mL/min for 8.0mm I.D. and 0.33mL/min for 4.6mm I.D. columns. Column temperature was 25° C, and injection volumes were 30µL for 8.0mm I.D. and 10µL for 4.6mm I.D. columns.

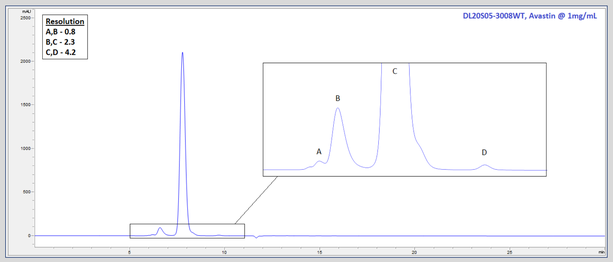
Results and Discussion

This work focused on the scaling-down of a monoclonal antibody SEC method, starting with a typical analytical-size SEC column (YMC-Pack Diol 5µm, 300Å, 300x8.0mm) and ending with a small particle, shorter length, narrower-bore column (YMC-pack Diol 2µm, 300Å, 150x4.6mm). A smaller pore size 200Å, 5µm column was also run to showcase the separation characteristics of the two porosities in 300x8.0mm geometry. The purpose of this experiment was to detail the differing performance characteristics exhibited by each column as physical properties were altered, one variable at a time.

Changes in column geometry were addressed by scaling flowrate (1.0mL/min to 0.33mL/min) and injection volume (30uL to 10uL) accordingly as inner diameter was decreased from 8.0mm to 4.6mm. This allows for a method that is typically run at 1.0mL/min for 15 minutes to be run at 0.33mL/min for 10 minutes, cutting runtime by 1/3 and solvent consumption by almost 2/3. As the results in the bottom chart indicate, when applied to SEC analysis of monoclonal antibodies, smaller particle stationary phases in shorter columns can increase throughput while still providing adequate resolution that rivals the standard 300x8.0mm, 5µm columns.

Effects of Particle Size, Pore Size, and Column Geometry on Antibody SEC Analysis

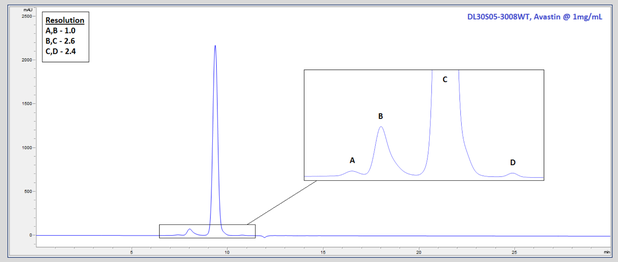
YMC-Pack Diol, 5µm, 200Å, 300x8.0mm



Same Particle Size
Same Geometry
Wider Porosity

Same Flowrate (1.0mL/min)
Same Inj. Volume (30µL)

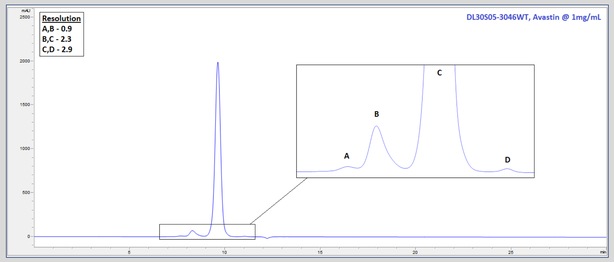
YMC-Pack Diol, 5µm, 300Å, 300x8.0mm



Same Particle Size
Same Pore Size
Smaller I.D.

Scaled Flowrate (0.33mL/min)
Scaled Inj. Volume (10µL)

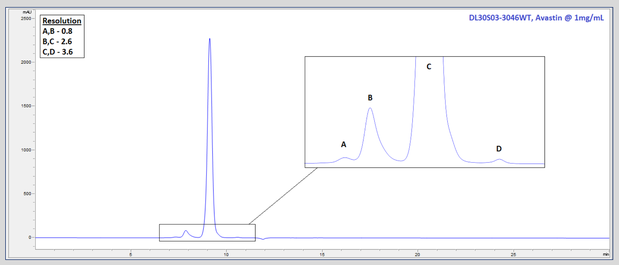
YMC-Pack Diol, 5µm, 300Å, 300x4.6mm



Same Geometry, Same Pore Size, Smaller Particle

Same Flowrate (0.33mL/min), Same Inj. Volume (10uL)

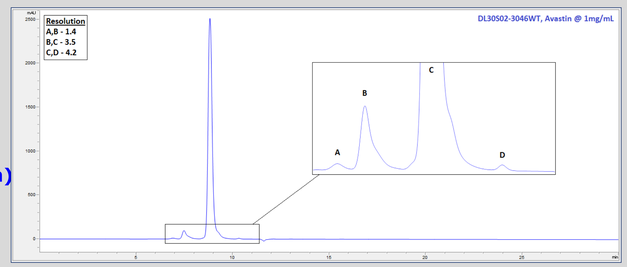
YMC-Pack Diol, 3µm, 300Å, 300x4.6mm



Same Pore Size
Same Geometry
Smaller Particle

Same Flowrate (0.33mL/min)
Same Inj. Volume (10µL)

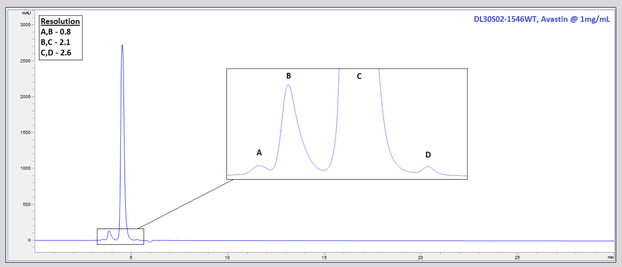
YMC-Pack Diol, 2µm, 300Å, 300x4.6mm



Same Pore Size
Same Particle Size
Shorter Length

Same Flowrate (0.33mL/min)
Same Inj. Volume (10µL)

YMC-Pack Diol, 2µm, 300Å, 150x4.6mm



	200Å, 5µm, 300x8.0mm	300Å, 5µm, 300x8.0mm	300Å, 5µm, 300x4.6mm	300Å, 3µm, 300x4.6mm	300Å, 2µm, 300x4.6mm	300Å, 2µm, 150x4.6mm
	DL20S05-3008WT	DL30S05-3008WT	DL30S05-3046WT	DL30S03-3046WT	DL30S02-3046WT	DL30S02-1546WT
Resolution Peak A-B:	0.8	1.0	0.9	0.8	1.4	0.8
Resolution Peak B-C:	2.3	2.6	2.3	2.6	3.5	2.1
Resolution Peak C-D:	4.2	2.4	2.9	3.6	4.2	2.6
%Area, Peak A	0.421	0.409	0.379	0.390	0.444	0.403
%Area, Peak B	4.148	4.011	4.154	4.325	4.880	4.425
%Area, Peak C	95.212	95.383	95.250	94.986	94.269	94.899
%Area, Peak D	0.219	0.197	0.218	0.299	0.406	0.273

Conclusions

- The use of smaller particles for SEC allows both column diameter and length to be shortened, increasing throughput and lowering solvent consumption, while providing resolution that is comparable to the larger 300x8.0mm columns typically used for these analyses.
- When %Area values were compared (see chart at left) all columns gave comparable results, indicating that the shorter, smaller particle columns are reliable for quantitative %aggregate analysis.