Use of Small Particle YMC SEC and IEX Materials for Improved Characterization of Monoclonal Antibodies

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Introduction

Continuously tightening quality requirements from regulatory authorities and a desire by biopharmaceutical companies to improve product characterization have resulted in the need for improved methods of analysis requiring greater resolution and reduced analysis time for mAb products. These new method requirements have prompted YMC to develop new stationary phases and smaller particles sizes in order to fulfill their customers' needs. This poster examines improvements in speed and resolution for ion-exchange (IEX) and size-exclusion (SEC) chromatography methods and the method variables affecting the characterization of antibody products.

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.

SEC Method Parameters

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 (CV) of mobile phase prior to 1st injection.

Instrument Parameters

HPLC System:	Jasco X-LC
Flowrate:	1.0 mL/min
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.

IEX Method Parameters

Mobile Phase

The dial-a-mix method was used via quaternary pump. Mobile phases were as follows:

Mobile Phase A:	0.1M Sodium Phosphate Monobasic
Mobile Phase B:	0.1M Sodium Phosphate Dibasic
Mobile Phase C:	1.0M Sodium Chloride
Mobile Phase D:	100% Water

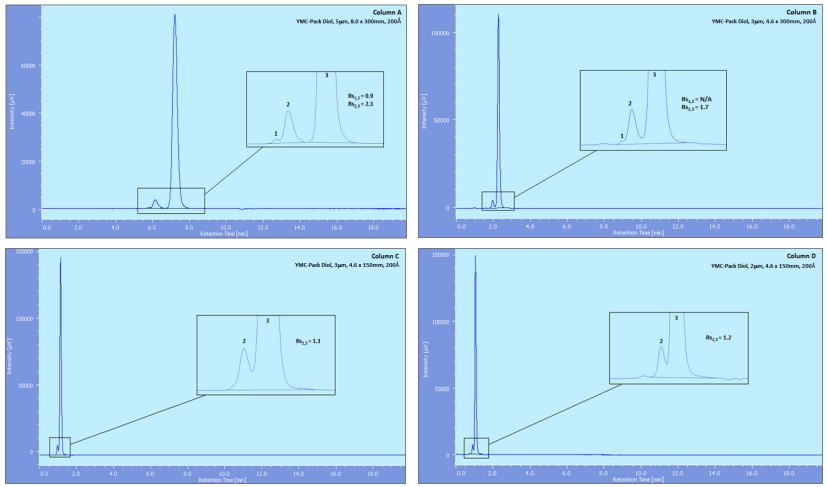
Antibody analyses (Avastin) were run using the dial-a-mix method at a pH = 6.5 using linear salt gradients of 0-200mM and 0-400mM (each spanning 55 minutes) as labeled in each chromatogram.

All columns were equilibrated with a minimum 10 column volumes of mobile phase prior to 1st injection.

Instrument Parameters

HPLC System:	Agilent 1100
Flowrate:	0.5mL/min
Column Temperature:	25°C
Detection λ:	215 nm
Injection Volume:	10µL

Effects of Particle Size on SEC



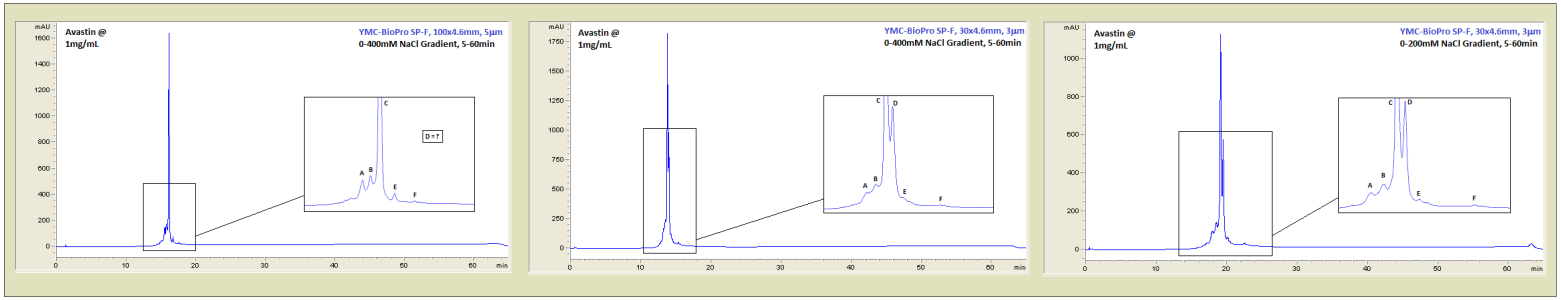
Column	Dimensions (mm)	Particle Size (µm)	Pore Size (Å)	Flow Rate (mL/min)	Resolution (Peak 2:3)	Monomer Peak RT (min)
A	8.0 x 300	5	200	1.0	2.3	7.23
B	4.6 x 300	3	200	1.0	1.7	2.27
C	4.6 x 150	3	200	1.0	1.2	1.21
D	4.6 x 150	2	200	1.0	1.2	1.17

Results and Discussion - SEC

The modern laboratory continuously sees the increased use of smaller particle sizes in order to speed up analysis time and simultaneously preserve resolution. When applied to SEC of monoclonal antibodies, smaller particles packed into shorter columns can increase throughput while providing adequate resolution between monomer and higher-order aggregates.

As seen in the chromatograms to the left and the chart above, flowrate was kept at 1.0mL/min while column inside diameter was decreased (increasing linear velocity) and column length was shortened, as particle size decreased. (Injection volume was adjusted for the change in column diameter) These results show that shorter columns can be used with smaller particles to increase throughput by greater than 6X while providing resolution of ≥1.2 between monomer and aggregates for Avastin.

Effects of Particle Size and Column Length on IEX



Results and Discussion – IEX

The use of smaller particles in IEX chromatography was investigated to determine if there would be any advantages for the analysis of charge variants in Avastin. As seen in the chromatograms above, a 30 x 4.6mm 3µm BioPro column shows an additional peak that was previously unresolved using the 100 x 4.6mm 5µm BioPro column, under the exact same method conditions (linear salt gradient of 0-400mM over 55 minutes). In addition, throughput was also increased by ~15%. The gradient was then shallowed (0-200mM over 55 minutes) and resolution was further increased for all peaks. These results indicate smaller particle sizes can be useful for increased resolution and throughput of charge variant analysis of monoclonal antibodies.

Conclusions

- In SEC, the use of smaller particles allows for column length to be shortened, increasing throughput, while still providing adequate resolution between monomer and aggregates in monoclonal antibody samples.
- In IEX, smaller particles show increased resolution of monoclonal antibody charge variants, enabling the use of shorter columns, allowing for improved separations and faster runtimes.