Fast Separation of Monoclonal Antibody and Dimer by SEC with Agilent Bio SEC

Application Note

BioPharma

Author

Tim Rice
Agilent Technologies, Inc.
2850 Centerville Rd.
Wilmington, DE 19808
USA

Abstract

The aggregation of protein therapeutics has become a major concern for the pharmaceutical industry and regulatory agencies. One of the main concerns with protein aggregates is that they may cause an adverse immune response *in vivo*. Size exclusion chromatography is the most widely used method to detect and separate protein aggregates from monomers. We present a method using Agilent Bio SEC-3, 300Å pore size silica columns to achieve a separation of monoclonal antibody aggregates from monomer in less than 5 min, significantly less time than normally achieved by conventional HPLC.

Introduction

Typical analytical SEC methods are run at flow rates at or below 1 mL/min on columns packed with 5- or 10-µm particles. The rigidity of Agilent's Bio SEC base silica allows for excellent stability under higher flow rates and higher salt concentration than normally used for size exclusion. The 3-µm particle size offers high efficiency for better resolution and allows for the use of shorter columns to achieve the desired separation.

Agilent Bio SEC-3 HPLC columns are packed with spherical, narrowly dispersed 3-µm silica particles coated with a proprietary hydrophilic layer. This thin polymeric layer is chemically bonded to pure, mechanically stable silica under controlled conditions, ensuring a highly efficient size exclusion particle. Agilent Bio SEC-3 HPLC columns are available in 100Å, 150Å and 300Å pore sizes to accommodate most peptide and protein size exclusion separations.

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Materials and Methods

Conditions without salted eluent

Column	Agilent Bio SEC-3, 300Å, 7.8 × 150 mm (p/n 5190-2512)
Sample	mAb (2 mg/mL)
Injection	5 µL
Flow rate	1.0 mL/min (56 bar), 1.5 mL/min (75 bar)
Eluent	150 mM sodium phosphate
Detection	220 nm

Conditions with salted eluent

Column	Agilent Bio SEC-3, 300Å, 7.8 × 150 mm
Sample	mAb (2 mg/mL)
Injection	5 µL
Flow rate	1.0 mL/min (56 bar), 1.5 mL/min (75 bar), 2 mL/min (105 bar)
Eluent	150 mM sodium phosphate + 100 mM Na sulfate
Detection	220 nm

Table 1. Monoclonal Antibody Monomer and Dimer Analysis Without Salted Eluent

Flow rate (mL/min)	Resolution ratio monomer:dimer	Monomer efficiency	Percentage dimer
1.0	1.58	3,684	0.65
1.5	1.31	2,574	0.70

Table 2. Monoclonal Antibody Monomer and Dimer Analysis With Salted Eluent

Flow rate (mL/min)	Resolution ratio monomer:dimer	Monomer efficiency	Percentage dimer
1.0	1.53	3,510	0.64
1.5	1.43	2,502	0.47
2.0	1.13	1,917	0.64

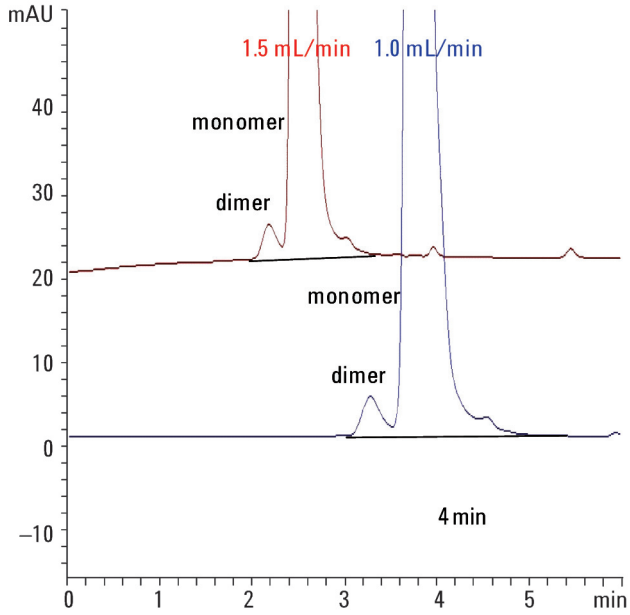

Figure 1. Fast size exclusion chromatography of monoclonal antibody without salted eluent using an Agilent Bio SEC-3 column.

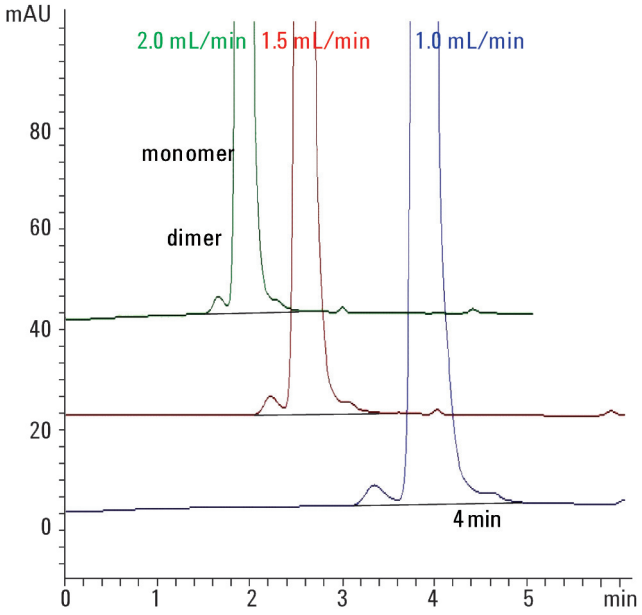

Figure 2. Fast size exclusion chromatography of monoclonal antibody with salted eluent using an Agilent Bio SEC-3 column.

Comparison of Agilent Bio Sec-3 and Competitor Column in the Analysis of a Monoclonal Antibody

In a column comparison test, the improvement in data quality produced by the Agilent Bio SEC-3 column is evident in Table 3 and Figure 3. The latter demonstrates the value of the column by revealing the presence of monoclonal antibody fragment that is missed by the competitor column, under salt and no-salt conditions.

Conditions

Column	Agilent Bio SEC-3, 300Å, 7.8 × 300 mm (p/n 5190-2511)
Column	Competitor 7.8 × 300 mm
Sample	mAb (2 mg/mL)
Injection	5 µL
Flow rate	1.0 mL/min
Eluent	150 mM sodium phosphate + 100 mM Na sulfate
Eluent	150 mM sodium phosphate
Detection	220 nm

Table 3. Monoclonal Antibody Monomer and Dimer Analysis Using Agilent Bio Sec-3 and a Competitor Column

Eluent	Column	Resolution ratio monomer:dimer	Monomer efficiency	Percentage dimer
Salt	Agilent	2.04	7,518	0.59
Salt	Competitor	1.88	3,967	0.57
No salt	Agilent	2.08	7,942	0.60
No salt	Competitor	1.92	4,164	0.57

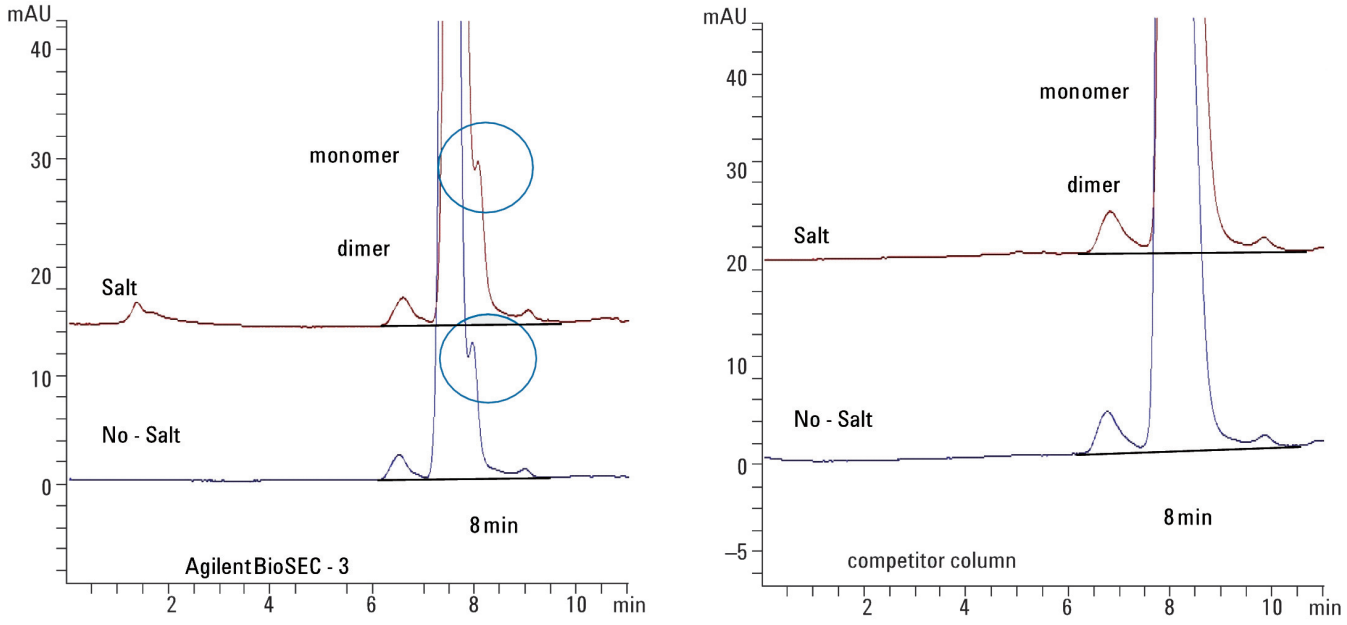

Figure 3. The Agilent Bio SEC-3 column reveals the presence of mAb aggregates missed by the competitor column.

Results and Discussion

It is evident from Table 1 and 2, and Figures 1 and 2, that the mAb separation is not dependent on salt concentration in the eluent. The Bio-3 SEC column is very robust so that different salt eluents can be used to suit the stability of the mAb under investigation, with no loss of chromatographic of performance.

The ability to use higher flow rates and shorter columns helps to reduce run times while maintaining backpressures well below the maximum for standard HPLC systems and the packing material. The Bio SEC-3 column can be run at flow rates up to 2 mL/min to achieve separation of monomer and aggregate in less than 5 min (Figure 2).

Conclusions

The Agilent Bio SEC-3 column delivers efficient separation of monoclonal antibody aggregates from monomers on conventional HPLC instrumentation in less than 5 min. Resolution ratio, monomer efficiency and percentage dimer are all greater on Agilent Bio SEC-3 than on a competitor's SEC column.

For More Information

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