

Improving Recovery and Resolution of mAb Aggregates using Mobile Phase Additives in SEC

Introduction

Size exclusion chromatography (SEC) is well established for mAb aggregate analysis. As the technique has been used since the early days of mAb development for pharmaceutical purposes, various method improvements have evolved. The possibilities of mobile phase enhancement are often overlooked in SEC, since it is thought to leave less room for improvement when compared to other chromatographic conditions, such as particle size, packing quality and column length.

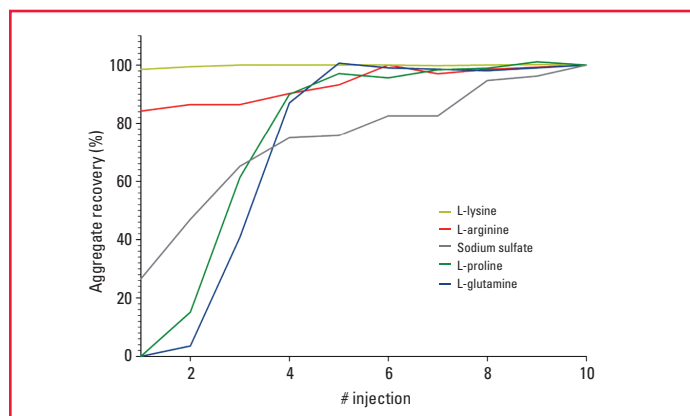
Once certain mobile phase parameters have been set, such as pH, stationary phase, and ionic strength, significant improvements can, in fact, be made to mAb aggregate separation by mobile phase additives. This application note will show how SEC analysis of mAb aggregate samples using a TSKgel® UltraSW Aggregate column are improved by amino acid additives in the mobile phase.

Results and Discussion

A mAb was aggregated by incubation at 75 °C for 5 minutes. The sample was then analyzed using a TSKgel UltraSW Aggregate column, 7.8 mm ID × 30 cm, with different mobile phases. 0.2 mol/L lysine, arginine, proline, glutamine or sodium sulfate (as a reference) were added to 0.1 mol/L sodium phosphate buffer, pH 6.7, respectively. A flow rate of 1 mL/min was applied and 20 µL and 100 µg of the aggregated mAb sample were injected. The columns were equilibrated for at least 10 column volumes.

Figure 1 illustrates the results on aggregate recovery. Lysine and arginine allow almost complete aggregate recovery starting with injection #1, while proline and glutamine lead to reduced aggregate recovery compared to sodium sulfate. The inter-injection variability is low, depicting the complete aggregate content for all of the injections.

Figure 1. Aggregate Recovery in Analytical SEC on TSKgel UltraSW Aggregate Column, 7.8 mm ID × 30 cm



In addition to aggregate recovery, the improvement of resolution is a top consideration in SEC analysis, particularly of the monomer and different aggregates. Figures 2A and 2B depict the separation profile of an aggregated mAb sample on a TSKgel UltraSW Aggregate column using 0.1 mol/L sodium phosphate buffer, pH 6.7, with an addition of either 0.2 mol/L arginine (A), or 0.2 mol/L proline (B). 10 injections with the respective amino acid buffer were followed by 10 injections applying sodium phosphate buffer with an addition of 0.2 mol/L sodium sulfate, in order to compare the two buffers. Injection #10 of the corresponding mobile phases are presented in the chromatograms. Monomer aggregate resolution, as well as monomer fragment resolution is slightly improved for the two amino acid buffers.

Figure 2A & 2B. Separation of a mAb Sample on TSKgel UltraSW Aggregate Column, 7.8 mm ID × 30 cm, using 0.2 mol/L Arginine (A) and 0.2 mol/L Proline (B) in the Mobile Phase

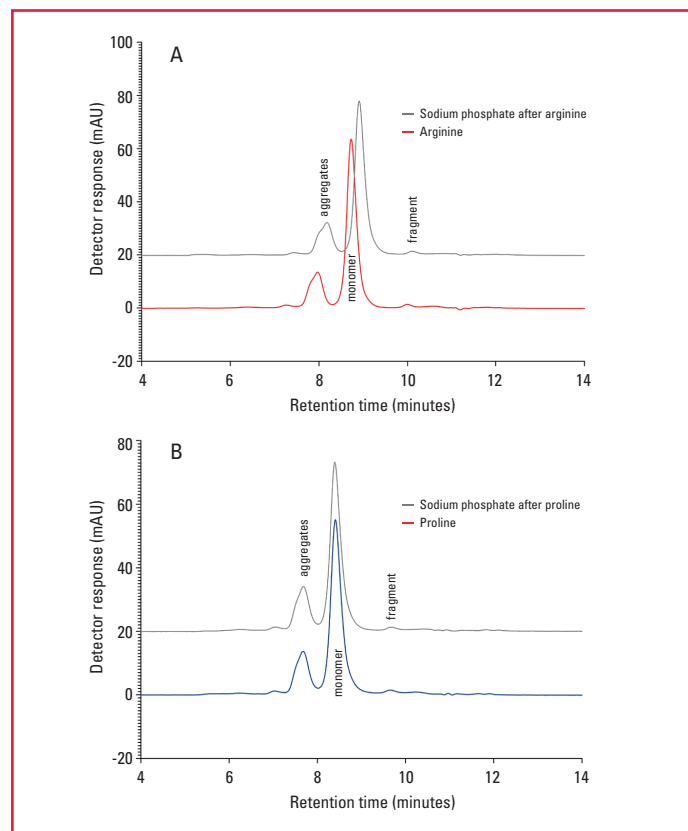


Table 1 lists the resolutions for some amino acid buffers and the results for the corresponding columns applying sodium phosphate buffer containing 0.2 mol/L sodium sulfate. Arginine, proline and glutamine provide slightly increased monomer aggregate resolution. For arginine, the fragment monomer resolution is also improved.

Table 1. Resolutions of Amino Acid Buffers

Buffer	Mean Rs Monomer - Aggregates	Mean Rs Monomer - Fragment
Arginine	1.6	3.2
NaP after arginine	1.4	3.0
Proline	1.5	3.0
NaP after proline	1.3	3.1
Glutamine	1.4	3.0
NaP after glutamine	1.3	3.0
Lysine	1.3	3.0
NaP after lysine	1.4	3.1

The average resolution of 10 injections with the respective mobile phase is listed in the table. Column: TSKgel UltraSW Aggregate, flow: 1 mL/min, injected volume: 20 µL, injected mass: 100 µg, detection: UV @ 280 nm.

Conclusions

While particle size, packing quality and column length are all important considerations in optimizing mAb aggregate analysis, mobile phase additives can also greatly contribute to improving your separation. The addition of amino acids such as lysine and arginine to the mobile phase, increase aggregate recovery, while glutamine, proline and arginine improve monomer aggregate resolution, as well as monomer fragment resolution. Although the increases in resolution are not drastic, they confirm that increased resolution due to the use of an advanced mobile phase is possible and, most importantly, that mobile phase testing can contribute to a more reliable and robust aggregate analysis.

The TSKgel UltraSW Aggregate column is designed for the recovery of mAb aggregates due to its smaller 3 µm particle size and higher exclusion limit. Coupled with an optimized mobile phase, this column delivers superior recovery and resolution of mAb aggregates.

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