Arginine as a Mobile Phase Co-solvent to Improve High Molecular Weight Aggregate Recovery for Size Exclusion Chromatography

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Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Silica-based SEC columns are modified with a hydrophilic stationary phase, typically to minimize electrostatic interactions of positive moieties on proteins and other analytes. However, depending on the physicochemical properties of the analyte, it may be prudent to investigate different mobile phase co-solvents to improve separation and sample recovery. One co-solvent that has been studied extensively is arginine, a common reagent used for protein refolding.[1] In this application note, we explore the impact of sample (i.e. aggregate) recovery on NIST mAb when modulating arginine concentration in mobile phase.

The mobile phase consisted of 200 mM Potassium Phosphate, pH 6.2, with arginine concentration assessed at 100 mM, 200 mM, and 250 mM. For NIST mAb, aggregate recovery was significantly less for 100 mM (80851 mAU) when compared to 200 and 250 mM (96818 and 97146, respectively). Further, percent high molecular weight (HMW) aggregate by peak area was 2.16% for 100 mM, which was again significantly lower than higher concentrations. Visually, post-peak fragment separation was slightly worse with 100 mM. HMW aggregate percentages and resolution values of monomer and dimer are summarized in **Table 1**.

In summary, arginine may be a useful co-solvent for SEC applications for large molecules. Particularly, arginine may improve high molecular weight aggregate recovery for monoclonal antibodies.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: 00H-4787-E0
Dimensions: 300 x 4.6 mm
Mobile Phase: 200 mM Potassium Phosphate, Arginine as indicated, pH 6.2
Flow Rate: 0.35 mL/min
Detection: UV @ 280 nm
Temperature: 25 °C
Sample: NIST mAb (30 µg)

1. Arakawa, Tsutomu et al. "Suppression of protein interactions by arginine: a proposed mechanism of the arginine effects." *Biophysical chemistry* vol. 127,1-2 (2007): 1-8. doi:10.1016/j.bpc.2006.12.007

Figure 1. SEC chromatographic overlays for ADC mimic, demonstrating the effect of organic solvent to peak shape and recovery.

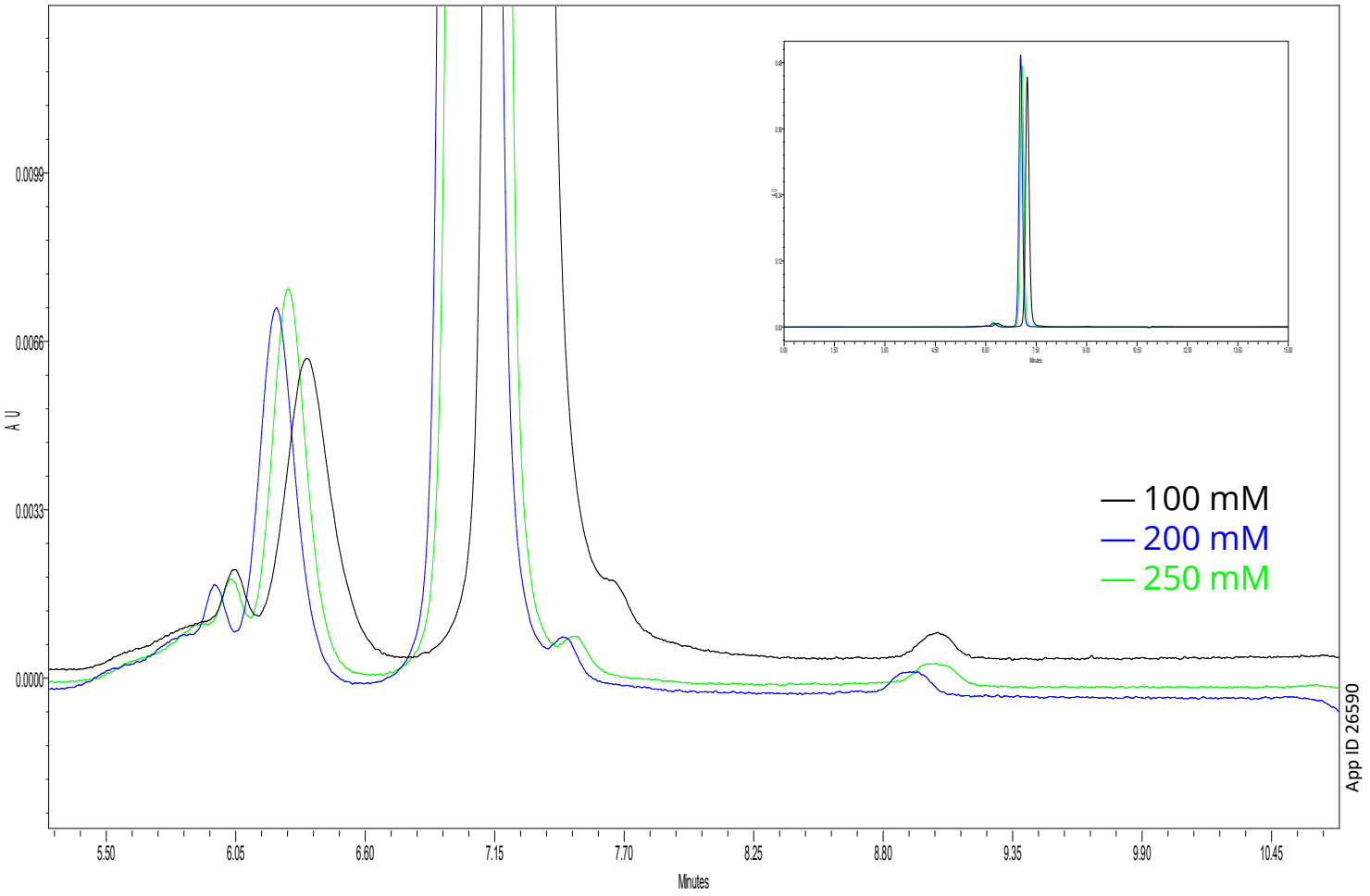

Table 1: Assessment of chromatographic parameters

Arginine Concetration	HMW Area Count (mAU)	Monomer Peak Height	% HMW	Rs 1,2
100 mM	80851	453324	2.16	3.02
200 mM	96818	493990	2.56	3.26
250 mM	97146	473197	2.58	3.25

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