# Optimizing Phosphate Concentration for Size Exclusion Chromatography Aggregate Analysis

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## Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating 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.

Because it is protein stabilizing and ubiquitous in biochemistry labs, phosphate is the common buffer used for SEC methods, especially for monoclonal antibodies. However, phosphate may also cause unwanted hydrophobic interactions. Consequently, exploring how phosphate concentration affects sample recovery and resolution of monomer and aggregate.

**Figure 1** shows an overlay of separation of trastuzumab, modulating the concentration of potassium phosphate. As shown in **Table 1**, there is minimal impact to retention time, resolution of monomer and dimer, and percent purity. **Figure 2** shows NIST mAb RM8671 SEC chromatogram overlays; again, only nominal differences are observed for retention time, resolution and percent purity.

In summary, assessing phosphate concentration may be necessary to ensure that no differences are observed with modulating concentration. This ensures proper sample recovery and demonstrates robustness of the method.

## LC Conditions

**Column:** Biozen™ 1.8 µm dSEC-2, 200 Å
**Dimensions:** 300 x 4.6 mm
**Part No.:** 00H-4787-E0
**Mobile Phase:** Potassium Phosphate (as indicated) + 250 mM Potassium Chloride, pH 6.2
**Flow Rate:** 0.35 mL/min
**Temperature:** 25 °C
**Detection:** UV @ 280 nm
**Sample:** Trastuzumab (30 µg)
NIST mAb (30 µg)

**Figure 1. SEC chromatographic overlays for Trastuzumab, modulating concentration of potassium phosphate.**

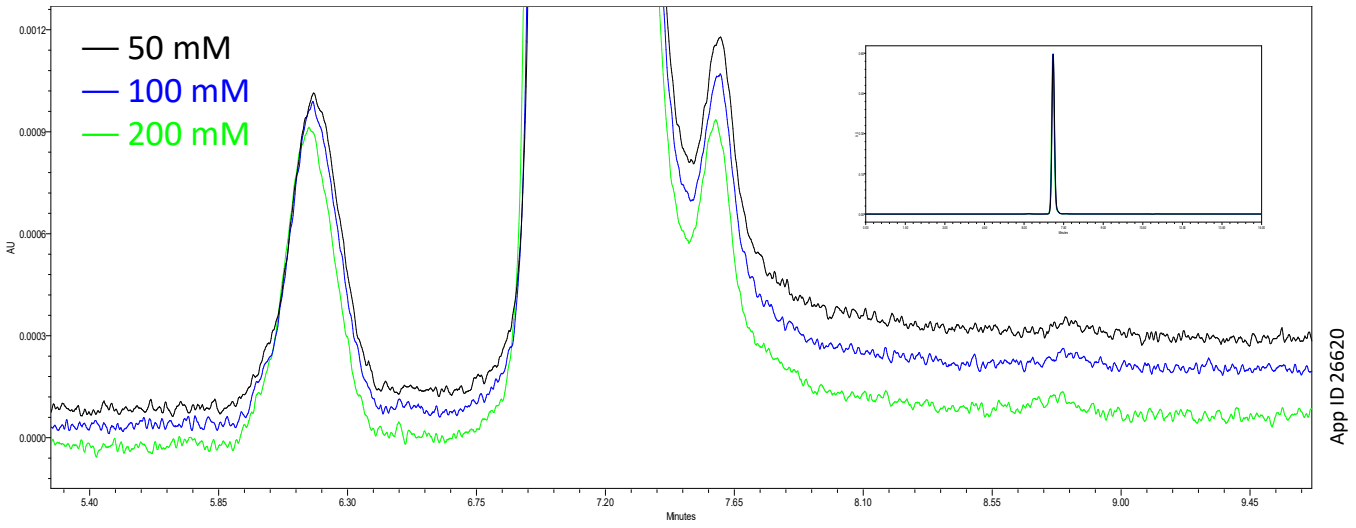

**Figure 2. SEC chromatographic overlays for NIST mAb, modulating concentration of potassium phosphate.**

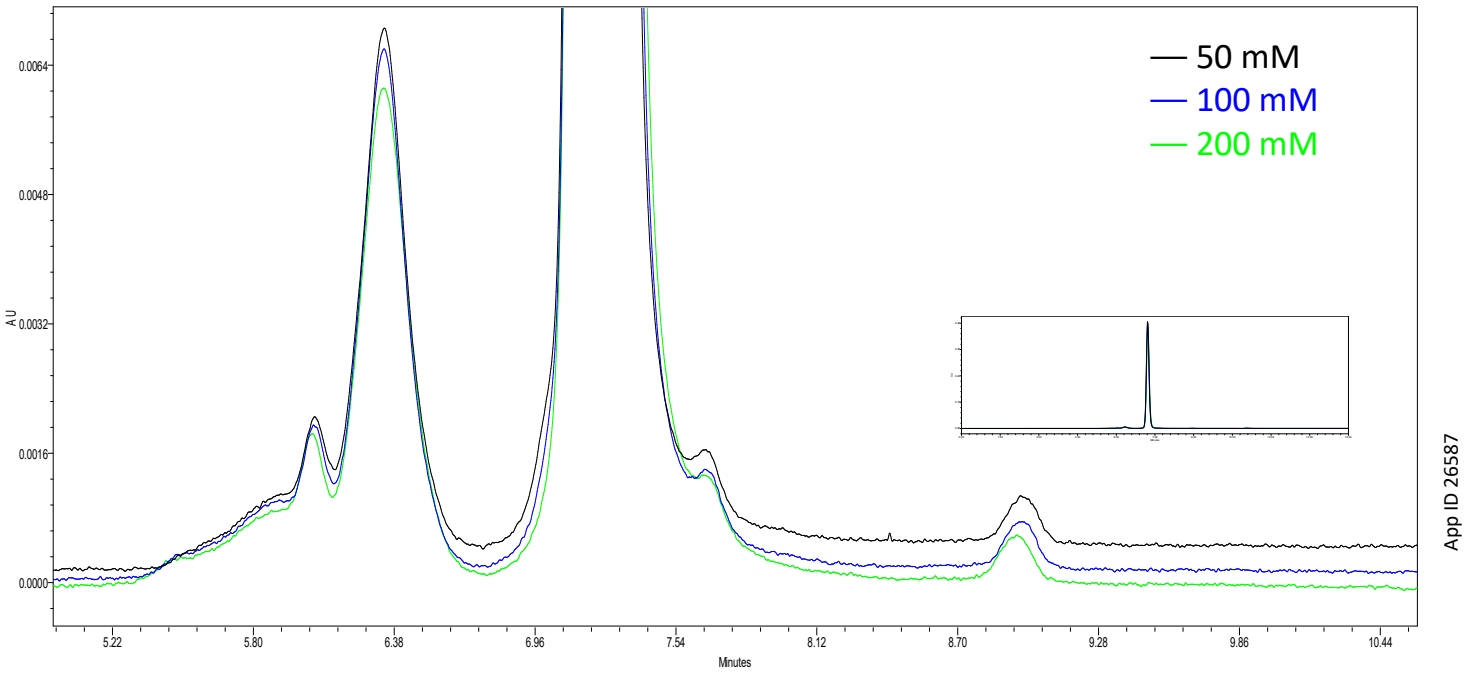


**Table 1: Assessment of chromatographic parameters**

| Sample | Phosphate Concentration (mM) | Monomer Retention Time (min) | Resolution 1,2 | % Purity Monomer |
|---|---|---|---|---|
| Trastuzumab | 50 | 7.048 | 3.56 | 99.31 |
| | 100 | 7.058 | 3.55 | 99.39 |
| | 200 | 7.058 | 3.78 | 99.49 |
| NIST mAb | 50 | 7.214 | 3.01 | 96.81 |
| | 100 | 7.219 | 3.02 | 96.4 |
| | 200 | 7.224 | 2.91 | 96.47 |

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