

Mobile Phase Optimization for the Analysis of an Antibody-Based Fusion Protein and Aggregates using a TSKgel Super SW3000 Size-Exclusion Column

TSK-GEL
APPLICATION NOTE

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Introduction:

During method development, many variables are examined to ensure method robustness. Factors such as elution profile, peak shape, and recovery are required to be consistent by GMP/GLP protocols. During a recent method re-qualification at Lexigen Pharmaceuticals, several variables were investigated to eliminate non-specific binding and increase the robustness of an established antibody separation method. Previous protocol required an unacceptable two-week "break-in" period to equilibrate the column with the mobile phase. Reduction of non-specific binding of proteins to SEC stationary phases can be achieved by exploring variables that affect either the ionic strength or hydrophobicity of the mobile phase. Typically, changes in mobile phase composition, such as buffer type and pH, salt concentration, the addition of organic modifiers, as well as the evaluation of competitive columns are the usual strategies to correcting non-specific adsorption. In this case, these options did not result in an improvement. Thus, an alternative approach to increase the chaotropic nature of the mobile phase was investigated. Concurrently, the buffer concentration was reduced to a minimum buffering capacity and the modifying salt was changed to NaClO₄. A drastic improvement on the performance of the method was evident.

Experimental Conditions:

Gel filtration was performed with a 4.6mmID x 30cm TSKgel Super SW3000 column packed with 4µm particles containing 250Å pores. The original running buffer consisted of 0.1M NaH₂PO₄ (pH 6.0) to which was added 15% acetonitrile and 0.2M NaCl. In the redesigned buffer system, NaCl was replaced by NaClO₄ and the phosphate buffer concentration was reduced to 0.05M. Initially, the concentration of sodium perchlorate investigated was 0.2M, however, best results were obtained at 0.4M. The flow rate of 0.35mL/min was left unchanged. Note that recent work has shown that acetonitrile can be omitted from the mobile phase without affecting separation quality or method robustness.

Results:

As shown in *Figure 1*, excessive peak tailing of "fusion protein 1" is evident with the use of 0.2M NaCl. Additionally, the expected protein dimer and trimer aggregates are not visible in the chromatogram. By switching from 0.2M sodium chloride to 0.2M of the more chaotropic sodium perchlorate salt, together with a two-fold reduction in the buffer concentration, less peak tailing and distinct peaks for the dimer and trimer species of MAb1 resulted. Doubling the perchlorate concentration to 0.4M provided further improvement in the peak shape of fusion protein 1 and associated aggregate species. Additionally, the

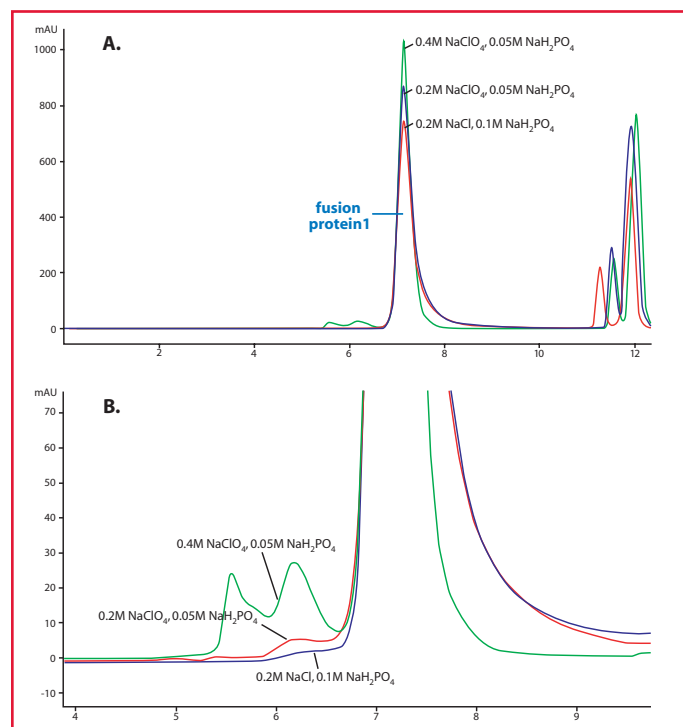
re-development increased the methods' robustness allowing for a substantial decrease in column "break-in" time from two weeks to 4 days as well as better peak area reproducibility and linearity of both monomer and aggregate species.

As a precaution, a more rigorous cleaning protocol consisting of flushing the system with DI water was implemented during periods of instrument inactivity. Although perchlorate is slightly less corrosive to stainless steel components relative to chloride, it is considered more aggressive to chemically bonded stationary phases.

Conclusions:

The selection of sodium perchlorate as a mobile phase component substantially improved the analysis and quantification of an experimental monoclonal antibody and its aggregates by SEC, thus providing the required level of method robustness.

Figure 1. A. Overlays of monoclonal antibody separation with 0.2M NaCl, 0.2M NaClO₄ and 0.4M NaClO₄. B. Enlargement of fusion protein 1's baseline region shows improved peak shape of the dimer and trimer aggregates with 0.4M NaClO₄.





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