Improve Recovery and Reproducibility with Pre-Treatment of Analytical TSKgel G3000SW$_{XL}$ Size Exclusion Columns

TSKgel
APPLICATION NOTE

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Abstract:

During a recent method optimization and investigation at Wyeth BioPharma, pre-treatment with bovine serum albumin improved reproducibility and recovery during the analytical SEC analysis of fractions pulled from a multi-step orthogonal purification of a 140 kDa apparent molecular weight protein from cell culture feedstock.

Introduction:

Ideally in size exclusion chromatography the only mechanism that provides separation of sample components is a sieving process based on the hydrodynamic radius of the sample components in combination with a porous and non-interactive stationary phase. For mono-dispersed samples such as proteins, spherical 5 micron silica-based materials derivatized with proprietary hydrophilic diol-type coatings have been widely used since their introduction by Kato et al. in 1987. Although exhaustively derivatized, unreacted silanol groups and the hydrophilic coating may contribute to unwanted ionic or hydrophobic interactions between the bonded phase and sample components. One technique to reduce the impact of such "secondary" interactions is to inject bovine serum albumin to occupy accessible reactive sites on the stationary phase.

At Wyeth BioPharma, a SEC analysis is employed at every stage of a three step cell culture purification to determine the purity and recovery of the high molecular weight species of the target protein. The cell culture is purified using affinity chromatography (protein A) and subsequent chromatographic polishing steps. The recovery and reproducibility of the peaks eluting in the region of high molecular weight species during SEC analysis of the post-protein A fraction was highly variable, signaling possible adsorption of the sample to the packing materials. A pre-treatment experimental protocol with BSA was administered to reduce the variability.

Conditions of SEC analysis:

Column: TSKgel G3000SW$_{XL}$, 5 µm, 7.8mm ID x 30cm
Elution: Phosphate Buffered Saline, pH 6.5
Sample: 50 µL injection volume, 50 µg load
Detection: 280 nm
Instrument: Waters 2690 Alliance

Results:

As can be seen in *Figure 1*, recovery of the high molecular weight region, expressed as a percent of total area recovery (% HMW), of the protein A purified sample was 1.1%. Upon injecting 10 aliquots x 20 µL of 5 mg/mL bovine serum albumin (BSA) onto the column, the recovery of the %HMW species increased to 3.5%. In the absence of BSA treatment, protein A purified samples historically returned %HMW

Figure 1. Overlay plot of the Protein A sample pre and post-treatment with BSA

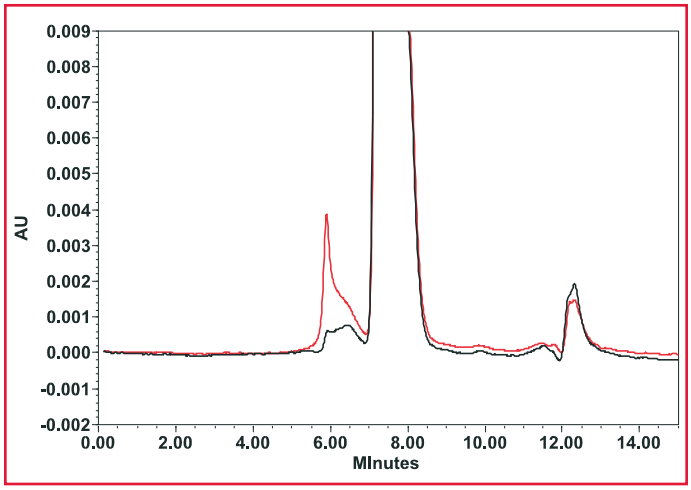

Table 1: Recoveries of all three types of samples pre and post-treatment with BSA

Sample	% HMW pre treatment	% HMW post treatment
Post Protein A	1.1	3.4
Post Polishing chromatographic step1	1.5	1.5
Post Polishing chromatographic step 2 (final product)	0.2	0.2

values of 1.0% to 1.4% when a column was new and gradually increased to 3.5% - 4.0% after as many as 60 injections. Interestingly, this result was only apparent with the protein A purified sample. *Table 1* shows that BSA pre-treatment had no effect on the samples obtained from the downstream chromatography steps. Further characterization work indicated that elevated levels of host cell proteins (HCP) were present in the protein A purified samples with respect to the post polishing samples. The HCP were found to elute in the HMW region of the analytical SEC profile. Furthermore, when the protein A purified samples were analyzed by analytical SEC after BSA treatment, the HMW region was found to be enriched in host cell protein when compared to the HMW region of the same sample run on analytical SEC prior to BSA treatment. This suggests that the association between sample and column packing was most prevalent with the HCP as opposed to target molecule HMW species.

Conclusions:

Pre-treatment of SEC columns with BSA is a viable option to reduce secondary interactions and improve consistency of results between analyses. In this particular protocol, the dramatic improvement in the

consistency of results associated with the protein A purified fraction, in contrast to the lack of effect seen on recovery of HMW species from downstream purification steps (chromatographic polishing steps 1 and 2), has provided the opportunity to further characterize the high molecular weight fractions of the protein A purified sample. This characterization has led to the conclusion that the results of SEC performed on samples from early purification steps may be negatively influenced by non-product related impurities derived from the cell culture environment.

References:

Y. Kato et al; *J. of Chromatrogr.* **404** (1987) 333-339

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