TSKgel G2000SWXL Columns for the Reproducible Analysis of Bovine Serum Albumin

TSKgel
APPLICATION NOTE

Introduction

TSKgel G2000SWXL columns feature high pore volume per unit column volume, low sample adsorption and excellent column efficiency, all contributing to unsurpassed sample resolution. With 5 µm particles and 125Å pores, these columns are an excellent choice for small proteins and peptide separations. The analysis of a Bovine Serum Albumin (BSA) sample was conducted on a TSKgel G2000SWXL, 7.8 mm ID x 30 cm column from Tosoh Bioscience. BSA (66.5 kDa) is often used as a protein concentration standard.

Experimental Conditions

Column:	TSKgel G2000SWXL, 5 µm, 7.8 mm ID x 30 cm
Mobile Phase:	100 mmol/L KH_2PO_4/Na_2HPO_4, pH 6.7, 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Detection:	UV@280 nm
Temperature:	ambient
Flow rate:	1.0 mL/min
Injection vol.:	20 µL
Sample:	Bovine Serum Albumin (Sigma Aldrich A7906, Lot # 080M1251V; >98% purity)

Results and Discussion

Figure 1 shows three consecutive runs of 102 µg of BSA on the TSKgel G2000SWXL column. These runs were highly reproducible. The monomer peak was clearly resolved from the dimer peak followed by aggregate peaks. A further study of the loading capacity of the column using a 10 times higher load of BSA also yielded a well resolved peak without any splitting, as shown in *Figure 2*.

Figure 1. Analysis of BSA Using a TSKgel G2000SWXL column

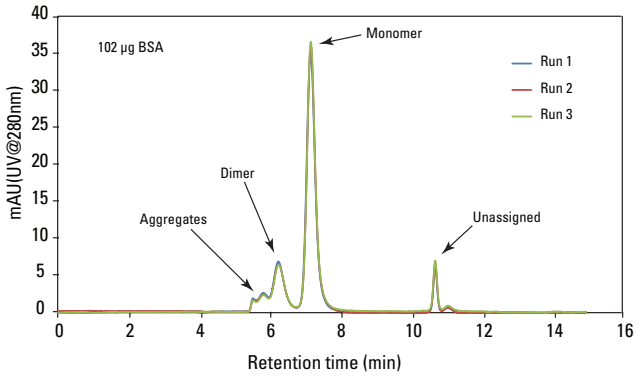
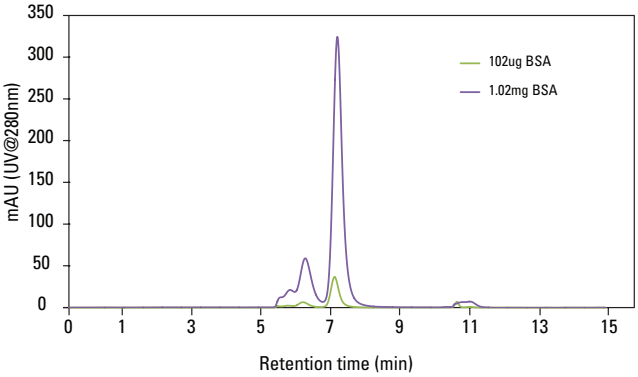


Figure 2. BSA Loading Capacity Study



Prior to this analysis the column was tested using a standard protein mixture (data not shown here) and compared with the recommended values mentioned in the operating conditions and specifications sheet supplied with the column. The efficiency of the column, as measured by the number of theoretical plates for PABA (para-amino benzoic acid), was 33944: well above the quality control specification value of >20,000. The asymmetry factor was 1.18, which is well within the quality control specification range of 0.7-1.6. Repeated injections produced excellent reproducibility.

Conclusions

A TSKgel G2000SWXL column is suitable for the separation of protein monomer from dimer and aggregates, as shown in this note using Bovine Serum Albumin. In all runs conducted, the monomer peak of BSA was clearly resolved from the dimer peak even with a ten-fold increase in BSA load. The analysis yielded excellent reproducibility, making the TSKgel G2000SWXL column an excellent choice for protein separation.

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