

The Characterization of Biological Samples by Microbore TSKgel® SuperSW3000 Size Exclusion Columns

TSKgel
APPLICATION NOTE

Introduction

Size exclusion chromatography (SEC) in an aqueous mobile phase, commonly referred to as gel filtration chromatography (GFC), is a powerful tool for the analysis of biological polymers like peptides, proteins and nucleic acids. Characterization of proteins and antibodies during the development and quality control of new biological medicines is very important in the biopharmaceutical industry. TSKgel SW SEC columns are also routinely employed in proteomics, where the ability to detect very small amounts of proteins is a critical need. TSKgel SuperSW3000 microbore columns (1mm ID and 2mm ID) were developed to provide optimum efficiency, excellent sensitivity and high recovery for the analysis of trace amounts of proteins.

TSKgel SuperSW3000 columns are packed with 4µm spherical silica particles. As the popular TSKgel SW and SW_{XL} columns, TSKgel SuperSW3000 columns are functionalized with hydrophilic diol groups. They offer low adsorption and a well-defined pore size distribution necessary for high performance size exclusion chromatography. The 4µm particle size of the TSKgel SuperSW3000 columns, coupled with a narrow column diameter, are valuable for applications where sample quantity is limited and both increased resolution and high sensitivity are needed, as in proteomic applications. Additionally the smaller particle size results in 20% more theoretical plates per column than a 5µm TSKgel G3000SW_{XL} column of the same length. This benefit can be used to either shorten run times or to take advantage of higher resolution. The narrower bore column diameters make the TSKgel SuperSW3000 columns compatible with LC/MS applications.

Results

Figure 1 shows a comparative separation of several standard proteins at low level concentrations on a 2mm ID TSKgel SuperSW3000 column and on a competitive GFC column. As the results reveal, the TSKgel SuperSW3000 column is an excellent choice for the rapid separation of proteins at trace levels.

A 1mm ID TSKgel SuperSW3000 column was used to analyze proteins in human serum. A fraction of interest was then analyzed by off-line SELDI/TOF/MS to establish the presence of BSA aggregates and IgG. Figure 2 demonstrates the applicability of TSKgel SuperSW3000 columns for the trace analysis of biological components by LC/MS analysis.

Conclusion

TSKgel SuperSW3000 microbore columns were developed for the trace analysis of proteins. These columns offer increased resolution, excellent sensitivity and high recovery - necessary characteristics for proteomic applications. Available in 1mm ID and 2mm ID with 4µm silica particle size, the TSKgel SuperSW3000 columns are an excellent fit for LC/MS analyses. Like TSKgel SW and SW_{XL} columns, the TSKgel SuperSW3000 columns will exhibit long lifetimes and good reproducibility.

Figure 1. Separation of Standard Proteins

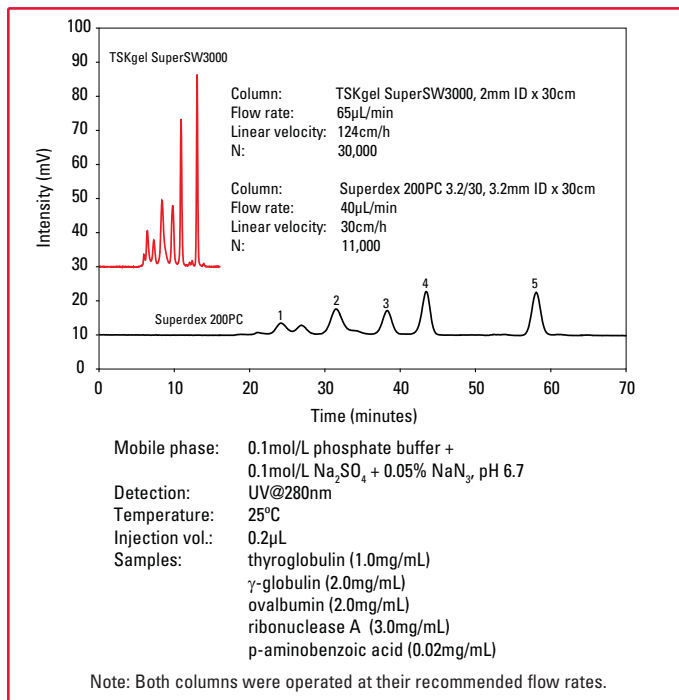
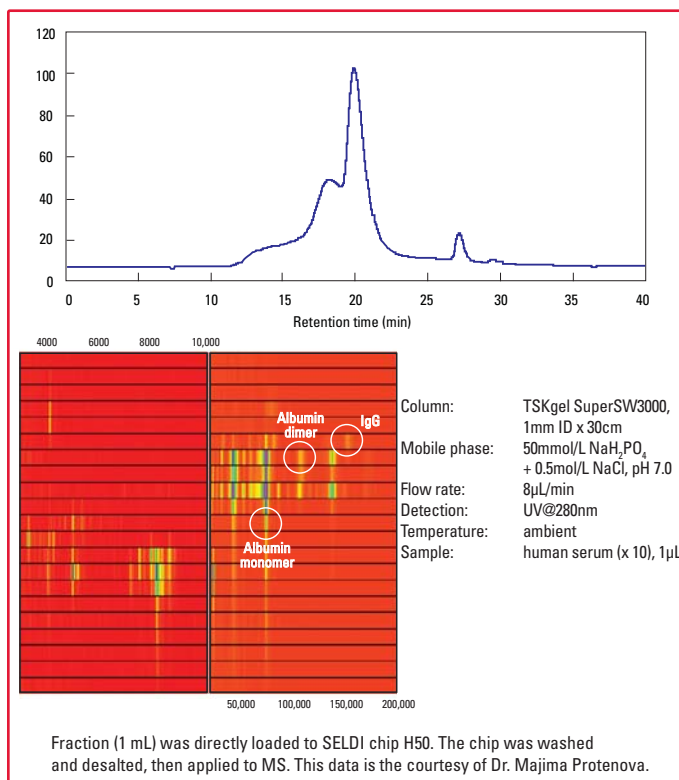


Figure 2. Separation of Human Serum Proteins





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