TOSOH

Application Note

TSKgel G3000SWXL COLUMNS FOR THE REPRODUCIBLE ANALYSIS OF mAbs AND PROTEINS

INTRODUCTION

Gel Filtration Chromatography (GFC) is popular among biochemists for the analysis of proteins and monoclonal antibodies. With 5 µm particles and 250 Å pores, TSKgel G3000SWXL columns are an excellent choice for protein separations. TSKgel SWXL columns feature rigid spherical silica particles, the surface of which has been shielded from interacting with proteins by chemical derivatization with ligands containing proprietary diol functional groups. Tosoh's proprietary surface chemistry provides an inertness, which allows for minimal adsorption of proteins and other protein aggregates.

TSKgel G3000SWXL columns also feature high pore volume per unit column volume, low sample adsorption, excellent column efficiency, and very well-defined pore size distribution. All of these factors contribute to unsurpassed sample resolution. This application note reports the analysis of monoclonal antibodies (mAb) using a TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm column, demonstrating the effectiveness of the proprietary surface chemistry.

EXPERIMENTAL CONDITIONS

Analyses were carried out using an Agilent 1200 HPLC system.

Column:	TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm; lot T03294-19S
Mobile phase:	100 mmol/L KH_2PO_4/Na_2HPO_4, pH 6.8, 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	20 µL
Sample:	monoclonal antibody (mAb-02)

High purity HPLC grade Sigma Aldrich chemicals were used in this study. All the standards and samples were filtered through a 0.45 µm membrane before injecting into the column.

RESULTS AND DISCUSSION

Figure 1 shows the overlay of the first two consecutive injections of a monoclonal antibody (mAb-02) onto a TSKgel G3000SWXL column. This brand new column was not pre-conditioned prior to this analysis. The monomer peak is baseline resolved from the dimer peak and the aggregate peaks. The overlay does not show any difference in the monomer and dimer peak areas of the first and second injections. There is no shift in retention time between the two consecutive injections and no difference in peak height is noticeable. These results clearly demonstrate that there was not any adsorption of the monoclonal antibody onto the column.

ANALYSIS OF mAb-02 USING A TSKgel G3000SWXL COLUMN - AN OVERLAY OF THE FIRST TWO INJECTIONS

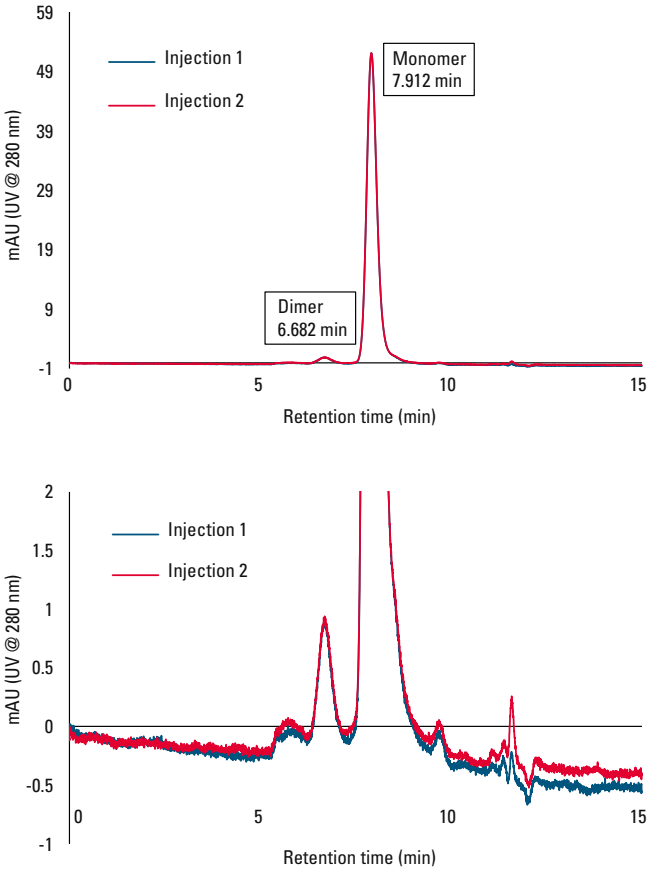

Figure 1

ANALYSIS OF mAb-02 USING A TSKgel G3000SWXL COLUMN - AN OVERLAY OF THE FIRST TWO INJECTIONS

MAB-02	RT (min)		PEAK AREA (mAU*S)	
	Dimer	Monmer	Dimer	Monomer
Injection 1	6.676	7.913	23.7	1029
Injection 2	6.682	7.912	24.2	1029.8

Table 1