Separation of a Monoclonal Antibody Monomer from its Impurities using New TSKgel® SW mAb Columns

TSKgel
APPLICATION NOTE

Introduction

The analysis of monoclonal antibodies (mAb) is growing in importance in the field of biotherapeutics for the treatment of a variety of diseases. Quality control of therapeutic mAb is essential, as the introduction of species to the body other than the monomer may induce toxic side effects. Therefore, the pure antibody monomer must be very well resolved from its dimer and higher molar mass aggregates, as well as the antibody fragments. Size exclusion chromatography (SEC) is the best choice for determining mAb monomers and their impurities, including aggregates, oligomers, and mAb fragments.

Tosoh Bioscience has answered the call for dedicated SEC columns for the high resolution separation of mAb with the new silica-based 4 µm TSKgel SuperSW mAb HR column, for high resolution separation of the monomer and dimer, and the 3 µm TSKgel UltraSW Aggregate column for the separation and quantification of mAb aggregates and oligomers. This application note demonstrates the superb performance of these new columns for the analysis of monoclonal antibodies.

Experimental Conditions

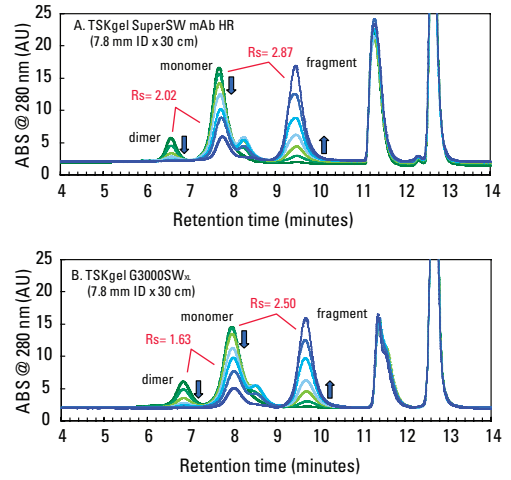
Column:	TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	200 mmol/L potassium phosphate buffer + 0.05% NaN_3, pH 6.7
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	10 g/L IgG digested with papain for 0-24 hr

Column:	TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm
Mobile phase:	100 mmol/L potassium phosphate buffer, 100 mmol/L sodium sulfate, pH 6.7 + 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	60 °C
Injection vol.:	20 µL
Sample:	BI-mAb-02 (4.6 mg/mL)

Results and Discussion

IgG monomer, dimer, and fragments from IgG digested by papain over a 24 hour period were analyzed using the TSKgel SuperSW mAb HR column *(Figure 1)*. The results exhibit the superior resolving power of this column for monomer/fragment and monomer/dimer separation (Rs = 2.87 and 2.02 respectively).

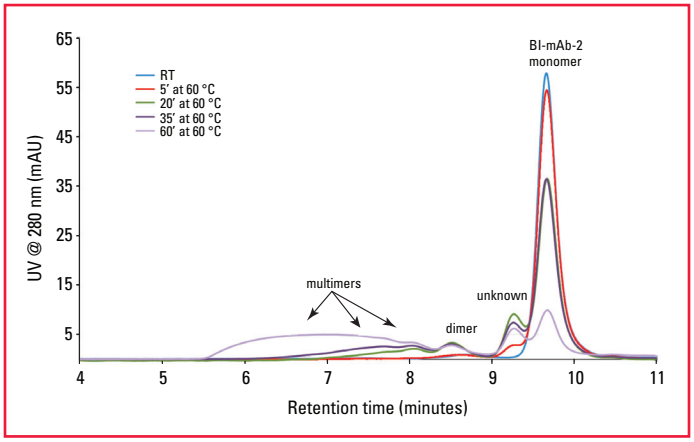
Figure 1. Separation IgG Monomer, Dimer, and Fragments from Papain Digested IgG by TSKgel SuperSW mAb HR and TSKgel G3000SW$_{XL}$ Columns



The results also show that the TSKgel SuperSW mAb HR column has superior performance of mAb separation in comparison to the TSKgel G3000SW$_{XL}$ column. While TSKgel G3000SW$_{XL}$ has set the standard for the separation of general proteins for more than 25 years, the new TSKgel SuperSW mAb HR column is more specifically suited for the analysis of mAb, as seen in the results of the analysis of IgG.

A heat denaturation study of a monoclonal antibody was conducted using a TSKgel UltraSW Aggregate column. The column was used to monitor the denaturation of the antibody as a function of time at pH 5.5 and 60 °C. Heating for one hour at 60 °C results in almost complete breakdown of the monoclonal antibody and the formation of very large aggregates that extend to the exclusion volume of the column. As seen in *Figure 2*, the efficient separation of aggregates from the monomer, induced by heat denaturation, could be achieved using the TSKgel UltraSW Aggregate column. Also shown in *Figure 2*, an 'unknown' aggregate peak of intermediate molecular weight between the monomer and dimer and several higher order aggregate peaks, in addition to the presumed dimer peak at 8.5 minutes, was seen.

Figure 2. Heat Denaturation Study of Monoclonal Antibody (BI-mAb-02) Using a TSKgel UltraSW Aggregate Column



Conclusions

The results of both analyses show the superb performance of the new TSKgel SuperSW mAb HR and UltraSW Aggregate columns for the analysis of monoclonal antibodies. The TSKgel SuperSW mAb HR column exhibited superior resolving power for IgG monomer, dimer, and fragments, while the TSKgel UltraSW Aggregate column demonstrated efficient separation of aggregates from the monomer peak. These new additions to the TSKgel SW-type column line are an excellent choice for your mAb analysis: TSKgel SuperSW mAb HR for high resolution monomer, dimer and fragment analysis, TSKgel UltraSW Aggregate for superior resolution of aggregates.

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