TOSOH

APPLICATION NOTE

ANALYSIS OF LARGE IMMUNOGLOBULIN AGGREGATES BY UHPLC

Antibody therapeutics are enjoying high growth rates, the major areas of therapeutic application being cancer and immune/inflammation-related disorders including arthritis and multiple sclerosis. Today, new antibody formats are entering clinical phases. Some of the new formats have a higher molecular weight than conventional antibodies. The characterization of these complex biomolecules is a major challenge in R & D, process monitoring and quality control. One of the main critical quality attributes is the content of high molecular weight (HMW) and low molecular weight (LMW) impurities.

Size exclusion chromatography (SEC) is the standard technology used in biopharmaceutical QC for mAb aggregate (HMW) and fragment (LMW) analysis. Silica based stationary phases with a pore size of 25 nm are established for decades for the analysis of conventional monoclonal antibodies. However, some of the next generation mAb formats, such as bispecific T-cell antibodies or antibody-cytokine fusion proteins that are larger than standard mAbs may require a slightly larger pore size for detailed analysis of their high molecular weight impurities.

ANALYSIS OF mAb AGGREGATES

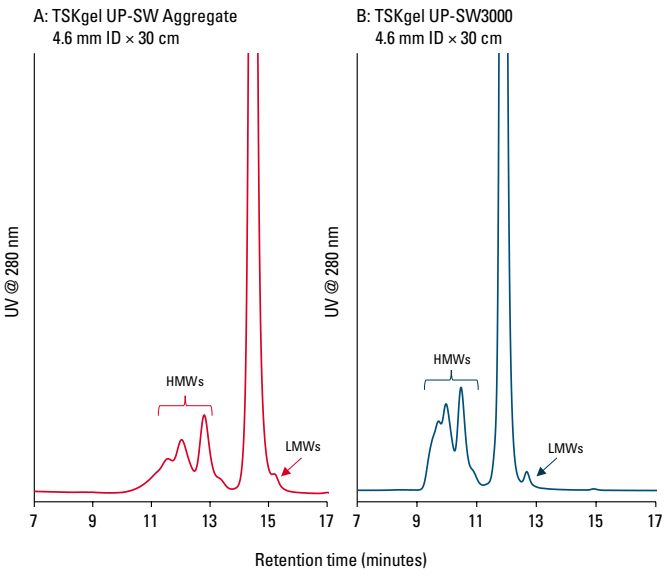

Figure 1

Separation of HMW and LMW impurities on TSKgel UP-SW columns with different pore sizes (30 nm UP-SW Aggregate; 25 nm UP-SW3000).

TSKgel UP-SW Aggregate, the latest addition to the UP-SW series of silica-based size exclusion chromatography (SEC) columns, provides a pore size of 30 nm. It is specifically designed to facilitate the analysis of very large proteins and higher antibody aggregates. This application note demonstrates the advantages of using a slightly larger pore size for the determination of HMW impurities for higher order aggregates of conventional antibodies and for larger immunoglobulin classes, such as IgM.

MATERIAL & METHODS

Columns :	A: TSKgel UP-SW Aggregate (3 µm, 4.6 mm x 30 cm, P/N 0023524) B: TSKgel UP-SW3000 (2 µm, 4.6 mm x 30 cm P/N 0023448) Figures 1 & 3
Eluent:	40 mmol/L phosphate buffer (pH 6.7) + 400 mmol/L sodium perchlorate + 0.05 % sodium azide
Flow rate:	0.20 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Inj. volume:	10 µL
Sample Figure 1:	mAb HMWs: dimer, aggregates; LMWs: fragments
Sample Figure 2:	heat aggregated mAb
Sample Figure 3:	IgM from human serum (Sigma-Aldrich P/N I8260)

RESULTS

HMW and LMW Impurities

The separation of HMW and LMW impurities of a monoclonal antibody was compared for two columns of the TSKgel UP-SW series, UP-SW3000, a column featuring the same pore size as the renowned TSKgel G3000SW$_{XL}$ SEC column, and UP-SW Aggregate featuring a larger pore size. While UP-SW3000 (B) is ideal to achieve good separation of both HMW and LMW impurities, UP-SW Aggregate allows a more detailed view on the higher order aggregates (Figure 1).

Higher Order Aggregates

The analysis of a heat denatured mAb using TSKgel UP-SW Aggregate is shown in Figure 2. The mAb sample was diluted 10-fold with 20 mmol/L sodium phosphate buffer (pH 7.2) + 150 mmol/L sodium chloride and dispensed into aliquots. Each aliquot was stored at 70 °C; 73 °C, 77 °C or 80 °C respectively for 2 hours to force mAb aggregation formation.

The content of higher order aggregates increases with rising temperature. Changes in the aggregate peak profile at four different temperature points are easily discerned between 70 - 80 °C.

ANALYSIS OF HEAT FORCED mAb AGGREGATION

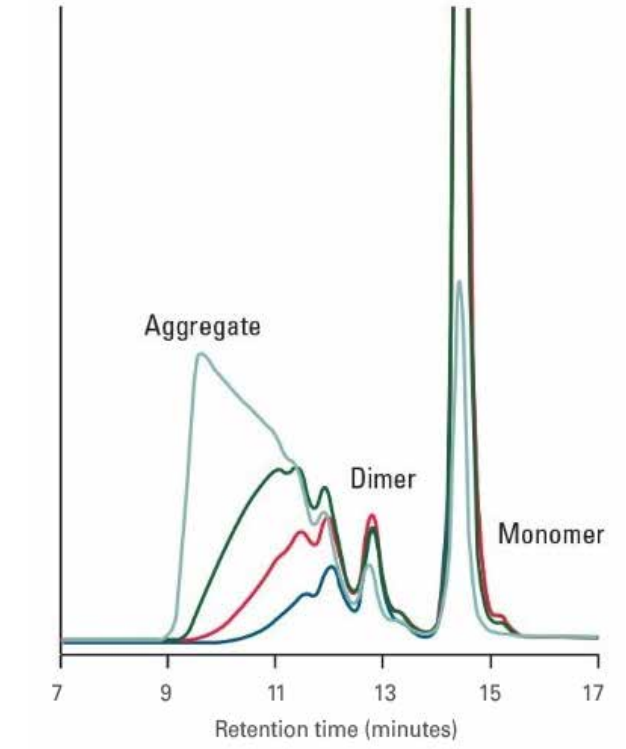

Figure 2

Dark blue: 70 °C; red: 73 °C; green: 77 °C; light blue: 80 °C

Analysis of human IgM

Figure 3 depicts the analysis of human Immunoglobulin M (IgM) on TSKgel UP-SW3000 and TSKgel UP-SW Aggregate. The molecular weight of IgM (970 kDa) is much higher than that of IgG (150 kDa). This is the reason why the larger pore size UP-SW Aggregate column is better suited to analyze the HMW impurities of IgM. While TSKgel UP-SW3000 does not resolve monomer and aggregates, TSKgel UP-SW Aggregate allows determination of the aggregate content.

CONCLUSION

TSKgel UP-SW Aggregate features the largest pore size of the UP-SW series size exclusion columns and was designed to meet the requirements of users analyzing biomolecules with higher molecular weight than standard IgG.

This application note demonstrates that compared to the 25 nm pore size of the TSKgel UP-SW3000 column, which is typically applied to analyze monoclonal antibodies, the 30 nm pore size TSKgel UP-SW Aggregate column is the better choice when analyzing higher order aggregates and large molecules.

SEPARATION OF IGM MONOMER AND AGGREGATES

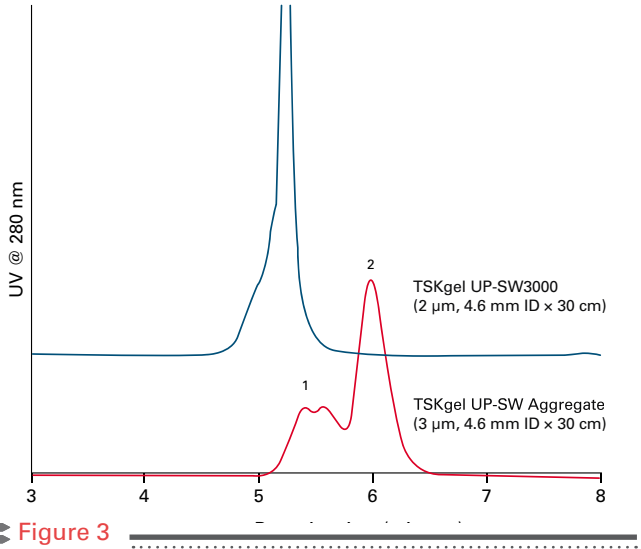

Figure 3

1: IgM Aggregates, 2: IgM Monomer