TOSOH

Application Note

Analysis of Large Immunoglobulin Aggregates by UHPLC-SEC

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 have been 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.

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

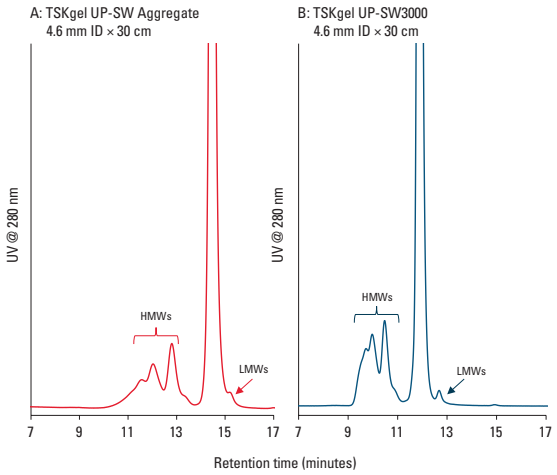
Materials and Methods:

Columns:	A: TSKgel UP-SW Aggregate, 3 µm, 4.6 mm ID × 30 cm B: TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 30 cm (Figures 1 & 3)
Mobile phase:	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
Injection vol.:	10 µL
Sample figure 1:	mAb HMWs: dimer, aggregates; LMWs: fragments
Sample figure 2:	heat aggregated mAb
Sample figure 3:	IgM from human serum (MilliporeSigma P/N I8260)

Results

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 G3000SWXL SEC column and the UP-SW Aggregate column featuring a larger pore size. While TSKgel UP-SW3000 (Panel B) is ideal to get good separation of both HMW and LMW impurities, the TSKgel UP-SW Aggregate column (Panel A) allows a more detailed view on the higher order aggregates *(Figure 1)*.

Figure 1. Analysis of mAb aggregates



The analysis of a heat denatured mAb using the TSKgel UP-SW Aggregate column 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.

Figure 2. Analysis of heat forced mAb aggregation

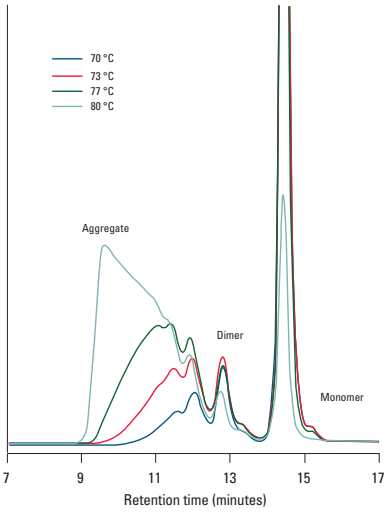
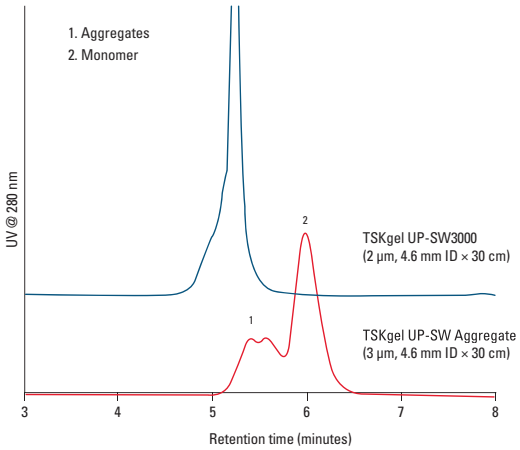


Figure 3 depicts the analysis of human Immunoglobulin M (IgM) on the TSKgel UP-SW3000 and TSKgel UP-SW Aggregate columns. The molecular weight of IgM is much higher than that of IgG. This is the reason why the larger pore size UP-SW Aggregate column is better suited to analyze the HMW impurities of IgM.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

Figure 3. Separation of IgM monomer and aggregates



Conclusions

The TSKgel UP-SW Aggregate column 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 TSKgel UP-SW Aggregate column is the better choice when analyzing high order aggregates and large molecules.