TOSOH

TSKgel® UP-SW AGGREGATE COLUMNS

PROVIDING SUPERIOR SEPARATION FOR HIGH ORDER AGGREGATES AND MACROMOLECULES

INTRODUCTION

Aqueous size exclusion chromatography (SEC) is a widely applied technique for protein characterization and quality control. The main application is the quantitative determination of monoclonal antibody aggregates. The biological phenomenon of protein aggregation is a major issue in therapeutic protein development, since the presence of these impurities reduces the potency of the drug formulation, even if non-toxic. Therapeutic antibodies must be free from these aggregate impurities. In order to fully evaluate the aggregates, a size exclusion column that has a large enough pore size is needed so that the higher order aggregates are not excluded in the void but separated as a function of hydrodynamic volume.

SEPARATION OF STANDARD PROTEINS

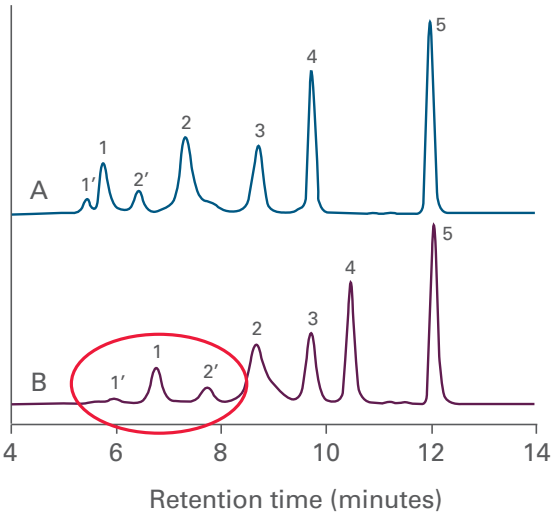

Figure 1

Column: A. TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm L
B. TSKgel UP-SW Aggregate, 3 µm, 4.6 mm ID × 30 cm L
Mobile phase: 100 mmol/L sodium phosphate buffer, pH 6.7,
+ 100 mmol/L sodium sulfate + 0.05 % sodium azide
Flow rate: 0.35 mL/min
Detection: UV @ 280 nm
Temperature: 25 °C
Injection vol.: 10 µL
Samples: 1. thyroglobulin (MW 640,000) (1′. thyroglobulin dimer)
2. γ - globulin (MW 155,000) (2′. γ - globulin dimer)
3. ovalbumin (MW 47,000)
4. ribonuclease A (MW 13,700)
5. p-amino benzoic acid (MW 137)

TSKgel UP-SW Aggregate columns are 3 µm, 30 nm pore size SEC analytical columns that have been designed with a higher exclusion limit than other TSKgel UP-SW columns. With a separation range of 10-2,000 kDa, these columns are ideal for the separation of mAb aggregates, high molecular weight proteins and nucleic acids. Available in 4.6 mm ID x 15 and 30 cm lengths, TSKgel UP-SW Aggregate columns are compatible with both UHPLC and HPLC systems and require less sample while delivering higher sensitivity compared to the 7.8 mm ID TSKgel UltraSW Aggregate column. These columns feature high pore volume per unit column volume, low sample adsorption and excellent column efficiency, all contributing to unsurpassed sample resolution.

The lifetime of the TSKgel UP-SW Aggregate columns are superior and can be maintained and further improved when using the corresponding guard columns. A “direct connect” (DC) guard column allows the minimization of extra column dead volume.

HIGHLIGHTS

- Proven TSKgel SW SEC quality
- Separation range of 10-2,000 kDa, ideal for high molecular weight (MW) proteins and mAb aggregates
- High sensitivity and excellent column efficiency
- Compatible with both HPLC and UHPLC systems
- Rapid analysis with use of short columns

APPLICATIONS

Figure 1 demonstrates the superior resolution of a TSKgel UP-SW Aggregate column for larger MW proteins. The pore characteristics of this column allow the widest separation range in the mAb dimer and higher MW regions, as noted by the circled areas in the figure. Compared to the 25 nm pore size of the TSKgel UP-SW3000 column, the 30 nm TSKgel UP-SW Aggregate column is the better choice when analyzing higher order aggregates and large molecules.

The analysis of a heat denatured mAb using the TSKgel UP-SW Aggregate column is shown in Figure 2. Thermal denaturation was employed to force mAb aggregation formation. Changes in the aggregate peak profile at four different temperature points are easily discerned between 70 - 80 °C, demonstrating the applicability of the TSKgel UP-SW Aggregate column for the separation of mAb aggregates.

Figure 3 shows the use of a TSKgel UP-SW Aggregate column for the separation of a DNA molecule over a wide molecular weight range. Excellent peak shape was obtained for a DNA fragment with up to 500 base pairs.

SEPARATION OF AGGREGATE PEAKS AT VARYING TEMPERATURES

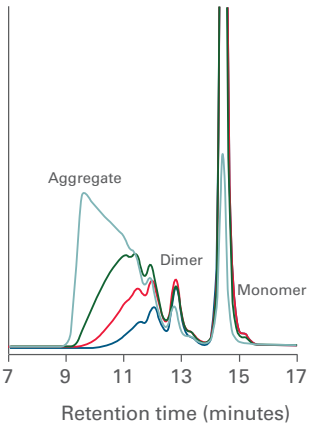

Figure 2

Column: TSKgel UP-SW Aggregate, 3 µm, 4.6 mm ID × 30 cm L
Mobile phase: 40 mmol/L sodium phosphate buffer, pH 6.7, + 400 mmol/L sodium perchlorate + 0.05 % sodium azide
Flow rate: 0.2 mL/min
Detection: UV @ 280 nm
Temperature: 25 °C
Injection vol.: 10 µL
Sample: mAb

* The sample was diluted by 10-fold with 20 mmol/L sodium phosphate buffer (pH 7.2) + 150 mmol/L sodium chloride and dispensed to small aliquots. Each aliquot was stored at following temperature respectively for 2 hours.
70 °C (blue chromatogram)
73 °C (red chromatogram)
77 °C (green chromatogram)
80 °C (light blue chromatogram)

SEPARATION OF DNA FRAGMENTS

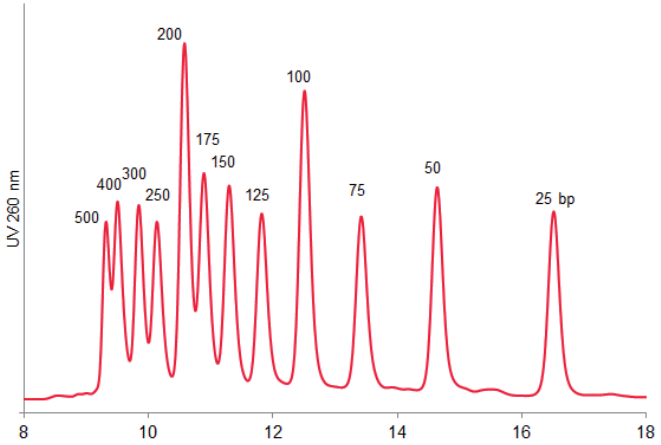

Figure 3

Column: TSKgel UP-SW Aggregate, 3 µm, 4.6 mm ID × 30 cm L
Mobile phase: 100 mmol/L sodium phosphate buffer, pH 6.7, + 300 mmol/L sodium chloride + 0.05 % sodium azide
Flow rate: 0.2 mL/min
Detection: UV @ 260 nm
Temperature: 25 °C
Injection vol.: 10 µL
Sample: DNA ladder

Ordering information

TSKgel UP-SW Aggregate

Part-No	Description	Matrix	Housing	Dimensions
0023524	TSKgel UP-SW Aggregate	Silica	Stainless Steel	4.6 mm ID x 30 cm L
0023525	TSKgel UP-SW Aggregate	Silica	Stainless Steel	4.6 mm ID x 15 cm L
0023526	TSKgel UP-SW Aggregate Guard	Silica	Stainless Steel	4.6 mm ID x 2.0 cm L
0023527	TSKgel UP-SW Aggregate Guard DC	Silica	Stainless Steel	4.6 mm ID x 2.0 cm L