TOSOH

Product Overview

TSKgel® UP-SW Aggregate Column

Providing superior separation for high order aggregates and macromolecules

Introduction

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. Monoclonal antibodies, widely used in the field of biotherapeutics, 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.

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 (MW) proteins and nucleic acids. Available in 4.6 mm ID × 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 (due to the derivatization of the particle surface with ligands containing diol functional groups) 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.

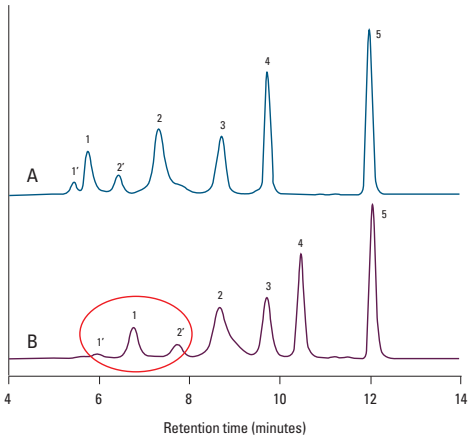
Highlights

- Proven TSKgel SW SEC quality
- Separation range of 10-2,000 kDa, ideal for high MW proteins and mAb aggregates
- Provides 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.

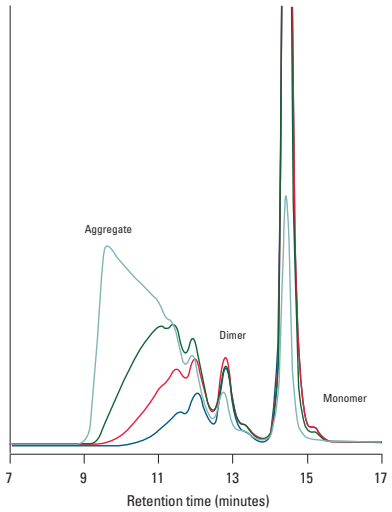
Figure 1. Separation of standard proteins



Column:	A. TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm B. TSKgel UP-SW Aggregate, 3 µm, 4.6 mm ID × 30 cm
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)

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 2. Separation of aggregate peaks at varying temperatures

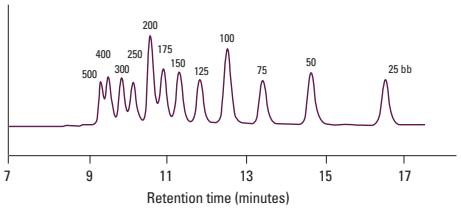


Column:	TSKgel UP-SW Aggregate, 3 µm, 4.6 mm ID × 30 cm
Mobile phase:	40 mmol/L sodium phosphate buffer, pH 6.7, + 400 mmol/L sodium perchlorate + 0.05 % sodium azide
Flow rate:	0.2 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	mAb

* 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 (black chromatogram)
73 °C (red chromatogram)
77 °C (green chromatogram)
80 °C (light blue chromatogram)

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.

Figure 3. Separation of DNA fragments



Column:	TSKgel UP-SW Aggregate, 3 µm, 4.6 mm ID × 30 cm
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

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
23524	TSKgel UP-SW Aggregate	Silica	Stainless Steel	4.6	30
23525	TSKgel UP-SW Aggregate	Silica	Stainless Steel	4.6	15
23526	TSKgel guard column UP-SW Aggregate	Silica	Stainless Steel	4.6	2
23527	TSKgel guard column UP-SW Aggregate DC*	Silica	Stainless Steel	4.6	2

*The guard column can be directly connected to the analytical column without tubing between the two columns. A male-type outlet endfitting on the guard column enables the direct connection to the screw-type endfitting of the analytical column.