TOSOH

Application Note

Rapid and accurate therapeutic mAb aggregate analysis using a TSKgel® UP-SW3000, 2 µm, SEC Column

Introduction

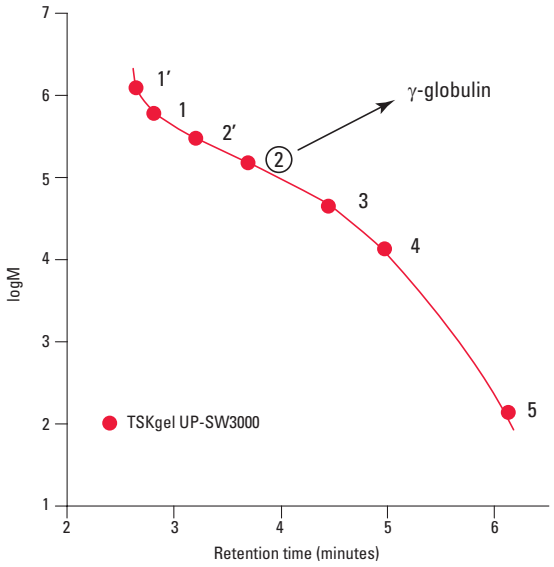
In the analysis of biomolecules, particularly HPLC analytical size exclusion chromatography (SEC) columns are widely used to determine the ratio of aggregates, dimers, monomers, and fragments in monoclonal antibodies (mAbs). Columns are expected to deliver high resolution, excellent reproducibility in a short analysis time. In order to achieve these parameters, SEC columns must have the appropriate particle size, pore size, good bonding chemistry, and suitable column dimensions. In addition, the columns must be packed well. Traditionally, SEC columns with 30 cm length are used for high resolution analysis because the length allows different molecular sizes to be separated with a longer run time. However, because of the long length, a typical analysis can take up to 30-40 minutes for each analysis. With the demands for high sample throughput, there is a need for shorter analysis time. There are many available SEC columns with 15 cm length currently available for this usage. However, these columns typically suffer from low resolution.

This application describes the use of a 4.6 mm ID × 15 cm TSKgel UP-SW3000 SEC column for fast and accurate mAb aggregate analysis without compromising the quality of the aggregate determination or reproducibility. Unlike many other available 15 cm length SEC columns, these columns are packed such that they can be operated with both HPLC and UHPLC systems. The 4.6 mm ID × 15 cm TSKgel UP-SW3000 SEC column has a particle size of 2 µm and a 25 nm pore size. The particles are coated with a hydrophilic diol-type bonded phase in order to minimize the interaction between the silica surface and proteins. The column is designed to be operated with a simple and well established method (sodium phosphate mobile phase, pH 6.8). A comparison study was done between a TSKgel UP-SW3000, 15 cm column and a 30 cm length column, both 4.6 mm ID. Results show that the run time of the 15 cm column was completed in 4 minutes without compromising the resolution of the chromatogram.

Experimental Conditions

Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.8, + 100 mmol/L sodium sulfate + 0.05% sodium azide
Gradient:	Isocratic
Flow rate:	as indicated in each chromatogram
LC system:	Ultimate® 3000RS UHPLC system
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	mAb (0.4 mg/mL)

Figure 1. Standard calibration curve of QC protein standard mixture generated by TSKgel UP-SW3000, 4.6 mm ID × 15 cm column



Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm
Mobile phase:	100 mmol/L phosphate buffer, pH 6.7, + 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol:	5 µL
Samples:	1' Thyroglobulin dimer 1. Thyroglobulin, 640,000 Da 2' γ-globulin dimer 2. γ-globulin, 155,000 Da 3. Ovalbumin, 47,000 Da 4. Ribonuclease A, 13,000 Da 5. p-aminobenzoic acid, 137 Da

Results

Figure 1 shows the protein standard calibration curve data that was generated using the TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 15 cm SEC column. The column was run with a simple aqueous mobile phase (sodium phosphate buffer, pH 6.8) as typically reported in literature for SEC separations. The data demonstrates that the TSKgel UP-SW3000 column has a broad and linear resolving range of molecular weights. The shallow slope around the molecular weights of thyroglobulin, γ-globulin to p-aminobenzoic acid suggests that the particles of the column have an optimized pore size for separating aggregates, dimer, monomer, and fragments of proteins with a molecular weight of approximately 150 kDa such as mAb.

Figure 2 shows the separation comparison data for mAb between a 30 cm TSKgel UP-SW3000 column and a 15 cm length. Both columns were operated under the same mobile phase conditions and flow rate. The results indicate that the 15 cm TSKgel UP-SW3000 column provides a similar profile to the 30 cm column with 50% less run time and 50% lower backpressure at a typical flow rate of 0.35 mL/min (See *Figure 2*). The resolution between dimer and monomer is slightly less with the 15 cm column but it is still above the resolution guidelines from the USP monogram (1.2 resolution is acceptable). In addition, when the 15 cm column is operated at the typical flow rate of 0.35 mL/min, the backpressure is only 11 MPa. Therefore, these columns can be used with both HPLC and UHPLC systems.

Figure 2. Comparison of mAb aggregates analysis between TSKgel UP-SW3000, 15 cm and 30 cm columns using the same mobile phase conditions and flow rate

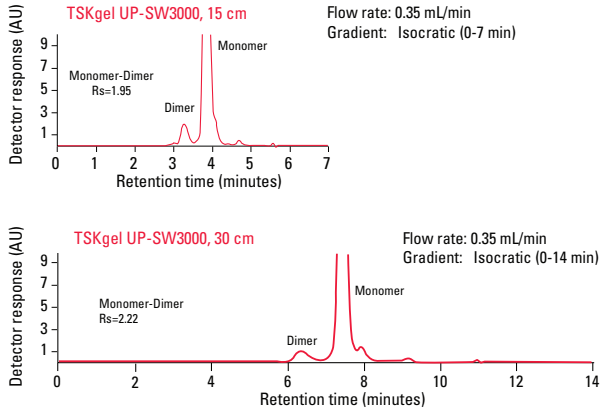


Figure 3 demonstrates the rapid aggregate determination of a mAb using a TSKgel UP-SW3000, 4.6 mm ID × 15 cm column operated at 0.5 mL/min. The figure shows that the analysis was completed in only 4 minutes, nearly a 4 times faster run time than the 30 cm length column (compare the run time of *Figure 2*, bottom panel to *Figure 3*) and nearly 8 times faster than a traditional SEC column run time of 30 minutes at 1 mL/min (data not shown). The resolution profile of the aggregates and monomer of mAb (Rs = 1.97) is still maintained at the acceptable range in the USP guideline. Results from 10 consecutive injections (*Table 1*) show that the TSKgel UP-SW3000, 15 cm column provides high reproducibility at a fast run time.

Figure 3. Fast analysis of mAb sample using TSKgel UP-SW3000, 4.6 mm ID × 15 cm column

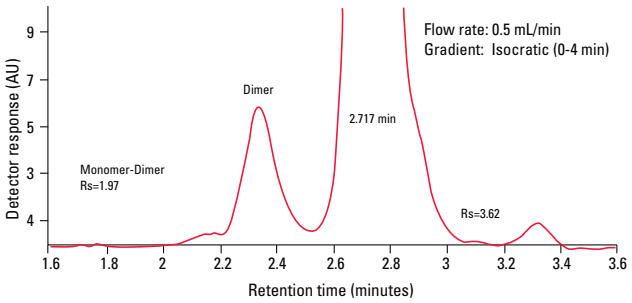


Table 1. 10 consecutive runs (of mAb sample) yielded excellent reproducibility.

Monomer peak						
Injection #	Ret. time min.	Area mAU min	Height mAU	Width (50%) min	Asym. EP	Plates EP
1	2.717	16.72	155.460	0.093	1.26	4754
2	2.717	16.58	155.440	0.093	1.26	4762
3	2.717	16.62	155.780	0.093	1.26	4762
4	2.717	16.87	156.750	0.093	1.26	4740
5	2.717	16.91	157.360	0.093	1.26	4748
6	2.717	16.90	157.310	0.093	1.26	4749
7	2.717	16.75	157.190	0.093	1.26	4770
8	2.717	16.92	157.540	0.093	1.27	4758
9	2.717	16.94	157.910	0.093	1.27	4762
10	2.717	16.85	157.400	0.092	1.27	4780
11	2.717	16.77	156.840	0.093	1.28	4787
12	2.717	16.64	154.700	0.093	1.26	4748
13	2.717	16.73	155.360	0.093	1.26	4747
15	2.717	16.82	156.090	0.093	1.26	4742
Average	2.717	16.787	156.509	0.093	1.264	4758
Std Dev	0.000	0.119	1.014	0.000	0.006	13.907
%RSD	0.000	0.707	0.648	0.391	0.501	0.292

Conclusion

The above results demonstrate the broad and linear molecular weight resolving range of TSKgel UP-SW3000, 2 µm SEC columns. This, in turn, drives the accuracy, reliability and reproducibility for molecules of interest such as the monomer, dimer, and aggregates of mAbs. The comparison between a 15 cm and 30 cm TSKgel UP-SW3000 column using the same flow rate and operating mobile phase conditions showed that the 15 cm length column generates similar and acceptable resolution for aggregate analysis. At 0.5 mL/min flow rate, analysis can be completed within 4 minutes with acceptable resolution and at a low backpressure that allows TSKgel UP-SW3000 columns to be run in both HPLC and UHPLC systems.