About: TSKgel Alpha and SuperAW Size Exclusion Columns

TSKgel Alpha and SuperAW columns were developed for the GPC analysis of polymers of intermediate polarity. As in the TSKgel PW and PW$_{XL}$ columns, the particles in these TSKgel columns have a hydroxylated methacrylate polymer backbone, but they differ in that they are crosslinked to a higher degree to minimize swelling in polar organic solvents (methanol, acetonitrile, DMSO, isopropanol, THF, and HFIP). The TSKgel Alpha and SuperAW columns provide accurate molar mass determination and exhibit normal retention of polystyrene polymers in dimethyl formamide (DMF) solvent. Unlike TSKgel PW columns, which are stable to a 50% organic mixed with water at most, TSKgel SuperAW and Alpha columns are stable in a wide variety of organic solvents at concentrations up to 100%. TSKgel Alpha and SuperAW columns are offered in 5 discrete exclusion ranges and as a mixed bed column. Both column types can accommodate polymer standards up to several million Dalton molar mass.

- Use TSKgel Alpha columns when throughput is not critical, when sample mass is not limited, to collect fractions, and to obtain maximum number of plates (at the expense of analysis time). The main application area for TSKgel Alpha columns is the analysis of polymers that are soluble in polar organic solvents. Examples include cellulose derivatives, polyimide, and sodium dodecylsulfate (all in 10 mmol/L LiBr in DMF), cleansing gel in methanol, and degree of saponification of polyvinylalcohol in hexafluoroisopropanol (HFIP).

  The TSKgel Alpha Series consists of six columns with three particle sizes: 7, 10, and 13 µm. These columns span a wide molar mass separation range, from 100 to more than 1 × 10^6 Da, when using polyethylene oxide (PEO) as a molar mass standard. There is one mixed bed column within the TSKgel Alpha line, TSKgel Alpha-M, which has an extended linear calibration range and is suitable for samples with a broad molar mass distribution, as well as samples with unknown molar mass.

TSKgel Alpha columns include:

- TSKgel Alpha-2500
- TSKgel Alpha-3000
- TSKgel Alpha-4000
- TSKgel Alpha-5000
- TSKgel Alpha-6000
- TSKgel Alpha-M

- Use TSKgel SuperAW columns for high throughput applications, to reduce solvent consumption and to reduce solvent disposal cost. TSKgel SuperAW columns contains a similar chemistry as the TSKgel Alpha columns but offer the benefit of smaller particle sizes (4, 6, 7, and 9 µm), smaller column dimensions, and equivalent resolution. Reductions in analysis time and mobile phase consumption make TSKgel SuperAW columns ideal for high throughput applications.

  The TSKgel SuperAW column line consists of five columns and a mixed bed column. These high efficiency columns are only available in 6.0 mm ID × 15 cm dimensions.

TSKgel SuperAW columns include:

- TSKgel SuperAW2500
- TSKgel SuperAW3000
- TSKgel SuperAW4000
- TSKgel SuperAW5000
- TSKgel SuperAW6000
- TSKgel SuperAWM-H

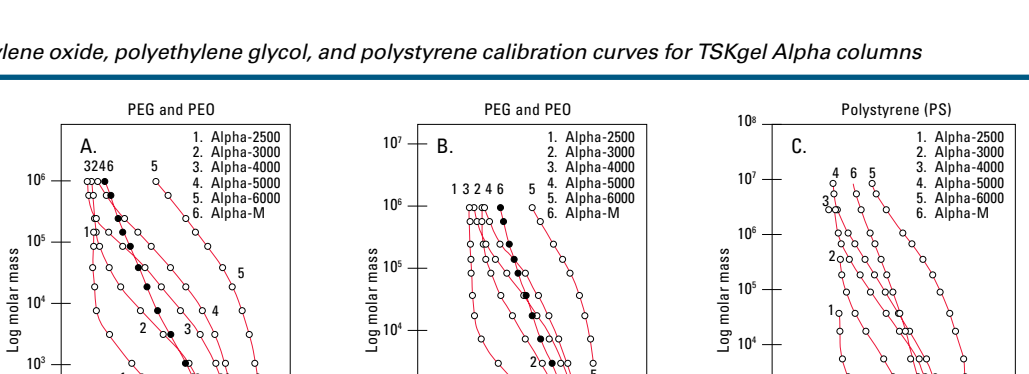
Attributes and Applications:

Product attributes of the TSKgel Alpha and SuperAW columns are shown in Table 19. These columns are for the analysis of polymers that are soluble in methanol, acetonitrile, DMSO, isopropanol, or THF and can also be used for water-soluble polymers. Figures 72-73 show the calibration curves for the TSKgel Alpha and SuperAW columns. Unlike TSKgel PW/PW$_{XL}$ columns, some of which are stable up to 50% organic mixed with water, TSKgel SuperAW and Alpha columns are stable in a wide variety of organic solvents at concentrations up to 100%. As shown in Figure 74, efficiency of all TSKgel SuperAW columns is maintained when changing solvents from water via acetonitrile, DMF, DMSO, THF to HFIP. Suitable solvents for TSKgel Alpha columns are shown in Figure 75.

Table 19: Product attributes

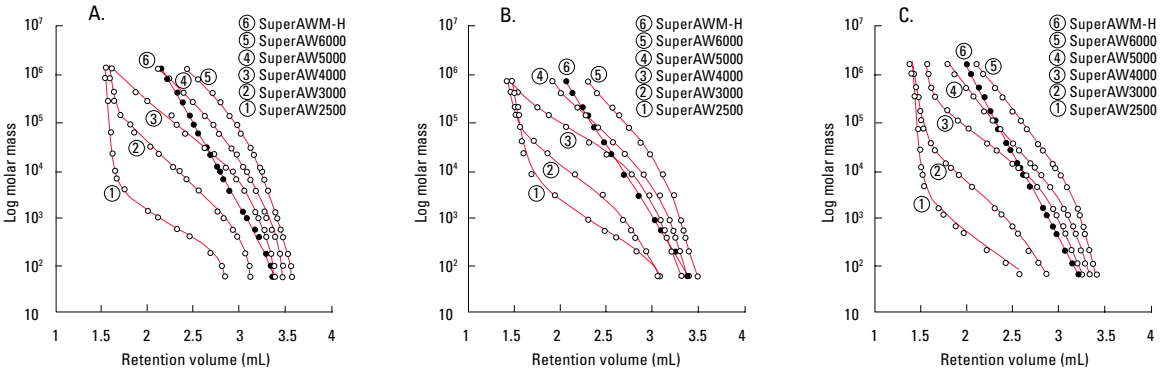
			Exclusion limit (Da) for various standards & eluents		
TSKgel column	**Particle size**	**Pore size**	**PEO in H_2O**	**PS in DMF with 10 mmol/L LiBr**	**PEG in MeOH with 10 mmol/L LiBr**
Alpha-2500	7 µm	2.5 nm	5,000	1×10^4	1×10^4
Alpha-3000	7 µm	15 nm	9×10^4	1×10^5	6×10^4
Alpha-4000	10 µm	45 nm	4×10^5	1×10^6	3×10^6
Alpha-5000	10 µm	100 nm	1×10^6	7×10^6	$>3 \times 10^5$
Alpha-6000	13 µm	>100 nm	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$
Alpha-M	13 µm	mixed bed	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$
SuperAW2500	4 µm	2.5 nm	5,000	1×10^4	1×10^4
SuperAW3000	4 µm	15 nm	9×10^4	1×10^5	6×10^4
SuperAW4000	6 µm	45 nm	4×10^5	1×10^6	3×10^6
SuperAW5000	7 µm	100 nm	1×10^6	7×10^6	$>3 \times 10^5$
SuperAW6000	9 µm	>100 nm	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$
SuperAWM-H	9 µm	mixed bed	$>1 \times 10^7$	$>1 \times 10^7$	$>3 \times 10^5$

Figure 72: Polyethylene oxide, polyethylene glycol, and polystyrene calibration curves for TSKgel Alpha columns



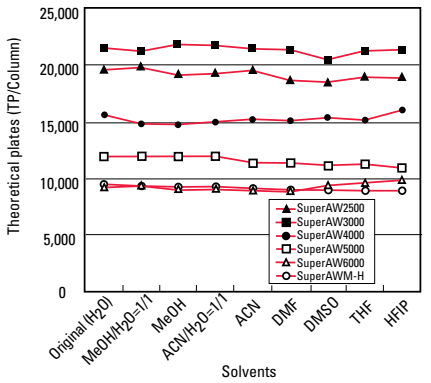
Column: **TSKgel Alpha columns, 7.8 mm ID × 30 cm**
Mobile phase: A. H_2O
B. MeOH with 10 mmol/L LiBr
C. DMF with 10 mmol/L LiBr
Flow rate: 1.0 mL/min
Detection: RI
Temperature: A. 25 °C B. 25 °C C. 40 °C
Samples: A, B: polyethylene glycol (PEG) and polyethylene oxide (PEO) standards
C. polystyrene (PS) standards

Figure 73: Polyethylene oxide, polyethylene glycol, and ethylene glycol calibration curves for TSKgel SuperAW columns



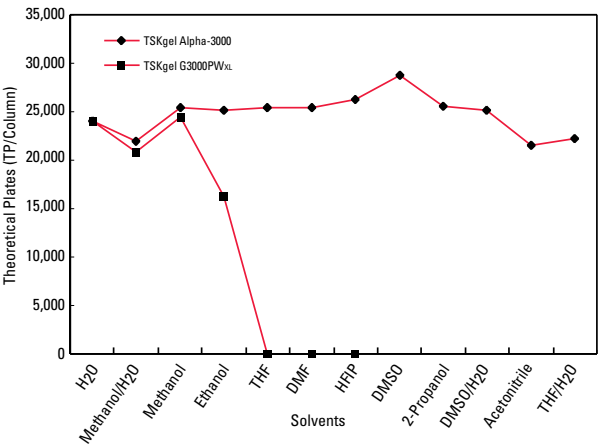
Column: **TSKgel SuperAW columns, 6.0 mm ID × 15 cm**
Mobile phase: A. H_2O
B. MeOH with 10 mmol/L LiBr
C. DMF with 10 mmol/L LiBr
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25 °C
Samples: polyethylene oxide (PEO), polyethylene glycol (PEG), and ethylene glycol (EG) standards

Figure 74: Column efficiency of TSKgel SuperAW columns



Column: **TSKgel SuperAW columns, 6.0 mm ID × 15 cm**
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25 °C
Injection vol.: 5 µL (2.5 g/L)
Sample: ethylene glycol

Figure 75: Solvent compatibility for TSKgel Alpha-3000 for organic solvents



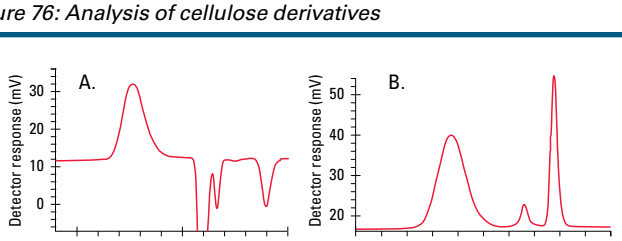
Conditions of solvent change
Flow Rate: 1.0 mL/min
Temperature: 25 °C
Time for purge: 8 h

Conditions for TP measurement
Flow Rate: 1.0 mL/min
Detection: RI
Temperature: 25 °C
Sample: ethylene glycol

Cellulose Derivatives

The versatility of using TSKgel Alpha columns with various polar solvents is illustrated in Figure 76 for the analysis of cellulose derivatives. A TSKgel Alpha-M column was used to separate ethylcellulose with the polar solvent DMF and ethylhydroxyethyl cellulose with methanol.

Figure 76: Analysis of cellulose derivatives

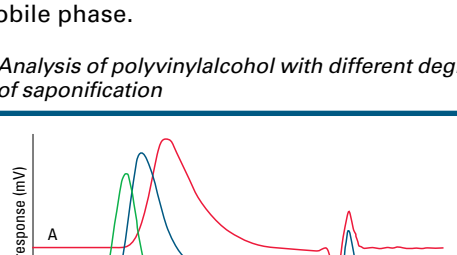


Column:	**TSKgel Alpha-M, 13 µm, 7.8 mm ID × 30 cm**
Mobile phase:	A. DMF with 10 mmol/L LiBr B. MeOH with 10 mmol/L LiBr
Flow rate:	0.5 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol:	50 µL
Samples:	A. ethyl cellulose, 0.1% B. ethyl hydroxyethyl cellulose, 0.1%

Polyvinylalcohol Characterization

The separation of polyvinylalcohol with different degrees of saponification is shown in Figure 77. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol (HFIP) mobile phase.

Figure 77: Analysis of polyvinylalcohol with different degrees of saponification

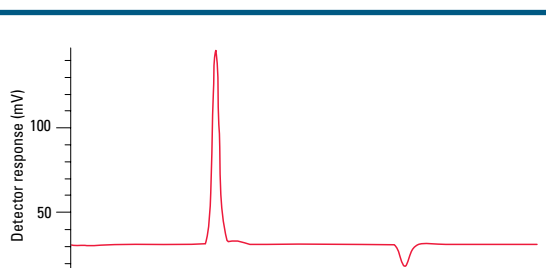


Column:	**TSKgel Alpha-5000 and Alpha-3000, 7.8 mm ID × 30 cm in series**
Mobile phase:	hexafluoroisopropanol (HFIP)
Flow rate:	0.5 mL/min
Detection:	RI
Temperature:	40 °C
Samples:	degree of saponification of polyvinyl alcohol: A. 75% B. 88% C. 100%

Glyceryl tri(2-ethylhexanoate)

Glyceryl tri(2-ethylhexanoate) is used as a plastic lubricant and as a cosmetic base. The analysis of this compound using a TSKgel Alpha-2500 column is shown in Figure 78.

Figure 78: Analysis of glyceryl tri(2-ethylhexanoate)

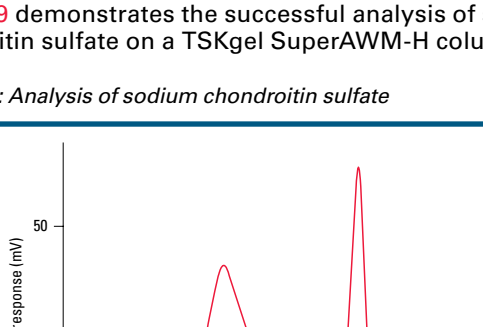


Column:	**TSKgel Alpha-2500, 7 µm, 7.8 mm ID × 30 cm**
Mobile phase:	MeOH
Flow rate:	0.5 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol:	50 µL
Sample:	glyceryl tri-2-ethylhexanoate
Sample load:	0.10%

Sodium Chondroitin Sulfate

Figure 79 demonstrates the successful analysis of sodium chondroitin sulfate on a TSKgel SuperAWM-H column.

Figure 79: Analysis of sodium chondroitin sulfate

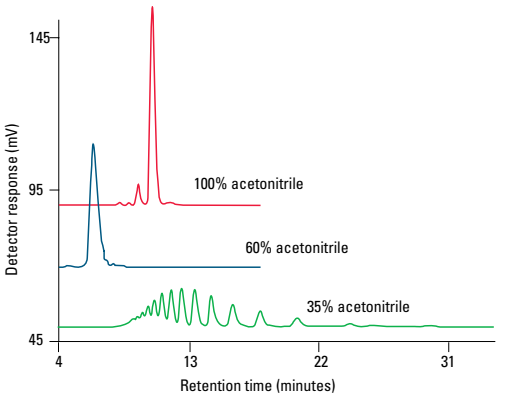


Column:	**TSKgel SuperAWM-H, 9 µm, 6.0 mm ID × 15 cm × 2**
Mobile phase:	H_2O with 0.2 mol/L sodium nitrate
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	25 °C
Injection vol.:	5 µL (2.5 g/L)
Sample:	chondroitin sulfate

Applications in Non-SEC Mode

Since TSKgel SuperAW columns have excellent solvent compatibility, it is possible to obtain different chromatograms by changing the mobile phase composition of a sample. An example of this is shown in Figure 80 for the analysis of a surfactant using a TSKgel SuperAW2500 column. The sample is separated based on the molecular size (SEC mode) when using a mobile phase with a 60% acetonitrile solution, while it is retained in other mobile phase compositions (separated by a non-SEC mode). Thus it is possible to set up the mobile phase conditions to suit the purpose of measurement (molar mass measurement, assay, separation) in one column.

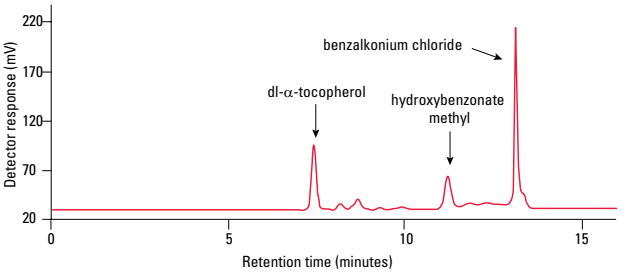
Figure 80: Analysis of a surfactant



Column:	**TSKgel SuperAW2500, 7 µm, 6.0 mm ID × 15 cm**
Mobile phase:	ACN, ACN solution
Flow rate:	0.6 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	20 µL

An application of measuring a formulated drug is shown in Figure 81. Additives are retained on the TSKgel SuperAW2500 column and separated. The effectiveness of this column for the separation of samples containing low molar mass to high molar mass components is apparent.

Figure 81: Analysis of medicated cream



Column:	**TSKgel SuperAW2500, 7 µm, 6.0 mm ID × 15 cm × 2**
Mobile phase:	ethanol
Flow rate:	0.6 mL/min
Detection:	UV @ 275 nm
Temperature:	40 °C
Injection vol.:	10 µL