TOSOH

SEPARATION REPORT NO. 110

HIGH-PERFORMANCE AQUEOUS SEC SEMI-MICRO COLUMNS: TSKgel SuperMultipore PW SERIES AND TSKgel SuperOligoPW

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1. Introduction

The convenience, simplicity, and superb reproducibility of size exclusion chromatography (SEC) have advanced its adoption as an analytical method across a broad range of fields, such as chemistry, food products, biosciences, and pharmaceuticals. SEC can be used to determine the molar mass distribution of both water-soluble and water-insoluble synthetic and natural polymers, thus the method is being widely adopted by researchers, developers, and analysts around the world.

The observed differences between an approximate and an actual calibration curve when using a typical SEC column depends on: particle and pore size of the column packing material, column exclusion limit, and column separation range. Similarly, the chromatogram of polymer samples also depends on the characteristics of the SEC column. For example, the use of multiple packing materials with different pore sizes may create distortions in chromatograms of polymer samples. Since molar mass is directly calculated from calibration curves made using polymer standards s and the chromatogram of the polymer sample, deviations in either parameter can make accurate molar mass determination by SEC difficult.

In order to resolve these issues, Tosoh developed two series of multipore columns for organic SEC: the TSKgel MultiporeHXL-M[1,2] and the TSKgel SuperMultiporeHZ series[3]. The TSKgel MultiporeHXL-M and SuperMultiporeHZ series columns have one packing material comprising of a single particle type with a broad range of pore sizes. The differences between the two column series is that the TSKgel MultiporeHXL-M column has a length of 30 cm while the SuperMultiporeHZ series columns are 15 cm in length. These products are highly regarded on the market.

Recently, Tosoh established a synthesis method for a new multipore column packing material with a broad range of pore sizes on a single particle for aqueous SEC. This method was established in a similar manner as that used for the TSKgel MultiporeHXL-M and TSKgel SuperMultiporeHZ series columns. Consequently, a high performance packing material for aqueous SEC enabling both the separation of polymers and oligomers was developed and marketed.

This report describes the fundamental properties and applications of the new TSKgel SuperMultiporePW series aqueous SEC semi-micro columns and the TSKgel SuperOligoPW column, a high performance aqueous SEC semi-micro column for oligomer separation.

2. Features of TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW columns

Conventional SEC has optimized the molar mass separation range of aqueous- and organic-soluble polymers by either coupling multiple columns with packing materials of different pore sizes, or by using mixed-bed columns that have modified pore characteristics (molar mass separation range and calibration curve linearity) with the optimum ratio of packing materials with different pore sizes. These methods, however, have inflection points in the calibration curve or distortions on the chromatograms for some samples. Thus, accuracy and analytical precision are insufficient. To alleviate these problems, which directly affect molar mass determination, Tosoh has marketed the TSKgel MultiporeHXL-M and the TSKgel SuperMultiporeHZ series SEC columns for organic solvents, and gained favorable reviews for them.

The TSKgel SuperMultiporePW series aqueous SEC semi-micro columns were developed under a new synthesis method and have some of the same features as the organic SEC columns. Because the new product is a semi-micro column packed with monodisperse fine particles, separation comparable to the conventional TSKgel PWXL series columns for aqueous SEC is achieved in half the analysis time and the volume of solvent required is reduced.

For oligomer separation, the product line also includes the high performance TSKgel SuperOligoPW aqueous SEC semi-micro column, which has outstanding separation performance for oligomers and low molar mass polymers.

3. Fundamental properties of TSKgel SuperMultiporePW series and TSKgel SuperOligoPW columns

The fundamental properties of TSKgel SuperMultiporePW series and TSKgel SuperOligoPW are presented in **Tables 1 and 2** and the features are summarized in **Table 3**. The TSKgel SuperMultiporePW series is comprised of three columns differing in particle size and molar mass separation range. The appropriate column can be selected based on the molar mass range of the polymer to be analyzed. The TSKgel SuperOligoPW column has the smallest particle size and molar mass exclusion limit, and is thus suited for oligomer separation.

Table 1 Physical characteristics of TSKgel SuperMultiporePW and TSKgel SuperOligoPW columns

	SuperMultiporePW-N	SuperMultiporePW-M	SuperMultiporePW-H	SuperOligoPW
Packing material substrate	Polymethacrylate	Polymethacrylate	Polymethacrylate	Polymethacrylate
Particle size	4 μm	5 μm	8 μm	3 μm
Molar mass exclusion limit (PEO, PEG/H_2O)	120,000	1,000,000	10,000,000*	6,000
Molar mass separation range (PEO, PEG/H_2O)	300 to 50,000	500 to 1,000,000	1,000 to 10,000,000	100 to 3,000
Column theoretical plates	16,000 TP/15 cm	12,000 TP/15 cm	7,000 TP/15 cm	16,000 TP/15 cm
Column size	6.0 mm ID x 15 cm	6.0 mm ID x 15 cm	6.0 mm ID x 15 cm	6.0 mm ID x 15 cm
Guard column size	4.6 mm ID x 3.5 cm	4.6 mm ID x 3.5 cm	4.6 mm ID x 3.5 cm	4.6 mm ID x 3.5 cm

*Estimated value
PEO: polyethylene oxide
PEG: polyethylene glycol

Table 2 Specification of TSKgel SuperMultiporePW series and TSKgel SuperOligoPW columns

Product Name	Theoretical plates (TP/column) (Guaranteed)	Asymmetry coefficient	Column size (ID, mm x length, cm)	Particle size (μm)
TSKgel SuperMultiporePW-N	16,000 TP/15 cm	0.7 to 1.6	6.0 x 15	4
TSKgel SuperMultiporePW-M	12,000 TP/15 cm	0.7 to 1.6	6.0 x 15	5
TSKgel SuperMultiporePW-H	7,000 TP/15 cm	0.7 to 1.6	6.0 x 15	8
TSKgel SuperOligoPW	16,000 TP/15 cm	0.7 to 1.6	6.0 x 15	3

Separation Conditions

Column size: 6.0 mm ID x 15 cm
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Temperature: 25˚C
Detection: RI (microcell)
Sample: ethylene glycol, 5 g/L
Injection volume: 2 μL

Table 3 Features of TSKgel SuperMultiporePW series and TSKgel SuperOligoPW columns

Features	Benefits
1) Multipore packing material (Each particle has a broad pore size distribution)	• Superior calibration curve linearity • No chromatogram distortion observed in analyzed samples → Improved accuracy and reproducibility of molecular mass data
2) Finer particle size of packing material (monodisperse particles)	• Enables reduced separation time with high separation performance in analysis → Comparable resolution to conventional column (30 cm) achieved in half the time • Resolution is steady under high flow-rate analysis • Improved column performance stability
3) Semi-micro column	• Reduced solvent volume → Solvent volume is 1/3 that of conventional columns (30 cm)
4) Adoption of highly hydrophilic substrate	• Applicable to wide variety of sample types

3-1. Pore Characteristics

Figure 1 shows the calibration curves obtained for the TSKgel SuperMultiporePW series and TSKgel SuperOligoPW column based on polyethylene oxide (PEO), polyethylene glycol (PEG), and ethylene glycol (EG) standards.

The measurable molar mass separation range for these columns is as follows: TSKgel SuperMultiporePW-N, 50,000 to 300 g/mol; TSKgel SuperMultiporePW-M, 1,000,000 to 500 g/mol; and SuperMultiporePW-H, 10,000,000 to 1,000 g/mol. Calibration curves for the TSKgel SuperMultiporePW column series show superior linearity for their measurable molar mass separation ranges. The molecular mass separation range on TSKgel SuperOligoPW for oligomers is 3,000 to 100 g/mol.

Figure 2 compares the calibration curves obtained on TSKgel SuperMultiporePW-M with that obtained from a column set of multiple conventional columns (TSKgel G5000PW$_{XL}$ coupled with G3000PW$_{XL}$) with different pore sizes (comparison of identical column sizes). Linearity (correlation coefficient) of straight-line sections of the calibration curve on TSKgel SuperMultiporePW-M is superior to that of the conventional column set.

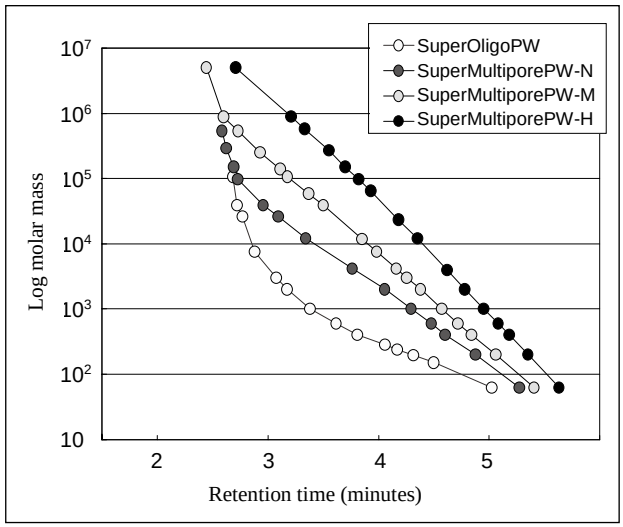

Figure 1 Calibration curves on TSKgel SuperMultiporePW series and TSKgel SuperOligoPW columns

Columns:	TSKgel SuperMultiporePW series and TSKgel SuperOligoPW (6.0 mm ID x 15 cm)
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	room temperature
Injection volume:	20 µL
Sample:	PEO, PEG, EG standards

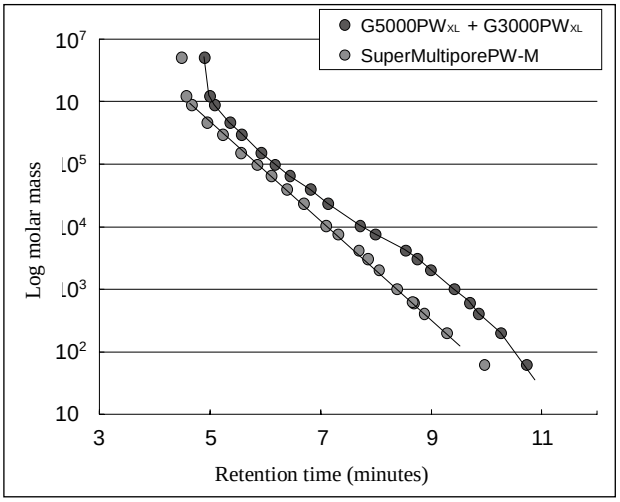

Figure 2 Calibration curves on TSKgel SuperMultiporePW-M and TSKgel PW$_{XL}$ columns

Columns:	TSKgel SuperMultiporePW-M and TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$ (6.0 mm ID x 15 cm x 2)
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	room temperature
Injection volume:	20 µL
Sample:	PEO, PEG, EG standards

3-2. Resolution

The TSKgel SuperOligoPW for oligomer separation uses a packing material with a particle size of 3 µm, and thus has twice the number of theoretical plates (per unit length) as the conventional TSKgel G-Oligo-PW. As shown in **Figure 3**, TSKgel SuperOligoPW achieves comparable resolution to a conventional column in half the analysis time.

The TSKgel SuperMultiporePW-N column uses a packing material with a particle size of 4 µm, and has twice the number of theoretical plates per unit length compared to its conventional counterpart, the TSKgel G3000PW$_{XL}$ column. Chromatograms of the PEO and PEG mixture obtained on both columns are shown in **Figure 4**. As shown in the figure, TSKgel SuperMultiporePW-N column achieves comparable resolution to that of the TSKgel G3000PW$_{XL}$ column in half the analysis time.

The TSKgel SuperMultiporePW-M uses a packing material with a particle size of 5 µm. Compared to a column system of multiple, coupled conventional columns with different pore sizes (TSKgel G5000PW$_{XL}$ and G3000PW$_{XL}$), the former achieves comparable resolution in half the analysis time, as shown on the chromatograms of the PEO and PEG mixture in **Figure 5**.

In **Figure 6**, the chromatograms of the PEO and PEG mixture are shown for TSKgel SuperMultiporePW-H and the mixed-bed type conventional column TSKgel GMPW$_{XL}$. TSKgel SuperMultiporePW-H achieves comparable resolution to the conventional column in half the analysis time.

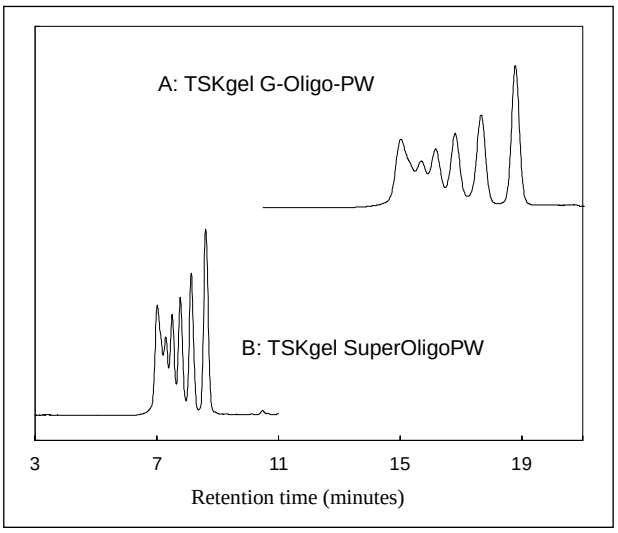

Figure 3 Separation of hydrolyzed product of β-cyclodextrin on TSKgel SuperOligoPW and TSKgel G-Oligo-PW columns

Columns:	A: TSKgel G-Oligo-PW (7.8 mm ID x 30 cm x 2)
	B: TSKgel SuperOligoPW (6.0 mm ID x 15 cm x 2)
Mobile phase:	H_2O
Flow rate:	A: 1.0 mL/min
	B: 0.6 mL/min
Detection:	RI
Temperature:	25˚C
Injection volume:	A: 100 µL
	B: 20 µL
Sample:	hydrolysate of β-cyclodextrin, 20 g/L

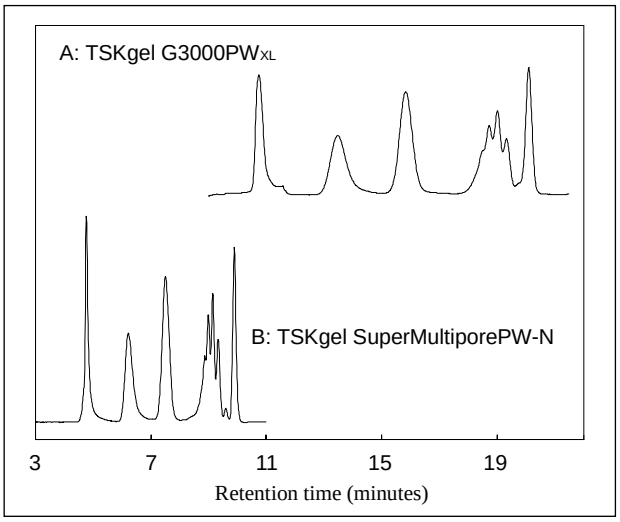

Figure 4 Separation of PEO mixture on TSKgel SuperMultiporePW-N and TSKgel G3000PW$_{XL}$ columns

Columns:	A: TSKgel G3000PW$_{XL}$ (7.8 mm ID x 30 cm x 2)
	B: TSKgel SuperMultiporePW-N (6.0 mm ID x 15 cm x 2)
Mobile phase:	H_2O
Flow rate:	A: 1.0 mL/min
	B: 0.6 mL/min
Detection:	RI
Temperature:	25˚C
Injection volume:	A: 100 µL
	B: 20 µL
Sample:	PEO and PEG standards

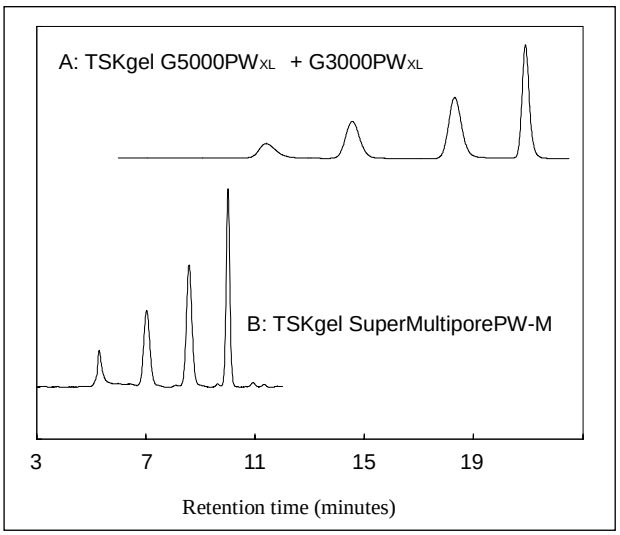

Figure 5 Separation of PEO mixture on TSKgel SuperMultiporePW-M and TSKgel PWXL columns

Columns:	A: TSKgel G5000PWXL + G3000PWXL (7.8 mm ID x 30 cm x 2)
	B: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm x 2)
Mobile phase:	H_2O
Flow rate:	A: 1.0 mL/min
	B: 0.6 mL/min
Detection:	RI
Temperature:	25˚C
Injection volume:	A: 100 µL
	B: 20 µL
Sample:	PEO and PEG standards

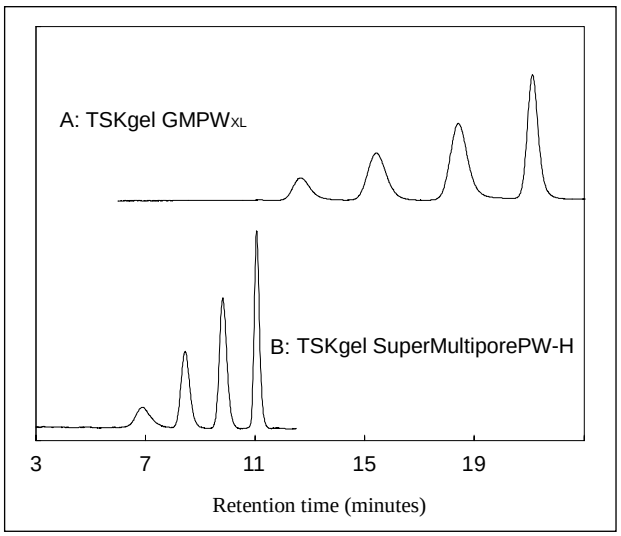

Figure 6 Separation of PEO mixture on TSKgel SuperMultiporePW-H and TSKgel GMPWXL columns

Columns:	A: TSKgel GMPWXL (7.8 mm ID x 30 cm x 2)
	B: TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm x 2)
Mobile phase:	H_2O
Flow rate:	A: 1.0 mL/min
	B: 0.6 mL/min
Detection:	RI
Temperature:	25˚C
Injection volume:	A: 100 µL
	B: 20 µL
Sample:	PEO and PEG standards

3-3. Flow rate dependence of height equivalent to a theoretical plate (HETP)

Results confirming flow rate dependence of height equivalent to a theoretical plate (HETP) are shown in **Figure 7** for low molecular mass sample ethylene glycol (EG) on TSKgel SuperOligoPW (particle size: 3 µm) and SuperMultiporePW-N, -M and -H (particle size: 4, 5 and 8 µm, respectively). As shown, the optimum flow rate (minimum HETP) for these columns range from 0.5 to 0.6 mL/min. For flow rates outside of this range, HETP gradually increases while column efficiency decreases.

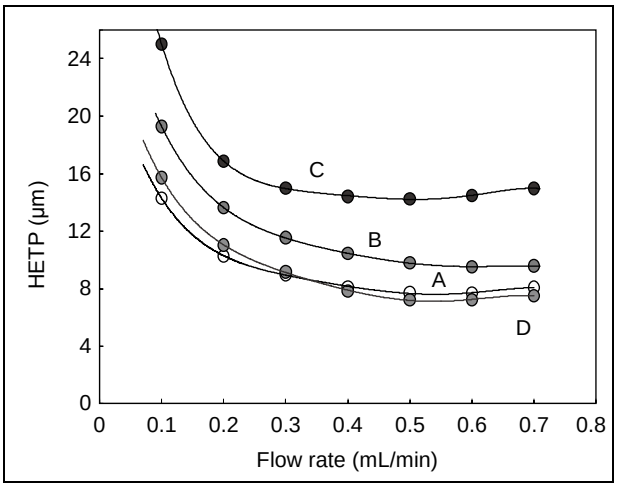

Figure 7 Relationship between flow rate and HETP for TSKgel SuperMultiporePW and TSKgel SuperOligoPW columns

Columns: A: TSKgel SuperMultiporePW-N (6.0 mm ID x 15 cm)
B: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
C: TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm)
D: TSKgel SuperOligoPW (6.0 mm ID x 15 cm)
Mobile phase: H_2O
Flow rate: 0.10 to 0.70 mL/min
Detection: RI
Temperature: 25˚C
Injection volume: 2 µL
Sample: EG standard, 5 g/L

3-4. Temperature dependence of theoretical plates

The effect of analysis temperature on the number of theoretical plates was investigated using a low molar mass polymer sample, ethylene glycol (EG), on the TSKgel SuperMultiporePW series and TSKgel SuperOligoPW columns (**Figure 8**).

As temperature increases, the number of theoretical plates decreased for TSKgel SuperMultiporePW-N, -M and TSKgel SuperOligoPW. The theoretical plates for the largest particle size column, TSKgel SuperMultiporePW-H, were only slightly affected by changes in temperature, and thus are shown to have little temperature dependence.

For actual analysis of high molar mass polymer samples, nevertheless, lowering operating pressure is considered more appropriate by increasing sample diffusion speed and lowering eluent viscosity with a relatively high column temperature. (Samples with temperature-dependent characteristics are not limited to these effects.)

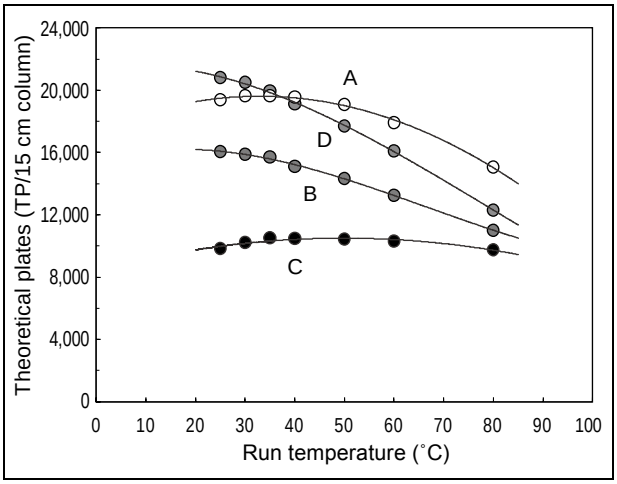

Figure 8 Relationship between temperature and theoretical plates for TSKgel SuperMultiporePW and TSKgel SuperOligoPW columns

Columns: A: TSKgel SuperMultiporePW-N (6.0 mm ID x 15 cm)
B: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
C: TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm)
D: TSKgel SuperOligoPW (6.0 mm ID x 15 cm)
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25 to 80˚C
Injection volume: 2 µL
Sample: EG standard, 5 g/L

3-5. Effect of sample injection volume

Sample injection volume is well known to have a significant effect on resolution and molar mass distribution data. Generally, maximum sample injection volume decreases as column size (volume) or packing material particle size becomes smaller.

In **Figure 9**, the dependence of HETP on injection volume of a low molar mass sample (EG) on the TSKgel SuperMultiporePW series and TSKgel SuperOligoPW column is shown. TSKgel SuperMultiporePW series and TSKgel SuperOligoPW high performance semi-micro columns are packed with fine particles. As shown, the maximum sample injection volume per column should be about 20 µL.

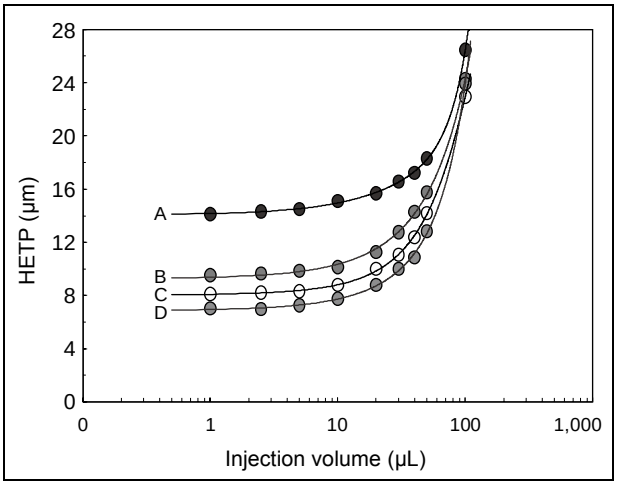

Figure 9 Relationship between sample injection volume and HETP for TSKgel SuperMultiporePW and TSKgel SuperOligoPW columns

Columns:
- A: TSKgel SuperMultiporePW-N (6.0 mm ID x 15 cm)
- B: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
- C: TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm)
- D: TSKgel SuperOligoPW (6.0 mm ID x 15 cm)

Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25˚C
Injection volume: 1 to 100 µL
Sample: EG standard, 5 g/L

3-6. Effect of sample concentration

Differences and fluctuations in the sample injection volume affect retention time (retention volume) of the sample and lead to variance in molar mass measurements and a decline in resolution. The impact is more pronounced under conditions where the maximum sample injection volume is exceeded. Moreover, even if the maximum sample injection volume is not exceeded, separation conditions (analysis flow rate, temperature, molar mass, and distribution), packing material characteristics, or the column may impose an adverse impact as described previously.

Even if the sample injection volume and other conditions are optimized, retention time (retention volume) becomes longer (greater) as sample concentrations increase. This phenomenon is called the concentration effect, which generally tends to become more pronounced as particle size of the packing material becomes smaller or molar mass of the sample becomes larger.

Chromatograms and weight-average molar mass (M_w) obtained on the TSKgel SuperMultiporePW-H column for polyacrylamide (molar mass approximately 300,000 g/mol) of different sample concentrations are shown in **Figure 10 and Figure 11.** Because the sample concentration was low (0.1-1.6 g/L), no change in weight-average molar mass (M_w) was observed.

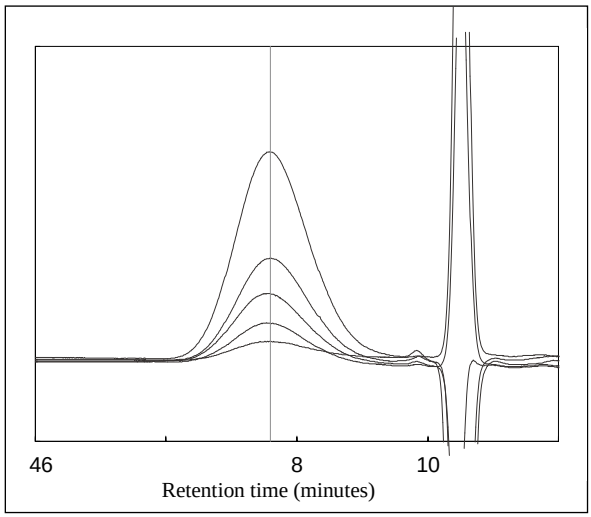

Figure 10 Concentration dependence of polyacrylamide on TSKgel SuperMultiporePW-H column

Column:	TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm x2)
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	25˚C
Injection volume:	30 µL
Concentration:	0.1, 0.2, 0.4, 0.8, 1.6 g/L
Sample:	polyacrylamide

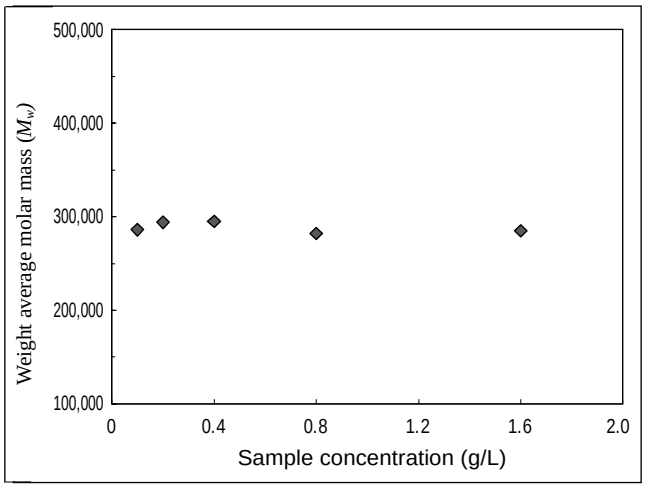

Figure 11 Concentration dependence of polyacrylamide weight-average molar mass on TSKgel SuperMultiporePW-H column

Column:	TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm x 2)
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	25˚C
Injection volume:	30 µL
Concentration:	0.1, 0.2, 0.4, 0.8, 1.6 g/L
Sample:	polyacrylamide

3-7. Effect of column temperature

In **Figure 12,** the dependence of temperature on PEO/PEG relative calibration curves is shown for both a TSKgel SuperMultiporePW-M column and a competitive mixed bed type column. For the other company's product, sample elution slows (or adsorbs without elution) as temperature increases thus, calibration curve preparation becomes difficult. Elution of real samples was predicted to sustain an impact, and thus to confound normal SEC analysis.

In contrast, the temperature dependence on sample elution for TSKgel SuperMultiporePW-M column was extremely small. Interaction between sample and packing material was confirmed to be weak.

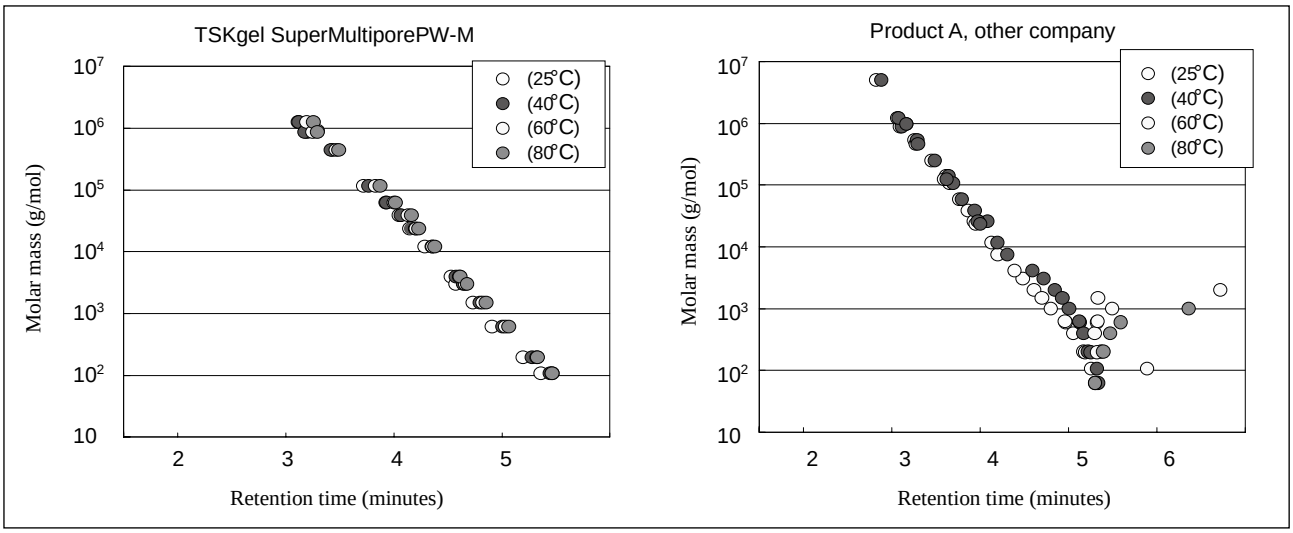

Figure 12 Temperature dependence of calibration curves for TSKgel SuperMultiporePW-M and a competitive column

Column: TSKgel SuperMultiporePW-M and product A, other company (6.0 mm ID x 15 cm x 2)
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25, 40, 60, 80˚C
Injection volume: 20 µL
Sample: PEO, PEG, and EG standards, 5 g/L

3-8. Effect of various separation conditions on calibration curves

PEO/PEG calibration curves under various elution conditions for TSKgel SuperMultiporePW series and TSKgel SuperOligoPW are shown in **Figures 13 to 17**.

Variances in salt concentration, column temperature, pH, organic solvent concentration in eluent, and type of buffer solution of eluent caused slight changes in all cases for the PEO/PEG elution time. The calibration curves changed, but linearity was not greatly affected.

The calibration curves for a polysaccharide in an aqueous eluent are shown in **Figure 18**. For each column within the TSKgel SuperMultiporePW series, calibration curves with high linearity are obtained for the appropriate molar mass separation ranges.

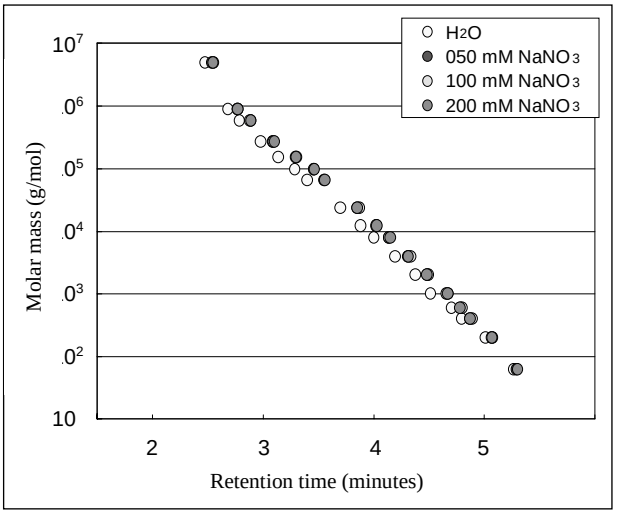

Figure 13 Calibration curve for TSKgel SuperMultiporePW-M column (salt concentration dependence)

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase: H_2O; 50, 100, 200 mmol/L $NaNO_3$
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PEO, PEG, and EG standards

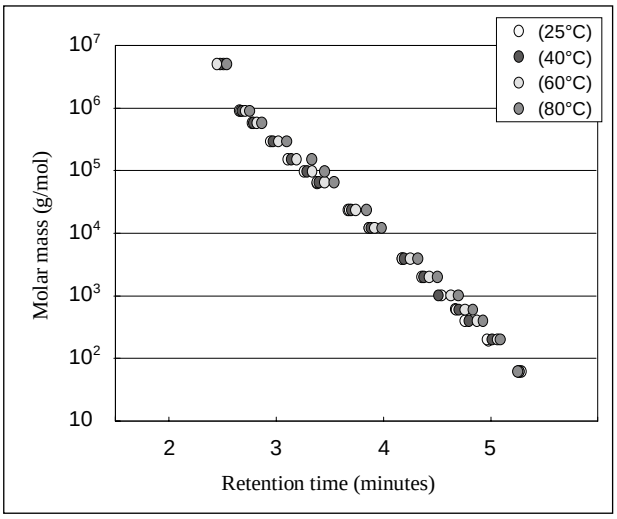

Figure 14 Calibration curve for TSKgel SuperMultiporePW-M column (temperature dependence)

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25, 40, 60, 80˚C
Injection volume: 20 µL
Sample: PEO, PEG, and EG standards

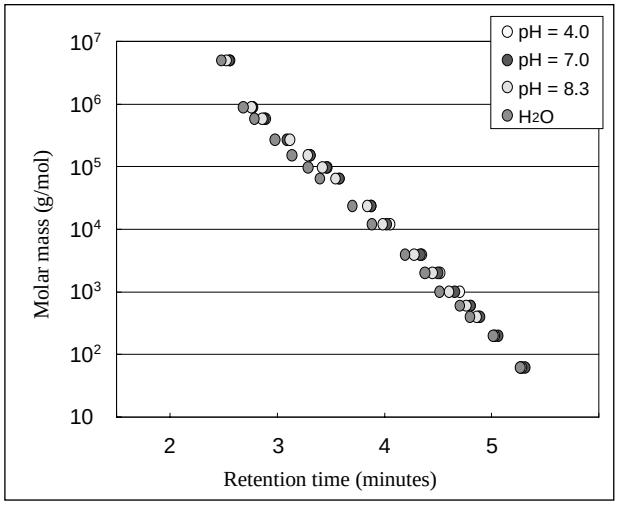

Figure 15 Calibration curves for TSKgel SuperMultiporePW-M column (pH dependence)

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase: 100 mmol/L acetate buffer solution
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PEO, PEG, and EG standards

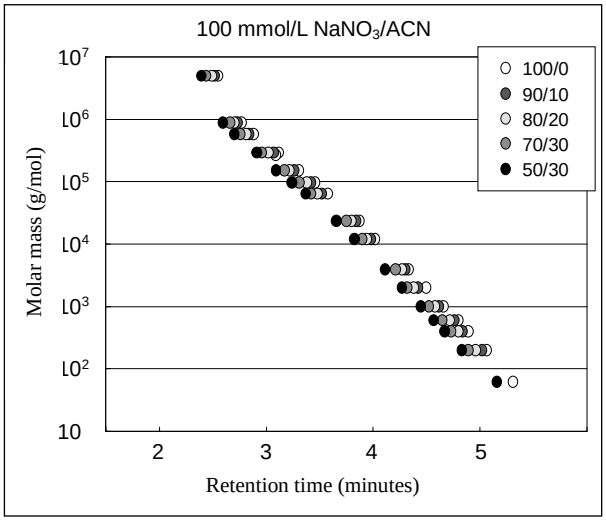

Figure 16 Calibration curves for TSKgel SuperMultiporePW-M column (acetonitrile concentration dependence)

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase: 100 mmol/L $NaNO_3$/acetonitrile
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PEO, PEG, and EG standards

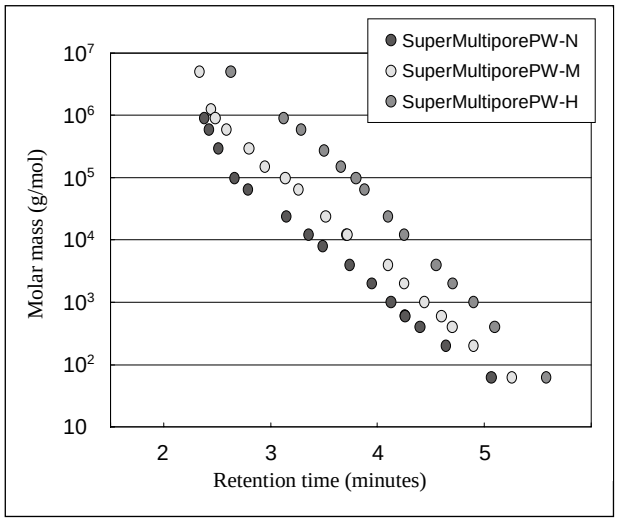

Figure 17 Calibration curves for TSKgel SuperMultiporePW series columns (carbonate buffer solution)

Column: TSKgel SuperMultiporePW series (6.0 mm ID x 15 cm)
Mobile phase: 100 mmol/L carbonate buffer solution (pH=10.6)
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25˚C
Injection volume: 20 µL
Sample: PEO, PEG, and EG standards

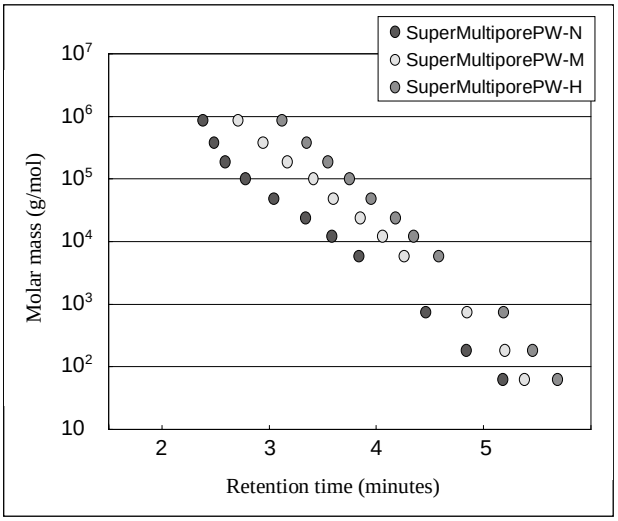

Figure 18 Calibration curves for TSKgel SuperMultiporePW series columns (polysaccharide/H_2O)

Column: TSKgel SuperMultiporePW series (6.0 mm ID x 15 cm)
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 25˚C
Injection volume: 20 µL
Sample: polysaccharide standards

3-9. Chromatogram distortion

Generally, SEC involves coupling multiple columns of different particle size and pore sizes, or mixed-bed columns that combine particle size with different pore sizes. Depending on the samples in these cases, distortions may be observed on the chromatograms. These distortions are not recognized on chromatograms when the TSKgel SuperMultiporePW series comprising multipore packing columns are used. This is due to their pore characteristic (pore structure), which is their most prominent feature.

Chromatograms of polyvinylpyrrolidone (PVP) and dextran as obtained on TSKgel SuperMultiporePW-M and TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$ column sets are shown in **Figures 19 and 20,** respectively. For PVP the TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$ column set column reveals distortion on its chromatogram, while this phenomenon is not observed for TSKgel SuperMultiporePW-M. For the dextran sample distortion occurs at a specific elution time, around 6.5 minutes on the TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$ column set. No chromatogram distortion is observed for the TSKgel SuperMultiporePW-M with dextran.

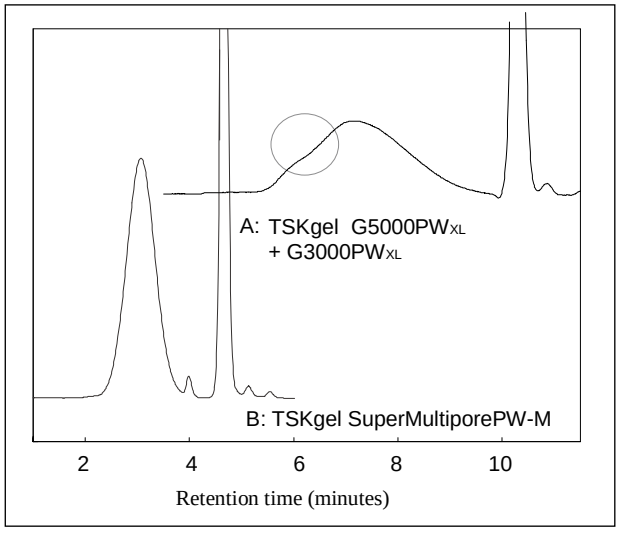

Figure 19 Polyvinylpyrrolidone separation on TSKgel SuperMultiporePW-M and TSKgel PW$_{XL}$ columns

Columns:	A:	TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$ (7.8 mm ID x 30 cm)
	B:	TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase:	100 mmol/L $NaNO_3$	
Flow rate:	A:	1.0 mL/min
	B:	0.6 mL/min
Detection:	RI	
Temperature:	25˚C	
Injection volume:	A:	100 µL
	B:	20 µL
Sample:	polyvinylpyrrolidone, 3 g/L	

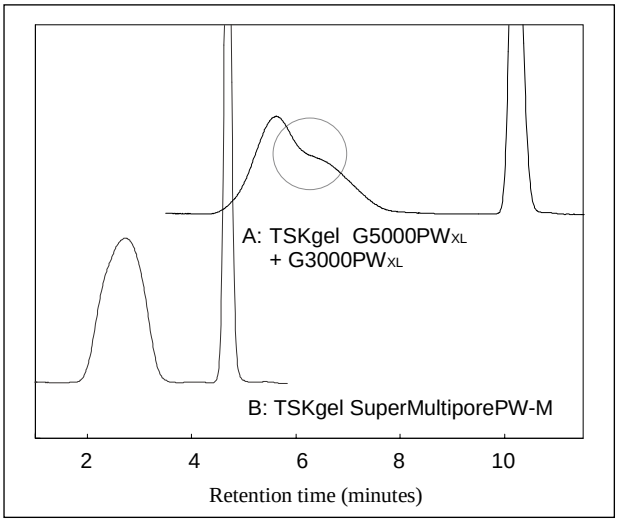

Figure 20 Dextran separation on TSKgel SuperMultiporePW-M and TSKgel PW$_{XL}$ columns

Columns:	A:	TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$ (7.8 mm ID x 30 cm)
	B:	TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase:	100 mmol/L $NaNO_3$	
Flow rate:	A:	1.0 mL/min
	B:	0.6 mL/min
Detection:	RI	
Temperature:	25˚C	
Injection volume:	A:	100 µL
	B:	20 µL
Sample:	dextran, 3 g/L	

4. Effect of various separation conditions on chromatograms

4-1. Salt concentration of eluent

Chromatograms of PVP and dextran using TSKgel SuperMultiporePW-M with mobile phases of varying concentrations of sodium nitrate ($NaNO_3$) are shown in **Figures 21 and 22**. Nearly uniform, quite satisfactory chromatograms were obtained under the respective conditions. Differences can be seen for molar mass, since sample molecule sizes differ according to salt concentration of the mobile phase.

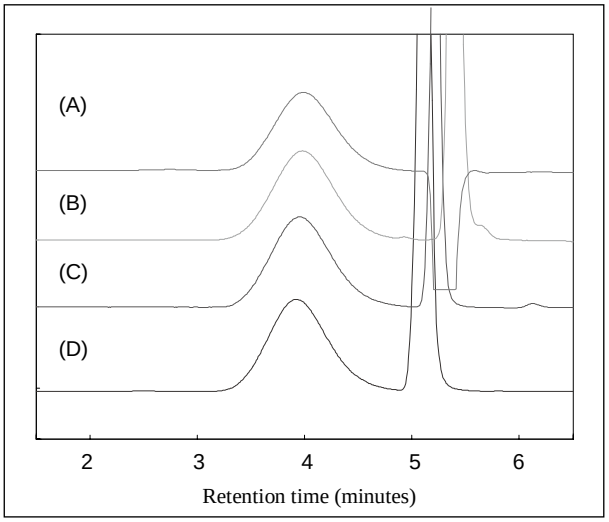

Figure 21 Salt concentration dependence of polyvinylpyrrolidone chromatogram and molar mass for TSKgel SuperMultiporePW-M column

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase: 25, 50, 100, 200 mmol/L $NaNO_3$
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PVP, 3 g/L

	$NaNO_3$ concentration (mmol/L)	Molar Mass (g/Mol)
(A)	200	19,000
(B)	100	19,000
(C)	50	21,000
(D)	25	22,000

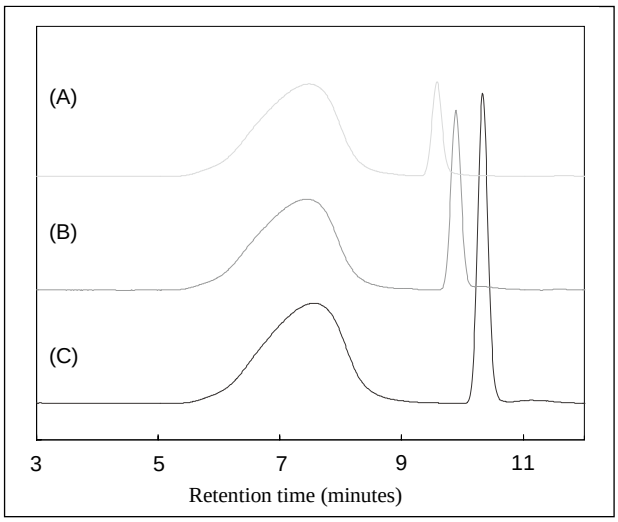

Figure 22 Salt concentration dependence of dextran chromatogram and molar mass for TSKgel SuperMultiporePW-M column

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm x 2)
Mobile phase: 25, 50, 100 mmol/L $NaNO_3$
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: dextran, 3 g/L

	$NaNO_3$ concentration (mmol/L)	Molar Mass (g/mol)
(A)	100	92,000
(B)	50	91,000
(C)	25	90,000

4-2. Column temperature

Temperature dependence data of the chromatogram and weight-average molecular mass (M_w) when using a TSKgel SuperMultiporePW-M column to measure PVP is shown in **Figure 23**.

No significant impact was observed for M_w or the chromatogram for temperatures ranging from 25 to 60˚C.

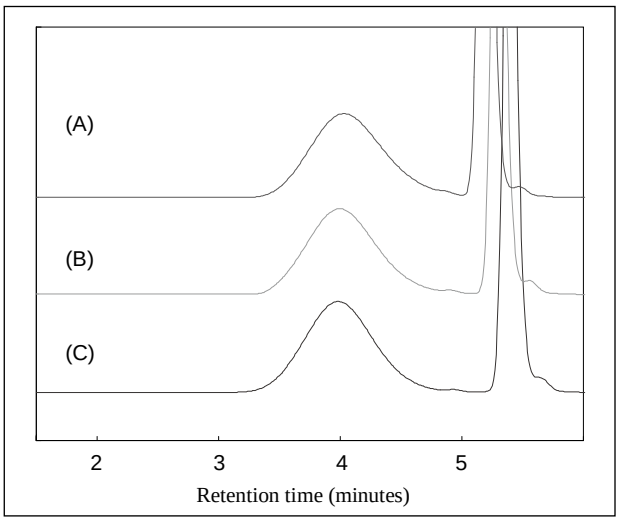

Figure 23 Temperature dependence of polyvinylpyrrolidone chromatogram and molar mass for TSKgel SuperMultiporePW-M column

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase: 100 mmol/L $NaNO_3$
Flow rate: 0.6 mL/min
Detection: RI
Temperature: **25, 40, 60°C**
Injection volume: 20 µL
Sample: PVP, 3 g/L

	Temperature (˚C)	M_w (g/mol)
(A)	60	20,000
(B)	40	19,000
(C)	25	19,000

4-3. Organic solvent concentration of eluent

Separation of PVP using a mobile phase with various organic solvent concentrations is shown in **Figure 24** for TSKgel SuperMultiporePW-H and in **Figure 25** for TSKgel GMPWXL.

The effect of organic solvent concentration on the chromatogram is negligible for the TSKgel SuperMultiporePW-H. In contrast, interaction between sample and packing material using the conventional TSKgel GMPWXL column increased in conditions where organic solvent concentration was 0 or 50%. Sample elution was consequently delayed and M_w became smaller.

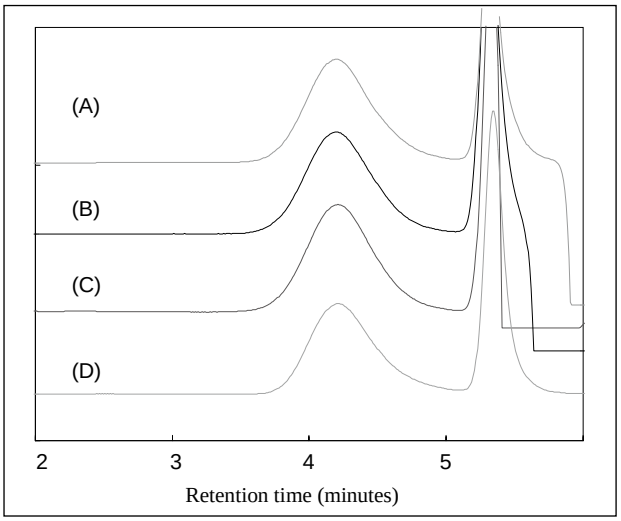

Figure 24 Organic solvent (ACN) concentration dependence of polyvinylpyrrolidone chromatogram and molar mass for TSKgel SuperMultiporePW-H column

Column: TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm)
Mobile phase: 100 mmol/L $NaNO_3$/acetonitrile
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PVP, 3 g/L

	100 mmol/L $NaNO_3$/acetonitrile	M_w (g/mol)
(A)	70/30	24,000
(B)	80/20	20,000
(C)	90/10	20,000
(D)	100/0	19,000

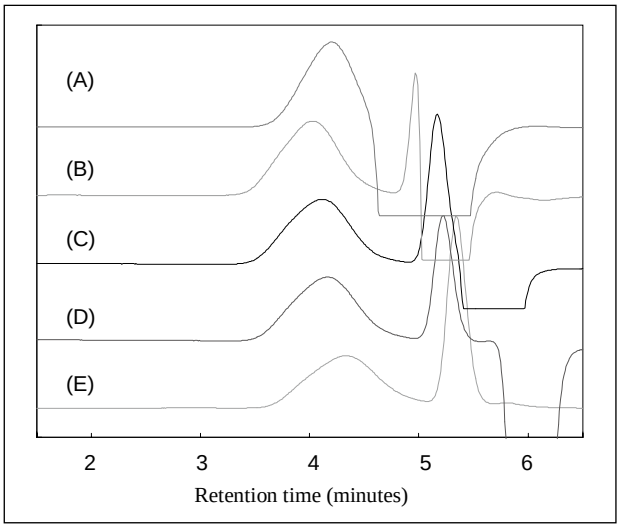

Figure 25 Organic solvent (ACN) concentration dependence of polyvinylpyrrolidone chromatogram and molar mass for TSKgel GMPW$_{XL}$ column

Column: TSKgel GMPW$_{XL}$ (6.0 mm ID x 15 cm)
Mobile phase: 100 mmol/L $NaNO_3$/acetonitrile
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PVP, 3 g/L

	100 mmol/L $NaNO_3$/acetonitrile	M_w (g/mol)
(A)	50/50	15,000
(B)	70/30	23,000
(C)	80/20	21,000
(D)	90/10	20,000
(E)	100/0	13,000

4-4. Eluent pH

Separation of PVP with mobile phases of various pH is shown in **Figure 26** for a TSKgel SuperMultiporePW-M column set. The retention time of the peak apex and the peak shape of the polymer sample differ according to pH. The cause for these differences is believed to be a change in surface characteristics of the packing material that results from a change in pH. The molar mass of the sample also differs as the interactions between packing material and sample change.

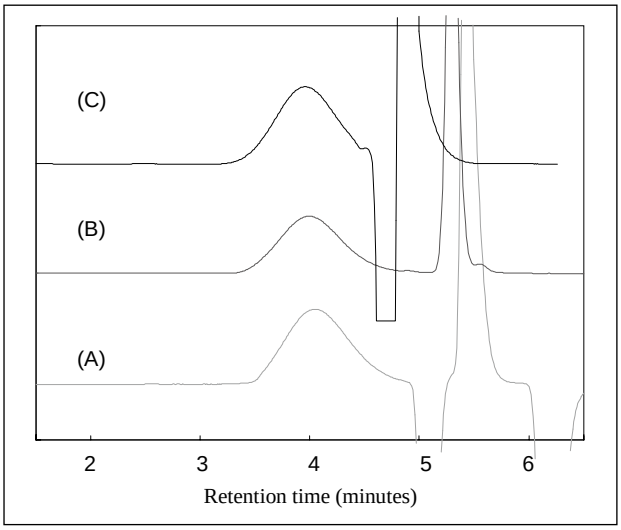

Figure 26 H dependence of polyvinylpyrrolidone chromatogram and molar mass for TSKgel SuperMultiporePW-M column

Column: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm)
Mobile phase: 100 mmol/L acetate buffer solution
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PVP, 3 g/L

	pH	M_w (g/mol)
(A)	4.0	17,000
(B)	7.0	19,000
(C)	8.3	22,000

5. Application of TSKgel SuperMultiporePW series

PVP chromatograms and M_w values of PVP separated with 100 mmol/L $NaNO_3$ mobile phase on TSKgel SuperMultiporePW-H and on two separate conventional columns (TSKgel GMPWXL and a competitive product) are shown in **Figure 27**. Sample molar mass data and satisfactory chromatograms were obtained on TSKgel SuperMultiporePW-H. In contrast, the effect of interaction between sample and packing material caused a delay in sample elution and resulted in a comparatively small molar mass computation for the TSKgel GMPWXL columns. Distortion was also observed in the chromatogram of the competitor's product, although the computed molar mass was not smaller than expected.

Various polymers were separated with 100 mmol/L $NaNO_3$ mobile phase on TSKgel SuperMultiporePW-M and conventional columns (TSKgel G5000PWXL, G3000PWXL). The resulting chromatograms and M_w values are shown in **Figure 28**. The effect of the interaction between sample and packing material for the conventional columns (TSKgel G5000PWXL, G3000PWXL) caused delays in elution and relatively small molar mass computation in some cases. Chromatogram distortions were also observed for some samples.

Table 4 shows the reproducibility of M_w data for samples separated as shown in **Figure 28**. Reproducibility of M_w data (same day) obtained with TSKgel SuperMultiporePW-M was confirmed to be superior to the conventional products.

Various polymers were separated with 100 mmol/L $NaNO_3$ eluent on TSKgel SuperMultiporePW-H. The resulting chromatograms and M_w values are shown in **Figure 29**.

In the same manner as for TSKgel SuperMultiporePW-M in **Figure 28**, the chromatograms and M_w values for various polymers were obtained quite satisfactorily for TSKgel SuperMultiporePW-H.

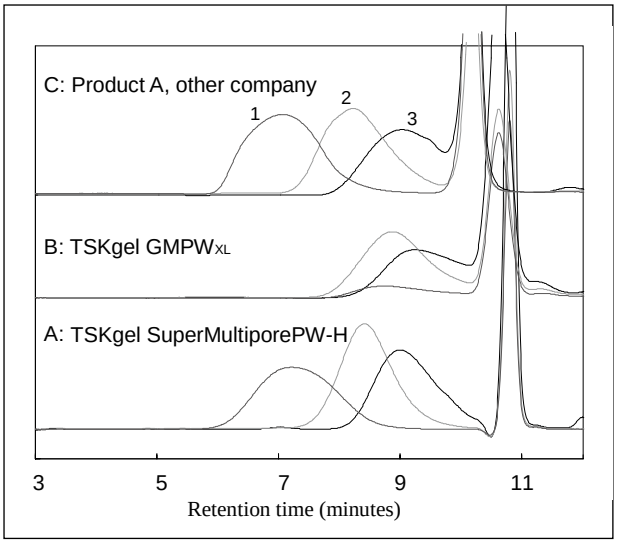

Figure 27 Polyvinylpyrrolidone chromatograms and molar mass for TSKgel SuperMultiporePW-H and current columns

Columns: A: TSKgel SuperMultiporePW-H (6.0 mm ID x 15 cm x 2)
B: TSKgel GMPWXL (6.0 mm ID x 15 cm x 2)
C: Product A, other company (6.0 mm ID x 15 cm x 2)
Mobile phase: 100 mmol/L $NaNO_3$
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PVP, 3 g/L

	M_w (g/mol)		
	(A)	(B)	(C)
1. PVP (K-15)	6,500	2,600	4,500
2. PVP (K-30)	25,000	5,200	21,000
3. PVP (K-90)	340,000	5,000	300,000

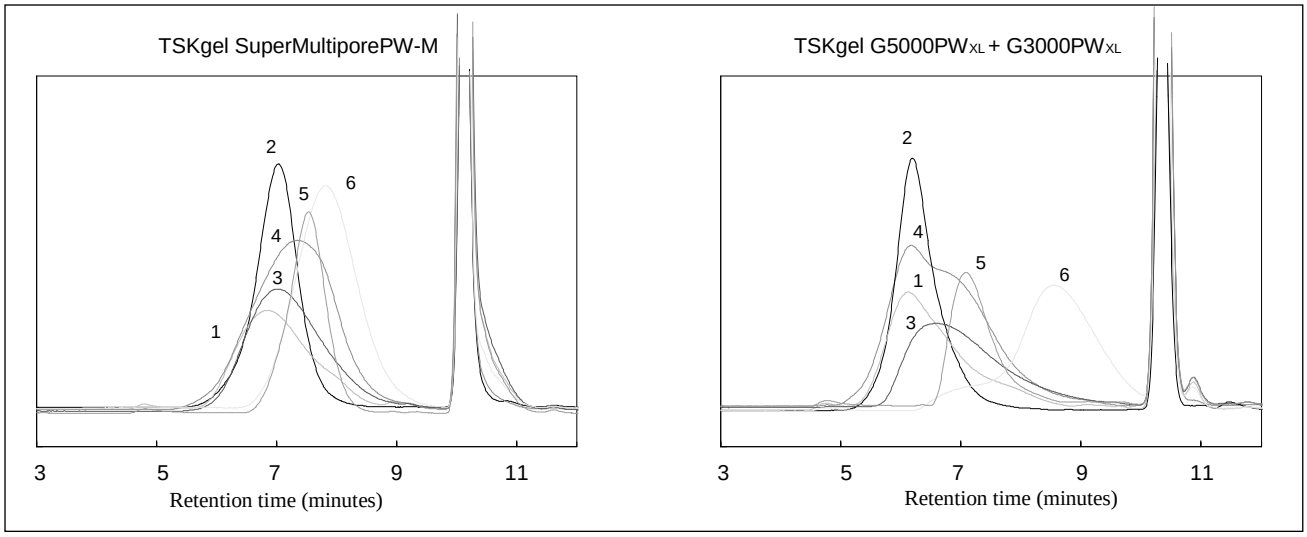

Figure 28 Chromatograms and molar mass of various polymers for TSKgel SuperMultiporePW-M and TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$ columns

Column: (6.0 mm ID x 15 cm x 2)
Mobile phase: 100 mmol/L $NaNO_3$
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PVP, 3 g/L

	M_w(g/mol)	
	SuperMultiporePW-M	G5000PW$_{XL}$ + G3000PW$_{XL}$
1. Carboxymethyl cellulose	134,000	115,000
2. Gum arabic	126,000	125,000
3. Hydroxypropyl celluose	108,000	55,000
4. Dextran	105,000	105,000
5. Sodium chondroitin sulfate	48,000	28,000
6. Polyvinylpyrrolidone (K-30)	26,000	9,300

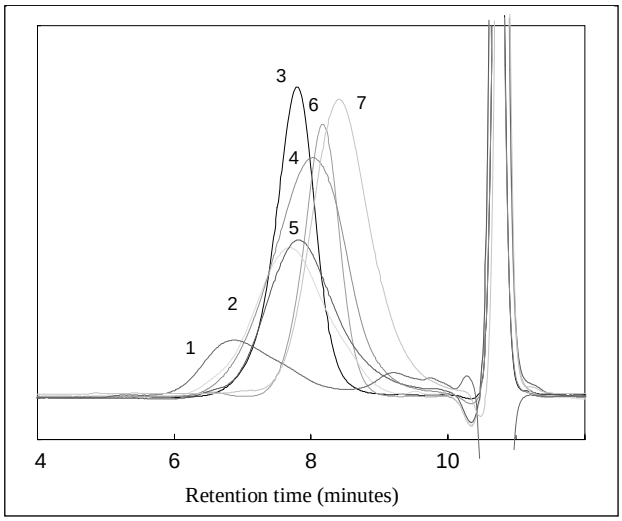

Figure 29 Chromatograms and M_w of various polymers for TSKgel SuperMultiporePW-H column

Column: TSKgel SuperMultiporePW-H
(6.0 mm ID x 15 cm x 2)
Mobile phase: 100 mmol/L $NaNO_3$
Flow rate: 0.6 mL/min
Detection: RI
Temperature: 40˚C
Injection volume: 20 µL
Sample: PVP, 3 g/L

	M_w (g/mol)
1. Dextran (200)	470,000
2. Carboxymethyl cellulose	150,000
3. Gum arabic	103,000
4. Dextran (EH-7114)	95,000
5. Hydroxypropyl cellulose	91,000
6. Sodium chondroitin sulfate	41,000
7. Polyvinylpyrrolidone (K-30)	29,000

Table 4 Reproducibility of M_w data of various samples on TSKgel SuperMultiporePW-M and conventional columns (same-day analysis) (n=5)

	Chondroitin $NaSO_4$		Gum arabic		Carboxymethyl cellulose	
	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
TSKgel SuperMultiporePW-M	48,000	0.49	126,000	0.35	134,000	0.67
TSKgel PW_{XL} series (TSKgel $G5000PW_{XL}$ + $G3000PW_{XL}$)	34,600	1.89	125,000	0.57	115,000	1.05

Separation conditions

Columns: TSKgel SuperMultiporePW-M (6.0 mm ID x 15 cm x 2) TSKgel $G5000PW_{XL}$ + $G3000PW_{XL}$ (7.8 mm ID x 30 cm x 2)

Mobile phase: 100 mmol/L $NaNO_3$

Flow rate: 0.6 mL/min (TSKgel SuperMultiporePW-M) 1.0 mL/min (TSKgel $G5000PW_{XL}$ + $G3000PW_{XL}$)

Temperature: 40˚C

Detection: RI

Injection volume: 20 µL (TSKgel SuperMultiporePW-M) 100 µL (TSKgel $G5000PW_{XL}$ + $G3000PW_{XL}$)

6. Fundamental properties and application of TSKgel SuperOligoPW

Polyethylene glycol (PEG 200) was separated on TSKgel SuperOligoPW and a conventional column (TSKgel G-Oligo-PW). The resulting chromatograms are shown in **Figure 30.** As shown, TSKgel SuperOligoPW has achieved comparable resolution as the conventional column in half the analysis time.

Chromatograms of maltopentaose degradation product, malto-oligosaccharide, and cello-oligosaccharide as separated on TSKgel SuperOligoPW and a conventional column (TSKgel G-Oligo-PW).are shown in **Figures 31**, **32**, and **33**, respectively. As shown, TSKgel SuperOligoPW has achieved comparable resolution as the conventional column for each of the samples in half the analysis time.

Separation of malto-oligosaccharide on TSKgel SuperOligoPW with various column temperatures (25, 40, 60, 80 and 95˚C) is shown in **Figure 34**. Elution of samples was confirmed to be more rapid with higher resolution as temperature was increased. Improvement of resolution was particularly significant for high polymer samples.

Chromatograms for alcohols separated on TSKgel SuperOligoPW under optimized conditions are shown in **Figure 35**. Analysis under these conditions results in elution via a Hydrophobic Interaction Chromatography (HIC) mechanism, rather than SEC mechanism.

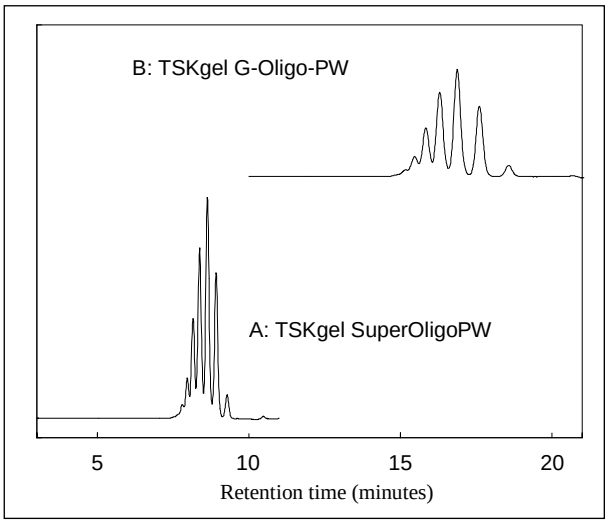

Figure 30 Polyethylene glycol chromatograms on TSKgel SuperOligoPW and TSKgel G-Oligo-PW

Columns:	A:	TSKgel SuperOligoPW (6.0 mm ID x 15 cm x 2)
	B:	TSKgel G-Oligo-PW (7.8 mm ID x 30 cm x 2)
Mobile phase:	H_2O	
Flow rate:	A:	0.6 mL/min
	B:	1.0 mL/min
Detection:	RI	
Temperature:	40˚C	
Injection volume:	A:	20 µL
	B:	100 µL
Sample:	PEG 200 standard	

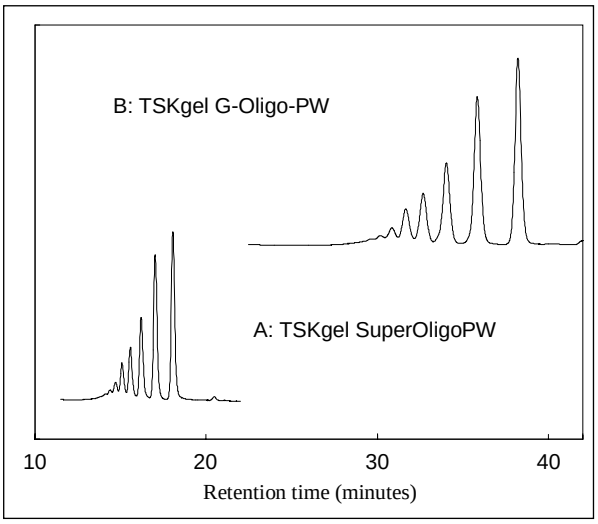

Figure 31 Maltopentaose chromatograms for TSKgel SuperOligoPW and TSKgel G-Oligo-PW columns

Columns:	A:	TSKgel SuperOligoPW (6.0 mm ID x 15 cm x 4)
	B:	TSKgel G-Oligo-PW (7.8 mm ID x 30 cm x 4)
Mobile phase:	H_2O	
Flow rate:	A:	0.6 mL/min
	B:	1.0 mL/min
Detection:	RI	
Temperature:	25˚C	
Injection volume:	A:	10 µL
	B:	50 µL
Sample:	maltopentaose degradation product	

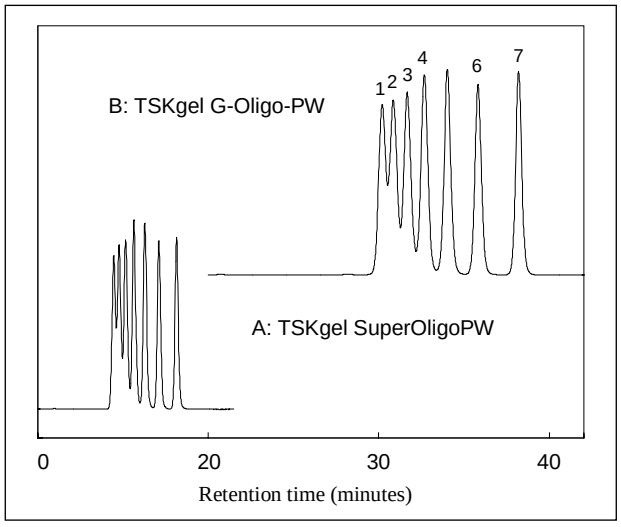

Figure 32 Malto-oligosaccharide chromatograms for TSKgel SuperOligoPW and TSKgel G-Oligo-PW

Columns: A: TSKgel SuperOligoPW (6.0 mm ID x 15 cm x 4)
B: TSKgel G-Oligo-PW (7.8 mm ID x 30 cm x 4)
Mobile phase: H_2O
Flow rate: A: 0.6 mL/min
B: 1.0 mL/min
Detection: RI
Temperature: 25˚C
Injection volume: A: 10 µL
B: 50 µL
Samples: 1. maltoheptaose
2. maltohexaose
3. maltopentaose
4. maltotetraose
5. maltotriose
6. maltose
7. glucose

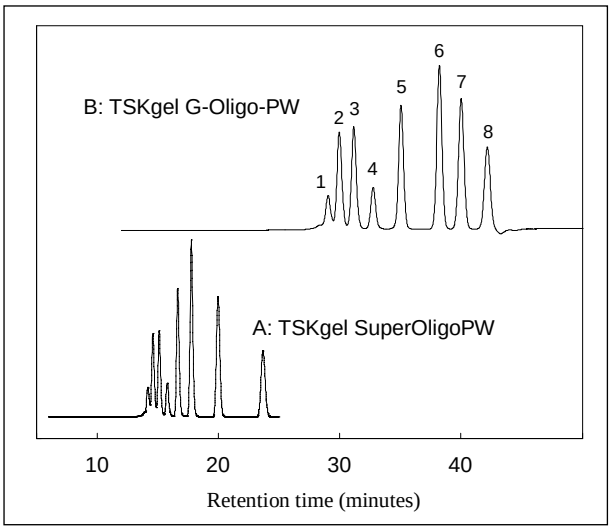

Figure 33 Cello-oligosaccharide chromatograms for TSKgel SuperOligoPW and TSKgel G-Oligo-PW columns

Columns: A: TSKgel SuperOligoPW (6.0 mm ID x 15 cm x 4)
B: TSKgel G-Oligo-PW (7.8 mm ID x 30 cm x 2)
Mobile phase: H_2O
Flow rate: A: 0.3 mL/min
B: 0.5 mL/min
Detection: RI
Temperature: 55˚C
Injection volume: A: 5 µL
B: 20 µL
Samples: 1. cellohexaose
2. cellopentaose
3. cellotetraose
4. cellotriose
5. cellobiose
6. glucose
7. ethylene glycol
8. ethanol

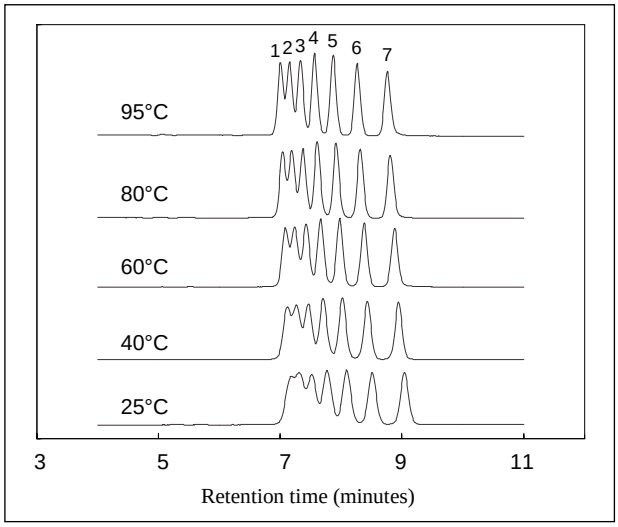

Figure 34 Temperature dependence of malto-oligosaccharide chromatograms for TSKgel SuperOligoPW column

Column:	TSKgel SuperOligoPW (6.0 mm ID x 15 cm x2)
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	25, 40, 60, 80, 95˚C
Injection volume:	10 μL
Samples:	1. maltoheptaose 2. maltohexaose 3. maltopentaose 4. maltotetraose 5. maltotriose 6. maltose 7. glucose

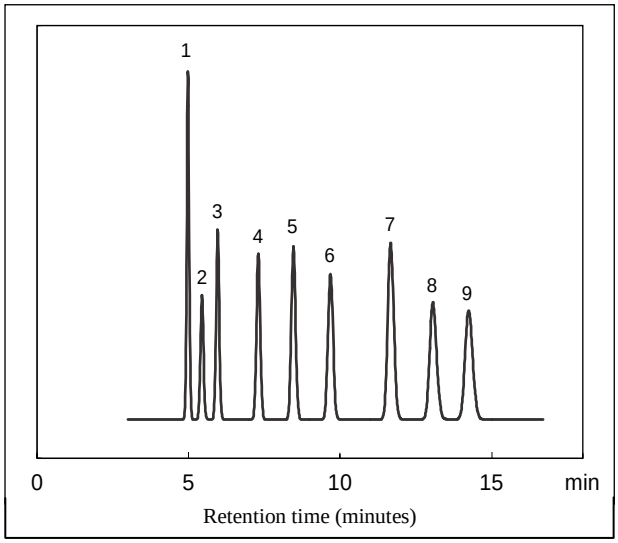

Figure 35 Chromatograms of alcohols for TSKgel SuperOligoPW column

Column:	TSKgel SuperOligoPW (6.0 mm ID x 15 cm)
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	80˚C
Injection volume:	2 μL
Sample:	1. ethylene glycol 2. methanol 3. ethanol 4. n-propanol 5. 2-butanol 6. n-butanol 7. sec-amyl alcohol 8. iso-amyl alcohol 9. n-amyl alcohol

7. Conclusion

The TSKgel SuperMultiporePW series comprises of a set of high performance aqueous SEC semi-micro columns packed with multipore packing material. Compared to conventional coupling methods of columns with different pore sizes or mixed-bed columns, the TSKgel SuperMultiporePW series yields ideal chromatograms. The packing material of the TSKgel SuperMultiporePW series columns is a result of a new synthesis method which yields monodisperse fine particles that retain resolution and enable high speed analysis.

The results shown in the report display that molar mass distribution data with high reproducibility and accuracy can be obtained using the TSKgel SuperMultiporePW series columns. Additionally, the TSKgel SuperOligoPW column, specifically designed for oligomer separation, can be used to achieve resolution comparable to the conventional oligomer columns in half the analysis time.

The TSKgel SuperMultiporePW series columns and TSKgel SuperOligoPW column have reduced dimensions that decrease solvent consumption. For SEC, the use of these columns is recommended in combination with the EcoSEC® GPC System, a dedicated high speed SEC system instrument with superior pump-line repeatability and baseline stability.

References

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[2]**TOSOH Research & Technology Review Vol.41 (1997),** Development of Multipore-type GPC columns: TSKgel MutiporeH series

[3] Tosoh Separation Report Nos.103, 105: TSKgel SuperMultiporeHZ Series: a High Performance SEC Semi-micro Column with Wide Pore Size Distribution