About: TSKgel PW Series Size Exclusion Columns

TSKgel PW and PWXL columns are recommended for analyses of water-soluble polymers and are prepared from hydrophilic polymethacrylate resin. TSKgel PWXL-CP columns are prepared from the same base resin as the TSKgel PWXL columns and were specifically developed for the analysis of water-soluble cationic polymers. TSKgel SuperMultiporePW columns are packed with particles containing a wide range of pore sizes for the analysis of water-soluble polymers with a wide molar mass range.

Stable from pH 2 to 12, TSKgel PW series columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 ˚C (50 ˚C for TSKgel G-DNA-PW column).

- Use TSKgel PW columns when analysis time is not critical, when sample mass is not limited, to collect fractions, or to obtain maximum number of plates (at the expense of analysis time). Particle sizes range from 12 µm for the smaller pore size columns (>10 nm - 20 nm) to 17 µm for the larger pore size columns (20 nm - >100 nm).

  The TSKgel GMPW column, within the TSKgel PW column line, is a mixed bed column containing a mixture of different pore sizes that has an extended linear calibration range, suitable for samples with a broad MM distribution as well as unknown samples.

  A TSKgel G6000PW column is available in PEEK column hardware, TSKgel BioAssist G6PW, when ultra-low sample adsorption is required, such as in virus analysis.

- Use higher efficiency TSKgel PWXL columns for optimal resolution, to reduce analysis time or in sample-limited applications. TSKgel PWXL columns have smaller particle sizes than TSKgel PW columns, resulting in improved resolution.

  The TSKgel PWXL product line also offers specialty columns for analyzing carbohydrate oligomers (TSKgel G-Oligo-PW) and DNA and RNA fragments of 500-5000 base pairs (TSKgel G-DNA-PW). TSKgel GMPWXL is a mixed bed scouting column for aqueous water-soluble linear polymers. Its pore volume is accessible to polymers ranging from molar masses of 500 up to 8.0 × 10^6 Da.

- Cationic groups were introduced on the surface of the TSKgel PWXL-CP packing material to prevent adsorption of cationic polymers and allow elution under low salt conditions. These columns show high theoretical plate numbers, linear calibration curves and excellent durability. The base resin is the same as that used in the TSKgel PWXL columns.

  Three columns are available within the TSKgel PWXL-CP line, each with a different particle size, separation range and exclusion limit, allowing polymers within a wide molar mass range to be separated and characterized.

- A wide molar mass range can be analyzed with the three different TSKgel SuperMultiporePW columns, from high molar mass water-soluble polymers to oligomers. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PWXL columns, which further reduces the chance of adsorption of hydrophilic polymers.

The range of pore sizes in which TSKgel PW and TSKgel PWXL columns are available permits a wide spectrum of water-soluble substances to be analyzed. The properties and molar mass separation ranges for all TSKgel PW series columns are summarized in Table 12.

The mechanism of SEC separation is based on the difference of apparent molecular size with no additional interaction between the column matrix and the sample molecules. In practice, however, a small number of weakly charged groups on the surface of all TSKgel PW series packings can cause changes in elution order from that of an ideal system. Fortunately, the mobile phase composition can vary greatly with TSKgel PW series columns to be compatible with a wide range of neutral, polar, anionic, and cationic samples. Table 13 lists appropriate mobile phases for GFC of major polymer types on TSKgel PW series columns.

For some nonionic, nonpolar polymers, such as polyethylene glycols, ideal size exclusion behavior can be obtained by using distilled water as the mobile phase. More polar ionic polymers may exhibit abnormal peak shapes or minor peaks near the void volume when eluted with distilled water due to ionic interactions between the sample and residual charged groups on the resin surface. To eliminate ionic interactions, a neutral salt such as sodium nitrate or sodium sulfate should be added to the aqueous eluent. Generally, a salt concentration of 0.1 mol/L to 0.5 mol/L is needed to overcome undesirable ionic interactions.

TSKgel PW resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. Depending on the sample, this can lead to hydrophobic interaction as a secondary retention mechanism. The extent of hydrophobic interaction increases as the salt concentration of the eluent increases, but it can be reduced by the addition of an organic modifier such as acetonitrile. Water-soluble organic solvents are frequently used as modifiers to suppress hydrophobic interactions between the sample and the resin surface.

Modifiers are also used for optimizing the elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in Table 13 below. All TSKgel PW series packings are compatible with 20% aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethylformamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition, these columns can be operated in 50% aqueous acetone.

Table 12: Properties and separation ranges of TSKgel PW, PW$_{XL}$, PW$_{XL}$-CP, and SuperMultiporePW columns

			Molar mass of samples (Da)
TSKgel column	**Particle size**	**Pore size**	**Polyethylene glycols & oxides**
SuperMultiporePW-N	4 µm	20 nm	300 – 5 × 10^4
SuperMultiporePW-M	5 µm	100 nm	500 – 1 × 10^6
SuperMultiporePW-H	8 µm	>100 nm	1,000 – 1 × 10^7
G2000PW	12 µm	12.5 nm	<3,000
G2500PW	12 µm and 17 µm	12.5 nm	<3,000
G3000PW	12 µm and 17 µm	20 nm	<5 × 10^4
G4000PW	17 µm	50 nm	<3 × 10^5
G5000PW	17 µm	100 nm	<1 × 10^6
G6000PW BioAssist G6PW	17 µm	>100 nm	<8 × 10^6
GMPW	17 µm	mixed pore sizes	1,000 – 8 × 10^6
G2500PW$_{XL}$	7 µm	12.5 nm	<3,000
G3000PW$_{XL}$	7 µm	20 nm	<5 × 10^4
G4000PW$_{XL}$	10 µm	50 nm	<3 × 10^5
G5000PW$_{XL}$	10 µm	100 nm	<1 × 10^6
G6000PW$_{XL}$	13 µm	>100 nm	<8 × 10^6
G-DNA-PW	10 µm	>100 nm	<8 × 10^6
GMPW$_{XL}$	13 µm	mixed pore sizes	1,000 – 8 × 10^6
SuperOligoPW	3 µm	12.5 nm	100 – 3,000
G-Oligo-PW	7 µm	12.5 nm	<3,000
G3000PW$_{XL}$-CP	7 µm	20 nm	200 – 5 × 10^4
G5000PW$_{XL}$-CP	10 µm	100 nm	400 – 5 × 10^5
G6000PW$_{XL}$-CP	13 µm	>100 nm	1,000 – 1 × 10^7

Columns: TSKgel PW columns, 7.5 mm ID × 60 cm
TSKgel PW$_{XL}$, G-Oligo-PW and G-DNA-PW columns, 7.8 mm ID × 30 cm
TSKgel SuperMultiporePW and SuperOligoPW columns, 6.0 mm ID × 15 cm
Mobile phase: polyethylene glycols and oxides (PEOs): distilled water
Flow rate: 1.0 mL/min, except for TSKgel SuperMultiporePW and SuperOligoPW columns: 0.6 mL/min

Table 13: Recommended mobile phases for GFC of water-soluble polymers on TSKgel PW, PW$_{XL}$, PW$_{XL}$-CP, and SuperMultiporePW columns

Type of polymer	Typical sample	Suitable mobile phase
Nonionic hydrophilic	polyethylene glycol	Distilled water
	soluble starch, methyl cellulose, pullulan	0.01 mol/L NaOH
	dextran, hydroxyethyl cellulose	20% DMSO (dimethyl sulfoxide)
	polyvinyl alcohol, polyacrylamide	Buffer or salt solution (e.g. 0.1-0.5 mol/L $NaNO_3$)
Nonionic hydrophobic	polyvinylpyrrolidone	Buffer or salt solution with organic solvent (e.g. 20% CH_3CN in 0.1 mol/L $NaNO_3$)
Anionic hydrophilic	sodium chondroitin sulfate, sodium alginate, carboxymethyl cellulose, sodium polyacrylate, sodium hyaluronate	Buffer or salt solution (e.g. 0.1 mol/L $NaNO_3$)
Anionic hydrophobic	sulfonated lignin sodium salt, sodium polystyrenesulfonate	Buffer or salt solution with organic solvent (e.g. 20% CH_3CN in 0.1 mol/L $NaNO_3$)
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethylene imine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4 or 0.8 mol/L $NaNO_3$
Cationic hydrophobic	poly(4-vinylbenzyltrimethylammonium chloride), poly(N-methyl-2-vinylpyridinium) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4
Amphoteric hydrophilic	peptides, proteins, poly- and oligosaccharides, DNA, RNA	Buffer or salt solution (e.g. 0.1 mol/L $NaNO_3$)
Amphoteric hydrophobic	blue dextran, collagen, gelatin, hydrophobic proteins, hydrophobic peptides	Buffer or salt solution with organic solvent (e.g. 20% CH_3CN in 0.1 mol/L $NaNO_3$ or 35-45% CH_3CN in 0.1% TFA)

About: TSKgel PW Size Exclusion Columns

TSKgel PW columns are composed of spherical, hydrophilic polymethacrylate beads. Particle sizes range from 12 µm for the smaller pore size columns to 17 µm for the larger pore size columns. Stable from pH 2 to 12, TSKgel PW columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 ˚C.

The TSKgel PW column line consists of the following columns:

- TSKgel G2000PW
- TSKgel G2500PW
- TSKgel G3000PW
- TSKgel G4000PW
- TSKgel G5000PW
- TSKgel G6000PW
- TSKgel GMPW

The mixed bed column, TSKgel GMPW, has an extended linear calibration range, suitable for samples with a broad molar mass distribution, as well as for unknown samples. The pore volume can be accessed by polymers ranging in molar mass from 500 to 8.0×10^6 Da. By quickly categorizing the molar mass profile of an unknown sample, the column enables a fast selection of the best TSKgel PW column for routine analysis.

Attributes and Applications

Product attributes of all eight TSKgel PW columns are shown in Table 14. All TSKgel PW columns have a base material of hydroxylated polymethacrylate, can be used in a maximum of 20% organic, and are shipped in water. The main application area for TSKgel PW columns is the analysis of water-soluble polymers, such as celluloses, acrylamides, glycols, dextrans, polyvinylalcohol, and oligosaccharides. TSKgel G2000PW, the larger particle size equivalent of TSKgel G-Oligo-PW, is most suitable for semi-preparative and preparative isolation of oligosaccharides. Representative application examples for the PW columns are illustrated in Table 15. The calibration curve for polyethylene glycol and oxides for the TSKgel PW columns is shown in Figure 49.

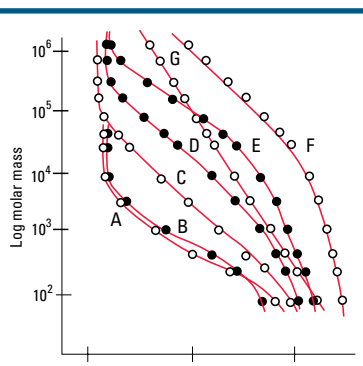
Table 14: Product attributes

TSKgel column	Particle size (mean)	Pore size (mean)	Calibration range
G2000PW	12 µm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
G2500PW	12 µm and 17 µm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
G3000PW	12 µm and 17 µm	20 nm	Up to 5.0×10^4 Da (polyethylene glycols and oxides)
G4000PW	17 µm	50 nm	Up to 3.0×10^5 Da (polyethylene glycols and oxides)
G5000PW	17 µm	100 nm	Up to 1.0×10^6 Da (polyethylene glycols and oxides)
G6000PW	17 µm	>100 nm	Up to 8.0×10^6 Da (polyethylene glycols and oxides)
GMPW	17 µm	mixed pore sizes	1,000 - 8.0×10^6 Da (polyethylene glycols and oxides)

Table 15: Representative application examples for TSKgel PW columns

Classification	Examples
1. Synthetic polymers • Nonionic • Cationic • Anionic	• PEG, polyglycerin, polyacrylamide • Polyethyleneimine, polyvinylpyrolidine • Poly (sodium acrylate), Poly (sodium styrene sulfonate)
2. Polysaccharides and derivatives	• Standard dextran, clinical dextran, pullulan, inulin, heparin, chitosan • Carboxymethylcellulose
3. Very large biopolymers • Polynucleotides • Viruses • Proteins	• DNA fragments • TMV, SBMV, TBSV • Lipoprotein (VLDL, LDL), apoferritin, gelatin, sea worm chlorocruorin
4. Small molecules • Oligomers • Others	• oligosaccharides (dextran hydrolysate, cyclodoxtrin • hydrolysate), cyclodextrins • oligopeptides • oligonucleotides

Figure 49: Polyethylene glycol and oxide calibration curves for TSKgel PW columns

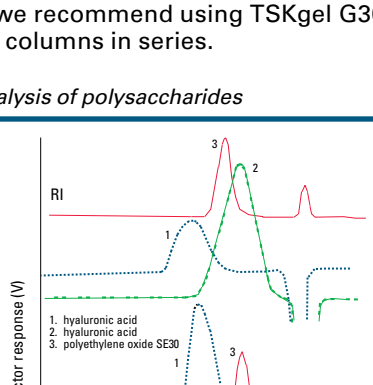


Column:	**A. G2000PW B. G2500PW C. G3000PW D. G4000PW E. G5000PW F. G6000PW G. GMPW all 7.5 mm ID × 60 cm**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	RI

Oligosaccharides

TSKgel PW columns are recommended for polysaccharide analysis due to their ability to separate a wide molar mass distribution. An effective separation of the anionic hydrophilic glucosaminoglycan, hyaluronic acid, is shown in Figure 50 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase. To obtain shorter analysis time and similar resolution, we recommend using TSKgel G3000PWXL and G4000PWXL columns in series.

Figure 50: Analysis of polysaccharides

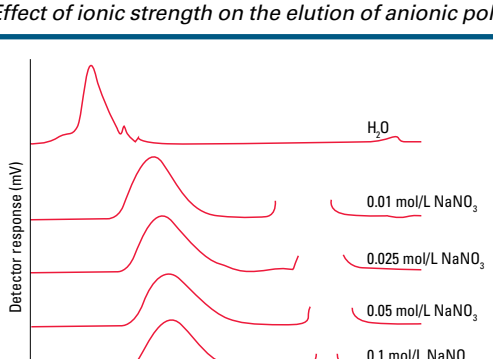


Columns:	**TSKgel G6000PW + G4000PW, two 7.5 mm ID × 60 cm columns in series**
Mobile phase:	H_2O with 0.2 mol/L NaCl
Flow rate:	0.9 mL/min
Temperature:	40 °C
Sample:	hyaluronic acid, polyethylene oxide

Polymers

Sodium polyacrylate, an anionic polymer, is effectively separated on two TSKgel GMPW columns in Figure 51. The addition of 0.01 mol/L $NaNO_3$ results in normal elution and peak shape overcoming the ionic repulsion between the anionic sample and the resin.

Figure 51: Effect of ionic strength on the elution of anionic polymers



Column:	**TSKgel GMPW, 17 µm, 7.5 mm ID × 60 cm × 2**
Mobile phase:	H_2O with 0.01 mol/L, 0.025 mol/L, 0.05 mol/L or 0.1 mol/L $NaNO_3$
Flow rate:	0.5 mL/min
Detection:	RI
Sample:	0.5 mL of 0.05-0.1% of the sodium salt of polyacrylic acid, an anionic polymer

About: TSKgel PWXL Size Exclusion Columns

TSKgel PWXL columns are composed of spherical, hydrophilic polymethacrylate beads. The smaller particle size of TSKgel PWXL columns provide 1.7x higher resolution than their TSKgel PW columns counterpart, making TSKgel PWXL columns more suitable for analytical purposes. Four specialty columns are included in the TSKgel PWXL column line.

The TSKgel G-DNA-PW column is designed for the separation of large polynucleotides such as DNA and RNA fragments of 500 - 5,000 base pairs. This column is a smaller particle size version of the TSKgel G6000PWXL column. The TSKgel G-Oligo-PW column is designed for high resolution separations of aqueous nonionic and cationic oligomers, and oligosaccharides such as hydrolyzed cyclodextrins. Because of the presence of cationic groups on the gel matrix, this column is not suitable for separating anionic polymers. The TSKgel G-Oligo-PW column has a PEG and PEO calibration curve identical to that of the TSKgel G2500PWXL column. The mixed-mode column, TSKgel GMPWXL, has an extended linear calibration range, suitable for samples with a broad MM distribution and unknowns.

The TSKgel SuperOligoPW column is designed for the determination of molar mass of aqueous oligomers, particularly oligosaccharides, and low molar mass aqueous polymers. The combination of the decreased particle size and semi-micro dimensions of the TSKgel SuperOligoPW column enables high speed separation with high resolution and lowered solvent consumption. Since the packing material in the TSKgel SuperOligoPW columns is more hydrophilic compared with TSKgel G-Oligo-PW columns, an even wider range of water-soluble polymers can be analyzed without the need to add organic solvent to the eluent.

The following TSKgel PWXL columns are offered:

- TSKgel G2500PWXL
- TSKgel G3000PWXL
- TSKgel G4000PWXL
- TSKgel G5000PWXL
- TSKgel G6000PWXL
- TSKgel G-DNA-PW
- TSKgel GMPWXL
- TSKgel G-Oligo-PW
- TSKgel SuperOligoPW

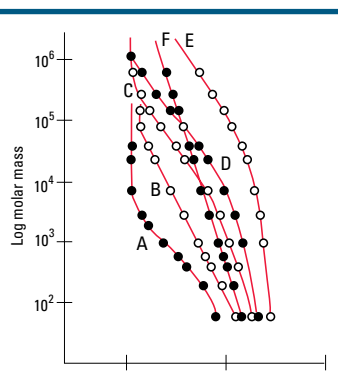
Attributes and Applications

The main application area for TSKgel PWXL columns is the analysis of water-soluble polymers, such as celluloses, acrylamides, glycols, dextrans, polyvinylalcohol, and oligosaccharides. Because of the presence of cationic groups on the base bead of TSKgel G2500PWXL, this column is not suited for separating anionic polymers. Product attributes of all of the TSKgel PWXL columns are shown in Table 16. All TSKgel PWXL columns have a base material of hydroxylated polymethacrylate, can be used in a maximum of 20% organic and are shipped in water. Figures 52 - 56 show the calibration curves for all of the TSKgel PWXL columns.

Table 16: Product attributes

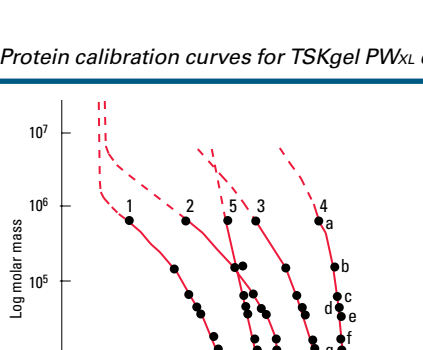
TSKgel column	Particle size (mean)	Pore size (mean)	Calibration range
G2500PWXL	7 µm	12.5 nm	<3,000 Da (polyethylene glycols and oxides)
G3000PWXL	7 µm	20 nm	$<4.0 \times 10^4$ Da (polyethylene glycols and oxides)
G4000PWXL	10 µm	50 nm	2,000 - 3.0×10^5 Da (polyethylene glycols and oxides)
G5000PWXL	10 µm	100 nm	4,000 - 8.0×10^5 Da (polyethylene glycols and oxides)
G6000PWXL	13 µm	>100 nm	4.0×10^4 - 8.0×10^6 Da (polyethylene glycols and oxides)
G-DNA-PW	10 µm	>100 nm	4.0×10^4 - 8.0×10^6 Da (polyethylene glycols and oxides)
GMPWXL	13 µm	mixed pore sizes	1,000 - 8.0×10^6 Da (polyethylene glycols and oxides)
G-Oligo-PW	7 µm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
SuperOligoPW	3 µm	12.5 nm	Up to 3,000 Da ($PEO, PEG/H_2O$)

Figure 52: Polyethylene glycol and oxide calibration curves for TSKgel PW$_{XL}$ columns



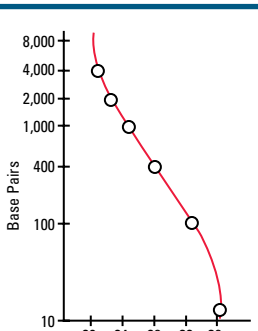
Column:	**A. G2500PW$_{XL}$ B. G3000PW$_{XL}$ C. G4000PW$_{XL}$ D. G5000PW$_{XL}$ E. G6000PW$_{XL}$ F. GMPW$_{XL}$ all 7.8 mm ID × 30 cm**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	RI

Figure 53: Protein calibration curves for TSKgel PW$_{XL}$ columns



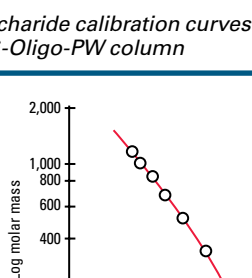
Column:	**1. TSKgel G3000PW$_{XL}$ 2. TSKgel G4000PW$_{XL}$ 3. TSKgel G5000PW$_{XL}$ 4. TSKgel G6000PW$_{XL}$ 5. TSKgel GMPW$_{XL}$ all 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.8
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Samples:	a. thyroglobulin (6.6×10^5 Da) b. γ-globulin (1.5×10^5 Da) c. albumin (6.7×10^4 Da) d. ovalbumin (4.3×10^4 Da) e. β-lactoglobulin (3.6×10^4 Da) f. myoglobin (1.69×10^4 Da) g. cytochrome C (1.24×10^4 Da)

Figure 54: Double stranded DNA calibration curves for TSKgel G-DNA-PW column



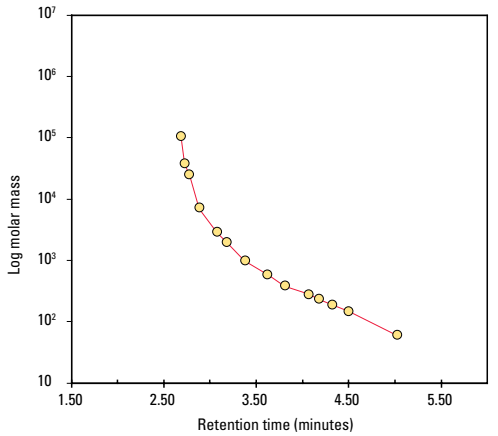
Column:	**TSKgel G-DNA-PW, 10 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	H_2O with 0.3 mol/L NaCl in 0.1 mol/LTris-HCl, pH 7.5, + 1 mmol/L EDTA
Flow rate:	0.15 mL/min
Detection:	UV @ 260 nm
Sample:	*Eco* RI and *Bst* NI-cleaved pBR322 DNA, void volume determined with λ-DNA

Figure 55: Oligosaccharide calibration curves for TSKgel G-Oligo-PW column



Column:	**TSKgel G-Oligo-PW, 7 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Sample:	hydrolyzed β-cyclodextrin

Figure 56: Polyethylene glycol, oxide and ethylene glycol calibration curve for TSKgel SuperOligoPW column

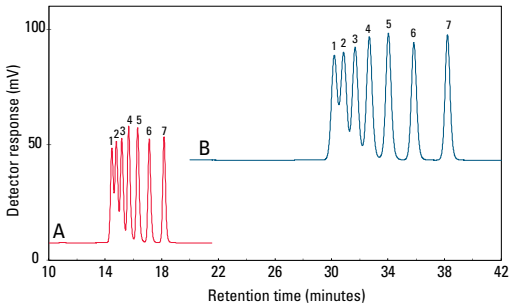


Column:	**TSKgel SuperOligoPW, 6.0 mm ID × 15 cm**
Mobile phase:	H_2O
Flow rate:	0.60 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	PEO, PEG and ethylene glycol

Oligosaccharides

Figure 57 demonstrates the high speed analysis of maltose oligomers using a TSKgel SuperOligoPW column compared to a TSKgel G-Oligo-PW column. The faster analysis time is due to the semi-micro dimensions (6.0 mm ID × 15 cm) and the small particle size (3 µm) of the TSKgel SuperOligoPW column compared to the 7.8 mm ID × 30 cm size and 7 µm particle size of the TSKgel G-Oligo-PW column.

Figure 57: Analysis of maltose oligomers

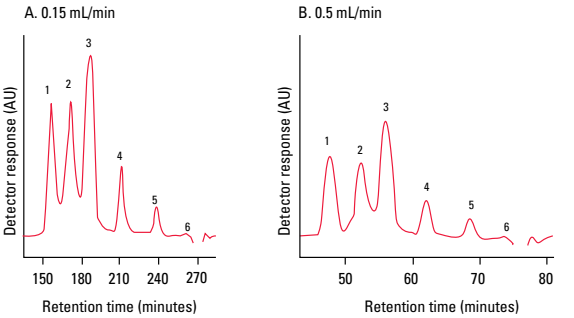


Columns:	**A. TSKgel SuperOligoPW, 3 µm, 6.0 mm ID × 15 cm × 4** **B. TSKgel G-Oligo-PW, 7 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	H_2O
Flow rate:	A: 0.6 mL/min B: 1.0 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	A: 10 µL B: 50 µL
Samples:	1. maltoheptose 2. maltohexose 3. maltopentose 4. maltotetraose 5. maltotriose 6. maltose 7. glucose

Large DNA Fragments

For the separation of large DNA fragments greater than 1,000 base pairs, a four column system is typically required. Baseline resolution of DNA fragments up to 7,000 base pairs can be achieved, provided there is a two-fold difference in the chain length of the fragments. Figure 58A shows the elution of double stranded DNA fragments, obtained from pBR322 DNA cleaved by both EcoRI and BstNI, on four TSKgel G-DNA-PW columns in series. The eluted peaks were collected and subjected to polyacrylamide gel electrophoresis, which showed almost complete separation of the 1060, 1857, and 4362 base pair fragments. Although lower flow rates typically yield better separations of most fragments, the resolution of the 1857 and 4362 base pair fragments was slightly greater at the higher flow rate, as shown in Figure 58B.

Figure 58A and 58B: Analysis of large DNA fragments

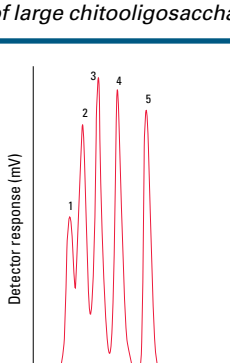


Column:	**TSKgel G-DNA-PW, 10 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	H_2O with 0.3 mol/L NaCl in 0.1 mol/LTris-HCl, pH 7.5, + 1 mmol/L EDTA
Flow Rate:	A. 0.15 mL/min B. 0.5 mL/min
Detection:	UV @ 260 nm
Samples:	60 µL of Eco RI and Bst NI - cleaved pBR322 DNA, base pairs: 1. 4362 2. 1857 3. 1060 & 928 4. 383 5. 121 6. 13

Oligomers

The TSKgel G-Oligo-PW column is designed for high resolution separations of nonionic and cationic oligomers. Figure 59 demonstrates excellent resolution of chito-oligosaccharides obtained by using the smaller, 6 µm particle size packing in the TSKgel G-Oligo-PW column.

Figure 59: Analysis of large chitooligosaccharides

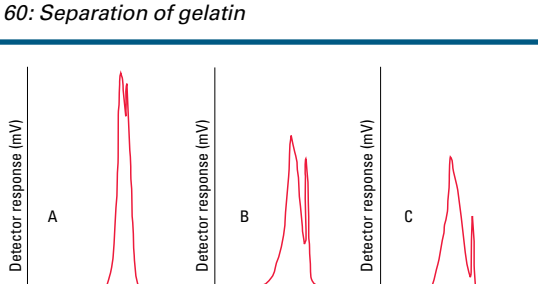


Column:	**TSKgel G-Oligo-PW, 7 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	RI
Samples:	1. chitohexaose 2. chitopentaose 3. chitotetraose 4. chitotriose 5. chitobiose

Complex Polymers

An example on the influence of pore size on the separation of complex polymers is shown in Figure 60. While on the large pore TSKgel G6000PWXL column, gelatin elutes in one narrow peak, on the G4000PWXL column the peak is much broader and the shoulder nearly separated from the main peak. This allows better determination of M_w/M_n and M_z/M_w.

Figure 60: Separation of gelatin

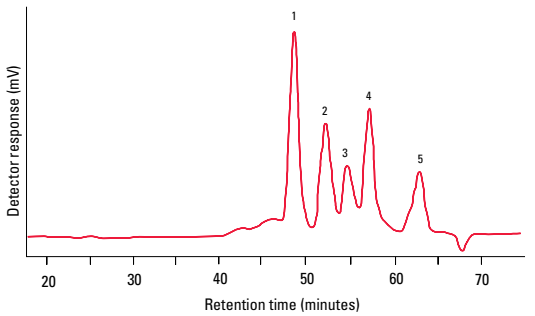


Columns:	**A. TSKgel G6000PWXL B. TSKgel G5000PWXL C. TSKgel G4000PWXL; all 7.8 mm ID × 30 cm**
Mobile phase:	0.2 mol/L phoshpate buffer, pH 6.0
Flow rate:	1.0 mL/min
Detection:	RI
Sample:	gelatin

Small Peptides

Figure 61 demonstrates that the separation of small peptides is possible on a TSKgel G3000PW$_{XL}$ column under denaturing conditions. Using an aqueous eluent containing 45% acetonitrile and 0.1% trifluoroacetic acid, the peptides were retained on the column using a size exclusion mechanism. An advantage of this method is that the eluent is volatile.

Figure 61: Analysis of small peptides

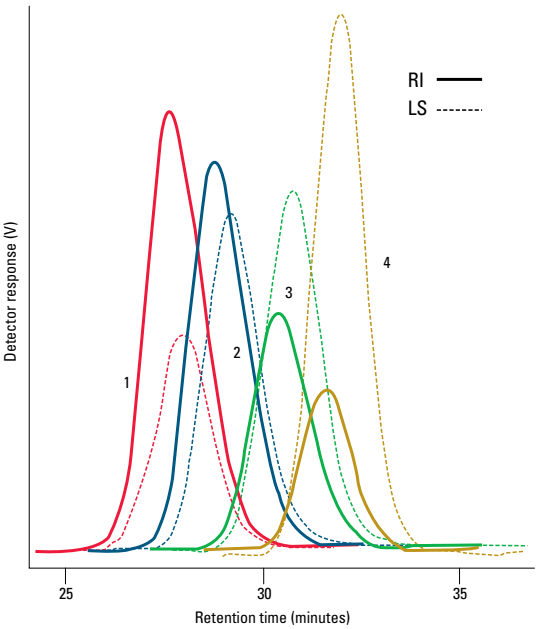


Column:	**TSKgel G3000PW$_{XL}$, 6 µm, 7.8 mm ID × 30 cm**
Mobile phase:	0.1% TFA / 45% CH_3CN
Flow rate:	1.0 mL/min
Samples:	peptides
	1. aprotinin
	2. insulin β-chain
	3. α-MSH
	4. bradykinin potentiator C
	5. glutathione

Molar Mass

Pullulan standard samples with a narrow molar mass distribution are commercially available. The molar mass of pullulan was analyzed by GFC/LALLS using a TSKgel GMPW$_{XL}$ column (Figure 62).

Figure 62: Analysis of pullulan

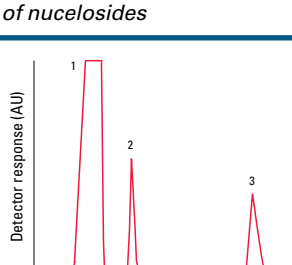


Column:	**TSKgel GMPW$_{XL}$, 13 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	0.1 mol/L sodium chloride
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	RI
	LS
Injection vol:	500 µL
Samples:	1. pullulan P400
	2. pullulan P200
	3. pullulan P100
	4. pullulan P50

Nucleic Acids

Desalting of nucleosides can be accomplished using the TSKgel G2500PW$_{XL}$ as depicted in Figure 63. Clearly, adenosine elutes after the void volume in the un-buffered water mobile phase.

Figure 63: Desalting of nucelosides

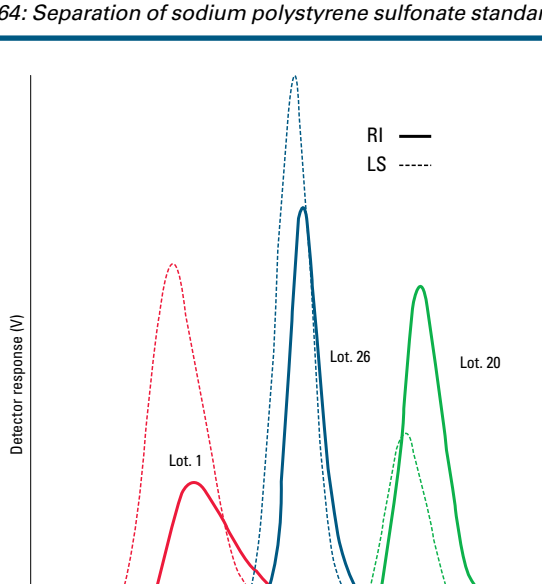


Column:	**TSKgel G2500PW$_{XL}$, 7 µm, 7.8 mm ID × 30 cm**
Mobile phase:	distilled H_2O
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Samples:	1. 0.5 mol/L NaCl 2. uridine 3. adenosine

Sodium Polystrene

Separation of sodium polystyrene sulfonate standards by GFC requires the addition of at least 10% acetonitrile or methanol to a 0.2 mol/L Na_2SO_4 mobile phase. Figure 64 shows chromatograms for sodium polystyrene sulfonate standards using a TSKgel GMPW$_{XL}$ column. Peak shapes for sodium polystyrene sulfonate samples obtained by adding 10% acetonitrile to a 0.2 mol/L Na_2SO_4 mobile phase remained constant upon addition of more acetonitrile.

Figure 64: Separation of sodium polystyrene sulfonate standards



Column:	**TSKgel GMPW$_{XL}$, 13 µm, 7.8 mm ID × 30 cm × 4**
Mobile phase:	ACN/0.2 mol/L sodium sulfate = 10/90
Flow rate:	1.0 mL/min
Detection:	RI LS
Temperature:	40 °C
Injection vol:	500 µL
Sample:	sodium poly(styrene sulfonates)

About: TSKgel PWXL-CP Size Exclusion Columns

TSKgel PWXL-CP columns were specifically developed for the analysis of water-soluble cationic polymers. Composed of polymethacrylate beads, cationic groups are introduced on the surface of the TSKgel PWXL-CP packing material to prevent adsorption of cationic polymers and allow elution under low salt conditions. These columns show high theoretical plate numbers, linear calibration curves, and high durability because the base resin is the same as that used in the TSKgel PWXL columns.

Three columns are available within the TSKgel PWXL-CP series, each with a different particle size, separation range, and exclusion limit, allowing polymers within a wide molar mass range to be separated and characterized.

- TSKgel G3000PWXL-CP
- TSKgel G5000PWXL-CP
- TSKgel G6000PWXL-CP

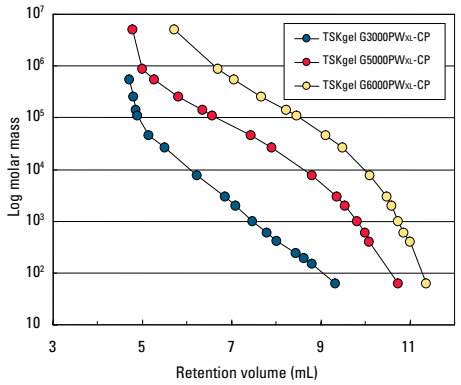
Attributes and Applications:

Table 17 shows the product attributes for each of the three TSKgel PWXL-CP columns. Figure 65 shows calibration curves produced with standard polyethylene oxide and polyethylene glycol in a 0.1 mol/L aqueous solution of sodium nitrate.

Table 17: Product attributes

TSKgel column	G3000PWXL-CP	G5000PWXL-CP	G6000PWXL-CP
Base material	polymethacrylate	polymethacrylate	polymethacrylate
Particle size	7 µm	10 µm	13 µm
Pore size	20 nm	100 nm	>100 nm
Exclusion limit	1.0×10^5 Da	1.0×10^6 Da	2.0×10^7 Da
Separation range (PEO, PEG)	200 ~ 5.0×10^4 Da	400 ~ 5.0×10^5 Da	1,000 ~ 1.0×10^7 Da
Theoretical plates	16,000	10,000	7,000

Figure 65: Polyethylene glycol and oxide calibration curves for TSKgel PWXL-CP columns

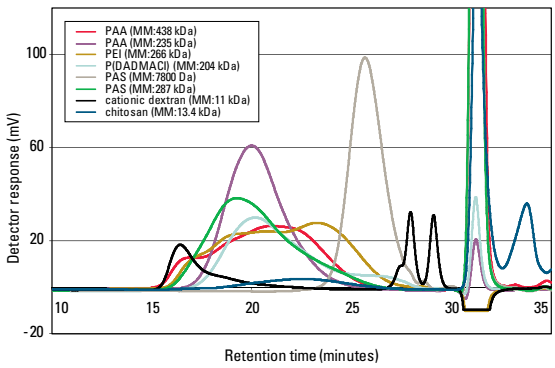


Columns: **TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID × 30 cm**
TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID × 30 cm
TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID × 30 cm
Mobile phase: H_2O with 0.1 mol/L $NaNO_3$
Flow Rate: 1 mL/min
Detection: RI
Temperature: 25 °C
Samples: polyethylene oxides (PEO) standards
polyethylene glycols (PEG) standards

Cationic Polymers

Various cationic polymers with different functional groups and molar masses were injected on the three TSKgel PWXL-CP columns (TSKgel G6000PWXL-CP, G5000PWXL-CP, and G3000PWXL-CP) connected in series. Figure 66 demonstrates that these SEC columns can be utilized for the analysis of a wide variety of cationic polymers.

Figure 66: Analysis of cationic polymers

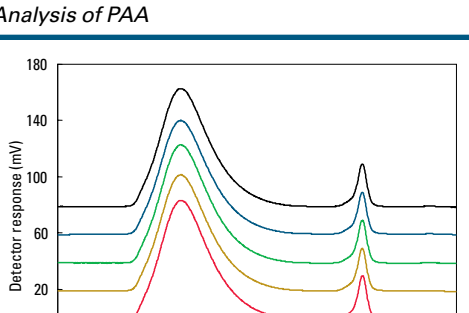


Columns: **TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID × 30 cm**
TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID × 30 cm
TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID × 30 cm
Mobile phase: H_2O with 0.1 mol/L $NaNO_3$
Flow Rate: 1 mL/min
Detection: RI
Temperature: 25 °C
Sample Load: 3 g/L, 100 µL

PAA

The TSKgel PWXL-CP columns eliminate ionic adsorption onto the particle by incorporating a cationic functionality on the particle surface. This is demonstrated in Figure 67 below. PAA [poly(acrylic acid)] was injected onto a TSKgel G5000PWXL-CP column. Each chromatogram, from the first injection (red) to the fifth injection (black), showed similar elution profiles without any adsorption of the polymer.

Figure 67: Analysis of PAA

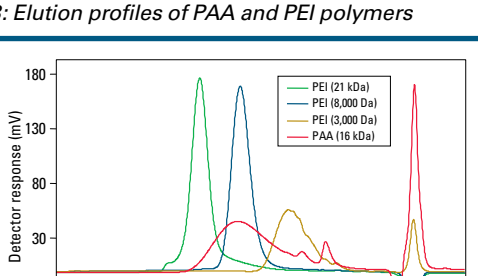


Column:	**TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID × 30 cm**
Mobile phase:	H_2O with 0.1 mol/L $NaNO_3$
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	25 °C
Sample:	polyallylamine-HCl (PAA)
Sample load:	3 g/L, 100 µL

Small Molar Mass Cationic Polymers

Small molar mass cationic polymers were analyzed on two TSKgel G3000PWXL-CP columns in series. As Figure 68 shows, these narrow molar mass cationic polymers eluted in order of their molar masses.

Figure 68: Elution profiles of PAA and PEI polymers



Column:	**TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	H_2O with 0.1 mol/L $NaNO_3$
Flow rate:	1.0 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	polyethyleneimine (PEI) polyallylamine-HCl (PAA)

About: TSKgel SuperMultiporePW Size Exclusion Columns

The innovative multi-pore particle synthesis technology*, pioneered by Tosoh scientists, is incorporated into TSKgel SuperMultiporePW columns for water-soluble polymer analysis. Three semi-micro columns varying in linear range are available within this series, enabling high speed and high resolution analysis with lowered solvent consumption. The base material of each TSKgel SuperMultiporePW column is polymethacrylate.

A wide molar mass range can be analyzed with the three different TSKgel SuperMultiporePW columns, from high molar mass water-soluble polymers to oligomers. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PW$_{XL}$ series columns, which further reduces the chance of adsorption of hydrophilic polymers.

- TSKgel SuperMultiporePW-N
- TSKgel SuperMultiporePW-M
- TSKgel SuperMultiporePW-H

*Using this proprietary technology, Tosoh can manufacture particles, each containing a broad range of pore sizes. This innovative approach essentially creates a linear calibration curve within each particle. As a result, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes.

Attributes and Applications:

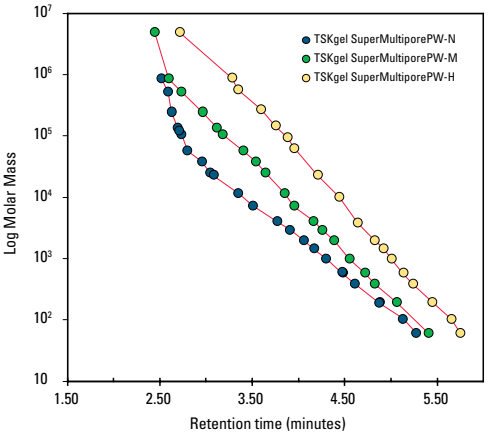
Table 18 shows the product attributes for each of the three TSKgel SuperMultiporePW columns. Figure 69 shows polyethylene glycol, oxide and ethylene glycol calibration curves for each of the TSKgel SuperMultiporePW columns.

Table 18: Product attributes

TSKgel column	SuperMultipore PW-N	SuperMultipore PW-M	SuperMultipore PW-H
Base material	polymethacrylate		
Particle size	4 µm*	5 µm*	8 µm*
Pore size	20 nm	100 nm	>100 nm
Exclusion limit (PEO, PEG/H_2O)	1.0×10^5 - 1.5×10^5 Da	6.0×10^5 - 1.5×10^6 Da	-
Separation range	300 ~ 5.0×10^4 Da	500 ~ 1.0×10^6 Da	1,000 ~ 1.0×10^7 Da
Theoretical plates/15cm column	>16,000	>12,000	>7,000

* Particle size distribution is monodisperse.

Figure 69: Polyethylene glycol, oxide, and ethylene glycol calibration curves for TSKgel SuperMultiporePW columns

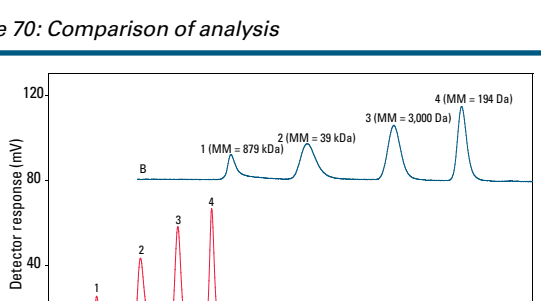


Columns:	**TSKgel SuperMultiporePW-N, 6.0 mm ID × 15 cm** **TSKgel SuperMultiporePW-M, 6.0 mm ID × 15 cm** **TSKgel SuperMultiporePW-H, 6.0 mm ID × 15 cm**
Mobile phase:	H_2O
Flow rate:	0.60 mL/min
Detection:	RI
Temperature:	25 °C
Samples:	polyethylene oxides (PEO) standards polyethylene glycols (PEG) standards ethylene glycol (EG) standards

Comparison with Conventional GPC Columns

A mixture of polyethylene oxide (PEO) and polyethylene glycol (PEG) was analyzed on a semi-micro TSKgel SuperMultiporePW-M column and on conventional-sized TSKgel G3000PWXL and TSKgel G5000PWXL columns in series. As shown in Figure 70, the analysis using the TSKgel SuperMultiporePW-M column was completed in ½ the time and with higher resolution than the analysis performed using the TSKgel G3000PWXL and TSKgel G5000PWXL columns. This is due to the semi-micro dimensions (6.0 mm ID × 15 cm) and the smaller particle size (4 µm) of the TSKgel SuperMultiporePW-M column compared to the 7.8 mm ID × 30 cm size and 7 and 10 µm particle size of the TSKgel G3000PWXL and TSKgel G5000PWXL columns respectively.

Figure 70: Comparison of analysis



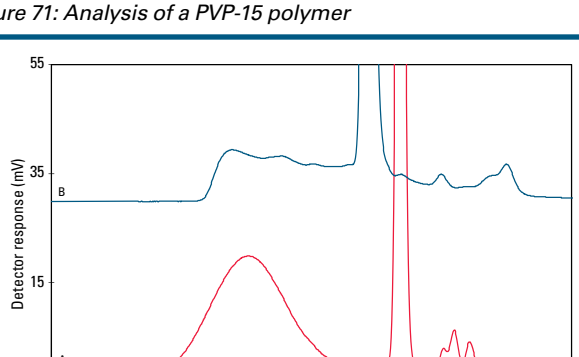
Resolution	TSKgel PWXL	TSKgel SuperMultiporePW-M
Peak 1/Peak 2	3.45	4.25
Peak 2/Peak 3	3.29	3.17
Peak 3/Peak 4	3.30	3.39

Columns:	A: **TSKgel SuperMultiporePW-M, 6.0 mm ID × 15 cm** **B: TSKgel G5000PWXL + G3000PWXL, each 6.0 mm ID × 15 cm**
Mobile phase:	H_2O
Flow rate:	0.6 mL/min
Detection:	RI
Temperature:	25 °C
Injection vol.:	A: 20 µL B: 100 µL
Samples:	mixture of PEO and PEG

PVP

Figure 71 demonstrates the lower hydrophobicity of the TSKgel SuperMultiporePW columns compared to the conventional TSKgel PWXL columns. Hydrophobic interaction causes partial adsorption of PVP-15 polymer on the TSKgel G3000PWXL and TSKgel G2500PWXL columns, while the absence of adsorption on the TSKgel SuperMultiporePW-N column suggests that the internal particle surface is more hydrophilic than the conventional columns.

Figure 71: Analysis of a PVP-15 polymer



Columns:	**A. TSKgel SuperMultiporePW-N, 6.0 mm ID × 15 cm × 2** **B. TSKgel G3000PWXL+G2500PWXL, 6.0 mm ID × 15 cm × 2**
Mobile phase:	100 mmol/L $NaNO_3$
Flow Rate:	0.60 mL/min
Detection:	RI
Temperature:	40 °C
Injection vol.:	20 µL
Samples:	PVP(K-15)