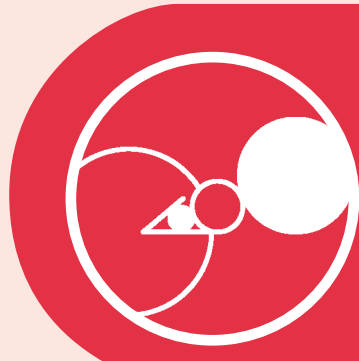
Size Exclusion Chromatography

TSKgel SW-type
- SW
- SW_{XL}
- SuperSW

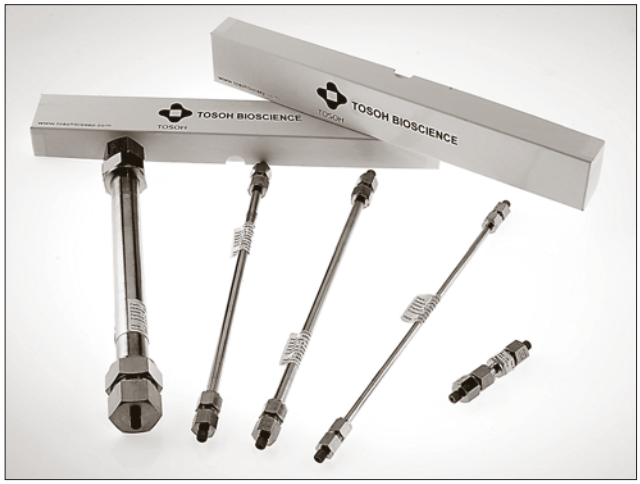
TSKgel PW-type
- PW
- PW_{XL}

TSKgel Alpha-type
- Alpha
- SuperAW

TSKgel H-type
- H_{XL}
- H_{HR}
- SuperH
- SuperHZ



Introduction to TSK-GEL Size Exclusion Chromatography Columns



Tosoh Bioscience provides TSK-GEL columns for both modes of Size Exclusion Chromatography (SEC), which are Gel Filtration Chromatography (GFC) and Gel Permeation Chromatography (GPC). GFC refers to the SEC separation of water soluble polymers in aqueous mobile phases, while GPC refers to the SEC separation of organic soluble polymers using an organic solvent as mobile phase. From a sample perspective, the TSK-GEL SW-type column line, which is based on spherical porous silica particles, is most suitable for analyzing proteins and peptides by gel filtration. Polymer-based TSK-GEL PW columns are best used for GFC analysis of other water soluble polymers, such as oligosaccharides, acrylic acid, etc. Polymer-based TSK-GEL Alpha and SuperAW columns can be used in aqueous solvents, and also with polar organic solvents, thus bridging the gap between GFC and GPC. Organic soluble polymers are best separated by GPC using polystyrene/divinylbenzene-based TSK-GEL H_{XL}, H_{HR} , SuperH or SuperHZ columns. *Table I* compares the characteristics of the various TSK-GEL column lines for SEC.

Tosoh Corporation has a proud history of innovation in size exclusion chromatography. The porous silica-based SW columns were originally introduced in 1978 as TSKgel G2000SW, G3000SW and G4000SW, using 10 and 13 µm spherical particles. The second generation of SEC columns for biopolymer analysis was introduced in 1987. By reducing the particle size from 10 to 5 µm for G2000SW_{XL} and G3000SW_{XL}, and from 13 to 8 µm for G4000SW_{XL}, analysis times were cut in half without sacrificing resolution. The third generation of SW-type columns was introduced in 1998 under the name SuperSW, a column line that features 4 µm spherical particles packed in 4.6 mm ID columns rather than the wider (7.8 mm ID) SW_{XL} columns. TSK-GEL SuperSW columns are available in two pore sizes that mimic the pore structure of the G3000SW_{XL} and G2000SW_{XL} columns. Due to their higher column efficiency and smaller column volume, these columns provide better resolution and higher sensitivity in sample limited cases. This trend towards smaller, more efficient particles packed into narrower bore columns has been expanded to include columns for the analysis of non-biological aqueous and non aqueous polymers. SuperAW, SuperH and SuperHZ columns represent the state of the art column technology for high throughput SEC analysis.

Bulk polymeric Toyopearl GFC resins for process scale separations are available in convenient LABPAK samplers (up to 150 mL) and in bulk quantities of up to 500 mL (see the Bulk Resins section of this catalog). For larger volumes of Toyopearl GFC media, please request a copy of the process media catalog.

Column Selection

The complete TSK-GEL SW, PW, Alpha and SuperAW column lines for GFC consist of many packings, available in various pore and particle sizes. The main criterion in choosing between the TSK-GEL SW, PW, Alpha and SuperAW SEC columns is the molecular weight of the sample and its solubility. The fact that the TSK-GEL SW columns are based on silica and the TSK-GEL PW, Alpha and SuperAW columns are derived from a hydrophilic polymer network has less impact on the separation than the particle and pore size differences. See the Molecular Weight Range Tables in each column type section to determine the best column choice based on pore size.

Due to their higher resolving power, the TSK-GEL SW columns are suitable for the separation of monodisperse biopolymers such as proteins and nucleic acids. TSK-GEL PW columns are commonly used for the separation of synthetic water-soluble polymers because they exhibit a much larger separation range, better linearity of calibration curves, and less adsorption than the TSK-GEL SW columns. While a TSK-GEL SW column is typically the first column to try for biopolymers, TSK-GEL PW columns have demonstrated good results for smaller peptides (<1,000 Da), protein aggregates, DNA fragments, and viruses.

The TSK-GEL Alpha Series columns offer a new alternative for performing SEC. Their compatibility with a wide range of solvents makes them useful for both gel filtration chromatography (GFC) and gel permeation chromatography (GPC). TSK-GEL SuperAW columns are based upon the the same chemistry as Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high-throughput applications. Unlike the Alpha columns, mixed bed formats are included in the SuperAW product offerings for samples with wide ranges of molecular weights/hydrodynamic radius. See *Figure 1* for more information on compatibility of all TSK-GEL SEC columns and solvents.

Non-aqueous GPC separations are performed with TSK-GEL H-type pre-packed columns. Our TSKgel MultiporeH_{XL}-M column contains porous polystyrene divinylbenzene packings with various sized pores on a single bead. TSK-GEL SuperH and SuperHZ columns are offered for high throughput applications where reductions in run time and solvent consumption are critical. *Table XII (see p. 61)* provides a list of possible shipping solvents along with compatible solvents for each H-type packing.

See *Table II* for suggestions on how to choose between TSK-GEL SW, TSK-GEL PW and TSK-GEL Alpha column types.

Features	Benefits
Rigid hydrophilic packings	Minimal swelling and excellent physical strength Low adsorption resulting in high mass recovery
Four series of SEC columns with different ranges of solvent compatibility *(Figure 1)*	Suitable for aqueous GFC and non-aqueous GPC
Easy scale up	Analytical and preparative pre-packed SEC columns

Table I

Characteristics of TSK-GEL Size Exclusion Column Lines

Column Line	TSK-GEL SW / SW_{XL} / SuperSW	TSK-GEL PW / PW_{XL}	TSK-GEL Alpha / SuperAW	TSK-GEL H_{XL} / H_{HR} / SuperH / SuperHZ
Resin Type	Silica	Methacrylate	Methacrylate	PS-DVB
No. of Available Pore Sizes	3	7	5	8
pH stability	2.5 - 7.5	2.0 - 12.0	2.0 - 12.0	1.0 - 14.0
Solvent Compatibility	100% polar	50% polar	100% polar and nonpolar	100% nonpolar, limited polar
Max. Temperature	30°C	80°C*	80°C	60°C (1000H-3000H) 80°C (4000H-GMH) 140°C (H_{HR},H- HT)
Max. Flow Rate (mL/min)	0.4 (SuperSW) 1.2 (SW and SW_{XL})	1.0 (PW_{XL}) 1.2 (PW)	0.6 (SuperAW) 1.0 (Alpha)	1.0 (H_{HR}) 1.2 (H_{XL}) 0.8 (SuperH) 0.7 (SuperHZ 6.0 mmID) 0.4 (SuperHZ 4.6 mmID)
Pressure ** (kg/cm^2)**	8-120	10-40	20-40	10-60
Application Focus	proteins	water-soluble polymers	intermediate polar polymers	organic-soluble polymers

* *Except for the TSKgel G-DNA-PW, which can be operated up to 50˚C and the 55 mm ID TSK-GEL PW-type columns, which can be operated up to 60˚C. When operating below 10˚C, reduce the flow rate to ensure that the maximum pressure is not exceeded.*

** *Depends on column dimensions and particle size*

Note: The operating conditions and specifications for each column are listed on the Operating Conditions and Specifications sheet (OCS) shipped with the column and in the Ordering Information section at the end of each section.

Figure 1

Solvent compatibility for TSK-GEL Size Exclusion Chromatography columns

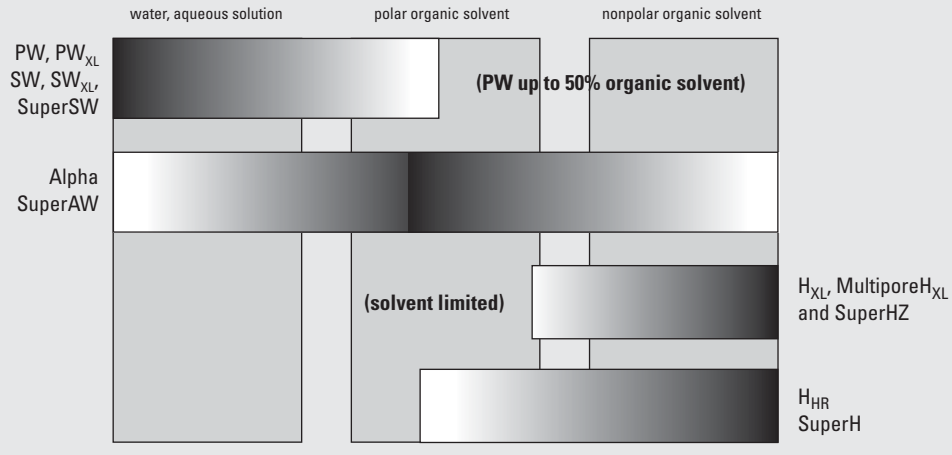


Table II

Column selection guide for high performance Gel Filtration Chromatography

Sample			*Column selection*		*Selection criteria*
			First choice	*Alternative*	
Carbo-hydrates	polysaccharides		TSKgel GMPW$_{XL}$	G5000PW$_{XL}$ and G3000PW$_{XL}$	large pore size, linear calibration curve, small particles, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW or TSKgel G2000PW	G2500PW$_{XL}$	small particles, high resolving power
Nucleic acids	DNA fragments	large	TSKgel G-DNA-PW or TSKgel G5000PW$_{XL}$	—	large pore size, small particles, high resolving power
		medium and small	TSKgel G4000SW$_{XL}$ / SW/ TSKgel BioAssist G4SW$_{XL}$, TSKgel SuperSW3000, or G3000SW$_{XL}$ / SW/ TSKgel BioAssist G3SW$_{XL}$	—	suitable pore sizes
	RNA		TSKgel G4000SW$_{XL}$ / SW/ TSKgel BioAssist G4SW$_{XL}$ TSKgel SuperSW3000, or G3000SW$_{XL}$ / SW/ TSKgel BioAssist G3SW$_{XL}$		suitable pore sizes
	oligonucleotides		TSKgel G2500PW$_{XL}$	—	small pore size, ionic interaction
Proteins	normal size small-medium proteins		TSKgel SuperSW3000, G3000SW$_{XL}$ / SW/ TSKgel BioAssist G3SW$_{XL}$ TSKgel G4000SW$_{XL}$ / SW, TSKgel BioAssist G4SW$_{XL}$ TSKgel SuperSW2000, or G2000SW$_{XL}$ / SW/ TSKgel BioAssist G2SW$_{XL}$	G3000PW$_{XL}$ or G4000PW$_{XL}$	small particles small to medium range pore sizes
	large proteins	low density lipoprotein	TSKgel G6000PW$_{XL}$ **or** TSKgel G5000PW$_{XL}$	—	large pore sizes
		gelatin	TSKgel GMPW$_{XL}$	G5000PW$_{XL}$ and G3000PW$_{XL}$	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000, G3000SW$_{XL}$ / SW/ TSKgel BioAssist G3SW$_{XL}$ or G2000SW$_{XL}$ / SW/ TSKgel BioAssist G2SW$_{XL}$	SuperSW2000 or G3000PW$_{XL}$	small to medium range pore size, versatile
	small		TSKgel G2500PW$_{XL}$	SuperSW2000 or G2000SW$_{XL}$ / SW	linear calibration curve, high resolving power
Viruses			TSKgel G6000PW$_{XL}$ or TSKgel G5000PW$_{XL}$	—	large pore size, high resolving power
Synthetic polymers			TSKgel GMPW$_{XL}$ **or** TSKgel Alpha-M	G5000PW and G3000PW$_{XL}$ or Alpha-5000 and Alpha-3000	large pore size, low adsorption, linear calibration curve
Synthetic oligomers	nonionic and cationic		TSKgel G-Oligo-PW, TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, high resolving power
	anionic		TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, ionic interaction

Applications

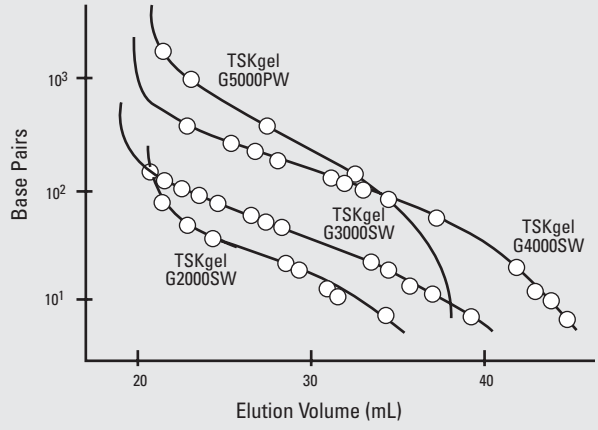
Polynucleotides

TSKgel G2000SW, G3000SW, G4000SW, and G5000PW columns are effective in separating double-stranded DNA fragments and ribosomal and transfer RNA. The choice of column is dependent on sample molecular weight. Small nucleic acids are adequately analyzed by using TSK-GEL SW columns. Larger nucleic acids should be analyzed with TSK-GEL PW columns of larger pore size, such as the TSKgel G-DNA-PW and TSKgel G5000PW columns. Calibration curves for double-stranded DNA fragments on TSK-GEL SW type columns and a TSKgel G5000PW column are shown in *Figure 2; Table III* lists the recommended TSK-GEL SW and TSK-GEL PW columns for separating double-stranded DNA and RNA fragments.

Separation of four *E. coli* RNAs, shown in *Figure 3*, confirms the better performance of TSK-GEL SW columns for samples with a wide molecular weight range. The sample consists of 4S tRNA (25,000 Da), 5S rRNA (39,000 Da), 16S rRNA (560,000 Da), and 23S rRNA (1,100,000 Da). All four polynucleotides are within the molecular weight range recommended for TSK-GEL SW type columns. The two chromatograms demonstrate a superior separation with the TSKgel G4000SW column.

Figure 2

Calibration curves for double-stranded DNA fragments on TSK-GEL SW and TSK-GEL PW columns



Column:	TSK-GEL SW or TSK-GEL PW, two 7.5 mm ID x 60 cm L columns in series
Sample:	22 fragments from *Hae* III-cleaved pBR322 DNA and 6 fragments from *Eco* RI-cleaved pBR322 DNA
Elution:	0.1 M NaCl in 0.1 M phosphate buffer, pH 7.0, plus 1 mM EDTA
Flow Rate:	1.0 mL/min
Detection:	UV @ 260 nm

Table III

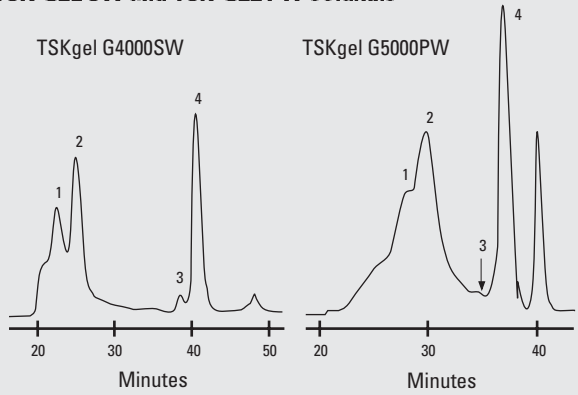
Recommended TSK-GEL SW and TSK-GEL PW columns for separating double-stranded DNA and RNA fragments

Base pairs of DNA	*Recommended column*
< 55	TSKgel G2000SW_{XL}/SW or G3000SW_{XL}/SW or SuperSW2000 or 3000
55 – 110	TSKgel G3000SW_{XL}/SW or SuperSW3000
110 – 375	TSKgel G4000SW_{XL}/SW
375 – 1500	TSKgel G5000PW_{XL}/PW
1000 – 7000	TSKgel G-DNA-PW
RNA (Da) Molecular Weight	
< 60,000	TSKgel G2000SW_{XL}/SW or G3000SW_{XL}/SW or SuperSW2000 or 3000
60,000 – 120,000	TSKgel G3000SW_{XL}/SW or SuperSW3000
120,000 – 1,200,000	TSKgel G4000SW_{XL}/SW
1,200,000 – 10,000,000	TSKgel G5000PW_{XL}

Note: To determine the approximate molecular weight (Da) of a DNA fragment, multiply the number of base pairs by 650.

Figure 3

Separation of total *E. coli* RNA on TSK-GEL SW and TSK-GEL PW columns



Column:	TSKgel G4000SW, two 13 μm, 7.5 mm ID x 30 cm L columns in series TSKgel G5000PW, two 17 μm, 7.5 mm ID x 30 cm L columns in series
Sample:	0.1 mL of 1:10 diluted solution of total *E. coli* RNA: 1. 23 s rRNA (1,100,000 Da), 2. 16 s rRNA (560,000 Da), 3. 5 s rRNA (39,000 Da), 4. 4 s tRNA (25,000 Da)
Elution:	0.1 M NaCl in 0.1 M phosphate buffer, pH 7.0, plus 1 mM EDTA
Flow Rate:	1.0 mL/min
Detection:	UV @ 260 nm

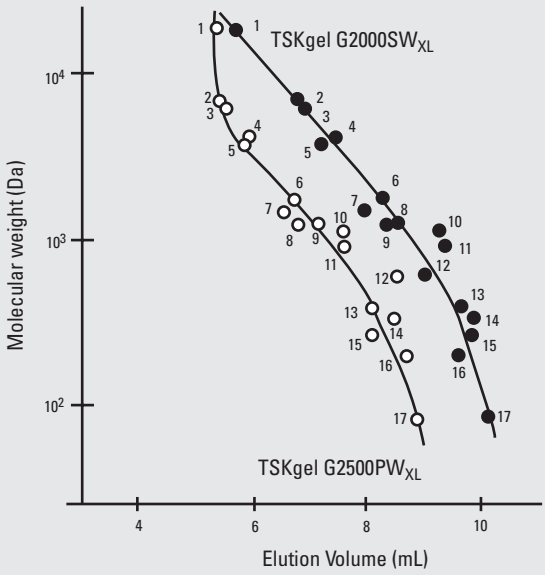
Peptides

As *Figure 4* shows, the calibration curves for TSKgel G2500PW$_{XL}$ and TSKgel G2000SW$_{XL}$ are very similar for samples below 3,000 Da. The data was generated using 17 samples ranging in size from myoglobin (17,800 Da) to glycine (75 Da). While the curves are similar in shape through this range of sample sizes, each sample molecule behaved differently on the two columns, indicating additional sample-resin interaction. For example, although an organic solvent was used to reduce hydrophobic effects, the elution of hydrophobic peptide leu-enkephalin was delayed on the TSKgel G2500PW$_{XL}$ column.

Small peptides may be difficult to chromatograph by aqueous GFC, due to complex non-size effects such as ionic and hydrophobic interactions. The addition of organic solvents and buffered salt solutions overcome these effects. *Figure 5* compares the separation of two mixtures of peptides on both TSKgel G2500PW$_{XL}$ and TSKgel G2000SW$_{XL}$ columns to demonstrate which might be superior for a particular type of peptide. The first group of peptides had molecular weights ranging from 6,500 Da to 555 Da. In the second group, the range was from 75 Da to 17,800 Da. The chromatograms confirm that TSKgel G2000SW$_{XL}$ columns give higher resolution for most peptide mixtures, but do not perform as well as TSKgel G2500PW$_{XL}$ columns at peptide molecular weights lower than 1,000 Da. For very small peptides, the TSK-GEL PW$_{XL}$ column type is preferable.

Figure 4

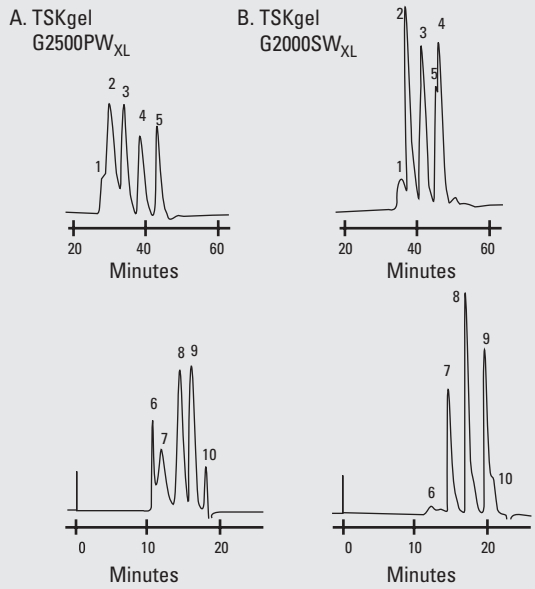
Calibration curves for peptides on TSKgel G2500PW$_{XL}$ and TSKgel G2000SW$_{XL}$ columns



Column: TSKgel G2500PW$_{XL}$, 6 μm, 7.8 mm ID x 30 cm L (○)
TSKgel G2000SW$_{XL}$, 5 μm, 7.8 mm ID x 30 cm L (●)
Sample: 1. myoglobin (17,800 Da), 2. aprotinin (6500 Da), 3. insulin (5807 Da), 4. big gastrin (3849 Da), 5. glucagon (3482 Da), 6. bombesin (1619 Da), 7. substance P (1347 Da), 8. bradykinin-potentiator B (1182 Da), 9. LH-RH (1182 Da), 10. oxytocin (1007 Da), 11. DSIP (848 Da), 12. leu-enkephalin (555 Da), 13. TRH (362 Da), 14. glutathione (307 Da), 15. tetraglycine (246 Da), 16. alanylvaline (188 Da), 17. glycine (75 Da)
Elution: 45% CH_3CN in 0.1% TFA, pH 3
Flow Rate: 1.0 mL/min
Detection: UV @ 215 nm

Figure 5

Separations of peptide mixtures on TSKgel G2500PW$_{XL}$ and TSKgel G2000SW$_{XL}$ columns



Column: A. TSKgel G2500PW$_{XL}$, 6 μm, 7.8 mm ID x 30 cm L
B. TSKgel G2000SW$_{XL}$, 5 μm, 7.8 mm ID x 30 cm L
Sample: approximately 2.5 μg/40 μL each of: 1. aprotinin (6500 Da), 2. big gastrin (3849 Da), 3. bombesin (1619 Da), 4. oxytocin (1007 Da), 5. leu-enkephalin (555 Da), 6. myoglobin (17,800 Da), 7. glucagon (3482 Da), 8. LH-RH (1182 Da), 9. TRH (362 Da), 10. glycine (75 Da)
Elution: 45% CH_3CN in 0.1% TFA
Flow Rate: 0.2 mL/min (top pair); 0.5 mL/min (bottom pair)
Detection: UV @ 215 nm

Proteins

In general, TSK-GEL SW columns have a fractionation range that matches the molecular weight range of most proteins and peptides. However, analytical methods that utilize combinations of TSK-GEL SW and TSK-GEL PW columns in series for isolating large lipoproteins (high and low density), chylomicron, etc. are illustrated in *Figure 6*.

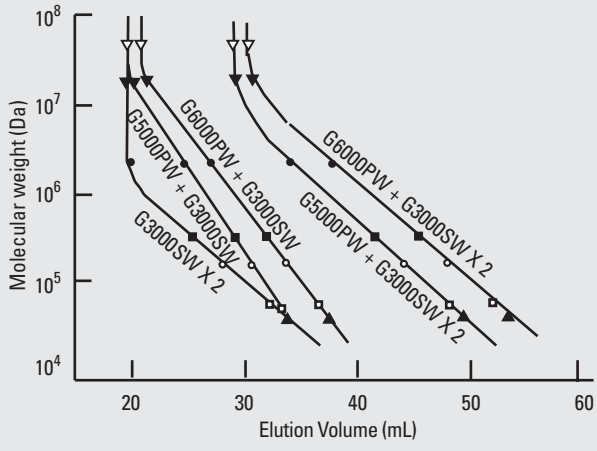
As shown in *Figure 7*, successful elution of the hydrophobic amphoteric polymer collagen (a connective tissue protein) and gelatin on the TSKgel GMPW column requires the addition of 20% CH_3CN to 0.1M $NaNO_3$. Peak areas are reduced and elution is not reproducible when the organic solvent is omitted from the elution buffer for hydrophobic samples.

IgG

The most suitable column and mobile phase will depend on the particular components that need to be measured. TSK-GEL SW_{XL} columns can provide fast, simplified quality control analyses of proteins, peptides and other large-size biopharmaceuticals. For example, a therapeutic solution of intravenous IgG may contain albumin as a stabilizer, and both proteins must be quantified following manufacture. Although literature reports describe the separation of these two proteins by many other chromatographic methods, long analysis times and complex gradient elutions are required. A method developed on TSKgel G3000SW_{XL} provides quantitative separation of the two proteins in 15 minutes with a simple, isocratic elution system. As shown in *Figure 8*, human albumin can be separated from a 20-fold excess of IgG, and quantified using an optimized elution buffer. This simple separation method can be applied to the isolation of other IgGs, such as monoclonal antibodies in ascites fluid.

Figure 6

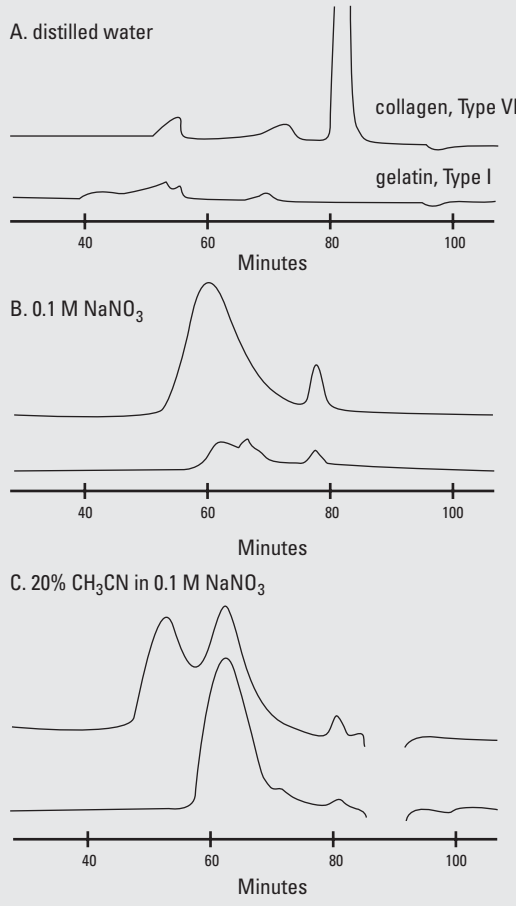
The relation between molecular weight of lipoproteins and elution volume for the combination GFC columns



Column: 7.5 mm ID x 60 cm L
Sample: ▽ chylomicron
▼ VLDL
● LDL
■ HDL_2
○ HDL_3
□ albumin
▲ ovalbumin
Elution: 0.1 M Tris-HCl buffer, pH 7.4
Flow Rate: 1.0 mL/min
Detection: on-line post column reaction

Figure 7

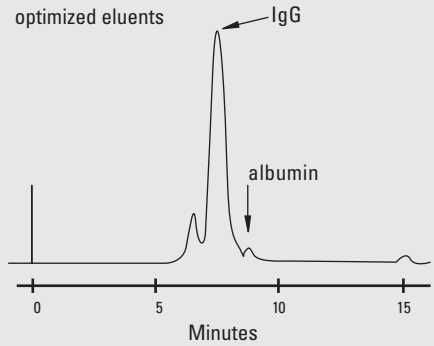
Use of mobile phase additives to improve the elution profile of amphoteric polymers



Column: TSKgel GMPW, two 17 μm, 7.5 mm ID x 60 cm L columns in series
Sample: 0.5 mL of 0.05-0.1% collagen, Type VI, or gelatin,Type I
Elution: A. distilled water, B. 0.1 M $NaNO_3$ in water, C. 20% CH_3CN in 0.1 M $NaNO_3$
Flow Rate: 0.5 mL/min
Detection: RI

Figure 8

QC test for albumin in intravenous human IgG



Column: TSKgel G3000SW_{XL}, 5 μm, 7.8 mm ID x 30 cm L
Sample: 5 μL of Venilon®, containing 237.5 mg of IgG and 12.5 mg of albumin
Elution: 0.1 M Na_2SO_4 in 0.05 M sodium phosphate buffer, pH 5.0
Flow Rate: 1.0 mL/min
Detection: UV @ 280 nm

Cleaning Procedures

Please see Appendix A for instructions on how to maintain and clean TSK-GEL Size Exclusion columns.

Improving resolution with SEC columns

- Verify that the column is not overloaded (refer to sample load information in the SW column section).
- Decrease the dead volume in the HPLC system by using the shortest tubing lengths and the smallest tubing ID possible without exceeding the maximum pressure for the column.
- Decrease the flow rate, but not lower than 0.3 mL/min for TSK-GEL SW and SW_{XL} columns because increased diffusion will occur.

  Note: TSK-GEL SuperSW columns show optimum resolution at flow rates below 0.3mL/min because of their small diameter.

- If using a 30 cm column, add an additional 30 cm column or switch to a $TSK\text{-}GEL_{XL}$-type column that has a smaller particle size. Resolution will improve more than two-fold. Alternatively, if using the SW_{XL}-type columns, switch to the TSK-GEL SuperSW that offer increased sensitivity and resolution due to 50% more theoretical plates.

Literature

For additional information describing applications of Size Exclusion Chromatography columns, or how to select the optimal Size Exclusion Chromatography column, please contact our Technical Service specialists or refer to Tosoh HPLC database on our website: www.tosohbioscience.com.

Silica-based TSK-GEL SW, TSK-GEL SW_{XL} and TSK-GEL SuperSW columns for aqueous Gel Filtration Chromatography of proteins and peptides

Highlights

- -NEW- PEEK column hardware available for SW_{XL} packings.
- TSK-GEL SW-type packings are comprised of rigid spherical silica gel chemically bonded with hydrophilic compounds.
- Low adsorption and well defined pore size distribution, which are required for high performance SEC.
- Particles having three different pore sizes are available packed as the standard SW or SW_{XL} (higher resolution) columns.
- TSK-GEL SuperSW columns feature a 4 µm particle size packing in a narrower 4.6 mm ID column for the highest resolution and sensitivity in the SW series.
- The TSK-GEL SuperSW as well as the larger particle size TSK-GEL QC-PAK (fast analysis) columns are available in two pore sizes. (The properties and molecular weight ranges of these packings are summarized in *Table IV*).
- Semi-preparative (21.5 mm ID) and preparative (55 mm, 108 mm ID) stainless steel columns are available for precise scale-up to commercial production.

Column selection

The three different pore sizes for the TSK-GEL SW packings results in different exclusion limits for several sample types, as shown by the calibration curves in *Figure 9*. From this data, recommended separation ranges for different sample types can be made for each column (Table *IV*). The ranges for polyethylene glycol (PEG) should be consulted when choosing a column for straight chain molecules, dextran for branched molecules, and globular proteins for globular molecules.

Standard TSK-GEL SW columns are packed with 10 µm particles (13 µm particles in G4000SW columns). The higher performance TSK-GEL SW_{XL} versions are packed with 5 µm particles (8 µm particles in G4000SW_{XL} columns). *Figure 10* shows the improved resolution and reduced analysis time with a protein standard mixture on the TSK-GEL SW_{XL} compared with SW analytical columns. The calibration curves for protein standards for the three types of SW columns are compared on the TSK-GEL SW_{XL} packings in *Figure 11*.

For preliminary research or reducing quality control testing time, the 15 cm long QC-PAK columns provide analysis times half as long as those on standard 30 cm columns, while retaining baseline resolution of many protein mixtures. They are available in glass or stainless steel packed with the high performance 5 µm TSK-GEL SW_{XL} packings. TSKgel QC-PAK GFC 200 columns contain the G2000SW_{XL} packing, while TSKgel QC-PAK GFC 300 columns contain the G3000SW_{XL} packing.

Table IV

Properties and separation ranges for TSK-GEL SW-type packings

TSK-GEL packing	*Particle Size (µm)*	*Pore Size (Å)*	*Molecular weight of sample (Da)*		
			Globular proteins	*Dextrans*	*Polyethylene glycols and oxides*
SuperSW2000	4	125	5,000–1.5 x 10^5	1,000–3 x 10^4	500–15,000
G2000SW_{XL}	5	125	5,000–1.5 x 10^5	1,000–3 x 10^4	500–15,000
BioAssist G2SW_{XL}	5	125	5,000–1.5 x 10^5	1,000–3 x 10^4	500–15,000
QC-PAK GFC 200	5	125	5,000–1.5 x 10^5	1,000–3 x 10^4	500–15,000
G2000SW	10, 13	125	5,000–1 x 10^5	1,000–3 x 10^4	500–15,000
SuperSW3000	4	250	1 x 10^4–5 x 10^5	2,000–7 x 10^4	1,000–3.5 x 10^4
G3000SW_{XL}	5	250	1 x 10^4–5 x 10^5	2,000–7 x 10^4	1,000–3.5 x 10^4
BioAssist G3SW_{XL}	5	250	1 x 10^4–5 x 10^5	2,000–7 x 10^4	1,000–3.5 x 10^4
QC-PAK GFC 300	5	250	1 x 10^4–5 x 10^5	2,000–7 x 10^4	1,000–3.5 x 10^4
G3000SW	10	250	1 x 10^4–5 x 10^5	2,000–7 x 10^4	1,000–3.5 x 10^4
G4000SW_{XL}	8	450	2 x 10^4–7 x 10^6	4,000–5 x 10^5	2,000–2.5 x 10^5
BioAssist G4SW_{XL}	8	450	2 x 10^4–7 x 10^6	4,000–5 x 10^5	2,000–2.5 x 10^5
G4000SW	13, 17	450	2 x 10^4–7 x 10^6	4,000–5 x 10^5	2,000–2.5 x 10^5

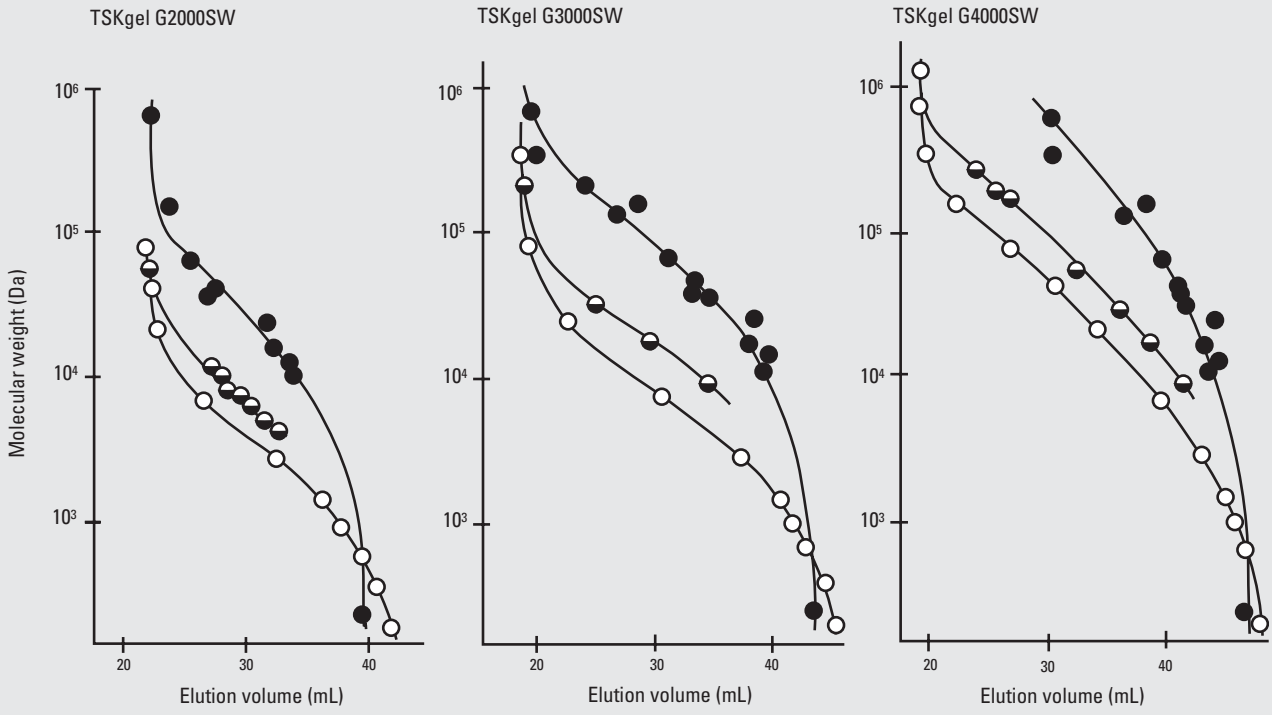
Data generated using the following conditions:

Columns: *Two 4 µm, 4.6 mm ID x 30 cm L TSK-GEL SuperSW columns in series; two 5 µm, 7.8mm ID x 30 cm L TSK-GEL SW_{XL} columns in series; two 10 µm, 7.5 mm ID x 60 cm L TSK-GEL SW columns in series*

Elution: *Globular proteins: 0.3 M NaCl in 0.1 M (0.05 M for SW_{XL} columns) phosphate buffer, pH 7*
Dextrans and polyethylene glycols and oxides (PEOs): distilled water

Figure 9

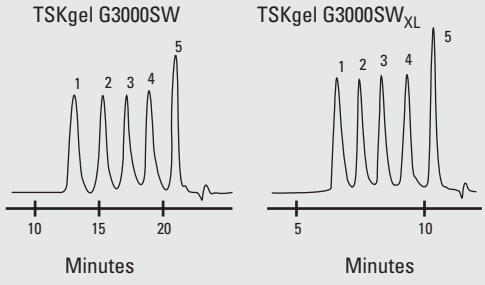
Polyethylene oxide, dextran and protein calibration curves for TSK-GEL SW columns



Column: TSK-GEL SW, two 7.5 mm ID x 60 cm L columns in series
Sample: ● proteins, ○ polyethylene oxides, ◒ dextrans
Elution: dextrans and polyethylene oxides: distilled water; proteins: 0.3 M NaCl in 0.1 M phosphate buffer, pH 7
Flow Rate: 1.0 mL/min
Detection: UV @ 220 nm and RI

Figure 10

Higher resolution with 5 μm TSK-GEL SW_{XL} compared with 10 μm TSK-GEL SW columns



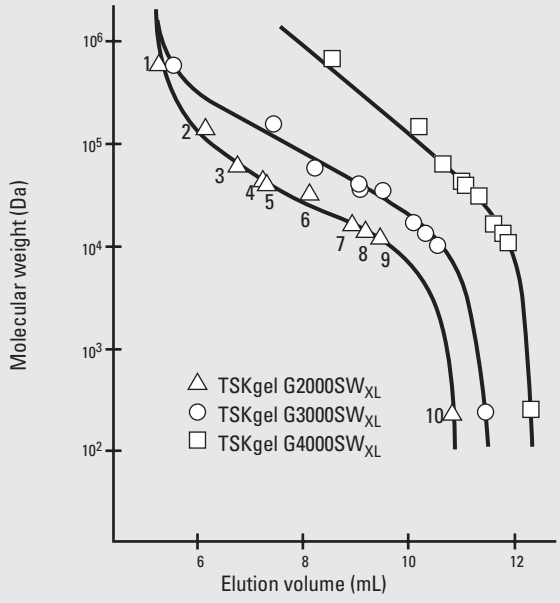
Column: Left: TSK-GEL SW, two 10 μm 7.5 mm ID x 30 cm L columns in series
Right: TSK-GEL SW_{XL}, one 5 μm, 7.8 mm ID x 30 cm L column
Sample: 1. glutamate dehydrogenase (55,000 Da)
2. lactate dehydrogenase (36,500 Da)
3. enolase (67,000 Da)
4. adenylate kinase (6,000 Da)
5. cytochrome C (12,400 Da)
Elution: 0.3 M NaCl in 0.05 M phosphate buffer, pH 6.9
Flow Rate: 1.0 mL/min
Detection: UV @ 220 nm

To further improve efficiency and resolution, TSK-GEL SuperSW columns are filled with 4μm particles. These columns feature a 4.6 mm ID to provide better sensitivity in sample-limited cases. A minimum of 30,000 plates/column is guaranteed. Two pore sizes are available, 125 Å and 250 Å, which provide molecular weight ranges as shown in *Table IV*. *Figure 12* shows similarities of the calibration curves of the TSK-GEL SuperSW and SW_{XL}. Chromatograms of a standard protein mixture in *Figure 13* illustrate the increased sensitivity and resolution of the TSKgel SuperSW3000 compared with the TSKgel G3000SW_{XL}. *Figure 14* depicts the same benefits on the SuperSW2000.

The eluent plays an important role in determining the appropriate column for a separation. Since the structure of globular proteins is compact, the molecular weight exclusion limit for proteins is higher than for linear polymers such as polyethylene oxides, dextrans, or double-stranded DNA. However, when denaturing eluents are used, the exclusion limit for proteins becomes more like the exclusion limit for linear polymers. *Table V* lists the suggested TSK-GEL SW and TSK-GEL PW columns for the separation of proteins and peptides with different eluents. *Figure 15* shows a calibration curve for proteins denatured with 0.1% SDS on the SuperSW3000.

Figure 11

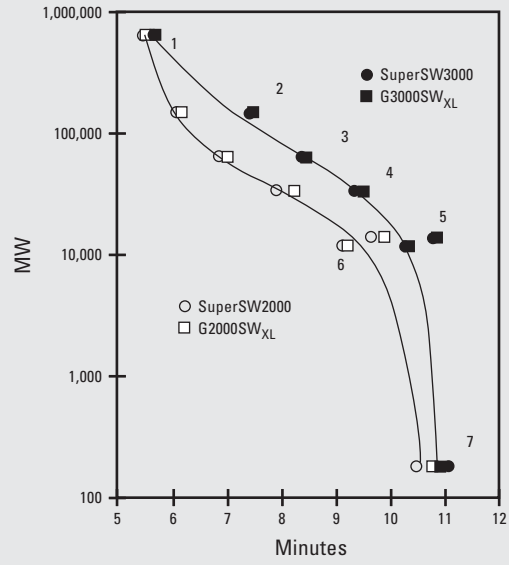
Protein calibration curves for TSK-GEL SW_{XL} columns



Column: TSK-GEL SW_{XL} columns, 5 or 8 μm, 7.8 mm ID x 30 cm L
Sample: 1. thyroglobulin (660,000 Da); 2. IgG (156,000 Da); 3. bovine serum albumin (67,000 Da); 4. ovalbumin (43,000 Da); 5. peroxidase (40,200 Da); 6. β-lactoglobulin (35,000 Da); 7. myoglobin (16,900 Da); 8. ribonuclease A (13,700 Da); 9. cytochrome C (12,400 Da); 10. glycine tetramer (246 Da)
Elution: 0.3 M NaCl in 0.1 M sodium phosphate buffer, pH 7
Detection: UV @ 220 nm

Figure 12

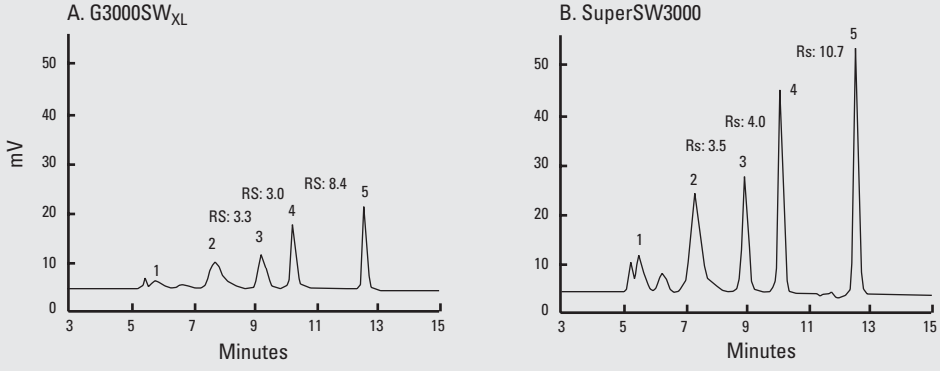
Calibration curves for TSK-GEL SuperSW and SW_{XL}



Sample: proteins: 1. thyroglobulin (670,000 Da); 2. γ-globulin (155,000 Da); 3. BSA (66,300 Da); 4. β-lactoglobulin (18,400 Da); 5. lysozyme (14,300 Da); 6. cytochrome C (12,400 Da); 7. triglycine (189 Da)
Elution: 0.15 M phosphate buffer (pH 6.8)
Flow Rate: 0.35 mL/min for SuperSW; 1.0 mL/min for SW_{XL}
Temperature: 25°C
Detection: UV @ 280 nm (220 nm for triglycine)

Figure 13

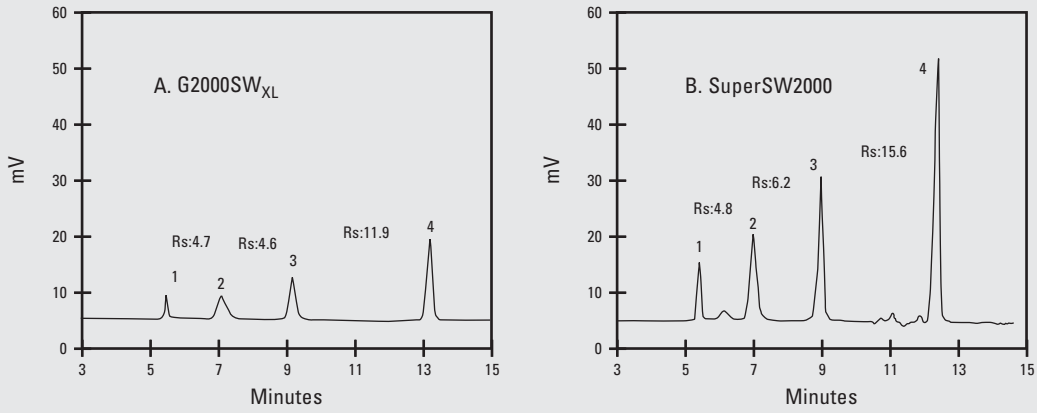
Comparison of TSKgel SuperSW3000 and TSKgel G3000SW $_{XL}$ for the separation of proteins



Column: A.TSKgel $G3000SW_{XL}$, 7.8mm ID x 30 cm L; B. TSKgel Super SW3000, 4.6 mm ID x 30 cm L
Sample: 5 μL of a mixture of 1. thyroglobulin, 0.5 mg/mL (660,000 Da); 2. γ-globulin, 1.0 mg/mL; (150,000 Da); 3. ovalbumin, 1.0 mg/mL (43,000 Da); 4. ribonuclease A, 1.5 mg/mL (12,600 Da); 5. *p*-aminobenzoic acid, 0.01 mg/mL (137 Da)
Elution: 0.1 M Na_2SO_4 in 0.1 M phosphate buffer with 0.05% NaN_3, pH 6.7
Flow Rate: 1.0 mL/min for $G3000SW_{XL}$; 0.35 mL/min for SuperSW3000
Temperature: 25°C
Detection: UV @ 220 nm

Figure 14

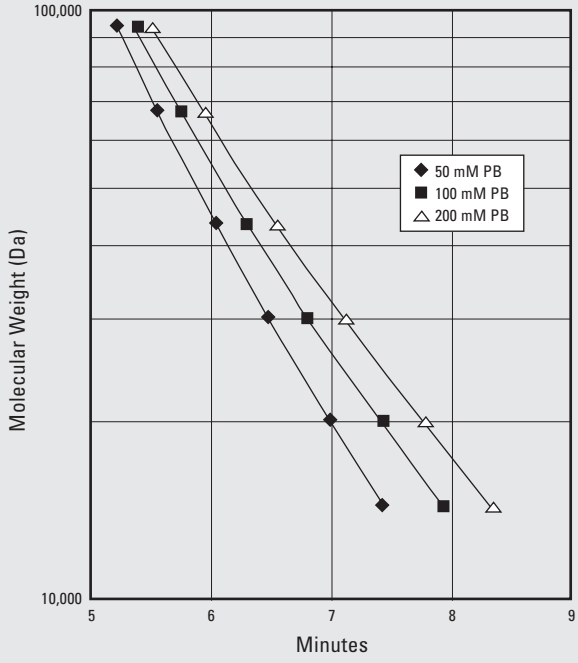
Comparison of chromatograms of proteins on TSKgel SuperSW2000 and TSKgel G2000SW$_{XL}$



Column:	A. TSKgel G2000SW$_{XL}$, 7.8 mm ID x 30 cm L; B. TSKgel SuperSW2000, 4.6 mm ID x 30 cm L
Sample:	1. thyroglobulin (0.2 mg/mL); 2. albumin (1.0 mg/mL); 3. ribonuclease A (1.0 mg/mL); 4. *p*-aminobenzoic acid (0.01 mg/mL)
Injection Volume:	5 µL
Elution:	0.1 M phosphate buffer + 0.1 M Na_2SO_4 + 0.05% NaN_3 (pH 6.7)
Flow Rate:	0.35 mL/min for SuperSW2000; 1.0 mL/min for G2000SW$_{XL}$
Temperature:	25°C
Detection:	UV @ 280 nm

Figure 15

Effect of buffer concentration on calibration curves of proteins on SuperSW3000 in 0.1% SDS eluent



Sample:	proteins*: 1. phosphorylase (94,000 MW); 2. BSA (67,000 MW); 3. ovalbumin (43,000 MW); 4. carbonic anhydrase (30,000 MW); 5. soybean trypsin inhibitor (20,100 MW); 6. α-lactalbumin (14,400 MW)
Eluent:	50 mM-200 mM phosphate buffer (pH 6.8) containing 0.1
Flow Rate:	0.35 mL/min
Temperature:	25°C
Detection:	UV @ 280 nm

* Proteins are denatured in phosphate buffer containing SDS and DTT at 40°C during 15 min.

Table V

Recommended TSK-GEL SW and TSK-GEL PW columns for separating peptides and proteins

Sample (Da)	*Recommended column*
Peptides	
with 45% CH_3CN in 0.1% TFA[a]	
500 – 2.5 x 10^4	TSKgel G2000SW$_{XL}$[b]/BioAssist G2SW$_{XL}$[b]/ SuperSW2000[b]
100 – 5 x 10^3	TSKgel G2500PW$_{XL}$[c]
1,000 – 5 x 10^4	TSKgel G3000PW$_{XL}$[d]
Peptides and proteins	
with 0.1% SDS in 0.1 M phosphate, pH 7.0	
1,000 – 2.5 x 10^4	TSKgel G2000SW$_{XL}$/SW/BioAssist G2SW$_{XL}$ or SuperSW2000
10,000 – 7 x 10^4	TSKgel G3000SW$_{XL}$/SW/BioAssist G3SW$_{XL}$ or SuperSW3000
15,000 – 3 x 10^5	TSKgel G4000SW$_{XL}$ or SW
with 6M guanidine-HCl in 0.1M phosphate, pH 6.0	
1,000 – 2.5 x 10^3	TSKgel G2000SW$_{XL}$/SW/BioAssist G2SW$_{XL}$ or SuperSW2000
2,000 – 7 x 10^4	TSKgel G3000SW$_{XL}$/SW[e]/BioAssist G3SW$_{XL}$ or SuperSW3000
3,000 – 4 x 10^5	TSKgel G4000SW$_{XL}$/SW
Proteins	
with 0.3 M NaCl in 0.05 ~ 0.1 M phosphate, pH 7.0	
5 x 10^3 – 1 x 10^5	TSKgel G2000SW$_{XL}$/SW/BioAssist G2SW$_{XL}$ or SuperSW2000
1 x 10^4 – 5 x 10^5	TSKgel G3000SW$_{XL}$/SW/BioAssist G3SW$_{XL}$ or SuperSW3000
2 x 10^4 – 7 x 10^6	TSKgel G4000SW$_{XL}$ or SW

Notes:

a Proteins may denature in this eluent, increasing the apparent molecular weight.

b Recommended as first column for peptide separation since it offers high resolution across a wide fractionation range.

c Recommended for small peptides. Lower overall resolution than the SW column, but durable to alkaline solutions.

d Recommended for larger peptides. TSKgel G3000SW$_{XL}$ is also effective with mild buffer for peptide fragments with molecular weight >10,000 Da.

e TSKgel G4000SW or SW$_{XL}$ is recommended for polypeptides above 60,000 Da.

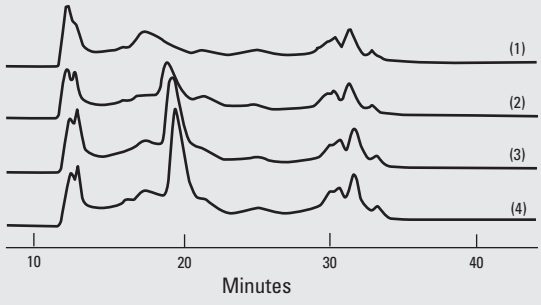
Applications

Proteins

The effect of different concentrations of surfactant on the separation of membrane proteins is seen in *Figure 16*. As the concentration of octaethyleneglycol dodecylether increases to 0.05%, the main peak becomes sharper and recovery increases. The TSKgel SuperSW3000 provides an excellent high resolution separation of IgG_1 from mouse ascites fluid as can be seen in *Figure 17*.

Figure 16

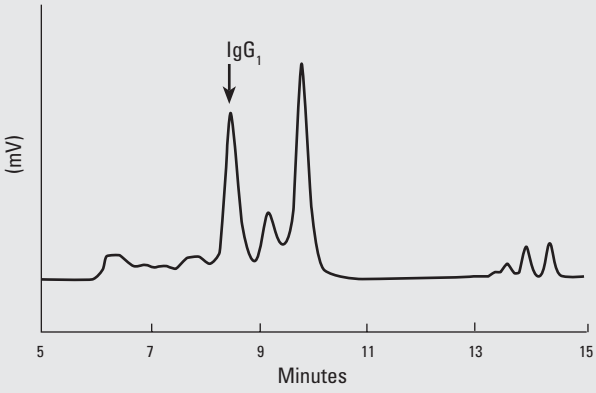
Separation of membrane protein by SEC with different surfactant concentration in the eluent



Column:	TSKgel G3000SW, 7.5 mm ID x 60 cm L
Sample:	Membrane protein from a crude extract from rat liver microsome
Elution:	(0.2 M sodium chloride + 20% glycerol + octaethyleneglycol dodecylether) in 50 mM phosphate buffer, pH 7.0. Note: concentration of surfactant: (1) 0.005%, (2) 0.01%, (3) 0.025%, (4) 0.05%
Flow Rate:	1.0 mL/min
Detection:	UV @ 280 nm

Figure 17

The separation of monoclonal antibody (IgG_1) from mouse ascites fluid



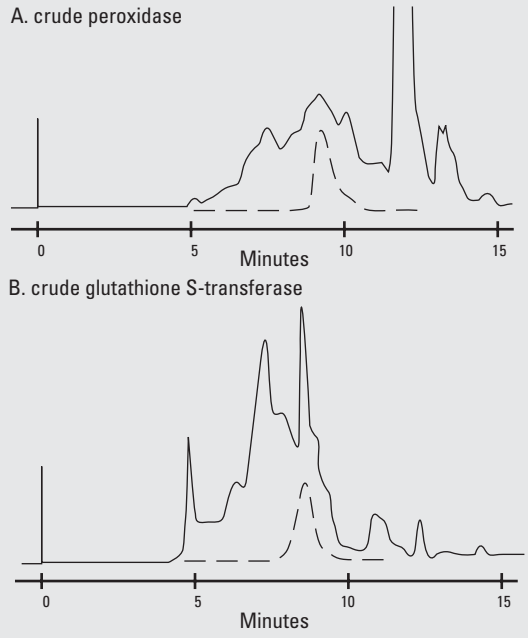
Column:	TSKgel SuperSW3000, 4.6 mm ID x 30 cm L
Sample:	5 μM of IgG_1 from mouse ascites
Elution:	0.2 M phosphate buffer, pH 6.7
Flow Rate:	0.35 mL/min
Detection:	UV @ 280 nm micro flow cell

Enzymes

Mobile phase conditions in GFC are optimized to ensure none or minimal interaction of the sample with the packing material. This gentle technique allows for high recovery of enzymatic activity. For example, crude samples of peroxidase and glutathione S-transferase were separated in only 15 minutes on a TSKgel $G3000SW_{XL}$ column, and activity recovery was 98% and 89% respectively. The elution profiles of the separations in *Figure 18* show that all of the activity eluted in a narrow band of about 1.5 mL.

Figure 18

Separation of crude protein samples on TSKgel $G3000SW_{XL}$



Column:	TSKgel $G3000SW_{XL}$, 5 μm, 7.8 mm ID x 30 cm L
Sample:	A. crude peroxidase from Japanese radish, 0.15 mg in 0.1 mL B. crude glutathione S-transferase from guinea pig liver extract, 0.7 mg in 0.1 mL
Elution:	0.3 M NaCl in 0.05 M phosphate buffer, pH 7
Flow Rate:	1.0 mL/min
Detection:	UV @ 220 nm (solid line) and enzyme assay tests (dashed line)
Recovery:	enzymatic activity recovered was 98% in A and 89% in B

Optimizing GFC with TSK-GEL SW, SW_{XL} and SuperSW Columns

Sample Load

In all modes of chromatography, high sample loads distort peak shapes and cause an overall decrease in efficiency due to column overload. Sample loads may be increased by using organic solvents to enhance the solubility of the sample or by using higher column temperatures to lower the viscosity of the mobile phase. When recovering protein activity, however, these techniques are usually not recommended.

The sample load limitations of TSK-GEL SW and TSK-GEL SW_{XL} analytical and semi-preparative columns are shown in *Figure 19*. At protein injections higher than 0.8 mg, HETP increases rapidly and column efficiency decreases with TSK-GEL SW and TSK-GEL SW_{XL} analytical columns. Sample concentrations in the range of 1-20 mg/mL are recommended, but proteins can be loaded at higher concentrations and higher total loads than synthetic macromolecules. For example, a TSKgel G3000SW semi-preparative column can provide high efficiency for 100 mg of bovine serum albumin (BSA) shown in *Figure 19*. For a synthetic, linear polyethylene glycol (7,500 Da), sample loads higher than 20 mg cause a rapid increase in HETP.

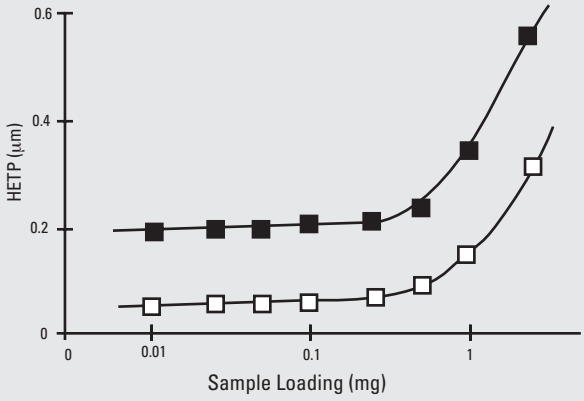
SuperSW columns provide excellent resolution at a sample load of less than 0.3 mg of BSA as seen in *Figure 20*. A sample volume less than 20 µL is recommended for the SuperSW2000 column, and less than 80 µL for the SuperSW3000 column, illustrated in *Figure 21*. However, a 5 µL injection ensures optimal results.

If a larger sample load is necessary, a 7.5 mm ID x 60 cm L SW column has twice the capacity of a 30 cm SW_{XL} column with very little loss of resolution.

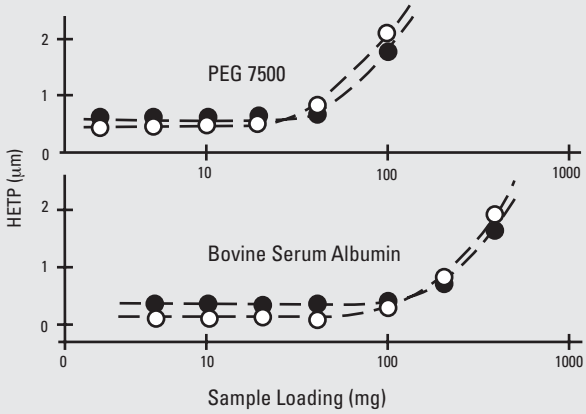
Figure 19

Effect of sample load on efficiency

A. Analytical TSK-GEL SW and TSK-GEL SW_{XL} Columns



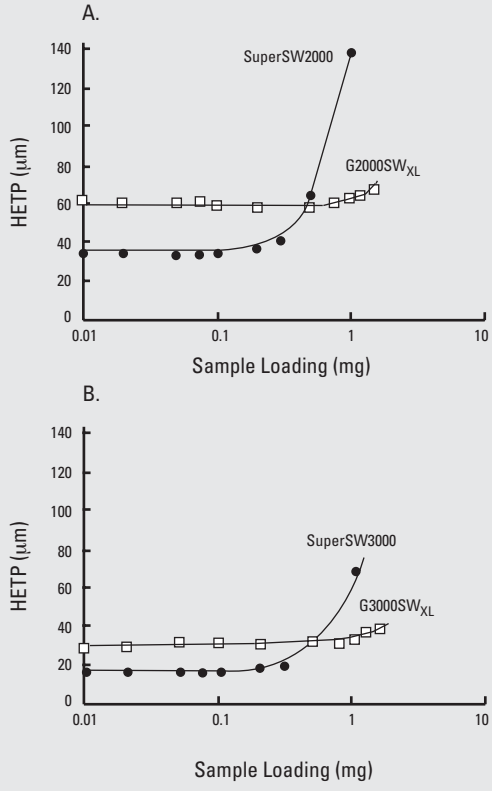
B. Semipreparative TSK-GEL SW Columns



Column: A. ☐ TSKgel $G3000SW_{XL}$, 5 µm, 7.8 mm ID x 30 cm L
■ TSKgel G3000SW, 10 µm, 7.5 mm ID x 30 cm L
B. TSKgel G3000SW, 13 µm, 21.5 mm ID x 60 cm L, two columns in series
Sample: A. bovine serum albumin in 100 µL
B. PEG 7500 or bovine serum albumin in 4 mL
Elution: A. 0.3 M NaCl in 0.05 M phosphate buffer, pH 7
B. 0.3 M NaCl in 0.1 M phosphate buffer, pH 7
Flow Rate: A. 1.0 mL/min,
B. ○ 4.0 mL/min, ● 16.0 mL/min
Detection: BSA: UV @ 220 nm, PEG 7500: RI

Figure 20

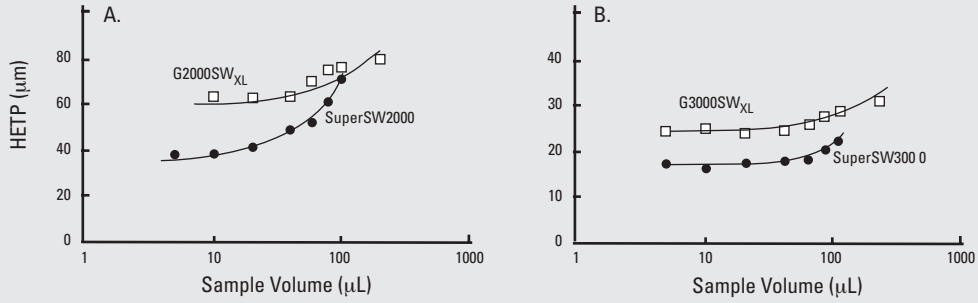
Comparison of sample loading on TSKgel SuperSW and TSKgel SW_{XL}



Column: A. TSKgel SuperSW2000, 4.6 mm IDx 30 cm L and TSKgel $G2000SW_{XL}$, 7.8 mm ID x 30cm L
B. TSKgel SuperSW3000, 4.6 mm ID x 30 cm L and TSKgel $G3000SW_{XL}$, 7.8 mm ID x 30 cm L
Sample: 10 µL of bovine serum albumin
Elution: 0.1 M NaCl in 50 mM phosphate buffer (pH 6.7)
Flow Rate: 0.2 mL/min for SuperSW; 0.57 mL/min for SW_{XL}
Detection: UV @ 280 nm

Figure 21

Comparison of sample volume on TSKgel SuperSW and TSKgel SW $_{XL}$



Column: A. TSKgel SuperSW2000, 4.6 mm ID x 30 cm L and TSKgel G2000SW$_{XL}$, 7.8 mm ID x 30 cm L
B. TSKgel SuperSW3000, 4.6 mm ID x 30 cm L and TSKgel G3000SW$_{XL}$, 7.8 mm ID x 30 cm L
Sample: 1 mg/mL of bovine serum albumin
Elution: 0.1 M Na_2SO_4 in 50 mM phosphate buffer (pH 6.7)
Flow Rate: 0.20 mL/min for Super SW; 0.57 mL/min for SW$_{XL}$
Detection: UV @ 280 nm

Selecting mobile phase buffers

Proper selection of elution conditions is necessary to maximize molecular sieving mechanisms and to minimize secondary effects, such as ionic and hydrophobic interactions between the sample and the column packing material. Under conditions of high ionic strength (>1.0 M), hydrophobic interactions may occur. Under low ionic strength (<0.1 M), ionic interactions are more likely to occur. Secondary interactions take place more often with small solutes since the residual silanol sites of TSK-GEL SW packings are mainly located in small pores, which are accessible to small solutes. In general, the use of relatively high ionic strength buffers can be used to overcome secondary interactions. A neutral salt, such as sodium sulfate, is often added to increase buffer ionic strength.

While the extent of secondary interactions is not as great for proteins as for smaller molecules, complex interactions can occur. For each protein, there will be an optimum buffer type and concentration that results in the highest resolution and recovery. As shown in *Table VI*, resolution among protein samples varies according to buffer type and strength. Although each salt used has the same molar concentration in the mobile phase, each provides a different ionic strength, from the lowest in strength, sodium chloride, to the highest, ammonium phosphate.

The ionic species of the mobile phase will also affect the separation, shown in *Table VI*, by the difference in resolution values for the different salts. In addition, calibration curves for proteins in potassium phosphate buffers are more shallow than those generated in sodium phosphate buffers, while the slope of the curve in Sorenson buffer (containing both Na^+ and K^+) is midway between the slopes generated with either cation alone. *Table VII* illustrates the impact of different buffer conditions on mass recovery for six sample proteins. In this case, the mass recovery of proteins is higher with sodium or potassium phosphate buffers (pH 6.9) than with Tris-HCl buffers (pH 7.8).

If hydrophobic interaction occurs between the sample and the column matrix, up to 100% water soluble organic, such as acetonitrile, acetone, methanol or ethanol, can be added to the mobile phase. Alternatively, 1% nonionic detergent (0.1% SDS), 8M urea, or 6 M guanidine can be used. Viscous mobile phases may require a flow rate reduction if the maximum pressure is exceeded (listed on the Operating Condition and Specification [OCS] sheet or the Ordering Information section at the end of this catalog section).

Choice of mobile phase pH

The optimal buffer pH for a separation depends on the protein's isoelectric point (pI) and the stability of the packing. In general, Tosoh Bioscience recommends the use of a buffer with a pH that is different from the pI of the protein so the protein will carry an overall net positive or negative charge. This generally increases the protein's stability. The pH stability of TSK-GEL SW packings ranges from 2.5 to 7.5. If the separation must be performed under basic conditions, the pH stable polymer-based TSK-GEL PW or TSK-GEL Alpha columns should be used.

Table VI

Resolution factors (R_S) of proteins with various ionic species added to the elution buffer

	Da	*0.2 M $(NH_4)_2HPO_4$*	*0.2 M Na_2SO_4*	*0.2 M $(NH_4)_2SO_4$*	*0.2 M $MgCl_2$*	*0.2 M NaCl*
Glutamate dehydrogenase	280,000					
		1.4	1.1	1.5	1.3	1.5
Alcohol dehydrogenase	150,000					
		0.4	0.5	0.2	0.5	0.6
Glutathione reductase	113,000					
		0.9	1.1	0.9	0.9	1.2
Enolase	67,000					
		1.1	0.8	1.3	0.8	0.4
Ovalbumin	43,000					
		1.3	1.3	1.4	1.9	2.2
Trypsinogen	24,000					
		1.0	0.5	0.2	0.8	0.9
Cytochrome C	12,400					

Column: *TSKgel G3000SW, 7.5 mm ID x 30 cm L*
Elution: *0.05 M GTA buffer, pH 7.0 (G: 3,3-dimethylglutaric acid, T: trishydroxyaminomethane, A: 2-amino-2-methyl-1,3-propanediol) plus indicated salt*
Detection: *UV @ 220 nm*

Table VII

Effect of buffer composition on mass recovery of proteins on TSKgel G3000SW

Protein	*Recovery (%)*		
	A. Sodium phosphate buffer	*B. Potassium phosphate buffer*	*C. Tris-HCl buffer*
Cytochrome C	98	101	92
Lysozyme	92	96	75
α-Chymotrypsinogen	95	98	90
IgG	95	98	88
Thyroglobulin	94	94	85
Ovalbumin	96	92	66

Column: *TSKgel G3000SW, 7.5 mm ID x 60 cm L*
Elution: *A. 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4, pH 6.9;*
B. 0.2 M KH_2PO_4 and 0.2 M K_2HPO_4, pH 6.9;
C. 0.2 M NaCl and 0.05 M Tris-HCl, pH 7.8
Flow Rate: *1.0 mL/min*
Detection: *UV @ 220 nm*

Ordering Information

Analytical and preparative TSK-GEL Size Exclusion silica-based column products: typical properties
See Appendix A for total column volumes and void volumes for each column size

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle Size (µm)*	*Min. Number Theoretical Plates*	*Flow Rate (mL/min) Range*	*Max.*	*Maximum Pressure Drop (kg/cm²)*
Glass columns								
16214	QC-PAK GFC 200, Glass, 125 Å	8.0	15	5	10,000	0.5 – 1.0	1.2	40
16216	QC-PAK GFC 300, Glass, 250 Å	8.0	15	5	10,000	0.5 – 1.0	1.2	40
08799	G2000SW, Glass, 125 Å	8.0	30	10	10,000	0.4 – 0.8	0.8	20
08800	G3000SW, Glass, 250 Å	8.0	30	10	10,000	0.4 – 0.8	0.8	20
08801	G4000SW, Glass, 450 Å	8.0	30	13	8,000	0.4 – 0.8	0.8	20
14464	G3000SW, Glass, 250 Å	20.0	30	13	6,000	3.0 – 6.0	8.0	8
Stainless steel columns								
18674	SuperSW2000, 125 Å	4.6	30	4	30,000	0.1 – 0.35	0.4	120
18675	SuperSW3000, 125 Å	4.6	30	4	30,000	0.1 – 0.35	0.4	120
08540	G2000SW$_{XL}$, 125 Å	7.8	30	5	20,000	0.5 – 1.0	1.2	70
08541	G3000SW$_{XL}$, 250 Å	7.8	30	5	20,000	0.5 – 1.0	1.2	70
08542	G4000SW$_{XL}$, 450 Å	7.8	30	8	16,000	0.5 – 1.0	1.2	35
16215	QC-PAK GFC 200, 125 Å	7.8	15	5	10,000	0.5 – 1.0	1.2	40
16049	QC-PAK GFC 300, 250 Å	7.8	15	5	10,000	0.5 – 1.0	1.2	40
05788	G2000SW, 125 Å	7.5	30	10	10,000	0.5 – 1.0	1.2	20
05789	G3000SW, 250 Å	7.5	30	10	10,000	0.5 – 1.0	1.2	25
05790	G4000SW, 450 Å	7.5	30	13	8,000	0.5 – 1.0	1.2	15
05102	G2000SW, 125 Å	7.5	60	10	20,000	0.5 – 1.0	1.2	40
05103	G3000SW, 250 Å	7.5	60	10	20,000	0.5 – 1.0	1.2	50
05104	G4000SW, 450 Å	7.5	60	13	16,000	0.5 – 1.0	1.2	30
06727	G2000SW, 125 Å	21.5	30	13	10,000	3.0 – 6.0	8.0	10
06728	G3000SW, 250 Å	21.5	30	13	10,000	3.0 – 6.0	8.0	15
06729	G4000SW, 450 Å	21.5	30	17	8,000	3.0 – 6.0	8.0	10
05146	G2000SW, 125 Å	21.5	60	13	20,000	3.0 – 6.0	8.0	20
05147	G3000SW, 250 Å	21.5	60	13	20,000	3.0 – 6.0	8.0	30
05148	G4000SW, 450 Å	21.5	60	17	16,000	3.0 – 6.0	8.0	20
07428	G2000SW, 125 Å	55.0	30	20	4,000	15.0 – 25.0	50.0	10
07481	G3000SW, 250 Å	55.0	30	20	4,000	15.0 – 25.0	50.0	10
07429	G2000SW, 125 Å	55.0	60	20	9,000	15.0 – 25.0	50.0	15
07482	G3000SW, 250 Å	55.0	60	20	9,000	15.0 – 25.0	50.0	15
PEEK Columns								
20027	BioAssist G2SW$_{XL}$ -NEW-	7.8	30	5	20,000	0.5 – 1.0	1.2	70
20026	BioAssist G3SW$_{XL}$ -NEW-	7.8	30	5	20,000	0.5 – 1.0	1.2	70
20027	BioAssist G4SW$_{XL}$ -NEW-	7.8	30	8	16,000	0.5 - 1.0	1.2	35

Pore Sizes: SuperSW2000, QC-PAK GFC 200, G2000SW, G2000SW$_{XL}$ and BioAssist G2SW$_{XL}$= 125 Å; SuperSW3000, QC-PAK GFC 300, G3000SW, G3000SW$_{XL}$ and BioAssist G3SW$_{XL}$= 250 Å; G4000SW, G4000SW$_{XL}$ and BioAssist G4SW$_{XL}$ = 450 Å.

Ordering Information

Analytical and preparative TSK-GEL Size Exclusion silica-based column products: typical properties *(continued)*
See Appendix A for total column volumes and void volumes for each column size

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle Size (µm)*	
Guard columns					
08805	SW Guard column, Glass	8.0	4.0	10	For all 8 mm ID SW and QC-PAK glass
14465	SW Guard column, Glass	20.0	4.0	20	For P/N 14464 SW glass
18762	SuperSW Guard column	4.6	3.5	4	For 4.6 mm ID SuperSW (contains SuperSW3000 packing)
08543	SW_{XL} Guard column	6.0	4.0	7	For all SW_{XL} and P/Ns 16215 and 16049 (contains 3000SW_{XL} packing)
18008	SW_{XL} Guard column, PEEK	6.0	4.0	7	For all BioAssist SW_{XL}, PEEK columns
05371	SW Guard column	7.5	7.5	10	For all 7.5 mm ID SW (contains 3000SW packing)
05758	SW Guard column	21.5	7.5	13	For all 21.5 mm ID SW
07427	SW Guard column	45.0	5.0	20	For 55 mm ID SW
Bulk packing					
08544	SW_{XL} Top-Off, 1g wet gel			5	For SW_{XL} and QC-PAK
06819	SW Top-Off, 1g wet gel			10	For all 7.5 mm ID SW

Polymer-based TSK-GEL PW and TSK-GEL PW_{XL} columns for Gel Filtration Chromatography of water soluble polymers

Highlights

- -NEW- PEEK column hardware available for G6000PW packings for ultra-low sample adsorption during virus analysis.
- Hydrophilic, rigid, spherical, porous methacrylate beads.
- Excellent chemical and mechanical stability.
- pH range of 2 to 12, with up to 50% organic solvent
- Temperatures up to 80˚C (50˚C for TSKgel G-DNA-PW).
- Wide molecular weight separation range up to 8 x 10^6 Da for linear polymers
- Available in analytical (7.5 mm & 7.8 mm ID), semi-preparative (21.5 mm ID) and preparative (55 mm and 108 mm ID) columns.

Polymeric TSK-GEL PW and TSK-GEL PW_{XL} columns are designed for Gel Filtration Chromatography (GFC) of water soluble organic polymers, proteins, peptides, polysaccharides, oligosaccharides, DNA, and RNA.

Column Selection

The properties and molecular weight separation ranges for all TSK-GEL PW type columns are summarized in *Table VIII*. Resins with six pore sizes are available. Particle sizes of resins packed in TSK-GEL PW columns are 10 µm, 17 µm, 20 µm, or 22 µm. The resins in TSK-GEL PW_{XL} columns are 6 µm, 10 µm, or 13 µm. Thus, PX_{XL} resins are higher performance versions of the TSK-GEL PW resins due to the smaller particle sizes. Specialty resin-based columns include the mixed-bed TSKgel GMPW and TSKgel $GMPW_{XL}$ for samples with a broad molecular weight range. They also include TSKgel G-Oligo-PW and TSKgel G-DNA-PW columns for oligosaccharides and for DNA or RNA respectively.

The eluent plays an important role in determining the appropriate column for a separation. Since the structure of globular proteins is compact, the molecular weight exclusion limit for proteins is higher than for linear polymers such as polyethylene oxides, dextrans or double-stranded DNA. However, when denaturing eluents are used, the exclusion limit for proteins is comparable to the exclusion limit for linear polymers. Therefore, consult *Table V* in the SW section for help in choosing a column. Use the molecular weight ranges for PEO in *Table VIII* when choosing a column for linear molecules; dextran for branched molecules, and globular proteins for globular molecules. Calibration curves for polyethylene glycols chromatographed on TSK-GEL PW and TSK-GEL PW_{XL} columns are shown in *Figure 22*. Protein calibration curves on TSKgel PW_{XL} columns are presented in *Figure 23*. Although many methods for polymer analysis have been satisfactorily developed on TSK-GEL PW columns, higher resolution can be achieved with a TSK-GEL PW_{XL} column. The smaller particle sizes of the TSK-GEL PW_{XL} columns provide almost 2.5 times the resolution of their TSK-GEL PW counterparts. In addition, with shorter TSK-GEL PW_{XL} columns, higher resolution separations are possible in less than half the time, as shown in *Figure 24* with polyethylene glycol and polyethylene oxide standards.

For analytical purposes, the TSK-GEL PW_{XL} columns are preferred. For preparative work, or for other cases in which large amounts of sample must be used, the 60 cm TSK-GEL PW columns are recommended because of their increased loading capacity.

Table VIII

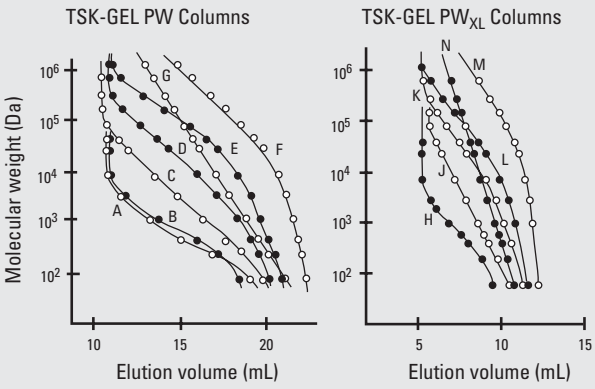
Properties and molecular weight separation ranges for TSK-GEL PW packings

			Molecular weight of sample (Da)		
TSKgel column	*Particle Size* (µm)*	*Average pore Size (Å)*	*Polyethylene glycols & oxides*	*Dextrans***	*Globular proteins***
G1000PW	10	<100	up to 1,000	—	<2,000
G2000PW	10, 17, 20	125	up to 2,000	—	<5,000
$G2500PW_{XL}$ G2500PW	6 10, 17, 20	<200	up to 3,000	—	<8,000
$G3000PW_{XL}$ G3000PW	6 10, 17, 20	200	up to 5 x 10^4	up to 6 x 10^4	500–8 x 10^5
$G4000PW_{XL}$ G4000PW	10 17, 22	500	2,000–3 x 10^4	1,000–7 x 10^5	1 x 10^4–1.5 x 10^6
$G5000PW_{XL}$ G5000PW	10 17, 20, 22	1000	4,000–1 x 10^6	5 x 10^4–2.5 x 10^6	<1 x 10^7
$G6000PW_{XL}$/BioAssist $G6PW_{XL}$ G6000PW	13 17, 25	>1000	4 x 10^4–8 x 10^6	5 x 10^5–5 x 10^7	<2 x10^8
$GMPW_{XL}$ GMPW	13 17	<100–1000	500–8 x 10^6	<5 x 10^7	<2 x 10^8
G-Oligo-PW G-DNA-PW	6 10	125 >1000	up to 3,000 4 x 10^4–8 x 10^6	— <5 x 10^7	<3,000 <2 x 10^8

Column: *TSK-GEL PW columns, 7.5 mm ID x 60 cm L; TSKgel PW_{XL}, G-Oligo-PW & G-DNA-PW, 7.8 mm ID x 30 cm L*
Elution: *Polyethylene glycols and oxides: distilled water; dextrans and proteins: 0.2 M phosphate buffer, pH 6.8*
Flow Rate: *1.0 mL/min*
Note: **Larger particle sizes of each group are for 21.5 mm ID x 60 cm L semi-preparative and 55 mm or 108 mm ID x 60 cm L preparative columns.*
***Maximum separation range determined from estimated exclusion limits.*

Figure 22

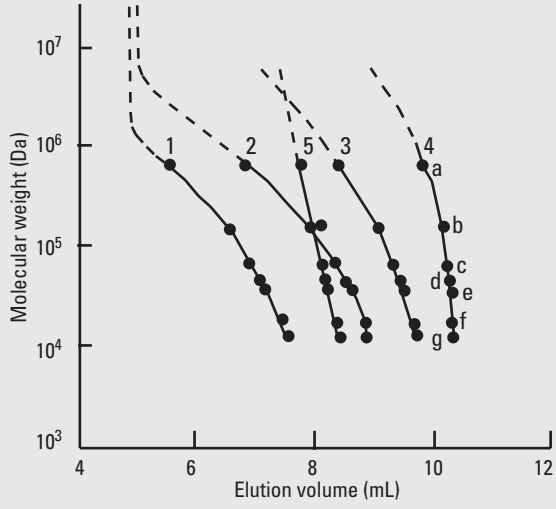
Polyethylene glycol and oxide calibration curves on TSK-GEL PW and TSK-GEL PW_{XL} columns



Column: TSK-GEL PW columns: A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5 mm ID x 60 cm L
TSK-GEL PW_{XL} columns: H. $G2500PW_{XL}$, J. $G3000PW_{XL}$, K. $G4000PW_{XL}$, L. $G5000PW_{XL}$, M. $G6000PW_{XL}$, N. $GMPW_{XL}$, all 7.8 mm ID x 30 cm L
Elution: distilled water
Flow Rate: 1.0 mL/min
Detection: RI

Figure 23

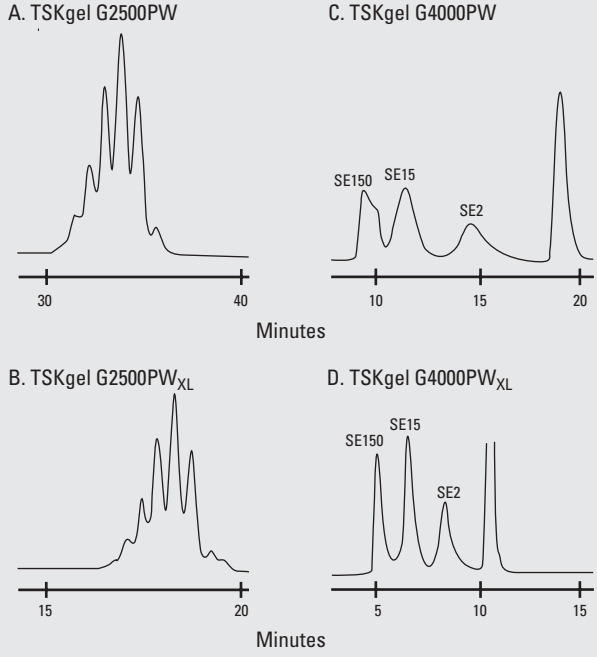
Protein calibration curves on TSKgel PW_{XL} columns



Column: 1. $G3000PW_{XL}$
2. $G4000PW_{XL}$
3. $G5000PW_{XL}$
4. $G6000PW_{XL}$
5. $GMPW_{XL}$
Sample: a. thyroglobulin (660,000 Da)
b. γ-globulin (150,000 Da)
c. albumin (67,000 Da)
d. ovalbumin (43,000 Da)
e. β-lactoglobulin (36,000 Da)
f. myoglobin (16,900 Da)
g. cytochrome C (12,400 Da)
Elution: 0.2 M phosphate buffer (pH 6.8)
Flow Rate: 1.0 mL/min
Detection: UV @ 280 nm

Figure 24

Faster analysis and higher resolution with TSK-GEL PW_{XL} columns



Column: A. TSKgel G2500PW, two 10 μm, 7.5 mm ID x 60 cm L columns in series
B. TSKgel $G2500PW_{XL}$, two 6 μm, 7.8 mm ID x 30 cm L columns in series
C. TSKgel G4000PW, 17 μm, 7.5 mm ID x 60 cm L
D. TSKgel $G4000PW_{XL}$, 10 μm, 7.8 mm ID x 30 cm L
Sample: A. & B.: polyethylene glycol 200
C. & D.: polyethylene oxide standards: SE-150, SE-15 and SE-2 in 100 μL
Elution: A. & B.: distilled water; C. & D.: 0.1 M NaCl
Flow Rate: 1.0 mL/min
Temperature: A. & B.: 25°C; C. & D.: 50°C
Detection: RI

TSKgel Oligo PW

The specialty column TSKgel Oligo PW is designed for high resolution separations of nonionic and cationic oligomers. Because of the presence of residual cationic groups, this column is not recommended for separating anionic materials. The polyethylene glycol and polythylene oxide calibration curves for TSKgel G-Oligo-PW (not shown) are identical to TSKgel G2500PW$_{XL}$ shown in *Figure 22*.

TSKgel G-DNA-PW

The TSKgel G-DNA-PW column is dedicated to the separation of large polynucleotides, such as DNA and RNA fragments of 500 to 5,000 base pairs. *Figure 25* shows a calibration curve for double-stranded DNA on the G-DNA-PW column. The exclusion limits for double-stranded DNA fragments are lower than those for rRNAs, indicating that double-stranded DNA fragments have a larger effective molecular weight in solution than rRNAs of the same molecular weight. The packing has very large pores (>1000 Å) and a small particle size (10 µm).

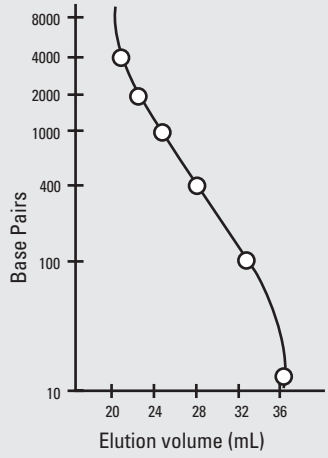
For the separation of large DNA fragments greater than 1,000 base pairs, a four-column system is typically required. Baseline resolution of DNA fragments up to 7,000 base pairs can be achieved, provided there is a two-fold difference in the chain length of the fragments. *Figure 26* shows the elution of double-stranded DNA fragments, obtained from pBR322 DNA cleaved by both *Eco* RI and *Bst* NI, 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 26B*.

TSKgel GMPW and TSKgel GMPW$_{XL}$

When the molecular weight range of the sample is broad or unknown, Tosoh Bioscience offers two mixed-bed columns, TSKgel GMPW and TSKgel GMPW$_{XL}$, for analysis. The TSKgel GMPW column and its high resolution counterpart, TSKgel GMPW$_{XL}$, are packed with the G2500, G3000 and G6000 PW or corresponding PW$_{XL}$ resins. They offer a broad molecular weight separation range. As shown in *Figure 22*, the calibration curve for polyethylene glycols and oxides on these mixed-bed columns is linear over the range of 100-1,000,000 Da.

Figure 25

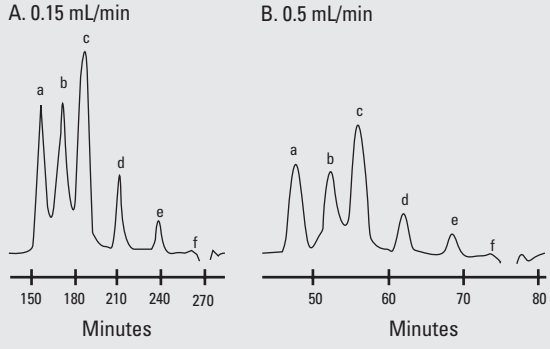
Calibration curve for double-stranded DNA



Column: TSKgel G-DNA-PW, four 10 µm, 7.8 mm ID x 30 cm L columns in series
Sample: *Eco* RI and *Bst* NI-cleaved pBR322 DNA, void volume determined with λ-DNA
Elution: 0.3 M NaCl in 0.1 M Tris-HCl, pH 7.5, plus 1 mM EDTA
Flow Rate: 0.15 mL/min
Detection: UV @ 260 nm

Figure 26

Separation of large DNA fragments on a TSKgel G-DNA-PW column



Column: TSKgel G-DNA-PW, four 10 µm, 7.8 mm ID x 30 cm L columns in series
Sample: 60 µL of *Eco* RI and *Bst* NI - cleaved pBR322 DNA, base pairs: a. 4362, b. 1857, c. 1060 & 928, d. 383, e. 121, f. 13
Elution: 0.3 M NaCl in 0.1 M Tris-HCl, pH 7.5, plus 1mM EDTA
Flow Rate: A. 0.15 mL/min, B. 0.5 mL/min
Detection: UV @ 260 nm

Applications

Nucleic acids

Desalting of nucleosides can be accomplished using the TSKgel G2500PW$_{XL}$ as depicted in *Figure 27*. In this case secondary interaction effects induce adenosine and uridine to elute after the void volume in the unbuffered water mobile phase.

Polysaccharides

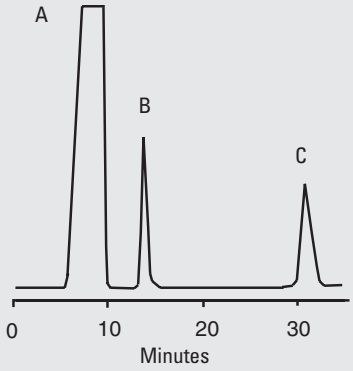
TSK-GEL PW columns are recommended for polysaccharide analysis, due to their ability to separate a wide molecular weight distribution. Nonionic polysaccharides are the least complicated molecules to analyze by size exclusion chromatography because they seldom exhibit secondary interactions with solid support. Two TSK-GEL PW columns (TSKgel G5000PW and TSKgel G3000PW) in series are effective for the characterization of clinical dextran.

Cationic samples can be adsorbed on the resin by electrostatic interaction. If the polymer is strongly cationic, a fairly high salt concentration is required to prevent ionic interactions. *Figure 28* demonstrates the effect of increasing sodium nitrate concentration on peak shapes for a cationic polymer, DEAE-dextran. A mobile phase of 0.5 M acetic acid with 0.3 M Na_2SO_4 can also be used.

An effective separation of the anionic hydrophilic glucosaminoglycan, hyaluronic acid, is shown in *Figure 29* on a TSKgel G6000PW and TSKgel G4000PW column in series with 0.2 M sodium chloride.

Figure 27

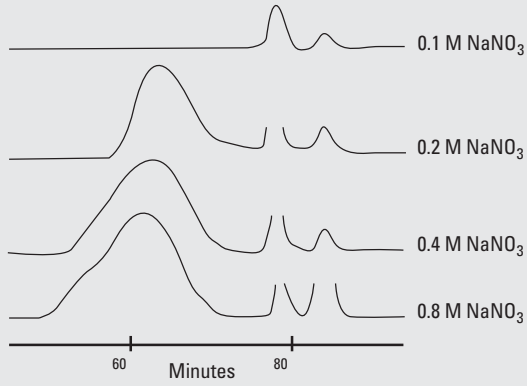
Desalting of nucleosides



Column:	TSKgel G2500PW$_{XL}$, 7.8 mm ID x 30 cm L
Sample:	A. 0.5 NaCl; B. uridine; C. adenosine
Eluent:	distilled water
Flow Rate:	1.0 mL/min
Detection:	UV @ 260 nm

Figure 28

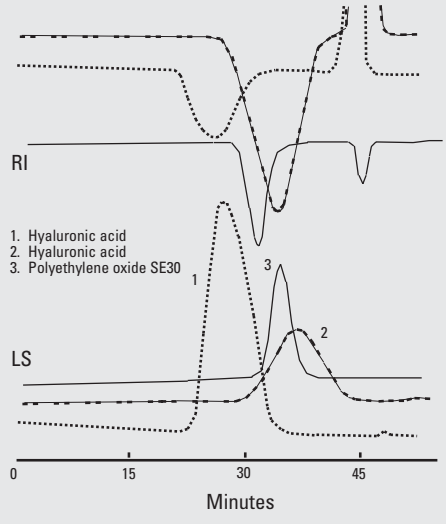
Use of sodium nitrate to elute cationic polymers



Column:	TSKgel GMPW, two 17 µm, 7.5 mm ID x 60 cm L columns in series
Sample:	0.5 mL of 0.05-0.1% of the DEAE-dextran cationic polymer
Elution:	0.1 M, 0.2 M, 0.4 M or 0.8 M $NaNO_3$ in water
Flow Rate:	0.5 mL/min
Detection:	RI

Figure 29

Separation of hyaluronic acid



Column:	TSKgel G6000PW + G4000PW, two 7.5 mm ID x 60 cm L columns in series
Sample:	hyaluronic acid
Elution:	0.2 M NaCl
Flow Rate:	0.9 mL/min
Temperature:	40˚C

Oligomers

The TSKgel G-Oligo-PW column is designed for high resolution separations of nonionic and cationic oligomers. *Figure 30* demonstrates excellent resolution of chito-oligosaccharides obtained by using the smaller, 6 µm particle size packing in TSKgel G-Oligo-PW columns as compared with the resolution obtained with a TSKgel G2000PW column. The pore sizes in both TSKgel G-Oligo-PW and TSKgel G2000PW columns are about 125 Å and both resins bear approximately 0.2 µeq/mL of cationic groups. Because of the presence of cationic groups, neither column is recommended for separating anionic materials. However, for nonionic oligomers, TSKgel G-Oligo-PW columns provide higher resolution than TSKgel G2500PW$_{XL}$ columns. The calibration curve for hydrolyzed β-cyclodextrin on two TSKgel G-Oligo-PW columns in series is shown in *Figure 31*.

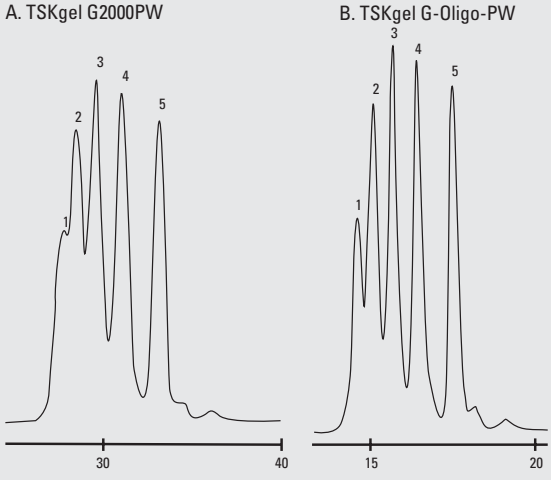
Polymers

Sodium polyacrylate, an anionic polymer, is effectively separated on two TSKgel GMPW columns in *Figure 32*. The addition of 0.01 M $NaNO_3$ results in normal elution and peak shape overcoming the ionic repulsion between the anionic sample and the resin.

Polyethyleneimine, a cationic polymer, is separated in *Figure 33* on two TSKgel GMPW$_{XL}$ columns with a 0.5 M acetic acid and 0.5 M sodium acetate as mobile phase.

Figure 30

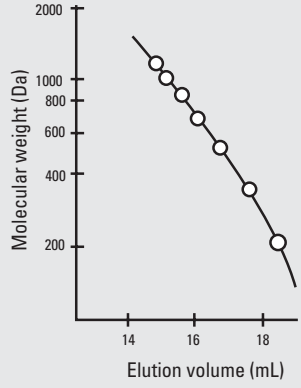
Faster analysis and higher resolution of chito-oligosaccharides on a TSKgel G-Oligo-PW column



Column: A. TSKgel G2000PW, two 10 µm, 7.5 mm ID x 60 cm L columns in series
B. TSKgel G-Oligo-PW, two 6 µm, 7.8 mm ID x 30 cm L columns in series
Sample: 1. chitohexaose, 2. chitopentaose, 3. chitotetraose, 4. chitotriose, 5. chitobiose.
Elution: distilled water
Flow Rate: 1.0 mL/min
Detection: RI

Figure 31

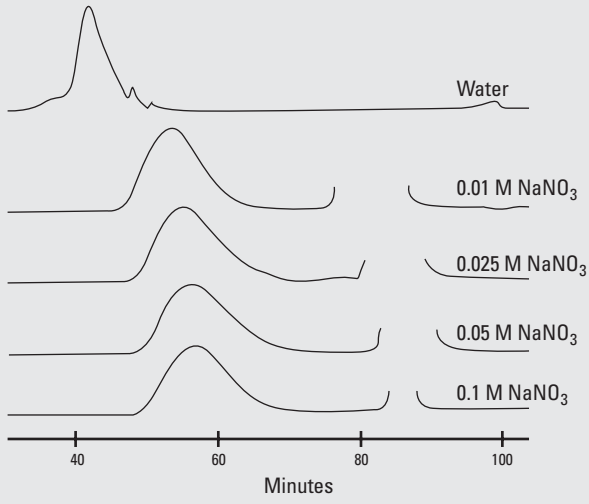
Calibration curve for oligosaccharides



Column: TSKgel G-Oligo-PW, two 6 µm, 7.8 mm ID x 30 cm L columns in series
Sample: hydrolyzed β-cyclodextrin
Elution: distilled water
Flow Rate: 1.0 mL/min
Detection: UV @ 260 nm

Figure 32

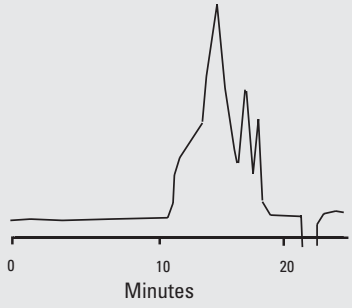
Effect of ionic strength on the elution of anionic polymers



Column:	TSKgel GMPW, two 17 μm, 7.5 mm ID x 60 cm L columns in series
Sample:	0.5 mL of 0.05-0.1% of the sodium salt of polyacrylic acid, an anionic polymer
Elution:	water, 0.01 M, 0.025 M, 0.05 M or 0.1 M $NaNO_3$ in water
Flow Rate:	0.5 mL/min
Detection:	RI

Figure 33

Separation of polyethyleneimine



Column:	TSKgel $GMPW_{XL}$, two 7.8 mm ID x 30 cm L in series
Sample:	polyethyleneimine
Eluent:	0.5 M acetic acid + 0.5 M sodium acetate
Flow Rate:	1.0 mL/min
Detection:	RI

Optimizing GFC with TSK-GEL PW and PW_{XL}

Selecting mobile phase buffers

In an ideal SEC separation, the mechanism is pure sieving, with no chemical interaction between the column matrix and the sample molecules. In practice, however, a small number of weakly charged groups on the surface of all TSK-GEL PW type packings can cause changes in elution order from that of an ideal system. Fortunately, the eluent composition can vary greatly with TSK-GEL PW columns, to be compatible with a wide range of neutral, polar, anionic and cationic samples. *Table IX* lists appropriate eluents for GFC of all polymer types on TSK-GEL PW type columns.

For some nonionic, nonpolar polymers, such as polyethylene glycols, normal chromatograms can be obtained by using distilled water. 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 is added to the aqueous eluent. Generally, a salt concentration of 0.1 M to 0.5 M is sufficient to overcome undesirable ionic interactions.

Hydrophobic samples

TSK-GEL PW-type resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. 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 solvent 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 IX*. All TSK-GEL PW-type column packings are compatible with 20% aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethyl formamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition these columns can be run in 50% aqueous acetone.

Table IX

Recommended eluents for GFC of water-soluble polymers on TSK-GEL PW type columns

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

Ordering Information

Analytical and preparative TSK-GEL Size Exclusion polymer-based column products: typical properties

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle Size (µm)*	*Min. Number Theoretical Plates*	*Flow Rate (mL/min) Range*	*Max.*	*Maximum Pressure Drop (kg/cm²)*
Stainless steel columns								
08031	G-Oligo-PW, 125 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08032	G-DNA-PW, >1.000 Å	7.8	30	10	10,000	0.2 – 0.5	0.6	20
08020	G2500PW$_{XL}$, <200 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08021	G3000PW$_{XL}$, 200 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08022	G4000PW$_{XL}$, 500 Å	7.8	30	10	10,000	0.3 – 0.8	1.0	20
08023	G5000PW$_{XL}$, 1.000 Å	7.8	30	10	10,000	0.3 – 0.8	1.0	20
08024	G6000PW$_{XL}$, >1.000 Å	7.8	30	13	7,000	0.3 – 0.8	1.0	20
08025	GMPW$_{XL}$, 100-1.000 Å	7.8	30	13	7,000	0.3 – 0.8	1.0	20
05760	G1000PW, <100 Å	7.5	30	10	5,000	0.5 – 1.0	1.2	20
05761	G2000PW, 125 Å	7.5	30	10	5,000	0.5 – 1.0	1.2	20
08028	G2500PW, <200 Å	7.5	30	10	5,000	0.5 – 1.0	1.2	20
05762	G3000PW, 200 Å	7.5	30	10	5,000	0.5 – 1.0	1.2	20
05763	G4000PW, 500 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05764	G5000PW, 1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05765	G6000PW, >1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
08026	GMPW, 100-1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05105	G2000PW, 125 Å	7.5	60	10	10,000	0.5 – 1.0	1.2	40
08029	G2500PW, <200 Å	7.5	60	10	10,000	0.5 – 1.0	1.2	40
05106	G3000PW, 200 Å	7.5	60	10	10,000	0.5 – 1.0	1.2	40
05107	G4000PW, 500 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05108	G5000PW, 1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05109	G6000PW, >1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
08027	GMPW, 100-1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05150	G2000PW, 125 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
08030	G2500PW, <200 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
05151	G3000PW, 200 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
05152	G4000PW, 500 Å	21.5	60	22	6,000	1.0 – 6.0	8.0	20
05153	G5000PW, 1.000 Å	21.5	60	22	6,000	1.0 – 6.0	8.0	20
05154	G6000PW, >1.000 Å	21.5	60	25	6,000	1.0 – 6.0	8.0	20
07926	G3000PW, 200 Å	55.0	60	20	4,500	15.0 – 25.0	30.0	15
07927	G5000PW , 1.000 Å	55.0	60	20	4,500	15.0 – 25.0	30.0	15
Guard columns								
08034	Oligo Guard column	6.0	4.0	12.0	For G-Oligo-PW			
08033	PW$_{XL}$ Guard column	6.0	4.0	12.0	For 7.8 mm ID PW$_{XL}$ and G-DNA-PW (contains 3000PW packing)			
06763	PW-L Guard column	7.5	7.5	12.0	For 7.5 mm ID G1000PW and G2000PW (contains 2000PW packing)			
06762	PW-H Guard column	7.5	7.5	12.0	For 7.5 mm ID G2500PW - G6000PW + GMPW (contains 3000PW packing)			
06757	PW-L Guard column	21.5	7.5	17.0	For 21.5 mm ID G2000PW			
06758	PW-H Guard column	21.5	7.5	17.0	For 21.5 mm ID G2500PW through G6000PW			
07924	PW Guard column	45.0	5.0	20.0	For 55 mm ID G3000PW + G5000PW			
Bulk packing								
08035	PW$_{XL}$ Top-Off, 1g wet resin			10.0	For all PW$_{XL}$ and G-DNA-PW			

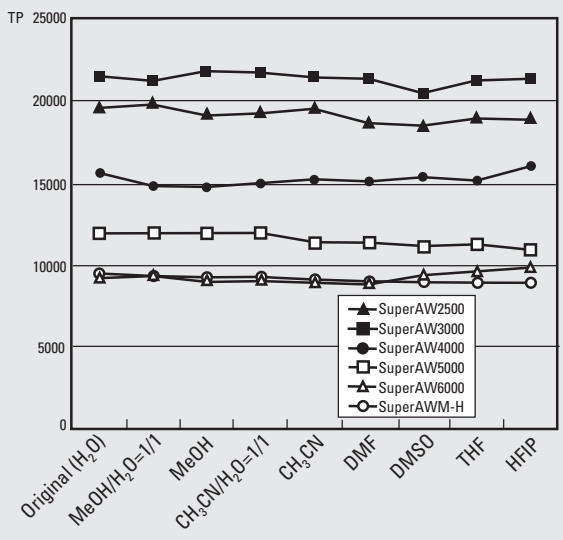
Polymer based TSK-GEL Alpha and SuperAW columns for Gel Filtration and Gel Permeation Chromatography of water soluble and organic soluble polymers

Highlights

- A unique hydrophilic, polyvinyl resin.
- Exhibits strong mechanical stability and minimal swelling characteristics.
- A wide range of solvent compatibility, from 100% water to 100% non-polar organic solvents (*Figure 34 and Figure 35*).
- The reduced particle size and shorter column length of TSK-GEL SuperAW columns provides equivalent resolution in ½ the time for high throughput applications.
- Unlike polystyrene-divinylbenzene (PS-DVB) resins that may adsorb polymers due to hydrophobic interaction, both the TSK-GEL Alpha and SuperAW columns allow for the separation of polymers soluble in methanol.
- Provide accurate molecular weight (MW) determination of samples in dimethyl formamide and exhibit normal retention of polystyrene polymers.
- System peaks from salts in the eluent elute away from the oligomer of interest (*Figure 36*), providing accurate MW determinations.

Figure 35

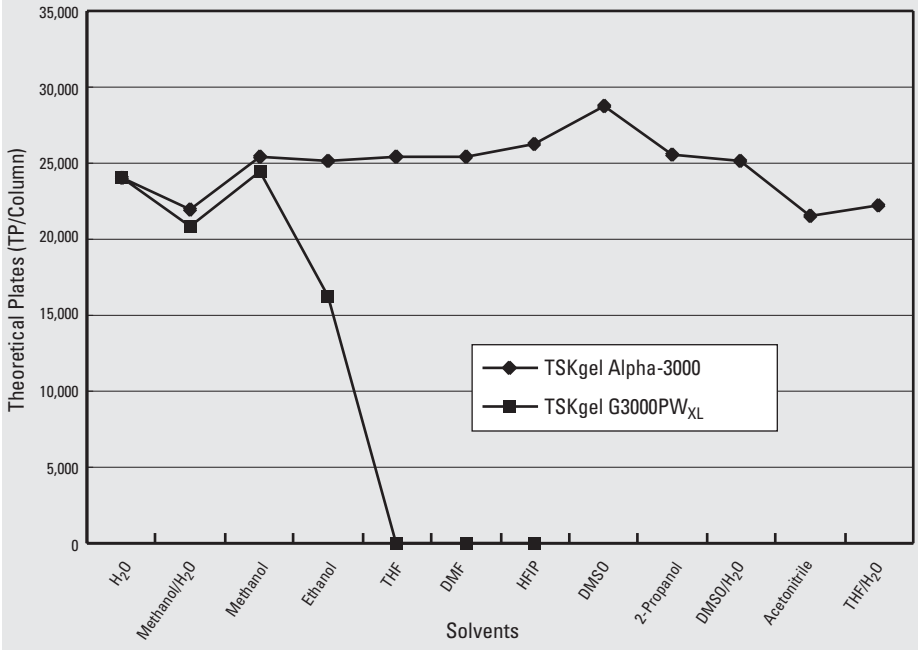
Solvent Compatibility of TSK-GEL SuperAW Series



Column:	TSK-GEL SuperAW Series (6.0 mm ID x 15 cm L)
Eluent:	Water
Flow rate:	0.6 mL/min
Temperature:	25°C
Detection:	Refractive index detector
Sample:	Ethylene glycol
Inj. volume:	5 µL (2.5 g/L)

Figure 34

Solvent compatibility for TSKgel Alpha-3000 for organic solvents



Conditions of solvent change

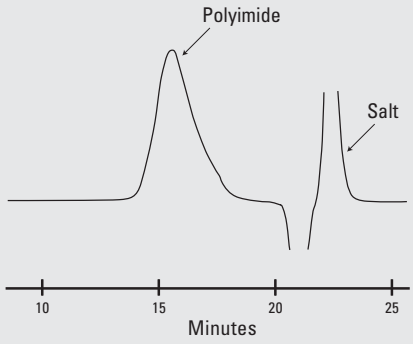
Flow Rate:	1.0 mL/min
Temperature:	25°C
Time for purge:	8h

Conditions for TP measurement

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

Figure 36

Effect of salt peak on chromatogram



Column:	TSKgel Alpha-M, two 7.8 mm ID x 30 cm L columns in series
Sample:	Polyimide
Elution:	60 mM LiBr in DMF
Flow Rate:	1.0 mL/min
Temperature:	40˚C
Detection:	RI

Column Selection

The TSK-GEL Alpha Series consists of six columns with three particle sizes: 7, 10, and 13 µm. These columns span a wide MW separation range from 100 to more than 1 x 10^6 Da when using polyethylene oxide (PEO) as a MW standard. Exclusion limits for the TSK-GEL Alpha columns for PEO in water and polystyrene (PS) in dimethylformamide (DMF) are shown in *Table X*. Calibration curves for the TSK-GEL Alpha Series columns are shown in *Figure 37* for polyethylene oxide, polyethylene glycol and polystyrene standards.

The TSK-GEL SuperAW series contains a similar chemistry as the Alpha series but offers the benefit of smaller particle sizes (4 µm to 9 µm) and smaller column dimensions. Reductions in analysis time and mobile phase consumption result making these columns ideal for high throughput applications. TSK-GEL SuperAW columns are offered in 5 discrete exclusion ranges and 1 mixed bed, polymers up accommodate PEO polymers standards over several million Dalton. (see *Figure 38*)

Table X

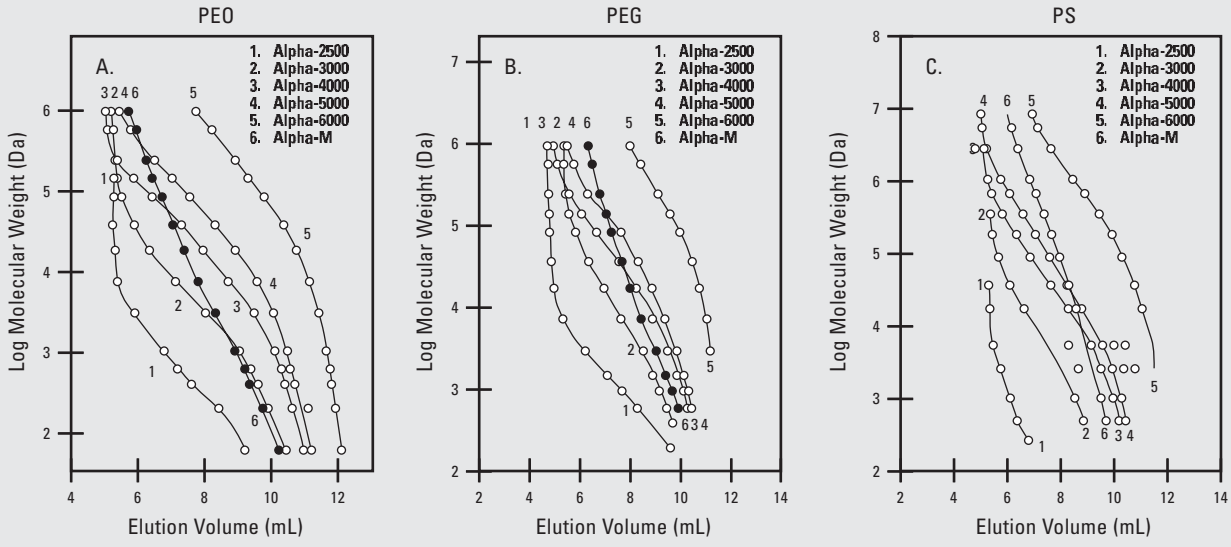
Exclusion limits for TSK-GEL Alpha Series and SuperAW Series columns

Column	***Particle Size (µm)***	***Exclusion limit (Da) for various standards and eluents***		
		PEO[a]/H_2O	*PS[b]/10mM LiBr in DMF*	*PEG[c]/10mM LiBr in MeOH*
Alpha-2500	7	5 x 10^3	1 x 10^4	1 x 10^4
Alpha-3000	7	9 x 10^4	1 x 10^5	6 x 10^4
Alpha-4000	10	4 x 10^5	1 x 10^6	3 x 10^6
Alpha-5000	10	1 x 10^6	7 x 10^6	N.D.
Alpha-6000	13	>1 x 10^7	>1 x 10^7	N.D.
Alpha-M	13	>1 x 10^7	>1 x 10^7	N.D.
SuperAW2500	4	5 x 10^3	8 x 10^3	1 x 10^4
SuperAW3000	4	9 x 10^4	8 x 10^4	1 x 10^5
SuperAW4000	6	1 x 10^6	6 x 10^5	6 x 10^5
SuperAW5000	7	1 x 10^6*	N.D.	N.D.
SuperAW6000	9	1 x 10^7*	N.D.	N.D.
SuperAWM-H	9	1 x 10^7*	N.D.	N.D.

N.D.= not determined a Polyethylene oxide b Polystyrene Divinyl Benzene c Polyethylene glycol
** Exclusion limit for SuperAW5000, SuperAW6000, and SuperAWM-H are estimated, respectively*

Figure 37

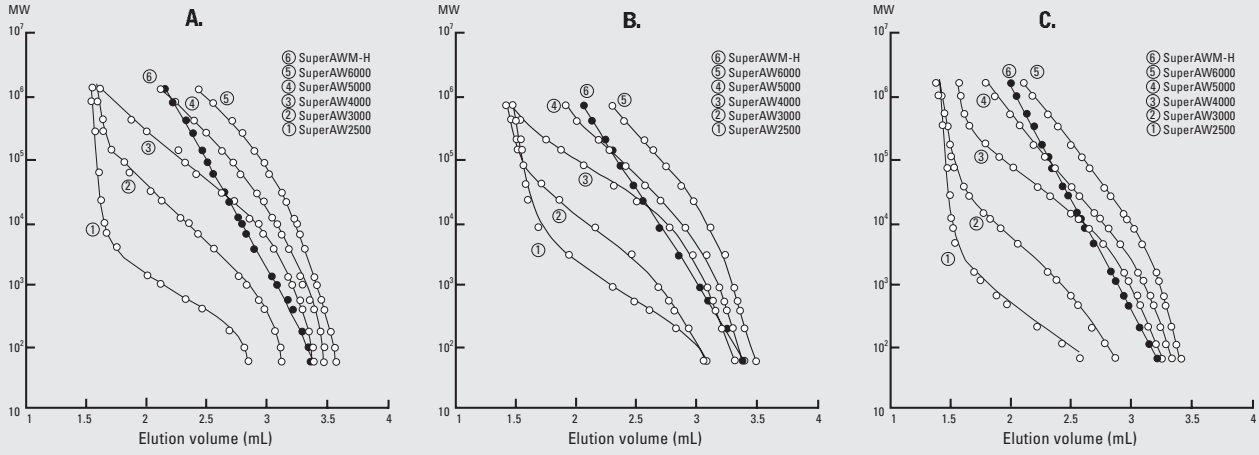
Polyethylene oxide (PEO), polyethylene glycol (PEG) and polystyrene (PS) calibration curves for TSK-GEL Alpha columns



Column: Alpha Series, 7.8 mm ID x 30 cm L
Eluent: A. H_2O; B. 10 mM LiBr in Methanol; C. 10 mM LiBr in DMF
Flow Rate: 1.0 mL/min
Temperature: A. 25°C; B. 25°C; C. 40°C
Detection: RI

Figure 38

Polyethylene oxide (PEO), ploye thylene glycol (PEG) and ethylene glycol (EG) calibration curves for TSK-GEL SuperAW Series



Column: TSK-GEL SuperAW Series (6.0 mm ID x 15 cm L)
Eluent: A. Water; B. MeOH containing 10mM LiBr; C. DMF containing 10 mM LiBr
Flow rate: 0.6 mL/min
Temperature: 25°C
Detection: Refractive index detector
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol

Applications

The versatility of using TSK-GEL Alpha columns with various polar solvents is illustrated in *Figure 39* 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.

The separation of polyvinylalcohol with different degrees of saponification is shown in *Figure 40*. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol mobile phase.

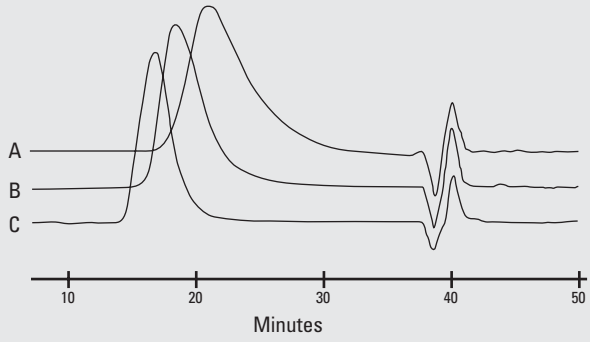
Separation of the components in cleansing gel are shown in *Figure 41*.

A separation of sodium dodecyl sulfate is shown in *Figure 43* on three Alpha columns in series: the TSKgel Alpha-4000, 3000 and 2500. This separation is performed in 50 mM lithium bromide in DMF.

The separation of dextran in *Figure 42* indicates the decreased run time that results when using TSKgel SuperAW2500 in comparison to a conventional 6 µm TSKgel G2500 PW_{XL}.

Figure 40

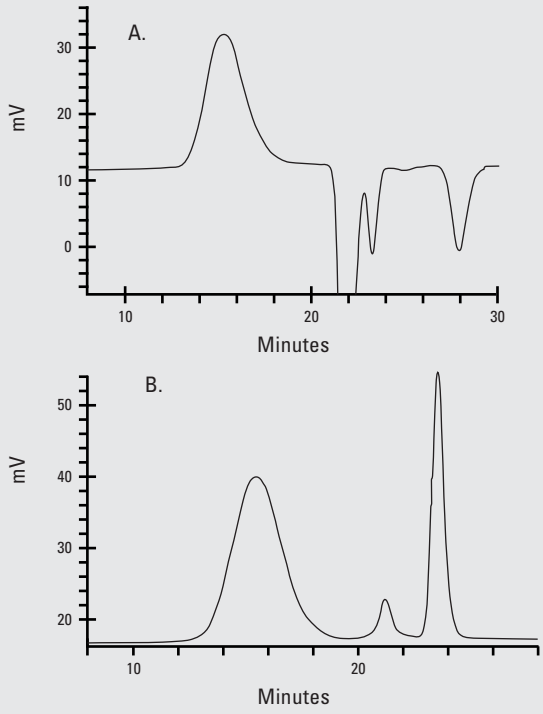
Polyvinylalcohol characterization using TSKgel Alpha-5000 and Alpha-3000 columns in series



Column:	TSKgel Alpha-5000 and Alpha-3000, 7.8 mm ID x 30 cm L in series
Sample:	degree of saponification of polyvinyl alcohol: A. 75%; B. 88%; C. 100%
Eluent:	hexafluoroisopropanol (HFIP)
Flow Rate:	0.5 mL/min
Temperature:	40°C
Detection:	RI

Figure 39

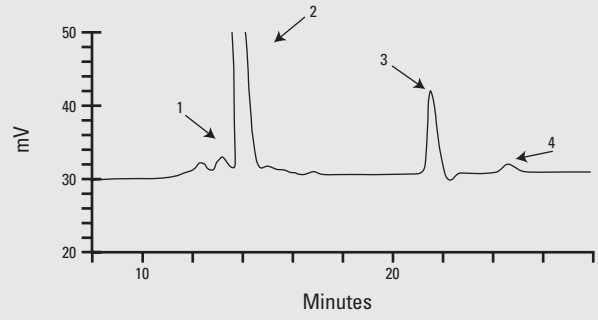
TSKgel Alpha-M separation of cellulose derivatives



Column:	TSKgel Alpha-M, 7.8 mm ID x 30 cm L
Sample:	A. 50 µL ethylcellulose, 0.1%; B. 50 µL ethylhydroxyethylcellulose, 0.1%
Elution:	A. 10 mM LiBr in DMF; B. 10 mM LiBr in methanol
Flow Rate:	0.5 mL/min
Temperature:	40°C
Detection:	RI

Figure 41

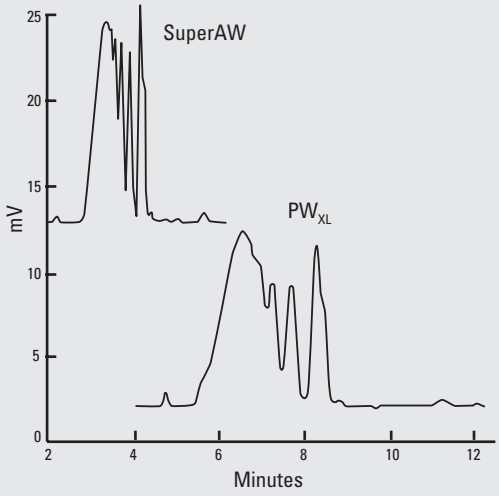
TSKgel Alpha-2500 separation of cleansing gel



Column:	TSKgel Alpha-2500, 7.8 mm ID x 30 cm L
Sample:	cleansing gel: 1. glyceryl monostearate POE (20); 2. glyceryl tri 2-ethylhexanoate; 3. glycerin; 4. sorbitol
Eluent:	methanol
Flow Rate:	0.5 mL/min
Temperature:	40°C
Detection:	RI

Figure 42

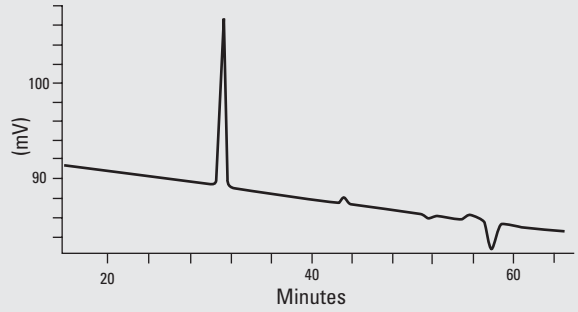
Comparison of TSKgel SuperAW2500 and TSKgel G2500PW$_{XL}$



Column:	TSKgel SuperAW2500 (6.0 mm ID x 15 cm L) TSKgel G2500PW$_{XL}$ (7.8 mm ID x 30 cm L)
Eluent:	Water
Flow rate:	0.6 mL/min (TSKgel SuperAW2500) 1.0 mL/min (TSKgel G2500PW$_{XL}$)
Temperature:	25°C
Detection:	Refractive index detector
Sample:	Dextran T-40 hydrolysate

Figure 43

Separation of sodium dodecyl sulfate



Columns:	(1) TSKgel Alpha-4000, 7.8 mm ID x 30 cm L (2) TSKgel Alpha-3000, 7.8 mm ID x 30 cm L (3) TSKgel Alpha-2500, 7.8 mm ID x 30 cm L in series
Sample:	200 μL of 1.7% sodium dodecyl sulfate
Elution:	50 mM lithium bromide in DMF
Flow Rate:	1.0 mL/min
Detection:	RI

Ordering Information

TSK-GEL Alpha Series and TSK-GEL SuperAW Series

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle Size (μm)*	*Min. Number Theoretical Plates*	*Flow Rate (mL/min) Range*	*Max.*	*Maximum Pressure Drop (kg/cm²)*
Stainless steel columns								
18339	Alpha-2500	7.8	30	7	16,000	0.5 – 0.8	1.0	40
18340	Alpha-3000	7.8	30	7	16,000	0.5 – 0.8	1.0	40
18341	Alpha-4000	7.8	30	10	10,000	0.3 – 0.6	1.0	30
18342	Alpha-5000	7.8	30	10	10,000	0.3 – 0.6	1.0	30
18343	Alpha-6000	7.8	30	13	7,000	0.3 – 0.6	1.0	20
18344	Alpha-M (mixed bed)	7.8	30	13	7,000	0.3 – 0.6	1.0	20
Guard columns								
18345	Alpha Guard column	6	4	13				
Stainless steel columns								
19315	SuperAW2500 -NEW-	6.0	15	4	16,000	0.3 – 0.6	0.6	60
19316	SuperAW3000 -NEW-	6.0	15	4	16,000	0.3 – 0.6	0.6	60
19317	SuperAW4000 -NEW-	6.0	15	6	10,000	0.3 – 0.6	0.6	40
19318	SuperAW5000 -NEW-	6.0	15	7	10,000	0.3 – 0.6	0.6	30
19319	SuperAW6000 -NEW-	6.0	15	9	7,000	0.3 – 0.6	0.6	20
19320	SuperAWM-H -NEW-	6.0	15	9	7,000	0.3 – 0.6	0.6	20
Guard columns								
19321	SuperAW-L Guard Column -NEW-		4.6	3.5	7			
19322	SuperAW-H Guard Column -NEW-		4.6	3.5	23			

Polymer-based TSK-GEL H_{XL}, TSK-GEL H_{HR}, TSK-GEL SuperH, TSK-GEL SuperHZ columns for Gel Permeation Chromatography of organic soluble polymers

Highlights

- -NEW- Reduce solvent consumption during high throughput analysis with narrow bore SuperH (6.0 mm ID) and SuperHZ (4.6 and 6.0 mm ID). *(Figure 45)*
- Porous, highly cross-linked, spherical polystyrene divinylbenzene (PS-DVB) resin.
- Four different TSK-GEL H-type columns are available. Each of these are packed with different particle sizes (*Table XI*).
- H-type packings are available in eight pore sizes.
- Expanded molecular weight ranges with exclusion limits from 1,000 Da to an estimated 4 x 10^8 Da.
- Minimal shrinking and swelling of the column bed.
- Chemically and thermally stable.
- Novel multi-pore distribution in the TSKgel MultiporeH_{XL}-M column provides linear calibration curves over a wider MW range.
- Mixed bed columns with optimized particle and pore sizes to prevent polymer sheering.

Column Selection

TSK-GEL H-type packings are available in eight pore sizes and span four different chemistries. The exclusion limits, pore sizes and application areas for the four chemistries of TSK-GEL H-type columns are listed in *Table XI*. For polymer samples with a broad molecular weight range, packings of several pore sizes are provided in the mixed-bed columns: TSK-GEL SuperHZM series, TSK-GEL SuperHM series, TSKgel GMH_{XL}, TSKgel GMH_{HR} and selected high temperature versions provide linear calibration curves up to several million Daltons. Additionally, TSK-GEL SuperHZM and SuperHM series are offered in several particle sizes appropriately matched to the molecular weight of the polymer sample in order to prevent sheering during the analysis. The "Super" prefix refers to the efficiency of the column. The super series columns contain ultra efficient particles as low as 3 µm, housed in 15 cm length columns. The smaller particle allows for equivalent resolution to conventional H_{XL} columns, with 50% less run time due to the shorter column length. The super series columns are an excellent choice for high throughput polymer analysis. Calibration curves for all four H-type columns are provided in *Figures 46, 47, 48, 49*. A comparison of the 3µm TSKgel SuperHZ columns to conventional larger particle TSKgel H_{XL} is shown in *Figure 44*.

Table XI

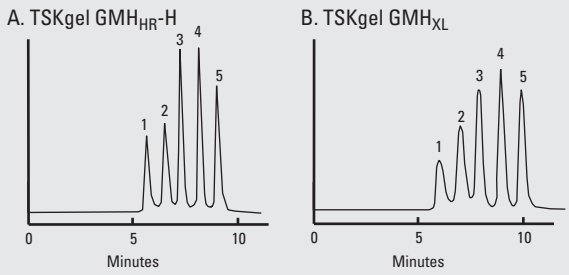
Comparison of TSK-GEL H-type resins

Series Type	SuperHZ	H_{XL}	SuperH	H_{HR}
Application focus	High-throughput polymer analysis with ultra low polymer adsorption, limited solvent compatibility range.	Conventional polymer analysis with ultra low polymer adsorption, limited solvent compatibility range.	High-throughput polymer analysis with expanded solvent compatibility.	Conventional polymer analysis with expanded solvent compatibility range.
Particle size	3, 5 and 10 µm, depending on pore size	5 and 9 µm, depending on pore size	3 and 5 µm, depending on pore size	5 µm
Theoretical plates[1]	16,000/15 cm column	16,000/30 cm column	16,000/15 cm column	16,000/30 cm column
Maximum temperature	G1000 - G4000 60°C G5000 - mixed 80°C	G1000 - G4000 60°C G5000 - mixed 80°C	140°C	140°C
Standard shipping solvent	THF	THF[2]	THF[2]	THF[2]
THF can be switched to	benzene, chloroform, toluene, xylene, dichloromethane[3] and dicholoroethane[3]		see *Table XII (page 61)*	
Other shipping solvents available?	yes[4]		no	
Number of solvent substitutions	One time only		Several[5]	
Solvent exchange instructions	Linear gradient with a 2%/min rate of change with a flow rate <0.25 mL/min.	Linear gradient with a 2%/min rate of change with a flow rate <0.5 mL/min.	Linear gradient with a 2%/min rate of change according to flow rates listed in *Table XIII*	

1) Theoretical plates listed are based on smallest particle size listed
2) High-temperature columns (HT) are shipped with ODCB (Orthochlorodivinylbenzene) as standard shipping solvent.
3) Dichloromethane and Dichloroethane are not available for G1000 pore size columns.
4) See Table XII for available shipping solvents
5) After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is not recommended.

Figure 44

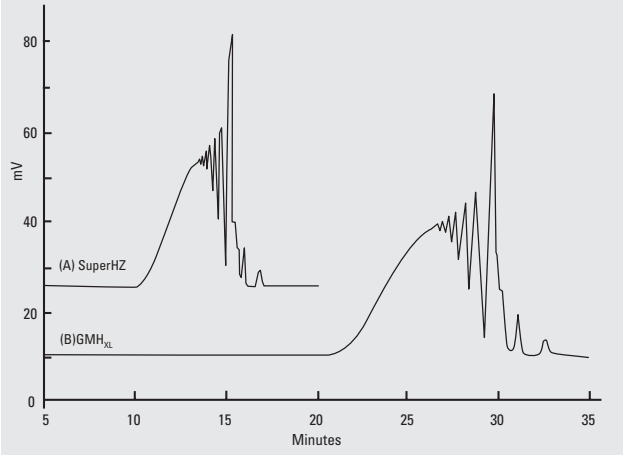
Comparison of resolution on TSKgel H_{HR} and H_{XL} columns



Column:	A: TSKgel GMH_{HR}-H, 7.8 mm ID x 30 cm L B: TSKGgel GMH_{XL}, 7.8 mm ID x 30 cm L
Sample:	polystyrene standards 5 μL
Elution:	THF
Temperature:	25°C
Detection:	UV @ 254 nm

Figure 45

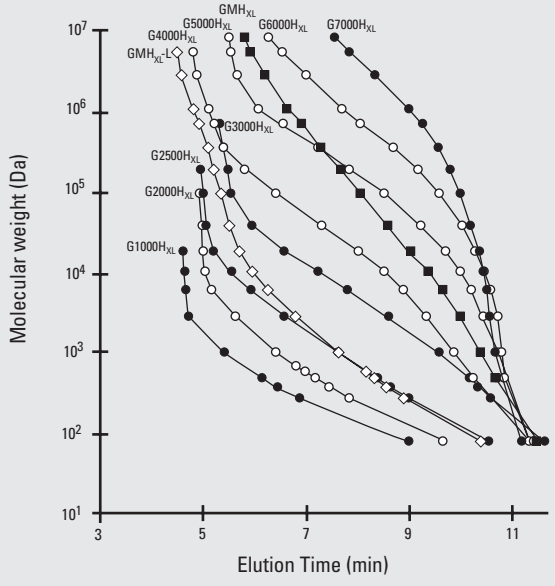
Comparison between SuperHZ and GMH_{XL}



Columns:	(A) TSKgel SuperHZ (4000, 3000, 2500) (4.6 mm ID x 15cm L) (B) TSKgel GMH_{XL} (4000, 3000, 2500) (7.8 mm ID x 30 cm L)
Eluent:	THF
Flow rate:	(A) 0.35 mL/min (B) 1.0 mL/min
Temperature:	40°C
Detection:	RI
Sample:	Phenolic resin
Inj. volume:	(A) 5 μL, (B) 30 μL

Figure 46

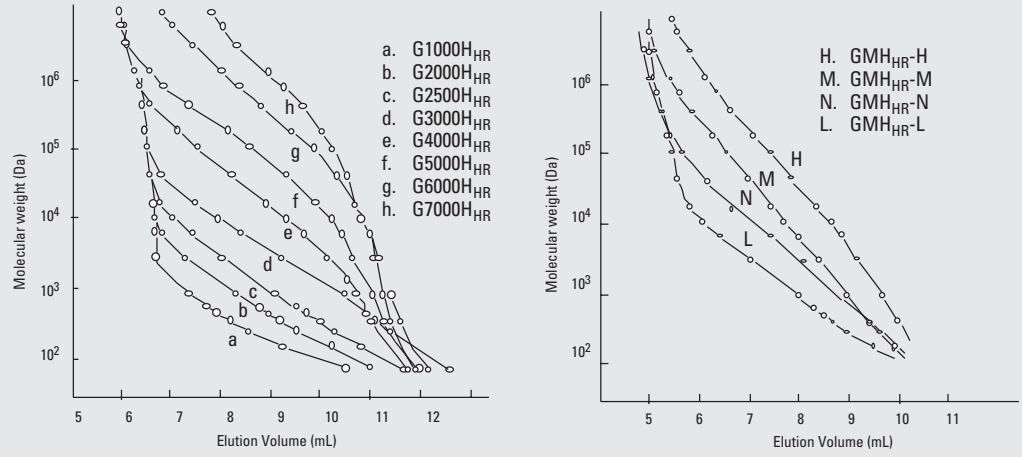
Calibration curves for TSKgel H_{XL} columns with polystyrene standards



Column size:	7.8 mm ID x 30 cm L
Sample:	polystyrene standards
Eluent:	THF
Flow Rate:	1.0 mL/min
Temperature:	25°C
Detection:	UV @ 254 nm

Figure 47

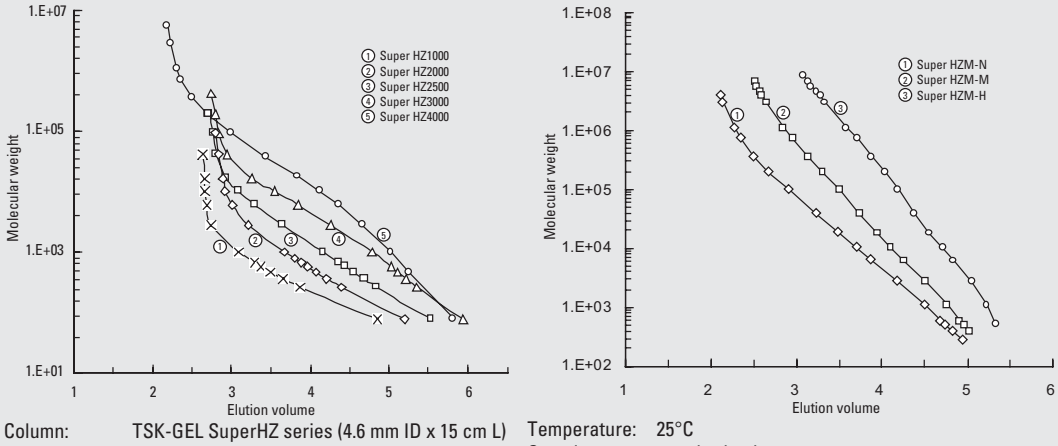
Calibration curves for TSKgel H_{HR} columns with polystyrene standards



Column:	TSKgel H_{HR} series (7.8 mm ID x 30 cm L)	Flow Rate:	1.0 mL/min
Sample:	polystyrene standards	Temperature:	25°C
Elution:	THF	Detection:	UV @ 254 nm

Figure 48

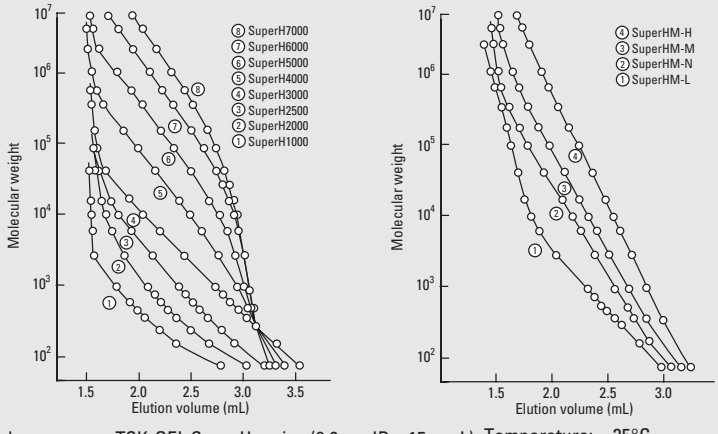
Calibration curves for TSK-GEL SuperHZ columns with polystyrene standards



Column:	TSK-GEL SuperHZ series (4.6 mm ID x 15 cm L)	Temperature:	25°C
Eluent:	THF	Sample:	standard polystyrene
Flow rate:	0.35 mL/min	Inj. volume:	2 μL

Figure 49

Calibration curves for TSK-GEL SuperH columns with polystyrene standards



Column:	TSK-GEL SuperH series (6.0 mmID x 15 cm L)	Temperature:	25°C
Eluent:	THF	Detection:	UV@254 nm
Flow rate:	0.6 mL/min	Sample:	polystyrene standards

Table XII

Solvent compatibility for TSK-GEL H-type columns

The column shipping solvent limits the types of solvents that can be used with each column type.

Column type	Shipping solvent *	Can be replaced with
SuperHZ and H_{XL}[1]	Tetrahydrofuran [3,4]	benzene, chloroform, toluene, xylene, dichloromethane, dichloroethane
	Acetone**	carbon tetrachloride[5], *o*-dichlorobenzene, dimethylformamide, dodecane, dimethyl sulfoxide, dioxane, ethylacetate, FC-113, hexane, pyridine, hexafluoroisopropanol/chloroform, methyl ethyl ketone, quinoline, cyclohexane,
	Chloroform**	*m*-cresol in chloroform, up to 10% hexafluoroisopropanol/chloroform
	Dimethylformamide**	dimethyl sulfoxide, dioxane, tetrahydrofuran, toluene
	o-Dichlorobenzene**	1-chloronaphthalene, trichlorobenzene
SuperH and H_{HR}[2]	Tetrahydrofuran [3]	acetone, decahydronaphthalene, ethanol, quinoline, benzene, *o*-dichlorobenzene, ethyl acetate, tetrafluoroethylene, carbon tetrachloride[5], dichloromethane, n-hexane, tetrahydrofuran, chloroform, 1,4-dioxane, hexafluoroisopropanol, toluene, 1-chloronaphthalene, dimethylacetoacetamide, methyl ethyl ketone, trichlorobenzene, *m*-cresol, dimethylformamide, -methylpyrrolidone, *o*-chlorophenol/chloroform, methyl sulfoxide, pyridine

Notes:

1 In case of SuperHZ and H_{XL}, keep flow rate below <0.5 mL/min during solvent change. Solvent can be changed one way/one time only.

2 In case of SuperH and H_{HR}, see table XIII for appropriate flow rates for solvent exchange. After switching to a very polar solvent, switching to a nonpolar is not recommended.

3 All TSK-GEL H_{XL}, H_{HR}, SuperHZ, SuperH and GMH analytical columns are shipped containing tetrahydrofuran (THF), except GMH-HT columns, which contain o-dichlorobenzene.

4 THF in G1000H_{XL} columns cannot be replaced with dichloromethane or dichloroethane.

5 Prolonged exposure to carbon tetrachloride can corrode the stainless steel parts of a column and an HPLC system.

** 100% methanol cannot be used with H-type columns; use this solvent with TSK-GEL SW-type columns or Alpha-type columns.*

*** TSK-GEL H columns may be specially ordered with this shipping solvent.*

Table XIII

Recommended flow rates for solvent exchange in SuperH and H_{HR} columns

Solvent	Flow Rate (mL/min)	
	SuperH	H_{HR}
	6.0 mm ID x 15 cm L	7.8 mm ID x 30 cm L
n-Hexane	0.5	0.9
methyl ethyl ketone	0.4	0.7
dichloromethane, ethyl acetate	0.35	0.6
toluene, chloroform	0.3	0.5
dimethylformamide	0.2	0.4
carbon tetrachloride, pyridine	0.15	0.3
dimethyl sulfoxide, dioxane, ethanol, N-methylpyrrolidone, *o*-dichlorobenzene	0.1	0.2
quinoline, hexafluoroisopropanol, 1-chloronaphthalene	0.05	0.1

The TSKgel MultiporeH$_{XL}$-M column offers a unique packing material and new strategy for the precise analysis of polymers by Gel Permeation Chromatography (GPC). Until now, the GPC separation of a sample containing a wide range of molecular weight polymers was performed by one of two strategies. One strategy combines columns with different pore sizes of packing material in series. The other strategy employs a single column with a blend of different pore sizes packing materials, commonly referred to as a mixed bed. Mixed bed columns do not always provide linear calibration curves, which may result in broad or split peaks. With the introduction of the TSKgel MultiporeH$_{XL}$-M column, a new strategy has been developed using a single column containing a novel polystyrene packing material with a multi-pore size distribution. *Figure 50* gives an illustration of the three strategies. A scanning electron microscope photograph of the TSKgel Multipore column in *Figure 51* shows several different pore sizes with continuous distribution in every bead. This results in sharper peaks without inflections that may be observed using mixed-bed columns.

The pore size distributions of the TSKgel MultiporeH$_{XL}$-M column and a mixed-bed column are shown in *Figure 52* . The mixed-bed column shows a sharp maximum for pores with a diameter of 0.08 µm, though the overall pore size distribution ranges from 0.006 to 0.6 µm in diameter. In the case of the TSKgel MultiporeH$_{XL}$-M column, the pore size distribution exhibits a wider maximum range from 0.02 to 0.1 µm in diameter. This difference in pore size distribution may explain the reason for the inflection phenomenon. A comparison of calibration curves for polystyrene standards on the TSKgel MultiporeH$_{XL}$-M column, the TSKgel GMH$_{HR}$-H and PLgel Mixed-C, are shown in *Figure 53*. Both the TSKgel GMH$_{HR}$-H and PLgel Mixed-C columns are mixed-bed columns.

Figure 50

Strategies for wide range separation using Size Exclusion Chromatography

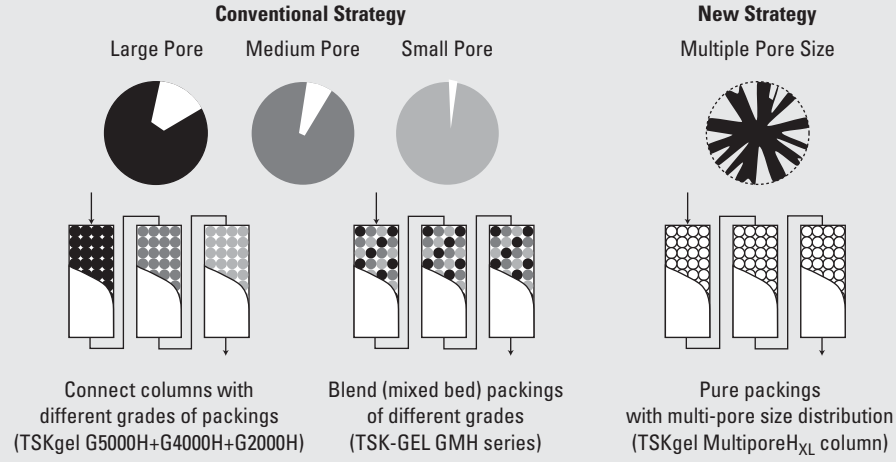


Figure 51

SEM photograph of the TSKgel multipore packing

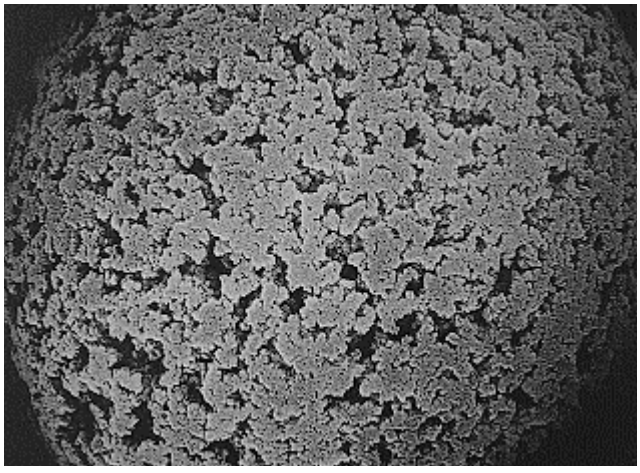


Figure 52

Pore size distribution of TSKgel MultiporeH$_{XL}$-M column and a mixed-bed column

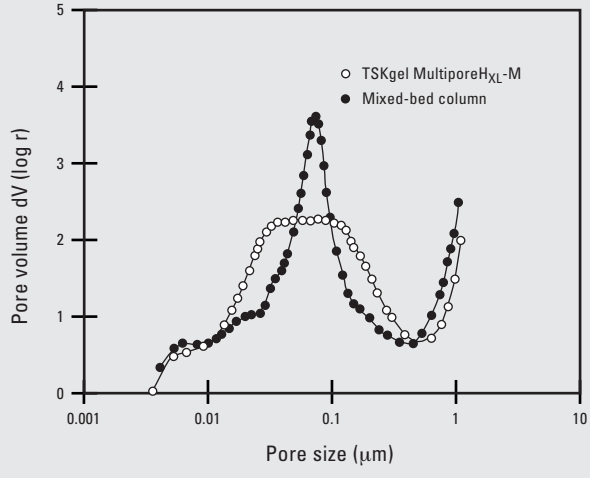
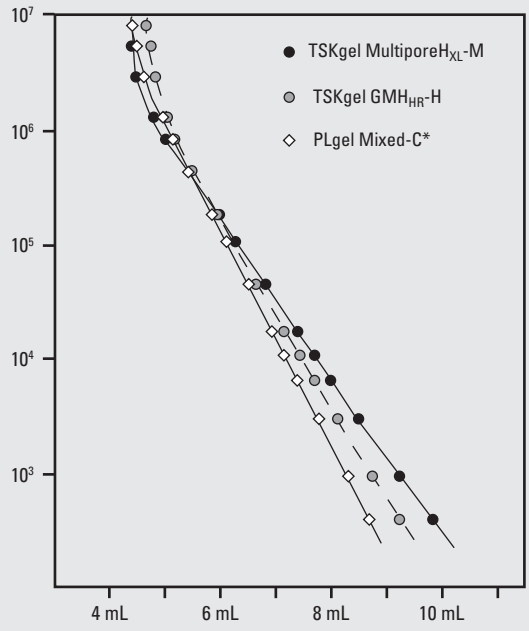


Figure 53

Calibration curves for Multipore and mixed-bed columns



Column: TSKgel Multipore H_{XL}-M, 7.8 mm ID x 30 cm L;
TSKgel GMH_{HR}-H, 7.8 mm ID x 30 cm L;
PLgel Mixed-C, 7.5 mm ID x 30 cm L
Sample: polystyrene standards
Elution: THF
Flow Rate: 1.0 mL/min
Temperature: 40˚C
Detection: UV @ 254 nm
*PLgel Mixed-C is a product from Polymer Laboratories Ltd.

Optimizing GPC with TSK-GEL H-type columns

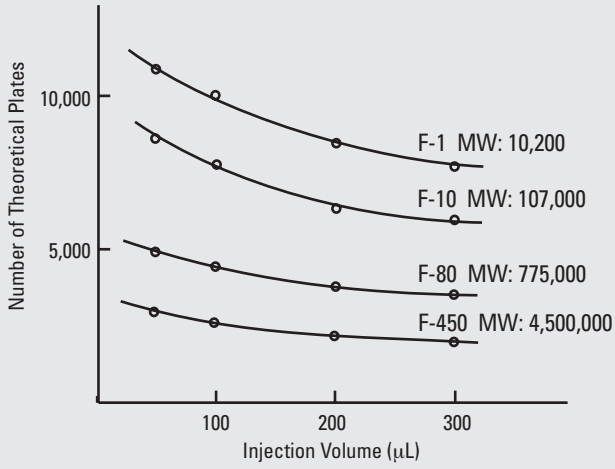
Sample loading

In general, column efficiency improves with decreasing sample concentration and decreasing sample volume. When particle size decreases, all other conditions constant, these trends are more pronounced. Similarly, reducing column volume by either reducing column length or column diameter also results in reduced sample volumes and concentrations at which efficiency starts to deteriorate.

Efficiency decreases rapidly as the injection volume increases from 100 µL to 300 µL for polystyrene with MW <110,000 Da as seen with the mixed bed TSKgel GMH_{XL} in *Figure 54.* Sample concentrations from 0.001-0.5 mg can be loaded on the 7.5 or 7.8 mm ID x 30 cm L column for an analytical separation *(Figure 55).* The smaller, more efficient particles in the Super series columns are more sensitive to injection volumes and sample concentrations. *Figure 56* shows that injection volumes of 1 µL to 10 µL are recommended for TSKgel SuperHZ2500. Recommended sample concentrations vary dependant on sample molecular weight. For polymers below $1x10^6$ Da concentrations can range up to 1 g/L. Larger polymers should not exceed 0.1 g/L to 0.5 g/L according to *Figure 57.*

Figure 54

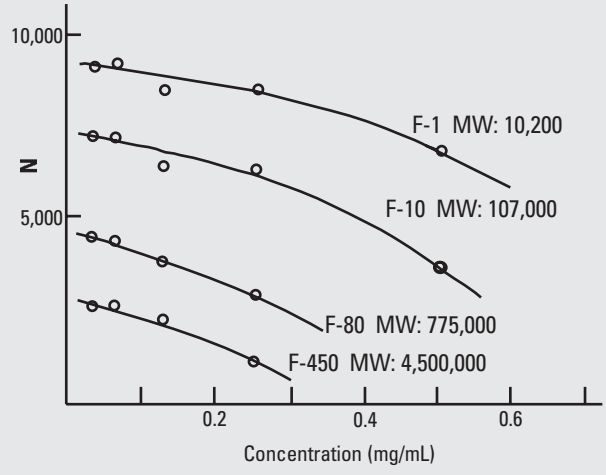
Dependence of the number of theoretical plates (N) on injection volume in the separation of polystyrene on TSKgel GMH_{XL}



Column: TSKgel GMH_{XL}, 7.8 mm ID x 30 cm L
Sample: polystyrene, F-1, 10, 80, 450 (0.125 mg/mL)
Elution: THF
Flow Rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 40˚C

Figure 55

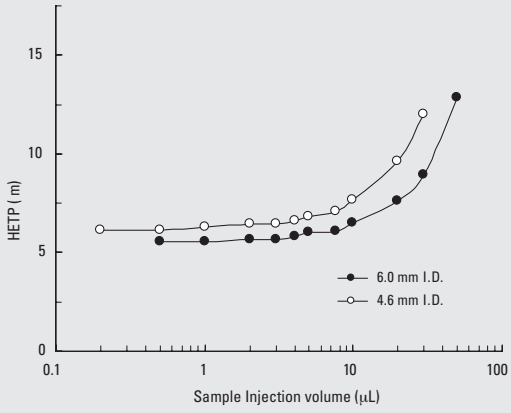
Dependence of the number of theoretical plates (N) on sample concentration in the separation of polystyrene on TSKgel GMH_{XL}



Column: TSKgel GMH_{XL}, 7.8 mm ID x 30 cm L
Sample: polystyrene, F-1, 10, 80, 450 (0.125 mg/mL)
Elution: THF
Flow Rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 40˚C

Figure 56

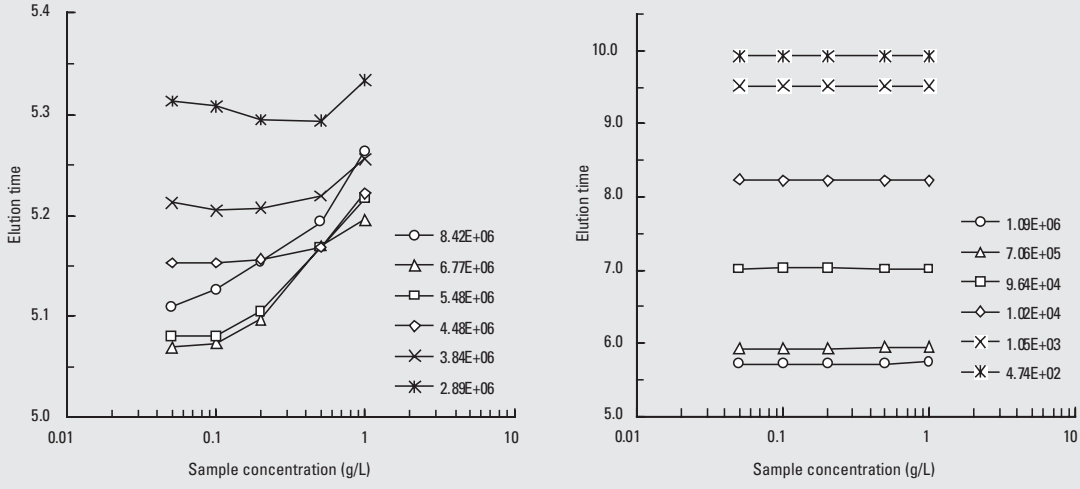
Relationship between HETP and Sample injection volume in SuperHZ2500



Column:	TSKgel SuperHZ2500 6.0 mm ID x 15 cm L, 4.6 mm ID x 15 cm L
Eluent:	THF
Flow rate:	0.6 mL/min (6.0 mm ID x 15 cm L), 0.35 mL/min (4.6 mm ID x 15 cm L)
Temperature:	25°C
Sample:	DCHP

Figure 57

Effect of sample concentration on elution time with SuperHZ-M



Column:	TSKgel SuperHZM-M (4.6 mm ID x 15 cm L x 2)
Eluent:	THF
Flow rate:	0.35 mL/min
Temperature:	40°C
Detection:	RI
Sample:	Standard polystyrene
Injection vol:	10 μL

Applications for TSK-GEL H-type columns

Many industrial applications detailing analyses of organic polymers have been developed with TSK-GEL H-type columns. *Table XV* gives examples of the recommended solvent for each application.

Table XV

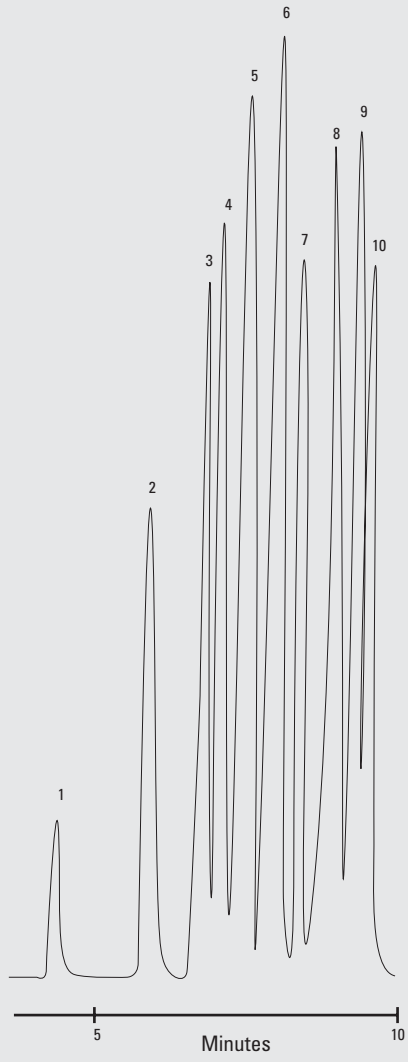
Recommended solvent	Application
THF	polystyrene, epoxy resin, phenoxy resin, polycarbonate, polyisoprene, polyvinyl acetate, polyvinyl chloride, monoglycerides, fatty acids, poly (butadiene-as) {as resin}, polybutadiene, poly (methyl methacrylate), poly (styrene-butadiene), poly (styrene-acrylonitrile) {as resin}
N,N-Dimethylformamide (DMF) +/- 5mM LiBr	polyvinyl chloride, polyvinyl fluoride, urea resins, polyurethane, polystyrene, polyester, polyimido ether, polyimido ester, polyphenol (aqueous solution), polyacrylonitrile
o-Dichlorobenzene (ODCB)	polyethylene, polypropylene
Chloroform	polycarboxylic ether, acrylic resin, epoxy resin, polystyrene
m-Cresol/Chloroform	nylon, polyester, polyamide, poly (ethylene terephthalate)
Toluene	polybutadiene, polysiloxane

Phthalate esters

Figure 58 demonstrates the high resolving power of a TSKgel G1000H$_{XL}$ column for low molecular weight phthalate esters. Resolution was close to baseline, even though the molecular weights of the esters differed by less than 50 Da.

Figure 58

High resolution of phthalate esters on TSKgel G1000H$_{XL}$



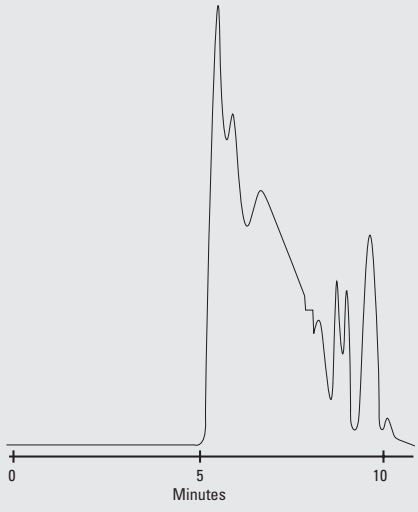
Column: TSKgel G1000H$_{XL}$, 7.8 mm ID x 30 cm L
Sample: 1. polystyrene (10,200 Da), 2. dioctylphthalate (391 Da), 3. dibutylphthalate (278 Da), 4.dipropylphthalate (250 Da), 5. diethylphthalate (222 Da), 6. dimethylphthalate (194 Da), 7. n-propylbenzene (120 Da), 8. ethylbenzene (116 Da), 9. toluene (92 Da), 10. benzene (78 Da)
Elution: THF
Flow Rate: 1.0 mL/min
Detection: UV @ 254 nm

Phenol resin

The TSKgel GMH_{XL}-L column has been designed to provide a complete profile for high molecular weight samples that contain low molecular weight additives. The calibration curve for this mixed-bed column is shallow in the low molecular weight range of oligomers. Sample adsorption is not observed. For example, the complete profile of a phenol resin, with high resolution of the low molecular weight components, is shown in *Figure 59*. Other applications for the TSKgel GMH_{XL}-L column include analyses of paint materials, bond and adhesive components, and synthetic polymer additives.

Figure 59

Separation of phenol resin on TSKgel GMH_{XL}-L



Column:	TSKgel GMH_{XL}-L, 7.8 mm ID x 30 cm L
Sample:	phenol resin
Elution:	THF
Flow Rate:	1.0 mL/min
Detection:	UV @ 254 nm

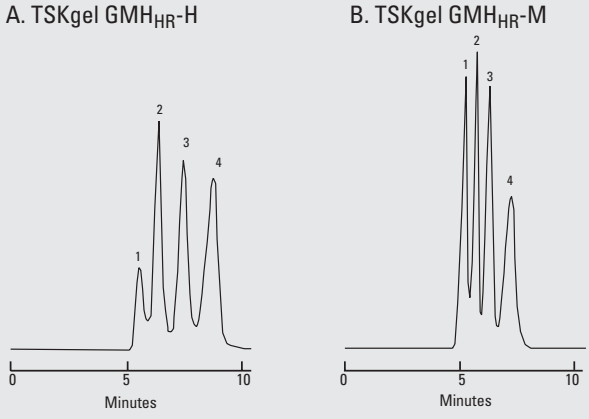
Polymethylmethacyrlate

The effect of different pore size distributions in the mixed beds of TSKgel GMH_{HR}-H and TSKgel GMH_{HR}-M is illustrated in *Figure 60*. The TSKgel GMH_{HR}-M produces sharper peaks in the 8×10^5 to 1×10^4 Da range.

Figure 61 shows two TSKgel Super HZM columns in series for the separation of polymethyl methacrylate. A low dead volume HPLC system as well as small sample volume and concentration are required to achieve optimum efficiency using the 4.6 mmID columns.

Figure 60

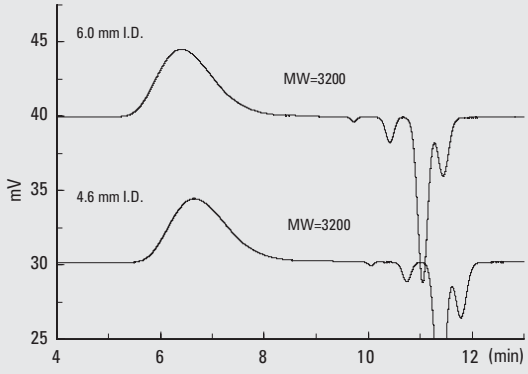
Comparison of TSKgel GMH_{HR}-H and -M columns with polymethylmethacrylate standards



Column:	A. TSKgel GMH_{HR}-H, 7.8 mm ID x 30 cm L; B. TSKgel GMH_{HR}-M, 7.8 mm ID x 30 cm L
Sample:	polymethylmethacrylate: 1. 820,000 Da, 2. 67,000 Da, 3. 10,200 Da, 4. 1,950 Da
Solvent:	5 mM sodium trifluoroacetate in hexafluoroisopropanol
Flow Rate:	1.0 mL/min
Detection:	UV @ 220 nm
Temperature:	40°C

Figure 61

Chromatogram of polymethyl methacrylate



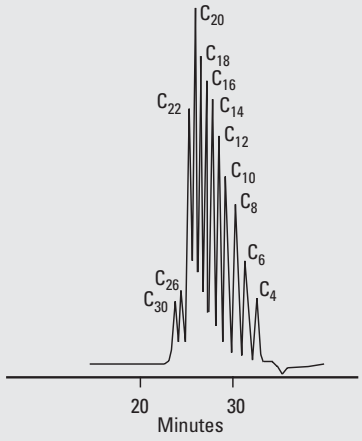
Column:	TSKgel SuperHZM-M x 2 (15 cm L each)
Eluent:	THF
Flow rate:	0.35 mL/min (4.6 mm ID) 0.6 mL/min (6.0 mm ID)
Temperature:	40°C
Detection:	RI
Sample:	Polymethyl methacrylate (1 g/L)
Inj. volume:	5 µL (4.6 mm ID) 9 µL (6.0 mm ID)

Fatty acids

In *Figure 62*, two TSKgel G2000H_{XL} columns in series separate a mixture of fatty acids ranging from C_4 to C_{30}.

Figure 62

Separation of fatty acids



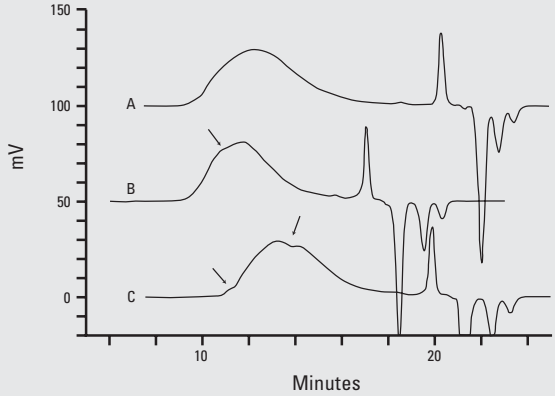
Column:	TSKgel G2000H_{XL}, two 7.8 mm ID x 30 cm L in series
Sample:	fatty acids
Elution:	THF
Flow Rate:	1.0 mL/min
Detection:	RI

Acrylic polymer

Figure 63 shows the separation of an acrylic polymer on the TSKgel MultiporeH_{XL}-M column compared with two commercially available mixed-bed columns. The arrows illustrate the inflections seen in the chromatographic separations on mixed-bed columns and the improvement achieved when using the TSKgel MultiporeH_{XL}-M column. For faster run times, the TSKgel SuperHZM can perform the separation in under 10 minutes *(Figure 65)*.

Figure 63

Separation of acrylic resin by size exclusion chromatography on TSKgel MultiporeH_{XL}-M and mixed-bed type columns



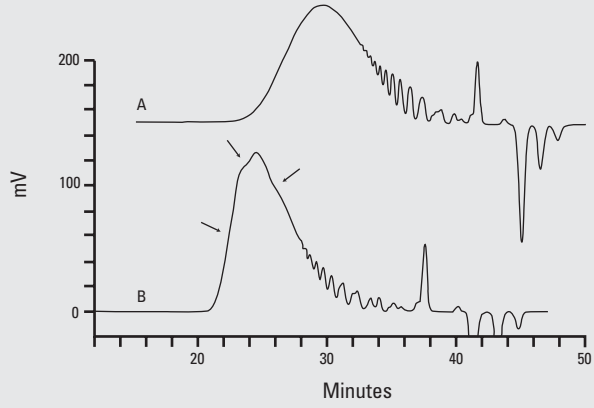
Column:	A. TSKgel MultiporeH_{XL}-M, two 7.8 mm ID x 30 cm L columns in series; B. Competitor P, two 7.5 mm ID x 30 cm L columns in series, mixed-bed type; C. Competitor S, two 8.0 mm ID x 30 cm L columns in series, mixed-bed type
Sample:	acrylic polymer (0.1%, 50 µL)
Elution:	THF
Flow Rate:	1.0 mL/min
Temperature:	40°C
Detection:	RI

Epoxy resin

Figure 64 compares the separation of epoxy resin on the TSKgel MultiporeH_{XL}-M and TSK-GEL H_{XL} columns. The multi-pore size distribution of the TSKgel MultiporeH_{XL}-M column gives a broad range of separation and linearity. Four TSK-GEL H_{XL} columns with different pore sizes are required to achieve the same broad range of separation. Note the appearance of the inflections in the chromatogram when using the TSK-GEL H_{XL} columns.

Figure 64

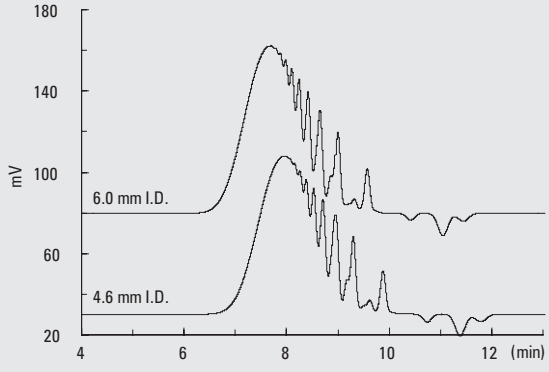
Comparison of epoxy resin separation on TSKgel MultiporeH_{XL}-M and conventional TSK-GEL H_{XL} columns



Column:	A. TSKgel MultiporeH_{XL}-M, four 7.8 mm ID x 30 cm L col. in series; B. TSKgel G4000H_{XL}+ G3000H_{XL} + G2500H_{XL} + G2000H_{XL}, four 7.8 mm ID x 30 cm L columns in series
Sample:	Epoxy resin, 50 µL
Elution:	THF
Flow Rate:	1.0 mL/min
Temperature:	40°C
Detection:	RI

Figure 65

Chromatogram of epoxy resin



Column:	TSKgel SuperHZM-M x 2
Eluent:	THF
Flow rate:	0.35 mL/min (4.6 mm ID) 0.6 mL/min (6.0 mm ID)
Temperature:	40°C
Detection:	RI
Sample:	Epoxy resin (10 g/L)
Inj. volume:	5 µL (4.6 mm ID) 9 µL (6.0 mm ID)

Ordering Information

Analytical and preparative TSK-GEL GPC column products: typical properties

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Min. Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
17352	G1000H_{HR}	7.8	30	5	16,000	0.5 – 1.0	2.0	50
17353	G2000H_{HR}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17354	G2500H_{HR}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17355	G3000H_{HR}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17356	G4000H_{HR}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17357	G5000H_{HR}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17358	G6000H_{HR}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17359	G7000H_{HR}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17362	GMH_{HR}-L mixed-bed	7.8	30	5	16,000	0.5 – 1.0	1.0	50
18055	GMH_{HR}-N mixed-bed	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17392	GMH_{HR}-M mixed-bed	7.8	30	5	16,000	0.5 – 1.0	1.0	50
17360	GMH_{HR}-H mixed-bed	7.8	30	5	16,000	0.5 – 1.0	1.0	50
16131	G1000H_{XL}	7.8	30	5	16,000	0.5 – 1.0	1.0	50
16134	G2000H_{XL}	7.8	30	5	16,000	0.5 – 1.0	1.2	50
16135	G2500H_{XL}	7.8	30	5	16,000	0.5 – 1.0	1.2	50
16136	G3000H_{XL}	7.8	30	6	16,000	0.5 – 1.0	1.2	35
16137	G4000H_{XL}	7.8	30	6	16,000	0.5 – 1.0	1.2	35
16652	GMH_{XL}-L mixed-bed	7.8	30	6	16,000	0.5 – 1.0	1.2	35
16138	G5000H_{XL}	7.8	30	9	14,000	0.5 – 1.0	1.2	15
16139	G6000H_{XL}	7.8	30	9	14,000	0.5 – 1.0	1.2	15
16140	G7000H_{XL}	7.8	30	9	14,000	0.5 – 1.0	1.2	15
16141	GMH_{XL} mixed-bed	7.8	30	9	14,000	0.5 – 1.0	1.2	15
07112	GMH_{XL}-HT	7.8	30	13	5,500	0.5 – 1.0	1.2	15
18403	Multipore H_{XL}-M	7.8	30	5	16,000	0.5 – 1.0	1.0	35
17990	TSKgel SuperH1000 -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	70
17991	TSKgel SuperH2000 -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	60
17992	TSKgel SuperH2500 -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	60
17993	TSKgel SuperH3000 -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	40
17994	TSKgel SuperH4000 -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	40
17995	TSKgel SuperH5000 -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	40
17996	TSKgel SuperH6000 -NEW-	6.0	15	5	10,000	0.3 – 0.6	0.8	30
17997	TSKgel SuperH7000 -NEW-	6.0	15	5	10,000	0.3 – 0.6	0.8	30
17998	TSKgel SuperHM-L -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	60
17999	TSKgel SuperHM-N -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	40
18000	TSKgel SuperHM-M -NEW-	6.0	15	3	16,000	0.3 – 0.6	0.8	40
18001	TSKgel SuperHM-H -NEW-	6.0	15	3 and 5	16,000	0.3 – 0.6	0.8	40

Ordering Information

Analytical and preparative TSK-GEL GPC column products: typical properties (continued)

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle Size (µm)*	*Min. Number Theoretical Plates*	*Flow Rate (mL/min) Range*	*Max.*	*Maximum Pressure Drop (kg/cm²)*
Stainless steel columns								
19309	TSKgel SuperHZ1000 -NEW-	4.6	15	3	16,000	0.15 – 0.35	0.4	56
19302	TSKgel SuperHZ1000 -NEW-	6.0	15	3	16,000	0.25 – 0.6	0.7	56
19310	TSKgel SuperHZ2000 -NEW-	4.6	15	3	16,000	0.15 – 0.35	0.4	50
19303	TSKgel SuperHZ2000 -NEW-	6.0	15	3	16,000	0.25 – 0.6	0.7	50
19311	TSKgel SuperHZ2500 -NEW-	4.6	15	3	16,000	0.15 – 0.35	0.4	40
19304	TSKgel SuperHZ2500 -NEW-	6.0	15	3	16,000	0.25 – 0.6	0.7	40
19312	TSKgel SuperHZ3000 -NEW-	4.6	15	3	16,000	0.15 – 0.35	0.4	30
19305	TSKgel SuperHZ3000 -NEW-	6.0	15	3	16,000	0.25 – 0.6	0.7	30
19313	TSKgel SuperHZ4000 -NEW-	4.6	15	3	16,000	0.15 – 0.35	0.4	35
19306	TSKgel SuperHZ4000 -NEW-	6.0	15	3	16,000	0.25 – 0.6	0.7	35
19960	TSKgel SuperHZM-N -NEW-	4.6	15	3	16,000	0.15 – 0.35	0.4	35
19961	TSKgel SuperHZM-N -NEW-	6.0	15	3	16,000	0.25 – 0.6	0.7	35
19962	TSKgel SuperHZM-M -NEW-	4.6	15	3 and 5	16,000	0.15 – 0.35	0.4	20
19963	TSKgel SuperHZM-M -NEW-	6.0	15	3 and 5	16,000	0.25 – 0.6	0.7	20
19964	TSKgel SuperHZM-H -NEW-	4.6	15	10	9,000	0.15 – 0.35	0.4	10
19965	TSKgel SuperHZM-H -NEW-	6.0	15	10	9,000	0.25 – 0.6	0.7	10
Guard columns								
18404	MultiporeH_{XL}-M Guard column	6.0	4.0	5	For P/N 18403			
07113	H_{XL}-L Guard column	6.0	4.0	7	For G1000H_{XL} - G4000H_{XL}, GMH_{XL}-L			
13727	H_{XL}-H Guard column	6.0	4.0	13	For G5000H_{XL} - G7000H_{XL} + GMH_{XL}			
17368	Guard column H_{HR}-L and H_{HR}	6.0	4.0	5	For G1000-4000H_{HR} and P/N 17362			
17369	Guard column H_{HR}-H and H_{HR}	6.0	4.0	5	For G5000-7000H_{HR} and P/Ns 18055, 17392 and 17360			
19314	SuperHZ Guard Column -NEW-	4.6	2.0	4	For 4.6 mm ID columns of SuperHZ1000-4000 and HZM-N &-M			
19668	SuperHZ Guard Column -NEW-	4.6	2.0	10	For 4.6 mm ID columns of SuperHZM-H			
19666	SuperHZ Guard Column -NEW-	4.6	3.5	4	For 6.0 mm ID columns of SuperHZ1000-4000 and HZM-N &-M			
19667	SuperHZ Guard Column -NEW-	4.6	3.5	10	For 6.0 mm ID columns of SuperHZM-H			