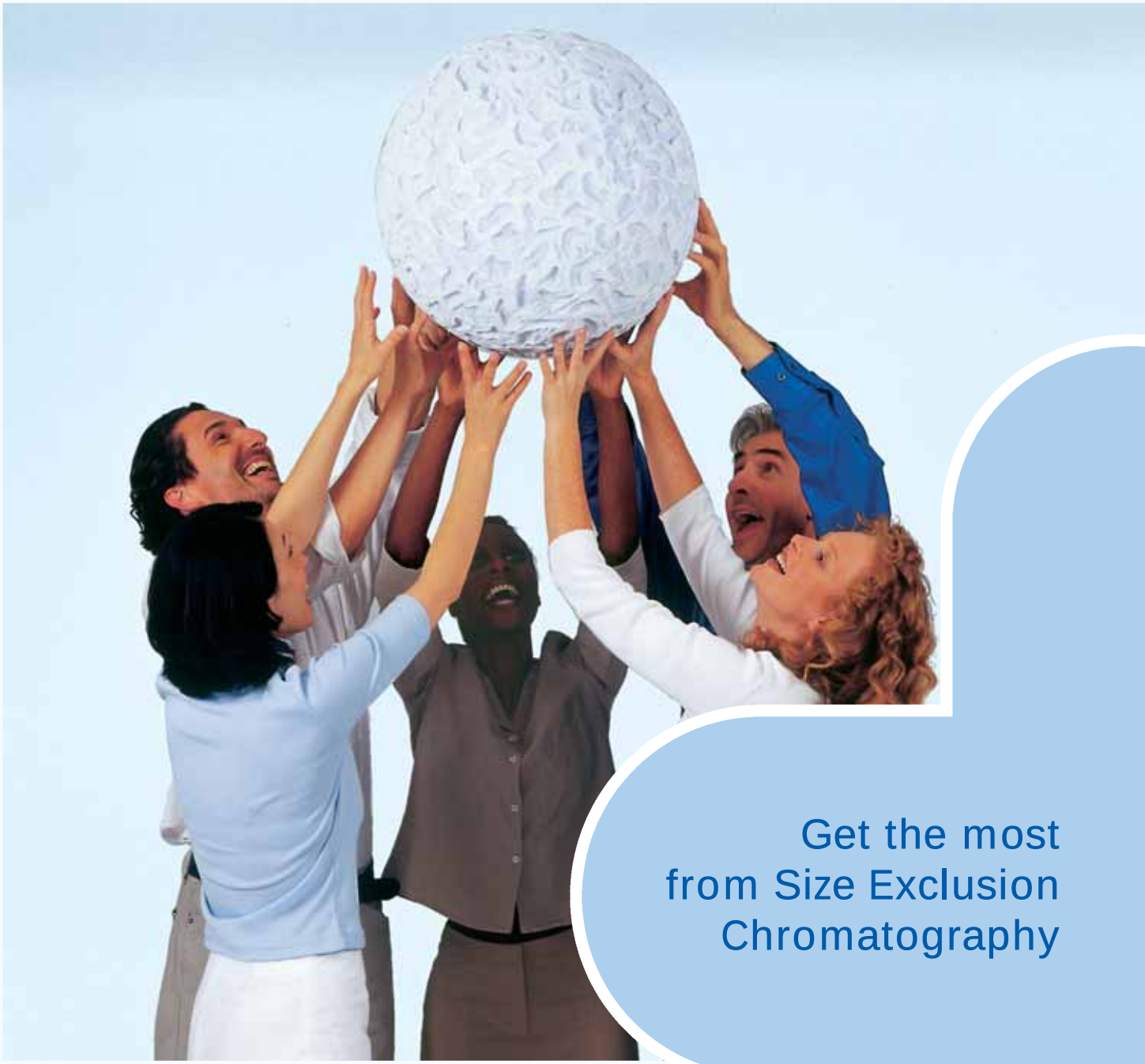
TOSOH BIOSCIENCE

Separations Business Unit

TSK-GEL SEC Columns



Get the most from Size Exclusion Chromatography

Tosoh Bioscience GmbH

Zettachring 6, 70567 Stuttgart, Germany
Tel: +49 (0)711 13257-0
Fax: +49 (0)711 13257-89
info.sep.eu@tosoh.com
www.tosohbioscience.de

Tosoh Bioscience LLC

156 Keystone Drive
Montgomeryville, PA 18936-9637
Tel: (215)283-5000 Fax: (215)283-5035
info.tbl@tosoh.com
www.tosohbioscience.com

TOSOH

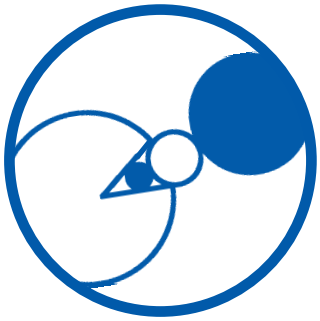
The Tosoh logo symbolizes the Corporate Philosophy or Tosoh's "vision of the ideal". The curved lines represent "the realization of happiness", reflecting Tosoh's management philosophy of putting people first. The square in the center expresses the advanced nature of Tosoh's technology and also represents the outstanding quality of Tosoh's products. The right-angle cut at the top portrays an image of contributing to society, Tosoh's basic stance towards the outside world. The red corporate color symbolizes the "Tosoh Spirit", which guides the ceaseless efforts to realize the ideal.

Nanyo Complex

Toyopearl resins are produced at the Tosoh Nanyo Complex – a sprawling 3 million square meter facility, that is Japan's largest chemical manufacturing complex.

Tokyo Research Center

Tosoh Bioscience is part of the Scientific Instruments division, located within Tosoh's Tokyo Research Center, Kanagawa-Ken, Japan.



TSK-GEL SEC Brochure

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TSK-GEL Column Selection

Column selection guide for high performance Size Exclusion Chromatography

Sample			Column selection		Selection criteria
			First choice	Alternative	
Carbo-hydrates	polysaccharides		TSKgel GMPWXL	G5000PWXL and G3000PWXL	large pore size, linear calibration curve, small particles, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW or TSKgel G2000PW	G2500PWXL	small particles, high resolving power
Nucleic acids	DNA fragments	large	TSKgel G-DNA-PW or TSKgel G5000PWXL	—	large pore size, small particles, high resolving power
		medium and small	TSKgel G4000SWXL / SW/ TSKgel BioAssist G4SWXL, TSKgel SuperSW3000, or TSKgel G3000SWXL / SW/ TSKgel BioAssist G3SWXL	—	suitable pore sizes
	RNA		TSKgel G4000SWXL / SW/ TSKgel BioAssist G4SWXL TSKgel SuperSW3000, or TSKgel G3000SWXL / SW/ TSKgel BioAssist G3SWXL		suitable pore sizes
	oligonucleotides		TSKgel G2500PWXL	—	small pore size, no ionic interaction
Proteins	normal size small-medium proteins		TSKgel SuperSW3000, TSKgel G3000SWXL / SW/ TSKgel BioAssist G3SWXL TSKgel G4000SWXL / SW, TSKgel BioAssist G4SWXL TSKgel SuperSW2000, or G2000SWXL / SW/ TSKgel BioAssist G2SWXL	G3000PWXL or G4000PWXL	small particles small to medium range pore sizes
	large proteins	low density lipoprotein	TSKgel G6000PWXL or TSKgel G5000PWXL	—	large pore sizes
		gelatin	TSKgel GMPWXL	G5000PWXL and G3000PWXL G4000SWXL	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000, TSKgel G3000SWXL / SW/ TSKgel BioAssist G3SWXL or TSKgel SuperSW2000, TSKgelG2000SWXL / SW/ TSKgel BioAssist G2SWXL	G3000PWXL	small to medium range pore size, versatile
	small		TSKgel G2500PWXL	SuperSW2000 or G2000SWXL / SW	linear calibration curve, high resolving power
Viruses			TSKgel G6000PWXL or TSKgel G5000PWXL	—	large pore size, high resolving power
Synthetic polymers			TSKgel GMPWXL or TSKgel Alpha-M	G5000PW and G3000PWXL or Alpha-5000 and Alpha-3000	large pore size, low adsorption, linear calibration curve
Synthetic oligomers	nonionic and cationic		TSKgel G-Oligo-PW, TSKgel G2500PWXL or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, high resolving power
	anionic		TSKgel G2500PWXL or TSKgel Alpha-2500	G2500PW or SuperAW2500	small pore size, no ionic interaction

TSK-GEL SEC Columns

Tosoh Corporation has a proud history of innovation in Size Exclusion Chromatography. TSK-GEL SEC columns are known worldwide for their reliability and suitability for the analysis of proteins, peptides and other biological macro-molecules. The complete TSK-GEL SW, PW, Alpha and SuperAW column lines consist of either silica based or polymeric based packings, ranging in particle size from 4 µm to 25 µm. Columns are available in analytical through preparative size, in stainless steel, PEEK or glass.

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.

Due to the 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 GFC and GPC. TSK-GEL SuperAW columns are based on the same chemistry as Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high throughput applications.

Size exclusion chromatography (SEC) is the general name for the chromatographic mode, in which components of a mixture are separated according to their molecular size, based on the flow of the sample through a porous packing.

The principal feature of Gel Filtration Chromatography (GFC), a subgroup of SEC, is its gentle non-interaction with the sample, enabling high retention of biomolecular enzymatic activity while separating multimers that are not easily distinguished by other chromatographic methods. However, SEC has limited separation capacity requiring that the molecular weights of the biomolecules differ by at least twofold.

Characteristics of TSK-GEL Size Exclusion column lines

Column line	TSK-GEL SW / SWXL / SuperSW	TSK-GEL PW / PWXL	TSK-GEL Alpha / SuperAW
Resin type	Silica	Methacrylate	Methacrylate
No. of available pore sizes	3/2	7	5
PH stability	2.5 - 7.5	2.0 - 12.0	2.0 - 12.0
Solvent compatability	100% polar	50% polar	100% polar, and nonpolar
Max. temp.	30°C	80°C*	80°C
Max. flow rate (mL/min)	1.2 (SW, SWXL) 0.4 (SuperSW)	1.2 (PW) 1.0 (PWXL)	1.0 (Alpha) 0.6 (SuperAW)
Pressure**(MPa)	0.8 - 1.2	1.0 - 4.0	2.0 - 4.0
Application focus	Proteins	Water soluble polymers	Intermediate polar 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.

Table 1

TSK-GEL SW Columns

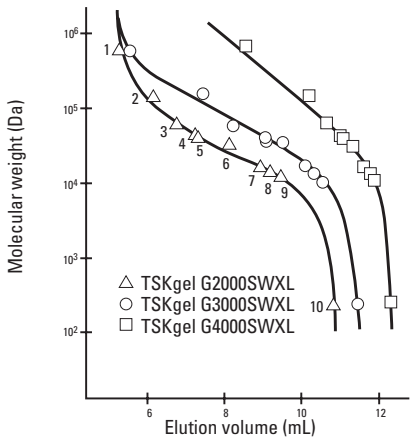
HIGHLIGHTS

- Rigid spherical silica gel chemistry bonded with hydrophilic groups
- Well defined pore size distribution
- Low adsorption properties
- TSK-GEL SuperSW columns for highest resolution and sensitivity
- NEW – PEEK column hardware for SWXL packings
- Short TSK-GEL QC-PAK columns for fast analysis
- Preparative stainless steel columns for precise scale up to commercial production

CHARACTERISTICS

TSK-GEL SW, SWXL and SuperSW packings are stable from pH 2.0 to 7.5 and have excellent solvent stability up to 100% organic solvents. Three different pore sizes of the SW and SWXL packings result in different exclusion limits for several sample types, as shown by the calibration curve in Figure 1. From this data, recommended separation ranges for globular proteins can be made for each column (see Table 2, for branched and linear molecules, calibration curves and separation ranges consult the Tosoh Bioscience Laboratory Products Catalogue). Different particle sizes, column dimensions and body materials are available (Table 2).

Protein calibration curves for TSK-GEL SWXL columns



Column: TSKgel SWXL column, 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. ß-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 sodiumphosphate buffer (pH 7.0)
Detection: UV @ 220 nm

Figure 1

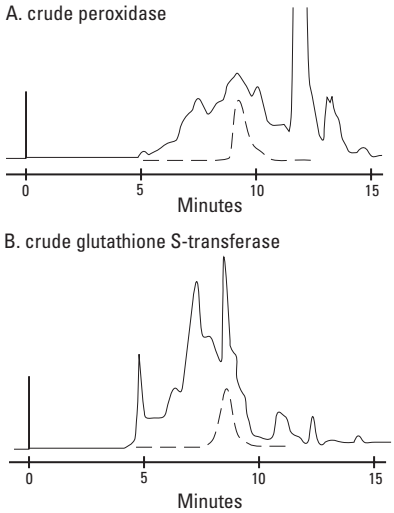
The resulting differences in column characteristics allow the scientist to select the appropriate column to his individual separation requirements.

Properties and separation ranges for TSK-GEL SW type packings

TSK-GEL column	ID (mm) x length (cm)	Particle size (µm)	Pore size (Å)	Min. no. theoret. plates	Flow rate (ml/min)	Max. pressure (MPa)	Molecular weight of proteins (Da)
SuperSW2000	4.6 x 30	4	125	30,000	0.1-0.4	12.0	5,000–1.5 x 10^5
G2000SWXL	7.8 x 30	5	125	20,000	0.5-1.2	7.0	5,000–1.5 x 10^5
BioAssist G2SWXL	7.8 x 30	5	125	20,000	0.5-1.2	7.0	5,000–1.5 x 10^5
QC-PAK GFC 200	7.8 x 15	5	125	10,000	0.5-1.2	4.0	5,000–1.5 x 10^5
G2000SW	7.5 x 30/60	10	125	10,000/20,000	0.5-1.2	2.0/4.0	5,000–1.0 x 10^5
	21.5 x 30/60	13	125	10,000/20,000	3.0-8.0	1.0/2.0	5,000–1.0 x 10^5
	55.0 x 30/60	20	125	-	-	-	5,000–1.0 x 10^5
SuperSW3000	1.0 x 30	4	250	>18,000	0.01-0.02	12.0	1 x 10^4–5 x 10^5
	2.0 x 30	4	250	>25,000	0.05-0.075	12.0	1 x 10^4–5 x 10^5
	4.6 x 30	4	250	30,000	0.1-0.4	12.0	1 x 10^4–5 x 10^5
G3000SWXL	7.8 x 30	5	250	20,000	0.5-1.2	7.0	1 x 10^4–5 x 10^5
BioAssist G3SWXL	7.8 x 30	5	250	20,000	0.5-1.2	7.0	1 x 10^4–5 x 10^5
QC-PAK GFC 300	7.8 x 15	5	250	10,000	0.5-1.2	4.0	1 x 10^4–5 x 10^5
G3000SW	7.5 x 30/60	10	250	10,000/20,000	0.5-1.2	2.5/5.0	1 x 10^4–5 x 10^5
	21.5 x 30/60	13	250	10,000/20,000	3.0-8.0	1.5/3.0	1 x 10^4–5 x 10^5
	55.0 x 30/60	20	250	-	-	-	1 x 10^4–5 x 10^5
G4000SWXL	7.8 x 30	8	450	16,000	0.5-1.2	3.5	2 x 10^4–7 x 10^6
BioAssist G4SWXL	7.8 x 30	8	450	16,000	0.5-1.2	3.5	2 x 10^4–7 x 10^6
G4000SW	7.5 x 30/60	13	450	8,000/16,000	0.5-1.2	1.5/3.0	2 x 10^4–7 x 10^6
	21.5 x 30/60	17	450	8,000/16,000	3.0-8.0	1.0/2.0	2 x 10^4–7 x 10^6

Table 2

Separation of crude protein sample on TSKgel G3000SWXL



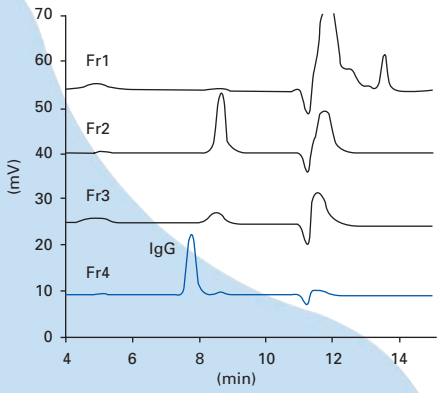
Column:	TSKgel G3000SWXL, 5 µm, 7.8 mmm ID x 30 cm L
Sample:	A. crude peroxidase from Japanese radish, 0.15 mg in 0.1 ml B. crude glutathione S-transferase from guinea pig liver extract, 0.7 mg in 0.1 ml
Elution:	0.3 M NaCl in 0.05 M phosphate buffer, pH 7.0
Flow rate:	1.0 ml/min
Detection:	UV @ 220 nm (solid line) and enzyme essay tests (dashed line)
Recovery:	enzymatic activity recovered was 98% in A and 89% in B

Figure 2

APPLICATIONS

SEC as a gentle separation technique allows high recovery of enzymatic activities. For example a crude sample of peroxidase and glutathione S-transferase was separated in only 15 minutes on a TSKgel G3000SWXL column.

Purity of an antibody from a cell culture supernatant (Anti TSH)



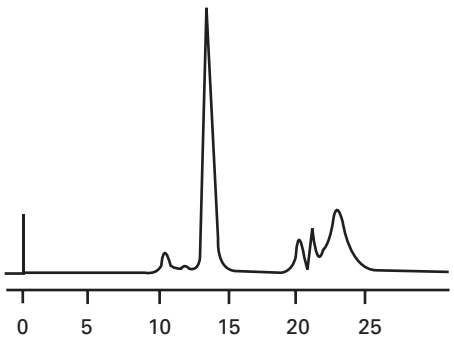
SEC assay of collected fractions from a HIC purification step

Column:	TSKgel BioAssist G3SWXL, 5 µm, 7.8 mm ID x 30 cm L
Elution:	0.3 mol/l phosphate buffer (pH 7.0)
Flow rate:	1 ml/min
Inj. Volume:	50 µl

Figure 3

The elution profile in Figure 2 shows that all of the activity eluted in a narrow band of only 1.5 min with high recoveries. When the analysis of proteins needs to be performed in a metal free environment, the BioAssistSW series offers TSK-GEL SWXL packings in PEEK housings featuring the same performance as with stainless steel columns (see Figure 3). For low pressure applications a TSK-GEL SW Glass column may be the right choice (see Figure 4). For production purposes, results from analytical columns can easily be scaled up to preparative columns. Figure 5 demonstrates the increase in sample volume without sacrificing resolution.

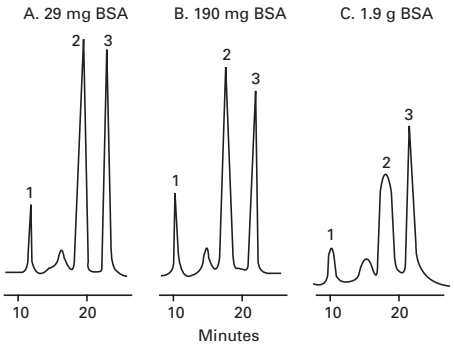
Separation of antithrombin III with TSK-GEL glass column



Column:	TSKgel G2000SW Glass, 10 µm, 8.0 mm ID x 30 cm L
Elution:	50 mM phosphate buffer + 0.3 M NaCl (pH 7.0)
Flow rate:	0.6 ml/min
Detection:	UV @ 280 nm

Figure 4

Purification of protein on preparative scale TSK-GEL columns



Columns:	TSKgel G3000SW columns, A. 13 µm, 21.5 mm ID x 60 cm L B. and C., 20 µm, 55 mm ID x 60 cm L
Sample:	1. blue dextran, 2. bovine serum albumin (BSA), 3. myoglobin, BSA/myoglobin = 4/1 w/w
Elution:	0.1 M NaCl in 0.1 M phosphate buffer, pH 6.8
Flow rate:	A. 7.2 ml/min (119 cm/h); B. & C.: 48 ml/min (121 cm/h)
Detection:	UV @ 280 nm

Figure 5

TSK-GEL SuperSW Columns

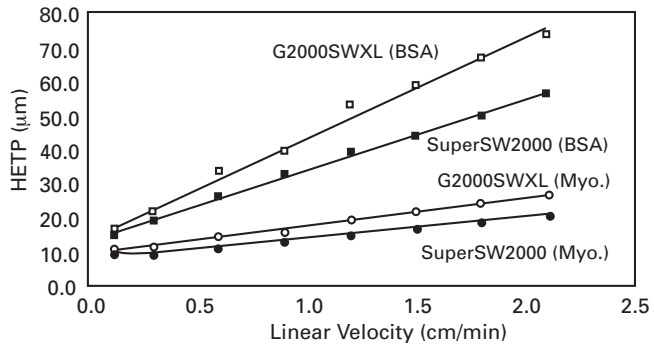
To further improve efficiency and sensitivity, TSK-GEL SuperSW columns packed with 4 µm spherical silica particles were developed.

CHARACTERISTICS

TSK-GEL SuperSW columns are available in two pore sizes, 125 Å and 250 Å, both featuring a minimum plate height of 30.000 plates/column. As demonstrated in Figure 6, dependability of height equivalent of theoretical Plate (HETP) values from flow rates is less than on the TSK-GEL SWXL column. The columns with 4.6 mm ID provide 300% increased sensitivity which is especially helpful for limited sample quantities or analysis of byproducts or aggregates (see Figure 8).

However, to benefit from the improved features of the TSK-GEL SuperSW columns, the operating system should be optimized. Optimal operating conditions are described in Table 3. With this set and a good sample preparation, very long column lifetimes are possible as demonstrated in figure 7. The drop in efficiency below 21.000 indicated a necessary replacement of the guard column. The plate count was restored after a new guard column was put into use.

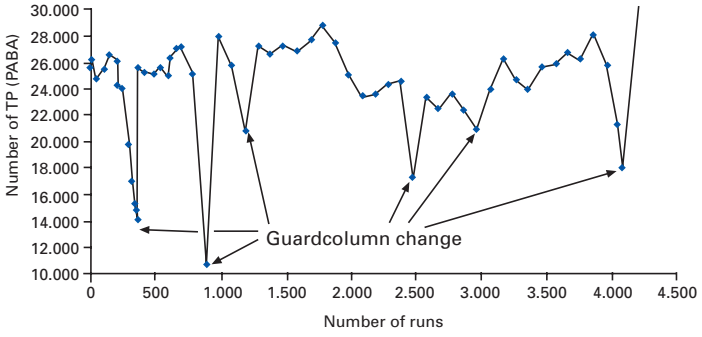
Relationship between flow rate and HETP in TSK-GEL SuperSW Series and TSK-GEL SWXL Series



Column:	TSKgel SuperSW, 4 µm, 4.6 mm ID x 30 cm L TSKgel SWXL, 5 µm, 7.8 mm ID x 30 cm L
Sample:	Standard proteins (5 µl) Bovine serum albumin (1 g/l) Myoglobin (1 g/l)
Elution:	0.2 mol/l phosphate buffer (pH 6.7)
Detection:	UV @ 280 nm, micro flow cell

Figure 6

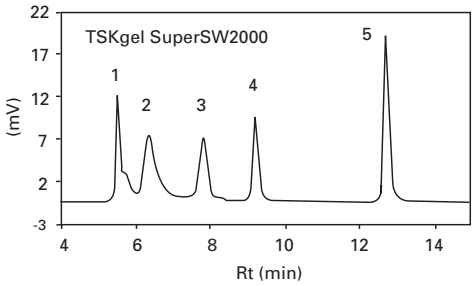
Stability of theoretical plates on a TSKgel SuperSW3000

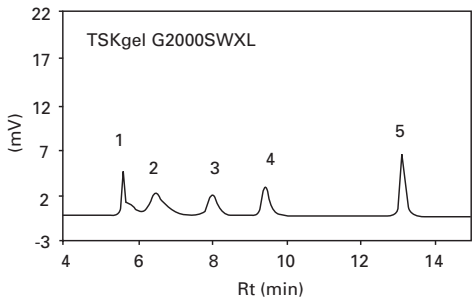


Column:	TSKgel SuperSW3000, 4 µm, 7.8 mm I.D. x 30 cm L. + guardcolumn
Sample:	g-Globulin (156 KD), 1 mg/ml eluent; Ovalbumin (43 KD), 1 mg/ml eluent; Cytochrome C (12.4 KD), 0.5 mg/ml eluent; PABA (137 D), 0.01 mg/ml eluent
Elution:	0,1 M P.B. + 0,1 M Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35 ml/min
Temperature:	ambient
Detection:	UV@280 nm, cell volume 1 µl, response time 0.2 sec.

Figure 7

Comparison of sensitivity between TSKgel SuperSW2000 and TSKgel G2000SWXL





Column:	TSKgel SuperSW2000, 4 µm, 4.6mm ID x 30 cm L TSKgel G2000SWXL, 5 µm, 7.8 mm ID x 30 cm L
Sample:	Standard proteins (5 µl) 1. Thyroglobulin (0.5 g/l) 2. Gamma-globulin (1 g/l) 3. Ovalbumin (1 g/l) 4. Ribonuclease A (1 g/l) 5. p-aminobenzoic acid (0.01 g/l)
Elution:	0.2 mol/l phosphate buffer (pH 6.7)
Flow rate:	0.35 ml/min (TSKgel SupperSW2000) 1.00 ml/min (TSKgel G2000SWXL)
Detection:	UV @ 280 nm, micro flow cell

Figure 8

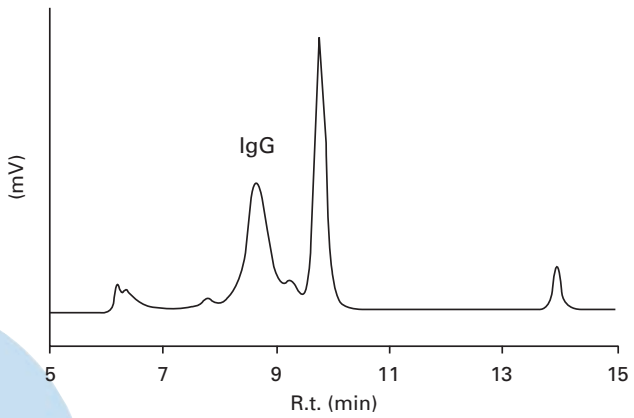
TSK-GEL SuperSW2000 and SuperSW3000 Operating Conditions

For best results, it is recommended to use the following experimental conditions for TSK-GEL SuperSW columns:

Connections *Tubing*	The conventional 0.1 mm tubing may be used, but length should be kept as short as possible. Void volume between the column and detector cell should be less than 20 µl.
Sample volume	Sample volume should be 5 µl or less.
Guard column	A guard column is highly recommended to reduce clogging and contamination.
Detector *Flow cell*	For best results, use a flow cell with a maximum of 2 µl. The 2 µl flow cell will give the highest efficiencies. A 2-10 µl flow cell can be used. If using a 10 µl flow cell, remove the heat coil to maintain high column efficiencies.
Time constant	The smallest time constant (less than 0.5 sec) is needed to achieve best column performance.
Pump	A pump capable of accurately delivering a flow rate between 0.05 ml/min and 0.35 ml/min is recommended

Table 3

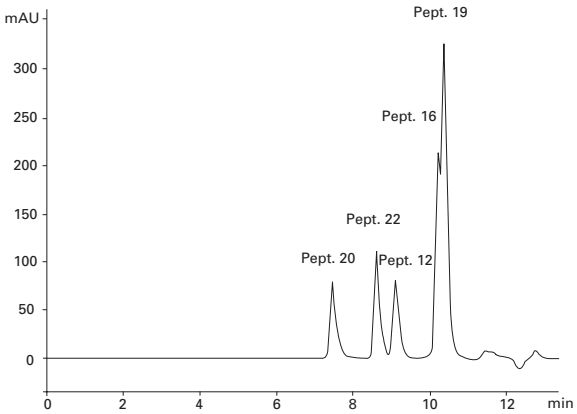
Separation of human serum albumin on a TSKgel SuperSW3000



Column:	TSKgel SuperSW3000, 4 µm, 4.6 mm ID x 30 cm L
Sample:	Human serum
Elution:	0.2 M phosphate buffer (pH 6.7)
Flow rate:	0.35 ml/min
Injection vol:	5 µl
Detection:	UV @ 280 nm

Figure 9

Separation of a peptide mixture on a TSKgel SuperSW2000



Column:	TSKgel SuperSW2000, 4 µm, 4.6 mm ID x 30 cm L
Sample:	Peptide P12: Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu Peptide P16: Trp-Gly-Gly-Tyr Peptide P19: Gly-Trp-Gly Peptide P20: H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Gly-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH Peptide P22: H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH
Elution:	0.1% TFA in 45% aq. ACN
Flow rate:	0.35 ml/min
Injection vol:	3 µl
Detection:	UV @ 210 nm

Figure 10

APPLICATIONS

Two application examples demonstrate the usability of the TSK-GEL SuperSW columns. Figure 9 shows an analysis of human serum. For solving a separation problem, also the eluent plays an important role.

Figure 10 demonstrates that very small molecules can be separated efficiently on a SuperSW column under non SEC conditions. Although the peptides 16 and 19 do not elute according to their molecular weight, a separation was possible with only one amino acid difference (based on different interaction with the gel surface).

TSK-GEL PW Columns

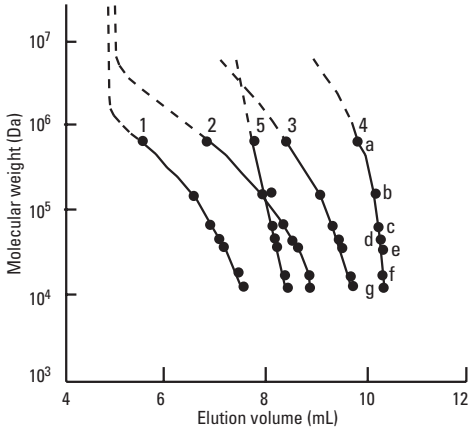
HIGHLIGHTS

- Hydrophilic, spherical polymeric methacrylate resin
- pH range of 2 to 12, with up to 50% organic solvent
- Temperatures up to 80°C
- Six different pore sizes allowing wide molecular weight separation ranges
- PEEK column hardware for G6000PW packings for ultra-low sample adsorption during virus analysis
- Preparative stainless steel columns for precise scale up

CHARACTERISTICS

The properties for all TSK-GEL PW and PWXL type columns are summarized in Table 4. Use the molecular weight ranges for polyethylene glycols/oxides (PEG/PEO) when choosing a column for linear molecules; calibration curves for globular molecules separated on TSK-GEL PWXL columns are shown in Figure 11. Specialty resin based columns include the mixed-bed TSK-GEL GMPW and GMPWXL for samples with a broad molecular weight range. They include TSK-GEL G-Oligo-PW and G-DNA-PW columns for oligo-saccharides and for DNA or RNA respectively.

Protein calibration curves on TSK-GEL PWXL columns



Column: 1. TSKgel G3000PWXL, 2. TSKgel G4000PWXL, 3. TSKgel G5000PWXL , 4. TSKgel G6000PWXL, 5. TSKgel GMPWXL
Sample: a. thyroglobulin (660,000 Da), b. gamma-globulin (150,000 Da), c. albumin (67,000 Da), d. ovalbumin (43,000 Da), e. ß-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

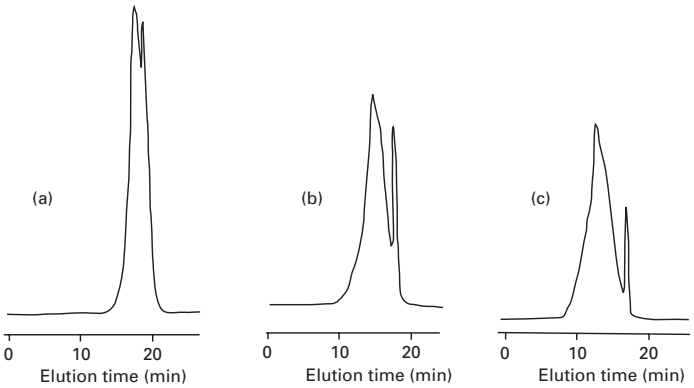
Figure 11

Analytical and preparative TSK-GEL size exclusion polymer based columns: typical properties

TSKgel column	ID (mm) X length (cm)	Particle size (µm)	Pore size (Å)	Min. no. theoret. plates	Flow rate (ml/min)	Max. pressure (MPa)	MW PEG/ PEO (Da)
G2000PW	7.5 x 30/60	12	125	5.000/10.000	0.5-1.2	2.0/4.0	up to 2,000
G2500PWXL	7.8 x 30	6	<200	14.000	0.5-1.0	4.0	up to 3,000
G2500PW	7.5 x 30/60	10	<200	5.000/10.000	0.5-1.2	2.0/4.0	up to 3,000
	21.5 x 60	17	<200	10.000	1.0-8.0	2.0	up to 3,000
G3000PWXL	7.8 x 30	6	200	14.000	0.5-1.0	4.0	up to 5 x 10^4
G3000PW	7.5 x 30/60	10	200	5.000/10.000	0.5-1.2	2.0/4.0	up to 5 x 10^4
	21.5 x 60	17	200	10.000	1.0-8.0	2.0	up to 5 x 10^4
	55.0 x 60	20	200	-	15.0-25.0	225 psi	up to 5 x 10^4
G4000PWXL	7.8 x 30	10	500	10.000	0.3-1.0	2.0	2,000 – 3 x 10^5
G4000PW	7.5 x 30/60	17	500	3.000/6.000	0.5-1.2	1.0/2.0	2,000 – 3 x 10^5
	21.5 x 60	22	500	6.000	1.0-8.0	2.0	2,000 – 3 x 10^5
G5000PWXL	7.8 x 30	10	1000	10.000	0.3-1.0	2.0	4,000 – 1 x 10^6
G5000PW	7.5 x 30/60	17	1000	3.000/6.000	0.5-1.2	1.0/2.0	4,000 – 1 x 10^6
	21.5 x 60	22	1000	6.000	1.0-8.0	2.0	4,000 – 1 x 10^6
	55.0 x 60	22	1000	-	-	-	4,000 – 1 x 10^6
G6000PWXL	7.8 x 30	13	>1000	7.000	0.3-1.0	2.0	4 x 10^4 – 8x 10^6
BioAssist G6PW	7.8 x 30	17	>1000	3.000	0.5-1.2	1.0	4 x 10^4 – 8x 10^6
G6000PW	7.5 x 30/60	17	>1000	3.000/6.000	0.5-1.2	1.0/2.0	4 x 10^4 – 8x 10^6
	21.5 x 60	25	>1000	6.000	1.0-8.0	2.0	4 x 10^4 – 8x 10^6
GMPWXL	7.8 x 30	13	<100-1000	7.000	0.3-1.0	2.0	500 - 8 x 10^6
GMPW	7.5 x 30/60	17	<100-1000	3.000/6.000	0.5-1.2	1.0/2.0	500 – 8 x 10^6
G-Oligo-PW	7.8 x 30	6	125	14.000	0.5-1.0	4.0	up to 3,000
G-DNA-PW	7.8 x 30	10	>1000	10.000	0.2-0.6	2.0	4 x 10^4 – 8 x 10^6

Table 4

Separation of gelatin on TSK-GEL PWXL Columns



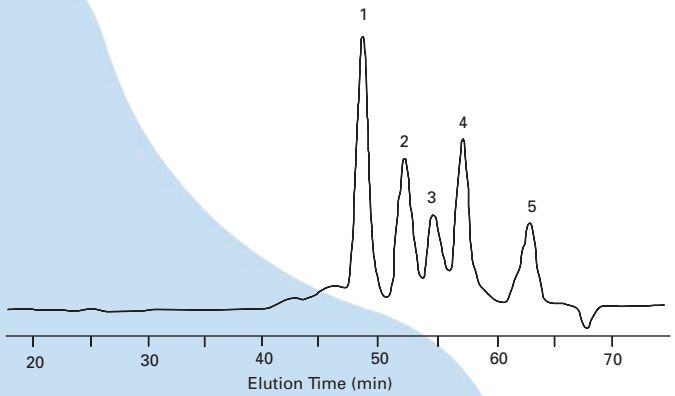
Column:	(a) TSkgel G6000PWXL, (b) TSKgel G5000PWXL, (c) TSKgel G4000PWXL; all 7.8 mm ID x 30 cm L
Sample:	Gelatin
Elution:	0.2 M phoshpate buffer (pH 6.0)
Flow rate:	1.0 ml/min
Detection:	RI

Figure 12

APPLICATIONS

An example on the influence of pore size on the separation of complex polymers is shown in Figure 12. While on the large pore TSKgel G6000PWXL column gelatine elutes in one narrow peak, on the G4000PWXL the peak is much broader and the shoulder nearly separated from the main peak. This allows better determination of Mw/Mn and Mz/Mw.

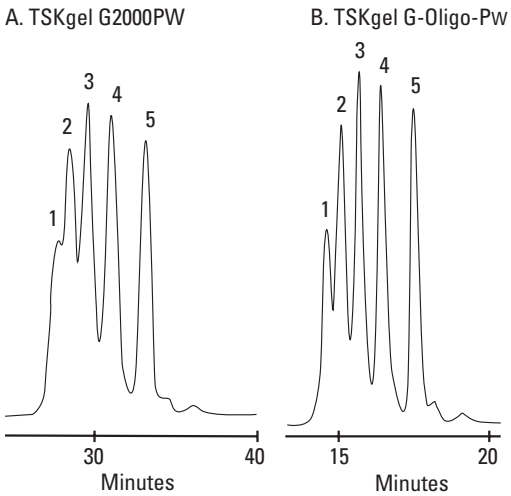
Elution curve for a peptide mixture on TSKgel G3000PWXL



Column:	TSKgel G3000PWXL, 6 µm, 7.8 mm ID x 30 cm L
Sample:	peptides; 1= aprotinin, 2= insulin B-chain, 3= a-MSH, 4= bradykinin potentiator C, 5= glutathione
Elution:	0.1% TFA / 45% CH_3CN
Flow rate:	1.0 ml/min

Figure 13

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

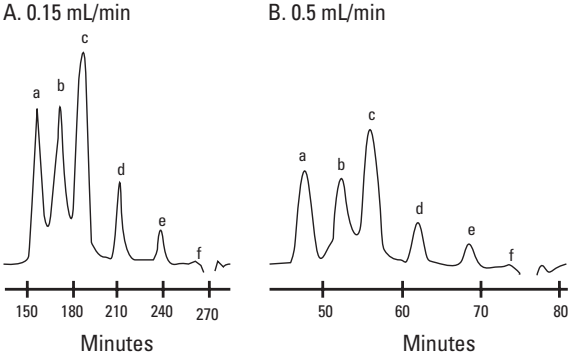


Column:	A. TSKgel G2000PW, two 10 µm, 7.5 mm ID x 60 cm L in series B. TSKgel G-Oligo-PW, two 6 µm, 7.8 mm ID x 30 cm L in series
Sample:	1. chitohexaose, 2. chitopentaose, 3. chitotetraose, 4. chitotriose, 5. chitobiose
Elution:	destilled water
Flow rate:	1.0 ml/min
Detection:	RI

Figure 14

The influence of particle size on resolution and analysis time can be seen in Figure 14. It compares the separation of chito-oligosaccharides on a TSKgel G2000PW column with 10 µm beads and a TSKgel G-Oligo-PW with a 6 µm material. Figure 13 demonstrates the separation of small peptides possible on a TSKgel G3000PWXL column under denaturating conditions. Very large molecules, however, can be separated nicely on the G-DNA-PW column as depicted in Figure 15.

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



Column:	TSKgel G-DNA-PW, 10 µm, 4 x 7.8 mm ID x 30 cm L
Sample:	60 ml 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 ris-HCl, pH 7.5, plus 1 mM EDTA
Flow rate:	A. 0.15 ml/min, B. 0.5 ml/min
Detection:	UV @ 260 nm

Figure 15

TSK-GEL Alpha/SuperAW Columns

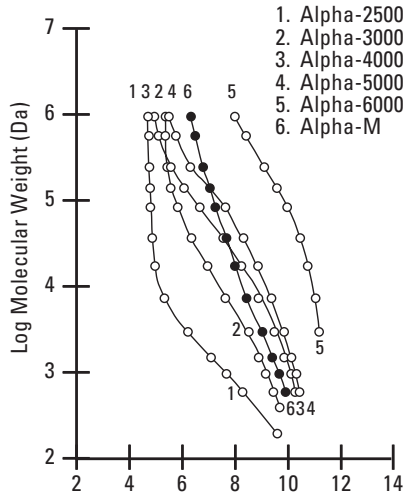
HIGHLIGHTS

- Unique hydrophilic polyvinyl resin with rigid spherical beads
- Minimal swelling characteristics from 100% water to 100% non-polar solvents
- Excellent mechanical and chemical stability
- TSKgel SuperAW columns with reduced particle size and shorter column length provide short analysis times and high resolution power
- Mixed mode applications possible for small molecules.

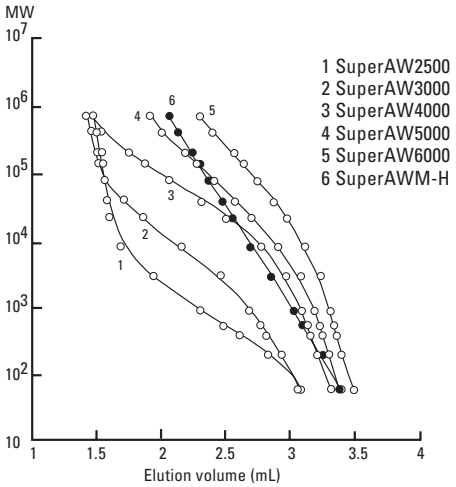
CHARACTERISTICS

The TSK-GEL Alpha and SuperAW column series offer a new alternative for performing SEC. The columns are packed with a hydrophilic, highly crosslinked vinyl polymer which is compatible to a wide range of solvents ranging from pure aqueous up to 100 % organic mobile phases (see Figure 17). Both series consist of six columns with different pore sizes, spanning a wide MW separation range from 100 to over 1,000,000 Da when using polyethylene glycol (PEG) as a standard (see Figure 16).

Calibration curves of TSK-GEL Alpha and SuperAW Series



Column: TSKgel Alpha Series, 7.8 mm ID x 30 cm L
Sample: Standard polyethylene oxide, polyethylene glycol, ethylene glycol
Elution: MeOH containing 10 mM LiBr
Flow rate: 1.0 ml/min
Temperature: A. 25°C; B. 25°C; C. 40°C
Detection: RI



Column: TSKgel SuperAW Series, 6.0 mm ID x 15 cm L
Sample: Standard polyethylene oxide, polyethylene glycol, ethylene glycol
Elution: MeOH containing 10 mM LiBr
Flow rate: 0.6 ml/min
Temperature: 25°C
Detection: RI

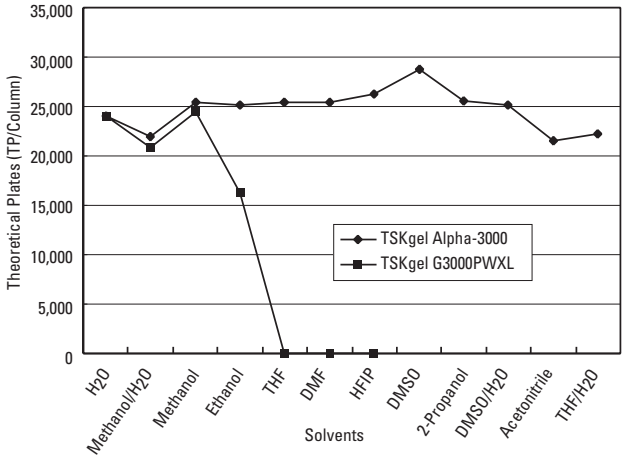
Figure 16

List of TSK-GEL Alpha and SuperAW-Series

TSK-GEL Column	ID (mm) x length (cm L)	Particle size (µm)	Min no. theoret. plates	Flow rate (ml/min)	Max. pressure (MPa)	Exclusion limit (PEO/H_2O)
Alpha-2500	7.8 x 30	7	16,000	0.5-1.0	40	5×10^3
Alpha-3000	7.8 x 30	7	16,000	0.5-1.0	40	9×10^4
Alpha-4000	7.8 x 30	10	10,000	0.3-1.0	30	4×10^5
Alpha-5000	7.8 x 30	10	10,000	0.3-1.0	30	1×10^6
Alpha-6000	7.8 x 30	13	7,000	0.3-1.0	20	$>1 \times 10^7$
Alpha-M	7.8 x 30	13	7,000	0.3-1.0	20	$>1 \times 10^7$
TSKgel SuperAW2500	6.0 x 15	4	>16,000	0.3-0.6	60	5×10^3
TSKgel SuperAW3000	6.0 x 15	4	>16,000	0.3-0.6	60	9×10^4
TSKgel SuperAW4000	6.0 x 15	6	>10,000	0.3-0.6	40	1×10^6
TSKgel SuperAW5000	6.0 x 15	7	>10,000	0.3-0.6	30	1×10^6
TSKgel SuperAW6000	6.0 x 15	9	>6,000	0.3-0.6	20	1×10^7
TSKgel SuperAWM-H	6.0 x 15	9	>6,000	0.3-0.6	20	1×10^7

Table 5

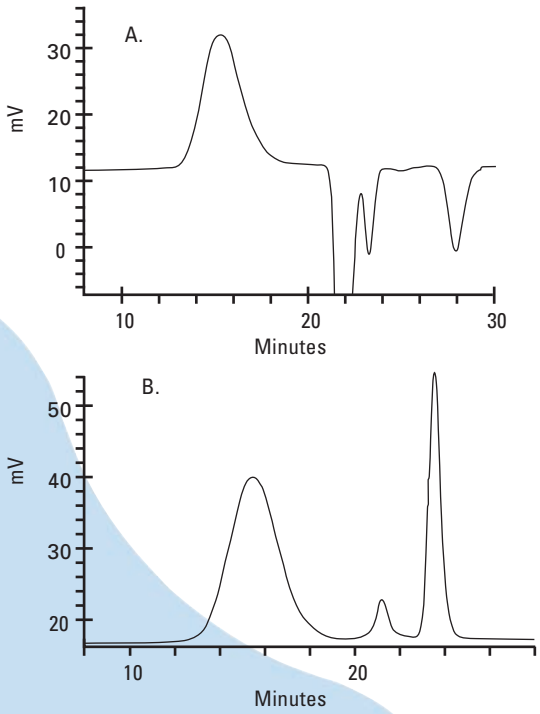
Solvent compatability for TSKgel Alpha-3000 for organic solvent



Conditions for solvent change:		Conditions for TP mearsurement:	
Flow rate:	1.0 ml/min	Sample:	ethylene glycol
Temperature:	25°C	Flow rate:	1.0 ml/min
Time for purge.	8 h	Temperature:	25°C
		Detection:	RI

Figure 17

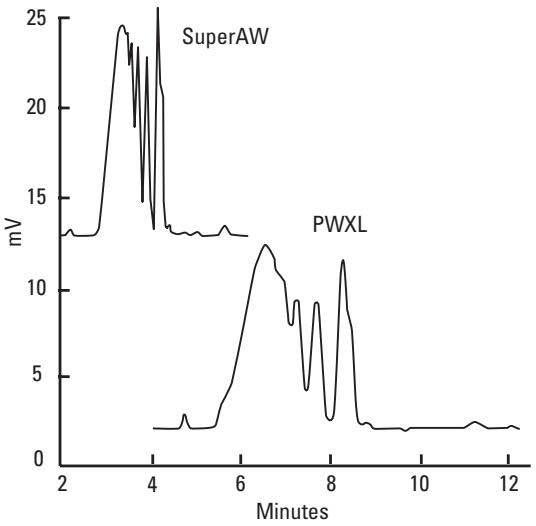
TSKgel Alpha-M separation of cellulose derivatives



Column:	TSKgel Alpha-M, 13 µm, 7.8 mm ID x 30 cm L
Sample:	A. 50 ml ethylcellulose, 0.1%; B. 50 ml 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 18

Comparison of TSKgel SuperAW2500 and G2500PWXL



Column:	TSKgel SuperAW2500, 6.0 mm ID x 15 cm L; TSKgel G2500PWXL, 7.8 mm ID x 30 cm L
Sample:	Dextran T-40 hydrolysate
Elution:	H_2O
Flow rate:	0.6 ml/min (TSKgel SuperAW2500) 1.0 ml/min (TSKgel G2500PWXL)
Temperature:	25°C
Detection:	RI

Figure 19

Exclusion limits for polyethylene oxides in water and other physical properties for the Alpha and SuperAW columns are listed in Table 5.

For samples with big differences in molecular weights, the mixed bed columns TSKgel Alpha-M and TSKgel SuperAWM-H show linear calibration curves over the whole range.

APPLICATIONS

The versatility of using TSK-GEL Alpha columns with various organic solvents is illustrated in Figure 18. A TSKgel Alpha-M column was used to separate ethyl-cellulose with the polar solvent DMF and ethylhydroxy-ethyl cellulose with methanol. Figure 19 illustrates the decreased analysis time when using TSKgel SuperAW in comparison to a conventional TSKgel G2500PWXL column.

Moreover the TSK-GEL Alpha and SuperAW column series can be used for separations of synthetic polymers, oligomers, additives and detergents as well as for saccharides, nucleic acids and peptides.

Optimizing SEC

SAMPLE LOAD

As SEC is a partition chromatography, sample load on the column is limited. High sample loads distort peak shapes and cause an overall decrease in efficiency due to column overload.

Optimal sample load highly depends on the sample properties (sample matrix) and the separation task. For analytical columns, sample concentrations of 1-20 mg/mL are recommended. Proteins can be loaded at higher concentrations and higher total loads than synthetic macromolecules. For preparative purposes for example, 100 mg of BSA can be loaded on two 21.5 mm ID x 60 cm L TSKgel G3000SW columns, but only 20 mg of PEG 7500.

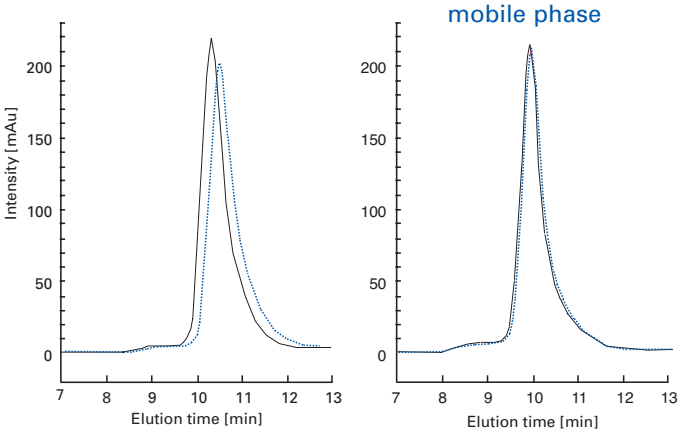
Sample volume depends very much on the type of column. On TSK-GEL SuperSW columns for example, a 5 µL injection volume ensures optimal results. Standard injection volumes for 7.5 and 7.8 mm ID columns are 20-100 µl, whereas for preparative purposes on 21.5 mm ID columns, injection volumes may be raised up to 2 ml.

MOBILE PHASE

Proper selection of the mobile phase is necessary to maximize molecular sieving mechanism and to minimize secondary effects such as ionic and hydrophobic interaction between the sample and the column packing material. For each sample there will be an optimum buffer type and concentration that results in the highest resolution and recovery.

SEC of pegylated proteins - no ethanol in mobile phase

SEC of pegylated proteins - with 10% ethanol in mobile phase



Column: G3000SWXL, 5 µm, 7.8 mm ID x 30 cm L
Sample: 10 ml of PEG-r-HuMGDF
——— initial Injection
····· after 150 injections
Mobile phase: 0.1 M sodium phosphate, pH 6.9, with 0.5 M NaCl
Flow rate: 0.7 ml/minute
Detection: UV @ 215 nm

Figure 20

For TSK-GEL SW columns mobile phases a buffer concentration between 0.1 M and 0.5 M is recommended. Under low ionic strength (< 0.1 M), ionic interactions between the sample molecules and the silica surface may occur. Under conditions of high ionic strength (>1.0 M), hydrophobic interactions are more likely to occur. A neutral salt, such as sodium sulphate may be added to the buffer to increase buffer ionic strength. Also the ionic species of the buffer has an effect on the separation. As a good starting point, a 0.1 M sodium phosphate buffer together with 0.1 M sodium sulphate has proved to be of value.

As the polymeric TSK-GEL PW and Alpha-type resins carry less residual charged groups on the surface than silica gels, salt concentration of the mobile phase can be lower. Non-ionic, non-polar compounds such as polyethylene glycols can simply be analysed with distilled water. For ionic polymeric compounds, a neutral salt such as sodium nitrate is added to the aqueous eluent. Generally, a concentration of 0.1 M to 0.2 M is sufficient to overcome undesirable ionic interactions.

If hydrophobic interaction occurs between the sample and the column matrix, a water soluble organic solvent can be added to the mobile phase. The addition of acetonitrile, acetone, ethanol or methanol up to a concentration of 20% may also prevent columns from fouling by suppressing interaction of hydrophobic impurities of the sample. An example is shown in Figure 20 with the analysis of a pegylated protein on a TSKgel G3000SWXL column. As pegylated products are more hydrophobic, they tend to interact with the column matrix. Over time enough of the pegylated product can foul the column, which is indicated by shifts of retention time and decreasing separation performance. By adding 10% of ethanol to the elution buffer, this problem is overcome. Figure 20 shows no differences in performance at the first and the 150th injection. (curtesy of J.J. Ratto et al. Amgen Inc., 1996)

COLUMN PROTECTION

To protect the column and increase its lifetime, the use of a guard column is strongly recommended. An example for the influence of the guard column on column lifetime is depicted on page 6 in Figure 7.

Sample purity, sample load and the composition of the mobile phase have an influence on column lifetime as already demonstrated in Figure 20.

For closer information on TSK-GEL SEC columns, please consult the Tosoh Bioscience Laboratory Products Catalogue!

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TOSOH BIOSCIENCE GmbH
Zettachring 6
70567 Stuttgart, Germany
Phone: +49 (0) 711 13257-0, Fax: +49 (0) 711 13257-89
info.sep.eu@tosoh.com, www.tosohbioscience.de

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