Application Overview

Gel Filtration Chromatography with TSKgel columns

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TOSOH

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Column Selection

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 TSKgel G3000SW$_{XL}$ / SW/ TSKgel BioAssist G3SW$_{XL}$	—	suitable pore sizes
	RNA		TSKgel G4000SW$_{XL}$ / SW/ TSKgel BioAssist G4SW$_{XL}$ TSKgel SuperSW3000, or TSKgel G3000SW$_{XL}$ / SW/ TSKgel BioAssist G3SW$_{XL}$		suitable pore sizes
	oligonucleotides		TSKgel G2500PW$_{XL}$	—	small pore size, no ionic interaction
Proteins	normal size small-medium proteins		TSKgel SuperSW3000, TSKgel 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$_{XL}$ G4000SW$_{XL}$	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000, TSKgel G3000SW$_{XL}$ / SW/ TSKgel BioAssist G3SW$_{XL}$ or TSKgel SuperSW2000, TSKgelG2000SW$_{XL}$ / SW/ TSKgel BioAssist G2SW$_{XL}$	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, no ionic interaction

TSKgel Gel Filtration Columns

Tosoh Corporation has a proud history of innovation in Size Exclusion Chromatography (SEC). TSKgel SEC columns are known worldwide for their reliability and suitability for the analysis of proteins, peptides and other biological macro-molecules. In this brochure, the focus is on the branch of SEC called Gel Filtration Chromatography (GFC). In this mode the mobile phase is an aqueous buffer. a mixture of water and organic modifiers or a polar organic solvent. The complete TSKgel SW, PW, Alpha and SuperAW column lines consist of either silica based or polymeric based packings, ranging in particle size from 4µm to 25µm. Columns are available in analytical through preparative size, in stainless steel, PEEK or glass.

The main criterion in choosing between the TSKgel SW, PW, Alpha and SuperAW SEC columns is the molecular weight of the sample and its solubility. The fact that the TSKgel SW columns are based on silica and the TSKgel 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 TSKgel SW columns are suitable for the separation of monodisperse biopolymers such as proteins and nucleic acids. TSKgel 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 TSKgel SW columns. While a TSKgel SW column is typically the first column to try for biopolymers, TSKgel PW columns have demonstrated good results for smaller peptides (<1,000 Da), protein aggregates, DNA fragments, and viruses.

The TSKgel 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. TSKgel 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 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.

Table 1

Characteristics of TSKgel Gel Filtration column lines

Column line	TSKgel SW / SW_{XL} / SuperSW	TSKgel PW / PW_{XL}	TSKgel 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, SW_{XL}) 0.4 (SuperSW)	1.2 (PW) 1.0 (PW_{XL})	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 TSKgel 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.

TSKgel SW Columns

HIGHLIGHTS

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

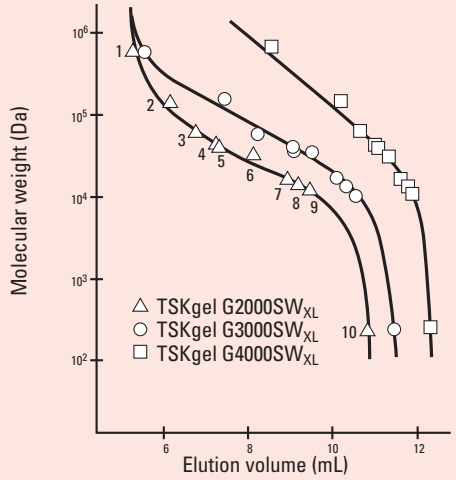
CHARACTERISTICS

TSKgel SW, SW_{XL} 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 TSKgel SW and SW_{XL} 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 Catalog).

Furthermore, different particle sizes, column dimensions and body materials are available (Table 2).

Figure 1

Protein calibration curves for TSKgel SW_{XL} columns



Column: TSKgel SW_{XL} 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

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

Table 2

Properties and separation ranges for TSKgel SW type packings

TSKgel 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
$G2000SW_{XL}$	7.8 x 30	5	125	20,000	0.5-1.2	7.0	5,000–1.5 x 10^5
BioAssist $G2SW_{XL}$	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
SuperSW3000	4.6 x 30	4	250	30,000	0.1-0.4	12.0	1 x 10^4–5 x 10^5
$G3000SW_{XL}$	7.8 x 30	5	250	20,000	0.5-1.2	7.0	1 x 10^4–5 x 10^5
BioAssist $G3SW_{XL}$	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
$G4000SW_{XL}$	7.8 x 30	8	450	16,000	0.5-1.2	3.5	2 x 10^4–7 x 10^6
BioAssist $G4SW_{XL}$	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

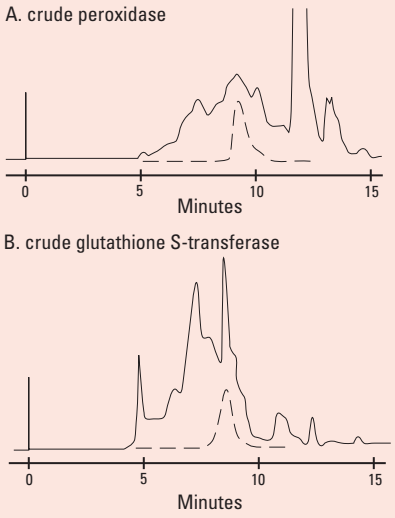
TSKgel SW Columns

APPLICATIONS

GFC 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 G3000SW$_{XL}$ column. 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 TSKgel SW$_{XL}$ packings in PEEK housings featuring the same performance as with stainless steel columns (see Figure 3). For low pressure applications a TSKgel G2000SW 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.

Figure 2

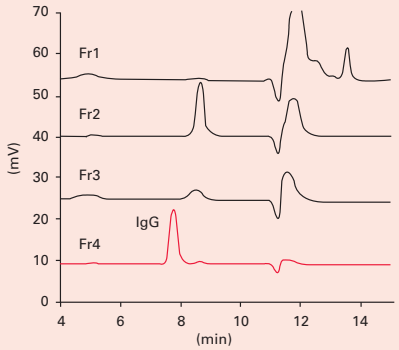
Separation of crude protein sample on TSKgel G3000SW$_{XL}$



Column:	TSKgel G3000SW$_{XL}$, 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 3

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

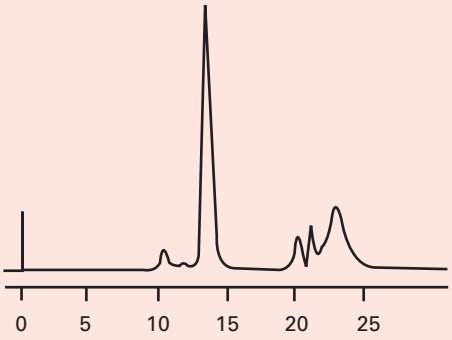


SEC assay of collected fractions from a HIC purification step

Column:	TSKgel BioAssist G3SW$_{XL}$, 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 4

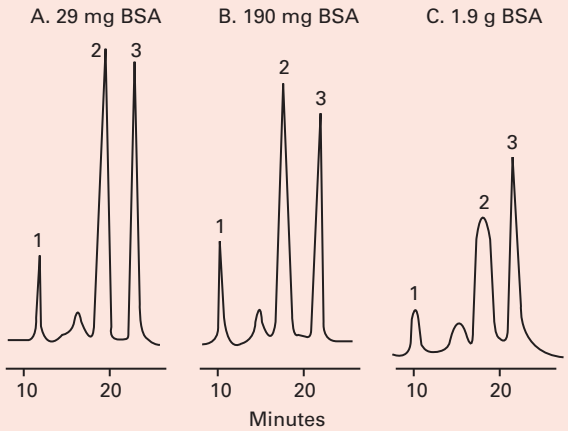
Separation of antithrombin III with TSKgel glass column



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 5

Purification of protein on preparative scale TSKgel 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

TSKgel SuperSW Columns

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

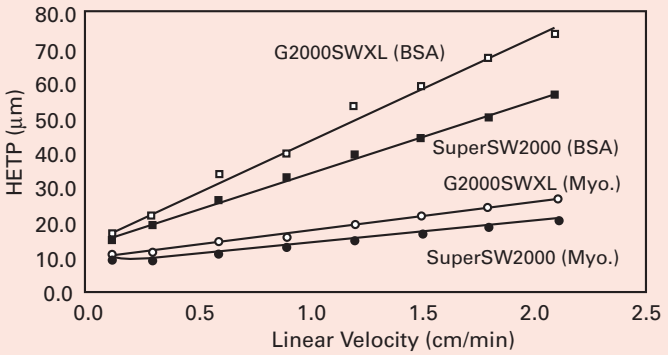
CHARACTERISTICS

TSKgel 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 TSKgel SW_{XL} 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 TSKgel 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.

Figure 6

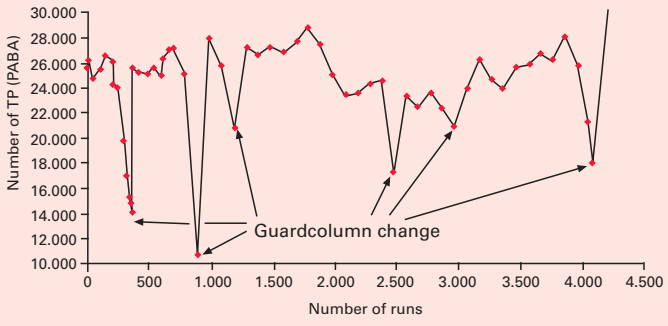
Relationship between flow rate and HETP in TSKgel SuperSW Series and TSKgel SW_{XL} Series



Column:	TSKgel SuperSW series, 4 µm, 4.6 mm ID x 30 cm L TSKgel SW_{XL} series, 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 7

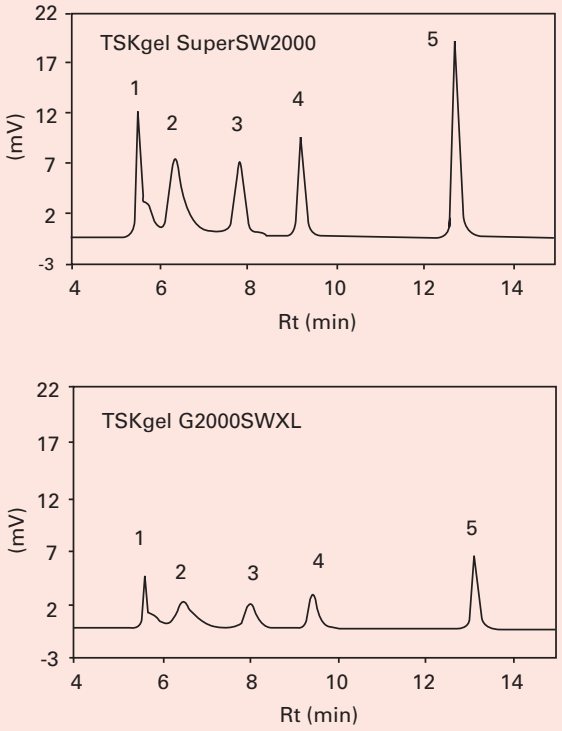
Stability of theoretical plates on a TSKgel SuperSW3000



Column:	TSKgel SuperSW3000, 4 µm, 7.8 mm I.D. x 30 cm L. + guardcolumn
Sample:	γ-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 8

Comparison of sensitivity between TSKgel SuperSW2000 and TSKgel $G2000SW_{XL}$



Column:	TSKgel SuperSW2000, 4 µm, 4.6mm ID x 30 cm L TSKgel $G2000SW_{XL}$, 5 µm, 7.8 mm ID x 30 cm L
Sample:	Standard proteins (5 µL) 1. Thyroglobulin (0.5 g/L) 2. Gamma-globulin (1g/L) 3. Ovalbumin (1g/L) 4. Ribonuclease A (1g/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 $G2000SW_{XL}$)
Detection:	UV @ 280 nm, micro flow cell

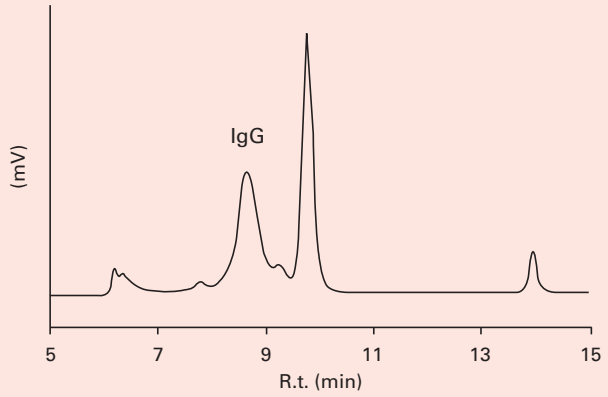
TSKgel SuperSW Columns

APPLICATIONS

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

Figure 9

Separation of human serum albumin on a TSKgel SuperSW3000

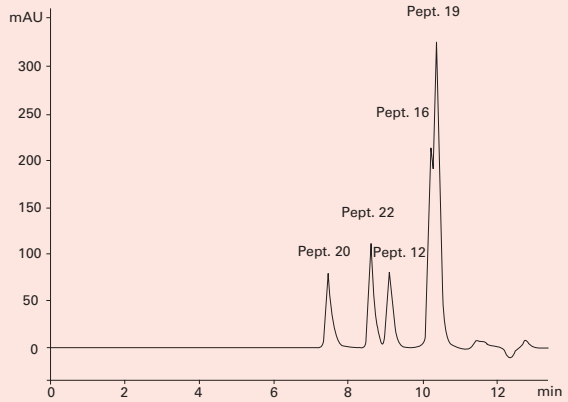


Column:	TSKgel SuperSW3000, 4 µm, 4.6 mm ID x 30 cm L
Sample:	Human serum
Elution:	0.2 M phosphate buffer (pH 6.7)
Flow rate:	0.35 mL/min
Injection vol:	5 µL
Detection:	UV @ 280 nm

Figure 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).

Figure 10

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

Table 3

TSKgel SuperSW2000 and SuperSW3000 Operating Conditions

For best results, it is recommended to use the following experimental conditions for TSKgel 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

TSKgel PW Columns

HIGHLIGHTS

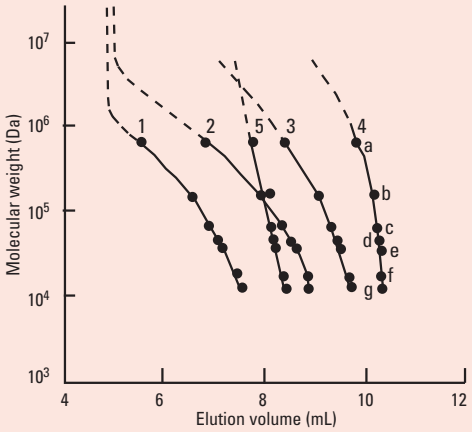
- Hydrophilic, spherical polymeric methacrylate resin pH range of 2 to 12, with up to 50% organic solvent
- Temperatures up to 80°C
- Six different pore sizes allowing wide molecular weight separation ranges
- PEEK column hardware for G6000PW packings for ultra-low sample adsorption during virus analysis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

CHARACTERISTICS

The properties for all TSKgel PW and PW_{XL} 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 TSKgel PW_{XL} columns are shown in Figure 11. Specialty resin-based columns include the mixed-bed TSKgel GMPW and TSKgel $GMPW_{XL}$ for samples with a broad molecular weight range. They include TSKgel G-Oligo-PW and TSKgel G-DNA-PW columns for oligosaccharides and for DNA or RNA respectively.

Figure 11

Protein calibration curves for TSKgel PW_{XL} columns



Column: 1. TSKgel $G3000PW_{XL}$, 2. TSKgel $G4000PW_{XL}$, 3. TSKgel $G5000PW_{XL}$, 4. TSKgel $G6000PW_{XL}$, 5. TSKgel $GMPW_{XL}$
Sample: a. thyroglobulin (660,000 Da), b. γ-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

Table 4

Properties and separation ranges for TSKgel PW type packings

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	10	125	5.000/10.000	0.5-1.2	2.0/4.0	up to 2,000
	21.5 x 60	17	125	10.000	1.0-8.0	2.0	up to 2,000
$G2500PW_{XL}$	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
$G3000PW_{XL}$	7.8 x 30	6	200	14.000	0.5-1.0	4.0	up to 5×10^4
G3000PW	7.5 x 30/60	10	200	5.000/10.000	0.5-1.2	2.0/4.0	up to 5×10^4
	21.5 x 60	17	200	10.000	1.0-8.0	2.0	up to 5×10^4
$G4000PW_{XL}$	7.8 x 30	10	500	10.000	0.3-1.0	2.0	$2,000 - 3 \times 10^5$
G4000PW	7.5 x 30/60	17	500	3.000/6.000	0.5-1.2	1.0/2.0	$2,000 - 3 \times 10^5$
	21.5 x 60	22	500	6.000	1.0-8.0	2.0	$2,000 - 3 \times 10^5$
$G5000PW_{XL}$	7.8 x 30	10	1000	10.000	0.3-1.0	2.0	$4,000 - 1 \times 10^6$
G5000PW	7.5 x 30/60	17	1000	3.000/6.000	0.5-1.2	1.0/2.0	$4,000 - 1 \times 10^6$
	21.5 x 60	22	1.000	6.000	1.0-8.0	2.0	$4,000 - 1 \times 10^6$
$G6000PW_{XL}$	7.8 x 30	13	>1000	7.000	0.3-1.0	2.0	$4 \times 10^4 - 8 \times 10^6$
BioAssist G6PW	7.8 x 30	17	>1000	3.000	0.5-1.2	1.0	$4 \times 10^4 - 8 \times 10^6$
G6000PW	7.5 x 30/60	17	>1000	3.000/6.000	0.5-1.2	1.0/2.0	$4 \times 10^4 - 8 \times 10^6$
	21.5 x 60	25	>1000	6.000	1.0-8.0	2.0	$4 \times 10^4 - 8 \times 10^6$
$GMPW_{XL}$	7.8 x 30	13	<100-1000	7.000	0.3-1.0	2.0	$500 - 8 \times 10^6$
GMPW	7.5 x 30/60	17	<100-1000	3.000/6.000	0.5-1.2	1.0/2.0	$500 - 8 \times 10^6$
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	$4 \times 10^4 - 8 \times 10^6$

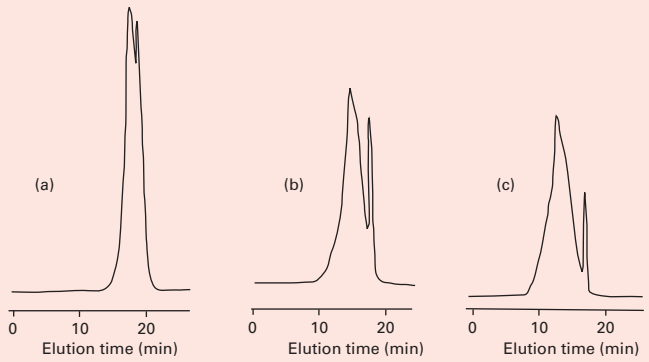
TSKgel PW Columns

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 G6000PW$_{XL}$ column gelatine elutes in one narrow peak, on the G4000PW$_{XL}$ the peak is much broader and the shoulder nearly separated from the main peak. This allows better determination of Mw/Mn and Mz/Mw.

Figure 12

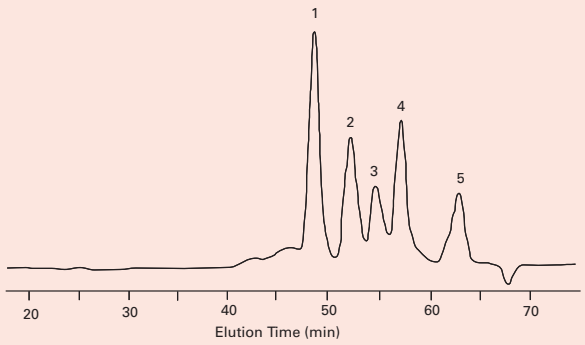
Separation of gelatin on TSKgel PW$_{XL}$ columns



Column:	(a) TSKgel G6000PW$_{XL}$, (b) TSKgel G5000PW$_{XL}$, (c) TSKgel G4000PW$_{XL}$; 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 13

Elution curve for a peptide mixture on TSKgel G3000PW$_{XL}$

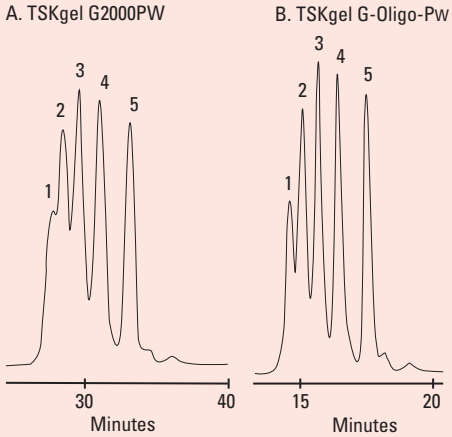


Column:	TSKgel G3000PW$_{XL}$, 6 µm, 7.8 mm ID x 30 cm L
Sample:	peptides; 1= aprotinin, 2= insulin B-chain, 3= a-MSH, 4= bradykinin potentiator C, 5= glutathione
Elution:	0.1% TFA / 45% CH_3CN
Flow rate:	1.0 mL/min

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 G3000PW$_{XL}$ column under denaturating conditions. Very large molecules, however, can be separated nicely on the G-DNA-PW column as depicted in Figure 15.

Figure 14

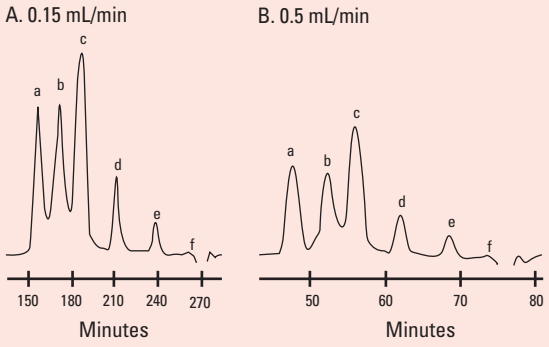
Faster analysis and higher resolution of chito-oligosaccharides on a TSKgel G-Oligo-PW column



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 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

TSKgel Alpha/SuperAW Columns

HIGHLIGHTS

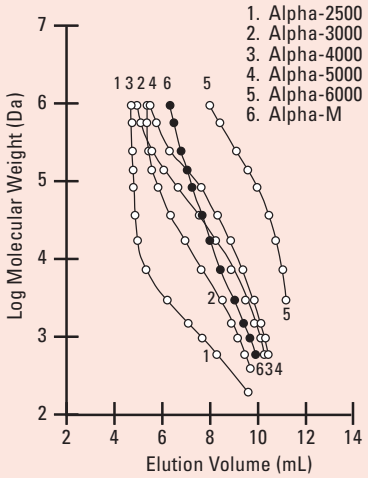
- Unique hydrophilic polyvinyl resin with rigid spherical beads
- Minimal swelling characteristics from 100% water to 100% non-polar solvents
- Excellent mechanical and chemical stability
- TSKgel SuperAW columns with reduced particle size and shorter column length provide short analysis times and high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

CHARACTERISTICS

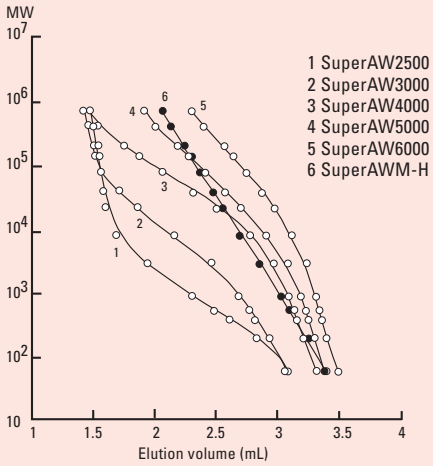
The TSKgel Alpha and the TSKgel SuperAW column series offer a new alternative for performing SEC. The columns are packed with a hydrophilic, highly crosslinked vinyl polymer which is compatible to a wide range of solvents ranging from pure aqueous up to 100 % organic mobile phases (see Figure 17). Both 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).

Figure 16

Calibration curves for TSKgel Alpha and SuperAW columns



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 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

Table 5

Typical properties of TSKgel Alpha and SuperAW type packings

TSKgel 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

TSKgel Alpha/SuperAW Columns

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.

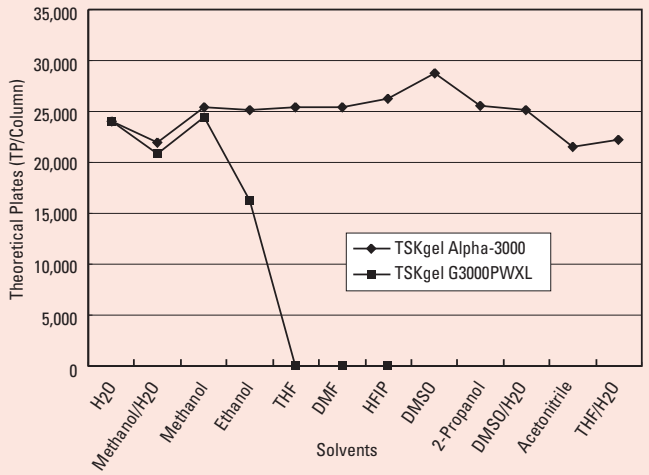
APPLICATIONS

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

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

Figure 17

Solvent compatability for TSKgel Alpha-3000 for organic solvents

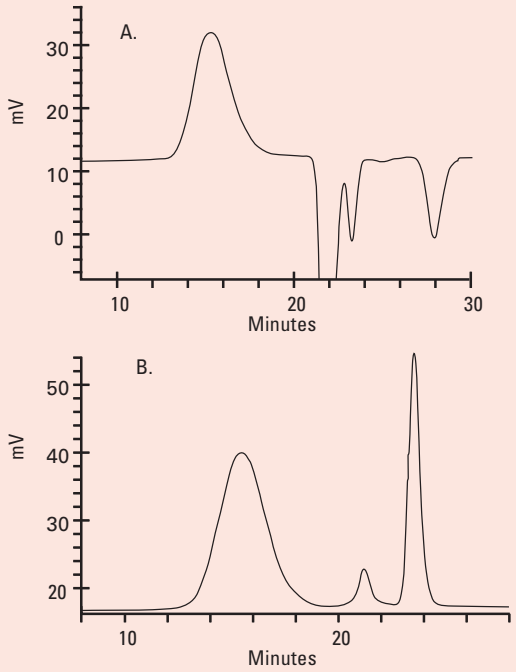


Conditions for solvent change:	
Flow rate:	1.0 mL/min
Temperature:	25°C
Time for purge.	8 h

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

Figure 18

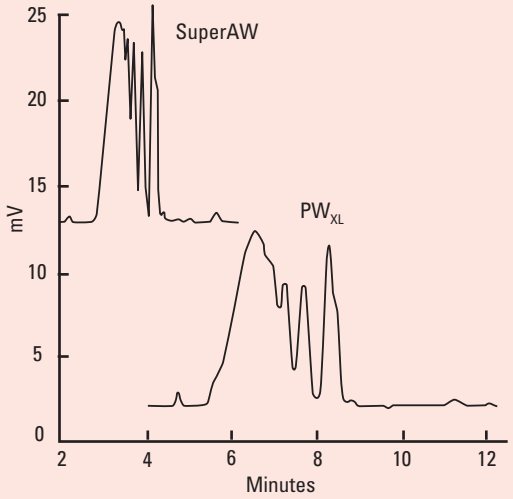
TSKgel Alpha-M separation of cellulose derivatives



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 19

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



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

Optimizing SEC

SAMPLE LOAD

In SEC, sample load on the column is limited due to the absense of a staionary phase that participates in the retention process. 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 mmID 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 TSKgel SuperSW columns for example, a 5 µL injection volume ensures optimal results. Standard injection volumes for 7.5 and 7.8 mmID columns are 20-100 µL, whereas for preparative purposes on 21.5mmID 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.

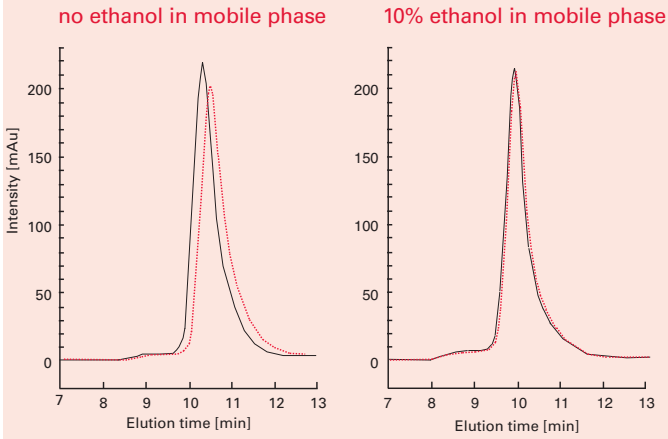
For TSKgel SW columns' mobile phases a buffer concentration between 0.1 M and 0.5 M is recommended. Under low ionic strength (< 0.1 M), ionic interactions between the sample molecules and the silica surface may occur. Under conditions of high ionic strength (>1.0 M), hydrophobic interactions are more 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 TSKgel 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 G3000SW$_{XL}$ 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. (courtesy of J.J. Ratto et al. Amgen Inc., 1996)

Figure 20

SEC of pegylated proteins



Column:	G3000SW$_{XL}$, 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/min
Detection:	UV @ 215 nm

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 additional information on TSKgel Size Exclusion columns for GFC, please consult the Tosoh Bioscience Laboratory Products Catalog.

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