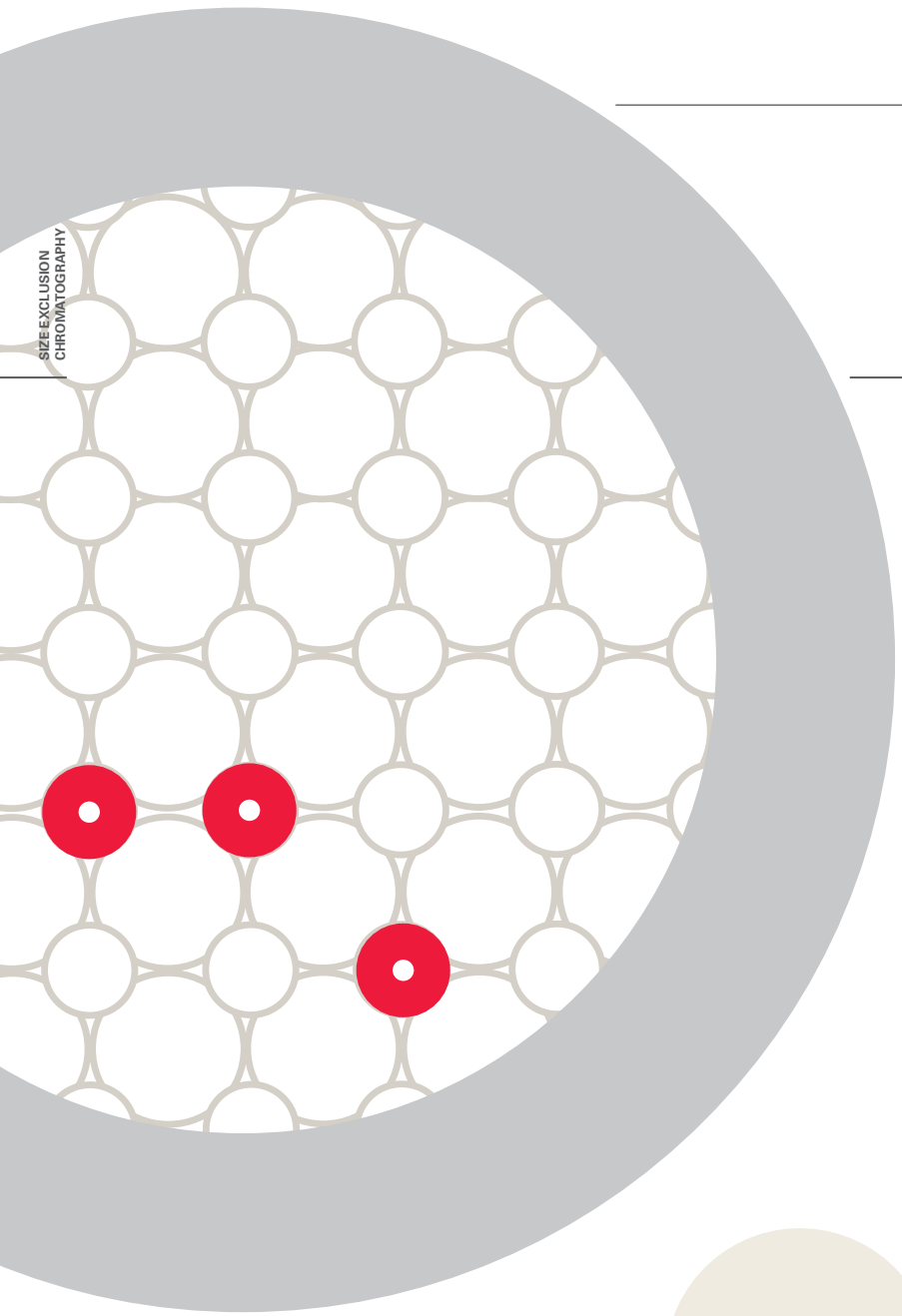
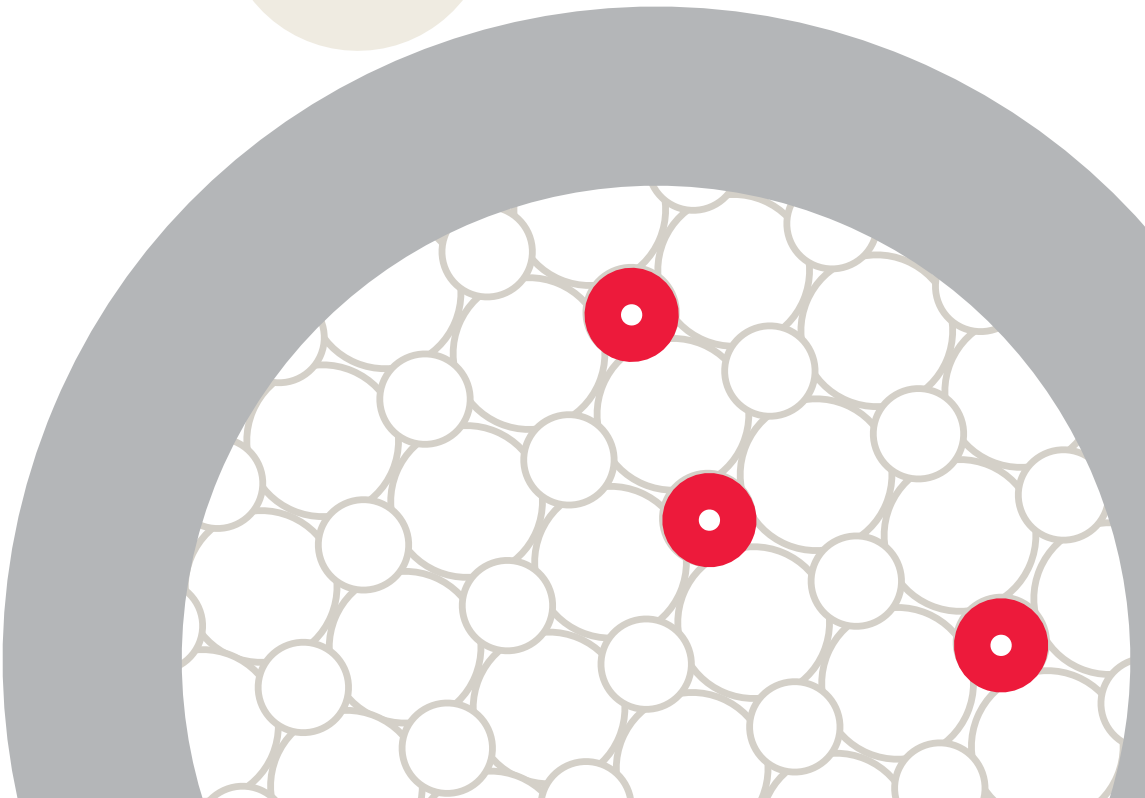




SEC
SIZE EXCLUSION CHROMATOGRAPHY

SEC PRODUCTS

TSKgel SW-type

TSKgel SW
TSKgel SWXL
TSKgel SuperSW
TSKgel SuperSW mAb
TSKgel UltraSW Aggregate

TSKgel PW-type

TSKgel PW
TSKgel PWXL
TSKgel PWXL-CP
TSkgel SuperMultiporePW
TSkgel SuperOligo PW

TSKgel Alpha-type

TSKgel Alpha
TSKgel SuperAW
TSKgel VMpak

TSKgel H-type

TSKgel HXL
TSKgel HHR
TSKgel HHR-HT
TSKgel SuperH
TSKgel SuperHZ
TSKgel Super MultiporeHZ
TSKgel MultiporeHXL

TSKgel SEC Standards

TOSOH FACT

Tosoh has a long history in size exclusion chromatography (SEC). In 1978 Tosoh first introduced porous silica-based SW columns for the isolation of proteins using LC. These first gels had particle sizes from 10 to 13 µm and were quickly adopted and referred to as the standard for analytical SEC on FPLC and HPLC systems.

As new packing materials were discovered and new bonding chemistries developed, the SEC product line has grown into four major classes of SEC columns. The following pages will help you choose the best column for your application.

INTRODUCTION TO TSKgel SIZE EXCLUSION COLUMNS

Size Exclusion Chromatography (SEC) is the dominant mode of separation for polymers. 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 column packed with porous particles. Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access all or a larger number of pores. In SEC, large molecules elute from the column first followed by smaller molecules, and the smallest molecules that can access all the pores elute last from the column. Size exclusion chromatography is the only mode of chromatography that does not involve interaction with a stationary phase by means of adsorption or partitioning of the solutes.

The terms SEC, GFC (gel filtration chromatography) and GPC (gel permeation chromatography) all refer to the same chromatographic technique. In GFC, an aqueous mobile phase is used, while an organic mobile phase is employed in GPC. The general term SEC covers both uses. Available TSKgel products are classified by application area and particle composition.

GEL FILTRATION CHROMATOGRAPHY (GFC)

The principal feature of GFC is its gentle non-interaction with the sample, enabling retention of enzymatic activity while separating multimers that are not easily distinguished by other chromatographic methods. SEC has limited peak capacity, however, requiring that the molar mass of the biomolecules differ by at least twofold. GFC is popular among biochemists for the isolation of proteins, for the removal of aggregates, to desalt a protein sample, to separate nucleic acid fractions, or to characterize water soluble polymers used in food products, paints, pharmaceutical preparations, etc.

TSKgel columns for GFC analysis consist of the TSKgel SW and PW series column lines. The main criterion in choosing between these TSKgel columns is the molar mass of the sample and its solubility. The fact that the TSKgel SW columns are based on silica and the TSKgel PW columns are derived from a hydrophilic polymer network has less impact on the separation than the particle and pore size differences between the column lines. 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.

Application area: Proteins and other biopolymers

Base material: silica

- SW
- SW$_{XL}$
- SuperSW/SuperSW mAb
- UltraSW

Due to higher resolving power, the TSKgel SW series columns are ideal for proteins and nucleic acids using an aqueous buffer as mobile phase. The TSKgel SW mAb columns within the TSKgel SW series are designed specifically for the analysis of monoclonal antibodies.

Application area: Water soluble polymers

Base material: polymethacrylate

- PW
- SuperMultiporePW
- SuperOligoPW
- PW$_{XL}$
- PW$_{XL}$-CP

TSKgel PW series columns are commonly used for the separation of synthetic polymers, oligosaccharides, nucleic acids and small viruses using aqueous buffer or salt solutions as mobile phase. The TSKgel SuperMultiporePW semi-micro SEC columns provide near linear calibration curves and are ideally suited to analyze the MW distribution of water soluble polymers with a wide range of molecular weights. The SuperOligoPW semi-micro column featuring a small particle size has been designed for fast analysis of oligosaccharides and other oligomers. The PW$_{XL}$-CP columns are developed to facilitate SEC separation of cationic polymer under low salt conditions.

FEATURES

- Rigid hydrophilic and hydrophobic packings
- Four series of SEC columns with diferent ranges of solvent compatibility
- Easy scale up

BENEFITS

- Minimal swelling and excellent physical strength
- Low adsorption resulting in high mass recovery
- Suitable for both types of size exclusion, aqueous (GFC) and non-aqueous (GPC)
- Analytical and preparative pre-packed SEC column

SEC

GEL PERMEATION CHROMATOGRAPHY (GPC)

GPC plays an important role in the characterization of polar organic-soluble and organic-soluble polymers in consumer, chemical, and petrochemical industries. GPC is often used to determine the relative molar mass of polymer samples as well as the distribution of molar masses.

Application area: Water- and organic-soluble polymers

Base material: highly crosslinked polymethacrylate

- Alpha
- SuperAW

TSKgel Alpha and SuperAW columns are compatible with a wide range of solvents and were developed for the GPC analysis of polymers of intermediate polarity, soluble in water, buffers and many organic solvents. TSKgel SuperAW columns are based on the same chemistry as TSKgel Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high throughput applications.

For the GPC analysis of organic-soluble polymers, Tosoh developed TSKgel H series, filled with polystyrene/divinylbenzene polymer particles. Each line of columns within the TSKgel H series differs in degree of inertness and operating temperature range. The proprietary multi-pore particle technology applied in some linear GPC columns ensures a wide pore size distribution in each particle leading to calibration curves with excellent linearity.

Application area: Organic-soluble polymers

Base material: polystyrene

Ultra-low adsorption columns with limited solvent range

- SuperHZ (high throughput)
- SuperMultiporeHZ
- HXL (conventional)

Low adsorption columns with expanded solvent range

- SuperH (high throughput)
- HHR (conventional)

High temperature GPC columns

- GMHHR HT/HT2

SUMMARY OF TSKgel SIZE EXCLUSION COLUMN LINES

Column line	TSKgel SW / SWXL / SuperSW / UltraSW	TSKgel PW / PWXL	TSKgel Alpha / TSKgel SuperAW	TSKgel H
Particle composition	Silica	Polymethacrylate	highly crosslinked Poly-methacrylate	PS-DVB
No. of available pore sizes	3/2/1	7	5	6
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	60-80°C (HXL, SuperHZ) 140°C (HHR and SuperH) 220°C HHR HT2
Pressure (MPa)**	1.0-12.0	1.0 - 4.0	2.0 - 4.0	1.5-6.0
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 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 and in the Ordering Information section at the end of each section.

COLUMN SELECTION GUIDE FOR TSKgel GEL FILTRATION COLUMNS

SAMPLE			COLUMN SELECTION		SELECTION CRITERIA
			FIRST CHOICE	ALTERNATIVE	
Carbohydrates	polysaccharides		TSKgel GMPW$_{XL}$ TSKgel SuperMultiporePW	TSKgel G5000PW$_{XL}$ & TSKgel G3000PW$_{XL}$	large pore size, small particles, linear calibration curve, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW TSKgel SuperOligoPW	TSKgel 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$_{XL}$, TSKgel BioAssist G4SW$_{XL}$ TSKgel SuperSW3000 or TSKgel G3000SW$_{XL}$	 TSKgel BioAssist G3SW$_{XL}$	suitable pore sizes
	RNA		TSKgel G4000SW$_{XL}$ TSKgel SuperSW3000 or TSKgel G3000SW$_{XL}$	TSKgel BioAssist G4SW$_{XL}$ TSKgel BioAssist G3SW$_{XL}$	suitable pore sizes
	oligonucleotides		TSKgel G2500PW$_{XL}$		small pore size, ionic interaction
Proteins	small to medium sized proteins		TSKgel SuperSW3000 TSKgel G3000SW$_{XL}$ TSKgel BioAssist G3SW$_{XL}$ TSKgel G4000SW$_{XL}$ TSKgel SuperSW2000 or TSKgel G2000SW$_{XL}$	TSKgel G3000PW$_{XL}$ / G4000PW$_{XL}$ TSKgel BioAssist G4SW$_{XL}$ TSKgel BioAssist G2SW$_{XL}$	small particles small to medium range pore sizes
	antibodies		TSKgel SuperSW mAB HR/HTP TSKgel UltraSW Aggregate		fragments/monomer & dimer higher aggregates
	large proteins	low density lipoprotein	TSKgel G6000PW$_{XL}$ or TSKgel G5000PW$_{XL}$		large pore sizes
		gelatin	TSKgel GMPW$_{XL}$ TSKgel SuperMultiporePW-M TSKgel G3000SW$_{XL}$	TSKgel G5000PW$_{XL}$ & G3000PW$_{XL}$	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000 TSKgel G3000SW$_{XL}$ TSKgel BioAssist G3SW$_{XL}$ or TSKgel G2000SW$_{XL}$	TSKgel SuperSW2000 / TSKgel G3000PW$_{XL}$ TSKgel BioAssist G2SW$_{XL}$	small to medium range pore size, versatile
	small		TSKgel G2500PW$_{XL}$	TSKgel SuperSW2000 / TSKgel G2000SW$_{XL}$	linear calibration curve, high resolving power
Viruses			TSKgel G6000PW$_{XL}$ or TSKgel G5000PW$_{XL}$ TSKgel SuperMultiporePW-H		large pore size, high resolving power
Synthetic polymers			TSKgel GMPW$_{XL}$ or TSKgel Alpha-M TSKgel SuperMultiporePW	TSKgel G5000PW$_{XL}$ & G3000PW$_{XL}$ / TSKgel Alpha-5000 & Alpha-3000	large pore size, low adsorption, linear calibration curve
	cationic		TSKgel G3000PW$_{XL}$-CP TSKgel G5000PW$_{XL}$-CP TSKgel G6000PW$_{XL}$-CP		medium to large pore size, low adsorption, linear calibration curve
Synthetic oligomers	nonionic		TSKgel G-Oligo-PW TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500 TSKgel SuperOligoPW and TSKgel SuperMultiporePW-N	TSKgel G2500PW / TSKgel SuperAW2500	small pore size, high resolving power
	anionic		TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500	TSKgel G2500PW / TSKgel SuperAW2500	small pore size, ionic interaction

SEC

TSKgel SW, SWXL AND SuperSW GEL FILTRATION COLUMNS

HIGHLIGHTS

- Dedicated columns for the analysis of monoclonals available
- TSKgel SW-type columns are all based on spherical silica particles with very high internal pore volumes.
- Silica particles in SW-type columns are chemically bonded with polar diol groups.
- SW-type columns feature low residual adsorption, which is essential for gel filtration analysis.
- Various pore sizes ranges available.
- Stainless steel, glass and PEEK column hardware available.

Tosoh recently added three TSKgel SW mAb columns to the renowned line of TSKgel SW series SEC columns. The TSKgel SW mAb columns meet the growing demand for the higher resolution and high throughput separation of monoclonal antibody (mAb) monomer and dimer/fragment, as well as higher resolution of mAb aggregates. While mAbs can be analyzed using many different modes of HPLC, size exclusion is best for determination of aggregate and fragment content.

TSKgel SW series columns contain a large pore volume per unit column volume, which results in either higher MW selectivity or better resolution when analyzing proteins. They are based on highly porous silica particles, the surface of which has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups. TSKgel SW series columns stand out from other silica- or polymer-based high performance size exclusion columns by virtue of their large pore volumes and low residual adsorption.

TSKgel SW mAb, SW, SuperSW and Ultra SW columns are stable from pH 2.5 to 7.5 and can be used in 100% aqueous conditions. The different pore sizes of the TSKgel SW series columns result in different exclusion limits for globular proteins, polyethylene oxides and dextrans, as summarized in TABLE I. Furthermore, different particle sizes, column dimensions and housing materials are available for each of the TSKgel SW series columns. When the protein analysis needs to be performed in a metal free environment, the BioAssistSW series offers TSKgel SW packings in PEEK housings, featuring the same performance as stainless steel columns.

RECOMMENDATIONS FOR TSKgel SW SERIES SELECTION

Samples of known molecular weight

Calibration curves for each TSKgel SW series column are provided in this catalog. Each curve represents a series of various standards (protein, PEO, or globular proteins, for example) with known molar masses. The molar mass range of the compound to be analyzed should be within the linear range of the calibration curve and similar to the chemical composition and architecture of the calibration standards.

Samples of unknown molecular weight

TSKgel G3000SWXL is the ideal scouting column. If the protein of interest elutes near the exclusion volume, then G4000SWXL is the logical next step. conversely, if the protein of interest elutes near the end of the chromatogram, try the G2000SWXL.

TABLE I

Properties and separation ranges for TSKgel SW-type packings

			Molecular weight of sample (Da)		
TSKgel packing	**Particle size (µm)**	**Pore size (nm)**	**Globular proteins**	**Dextrans**	**Polyethylene glycols and oxides**
SuperSW2000	4	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
G2000SWXL/BioAssist G2SWXL	5	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
QC-PAK TSK 200	5	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
G2000SW	10, 13, 20	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
SuperSW3000	4	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
SuperSW mAb	4	25	$1 \times 10^4 – 5 \times 10^5$		
G3000SWXL/BioAssist G3SWXL	5	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
QC-PAK TSK 300	5	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
G3000SW	10, 13, 20	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
UltraSW Aggregate	3	30	$1 \times 10^4 – 2 \times 10^6$		
G4000SWXL/BioAssist G4SWXL	8	45	$2 \times 10^4 – 7 \times 10^6$	$4 \times 10^3 – 5 \times 10^5$	$2 \times 10^3 – 2.5 \times 10^5$
G4000SW	13, 17	45	$2 \times 10^4 – 7 \times 10^6$	$4 \times 10^3 – 5 \times 10^5$	$2 \times 10^3 – 2.5 \times 10^5$

Data generated using the following conditions:

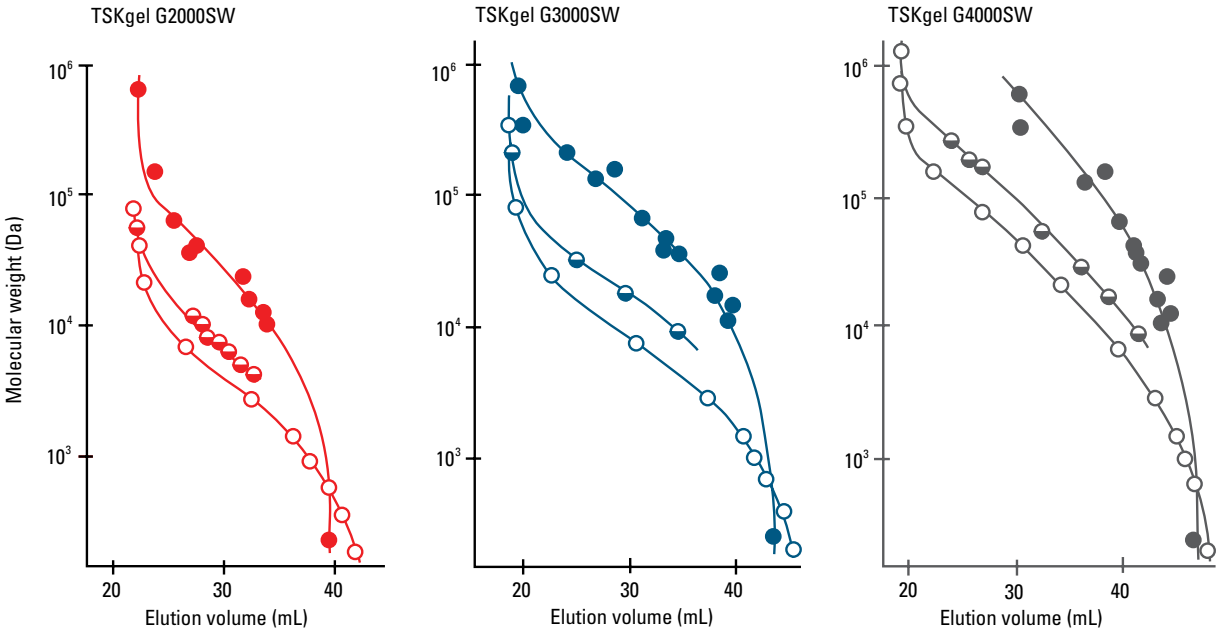
Columns: *Two 4 µm, 4.6 mm ID x 30 cm L TSKgel SuperSW columns in series; two 5 µm, 7.8 mm ID x 30 cm L TSKgel SWXL columns in series; two 10 µm, 7.5 mm ID x 60 cm L TSKgel SW columns in series*

Elution: *Globular proteins: 0.3 mol/L NaCl in 0.1 mol/L (0.05 mol/L for SWXL columns) phosphate buffer, pH 7.0*
Dextrans and polyethylene glycols and oxides (PEOs): distilled water

CALIBRATION CURVES FOR TSKgel SW-TYPE GEL FILTRATION COLUMNS

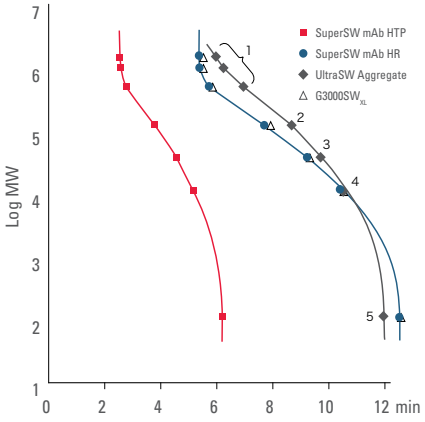
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Polyethylene oxide, dextran and protein calibration curves for TSKgel SW columns

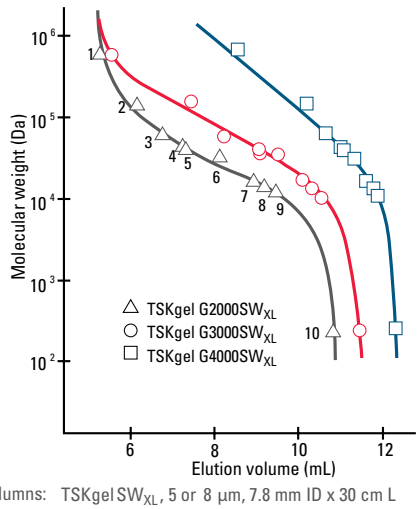


Column: TSK-GEL SW, two 7.5 mm ID x 60 cm L columns in series
Sample: ● proteins, ○ polyethylene oxides, ◒ dextrans
Elution: dextrans and polyethylene oxides: distilled water; proteins: 0.3 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0
Flow Rate: 1.0 mL/min
Detection: UV @ 220 nm and RI

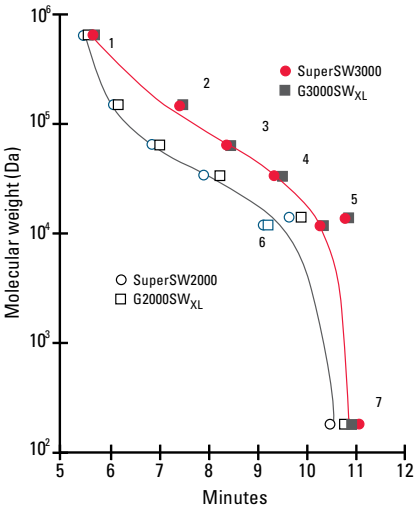
Calibration curves for TSKgel SW columns



Columns: TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm
TSKgel SuperSW mAb HR, 4 µm
TSKgel UltraSW Aggregate, 3 µm
TSKgel G3000SWXL, 4 µm, (all 7.8 mm ID × 30 cm)
Sample: 1. Thyroglobulin (MW 640,000), 2. γ-Globulin (MW 155,000), 3. Ovalbumin (MW 47,000), 4. Ribonuclease A (MW 13,700), 5. p-Aminobenzoic acid (MW 137)
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7, 0.05% NaN_3
Flow rate: 1.0 mL/min, 0.35 mL/min (SuperSW mAb HTP)
Temp.: 25°C
Detection: UV @ 280 nm
Inj. vol.: 10 µL, 5 µL (SuperSW mAb HTP)



Columns: TSKgel SW_{XL}, 5 or 8 µm, 7.8 mm ID x 30 cm L
Sample: 1. thyroglobulin (660,000 Da); 2. IgG (160,000 Da) 3. BSA (67,000 Da); 4. ovalbumin (43,000 Da); 5. peroxidase (40,200 Da); 6. β-lactoglobulin (18,400 Da); 7. myoglobin (16,900 Da) 8. ribonuclease A (12,600 Da); 9. cytochrome C (12,400 Da); 10. glycine tetramer (246 Da)
Elution: 0.3 mol/L NaCl in 0.1 mol/L sodium phosphate buffer, pH 7.0
Detection: UV @ 220 nm



Columns: TSKgel SWXL, 5µm, 7.8 mm ID x 30 cm, TSKgel SuperSW, 4 µm, 4.6 mm ID x 30 cm
Sample: proteins: 1. thyroglobulin (660,000 Da); 2. γ-globulin (150,000 Da); 3. BSA (67,000 Da); 4. β-lactoglobulin (18,400 Da); 5. lysozyme (14,500 Da); 6. cytochrome C (12,400 Da); 7. triglycine (189 Da)
Elution: 0.15 mol/L phosphate buffer (pH 6.8)
Flow rate: 0.35 mL/min for SuperSW; 1.0 mL/min for SW_{XL}
Temperature: 25°C

SEC

TSKgel SW-TYPE GEL FILTRATION COLUMNS

Proteins (general)
Choose one of the TSKgel SWXL columns using the calibration curves on PAGE 12 to select the appropriate pore size based on knowledge or estimate of protein size.

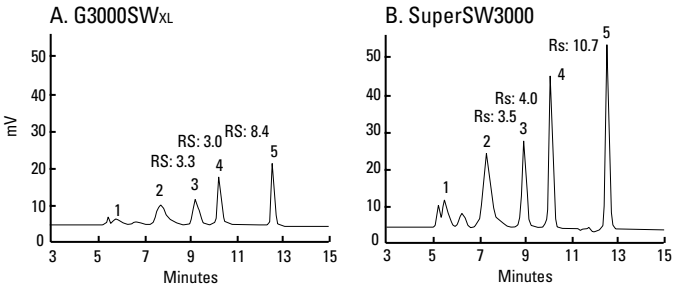
Monoclonal antibodies
TSKgel SuperSW mAb columns have been developed for the analysis of monoclonal antibodies. They provide higher resolution (HR) or faster analysis (HTP) than the TSKgel G3000SWXL which is traditionally used for quality control in many QC labs. TSKgel SuperSW3000 is utilized when sample is limited or at very low concentration.

Peptides
TSKgel G2000SWXL is the first selection for the analysis of peptides. TSKgel SuperSW2000 is utilized when sample is limited or at very low concentration.

Other
The use of TSKgel SuperSW columns requires optimization of the HPLC system with respect to extra-column band broadening. Capillary tubing ID, injection volume, detector cell volume, and detector time constant all need to be reduced to fully benefit from the high column efficiency and small peak volumes of the SuperSW columns. Use SW columns when not sample limited or when larger amounts of sample need to be isolated.

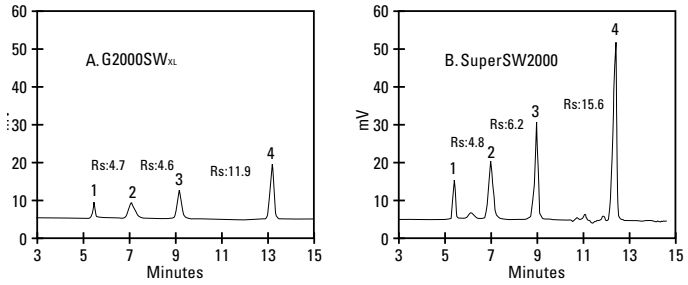
COMPARING TSKgel SW, SWXL AND SuperSW GEL FILTRATION COLUMNS
FIGURE 1 & FIGURE 2 show the increased resolution and sensitivity of the TSKgel SuperSW columns compared to TSKgel SWXL columns. This is due to the smaller particle size (4 vs. 5 µm) and the narrow column diameter (4.6 mm ID).

FIGURE 1
Comparison of TSKgel Super SW3000 and TSKgel G3000SWXL for the separation of proteins



Column: A. TSKgel G3000SWXL, 7.8 mm ID x 30 cm L;
B. TSKgel SuperSW3000, 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 mol/L $NaSO_4$ in 0.1 mol/L in phosphate buffer with 0.05 % NaN_3, pH 6.7; Flow rate: 1.0 mL/min for G3000SWXL; 0.35 mL/min for SuperSW3000;
Temp: 25°C; Detection: UV @ 220 nm

FIGURE 2
Comparison of TSKgel Super SW2000 and TSKgel G3000SW for the separation of Proteins



Column: A. TSKgel G2000SWXL, 7.8 mm ID x 30 cm L;
B. TSKgel SuperSW2000, 4.6 mm ID x 30cm 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);
Inj. Volume: 5 µL; Elution: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05 % NaN_3 (pH 6.7);
Flow rate: 0.35 mL/min for SuperSW2000; 1.0 mL/min for G2000SWXL;
Temp: 25°C; Detection: UV @ 280 nm

APPLICATIONS OF TSKgel SW-TYPE GEL FILTRATION COLUMNS

ANALYSIS OF MONOCLONAL ANTIBODIES:

The TSKgel SuperSW mAb size exclusion series consists of three specialized columns designed for the separation and analysis of monoclonal antibodies (mAb). Compared to competitive columns, these new stainless steel, silica-based TSKgel columns offer reduced lot-to-lot variation, long column life, reduction of unspecified adsorption, and superior recovery of aggregates. TSKgel mAb columns are compatible with both HPLC and UHPLC systems.

These columns are available within the TSKgel SW mAb column line:

- TSKgel SuperSW mAb HR
- TSKgel SuperSW mAb HTP
- TSKgel UltraSW Aggregate

TSKgel SuperSW mAb HR and SuperSW mAb HTP both contain 4 µm particles. The HR designation represents the high resolution analysis of mAb monomer, dimer, and fragments, while the HTP stands for "high throughput" due to the smaller dimensions (4.6 mm ID × 15 cm). The TSKgel UltraSW Aggregate column is packed with particles featuring a smaller particle size, 3 µm, and slightly larger pore size. It offers high resolution separation of mAb multimers.

These columns utilize a unique technology, which produces a shallow calibration curve in the molar mass region of a typical antibody. The calibration curve for the TSKgel SuperSW mAb HR column is similar to that of TSKgel G3000SW$_{XL}$. It has a shallower slope than the TSKgel UltraSW Aggregate column around the molar mass range of γ-globulin resulting in a higher resolution for that mass range.

HIGH SPEED ANALYSIS OF THERAPEUTIC mAb

A shorter column length allows the TSKgel SuperSW mAb HTP column to provide fast and efficient run times in the high resolution separation of a mAb monomer and dimer. FIGURE 3 shows no loss in resolution in the analysis of a therapeutic mAb at a 0.50 mL/min flow rate and an increased pressure of 5.0 MPa.

HIGH RESOLUTION SEPARATION OF MONOMER & DIMER

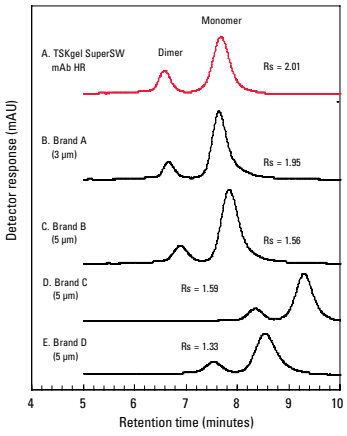
FIGURE 4 demonstrates the superior resolution of the TSKgel SuperSW mAb HR column compared to four competitive columns in the analysis of a mAb monomer and dimer. TSKgel SuperSW mAb HR shows excellent resolution of gamma-globulin dimer and monomer.

DURABILITY OF SuperSW mAb COLUMNS

FIGURE 5 demonstrates the good durability of the TSKgel SuperSW mAb HR column through the reproducibility of resolution for a γ-globulin sample injection.

FIGURE 4

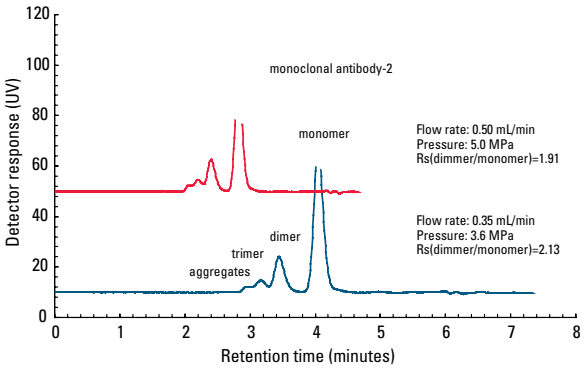
Comparison of resolution of mAb monomer and dimer



Columns: A. TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm;
B. Brand A, 3 µm, 7.8 mm ID × 30 cm; C. Brand B, 5 µm, 7.8 mm ID × 30 cm;
D. Brand C, 5 µm, 8.0 mm ID × 30 cm; E. Brand D, 5 µm, 8.0 mm ID × 30 cm
Mobile phase: 200 mmol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
Temperature: 25 °C; Injection vol.: 10 µL
Sample: IgG (human polyclonal), 1.0 g/L

FIGURE 3

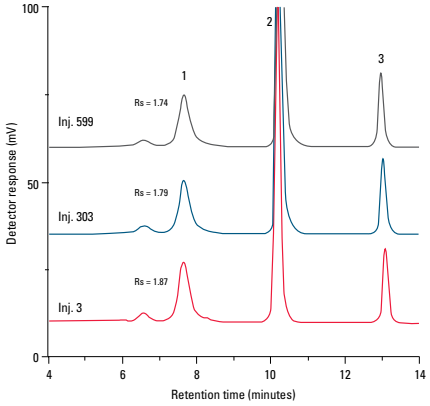
High speed separation of therapeutic mAb



Column: TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 0.50 mL/min, 0.35 mL/min; Detection: UV @ 280 nm
Temperature: 25 °C; Sample: monoclonal antibody-2
(mouse-human chimeric IgG, Erbitux®), 5 µL

FIGURE 5

High durability of TSKgel SuperSW mAb HR column



Column: TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 0.8 mL/min; Detection: UV @ 280 nm
Injection vol.: 10 µL
Samples: 1. γ-Globulin; 2. Cytochrome C; 3. DNP-L-Alanine

SEC

APPLICATIONS OF TSKgel SW-TYPE GEL FILTRATION COLUMNS

SEPARATION OF HIGHER AGGREGATES

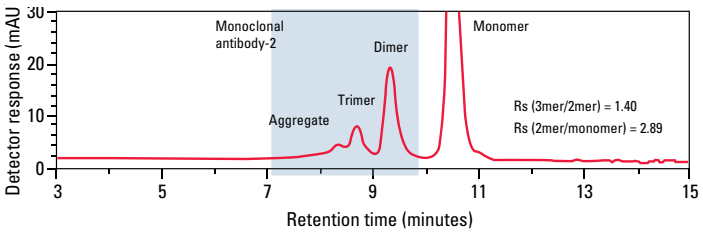
TSKgel UltraSW Aggregate has a smaller particle size than the SuperSW material, and offers high resolution separation of mAb multimers. FIGURE 6 shows the analysis of a mouse-human chimeric IgG using the TSKgel UltraSW Aggregate column. Superior resolution of the mAb trimer and dimer is obtained. The smaller particle size (3 µm) and higher molecular weight exclusion limit (2,500 kDa, globular proteins) of the TSKgel UltraSW Aggregate column, compared to the TSKgel SuperSW mAb HR and HTP columns, allows for high resolution separation of mAb multimers and aggregates.

SEPARATION OF LARGE PROTEINS

TSKgel UltraSW Aggregate provides a larger pore size than TSKgel SuperSW3000. It is therefore not only suited for the analysis of mAb aggregates but can also be used for the analysis of other large proteins and their aggregates. The analysis of a heat denatured, large hydrophobic metalloprotein, apoferritin, is shown in FIGURE 7. A set of six, 0.3 mL HPLC vials each containing 100 µL stock solution of apoferritin was used for protein thermal denaturation. Thermal denaturation was carried out at 60°C using an electric heating block. Individual sample vials were tightly capped and exposed to the heat for 5, 20, 30, 45, and 60 minutes. Samples were analyzed using a TSKgel UltraSW Aggregate column at the end of each incubation time period. The TSKgel Ultra SW Aggregate column yielded high resolution between the monomer and dimer. The trimer, tetramer and higher order aggregates of apoferritin were well separated.

FIGURE 6

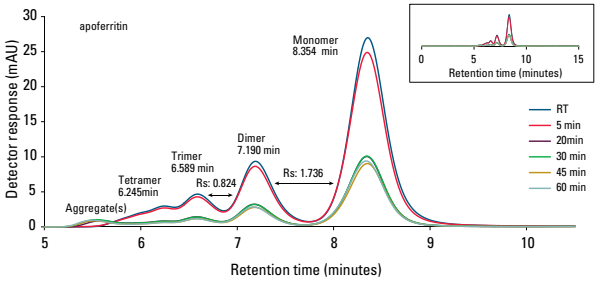
Separation of mAb trimer and dimer



Column: TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 0.8 mL/min; Detection: UV @ 280 nm
Temperature: 25 ˚C; Sample: monoclonal antibody-2 (mouse-human chimeric IgG, Erbitux), 10 µL

FIGURE 7

Analysis of heat induced forced denatured, large hydrophobic metalloprotein, apoferritin



Protein	Molecular weight (kDa)			
	Monomer	Dimer	Trimer	Tetramer
ferritin and apoferritin	450	900	1350	1800

Column: TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm
Mobile phase: 50 mmol/L potassium phosphate (monobasic), 50 mmol/L sodium phosphate (dibasic), 100 mmol/L sodium sulfate, 0.05% NaN_3, pH 6.7
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
Temperature: 30 °C; Injection vol.: 10 µL
Samples: ferritin – Sigma, 4.7 g/L, in saline (0.9% NaCl in water) solution, stored at 2-8 °C apoferritin – Sigma, 5.0 g/L, in 50% glycerol and 0.075 mol/L sodium chloride, stored at -20 °C

APPLICATIONS OF TSKgel SW-TYPE GEL FILTRATION COLUMNS

MEMBRANE PROTEINS

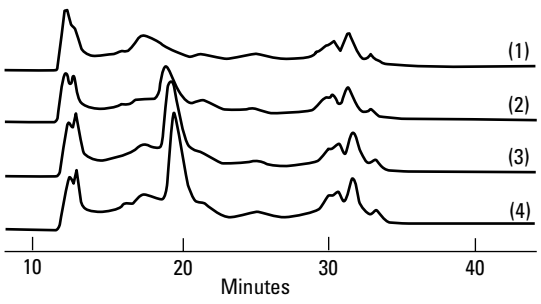
A TSKgel G3000SW column was used to study the effect of different concentrations of the non-ionic surfactant octaethyleneglycol dodecylether on the analysis of membrane proteins from a crude extract from rat liver microsome. The effect of different concentrations of surfactant on the separation of membrane proteins is seen in FIGURE 8. As the concentration of octaethyleneglycol dodecylether increases to 0.05%, the main peak becomes sharper and recovery increases. Caution: we recommend that columns that have been used with a surfactant-containing mobile phase are dedicated for that particular use.

NUCLEIC ACIDS

Separation of four E. coli RNAs, shown in FIGURE 9, confirms the high performance of TSKgel G4000SW columns for samples with a wide high molar mass range. The sample consists of 4S tRNA (2.5 × 10^4 Da), 5S rRNA (3.9 ×10^4 Da), 16S rRNA (5.6 × 10^5 Da), and 23S rRNA (1.1 × 10^6 Da). All four polynucleotides are within the molar mass range recommended for this TSKgel SW column.

FIGURE 8

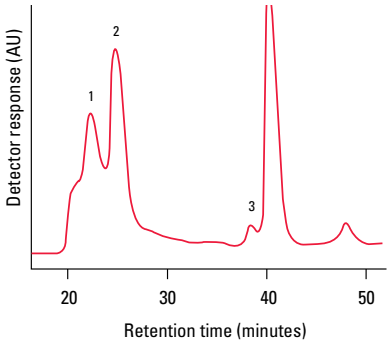
Analysis of membrane protein with differing surfactant concentrations in the mobile phase



Column: TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm
Mobile phase: (0.2 mol/L sodium chloride + 20% glycerol + octaethylene glycol dodecylether) in 50 mmol/L 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
Sample: membrane protein from a crude extract from rat liver microsome

FIGURE 9

Separation of total E. coli RNA



Columns: TSKgel G4000SW, 13 µm, 7.5 mm ID × 30 cm × 2
Mobile phase: 0.13 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0, + 1 mmol/L EDTA
Flow rate: 1.0 mL/min; Detection: UV @ 260 nm; Injection vol.: 5 µg
Sample: 0.1 mL of 1:10 diluted solution of total E. coli RNA:
1. 23s rRNA (1.1 × 106 Da); 2. 16s rRNA (5.6 × 105 Da)
3. 5s rRNA (3.9 × 104 Da); 4. 4s rRNA (2.5 × 104 Da)

SEC

ENZYMES

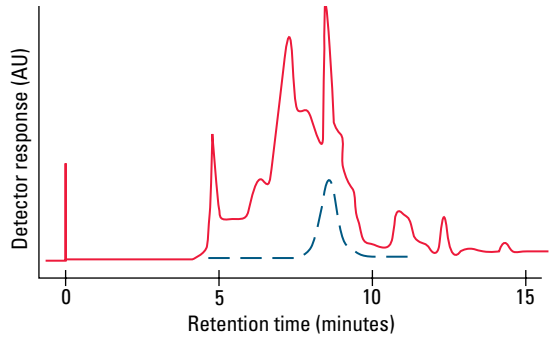
Mobile phase conditions in gel filtration are optimized to ensure little or no interaction of the sample with the packing material. This gentle technique allows for high recovery of enzymatic activity. A crude sample of glutathione S-transferase was separated in only 15 minutes on a TSKgel G3000SWXL column and activity recovery was 98% and 89%, respectively. The elution profile of the separation in FIGURE 10 shows that all of the activity eluted in a narrow band of about 1.5 mL..

SEC-MALS ANALYSIS OF PROTEIN AGGREGATION

TSKgel G3000SWXL is the industry standard for aggregation analysis in quality control of monoclonal antibodies. FIGURE 11 depicts the analysis of mAb Aggregates with UV, refractive index (RI) and multi angle light scattering (MALS) detection.

FIGURE 10

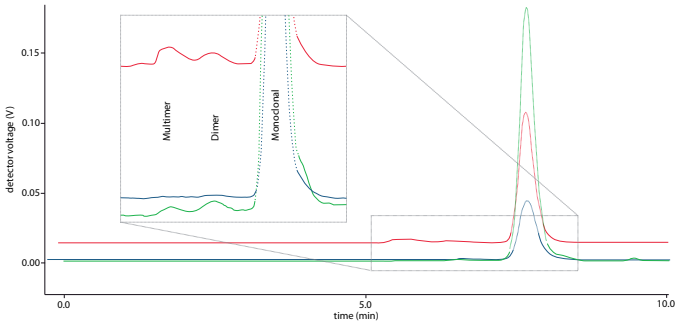
Separation of crude protein sample on TSKgel G3000SWXL



Column: TSKgel G3000SWXL 5 µm, (7.8 mm ID x 30 cm L); Sample: crude glutathione S-transferase from guinea pig liver extract, 0.7 mg in 0.1 mL;
Elution: 0.3 mol/L NaCl in 0.05 mol/L phosphate buffer, pH 7;
Flow rate: 1.0mL/min; Detection: UV@220 nm (solid line) and enzyme assay tests (dashed line); Recovery: enzymatic activity recovered was 89 %

FIGURE 11

SEC-Mals-UV-RI analysis of mAb aggregates



Column: TSKgel G3000SWXL column, 5 µm, 7.8 mm ID x 30 cm L
Sample: monoclonal antibody, Inj.volume: 20 µL;
Mobile phase: phosphate buffered saline (PBS); Flow rate: 1 mL/min;
Detection: MALS (red), refractive index (blue) & UV @ 280 nm (green);
HPLC System: LC-20A prominence, Shimadzu;
MALS detector: miniDAWN™ TREOS, Wyatt Techn. Corp.

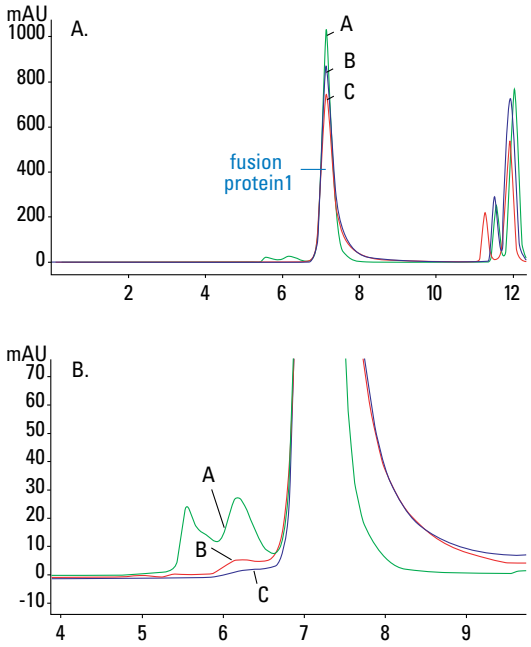
HIGH RESOLUTION ANALYSIS OF FUSION PROTEINS

During method development, many variables are examined to ensure method robustness. Factors such as elution profile, peak shape, and recovery are required to be consistent. During a method re-qualification several variables were investigated to eliminate non-specific binding and increase the robustness of an established QC method using a TSKgel SuperSW3000 column.

As shown in FIGURE 12, excessive peak tailing of "fusion protein 1" is evident with the use of 0.2 mol/L NaCl (chromatogram C). Additionally, the expected protein dimer and trimer aggregates are not visible. By switching from 0.2 mol/L sodium chloride to 0.2 mol/L of the more chaotropic sodium perchlorate salt, together with a two-fold reduction in the buffer concentration, less peak tailing and distinct peaks for the dimer and trimer species of mAb 1 resulted (chromatogram B). Doubling the perchlorate concentration to 0.4 mol/L provided further improvement in the peak shape of fusion protein 1 and associated aggregate species (chromatogram A). FIGURE 12B is an enlargement of the baseline region, showing an improved peak shape of the dimer and trimer aggregates with the use of 0.4 mol/L $NaClO_4$.

FIGURE 12

Overlays of antibody fusion protein analysis



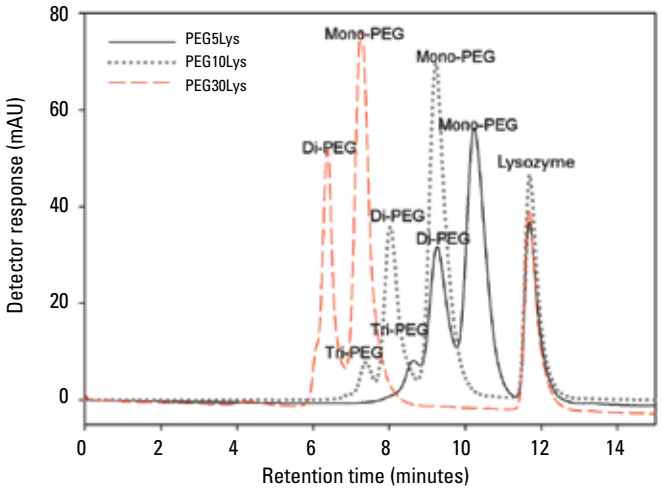
Column: TSKgel SuperSW3000, 4 µm, 4.6 mm ID x 30 cm L;
Mobile phase: c: 0.4 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4, b: 0.2 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4, a: 0.2 mol/L NaCl, 0.1 mol/L NaH_2PO_4;
Flow rate: 0.35 mL/min; Detection: UV @ 214 nm; Injection vol.: 5 µL;
Samples: antibody fusion protein

PEGYLATED PROTEINS

Chemical modification of therapeutic proteins is of increasing interest. One of the most frequently used protein modification methods, PEGylation, changes the biochemical and physicochemical properties of the protein, which can result in several important benefits, among them more effective target delivery, slower in vivo clearance, and reduced toxicity and immunogenicity of therapeutic proteins. After PEGylation reaction the mixture has to be purified in order to remove non-reacted protein and undesired reaction products. A TSKgel G3000SWXL column was used for the characterization of PEGylated lysozyme, as shown in FIGURE 13. A random PEGylation of lysozyme using methoxy PEG aldehyde of sizes 5 kDa, 10 kDa and 30 kDa was performed. The retention volumes of PEGylated lysozymes were used to assign the peaks based on a standard calibration curve. As a result of PEGylation, a large increase in the size of lysozyme by size exclusion chromatography was observed. The SEC elution position of lysozyme modified with a 30 kDa PEG was equivalent to that of a 450 kDa globular protein. There was a linear correlation between the theoretical molar mass of PEGylated protein and the molar mass calculated from SEC. This result illustrates the strong effect that PEG has on the hydrodynamic radius of the resulting PEGylated protein.

FIGURE 13

SEC analysis of PEGylation reaction mixtures



Column: TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm
Mobile phase: 0.1 mol/L phosphate buffer, 0.1 mol/L Na_2SO_4, pH 6.7
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm; Injection vol.: 20 µL
Sample: 5, 10, 30 kDa methoxy PEG aldehyde

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min)	Maximum pressure drop (MPa)
TSKgel Stainless steel columns							
0018674	SuperSW2000	4.6	30	4	≥ 30,000	0.1 -0.35	12.0
0021845	SuperSW3000	1.0	30	4	≥ 18,000	0.016	12.0
0021485	SuperSW3000	2.0	30	4	≥ 25,000	0.065	12.0
0018675	SuperSW3000	4.6	30	4	≥ 30,000	0.1 - 0.35	12.0
0022854	SuperSW mAb HR - NEW -	7.8	30	4	≥ 30,000	0.5 -1.0	12.0
0022855	SuperSW mAb HTP - NEW -	4.6	15	4	≥ 15,000	0.1 -0.35	8.0
0022856	UltraSW Aggregate - NEW -	7.8	30	3	≥ 35,000	0.5 -1.0	12.0
0008540	G2000SWXL	7.8	30	5	≥ 20,000	0.5 -1.0	7.0
0008541	G3000SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	7.0
0008542	G4000SWXL	7.8	30	8	≥ 16,000	0.5 - 1.0	3.5
0016215	QC-PAK GFC 200	7.8	15	5	≥ 10,000	0.5 -1.0	4.0
0016049	QC-PAK GFC 300	7.8	15	5	≥ 10,000	0.5 -1.0	4.0
0005788	G2000SW	7.5	30	10	≥ 10,000	0.5 -1.0	2.0
0005789	G3000SW	7.5	30	10	≥ 10,000	0.5 -1.0	2.5
0005790	G4000SW	7.5	30	13	≥ 8,000	0.5 -1.0	1.5
0005102	G2000SW	7.5	60	10	≥ 20,000	0.5 -1.0	4.0
0005103	G3000SW	7.5	60	10	≥ 20,000	0.5 -1.0	5.0
0005104	G4000SW	7.5	60	13	≥ 16,000	0.5 -1.0	3.0
0006727	G2000SW	21.5	30	13	≥ 10,000	3.0 -6.0	1.0
0006728	G3000SW	21.5	30	13	≥ 10,000	3.0 -6.0	1.5
0006729	G4000SW	21.5	30	17	≥ 8,000	3.0 - 6.0	1.0
0005146	G2000SW	21.5	60	13	≥ 20,000	3.0 -6.0	2.0
0005147	G3000SW	21.5	60	13	≥ 20,000	3.0 -6.0	3.0
0005148	G4000SW	21.5	60	17	≥ 16,000	3.0 -6.0	2.0
TSKgel PEEK Columns							
0020027	BioAssist G2SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	7.0
0020026	BioAssist G3SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	7.0
0020025	BioAssist G4SWXL	7.8	30	8	≥ 16,000	0.5 - 1.0	3.5
TSKgel Glass Columns							
0008800	G3000SW, Glass	8.0	30	10	≥ 10,000	0.4 - 0.8	2.0
0008801	G4000SW, Glass	8.0	30	13	≥ 8,000	0.4 - 0.8	2.0

Suitable SEC guard columns are listed on page 20.

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	
Guard column products					
0008805	SW Guard column, Glass	8.0	4.0	10	For all 8 mm ID SW glass columns
0018762	SuperSW Guard column	4.6	3.5	4	For 4.6 mm ID SuperSW columns
0002857	SuperSW mAb Guard column - NEW -	6.0	4.0	4	For all 8 mm ID SW glass columns
0002858	SuperSW mAb Guard column - NEW -	3.0	4.0	4	For all 8 mm ID SW glass columns
0002859	UltraSW Guard column - NEW -	6.0	4.0	3	For all 8 mm ID SW glass columns (contains SuperSW3000 packing)
0008543	SWXL Guard column	6.0	4.0	7	For all SWXL columns and P/Ns 0016215 and 0016049 (contains 3000SWXL packing)
0018008	BioAssist SWXL Guard column	6.0	4.0	7	For all BioAssist SWXL, PEEK columns
0005371	SW Guard column	7.5	7.5	10	For all 7.5 mm ID SW columns (contains 3000SW packing)
0005758	SW Guard column	21.5	7.5	13	For all 21.5 mm ID SW columns
Bulk packing					
0008544	SWXL Top-Off, 1g wet gel			5	For SWXL and QC-PAK columns

SEC

TSKgel PW and TSKgel PWXL columns - Gel Filtration Chromatography of water soluble polymers

HIGHLIGHTS

- Hydrophilic, rigid, spherical, porous methacrylate beads 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 separation range up to 2 x 10^7 Da for linear polymers
- Linear SEC column line incorporating proprietary multi-pore technology
- Specialty columns for low salt separation of cationic polymers

Polymeric TSKgel PW and high resolution TSKgel PWXL columns are designed for SEC of water soluble organic polymers, polysaccharides, DNA, and RNA. They are based on a hydrophilic polymethacrylate matrix. The range of pore sizes in which TSKgel PW and TSKgel PWXL columns are available permits a wide spectrum of water soluble substances to be analyzed. The properties and molar mass separation ranges for all TSKgel PW series columns are summarized in TABLE II.

Stable from pH 2 to 12, TSKgel PW series columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 °C (50 °C for TSKgel G-DNA-PW column). For analytical purposes the TSKgel PWXL columns are preferred because of their higher resolution whereas for preparative work the 60 cm TSKgel PW columns are recommended because higher sample amounts can be applied. For the analysis of proteins and peptides we recommend to use silica based SW type columns.

A number of specialty columns include columns for oligosaccharides, nucleic acids, and samples with a broad molecular weight range. A large pore G6000PW phase is available in PEEK column hardware (TSKgel BioAssist G6PW) for ultra-low sample adsorption during virus analysis. TSKgel PWXL-CP columns are especially suited for the separation of cationic polymers.

The latest additions to the TSKgel PW family are high resolution semi-micro SEC columns: TSKgel SuperoligoPW for oligomer analysis and TSKgel SuperMultiporePW columns for MW distribution analysis by linear SEC. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PWXL columns, which further reduces the risk of adsorption of hydrophilic polymers.

TABLE II

Properties and separation ranges for TSKgel PW-type packings

TSKgel Column	Particle size (µm)	Pore size (nm)	MW range (PEG/PEO)	MW range Dextrans*	MW range Globular Proteins
G2000PW	12	12.5	< 2 x 10^3	-	< 5 x 10^3
G2500PW	12, 17	< 20	< 3 x 10^3	< 3 x 10^3	< 8 x 10^3
G3000PW	12, 17	20	< 5 x 10^4	< 6 x 10^4	5 x 10^2 - 8 x 10^5
G4000PW	17	50	< 3 x 10^5	1 x 10^3 - 7 x 10^5	1 x 10^4 - 1.5 x 10^6
G5000PW	17	100	< 1 x 10^6	5 x 10^4 - 2.5 x 10^6	< 1 x 10^8
G6000PW/ BioAssist G6PW	17	> 100	< 8 x 10^6	5 x 10^5 - 5 x 10^7	< 2 x 10^8
GMPW	17	< 10 - 100	5 x 10^2 - 8 x 10^6	< 5 x 10^7	< 2 x 10^8
G2500PWXL	7	< 20		< 3 x 10^3	< 8 x 10^3
G3000PWXL	7	20	< 5 x 10^4	< 6 x 10^4	5 x 10^2 - 8 x 10^5
G4000PWXL	10	< 50	< 3 x 10^5	1 x 10^3 - 7 x 10^5	1 x 10^4 - 1.5 x 10^6
G5000PWXL	10	100	< 1 x 10^6	5 x 10^4 - 2.5 x 10^6	< 1 x 10^8
G6000PWXL	13	> 100	< 8 x 10^6	5 x 10^5 - 5 x 10^7	< 2 x 10^8
G-DNA-PW	10	> 100	< 8 x 10^6	< 5 x 10^7	
GMPWXL	13	10 - 100	5 x 10^2 - 8 x 10^6	< 5 x 10^7	< 2 x 10^8
G-Oligo-PW	7	12.5	0< 3 x 10^3		< 5 x 10^3
SuperMultiporePW-N	4	n/a	3 x 10^2 - 5 x 10^4		
SuperMultiporePW-M	5	n/a	5 x 10^2 - 1 x 10^6		
SuperMultiporePW-H	8 (6-10)	n/a	1 x 10^3 - 1 x 10^7		
SuperOligoPW	3	n/a	1 x 10^2 - 3 x 10^3		
G3000PWXL-CP	7	20	< 9 x 10^4		
G5000PWXL-CP	10	100	< 1 x 10^6		
G6000PWXL-CP	13	> 100	< 2 x 10^7		

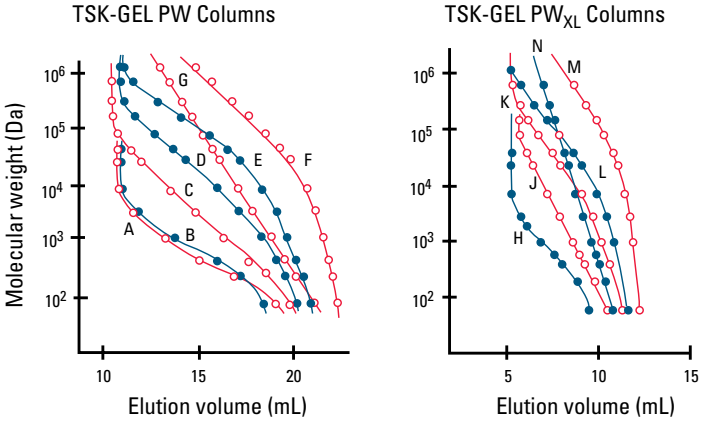
Column: TSKgel PW columns, 7.5 mm ID x 60 cm L; TSKgel PWXL, TSKgel PWXL-CP, G-Oligo-PW & G-DNA-PW, 7.8 mm ID x 30 cm L
Elution: Polyethylene glycols and oxides: distilled water; dextrans: 0.2 mol/L phosphate buffer, pH 6.8
Flow rate: 1.0 mL/min, except for TSKgel SuperMultiporePW and TSKgel SuperOligoPW columns: 0.6 mL/min
*Note: *Maximum separation range determined from estimated exclusion limits*

CALIBRATION CURVES FOR TSKgel PW / SuperMultiporePW GEL FILTRATION COLUMNS

The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

FIGURE 14

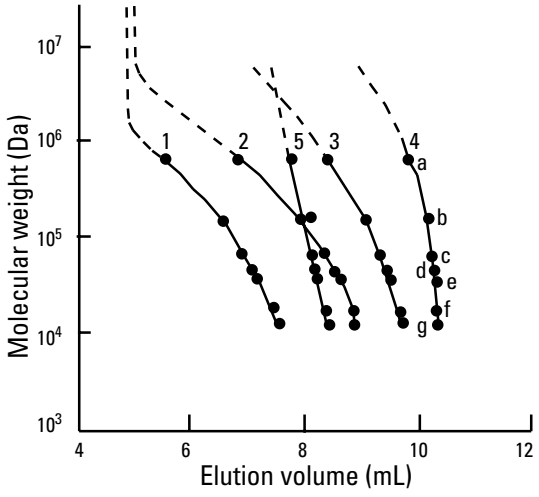
Polyethylene glycol and oxide calibration curves on TSKgel PW and TSKgel PW$_{XL}$ columns



Column: TSKgel PW columns: A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5mm ID x 60 cm L TSKgel 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 m L/min; Detection: RI

FIGURE 15

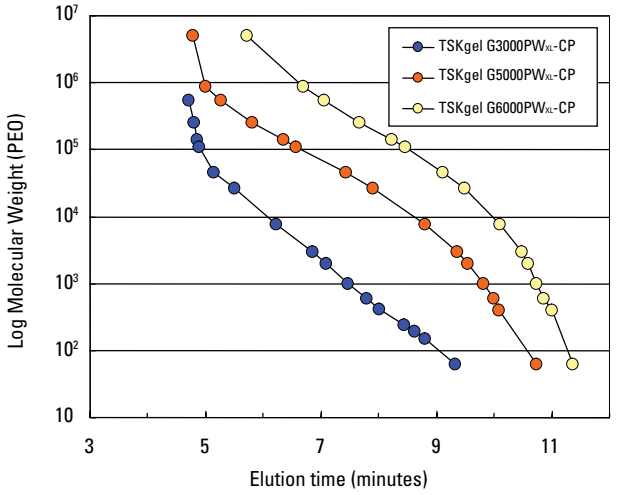
Protein calibration curves on TSKgel PW$_{XL}$ columns



Column: 1. TSKgel 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 mol/L phosphate buffer (pH 6.8); Flow rate: 1.0 mL/min; Detection: UV @ 280 nm

FIGURE 16

Polyethylene glycol and oxide calibration curves for TSKgel PW$_{XL}$-CP columns

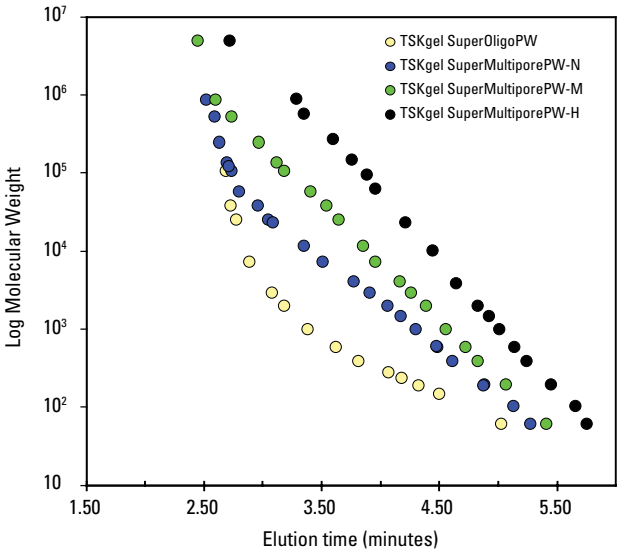


Columns: TSKgel G3000PW$_{XL}$-CP, 7 µm, 7.8 mm ID x 30 cm L, TSKgel G5000PW$_{XL}$-CP, 10 µm, 7.8 mm ID x 30 cm L, TSKgel G6000PW$_{XL}$-CP, 13 µm, 7.8 mm ID x 30 cm L

Mobile phase: 0.1 mol/L $NaNO_3$; Flow rate: 1 mL/min; Detection: RI; Temperature: 25°C; Samples: polyethylene oxides (PEO) standards, polyethylene glycols (PEG) standards

FIGURE 17

Polyethylene glycol, oxide and ethylene glycol calibration curves for TSKgel SuperMultiporePW and SuperOligoPW



Columns: TSKgel SuperOligoPW, SuperMultiporePW-N, SuperMultiporePW-M, SuperMultiporePW-H (each 6.0 mm ID x 15 cm L);

Mobile phase: H_2O; Flow rate: 0.60 mL/min; Detection: RI; Temperature: 25°C; Samples: polyethylene oxides (PEO) standards, polyethylene glycols (PEG) standards, ethylene glycol (EG) standards

SEC

COLUMNS FOR SPECIFIC APPLICATIONS

TSKgel PW$_{XL}$-CP

The new TSKgel PW$_{XL}$-CP columns are designed to facilitate the separation of cationic polymers by SEC at low salt conditions. They are based on the well known PW-type of polymeric resins for aqueous SEC. Cationic surface modification enables low salt elution of cationic polymers with high recoveries. The columns show high theoretical plate numbers, linear calibration curves and high durability. They are produced with three pore sizes for diffrent ranges (G3000-, G5000- and G6000PW$_{XL}$-CP). FIGURE 16 shows the analysis of various cationic polymers on a series of TSKgel PW$_{XL}$-CP columns.

TSKgel SuperOligoPW & G-Oligo-PW

The new TSKgel SuperOligoPW column was developed for the fast determination of molecular mass of aqueous oligomers, particularly oligosaccharides, and low molecular weight aqueous polymers. This is a semi-micro column (6.0 mm ID x 15 cm L) packed with spherical monodisperse polymethacrylate 3 µm particles. The combination of the decreased particle size and small dimensions of the TSKgel SuperOligoPW column enables high speed separation with high resolution - half of the separation time with the same resolution compared to conventional size exclusion columns. An added benefit of the semi-micro and small particle size is lower solvent consumption compared to conventional columns.

TSKgel G-Oligo-PW was designed for high resolution separations of nonionic and cationic oligomers and oligosaccharides such as hydrolyzed cyclodextrins. 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 the calibration curve for TSKgel G2500PW$_{XL}$ (shown on the previous page. FIGURE 18 shows the calibration curve for double stranded DNA for the TSKgel G-DNA-PW column.

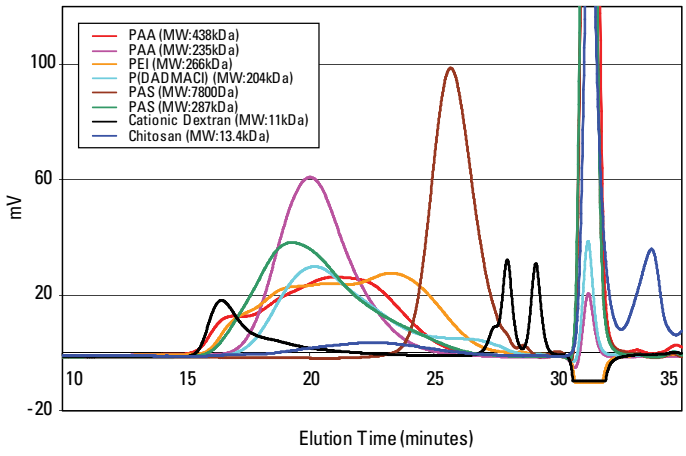
TSKgel G-DNA-PW

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. The exclusion limits for double-stranded DNA fragments are lower than those for rRNAs. The packing of the TSKgel G-DNA-PW column has very large pores (>100 nm) 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 18

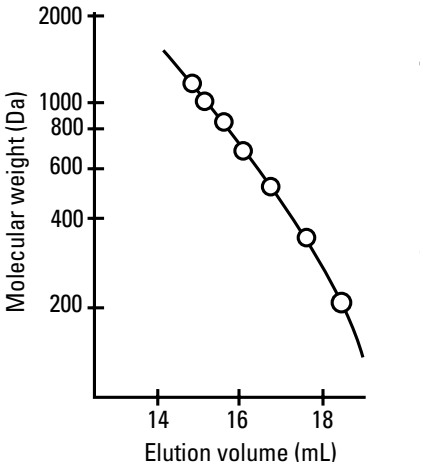
Double stranded DNA calibration curve for TSKgel G-DNA-PW column



Columns: TSKgel G3000PW$_{XL}$-CP, 7 µm (7.8 mm ID x 30 cm L), TSKgel G5000PW$_{XL}$-CP, 10 µm (7.8 mm ID x 30 cm L), TSKgel G6000PW$_{XL}$-CP, 13 µm (7.8 mm ID x 30 cm L); Eluent: 0.1 mol/L $NaNO_3$; Flow rate: 1 mL/min; Detection: RI; Temperature: 25°C; Sample Load: 3 g/L, 100 µL

FIGURE 19

Oigosaccharides calibration curve for TSKgel G-Oligo-PW column



Column: TSKgel G-Oligo-PW, two 6 µm, 7.8 mm ID x 30 cm L columns in series; Mobile phase: distilled H_2O; Flow rate: 1.0 mL/min; Detection: UV @ 260 nm; Sample: hydrolyzed -cyclodextrin

COLUMNS FOR SPECIFIC APPLICATIONS

TSKgel GMPW AND TSKgel GMPWXL

When the molecular weight range of the sample is broad or unknown, Tosoh Bioscience offers mixed-bed and multipore columns for analysis. The mixed bed column TSKgel GMPW and its high resolution counterpart, TSKgel GMPWXL, are packed with the G2500, G3000 and G6000 PW or corresponding PWXL resins. They offer a broad molecular weight separation range. As shown on page 42, the calibration curve for polyethylene glycols and oxides on these columns is fairly shallow and is linear over the range of 100-1,000,000 Da. The introduction of mixed-bed columns has facilitated the analysis of polydisperse samples. Previously, two-column systems such as TSKgel G3000PW and TSKgel G6000PW, were required to achieve good resolution with wide MW-range samples. The substitution of a TSKgel GMPW series column can save both time and money compared with multi-column systems.

TSKgel SuperMultiporePW

TSKgel SuperMultiporePW columns incorporate the multi-pore particle synthesis technology developed by Tosoh scientists in which monodisperse particles exhibit a broad range of pore sizes. See page 54 for additional information on multipore technology. Each particle, by design, has an extended linear calibration curve, thereby greatly diminishing the appearance of chromatograms with inflection points. This allows better reproducibility when determining molecular mass and molecular mass distribution of polymers.

Three semi-micro (6.0 mm ID x 15 cm L) columns are available within the TSKgel SuperMultiporePW series containing 4, 5 or 8 µm particles. This enables high speed separation for aqueous polymers and low solvent consumption compared to the conventional SEC columns. In addition, a wide separation range can be analyzed with the three different columns, from high molecular mass aqueous polymers to oligomers.

Multi-pore, semi-micro SEC columns provide high resolution and smooth peak shapes without shoulders of inflection points. This leads to better accuracy and reproducibility when determining the molecular mass distribution of water soluble polymers

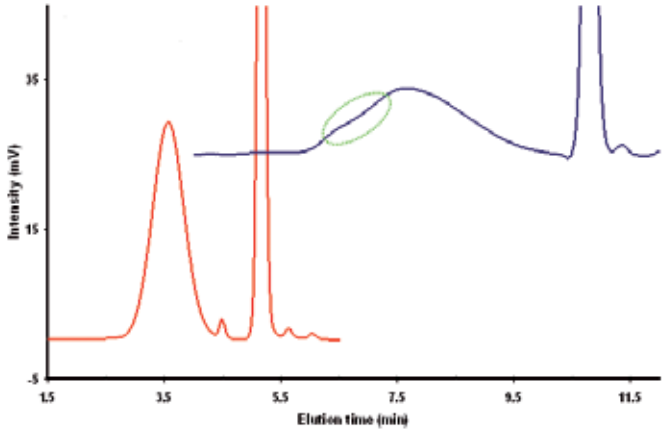
COMPARISON WITH CONVENTIONAL GPC COLUMNS

FIGURE 20 shows the SEC analysis of a real sample Polyvinyl-pyrrolidone (PVP) K-30- on a series of conventional TSKgel G3000PWXL and G5000PWXL columns compared to the one obtained with a single TSKgel SuperMultiporePW-M linear SEC column (MW range 600,000 – 1,500,000). On a series of conventional SEC columns the Polyvinylpyrrolidone peak shows an inflection point, which does not appear on the SuperMultiporePW-M column. Analysis is much faster and more sensitive when applying the new multi-pore packing.

A mixture of polyethylene oxide (PEO) and polyethylene glycol (PEG) was analyzed on a semi-micro TSKgel SuperMultiporePW-M column and on conventional-sized TSKgel G3000PWXL and TSKgel G5000PWXL columns in series. As shown in FIGURE 21, the analysis using the TSKgel SuperMultiporePW-M column was completed in half the time and with higher resolution than the analysis performed using the TSKgel G3000PWXL and TSKgel G5000PWXL columns. This is due to the semi-micro dimensions (6.0 mm ID x 15 cm L) and the smaller particle size (5 µm) of the TSKgel SuperMultiporePW-M column compared to the 7.8 mm ID x 30 cm L size and 7 and 10 µm particle size of the TSKgel G3000PWXL and TSKgel G5000PWXL columns respectively.

FIGURE 20

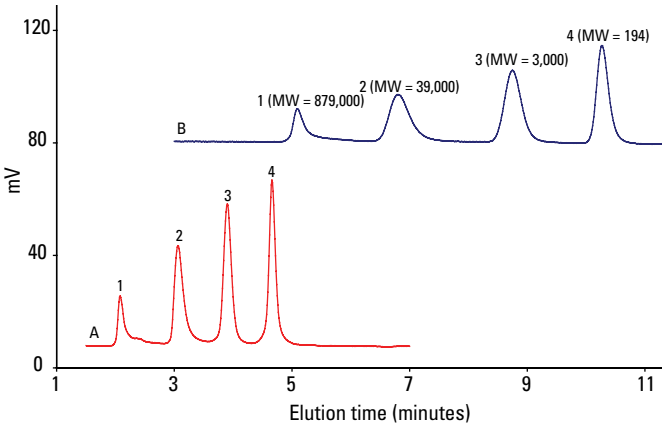
Analysis of polyvenylpyrrolidone



Columns: TSKgel SuperMultiporePW-M, 6 mm ID x 15 cm L x 1 (red) TSKgel G3000PWXL & G5000PWXL, each 7.8 mm ID x 30 cm L in line (blue); Sample: Polyvinylpyrrolidone (K-30); Mobile phase: 0.1 mol/L $NaNO_3$; Flow rate: 0.6 mL/min; Detection: RI

FIGURE 21

Comparison of analysis of a mixture of PEO and PEG



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

SEC

OPTIMIZING GEL FILTRATION WITH TSKgel PW AND TSKgel PWXL COLUMNS

SELECTING MOBILE PHASE BUFFERS

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

For some nonionic, nonpolar polymers, such as polyethylene glycols, ideal size exclusion behavior 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 should be added. Generally, a salt concentration of 0.1 to 0.5 mol/L is sufficient to overcome undesirable ionic interactions.

HYDROPHOBIC SAMPLES

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

Modifiers are also used for optimizing the elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in the table below. All TSKgel 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 operated in 50 % aqueous acetone.

TABLE III

Recommended eluents for GFC of water soluble polymer on TSKgel 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 0.01N NaOH 20% DMSO Buffer or salt solution (e.g., 0.1–0.5 mol/L $NaNO_3$)
Nonionic hydrophobic	polyvinylpyrrolidone	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1mol/L $NaNO_3$)
Anionic hydrophilic	sodium chondroitin sulfate, sodium alginate, carboxymethyl cellulose, sodium polyacrylate, sodium hyaluronate	Buffer or salt solution (e.g., 0.1 mol/L $NaNO_3$)
Anionic hydrophobic	sulfonated lignin sodium salt, sodium polystyrenesulfonate	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L $NaNO_3$)
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethyleneimine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4, or 0.8 mol/L $NaNO_3$ (0.1 mol/L $NaNO_3$ for PWXL-CP type)
Cationic hydrophobic	poly(4-vinylbenzyltrimethylammonium chloride), poly(N-methyl-2-vinylpyridinium) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4
Amphoteric hydrophilic	peptides, proteins, poly-and oligosaccharides, DNA, RNA	Buffer or salt solution (e.g., 0.1 mol/L $NaNO_3$)
Amphoteric hydrophobic	blue dextran, collagen, gelatin, hydrophobic proteins hydrophobic peptides	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L $NaNO_3$ or 35 - 45% ACN in 0.1% TFA)

APPLICATIONS OF TSKgel PW-TYPE GEL FILTRATION COLUMNS

POLYSACCHARIDES

TSKgel 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 SEC because they seldom exhibit secondary interactions with the solid support. 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 with conventional SEC packings. A mobile phase of 0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4 can also be used.

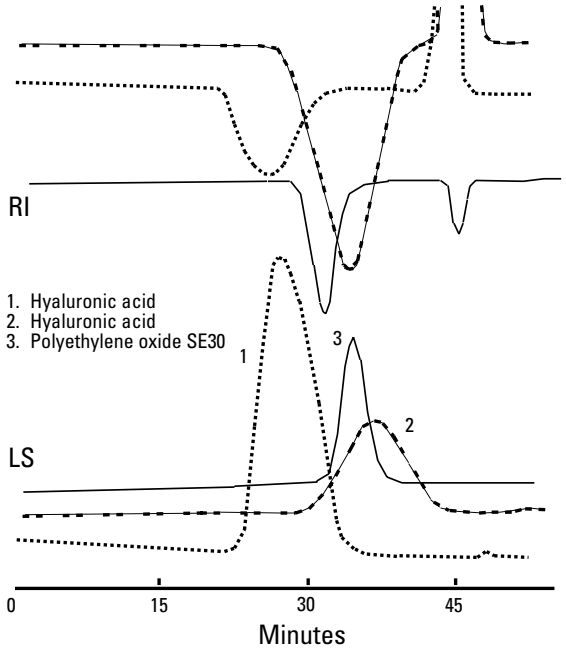
The new TSKgel PW$_{XL}$-CP series enables elution of water soluble, cationic polymers under low salt conditions (e.g. 0.1 mol/L $NaNO_3$). An effective separation of the anionic hydrophilic gluco-saminoglycan, hydraluronic acid, is shown in FIGURE 22 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase.

OLIGOSACCHARIDES

FIGURE 23 shows the rapid analysis of maltose oligomers using a TSKgel SuperOligoPW column compared to a TSKgel G-Oligo-PW column. The faster analysis time is due to the semi-micro dimensions (6.0 mm ID x 15 cm L) and the small particle size (3 µm) of the TSKgel SuperOligoPW column compared to the 7.8 mm ID x 30 cm L size and 7 µm particle size of the TSKgel G-Oligo-PW column.

FIGURE 22

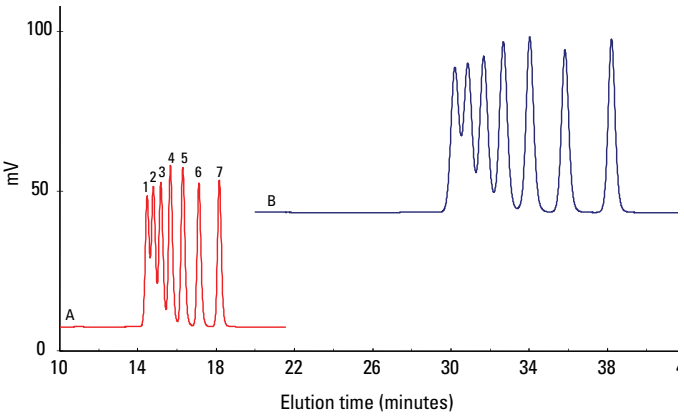
Analysis of oligosaccharides



Column: TSKgel G6000PW + G4000PW, two 7.5 mm ID x 60 cm L columns in series; Mobile phase: 0.2 mol/L NaCl; Flow rate: 0.9 mL/min
Temperature: 40°C; Samples: hyaluronic acid

FIGURE 23

Analysis of maltose oligomers



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

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Stainless Steel Columns							
0022789	SuperMultiporePW-N	6.0	15	4	>16,000	0.3 - 0.6	4.5
0022790	SuperMultiporePW-M	6.0	15	5	>12,000	0.3 - 0.6	2.7
0022791	SuperMultiporePW-H	6.0	15	8	>7,000	0.3 - 0.6	0.9
0022792	SuperOligoPW	6.0	15	3	>16,000	0.3 - 0.6	5.0
0008031	G-Oligo-PW	7.8	30	7	≥ 16,000	0.5 - 0.8	4.0
0008032	G-DNA-PW	7.8	30	10	≥ 10,000	0.2 - 0.5	2.0
0008020	G2500PW$_{XL}$	7.8	30	7	≥ 16,000	0.5 - 0.8	4.0
0008021	G3000PW$_{XL}$	7.8	30	7	≥ 16,000	0.5 - 0.8	4.0
0008022	G4000PW$_{XL}$	7.8	30	10	≥ 10,000	0.3 - 0.6	2.0
0008023	G5000PW$_{XL}$	7.8	30	10	≥ 10,000	0.3 - 0.6	2.0
0008024	G6000PW$_{XL}$	7.8	30	13	≥ 7,000	0.3 - 0.6	2.0
0008025	GMPW$_{XL}$	7.8	30	13	≥ 7,000	0.3 - 0.6	2.0
0021873	G3000PW$_{XL}$-CP	7.8	30	7	≥ 16,000	1.0	
0021874	G5000PW$_{XL}$-CP	7.8	30	10	≥ 10,000	1.0	
0021875	G6000PW$_{XL}$-CP	7.8	30	13	≥ 7,000	1.0	
0005761	G2000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	2.0
0008028	G2500PW	7.5	30	12	≥ 5,000	0.5 - 1.0	2.0
0005762	G3000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	2.0
0005763	G4000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0005764	G5000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0005765	G6000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0008026	GMPW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0005105	G2000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	4.0
0008029	G2500PW	7.5	60	12	≥ 10,000	0.5 - 1.0	4.0
0005106	G3000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	4.0
0005107	G4000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0005108	G5000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0005109	G6000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0008027	GMPW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0008030	G2500PW	21.5	60	17	≥ 10,000	1.6 - 6.0	2.0
PEEK							
0020024	BioAssist G6PW	7.8	30	17	≥ 3,000	0.5 - 1.0	10
Guard columns							
0022793	SuperMP (PW)-N Guard column	4.6	3.5	4			
0022794	SuperMP (PW)-M Guard column	4.6	3.5	5			
0022795	SuperMP (PW)-H Guard column	4.6	3.5	8			
0022796	SuperOligoPW Guard column	4.6	3.5	3			
0008034	Oligo Guard column	6.0	4.0	13	For 7.8 mm ID G-Oligo-PW columns		
0008033	PW$_{XL}$ Guard column	6.0	4.0	12	For 7.8 mm ID PW$_{XL}$& G-DNA-PW (TSKgel G3000PW packing)		
0021876	PW$_{XL}$-CP Guard column	6.0	4.0	13	For 7.8 mm ID PW$_{XL}$-CP columns		
0006763	PW-L Guard column	7.5	7.5	13	For 7.5 mm ID G2000PW (TSKgel G2000PW packing)		
0006762	PW-H Guard column	7.5	7.5	13	For 7.5 mm ID G2500PW through GMPW columns		
0006758	PW-H Guard column	21.5	7.5	17	For 21.5 mm ID G2500PW through G5000PW columns		
Bulk packing							
0008035	PW$_{XL}$ Top-Off, 1 g wet resin			10	For all PW$_{XL}$ and G-DNA-PW columns		

TSKgel ALPHA AND SuperAW GEL FILTRATION COLUMNS

Gel Filtration and Gel Permeation Chromatography of water soluble and polar organic-soluble polymers

HIGHLIGHTS

- A unique hydrophilic, polymer resin is available in conventional column dimensions (Alpha) and high throughput column format (SuperAW).
- Exhibits strong mechanical stability and minimal swelling characteristics
- A wide range of solvent compatibility, from 100% water to 100% non-polar organic solvents
- The reduced particle size and shorter column length of TSKgel SuperAW columns provide equivalent resolution in one half the time for high throughput applications.
- Unlike polystyrene-divinylbenzene (PS-DVB) resins that may adsorb polymers due to hydrophobic interaction, both the TSKgel Alpha and
- SuperAW columns allow for the separation of polymers soluble in methanol.
- Provide accurate molecular weight 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, providing accurate MW determinations.

COLUMN SELECTION

The TSKgel Alpha Series consists of six columns with three particle sizes: 7, 10, and 13 µm. These columns span a wide MW separation range from 10^2 to more than 1 x 10^6 Da when using polyethylene oxide (PEO) as a MW standard. Exclusion limits for the TSKgel Alpha columns for polyethylene oxide (PEO), polyethylene glycols (PEG) and polystyrenes (PS) are shown in the table below. Calibration curves for the TSKgel Alpha Series columns are shown on the next page for polyethylene oxide, polyethylene glycol and polystyrene standards.

The TSKgel SuperAW series contains a similar chemistry as the TSKgel 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 make SuperAW columns ideal for high throughput applications. TSKgel Alpha and SuperAW columns are offered in 5 discrete exclusion ranges and 1 mixed bed. Both column types can accommodate polymer standards up to several million Dalton molecular weight (see calibration curves on the next page

TABLE IV

Exclusion limits for TSKgel Alpha Series and SuperAW Series columns

TSKgel Column	Particle size (µm)	Exclusion limit (Da) for various standards and eluents		
		PEO[a]/H_2O	PS[b]/10 mmol/L LiBr in DMF	PEG[c]/10 mmol/L 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

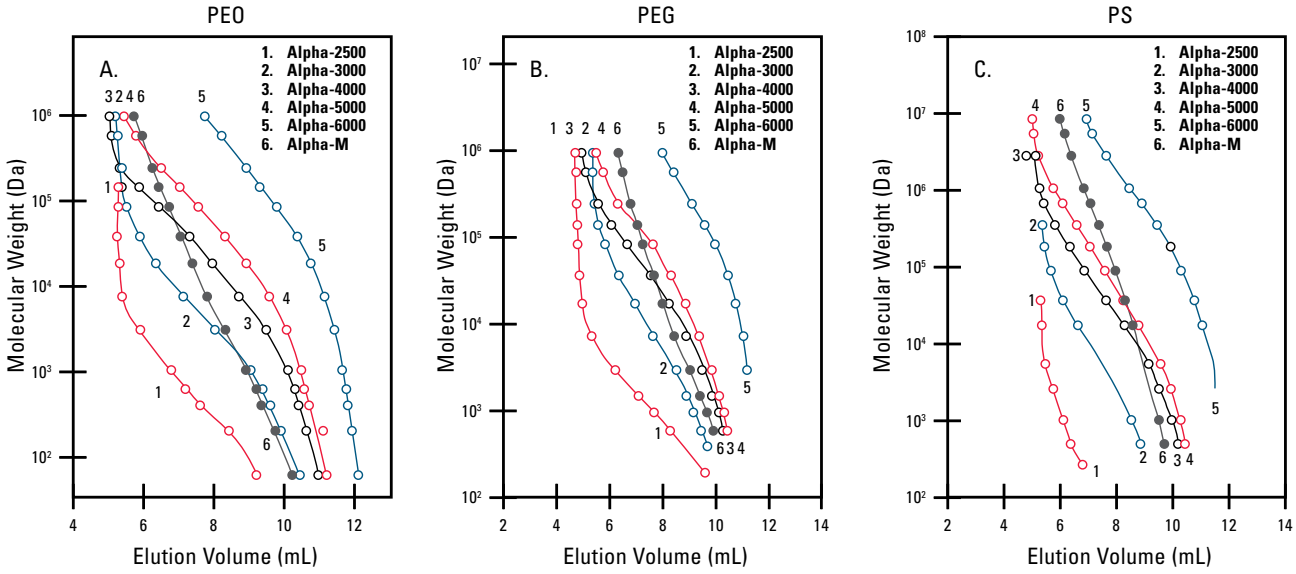
** Exclusion limit for SuperAW5000, SuperAW6000, and SuperAWM-H are estimated, respectively*

SEC

CALIBRATION CURVES FOR TSKgel ALPHA AND SuperAW GEL FILTRATION COLUMNS

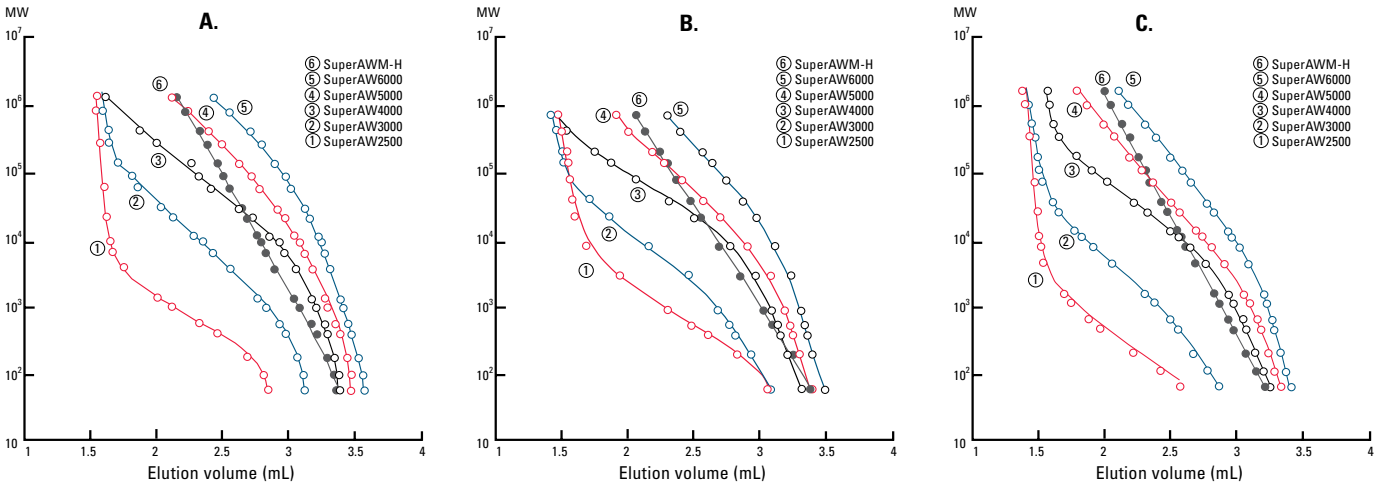
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

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



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

Calibration curves for TSKgel SuperAW series in different solvents with different polarity



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

APPLICATIONS OF TSKgel ALPHA AND SuperAW GEL FILTRATION COLUMNS

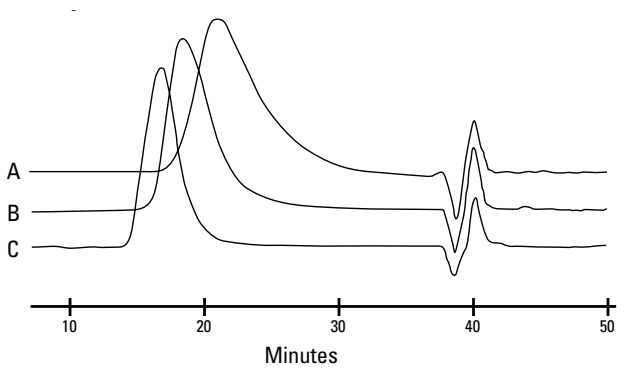
The versatility of using TSKgel Alpha columns with various polar solvents is illustrated in FIGURE 24 for the analysis of cellulose derivatives. A TSKgel Alpha-M column was used to separate ethylcellulose with the polar solvent DMF and ethylhydroxyethyl cellulose with methanol.

The separation of polyvinylalcohol with different degrees of saponification is shown in FIGURE 25. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol mobile phase.

FIGURE 26 shows that the column efficiency of TSKgel SuperAW series columns is maintained in a wide variety of polar organic solvents.

FIGURE 25

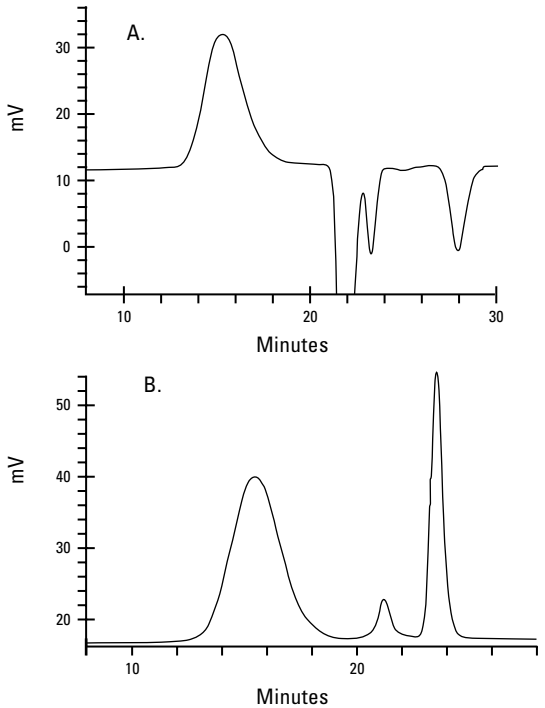
Polyvinylalcohol characterization using TSKgel Alpha-5000 and Alpha-3000 columns in series



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 24

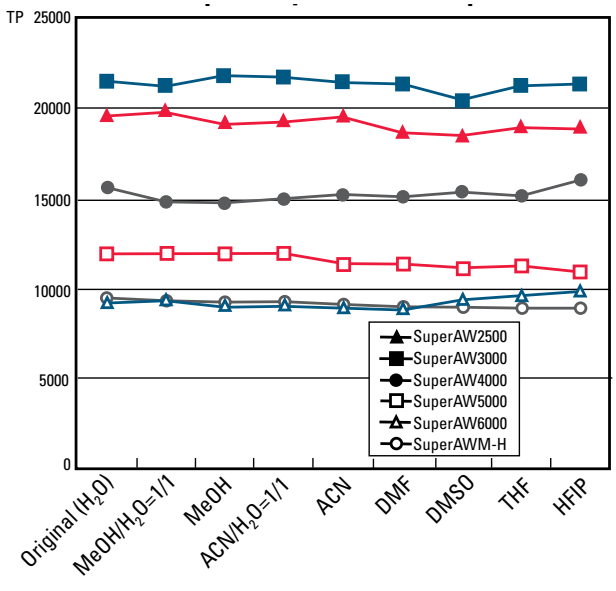
TSKgel Alpha-M separation of cellulose derivatives



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 mmol/L LiBr in DMF; B. 10 mmol/L LiBr in methanol; Flow rate: 0.5 mL/min; Temperature: 40°C; Detection: RI

FIGURE 26

Solvent compatibility of TSKgel SuperAW series



Column: TSKgel 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)

SEC

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min)*	*Maximum pressure drop (MPa)*	
TSKgel Stainless Steel Columns								
0018339	Alpha-2500	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
0018340	Alpha-3000	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
0018341	Alpha-4000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	3.0
0018342	Alpha-5000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	3.0
0018343	Alpha-6000	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
0018344	Alpha-M (mixed bed)	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
Guard columns								
0018345	Alpha Guard column	6	4	13	For all Alpha columns			
TSKgel VMpak columns*								
0020011	VMpak-25	2.0	5	7	≥ 1,000	0.1 - 0.2	0.25	2.0
0020012	VMpak-25	2.0	15	7	≥ 3,000	0.1 - 0.2	0.25	6.0
TSKgel Stainless Steel Columns								
0019315	SuperAW2500	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	6.0
0019316	SuperAW3000	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	6.0
0019317	SuperAW4000	6.0	15	6	≥ 10,000	0.3 - 0.6	0.6	4.0
0019318	SuperAW5000	6.0	15	7	> 10,000	0.3 - 0.6	0.6	3.0
0019319	SuperAW6000	6.0	15	9	> 7,000	0.3 - 0.6	0.6	2.0
0019320	SuperAWM-H	6.0	15	9	> 7,000	0.3 - 0.6	0.6	2.0
Guard columns								
0019321	SuperAW-L Guard Column	4.6	3.5	7	For SuperAW2500-4000 columns.			
0019322	SuperAW-H Guard Column	4.6	3.5	13	For SuperAW5000-AWM-H columns			

**TSKgel VMpak-25 series contains a similar packing as TSKgel Alpha-2500. It can be used for multimodal LC/LC-MS separations.*

TSKgel HXL, HHR, SuperH AND SuperHZ GEL PERMEATION COLUMNS

Polymer-based columns for Gel Permeation Chromatography of organic-soluble polymers

HIGHLIGHTS

- Porous, highly cross-linked, spherical polystyrene divinylbenzene (PS-DVB) resin.
- Five different TSKgel H-type columns are available
- Expanded molecular weight ranges with exclusion limits from 1,000 g/mol to an estimated 4 x 10^8 g/mol
- Minimal shrinking and swelling of the column bed
- Chemically and thermally stable
- Semi-micro SuperMultiporeHZ, SuperHZ and Super H columns for reduced solvent consumption in high throughput analysis
- Multipore columns provide linear calibration curves over a wider MW range for conventional GPC (Multipore HXL) and semi-micro GPC (SuperMultiporeHZ)
- Mixed bed GPC columns for ultra-high temperature GPC up to 220°C

TSKgel H Series columns are recommended for the analysis of organic-soluble polymers and are packed with spherical particles composed of polystyrene cross-linked with divinylbenzene (PS-DVB). Each line of columns within this series differs in degree of inertness and operating temperature range. The packings are available in eight pore sizes and span four different column chemistries. For polymer samples with a broad molecular range, packing of several pore sizes are provided in the mixed bed columns: TSKgel SuperHZM series, TSKgel SuperHM series, TSKgel GMHXL, TSKgel GMHHR, and selected high temperature versions provide linear calibration curves up to several million Daltons (see page 53).

COLUMN SELECTION

Best results are obtained when selecting a column with the sample's molar mass in the linear portion of the calibration curve. The Super prefix refers to the efficiency of the column. The Super series columns contain ultra efficient particles as small as 3 µm, housed in 15 cm length columns. The smaller particle allows for equivalent resolution to conventional HXL 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.

TABLE V

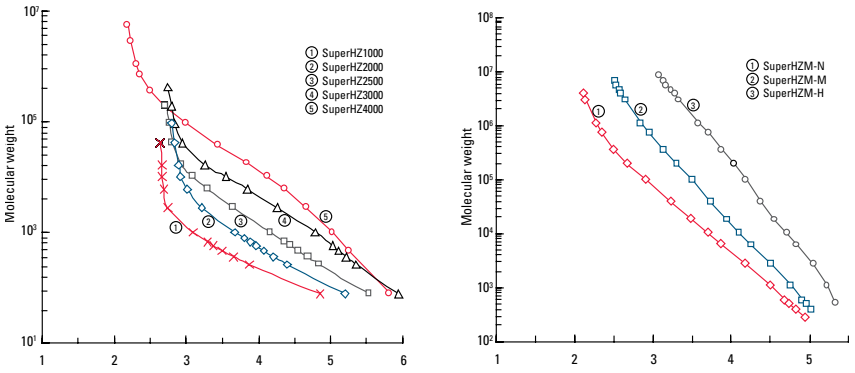
Series Type	SuperMultiporeHZ	SuperHZ	HXL	SuperH	HHR
Application focus	Ultra-high performance with a low dead volume and a wide pore distribution in each particle for superior linearity	High-throughput polymer analysis with ultra low polymer adsorption. Limited solvent compatibility range.	Conventional polymer analysis with ultra low polymer adsorption. Ltd solvent compatibility range.	High-throughput polymer analysis with expanded solvent compatibility.	Conventional polymer analysis with expanded solvent compatibility range.
Particle size	3, 4 and 6 µm, depending on pore size	3, 5 and 10 µm, depending on pore size	5, 9 and 13 µm, depending on pore size	3 and 5 µm, depending on pore size	5 µm
Theoretical plates[1]	20,000/15 cm column	16,000/15 cm column	16,000/30 cm column	16,000/15 cm column	16,000/30 cm column
Maximum temperature	60°C	G1000 - G4000 60°C G5000 - mixed 80°C	G1000 - G4000 60°C G5000 - mixed 80°C	140°C	140°C 220°C for HHR HT2
Standard shipping solvent	THF	THF	THF[2]	THF[2]	THF[2]
THF can be switched to	none	benzene, chloroform, toluene, xylene, dichloromethane[3] and dicholoroethane[3]		see our website for detailed information	
Other shipping solvents available?	yes[4]	yes[4]		no	
Number of solvent substitutions	-	One time only	One time only	Several[5]	Several[5]
Solvent exchange instructions		Linear gradient with a 2 %/min rate of change at a flow rate <0.25 mL/min	Linear gradient with a 2 %/min rate of change at a flow rate <0.5 mL/min	Linear gradient with a 2%/min rate of change according to flow rates listed on our website	

1) Theoretical plates listed are based on smallest particle size listed
2) High-temperature columns (HT) are shipped with OCDB (Orthochlorodivinylbenzene) as standard shipping solvent.
3) Switching from THF to dichloromethane and dichloroethane is not recommended for G1000 pore size columns.
4) See our website for available shipping solvents
5) After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is not recommended.

SEC

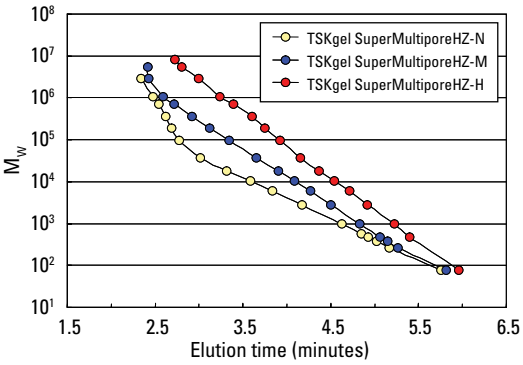
CALIBRATION CURVES FOR TSKgel H-TYPE GEL PERMEATION COLUMNS

Calibration curves for TSKgel SuperHZ columns with polystyrene standards



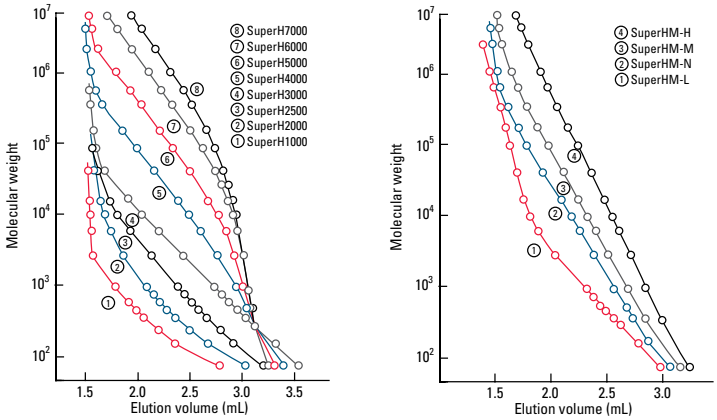
Column: TSKgel SuperHZ series (4.6 mm ID x 15 cm L); Eluent: THF; Flow rate: 0.35 mL/min; Temp.: 25°C; Sample: polystyrene standards; Inj. volume: 2 µL

Calibration curves for TSKgel SuperMultiporeHZ-M, H and N columns



Columns: TSKgel SuperMultiporeHZ-N, 3 µm, 4.6 mm ID x 15 cm L, TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID x 15 cm L, TSKgel SuperMultiporeHZ-H, 6 µm, 4.6 mm ID x 15 cm L; Mobile phase: THF; Flow rate: 0.35 mL/min; Detection: UV @ 254 nm; Temp.: 25°C; Samples: polystyrene standards

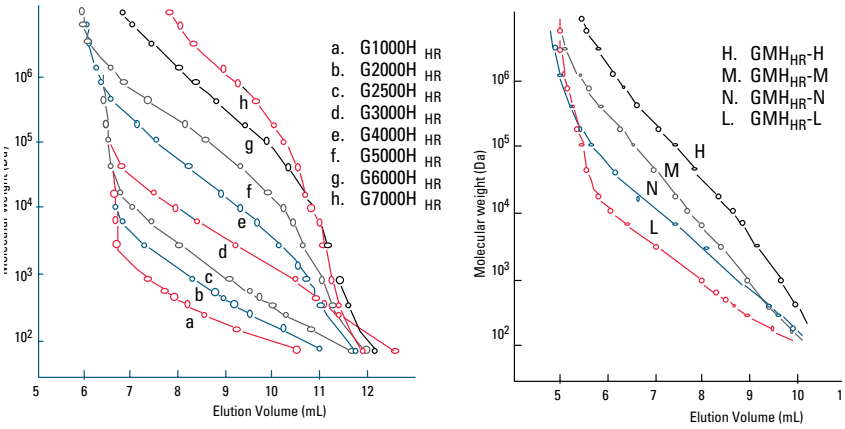
Calibration curves for TSKgel SuperH columns with polystyrene standards



Column: TSKgel SuperH series (6.0 mm ID x 15 cm L); Eluent: THF; Flow rate: 0.6 mL/min; Temp.: 25°C; Detection: UV @ 254 nm; Sample: polystyrene standards

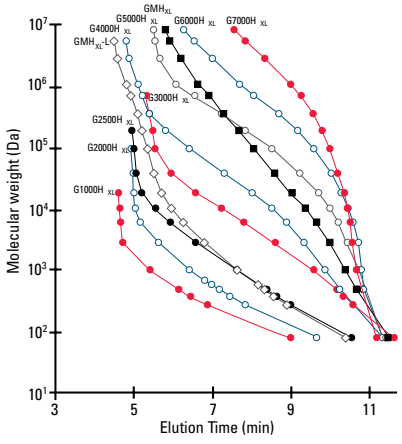
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

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



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

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; Temp.: 25°C; Detection: UV @ 254 nm

MULTI-PORE SIZE DISTRIBUTION IN A POLYSTERENE PACKING MATERIAL

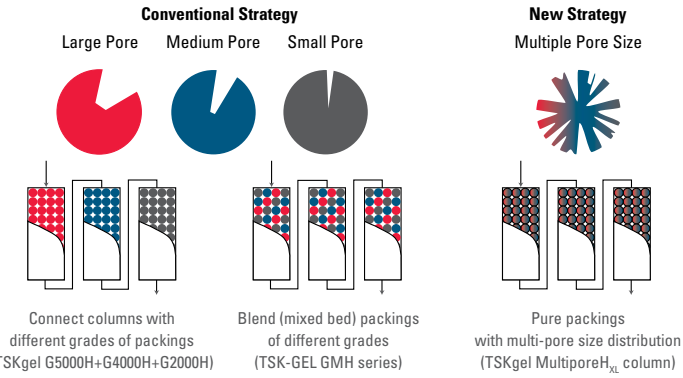
Novel approach to GPC of samples with a wide range of molecular weights

Prior to the introduction of TSKgel MultiporeHXL and SuperMultiporeHZ columns, scientists separating polymers with a wide range of molecular weights were left with two options. One option is to use multiple columns of different pore sizes linked together in series. A second is to use a column packed with a mixed bed resin of different pore sizes at an optimized mix ratio. However, problems can occur with both of these methods, which include distortion of the chromatogram or deviations between the actual calibration curve and the calibration curve approximated from data obtained from the molecular weight standards.

As is shown in FIGURE 27, a novel approach to solve this problem was developed by Tosoh scientists and is incorporated in TSKgel MultiporeHXL and SuperMultiporeHZ Series columns.

FIGURE 27

Strategies for wide range separation using SEC



These columns are packed with particles of uniform size synthesized with a broad distribution of pore sizes. This novel approach creates a linear calibration curve within each particle. Therefore, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes. This results in sharper peaks without inflection points that may be observed using mixed-bed columns.

The pore size distributions of the TSKgel MultiporeHXL-M column and a mixed-bed column are shown in FIGURE 28. 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 MultiporeHXL-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.

The small ID (4.6 mm) and length (15 cm) of the SuperMultiporeHZ columns reduces solvent consumption and results in quick run times, and offers high throughput capabilities. FIGURE 29 demonstrates that inflection points are no longer observed with semi-micro columns packed from particles prepared by multi-pore technology.

TSKgel H series columns can be applied to analyse the molecular mass distribution of a broad variety of organic-soluble polymers. TABLE V lists the recommended solvents by application for TSKgel H series columns. Super H columns are ideally suited to save analysis time and solvent by semi-micro GPC. For optimum performance they should be used in combination with a low dead volume GPC instrument such as the all-in-one EcoSEC system. TABLE VI suggests optimum flow rates to be applied for TSKgel SuperH and TSKgel HHR columns for various solvents.

FIGURE 28

Pore size distribution of TSKgel MultiporeHXL-M column and a mixed-bed column

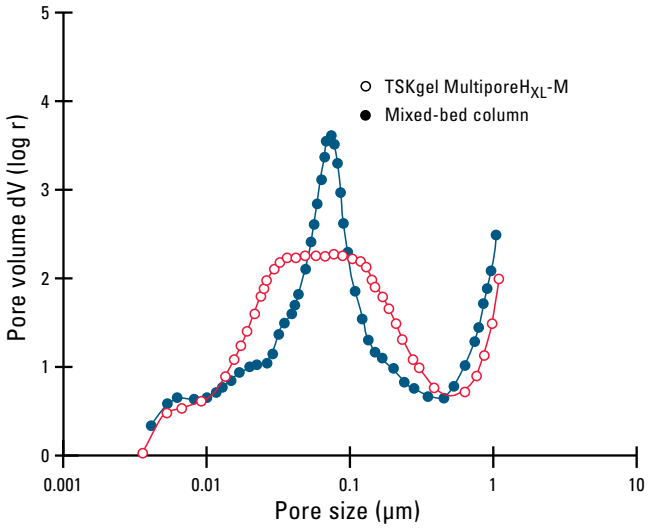
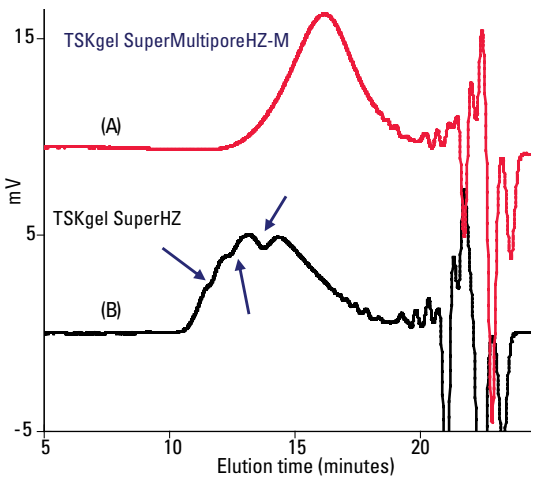


FIGURE 29

Comparison of TSKgel SuperMultiporeHZ-M and TSKgel SuperHZ for separation of Acrylic resin



Column: (A) TSKgel SuperMultiporeHZ-M,4.6 mm ID x 15 cm L, x 4;
(B) TSKgel SuperHZ4000+3000+2500+2000, 4.6 mm ID x 15 cm L x 4
Mobile phase: THF; Detection: RI; Temperature: 40°C; Injection vol.: 10 µL
Samples: acrylic resin

SEC

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

SOLVENTS AND FLOW RATES

TSKgel H series columns can be applied to analyse the molecular mass distribution of a broad variety of organic-soluble polymers. Table 5 lists the recommended solvents by application for TSKgel H series columns. Super H columns are ideally suited to save analysis time and solvent by semi-micro GPC. For optimum performance they should be used in combination with a low dead volume GPC instrument such as the all-in-one EcoSEC system. Table 6 suggests optimum flow rates to be applied for TSKgel SuperH and TSKgel HHR columns for various solvents.

TABLE V

Recommended flow rates (mL/min) for TSKgel SuperH and HHR columns

Solvent	TSKgel SuperH 6.0 mm ID × 15 cm	TSKgel HHR 7.8 mm ID × 30 cm
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

TABLE VI

Recommended solvents by application for TSKgel H series columns

Solvent	Application
THF	polystyrene, epoxy resin, phenoxy resin, polycarbonate, polyisoprene, polyvinyl acetate, polyvinyl chloride, monoglycerides, fatty acids, polybutadiene, poly(methyl methacrylate), poly(styrene-butadiene), poly(styrene-acrylonitrile)
n,n-Dimethylformamide (DMF) + 5 mmol/L 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

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

PHTHALATE ESTERS

FIGURE 30 demonstrates the high efficiency separation on a TSKgel G1000HXL 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.

PHENOL RESIN

The TSKgel GMHXL-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.

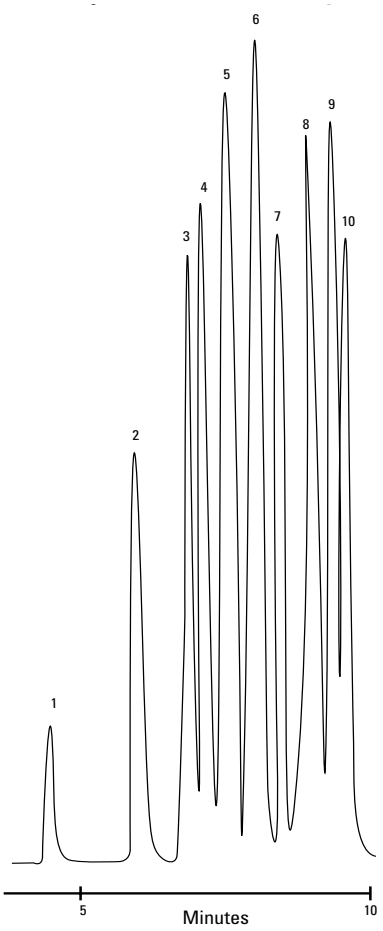
For example, the complete profile of a phenol resin, with high resolution of the low molecular weight components, is shown in FIGURE 31. Other applications for the TSKgel GMHXL-L column include analyses of paint materials, bond and adhesive components and synthetic polymer additives.

FATTY ACIDS

In FIGURE 32, two TSKgel G2000HXL columns in series separate a mixture of fatty acids ranging from C4 to C30.

FIGURE 30

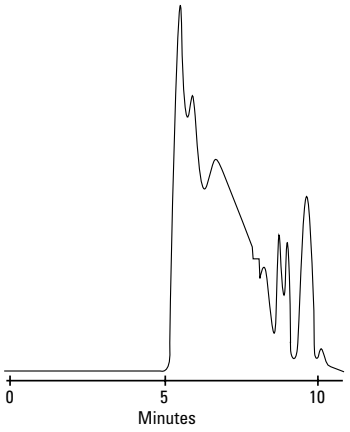
High resolution of phtalate ester on TSKgel G1000HXL



Column: TSKgel G1000HXL, 7.8 mm ID x 30 cm L;
Sample: 1. polystyrene (10,200Da), 2. dioctylphthalate (391Da), 3. dibutylphthalate (278Da), 4.dipropylphthalate (250Da), 5. diethylphthalate (222Da), 6. dimethylphthalate (194Da), 7. n-propylbenzene (120Da), 8. ethylbenzene (116Da), 9. toluene (92Da), 10. benzene (78Da); Elution: THF; Flow rate: 1.0 mL/min; Detection: UV @ 254 nm

FIGURE 31

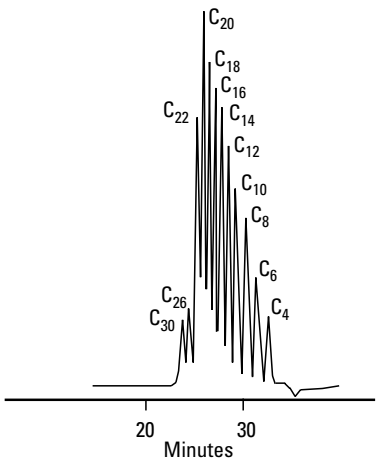
Separation of phenol resin on TSKgel GMHXL-L



Column: TSKgel GMHXL-L, 7.8 mm ID x 30 cm L;
Sample: phenol resin; Elution: THF; Flow rate: 1.0 mL/min;
Detection: UV @ 254 nm

FIGURE 32

Separation of fatty acid



Column: TSKgel G2000HXL, two 7.8 mm ID x 30 cm L in series;
Sample: fatty acids; Elution: THF; Flow rate: 1.0 mL/min; Detection: RI

SEC

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

SHEAR DEGRADATION

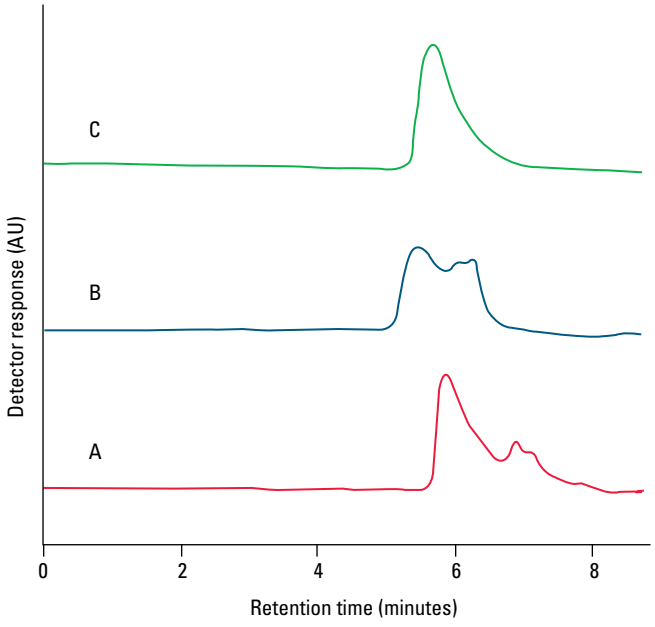
Shear degradation is observed especially when ultra-high molar mass compounds are analyzed. It tends to occur when analysis is carried out at high flow rates using a micro-particle size packing material. FIGURE 33 demonstrates the relationship between shear degradation and particle size of the packing material, when TSKgel GMH columns were used. When the flow rate is 1.0 mL/min, normal elution of an ultra-high molar mass sample (2.06 × 107 Da) is only possible with the TSKgel GMH$_{HR}$-H(S) column, which has a large particle size. However, with the TSKgel GMH$_{XL}$ and GMH$_{HR}$-H columns, shear degradation does take place and new peaks appear in the chromatogram on the smaller molar mass side.

ACRYLIC POLYMER

FIGURE 34 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 chromatograms from mixed-bed columns and the improvement achieved when using the TSKgel MultiporeH$_{XL}$-M column.

FIGURE 33

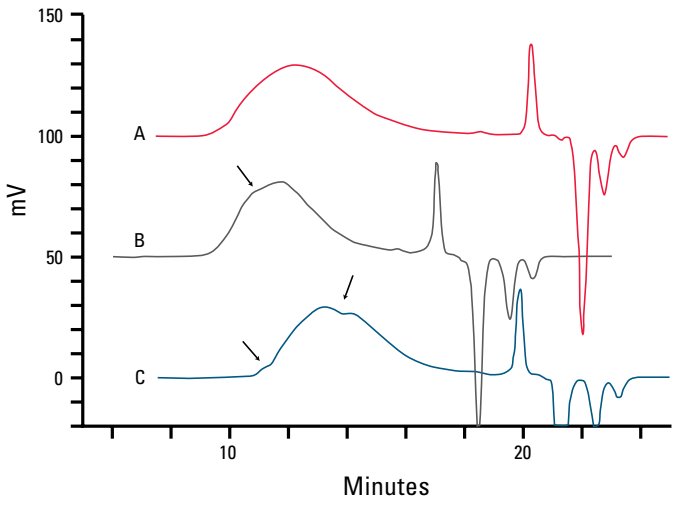
Shear degradation comparison



TSKgel GMH$_{HR}$-H, 5 µm, 7.8 mm ID × 30 cm L; B: TSKgel GMH$_{XL}$, 9 µm, 7.8 mm ID × 30 cm L; C: TSKgel GMH$_{HR}$-H(S), 13 µm, 7.8 mm ID × 30 cm L
Mobile phase: THF; Flow rate: 1.0 mL/min
Detection: UV @ 254 nm; Temperature: 25 °C
Sample: polystyrene standard F2000 (2.06 x 10^7 Da) 20 µL (0.025%)

FIGURE 34

Separation of acrylic resin by SEC 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 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

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

POLYMETHYLMETHACRYLATE

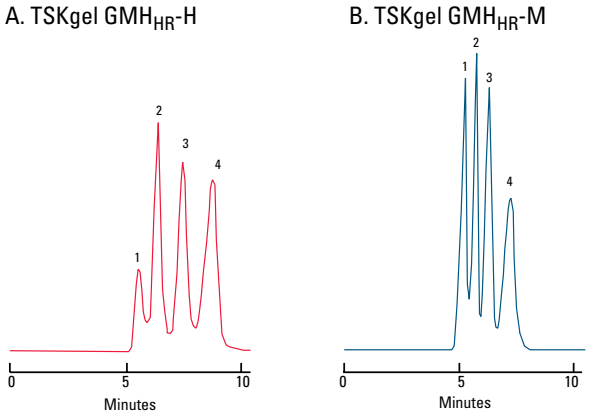
The effect of different pore size distributions in the mixed beds of TSKgel GMH$_{HR}$-H and TSKgel GMH$_{HR}$-M is illustrated in FIGURE 35. The TSKgel GMH$_{HR}$-M produces better resolution in the 8 x 10^5 to 1 x 10^4 Da range.

SEMI-MICRO GPC

Semi-micro columns are referred to as such since their dimensions are smaller than conventional columns in terms of internal diameter as well as in length: 4.6 mm or 6 mm ID x 15 cm vs. 7.8 mm ID x 30 cm of conventional GPC columns. As shown in FIGURE 36, a TSKgel SuperMultiporeHZ-N column provides the same or higher resolution at a much shorter analysis time than multiple conventional sized columns linked together.

FIGURE 35

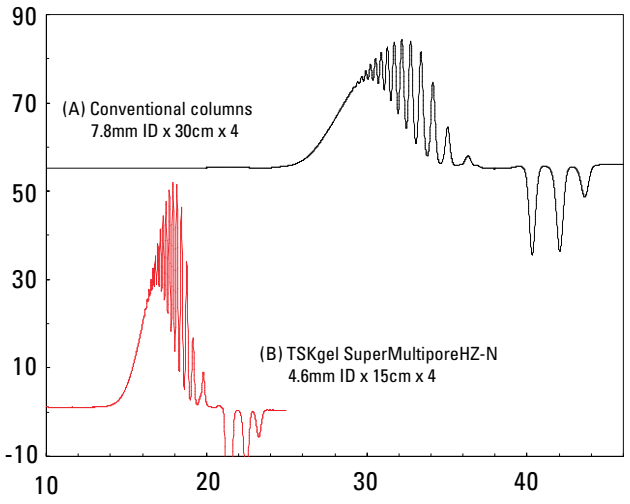
Comparison of TSKgel GMH$_{HR}$-H and -M columns with polymethylmethacrylate standards



Columns: 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 mmol/L sodium trifluoroacetate in hexafluoroisopropanol; Flow rate: 1.0 mL/min; Detection: UV @ 220 nm; Temperature: 40°C

FIGURE 36

PTMEG Analysis on conventional and semi-micro TSKgel Columns



Columns: A. Conventional columns, 7.8 mm ID x 30 cm L x 4, B. TSKgel SuperMultiporeHZ-N, 4.6 mm ID x 15 cm L x 4;
Mobile phase: THF; Flow rate: (A) 1.0 mL/min (B) 0.35 mL/min; Temperature: 40°C; Injection vol.: (A) 60 µL (B) 10 µL; Sample: poly(teramethylene ether glycol), (PTMEG 650), 10 µg/µL

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Stainless Steel Columns							
0017352	G1000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017353	G2000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017354	G2500H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017355	G3000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017356	G4000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017357	G5000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017358	G6000H$_{HR}$	7.8	30	5	≥ 10,000	0.5 - 1.0	5.0
0017359	G7000H$_{HR}$	7.8	30	5	≥ 10,000	0.5 - 1.0	5.0
0017362	GMH$_{HR}$-L mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0018055	GMH$_{HR}$-N mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017392	GMH$_{HR}$-M mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017360	GMH$_{HR}$-H mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0018393	GMH$_{HR}$-H(S)HT mixed-bed	7.8	30	13	≥ 8,000	5.0 - 1.0	2.0
0018391	GMH$_{HR}$-H(30)HT mixed-bed	7.8	30	30	≥ 4,000		
0018392	GMH$_{HR}$-H(20)HT mixed-bed	7.8	30	20	≥ 6,000		
0016131	G1000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0016134	G2000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0016135	G2500H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0016136	G3000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0016137	G4000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0016138	G5000H$_{XL}$	7.8	30	9	≥ 14,000	0.5 - 1.0	1.5
0016139	G6000H$_{XL}$	7.8	30	9	≥ 14,000	0.5 - 1.0	1.5
0016140	G7000H$_{XL}$	7.8	30	9	≥ 14,000	0.5 - 1.0	1.5
0016141	GMH$_{XL}$ mixed-bed	7.8	30	9	≥ 16,000	0.5 - 1.0	1.5
0016652	GMH$_{XL}$-L mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0018403	Multipore H$_{XL}$-M	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0017990	SuperH1000	6.0	15	3	≥ 16,000	0.3 - 0.6	6.0
0017991	SuperH2000	6.0	15	3	≥ 16,000	0.3 - 0.6	6.0
0017992	SuperH2500	6.0	15	3	≥ 16,000	0.3 - 0.6	6.0
0017993	SuperH3000	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017994	SuperH4000	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017995	SuperH5000	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017996	SuperH6000	6.0	15	5	≥ 16,000	0.3 - 0.6	4.0
0017997	SuperH7000	6.0	15	5	≥ 16,000	0.3 - 0.6	4.0
0017998	SuperHM-L	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017999	SuperHM-N	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0018000	SuperHM-M	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0018001	SuperHM-H	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Stainless Steel Columns							
0019309	TSKgel SuperHZ1000	4.6	15	3	≥ 16,000	0.15 - 0.35	5.6
0019302	TSKgel SuperHZ1000	6.0	15	3	≥ 16,000	0.25 - 0.60	5.6
0019310	TSKgel SuperHZ2000	4.6	15	3	≥ 16,000	0.15 - 0.35	5.0
0019303	TSKgel SuperHZ2000	6.0	15	3	≥ 16,000	0.25 - 0.60	5.0
0019311	TSKgel SuperHZ2500	4.6	15	3	≥ 16,000	0.15 - 0.35	4.0
0019304	TSKgel SuperHZ2500	6.0	15	3	≥ 16,000	0.25 - 0.60	4.0
0019312	TSKgel SuperHZ3000	4.6	15	3	≥ 16,000	0.15 - 0.35	3.0
0019305	TSKgel SuperHZ3000	6.0	15	3	≥ 16,000	0.25 - 0.60	3.0
0019313	TSKgel SuperHZ4000	4.6	15	3	≥ 16,000	0.15 - 0.35	3.5
0019306	TSKgel SuperHZ4000	6.0	15	3	≥ 16,000	0.25 - 0.60	3.5
0019660	TSKgel SuperHZM-N	4.6	15	3	≥ 16,000	0.15 - 0.35	3.5
0019661	TSKgel SuperHZM-N	6.0	15	3	≥ 16,000	0.25 - 0.60	3.5
0019662	TSKgel SuperHZM-M	4.6	15	3 and 5	≥ 16,000	0.15 - 0.35	2.0
0019663	TSKgel SuperHZM-M	6.0	15	3 and 5	≥ 16,000	0.25 - 0.60	2.0
0019664	TSKgel SuperHZM-H	4.6	15	10	≥ 9,000	0.15 - 0.35	1.0
0019665	TSKgel SuperHZM-H	6.0	15	10	≥ 9,000	0.25 - 0.60	1.0
0021488	SuperMultiporeHZ-M	4.6	15	4	≥ 16,000	0.15 - 0.35	2.4
0021815	SuperMultiporeHZ-N	4.6	15	3	≥ 20,000	0.15 - 0.35	4.0
0021885	SuperMultiporeHZ-H	4.6	15	6	≥ 11,000	0.15 - 0.35	1.0
Guard columns							
0018404	MultiporeHXL-M Guard	6.0	4.0	5	For P/N 0018403		
0007113	HXL-L Guard Column	6.0	4.0	7	For G1000HXL through G4000HXL columns		
0013727	HXL-H Guard Column	6.0	4.0	13	For G5000HXL through GMHXL-L mixed-bed columns		
0017368	HHR-L Guard Column	6.0	4.0	13	For G1000-4000HHR and GMHHR-L columns		
0017369	HHR-H Guard Column	6.0	4.0	5	For G5000-7000HHR and and GMHHR-M; -N; -H columns		
0018002	SuperH-L Guard Column	4.6	3.5	3	For SuperH1000-4000		
0018003	SuperH-H Guard Column	4.6	3.5	3	For SuperH5000-7000 and HM-L;-N;-M;-H columns		
0018004	SuperH-RC Ref. Column	6.0	15	4	For EcoSEC		
0019314	SuperHZ-L Guard Column	4.6	2.0	4	For 4.6 mm ID SuperHZ1000-4000 and HZM-N &-M		
0019668	SuperHZ-H Guard Column	4.6	2.0	10	For 4.6 mm ID SuperHZM-H columns		
0019666	SuperHZ-L Guard Column	4.6	3.5	4	For 6.0 mm ID SuperHZ1000-4000 and HZM-N &-M columns		
0019667	SuperHZ-H Guard Column	4.6	3.5	10	For 6.0 mm ID SuperHZM-H columns		
0021489	SuperMP-M Guard	4.6	2.0	4	For SuperMultipore HZ-M P/N 0021488		
0021816	SuperMP-N Guard	4.6	2.0	3	For SuperMultipore HZ-N P/N 0021815		
0021886	SuperMP-H Guard	4.6	2.0	6	For SuperMultipore HZ-H P/N 0021887		
TSKgel GPC columns for high temperature GPC							
0022887	GMHHR-H (30) HT2**	7,8	30		For HT-GPC up to 220°C		
0022888	GMHHR-H (20) HT2**	7.8	30		For HT-GPC up to 220°C		
0022889	GMHHR-H (S) HT2**	7,8	30		For HT-GPC up to 220°C		
0022890	G2000HHR (20) HT2**	7,8	30		For HT-GPC up to 220°C		
0018391	GMHHR-H (30)HT*	7,8	30		For HT-GPC		
0018392	GMHHR-H (20)HT*	7,8	30		For HT-GPC		
0018393	GMHHR-H (S)HT*	7,8	30		For HT-GPC		
Guard columns for high temperature GPC							
0022891	HHR (30) HT2** guardcolumn	7,5	7,5		For HT-GPC up to 220°C		
0022892	HHR (S) HT2** guardcolumn	7,5	7,5		For HT-GPC up to 220°C		
0018397	GMHHR-H (S)HT* guardcolumn	7,5	7,5		For HT-GPC		
0022893	HHR HT-RC Ref. Column	7,5	7,5		For EcoSEC HT		

HHR-HT/HT2 and HXL-HT/HT2 columns are packed in ODCB, HT* Temp. max 170 °C; HT2** Temp. max 220°C

SEC

AMBIENT AND HIGH TEMPERATURE EcoSEC GPC SYSTEM - BASED ON 40 YEARS EXPERIENCE

EcoSEC is a compact, all-in-one GPC system for fast, high resolution, semi-micro GPC. Comprising a precision solvent delivery system, automatic injector, column oven and a high performance refractive index detector, the design of the system components, their configuration and the optimized flow line provides outstanding performance with minimized dead volume. This makes EcoSEC the ideal instrument to be used in combination with the well respected TSKgel semi-micro GPC/SEC columns.

The EcoSEC High Temperature GPC System was issued to meet the demands for reliable results and reproducibility all combined in an easy to use and save instrument specifically for high temperature analyses. The EcoSEC High Temperature GPC System incorporates the proven design and technology used in the ambient EcoSEC GPC system.

For a detailed description of the ambient and high temperature EcoSEC instruments plese refer to our brochures ***EcoSEC GPC/SEC System*** and ***EcoSEC High Temperature GPC System.*** Request a printed copy at sales-marketing.tbg@tosoh.com or visit us at www.ecosec.eu.

ORDERING INFORMATION

TSKgel polymer standards: typical properties

Polystyrene

To calibrate TSKgel SuperMultiporeHZ columns

Part #	Description	Nominal MW (Da)	Amount
0021912	PStQuick MP-N	5.3×10^2 - 4.4×10^4	60 vials
0021913	PStQuick MP-M	5.3×10^2 - 8.0×10^5	60 vials
0021914	PStQuick MP-H	9.5×10^2 - 5.5×10^6	60 vials

To calibrate TSKgel H-type mixed-bed columns

Part #	Description	Nominal MW (Da)	Amount
0021915	PStQuick Kit-L	5.3×10^2 - 4.2×10^5	40 vials
0021916	PStQuick Kit-M	5.3×10^2 - 2.9×10^6	40 vials
0021917	PStQuick Kit-H	5.3×10^2 - 8.4×10^6	60 vials

To calibrate standard TSKgel GPC columns

Part #	Description	Amount
0021911	PStQuick A (A-2500, F-2, F-20, F-128, F-850)	20 vials
0021910	PStQuick B (A-1000, F-1, F-10, F-80, F-550)	20 vials
0021909	PStQuick C (A-500, A-5000, F-4, F-40, F-288)	20 vials
0021908	PStQuick D (A-2500, F-2, F-20, F-128)	20 vials
0021907	PStQuick E (A-1000, A-5000, F-4, F-40)	20 vials
0021906	PStQuick F (A-500, A-2500, F-2, F-20)	20 vials

Part #	Description	Nominal MW (Da)	Amount
0005202	A-300		10 g
0005203	A-500	530 MW	10 g
0005204	A-1000	950 MW	10 g
0005205	A-2500	2.800 MW	5 g
0005206	A-5000	6.200 MW	5 g
0005207	F-1	10.300 MW	5 g
0005208	F-2	16.700 MW	5 g
0005209	F-4	43.900 MW	5 g
0005210	F-10	102.000 MW	5 g
0005211	F-20	186.000 MW	5 g
0005212	F-40	422.000 MW	5 g
0005213	F-80	775.000 MW	5 g
0005214	F-128	1260.000 MW	1 g
0005215	F-288	2.890.000 MW	1 g
0005216	F-380	3.840.000 MW	1 g
0005217	F-450	4.480.000 MW	1 g
0005218	F-550	5.480.000 MW	1 g
0005219	F-700	6.770.000 MW	1 g
0005220	F-850	8.420.000 MW	1 g
0005221	F-2000	20.600.000 MW	1 g

0006476 Oligomer Kit, A-500 thru F-12812 x 1 g
0006477 High MW Kit, F-10 thru F-200012 x 1 g

Polyethylene oxide

Part #	Description	Nominal MW (Da)	Amount
0006211	SE-2	18.000 MW	0.5 g
0006212	SE-5	39.000 MW	0.5 g
0006213	SE-8	86.000 MW	0.5 g
0006214	SE-15	145.000 MW	0.5 g
0006215	SE-30	252.000 MW	0.5 g
0006216	SE-70	594.000 MW	0.5 g
0006217	SE-150	996.000 MW	0.5 g

0005773 Polyethylene Oxide Kit, SE-2 thru SE-150 7 x 0.2 g

The above molecular weights are determined by light scattering except for A-300, A-500, and A-1000, which are based on size exclusion chromatography. Results may vary among individual batches.