TOSOH

SEC COLUMNS

TOSOH BIOSCIENCE

YOUR SPECIALIST IN SEPARATION

SEC
SIZE EXCLUSION CHROMATOGRAPHY

Size exclusion chromatography (SEC) separates molecules based on their size, or more precisely, their hydrodynamic volume. It is usually applied to large molecules such as proteins or synthetic polymers. When an aqueous mobile phase is used, SEC is also referred to as gel filtration chromatography (GFC). When an organic eluent is applied, SEC is referred to as gel permeation chromatography (GPC). GPC is typically used to determine the molecular weight (MW) and the MW distribution of synthetic polymers while GFC is used to separate biopolymers based on their size.

Aqueous SEC is a popular technique for the separation and purification of proteins because of its effectiveness and non-denaturing mobile phase conditions. It is popular for the isolation of proteins, removal of aggregates, desalting or characterization of water-soluble polymers used in food products, paints, pharmaceutical formulations and the like. Stationary phases for aqueous SEC range from soft packing materials, such as dextran or agarose, over hydrophilic polymers to silica. Soft particles were employed as stationary phases for early GFC whereas today porous silica particles with high mechanical strength are applied for aqueous SEC in high performance liquid chromatography (HPLC).

Tosoh Bioscience offers a broad portfolio SEC columns packed with silica or polymer based porous beads. They are well suited for a wide range of applications in R&D, method development and quality control. TSKgel SW and SWXL are silica SEC phases with pore size distributions suited to protein separations. TSKgel SW-type packings feature low adsorption and well-defined pore size distribution. It is the leading SEC column series for HPLC due to its excellent resolution.

Polymeric TSKgel PW and PWXL columns are designed for GFC of water soluble organic polymers, polysaccharides, oligosaccharides, DNA and RNA. The TSKgel Alpha and SuperAW series, based on a unique hydrophilic, polyvinyl resin, is suited for SEC of water-soluble and polar organic-soluble polymers. TSKgel columns for gel permeation chromatography of organic soluble polymers are described in a separate brochure on GPC columns.

Tosoh Corporation employs state-of-the-art manufacturing techniques that result in uniformly bonded packing materials with narrow pore size distributions and well-defined particle sizes to ensure high performance and efficiency.

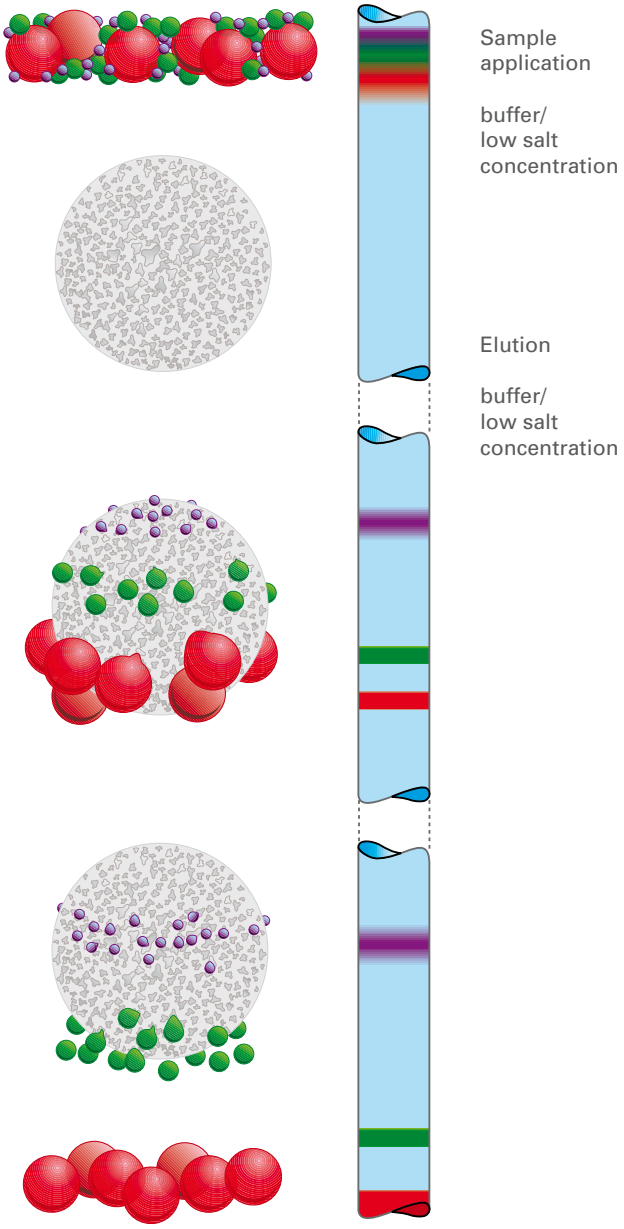
SEC
HOW IT WORKS

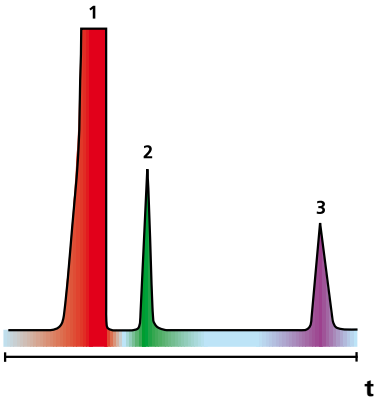
Size exclusion chromatography (SEC) is a method in which components of a mixture are separated according to their molecular size, based on the flow of the sample through a porous packing. In contrast to all other modes of liquid chromatography the prerequisite for SEC is that the analyte does not interact with the surface of the stationary phases. Differences in elution time are ideally based solely on the volume the analyte passes.

Large biomolecules that cannot penetrate the pores of the packing material elute first from the column. They are said to be excluded from the packing; they flow with the mobile phase in the interparticle space of the packed column. The exclusion limit characterizes the upper limit of molecular weight (or size), beyond which molecules will elute at the same retention volume called the exclusion or void volume of the column. Many SEC columns are referred to by their exclusion limit.

Smaller molecules can partially or completely enter the porous particles. Because these smaller molecules have to flow through the interparticle space, as well as through the pore volume, they will elute from the column after the excluded sample components. Molecules small enough to penetrate the whole pore system of the stationary phase will pass the entire pore and interparticle volume, and will elute late. Their retention volume is referred to as 'total permeation' in SEC, whereas it is interpreted as 'unretained peak' in conventional LC modes.

SEC is a very simple method for separating biomolecules, because it is not necessary to change the composition of the mobile phase during elution. However, the separation capacity of this method is limited. For a baseline separation it is necessary that the molecular weights of the molecules differ by at least 10 to 20 %.





SEC
TSKgel SEC COLUMNS

Tosoh Corporation has a proud history of innovation in size exclusion chromatography. TSKgel SEC columns are known worldwide for their reliability and suitability for the analysis of proteins, peptides and other biological macro-molecules. The complete TSKgel SW, PW, Alpha and SuperAW column lines consist of either silica based or polymer based packings, ranging in particle size from 4 µm to 20 µm. Columns are available in analytical through semi- preparative size, in stainless steel, PEEK or glass.

COLUMN SELECTION

The main criterion in choosing between the TSKgel SW, PW, Alpha and SuperAW SEC columns is the molecular weight of the sample and its solubility. The fact that the TSKgel SW columns are based on silica and the TSKgel PW, Alpha and SuperAW columns are derived from a hydrophilic polymer network has less impact on the separation than the particle and pore size differences.

TSKgel SW SERIES

Tosoh Bioscience TSKgel SW and SWXL series are silica SEC phases with pore size distributions suited to protein separations. A hydrophilic diol-type bonded phase shields the silica surface from interacting with protein samples. Due to their high resolving power, the TSKgel SW columns are suitable for the separation of mono-disperse biopolymers such as proteins and nucleic acids. TSKgel SW-type packings feature low adsorption and well-defined pore size distribution. They are the leading SEC columns in bioanalysis due to its excellent resolution.

TSKGEL PW SERIES

TSKgel PW and PWXL columns are packed with hydrophilic, rigid polymethacrylate beads. They are commonly used for the separation of synthetic water soluble polymers because they exhibit a much larger separation range, better linearity of calibration curves, and less adsorption than the TSKgel SW columns. While a TSKgel SW column is typically the first column to try for biopolymers, TSKgel PW columns have demonstrated good results for smaller peptides (<1,000 Da), protein aggregates, DNA fragments, and viruses. TSKgel PWXL-CP columns are especially suited for the separation of cationic polymers at low salt.

TSKgel AW/ALPHA SERIES

The TSKgel Alpha series columns are packed with polyvinyl beads and offer a new alternative for performing SEC. Their compatibility with a wide range of solvents makes them useful for both GFC and GPC. TSKgel SuperAW columns are based on the same chemistry as Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high throughput applications.

TABLE 1

CHARACTERISTICS OF TSKgel SIZE EXCLUSION COLUMN LINES

Column line	TSKgel SW / SWXL /SuperSW	TSKgel PW / PWXL	TSKgel Alpha / SuperAW
Resin type	Silica	Polymethacrylate	highly crosslinked Polymethacrylate
No. of available pore sizes	3/2	7	5
PH stability	2.5 - 7.5	2.0 - 12.0	2.0 - 12.0
Solvent compatability	100% polar	50% polar	100% polar, and nonpolar
Max. temp.	30°C	80°C*	80°C
Max. flow rate (mL/min)	6.0 (SW) 1.2 (SWXL) 0.4 (SuperSW)	1.2 (PW) 1.0 (PWXL)	1.0 (Alpha) 0.6 (SuperAW)
Pressure**(MPa)	1.0 - 12.0	1.0 - 4.0	2.0 - 4.0
Application focus	Proteins	Water-soluble polymers	Intermediate polar polymers

* Except for the TSKgel G-DNA-PW, which can be operated up to 50°C. 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.

SEC
TSKgel SEC COLUMN SELECTION

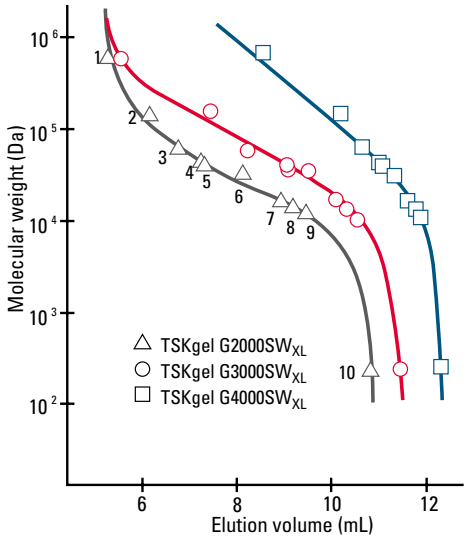
SAMPLE			COLUMN SELECTION		SELECTION CRITERIA
			FIRST CHOICE	ALTERNATIVE	
Carbohydrates	polysaccharides		TSKgel GMPWXL TSKgel SuperMultiporePW	TSKgel G5000PWXL and TSKgel G3000PWXL	large pore size, linear calibration curve, small particles, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW TSKgel SuperOligoPW	TSKgel G2500PWXL	small particles, high resolving power
Nucleic acids	DNA fragments	large	TSKgel G-DNA-PW or TSKgel G5000PWXL		large pore size, small particles, high resolving power
		medium and small	TSKgel G4000SWXL, TSKgel BioAssist G4SWXL TSKgel SuperSW3000 or TSKgel G3000SWXL TSKgel BioAssist G3SWXL		suitable pore sizes
	RNA		TSKgel G4000SWXL TSKgel BioAssist G4SWXL TSKgel SuperSW3000 or TSKgel G3000SWXL TSKgel BioAssist G3SWXL		suitable pore sizes
	oligonucleotides		TSKgel G2500PWXL		small pore size, ionic interaction
Proteins	normal size small-medium proteins		TSKgel SuperSW3000 TSKgel G3000SWXL TSKgel BioAssist G3SWXL TSKgel G4000SWXL TSKgel BioAssist G4SWXL TSKgel SuperSW2000 or TSKgel G2000SWXL TSKgel BioAssist G2SWXL	TSKgel G3000PWXL or G4000PWXL	small particles small to medium range pore sizes
	large proteins	low density lipoprotein	TSKgel G6000PWXL or TSKgel G5000PWXL		large pore sizes
		gelatin	TSKgel GMPWXL TSKgel SuperMultiporePW-M TSKgel G3000SWXL	TSKgel G5000PWXL and G3000PWXL	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000 TSKgel G3000SWXL TSKgel BioAssist G3SWXL or TSKgel G2000SWXL TSKgel BioAssist G2SWXL	TSKgel SuperSW2000 or G3000PWXL	small to medium range pore size, versatile
	small		TSKgel G2500PWXL	TSKgel SuperSW2000 or G2000SWXL	linear calibration curve, high resolving power
Viruses			TSKgel G6000PWXL or TSKgel G5000PWXL TSKgel SuperMultiporePW-H		large pore size, high resolving power
Synthetic polymers			TSKgel GMPWXL or TSKgel Alpha-M TSKgel SuperMultiporePW	TSKgel G5000PWXL and G3000PWXL or TSKgel Alpha-5000 and Alpha-3000	large pore size, low adsorption, linear calibration curve
	cationic		TSKgel G3000PWXL-CP TSKgel G5000PWXL-CP TSKgel G6000PWXL-CP		medium to large pore size, low adsorption, linear calibration curve
Synthetic oligomers	nonionic		TSKgel G-Oligo-PW TSKgel G2500PWXL or TSKgel Alpha-2500 TSKgel SuperOligoPW and TSKgel SuperMultiporePW-N	TSKgel G2500PW or SuperAW2500	small pore size, high resolving power
	anionic		TSKgel G2500PWXL or TSKgel Alpha-2500	TSKgel G2500PW or SuperAW2500	small pore size, ionic interaction

SEC
TSKgel SW SERIES

TSKgel SW-type columns (SW, SWXL and SuperSW) are all based on spherical silica particles with very high internal pore volume. They are stable from pH 2.0 to 7.5 and have excellent solvent stability up to 100% polar organic solvents. Three different pore sizes of the SW and SWXL packings result in different exclusion limits for several sample types, as shown by the calibration curve in Figure 1. From this data, recommended separation ranges for globular proteins can be made for each column (see Table 2). Different particle sizes, column dimensions and column hardware materials are available.

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

FIGURE 1



PROTEIN CALIBRATION CURVES FOR TSKgel SWXL COLUMNS

Column: TSKgel SWXL columns, 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)
Mobile phase: 0.3 mol/L NaCl in 0.1 mol/L sodium phosphate buffer, pH 7.0; Detection: UV @ 220 nm

HIGHLIGHTS

- Rigid spherical silica gel chemistry bonded with hydrophilic groups
- Well defined pore size distribution
- Low non specific adsorption
- Highest resolution and sensitivity
- PEEK column hardware for SWXL packings
- Short TSKgel QC-PAK columns for fast analysis
- Semi-preparative stainless steel columns for precise scale up

TABLE 2

PROPERTIES AND SEPARATION RANGES FOR TSKgel SW TYPE PACKINGS

TSKgel COLUMN	ID (MM) X LENGTH (CM L)	PARTICLE SIZE (µM)	PORE SIZE (Å)	MIN. NO. THEORET. PLATES	MOLECULAR WEIGHT OF PROTEINS (DA)
SuperSW2000	4.6 x 30	4	125	30,000	5 x 10^3–1.5 x 10^5
G2000SWXL	7.8 x 30	5	125	20,000	5 x 10^3–1.5 x 10^5
BioAssist G2SWXL	7.8 x 30	5	125	20,000	5 x 10^3–1.5 x 10^5
QC-PAK GFC 200	7.8 x 15	5	125	10,000	5 x 10^3–1.5 x 10^5
G2000SW	7.5 x 30/60 21.5 x 30/60	10 13	125 125	10,000/20,000 10,000/20,000	5 x 10^3–1.5 x 10^5 5 x 10^3–1.5 x 10^5
SuperSW3000	4.6 x 30	4	250	30,000	1 x 10^4–5 x 10^5
G3000SWXL	7.8 x 30	5	250	20,000	1 x 10^4–5 x 10^5
BioAssist G3SWXL	7.8 x 30	5	250	20,000	1 x 10^4–5 x 10^5
QC-PAK GFC 300	7.8 x 15	5	250	10,000	1 x 10^4–5 x 10^5
G3000SW	7.5 x 30/60 21.5 x 30/60	10 13	250 250	10,000/20,000 10,000/20,000	1 x 10^4–5 x 10^5 1 x 10^4–5 x 10^5
G4000SWXL	7.8 x 30	8	450	16,000	2 x 10^4–7 x 10^6
BioAssist G4SWXL	7.8 x 30	8	450	16,000	2 x 10^4–7 x 10^6
G4000SW	7.5 x 30/60 21.5 x 30/60	13 17	450 450	8,000/16,000 8,000/16,000	2 x 10^4–7 x 10^6 2 x 10^4–7 x 10^6

SEC
TSKgel SW SERIES APPLICATIONS

AGGREGATE ANALYSIS

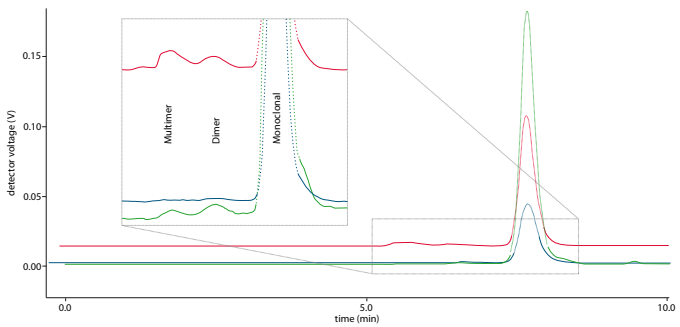
Protein aggregation is a common issue encountered during expression, purification and formulation of protein biotherapeutics, which needs to be characterized and controlled during the development and production of protein pharmaceuticals such as monoclonal antibodies (mAbs). Even small amounts of aggregates can alter the therapeutic function. TSKgel G3000XL columns are the industry standard for quality control of MAbs by SEC. Besides the traditional detection of proteins using their UV absorption at 280 nm, multi angle light scattering (MALS) detection gains more and more interest in protein analysis. Being a universal detection method, MALS can deliver valuable additional information. As it will also detect several other impurities, pure solvents and samples are of utmost importance. This also applies to the stationary phase, which should not generate interfering baseline noise under the conditions used for analysis. Figure 2 shows the analysis of MAb aggregates of a commercial monoclonal antibody with UV, refractive index (RI) and MALS detection. Separation was performed on a TSKgel G3000SWXL column under standard conditions.

When the analysis of proteins needs to be performed in a metal free environment, the BioAssistSW series offers TSKgel SWXL packings in PEEK housings, featuring the same performance as stainless steel columns. Figure 3 shows a typical separation performed with a BioAssist SW PEEK column.

USE OF DETERGENTS

Some SEC separations require denaturing conditions like sodiumdodecylsulfate (SDS) containing eluents. In other cases the formulations of biopharmaceuticals contain some detergents (e.g. Tween 20 or Triton). TSKgel SW type columns can be operated under these conditions although certain amounts of the detergent will stick to the column, affecting column lifetime and the future use of the column. If analysis under denaturing conditions was performed once, the affected column should be used with detergent containing eluents only. Regular maintenance of the column, the use of guard columns and monitoring of the column status by analyzing control samples are recommended as well.

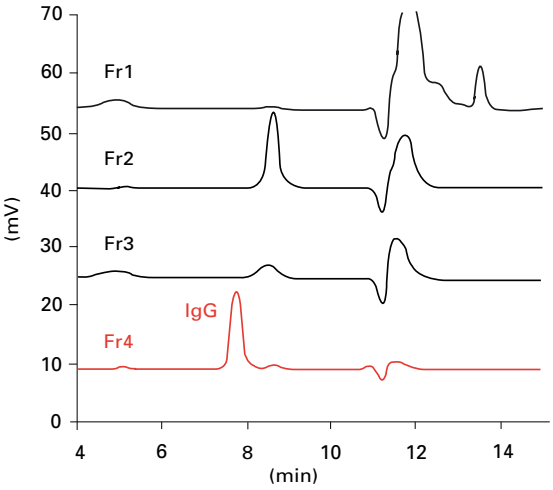
FIGURE 2



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.

FIGURE 3



QC ANALYSIS OF AN ANTI-TSH ANTIBODY PURIFIED FROM CELL CULTURE SUPERNATANT

Column: TSKgel BioAssist G3SWXL, 5 µm, 7.8 mm ID x 30 cm L
Mobile phase: 0.3 mol/L phosphate buffer, pH 7.0
Flow rate: 1.0 mL/min; Inj. volume: 50 µL

SEC
TSKgel SuperSW SERIES

Speed and resolution is an increasing demand in liquid chromatography. The need for high sensitivity applicable to trace analysis is increasing as sample size or sample concentrations become limited. To meet the needs of high sensitivity and high resolution protein analysis Tosoh Bioscience developed TSKgel SuperSW columns packed with 4 µm spherical silica particles. TSKgel SuperSW columns are available in two pore sizes, 125 Å and 250 Å, both featuring a minimum of 30,000 theoretical plates / column. Compared to the well established TSKgel SWXL (5 µm) series, SuperSW columns show higher resolution due to a 50 percent increase in theoretical plate numbers (Table 3).

To further improve performance, TSKgel SuperSW media are packed into columns with smaller inner diameter (1.0, 2.0, 4.6 mm ID). The smaller diameters are one reason for increased peak heights. In addition, the high resolution of the 4 µm particles and accordingly smaller peak widths further increase peak height provided the HPLC system is optimized with regard to dead volume.

HIGHLIGHTS

- 4 µm particle size featuring superior resolution and highest sensitivity
- Low non-specific adsorption
- High reproducibility due to well-defined pore size distribution
- 30,000 theoretical plates / column (4.6 mm ID)
- Microbore columns for increased sensitivity and reduced buffer consumption

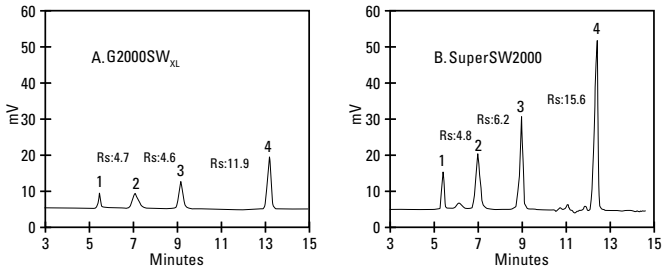
Figure 4 demonstrates the superior sensitivity reached with TSKgel SuperSW2000 compared to a TSKgel G2000SWXL column of the same length but larger inner diameter. TSKgel SuperSW can yield peak heights approximately 4 times that of TSKgel SWXL due to downsizing in column diameter and increased theoretical plates.

TABLE 3

SPECIFICATIONS OF TSKgel SuperSW SERIES COMPARED TO TSKgel SWXL SERIES

TSKgel COLUMN	PARTICLE SIZE (µM)	COLUMN SIZE (mm ID X cm L)	GUARANTEED THEOR. PLATES
TSKgel SuperSW2000	4	4.6 x 30	30,000
TSKgel SuperSW3000	4	4.6 x 30	30,000
TSKgel G2000SWXL	5	7.8 x 30	20,000
TSKgel G3000SWXL	5	7.8 x 30	20,000

FIGURE 4



COMPARISON OF TSKgel SuperSW2000 AND TSKgel G2000SWXL 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 30 cm L
Sample: 1. thyroglobulin (0.2 mg/mL); 2. albumin (1.0 mg/mL); 3. ribonuclease A (1.0 mg/mL); 4. p-aminobenzoic acid (0.01 mg/mL)
Inj. volume: 5 µL
Mobile phase: 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 (SuperSW2000), 1.0 mL/min (G2000SWXL)
Temp: 25°C; Detection: UV @ 280 nm

SEC
TSKgel SuperSW SERIES

SEPARATION RANGE OF TSKgel SuperSW

The TSKgel SuperSW series has the same pore sizes as the conventional TSKgel SWXL series with equivalent grade. Therefore it has similar calibration curves and separation ranges as well. Method transfer from conventional SEC to high resolution SEC is very straight forward. TSKgel SuperSW columns are available in two pore sizes, 125 Å (TSKgel SuperSW2000) and 250 Å (TSKgel SuperSW3000). Figure 5 shows the SEC calibration curves for standard proteins. In general, TSKgel SuperSW2000 is suited to separate proteins with molecular weights of 150 KDa or smaller. TSKgel SuperSW3000 can be used for the separation of proteins with molecular weights up to 500 KDa.

INCREASED DETECTION LIMIT

Table 4 shows the detection limits for some proteins. The high sensitivity allows for analysis of nanogram sample amounts. If sample amount is limited a reduction of column inner diameter can further enhance sensitivity. TSKgel SuperSW3000 columns are available with 4.6; 2 and 1 mm ID. Figure 6 shows the levels of sensitivity which can be reached with semi-micro or micro columns. When limited sample amount is an issue (e.g. in proteomics research) enhancing detection limits by using a micro column can increase the number of hits.

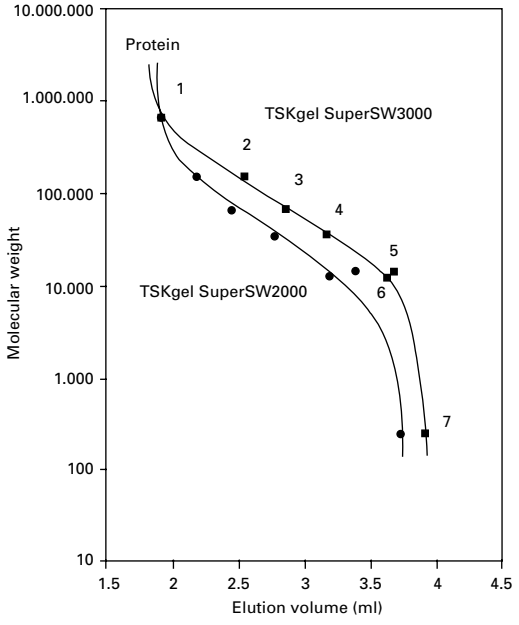
TABLE 4

DETECTION LIMIT FOR PROTEINS (S/N=3)

	TSKgel SuperSW	TSKgel SWXL
FLOW CELL	STANDARD CELL (LOW DEAD VOLUME TYPE)	STANDARD CELL (LOW DEAD VOLUME TYPE)
Light path length	10 mm	10 mm
Thyro-globulin	70 ng	200 ng
γ-globulin	50 ng	100 ng
Bovine serum albumin	70 ng	200 ng
Ovalbumin	50 ng	100 ng
Myoglobin	15 ng	30 ng

Column: TSKgel SuperSW3000, 4.6 mm ID x 30 cm L;
TSKgel G3000SWXL, 7.8 mm ID x 30 cm L
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7
Detection: UV @ 220 nm

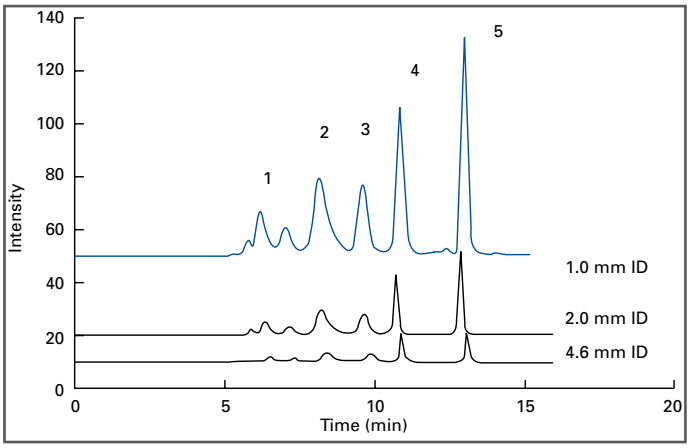
FIGURE 5



PROTEIN CALIBRATION CURVES FOR TSKgel SuperSW

Column: TSKgel SuperSW Series, 4.6 mm ID X 30 cm L
Sample: Standard proteins (5 µL, 0.1 g/L each);
1.thyroglobulin 2. γ-globulin 3. bovine serum albumin
4. ß-lactoglobulin 5. lysozyme 6. cytochrome C 7. glycine tetramer
Mobile phase: 0.2 mol/L phosphate buffer (pH 6.7)
Flow rate: 0.35 mL/min; Detection: UV @ 280 nm

FIGURE 6



ESTIMATION OF SENSITIVITY

Column: TSKgel SuperSW3000, 1.0, 2.0, 4.6 mm ID x 30 cm L
Sample: 1. thyroglobulin (1.0 g/L), 2. γ-globulin (2.0 g/L), 3. ovalbumin (2.0 g/L), 4. ribonuclease A (3.0 g/L), 5. p-aminobenzoic acid (0.02 g/L)
Mobile phase: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3
Flow rate: 16 µL/min (1 mm), 65 µL/min (2 mm); 350 µL/min (4.6 mm)
Inj.volume: 0.2 µL; Temperature.: 25 °C
Detection: UV @ 280 nm, cell vol. 2 µL (4.6 mm ID), 35 nL (1.0, 2.0 mm ID)

SEC
TSKgel SuperSW APPLICATIONS

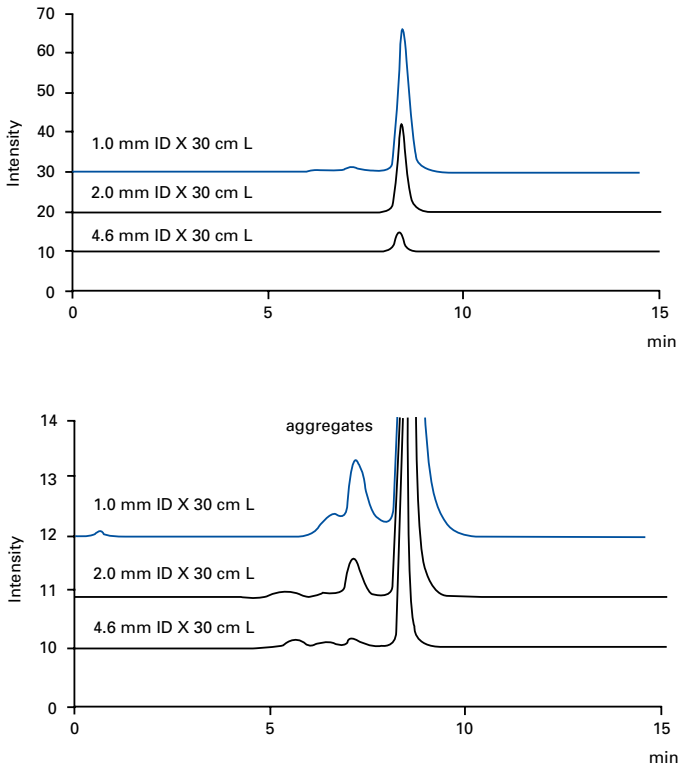
QC ANALYSIS OF ANTIBODIES

Thermally induced denaturation or aggregation of therapeutic antibodies can be a significant problem during different stages of its production and formulation, since aggregates affect the efficiency of the biotherapeutic. Thus the quantification of aggregates is an important parameter in the quality control analysis of biopharmaceuticals. Using TSKgel SuperSW3000 columns the amounts of tri-, di- and monomers of monoclonal antibodies can be monitored. Quantification is facilitated by using smaller inner diameter columns since peak height is significantly increased (Figure 7).

SEC-ESI-MS ANALYSIS OF PROTEINS

Hyphenated separation techniques like HPLC-MS or HPLC-ELSD allow sensitive analysis of samples with very low analyte concentrations. Moreover MS/MS detection is a powerful tool to provide further structural information about the compounds. These detection methods require the use of volatile buffer systems because the solvent must be evaporated before the sample molecules enter the detection system. For LC/MS analysis TSKgel SuperSW columns can be run with formate buffers as mobile phase, instead of the common phosphate buffers. Figure 8 demonstrates that at least 300 mM ammonium formate is necessary to reach separation efficiencies comparable to 100 mM phosphate buffer.

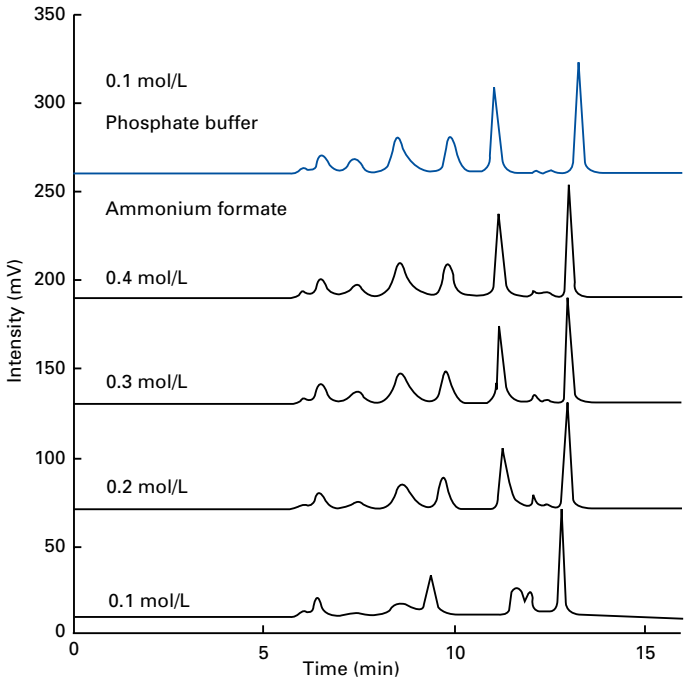
FIGURE 7



SEPARATION OF IgG ON TSKgel SuperSW3000

Column: TSKgel SuperSW3000, 1.0 mm ID x 30 cm L
Sample: IgG (mouse, mAb, 1.0 g/L)
Mobile phase: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4, + 0.05 NaN_3; Flow rate: 16 µL/min (1 mm ID), 65 µL/min (2 mm ID), 350 µL/min (4.6 mm ID); Inj.Vol.: 0.2 µL; Temp.: 25 °C
Detection: UV @ 280 nm, cell vol. 2 µL (4.6 mm), 35 nL (1.0, 2.0 mm)

FIGURE 8



SEPARATION OF PROTEINS WITH AMMONIUM FORMATE ELUENT ON TSKgel SuperSW3000

Column: TSKgel SuperSW3000, 1.0, 2.0, 4.6 mm ID x 30 cm L
Sample: 1. tyroglobulin (1.0 g/L), 2. γ-globulin (2.0 g/L), 3. ovalbumin (2.0 g/L), 4. ribonuclease A (3.0 g/L), 5. p-aminobenzoic acid (0.02 g/L)
Mobile phase: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3
Flow rate: 16 µL/min (1 mm ID), 65 µL/min (2 mm ID), 350 µL/min (4.6 mm ID)
Inj.volume: 0.2 µL; Temp.: 25 °C
Detection: UV @ 280 nm, cell vol. 2 µL (4.6 mm ID), 35 nL (1.0, 2.0 mm ID)

SEC
TSKgel SuperSW SYSTEM REQUIREMENTS

OPTIMIZATION OF HPLC EQUIPMENT

To benefit from the improved features of TSKgel SuperSW columns the HPLC system should be optimized and extra column peak broadening reduced. This means reduction of dead volume and adjustment of sample concentration and injection volume.

SYSTEM DEAD VOLUME

Key components of the HPLC system with regard to dead volume reduction are the void volume of tubings, the cell volume of the detector cell and the void volume of the injection unit. Modern UHPLC systems designed for use with sub 2 µm particles exhibit extremely small dead volumes and can be used for SEC analysis without modification.

VOID VOLUME OF THE TUBING

The volume of tubing from injector to column, column to detector influences the diffusion within the tubing and the column efficiency. Column efficiency starts deteriorating remarkably when the volume of the tubing exceeds 10 µL (e.g. 0.1 mm ID x 150 cm L). Shortening of tubings of 0.1 or 0.125 mm inner diameter is often better than using longer capillaries with smaller inner diameters. The backpressure increases with smaller inner diameters and the system becomes more susceptible towards clogging.

DETECTOR CELL VOLUME

The detector cell volume also contributes to the dead volume of the system and might impair peak resolution. For most separations with 4.6 mm ID TSKgel SuperSW columns a 8-10 µL standard detector cell might be sufficient but for semi-micro (2 mm ID) or micro columns (1 mm ID), we strongly recommend using semi-micro detector cells.

INJECTOR

The maximum number of theoretical plates in isocratic separations can be reached when using a low diffusion type manual injector like the Rheodyne 8125. All kinds of automated HPLC injectors will deteriorate column efficiency to a certain extend but due to practical reasons, auto-samplers are nowadays standard. All the more it is important to select an auto-sampler capable of trace injection mode. Dead volume of the outlet capillary should be minimized to the utmost (as short as possible, 0.1 mm ID). Figure 9 shows the effect of injector tubings on column efficiency for a 1 mm ID column.

TSKgel SuperSW2000 AND SuperSW3000 OPERATING CONDITIONS

For best results, it is recommended to use the following experimental conditions for TSKgel SuperSW columns:	
CONNECTIONS	
Tubing	The conventional 0.1 mm tubing may be used, but length should be kept as short as possible. Void volume between the column and detector cell should be less than 20 µL.
INJECTOR	Best results are obtained with a low diffusion type manual injector (Rheodyne 8152). Autosampler outlet void volume should be as low as possible.
SAMPLE VOLUME	Sample volume should be 10 µL or less. Sample load should be less than 100 µg (4.6 mm ID column).
GUARD COLUMN	A guard column or an inline filter is highly recommended to reduce clogging and contamination.
DETECTOR	
Flow Cell	For best results, use a flow cell with a maximum of 2 µL. The 2 µL flow cell will give the highest efficiencies. A 2-10 µL flow cell can be used for 4.6 mm ID columns. However, theoretical plates will be reduced.
Time Constant	A small time constant (less than 0.5 sec) is needed to achieve best column performance.
PUMP	A pump capable of accurately delivering a flow rate between 0.01 mL/min and 0.35 mL/min is recommended.

SEC
TSKgel SuperSW SYSTEM REQUIREMENTS

SAMPLE LOAD AND INJECTION VOLUME

Although the efficiency of TSKgel SuperSW columns is high, it is obvious that it decreases at high sample loads. Figure 10 shows that sample load should not exceed 100 µg for a TSKgel SuperSW3000 column of 4.6 mm ID x 30 cm L. On the other hand the injection volume itself is a critical parameter. As for all HPLC applications injection volume should be as small as possible. If injection volume exceeds 20 µL on a 4.6 mm ID column, a considerable deterioration of column efficiency is observed for TSKgel SuperSW2000 (80 µL for TSKgel SuperSW3000). In general the sample load should be less than 100 µg in less than 10 µL injection volume for a 4.6 mm ID TSKgel SuperSW column.

FLOW RATE DEPENDENCE

The effect of flow rate on column efficiency depends on particle size of packing materials, sample molecular size, eluent viscosity, etc. The appropriate flow rate for TSKgel SuperSW columns is up to 0.4 mL/min for a 4.6 mm ID column, up to 75 µL/min for a 2 mm ID column, and up to 20 µL/min for a 1 mm ID column, respectively. If higher resolution is required the flow rate can be lowered.

MOBILE PHASE

The eluent plays an important role in SEC separations. When denaturing agents are used, the exclusion limits for proteins become smaller since they lose their compact globular structure. Proper selection of eluting conditions is necessary to maximize the molecular sieving mechanism and to minimize secondary effects, such as ionic and hydrophobic interactions between the sample and the column packing material. In general, the use of relatively high ionic strength buffers is recommended for most protein applications. A neutral salt is often added to increase ionic strength.

RECOVERY OF PROTEIN

TSKgel SuperSW series is capable of obtaining high protein recovery even in trace analysis with sample load of 1 µg or lower. Most proteins are recovered quantitatively with TSKgel SuperSW series, but it is important to make sure that samples in small concentrations are not adsorbed to the sample vial or to the HPLC system itself. Similar samples should be injected several times before measurement so that adsorption points within the system are inactivated in advance when trace analysis is performed.

FIGURE 9

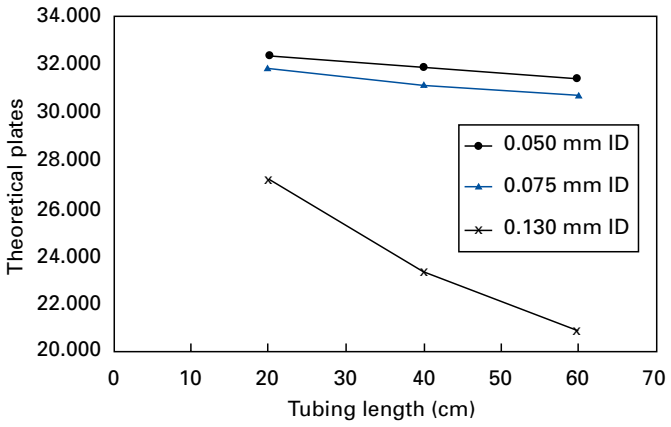
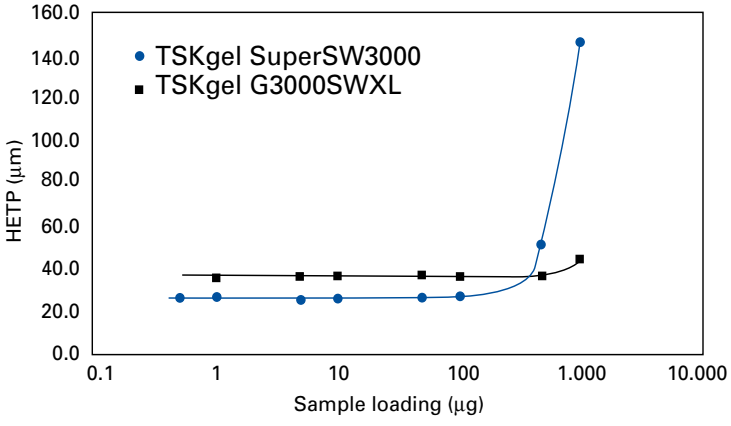


FIGURE 10



INFLUENCE OF TUBING (INJECTOR TO COLUMN)

Column: TSKgel SuperSW3000 1.0 mm ID x 30 cm L
Mobile phase: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05 % NaN_3
Flow rate: 16 µL/min; Inj.volume: 0.2 µL; Temp.: 25 °C
Detection: UV @ 280 nm
Sample: p-Aminobenzoic acid (20mg/L)
Tubing: ID (mm) x L (cm), Vol.
0.050 x 20, 393 nL; 0.050 x 40, 785 nL; 0.050 x 60, 1178 nL;
0.075 x 20, 883 nL; 0.075 x 40, 1766 nL; 0.075 x 60, 2469 nL;
0.130 x 20, 2653 nL; 0.130 x 40, 5307 nL; 0.130 x 60, 7960 nL

EFFECT OF SAMPLE LOAD

Column: TSKgel SuperSW series, 4.6 mm ID x 30 cm L;
TSKgel SWXL series, 7.8 mm ID x 30 cm L
Sample: Bovine serum albumin
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7
Flow rate: 0.35 mL/min (SuperSW series), 1.00 mL/min (SWXL series)
Temp.: 25 °C; Detection: UV @ 280 nm, micro flow cell

SEC
TSKgel SW SERIES ORDERING INFORMATION

ORDERING INFORMATION

PART #	DESCRIPTION	ID (MM)	LENGTH (CM)	PARTICLE SIZE (µM)	NUMBER THEORETICAL PLATES	FLOW RATE (ML/MIN) RANGE	FLOW RATE (ML/MIN) MAX.	MAXIMUM PRESSURE DROP (MPa)
GLASS COLUMNS								
16214	QC-PAK GFC 200GL	8.0	15	5	≥ 10,000	0.5 - 1.0	1.2	4.0
16216	QC-PAK GFC 300GL	8.0	15	5	≥ 10,000	0.5 - 1.0	1.2	4.0
08800	G3000SW, Glass	8.0	30	10	≥ 10,000	0.4 - 0.8	0.8	2.0
08801	G4000SW, Glass	8.0	30	13	≥ 8,000	0.4 - 0.8	0.8	2.0
STAINLESS STEEL COLUMNS								
18674	SuperSW2000	4.6	30	4	≥ 30,000	0.1 - 0.35	0.4	12.0
21845	SuperSW3000 -NEW-	1.0	30	4	≥ 18,000	0.016	0.02	12.0
21485	SuperSW3000 -NEW-	2.0	30	4	≥ 25,000	0.065	0.075	12.0
18675	SuperSW3000	4.6	30	4	≥ 30,000	0.1 - 0.35	0.4	12.0
08540	G2000SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	1.2	7.0
08541	G3000SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	1.2	7.0
08542	G4000SWXL	7.8	30	8	≥ 16,000	0.5 - 1.0	1.2	3.5
16215	QC-PAK GFC 200	7.8	15	5	≥ 10,000	0.5 - 1.0	1.2	4.0
16049	QC-PAK GFC 300	7.8	15	5	≥ 10,000	0.5 - 1.0	1.2	4.0
05788	G2000SW	7.5	30	10	≥ 10,000	0.5 - 1.0	1.2	2.0
05789	G3000SW	7.5	30	10	≥ 10,000	0.5 - 1.0	1.2	2.5
05790	G4000SW	7.5	30	13	≥ 8,000	0.5 - 1.0	1.2	1.5
05102	G2000SW	7.5	60	10	≥ 20,000	0.5 - 1.0	1.2	4.0
05103	G3000SW	7.5	60	10	≥ 20,000	0.5 - 1.0	1.2	5.0
05104	G4000SW	7.5	60	13	≥ 16,000	0.5 - 1.0	1.2	3.0
06727	G2000SW	21.5	30	13	≥ 10,000	3.0 - 6.0	8.0	1.0
06728	G3000SW	21.5	30	13	≥ 10,000	3.0 - 6.0	8.0	1.5
06729	G4000SW	21.5	30	17	≥ 8,000	3.0 - 6.0	8.0	1.0
05146	G2000SW	21.5	60	13	≥ 20,000	3.0 - 6.0	8.0	2.0
05147	G3000SW	21.5	60	13	≥ 20,000	3.0 - 6.0	8.0	3.0
05148	G4000SW	21.5	60	17	≥ 16,000	3.0 - 6.0	8.0	2.0
PEEK COLUMNS								
20027	BioAssist G2SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	1.2	7.0
20026	BioAssist G3SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	1.2	7.0
20025	BioAssist G4SWXL	7.8	30	8	≥ 16,000	0.5 - 1.0	1.2	3.5

SEC
TSKgel PW SERIES

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. Stable from pH 2 to 12, TSKgel PW series columns can be used in mobile phases of water or buffer (up to 50% polar organic solvent). A large pore G6000PW phase is available in PEEK column hardware (TSKgel BioAssist G6PW) for ultra-low sample adsorption during virus analysis. The properties of all TSKgel PW columns are summarized in Table 5.

When the molecular weight range of the sample is broad or unknown, Tosoh Bioscience offers two mixed-bed columns: The TSKgel GMPW column and its high resolution counterpart, TSKgel GMPWXL, are packed with the G2500, G3000 and G6000 PW or corresponding PWXL resins.

The new generation of TSKgel SuperMultiporePW columns for semi-micro SEC provide near linear calibration curves. They are packed with spherical, mono-disperse particles incorporating a proprietary multi-pore particle technology. They are ideally suited to analyze water soluble polymers, such as polyvinylpyrrolidones or dextrans.

The TSKgel PWXL product line also offers specialty columns for analyzing carbohydrate oligomers (TSKgel G-Oligo-PW) and DNA and RNA fragments of 500-5000 base pairs (TSKgel G-DNA-PW). The new SuperOligoPW semi-micro SEC column featuring a small particle size has been designed to enable fast analysis of oligosaccharides and other water soluble oligomers.

TSKgel PWXL-CP columns have the same base matrix as the PWXL columns and were specifically developed for the analysis of water-soluble cationic polymers.

HIGHLIGHTS

- Hydrophilic spherical polymethacrylate particles
- pH range of 2-12 with up to 50% polar organic solvent
- Seven different TSKgel PW pore sizes
- Linear SEC column line encorporating proprietary multipore technology
- Speciality columns for challenging SEC separations

TABLE 5

PROPERTIES AND SEPARATION RANGES OF TSKgel PW, PWXL AND PWXL-CP COLUMNS

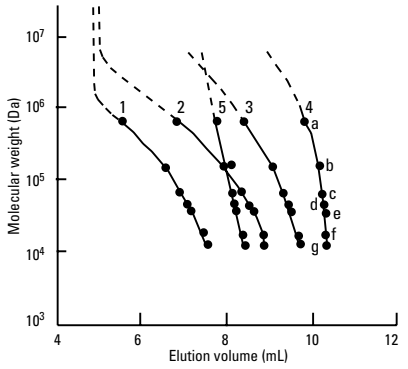
TSKgel COLUMN	PARTICLE SIZE (µM)	PORE SIZE (Å)	MW RANGE (PEG/PEO)
G1000PW	12	<100	$<1 \times 10^3$
G2000PW	12	125	$<2 \times 10^3$
G2500PW	12, 17	<200	$<3 \times 10^3$
G3000PW	12, 17	200	$<5 \times 10^4$
G4000PW	17	500	$<3 \times 10^5$
G5000PW	17	1,000	$<1 \times 10^6$
G6000PW/ BioAssist G6PW	17	>1,000	$<8 \times 10^6$
GMPW	17	<100-1,000	5×10^2 - 8×10^6
G2500PWXL	7	<200	$<3 \times 10^3$
G3000PWXL	7	200	$<5 \times 10^4$
G4000PWXL	10	<500	$<3 \times 10^5$
G5000PWXL	10	1000	$<1 \times 10^6$
G6000PWXL	13	>100	$<8 \times 10^6$
G-DNA-PW	10	>1,000	$<8 \times 10^6$
GMPWXL	13	100-1,000	5×10^2 - 8×10^6
G-Oligo-PW	7	125	$<5 \times 10^3$
SuperMultiporePW-N	4	n/a	3×10^2 - 5×10^4
SuperMultiporePW-M	5	n/a	5×10^2 - 1×10^6
SuperMultiporePW-H	8 (6-10)	n/a	1×10^3 - 1×10^7
SuperOligoPW	3	n/a	1×10^2 - 3×10^3
G3000PWXL-CP	7	200	$< 9 \times 10^4$
G5000PWXL-CP	10	1,000	$< 1 \times 10^6$
G6000PWXL-CP	13	>1,000	$< 2 \times 10^7$

SEC
TSKgel PW SERIES

CALIBRATION CURVES

Figure 11 shows the calibration curves for polyethylene glycol (PEG) and oxides (PEO) for TSKgel PW and TSKgel PWXL columns, respectively. In general silica based SW type columns are recommended for the analysis of proteins, but for special applications, e.g. at basic pH or for large molecular weight proteins, PW type columns can be applied (Figure 12). Figure 13 shows the near linear calibration curves for PEG/PEO on TSKgel SuperMultiporePW columns.

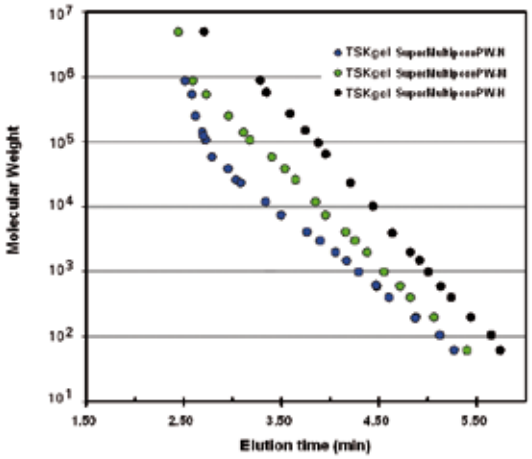
FIGURE 12



PROTEIN CALIBRATION CURVES ON TSKgel PWXL COLUMNS

Column: 1. TSKgel G3000PWXL, 2. TSKgel G4000PWXL, 3. TSKgel G5000PWXL, 4. TSKgel G6000PWXL, 5. TSKgel GMPWXL
Sample: a. thyroglobulin (660,000 Da), b. γ-globulin (150,000 Da), c. albumin (67,000 Da), d. ovalbumin (43,000 Da), e. ß-lactoglobulin (36,000 Da), f. myoglobin (16,900 Da), g. cytochrome C (12,400 Da)
Mobile phase: 0.2 M phosphate buffer (pH 6.8); Flow rate: 1.0 mL/min; Detection: UV @ 280 nm

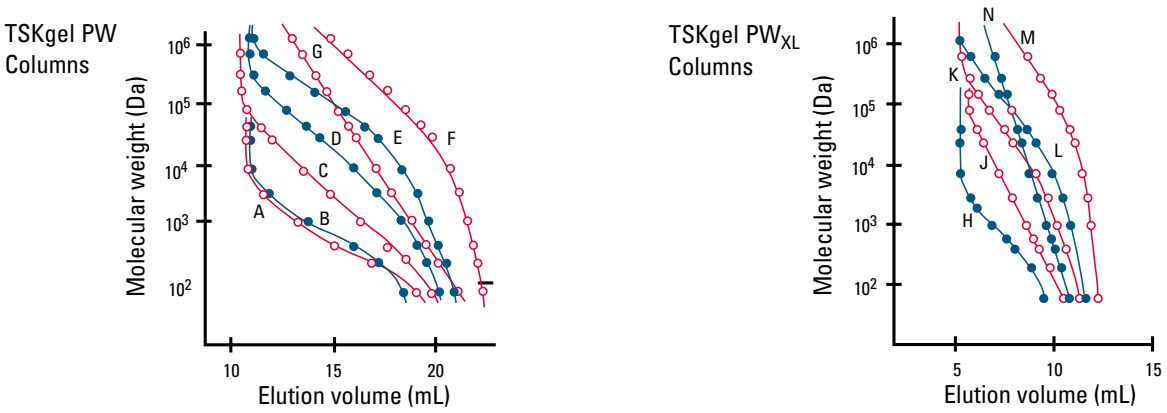
FIGURE 13



CALIBRATION CURVES FOR TSKgel SuperMultiporePW

Sample: PEO & PEG standards;
Mobile phase: H_2O; Flow rate: 0.6 mL/min; Detection: RI;
Temperature: 25 °C

FIGURE 11



POLYETHYLENE GLYCOL AND OXIDE CALIBRATION CURVES ON TSKgel PW AND TSKgel PWXL COLUMNS

Column: TSKgel PW columns: A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5 mm ID x 60 cm L
Mobile phase: distilled water; Flow rate: 1.0 mL/min; Detection: RI

TSKgel PWXL columns: H. G2500PWXL, J. G3000PWXL, K. G4000PWXL, L. G5000PWXL, M. G6000PWXL, N. GMPWXL, all 7.8 mm ID x 30 cm L

SEC
TSKgel PW SERIES APPLICATIONS

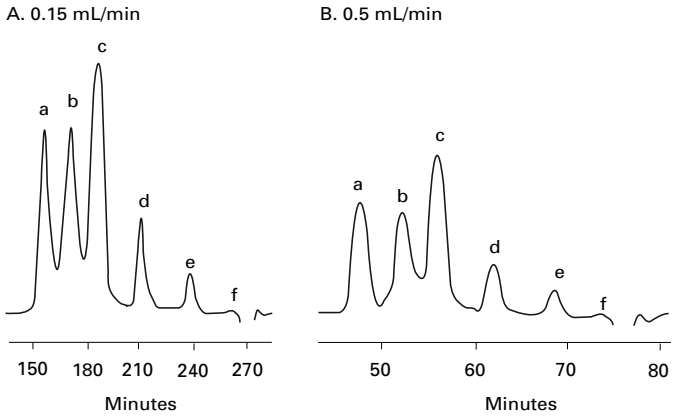
LARGE DNA FRAGMENTS

For the separation of large DNA fragments greater than 1,000 base pairs, a four-column system is typically required. Baseline resolution of DNA fragments up to 7,000 base pairs can be achieved, provided there is a two-fold difference in the chain length of the fragments. Figure 14A shows the elution of double stranded DNA fragments, obtained from pBR322 DNA cleaved by both Eco RI and Bst NI, on four TSKgel G-DNA-PW columns in series. The eluted peaks were collected and subjected to polyacrylamide gel electrophoresis, which showed almost complete separation of the 1060, 1857, and 4362 base pair fragments. Although lower flow rates typically yield better separations of most fragments, the resolution of the 1857 and 4362 base pair fragments was slightly greater at the higher flow rate, as shown in Figure 14B.

OLIGOMERS

The influence of particle size on resolution and analysis time can be seen in Figure 15. It compares the separation of PEG 200 on two TSKgel G-Oligo-PW columns in series with 7 µm beads and two newly developed TSKgel SuperOligoPW semi-micro columns with a 3 µm material. The TSKgel SuperOligoPW column is designed for high resolution separations water soluble oligomers. Figure 15 demonstrates excellent resolution of the PEG 200 obtained by using the smaller, 3 µm particle size packing in the TSKgel SuperOligoPW column.

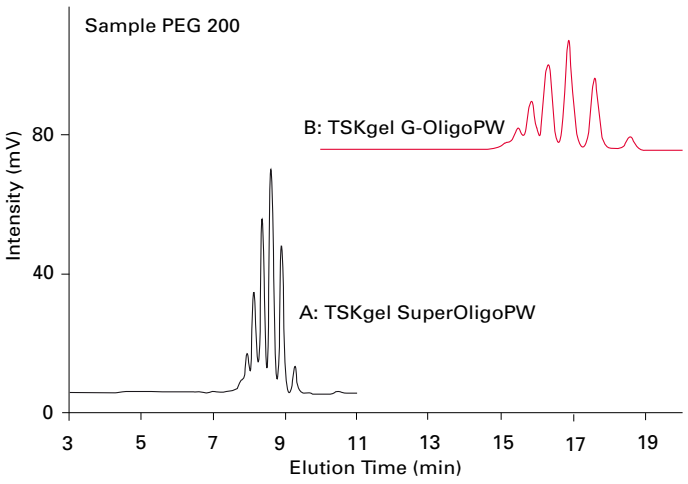
FIGURE 14



SEPARATION OF LARGE DNA FRAGMENTS ON TSKgel G-DNA-PW

Column: TSKgel G-DNA-PW, 10 µm, 4 x 7.8 mm ID x 30 cm L
Sample: 60 µL of Eco RI and Bst NI cleaved pBR322 DNA, Base pairs: a. 4362, b. 1857, c. 1060 & 928, d. 383, e. 121, f. 13
Mobile phase: 0.3 M NaCl, 1 mM EDTA, in 0.1 M Tris-HCl, pH 7.5, Flow rate: A. 0.15 mL/min, B. 0.5 mL/min; Detection: UV @ 260 nm

FIGURE 15



ANALYSIS OF PEG 200. COMPARISON BETWEEN TSKgel SuperOligoPW AND TSKgel G-OLIGO-PW

Column: A. TSKgel SuperOligoPW, 6.0 mm ID x 15 cm L x 2
B. TSKgel G-Oligo-PW, 7.8 mm ID x 30 cm L x 2
Mobile phase: H_2O; Flow rate: A: 0.6 mL/min, B: 1.0 mL/min
Detection: RI; Temp.: 25°C; Inj. vol.: A: 20 µL, B: 100 µL

SEC
TSKgel PW-CP SERIES

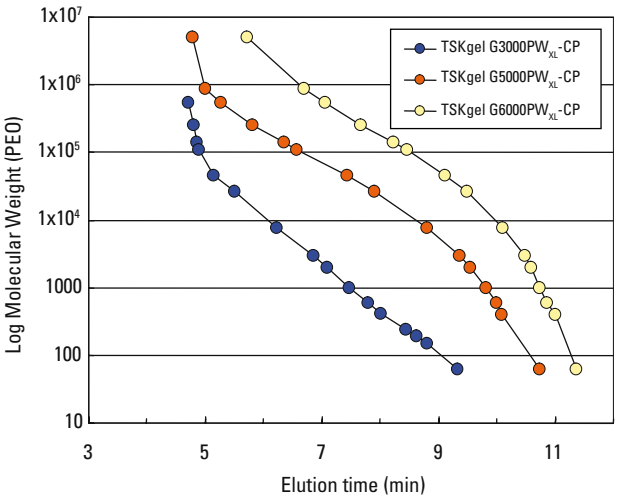
TSKgel PWXL-CP size exclusion columns were specifically developed for the analysis of water soluble cationic polymers. Three columns are available within the TSKgel PWXL-CP series, each with a different particle size, separation range and exclusion limit, allowing polymers within a wide molecular mass range to be separated and characterized.

When using conventional SEC columns the analysis of cationic polymers requires a high salt concentration in the mobile phase to prevent adsorption of the polymers onto the particles in SEC columns. The TSKgel PWXL-CP columns eliminate ionic adsorption onto the particle by incorporating a cationic functionality on the particle surface. This modification results in high recovery for cationic polymers and enables elution under low salt conditions.

These columns show high theoretical plate numbers, linear calibration curves and high durability. The base resin is the same as that used in the TSKgel PWXL columns. Figure 16 shows the calibration curves for PEG/PEO calibration curves obtained with TSKgel PWXL-CP columns.

Figure 17 demonstrates that these SEC columns can be utilized for the analysis of a wide variety of cationic polymers. Various cationic polymers with different functional groups and molecular weights were injected on the three TSKgel PWXL-CP columns (TSKgel G6000PWXL-CP, G5000PWXL-CP and G3000PWXL-CP, connected in series).

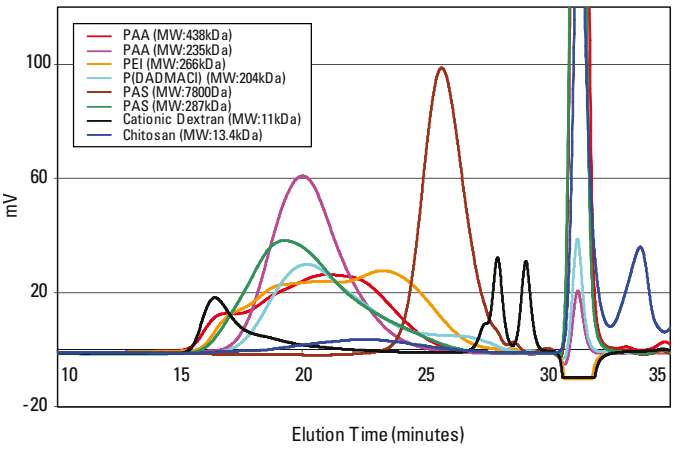
FIGURE 16



CALIBRATION CURVE FOR TSKgel PWXL-CP COLUMNS

Columns: TSKgel G3000PWXL-CP, 7 µm; TSKgel G5000PWXL-CP, 10 µm; TSKgel G6000PWXL-CP, 13 µm
Samples: polyethylene oxides (PEO) standards; polyethylene glycols (PEG) standards
Mobile phase: 0.1 mol/L $NaNO_3$; Flow rate: 1 mL/min;
Detection: RI; Temp: 25 °C

FIGURE 17



ANALYSIS OF CATIONIC POLYMERS

Columns: TSKgel G3000PWXL-CP, 7 µm (7.8 mm ID x 30 cm L), TSKgel G5000PWXL-CP, 10 µm (7.8 mm ID x 30 cm L), TSKgel G6000PWXL-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
Sample Load: 3 g/L, 100 µL

SEC
TSKgel SuperMultipore SERIES

The new TSKgel SuperMultiporePW column line is incorporating Tosoh's proprietary multi-pore particle technology. These semi-micro SEC columns provide near linear calibration curves. They are ideally suited to analyze the molecular weight and the MW distribution of water soluble polymers, such as polyvinylpyrrolidones or dextrans.

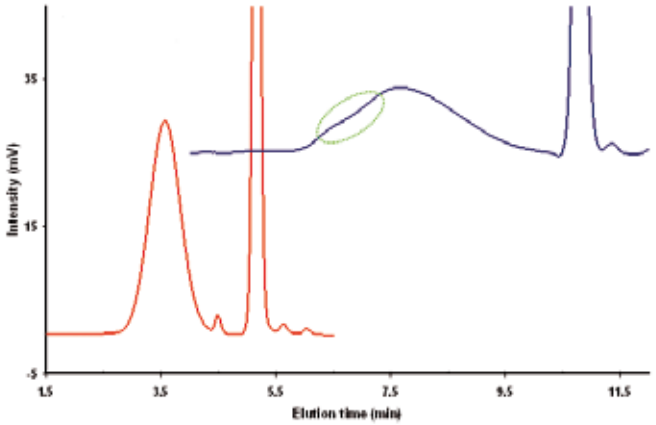
TSKgel SuperMultiporePW columns are packed with spherical mono-disperse polymethacrylate particles, each containing a wide range of pore sizes. They belong to the semi-micro type of SEC columns (6 mm ID, 15 cm length) providing high theoretical plate numbers at half of the length of a conventional SEC column. The TSKgel SuperMultiporePW series comprises of three column types covering different molecular weight ranges (PW-N; PW-M, PW-H).

Multi-pore particle technology is the most elegant way to achieve near linear SEC calibration curves. It solves the known problem of peak disturbances/inflection points, which typically occur due to a mismatch of pore sizes when columns with different molecular weight ranges are coupled. Particles produced by multi-pore technology contain a broad range of pore sizes in a single polymeric bead. This innovative approach essentially creates a linear calibration curve within each particle (Figure 18).

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

Figure 19 shows the SEC analysis of a real sample - Polyvinylpyrrolidone (PVP) K-30 - on a series of conventional TSKgel G3000PWXL and G5000PWXL columns compared to the one obtained with a single TSKgel SuperMultiporePW-M semi-micro 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.

FIGURE 19

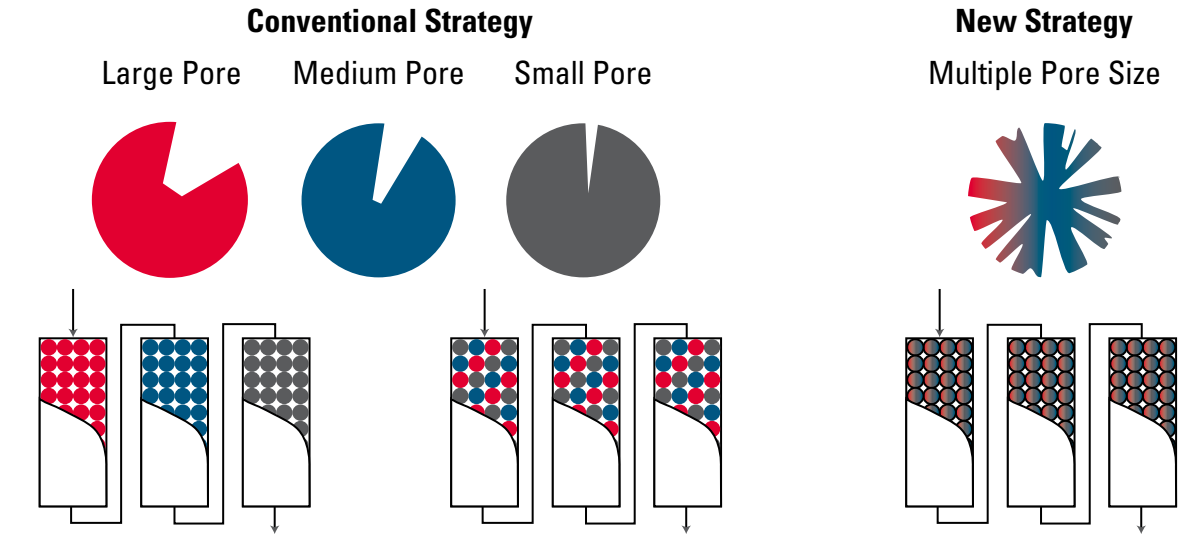


ANALYSIS OF POLYVINYLPYRROLIDONE

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 18

STRATEGIES FOR WIDE RANGE SEPARATION USING SIZE EXCLUSION CHROMATOGRAPHY



SEC
TSKgel PW SERIES ORDERING INFORMATION

ORDERING INFORMATION

PART #	DESCRIPTION	ID (MM)	LENGTH (CM)	PARTICLE SIZE (µM)	NUMBER THEORETICAL PLATES	FLOW RATE (ML/MIN) RANGE	FLOW RATE (ML/MIN) MAX.	MAXIMUM PRESSURE DROP (MPA)
STAINLESS STEEL COLUMNS								
22789	SuperMultiporePW-N	6.0	15	4	>16,000	0.3 - 0.6	0.6	4.5
22790	SuperMultiporePW-M	6.0	15	5	>12,000	0.3 - 0.6	0.6	2.7
22791	SuperMultiporePW-H	6.0	15	8 (6-10)	>7,000	0.3 - 0.6	0.6	0.9
22792	SuperOligoPW	6.0	15	3	>16,000	0.3 - 0.6	0.6	5.0
08031	G-Oligo-PW	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
08032	G-DNA-PW	7.8	30	10	≥ 10,000	0.2 - 0.5	0.6	2.0
08020	G2500PWXL	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
08021	G3000PWXL	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
08022	G4000PWXL	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	2.0
08023	G5000PWXL	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	2.0
08024	G6000PWXL	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
08025	GMPWXL	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
21873	G3000PWXL-CP	7.8	30	7	≥ 16,000		1.0	5.5
21874	G5000PWXL-CP	7.8	30	10	≥ 10,000		1.0	2.5
21875	G6000PWXL-CP	7.8	30	13	≥ 7,000		1.0	2.0
05760	G1000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	2.0
05761	G2000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	2.0
08028	G2500PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	2.0
05762	G3000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	1.2	2.0
05763	G4000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	1.0
05764	G5000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	1.0
05765	G6000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	1.0
08026	GMPW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.2	1.0
05105	G2000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	4.0
08029	G2500PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	4.0
05106	G3000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	1.2	4.0
05107	G4000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	2.0
05108	G5000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	2.0
05109	G6000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	2.0
08027	GMPW	7.5	60	17	≥ 6,000	0.5 - 1.0	1.2	2.0
08030	G2500PW	21.5	60	17	≥ 10,000	1.6 - 6.0	8.0	2.0
PEEK								
20024	BioAssist G6PW	7.8	30	17	≥ 3,000	0.5 - 1.0	1.2	10
GUARD COLUMNS								
22793	SuperMP (PW)-N Guard column	4.6	3.5	4				
22794	SuperMP (PW)-M Guard column	4.6	3.5	5				
22795	SuperMP (PW)-H Guard column	4.6	3.5	8				
22796	SuperOligoPW Guard column	4.6	3.5	3				
08034	Oligo Guard column	6.0	4.0	13	For 7.8 mm ID G-Oligo-PW columns			
08033	PWXL Guard column	6.0	4.0	12	For 7.8 mm ID PWXL & G-DNA-PW (TSKgel G3000PW packing)			
21876	PWXL-CP Guard column	6.0	4.0	13	For 7.8 mm ID PWXL-CP columns			
06763	PW-L Guard column	7.5	7.5	13	For 7.5 mm ID G1000PW & G2000PW (TSKgel G2000PW packing)			
06762	PW-H Guard column	7.5	7.5	13	For 7.5 mm ID G2500PW through GMPW columns			
06758	PW-H Guard column	21.5	7.5	17	For 21.5 mm ID G2500PW through G5000PW columns			

SEC
TSKgel Alpha & SuperAW SERIES

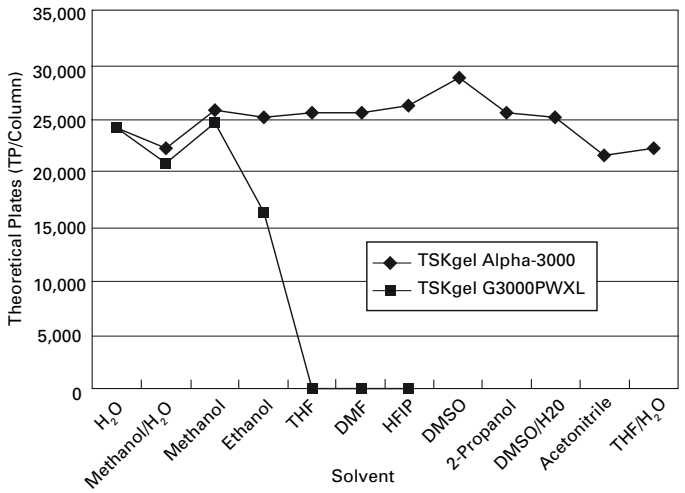
The TSKgel Alpha and SuperAW column series offer a new alternative for performing SEC. The columns are packed with a hydrophilic, highly crosslinked polymer which is compatible to a wide range of solvents ranging from pure aqueous up to 100 % organic mobile phases (see Figure 20). Both series consist of six columns with different pore sizes, spanning a wide MW separation range from 100 to over 1,000,000 Da when using polyethylene glycol (PEG) as a standard. Exclusion limits for polyethylene oxides in water and other physical properties for the Alpha and SuperAW columns are listed in Table 6.

The TSKgel Alpha and SuperAW column series can be used for separations of synthetic polymers, oligomers, additives and detergents as well as for saccharides, nucleic acids and peptides. TSKgel SuperAW columns with reduced particle size and semi-micro column dimensions of 6 mm ID and 15 cm length provide short analysis times and higher resolution power. For samples with big differences in molecular weights, the mixed bed columns TSKgel Alpha-M and TSKgel SuperAWM-H show linear calibration curves over the whole range.

HIGHLIGHTS

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

FIGURE 20



SOLVENT COMPATABILITY OF TSKgel Alpha-3000 WITH ORGANIC SOLVENT

Conditions for solvent change: Flow rate: 1.0 mL/min
Temp.: 25 °C; Time for purge: 8 h
Conditions for TP measurement: Sample: ethylene glycol
Flow rate: 1.0 mL/min; Temp.: 25 °C; Detection: RI

TABLE 6

PROPERTIES AND SEPARATION RANGES OF TSKgel Alpha AND SuperAW-SERIES

TSKgel COLUMN	ID (MM) X LENGTH (CM L)	PARTICLE SIZE (µM)	MIN NO. THEORET. PLATES	EXCLUSION LIMIT (PEO/H_2O)
Alpha-2500	7.8 x 30	7	16,000	5×10^3
Alpha-3000	7.8 x 30	7	16,000	9×10^4
Alpha-4000	7.8 x 30	10	10,000	4×10^5
Alpha-5000	7.8 x 30	10	10,000	1×10^6
Alpha-6000	7.8 x 30	13	7,000	$>1 \times 10^7$
Alpha-M	7.8 x 30	13	7,000	$>1 \times 10^7$
SuperAW2500	6.0 x 15	4	>16,000	5×10^3
SuperAW3000	6.0 x 15	4	>16,000	9×10^4
SuperAW4000	6.0 x 15	6	>10,000	1×10^6
SuperAW5000	6.0 x 15	7	>10,000	1×10^6
SuperAW6000	6.0 x 15	9	>6,000	1×10^7
SuperAWM-H	6.0 x 15	9	>6,000	1×10^7

SEC TSKgel Alpha & SuperAW SERIES ORDERING INFORMATION

ORDERING INFORMATION

PART #	DESCRIPTION	ID (MM)	LENGTH (CM)	PARTICLE SIZE (µM)	NUMBER THEORETICAL PLATES	FLOW RATE (ML/MIN) RANGE	FLOW RATE (ML/MIN) MAX.	MAXIMUM PRESSURE DROP (MPA)
STAINLESS STEEL COLUMNS								
18339	Alpha-2500	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
18340	Alpha-3000	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
18341	Alpha-4000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	3.0
18342	Alpha-5000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	3.0
18343	Alpha-6000	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
18344	Alpha-M (mixed bed)	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
GUARD COLUMNS								
18345	Alpha Guard column	6.0	4	13	For all Alpha columns			
VMPAK COLUMNS*								
20011	VMpak-25	2.0	5	7	≥ 1,000	0.1 - 0.2	0.25	2.0
20012	VMpak-25	2.0	15	7	≥ 3,000	0.1 - 0.2	0.25	6.0
STAINLESS STEEL COLUMNS								
19315	SuperAW2500	6.0	15	4	≥ 16,000	0.3 - 0.6		6.0
19316	SuperAW3000	6.0	15	4	≥ 16,000	0.3 - 0.6		6.0
19317	SuperAW4000	6.0	15	6	≥ 10,000	0.3 - 0.6		4.0
19318	SuperAW5000	6.0	15	7	>10,000	0.3 - 0.6		3.0
19319	SuperAW6000	6.0	15	9	>7,000	0.3 - 0.6		2.0
19320	SuperAWM-H	6.0	15	9	>7,000	0.3 - 0.6		2.0
GUARD COLUMNS								
19321	SuperAW-L Guard Column	4.6	3.5	7	For SuperAW2500-4000 columns.			
19322	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 and LC-MS separations.

SEC
OPTIMIZING SEC

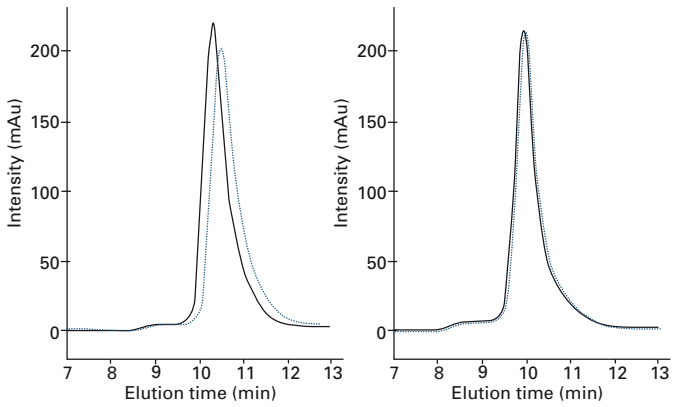
SAMPLE LOAD

As SEC is a partition chromatography, sample load on the column is limited. High sample loads distort peak shapes and cause an overall decrease in efficiency due to column overload. Optimal sample load highly depends on the sample properties (sample matrix) and the separation task. For analytical columns, sample concentrations of 1-20 mg/ml are recommended. Proteins can be loaded at higher concentrations and higher total loads than synthetic macromolecules. For preparative purposes for example, 100 mg of BSA can be loaded on two 21.5 mm ID x 60 cm L TSKgel G3000SW columns, but only 20 mg of PEG 7500.
Sample volume depends very much on the type of column. On TSKgel SuperSW columns for example, a 5 µL injection volume ensures optimal results. Standard injection volumes for 7.5 and 7.8 mm ID columns are 20-100 µl, whereas for preparative purposes on 21.5 mm ID columns, injection volumes may be raised up to 2 ml.

MOBILE PHASE

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

FIGURE 22



INFLUENCE OF MOBILE PHASE

A: No ethanol in mobile phase; B: 10% ethanol in mobile phase
Column: TSKgel G3000SWXL columns, 5 µm, 7.8 mm ID x 30 cm L
Sample: 10 mL PEG r-HuMGDF;
—— initial injection; ····· after 150 injections
Mobile phase: 0.1 M sodium phosphate, pH 6.9, 0.5 M NaCl
Flow rate: 0.7 mL/min; Detection: UV @ 220 nm

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

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

If hydrophobic interaction occurs between the sample and the column matrix, a water soluble organic solvent can be added to the mobile phase. The addition of acetonitrile, acetone, ethanol or methanol up to a concentration of 20% may also prevent columns from fouling by suppressing interaction of hydrophobic impurities of the sample. An example is shown in Figure 22 with the analysis of a pegylated protein on a TSKgel G3000SWXL column. As pegylated products are more hydrophobic, they tend to interact with the column matrix. Over time the pegylated product can foul the column, which is indicated by shifts of retention time and decreasing separation performance. By adding 10% of ethanol to the elution buffer, this problem is overcome and no differences in performance at the first and the 150th injection are observed (courtesy of J.J. Ratto et al. Amgen Inc., 1996).

COLUMN PROTECTION

To protect the column and increase its lifetime, the use of a guard column is strongly recommended. Sample purity, sample load and the composition of the mobile phase have an influence on column lifetime. For information on TSKgel SEC columns for GPC analysis of organic polymers please refer to the TSKgel GPC column brochure.

TOSOH

TSKgel® UP-SW3000 UHPLC COLUMNS

TSKgel UP-SW3000 columns packed with 2 µm silica based particles are the latest addition to the popular TSKgel SW series, the gold standard for QC analysis of antibody therapeutics. The new silica-based UHPLC columns are based on the proven proprietary surface technology of the renowned TSKgel SW series and facilitate the transfer of existing HPLC methods to UHPLC systems.

Aqueous size exclusion chromatography (SEC) is the method of choice for the analysis of proteins fragments, monomers, and aggregates under non-denaturing conditions. Based on the flow of the sample through a porous stationary phase SEC separates molecules according to their size, or more precisely, their hydrodynamic volume. In aqueous elution systems SEC is also referred to as gel filtration chromatography (GFC). TSKgel G3000SWXL columns have been the industry's standard for quality control of monoclonals by SEC for decades.

HIGHLIGHTS

- Proven TSKgel SW SEC quality
- Virtual absence of nonspecific interaction
- Easy transfer of existing HPLC methods
- Optimized for mAb quality control

The new columns can be used with modern HPLC and UHPLC systems and are available with 15 or 30 cm length. The short one enables short analysis times; the long one provides higher resolution for mAb analysis. The lifetime of the columns can be improved when using the corresponding guard columns. A "direct connect" (DC) guard column allows minimizing extra column dead volume.

COMPARISON OF CALIBRATION CURVES

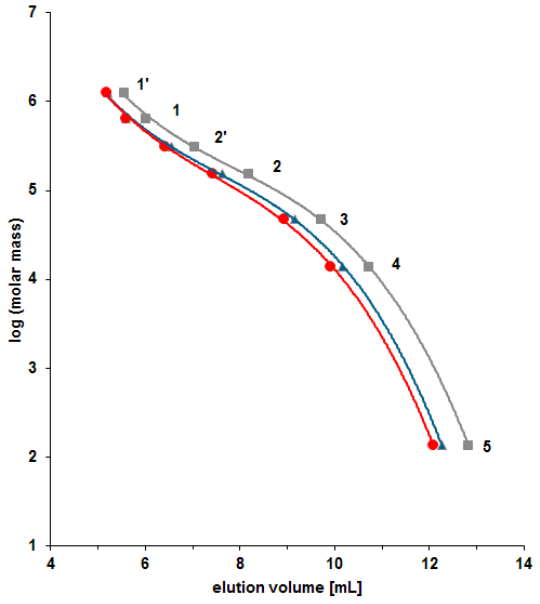


Figure 1

Columns: A: TSKgel UP-SW3000 (4.6 mm ID × 30 cm, red)
B: TSKgel SuperSW3000 (4.6 mm ID × 30 cm, blue)
C: TSKgel G3000SWXL (7.8 mm ID × 30 cm, grey)
Mobile phase: 100 mmol/L phosphate buffer (pH 6.7) + 100 mmol/L sodium sulfate + 0.05% NaN_3
Flow rate: A & B: 0.35 mL/min; C: 1.0 mL/min
Temperature: 25°C; Detection: UV @ 280 nm
Injection vol.: 10 µL
Samples: 1. thyroglobulin (640,000 Da); (1' thyroglobulin aggregate);
2. γ-globulin (155,000 Da); (2' γ-globulin dimer);
3. ovalbumin (47,000 Da);
4. ribonuclease A (13,700 Da);
5. p-aminobenzoic acid (137 Da)

COMPARISON OF TSKgel SW COLUMN SERIES

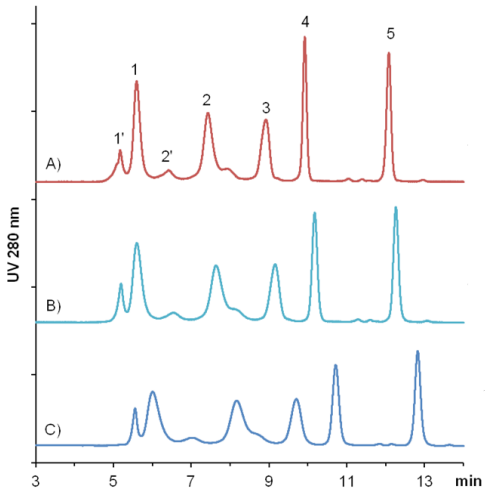


Figure 2

Column	Particle size	N (peak 4)	AS (peak 4)
A: TSKgel UP-SW3000	2 µm	45,625	0.95
B: TSKgel SuperSW3000	4 µm	24,419	1.02
C: TSKgel G3000SWXL	5 µm	18,325	1.05

Columns: A: TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 30 cm
B: TSKgel SuperSW3000, 4 µm, 4.6 mm ID x 30 cm
C: TSKgel G3000SWXL, 5 µm, 7.8 mm ID x 30 cm
Mobile phase: 100 mmol/L phosphate buffer (pH 6.7) + 100 mmol/L sodium sulfate + 0.05 % NaN3
Flow rate: A, B: 0.35 mL/min, C: 1.0 mL/min
Temperature: 25 °C
Detect.: UV @ 280 nm (A,B: micro flow cell, C: standard flow cell)
Injection vol.: 10 µL
Sample: 1. thyroglobulin, 640,000 Da (1' thyroglobulin dimer)
2. γ-globulin, 155,000 Da (2' γ-globulin dimer)
3. ovalbumin, 47,000 Da; 4. ribonuclease A, 13,700 Da
5. p-aminobenzoic acid, 137 Da

MASS RANGE

Figure 1 shows the calibration curve and the molecular weight range of the new 2 µm TSKgel UP-SW3000 compared to those of 5 micron TSKgel G3000SWXL and 4 micron TSKgel SuperSW3000. Calibration curves and mass ranges are almost identical which facilitates transfer of existing methods.

TSKgel UP-SW3000 has the same molecular mass separation range as the equivalent grades of conventional TSKgel SW-type columns but much higher column efficiency: Figure 2 shows the increase in resolution achieved by reducing the particle size from 5 (respectivly 4) micron to 2 micron.

REPRODUCIBLE PERFORMANCE

TSKgel SW SEC columns are known for their outstanding quality and reproducibility. 40 years of expertise in development and production of gel filtration columns have paved the road to UHP-SEC. The good lot-to-lot reproducibility of TSKgel UP-SW3000 is proved in Figure 3.

APPLICATION

TSKgel UP-SW3000 is suited for the separation of antibody dimer, monomer, and fragments in one run with ultra-high resolution (Figure 4). One TSKgel UP-SW3000 achieves even higher resolution than two TSKgel G3000SWXL columns connected in series.

BATCH-TO-BATCH REPRODUCIBILITY

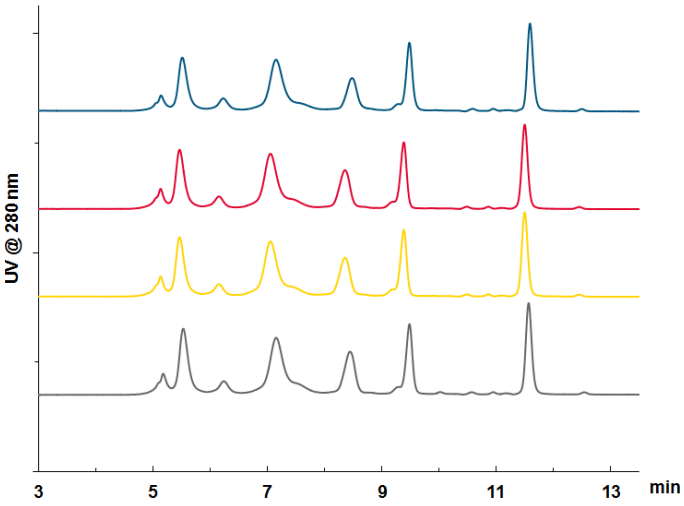


Figure 3

Column: TSKgel UP-SW3000 4.6 mm ID x 30 cm from 4 different batches
Condition and samples: see Figure 1 A

ANALYSIS OF MONOCLONAL ANTIBODIES

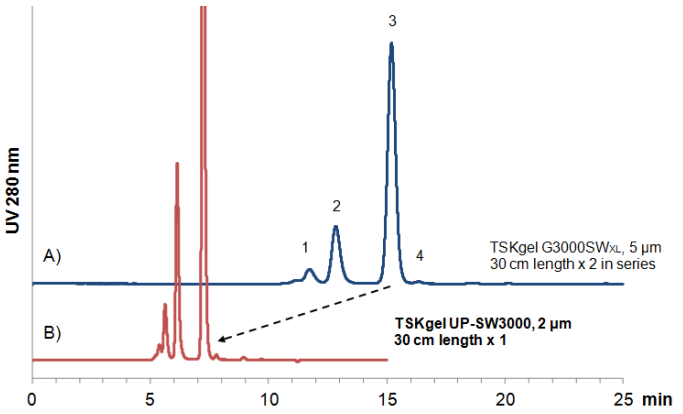


Figure 4

Column	Rs (peak 1/2)	Rs (peak 2/3)	Rs (peak 3/4)
A: TSKgel G3000SWXL x2	1.60	3.63	1.77
B: TSKgel UP-SW3000	2.16	5.02	2.56

Columns: A) TSKgel G3000SWXL, 5 µm, 7.8 mm ID x 30 cm x 2;
B) TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 30 cm
Mobile phase: 100 mmol/L phosphate buffer + 100 mmol/L sodium sulfate + 0.05% sodium azide, pH 6.7
Flow rate: A) 1.0 mL/min, B) 0.35 mL/min; Temperature: 25°C
Detection: UV @ 280 nm; Injection vol.: 10 µL
Sample: mouse-human chimeric IgG, monoclonal
1 trimer, 2 dimer, 3 monomer, 4 fragment

Ordering information

Part-No	Description	Matrix	Housing	Dimensions
0023449	TSKgel UP-SW3000, 2 µm	Silica	Stainless steel	4.6 mm ID x 15.0 cm L
0023448	TSKgel UP-SW3000, 2 µm	Silica	Stainless steel	4.6 mm ID x 30.0 cm L
0023450	TSKgel Guardcolumn UP-SW	Silica	Stainless steel	4.6 mm ID x 2.0 cm L
0023451	TSKgel Guardcolumn UP-SW DC	Silica	Stainless steel	4.6 mm ID x 2.0 cm L

TSK-GEL PW Brochure

Table of Contents

TSK-GEL PW Series

HPLC columns for the size separation of biological macromolecules

TSK-GEL PW columns are designed for aqueous, Size Exclusion Chromatography (SEC) of proteins, polysaccharides, oligosaccharides, DNA and RNA. The column packing materials are porous, hydrophilic, rigid, polymer beads with particle sizes ranging from 6 µm to 22 µm. They exhibit excellent chemical and mechanical stability, have been used from pH 2.0 to 12.0, and can be cleaned with 0.5 M NaOH.
They are particularly useful at pH extremes which would adversely affect silica SEC resins (TSK-GEL SW Series). Depending on which of the six pore size ranges is selected, these columns have 10,000 to 30,000 plates/m and are available in both analytical and preparative housings.

TSK-GEL PWXL columns are a smaller particle size version of the TSK-GEL PW polymer packing. These columns are used where the highest resolution of sample peaks is required. There are five pore size ranges available with chromatographic efficiencies varying from 23,000 to 50,000 plates/m.

TSK-GEL PW specialty columns

TSK-GEL GMPW is a mixed-bed SEC column comprised of various pore sizes and 17 µm particles. This maximizes the range of molecular weights that will elute between the excluded volume and the permeation volume.

Polyethylene glycol and oxide calibration curves for TSK-GEL PW and TSK-GEL PWXL columns

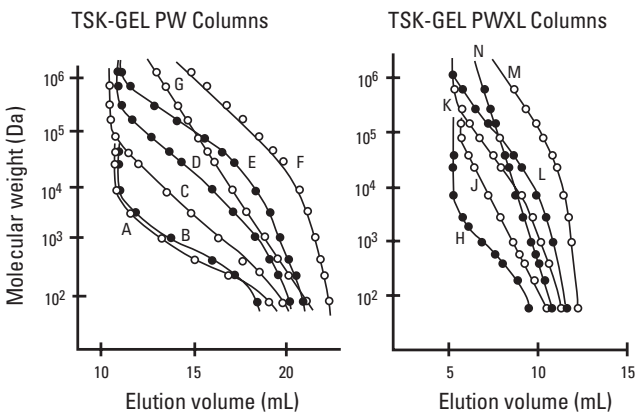


Figure 1
Column: TSK-GEL PW columns: A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5 mm L x 60 cm L
TSK-GEL PWXL columns: H. G2500PWXL, J. G3000PWXL, K. G4000PWXL, L. G5000PWXL, M. G6000PWxL, N. GMPWXL, all 7.8 mm ID x 30 cm L
Sample: PEO and PEG
Elution: distilled water
Flow rate: 1.0 ml/min
Detection: RI

TSK-GEL GMPWXL is the 13 µm high resolution version of TSK-GEL GMPW (an example is shown in Figure 9).

TSK-GEL G-Oligo-PW is specially designed for oligosaccharides and other aqueous nonionic polar oligomers of less than 3,000 Daltons. This column is based on a 6 µm version of the TSK-GEL G2000PW packing material (see Figure 3, 7, 6).

TSK-GEL G-DNA-PW is also a specially designed column but for the separation of DNA and RNA molecules of less than 7,000 base pairs and other large molecules (see Figure 4 and 8).

Protein calibration curves on TSK-GEL PWXL columns

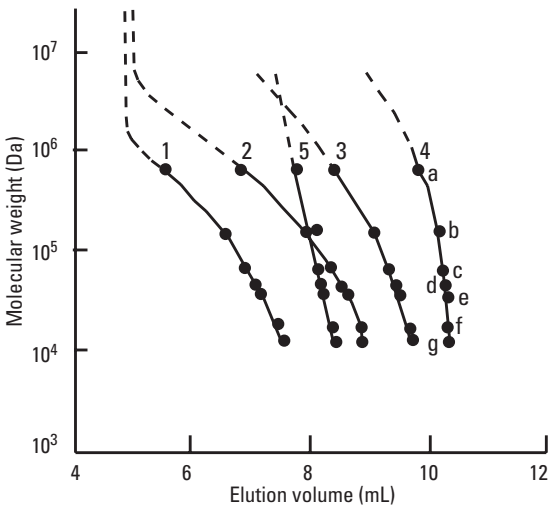


Figure 2
Column: 1. G3000PWXL, 2. G4000PWXL, 3. G5000PWXL 4. G6000PWXL, 5. GMPWXL
Sample: a. thyroglobulin, b. y-globulin, c. albumin, d. ovalbumin, e. ß-lactoglobulin, f. myoglobin, g. cytochrome C
Elution: 0.2 M phosphate buffer, pH 6.8
Flow rate: 1.0 ml/min
Detection: UV @ 280 nm

TSK-GEL PW Series

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

TSKgel column	Particle Size* (µm)	Average pore Size (Å)	Molecular weight of sample (Da)		
			Polyethylene glycols & oxides	Dextrans**	Globular proteins**
G2000PW	12, 17, 20	125	up to 2,000	—	<5,000
$G2500PW_{XL}$ G2500PW	6 12, 17, 20	<200	up to 3,000	—	<8,000
$G3000PW_{XL}$ G3000PW	6 12, 17, 20	200	up to 5×10^4	up to 6×10^4	$500 - 8 \times 10^5$
$G4000PW_{XL}$ G4000PW	10 17, 22	500	$2{,}000–3 \times 10^4$	$1{,}000 – 7 \times 10^5$	$1 \times 10^4 – 1.5 \times 10^6$
$G5000PW_{XL}$ G5000PW	10 17, 20, 22	1000	$4{,}000–1 \times 10^6$	$5 \times 10^4 – 2.5 \times 10^6$	$<1 \times 10^7$
$G6000PW_{XL}$/BioAssist $G6PW_{XL}$ G6000PW	13 17, 25	>1000	$4 \times 10^4 – 8 \times 10^6$	$5 \times 10^5 – 5 \times 10^7$	$<2 \times 10^8$
$GMPW_{XL}$ GMPW	13 17	<100–1000	$500–8 \times 10^6$	$<5 \times 10^7$	$<2 \times 10^8$
G-Oligo-PW G-DNA-PW	6 10	125 >1000	up to 3,000 $4 \times 10^4 – 8 \times 10^6$	— $<5 \times 10^7$	<3,000 $< 2 \times 10^8$

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

Table I

Calibration curve for TSK-GEL G-Oligo-PW

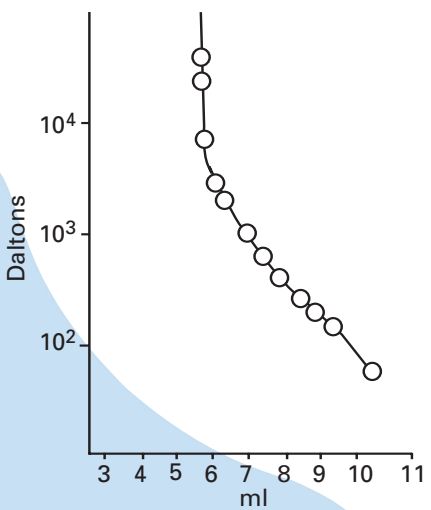


Figure 3
Column: TSKgel G-Oligo-PW, 7.8 mm ID X 30 cm L
Sample: PEG and PEO standards
Flow rate: 1.0 ml/min
Detection: RI

Calibration curve for double-stranded DNA

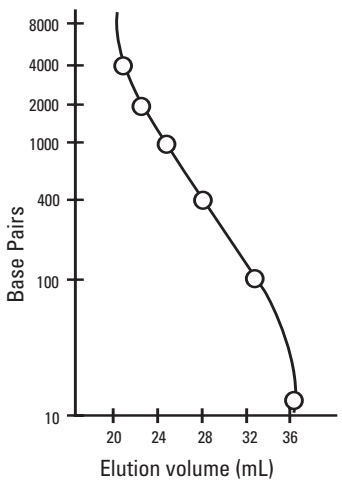


Figure 4
Column: TSKgel G-DNA-PW, four 10 µm, 7.8 mm ID X 30 cm L columns in series
Sample: double-stranded DNA fragments: EcoR I and BstN I cleaved pBR322 DNA, void volume determined with DNA
Elution: 0.3 M NaCl in 0.1 M Tris-HCl, pH 7.5
Flow rate: 15 ml/min
Detection: UV @ 260 nm

TSK-GEL PW Series

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

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

Table II

PW Columns are compatible with*:

Up to 50% Polar Organics

Acetonitrile
Acetone
Isopropanol
Methanol
Ethanol
DMF
DMSO

Up to 20% Nonpolar Organics

THF

*Switch to these buffers at 50% of the standard flow rate.

Detergents

up to 0.1% SDS
up to 1% Tween, Triton

Chaotropic Agents

8 M Urea
6 M Guanidine

Not Compatible

Toluene (not water soluble)
and other not water soluble solvents

TSK-GEL PW Series

Elution curves for PEG standards on TSK-GEL G25000PWXL

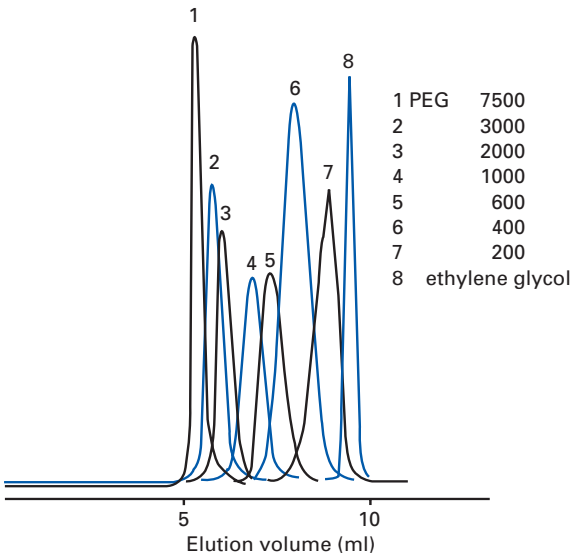


Figure 5
Column: TSKgel G2500PWXL, 7.8 mm ID X 30 cm L
Sample: polyethylene glycols
Elution: destilled water
Flow rate: 1.0 ml/min
Detection: RI

Separation of chito oligosaccharides

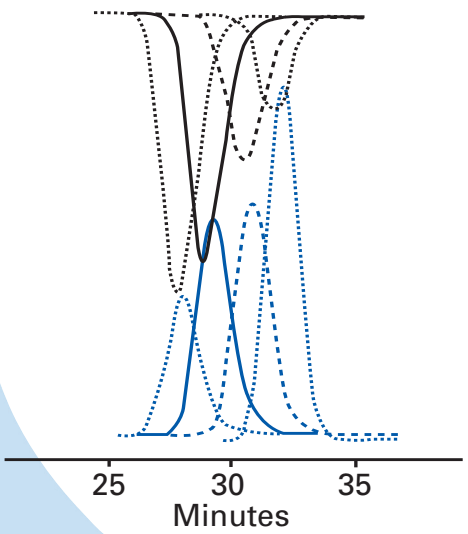


Figure 6
Column: TSKgel G-Oligo-PW, four 7.8 mm ID X 30 cm L in series
Sample: chito oligosaccharides
Elution: destilled water
Detection: RI

Separation on hydrolyzed ß-cyclodextrin

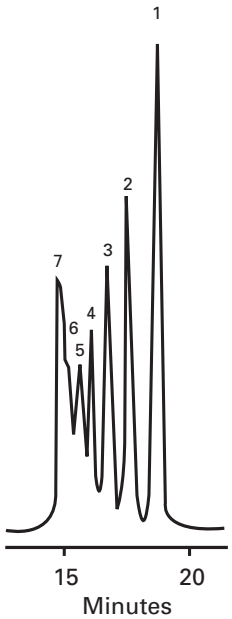


Figure 7
Column: TSKgel G-Oligo-PW, two 6 µm, 7.8 mm ID X 30 cm L columns in series
Sample: hydrolyzed ß-cyclodextrin
Elution: destilled water
Flow rate: 1.0 ml/min
Temperature: 60 °C
Detection: RI

Separation of EcoR I and BstN I cleaved pBR322 DNA

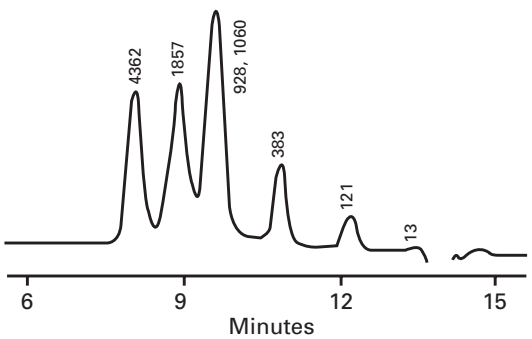


Figure 8
Column: TSKgel G-DNA-PW, four 10 µm, 7.8 mm ID X 30 cm L in series
Sample: 1.7 µg of EcoR I cleaved pBR322 DNA and 8.0 µg of BstN I cleaved pBR322 DNA
Elution: 0.3 M NaCl in 0.1 Tris-HCl, pH 7.5 and 1 mM EDTA
Flow Rate: 0.3 ml/min
Detection: UV @ 260 nm

Separation of pullulan

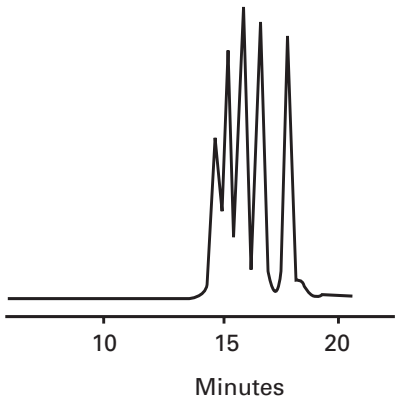


Figure 9
Column: TSKgel GMPWXL, four 7.8 mm ID X 30 cm L in series
Sample: pullulan
Elution: 0.1 M NaCl
Flow Rate: 1.0 ml/min
Detection: RI, LS

Separation of gelatin

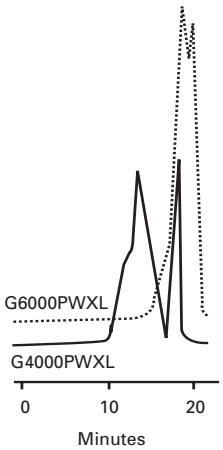


Figure 10
Column: ——— TSKgel G4000PWXL
..... TSKgel G6000PWXL
Sample: gelatin
Elution: 0.2 mM phosphate buffer, pH 6.9
Flow Rate: 1.0 ml/min
Detection: RI

Troubleshooting and cleaning TSK-GEL PW columns

Selecting mobile phase buffers

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

For some nonionic, nonpolar polymers, such as poly-ethylene glycols, normal chromatograms can be obtained by using distilled water. Some more polar nonionic polymers exhibit abnormal peak shapes or minor peaks near the void volume when eluted with distilled water, due to ionic interactions between the sample and the charged groups on the resin surface. To eliminate ionic interactions, a neutral salt, such as sodium nitrate or sodium sulfate, is added to the aqueous eluent. Generally, a salt concentration of 0.1 M to 0.5 M is sufficient to overcome undesired ionic interactions.

TSK-GEL PW-type resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. The hydrophobic interaction increases as the salt concentration of the eluent increases. This hydrophobic interaction between the sample and the resin surface can be suppressed by the addition of a water-soluble organic solvents modifier (e.g. acetonitrile, isopropanol).

Modifiers are used for proper elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in Table II. All TSK-GEL PW-type column packings are compatible with up to 50% aqueous solutions of methanol, ethanol, propanol, acetonitrile, formic acid, acetic acid, dimethyl formamide, dimethyl sulfoxide, or acetone. Solvent exchange must be carried out slowly.

Improving column performance

Listed below are the four most common causes for poor column performance and the methods you can use to prevent these problems:

1. Void or dead volume at the column inlet, channeling, denaturation of the packing surface

Sudden pressure surges and higher than recommended flow rates can compress the column packing and cause a void. We recommend continuous flow injection with a loop injector and installation of a pulse damper to suppress the sudden pressure surges encountered with quick return pumps. Bulk packing is available to refill voids in the analytical and semi-preparative columns. A guard column will protect your analytical column from pressure surges and irreversibly binding contaminants. Upon injection, the sample can cause a change in the eluent pH. The guard column will allow the pH to equilibrate with the mobile phase before it reaches the analytical column.

2. Air on the column

The column should be tightly capped when not in use. Mobile phases should be sparged to prevent air from entering the column. If air does enter the column, follow the rehydration method.

3. Column contamination or incomplete recovery of the sample

The cleaning directions which follow give suggestions for removing ionic, hydrophobic or absorbed contaminants. Refer to "Selecting mobile phase buffers" for advice on modifiers that will prevent nonspecific binding to the media matrix.

4. Frit plugging and high pressure

Solvents and samples should be filtered though a 0.45 µm filter to prevent clogging of the column frits. If the frit becomes partially plugged, the result may be split peaks or high pressure. The entire end-fitting can be removed and sonicated in 6 M nitric acid. Be careful not to disturb the packing and rinse well after cleaning. Alternatively, we stock replace-ment end-fittings according to the column ID. Furthermore, we recommend placing an in-line filter before the injector, to prevent particles created by pump seal wear from reaching the analytical column.

5. Column overload

Column overload can cause peak splitting and poor resolution. Analytical work necessitates an injection volume less than 1% of the total column volume with a sample concentration less than 10 mg/ml.

Improving resolution

1. Verify that the column is not being overloaded.

2. Decrease the dead volume in your HPLC system by using the shortest tubing lengths and the smallest tubing ID possible without exceeding the maximum pressure for the column.

3. Decrease the flow rate, but not lower than 0.3 ml/min because diffusion will increase.

4. If using a 30 cm PW column, add an additional 30 cm column or switch to an XL-type column which has a smaller particle size and will improve resolution more than two fold.

Cleaning

If the column becomes contaminated, the following cleaning procedures are suggested with the column in reverse flow at half the recommended maximum flow rate (see also ODS-sheet that comes with the column or the TSK-GEL Instruction Manual):

1. If the column is contaminated with basic com-pounds, rinse with 3-5 column volumes (CV) of 0.5-1.0 M neutral salt or a buffered solution at low pH (pH 2-3) or high pH (0.1-0.2 N NaOH,pH 13.0).

2. If the column is contaminated with hydrophobic compounds, rinse with 3-5 CV of 20% organic methanol, acetonitrile, THF etc.)
3. For precipitated protein, chaotropic reagents such as 0.1 % SDS, 8 M urea or 6 M guanidine, or proteolytic enzymes such as pepsin may be used. However, an extended washing with buffer is required to remove SDS and guanidine.

Unexpected elution behavior can occur if these reagents are not completely removed.
Rinse with 5 CV of D. I. water after each cleaning step before proceeding to the next step.

Storage

When the column will be used the next day, allow it to run overnight at a low flow rate in a buffer that does not contain a halide. When the column will not be used for more than a day, flush salt from the column and store in 0.05% sodium azide or 20% ethanol. Seal tightly to prevent drying out.

Rehydration

Dehydration of the column may occur after long term storage or from inadvertently pumping air over a column. Rehydrate the column with the following procedure:

1. Connect the column to your LC pump, but not to the detector, in reverse flow direction.

2. Pump a filtered mobile phase of 20% methanol in distilled, deionized water over the column at half the recommended maximum flow rate listed in the ordering information for your column.

3. Continue this procedure for several hours until you are confident that the column has been rehydrated.

4. Reconnect the column to your LC in the proper flow direction.

5. Rinse well with distilled, deionized water then equilibrate with your normal mobile phase.

Perform the recommernded QC test to ensure that the column is performing properly.

TSK-GEL PW Series

Ordering Information

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

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Min. Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm2)
Stainless steel columns								
08031	G-Oligo-PW, 125 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08032	G-DNA-PW, >1.000 Å	7.8	30	10	10,000	0.2 – 0.5	0.6	20
08020	G2500PWXL, <200 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08021	G3000PWXL, 200 Å	7.8	30	6	14,000	0.5 – 0.8	1.0	40
08022	G4000PWXL, 500 Å	7.8	30	10	10,000	0.3 – 0.8	1.0	20
08023	G5000PWXL, 1.000 Å	7.8	30	10	10,000	0.3 – 0.8	1.0	20
08024	G6000PWXL, >1.000 Å	7.8	30	13	7,000	0.3 – 0.8	1.0	20
08025	GMPWXL, 100-1.000 Å	7.8	30	13	7,000	0.3 – 0.8	1.0	20
05761	G2000PW, 125 Å	7.5	30	12	5,000	0.5 – 1.0	1.2	20
08028	G2500PW, <200 Å	7.5	30	12	5,000	0.5 – 1.0	1.2	20
05762	G3000PW, 200 Å	7.5	30	12	5,000	0.5 – 1.0	1.2	20
05763	G4000PW, 500 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05764	G5000PW, 1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05765	G6000PW, >1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
08026	GMPW, 100-1.000 Å	7.5	30	17	3,000	0.5 – 1.0	1.2	10
05105	G2000PW, 125 Å	7.5	60	12	10,000	0.5 – 1.0	1.2	40
08029	G2500PW, <200 Å	7.5	60	12	10,000	0.5 – 1.0	1.2	40
05106	G3000PW, 200 Å	7.5	60	12	10,000	0.5 – 1.0	1.2	40
05107	G4000PW, 500 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05108	G5000PW, 1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05109	G6000PW, >1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
08027	GMPW, 100-1.000 Å	7.5	60	17	6,000	0.5 – 1.0	1.2	20
05150	G2000PW, 125 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
08030	G2500PW, <200 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
05151	G3000PW, 200 Å	21.5	60	17	10,000	1.0 – 6.0	8.0	20
05152	G4000PW, 500 Å	21.5	60	22	6,000	1.0 – 6.0	8.0	20
05153	G5000PW, 1.000 Å	21.5	60	22	6,000	1.0 – 6.0	8.0	20
05154	G6000PW, >1.000 Å	21.5	60	25	6,000	1.0 – 6.0	8.0	20
07926	G3000PW, 200 Å	55.0	60	20	4,500	15.0 – 25.0	30.0	15
07927	G5000PW , 1.000 Å	55.0	60	20	4,500	15.0 – 25.0	30.0	15
Guard columns								
08034	Oligo Guard column	6.0	4.0	13	For G-Oligo-PW			
08033	PWXL Guard column	6.0	4.0	12	For 7.8 mm ID PWXL and G-DNA-PW (contains 3000PW packing)			
06763	PW-L Guard column	7.5	7.5	13	For 7.5 mm ID G2000PW, (contains 2000PW packing)			
06762	PW-H Guard column	7.5	7.5	13	For 7.5 mm ID G2500PW-G6000PW + GMPW (contains 3000PW packing)			
06757	PW-L Guard column	21.5	7.5	17	For 21.5 mm ID G2000PW			
06758	PW-H Guard colum	21.5	7.5	17	For 21.5 mm ID G2500PW through G6000PW			
07924	PW Guard colum	45.0	5.0	20	For 55 mm ID G3000PW + G5000PW			
Bulk packing								
08035	PWXL Top-Off, 1g wet resin			10	For all PWXL and G-DNA-PW			

TSK-GEL® PWXL-CP

Size Exclusion Chromatography Columns for Cationic Polymer Analysis

PRODUCT HIGHLIGHTS

- High recoveries for cationic polymers (see Table 2)
- High reproducibility over time without adsorption (see Figure 1)
- Column offering allows separation of wide MW range of polymers
- Elution under low salt conditions

INTRODUCTION

TSK-GEL PWXL-CP size exclusion columns were specifically developed for the analysis of water soluble cationic polymers. Three columns are available within the TSK-GEL PWXL-CP series, each with a different particle size, separation range and exclusion limit, allowing polymers within a wide molecular mass range to be separated and characterized.

The analysis of cationic polymers requires a high salt concentration in the mobile phase to prevent adsorption of the polymers onto the particles in SEC columns. As a result, many polymer researchers encounter low recovery when analyzing cationic polymers, as well as poor reproducibility from run to run.

The TSK-GEL PWXL-CP columns eliminate ionic adsorption onto the particle by incorporating a cationic functionality on the particle surface. This modification results in high recovery for cationic polymers and enables elution under low salt conditions.

PROPERTIES OF TSK-GEL PWXL-CP COLUMNS

	G3000 PWXL-CP	G5000 PWXL-CP	G6000 PWXL-CP
Base material	Poly methacrylate	Poly methacrylate	Poly methacrylate
Particle size	7 µm	10 µm	13 µm
Exclusion limit (Da)	100 000	1 000000	20 000 000
Separation range (Da), (PEO, PEG)	200 - 50 000	400 - 500 000	1 000 - 10 000 000
Theoretical plates	16 000	10 000	7 000

TABLE 1

Column	Recovery
G3000 PWXL-CP	100,2%
G5000 PWXL-CP	98,8%
G6000 PWXL-CP	97,4%

TABLE 2

These columns show high theoretical plate values, linear calibration curves and high durability because the base resin is the same as that used in the TSK-GEL PWXL series columns.

PAA was injected onto a TSKgel G5000PWXL-CP column. Each chromatogram, from the first injection (red) to the fifth injection (black), showed similar elution profiles without any adsorption of the polymer.

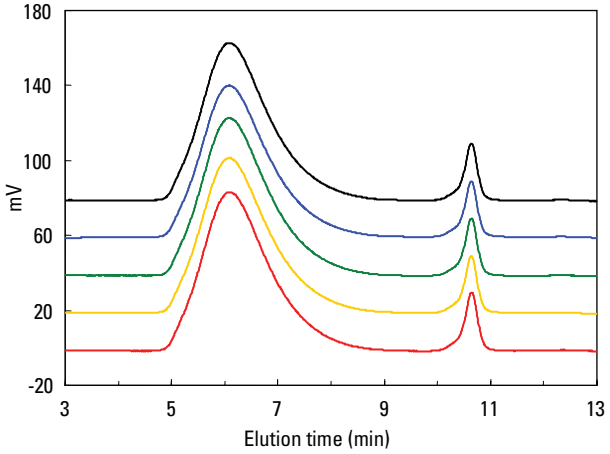


FIGURE 1

Column: TSKgel G5000PWXL-CP
Eluent: 0.1 mol/l $NaNO_3$
Flow rate: 1.0 ml/min
Detection: RI
Temperature: 25°C
Sample: polyallyamine-HCl (PAA)
Sample load: 3 g/l, 100 µl

Application

Various cationic polymers with different functional groups and molecular weights were injected on the TSK-GEL PWXL-CP columns (TSKgel G6000PWXL-CP, G5000PWXL-CP and G3000PWXL-CP, connected in series). Figure 2 demonstrates that these new SEC columns can be utilized for the analysis of a wide variety of cationic polymers.

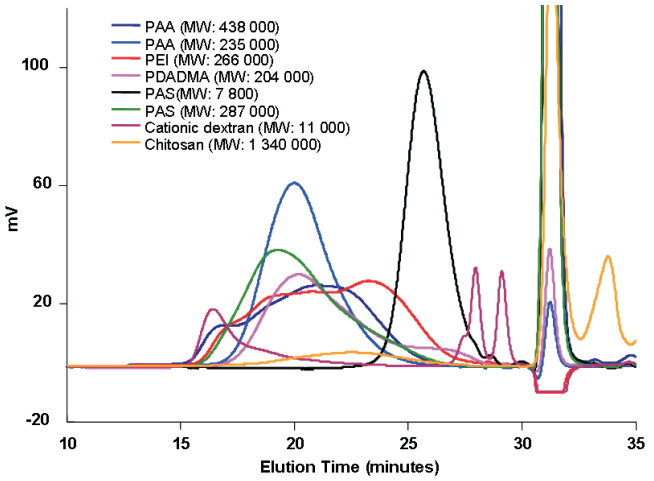


FIGURE 2

Column: TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID X 30 cm L
TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID X 30 cm L
TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID X 30 cm L
Eluent: 0.1 mol/l $NaNO_3$
Flow rate: 1.0 ml/min
Detection: RI
Temp.: 25°C
Sample load: 3 g/l, 100 µl

For further details of choice and selection of the TSK-GEL® column that best suits your particular process purification needs, please contact us:

Tel. + 49 (0) 711 13257 0
or
info.sep.eu@tosoh.com
or
www.tskgel.com

Ordering information

TSKgel PWXL-CP

Part-No	Description	Matrix	Housing	Dimensions
21873	TSKgel G3000PWXL-CP, 7 µm, 200 Å	Polymer	Stainless steel	7.8 mm ID x 30 cm L
21874	TSKgel G5000PWXL-CP, 10 µm, 200 Å	Polymer	Stainless steel	7.8 mm ID x 30 cm L
21875	TSKgel G6000PWXL-CP, 13 µm, 200 Å	Polymer	Stainless steel	7.8 mm ID x 30 cm L
21876	TSKgel PWXL-CP Guardcolumn	Polymer	Stainless steel	6.0 mm ID x 4.0 cm L

TOSOH BIOSCIENCE

NEW universal steric exclusion columns

NEW SEC columns and capillaries

NEW High resolution columns

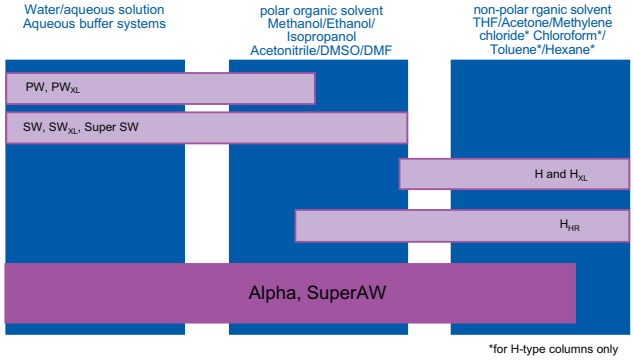
New high performance polymeric analytical columns for SEC and GPC

Take advantage of the versatile and rigid TSKgel SuperAW columns. Speed up your method development and let the column do what **YOU** want!

Introduction

Size exclusion chromatography (SEC) as a method to separate compounds by their molecular sizes is widely used for the separation, purification and determination of molecular weight distributions of hydrophilic as well as of hydrophobic substances.
Usually hydrophobic compounds are analyzed with organic solvents on a polystyrene-divinylbenzene matrix (represented by the H-type column series), whereas SEC of polar compounds needs aqueous solvents and a hydrophilic matrix, based either on silica (the SW and SWXL series) or on polymethacrylate (represented by the PW and PWXL series).
Analysis of polar, non water soluble macromolecules always was difficult on both resin types, due to their limited solvent compatibility.

Table 1 Solvent Compatibilities for TSK-GEL SEC Matrices



With the introduction of Alpha-Series, and now the TSKgel SuperAW column series, this restriction was overcome due to a resin based on a hydrophilic, highly crosslinked vinyl polymer. This resin, which is solvent compatible from pure aqueous up to 100 % organic mobile phases*1), enables SEC of various polymers soluble either in aqueous solutions or in organic solvents.

Table 2 Features of TSKgel Super AW columns

Grade	Exclusion limit (PEO/DMF)	Particle size (µm)	Theoretical Plates	Dimension (mm ID x cm L)
TSKgel SuperAW2500	2×10^3	4	> 16,000	6.0 x 15
TSKgel SuperAW3000	6×10^4	4	> 16,000	6.0 x 15
TSKgel Super AW4000	4×10^5	6	> 10,000	6.0 x 15
TSKgel SuperAW5000	4×10^6	7	> 10,000	6.0 x 15
TSKgel SuperAW6000	> 4×10^7	9	> 6,000	6.0 x 15
TSKgel SuperAWM-H	> 4×10^7	9	> 6,000	6.0 x 15

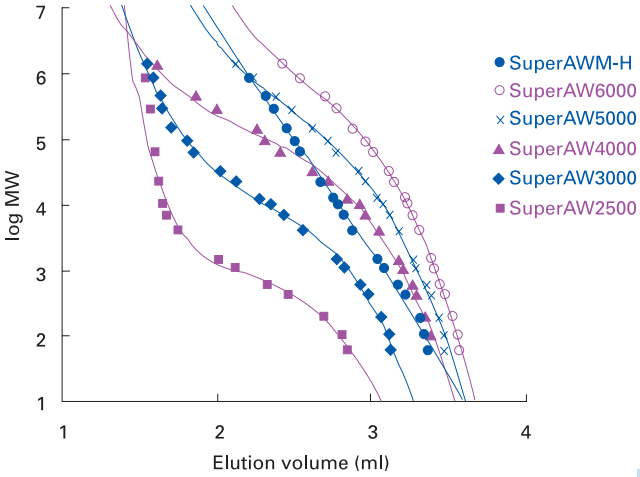
New TSKgel SuperAW column series

The TSKgel SuperAW column series consists of six SEC-column types. The analytical columns are packed with a micro-particle sized, mechanically stable hydrophilic polymer with different pore sizes.

*1) Exception: Chloroform, Toluene, Hexane

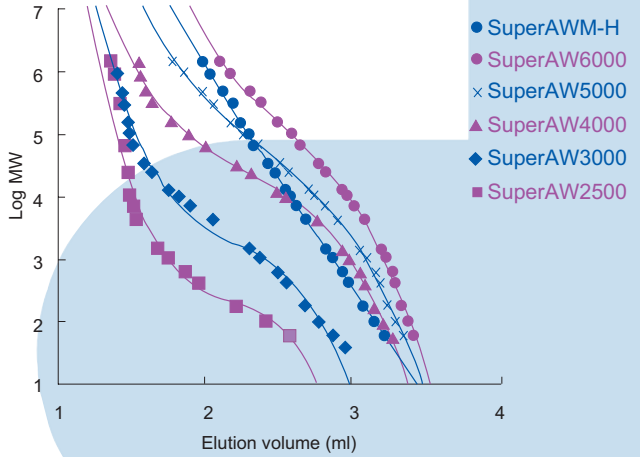
The different pore sizes of the resins allow for a wide separation range of 100-1.000.000 Da using Polyethylene oxides (PEO), and, in addition, each column type shows a high degree of linearity considering the specific calibration range for the respective column.

Figure 1 Calibration curves of PEO and PEG in water



Column: 6.0 mm ID x 15 cm L
Eluent: Water
Flow rate: 0.6 ml/min
Temp.: ambient
Detect.: RI
Sample: Standard polyethylene oxide, polyethylene glycol, ethylene glycol
Sample conc.: 0.04-0.1% each

Figure 2 Calibration curves of PEO and PEG in DMF

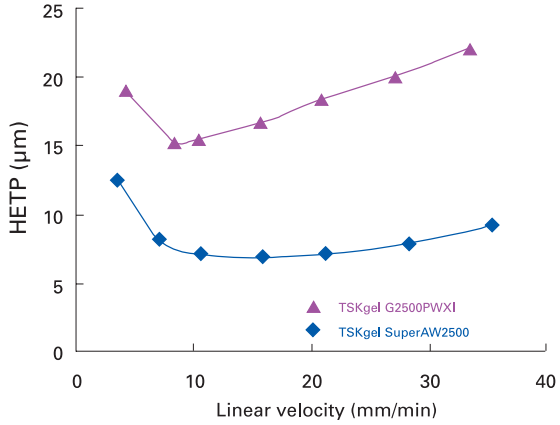


Column: 6.0 mm ID x 15 cm L
Eluent: 10mM LiBr in DMF
Flow rate: 0.6 ml/min
Temp.: 40 °C
Detect.: RI
Sample: Standard polyethylene oxid, polyethylene glycol, ethylene glycol
Sample conc.: 0.04-0.1% each
Inj. Vol.: 10 µl

The combination of small particle sized resins and short column dimensions results in a superior separation performance of TSK-GEL SuperAW columns compared to conventional SEC-columns.

As demonstrated in the plot in figure 3, showing column performance in relation to flow rates, HETP values are smaller and less dependent on flow rate than with conventional columns.

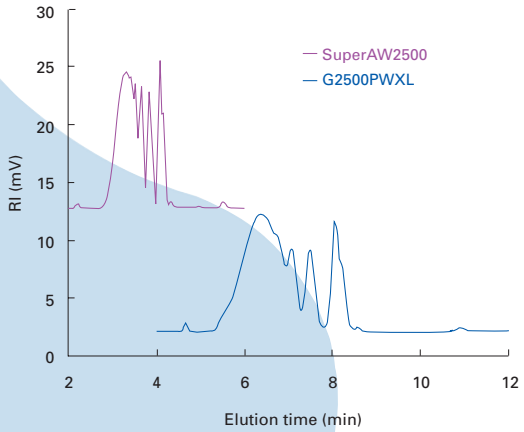
Figure 3 Plots of HETP vs. linear velocity



Columns: TSKgel SuperAW2500 (6.0 mm ID x 15 cm L)
TSKgel G2500PWXL (7.8 mm ID x 30 cm L)
Eluent: Water
Temp.: ambient
Detect.: RI
Sample: Ethylene glycol, 2.5g/l
Inj. Vol.: 5 µl (Super AW2500)
20 µl (G2500PWXL)

This, in practice means that the 15 cm TSKgel SuperAW column provides almost the same theoretical plate number than conventional SEC-columns with 30 cm length, enabling shorter analysis times followed by reduced solvent consumption.

Figure 4 Performance Comparison of TSKgel SuperAW2500 vs. TSKgel G2500PWXL

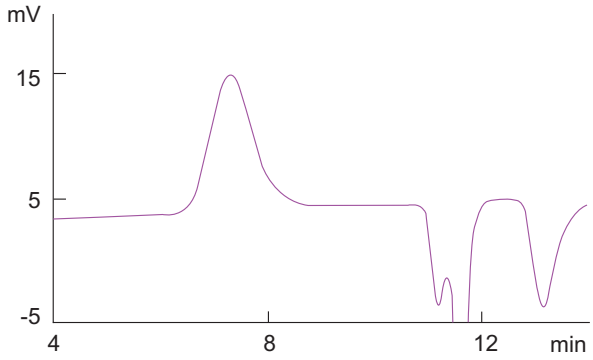


Column: TSKgel SuperAW2500 (6.0 mm ID x 15 cm L)
Eluent: Water
Sample: hydrolysate of dextran T-40
Flow rate: 0.6 ml/min
Sample load: 5 µl
Column: TSKgel G2500PWXL (7.8 mm ID x 30 cm L)
Flow rate: 1.0 ml/min
Sample load: 10 µl

Applications

Various applications of the TSKgel SuperAW columns are available on request. As an example the analysis of Polyacrylonitrile in DMF containing 10 mmol Lithium is shown (Figure 5).

Figure 5 Analysis of Polyacrylonitrile in DMF

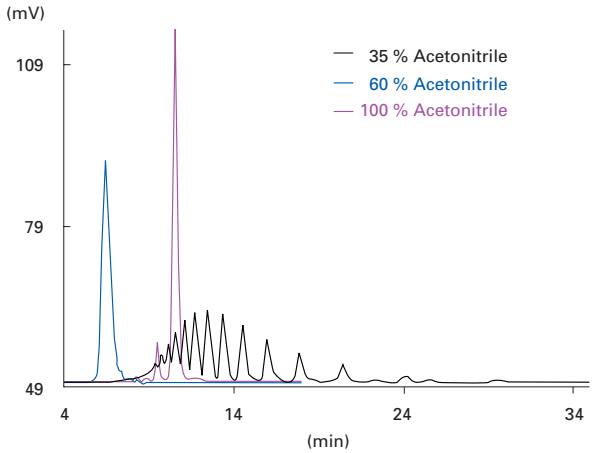


Column: TSKgel SuperAWM-H (6.0 mm ID x 15 cm L, two in series)
Eluent: DMF containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40 °C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

Separation by Non-SEC mode

The excellent solvent compatibility of the TSKgel SuperAW also enables analysis of small or complex compounds in different **Non-SEC-modes**. As shown in figure 7 different chromatograms can be obtained from a surfactant by changing the eluent from 35% of acetonitrile to 100% of acetonitrile. The sample is separated based on molecular size with 60% acetonitrile and it is retained on the column in other eluent compositions based on either hydrophobic or ionic interactions.
Thus it is possible to set up different elution conditions to suit the purpose of measurement in **one** column.

Figure 6 Separation of Triton X-100 by non-SEC mode



Column: TSKgel SuperAW2500 (6.0 mm ID x 15cm L, two in series)
Sample: Triton X-100
Eluent: dif. Acetonitrile / Water solutions
Flow rate: 0.6 ml/min
Temperature: 40°C
Detection: UV 280nm
Injection volume: 20 µl
Sample concentr.: 1.0 g/l

Conclusions

- TSKgel SuperAW columns are allround SEC columns for initial analysis of unknown samples
- Hydrophilic resin with little swelling and shrinkage is compatible to wide range of solvent (water to organic solvent)
- Rigid resin with excellent mechanical stability leads to pressure stablility up to 600psi = 4.0Mpa
- Micro-particle gel packed into analytical columns allows short analysis times and high resolution power
- Five pore sizes and a mixed-bed enable a wide separation range
- Separations possible by SEC, non-SEC or mixed mode allow to optimize the chromatographic method to analytical needs

For further details of choice and selection of the suitable TSK-GEL column that best meets your particular needs. please contact us:

Tel.: +49 (0)711 132570
or
info.sep.eu@tosoh.com
or
www.tosohbioscience.com

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Separations Business Unit

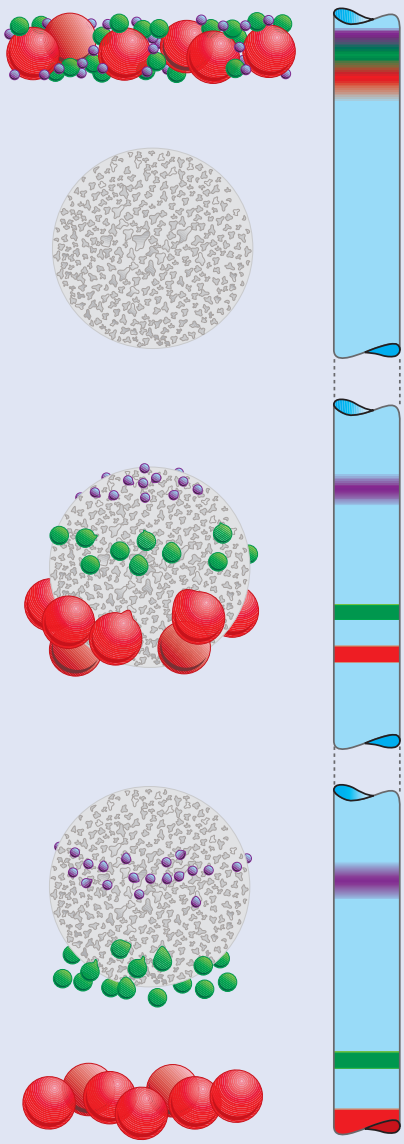
Size Exclusion with TSKgel® SuperSW

Get the most from Size Exclusion Chromatography!

Why we are different

- Tosoh Bioscience is a global leader in the field of bioseparations
- Tosoh Bioscience provides comprehensive technical and regulatory support
- Tosoh Bioscience offers the broadest range of columns for aqueous and organic SEC
- Tosoh Bioscience is the leading manufacturer of silica based SEC columns

Size Exclusion Chromatography

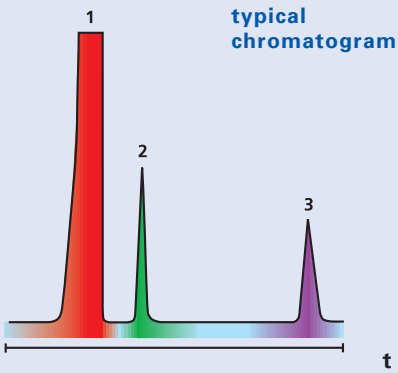


Sample application

buffer/low salt concentration

Elution

buffer/low salt concentration



typical chromatogram

Size Exclusion Chromatography (SEC)

is the general name for the chromatographic mode also referred to as gel permeation chromatography (GPC) for non-aqueous elution systems or gel filtration chromatography (GFC) for aqueous systems. SEC is a method in which components of a mixture are separated according to their molecular size, based on the flow of the sample through a porous packing. Large biomolecules that cannot penetrate the pores of the packing material elute first from the column. These large biomolecules are said to be excluded from the packing; they flow with the mobile phase in the interparticle space of the packed column. Smaller molecules can partially or completely enter the packing particles. Because these smaller molecules have to flow through the interparticle space, as well as through the pore volume, they will elute from the column after the excluded sample components.

SEC is a very simple method for separating biomolecules, because it is not necessary to change the composition of the mobile phase during elution. However, the separation capacity of this method is limited. For a baseline separation it is necessary that the molecular weights of the biomolecules differ at least 10 to 20 %.

TSK-GEL® Columns for SEC

TSKgel SW-Series
TSKgel PW-Series
TSKgel Alpha-Series
TSKgel SuperAW-Series
TSKgel H-Series

High resolution SEC with TSKgel SuperSW

Features & Benefits of TSKgel SuperSW columns

- 4 µm particle size featuring superior resolution and highest sensitivity
- Low non-specific adsorption
- High reproducibility due to well-defined pore size distribution
- > 30.000 theoretical plates/column (4.6 mm ID)
- Microbore columns for increased sensitivity and reduced buffer consumption

Size exclusion chromatography (SEC)

SEC separates molecules based on their size, or more precisely, their hydrodynamic volume. It is usually applied to large molecules such as proteins or industrial polymers. When an aqueous eluent is used, SEC is also referred to as gel filtration chromatography (GFC).

SEC is a well-known technique for the separation and purification of biopolymers because of its effectiveness and non-denaturing mobile phase conditions. It is popular among biochemists for the isolation of proteins, removal of aggregates, desalting or characterization of water-soluble polymers used in food products, paints, pharmaceutical formulations and the like. While soft packing materials such as dextran or agarose were employed as stationary phases for early GFC, porous silica particles with high mechanical strength also have come to be employed for SEC in high performance liquid chromatography (HPLC).

Tosoh Bioscience TSKgel SW and SWXL series are silica SEC phases with pore size distributions suited to protein separations. A hydrophilic diol-type bonded phase shields the silica surface from interacting with protein samples.

	Particle size (µm)	Column size	Guaranteed theoretical plates
TSKgel SuperSW2000	4	4.6 mm ID x 30 cm L	30,000
TSKgel SuperSW3000	4	4.6 mm ID x 30 cm L	30,000
TSKgel G2000SWXL	5	7.8 mm ID x 30 cm L	20,000
TSKgel G3000SWXL	5	7.8 mm ID x 30 cm L	20,000

Table 1 Specifications of TSKgel SuperSW series compared to TSKgel SWXL series

TSKgel SW-type packings feature low adsorption and well-defined pore size distribution. It is the leading SEC column series for HPLC due to its excellent resolution.

High resolution SEC

Speed and resolution is an increasing demand in liquid chromatography. The need for high sensitivity applicable to trace analysis, is increasing as sample size or sample concentration become limited. To meet the needs of high sensitivity and high resolution protein analysis Tosoh Bioscience developed TSKgel SuperSW columns packed with 4 µm spherical silica particles. Compared to the well established TSKgel SWXL (5 µm) columns, SuperSW columns show higher resolution due to a 50 percent increase in theoretical plate numbers (Table 1).

Comparison of TSKgel SuperSW3000 and Dextran/Agarose-type resin

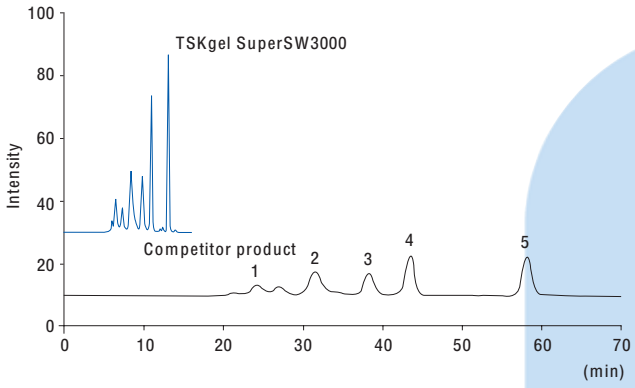


Figure 1
Column: A. TSKgel SuperSW3000, 2 mm ID x 30 cm L;
B. Competitor product, 3.2 mm ID x 30 cm L
Sample: 0.2 µL, 1. thyroglobulin, 1.0 mg/mL; 2. γ-globulin, 2.0 mg/mL;
3. ovalbumin, 2.0 mg/mL; 4. ribonuclease A, 3.0 mg/mL;
5. *p*-aminobenzoic acid, 0.02 mg/mL
Flow rate: A. 65 µL/min N = 30.000; B. 40 µL/min N = 11.000
Temperature: 25°C
Detection: UV @ 280 nm

TSKgel SuperSW Series

Compared to polysaccharide based gel filtration media the increase in resolution, sensitivity and speed is even higher. Figure 1 compares the separation of a protein standard on a dextran/ agarose resin to a TSKgel SuperSW3000 column.

Increased detection limits

To further improve performance, TSKgel SuperSW media are packed into columns with smaller inner diameter (1, 2, 4.6 mm ID). The smaller column diameters are one reason for increased peak heights. In addition, the high resolution of the 4 µm TSKgel SuperSW resins and accordingly the smaller peak widths further increase peak height, provided the HPLC system is optimized with regard to dead volume.

Comparison of TSKgel SuperSW3000 and G3000SWXL for the separation of proteins

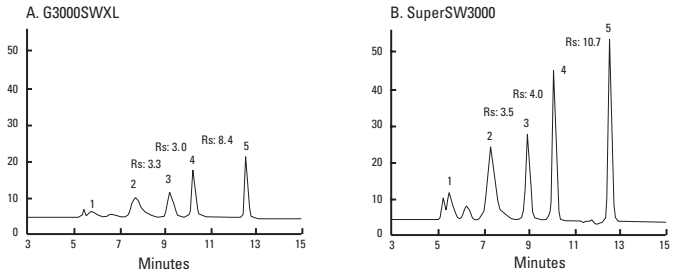


Figure 2
Column: A.TSKgel G3000SWXL, 7.8 mm ID x 30 cm L;
B. TSKgel Super SW3000, 4.6 mm ID x 30 cm L
Sample: 5 µL of a mixture of 1. thyroglobulin, 0.5 mg/mL (660,000 Da); 2. γ-globulin, 1.0 mg/mL; (150,000 Da); 3. ovalbumin, 1.0 mg/mL (43,000 Da); 4. ribonuclease A, 1.5 mg/mL (12,600 Da); 5. *p*-aminobenzoic acid, 0.01 mg/mL (137 Da)
Elution: 0.1 M Na_2SO_4 in 0.1 M phosphate buffer with 0.05% NaN_3, pH 6.7
Flow rate: 1.0 mL/min for G3000SWXL; 0.35 mL/min for SuperSW3000
Temperature: 25°C
Detection: UV @ 220 nm

	TSKgel SuperSW		TSKgel G3000SWXL
Flow cell	Standard cell (low dead volume type)	Micro flow cell	Standard cell (low dead volume type)
Light path length	10 mm	4 mm	10 mm
Thyroglobulin	70 ng	300 ng	200 ng
γ-globulin	50 ng	100 ng	100 ng
Bovine serum albumin	70 ng	300 ng	200 ng
Ovalbumin	50 ng	100 ng	100 ng
Myoglobin	15 ng	50 ng	30 ng
Column:	TSKgel SuperSW, 4.6 mm ID x 30 cm L		
Eluent:	0.2 mol/L phosphate buffer, pH 6.7		
Detection:	UV @ 220 nm		

Table 2 Detection limit for proteins (S/N=3)

Figure 2 demonstrates the superior sensitivity reached with TSKgel SuperSW3000 compared to a TSKgel G3000SWXL column of the same length but larger inner diameter. TSKgel SuperSW can yield peak heights approximately 4 times that of TSKgel SWXL due to downsizing in column diameter and increased theoretical plates. Table 2 shows the detection limits for major proteins. The high sensitivity allows for analysis of nanogram sample amounts.

Separation range of TSKgel SuperSW series

TSKgel SuperSW columns are available in two pore sizes, 125 Å (TSKgel SuperSW2000) and 250 Å (TSKgel SuperSW3000) covering different separation ranges. Table 3 shows the separation ranges for polyethylene glycol (PEG), dextran and typical proteins. Figure 3 shows the SEC calibration curves of TSKgel SuperSW series for standard proteins.

	Molecular weight separation range	
	TSKgel SuperSW2000	TSKgel SuperSW3000
Polyethylene glycol	500 - 15,000	1,000 - 35,000
Dextran	1,000 - 30,000	2,000 - 70,000
Protein	5,000 - 150,000	10,000 - 500,000

Table 3 Molecular weight separation range of TSKgel SuperSW series

Protein calibration curves for TSKgel SuperSW

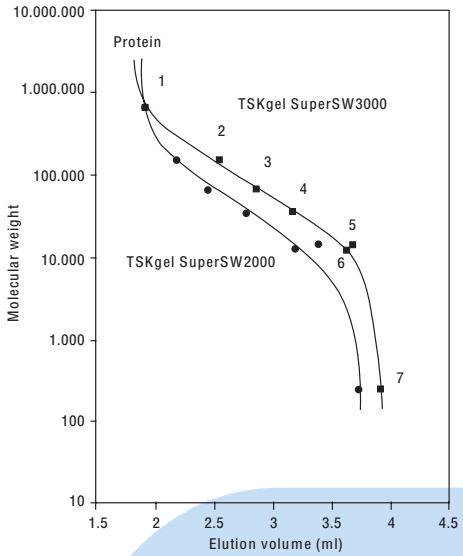


Figure 3
Column: TSKgel SuperSW Series, 4.6 mm ID X 30 cm L
Sample: Standard proteins (5 µL, 0.1g/L each); 1. thyroglobulin 2. γ-globulin 3. bovine serum albumin 4. ß-lactoglobulin 5. lysozyme 6. cytochrome C 7. glycine tetramer
Eluent: 0.2 mol/L phosphate buffer (pH 6.7)
Flow rate: 0.35 mL/min
Detection: UV @ 280 nm

TSKgel SuperSW Series

P/N	Column size	Min. theoretical plates	Asymmetry factor	Flow rate
18675	4.6 mm ID x 30 cm L	30,000	0.70-1.60	Max. 350 µL/min (Max. 12 Mpa)
21485	2.0 mm ID x 30 cm L	25,000	0.70-1.60	Max. 75 µL/min (Max. 12 Mpa)
21845	1.0 mm ID x 30 cm L	18,000	0.70-1.60	Max. 20 µL/min (Max.12 Mpa)

Table 4: Specifications of TSKgel SuperSW3000 series

The TSKgel SuperSW series has the same pore sizes as the conventional TSKgel SWXL series with equivalent grade. Therefore it has similar calibration curves and separation ranges as well. Thus method transfer from conventional SEC to high resolution SEC is very straight forward. In general, TSKgel SuperSW2000 is suited for the separation of proteins with molecular weights of 150,000 Da or smaller. TSKgel SuperSW3000 can be used for the separation of proteins with molecular weights up to 500,000 Da.

Microbore TSKgel SuperSW columns

If sample amount is limited a reduction of column inner diameter can enhance sensitivity. To meet these requests TSKgel SuperSW3000 columns are available with 2 and 1 mm ID. Table 4 shows the specifications of the TSKgel SuperSW3000 columns.

Applications

The small particle size and well-defined pore sizes of TSKgel SuperSW columns provide fast separations with guaranteed efficiencies of 30,000 theoretical plates per 30 cm column (4.6 mm ID). This is the reason why TSKgel SuperSW columns are widely used for peptide and protein analysis in research and development. Whenever limited sample amount is an issue, TSKgel SuperSW columns are the first choice for gel filtration HPLC analysis. In addition to the increased sensitivity, the narrow bore column design involves a remarkable reduction in solvent consumption.

Selection of column dimension

As a result of smaller particle size and accordingly higher number of theoretical plates, sensitivity is increased when using TSKgel SuperSW columns compared to TSKgel SW or SWXL columns. Sensitivity can be further enhanced by reducing inner diameter of SuperSW columns.

Estimation of sensitivity (standard proteins)

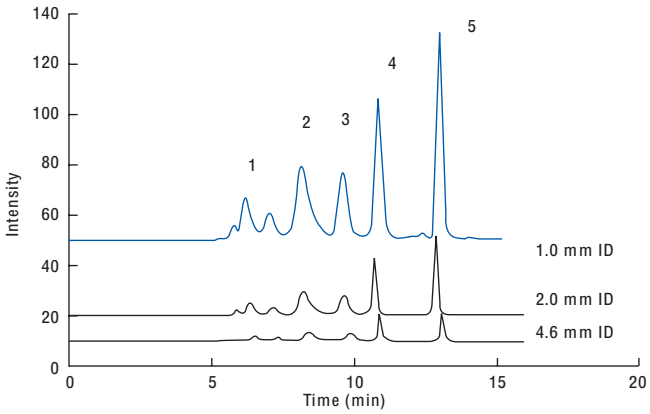


Figure 4
Column: TSKgel SuperSW3000, 1.0, 2.0, 4.6 mm ID x 30 cm L
Sample: 1. tyroglobulin (1.0 g/L), 2. γ-globulin (2.0 g/L), 3. ovalbumin (2.0 g/L), 4. ribonuclease A (3.0 g/L), 5. *p*-aminobenzoic acid (0.02 g/L)
Eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3
Flow rate: 16 µL/min (1 mm ID), 65 µL/min (2 mm ID), 350 µL/min (4.6 mm ID)
Inj.volume: 0.2 µL
Temp.: 25 °C
Detection.: UV @ 280 nm, cell vol. 2 µL (4.6 mm ID), 35 nL (1.0, 2.0 mm ID)

Figure 4 shows the levels of sensitivity which can be reached with semi-micro or micro columns. In the emerging research fields of proteomics, limited sample amount is an issue for most of the separations. In such cases enhancing detection limits by using a micro column can increase the number of hits.

SEC analysis of antibodies

Thermally induced denaturation or aggregation of therapeutic antibodies can be a significant problem during different stages of its production and formulation, since aggregates affect the efficiency of the formulation. Thus the quantification of aggregates is an important parameter in the quality control analysis of biopharmaceuticals. Using TSKgel SuperSW3000 columns the amounts of tri-, di- and monomers of IgG monoclonal antibodies can be monitored.

Applications

Quantification is facilitated by using smaller inner diameter columns since peak height is significantly increased (Figure 5).

Estimation of sensitivity (IgG)

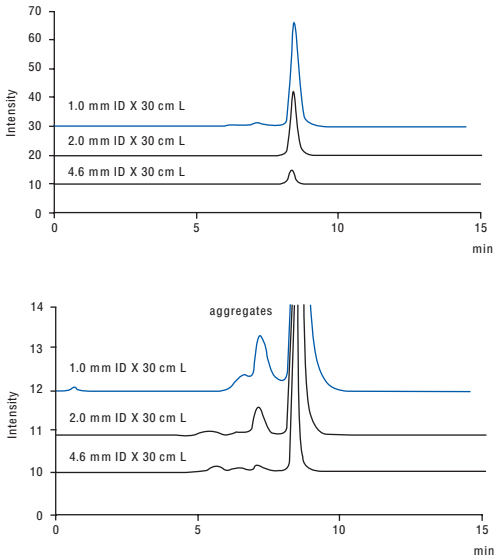


Figure 5
Column: TSKgel SuperSW3000, 1.0 mm ID x 30 cm L
Sample: IgG (mouse, mAb, 1.0 g/L)
Eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3
Flow rate: 16 µL/min
Inj.volume: 0.2 µL
Temp.: 25 °C
Detection.: UV @ 280 nm, cell vol. 2 µL (4.6 mm ID), 35 nL(1.0, 2.0 mm ID)

Separation of IgG and albumin on TSKgel SuperSW3000

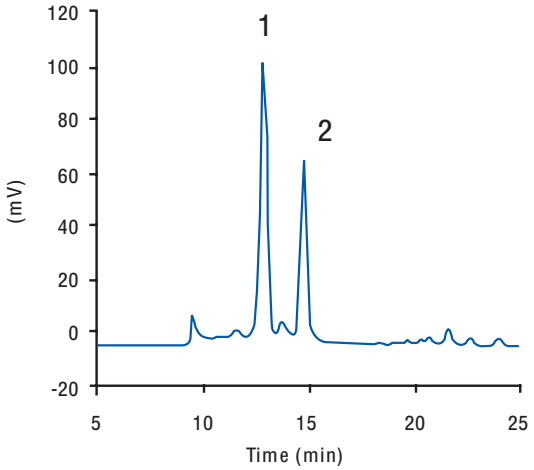


Figure 6
Column: TSKgel SuperSW3000, 4.6 mm ID x 30 cm L
Sample: 5 µL mouse ascites: 1. IgG; 2. albumin
Eluent: 50 mM phosphate buffer + 100 mM Na_2SO_4, pH 6.7
Flow rate: 0.2 µL/min
Temp.: 25 °C
Detection.: UV @ 280 nm

Separation of proteins with ammonium formate eluent on TSKgel SuperSW3000

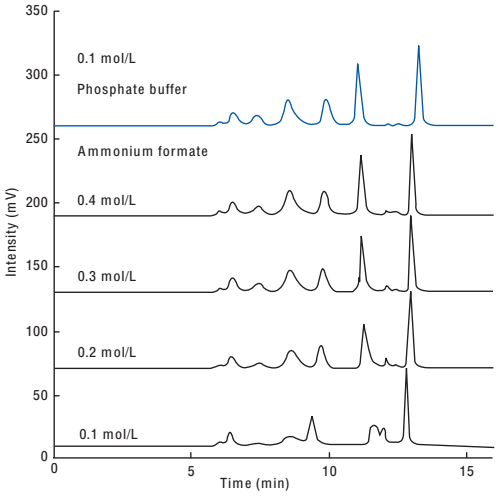


Figure 7 Same conditions and sample as in figure 4.

SEC-ESI-MS analysis of proteins

Hyphenated separation techniques like HPLC-MS or HPLC-ELSD allow sensitive analysis of samples with very low analyte concentrations. Moreover MS/MS detection is a powerful tool to provide further structural information about the compounds. These detection methods require the use of volatile buffer systems because the solvent must be evaporated before the sample molecules enter the detection system. For LC/MS analysis TSKgel SuperSW columns can be run with formate buffers as mobile phase, instead of the common phosphate buffers. Figure 7 demonstrates that at least 300 mM ammonium formate is necessary to reach separation efficiencies comparable to 100 mM phosphate buffer.

Protein analysis under denaturing conditions

Some SEC separations require denaturing conditions like sodiumdodecylsulfate (SDS) containing eluents. In other cases the formulations of biopharmaceuticals contain some detergents (e.g. Tween 20 or Triton). TSKgel SuperSW columns can be operated under these conditions although certain amounts of the detergent will stick to the column, effecting column lifetime and the future use of the column. If analysis under denaturing conditions was performed once, the affected column should be used with detergent containing eluents only. Regular maintenance of the column, the use of guard columns and monitoring of the column status by analysing control samples are recommended as well.

Hardware requirements

Optimization of HPLC equipment

Optimizing the HPLC system to minimize extra column peak broadening is strongly recommended to reach the highest separation power with a TSKgel SuperSW SEC column. This means minimization of dead volume and adjustment of sample concentration and injection volume. Key components of the HPLC system with regard to dead volume reduction are the void volume of tubings, the cell volume of the detector cell and the void volume of the injection unit. HPLC systems designed for use with modern sub 2 µm HPLC columns exhibit extremely small dead volumes. Currently these systems are not evaluated for high resolution SEC use. Using common SEC buffers with such a system might result in a high system backpressure or increased risk of clogging. In worst case the pressure could exceed the pressure limits of TSKgel SuperSW columns. We recommend to carefully evaluate the system's dead volume and the system's backpressure at the flow rates used for SEC analysis.

Void volume of the tubing

The volume of tubing between injector and column, and column and detector influences the diffusion within the tubing and the column efficiency.

Column efficiency starts deteriorating remarkably when the volume of the tubing exceeds 10 µl (e.g. 0.1 mm ID x 150 cm L). Shortening of tubings of 0.1 or 0.125 mm inner diameter to the minimum is often better than using long tubings with smaller inner diameters. The backpressure increases with smaller inner diameters and the system becomes more susceptible towards clogging.

Cell volume of the detector

The detector cell volume also contributes to the dead volume of the system and might impair peak resolution. Compared to a semi-micro detector cell with 2 to 3 µl cell volume, the standard cells of most high end HPLC instrument's UV or PDA detectors, having cell volumes of 10-12 µl and small inner diameter inlet capillaries do not have a big influence on the number of theoretical plates. The increase in efficiency by using a smaller cell is below 5 %. On the other hand the path length of semi-micro or micro cells is often shorter than for standard cells. Consequently some 40 to 60% loss of sensitivity might be the price for higher resolution (Table 2). For most separations with 4.6 mm ID TSKgel SuperSW columns a 10 µl standard detector cell is a good choice.

Recommended flow cells for common HPLC systems for UV/PDA detection

Detector model/Column ID	4.6 mm ID (max. sensitivity)	4.6 mm ID (max. resolution) & 2 mm ID	1 mm ID
Agilent Technologies 1200 VWD SL	Standard cell, 14 µl G1314C #018	Semi-micro cell, 5 µl G1314C #016	
Agilent Technologies 1200 DAD SL	Standard cell, 13 µl G1315C #018	Semi-micro cell, 5 µl G1315C #016	Micro cell, 2 µl G1315C #010
Dionex UltiMate VWD-3100/-3400	Standard cell, 11µl 6074.0250	Micro cell, 1.4 µl 6074.0260	U-Z-View Micro 180 nl 6074.0290
Dionex UltiMate PDA-3000	Standard cell, 13 µl 6080.0210	Semi-micro cell, 3.1 µl 6080.0230	
Waters 2489 UV/VIS	Standard cell, 10 µl WAS081140	Microbore cell 2.6 µl WAT081159	
Shimadzu Prominence UV/UV-VIS SPD-20A/-20AV	Standard cell, 12 µl Incl.	Semi-micro cell, 2.5 µl 228-45605-91	Dionex U-Z View Micro, 140 nl; 160239
Shimadzu Prominence PDA SPD-M20A	Standard cell, 10 µl Incl.	Semi-micro cell, 2.5 µl 228-45605-92	Dionex U-Z View Micro, 140 nl; 160239
VWR LaChrom Elite UV/UV-VIS L-2400/2420	Standard cell, 13 µl 890-0500	Semi-micro cell, 3.2 µl 890-0504	Micro cell, 0.9 µl 890-0506
VWR LaChrom Elite DAD L-2450	Standard cell, 13 µl 890-0550	Semi-micro cell, 3.2 µl 890-0554	Micro cell, 0.9 µl 890-0556

Table 5

Hardware requirements

In case that semi-micro (2 mm ID) or micro columns (1 mm ID) are used, we strongly recommend adjusting the cell volume accordingly. Table 5 shows the recommended flow cells for the most frequently used HPLC systems.

Injector

The maximum number of theoretical plates in isocratic HPLC separations is always reached using a low diffusion type manual injector like the Rheodyne 8125. A general-purpose injector like the Rheodyne 7125 will lead to the loss of 10% in efficiency. All kinds of automated HPLC injectors will deteriorate column efficiency as well. Due to practical reasons, auto-samplers are nowadays standard in HPLC systems. All the more it is important to select an auto-sampler capable of trace injection mode.

Dead volume of the outlet capillary should be minimized to the utmost (as short as possible, ID $\leq$ 0.1 mm). Figure 8 shows the effect of injector tubing on column efficiency for a 1 mm ID column.

Influence of tubing (injector to column)

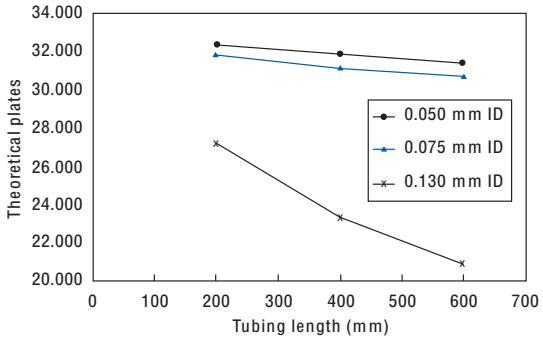


Figure 8
Column: TSKgel SuperSW3000 1.0 mm ID x 30 cm L
Eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05 % NaN_3
Flow rate: 16 µL/min
Inj.volume: 0.2 µL
Temp.: 25 °C
Detection.: UV @ 280 nm
Sample: p-Aminobenzoic acid (20mg/L)
Tubing:

ID (mm)	L (mm)	Vol. (nL)
0.050	200	393
	400	785
	600	1178
0.075	200	883
	400	1766
	600	2469
0.130	200	2653
	400	5307
	600	7960

Effect of sample load

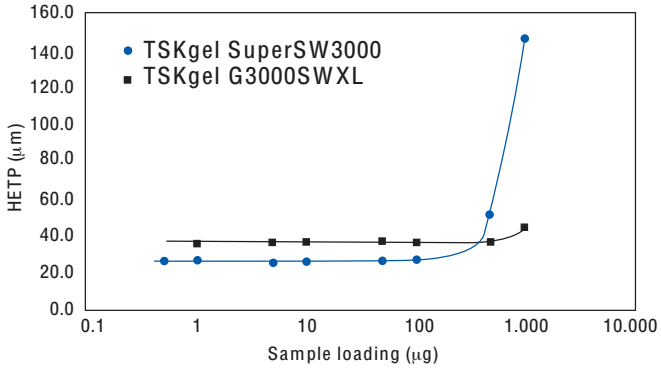


Figure 9
Column: TSKgel SuperSW series, 4.6 mm ID x 30 cm L,
TSKgel SWXL series, 7.8 mm ID x 30 cm L
Sample: Bovine serum albumin
Eluent: 0.2 mol/L phosphate buffer, pH 6.7
Flow rate: 0.35 mL/min (TSK-GEL SuperSW series)
1.00 ml/min (TSK-GEL SWXL series)
Temp.: 25 °C
Detection.: UV @ 280 nm, micro flow cell

Sample load and injection volume

Although the height equivalent to a theoretical plate (HETP) is small in TSKgel SuperSW series it is obvious that it increases at high sample loads. Figure 9 shows that sample load should not exceed 100 µg for a TSKgel SuperSW3000 column of 4.6 mm ID x 30 cm L. On the other hand the injection volume itself is a critical parameter.

Effect of injection volume on a 2 mm ID TSKgel SuperSW3000 column

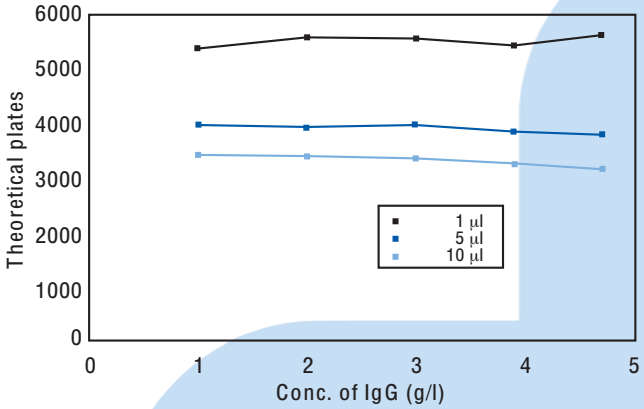


Figure 10
Column: TSKgel SuperSW3000 2.0 mm ID x 30 cm L
Sample: IgG (mouse, mAb) 1, 5, 10 µl
Eluent: 0.1 M phosphate buffer + 0.1 M Na_2SO_4 + 0.05 % NaN_3 (pH 6.7)
Flow rate: 0.065 mL/min
Temp.: 25 °C
Detection.: UV @ 280 nm

Hardware requirements

As for all HPLC applications injection volume should be as small as possible. If injection volume exceeds 20 µl on a 4.6 mm ID column, a considerable deterioration of column efficiency is observed for TSKgel SuperSW2000 (80 µl for TSKgel SuperSW3000).

The influence of injection volume is even higher when using microbore TSKgel SuperSW columns. Figure 10 demonstrates that a certain increase in sample concentration does not harm the efficiency of a microbore column if the injection volume is small. On the other hand an increase of the injection volume itself has a remarkable effect.

In general the sample load should be less than 100 µg as total amount and less than 10 µl as injection volume for a 4.6 mm ID TSKgel SuperSW column.

Mobile phase

The eluent plays an important role in SEC separations. When denaturing eluents are used, the exclusion limit for proteins become smaller since they lose their compact globular structure. Proper selection of eluting conditions is necessary to maximize molecular sieving mechanisms and to minimize secondary effects, such as ionic and hydrophobic interactions between the sample and the column packing material. Under conditions of high ionic strength (> 1.0 M), hydrophobic interactions may occur. Under low ionic strength (<0.1 M), ionic interactions are more likely to occur. In general, the use of relatively high ionic strength buffers is recommended for most applications. A neutral salt, such as sodium sulfate, is often added to increase ionic strength.

If hydrophobic interaction occurs between the sample and the matrix, up to 100% water soluble organic, such as acetonitrile, acetone, methanol or ethanol, can be added to the mobile phase. If mass spectrometric detection is applied it is necessary to change to a volatile buffer system.

Flow rate dependence

The effect of flow rate on HETP depends on particle size of packing materials, sample molecular size, eluent viscosity, etc. Since the particle size of TSKgel SuperSW is small, it has small HETP throughout a broad range of flow rates (Figure 11).

Van Deemter curve

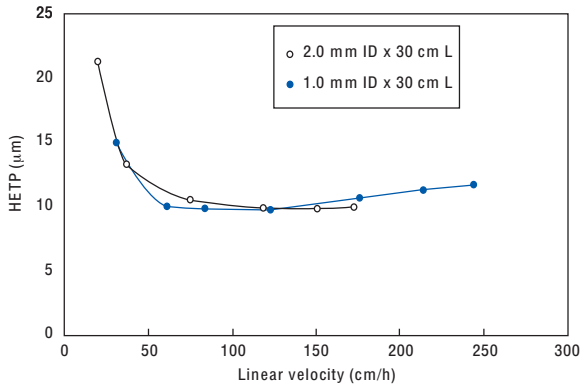


Figure 11
Column: TSKgel SuperSW3000, 1.0 mm ID x 30 cm L
TSKgel SuperSW3000, 2.0 mm ID x 30 cm L
Sample: *p*-Aminobenzoic acid (20 mg/L)
Eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3
Detection.: UV @ 280 nm
Temp.: 25 °C
Inj.volume: 0.2 µL (1.0 mm ID), 1.0 µL (2.0 mm ID)

The appropriate flow rate for TSKgel SuperSW columns is up to 0.4 ml/min for a 4.6 mm ID column, up to 75 µl/min for a 2 mm ID column, and up to 20 µl/min for a 1 mm ID column respectively. If higher resolution is required the flow rate can be lowered.

Recovery of protein

TSKgel SuperSW series is capable of obtaining high protein recovery even in trace analysis with sample load of 1 µg or lower. Table 6 shows the recovery of proteins at sample concentrations of 20 µg/mL (sample load 100 ng). Most proteins are recovered quantitatively with TSKgel SuperSW series, but it is important to make sure that samples in small concentrations are not adsorbed to the HPLC system (injector, tubing etc.) itself. Similar samples should be injected several times before measurement so that the adsorption point within the system is inactivated in advance when trace analysis is performed.

	TSKgel SuperSW2000	TSKgel SuperSW3000
Thyroglobulin	86%	97%
γ-globulin	90%	90%
BSA	99%	86%
Ovalbumin	97%	98%
Ribonuclease A	86%	87%
Myoglobin	93%	96%
Cytochrome C	85%	90%
Lysozyme	93%	89%

Table 6 Recovery of proteins

Ordering Information

Conclusion

TSKgel SuperSW series is a group of columns in which particle size and column size of the conventional TSKgel SWXL series have been reduced and at the same time to improve resolution and sensitivity. As additional benefit of the narrow column diameters buffer consumption is considerably reduced. TSKgel SuperSW series is ideal for sample-limited applications because it maintains high recovery even for sample injection at a low concentration. It is therefore suited to trace analysis of biopolymers by SEC.

As a result of the high manufacturing quality of TSKgel SuperSW resins these columns show an extremely low amount of column bleeding. Hence they can be used for SEC separation followed by mass spectrometric detection as well.

In order to exert the better performance of TSKgel SuperSW series, the use of equipment with minimized dead volume is recommended. Table 7 summarizes the cautions in using TSKgel SuperSW series columns. Under ideal conditions with a proper sample preparation and the use and regular exchange of guard columns a long column lifetime can be achieved. For micro and semi micro columns a line filter instead of a guard column is recommended to keep dead volume low.

Based on their high efficiency TSKgel SuperSW2000 and TSKgel SuperSW3000 columns are ideally suited for all highly sensitive gel filtration analysis in the fields of biotechnology, proteomics and in quality control of low dose biopharmaceuticals.

P/N	Column	Column size	Min. theoretical plates
18674	TSKgel SuperSW2000	4.6 mm ID x 30 cm L	30,000
21845	TSKgel SuperSW3000	1.0 mm ID x 30 cm L	18,000
21485	TSKgel SuperSW3000	2.0 mm ID x 30 cm L	25,000
18675	TSKgel SuperSW3000	4.6 mm ID x 30 cm L	30,000
18762	Super SW Guardcolumn, 4 µm, for P/N 18674 and 18675		

Notes to be made in using TSKgel SuperSW series

- Reduce peak broadening in tubing, detector, etc.
- Take care of sample overloading
- Take care of flow rate of pumping system since the required flow rate is low

Tubing:

- Use 0.1 mm ID tubing. It is recommended that the total tubing length is 100 cm or shorter
- Connection pipe set type L (product no. 018186; 0.1 mm ID x 40 cm L, 2 pieces) available, connection surface (both ends) with fine-cut finishing
- Sections requiring 0.1 mm ID tubing

  a) Between injection valve/column inlet, or auto-sampler/column inlet

  b) Between column outlet/detector inlet (tubing on inlet side of the detector)

Pumping system:

- Pumping system should be applicable to semi-micro HPLC
- Flow rate should be 0.01 - 0.35 ml/min

Injector:

- Low diffusion type injector (Reodyne 8125) is recommended

Guard column:

- Be sure to connect an in-line filter or a guard column (product no. 18762) to protect the column (A set of connection tubing is a standard accessory to the guard column)

Detector:

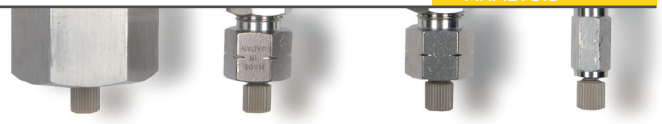
- For UV detectors, use micro flow cells or low dead volume type cells. Low dead volume type cells are effective in high-sensitivity analysis. Use of standard cell is also possible for 4.6 mm ID columns. However, theoretical plates will be reduced.

Sample:

- Sample injection volume should be 1 - 10 µl. sample load should be 100 µg or smaller.

If help is needed, contact our technical support specialists to offer you assistance at +49 (0)711 13257-0.

Table 8



TSKgel® SuperSW mAb HTP/HR
TSKgel® UltraSW AGGREGATE

INTRODUCTION

Aqueous size exclusion chromatography (SEC) is the method of choice for the analysis of protein fragments, monomers, and aggregates under non-denaturing conditions. Based on the flow of the sample through a porous stationary phase SEC separates molecules according to their size, or more precisely, their hydrodynamic volume. In aqueous elution systems SEC is also referred to as gel filtration chromatography (GFC). TSKgel G3000SWXL columns have been the industry's standard for quality control of monoclonals by SEC for decades. Based on the proven proprietary surface technology of the renowned TSKgel SW series, a new series of silica-based SEC columns was engineered to provide shorter analysis time or higher resolution for antibody analysis.

HIGHLIGHTS

- Optimized for antibody analysis
- Small particle size for UHPLC use
- Highest resolution with SuperSW mAb HR
- Fast separation with SuperSW mAb HTP
- Higher molecular weight range with UltraSW Aggregate

FEATURES

The new series of dedicated SEC columns for mAb analysis delivers significant advancements in resolution. It comprises of three different columns and their guards. Table 1 summarizes the characteristics of the mAb SEC columns and Figure 1 shows the calibration curves and the molecular weight range of the three columns.

The calibration curve of TSKgel SuperSW mAb HR is most similar to the one of TSKgel G3000SWXL, the current industrial standard for antibody analysis. The new columns are highly reproducible. The high Batch-to-Batch stability is proved in Figure 2 for TSKgel Super SW mAb HR. The lifetime of the columns can be further improved when using the corresponding guard columns.

CALIBRATION CURVE

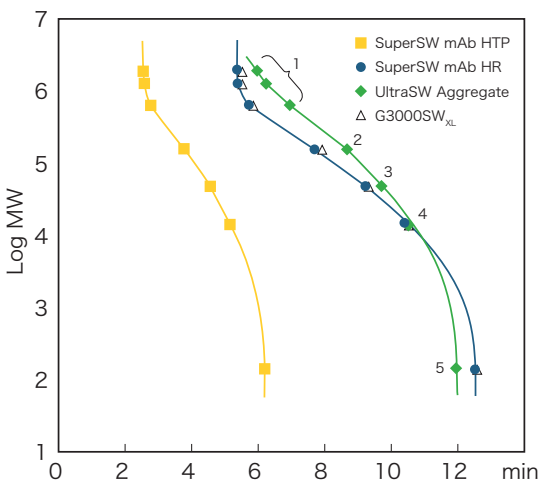


Figure 1

Columns: TSKgel SuperSW mAb HTP 4.6 mm ID × 15 cm, TSKgel SuperSW mAb HR, TSKgel UltraSW Aggregate, TSKgel G3000SWXL (all 7.8 mm ID × 30 cm)
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); Temperature: 25°C; Detection: UV 280 nm; Inj. vol.: 10 µL, 5 µL (SuperSW mAb HTP)
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)

CHARACTERISTICS OF TSKgel mAb COLUMNS

Column	TSKgel SuperSW mAb HR	TSKgel SuperSW mAb HTP	TSKgel UltraSW Aggregate
Dimension	7.8 mm ID x 30 cm	4.6 mm ID x 15 cm	7.8 mm ID x 30 cm
Theoretical plates	≥30.000	≥15.000	≥35.000
Base material	Silica gel		Silica gel
Particle size	4 µm		3 µm
Separation range (globular proteins)	10,000 - 500,000 Da		10,000 - 2,000,000 Da

Table 1

Each of the new SEC columns is tailored to a specific separation problem. TSKgel SuperSW mAb HTP - "HTP" standing for high throughput - was developed to enable an easy transfer of HPLC methods based on TSKgel SWXL to fast UHPLC analysis. Small particle size silica beads are packed in UHPLC column hardware with 4.6 mm inner diameter. This enables to double the throughput without compromising resolution (Figure 3).

TSKgel SuperSW mAb HR - "HR" indicating high resolution - delivers superior resolution over the whole range from fragments to aggregates when analysing monoclonals (Figure 4). It has the same column dimensions - 7.8 mm by 30 cm length - as the established TSKgel G3000SWXL. TSKgel Ultra SW Aggregate (7.8 mm ID x 30 cm) features a smaller particle size and a higher exclusion limit. It offers a wider separation window in the aggregate region (Figure 4C).

FAST ANALYSIS OF mAb AGGREGATION

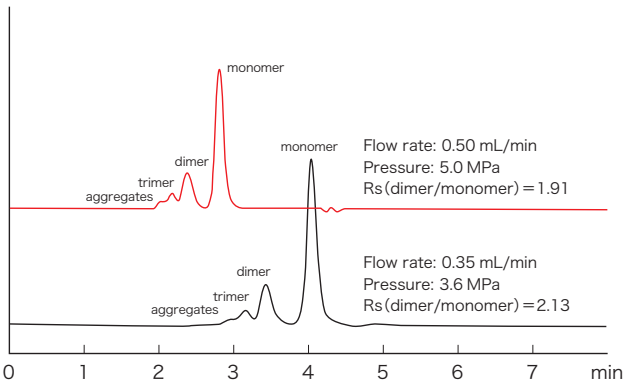


FIGURE 3

Column: TSKgel SuperSW mAb HTP (4.6 mm ID x 15 cm)
Elution: 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
Temp.: 25°C; Sample: monoclonal antibody (mouse-human chimeric IgG, Erbitux), 5 µL

BATCH TO BATCH REPRODUCIBILITY

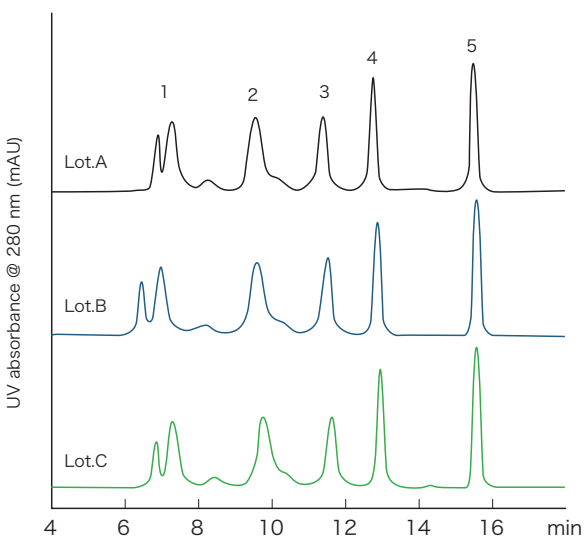


FIGURE 2

Column: TSKgel SuperSW mAb HR (7.8 mm ID x 30 cm)
Eluent: 0.2 mol/L phosphate buffer (pH 6.7)+ 0.05% NaN_3
Flow rate: 0.8 mL/min; Detection: UV @ 280 nm; Inj. volume: 10 µL;
Sample: 1. Thyrobulobulin, 2. γ-Globulin, 3. Ovalbumin, 4. Ribonuclease A, 5. p-Aminobenzoic acid

COMPARISON OF AGGREGATE ANALYSIS

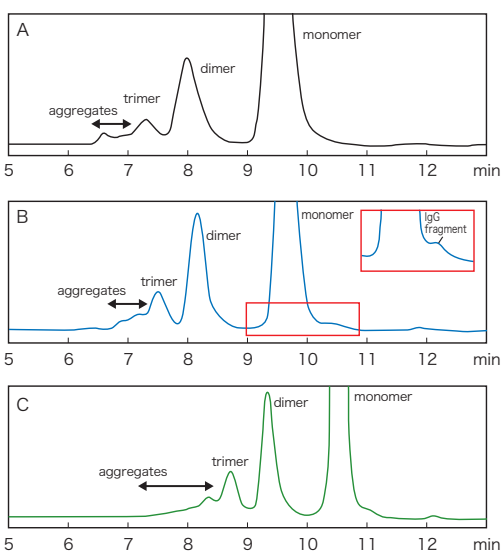


FIGURE 4

Columns: A. TSKgel G3000SWXL, B. TSKgel SuperSW mAb HR, C. TSKgel UltraSW Aggregate; Dimension: 7.8 mm ID × 30 cm;
Eluent: 0.2 mol/L phosphate buffer (pH 6.7) + 0.05% NaN_3
Flow rate: 0.8 mL/min; Detection: UV @ 280 nm; Temp.: 25°C;
Sample: monoclonal antibody, (mouse-human chimeric IgG, Erbitux), 10 µL

Ordering information

Part-No	Description	Matrix	Housing	Dimensions
22854	TSKgel SuperSW mAb HR, 4 µm, 250 Å	Silica	Stainless steel	7.8 mm ID x 30.0 cm L
22855	TSKgel SuperSW mAb HTP, 4 µm, 250 Å	Silica	Stainless steel	4.6 mm ID x 15.0 cm L
22856	TSKgel UltraSW Aggregate, 3 µm, 300 Å	Silica	Stainless steel	7.8 mm ID x 30.0 cm L
22857	TSKgel Guardcolumn SuperSW mAb, 4 µm	Silica	Stainless steel	6.0 mm ID x 4.0 cm L
22858	TSKgel Guardcolumn SuperSW mAb, 4 µm	Silica	Stainless steel	3.0 mm ID x 2.0 cm L
22859	TSKgel Guardcolumn UltraSW, 3 µm	Silica	Stainless steel	6.0 mm ID x 4.0 cm L