TOSOH

interchim®

SEC COLUMNS

TOSOH BIOSCIENCE

YOUR SPECIALIST IN SEPARATION

ABOUT US

WITH A GLOBAL PERSPECTIVE.

TOSOH BIOSCIENCE GmbH, Separations Business Unit, Stuttgart, is an acknowledged global leader in the field of bioseparations. Established as TosoHaas in 1987, the original joint venture between Tosoh Corporation of Japan and the Rohm and Haas Company, USA, has become synonymous with advanced products and quality support. In the year 2000, Tosoh Corporation acquired a 100% controlling interest changing the name to TOSOH BIOSEP. In the course of unifying all Tosoh affiliates, the new Brand Name Tosoh Bioscience evolved. Today, the two branches, Bioseparations and Diagnostics operate with the same name Tosoh Bioscience - Separations Business Unit and accordingly Diagnostics Business Unit. Tosoh manufacturing sites in Japan provide products to the sales and support subsidiaries in the U.S. and Europe, ensuring full global coverage. Over the last 30 years, TSKgel SW columns have become the worldwide industry standard for size exclusion chromatography of biomolecules.

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TOSOH HISTORY

1935	FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
1936	OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
1971	SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSK-GEL DEVELOPED BY TOSOH
1974	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
1979	TOSOH DEVELOPS TOYOPEARL MEDIA
1983	TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
1987	TOSOHAAS US OPERATIONS FORMED IN MONTGOMERYVILLE
1989	TOSOHAAS GMBH OPERATIONS FORMED IN STUTTGART
1995	TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
2000	IN NOVEMBER FORMER TOSOHAAS US OPERATIONS BECOMES TOSOH BIOSEP LLC, A 100% SUBSIDIARY OF TOSOH CORPORATION
2001	IN JANUARY FORMER TOSOHAAS GMBH EUROPEAN OPERATIONS BECOMES TOSOH BIOSEP GMBH, A 100% SUBSIDIARY OF TOSOH CORPORATION
2002/ 2003	TOSOH CORPORATION ANNOUNCES THAT ALL TOSOH AFFILIATED SCIENTIFIC AND DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE, WILL BE UNIFIED UNDER THE NEW NAME TOSOH BIOSCIENCE.
2008	ECOSEC , THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
2009	TOSOH BIOSCIENCE GMBH CELEBRATES ITS 20TH ANNIVERSARY IN STUTTGART
2010	TOSOH CELEBRATES ITS 75TH YEAR IN BUSINESS WITH THE OPENING OF FIVE NEW PLANTS, AND CONTINUED RAPID EXPANSION IN CHINA

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.

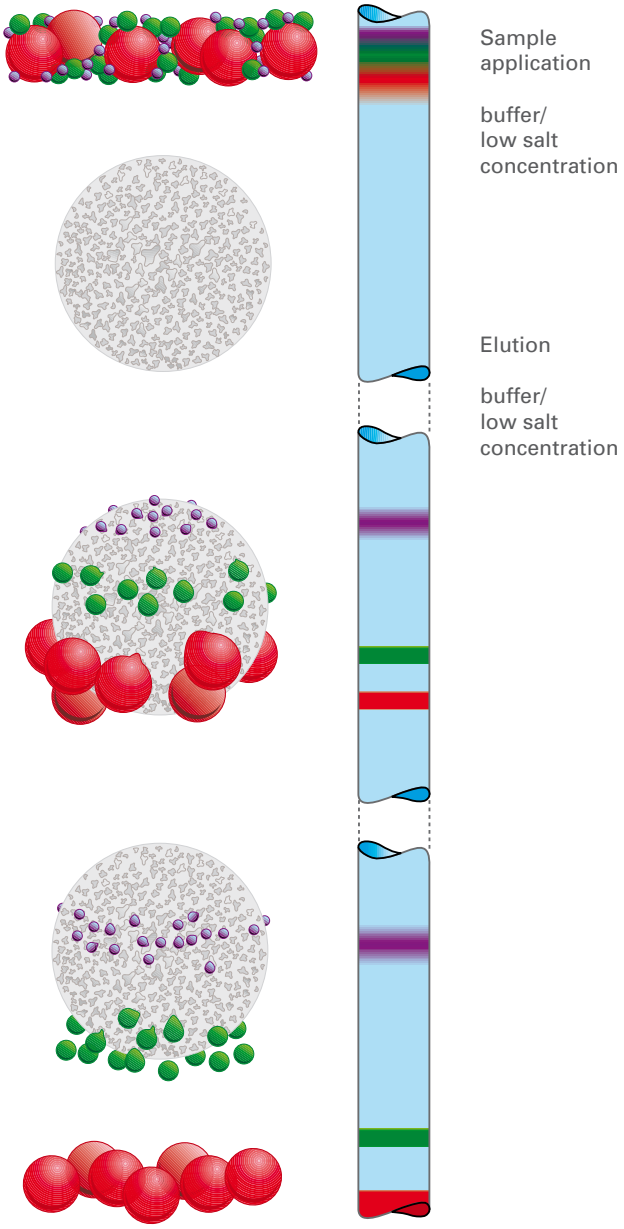
SEC
HOW IT WORKS

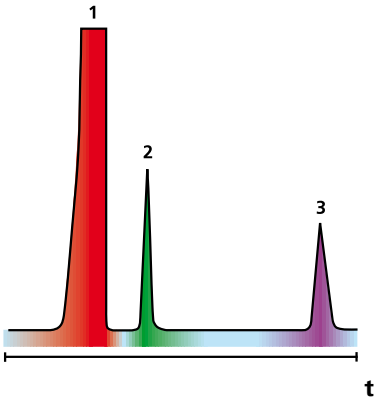
Size exclusion chromatography (SEC) is a method in which components of a mixture are separated according to their molecular size, based on the flow of the sample through a porous packing. In contrast to all other modes of liquid chromatography the prerequisite for SEC is that the analyte does not interact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

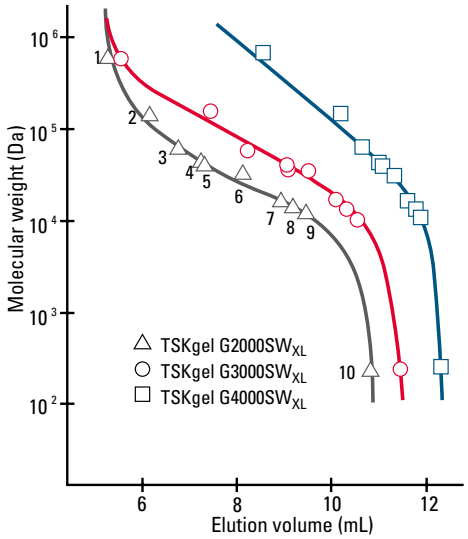
SAMPLE			COLUMN SELECTION		SELECTION CRITERIA
			FIRST CHOICE	ALTERNATIVE	
Carbohydrates	polysaccharides		TSKgel GMPWXL TSKgel SuperMultiporePW	TSKgel G5000PWXL and TSKgel G3000PWXL	large pore size, linear calibration curve, small particles, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW TSKgel 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

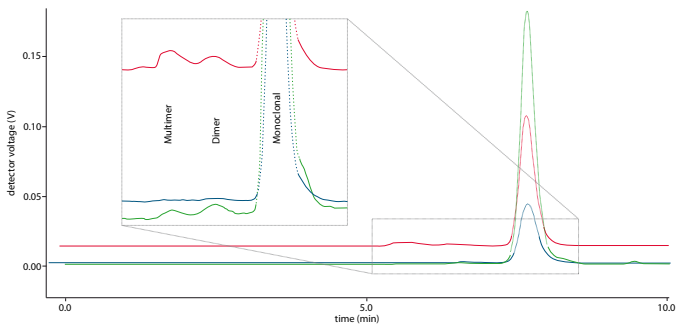
Protein aggregation is a common issue encountered during expression, purification and formulation of protein biotherapeutics, which needs to be characterized and controlled during the development and production of protein pharmaceuticals such as monoclonal antibodies (mAbs). Even small amounts of aggregates 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.

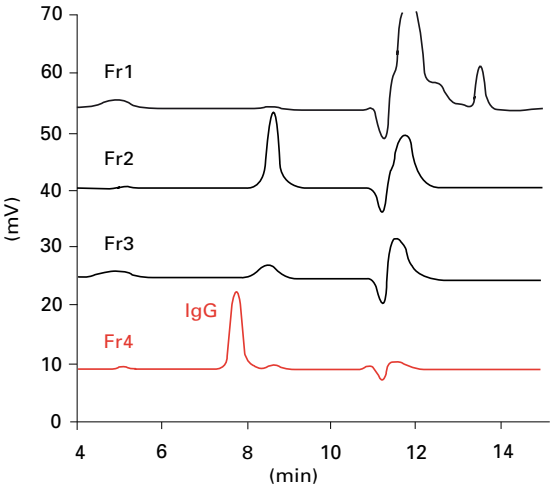
FIGURE 2



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

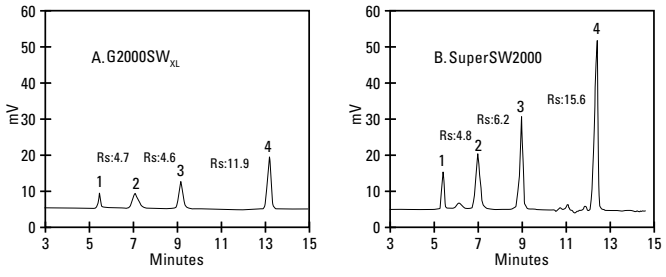
Figure 4 demonstrates the superior sensitivity reached with TSKgel SuperSW2000 compared to a TSKgel G2000SWXL column of the same length but larger inner diameter. TSKgel SuperSW can yield peak heights approximately 4 times that of TSKgel SWXL due to downsizing in column diameter and increased theoretical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

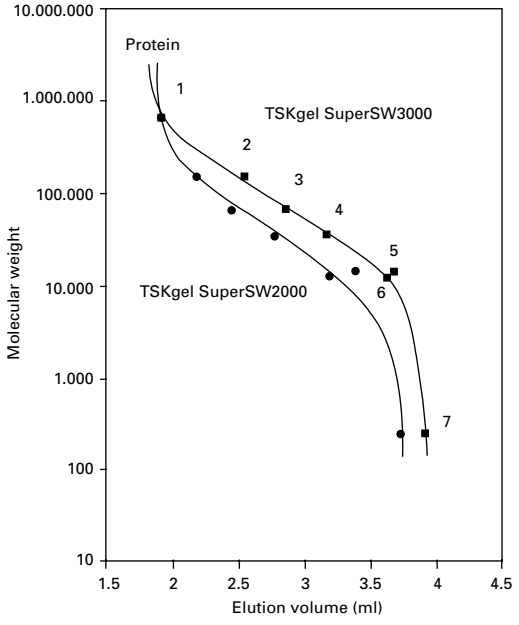
TABLE 4

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

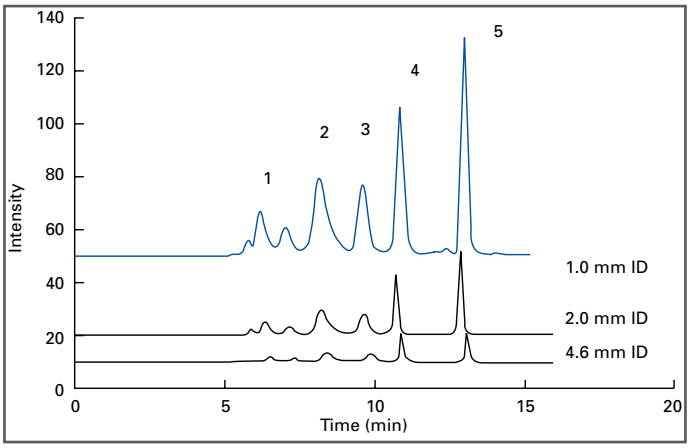
FIGURE 5



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

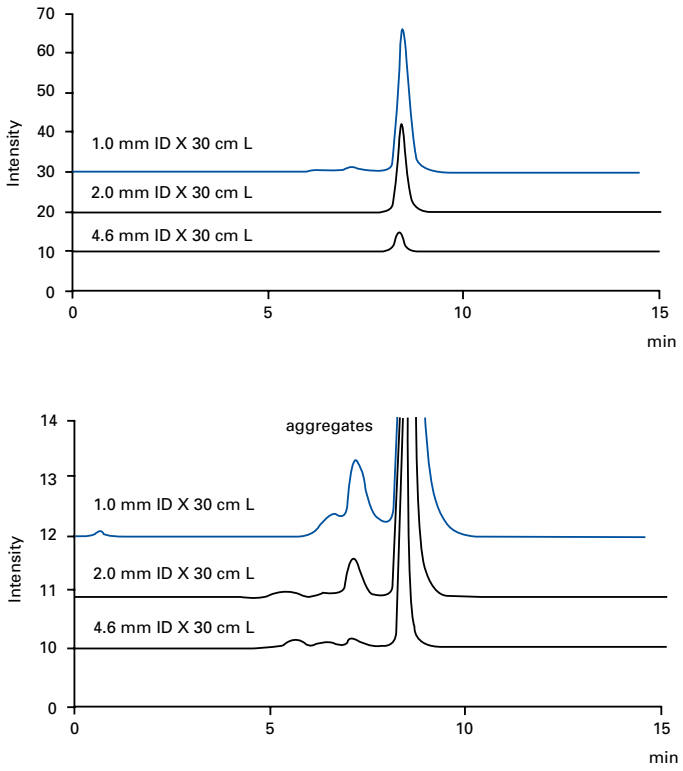
QC ANALYSIS OF ANTIBODIES

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.

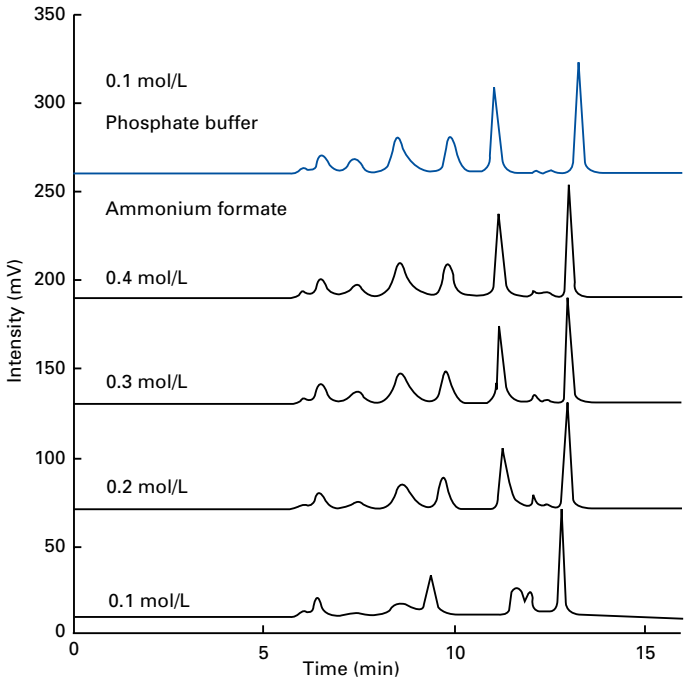
FIGURE 7



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

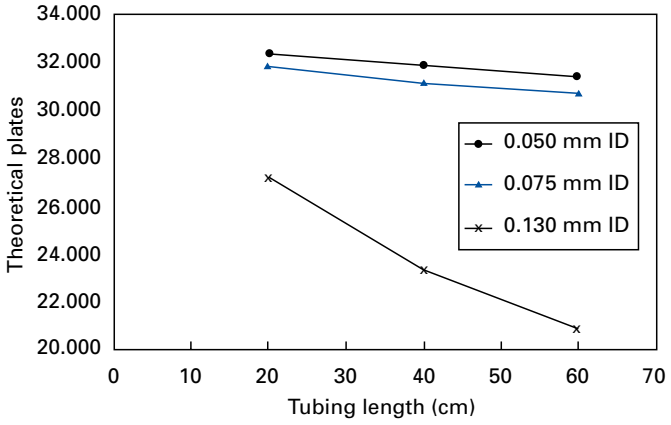
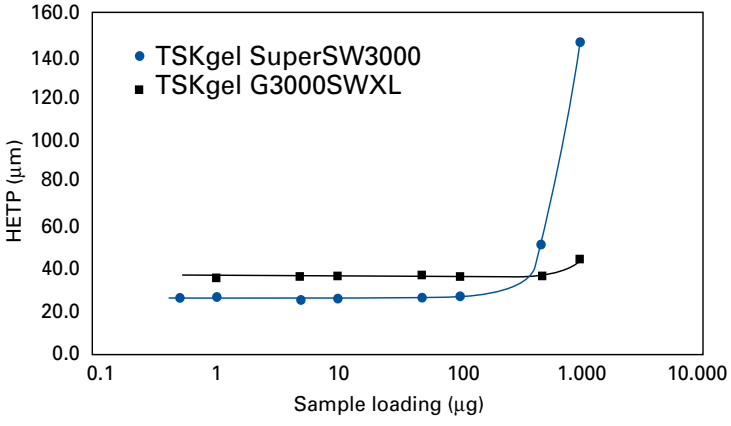


FIGURE 10



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

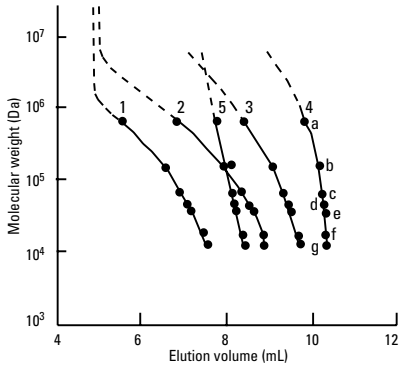
TSKgel COLUMN	PARTICLE SIZE (µM)	PORE SIZE (Å)	MW RANGE (PEG/PEO)
G1000PW	12	<100	$<1 \times 10^3$
G2000PW	12	125	$<2 \times 10^3$
G2500PW	12, 17	<200	$<3 \times 10^3$
G3000PW	12, 17	200	$<5 \times 10^4$
G4000PW	17	500	$<3 \times 10^5$
G5000PW	17	1,000	$<1 \times 10^6$
G6000PW/ BioAssist G6PW	17	>1,000	$<8 \times 10^6$
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.

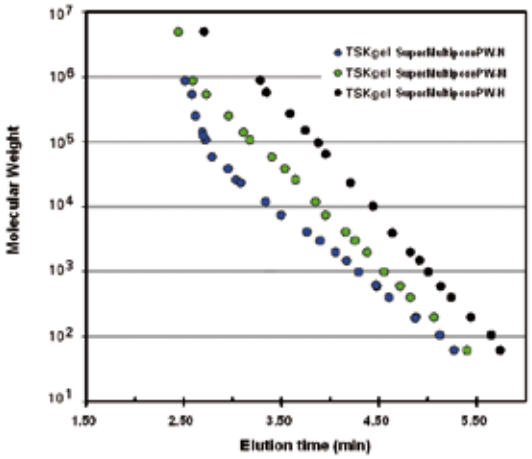
FIGURE 12



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

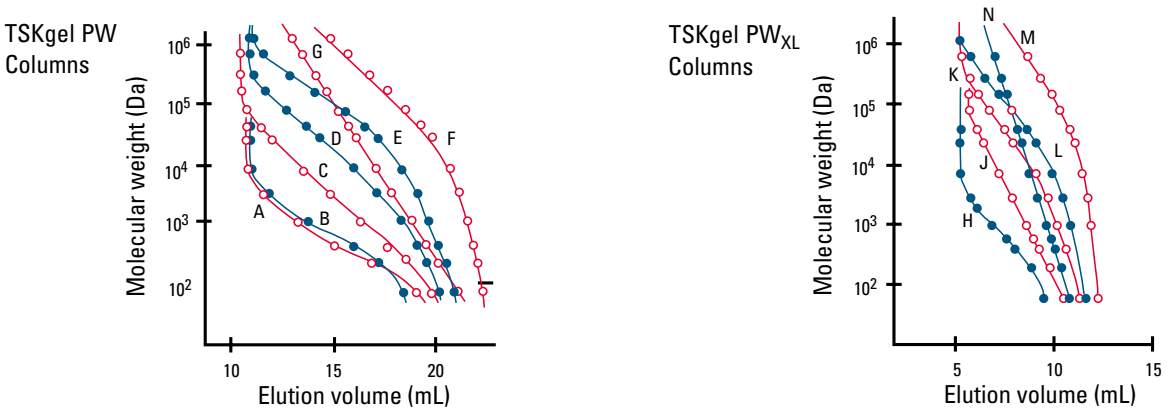
FIGURE 13



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

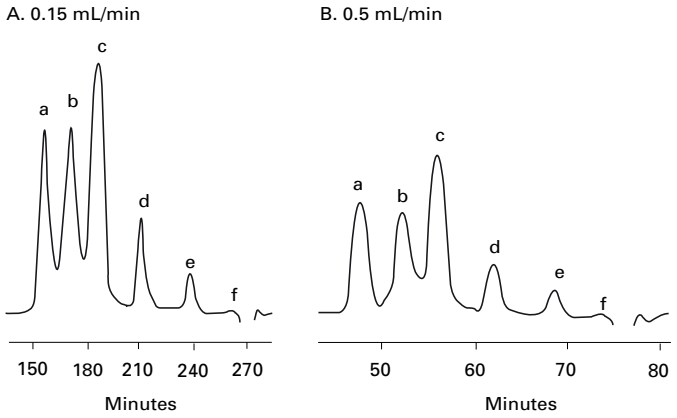
LARGE DNA FRAGMENTS

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.

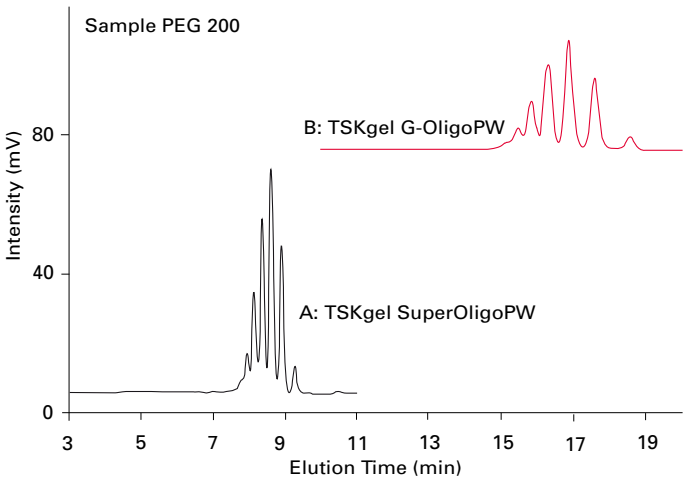
FIGURE 14



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

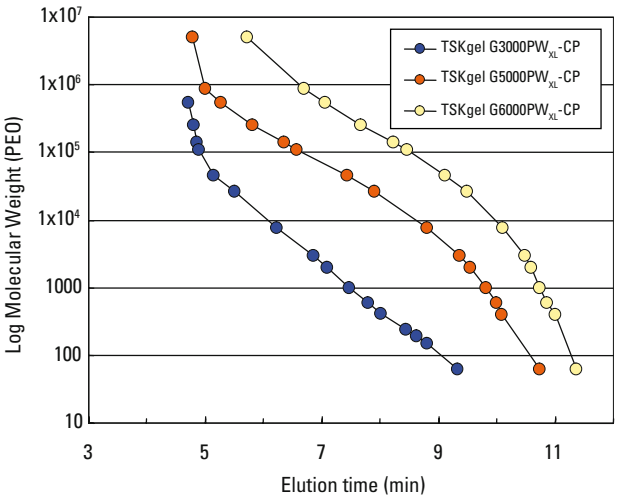
TSKgel PWXL-CP size exclusion columns were specifically developed for the analysis of water soluble cationic polymers. Three columns are available within the TSKgel PWXL-CP series, each with a different particle size, separation range and exclusion limit, allowing polymers within a wide molecular mass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

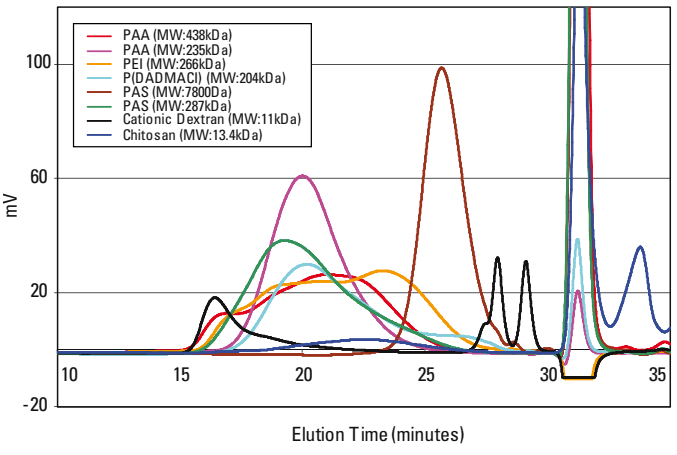
FIGURE 16



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.

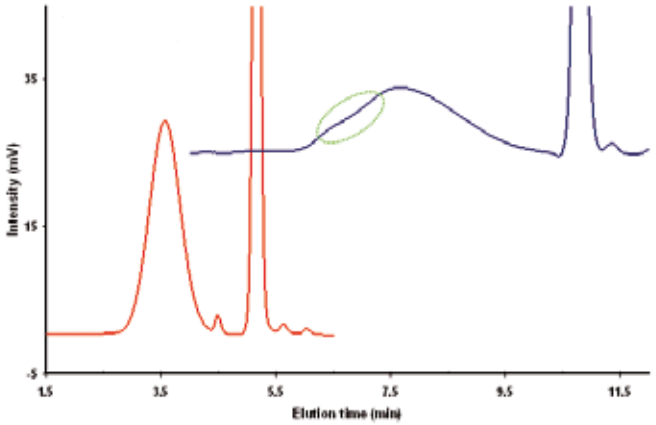
TSKgel SuperMultiporePW columns are packed with spherical mono-disperse polymethacrylate particles, each containing a wide range of pore sizes. They belong to the semi-micro type of SEC columns (6 mm ID, 15 cm length) providing high theoretical plate numbers at half of the length of a conventional SEC 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

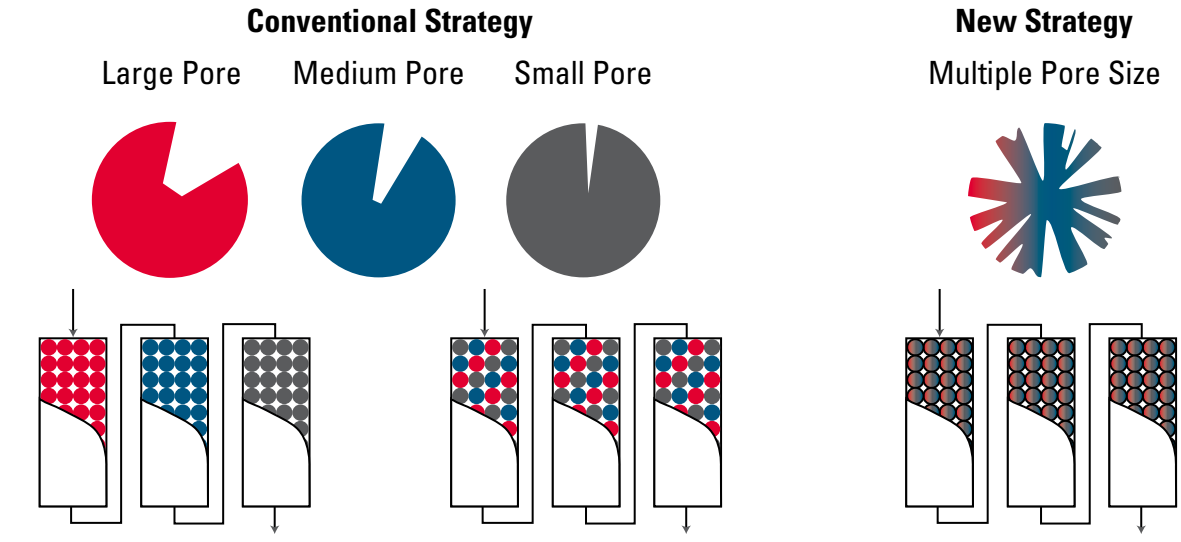


ANALYSIS OF POLYVINYLPYRROLIDONE

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

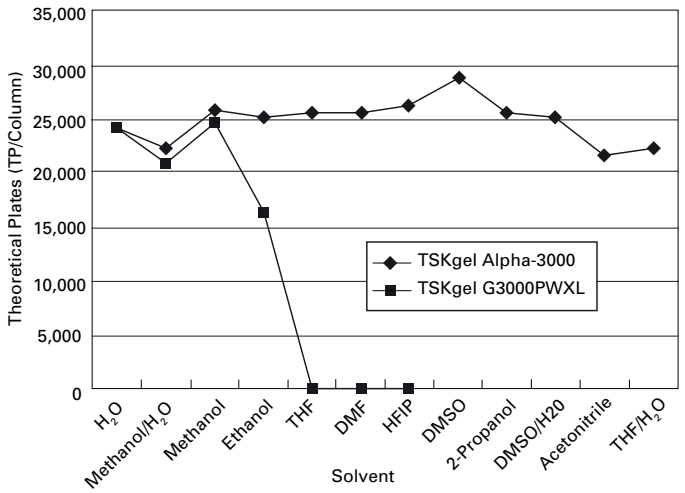
The TSKgel Alpha and SuperAW column series offer a new alternative for performing SEC. The columns are packed with a hydrophilic, highly crosslinked polymer which is compatible to a wide range of solvents ranging from pure aqueous up to 100 % organic mobile phases (see Figure 20). Both series consist 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

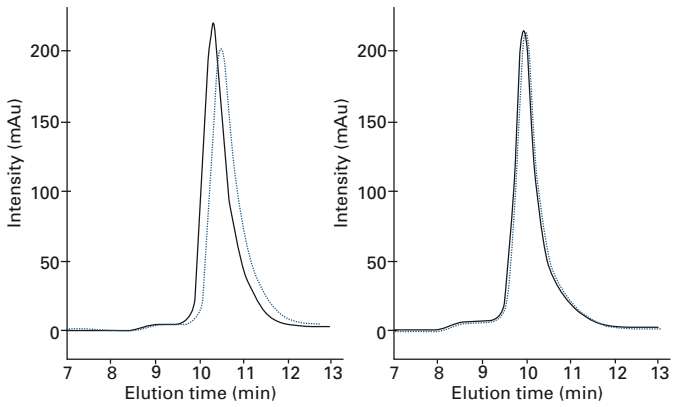
SAMPLE LOAD

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.