TOSOH BIOSCIENCE

Separations Business Unit

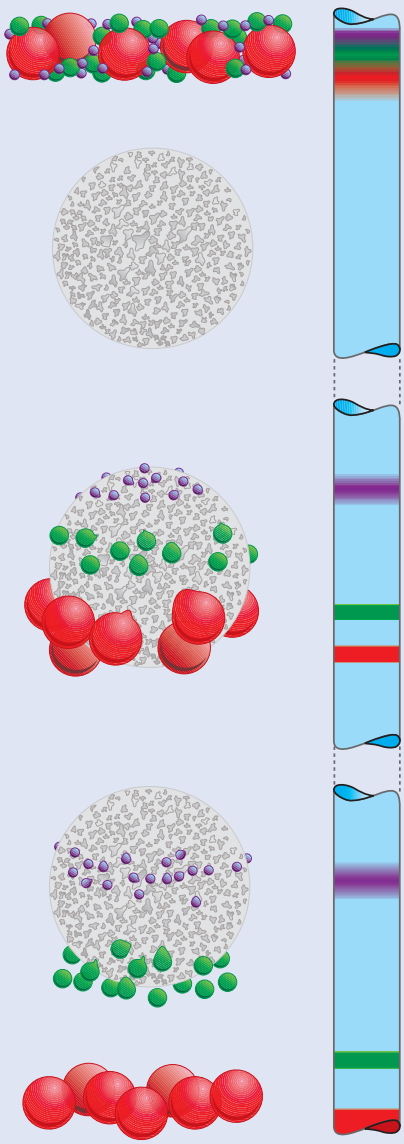
Size Exclusion with TSKgel® SuperSW

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

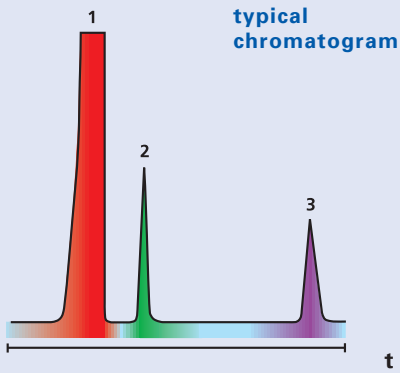


Sample application

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

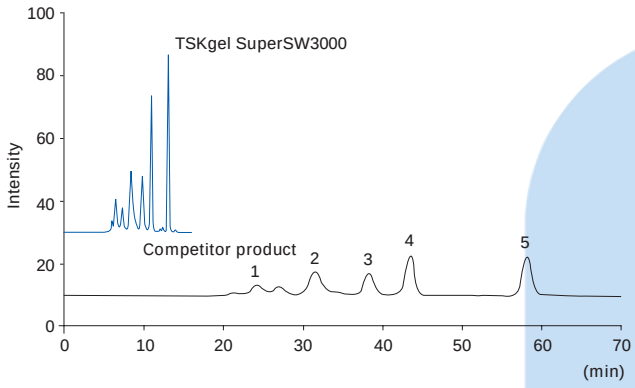


Figure 1
Column: A. TSKgel SuperSW3000, 2 mm ID x 30 cm L;
B. Competitor product, 3.2 mm ID x 30 cm L
Sample: 0.2 µL, 1. thyroglobulin, 1.0 mg/mL; 2. γ-globulin, 2.0 mg/mL;
3. ovalbumin, 2.0 mg/mL; 4. ribonuclease A, 3.0 mg/mL;
5. *p*-aminobenzoic acid, 0.02 mg/mL
Flow rate: A. 65 µL/min N = 30.000;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

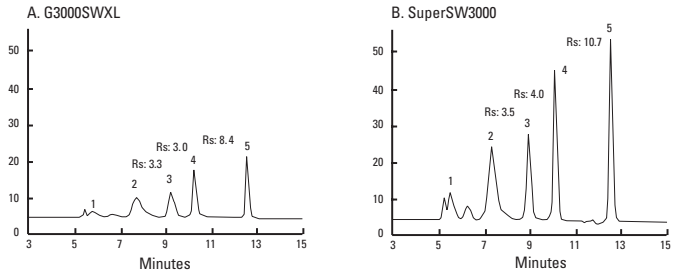


Figure 2
Column: A.TSKgel G3000SWXL, 7.8 mm ID x 30 cm L;
B. TSKgel Super SW3000, 4.6 mm ID x 30 cm L
Sample: 5 µL of a mixture of 1. thyroglobulin, 0.5 mg/mL (660,000 Da); 2. γ-globulin, 1.0 mg/mL; (150,000 Da); 3. ovalbumin, 1.0 mg/mL (43,000 Da); 4. ribonuclease A, 1.5 mg/mL (12,600 Da); 5. *p*-aminobenzoic 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

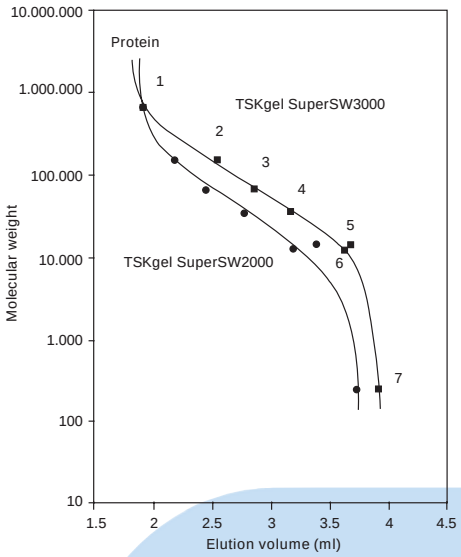


Figure 3
Column: TSKgel SuperSW Series, 4.6 mm ID X 30 cm L
Sample: Standard proteins (5 µL, 0.1g/L each); 1. thyroglobulin 2. γ-globulin 3. bovine serum albumin 4. ß-lactoglobulin 5. lysozyme 6. cytochrome C 7. glycine tetramer
Eluent: 0.2 mol/L phosphate buffer (pH 6.7)
Flow rate: 0.35 mL/min
Detection: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

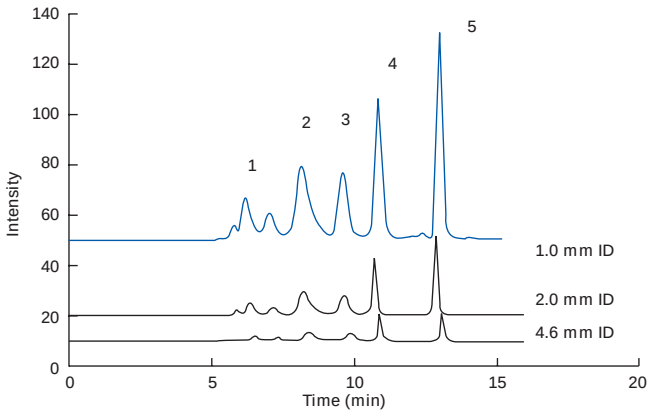


Figure 4
Column: TSKgel SuperSW3000, 1.0, 2.0, 4.6 mm ID x 30 cm L
Sample: 1. tyroglobulin (1.0 g/L), 2. γ-globulin (2.0 g/L), 3. ovalbumin (2.0 g/L), 4. ribonuclease A (3.0 g/L), 5. *p*-aminobenzoic acid (0.02 g/L)
Eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3
Flow rate: 16 µL/min (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)

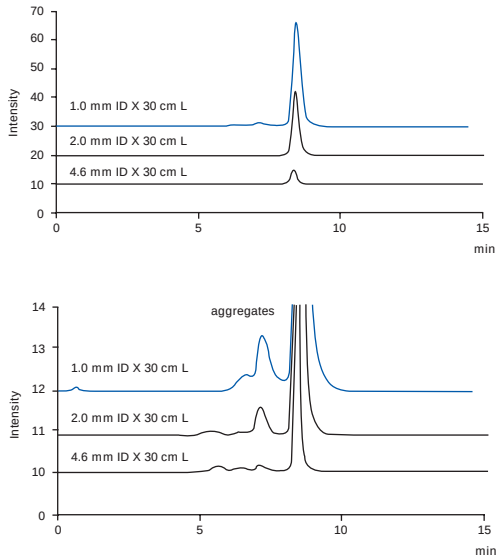


Figure 5

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

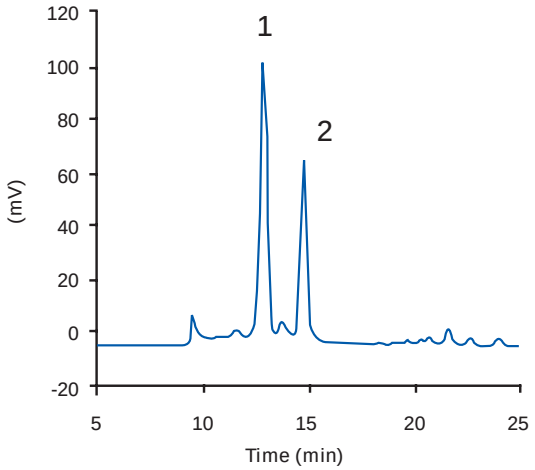


Figure 6

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

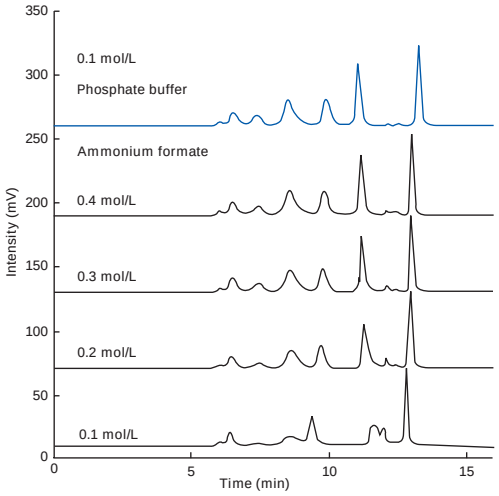


Figure 7 Same conditions and sample as in figure 4.

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 ≤ 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)

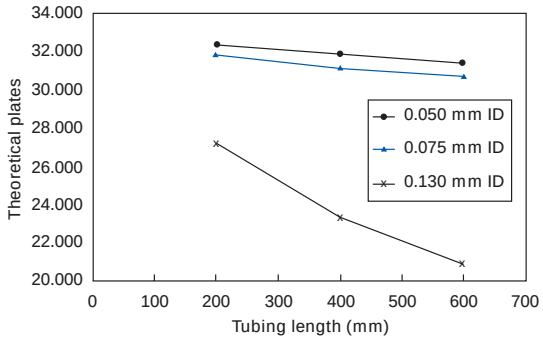


Figure 8
Column: TSKgel SuperSW3000 1.0 mm ID x 30 cm L
Eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05 % NaN_3
Flow rate: 16 µL/min
Inj.volume: 0.2 µL
Temp.: 25 °C
Detection.: UV @ 280 nm
Sample: p-Aminobenzoic acid (20mg/L)
Tubing:

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

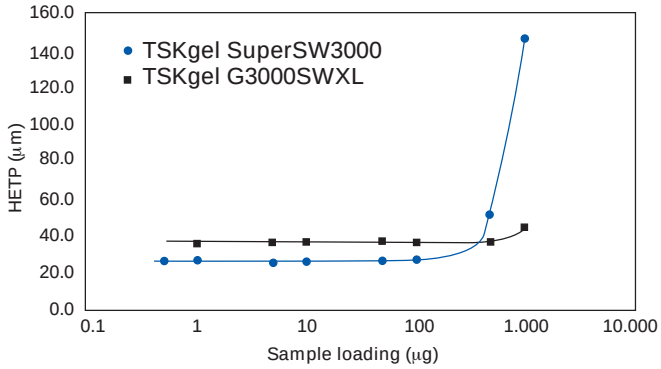


Figure 9
Column: TSKgel SuperSW series, 4.6 mm ID x 30 cm L, TSKgel SWXL series, 7.8 mm ID x 30 cm L
Sample: Bovine serum albumin
Eluent: 0.2 mol/L phosphate buffer, pH 6.7
Flow rate: 0.35 mL/min (TSK-GEL SuperSW series)
1.00 ml/min (TSK-GEL SWXL series)
Temp.: 25 °C
Detection.: UV @ 280 nm, micro flow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

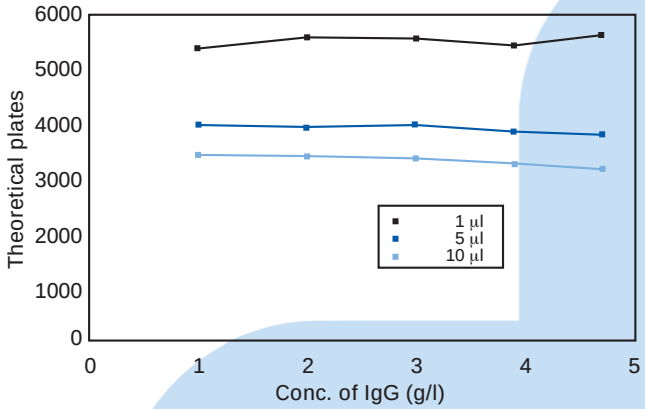


Figure 10
Column: TSKgel SuperSW3000 2.0 mm ID x 30 cm L
Sample: IgG (mouse, mAb) 1, 5, 10 µl
Eluent: 0.1 M phosphate buffer + 0.1 M Na_2SO_4 + 0.05 % NaN_3 (pH 6.7)
Flow rate: 0.065 mL/min
Temp.: 25 °C
Detection.: UV @ 280 nm

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

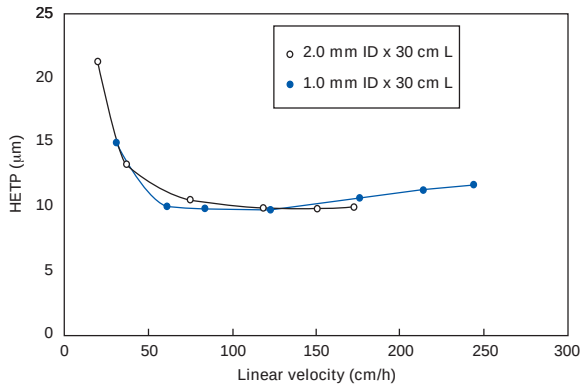


Figure 11
Column: TSKgel SuperSW3000, 1.0 mm ID x 30 cm L
TSKgel SuperSW3000, 2.0 mm ID x 30 cm L
Sample: *p*-Aminobenzoic acid (20 mg/L)
Eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3
Detection.: UV @ 280 nm
Temp.: 25 °C
Inj.volume: 0.2 µL (1.0 mm ID), 1.0 µL (2.0 mm ID)

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

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