

# TSK-GEL Super SW Series

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**TOSOH BIOSCIENCE LLC**  
*Separations Business Unit*

TOSOH

## 1. Introduction

High-performance liquid chromatography (HPLC) is widely used as a separation/purification method in the field of biopolymers due to its speed, ease of use and sensitivity. In particular, separations based on molecular size named size-exclusion chromatography (SEC), have been used in protein separation/purification as the technique of choice because of its effectiveness and non denaturing mobile phase conditions. While soft packing materials with reticulate structure such as dextran, agarose, etc. were employed as packing materials for early SEC, silica-type packing materials with high strength also have come to be employed for SEC in HPLC.

Our TSK-GEL SW series is a group of silica-type SEC packing materials with pore size distribution suited to protein separation, and is used throughout the world for its excellent resolution.

Speed and high resolution continue to be demanded in the field of HPLC. Most recently, demand for high sensitivity that is applicable to trace analysis is on the increase as sample size becomes limited and/or lower in concentration. In other HPLC separation modes including reverse phase chromatography (RPC), normal phase chromatography (NP) and ion exchange chromatography (IEX), semi-micro columns which are applicable to trace analysis have already been commercialized. Demand for high sensitivity, high resolution hplc columns also grows in the field of SEC along with trace analysis applications. This report describes the features, basic properties, and applications of TSK-GEL Super-SW series, in which particles have been made smaller than the conventional TSK-GEL SW series and the column has been downsized to provide high sensitivity and high resolution.

## 2. Features

Table-1 shows the specifications of TSK-GEL SuperSW and SW<sub>XL</sub> series. As noted, the smaller particle size of the TSK-GEL SuperSW provides approximately 1.5 times the number of theoretical plates than the conventional high performance TSK-GEL SW<sub>XL</sub> series. Additionally, in Table-2, separation ranges of TSK-GEL SuperSW series for polyethylene glycol (PEG), dextran, and protein are shown. In Figures-1 and -2, calibration curves of TSK-GEL SuperSW series for standard polyethylene glycols (PEG) and standard proteins are shown, respectively. Since TSK-GEL SuperSW series has the same calibration curve as the conventional TSK-GEL SW<sub>XL</sub> series with equivalent grade, it has the same molecular weight separation range. In general, TSKgel SuperSW2000 is suited for separation of proteins with molecular weight of 70,000 or smaller, and TSKgel SuperSW3000 is suited for separation of proteins with molecular weight of 70,000 to 300,000.

Figures-3 and -4 show the chromatograms of standard proteins on TSK-GEL SuperSW series and the conventional TSK-GEL SW<sub>XL</sub> series. A UV detector with a micro flow cell was used. Due to the smaller 4.6mmID bore of the TSK-GEL SuperSW columns, relative to the 7.8mmID TSK-GEL SW<sub>XL</sub>, it is evident that increased peak heights result. In Table-3, resolution (Rs) calculated from these chromatograms is shown. It is clear from the table that TSK-GEL SuperSW series has approximately 1.2 times better resolution compared to TSK-GEL SW<sub>XL</sub> series.

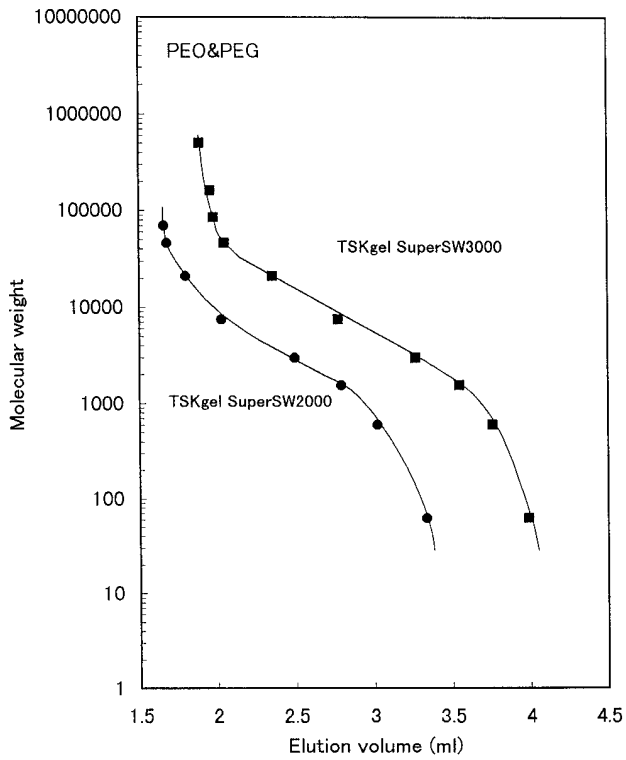
Figures-5 and -6 show comparison of analysis time under the condition that resolution is nearly equal between TSK-GEL SW<sub>XL</sub> series and TSK-GEL SuperSW series. The higher efficiency of TSK-GEL SuperSW series was used to decrease the separation time by 50% relative to SW<sub>XL</sub> without sacrificing resolution.

**Table-1 Specifications of TSK-GEL SuperSW Series and TSK-GEL SW<sub>XL</sub> Series**

	Particle size (μm)	Column size	Guaranteed theoretical plates
TSKgel SuperSW2000	4	4.6mm I.D. × 30cm	30,000
TSKgel SuperSW3000	4	4.6mm I.D. × 30cm	30,000
TSKgel G2000SW <sub>XL</sub>	5	7.8mm I.D. × 30cm	20,000
TSKgel G3000SW <sub>XL</sub>	5	7.8mm I.D. × 30cm	20,000

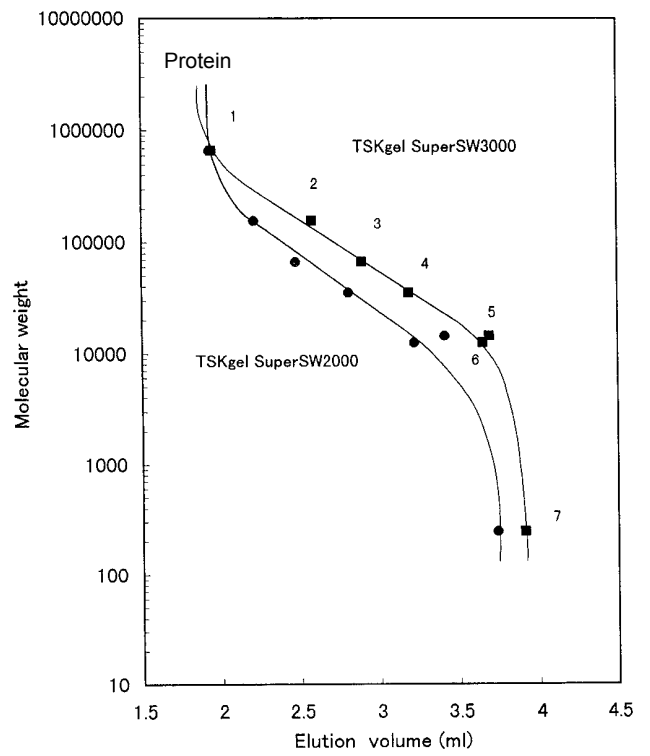
**Table-2 Molecular Weight Separation Range of TSK-GEL SuperSW Series**

	Molecular weight separation range	
	SuperSW2000	SuperSW3000
Polyethylene glycol	500- 15,000	1,000- 35,000
Dextran	1,000- 30,000	2,000- 70,000
Protein	5,000-100,000	10,000-500,000



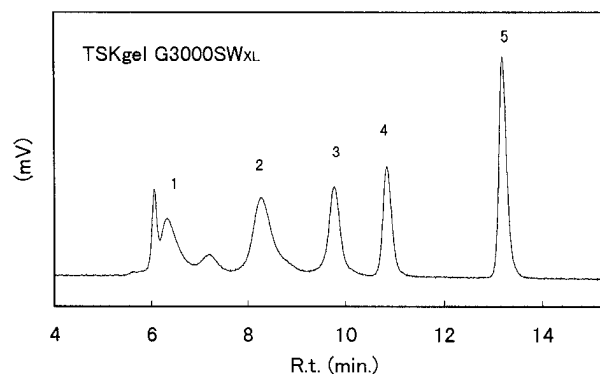
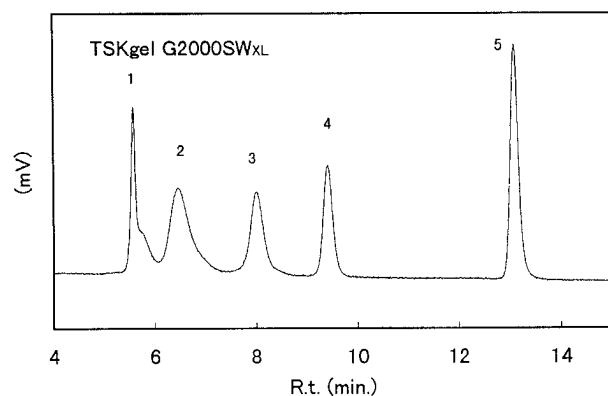
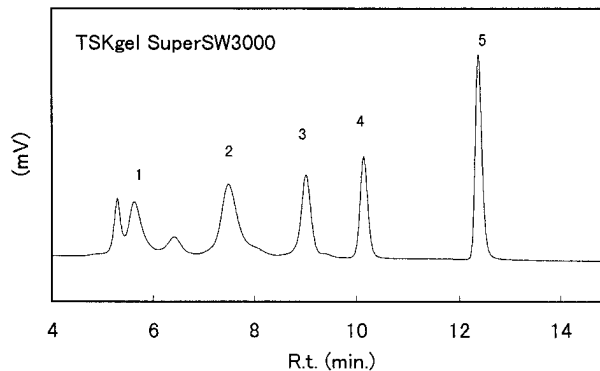
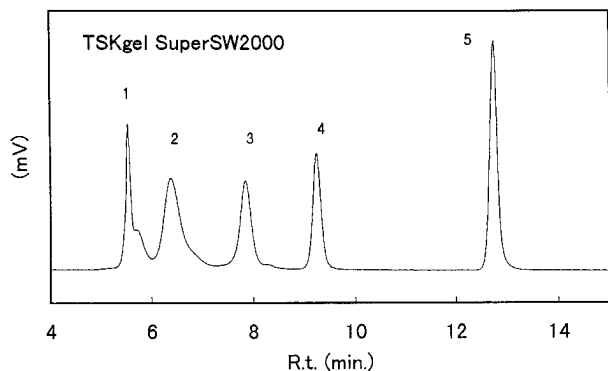
**Figure-1 PEO&PEG Calibration Curves for TSK-GEL SuperSW Series**

Column: TSK-GEL SuperSW Series  
(4.6mm I.D. × 30cm)  
Eluent: 0.05% sodium azide aqueous solution  
Flow rate: 0.35mL/min  
Temperature: 25°C  
Detection: Refractive index detector  
Samples: PEO, PEG (5μL)



**Figure-2 Protein Calibration Curves for TSK-GEL SuperSW Series**

Column: TSK-GEL SuperSW Series  
(4.6mm I.D. × 30cm)  
Eluent: 0.2mol/L phosphate buffer (pH6.7)  
Flow rate: 0.35mL/min  
Detection: UV (280nm)  
Samples: Standard proteins (5μL, 0.1g/L each)  
1. Thyroglobulin  
2. γ-globulin  
3. Bovine serum albumin  
4. β-lactoglobulin  
5. Lysozyme  
6. Cytochrome C  
7. Glycine tetramer



**Figure-3 Comparison between TSKgel SuperSW2000 and TSKgel G2000SW<sub>xL</sub>**

Column: TSKgel SuperSW2000 (4.6mm I.D. × 30cm)  
 TSKgel G2000SW<sub>xL</sub> (7.8mm I.D. × 30cm)  
 Eluent: 0.2mol/L phosphate buffer (pH6.7)  
 Flow rate: 0.35mL/min (TSKgel SuperSW2000)  
 1.00mL/min (TSKgel G2000SW<sub>xL</sub>)  
 Detection: UV (280nm), micro flow cell  
 Samples: Standard proteins (5μL)  
 1. Thyroglobulin (0.5g/L)  
 2. γ-globulin (1g/L)  
 3. Ovalbumin (1g/L)  
 4. Ribonuclease A (1g/L)  
 5. p-aminobenzoic acid (0.01g/L)

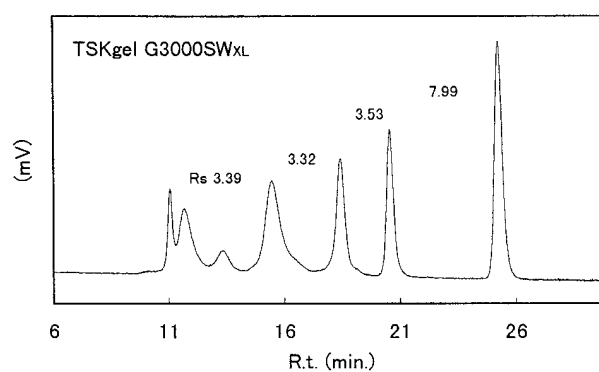
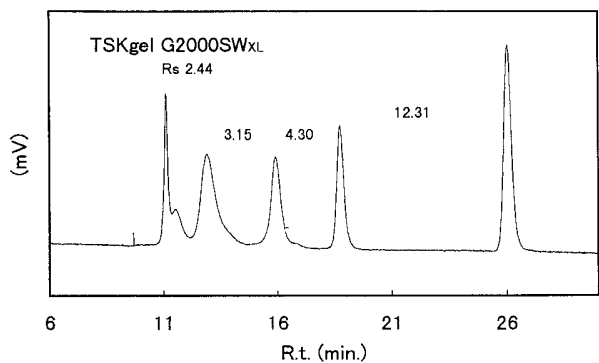
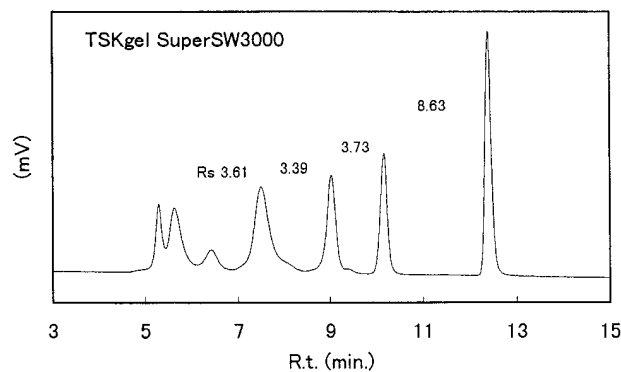
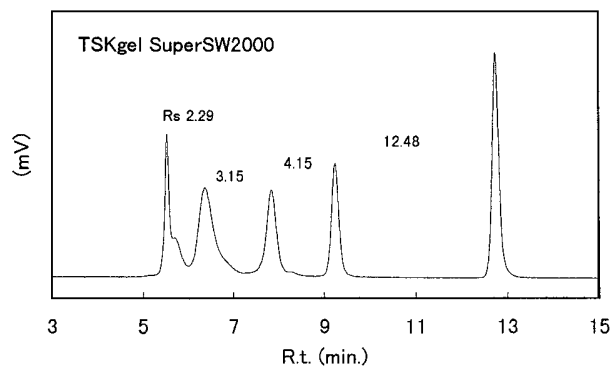
**Figure-4 Comparison between TSKgel SuperSW3000 and TSKgel G3000SW<sub>xL</sub>**

Column: TSKgel SuperSW3000 (4.6mm I.D. × 30cm)  
 TSKgel G3000SW<sub>xL</sub> (7.8mm I.D. × 30cm)  
 \* Separation conditions are the same as Figure-3.

**Table-3 Comparison of Resolution between TSK-GEL SuperSW Series and TSK-GEL SW<sub>xL</sub> Series**

	Resolution (Rs)*			
	SuperSW2000	G2000SW <sub>xL</sub>	SuperSW3000	G3000SW <sub>xL</sub>
Thyroglobulin	2.29	2.24	3.61	—
γ-globulin	3.15	2.85	3.39	2.79
Ovalbumin	4.15	3.55	3.73	2.94
Ribonuclease A	12.48	11.62	8.63	7.67
p-aminobenzoic acid				

\* UV detector with micro flow cell



**Figure-5 Comparison between TSKgel SuperSW2000 and TSKgel G2000SW<sub>XL</sub>**

Column: TSKgel SuperSW2000 (4.6mm I.D. × 30cm)  
 TSKgel G2000SW<sub>XL</sub> (7.8mm I.D. × 30cm)

Eluent: 0.2mol/L phosphate buffer (pH6.7)  
 Flow rate: 0.35mL/min (TSKgel SupperSW2000)  
 0.50mL/min (TSKgel G2000SW<sub>XL</sub>)

Detection: UV (280nm), micro flow cell

Samples: Standard proteins (5 $\mu$ L)  
 1. Thyroglobulin (0.5g/L)  
 2.  $\gamma$ -globulin (1g/L)  
 3. Ovalbumin (1g/L)  
 4. Ribonuclease A (1g/L)  
 5. p-aminobenzoic acid (0.01g/L)

**Figure-6 Comparison between TSKgel SuperSW3000 and TSKgel G3000SW<sub>XL</sub>**

Column: TSKgel SuperSW3000 (4.6mm I.D. × 30cm)  
 TSKgel G3000SW<sub>XL</sub> (7.8mm I.D. × 30cm)

\* Separation conditions are the same as Figure-5.

### 3. Basic Properties

#### 3-1 Optimization of Equipment

Although TSK-GEL SuperSW series is a high-performance SEC column with high resolution and high sensitivity, it is necessary to optimize the equipment especially the detector cell and tubing in order to maximize column performance. In this section, optimization of equipment is described.

In columns with small column volume such as TSK-GEL SuperSW series, the void volume of equipment has a large influence on column efficiency. The following 3 components should be examined to ensure the total void volume of the system is minimized.

- Void volume of tubing
- Cell volume of detector
- Void volume in injection unit

In TSK-GEL SuperSW series, it is necessary to suppress solute dispersion in these components to achieve the highest efficiency.

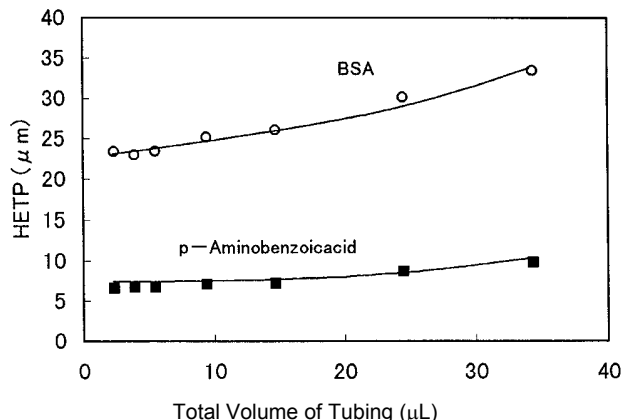
##### 3-1-1 Void Volume of Tubing

In Figure-7, the effect of volume of tubing between injector/column and column/detector on column efficiency is shown. As the volume of tubing increases, dispersion of solute within the tubing increases and deteriorates the column efficiency. With TSK-GEL SuperSW series, column efficiency begins to deteriorate when volume of tubing exceeds 10 $\mu$ L (0.1mm I.D.  $\times$  150cm). Therefore, we recommend that 0.1mm I.D.  $\times$  100cm or shorter tubing should be used between injector/column and column/detector with TSK-GEL SuperSW series. A set of two 0.1mm I.D.  $\times$  40cm pipes, "connection pipe set, type L," (including two pipes, product No. 018186) is available from our lineup.

##### 3-1-2 Cell Volume of Detector

Table-4 shows the effect of detector cell on efficiency. Although column efficiency deteriorates somewhat for low dead volume type cells (standard cells from which heat sink has been removed) the rate of deterioration can be suppressed within 5% relative to a micro flow cell designed with minimal dead volume. However, since a standard cell with heat sink contributes approximately 30 $\mu$ L of void volume. Column efficiency deteriorates of approximately 30% relative to a micro flow cell.

On the other hand, sensitivity is proportional to the length of light path in the cell. Figure-8 shows the chromatograms when a micro flow cell or low dead volume type cell is used. With a low dead volume type cell with light path length of 10mm, 2.5 times sensitivity is obtained compared to micro flow cell with light path length of 4mm. In TSK-GEL SuperSW series, it is necessary that micro flow cell should be used when high resolution is required, and low dead volume type cell should be used when high sensitivity is required. Furthermore, sensitivity of TSK-GEL SuperSW series is improved by approximately 3 times compared to TSK-GEL SW<sub>XL</sub> series even when a normal standard cell is used.



**Figure-7 Effect of Total Volume of Tubing on HETP**

Column: TSKgel SuperSW3000 (4.6mm I.D.  $\times$  30cm)  
 Eluent: 0.1mol/L phosphate buffer + 0.1mol/L sodium sulfate + 0.05% sodium azide (pH 6.7)  
 Flow rate: 0.35mL/min  
 Detection: UV (280nm)  
 Sample: Bovine serum albumin, p-aminobenzoic acid

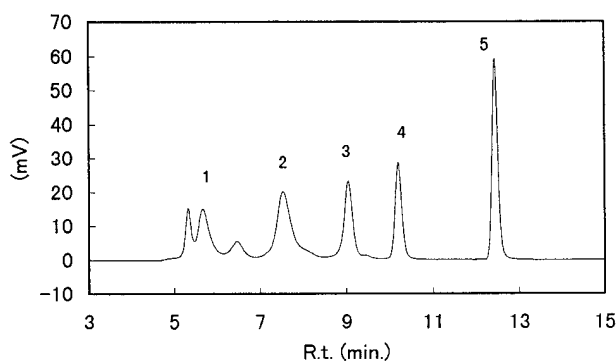
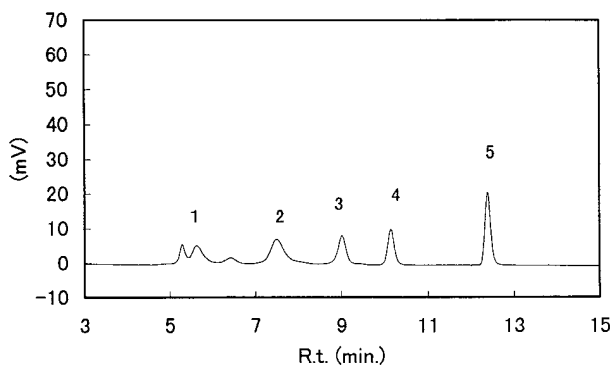
- The volume of tubing is the total volume between injector/column and column/detector.

**Table-4 Effect of Cell Volume on Column Efficiency**

Cell volume	Theoretical plates of column (rate of deterioration in theoretical plates)
2 $\mu$ L (micro flow cell)	41,199 (0%)
10 $\mu$ L (low dead volume type cell)	40,189 (2.5%)
30 $\mu$ L (standard cell)	30,855 (25%)

Low dead volume type: Standard cells from which heat sink has been removed (1mmID tubing is used.)

Column: TSKgel SuperSW3000  
 Eluent: 0.2mol/L phosphate buffer (pH 6.7), Sample: p-aminobenzoic acid

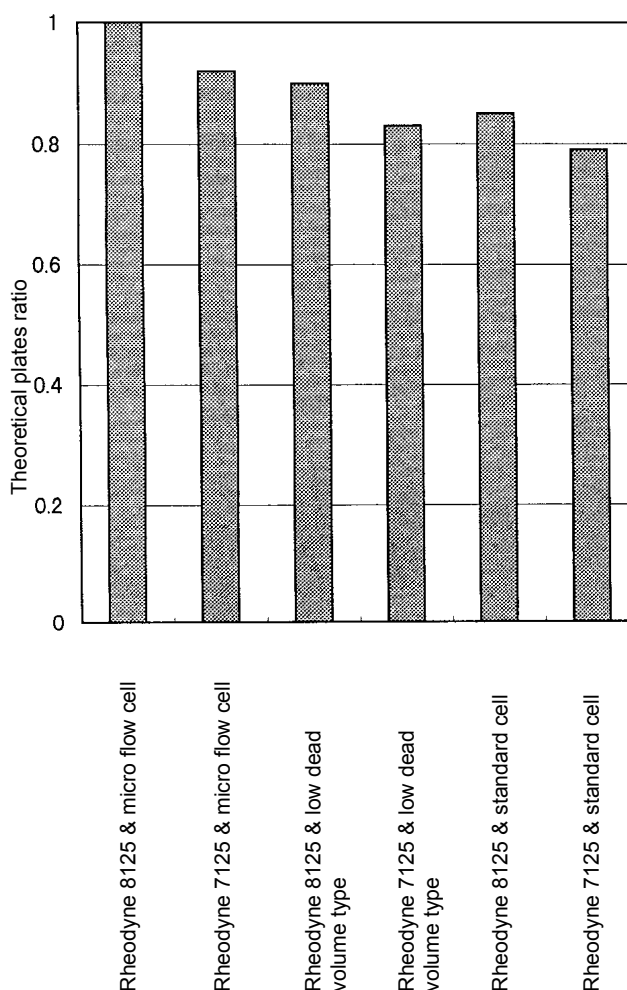


**Figure-8 Comparison of Peak Heights between the Different Cell**

Column: TSKgel SuperSW3000 (4.6mm I.D. × 30cm)  
 \* Separation conditions are the same as Figure-3.

### 3-1-3 Injector

Figure-9 shows the effect of injector and detector cell. The effect of injector and cell was examined, with the column efficiency when a low-diffusion type injector (Rheodyne 8125) and micro flow cell are used. This combination is expected to have the smallest peak broadening when set to 100%. It is clear that dispersion of solute inside the injector was large with the general-purpose injector (Rheodyne 7125). When a micro flow cell is used, column efficiency deteriorates by approximately 10%. In the case of combining general-purpose injector and standard cell, the column efficiency deteriorates by 20% or more. In order to exert the TSK-GEL SuperSW column performance sufficiently, it is desired that low-diffusion type injector is used. Furthermore, when an auto-sampler is required, the use of an auto-sampler capable of trace injection mode is recommended.



**Figure-9 Effect of Injector and Detector Cell on Column Efficiency**

Column: TSKgel SuperSW3000 (4.6mm I.D. × 30cm)  
 Eluent: 0.1mol/L phosphate buffer + 0.1mol/L sodium sulfate + 0.05% sodium azide (pH 6.7)  
 Flow rate: 0.35mL/min  
 Detection: UV (280nm)  
 Sample: p-aminobenzoic acid (5μL)

### 3-2 Sensitivity

Figures-10 and -11 show chromatograms to compare the peak height of standard proteins on TSK-GEL SuperSW series and TSK-GEL SW<sub>XL</sub> series. It is evident that TSK-GEL SuperSW series can yield peak height approximately 4 times that of TSK-GEL SW<sub>XL</sub> series due to downsizing in column and increased theoretical plates.

Table-5 shows the limits of detection for major proteins. Although limit of detection varies depending on sample, separation conditions, detection wavelength and light path length of the cell, it is approximately 1/2 – 1/3 of the SW<sub>XL</sub> series when a cell with a light path length of 10mm (low dead volume type) is used with TSK-GEL SuperSW series. The increased sensitivity allows for analysis of nanogram sample amounts. TSK-GEL SuperSW series can be recommended as a SEC column that is suited for trace analysis.

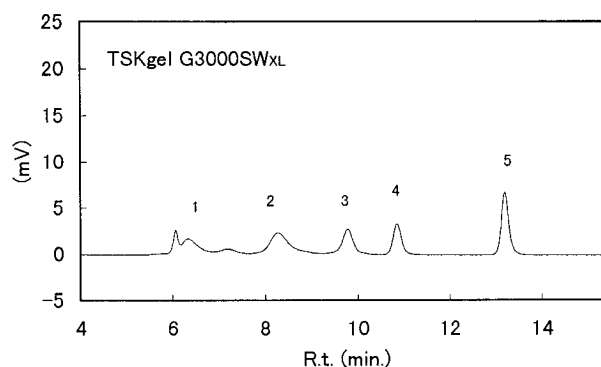
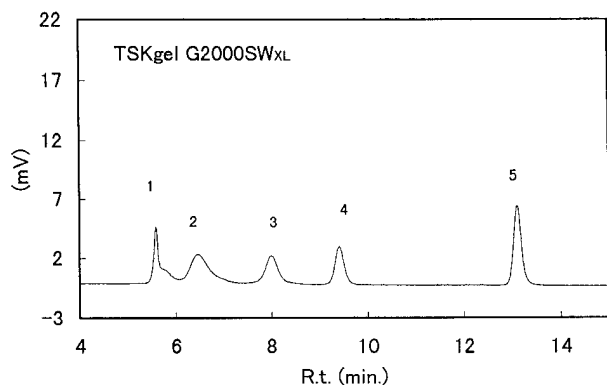
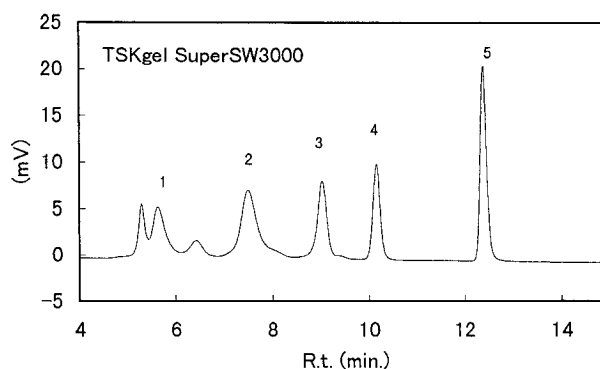
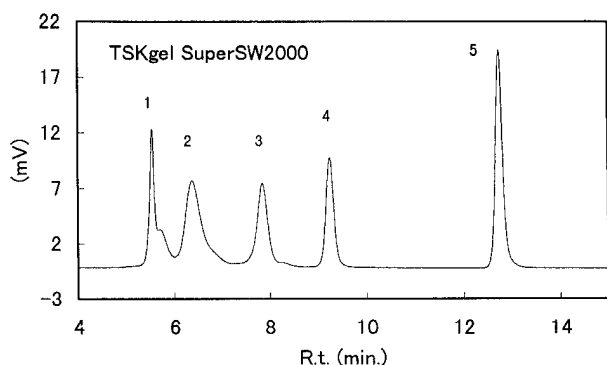
**Table-5 Limit of Detection for Proteins (S/N = 3)**

	SuperSW3000		G3000SW <sub>XL</sub>
	Standard cell (low dead volume type) Light path length 10mm	Micro flow cell 4mm	Standard cell (low dead volume type) 10mm
Thyroglobulin	70ng	300ng	200ng
γ-globulin	50ng	100ng	100ng
Bovine serum albumin	70ng	300ng	200ng
Ovalbumin	50ng	100ng	100ng
Myoglobin	15ng	50ng	30ng

Column: TSKgel SuperSW3000 (4.6mm I.D. × 30cm)

Eluent: 0.2mol/L phosphate buffer (pH 6.7)

Detection: UV (280nm)



**Figure-10 Comparison of Sensitivity between TSKgel SuperSW2000 and TSKgel G2000SW<sub>XL</sub>**

Column: TSKgel SuperSW2000 (4.6mm I.D. × 30cm)  
TSKgel G2000SW<sub>XL</sub> (7.8mm I.D. × 30cm)

\* Separation conditions are the same as Figure-3.

**Figure-11 Comparison of Sensitivity between TSKgel SuperSW3000 and TSKgel G3000SW<sub>XL</sub>**

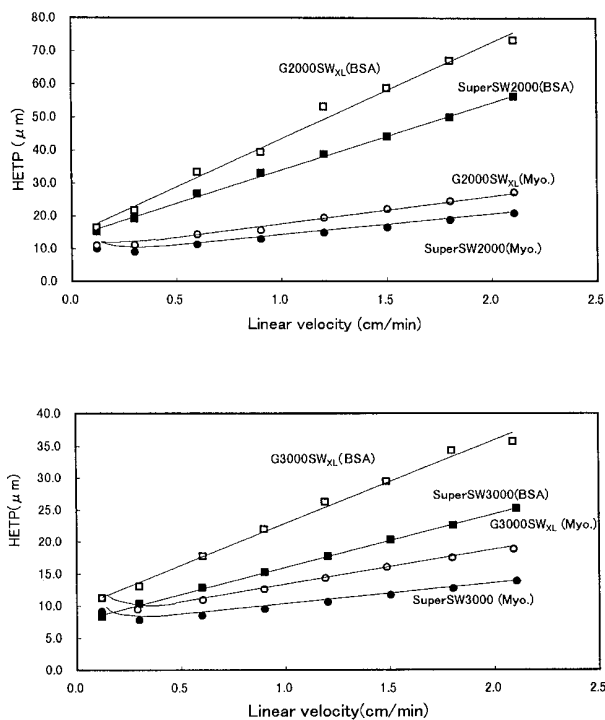
Column: TSKgel SuperSW3000 (4.6mm I.D. × 30cm)  
TSKgel G3000SW<sub>XL</sub> (7.8mm I.D. × 30cm)

\* Separation conditions are the same as Figure-3.

### 3-3 Flow Rate Dependence of Height Equivalent to a Theoretical Plate (HETP)

The effect of flow rate on height equivalent to a theoretical plate (HETP) depends on particle size of packing materials, sample molecular size, eluent viscosity, etc. A typical example of HETP's flow rate dependency on TSK-GEL SuperSW series and TSK-GEL SW<sub>XL</sub> series using bovine serum albumin (BSA) and myoglobin is shown in Figure-12. It is clear that TSK-GEL SuperSW series has small HETP throughout the full flow rate range and small flow rate dependence compared to TSK-GEL SW<sub>XL</sub> series since the particle size is small. The appropriate flow rate for TSK-GEL SuperSW series is 0.1 – 0.35mL/min.

In Figure-13, chromatograms of commercial molecular weight markers at various flow rates are shown. Table-6 shows the resolution (Rs) calculated from the chromatograms. When flow rate is lower, separation of high polymer protein is improved, and resolution calculated at flow rate of 0.35mL/min is twice of that of 0.05mL/min. Although TSK-GEL SuperSW series have smaller flow rate dependency than conventional ones, please use it at lower flow rate when higher resolution is required.

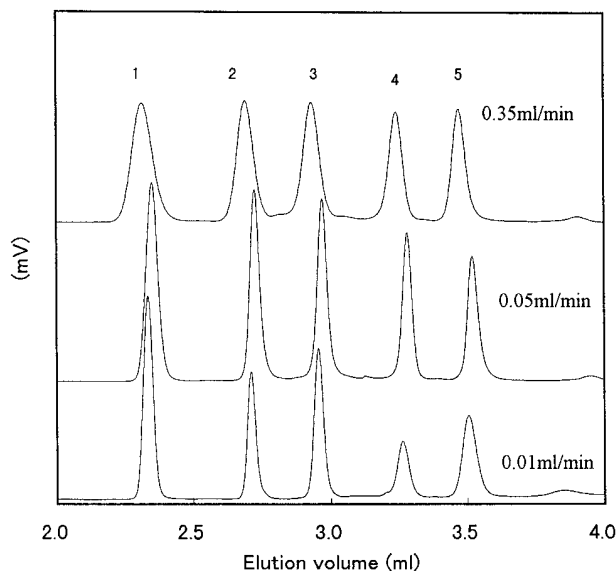


**Figure-12 Relationship between Flow Rate and HETP in TSK-GEL SuperSW Series and TSK-GEL SW<sub>XL</sub> Series**

Column: TSK-GEL SuperSW series (4.6mm I.D. × 30cm)  
 TSK-GEL SW<sub>XL</sub> series (7.8mm I.D. × 30cm)  
 Eluent: 0.2mol/L phosphate buffer (pH 6.7)  
 Detection: UV (280nm), micro flow cell  
 Samples: Standard proteins (5μL)  
 Bovine serum albumin (1g/L)  
 Myoglobin (1g/L)

**Table-6 Relationship between Flow Rate and Resolution**

	Resolution (Rs)		
	0.35mL/min	0.05mL/min	0.01mL/min
Glutamate dehydrogenase	2.91	5.10	6.13
Lactate dehydrogenase	2.13	3.78	4.12
Enolase	2.97	4.79	4.75
Adenylate kinase	2.44	3.50	3.18
Cytochrome c			

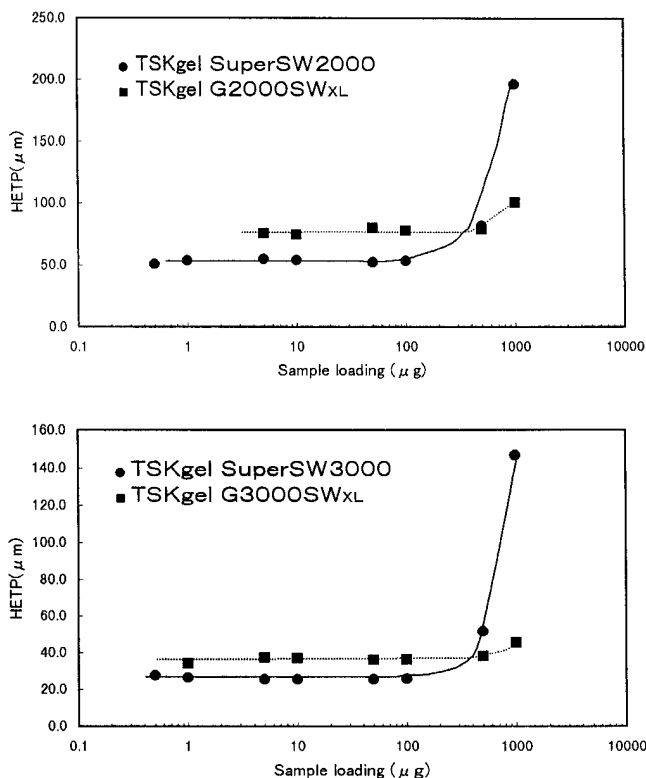


**Figure-13 Effect of Flow Rate on Separation**

Column: TSKgel SuperSW3000 (4.6mm I.D. × 30cm)  
 Eluent: 0.1mol/L phosphate buffer + 0.1mol/L sodium sulfate + 0.05% sodium azide (pH 6.7)  
 Flow rate: 0.01, 0.05, 0.35mL/min  
 Temperature: 25°C  
 Detection: UV (280nm), micro flow cell  
 Samples: 1. Glutamate dehydrogenase  
 2. Lactate dehydrogenase  
 3. Enolase  
 4. Adenylate kinase  
 5. Cytochrome C

### 3-4 Sample Load

Figure-14 shows the effect of sample load on HETP under a constant injection volume. Although HETP is small in TSK-GEL SuperSW series than in TSK-GEL SW<sub>XL</sub> series, it is obvious that it increases drastically at load of 100 $\mu$ g or larger. It is also apparent that TSK-GEL SuperSW series should be used under load of 100 $\mu$ g or smaller.

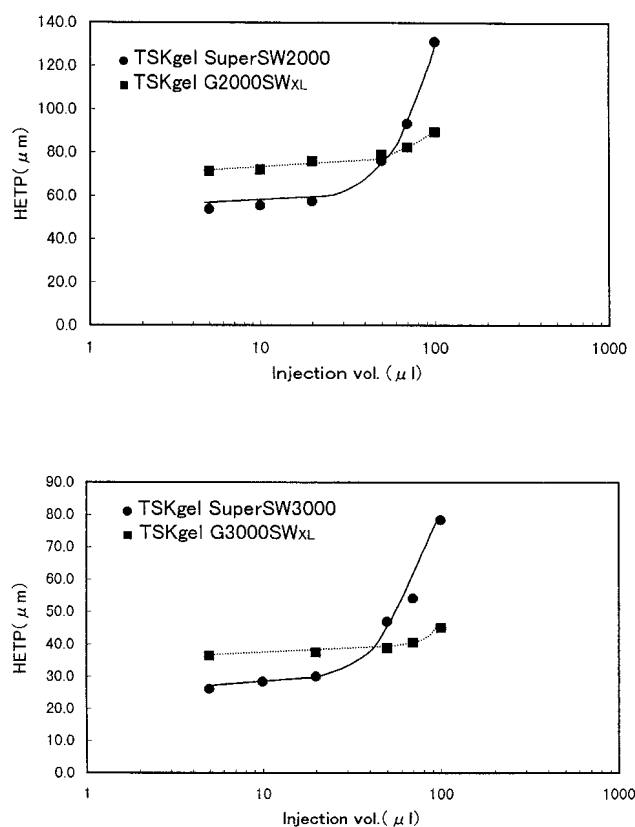


**Figure-14 Relationship between Sample Load and HETP in TSK-GEL SuperSW Series and TSK-GEL SW<sub>XL</sub> Series**

Column: TSK-GEL SuperSW series (4.6mm I.D.  $\times$  30cm)  
 TSK-GEL SW<sub>XL</sub> series (7.8mm I.D.  $\times$  30cm)  
 Eluent: 0.2mol/L phosphate buffer (pH 6.7)  
 Flow rate: 0.35mL/min (TSK-GEL SuperSW series)  
 1.00mL/min (TSK-GEL SW<sub>XL</sub> series)  
 Detection: UV (280nm), micro flow cell  
 Sample: Bovine serum albumin (5 $\mu$ L)

In Figure-15, the effect of injection volume on HETP under a constant sample concentration is shown. It is obvious that the injection volume at which HETP starts changing is approximately 10 $\mu$ L for TSK-GEL SuperSW series, and that it is smaller than that of TSK-GEL SW<sub>XL</sub> series.

It is surmised that the desired sample load of TSK-GEL SuperSW series are 100 $\mu$ g or smaller as total amount 10 $\mu$ L or smaller as injection volume.



**Figure-15 Relationship between Injection Volume and HETP in TSK-GEL SuperSW Series and TSK-GEL SW<sub>XL</sub> Series**

Column: TSK-GEL SuperSW series (4.6mm I.D.  $\times$  30cm)  
 TSK-GEL SW<sub>XL</sub> series (7.8mm I.D.  $\times$  30cm)  
 Eluent: 0.2mol/L phosphate buffer (pH 6.7)  
 Flow rate: 0.35mL/min (TSK-GEL SuperSW series)  
 1.00mL/min (TSK-GEL SW<sub>XL</sub> series)  
 Detection: UV (280nm), micro flow cell  
 Sample: Bovine serum albumin (0.2g/L)

### 3-5 Recovery of Protein

Table-7 shows the recovery of protein at sample concentration of 20 $\mu$ g/mL (sample load 100ng). With TSK-GEL SW<sub>XL</sub> series, recovery of thyroglobulin at the sample load of 1 $\mu$ g was 70% level. In addition, recovery deteriorated with sample load of 1 $\mu$ g or smaller (see our separation report No.46). On the other hand, it was found that most protein was recovered quantitatively with TSK-GEL SuperSW series even under the sample load of 100ng. TSK-GEL SuperSW series is capable of obtaining high protein recovery even in trace analysis with sample load of 1 $\mu$ g or smaller.

While TSK-GEL SuperSW series has high recovery even with small sample concentration, sample might be adsorbed by HPLC system other than the column (tubing, etc.) in HPLC trace analysis. It is important that similar sample is injected several times before measurement so that the adsorption point within the system is inactivated in advance when trace analysis is performed.

**Table-7 Recovery of Protein**

	SuperSW2000	SuperSW3000
Thyroglobulin	86%	97%
$\gamma$ -globulin	90%	90%
Bovine serum albumin	99%	86%
Ovalbumin	97%	98%
Ribonuclease A	86%	87%
Myoglobin	93%	96%
Cytochrome C	85%	90%
Lysozyme	93%	89%

Eluent: 0.2mol/L phosphate buffer (pH 6.7)

Flow rate: 0.35mL/min

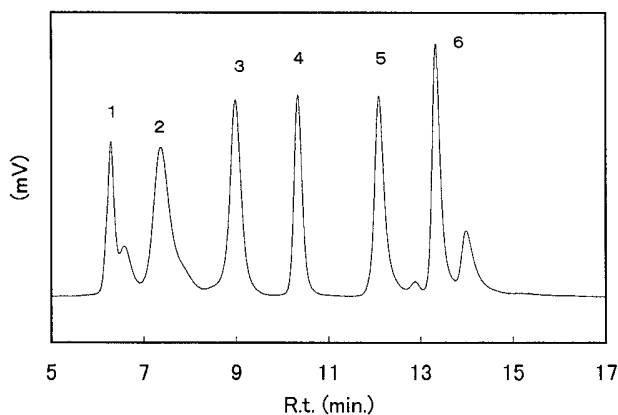
Detection: UV (280nm), micro flow cell

Sample: 100ng (20mg/L, 5 $\mu$ L)

### 4. Applications of TSK-GEL SuperSW Series

Figure-16 shows an example of peptide mixture separation on TSKgel SuperSW2000. Figures-17, -18 and -19 show chromatograms of commercial glutamic

acid-oxalacetic acid transaminase, mouse ascites monoclonal antibody (IgG1) and human serum on TSKgel SuperSW3000



**Figure-16 Separation of Mixture of Protein/peptide**

Column: TSKgel SuperSW2000 (4.6mm I.D.  $\times$  30cm)

Eluent: 0.2mol/L phosphate buffer (pH 6.7)

Flow rate: 0.35mL/min

Detection: UV (220nm), micro flow cell

Sample: Protein/peptide mixture (5 $\mu$ L)

1. Thyroglobulin (0.1g/L)

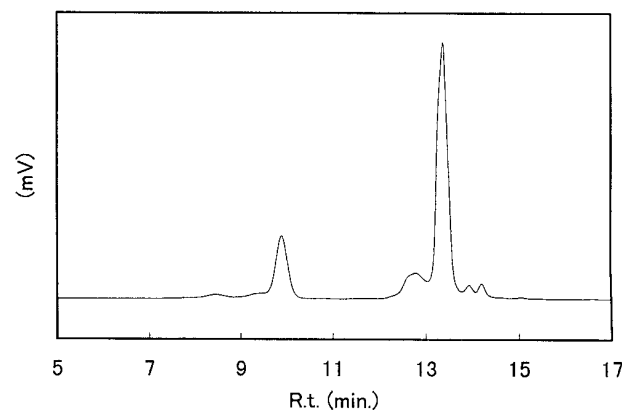
2.  $\gamma$ -globulin (0.2g/L)

3. Ovalbumin (0.2g/L)

4. Myoglobin (0.1g/L)

5. Insulin (0.1g/L)

6. Oxytocin (0.1g/L)



**Figure-17 Separation of Commercial Glutamic Acid-Oxalacetic Acid Transaminase**

Column: TSKgel SuperSW3000 (4.6mm I.D.  $\times$ 30cm)

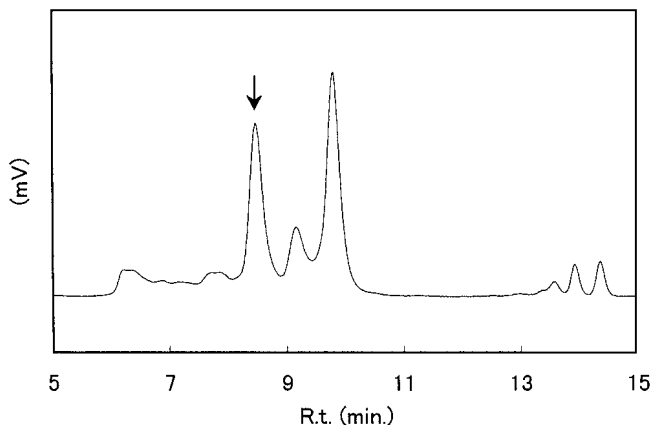
Eluent: 0.2mol/L phosphate buffer (pH 6.7)

Flow rate: 0.35mL/min

Detection: UV (280nm), micro flow cell

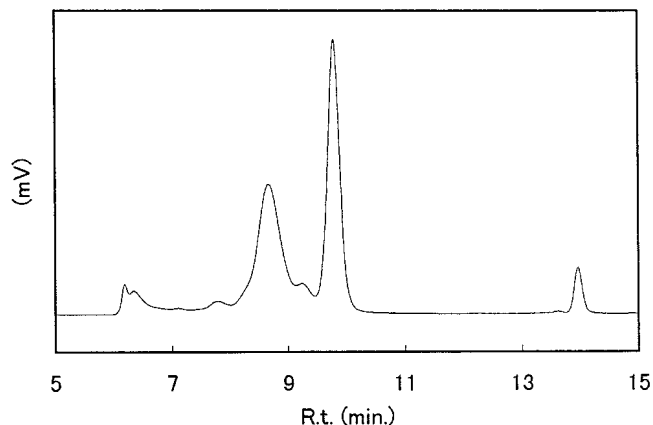
Sample: Glutamic acid-oxalacetic acid

transaminase (1g/L, 5 $\mu$ L)



**Figure-18 Separation of Mouse Ascites Monoclonal Antibody (IgG1)**

Column: TSKgel SuperSW3000 (4.6mm I.D. ×30cm)  
 Eluent: 0.2mol/L phosphate buffer (pH 6.7)  
 Flow rate: 0.35mL/min  
 Detection: UV (280nm), micro flow cell  
 Sample: Mouse ascites (5μL)



**Figure-19 Separation of Human Blood Serum**

Column: TSKgel SuperSW3000 (4.6mm I.D. ×30cm)  
 Eluent: 0.2mol/L phosphate buffer (pH 6.7)  
 Flow rate: 0.35mL/min  
 Detection: UV (280nm), micro flow cell  
 Sample: Human serum (5μL)

## 5. Conclusion

TSK-GEL SuperSW series is a group of columns in which particle size and column size of the conventional TSK-GEL SW<sub>XL</sub> series have been reduced at the same time to improve resolution and sensitivity. Resolution has been improved to 1.2 – 1.5 times, and sensitivity to approximately 2 – 3 times compared to the conventional TSK-GEL SW<sub>XL</sub> series. Furthermore, it maintains high recovery even for sample injection at a low concentration, and it is suited to trace analysis of biopolymers.

In order to exert the better performance of TSK-GEL SuperSW series, the use of equipment with minimized dead volume is recommended. Peak broadening outside the column is a major cause of deteriorated separation performance. Table-8 summarizes the cautions in using TSK-GEL SuperSW series.

**Table-8 Notes to Be Made in Using TSK-GEL Super SW Series**

\* Reduce peak broadening in tubing, detector, etc.

\* Take care of sample overloading.

\* Take care of flow rate of pumping system since its flow rate is low.

Tubing:

Use 0.1mm I.D. tubing. It is recommended that the total tubing length is 100cm or shorter.

Connection pipe set type L (product No. 018186: 0.1mm I.D. × 40cm, 2 pieces) available; connection surface (both ends) with fine-cut finishing

Sections requiring 0.1mm I.D. 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 0.1 – 0.35mL/min.

Injector:

Low-diffusion type injector (Rheodyne8125) is recommended.

Guard column:

Be sure to connect 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. However, theoretical plates will be approximately 80% of those with micro flow cell.)

Sample: Sample injection volume should be 1 – 10μL. Sample load should be 100μg or smaller.