TOSOH

SEPARATION REPORT NO. 95

TSKgel SuperSW SERIES COLUMNS

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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, known as 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.

TSKgel SW series columns are a group of silica-type SEC packing materials with pore size distribution suited for protein separation, and are used throughout the world for their 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 has also grown in the field of SEC, along with trace analysis applications.

This report describes the features, basic properties and applications of TSKgel SuperSW series columns, in which particles have been made smaller than the conventional TSKgel SW series columns, and the columns have been downsized to provide high sensitivity and high resolution.

2. Features

Table 1 shows the specifications of TSKgel SuperSW and SW_{XL} series columns. As noted, the smaller particle size of the TSKgel SuperSW columns provide approximately 1.5 times the number of theoretical plates than the conventional high performance TSKgel SW_{XL} series columns. Additionally, in Table 2, separation ranges of TSKgel SuperSW series columns for polyethylene glycol (PEG), dextran and protein are shown. In Figures 1 and 2, calibration curves of TSKgel SuperSW series columns for standard polyethylene glycols (PEG) and standard proteins are shown, respectively. Since TSKgel SuperSW series columns have the same calibration curve as the conventional TSKgel SW_{XL} series columns with equivalent grade, they have the same molecular weight separation range. In general, TSKgel SuperSW2000 is suited for the separation of proteins with molecular weights of 70,000Da or smaller and TSKgel SuperSW3000 is suited for the separation of proteins with molecular weights of 70,000 to 300,000Da.

Figures 3 and 4 show the chromatograms of standard proteins on TSKgel SuperSW series columns and the conventional TSKgel SW_{XL} series columns. A UV detector with a micro flow cell was used. Due to the smaller 4.6mm ID bore of the TSKgel SuperSW columns relative to the 7.8mm ID TSKgel SW_{XL} columns, 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 TSKgel SuperSW series columns have approximately 1.2 times better resolution compared to TSKgel SW_{XL} series columns.

Figures 5 and 6 show the comparison of analysis time under the condition that resolution is nearly equal between TSKgel SW_{XL} series and TSKgel SuperSW series columns. The higher efficiency of the TSKgel SuperSW series columns was used to decrease the separation time by 50% relative to TSKgel SW_{XL} columns without sacrificing resolution.

Table 1 Specifications of TSKgel SuperSW Series and TSKgel SW_{XL} Series Columns

TSKgel Column	Particle size (μm)	Column size	Guaranteed theoretical plates
SuperSW2000	4	4.6mm ID × 30cm	30,000
SuperSW3000	4	4.6mm ID × 30cm	30,000
G2000SW_{XL}	5	7.8mm ID × 30cm	20,000
G3000SW_{XL}	5	7.8mm ID × 30cm	20,000

Table 2 Molecular Weight Separation Range of TSKgel SuperSW Series Columns

	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

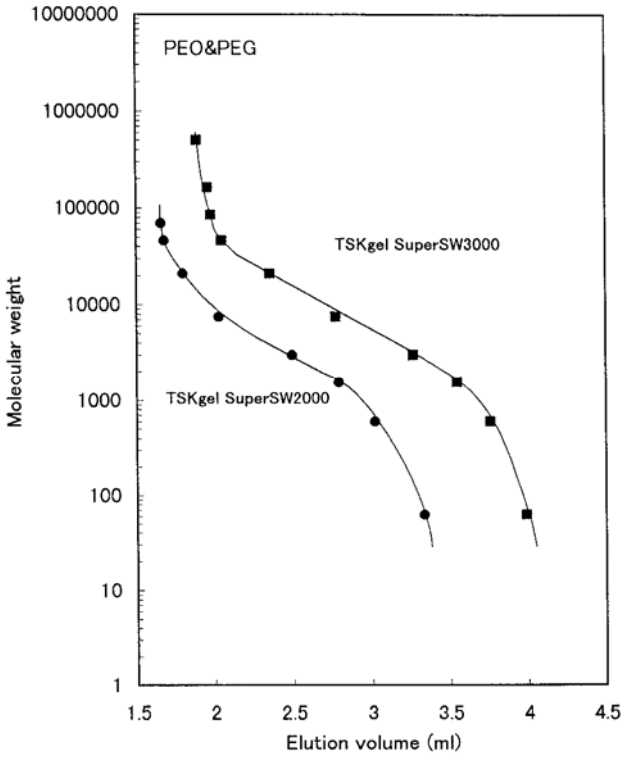

Figure 1 **PEO & PEG Calibration Curves for TSKgel SuperSW Series Columns**

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

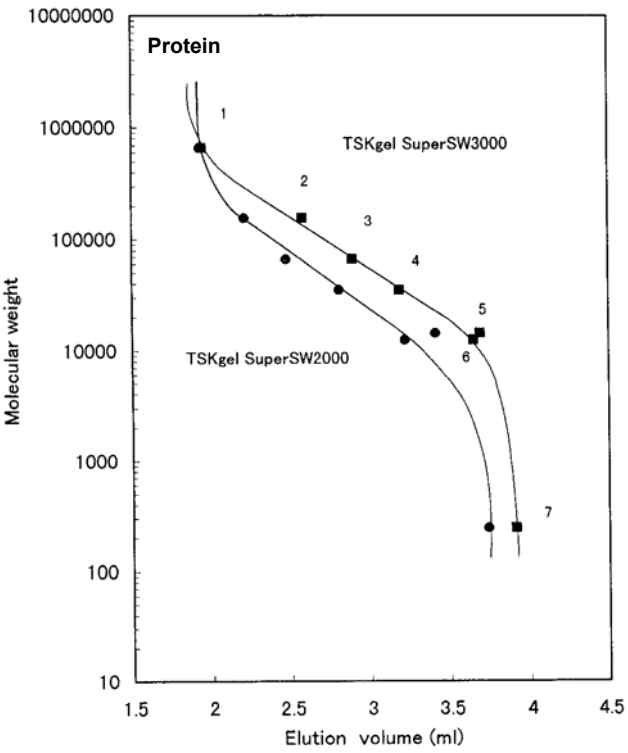

Figure 2 **Protein Calibration Curves for TSKgel SuperSW Series Columns**

Column: TSKgel SuperSW Series
(4.6mm ID × 30cm)
Eluent: 0.2mol/L phosphate buffer (pH6.7)
Flow rate: 0.35mL/min
Detection: UV@28 0nm
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

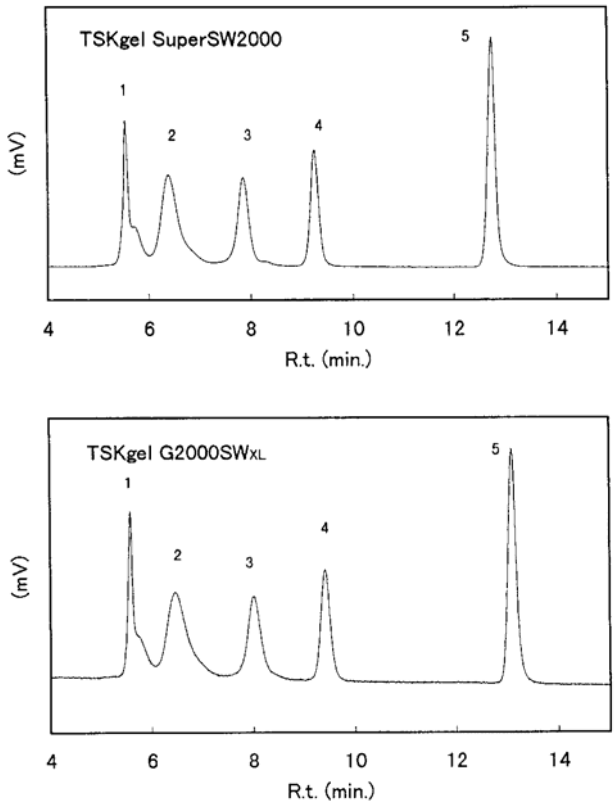

Figure 3 Comparison between TSKgel SuperSW2000 and TSKgel G2000SW$_{XL}$ Columns

Column: TSKgel SuperSW2000 (4.6mm ID × 30cm)
T SKgel G2000SW$_{XL}$ (7.8mm ID × 30cm)
Eluent: 0.2mol/L phosphate buffer (pH6.7)
Flow rate: 0.35mL/min (TSKgel SupperSW2000)
1.0mL/min (TSKgel G2000SW$_{XL}$)
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)

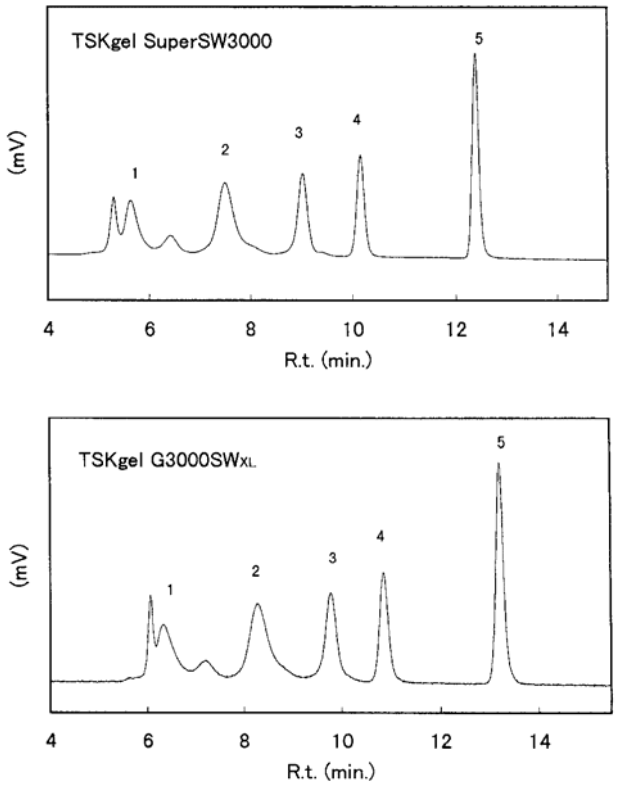

Figure 4 Comparison between TSKgel SuperSW3000 and TSKgel G3000SW$_{XL}$ Columns

Column: TSKgel SuperSW3000 (4.6mm ID × 30cm)
T SKgel G3000SW$_{XL}$ (7.8mm ID × 30cm)

Separation conditions are the same as Figure 3.

Table 3 Comparison of Resolution between TSKgel SuperSW Series and TSKgel SW$_{XL}$ Series Columns

	Resolution (Rs)*			
	SuperSW2000	G2000SW$_{XL}$	SuperSW3000	G3000SW$_{XL}$
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

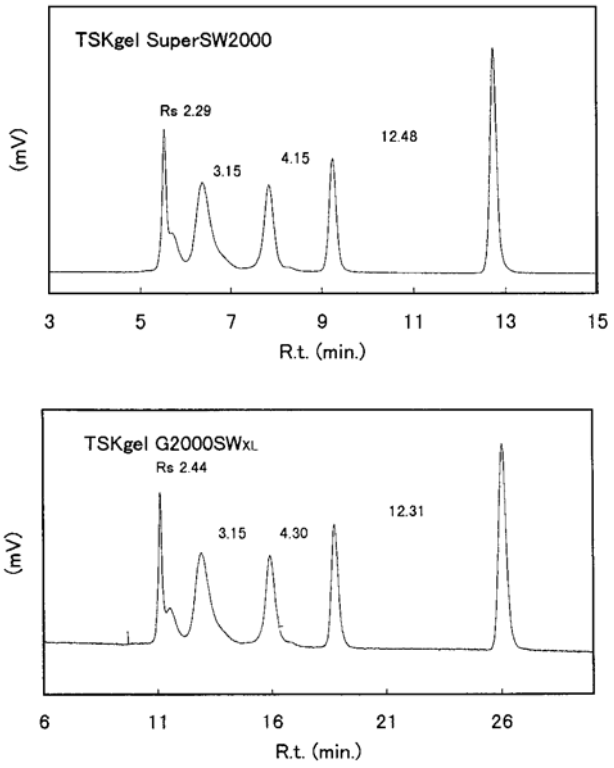

Figure 5 Comparison between TSKgel SuperSW2000 and TSKgel G2000SW$_{XL}$ Columns

Column: TSKgel SuperSW2000 (4.6mm ID × 30cm)
T SKgel G2000SW$_{XL}$ (7.8mm ID × 30cm)
Eluent: 0.2mol/L phosphate buffer (pH6.7)
Flow rate: 0.35mL/min (TSKgel SuperSW2000)
0.50mL/min (TSKgel G2000SW$_{XL}$)
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)

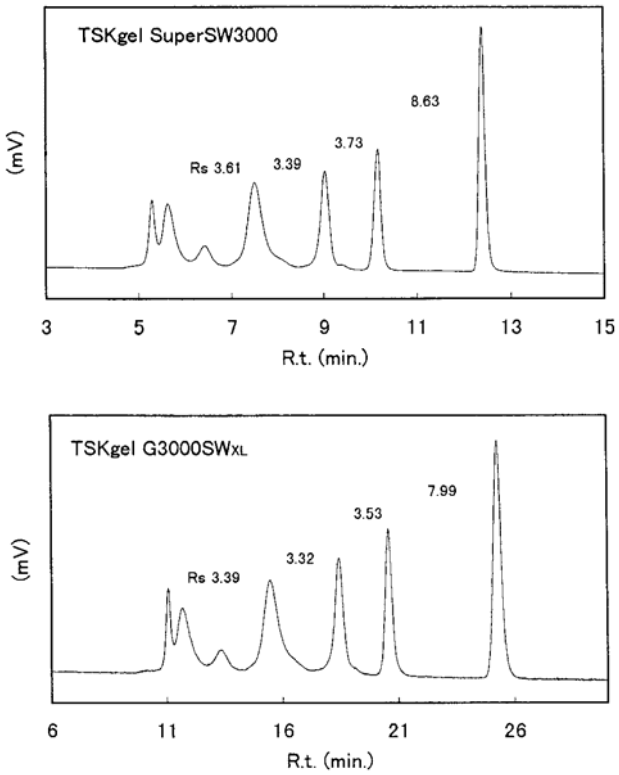

Figure 6 Comparison between TSKgel SuperSW3000 and TSKgel G3000SW$_{XL}$ Columns

Column: TSKgel SuperSW3000 (4.6mm ID × 30cm)
T SKgel G3000SW$_{XL}$ (7.8mm ID × 30cm)

Separation conditions are the same as Figure 5.

3. Basic Properties

3-1 Optimization of Equipment

Although the TSKgel SuperSW series consists of high performance SEC columns 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 the TSKgel SuperSW series, the void volume of the 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:

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

In the TSKgel SuperSW column series, it is necessary to suppress solute dispersion in these components to achieve the highest efficiency.

3-1-1 Connecting Tubing

In Figure 7, the effect of the volume of tubing between injector/column and column/detector on column efficiency is shown. As the volume of tubing increases, the dispersion of solute within the tubing increases and deteriorates the column efficiency. With TSKgel SuperSW series columns, column efficiency begins to deteriorate when the volume of tubing exceeds 10μL (0.1mm ID × 150cm). Therefore, we recommend that 0.1mm ID X 100cm or shorter tubing should be used between the injector/column and the column/detector with TSKgel SuperSW series columns.

3-1-2 Cell Volume of Detector

Table 4 shows the effect of the 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 30μL of void volume, column efficiency deteriorates 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 a light path length of 10mm, 2.5 times the sensitivity is obtained compared to a micro flow cell with a light path length of 4mm. In TSKgel SuperSW series columns, it is necessary that a micro flow cell be used when high resolution is required and a low dead volume-type cell be used when high sensitivity is required. Furthermore, sensitivity of TSKgel SuperSW series columns is improved by approximately 3 times compared to TSKgel SW_{XL} series columns even when a normal standard cell is used.

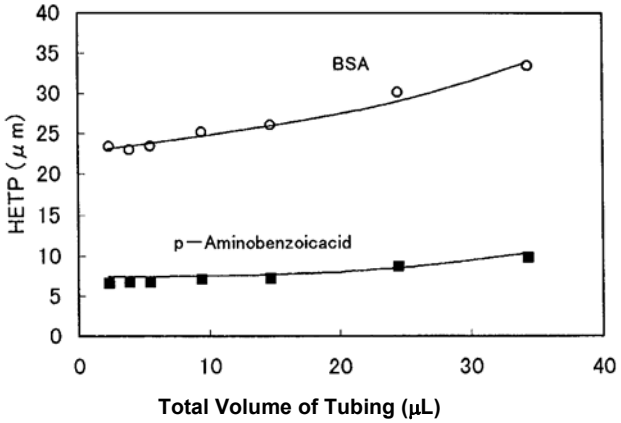

Figure 7 Effect of Total Volume of Tubing* on HETP

Column: TSKgel SuperSW3000 (4.6mm ID × 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@28 0nm
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)	
2μL (micro flow cell)	41,199	(0%)
10μL (low dead volume-type cell*)	40,189	(2.5%)
30μL (standard cell)	30,855	(25%)

* Low dead volume-type cell: Standard cells from which heat sink has been removed (1mm ID tubing is used.)

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

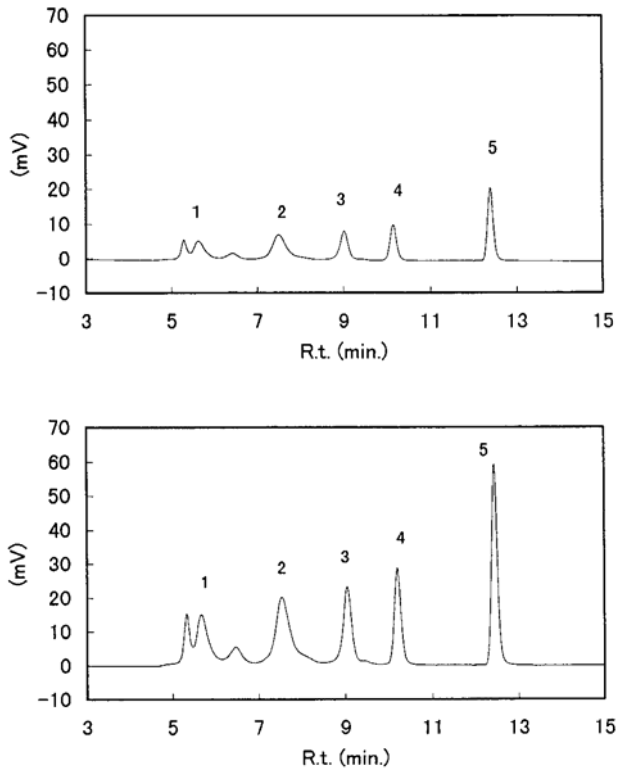

Figure 8 Comparison of Peak Heights between the Different Cell Types

Column: TSKgel SuperSW3000 (4.6mm ID × 30cm)
Upper Chromatogram: micro flow cell
Lower Chromatogram: low dead volume-type cell

Separation conditions are the same as Figure 3.

3-1-3 Injector

Figure 9 shows the effect of injector and detector cell on column efficiency. When a low-diffusion type injector (Rheodyne 8125) and micro flow cell are used, this combination has the smallest peak broadening. The value of the peak broadening for this combination was arbitrarily set at 100%. It is clear that the 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 a general-purpose injector and a standard cell, the column efficiency deteriorates by 20% or more.

In order to maximize the performance of TSKgel SuperSW columns, a low-diffusion type injector should be used. Furthermore, when an auto-sampler is required, the use of an auto-sampler capable of trace injection mode is recommended.

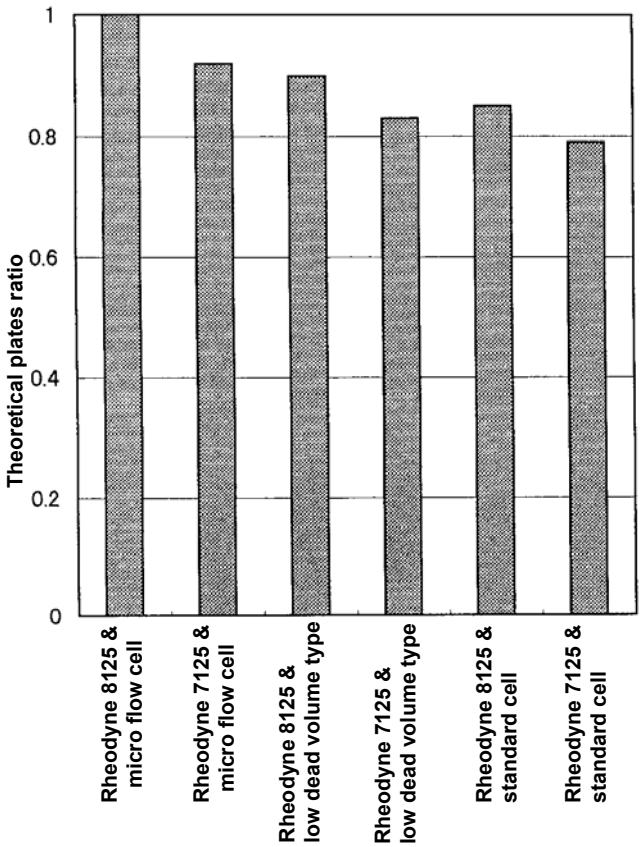

Figure 9 Effect of Injector and Detector Cell on Column Efficiency

Column: TSKgel SuperSW3000 (4.6mm ID × 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@28 0nm
Sample: p-aminobenzoic acid (5μL)

3-2 Sensitivity

Figures 10 and 11 compare the peak height of standard proteins when separated on TSKgel SuperSW series and TSKgel SW_{XL} series columns. It is evident that TSKgel SuperSW series columns yield peak heights that are approximately 4 times that of TSKgel SW_{XL} series columns. This can be attributed to the smaller internal diameter of the TSKgel SuperSW columns and increased theoretical plates.

Table 5 compares the limits of detection of TSKgel SuperSW3000 and TSKgel $G3000SW_{XL}$ columns for major proteins. Although the limit of detection varies depending on the sample analyzed, separation conditions, detection wavelength, and light path length of the cell, the TSKgel SuperSW3000 column, when used in combination with a cell with a light path length of 10mm (low dead volume type), has a limit of detection that is approximately 1/2 – 1/3 of the TSKgel $G3000SW_{XL}$ column. The increased sensitivity of the TSKgel SuperSW3000 column allows for analysis of nanogram levels of protein sample amounts. Therefore, TSKgel SuperSW series SEC columns are well suited for trace analysis. Note that further improvements in sensitivity can be achieved by using smaller bore columns, such as the TSKgel SuperSW3000 1mm and 2mm ID columns.

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

	SuperSW3000		$G3000SW_{XL}$
	Standard cell (low dead volume type)	Micro flow cell	Standard cell (low dead volume type)
Light path length	10mm	4mm	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 ID × 30cm)
Eluent: 0.2mol/L phosphate buffer (pH 6.7)
Detection: UV@280nm

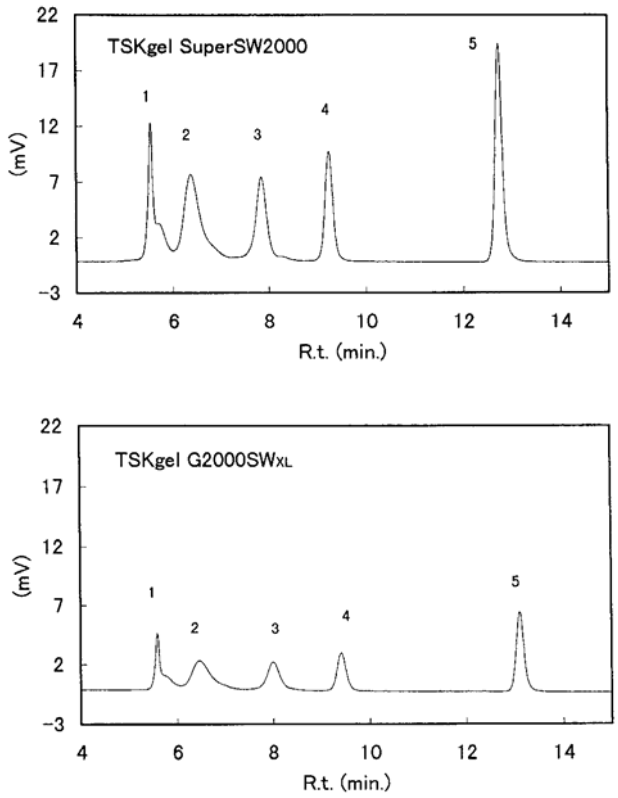

Figure 10 Comparison of Sensitivity between TSKgel SuperSW2000 and TSKgel $G2000SW_{XL}$ Columns

Column: TSKgel SuperSW2000 (4.6mm ID ×30cm)
T SKgel $G2000SW_{XL}$ (7.8mm ID × 30cm)

Separation conditions are the same as Figure 3.

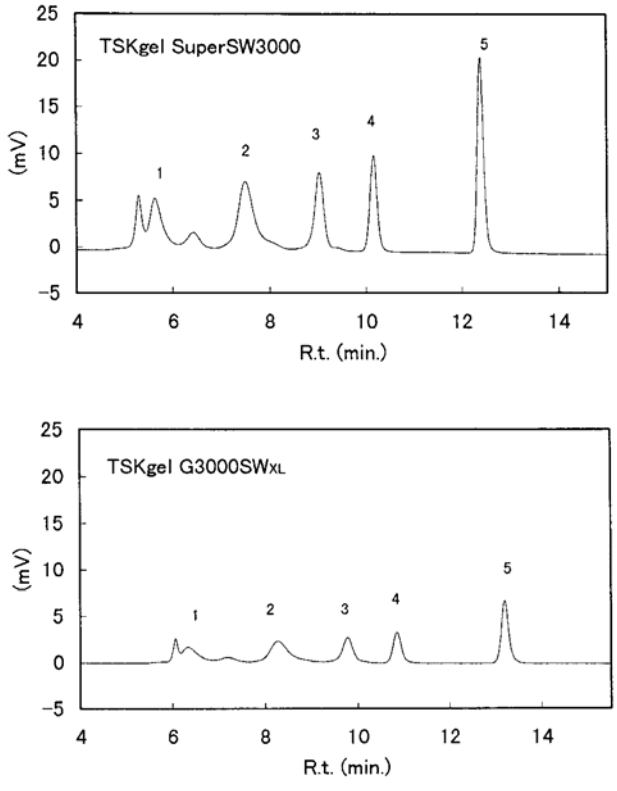

Figure 11 Comparison of Sensitivity between TSKgel SuperSW3000 and TSKgel $G3000SW_{XL}$ Columns

Column: TSKgel SuperSW3000 (4.6mm ID ×30cm)
T SKgel $G3000SW_{XL}$ (7.8mm ID × 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 the particle size of the packing materials, sample molecular size, eluent viscosity, etc. A typical example of HETP's flow rate dependency on TSKgel SuperSW series and TSKgel SW_{XL} series columns using bovine serum albumin (BSA) and myoglobin is shown in Figure 12. It is clear that TSKgel SuperSW series columns have low HETP throughout the full flow rate range and low flow rate dependence compared to TSKgel SW_{XL} series columns since the particle size is small. The appropriate flow rate for TSKgel SuperSW series columns 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 decreased, separation of high polymer protein is improved. Resolution calculated at a flow rate of 0.35mL/min is twice that of the resolution calculated at a flow rate of 0.05mL/min. Although TSKgel SuperSW series columns have a lower flow rate dependency than conventional columns, a lower flow rate should be used when higher resolution is required.

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

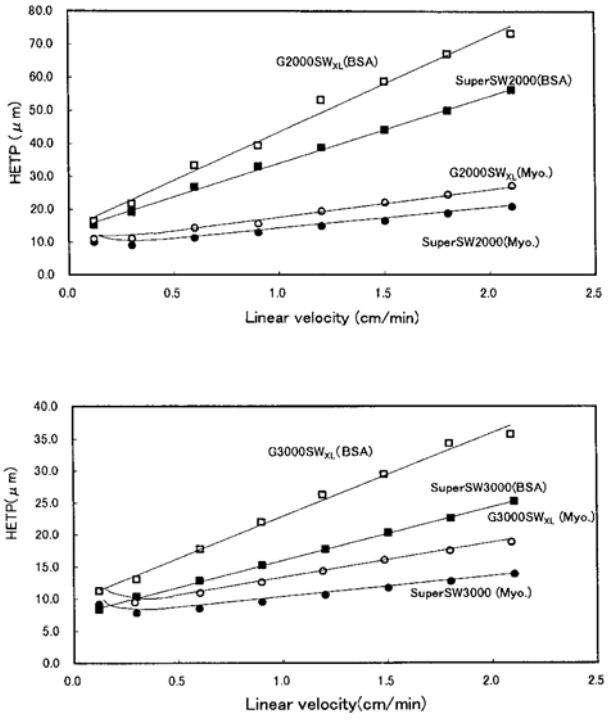

Figure 12 Relationship be tween Flow Ra te and HETP in TSKgel Super SW Se ries a nd TSKgel SW_{XL} Series Columns

Column: TSKgel SuperSW series (4.6mm ID × 30cm)
T SKgel SW_{XL} series (7.8mm ID × 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)
m yoglobin (1g/L)

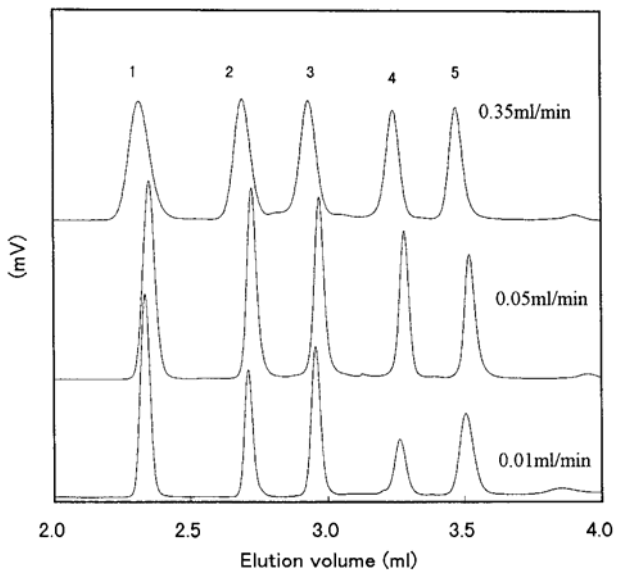

Figure 13 Effect of Flow Rate on Separation

Column: T SKgel SuperSW3000 (4.6mm ID × 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 lower in TSKgel SuperSW series columns than in TSKgel SW_{XL} series columns, it is obvious that it increases dramatically at loads of 100µg or greater. Therefore, TSKgel SuperSW series columns should be used under loads of 100µg or less.

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 10µL for TSKgel SuperSW series columns; this injection volume is less than that of TSKgel SW_{XL} series columns.

The desired sample load of TSKgel SuperSW series columns is 100µg or less of the total injection volume amount (10µL or less).

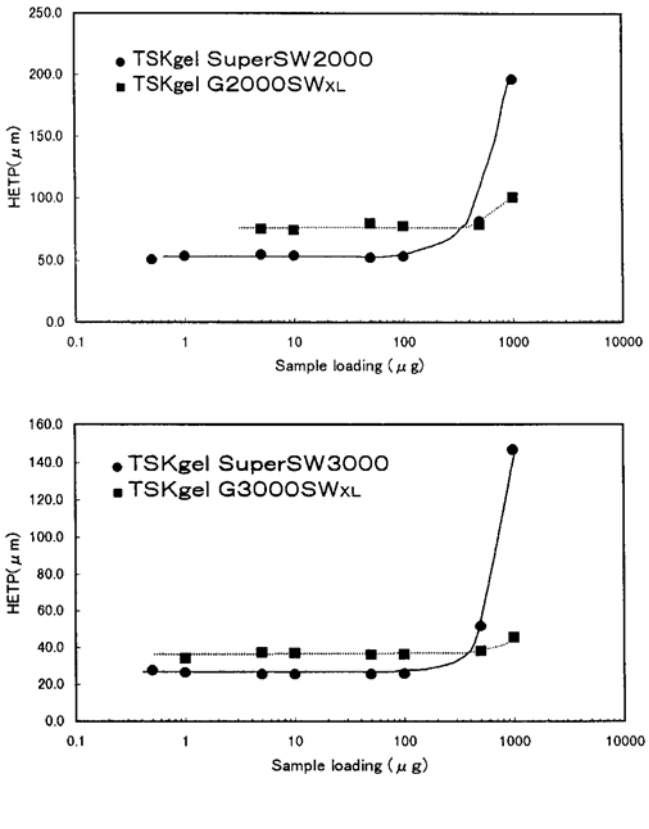

Figure 14 Relationship between Sample Load and HETP in TSKgel SuperSW Series and TSKgel SW_{XL} Series Columns

Column: TSKgel SuperSW series (4.6mm ID × 30cm)
T SKgel SW_{XL} series (7.8mm ID × 30cm)
Eluent: 0.2mol/L phosphate buffer (pH 6.7)
Flow rate: 0.35mL/min (TSKgel SuperSW series)
1.00mL/min (TSKgel SW_{XL} series)
Detection: UV@280nm, micro flow cell
Sample: bovine serum albumin (5µL)

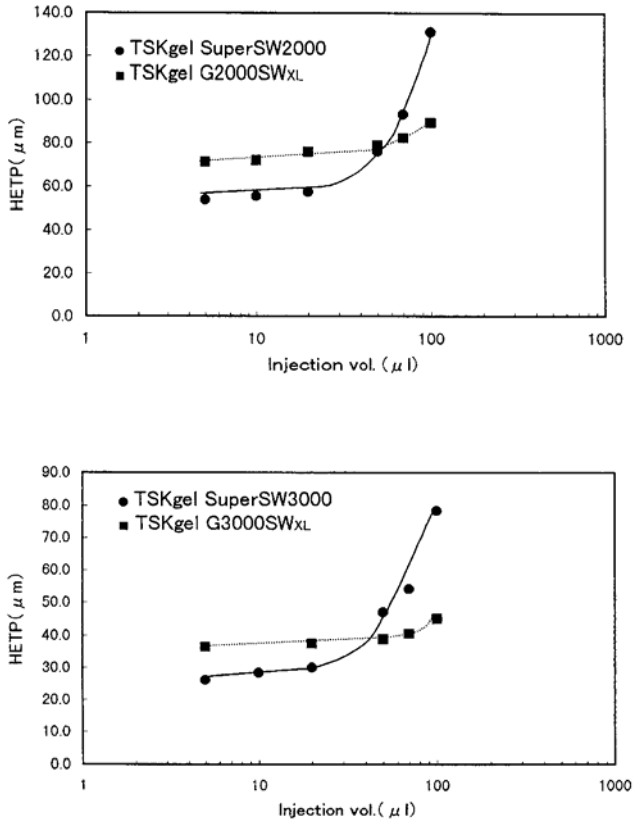

Figure 15 Relationship between Injection Volume and HETP in TSKgel SuperSW Series and TSKgel SWXL Series Columns

Column: TSKgel SuperSW series (4.6mm ID × 30cm)
T SKgel SW_{XL} series (7.8mm ID × 30cm)
Eluent: 0.2mol/L phosphate buffer (pH 6.7)
Flow rate: 0.35mL/min (TSKgel SuperSW series)
1.00mL/min (TSKgel SW_{XL} series)
Detection: UV@280nm, micro flow cell
Sample: bovine serum albumin (0.2g/L)

3-5 Recovery of Protein

Table 7 compares protein recovery rates of TSKgel SuperSW2000 and TSKgel SuperSW3000 columns at a sample concentration of 20µg/mL (sample load of 100ng). A majority of protein was recovered quantitatively with the TSKgel SuperSW columns, even at the low sample load of 100ng. With TSKgel SW_{XL} series columns, recovery of thyroglobulin at a sample load of 1µg was 70%. Recovery deteriorated even further when the sample load was 1µg or less (see Separation Report No. 46 for the data). As shown in Table 7, TSKgel SuperSW series columns are capable of obtaining high protein recovery rates, even when analyzing trace protein amounts with sample loads of 1µg or less.

While TSKgel SuperSW series columns have high protein recovery rates, even with low sample concentration loads, the sample may be adsorbed by components of the HPLC system other than the column (tubing, etc.) when analyzing trace amounts of protein. It is important when trace analysis is performed that several sample injections are made before measurement so that the adsorption point within the system is first inactivated.

Table 7 Protein Recovery

	SuperSW2000	SuperSW3000
Thyroglobulin	86%	97%
γ-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, 5µL)

4. Applications of TSK-GEL SuperSW Series

Figure 16 shows an example of a peptide mixture separation on a TSKgel SuperSW2000 column. Figures 17, 18 and 19 show separations of commercial glutamic acid-oxalacetic acid transaminase, mouse ascites monoclonal antibody (IgG1) and human serum on a TSKgel SuperSW3000 column.

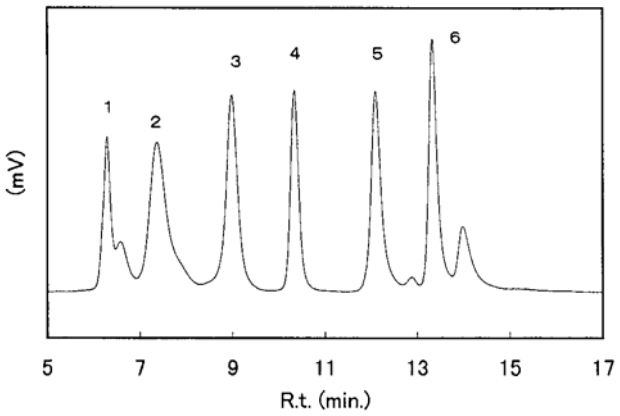

Figure 16 Separation of Mixture of Protein/Peptides

Column: TSKgel SuperSW2000 (4.6mm ID × 30cm)
Eluent: 0.2mol/L phosphate buffer (pH 6.7)
Flow rate: 0.35mL/min
Detection: UV@220nm, micro flow cell
Sample: protei n/peptide mixture (5µL)
1. thyroglobulin (0.1g/L)
2. γ-globulin (0.2g/L)
3. ovalbumin (0.2g/L)
4. myoglobin (0.1g/L)
5. insulin (0.1g/L)
6. oxytocin (0.1g/L)

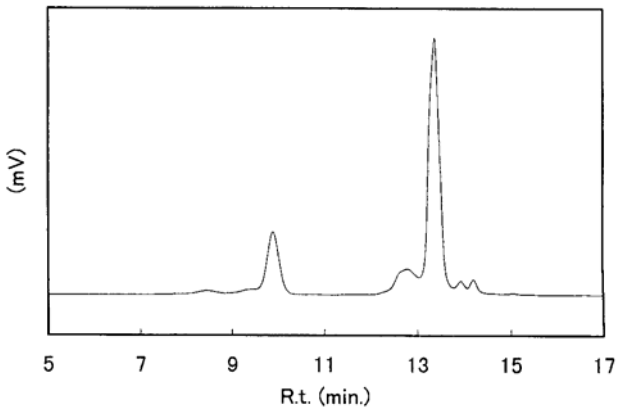

Figure 17 Separation of Commercial Glutamic Acid-Oxalacetic Acid Transaminase

Column: TSKgel SuperSW3000 (4.6mm ID ×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, 5µL)

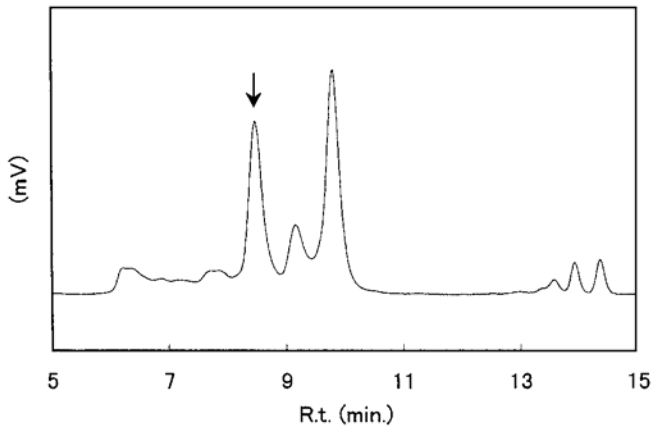

Figure 18 Separation of Mouse Ascites Monoclonal Antibody (IgG1)

Column: TSKgel SuperSW3000 (4.6mm ID ×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)

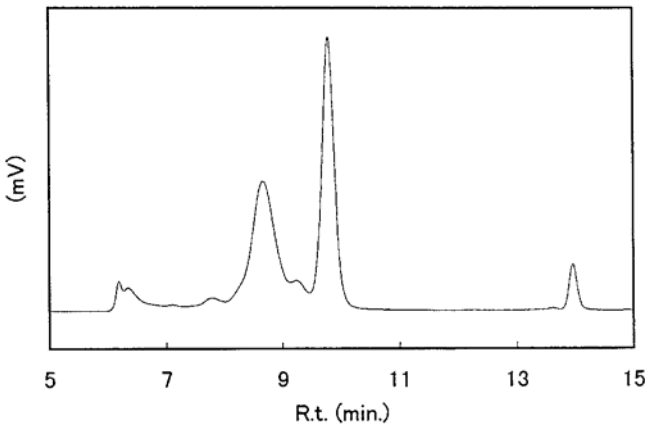

Figure 19 Separation of Human Blood Serum

Column: TSKgel SuperSW3000 (4.6mm ID ×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

TSKgel SuperSW series is a group of columns in which particle size and column size of the conventional TSKgel SW_{XL} series columns have been reduced - leading to improved resolution and sensitivity. Resolution has been improved 1.2 – 1.5 times and sensitivity to approximately 2 – 3 times compared to the conventional TSKgel SW_{XL} series columns. Furthermore, it maintains high recovery rates, even for sample injections at low concentrations. This makes the TSKgel SuperSW series columns well suited to trace analysis of biopolymers.

In order to ensure the best performance of TSKgel SuperSW series columns, 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 details the recommendations for use of TSKgel SuperSW series columns.

Table 8 Operating Instructions When Using TSKgel SuperSW Series Columns

In general:

- Suppress peak broadening in connecting tubing between injector, guard column, analytical column, and detector.
- Prevent the sample volume from causing extra-column band broadening due to volume overloading. You can test this by injecting half the sample volume and measuring peak efficiency.

Tubing:

- Use 0.004” or 0.005” ID (0.100mm or 0.125mm) tubing, when available. A length of 100cm or less is desired.
- Sections requiring 0.004” or 0.005” ID tubing
  - Between injection valve and guard column, and between guard column outlet and column
  - Between column outlet and detector inlet

Pumping system:

- Pumping system should be applicable to semi-micro HPLC. Flow rate should be in the range of 0.1 – 0.35mL/min.

Injector:

- A low dispersion injector (such as Rheodyne 8125) is recommended.

Guard column:

- We recommend that you install a guard column (Part No. 18762) to protect your TSKgel SuperSW column.

Detector:

- When working with a UV detector, install a micro flow cell or a low dead volume-type cell. Low dead volume-type cells are effective in high-sensitivity analysis. (Use of a standard cell is also possible. However, theoretical plates will be approximately 80% of those obtained with a micro flow cell.)

Sample:

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