

TSKgel Super AW Series High-performance SEC Semi-Micro Columns

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TOSOH BIOSCIENCE LLC
Separations Business Unit

TOSOH

1. Introduction

Size exclusion chromatography (SEC) is a method that separates compounds dissolved in aqueous solution or organic solvents by their molecular size. It is applicable to a wide range of substances from low molecular weight to high molecular weight and from hydrophilic to hydrophobic compounds. Since it enables not only separation and purification, but also molecular weight determinations and distributions, it has become an invaluable tool for the analysis of macromolecules in many fields of research and development.

To meet the varying needs of researchers studying synthetic macromolecules, Tosoh has developed several column lines to accommodate various SEC needs.

- For gel permeation chromatography (GPC) using organic solvents, the TSKgel H_{XL} is available. Other offerings in GPC include the TSKgel H_{HR} series which allows for solvent changes to many different organic solvents and TSKgel SuperHZ column series for ultra-fast GPC.
- For macromolecular analysis in the aqueous phase Tosoh has developed the TSKgel PW_{XL} series. In addition, the TSKgel Alpha series was introduced to provide additional flexibility in method development. The stationary phase of the TSKgel Alpha series is highly cross linked. This results in superior consistent performance with used with an aqueous or polar organic mobile phase.

Most recently, Tosoh commercialized TSKgel SuperAW series. TSKgel SuperAW series columns share similar including the hydrophilic matrix properties of the TSKgel PW_{XL} and the stability of the TSKgel Alpha series columns. However, the smaller particle size and semi micro column dimensions provide the capability for high throughput and more efficient analyses. In this article, the basic features and applications of TSKgel SuperAW series are introduced.

2. Properties of SuperAW Series

The polymer-type packing materials that have been developed up to present had large swelling or shrinkage properties depending on the difference in solvents chosen as an eluent. Of additional concern, these packings may have unwanted hydrophobic interactions with certain samples if used in conjunction with polar organic solvents. Therefore, it was necessary for columns to be selected based on the type of eluent and sample to be analyzed. TSKgel Alpha series was introduced to solve this problem. The ability of the TSKgel Alpha series to operate in a wide range to solvent systems ranging from 100% aqueous systems to 100% polar organic solvent systems without shrinking or swelling provided a general purpose SEC column for polymeric analysis.

TSKgel SuperAW series builds upon the flexibility of the TSKgel Alpha series by offering equivalent separations in 1/2 for analysis time using 1/3 the amount of solvent consumption compared to conventional columns. This high-throughput capability is a direct function of the recently developed micro particles and the smaller bore column housing. By using a similar highly-cross linked hydrophilic base material as in the TSKgel Alpha series, suppression of hydrophobic interactions with sample and SEC measurement in the presence of polar organic solvents is possible. Additionally, solvent replacements can be made from a wide range polar organic solvents to water without appreciable shrinking or swelling of the packing. The features of TSKgel SuperAW series are summarized in Table-1. TSKgel SuperAW series consists of 5 columns of different discreet separation ranges and 1 mixed bed column that blends several gel types to provide an expanded linear separation range. These 6 columns types cover a wide range of molecular weights, enabling selection of a column most suited to sample molecular weight or purpose of measurement. Table-2 shows the list of TSKgel SuperAW series products.

Table-1 Features of TSKgel SuperAW Series

Feature	Advantage
1) Micro-particle gel packed in semi-micro column	<ul style="list-style-type: none"> • SEC measurement with short time and high resolution possible → Resolution equivalent to PW_{XL} or α column (30cm) with nearly half the analysis time • Solvent-saving → Solvent consumption approximately 1/3 of conventional columns (30cm)
2) Hydrophilic gel with small swelling or shrinkage	<ul style="list-style-type: none"> • Solvent replacement from water to organic solvent possible • Small sample absorption in polar organic solvent • SEC possible in aqueous systems to polar organic solvent systems
3) High mechanical strength	<ul style="list-style-type: none"> • High durability

Table-2 List of TSKgel SuperAW Series

Grade	Exclusion limit (PEO/DMF)	Particle size (μm)	Theoretical plates	Column size (mm I.D. x cm)
TSKgel SuperAW2500	2×10^3	4	> 16,000	6.0 x 15
TSKgel SuperAW3000	6×10^4	4	> 16,000	6.0 x 15
TSKgel SuperAW4000	4×10^5	6	> 10,000	6.0 x 15
TSKgel SuperAW5000	4×10^6	7	> 10,000	6.0 x 15
TSKgel SuperAW6000	$> 4 \times 10^7$	9	> 6,000	6.0 x 15
TSKgel SuperAWM-H	$> 4 \times 10^7$	9	> 6,000	6.0 x 15

I.D. indicates the internal diameter.

3. Basic Properties of SuperAW Series

3-1. Solvent Compatibility

Figure-1 shows results of a solvent compatibility test with the TSKgel SuperAW series. In this test, theoretical plates were measured after switching from water to solvent and back to water. The substitution of from water to organic used a flow rate of 0.6mL/min for a minimum of 5 hours. After substitution, organic solvent remained stagnant for a minimum of 14 hours followed by a mobile phase replacement back to water at a of flow rate 0.6mL/min over 5 hours. The procedure was repeatedly performed using various test solvents. It is evident that regardless of column type, the change in theoretical plates was small even after exchanging with many solvents of varying polarities.

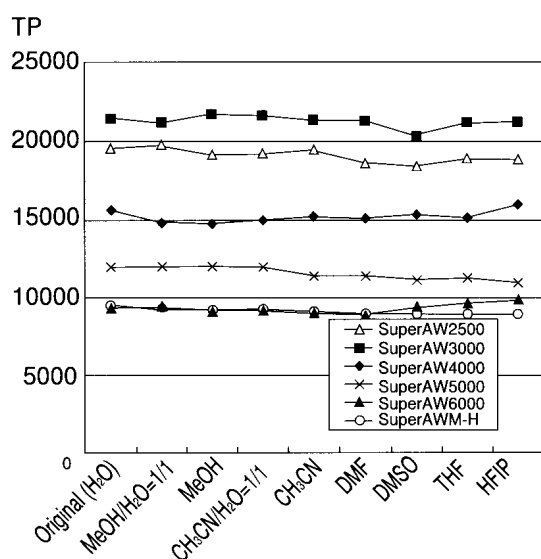


Figure-1 Solvent Compatibility of TSKgel SuperAW Series
(Theoretical Plates Measurement Conditions)

Column: TSKgel SuperAW Series
(6.0mm I.D. × 15cm)
Eluent: Water
Flow rate: 0.6mL/min
Temperature: 25°C
Detection: Refractive index detector
Sample: Ethylene glycol
Injection volume: 5µL (2.5g/L)

3-2. Calibration Curves with Various Solvents

Although TSKgel SuperAW series allows for exchange to various solvents, it is necessary that the calibration curves be constructed using standards suitable with the intended solvent for sample analysis. Figures-2 through 6 show calibration curves in water (standard sample PEO, PEG, and pullulan), methanol (PEO, PEG), DMF (PEO, PEG), and DMSO (pullulan). While a calibration curve with favorable linearity has been achieved in each solvent, some differences are evident in the molecular weight separation range and slope depending on the solvent type.

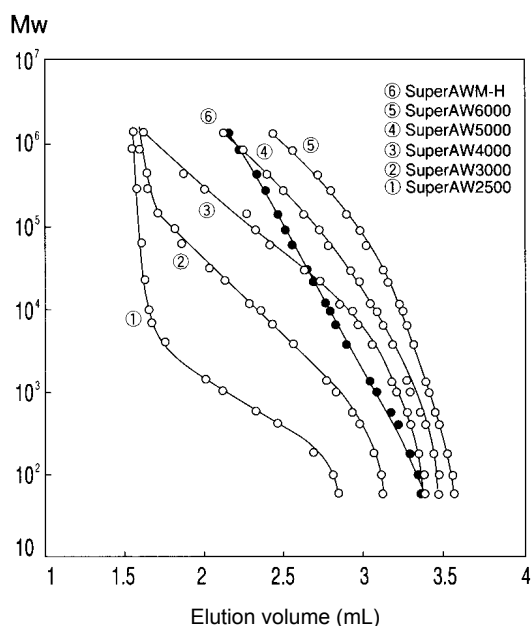


Figure-2 Calibration Curves of TSKgel SuperAW Series (1)

Column: TSKgel SuperAW Series
(6.0mm I.D. × 15cm)
Eluent: Water
Flow rate: 0.6mL/min
Temperature: 25°C
Detection: Refractive index detector
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol

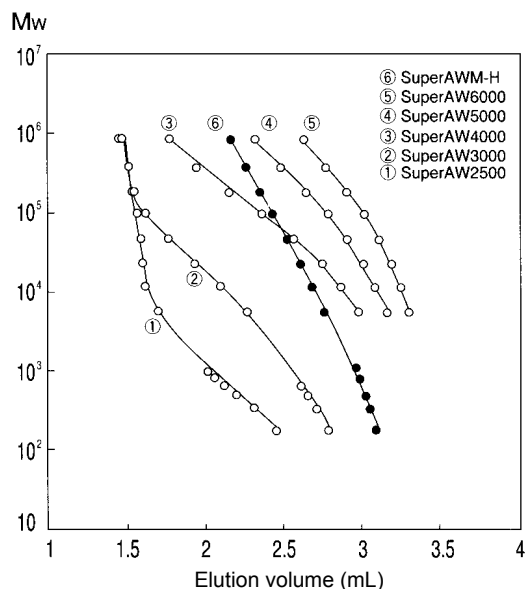


Figure-3 Calibration Curves of TSKgel SuperAW Series (2)

Column: TSKgel SuperAW Series (6.0mm I.D. × 15cm)
 Eluent: Water
 Flow rate: 0.6mL/min
 Temperature: 25°C
 Detection: Refractive index detector
 Samples: Standard pullulan, oligomaltose, glucose

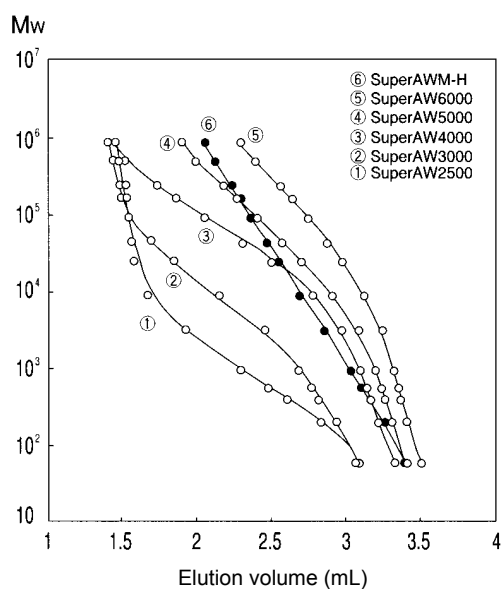


Figure-4 Calibration Curves of TSKgel SuperAW Series (3)

Column: TSKgel SuperAW Series (6.0mm I.D. × 15cm)
 Eluent: Methanol containing 10mmol/L LiBr
 Flow rate: 0.6mL/min
 Temperature: 25°C
 Detection: Refractive index detector
 Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol

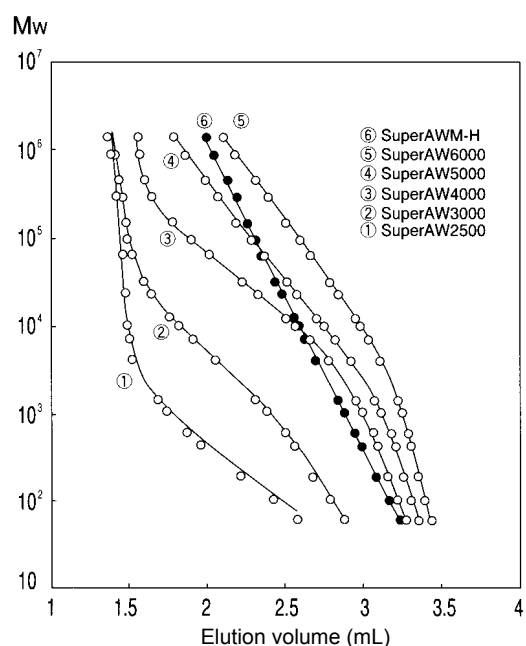


Figure-5 Calibration Curves of TSKgel SuperAW Series (4)

Column: TSKgel SuperAW Series (6.0mm I.D. × 15cm)
 Eluent: DMF containing 10mmol/L LiBr
 Flow rate: 0.6mL/min
 Temperature: 25°C
 Detection: Refractive index detector
 Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol

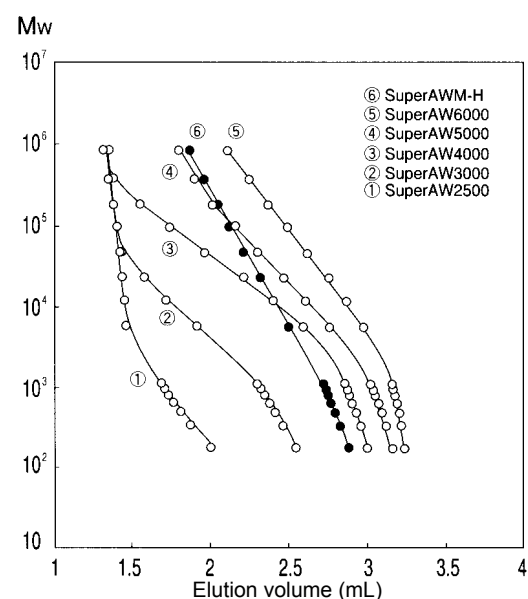


Figure-6 Calibration Curves of TSKgel SuperAW Series (5)

Column: TSKgel SuperAW Series (6.0mm I.D. × 15cm)
 Eluent: DMSO containing 10mmol/L NaNO₃
 Flow rate: 0.6mL/min
 Temperature: 25°C
 Detection: Refractive index detector
 Samples: Standard pullulan, oligomaltose, glucose

3-3. Resolution and Solvent Consumption

Compared to conventional columns, TSKgel SuperAW series has been packed with gel of smaller particle size. The column performance is a function of particle size, and the column efficiency increases as the particle size decreases. Thus, TSKgel SuperAW has twice the theoretical plates per unit length than conventional columns such as TSKgel PW_{XL}. Figure-7 shows chromatograms of dextran T-40 hydrolysate measured on TSKgel SuperAW 2500 and TSKgel G2500PW_{XL}. It is apparent that the TSKgel Super AW2500 column yields a equivalent separation in half the time.

Moreover, since the flow rate of SuperAW2500 is 60% of G2500PW_{XL} column (0.6mL/min compared to 1.0mL/min), the amount of solvent consumption in one sample measurement will be decreased by 1/3.

It is apparent for applications where speed and cost per analysis are critical, TSKgel SuperAW series provide the superior performance.

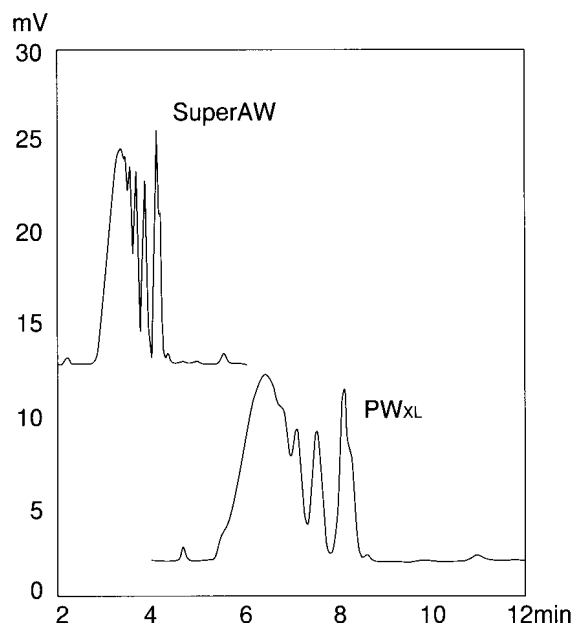


Figure-7 Comparison of Chromatograms

Column: TSKgel SuperAW2500 (6.0mm I.D. × 15cm)
TSKgel G2500PWXL (7.8mm I.D. × 30cm)
Eluent: Water
Flow rate: 0.6mL/min (TSKgel SuperAW2500)
1.0mL/min (TSKgel G2500PW_{XL})
Temperature: 25°C
Detection: Refractive index detector
Sample: Dextran T-40 hydrolysate

3-4. Effect of Flow Rate

Flow rate dependence of HETP (height equivalent to a theoretical plate) was confirmed. Normally, the optimal flow rate depends on the particle size and the molecular weight of the sample. Figure-8 shows the flow rate dependence of ethylene glycol on HETP for SuperAW2500 and G2500PW_{XL}. While the optimal linear velocity of the PW_{XL} column is 10mm/min (flow rate 0.5mL/min), the HETP changes drastically around this value. Alternatively, SuperAW column has a very flat response in HETP in the linear velocity range of 10 to 20mm/min (flow rate 0.3 to 0.6mL/min). That is,

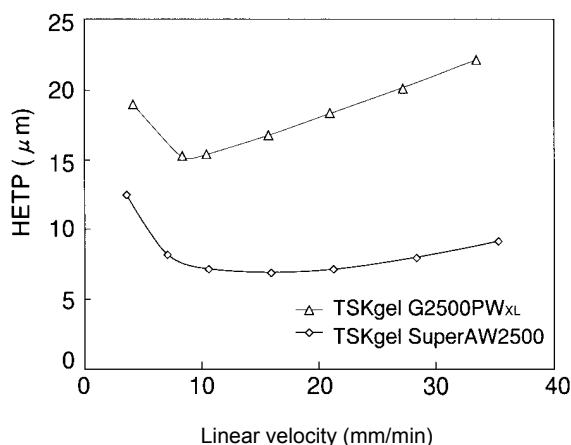


Figure-8 Relationship between HETP and Linear Velocity (1)

Column: TSKgel SuperAW2500
(6.0mm I.D. × 15cm)
TSKgel G2500PW_{XL}
(7.8mm I.D. × 30cm)
Eluent: Water
Temperature: 25°C
Sample: Ethylene glycol 2.5g/L
Injection volume: 5μL (TSKgel SuperAW2500)
10μL (TSKgel G2500PW_{XL})

SuperAW column retains its performance under high flow rate. Figure-9 shows the HETP flow rate dependence for high-molecular weight pullulan (P-20; molecular weight 23,700, P-5; molecular weight 5,800). In both PW_{XL} columns and SuperAW columns, HETP becomes larger as the linear velocity increases. However, it is clear that the rate of change is smaller in SuperAW columns. Based on the above, SuperAW series is evidently a group of columns which possess high column efficiency over a wide range of flow rates.

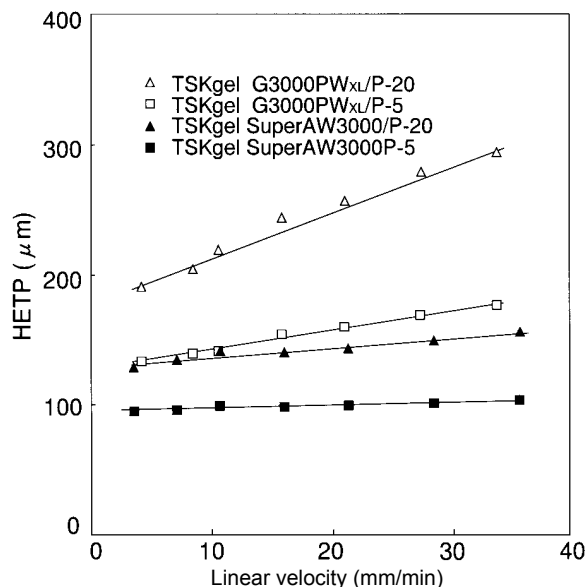


Figure-9 Relationship between HETP and Linear Velocity (2)

Column: TSKgel SuperAW3000
(6.0mm I.D. × 15cm)
TSKgel G3000PW_{XL}
(7.8mm I.D. × 30cm)
Eluent: Water
Temperature: 25°C
Samples: Pullulan P-5 (1g/L), pullulan P-20 (1g/L)
Injection volume: 5μL (TSKgel SuperAW3000)
10μL (TSKgel G3000PW_{XL})

3-5. Effect of Sample Injection Volume

When the sample injection volume is increased, the peak disperses and separation can be affected. The effect of injection volume on HETP using a low-molecular weight compound (ethylene glycol) and a molecular weight distribution of the macromolecule (pullulan) are shown in Figures 10 and 11 respectively. While it is expected a loss in efficiency will be evident upon sample overload, the point at which this occurs varies depending on the sample type. HETP increases drastically for ethylene glycol beyond 5 μ L on SuperAW column. On the other

hand, no steep increase in HETP is seen with pullulan until it reaches about 20 μ L on the SuperAW column. Thus, it is necessary for researchers to adjust their maximum injection volume depending on whether their analysis involves working with low-molecular weight substances or oligomers or macromolecules to 5 μ L or 20 μ L respectively. These recommended values are in turn lower than conventional columns due to the lower capacities of smaller, more efficient particles.

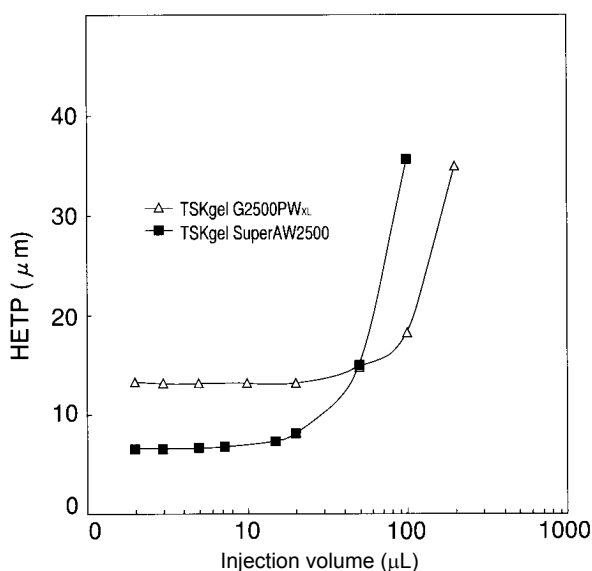


Figure-10 Relationship between HETP and Sample Injection Volume (1)

Column: TSKgel SuperAW2500 (6.0mm I.D. \times 15cm)
 TSKgel G2500PW_{XL} (7.8mm I.D. \times 30cm)
 Eluent: Water
 Flow rate: 0.6mL/min (TSKgel SuperAW2500)
 1.0mL/min (TSKgel G2500PW_{XL})
 Temperature: 25°C
 Sample: Ethylene glycol 2.5g/L

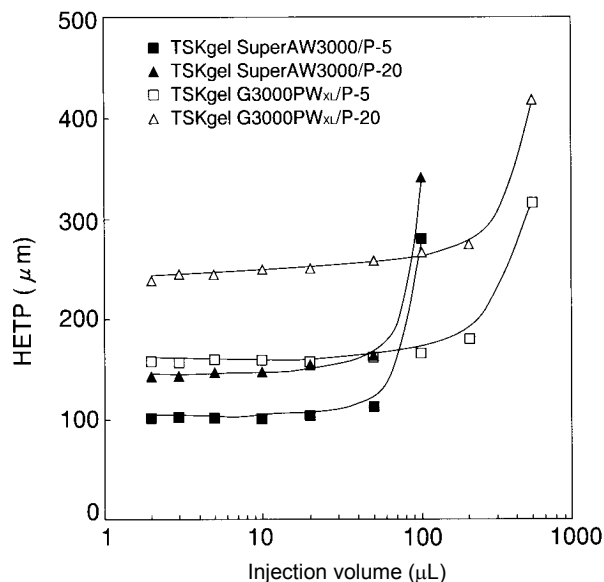


Figure-11 Relationship between HETP and Sample Injection Volume (2)

Column: TSKgel SuperAW3000 (6.0mm I.D. \times 15cm)
 TSKgel G3000PW_{XL} (7.8mm I.D. \times 30cm)
 Eluent: Water
 Flow rate: 0.6mL/min (TSKgel SuperAW3000)
 1.0mL/min (TSKgel G3000PW_{XL})
 Temperature: 25°C
 Sample: Pullulan P-5 (1g/L)
 Pullulan P-20 (1g/L)
 Injection volume: 5 μ L (TSKgel SuperAW3000)
 10 μ L (TSKgel G3000PW_{XL})

4. Applications

4-1. Applications of Various Polymer Measurements

A list of various samples measured on SuperAW column is shown in Table-3. Furthermore, chromatograms of these samples are shown in Figures-12 to-27.

Table-3 Applications of Various Samples on TSKgel SuperAW Series

Figure	Sample name	Column	Solvent
12	Sodium chondroitin sulfate	TSKgel SuperAWM-H	0.2mol/L sodium nitrate
13	Sodium alginate	TSKgel SuperAWM-H	0.2mol/L sodium nitrate
14	Carboxymethyl cellulose	TSKgel SuperAWM-H	0.2mol/L sodium nitrate
15	Sodium polystyrene sulfonate	TSKgel SuperAWM-H	0.2mol/L sodium nitrate/acetonitrile = 80/20
16	Polyvinyl pyrrolidone	TSKgel SuperAWM-H	0.2mol/L sodium nitrate/acetonitrile = 80/20
17	Gum arabic	TSKgel SuperAWM-H	0.2mol/L sodium nitrate/acetonitrile = 80/20
18	Ethylhydroxy-ethylcellulose	TSKgel SuperAWM-H	Methanol containing 10mmol/L LiBr
19	Vinyl alcohol/vinyl butyral copolymer	TSKgel SuperAWM-H	Methanol containing 10mmol/L LiBr
20	Hydroxypropylcellulose	TSKgel SuperAWM-H	Methanol containing 10mmol/L LiBr
21	Polymethyl vinyl ether	TSKgel SuperAWM-H	Methanol containing 10mmol/L LiBr
22	Cellulose acetate	TSKgel SuperAWM-H	DMF containing 10 mmol/L LiBr
23	N-isopropyl acrylamide	TSKgel SuperAWM-H	DMF containing 10 mmol/L LiBr
24	Polyacrylonitrile	TSKgel SuperAWM-H	DMF containing 10 mmol/L LiBr
25	Vinyl chloride/vinyl acetate copolymer	TSKgel SuperAWM-H	DMF containing 10 mmol/L LiBr
26	Styrene/allyl alcohol copolymer	TSKgel SuperAWM-H	DMF containing 10 mmol/L LiBr
27	Poly (p-phenylene ether sulfone)	TSKgel SuperAWM-H	DMF containing 10 mmol/L LiBr

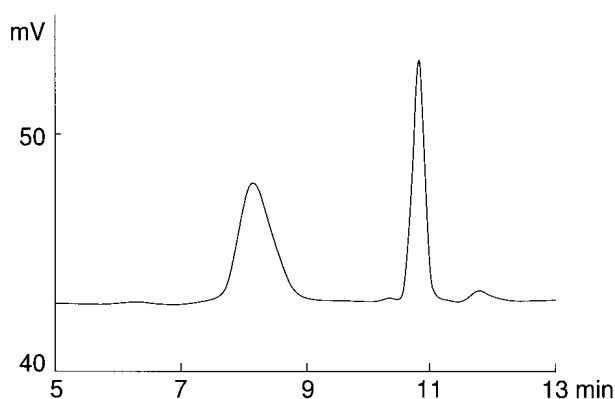


Figure-12 Chromatogram of Sodium Chondroitin Sulfate

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: 0.2mol/L sodium nitrate
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

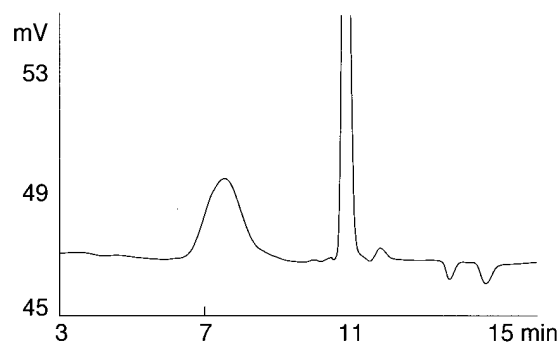


Figure-13 Chromatogram of Sodium Alginate

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: 0.2mol/L sodium nitrate
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

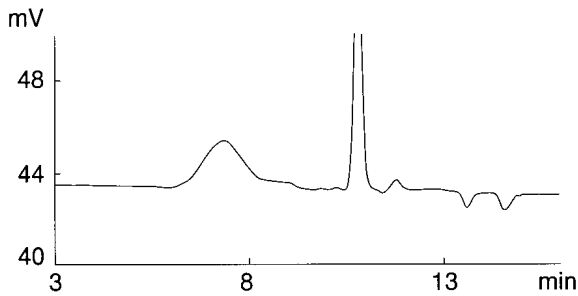


Figure-14 Chromatogram of Carboxymethyl Cellulose

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: 0.2mol/L sodium nitrate
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

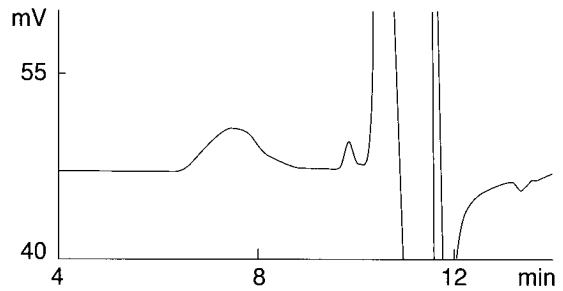


Figure-15 Chromatogram of Sodium Polystyrene Sulfonate

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: 0.2mol/L sodium nitrate/acetonitrile = 80/20
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

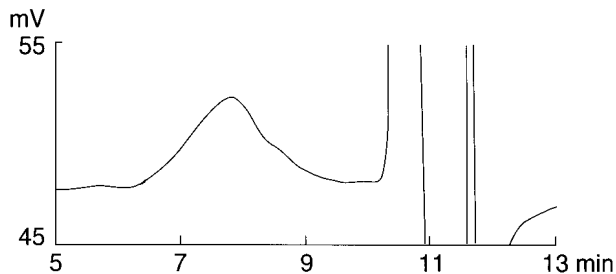


Figure-16 Chromatogram of Polyvinyl Pyrrolidone

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: 0.2mol/L sodium nitrate/acetonitrile = 80/20
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

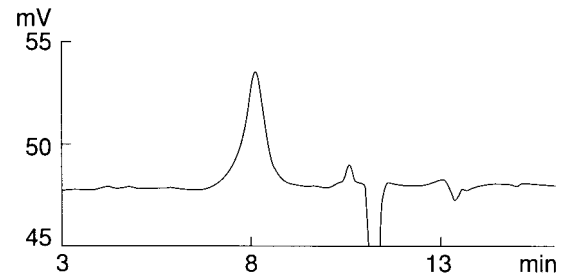


Figure-17 Chromatogram of Gum Arabic

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: 0.2mol/L sodium nitrate/acetonitrile = 80/20
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

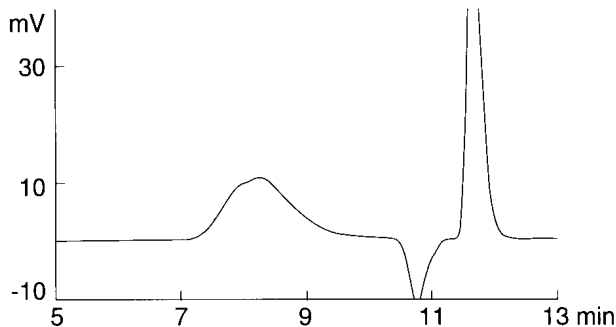


Figure-18 Chromatogram of Ethylhydroxy-ethylcellulose

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: Methanol containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

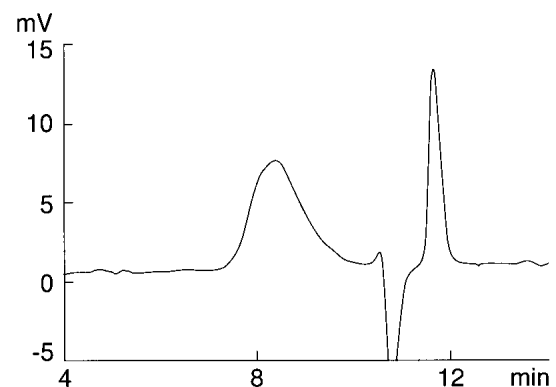


Figure-19 Chromatogram of Vinyl Alcohol/vinyl Butyral Copolymer

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: Methanol containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

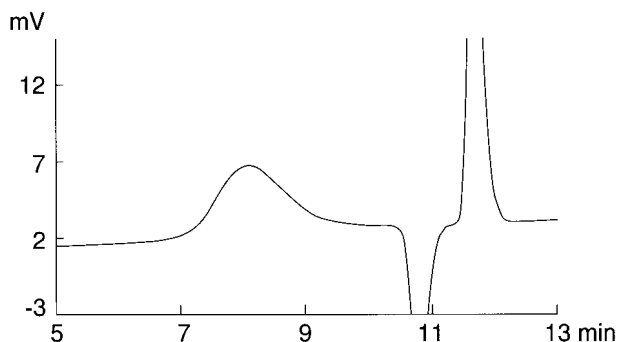


Figure-20 Chromatogram of Hydroxypropylcellulose

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: Methanol containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

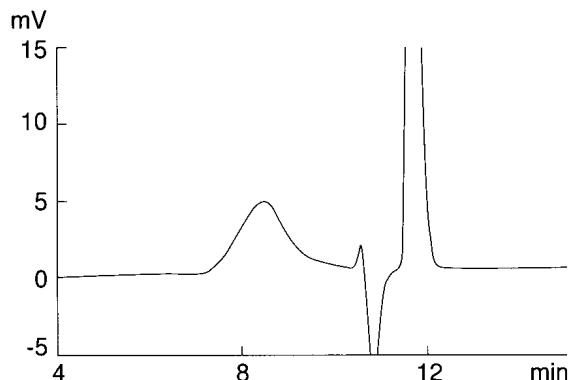


Figure-21 Chromatogram of Polymethyl Vinyl Ether

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: Methanol containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

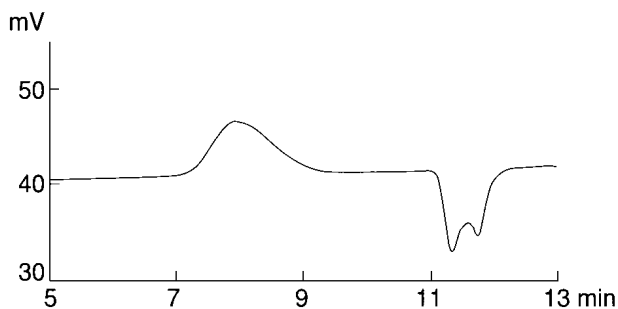


Figure-22 Chromatogram of Cellulose Acetate

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: DMF containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

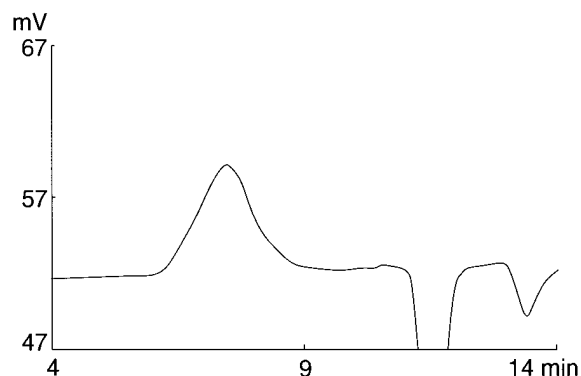


Figure-23 Chromatogram of N-isopropyl Acrylamide

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: Methanol containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

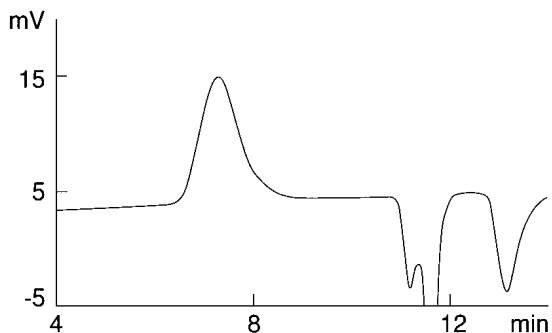


Figure-24 Chromatogram of Polyacrylonitrile

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: DMF containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

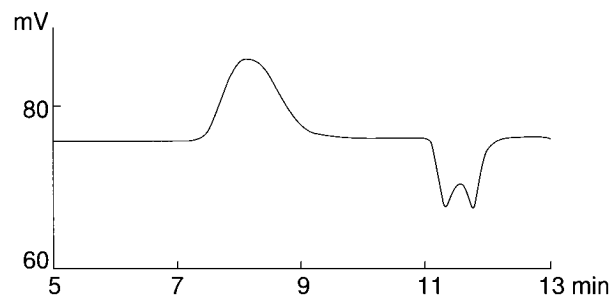


Figure-25 Chromatogram of Vinyl Chloride/vinyl Acetate Copolymer

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: DMF containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20µL (0.5g/L)

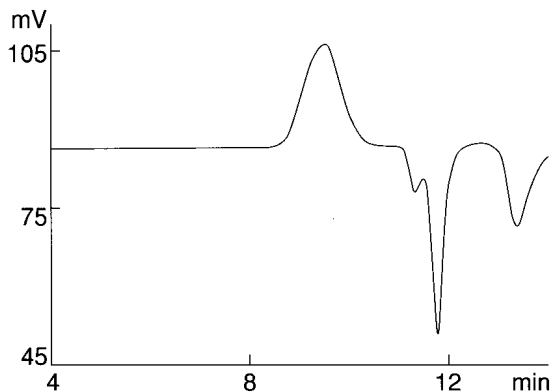


Figure-26 Chromatogram of Styrene/allylalcohol Copolymer

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: DMF containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20μL (0.5g/L)

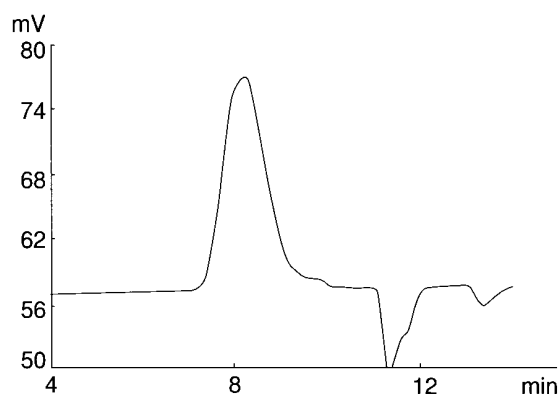


Figure-27 Chromatogram of Poly (p-phenylene Ether Sulfone)

Column: TSKgel SuperAWM-H
(6.0mm I.D. × 15cm × 2)
Eluent: DMF containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20μL (0.5g/L)

4-2. Applications in Non-SEC Mode

Since TSKgel SuperAW columns have excellent solvent compatibility as previously discussed, it is possible to obtain different chromatograms as shown in Figure-28 by changing the eluent composition on samples such as surfactant. That is, the sample is separated based on the molecular size (SEC mode) in 60% acetonitrile solution, and it is retained in other eluent compositions (non-SEC mode). Thus it is possible to set up the elution conditions to suit the purpose of measurement (molecular weight measurement, assay, separation) in one column. Applications of measuring formulated drugs are shown in Figures-29 and -30. In Figure-29, the low-molecular weight components of poultice are retained in the column and clearly separated. In Figure-30, additives are retained in the column and separated. It is apparently very effective in separation of samples containing from low-molecular weight to high-molecular weight components or several low-molecular weight components.

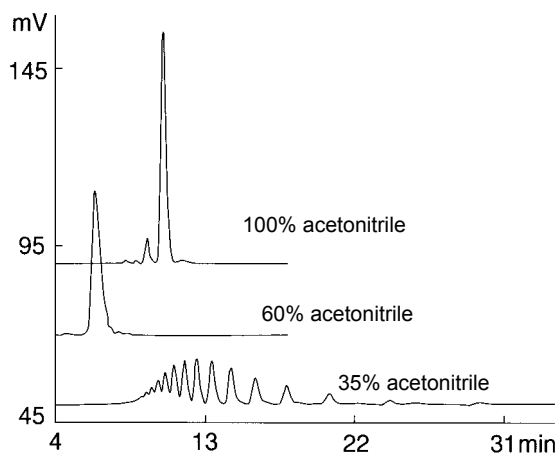


Figure-28 Measurement of a Surfactant

Column: TSKgel SuperAW2500
(6.0mm I.D. × 15cm)
Eluent: Acetonitrile, acetonitrile solution
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: UV (280nm)
Injection volume: 20μL

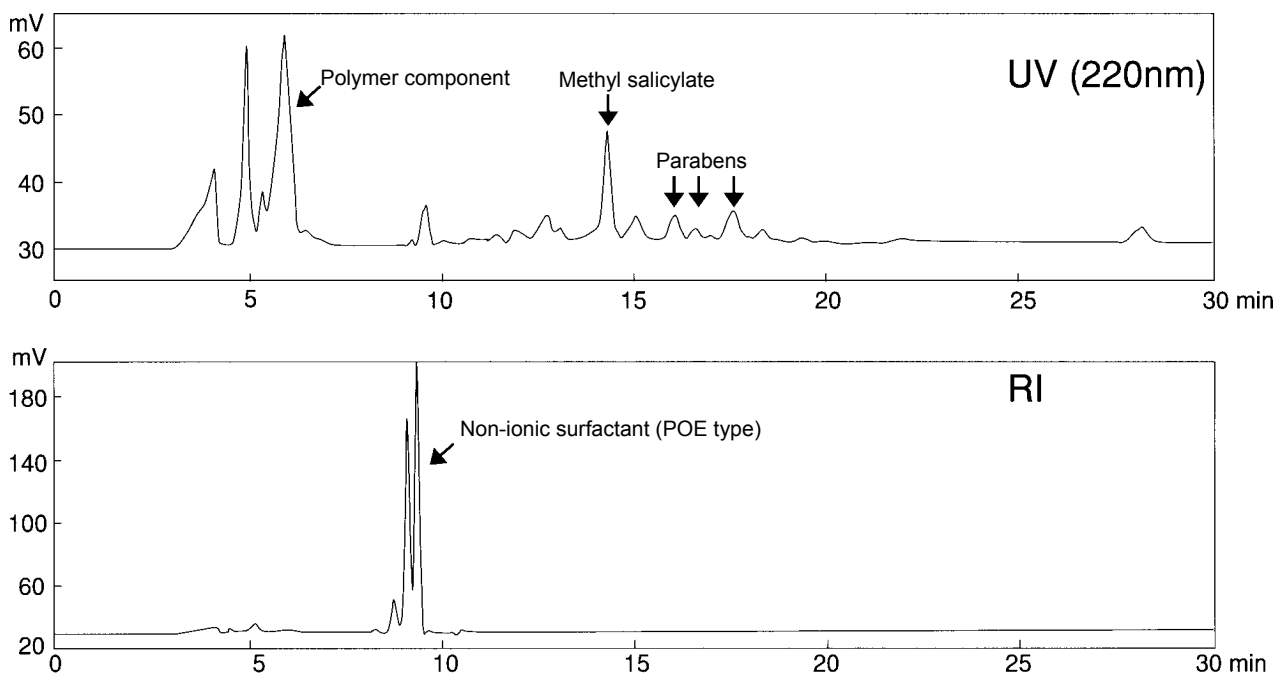


Figure-29 Application on Poultice

Column: TSKgel SuperAW2500
 (6.0mm I.D. × 15cm × 2)
 Eluent: Methanol/water = 60/40
 Flow rate: 0.6mL/min
 Temperature: 40°C
 Detection: UV (220nm), refractive index detector
 Injection volume: 10µL

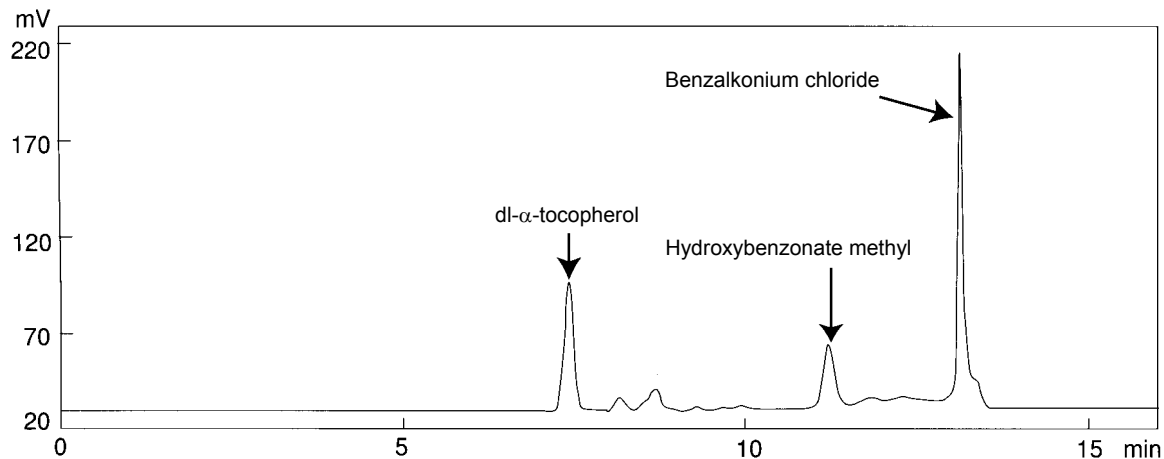


Figure-30 Application on Cream

Column: TSKgel SuperAW2500
 (6.0mm I.D. × 15cm × 2)
 Eluent: Ethanol
 Flow rate: 0.6mL/min
 Temperature: 40°C
 Detection: UV (275nm)
 Injection volume: 10µL

5. Conclusion

As described in the above sections, it should be clear that TSKgel SuperAW series is a group of columns that covers a wide range from aqueous SEC to polar organic solvent SEC. Analyses can be achieved at a high speed, high resolution and with considerably less solvent consumption compared to conventional columns. In addition, measurement in non-SEC mode is possible making TSKgel SuperAW columns an excellent first choice for measuring unknown samples.