No. 099

TOSOH

SEPARATION REPORT

High-performance SEC Semi-Micro Columns Compatible with both Aqueous SEC and Polar Organic SEC, TSKgel SuperAW Series

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TOSOH CORPORATION

1. Introduction

Size exclusion chromatography (SEC) is a method that separates compounds dissolved in aqueous solution or organic solvents by their molecular sizes. There is a wide range of applicable substances from low molecular weight to high molecular weight and from hydrophilic compounds to hydrophobic compounds, and it is used in various fields. Since it enables not only separation and purification, but also measuring molecular weight or molecular weight distribution of macromolecules, it has especially become an inevitable method in research of macromolecules.

In succession to its organic solvent SEC columns, TSKgel H_{XL} series, Tosoh has commercialized several groups of products, including H_{HR} series which allows the use of various organic solvents and SuperH column series for ultra-fast GPC. Meanwhile, PW_{XL} series has been commercialized as a group of aqueous SEC columns and α series as a group of SEC columns that can be used widely with aqueous solutions to polar organic solvents.

It has recently commercialized TSKgel SuperAW series, which has been developed by packing hydrophilic base materials with small particle size in semi-micro column and allows solvent replacement from aqueous solutions to organic solvents.

In this article, the basic features of TSKgel SuperAW series and applications on various samples are introduced.

Table-1 Features of TSKgel Super AW

Feature	Advantage
1) Micro-particle gel packed in semi-micro column	• 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	• 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	• High durability

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 used as eluent, and they may have interaction with samples according to its properties when specific packing material is used for various samples with different properties. Therefore, it was necessary that columns were selected for each type of eluent or sample to be analyzed. TSKgel α series was introduced as a column to solve these problems, which has been received well as a SEC column that covers from aqueous systems to polar organic solvent systems. TSKgel SuperAW series that has been newly developed is capable of achieving separation equivalent to conventional column with 1/2 for analysis time and 1/3 for solvent consumption, due to the micro-particle packing material that has been packed into semi-micro column. In addition, since hydrophilic base material has been used as the packing material as in α series, suppression of hydrophobic interaction with sample and SEC measurement under polar organic solvents became possible. With drastic improvements in swelling/shrinkage properties of the packing material, solvent can be replaced in a wide range from water (aqueous solutions) to organic solvents. The features of TSKgel SuperAW series are summarized in Table-1. TSK-Gel SuperAW series consists of 5 types of columns in which gels with different separation ranges and 1 type of mixed column. These 6 types of columns 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-2 List of TSKgel SuperAW Series

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

I.D. indicates the internal diameter.

3. Basic Properties of SuperAW Series

3-1. Solvent Compatibility

Figure-1 shows the results of solvent compatibility test on TSKgel SuperAW series. In this test, theoretical plates were measured after substituting from water to test solvent (flow rate: 0.6mL/min, 5 hours or longer), standing (14 hours or longer), and then substituting with water (flow rate: 0.6mL/min) again. This was repeatedly performed on various test solvents. It is evident that in the column of any grade, change in theoretical plates was small even after exchanging with various solvents and that they allow replacement of various solvents from aqueous solutions to polar organic solvents.

3-2. Calibration Curves with Various Solvents

Although TSKgel SuperAW series allows exchange to various solvents, it is necessary that the standard sample used should be selected to suit the solvent in actual use. Figures-2 to -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 difference was seen in molecular weight separation range and slope depending on the solvent type.

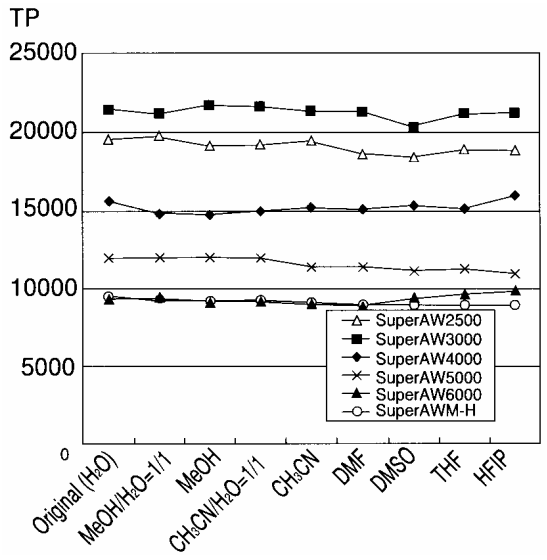

Figure-1 Solvent Compatibility of TSKgel SuperAW Series
(Theoretical Plates Measurement Conditions)

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)

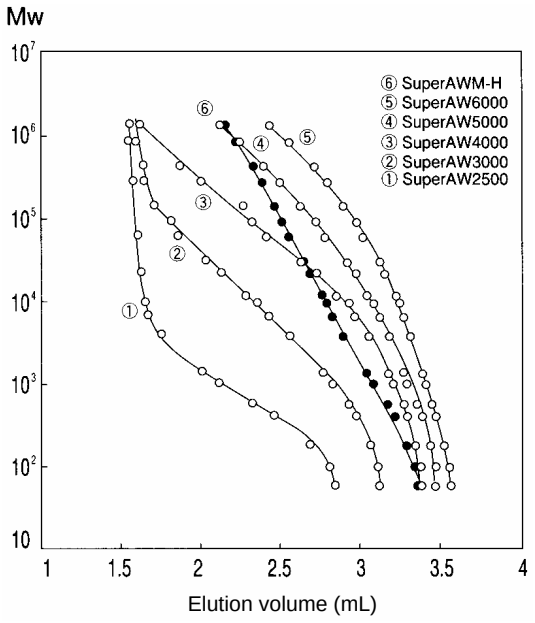

Figure-2 Calibration Curves of TSKgel SuperAW Series (1)

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

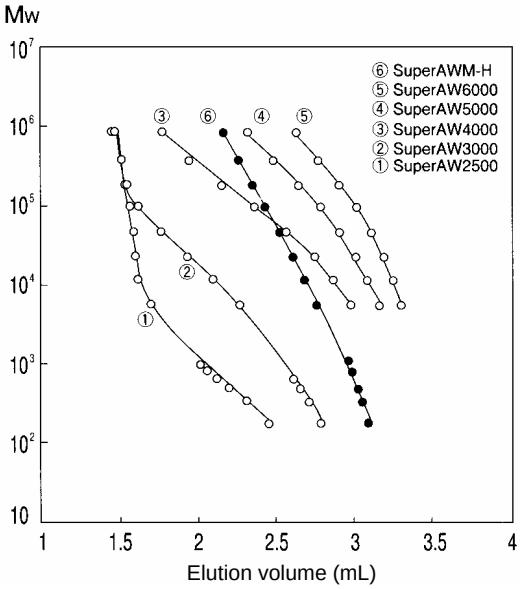

Figure-3 Calibration Curves of TSKgel SuperAW Series (2)

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

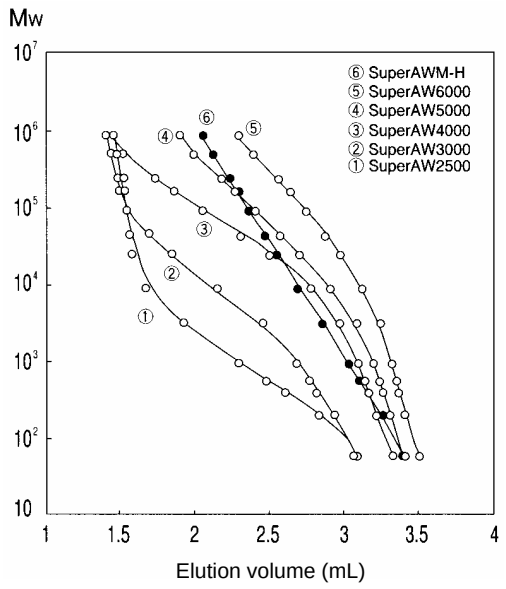

Figure-4 Calibration Curves of TSKgel SuperAW Series (3)

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

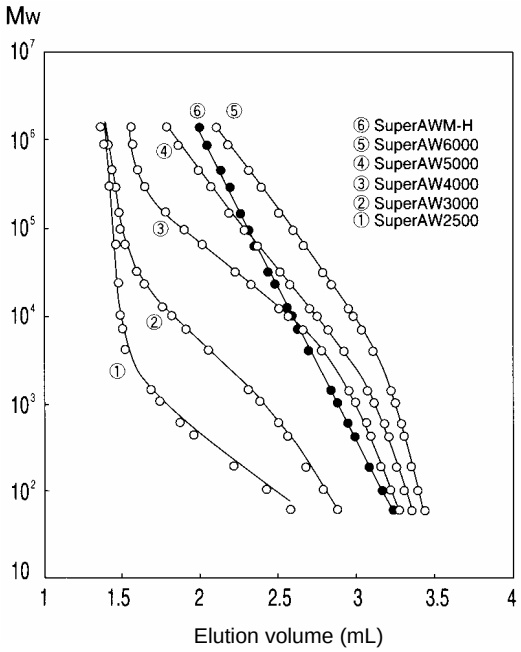

Figure-5 Calibration Curves of TSKgel SuperAW Series (4)

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

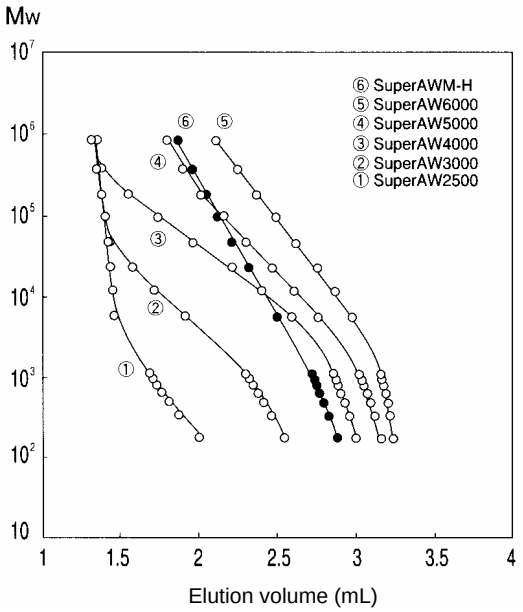

Figure-6 Calibration Curves of TSKgel SuperAW Series (5)

Column: TSKgel SuperAW Series (6.0mm I.D. × 15cm)
Eluent: DMSO containing 10mmol/L $NaNO_3$
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 the present TSKgel PW_{XL} series or TSKgel α series, TSKgel SuperAW series has been packed with gel with smaller particle size. The column performance depends on the particle size, and the column efficiency increases as the particle size decreases. Furthermore, it has theoretical plates per unit length that is twice compared to TSKgel PW_{XL} series since it is packed in a column with a small dead volume structure. Figure-7 shows chromatograms of dextran T-40 hydrolysate measured on TSKgel SuperAW2500 and TSKgel $G2500PW_{XL}$. It is apparent that SuperAW2500 column yields separation equivalent to that of the conventional column in approximately half the time.

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

As it is apparent, TSKgel SuperAW series can be called a group of columns with very high cost performance, which is capable of obtaining chromatograms equivalent to those of the conventional general SEC columns with 1/2 measurement time and 1/3 solvent consumption.

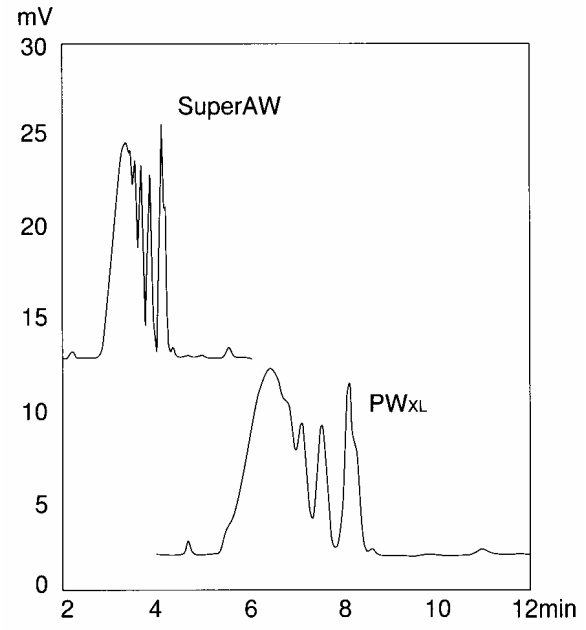

Figure-7 Comparison of Chromatograms

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 HETP on ethylene glycol in SuperAW2500 and G2500PW$_{XL}$. While the optimal linear velocity of PW$_{XL}$ column is 10mm/min (flow rate 0.5mL/min) and HETP changes drastically around this value, SuperAW column has smallest HETP in the linear velocity range of 10 to 20mm/min (flow rate 0.3 to 0.6mL/min), yielding almost no change. That is, SuperAW column indicates high theoretical plates in this flow rate range and has little deterioration in performance even 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 with extremely small effect of flow rate on sample separation, which can exert high column efficiency in a wide range of flow rates.

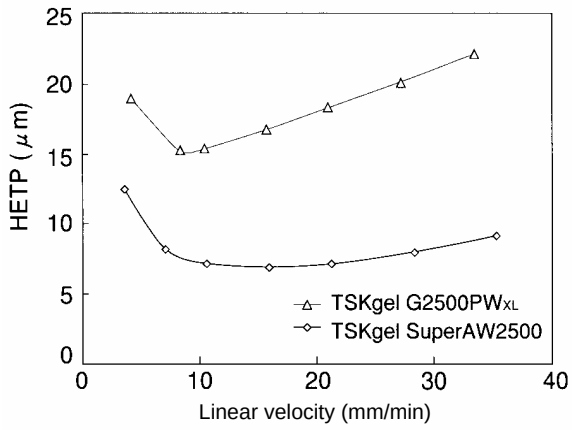

Figure-8 Relationship between HETP and Linear Velocity (1)

Column: TSKgel SuperAW2500 (6.0mm I.D. × 15cm) TSKgel G2500PWXL (7.8mm I.D. × 30cm)
Eluent: Water
Temperature: 25°C
Sample: Ethylene glycol 2.5g/L
Injection volume: 5μL (TSKgel SuperAW2500) 10μL (TSKgel G2500PW$_{XL}$)

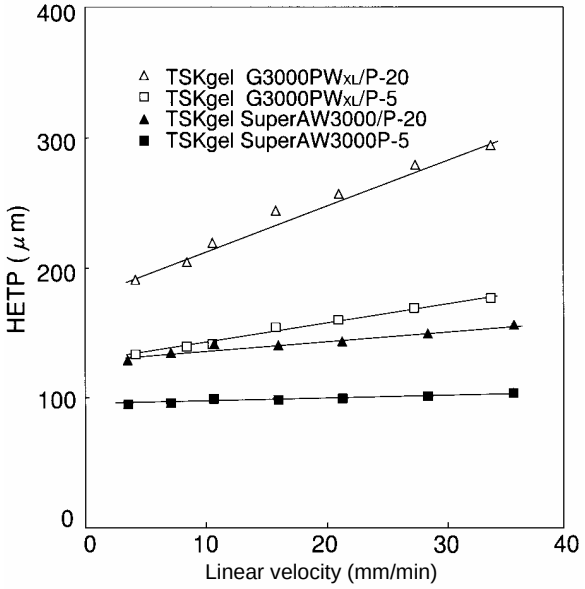

Figure-9 Relationship between HETP and Linear Velocity (2)

Column: TSKgel SuperAW3000 (6.0mm I.D. × 15cm) TSKgel G3000PWXL (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 affects separation. The effect of injection volume on HETP using a macromolecule (pullulan) with molecular weight distribution and low-molecular weight compound (ethylene glycol) is shown (Figures-10 and -11). While increase in HETP is seen on PW_{XL} column when it exceeds 20μL for ethylene glycol, HETP increases from a little beyond 5μL on SuperAW column. On the other hand, no steep increase in HETP is seen with pullulan until it reaches about 20μL on SuperAW column. Thus it is necessary that sample injection volume of 5μL per column is set when SuperAW column is used for separation of low-molecular weight substances or oligomers, and 20μL per column is set when it is used for measurement of molecular weight or molecular weight distribution of macromolecules.

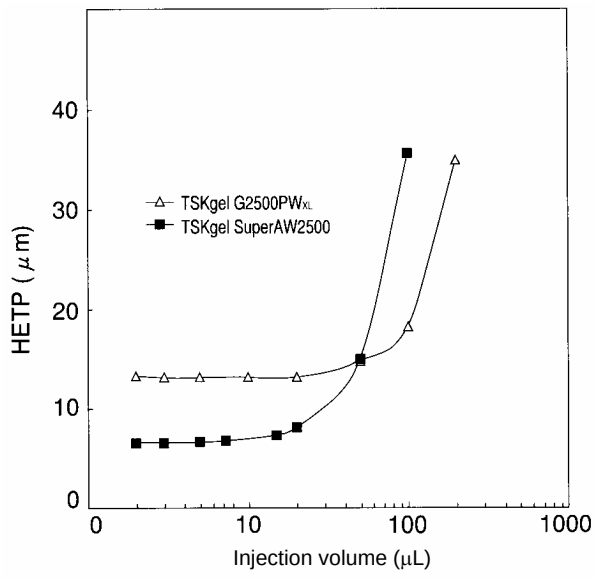

Figure-10 Relationship between HETP and Sample Injection Volume (1)

Column: TSKgel SuperAW2500 (6.0mm I.D. × 15cm)
TSKgel $G2500PW_{XL}$ (7.8mm I.D. × 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

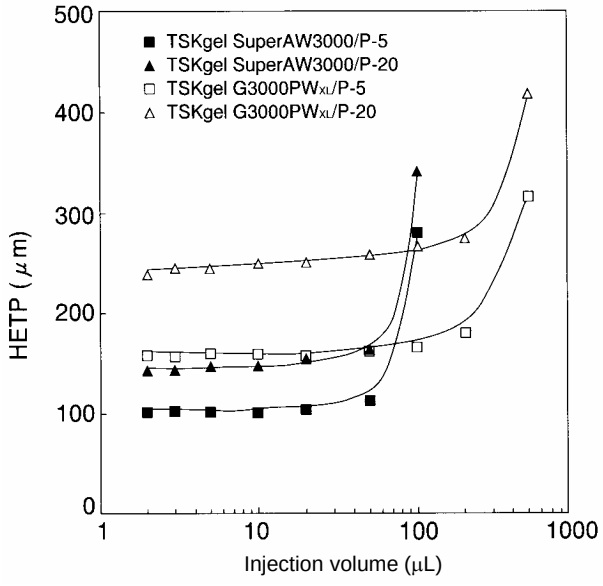

Figure-11 Relationship between HETP and Sample Injection Volume (2)

Column: TSKgel SuperAW3000
(6.0mm I.D. × 15cm)
TSKgel $G3000PW_{XL}$
(7.8mm I.D. × 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: 5μL (TSKgel SuperAW3000)
10μL (TSKgel $G3000PW_{XL}$)

4. Applications

4-1. Applications of Various Polymer Measurement

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/allylalcohol 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

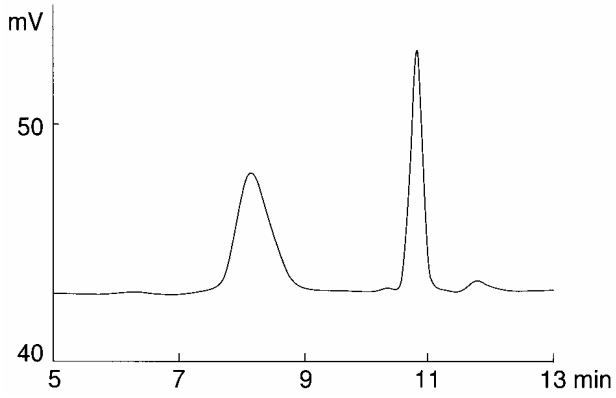

Figure-12 Chromatogram of Sodium Chondroitin Sulfate

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)

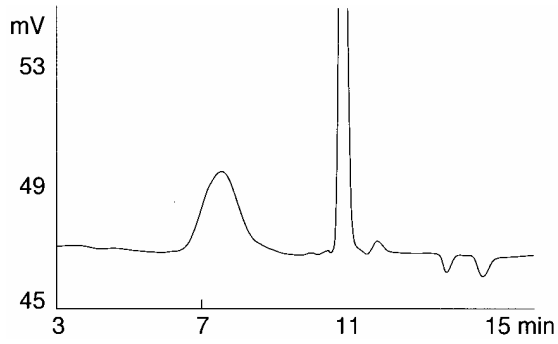

Figure-13 Chromatogram of Sodium Alginate

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)

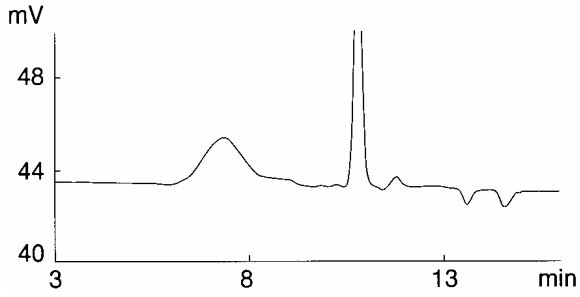

Figure-14 Chromatogram of Carboxymethyl Cellulose

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)

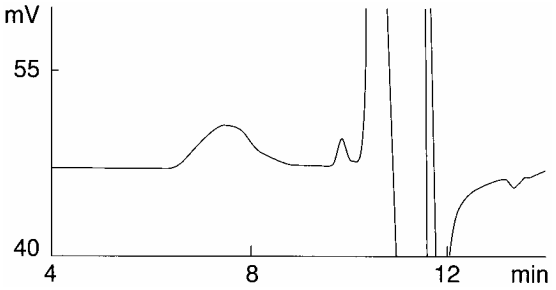

Figure-15 Chromatogram of Sodium Polystyrene Sulfonate

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)

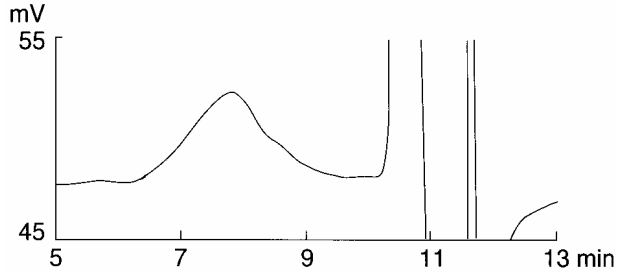

Figure-16 Chromatogram of Polyvinyl Pyrrolidone

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)

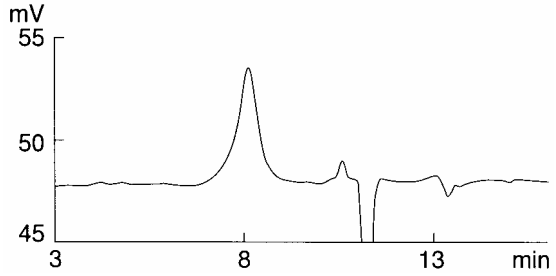

Figure-17 Chromatogram of Gum Arabic

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)

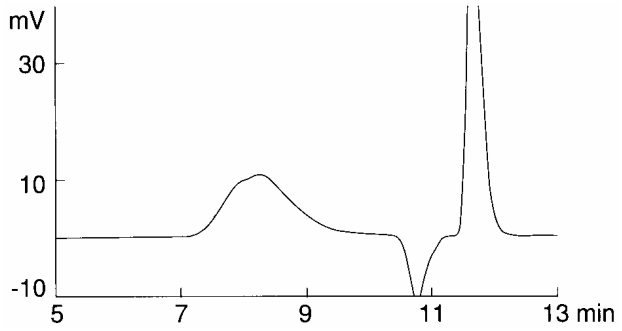

Figure-18 Chromatogram of Ethylhydroxy-ethylcellulose

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)

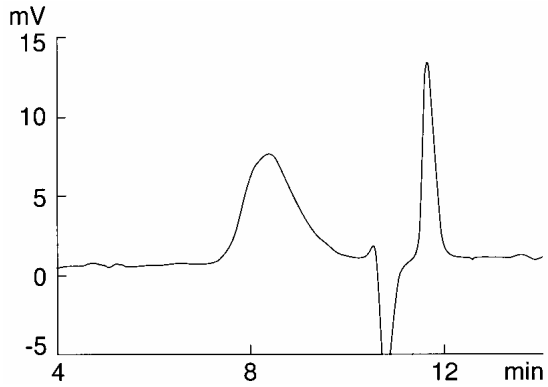

Figure-19 Chromatogram of Vinyl Alcohol/vinyl Butyral Copolymer

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)

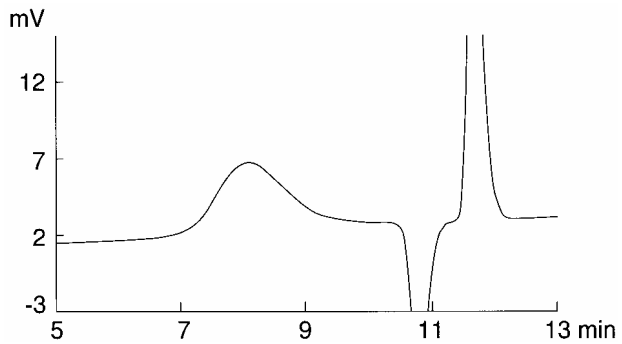

Figure-20 Chromatogram of Hydroxypropylcellulose

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)

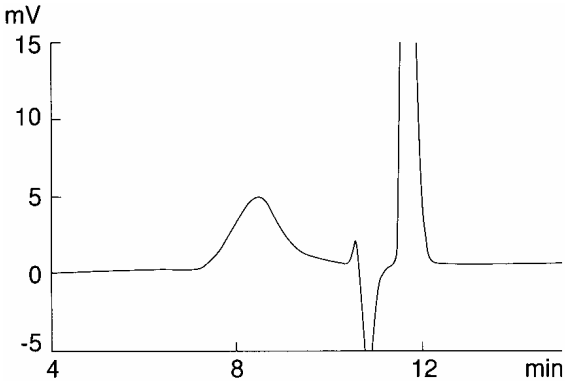

Figure-21 Chromatogram of Polymethyl Vinyl Ether

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)

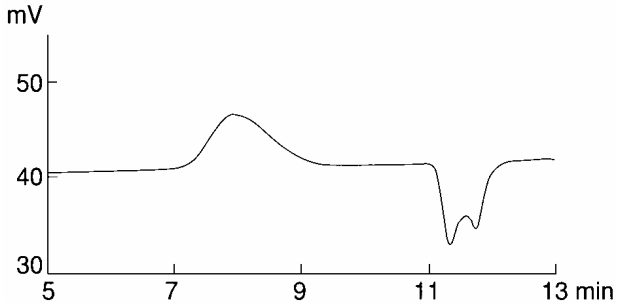

Figure-22 Chromatogram of Cellulose Acetate

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)

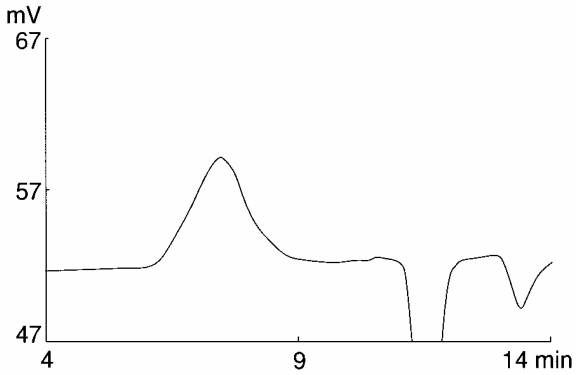

Figure-23 Chromatogram of N-isopropyl Acrylamide

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)

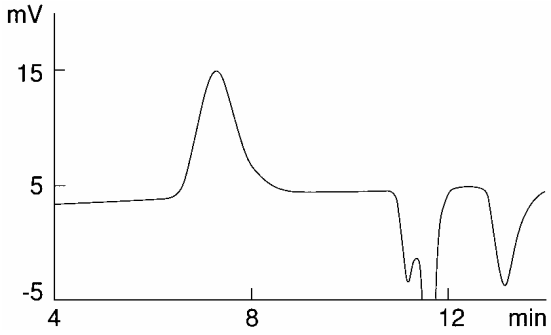

Figure-24 Chromatogram of Polyacrylonitrile

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)

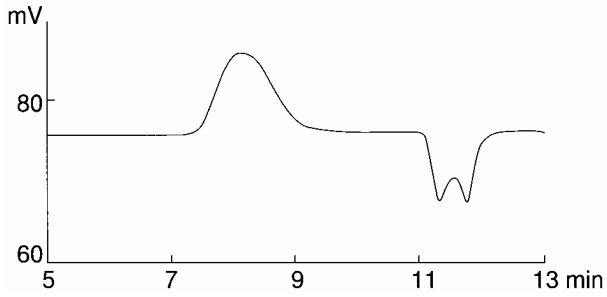

Figure-25 Chromatogram of Vinyl Chloride/vinyl Acetate Copolymer

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)

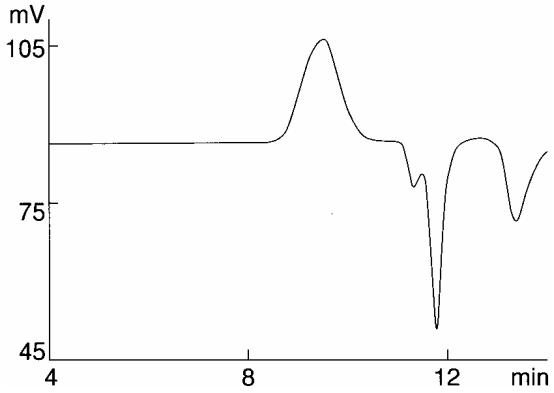

Figure-26 Chromatogram of Styrene/allylalcohol Copolymer

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)

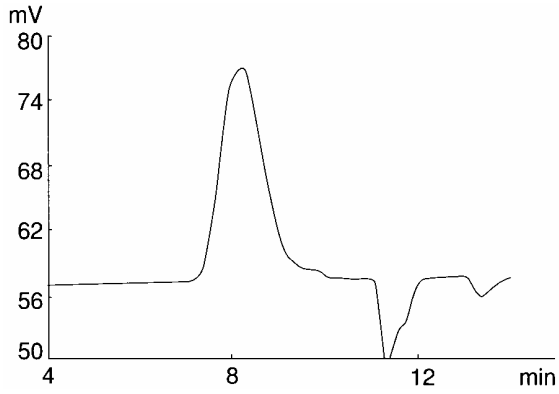

Figure-27 Chromatogram of Poly (p-phenylene Ether Sulfone)

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 it has excellent solvent compatibility, it is possible to obtain different chromatograms as shown in Figure-28 by changing the eluent 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.

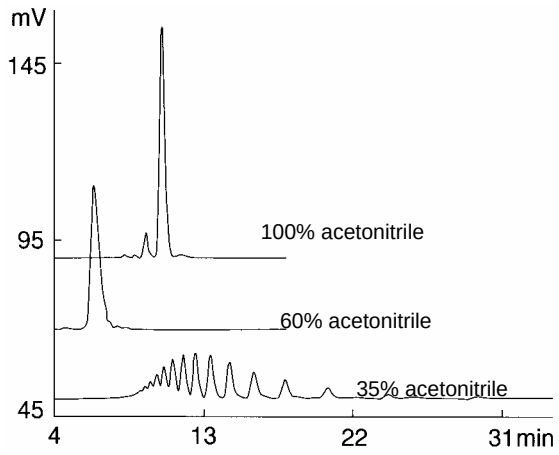

Figure-28 Measurement of a Surfactant

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

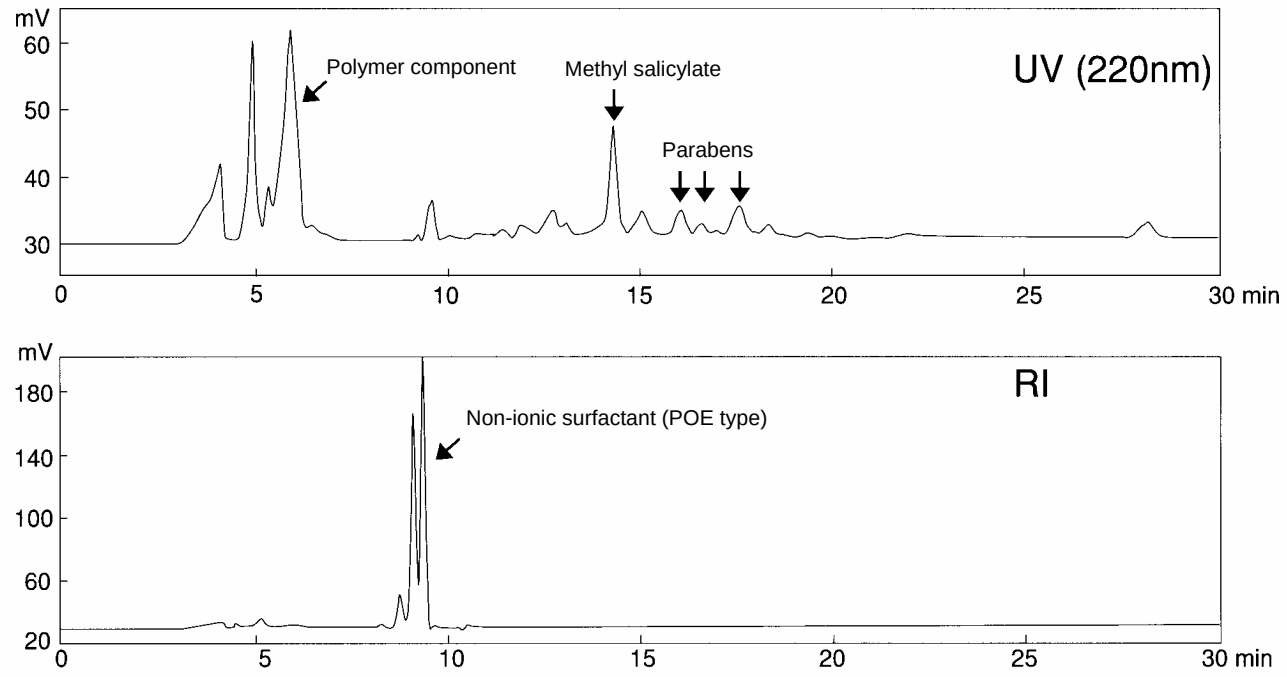

Figure-29 Application on Poultice

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

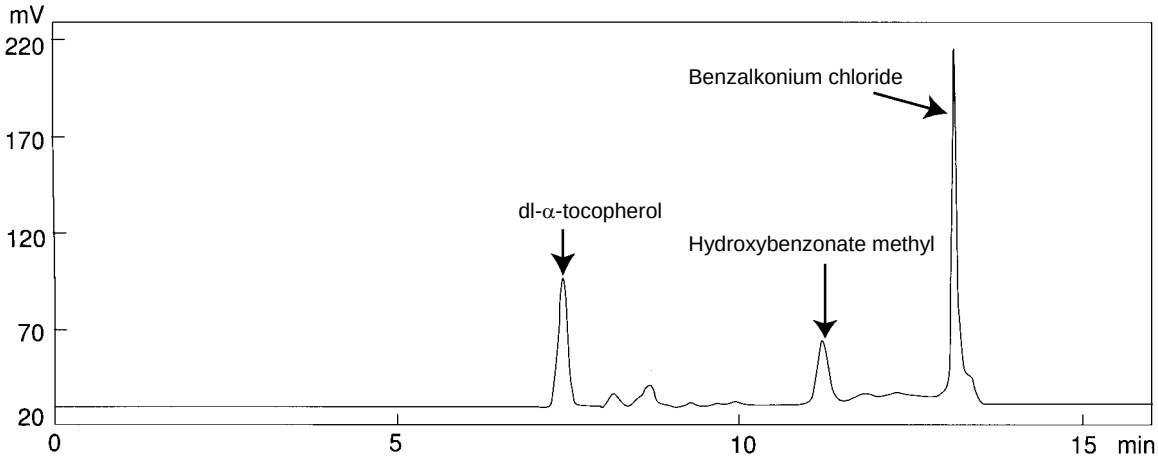

Figure-30 Application on Cream

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, and has high general versatility achieving high speed, high resolution, and solvent saving compared to conventional columns. In addition, measurement in non-SEC mode is easily examined, and it can be called a first choice column for measuring unknown samples.