Separation Report No. 094

Analyzing Polymers that are Soluble in Polar-Organic Solvents with TSKgel Alpha Series Columns

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TOSOH

TOSOH BIOSCIENCE LLC
Separations Business Unit

3604 Horizon Drive, Suite 100, King of Prussia, PA 19406
Phone: (484) 805-1219 FAX: (610) 272-3028
Orders and Technical Service: (800) 366-4875
Member of the TOSOH Group

1. Introduction

Gel permeation chromatography (GPC), now often referred to by the acronym SEC, which stands for size exclusion chromatography, is a procedure for separating a wide range of polymers, both polar and non-polar, based on the molecular size of the sample. SEC is typically used to measure molecular weight and analyze branching and distribution or other properties of polymers. Other applications include the separation and pattern analysis of oligomers and low molecular weight compounds.

Tosoh offers a large selection of high performance liquid chromatography columns for analyzing the properties of polymers, including the TSKgel H_{XL} Series, a line of high performance SEC columns for polymers that are soluble in organic solvents, the TSKgel H_{HR} Series of high performance SEC columns capable of withstanding change-over between organic solvents, and the TSKgel SuperH Series of ultra high performance SEC columns. These columns can be used for a broad range of polymers that are soluble in organic solvents. Tosoh also manufactures the TSKgel PW_{XL} Series of high performance SEC columns, which use an aqueous solvent or a mixture of water and a lower percentage organic solvent, and are primarily used to analyze water-soluble polymers. However, difficulties relating to sample solubility, adsorption of molecular weight standards and solvent compatibility of columns have occurred when conventional TSKgel H-type and PW-type columns were used for SEC of polar polymers.

The recently launched TSKgel Alpha Series of GPC columns are capable of solving these issues in analyzing polar polymers. This report describes the basic features of the TSKgel Alpha Series and presents examples of separations of polar polymers using a variety of solvents.

2. Features of Alpha Series Columns

Problems have occurred when polar solvents were used with packing materials composed of styrene divinylbenzene copolymer, including adsorption of low molecular weight polystyrene standards and hydrophobic interaction between samples and packing materials. Furthermore, when a hydrophilic polymer is used as the packing material, compatibility between the packing material and various types of polar solvents is not perfect. Consequently, it has been difficult to select the optimal column for use in SEC for polar polymer samples.
The TSKgel Alpha Series of columns for SEC was developed to resolve these conflicts. The features of TSKgel Alpha columns are summarized in Table 1. The TSKgel Alpha Series of columns provide a solution for the following two common problems encountered when characterizing polar polymers.

1) A hydrophilic packing material was used to minimize hydrophobic interaction between the column packing material and polar polymers.
   The use of a hydrophilic material eliminates adsorption of molecular weight standards, even with polar solvents, and permits accurate molecular weight determination. Also, because hydrophobic adsorption of the samples is minimized, reproducibility is improved and the types of polymers that can be studied are expanded.
2) TSKgel Alpha Series columns are compatible with an extensive variety of polar organic solvents. The packing material is much more resistant to swelling and shrinking compared to, for instance, TSKgel PW_{XL}-type columns.

Table 1 Features of TSKgel Alpha Series Columns

Feature	Benefits
1) Hydrophilic matrix	・ Limited hydrophobic adsorption even in the presence of polar organic solvents. ・ Accurate calibration curves in dimethylformamide (DMF), due to elimination of adsorption of standard polystyrene samples as seen when polar polymers are analyzed on TSKgel H-type columns in DMF-based solvents.
2) Solvent compatibility	・ Depending on the analyte, a wide range of solvents can be selected, from water to non-polar solvents. ・ When using UV detection, solvents that do not absorb short wavelength light can be used (for example, water, methanol, acetonitrile, and HFIP), and in-line use with RI detection is also possible. ・ Accurate molecular weight determination is possible in DMF-based solvent systems because salt peaks do not overlap with the peaks of low molecular weight polymers.
3) Other characteristics ・ Ability to handle changes in salt concentration ・ Superior mechanical strength ・ Stable up to 80°C	・ Column is not affected by changes in salt concentration, which simplifies method development. ・ Analysis can be performed at high flow rates. ・ When using solvents or samples of high viscosity, working at a higher temperature may offset the expected increase in pressure. ・ Increasing the temperature can also lead to improvements in column efficiency and column calibration.

With conventional GPC columns packed with hydrophilic matrices, switching from an aqueous solvent to an organic solvent may lead to shrinking or swelling of the packed resin. Therefore, the use of polar solvents is often greatly restricted. With the TSKgel Alpha Series of columns, the packing material was engineered to display minimal swelling and shrinking even when a wide variety of solvents (from water to mildly polar solvents) are used. Thus, column performance is maintained when changing solvents.

The TSKgel Alpha Series consists of a line of solvent-compatible SEC columns packed with hydrophilic polymer particles of six different separation ranges. Table 2 lists the grades available in the TSKgel Alpha Series. Because the TSKgel Alpha Series covers a broad separation range and permits the selection of solvent that best dissolves the sample, it can be used with a wide range of polymers that are soluble in water or various polar solvents. However, the calibration curves of the various columns will differ depending on the solvent used, and thus as discussed below, it is necessary to prepare calibration curves using the appropriate molecular weight standards for the solvent system employed.

Table 2 TSKgel Alpha Series Grades

Grade	Column size (mm ID×cm)	Minimum number of plates[1] (30cm)	Exclusion limit molecular weight (Da) PEO/H_2O[2]	PS/DMF[3]
Alpha-2500	7.8×30	16,000	5×10^3	1×10^4
Alpha-3000	7.8×30	16,000	9×10^4	1×10^5
Alpha-4000	7.8×30	10,000	4×10^5	1×10^6
Alpha-5000	7.8×30	10,000	1×10^6	7×10^6
Alpha-6000	7.8×30	7,000	1×10^7	1×10^7
Alpha-M	7.8×30	7,000	1×10^7	1×10^7

1) Eluent: H_2O
Flow rate: 1.0mL/min
Sample: ethylene glycol
Temperature: 25°C
Detection: RI
2) Eluent: H_2O
Flow rate: 1.0mL/min
Sample: standard poly(ethylene oxide)
Temperature: 25°C
Detection: RI
3) Eluent: dimethylformamide
Flow rate: 1.0mL/min
Sample: standard polystyrene
Temperature: 25°C
Detection: RI

3. Basic Characteristics of TSKgel Alpha Series Columns

1) Cha nging Solvents

Table 3 shows solvent compatibility of TSKgel Alpha Series columns for several solvents. There was little change in theoretical plate number after each of the solvents were changed, clearly demonstrating that the TSKgel Alpha Series has an outstanding stability to accommodate solvent changes. A single column can be used with a variety of solvents from aqueous to non-polar solvents, which offers the opportunity to choose a solvent based on the properties of the sample being analyzed.

Table 3 TSKgel Alpha Series: Solvent Compatibility

Solvent	Number of Theoretical Plates	
TSKgel	Alpha-2500	Alpha-3000
Methanol	27,700	25,370
Ethanol	16,760	25,120
THF	24,340	25,370
DMF	24,550	25,370
DMSO	25,840	28,800
Isopropanol	20,630	25,610
DMSO/H_2O = 1/1	24,450	25,120
Methanol/H_2O = 1/1	25,730	20,900
Acetonitrile/H_2O = 1/1	24,530	21,540
THF/H_2O = 1/1	23,850	22,200
HFIP	18,720	28,720

Solvent change conditions:

- Flow rate and temperature during change-over to test solvent: 1.0mL/min, 25°C
- Duration of flow-through after change-over from water to test solvent: 8h
- Flow rate and temperature during change-over from test solvent to water: 1.0mL/min, 25°C

Conditions for measuring number of theoretical plates:

- Eluent: H_2O
- Flow rate: 1.0mL/min
- Temperature: 25°C
- Detection: RI
- Sample: ethylene glycol

2) Calib ration curves

Although TSKgel Alpha columns are solvent-compatible, a few properties must be considered when selecting the molecular weight standard to use for construction of the calibration curve. These properties include the solubility of the polymer in the solvent and the potential of polymer adsorption by the packing material. Table 4 depicts the solvents and appropriate molecular weight standards used with the TSKgel Alpha Series columns. Calibration curves for various solvents are illustrated in Figures 1 through 8. It is clear that the calibration curve will differ depending on the type of solvent used in the column.

Table 4 Conditions for Generating Calibration Curves with TSKgel Alpha Series Columns

Eluent used	Molecular weight standard	Calibration curve	Notes
1) H_2O	PEO/PEG	Fig. 1	
2) H_2O	Pullulan	Fig. 2	
3) 0.1M $NaClO_4$/ACN	PNASS	Fig. 3	
4) Methanol (10mmol/L LiBr)	PEO/PEG	Fig. 4	Not readily soluble at high temps. (≥ 60ºC)
5) THF	Polystyrene	Fig. 5	
6) Polystyrene	Polystyrene	Fig. 6	
7) DMF (10mmol/L LiBr)	PEO/PEG	Fig. 7	
8) DMF (10mmol/L LiBr)	Polystyrene	Fig. 8	
9) H_2O	PEO/PEG	Figs. 9-11	Temp 25, 40, 60ºC
10) DMF	PEO/PEG	Figs. 12-14	Temp 25, 40, 60, 80ºC

* THF : tetrahydrofuran
DMF : dimethylformamide
DMSO : dimethylsulfoxide
HFIP : hexafluoroisopropanol
PEO : poly(ethylene oxide)
PEG : poly(ethylene glycol)
PNASS : poly(sodium styrene sulfonate)

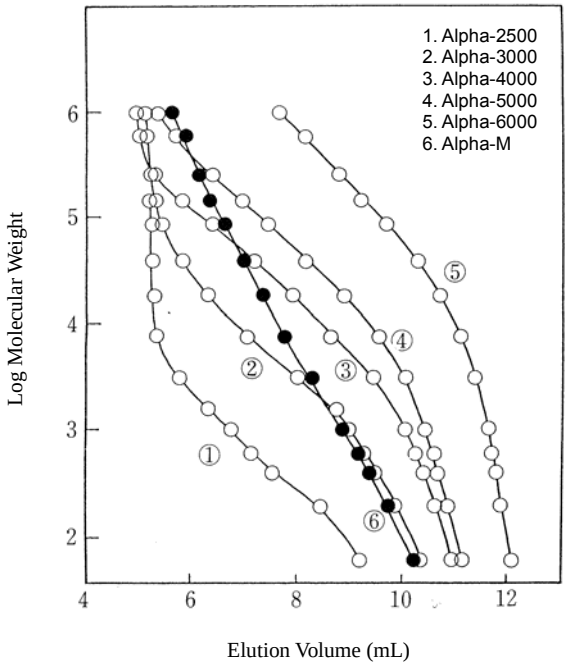

Fig. 1 TSKgel Alpha Series Calibration Curve (H_2O/PEO, PEG)

Column: TSKgel Alpha Series
Eluent: H_2O
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Samples: poly(ethylene oxide), poly(ethylene glycol) and ethylene glycol

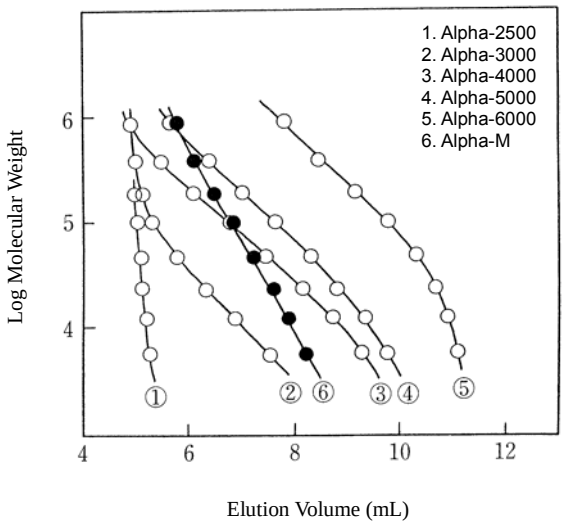

Fig. 2 TSKgel Alpha Series Calibration Curve (H_2O/pullulan)

Column: TSKgel Alpha Series
Eluent: H_2O
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Sample: pullulan

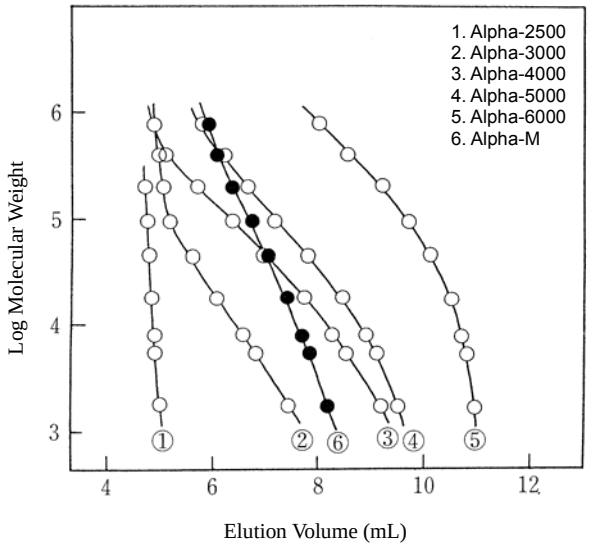

Fig. 3 TSKgel Alpha Series Calibration Curve (Aqueous solution/PNASS)

Column: TSKgel Alpha Series
Eluent: 0.1mmol/L sodium perchlorate in 22.5% acetonitrile
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Sample: poly(sodium polystyrene sulfonate)

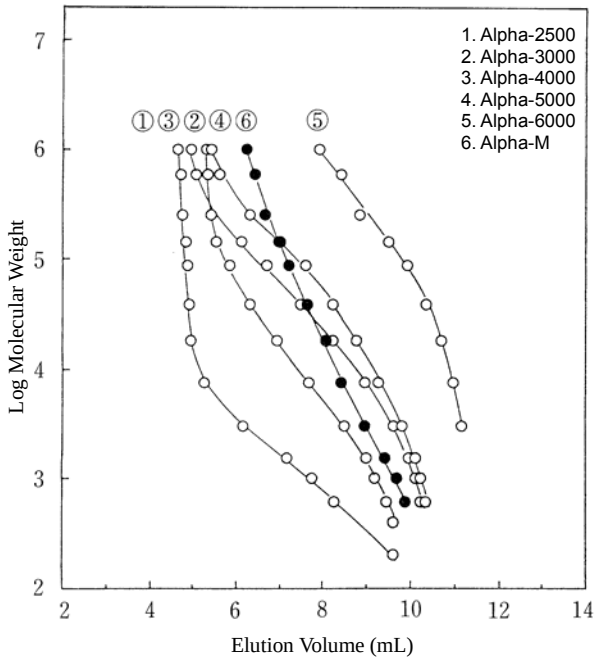

Fig. 4 TSKgel Alpha Series Calibration Curve (Methanol/PEO, PEG)

Column: TSKgel Alpha Series
Eluent: 10mmol/L LiBr in methanol
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Samples: standard poly(ethylene oxide), poly(ethylene glycol) and ethylene glycol

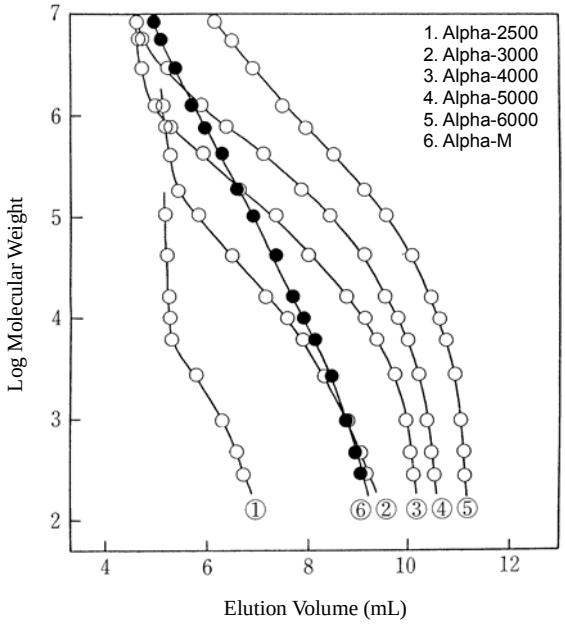

Fig. 5 TSKgel Alpha Series Calibration Curve (THF/PS)

Column: TSKgel Alpha Series
Eluent: tetrahydrofuran
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Sample: polystyrene

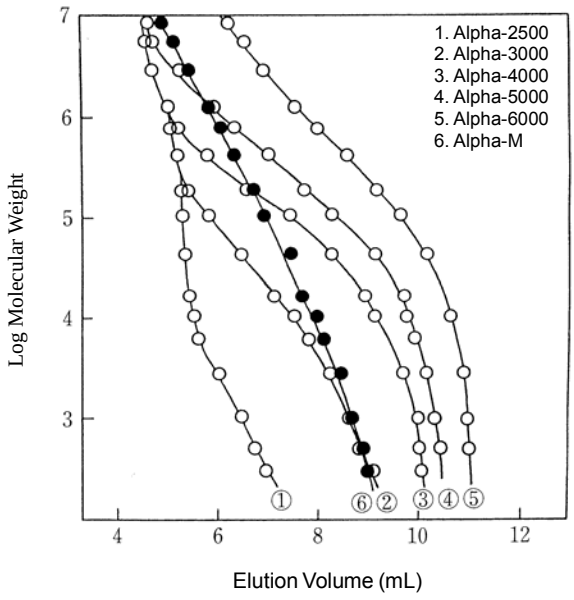

Fig. 6 TSKgel Alpha Series Calibration Curve ($CHCl_3$/PS)

Column: TSKgel Alpha Series
Eluent: chloroform
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Sample: polystyrene

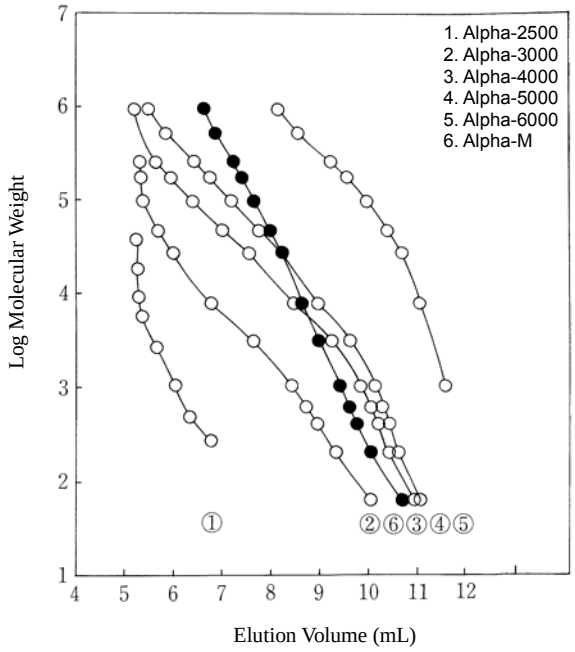

Fig. 7 TSKgel Alpha Series Calibration Curve (DMF/PEO, PEG)

Column: TSKgel Alpha Series
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Samples: poly(ethylene oxide), poly(ethylene glycol) and ethylene glycol

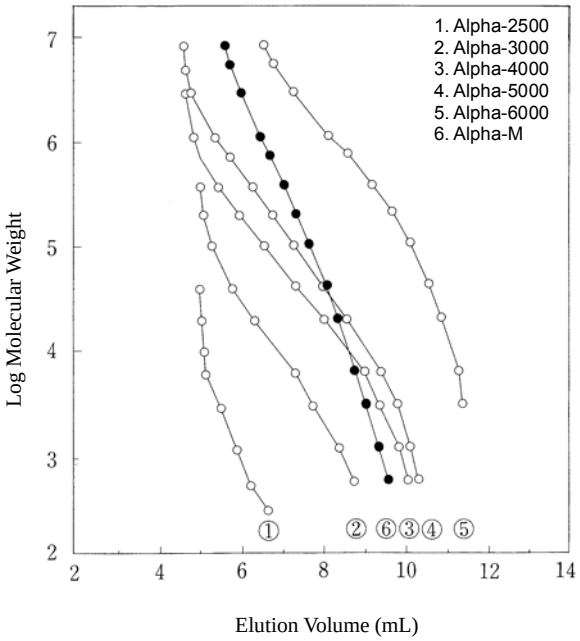

Fig. 8 TSKgel Alpha Series Calibration Curve (DMF/PS)

Column: TSKgel Alpha Series
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: RI
Sample: polystyrene

3) Effect of temperature

Temperature has various effects on SEC analysis. Figures 9 through 11 demonstrate the temperature dependence of calibration curves in water, while Figures 12 through 14 show the effects of temperature on the calibration curves in DMF. Standard poly(ethylene oxide) (PEO) and poly(ethylene glycol) (PEG) were used as the molecular weight standards. In H_2O/PEO systems the apparent pore size appeared to increase with elevation of temperature, but this is caused by a delay in the elution position due to adsorption of high-molecular PEO. With high temperature (80°C) analysis in a water mobile phase, it is difficult to create calibration curves for PEO and PEG due to adsorption of the molecular weight standard. However, when analysis is performed at high temperature (80°C) in a DMF mobile phase, a good calibration curve can be obtained for each grade without adsorption of the calibration standard. As a result, accurate molecular weight determination can be obtained by using PS or PEO depending on the type of polymer (see Figures 12 through 14).

In general, as temperature increases, the viscosity of the solvent decreases and solute diffusion coefficients increase, resulting in an increase in the number of theoretical plates. Figures 15 through 17 demonstrate the effect of temperature on the theoretical plate number of high molecular weight samples characterized in water and DMF systems. Although, as noted above, higher efficiencies can be obtained in water when operating at elevated temperatures, longer retention times due to adsorption of the molecular weight standard may occur under these conditions.

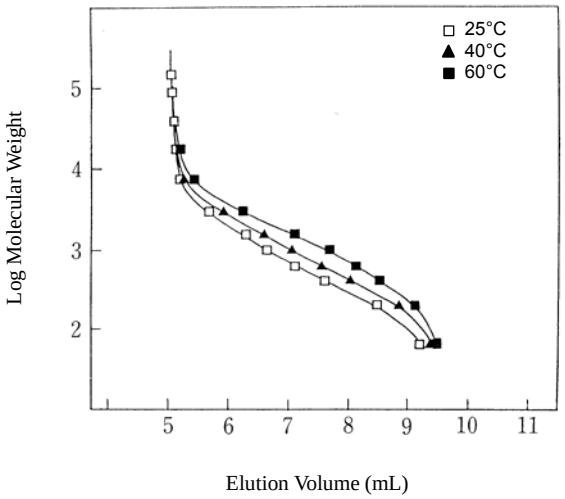

Fig. 9 Temperature Dependence of TSKgel Alpha-2500 Calibration Curve (H_2O/PEO, PEG)

Column: TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent: H_2O
Flow rate: 1.0mL/min
Temp.: 25°C to 80°C
Detection: RI
Samples: poly(ethylene oxide), poly(ethylene glycol) and ethylene glycol

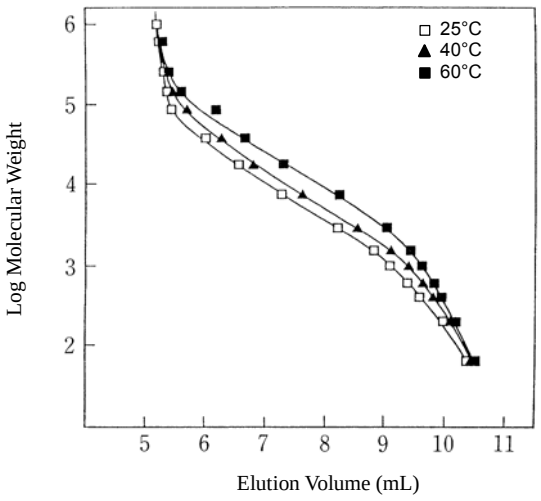

Fig. 10 Temperature Dependence of TSKgel Alpha-3000 Calibration Curve (H_2O/PEO, PEG)

Other analysis conditions are the same as in Fig. 9.

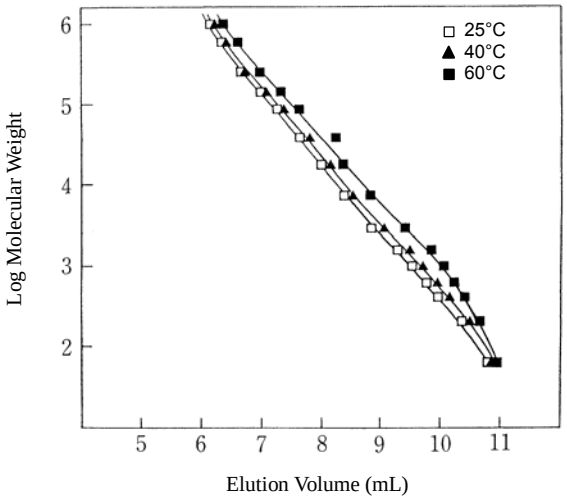

Fig. 11 Temperature Dependence of TSKgel Alpha-M Calibration Curve (H_2O/PEO, PEG)

Other analysis conditions are the same as in Fig. 9.

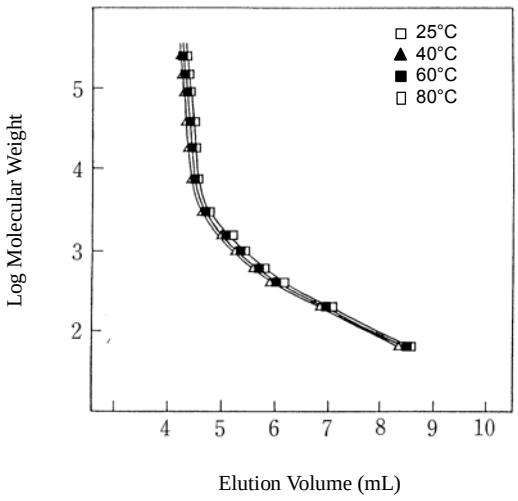

Fig. 12 Temperature Dependence of TSKgel Alpha-2500 Calibration Curve (DMF/PEO, PEG)

Column: TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent: dimethylformamide
Flow rate: 1.0mL/min
Temp.: 25°C to 80°C
Detection: RI
Samples: poly(ethylene oxide), poly(ethylene glycol) and ethylene glycol

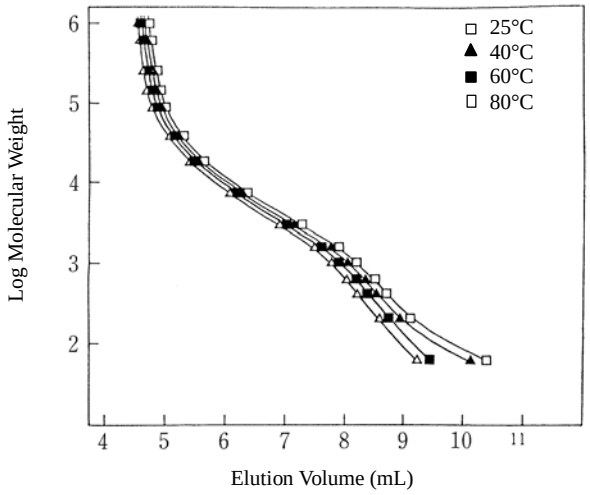

Fig. 13 Temperature Dependence of TSKgel Alpha-3000 Calibration Curve (DMF/PEO, PEG)

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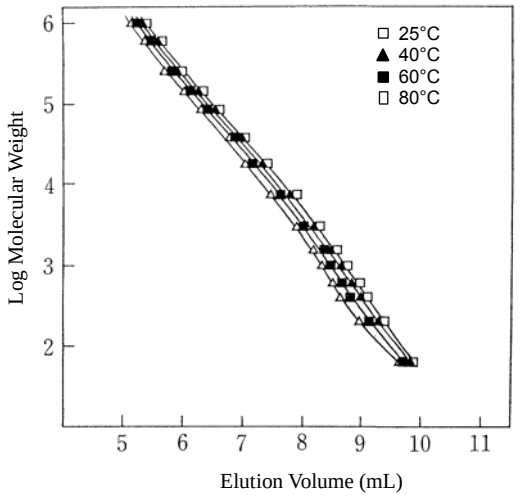


Fig. 14 Temperature Dependence of TSKgel Alpha-M Calibration Curve (DMF/PEO, PEG)

Other analysis conditions are the same as in Fig. 12.

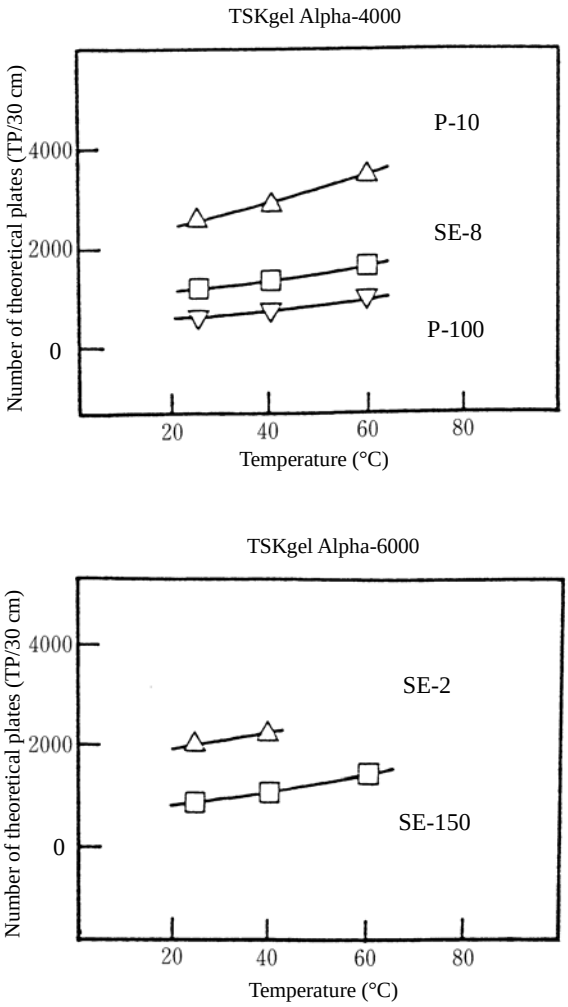
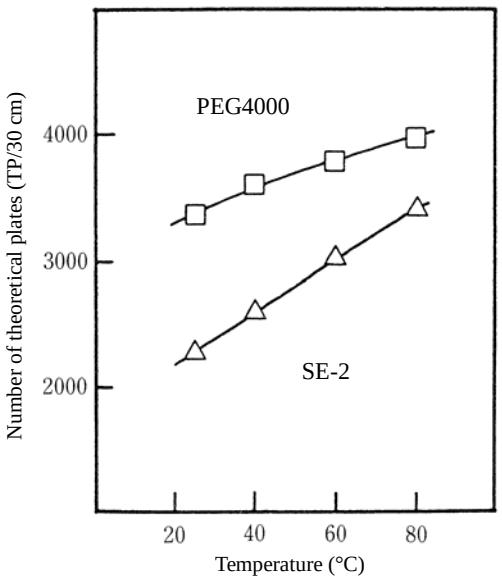


Fig. 15 Relationship between Temperature and Number of Theoretical Plates of Molecular Weight Standard in TSKgel Alpha Series

Columns:	TSKgel Alpha-4000, Alpha-6000, 7.8mm ID x 30cm
Eluent:	H_2O
Flow rate:	1.0mL/min
Temp.:	25°C to 80°C
Detection:	RI
Samples:	poly(ethylene oxide) SE-2,SE-8,SE-150 and pullulan (P-10, P-100)



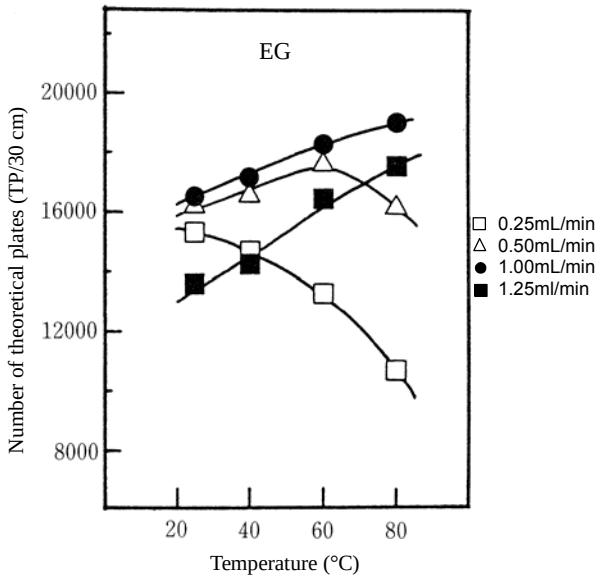


Fig. 16 Relationship between Temperature and Number of Theoretical Plates of Molecular Weight Standard in TSKgel Alpha-3000

Columns:	TSKgel Alpha-3000, 7.8mm ID x 30cm
Eluent:	dimethylformamide
Flow rate:	0.25mL/min to 1.25mL/min
Temp.:	25°C to 80°C
Detection:	RI
Samples:	poly(ethylene oxide) SE-2, poly(ethylene glycol) 4000 and ethylene glycol
Inj. vol.:	50µL

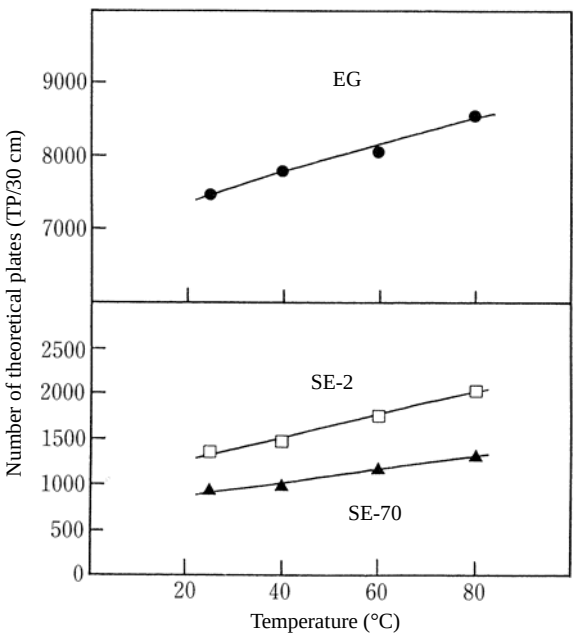


Fig. 17 Relationship between Temperature and Number of Theoretical Plates of Molecular Weight Standard in TSKgel Alpha-M

Columns:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	dimethylformamide
Flow rate:	1.0mL/min
Temp.:	25°C to 80°C
Detection:	RI
Sample:	poly(ethylene oxide) SE-2,SE-70
Inj. vol.:	50µL

4) Effect of flow rate

Figures 18 and 19 demonstrate the dependence of column plate number on the flow rate in water and DMF mobile phases. Typically, the optimum flow rate depends on the particle size of the packing material and the molecular weight of the molecule being examined. With low molecular weight samples in a water mobile phase, acceptable results are obtained at a flow rate around 0.7mL/min, while with high molecular weight polymers the best results are obtained at lower flow rates. The organic mobile phase produced very similar results. Because optimal flow rate depends on the viscosity of the solvent, for analysis to be performed under optimal conditions, it is critical to understand the relationship between flow rate and separation performance in the solvent used for analysis.

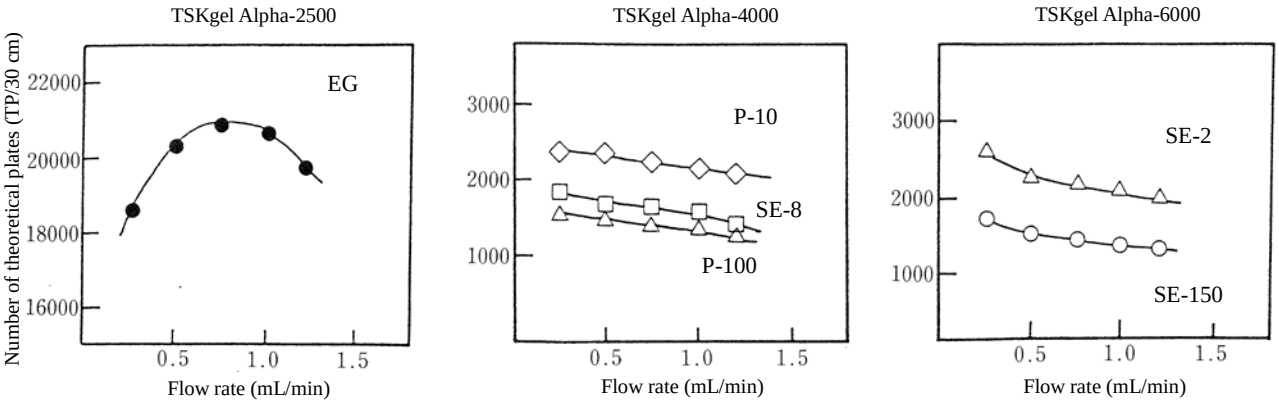


Fig. 18 Relationship between Flow Rate and Number of Theoretical Plates of Molecular Weight Standard in TSKgel Alpha Series Columns

Columns:	TSKgel Alpha-2500, Alpha-4000, Alpha-6000, 7.8mm ID x 30cm
Eluent: H_20	
Flow rate:	0.25mL/min to 1.3mL/min
Temperature:	25°C
Detection:	RI
Samples:	poly(ethylene oxide) SE-2,SE-8,SE-150, pullulan (P-10,P-100) and ethylene glycol

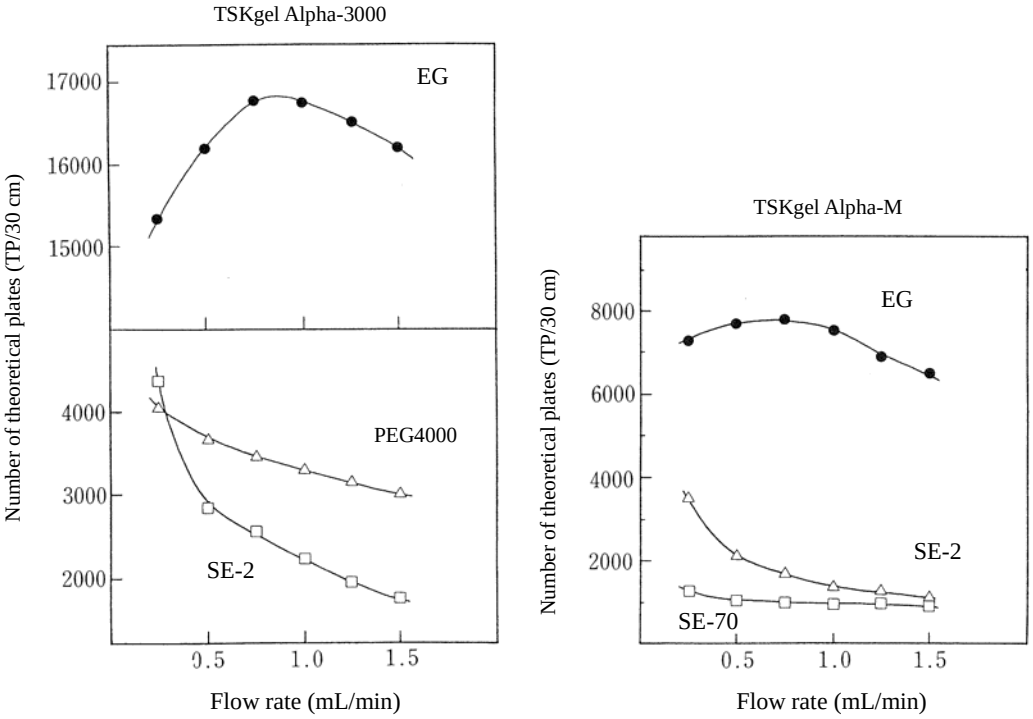


Fig. 19 Relationship between Flow Rate and Number of Theoretical Plates of Molecular Weight Standard in TSKgel Alpha Series Columns

Columns:	TSKgel Alpha-3000, Alpha-M, 7.8mm ID x 30cm
Eluent:	dimethylformamide
Flow rate:	0.25mL/min to 1.5mL/min
Temperature:	25°C
Detection:	RI
Samples:	poly(ethylene oxide) SE-2, poly(ethylene glycol) 4000 and ethylene glycols
Injection volume:	50µL

5) Effect of sample injection volume

Similar to what is seen in SEC analyses using THF mobile phases, the elution position of high molecular weight polymer also depends on the injection volume in polar solvents. Consequently, if strict quality control is an objective, the injection volume must remain constant. Figures 20 and 21 depict the effects of sample (PEO) injection volume on column theoretical plate number in a water mobile phase for the TSKgel Alpha-3000 and TSKgel Alpha-M columns, respectively. Figures 22 and 23 show the same point in a DMF mobile phase. In each of these solvent systems a decrease in the plate number is seen at around 100μL with both low and high molecular weight samples.

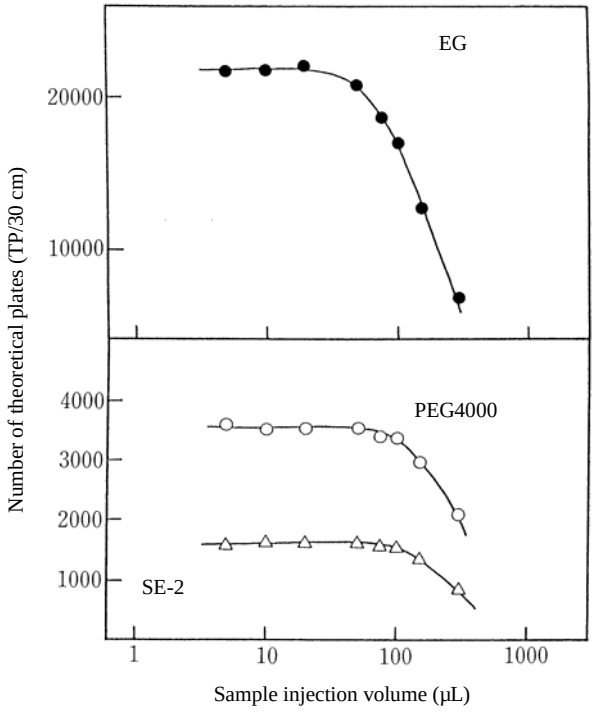


Fig. 20 Relationship between Number of Theoretical Plates and Injection Volume (Constant Concentration) of Molecular Weight Standard in TSKgel Alpha-3000 Column

Column:	TSKgel Alpha-3000, 7.8mm ID x 30cm
Eluent:	H_2O
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Samples:	poly(ethylene oxide) SE-2, poly(ethylene glycol) 4000 and ethylene glycol
Concentration:	ethylene glycol and poly(ethylene glycol) 4000 (0.05%); poly(ethylene oxide) SE-2, (0.1%)

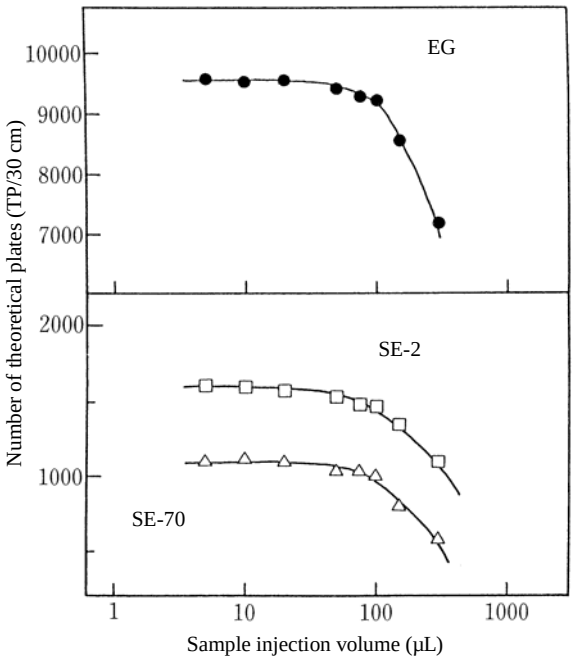


Fig. 21 Relationship between Number of Theoretical Plates and Injection Volume (Constant Concentration) of Molecular Weight Standard in TSKgel Alpha-M Column

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	H_2O
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Samples:	poly(ethylene oxide) SE-2,SE-70 and ethylene glycol
Concentration:	ethylene glycol (0.05%), poly(ethylene oxide)SE-70 (0.04%), poly(ethylene oxide) SE-2 (0.1%)

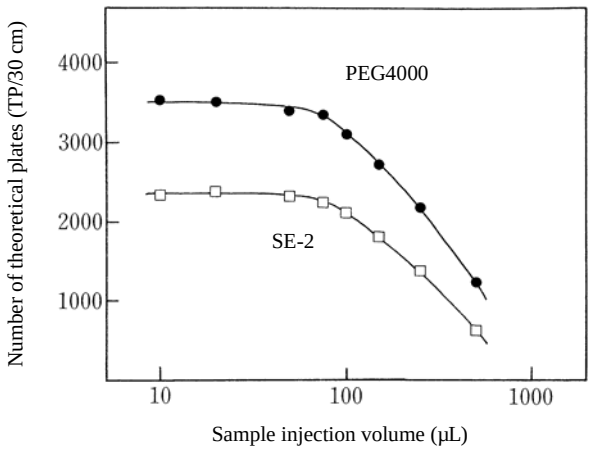


Fig. 22 Relationship between Number of Theoretical Plates and Injection Volume (Constant Concentration) of Molecular Weight Standard in TSKgel Alpha-3000 Column

Column:	TSKgel Alpha-3000, 7.8mm ID x 30cm
Eluent:	dimethylformamide
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Sample:	poly(ethylene oxide) SE-2 and poly(ethylene glycol) 4000
Concentration:	poly(ethylene oxide) SE-2, (0.15%), poly(ethylene glycol) 4000 (0.15%)

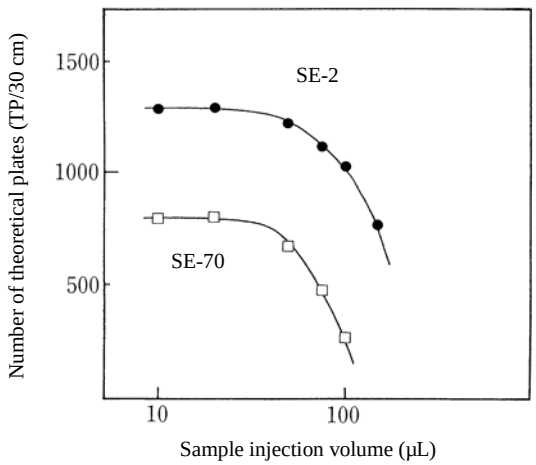


Fig. 23 Relationship between Number of Theoretical Plates and Injection Volume (Constant Concentration) of Molecular Weight Standard in TSKgel Alpha-M Column

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	dimethylformamide
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Sample:	poly(ethylene oxide) SE-2,SE-70 (2mg/mL)
Concentration:	SE-70 (0.1%), SE-2 (0.15%)

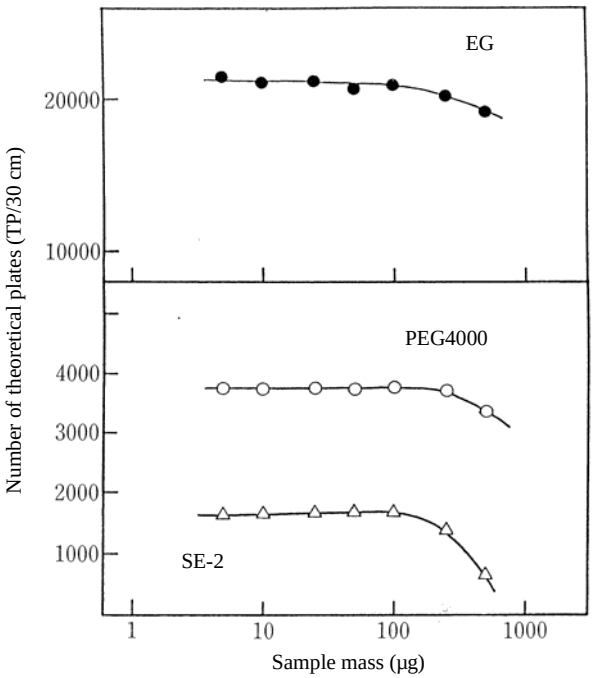


Fig. 24 Relationship between Number of Theoretical Plates and Sample Mass (at Constant Injection Volume) of Molecular Weight Standard in TSKgel Alpha-3000 Column

Column:	TSKgel Alpha-3000, 7.8mm ID x 30cm
Eluent:	H_2O
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Sample:	poly(ethylene oxide) SE-2, poly(ethylene glycol) 4000 and ethylene glycol
Injection volume:	50μL

6) Effect of sample injection concentration

As the concentration of the polymer increases, sample mass overload can occur starting at the column top, usually leading to shorter retention times and lower column efficiency. This phenomenon must be kept in mind when processing samples for preparative isolation. Figure 24 (TSKgel Alpha-3000 column) and Figure 25 (TSKgel Alpha-M column) show how sample concentration at constant injection volume affects column efficiency in a water mobile phase. In Figures 26 and 27 the same effects of sample concentration occur when a DMF mobile phase is used. The higher the molecular weight of the polymer, the less sample mass can be injected before overloading becomes noticeable by a loss in the number of theoretical plates.

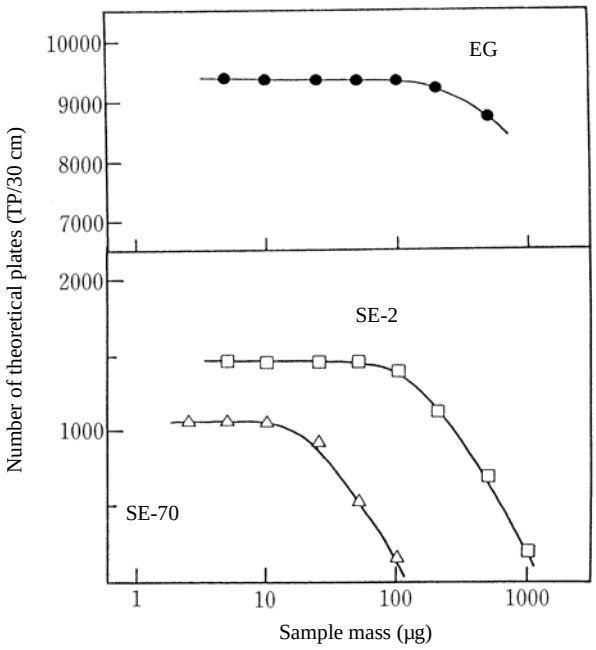


Fig. 25 Relationship between Number of Theoretical Plates and Sample Mass (Constant Injection Volume) of Molecular Weight Standard in TSKgel Alpha-M Column

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	H_2O
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Sample:	poly(ethylene oxide) SE-2, SE-70 and ethylene glycol (EG)
Injection volume:	50μL

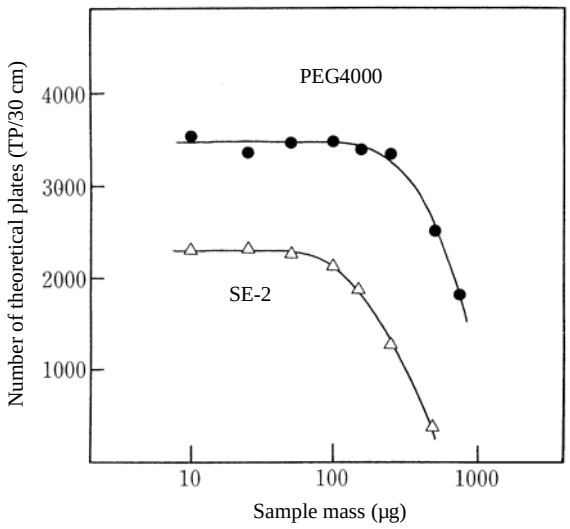


Fig. 26 Relationship between Number of Ttheoretical Plates and Sample Mass (Constant Injection Volume) of Molecular Weight Standard in TSKgel Alpha-3000 Column

Column:	TSKgel Alpha-3000, 7.8mm ID x 30cm
Eluent:	dimethylformamide
Flow rate:	0.25mL/min to 1.0mL/min
Temperature:	25°C
Detection:	RI
Sample:	standard poly(ethylene oxide) SE-2, poly(ethylene glycol) 4000 and ethylene glycol
Injection volume:	50μL

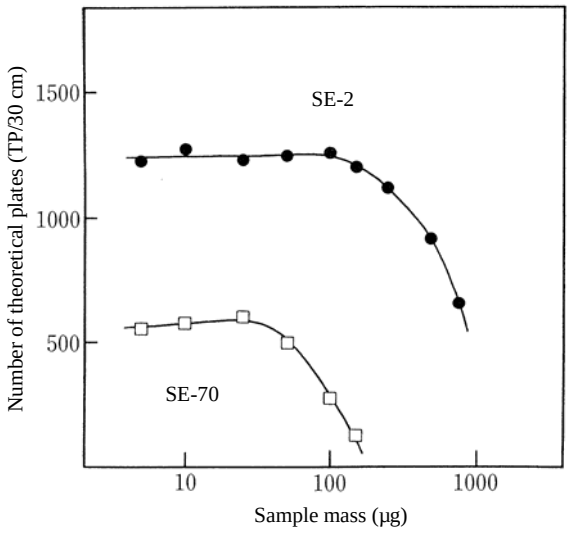


Fig. 27 Relationship between Number of Theoretical Plates and Sample Mass (Constant Injection Volume) of Molecular Weight Standard in TSKgel Alpha-M Column

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	dimethylformamide
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Sample:	poly(ethylene oxide) SE-2, SE-70
Injection volume:	50μL

7) Effect of adding salt to the solvent

In SEC analyses performed under polar mobile phase conditions, the sample being analyzed often has a dissociable group. When a salt is not present in the solvent, changes to the elution pattern frequently appear as a result of changes in molecular size caused by intramolecular repulsion or aggregation and interaction with the packing material. With polar solvents such as DMF and N-methylpyrrolidone (NMP), etc., interactions with basic impurities in the solvent have also been reported. In these cases, ionic adsorption can be suppressed by adding an appropriate salt to the solvent. Depending on the sample, solubility may also be improved by adding salt.

Figure 28 shows the effect of adding lithium bromide (LiBr) to DMF on the elution pattern of a phenol resin. In the case of this sample, a good separation pattern was obtained at a LiBr concentration of ~50mmol/L. The optimal concentration of salt to add will vary depending on the sample, thus tests must be performed to ascertain the salt concentration at which the separation pattern becomes constant. Be aware that calibration curves will vary depending on the presence of added salt and its concentration.

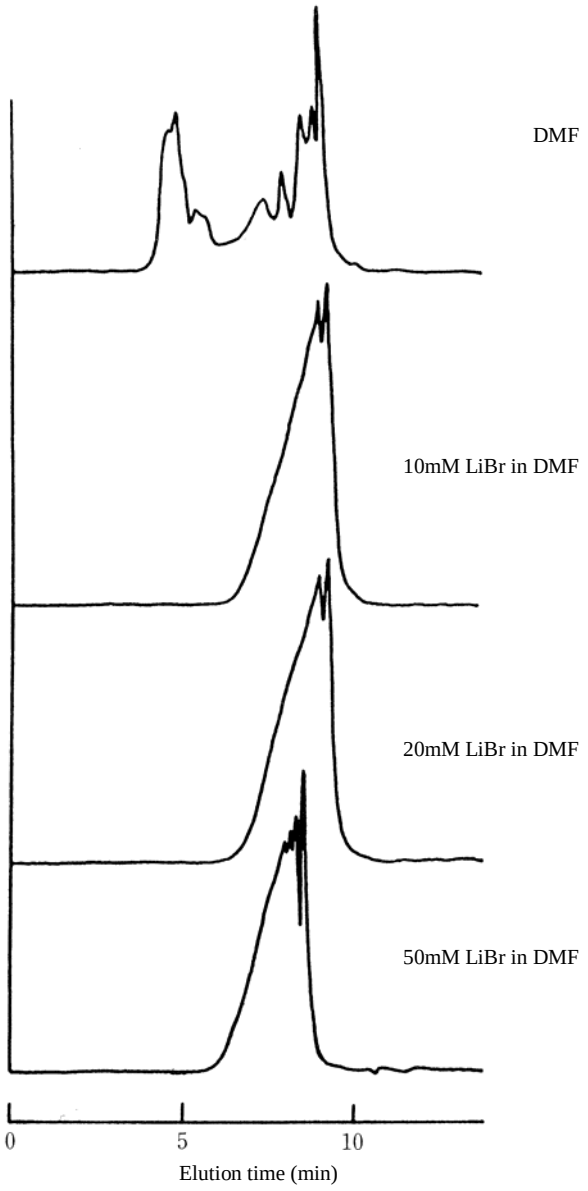


Fig. 28 Dependence of Phenol Resin Chromatogram on Salt Concentration in TSKgel Alpha-3000 Column

Column:	TSKgel Alpha-3000, 7.8mm ID x 30cm
Eluent:	0-50mmol/L LiBr in dimethylformamide
Flow rate:	0.25mL/min to 1.0mL/min
Temperature:	25°C
Detection:	UV@270nm
Sample:	phenol resin

8) Ionicity and hydrophobicity

Table 5 shows the results of analyses of the ionicity and hydrophobicity of the packing material of the TSKgel Alpha Series columns using an aqueous solvent system. The ionicity and hydrophobicity of the packing material in an aqueous solvent system were essentially the same as with the TSKgel PW_{XL} Series of water-system SEC columns. Adsorption of the molecular weight standard is a problem that can occur in polystyrene/divinylbenzene columns when a polar organic solvent system is used. Figure 29 compares the elution behavior of polystyrene standards on a TSKgel Alpha-3000 column versus a TSKgel $G3000H_{HR}$ column. When a polar organic solvent is used in a polystyrene column, adsorption of PS is observed, but with the Alpha Series, a good chromatogram is obtained without adsorption.

Table 5 Ionicity and Hydrophobicity of TSKgel Alpha Series Columns

Sample	Eluent	Capacity factor (k')*	
		TSKgel Alpha-2500	TSKgel Alpha-5000
beta-phenethyl alcohol	H_2O	7.66	5.05
	50% acetonitrile	0.00	0.07
tryptophan	H_2O	2.69	0.36

Analysis conditions: Eluent: H_20, 50% acetonitrile
Flow rate: 1.0mL/min; Detection: RI
* Calculated with ethylene glycol elution time as t_0.

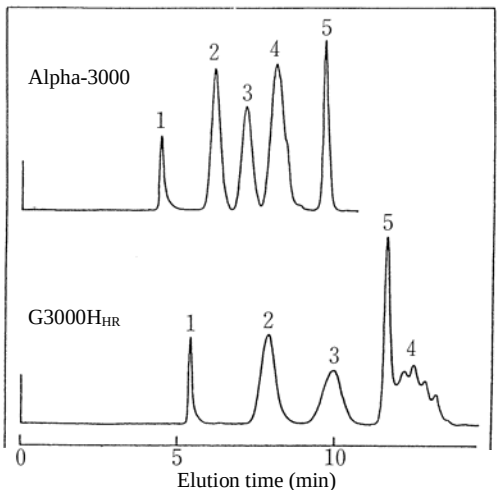


Fig. 29 Comparison of TSKgel Alpha-3000 and TSKgel G3000H$_{HR}$ Columns in the Separation of a Standard Polyethylene Mixture

Columns: TSKgel Alpha-3000, TSKgel G3000H$_{HR}$, 7.8mm ID x 30cm
Eluent: dimethylformamide
Flow rate: 1.0mL/min
Temperature: 25°C
Detection: UV@270nm
Samples: 1. Standard polyethylene (F-20) MW: 190,000
2. Standard polyethylene (F-1) MW: 9,100
3. Standard polyethylene (A-2500) MW: 2,800
4. Standard polyethylene (A-500) MW: 500
5. Acetone

9) Elution behavior of solvent peaks

Ghost peaks are another problem that occurs when a polar solvent is used in a polystyrene/divinylbenzene column, particularly when using DMF containing a salt, such as LiBr. Under such conditions ghost peaks from water, dissolved gas and salts such as LiBr appear in the low molecular weight region of the chromatogram, which can overlap the sample peaks and impede the accurate determination of molecular weight. However, with the TSKgel Alpha Series columns, when DMF containing LiBr is used as the solvent, ghost peaks derived from water or salts appear later than ghost peaks derived from dissolved gas, so the chromatogram of the sample is not affected. Figures 30 through 35 show the temperature dependency of the location of ghost peaks in various grades. Ghost peaks derived from dissolved gas and LiBr are eluted near polyethylene glycol (EG) and it is clear that the impact of temperature on elution time is minimal in each grade. However, in the TSKgel Alpha-2500, TSKgel Alpha-5000 and TSKgel Alpha-M columns, peaks from water appear to have a strong effect on the elution position and it is clear that temperature dependence varies depending on the grade.

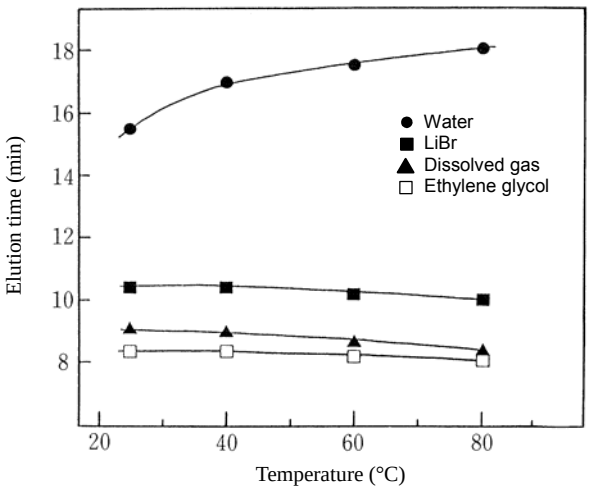


Fig. 30 Relationship between Temperature and Elution Time of System Peaks with TSKgel Alpha-2500 in DMF Mobile Phase

Column: TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent: dimethylformamide
Flow rate: 1.0mL/min
Temperature: 25°C to 80°C
Detection: RI
SampleS: dissolved gas, lithium bromide and water

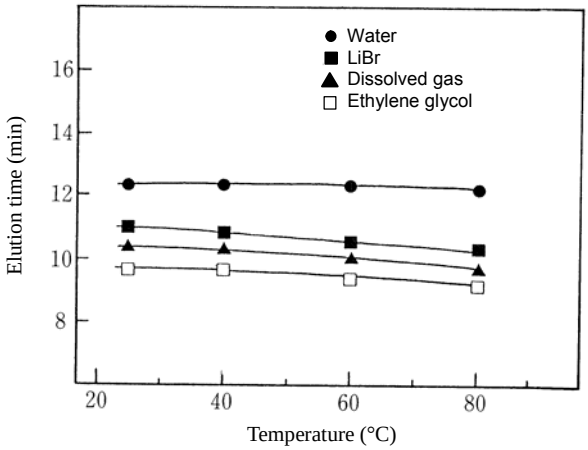


Fig. 31 Relationship between Temperature and Elution Time of System Peaks with TSKgel Alpha-3000 in DMF Mobile Phase

Column: TSKgel Alpha-3000, 7.8mm ID x 30cm
Other analysis conditions are the same as those in Fig. 30.

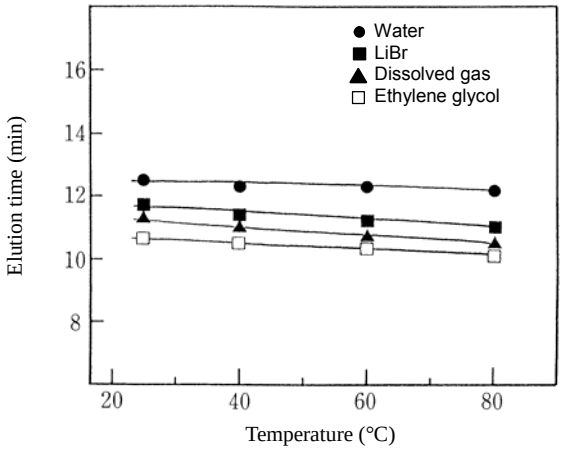


Fig. 32 Relationship between Temperature and Elution Time of System Peaks with TSKgel Alpha-4000 in DMF Mobile Phase

Column: TSKgel Alpha-4000, 7.8mm ID x 30cm

Other analysis conditions are the same as those in Fig. 30.

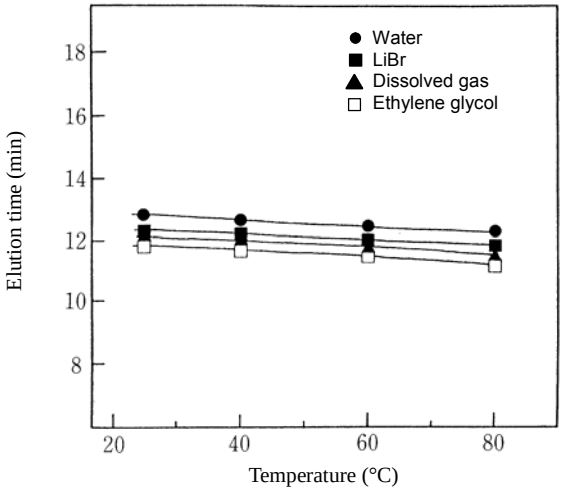


Fig. 34 Relationship between Temperature and Elution Time of System Peaks with TSKgel Alpha-6000 in DMF Mobile Phase

Column: TSKgel Alpha-6000, 7.8mm ID x 30cm

Other analysis conditions are the same as those in Fig. 30.

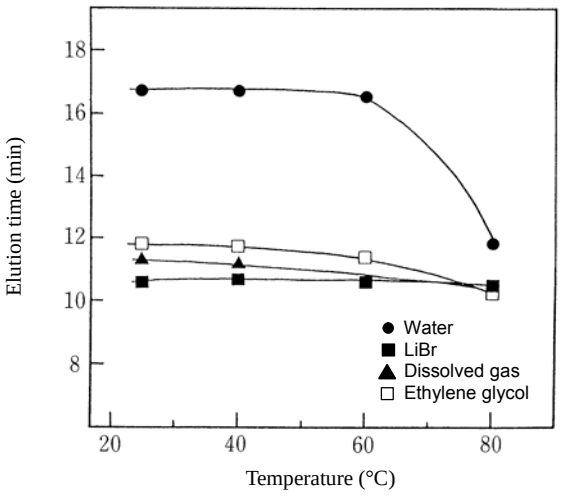


Fig. 33 Relationship between Temperature and Elution Time of System Peaks with TSKgel Alpha-5000 in DMF Mobile Phase

Column: TSKgel Alpha-5000, 7.8mm ID x 30cm

Other analysis conditions are the same as those in Fig. 30.

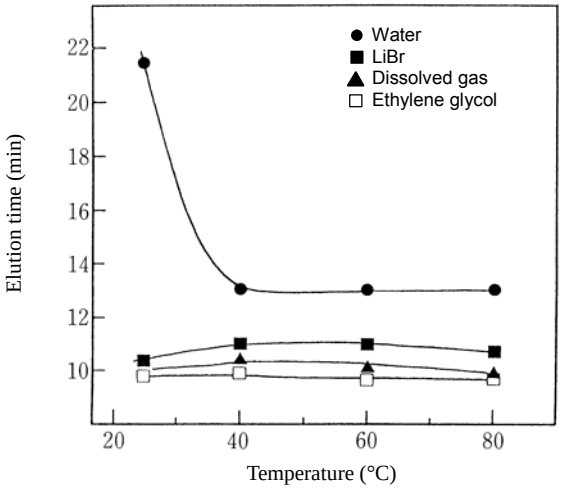


Fig. 35 Relationship between Temperature and Elution Time of System Peaks with TSKgel Alpha-M in DMF Mobile Phase

Column: TSKgel Alpha-M, 7.8mm ID x 30cm

Other analysis conditions are the same as those in Fig. 30.

4. Points to Consider before Conducting Analyses

Listed below are several points to consider when conducting general SEC analysis, from sample preparation to analysis.

1) Sample preparation procedures

1. Dissolve the sample (solubility test)

a) When it is unclear which solvent to use to dissolve the sample, first perform solubility tests using a number of different types of solvents (water, methanol, THF, DMF, etc.)

b) Prepare sample solutions of approximately 0.1% (w/v) and visually check the dissolved condition of the sample.

In general, polymers take longer to dissolve than low molecular weight compounds, and depending on the circumstances, a sample may need to be left standing at room temperature for 12 or more hours. Moreover, depending on the type of polymer and solvent used, dissolution may require temperatures above 40°C or below 10°C. When dissolved, the main chain of a polymer has the tendency to break (this tendency becomes more pronounced the higher the molecular weight). Consequently, avoid shaking/agitating sample solutions during and after dissolution.

2. Preparation of sample solutions

An organic solvent used as mobile phase should be selected from among solvents that dissolve the sample. When water is used as mobile phase, the type and concentration of salts, pH, etc., should be selected according to the properties of the sample (ionicity, hydrophobicity). Basically, solid samples should dissolve in the mobile phase. Liquid samples should be prepared by dilution with the mobile phase, but be aware that this can result in the formation of insoluble components.

A typical polymer concentration is about 0.1% (w/v) for samples, but may be modified depending on the molecular weight and sensitivity.

3. Filtration of sample solutions

Remove insoluble components (other than the target polymer of the sample) by filtration using a syringe filter. However, if the molecular weight of the targeted component is too large to pass through the filter, the insoluble components must be removed by centrifugation.

2) To obtain stable analysis results

1. Injection volume and injection concentration (overload)

It is common practice to inject high molecular weight polymers using a high volume and low concentration, while small sample injection volumes at high concentration are used for low molecular weight polymers.

For a 30cm x 7.8mm ID column, an injection volume of 50μL can be used as a rough guideline; however, this may need to be altered depending on the molecular weight of the sample. When analyzing a high molecular weight polymer that has a wide molecular weight distribution, the injection volume may need to be increased (50-100μL). Conversely, for low molecular weight samples or when peak separation of oligomers is targeted, decreased injection volumes are required (about 10 to 20μL with a 30cm column).

A rough guideline for sample concentration is 0.1% (w/v). However, a lower concentration is required when the sample has a high molecular weight. Thus, using a higher sample concentration, longer retention is likely to occur, caused by the tendency for the hydrodynamic volume of solvated polymers to decrease as the sample concentration is increased. In general, this becomes more noticeable as the molecular weight of the sample increases. Therefore, when deciding on the conditions for analyzing a new sample, analyses must be performed at a minimum of two concentration levels and the conditions (sample concentration and flow rate) under which elution time and peak shape (mean molecular weights: Mz, Mw, Mn) remain constant must be investigated.

2. Molecular weight standard and target sample

When analyzing a molecular weight standard to prepare a calibration curve, the mobile phase, instruments, column, injection volume, and other analysis conditions must be the same as those that will be used for the target sample. It is also advised to analyze the standard and target samples on the same day. To prepare molecular weight standards, standard compounds with different molecular weights are dissolved together in the same container and analyzed as a mixed sample. In general, the concentration of the molecular weight standard should be about one half the concentration of the target sample, but this may need to be modified depending on the molecular weights of each of the molecular weight standards. In short, the higher the molecular weight, the lower the concentration.

3. Column temperature

Due to the significant impact of temperature on sample elution time and detector (RI) baseline fluctuations, the temperature of the mobile phase and the column must be kept constant during the measurement. Analysis is generally performed between 35 and 40 ºC, but it is necessary to empirically determine the appropriate temperature when using high viscosity solvents and when analyzing samples with properties that can change as a function of temperature. Of course, it is important to note the boiling point of the organic solvent.

4. Flow rate

Since molecular weight is calculated from the elution time, it is critical to maintain a constant flow rate and have a pump capable of delivering a precise flow rate. In general, a flow rate of 1.0mL/min is used, but in the case of high molecular weight samples the flow rate must be reduced to 0.5mL/min or less.

5. Mobile phases

Typical mobile phases, based on the properties of the sample, are indicated below.

For nonionic and acidic polymers:

- Buffer solution: 0.2mol/L phosphate buffer (pH 6.8), etc.
- Salt solution: 0.2mol/L $NaNO_3$

For basic polymers:

- 1mol/L acetate buffer (pH 4.5)
- 0.3mol/L triethylamine-phosphoric acid (pH 2.9)

For hydrophobic polymers*:

- Aqueous salt solution containing polar organic solvent
- Polar organic solvent (methanol, DMF, THF, HFIP, etc.)

* When polar organic solvents other than THF are used as an eluent, the addition of an organic soluble salt such as LiBr is recommended.
* When an organic solvent is added to a salt solution, take care to avoid precipitation of the salt.

3) Selecting the solvent

A RI detector is commonly used in SEC analysis. The molecular weight distribution is typically calculated based on the assumption that the refractive index increment (change in the refractive index with the polymer concentration) is constant independent of molecular weight. The refractive index increment, however, depends on the molecular weight in the low molecular weight region and, as a result, the lower the molecular weight, the lower the detection sensitivity. Therefore, it is difficult to accurately calculate molecular weight distribution of low molecular weight polymers. However, when a solvent having low RI is used, the constant refractive index increment is obtained over a wider molecular weight range. Thus, the lower the RI of the mobile phase, the lower the molecular weight of the polymer that can be analyzed at constant sensitivity. Table 6 shows the sensitivity correction factors of various molecular weights of polyethylene glycol dissolved in polar solvents. As the molecular weight of the polymer decreases, the intensity of the RI response becomes smaller and a larger factor is required for the sensitivity correction. A polymer with a molecular weight as low as 2,500 and 1,200 can be detected in chloroform and THF, respectively, within a 5% difference in the sensitivity when compared to the intensity of the RI response of the polymer with a molecular weight of 20,000 as a reference. In contrast, in methanol, detection is possible down to a molecular weight of 550 within a 5% difference in the sensitivity. Using HFIP, which has an even lower refractive index, detection becomes possible at a molecular weight as low as 500.

Thus, by selecting a solvent with a lower refractive index, an accurate molecular weight distribution can be obtained, even when the relative sensitivity depending on the molecular weight is unknown. The TSKgel Alpha Series columns can be used in water, methanol, acetonitrile, and HFIP as mobile phases, which have comparatively low RI. Consequently this series of packed columns is expected to perform particularly well in component analyses of samples containing low molecular weight polymers, such as oligomers, etc.

5. Examples of Separation of Polar Compounds using Various Solvents

Table 7 shows examples of separations of various polar polymers conducted using different solvents with the TSKgel Alpha Series of columns. The chromatograms are displayed in Figures 36 to 75.Table-7 Examples of solvent systems for analyzing of polar polymers using TSKgel Alpha Series

Table 6 Sensitivity Correction Factor of Poly(ethylene glycol) by Solvent

Molecular mass m, quaternary structure	Refractive index[1] Poly(ethylene glycol)	Sensitivity correction factor				Required correction %
		Chloroform[1]	THF[1]	Methanol	HFIP	
106 (m=2)	1.4455	8.912	1.655	1.266	1.158	
150 (m=3)	1.4529	2.806	1.402	1.154	1.110	10% line
194 (m=4)	1.4563	2.134	1.310	1.124	1.089	
238 (m=5)	1.4589	1.804	1.248	1.102	1.073	
282 (m=6)	1.4597	1.722	1.230	1.093	1.069	
326 (m=7)	1.4610	1.603	1.201	1.085	1.061	
370 (m=8)	1.4619	1.530	1.183	1.078	1.056	
414 (m=9)	1.4623	1.500	1.174	1.074	1.054	
458 (m=10)	1.4630	1.450	1.160	1.069	1.050	5% line
500	1.4640	1.384	1.141	1.061	1.044	
550	1.4653	1.306	1.117	1.051	1.037	
600	1.4660	1.268	1.104	1.046	1.034	
650	1.4664	1.247	1.096	1.043	1.031	3% line
700	1.4668	1.227	1.090	1.040	1.029	
750	1.4670	1.217	1.086	1.038	1.028	
800	1.4674	1.198	1.079	1.035	1.026	
850	1.4676	1.188	1.076	1.034	1.025	
900	1.4678	1.179	1.073	1.033	1.024	
950	1.4680	1.170	1.069	1.031	1.023	
1,000	1.4682	1.161	1.066	1.030	1.022	
1,100	1.4686	1.143	1.059	1.027	1.020	
1,200	1.4689	1.131	1.054	1.025	1.018	
1,300	1.4692	1.118	1.049	1.022	1.016	
1,400	1.4694	1.110	1.046	1.021	1.015	
1,500	1.4696	1.102	1.043	1.020	1.014	
1,700	1.4700	1.086	1.037	1.017	1.012	
2,000	1.4704	1.071	1.030	1.014	1.010	
2,500	1.4708	1.056	1.024	1.011	1.008	
3,000	1.4710	1.048	1.021	1.010	1.007	
3,500	1.4713	1.038	1.016	1.008	1.006	
4,000	1.4715	1.031	1.013	1.006	1.005	
5,000	1.4718	1.020	1.009	1.004	1.003	
6,000	1.4720	1.013	1.006	1.003	1.002	
7,000	1.4721	1.010	1.004	1.002	1.002	
8,000	1.4722	1.007	1.003	1.001	1.001	
20,000	1.4724	1.000	1.000	1.000	1.000	

Refractive index of solvent (25 ºC) Chloroform : 1.4421 THF : 1.4044
Methanol : 1.3265 HFIP : 1.2752

Sensitivity correction factor: $\frac{n_{MW(PEG=20.000)}}{n_{MW}} \frac{n_{SOLVENT}}{n_{SOLVENT}}$

1. S. Mori, Anal.Chem, 50, 1639 (1978)

Table 7 Examples of Solvent Systems for Analyzing of Polar Polymers using TSKgel Alpha Series Columns

Fig.	Sample name	Column used	Sample used	Features
36	Acrylonitrile/styrene copolymer	TSKgel Alpha-M	DMF/10mmol/L LiBr	
37	Acrylonitrile/vinylidene chloride copolymer	TSKgel Alpha-M	DMF/10mmol/L LiBr	
38	Poly(N-isopropylacrylamide)	TSKgel Alpha-M	MeOH/10mmol/L LiBr	
39	Ethyl cellulose	TSKgel Alpha-M	DMF/10mmol/L LiBr	In methanol system, highly sensitive analysis is possible at low refractive index (RI)
40	Ethyl cellulose	TSKgel Alpha-M	MeOH/10mmol/L LiBr	Required sensitivity of target compound, solubility of coexistent compounds, DMF has better separation
41	Ethyl hydroxyethyl cellulose	TSKgel Alpha-M	MeOH/10mmol/L LiBr	With samples containing a surfactant, linked with low molecular weight grade, DMF is also a possible solvent
42	Vinyl chloride/vinyl acetate copolymer	TSKgel Alpha-M	DMF/10mmol/L LiBr	Poor peak shape with H type column
43	Benzalkonium chloride	TSKgel Alpha-2500	DMF/10mmol/L LiBr	
44	Carboxymethyl cellulose	TSKgel Alpha-5000	0.1mol/L phosphate buffer (ph 6.8)	Addition system, separation performance improves under heated analysis
45	Cleansing gel (model system)	TSKgel Alpha-2500	MeOH	
46	Cellulose acetate	TSKgel Alpha-M	DMF/10mmol/L LiBr	Analysis also possible with H type
47	Styrene/allyl alcohol resin	TSKgel Alpha-M	DMF/10mmol/L LiBr	
48	Sodium dodecylbenzene sulfonate (hard)	TSKgel Alpha-2500	DMF/10mmol/L LiBr	Highly sensitive detection possible with methanol + 10 mmol/L LiBr/UV (215 nm)
49	Sodium dodecyl sulfate (SDS)	TSKgel Alpha-4000 + Alpha-3000 + Alpha-2000 × 2	DMF/10mmol/L LiBr	
50	Glyceryl tri(2-ethylhexanoate)	TSKgel Alpha-2500	MeOH	
51	Triton X-100	TSKgel Alpha-4000 + Alpha -3000 + Alpha-2000 × 2	DMF/50mmol/L LiBr	
52	Urea resin	TSKgel Alpha-M	DMF	
53	Hydroxypropyl cellulose	TSKgel Alpha-M	MeOH/10mmol/L LiBr	In methanol system highly sensitive analysis of low molecular weight compounds is possible, also possible in DMF
54	N-vinylpyrrolidone/vinyl acetate copolymer	TSKgel Alpha-M	MeOH/10mmol/L LiBr	
55	N-vinylpyrrolidone/vinyl acetate copolymer	TSKgel Alpha-M	DMF/10mmol/L LiBr	
56	Brij-35	TSKgel Alpha-4000 + Alpha -3000 + Alpha-2000 × 2	DMF/50mmol/L LiBr	
57	Sodium polyacrylate	TSKgel Alpha-M	0.2mol/L NaNO3	
58	Polyacrylonitrile (PAN)	TSKgel Alpha-M	DMF/10mmol/L LiBr	Analysis also possible with H type
59	Poly(amic acid)	TSKgel Alpha-M	DMF/30 mmol/L LiBR/60 mmol/L phosphoric acid	Analysis also possible with H type
60	Poly(amide-imide)	TSKgel Alpha-M	NMP/10mmol/L LiBr	
61	Polyimide	TSKgel Alpha-M	NMP/10mmol/L LiBr	
62	Poly(ethylene glycol mono p-octylphenyl ether)	TSKgel Alpha-2500	DMF/10mmol/L LiBr	Highly sensitive detection possible with methanol + 10 mmol/L LiBr/UV (215 nm)
63	Poly(vinyl alcohol)	TSKgel Alpha-5000 + Alpha-3000	HFIP	Analysis possible regardless of degree of saponification, from vinyl acetate to PVA; low wavelength analysis possible with HFIP
64	Poly(vinyl alcohol)	TSKgel Alpha-5000×2	0.1mol/L NaCl/MeOH	Analysis of copolymer ratio of compound containing low vinyl acetate is possible with combined use of UV/RI
65	Polyvinylpyrrolidone (PVP79)	TSKgel Alpha-M	MeOH/50mmol/L NaNO3=6/4	
66	Poly(vinyl butyral) (butyral resin)	TSKgel Alpha-M	DMF/10mmol/L LiBr	No interference from ghost peaks from water or salts
67	Polyvinylmethylether	TSKgel Alpha-M	MeOH/10mmol/L LiBr	Possible to select solvent based on sensitivity and separation from coexisting compounds
68	Poly(vinyl methyl ether)	TSKgel Alpha-M	DMF/10mmol/L LiBr	
69	Poly(p-phenylene ether sulfone)	TSKgel Alpha-M	DMF/10mmol/L LiBr	Polystyrene can be used as a molecular weight standard
70	Poly(vinylidene fluoride)	TSKgel Alpha-M	DMF/10mmol/L LiBr	Depending on solvent, the refractive index is low, detected as negative peak
71	Poly (methylmethacrylate / methacrylic acid) copolymer	TSKgel Alpha-M	DMF/10mmol/L LiBr	
72	Methylvinylether/maleic acid copolymer	TSKgel Alpha-M	DMF/30 mmol/L LiBR/60 mmol/L phosphoric acid	
73	N-methoxymethylated polyamide	TSKgel Alpha-M	MeOH/10mmol/L LiBr	
74	Melamine resin	TSKgel Alpha-M	DMF/LiBr	
75	Melamine-modified urea resin	TSKgel Alpha-M	DMF/10mmol/L LiBr	

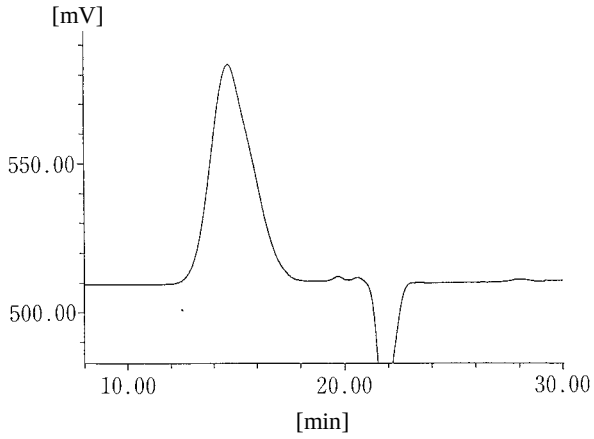


Fig. 36 Separation of acrylonitrile/styrene copolymer

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: acrylonitrile/styrene copolymer (0.1%, 50µL)

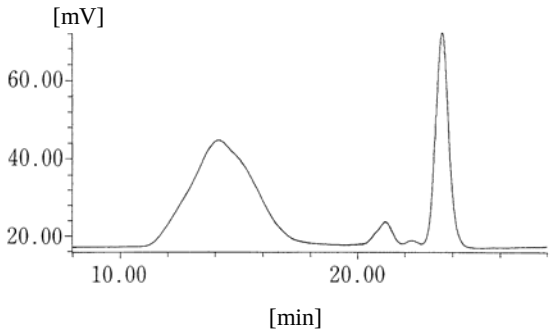


Fig. 38 Separation of poly(N-isopropylacrylamide)

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: N-isopropylacrylamide (0.1%, 50µL)

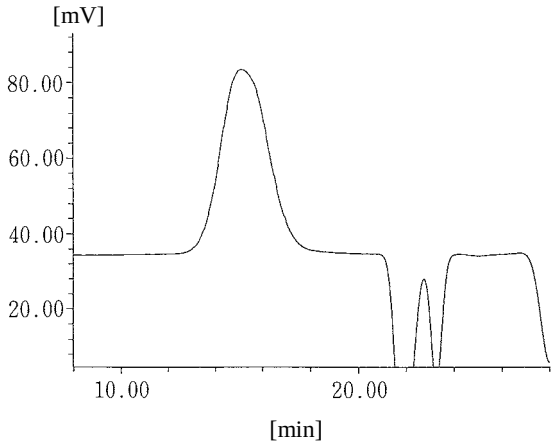


Fig. 37 Separation of acrylonitrile/vinylidene chloride copolymer

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: acrylonitrile/vinylidene chloride copolymer (0.1%, 50µL)

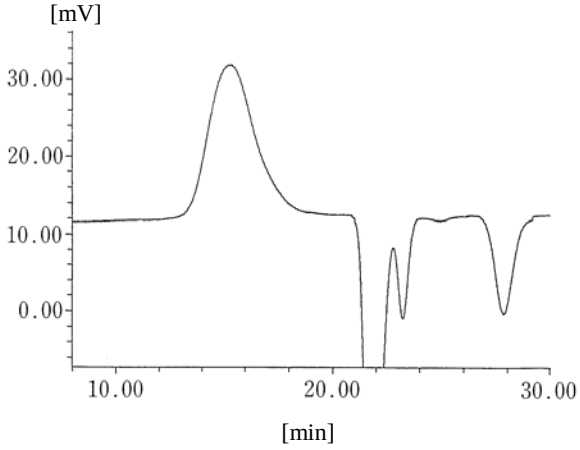


Fig. 39 Separation of ethyl cellulose

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: ethyl cellulose (0.1%, 50µL)

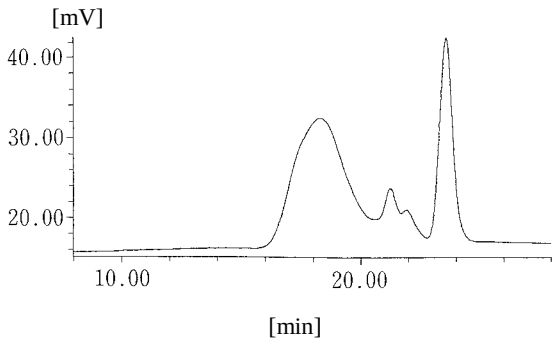


Fig. 40 Separation of ethyl celluose

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: ethyl cellulose (0.1%, 50µL)

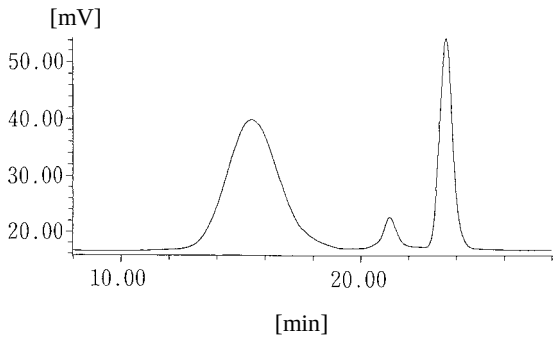


Fig. 41 Separation of ethyl hydroxyethyl cellulose

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: ethyl hydroxyethyl cellulose (0.1%, 50µL)

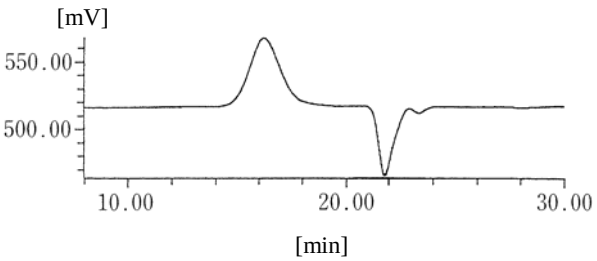


Fig. 42 Separation of vinyl chloride/vinyl acetate copolymer

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: vinyl chloride/vinyl acetate copolymer (0.1%, 50µL)

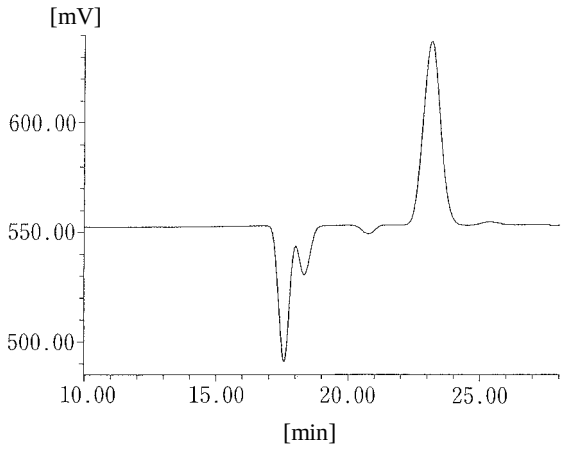


Fig. 43 Separation of benzalkonium chloride

Column: TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: benzalkonium chloride (0.1%, 50µL)

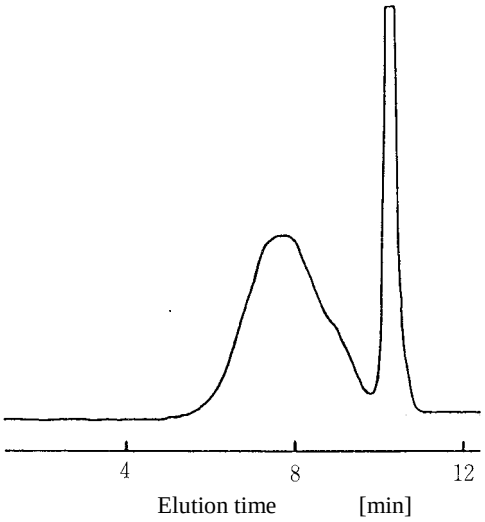


Fig. 44 Separation of carboxymethyl cellulose

Column:	TSKgel Alpha-5000, 7.8mm ID x 30cm
Eluent:	0.1mol/L phosphate (pH 6.8)
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	RI
Sample:	carboxymethyl cellulose

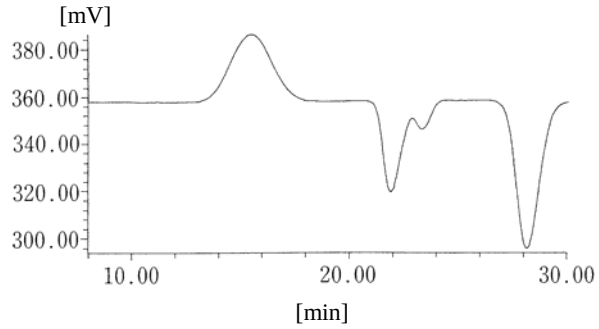


Fig. 46 Separation of cellulose acetate

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	10mmol/L LiBr in dimethylformamide
Flow rate:	0.5mL/min
Temperature:	40°C
Detection:	RI
Sample:	cellulose acetate (0.1%, 50µL)

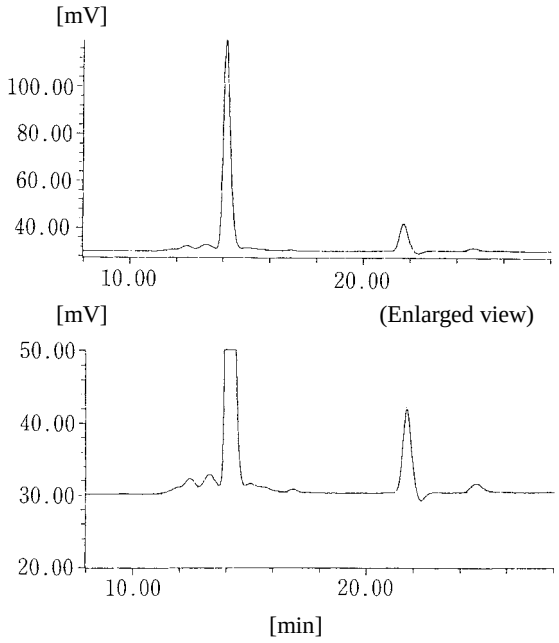


Fig. 45 Separation of cleansing gel (model system)

Column:	TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent:	methanol
Flow rate:	0.5mL/min
Temperature:	40°C
Detection:	RI
Sample:	Cleansing gel (model system) (0.1%, 50µL)

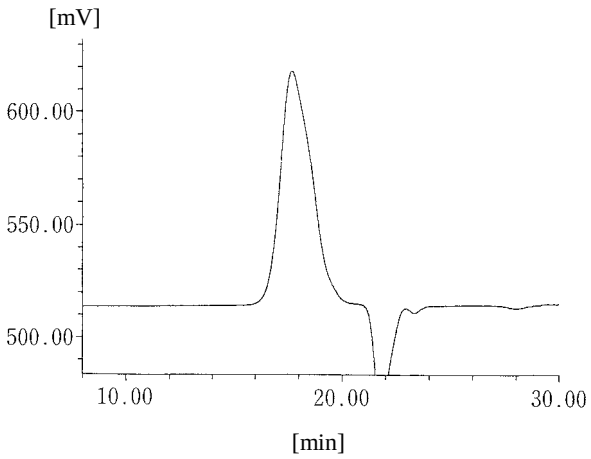


Fig. 47 Separation of styrene/allyl alcohol resin

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	10mmol/L LiBr in dimethylformamide
Flow rate:	0.5mL/min
Temperature:	40°C
Detection:	RI
Sample:	styrene/allyl alcohol copolymer (0.1%, 50µL)

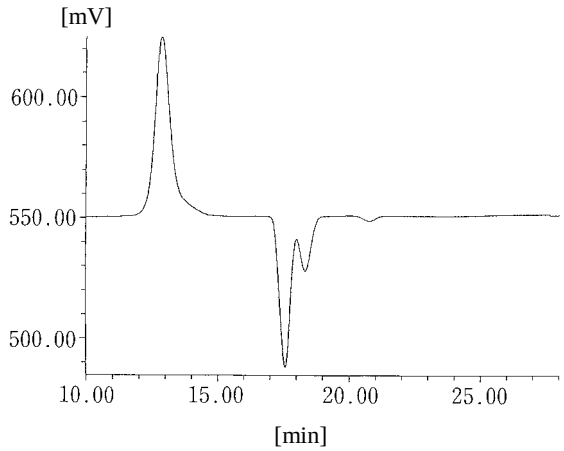


Fig. 48 Separation of sodium dodecylbenzene sulfonate (hard)

Column: TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: sodium dodecylbenzene sulfonate (hard) (0.1%, 50µL)

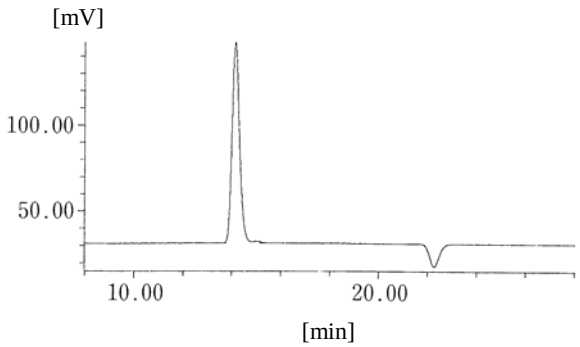


Fig. 50 Separation of glyceryl tri(2-ethylhexanoate)

Column: TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent: methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: glyceryl tri(2-ethylhexanoate) (0.1%, 50µL)

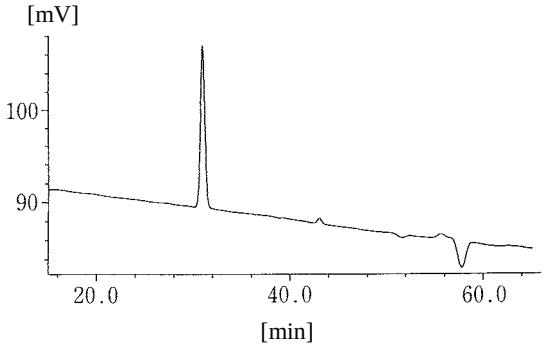


Fig. 49 Separation of sodium dodecyl sulfate

Column: TSKgel Alpha-4000 + Alpha-3000 + Alpha-2000 x 2, 7.8mm ID x 30cm
Eluent: 50mmol/L LiBr in dimethylformamide
Flow rate: 1.0mL/min
Temperature: 40°C
Detection: RI
Sample: sodium dodecyl sulfate (1.7%, 200µL)

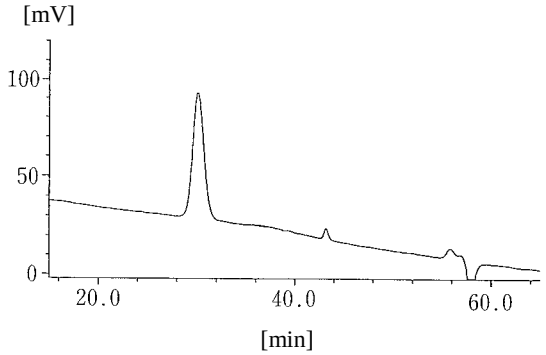


Fig. 51 Separation of Triton X-100

Other elution conditions are the same as those in Fig. 53.

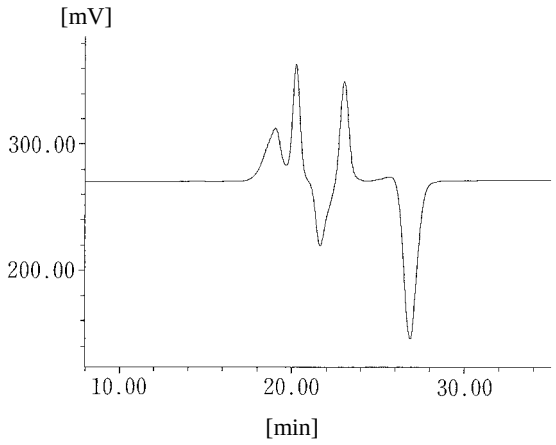


Fig. 52 Separation of urea resin

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 50mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: urea resin (0.1%, 50μL)

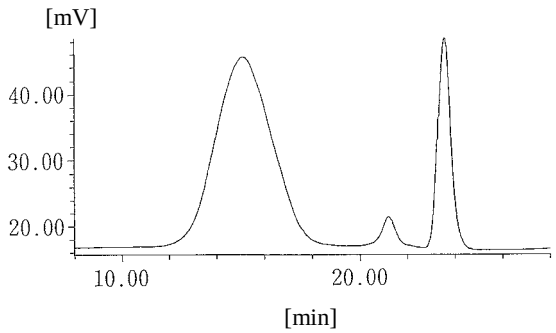


Fig. 53 Separation of hydroxypropyl cellulose

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: hydroxypropyl cellulose (1.7%, 200μL)

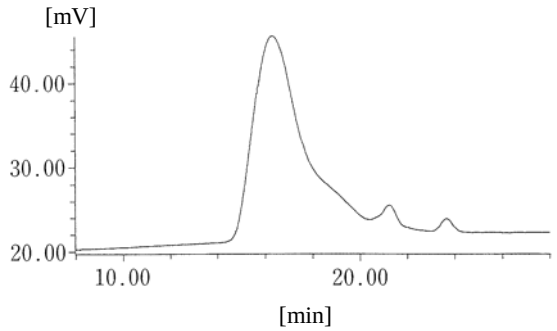


Fig. 54 Separation of N-vinylpyrrolidone/vinyl acetate copolymer

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: N-vinylpyrrolidone/vinyl acetate copolymer (0.1%, 50μL)

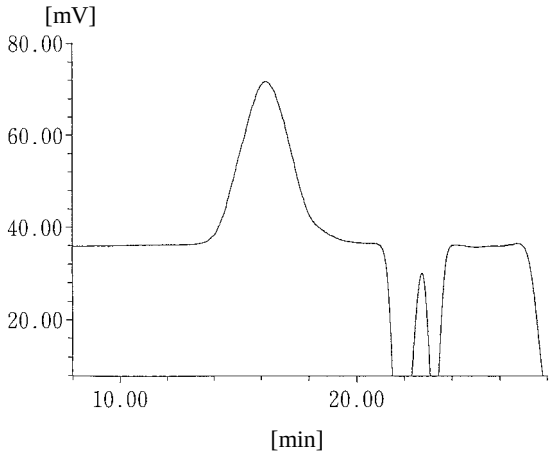


Fig. 55 Separation of N-vinylpyrrolidone/vinyl acetate copolymer

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: N-vinylpyrrolidone/vinyl acetate copolymer (0.1%, 50μL)

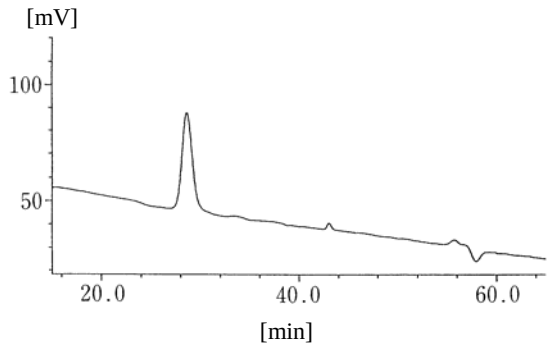


Fig. 56 Separation of Brij-35

Other elution conditions are the same as those in Fig. 53.

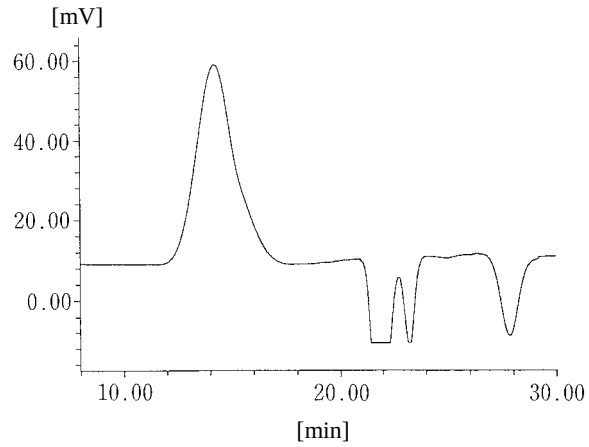


Fig. 58 Separation of polyacrylonitrile (PAN)

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	10mmol/L LiBr in dimethylformamide
Flow rate:	0.5mL/min
Temperature:	40°C
Detection:	RI
Sample:	poly(acrylonitrile) (0.1%, 50μL)

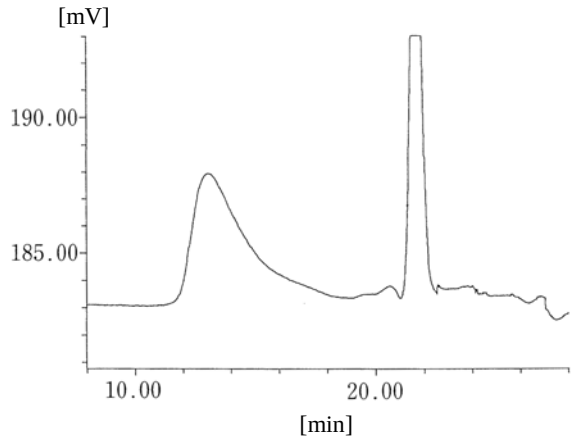


Fig. 57 Separation of sodium polyacrylate

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	0.2mol/L $NaNO_3$
Flow rate:	0.5mL/min
Detection:	RI
Temperature:	40°C
Sample:	sodium polyacrylate (50μL)

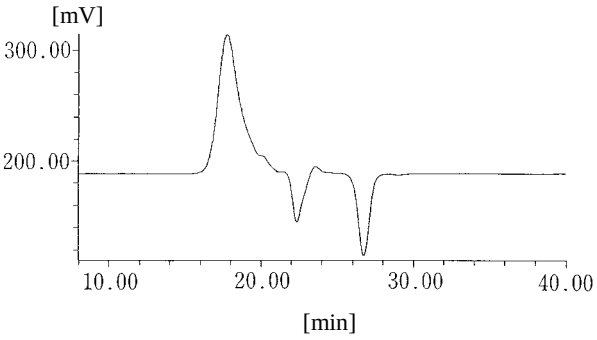


Fig. 59 Separation of polyamic acid

Column:	TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent:	30mmol/L LiBr + 60mmol/L H_3PO_4 in dimethylformamide
Flow rate:	0.5mL/min
Temperature:	40°C
Detection:	RI
Sample:	poly(amic acid) (0.1%, 50μL)

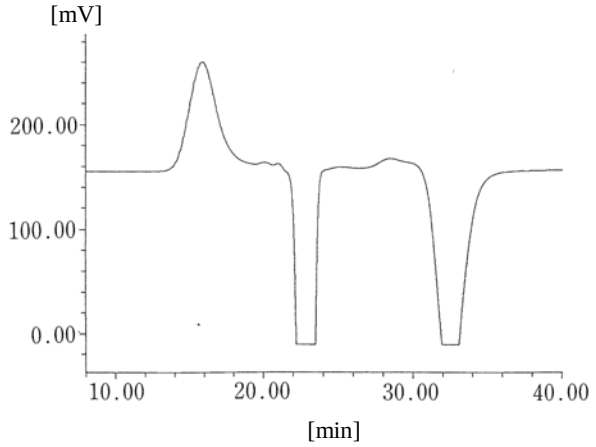


Fig. 60 Separation of polyamide-imide

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in N-methylpyrrolidone
Flow rate: 0.5mL/min
Detection: RI
Temperature: 40°C
Sample: poly(amide-imide) (0.1%, 50µL)

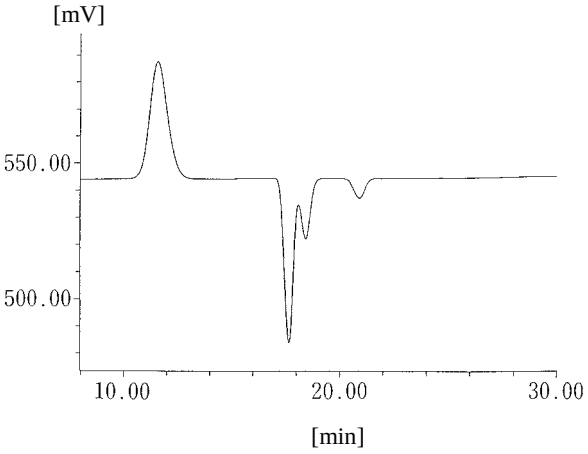


Fig. 62 Separation of polyethylene glycol mono p-octylphenyl ether

Column: TSKgel Alpha-2500, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(ethylene glycol mono p-octylphenyl ether) (0.1%, 50µL)

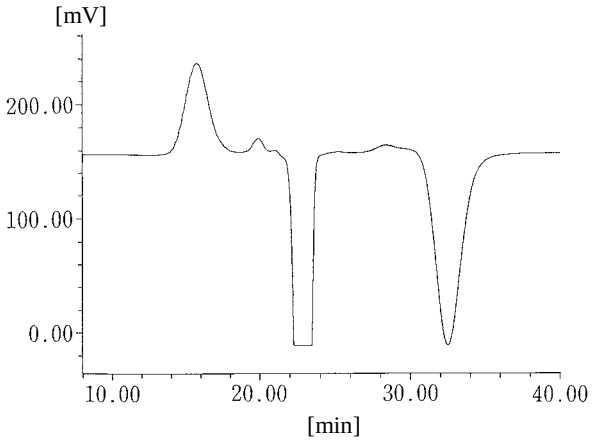


Fig. 61 Separation of polyimide

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in N-methylpyrrolidone
Flow rate: 0.5mL/min
Detection: RI
Temperature: 40°C
Sample: polyimide (0.1%, 50µL)

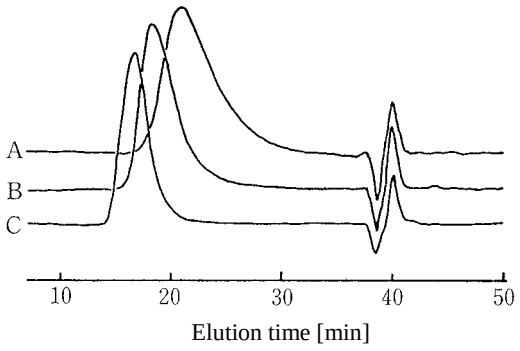


Fig. 63 Separation of polyvinyl alcohol

Column: TSKgel Alpha-5000 + Alpha-3000, 7.8mm ID x 30cm x 2
Eluent: Hexafluoroisopropanol (HFIP)
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Samples: (A) poly(vinyl alcohol) (degree of saponification: 75%)
(B) poly(vinyl alcohol) (degree of saponification: 88%)
(C) poly(vinyl alcohol) (degree of saponification: 100%)

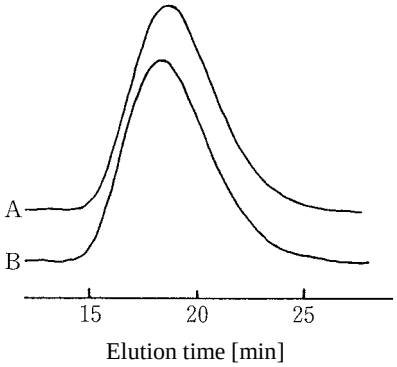


Fig. 64 Separation of polyvinyl alcohol

Column: TSKgel Alpha-5000, 7.8mm ID x 30cm x 2
Eluent: 0.1mol/L NaCl/MeOH = 1/1
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: (A) RI (B) UV@210nm
Samples: (A) poly(vinyl alcohol) (degree of saponification: 75%)
(B) poly(vinyl alcohol) (degree of saponification: 88%)

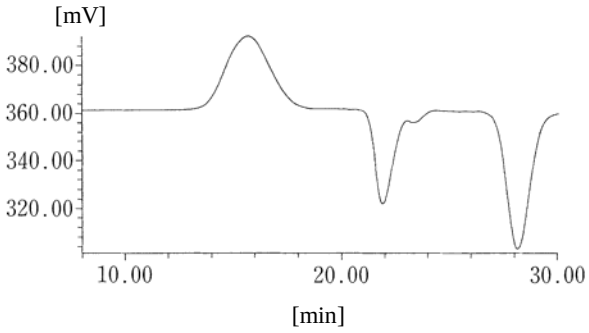


Fig. 66 Separation of polyvinylbutyral

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(vinyl butyral) (0.1%, 50µL)

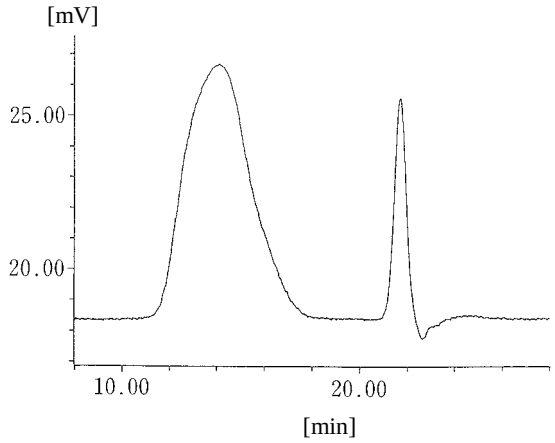


Fig. 65 Separation of polyvinylpyrrolidone (PVP79)

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 0.1mol/L NaCl/MeOH = 1/1
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(vinyl pyrrolidone) (50µL)

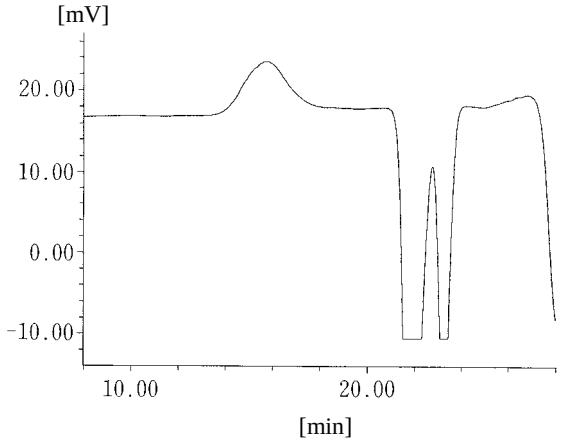


Fig. 67 Separation of polyvinylmethyl ether

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(vinyl methyl ether) (0.1%, 50µL)

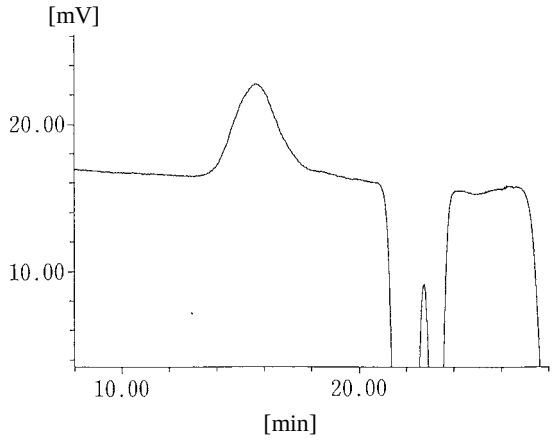


Fig. 68 Separation of polyvinylmethyl ether

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(vinyl methyl ether) (0.1%, 50µL)

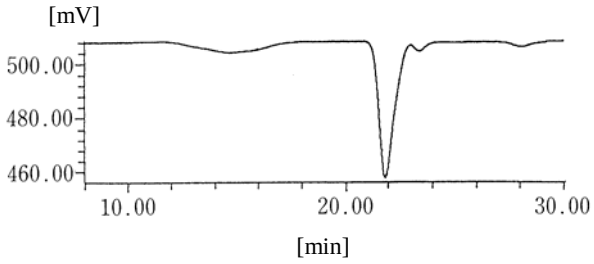


Fig. 70 Separation of polyvinylidene fluoride

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(vinylidene fluoride) (0.1%, 50µL)

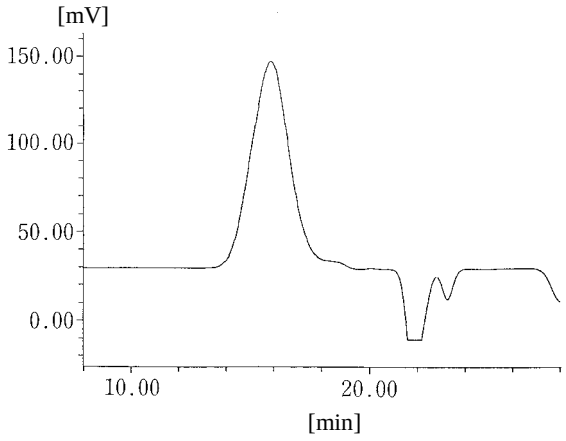


Fig. 69 Separation of poly(p-phenylene ether sulfone)

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(p-phenylene ether sulfone) (0.1%, 50µL)

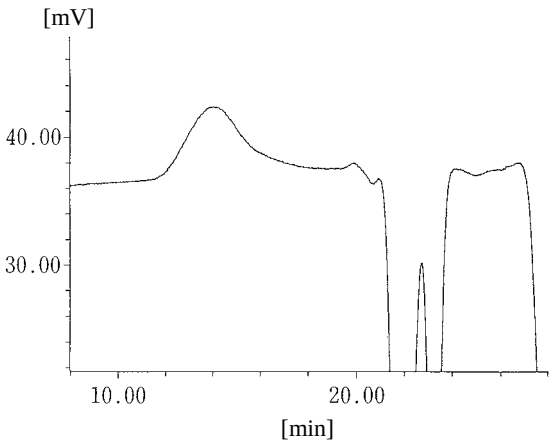


Fig. 71 Separation of poly (methylmethacrylate/methacrylic acid) copolymer

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: poly(methyl methacrylate/methacrylic acid) copolymer (0.1%, 50µL)

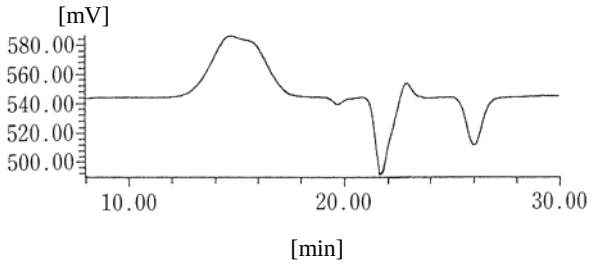


Fig. 72 Separation of methylvinylether/maleic acid copolymer

Column: TSKgel Alpha-M, 7.8mm ID x 30cm x 2
Eluent: 30mmol/L LiBr + 60mmol/L phosphoric acid in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: methylvinylether/maleic acid copolymer (0.1%, 50µL)

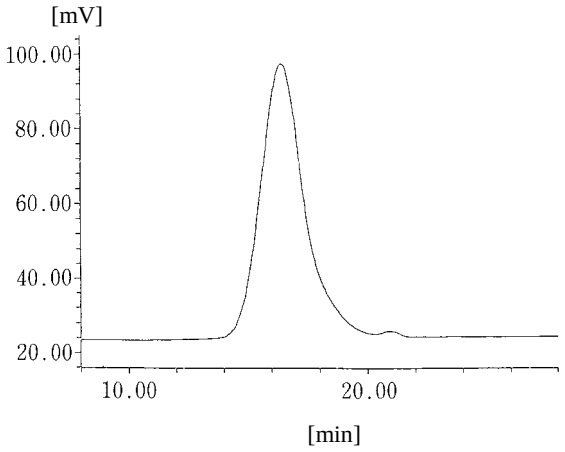


Fig. 73 Separation of N-methoxymethylated polyamide

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in methanol
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: N-methoxymethylated polyamide (50µL)

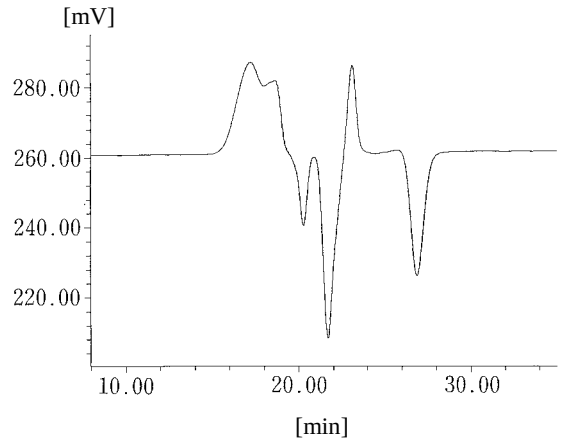


Fig. 74 Separation of melamine resin

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: butylated melamine resin (0.1%, 50µL)

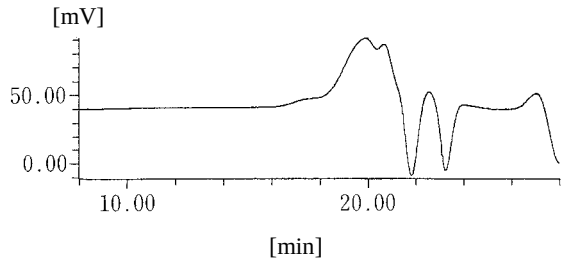


Fig. 75 Separation of melamine-modified urea resin

Column: TSKgel Alpha-M, 7.8mm ID x 30cm
Eluent: 10mmol/L LiBr in dimethylformamide
Flow rate: 0.5mL/min
Temperature: 40°C
Detection: RI
Sample: melamine-modified urea resin (0.1%, 50µL)

6. Summary

Size exclusion columns are usually classified into the following types: columns for aqueous solvent systems, in which the matrix is composed of a hydrophilic synthetic polymer, and columns used with an organic solvent system, in which a hydrophobic synthetic polymer such as styrene divinylbenzene is the matrix. However, multiple problems have occurred with each of these types of columns when used to study polar polymer molecules. These problems include (1) the ability of the packing material to withstand replacing one organic solvent with another, (2) adsorption of standard polymers in the presence of polar solvents, and (3) the solubility of the sample being characterized. However, with the TSKgel Alpha Series of columns discussed in this report, a variety of solvents can be chosen ranging from aqueous solutions to organic solvents, making it possible to set the conditions for investigation based on the solubility of the chemical and the molecular weight standard. As a result, the TSKgel Alpha Series of columns can be used when analyzing polar polymer samples that have been so cumbersome to characterize with traditional SEC columns in the past.