



# SEPARATION REPORT NO. 94


## ANALYZING POLYMERS THAT ARE SOLUBLE IN POLAR-ORGANIC SOLVENTS WITH TSKGEL ALPHA SERIES COLUMNS

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### Contact us today for more information

EMEA  +49 6155 704 37 12

USA  +1-800-366-4875 option #3



[www.tosohbioscience.com](http://www.tosohbioscience.com)



[techsupport.tb@tosoh.com](mailto:techsupport.tb@tosoh.com)

[techservice.tbl@tosoh.com](mailto:techservice.tbl@tosoh.com)

## 1. Introduction

Gel permeation chromatography (SEC), now often called size exclusion chromatography, is a procedure for separating a wide range of samples, both polar and non-polar, based on the molecular size of the sample. Consequently, SEC is widely used to measure molecular weight and analyze branching and distribution of other properties of polymers, as well as in separation and pattern analysis of oligomers and low molecular weight compounds.

We sell a wide variety of high-performance liquid chromatography columns for analyzing the properties of polymers including the TSK-GEL H<sub>XL</sub> series, an organic solvent line of high-performance SEC columns; the TSK-GEL H<sub>HR</sub> series of high performance SEC columns capable of withstanding conversion between organic solvents, and the Super H series of ultra-high-performance SEC columns. These columns can be used for a wide range of sample groups dissolved in organic solvents. We also market the TSK-GEL PW<sub>XL</sub> series of high-performance SEC columns, which use an aqueous solvent or a mixture of water and an organic solvent, and are primarily used to analyze water-soluble polymers. However, problems relating to sample solubility, adsorption of molecular weight standards, and solvent compatibility of columns, have occurred when conventional H type and PW type columns were used for SEC of polar samples.

The recently launched TSK-GEL Alpha series of SEC columns is capable of solving these problems in analyzing polar samples. This report introduces the basic features of the TSK-GEL Alpha series, and presents examples of separations of polar polymers using a variety of solvents.

## 2. Features of Alpha Series

Problems have occurred when polar solvents were used with styrene divinylbenzene copolymer types of packing materials, including adsorption of low molecular weight polystyrene standard compounds and hydrophobic interactions between the matrix and the sample. Moreover, when a hydrophilic polymer is used as the packing material, compatibility between various types of polar solvents is not perfect. Consequently, it has been difficult to select the optimal packed column for use in SEC for polar polymer samples.

The Alpha series of packed columns for SEC was developed to resolve these problems. The features are summarized in [Table 1](#). The Alpha series emphasizes resolving the following two issues.

1) To minimize hydrophobic interactions between the column matrix and polar polymers, a hydrophilic matrix was used as the packing material.

The use of a hydrophilic material eliminates adsorption of molecular weight standards, even with polar solvents, and enables accurate molecular weight determination. Also, because hydrophobic adsorption of the samples can be minimized, data reproducibility is improved, and the range of samples that can be analyzed is expanded.

2) Greatly improves swelling and shrinking of the packing material.

**Table-1 Features of TSK-GEL Alpha Series**

Feature	Benefits
Use of hydrophilic matrix	<ul style="list-style-type: none"><li>• Little hydrophilic adsorption even in the presence of polar organic solvents.</li><li>• Provides accurate calibration curves when used in dimethylformamide (DMF) systems, due to elimination of adsorption of standard polystyrene samples that is observed with H-type columns.</li></ul>
Swelling and shrinking	<ul style="list-style-type: none"><li>• Depending on the sample, a wide range of solvents can be selected, from water to non-polar solvents.</li></ul>
Solvent compatible type	<ul style="list-style-type: none"><li>• In UV detection, solvents that are active in the low-wavelength region can be used (including water, methanol, acetonitrile, and HFIP), and joint use with RI is also possible.</li><li>• Accurate molecular weight determination is possible in DMF systems, because salt peaks do not overlap on chromatograms in the low molecular weight regions.</li></ul>
Ability to handle changes in salt concentration	<ul style="list-style-type: none"><li>• Column is highly resistant to changes in salt concentration, so it is easy to investigate analysis conditions.</li><li>• Analysis can be performed under high flow rates.</li></ul>
Superior mechanical strength	<ul style="list-style-type: none"><li>• Temperature can be increased.</li><li>• When solvents with high viscosity are used or when samples with a high level of viscosity are used, a reduction in pressure can be expected due to increased temperature. Moreover, separation performance can also be improved due to improvement in column efficiency and improved calibration curves by increasing the temperature.</li></ul>

With conventional columns packed with hydrophilic matrices, in the presence of various organic solvents, it is difficult to convert from an aqueous solvent to an organic solvent due to differences in swelling and shrinking that occur. Moreover, the use of polar solvents is greatly restricted. With the Alpha series of columns, the packing material was engineered to minimize swelling and shrinking, which, with no reduction in column performance, results in a carrier in which conversion between solvents is possible, even when a wide range of solvents (from water to mildly polar solvents) is used.

The TSK-GEL Alpha Series consists of a line of solvent-compatible SEC columns packed with hydrophilic polymer matrices that have six different separation ranges.

Table 2 lists the grades available in the TSK-GEL Alpha series.

Because the Alpha series covers a wide separation range and permits selection of the solvent that best dissolves the sample, it can be used with a wide range of compounds ranging from aqueous to mildly polar. 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 TSK-GEL Alpha Series Grades**

Grade	Column size (mm ID× cm L)	Guaranteed no. of theor. plates <sup>1)</sup> (TP/30cm)	Exclusion limit molecular weight	
			PEO/H <sub>2</sub> O <sup>2)</sup>	PS/DMF <sup>3)</sup>
Alpha-2500	7.8 × 30	16,000	5×10 <sup>3</sup>	1×10 <sup>4</sup>
Alpha-3000	7.8 × 30	16,000	9×10 <sup>4</sup>	1×10 <sup>5</sup>
Alpha-4000	7.8 × 30	10,000	4×10 <sup>5</sup>	1×10 <sup>6</sup>
Alpha-5000	7.8 × 30	10,000	1×10 <sup>6</sup>	7×10 <sup>6</sup>
Alpha-6000	7.8 × 30	7,000	> 1×10 <sup>7</sup>	> 1×10 <sup>7</sup>
Alpha-M	7.8 ×30	7,000	> 1×10 <sup>7</sup>	> 1×10 <sup>7</sup>

- 1) Eluent: H<sub>2</sub>O  
Flow rate: 1.0 mL/min  
Sample: Ethylene glycol  
Temperature: 25 °C  
Detection: Differential refractometer
- 2) Eluent: H<sub>2</sub>O  
Flow rate: 1.0 mL/min  
Sample: Standard polyethylene oxide  
Temperature: 25 °C  
Detection: Differential refractometer
- 3) Eluent: Dimethylformamide  
Flow rate: 1.0 mL/min  
Sample: Standard polystyrene  
Temperature: 25 °C  
Detection: Differential refractometer

**Table-3 TSK-GEL Alpha Series: Solvent compatibility**

Solvent	Theoretical number of plates	
	TSKgel Alpha-2500	TSKgel Alpha-3000
Methanol	27700	25370
Ethanol	16760	25120
THF	24340	25370
DMF	24550	25370
DMSO	25840	28800
Isopropanol	20630	25610
DMSO/H <sub>2</sub> O = 1/1	24450	25120
Methanol/H <sub>2</sub> O = 1/1	25730	20900
Acetonitrile/H <sub>2</sub> O = 1/1	24530	21540
THF/H <sub>2</sub> O = 1/1	23850	22200
HFIP	18720	28720

Solvent conversion conditions:

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

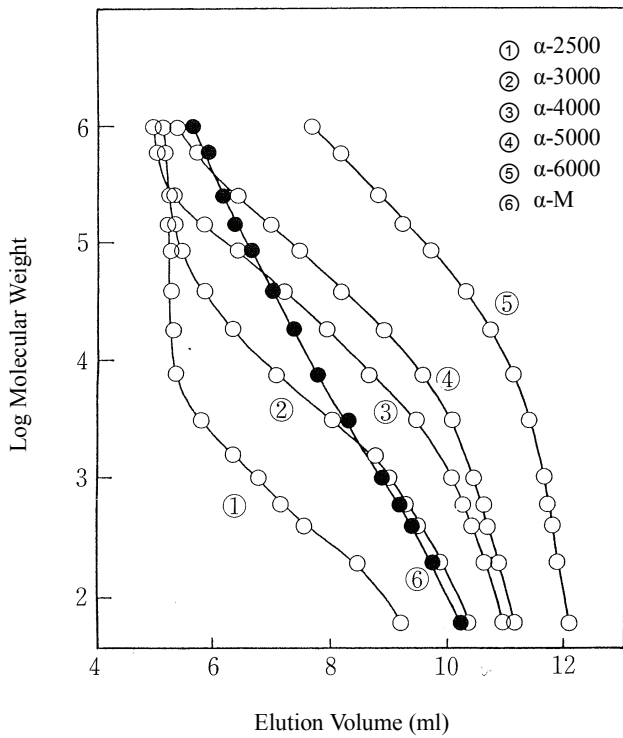
Conditions for measuring number of theoretical plates

- Eluent: H<sub>2</sub>O
- Flow rate: 1.0 mL/min
- Temperature: 25 °C
- Detection: Differential refractometer
- Sample: Ethylene glycol

**Table-4 Conditions for analyzing calibration curves in TSK-GEL Alpha Series**

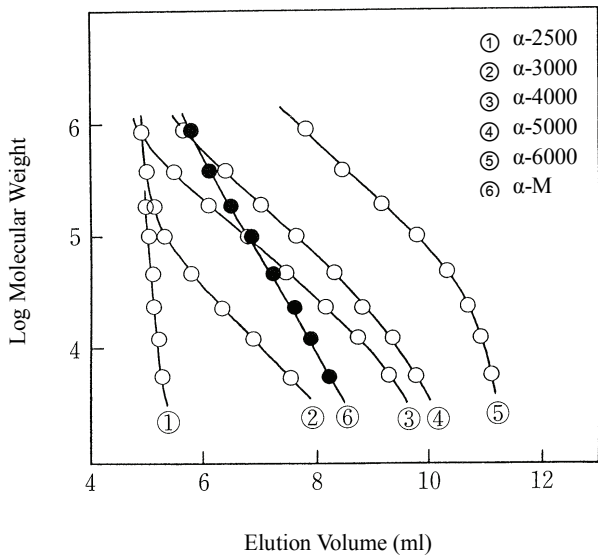
Eluent used	Molecular weight standard	Calibration curve	Notes
1) H <sub>2</sub> O	PEO/PEG	Fig. 1	
2) H <sub>2</sub> O	Pullulan	Fig. 2	
3) 0.1M NaClO <sub>4</sub> /AcCN	PNASS	Fig. 3	
4) Methanol (10 mM LiBr)	PEO/PEG	Fig. 4	Not readily soluble at high temperatures (≥60 °C)
5) THF	Polystyrene	Fig. 5	
6) Polystyrene	Polystyrene	Fig. 6	
7) DMF (10 mM LiBr)	PEO/PEG	Fig. 7	
8) Polystyrene	Polystyrene	Fig. 8	
9) H <sub>2</sub> O	PEO/PEG	Figs. 9-11	Temp 25, 40, 60 °C
10) DMF	PEO/PEG	Figs. 12-14	Temp 25, 40, 60, 80 °C

- THF : Tetrahydrofuran
- DMF : Dimethylformamide
- DMSO : Dimethylsulfoxide
- HFIP : Hexafluoroisopropanol
- PEO : Polyethylene oxide
- PEG : Polyethylene glycol
- PNASS : Poly(sodium styrene sulfonate)



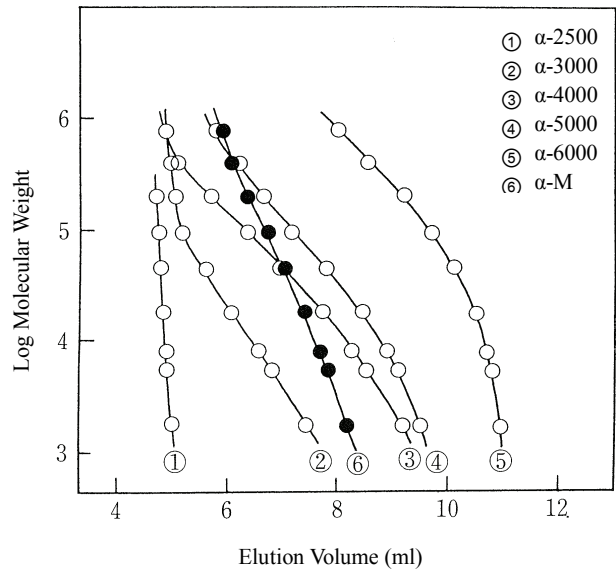
**Fig. 1** TSK-GEL Alpha Series Calibration Curve (H<sub>2</sub>O/PEO, PEG)

Column: TSK-GEL Alpha Series  
 Eluent: H<sub>2</sub>O  
 Flow rate: 1.0 mL/min  
 Temp.: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG



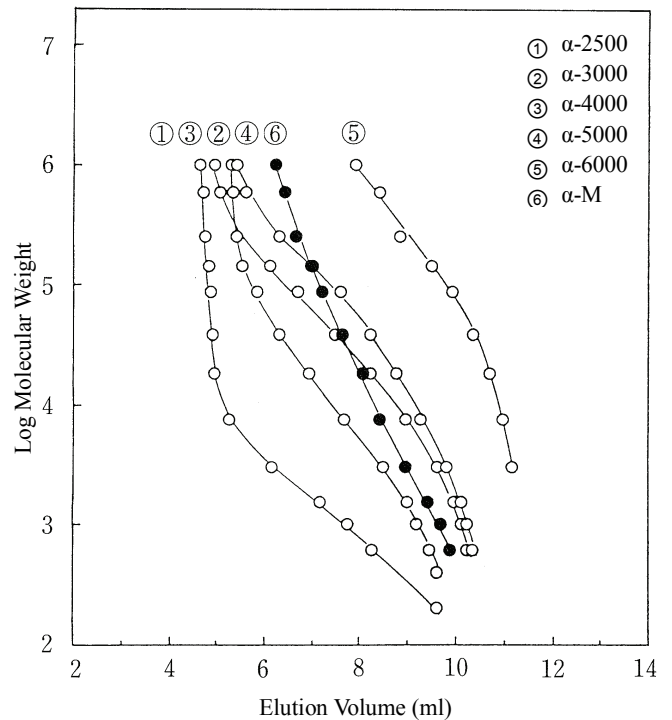
**Fig. 2** TSK-GEL Alpha Series Calibration Curve (H<sub>2</sub>O/pullulan)

Column: TSK-GEL Alpha Series  
 Eluent: H<sub>2</sub>O  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard pullulan



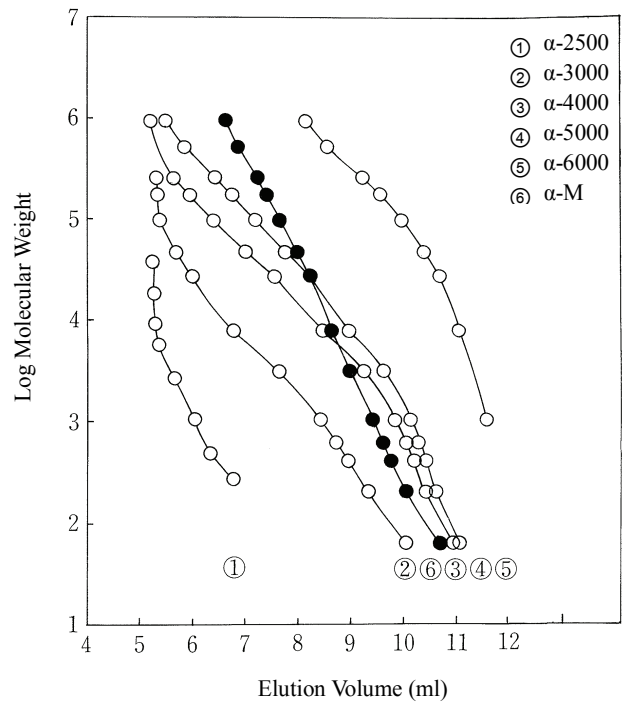
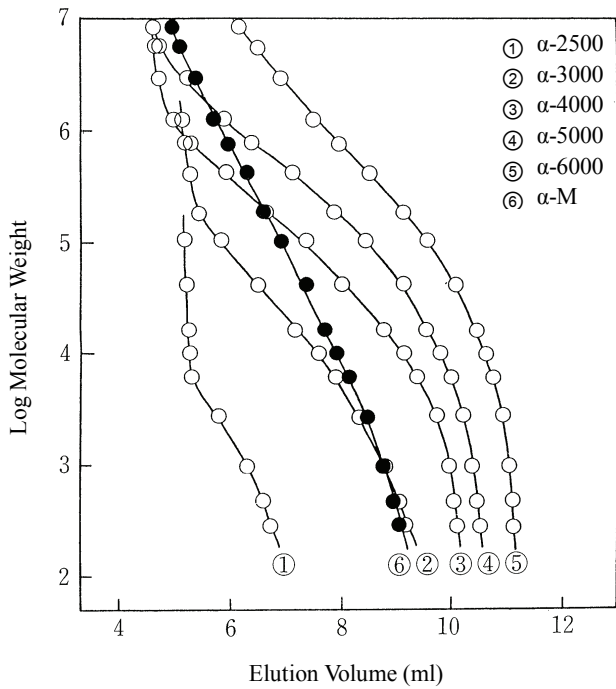
**Fig. 3** TSK-GEL Alpha Series Calibration Curve (Aqueous solution/PNASS)

Column: TSK-GEL Alpha Series  
 Eluent: 0.1 M sodium perchlorate in 22.5% acetonitrile  
 Flow rate: 1.0 mL/min  
 Temp.: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard sodium polystyrene sulfonate



**Fig. 4** TSK-GEL Alpha Series Calibration Curve (Methanol/PEO, PEG)

Column: TSK-GEL Alpha Series  
 Eluent: 10 mM LiBr in methanol  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG



**Fig. 5** TSK-GEL Alpha Series Calibration Curve

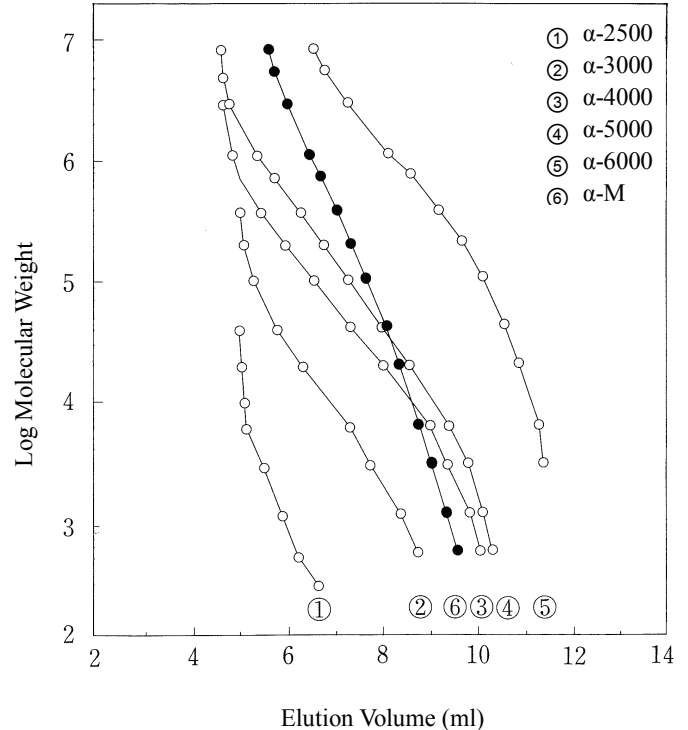
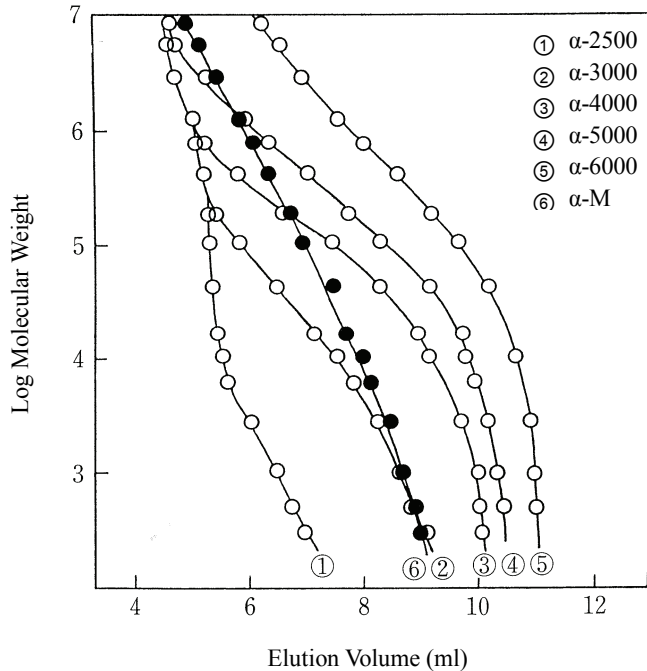
(THF/PS)

Column: TSK-GEL Alpha Series  
 Eluent: Tetrahydrofuran  
 Flow rate: 1.0 mL/min  
 Temp.: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polystyrene

**Fig. 7** TSK-GEL Alpha Series Calibration Curve

(DMF/PEO, PEG)

Column: TSK-GEL Alpha Series  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 1.0 mL/min  
 Temp.: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG, EG



**Fig. 6** TSK-GEL Alpha Series Calibration Curve

(CHCl<sub>3</sub>/PS)

Column: TSK-GEL Alpha Series  
 Eluent: Chloroform  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polystyrene

**Fig. 8** TSK-GEL Alpha Series Calibration Curve (DMF, PS)

Column: TSK-GEL Alpha Series  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polystyrene

### 3. Basic Characteristics of Alpha Series

#### 3.1 Conversion between Eluents

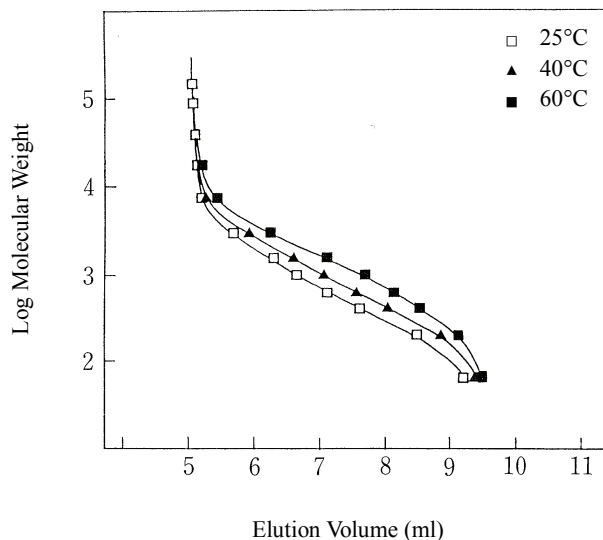
Table 3 shows the results of solvent compatibility tests using various solvents. There was little change in theoretical plate number after any of the solvents were changed, clearly demonstrating that the TSK-GEL Alpha series has an outstanding ability to accommodate conversion between solvents. A single column can be converted to accommodate a variety of solvents from aqueous solvents to nonpolar solvents, which greatly expands the ability to choose a solvent appropriate to the properties of the sample being analyzed.

#### 3.2 Calibration curves

Although TSKgel Alpha is presented as a solvent-compatible series of columns, when selecting the molecular weight standard to use for calibration curves, various properties must be considered, including the solubility in the solvent and the capacity for adsorption by the packing material. Table 4 shows the solvents and appropriate molecular weight standards used with the TSK-GEL Alpha series, and calibration curves for various solvents are shown in Figs. 1 to 8. It is clear that the calibration curve will differ depending on the type of solvent used.

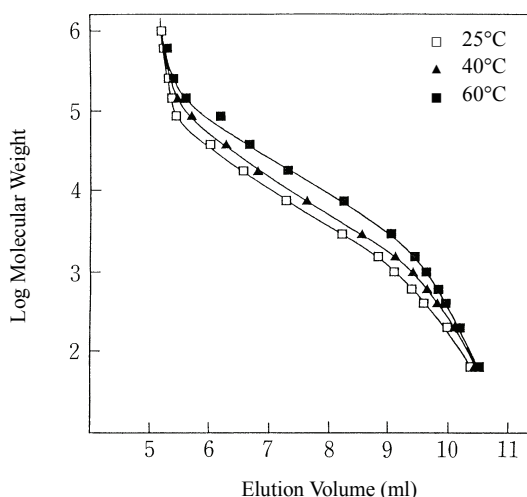
#### 3.3 Effect of temperature

Temperature has various effects on SEC analysis. Figs. 9 to 11 show the temperature dependence of calibration curves in aqueous solvents, while Figs. 12 to 14 show the temperature dependence of calibration curves in DMF solvent systems. Standard polyethylene oxide (PEO) and polyethylene glycol (PEG) were used as the molecular weight standards. In H<sub>2</sub>O/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 water systems, 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 system, a good calibration curve can be obtained at each grade without adsorption of the sample on PEO or PS. As a result, accurate molecular weight determination can be performed by using PS and PEO separately (See Figs. 12 to 14).



**Fig. 9** Temperature dependence of TSKgel Alpha-2500 calibration curve (H<sub>2</sub>O/PEO, PEG)

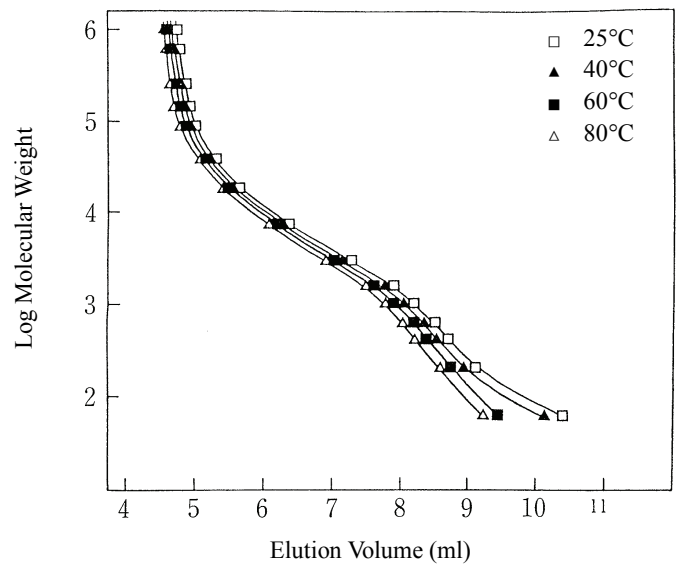
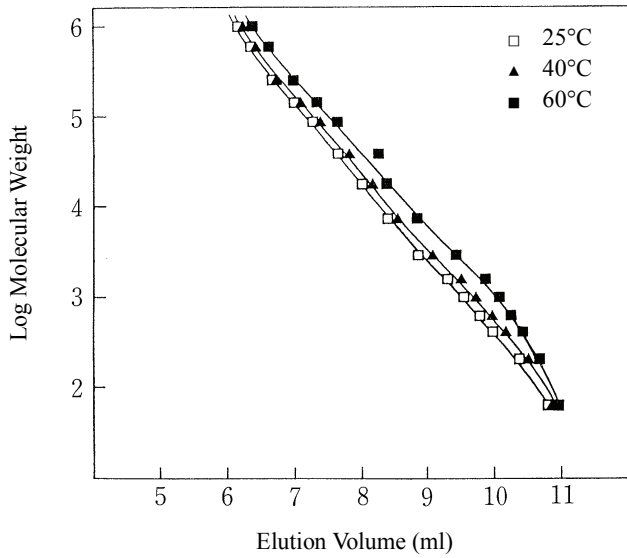
Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)  
Eluent: H<sub>2</sub>O  
Flow rate: 1.0 mL/min  
Temp.: 25 °C to 80 °C  
Detection: Differential refractometer  
Sample: Standard polyethylene oxide, PEG and EG



**Fig. 10** Temperature dependence of TSKgel Alpha-3000 calibration curve (H<sub>2</sub>O/PEO, PEG)

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



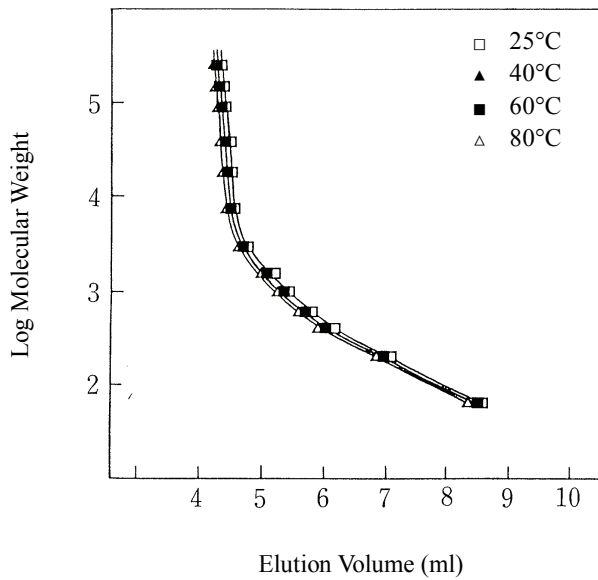


**Fig. 11** Temperature dependence of TSKgel Alpha-M calibration curve (H<sub>2</sub>O/PEO, PEG)

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

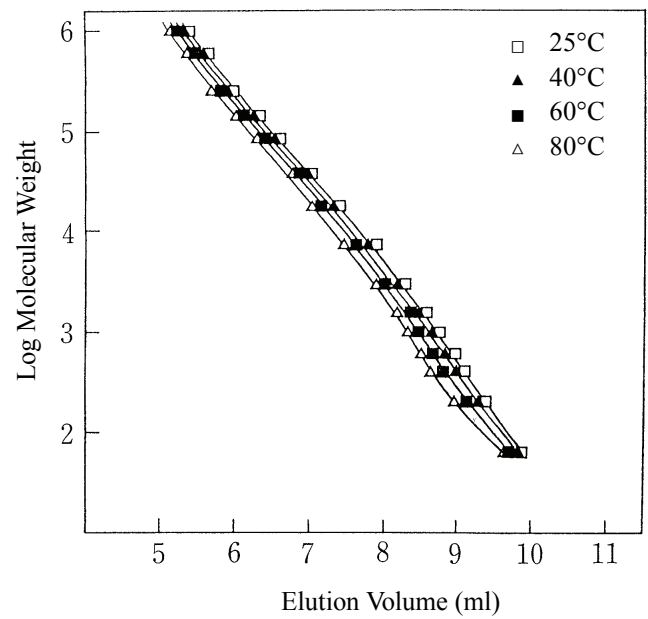
**Fig. 13** Temperature dependence of TSKgel Alpha-3000 calibration curve (DMF/PEO, PEG)

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



**Fig. 12** Temperature dependence of TSKgel Alpha-2500 calibration curve (DMF/PEO, PEG)

Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 1.0 mL/min  
 Temp.: 25 °C to 80 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG



**Fig. 14** Temperature dependence of TSKgel Alpha-M calibration curve (DMF/PEO, PEG)

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

In general, solvent viscosity decreases as the temperature is increased, causing an increase in the number of theoretical plates. Figures 15 to 17 show the temperature dependence of the theoretical plate number of high molecular weight samples in water and DMF systems. Although increases in the theoretical plate number are observed when the temperature is increased, as noted above, in aqueous solvents at high temperatures, a delay is observed due to adsorption of the molecular weight standard. Thus, the temperature under which analysis is performed must be given careful consideration based on the solvent system used.

### 3.4 Effect of flow rate

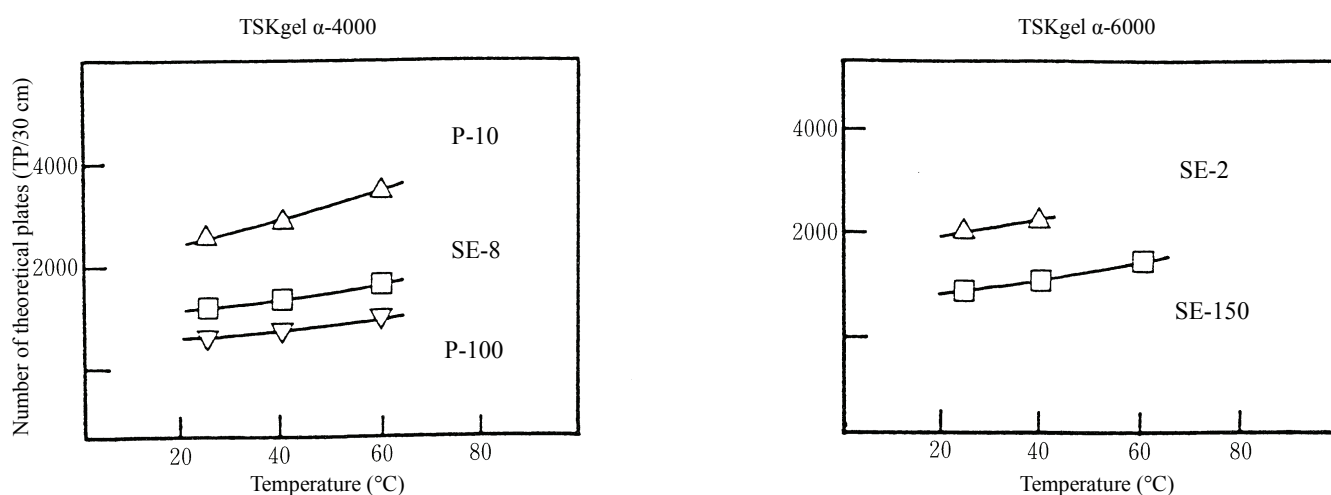
Figures 18 and 19 show the dependence of column plate number on the flow rate in aqueous solvent and DMF systems. Normally, the optimal flow rate depends on the particle size of the packing material and the molecular weight of the sample being analyzed. With low molecular weight samples, good results are obtained at a flow rate around 0.7 mL/min, while with high molecular weight samples the best results are obtained at lower flow rates. Organic solvents 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 necessary to understand the relationship between flow rate and separation performance for the solvent being used for analysis.

### 3.5 Effect of sample injection volume

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

### 3.6 Effect of sample injection concentration

As the concentration of the sample increases, a sample overload effect can appear, reducing column efficiency. This issue must be kept in mind if the objective is to process samples in batches. Figure 24 (TSKgel Alpha 3000) and Figure 25 (TSKgel Alpha-M) show the effects of sample concentration at a constant injection volume on column efficiency in a water system, while Figs. 26 and 27 show the same when a DMF system is used. The load decreases as the molecular weight of the sample increases.

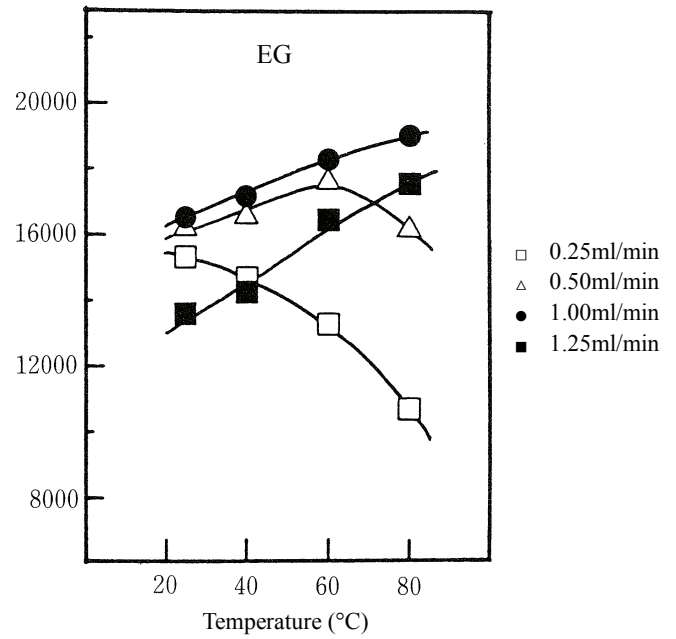
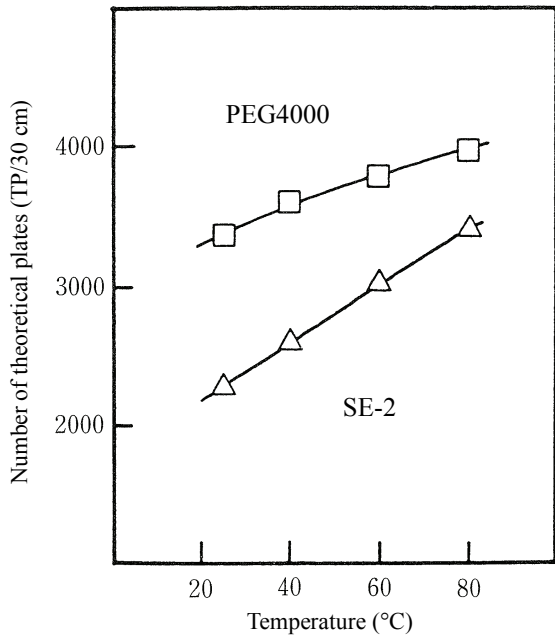


**Fig. 15 Relationship between temperature and number of theoretical plates of molecular weight standard in TSK-GEL**

#### Alpha Series

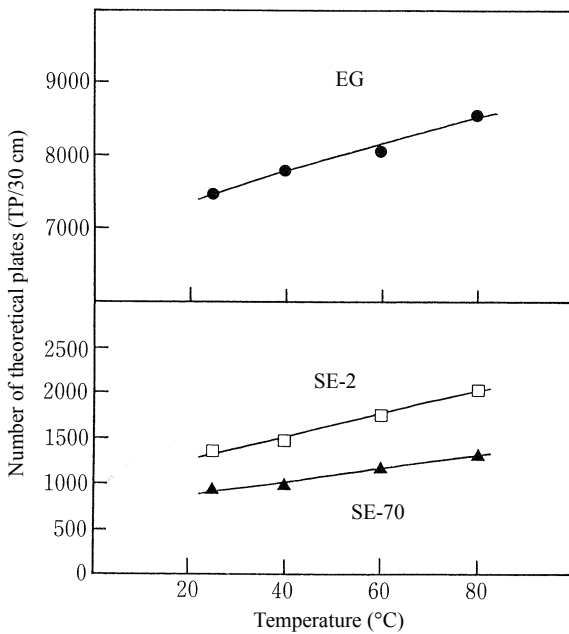
Columns: TSKgel Alpha-4000, Alpha-6000 (7.8 mm ID x 30 cm L)  
 Eluent: H<sub>2</sub>O  
 Flow rate: 1.0 mL/min  
 Temp.: 25 °C to 80 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide (SE) and standard pullulan (P)





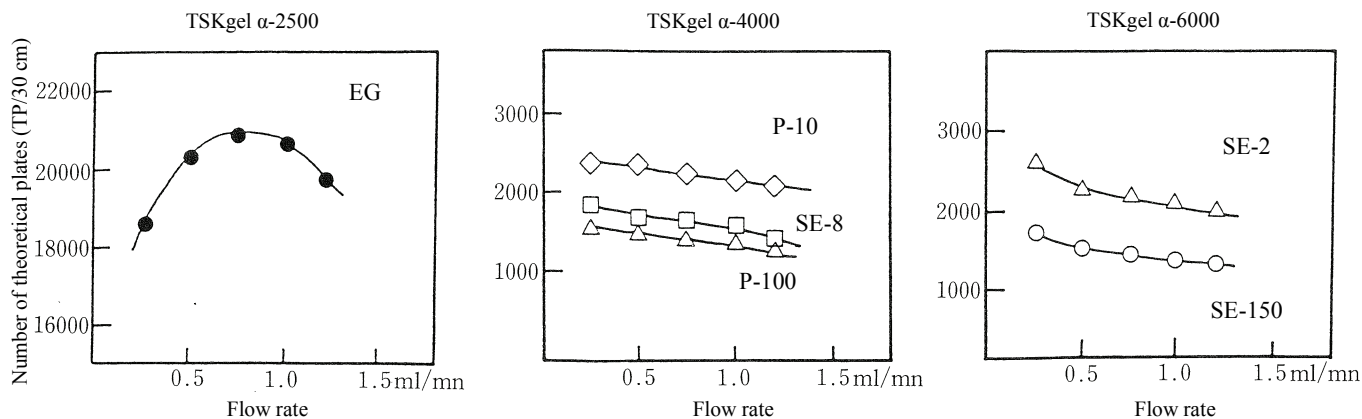
**Fig. 16** Relationship between temperature and number of theoretical plates of molecular weight standard in TSKgel Alpha-3000

Analysis conditions  
 Columns: TSKgel Alpha-3000 (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 0.25 mL/min to 1.25 mL/min  
 Temperature: 25 °C to 80 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG  
 Injection volume: 50 µL



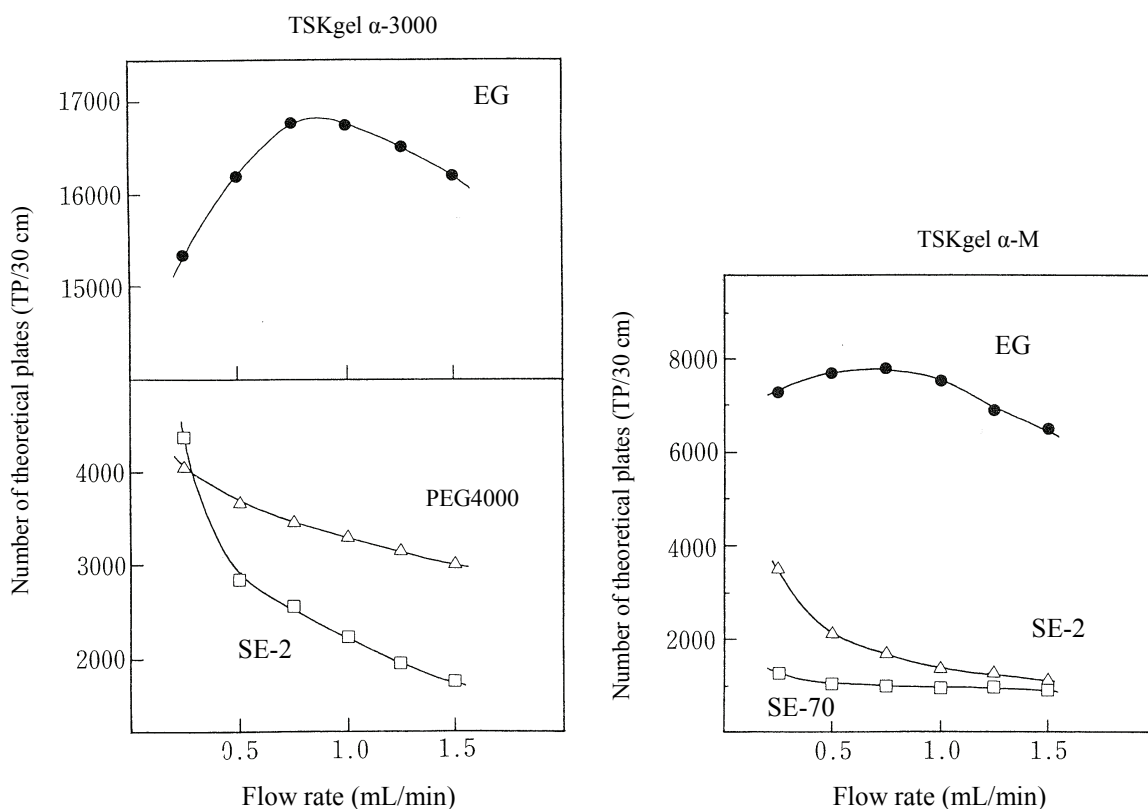
**Fig. 17** Relationship between temperature and number of theoretical plates of molecular weight standard in TSKgel Alpha-M

Columns: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C to 80 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide  
 Injection volume: 50 µL



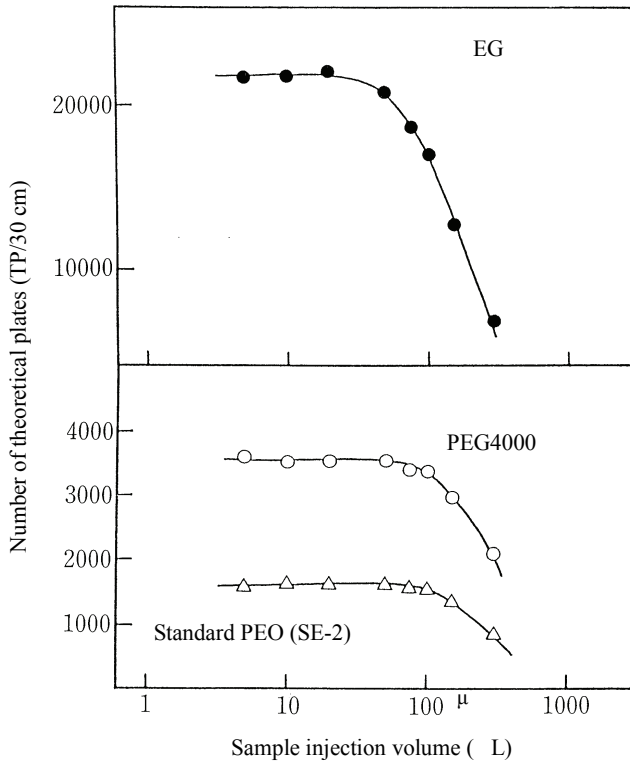
**Fig. 18** Relationship between flow rate and number of theoretical plates of molecular weight standard in TSK-GEL Alpha series columns

Columns: TSKgel Alpha-2500, Alpha-4000, Alpha-6000 (7.8 mm ID x 30 cm L)  
 Eluent: H<sub>2</sub>O  
 Flow rate: 0.25 mL/min to 1.3 mL/min  
 Temp.: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, standard pullulan and EG



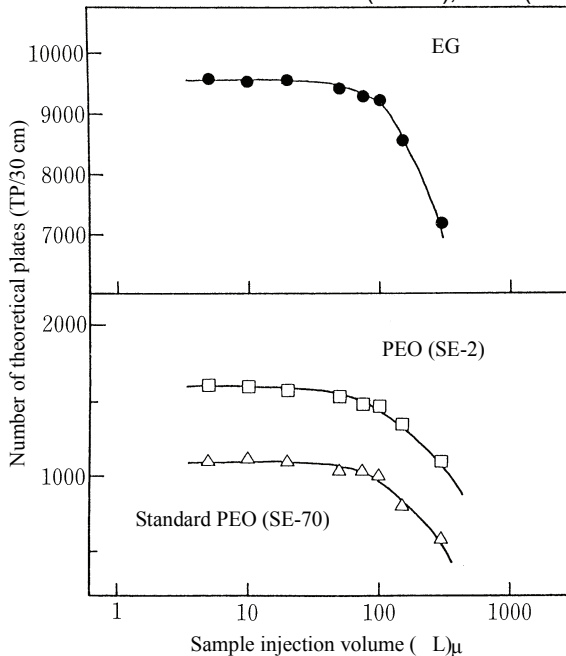
**Fig. 19** Relationship between flow rate and number of theoretical plates of molecular weight standard in TSK-GEL Alpha series columns

Columns: TSKgel Alpha-3000, Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 0.25 mL/min to 1.5 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG  
 Injection volume: 50  $\mu$ L



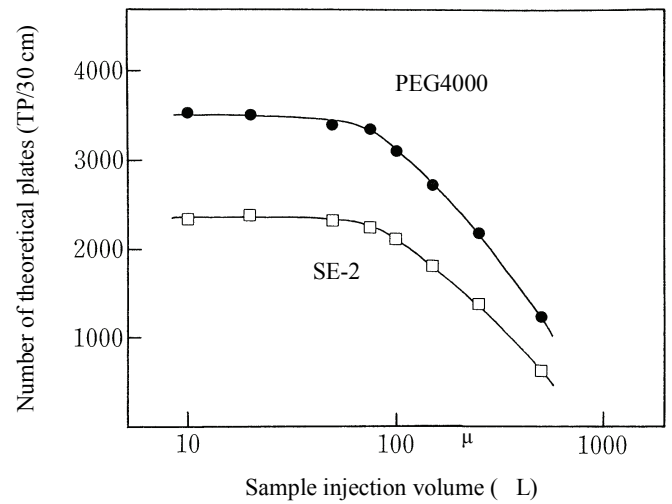
**Fig. 20** Relationship between number of theoretical plates and injection volume (constant concentration) of molecular weight standard in TSKgel Alpha-3000

Column: TSKgel Alpha-3000 (7.8 mm ID x 30 cm L)  
 Eluent: H<sub>2</sub>O  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG  
 Concentration: EG and PEG4000 (0.05%); SE-2 (0.1%)



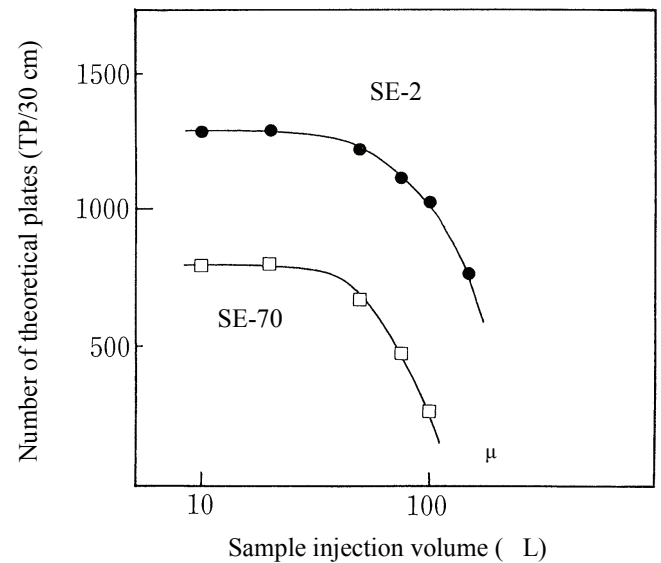
**Fig. 21** Relationship between number of theoretical plates and injection volume (constant concentration) of molecular weight standard in TSKgel Alpha-M

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: H<sub>2</sub>O  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide and EG  
 Concentration: EG (0.05%), SE-70 (0.04%), SE-2 (0.1%)



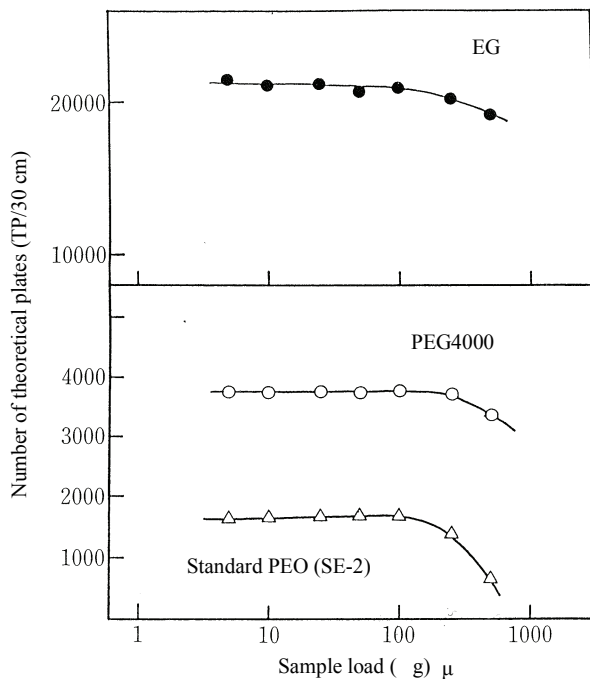
**Fig. 22** Relationship between number of theoretical plates and injection volume (constant concentration) of molecular weight standard in TSKgel Alpha-3000

Column: TSKgel Alpha-3000 (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide and PEG  
 Concentration: SE-2 (0.15%), PEG4000 (0.15%)



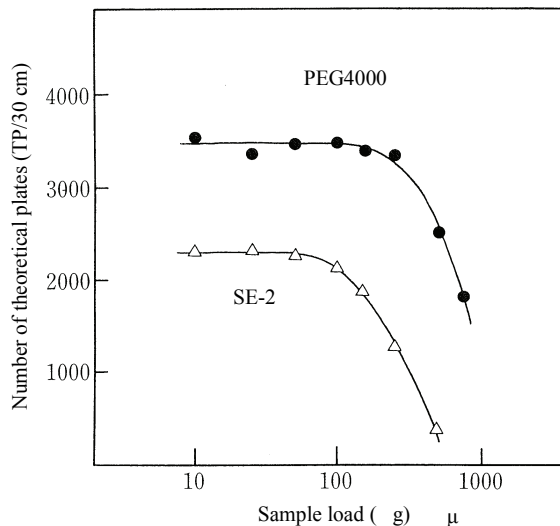
**Fig. 23** Relationship between number of theoretical plates and injection volume (constant concentration) of molecular weight standard in TSKgel Alpha-M

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide (2 mg/mL)  
 Concentration: SE-70 (0.1%), SE-2 (0.15%)



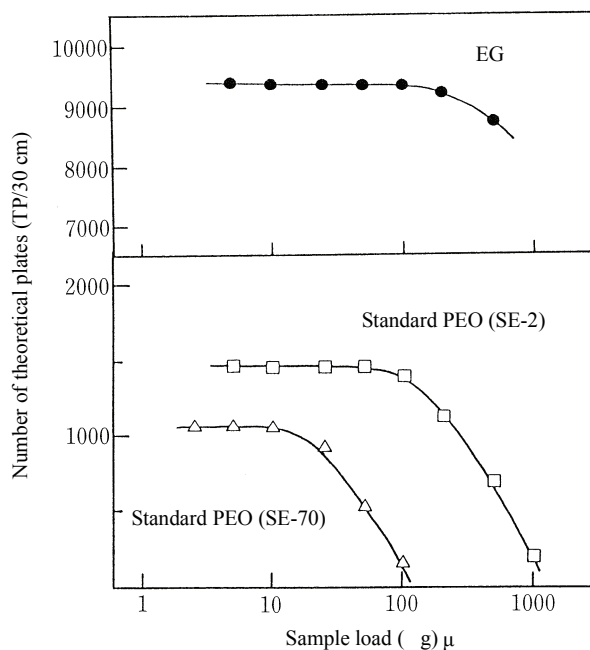
**Fig. 24** Relationship between number of theoretical plates and load (constant injection volume) of molecular weight standard in TSKgel Alpha-3000

Column: TSKgel Alpha-3000 (7.8 mm ID x 30 cm L)  
 Eluent: H<sub>2</sub>O  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG  
 Injection volume: 50 μL



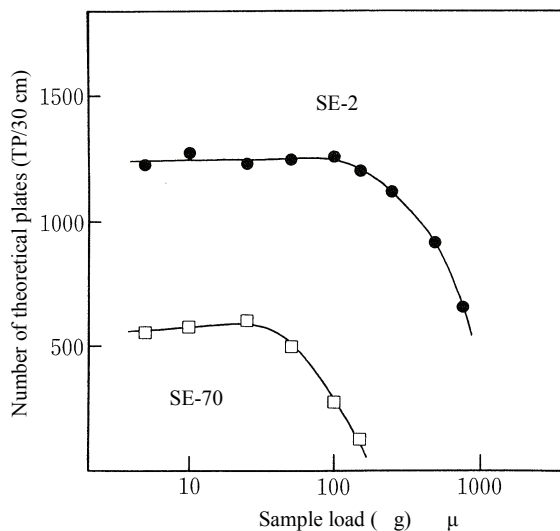
**Fig. 26** Relationship between number of theoretical plates and load (constant injection volume) of molecular weight standard in TSKgel Alpha-3000

Column: TSKgel Alpha-3000 (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 0.25 mL/min to 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide, PEG and EG  
 Injection volume: 50 μL



**Fig. 25** Relationship between number of theoretical plates and load (constant injection volume) of molecular weight standard in TSKgel Alpha-M

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: H<sub>2</sub>O  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide and EG  
 Injection volume: 50 μL



**Fig. 27** Relationship between number of theoretical plates and load (constant injection volume) of molecular weight standard in TSKgel Alpha-M

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: Dimethylformamide  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: Differential refractometer  
 Sample: Standard polyethylene oxide  
 Injection volume: 50 μL

### 3.7 Effect of adding salt to the solvent

In SEC analyses conducted using polar solvents, 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, aggregation, and interaction with the packing material. With polar solvents such as DMF and N-methylpyrrolidone (NMP), etc., interactions with basic impurities within the solvent have also been reported. In such cases, ionic adsorption can be suppressed by adding the appropriate salts to the solvent, and depending on the sample, solubility may also be improved.

Fig. 28 shows the effect of adding LiBr to DMF on the elution pattern of a phenol resin. The chromatography pattern changes with the addition of a large amount of LiBr, and in the case of this sample, a good separation pattern was obtained at a concentration around 50 mM. 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.

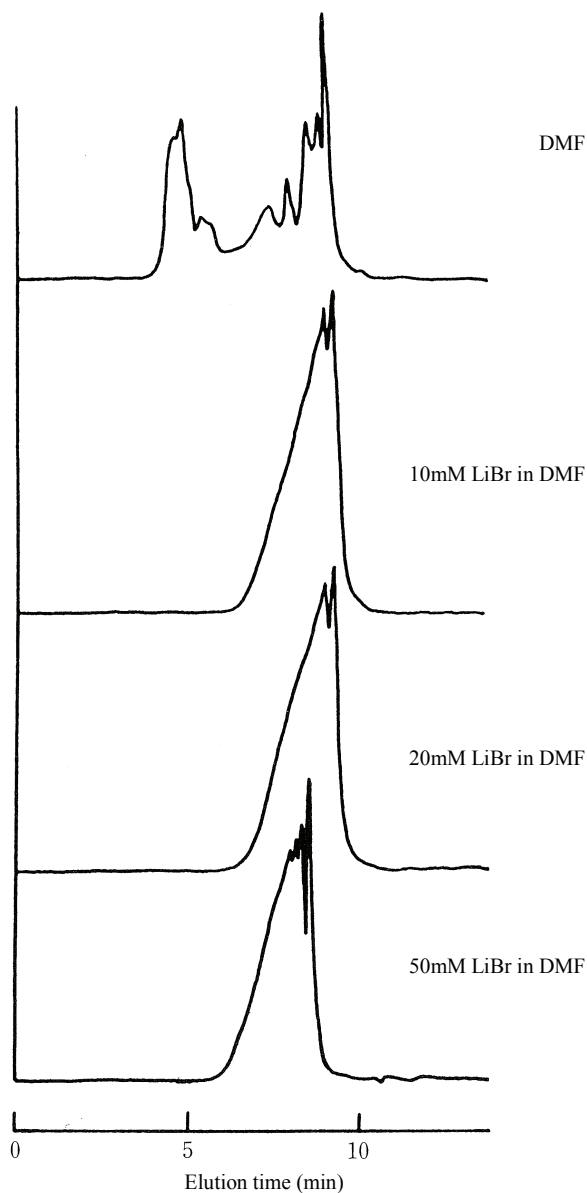
### 3.8 Ionicity and hydrophobicity

Table 5 shows the results of analyses of the ionicity and hydrophobicity of the packing material of the TSK-GEL Alpha series 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<sub>XL</sub> series of water-system SEC columns. Adsorption of the molecular weight standard is a problem that can occur in styrene vinyl benzene columns when a polar organic solvent system is used. Fig. 29 compares the elution behavior of standard PS in the TSKgel Alpha-3000 versus the TSKgel G3000H<sub>HR</sub>. When a polar organic solvent is used in a styrene column, adsorption of PS is observed, but with the Alpha series, a good chromatogram is obtained without adsorption.

### 3.9 Elution behavior of solvent peaks

Another drawback occurs when a polar solvent is used in a styrene column, particularly when DMF containing a salt, such as LiBr, etc., is used. 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 molecular weight determination. However, with the TSK-GEL Alpha series, 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. Figs. 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 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. 28** Dependence of phenol resin chromatogram on salt concentration in TSKgel Alpha-3000

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

**Table-5 Ionicity and hydrophobicity of TSK-GEL Alpha Series**

Sample	Eluent	Capacity factor (k')*	
		TSKgel Alpha-2500	TSKgel Alpha-5000
Beta-phenethyl alcohol	H <sub>2</sub> O	7.66	5.05
	50% acetonitrile	0.00	0.07
Tryptophan	H <sub>2</sub> O	2.69	0.36

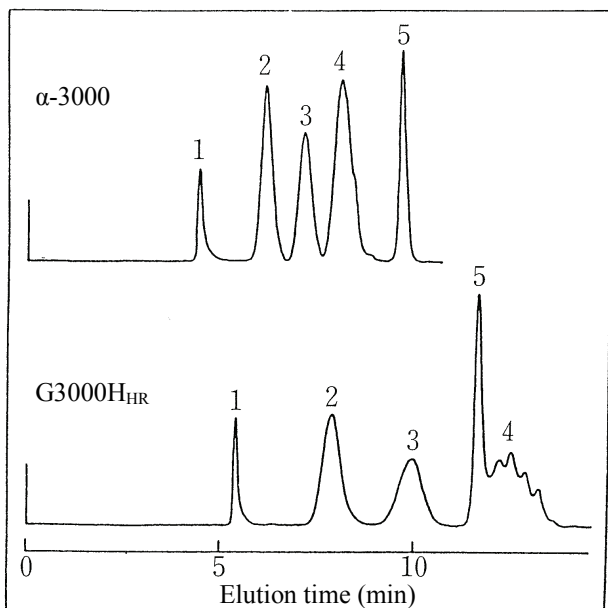
Analysis conditions:

Eluent: H<sub>2</sub>O, 50% acetonitrile

Flow rate: 1.0 mL/min;

Detection: Differential refractometer

\* Calculated with ethylene glycol elution time as t<sub>0</sub>.



**Fig. 29 Comparison of TSKgel Alpha-3000 and G3000HR in separation of standard polyethylene mixture**

Columns: TSKgel Alpha-3000, TSKgel G3000HR (7.8 mm ID x 30 cm L)

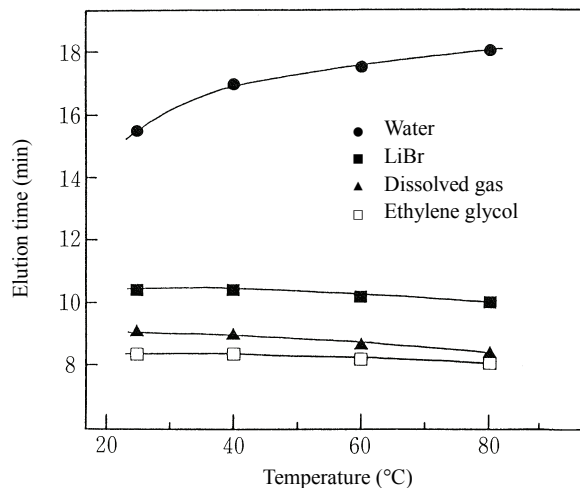
Eluent: Dimethylformamide

Flow rate: 1.0 mL/min

Temperature: 25 °C

Detection: UV (270 nm)

Sample: 1. Standard polyethylene (F-20) MW: 190,000  
 2. " (F-1) " 9,100  
 3. " (A-2500) " 2,800  
 4. " (A-500) " 500  
 5. Acetone



**Fig. 30 Relationship between temperature and elution time of system peak with TSKgel Alpha-2500 in DMF mobile phase**

Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)

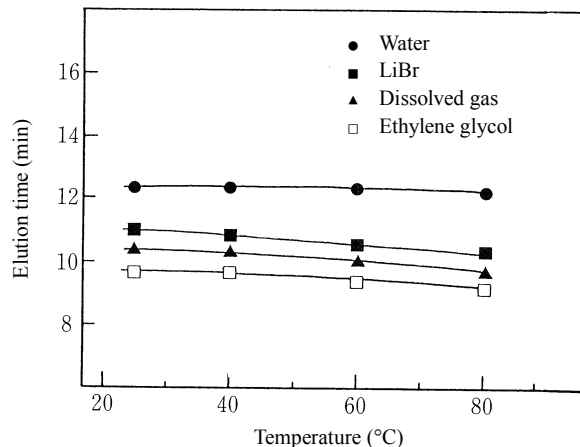
Eluent: Dimethylformamide

Flow rate: 1.0 mL/min

Temp.: 25 °C to 80 °C

Detection: Differential refractometer

Sample: Dissolved gas, lithium bromide and water

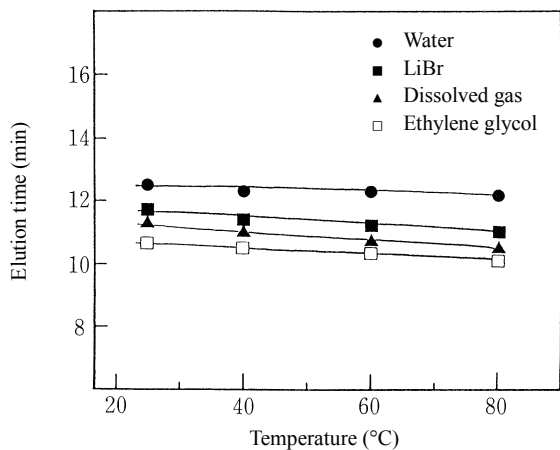


**Fig. 31 Relationship between temperature and elution time of system peak with TSKgel Alpha-3000 in DMF mobile phase**

Column: TSKgel Alpha-3000 (7.8 mm ID x 30 cm L)

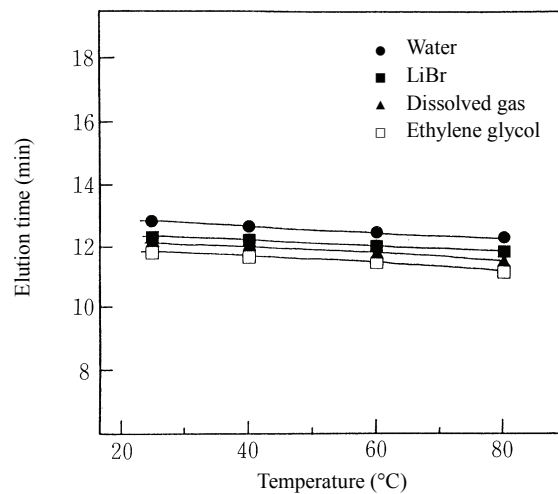
Other analysis conditions are the same as those in Fig. 30.





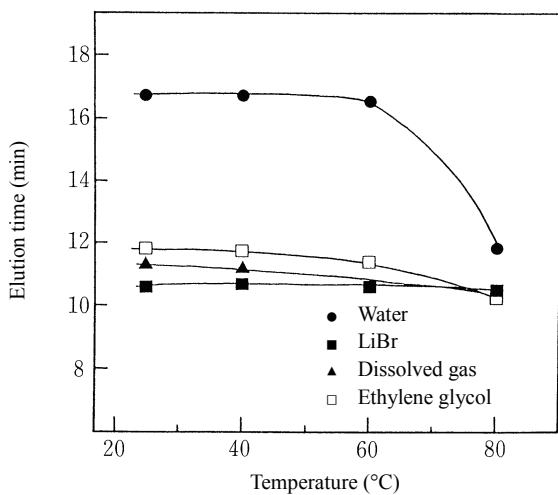
**Fig. 32 Relationship between temperature and elution time of system peak with TSKgel Alpha-4000 in DMF mobile phase**

Column: TSKgel Alpha-4000 (7.8 mm ID x 30 cm L)  
Other analysis conditions are the same as those in Fig. 30.



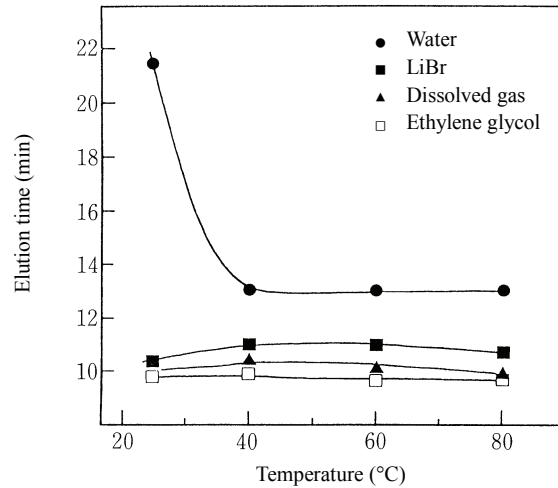
**Fig. 34 Relationship between temperature and elution time of system peak with TSKgel Alpha-6000 in DMF mobile phase**

Column: TSKgel Alpha-6000 (7.8 mm ID x 30 cm L)  
Other analysis conditions are the same as those in Fig. 30.



**Fig. 33 Relationship between temperature and elution time of system peak with TSKgel Alpha-5000 in DMF mobile phase**

Column: TSKgel Alpha-5000 (7.8 mm ID x 30 cm L)  
Other analysis conditions are the same as those in Fig. 30.



**Fig. 35 Relationship between temperature and elution time of system peak with TSKgel Alpha-M in DMF mobile phase**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
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 preparation of samples until analysis.

### 4.1 Sample preparation procedures

#### 4.1.1 Dissolving of 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, dissolving may require temperatures above 40 °C or below 10 °C. When dissolved, the main chain of a polymer tends to break (this tendency becomes more marked the higher the molecular weight). Consequently, avoid shaking/agitating sample solutions during and after dissolution.

#### 4.1.2 Preparation of sample solutions

When an organic solvent is used as the solvent, fundamentally, the solvent should be selected from among solvents that dissolve the sample. When a water system is used as the solvent, the solvent should be selected according to the properties of the sample (ionicity, hydrophobicity). Basically, solid samples should dissolve in the solvent. Solution samples should be prepared by diluting with the solvent, but be aware that this can cause insoluble components to be produced.

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

#### 4.1.3 Filtration of sample solutions

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

### 4.2 To obtain stable analysis results

#### 4.2.1 Injection volume and injection concentration (overload)

Basically, high molecular weight samples are injected at high volumes and low concentration levels, while for low molecular weight compounds, a low volume of injection and a high concentration are used.

For a 30-cm column, an injection volume of 50  $\mu\text{L}$  is a rough standard, however, this may need to be altered depending on the molecular weight of the sample. When analyzing polymers that have a high molecular weight distribution, the injection volume may need to be increased (50-100  $\mu\text{L}$ ). Conversely, for low molecular weight samples and when peak separation of oligomers is targeted, decreased injection volumes are required (about 10 to 20  $\mu\text{L}$  with a 30-cm column).

A rough standard for the sample concentration is 0.1% (w/v). However, a lower concentration is required when the sample has a high molecular weight. The higher the concentration level of the sample, the greater the delay in elution time. This is 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. Consequently, 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/peak shape (mean molecular weights:  $M_z$ ,  $M_w$ ,  $M_n$ ) remain constant must be investigated.

#### 4.2.2 Molecular weight standard and target sample

When analyzing a molecular weight standard to prepare a calibration curve, the solvent, 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 preferable if the standard and target samples are analyzed on the same day. For normal molecular weight standards, a number of types of 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 is about one half the target sample, but this may need to be changed depending on the molecular weights of each of the molecular weight standards. In short, the higher the molecular weight, the lower the concentration.

#### 4.2.3 Column temperature

Due to the significant impact of temperature on sample elution time and detector (RI) baseline variations, the temperature must be kept constant in a column constant-temperature bath. Analysis is generally performed between 35 and 40 °C, but it is necessary to determine the appropriate temperature when using solvents with high viscosity and when analyzing samples with properties that can change depending on the temperature. Moreover, it is necessary to pay attention to boiling point when increasing the temperature when an organic solvent is used as the solvent.

#### 4.2.4 Flow rate

Because molecular weight is calculated from the elution time, the flow rate is crucial, and a pump with an extremely precise flow rate is required. In general, a flow rate of 1.0 mL/

min is used, but in the case of high molecular weight samples, the flow rate must be reduced to 0.5 mL/min or less.

#### 4.2.5 Solvents

Typical solvents based on the properties of the sample are indicated below.

- Nonionic and acidic compounds:

Buffer solution: 0.2 M phosphate buffer (pH 6.8), etc

Salt solution: 0.2 M NaO<sub>3</sub>

- Basic compounds:

1 M Acetic acid buffer (pH 4.5)

0.3 M Triethylamine-phosphoric acid (pH 2.9)

- Hydrophobic compounds

Polar organic solvent (acetonitrile, methanol) added to basic solution

Polar organic solvent (methanol, DMF, THF, HFIP, etc.)

\* When a polar organic solvent is used, a salt must be added (DMF, methanol, HFIP, etc.)

\* When an organic solvent is added to a basic solution, take care to avoid precipitation of the salt

#### 4.3 Selecting the solvent

A differential refractometer (RI detector) is often used in SEC analysis. When calculating molecular weight distribution, in general, the change in the refractive index per unit weight (concentration) is constant, but the change in this refractive index becomes less stable as molecular weight decreases. Under such circumstances it becomes impossible to accurately calculate molecular weight distribution, or the quantitative values for low molecular weight components differ. In normal analysis of polymers, particularly when a non-aromatic solvent is used, the refractive index of the sample increases, and the difference with respect to the solvent can be detected. In this type of detection system, the lower the refractive index of the solvent, the lower the molecular weight of the compound that can be analyzed at a constant sensitivity. Table 6 shows the relative sensitivity of various molecular weights of polyethylene glycol dissolved in a polar solvent. Using a sample with a molecular weight of 20,000 as the standard, with chloroform, which has a comparatively high refractive index, the molecular weight sensitivity is 2500, and with THF, a molecular weight of 1200 can be analyzed, but a 5% difference in sensitivity is produced. However, with methanol, analysis is possible down to a molecular weight of 550 (at a relative sensitivity within 5%), and with HFIP, which has an even lower refractive index, analysis becomes possible at a molecular weight as low as 500.

When used with samples for which analysis is possible with a relatively wide range of solvents and by choosing a solvent with a lower refractive index, the quantitative values obtained will be the true value or distributed in the vicinity of the true value, even when the relative sensitivity is unknown. The TSK-GEL Alpha series is able to conduct

analyses using water, methanol, acetonitrile and HFIP as solvents, which have comparatively low indices of refraction, and consequently this series of packed columns is expected to perform particularly well in component analyses of samples containing low molecular weight components, such as oligomers, etc.

## 5 Examples of Separation of Polar Compounds using Various Solvents

Table 7 shows examples of separations of various polar compounds conducted using various solvents with the TSK-GEL Alpha series of columns. Usage examples are shown in Figs. 36 to 75.

**Table-6 Sensitivity correction factor of polyethylene glycol by solvent**

Molecular mass m, quaternary structure	Refractive index <sup>1)</sup> (Polyethylene glycol)	Sensitivity correction factor				Required correction %	
		Chloroform <sup>1)</sup>	THF <sup>1)</sup>	Methanol	HFIP		
106 (m=2)	1.4455	8.912	1.655	1.266	1.158	10% line	
150 (m=3)	1.4529	2.806	1.402	1.154	1.110		
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		

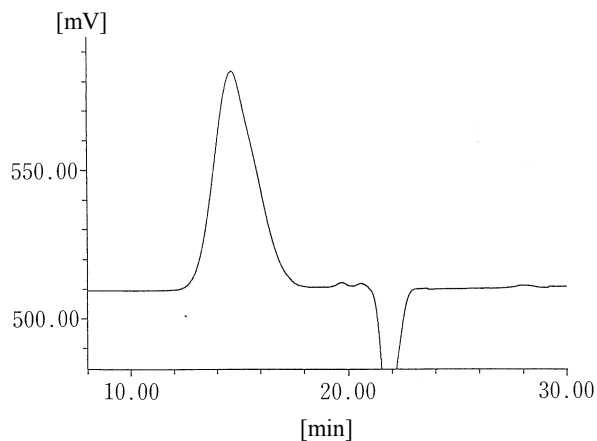
Refractive index of solvent (25 °C) Chloroform : 1.4421 THE : 1.4044  
Methanol : 1.3265 HFIP : 1.2752

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

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

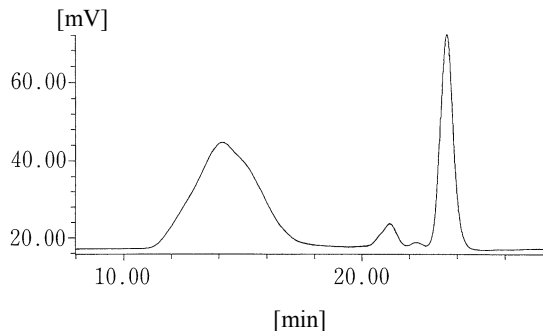
**Table-7 List of separation examples of polar macromolecular compounds using TSK-GEL Alpha Series**

Figure	Sample name	Column used	Sample used	Features
36	Acrylonitrile/styrene copolymer	TSKgel Alpha-M	DMF/10mM LiBr	
37	Acrylonitrile/vinylidene chloride copolymer	TSKgel Alpha-M	DMF/10mM LiBr	
38	Poly(N-isopropylacrylamide)	TSKgel Alpha-M	MeOH/10mM LiBr	
39	Ethyl cellulose	TSKgel Alpha-M	DMF/10mM LiBr	In methanol system, highly sensitive analysis is possible at low refractive index (RI)
40	Ethyl cellulose	TSKgel Alpha-M	MeOH/10mM LiBr	Required sensitivity of target compound, solubility of coexistent compounds, DMF has better separation
41	Ethyl hydroxyethyl cellulose	TSKgel Alpha-M	MeOH/10mM 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/10mM LiBr	Peak shape not good with H type column
43	Benzalkonium chloride	TSKgel Alpha-2500	DMF/10mM LiBr	
44	Carboxymethyl cellulose	TSKgel Alpha-5000	0.1 M phosphate buffer (pH 6.8)	Addition system, separation performance improves under heat analysis
45	Cleansing gel (model system)	TSKgel Alpha-2500	MeOH	
46	Cellulose acetate	TSKgel Alpha-M	DMF/10mM LiBr	Analysis also possible with H type
47	Styrene/allyl alcohol resin	TSKgel Alpha-M	DMF/10mM LiBr	
48	Sodium dodecylbenzene sulfonate (hard)	TSKgel Alpha-2500	DMF/10mM LiBr	Highly sensitive detection possible with methanol + 10 mM LiBr/UV (215 nm)
49	Sodium dodecyl sulfate (SDS)	TSKgel Alpha-4000+Alpha-3000+Alpha-2000*2	DMF/10mM LiBr	
50	Glycerol tri(2-ethylhexanoate)	TSKgel Alpha-2500	MeOH	
51	Triton X-100	TSKgel Alpha-4000+Alpha-3000+Alpha-2000*2	DMF/50mM LiBr	
52	Urea resin	TSKgel Alpha-M	DMF	
53	Hydroxypropyl cellulose	TSKgel Alpha-M	MeOH/10mM 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/10mM LiBr	
55	N-vinylpyrrolidone/vinyl acetate copolymer	TSKgel Alpha-M	DMF/10mM LiBr	
28	Phenol resin	TSKgel Alpha-3000	DMF/50mM LiBr	
56	Brij-35	TSKgel Alpha-4000+Alpha-3000+Alpha-2000*2	DMF/50mM LiBr	
57	Sodium polyacrylate	TSKgel Alpha-M	0.2M NaNO <sub>3</sub>	
58	Polyacrylonitrile (PAN)	TSKgel Alpha-M	DMF/10mM LiBr	Analysis also possible with H type
59	Polyamic acid	TSKgel Alpha-M	DMF/30 mM LiBR/69 mM phosphoric acid	Analysis also possible with H type
60	Polyamide-imide	TSKgel Alpha-M	NMP/10mM LiBr	
61	Polyimide	TSKgel Alpha-M	NMP/10mM LiBr	
62	Polyethylene glycol mono p-octylphenyl ether	TSKgel Alpha-2500	DMF/10mM LiBr	Highly sensitive detection possible with methanol + 10 mM LiBr/UV (215 nm)
63	Polyvinyl 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	Polyvinyl alcohol	TSKgel Alpha-5000*2	0.1M 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/50mM NaNO <sub>3</sub> =6/4	
66	Polyvinylbutyral (butyral resin)	TSKgel Alpha-M	DMF/10mM LiBr	No interference from ghost peaks from water or salts
67	Polyvinylmethylether	TSKgel Alpha-M	MeOH/10mM LiBr	Possible to select solvent based on sensitivity and separation from coexisting compounds
68	Polyvinylmethylether	TSKgel Alpha-M	DMF/10mM LiBr	
69	Poly(p-phenylene ether sulfone)	TSKgel Alpha-M	DMF/10mM LiBr	Polystyrene can be used as a molecular weight standard
70	Polyvinylidene fluoride	TSKgel Alpha-M	DMF/10mM LiBr	Depending on solvent, the refractive index is low, detected as negative peak
71	Poly (methylmethacrylate / methacrylic acid) copolymer	TSKgel Alpha-M	DMF/10mM LiBr	
72	Methylvinylether/maleic acid copolymer	TSKgel Alpha-M	DMF/30 mM LiBR/69 mM phosphoric acid	
73	N-methoxymethylated polyamide	TSKgel Alpha-M	MeOH/10mMLiBr	
74	Melamine resin	TSKgel Alpha-M	DMF/LiBr	
75	Melamine-modified urea resin	TSKgel Alpha-M	DMF/10mM LiBr	



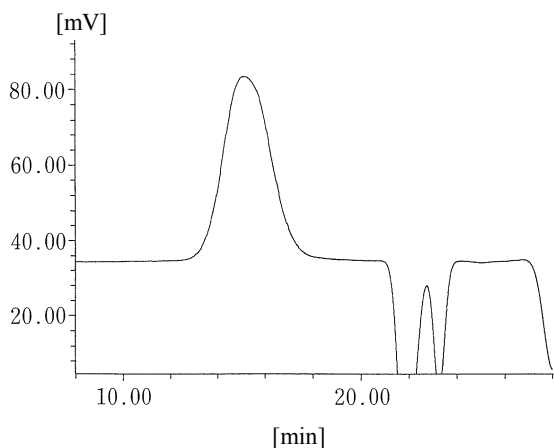
**Fig. 36 Separation of acrylonitrile/styrene copolymer**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Acrylonitrile/styrene copolymer (0.1%, 50 µL)



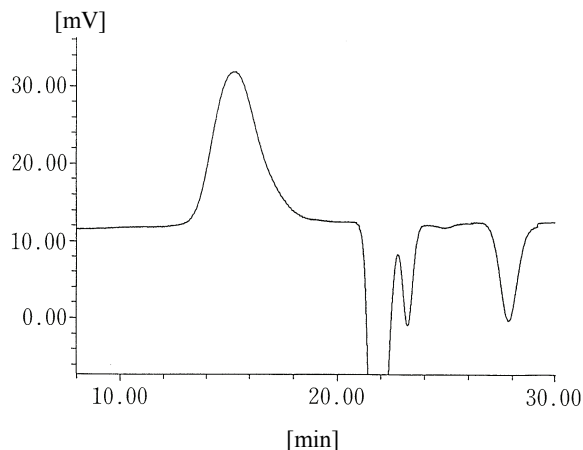
**Fig. 38 Separation of poly(N-isopropylacrylamide)**

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



**Fig. 37 Separation of acrylonitrile/vinylidene chloride copolymer**

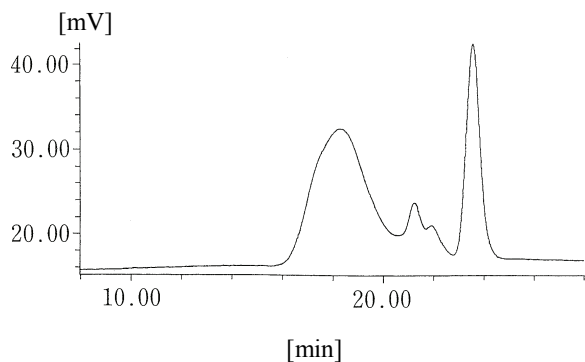
Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Sample: Acrylonitrile/vinylidene chloride copolymer (0.1%, 50 µL)



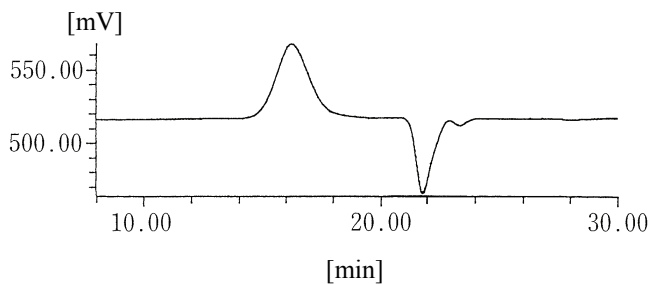
**Fig. 39 Separation of ethyl cellulose**

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

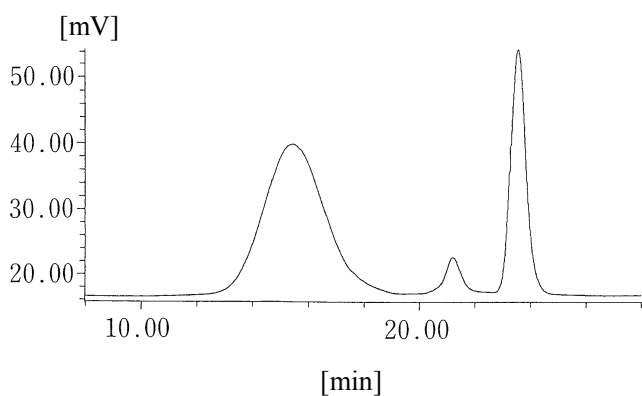




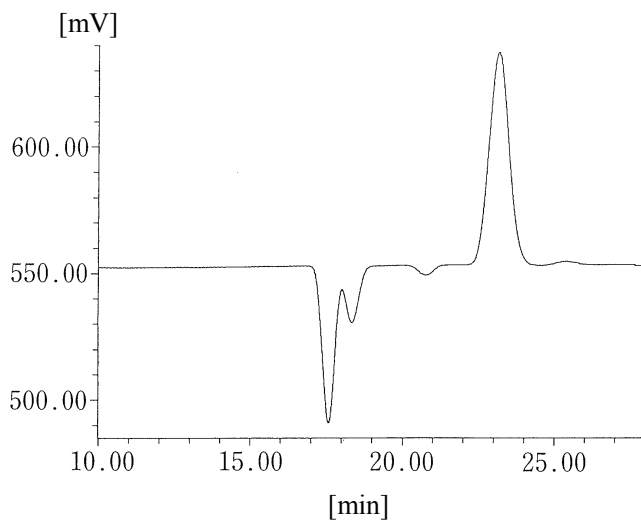
**Fig. 40 Separation of ethyl cellulose**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in methanol  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Ethyl cellulose (0.1%, 50 µL)



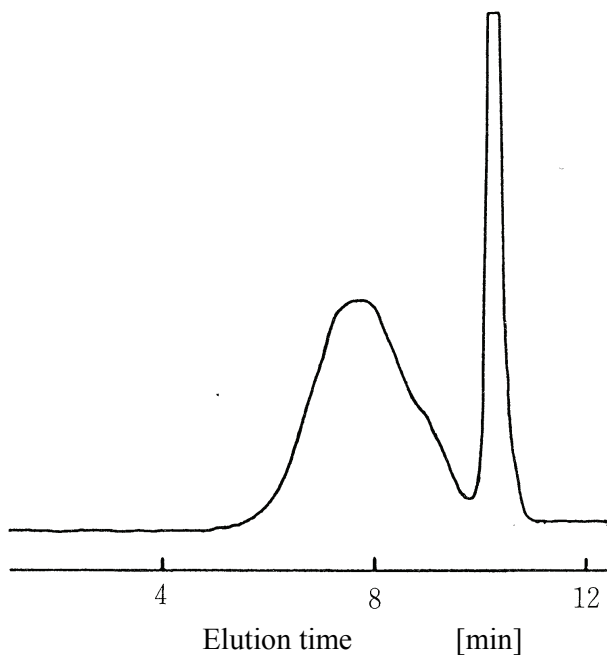
**Fig. 42 Separation of vinyl chloride/vinyl acetate copolymer**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Vinyl chloride/vinyl acetate copolymer (0.1%, 50 µL)



**Fig. 41 Separation of ethyl hydroxyethyl cellulose**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in methanol  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Sample: Ethyl hydroxyethyl cellulose (0.1%, 50 µL)

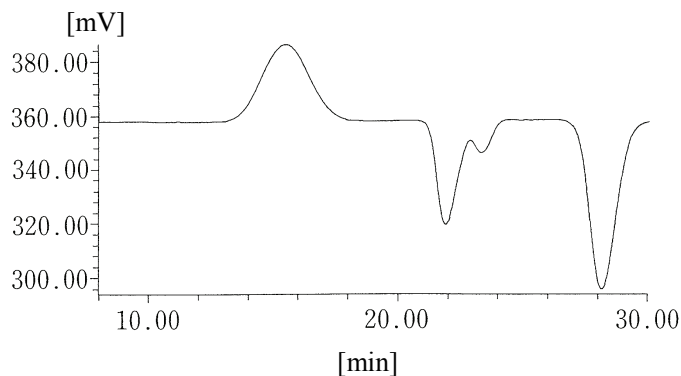


**Fig. 43 Separation of benzalkonium chloride**  
 Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Sample: Benzalkonium chloride (0.1%, 50 µL)



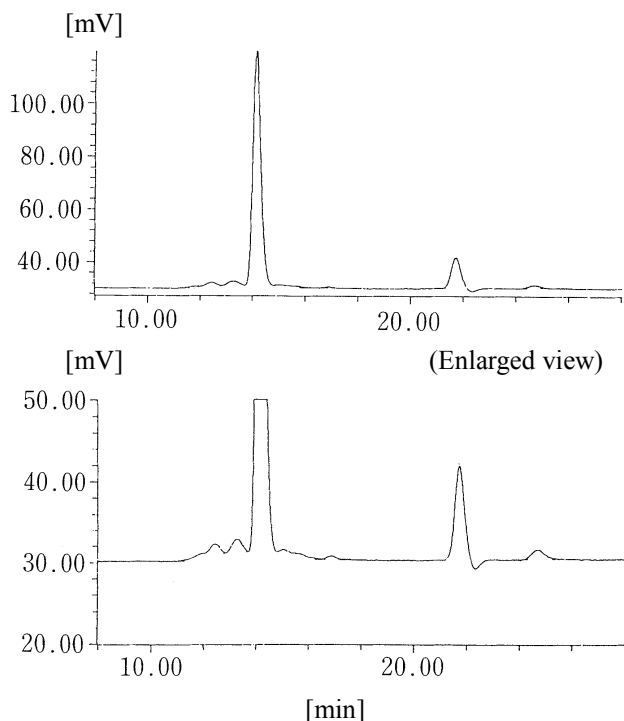
**Fig. 44 Separation of carboxymethyl cellulose**

Column: TSKgel Alpha-5000 (7.8 mm ID x 30 cm L)  
 Eluent: 0.1 M phosphate (pH 6.8)  
 Flow rate: 1.0 mL/min  
 Temp.: 25 °C  
 Detection: Differential refractometer  
 Sample: Carboxymethyl cellulose



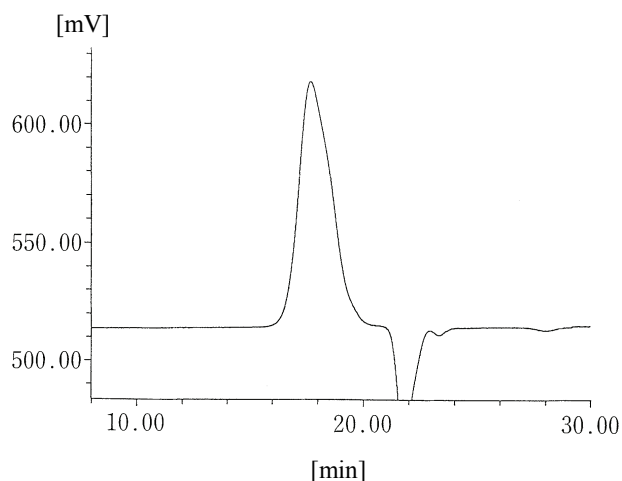
**Fig. 46 Separation of cellulose acetate**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Cellulose acetate (0.1%, 50  $\mu$ L)



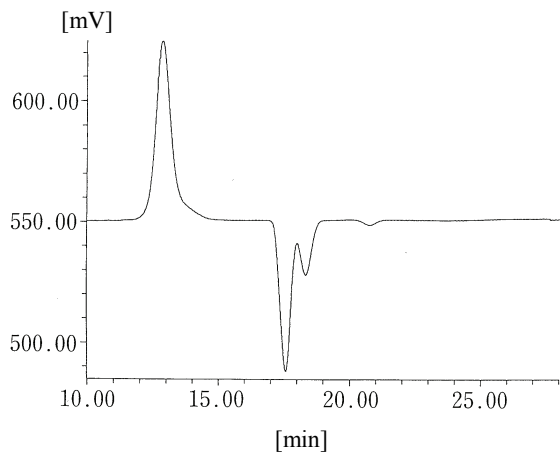
**Fig. 45 Separation of cleansing gel (model system)**

Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)  
 Eluent: Methanol  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Sample: Cleansing gel (model system) (0.1%, 50  $\mu$ L)



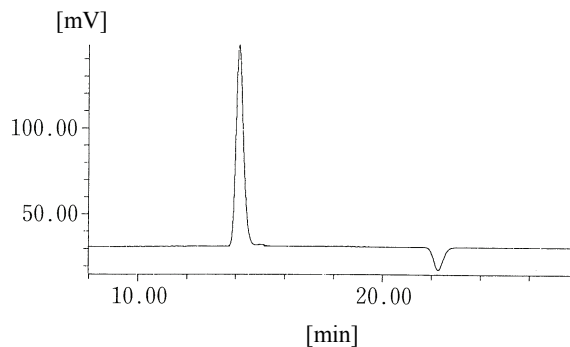
**Fig. 47 Separation of styrene/allyl alcohol resin**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Sample: Styrene/allyl alcohol copolymer (0.1%, 50  $\mu$ L)



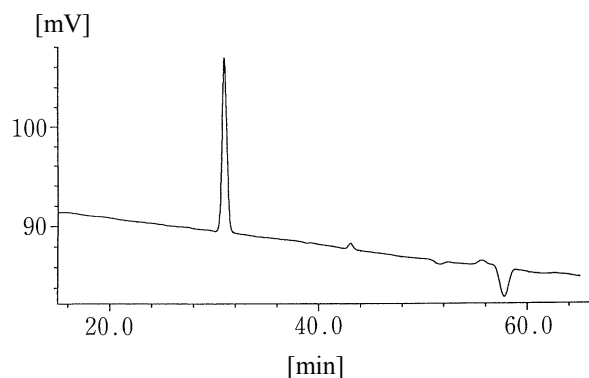
**Fig. 48 Separation of sodium dodecylbenzene sulfonate (hard)**

Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Sodium dodecylbenzene sulfonate (hard) (0.1%, 50 µL)



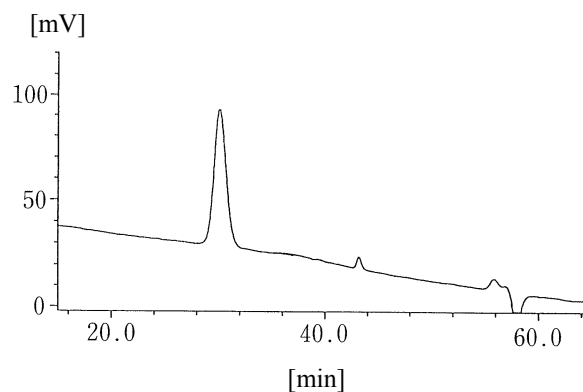
**Fig. 50 Separation of glyceryl tri(2-ethylhexanoate)**

Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)  
 Eluent: Methanol  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Glyceryl tri(2-ethylhexanoate) (0.1%, 50 µL)



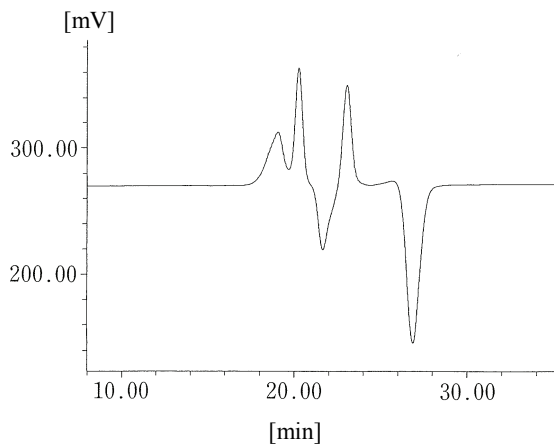
**Fig. 49 Separation of sodium dodecyl sulfate**

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

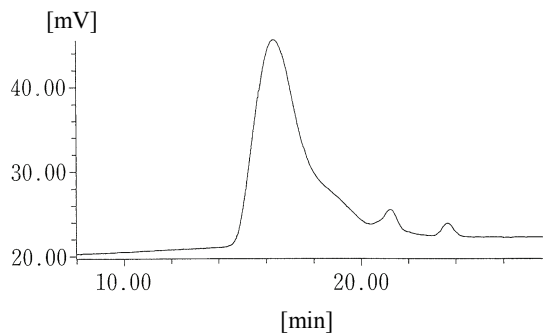


**Fig. 51 Separation of Triton X-100**

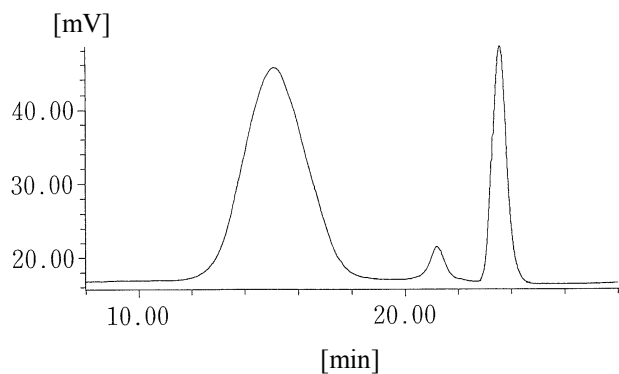
Other elution conditions are the same as those in Fig. 53.



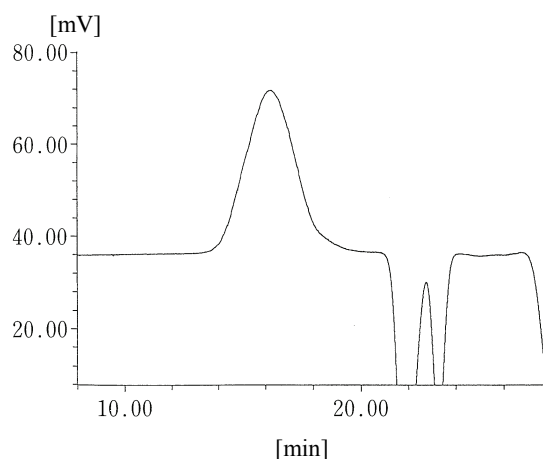
**Fig. 52 Separation of urea resin**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 50 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Urea resin (0.1%, 50  $\mu$ L)



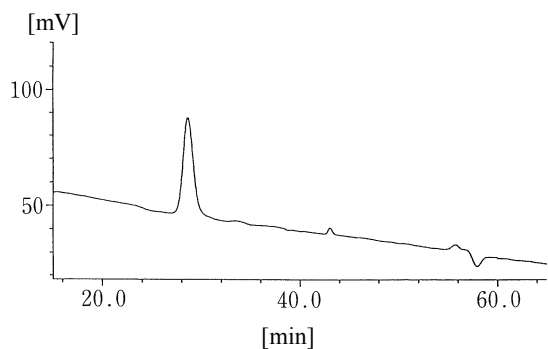
**Fig. 54 Separation of N-vinylpyrrolidone/vinyl acetate copolymer**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in methanol  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: N-vinylpyrrolidone/vinyl acetate copolymer (0.1%, 50  $\mu$ L)



**Fig. 53 Separation of hydroxypropyl cellulose**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in methanol  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Hydroxypropyl cellulose (1.7%, 200  $\mu$ L)

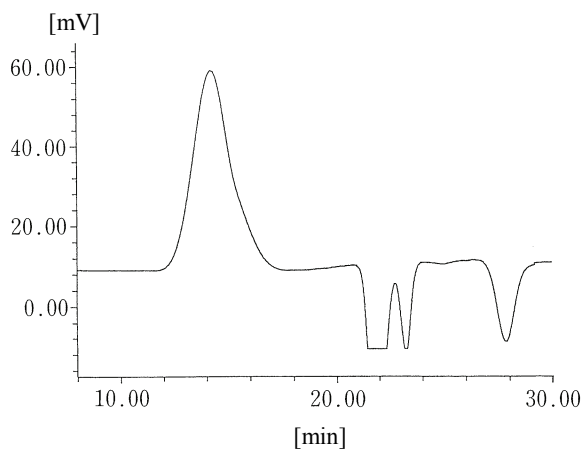


**Fig. 55 Separation of N-vinylpyrrolidone/vinyl acetate copolymer**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: N-vinylpyrrolidone/vinyl acetate copolymer (0.1%, 50  $\mu$ L)



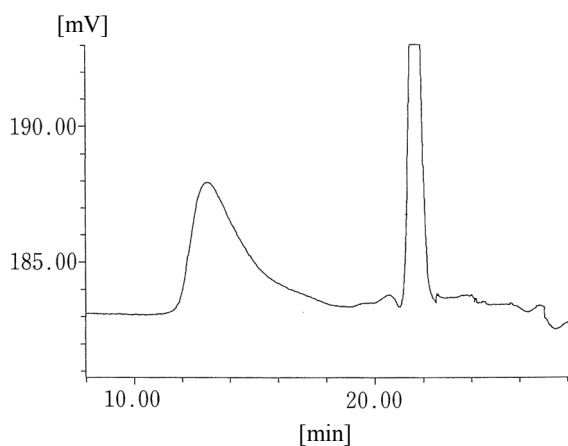
**Fig. 56 Separation of Brij-35**

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



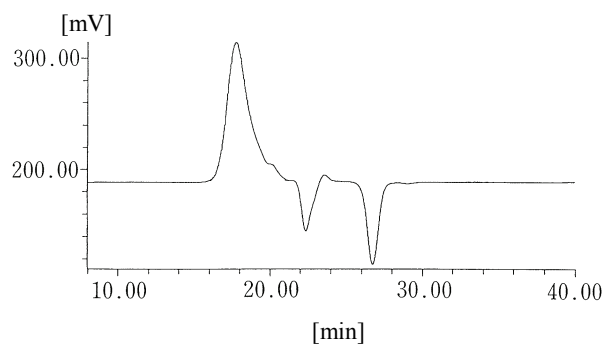
**Fig. 58 Separation of polyacrylonitrile (PAN)**

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



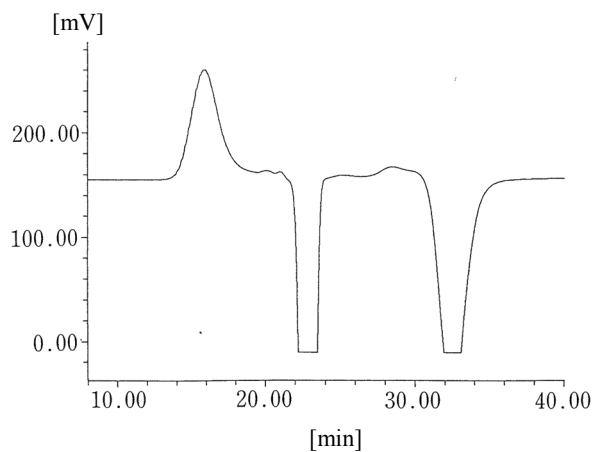
**Fig. 57 Separation of sodium polyacrylate**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 0.2 M NaNO<sub>3</sub>  
 Flow rate: 0.5 mL/min  
 Detection: Differential refractometer  
 Temp.: 40 °C  
 Injection volume: 50 µL



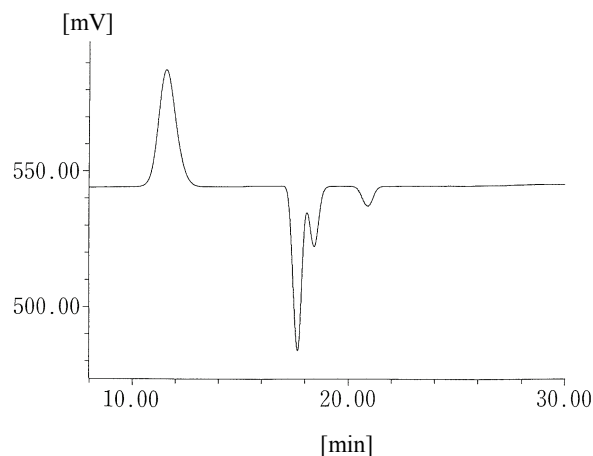
**Fig. 59 Separation of polyamic acid**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 30 mM LiBr + 60 mM H<sub>3</sub>PO<sub>4</sub> in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Polyamic acid (0.1%, 50 µL)



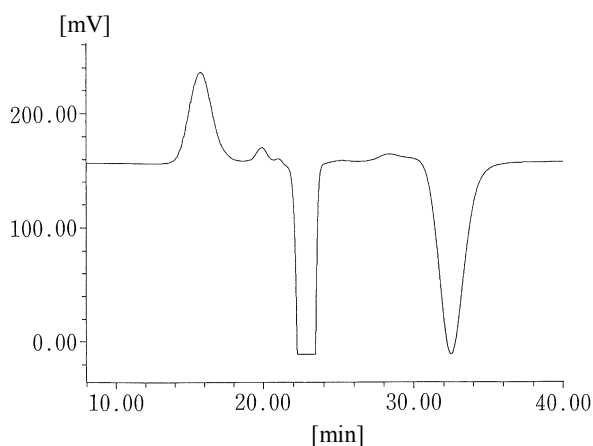
**Fig. 60 Separation of polyamide-imide**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in N-methylpyrrolidone  
 Flow rate: 0.5 mL/min  
 Detection: Differential refractometer  
 Temp.: 40 °C  
 Sample: Polyamide-imide (0.1%, 50 µL)



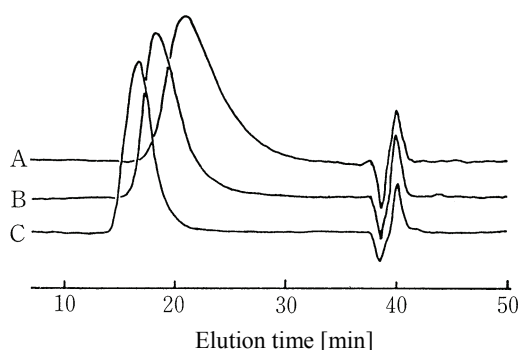
**Fig. 62 Separation of polyethylene glycol mono p-octylphenyl ether**

Column: TSKgel Alpha-2500 (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Polyethylene glycol mono p-octylphenyl ether (0.1%, 50 µL)



**Fig. 61 Separation of polyimide**

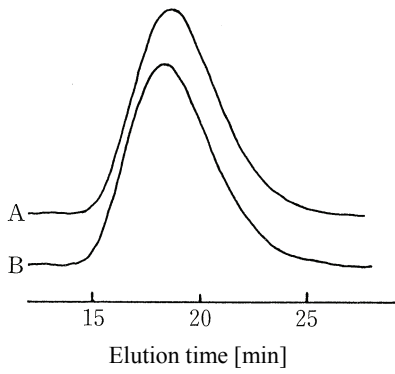
Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in N-methylpyrrolidone  
 Flow rate: 0.5 mL/min  
 Detection: Differential refractometer  
 Temp.: 40 °C  
 Sample: Polyimide (0.1%, 50 µL)



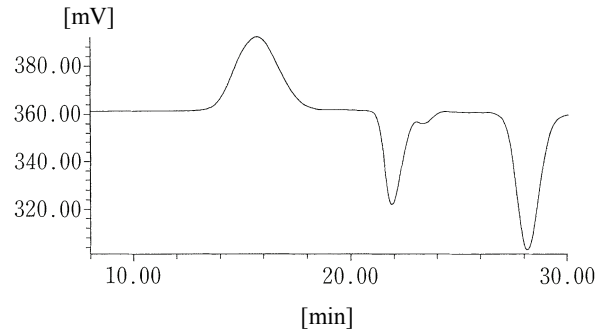
**Fig. 63 Separation of polyvinyl alcohol**

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

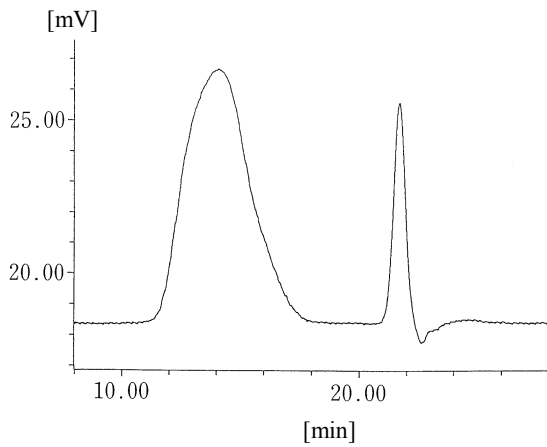




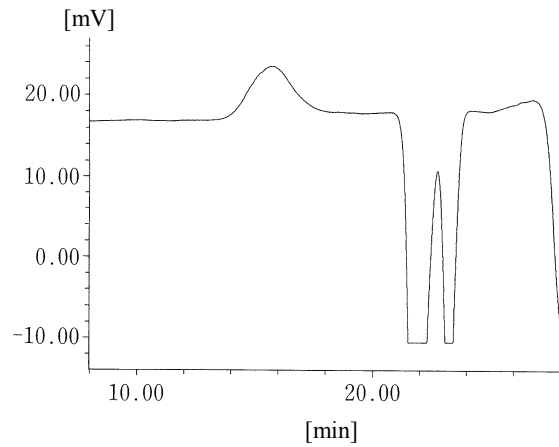
**Fig. 64 Separation of polyvinyl alcohol**  
 Column: TSKgel Alpha-5000 (7.8 mm ID x 30 cm L x 2)  
 Eluent: 0.1 M NaCl/methanol = 1/1  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: (A) Differential refractometer  
 (B) UV (210 nm)  
 Sample: (B) Polyvinyl alcohol (degree of saponification: 88%)



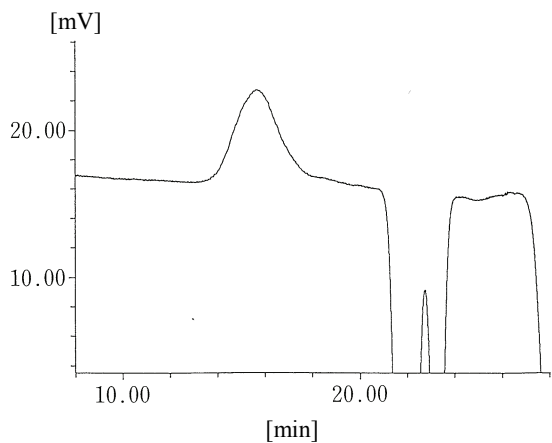
**Fig. 66 Separation of polyvinylbutyral**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Polyvinylbutyral (0.1%, 50 µL)



**Fig. 65 Separation of polyvinylpyrrolidone (PVP79)**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 0.1 M NaCl aq/methanol = 1/1  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Polyvinylpyrrolidone  
 Injection volume: 50 µL

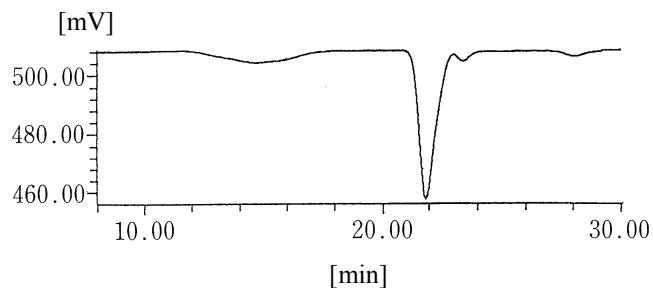


**Fig. 67 Separation of polyvinylmethylether**  
 Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in methanol  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Samples: Polyvinylmethyl ether (0.1%, 50 µL)



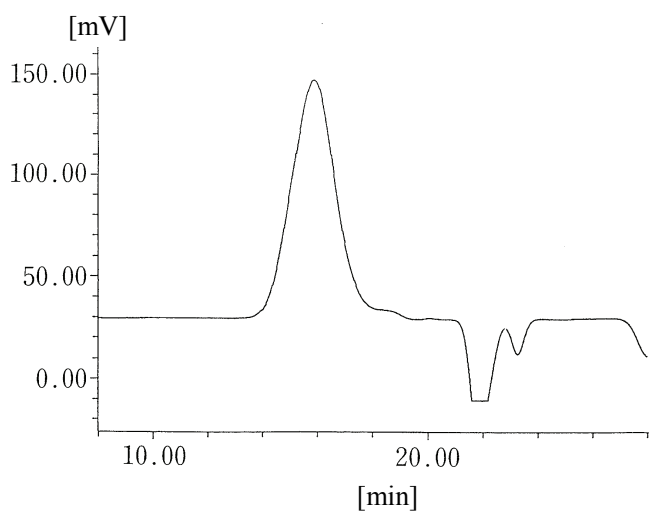
**Fig. 68 Separation of polyvinylmethylether**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Polyvinylmethylether (0.1%, 50  $\mu$ L)



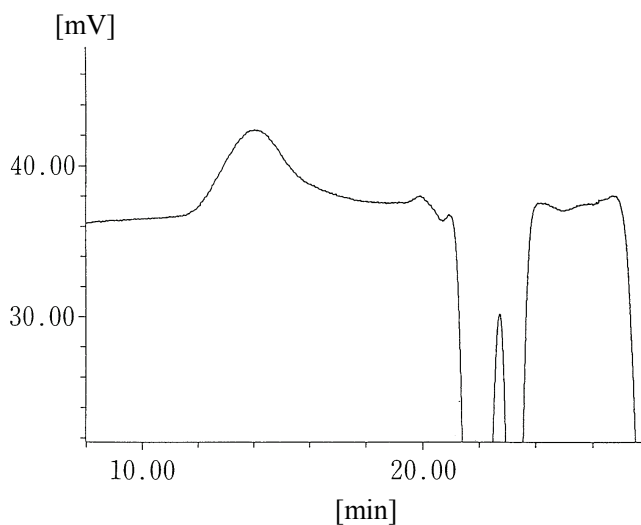
**Fig. 70 Separation of polyvinylidene fluoride**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Polyvinylidene fluoride (0.1%, 50  $\mu$ L)



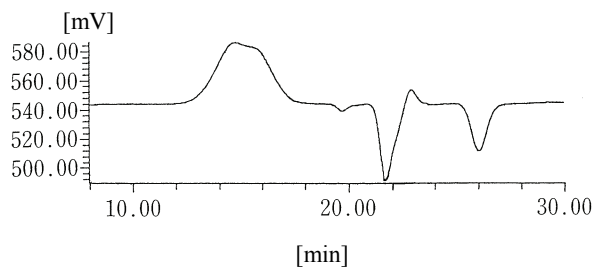
**Fig. 69 Separation of poly(p-phenylene ether sulfone)**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Sample: Poly (p-phenylene ether sulfone) (0.1%, 50  $\mu$ L)



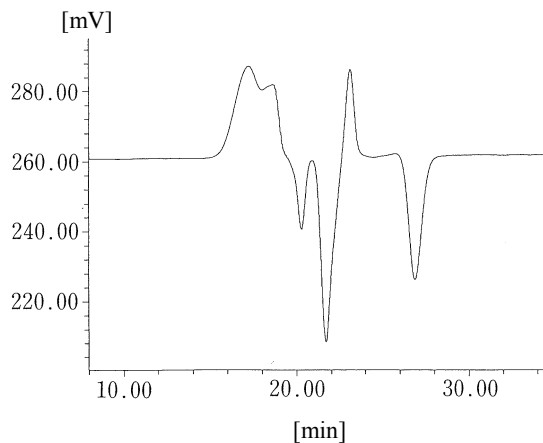
**Fig. 71 Separation of poly(methylmethacrylate/methacrylic acid) copolymer**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Samples: Poly(methylmethacrylate/methacrylic acid) copolymer (0.1%, 50  $\mu$ L)



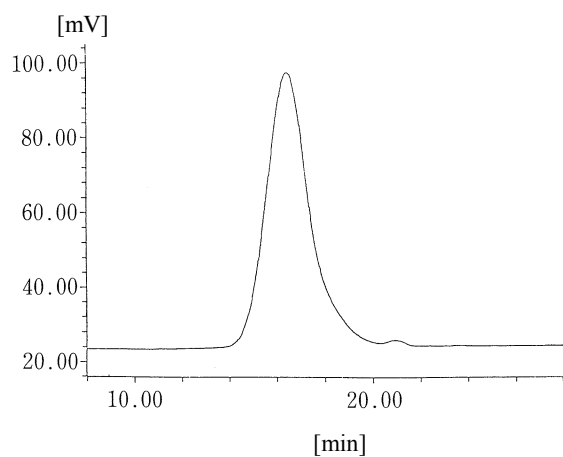
**Fig. 72 Separation of methylvinylether/maleic acid copolymer**

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



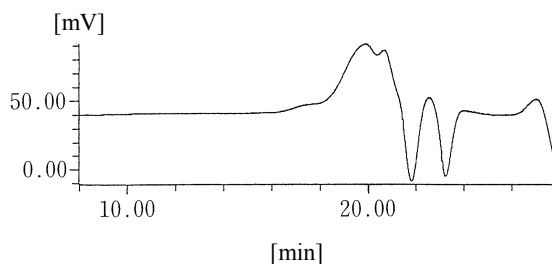
**Fig. 74 Separation of melamine resin**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Sample: Butylated melamine resin (0.1%, 50 µL)



**Fig. 73 Separation of N-methoxymethylated polyamide**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L L)  
 Eluent: 10 mM LiBr in methanol  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: Differential refractometer  
 Injection volume: 50 µL



**Fig. 75 Separation of melamine-modified urea resin**

Column: TSKgel Alpha-M (7.8 mm ID x 30 cm L)  
 Eluent: 10 mM LiBr in dimethylformamide  
 Flow rate: 0.5 mL/min  
 Temp.: 40 °C  
 Detection: Differential refractometer  
 Samples: Melamine-modified urea resin (0.1%, 50 µL)

## Summary

SEC packed columns can be generally classified into the following types: water-system columns in which the matrix is composed of a hydrophilic synthetic polymer; and columns used with an organic Solvent system, with a non-aqueous synthetic polymer such as styrene divinylbenzene as the matrix. However, various problems have occurred with each of these types of columns when used to analyze polar polymer samples. These problems concern the capacity of the packing material to withstand conversion between organic solvents, the adsorption of standard compounds by polar solvents, and the solubility of the sample being analyzed. However, with the newly developed TSK-GEL Alpha series of columns discussed here, a wide range of solvents can be selected ranging from aqueous solutions to organic solvents, making it possible to set the conditions for analysis based on the solubility of the sample and the molecular weight standard. As a result, this series of columns can be counted on for use in analyzing polar polymer samples that have been so difficult to analyze with conventional SEC columns in the past.