Separation Report No. 088

High Speed and High Performance Semi-micro GPC Columns: TSKgel SuperH Series

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1. Introduction

With advancements in polymer science, a wide variety of new polymers have been created. Today, with the many ways in which products engineered from these substances are used, it is important to thoroughly understand the properties of these polymer substances from the perspective of usage, development and quality control.

It goes without saying that average molecular weight (mass) and molecular weight distribution are among the properties of polymers that are most important to understand. A number of methods have been used over the years for determining average molecular weight and molecular weight distribution including ebulliometry, light scattering techniques, osmometry, viscosity, and absolute methods such as ultracentrifugation. Currently, size exclusion chromatography (SEC), a mode of liquid chromatography, is the most widely used of these methods because SEC is relatively simple and quick and has good reproducibility.

Gel filtration chromatography (GFC) using polysaccharide gels was first used in the field of biopolymers as a method of size exclusion in which molecules are separated based on their size. On the other side of the solvent spectrum, J.C. Moore[1] in 1964 coined the phrase gel permeation chromatography (GPC) when analyzing synthetic polymers in an organic solvent system with a cross-linked polystyrene gel; since that time the use of SEC has rapidly increased.

As shown in Table 1, TOSOH commercialized the TSKgel S type series packed columns for GPC in organic solvent systems in 1971. Since that time, the performance and speed of analysis of TSKgel GPC columns has continuously improved.

In 1992, TOSOH developed the HR series of TSKgel H-type columns (hereafter referred to as the TSKgel H_{HR} series) which had superior physical stability and an ability to withstand solvent conversion that surpassed that of previous product lines. Today, the particle size in the TSKgel H_{HR} series columns has been reduced to the micro-particulate level, culminating in a series of highly durable, ultra-high performance semi-micro GPC columns: the TSKgel SuperH series columns (hereafter referred to as TSKgel SuperH series) of GPC columns. Moreover, in response to environmental concerns, this series of columns was developed to reduce organic solvent consumption and the cost of solvent disposal.

In this report, the features and basic characteristics of the TSKgel SuperH Series are introduced together with examples of their application in polymer analyses.

2. Features

The packing material in the TSKgel H_{HR} series was reduced to 3μm and was packed into a stainless steel column (6.0mm ID x 15cm) using advanced packing technology. Consequently, the TSKgel SuperH series maintains the same pore size characteristics as the TSKgel H_{HR} series, allows the user to repeatedly change mobile phase solvents, and has twice the number of theoretical plates as TSKgel H_{HR} series columns of the same length.

TSKgel SuperH series columns possess the following features:

(1) Similar separation performance as the conventional TSKgel H_{HR} and H_{XL} series columns in half the time.
(2) The relative sensitivity is improved 3- to 4-fold over columns with conventional internal diameters (7.8mm ID) in sample limited cases.
(3) Solvent consumption is reduced to one-third that of conventional columns which proportionally reduces the solvent cost per analysis as well as the disposal cost.
(4) Due to the small particle size of the packing material, separation performance depends less on flow rate than conventional columns. At higher flow rates there is very little reduction in separation performance.
(5) The TSKgel H_{HR} columns in the TSKgel SuperH series maintain column efficiency when changing from one organic solvent to another.
(6) Mixed bed columns with superior calibration curve linearity are available in 4 grades which allows the appropriate column to be selected based on the molecular weight and molecular weight distribution of the sample.

Table 2 shows a comparison of the performance characteristics of the TSKgel SuperH and H_{HR} series columns. Calibration curves of the TSKgel SuperH series columns are shown in Figures 1 and 2 determined with polystyrene standards in THF solvent.

Table 1 Steps in the development and commercialization of packed columns for GPC using organic solvent systems

Year	Product name	Column length (cm)	Particle size (μm)	Number of theoretical plates (30cm column)
1971	TSKgel S Type	120	40	1,500
1972	TSKgel H Type	60	10 13	8,000 6,00
1983	TSKgel H_{XL} Series	30	5 13	16,000 8,000
1987	TSKgel H_{XL} New Series	30	5 10	16,000 14,000
1992	TSKgel H_{HR} Series	30	5 13	16,000 8,000
1993	TSKgel SuperH Series	15	3	32,000

Table 2 Comparison of performance of TSKgel SuperH and H_{HR} Series columns

TSKgel SuperH series			TSKgel H_{HR} series		
Grade	Particle size (μm)	Guaranteed number of theoretical plates (15cm column)	Grade	Particle size (μm)	Guaranteed number of theoretical plates (30cm column)
SuperH1000	3	16,000	G1000H_{HR}	5	16,000
SuperH2000	3	16,000	G2000H_{HR}	5	16,000
SuperH2500	3	16,000	G2500H_{HR}	5	16,000
SuperH3000	3	16,000	G3000H_{HR}	5	16,000
SuperH4000	3	16,000	G4000H_{HR}	5	16,000
SuperH5000	3	16,000	G5000H_{HR}	5	16,000
SuperH6000	5	10,000	G6000H_{HR}	5	16,000
SuperH7000	5	10,000	G7000H_{HR}	5	16,000
SuperHM-L	3	16,000	GMH_{HR}-L	5	16,000
SuperHM-N	3	16,000	GMH_{HR}-N	5	16,000
SuperHM-M	3	16,000	GMH_{HR}-M	5	16,000
SuperHM-H	3	16,000	GMH_{HR}-H	5	16,000

Conditions for measuring theoretical plates

Columns: TSKgel SuperH series, 6.0mm ID x 15cm
TSKgel H_{HR} series, 7.8mm ID x 30cm
Eluent: tetrahydrofuran (THF)
Flow rate: TSKgel SuperH (0.6mL/min)
TSKgel H_{HR} (1.0mL/min)
Temperature: 25ºC
Detection: UV@254nm
Samples: TSKgel SuperH100 (*p* -hydroxybenzyl alcohol)
TSKgel SuperH2000-H7000 and SuperHM (dicyclohexyl phthalate)
TSKgel G1000H_{HR}-G2500H_{HR} (benzene)
TSKgel G3000H_{HR}-G4000H_{HR}, GMH_{HR}-L and -M (*n*-butylbenzene)
TSKgel G5000H_{HR}-G7000H_{HR}, GMH_{HR}-M and -H (dicyclohexyl phthalate)

Polystyrene standards were used for the calibration of TSKgel SuperH and SuperHM columns.

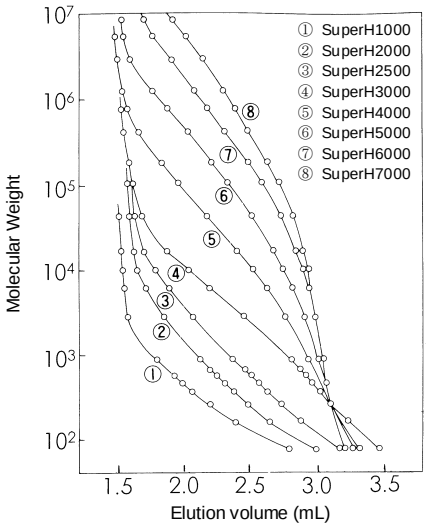

Fig. 1 Calibration curves of TSKgel SuperH series columns

Column size: 6.0mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: polystyrene standards

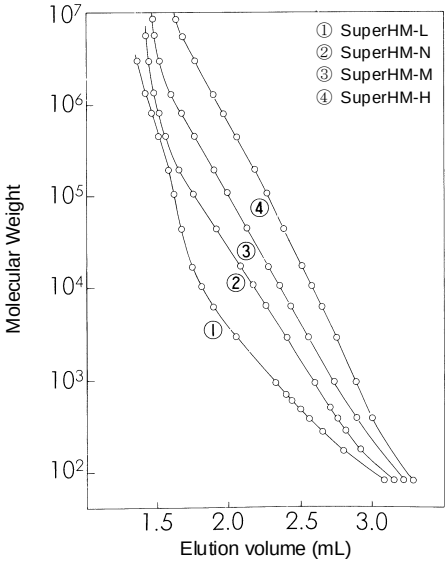

Fig. 2 Calibration curves of TSKgel SuperHM series columns

Column size: 6.0mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: polystyrene standards

3. Basic characteristics

3-1. Separation performance

As shown in Table 2, the particle size of the packing materials in the TSKgel SuperH1000-SuperH5000, SuperHM-L, SuperHM-N, SuperHM-M, and SuperHM-H columns was reduced from 5µm to 3µm. This reduction in particle size, when compared to the conventional TSKgel H_{HR} series of columns, results in a 2-fold increase in the number of theoretical plates per unit length. Since the number of theoretical plates (column efficiency) is proportional to column length, the 15cm TSKgel SuperH series columns can achieve the same separation performance as 30cm TSKgel H_{HR} series columns in half the analysis time.

Figure 3 compares chromatograms of epoxy resin separated using the TSKgel SuperH3000 and G3000H_{HR} columns. Figure 4 compares chromatograms of a mixed standard polystyrene sample separated using the TSKgel SuperHM-H, GMH_{HR}-H and GMH_{XL} columns. These figures clearly show that the TSKgel SuperH series columns can achieve the separation performance of the conventional TSKgel H_{HR} and H_{XL} series columns in half the analysis time.

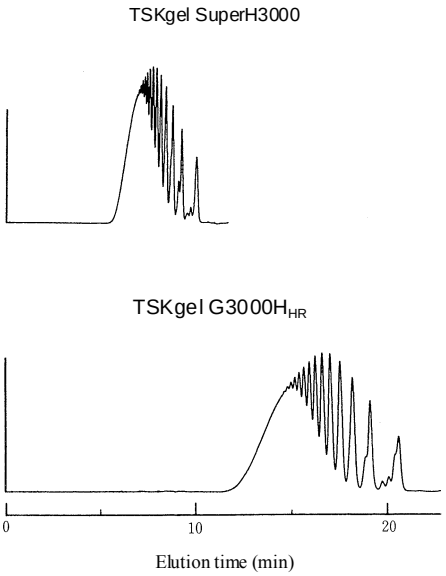

Fig. 3 Separation of epoxy resin using TSKgel SuperH3000 and TSKgel G3000H_{HR} columns

Columns: TSKgel SuperH3000, 6.0mm ID x 15cm x 2
TSKgel G3000H_{HR}, 7.8mm ID x 30cm x 2
Eluent: THF
Flow rate: TSKgel SuperH3000 (0.6mL/min)
TSKgel G3000H_{HR} (1.0mL/min)
Temperature: 25ºC
Detection: UV@254nm
Sample: epoxy resin

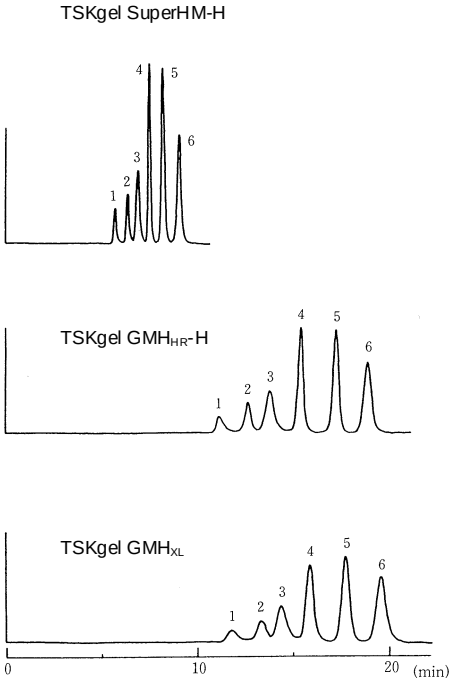

Fig. 4 Separation of polystyrene mixture using TSKgel SuperHM-H and conventional columns

Columns: TSKgel SuperHM-H, 6.0mm ID x 15cm x 2
TSKgel GMH_{HR}-H, GMH_{XL}, 7.8mm ID x 30cm x 2
Eluent: THF
Flow rate: TSKgel SuperHM-H (0.6mL/min)
TSKgel GMH_{HR}-H, GMH_{XL} (1.0mL/min)
Temperature: 25ºC
Detection: UV@254nm
Samples: polystyrene standards
1. MW 8,420,000 2. MW 1,260,000
3. MW 422,000 4. MW 107,000
5. MW 16,700 6. MW 2,800

3-2. Column efficiency as a function of flow rate

3-2-1. Flow-rate-dependence of HETP with low molecular weight samples

The effect of flow rate* on the height equivalent to a theoretical plate (HETP) depends on the particle size of the packing material, sample type and molecule size, solvent type, viscosity, and temperature.

Figure 5 compares the flow rate dependence of HETP in the TSKgel SuperHM-H columns versus conventional columns using dicyclohexyl phthalate (DCHP) as the sample and Figure 6 compares the flow rate dependence of HETP with the TSKgel SuperH2500 (sample: DCHP) versus the TSKgel G2500H_{XL} and TSKgel G2500H_{HR} (sample: benzene).

It is well known that the dependence of HETP on linear velocity decreases as the particle size of the packing material becomes smaller. In conventional columns, the flow-rate dependence of the HETP is very high in the high flow rate region (the region in which the linear velocity ≥ 0.045cm/sec and the flow rate ≥ 1.2mL/min). In TSKgel SuperH columns, however, in which the particle size of the packing material has been reduced, HETP depends very little on flow rate. As a result, when a low molecular weight sample such as DCHP is analyzed, the analysis time can be decreased by increasing the flow rate (linear velocity: 0.07cm/sec, flow rate: 1.2mL/min) without affecting peak resolution.

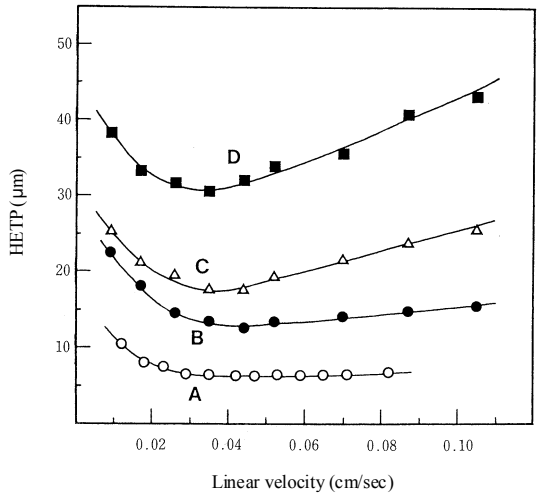

Fig. 5 Relationship between HETP and flow rate for TSKgel SuperH and conventional columns

Columns: A. TSKgel SuperHM-H, 6mm ID x 15cm
B. TSKgel GMH_{HR}-H, 7.8mm ID x 30cm
C. TSKgel GMH_{XL}, 7.8mm ID x 30cm
D. TSKgel GMH_{HR}-H(S), 7.8mm ID x 30cm
Eluent: THF
Temperature: 25 ºC
Detection: UV@254nm
Samples: dicyclohexyl phthalate (DCHP) (0.1%)
A: 3μL; B, C, and D: 20μL

* To compare the performance of columns that vary in internal diameter, flow rate is converted to linear velocity, which is equal to the time it takes for a small molecular weight compound to elute from the column ($\langle v \rangle = L/t_R$ in which L is column length (cm) and t_R is retention time of a small MW compound).

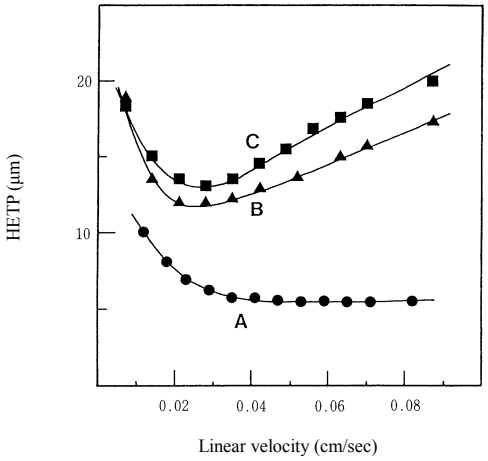

Fig. 6 Relationship between HETP and flow rate for TSKgel SuperH and conventional columns

Columns: A. TSKgel SuperH2500, 6mm ID x 15cm
B. TSKgel G2500H_{HR}, 7.8mm ID x 30cm
C. TSKgel G2500H_{XL}, 7.8mm ID x 30cm
Eluent: THF
Temperature: 25 ºC
Detection: UV@254nm
Samples: A: DCHP (0.1%), 3μL
B, C: Benzene (0.1%), 20μL

Figure 7 shows the relationship between linear velocity and resolution of standard polystyrene A-500 in chromatograms produced by TSKgel SuperH2500 and G2500H_{XL} columns. With low molecular weight samples such as A-500, the separation performance of the TSKgel SuperH2500 column is essentially independent of flow rate, as a high level of separation is maintained even in the high flow rate region. On the other hand, with the TSKgel G2500H_{XL} column, separation performance decreases as flow rate increases.

3-2-2. Flow-rate dependence of HETP with polymer samples

Figure 8 shows the flow rate dependence of HETP in a TSKgel SuperHM-H column for standard polystyrenes.

With low molecular weight samples, no flow rate dependence of HETP was observed at high flow rates. When a polymer sample is used, HETP increases with the flow rate, confirming the dependency of HETP on flow rate. This effect increases with increasing molecular weight.

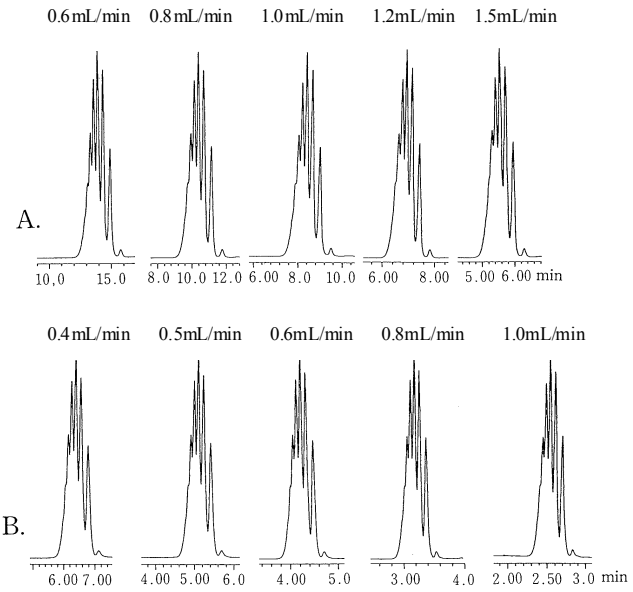

Fig. 7 **Flow rate dependence of separation of standard polystyrenes in TSKgel SuperH2500 and G2500H$_{XL}$ columns**

Columns: A. TSKgel G2500H$_{XL}$, 7.8mm ID x 30cm
B. TSKgel SuperH2500, 6mm ID x 15cm

Eluent: THF

Temperature: 25ºC

Detection: UV@254nm

Sample: standard polystyrene A-500 (0.1%), 10µL

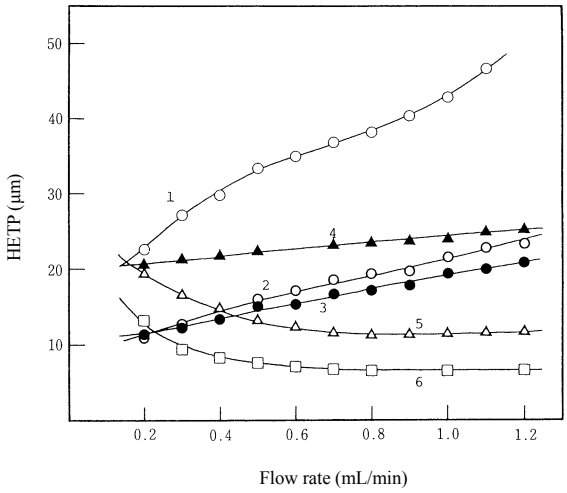

Fig. 8 **Relationship between HETP and flow rate at various molecular weights for TSKgel SuperHM-H columns**

Column: TSKgel SuperHM-H, 6mm ID x 15cm

Eluent: THF

Temperature: 25 ºC

Detection: UV@254nm

Sample: standard polystyrene
1. MW 1,260,000 (○) 2. MW 107,000 (○)
3. MW 16,700 (●) 4. MW 2,800 (▲)
5. MW 500 (△) 6. DCHP (□)

Figure 9 shows the relationship between flow rate and resolution of standard polystyrenes for a TSKgel SuperHM-H column. Resolution* is clearly dependent on flow rate, as separation performance decreases with increasing flow rate. Thus, when polymer samples are analyzed, the lower the flow rate, the better the separation performance.

Figure 10 shows the flow rate dependence on resolution for epoxy resin in chromatograms from TSKgel SuperH columns, while Figure 11 shows how resolution depends on flow rate for standard polystyrenes in chromatograms from the TSKgel SuperHM-H column. In TSKgel SuperH columns, separation performance depends little on flow rate in comparison to the conventional TSKgel H_{HR} and H_{XL} series columns, making analysis at high flow rates possible. However, better separation performance is obtained at lower flow rates when polymer samples are analyzed with this column.

Consequently, when analyzing a polymer sample with the TSKgel SuperH series, the flow rate should be between 0.3 and 0.6mL (equivalent to 0.5 to 1.0mL with conventional TSKgel H_{HR} and H_{XL} series columns) and when analyzing oligomers and low molecular weight samples, the optimum flow rate is about 0.6mL/min.

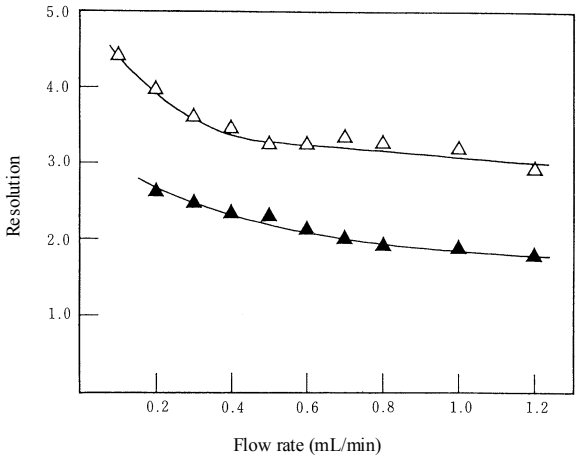

Fig. 9 Relationship between flow rate and Resolution for standard polystyrenes on TSKgel SuperHM-H columns

Column: TSKgel SuperHM-H, 6mm ID x 15cm x 2
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrenes
Separation performance:
△: F-550 (0.02%)/F-80 (0.022%)
▲: F-40 (0.035%)/F-10 (0.05%)
F-550 (MW: 5,480,000)
F-80 (MW: 706,000)
F-40 (MW: 422,000)
F-10 (MW: 107,000)

* As a reminder, Resolution is defined as the ratio of the difference in retention times of two neighboring peaks and the sum of the standard deviations of those peaks.

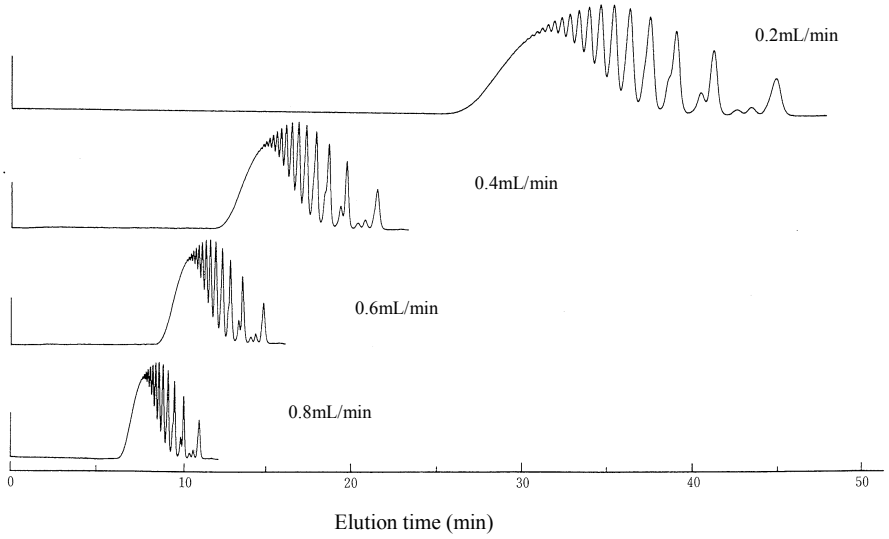

Fig. 10 **Relationship between flow rate and Resolution for epoxy resin on TSKgel SuperH columns**

Column: TSKgel SuperH3000 x 2 + TSKgel SuperH2500, 6mm ID x 15cm x 3
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: epikote 1004 (0.1%), 10µL

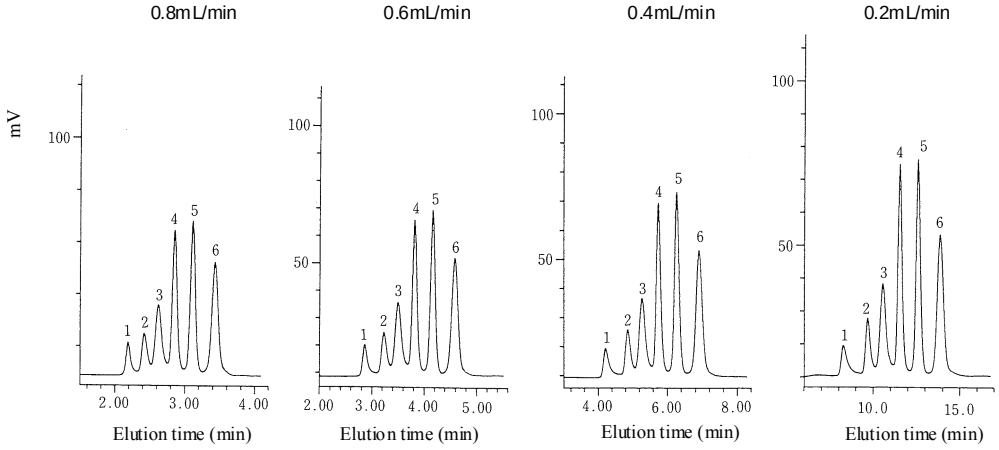

Fig. 11 **Effect of flow rate on Resolution for standard polystyrenes on a TSKgel SuperHM-H column**

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene
1. MW 8,420,000 2. MW 1,260,000
3. MW 422,000 4. MW 107,000
5. MW 16,700 6. MW 2,800

3-3. Peak detection sensitivity

The dimensions of TSKgel SuperH series columns have been reduced from 7.8mm ID x 30cm to 6.0mm ID x 15cm to create a semi-micro column with ultra-high performance capability. As a result, peaks elute from TSKgel SuperH columns in smaller peak volumes, which translates into higher detection sensitivity, when injecting the same sample volume than what can be obtained on conventional TSKgel H_{HR} and H_{XL} series of columns.

Figure 12 shows the relationship between the peak height and sample injection volume for standard polystyrene A-500 on TSKgel SuperH2500 and G2500H_{XL} columns. Figure 13 shows the relationship between peak height and injection volume for polystyrene using the TSKgel SuperHM-H and GMH_{HR}-H columns.

Figure 14 compares chromatograms of the separation of standard polystyrene A-500 on the TSKgel SuperH2500 and G2500H_{XL} columns. Figures 15 and 16 compare the chromatograms of a standard polystyrene mixture and commercial polystyrene using the TSKgel SuperHM-H GMH_{HR}-H and GMH_{XL} columns.

It is clear from these figures that the detection sensitivity is increased 3- to 4-fold relative to that of conventional columns. Of course, one must not overload the narrower (6 vs. 7.8mm ID) and shorter (15 vs. 30cm) TSKgel SuperH columns. Mass or volume overloading causes the peaks to broaden and resolution to decrease. The simplest way to avoid overloading is to reduce sample volume and mass by a factor of 3-4 compared to the quantity used with conventional columns.

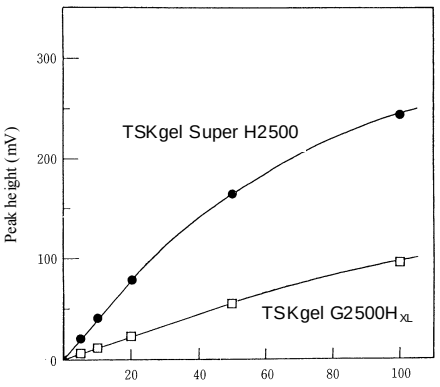

Fig. 12 Relationship between peak height and injection volume of polystyrene using TSKgel SuperH2500 and G2500H_{XL} columns

Columns: TSKgel SuperH2500, 6.0mm ID x 15cm x 2
TSKgel G2500H_{XL}, 7.8mm ID x 30cm x 2
Eluent: THF
Flow rate: TSKgel SuperH2500 (0.6mL/min)
TSKgel G2500H_{XL} (1.0mL/min)
Sample: standard polystyrene A-500

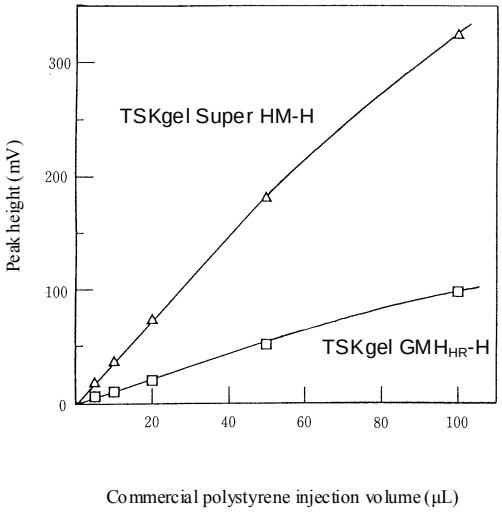

Fig. 13 Relationship between peak height and injection volume of polystyrene using TSKgel SuperHM-H and GMH_{HR} columns

Columns: TSKgel SuperHM-H, 6.0mm ID x 15cm x 2
GMH_{HR}-H, 7.8mm ID x 30cm x 2
Eluent: THF
Flow rate: TSKgel SuperHM-H (0.6mL/min)
TSKgel GMH_{HR}-H (1.0mL/min)
Sample: commercial polystyrene (0.5%)

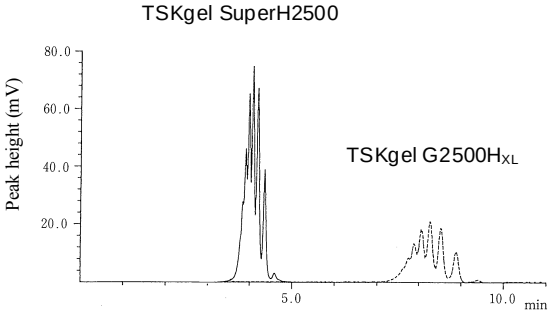

Fig. 14 Comparison of peak heights of standard polystyrene (A-500) on TSKgel SuperH2500 and G2500H_{XL} columns

Columns: TSKgel SuperH2500, 6mm ID x 15cm
TSKgel G2500H_{XL}, 7.8mm ID x 30cm
Eluent: THF
Flow rate: TSKgel SuperH2500 (0.6mL/min)
TSKgel G2500H_{XL} (1.0mL/min)
Temperature: 25 ºC
Detection: UV@254nm
Sample: standard polystyrene (A-500), 0.1%, 10μL

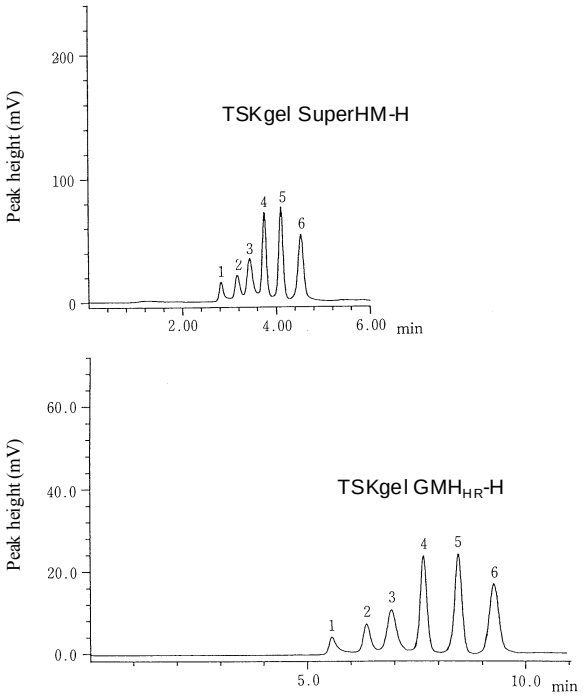

Fig. 15 Separation of standard polystyrenes by TSKgel SuperHM-H and GMH$_{HR}$-H columns

Columns: TSKgel SuperHM-H, 6mm ID x 15cm
TSKgel GMH$_{HR}$-H, 7.8mm ID x 30cm
Eluent: THF
Flow rate: TSKgel SuperHM-H (0.6mL/min)
TSKgel GMH$_{HR}$-H (1.0mL/min)
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene, 10µL
1. MW 8,420,000, (0.02%)
2. MW 1,260,000 (0.035%)
3. MW 422,000 (0.06%)
4. MW 107,000 (0.09%)
5. MW 16,700 (0.1%)
6. MW 2,800 (0.1%)

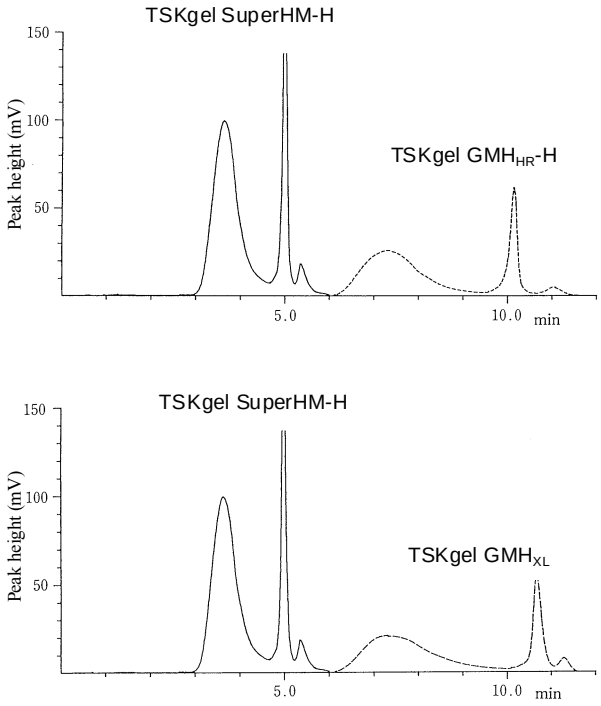

Fig. 16 Comparison of commercial polystyrene peak heights produced by TSKgel SuperHM-H and conventional columns

Columns: TSKgel SuperHM-H, 6mm ID x 15cm
TSKgel GMH$_{HR}$-H, TSKgel GMH$_{XL}$,
7.8mm ID x 30cm
Eluent: THF
Flow rate: TSKgel SuperHM-H (0.6mL/min)
TSKgel GMH$_{HR}$-H, TSKgel GMH$_{XL}$
(1.0mL/min)
Temperature: 25ºC
Detection: UV@254nm
Sample: commercial polystyrene (0.25%), 20µL

3-4. Sol vent compatibility

Because the TSKgel SuperH series columns are filled with particles of the same chemistry, albeit of smaller size than the particles used in the TSKgel H$_{HR}$ series, it is expected that shrinkage and swelling of the packing materials in various solvents will be equivalent to the properties of the TSKgel H$_{HR}$ series columns.

Table 3 compares shrinkage and swelling properties of the TSKgel SuperH2000, G2000 H$_{HR}$ and G2000H$_{XL}$ columns with various organic solvents. Based on this data it is clear that the solvent can be converted from the initially loaded solvent (THF) to virtually any of the organic solvents listed in Table 4.

Table 3 Comparison of shrinkage and swelling properties of TSKgel SuperH2000, G2000 H_{HR} and G2000H_{XL} columns

Solvent	Shrinkage/Swelling		
	SuperH2000	G2000H_{HR}	G2000H_{XL}
Toluene	1.00	1.01	1.06
Benzene	1.01	1.00	ND
THF	1.00	1.00	1.00
Dimethylformamide (DMF)	1.00	0.99	0.86
Acetone	0.99	0.99	0.86
Methanol (MeOH)	0.98	0.98	0.67
THF/water = 1/1	0.97	0.98	ND
MeOH/water = 1/1	0.92	0.93	ND
Water	0.85	0.86	0.52

*Shrinkage/swelling occurring with various organic solvents relative to the volume in THF (1.00).

Table 4 Solvents that can be exchanged in TSKgel SuperH series columns

Toluene, xylene, chloroform ($CHCl_3$), benzene, dichloromethane, dichloroethane, dimethylformamide (DMF), dimethylsulfoxide (DMSO), dioxane, N-methylpyrrolidone (NMP), *m*-cresol/$CHCl_3$, quinoline, methyl ethyl ketone (MEK), *o*-dichlorobenzene (ODCB), trichlorobenzene (TCB), hexafluoroisopropanol (HFIP), HFIP/$CHCl_3$, *o*-chlorophenol (OCP), OCP/$CHCl_3$, pyridine, carbon tetrachloride, ethyl acetate, methanol (MeOH), MeOH/$CHCl_3$, THF/MeOH, acetone, ethanol, dimethylacetamide, n-hexane, dodecane, 1-chloronaphthalene, FC-113, trichloroethane

Figure 17 shows the changes resulting when solvents in TSKgel SuperH columns (TSKgel SuperH2000, SuperH3000, and SuperHM-H) were directly converted from THF to an organic solvent (from toluene to ethanol) expressed as the ratio of the number of theoretical plates as measured in THF after solvent conversion versus the number of theoretical plates in THF before solvent conversion. In this test, direct conversion from THF to one of a number of organic solvents was performed. After leaving the new solvent in the columns for one week, the solvent was converted back to THF and then converted again to a new organic solvent. With this test method, the changes to the packing properties of the column (column efficiency) were recorded under continuous conversion of the solvent to various other organic solvents.

The results show that for each TSKgel SuperH columns there was no change in the number of theoretical plates after conversion to any other organic solvent, which clearly demonstrates that the TSKgel SuperH packing material is very stable and robust and possesses the same outstanding ability to withstand conversion between solvents as the packing material in TSKgel H_{HR} series columns.

Figure 18 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperH2500 column with various organic solvents (THF, $CHCl_3$, DMF, and CCl_4) and Figure 19 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperHM-H column with various organic solvents.

Due to the interaction between the packing material and standard polystyrene when using DMF as the mobile phase[2], the elution volume of standard polystyrenes is greater than it is with "good" solvents such as THF and $CHCl_3$. This effect is particularly noticeable with TSKgel SuperH2500, a column for the analysis of low molecular weight samples. Under these circumstances, polyethylene oxide (PEO) is recommended as the standard sample, as this reacts very little with the packing material.

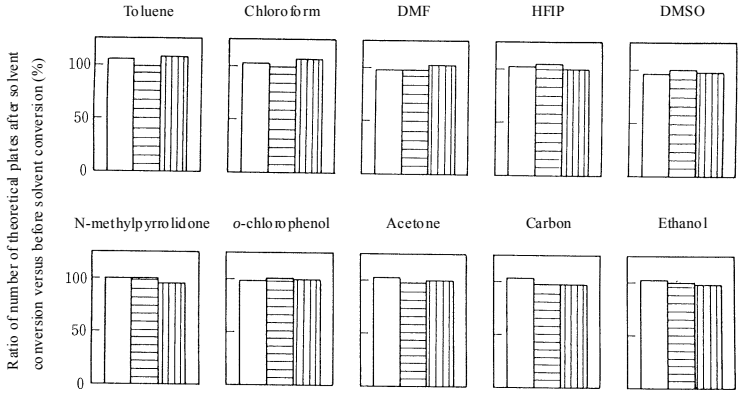

Fig. 17 Solvent compatibility of TSKgel SuperH Series columns

<Solvent conversion conditions>
Flow rate for conversion to test solvent: 0.2mL/min
Temperature during conversion to test solvent: 25ºC
Duration of conversion from THF to test solvent: 16 hours
Time left at rest with test solvent: 1 week
Flow rate, temperature and time elapsed during conversion from test solvent to THF: 0.2mL/min, 25ºC and 8 hours

<Conditions for measuring number of theoretical plates>
Eluent: THF Flow rate: 0.6mL/min
Temperature: 25ºC Detection: UV@254nm
Sample: DCHP (0.1%), 2µL
TSKgel SuperH2000: □
TSKgel SuperH3000: ▤
TSKgel SuperHM-H: ▥

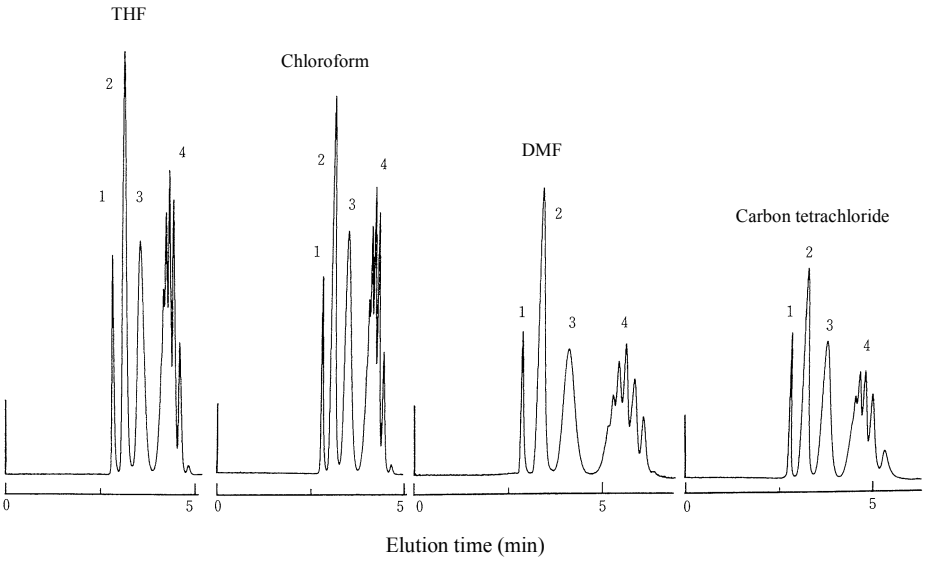

Fig. 18 **Separation of standard polystyrenes compared using various solvents on a TSKgel SuperH2500 column**

Column: 6.0mm ID x 15cm
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm or 270nm
Sample: 1. MW 190,000
2. MW 9,100
3. MW 2,800
4. A-500

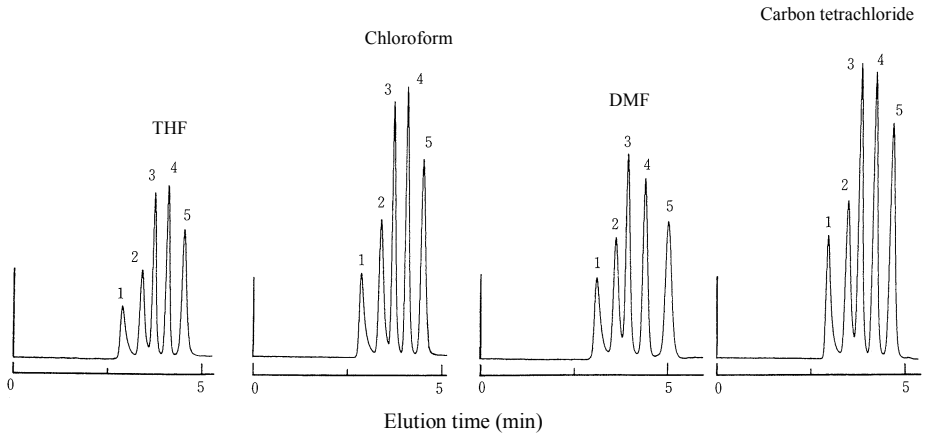

Fig. 19 **Separation of standard polystyrenes compared using various solvents on a TSKgel SuperHM-H column**

Column: 6.0mm ID x 15cm
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm or 270nm
Sample: 1. MW 2,890,000 2. MW 422,000
3. MW 107,000 4. MW 16,700
5. MW 2,800

3-5. Samp le load

Analysis conditions include some factors involved in maximizing the high performance of semi-micro columns represented by the TSKgel SuperH series, and the most important of these conditions is the sample load (sample injection volume and sample concentration). As in other modes of chromatography analysts try to balance the need for sensitivity with sample overload of the column by either volume or mass of the sample injected.

Sample load depends on sample molecular weight and type, mobile phase, flow rate, temperature, column size, and particle size of the packing material. In particular, sample load decreases as the viscosity and molecular weight of the sample increases, and in high performance columns the sample load decreases with particle size. Consequently, to analyze molecular weight distribution with good repeatability at a high degree of separation, it is important to thoroughly understand the sample load in the column being used.

3-5-1. Samp le concentration

Figure 20 shows the relationship between sample concentration, sample load and HETP of various standard polystyrenes using a TSKgel SuperHM-H column. Figure 21 shows the relationship between sample concentration, sample load and elution volume of various standard polystyrenes. The maximum sample concentration and sample load will differ depending on the polystyrene standard used. The dependence of HETP and elution volume on sample concentration and sample load will increase as the molecular weight of the sample increases. Table 5 shows the maximum sample concentration and maximum sample load for various standard polystyrenes based on the results shown in Figures 20 and 21.

Table 5 Maximum sample concentrations and sample loads in TSKgel SuperH columns

Molecular weight	Maximum sample concentration (%)	Maximum sample load (μg)
~10,000	2.0	200
10,000~50,000	1.0	100
50,000~200,000	0.2	20
200,000~500,000	0.1	10
500,000~1,000,000	0.05	5
1,000,000~5,000,000	0.02	2
>5,000,000	0.01	1

Column: 6.0mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene, 10μL

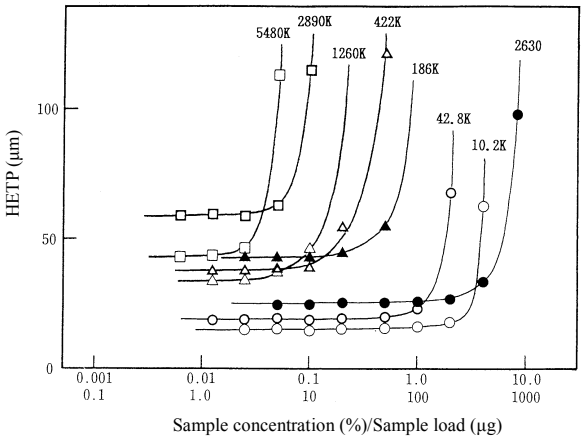

Fig. 20 Relationship between sample concentration (sample load) and HETP for standard polystyrenes on a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene, 10μL

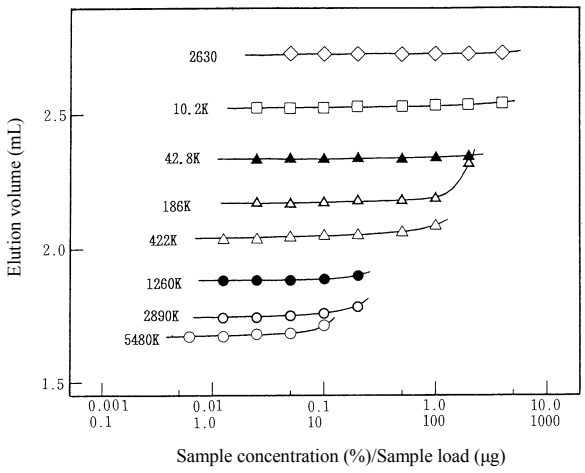

Fig. 21 Relationship between sample concentration (sample load) and elution volume for standard polystyrenes on a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene, 10μL

3-5-2. Samp le injection volume

Figure 22 shows the relationship between sample injection volume, sample load and HETP for various standard polystyrenes using a TSKgel SuperHM-H column and Figure 23 shows the relationship between sample injection volume, sample load and elution volume for various standard polystyrenes. The dependence of HETP and elution volume on sample concentration and sample load increases with the injection volume. However, unlike sample concentration, there is essentially no correlation between the sample injection volume and molecular weight and it is understood that the maximum injection volume is 20μL (20μg).

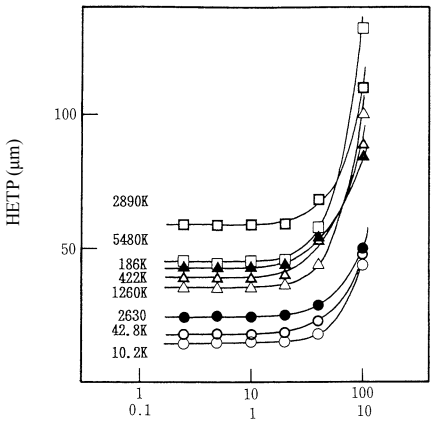

Fig. 22 Relationship between sample injection volume (sample load) and HETP for standard polystyrenes on a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene
Sample concentration: 0.01%

Moreover, in this column the maximum sample injection volume is essentially unrelated to the molecular weight since as the sample injection volume reaches 20μL (2μg), HETP will depend largely on the sample injection volume. One conventional method to increase the sample load is to decrease the sample concentration while increasing the sample injection volume.[3,4] However, the best method to increase the maximum sample load in TSKgel SuperH columns is to decrease the sample injection volume (≤ 10μL) and increase the sample concentration up to the maximum concentration (amount listed in Table 5).

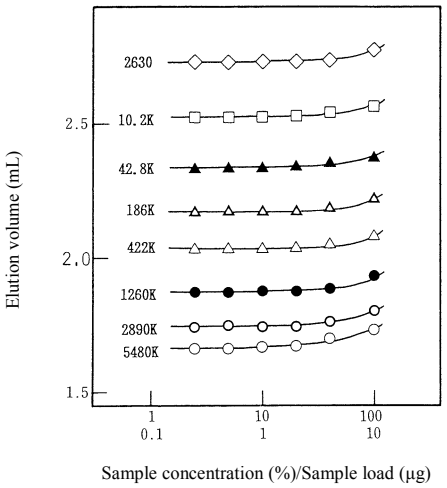

Fig. 23 Relationship between sample concentration (sample load) and elution volume for standard polystyrenes on a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene (10μL)
Sample concentration: 0.01%

Figure 24 shows the relationship between sample injection volume and separation performance for standard polystyrenes with molecular weights of 8,420,000 and 1,260,000 using 1 to 4 TSKgel SuperHM-H columns in series. It is clear that the effect of injection volume on separation performance decreases, as expected, as the number of columns is increased.

Figures 25 to 27 show how separation performance is dependent on sample injection volume in chromatograms of a standard polystyrene mixture produced with 1, 2, and 4 TSKgel SuperHM-H columns in series.

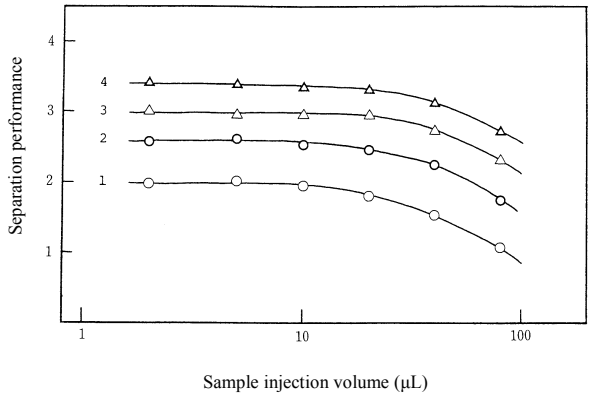

Fig. 24 **Relationship between separation performance and sample load of standard polystyrenes on TSKgel SuperHM-H columns**

Columns: TSKgel SuperHM-H
6mm ID x 15cm (○)
6mm ID x 15cm x 2 (○)
6mm ID x 15cm x 3 (△)
6mm ID x 15cm x 4 (△)
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: standard polystyrene (10μL)
MW 8,420,000 (0.02%)
MW 1,260,000 (0.035%)

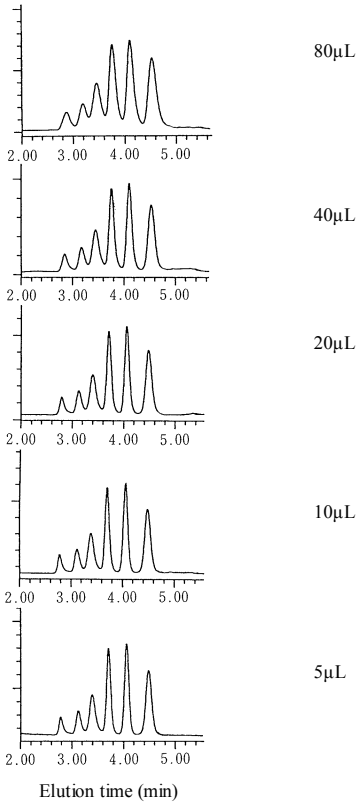

Fig. 25 **Effect of sample load on separation of standard polystyrene using a TSKgel SuperHM-H column**

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene mixture

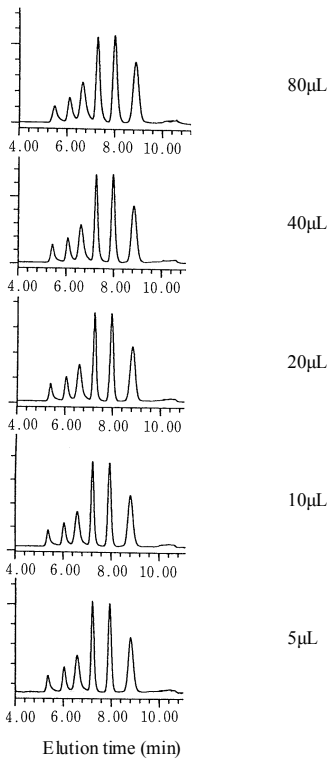

Fig. 26 **Effect of sample load on separation of standard polystyrene using a TSKgel SuperHM-H column**

Column: TSKgel SuperHM-H, 6mm ID x 15cm x 2
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene mixture

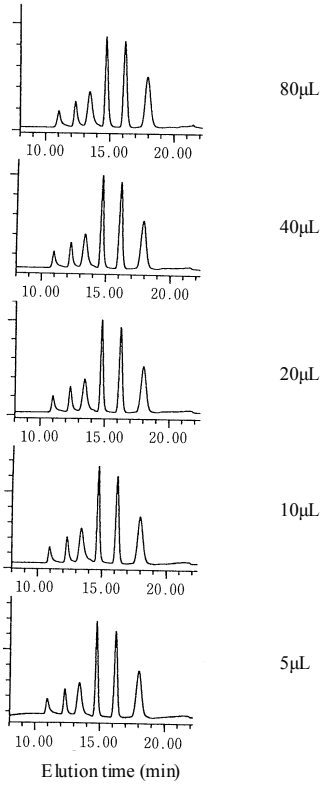

Fig. 27 **Effect of sample load on separation of standard polystyrene using a TSKgel SuperHM-H column**

Column: TSKgel SuperHM-H, 6mm ID x 15cm x 4
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene mixture

3-6. Sh ear degradation

Shear degradation is frequently observed, particularly when analyzing very high molecular weight samples, when using packing materials composed of very small particles, and when operating at high flow rates.[5]

Figure 28 shows the dependence of shear degradation of standard polystyrene F-2000 (MW: 20,600,000) on flow rate in the TSKgel SuperHM-H column. With the TSKgel SuperHM-H column, shear degradation is observed at each flow rate and elution does not proceed normally.

Therefore, to conduct normal GPC analysis of very high molecular weight samples such as F-2000, a flow rate of 0.8mL/min or less is recommended using a TSKgel GMH_{HR}-H (S) column (particle size: 13μm). This is shown in Figure 29.

Figure 30 shows the dependence of shear degradation of standard polystyrene F-850 (MW: 8,420,000) on flow rate in the TSKgel SuperHM-H column and Figure 31 shows the dependence of elution volume on flow rate of various standard polystyrenes in the TSKgel SuperHM-H column. Polystyrene standard F-850 can be analyzed normally at 0.6mL/min or less, but shear degradation occurs at flow rates of 0.8mL/min and above.

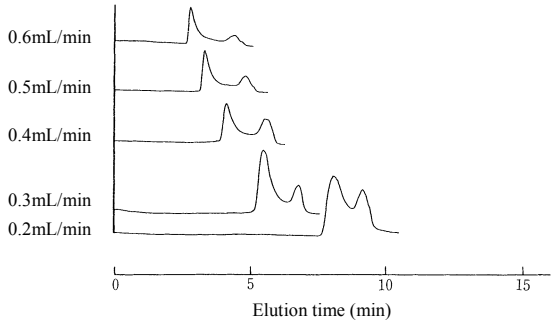

Fig. 28 Dependence of shear degradation on flow rate using a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene
F-2000 (MW 20,600,000, 0.015%)

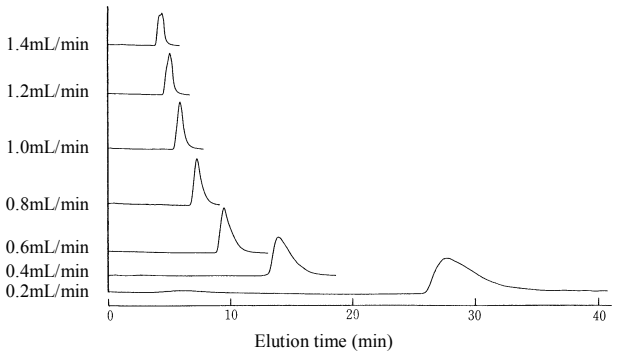

Fig. 29 Dependence of shear degradation on flow rate using a TSKgel GMH_{HR}-H (S) column

Column: TSKgel GMH_{HR}-H (S), 7.8mm ID x 30cm
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene
F-2000 (MW 20,600,000, 0.015%)

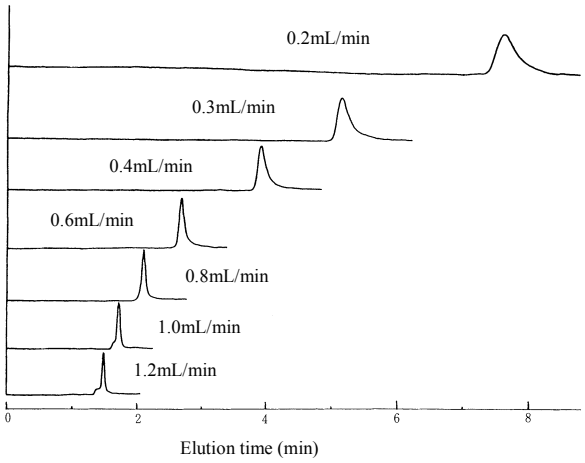

Fig. 30 Dependence of shear degradation on flow rate of standard polystyrene (F-850) using a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene F-850 (0.01%), 10μL

Figure 32 compares the shear degradation of standard polymer F-850 (MW: 8,420,000) using TSKgel SuperHM-H, SuperHM-M and SuperHM-N columns. As is evident here, shear degradation depends on sample pore size and the effect becomes stronger the smaller the pore size. It is strongest with the TSKgel SuperHM-N column. Consequently, it is crucial to be aware of the potential for shear degradation when using TSKgel SuperHM-M and SuperHM-N grade columns (see Table 8).

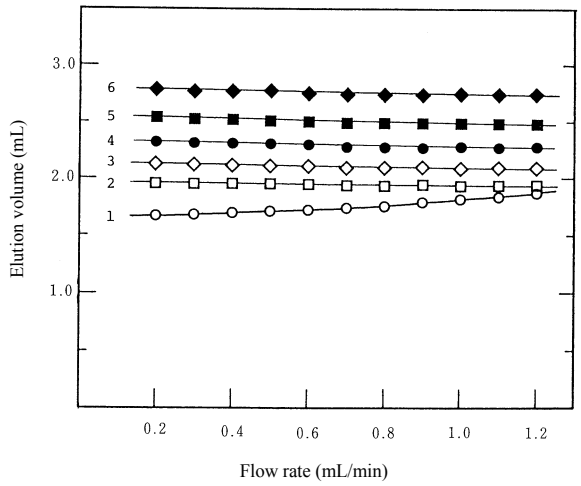

Fig. 31 Relationship between elution volume and flow rate of standard polystyrenes using a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene
1. MW 8,420,000 (F-850, ○)
2. MW 1,260,000 (F-128, □)
3. MW 422,000 (F-40, ◇)
4. MW 107,000 (F-10, ●)
5. MW 16,700 (F-2, ■)
6. MW 2,800 (A-2500, ◆)

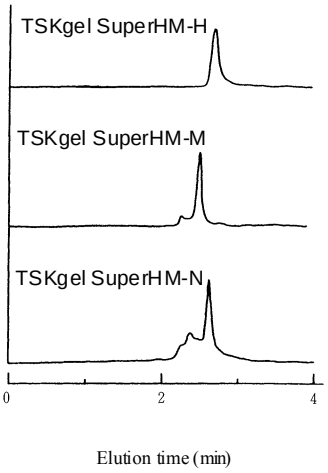

Fig. 32 Comparison of shear degradation of standard polystyrene F-850 using TSKgel SuperHM series columns

Column size: 6.0mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene F-850 (0.01%), 10µL

3-7. Co lumn temperature

The following advantages are gained by conducting analysis at high temperature:

(1) Peaks become sharp and separation performance is increased. This is especially noticeable at higher flow rates.

(2) Sample elution volume decreases, shortening analysis time.

(3) Viscosity of the mobile phase is lowered and operating pressure is decreased. This is an especially effective method with high-viscosity solvents such as DMSO, DMF, HFIP, etc.

Figures 33 and 34 demonstrate the temperature dependence of the separation of epoxy resin and a standard polystyrene mixture in TSKgel SuperH columns.

Figure 35 shows the temperature dependence of the separation of standard polystyrenes at various flow rates on the TSKgel SuperHM-H column. Moreover, although shear degradation of the sample occurs at flow rates of 0.8mL/min and above (see 3-6), shear degradation occurs less readily as the temperature increases.

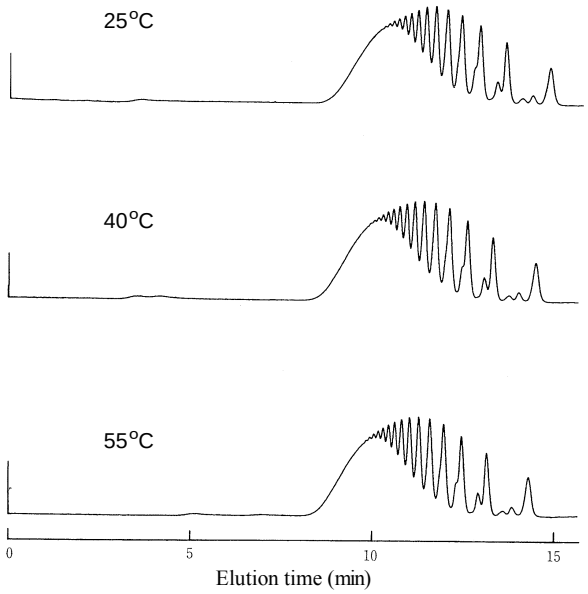

Fig. 33 Temperature dependence of separation of epoxy resin using TSKgel SuperH columns

Columns: TSKgel SuperH3000 x 2 + TSKgel SuperH2500
6mm ID x 15cm x 3
Eluent: THF
Flow rate: 0.6mL/min
Detection: UV (254nm)
Sample: Epikote 1004 (0.01%), 10µL

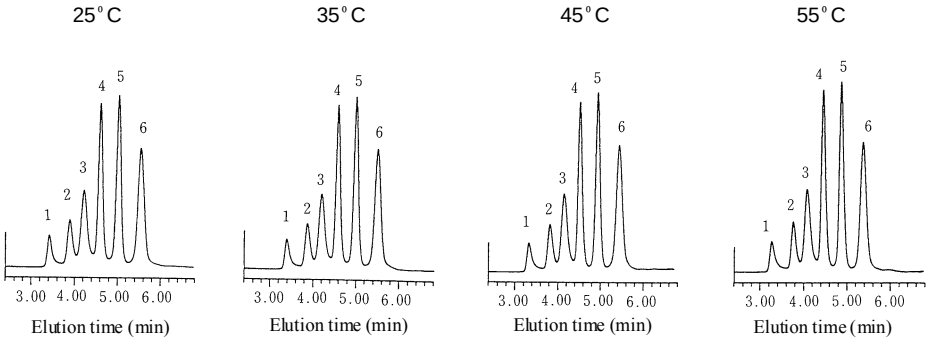

Fig. 34 Temperature dependence of separation of standard polystyrene using a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.5mL/min
Detection: UV@254nm
Sample: standard polystyrene

1. MW 8,420,000
2. MW 1,260,000
3. MW 422,000
4. MW 107,000
5. MW 16,700
6. MW 2,800

3-8. Op timization of hardware (system)

To maximize column performance it is extremely important to optimize the analysis conditions, including the solvent and the software, as discussed above. In addition to optimizing these conditions when using such high performance columns as TSKgel SuperH columns, it is important to minimize broadening of the sample peak outside the column. Specifically, suppressing band spreading in the detector, sample injector and tubing is extremely important with high efficiency columns.

MacDonald[6] uses the following equation to express the sample band spreading detected that occurs in actual GPC analysis:

$$\omega t^2 = \omega i^2 + \omega a^2 + \omega j^2 + \omega f^2 + \omega c^2$$

where ωt^2 = total band spreading; ωi^2 = spreading in sample injector; ωa^2 = spreading between sample injector and column inlet and between column outlet and detector inlet; ωj^2 = spreading at joints between columns; ωf^2 = spreading in flow cells (detectors); and ωc^2 = spreading within the column.

From this equation it is clear that while band spreading within the column occurs, overall peak volume can be significantly affected by extra-column effects.

3-8-1. Ba nd broadening in the detector

Table 6 compares the number of theoretical plates for a low molecular weight sample (DCHP) using a TSKgel SuperH2500 column with various types of UV detectors and different flow cell volumes. Figure 36 compares the separation performance of each of these using standard polystyrene A-500 and epoxy resin samples. Based on these results, it is clear that the number of theoretical plates and the separation performance of the TSKgel SuperH column are significantly affected by band broadening in the detector, including the size of the flow cell. In analyses performed with a TSKgel SuperH column, a UV-8020 microcell with reduced dead volume (or an equivalent device) must be used as the detector.

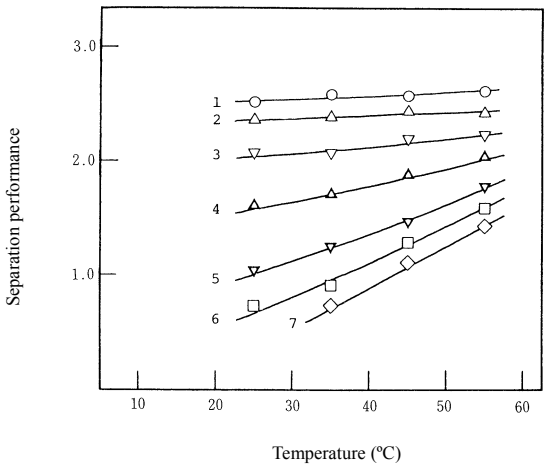

Fig. 35 Relationship between separation performance and temperature at various flow rates using a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: THF
Flow rate: 1. 0.2mL/min 2. 0.4mL/min
3. 0.6mL/min 4. 0.8mL/min
5. 1.0mL/min 6. 1.1mL/min
7. 1.2mL/min
Temperature: 25ºC-55ºC
Detection: UV@254nm
Samples: standard polystyrene 0.02%, 10µL
MW 8,420,000 and MW 1,260,000

Table 6 Comparison of number of theoretical plates with various detectors

Number of theoretical plates (TP/15cm)		
UV-8020[*1]	UV-8010[*2]	UV-8010[*3]
28,100	23,860	17,890

Column: TSKgel SuperH2500, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: DCHP 0.1%, 2µL

[*1] flow cell volume: 2µL microcell
[*2] flow cell volume: 10µL low dead volume type
[*3] flow cell volume: 10µL

Table 7 compares the number of theoretical plates resulting from differences in the *response* of the UV detector (UV-8020 microcell specifications) on the TSKgel SuperH2500 column. When the response time is *slow*, the number of theoretical plates decreases to less than half compared to when it is set to *fast*. Thus by setting the time constant to *fast*, adequate performance can be obtained in analyses conducted using TSKgel SuperH columns.

Table 7 Effect of time constant of detector on number of theoretical plates of TSKgel SuperH2500

Number of theoretical plates (N)		
Time constant (RESPONSE)		
FAST	STD	SLOW
28,100	21,960	12,400

Column: TSKgel SuperH2500, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV-8020 (microcell), 254nm
Sample: DCHP (0.1%), 2µL

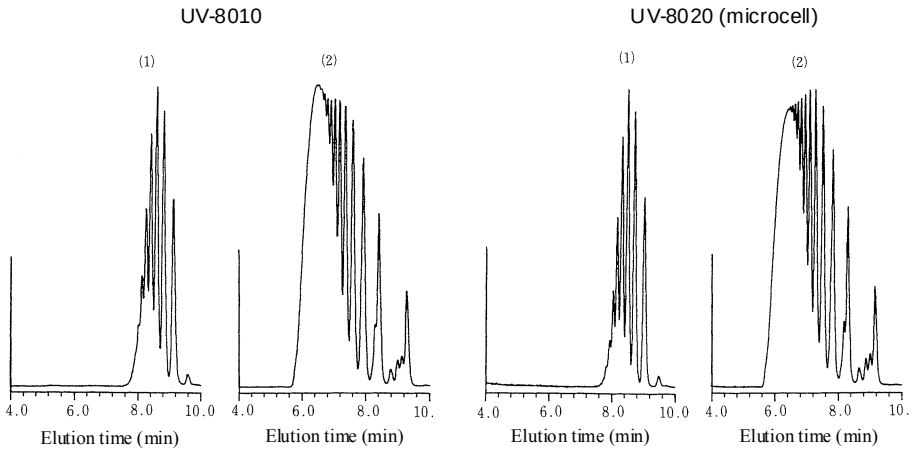

Fig. 36 Dependence of separation performance on band spreading in detector in TSKgel SuperH2500 column

Column: TSKgel SuperH2500, 6mm ID x 15cm x 2
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: (1) standard polystyrene A-500 (0.1%), 10µL
(2) Epikote 1004 (0.1%), 10µL

3-8-2. Band broadening from tubing at column inlet and outlet

According to Scott[7], band spreading of the sample that occurs within the connecting tubing (σ_i^2) can be expressed by the following equation:

$$\sigma_i^2 = \frac{d^4FL}{24Dm}$$

where d = inside diameter of tubing (cm); F = flow (mL/s); L = length of tubing (cm); and Dm = diffusion coefficient of the sample in mobile phase (cm^2/s).

It is clear from this equation that band spreading depends on the inside diameter and length of the connecting tubing. In particular, the larger the inside diameter of the tubing the greater the band spreading of the sample.

Using a TSKgel SuperH2500 column, Figure 37 shows how the number of theoretical plates is affected by the length of the connecting tubing between the sample injector and the column inlet (inside diameter 0.2mm). Figure 38 shows the effect of the length of the connecting tubing between the column outlet and the detector (inside diameter 0.2mm) on the number of theoretical plates.

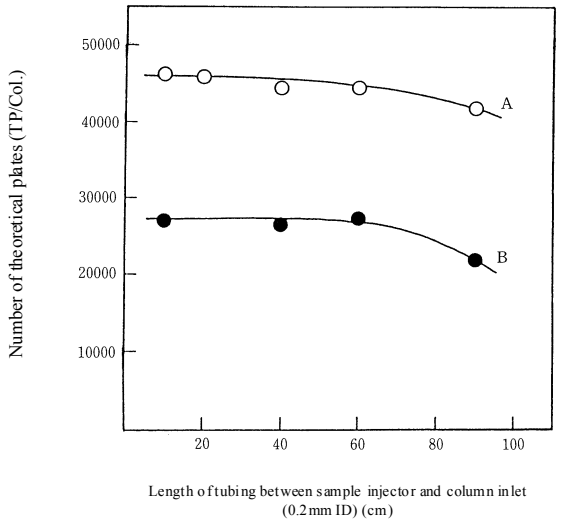

Fig. 37 Relationship between theoretical plates and length of column inlet tubing

Column: TSKgel SuperH2500
A. 6mm ID x 15cm x 2 B. 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm, UV-8020 (microcell)
Sample: DCHP (0.1%), 10µL

In addition, Figure 39 shows the effect of the length of the connecting tubing between the sample injector and the column inlet on the number of theoretical plates on the inside diameter.

When tubing with an inside diameter of 0.2mm is used, the number of theoretical plates is affected if the length of the tubing between the sample injector and the column inlet or between the column outlet and detector is longer than 60cm. Tubing with an inside diameter of 0.1mm is not linked to a decreased number of theoretical plates up to a length of 80cm. On the other hand, if the tubing diameter is increased to 0.3mm, column performance is markedly affected, and it is clear that tubing of 20cm or longer is not recommended.

Consequently, although the narrower and shorter the connecting tubing the better, for practical applications the recommended tubing dimensions are 0.2mm ID x 40 to 50cm.

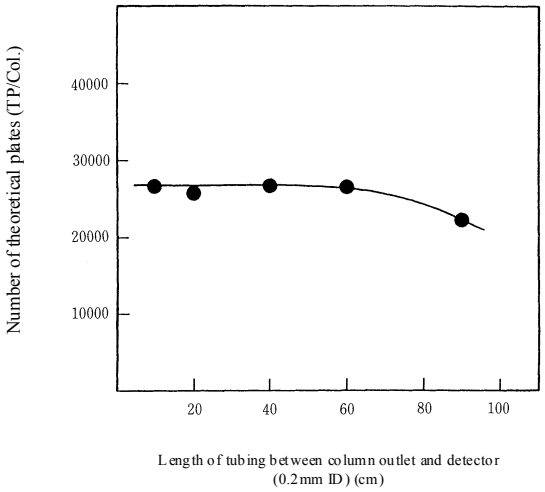

Fig. 38 Relationship between theoretical plates and length of column outlet tubing

Column: TSKgel SuperH2500, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm, UV-8020 (microcell)
Sample: DCHP (0.1%), 10µL

3-8-3. Band broadening in connecting tubing between columns

Figure 40 shows the effect of the dimensions of the connecting tubing between columns on the number of theoretical plates using two TSKgel SuperH2500 columns. Normally, the length of connecting tubing between columns must be around 10cm, and at this length the column performance is not affected if the tubing has an inside diameter of 0.1 or 0.2mm. However, if the inside diameter is 0.3mm, column performance decreases even when the length of the tubing is 10cm.

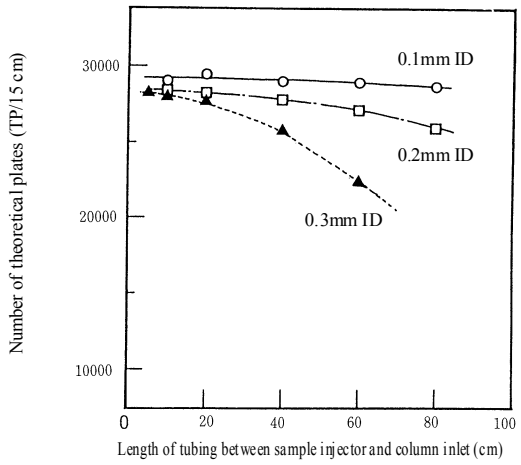

Fig. 39 Relationship between number of theoretical plates and inside diameter and length of column inlet tubing

Column: TSKgel SuperH2500, 6mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm, UV-8020 (microcell)
Sample: DCHP (0.1%), 2µL

3-8-4. Band broadening in other connecting components

Band broadening of the sample will also occur for the following reasons, in addition to those discussed in sections 3-8-1 to 3-8-3, above:

(1) Band broadening from joints between tubing (connecting joints).
(2) Band broadening due to gaps (dead volume) between all connecting components.
(3) Band broadening due to machining defects at the ends of the tubing.

Thus, as explained above, extra-column band broadening must be minimized to take full advantage of the high performance TSKgel SuperH series columns. For optimal use, please consult this Separation Report and the column User's Guide.

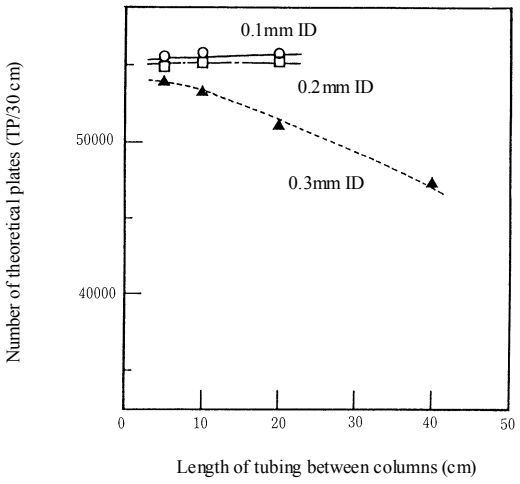

Fig. 40 Relationship between number of theoretical plates and inside diameter of tubing and length of connecting tubing between columns

Column: TSKgel SuperH2500, 6mm ID x 15cm x 2
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25 ºC
Detection: UV@254nm, UV-8020 (microcell)
Sample: DCHP (0.1%), 2µL

3-9. Mixed bed columns (linear)

The TSKgel SuperH series column line also features four mixed-bed columns with linear calibration curves. The TSKgel SuperHM-H and SuperHM-M columns are used to analyze the molecular weight distribution of polymers while the TSKgel SuperHM-N column is best suited for GPC analysis of samples that have a relatively low molecular weight. The TSKgel SuperHM-L column was developed to analyze oligomers and low molecular weight samples. It also was optimally designed for pattern analysis of samples from the high molecular weight range to the oligomer range.

Table 8 lists the molecular weight ranges and linear range of the calibration curve in each of the grades. Figure 2 shows the polystyrene calibration curve in THF.

Table 8 Molecular weight fractionation range of mixed-bed columns

TSKgel Grade	Molecular weight fractionation range	Linear component of calibration curve
SuperHM-L	100~3,000,000	200~10,000
SuperHM-N	100~1,000,000	300~200,000
SuperHM-M	300~3,000,000	300~1,000,000
SuperHM-H	500~10,000,000	1,000~8,000,000

Figures 41 to 44 compare the elution curves of various types of standard polystyrenes for the TSKgel SuperHM series columns.

Figures 45 and 46 compare chromatograms of standard polystyrene polymers separated on TSKgel SuperHM series columns.

Figures 47 and 48 compare TSKgel SuperHM series columns for the separation of an epoxy resin sample.

Figure 49 compares the separation of phenol resin on TSKgel SuperHM series columns.

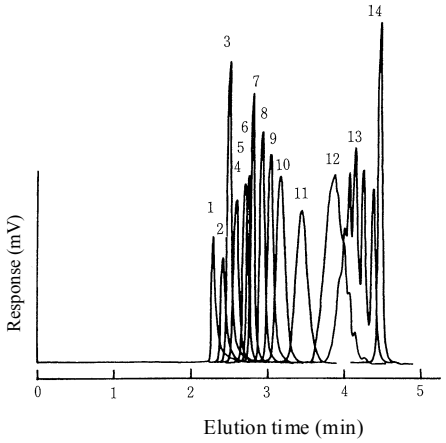

Fig. 41 Elution curve of standard polystyrene using TSKgel SuperHM-L

Column:	TSKgel SuperHM-L, 6.0mm ID x 15cm	
Eluent:	THF	
Flow rate:	0.6mL/min	
Temperature:	25ºC	
Detection:	UV@254nm	
Sample:	1. MW 2,890,000	2. MW 1,260,000
	3. MW 775,000	4. MW 422,000
	5. MW 186,000	6. MW 107,000
	7. MW 42,800	8. MW 16,700
	9. MW 10,200	10. MW 6,200
	11. MW 2,800	12. A-1,000
	13. A-500	14. DCHP

4. Applications

Figures 50 to 56 show examples of analyses performed on various polymer samples.

Figure 57 shows an example of a chromatogram produced when Epikote 1004 was separated using a high speed GPC instrument (HLC-8120PC).

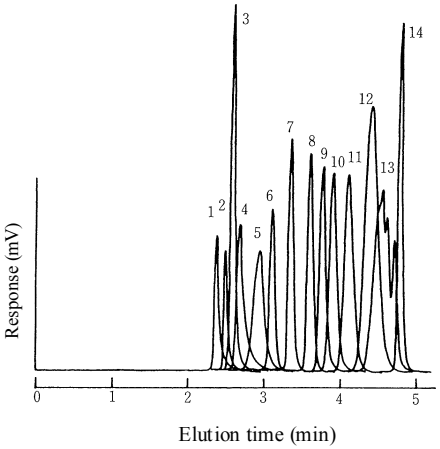

Fig. 42 Elution curve of standard polystyrenes using a TSKgel SuperHM-N column

Column: TSKgel SuperHM-N, 6.0mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample:

1. MW 2,890,000	2. MW 1,260,000
3. MW 775,000	4. MW 422,000
5. MW 186,000	6. MW 107,000
7. MW 42,800	8. MW 16,700
9. MW 10,200	10. MW 6,200
11. MW 2,800	12. A-1,000
13. A-500	14. DCHP

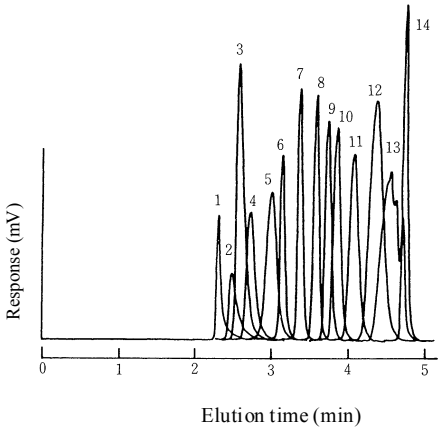

Fig. 43 Elution curve of standard polystyrene using a TSKgel SuperHM-M column

Column: TSKgel SuperHM-M, 6.0mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample:

1. MW 2,890,000	2. MW 1,260,000
3. MW 775,000	4. MW 422,000
5. MW 186,000	6. MW 107,000
7. MW 42,800	8. MW 16,700
9. MW 10,200	10. MW 6,200
11. MW 2,800	12. A-1,000
13. A-500	14. DCHP

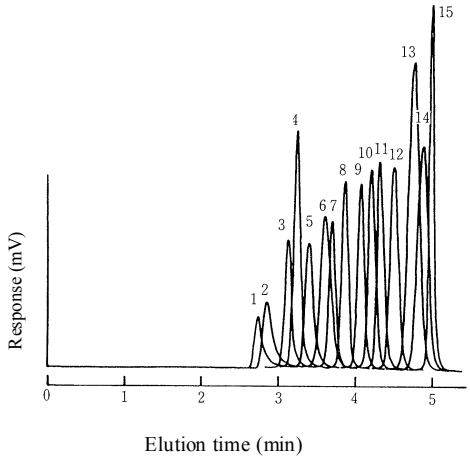

Fig. 44 Elution curve of standard polystyrene using a TSKgel SuperHM-H column

Column: TSKgel SuperHM-H, 6.0mm ID x 15cm
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample:

1. MW 5,480,000	2. MW 2,890,000
3. MW 1,260,000	4. MW 775,000
5. MW 422,000	6. MW 186,000
7. MW 107,800	8. MW 42,800
9. MW 16,700	10. MW 10,200
11. MW 6,200	12. MW 2,800
13. A-1,000	14. A-500
15. DCHP	

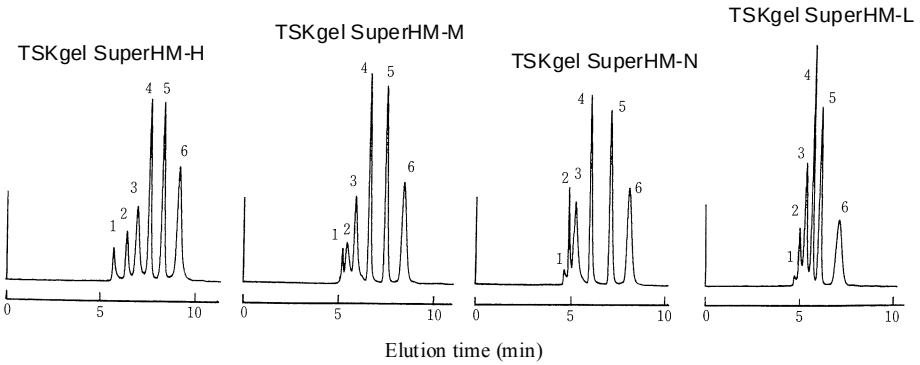

Fig. 45 **Comparison of separations of standard polystyrenes on TSKgel SuperHM series columns**

Columns: TSKgel SuperHM-H, SuperHM-M, SuperHM-N, SuperHM-L
All: 6mm ID x 15cm x 2
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene
1. MW 8,420,000 2. MW 1,260,000
3. MW 422,000 4. MW 107,000
5. MW 16,700 6. MW 2,800

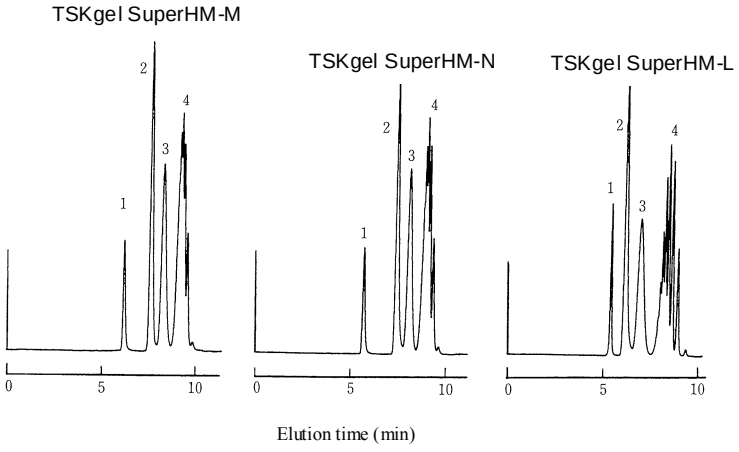

Fig. 46 **Comparison of separations of standard polystyrenes on TSKgel SuperHM series columns**

Columns: TSKgel SuperHM-M, SuperHM-N, SuperHM-L
All: 6mm ID x 15cm x 2
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene
1. MW 190,000 2. MW 9,100
3. MW 2,800 4. A-500

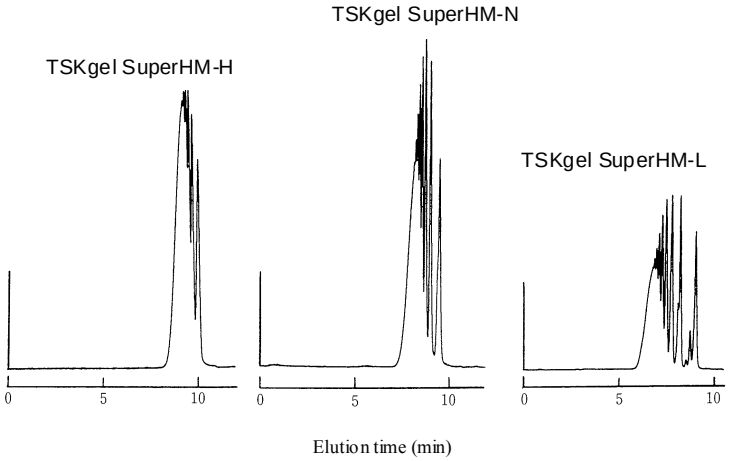

Fig. 47 **Comparison of separations of epoxy resin on TSKgel SuperHM series columns**

Columns: TSKgel SuperHM-H, SuperHM-N, SuperHM-L
All: 6mm ID x 15cm x 2
Eluent: THF
Temperature: 25ºC
Detection: UV@254nm
Sample: epoxy resin (Ep100)

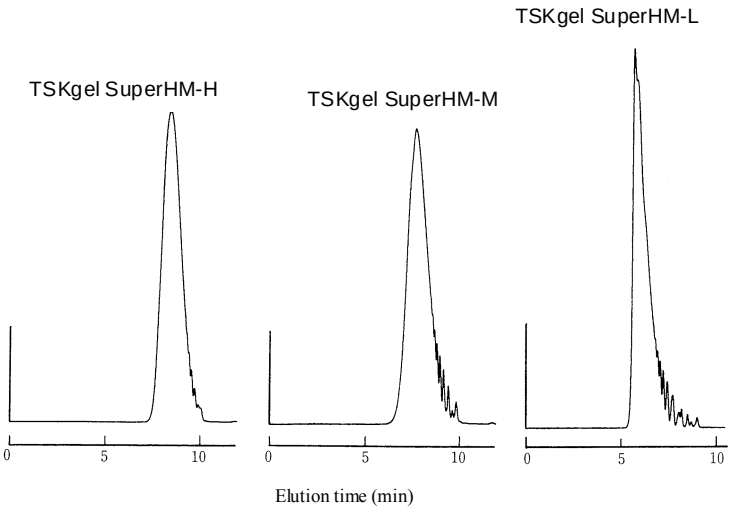

Fig. 48 **Comparison of separations of epoxy resin on TSKgel SuperHM series columns**

Columns: TSKgel SuperHM-H, SuperHM-M, SuperHM-L
All: 6mm ID x 15cm x 2
Eluent: THF
Temperature: 25 ºC
Detection: UV@254nm
Sample: epoxy resin (Ep100)

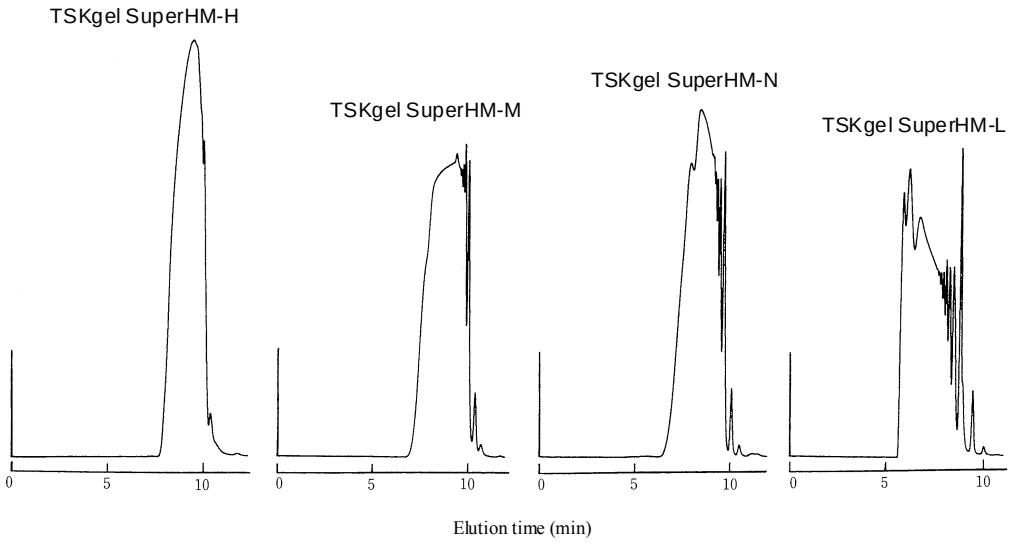

Fig. 49 **Comparison of separations of phenol resin with the TSKgel SuperHM series columns**

Columns: TSKgel SuperHM-H, SuperHM-M, SuperHM-N, SuperHM-L
All: 6mm ID x 15cm x 2
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: phenol resin

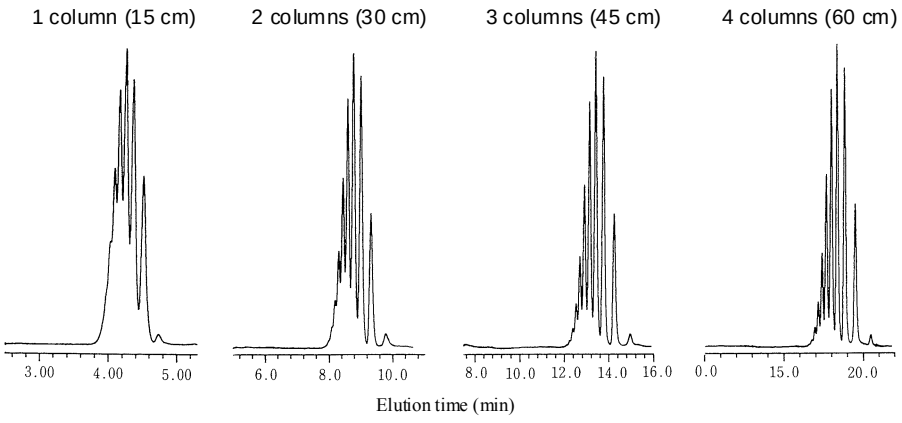

Fig. 50 **Separation of standard polystyrene (A-500) as a function of column length on a TSKgel SuperH2500 column**

Column: TSKgel SuperH2500, 6mm ID x 15cm x 1-4
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: standard polystyrene A-500 (0.1%, 10µL)

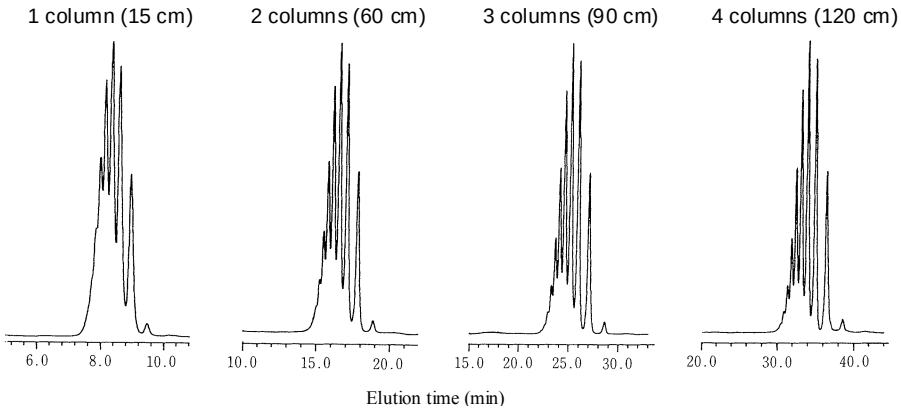

Fig. 51 **Separation of standard polystyrene (A-500) as a function of column length on a TSKgel G2500H$_{XL}$ column**

Column:	TSKgel G2500H$_{XL}$, 7.8mm ID x 30cm x 1-4
Eluent:	THF
Flow rate:	1.0mL/min
Temperature:	25ºC
Detection:	UV@254nm
Sample:	standard polystyrene A-500 (0.1%), 20µL

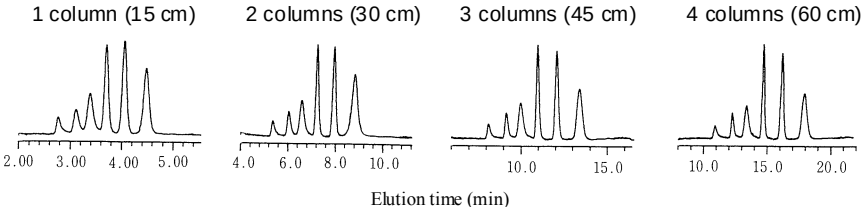

Fig. 52 **Separation of standard polystyrene as a function of column length on a TSKgel SuperHM-H column**

Column:	TSKgel SuperHM-H, 6mm ID x 15cm x 1-4
Eluent:	THF
Flow rate:	0.6mL/min
Temperature:	25ºC
Detection:	UV@254nm
Sample:	standard polystyrene

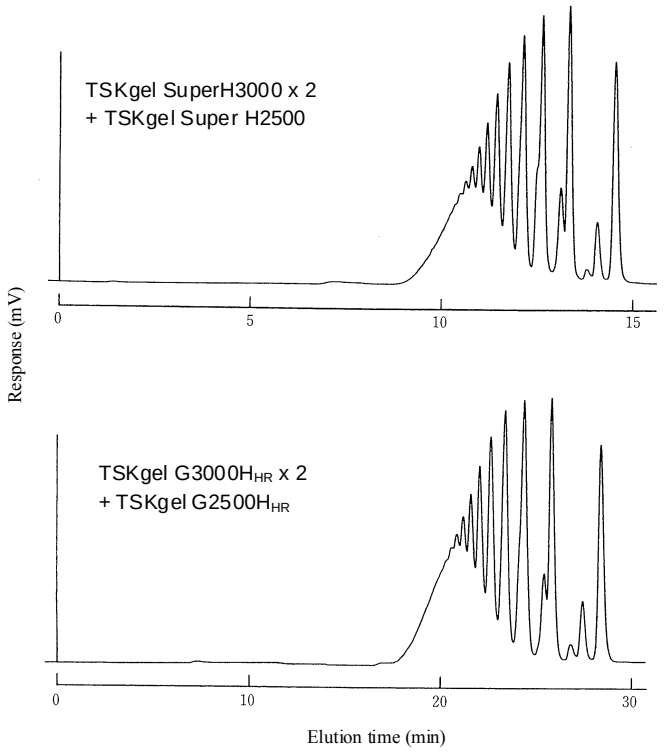

Fig. 53 **Comparison of separation performance of TSKgel SuperH and H_{HR} columns**

Columns: TSKgel SuperH3000 x 2 + TSKgel SuperH2500, 6mm ID x 15cm x 3
TSKgel G3000H_{HR} x 2 + TSKgel G2500H_{HR}, 7.8mm ID x 30cm x 3
Eluent: THF
Flow rate: TSKgel SuperH (0.6mL/min)
TSKgel H_{HR} (1.0mL/min)
Temperature: 25ºC
Detection: UV@254nm
Sample: Epikote 1001 (0.1%), 10µL

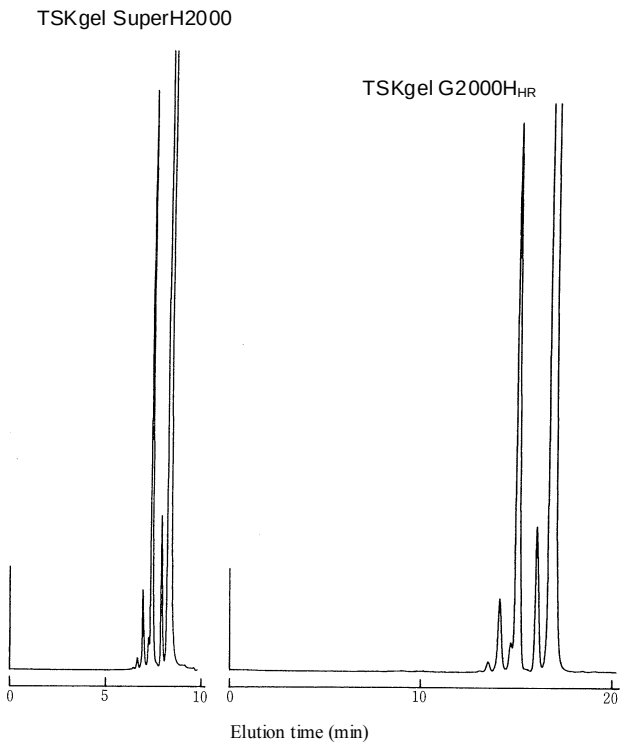

Fig. 54 **Comparison of separation of epoxy resin on TSKgel SuperH2000 and TSKgel G2000 H_{HR} columns**

Columns: TSKgel SuperH2000, 6mm ID x 15cm x 2
TSKgel G2000H_{HR}, 7.8mm ID x 30cm x 2
Eluent: THF
Flow rate: TSKgel SuperH2000 (0.6mL/min)
TSKgel G2000 H_{HR} (1.0mL/min)
Temperature: 25ºC
Detection: UV@254nm
Sample: Epikote 828 (0.1%), 10µL

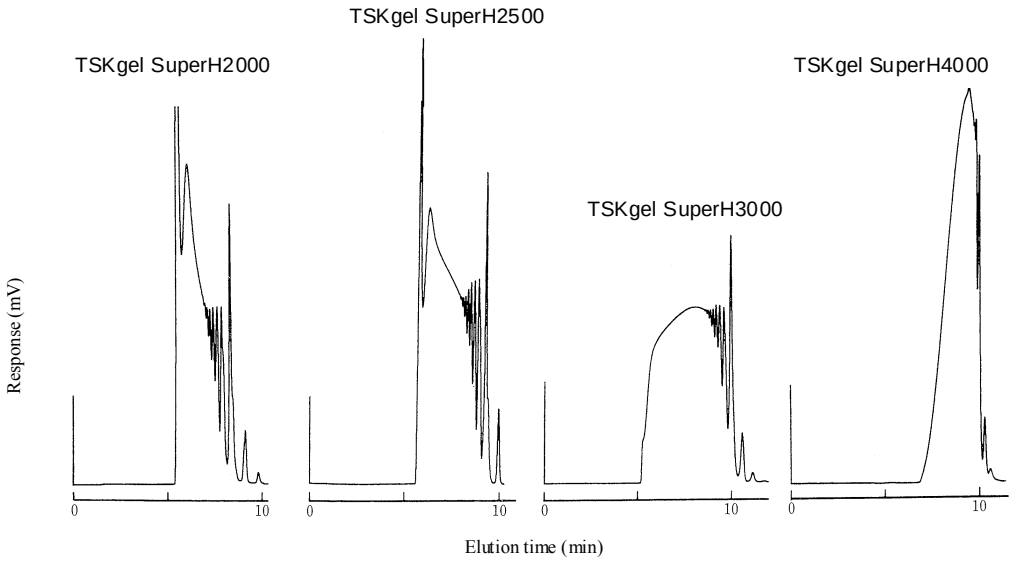

Fig. 55 Comparison of separation of phenol resin in TSKgel SuperH series columns

Columns: TSKgel SuperH2000 - SuperH4000, 6mm ID x 15cm x 2, respectively
Eluent: THF
Flow rate: 0.6mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: phenol resin (0.1%), 5µL

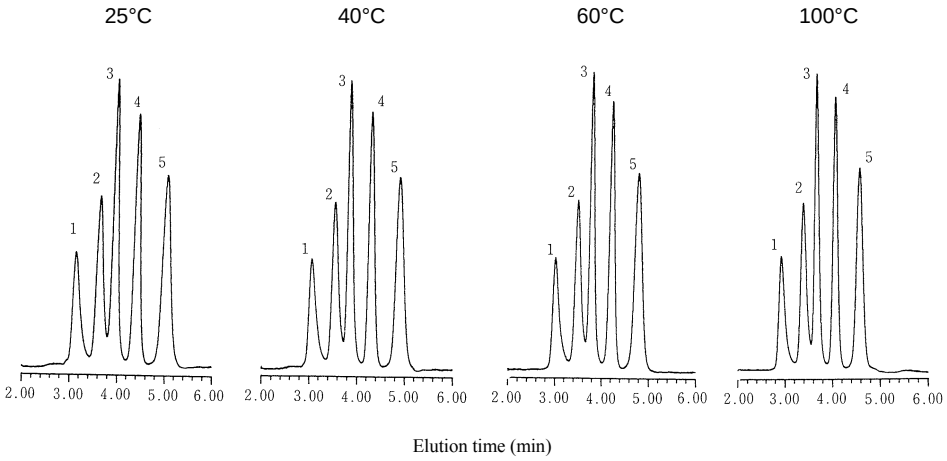

Fig. 56 Temperature dependence of separation of standard polystyrene on TSKgel SuperHM-H columns

Column: TSKgel SuperHM-H, 6mm ID x 15cm
Eluent: 10mmol LiBr in DMF
Flow rate: 0.6mL/min
Detection: UV@270 nm
Sample: standard polystyrene
1. MW 2,890,000 2. MW 422,000
3. MW 107,000 4. MW 16,700
5. MW 2,800

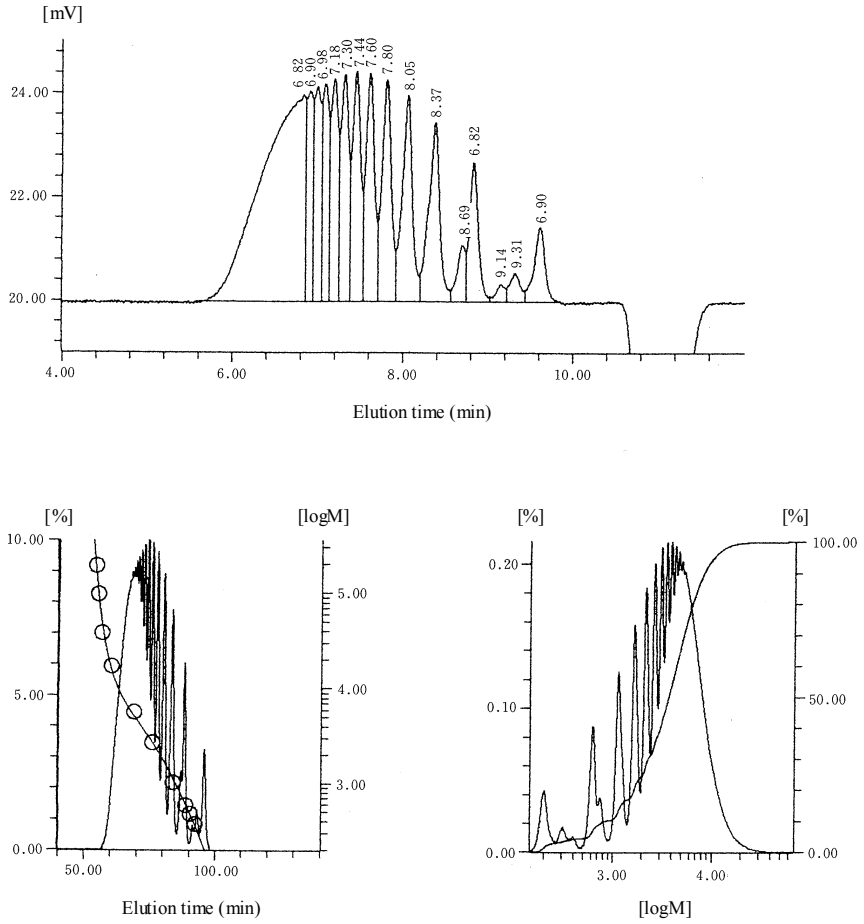

Fig. 57 Separation of Epikote 1004 (E1004) using a high speed GPC instrument (EcoSEC GPC System)

Columns:	TSKgel SuperH3000 + TSKgel SuperH2500, 6mm ID x 15cm x 2
Temperature:	25 ºC
Eluent:	THF
Flow rate:	0.6mL/min
Detection:	RI
Sample:	Epikote 1004 (EP1004) (0.1%), 10μL

5. Conclusions

With the semi-micro, high performance TSKgel SuperH series GPC columns, the separation performance of conventional TSKgel H_{HR} and H_{XL} series columns can be achieved in half the analysis time.

The TSKgel SuperH series column line was designed to minimize sample band broadening in the column. Consequently, to maximize the performance of these columns it is important to optimize analysis conditions and decrease dead volume in the system as described in the text above (Section 3-8).

The GPC system must be optimized to reduce extra-column band broadening. The high performance of TSKgel SuperH series columns can be fully and conveniently exploited by using Tosoh's EcoSEC GPC System or equivalent.

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