Solvent-Compatible High Performance GPC Columns: TSKgel H_{HR} Series

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Message for Sigma-Aldrich/Supelco customers:

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For the availability of TSKgel columns in Asia and Australia, please consult the website of Tosoh Corporation.

Note: On the Sigma-Aldrich website and in the Supelco catalog TSKgel part numbers are preceded by the number 8, for example:
817356 for TSKgel G4000Hhr, 7.8mmID x 30cm, 5µm

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1. Introduction

Gel permeation chromatography (GPC), a branch of size exclusion chromatography (SEC), is the dominant analytical method used to investigate the molecular properties of polymer substances, such as molecular weight and molecular weight distribution, and is widely used given the excellent repeatability of the procedure, as well as its relative simplicity and speed.

In 1983, Tosoh developed and produced the TSKgel H_{XL} columns of high performance GPC columns for use with organic solvent systems. The positive characteristics of these columns have been recognized by researchers in industry as well as academia.

In recent history, high performance functional polymers, such as engineering plastics, have been developed and commercialized. Due to the high performance and high functionality of these polymers, they are not readily soluble (or are insoluble) in solvents conventionally used in GPC such as tetrahydrofuran (THF) and chloroform. As a result, a variety of solvents are now being used for GPC analysis of these polymers as replacements for THF and chloroform. Examples of such solvents include: fluorinated alcohols such as 2,2,2-trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP),[1-4] pentafluorophenol (PFP)/chloroform,[5] N-methylpyrrolidone (NMP), dimethylformamide (DMF) and dimethylsulfoxide (DMSO). For the most part, these solvents are more polar and more viscous than THF and chloroform and are difficult to use with conventional TSKgel H_{XL} columns from the perspective of column durability and stability. Moreover, conversion to these solvents from THF has been a problem with TSKgel H_{XL} columns, which has limited their use. Furthermore, using an unsuitable solvent (mobile phase) during analysis can result in adsorption of one or more sample components.

Thus, it has now become difficult to assure the repeatability, stability, and accuracy of GPC analyses of various types of polymers under conventional analysis conditions alone (for example, using a TSKgel H_{XL} column with THF as the solvent).

The TSKgel H_{HR} columns are a series of GPC columns that are filled with robust packing materials that can withstand a wider range of organic solvents. This series of columns not only can withstand conversion from one solvent to another; it also provides more stable analysis under specialized conditions or with specialized solvents, in comparison to the TSKgel H_{XL} columns.

In this report, the features and basic characteristics of the TSKgel H_{HR} columns will be introduced and application examples are presented.

2. Features

The TSKgel H_{HR} columns contain a highly cross-linked styrene-vinyl benzene copolymer that maintains the pore properties of the TSKgel H_{XL} columns while at the same time is extremely robust, with virtually no swelling or shrinkage, and which, as a result, can withstand solvent changes, and improves the quality of polymer analysis.

Table 1 compares the characteristics of the TSKgel H_{HR} and H_{XL} columns. Grades used for polymer analysis (TSKgel G5000H_{HR} – G7000H_{HR} and TSKgel GMH_{HR}-M, and –H) use a 5μm packing material, in contrast to the 10μm size packing material of the conventional TSKgel H_{XL} columns. As a result, the guaranteed number of theoretical plates is raised from 14,000/30cm to 16,000/30cm and sample resolution is improved. Moreover, the grades used for analyzing low molecular weight substances (TSKgel G1000H_{HR} - G4000H_{HR} and TSKgel GMH_{HR}-L) maintain the same efficiency levels as the corresponding columns in the TSKgel H_{XL} series. In addition, an S-type grade has been prepared for high temperature analysis of ultra high molecular weight substances for use in the separation of polymers.

Table 2 lists the solvents that the TSKgel H_{HR} columns can accommodate. As shown in this table, the TSKgel H_{HR} columns can accommodate a variety of organic solvents and has excellent durability with each of these solvents. In addition, this series of columns is shipped in THF, making it possible to directly convert to a variety of other organic solvents.

Figures 1 to 8 show calibration curves of the TSKgel H_{HR} columns produced when different standard samples were analyzed in an assortment of solvents.

Table 1 Comparison of performance of TSKgel H_{HR} and H_{XL} columns

TSKgel Column	TSKgel H_{HR}		TSKgel H_{XL}	
	Particle size (µm)	Guaranteed number of theoretical plates (N/column)	Particle size (µm)	Guaranteed number of theoretical plates (N/column)
G1000H	5	16,000	5	16,000
G2000H	5	16,000	5	16,000
G2500H	5	16,000	5	16,000
G3000H	5	16,000	5	16,000
G4000H	5	16,000	5	16,000
G5000H	5	16,000	10	14,000
G6000H	5	16,000	10	14,000
G7000H	5	16,000	10	14,000
G5000H (S)	13	8,000	—	——
G6000H (S)	13	8,000	—	——
G7000H (S)	13	8,000	—	——
GMH-H	5	16,000	—	——
GMH-M	5	16,000	—	——
GMH-L	5	16,000	5	16,000
GMH	—	——	10	14,000
GMH-H (S)	13	8,000	—	——
GMH-M (S)	13	8,000	—	——

Conditions for analyzing column efficiency

Column size: 7.8mm ID x 30cm

Solvent: tetrahydrofuran (THF)

Flow rate: 1.0mL/min

Samples: benzene: TSKgel G1000H - G2500H

n-butylbenzene: TSKgel G3000H, G4000H and GMH-L

dicyclohexylphthalate: TSKgel G5000H to G7000H, GMH-M, and GMH-H

Table 2 Solvents that can be used in TSKgel H_{HR} columns

Compatible solvents
toluene, xylene, chloroform, benzene, dichloromethane, dichloroethane, dimethylformamide, dimethylsulfoxide, dioxane, N-methylpyrrolidone, m-cresol/chloroform, quinoline, methyl ethyl ketone (MEK), o-dichlorobenzene, trichlorobenzene, hexafluoroisopropanol (HFIP), HFIP/chloroform, o-chlorophenol/chloroform, pyridine, carbon tetrachloride, ethyl acetate, methanol/chloroform, THF/methanol, acetone, ethanol, dimethylacetamide, n-hexane, dodecane, 1-chloronaphthalene, FC-113, trichloroethane

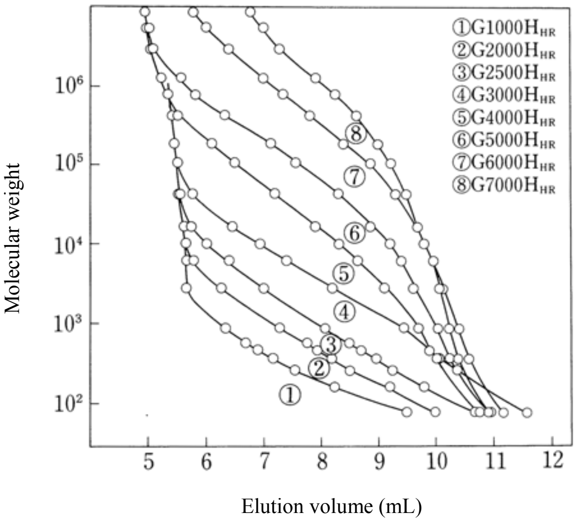

Figure 1 **Calibration curves produced by polystyrene in THF**

Columns: TSKgel H_{HR} series, 7.8mm ID x 30cm
Solvent: tetrahydrofuran (THF)
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: standard polystyrenes

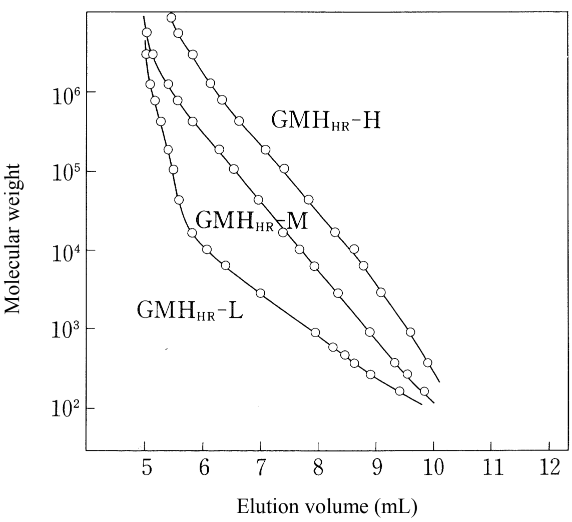

Figure 2 **Calibration curves produced by polystyrene in THF**

Columns: TSKgel H_{HR} series, 7.8mm ID x 30cm
Solvent: THF
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: standard polystyrene

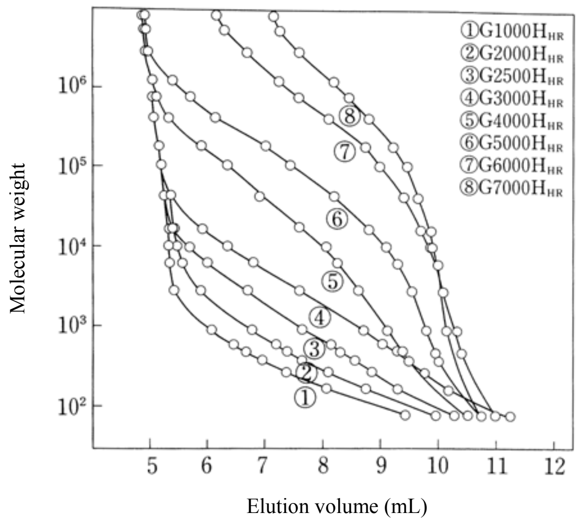

Figure 3 **Calibration curves produced by polystyrene in chloroform**

Columns: TSKgel H_{HR} series, 7.8mm ID x 30cm
Solvent: chloroform
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: standard polystyrenes

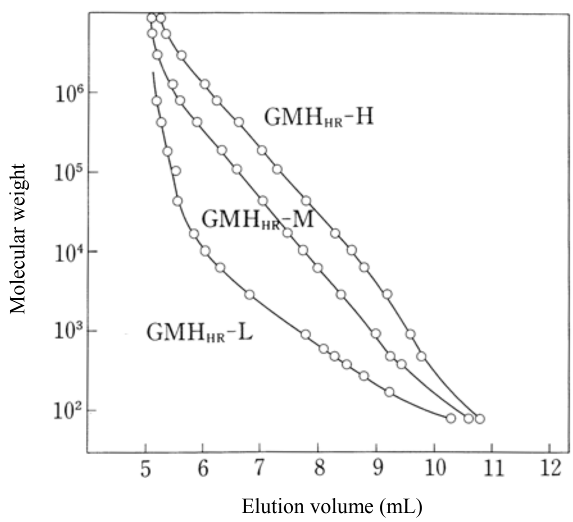

Figure 4 **Calibration curves produced by polystyrene in chloroform**

Columns: TSKgel H_{HR} series, 7.8mm ID x 30cm
Solvent: chloroform
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: standard polystyrene

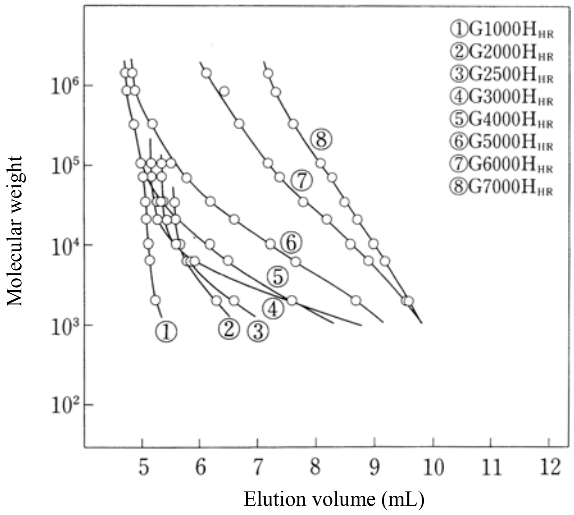

Figure 5 Calibration curves produced by polymethylmethacrylate in HFIP

Columns:	TSKgel H_{HR} series, 7.8mm ID x 30cm
Solvent:	5mmol/L sodium trifluoroacetate in HFIP
Flow rate:	1.0mL/min
Temperature:	40ºC
Detection:	UV@220nm
Samples:	standard polymethylmethacrylates

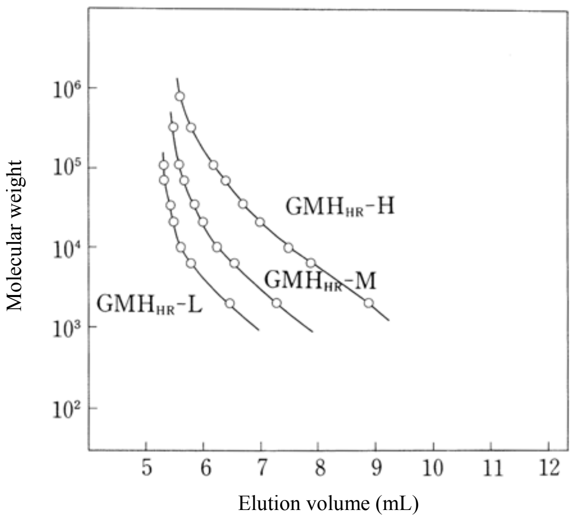

Figure 6 Calibration curves produced by polymethylmethacrylate in HFIP

Columns:	TSKgel H_{HR} series. 7.8mm ID x 30cm
Solvent:	5mmol/L sodium trifluoroacetate in HFIP
Flow rate:	1.0mL/min
Temperature:	40ºC
Detection:	UV@220nm
Samples:	standard polymethylmethacrylates

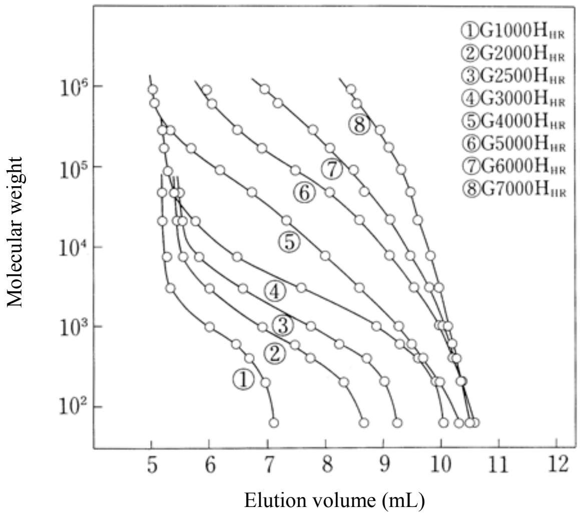

Figure 7 Calibration curves produced by PEO and PEG in DMF

Columns:	TSKgel H_{HR} series, 7.8mm ID x 30cm
Solvent:	10mmol/L lithium bromide in DMF
Flow rate:	10mL/min
Temperature:	25ºC
Detection:	RI
Samples:	standard polyethylene oxides and polyethylene glycols

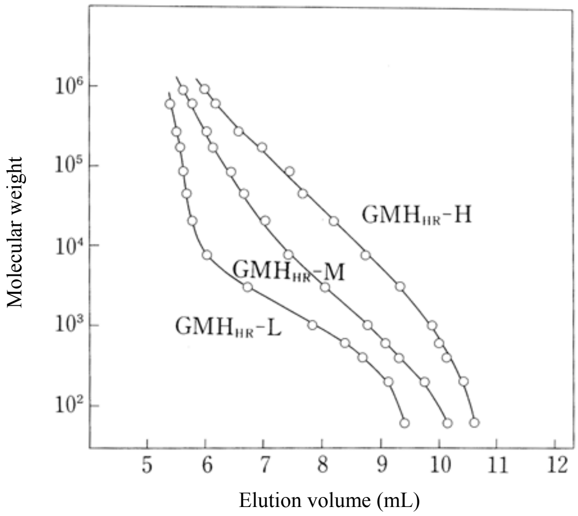

Figure 8 Calibration curves produced by PEO and PEG in DMF

Columns:	TSKgel H_{HR} series, 7.8mm ID x 30cm
Solvent:	10mmol/L lithium bromide in DMF
Flow rate:	1.0mL/min
Temperature:	25ºC
Detection:	RI
Samples:	standard polyethylene oxides and polyethylene glycols

3. Basic Characteristics

3-1. Resolution of grades for polymer analysis

Table 1 catalogs the grades used for polymer analysis: TSKgel G5000H_{HR} - G7000H_{HR}, GMH_{HR}-M and GMH_{HR}-H. The particle size of the packing material has been decreased to 5µm, realizing better resolution compared to the TSKgel H_{XL} columns.

Figure 9 shows chromatograms of the separation of a mixed polystyrene sample obtained using the TSKgel G5000H_{HR} and G5000H_{XL} columns. Figure 10 compares chromatograms of the separation of a mixed polystyrene sample obtained using the TSKgel GMH_{HR}-H, GMH_{XL} and GMH_{HR}-H (S) columns. It is clear that in both grades better separation is achieved with the TSKgel H_{HR} columns than the conventional TSKgel H_{XL} columns.

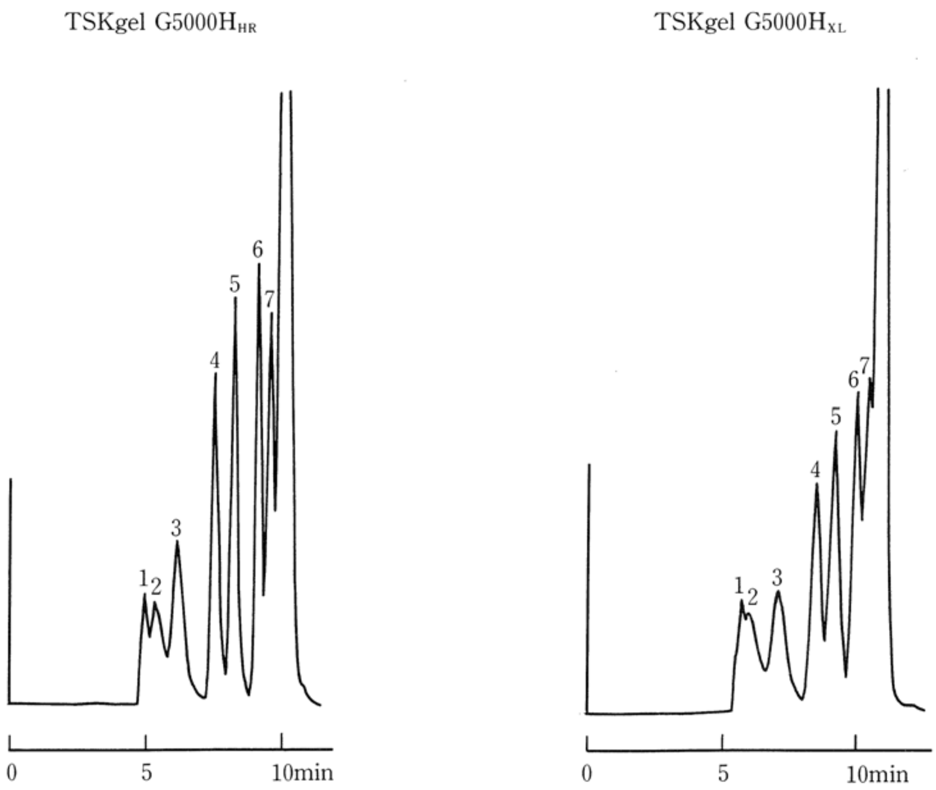

Figure 9 Comparison of resolution of TSKgel G5000H_{HR} and TSKgel G5000H_{XL} columns

Columns: TSKgel G5000H_{HR} and TSKgel G5000H_{XL}, both 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Samples: standard polystyrenes
1. 2,890,000 Da 2. 1,260,000 Da
3. 422,000 Da 4. 107,000 Da
5. 42,800 Da 6. 10,200 Da
7. 2,800 Da

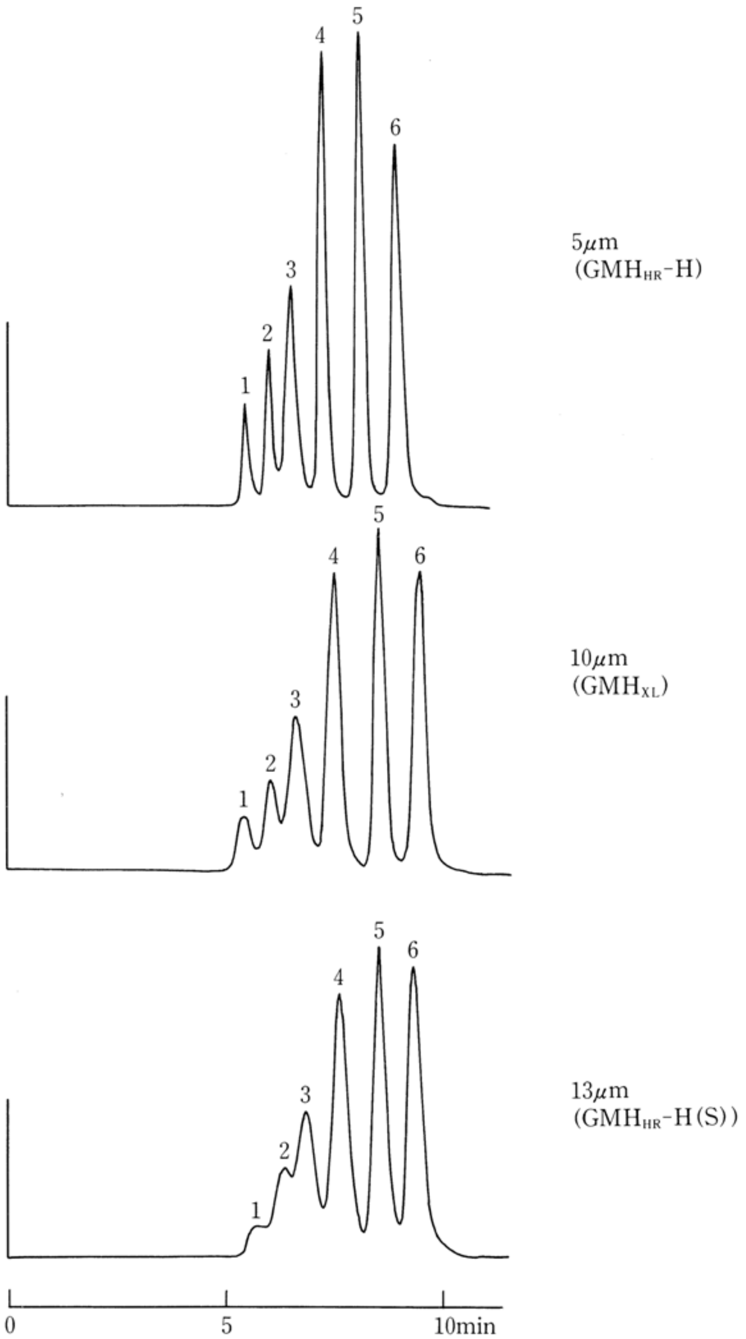

Figure 10 Separations of polystyrene mixtures by TSKgel GMH columns

Columns: TSKgel GMH$_{HR}$-H, TSKgel GMH$_{XL}$, TSKgel GMH$_{HR}$-H(S), all 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Samples: standard polystyrene

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

3-2. Dependence of height equivalent to theoretical plate on flow rate

The height equivalent to a theoretical plate (HETP) depends greatly on the particle size of the packing material, the molecular size of the sample, and the flow rate and viscosity of the solvent, etc. Figure 11 shows a comparison of the flow rate dependency of HETP for a TSKgel GMH_{HR}-H (5μm) column versus a TSKgel GMH_{XL} (10μm) and TSKgel GMH_{HR}-H(S) (13μm) column using dicyclohexyl phthalate (DCHP) as the sample.

When separating DCHP, a low molecular weight sample, on the TSKgel GMH_{HR}-H column, there is little change in HETP even when the flow rate is increased. On the other hand the TSKgel GMH_{XL} and GMH_{HR}-H(S) columns are affected by flow rate, as HETP increases at high flow rates (1.5mL/min or more).

Thus, with the TSKgel GMH_{HR}-H column, containing a packing material comprised of small-sized particles, HETP is impacted slightly by flow rate when a low molecular weight sample (DCHP) is analyzed. Therefore, analysis time can be reduced by using a high flow rate (1.5 to 2.0mL/min).

Figure 12 depicts the relationship between flow rate and resolution of polymer samples using TSKgel GMH_{HR}-H, GMH_{HR}-H(S), and GMH_{XL} columns. Resolution was least affected by flow rate for the TSKgel GMH_{HR}-H (5μm) column, but regardless of the particle size of the packing material, resolution decreased as flow rate increased. Consequently, a suitable flow rate should be 0.5mL/min or less when analyzing high molecular weight polymer samples.

Figures 13 to 15 demonstrate how the separation of a standard polyethylene mixture depends on flow rate for the TSKgel GMH_{HR}-H, GMH_{XL} and GMH_{HR}-H(S) columns. The TSKgel GMH_{HR}-H column shows little flow rate dependence and is suitable for analysis at high flow rate, even with a polymer sample with a molecular weight around 3,000,000 Da. However, in general, a flow rate between 0.5 and 1.0mL/min is considered optimal from the perspective of individual peak sharpness.

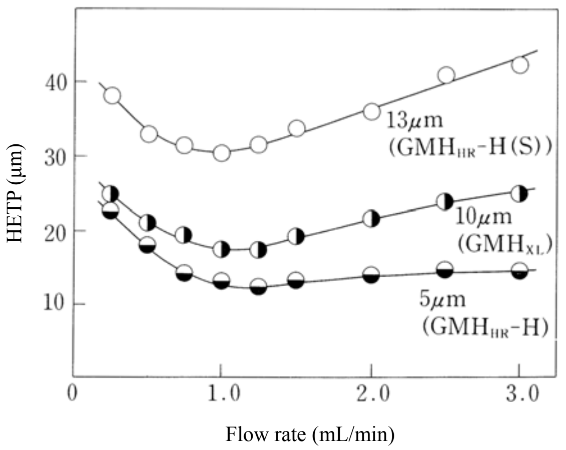

Figure 11 Relationship between flow rate and HETP for a TSKgel GMH column

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{XL}, TSKgel GMH_{HR}-H(S), all 7.8mm ID x 30cm

Solvent: THF
Flow rate: 0.25mL/min to 3.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: dicyclohexyl phthalates (DCHP)

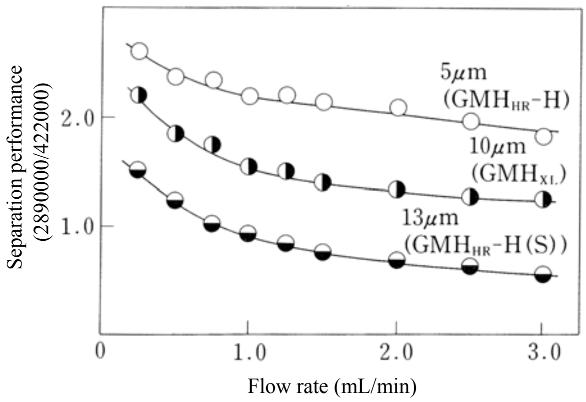

Figure 12 Relationship between flow rate and resolution for a TSKgel GMH column

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{XL}, TSKgel GMH_{HR}-H(S), all 7.8mm ID x 30cm

Solvent: THF
Flow rate: 0.25mL/min to 3.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Samples: standard polystyrenes 2,890,000 Da and 422,000 Da

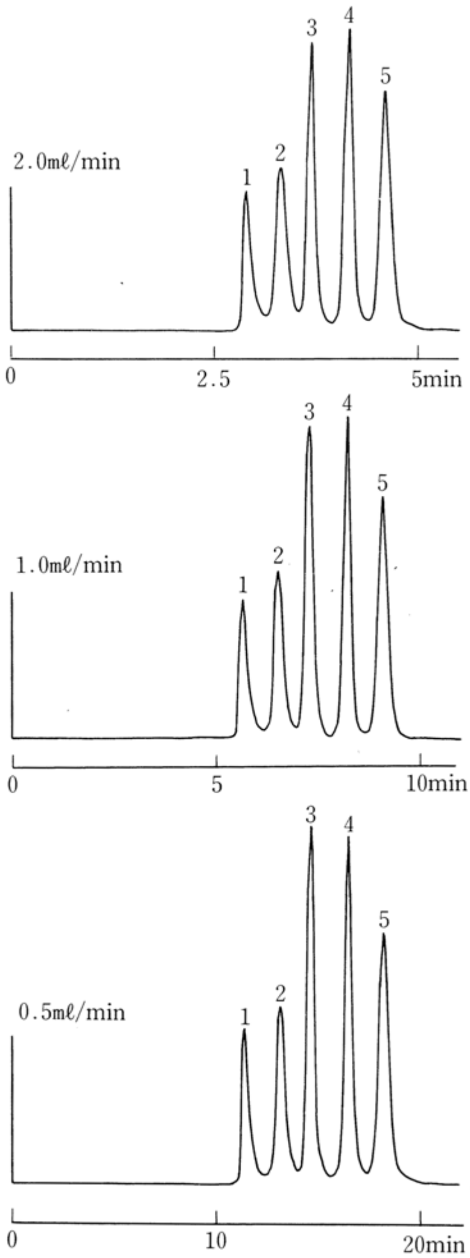

Figure 13 **Flow rate dependence for standard polystyrenes on a TSKgel GMH$_{HR}$-H column**

Columns: TSKgel GMH$_{HR}$-H, 7.8mm ID x 30cm

Solvent: THF

Flow rate: 0.5, 1.0, and 2.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Samples: standard polystyrenes
1. 2,890,000 Da 2. 422,000 Da
3. 107,000 Da 4. 16,700 Da
5. 2,800 Da

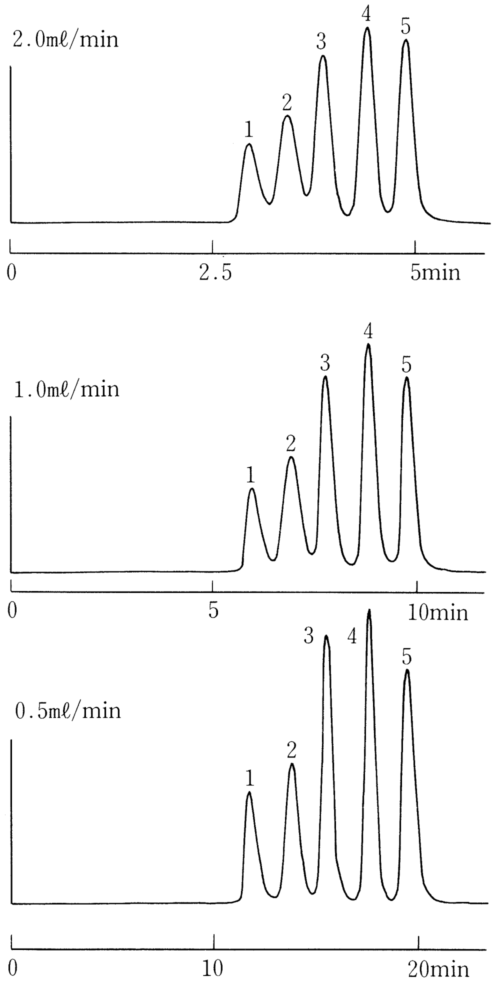

Figure 14 **Flow rate dependence for standard polystyrenes on a TSKgel GMH$_{XL}$ column**

Columns: TSKgel GMH$_{XL}$, 7.8mm ID x 30cm

Solvent: THF

Flow rate: 0.5, 1.0, and 2.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Samples: standard polystyrenes
1. 2,890,000 Da 2. 422,000 Da
3. 107,000 Da 4. 16,700 Da
5. 2,800 Da

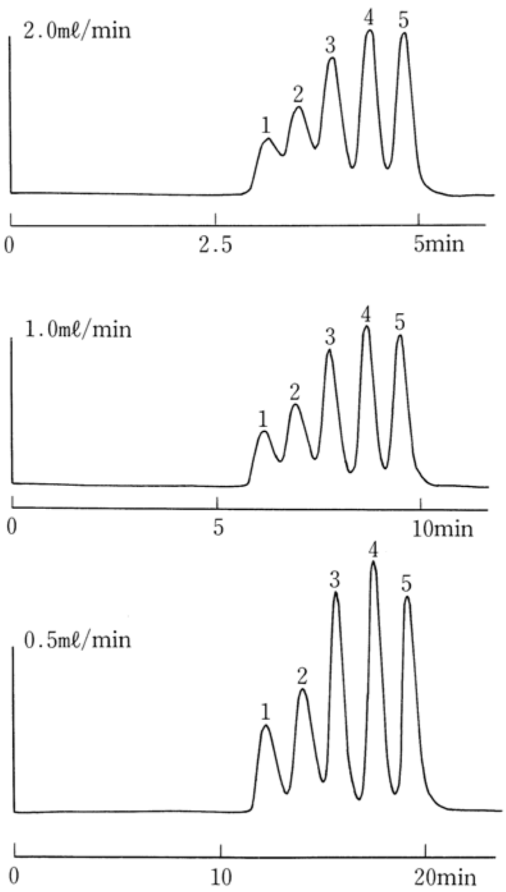

Figure 15 Flow rate dependence of separation of standard polystyrenes using a TSKgel GMH_{HR}-H(S) column

Columns: TSKgel GMH_{HR}-H(S), 7.8mm ID x 30cm

Solvent: THF

Flow rate: 0.5, 1.0, and 2.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Samples: standard polystyrene
1. 2,890,000 Da 2. 422,000 Da
3. 107,000 Da 4. 16,700 Da
5. 2,800 Da

3-3. Solvent compatibility

Table 3 compares the swelling and shrinkage properties of the packing materials for TSKgel $G2000H_{HR}$ and $G2000H_{XL}$ columns in various organic solvents. The packing materials in the TSKgel H_{HR} columns experience much less swelling and shrinkage in each of the solvents than those in the TSKgel H_{XL} columns. This logically suggests that with these columns it would be possible to replace THF, which is in the column at the time of shipment, with a variety of other solvents.

Table 3 Comparison of swelling and shrinkage properties of TSKgel $G2000H_{HR}$ and $G2000H_{XL}$ columns

Solvent	Swelling/shrinkage*	
	TSKgel $G2000H_{HR}$	TSKgel $G2000H_{XL}$
Toluene	1.01	1.06
Benzene	1.00	——
THF	1.00	1.00
DMF	0.99	0.86
Acetone	0.99	0.86
Methanol (MeOH)	0.98	0.67
THF/water = 1/1	0.98	——
MeOH/water = 1/1	0.93	——
Water	0.86	0.52

* Swelling or shrinkage with each solvent calculated based on 1.00 as the index for the swelling volume that occurs with THF.

Tables 4 and 5 depict the changes in the number of theoretical plates when the solvent in the column is directly converted from THF to other organic solvents. The following procedure for switching between solvents was followed: (1) the solvent was directly converted from THF to an organic solvent, then (2) after leaving this solvent in the column for 1 week it was converted back to THF, and then (3) altered again to another solvent. In other words, solvent conversion was performed continuously as the solvent was converted from THF to a different organic solvent and back again, while examining changes in the properties of the packing material under these conditions.

Table 4 shows that for the TSKgel $G2000H_{HR}$ column, despite converting to 8 different organic solvents, the number of theoretical plates for benzene changed very little and column integrity remained intact even when repeatedly changing solvents.

Table 5 compares solvent compatibility for TSKgel G1000H and G2500H columns in the TSKgel H_{HR} and H_{XL} series. In the TSKgel H_{XL} columns, the solvent could be converted to chloroform or DMF, but when it was converted to HFIP a void developed on the column inlet side due to shrinkage of the the packing material.

On the other hand, in the TSKgel H_{HR} columns, the solvent could be directly converted from THF to another solvent, even to HFIP, and could also withstand the continuous susbstitutions of solvents thereafter up through ethanol. However, with the TSKgel G2500H_{HR} column, after the solvent was converted to ethanol, the number of theoretical plates for benzene dropped noticeably below 16,000, the guaranteed number of plates. Thus, TSKgel H_{HR} columns can be converted from a good solvent (THF, chloroform, etc.) to a poor solvent (DMF, HFIP, etc.), which is not possible with the conventional TSKgel H_{XL} columns. Moreover, solvent exchange can also be performed at the same flow rate as analysis (0.5 to 1.0mL/min). Furthermore, conversion from a poor solvent to another poor solvent (for example, from DMSO to DMF, or from acetone to methanol, etc.) is also possible. When switching to a high viscosity solvent it is recommended to reduce the flow rate to 0.3mL/min or less.

Because TSKgel H_{HR} columns can be converted to accommodate various organic solvents, these columns are best suited for use when analyzing unknown samples, which positions them as the preferred tool in your GPC column toolbox.

Table 4 Solvent compatibility of TSKgel G2000H_{HR} column

Solvent	Column No. 1	Column No. 2
	Number of theoretical plates (TP/30cm)	
THF	22,200	20,900
Chloroform		
THF	21,400	20,300
DMF		
THF	21,800	23,400
DMSO		
THF	24,100	21,200
Dioxane		
THF	22,300	20,700
ODCB		
THF	22,300	21,100
Acetone		
THF	22,400	19,800
HFIP		
THF	22,000	19,300
Quinoline		
THF	21,700	19,900

Solvent conversion conditions:

Flow rate during conversion to test solvent: 1.0mL/min
Temperature during conversion to test solvent: 25ºC
Duration of conversion from THF to test solvent: 16 hours
Time test solvent remained in column: 1 week
Flow rate during conversion from test solvent to THF: 1.0mL/min
Duration of conversion from test solvent to THF: 1 hour

Conditions for measuring number of theoretical plates:

Solvent: THF
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: benzene, 20µL

Table 5 Comparison of solvent compatibility of TSKgel H_{HR} and H_{XL} columns

Solvent	TSKgel H_{HR}		TSKgel H_{XL}	
	G1000	G2500	G1000	G2000
	Number of theoretical plates (TP/30cm)			
THF	18,700	20,700	20,300	20,550
Chloroform				
THF	19,000	17,800	20,400	20,800
DMF				
THF	18,700	18,200	17,200	21,300
HFIP			Void*	Void*
THF	18,200	16,600		
Methyl cellosolve			Void*	Void*
THF	18,500	17,400		
DMSO				Void*
THF	16,900	18,200		
Carbon tetrachloride				
THF	19,500	17,200		
Methanol				Void*
THF	17,200	15,400		

*: Void occurred on the column inlet side.

Solvent conversion conditions:

Flow rate during conversion to test solvent: 1.0mL/min
Temperature during conversion to test solvent: 25ºC
Duration of conversion from THF to test solvent: 24 hours
Time test solvent remained in column: 1 week
Flow rate during conversion from test solvent to THF: 1.0mL/min
Duration of conversion from test solvent to THF: 1 hour

Conditions for measuring number of theoretical plates:

Solvent: THF
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@254nm
Sample: benzene, 20µL

3-4. Mech anical strength

Figure 16 shows changes in column efficiency when a sample was passed through the TSKgel G2000H_{HR}, G2500H_{HR}, G2000H_{XL} and G2500H_{XL} columns at a flow rate of 2.5mL/min. In the TSK-GEK H_{XL} columns, at a high flow rate the number of theoretical plates decreases during the lifetime of the column. However, for the TSKgel H_{HR} columns there is no change in the number of theoretical plates even after 400 hours, and it is clear that the TSKgel H_{HR} columns not only have the ability to withstand solvent conversion, but also have excellent mechanical strength. Conditions for measuring number of theoretical plates are listed in Table 4.

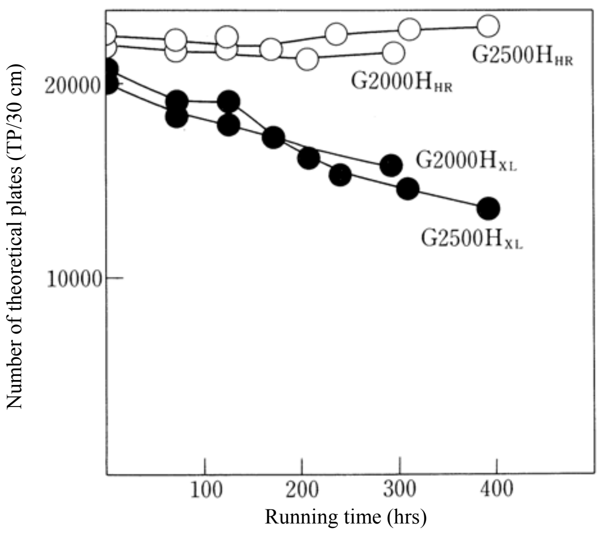

Figure 16 Change in efficiency of TSKgel H_{HR} and H_{XL} columns as a function of running time

Columns: TSKgel G2000H_{HR}, TSKgel G2500H_{HR}, TSKgel G2000H_{XL} and TSKgel G2500H_{XL}, all 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Sample: benzene

3-5. Separation of ultra high molecular weight polymers

Reducing the particle size of the packing material is a requirement to achieve more efficient separations and/or shorter analysis times. However, to accurately measure the average molecular weight and mean molecular distribution of ultra high molecular weight polymers, it is vital to be aware of the following issues:

(1) Sample load (concentration, injection volume)
(2) Overloading
(3) Shear degradation
(4) Column temperature

3-5-1 Samp le load

When injecting a sample on a column, it is important to limit injection volume (and sample mass) to prevent broadening of the peak and subsequent loss of resolution. This is true for all modes of chromatography, but is especially important in GPC as a change in the peak volume will also result in a shift of the peak maximum and thus a change in MW as estimated from the calibration curve. The maximum sample volume (before peak distortion takes place) depends on the type and molecular weight of the sample, the mobile phase, and the particle size of the packing material. The maximum sample volume decreases with (1) increasing molecular weight of the polymer and with (2) decreasing particle size of the packing material. Moreover, in the case of polymer samples, one can inject a higher volume by reducing the sample concentration as much as possible (0.02% or below)[6,7].

Figure 17 shows the relationship between sample injection volume and resolution (separation performance) for standard polystyrenes (2,890,000 Da and 422,000 Da) using TSKgel GMH$_{HR}$-H, GMH$_{XL}$, and GMH$_{HR}$-H(S) columns. The smaller the particle size, the smaller the sample injection volume at which volume overloading takes place. The maximum sample volume that does not cause a loss of resolution is about 10μL with the TSKgel GMH$_{HR}$-H column, about 20μL with the TSKgel GMH$_{XL}$ column, and around 100μL for the TSKgel GMH$_{HR}$-H(S) column. In addition, with the TSKgel GMH$_{HR}$-H column, assuming that no mass overloading occurs, the loss of resolution is minimal even when injecting a 50μL sample. It is also clear that the large particle size TSKgel GMH$_{HR}$-H(S) column is the preferred option when an injection volume larger than 50μL is required.

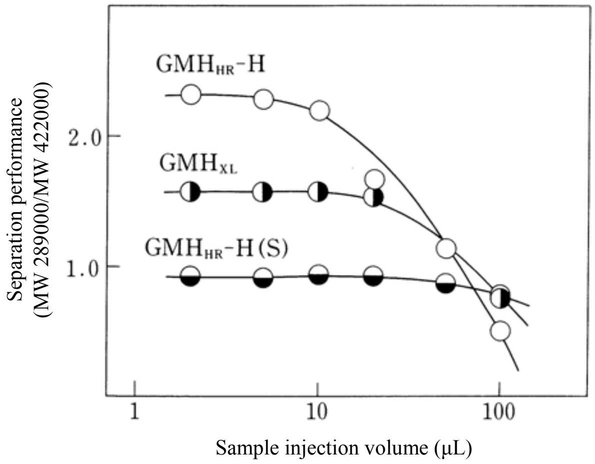

Figure 17 **Relationship between resolution and sample injection volume in TSKgel GMH columns**

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{XL}, TSKgel GMH_{HR}-H(S), each 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Samples: standard polystyrene (2,890,000 Da, 422,000 Da)

As mentioned above, volume overload takes place when the injection volume is increased when separating polymer samples. Figures 18 to 20 demonstrate how sample injection volume affects packing materials of different particle sizes. As illustrated in Figure 18, overloading of the TSKgel GMH_{HR}-H column takes place when a 2,890,000 Da compound is analyzed at an injection volume of 20µL and higher. Using the same column, when a 422,000 Da sample is analyzed at an injection volume of 100µL, peaks broaden substantially and compounds elute later than their normal positions. Figure 19 shows similar results for the TSKgel GMH_{XL} column when a 100µL sample is injected.

On the other hand, on the TSKgel GMH_{HR}-H(S) column, which has a large particle size (13µm), overloading is not noticeable, and as discussed above, the TSKgel GMH_{HR}-H(S) column is preferred when there is a need to increase the injection volume of a polymer sample.

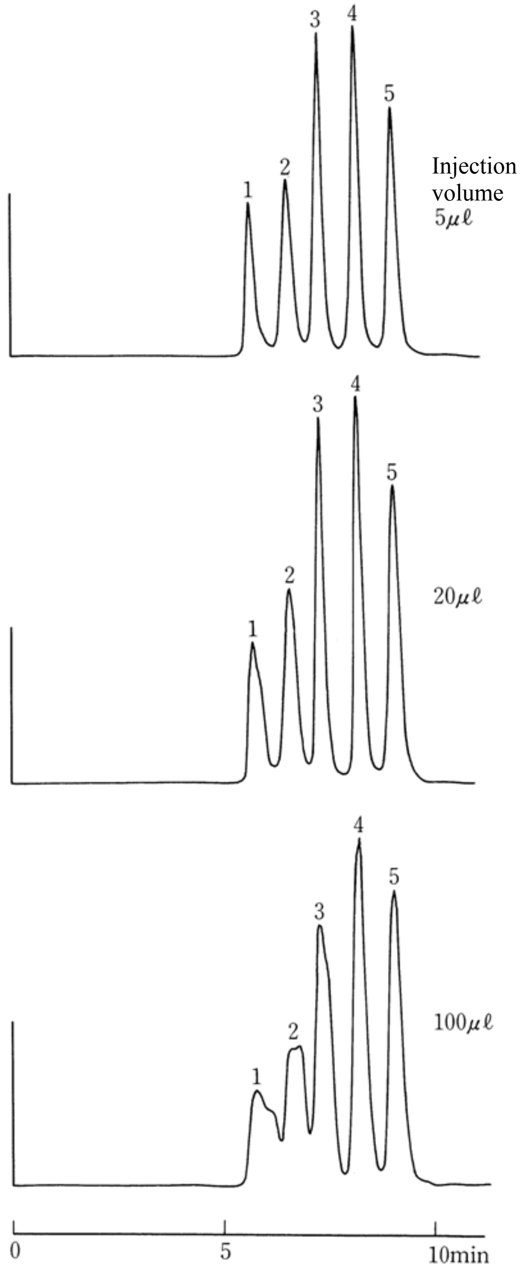

Figure 18 **Effect of sample volume on separation of polystyrenes by a TSKgel GMH_{HR}-H column**

Column: TSKgel GMH_{HR}-H, 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Samples: standard polystyrenes (5µL, 20µL, 100µL)
1. 2,890,000 Da (0.5mg/mL)
2. 422,000 Da (0.7mg/mL)
3. 107,000 Da (1.0mg/mL)
4. 16,700 Da (1.0mg/mL)
5. 2,800 Da (1.0mg/mL)

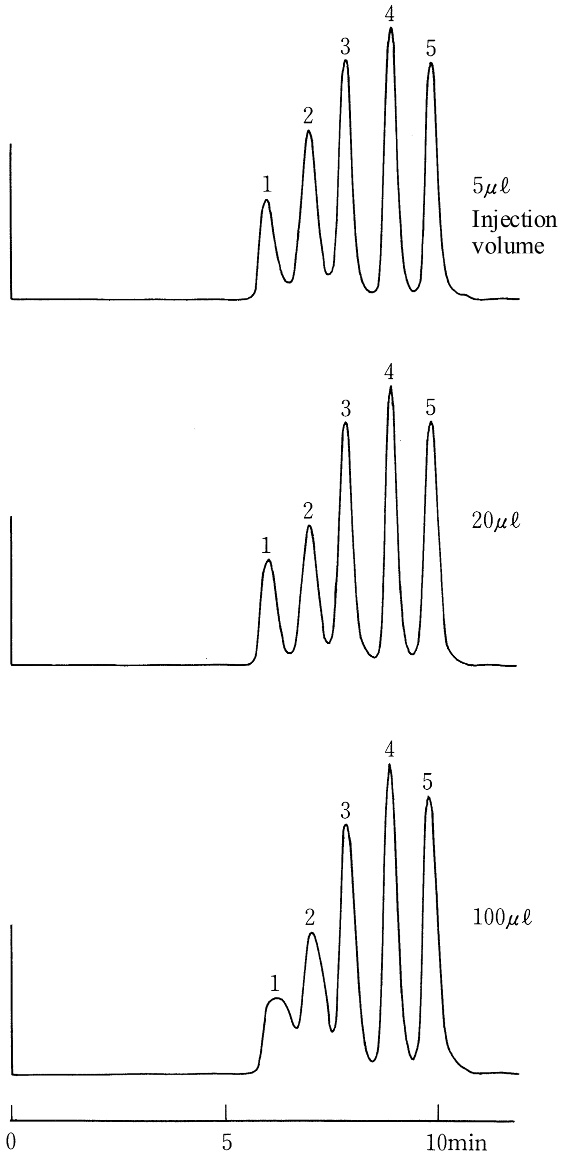

Figure 19 **Effect of sample volume on separation of polystyrenes by a TSKgel GMH_{XL} column**

Column: TSKgel GMH_{XL}, 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25°C

Detection: UV@254nm

Samples: standard polystyrenes (5μL, 20μL, 100μL)
1. 2,890,000 Da (0.5mg/mL)
2. 422,000 Da (0.7mg/mL)
3. 107,000 Da (1.0mg/mL)
4. 16,700 Da (1.0mg/mL)
5. 2,800 Da (1.0mg/mL)

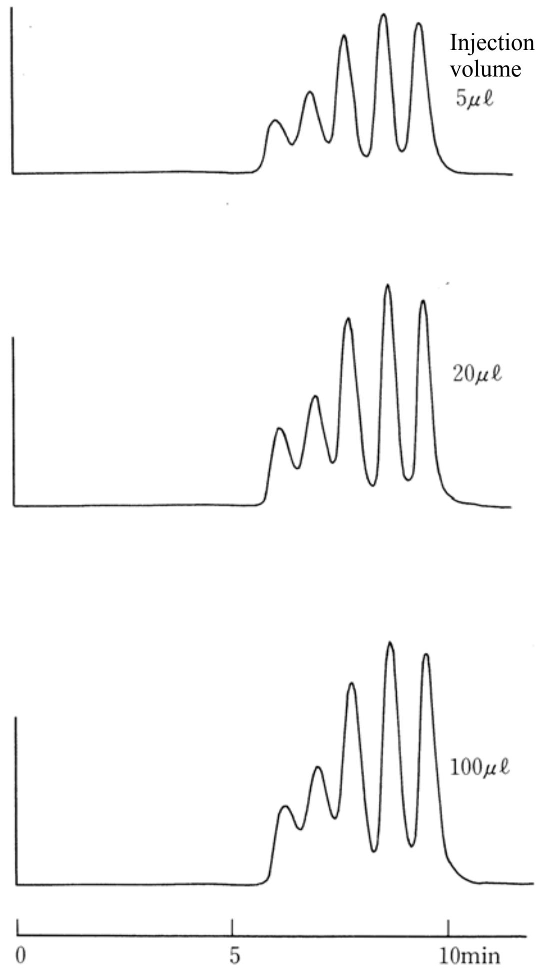

Figure 20 **Effect of sample volume on separation of polystyrenes by a TSKgel GMH_{HR}-H(S) column**

Column: TSKgel GMH_{HR}-H(S), 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25°C

Detection: UV@254nm

Samples: standard polystyrenes (5μL, 20μL, 100μL)
1. 2,890,000 Da (0.5mg/mL)
2. 422,000 Da (0.7mg/mL)
3. 107,000 Da (1.0mg/mL)
4. 16,700 Da (1.0mg/mL)
5. 2,800 Da (1.0mg/mL)

3-5-2. Sh   ear degradation

This phenomenon is observed especially when ultra-high molecular weight compounds are analyzed. Shear degradation tends to occur when analysis is carried out at high flow rates using a microparticle size packing material.

Figure 21 demonstrates the relationship between shear degradation and particle size of the packing material, when TSKgel GMH columns were used. When the flow rate is 1.0mL/min, normal elution of an ultra-high molecular weight sample (20,600,000 Da) is only possible with the TSKgel GMH_{HR}-H(S) column, which has a large particle size. However, with the TSKgel GMH_{XL} and GMH_{HR}-H columns, shear degradation does take place and new peaks appear in the chromatogram on the smaller molecular weight side.

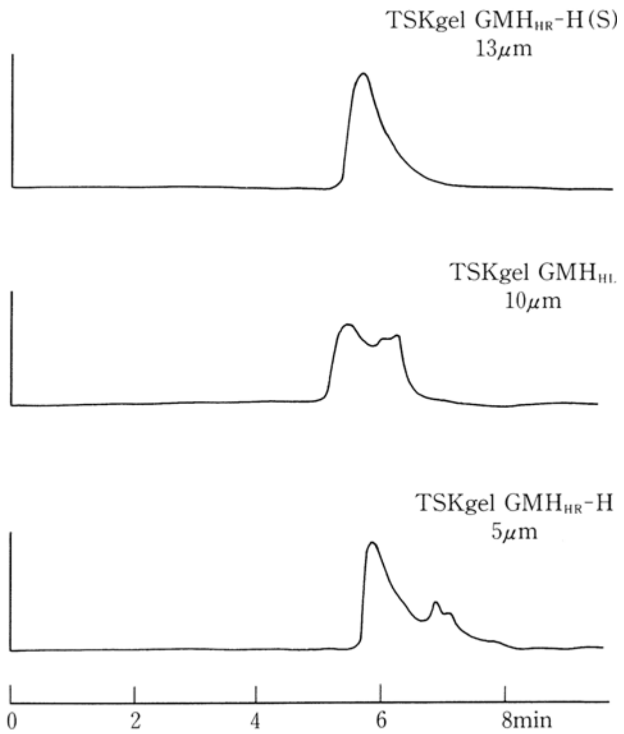

Figure 21	**Dependence of shear degradation of polymer samples on particle size in TSKgel GMH columns**
Columns:	TSKgel GMH_{HR}, TSKgel GMH_{XL}, TSKgel GMH_{HR}-H(S), each 7.8mm ID x 30cm
Solvent:	THF
Flow rate:	1.0mL/min
Temperature:	25ºC
Detection:	UV@254nm
Sample:	standard polystyrene F-2000 (20,600,000 Da), 20μL (0.025%)

Figure 22 shows the effect of shear degradation on flow rate. For the TSKgel GMH_{XL} columns, shear degradation can be suppressed by lowering the flow rate to 0.6mL/min or less. However, with the TSKgel GMH_{HR}-H column, shear degradation cannot be prevented, even at a flow rate of 0.4mL/min. Consequently, when analyzing ultra-high molecular weight samples with TSKgel H_{HR} columns, it is better to use the (S)- type column.

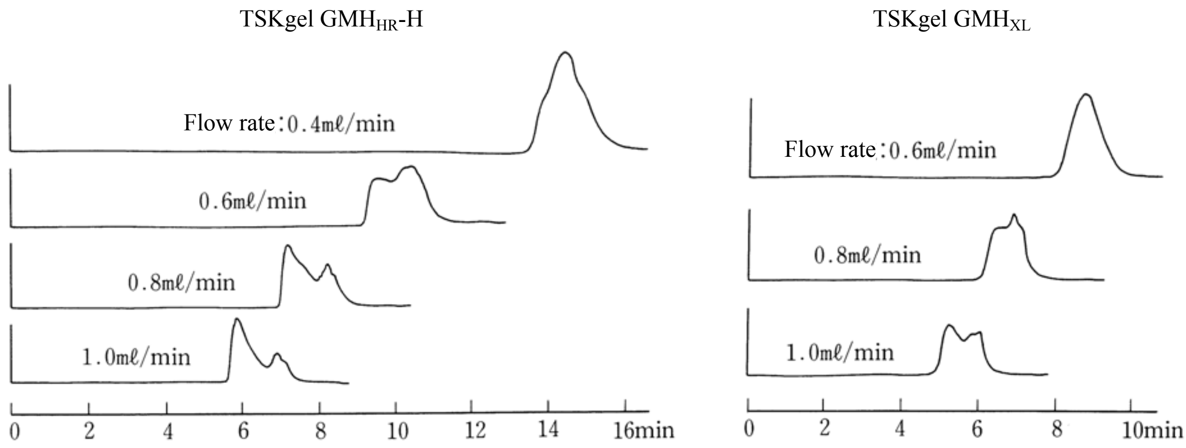

Figure 22	**Flow-rate dependence of shear degradation of samples using TSKgel GMH_{HR}-H and TSKgel GMH_{XL} columns**
Columns:	TSKgel GMH_{HR}-H, TSKgel GMH_{XL}, both 7.8mm ID x 30cm
Solvent:	THF
Flow rate:	0.4 to 1.0mL/min
Temperature:	25ºC
Detection:	UV@254nm
Sample:	standard polystyrene F-2000 (20,600,000 Da), 20μL (0.025%)

3-5-4. Co lumn temperature

In general, as the column temperature is raised, sample elution occurs more rapidly due to changes in the distribution coefficient (Kd).[8] In Figure 23 the temperature dependence of calibration curves of standard polystyrenes produced using a TSKgel GMH_{HR}-H column is shown.

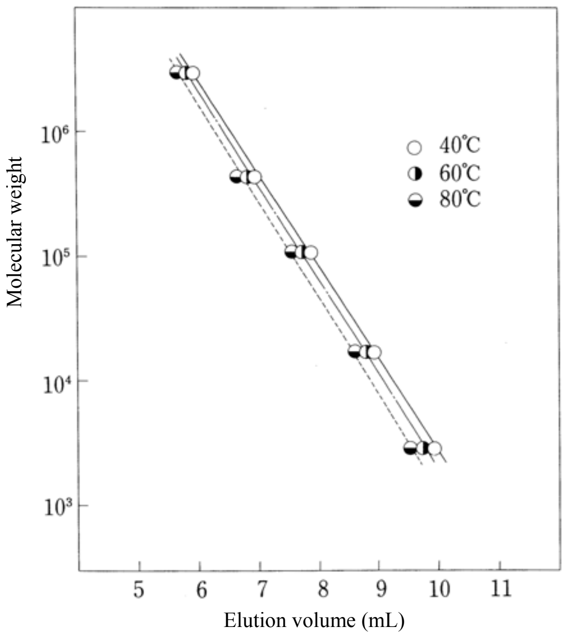

Figure 23 Temperature dependence of the calibration curve for a TSKgel GMH_{HR}-H column

Column:	TSKgel GMH_{HR}-H, 7.8mm ID x 30cm
Solvent:	DMF
Flow rate:	1.0mL/min
Temperature:	40ºC to 80ºC
Detection:	RI
Sample:	standard polystyrene

3-6. Mixed column (linear type)

Three linear type, mixed bed columns are available in the TSKgel H_{HR} series columns. The TSKgel GMH_{HR}-H column exhibits a linear calibration curve in the molecular weight separation range of 10^3 to 10^7, while the TSKgel GMH_{HR}-M column has a linear calibration curve within a separation range of 500 to 10^6 Dalton. The TSKgel GMH_{HR}-L column was designed for analysis of oligomers and low molecular weight samples in the range of 10^4 Dalton or below. This range is optimal for pattern analysis, in which the oligomer region is well separated, while at the same time maintaining separation in the polymer region. Furthermore, the linear TSKgel GMH_{HR}-H(S) and GMH_{HR}-M(S) columns are used for analyzing ultra-high molecular weight substances.

The calibration curves shown in Figure 2 were obtained with THF as the solvent and standard polystyrene as the sample. Figures 4, 6 and 8 show calibration curves obtained with chloroform, HFIP and DMF as solvents.

Figures 24 to 27 compare chromatograms of the separation of a standard polystyrene mixture with TSKgel GMH_{HR} columns.

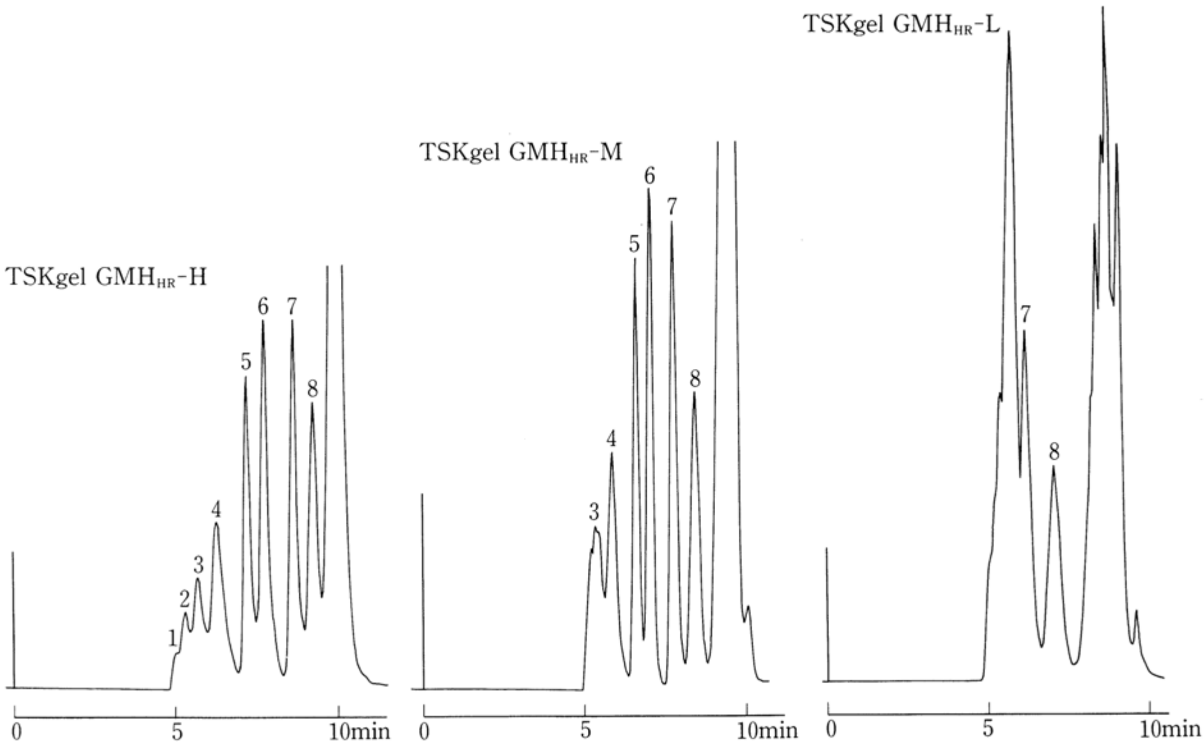

Figure 24 Comparison of separation of standard polystyrene using TSKgel GMH$_{HR}$ columns

Columns:	TSKgel GMH$_{HR}$-H, TSKgel GMH$_{HR}$-M, TSKgel GMH$_{HR}$-L, each 7.8mm ID x 30cm
Solvent:	THF
Flow rate:	1.0mL/min
Temperature:	25ºC
Detection:	UV@254nm
Sample:	standard polystyrene

1. 8,420,000 Da 2. 2,890,000 Da
3. 1,260,000 Da 4. 422,000 Da
5. 107,000 Da 6. 42,800 Da
7. 10,200 Da 8. 2,800 Da

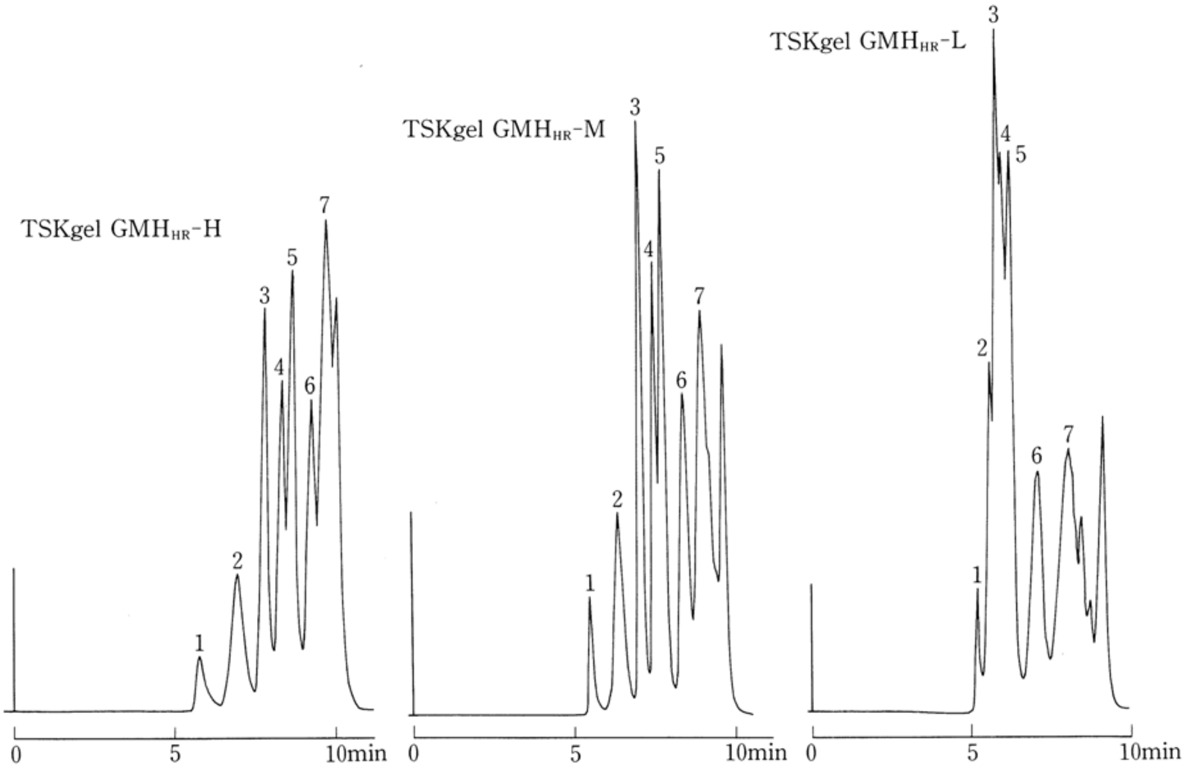

Figure 25 Comparison of separation of standard polystyrene using TSKgel GMH_{HR} columns

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{HR}-M, TSKgel GMH_{HR}-L, each 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Sample: standard polystyrene

1. 1,260,000 Da 2. 186,000
3. 42,800 Da 4. 16,700 Da
5. 10,200 Da 6. 2,800 Da
7. A-1000 Da

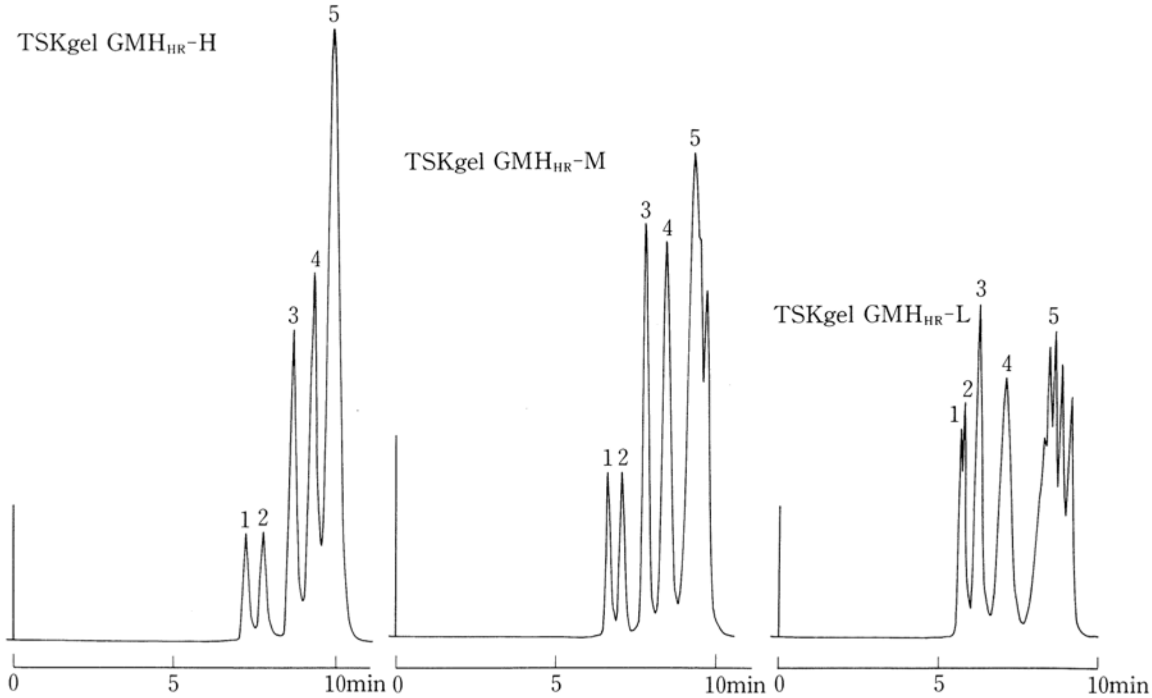

Figure 26 **Comparison of separation of standard polystyrene using TSKgel GMH$_{HR}$ columns**

Columns: TSKgel GMH$_{HR}$-H, TSKgel GMH$_{HR}$-M, TSKgel GMH$_{HR}$-L, each 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Sample: standard polystyrene
1. 107,000 Da 2. 42,800 Da
3. 10,200 Da 4. 2,800 Da
5. A-500 Da

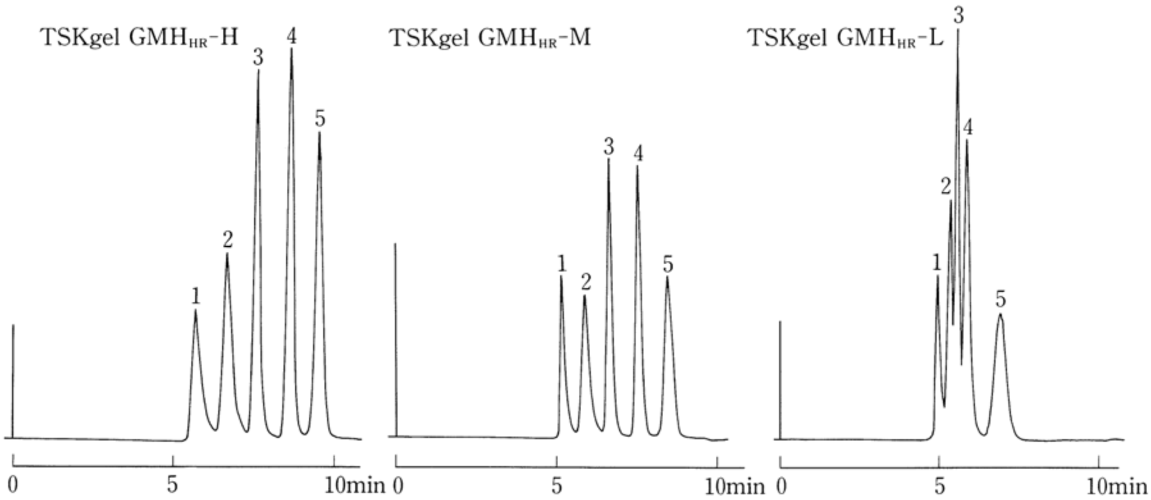

Figure 27 Comparison of separation of standard polystyrene using TSK gel GMH$_{HR}$ columns

Columns:	TSKgel GMH$_{HR}$-H, TSKgel GMH$_{HR}$-M, TSKgel GMH$_{HR}$-L, each 7.8mm ID x 30cm
Solvent:	THF
Flow rate:	1.0mL/min
Temperature:	25ºC
Detection:	UV@254nm
Sample:	standard polystyrene 1. 2,890,000 Da 2. 422,000 Da 3. 107,000 Da 4. 16,700 Da 5. 2,800 Da

Figure 28 compares chromatograms of the separation of an epoxy resin (Epikote 1001).

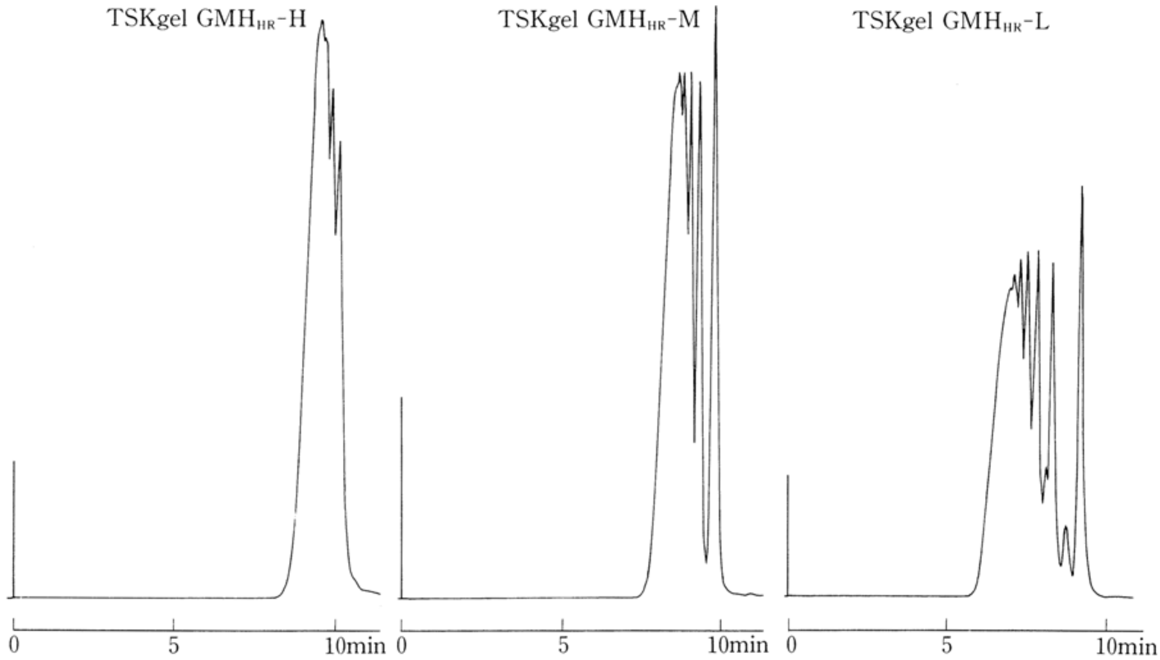

Figure 28 Comparison of separation of an epoxy resin (Epikote 1001) using TSKgel GMH$_{HR}$ columns

Columns: TSKgel GMH$_{HR}$-H, TSKgel GMH$_{HR}$-M, TSKgel GMH$_{HR}$-L, each 7.8mm ID x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Sample: epoxy resin (Epikote 1001)

Figure 29 compares chromatograms of the separation of phenol resin.

As is indicated from these figures, the optimal fractionation range will vary with each grade.

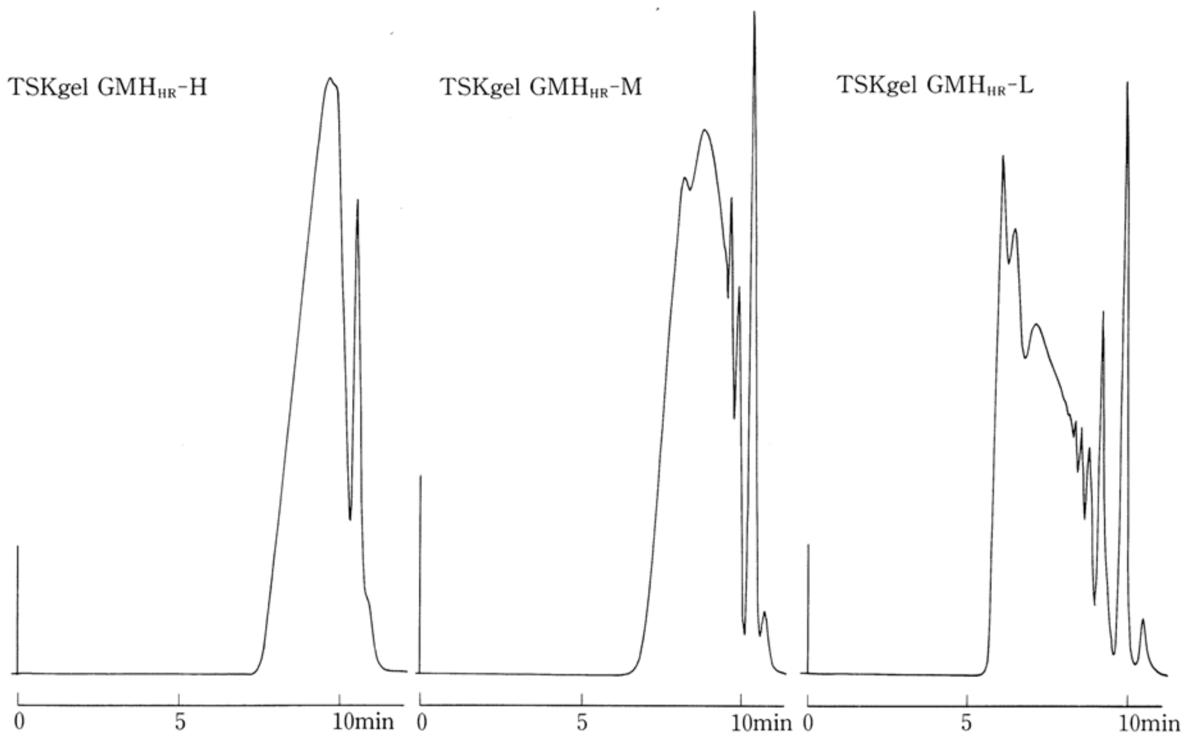

Figure 29 TSK **Comparison of separation of phenol resin using the gel GMH$_{HR}$ columns**

Columns: TSKgel GMH$_{HR}$-H, TSKgel GMH$_{HR}$-M, TSKgel GMH$_{HR}$-L, each 7.8mm x 30cm

Solvent: THF

Flow rate: 1.0mL/min

Temperature: 25ºC

Detection: UV@254nm

Sample: phenol resin

3-7. Effect of adding salt to special solvents

When a DMF mobile phase is used to analyze a sample containing a dissociable ionic group such as a sulfonate group or carboxyl group within the polymer molecule or at the terminus, the elution position of the sample may appear very early. Various explanations have been offered for the cause: expansion of the polymer coil due to electrostatic effects, swelling of the polymer structure due to intramolecular assembly, bipolar interactions between a polar group and DMF, and ionic repulsion between the packing material and a dissociable group in the sample. The addition of a lithium salt (LiBr or LiCl) to the DMF solvent has been proposed as a method for suppressing this phenomenon.[9-11]

A similar phenomenon is also observed with phenol resins,[12] urea-formaldehyde resins,[13] and polymers that have a carboxyl group.[14-17] Moreover, Hann et al.[18] has noted that adsorption can occur with a quaternized polyurethane.

Accordingly, the elution behavior of alkyd resins, phenol resins, and polyetherimide was investigated using the TSKgel H_{HR} columns, in systems prepared by adding a salt (LiBr, sodium trifluoroacetate) to a DMF solvent, a THF/methanol mixed solvent, and a HFIP solvent.

Using the TSKgel G3000H_{HR} column, Figure 30 shows the elution behavior of alkyd resin and phenol resin analyzed using a DMF solvent, as well as the effects of adding LiBr to DMF.

With the DMF solvent, both resins eluted abnormally early from the column due to a static electric interaction. However, by adding LiBr to the DMF solvent, a normal chromatogram is obtained. Normal elution behavior of alkyd resins is possible when the LiBr concentration is about 30mmol/L, and with phenol resins, when the concentration of the salt is around 10mmol/L.

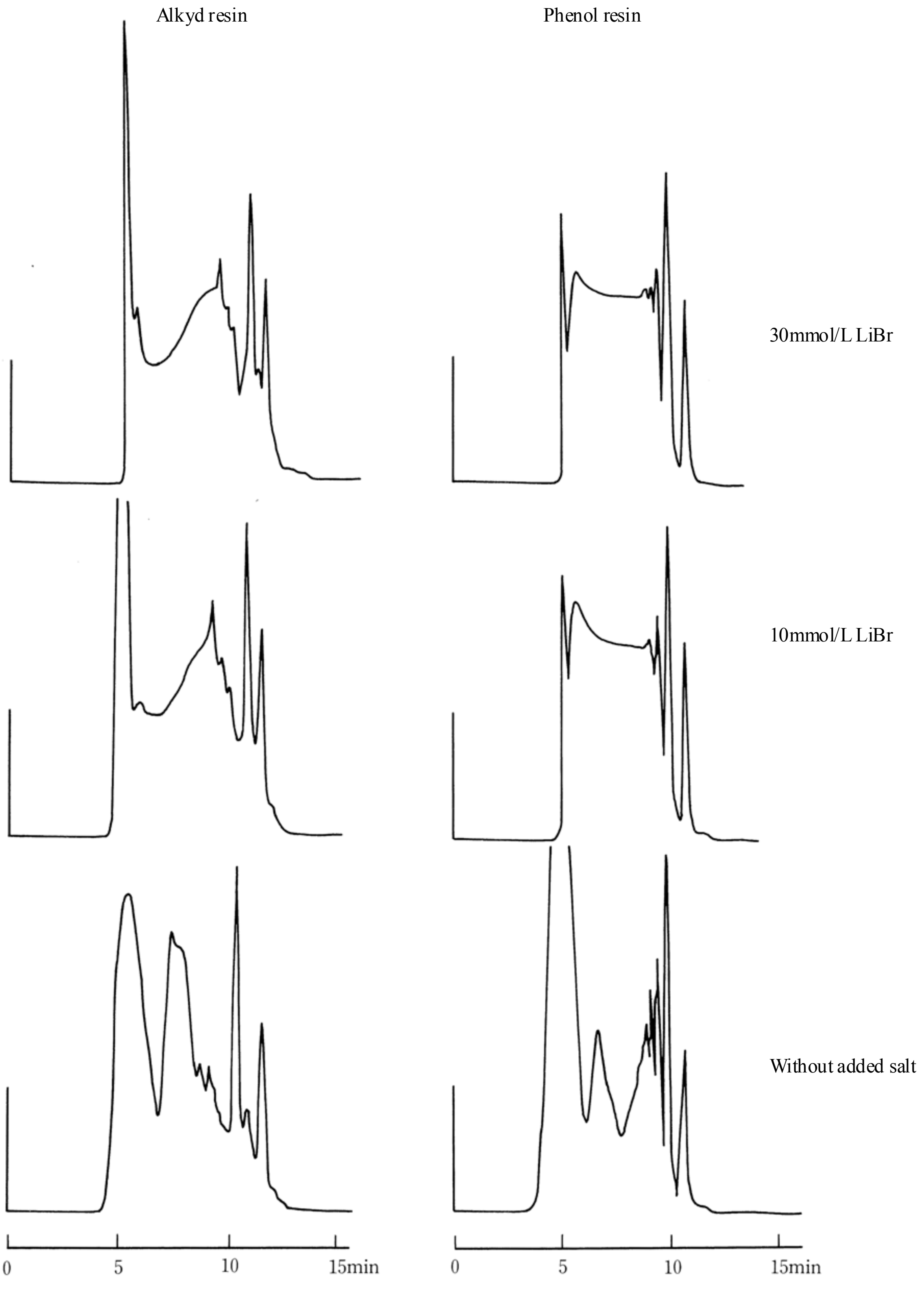

Figure 30 **Separation of alkyd resin and phenol resin on a TSKgel G3000H$_{HR}$ column**

Column:	TSKgel G3000H$_{HR}$, 7.8mm ID x 30cm
Solvent:	DMF (containing LiBr)
Flow rate:	1.0mL/min
Temperature:	25ºC
Detection:	UV@254nm
Samples:	alkyd resin, phenol resin

Using the TSkgel G3000H_{HR} column, Figure 31 illustrates the elution behavior of alkyd resin and phenol resin in a THF/methanol (60/40) solvent, as well as the results after adding 20mmol/L LiBr. With the THF/methanol = 6/4 mixed solvent, as in the DMF solvent, both resins eluted abnormally early due to a static electric interaction, but after adding 20mmol/L LiBr to the THF/methanol = 6/4 mixed solvent, a normal chromatogram was obtained.

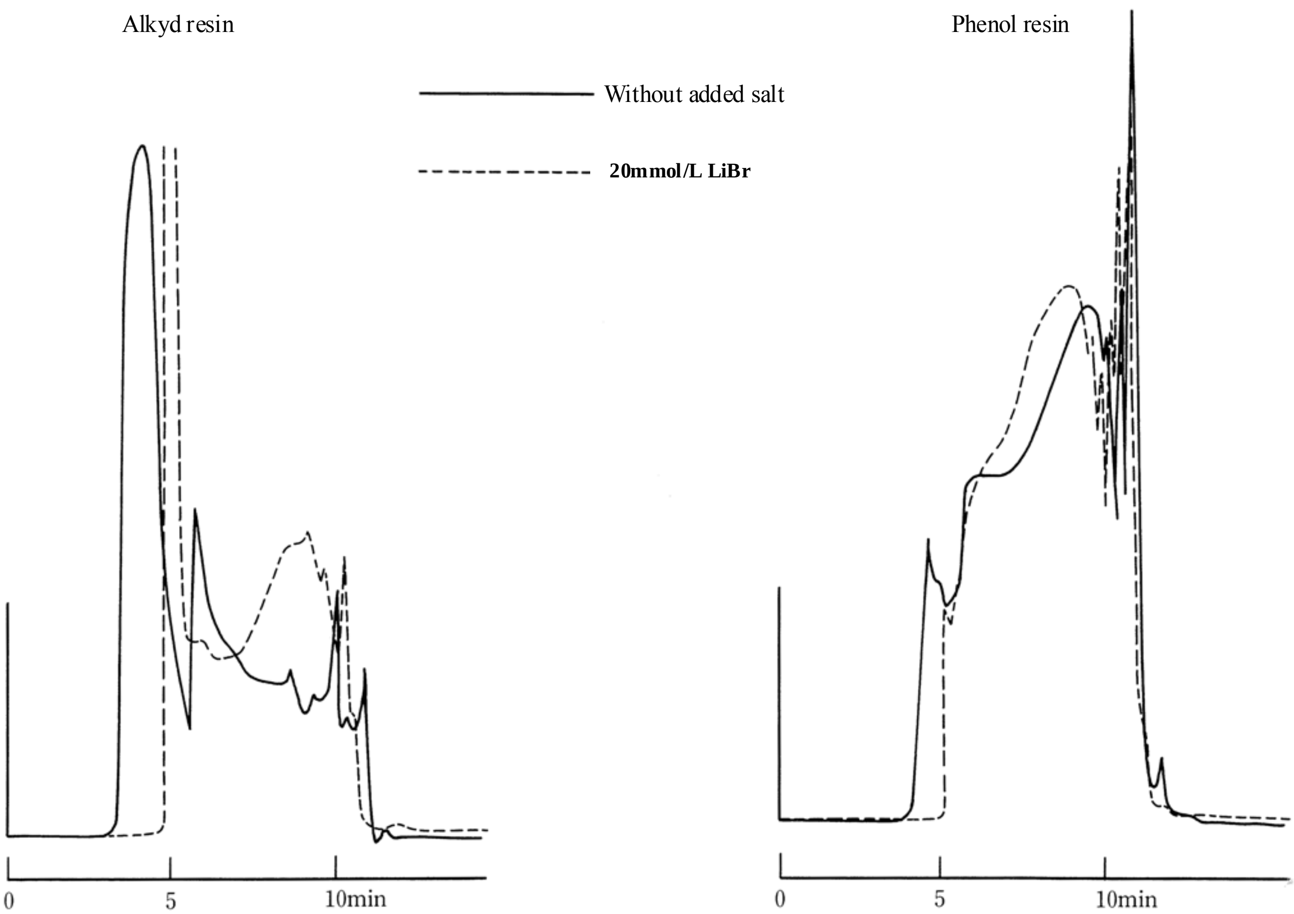

Figure 31 Separation of alkyd resin and phenol resin on a TSKgel G3000H_{HR} column

Column: TSKgel G3000H_{HR}, 7.8mm ID x 30cm
Solvent: THF/methanol = 6/4 (containing LiBr)
Flow rate: 1.0mL/min
Temperature: 25 ºC
Detection: UV (254nm)
Sample: alkyd resin and phenol resin

Using the TSKgel G4000H_{HR} and G5000H_{HR} columns, Figure 32 shows the elution behavior of polyetherimide in HFIP solvent, as well as the effect of adding 10mmol/L sodium trifluoroacetate. The resulting chromatograms are different from those obtained using DMF and THF/methanol mobile phases as discussed earlier in Figures 30 and 31. Without the added salt, adsorption was observed, while normal GPC behavior was established after adding 10mmol/L sodium trifluoroacetate to the HFIP solvent.

Thus as described above, elution behavior of samples can become abnormal when conducting analyses using special solvent systems, but in some cases a normal chromatogram can be produced by including a salt to the solvent.

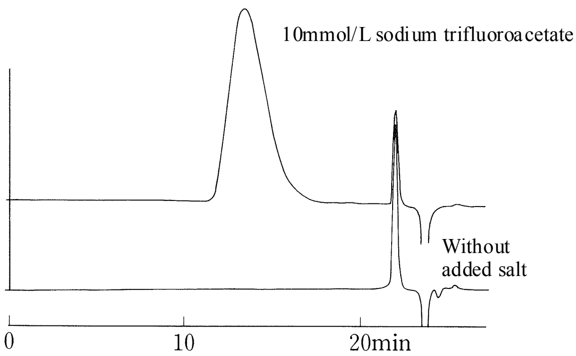

Figure 32 Separation of polyetherimide by TSKgel H_{HR} columns

Columns: TSKgel G5000H_{HR} + TSKgel G4000H_{HR}, 7.8mm ID x 30cm x 2
Solvent: HFIP (containing sodium trifluoroacetate)
Flow rate: 1.0mL/min
Temperature: 40ºC
Detection: RI
Sample: polyetherimide

4. Applications

Figure 33 compares chromatograms produced using multiple columns coupled in series.

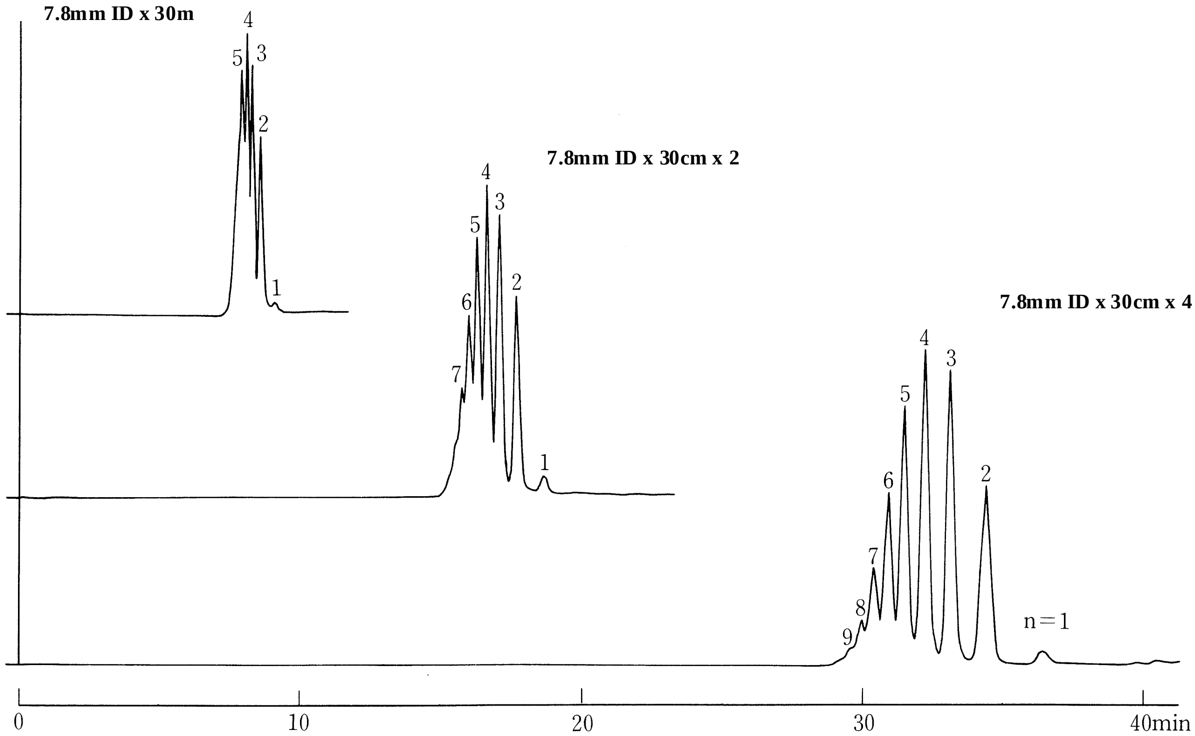

Figure 33 Separation of standard polyethylene A-500 using TSKgel G2500H_{HR} columns

Columns:	TSKgel G2500H_{HR}, 7.8mm ID x 30 cm (x 1, x 2, x 4)
Solvent:	THF
Flow rate:	1.0mL/min
Temperature:	25ºC
Detection:	UV@254nm
Sample:	standard polyethylene A-500

Figures 34 to 43 show analyses of various resins and polymers using a variety of organic solvents.

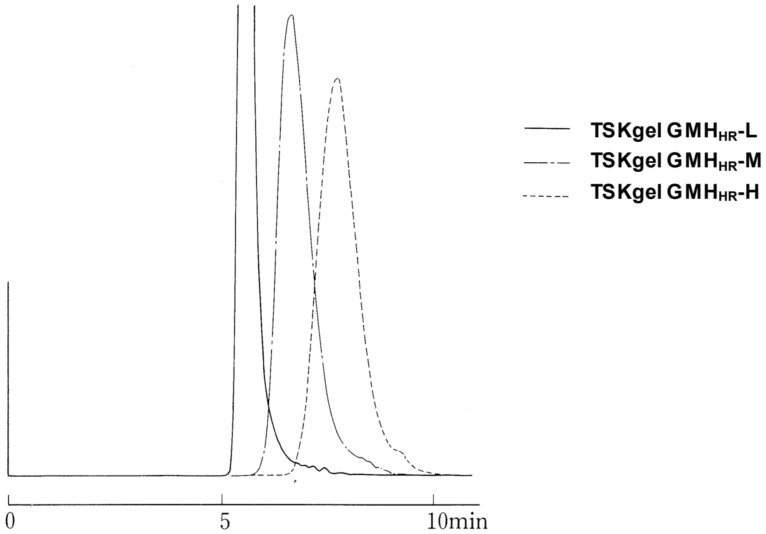

Figure 34 Separation of polycarbonate using the TSKgel GMH_{HR} columns

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{HR}-M, TSKgel GMH_{HR}-L, each 7.8mm ID x 30cm

Solvent: chloroform
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@254nm

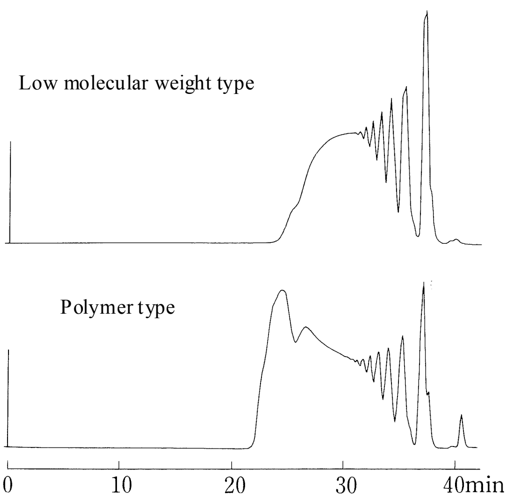

Figure 35 Separation of phenol resin (novolak type) by TSKgel H_{HR} columns

Columns: TSKgel G4000H_{HR} + TSKgel G3000H_{HR} + TSKgel G2000H_{HR} x 2, 7.8mm ID x 30cm x 4

Solvent: 10mmol/L LiBr in DMF
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@270nm
Samples: low molecular weight type and polymer type phenol resins (novolak type)

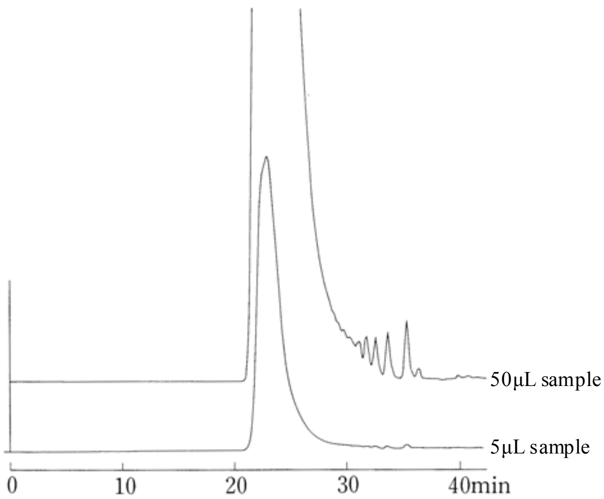

Figure 36 Separation of polyethersulfone by TSKgel H_{HR} columns

Columns: TSKgel G4000H_{HR} + TSKgel G3000H_{HR} + TSKgel G2000H_{HR} x 2, 7.8mm ID x 30cm x 4

Solvent: 10mmol/L LiBr in DMF
Flow rate: 1.0mL/min
Temperature: 25ºC
Detection: UV@270nm
Sample: polyethersulfone

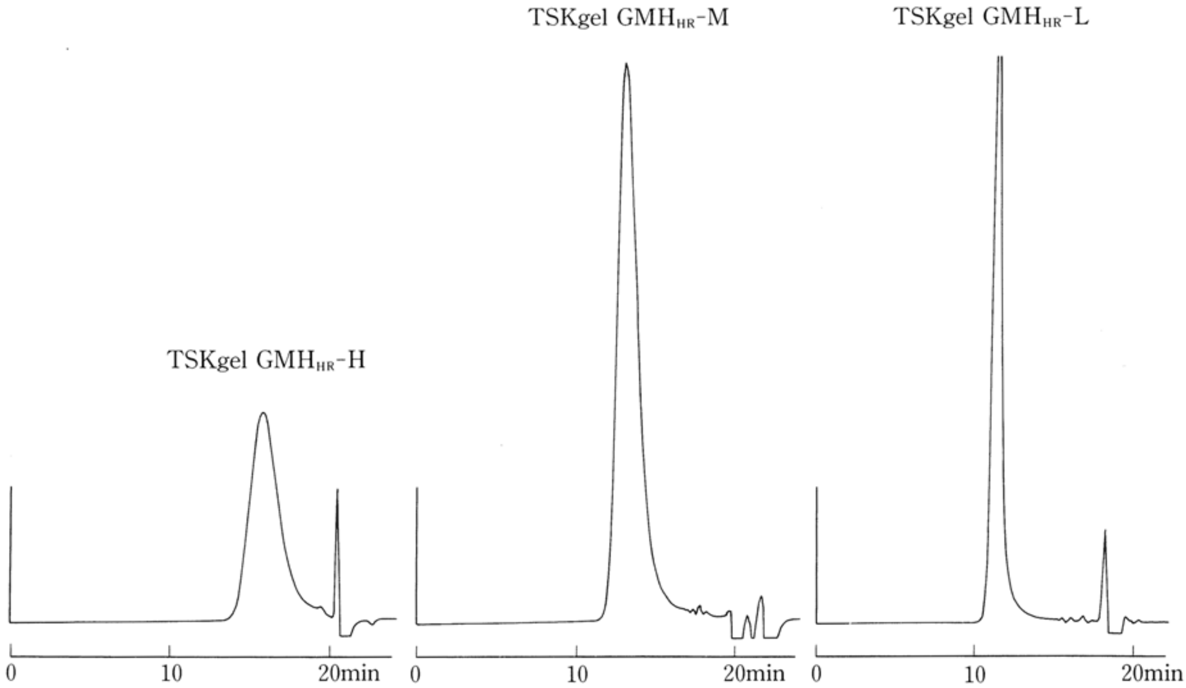

Figure 37 **Separation of polyethersulfone using TSKgel GMH_{HR} columns**

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{HR}-M, TSKgel GMH_{HR}-L, each 7.8mm ID x 30cm x 2

Solvent: 10mmol/L LiBr in DMF
Flow rate: 1.0mL/min
Temperature: 80ºC
Detection: RI

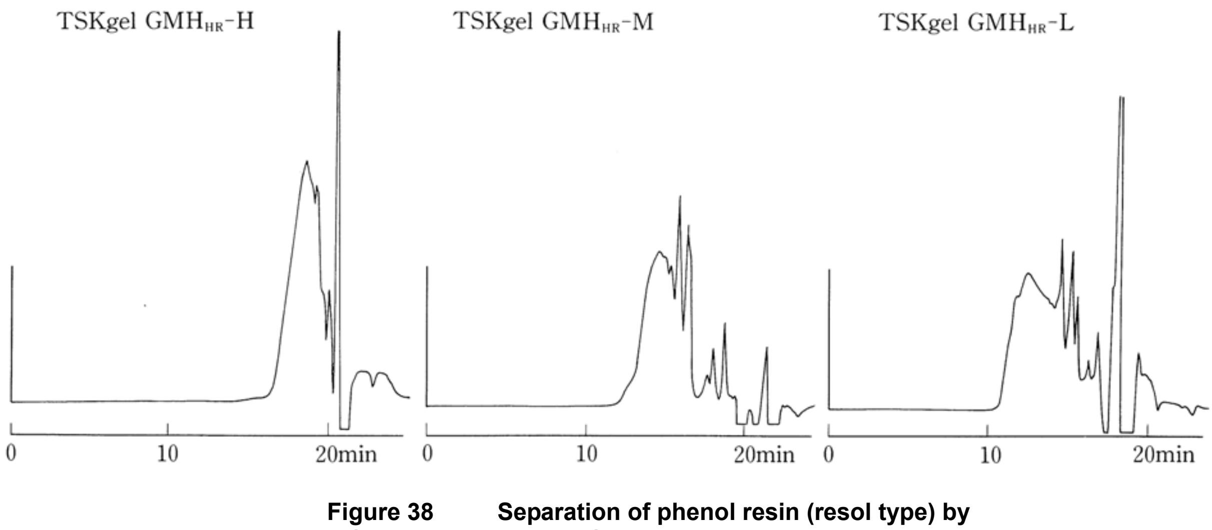

Figure 38 TSK **Separation of phenol resin (resol type) by gel GMH_{HR} columns**

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{HR}-M, TSKgel GMH_{HR}-L, each 7.8mm ID x 30cm x 2

Solvent: 10mmol/L LiBr in DMF
Flow rate: 1.0mL/min
Temperature: 80ºC
Detection: RI

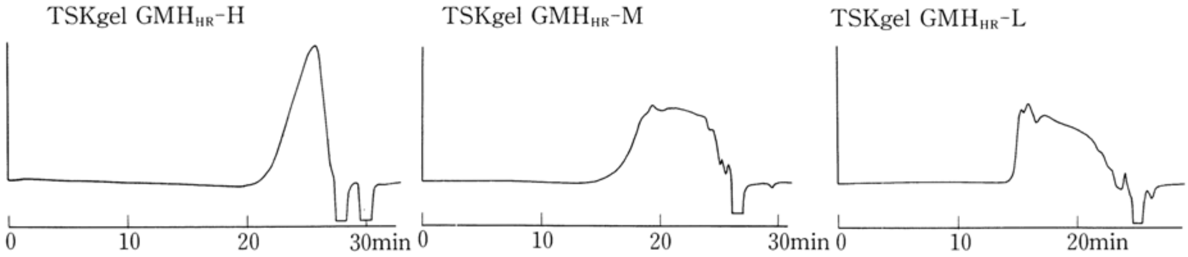

Figure 39 **Separation of melamine resin by TSKgel GMH_{HR} columns**

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{HR}-M, TSKgel GMH_{HR}-L, each 7.8mm ID x 30cm x 2

Solvent: 10mmol/L LiBr in DMSO

Flow rate: 0.75mL/min

Temperature: 80 ºC

Detection: RI

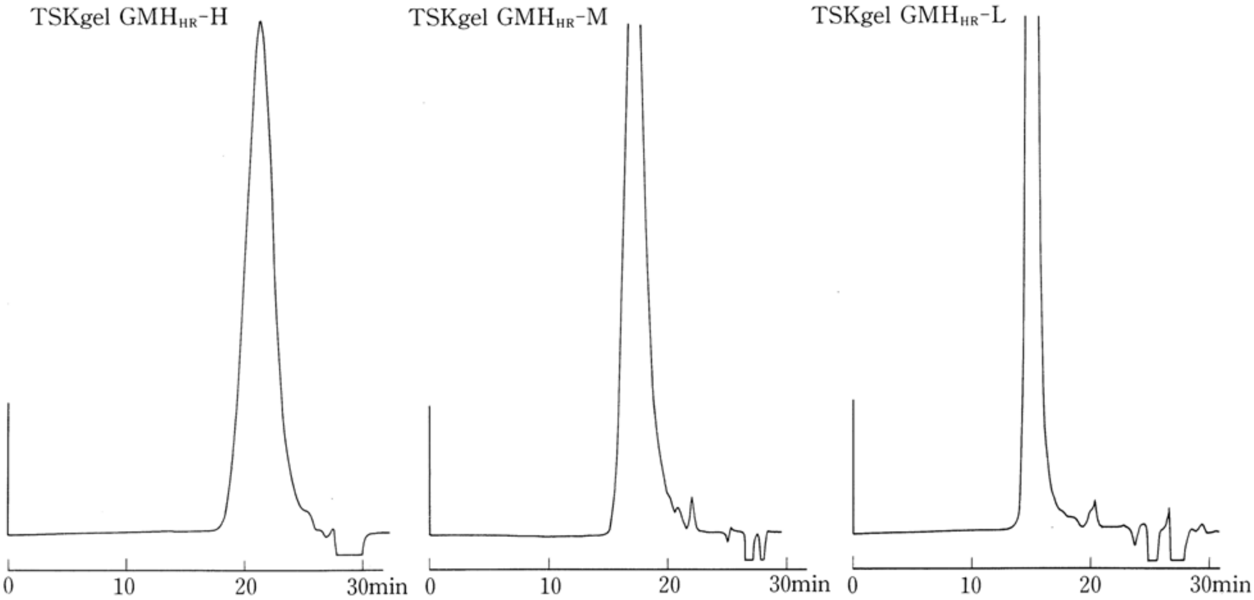

Figure 40 **Separation of polyetherimide by TSKgel GMH_{HR} columns**

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{HR}-M, TSKgel GMH_{HR}-L, each 7.8mm ID x 30cm

Solvent: 10mmol/L LiBr in N-methylpyrrolidone

Flow rate: 0.75mL/min

Temperature: 80ºC

Detection: RI

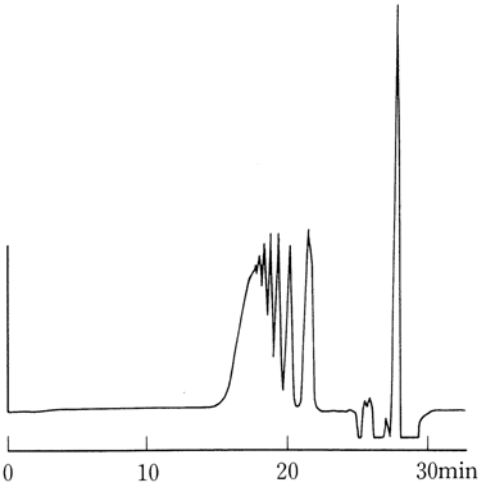

Figure 41 **Separation of Dextran T-40 hydrolysate by TSKgel H_{HR} columns**

Columns: TSKgel G3000H_{HR} + TSKgel G2500H_{HR}, 7.8mm ID x 30cm x 2

Solvent: 10mmol/L LiBr in N-methylpyrrolidone

Flow rate: 0.75mL/min

Temperature: 80ºC

Detection: RI

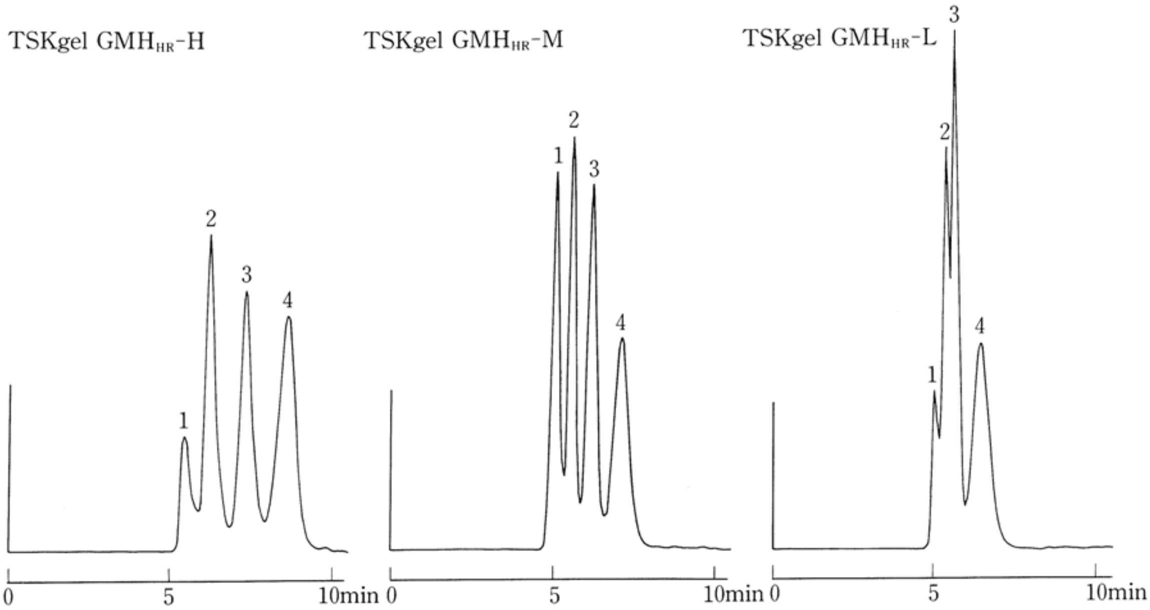

Figure 42 **Separation of polymethylmethacrylate mixture by TSK gel GMH_{HR} columns**

Columns: TSKgel GMH_{HR}-H, TSKgel GMH_{HR}-M, TSKgel GMH_{HR}-L, each 7.8mm ID x 30cm

Solvent: 5mmol/L sodium trifluoroacetate in HFIP

Flow rate: 1.0mL/min

Temperature: 40ºC

Detection: UV@220nm

Samples: polymethylmethacrylate

1. 820,000 Da 2. 67,000 Da
3. 10,200 Da 4. 1,950 Da

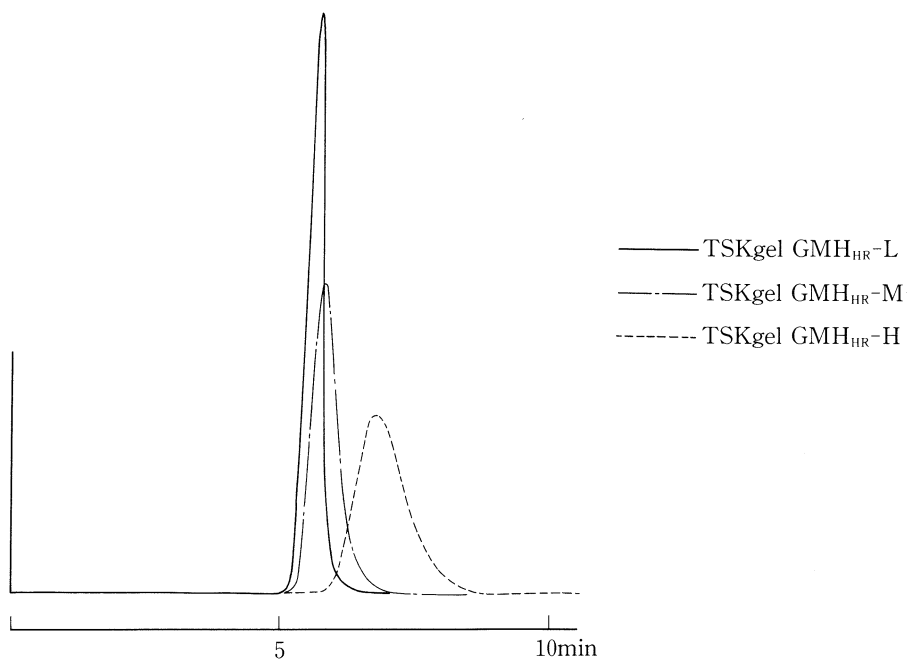

Figure 43 Separation of Nylon 66 on TSKgel GMH$_{HR}$ columns

Columns: TSKgel GMH$_{HR}$-H, TSKgel GMH$_{HR}$-M, TSKgel GMH$_{HR}$-L, each 7.8mm ID x 30cm

Solvent: 5mmol/L sodium trifluoroacetate in HFIP

Flow rate: 1.0mL/min

Temperature: 40°C

Detection: RI

Sample: Nylon 66

5. Conc lusions

By greatly increasing the strength of the packing material, the TSKgel H_{HR} columns significantly expand performance over what can be achieved with the conventional TSKgel H_{XL} columns. As a result, the following improvements are realized: (a) enhanced ability to accommodate solvent conversion, (b) better durability when used with specialized solvents, and (c) more stable analysis at high flow rates.

In conclusion, the TSKgel H_{HR} columns allow analysis under a broader range of conditions and with a wider variety of solvents, which makes these columns the best choice to tackle new and unknown analytical challenges.

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Message for Sigma-Aldrich/Supelco customers:

Sigma-Aldrich Corporation is a non-exclusive distributor of TSKgel columns and TOYOPEARL bulk resins (< 1 L) in North, South and Central America, Europe, Africa and the Middle East.

For the availability of TSKgel columns in Asia and Australia, please consult the website of Tosoh Corporation.

Note: On the Sigma-Aldrich website and in the Supelco catalog TSKgel part numbers are preceded by the number 8, for example:
817356 for TSKgel G4000Hhr, 7.8mmID x 30cm, 5µm

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