No.103

TOSOH

SEPARATION REPORT

TSKgel SuperMultiporeHZ Series: a High Performance SEC Semi-microcolumn with Wide Pore Size Distribution

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1. Introduction

Size exclusion chromatography (SEC) is a method of determining relative average molecular weight and relative average molecular weight distributions; however; owing to its reproducibility and convenience as a measurement method (in terms of ease of automation and measurement time), it has also gained widespread use as a method of measuring average molecular weight in high polymers in a wide range of fields, including chemistry, biochemistry, food products, and pharmaceuticals.

In SEC, because average molecular weight is calculated based on a calibration curve constructed from a standard sample, in some instances there are differences between the approximate calibration curve and the accurate calibration curve, depending upon the column grade selected. Also, the use of multiple packing materials with different pore sizes results in distortion in the chromatograms of some polymer samples.

Tosoh has already commercialized a columns with wide pore size distribution (TSKgel MultiporeHXL-M)[1,2] that resolves these problems by incorporating a wide range of pore size distribution within a single particle.

In the latest SEC column, a packing material with monodisperse particle, produced using a new synthesis method, is packed into a semi-microcolumn (4.6 mm ID × 15 cm), resulting in a reduction in the solvent consumption, while the characteristics of TSKgel MultiporeHXL-M are maintained. Further, a new packing material with a pore size distribution suitable for low molecular weight grades, which focuses on oligomers and samples of low molecular weight, has been developed and commercialized.

In this study, the basic characteristics and applications of the TSKgel SuperMultiporeHZ Series, a novel multipore type semi-micro SEC column for organic solvent, are introduced.

2. Characteristics of the TSKgel SuperMultiporeHZ Series

SEC in the past have generally employed methods in which several columns, each with different packing material with different pore size, are connected, and the molecular weight separation range is optimized. An alternative approach is to use a so-called mixed-bed type column, in which pore characteristics (molecular weight separation range and linearity of the calibration curve) are improved by blending several packing materials with different pore sizes in optimal proportions.

However, in these methods, errors may arise between the actual data points obtained from measurements of a standard sample of known molecular weight and the calibration curve approximated by a higher order equation. Also, an inflection point appears in the calibration curve due to the mixing (or connecting) of packing materials (or packed columns) of different pore sizes (molecular weight exclusion limit), and distortion is observed in the chromatogram, depending upon the sample. In other words, problems can arise with respect to accuracy and precision. The multipore column TSKgel MultiporeHXL-M has been commercialized as a column that overcomes these problems and has received favorable criticism.

In the TSKgel SuperMultiporeHZ Series, which was developed based on a novel synthesis method, a semi-microcolumn is packed with a packing material of monodisperse microparticles, and can therefore achieve separations equal to those of conventional columns in half the measurement time and reduce the amount of solvent used by 1/6th, while maintaining the features inherited from the existing product.

Also available is the TSKgel SuperMultiporeHZ-N, a multipore column for new low molecular weight grades, which exhibits superior separation of oligomers and low molecular weight samples.

The fundamental properties of the TSKgel SuperMultiporeHZ Series are shown in **Tables 1** and **2**; and its features are tabulated in **Table 3**

Table 1. Summary of Physical Properties of the TSKgel SuperMultiporeHZ Series

Physical Attributes	TSKgel SuperMultiporeHZ-N	TSKgel SuperMultiporeHZ-M
Packing material	Poly(styrene/divinylbenzene)	
Particle size	3 μm (monodisperse particles)	4 μm (monodisperse particles)
Molecular wt. exclusion limit	120,000	2,000,000
Central pore diameter	8 nm	14 nm
Molecular wt. separation range	50,000 ~ 500	1,000,000 ~ 500
Number of theoretical plates	20,000 plates/15 cm	16,000 plates/15 cm
Column size (analytical column)	4.6 mm ID × 15 cm	
Column size (guard column)	4.6 mm ID × 2 cm	

Table 2. Performance Summary of Multipore-type SEC Columns

Product Name	Number of Theoretical Plates (TP/Column)	Asymmetry Coefficient	Column Size (mm ID × cm)	Particle size (μm)
TSKgel SuperMultiporeHZ-N	20,000/15 cm	0.7 ~ 1.4	4.6 × 15	3.0
TSKgel SuperMultiporeHZ-M	16,000/15 cm	0.7 ~ 1.4	4.6 × 15	4.0
TSKgel MultiporeHXL-M	16,000/30 cm	0.7 ~ 1.4	7.8 × 30	6.0

Conditions

Eluent: THF
Flow Rate: 0.35 mL/min (4.6mmID x 15cm)
1.0 mL/min (7.8mmID x 30cm)
Detection: UV254nm (micro-cell)
Temperature: 25ºC
Sample: DCHP (0.5%)
Injection Vol.: 1 μL (4.6mmID x 15cm)
20μL (7.8mmID x 30cm)

* DCHP: Dicyclohexyl phthalete

3. Fundamental Properties of the TSKgel SuperMultiporeHZ Series

3.1. Pore Characteristics

As shown in **Table 1** and **Table 2**, the TSKgel SuperMultiporeHZ Series consists of 2 grades, namely low molecular weight and high molecular weight.

In **Figure 1**, a calibration curve for standard polystyrene in THF as eluent is shown. The measurable molecular weight separation range is 50,000 ~ 500 for TSKgel SuperMultiporeHZ-N, the low molecular weight grade, and 1,000,000 ~ 500 for the high molecular weight grade TSKgel SuperMultiporeHZ-M.

The calibration curves for both grades possess superior linearity in their measurable molecular weight separation ranges.

Figure 2 shows a comparison of the calibration curve of TSKgel SuperMultiporeHZ-N with one that is typical of multiple-type columns with differing pore sizes used to make oligomer measurements (TSKgel SuperHZ 4000 + 3000 + 2500 + 2000). The slope of the calibration curve of TSKgel SuperMultiporeHZ-N is seen to be shallower in the low molecular weight range compared with the existing column series, and therefore superior for separation.

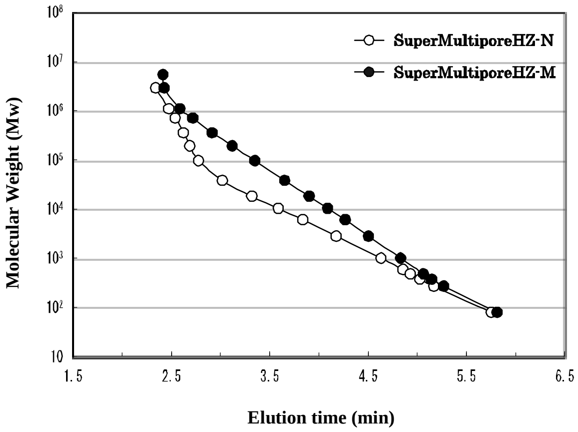

Figure 1. Calibration Curves for the TSKgel SuperMultiporeHZ Series.

Column: TSKgel SuperMultiporeHZ Series (4.6 mm ID × 15 cm)
Eluent: THF
Flow Rate: 0.35 mL/min
Temperature: Room temperature
Injection Vol.: 5 μL
Samples: Standard polystyrene (TSK Standard Polystyrene F-550 ~ A-550), Benzene

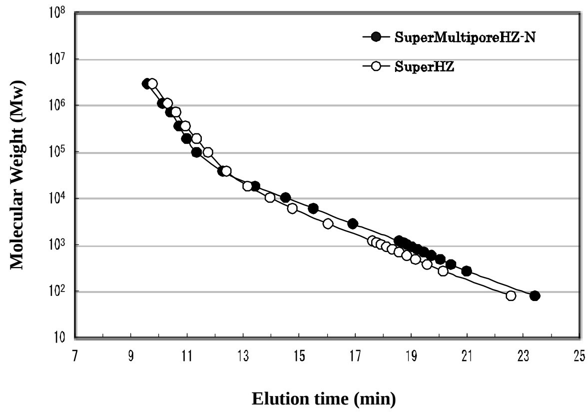

Figure 2. Calibration Curves for the TSKgel SuperMultiporeHZ and TSKgel SuperHZ Columns.

Column: TSKgel SuperMultiporeHZ-N × 4 pcs
TSKgel SuperHZ 4000+3000+2500+2000 (4.6 mm ID × 15 cm × 4 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Temperature: Room temperature
Injection Vol: 5 μL
Samples: Standard polystyrene (TSK Standard Polystyrene F-288 ~ A-500), Benze

Table 3. Features of the TSKgel SuperMultiporeHZ Series

Feature	Advantages
1) Multipore-type column packing material (wide pore size distribution within monodisperse particles)	• Superior linearity of calibration curve. • Distortion in chromatogram of polymer sample is not observed. → Increased accuracy of molecular weight measurement, improved reproducibility.
2) Miniaturization of packing materials (monodisperse particles)	• SEC conducted in short times with high resolution. → The same resolution as a conventional column (30 cm) is attainable in half the measurement time. • Resolution is not reduced even for high velocity measurements. • Improved stability of column performance.
3) Semi-microcolumn	• Reduced solvent consumption. → Solvent consumption is reduced by 1/6th relative to a conventional column (30 cm).
4) Low adsorptivity	• Applicable to a wide variety of polymer samples.

3.2. Separation Properties

TSKgel SuperMultiporeHZ-N, which is a low molecular weight grade, employs a packing material with a particle size of 3 μm, and possesses twice the number of theoretical plates per unit length compared with the TSKgel HXL Series, which is also a low molecular weight grade. **Figure 3** shows a chromatogram of an oligomer (PTMEG 650) analyzed on both types of column. It can be seen that the TSKgel SuperMultiporeHZ-N attains a resolution equal to that of the HXL Series in half the measurement time.

Figure 4 shows a chromatogram in which PTMEG 650 was measured using the TSKgel SuperHZ Series (TSKgel SuperHZ 4000 + 3000 + 2500 + 2000 and TSKgel SuperHZ-M/N) and TSKgel SuperMultiporeHZ-N. Compared with the current TSKgel SuperHZ Series (chromatograms B & C), TSKgel SuperMultiporeHZ-N (chromatogram A) shows higher resolution.

High molecular weight grade, TSKgel SuperMultiporeHZ-M uses a packing material with a particle size of 4 μm and possesses twice the number of theoretical plates per unit length compared with TSKgel MultiporeHXL-M. **Figure 5** shows an overlap of the elution curves for standard polystyrene measured on both types. It can be seen that TSKgel SuperMultiporeHZ-M achieves the same separation as MultiporeHXL-M in half the measurement time.

Figure 6 shows a chromatogram in which a standard polystyrene oligomer (TSK standard polystyrene A-500: Mw approx. 500) is analyzed using both TSKgel SuperMultiporeHZ-N and TSKgel SuperMultiporeHZ-M. From the shallow slope of the calibration curve, it is clear that SuperMultiporeHZ-N, which has a smaller particle size, shows higher resolution.

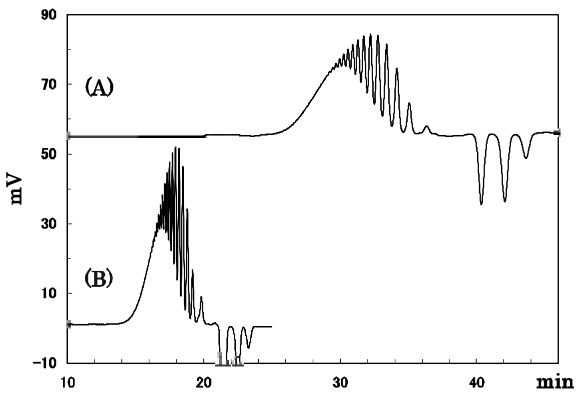

Figure 3. PTMEG Separation by TSKgel SuperMultiporeHZ-N and HXL Columns.

Column: (A) TSKgel G 4000 + 3000 + 2500 + 2000HXL (7.8 mm ID × 30 cm × 4 pcs)
(B) TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 4 pcs)
Eluent: THF
Flow Rate: (A) 1.0 mL/min
(B) 0.35 mL/min
Detection: RI
Temperature: 40°C
Injection Vol.: (A) 50 μL
(B) 10 μL
Sample: Polytetramethylene ether glycol (PTMEG 650)

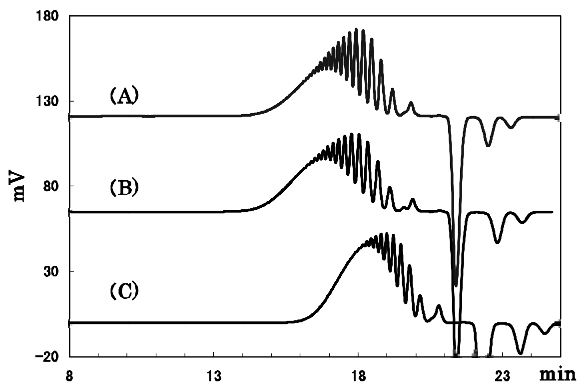

Figure 4. PTMEG Separation by TSKgel SuperMultiporeHZ-N and SuperHZ.

Column: (A) TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 4 pcs)
(B) TSKgel SuperHZ 4000 + 3000 + 2500 + 2000 (4.6 mm ID × 15 cm × 4 pcs)
(C) TSKgel SuperHZ-M-N (4.6 mm ID × 15 cm × 4 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40°C
Injection Vol.: 10 μL
Sample: Polytetramethylene ether glycol (PTMEG 650)

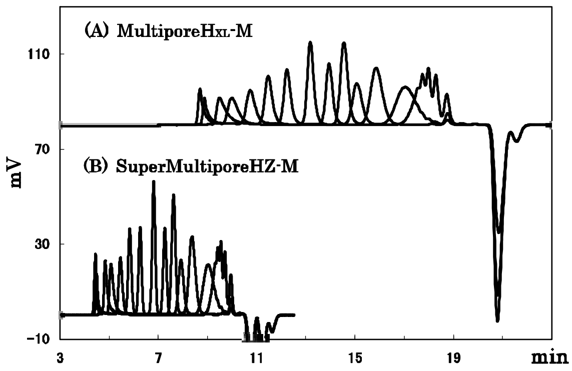

Figure 5. Standard polystyrene elution curve of TSKgel SuperMultiporeHZ-M and MultiporeHXL-M.

Column: (A) TSKgel MultiporeHXL-M (7.8 mm ID × 30 cm × 2 pcs)
(B) TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: (A) 1.0 mL/min
(B) 0.35 mL/min
Detection: RI
Temperature: 40°C
Injection Vol.: (A) 50 μL
(B) 10 μL
Sample: Standard polystyrene (TSK Standard Polystyrene F-288 ~ A-500)

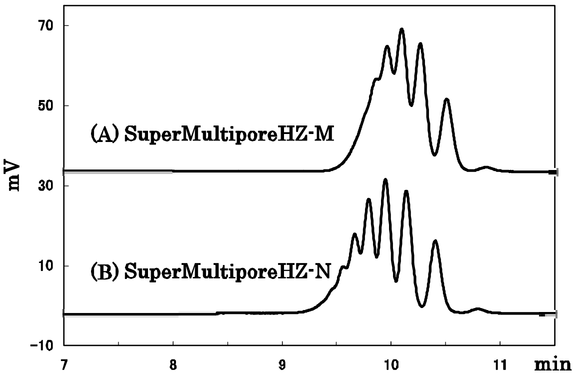

Figure 6. Separation of standard polystyrene oligomer by TSKgel SuperMultiporeHZ-M and SuperMultiporeHZ-N.

Column: (A) TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
(B) TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: UV 254 nm
Temperature: Room temperature
Injection Vol.: 5 μL
Sample: Standard polystyrene oligomer (TSK standard polystyrene A-500)

3.3. Dependence of Height Equivalent to a Theoretical Plate (HETP) on linear velocity

Using low molecular weight monodisperse sample (Dicyclohexyl phthalate, DCHP), **Figure 7** shows results confirming the dependence of HETP on the linear velocity for TSKgel MultiporeHXL-M (particle size: 6 μm) and TSKgel SuperMultiporeHZ-N and -M (particle size: 3 μm and 4 μm).

It can be seen that the optimal linear velocity (resulting in a minimum HETP) for the larger-particle-size TSKgel MultiporeHXL-M is roughly 0.035 cm/sec (7.8 mm ID column approx. 1.0 mL/min), and that at linear velocities higher than this, HETP gradually increases and column efficiency drops. In contrast, the TSKgel SuperMultiporeHZ series, with its micro particles, has an optimal linear velocity in a higher range (0.035 ~ 0.04 cm/sec: approx. 0.35 ~ 0.4 mL/min) than TSKgel MultiporeHXL-M, and analysis is possible even at high velocities, a condition under which column efficiency drops and flow rate is high and difficult to analyze.

Figure 8 shows the relationship between the HETP of TSKgel SuperMultiporeHZ-M and the linear velocity for a standard high molecular weight sample (TSK standard polystyrene F-40, Mw = 355,000; F-2, Mw = 18,100). The smallest HETP values (i.e., highest column efficiencies) are obtained for samples of small molecules (C) (0.035 ~ 0.04 cm/sec), while for high molecular weight molecules (A, B), HETP gradually drops as the linear velocity drops and column efficiency increases. This trend becomes more pronounced as the molecular weight of the analyte increases. Consequently, for samples with an average molecular weight of 10,000 or less, high-velocity measurement is feasible. However, for samples with a high molecular weight of 50,000 or more, low-velocity measurement is recommended.

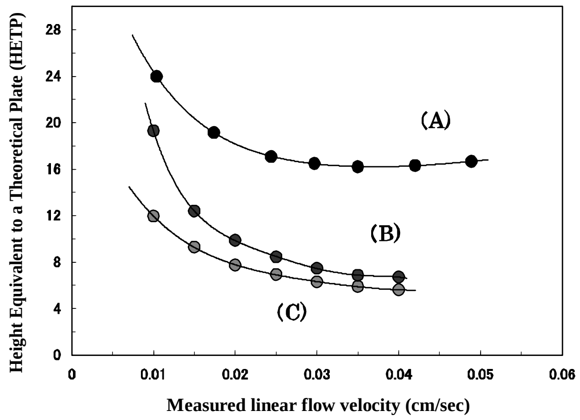

Figure 7. Relationship between linear velocity and HETP in TSKgel SuperMultipore series.

Column: (A) TSKgel MultiporeHXL-M (7.8 mm ID × 30 cm)
(B) TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm)
(C) TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm)
Eluent: THF
Flow Rate: 0.10 ~ 1.40 mL/min
Detection: UV 254 nm
Temperature: Room temperature
Injection Vol.: (A) 20 μL
(B, C) 1 μL
Sample: Dicyclohexyl phthalate (DCHP)

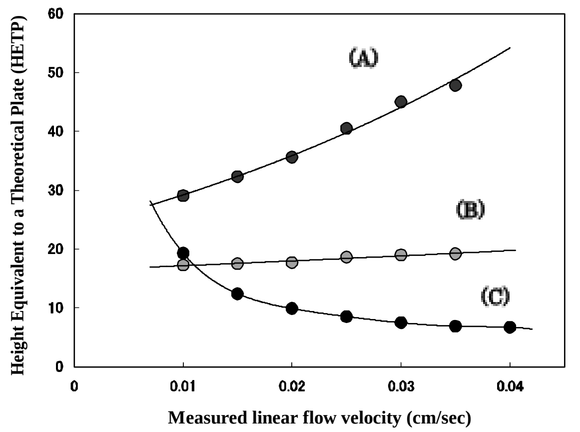

Figure 8. Relationship between linear velocity and HETP in TSKgel SuperMultiporeHZ-M.

Column: TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm)
Eluent: THF
Flow Rate: 0.10 ~ 0.40 mL/min
Detection: UV 254 nm
Temperature: Room temperature
Injection Vol.: (A, B) 5 μL
(C) 1 μL
Sample: (A) Standard polystyrene (Mw: 355,000)
(B) Standard polystyrene (Mw: 18,1000)
(C) DCHP

3.4. Dependence of Calibration Curve on Flow Rate

For the TSKgel SuperMultiporeHZ Series, the influence of changes in the measurement flow rate on the calibration curve was investigated (**Figure 9** and **Figure 10**).

From standard polystyrene calibration curve data obtained by varying the measurement flow rate in the range 0.1 mL/min to 0.35 mL/min, it was found that the dependence on measurement flow rate was negligible for both grades of pore characteristics.

From this result, it is clear that in the measured range of giant molecules of standard polystyrene (Mw of TSKgel SuperMultiporeHZ-N, 1,080,000 or less; Mw of TSKgel SuperMultiporeHZ-M, 2,890,000 or less), analysis may be conducted at a measurement flow rate in the range 0.1 ~ 0.35 mL/min. There were few overload effects or changes in the hydrodynamic volume of the sample due to cleaving of molecular chains, etc., and suitable chromatograms were obtainable.

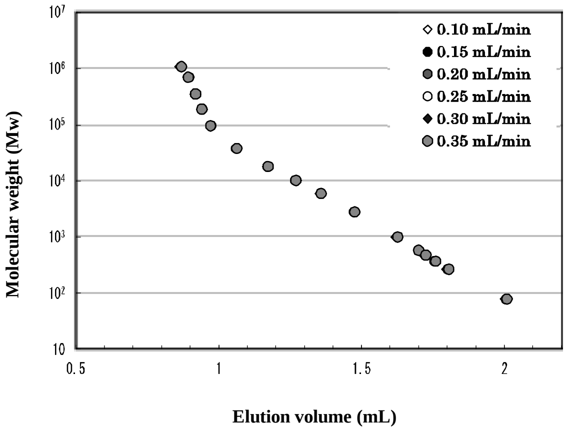

Figure 9. Dependence of Calibration Curve on Flow Rate for TSKgel SuperMultiporeHZ-N

Column: (A) TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm)
Eluent: THF
Flow Rate: 0.10 ~ 0.35 mL/min
Temperature: Room temperature
Injection Vol.: 5 μL
Sample: Standard polystyrene (TSK standard polystyrene F-128 ~ A-500), benzene

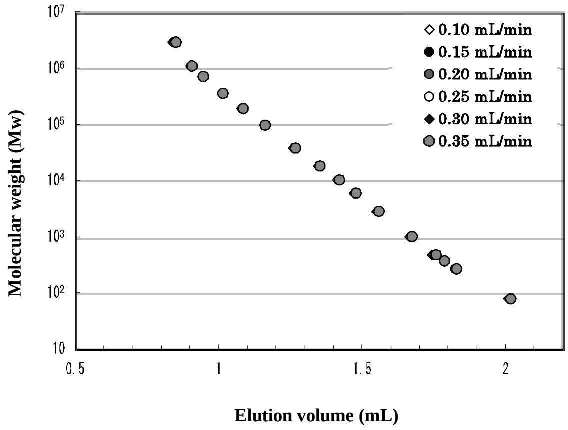

Figure 10. Dependence of Calibration Curve on Flow Rate for TSKgel SuperMultiporeHZ-M

Column: TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm)
Eluent: THF
Flow Rate: 0.10 ~ 0.35 mL/min
Temperature: Room temperature
Injection Vol.: 5 μL
Sample: Standard polystyrene (TSK standard polystyrene F-288 ~ A-500), benzene

3.5. Influence of Sample Injection Volume

It is known that sample injection volume has a significant influence on separation and molecular weight distribution. Generally, the smaller the particle size of the packing material when column size is small, the smaller the maximum sample injection volume.

Figure 11 shows the dependence of HETP on injection volume for a low molecular weight monodisperse sample (DCHP) using the TSKgel SuperMultiporeHZ series. The maximum sample injection volume is 5 μL per column for the TSKgel SuperMultiporeHZ series, which is packed with micro particles, and 50 μL for TSKgel MultiporeHXL-M.

The maximum sample injection volume of 5 μL can also be deduced from observation of the dependence of separation on injection volume for standard polystyrene oligomers (TSK standard polystyrene A-500) using TSKgel SuperMultiporeHZ-N (**Figure 12** and **Figure 13**).

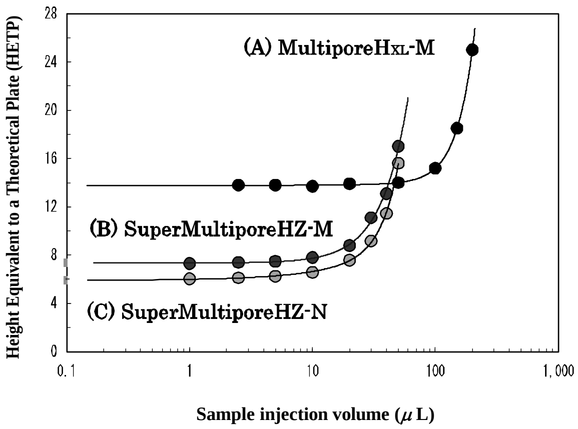

Figure 11. Relationship between sample injection volume and HETP for TSKgel SuperMultiporeHZ series and TSKgel MultiporeHXL-M

Column: (A) TSKgel MultiporeHXL-M (7.8 mm ID × 30 cm × 2 pcs)
(B) TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
(C) TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: (A) 0.10 mL/min
(B, C) 0.35 mL/min
Detection: UV 254 nm
Temperature: Room temperature
Injection Vol.: 1 ~ 200 μL
Sample: Dicyclohexyl phthalate (DCHP), 5 g/L

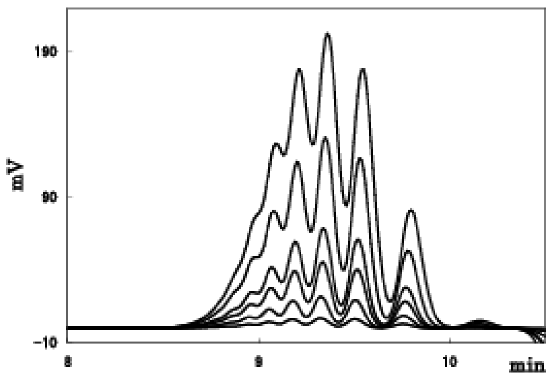

Figure 12. Dependence of the chromatogram of a standard polystyrene oligomer on injection volume for TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 2, 5, 10, 15, 30, 50 μL
Sample: Standard polystyrene oligomer (TSK standard polystyrene A-500), 3 g/L

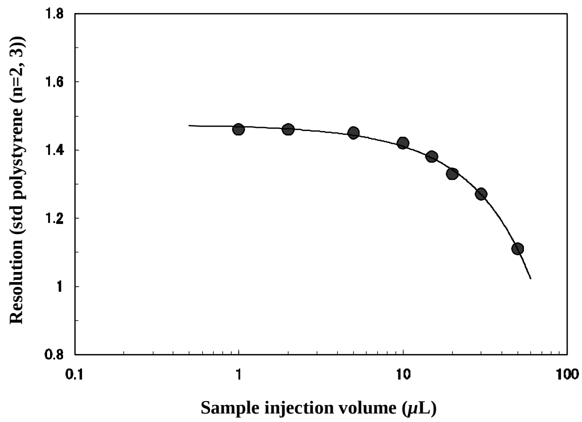

Figure 13. Dependence of resolution on injection volume for a standard polystyrene oligomer (2 and 3 volumes) in TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 1, 2, 5, 10, 15, 20, 30, 50 μL
Sample: Standard polystyrene (A-500), 3 g/L

3.6. Effect of Sample Concentration

Differences in the sample injection volume can affect the elution time of the sample (elution volume), which can cause variations in molecular weight measurement and fluctuations or reductions in resolution. Further, under conditions exceeding the maximum sample injection volume, this influence becomes pronounced. It has been reported previously that even if the maximum sample injection volume is not exceeded, the conditions (flow rate, temperature, molecular weight and distribution) and characteristics of the packing material or packed column can have a negative influence.

High sample concentration causes a decrease in hydrodynamic volume and increase elution time (increase elution volume), even if fixed conditions such as sample injection volume are optimized. This phenomenon is referred to as ‘concentration effect’ and generally tends to strengthen as particle size of the packing material is reduced and sample molecular weight is increased.

Figure 14 and **Figure 15** show, respectively, a chromatogram for low molecular weight standard polystyrene, and the dependence of sample concentration on the resolution between dimmer (n = 2) and trimmer (n = 3) of low molecular weight grade TSKgel SuperMultiporeHZ-N. These results show that if sample concentration is 10 g/L or less, suitable resolution can be stably maintained.

Figure 16 and **Figure 17** show the chromatograms and molecular weights obtained for different concentrations of phenolic resin (Mw approx. 5,000) on TSKgel SuperMultiporeHZ-N. **Figure 18** and **Figure 19** show the chromatograms and molecular weights obtained for epoxy resin (Mw approx. 8,000) under the same conditions. Since the molecular weights of both samples were relatively low, as long as the sample concentration remains upto 20 g/L, no fluctuations in the average molecular weight are found.

Chromatograms and data showing the dependence of the obtained molecular weights on sample concentration are shown in **Figures 20** to **23** for epoxy resin and polystyrene (SRM706) using TSKgel SuperMultiporeHZ-M. For epoxy resin, with an average molecular weight (Mw) of approximately 20,000, no concentration effect is found at low sample concentrations (4 g/L or lower); however, for polystyrene, with an average molecular weight (Mw) of approximately 260,000, concentrations of more than 2 g/L result in a increase of elution time, and a consequent reduction in molecular weight is observed. Thus it can be seen that even at molecular weights such as these, suitable sample concentrations differ, and sample concentration optimization becomes a primary concern.

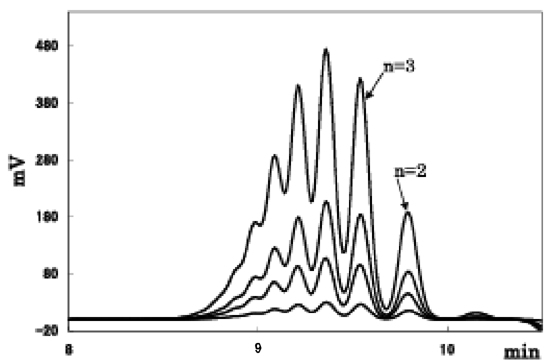

Figure 14. dependence of chromatogram of standard polystyrene oligomer on sample concentration using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 1.2, 5, 10, 20 g/L
Sample: Standard polystyrene oligomer (TSK standard polystyrene A-500)

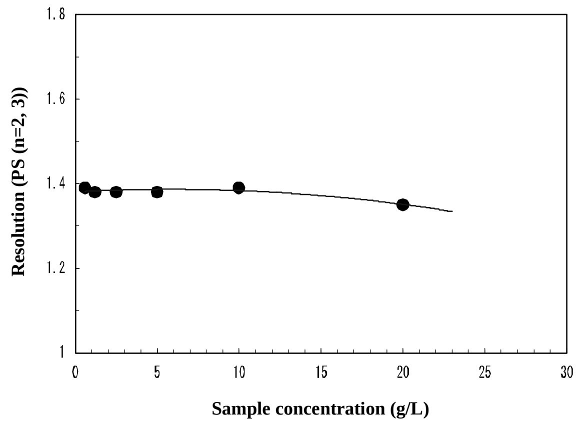

Figure 15. Effect of sample concentration on resolution for a standard polystyrene oligomer using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.6, 1.2, 2.5, 5, 10, 20 g/L
Sample: Standard polystyrene oligomer (TSK standard polystyrene A-500

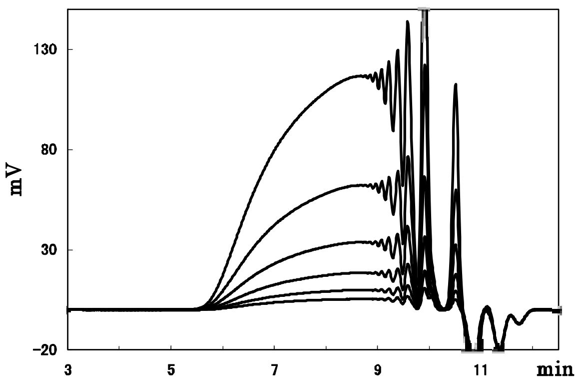

Figure 16. Dependence of chromatogram of phenolic resin on sample concentration using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.6, 1.2, 2.5, 5, 10, 20 g/L
Sample: Phenolic resin (Mw: approx. 5,000)

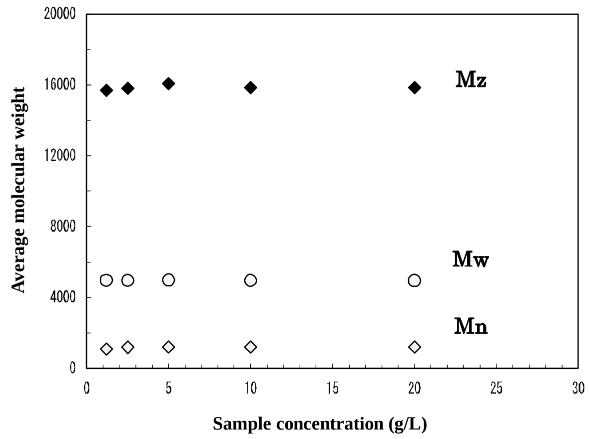

Figure 17. Dependence of molecular weight measurement for phenolic resin on sample concentration using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 1.2, 2.5, 5, 10, 20 g/L
Sample: Phenolic resin (Mw: approx. 5,000)

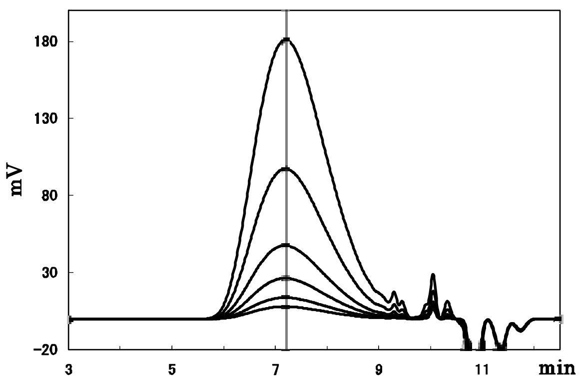

Figure 18. Dependence of chromatogram of epoxy resin on sample concentration using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.6, 1.2, 2.5, 5, 10, 20 g/L
Sample: Epoxy resin (Mw: approx. 8,000)

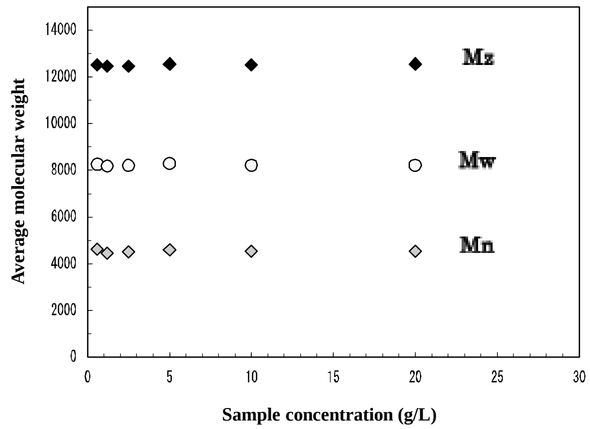

Figure 19. Dependence of molecular weight measurement for epoxy resin on sample concentration using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.6, 1.2, 2.5, 5, 10, 20 g/L
Sample: Epoxy resin (Mw: approx. 8,000)

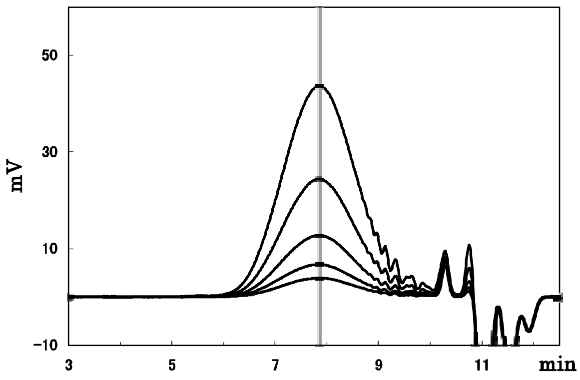

Figure 20. Dependence of chromatogram of epoxy resin on sample concentration using TSKgel SuperMultiporeHZ-M

Column: TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.5, 1, 1.5, 3, 5 g/L
Sample: Epoxy resin (Mw: approx. 20,000)

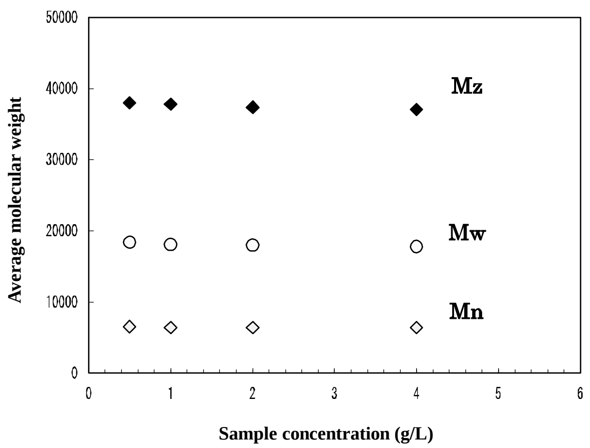

Figure 21. Dependence of molecular weight measurement for epoxy resin on sample concentration using TSKgel SuperMultiporeHZ-M

Column: TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.5, 1, 2, 4 g/L
Sample: Epoxy resin (Mw: approx. 20,000)

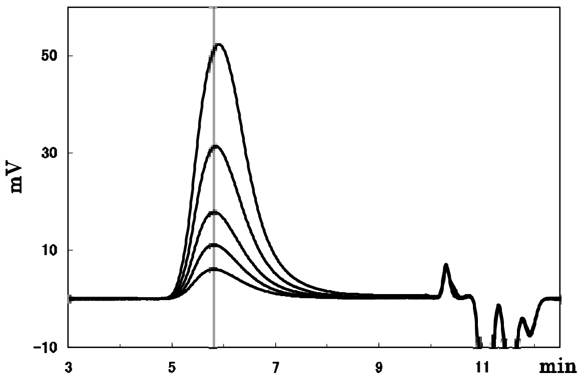

Figure 22. Dependence of chromatogram of polystyrene on sample concentration using TSKgel SuperMultiporeHZ-M

Column: TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.5, 1, 1.5, 3, 5 g/L
Sample: Standard polystyrene (NIST SRM 706: Mw: approx. 258,000)

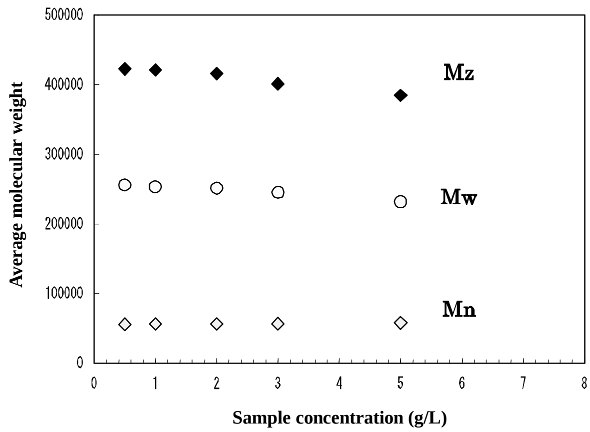

Figure 23. Dependence of molecular weight measurement for polystyrene on sample concentration using TSKgel SuperMultiporeHZ-M

Column: TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 15 μL
Concentration: 0.5, 1, 1.5, 3, 5 g/L
Sample: Standard polystyrene (NIST SRM 706: Mw: approx. 258,000)

3.7. Influence of Measurement Temperature

Figure 24 and **Figure 25** show dependence of the calibration curve of standard polystyrene on temperature using the TSKgel SuperMultiporeHZ series. In both cases, as the measurement temperature rises, each type of standard polystyrene exhibits faster elution and calibration curve consequently shifts to early elution time.

Figure 26 shows the relationship between HETP and measurement temperature for each type of standard polystyrene using TSKgel SuperMultiporeHZ-N. It is known that as the temperature rises, sample diffusion is reduced; however, it is clear that this trend becomes more pronounced in high molecular weight samples.

When measurements are made at high temperatures, high resolution and high-speed analysis is anticipated, due to faster sample elution, a reduction in sample diffusion, and an increase in the optimal flow rate for sample.

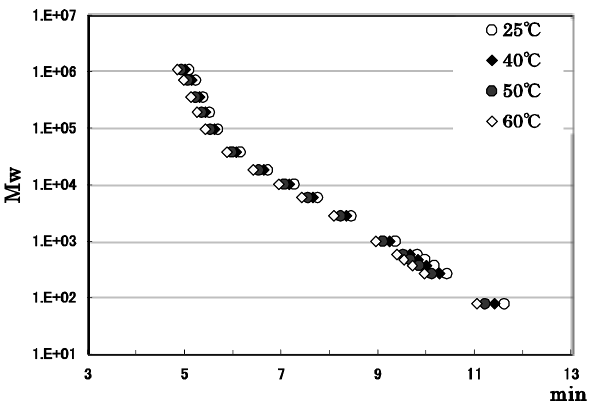

Figure 24. Dependence of calibration curve on temperature using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Temperature: 25, 40, 50, 60ºC
Injection Vol.: 5 μL
Sample: Standard polystyrene (TSK standard polystyrene F-128 ~ A-500)

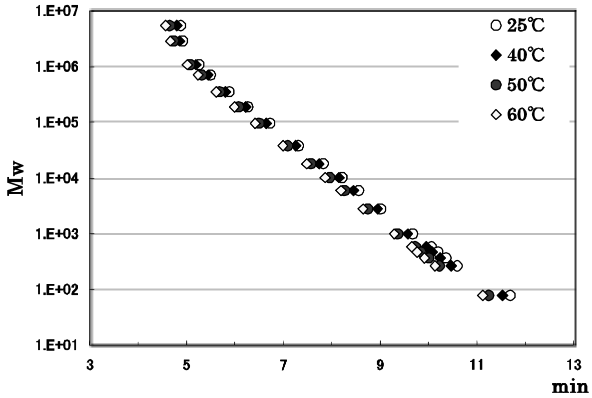

Figure 25. Dependence of calibration curve on temperature using TSKgel SuperMultiporeHZ-M

Column: TSKgel SuperMultiporeHZ-M (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Temperature: 25, 40, 50, 60ºC
Injection Vol.: 5 μL
Sample: Standard polystyrene (TSK standard polystyrene F-550 ~ A-500)

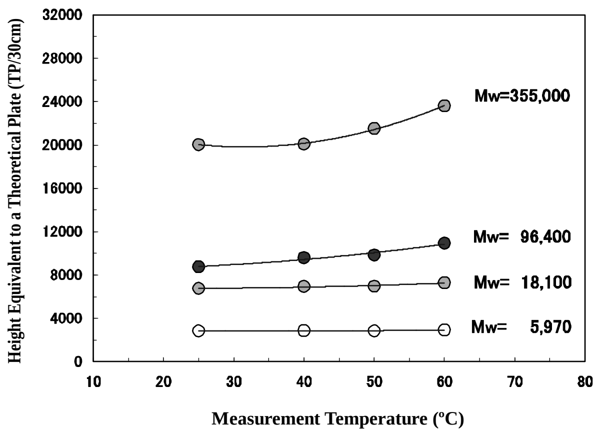

Figure 26. Dependence of HETP of standard polystyrene on temperature using TSKgel SuperMultiporeHZ-N

Column: TSKgel SuperMultiporeHZ-N (4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Temperature: 25, 40, 50, 60ºC
Injection Vol.: 5 μL
Sample: Standard polystyrene (TSK standard polystyrene F-40, F-10, F-2, A-5000)

3.8. Chromatogram Distortion

In SEC, measurements are generally made by use of multiple connections of different-grade columns with different pore sizes, or by using mixed-bed type columns, in which grades of different pore sizes are mixed. In this case, distortion may be observed in the chromatogram, depending upon the sample. In contrast, the use of a multipore-type column (TSKgel SuperMultiporeHZ series, TSKgel MultiporeHXL-M) eliminates distortion in the chromatogram, due to the pore characteristics (pore structure) that constitute the predominant feature of this column.

Figure 27 shows chromatograms of phenolic resin on TSKgel SuperMultiporeHZ-N and on TSKgel SuperHZ

Table 4 shows molecular weights and polydispersity measurements on TSKgel SuperMultiporeHZ-N and on commercial (4000 and 2000 grade connection) columns with packing material of different lots (in the case of commercial columns, lot of 4000 grade used was identical and that of 2000 grade was different) to obtain measurements of silicon resin. It can be seen that, compared with the commercial columns, the multipore-type columns exhibit relatively small differences among packing material lots in terms of the molecular weights measurement. **Figure 30** shows chromatograms of silicon on TSKgel SuperMultiporeHZ-N. No significant differences can be seen among the chromatograms, and the differences among lots are small.

Figure 31 and **Figure 32** show chromatograms of phenolic resin on TSKgel SuperMultiporeHZ-M, TSKgel G (4000 + 3000 + 2500 + 2000) HXL, and TSKgel SuperHZ (4000 + 3000 + 2500 + 2000). With conventional products, distortion can be seen in the chromatogram; however, with TSKgel SuperMultiporeHZ-M, this phenomenon is not observed.

3000 + 2500 + 2000. Distortion is seen in the chromatogram on the TSKgel SuperHZ series; however, for TSKgel SuperMultiporeHZ-N, this phenomenon is not observed.

Figure 28 and **Figure 29** show chromatograms of different types of phenolic resin on TSKgel SuperMultiporeHZ-N and on TSKgel SuperHZ 3000 + 2000. In the chromatograms on TSKgel SuperHZ 3000 + 2000 (**Figure 29**), distortion within a specific elution time is observed for samples of certain molecular weights. In contrast, in the case of TSKgel SuperMultiporeHZ-N, no distortion is seen in the chromatogram regardless of the sample used.

Figure 33 shows chromatograms obtained from both column grades using acrylic resin as a sample. For acrylic resin, as in the case of phenolic resin, distortion is observed in the chromatogram obtained using conventional products; however, with TSKgel SuperMultiporeHZ-M, this phenomenon is not observed.

Figure 34 shows chromatograms of phenolic resin on TSKgel SuperHZM-M, which is a mixed-bed column with an optimized mixing ratio of packing materials of different pore sizes, and TSKgel SuperMultiporeHZ-M, the multipore-type column. It can be seen that even the mixed-bed column, in which pore characteristics are improved by optimizing the packing material mixing ratio, exhibits distortion in the chromatogram.

Figure 34 shows chromatograms on TSKgel SuperMultiporeHZ-M and a third-party mixed-bed type column of the same type as TSKgel SuperHZM-M. In the case of the mixed-bed type column, distortion is seen in the chromatogram.

Table 4. Average molecular weight of silicon resin using TSKgel SuperMultiporeHZ-N and SuperHZ columns and packing materials of different lots.

Column (Gel Lot)	Average Molecular Weight			Polydispersion	
	Mw	Mn	Mz	Mz/Mw	Mw/Mn
TSKgel SuperMultiporeHZ-N (A)	3,410	1,340	7,750	2.27	2.54
TSKgel SuperMultiporeHZ-N (B)	3,400	1,340	7,740	2.28	2.54
TSKgel SuperMultiporeHZ-N (C)	3,430	1,350	7,850	2.29	2.54
Average	3,410	1,340	7,780	2.28	2.54
(RSD)	(0.37%)	(0.35%)	(0.64%)	(0.36%)	(0.00%)
Commercial Column (4000 + 2000 grade) (A)	3,430	1,330	7,640	2.23	2.58
Commercial Column (4000 + 2000 grade) (B)	3,480	1,310	7,990	2.30	2.66
Commercial Column (4000 + 2000 grade) (C)	3,370	1,270	7,850	2.33	2.65
Commercial Column (4000 + 2000 grade) (D)	3,540	1,320	7,710	2.18	2.68
Average	3,455	1,310	7,800	2.26	2.64
(RSD)	(1.81%)	(1.310%)	(1.72%)	(2.60%)	(1.43%)

Conditions
Eluent: THF, Flow Rate: 0.35 mL/min, Temperature: 40ºC
Detection: RI (HLC-8220GPC), Injection Vol.: 10 μL, Sample: Silicon resin (3 g/L) 10 μL

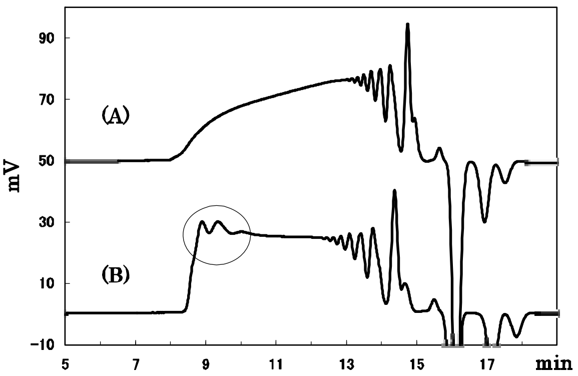

Figure 27. Separation of phenolic resin by TSKgel SuperMultiporeHZ-N and TSKgel SuperHZ columns.

Column: (A) TSKgel SuperMultiporeHZ-N
(4.6 mm ID × 15 cm × 3 pcs)
(B) TSKgel SuperHZ 3000 + 2500 + 2000
(4.6 mm ID × 15 cm × 3 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Phenolic resin (3 g/L)

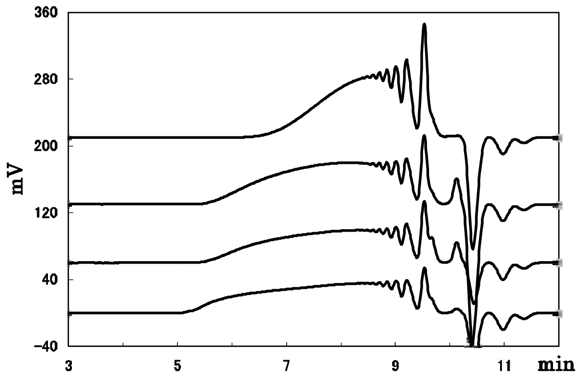

Figure 28. Separation of different types of phenolic resin by TSKgel SuperMultiporeHZ-N.

Column: TSKgel SuperMultiporeHZ-N
(4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Phenolic resin (3 g/L)

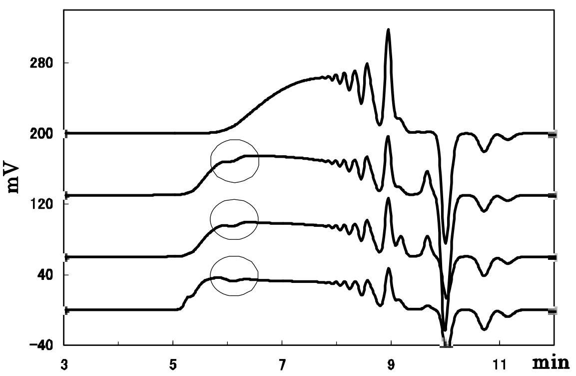

Figure 29. Separation of different types of phenolic resin by TSKgel SuperHZ.

Column: TSKgel SuperMultiporeHZ 3000 + 2000
(4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Phenolic resin (3 g/L)

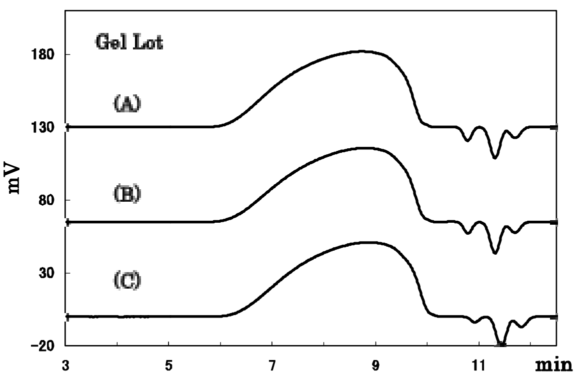

Figure 30. Separation of silicon-based resin by TSKgel SuperMultiporeHZ-N using packing materials of different lots.

Column: TSKgel SuperMultiporeHZ-N
(4.6 mm ID × 15 cm × 2 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Silicon-based resin

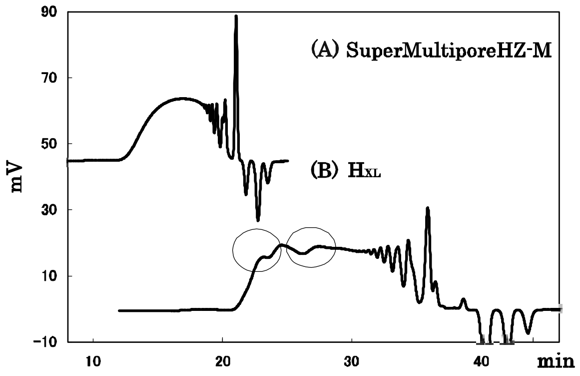

Figure 31. Separation of phenolic resin by TSKgel SuperMultiporeHZ-M and HXL column series.

Column: (A) TSKgel SuperMultiporeHZ-M
(4.6 mm ID × 15 cm × 4 pcs)
(B) TSKgel G 4000 + 3000 + 2500 + 2000 HXL
(7.8 mm ID × 30 cm × 4 pcs)
Eluent: THF
Flow Rate: (A) 0.35 mL/min
(B) 1.0 mL/min
Temperature: 40ºC
Detection: RI
Sample: Phenolic resin (3 g/L)
Injection Vol.: (A) 10 μL
(B) 50 μL

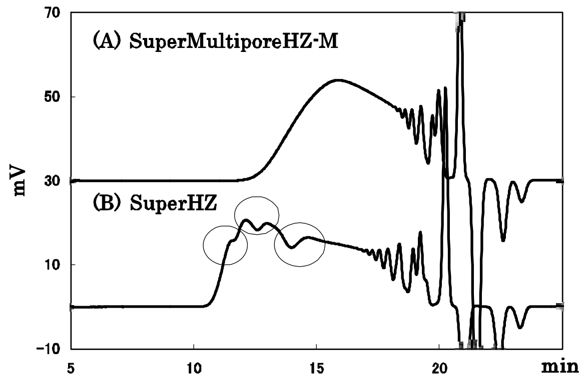

Figure 32. Separation of phenolic resin by TSKgel SuperMultiporeHZ-M and SuperHZ columns.

Column: (A) TSKgel SuperMultiporeHZ-M
(4.6 mm ID × 15 cm × 4 pcs)
(B) TSKgel SuperHZ 4000 + 3000 + 2500 + 2000
(4.6 mm ID × 15 cm × 4 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Phenolic resin (3 g/L)

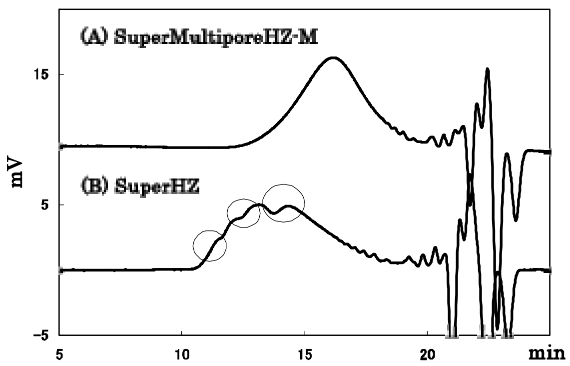

Figure 33. Separation of acrylic resin by TSKgel SuperMultiporeHZ-M and SuperHZ columns.

Column: (A) TSKgel SuperMultiporeHZ-M
(4.6 mm ID × 15 cm × 4 pcs)
(B) TSKgel SuperHZ 4000 + 3000 + 2500 + 2000
(4.6 mm ID × 15 cm × 4 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Acrylic resin (3 g/L)

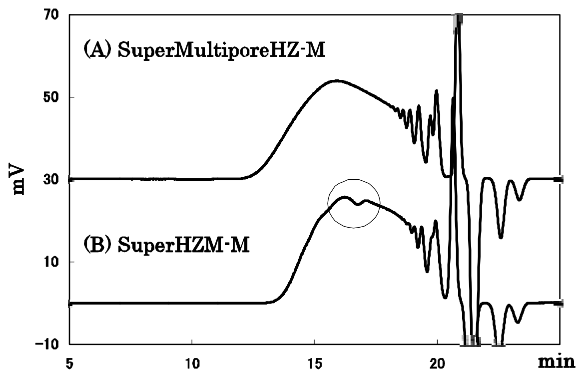

Figure 34. Separation of phenolic resin by TSKgel SuperMultiporeHZ-M and SuperHZM-M column series.

Column: (A) TSKgel SuperMultiporeHZ-M
(4.6 mm ID × 15 cm × 4 pcs)
(B) TSKgel SuperHZM-M
(4.6 mm ID × 15 cm × 4 pcs)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Phenolic resin (3 g/L)

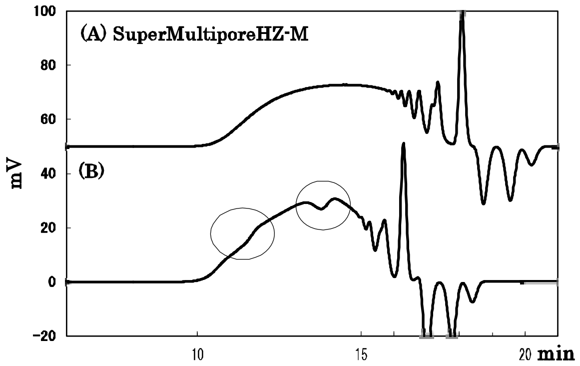

Figure 35. Separation of phenolic resin by TSKgel SuperMultiporeHZ-M and mixed-bed column made by another company.

Column: (A) TSKgel SuperMultiporeHZ-M (4.6 mm ID × 25 cm)
(B) Other company's mixed-bed column (4.6 mm ID × 25 cm)
Eluent: THF
Flow Rate: 0.35 mL/min
Detection: RI
Temperature: 40ºC
Injection Vol.: 10 μL
Sample: Phenolic resin (3 g/L)

4. Conclusion

The TSKgel SuperMultiporeHZ series is an organic soluble high performance SEC semi-microcolumn containing a packing material with wide pore size distribution, which provides superior chromatograms compared with those obtained from conventional mixed-bed type columns. The results of extensive testing show that measurement reproducibility is high and accurate molecular weight distribution data is attainable.

The production of micro and monodisperse particles was achieved by a novel synthesis method. The use of this packing material allows high speed analysis while maintaining conventional resolution. Further, this semi-micro column contributes reduction of solvent consumption. The column is recommended for SEC measurements in conjunction with the HLC-8220GPC, which is a specialized piece of equipment for a high speed SEC system with low dead volume and superior fluid delivery reproducibility.

Reference

1) M.Nagata, T.Kato,H. Furutani, *J.Liq.Chrom & Rel.Technol.*, 21 (10) 1471-1484 (1998)
2) K. Tokunaga, H. Moriyama, *J. TOSOH Res.*, 41 (1997)