Separation Report No. 105

Semi-micro Columns for Size Exclusion Chromatography in Organic Solvent Systems: TSKgel SuperMultiporeHZ Series

Table of Contents

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TOSOH BIOSCIENCE

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1. Introduction

Size exclusion chromatography (SEC) is widely used for determining the molecular weight and molar mass distribution of polymers.

SEC is a method for calculating molecular weight from a calibration curve created using molecular weight standards. However, when SEC is used to analyze polymers with a wide molar mass distribution, it is necessary to use either a method where multiple columns of different pore sizes are linked together, or a method where a mixed bed column is used in which the column is packed with materials of different pore sizes at an optimized mix ratio. Problems can occur with both of these methods, including distortion of the chromatogram or deviations between the actual calibration curve and the calibration curve approximated from data obtained from the molecular weight standards. To solve these problems Tosoh first developed a multi-pore packing material in which a wide range of pore sizes are contained within a single particle (TSKgel MultiporeHXL-M).[1]

With the introduction of the new TSKgel SuperMultiporeHZ Series product line, Tosoh further increased performance while still maintaining the basic properties of TSKgel MultiporeHXL-M by using a packing material composed of monodisperse particles and by reducing column dimensions to the semi-micro level (4.6mm ID x 15cm), which cuts down on solvent consumption. In addition, the new TSKgel SuperMultiporeHZ Series columns contain a column for low molecular weight samples and oligomers, as well as a column for use with high MW polymers.

In this report, the features and basic characteristics of the TSKgel SuperMultiporeHZ Series columns are introduced and application examples are presented.

2. Features

The TSKgel SuperMultiporeHZ Series of semi-micro columns for size exclusion chromatography (SEC) for use with organic solvent systems are packed with a material comprised of monodisperse particles. While maintaining the features of the TSKgel MultiporeHXL-M, this series of columns is able to achieve the same level of separation as the TSKgel MultiporeHXL-M column but in half the time and consuming 1/6th the volume of solvent.

The basic properties of the TSKgel SuperMultiporeHZ Series are shown in *Tables 1* and *2*, and the features are summarized in *Table 3*.

Table 1: Physical properties of TSKgel SuperMultiporeHZ Series Columns

	TSKgel SuperMultiporeHZ-N	TSKgel SuperMultiporeHZ-M	TSKgel SuperMultiporeHZ-H
Packing material	poly (styrene/divinylbenzene)	poly (styrene/divinylbenzene)	poly (styrene/divinylbenzene)
Particle size	3µm (monodisperse)	4µm (monodisperse)	6µm (monodisperse)
Molecular weight exclusion limit*	120,000	2,000,000	40,000,000*
Average pore size	80Å	140Å	-
Molecular mass fractionation range*	300 ~ 50,000	500 ~ 1,000,000	1,000 ~ 10,000,000
Theoretical number of plates	16,000 TP/15cm	20,000 TP/15cm	11,000 TP/15cm
Column size	4.6mm ID x 15cm	4.6mm ID x 15cm	4.6mm ID x 15cm
Guard column size	4.6mm ID x 2cm	4.6mm ID x 2cm	4.6mm ID x 2cm

*Applicable to polystyrene in THF

Table 2: Properties of SEC columns based on the multi-pore particle synthesis technology developed by Tosoh

Product name	Number of theoretical plates (15cm column)	Asymmetry factor	Column dimensions (mm ID x cm)	Particle size (µm)
TSKgel SuperMultiporeHZ-N	20,000/15cm	0.7 ~ 1.4	4.6 x 15	3.0
TSKgel SuperMultiporeHZ-M	16,000/15cm	0.7 ~ 1.4	4.6 x 15	4.0
TSKgel SuperMultiporeHZ-H	11,000/15cm	0.7 ~ 1.4	4.6 x 15	6.0
TSKgel MultiporeHXL-M	16,000/30cm	0.7 ~ 1.4	7.8 x 30	6.0

Conditions

Eluent:	THF	Detection:	UV@254nm (UV-8020 microcell)
Flow rate:	0.35mL/min (4.6mm ID x 15cm) 1.0mL/min (7.8mm ID x 30cm)	Sample:	Dicyclohexyl phthalate (DCHP) (0.5%)
Temperature:	25°C	Inj. volume:	1µL (4.6mm ID x 15cm) 20µL (7.8mm ID x 30cm)

3. Basic Characteristics

3-1. Pore characteristics

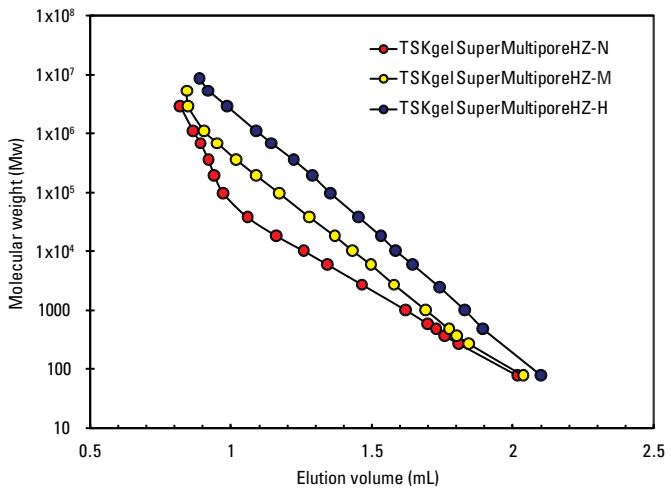
As shown in *Tables 1* and *2*, the TSKgel SuperMultiporeHZ Series consists of a total of 3 types of columns: one grade for low molecular weight samples and two columns with different molecular mass fractionation ranges for polymers.

Figure 1 shows calibration curves created with a standard polystyrene kit, PStQuick, (developed by Tosoh) using THF as the solvent.

Samples within the following molecular mass fractionation ranges can be analyzed: the TSKgel SuperMultiporeHZ-N column can analyze low molecular weight samples between approximately 50,000Da to 500kDa; the TSKgel SuperMultiporeHZ-M column: polymers between about 1,000,000Da and 500kDa; and the TSKgel SuperMultiporeHZ-H column can analyze samples that are approximately 10,000,000Da to 1,000kDa. Linear calibration curves are produced within each of these molecular mass fractionation ranges.

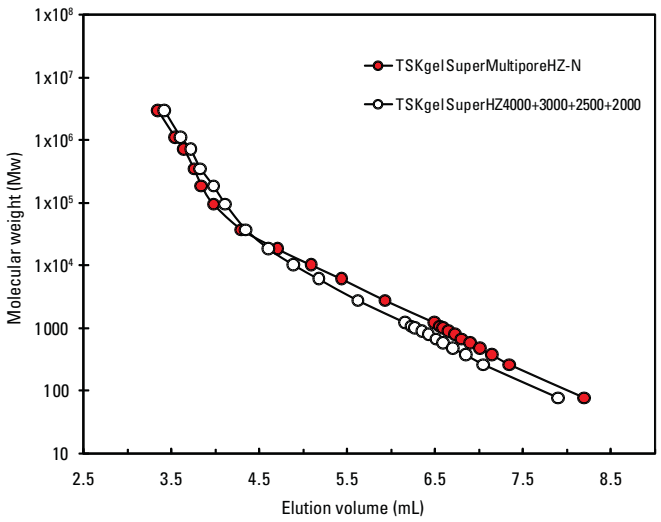
Figure 2 compares calibration curves produced with the TSKgel SuperMultiporeHZ-N column for low molecular weight samples with a series of multiple connected columns of different pore sizes (TSKgel SuperHZ4000 + 3000 + 2500 + 2000). The slope of the calibration curve in the low molecular weight region is more gradual with the TSKgel SuperMultiporeHZ-N column than in the series of multiple connected columns from the TSKgel SuperHZ Series.

Figure 1: Calibration curves for TSKgel SuperMultiporeHZ Series



Columns:	TSKgel SuperMultiporeHZ Series (4.6mm ID x 15cm)
Solvent:	THF
Flow rate:	0.35mL/min
Temp.:	25°C
Sample:	Standard polystyrene kit (PStQuick kit-H) and benzene
Inj. vol.:	5µL

Figure 2: Calibration curves with TSKgel SuperMultiporeHZ-N and TSKgel SuperHZ Series



Columns:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) TSKgel SuperHZ4000+3000+2500+2000 (4.6mm ID x 15cm) x 4
Solvent:	THF
Flow rate:	0.35mL/min
Temp.:	25°C
Sample:	Standard polystyrene kit (PStQuick kit-H) and benzene
Inj. vol.:	5µL

Table 3: Features of TSKgel SuperMultiporeHZ Series Columns

Features	Advantages
Multi-pore packing material (wide range of pores contained in single particle)	• Calibration curves with superior linearity • No observable distortion of chromatograms • Improved accuracy and repeatability of molecular weight data →Capable of rapid analysis with high separation performance
Smaller particle size (monodisperse particles)	• Capable of achieving the same separation performance as conventional columns (30cm) in half the analysis time • No reduction in separation performance even in analysis at high flow rates • Improved robustness of column performance
Semi-micro column	• Reduced solvent consumption →1/6th the consumption of conventional (30cm) columns
Low adsorption packing material	• Can be used for a wide variety of samples

3-2. Separation performance

The new TSKgel SuperMultiporeHZ column line consists of three columns: -N, -M and -H for the analysis of polymers of low, medium and high molecular weights, respectively.

The TSKgel SuperMultiporeHZ-N column, for low molecular weight samples, uses a 3µm particle size packing material and has twice the number of theoretical plates per unit length as the general purpose TSKgel H_{XL} Series of columns for low molecular weight use. As shown in *Figure 3*, the TSKgel SuperMultiporeHZ-N column achieves the same separation performance as the existing TSKGEL H_{XL} Series columns in half the analysis time.

Figure 4 compares the separation of oligomers in poly(tetramethylene ether glycol) (PTMEG) 650 with the TSKgel SuperMultiporeHZ-N column versus the TSKgel SuperHZ Series (TSKgel SuperHZ 4000 + 3000 + 2500 + 2000 and TSKgel SuperHZM-N). It is clear that the TSKgel SuperMultiporeHZ-N column has better separation performance than either multiple columns from the TSKgel SuperHZ Series linked together or the mixed bed-type column alone.

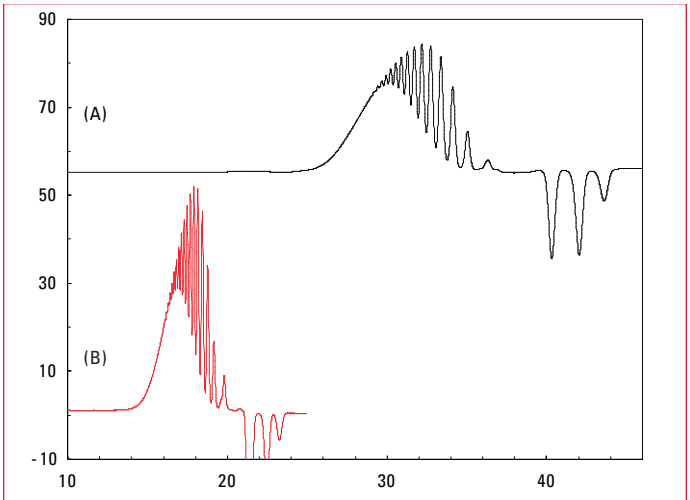
The TSKgel SuperMultiporeHZ-M column contains 4µm spherical particles and has twice the number of theoretical plates per unit length as the TSKgel MultiporeH$_{XL}$-M column. *Figure 5* compares elution curves for a standard polyethylene kit (PStQuick) analyzed on both column types. The TSKgel SuperMultiporeHZ-M column provides a separation that is equivalent to that of the TSKgel MultiporeH$_{XL}$-M column in half the analysis time.

Figure 6 compares separation on the TSKgel SuperMultiporeHZ-N column versus the TSKgel SuperMultiporeHZ-M column in the low molecular weight region (standard polystyrene A-500). The calibration curve for the TSKgel SuperMultiporeHZ-N column is not as steep and better separation is provided in the low molecular weight region due to the smaller particle size (higher number of theoretical plates) of the TSKgel SuperMultiporeHZ-N column.

Figure 7 is a chromatogram of an epoxy resin (Mw approximately 6,000) created using the TSKgel SuperMultiporeHZ Series of columns.

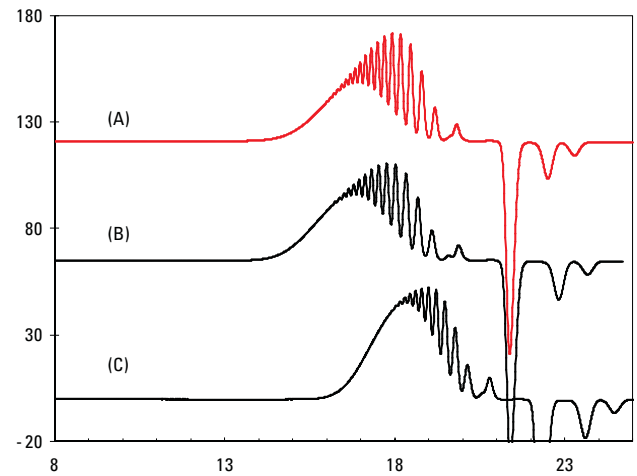
The best separation performance is shown by the TSKgel SuperMultiporeHZ-N column, the grade used for low molecular weight samples, and it is clear that the TSKgel SuperMultiporeHZ-H polymer-grade column does not provide adequate separation performance.

Figure 3: Separation of PTMEG by TSKgel SuperMultiporeHZ-N and TSKgel H_{XL} Series



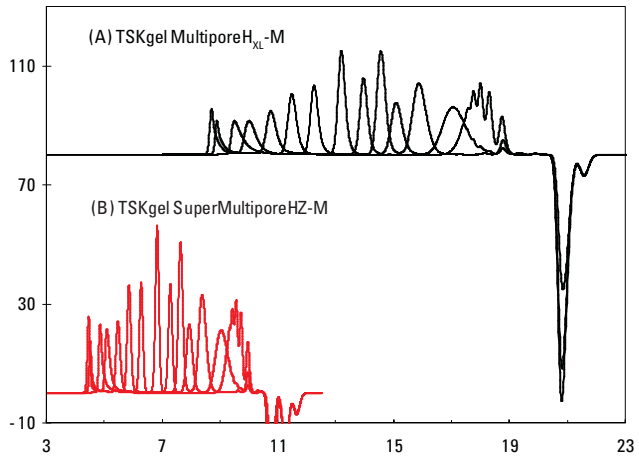
Columns:	(A) TSKgel G1000H$_{XL}$+3000+2500+2000 (7.8mm ID x 30cm) x 4 (B) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 4
Solvent:	THF
Flow rate:	(A) 1.0mL/min (B) 0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	poly(tetramethylene ether glycol) (PTMEG) 650 (10µg/µL)
Inj. vol.:	(A) 50µL (B) 10µL

Figure 4: Separation of PTMEG by TSKgel SuperMultiporeHZ-N, TSKgel SuperHZM-N and TSKgel SuperHZ Series



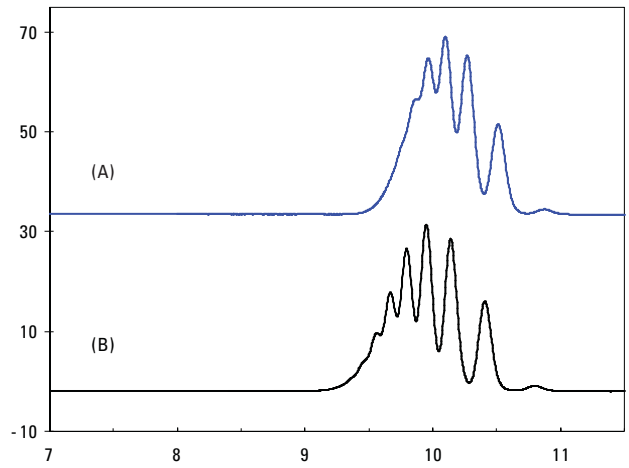
Columns:	(A) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 4 (B) TSKgel SuperHZ4000+3000+2500+2000 (4.6mm ID x 15cm) x 4 (C) TSKgel SuperHZM-N (4.6mm ID x 15cm) x 4
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	PTMEG 650 (10µg/µL)
Inj. vol.:	10µL

Figure 5: Chromatograms of standard polystyrene in TSKgel SuperMultiporeHZ-M and TSKgel MultiporeH$_{XL}$-M



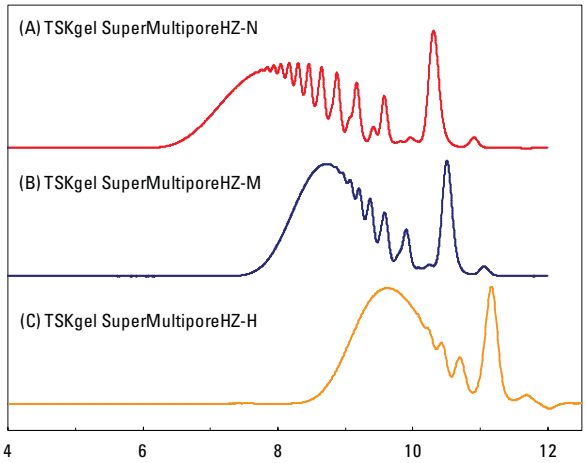
Columns:	(A) TSKgel MultiporeH$_{XL}$-M (7.8mm ID x 30cm) x 2 (B) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 4
Solvent:	THF
Flow rate:	(A) 1.0mL/min (B) 0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Standard polystyrene kit (PStQuick kit-H)
Inj. vol.:	(A) 50µL (B) 10µL

Figure 6: Separation of standard polystyrene by the TSKgel SuperMultiporeHZ-N and TSKgel SuperMultiporeHZ-M



Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 2 (B) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	UV@254nm
Temp.:	25°C
Sample:	Standard polystyrene oligomer (TSK standard polystyrene A-500) (5µg/µL)
Inj. vol.:	5µL

Figure 7: Chromatograms of epoxy resin produced using TSKgel SuperMultiporeHZ Series



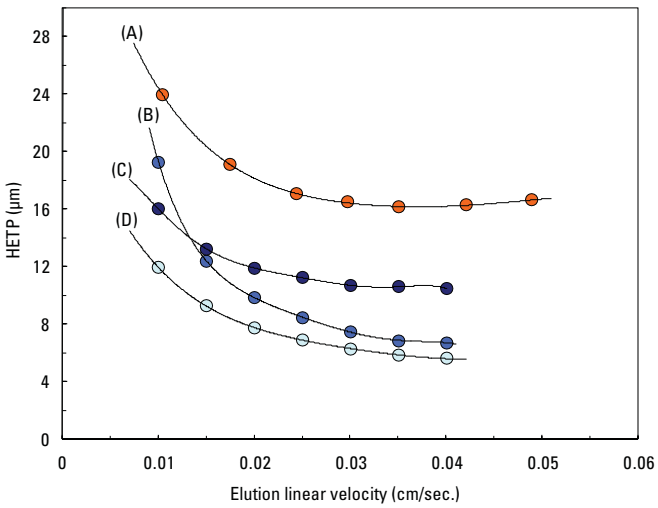
Columns:	(A) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2 (B) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 2 (C) TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	UV@254nm
Temp.:	25°C
Sample:	Epoxy resin (3µg/µL)
Inj. vol.:	10µL

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

Figure 8 shows the relationship between column efficiency (HETP or height equivalent of a theoretical plate) and linear velocity for the TSKgel MultiporeH$_{XL}$-M column (particle size: 6µm) and TSKgel SuperMultiporeHZ-N, -M and -H columns (particle sizes: 3µm, 4µm, and 6µm) using a low molecular weight sample [dichlorohexyl phthalate]. The minimum HETP value for the TSKgel MultiporeH$_{XL}$-M column (particle size: 6µm) is achieved at the linear velocity of approximately 0.035cm/sec. At higher linear velocities, HETP values increase and column efficiency declines. On the other hand, for the TSKGEL SuperMultiporeHZ Series columns, in which the packing consists of monodisperse particles, the optimal linear velocity is higher than that for the TSKgel MultiporeH$_{XL}$-M column, making high-speed analysis possible.

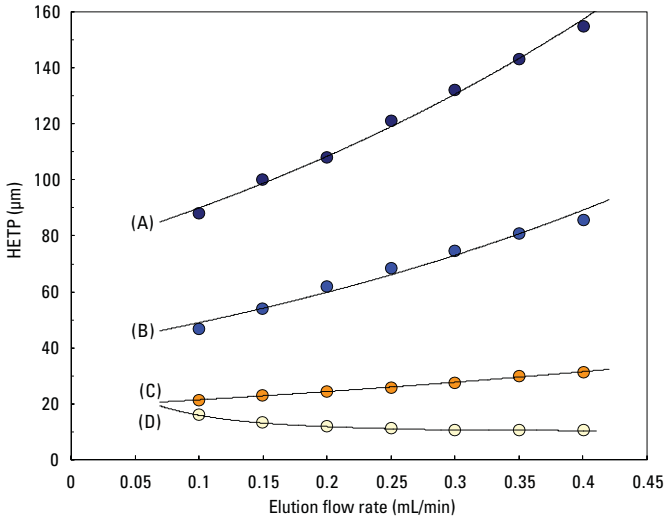
Figure 9 shows the relationship between HETP and flow rate with the TSKgel SuperMultiporeHZ-H column when high molecular weight samples [standard polystyrene F-128 (Mw: 1,090,000), F-20 (Mw: 190,000), F-2 (Mw: 18,100)] and low molecular weight samples [dichlorohexyl phthalate] are used. With a low molecular weight sample (denoted as D in the figure), column efficiency is maintained even at a high flow rate, but as the molecular weight increases, the optimum flow rate decreases. In general, samples with an average molecular weight of 10,000 or less can be analyzed at high flow rates, but polymer samples with a molecular weight of 50,000 or more should be analyzed at lower flow rates.

Figure 8: Relationship between HETP and linear velocity for TSKgel SuperMultiporeHZ Series columns and a TSKgel MultiporeH$_{XL}$-M column



Columns:	(A) TSKgel MultiporeH$_{XL}$-M (7.8mm ID x 30cm) (B) TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) (C) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) (D) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm)
Solvent:	THF
Flow rate:	0.10 to 1.40mL/min
Detection:	UV@254nm
Temp.:	25°C
Sample:	Dicyclohexyl phthalate (DCHP) (5µg/µL)
Inj. vol.:	(A) 20µL (B), (C) and (D) 1µL

Figure 9: Relationship between HETP and flow rate in TSKgel SuperMultiporeHZ-H

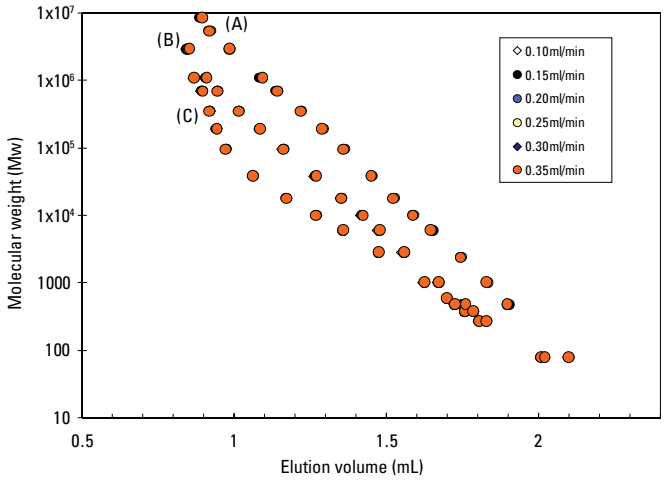


Columns:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm)
Solvent:	THF
Flow rate:	0.10 to 0.40mL/min
Detection:	UV@254nm
Temp.:	25°C
Samples:	(A) Standard polystyrene (Mw: 1,090,000) (0.25µg/µL) (B) Standard polystyrene (Mw: 190,000) (0.40µg/µL) (C) Standard polystyrene (Mw: 18,100) (0.50µg/µL) (D) DCHP (5µg/µL)
Inj. vol.:	(A), (B), (C) 5µL (D) 1µL

3-4. Calibration curve as a function of flow rate

Figure 10 shows calibration curves obtained using standard polystyrene samples when the flow rate was varied from 0.1mL/min to 0.35mL/min, using the TSKgel SuperMultiporeHZ Series of columns. Within the confirmed flow rate range, no overloading or shear degradation problems were observed. The fact that all the points are red, representing 0.35mL/min flow rate, illustrates the point that for each TSKgel SuperMultiporeHZ column, calibration is independent of flow rate within the flow rate range of 0.10mL/min to 0.35mL/min.

Figure 10: Flow rate dependence of the calibration curves of TSKgel SuperMultiporeHZ Series columns



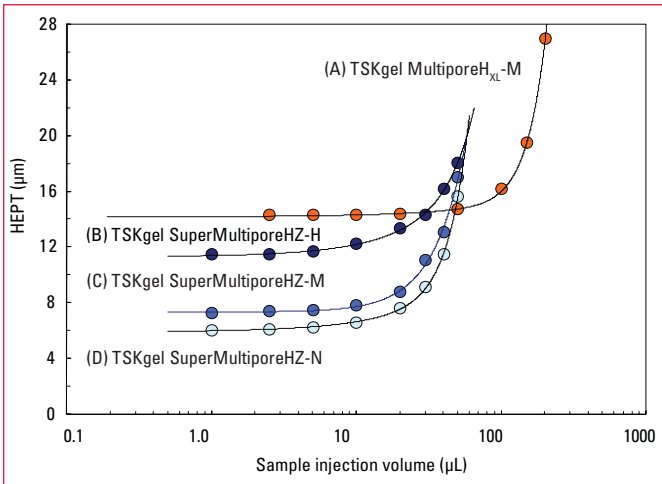
Columns:	(A) TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm)
	(B) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm)
	(C) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm)
Solvent:	THF
Flow rate:	0.10 to 0.35mL/min
Temp.:	25°C
Sample:	Standard polystyrene kit (PStQuick MP-H, MP-M, and MP-N)
Inj. vol.:	5µL

3-5. Effect of sample injection volume

It is well known that sample injection volume may affect separation performance and molar mass distribution data. In general, the maximum sample injection volume decreases as the column size and particle size of the packing material decrease.

Figure 11 shows the relationship between sample injection volume and HETP when a low molecular weight compound (DCHP) is injected on the TSKgel SuperMultiporeHZ Series columns. For the 7.8mm ID x 30cm TSKgel MultiporeH$_{XL}$-M column, the maximum sample injection volume is about 50µL, while the maximum injection volume is 5µL for the 4.6mm ID x 15cm TSKgel SuperMultiporeHZ Series columns.

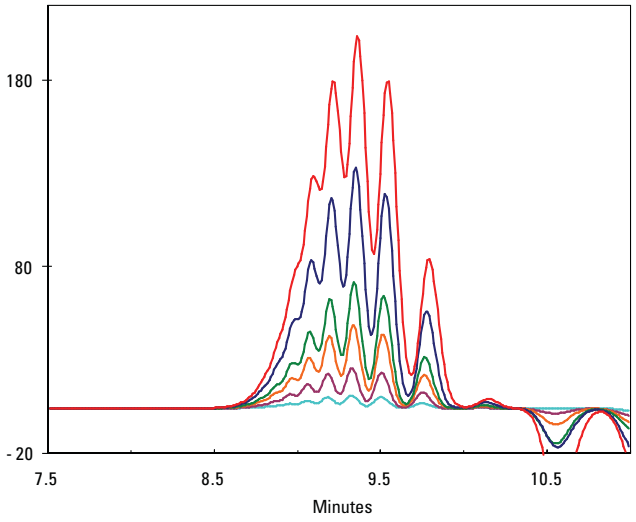
Figure 11: Relationship between HETP and sample injection volume for TSKgel SuperMultiporeHZ Series columns and a TSKgel MultiporeH$_{XL}$-M column



Columns:	(A) TSKgel MultiporeH$_{XL}$-M (7.8mm ID x 30cm) x 2
	(B) TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 2
	(C) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 2
	(D) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	(A) 1.0mL/min (B), (C) & (D) 0.35mL/min
Detection:	UV@254nm
Temp.:	Room temperature
Sample:	DCHP (5µg/µL)
Inj. vol.:	1 to 200µL

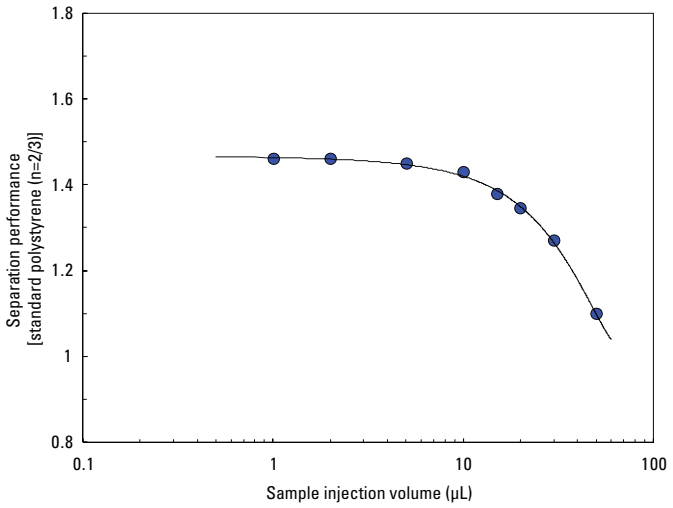
Figures 12 and *13* show chromatograms and the separation performance of a standard polystyrene dimer/trimer, when a low molecular weight standard polystyrene (A-500) was analyzed at various injection volumes using the TSKgel SuperMultiporeHZ-N column. The results shown here also indicate a maximum sample injection volume of around 5µL.

Figure 12: Injection-volume dependence of chromatograms of standard polystyrene oligomer with TSKgel SuperMultiporeHZ-N column



Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Standard polystyrene oligomer (TSK standard polystyrene A-500)
Inj. vol.:	2, 5, 10, 15, 30, and 50µL

Figure 13: Injection-volume dependence of separation performance of standard polystyrene oligomer (dimer and trimer) with the TSKgel SuperMultiporeHZ-N column



Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Standard polystyrene oligomer (TSK standard polystyrene A-500)
Inj. vol.:	1, 2, 5, 10, 15, 20, 30, and 50µL

3-6. Effect of sample concentration

Changes in the sample concentration may cause differences in average molecular weight analysis results as well as fluctuations in separation performance. The smaller the particle size of the packing material and the larger the molecular weight of the sample, the more readily analysis results tend to be affected. In addition, with polymers it has been observed that hydrodynamic volume decreases and elution is delayed as the sample concentration increases.

Figures 14 and *15* show chromatograms and dimer/trimer separation performance with standard polystyrene (A-500) samples of varying concentration using the TSKgel SuperMultiporeHZ-N column. The results shown here indicate that stable and good separation performance can be obtained if the sample concentration is 10µg/µL or less.

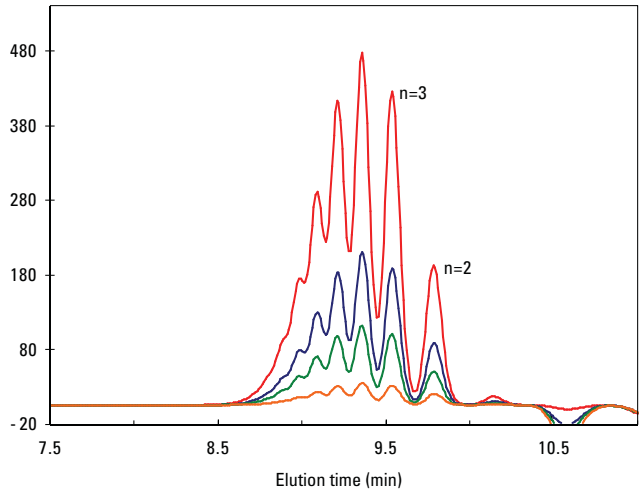
Figures 16 to *19* show average molecular weights and chromatograms obtained of a phenol resin and an epoxy resin when analyzed at different sample concentrations. The figures show that consistent average molecular weight values could be obtained up to a sample concentration of 20µg/µL.

Figures 20 to *23* show average molecular weight data and chromatograms obtained when an epoxy resin and a polystyrene mixture (NIST SRM706) were analyzed using the TSKgel SuperMultiporeHZ-M column with different sample concentrations. When an epoxy resin with an average molecular weight (Mw) of approximately 20,000 was analyzed, results depended very little on sample concentration up to 4µg/µL. However, when polystyrene with an average molecular weight (Mw) of approximately 250,000 was analyzed, delayed elution and, as a result, decreased average molecular weights were observed at sample concentrations greater than 2µg/µL.

Figures 24 and *25* show how the chromatograms and average molecular weight data vary depending on the sample concentration when an acrylic resin [average molecular weight (Mw) approximately 600,000] is analyzed using the polymer grade TSKgel SuperMultiporeHZ-H column. Even with the polymer grade column, the recommended sample concentration for a high molecular weight sample is less than 2µg/µL.

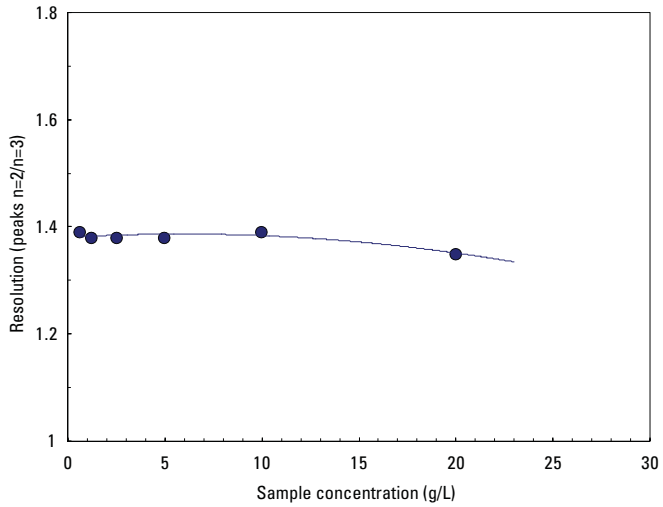
Thus, the appropriate sample concentration will vary depending on the molecular weight of the sample, a crucial factor in optimizing the sample concentration.

Figure 14: Chromatograms of standard polystyrene oligomer as a function of sample concentration on a TSKgel SuperMultiporeHZ-N column



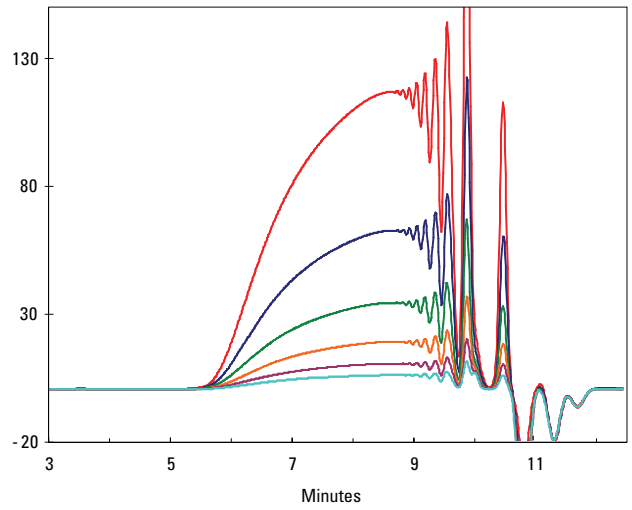
Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Standard polystyrene oligomer (TSK standard polystyrene A-500)
Inj. vol.:	15µL
Sample conc.:	1.2, 5, 10 and 20µg/µL

Figure 15: Resolution as a function of the sample concentration of a standard polystyrene oligomer on a TSKgel SuperMultiporeHZ-N column



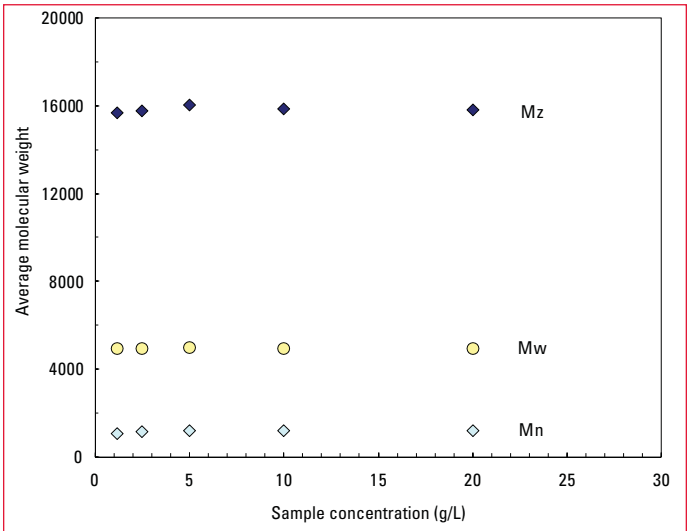
Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Standard polystyrene oligomer (TSK standard polystyrene A-500)
Inj. vol.:	15µL
Sample conc.:	0.6, 1.2, 2.5, 5, 10 and 20µg/µL

Figure 16: Phenol resin chromatograms as a function of sample concentration on a TSKgel SuperMultiporeHZ-N column



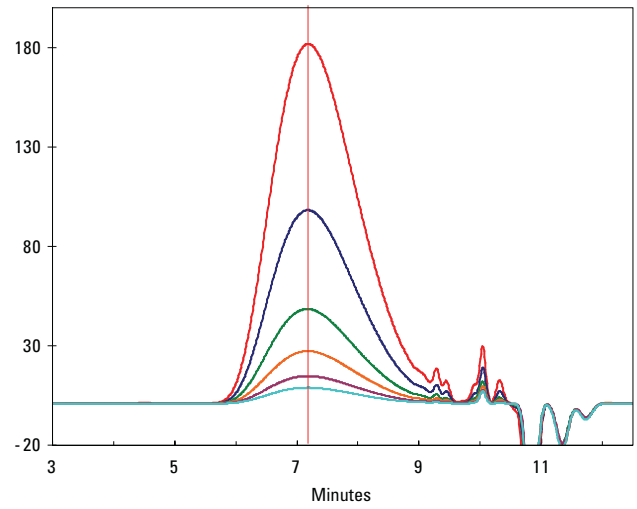
Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Phenol resin (Mw: approximately 5,000)
Inj.vol.:	15µL
Sample conc.:	0.6, 1.2, 2.5, 5, 10 and 20µg/µL

Figure 17: Molecular weight as a function of sample concentration of phenol resin on a TSKgel SuperMultiporeHZ-N column



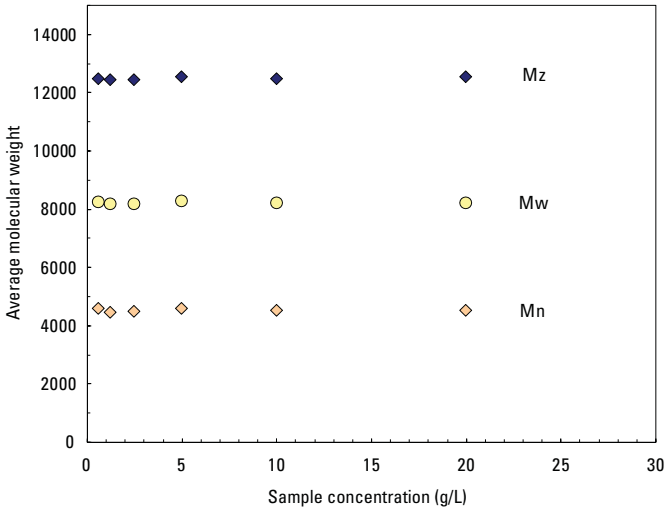
Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Phenol resin (Mw: approximately 5,000)
Inj. vol.:	15µL
Sample conc.:	0.6, 1.2, 2.5, 5, 10 and 20µg/µL

Figure 18: Epoxy resin chromatograms as a function of sample concentration on a TSKgel SuperMultiporeHZ-N column



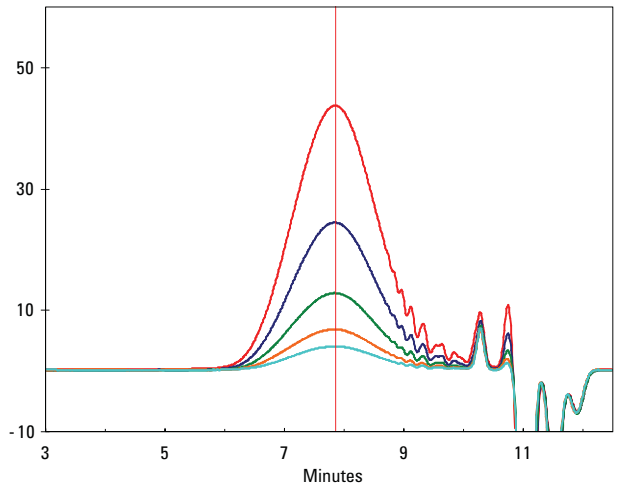
Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Epoxy resin (Mw: approximately 8,000)
Inj. vol.:	15µL
Sample conc.:	0.6, 1.2, 2.5, 5, 10 and 20µg/µL

Figure 19: Molecular weight of epoxy resin as a function of sample concentration on a TSKgel SuperMultiporeHZ-N column



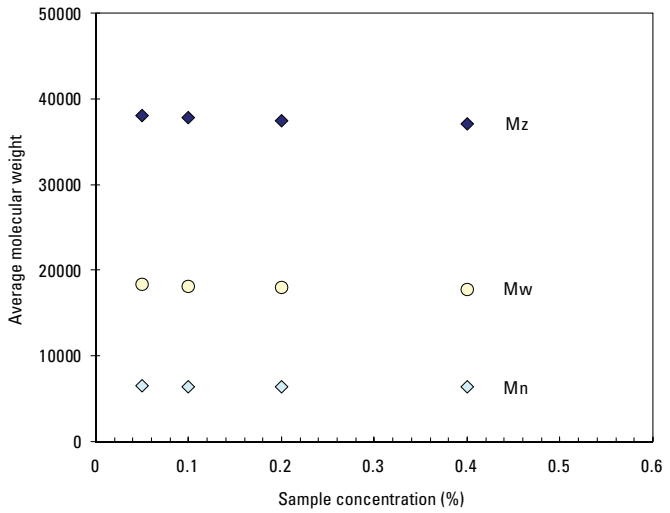
Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Epoxy resin (Mw: approximately 8,000)
Inj. vol.:	15µL
Sample conc.:	0.6, 1.2, 2.5, 5, 10 and 20µg/µL

Figure 20: Epoxy resin chromatograms as a function of sample concentration on a TSKgel SuperMultiporeHZ-M column



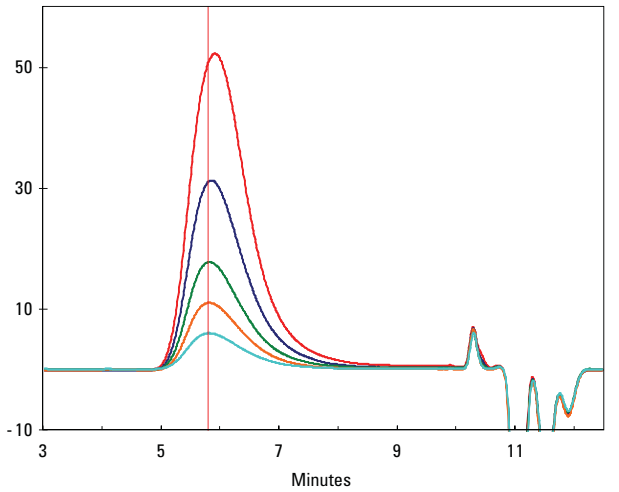
Column:	TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Epoxy resin (Mw: approximately 20,000)
Inj. vol.:	15µL
Sample conc.:	0.5, 1, 1.5, 3, and 5µg/µL

Figure 21: Epoxy resin molecular weight as a function of sample concentration on a TSKgel SuperMultiporeHZ-M column



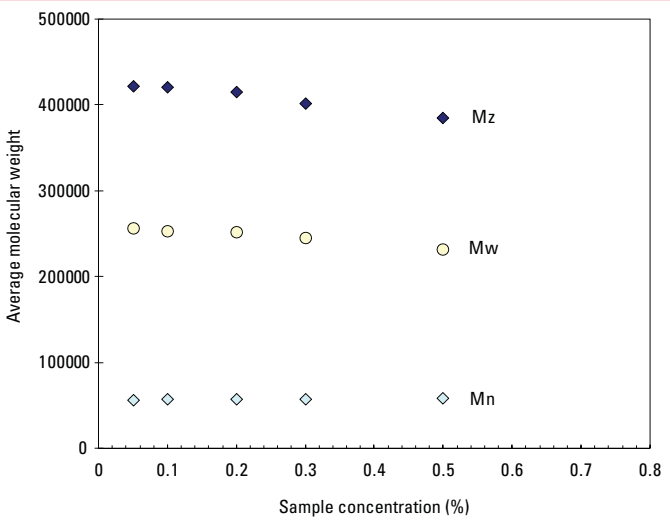
Column:	TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Epoxy resin (Mw: approximately 20,000)
Inj. vol.:	15µL
Sample conc.:	0.5, 1, 2 and 4µg/µL

Figure 22: Polystyrene chromatograms as a function of sample concentration on a TSKgel SuperMultiporeHZ-M column



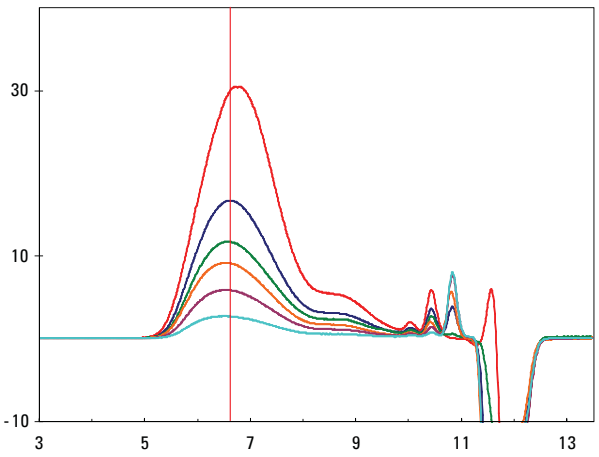
Column:	TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Standard polystyrene (NIST SRM 706; Mw: approximately 258,000)
Inj. vol.:	15µL
Sample conc.:	0.5, 1, 2, 3 and 5µg/µL

Figure 23: Polystyrene molecular weight as a function of sample concentration on a TSKgel SuperMultiporeHZ-M column



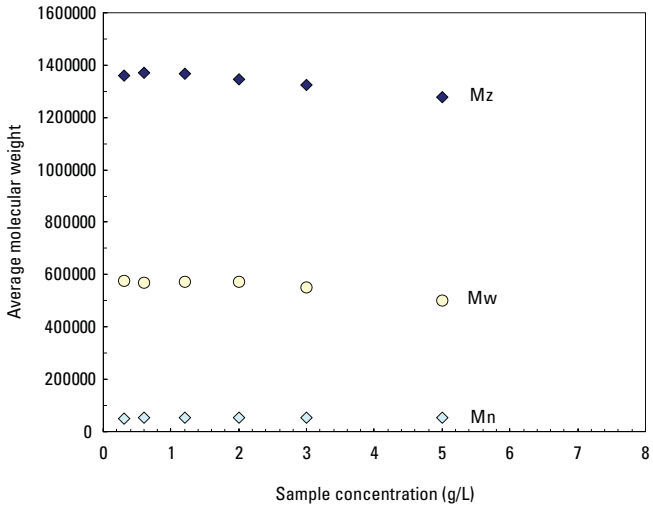
Column:	TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Standard polystyrene (NIST SRM 706; Mw: approximately 258,000)
Inj. vol.:	15µL
Sample conc.:	0.5, 1, 2, 3 and 5µg/µL

Figure 24: Acrylic resin chromatograms as a function of sample concentration on a TSKgel SuperMultiporeHZ-H column



Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Acrylic resin (Mw: approximately 600,000)
Inj. vol.:	15µL
Sample conc.:	0.3, 0.6, 1.2, 2, 3, and 5µg/µL

Figure 25: Acrylic resin molecular weight as a function of sample concentration on a TSKgel SuperMultiporeHZ-H column



Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Temp.:	40°C
Detection:	RI
Sample:	Acrylic resin (Mw: approximately 600,000)
Inj. vol.:	15µL
Sample conc.:	0.3, 0.6, 1.2, 2, 3, and 5µg/µL

3-7. Irregular chromatograms

The most important feature of multi-pore SEC columns is that their pore characteristics (pore structure) eliminate distortions that are observed in chromatograms produced by connecting multiple columns, each with a different pore size, or by multiple units of a mixed-bed type column, each containing a mixture of packing materials of various pore sizes.

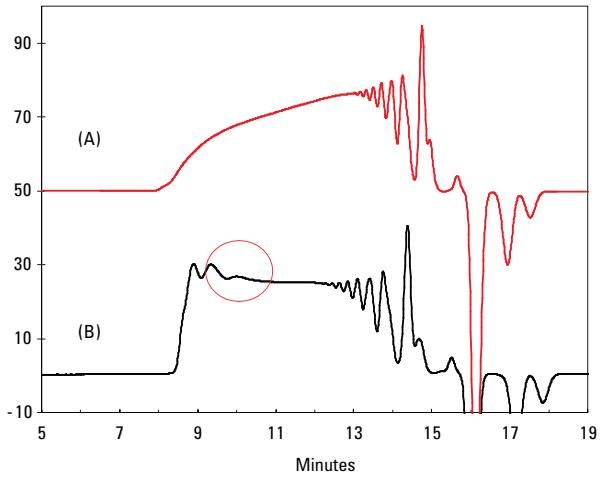
3-7-1. TSKgel SuperMultiporeHZ-N

Figure 26 shows chromatograms of phenol resin obtained with multiple units of the TSKgel SuperMultiporeHZ-N column and with TSKgel SuperHZ (3000 + 2500 + 2000) columns in series. Distortions appear on the chromatogram obtained with the TSKgel SuperHZ Series columns (B), but not on the chromatogram obtained on the TSKgel SuperMultiporeHZ-N column (A).

Figures 27 and *28* show chromatograms of various phenol resins obtained with the TSKgel SuperMultiporeHZ-N column and the TSKgel SuperHZ (3000 + 2000) Series columns. In *Figure 28*, which was created using two TSKgel SuperHZ (3000 + 2000) columns in series, distortions are seen on the chromatogram at a specific elution time with each molecular weight sample. On the other hand, with the TSKgel SuperMultiporeHZ-N column (*Figure 27*), no distortion is observed on the chromatogram with any of the samples.

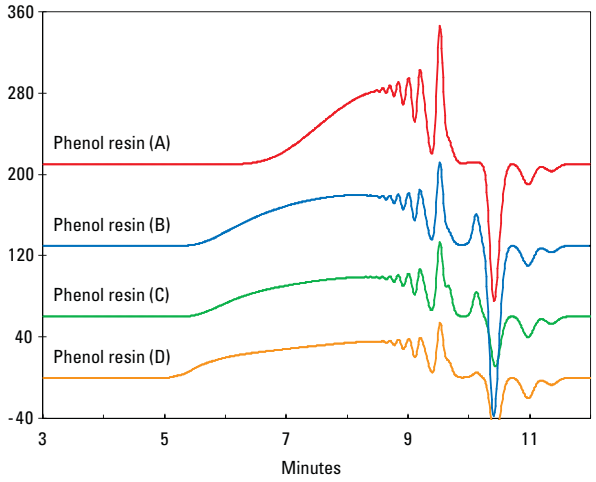
Table 4 shows average molecular weight and polydispersity data when various silicone resins are analyzed with the TSKgel SuperMultiporeHZ-N column, as well as with TSKgel SuperHZ columns in series (4000 + 2000) using packing materials of TSKgel SuperHZ2000 from different lots. As is clear from the table, there is less difference in the average molecular weight data resulting from using packing material of different lots with TSKgel SuperMultiporeHZ-N, a multi-pore type column, than occurs with the TSKgel SuperHZ Series columns. Moreover, *Figure 29* shows chromatograms of silicone resin obtained on columns produced from three lots of TSKgel SuperMultiporeHZ-N packing material. No marked differences between the chromatograms are apparent and it is clear that there is very little difference between lots.

Figure 26: Chromatograms of phenol resins created with the TSKgel SuperMultiporeHZ-N and TSKgel SuperHZ Series columns



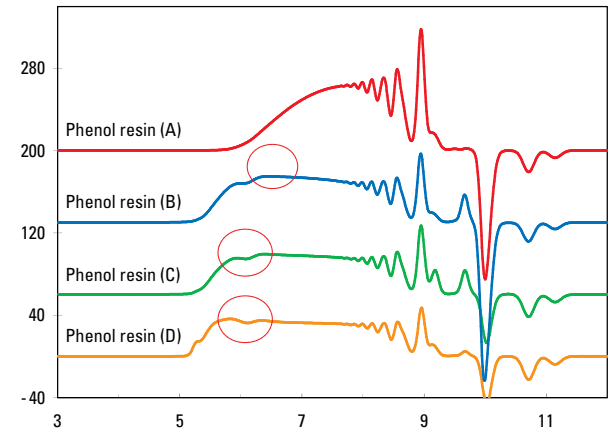
Column:	(A) TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 3 (B) TSKgel SuperHZ 3000+2500+2000 (4.6mm ID x 15cm) x 3
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Phenol resin (3µg/µL)
Inj. vol.:	10µL

Figure 27: Chromatograms of various phenol resins created with the TSKgel SuperMultiporeHZ-N column



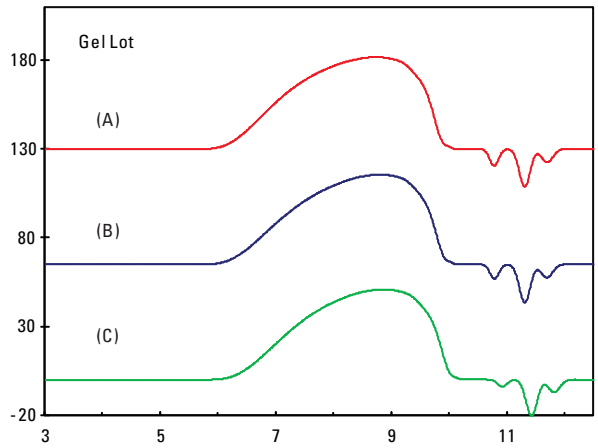
Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.: 4	0°C
Sample:	Phenol resin (3µg/µL)
Inj. vol.:	10µL

Figure 28: Chromatograms of various phenol resins with TSKgel SuperHZ Series columns



Column:	TSKgel SuperHZ3000+2000 (4.6mm ID×15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Phenol resin (3µg/µL)
Inj. vol.:	10µL

Figure 29: Chromatograms of silicone resins obtained on three lots of TSKgel SuperMultiporeHZ-N packing material



Column:	TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Silicone resin (3µg/µL)
Inj. vol.:	10µL

Table 4: Average molecular weight of silicone resin analyzed on multiple lots of TSKgel SuperMultiporeHZ-N columns and TSKgel SuperHZ Series columns

Column (Gel Lot)	Average molecular weight			Polydispersity	
	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
Ave. (RSD)	3,410 (0.37%)	1,340 (0.35%)	7,780 (0.64%)	2.28 (0.36%)	2.54 (0.00%)
TSKgel SuperHZ4000 + TSKgel SuperHZ2000 (A)	3,430	1,330	7,640	2.23	2.58
TSKgel SuperHZ4000 + TSKgel SuperHZ2000 (B)	3,480	1,310	7,990	2.3	2.66
TSKgel SuperHZ4000 + TSKgel SuperHZ2000 (C)	3,370	1,270	7,850	2.33	2.65
TSKgel SuperHZ4000 + TSKgel SuperHZ2000 (D)	3,540	1,320	7,710	2.18	2.68
Ave. (RSD)	3,455 (1.81%)	1,310 (1.74%)	7,800 (1.72%)	2.26 (2.60%)	2.64 (1.43%)

Columns:	(4.6mm ID x 15cm) x 2	Temp.:	40°C
Solvent:	THF	Sample:	Silicone resin (3µg/µL)
Flow rate:	0.35mL/min	Inj. vol.:	10µL
Detection:	RI		

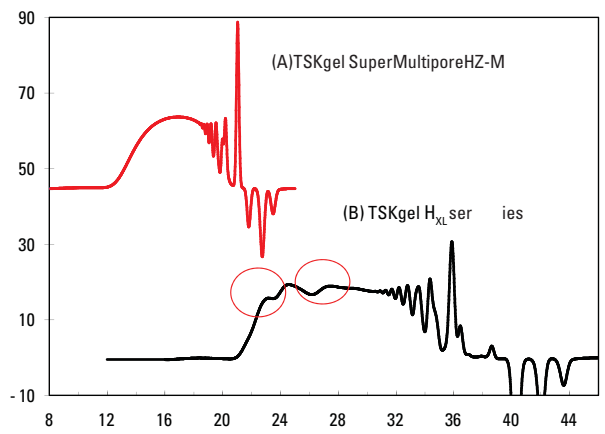
3-7-2. TSKgel SuperMultiporeHZ-M

Figures 30 and *31* show chromatograms of phenol resin produced with the TSKgel SuperMultiporeHZ-M column in comparison with the TSKgel H_{XL} columns in series (grades 4000 + 3000 + 2500 + 2000) and the TSKgel SuperHZ columns in series (grades 4000 + 3000 + 2500 + 2000). Distortion appears on the chromatograms produced with the TSKgel H_{XL} and SuperHZ Series, which does not appear on the TSKgel SuperMultiporeHZ-M column.

Figure 32 shows chromatograms of both grades using an acrylic resin sample. With the acrylic resin, as with the phenol resin sample, distortion appears on the chromatogram produced with the TSKgel H_{XL} Series columns that is not observed with the TSKgel SuperMultiporeHZ-M column.

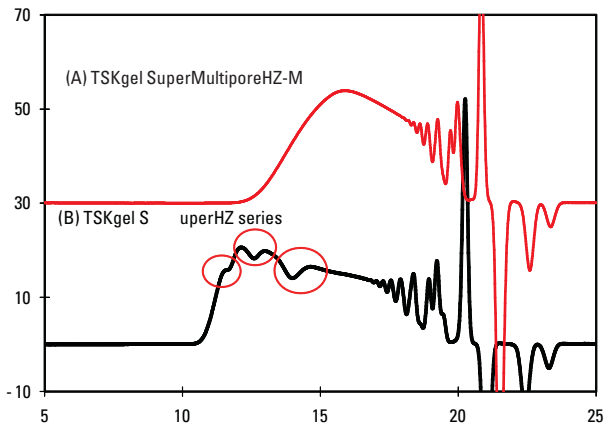
Figure 33 shows chromatograms of phenol resin produced with TSKgel SuperHZM-M, a mixed bed column in which packing materials of different pore sizes are combined in an optimized mixing ratio, and with TSKgel SuperMultiporeHZ-M, a multi-pore type column. Even in a mixed bed column, in which the pore characteristics have been improved by optimizing the packing material mixture, the same type of distortion appears on the chromatogram.

Figure 30: Chromatograms of phenol resin on a TSKgel SuperMultiporeHZ-M column and TSKgel H_{XL} Series columns



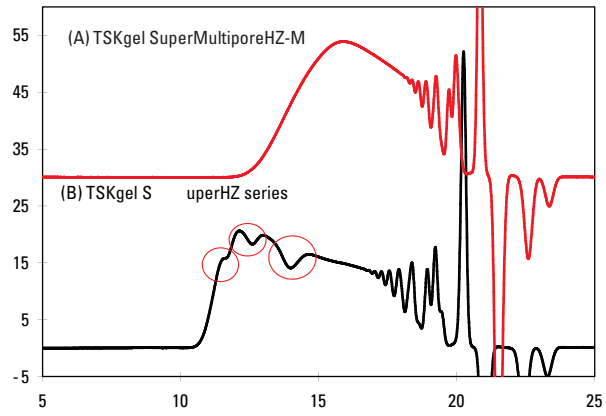
Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 4 (B) TSKgel G4000H_{XL}+3000+2500+2000 (7.8mm ID x 30cm) x 4
Solvent:	THF
Flow rate:	(A) 0.35mL/min (B) 1.0mL/min
Detection:	RI
Temp.:	40°C
Sample:	Phenol resin (3µg/µL)
Inj. vol.:	(A) 10µL (B) 50µL

Figure 31: Chromatograms of phenol resin on a TSKgel SuperMultiporeHZ-M column and TSKgel SuperHZ Series columns



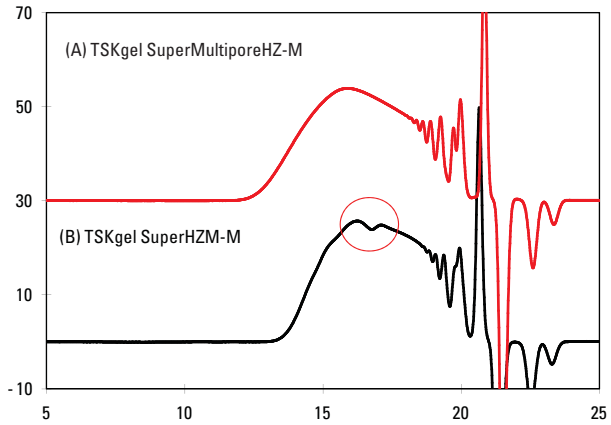
Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 4 (B) TSKgel SuperHZ4000+3000+2500+2000 (4.6mm ID x 15cm) x 4
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Phenol resin (3µg/µL)
Inj. vol.:	10µL

Figure 32: Chromatograms of acrylic resin on a TSKgel SuperMultiporeHZ-M column and TSKgel SuperHZ Series columns



Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 4 (B) TSKgel SuperHZ4000+3000+2500+2000 (4.6mm ID x 15cm) x 4
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Acrylic resin (3µg/µL)
Inj. vol.:	10µL

Figure 33: Chromatograms of phenol resin created with a TSKgel SuperMultiporeHZ-M column and a TSKgel SuperHZM-M column



Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm) x 4 (B) TSKgel SuperHZM-M (4.6mm ID x 15cm) x 4
Solvent:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temp.:	40°C
Sample:	Phenol resin (3µg/µL)
Inj. vol.:	10µL

3-7-3. TSKgel SuperMultiporeHZ-H

Figure 34 shows chromatograms of a styrene-acrylic resin copolymer obtained using TSKgel SuperHZM-H, a mixed bed type of column in which packing materials of different pore sizes are combined at an optimized mixing ratio, and TSKgel SuperMultiporeHZ-H, a multi-pore type of column for polymer analysis.

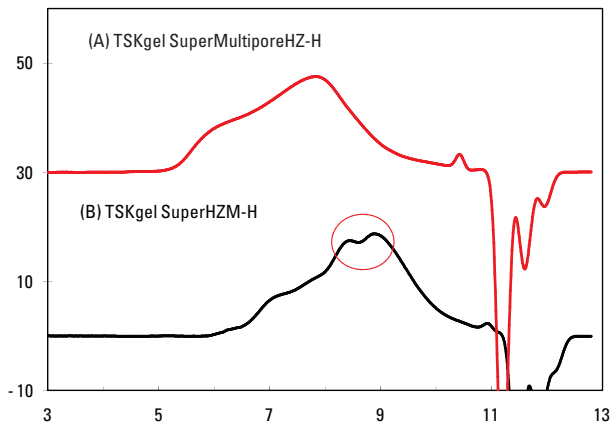
Even for TSKgel SuperHZM-H, a mixed bed column in which the pore characteristics have been improved by optimizing the packing material mixture, distortion of the chromatogram is confirmed, similar to what was observed with the TSKgel SuperHZM-M column.

3-7-4. Comparison to other commercially available product

Figure 35 shows chromatograms of phenol resin obtained on the TSKgel SuperMultiporeHZ-M column and with a mixed bed column from another company, which is similar to the TSKgel SuperHZM-M column.

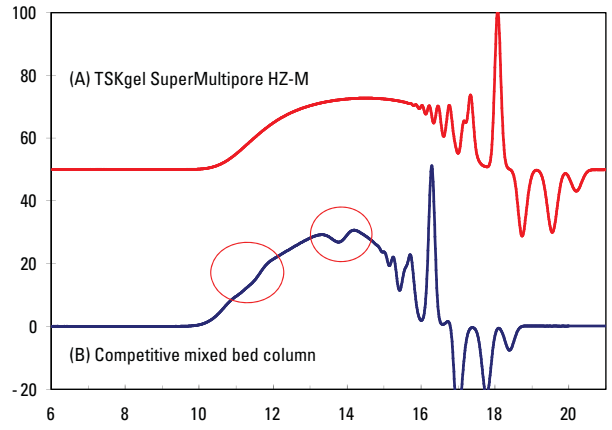
The same type of distortions seen in *Figure 33* can also be observed here with this mixed bed type of column.

Figure 34: Chromatograms of a styrene-acrylic resin copolymer obtained with a TSKgel SuperMultiporeHZ-H column and a TSKgel SuperHZM-H column



Columns:	(A) TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 2 (B) TSKgel SuperHZM-H (4.6mm ID x 15cm) x 2
Solvent:	THF
Flow rate:	0.35mL/min
Temp.:	40°C
Detection:	RI
Sample:	Styrene-acrylic resin copolymer (3µg/µL)
Inj. vol.:	10µL

Figure 35: Chromatograms of phenol resin obtained with a TSKgel SuperMultiporeHZ-M column and a mixed bed column from a competitor

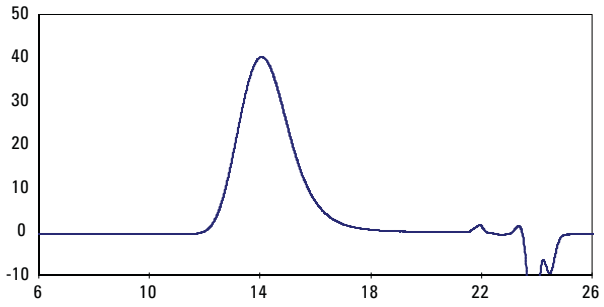


Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 25cm) (B) Competitive mixed bed column (4.6mm ID x 25cm)
Solvent:	THF
Flow rate:	0.35mL/min
Temp.:	40°C
Detection:	RI
Sample:	Phenol resin (3µg/µL)
Inj. vol.:	10µL

4. Application examples

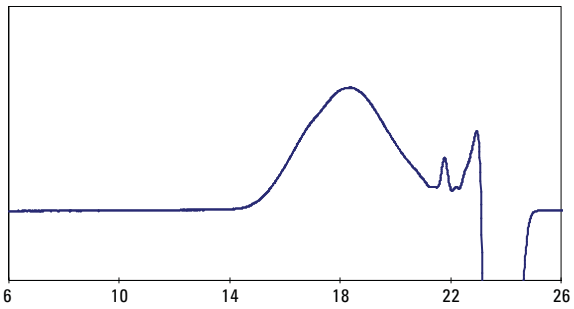
Figures 36 to *43* show chromatograms of various polymers analyzed using the TSKgel SuperMultiporeHZ-H column. Smooth chromatograms without any distortion are obtained for each of these polymers.

Figure 36: Separation of polyisobutylene on a TSKgel SuperMultiporeHZ-H column



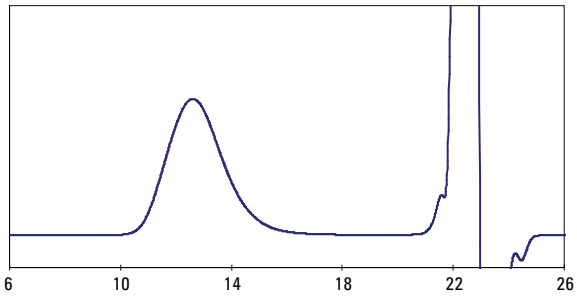
Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Polyisobutylene (3µg/µL)
Inj. vol.:	10µL

Figure 37: Separation of acrylic resin (1) on a TSKgel SuperMultiporeHZ-H column



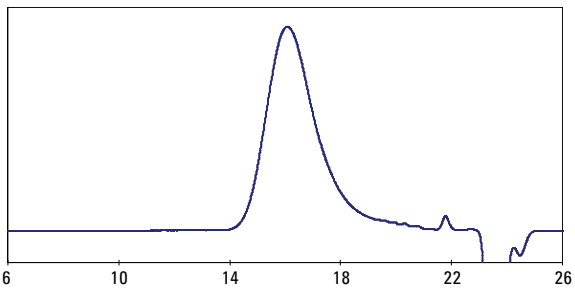
Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Acrylic resin (1) (3µg/µL)
Inj. vol.:	10µL

Figure 38: Separation of polystyrene (NIST SRM 706) on a TSKgel SuperMultiporeHZ-H column



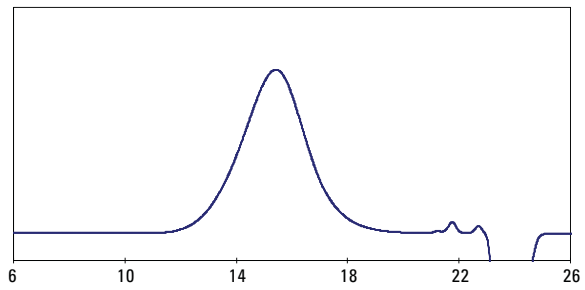
Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Polystyrene (NIST SRM 706) (3µg/µL)
Inj. vol.:	10µL

Figure 39: Separation of polybutadiene on a TSKgel SuperMultiporeHZ-H column



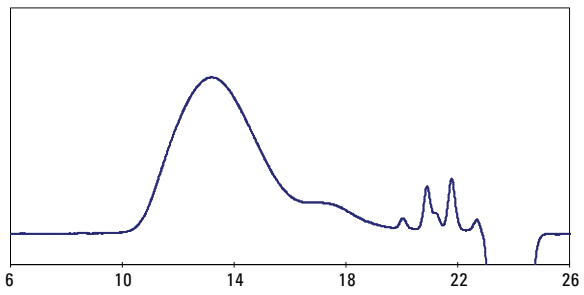
Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Polybutadiene (3µg/µL)
Inj. vol.:	10µL

Figure 40: Separation of polyvinylbutyral on a TSKgel SuperMultiporeHZ-H column



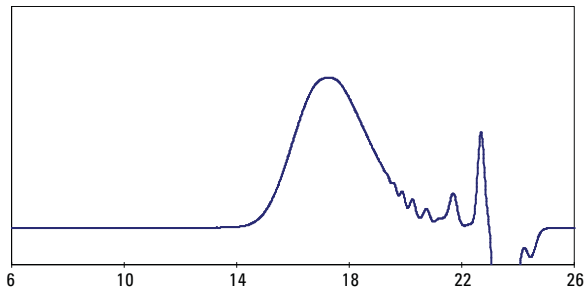
Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Polyvinylbutyral (3µg/µL)
Inj. vol.:	10µL

Figure 41: Separation of polycarbonate on a TSKgel SuperMultiporeHZ-H column



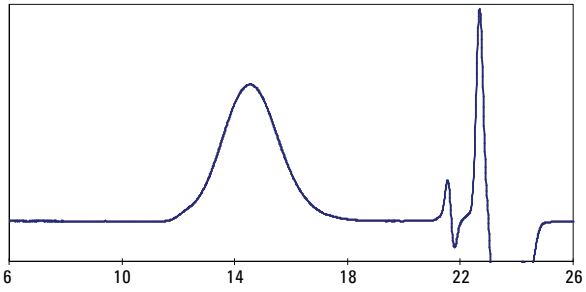
Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Polycarbonate (3µg/µL)
Inj. vol.:	10µL

Figure 42: Separation of epoxy resin on a TSKgel SuperMultiporeHZ-H column



Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Epoxy resin (3µg/µL)
Inj. vol.:	10µL

Figure 43: Separation of acrylic resin (2) on a TSKgel SuperMultiporeHZ-H column



Column:	TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm) x 4
Solvent:	THF
Detection:	RI
Flow rate:	0.35mL/min
Temp.:	40°C
Sample:	Acrylic resin (2) (3µg/µL)
Inj. vol.:	10µL

5. Conclusions

The TSKgel SuperMultiporeHZ-type columns are a new series of semi-micro multi-pore columns for use in SEC with organic solvent systems that are capable of producing ideal chromatograms. In addition to maintaining the positive features of the TSKgel MultiporeH$_{XL}$-M columns, this new series of columns is capable of high-speed analysis by applying packing materials composed of monodisperse particles, while at the same time cutting down on solvent consumption by reducing column dimensions to a semi-micro level. To achieve optimum precision, it is recommended that TSKgel SuperMultiporeHZ-type columns be used in combination with the EcoSEC HLC-8320 GPC system, a high speed GPC instrument that is compatible with semi-micro columns.

Reference

M. Nagata, T. Kato, H. Furutani, J. Liq. Chrom & Rel. Technol., 21 (10) 1471-1484 (1998)