A new sophisticated GPC/SEC instrument for high-throughput and semi-micro SEC

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Introduction

EcoSEC is a new sophisticated HPLC instrument from Tosoh that was developed for semi-micro and high throughput size exclusion chromatography (SEC), specifically gel permeation chromatography (GPC).

The EcoSEC instrument consists of:

- on-line degasser
- auto-injector
- temperature-controlled pumping system
- column oven
- refractive-index (RI) detector
- optional UV detector

The EcoSEC system is contained in a single chassis and is computer-controlled.
The performance of this new GPC/SEC instrument was evaluated by testing:

- flow rate reproducibility
- precision of temperature control in the pumping unit, column oven and RI detector
- base line stability
- sensitivity of the RI detector
- other system characteristics

In addition, the precision of measuring molar mass and molar mass distribution was evaluated in combination with semi-micro SEC columns. TSK-GEL SuperMultiporeHZ type columns are packed with monodisperse polystyrene particles which contain a broad pore size distribution. The EcoSEC system, in combination with TSK-GEL SuperMultiporeHZ type columns, was assessed for its performance of the analysis of various polymer samples.

Experimental

Instrumentation

HLC-8320GPC, or EcoSEC (Tosoh, Japan), was used as a dedicated GPC/SEC system and contains an on-line degasser, a pumping unit, an auto-injector, a column oven, and a refractive index (RI) detector. To limit baseline fluctuations, the pumping system and the RI detector are positioned in a temperature-controlled environment.

Chemicals and Materials

Stabilized analytical grade tetrahydrofuran (THF) was used as an eluent without further treatment (Wako, Japan). Synthetic polymers and other reference standards were obtained from Wako and other resin suppliers. Polystyrene standards with narrow molecular mass distribution were obtained from Tosoh.

Experimental

Columns

TSKgel SuperMultiporeHZ-H (4.6mm ID x 15cm),
TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm),
TSKgel SuperMultiporeHZ-N (4.6mm ID x 15cm),
TSKgel MultiporeH_{XL}-M (7.8 mm ID x 30cm), and
TSK-GEL SuperHZ type columns (4.6mm ID x 15cm) were all obtained from Tosoh (Tokyo, Japan).

Commercially available columns

Mixed-bed type columns (4.6mm ID x 25cm) were obtained from Polymer Laboratories (UK).

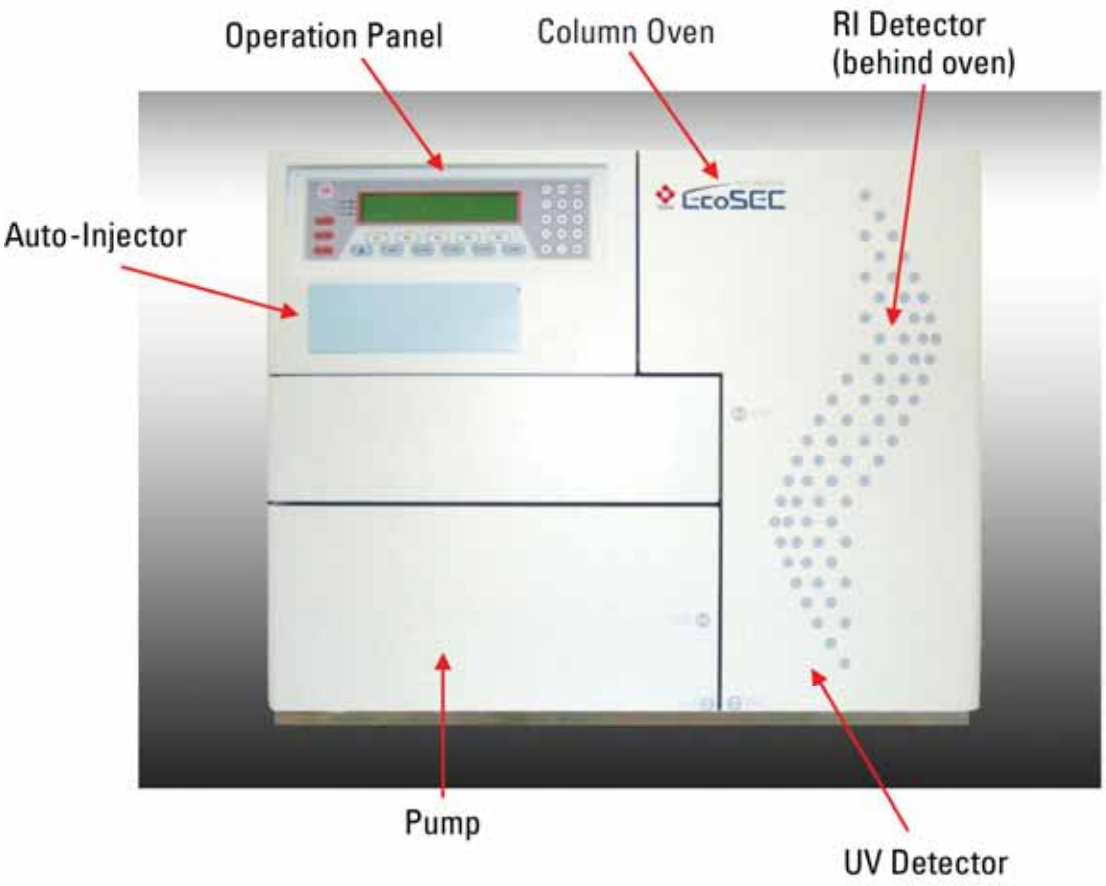
Conditions

Eluent: THF, Flow rate: 0.35mL/min, Temperature: 40ºC
Detection: RI and UV@254nm

Preparation of sample solutions

Synthetic polymers were dissolved in THF at concentrations of 0.1- 0.0125g/L. Polystyrene standards were dissolved in THF at concentrations of 0.2-1.0g/L and gently stirred for a period of 12-24 hours prior to use.

Figure 1: Features and Specifications of EcoSEC



Specifications

1. Pump

Flow Rate:	10 to 2,000µL/min
Accuracy:	+/- 2%
Precision:	+/- 0.2%
Max pressure:	25MPa

2. Auto-injector

Injection vol.:	1 to 1,500µL
# of samples:	100

3. Column oven

Temp range:	ambient +10 to 60°C
Capacity:	eight, 7.8mm ID*30cm columns

4. Detector(RI)

Type:	Dual flow type
Cell Volume:	2.5µL

5. Detector(UV): optional

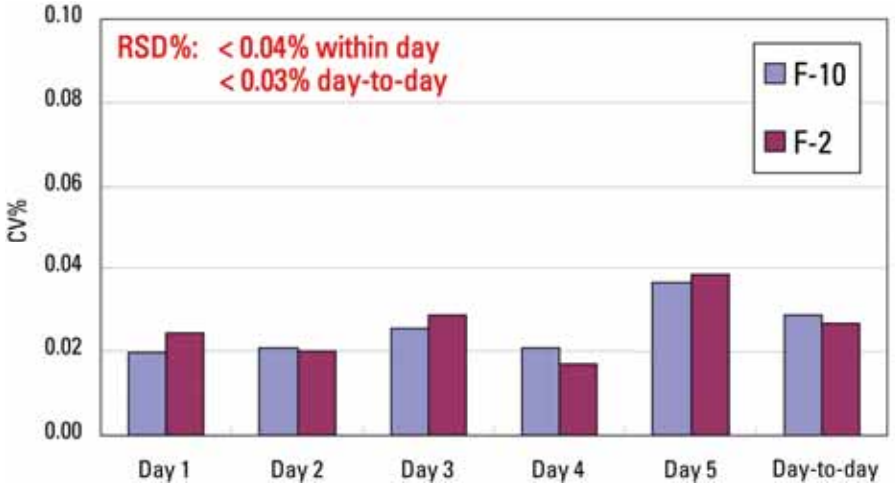
Wave length:	195 to 350nm
Cell volume:	2µL

6. Dimensions

680(W) x 500(D) x 580(H)mm

The EcoSEC system consists of an on-line degasser, a temperature-controlled pump, an auto-injector, a column oven, a temperature controlled RI detector, and an optional UV detector.

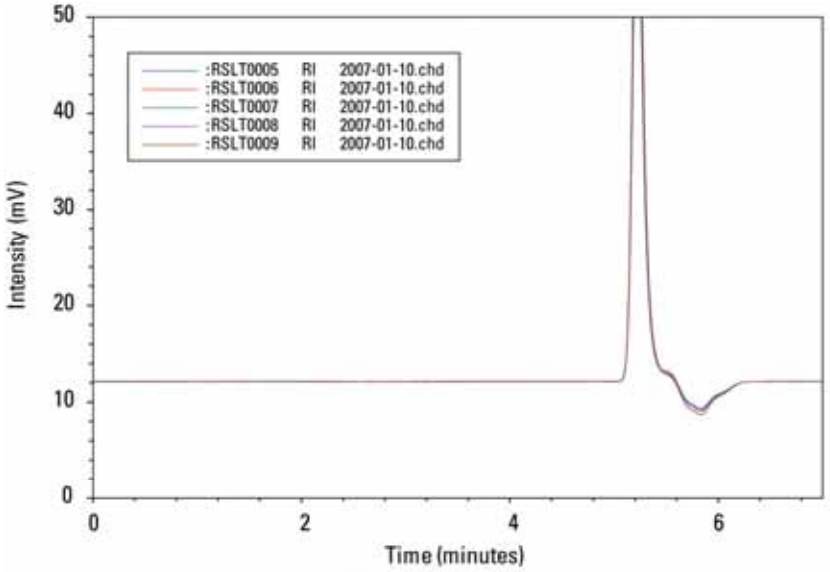
Figure 2: Reproducibility of Polystyrene Retention



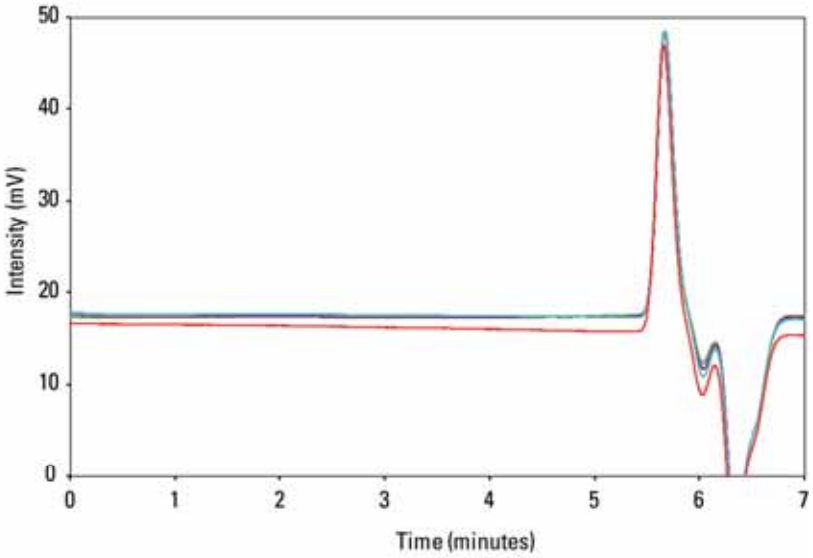
Column:	TSKgel SuperMultiporeHZM-M x 2 (4.6mm ID x 15cm)
Eluent:	THF
Flow Rate:	0.35mL/min
# of injections:	10/day
Samples:	F-10: polystyrene standard (~100,000 Dalton)
	F-2: polystyrene standard (~10,000 Dalton)

Excellent reproducibility of retention times can be achieved by controlling the temperature of the pumping system and RI detector. Relative standard deviations (RSD) for within-day and day-to-day runs were less than 0.04% and less than 0.03%, respectively.

Figure 3: Comparison of RI Baseline Variation between EcoSEC and Commercial Component GPC System



EcoSEC

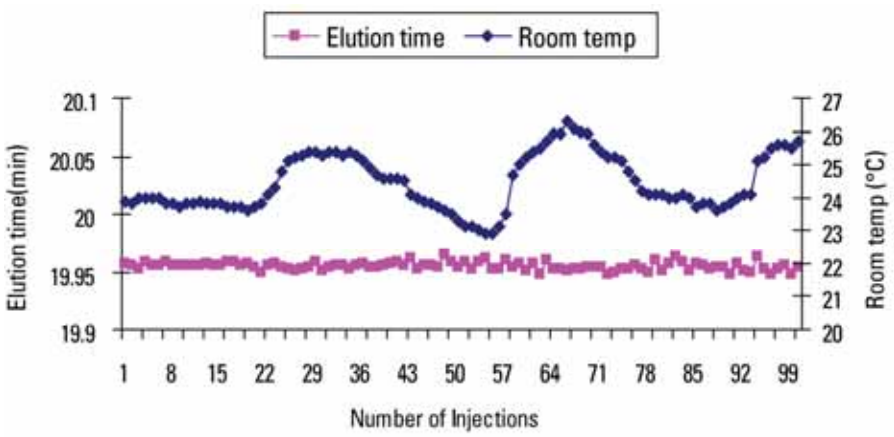


Component GPC system

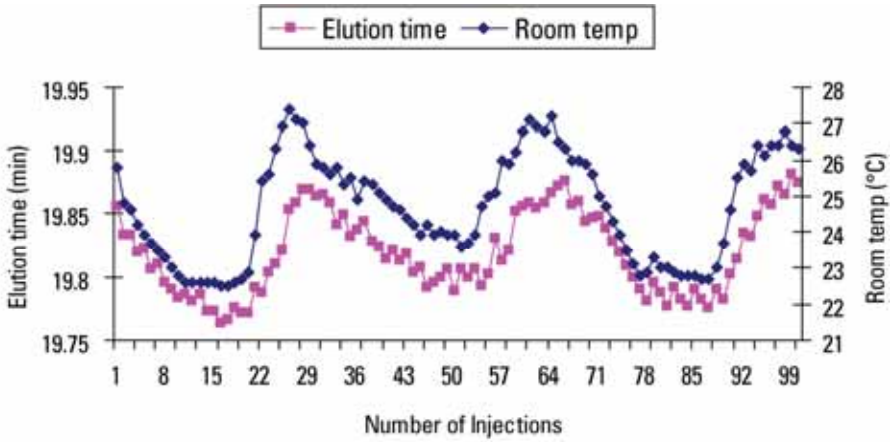
Column:	TSKgel SuperHZ-H (4.6mm ID x 15cm)
Eluent:	THF
Flow rate:	0.35mL/min
Sample:	dicyclohexyl phthalate
# of injections:	5
x-axis scale:	7min
y-axis full scale:	50mV

The EcoSEC system contains a dual-flow type RI detector, which contributes to the excellent baseline stability compared with a GPC system assembled from individual components.

Figure 4: Comparison of Effect of Laboratory Temperature on Retention Time between EcoSEC and Component GPC System



EcoSEC

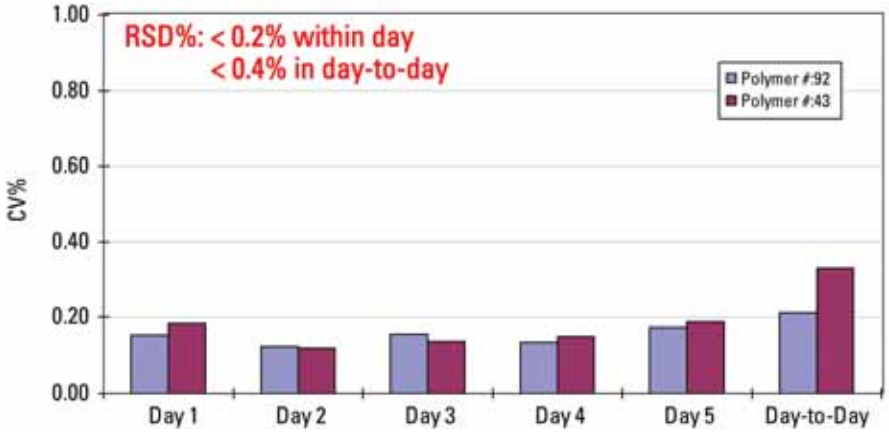


Component GPC system

Column:	TSKgel SuperHM x 2 (4.6mm ID x 15cm)
Eluent:	THF
Flow Rate:	0.35mL/min
Sample:	dicyclohexyl phthalate

Excellent reproducibility of retention times on the EcoSEC system were achieved by controlling the temperature of the pumping system and the RI detector and were not affected by normal variations in laboratory temperature.

Figure 5: Reproducibility of Molecular Mass Measurement



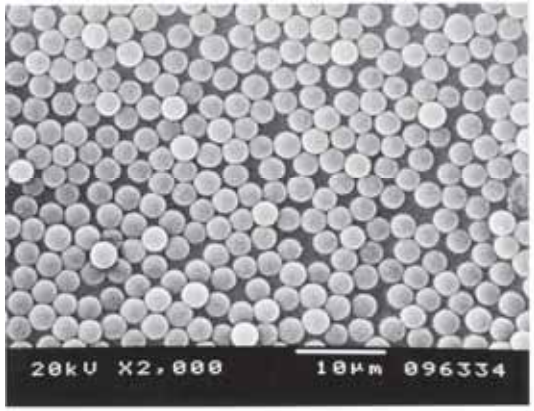
Column:	TSKgel SuperHM x 2 (4.6mm ID x 15cm)
Eluent:	THF
Flow rate:	0.35mL/min
# of injection:	10/day
Samples:	Polymer #: 92: vinylchloride/vinylacetate copolymer (MW 2.4×10^4 Da)
	Polymer #: 43: polycaprolactone (MW 1.5×10^4 Da)

As a result of the low RSD values for retention times, excellent precision of molar mass (MW) measurements were obtained for two polymer standard samples. The RSD values for molar mass measurements within the same day and from day-to-day were less than 0.2% and less than 0.4%, respectively.

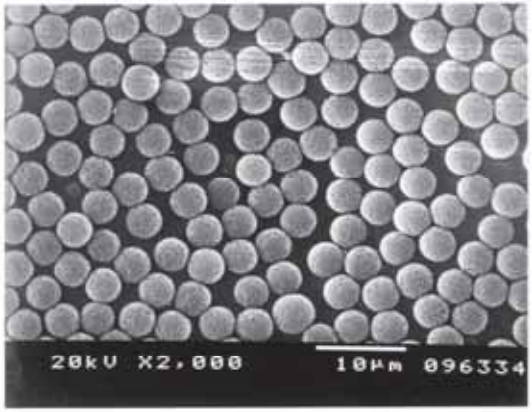
Table 1: Physical Properties of TSKgel SuperMultiporeHZ-M and TSKgel SuperMultiporeHZ-N Columns

Parameter	TSKgel SuperMultiporeHZ-N	TSKgel SuperMultiporeHZ-M
Base material	Poly(Styrenedivinylbenzene)	
Particle diameter	3µm	4µm
Max. exclusion limit MW	120,000	2,000,000
Mean pore size	8nm	14nm
Range of polystyrene sample	50,000 ~ 500	1,000,000 ~ 500
Theoretical plates	18,000/15cm	16,000/15cm
Column size (Analytical)	4.6mm ID x 15cm	
Column size (Guard)	4.6mm ID x 23cm	

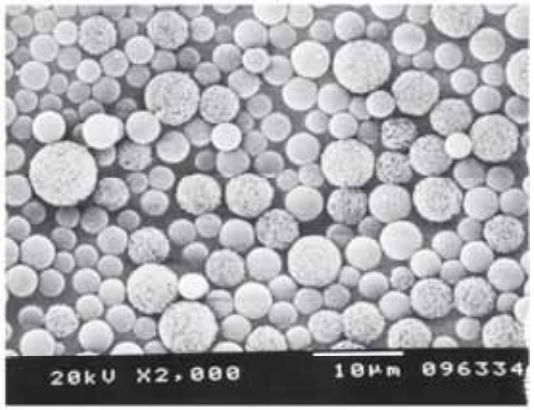
Figure 6: SEM Pictures of TSK-GEL SuperMultiporeHZ Type and Mix-bed Type Particles



TSKgel SuperMultiporeHZ-N
(3 micron particles)



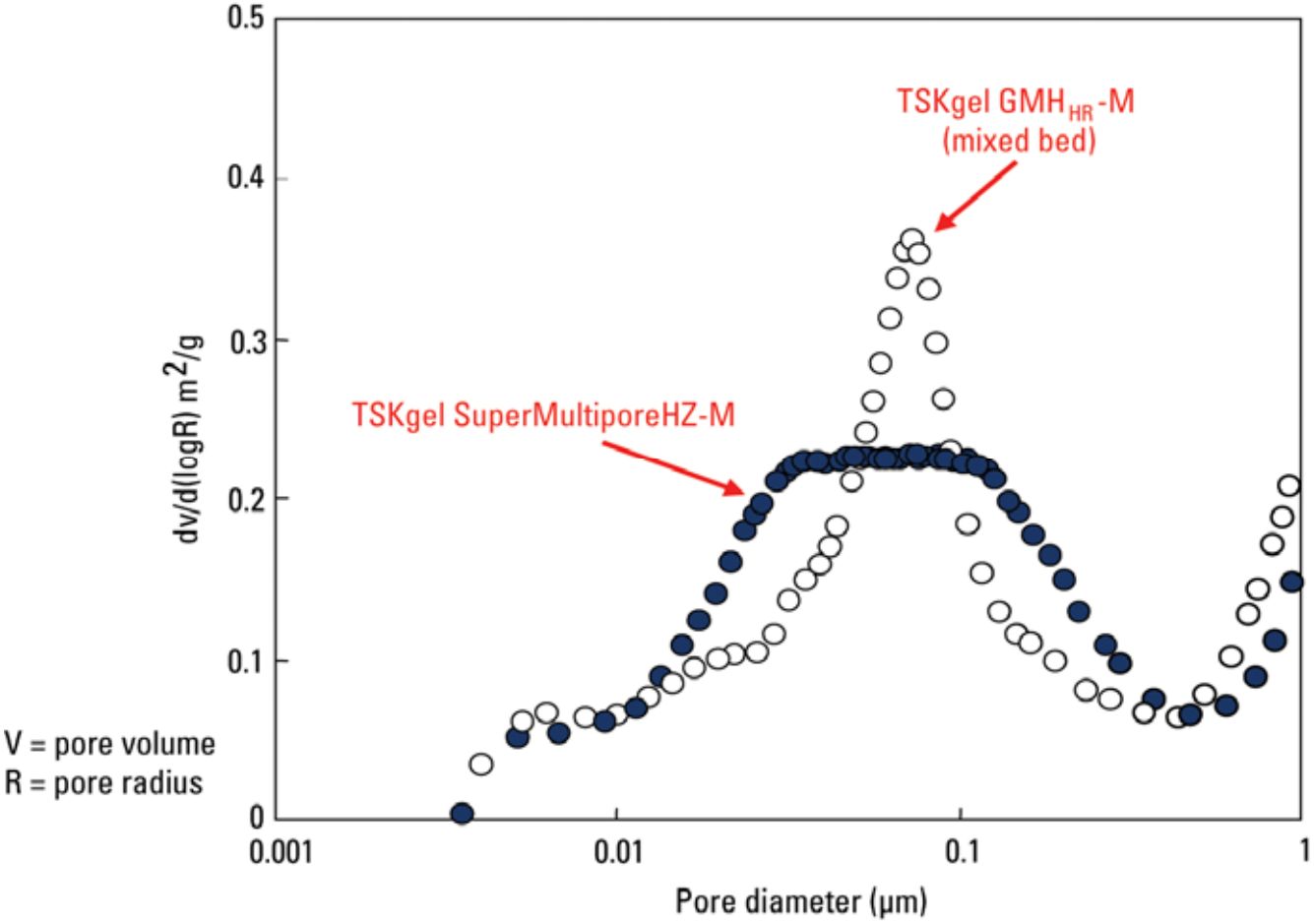
TSKgel SuperMultiporeHZ-M
(4 micron particles)



TSKgel SuperHZM-M (mixed-bed)
(4-7 micron particles)

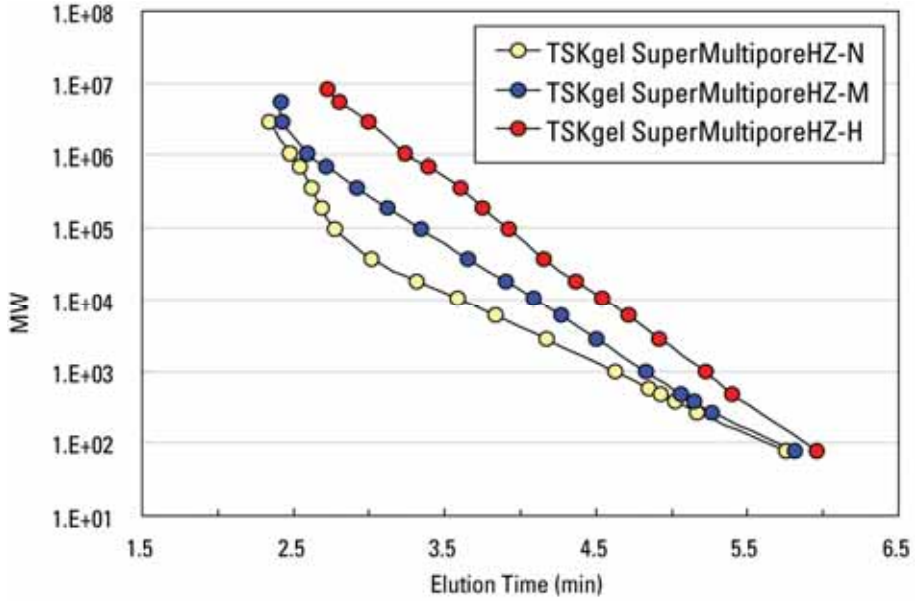
In recent years Tosoh scientists developed novel multi-pore particles in an effort to improve the linearity of so-called mixed-bed columns. TSK-GEL SuperMultiporeHZ-M and SuperMultiporeHZ-N columns are packed with spherical monodispersed PS-DVB particles of 3 and 4 micron size, respectively. Column dimensions have been minimized to allow for fast high-throughput analyses and to reduce solvent consumption.

Figure 7: Pore Characteristics of Particles in TSK-GEL SuperMultiporeHZ-M and Mixed-bed Type Columns



The surface area in the particles of a TSKgel SuperMultiporeHZ-M column is distributed over a much broader range of pore diameters than what is found in a representative conventional GPC column.

Figure 8: Calibration Curves of TSKgel SuperMultiporeHZ Type Columns

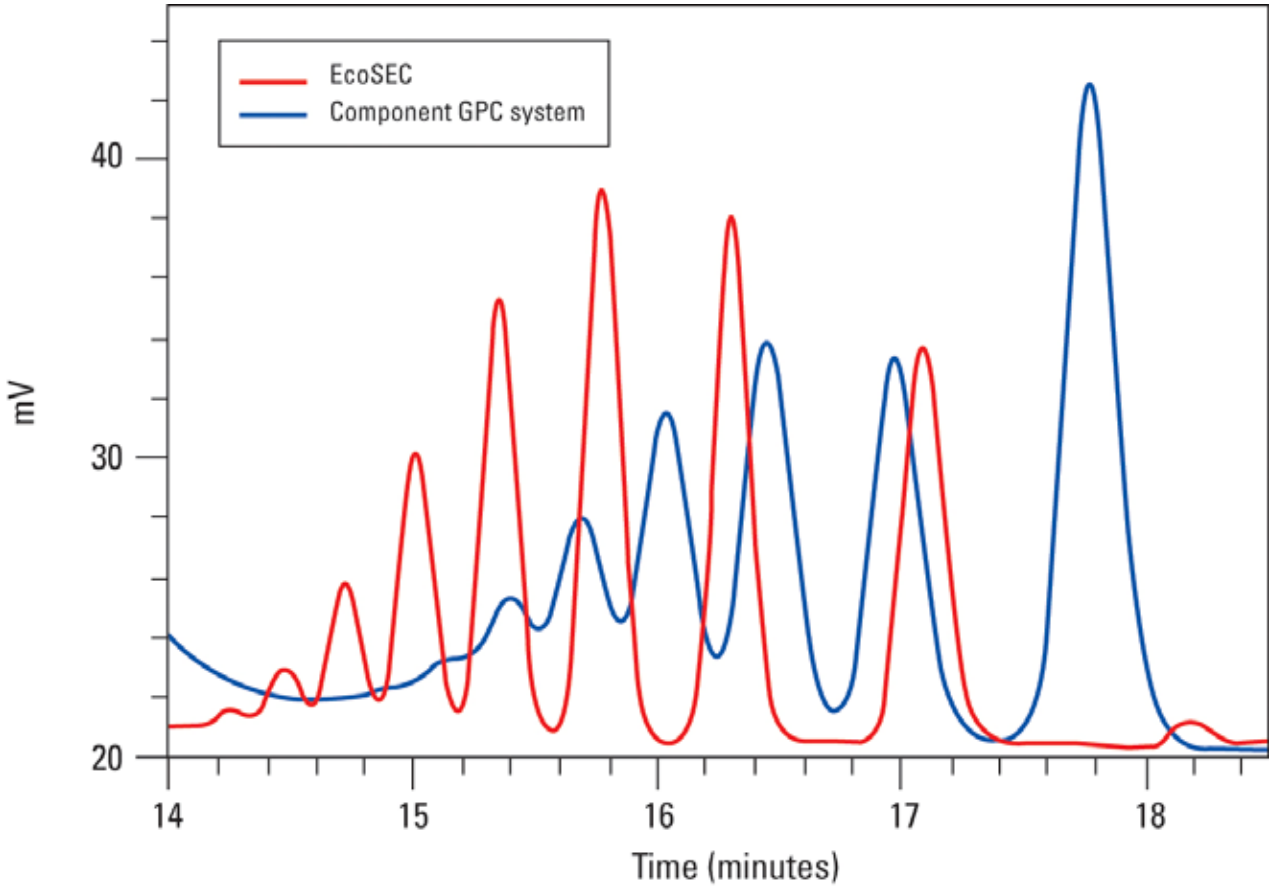


Columns:	4.6mm ID x 15cm
Eluent:	THF
Flow rate:	0.35mL/min
Temperature:	25°C
Detection:	UV@254nm
UV cell:	UV-8020 microcell
Samples:	polystyrene standards

	Linearity	Slope	MW limit (Da)	Porosity	Pore Size (Å)
SuperMultiporeHZ-N	0.9996	-2.8131	125,000	70.3	84
SuperMultiporeHZ-M	0.9998	-3.8211	1,810,000	72.6	138
SuperMultiporeHZ-H	0.9992	-4.4924			

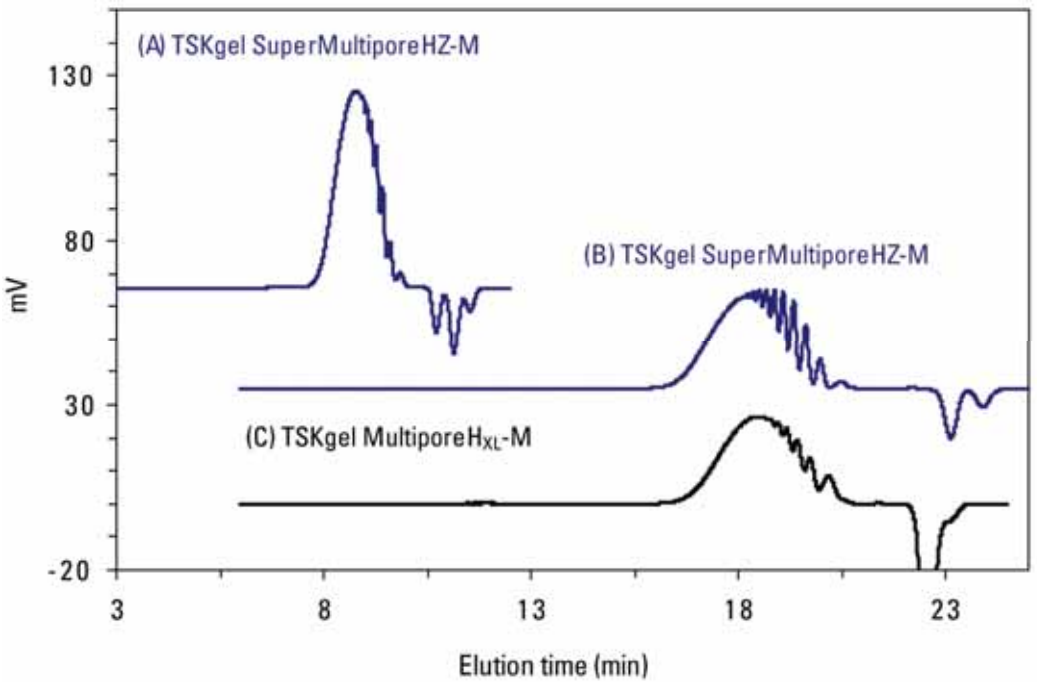
TSK-GEL SuperMultiporeHZ type columns show good linearity of the calibration curves of standard polystyrenes in THF. For TSKgel SuperMultiporeHZ-N, the linear range extends from MW 500 to MW 38,000 Dalton, while the TSKgel SuperMultiporeHZ-M column exhibits a linear range from MW 500 to 1 million Dalton.

Figure 9: Effect of System Dead Volume on Peak Resolution



As expected, it is critical to minimize system dead volume in order to take full advantage of high performance semi-micro GPC columns.

Figure 10: Chromatograms of PTMEG on TSKgel SuperMultiporeHZ-M and TSKgel MultiporeH$_{XL}$-M Columns

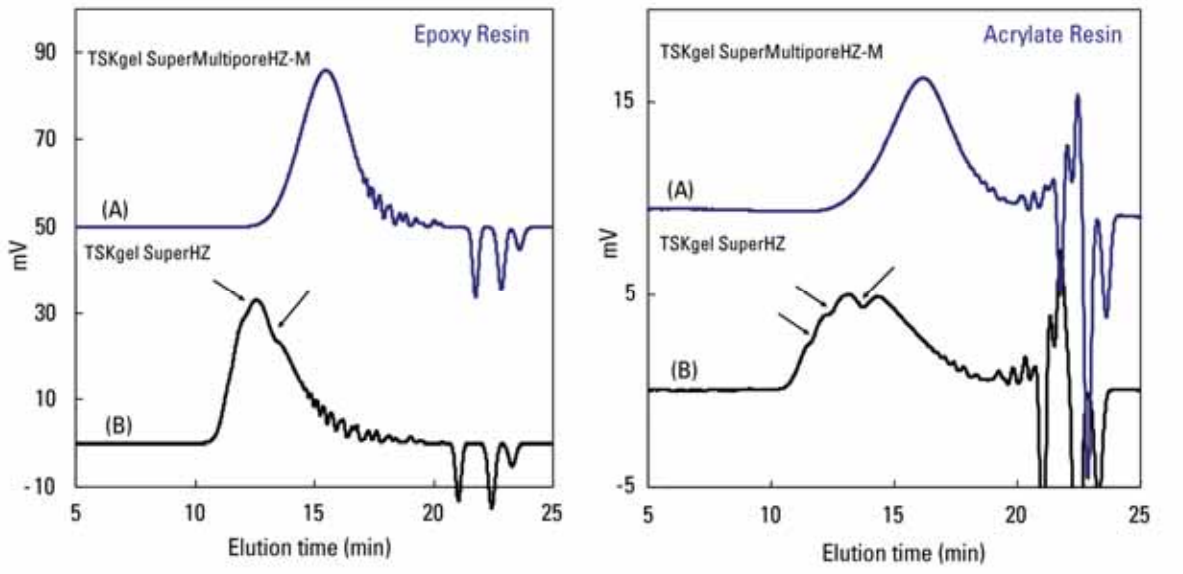


Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm x 2)
	(B) TSKgel SuperMultiporeHZ-M (4.6mm ID x 15cm x 4)
	(C) TSKgel MultiporeHXL-M (7.8mm ID x 30cm x 2)
Eluent:	THF
Flow rate:	(A): 0.35mL/min
	(B): 0.35mL/min
	(C): 1.0mL/min
Temp.:	40°C
Detection:	RI (HLC-8120GPC)
Sample:	PTMEG* 650 (1.0%)
Inj. Vol.:	(A) & (B): 10µL
	(C): 35µL

* Polytetramethylene ether glycol

While the eluent consumption on semi-micro columns is about 1/6th of that for a conventional 7.8mm ID column, similar resolution can be obtained in half the analysis time, or improved resolution can be obtained in the same analysis time, as that for conventional GPC columns.

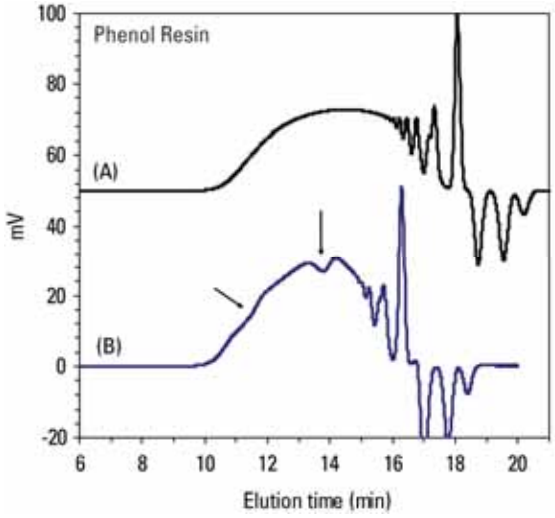
Figure 11: Comparison of Chromatogram between TSK-GEL SuperMultiporeHZ-M and Commercial Columns

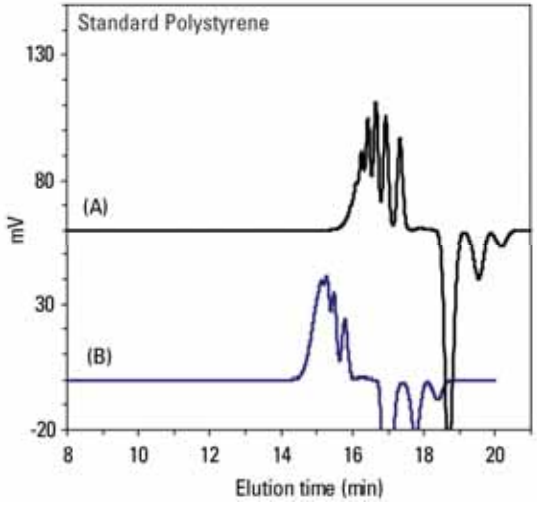


Columns:	(A) SuperMultiporeHZ-M (4.6mm ID x 15cm x 4) (B) TSkgel SuperHZ4000 + SuperHZ3000 + SuperHZ2500 + SuperHZ2000 (4.6mm ID x 15cm x 4)
Eluent:	THF
Flow rate:	0.35mL/min
Temp.:	40°C
Detection:	RI (EcoSEC)
Samples:	epoxy resin (Epikote 1009), 0.3% acrylic resin, 0.3%
Inj. Vol.:	10µL

Various polymers were analyzed on the TSK-GEL SuperMultiporeHZ type columns. The separations were completed in half the time required for conventional columns and with better resolution when obtained on the EcoSEC system. Chromatograms of broad molecular weight distribution polymers on a TSKgel SuperMultiporeHZ-M column were found to be very smooth without any inflection points, in contrast to those obtained on commercial columns.

Figure 12: Comparison of Chromatogram between TSKgel SuperMultiporeHZ-M and Commercial Mixed-bed Type Columns





Columns:	(A) TSKgel SuperMultiporeHZ-M (4.6mm ID x 25cm x 2)
	(B) Commercial mixed-bed type column (4.6mm ID x 25cm x 2)
Eluent:	THF
Flow rate:	0.35mL/min
Temperature:	40°
Detection:	RI (EcoSEC)
Inj. vol.:	10µL
Samples:	phenolic resin, 0.5%
	standard polystyrene, 0.2%

Chromatograms of synthetic polymers on semi-micro TSK-GEL SuperMultiporeHZ columns using the new EcoSEC instrument can be obtained without any inflection points for polymers of broad molecular weight distribution, in contrast to those obtained on commercial columns. Moreover, the TSKgel SuperMultiporeHZ-M column was found to provide higher resolution for polystyrene oligomers than what can be obtained on commercial mixed-bed type columns.

Table 2 Recovery & Repeatability of MTX Analysis using TSKgel BSA-ODS/V Column

Concentration*	Recovery**	Repeatability (RSD, %, n=6)	
(µg/mL)	(%)	Standard	Spiked serum
0.2	116.3	1.29%	2.48%
2	106	0.72%	2.45%

* Two-fold dilution
** Average (n=6)