



EcoSEC GPC System

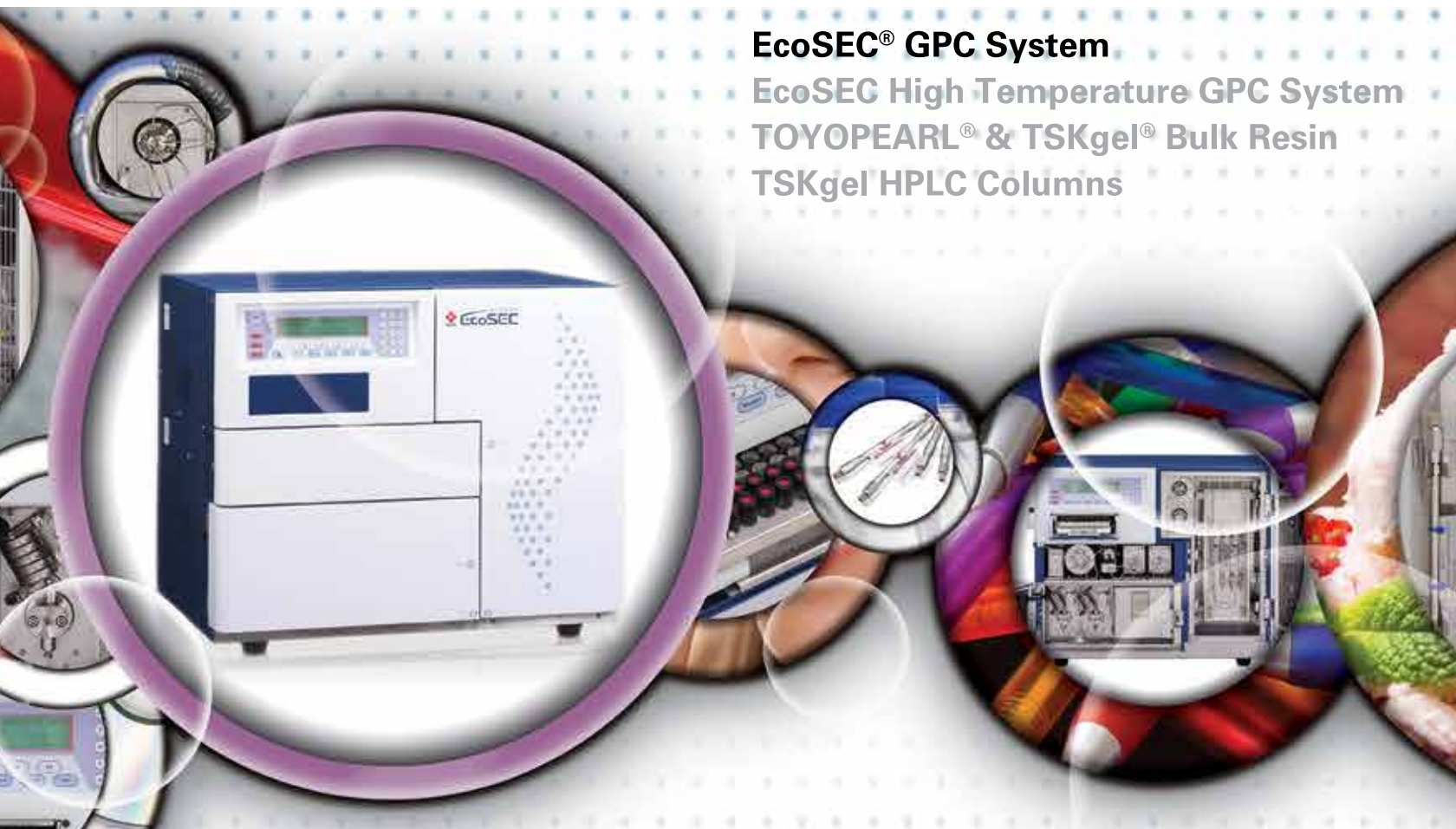
Experts in Chromatography

EcoSEC® GPC System

EcoSEC High Temperature GPC System

TOYOPEARL® & TSKgel® Bulk Resin

TSKgel HPLC Columns



2015

Product Guide

TOSOH BIOSCIENCE

GPC Sample Analysis Program



With our well-equipped labs and expert GPC scientists, Tosoh provides a broad range of analytical services to help your team with extensive analysis of organic and aqueous soluble polymers using our EcoSEC GPC System and EcoSEC High Temperature GPC System.

The GPC Sample Analysis Program offers:

- Peak position calibration molar mass determination by GPC/RI or GPC/UV
- Universal calibration molar mass determination by GPC/RI/VISC
- Absolute molar mass determination by GPC/RI/MALS
- Polymeric size (radius of gyration) determination by GPC/RI/MALS
- Polymer size (viscometric radius) determination by GPC/RI/VISC
- Conformation/Architecture (dimensionless ratios and Mark-Houwink plots) by GPC/RI/MALS/VISC
- dn/dc determination

To learn more about our GPC Sample Analysis Program/Pricing contact us at:

Email: TBLEcoSEC@tosoh.com

Phone: 1-800-366-4875, option #4

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The experience gained from 40+ years of Gel Permeation Chromatography (GPC) instrumentation development is clearly visible in the All-In-One System architecture of the EcoSEC GPC System. This design concept is the foundation on which the benefits of the system rest: low dead volume for improved resolution and molar mass distribution accuracy, temperature controlled pumps for excellent flow rate precision regardless of changes in laboratory temperature, and dual flow RI (refractive index) detection for unmatched baseline stability.

Time and solvent can be saved using the EcoSEC GPC System with optional semi-micro columns due to the system's low dead volume. The dead volume of the EcoSEC GPC System (<20 μ L) is less than half the dead volume of conventional GPC systems.

All-In-One-System

- **Superior performance**
 - Unmatched baseline stability due to unique dual flow RI detector design
 - High degree of precision in retention time and molar mass determination due to advanced temperature controlled pumps
 - Exceptional reproducibility day to day, system to system, and site to site
- **Increased throughput**
 - Stable RI baseline with low baseline drift in THF obtained within 90 minutes of startup
 - Unattended operation with built-in autosampler
- **Unparalleled versatility**
 - Column switching valve reduces time between column changes and rapidly establishes a stable baseline (within 15 minutes)
 - Easy to use, intuitive software specific to GPC analysis
 - Optional UV detector for measurement of UV-absorbing polymers
 - Compatible with external viscometry and multi-angle light scattering detectors
- **Optional semi-micro columns**
 - 50% reduction in run times and solvent cost savings of 85% due to low dead volume design
 - TSKgel SuperMultiporeHZ columns are packed with particles synthesized with a range of pore sizes, resulting in no inflection points in the calibration curve. The lack of inflection points allows better accuracy and reproducibility when determining the molar mass distribution of polymers.

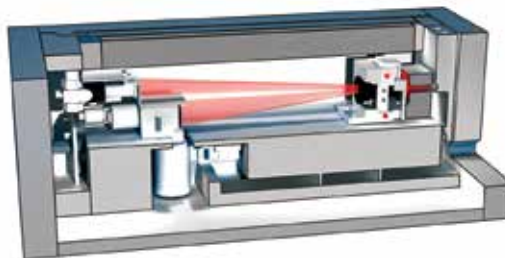


Component	Description	Benefit
All-in-one design	The EcoSEC GPC System is designed with low dead volume (<20 μL), temperature controlled pumps, and dual flow RI detection.	Improved resolution and molar mass distribution accuracy, excellent flow rate precision regardless of changes in laboratory temperature, and unmatched baseline stability.
Control panel	Allows the system to be controlled manually and at the discretion of the operator.	Saves time by controlling a series of operations without the use of the computer or software.
Autosampler	100 sample capacity, 1 to 1,500 μL per injection.	Automatic sample injection for unattended, around the clock operation.
Purge unit and degasser	20 and 40 mL solvent volume; variable degassing capacity (for semi-micro or 30 cm column).	Saves time with rapid solvent changes via purge valve eliminating solvent replacement and other time-consuming manual operations.
Temperature controlled pumps	Pump heads and solvent lines are maintained at a constant temperature.	Improves baseline stability by removing the effect of temperature fluctuations. This results in consistent and accurate flow rates and reproducible molar mass determinations.
Column oven	Engineered for precise (± 0.02 $^{\circ}\text{C}$) column temperature; oven can accommodate up to 8, 30 cm length columns.	Constant column temperature ensures precise and reproducible molar mass determinations.
RI detector	Low dead volume flow cell, 2.5 μL . Solvent flows through a separate reference cell.	Enhanced baseline stability from dual flow cell RI detector.
UV detector (optional)	Low dead volume flow cell, 2 μL . Wavelength range from 195-350 nm.	Option for measuring UV-absorbing polymers.
Light Scattering detector (optional)	Various technologies available.	Absolute molar mass and polymer size determination.
Viscometry detector (optional)	Various designs available.	Universal calibration, Mark-Houwink plot, determination of intrinsic viscosity and polymer size.

Superior Performance

Unmatched Baseline Stability

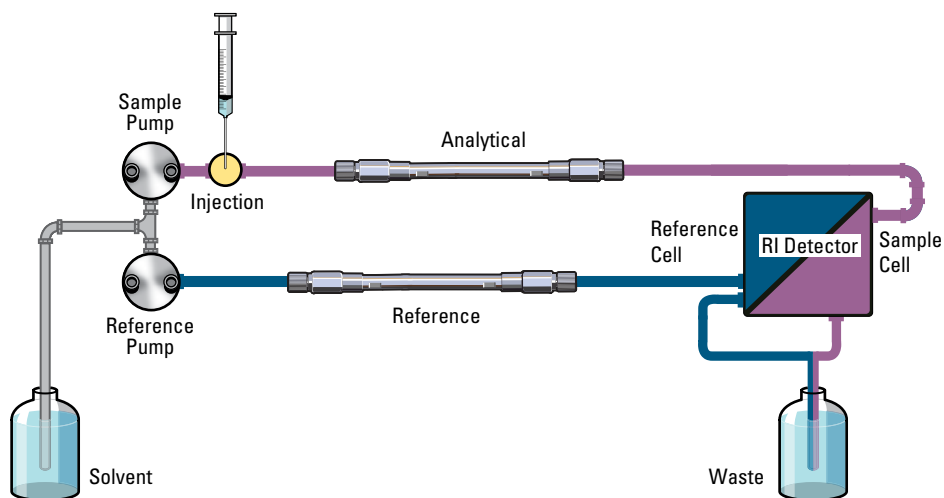
- Dual flow RI cell and pump design
- Continuous correction of RI baseline drift due to solvent instability
- Improved molar mass precision and accuracy
- Rapid baseline stability at startup



Dual Flow Pump Design

The EcoSEC GPC System has a unique dual flow design which includes the use of two pumps. **Figure 1** demonstrates the flow paths of the sample and reference pumps. The sample pump flows solvent from the solvent reservoir through the following system components in sequence: autosampler, analytical column, sample side of RI detector cell, and waste container. The solvent flows via the reference pump from the solvent reservoir through a reference column, the reference side of the RI detector cell, and then the waste container.

Figure 1: Flow paths of sample and reference pumps in the EcoSEC GPC System



Dual Flow Refractive Index Detector

The refractive index detector in the EcoSEC GPC System is unlike any other refractive index detector on the market due to its unique dual flow design. The EcoSEC GPC System RI flow cell is constructed in such a way that there are two sides: (1) the reference side, containing a flowing stream of pure solvent; and (2) a sample side, containing a flowing stream of analyte in the same solvent as in the reference side (Figure 2).

The unique dual flow design of the EcoSEC GPC System results in superb RI baseline stability and reduced RI baseline drift. In a conventional RI detector, over time, the refractive index of the stagnant pure solvent in the reference side will slowly change and the two photodiodes will no longer produce equal signals, thus the contents of the reference and sample sides have different refractive indices and will produce a voltage difference similar to that of an analyte in solution. For example, the refractive index of THF slowly alters over time, due to the buildup of peroxide-related compounds, resulting in baseline drift (Figure 3). The dual flow design of the RI detector in the EcoSEC GPC System compensates for the changes in refractive index of the solvent over time by continuously flowing pure solvent through the reference side of the flow cell.

Another benefit of the dual flow cell is rapid attainment of baseline stability when the instrument is first started, as purging is not required. A stable baseline can be achieved by flowing only 50 mL of solvent through the instrument. Additionally, the reference side mobile phase can be sent to waste or recycled back to the solvent bottle.

Figure 2: Depiction of dual flow RI detector in the EcoSEC GPC System, showing the compensation of the changes in refractive index of the solvent over time

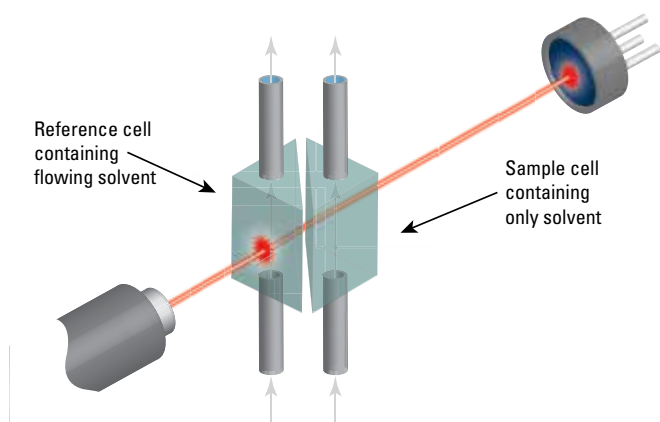
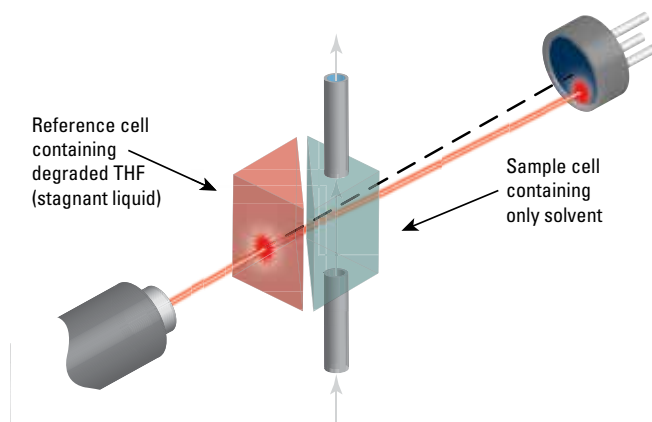


Figure 3: Depiction of RI detector flow cell showing the effects of THF degradation in the stagnant reference side of a conventional GPC system



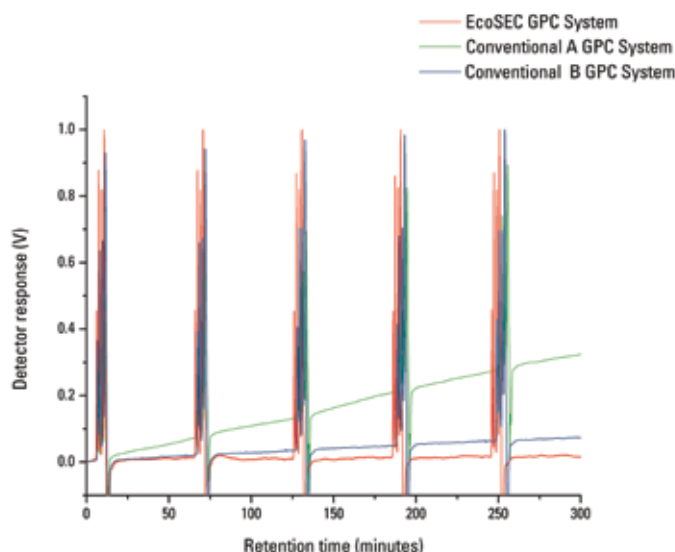
Comparison of Baseline Stability

The EcoSEC GPC System offers unmatched baseline stability because it is the only GPC system which uses a dual flow refractive index detector and temperature controlled pumps. Baseline stability is essential for the accurate calculation of polymer molar mass averages. For example, computer simulations predict a polymer with a polydispersity index (*PDI*) of 5 will have an 18% error for M_z if baseline instability leads to a 4% error in peak width determination. In addition, a 2% uncertainty in baseline height will result in a 20% error in M_z .¹

A study was done to demonstrate the superb baseline stability of the EcoSEC GPC System compared to that of two conventional GPC systems using both 15 cm and 30 cm columns over a five hour time period. The figures below demonstrate that the EcoSEC GPC System maintains the efficiency of semi-micro columns and maintains a stable RI baseline when both conventional and semi-micro GPC columns are used.

As shown in **Figures 4A and 4B**, five consecutive injections of polystyrene standards with run times deliberately extended to one hour without auto zeroing the detectors between injections, resulted in an extremely stable baseline with low baseline drift on the EcoSEC GPC System and a significantly drifting baseline on the two conventional GPC systems. In comparison to the conventional GPC systems, the EcoSEC GPC System has both a lower baseline drift and a better signal to noise ratio.

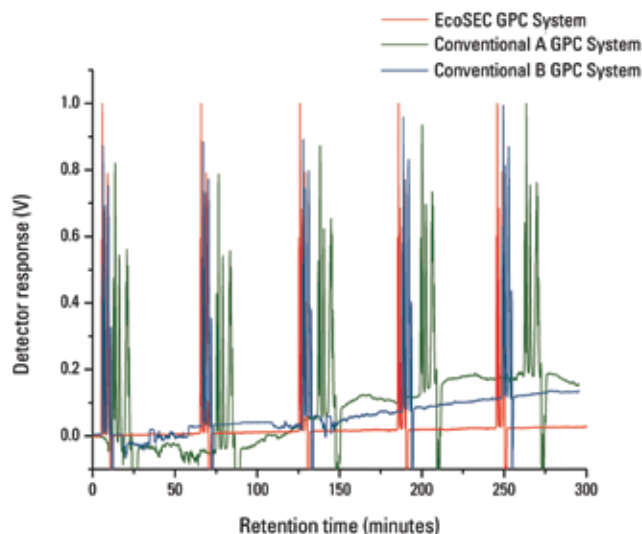
Figure 4A: Comparison of baseline drift of the dual flow refractive index detector of the EcoSEC GPC System and two conventional GPC systems using semi-micro columns



Column: TSKgel SuperMultiporeHZ-M, 4 μ m, 4.6 mm ID \times 15 cm \times 2
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: polystyrene standards, PStQuick MP-M series

¹Tcjr, W.J.; Rudin, A.; and Fyfe, C.A. Effects of data analysis on accuracy and precision of GPC results. *J. Polym. Sci. Polym. Phys. Ed.* **1982**, 20, (8), 1443-1451.

Figure 4B: Comparison of baseline drift of the dual flow refractive index detector of the EcoSEC GPC System and two conventional GPC systems using conventional columns



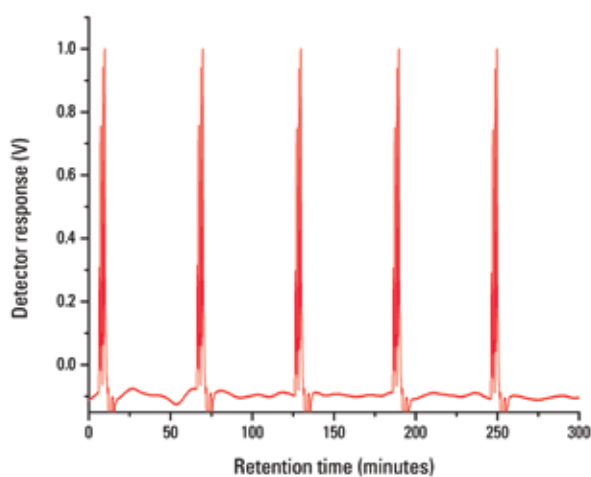
Column: TSKgel GMHXL-L, 5 μ m, 7.8 mm ID \times 30 cm \times 2
Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: polystyrene standards, PStQuick MP-M series

Baseline Stability in Various Solvents

The EcoSEC GPC System displays an extremely stable baseline with low baseline drift when analyzing polymers in neat, mixed, and complex solvent systems.

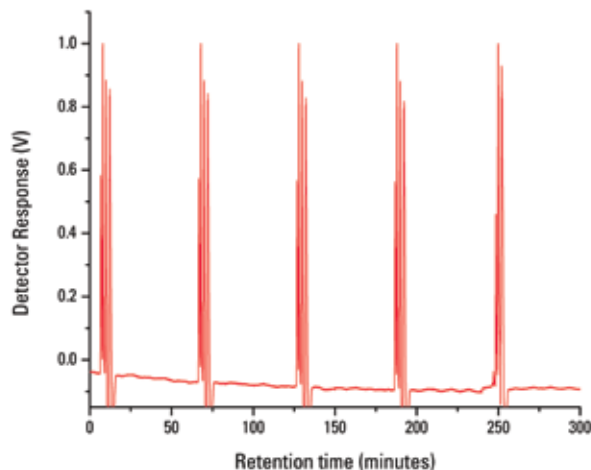
The following figures show five consecutive injections of polystyrene standards in chloroform (Figure 5), DMAc with 0.02 mol/L LiBr (Figure 6), and 95:5 Dichloromethane:HFIP with 5 mmol/L tetraethylammonium bromide (Figure 7) on semi-micro TSKgel GPC columns. The run times were deliberately extended to one hour without auto zeroing the detector between injections for a total of five hours at a flow rate of 0.35 mL/min.

Figure 5: Baseline stability of the EcoSEC GPC System in chloroform



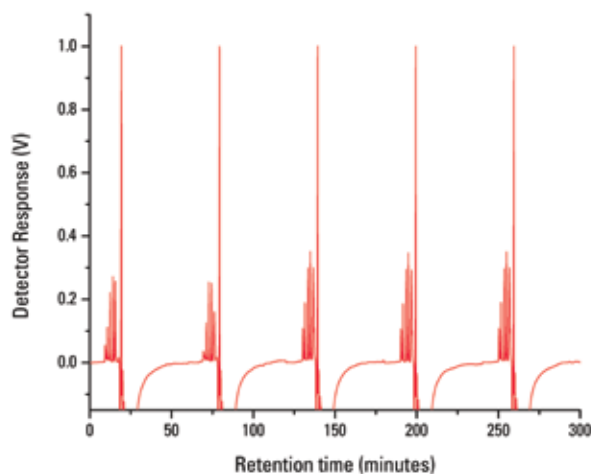
Column: TSKgel SuperH2M-M, 3 μ m, 4.6 mm ID \times 15 cm \times 2
Mobile phase: chloroform
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: polystyrene standards, PStQuick MP-M series

Figure 6: Baseline stability of the EcoSEC GPC System in DMAc with 0.02 mol/L LiBr



Column: TSKgel SuperH2M-M, 3 μ m, 4.6 mm ID \times 15 cm \times 2
Mobile phase: DMAc with 0.02 mol/L LiBr
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: polystyrene standards, PStQuick MP-M series

Figure 7: Baseline stability of the EcoSEC GPC System in 95:5 dichloromethane:HFIP with 5 mmol/L tetraethylammonium bromide



Column: TSKgel SuperHM-H, 3 μ m, 6 mm ID \times 15 cm \times 2
Mobile phase: 95:5 dichloromethane:HFIP with 5 mmol/L tetraethylammonium bromide
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: polystyrene standards, PStQuick B + PStQuick C

Comprehensive Temperature Control

Elution Time Precision

To assess the influence of environmental conditions within the laboratory on solvent flow, a study was done in which the EcoSEC GPC System and a conventional GPC system were placed in a chamber where the temperature was cycled between 23 °C and 26 °C. A series of 99 injections of polystyrene were made over a time period of ten hours. For each instrument the elution volume at peak maximum was measured; the resulting data is shown in Figures 8A and 8B below. The retention time drift of the EcoSEC GPC System was about 20% lower than that of the conventional GPC system.

The results shown demonstrate that the engineering design concepts of the EcoSEC GPC System result in a high degree of reproducibility of retention time and molar mass determination.

Figure 8A: Mobile phase delivery reproducibility of the EcoSEC GPC System with ambient temperature changes

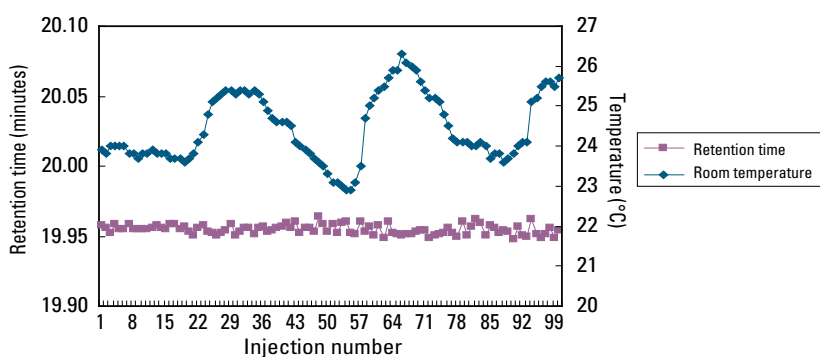
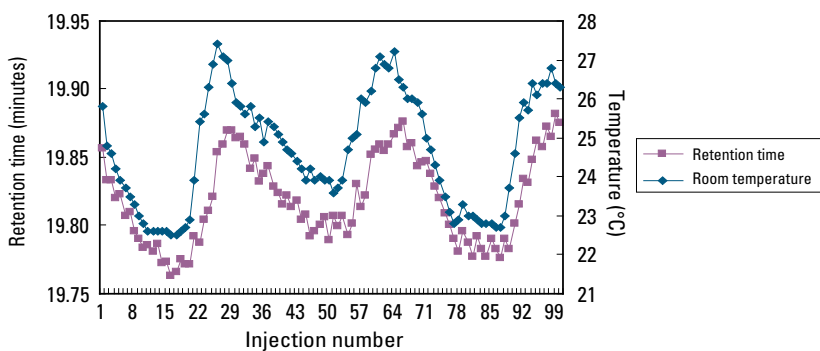


Figure 8B: Mobile phase delivery reproducibility of a conventional system with ambient temperature changes



M_w Precision

Molar mass averages can be affected by changes in the environment and measuring conditions. Generally, these variations are the result of one or more factors including flow rate reproducibility, baseline drift and injection reproducibility. In addition to controlling column temperature, Tosoh engineers added temperature control for both pumps and inlet and outlet tubing on the EcoSEC GPC System to deliver top GPC analysis performance.

Figure 9 demonstrates the superiority of the EcoSEC GPC System for the determination of weight-average molar masses.

Figure 9: Reproducibility of M_w analysis

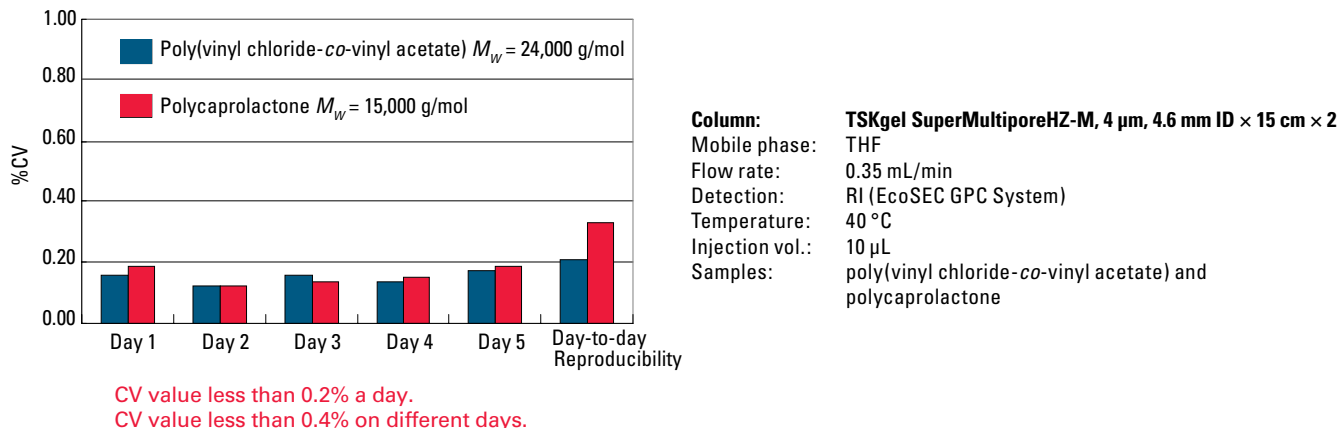
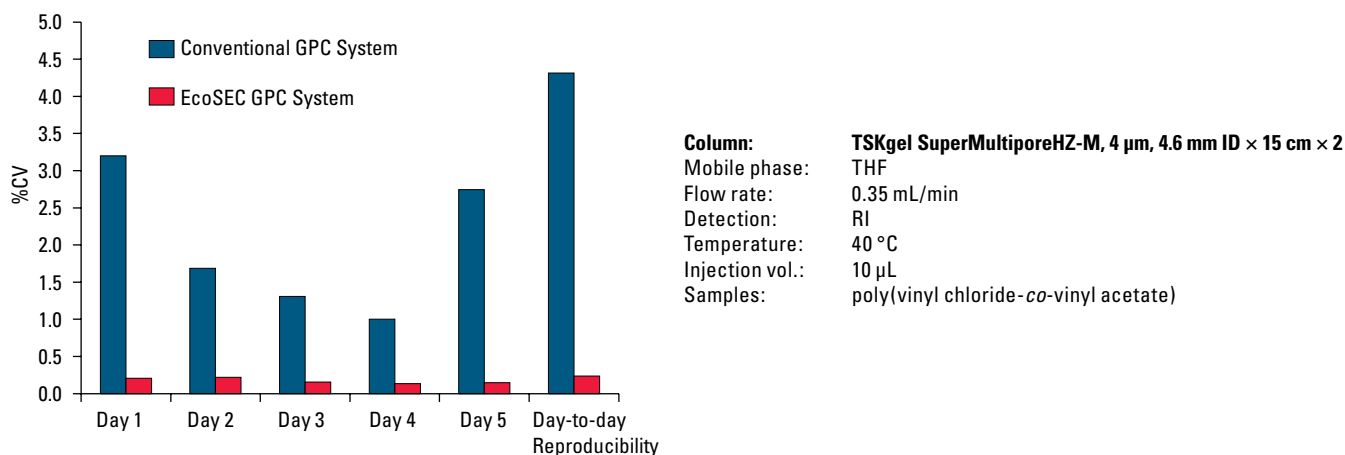


Figure 10 shows a comparison of M_w reproducibility for a sample injected 10 times a day for 5 days on the EcoSEC GPC System compared to a conventional GPC system. The reproducibility of the EcoSEC GPC System was superior by a factor of 3 to that of the conventional GPC system.

Figure 10: Comparing M_w reproducibility of the EcoSEC GPC System and a conventional GPC system

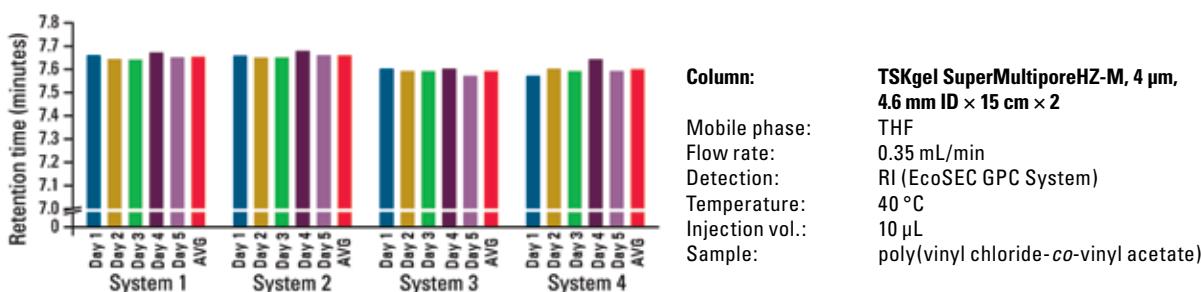


System-to-System Reproducibility

Often measurements can be reproduced using the same equipment but results differ when an instrument from the same or another manufacturer is used. Among the system-specific factors which can influence the results of GPC analysis, fluctuations in elution time, in particular, can have a significant effect.

A study was performed using a polydisperse poly(vinyl chloride-*co*-vinyl acetate) sample run on four different EcoSEC GPC Systems by different operators to assess system reproducibility. The results are shown in **Figure 11**. The high precision of the EcoSEC GPC System results in minimal variation among instruments and from day-to-day.

Figure 11: Day-to-day reproducibility



Four EcoSEC GPC Systems, 4 operators,
4 column sets, 4 conditions, one location

Site-to-Site Reproducibility

To test site reliability, a round-robin study was undertaken in which the same polydisperse poly(vinyl chloride-*co*-vinyl acetate) sample was run on EcoSEC GPC Systems located at four different sites. The results are displayed in **Table 1**.

Reproducibility from system-to-system and location-to-location is exceptional with the EcoSEC GPC System. Coefficients of variations for all molar mass averages were all well below 1%. Because of the high instrument-to-instrument reproducibility of the EcoSEC GPC System, methods developed at one location, *e.g.*, an R&D laboratory, can be reliably transferred to a second site, *e.g.*, a QC lab at a manufacturing site, and so on.

Table 1: Site-to-site reproducibility

	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)
Site A	1.30×10^4	2.98×10^4	5.37×10^4
Site B	1.37×10^4	2.99×10^4	5.43×10^4
Site C	1.36×10^4	2.98×10^4	5.32×10^4
Site D	1.37×10^4	3.02×10^4	5.41×10^4
Average	1.37×10^4	2.99×10^4	5.38×10^4
Deviation	70	160	420
%CV	0.52	0.55	0.78

Column: TSKgel SuperMultiporeHZ-M, 4 μ m, 4.6 mm ID \times 15 cm \times 2
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: poly(vinyl chloride-*co*-vinyl acetate)
 Average of values measured with each instrument (n = 10).

Four EcoSEC GPC Systems, 4 operators,
4 column sets, 4 conditions, 4 locations

Column Switching Valve

- Reduce column switching time
- Easily switch between low MM and high MM range columns
- Eliminate temperature related baseline drift following column change

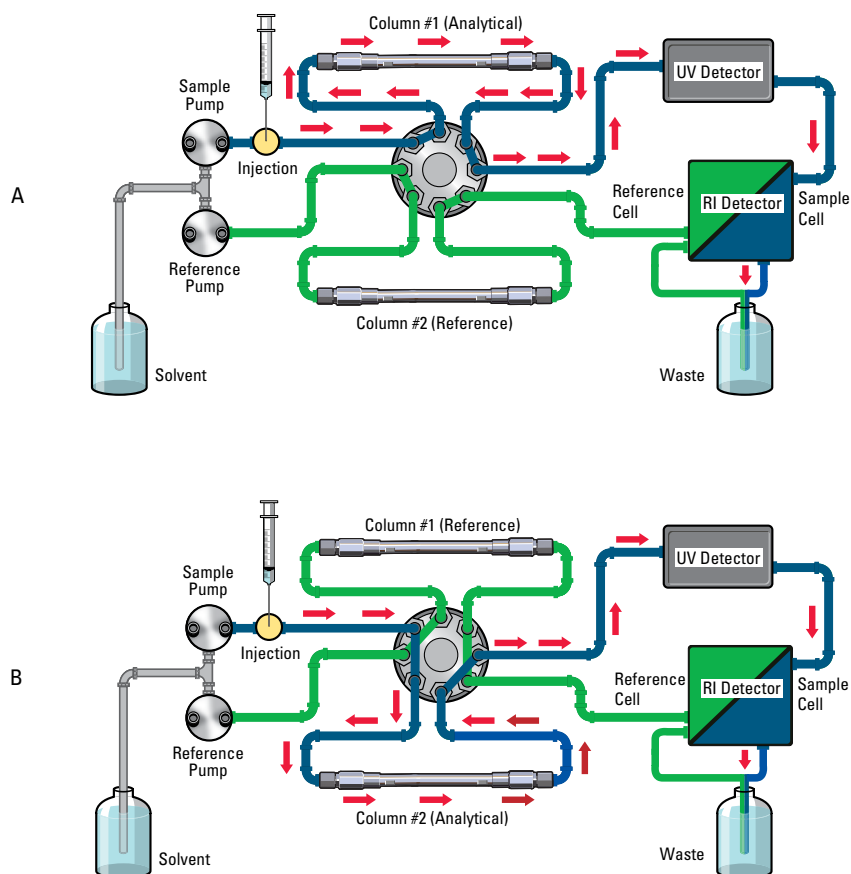


Rapid Column Switching

The EcoSEC GPC System contains two pumps: a sample pump to deliver sample and solvent through the analytical column and the sample side of the RI detector flow cell and a reference pump to flow solvent (via a reference column) to the reference side of the RI detector flow cell. By installing an optional column switching valve and replacing the reference column with another analytical column, an analysis can be performed on column 1 while equilibrating column 2. After switching the valve, column 2 becomes the analytical column while column 1 will be in the flow path to the reference side of the RI detector flow cell (Figure 12).

Since the column switching valve changes column sets while the oven door remains closed and switches to an already equilibrated column set, a stable baseline is rapidly established.

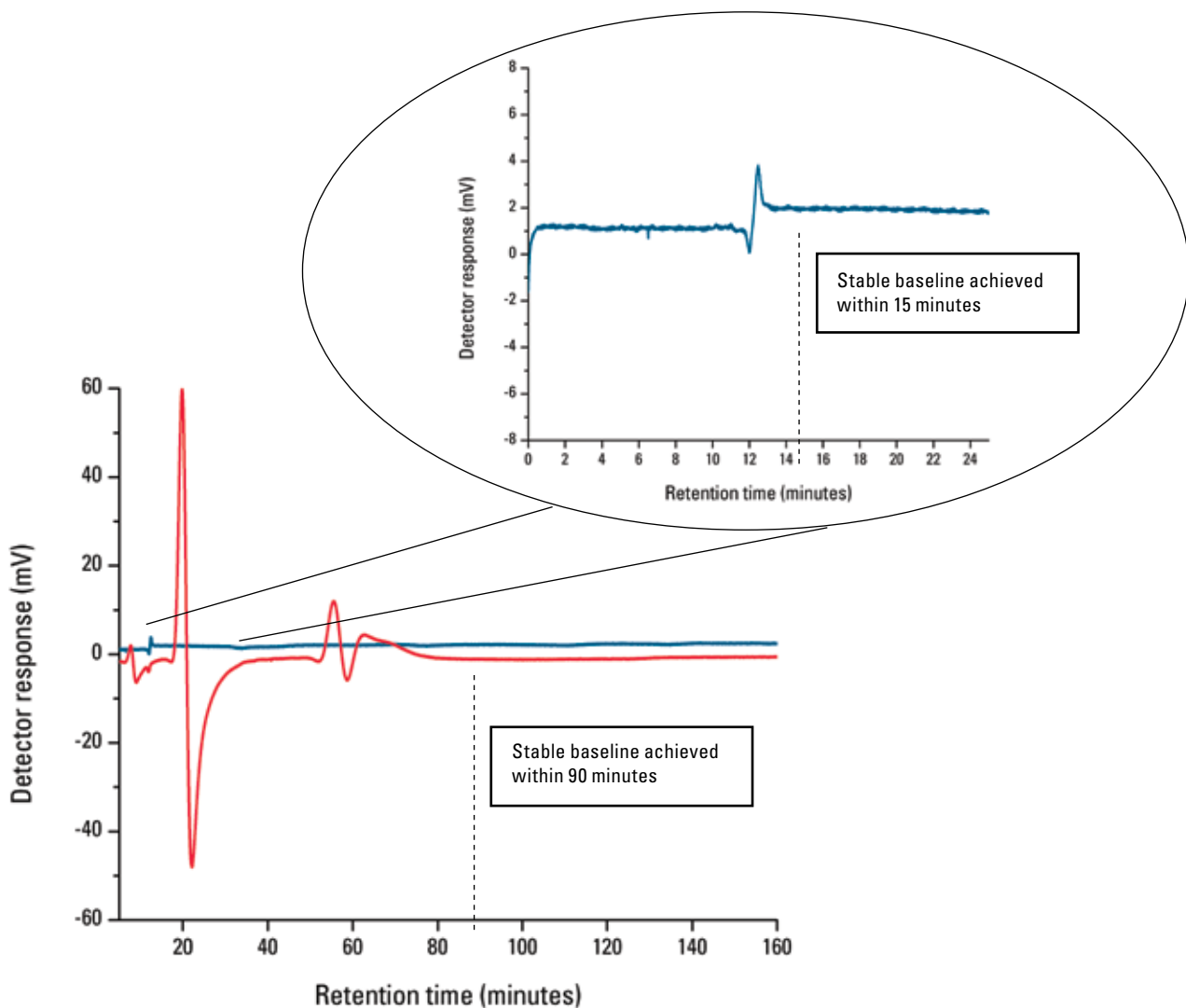
Figure 12: A: Flow path with column 1 as the analytical column B: Flow path with column 2 as the analytical column



Comparison of Time to Baseline Stability with and without the Column Switching Valve

On the EcoSEC GPC System the RI baseline is considered stabilized when the drift in signal is 1×10^{-7} RIU/h or less (based on THF at a flow rate of 1.0 mL/min). When a new set of columns is manually placed on the EcoSEC GPC System and the flow rate is started, the RI baseline stabilizes within 80 - 90 minutes. When a new column set is brought online using the column switching valve, the baseline stabilizes within 15 minutes. (Experimental conditions: THF, 35 °C, 0.35 mL/min, 20 min warm-up at 50% flow rate). **Figure 13** clearly demonstrates the 65 - 75 minute savings in time required to reach a stable baseline when the columns are switched using the column switching valve compared to manually changing columns.

Figure 13: Overlay of refractive index detector signals during equilibration following a column change using the column switching valve (blue) and without use of the column switching valve (red)

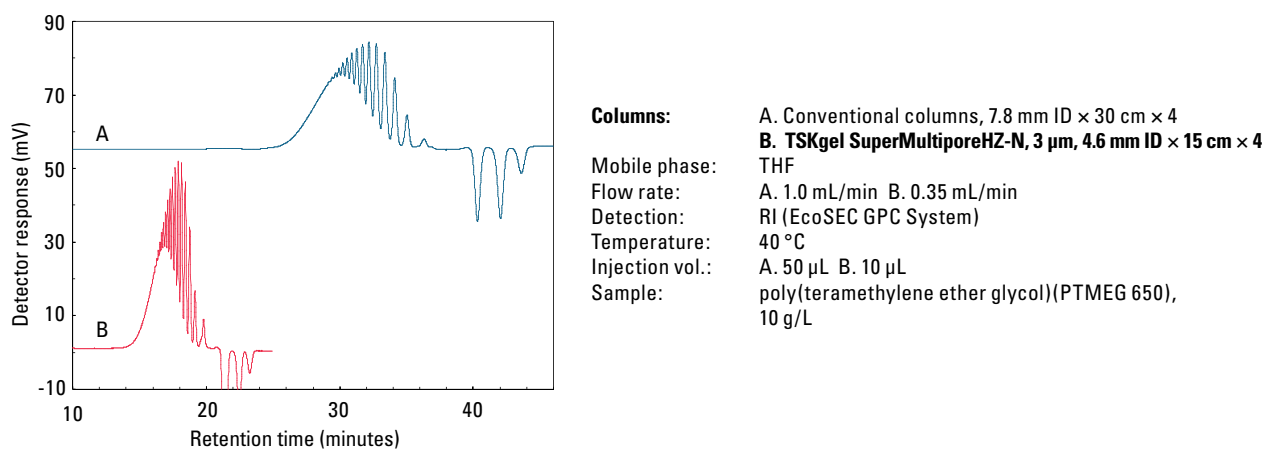


Increased Throughput and Lower Solvent Costs

Minimal extra-column band broadening is required to take full advantage of the highest efficiency GPC columns. The EcoSEC GPC System is engineered to minimize system dead volume. The semi-micro design allows the use of GPC columns with smaller ID (4.6 mm) and shorter lengths (15 cm) such as the TSKgel SuperMultiporeHZ columns. Together with a small stroke volume pump and a 2.5 μ L RI flow cell, the EcoSEC GPC System allows accurate and precise molar mass measurements, particularly when benefiting from state-of-the-art column technology.

As shown in **Figure 14**, when run on the EcoSEC GPC System, the TSKgel SuperMultiporeHZ-N (4.6 mm ID \times 15 cm) column achieves separation efficiency equivalent to that of a conventional high speed column (7.8 mm ID \times 30 cm), but analysis time is reduced to half that of a conventional column and one-sixth the amount of solvent is consumed.

Figure 14: Comparing semi-micro and conventional GPC columns

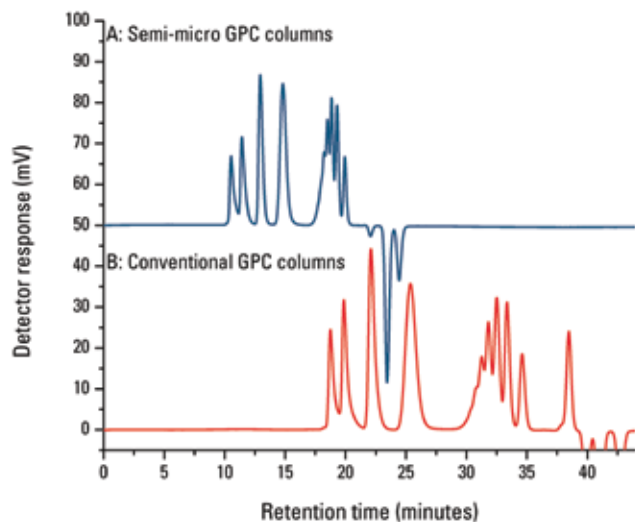


A comparison of chromatograms obtained from conventional and semi-micro TSKgel HxL and SuperHZ series columns are shown in **Figures 15 and 16**. TSKgel HxL and SuperHZ series columns have similar separation performance, solvent compatibility, stationary phase composition, and column efficiency. The differences between the two column series are particle size and column length.

A direct comparison between chromatograms obtained, under optimal operating conditions for each column length, for a mixture of polystyrene standards ranging in molar mass from 530 to 2.9×10^6 g/mol are shown in **Figure 15**. The resolution obtained via both column sets is virtually identical, the monomer, dimer, trimer, and tetramer of the lowest molar mass standard, 530 g/mol, can all be identified on both column lengths. Separation of the polystyrene standards using semi-micro GPC columns, **Figure 15A**, occurs in less than thirty minutes, approximately half the time required to obtain an identical separation using conventional GPC columns, **Figure 15B**.

The GPC chromatogram of a real world polymer sample composed primarily of propylene glycol monomethyl ether acetate as obtained using the EcoSEC GPC System with semi-micro and conventional GPC columns was also compared. As can be seen in **Figures 16A and 16B**, a slight increase in resolution is observed towards the low molar mass, longer retention time region of the GPC chromatogram obtained using conventional GPC columns compared to semi-micro GPC columns. The combination of the low dead volume of the EcoSEC GPC System and the semi-micro GPC columns allowed for complete analysis in approximately 25 minutes, whereas analysis using conventional columns and the EcoSEC GPC System required an analysis times close to 45 minutes.

Figure 15: Elution profiles of polystyrene standards as monitored by RI on the EcoSEC GPC System with A: semi-micro GPC columns and B: conventional GPC columns



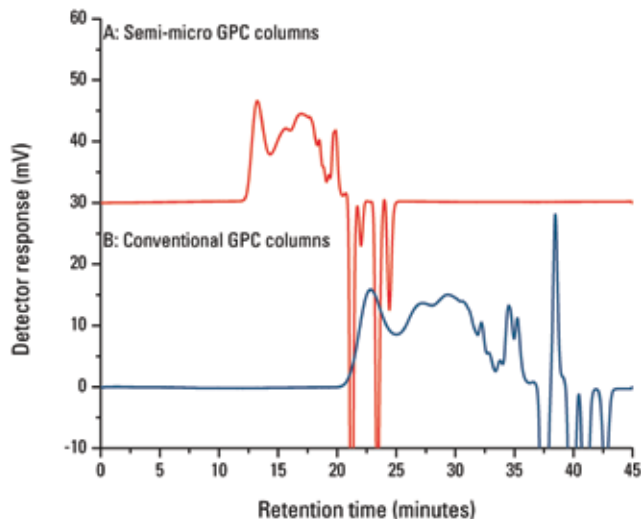
A Columns: TSKgel SuperHZ1000, SuperHZ2000, SuperHZ3000, SuperHZ4000, 3 μ m, 4.6 mm ID \times 15 cm

Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: UV @ 248 nm, RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 30 μ L
Samples: polystyrene standards, PStQuick MP-M series

B Columns: TSKgel G1000H_{XL}, G2000H_{XL}, G3000H_{XL}, G4000H_{XL}, 5 μ m, 7.8 mm ID \times 30 cm

Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: UV @ 248 nm, RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 150 μ L
Samples: polystyrene standards, PStQuick MP-M series

Figure 16: Elution profiles of a real-world polymer sample as monitored by RI on the EcoSEC GPC System with A: semi-micro GPC columns and B: conventional GPC columns



A Columns: TSKgel SuperHZ1000, SuperHZ2000, SuperHZ3000, SuperHZ4000, 3 μ m, 4.6 mm ID \times 15 cm

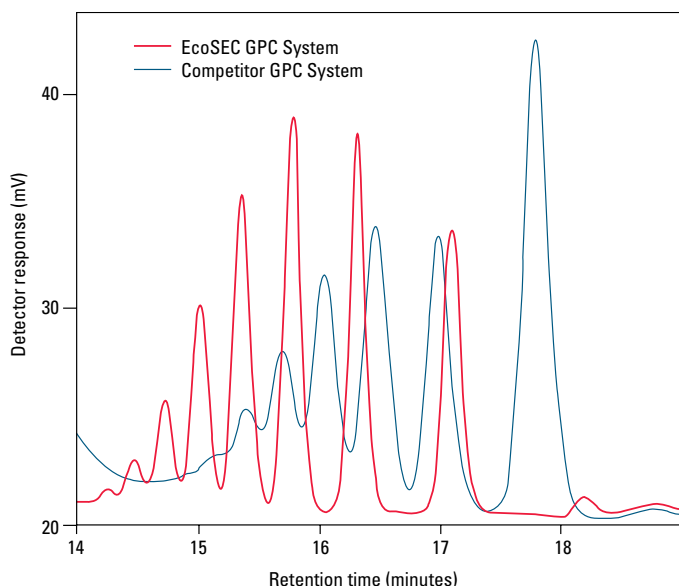
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: UV @ 248 nm, RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 30 μ L
Sample: real world polymer sample

B Columns: TSKgel G1000H_{XL}, G2000H_{XL}, G3000H_{XL}, G4000H_{XL}, 5 μ m, 7.8 mm ID \times 30 cm

Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: UV @ 248 nm, RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 150 μ L
Sample: real world polymer sample

Figure 17 shows an example of an oligomer (A-500) separation using four TSKgel SuperHZ2000 GPC columns in tandem on an EcoSEC GPC System and a conventional GPC system. A faster analysis and improved resolution is achieved with the EcoSEC GPC System as a result of the advanced engineering design of the system.

Figure 17: Comparison of resolution of a semi-micro column run on an EcoSEC GPC System and a conventional GPC system



Column: TSKgel SuperHZ2000, 3 μm, 4.6 mm ID × 15 cm × 4
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI
Temperature: 40 °C
Injection vol.: 10 μL
Sample: styrene oligomer (A-500), 0.2 g/L

The combination of the EcoSEC GPC System and semi-micro columns provides significant solvent related cost savings while doubling sample throughput without compromising resolution. As shown in Table 2, the solvent related cost savings are extraordinary for samples requiring expensive solvents such as hexafluoroisopropanol.

Table 2: Annual solvent cost saving with semi-micro columns and the EcoSEC GPC System

Solvent	Competitive GPC System	EcoSEC GPC System	Savings
Chloroform (\$17/L)	\$1,830	\$295	\$1,535
DMF* (\$25/L)	\$2,600	\$416	\$2,184
NMP* (\$30/L)	\$3,082	\$493	\$2,589
THF* (\$40/L)	\$4,160	\$666	\$3,494
HFIP* (\$1,000/L)	\$96,493	\$15,439	\$81,054

* DMF: dimethylformamide; NMP: N-methylpyrrolidone; THF: tetrahydrofuran; HFIP: hexafluoroisopropanol

EcoSEC GPC System Specifications

Pump	Specification
Flow rate	0.010 to 2.000 mL/min in 0.001 mL/min steps
Accuracy	± 2%
Precision	± 0.2%
Maximum pressure	25 MPa or 3,500 psi
Safety features	Liquid supply stops if pressure rises above the upper limit or drops below the lower limit, Plunger drive count monitoring, Pan for liquid leakage
Stroke volume	7.51 µL
Auto-injector	
Injection volume	1 to 1,500 µL in 1 µL increments
Number of samples	100, 2 mL injection vials
Column Oven	
Temperature range	Ambient plus 10 °C to 60 °C
Capacity	7.8 mm ID × 30 cm × 8 columns
Accuracy	± 0.5 °C
Precision	± 0.2 °C
RI Detector	
Type	Bryce (dual flow type), Tungsten light source (1.00-1.80 RI range)
Optics	Deflection
Cell volume	2.5 µL
Cell pressure limit	0.5 MPa
Noise	2×10^{-9} RIU
Drift	1×10^{-7} RIU/h (THF, 1.0 mL/min)
Dynamic range	$\pm 2.5 \times 10^{-4}$ RIU
Temperature control	Off, 35 °C, 40 °C, 45 °C
Analog out	For connection to third party light scattering and viscometry detectors
Safety features	Leak sensor and thermal fuse for circuit block
Instrument	
Dimensions	680 (W) × 500 (D) × 550 (H) mm = 2.2' × 1.6' × 1.8'
Weight	95 kg = 210 lbs
Dead volume	<20 µL



Unparalleled Versatility

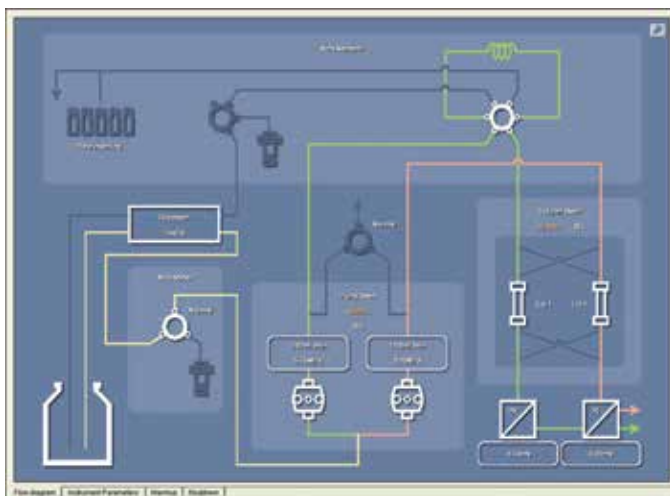
- GPC-specific EcoSEC GPC System software to simplify system control and data handling
- Controls up to 2 EcoSEC GPC Systems
- Excellent data handling and report generation
- Fully featured data handling system; analyze data from two detectors
- Start and stop system automatically
- One license for multiple locations

Features include:

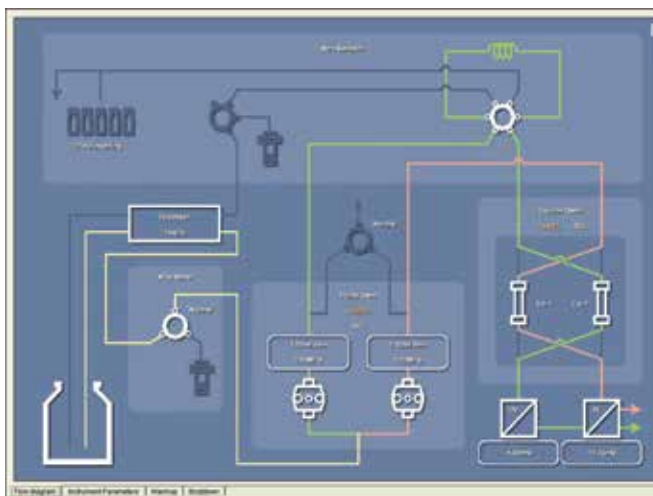
Flow Diagram

- Unique screen allows you to easily modify running conditions of an individual component

Typical flow

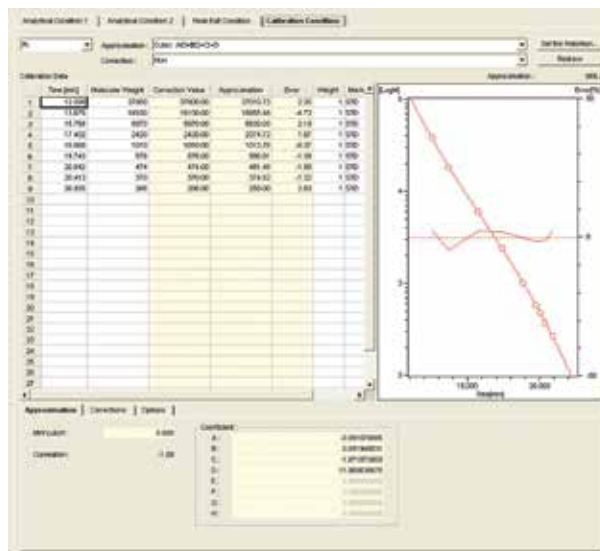


With use of column switching valve



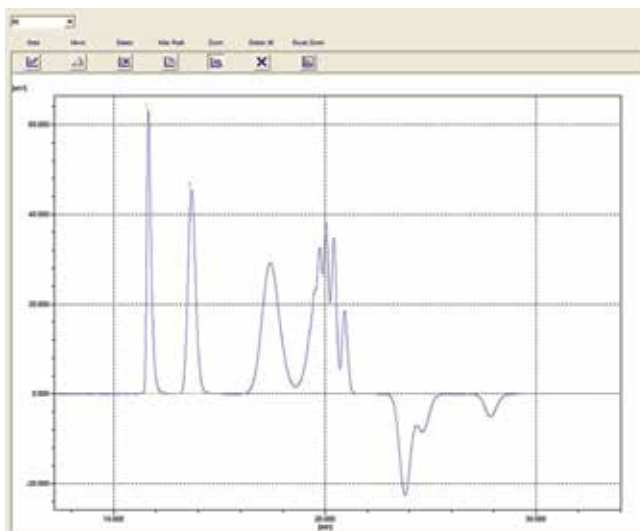
Method

- All parameters for data acquisition and peak integration, including baseline operations, are saved in the template method
- One click switching between calibration curves



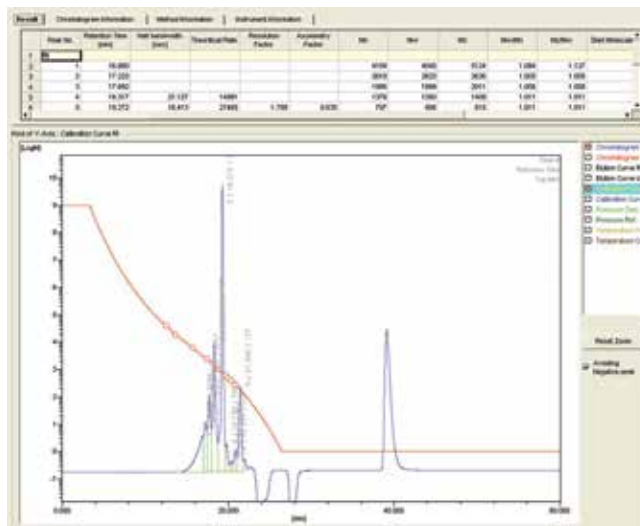
Peak Editing

- Full editing functionality including baseline setting and peak splitting using the mouse
- Automated peak editing



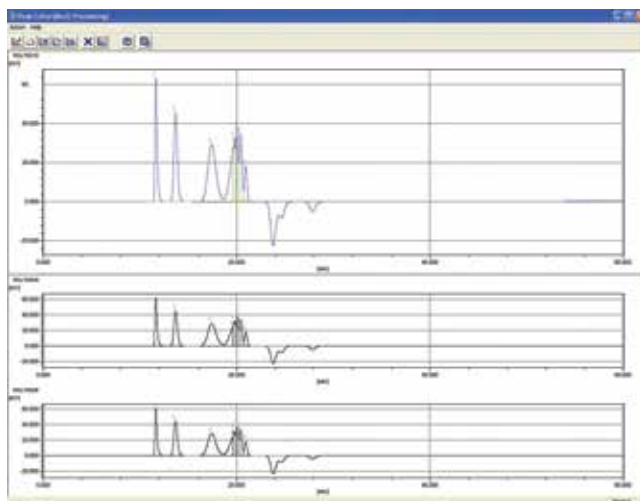
Data Management

- Allows viewing of chromatograms, elution curve, flow rate, pressure, and temperature



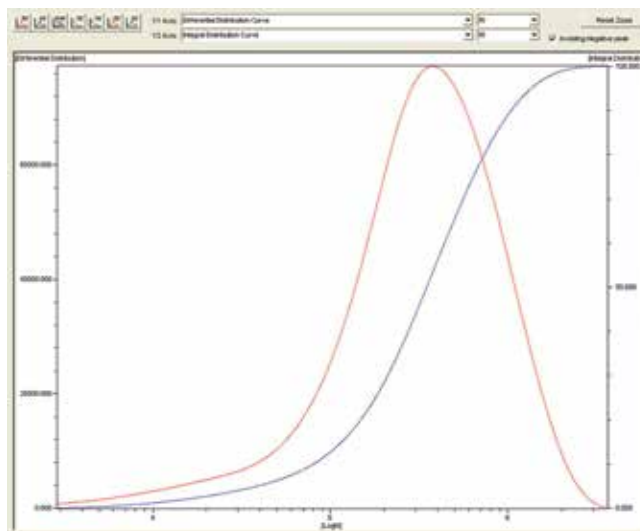
Multiprocessing Function

- Automatically applies exact set of peak detection and integration parameters to all chromatograms in a list
- Similar chromatograms are processed identically for enhanced reproducibility



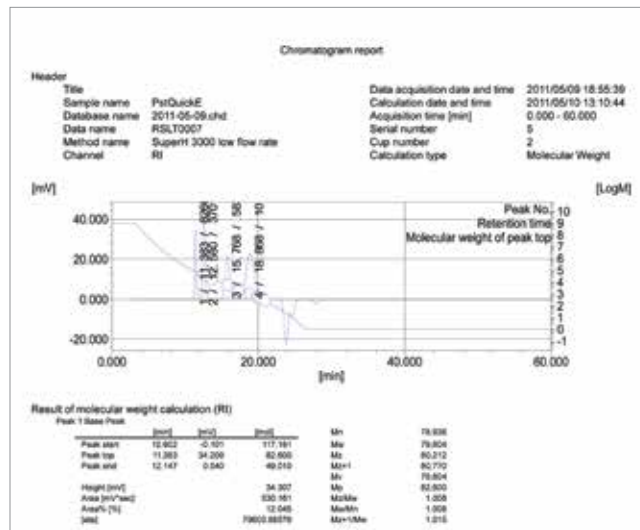
GPC Specific Quantitative Calculations

- M_n , M_w and M_z molar mass averages
- Cumulative and differential molar mass plotting



Report Generation

- Large number of built in reports
- Customizable reports
- Easily export data into text or pdf files



Software Specifications

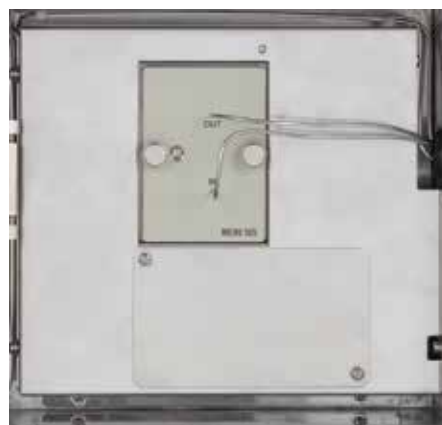
Feature	Description
Software	Provided on CD-ROM
Data acquisition	2-channel (RI,UV)/1-system USB connection
Acquisition time	0.0 to 999.9 minutes
Acquisition interval	50 ms or more (10 ms steps) Upper limit: 1000 ms
Calibration curve approximation	<ul style="list-style-type: none"> • First-degree expression • 3rd-degree expression • 3rd-degree expression + hyperbola • 5th-degree expression • 7th-degree expression • 7th-degree expression (odd power) • 7th-degree expression (odd power) + hyperbola
Calibration curve correction	<ul style="list-style-type: none"> • Mark-Houwink • Q factor • Polymerization degree • USP
Quantitative calculation specific to GPC	<ul style="list-style-type: none"> • Molar mass averages (M_n, M_w, and M_z) • Polydispersity Index (PDI) • Cumulative/differential molar mass distributions • Concentration ratio
Special calculation function	<ul style="list-style-type: none"> • Internal standard correction function • Copolymer analysis • Molar mass fraction specific calculation • Calculation range specification • Lag time correction
Column test	<ul style="list-style-type: none"> • Theoretical plate number • Resolution • Symmetry factor • Half bandwidth
Calculation standard	<ul style="list-style-type: none"> • ASTM® • DIN® • USP • JIS • JP • ISO 16014 • Tosoh Standard
FDA 21 CFR Part 11	Software validation, authentication by user ID and password, log out, and audit trail
Warm up and shut down timers	<ul style="list-style-type: none"> • Daily • Weekly
RI and UV detector auto balance	Optional prior to injection

The standard EcoSEC GPC System consists of the following:

- EcoSEC GPC System instrument
- EcoSEC GPC Workstation Software
- Dual flow RI detector
- Optional 2-way column switching valve (see page 12)
- Optional UV detector

UV Detector

- Variable UV; 195 – 350 nm
- Semi-micro flow cell (2 μ L)
- Factory installed option



The optional UV detector is variable from 195 to 350 nm and the detector flow path and electronics are optimized for the use of semi-micro columns. The volume of the flow cell is reduced to 2 μ L and the shortest time constant is 0.5 seconds.

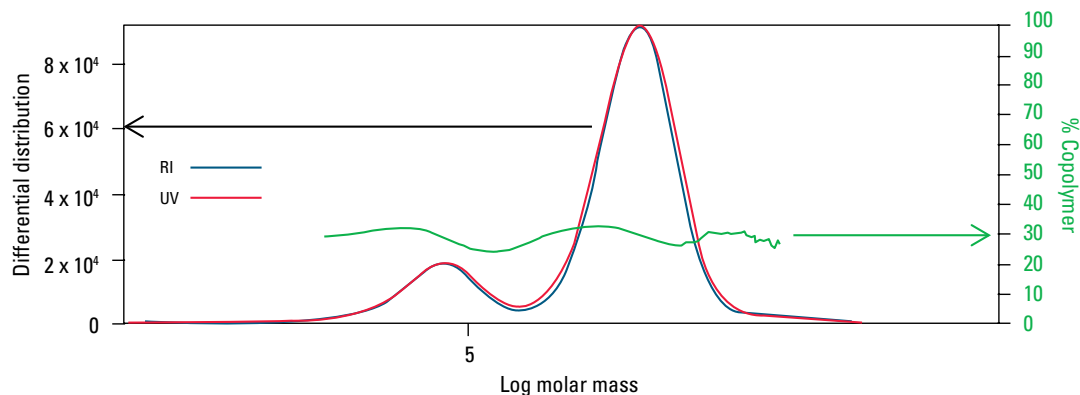
UV Detector Specifications

UV Detector	Specification
System	Dual beam, single flow cell
Light source	Deuterium lamp
Wavelength range	195 to 350 nm
Wavelength accuracy	± 2 nm
Bandwidth	8 nm
Range (FS)	0.5, 1, 2, 4 AU/1 V
Response	0.5, 1.0, 3.0 seconds
Drift	3×10^{-4} AU/h (254 nm, air in cell, response: 1.0 s)
Noise	2.5×10^{-5} AU (254 nm, air in cell, response: 1.0 s)
Flow cell volume	2 μ L
Safety mechanism	Liquid leakage sensor; lighting time monitoring

Copolymer Analysis

The EcoSEC GPC System equipped with both RI and UV detectors can be used to determine the structural composition of an unknown copolymer, in which the copolymer contains one UV visible and one non-UV visible component. At least one copolymer of known composition must be available to create a copolymer calibration curve. The final result is a plot of the structural composition at each molar mass. This composition curve overlaid on the chromatogram, as seen in **Figure 1**, can be generated using the EcoSEC GPC Workstation Software. The software allows for the creation and use of separate UV and RI specific calibration curves while correcting for the inter detector delay volume.

Figure 1: Copolymer analysis of polystyrene-*b*-polybutene



Column: TSKgel SuperMultiporeHZ-M, 4 μ m, 4.6 mm ID \times 15 cm \times 2
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI, UV @ 254 nm (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Samples: PS-*b*-PB, 0.2 wt%

Enhanced EcoSEC GPC System Analysis

The addition of multiple detection methods to the EcoSEC GPC System allows for the characterization of a variety of polymer properties. A multi-detector GPC set up can be used to determine:

- Polystyrene relative molar mass averages based on RI or UV detection
- Copolymer compositional drift with RI and UV detection
- Universal calibration, intrinsic viscosity and viscometric radius with viscometry detection
- Absolute molar mass averages and radius of gyration with multi-angle light scattering (MALS) detection
- Hydrodynamic radius determination with quasi-elastic light scattering (QELS) detection

Summary of Detector Capabilities

Detector	Molar Mass Determination	Detects Most Polymers	Required For Copolymer Composition Analysis	Application Example
RI	Relative	Yes	Yes	Pages 24, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41, 42
UV	Relative	No	Yes	Pages 24, 35, 36, 38

Static Light Scattering Detectors

Detector	Molar Mass Range (g/mol)	Radius of Gyration (R_g) range (nm)	Angular placement of photodiode(s) (deg)	Options/ Features	Application Example
Low Angle Light Scattering (LALS)	10^3 to $>10^6$	10 to 150	7	90° measurement for SEC ³ (RALS +VISC+RI)	
Right Angle Light Scattering (RALS) for SEC ³	10^3 to $>10^7$	Only with viscometer (calculated, not measured R_g)	90	7° measurement for LALS	
Two-Angle	10^3 to $>10^6$	10 to 200	15, 90	QELS	
Three-Angle	10^3 to 10^6	10 to 50	45, 90, 135	QELS at 90°	Page 35
Seven-Angle	10^3 to 10^8	10 to 150	35, 50, 75, 90, 105, 130, 145	Self cleaning CCD's attached with no glass-glass reflectance	Page 37
Eight-Angle	$< 10^3$ to 10^7	10 to 300	17, 36, 52, 70, 90, 110, 132, 151	Variable-angle QELS; integrated ultrasonic cell cleaner	Page 27, 30
18-Angle	10^3 to 10^9	10 to 500	22, 28, 32, 38, 44, 50, 57, 64, 72, 81, 90, 99, 108, 117, 126, 134, 141, 147	Variable-angle QELS; polarization option, integrated ultrasonic cell cleaner	Page 35

Adapted from: Striegel, A.M.; Yau, W.W.; Kirkland, J.J.; Bly, D.D. *Modern Size Exclusion Liquid Chromatography 2nd ed*; Wiley: New York, 2009.

Viscometry Detectors

Detector	Split Ratio	S/N	Recovery column volumes between runs	Obtainable Measurements	Application Example
Single Capillary Viscometer	Not applicable	poor	1	<ul style="list-style-type: none"> • Molar mass via universal calibration curves • Specific and intrinsic viscosity and their distributions • Viscometric radius and its distribution • Mark-Houwink plots • Branching information • Dilute solution conformation 	
4-Capillary Viscometer	50/50	good	1		Pages 28, 35, 39
4-Capillary Viscometer	80/20	excellent	0		Page 37

Tosoh Bioscience can tailor a system to meet your application needs.

Does your analysis require additional detectors beyond RI and UV?

The EcoSEC GPC System provides easy and effortless connectivity when using multi-detector configurations. We offer external light scattering and viscometry detectors.

Contact us for a quote!

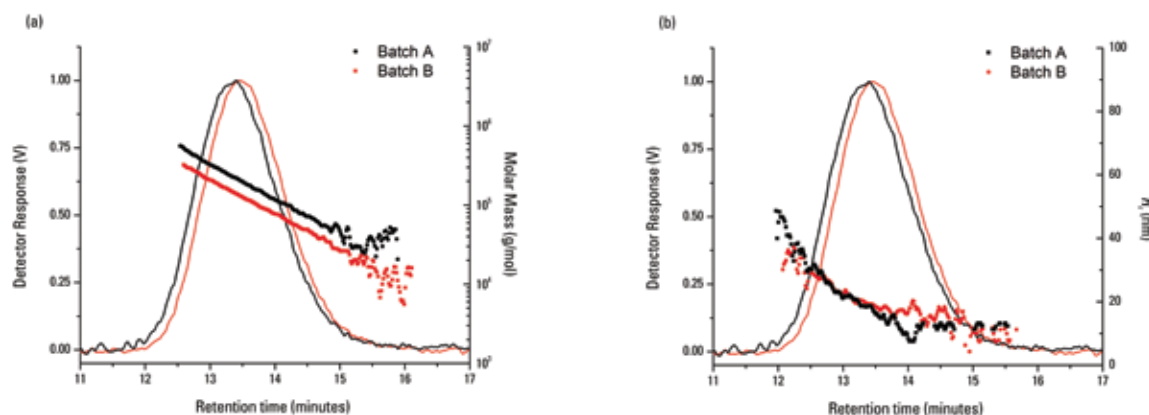
Renewable-Based Polymers

The demand for renewable or bio-based polymers continues to rise exponentially as manufacturers within the automotive, footwear, carpet, and furniture sectors seek to sell more sustainable products. One group of polymers gaining a great deal of interest is thermoplastic polyurethanes or TPUs. A TPU is an elastomer that resembles rubber in consistency and feel but by nature has outstanding abrasion resistance, great low temperature flexibility, resistance to oil, and a high threshold for support weight, in addition to being very bondable, durable, paintable, and impact resistant. The specific end-use properties, such as tensile strength, elongation, conductivity, chemical resistance, and toughness, of a batch of TPUs depends on macromolecular properties such as molar mass, branching, degree of crosslinking, and polymeric size. Two different batches of TPUs were characterized based on molar mass and polymeric size using the EcoSEC GPC System coupled to a multi-angle light scattering detector (MALS).

The molar mass distributions of two different batches of TPUs as plotted across the GPC elution profile, as monitored by the 90° light scattering signal, are shown in **Figure 1A**. The absolute weight average molar mass, M_w , is slightly higher for A than B, 1.64×10^5 and 1.42×10^5 g/mol, respectively. Both batches of TPUs, A and B, show a polydispersity in molar mass as the molar mass decreases as a function of increasing retention time, **Figure 1A**. Additionally, the molar mass polydispersity index, PDI , of the two batches indicate samples polydisperse in molar mass as $PDI = 1.6$ for both batches.

The addition of a MALS detector to the EcoSEC GPC System also permits for the determination of a polymeric sizing parameter, the root-mean-square radius or radius of gyration, R_g . The radius of gyration for both TPUs, A and B, were identical, $R_g = 20$ nm. The radius of gyration distribution as plotted across the GPC elution profile, as monitored by the 90° light scattering signal, as well as the radius of gyration polydispersity index values can be used to determine the size polydispersity of the sample which may influence end-use properties of the TPUs. As seen in **Figure 1B** the size of both TPUs decreases as a function of increasing retention time, an indication that the samples are polydisperse with respect to size. The size PDI value for batch A is slightly greater than that of batch B, 1.3 and 1.1, respectively. **Figure 1B** also provides evidence that the TPU samples are eluting from the GPC column by a true size exclusion mechanism as the polymeric size is decreasing as a function of increasing retention time.

Figure 1: Thermoplastic polyurethanes



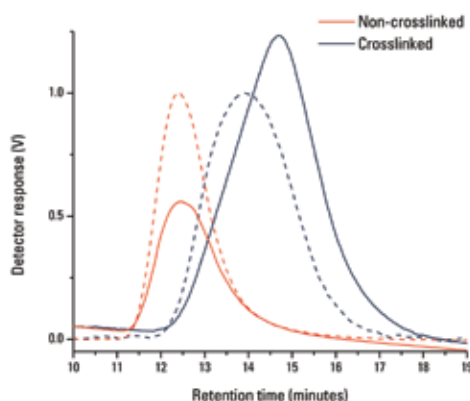
Column: TSKgel GMH_{HR}-H, mixed bed, 7.8 mm ID × 30 cm × 2
Mobile phase: DMF with 0.01% LiBr
Flow rate: 1.0 mL/min
Detectors: RI (EcoSEC GPC System), MALS (Wyatt DAWN® 8+)
Temperature: 50 °C
Injection vol.: 100 µL
Sample: thermoplastic polyurethanes

Dual Detector Hyaluronic Acid Analysis

Hyaluronic acid or hyaluronan is a naturally occurring linear polysaccharide composed of alternating repeating D-glucuronic acid and D-N-acetylglucosamine units. There are two forms of hyaluronic acid, linear and cross-linked, which are common components of cosmetics, personal care products, dietary supplements, medical products, and medical devices. Different applications of hyaluronic acid require different configurations, e.g. hyaluronic acid must adopt different degrees of cross-linking depending on the application of interest. The EcoSEC GPC System and a ViscoStar[®] differential viscometer were used to determine the molar mass averages and polydispersity of a crosslinked and non-crosslinked hyaluronic acid sample. In addition to the molar mass averages, the dual detector set-up allows for the determination of other physicochemical properties such as polymeric size, confirmation, and intrinsic viscosity.

Chromatograms of the two hyaluronic acid samples, as monitored by the RI and VISC, are shown in Figure 2. The elution profile as measured by both detection methods displays a shorter retention time for the crosslinked hyaluronic acid sample than the non-crosslinked hyaluronic acid sample. The difference in retention times between the two samples is an indication that the crosslinked hyaluronic acid sample is smaller in polymeric size than the non-crosslinked hyaluronic acid sample.

Figure 2: Elution profile of non-crosslinked (red) and crosslinked (blue) hyaluronic acid as monitored by the RI (solid) and VISC (dash)



Column: TSKgel GMPW_{XL}, 13 μm, 7.8 mm ID × 30 cm × 2
Mobile phase: H₂O with 0.1 mol/L NaNO₃ and 0.02% NaN₃
Flow rate: 1.0 mL/min
Detectors: RI (EcoSEC GPC System), VISC (Wyatt ViscoStar[®])
Temperature: 35 °C
Injection vol.: 250 μL
Sample: hyaluronic acid

The molar mass averages and polydispersity index of the hyaluronic acid samples were determined via universal calibration via the dual detector experimental set-up and are given in Table 1. The addition of the differential viscometer to the EcoSEC GPC System allowed for the determination of several parameters that can provide structural comparisons between the two hyaluronic acid samples, e.g. intrinsic viscosity and viscometric radius, Table 1.

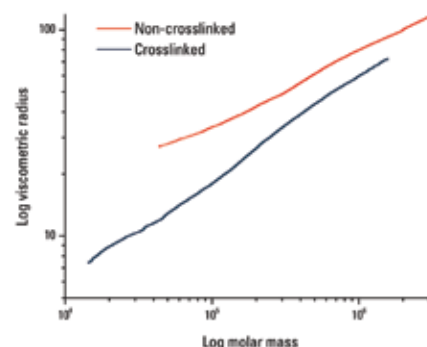
The structural differences of the two hyaluronic acid samples were determined as a function of molar mass through a conformation plot, Figure 3, which plots the viscometric radius vs the molar mass. At any given molar mass the viscometric radius for the crosslinked hyaluronic acid sample is smaller than that of the non-crosslinked hyaluronic acid sample. The non-crosslinked hyaluronic acid sample was determined to have a larger viscometric radius and higher intrinsic viscosity than the crosslinked hyaluronic acid sample, two indications that the non-crosslinked hyaluronic acid is more extended than the crosslinked hyaluronic acid.

Table 1: Molar mass averages, polydispersity index, and viscometry values of hyaluronic acid samples

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI^a	$[\eta]$ (mL/g)	R_{η} (nm)
Non-crosslinked	3.18×10^5	8.58×10^5	1.50×10^6	2.72	3,000	68
Crosslinked	7.22×10^4	2.14×10^5	4.96×10^5	2.97	500	25

^a $PDI = M_w/M_n$

Figure 3: Conformation plot of non-crosslinked (red) and crosslinked (blue) hyaluronic acid

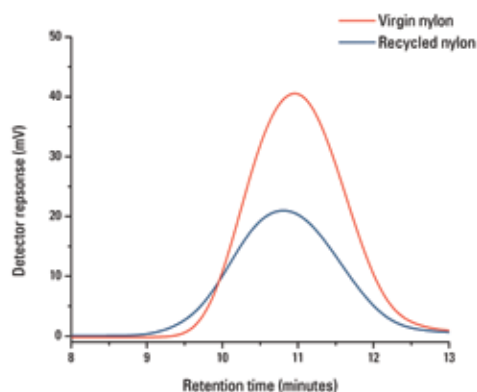


Environmentally Friendly Analysis of Nylon

Green initiatives are continuously approaching the polymer science discipline from all sides as companies are not only interested in greener products and additives but greener and more cost effective synthesis and characterization methods. One class of polymers that is of high interest is polyamides, more specifically nylons, as these plastics are common materials in everyday life which produce large quantities of environmental contaminants.¹ It is critical to be able to characterize virgin and recycled nylon as the recycling process of nylon can result in the reduction of physical-mechanical properties as well as changes in morphology resulting in different end-use properties. A greener and more cost effective method for the characterization of the molar mass averages and distributions of nylon in hexafluoroisopropanol (HFIP) was employed by using an EcoSEC GPC System and semi-micro GPC columns. The combination of the low dead volume of the EcoSEC GPC System and semi-micro GPC columns provides significant solvent related costs while doubling sample throughput without compromising resolution.

The GPC experiments provide two forms of comparison between the virgin and recycled nylon samples: GPC chromatograms and poly(methyl methacrylate) (PMMA) relative molar mass averages and molar mass distributions. The GPC elution profiles of the virgin and recycled nylon as monitored by the RI detector are shown in Figure 4. The virgin nylon elutes after the recycled nylon. The longer retention time of the virgin nylon indicates that the virgin material is slightly smaller in polymeric size compared to the recycled material: as elution order in GPC is that of an “inverse-sieving” technique, smaller analytes elute after the larger analytes.

Figure 4: GPC elution profile of virgin nylon (red), and recycled nylon (blue) as monitored by RI



Column: TSKgel SuperAWM-H, 9 μ m, 6.0 mm ID \times 15 cm \times 2
Mobile phase: HFIP
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 20 μ L
Samples: virgin and recycled nylon

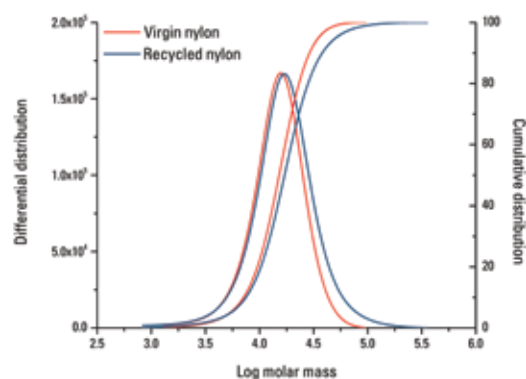
The molar mass averages and polydispersity index, *PDI*, as determined via a PMMA RI calibration curve are given in Table 2. A comparison of the molar mass averages and molar mass distribution, Figure 5, of the virgin nylon material with the recycled nylon material reveals an increase in the molar mass averages and breadth of the distribution curve of the recycled nylon compared to the molar mass averages of the virgin nylon. The molar mass averages and distributions of the virgin and recycled nylon samples obtained by GPC are different enough to distinguish the two products from one another but similar enough to both create successful products with the same end-use properties.

Table 2: Molar mass averages and polydispersity index of nylon samples via RI

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	<i>PDI</i> ^a
Virgin nylon	1.22×10^4 $\pm 46^b$	1.71×10^4 ± 75	2.29×10^4 ± 346	1.41 ± 0.01
Recycled nylon	1.33×10^4 ± 438	2.17×10^4 ± 210	3.93×10^4 $\pm 1,105$	1.62 ± 0.05

^a $PDI = M_w/M_n$; ^b Standard deviations from six injections

Figure 5: Differential and cumulative distributions of nylon (red) and recycled nylon (blue)



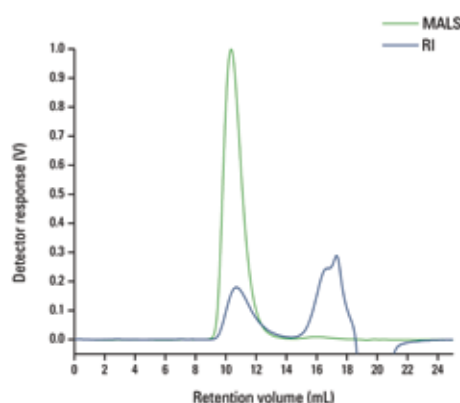
¹Crespo, J.E.; Parres, E.; Peydro, M.A.; Navarro, R. *Polym. Eng. Sci.*, **2013**, 53, 679-688.

Additives and Fillers in Commercial Polymers

Small quantities of additives and fillers are embedded in most commercial polymers in order to obtain certain desirable end-use properties. Typically additives and fillers are added to commercial polymers to improve compatibility of dissimilar elastomers, mixing, processing and surface tack, extrusion rates, appearance, and reinforcement. Commercial polymers can contain a wide variety of additives and fillers, some of which can easily be removed from the commercial polymer through filtering while others may require a separation method such as GPC. The ability to separate a commercial polymer from the various additives and fillers is necessary when analyzing the molar mass averages and distributions of a polymer as the additives and fillers can skew the molar mass averages and distributions.

An EcoSEC GPC System with a dual flow RI detector coupled to a multi-angle light scattering detector (MALS) was used to separate and identify the presence of an additive in a commercial rubber sample. **Figure 6** shows the overlay of the GPC traces from the RI and MALS detectors. The RI detector shows two baseline resolved peaks while the MALS detector shows a single peak. The later eluting species, present only in the RI detector, are indicative of the additive, as materials polymeric in nature would be detectable by both the MALS and RI detectors. Additives are generally molecules low in molar mass and approaching the detection limit of the MALS detector (~1,000 g/mol) but present at a fairly high concentration, thus detectable by the concentration sensitive detector.

Figure 6: GPC elution profile of a rubber sample with additives as monitored by RI (blue) and MALS (green)



Column: TSKgel SuperMultiporeHZ-M, 4 μ m, 4.6 mm ID \times 15 cm \times 3
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System), MALS (Wyatt DAWN 8+)
Temperature: 35 $^{\circ}$ C
Injection vol.: 30 μ L
Sample: rubber

The baseline separation of the rubber from the additive allows for the determination of the polystyrene relative molar mass averages of both species and the absolute molar mass averages of the rubber, **Table 3**. The polystyrene relative and absolute molar mass averages obtained for the rubber are not expected to match, as the polystyrene relative values are dependent on the chemistry and architecture of the sample and standards. The dual detector GPC set-up allows for the identification of the presence of an additive and determination of the molar mass averages of both the rubber and additive within the commercial polymer sample.

Table 3: Molar mass averages and polydispersity index of a rubber sample and additive via RI and MALS

Sample (Detection Method)	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI^a
Rubber (RI)	$1.33 \times 10^5 \pm 0.02^b \times 10^5$	$3.10 \times 10^5 \pm 0.02 \times 10^5$	$4.80 \times 10^5 \pm 0.03 \times 10^5$	2.33 ± 0.01
Additive (RI)	455 ± 6	$1.06 \times 10^3 \pm 0.01 \times 10^3$	$2.42 \times 10^3 \pm 0.04 \times 10^3$	2.33 ± 0.02
Rubber (MALS)	$3.98 \times 10^5 \pm 0.39 \times 10^5$	$7.34 \times 10^5 \pm 0.21 \times 10^5$	$1.08 \times 10^6 \pm 0.21 \times 10^5$	1.849 ± 0.126

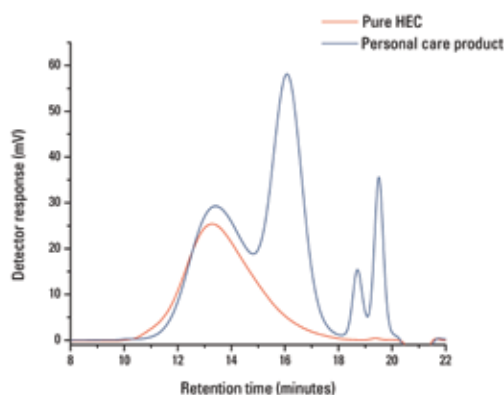
^a $PDI = M_w/M_n$; ^b Standard deviations from four injections

Polymers in Personal Care Products

Cosmetic and personal care companies are interested in the ability to characterize one of the most highly used non-ionic, water soluble polymers in their formulations, hydroxyethylcellulose (HEC). HEC is derived from cellulose and used in products such as shampoos, body washes, shower gels, and eye drops as it has the ability to thicken solutions and reduce the amount of suds or foam they form. The characterization of pure HEC and HEC within a personal care product was performed utilizing the EcoSEC GPC System with an internal dual flow RI detector and semi-micro columns for polymer analysis in an aqueous mobile phase.

The chromatograms of the pure HEC and the HEC within a personal care product, as monitored by the RI detector, are shown in Figure 7. The elution profile of the pure HEC displays the presence of one species while the personal care product displays a distinctive bimodal distribution in the location of the pure HEC as well as two additional components in the low molar mass region of the chromatogram. The bimodal distribution in the HEC region of the chromatogram for the personal care product could be a result of either two completely different polymer species in the product or the presence of two distinctive size (molar mass) distributions of HEC in the product with the lower molar mass portion of the HEC being present at a higher concentration than the high molar mass portion. The two later eluting species in the chromatogram for the personal care product are two additional components of the product that are significantly smaller in size than the main polymeric components of the product.

Figure 7: Elution profile of pure hydroxyethylcellulose and hydroxyethylcellulose in a personal care product



Column: TSKgel SuperMultiporePW-H, 8 μm , 4.6 mm ID \times 15 cm \times 3
Mobile phase: H₂O with 0.1 mol/L NaNO₃ and 0.02% NaN₃
Flow rate: 0.50 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 35 $^{\circ}\text{C}$
Injection vol.: 25 μL
Sample: hydroxyethylcellulose

The polyethylene oxide and polyethylene glycol RI relative molar mass averages of the pure HEC and the HEC within a personal care product are given in Table 4. The molar mass averages for the HEC within the personal care product were shown to vary from that of the pure HEC when the molar mass averages of both components in the HEC region of the chromatogram for the personal care product were determined collectively and separately. The molar mass distribution of the pure HEC and the HEC region of the personal care product indicate a polydisperse polymer as $PDI=9.82$ and $PDI=12.64$ (collectively) or $PDI=2.27$ and 1.59 (separately), respectively.

Table 4: Molar mass averages and polydispersity index of pure hydroxyethylcellulose and hydroxyethylcellulose in a personal care product

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI^a
Pure HEC	$1.50 \times 10^5 \pm 0.04^b \times 10^5$	$1.47 \times 10^6 \pm 0.01 \times 10^6$	$5.93 \times 10^6 \pm 0.01 \times 10^6$	9.82 ± 0.20
HEC in a personal care product (collectively)	$4.67 \times 10^4 \pm 0.01 \times 10^4$	$5.89 \times 10^5 \pm 0.02 \times 10^5$	$2.78 \times 10^6 \pm 0.06 \times 10^6$	12.61 ± 0.03
HEC in a personal care product (separately)	$5.21 \times 10^5 \pm 0.06 \times 10^5$	$1.12 \times 10^6 \pm 0.04 \times 10^6$	$2.47 \times 10^5 \pm 0.16 \times 10^5$	2.29 ± 0.01
	$2.69 \times 10^4 \pm 0.07 \times 10^4$	$4.32 \times 10^4 \pm 0.09 \times 10^4$	$6.38 \times 10^4 \pm 0.01 \times 10^4$	1.61 ± 0.23

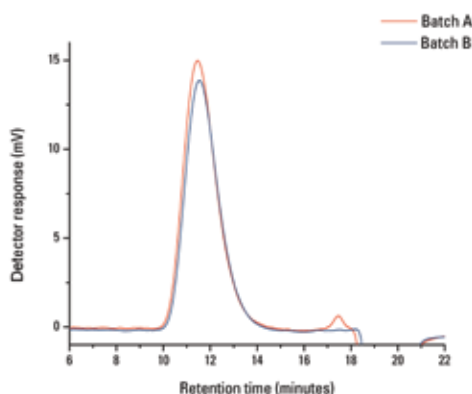
^a $PDI = M_w/M_n$; ^b Standard deviations from four injections

Utilities of GPC in Industry

One of the primary focuses of the polymer and plastics industries is the ability to differentiate polymers in a sustainable and time effective manner. Currently GPC methods are being used to distinguish polymers based on molar mass or hydrodynamic volume (size) in solution, as GPC is a fast, reliable, and robust method for polymer characterization. Most companies involved in the manufacturing and development of end-use products that involve polymers rely heavily on GPC. Throughout the polymer and plastics industries, the EcoSEC GPC System is used to detect differences from batch-to-batch or lot-to-lot of a given polymer, to monitor reaction processes, to determine variations in molar mass averages obtained through different synthesis routes, and to distinguish between polymers with the same chemical compositions but different end-use properties, to name a few.

Some of the utilities of the EcoSEC GPC System in the polymer and plastics industries are shown in Figures 8-10. Figure 8 compares the GPC elution profiles of two different batches of a PMMA based molding resin that can be used in automotive, home appliances, and electronics. Batch A extends further in the larger polymeric size, shorter retention time direction of the GPC elution profile than Batch B, an indication that the two batches differ in polymeric size. The slight variation in the GPC elution profile results in an approximately 10% difference in the poly(methyl methacrylate) molar mass averages between the two batches, Table 5. The difference in molar mass averages between Batch A and Batch B may or may not affect the end-use properties of a given polymer as the polydispersity index, *PDI*, remains essentially constant amongst the two batches.

Figure 8: GPC elution profiles of two different batches of a PMMA based molding resin



Columns: TSKgel SuperMultiporeHZ-M, 4 μm, 4.6 mm ID × 15 cm × 2 + TSKgel SuperHZ2500, 3 μm, 4.6 mm ID × 15 cm × 1
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 35 °C
Injection vol.: 20 μL
Sample: PMMA based molding resin

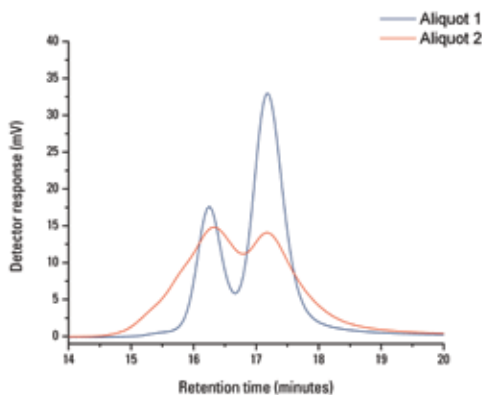
Table 5: Molar mass averages and polydispersity index of two different batches of a PMMA based molding resin

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	<i>PDI</i> ^a
Batch A	$6.59 \times 10^4 \pm 0.15^b \times 10^4$	$1.38 \times 10^5 \pm 0.02 \times 10^5$	$2.24 \times 10^5 \pm 0.03 \times 10^5$	2.11 ± 0.02
Batch B	$5.90 \times 10^4 \pm 0.10 \times 10^4$	$1.24 \times 10^5 \pm 0.01 \times 10^5$	$2.02 \times 10^5 \pm 0.03 \times 10^5$	2.11 ± 0.03

^a *PDI* = M_w/M_n ; ^b Standard deviations from four injections

An example of using the EcoSEC GPC System to monitor a reaction process is shown in Figure 9 by overlaying aliquots of a reaction collected thirty minutes apart. Each aliquot produces a different GPC elution profile which can be used to determine if the reaction process taking place is correct through a comparison process with known GPC elution profiles for various stages of the reaction. In general for this sample as the reaction process progresses the two individual components, indicated by the distinctive bimodal GPC elution profile of aliquot 1, blend to become one component in the final product, indicated by the decrease in the bimodality of aliquot 2.

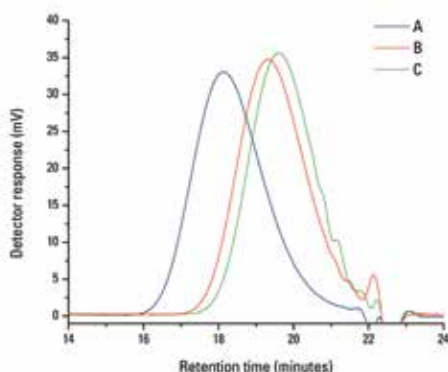
Figure 9: GPC elution profile of aliquots of a reaction collected thirty minutes apart



Column: TSKgel GMHXL, 9 μ m, 7.8 mm ID \times 30 cm \times 1
Mobile phase: THF
Flow rate: 1.0 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 35 $^{\circ}$ C
Injection vol.: 100 μ L
Sample: synthetic rubber

The use of the EcoSEC GPC System to distinguish between polymers obtained through different synthesis routes with the same chemical composition but different end-use properties is shown in Figure 10. The GPC elution profile for three polyimide samples shows a variation in retention time, thus also in the molar mass averages, Table 6. While these three polyimide samples are composed of the same chemical composition, the samples are shown to have different end-use properties due to differences in their molar mass averages and molar mass distributions.

Figure 10: GPC elution profile of polymers with the same chemical composition but different end-use properties



Column: TSKgel GMHXL, 9 μ m, 7.8 mm ID \times 30 cm \times 2
Mobile phase: DMF with 0.02 mol/L LiBr
Flow rate: 1.0 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 35 $^{\circ}$ C
Injection vol.: 100 μ L
Sample: polyimides

Table 6: Molar mass averages and polydispersity index of polymers with the same chemical composition but different end-use properties

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI ^a
A	$3.98 \times 10^4 \pm 0.01^b \times 10^4$	$6.47 \times 10^4 \pm 0.01 \times 10^4$	$8.98 \times 10^4 \pm 0.01 \times 10^4$	1.62 ± 0.02
B	$1.86 \times 10^4 \pm 0.01 \times 10^4$	$2.87 \times 10^4 \pm 0.01 \times 10^4$	$3.95 \times 10^4 \pm 0.01 \times 10^4$	1.54 ± 0.01
C	$1.53 \times 10^4 \pm 0.01 \times 10^4$	$2.34 \times 10^4 \pm 0.01 \times 10^4$	$3.20 \times 10^4 \pm 0.01 \times 10^4$	1.52 ± 0.01

^a PDI = M_w/M_n ; ^b Standard deviations from four injections

Polymer-Based Therapeutics

Polymer-based drug and gene delivery systems began to emerge from the laboratory benches about 30 years ago as a promising therapeutic strategy for treatment of devastating human diseases. Polymeric materials are useful for solving drug delivery problems as they are relatively large compared to low molar mass drugs, and when combined with these drugs they can augment the drug's performance and change their bioavailability.² The use of synthetic polymers in therapeutics is continuously growing, thus increasing the need for a method to characterize the molar mass averages and molar mass distributions of these polymers as variations in molar mass averages and molar mass distributions can affect aspects of the therapeutic such as in vitro binding activity and biodegradation.² The molar mass averages and molar mass distributions of a polymer being used in therapeutics is critical for designing an effective polymer-based therapeutic and is most commonly characterized using GPC.

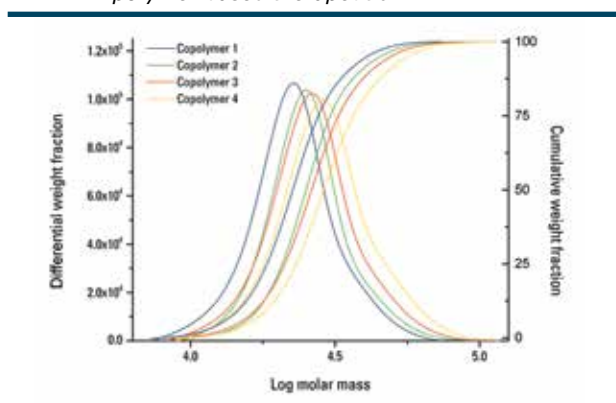
The EcoSEC GPC System was used to determine the molar mass averages and distributions of four block copolymers intended to be used in polymer-based drug or gene delivery systems. The polystyrene relative molar mass averages, M_n , M_w , and M_z , are given in Table 7. The variation of the molar mass averages for the four block copolymers may be great enough to affect the role the polymer plays in the polymer-based therapeutic within the body. For example, the molar mass of the polymer can influence the biodegradation of synthetic polymer in the body, thus resulting in the production of lower molar mass polymer that has different biological effects. In addition to the molar mass averages, the molar mass distribution can also influence various properties of therapeutics. The molar mass distributions of the four block copolymers are compared in Figure 11.

Table 7: Molar mass averages and polydispersity index of four block copolymers for use in a polymer-based therapeutic

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI^a
Copolymer 1	2.09×10^4 $\pm 0.01^b \times 10^4$	2.38×10^4 $\pm 0.01 \times 10^4$	2.70×10^4 $\pm 0.01 \times 10^4$	1.13 ± 0.01
Copolymer 2	2.38×10^4 $\pm 0.01 \times 10^4$	2.64×10^4 $\pm 0.01 \times 10^4$	2.93×10^4 $\pm 0.01 \times 10^4$	1.11 ± 0.01
Copolymer 3	2.48×10^4 $\pm 0.01 \times 10^4$	2.81×10^4 $\pm 0.01 \times 10^4$	3.22×10^4 $\pm 0.01 \times 10^4$	1.14 ± 0.01
Copolymer 4	2.74×10^4 $\pm 0.01 \times 10^4$	3.10×10^4 $\pm 0.01 \times 10^4$	3.55×10^4 $\pm 0.01 \times 10^4$	1.14 ± 0.01

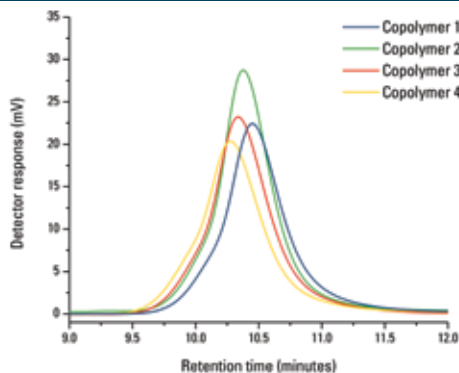
^a $PDI = M_w/M_n$; ^b Standard deviations from four injections

Figure 11: Overlay of cumulative and differential molar mass distribution of four block copolymers for use in a polymer-based therapeutic



Information regarding the differences between the four block copolymers for use in a polymer-based therapeutic can be seen by comparing their GPC elution profiles, Figure 12. The shift in GPC retention time amongst the four block copolymers indicates a variation in polymeric size between the block copolymers, as elution order in GPC is that of an "inverting-sieving" technique, large analytes sample a smaller pore volume than smaller analytes resulting in the larger analytes eluting from the column prior the smaller analytes. Variations in polymeric size within a polymer-based therapeutic can dramatically affect its behavior within a biological system.

Figure 12: GPC elution profile of four block copolymers for use in a polymer-based therapeutic



Columns: TSKgel SuperHZ4000, 3 μ m, 4.6 mm ID \times 15 cm +
TSKgel SuperHZ3000, 3 μ m, 4.6 mm ID \times 15 cm +
TSKgel SuperHZ2000, 3 μ m, 4.6 mm ID \times 15 cm
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 35 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: block copolymer

²Kabanov, A.V.; Okano, T. Challenges in Polymer Therapeutics. In *Polymer Drugs in the Clinical Stage: Advantages and Prospects*, Volume 519; Maeda, H.; Kabanov, A.V.; Kataoka, K., Okano, T. eds.; Academic Press: New York, 2003; pp 1-20.

Sugar Beet Pectin

Members of the Dairy and Functional Food Unit at the U S Department of Agriculture,[†] Eastern Region Research Center, Agricultural Research Service, are interested in the detailed characterization of sugar beet pectin (SBP), as it has the potential to be used in the production of industrial products, *e.g.*, as an emulsifying agent in food systems.³ The characterization of SBP was performed utilizing the EcoSEC GPC System with an internal dual flow RI detector and UV detector coupled to multi-angle light scattering (MALS), quasi-elastic light scattering (QELS), and differential viscometry (VISC).

The results of the experiments are given in Table 8. The weight-average molar mass for the SBP was determined to be approximately 1.1×10^6 g/mol and shown to vary less than 7% amongst the four concentration sensitive detectors (UV @ 310, 278, and 250 nm and RI). The addition of the external detectors to the EcoSEC GPC System allows for the determination of the molar mass of SBP, in a calibrant-independent fashion, as well as several sizing parameters (R_G and R_H), and intrinsic viscosity, also given in Table 8.

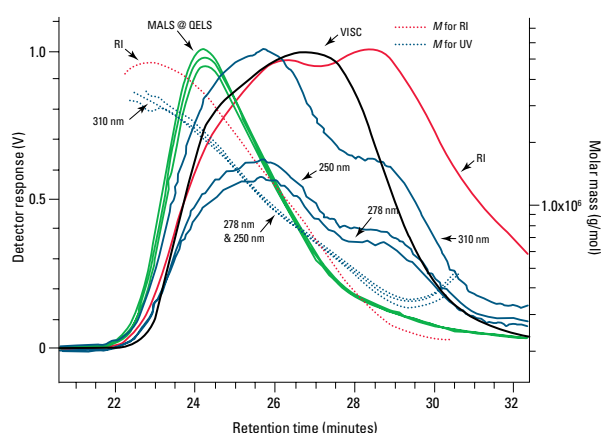
Table 8: Molar mass, R_G , R_H , and intrinsic viscosity measurements for sugar beet pectin

	Detection Method			
	RI	UV @ 250 nm	UV @ 278 nm	UV @ 310 nm
M_w (g/mol) ^a	$1.098 \times 10^6 \pm 0.003^b \times 10^6$	$1.097 \times 10^6 \pm 0.004 \times 10^6$	$1.147 \times 10^6 \pm 0.004 \times 10^6$	$1.187 \times 10^6 \pm 0.002 \times 10^6$
$R_{G,z}$ (nm) ^a	43 ± 1	43 ± 1	45 ± 1	42 ± 1
$R_{H,z}$ (nm) ^c	53 ± 1	43 ± 1	43 ± 1	44 ± 1
$[\eta]_w$ (dL/g)	3.5 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.5 ± 0.1

^a with MALS; ^b Standard deviation; ^c with QELS

Figure 13 shows the chromatograms of SBP, as monitored by the individual detectors. The elution profile of the SBP, as measured by the four concentration sensitive detectors, displays a distinct bimodal distribution. Evidence of molar mass polydispersity of SBP is seen in Figure 13, as the molar mass of the SBP decreases by an order-of-magnitude with increasing elution volume. It should also be noted that if one compares the molar mass distributions of the four concentration sensitive detectors, the molar mass of the SBP is higher at lower elution volumes and lower at larger elution volumes via the RI detector than via the UV detector. The difference in molar masses between the various detection methods is an indication that the particles with higher molar masses have fewer UV absorbing molecules associated with them than their lower molar mass counterparts.

Figure 13: Elution profile of sugar beet pectin



Column: TSKgel GMPWxl, 13 μ m, 7.8 mm ID \times 30 cm \times 2
Mobile phase: H₂O with 0.05 mol/L NaNO₃ and 0.1% NaN₃
Flow rate: 0.7 mL/min
Detection: RI (EcoSEC GPC System), UV (EcoSEC GPC System @ 310, 278, and 250 nm), MALS (Wyatt DAWN HELEOS® II), QELS (Wyatt QELS™), VISC (Wyatt ViscoStar)
Temperature: 35 °C
Injection vol.: 200 μ L
Sample: sugar beet pectin

³Fishman, M. L.; Chau, H. K.; Qi, P. X.; Hotchkiss Jr., A. T.; Yadav, M. P. *Carbohydr. Polym.* **2013**, 92, (2), 2257-2266.

[†]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Quality Control Procedures

One of the most common utilities of GPC is the determination of molar mass averages and distributions of polymers using peak position calibration involving polystyrene standards of known molar mass and chemistry for quality control procedures. It is essential for polymer manufactures to differentiate between “good” and “bad” batches of products, as differences in molar mass averages and distributions between batches can lead to changes in a polymer’s end-use properties, e.g. tensile strength, elongation, brittleness, etc. The EcoSEC GPC System is currently used in numerous quality control laboratories to differentiate between “good” and “bad” batches of products through monitoring molar mass averages and GPC elution profiles.

Figure 14 shows an example of how the EcoSEC GPC System can be used to distinguish between two batches of the same polymer. The sample labeled “good” is a batch of polymer that performs at or above standards when used in its end-use application while the sample labeled “bad” has shown to perform below standards in the same end-use application. The overlap of both the RI and UV GPC elution profiles of the “good” and “bad” samples shows that the two samples are significantly different from one another. The “good” sample has a shorter retention time than that of the “bad” sample. The difference in retention time indicates that the “good” sample is larger in polymeric size than its “bad” counterpart. Additionally, the molar mass averages and polydispersity index, *PDI*, of the “good” and “bad” samples, Table 9, provide evidence that the two batches of polymer are significantly different from one another. The molar mass averages M_n , M_w and M_z for the “good” sample are larger than those for “bad” sample. The GPC elution profile and molar mass averages provide significant information about the difference between the “good” and “bad” samples.

Figure 14: GPC elution profiles of “good” and “bad” batches of product as monitored by RI (red) and UV (blue)

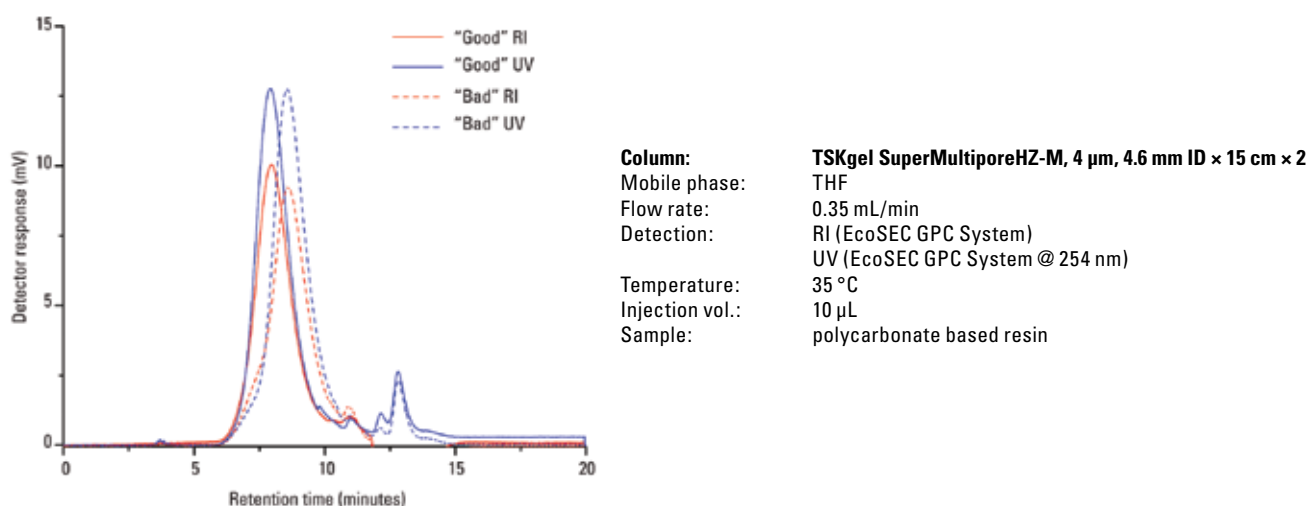


Table 9: Molar mass averages and polydispersity index of “good” and “bad” batches of product

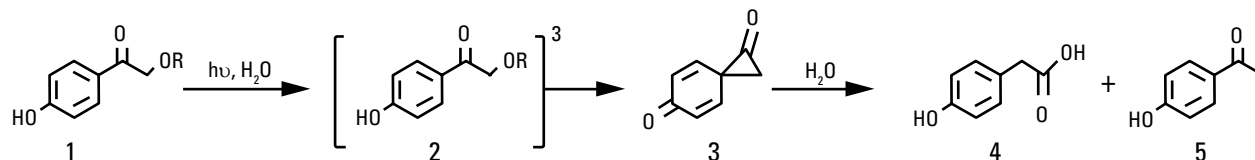
Sample (Detection Method)	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	<i>PDI</i> ^a
“Good”(RI)	$1.100 \times 10^4 \pm 335^b$	$5.199 \times 10^4 \pm 752$	$1.339 \times 10^5 \pm 3,072$	4.73 ± 0.08
“Good”(UV)	$1.123 \times 10^4 \pm 333$	$4.367 \times 10^4 \pm 402$	$1.064 \times 10^5 \pm 1,698$	3.89 ± 0.09
“Bad”(RI)	$6,064 \pm 35$	$3.036 \times 10^4 \pm 260$	$1.259 \times 10^5 \pm 1,465$	5.01 ± 0.02
“Bad”(UV)	$5,364 \pm 38$	$2.162 \times 10^4 \pm 120$	$9.635 \times 10^4 \pm 1,154$	4.03 ± 0.02

^a $PDI = M_w/M_n$; ^b Standard deviations from six injections

Photodegradable Polymer Degradation Analysis

Due to the need for polymers that are both photodegradable and biodegradable, Dr. Abraham Joy and his colleagues at the University of Akron have developed polycarbonate materials based on the alkoxyphenacyl photoactive moiety.⁴ This new class of polymers is mechanically robust, biodegradable, and stable to high temperatures in the absence of light with potential applications in controlled drug release devices, ocular implants, and dermal patches. Upon radiation, the photoactive moiety undergoes a Favorski type of rearrangement, resulting in two major products, the phenylacetic acid derivative and the reduced acetophenone (Figure 16).⁵

Figure 16: Mechanism for the photo-rearrangement of hydroxyphenacyl esters



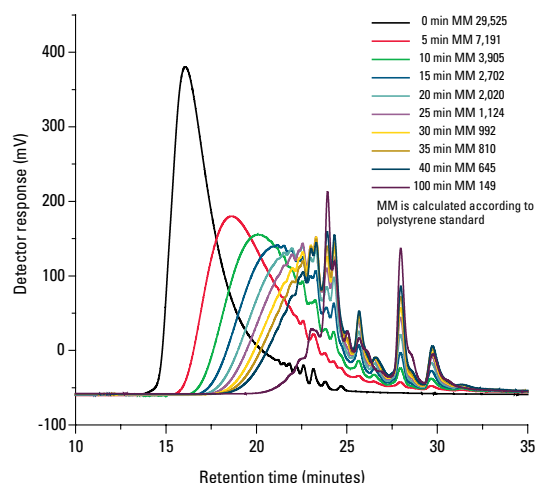
The EcoSEC GPC System was used to determine the polystyrene relative molar mass averages, M_n and M_w , and the polydispersity index, PDI , of an alkoxyphenacyl-based polycarbonate homopolymer, 5% PEG copolymer, and 10% PEG copolymer, all given in Table 11. The PDI s of the 5% and 10% PEG copolymer are smaller than the PDI of the homopolymer because the PEG copolymer samples were fractionated twice and the homopolymer was fractionated only once.

Table 11: Molar mass distributions and polydispersity index for homopolymer and copolymers

Composition	M_n (g/mol)	M_w (g/mol)	PDI
Homopolymer	1.29×10^4	2.95×10^4	2.3
5% PEG	2.27×10^4	2.63×10^4	1.2
10% PEG	8,810	1.04×10^4	1.2

Photodegradation of the homopolymer and copolymers was investigated by irradiation of the polymers in chloroform in a Rayonet reactor at 300 nm. Figure 17 shows GPC traces indicating time-dependent degradation with a 75% reduction in average molar mass within 5 minutes of irradiation. Subsequent analysis (data not shown) shows similar degradation for all three polymers.

Figure 17: GPC traces showing decrease in molar mass (M_w) with increasing radiation time for the alkoxyphenacyl-based polycarbonate homopolymer.



Columns: TSKgel SuperH3000, 3 μ m, 6.0 mm ID x 15 cm x 2 + TSKgel SuperH4000, 3 μ m, 6.0 mm ID x 15 cm x 1
Mobile phase: chloroform
Flow rate: 0.38 mL/min
Detection: UV (EcoSEC GPC System @ 278 nm)
Temperature: 40 °C
Injection vol.: 10 μ L
Sample: alkoxyphenacyl polycarbonate homopolymer, 1 - 2 mg/mL

⁴Sun, S.; Chamsaz, E. A.; Joy, A. *Macro Lett.*, **2012**, 1 (10), 1184–1188.

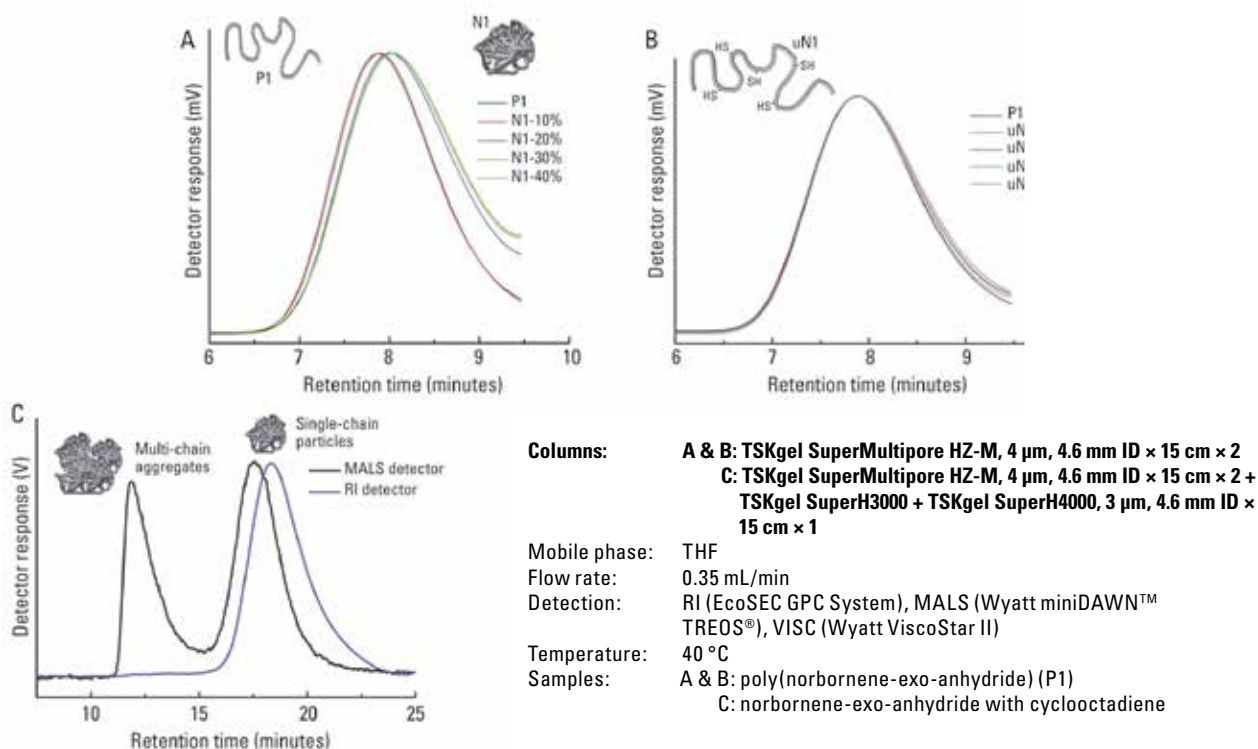
⁵Givens, R. S.; Heger, D.; Hellrung, B.; Kamdzhilov, Y.; Mac, M.; Conrad, P. G.; Cope, E.; Lee, J. I.; Mata-Segreda, J. F.; Schowen, R. L.; Wirz, J. J. *Am. Chem. Soc.* **2008**, 130, 3307-3309.

Single-chain Polymer Nanoparticles

Dr. Erik Berda's research group at the University of New Hampshire is working on the fabrication and characterization of single-chain polymer nanoparticles (SCNPs) that can reversibly undergo a coil to particle transition via formation and cleavage of intramolecular disulfide cross-links.⁶ In their initial studies Dr. Berda's group synthesized poly(norbornene-exo-anhydride) (P1), via ROMP using third generation Grubbs catalyst as an initiator and controlled the degree of collapse that occurs during nanoparticle (N1) formation by varying the amount of difunctional cross-linker added. The coil to particle transition was then characterized using the EcoSEC GPC System with dual flow RI via polystyrene relative molar mass averages. **Figure 18A** shows a series of GPC traces for P1 and its corresponding N1 after various extents of intramolecular cross-linking. As expected, an increase in GPC retention time is observed as the intramolecular cross-linking reaction progresses. This is due to a decrease in hydrodynamic volume that occurs as the coil collapses. Once the folding of the chains into SCNPs was confirmed via the GPC retention times, dithioerithol was introduced to unfold the N1 back to their original conformation. The transition from particle to coil was also confirmed via decreased GPC retention time, signifying an increase in hydrodynamic volume, **Figure 18B**.

To complement their initial studies Dr. Berda's group synthesized a second polymer, norbornene-exo-anhydride with cyclooctadiene (COD) (P2), to characterize via triple-detector GPC. For the characterization of P2, the EcoSEC GPC System with dual flow RI was coupled to multi-angle light scattering (MALS) and differential viscometry (VISC). The effectiveness of the triple-detector GPC system was highlighted by determining the difference between single-chain and multi-chain behavior. **Figure 18C** shows an overlay of the MALS and RI traces when the intra-molecular cross-linking reaction was extended with a slight excess of the cross-linker to encourage intermolecular coupling. The RI detector shows a single peak that can be attributed to single-chain particles, while the MALS detector shows two peaks of nearly equal intensity. The later eluting MALS peak corresponds to the single-chain particles while the early eluting peak is that of multi-chain aggregates, which are present at a negligible concentration as indicated by the RI detector. For this particular sample analysis, single-detector GPC would not have revealed the presence of the larger aggregates.

Figure 18: Single-chain polymer nanoparticles



⁶Tuten, B.T.; Chao, D.; Lyon C.K.; Berda, E.B. *Polym. Chem.* **2012**, *3*, 3068-3071.

HFIP Reproducibility

Dr. Li Jia and co-workers at the University of Akron are investigating different synthetic routes for the formation of polypeptoids with alternating block structures. Highly reproducible data is needed to obtain subtle molar mass distribution trends from the various synthetic routes. The EcoSEC GPC System and a set of TSKgel mixed bed columns were used successfully to obtain high quality molar mass distribution (MMD) data of a series of Dr. Jia's block poly- β -alkylalanoids with hexafluoroisopropanol (HFIP) as the mobile phase in under 15 minutes.

As shown in Table 12, percent standard deviations are more than 10x lower than values previously reported for polyamides in HFIP.⁷ Percent relative standard deviation of the polydispersity index (*PDI*) ranged from 0.1 to 0.5%, permitting one to report *PDI*s within three significant figures. The high precision of the EcoSEC GPC System allows for the detailed study of polymerization reactions.

Table 12: Averaged values from three consecutive injections and the percent relative standard deviations

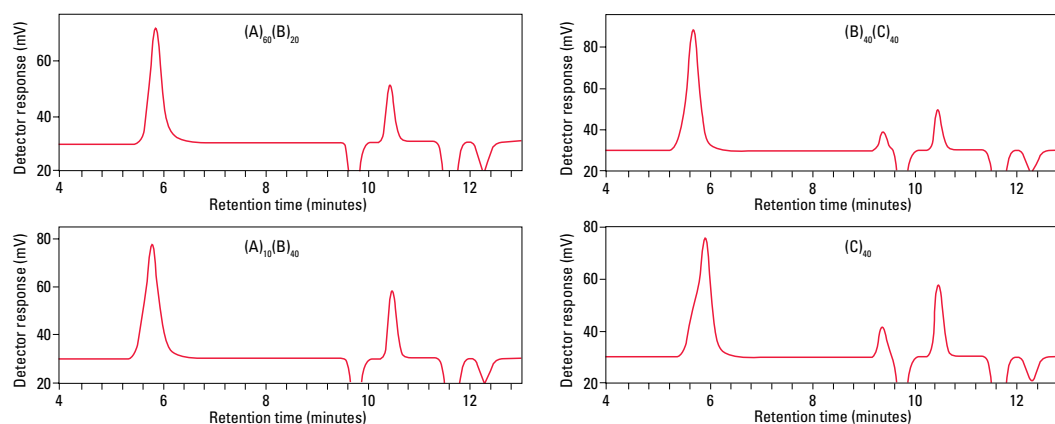
Sample ^a	M_n^b (g/mol)		M_w^b (g/mol)		<i>PDI</i> ^b	
		Rel std dev		Rel std dev		Rel std dev
(A) ₁₀ (B) ₄₀	$2.65 \times 10^4 \pm 10$	0.04%	$3.03 \times 10^4 \pm 30$	0.11%	1.14 ± 0.01	0.09%
(A) ₆₀ (B) ₂₀	$3.33 \times 10^4 \pm 170$	0.52%	$4.07 \times 10^4 \pm 28$	0.07%	1.22 ± 0.01	0.50%
(A) ₄₀ (B) ₄₀	$4.87 \times 10^4 \pm 220$	0.45%	$6.09 \times 10^4 \pm 160$	0.26%	1.25 ± 0.01	0.10%
(C) ₄₀	$3.01 \times 10^4 \pm 50$	0.18%	$3.64 \times 10^4 \pm 140$	0.37%	1.21 ± 0.01	0.39%

^a Block lengths were determined by Dr. Jia from independent measurements. Chemical composition of blocks A, B and C will be published by L. Jia.

^b Molar mass data were obtained from a PMMA calibration curve. Molar mass averages given in the table are averages of three sequential injections per sample. Based on block lengths, MMD are significantly overestimated.

Sample chromatograms from 4 selected poly- β -alkylalanoid samples run on an EcoSEC GPC System using two TSKgel GMHHR-M, 5 μ m, 4.6 mm ID \times 15 cm columns are shown in Figure 19. Sample profiles display very little tailing and no baseline drift, allowing for highly precise data not available with conventional systems. All samples, with the exception of (C)₄₀, contain almost symmetrical, narrow polymer profiles eluting around 6 minutes. The shoulder seen in (C)₄₀ is indicative of another population of a high MM polymer component in the sample.

Figure 19: Poly- β -alkylalanoid samples



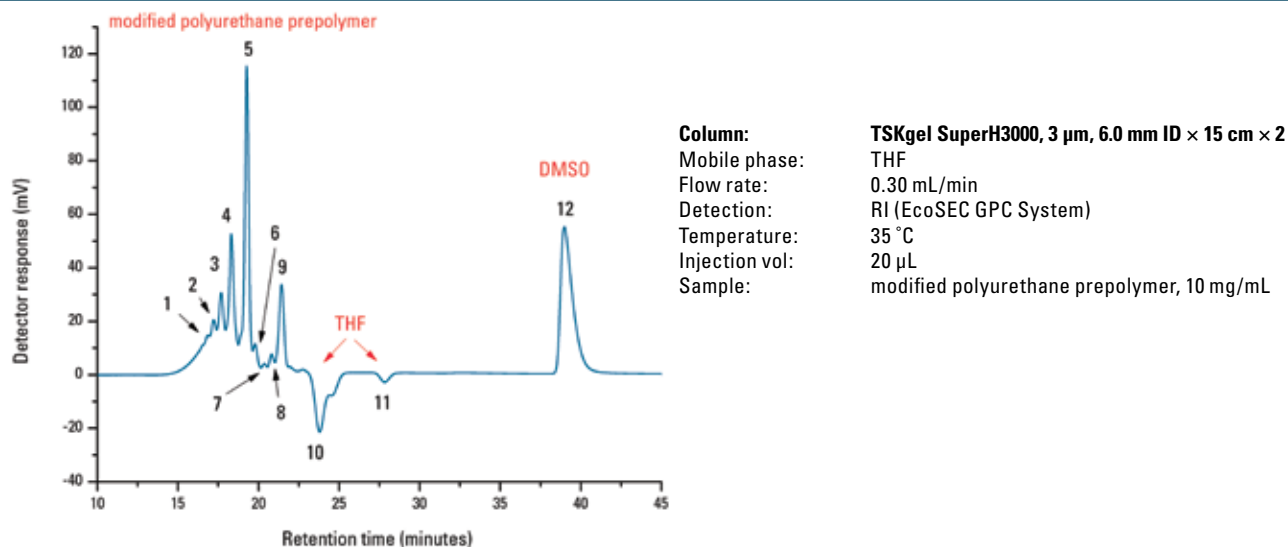
Column: TSKgel GMHHR-M, 5 μ m, 4.6 mm ID \times 15 cm \times 2 packed in HFIP
Mobile phase: HFIP containing 5 mmol/L sodium trifluoroacetate
Flow rate: 0.35 mL/min
Detection: RI (EcoSEC GPC System)
Temperature: 40 $^{\circ}$ C
Injection vol.: 10 μ L
Samples: selection of poly- β -alkylalanoid samples

⁷Robert, E. C.; Bruessau, R.; Dubois, J.; Jacques, B.; Meijerink, N.; Nguyen, T. Q.; Niehaus, D. E.; Tobisch, W. A. *Pure Appl. Chem.* **2004**, *76*, 2009–2025.

Modified Polyurethane Prepolymer Analysis

An EcoSEC GPC System was used to analyze an isocyanate modified polyurethane prepolymer with residual DMSO. As shown in Figure 20, separation of the sample by GPC results in ten positive chromatographic peaks and two negative chromatographic peaks. The first nine chromatographic peaks correspond to components of the modified polyurethane prepolymer while the two negative chromatographic peaks are indicative of the solvent, THF. The latest eluting peak is a result of the residual DMSO present in the sample and is retained by a non-SEC retention mechanism, as it elutes after the void volume of the column.

Figure 20: Modified polyurethane prepolymer sample



The molar mass averages M_n , M_w , and M_z , of the sample, given in Table 13, were determined via a polystyrene relative calibration curve. The sample was found to have a weight average molar mass, M_w , ranging from 4,199 to 178 g/mol. The polydispersity index (*PDI*) shown in Table 13 for the entire sample, *e.g.*, peaks 1 through 9, was 2.26 while the individual components of the polyurethane prepolymer had *PDI* values ranging from 1.01 to 1.09. From the *PDI* values it can be concluded that collectively the sample is polydispersed with respect to molar mass, but the nine visible components within the sample are virtually monodispersed with respect to molar mass.

Table 13: Molar mass averages and polydispersity index for modified polyurethane prepolymer sample in THF at 0.3 mL/min

Peak	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	<i>PDI</i> ^a
1	4,199 \pm 46 ^b	4,606 \pm 67	5,214 \pm 109	1.09 \pm 0.01
2	2,643 \pm 19	2,655 \pm 18	2,667 \pm 18	1.01 \pm 0.01
3	2,011 \pm 16	2,024 \pm 16	2,038 \pm 16	1.01 \pm 0.01
4	1,387 \pm 10	1,403 \pm 10	1,418 \pm 10	1.01 \pm 0.01
5	798 \pm 4	808 \pm 5	817 \pm 5	1.01 \pm 0.01
6	551 \pm 9	554 \pm 9	557 \pm 9	1.01 \pm 0.01
7	391 \pm 9	394 \pm 9	397 \pm 10	1.01 \pm 0.01
8	278 \pm 3	280 \pm 3	282 \pm 3	1.01 \pm 0.01
9	178 \pm 1	181 \pm 1	183 \pm 1	1.01 \pm 0.01
All	676 \pm 9	1,531 \pm 31	2,873 \pm 83	2.26 \pm 0.02

^a. *PDI* = M_w/M_n ; ^b. Standard deviations from six injections

Analysis of Styrene and Isoprene Block Copolymers

Dr. Jimmy Mays' group from the Department of Chemistry at the University of Tennessee, Knoxville, is synthesizing and characterizing the bulk morphology of fluorinated and sulfonated block copolymers. Well-defined block copolymers of sulfonated polystyrene-*b*-fluorinated polyisoprene (sPS-*b*-fPI), **Figure 21**, were synthesized by anionic polymerization followed by fluorination and sulfonation.⁸ The EcoSEC GPC System, equipped with TSKgel SuperMultiporeHZ columns, was then used to determine the number-average molar mass, M_n , and the polydispersity index, *PDI*, of sPS-*b*-fPI, as well as that of the precursor polymer (PS-*b*-PI), **Table 14**. As seen in **Figure 22**, complete analysis of sPS-*b*-fPI was obtained in less than 10 minutes with excellent resolution using the EcoSEC GPC System.

Figure 21: Structure of sulfonated polystyrene-*b*-fluorinated polyisoprene (sPS-*b*-fPI)

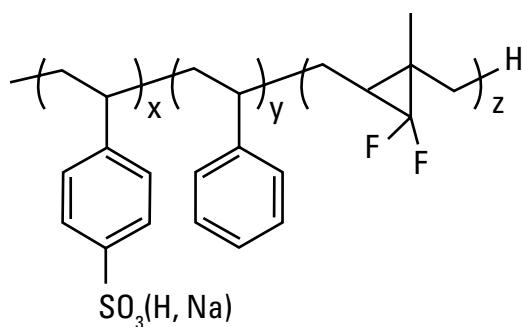
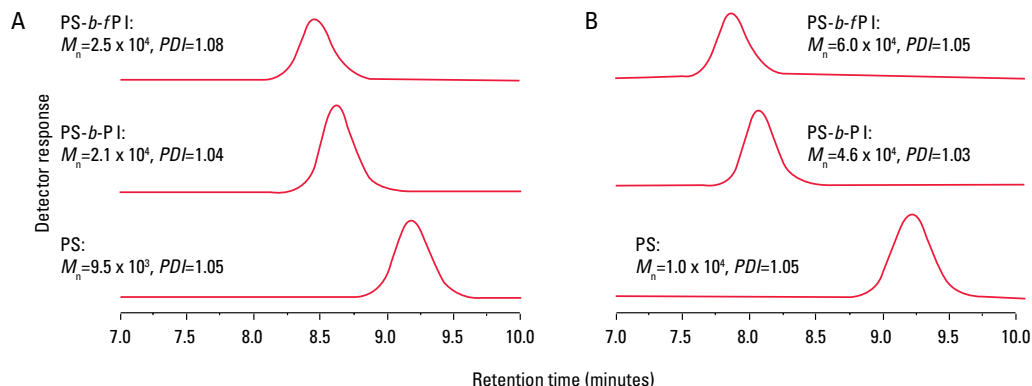


Table 14: Number-average molar mass, M_n , and the polydispersity index (*PDI*) of sPS-*b*-fPI and the precursor polymer (PS-*b*-PI)

Series ^a	PS- <i>b</i> -PI		sPS- <i>b</i> -fPI	
	M_n (g/mol)	<i>PDI</i>	M_n (g/mol)	<i>PDI</i>
1	2.1×10^4	1.04	2.5×10^4	1.08
2	4.6×10^4	1.03	6.0×10^4	1.05

^aseries 1 in acid form; series 2 in Na form

Figure 22: Sulfonated polystyrene-*b*-fluorinated polyisoprene precursor samples



Column: TSKgel SuperMultiporeHZ-M, 4 μ m, 4.6 mm ID x 15 cm
 Mobile phase: THF
 Flow rate: 0.35 mL/min
 Detection: RI (EcoSEC GPC System)
 Temperature: 35 °C
 Injection vol: 20 μ L
 Samples: A. series 1, table 13 B. series 2, table 13

⁸Wang, X.; Hong, K.; Baskaran, D.; Goswami, M.; Sumpter, B.; Mays, J. *Soft Matter*, **2011**, *7*, 7960.

TSKgel GPC Columns

Tosoh introduced its first line of GPC columns in 1971. Ever since, Tosoh scientists have made important contributions to advances in polymer analysis by developing state-of-the-art GPC columns for the most demanding applications.

Semi-micro columns are the TSKgel columns of choice for use with the EcoSEC GPC System.

They are referred to as such since their dimensions are smaller than conventional columns in terms of internal diameter as well as in length: 4.6 mm or 6 mm ID x 15 cm vs. 7.8 mm ID x 30 cm.

GPC columns for polymers soluble in organic solvents

Semi-micro columns (4.6 or 6.0 mm ID x 15 cm)

- TSKgel SuperMultiporeHZ columns
- TSKgel SuperHZ columns for ultra-low adsorption
- TSKgel SuperH columns for low adsorption

Conventional columns (7.8 mm ID x 30 cm)

- TSKgel H_{XL} columns for ultra-low adsorption
- TSKgel H_{HR} columns for low adsorption
- TSKgel H_{HR} HT and HT2 columns for high temperature analysis



GPC columns for polymers soluble in polar organic solvents

Semi-micro columns (6.0 mm ID x 15 cm)

- TSKgel SuperAW columns

Conventional columns (7.8 mm ID x 30 cm)

- TSKgel Alpha columns

GPC columns for polymers soluble in aqueous solvents

Semi-micro columns (6.0 mm ID x 15 cm)

- TSKgel SuperMultiporePW columns

Conventional columns (7.5 or 7.8 mm ID x 30 or 60 cm)

- TSKgel PW columns
- TSKgel PW_{XL} columns for higher efficiency
- TSKgel PW_{XL}-CP columns for analysis of cationic polymers

TSKgel H Series Size Exclusion Columns

TSKgel H series columns are recommended for the analysis of organic-soluble polymers and are packed with spherical particles composed of polystyrene crosslinked with divinylbenzene (PS-DVB). This series includes TSKgel H_{XL}, H_{HR}, SuperH, SuperHZ, and SuperMultiporeHZ columns. Each line of columns within this series differs in degree of inertness and operating temperature range.

The Super prefix designates short (15 cm) columns packed with particles as small as 3 μm. The smaller particle allows for equivalent resolution to conventional TSKgel H_{XL} columns, with 50% reduction in analysis time due to the shorter column length. The TSKgel Super series columns are an excellent choice for high throughput polymer analysis.

- The TSKgel H_{XL} columns are conventional GPC columns of 7.8 mm ID × 30 cm. The column line consists of eight columns with different pore sizes, TSKgel G1000H_{XL} through TSKgel G7000H_{XL}, and three columns with an extended linear range of the calibration curve, TSKgel GMH_{XL}, TSKgel GMH_{XL}-L and TSKgel MultiporeH_{XL}-M. The 5 μm particles in the TSKgel MultiporeH_{XL}-M column contain a broad range of pore sizes. This innovative approach essentially creates a linear calibration curve within each particle. As a result, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes.

The main characteristics of TSKgel H_{XL} columns are: ultra-low sample adsorption, i.e., the columns show true size exclusion behavior for most polymers, limited solvent range, and a maximum operating temperature of 60 °C for TSKgel G1000H_{XL} - G3000H_{XL}, and 80 °C for the remaining columns in the TSKgel H_{XL} column line.

- The TSKgel H_{HR} column line consists of eight conventional GPC columns of 7.8 mm ID × 30 cm with different pore sizes, TSKgel G1000H_{HR} through TSKgel G7000H_{HR}, and seven mixed bed columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel GMH_{HR}-L, GMH_{HR}-N, GMH_{HR}-M, to GMH_{HR}-H. The main characteristic of these TSKgel H_{HR} columns is a broad solvent range.

In addition, nine TSKgel H_{HR} mixed bed columns are available for high temperature analysis. The maximum operating temperature of the TSKgel H_{HR} HT columns is 140 °C and the maximum operating temperature of the TSKgel H_{HR} HT2 columns is 220 °C.

- The TSKgel SuperH column line consists of eight columns of 6.0 mm ID × 15 cm with different pore sizes, TSKgel SuperH1000 through TSKgel SuperH7000, and four mixed bed columns with an extended linear range of the calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHM-L, SuperHM-N, SuperHM-M, to SuperHM-H. TSKgel SuperH columns are high efficiency/high throughput versions of the conventional TSKgel H_{HR} columns. Both column types are based on the same bead chemistry.

The main characteristics of TSKgel SuperH columns are: a maximum operating temperature of 140 °C and the ability to use a broad range of solvents.

- The TSKgel SuperHZ column line consists of five columns of 4.6 mm ID × 15 cm and 6.0 mm ID × 15 cm with different pore sizes, TSKgel SuperHZ1000 through TSKgel SuperHZ4000, and three columns with an extended linear range of the calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHZM-L, SuperHZM-N to SuperHZM-H.

The main characteristics of TSKgel SuperHZ columns are: developed for high throughput, high efficiency GPC applications such as those encountered in combinatorial chemistry experiments, ultra-low sample adsorption, limited solvent range, and a maximum operating temperature of 60 °C for TSKgel SuperHZ1000 - SuperHZ3000 and 80 °C for the remaining columns in the TSKgel SuperHZ line.

- The TSKgel SuperMultiporeHZ column line consists of three columns of 4.6 mm ID × 15 cm with particles sizes of 3, 4 and 6 μm. The particles in TSKgel SuperMultiporeHZ columns are monodisperse in size and exhibit a broad range of pore sizes. Each particle, by design, has an extended linear calibration curve, thereby greatly diminishing chromatograms with inflection points.

A comparison of TSKgel H series columns is detailed in [Table 1](#). The cross-linking of the polystyrene particles in TSKgel H series columns ensures minimal shrinking and swelling of the column bed when the organic solvent is changed according to the solvent recommendations outlined in [Table 2](#). Suggested flow rates for solvent exchange in TSKgel SuperH and H_{HR} columns are outlined in [Table 3](#). [Table 4](#) lists the recommended solvents by application for TSKgel H series columns.

Table 1: Comparison of TSKgel H series columns

TSKgel series	SuperMultiporeHZ	SuperHZ	SuperH	H _{XL}	H _{HR}
Application focus	Ultra-high performance with a low dead volume and a wide pore distribution in each particle for superior linearity	High throughput polymer analysis with ultra-low polymer adsorption, limited solvent compatibility range	High throughput polymer analysis with expanded solvent compatibility range	Conventional polymer analysis with ultra-low polymer adsorption, limited solvent compatibility range	Conventional polymer analysis with expanded solvent compatibility range
Particle size	3 μm, 4 μm, and 6 μm, depending on pore size	3 μm, 5 μm, and 10 μm, depending on pore size	3 μm and 5 μm, depending on pore size	5 μm, 6 μm, 9 μm, and 13 μm, depending on pore size	5 μm, 13 μm, 20 μm, and 30 μm
Particle matrix	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)	Polystyrene divinylbenzene (PS-DVB)
Number of solvent substitutions	None	One time only	Several ¹	One time only	Several ¹

¹ After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is not recommended.

Table 2: Solvent compatibility for TSKgel H series columns

TSKgel series	Shipping solvent*	Can be replaced with:
SuperHZ and H _{XL} ¹	Tetrahydrofuran ^{3,4}	benzene, chloroform, toluene, xylene, dichloromethane, dichloroethane
	Acetone**	carbon tetrachloride ⁵ , <i>o</i> -dichlorobenzene, dimethylformamide, dodecane, dimethyl sulfoxide, dioxane, ethylacetate, FC-113, hexane, pyridine, hexafluoroisopropanol/chloroform, methyl ethyl ketone, quinoline, cyclohexane
	Chloroform**	<i>m</i> -cresol in chloroform, up to 10% hexafluoroisopropanol/chloroform
	Dimethylformamide	dimethyl sulfoxide, dioxane, tetrahydrofuran, toluene
SuperH and H _{HR} ²	Tetrahydrofuran ³	acetone, ethanol, quinoline, benzene, <i>o</i> -dichlorobenzene, ethyl acetate, dodecane, FC-113, carbon tetrachloride ⁵ , dichloromethane, dichloroethane, trichloroethane, <i>n</i> -hexane, cyclohexane, xylene, tetrahydrofuran, chloroform, 1,4-dioxane, hexafluoroisopropanol, toluene, 1-chloronaphthalene, <i>N,N</i> -dimethylacetoacetamide, methyl ethyl ketone, trichlorobenzene, <i>m</i> -cresol, dimethylformamide, methylpyrrolidone, <i>o</i> -chlorophenol/chloroform, dimethyl sulfoxide, pyridine
SuperMultiporeHZ	Tetrahydrofuran ³	Cannot be replaced. TSKgel SuperMultiporeHZ columns can be used only in tetrahydrofuran

¹ In case of TSKgel SuperHZ and H_{XL}, keep flow rate as mentioned below during solvent change. Solvent can be changed one way/one time only.

TSKgel H_{XL}: below <0.5 mL/min

TSKgel SuperHZ (4.6 mm ID): below <0.15 mL/min

TSKgel SuperHZ (6.0 mm ID): below <0.3 mL/min

² In case of TSKgel SuperH and H_{HR}, see Table 3 for appropriate flow rates for solvent exchange. After switching to a very polar solvent, switching to a nonpolar solvent is not recommended.

³ All TSKgel H_{XL}, H_{HR}, SuperHZ, SuperH, SuperMultipore, and GMH analytical columns are shipped containing tetrahydrofuran (THF), except the TSKgel high temperature columns, which contain *o*-dichlorobenzene (ODCB).

⁴ THF in TSKgel G1000H_{XL} columns cannot be replaced with dichloromethane or dichloroethane.

⁵ Prolonged exposure to carbon tetrachloride can corrode the stainless steel parts of a column and an HPLC system.

* 100% methanol cannot be used with TSKgel H series columns; use this solvent with TSKgel SW or Alpha columns.

** TSKgel H series columns may be specially ordered with this shipping solvent.

Table 3: Recommended flow rates (mL/min) for TSKgel SuperH and HHR columns

Solvent	TSKgel SuperH 6.0 mm ID × 15 cm	TSKgel HHR 7.8 mm ID × 30 cm
<i>n</i> -Hexane	0.5	0.9
methyl ethyl ketone	0.4	0.7
dichloromethane, ethyl acetate	0.35	0.6
toluene, chloroform	0.3	0.5
dimethylformamide	0.2	0.4
carbon tetrachloride, pyridine	0.15	0.3
dimethyl sulfoxide, dioxane, ethanol, N-methylpyrrolidone, <i>o</i> -dichlorobenzene	0.1	0.2
quinoline, hexafluoroisopropanol, 1-chloronaphthalene	0.05	0.1

Table 4: Recommended solvents by application for TSKgel H series columns

Recommended solvent	Application
THF	polystyrene, epoxy resin, phenoxy resin, polycarbonate, polyisoprene, polyvinyl acetate, polyvinyl chloride, monoglycerides, fatty acids, polybutadiene, poly(methyl methacrylate), poly(styrene-butadiene), poly(styrene-acrylonitrile)
N,N-Dimethylformamide (DMF) + 5 mmol/L LiBr	polyvinyl chloride, polyvinyl fluoride, urea resins, polyurethane, polystyrene, polyester, polyimido ether, polyimido ester, polyphenol (aqueous solution), polyacrylonitrile
<i>o</i> -Dichlorobenzene (ODCB)	polyethylene, polypropylene
chloroform	polycarboxylic ether, acrylic resin, epoxy resin, polystyrene
<i>m</i> -Cresol/Chloroform	nylon, polyester, polyamide, poly(ethylene terephthalate)
toluene	polybutadiene, polysiloxane



TSKgel H_{XL} Size Exclusion Columns

TSKgel H_{XL} columns are conventional GPC columns of 7.8 mm ID × 30 cm containing 5, 6, 9, or 13 μm particles composed of PS-DVB. The TSKgel H_{XL} column lines consists of eight columns with different pore sizes, TSKgel G1000H_{XL} through TSKgel G7000H_{XL}, and three columns with an extended linear range of the calibration curve, TSKgel GMH_{XL}, TSKgel GMH_{XL}-L and TSKgel MultiporeH_{XL}-M.

The TSKgel H_{XL} column line consists of the following columns:

- TSKgel G1000H_{XL}
- TSKgel G2000H_{XL}
- TSKgel G2500H_{XL}
- TSKgel G3000H_{XL}
- TSKgel G4000H_{XL}
- TSKgel G5000H_{XL}
- TSKgel G6000H_{XL}
- TSKgel G7000H_{XL}
- TSKgel GMH_{XL} mixed bed
- TSKgel GMH_{XL}-L mixed bed
- TSKgel MultiporeH_{XL}-M

Three of the linear columns are mixed bed columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The remaining column is a multi-pore column, in which each particle contains a range of pore sizes that provide a linear calibration curve. The innovative multi-pore approach, pioneered by Tosoh, is a synthetic chemistry answer to the question of how to obtain a column with an extended linear calibration curve, while mixed bed columns represent a mechanical way of obtaining a linear calibration curve. In general, Multipore columns have a smoother, more linear, calibration curve.

Attributes and Applications:

Product attributes of all of the TSKgel H_{XL} columns are shown in [Table 5](#). These columns are for the use of conventional polymer analysis and show ultra-low polymer absorption, i.e., the columns show true size exclusion behavior for most polymers. TSKgel H_{XL} columns are shipped in THF. These columns can be exchanged for a limited number of organic solvents. See the table within the TSKgel H series column overview for a listing of these solvents. [Figures 1-2](#) show the calibration curves for the TSKgel H_{XL} columns.

Table 5: Product attributes

TSKgel column	Particle size	Pore size	Exclusion limit	Max. temp.
G1000H _{XL}	5 μm	1.5 nm	1,000 Da	60 °C
G2000H _{XL}	5 μm	2 nm	1.0 × 10 ⁴ Da	60 °C
G2500H _{XL}	5 μm	3 nm	2.0 × 10 ⁴ Da	60 °C
G3000H _{XL}	5 μm	7.5 nm	6.0 × 10 ⁴ Da	60 °C
G4000H _{XL}	5 μm	20 nm	4.0 × 10 ⁵ Da	80 °C
G5000H _{XL}	9 μm	65 nm	4.0 × 10 ⁶ Da	80 °C
G6000H _{XL}	9 μm	>65 nm	4.0 × 10 ⁷ Da	80 °C
G7000H _{XL}	9 μm	>65 nm	4.0 × 10 ⁸ Da	80 °C
GMH _{XL}	9 μm	mixed pore sizes	4.0 × 10 ⁸ Da	80 °C
GMH _{XL} -L	5 μm	mixed pore sizes	4.0 × 10 ⁶ Da	80 °C
MultiporeH _{XL} -M	5 μm	broad distribution of pore size in each particle	2.0 × 10 ⁶ Da	60 °C

Figure 1: Calibration curves of TSKgel H_{XL} columns

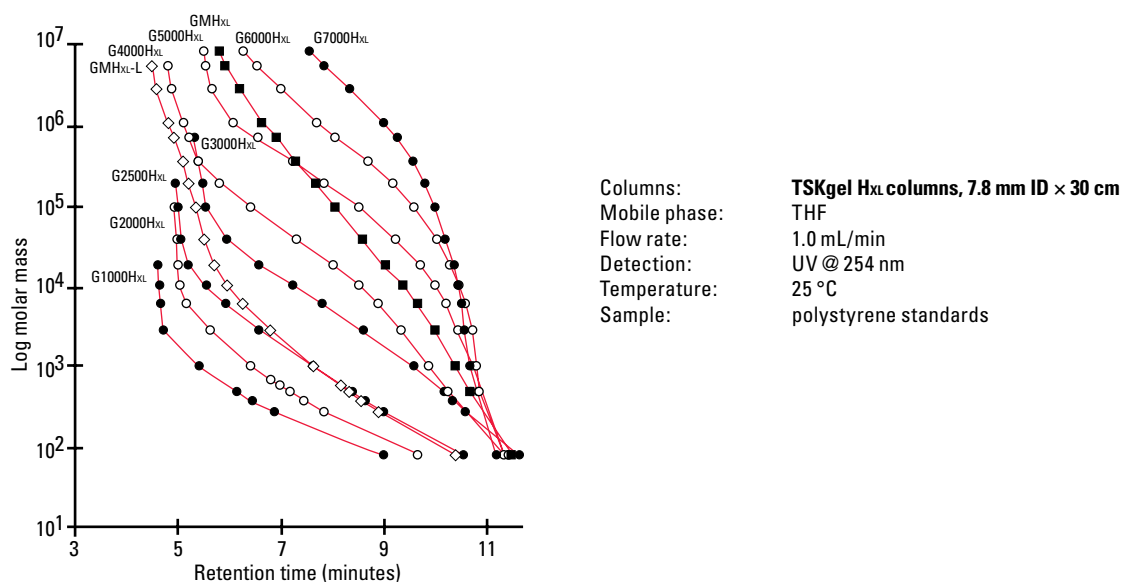
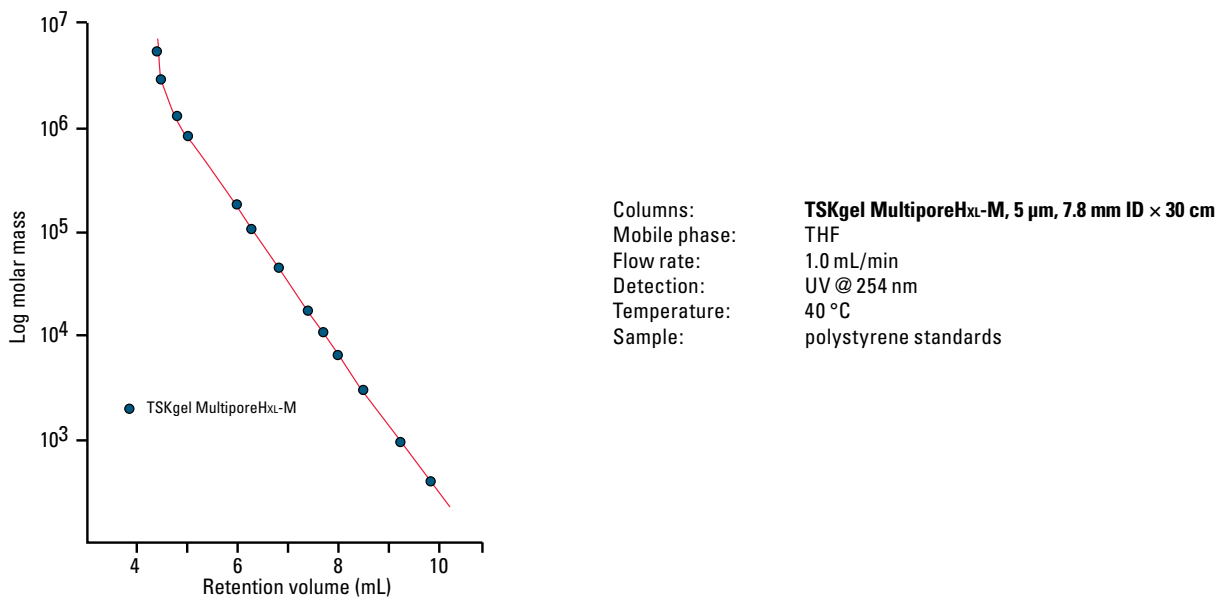


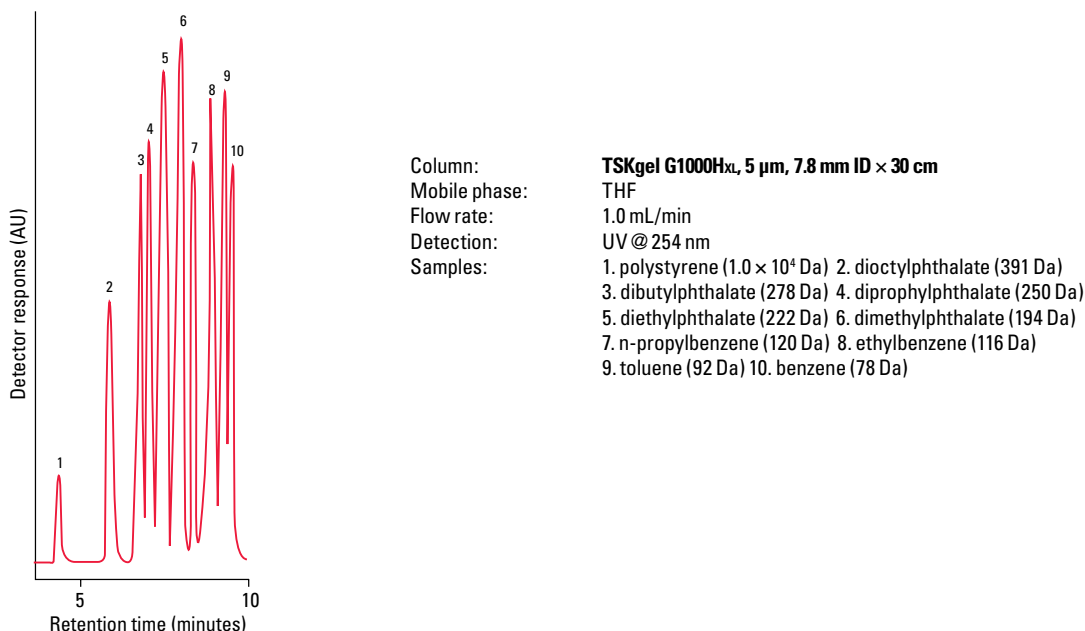
Figure 2: Calibration curve of TSKgel MultiporeH_{XL}-M column



Phthalate Esters

Figure 3 demonstrates the high efficiency separation on a TSKgel G1000H_{XL} column for low molar mass phthalate esters. Resolution was close to baseline even though the molar masses of the esters differed by less than 50 Da.

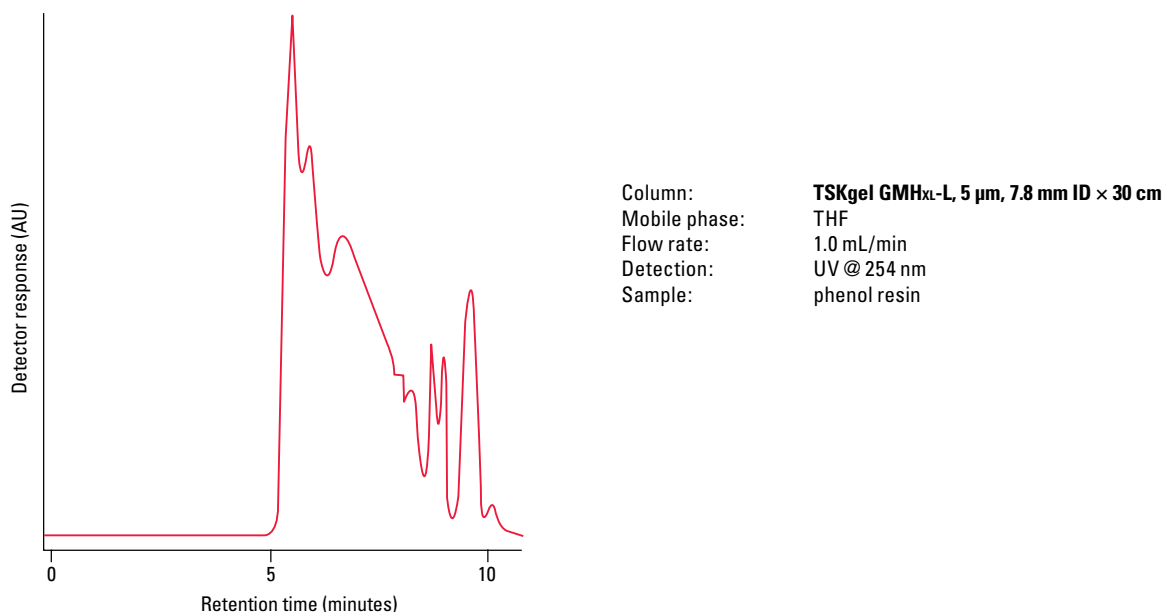
Figure 3: High resolution of phthalate esters



Phenol Resin

The TSKgel GMH_{XL}-L column has been designed to provide a complete profile for high molar mass samples that contain low molar mass additives. The calibration curve for this mixed bed column is shallow in the low molar mass range of oligomers. Sample adsorption is not observed. For example, the complete profile of a phenol resin, with high resolution of the low molar mass components, is shown in Figure 4. Other applications for the TSKgel GMH_{XL}-L column include analyses of paint materials, bond and adhesive components and synthetic polymer additives.

Figure 4: Separation of phenol resin



TSKgel H_{HR} Size Exclusion Columns

TSKgel H_{HR} columns are conventional GPC columns with dimensions of 7.8 mm ID × 30 cm containing spherical particles composed of PS-DVB. The TSKgel H_{HR} column line consists of eight columns with different pore sizes, TSKgel G1000H_{HR} through TSKgel G7000H_{HR}, and ten columns with an extended linear range of the calibration curve.

The TSKgel H_{HR} column line consists of the following columns:

- TSKgel G1000H_{HR}
- TSKgel G2000H_{HR}
- TSKgel G2500H_{HR}
- TSKgel G3000H_{HR}
- TSKgel G4000H_{HR}
- TSKgel G5000H_{HR}
- TSKgel G6000H_{HR}
- TSKgel G7000H_{HR}
- TSKgel G2000H_{HR} (20) HT
- TSKgel GMH_{HR}-H mixed bed
- TSKgel GMH_{HR}-L mixed bed
- TSKgel GMH_{HR}-M mixed bed
- TSKgel GMH_{HR}-N mixed bed
- TSKgel GMH_{HR}-H HT mixed bed
- TSKgel GMH_{HR}-H (S) HT mixed bed
- TSKgel GMH_{HR}-H HT2 mixed bed
- TSKgel GMH_{HR}-H (S) HT2 mixed bed
- TSKgel G2000H_{HR} (20) HT2

The linear, or mixed bed columns, contain particles with different pore sizes that are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel GMH_{HR}-L, GMH_{HR}-N, GMH_{HR}-M, to GMH_{HR}-H. All of the TSKgel high temperature mixed bed columns are shipped in ODCB (*o*-dichlorobenzene).

The TSKgel H_{HR} HT2 mixed bed columns are available for ultra-high temperature analysis. Packed with PS-DVB beads, the maximum operating temperature of these columns is 220 °C.

The issue of shearing that occurs with the analysis of ultra-high molar mass polymers is overcome by the TSKgel GMH_{HR}-M (S), GMH_{HR}-H (S), GMH_{HR}-H (S) HT and GMH_{HR}-H (S) HT2 columns. The (S) is a reference to this shearing effect.

Attributes and Applications:

The product attributes for all of the TSKgel H_{HR} columns is shown in [Table 6](#). TSKgel H_{HR} columns have a broad solvent range and are shipped in THF, except for the high temperature mixed bed columns, which are shipped in ODCB. THF can be exchanged for a wide variety of organic solvents. See the table within the TSKgel H series column overview for a listing of these solvents. [Figures 5-9](#) show the calibration curves for the TSKgel H_{HR} columns.

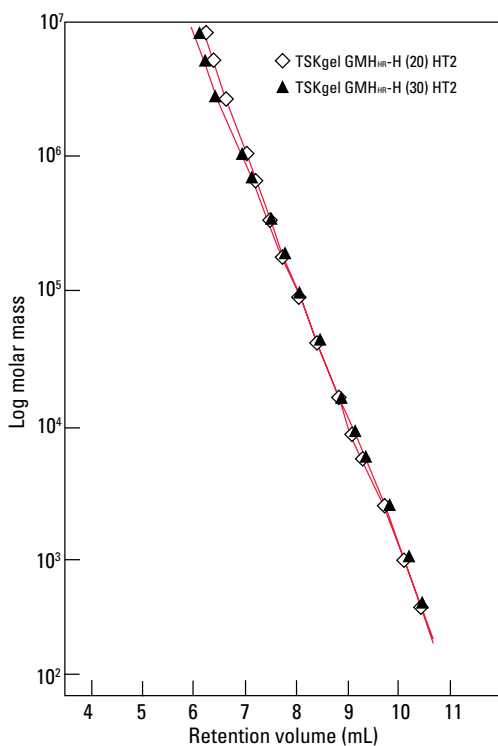
Table 6: Product attributes

TSKgel column	Particle size	Pore size	Exclusion limit	Max. temp.
G1000H _{HR}	5 μm	1.5 nm	1,000 Da	140 °C
G2000H _{HR}	5 μm	2 nm	1.0 × 10 ⁴ Da	140 °C
G2500H _{HR}	5 μm	3 nm	2.0 × 10 ⁴ Da	140 °C
G3000H _{HR}	5 μm	7.5 nm	6.0 × 10 ⁴ Da	140 °C
G4000H _{HR}	5 μm	20 nm	4.0 × 10 ⁵ Da	140 °C
G5000H _{HR}	5 μm	65 nm	4.0 × 10 ⁶ Da	140 °C
G6000H _{HR}	5 μm	>65 nm	4.0 × 10 ⁷ Da	140 °C
G7000H _{HR}	5 μm	>65 nm	4.0 × 10 ⁸ Da	140 °C
GMH _{HR} -H	5 μm, 13 μm, 20 μm, 30 μm	mixed pore sizes	4.0 × 10 ⁸ Da	80 °C
GMH _{HR} -L	5 μm	mixed pore sizes	4.0 × 10 ⁶ Da	80 °C
GMH _{HR} -M	5 μm, 13 μm	mixed pore sizes	4.0 × 10 ⁶ Da	80 °C
GMH _{HR} -N	5 μm	mixed pore sizes	4.0 × 10 ⁵ Da	80 °C
GMH _{HR} -H HT	5 μm	mixed pore sizes	4.0 × 10 ⁸ Da	140 °C
GMH _{HR} -H (20) HT	20 μm	mixed pore sizes	4.0 × 10 ⁸ Da	140 °C
GMH _{HR} -H (30) HT	30 μm	mixed pore sizes	4.0 × 10 ⁸ Da	140 °C

Table 6: Product attributes, continued

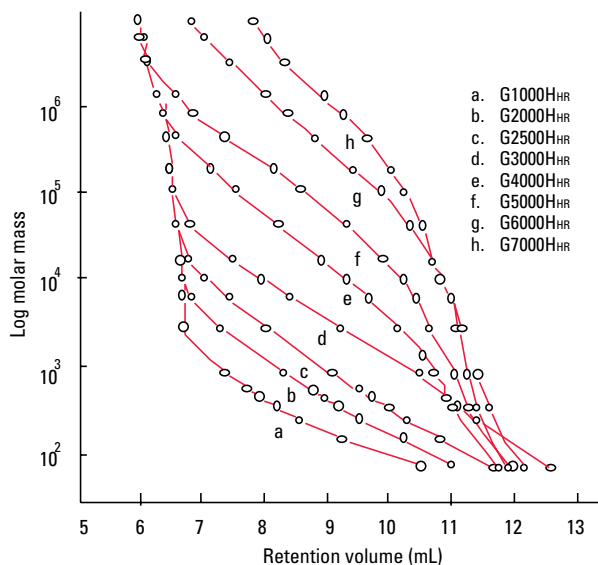
TSKgel column	Particle size	Pore size	Exclusion limit	Max. temp.
GMH _{HR} -H (S) HT	13 μm	mixed pore sizes	4.0 × 10 ⁸ Da	140 °C
G2000H _{HR} (20) HT	20 μm	2 nm	1.0 × 10 ⁴ Da	140 °C
GMH _{HR} -H (20) HT2	20 μm	mixed pore sizes	4.0 × 10 ⁸ Da	220 °C
GMH _{HR} -H (30) HT2	30 μm	mixed pore sizes	4.0 × 10 ⁸ Da	220 °C
GMH _{HR} -H (S) HT2	13 μm	mixed pore sizes	4.0 × 10 ⁸ Da	220 °C
G2000H _{HR} (20) HT2	20 μm	2 nm	1.0 × 10 ⁴ Da	220 °C

Figure 5: Calibration curves of TSKgel GMH_{HR}-H HT2 columns



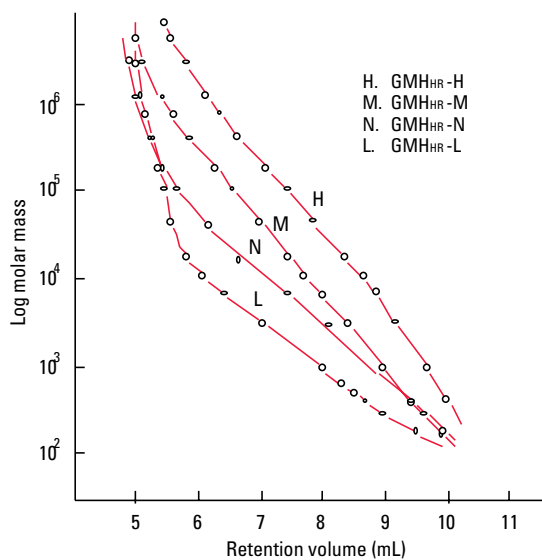
Columns: **TSKgel GMH_{HR}-H (20) HT2, 20 μm, 7.8 mm ID × 30 cm**
TSKgel GMH_{HR}-H (30) HT2, 30 μm, 7.8 mm ID × 30 cm
 Mobile phase: ODCB with 0.05% BHT
 Flow rate: 1.0 mL/min
 Detection: RI
 Temperature: 135 °C
 Sample: polystyrene standards

Figure 6: Calibration curves of TSKgel H_{HR} columns



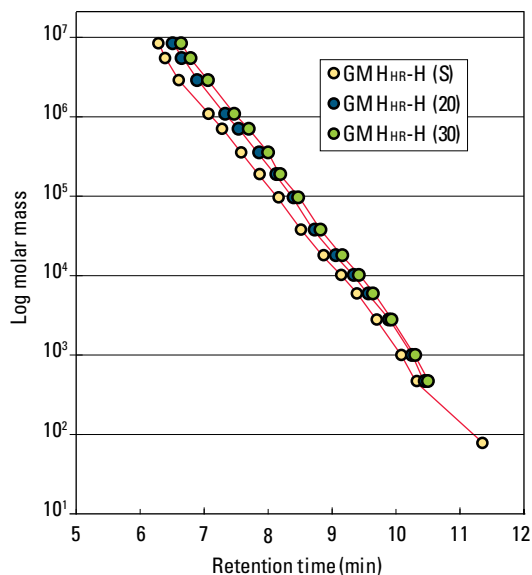
Columns: **TSKgel H_{HR} columns, 7.8 mm ID × 30 cm**
 Mobile phase: THF
 Flow rate: 1.0 mL/min
 Detection: UV @ 254 nm
 Temperature: 25 °C
 Samples: polystyrene standards

Figure 7: Calibration curves of TSKgel H_{HR} mixed bed columns



Columns: **TSKgel H_{HR} columns, 7.8 mm ID × 30 cm**
 Mobile phase: THF
 Flow rate: 1.0 mL/min
 Detection: UV @ 254 nm
 Temperature: 25 °C
 Samples: polystyrene standards

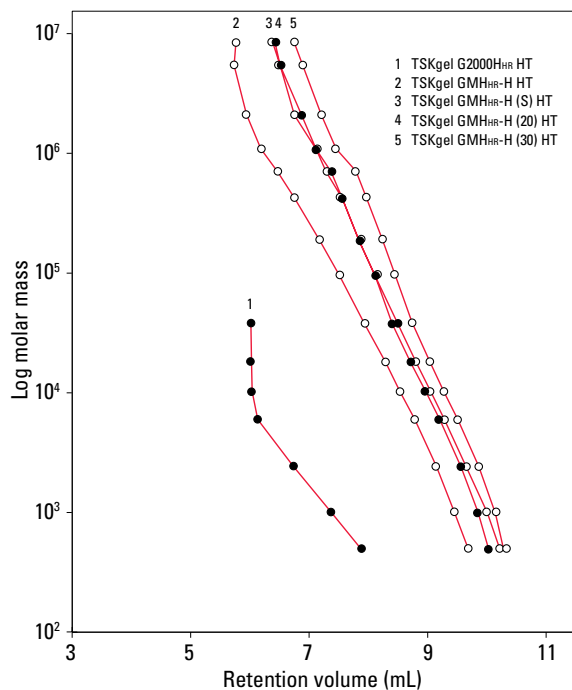
Figure 8: Calibration curves of TSKgel H_{HR}-H columns



Columns: TSKgel GMH_{HR}-H (S), 13 μm, 7.8 mm ID × 30 cm
 TSKgel GMH_{HR}-H (20), 20 μm, 7.8 mm ID × 30 cm
 TSKgel GMH_{HR}-H (30), 30 μm, 7.8 mm ID × 30 cm

Mobile phase: THF
 Flow rate: 1.0 mL/min
 Detection: UV @ 254 nm
 Temperature: 25 °C
 Sample: polystyrene standards

Figure 9: Calibration curves of TSKgel HT columns



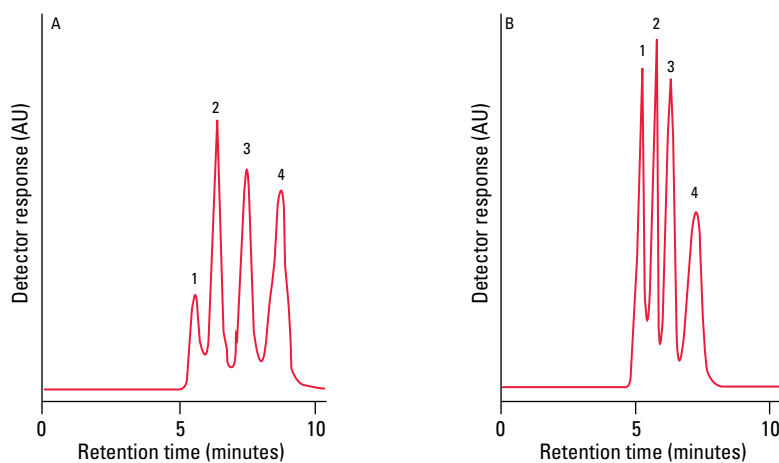
Columns: TSKgel G2000H_{HR} (20) HT, 20 μm, 7.8 mm ID × 30 cm
 TSKgel GMH_{HR}-H HT, 5 μm, 7.8 mm ID × 30 cm
 TSKgel GMH_{HR}-H (S) HT, 13 μm, 7.8 mm ID × 30 cm
 TSKgel GMH_{HR}-H (20) HT, 20 μm, 7.8 mm ID × 30 cm
 TSKgel GMH_{HR}-H (30) HT, 30 μm, 7.8 mm ID × 30 cm

Mobile phase: ODCB with 0.05% BHT
 Flow rate: 1.0 mL/min
 Detection: RI (EcoSEC High Temperature GPC System)
 Temperature: 135 °C
 Injection vol.: 300 μL
 Sample: polystyrene

Polymethyl methacrylate

The effect of different pore size distributions in the mixed beds of TSKgel GMH_{HR}-H and TSKgel GMH_{HR}-M is illustrated in **Figure 10**. The TSKgel GMH_{HR}-M produces sharper polymethyl methacrylate peaks in the 8.0×10^5 to 1.0×10^4 Da range.

Figure 10: Comparison of standard polymethylmethacrylate mixture



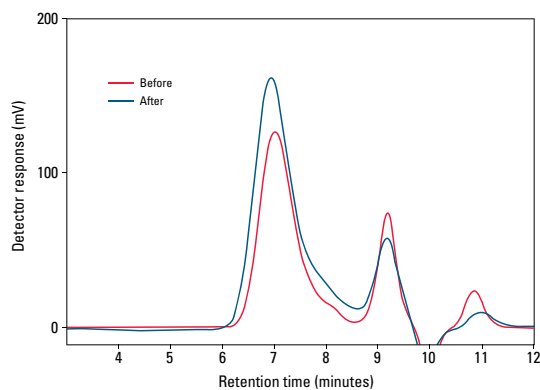
Columns:	A. TSKgel GMH_{HR}-H, 5 μm, 7.8 mm ID \times 30 cm
	B. TSKgel GMH_{HR}-M, 5 μm, 7.8 mm ID \times 30 cm
Mobile phase:	5 mmol/L sodium trifluoroacetate in HFIP
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Temperature:	40 $^{\circ}$ C
Sample:	standard polymethylmethacrylate
	1. 8.2×10^5 Da
	2. 6.7×10^4 Da
	3. 1.02×10^4 Da
	4. 1,950 Da

Column Durability at 220 °C

Column durability in high temperature GPC polymer analysis is essential as these columns are continuously exposed to harsh organic solvents, extremely elevated temperatures and temperature cycling as GPC systems are turned on and off. The durability of a high temperature GPC column directly influences the quality, applicability and selectivity, or resolution, of the GPC column, thus the accuracy of the molar mass averages obtained. As a high temperature GPC column begins to fail or lose resolution due to the extreme experimental conditions required for high temperature GPC polymer analysis, the number- and z-average molar mass values obtained become inflated and the GPC elution profile begins to shift due to a decrease in multiple factors that affect the ability of the columns to separate species varying in hydrodynamic volume.

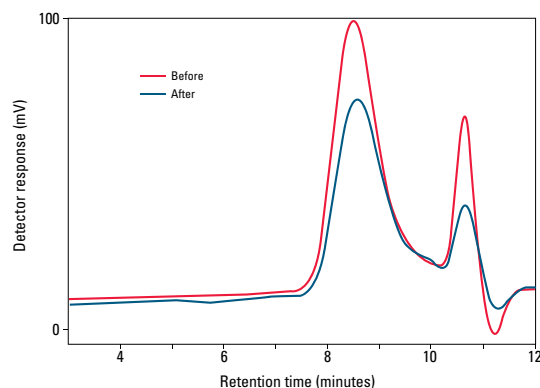
A durability and stability study of a TSKgel GMH_{HR}-H (S) HT high temperature GPC column was performed and the results were compared to another commercially available column for polymer analysis at 220 °C. The deterioration of the commercially available high temperature GPC column is observed in the GPC elution profiles, **Figure 11**, as the resolution between the sample and solvent peaks decreases after the column is exposed to temperature cycling. The GPC elution profiles obtained for the TSKgel GMH_{HR}-H (S) HT column before and after temperature cycling remain superimposable, **Figure 12**.

Figure 11: GPC elution profile for a polymer before and after temperature cycling obtained using a commercially available high temperature GPC column



Column: Commercially available high temperature GPC column,
13 μ m, 7.8 mm ID \times 30 cm
Mobile phase: 1-chloronaphthalene
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 220 °C
Injection vol.: 200 μ L
Sample: synthetic polymer

Figure 12: GPC elution profile for a polymer before and after temperature cycling obtained using a TSKgel GMH_{HR}-H (S) HT column



Column: **TSKgel GMH_{HR}-H (S) HT, 13 μ m, 7.8 mm ID \times 30 cm**
Mobile phase: 1-chloronaphthalene
Flow rate: 1.0 mL/min
Detection: RI
Temperature: 220 °C
Injection vol.: 200 μ L
Sample: synthetic polymer

SuperH Size Exclusion Columns

TSKgel SuperH columns are conventional GPC columns with dimensions of 6.0 mm ID × 15 cm containing spherical particles composed of PS-DVB. The TSKgel SuperH column line consists of eight columns with different pore sizes, TSKgel SuperH1000 through TSKgel SuperH7000, and four columns with an extended linear range of the calibration curve.

TSKgel SuperH columns are high efficiency/high throughput versions of the conventional TSKgel H_{HR} columns. Both column types are based on the same bead chemistry.

The TSKgel SuperH line consists of the following columns:

- TSKgel SuperH1000
- TSKgel SuperH2000
- TSKgel SuperH2500
- TSKgel SuperH3000
- TSKgel SuperH4000
- TSKgel SuperH5000
- TSKgel SuperH6000
- TSKgel SuperH7000
- TSKgel SuperHM-H mixed bed
- TSKgel SuperHM-L mixed bed
- TSKgel SuperHM-M mixed bed
- TSKgel SuperHM-N mixed bed

The TSKgel SuperH product line contains four linear or mixed bed columns, in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHM-L, SuperHM-M, SuperHM-N, to SuperHM-H.

The volume of a 6 mm ID × 15 cm TSKgel SuperH column is 3.4 times smaller than that of a conventional 7.8 mm ID × 30 cm column. As a result, peak volumes will be proportionally smaller on TSKgel SuperH columns compared to a corresponding TSKgel H_{HR} column. Thus, your HPLC system may require optimization of components that can give rise to extra-column band broadening, such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant.

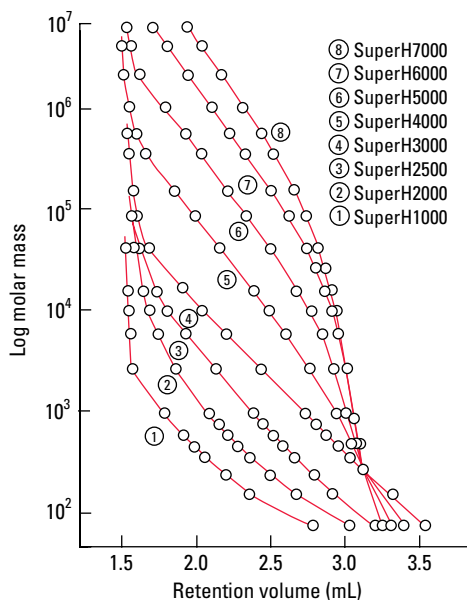
Attributes and Applications:

Table 7 shows product attributes of TSKgel SuperH columns. The maximum operating temperature for TSKgel SuperH columns is 140 °C. All TSKgel SuperH columns are shipped in THF, which can be exchanged for a wide variety of organic solvents. See the table within the TSKgel H series column overview for a listing of these solvents. **Figures 13-14** show the calibration curves for the TSKgel SuperH columns.

Table 7: Product attributes

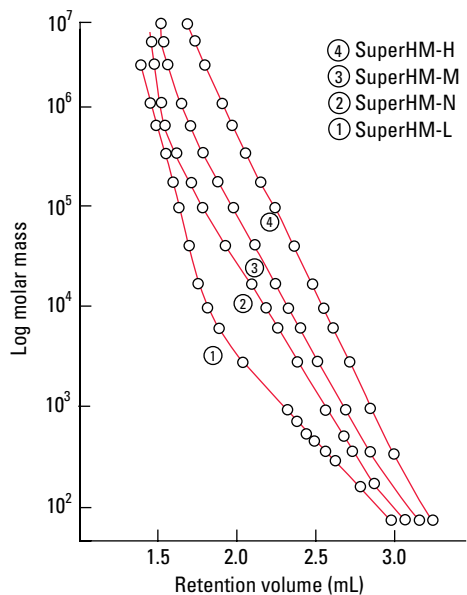
TSKgel column	Particle size (mean)	Pore size (mean)	Exclusion limit
SuperH1000	3 μm	1.5 nm	1,000 Da
SuperH2000	3 μm	2 nm	1.0 × 10 ⁴ Da
SuperH2500	3 μm	3 nm	2.0 × 10 ⁴ Da
SuperH3000	3 μm	7.5 nm	6.0 × 10 ⁴ Da
SuperH4000	3 μm	20 nm	4.0 × 10 ⁵ Da
SuperH5000	3 μm	65 nm	4.0 × 10 ⁶ Da
SuperH6000	5 μm	>65 nm	4.0 × 10 ⁷ Da
SuperH7000	5 μm	>65 nm	4.0 × 10 ⁸ Da
SuperHM-H	3 μm	mixed pore sizes	4.0 × 10 ⁸ Da
SuperHM-L	3 μm	mixed pore sizes	4.0 × 10 ⁶ Da
SuperHM-M	3 μm	mixed pore sizes	4.0 × 10 ⁶ Da
SuperHM-N	3 μm	mixed pore sizes	4.0 × 10 ⁵ Da

Figure 13: Calibration curves for TSKgel SuperH columns



Columns: **TSKgel SuperH columns, 6.0 mm ID × 15 cm**
 Mobile phase: THF
 Flow rate: 0.6 mL/min
 Detection: UV @ 254 nm
 Temperature: 25 °C
 Sample: polystyrene standards

Figure 14: Calibration curves for TSKgel SuperH mixed bed columns



Columns: **TSKgel SuperH columns, 6.0 mm ID × 15 cm**
 Mobile phase: THF
 Flow rate: 0.6 mL/min
 Detection: UV @ 254 nm
 Temperature: 25 °C
 Sample: polystyrene standards

Polystyrene Mixtures

Figure 15 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperH2500 column with various organic solvents (THF, CHCl₃, DMF, and CCl₄) and Figure 16 compares chromatograms of standard polystyrene mixtures separated using a TSKgel SuperHM-H column with various organic solvents.

Due to the interaction between the packing material and standard polystyrene when using DMF as the mobile phase, the elution volume of standard polystyrenes is greater than it is with “good” solvents such as THF and CHCl₃. This effect is particularly noticeable with TSKgel SuperH2500, a column for the analysis of low molar mass samples. Under these circumstances, polyethylene oxide (PEO) is recommended as the standard sample, as this reacts very little with the packing material.

Figure 15: Separation of standard polystyrenes using a TSKgel SuperH2500 column

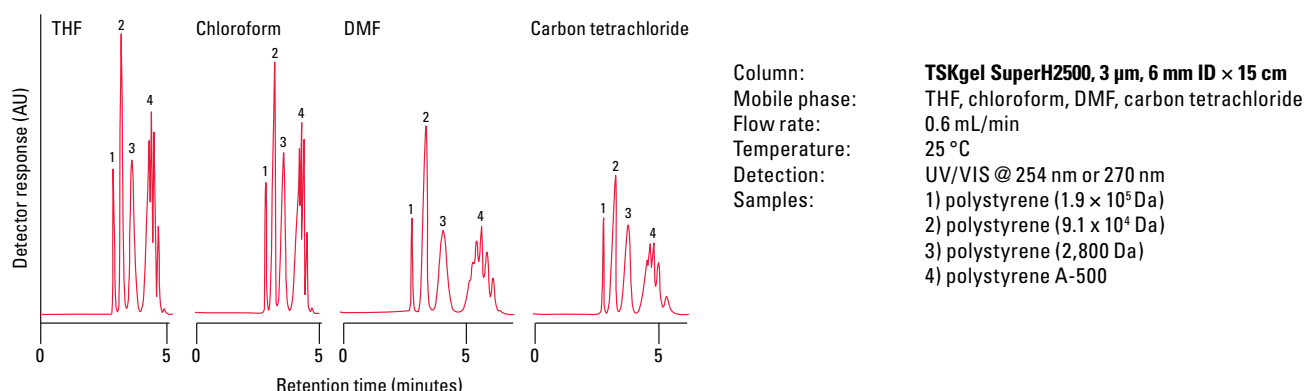
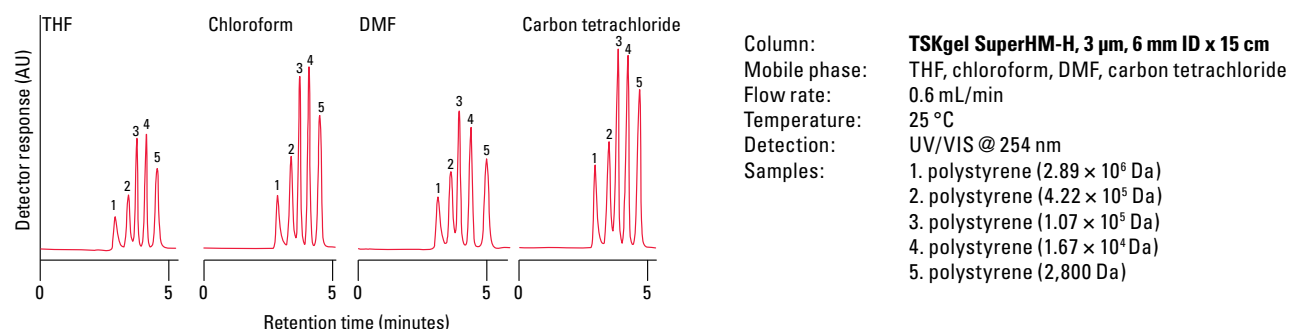


Figure 16: Separation of standard polystyrenes using a TSKgel SuperHM-H column



TSKgel SuperHZ Size Exclusion Columns

The TSKgel SuperHZ column line consists of five columns of 4.6 mm ID and 6.0 mm ID × 15 cm containing spherical particles composed of PS-DVB, TSKgel Super HZ1000 – 4000. Each column consists of a different pore size packing material. Subsequently, a unique separation range for each column exists, allowing researchers to choose a column that is designed for the sample type being analyzed.

The TSKgel SuperHZ column line also contains three linear, or mixed bed columns in which particles with different pore sizes are blended to provide an extended linear calibration curve. The mixed bed columns feature increasing linear calibration ranges, from TSKgel SuperHSM-M to SuperHSM-N to SuperHSM-H. The mixed bed columns are also available in 4.6 mm ID and 6.0 mm ID × 15 cm.

The following eight columns are available within the TSKgel SuperHZ column line:

- TSKgel SuperHZ1000
- TSKgel SuperHZ2000
- TSKgel SuperHZ2500
- TSKgel SuperHZ3000
- TSKgel SuperHZ4000
- TSKgel SuperHSM-H mixed bed
- TSKgel SuperHSM-M mixed bed
- TSKgel SuperHSM-N mixed bed

TSKgel SuperHZ column dimensions are 6 mm ID × 15 cm and 4.6 mm ID × 15 cm versus 7.8 mm ID × 30 cm for conventional GPC columns. The smaller column dimensions translate to a reduction of peak volume by a factor of 3.4 (6 mm ID) and a factor of 5.8 (4.6 mm ID) versus the same component eluting from a corresponding TSKgel HXL column. Thus, your HPLC system may require optimization of components that can give rise to extra-column band broadening, such as connecting tubing, injector, injection volume, detector cell volume, and detector time constant.

Attributes and Applications:

TSKgel SuperHZ columns have been developed for high throughput, high efficiency GPC applications such as those encountered in combinatorial chemistry experiments. These columns feature ultra-low sample adsorption, i.e., the columns show true size exclusion behavior for most polymers.

TSKgel SuperHZ1000 – 4000 columns are capable of measuring monomers, polymer additives, oligomers and polymers up to a molar mass of several hundred thousand with proper selection of pore size. Ultra-fine particles (3 µm) have been developed to provide high resolution over the entire molar mass range. This is especially important for the separation of low molar mass compounds.

Additionally, the mixed bed columns (TSKgel SuperHSM-N, M-M, and M-H) are capable of measuring oligomers and polymers with molar masses up to tens of millions with proper selection of the pore size. The various particle sizes of the mixed bed packing materials have been optimized to ensure resolution in the low molar mass range while avoiding shear degradation of polymers in the high molar mass region.

The columns are shipped in THF, which can be exchanged for a limited number of organic solvents as shown in the table within the TSKgel H series column overview.

Table 8 shows the product attributes of TSKgel SuperHZ columns, while **Table 9** lists the features of the TSKgel SuperHZ column line and the corresponding benefits. The calibration curves for the TSKgel SuperHZ columns are shown in **Figures 17-18**.

Table 8: Product attributes

TSKgel column	Particle size	Pore size	Exclusion limit	Max. temp.
SuperHZ1000	3 µm	1.5 nm	1,000 Da	60 °C
SuperHZ2000	3 µm	2 nm	1.0 × 10 ⁴ Da	60 °C
SuperHZ2500	3 µm	3 nm	2.0 × 10 ⁴ Da	60 °C
SuperHZ3000	3 µm	7.5 nm	6.0 × 10 ⁴ Da	60 °C
SuperHZ4000	3 µm	20 nm	4.0 × 10 ⁵ Da	80 °C
SuperHSM-H	10 µm	mixed pore sizes	4.0 × 10 ⁸ Da	80 °C
SuperHSM-M	3 µm	mixed pore sizes	4.0 × 10 ⁶ Da	80 °C
SuperHSM-N	3 µm	mixed pore sizes	7.0 × 10 ⁵ Da	80 °C

Table 9: Features and benefits of TSKgel SuperHZ columns

Feature	Benefit
Ultra-fine particles used in packing material	<ul style="list-style-type: none"> Short measurement time is achieved. Resolution equivalent to conventional columns (30 cm) can be obtained in 1/2 measurement time Resolution does not deteriorate even under a high flow rate.
Semi-micro columns (4.6 mm ID and 6.0 mm ID)	<ul style="list-style-type: none"> Reduction in solvent consumption (running costs, effluent processing costs) 1/6 to 1/3 solvent consumption compared to conventional columns
Optimization of particle size in the packing materials	<ul style="list-style-type: none"> Shear degradation in polymers with high molar mass can be prevented
Adoption of low-adsorption packing materials	<ul style="list-style-type: none"> Applicable to wide range of samples

Figure 17: Calibration curves for TSKgel SuperHZ columns

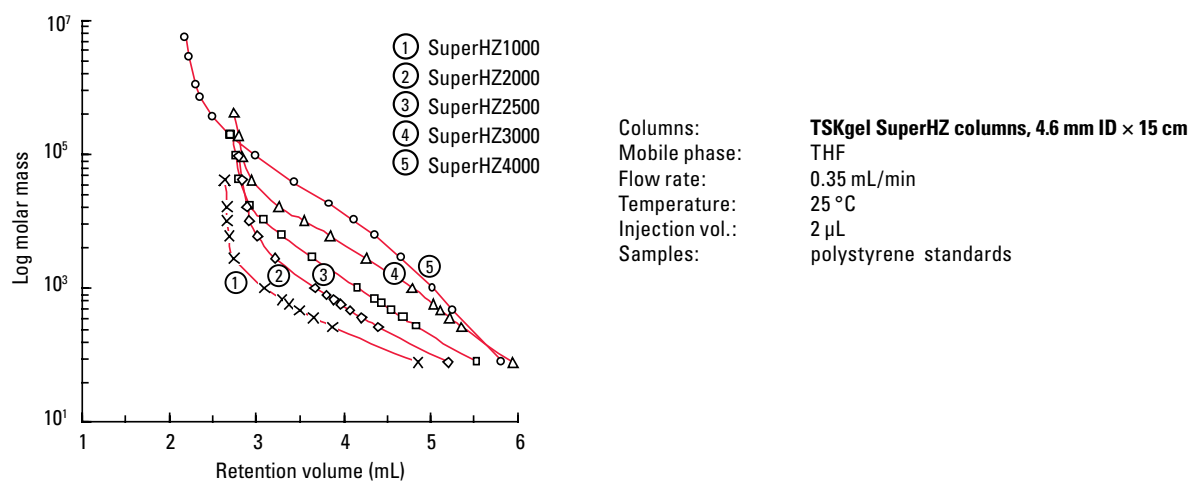
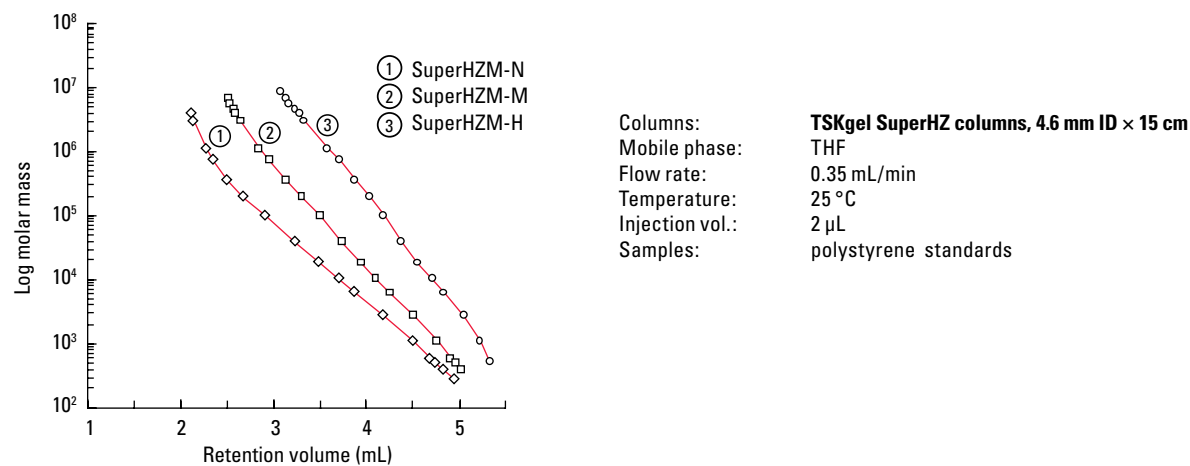


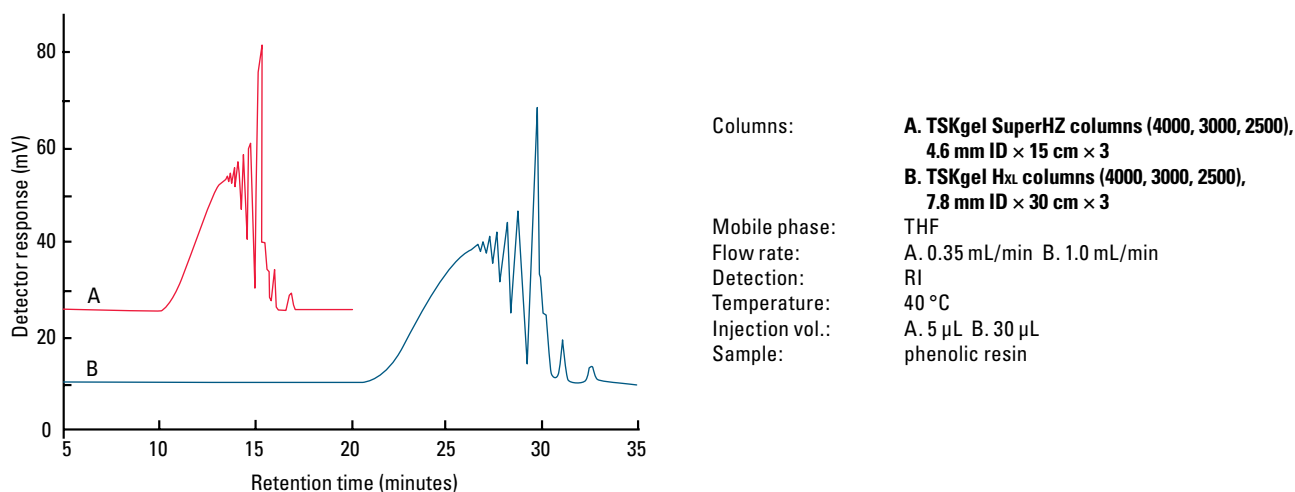
Figure 18: Calibration curves for TSKgel SuperHZ mixed bed columns



Faster Analysis

TSKgel SuperHZ1000-SuperHZ4000 columns are packed with 3 µm particles. The ultra-fine particles allow for high efficiency separations of low molar mass substances such as oligomers. These columns have theoretical plate values (per unit length) which are twice those of the conventional 5 µm columns. As a result, equal resolution can be obtained within half the analysis time. An example showing the analysis of phenolic resin is demonstrated in **Figure 19**.

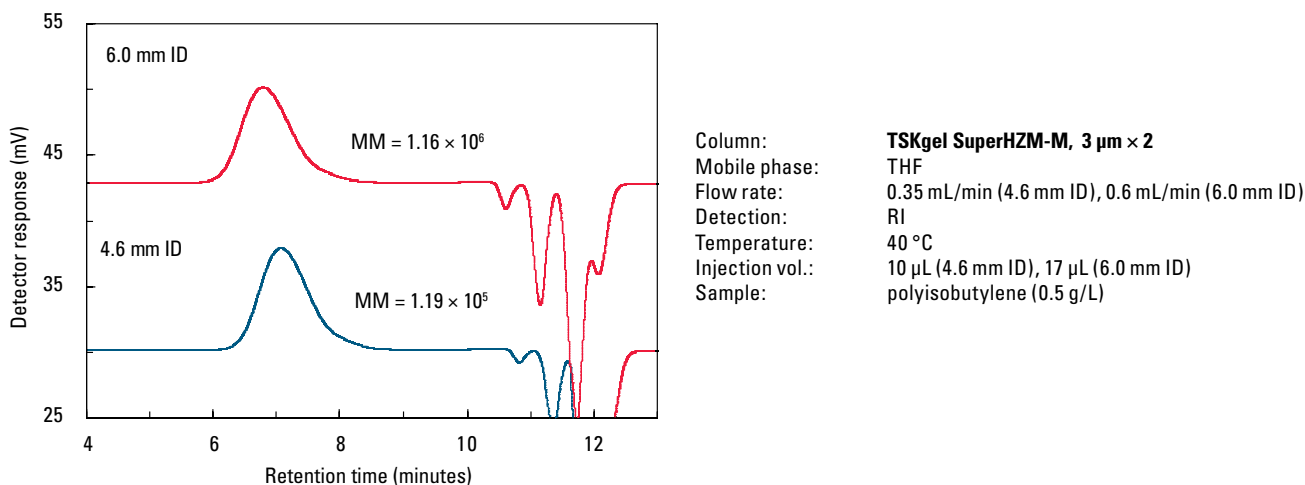
Figure 19: Comparison of analysis on TSKgel SuperHZ and TSKgel HxL columns



Polyisobutylene

The chromatogram in **Figure 20** shows the analysis of polyisobutylene using two TSKgel SuperHZM-M columns in series.

Figure 20: Analysis of polyisobutylene



TSKgel SuperMultiporeHZ Size Exclusion Columns

TSKgel SuperMultiporeHZ columns represent a new strategy for the separation of polymers with a wide range of molar masses. These columns are packed with particles of a uniform size, with each particle having a very broad pore size distribution. This innovative multi-pore approach, pioneered by Tosoh Bioscience, essentially creates a linear calibration curve within each particle. The spherical monodisperse, 3, 4 or 6 μm particles consist of cross-linked polystyrene/divinylbenzene copolymer. This base material, coupled with the semi-micro column dimensions (4.6 mm ID \times 15 cm), offers users high speed and low solvent consumption analyses with precise results. Three columns are available within the TSKgel SuperMultiporeHZ series, each with a different particle size and separation range.

The TSKgel SuperMultiporeHZ columns offered include:

- TSKgel SuperMultiporeHZ-N
- TSKgel SuperMultiporeHZ-M
- TSKgel SuperMultiporeHZ-H

Multi-pore Technology

Prior to the introduction of TSKgel SuperMultiporeHZ columns, scientists separating polymers with a wide range of molar masses were left with two options. One option was to use multiple columns of different pore sizes linked together in series. A second was to use a column packed with a mixed bed resin of different pore sizes at an optimized mix ratio. However, problems can occur with both of these methods, which include distortion of the chromatogram or deviations between the actual calibration curve and the calibration curve approximated from data obtained from the molar mass standards.

As is shown in **Figure 21**, a novel approach to solve this problem was developed by Tosoh scientists and is incorporated in TSKgel SuperMultiporeHZ columns. Small particles of uniform size are synthesized with a broad distribution of pore sizes. This novel approach creates a linear calibration curve within each particle. Therefore, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes.

Figure 21: Graphical representations illustrate the multi-pore particle synthesis technology

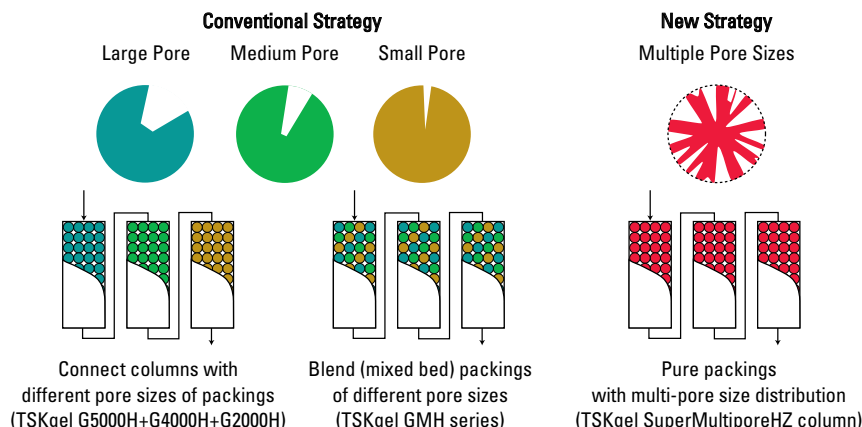
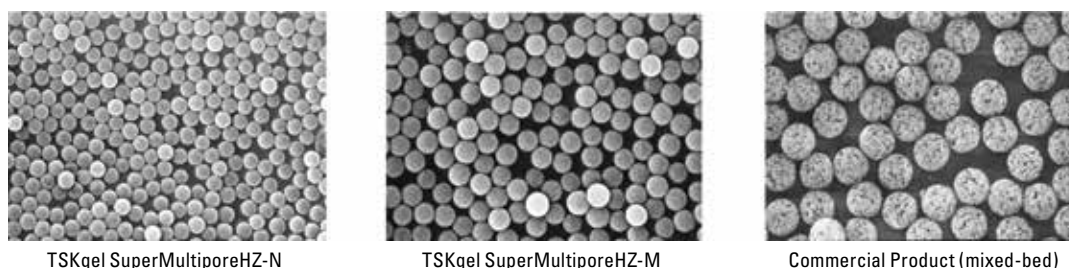


Figure 22 shows the monodispersity of the particle size distribution of TSKgel SuperMultiporeHZ columns compared to a conventional mixed-bed column.

Figure 22: TSKgel SuperMultiporeHZ columns are packed with monodisperse particles



Attributes and Applications:

Product attributes for the TSKgel SuperMultiporeHZ columns are listed in Table 10. Table 11 lists features and benefits of these columns. TSKgel SuperMultiporeHZ columns can be utilized for the analysis of polymers with a wide MM distribution range. The columns are shipped in THF, which cannot be replaced for any other organic solvent. Figure 23 shows the calibration curves for the TSKgel SuperMultiporeHZ columns.

Table 10: Product attributes

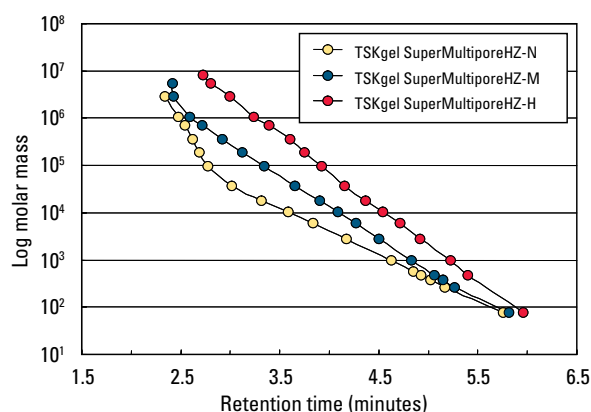
TSKgel column	SuperMultipore HZ-N	SuperMultipore HZ-M	SuperMultipore HZ-H
Base material	PS-DVB	PS-DVB	PS-DVB
Particle size	3 μm^*	4 μm^*	6 μm^*
Pore size	8 nm	14 nm	>14 nm
Exclusion limit (PST/THF)	1.2×10^5 Da	2.0×10^6 Da	4.0×10^7 Da
Separation range	300 ~ 5.0×10^4 Da	500 ~ 1.0×10^6 Da	1,000 ~ 1.0×10^7 Da
Theoretical plates/15 cm column	20,000	16,000	11,000

* Particle size distribution is monodisperse.

Table 11: Features and benefits

Feature	Benefit
Multi-pore packing material (wide range of pores contained in single particle)	<ul style="list-style-type: none"> • Calibration curves with superior linearity • No observable distortion of chromatograms • Improved accuracy and repeatability of molar mass data • Capable of rapid analysis with high separation performance
Smaller particle size (monodisperse particles)	<ul style="list-style-type: none"> • Capable of achieving the same separation performance as conventional columns (30 cm) in half the analysis time • No reduction in separation performance even for analysis at high flow rates • Improved robustness of column performance
Semi-micro column	<ul style="list-style-type: none"> • Reduced solvent consumption • 1/6th the consumption of conventional (30 cm) columns
Low adsorption packing material	<ul style="list-style-type: none"> • Can be used for a wide variety of samples

Figure 23: Calibration curves for TSKgel SuperMultiporeHZ columns



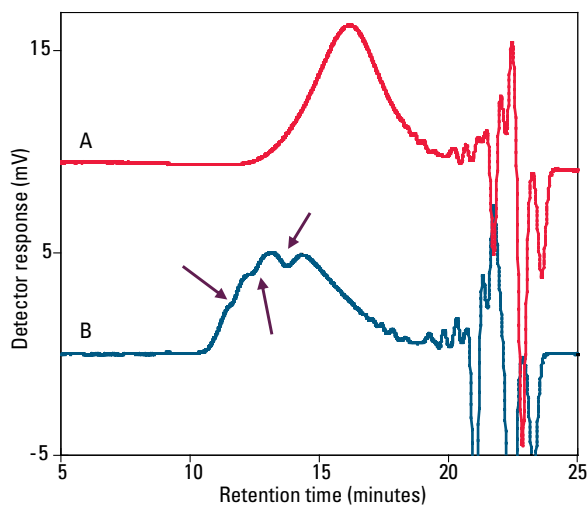
Columns: TSKgel SuperMultiporeHZ-N, 3 μm , 4.6 mm ID \times 15 cm
 TSKgel SuperMultiporeHZ-M, 4 μm , 4.6 mm ID \times 15 cm
 TSKgel SuperMultiporeHZ-H, 6 μm , 4.6 mm ID \times 15 cm

Mobile phase: THF
 Flow rate: 0.35 mL/min
 Detection: UV @ 254 nm
 Temperature: 25 $^{\circ}\text{C}$
 Samples: PStQuick polystyrene standards

Acrylic Resin

Figure 24 demonstrates that inflection points are no longer observed with columns packed from particles prepared by multi-pore technology.

Figure 24: Comparison for separation of acrylic resin



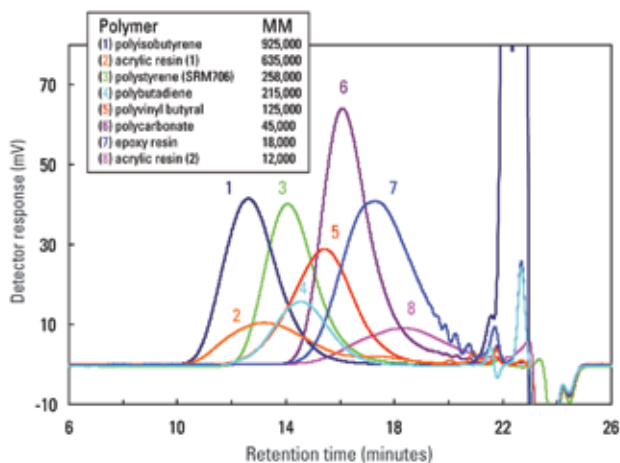
Columns: **A. TSKgel SuperMultiporeHZ-M, 4.6 mm ID × 15 cm × 4**
B. TSKgel SuperHZ4000+3000+2500+2000, 4.6 mm ID × 15cm × 1

Mobile phase: THF
 Detection: RI
 Temperature: 40 °C
 Injection vol.: 10 µL
 Samples: acrylic resin

Various Polymers

Various polymers were analyzed on four TSKgel SuperMultiporeHZ-M columns in series. The superimposed chromatograms in Figure 25 clearly demonstrate that these new GPC columns can be utilized for the analysis of polymers with a wide *MMD*.

Figure 25: Separation of various polymers



Columns: **SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 4**

Mobile phase: THF
 Flow rate: 0.35 mL/min
 Detection: RI
 Temperature: 25 °C
 Injection vol.: 10 µL
 Sample conc.: 0.3%

TSKgel Alpha and SuperAW Size Exclusion Columns

TSKgel Alpha and SuperAW columns were developed for the GPC analysis of polymers of intermediate polarity. As in the TSKgel PW and PW_{XL} columns, the particles in these TSKgel columns have a hydroxylated methacrylate polymer backbone, but they differ in that they are crosslinked to a higher degree to minimize swelling in polar organic solvents (methanol, acetonitrile, DMSO, isopropanol, THF, and HFIP). The TSKgel Alpha and SuperAW columns provide accurate molar mass determination and exhibit normal retention of polystyrene polymers in dimethyl formamide (DMF) solvent. Unlike TSKgel PW columns, which are stable to a 50% organic mixed with water at most, TSKgel SuperAW and Alpha columns are stable in a wide variety of organic solvents at concentrations up to 100%. TSKgel Alpha and SuperAW columns are offered in 5 discrete exclusion ranges and as a mixed bed column. Both column types can accommodate polymer standards up to several million Dalton molar mass.

- Use TSKgel Alpha columns when throughput is not critical, when sample mass is not limited, to collect fractions, and to obtain maximum number of plates (at the expense of analysis time). The main application area for TSKgel Alpha columns is the analysis of polymers that are soluble in polar organic solvents. Examples include cellulose derivatives, polyimide, and sodium dodecylsulfate (all in 10 mmol/L LiBr in DMF), cleansing gel in methanol, and degree of saponification of polyvinylalcohol in hexafluoroisopropanol (HFIP).

The TSKgel Alpha Series consists of six columns with three particle sizes: 7, 10, and 13 μm . These columns span a wide MM separation range, from 100 to more than 1×10^6 Da, when using polyethylene oxide (PEO) as a MM standard. There is one mixed bed column within the TSKgel Alpha line, TSKgel Alpha-M, which has an extended linear calibration range and is suitable for samples with a broad MM distribution, as well as samples with unknown molar mass.

TSKgel Alpha columns include:

- TSKgel Alpha-2500
- TSKgel Alpha-3000
- TSKgel Alpha-4000
- TSKgel Alpha-5000
- TSKgel Alpha-6000
- TSKgel Alpha-M

- Use TSKgel SuperAW columns for high throughput applications, to reduce solvent consumption and to reduce solvent disposal cost. TSKgel SuperAW columns contains a similar chemistry as the TSKgel Alpha columns but offer the benefit of smaller particle sizes (4, 6, 7, and 9 μm), smaller column dimensions, and equivalent resolution. Reductions in analysis time and mobile phase consumption make TSKgel SuperAW columns ideal for high throughput applications.

The TSKgel SuperAW column line consists of five columns and a mixed bed column. These high efficiency columns are only available in 6.0 mm ID \times 15 cm dimensions.

TSKgel SuperAW columns include:

- TSKgel SuperAW2500
- TSKgel SuperAW3000
- TSKgel SuperAW4000
- TSKgel SuperAW5000
- TSKgel SuperAW6000
- TSKgel SuperAWM-H

Attributes and Applications:

Product attributes of the TSKgel Alpha and SuperAW columns are shown in [Table 12](#). These columns are for the analysis of polymers that are soluble in methanol, acetonitrile, DMSO, isopropanol, or THF and can also be used for water-soluble polymers. [Figures 26 - 27](#) show the calibration curves for the TSKgel Alpha and SuperAW columns. Unlike TSKgel PW/PW_{XL} columns, some of which are stable up to 50% organic mixed with water, TSKgel SuperAW and Alpha columns are stable in a wide variety of organic solvents at concentrations up to 100%. As shown in [Figure 28](#), efficiency of all TSKgel SuperAW columns is maintained when changing solvents from water via acetonitrile, DMF, DMSO, THF to HFIP. Suitable solvents for TSKgel Alpha columns are shown in [Figure 29](#).

Table 12: Product attributes

TSKgel column	Particle size	Pore size	Exclusion limit (Da) for various standards & eluents		
			PEO in H ₂ O	PS in DMF with 10 mmol/L LiBr	PEG in MeOH with 10 mmol/L LiBr
Alpha-2500	7 μm	2.5 nm	5,000	1 × 10 ⁴	1 × 10 ⁴
Alpha-3000	7 μm	15 nm	9 × 10 ⁴	1 × 10 ⁵	6 × 10 ⁴
Alpha-4000	10 μm	45 nm	4 × 10 ⁵	1 × 10 ⁶	3 × 10 ⁶
Alpha-5000	10 μm	100 nm	1 × 10 ⁶	7 × 10 ⁶	>3 × 10 ⁵
Alpha-6000	13 μm	>100 nm	>1 × 10 ⁷	>1 × 10 ⁷	>3 × 10 ⁵
Alpha-M	13 μm	mixed bed	>1 × 10 ⁷	>1 × 10 ⁷	>3 × 10 ⁵
SuperAW2500	4 μm	2.5 nm	5,000	1 × 10 ⁴	1 × 10 ⁴
SuperAW3000	4 μm	15 nm	9 × 10 ⁴	1 × 10 ⁵	6 × 10 ⁴
SuperAW4000	6 μm	45 nm	4 × 10 ⁵	1 × 10 ⁶	3 × 10 ⁶
SuperAW5000	7 μm	100 nm	1 × 10 ⁶	7 × 10 ⁶	>3 × 10 ⁵
SuperAW6000	9 μm	>100 nm	>1 × 10 ⁷	>1 × 10 ⁷	>3 × 10 ⁵
SuperAWM-H	9 μm	mixed bed	>1 × 10 ⁷	>1 × 10 ⁷	>3 × 10 ⁵

Figure 26: Polyethylene oxide, polyethylene glycol, and polystyrene calibration curves for TSKgel Alpha columns

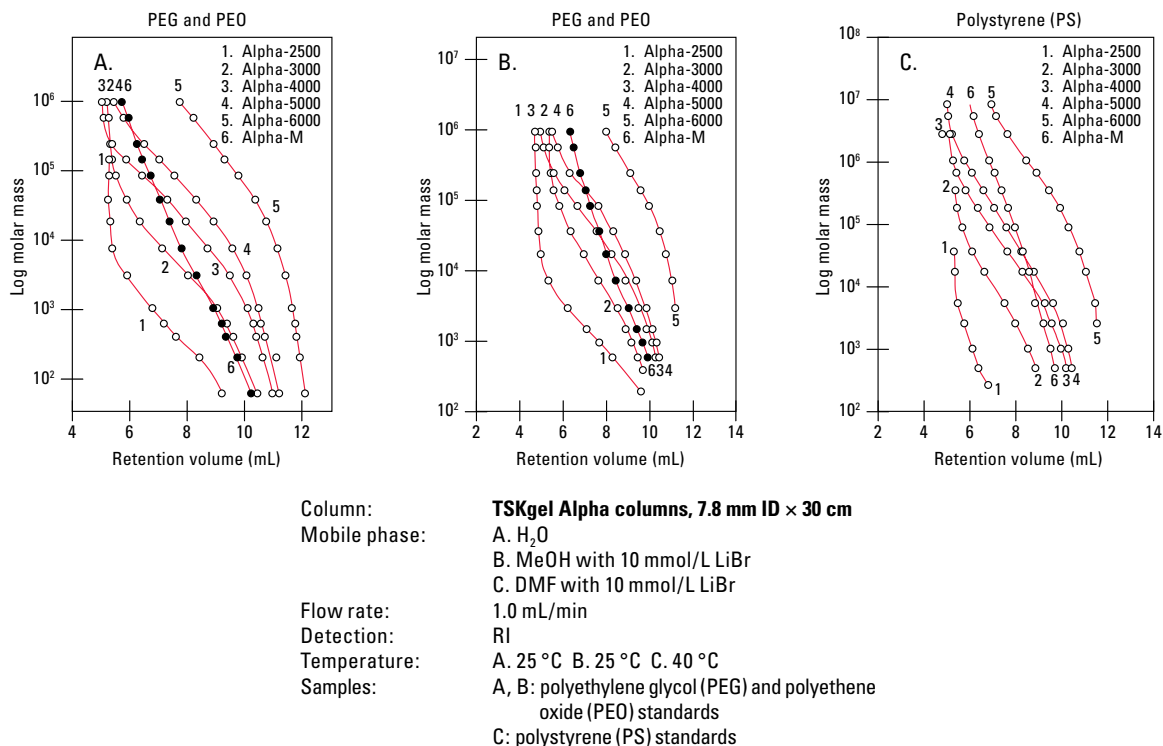


Figure 27: Polyethylene oxide, polyethylene glycol, and ethylene glycol calibration curves for TSKgel SuperAW columns

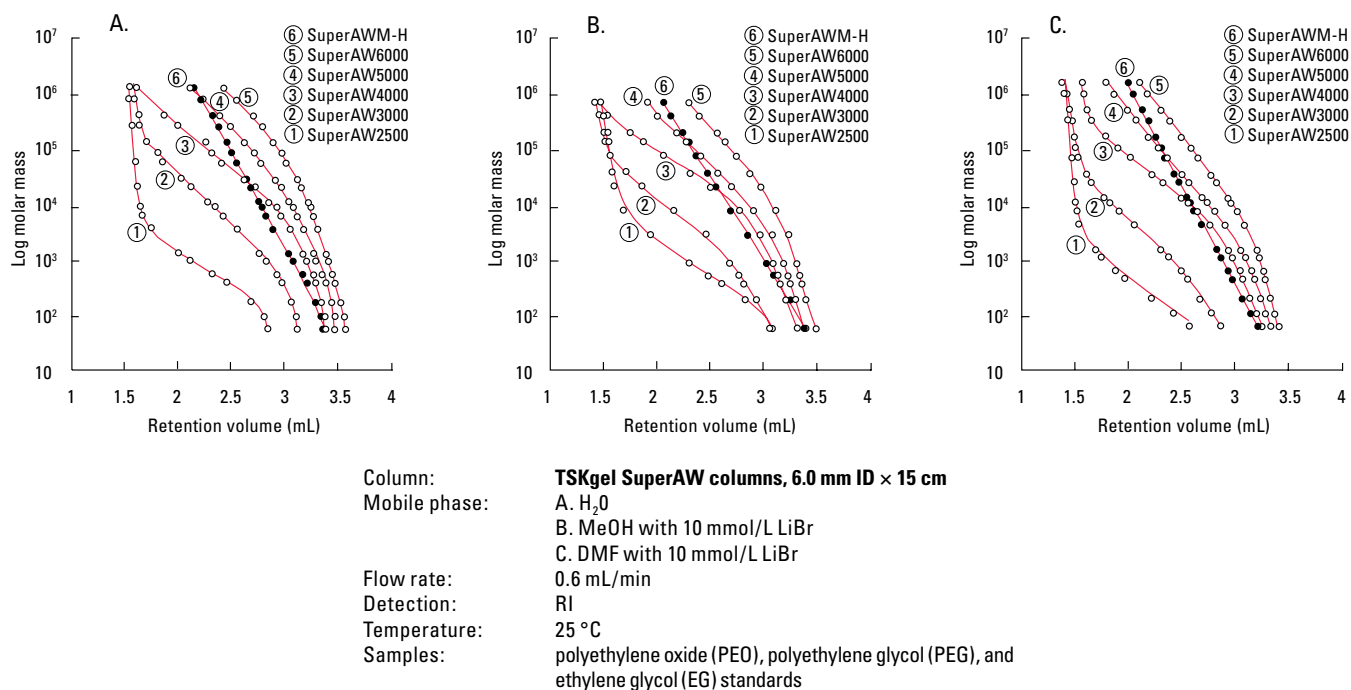


Figure 28: Column efficiency of TSKgel SuperAW columns

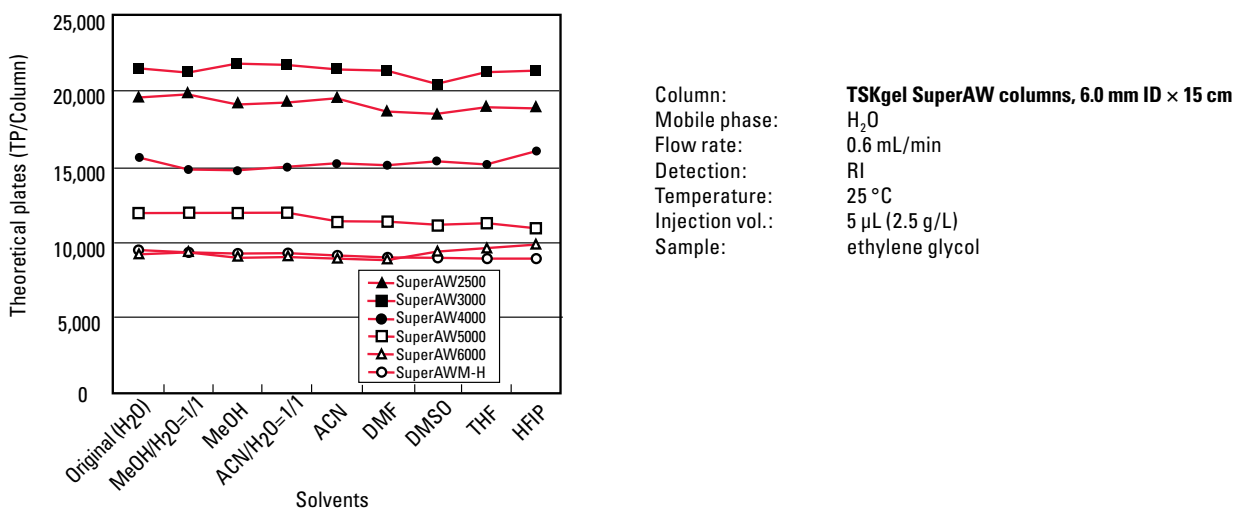
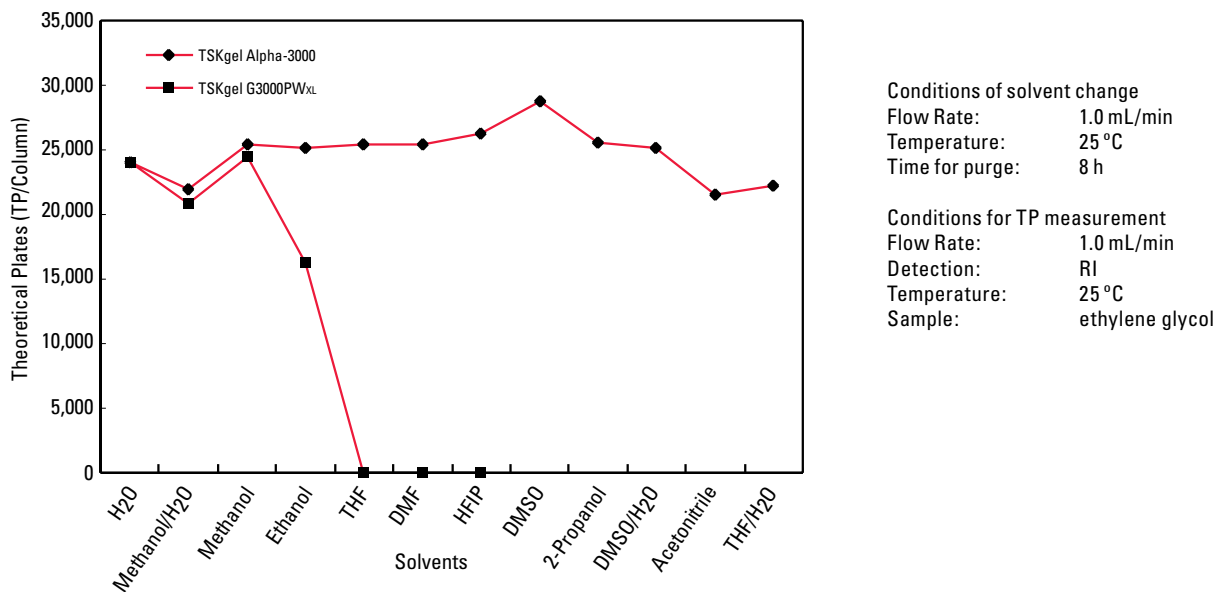


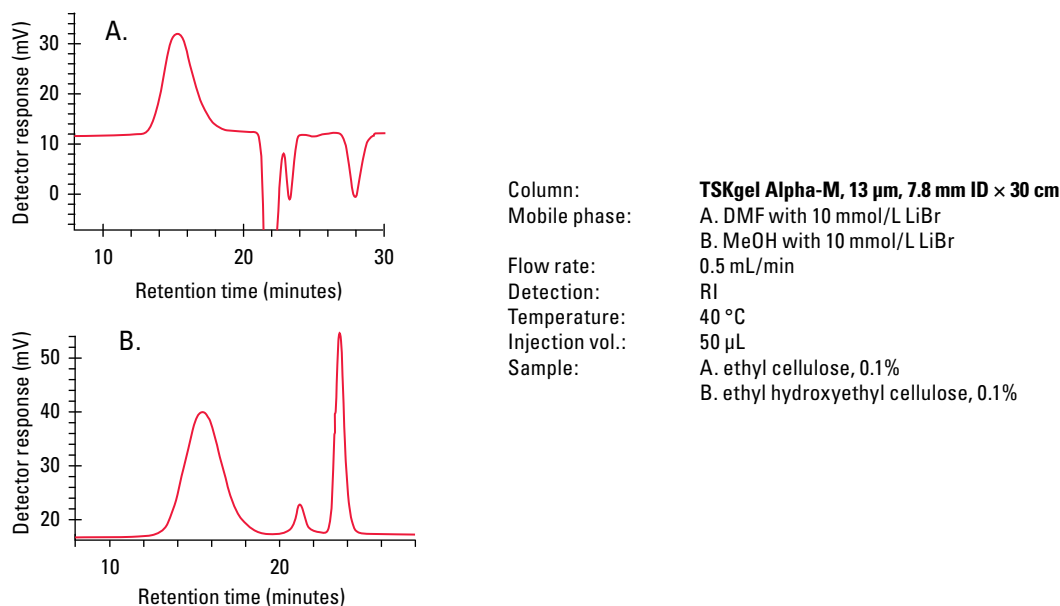
Figure 29: Solvent compatibility for TSKgel Alpha-3000 for organic solvents



Cellulose Derivatives

The versatility of using TSKgel Alpha columns with various polar solvents is illustrated in Figure 30 for the analysis of cellulose derivatives. A TSKgel Alpha-M column was used to separate ethylcellulose with the polar solvent DMF and ethylhydroxyethyl cellulose with methanol.

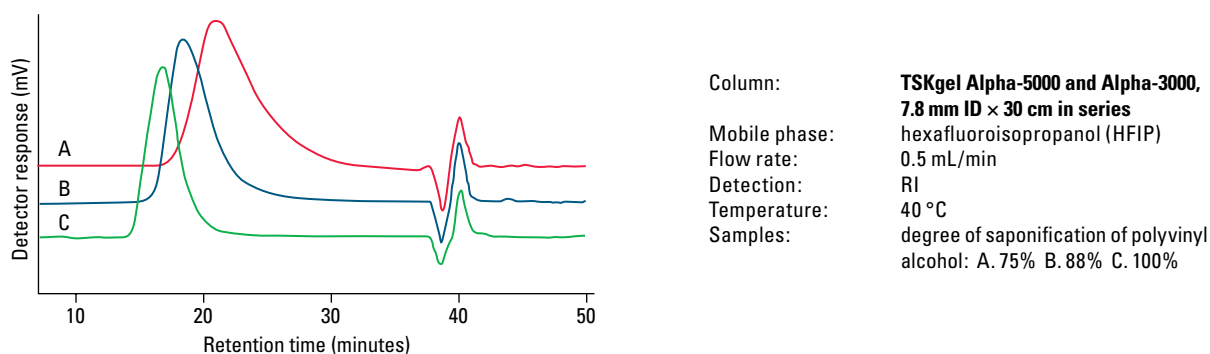
Figure 30: Analysis of cellulose derivatives



Polyvinylalcohol Characterization

The separation of polyvinylalcohol with different degrees of saponification is shown in Figure 31. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol (HFIP) mobile phase.

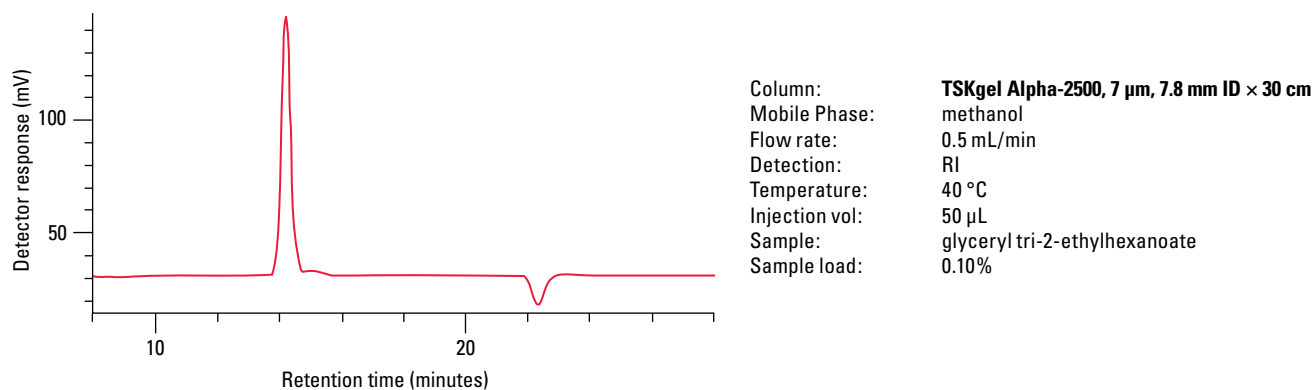
Figure 31: Analysis of polyvinylalcohol with different degrees of saponification



Glyceryl tri(2-ethylhexanoate)

Glyceryl tri(2-ethylhexanoate) is used as a plastic lubricant and as a cosmetic base. The analysis of this compound using a TSKgel Alpha-2500 column is shown in **Figure 32**.

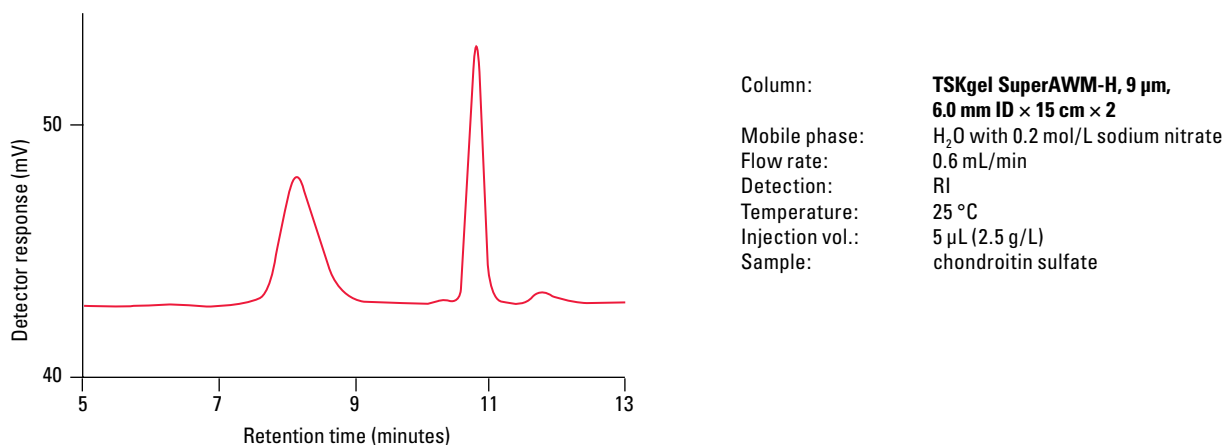
Figure 32: Analysis of glyceryl tri(2-ethylhexanoate)



Sodium Chondroitin Sulfate

Figure 33 demonstrates the successful analysis of sodium chondroitin sulfate on a TSKgel SuperAWM-H column.

Figure 33: Analysis of sodium chondroitin sulfate



TSKgel PW Series Size Exclusion Columns

TSKgel PW and PW_{XL} columns are recommended for analyses of water-soluble polymers and are prepared from hydrophilic polymethacrylate resin. TSKgel PW_{XL}-CP columns are prepared from the same base resin as the TSKgel PW_{XL} columns and were specifically developed for the analysis of water-soluble cationic polymers. TSKgel SuperMultiporePW columns are packed with particles containing a wide range of pore sizes for the analysis of water-soluble polymers with a wide molar mass range.

Stable from pH 2 to 12, TSKgel PW series columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 °C (50 °C for TSKgel G-DNA-PW column).

- Use TSKgel PW columns when analysis time is not critical, when sample mass is not limited, to collect fractions, or to obtain maximum number of plates (at the expense of analysis time). Particle sizes range from 12 µm for the smaller pore size columns (12.5 nm) to 17 µm for the larger pore size columns (20 nm - >100 nm).

The TSKgel GMPW column, within the TSKgel PW column line, is a mixed bed column containing a mixture of different pore sizes that has an extended linear calibration range, suitable for samples with a broad MM distribution as well as unknown samples.

A TSKgel G6000PW column is available in PEEK column hardware, TSKgel BioAssist G6PW, when ultra-low sample adsorption is required, such as in virus analysis.

- Use higher efficiency TSKgel PW_{XL} columns for optimal resolution, to reduce analysis time or in sample-limited applications. TSKgel PW_{XL} columns have smaller particle sizes than TSKgel PW columns, resulting in improved resolution.

The TSKgel PW_{XL} product line also offers specialty columns for analyzing carbohydrate oligomers (TSKgel G-Oligo-PW) and DNA and RNA fragments of 500-5000 base pairs (TSKgel G-DNA-PW). TSKgel GMPW_{XL} is a mixed bed scouting column for aqueous water-soluble linear polymers. Its pore volume is accessible to polymers ranging from molar masses of 1,000 up to 8.0×10^6 Da.

- Cationic groups were introduced on the surface of the TSKgel PW_{XL}-CP packing material to prevent adsorption of cationic polymers and allow elution under low salt conditions. These columns show high theoretical plate numbers, linear calibration curves and excellent durability. The base resin is the same as that used in the TSKgel PW_{XL} columns.

Three columns are available within the TSKgel PW_{XL}-CP line, each with a different particle size, separation range and exclusion limit, allowing polymers within a wide molar mass range to be separated and characterized.

- A wide molar mass range can be analyzed with the three different TSKgel SuperMultiporePW columns, from high molar mass water-soluble polymers to oligomers. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PW_{XL} columns, which further reduces the chance of adsorption of hydrophilic polymers.

The range of pore sizes in which TSKgel PW and TSKgel PW_{XL} columns are available permits a wide spectrum of water-soluble substances to be analyzed. The properties and molar mass separation ranges for all TSKgel PW series columns are summarized in [Table 13](#).

The mechanism of SEC separation is based on the difference of apparent molecular size with no additional interaction between the column matrix and the sample molecules. In practice, however, a small number of weakly charged groups on the surface of all TSKgel PW series packings can cause changes in elution order from that of an ideal system. Fortunately, the mobile phase composition can vary greatly with TSKgel PW series columns to be compatible with a wide range of neutral, polar, anionic, and cationic samples. [Table 14](#) lists appropriate mobile phases for GFC of major polymer types on TSKgel PW series columns.

For some nonionic, nonpolar polymers, such as polyethylene glycols, ideal size exclusion behavior can be obtained by using distilled water as the mobile phase. More polar ionic polymers may exhibit abnormal peak shapes or minor peaks near the void volume when eluted with distilled water due to ionic interactions between the sample and residual charged groups on the resin surface. To eliminate ionic interactions, a neutral salt such as sodium nitrate or sodium sulfate should be added to the aqueous eluent. Generally, a salt concentration of 0.1 mol/L to 0.5 mol/L is needed to overcome undesirable ionic interactions.

TSKgel PW resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. Depending on the sample, this can lead to hydrophobic interaction as a secondary retention mechanism. The extent of hydrophobic interaction increases as the salt concentration of the eluent increases, but it can be reduced by the addition of an organic modifier such as acetonitrile. Water-soluble organic solvents are frequently used as modifiers to suppress hydrophobic interactions between the sample and the resin surface.

Table 13: Properties and separation ranges of TSKgel PW, PW_{XL}, PW_{XL}-CP, and SuperMultiporePW columns

			Molar mass of samples (Da)
TSKgel column	Particle size	Pore size	Polyethylene glycols & oxides
SuperMultiporePW-N	4 µm	20 nm	300 – 5 × 10 ⁴
SuperMultiporePW-M	5 µm	100 nm	500 – 1 × 10 ⁶
SuperMultiporePW-H	8 µm	>100 nm	1,000 – 1 × 10 ⁷
<hr/>			
G2000PW	12 µm	12.5 nm	<3,000
G2500PW	12 µm and 17 µm	12.5 nm	<3,000
G3000PW	12 µm and 17 µm	20 nm	<5 × 10 ⁴
G4000PW	17 µm	50 nm	<3 × 10 ⁵
G5000PW	17 µm	100 nm	<1 × 10 ⁶
G6000PW BioAssist G6PW	17 µm	>100 nm	<8 × 10 ⁶
GMPW	17 µm	mixed pore sizes	1,000 – 8 × 10 ⁶
<hr/>			
G2500PW _{XL}	7 µm	12.5 nm	<3,000
G3000PW _{XL}	7 µm	20 nm	<5 × 10 ⁴
G4000PW _{XL}	10 µm	50 nm	<3 × 10 ⁵
G5000PW _{XL}	10 µm	100 nm	<1 × 10 ⁶
G6000PW _{XL}	13 µm	>100 nm	<8 × 10 ⁶
G-DNA-PW	10 µm	>100 nm	<8 × 10 ⁶
GMPW _{XL}	13 µm	mixed pore sizes	1,000 – 8 × 10 ⁶
SuperOligoPW	3 µm	12.5 nm	<3,000
G-Oligo-PW	7 µm	12.5 nm	<3,000
<hr/>			
G3000PW _{XL} -CP	7 µm	20 nm	200 – 5 × 10 ⁴
G5000PW _{XL} -CP	10 µm	100 nm	400 – 5 × 10 ⁵
G6000PW _{XL} -CP	13 µm	>100 nm	1,000 – 1 × 10 ⁷
Columns: TSKgel PW columns, 7.5 mm ID × 60 cm TSKgel PW _{XL} , G-Oligo-PW and G-DNA-PW columns, 7.8 mm ID × 30 cm TSKgel SuperMultiporePW and SuperOligoPW columns, 6.0 mm ID × 15 cm Mobile phase: polyethylene glycols and oxides (PEOs): distilled water Flow rate: 1.0 mL/min, except for TSKgel SuperMultiporePW and SuperOligoPW columns: 0.6 mL/min			

Modifiers are also used for optimizing the elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in **Table 14**. All TSKgel PW series packings are compatible with 20% aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethyl formamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition, these columns can be operated in 50% aqueous acetone.

Table 14: Recommended mobile phases for GFC of water-soluble polymers on TSKgel PW, PW_{XL}, PW_{XL}-CP, and SuperMultiporePW columns

Type of polymer	Typical sample	Suitable mobile phase
Nonionic hydrophilic	polyethylene glycol	Distilled water
	soluble starch, methyl cellulose, pullulan	0.01 mol/L NaOH
	dextran, hydroxyethyl cellulose	20% DMSO (dimethyl sulfoxide)
	polyvinyl alcohol, polyacrylamide	Buffer or salt solution (e.g. 0.1-0.5 mol/L NaNO ₃)
Nonionic hydrophobic	polyvinylpyrrolidone	Buffer or salt solution with organic solvent (e.g. 20% CH ₃ CN in 0.1 mol/L NaNO ₃)
Anionic hydrophilic	sodium chondroitin sulfate, sodium alginate, carboxymethyl cellulose, sodium polyacrylate, sodium hyaluronate	Buffer or salt solution (e.g. 0.1 mol/L NaNO ₃)
Anionic hydrophobic	sulfonated lignin sodium salt, sodium polystyrenesulfonate	Buffer or salt solution with organic solvent (e.g. 20% CH ₃ CN in 0.1 mol/L NaNO ₃)
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethylene imine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na ₂ SO ₄ or 0.8 mol/L NaNO ₃
Cationic hydrophobic	poly(4-vinylbenzyltrimethylammonium chloride), poly(N-methyl-2-vinylpyridinium) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na ₂ SO ₄
Amphoteric hydrophilic	peptides, proteins, poly- and oligosaccharides, DNA, RNA	Buffer or salt solution (e.g. 0.1 mol/L NaNO ₃)
Amphoteric hydrophobic	blue dextran, collagen, gelatin, hydrophobic proteins, hydrophobic peptides	Buffer or salt solution with organic solvent (e.g. 20% CH ₃ CN in 0.1 mol/L NaNO ₃ or 35-45% CH ₃ CN in 0.1% TFA)

TSKgel PW Size Exclusion Columns

TSKgel PW columns are composed of spherical, hydrophilic polymethacrylate beads. Particle sizes range from 12 μm for the smaller pore size columns to 17 μm for the larger pore size columns. Stable from pH 2 to 12, TSKgel PW columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 °C.

The TSKgel PW column line consists of the following columns:

- TSKgel G2000PW
- TSKgel G2500PW
- TSKgel G3000PW
- TSKgel GMPW
- TSKgel G4000PW
- TSKgel G5000PW
- TSKgel G6000PW

The mixed bed column, TSKgel GMPW, has an extended linear calibration range, suitable for samples with a broad MM distribution, as well as for unknown samples. The pore volume can be accessed by polymers ranging in molar mass from 1,000 to 8.0×10^6 Da. By quickly categorizing the MM profile of an unknown sample, the column enables a fast selection of the best TSKgel PW column for routine analysis.

Attributes and Applications

Product attributes of all eight TSKgel PW columns are shown in [Table 15](#). All TSKgel PW columns have a base material of hydroxylated polymethacrylate, can be used in a maximum of 20% organic, and are shipped in water. The main application area for TSKgel PW columns is the analysis of water-soluble polymers, such as celluloses, acrylamides, glycols, dextrans, polyvinylalcohol, and oligosaccharides. TSKgel G2000PW, the larger particle size equivalent of TSKgel G-Oligo-PW, is most suitable for semi-preparative and preparative isolation of oligosaccharides. Representative application examples for the PW columns are illustrated in [Table 16](#). The calibration curve for polyethylene glycol and oxides for the TSKgel PW columns is shown in [Figure 34](#).

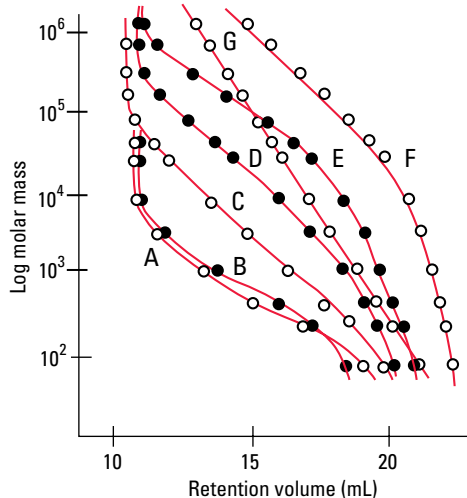
Table 15: Product attributes

TSKgel column	Particle size (mean)	Pore size (mean)	Calibration range
G2000PW	12 μm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
G2500PW	12 μm and 17 μm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
G3000PW	12 μm and 17 μm	20 nm	Up to 5.0×10^4 Da (polyethylene glycols and oxides)
G4000PW	17 μm	50 nm	Up to 3.0×10^5 Da (polyethylene glycols and oxides)
G5000PW	17 μm	100 nm	Up to 1.0×10^6 Da (polyethylene glycols and oxides)
G6000PW	17 μm	>100 nm	Up to 8.0×10^6 Da (polyethylene glycols and oxides)
GMPW	17 μm	mixed pore sizes	1,000 - 8.0×10^6 Da (polyethylene glycols and oxides)

Table 16: Representative application examples for TSKgel PW columns

Classification	Examples
1. Synthetic polymers <ul style="list-style-type: none"> • Nonionic • Cationic • Anionic 	<ul style="list-style-type: none"> • PEG, polyglycerin, polyacrylamide • Polyethyleneimine, polyvinylpyrrolidone • Poly (sodium acrylate), Poly (sodium styrene sulfonate)
2. Polysaccharides and derivatives	<ul style="list-style-type: none"> • Standard dextran, clinical dextran, pullulan, inulin, heparin, chitosan • Carboxymethylcellulose
3. Very large biopolymers <ul style="list-style-type: none"> • Polynucleotides • Viruses • Proteins 	<ul style="list-style-type: none"> • DNA fragments • TMV, SBMV, TBSV • Lipoprotein (VLDL, LDL), apoferritin, gelatin, sea worm chlorocruorin
4. Small molecules <ul style="list-style-type: none"> • Oligomers • Others 	<ul style="list-style-type: none"> • oligosaccharides (dextran hydrolysate, cyclodextrin hydrolysate), cyclodextrins • oligopeptides • oligonucleotides

Figure 34: Polyethylene glycol and oxide calibration curves for TSKgel PW columns

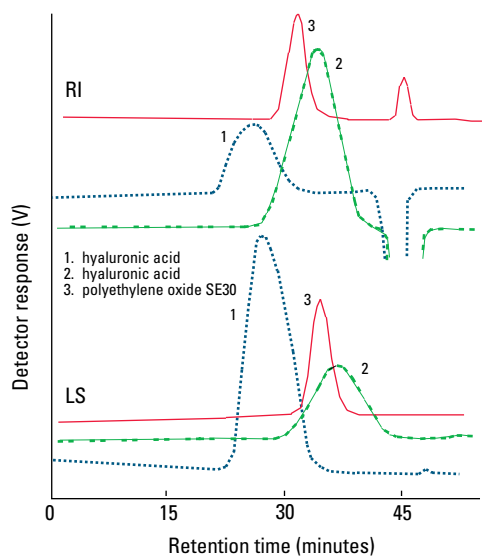


Column: **A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5 mm ID × 60 cm**
 Mobile phase: distilled H₂O
 Flow rate: 1.0 mL/min
 Detection: RI

Oligosaccharides

TSKgel PW columns are recommended for polysaccharide analysis due to their ability to separate a wide molar mass distribution. An effective separation of the anionic hydrophilic glucosaminoglycan, hyaluronic acid, is shown in Figure 35 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase. To obtain shorter analysis time and similar resolution, we recommend using TSKgel G3000PW_{XL} and G4000PW_{XL} columns in series.

Figure 35: Analysis of polysaccharides

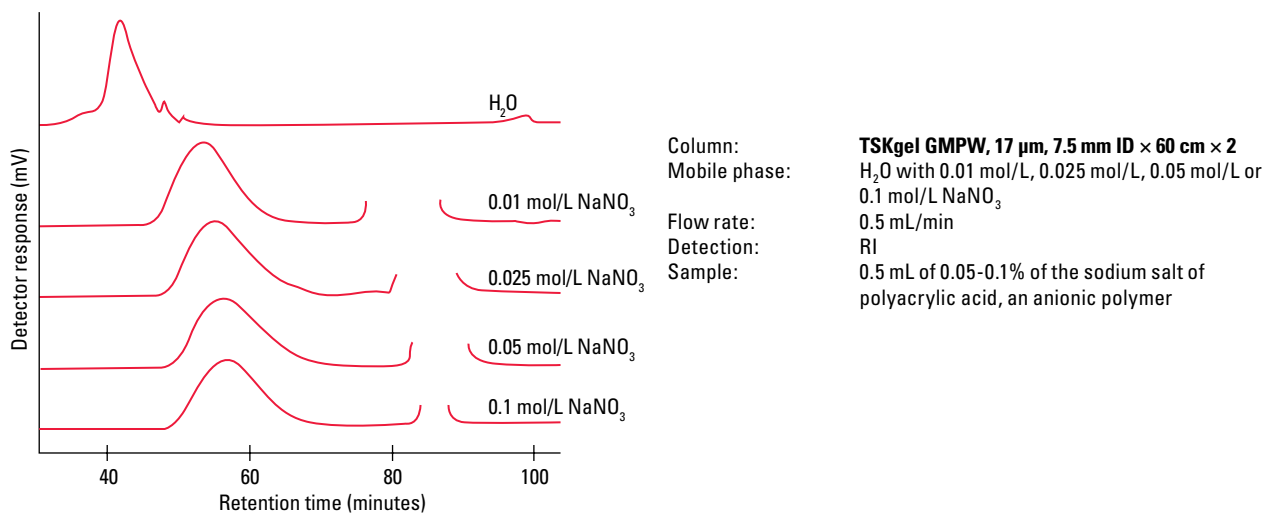


Columns: **TSKgel G6000PW + G4000PW, two 7.5 mm ID × 60 cm columns in series**
 Mobile phase: H₂O with 0.2 mol/L NaCl
 Flow rate: 0.9 mL/min
 Temperature: 40 °C
 Sample: hyaluronic acid, polyethylene oxide

Polymers

Sodium polyacrylate, an anionic polymer, is effectively separated on two TSKgel GMPW columns in **Figure 36**. The addition of 0.01 mol/L NaNO₃ results in normal elution and peak shape overcoming the ionic repulsion between the anionic sample and the resin.

Figure 36: Effect of ionic strength on the elution of anionic polymers



TSKgel PW_{XL} Size Exclusion Columns

TSKgel PW_{XL} columns are composed of spherical, hydrophilic polymethacrylate beads. The smaller particle size of TSKgel PW_{XL} columns provide 1.7x higher resolution than their TSKgel PW columns counterpart, making TSKgel PW_{XL} columns more suitable for analytical purposes. Four specialty columns are included in the TSKgel PW_{XL} column line.

The TSKgel G-DNA-PW column is designed for the separation of large polynucleotides such as DNA and RNA fragments of 500 - 5,000 base pairs. This column is a smaller particle size version of the TSKgel G6000PW_{XL} column. The TSKgel G-Oligo-PW column is designed for high resolution separations of aqueous nonionic and cationic oligomers, and oligosaccharides such as hydrolyzed cyclodextrins. Because of the presence of cationic groups on the gel matrix, this column is not suitable for separating anionic polymers. The TSKgel G-Oligo-PW column has a PEG and PEO calibration curve identical to that of the TSKgel G2500PW_{XL} column. The mixed-mode column, TSKgel GMPW_{XL}, has an extended linear calibration range, suitable for samples with a broad MM distribution and unknowns.

The TSKgel SuperOligoPW column is designed for the determination of molar mass of aqueous oligomers, particularly oligosaccharides, and low molar mass aqueous polymers. The combination of the decreased particle size and semi-micro dimensions of the TSKgel SuperOligoPW column enables high speed separation with high resolution and lowered solvent consumption. Since the packing material in the TSKgel SuperOligoPW columns is more hydrophilic compared with TSKgel G-Oligo-PW columns, an even wider range of water-soluble polymers can be analyzed without the need to add organic solvent to the eluent.

The following TSKgel PW_{XL} columns are offered:

- TSKgel G2500PW_{XL}
- TSKgel G3000PW_{XL}
- TSKgel G4000PW_{XL}
- TSKgel G5000PW_{XL}
- TSKgel G6000PW_{XL}
- TSKgel G-DNA-PW
- TSKgel GMPW_{XL}
- TSKgel G-Oligo-PW
- TSKgel SuperOligoPW

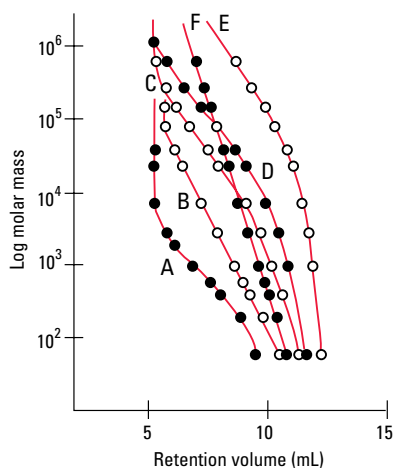
Attributes and Applications

The main application area for TSKgel PW_{XL} columns is the analysis of water-soluble polymers, such as celluloses, acrylamides, glycols, dextrans, polyvinylalcohol, and oligosaccharides. Because of the presence of cationic groups on the base bead of TSKgel G2500PW_{XL}, this column is not suited for separating anionic polymers. Product attributes of all of the TSKgel PW_{XL} columns are shown in **Table 17**. All TSKgel PW_{XL} columns have a base material of hydroxylated polymethacrylate, can be used in a maximum of 20% organic and are shipped in water. **Figures 37 - 41** show the calibration curves for all of the TSKgel PW_{XL} columns.

Table 17: Product attributes

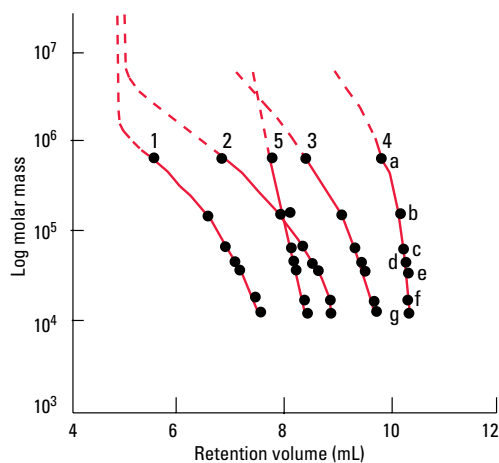
TSKgel column	Particle size (mean)	Pore size (mean)	Calibration range
G2500PW _{XL}	7 µm	12.5 nm	<3,000 Da (polyethylene glycols and oxides)
G3000PW _{XL}	7 µm	20 nm	<4.0 × 10 ⁴ Da (polyethylene glycols and oxides)
G4000PW _{XL}	10 µm	50 nm	2,000 - 3.0 × 10 ⁵ Da (polyethylene glycols and oxides)
G5000PW _{XL}	10 µm	100 nm	4,000 - 8.0 × 10 ⁵ Da (polyethylene glycols and oxides)
G6000PW _{XL}	13 µm	>100 nm	4.0 × 10 ⁴ - 8.0 × 10 ⁶ Da (polyethylene glycols and oxides)
G-DNA-PW	10 µm	>100 nm	4.0 × 10 ⁴ - 8.0 × 10 ⁶ Da (polyethylene glycols and oxides)
GMPW _{XL}	13 µm	mixed pore sizes	1,000 - 8.0 × 10 ⁶ Da (polyethylene glycols and oxides)
G-Oligo-PW	7 µm	12.5 nm	Up to 3,000 Da (polyethylene glycols and oxides)
SuperOligoPW	3 µm	12.5 nm	<3,000 Da (PEO,PEG/H ₂ O)

Figure 37: Polyethylene glycol and oxide calibration curves for TSKgel PW_{XL} columns



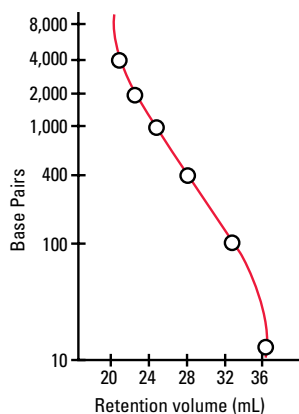
Column: **A. G2500PW_{XL}, B. G3000PW_{XL}, C. G4000PW_{XL}, D. G5000PW_{XL}, E. G6000PW_{XL}, F. GMPW_{XL}, all 7.8 mm ID × 30 cm**
 Mobile phase: distilled H₂O
 Flow rate: 1.0 mL/min
 Detection: RI

Figure 38: Protein calibration curves for TSKgel PW_{XL} columns



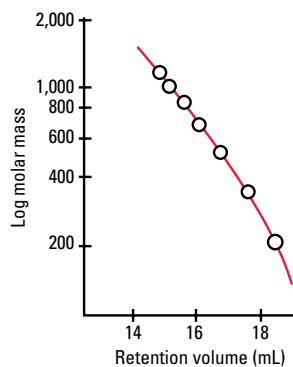
Column: **1. TSKgel G3000PW_{XL}, 2. TSKgel G4000PW_{XL}, 3. TSKgel G5000PW_{XL}, 4. TSKgel G6000PW_{XL}, 5. TSKgel GMPW_{XL}, all 7.8 mm ID × 30 cm**
 Mobile phase: 0.2 mol/L phosphate buffer, pH 6.8
 Flow rate: 1.0 mL/min
 Detection: UV @ 280 nm
 Sample: a. thyroglobulin (6.6 × 10⁵ Da)
 b. γ-globulin (1.5 × 10⁵ Da)
 c. albumin (6.7 × 10⁴ Da)
 d. ovalbumin (4.3 × 10⁴ Da)
 e. β-lactoglobulin (3.6 × 10⁴ Da)
 f. myoglobin (1.69 × 10⁴ Da)
 g. cytochrome C (1.24 × 10⁴ Da)

Figure 39: Double stranded DNA calibration curves for TSKgel G-DNA-PW column



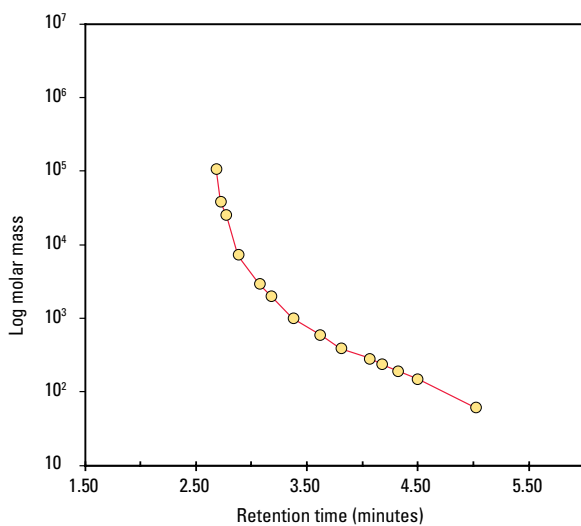
Column: **TSKgel G-DNA-PW, 10 μm, 7.8 mm ID × 30 cm × 4**
 Mobile phase: H₂O with 0.3 mol/L NaCl in 0.1 mol/L Tris-HCl, pH 7.5, + 1 mmol/L EDTA
 Flow rate: 0.15 mL/min
 Detection: UV @ 260 nm
 Sample: *Eco*RI and *Bst*NI-cleaved pBR322 DNA, void volume determined with λ-DNA

Figure 40: Oligosaccharide calibration curve for TSKgel G-Oligo-PW column



Column: **TSKgel G-Oligo-PW, 7 μ m, 7.8 mm ID \times 30 cm \times 2**
 Mobile phase: distilled H₂O
 Flow rate: 1.0 mL/min
 Detection: UV @ 260 nm
 Sample: hydrolyzed β -cyclodextrin

Figure 41: Polyethylene glycol, oxide and ethylene glycol calibration curve for TSKgel SuperOligoPW column

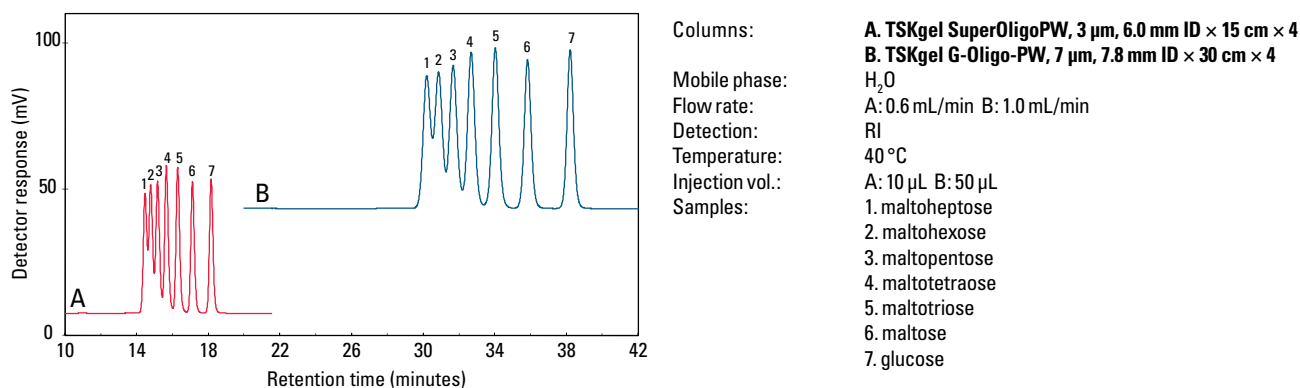


Columns: **TSKgel SuperOligoPW, 3 μ m, 6.0 mm ID \times 15 cm**
 Mobile phase: H₂O
 Flow rate: 0.60 mL/min
 Detection: RI
 Temperature: 25 °C
 Samples: PEO, PEG and ethylene glycol

Oligosaccharides

Figure 42 demonstrates the high speed analysis of maltose oligomers using a TSKgel SuperOligoPW column compared to a TSKgel G-Oligo-PW column. The faster analysis time is due to the semi-micro dimensions (6.0 mm ID × 15 cm) and the small particle size (3 μm) of the TSKgel SuperOligoPW column compared to the 7.8 mm ID × 30 cm size and 7 μm particle size of the TSKgel G-Oligo-PW column.

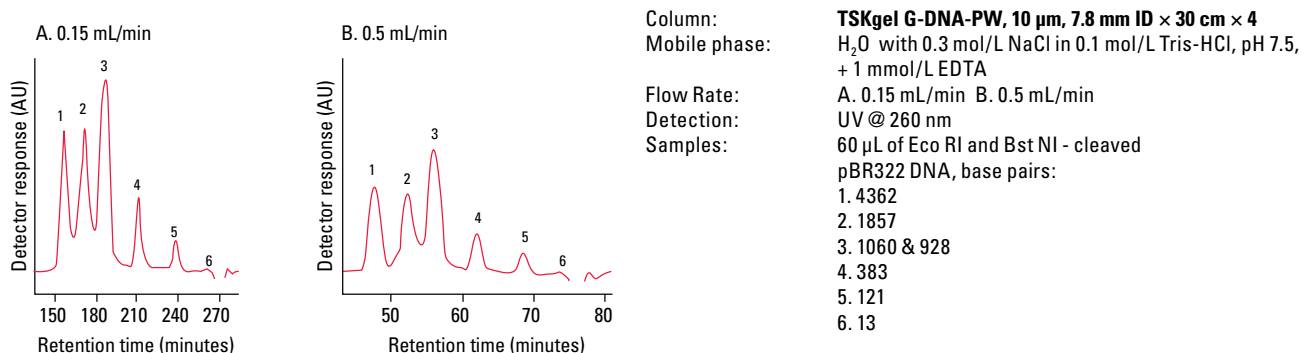
Figure 42: Analysis of maltose oligomers



Large DNA fragments

For the separation of large DNA fragments greater than 1,000 base pairs, a four column system is typically required. Baseline resolution of DNA fragments up to 7,000 base pairs can be achieved, provided there is a two-fold difference in the chain length of the fragments. **Figure 43A** shows the elution of double stranded DNA fragments, obtained from pBR322 DNA cleaved by both EcoRI and BstNI, on four TSKgel G-DNA-PW columns in series. The eluted peaks were collected and subjected to polyacrylamide gel electrophoresis, which showed almost complete separation of the 1060, 1857, and 4362 base pair fragments. Although lower flow rates typically yield better separations of most fragments, the resolution of the 1857 and 4362 base pair fragments was slightly greater at the higher flow rate, as shown in **Figure 43B**.

Figure 43A & 43B: Analysis of large DNA fragments



TSKgel PW_{XL}-CP Size Exclusion Columns

TSKgel PW_{XL}-CP columns were specifically developed for the analysis of water-soluble cationic polymers. Composed of polymethacrylate beads, cationic groups are introduced on the surface of the TSKgel PW_{XL}-CP packing material to prevent adsorption of cationic polymers and allow elution under low salt conditions. These columns show high theoretical plate numbers, linear calibration curves, and high durability because the base resin is the same as that used in the TSKgel PW_{XL} columns.

Three columns are available within the TSKgel PW_{XL}-CP series, each with a different particle size, separation range, and exclusion limit, allowing polymers within a wide molar mass range to be separated and characterized.

- TSKgel G3000PW_{XL}-CP
- TSKgel G5000PW_{XL}-CP
- TSKgel G6000PW_{XL}-CP

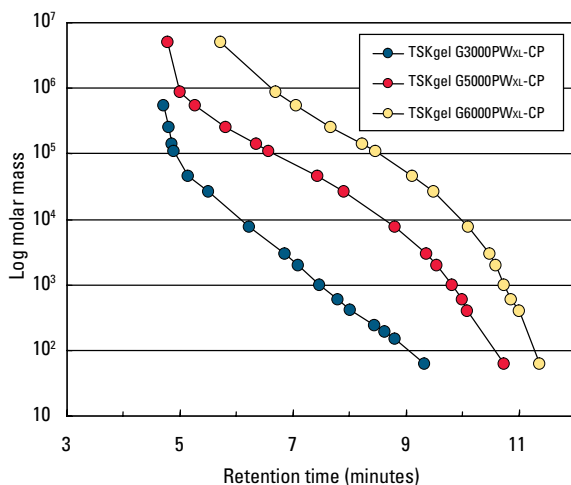
Attributes and Applications:

Table 18 shows the product attributes for each of the three TSKgel PW_{XL}-CP columns. Figure 44 shows calibration curves produced with standard polyethylene oxide and polyethylene glycol in a 0.1 mol/L aqueous solution of sodium nitrate.

Table 18: Product attributes

TSKgel column	G3000PW _{XL} -CP	G5000PW _{XL} -CP	G6000PW _{XL} -CP
Base material	polymethacrylate	polymethacrylate	polymethacrylate
Particle size	7 μm	10 μm	13 μm
Pore size	20 nm	100 nm	>100 nm
Exclusion limit	1.0 × 10 ⁵ Da	1.0 × 10 ⁶ Da	2.0 × 10 ⁷ Da
Separation range (PEO, PEG)	200 ~ 5.0 × 10 ⁴ Da	400 ~ 5.0 × 10 ⁵ Da	1,000 ~ 1.0 × 10 ⁷ Da
Theoretical plates	16,000	10,000	7,000

Figure 44: Polyethylene glycol and oxide calibration curves for TSKgel PW_{XL}-CP columns



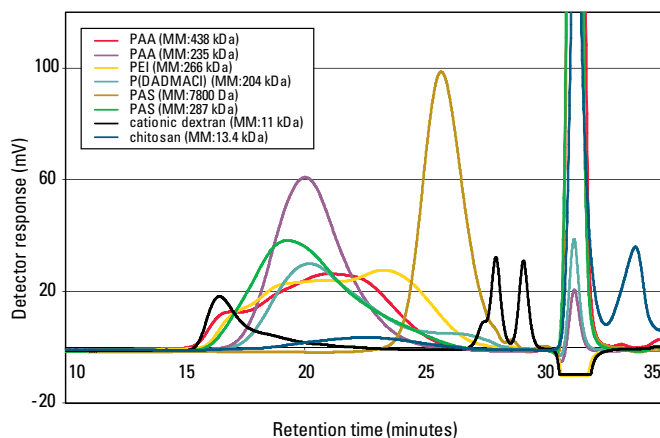
Columns: TSKgel G3000PW_{XL}-CP, 7 μm, 7.8 mm ID × 30 cm
 TSKgel G5000PW_{XL}-CP, 10 μm, 7.8 mm ID × 30 cm
 TSKgel G6000PW_{XL}-CP, 13 μm, 7.8 mm ID × 30 cm

Mobile phase: H₂O with 0.1 mol/L NaNO₃
 Flow Rate: 1 mL/min
 Detection: RI
 Temperature: 25 °C
 Samples: polyethylene oxides (PEO) standards
 polyethylene glycols (PEG) standards

Cationic Polymers

Various cationic polymers with different functional groups and molar masses were injected on the three TSKgel PW_{XL}-CP columns (TSKgel G6000PW_{XL}-CP, G5000PW_{XL}-CP, and G3000PW_{XL}-CP) connected in series. **Figure 45** demonstrates that these SEC columns can be utilized for the analysis of a wide variety of cationic polymers.

Figure 45: Analysis of cationic polymers



Columns: **TSKgel G3000PW_{XL}-CP, 7 μm, 7.8 mm ID × 30 cm**
TSKgel G5000PW_{XL}-CP, 10 μm, 7.8 mm ID × 30 cm
TSKgel G6000PW_{XL}-CP, 13 μm, 7.8 mm ID × 30 cm

Mobile phase: H₂O with 0.1 mol/L NaNO₃
 Flow Rate: 1 mL/min
 Detection: RI
 Temperature: 25 °C
 Sample Load: 3 g/L, 100 μL

TSKgel SuperMultiporePW Size Exclusion Columns

The innovative multi-pore particle synthesis technology*, pioneered by Tosoh scientists, is incorporated into TSKgel SuperMultiporePW columns for water-soluble polymer analysis. Three semi-micro columns varying in linear range are available within this series, enabling high speed and high resolution analysis with lowered solvent consumption. The base material of each TSKgel SuperMultiporePW column is polymethacrylate.

A wide molar mass range can be analyzed with the three different TSKgel SuperMultiporePW columns, from high molar mass water-soluble polymers to oligomers. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PW_{XL} series columns, which further reduces the chance of adsorption of hydrophilic polymers.

- TSKgel SuperMultiporePW-N
- TSKgel SuperMultiporePW-M
- TSKgel SuperMultiporePW-H

*Using this proprietary technology, Tosoh can manufacture particles, each containing a broad range of pore sizes. This innovative approach essentially creates a linear calibration curve within each particle. As a result, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes.

Attributes and Applications:

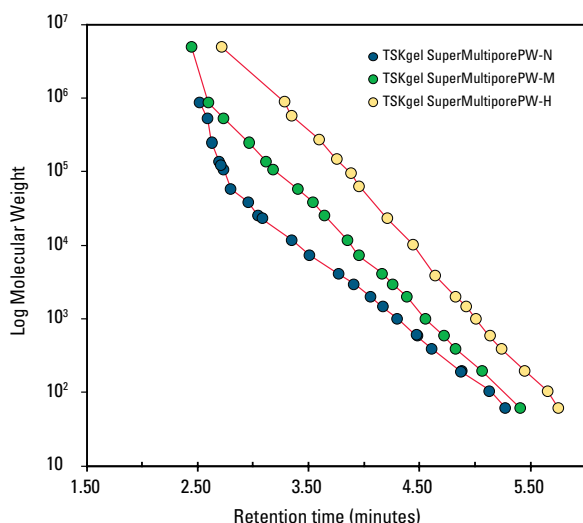
Table 19 shows the product attributes for each of the three TSKgel SuperMultiporePW columns. Figure 46 shows polyethylene glycol, oxide and ethylene glycol calibration curves for each of the TSKgel SuperMultiporePW columns.

Table 19: Product attributes

TSKgel column	SuperMultiporePW-N	SuperMultiporePW-M	SuperMultiporePW-H
Base material	polymethacrylate	polymethacrylate	polymethacrylate
Particle size	4 μm*	5 μm*	8 μm*
Pore size	20 nm	100 nm	>100 nm
Exclusion limit (PEO, PEG/H ₂ O)	1.0 × 10 ⁵ - 1.5 × 10 ⁵ Da	6.0 × 10 ⁵ - 1.5 × 10 ⁶ Da	-
Separation range	300 ~ 5.0 × 10 ⁴ Da	500 ~ 1.0 × 10 ⁶ Da	1,000 ~ 1.0 × 10 ⁷ Da
Theoretical plates/15cm column	>16,000	>12,000	>7,000

* Particle size distribution is monodisperse.

Figure 46: Polyethylene glycol, oxide, and ethylene glycol calibration curves for TSKgel SuperMultiporePW columns



Columns: **TSKgel SuperMultiporePW-N, 4 μm, 6.0 mm ID × 15 cm**
TSKgel SuperMultiporePW-M, 5 μm, 6.0 mm ID × 15 cm
TSKgel SuperMultiporePW-H, 8 μm, 6.0 mm ID × 15 cm

Mobile phase: H₂O
 Flow rate: 0.60 mL/min
 Detection: RI
 Temperature: 25 °C
 Samples: polyethylene oxides (PEO) standards
 polyethylene glycols (PEG) standards
 ethylene glycol (EG) standards