The EcoSEC® GPC System Delivers High Performance with Either Semi-Micro or Conventional Gel Permeation Chromatography Columns

Introduction

Traditionally, gel permeation chromatography (GPC) systems have implemented columns having dimensions of 7.8 mm ID × 30 cm. Column dimensions were dictated by large detector cell volumes, a lack of accuracy and precision of injectors at low volumes, and dead volume in connecting tubing, ports, and joints of the GPC systems.[1] The reduction of detector cell volumes, the optimization of tubing, connectors, ports, and joints, and the increased accuracy and precision of low volume injectors over the years has led to the development and applicability of semi-micro GPC columns. Semi-micro columns are environmentally and economically friendly as they allow for a reduction in organic solvent consumption and a decrease in solvent related costs by operating at lower flow rates and requiring shorter analysis times. Tosoh introduced semi-micro GPC columns over twenty years ago having dimensions of 4.6 or 6.0 mm ID × 15 cm with similar physical stability and chromatographic resolution as conventional GPC columns.

For over 40 years Tosoh scientists have been perfecting GPC instrumentation through the progression of the EcoSEC GPC System. The foundation of the EcoSEC GPC System is an all-in-one System designed with: low dead volume for improved resolution and molar mass distribution accuracy *regardless* of GPC column length, temperature controlled pumps for excellent flow rate precision independent of changes in laboratory temperature, and a dual flow refractive index detector (RI) for unmatched baseline stability. The EcoSEC GPC System is designed to eliminate the issues associated with the use of semi-micro GPC columns since the detectors within the EcoSEC GPC System have low dead volume flow cells, 2.5 µL and 2.0 µL for the RI and UV detectors, respectively. The system is optimized to have a total system dead volume of < 20 µL and the injector is accurate and precise from 1 to 1,500 µL. These features of the EcoSEC GPC System allow for the use of *both* conventional (30 cm) and semi-micro (15 cm) GPC columns without compromising resolution and molar mass accuracy.

The ability to use conventional and semi-micro GPC columns on the same GPC system allows the user to compare and contrast current methods involving conventional GPC columns with new more environmentally and economically friendly methods encompassing semi-micro GPC columns. Here we compare the chromatograms as well as the molar mass averages obtained from conventional and semi-micro TSKgel® HXL and SuperHZ series columns using the EcoSEC GPC System for both polystyrene standards and real-world polymer samples.

Experimental Conditions

Sample analysis was performed on a system consisting of an EcoSEC GPC System (HLC-8320) equipped with a UV and dual flow RI detector. The UV absorbance was monitored at a wavelength of 248 nm. Semi-micro GPC separation of unfiltered 30 µL injections occurred over a column bank consisting of one 4.6 mm ID × 15 cm, 3 µm particle size TSKgel SuperHZ4000, one 4.6 mm ID × 15 cm, 3 µm particle size TSKgel SuperHZ3000, one 4.6 mm ID × 15 cm, 3 µm particle size TSKgel SuperHZ2000, and one 4.6 mm ID × 15 cm, 3 µm particle size TSKgel SuperHZ1000 preceded by the appropriate guard column (Tosoh Bioscience LLC).

Conventional GPC separation of unfiltered 150 µL injections occurred over a column bank consisting of one 7.8 mm ID × 30 cm, 5 µm particle size TSKgel G4000HXL, 7.8 mm ID × 30 cm, 5 µm particle size TSKgel G3000HXL, 7.8 mm ID × 30 cm, 5 µm particle size TSKgel G2000HXL, 7.8 mm ID × 30 cm, 5 µm particle size TSKgel G1000HXL preceded by the appropriate guard column (Tosoh Bioscience LLC). The mobile phase and solvent was tetrahydrofuran (THF) (BDH) at a flow rate of 0.35 mL/min for semi-micro GPC analysis and 1.0 mL/min for conventional GPC analysis. Detector, pump oven, and column oven were maintained at 40 °C. For all chromatographic determinations, results are averages of four injections. Data was processed with the EcoSEC GPC Workstation software, version 1.08.

A calibration curve was created for the RI and UV at 40 °C for both column sets using PStQuick Kit-H polystyrene mix standard (Tosoh Bioscience LLC) with a molar mass ranging from 500 to 8.4 × 10^6 g/mol. Polystyrene standards were prepared by adding 1.750 mL of THF to each PStQuick vial. Calibration curves were created for each column set and individual detector ranging from 266 to 1.9 × 10^5 g/mol. Polystyrene standards were analyzed under the same conditions as those used for sample analysis as described above. Calibration curve data for 0.35 mL/min and 1.0 mL/min was fitted with a cubic function and error values were less than 6%.

Results and Discussion

As mentioned in the "Introduction", the EcoSEC GPC System is designed to be used in conjunction with either conventional or semi-micro GPC columns without compromising the benefits and features of the all-in-one System. The applicability of both column lengths with the EcoSEC GPC System are shown for a mixture of polystyrene standards and a real-world polymer sample through the comparison of chromatograms obtained from conventional and semi-micro TSKgel HXL and SuperHZ series columns, *Figures 1 and 2*. 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 1*. 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 1A*, occurs in less than thirty minutes, approximately half the time required to obtain an identical separation using conventional GPC columns, *Figure 1B*.

The GPC chromatogram as well as weight-average molar mass 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 were compared. As can be seen in *Figure 2A and 2B*, the GPC chromatograms obtained via both column lengths are very similar to one another. 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.

Figure 1. 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.

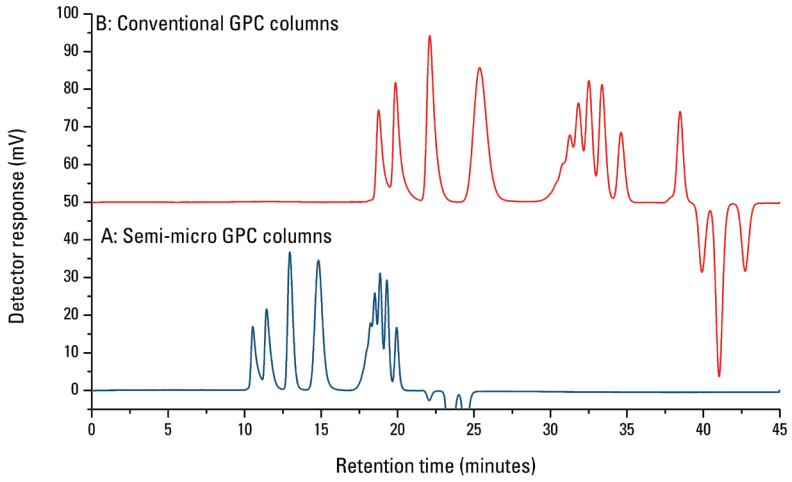
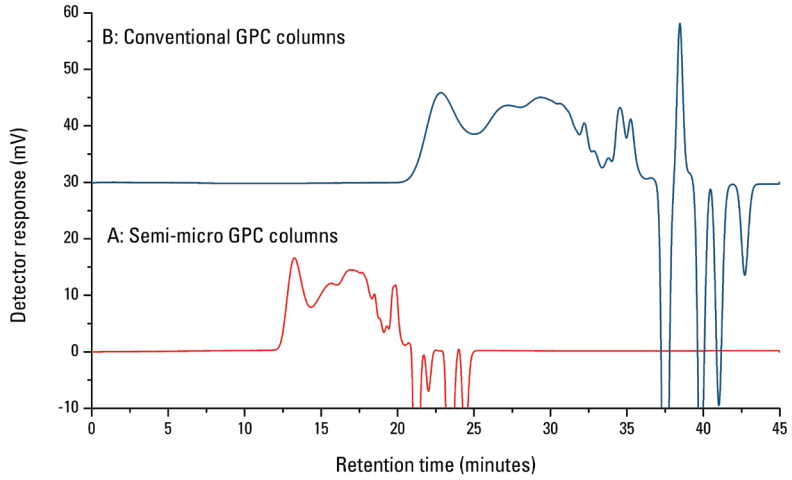


Figure 2. Elution profiles of a real-world polymer sample as monitored by RI on the EcoSEC GPC System with (A) semi-micro GPC columns and (B) conventional GPC columns.



The difference in resolution can be attributed to the fact that the separation range of the conventional GPC columns extends slightly further in the low molar mass region than the semi-micro GPC columns. The slight difference in resolution between the two column sets does not impact the polystyrene relative molar mass averages obtained for the sample. The weight-average molar mass for the sample was determined to be 1,060 and 1,040 g/mol for the semi-micro and conventional GPC columns, respectively, a difference of less than 2%. The greatest benefits of using the semi-micro GPC columns are the decrease in solvent consumption and analysis time. The use of semi-micro GPC columns instead of conventional GPC columns reduced the analysis time by approximately fifty percent without sacrificing resolution or affecting the polystyrene molar mass values. Both types of columns take advantage of the dual flow refractive index design in the EcoSEC GPC System and produced reliable and reproducible results.

Conclusions

The EcoSEC GPC System in conjunction with conventional and semi-micro GPC columns was shown to be comparable for the analysis of polymer standards and real-world samples. The features of the EcoSEC GPC System, low dead volume, temperature controlled pumps and a dual flow refractive index detector, were found to be independent of column length. The use of semi-micro columns was shown to enhance the low system dead volume and the dual flow refractive index detector baseline stability while increasing sample throughput and decreasing solvent consumption, i.e. solvent related cost. The chromatographic resolution obtained on the conventional and semi-micro GPC columns was found comparable to one another while the molar mass averages obtained using the two experimental set-ups were within experimental error. The combination of the low dead volume of the EcoSEC GPC System and the semi-micro GPC columns allowed for complete analysis in less than 30 minutes, whereas analysis using conventional columns and the EcoSEC GPC System requires analysis times close to 60 minutes. The ability to use conventional and semi-micro GPC columns on the same GPC system allows the user to pick which column length is more suitable for a given application whether it be due to already established experimental procedures or the need for new more environmentally and economically friendly methods.

References

[1]Ishii, D.; Hibi, K.; Assai, K.; Jonokuchi, T. *J. Chrom. A.*, **1978**, 151, 147-154.

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