TOSOH

GPC APPLICATION NOTEBOOK

AMBIENT / HIGH TEMPERATURE / SEMI-MICRO

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02 EDITORIAL DEAR READER

Gel permeation chromatography (GPC) is a type of size exclusion chromatography (SEC) that separates molecules according to their hydrodynamic volume which is related to their molecular weight. The separation is based strictly on the size of the sample in solution, and there should be no interaction with the stationary phase of the GPC column. Elution order in GPC is that of an “inverse-sieving” technique, large molecules access a smaller pore volume than smaller molecules resulting in larger molecules eluting from the GPC column prior to the smaller molecules.

GPC can determine several important parameters. These include number average molecular weight (Mn), weight average molecular weight (Mw), Z weight average molecular weight (Mz), and the most fundamental characteristic of a polymer, its molecular weight distribution. These parameters are important, since they affect many of the characteristic physical properties of a polymer. Differences in these parameters can cause significant differences in the end-use properties of a polymer.

To prepare a sample for GPC analysis the polymer is first dissolved in an appropriate solvent. Most often a bank of two to four GPC columns is used to achieve high resolution analysis of the polymer sample. Today, modern semi-micro GPC column technology helps to reduce analysis time without compromising the resolution and to reduce the consumption of solvent. The latter being a great environmental as well as economic advantage if you have to determine the molecular weight of polymers that dissolves only in expensive solvents.

GPC/SEC is usually carried out at room temperature, but some polymers such as high molecular weight polyolefins need high temperature for effective dissolution. Hence, GPC analysis of these polymers needs to be performed at higher temperature. This application book compiles various examples of ambient and high temperature GPC analysis for a broad range of polymers.

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IMPRESSUM

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AMBIENT EcoSEC GPC SYSTEM

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Application Note

IMPROVE REPRODUCIBILITY OF MW MEASUREMENTS OF POLYPEPTOIDS

ABSTRACT

The EcoSEC GPC System and a set of TSKgel mixed-bed columns, both available from Tosoh Bioscience, were used successfully for obtaining high quality MWD data of a series of block poly-b-alkylalanoids with HFIP as the mobile phase in under 15 minutes. The apparent MW averages based of PMMA calibration ranged from 27,000 to 49,000 for M_n and from 30,000 to 61,000 for M_w with an average polydispersity of 1.20. Because of excellent flow rate and temperature control and baseline stability of the EcoSEC GPC System, average MW values ranged from 0.2 to 0.3% relative standard deviation, a ten-fold reproducibility improvement as compared to SEC literature data of polyamides using a similar mobile phase system.

INTRODUCTION

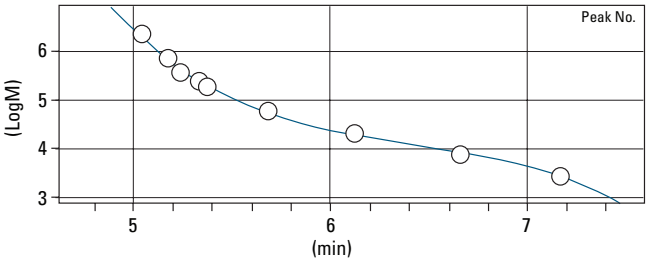
There is considerable attention placed on the synthesis and characterization of polypeptoids, a new class of synthetic polypeptide analogues. One of the groups most active in this area is Dr. Li Jia and co-workers at the University of Akron[1-4] who are investigating different synthetic routes for the formation of polypeptoids with alternating block structures. Peptoids are peptide isomers that differ in the connectivity of the side chain. These polyamides are similar to polypeptides; while the side chain of the amino acid residue in a peptide is attached to the alpha carbon, the side chain of a polypeptoid is attached to the nitrogen[5]. The resulting structure imparts proteolytic stability, may mimic polypeptide behavior, and could have novel polymer properties for commercial use, as they are close in structure to nylon-type polyamides.

Because of intense hydrogen-bonding among chains of these polymers, aggressive solvents must be used as the mobile phase to affect solubility. Furthermore, highly reproducible data is needed to obtain subtle molecular weight distribution trends. In view of this, an EcoSEC GPC System was used with TSKgel mixed-bed columns and hexafluoroisopropanol (HFIP) as the mobile phase. The EcoSEC GPC System is ideally suited for the detailed study of polymerizations owing to its high column resolution and superior instrument performance. The unprecedented reproducibility, accuracy and RI sensitivity of this System is due to its superior design: accurate temperature control, dual-compartment oven control, newly engineered pumping system, low RI dead volume, reference GPC column, and low injection volume.

This Application Note presents data obtained from a series of poly-b-alkylalanoids synthesized by Dr. Li Jia, which were obtained using living alternating copolymerization of N-alkylaziridines and carbon monoxide[5].

EXPERIMENTAL CONDITIONS

A series of block poly-b-alkylalanoids were provided by Dr. Li Jia[6] and SEC analysis was done at Tosoh's Tokyo Research Center in Japan. Polymers were dissolved as received in HFIP at a level of 0.5 mg/mL, and passed through a 0.5 µm membrane filter. The column set used was a series of two TSKgel GMH_{HR}-M (4.6 mm ID x 15 cm L) columns packed in HFIP. These mixed-bed columns have a separation range of about 10^2 to 4 x 10^6. The mobile phase consisted of HFIP containing 5 mmol/L sodium trifluoroacetate to help prevent sample adsorption.



Calibration Data (RI)

Time (min)	Molecular Weight	Error %	Weight	Mark	Data Name	Coefficient	
5.047	2,100,000	14.81379	1	STD	RSLT0001	A	-5.265665e-001
5.175	670,000	-0.65245	1	STD	RSLT0002	B	1.015145e+001
5.240	360,000	-20.96319	1	STD	RSLT0003	C	-6.581060e+001
5.328	245,000	-4.16016	1	STD	RSLT0001	D	1.475134e+002
5.375	185,000	-6.38593	1	STD	RSLT0002		
5.678	60,000	10.78541	1	STD	RSLT0003	Correlation	-0.967
6.122	20,850	11.23301	1	STD	RSLT0001		
6.663	7,500	-14.59096	1	STD	RSLT0002		
7.170	1,825	4.11967	1	STD	RSLT0003		

Figure 1

PMMA calibration curve with two TSKgel GMH_{HR}-M (4.6 mm ID x 15 cm L) columns at a flow rate of 0.35 mL/min using HFIP as the mobile phase.

Sample[1]	Mn[1]		Mw[2]		PD[2]	
		Rel std dev		Rel std dev		Rel std dev
$A_{10}B_{40}$	26,500±10	0.04%	30,300±30	0.11%	1.14±0.001	0.09%
$A_{60}B_{20}$	33,300±170	0.52%	40,700±28	0.07%	1.22±0.006	0.50%
$A_{40}B_{40}$	48,700±220	0.45%	60,900±160	0.26%	1.25±0.001	0.10%
C_{40}	30,100±50	0.18%	36,400±140	0.37%	1.21±0.005	0.39%

1. Block lengths were determined by Dr. Jia from independent measurements. Chemical composition of blocks A, B and C will be published by L. Jia.
2. Apparent MW data were obtained from a PMMA calibration curve. Apparent MW averages given in the table are apparent averages of three sequential injections per sample. Based on block lengths, MWD are significantly overestimated.

Table 1

Apparent SEC data were obtained using a PMMA calibration curve and an EcoSEC GPC System and two TSKgel mixed-bed columns (4.6 mm ID x 15 cm L) with HFIP containing 5 mmol/L NaTFA as the mobile phase. Flow rate was 0.35 mL/min. Injection volume was 10 µL of 0.5 mg/mL sample dissolved in HFIP. An RI detector set to 40°C was used. Samples were submitted by Dr. L. Jia (University of Akron).

An EcoSEC GPC System was used at a flow rate of 0.35 mL/min at a column and system temperature of 40°C. Injection volume was 10 µL. A refractive index monitor was used for detection.

Calibration was based on a series of nine poly(methyl methacrylate) (PMMA) standards (American Polymer Standard) ranging in molecular weight from 2,820 to 2,100,000. A cubic fit was used for the calibration curve (correlation -0.967) (Fig. 1).

RESULTS AND DISCUSSION

Samples of block poly-b-alkylalanoids were chosen that encompassed a wide selection of different block lengths and compositions. SEC chromatograms of four representative samples are shown in Figures 2 to 5 and molecular weights summarized in Table 1.
(Chemical compositions of the blocks will be published in a forthcoming publication by L. Jia.)

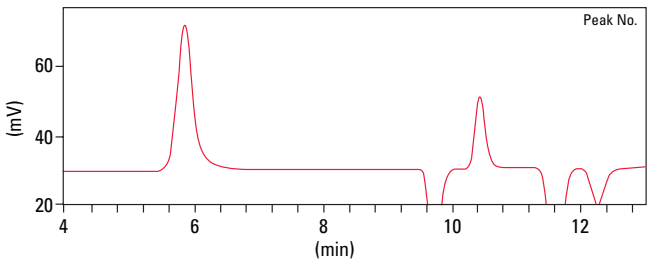


Figure 2

PMMA calibration curve with two TSKgel GMH_{HR}-M (4.6 mm ID x 15 cm L) columns at a flow rate of 0.35 mL/min using HFIP as the mobile phase.

As indicated, all samples had narrow polydispersity values of 1.2, as expected from living polymerization reactions. Since PMMA was used for calibration, which has a different composition than the blocks, reported molecular weight (MW) data are relative or apparent MW, and should only be used for comparisons among samples. In fact, these values are significantly higherthan calculated MW of samples computed from experimentally determined block lengths and block composition. Overestimation of MW implies that these blocks have highly extended conformations in HFIP as compared to PMMA in the mobile phase. In other words, these polypeptoids form strong hydrogen-bonds with HFIP. However, to obtain more accurate MW data, standards that are compositionally similar to polypeptoids, such as polyamide standards, e.g., nylon 66, should be used. Alternatively, an online viscometer with PMMA universal calibration or a light scattering detector should be used.

For this column set, the exclusion limit is about 1.75 mL (5 min), while the total permeation volume is close to 3.5 mL (10 min). As indicated by the chromatograms, except for C_{40} (Fig. 5), these samples contain almost symmetrical, narrow polymer profiles eluting in the range of about 6 min, while residual solvents, water and dissolved air elute in the 10 to 12 min range. There is very little tailing, and in all cases, the peak tail returns to the initial baseline with no baseline drift. This feature allows for highly accurate data not available with conventional GPC systems. (The shoulder seen in C_{40} (Fig. 5) is indicative of another population of a high MW polymer component in the sample.)

The MW data in Table 1 are averaged values from three consecutive injections; the percent relative standard deviations of each set of three injections are given in Table 2, along with grand averages. The average percent standard deviations range from about 0.04 to 0.5%, with grand average of 0.3% for M_n and 0.2% for M_w. These percent standard deviations are more than 10 x lower than the values reported for polyamides in HFIP mobile phase[7]. Lastly, the percent relative standard deviation of polydispersities (PD) ranges from 0.1 to 0.5%, which permits one to report PDs within three significant figures. The high accuracy allows for the detailed study of polymerization reactions.

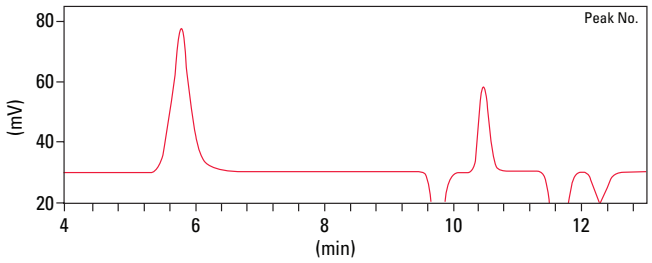


Figure 3

GPC chromatogram of $(A)_{60}(B)_{20}$ block poly-b-alkylalanoid using EcoSEC GPC System with two TSKgel GMH_{HR}-M (4.6 mm ID x 15 cm L) columns at a flow rate of 0.35 mL/min using HFIP as the mobile phase.

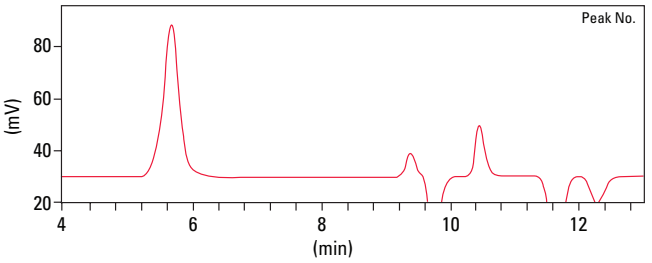


Figure 4

GPC chromatogram of $(B)_{40}(C)_{40}$ block poly-b-alkylalanoid using EcoSEC GPC System with two TSKgel GMH_{HR}-M (4.6 mm ID x 15 cm L) columns at a flow rate of 0.35 mL/min using HFIP as the mobile phase.

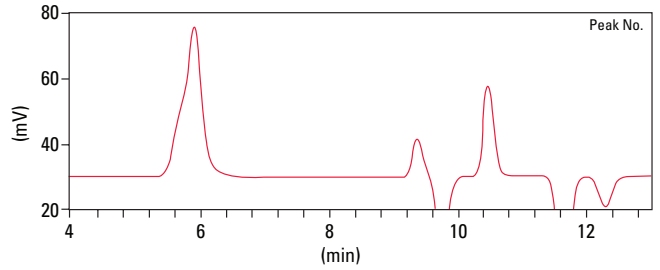


Figure 5

GPC chromatogram of $(C)_{40}$ block poly-b-alkylalanoid using EcoSEC GPC System with two TSKgel GMH_{HR}-M (4.6 mm ID x 15 cm L) columns at a flow rate of 0.35 mL/min using HFIP as the mobile phase.

CONCLUSIONS

These apparent MW data demonstrate that an HFIP mobile phase can be reliably used with the EcoSEC GPC System for determining the MWD of polypeptoids, specifically block poly-b-alkylalanoids. In addition, these results demonstrate that the EcoSEC GPC System has unprecedented baseline and flow rate stability, as compared to using conventional HPLC systems for GPC analysis. Even with using HFIP, a toxic, strong solvent capable of dissolving polypeptoids, the pooled percent relative standard deviation of number- and weight-average MWs of a set of block poly-b-alkylalanoids, was 0.2 to 0.3%, surpassing the reproducibility using conventional HPLC systems used for SEC. This method is suitable for the study and characterization of these interesting polypeptide analogues and for the optimization of polymerization conditions for block poly-b-alkylalanoids.

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TOSOH

Application Note

ANALYSIS OF ASPHALT BINDERS USING GEL PERMEATION CHROMATOGRAPHY

INTRODUCTION

Asphalt is a mixture of a wide variety of chemical compounds that include aliphatic hydrocarbons and highly fused aromatic ring systems. The components of asphalts are classified into two categories based on their molar mass. Medium molar mass asphalt components are classified as asphaltenes and low molar mass components are classified as maltenes. To improve the final properties of asphalts a high molar mass polymer known as the asphalt binder can also be added to the mixture of asphaltenes and maltenes, resulting in the formation of polymer-modified asphalt cements (PMACs). PMACs can be made from polystyrene-b-polybutadiene-b-polystyrene (SBS) and polystyrene-polybutadiene rubber (SBR). The addition of these types of polymers results in polymer-modified asphalt binders that are regarded as a true solution in which the polymer is homogeneously blended with the components of the base asphalt cement.

Polymer-modified asphalt binders are of great importance as unmodified, or neat, asphalt is sensitive to high and low temperatures when used in roads. Thus, during the summer, the asphalt softens, while during winter it becomes more rigid and tends to crack. These changes cause rutting and deformation of the asphalt hindering its ability to perform as it was designed. The addition of the high molar mass polymers to the asphalts ensures good performance as they make the asphalt more elastic over a larger temperature domain. Asphalts, just like other materials containing polymeric materials, must be characterized to determine their end-use properties or environments. One method that is ideal for the characterization of the three species of PMACs: asphaltenes, maltenes and polymer, is gel permeation chromatography (GPC).

Typical polymer asphalt GPC chromatograms have a clear separation of polymer from asphalt in the elution sequence being that the polymer has a higher molar mass than that of the other asphalt components. This difference in molar mass obtained via GPC also makes it easy to quantify the amount of polymer in a blend of polymer and asphalt or in PMAC materials. Here, we have utilized the EcoSEC GPC System to achieve three different objectives: (1) to separate the three species of PMACs, asphaltenes, maltenes and polymer molecules from one another and to characterize the molar mass averages based on polystyrene standards, (2) to compare the molar mass distributions and GPC elution profile PMACs created from polymer molecules from different suppliers, and (3) to analyze a large number of asphalts and correlating relative concentration values with binder properties to give a better understanding in the selection/modification of asphalt with better performance.

EXPERIMENTAL

Sample analysis was performed on a system consisting of an EcoSEC GPC System equipped with a RI detector. Samples were filtered through a 0.45 µm PTFE filter and injected onto a column bank consisting of a 6.0 mm ID × 15 cm, 3 µm TSKgel® SuperHZ4000 (exclusion limit 4 × 10^5 g/mol, PN 0019312), two 6.0 mm ID x 15 cm, 3 µm particle size TSKgel SuperHZ3000 (exclusion limit 6 × 10^4 g/mol, PN 0019311) and a 6.0 mm ID x 15 cm, 3 µm TSKgel SuperHZ2500 (exclusion limit 2 × 10^4 g/mol, PN 0019304). The mobile phase and eluent was THF stabilized with butylated hydroxytoluene (BHT) as a peroxide inhibitor at a flow rate of 0.35 mL/min. Detector, pump oven, and column oven were maintained at 40 °C.

For all chromatographic determinations, results are averages of two injections. The molar mass and molar mass distribution of the asphalt samples was determined based on a polystyrene calibration curve. The calibration curve was created for the RI at 40 °C using PSt Quick Kits B, E, and F, ranging in molar mass from 266 to 5.5 × 10^6 g/mol. Calibration curve data for 0.35 mL/min was fitted with a cubic function and error values were less than 5%.

RESULTS AND DISCUSSION

The EcoSEC GPC System with a dual flow refractive index detector was used for analysis of asphalts.

The separation of PMACs by GPC results in three distinctive component peaks. As seen in Figure 1, baseline resolution is obtained between the polymer molecules and the asphaltene and maltene components of the PMACs. The molar mass distributions as obtained by peak position calibration using polystyrene standards results in very distinctive molar mass ranges for each of the PMAC components, i.e., polymer molecule, asphaltenes and maltenes. The molar mass of the polymer molecule ranges from 1.9 × 10^4 to 5.5 × 10^6 g/mol, while the molar mass of the asphaltenes and maltenes ranges from 3.0 × 10^3 to 1.9 × 10^4 g/mol and 200 to 3.0 × 10^3 g/mol, respectively.

The final properties of PMACs can vary based on the chemistry and architecture of the polymer molecule added as well as the source of the polymers. Three different sources provided samples of SBS to use as the polymer modifier in PMACs. Figure 2 shows that the SBS from the three

different sources vary in polymeric size. Furthermore the SBS sample from Company B results in a single GPC peak while the SBS samples from Company A and Company F are bimodal. The molar mass of the SBS from Company A is twice as large in molar mass as that from Company B and Company F. The SBS polymer modifier varies between suppliers and thus could result in PMACs with different end-use properties.

The ability to reverse engineer asphalts is important thus a method to determine the amount of polymer in a PMAC is essential. To estimate the accuracy and feasibility of using GPC for the analysis of the percentage of polymer in an asphalt mixture compared to the asphaltenes and maltenes, different known amounts of SBS polymer from Company A were mixed with asphaltenes and maltenes and then analyzed via GPC to prove that the chromatogram accurately reflects the change in concentration.

GPC ELUTION PROFILE AND MOLAR MASS AVERAGES OF SBS POLYMER FROM THREE DIFFERENT SUPPLIERS

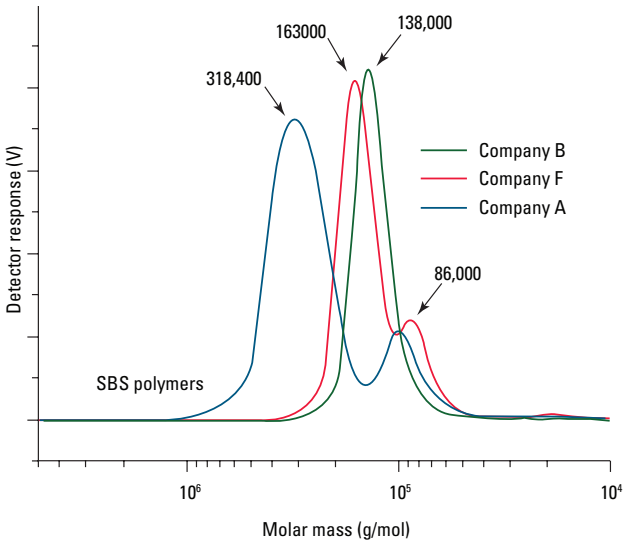

Figure 2

GPC ELUTION PROFILE AND MOLAR MASS AVERAGES OF PMAC COMPONENTS

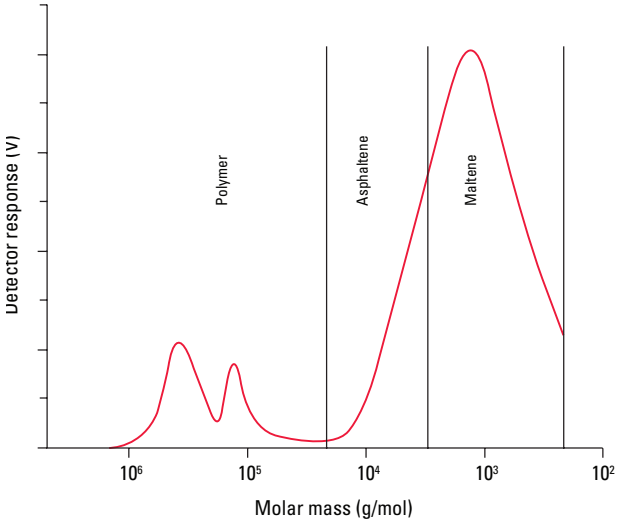

Figure 1

GPC ELUTION PROFILE AND MOLAR MASS AVERAGES OF ASPHALTS WITH DIFFERENT CONCENTRATIONS OF SBS POLYMER FROM COMPANY A

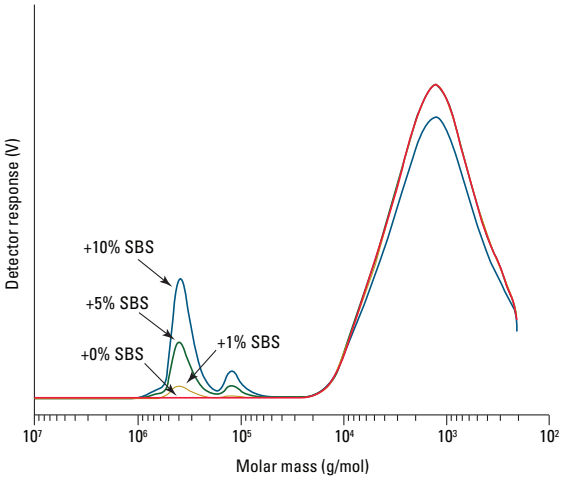

Figure 3

RELATIVE CONCENTRATIONS OF COMPONENTS IN SPIKED SBS ASPHALT MIXTURES

	1000-300K %	300-45K %	45-19K %	Total poymer (%)	Asphaltene (%)	Maltene (%)
ASPHALT	0	0	0.034	0.034	17.48	82.48
ASPHALT+ 1% SBS	0.72	0.18	0.09	0.99	17.24	81.77
ASPHALT+ 2% SBS	1.46	0.43	0.13	2.03	17.17	80.80
ASPHALT+ 5% SBS	3.48	1.08	0.20	4.76	16.17	78.47
ASPHALT+ 10% SBS	7.77	2.46	0.28	10.51	15.87	73.61

Table 1

The GPC chromatogram for each amount of SBS polymer can be seen in Figure 3 and the corresponding relative component concentrations is given in Table 1. The total percentage of SBS polymer calculated via GPC compares well to the amount of SBS polymer used in each sample.

CONCLUSIONS

The EcoSEC GPC System with a dual flow RI detector was successfully used to separate the three species of PMACs, asphaltenes, maltenes and polymer molecules from one another and to characterize the molar mass averages based on polystyrene standards. GPC analysis of asphalts results in a chromatogram with near base-line resolution between the polymer molecule and the asphaltenes and maltenes. The GPC elution profiles were used to compare SBS polymer from three different suppliers. The SBS from each supplier varied in elution profile, modality, and molar mass. Finally GPC was successfully used as a method for correlating relative concentration values of polymer molecule in asphalts. The use of GPC for the characterization of asphalts and PMACs proves to be a very versatile method.

TOSOH

Application Note

ANALYSIS OF POLYMERS FOR THERAPEUTICS USING THE EcoSEC® GPC SYSTEM

INTRODUCTION

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. Polymer therapeutics include rationally designed macromolecular drugs, polymer-drug and polymer-protein conjugates, polymeric micelles containing covalently bound drugs and polyplexes for DNA delivery.[1] Conceptually, polymer therapeutics share many of the same features as other macromolecular drugs, such as, proteins, antibodies, and oligonucleotides, with the added bonus of the versatility of the synthetic chemistry, which allows for the tailoring of the molar mass, addition of biomimetic features to the man-made therapeutic and even the possibility of including bioresponsive elements.

Increased synthesis control of polymer properties has permitted the production of polymer assemblies for targeted and controlled drug delivery. Polymeric materials have been deemed extremely useful for solving drug delivery problems as they are relatively large in molar mass compared to low molar mass drugs, and when combined with these drugs they can augment the drug's performance and change their bioavailability.[2] Furthermore, synthetic polymers are perfectly suited for producing formulations with biopolymers due their ability to self-assemble with these molecules.

The growing use of synthetic polymers in therapeutics increases the need for a method to characterize their molar mass averages and distributions as variations in molar mass averages and distributions can affect aspects of the therapeutic such as in vitro binding activity and biodegradation.[1] The molar mass averages and molar mass distributions of a polymer are commonly characterized using gel permeation chromatography (GPC). Here we report on the use of an all-in-one GPC system, the EcoSEC GPC System, for the analysis and differentiation of the molar mass averages and distributions of four block copolymers intended to be used in a polymer-based therapeutic.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on a system consisting of an EcoSEC GPC System equipped with a RI detector. Separation of unfiltered 10 µL injections occurred over a column bank consisting of a 4.6 mm ID × 15 cm, 3 µm TSKgel® SuperHZ4000 (exclusion limit 1 × 10^5 g/mol) (PN 0019313), a 4.6 mm ID × 15 cm, 3 µm particle size TSKgel SuperHZ3000 (exclusion limit 6 × 10^4 g/mol) (PN 0019312) and a 4.6 mm ID × 15 cm, 3 µm TSKgel SuperHZ2000 (exclusion limit 1 × 10^4 g/mol) (PN 0019310) preceeded by the appropriate guard column (PN 0019314). The mobile phase and solvent was tetrahydrofuran (THF) at a flow rate of 0.35 mL/min. Detector, pump oven, and column oven were maintained at 35°C.

Four block copolymers intended to be used in polymer-based drug or gene delivery system were analyzed: Block copolymer 1-4. Samples solutions were prepared by dissolving the samples in tetrahydrofuran through heating and stirring over twenty-four hours. Results are averages of four injections.

A calibration curve was created for the RI at 35°C using six polystyrene (PS) standards PS 1: 1,270 g/mol; PS 2: 3,180 g/mol; PS 3: 6,940 g/mol; PS 4: 2.2 × 10^4 g mol; PS 5: 5.2 × 10^4 g/mol and PS 6: 1.4 × 10^5 g/mol. All standards were prepared using the same heating and stirring procedure over a twenty-four hour period as the block copolymer samples. Calibration curve data for 0.35 mL/min was fitted with a cubic function and error values were less than 5%.

RESULTS AND DISCUSSION

The ability to characterize the molar mass averages and distributions of a polymer being used in therapeutics is critical as the molar mass averages and distributions can affect the biocompatibility, mechanical properties, and bioavaiability properties of the therapeutic. An EcoSEC GPC System encompassing a dual flow RI detector was used to perform GPC analysis on four block copolymer samples intended to be used in a polymer-based therapeutic that have the same chemical composition but different molar masses.

The molar mass averages, Mn, Mw, and Mz, as determined via a polystyrene RI calibration curve are given in Table 1. The molar mass averages increase gradually from block copolymer 1 to block copolymer 4. The difference in molar mass averages between the block copolymer with the lowest molar mass, block copolymer 1, and the block copolymer with the highest molar mass, block copolymer 4, is approximately 25% among the three molar mass averages. In general, the variation of the molar mass averages observed for the four block copolymers may be great enough to affect the role the polymer plays in the polymer-based therapeutic within the body. The molar mass of the polymer in a polymer-based therapeutic can influence the biodegradation of the synthetic polymer once within the body, thus resulting in the production of lower molar mass polymer that has different biological effects.

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF FOUR BLOCK COPOLYMERS

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI[a]
Block Copolymer 1	2.09×10^4 $\pm 0.01 \times 10^4$[b]	2.38×10^4 $\pm 0.01 \times 10^4$	2.70×10^4 $\pm 0.01 \times 10^4$	1.13 ± 0.01
Block 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
Block 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
Block Copolymer 1	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 = Mw/Mn* [b] *Standard deviations from three injections*

Table 1

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 1, thru the differential and cumulative molar mass distributions. The molar mass distributions of the four block copolymer vary in conjunction with the variations in the molar mass averages for the four block copolymers. However, the polydispersity index, PDI, for the four block copolymers remain virtually identical for the four block copolymers, PDI = 1.11-1.14. The consistency amongst the PDI values for the four block copolymers is an indication that any variation observed in the polymer-based therapeutic composed of these block copolymers is independent of the polydispersity of the polymer used in the therapeutic.

Information regarding the differences between the four block copolymers for use in a polymer-based therapeutic is also seen by comparing their GPC elution profiles, Figure 2. The shift in GPC retention time amongst the four block copolymers indicates a variation in polymeric size. Based on the GPC elution profiles of the four block copolymers it appears that block copolymer 1 is smallest in polymeric size and block copolymer 4 is the largest in polymeric size. Block copolymers 2 and 3 appear to be similar in polymeric size as the GPC elution profiles vary only slightly in their breadth and detector response, the latter being a direct function of sample concentration.

CUMULATIVE AND DIFFERENTIAL MOLAR MASS DISTRIBUTION OF FOUR BLOCK COPOLYMERS

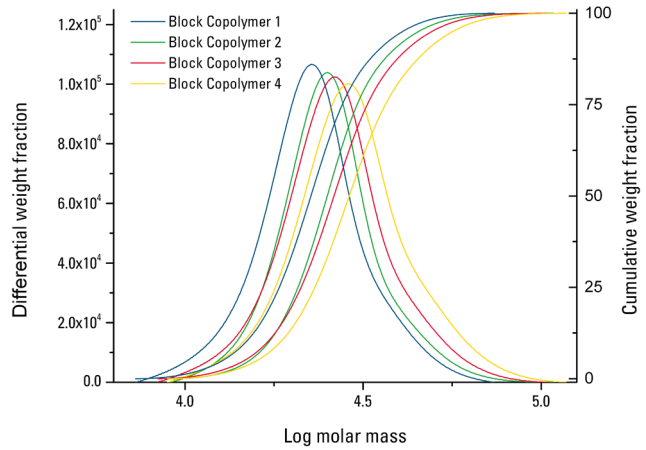

Figure 1

GPC ELUTION PROFILE OF FOUR BLOCK COPOLYMERS

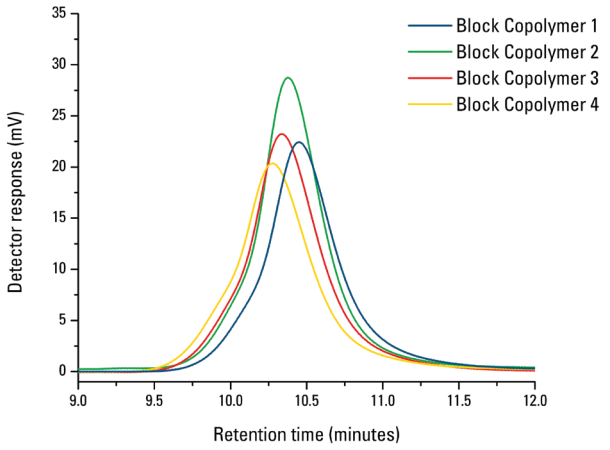

Figure 2

The difference in polymeric size observed between the four block copolymers based on the GPC elution profile has the possibility of dramatically affecting the behavior of the polymer-based therapeutic once within a biological system.

CONCLUSIONS

Four block copolymers intended to be used in polymer-based therapeutics were characterized based on the polystyrene relative molar mass averages and distributions as obtained by gel permeation chromatography using the EcoSEC GPC System. The polystyrene relative molar mass averages for the four block copolymers differed by no more than 25% between the highest and lowest molar mass block copolymers. The molar mass distributions of the four block copolymer varied in conjunction with the variations in the molar mass averages for the four block copolymers. Even though variations were observed in the molar mass averages and distributions, the molar mass polydispersity, PDI, amongst the four block copolymers remained constant. Additional differences in the four block copolymers were observed through the GPC elution profiles of the four block copolymers. The GPC elution profiles for the four block copolymers indicate noticeable variations in polymeric size amongst the copolymers. Overall, the use of the EcoSEC GPC System allowed for the determination of the molar mass averages and distributions as well as a comparison of relative polymeric size, based on GPC elution order, of four block copolymers.

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TOSOH

Application Note

AN APPROACH TO FAILURE ANALYSIS OF PC/ABS RESINS USED IN AUTOMOBILE PARTS: MOLAR MASS DETERMINATION VIA GPC

INTRODUCTION

For polymeric materials the molar mass and molar mass distribution play a vital role in the determination of mechanical, bulk, and solution properties. These properties govern polymer processing and the end-use performance of a given material.[1,2] Unlike small molecules, which have discrete molar mass distributions, synthetic polymers are typically composed of hundreds to thousands of chains of different molar mass that result in a distinctive molar mass distribution. The shape and breadth of a polymer's molar mass distribution will depend on the mechanism, kinetics and condition of the polymerization, and will dictate the end-use properties of the polymer. Polymer properties such as, hardness, tear strength, impact resistances, wear, brittleness, toughness, tackiness, etc., are important in determining the successes or failure of a given material.

One polymeric material of particular interest to the automotive industry is an alloyed grade thermoplastic: polycarbonate acrylonitrile-butadiene-styrene (PC/ABS). High-impact and heat-resistant grades of PC/ABS alloys are used in the instrument panels, armrests, interior trim panels, seatbelt retainers, glove compartment doors, and lift gates of automobiles, while plating grades of PC/ABS are used in wheel covers, grilles, headlight bezels, mirror housings, and decorative trim.[3] The numerous uses of PC/ABS in the automotive industry makes the characterization of the PC/ABS essential for the determination of whether an end-use material will be successful or not. The physical and chemical properties of PC/ABS resins are traditionally analyzed by techniques such as, infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), thermogravometric analysis (TGA), gel permeation chromatography (GPC), and melt flow.[4] Each of these techniques provides various details about the PC/ABS material being used to form the given automotive part, e.g. FT-IR, DSC and TGA all provide information about crosslinking, while GPC (depending on the detection methods implemented) can provide information regarding molar mass, molar mass distribution, and polymeric size.

The difference between a successful and unsuccessful polymer based material can be determined by observing the molar mass and molar mass distribution of the polymer(s) encompassing the end-use material. Here we have implemented the use of the EcoSEC® GPC System encompassing dual flow refractive index (RI) and UV detectors to perform failure analysis on two PC/ABS automobile parts. The use of GPC for the failure analysis allowed for determination of the molar mass averages, molar mass distributions, and a comparison of successful and unsuccessful PC/ABS automobile parts.

EXPERIMENTAL CONDITIONS

PC/ABS samples were prepared by dissolving shaved off portions of the sample in tetrahydrofuran (THF) (Fisher Chemical) for a final sample concentration of 1.0 g/L. Samples were shaken manually, allowed to sit overnight, and filtered using a 0.45 µm PTFE syringe filter (Acrodisc) before analysis was performed. Sample analysis was performed on a system consisting of an EcoSEC GPC System (HLC-8320) (Tosoh Bioscience) equipped with RI and UV detectors. The UV absorbance was monitored at a wavelength of 254 nm. Separation of filtered 10 µL injections occurred over a column bank consisting of two 4.6 mm ID × 15 cm, 4 µm particle size TSKgel® SuperMultiporeHZ-M column (exclusion limit 1,000,000 g/mol) proceeded by the appropriate guard column (Tosoh Bioscience). The mobile phase was THF at a flow rate of 0.35 mL/min. Detector, pump oven, and column oven were maintained at 35 °C.

For all chromatographic determinations, results are averages of three injections from two separate sample dissolutions. Data was processed with the EcoSEC GPC Workstation software, version 1.08. The molar mass and molar mass distribution of the PC/ABS samples was determined based on a polystyrene relative calibration curve created from a PStQuick MP-M polystyrene mix standard (Tosoh Bioscience) ranging in molar mass from 530 to 780,000 g/mol under the same experimental conditions as sample analysis. Calibration curve data was fitted with a linear function and error values were less than 1%.

RESULTS AND DISCUSSION

As mentioned in the "Introduction and Experimental" sections, an EcoSEC GPC System encompassing dual flow refractive index and UV detectors was used for failure analysis of PC/ABS automobile parts. The molar mass averages of two samples, successful and unsuccessful PC/ABS, were determined via GPC. The successful product was shown to perform up to standards while the unsuccessful product failed at some point during production or usage. The dual-detector GPC experiments provide two forms of

comparison between the successful and unsuccessful PC/ABS automobile parts: GPC chromatograms and polystyrene relative molar mass averages and distributions. The chromatograms of the successful and unsuccessful PC/ABS as monitored by both the RI and UV detectors are shown in Figure 1. The successful PC/ABS sample elutes prior to the unsuccessful PC/ABS. The shorter retention time of the successful PC/ABS indicates that the successful PC/ABS sample is larger in polymeric size than the unsuccessful PC/ABS sample; as the elution order in GPC is that of an "inverse-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. Thus, the GPC chromatogram alone provides sufficient indication that the successful and unsuccessful PC/ABS samples are different from one another.

The results of the experiments, in the form of polystyrene relative molar mass averages, are given in Table 1. The successful PC/ABS sample was determined to have a significantly higher number-, weight-, and z-average molar mass than the unsuccessful PC/ABS sample. The number-average molar mass, Mn, varies the greatest between the two samples, as Mn of the successful product is nearly twice that of the unsuccessful product. The difference in the molar mass averages is an important characteristic of any product as the molar mass averages dictates end-use properties such as tensile strength, elongation, brittleness, hardness, toughness, etc. For PC/ABS specifically, the molar mass averages directly influence the toughness and melt viscosity of the end-use material. Higher molar mass PC/ABS is tougher than their lower molar mass counterparts; thus, explaining one reason why the unsuccessful PC/ABS failed in the end-use material; the lower the molar mass, the weaker the end-use material. The difference in the molar mass averages between the successful and unsuccessful PC/ABS samples can also be observed in the form of molar mass distributions, MMD.

The MMDs of both the successful and unsuccessful PC/ABS samples, as obtained by GPC/RI, are shown in Figure 2. The successful PC/ABS sample extends significantly further in the high molar mass direction than the unsuccessful PC/ABS sample, while the unsuccessful PC/ABS contains a considerably higher quantity of low molar mass species than the successful PC/ABS sample. The molar mass averages and distributions between the two PC/ABS samples differ enough to result in a successful and an unsuccessful end-use product.

GPC ELUTION PROFILE OF SUCCESSFUL AND UNSUCCESSFUL PC/ABS AUTOMOBILE PARTS AS MONITORED BY RI (RED) AND UV (BLUE)

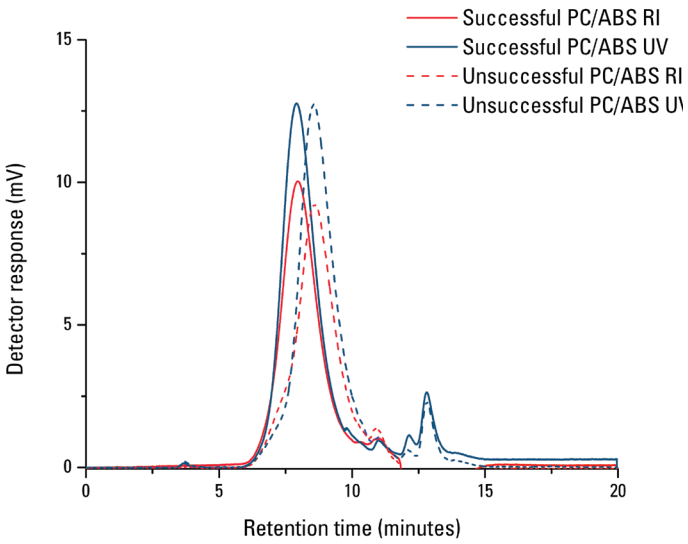

Figure 1

OVERLAY OF CUMULATIVE AND DIFFERENTIAL MOLAR MASS DISTRIBUTIONS OF SUCCESSFUL AND UNSUCCESSFUL PC/ABS AUTOMOBILE PARTS

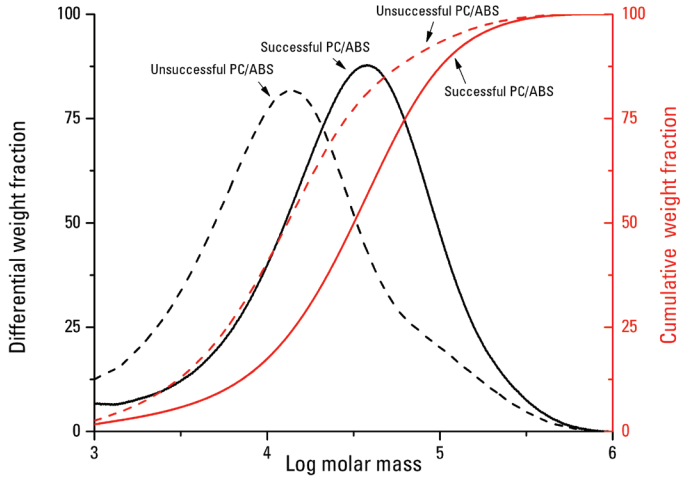

Figure 2

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF SUCCESSFUL AND UNSUCCESSFUL PC/ABS AUTOMOBILE PARTS

Sample (Detection Method)	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI[a]
Successful PC/ABS (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
Successful PC/ABS (UV)	$1.123 \times 10^4 \pm 333$	$4.367 \times 10^4 \pm 402$	$1.063 \times 10^5 \pm 1{,}698$	3.89 ± 0.09
Unsuccessful (RI)	$6{,}064 \pm 35$	$3.036 \times 10^4 \pm 260$	$1.259 \times 10^5 \pm 1{,}465$	5.01 ± 0.02
Unsuccessful (UV)	$5{,}364 \pm 38$	$2.161 \times 10^4 \pm 120$	$9.635 \times 10^4 \pm 1{,}154$	4.03 ± 0.02

[a] PDI = M_w/M_n; [b] Standard deviations from six injections

Table 1

CONCLUSIONS

The EcoSEC GPC System with dual flow refractive index and UV detection was used to perform failure analysis of PC/ABS resins used in automobile parts. PC/ABS samples, successful and unsuccessful, were compared via GPC chromatograms and polystyrene relative molar mass averages and distributions. The successful PC/ABS sample was determined to have a higher molar mass and larger polymeric size, based on polystyrene relative molar mass averages and the GPC elution profile, than the unsuccessful PC/ABS sample. The use of GPC for failure analysis allowed for immediate differentiation between the successful and unsuccessful PC/ABS samples based on the GPC elution profile, which was then confirmed through observed differences in the polystyrene relative molar mass averages and molar mass distribution of the successful and unsuccessful PC/ABS samples.

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TOSOH

Application Note

ANALYSIS OF HYDROXYETHYLCELLULOSE IN PERSONAL CARE PRODUCTS

INTRODUCTION

Hydroxyethylcellulose (HEC), Figure 1, is a water soluble polymer derived from alkali cellulose and ethylene oxide or ethylene chlorohydrin by etherification. HEC is the most hydrophilic and widely used cellulose ether. This non-ionic soluble cellulose ether is useful for thickening, suspending, dispersing, emulsifying, film-forming and water-protecting as well as providing other protective colloidal properties. HEC is widely used for applications such as oil exploitation, coatings, building, medicine, food, textile, and papermaking. In the personal care chemical industry HEC is found in products such as toothpaste, soap, lotions, and cosmetics, as HEC acts as a thickener, dispersing agent, binder and stabilizer to increase the density, lubrication or mercerized appearances of products. For example, in personal care products such as shampoos, body washes, shower gels, and eye drops HEC has the ability to thicken solutions and reduce the amount of suds or foam they form, thus increasing the cleaning effect.[1]

STRUCTURE OF HYDROXYETHYLCELLULOSE

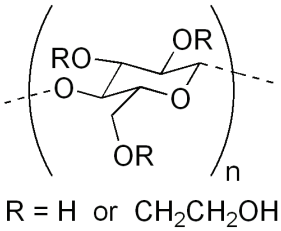

Figure 1

The ability to characterize a polymer in its end use environment is essential as the molar mass and size of a polymer can change depending on experimental conditions such as mobile phase. One powerful tool for the characterization of the molar mass of cellulose derived molecules is size exclusion chromatography (SEC).[1,2] Unlike cellulose itself, which is insoluble in water, HEC is water soluble thus can be characterized in an environment similar to that used in most personal care products. In general, the characterization of cellulose derived polymers by SEC can provide information regarding the molar mass distributions, and when coupled to various physical detection methods, can provide information regarding polymeric size and architecture. Here we report the use of the EcoSEC® GPC System with an internal dual flow RI detector and semi-micro SEC columns for the molar mass determination of pure HEC and HEC within two personal care products using conventional or peak-position calibration.

EXPERIMENTAL

Sample analysis was performed on a system consisting of an EcoSEC GPC System equipped with a RI detector. Separation of unfiltered 25 µL injections occurred over a column bank consisting of three 4.6 mm ID × 15 cm, 8 µm TSKgel® SuperMultiporePW-H columns (P/N 0022791 exclusion limit 1 × 10^7 g/mol) preceded by the appropriate guard column. The mobile phase and solvent was water with 0.1 mol/L $NaNO_3$ and 0.02% NaN_3 at a flow rate of 0.50 mL/min. Detector, pump, and column oven were maintained at 35 °C. Three samples were analyzed: hydroxyethylcellulose (HEC) and two personal care products with hydroxyethylcellulose.

For all chromatographic determinations, results are averages of three injections. The molar mass and molar mass distribution of the HEC samples was determined based on a polyethylene oxide and polyethylene glycol calibration curve. The calibration curve was created for the RI at 35 °C using polyethylene oxide and polyethylene glycol standards ranging in molar mass from 615 to 1.4 × 10^5 g/mol. All standards were prepared at a concentration of ~1 g/L. Calibration curve data for 0.50 mL/min was fitted with a cubic function and error values were less than 5%.

RESULTS AND DISCUSSION

Hydroxyethylcellulose is a cellulose derivative commonly used in personal care products such as toothpaste, shampoos, and eye drops that can be characterized based on molar mass by size exclusion chromatography. The chromatograms of the pure HEC and the HEC found within two personal care products, as monitored by the dual flow RI detector in the EcoSEC GPC System, are shown in Figure 2. The elution profile of the pure HEC displays the presence of one species while the two personal care products display two very different elution profiles. Personal care product 1 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. Personal care product 2 displays a single peak spanning a very similar elution window as does pure HEC as well as one of the two additional components observed in personal care product 1. The bimodal distribution in the HEC region of the chromatogram for personal care product 1 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 personal care product 1 and the one late eluting species in the chromatogram for personal care product 2 appear to be additional components that are significantly smaller in size than the main polymeric components of the personal care product,.

SEC ELUTION PROFILE OF PURE HYDROXYETHYLCELLULOSE AND HYDROXYETHYLCELLULOSE IN TWO PERSONAL CARE PRODUCTS

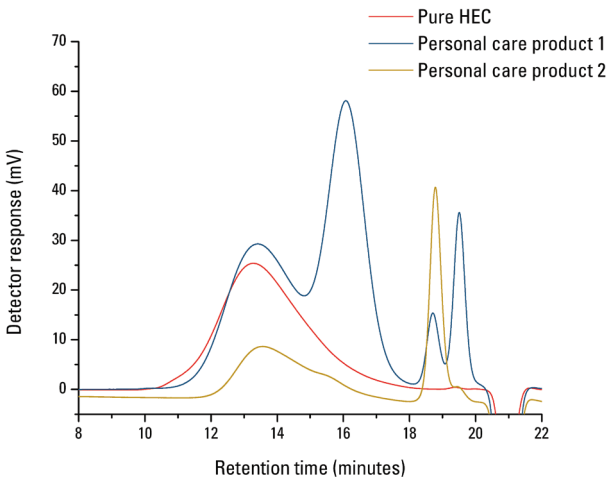

Figure 2

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF PURE HYDROXYETHYLCELLULOSE AND HYDROXYETHYLCELLULOSE IN TWO PERSONAL CARE PRODUCTS

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI[a]
Pure HEC	1.50×10^5 $\pm 0.04^b \times 10^5$	1.47×10^6 $\pm 0.01 \times 10^6$	5.93×10^6 $\pm 0.01 \times 10^6$	9.82 ±0.20
HEC in personal care product 1 (collectively)	4.67×10^4 $\pm 0.01 \times 10^4$	5.89×10^5 $\pm 0.02 \times 10^5$	2.78×10^6 $\pm 0.06 \times 10^6$	12.61 ±0.03
HEC in personal care product 1 (separately)	5.21×10^5 $\pm 0.06 \times 10^5$	1.12×10^6 $\pm 0.04 \times 10^6$	2.47×10^5 $\pm 0.16 \times 10^5$	2.29 ±0.01
	2.69×10^4 $\pm 0.07 \times 10^4$	4.32×10^4 $\pm 0.09 \times 10^4$	6.38×10^4 $\pm 0.01 \times 10^4$	1.61 ±0.23
HEC in personal care product 2	3.13×10^5 $\pm 0.01 \times 10^5$	1.69×10^6 $\pm 0.05 \times 10^6$	4.95×10^6 $\pm 0.01 \times 10^6$	5.43 ±0.23

[a] *PDI = M_w/M_n* [b] *Standard deviations from three injections*

Table 1

The polyethylene oxide and polyethylene glycol RI relative number-, weight-, and z-average molar mass values of the pure HEC and the HEC within a personal care product are given in Table 1. The molar mass averages for the HEC within the personal care product 1 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 detemined collectively and separately. The molar mass averages for the pure HEC are approximately 30-50% greater than that of the HEC found in the personal care product 1. The weight- and z-average molar mass values for personal care product 2 are relatively close to that of the pure HEC while the number-average molar mass was twice as large. The differences between the molar mass averages, not to mention SEC elution profile, between the two personal care products is an indication that the two personal care products will perform differently in their end use application.

The molar mass distribution of the pure HEC and the HEC region of the personal care products indicate a polydisperse polymer. For the pure HEC the polydispersity index value, PDI, is 9.82 and for personal care product 1 the PDI=12.64 (collectively) or PDI=2.27 and 1.59 (separately) and for personal care product 2 the PDI=5.43. Personal care product 1 has a slightly larger polydispersity than the pure HEC while personal care product 2 has a significantly smaller polydispersity than that of the pure HEC. From the SEC elution profile and molar mass averages and distributions of both products it appears that personal care product 2 is more similar to that of pure HEC than personal care product 1, but both products contain species with elution profiles and molar masses similar to HEC.

CONCLUSIONS

The EcoSEC GPC System with a dual flow RI detector was used to compare and contrast two personal care products containing hydroxyethylcellulose, a water soluble derivative of cellulose, based on SEC elution profile and polyethylene oxide and polyethylene glycol relative molar mass averages. The two personal care products have distinctive differences form one another and pure HEC. Personal care product 1 was bimodal with molar mass averages significantly less and a polydispersity slightly higher than that of pure HEC. Personal care product 2 had the same single SEC peak as that of HEC and weight- and z-molar mass averages relatively close to that of the pure HEC but a significantly higher number-average molar mass thus a much lower polydispersity index value.

The use of SEC/RI allowed for the characterization of a cellulose derived polymer, HEC, in an environment similar to its end use based on molar mass averages and distributions. The single detector SEC set-up used here could be coupled to various physical detection methods such as multi-angle or dynamic light scattering and differential viscometry to provide additional information regarding polymer size or architecture of the pure HEC and the HEC in personal care products.

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TOSOH

Application Note

ANALYSIS OF GRADIENT COPOLYMERS USING THE EcoSEC® GPC SYSTEM

INTRODUCTION

Gradient sequence copolymers are novel materials which have provoked interest due to their unique properties compared to their random, alternating and block equivalents. Unlike block copolymers which have an abrupt change in sequence, gradient sequence copolymers exhibit a gradual change in co-monomer composition from one type of monomer to another. An example of a gradient copolymer is poly(3-hexylthiophene-b-[1-hexane]), Figure 1, which is composed of poly(3-hexylthiophene) and poly(1-hexene).

Applications of these materials include making phase-separated polymer blends more compatible, impact dampeners and reinforcements[1]. π-conjugated gradient copolymers can affect the phase separation in polymer/polymer and polymer/fullerene blends. Therefore, the gradient copolymers must be characterized carefully and a need for a method to determine molar mass averages and molar mass distributions of these polymers is crucial.

A common way to characterize gradient copolymers is gel permeation chromatography (GPC). Here, we report the use of an all-inclusive GPC system, the EcoSEC GPC system for the analysis and differentiation of the molar mass averages and distributions of a gradient sequence π-conjugated block copolymer and its monomers.

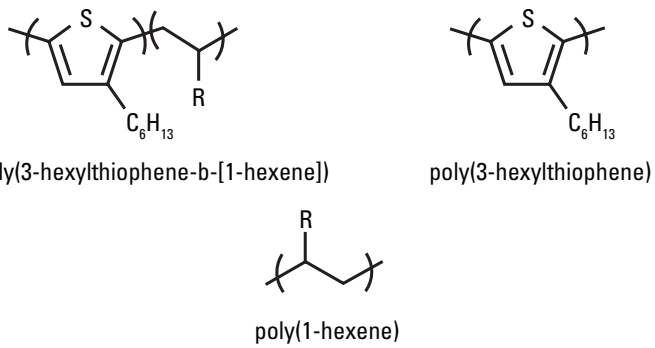

Figure 1

EXPERIMENTAL

Sample analysis was performed on a system consisting of an EcoSEC GPC System equipped with a dual flow RI detector and UV detector. The UV absorbance was monitored at 254 nm and 350 nm. Separation of unfiltered 10 µL injections occurred over a column bank consisting of two TSKgel® semi-micro SuperMultipore GPC columns and two TSKgel semi-micro mixed bead GPC columns. The mobile phase and solvent was THF (BDH) at a flow rate of 0.350 mL/min. Detector, pump oven, and column oven were maintained at 40 °C. For all chromatographic determinations, results are averages of three injections.

The molar mass and molar mass distributions of poly(3-hexylthiophene-b-[1-hexane]), poly(3-hexylthiophene) and poly(1-hexene) were determined based on a polystyrene calibration curve. The calibration was created for the RI and UV (254 nm) at 40 °C using polystyrene standards with a molar mass ranging from 500 to 7.0×10^5 g/mol. Calibration curve data at 0.35 mL/min was fitted with a cubic function for the SuperMultipore column set and a linear function for the mixed-bed columns. All error values were less than 5%.

RESULTS AND DISCUSSION

The ability to characterize the molar mass averages and distributions of a π-conjugated gradient copolymer is critical for designing polymer blends as molar mass averages and distributions affect the phase separation of polymer blends. An EcoSEC GPC System housing a dual flow refractive index detector was used to perform gel permeation chromatography analysis on poly(3-hexylthiophene-b-[1-hexane]), poly(3-hexylthiophene) and poly(1-hexene).

The GPC chromatograms of the copolymer, poly(3-hexylthiophene-b-[1-hexane]), and the two monomers, poly(3-hexylthiophene) and poly(1-hexene), as monitored by the dual flow RI detector and the UV detector, are shown in Figures 2-4, respectively. The copolymer, poly(3-hexylthiophene-b-[1-hexane]), displays a distinctive bimodal distribution while the two corresponding polymers have a mono-modal distribution.

As seen in Figures 2-4, by comparing the retention times of the RI response for the three samples, the later eluting species seen in Figure 2 has the same retention time as the poly(3-hexylthiophene), in Figure 3. The early eluting species seen in Figure 2 elutes earlier than that of poly(1-hexene) (Figure 4), an indication that the earlier elution species in Figure 2 is that of the copolymer. The copolymer elutes prior

to the corresponding polymers as it is larger in polymeric size than the polymers.

The number, weight, and z-average molar masses, M_n, M_w, and M_z, as determined using both RI and UV via a polystyrene calibration curve are shown in Tables 1 and 2, respectively. The identity of the two components of the bimodal distribution of the GPC elution profile of the copolymer, poly(3-hexylthiophene-b-[1-hexane]), is also supported in the comparison of the molar mass averages of copolymer to that of the two corresponding polymers. The molar mass of the later eluting species of the copolymer has molar mass averages similar to that of poly(3-hexylthiophene). The early eluting species of the copolymer has molar mass averages and a molar mass distribution greater than that of either of the pure polymers; an indication that through the synthesis a copolymer was made with a greater molar mass than that of the two corresponding pure polymers.

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF COPOLYMER AND CORRESPONDING POLYMERS AS DETERMINED BASED ON RI CALIBRATION.

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI (M_w/M_n)
poly(3-hexylthiophene-b-[1-hexane]	3.46×10^5 6.43×10^3	5.12×10^5 2.58×10^4	6.81×10^5 4.75×10^4	1.48 3.88
poly(3-hexylthiophene)	1.65×10^4	2.03×10^4	2.42×10^4	1.26
poly(1-hexene)	1.24×10^5	1.79×10^5	2.21×10^5	1.45

Table 1

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF COPOLYMER AND CORRESPONDING POLYMERS AS DETERMINED BASED ON UV CALIBRATION.

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI
poly(3-hexylthiophene-b-[1-hexane]	8.26×10^5 8.76×10^3	1.08×10^6 2.69×10^4	1.35×10^6 6.48×10^4	1.31 3.08
poly(3-hexylthiophene)	1.70×10^4	2.06×10^4	2.44×10^4	1.21

Table 2

ELUTION PROFILE OF POLY(3-HEXYLTHIOPHENE-B-[1-HEXANE], AS MONITORED BY RI (BLUE) AND UV (RED).

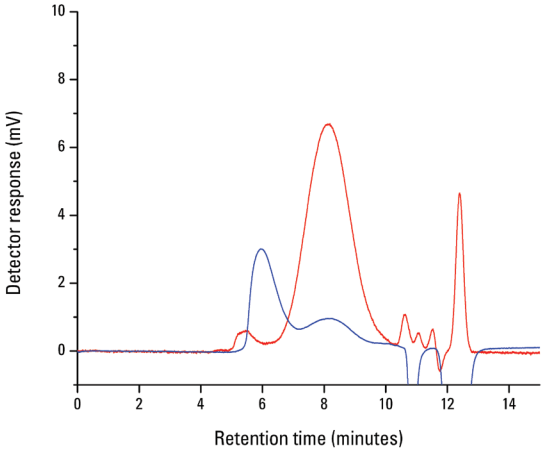

Figure 2

ELUTION PROFILE OF POLY(3-HEXYLTHIOPHENE), AS MONITORED BY THE RI (BLUE) AND UV (RED).

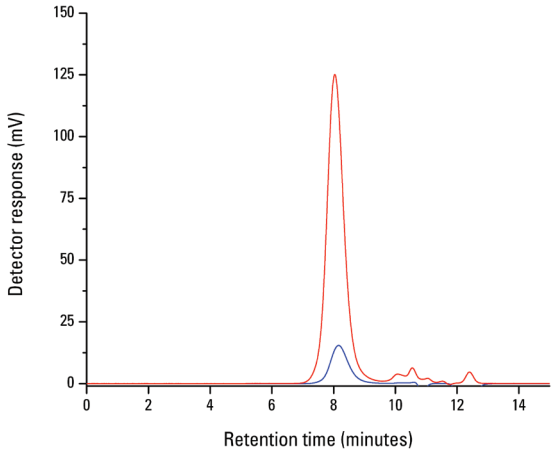

Figure 3

ELUTION PROFILE OF POLY(1-HEXENE), AS MONITORED BY THE RI (RED).

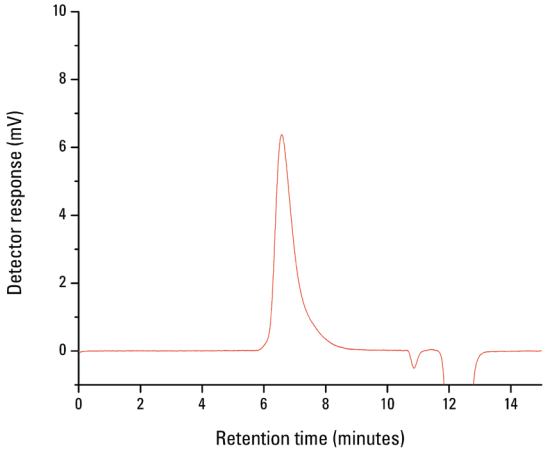

Figure 4

CONCLUSIONS

A copolymer intended to be used in polymer/polymer and polymer/fullerene blends was characterized based on the polystyrene relative molar mass averages and distributions as obtained by gel permeation chromatography using the EcoSEC GPC System with both an RI and UV detector and semi-micro TSKgel GPC columns. Through the comparison of the GPC elution profiles and the molar mass averages of the copolymer, poly(3-hexylthiophene-b-[1-hexane]), and the two polymers, poly(3-hexylthiophene) and poly(1-hexene) it can be concluded that the copolymer sample, poly(3-hexylthiophene-b-[1-hexane]), contains copolymer and excess amounts of poly(3-hexylthiophene). The GPC elution profiles and molar mass averages of the samples were obtained in less than fifteen minutes, thus providing a fast and reliable method for the analysis of copolymers.

TOSOH

Application Note

ANALYSIS OF HYALURONIC ACID USING THE EcoSEC® GPC SYSTEM

INTRODUCTION

Hyaluronic acid or hyaluronan is a naturally occurring linear polysaccharide composed of alternating repeating D-glucuronic acid and D-N-acetylglucosamine units. Hyaluronic acid contains between 500 and 50,000 monosaccharide units per molecule, thus has a molar mass that can range from 10^4 to 10^7 g/mol with a polydispersity index (PDI) >1.3.[1-3] This polysaccharide is widely distributed in mammalian cells and tissue but is primarily found in synovial fluid and loose connective tissues such as the umbilical cord, dermis and arterial walls. The roles which hyaluronic acid plays within the body and its unique physicochemical properties, e.g. viscoelastic properties and water retention capacity, have led to interest in the characterization of the polysaccharide, as hyaluronic acid has potential applications in drug delivery, as a surgical aid for ophthalmology and potential for treatment of arthritis.[1]

There are two forms of hyaluronic acid, linear and crosslinked, 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. Linear non-crosslinked hyaluronic acid is typically employed in ophthalmic device materials while crosslinked hyaluronic acid is employed in structure implants.[2] Along with the degree of cross-linking, the molar mass of hyaluronic acid plays a role in its elastic characteristics, as the higher the molar mass the higher the viscosity of the solution.

The unique physicochemical properties of hyaluronic acid are governed greatly by molar mass and molar mass distribution, thus a reliable method needed for determining the molar mass is important. Traditionally the molar mass of hyaluronic acid has been determined using peak-position calibration size exclusion chromatography (SEC) with a refractive index detector (RI) or UV detector based on the standards such as pullulan or dextran. Another way of determining molar mass, which is more absolute (independent of the chemistry or architecture of the samples and standards), is universal calibration. Here we have used a dual detector SEC set-up encompassing the dual flow RI in the EcoSEC GPC System and a ViscoStar® differential viscometer 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 SEC set-up allows for the determination of other physicochemical properties such as polymeric size, confirmation, and intrinsic viscosity.

EXPERIMENTAL CONDITIONS

Dual detector SEC analysis was performed using the EcoSEC GPC System equipped with a refractive index detector (RI) coupled in series to a ViscoStar Viscometer (Wyatt Technology Corporation). Separation of unfiltered 250 µL injections occurred over a column bank consisting of two 7.8 mm ID × 30 cm, 13 µm TSKgel® GMPWXL columns (separation range ~1,000 to 5 × 10^6 g/mol , PN 0008025). The mobile phase and solvent were water with 0.1 mol/L $NaNO_3$ and 0.02% NaN_3 at a flow rate of 1.0 mL/min. Detector, pump oven, and column oven were maintained at 35°C. Data was processed using Wyatt's ASTRA 6.1 software. Two hyaluronic acid samples, crosslinked and non-crosslinked, were prepared with a final concentration of 0.20 g/L. All chromatographic determinations are averages of three injections from one sample dissolution.

Molar mass averages obtained from dual detector SEC experiments were determined based on an universal calibration curve created using eight pullulan standards (Polymer Standard Service) ranging in molar mass from 5.0 × 10^3 to 7.1 × 10^5 g/mol under the same experimental conditions as sample analysis. The universal calibration curve data was fitted with a linear function and had a R^2 value of 0.999. For the dual detector SEC experiments the interdetector delays were calculated using a virtually monodisperse pullulan

SEC ELUTION PROFILE OF NON-CROSSLINKED (RED) AND CROSSLINKED (BLUE) HYALURONIC ACID AS MONITORED BY THE RI (SOLID) AND VISC (DASH)

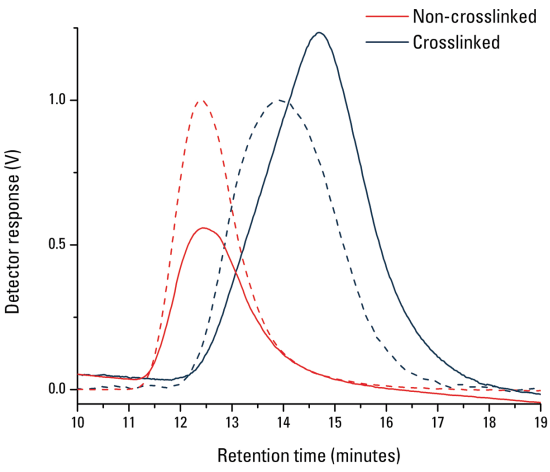

Figure 1

standard with a molar mass of 4.6 × 10^4 g/mol (Polymer Standard Service).

RESULTS AND DISCUSSION

As described above, an EcoSEC GPC System equipped with an internal dual flow refractive index (RI) detector was coupled to a differential viscometer (VISC) to characterize crosslinked and non-crosslinked hyaluronic acid samples. The SEC elution profile, as monitored by the RI and VISC, is shown in Figure 1. The SEC 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 in the GPC elution 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.

MOLAR MASS

The molar mass averages and polydispersity index of the hyaluronic acid samples were determined via universal calibration via dual detector SEC and are given in Table 1. The molar mass averages for the non-crosslinked hyaluronic acid sample were about an order of magnitude larger than that of the crosslinked hyaluronic acid sample. The molar mass distribution of the non-crosslinked hyaluronic acid sample extends further in the high molar mass direction, Mz, while the molar mass distribution of the crosslinked hyaluronic acid sample extends further in the low molar mass direction, Mn, Figure 2. For a majority of the molar mass distribution, the two hyaluronic acid samples have the same molar mass. The molar mass polydispersity was > 1, an indication that hyaluronic acid is polydisperse in molar mass no matter the degree of crosslinking.

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF HYALURONIC ACID SAMPLES VIA SEC/RI/VISC.

Sample	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	PDI
Non-crosslinked	3.18 x 10^5	8.58 x 10^5	1.50 × 10^6	2.727
Crosslinked	7.22 × 10^4	2.14 × 10^5	4.96 × 10^5	2.977

Table 1

STRUCTURE

The dual detector SEC experimental set-up employed here also allows for the determination of several parameters that can provide structural comparisons between the two hyaluronic acid samples, e.g. intrinsic viscosity, viscometric radius, conformation plots, and Mark-Houwink plots. The intrinsic viscosity of a sample is defined as the amount a dissolved molecule contributes to the overall viscosity of the solute and can be thought of as an inverse density. The intrinsic viscosity is an indication of how extended a polymer is in solution, the larger the value of the intrinsic viscosity the more extended or the less dense a polymer is in solution. As expected, the intrinsic viscosity of the hyaluronic acid identified as being non-crosslinked is significantly (almost an order of magnitude) larger, 3,000 g/mL, than the intrinsic viscosity of the hyaluronic acid sample identified as being crosslinked, 500 mL/g.

GPC ELUTION PROFILE OF NON-CROSSLINKED (RED) AND CROSSLINKED (BLUE) HYALURONIC ACID AND MOLAR MASS DISTRIBUTION ACROSS THE ELUTION PROFILES

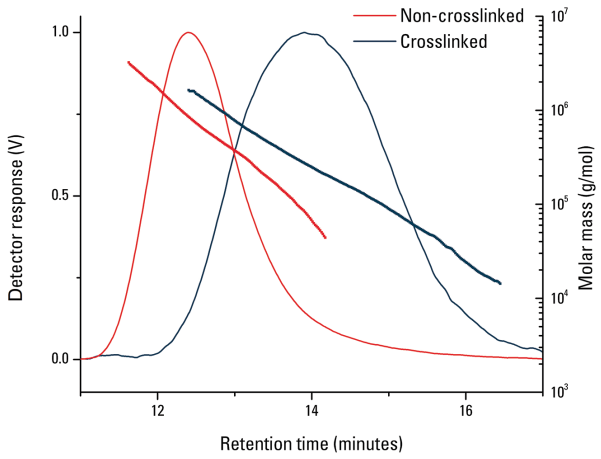

Figure 2

The viscometric radius of a sample scales proportional to the intrinsic viscosity, as the viscometric radius, R is the radius of a solid sphere that increase the viscosity of the fluid by the same amount as does a macromolecule. The viscometric radius of the non-crosslinked hyaluronic acid sample is almost three times that of the crosslinked hyaluronic acid, 68 and 25 nm, respectively. The distinct differences between the intrinsic viscosity and viscometric radius for the two hyaluronic acid samples confirm that the two samples vary in the degree of crosslinking. The structural differences between the two hyaluronic acid samples can also be seen through the 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.

CONCLUSIONS

Two hyaluronic acid samples were analyzed using a dual detector SEC set-up encompassing the dual flow refractive index detector in the EcoSEC GPC System and a ViscoStar differential viscometer. The molar mass of the two hyaluronic acid samples were determined using universal calibration. The two hyaluronic acid samples were determined to

CONFORMATION PLOT OF NON-CROSSLINKED (RED) AND CROSSLINKED (BLUE) HYALURONIC ACID

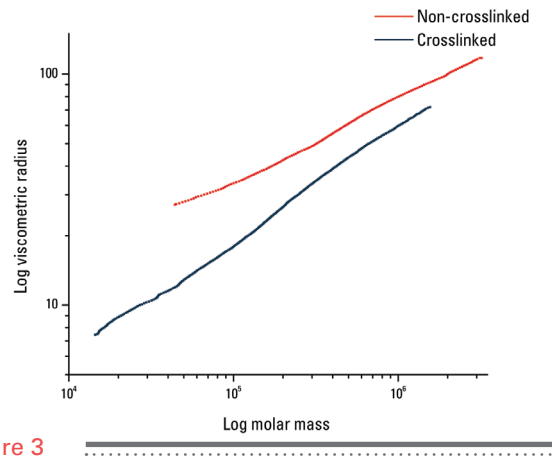

Figure 3

be similar in molar mass but different in structure. The addition of the differential viscometer to the EcoSEC GPC System allowed for the structural differences of the two hyaluronic acid samples to be determined as a function of molar mass. 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. The use of an SEC/RI/VISC experimental set-up permits for the determination of molar mass averages based on universal calibration, a method shown to be independent of the chemistry and architecture of a polymer, and physicochemical properties such as polymeric size, confirmation, and intrinsic viscosity.

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2. Waters, J.; Leiske, D. LCGC, 2005, 23, 302-310.
3. Vorvolakos, K.; Isayeva, I.; Do Luu, H-M,; Patwardhan, D.V.; Pollack, S.K., Med. Devices, 2010, 2011, 1-10.

TOSOH

Application Note

MONITORING SINGLE-CHAIN NANOPARTICLE FORMATION AND CHARACTERIZATION OF NANOPARTICLES

INTRODUCTION

Single-chain polymer nanoparticles (SCNPs) are well-defined linear polymers folded into nanostructures of defined architecture which can be used for tasks such as catalysis, sensors and nanomedicine. The general scheme to forming SCNPs is to take a linear polymer as a random coil with functionalities that allow cross-linking, and induce intra-chain, cross-linking to form a folded particle, as shown in the cartoon in Figure 1. The polymer can either require an external cross-linker or the cross-linking agent can be included in the polymer backbone as pendant functional groups. In order to ensure intramolecular cross-linking over intermolecular cross-linking, the cross-linking reaction is done under ultra-dilute conditions.

THE TRANSITION FROM RANDOM COIL TO A FOLDED NANOPARTICLE

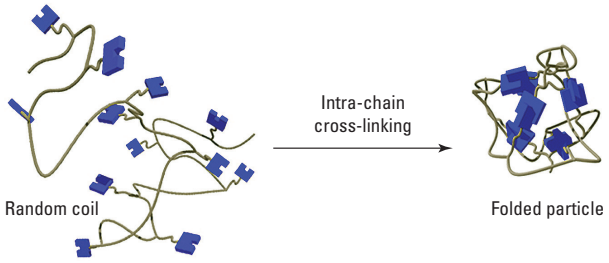

Figure 1

The folding and unfolding of the SCNPs from the random coil to globule structure can either be reversible or irreversible depending on the chemistry behind the cross-linking reaction. SCNPs can be formed using a variety of methods utilizing many different types of chemistry. For example, poly(norbornene-exo-anhydride) (P1) can be synthesized, via ROMP using third generation Grubbs catalyst as an initiator and the degree of collapse that occurs during nanoparticle (N1) formation can be controlled by varying the amount of difunctional cross-linker added.

The formation of SCNPs from the polymer coils can be characterized using gel permeation chromatography (GPC), based on polymer size and molar mass. GPC, a separation method which separates molecules based on their size in solution, is ideal for analyzing the nanoparticle formation as the size in solution of the random polymer varies from that of the collapsed nanoparticle, thus resulting in a variation of GPC retention times and elution profiles. GPC can also be used to monitor the unfolding of the SCNPs as the SCNPs can reversibly undergo a coil to particle transition through the formation and cleavage of intramolecular disulfide cross-links. Here we report the use of single and multi-detector GPC analysis, using the EcoSEC GPC System coupled to various detection methods for the monitoring of the folding and unfolding of SCNPs.

EXPERIMENTAL

Sample analysis was performed on an EcoSEC GPC System equipped with a dual flow refractive index (RI) detector coupled in series to a multi-angle light scattering detector, MALS (Wyatt miniDAWN™ TREOS®). Unfiltered 25 µL injections of 1 mg/mL samples were separated over a column bank consisting of two 4.6 mm ID x 15 cm, 4 µm TSKgel® SuperMultiporeHZ-M columns. The solvent and mobile phase were tetrahydrofuran (THF) at a flow rate of 0.35 mL/min. Detector, pump oven, and column oven were maintained at 35 °C.

The miniDAWN detector was normalized in-house using a polystyrene standard. Calculation of interdetector delays and interdetector band broadening correction were performed using polystyrene. Calibration of the MALS unit was performed using toluene. The differential refractive index increment values (dn/dc) were calculated online assuming 100% mass recovery (RI as the concentration detector) using the Astra 6.0 software package (Wyatt Technologies). Absolute molar mass and molar mass distributions were calculated using the Astra 6 software package. Relative molar masses were obtained against polystyrene standards (PStQuick MP-M) and calculated using the EcoSEC Workstation software.

RESULTS AND DISCUSSION

SCNPs can reversibly undergo a coil to particle transition via formation and cleavage of intramolecular disulfide cross-links.[1] The coil to particle transition of poly(norbornene-exo-anhydride) (P1), synthesized via ROMP using third generation Grubbs catalyst as an initiator was collapsed into nanoparticles (N1) of different degrees by varying the amount of difunctional cross-linker added.

The coil and particles were then characterized using the EcoSEC GPC System with dual flow RI via polystyrene relative molar mass averages.

Figure 2 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. The increase is GPC retention time is due to a decrease in hydrodynamic volume that occurs as the coil collapses, as the elution order in GPC is that of an "inverse-sieving" technique, smaller analytes elute after the larger analytes.

After formation of the nanoparticles by disulfide bridges was confirmed via GPC, redox chemistry was employed to reduce the disulfide linkages to corresponding thiols, thus unfolding the nanoparticle, N1, back to the polymer in a coil conformation, uN1. Using catalytic amounts of ferric chloride, the thiols were oxidized to re-fold the polymer to a globule-like conformation. The transition from particle to coil was confirmed via a decrease in GPC retention time, signifying an increase in hydrodynamic volume, as shown in Figure 3. The unfolded nanoparticle returns to the original size and conformation based on the identical trace of the GPC elution profile.

The next part of the SCNPs study was to differentiate between single-chain particles and aggregates of a few chains and to determine the absolute molar mass via dual-detector GPC (GPC/RI/MALS). Copolymerizing the anhydride monomer previously used with cyclooctadiene (COD) allowed control and a way to predict the amount of mass that should be added upon application of the folding mechanism. The folding reaction was repeated with the same cross-linking agent in excess and for an extended reaction time to encourage a small amount of intermolecular coupling.

Figure 4 shows an overlay of the MALS and RI traces when the intramolecular 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 single peak shifted to longer retention time in the RI trace can be attributed to the single-chain particles that were formed. The first peak in the MALS trace, with the shorter retention time, represents the small amount of multi-chain aggregates that formed. Light scattering is very sensitive to larger objects and they scatter light to a greater extent and therefore will give a stronger signal than something smaller in size. RI, on the other hand, is concentration sensitive. Therefore, the lack of the earlier eluting peak in the RI trace portrays the low concentration of multi-chain aggregates that formed compared to the much higher concentration of the single-chain particles. For this particular sample analysis, single-detector GPC would not have revealed the presence of the larger aggregates.

GPC ELUTION PROFILE AS MONITORED BY THE RI FOR THE POLYMER AND NANOPARTICLES FORMED WITH VARYING AM OUNTS OF CROSS-LINKER ADDED

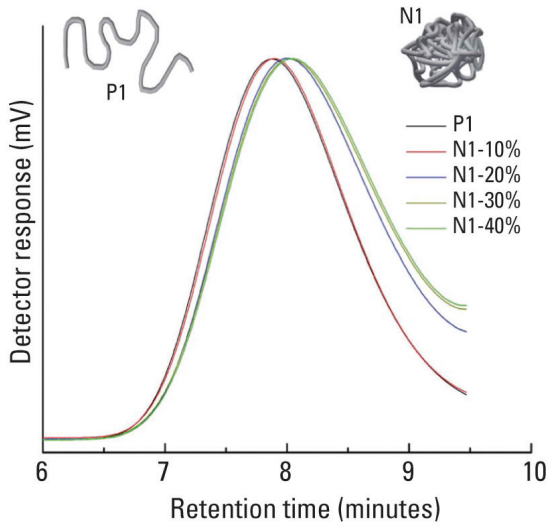

Figure 2

GPC ELUTION PROFILE AS MONITORED BY THE RI OF THE ORIGINAL POLYMER WITH UNFOLDED NANOPARTICLES

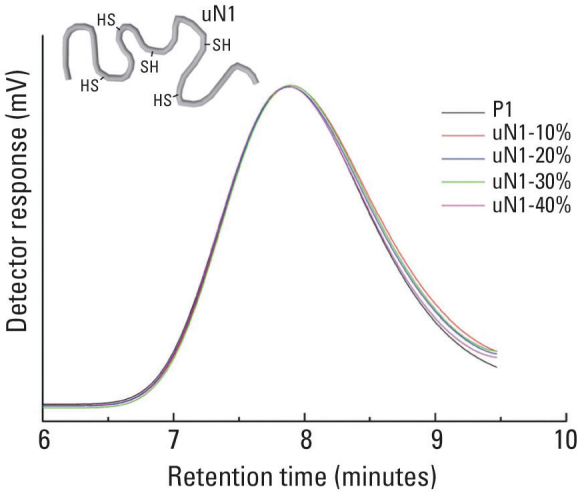

Figure 3

GPC ELUTION PROFILE AS MONITORED BY THE MALS (BLACK) AND RI (BLUE) DETECTORS FOR THE SCNP

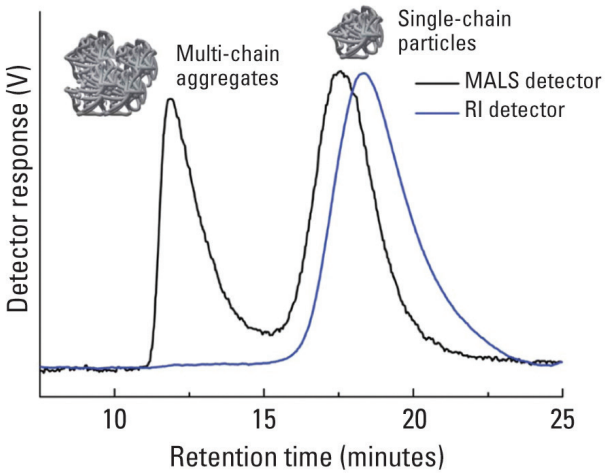

Figure 4

CONCLUSIONS

The transition of a random coil polymer to and from a folded single chain nanoparticle was monitored using the EcoSEC GPC System with a refractive index detector coupled to a multi-angle light scattering detector. The GPC elution profile was used to monitor the reaction progress and limitations of disulfide linkages formed. The formation or folding of the SCNPs results in an increase in GPC retention time as a direct result of a decrease in polymeric size. On the other hand the unfolding of the SCNPs back to their original random coil formation results in an increase in GPC retention time as the polymeric size is increasing. The dual detector set up was also used to monitor changes in the GPC elution profile when an intramolecular cross-linking reaction was extended with a slight excess of the cross-linker to encourage intermolecular coupling. The intermolecular coupling resulted in a single RI peak, while the MALS detector shows two peaks. The peak present in the MALS detector but not the RI detector is a result of a very low concentration of multi-chain aggregates and thus only revealed through a dual-detector GPC set up (GPC/RI/MALS). The SCNP experiment showed the versatility of the EcoSEC GPC System when coupled to MALS to determine polymer chain folding and unfolding, and interchain versus intra-chain coupling.

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TOSOH

Application Note

GOING GREEN: ANALYSIS OF VIRGIN AND RECYCLED NYLON 11 IN HFIP USING THE EcoSEC® GPC SYSTEM AND SEMI-MICRO GEL PERMEATION CHROMATOGRAPHY COLUMNS

INTRODUCTION

Over the past several decades green initiatives have been approaching the polymer science discipline from all sides. 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. They produce large quantities of scraps and wastes that contaminate the environment. One solution for decreasing nylon waste is plastic recycling, because recycling can decrease the cost and environmental contamination related to everyday use of nylons. The major caveats of recycling nylon are the reduction of physical-mechanical properties and changes in morphology which result from polymer degradation that occurs during the recycling processes.[1-2]

Due to the aforementioned caveats, the ability to accurately and precisely characterize virgin and recycled nylon materials is essential. One common method used for nylon characterization is the determination of molar mass averages and distributions by gel permeation chromatography (GPC). The use of GPC for the analysis of nylons has its own challenges, namely the poor solubility in common organic solvents and strong adsorptive interactions.[3] To solve the problems related to GPC analysis of nylons, the analysis is typically performed at extremely high temperatures using m-cresol or temperatures closer to ambient using very costly solvents such as hexafluoroisopropanol (HFIP).

The necessity to use HFIP for the analysis of nylons by GPC to determine the similarities and differences between the molar mass averages and distributions of virgin and recycled nylon materials results in very costly experiments. Here we report on the use of a low dead volume all-in-one GPC system, the EcoSEC GPC System, with semi-micro (6 mm ID × 15 cm) GPC columns for the analysis of virgin and recycled nylon material in HFIP. The combination of the low dead volume of the EcoSEC GPC System and semi-micro GPC columns provides significant solvent related cost savings while doubling sample throughput without compromising resolution.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on a system consisting of an EcoSEC GPC System (HLC-8320) equipped with a RI detector. Separation of unfiltered 20 µL injections occurred over a column bank consisting of two 6 mm ID × 15 cm, 9 µm particle size TSKgel® SuperAWM-H columns (P/N 0019320 exclusion limit ~1 × 10^7 g/mol). The mobile phase and solvent were hexafluoroisopropanol with 5 mmol/L sodium trifluoroacetate at a flow rate of 0.35 mL/min. Detector, pump, and column oven were maintained at 40 °C. Two Nylon 11 samples were analyzed: virgin nylon 11 and recycled nylon 11. Sample solutions were prepared by diluting the samples in mobile phase for a final sample concentration of 1.0 mg/mL. Samples were shaken manually for one minute and allowed to sit overnight before analysis was performed. For all chromatographic determinations, results are averages of six injections from two separate sample dissolutions. Data was processed with the EcoSEC GPC Workstation software.

A calibration curve was created for the RI at 40 °C using poly(methyl methacrylate) (PMMA) standards ranging in molar mass from 6,270 to 1.1 × 10^6 g/mol. PMMA standards were prepared by diluting nine individual PMMA standards in mobile phase, in separate vials, for a final sample concentration of 1.0 mg/mL.

GPC ELUTION PROFILE OF VIRGIN NYLON 11 (RED), AND RECYCLED NYLON 11 (BLUE) AS MONITORED BY RI.

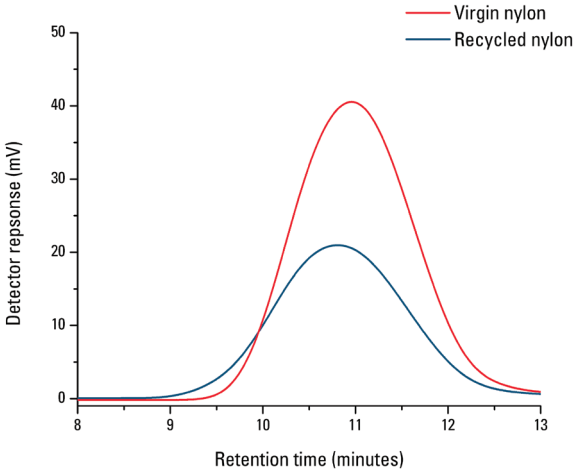

Figure 1

PMMA standards were analyzed under the same conditions as those used for sample analysis as described above. Calibration curve data for 0.35 mL/min was fitted with a linear function and error values were less than 5%.

RESULTS AND DISCUSSION

A greener and more cost effective method for the characterization of nylon 11 in hexafluoroisopropanol (HFIP) was employed by using an EcoSEC GPC System encompassing a dual flow refractive index detector and semi-micro gel permeation chromatography (GPC) columns. The combination of the low dead volume of the EcoSEC GPC System and semi-micro GPC columns provides significantly reduce solvent related costs.

Two nylon 11 samples, which produced successful final products: virgin nylon 11 and recycled nylon 11, were analyzed in HFIP. The GPC experiments provided two forms of comparison between the virgin and recycled nylon 11 samples: GPC chromatograms and poly(methyl methacrylate) relative molar mass averages and molar mass distributions.

The GPC elution profiles of the virgin and recycled nylon 11 as monitored by the dual flow RI detector are shown in Figure 1. The virgin nylon 11 elutes after the recycled nylon 11. The longer retention time of the virgin nylon 11 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 analysts. The GPC elution profiles of the two samples also vary in broadness, with the elution profile of the recycled nylon 11 extended further in the shorter retention time larger polymeric size direction than its virgin counterpart.

The molar mass averages and polydispersity index, PDI, as determined via a PMMA RI calibration curve are given in Table 1. A comparison of the molar mass averages of the virgin nylon 11 material with the recycled nylon 11 material reveals higher number-, weight-, and z-average molar mass values for the recycled nylon 11 compared to the virgin nylon 11. In general, an increase in the molar mass averages of the recycled nylon 11 compared to the molar mass averages of the virgin nylon 11 is expected, as the rate of increase between the molar mass values is dictated by probability of main-chain scission and crosslinking during the recycling process.[2]

The differences between the virgin and recycled nylon 11 can also be observed by comparing the PDI values, Table 1, and the differential and cumulative distributions, Figure 2. The recycling process of nylon 11 results in an increase in the polydispersity index, virgin material PDI=1.41 and recycled material PDI=1.62, thus a corresponding increase in the breadth of the distribution curves and molar mass range for the recycled nylon 11, Figure 2.

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF NYLON 11 SAMPLES VIA RI

Sample	Retention time	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI
Virgin nylon 11	10.959	1.215×10^4 ± 46[b]	1.713×10^4 ± 75	2.293×10^4 ± 346	1.48 3.88
Recycled nylon 11	10.802	1.334×10^4 ± 438	2.169×10^4 ± 210	3.932×10^4 ± 1,105	1.26

a PDI = Mw/Mn
b Standard deviations from six injections

Table 1

DIFFERENTIAL AND CUMULATIVE DISTRIBUTIONS OF NYLON 11 (RED) AND RECYCLED NYLON 11 (BLUE)

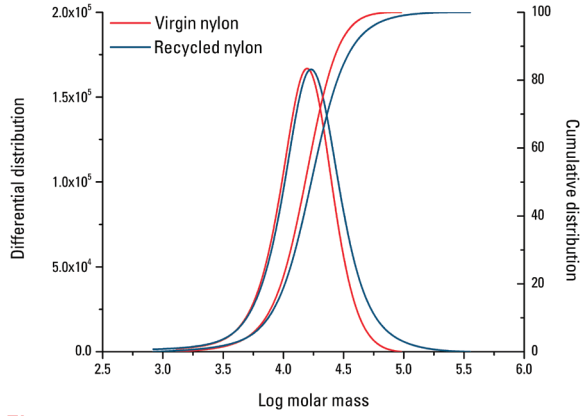

Figure 2

The molar mass averages and molar mass distributions of two nylon 11 samples: virgin nylon 11 and recycled nylon 11, were determined via a dual flow RI detector using the EcoSEC GPC System and semi-micro GPC columns in HFIP. The GPC elution profile for the virgin nylon 11 was determined to be narrower and eluted later than the recycled nylon 11. The molar mass averages, M_n, M_w, and M_z, as determined via PMMA relative calibration curves were found to be greater for recycled nylon 11 than for virgin nylon 11. Additional differences between virgin and recycled nylon 11 were observed by comparing the PDI values of the samples. The recycling process of nylon 11 results in an increase in the polydispersity index. The molar mass averages and distributions of the virgin and recycled nylon 11 samples obtained by GPC in this case were determined to be distinguishable from one another even though both nylon 11 samples can be used to create successful products with the same end-use properties. Additionally, the use of the EcoSEC GPC System with semi-micro GPC columns decreases the consumption of HFIP by ~85%. This equates to savings of over 70,000 EUR in solvent costs for a time period of one year. The end result is a greener and more cost effective method for the characterization of nylon 11.

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TOSOH

Application Note

THE CHARACTERIZATION OF SUGAR BEET PECTIN USING THE EcoSEC® GPC SYSTEM COUPLED TO MULTI-ANGLE LIGHT SCATTERING, QUASI-ELASTIC LIGHT SCATTERING, AND DIFFERENTIAL VISCOMETRY

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ABSTRACT

The need to increase the use of low valued co-products derived from the processing of sugar beets has prompted the investigation of the structure of the pectin extracted from sugar beet pulp. The characterization of sugar beet pectin is essential as it has the potential to be used in the production of industrial products, e.g., as an emulsifying agent in food systems. This added use of sugar beet pectin should be of help to sugar beet growers and processors by increasing the demand and value of their by-product without increasing the cost of sugar to the consumer. Here we discuss the characterization of sugar beet pectin utilizing the EcoSEC GPC System with an internal dual-flow differential refractive index detector and UV detector coupled to multi-angle light scattering, quasi-elastic light scattering, and differential viscometry. Implementing this multi-detector SEC technique allowed for the determination of the molar mass averages, in a calibrant-independent fashion, as well as several sizing parameters.

INTRODUCTION

An estimated 2 million tons of dry sugar beet pulp is generated annually by U.S. industries as a result of the extraction of sugar from sugar beets[1]. Currently sugar beet pulp is mainly dried and sold as low-value animal feed at little profit because of the costly energy required to dry it for storage and shipment. Sugar beet pulp, especially the high molar mass pectin portion, has the potential to be an enormous untapped source of a valuable polysaccharide for U.S. industry. The need to increase the utilization of low-value co-products derived from the processing of sugar beets has prompted the investigation of the structure of the pectin extracted from sugar beet pulp.

Sugar beet pulp on a dry weight basis is composed of about 67% plant cell wall polysaccharides, 19% of which are pectin, 21% pectin-associated arabinan, and 24% cellulose[1], all of which can potentially add value to the pulp if isolated and characterized. The pectin in sugar beets has different chemical features than that from other sources of pectin, i.e. citrus, as the former tends to have a higher degree of acetylation, a higher natural sugar content, and contains feruloyl groups. Furthermore, unlike citrus pectin which is currently used as a gelling and thickening agent, sugar beet pectin (SBP) has very poor gelling properties, thus the isolation and characterization of it could result in new applications, especially in the production of industrial products. Thus far, SBP has demonstrated the potential to be used as an emulsifying agent in food systems, as it has been found to reduce the interfacial tension between oil and water phases[1]. The capability of SBP to reduce surface tension has been attributed to the presence of acetyl groups and hydrophobic proteins in SBP preparations[2].

PHYSICAL PROPERTIES OF SUGAR BEET PECTIN

	Detection Method			
	RI	UV@250 nm	UV@278 nm	UV@ 310 nm
M_w (g/mol)[a]	1.098 x 10^6	1.097 x 10^6	1.147 x 10^6	1.187 x 10^6
	(0.003 x 10^6)[b]	(0.004 x 10^6)	(0.004 x 10^6)	(0.002 x 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

Table 1

In order to better understand the ability of SBP to act as an emulsifier, we have investigated the structure of SBP using size exclusion chromatography coupled to a multiplicity of physical detection methods, namely multi-angle light scattering (MALS), quasi-elastic light scattering (QELS), multi-wavelength UV, differential refractometry (RI), and differential viscometry (VISC), and corroborated these results with results from atomic force microscopy[1].

EXPERIMENTAL CONDITIONS

Sugar beet pectin was prepared using microwave-assisted flash-extraction as described in reference 1. Size exclusion chromatography analysis of the sugar beet pectin was performed on a system consisting of an EcoSEC GPC System (Tosoh Bioscience) with an internal dual-flow differential refractive index detector and UV detector connected in series to a HELEOS II MALS photometer (Wyatt Technology Corp.), a QELS photometer (Wyatt), and a ViscoStar differential viscometer (Wyatt). The UV absorbance was monitored at wavelenghts of 310, 278, and 250 nm. The solvent and mobile phases were water with 0.05 M $NaNO_3$ and 0.01% NaN_3, at a flow rate of 0.7 mL/min. 200 µL injections of 1 mg/mL solutions were injected onto a column bank consisting of three TSKgel GMPW$_{XL}$ columns (30 cm x 7.8 mm) with a particle size of 13 µm obtained from Tosoh Bioscience. These mixed-bed columns have a separation range, based on polyethylene oxides, of 1000 to 8 x 10^6 g/mol. Detectors, pump oven, and column oven were maintained at 35°C. Data acquisition and processing were performed using Wyatt's ASTRA 5.3.4.16 software.

The HELEOS II detector was normalized in-house using a pullulan standard, with a molar mass of 47,300 g/mol, while calculation of interdetector delays and interdetector band broadening correction were performed using BSA. Calibration of the MALS unit was performed using toluene. The ∂n/∂c of sugar beet pectin was determined previously to be 0.130 mL/g.

RESULTS AND DISCUSSION

As described above, the EcoSEC GPC System equipped with TSKgel SEC columns was coupled to a train of MALS, QELS, UV, RI, and VISC detectors to determine the molar mass and size of sugar beet pectin. The results of the experiments are given in Table 1. Figure 1 shows the chromatograms of the sugar beet pectin, as monitored by the individual detectors. The SEC elution profile of the SBP, as measured by both of the concentration-sensitive detectors, RI and UV, displays a distinct bimodal distribution. The weight-average molar mass values MW, given in Table 1, are calculated via:

$$M_w = \frac{\sum_i c_i M_i}{\sum_i c_i}$$

where, at each elution slice i, c_i is the concentration of the analyte provided by the concentration-sensitive detector and M_i is the molar mass of the analyte provided by the MALS detector after correction for interdetector delay. As indicated in Table 1, the weight-average molar mass for the SBP was determined to be approximately 1.1 x 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). Evidence of molar mass polydispersity of SBP is seen in Figure 2, where M is plotted across the SEC elution for each concentration sensitive detector. From the detector response for the four concentration-sensitive detectors it appears as if the chromatogram is bimodal. Additionally, if one observes the molar mass of the SBP as a function of SEC elution volume, it can be seen that the molar mass of the SBP decreases by an order-of-magnitude with increasing elution volume, an indication the SBP is polydisperse with respect to molar mass.

SEC ELUTION PROFILE OF SUGAR BEET PECTIN AS MONITORED BY MALS & QELS (GREEN), RI (BLUE), UV @ 250 NM (RED), AND VISC (BLACK) AT 0.7 ML/MIN.

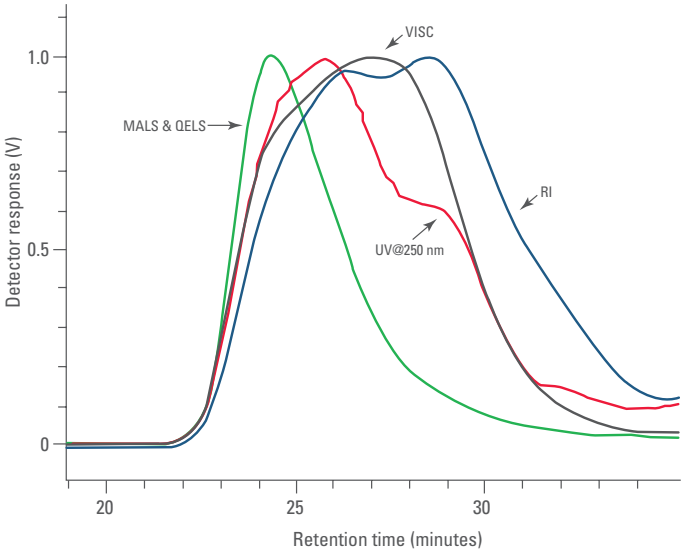

Figure 1

SEC ELUTION PROFILE OF SUGAR BEET PECTIN USING FOUR CONCENTRATION-SENSITIVE DETECTORS (UV @ 310, 278, AND 250 NM AND RI) AND MOLAR MASS DISTRIBUTIONS ACROSS THE ELUTION PROFILES AS DETERMINED BY MALS.

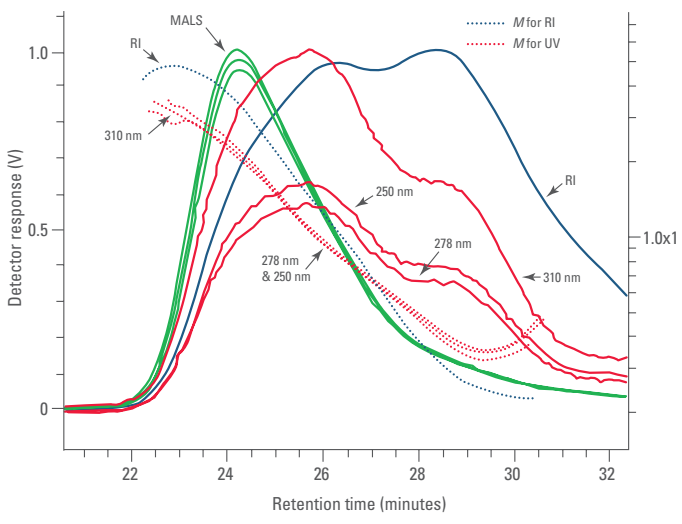

Figure 2

It should also been 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. The addition of the two light scattering detections, MALS and QELS, and viscometry detection to the EcoSEC GPC System not only allows for the determination of the molar mass of SBP in a calibrant-independent fashion but also for the determination of polymeric size and intrinsic viscosity. The radius of gyration RG and the hydrodynamic radius RH for the SBP were determined via MALS and QELS, respectively, and are given in Table 1. The difference in the value of RG for SBP as determined by the four concentration-sensitive detectors is minimal, ± 3 nm. Conversely, the difference in the value of RH for SBP as determined by the RI detector compared to the three UV wavelengths varies by 10 nm, a direct reflection of the dependence of concentration, as determined by the concentration-sensitive detector, on the calculation of RH.

CONCLUSIONS

The EcoSEC GPC System with refractive index and UV detection was coupled to MALS, QELS, and VISC detection to characterize the physicochemical properties of sugar beet pectin. The sugar beet pectin was determined to be polydisperse with respect to molar mass and to have a weight-average molar mass value around 1.1-million g/mol. Two polymeric size parameters were also determined, RG and RH, as well as the intrinsic viscosity. The coupling of the EcoSEC GPC System to external detectors was successful for the analysis of complex macromolecules based on molar mass, size, and intrinsic viscosity.

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*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.

TOSOH

Application Note

PHOTODEGRADABLE POLYMER DEGRADATION ANALYSIS USING THE EcoSEC® GPC SYSTEM

INTRODUCTION

Photoresponsive polymers have several advantages over other stimuli responsive materials due to the spatial and temporal control of the input.[1] Photoactivation can be used to influence various polymer properties such as release or capture of additives, change in viscosity, modulus and pH. Early efforts in the field of photoresponsive polymers were aimed primarily at decreasing the environmental impact of plastics in landfills and on marine life. However, the long degradation time and non-biodegradability of these materials make them unsuitable for biological applications. Currently there is a strong need in biomedical applications for polymers that are both photodegradable and biodegradable. Such polymers are being investigated as drug delivery devices and as platforms with phototunable physical and mechanical properties.

To address the needs of biological application, a new class of photodegradable polycarbonate materials has been developed based on the alkoxyphenacyl photoactive moiety that undergo controlled degradation to oligomers upon irradiation at 300 nm.[1] These polycarbonates are mechanically robust, biodegradable, and stable at high temperatures in the absence of light. The combination of the thermal and mechanical properties of these polymers promises usefulness in biomedical applications such as controlled drug release devices, ocular implants, and dermal patches.

The photodegradable polycarbonate homopolymer as well as different copolymers with poly(ethylene glycol) (PEG) were synthesized and characterized by gel permeation chromatography (GPC) using the EcoSEC GPC System. Here we report, through molar mass averages and polydispersity index values as determined by GPC, the photodegradation of the polycarbonate homopolymer, 5% PEG copolymer, and 10% PEG copolymer by irradiation of the polymers in chloroform in a Rayonet reactor at 300 nm as well as the hydrolytic degradation of the copolymers by incubation in phosphate buffered saline (PBS) at 37°C.

EXPERIMENTAL CONDITIONS

Analysis of the homopolymer, copolymers, photodegraded samples, and hydrolytically degraded samples were performed on an EcoSEC GPC System equipped with a dual flow refractive index detector (RI) and UV detector. The polymers under investigation have UV absorption from 250 to 320 nm with a λ_{max} at 280 nm. Separation occurred over a column bank consisting of two 6 mm ID × 15 cm, 3 µm TSKgel® SuperH3000 (exclusion limit 6 × 10^4 g/mol, PN 0017993) and one 6 mm ID × 15 cm, 3 µm TSKgel SuperH4000 (exclusion limit 5 × 10^5 g/mol, PN 0017994) columns. The mobile phase and solvent were chloroform ($CHCl_3$) at flow rate of 0.38 mL/min. Detector, pump oven, and column oven were maintained at 40°C. For all chromatographic determinations, results are those based on a polystyrene calibration curve.

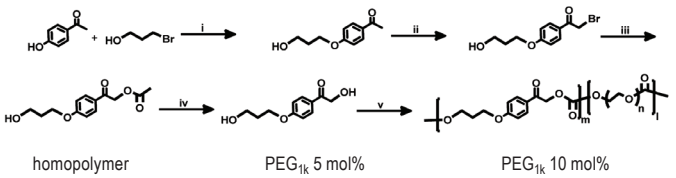
POLYMER PHOTODEGRADATION WITH TIME OF IRRADIATION

The photodegradable homopolymer poly(2-hydroxy-1-(4-(3-hydroxypropoxy)phenyl)ethanonecarbonate) (20 mg) was dissolved in chloroform and transferred to a quartz tube. The sample was irradiated in a Rayonet RPR-200 reactor at 300 nm where the polymer has a significant UV absorption (5.34 mW/cm^2). Every 5 minutes, the reactor was turned off and 1 mL of reaction mixture was taken out and filtered through a 0.45 µm PTFE filter. A total of 9 samples were taken in 40 minutes of irradiation and the rest of the solution was irradiated for an extra hour.

HYDROLYTIC DEGRADATION OF POLYMERS

Poly(2-hydroxy-1-(4-(3-hydroxypropoxy)phenyl)ethanone carbonate)-co-poly((poly(ethyleneglycol)diol)carbonate) copolymers were dissolved in $CHCl_3$. The solution was stirred for a few hours to allow the polymer to dissolve completely. Polymer films were prepared by solvent casting the above solution into a Teflon® dish and allowing the solvent to evaporate slowly overnight and were dried under vacuum prior to use. Hydrolytic degradation was monitored by immersing half of each polymer film (~36 mg) in a vial containing 5 mL of PBS solution in an incubator at 37°C. At the end of every week (total of 4 weeks) a small piece of film was taken from the vial, dried, dissolved in $CHCl_3$ and analyzed by GPC.

SYNTHESIS OF PHOTODEGRADABLE POLYMERS[1]



(i) K_2CO_3/18-crown-6, acetone reflux. (ii) $CuBr_2$, EtOH, $CHCl_3$ (over two steps: 75% yield). (iii) NaOAc, CH_3COOH, H_2O (99% yield). (iv) 1. NaOH, MeOH, 2. $NaHSO_4$ (46% yield). (v) Triphosgene, $CHCl_3$, pyridine, PEG_{1k}

Figure 1

MOLAR MASS DISTRIBUTIONS AND POLYDISPERSITY INDEX

Composition	*Mn (g/mol)*	*Mw (g/mol)*	*PDI*
Homopolymer	1.29 x 10^4	2.95 x 10^4	2.3
5% PEG	2.27 x 10^4	2.63 x 10^4	1.2
10% PEG	8,810	1.04 x 10^4	1.2

Table 1

RESULTS AND DISCUSSION

Polycarbonate homopolymer and copolymers were synthesized using the scheme shown in Figure 1. The polystyrene relative molar mass averages, Mn and Mw, and the polydispersity index, PDI, were determined using the EcoSEC GPC System with dual flow RI and UV detectors. Three polymers were synthesized and analyzed: an alkoxyphenacyl-based polycarbonate homopolymer, 5% PEG copolymer, and 10% PEG copolymer. The polystyrene relative molar mass averages, Mn and Mw, and the polydispersity index, PDI, for the initial homopolymer and copolymers are given in Table 1. The polystyrene weight-average molar mass values were also determined for the homopolymer and copolymers after photochemical and hydrolytic degradation.

The photochemical degradation of the homopolymer and copolymers was examined by irradiation in $CHCl_3$ in a Rayonet reactor at 300 nm. GPC elution profiles of the irradiated homopolymer showed that the polymer underwent controlled time-dependent chain scissions upon irradiation, Figure 2. Prior to irradiation the polystyrene relative weight-average, molar mass was determined to be 2.95 × 10^4 g/mol. Within five minutes of irradiation, there was a loss of three-fourths of the Mw,- of the polymer, Mw= 7.2 × 10^3 g/mol. After a hundred minutes of irradiation of the homopolymer the weight-average molar mass, Mw, of the polymer had decreased to 146 g/mol. The Mw values were shown to continuously decrease with irradiation time. The GPC elution profile shifts towards longer retention times with increasing irradiation time, indicating that the irradiated homopolymer is smaller in polymer size compared to the non-irradiated homopolymer. The photodegradation of the copolymer polymers showed a similar trend to that of the homopolymer.

DECREASE IN Mw-WITH IRRADIATION TIME FOR THE POLYCARBONATE HOMOPOLYMER

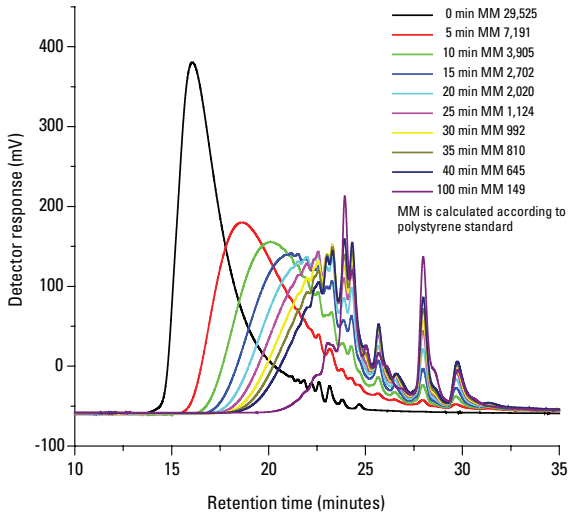


Figure 2

Hydrolytic degradation of the polymers was obtained by incubation of the copolymers, 5% and 10% PEG, in phosphate buffered saline at 37°C. For the copolymers, hydrolytic degradation is reflected by the molar mass loss with the time of incubation, Figure 3. For example, over a period of 28 days, the copolymer with 5% PEG showed increasing molar mass loss with time and ultimate loss of 61% of molar mass on day 28. As expected, the photochemical degradation is much faster – the polymer undergoes almost complete photodegradation within 30 minutes, Figure 2, while hydrolytic degradation over 28 days results in a 61% loss for the 5% PEG copolymer, Figure 3.

HYDROLYTIC DEGRADATION OF COPOLYMERS

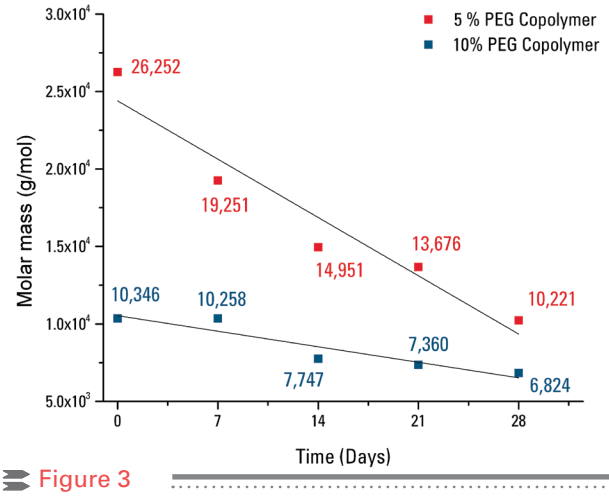


Figure 3

CONCLUSIONS

The synthesis and properties of a new class of photodegradable polymers that undergo controlled chain scission upon irradiation at 300 nm were analyzed using the EcoSEC GPC System. The polystyrene relative molar mass averages of a homopolymer and two copolymers were determined before, during and after photochemical and hydrolytic degradation. Additionally, the GPC elution profile was monitored during both photodegradation processes. The photodegradation results demonstrate that the polymers quickly lose their molar mass upon irradiation. These properties along with others determined via different characterization methods, e.g. NMR, make these polycarbonate homopolymer and copolymers valuable for many biomedical applications.[1] The low dispersion design of the EcoSEC GPC System combined with the use of 15 cm long TSKgel columns provided a fast and robust method for monitoring the degradation by GPC.

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TOSOH

Application Note

SYNTHESIS MONITORING AND OLIGOMERIC ANALYSIS OF PEGylated POLYMERS

INTRODUCTION

New roles and applications in the areas of science and technology are continuously being found for synthetic polymers. Specifically, the use of synthetic polymers in medicine is growing, as these polymers offer unique and versatile platforms for applications such as implants, medical devices, surgical adhesives, drug delivery vesicles, and injectable polymer-drug conjugates[1,2]. As the application of synthetic polymers increases, there is a manifested need for methods to accurately and precisely characterize the materials.

One of the most common and valuable tools employed for the analysis and characterization of polymers is size exclusion chromatography (SEC or gel permeation chromatography, GPC). The principle use of SEC, even a half-century after its inception, remains as determining the molar mass averages and distributions of natural and synthetic polymers through the application of calibration curves[3]. The applicability of SEC for synthetic polymers also extends into the realms of synthesis monitoring and oligomeric quantification. Synthesis monitoring using SEC not only allows for separation of polymeric material based on size but also provides information about the reactions, e.g., did the reaction go to completion, is the product uniform in terms of molar mass or size, did a byproduct form, etc. Oligomeric SEC plays an important role in the quantification of oligomeric content (i.e., low-molar mass species) of a polymer sample for the purposes of pre-manufacture notification (PMN) regulations for new chemical substances as well as import and export purposes[3].

The utility of SEC for synthesis monitoring and oligomeric analysis makes it an invaluable tool for characterizing synthetic polymeric material for use in medicine, as these materials require thorough characterization amongst other validations. Here we report on the use of an all-in-one dedicated GPC system, the EcoSEC® GPC System, to monitor the synthesis and to quantify the oligomeric content of two PEGylated synthetic polymers intended for use in medical applications.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on a system consisting of an EcoSEC GPC System (HLC-8320) equipped with a refractive index detector (RI). Separation of unfiltered 40 µL injections occurred over a column bank consisting of two 6.0 mm ID × 15 cm, 3 µm particle size TSKgel® SuperH3000 columns (exclusion limit 60,000 g/mol) preceded by the appropriate guard column (Tosoh Bioscience). The solvent and mobile phase were tetrahydrofuran (THF) (Fisher Chemical) at a flow rate of 0.3 mL/min. Detector, pump oven, and column oven were maintained at 35°C. Two PEGylated polymers and a starting material were analyzed: PEG-A, PEG-B and starting material C, respectively. For all chromatographic determinations, results are averages of three injections from two separate sample solutions. Sample solutions were prepared by diluting the sample (98% purity) with THF for a final sample concentration of approximately 10 to 15 mg/mL. Samples were shaken manually for a minute and allowed to sit for 3 hours before analysis was performed. Data was processed with the EcoSEC GPC Workstation Software version 1.08.

A calibration curve was created using PStQuick Kit-L polystyrene standards (Tosoh Bioscience) ranging in molar mass from 266 to 37,900 g/mol. Calibration curve data was fitted with a cubic function and error values were less than 5%.

SYNTHESIS MONITORING BY SEC. SEC ELUTION PROFILE OF PEG-A (RED), PEG-B (BLUE), AND STARTING MATERIAL C (GREEN) AS MONITORED BY RI

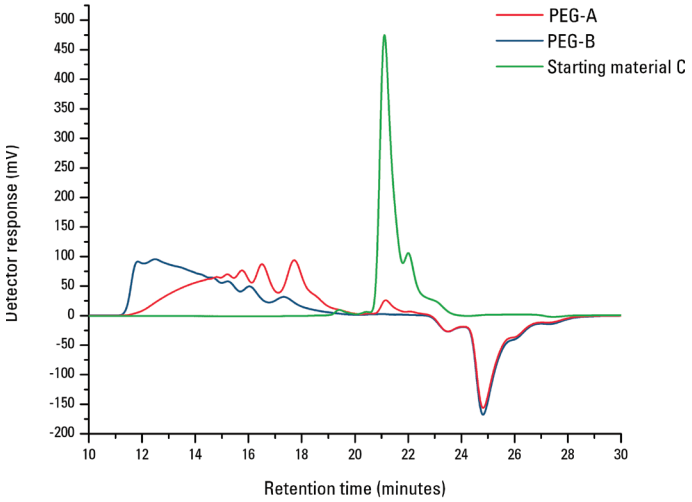

Figure 1

RESULTS AND DISCUSSION

As described above, an EcoSEC GPC System equipped with an internal dual-flow refractive index (RI) detector was used for the characterization of two PEGylated polymers. The detector response of the RI detector was used to monitor the synthesis of the formation of the two PEGylated polymers and to compare the chromatograms of both materials to that of one of the starting materials. Additionally, a polystyrene relative calibration curve was used to determine the peak-average molar masses M_p and the oligomeric content of the two samples.

SYNTHESIS MONITORING

SEC is an ideal tool for monitoring the formation of PEGylated polymers, as the method separates the polymers based on size while simultaneously providing information about the molar mass of the newly synthesized species. The two polymers analyzed here, PEG-A and PEG-B, are composed of the same basic components which vary in molar mass between the two samples. The molar mass difference of the starting material of PEG-A and PEG-B is reflected in the end-products, as PEG-A and PEG-B produce different chromatograms when separated by SEC, Figure 1. As can be seen in Figure 1, the peak shape of the two PEGylated polymers differs; the chromatogram for PEG-B has a greater RI detector response at the earlier elution volume, larger polymer size region of the chromatogram while the opposite is true for PEG-A.

The ability to identify all species within the PEGylated sample is essential in the validation of polymers intended for medical applications. Thus, the role of synthesis monitoring here is not only to compare the difference between the two species produced from similar procedures but to compare the two species to the starting material(s). As seen in Figure 1, by comparing the SEC chromatograms of starting material C with that of PEG-A and PEG-B, a fairly substantial amount of starting material C remains in PEG-A while all of starting material C has reacted in PEG-B.

OLIGOMERIC ANALYSIS

The characterization of the oligomers present in the PEGylated samples is best achieved by the peak-average molar mass M_p. The polystyrene relative peak-average molar mass values for each mode in the chromatograms for the PEG-A and PEG-B samples are given in Figures 2 and 3, respectively. The values for the peak-average molar mass between the two samples differ significantly. The M_p values of PEG-A range from approximately 2,000 to 10,000 g/mol indicating that most, if not all, of the species present are oligomeric in nature. Conversely, the M_p values of PEG-B range from approximately 2,500 to 60,000 g/mol indicating that low- and high-molar mass species are present. Additionally, oligomeric analysis of a sample can be extended beyond the determination of M_p to include the determination of the number-average molar mass M_n and quantitation of the percentage of the molar mass distribution below a certain molar mass value, data not presented here. However, it must be remembered that the detector response of the RI detector or any concentration-sensitive detector is generally not constant in the oligomeric region[3].

SEC ANALYSIS OF PEG-A AS MONITORED BY RI. NUMBERS ON GRAPH REPRESENT POLYSTYRENE RELATIVE PEAK-AVERAGE MOLAR MASS *MP* VALUES OF EACH MODE.

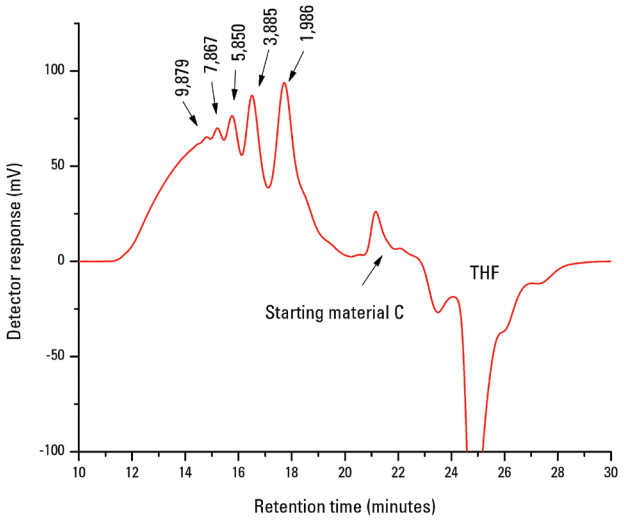

Figure 2

SEC ANALYSIS OF PEG-B AS MONITORED BY RI. NUMBERS ON GRAPH REPRESENT POLYSTYRENE RELATIVE PEAK-AVERAGE MOLAR MASS *MP* VALUES OF EACH MODE.

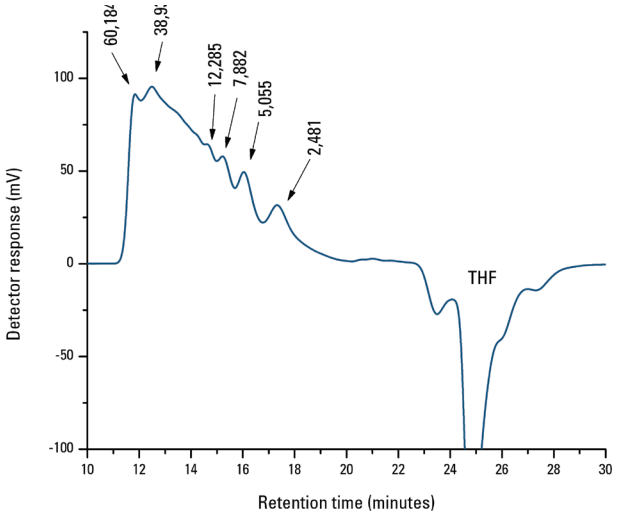

Figure 3

CONCLUSIONS

The use of the EcoSEC GPC System was extended beyond the principle use of SEC for determining the molar mass averages and distributions for synthetic polymers via polystyrene relative calibration curves to include synthesis monitoring and oligomeric content analysis. The synthesis process for two PEGylated synthetic polymers intended for use in medical applications was analyzed by comparing the SEC chromatograms of the two PEGylated polymers with that of one of the starting materials. From this comparison it was concluded that starting material remained in one of the PEGylated samples, PEG-A, and was absent in the other PEGylated sample, PEG-B. The SEC chromatograms of the PEGylated polymers also provided indication of differences in the molar mass distribution between the two PEGylated samples. Additionally, based on the peak-average molar masses *Mp* the oligomeric

content of the two PEGylated polymers were shown to differ, with PEG-A containing mainly oligomeric species and PEG-B containing both low- and high-molar mass species. Combining the oligomeric content information with the SEC chromatograms was shown to provide a more detailed picture about the distribution of the low-molar mass species within the two PEGylated samples, information beneficial in the validation and regulation of synthetic polymers. Finally, the advanced engineering design of the EcoSEC GPC System, e.g., low-dead volume, minimal extra-column band broadening, etc., is an added advantage of using the system for synthesis monitoring and oligomeric analysis as it provides increased resolution and separation efficiency in the oligomeric region compared to traditional GPC systems.

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TOSOH

Application Note

CHARACTERIZATION OF A PLASTIC ALTERNATIVE VIA GPC: POLYHYDROXYBUTYRATE

INTRODUCTION

Traditional plastics have two primary environmental disadvantages: (1) they are believed not to decompose very rapidly as they are not biodegradable but photodegradable and (2) they are made from petroleum which is a nonrenewable resource that contributes significantly to global climate changes. Fortunately, during the past several decades there have been many promising developments of eco-friendly plastics. However, a large majority of eco-friendly plastics are only semi-eco-friendly as they include various amounts of previously recycled, petroleum-based plastics and are still non-biodegradable. Truly eco-friendly plastics are those that are composed of biological material rather than fossil fuels and are biodegradable.

One promising biodegradable substitute for plastics that is made from renewable resources is a biopolymer known as polyhydroxybutyrate or PHB. PHB is a linear polyester of D(-)-3-hydroxybutyric acid which was first discovered in the mid-1920s. PHB is biosynthesized by several bacteria as a means of carbon storage and source of reducing equivalents.[1] PHB is usually produced under conditions of carbon oversupply and low levels of other nutrients including nitrogen, phosphate and oxygen and is dependent on at least three different enzymes. Commercial production of PHB has been successfully attempted using relatively cheap substrates such as methanol, beet molasses, ethanol, scratch and whey, cane molasses, and soy cake as unrefined carbon sources and refined sugars.[2]

The use of PHB in commercial products is reliant on the development of low cost processes that produce biodegradable plastics with properties similar or superior to their petrochemical counterparts. Once a process for the production of PHB is developed, the physicochemical properties of the PHB must be characterized, as variations in properties such as the molar mass, will dictate how the biodegradable plastics performs compared to the petrochemical plastic. The chemical and thermal properties of PHB are typically analyzed using a collection of methods, namely nuclear magnetic resonance spectroscopy (NMR), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC).[1-2] Here we have implemented the use of the EcoSEC® GPC System encompassing a dual flow refractive index detector to determine the molar mass averages and molar mass distribution of two PHB polymers produced from different processes.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on a system consisting of an EcoSEC GPC System equipped with RI detector. Separation of unfiltered 25 µL injections occurred over a column bank consisting of two 4.6 mm ID × 15 cm, 3 & 5 µm TSKgel® SuperHZM-M column (exclusion limit 4×10^6 g/mol, PN 0019663). The mobile phase and solvent were chloroform at a flow rate of 0.30 mL/min. Detector, pump oven, and column oven were maintained at 35 °C. For all chromatographic determinations, results are averages of five injections from two separate sample dissolutions. Data was processed with the EcoSEC GPC Workstation software, version 1.08.

The two polyhydroxybutyrate polymers (PHB A and PHB B) were dissolved in chloroform for a final sample concentration of 1.0 g/L. Samples were heated to 60 °C while being stirred with a magnetic stir bar for two hours and then cooled to room temperature prior to injection. Complete dissolution of the sample did not occur. Approximately 80 to 90% of the sample went into solution, which is expected for poly-hydroxybutyrate polymers in chloroform according to the literature.

A calibration curve was created for the RI at 35°C using PStQuick C polystyrene mix standard ranging in molar mass from 530 to 2.9×10^6 g/mol. Calibration curve data for 0.30 mL/min was fitted with a linear function and error values were less than 5%.

GPC ELUTION PROFILE OF A COMMERCIALLY AVAILABLE PHB SAMPLE (PHB A) AND A HOMEMADE PHB SAMPLE (PHB B)

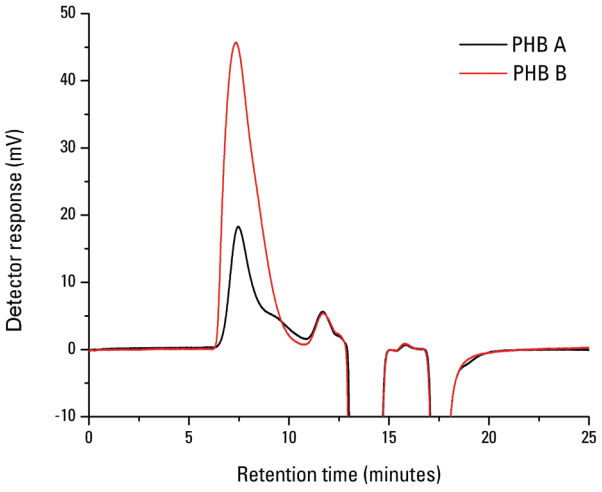

Figure 1

RESULTS AND DISCUSSION

The molar mass averages and molar mass distributions of PHB differ according to the organism, conditions of growth and method of extraction, and can vary from about 5×10^5 to well over a million, thus the ability to characterize the molar mass averages and molar mass distribution of the PHB is critical in assessing if the biodegradable plastics produced will have properties similar or superior to their petrochemical counterparts.[1] An EcoSEC GPC System encompassing a dual flow RI detector with semi-micro GPC columns was used to determine the molar mass averages and molar mass distribution of a commercially available PHB sample (PHB A) and a homemade PHB sample (PHB B).

The GPC chromatograms of the PHB samples as monitored by the RI detector are shown in Figure 1. The commercially available PHB sample (PHB A) elutes prior to the homemade PHB sample (PHB B). The slightly shorter retention time of the PHB A sample indicates that the commercially available PHB is larger in polymeric size than the homemade PHB. In addition to variations in elution time amongst the two samples, the shape of the GPC elution profile shows distinctive differences.

The PHB B sample has a fairly Gaussian shaped GPC elution profile while the PHB A sample GPC elution profile has a shoulder towards the later elution time, smaller analyte region of the chromatogram. The shoulder seen in the GPC elution profile of the PHB A sample is an indication that a second distinctive species is present within the sample. The additional species may be additional PHB with a different polymeric size or molar mass, an indication that the process for producing the PHB product was not completely finished or side products of the processing of the biodegradable material. Identification of the exact source of the shoulder on the PHB B GPC elution profile would require the use of additional detection methods.

The molar mass averages, Mn, Mw, and Mz, as determined via a polystyrene RI calibration curve are given in Table 1. The molar mass averages of the commercial available PHB (PHB A) and the homemade PHB (PHB B) are in agreement with the variations seen in the GPC elution profile, as the molar mass averages for PHB A are slightly less than those of PHB B. In general the molar mass averages are affected by the GPC elution profiles as the molar mass is determined for each slice eluting from the GPC column and then weighted averages are calculated based on the molar mass at each eluting slice and the RI detector response. The number- and weight-average molar masses vary the most between the two PHB samples while the Mz values are more comparable to one another. The Mn is influenced by the longer retention time portion of the GPC elution profile, which is very similar in retention times but not GPC elution profile shape, for PHB A and PHB B. The z-average molar mass is influenced by the shorter retention time portion of the GPC elution profile, which extends only slightly further towards the higher molar mass region for PHB B than PHB A.

In addition to comparing the molar mass averages of the two PHB samples, the polydispersity index, PDI, can also be compared. The polydispersity of the commercially available PHB, PHB A, is nearly double that of homemade PHB, PHB B, PDI=8.744 and PDI=4.863 for PHB A and PHB B, respectively.

CONCLUSIONS

Two polyhydroxybutyrate polymers, commercially available PHB sample (PHB A) and a homemade PHB sample (PHB B), where characterized based on the polystyrene relative molar mass averages, molar mass distributions, and GPC elution profiles as obtained using the EcoSEC GPC System with semi-micro GPC columns. The GPC elution profiles for the two PHB samples indicated a difference between the two samples as the peak shape, breadth and retention time differed. Variations between the commercially available PHB and the homemade PHB were also observed in the molar mass averages and molar mass polydispersity. The molar mass averages vary more towards the lower molar mass, smaller polymer size, longer retention time portion of the GPC elution profile as the values for the number- and weight-average molar masses differ more than the z-average molar mass values. Additionally the polydispersity of the commercially available PHB sample was twice that of the homemade PHB sample.

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF A COMMERCIALLY AVAILABLE PHB SAMPLE (PHB A) AND A HOMEMADE PHB SAMPLE (PHB B)

Sample	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	PDI[a]
PHB A	8.22×10^4 $\pm 0.49^b \times 10^4$	7.17×10^5 $\pm 0.01 \times 10^5$	1.44×10^6 $\pm 0.01 \times 10^6$	8.74 ± 0.38
PHB B	2.15×10^5 $\pm 0.14 \times 10^5$	1.04×10^6 $\pm 0.01 \times 10^6$	2.00×10^6 $\pm 0.01 \times 10^6$	4.86 ± 0.30

[a] PDI = Mw/Mn [b] Standard deviations from four injections

Table 1

The large difference in the PDI between samples may be of concern if the two PHB samples are intended to be identical, as large differences in PDI and molar mass averages can have large effects on the end-use properties of polymers. Variations in the molar mass averages and molar mass distributions of PHB can affect the thermoplasticity and biodegradability of the plastic thus the differences in the molar mass averages of the PHB A and PHB B samples may not only affect the end-use properties of the PHB but also the environmental impact of the a product made with PHB.[1]

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TOSOH

Application Note

MOLAR MASS MEASUREMENTS OF AN ISOCYANATE MODIFIED POLYURETHANE PREPOLYMER IN LESS THAN 1 HOUR

INTRODUCTION

Isocyanates are both highly reactive and highly toxic low molar mass chemicals. One common technique used to take advantage of isocyanate reactivity while eliminating safety concerns is to synthesize polyurethane prepolymers for use in subsequent polymerizations. The physical properties of the resultant polymer are influenced to a large degree by the size of the polyol chains in the prepolymer. Harder polymers are formed with larger polyol chains and softer polymers are formed with smaller polyol chains.1 Here we report on the use of the EcoSEC GPC System to determine the molar mass and molar mass averages of an isocyanate modified polyurethane prepolymer (IMPP) with residual dimethyl sulfoxide (DMSO). The low dead volume of the EcoSEC GPC System combined with the use of semi-micro GPC columns allowed for an efficient separation and characterization of the prepolymer sample in less than 1 hour.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on a system consisting of an EcoSEC GPC System (HLC-8320) equipped with a refractive index detector (RI). Separation of unfiltered 20 µL injections occurred over a column bank consisting of two 6.0 mm ID × 15 cm, 3 µm particle size TSKgel® SuperH3000 (PN 0017993) columns preceded by the appropriate guard column (PN 0018002) (Tosoh Bioscience). The solvent and mobile phase were tetrahydrofuran (THF) (Fisher Chemical) at flow rates of 0.3 and 0.6 mL/min. Detector, pump oven, and column oven were maintained at 35 °C. For all chromatographic determinations, results are averages of three injections from two separate sample dispersions. Sample solutions were prepared by diluting 99% pure sample with THF for a final sample concentration of approximately 10 mg/mL. Samples were shaken manually for a minute and allowed to sit for 3 hours before analysis was performed. Data was processed with the EcoSEC GPC Workstation Software version 1.08. A RI calibration curve was created using PStQuick Kit-L polystyrene standards (PN 0021915) ranging in molar mass from 266 to 37,900 g/mol (Figure 1).

RI CALIBRATION CURVE FOR PSTQUICK KIT-L POLYSTYRENE STANDARDS AT 0.3 ML/MIN IN THF AT 35 °C

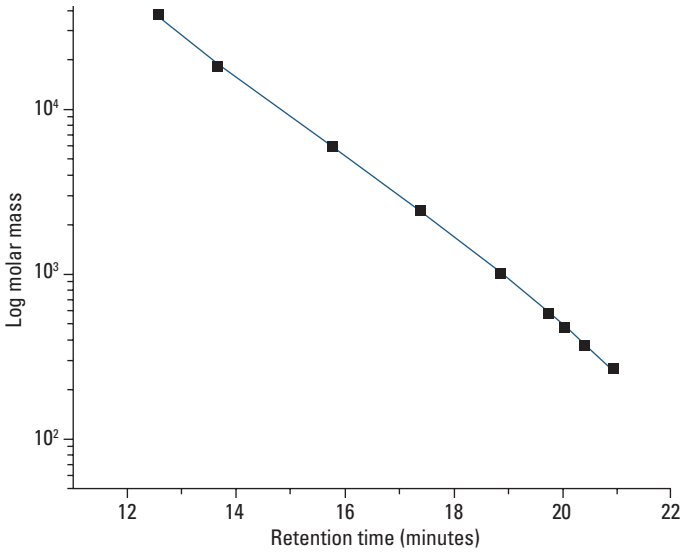

Figure 1

SEC ELUTION PROFILE OF IMPP SAMPLE AS MONITORED BY RI (BLUE) AT 0.3 ML/MIN IN THF AT 35 °C

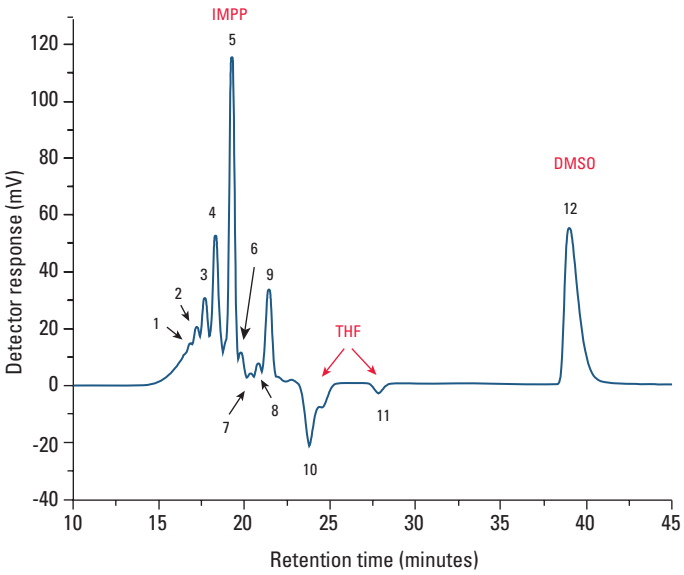

Figure 2

Calibration curve data for both 0.3 and 0.6 mL/min were fitted with a cubic function and error values were no greater than 5 %.

RESULTS AND DISCUSSION

An EcoSEC GPC System encompassing a refractive index detector was used to perform size exclusion chromatography analysis on an IMPP sample composed of 54% urethane prepolymer, 11.5% dimethyl sulfoxide (DMSO), and 34.5% 1,1,1,3,3 pentafluoropropane. As seen in Figure 2, separation of the sample by size exclusion chromatography results in ten positive and two negative chromatographic peaks. The nine positive chromatographic peaks eluting from the column between 14.0 and 22.5 minutes correspond to the urethane prepolymer and 1,1,1,3,3 pentafluoropropane. Peaks 1 through 5 correspond to the urethane prepolymer and peaks 6 through 9 correspond to either the urethane prepolymer or 1,1,1,3,3 pentafluoropropane. The identities of peaks 6 through 9 were not confirmed due to the lack of availability of 1,1,1,3,3 pentafluoropropane. The two negative peaks at 23.7 and 27.8 minutes are indicative of the sample solvent, THF. Additionally, the latest eluting peak at 39.0 minutes is a result of residual DMSO present in the IMPP sample. Note that the DMSO peak elutes after the void volume of the column (~26.5 minutes) and as such is retained by a non-SEC retention mechanism.

The RI calibrant-relative molar mass averages M_n, M_w, and M_z for the IMPP sample were calculated based on the RI calibration curve, see Figure 1, and are given in Table 1. The weight-average molar mass, M_W, of the IMMP sample ranges from 4,606 g/mol for the earliest eluting peak to 181 g/mol for the latest eluting peak, with an average weight-average molar mass value of 1,531 g/mol. Prior knowledge of the sample predicted the molar mass range to be from 1,000 to 5,000 g/mol.

CUMULATIVE AND DIFFERENTIAL MOLAR MASS DISTRIBUTION FOR IMPP SAMPLE IN THF AT 0.3 ML/MIN

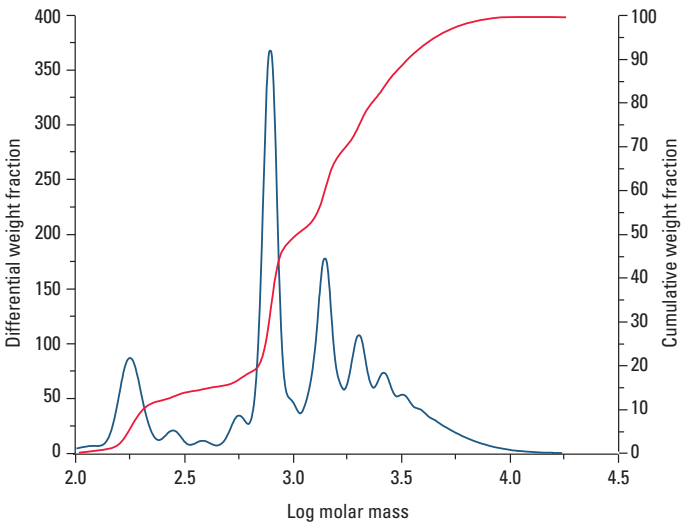

Figure 3

SEC ELUTION PROFILE OF IMPP SAMPLE AS MONITORED BY RI (BLUE) AT 0.6 ML/MIN IN THF AT 35 °C

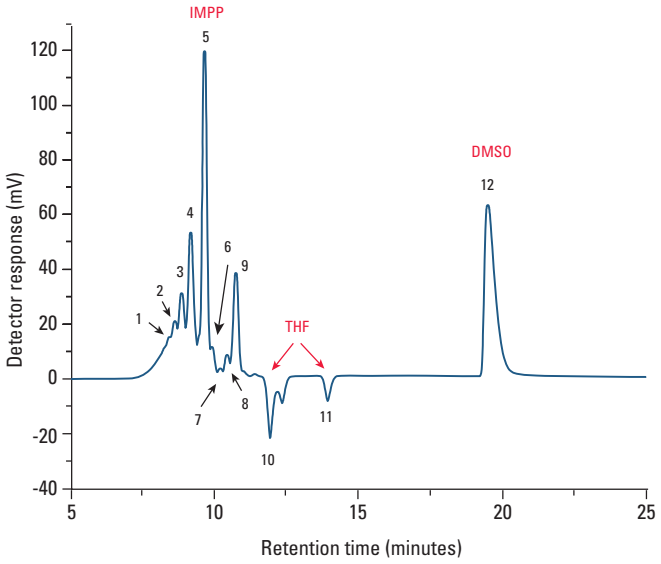

Figure 4

MOLAR MASS AVERAGES, AND POLYDISPERSITY INDEX FOR IMPP SAMPLE IN THF AT 0.3 ML/MIN

Peak	Retention Time (min)	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI[a]
1	16.858	4,199 ± 46b	4,606 ± 67	5,214 ± 109	1.09± 0.01
2	17.222	2,643 ± 19	2,655 ± 18	2,667 ± 18	1.01 ± 0.01
3	17.688	2,011 ± 16	2,024 ± 16	2,038 ± 16	1.01 ± 0.01
4	18.314	1,387 ± 10	1,403 ± 10	1,418 ± 10	1.01 ± 0.01
5	19.335	798 ± 4	808 ± 5	817 ± 5	1.01 ± 0.01
6	19.790	551 ± 9	554 ± 9	557 ± 9	1.01 ± 0.01
7	20.370	391 ± 9	394 ± 9	397 ± 10	1.01 ± 0.01
8	20.824	278 ± 3	280 ± 3	282 ± 3	1.01 ± 0.01
9	21.439	178 ± 1	181 ± 1	183 ± 1	1.01 ± 0.01
All		676 ± 9	1,531 ± 31	2,873 ± 83	2.26 ± 0.02

[a] PDI = M_w/M_n; [b] Standard deviations from six injections

Table 1

By combining the molar mass information found in Table 1 with prior knowledge of the sample it can be concluded that peaks 1 through 5 are indeed the urethane prepolymer and peak 9 is most likely 1,1,1,3,3 pentafluoropropane. The low molar masses corresponding to peaks 6 through 8 may be oligomers or unreactive material from the synthesis process of IMPP. The polydispersity index, PDI = M_w/M_n, for the entire urethane prepolymer sample including 1,1,1,3,3 pentafluoropropane (peaks 1 through 9) was 2.26, while the nine individual components had PDI values ranging from 1.01 to 1.09. From the PDI values it can be concluded that collectively the sample is polydisperse with respect to molar mass but the nine visible components within the IMPP sample are virtually monodisperse with respect to molar mass. The molar mass distribution for the IMPP sample, as obtained at 0.3 mL/min, is shown in Figure 3.

Analysis of the IMPP was initially performed at a flow rate of 0.3 mL/min (the lowest recommended flow rate for the TSKgel SuperH3000 columns) and total analysis was achieved in 45 minutes (Figure 2). In order to increase the throughput of the EcoSEC GPC System the flow rate was increased to 0.6 mL/min (the highest recommended flow rate for the TSKgel SuperH3000 columns) and total analysis was achieved in 22 minutes (Figure 4). As seen by comparing Figures 2 and 4, doubling the chromatographic flow rate had a minimal effect on the chromatographic resolution of the IMPP sample. The only noticeable difference between the chromatograms at 0.3 mL/min and 0.6 mL/min is a slight decrease in chromatographic resolution that occurs between peaks 5 and 6.

CONCLUSIONS

An IMPP sample was analyzed using an EcoSEC GPC System with a refractive index detector. The molar mass averages and polydispersity index of the IMPP sample was determined using a polystyrene relative calibration curve. The chromatogram of the IMPP displayed twelve distinctive peaks. Peaks 1 through 5 were determined to be the urethane prepolymer component of the IMPP and found to have a weight average molar mass ranging from 4,199 to 798 g/mol. The identity of peaks 6 through 9 were not confirmed but are hypothesized to be urethane prepolymer, unreactive species from the synthesis of the sample or 1,1,3,3 pentafluoropropane based on their molar mass range, Mw = 551-178 g/mol. Peaks 10 and 11 and peak 12 are due to the THF used to dilute the IMPP sample and the residual DMSO in the IMPP sample, respectively. The sample was analyzed at two different chromatographic flow rates, 0.3 and 0.6 mL/min. A flow rate of 0.6 mL/min compared to that of 0.3 mL/min resulted in a decrease in analysis time from 45 minutes to 22 minutes. In conclusion, the molar mass averages and polydispersity of the IMPP sample were successfully determined in less than 1 hour using the EcoSEC GPC System.

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TOSOH

Application Note

ANALYSIS OF RENEWABLE-BASED POLYMERS USING THE ECOSEC® GPC SYSTEM COUPLED TO MULTI-ANGLE LIGHT SCATTERING

INTRODUCTION

Scientist are continuously working on reducing the our reliance on fossil fuels by developing environmentally friendly and cost effective plastics from natural, sustainable and renewable materials, such as vegetable oils, starches, and sugars. The overall goal is to reduce the reliance on petroleum-based plastics and help mitigate environmental damage by designing materials that are compostable and less harmful while in use. Manufacturers within the automotive, footwear, carpet, and furniture sectors are starting to demand renewable or bio-based polymers as they seek to sell more sustainable products. A renewable or bio-base polymer is a plastic material that addresses the needs of a consumer without damaging our environment, health and economy. The feedstock of renewable polymers is typically a plant and production of such polymers uses less net water and non-renewable energy, emits less greenhouse gases and has a smaller carbon footprint than their non-renewable counterparts, while still being economically viable.

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 physical and chemical properties of renewable polymers can be analyzed using the same methods used for the analysis of non-renewable polymers such as infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), gel permeation chromatography (GPC), and melt flow. Each of these techniques provides details about the macromolecular properties and thus provides information about the end-use properties of the renewable polymer.

The specific end-use properties of a batch of TPUs, such as tensile strength, elongation, conductivity, chemical resistance, and toughness, depends on macromolecular properties such as molar mass, branching, degree of crosslinking, and polymeric size. The molar mass and polymeric size of TPUs can be determined using GPC coupled to a series of detection methods. Here we have used the EcoSEC GPC System encompassing a dual flow refractive index (RI) detector with a multi-angle light scattering detector (MALS) to determine the absolute molar mass, absolute molar mass distribution, and polymeric size of two different batches of TPUs.

EXPERIMENTAL

Dual detector GPC analysis was performed using the EcoSEC GPC System equipped with a refractive index detector (RI) coupled in series to a DAWN® 8+ MALS photometer (Wyatt Technology Corporation). Separation of unfiltered 100 µL injections occurred over a column bank consisting of two 7.8 mm ID × 30 cm TSKgel® GMH$_{HR}$-H columns (separation range ~500 to 4 × 10^8 g/mol) (PN 0017360). The mobile phase and solvent were dimethylformamide (DMF) with 0.01% LiBr at a flow rate of 1.0 mL/min. Detector, pump oven, and column oven were maintained at 45 and 50 °C depending on the component. Data was processed using Wyatt's ASTRA® 6.1 software. Two TPU samples were prepared with a final concentration of 1.0 g/L. All chromatographic determinations are averages of three injections from one sample dissolution.

The DAWN 8+ detector was normalized in-house using a polystyrene standard, with a molar mass of 3.79 × 10^4 g/mol. Calculations for interdetector delays and interdetector band broadening corrections were performed using the same polystyrene standard used to normalize the detector. Calibration of the MALS unit was performed using toluene. All MALS data were fitted using a Zimm model. The $\partial n/\partial c$ values for each sample were determined using an online 100% mass recovery method using the RI detector housed within the EcoSEC GPC System.

RESULTS AND DISCUSSION

Several macromolecular properties of renewable polymers can be characterized using GPC coupled to detection methods such as refractive index and multi-angle light scattering. The coupling of a size-based separation method such as GPC to an absolute detection method such as multi-angle light scattering allows for the determination of the absolute molar mass, absolute molar mass distribution and polymeric size of polymers such as TPUs. Two different batches of TPUs were analyzed in DMF. The GPC experiments provided several forms of comparison between the two different batches of TPUs: GPC chromatograms, absolute molar mass and molar distributions, and polymer size (radius of gyration) and polymer size distribution.

The GPC elution profiles of the two different batches of TPUs as monitored by the MALS detector is shown in Figure 1. TPU Batch B elutes after TPU Batch A. The longer

retention time of TPU Batch B indicates that TPU Batch B is slightly smaller in polymeric size compared to the TPU Batch A: as elution order in GPC is that of an "inverse-sieving" technique, smaller analytes elute after the larger analytes.

The polymeric size comparison can also be done quantitatively as the addition of a MALS detector to the EcoSEC GPC System permits for the determination of a polymeric sizing parameter, the root-mean-square radius or radius of gyration, RG. The average radius of gyration for both TPUs, A and B, were identical, RG = 20 nm, but TPU Batch A does contain some species larger in polymeric size than TPU Batch B. 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 1, the size of both TPUs decreases as a function of increasing retention time, an indication that the samples are polydisperse with respect to size.

GPC ELUTION PROFILE OF TWO BATCHES OF TPUS AS MONITORED BY MALS AND RADIUS OF GYRATION DISTRIBUTION ACROSS THE ELUTION PROFILES AS DETERMINED BY MALS

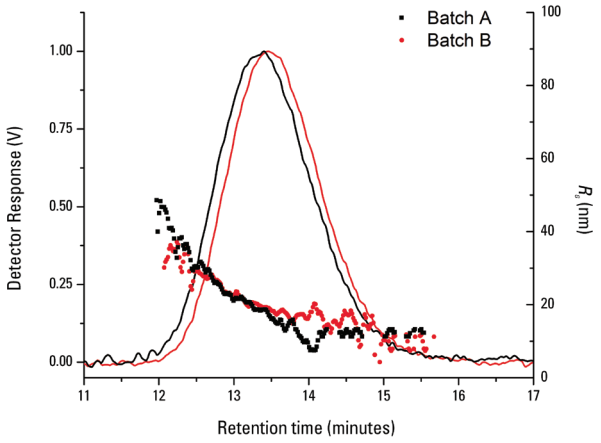

Figure 1

GPC ELUTION PROFILE OF TWO BATCHES OF TPUS AS MONITORED BY MALS AND ABSOLUTE MOLAR MASS DISTRIBUTION ACROSS THE ELUTION PROFILES AS DETERMINED BY MALS

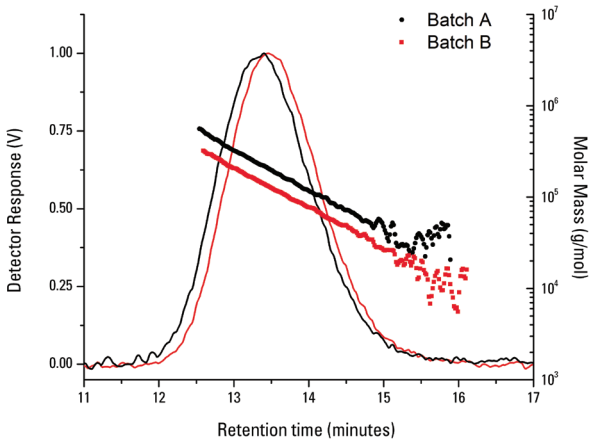

Figure 2

The size PDI value for batch A is slightly greater than that of batch B, 1.3 and 1.1, respectively.

Figure 1 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.

The absolute molar mass averages and the molar mass distributions of the two different batches of TPUs were also determined. The molar mass distribution of the two batches of TPUs as plotted across the GPC elution profile, as monitored by the 90° light scattering signal, are shown in Figure 2. 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 2. Additionally, the molar mass polydispersity index, PDI, of the two batches indicate samples polydisperse in molar mass as PDI = 1.6 for both batches. TPU Batches A and B when analyzed by GPC coupled to RI and MALS produce GPC elution profiles, absolute molar mass averages and distributions as well as polymeric size measurements similar enough to one another to create products with the same end-use properties.

CONCLUSIONS

Two batches of thermoplastic polyurethanes, a renewable-based polymer, was characterized based on the GPC elution profile, absolute molar mass averages and absolute molar mass distribution, and polymeric size and polymer size distribution using the EcoSEC GPC System with a dual flow refractive index detector coupled to a multi-angle light scattering detector. The GPC elution profiles of the two batches of TPUs were found to vary slight as TPU Batch A elutes slightly later than TPU Batch B. The absolute molar mass averages and absolute molar mass distribution of the two TPU samples were found to be similar to one another, with TPU Batch A being slightly higher in molar mass than TPU Batch B and both batches having identical polydispersity index values.

The polymeric size of the two TPU Batches was also determined to be identical as the radius of gyration and radius of gyration distribution were almost identical to one another. The coupling of the EcoSEC GPC System encompassing a dual flow refractive index detector with multi-angle light scattering detector (MALS) allowed for the determination of several macromolecular properties, e.g. absolute molar mass, absolute molar mass distribution, and polymeric size, which influence the end-use properties of a polymer.

TOSOH

Application Note

ANALYSIS OF WATER-SOLUBLE POLYMERS USING LINEAR SIZE EXCLUSION HPLC COLUMNS AND A SEMI-MICRO SEC SYSTEM

In recent years water-soluble polymers are gaining more and more interest in different applications. The molecular weight distribution of polymers is usually characterized by size exclusion chromatography (SEC) coupled with refractive index, viscometric or laser light scattering detection. Recent advances in SEC comprise semi-micro SEC and the design of linear columns providing wide molecular weight separation ranges and near-linear calibrations. We describe the separation of water-soluble polymers with a new generation of linear, polymer-based SEC columns using the compact EcoSEC SEC system.

INTRODUCTION

One of the most widely used water-soluble polymers is Polyvinylpyrrolidone (PVP). For more than 70 years it is applied in a variety of applications in medicine, pharmacy, cosmetics and industrial production. PVP binds to polar molecules exceptionally well. It supports the solubility of tablets in water, makes the glue stick adhesive and plays an essential role in the production of membranes. In ceramic, paper and dye applications, it produces visual effects such as gloss and brilliancy. As a food additive, PVP is a stabilizer (E1201). A characteristic feature of a certain polymer is its molecular weight distribution curve, which is usually obtained by SEC Analysis.

LINEAR SEC COLUMNS

The latest progress in size exclusion chromatography (SEC) column technology was the development of columns with an extended linear calibration range. A linear molecular weight calibration curve covering an extended molecular weight operation range can be obtained in different ways. The traditional and simplest way is to couple different columns, each containing particles with distinct pore size distributions. It is inevitable that the resulting calibration curve will deviate from linearity between each pore size range, resulting in irregularities in the chromatogram. Therefore mixed-bed columns were developed to overcome column mismatches. Mixed-bed columns are prepared by mixing batches of particles each containing a narrow pore size distribution. However, inflection points in chromatograms can still arise due to a mismatch of pore sizes. The latest development in SEC column design, the multi-pore particle synthesis technology can provide a real solution to avoid inflection points. Particles prepared by multi-pore technology contain a broad range of pore sizes in a single polymeric bead. This innovative approach essentially creates a linear calibration curve within each particle (Figure 1).

MULTI-PORE TECHNOLOGY

Strategies for wide range separation using Size Exclusion Chromatography

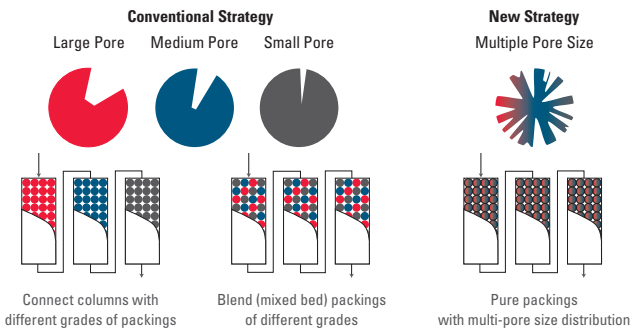

Figure 1

The multi-pore particle synthesis technology was used to design multi-pore particles of the polymethacrylate based TSKgel PW type packing for aqueous SEC. The new TSKgel SuperMultiporePW columns are packed with spherical mono-disperse polymethacrylate particles, each containing a wide range of pore sizes. Based on their small particle sizes it is possible to reach high theoretical plates at half of the length of a conventional SEC column of 30 cm length. The TSKgel SuperMultipore PW series comprises of three column types covering different molecular weight ranges (PW-N; PW-M, PW-H). They exhibit high resolution and good linearity of the calibration curves across a wide range of molecular mass of PEO and PEG standards in aqueous eluent as shown in Figure 2. With dimensions of 6 mm ID and 15 cm length they belong to the semi-micro type of SEC columns.

SEMI-MICRO SEC

Semi-micro column dimensions are advantageous in terms of shorter analysis time, lower solvent consumption and reduced solvent disposal cost. To fully exploit the advantages of semi-micro SEC columns the HPLC system needs to be optimized to minimize extra column peak broadening. This means first of all minimization of dead volume. Key components of the HPLC system with

regard to dead volume reduction are the void volumes of tubings, the detector cell volume and the void volume of the injection unit. EcoSEC is a compact, all-in-one SEC system that was designed to perform both, semi-micro and conventional SEC. Key features of the system are the low system dead volume, outstanding pump reproducibility and an extremely stable baseline of the dual flow refractive index (RI) detector. The RI detector is designed in a way that the pure mobile phase is permanently rinsing the reference site of the detector cell. The solvent in the sample site and the reference site of the cell has always the same quality and temperature. This leads to extremely stable baselines allowing a proper integration and processing of smallest peaks. The small size of the RI's detector cell (2.5 µl) minimizes peak broadening. The EcoSEC SEC system offers outstanding performance, high sensitivity and short analysis time when combined with TSKgel semi-micro SEC columns.

ANALYSIS OF WATER-SOLUBLE POLYMERS

Water-soluble synthetic polymers were analyzed using the EcoSEC SEC system with RI detection and the newly developed TSKgel SuperMultiporePW-M (5 µm particle size, 6 mm ID x 15 cm L) size exclusion column with 0.1 M $NaNO_3$ as mobile phase. Injection volume was 35 µl and flow rate 0.6 mL/min at 25 °C. Figure 3 shows the SEC analysis of a real sample - Polyvinylpyrrolidone (PVP) K-30 - on a series of conventional TSKgel G3000PW$_{XL}$ and G5000PW$_{XL}$ columns compared to the one obtained with a single TSKgel SuperMultiporePW-M semi-micro linear SEC column (MW range 600 000 – 1 500 000). When analyzed with a series of conventional SEC columns the Polyvinylpyrrolidone peak shows an inflection point, which does not appear on the SuperMultiporePW-M column. Due to its smaller particle size and the semi-micro column dimensions, analysis is much faster and more sensitive when applying the new multi-pore packing. Figure 4 shows the SEC elugrams of various water-soluble polymers obtained on a TSKgel SuperMultiporePW-M column.

CALIBRATION CURVES FOR TSKgel SuperMultiporePW

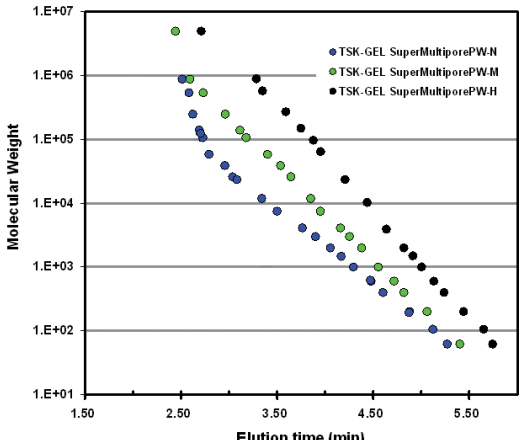

Figure 2

Mobile phase: H_2O, Flow rate: 0.6 mL/min, RI detection, 25°C, PEO & PEG standards

CONCLUSION

Chromatograms obtained on TSKgel SuperMultiporePW columns show high resolution and smooth peak shapes without shoulders or inflection points, thus allowing better accuracy and reproducibility when determining the molecular mass distribution of water soluble polymers. Various polymers and oligomers were analyzed on the TSKgel SuperMultiporePW semi-micro columns. Compared with conventional SEC columns, the TSKgel SuperMultiporePW columns delivered superior results at shorter run times and lower solvent consumption. The results illustrate the advantages of these materials over traditional columns for aqueous SEC.

ANALYSIS OF POLYVINYLPYRROLIDONE

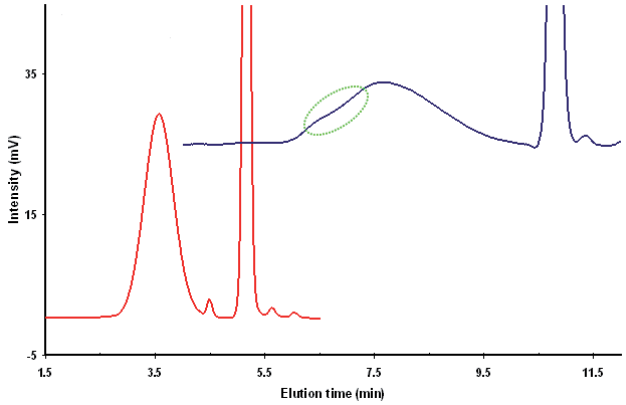

Figure 3

Columns: TSKgel SuperMultiporePW-M (6 mm ID x 15 cm L x 1) (red); TSKgel G3000PW$_{XL}$ & G5000PW$_{XL}$ (each 7.8 mm ID x 30 cm

ANALYSIS OF WATER-SOLUBLE POLYMERS

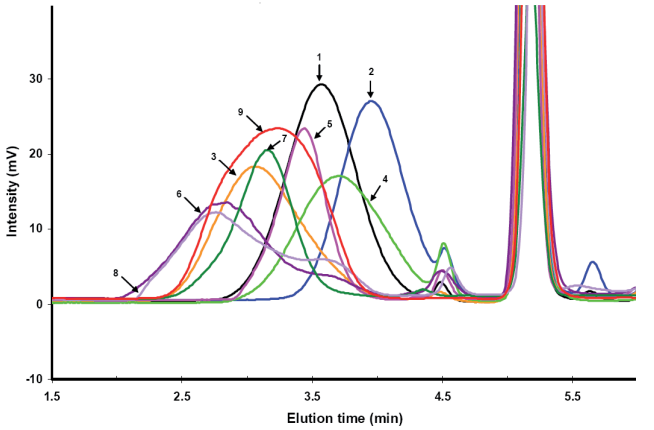

Figure 4

Column: TSKgel SuperMultiporePW-M (6 mm ID x 15 cm L), Samples: 1: polyvinylpyrrolidone (K-30), 2: polyvinylpyrrolidone (K-15), 3: hydroxypropylcellulose (200 K) 4: hydroxypropylcellulose (100 K), 5: chondroitin sulfate sodium salt, 6: carboxymethyl cellulose, 7: arabic gum, 8: poly(sulfopropyl methacrylate-co-acrylic acid), 9: dextran (200K)

TOSOH

Application Note

MOLAR MASS DETERMINATION OF A SYNTHETIC RUBBER RESIN VIA SINGLE-, DUAL- AND TRIPLE-DETECTOR GPC

INTRODUCTION

Natural and synthetic rubbers are key components to many applications, e.g. clothing, vehicles, toys, fire arms, etc. Currently synthetic rubbers constitute 75% of all rubber consumption worldwide. Due to the high consumption of synthetic rubber and synthetic rubber products, it is critical to understand the relationship between molecular structure and physical properties, as these properties directly affect how useful products perform under a variety of demanding end-use conditions. The molecular characteristics of greatest significance for the physical properties of synthetic rubbers are considered to be the nature of the monomer units, molar mass, cross-linking, chain structure, and chain branching.[1] The ability to characterize the molecular properties of rubber compounds present analysts with one of their most difficult challenges, as compounds may contain over 15 different ingredients,[2] some in very low concentrations, and analysis goals can range from quality control to reverse engineering to failure analysis.

The most preeminent technique analysts use for the characterization of physicochemical properties of natural and synthetic rubbers is gel permeation chromatography (GPC). One of the advantages of GPC is that it can be used to provide molar mass averages and molar mass distributions of both the raw polymers used in the rubber product and the final compounded product. Traditionally, single-detector GPC systems encompassing a refractive index detector (RI) has been used to determine polystyrene relative molar mass averages.[2] The developments and advances in dual- (RI and differential viscometry [VISC]) and triple- (RI, VISC, and multi-angle light scattering [MALS]) detector GPC systems over the past twenty years has resulted in the increased use of multi-detector GPC systems for the determination of "absolute" molar mass averages, as a single-detector GPC system can easily be expanded to a dual- or triple-detector GPC system with the addition of external VISC and MALS detectors.

Here we demonstrate the expansion of the EcoSEC GPC System, equipped with a dual flow RI detector for single-detector GPC experiments, to a dual- and triple-detector GPC system encompassing external VISC and MALS detectors for multi-detector GPC experiments. The multiplicity of detector combinations allows for the determination of the molar mass averages and distributions and various other polymer parameters, e.g. radius of gyration, viscometric radius, and intrinsic viscosity, of a synthetic rubber via three independent modes: (1) polystyrene relative calibration curve (GPC/RI), (2) universal calibration curve (GPC/RI/VISC), and (3) absolute molar mass (GPC/RI/VISC/MALS).

EXPERIMENTAL CONDITIONS

Single-detector GPC analysis was performed using the EcoSEC GPC System equipped with a refractive index detector (RI). Dual- and triple-detector GPC analysis was performed by either coupling in parallel an ETA-2010 Viscosity detector (Polymer Standards Service, PSS) or a SLD 7000 Light Scattering Photometer (PSS) and an ETA-2010 Viscosity detector (PSS) to the EcoSEC GPC System mentioned above.

Separation of unfiltered 25 µL injections occurred over a column bank consisting of three 4.6 mm ID × 15 cm, 6 µm particle size TSKgel® SuperMultiporeHZ-H columns (separation range ~1,000 to 1.0 × 10^7 g/mol, exclusion limit 4.0 × 10^7 g/mol , PN 0021885). The mobile phase and solvent were tetrahydrofuran (THF) at a flow rate of 0.35 mL/min. Detector, pump oven, and column oven were maintained at 35 °C. The synthetic rubber resin sample was prepared by diluting the sample in THF for a final concentration of ~1.6 to 2.6 g/L and left to sit for 3 hours before analysis was performed.

UNIVERSAL CALIBRATION CURVE FOR TEN POLYSTYRENE STANDARDS RANGING IN MOLAR MASS FROM 1.0 × 10^4 TO 6.8 × 10^6 g/mol

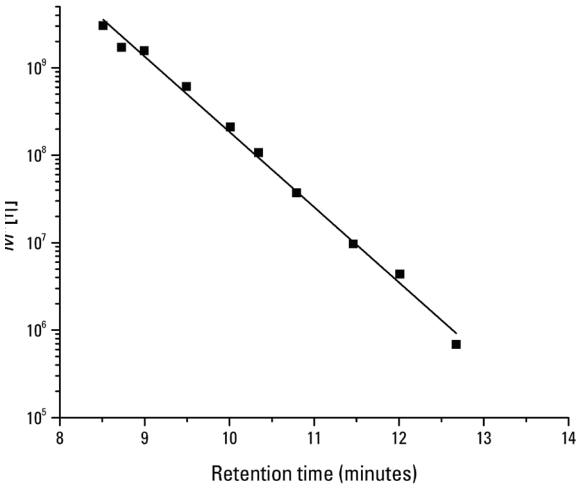

Figure 1

All chromatographic determinations are averages of six injections from two separate sample dissolutions. Data was processed with WinGPC® Unity software version 7.4.0.

Molar mass averages obtained from single-detector GPC experiments were determined based on a polystyrene relative calibration curve created from PStQuick Kit MP-H polystyrene mix standard ranging in molar mass from 1,110 to 5.5 × 10^6 g/mol under the same experimental conditions as sample analysis. The calibration curve data was fitted with a linear function and error values were less than 6%. Molar mass averages from dual-detector GPC experiments were determined based on an universal calibration curve created using ten polystyrene standards ranging in molar mass from 1.0 × 10^4 to 6.8 × 10^6 g/mol under the same experimental conditions as sample analysis, Figure 1. The universal calibration curve data was fitted with a linear function and had a R^2 value of 0.999. For triple-detector GPC experiments the normalization of SLD 7000 Light Scattering Photometer and calculation of interdetector delays were performed using a virtually monodisperse polystyrene standard with a molar mass of 9.6 × 10^4 g/mol. All MALS data were fitted using the Debye model for data from seven different angles ranging from 35° to 145°. The specific refractive index increment (∂n/∂c) value of the synthetic rubber resin sample was determined previously to be 0.118 mL/g.

RESULTS AND DISCUSSION

The molar mass averages of a synthetic rubber resin sample were determined using three independent methods: (1) polystyrene relative calibration via single-detector GPC, (2) universal calibration via dual-detector GPC and (3) absolute detection via triple-detector GPC. In general, the three methods are expected to provide different values for the molar mass averages and molar mass distributions as each method is dependent on different parameters and involves various assumptions. Molar mass averages obtained via single-detector GPC using calibrant-relative calibration are dependent on the chemistry and architecture of both the standards used to construct the curve and the analyte under analysis. Relative calibration curves are quite precise, but their accuracy is only good when the chemistry and architecture of the analyte are identical to those of the calibration standards.[3] Molar mass averages obtained via universal calibration are considered to be absolute in nature, that is independent of chemistry and architecture, but are dependent on solvent and temperature conditions. Universal calibration curves can be constructed using standards of chemistry and/or architecture different from those of the analyte under analysis, since the calibration is based on the product of the molar mass, M, and the intrinsic viscosity, [η], of the polymer sample. Molar mass averages obtained via multi-angle light scattering are absolute in nature, as they are independent of solvent and temperature conditions and calibration curves are not required to obtain quantitative information.

The molar mass averages and polydispersity index as obtained by the three methods mentioned above are given in Table 1. As mentioned previously the molar mass averages as determined by the three different methods are not expected to be equal. As seen in Table 1 the molar mass averages, M_n, M_w, and M_z, using all three methods are in fair agreement with one another. The similarity between the molar mass averages obtained via absolute detection (MALS) and the various calibration methods could be a result of several factors. The chemistry and architecture of the synthetic rubber resin may be fairly similar to that of the standard used for the calibrant-relative calibration (linear polystyrene) making the calibrant-relative calibration data more accurate. Additionally, the solvent and temperature conditions used for sample analysis may be classified as "good" thus increasing the reliability of the universal calibration. The molar mass polydispersity is also in agreement amongst the three methods as PDI > 1, an indication that the synthetic rubber resin is polydisperse with respect to molar mass.

The GPC chromatograms of the synthetic rubber resin, as monitored by the individual detectors in the triple-detector GPC experimental set-up, are given in Figure 2. It should be noted that the GPC chromatograms as monitored by the RI and VISC in single- and dual-detector GPC experiments are comparable to those of the triple-detector GPC experiments. Unlike the single- and dual-detector GPC

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF A SYNTHETIC RUBBER RESIN OBTAINED VIA GPC/RI, GPC/RI/VISC AND GPC/RI/VISC/MALS.

Detection Method	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI[a]
Single-detector GPC (GPC/RI)	1.185 × 10^5 ± 0.006[b] × 10^5	4.265 × 10^5 ± 0.031 × 10^5	7.657 × 10^5 ± 0.024 × 10^5	3.60 ± 0.04
Dual-detector GPC (GPC/RI /VISC)	1.082 × 10^5 ± 0.341 × 10^5	4.285 × 10^5 ± 0.337 × 10^5	9.094 × 10^5 ± 0.521 × 10^5	3.96 ± 0.15
Triple-detector GPC (GPC/RI/VISC/MALS)	1.327 × 10^5 ± 0.329 × 10^5	4.800 × 10^5 ± 0.609 × 10^5	9.387 × 10^5 ± 0.441 × 10^5	3.62 ± 0.18

[a] *PDI = M_w/M_n* [b] *Standard deviations from six injections*

Table 1

GPC ELUTION PROFILE OF A SYNTHETIC RUBBER RESIN AS MONITORED BY RI (RED), VISC (BLACK) AND MALS (GREEN) AND MOLAR MASS (RED) AND RADIUS OF GYRATION (GREEN)

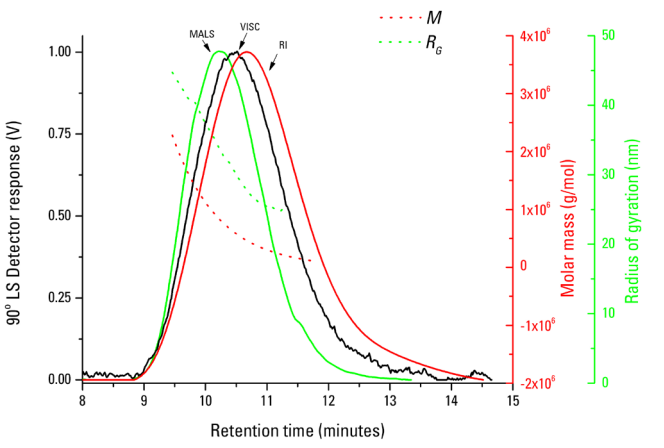

Figure 2

experiments the triple-detector GPC experiments also allow for the determination of the radius of gyration and its distribution. The radius of gyration was determined to be 33 ± 1 nm with a PDI = 1.2. The molar mass and size polydispersity of synthetic rubber resin can also be observed in Figure 2, as both the molar mass and radius of gyration, RG, decrease with increasing elution volume.

CONCLUSIONS

Single-, dual-, and triple-detector GPC experiments were performed using the EcoSEC GPC System encompassing a dual flow refractive index detector coupled either to differential viscometry or differential viscometry and multi-angle light scattering. The molar mass averages of a synthetic rubber resin were determined using three independent methods: (1) polystyrene relative calibration curve (GPC/RI), (2) universal calibration curve (GPC/RI/VISC), and (3) absolute molar mass (GPC/RI/VISC/MALS).

A comparison between the three methods showed the molar mass averages and polydispersity of the three methods were in fair agreement with one another. The use of the EcoSEC GPC System in conjunction with VISC and MALS for triple-detector GPC experiments allowed for the characterization of the synthetic rubber resin based on absolute molar mass and polymeric size, providing a more detailed picture of the molecular characteristics and thus the physical properties of the synthetic rubber resin.

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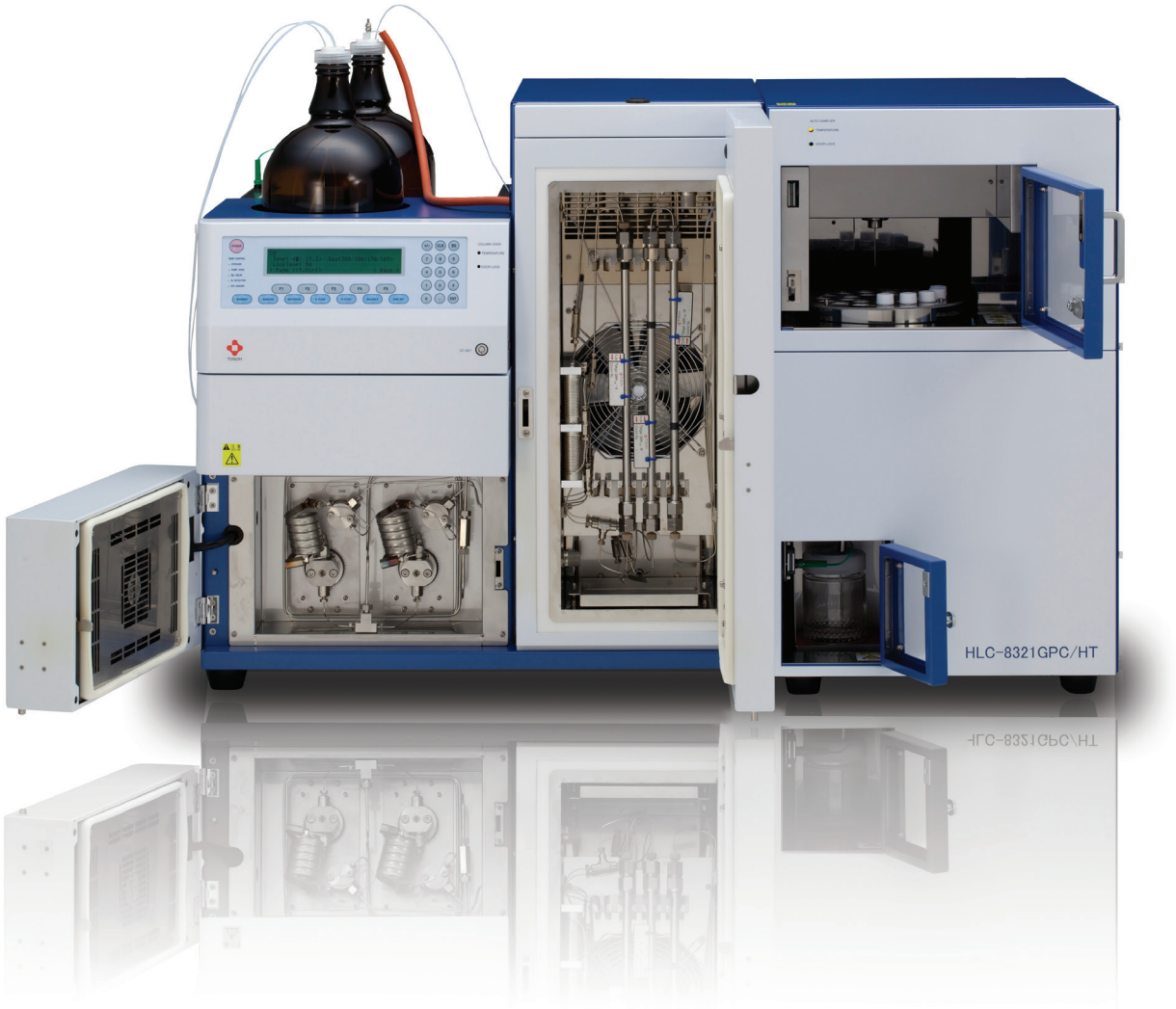
HIGH TEMPERATURE EcoSEC GPC SYSTEM



TOSOH

POLYPHENYLENE SULFIDE (PPS) ANALYSIS USING THE EcoSEC® HIGH TEMPERATURE GPC SYSTEM

INTRODUCTION

Polyphenylene Sulfide (PPS) has attracted a considerable amount of interest in the polymer industry due to its high tensile strength, dimensional stability, flame resistance, and excellent stability in organic liquids[1]. PPS is an engineered polymer with a rigid backbone of aromatic rings linked by sulfur atoms. The molecular structure of PPS makes the material useful for a number of applications, including filter fabrics, felts for paper making, ball valves, electrical sockets, and optical fiber cables. One of the main characteristic of PPS is its high resistance to chemical and thermal attacks, thus making PPS a useful structural material.

The same characteristics that make PPS an ideal polymer for various applications also hinder the ability to characterize the polymer, as it has very limited solubility. PPS is virtually insoluble in most organic solvents at ambient temperatures and thus can only be characterized in the solid state or by using elevated temperatures. The limited solubility of PPS makes it very difficult to determine properties such as molar mass averages and molar mass distributions. Traditionally, PPS has been characterized by infrared spectrometry and thermal analysis methods.[2] One method which can also be used to characterize PPS is high temperature gel permeation chromatography (GPC) as PPS is soluble in 1-chloronaphthanlene (1-CN) at extremely elevated temperatures (> 200 °C).

The ability to characterize the molar mass averages and distributions of PPS is essential as these properties play a vital role in the determination of mechanical, bulk and solution properties of the processing and end-use properties of a given material. For polymers which are used in textiles, such as PPS, the molar mass averages and distributions determined by high temperature GPC are a good tool for failure analysis investigations. Analysis of PPS using high temperature GPC is traditionally difficult as the analysis must be performed in 1-CN at 220 °C. 1-CN is a difficult solvent to use for analytical experiments as the solvent ambers over time and can cause havoc for detection methods such as refractive index. Here we have used the EcoSEC High Temperature GPC System encompassing a dual flow refractive index detector (RI) to determine the molar mass averages and molar mass distribution of two PPS samples that were exposed to different environments.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on a system consisting of an EcoSEC High Temperature GPC System (HLC-8321 GPC/HT) equipped with a RI detector. Separation of unfiltered 300 µL injections occurred over a column bank consisting of two 7.8 mm ID × 30 cm, 13 µm particle size TSKgel GMH$_{HR}$-H(S) HT2 columns (exclusion limit 4 × 10^8 g/mol) (PN 22889) followed by the corresponding guard. The mobile phase and solvent were 1-CN (Fisher) at a flow rate of 1.0 mL/min. Solvent reservoir and pup oven were maintained at 40°C. Detector, auto injector and column oven were maintained at 220°C. Two polyphenylene sulfide (PPS) felt samples were provided by Inovatia Laboratories (Fayette, MO). The polymer samples were dissolved in 1-CN at 220°C and shaken for two hours using the Tosoh Bioscience sample prep system (PN 23801). The final sample concentrations were approximately 2.0 g/L. Data was processed with the EcoSEC GPC Workstation software.

Molar mass averages were determined for each polymer sample using a calibration curve. A calibration curve for each column set was created for the RI detector at 220°C using Tosoh polystyrene standards, ranging in molar mass from 1,050 to 2.11 × 10^6 g/mol. Calibration curve data for polystyrene standards in 1-CN at 220°C at a flow rate of 1.0 mL/min was fitted with a cubic function and error values were less than 5%.

GPC ELUTION PROFILE OFNEW AND USED PPS SAMPLES AS MONITORED BY RI

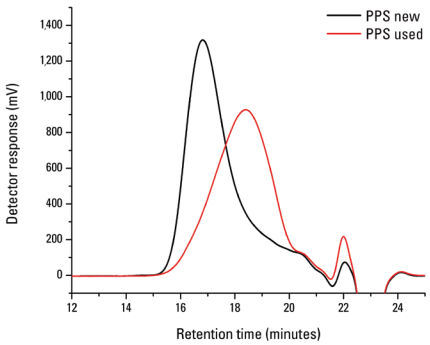


FIGURE 1

RESULTS AND DISCUSSION

As mentioned in the "Introduction and Experimental" sections, an EcoSEC High Temperature GPC System encompassing a dual flow refractive index detector was used for a material failure investigation. The molar mass averages and molar mass distributions of two PPS samples, one new and one used, were determined via GPC/RI. The new and used PPS materials were shown to have significant differences when analyzed by GPC/RI. The polystyrene relative molar mass averages, molar mass distributions, and GPC elution profiles o the new and used materials were compared to determine if usage of the PPS sample played a role in the failure of the product.

The GPC chromatograms of the new and used PPS samples as monitored by RI are shown in Figure 1. The new PPS sample elutes prior to the used PPS sample. The shorter retention time of the new PPS sample indicates that the new PPS sample is larger in polymeric size than the used PPS sample; as the elution order in GPC is that of an "inverse-sieving" technique, larger analytes sample a smaller pore volume than smaller analytes resulting in the larger analytes eluting from the GPC column prior the smaller analytes. The GPC chromatogram for the used PPS sample is shifted considerably towards the longer retention time, smaller polymeric size, compared to that of the new PPS sample. Thus, the GPC chromatograms alone provide sufficient evidence tat the use of the PPS sample has resulted in a decrease in polymeric size within the sample; possibly causing the failure of the product when in use for a predetermined amount of time.

The new and used PPS samples can also be compared for failure investigation through their polystyrene relative molar mass averages. As seen in Table 1, the new PPS sample was determined to have a higher number-, weight-, and z-average molar mass than the used PPS sample. Differences observed in the molar mass averages is important in any product failure investigation as the molar mass averages dictate the end-use properties of a products, such as tensile strength, elongation, brittleness, hardness, toughness, etc. The approximately 20 to 50% decrease in the molar mass averages observed between the new and the used PPS is potentially enough evidence to determine that after a predetermined amount of time the end-use product(s) made with this PPS sample will begin to fail or will no longer be able to perform to standards.

The differences in the molar mass averages observed between the new and the used PPS samples can also be observed through the molar mass distributions, MMD. The MMDs of both the new and used PPS samples, as determined using polystyrene relative calibration curves from GPC/RI, are shown in Figure 2. The new PPS sample MMD extends significantly further in the high and low molar mass directions than the used PPS sample. The used PPS sample has a considerably higher quantity of low molar mass species than the new PPS sample. The decreased breadth of the molar mass distribution of the used PPS sample compared to the new PPS sample can also be seen through the polydispersity index values,

OVERLAY OF CUMULATIVE AND DIFFERENTIAL MOLAR MASS DISTRIBUTION OF NEW AND USED PPS SAMPLES

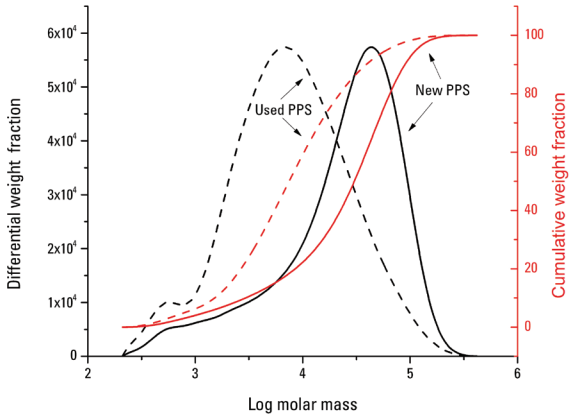


FIGURE 2

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF NEW AND USED PPS SAMPLES VIA GPC/RI

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI[a]
PPS new	5,790	3.91×10^4	7.19×10^4	6.746
PPS used	3,176	1.62×10^4	5.54×10^4	5.106

[a]PDI=M_w/M_n

The PDI of the new PPS sample is 25% greater than that of the used PPS, an indication that usage of the PPS results in a significant change in the molar mass distributon of the product.

CONCLUSIONS

The EcoSEC HT GPC System with a dual flow refractive index detector was used to perform failure analysis on two PPS samples, one new and one used. GPC/RI analysis was successfully performed using 1-CN at 220 °C using the EcoSEC High Temperature GPC System. The new and used PPS samples were compared via GPC chromatograms and polystyrene relative molar mass averages and distributions. The new PPS sample was determined to have a higher molar mass, broader molar mass distribution and a larger polymeric size than the used PPS sample. Using the PPS sample for a predetermined amount of time appears to decrease the molar mass averages and polymeric size of the polymer. The use of GPC/RI for the failure investigation of PPS allowed for immediate differentiation between the new and used PPS samples based on the GPC/RI elution profile, which was then confirmed through differences in the polystyrene relative molar mass averages and molar mass distributions. The dual flow refractive index design of the EcoSEC HighTemperature GPC System permitted the analysis of PPS in a traditionally difficult solvent system for GPC analysis, 1-CN at 220 °C.

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TOSOH

Application Note

ANALYSIS OF POLYPROPYLENE RANDOM COPOLYMER SAMPLES USING THE EcoSEC® HIGH TEMPERATURE GPC SYSTEM

INTRODUCTION

The polypropylene market is one of the largest and most versatile polymer markets today with over 50 million tons produced annually and sold into a wide variety of household and industrial applications. In the home, polypropylene can be found in everything from audio speakers to carpets and automotive components. Industrially, polypropylene is essential in living hinges, RF capacitors, medical devices, and contact lens molding.

The variety of products in which polypropylene is present require versatility in mechanical, thermal and chemical properties. For this reason, depending upon the application, three major categories of polypropylene exist; homopolymer, block copolymer, and random copolymer. While homopolymer is the general purpose grade of polypropylene, block copolymer usually contains 5-15% ethylene and exhibits enhanced impact resistance. Random copolymer containing 1-7% ethylene is more malleable and crystal clear. For this reason, it is often used in medical applications and contact lens production.

In this application note, the molar mass averages and polydispersity of two polypropylene random copolymer samples were determined via refractive index (RI) detection using the EcoSEC High Temperature GPC System and TSKgel columns. The enhanced thermal, flow rate and dual flow RI detector signal stability of the EcoSEC High Temperature GPC System in combination with the excellent resolving power of the TSKgel GMH$_{HR}$-H (20) HT2 high temperature GPC columns produce reliable and highly reproducible data for the two samples analyzed in triplicate.

EXPERIMENTAL GPC CONDITIONS

Column:	TSKgel GMH$_{HR}$-H (20) HT2, 20 µm, 7.8 mm ID × 30 cm × 2, PN 0022890
Mobile phase:	trichlorobenzene (butylated hydroxyl toluene (BHT) added, 200 ppm)
Flow rate:	1 mL/min
Detection:	RI
Temperature:	140°C
Injection vol.:	300 µL
Sample:	polypropylene random copolymer

RESULTS AND DISCUSSION

The EcoSEC High Temperature GPC System encompassing a dual flow refractive index detector was successfully used to perform gel permeation chromatography (GPC) analysis of two polymer samples using a series of TSKgel high temperature GPC columns. The number-, weight- and z-average molar mass values (M_n, M_w, and M_z) and polydispersity index, PDI, were calculated for a polypropylene equivalent via EcoSEC software by applying a Mark-Houwink constant. The obtained values are given in Tables 1 and 2. Overlays of triplicate analysis in Figure 1 and 2 indicate a very high level of reproducibility.

GPC ELUTION PROFILE OF 3 CONSECUTIVE INJECTIONS OF SAMPLE 1 (PP EQUIVALENT) AS MONITORED BY RI

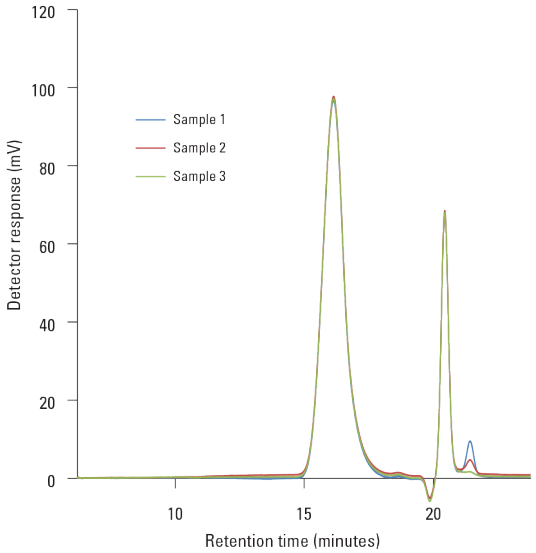

Figure 1

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF SAMPLE 1 (PP EQUIVALENT) VIA RI

Injection number	Retention time	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI (M_w/M_n)
1	16.532	54380	145630	286074	2.678
2	16.527	54153	145548	289290	2.688
3	16.548	54027	145195	286331	2.687
Average	16.537	54186.67	145457.70	287231.70	2.684
STDEV	0.011	178.89	231.14	1787.20	0.001
CV%	0.066	0.33	0.16	0.62	0.200

Table 1

Mark-Houwink constant for polypropylene (PP):
αPP = 0.750, logKPP = -3.8633

GPC ELUTION PROFILE OF 3 CONSECUTIVE INJECTIONS OF SAMPLE 2 (PP EQUIVALENT) AS MONITORED BY RI

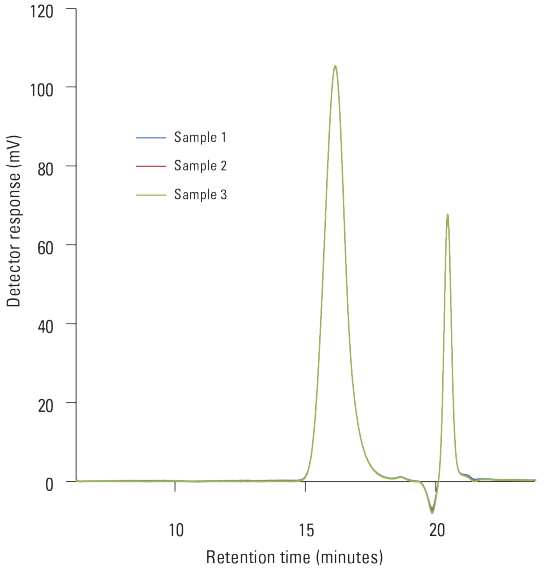

Figure 2

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF SAMPLE 2 (PP EQUIVALENT) VIA RI

Injection number	Retention time	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI (M_w/M_n)
1	16.532	52396	145040	292193	2.768
2	16.532	52519	145298	292904	2.767
3	16.533	54427	145729	291369	2.677
Average	16.532	53114	145355.70	292155.30	2.737
STDEV	0.001	1138.75	348.10	768.19	0.052
CV%	0.036	2.14	0.24	0.26	1.900

Table 2

CONCLUSIONS

An EcoSEC High Temperature GPC system was used to analyze two polypropylene random copolymers. The molar mass averages, M_n, M_w, and M_z, were determined via polystyrene calibration curves. The reproducibility and reliability of the dual flow refractive index detector in the EcoSEC High Temperature GPC System were shown through both the very low variation in sample retention and superb baseline stability observed when overlaying three consecutive RI injections of each sample. The molar mass values were calculated by EcoSEC software for PP equivalents based on Mark-Houwink constants.

TOSOH

Application Note

CHARACTERIZATION OF A CONDUCTING POLYMER USING THE ECOSEC® HIGH TEMPERATURE GPC SYSTEM

INTRODUCTION

Conducting polymers, such as polythiophenes (Figure 1), have been widely investigated over the past several decades due to their potential industrial applications based on their conductivity and organic light-emitting capability. Polythiophenes and their derivatives have recently become of growing interest as they have shown promise in electronic and optical applications, such as organic transistors and solar cells. Polythiophenes are polymerized thiophenes, i.e. the polymer backbone contains sulfur heterocyles that become conductive when electrons are added or removed through doping. To date polythiophenes have been used in the development of electronics, energy storage batteries, photochromic devices, and nonlinear optical devices. Due to the electron-rich character of the thiophene ring, polythiophenes can be easily and reversibly oxidized by chemical and electrochemical means to form highly conducting materials.[1, 2]

EXAMPLE OF MONOMER REPEAT UNIT OF POLYTHIOPHENE

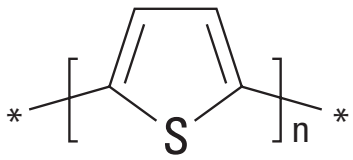

Figure 1

The heavy focus on synthesis of conducting polymers facilitates the need for characterization methods. Among the methods employed for the characterization of the intermediates and final conducting polymers are FT-IR, NMR, gel permeation chromatography (GPC), and microscopy. Some conducting polymers have limited solubility, thus require the use of a method such as high temperature GPC for determination of the molar mass averages and molar mass distributions. Similar to other polymers, the molar mass averages and molar mass distributions of conducting polymers play a role in determining the end-use properties of the polymer's applications.

Here we have used the EcoSEC High Temperature GPC System encompassing a dual flow refractive index detector (RI) to determine the molar mass averages and molar mass distribution of two conjugated polymers similar to polythiophenes.

EXPERIMENTAL

Sample analysis was performed on a system consisting of an EcoSEC High Temperature GPC System equipped with a RI detector. Separation of unfiltered 300 µL injections occurred over a column bank consisting of two 7.8 mm ID × 30 cm, 13 µm particle size TSKgel GMH$_{HR}$-H(S) HT2 columns (exclusion limit 4 × 10^8 g/mol) (PN 0022889) followed by the corresponding guard. The mobile phase and solvent were 1,2,3-trichlorobenzene (TCB) at a flow rate of 1.0 mL/min. Solvent reservoir and pump oven were maintained at 40 °C. Detector, auto injector and column oven were maintained at 145 °C. Two conducting polymers similar to polythiophene were dissolved in TCB at 145 °C and shaken for one hour using the Tosoh Bioscience sample prep system. The final sample concentrations were approximately 2.0 g/L.

Molar mass averages were determined for each polymer sample using a calibration curve. A calibration curve was created for the RI detector at 145 °C using Tosoh polystyrene standards, ranging in molar mass from 1,010 to 5.48 × 10^6 g/mol. Calibration curve data for polystyrene standards in TCB at 145 °C at a flow rate of 1.0 mL/min was fitted with a cubic function and error values were less than 5%.

RESULTS AND DISCUSSION

The polystyrene relative molar mass averages and molar mass distributions of two conducting polymers similar to polythiophene were determined via GPC/RI and can be compared to determine if there is a difference between macromolecular properties, and thus end-use properties, of the two different polythiophene-like conducting polymers synthesized through different mechanisms.

MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF TWO CONDUCTING POLYMER SAMPLES VIA RI

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)
Polymer A	2.58 × 10^4 ± 0.01 × 10^4	6.51 × 10^4 ± 0.02 × 10^4	1.34 × 10^5 ± 0.03 × 10^5
Polymer B	9.39 × 10^3 ± 0.01[a] × 10^3	1.26 × 10^4 ± 0.04 × 10^4	1.60 × 10^4 ± 0.01 × 10^4

[a] *Standard deviations from two injections*

Table 1

The polystyrene relative number-, weight-, and z-average molar mass values, M_n, M_w, and M_z, respectively, are given in Table 1. All three molar mass averages were determined to be greater for Polymer A than Polymer B. The variation between the molar mass averages of the two conducting polymers may be enough to change the conductivity of the polymers, thus their end-use applications. Differences observed in molar mass averages, as well as distribution, are important as they affect the applicable uses of a conductive polymer.

In addition to the molar mass averages, the molar mass distribution, MMD, can also influence various properties of conducting polymers. The molar mass distributions of the two conducting polymers, as determined using polystyrene relative calibration curves from GPC/RI, are compared in Figure 2. The molar mass distribution of Polymer A is significantly larger than that of Polymer B, as the MMD for Polymer A extends further in the high molar mass region compared to that of Polymer B.

OVERLAY OF CUMULATIVE AND DIFFERENTIAL MOLAR MASS DISTRIBUTION OF TWO CONDUCTING POLYMER SAMPLES

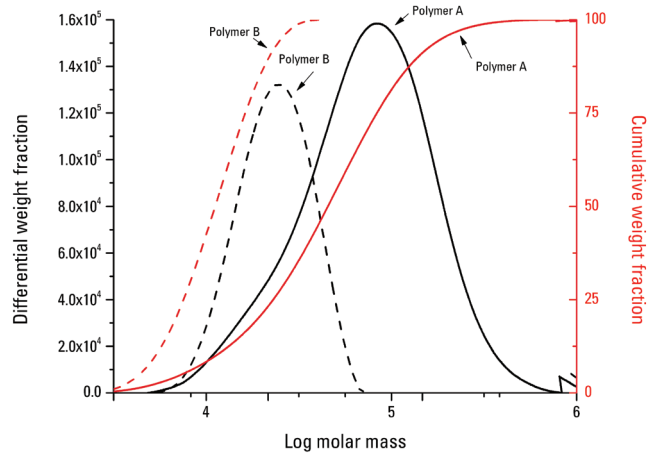

Figure 2

GPC ELUTION PROFILE OF TWO CONDUCTING POLYMERS AS MONITORED BY RI

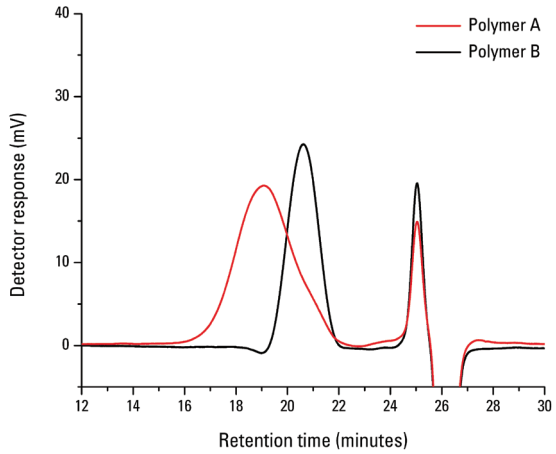

Figure 3

Additionally, the difference in the molar mass distributions between the two conducting polymers can be seen through the polydispersity index value for Polymer A and Polymer B and were determined to be 2.58 and 1.33, respectively.

Information regarding the difference between the two conducting polymers can be seen by comparing their GPC elution profiles, Figure 3. The shift in GPC retention time amongst the two conducting polymers indicates a variation in polymeric size between the two conducting polymers, as elution order in GPC is that of an "inverse-sieving" technique, large analytes sample a smaller pore volume than smaller analytes resulting in larger analytes eluting from the GPC column prior to the smaller analytes.

The GPC chromatogram of Polymer A is shifted considerably towards a longer retention time, thus smaller polymer size, compared to that of Polymer B. Thus, based on the GPC elution profile Polymer A is significantly larger in polymeric size than Polymer B.

CONCLUSIONS

The EcoSEC High Temperature GPC System with dual flow refractive index detector was used to characterize two conducting polymers similar to polythiophene. GPC/RI was successfully performed to determine the polystyrene relative molar mass averages and molar mass distributions of the two conducting polymers.

Polymer A was determined to have significantly higher molar mass averages as well as a larger molar mass distribution than Polymer B. The GPC/RI chromatogram also provided evidence that the polymeric size of Polymer A is greater than that of Polymer B. The use of GPC/RI for the comparison of two conducting polymers similar to polythiophene allows for an immediate differentiation between the two samples based on the GPC/RI elution profiles as well as the polystyrene molar mass averages and molar mass distributions.

The EcoSEC High Temperature GPC System encompassing a dual flow refractive index detector provides an efficient and reliable method for determining several macromolecular properties which influence the end-use applications of conducting polymers.

TOSOH

Application Note

TSKgel HIGH TEMPERATURE GPC COLUMN DURABILITY

In gel permeation chromatography (GPC), just like all chromatography modes, the heart of the separation lies in the quality, applicability and selectivity, or resolution, of the column. The selectivity of a GPC column is based on the ability of the pores in the column packing material to differentiate between species of varying hydrodynamic volume. The pores of the packing material within a GPC column are sampled by the analytes as they travel through the column in a size dependent manner. Due to their size, the larger components of the analyte sample either a smaller number of pores or, within a given pore, a smaller pore volume than the smaller components of the analyte, thus the larger components elute from the column prior to the smaller components.

GPC is considered a low resolution technique and does not provide infinite resolution of species with different hydrodynamic volume. As a result of the low resolution of the separation technique, each slice eluting from the GPC column has some residual polydispersity. This residual polydispersity, combined with extra dead volume in detectors and instrument tubing, leads to the overestimation of sample polydispersity because the peak eluting from the GPC column is broadened and appears to cover a wide molar mass range.[1] The superficial broadening of the molar mass range due to the resolution of a GPC column has a direct impact on accuracy of the molar mass averages. The accuracy of the number and z-average molar masses, M_n and respectively, can decrease by more than 10% as the resolution of a GPC column set decreases, while the accuracy of the weight-average molar mass, M_w, remains virtually unaffected by resolution.[1, 2]

The ability to obtain accurate molar mass averages for polymers by GPC, without superficially broadening the distribution, is essential as the molar mass averages and distributions affect the processing and end-use properties of materials. To obtain the best separation, thus most accurate molar mass averages possible, a GPC column must provide a linear calibration in the molar mass range of interest, narrow particle size distribution of the packing material, a large number of theoretical plates or high resolving power and durability.[2] Column manufactures, such as Tosoh, focus their innovations in GPC column technology on these characteristics as they directly affect column quality, applicability and selectivity, or resolution.

Column characteristics such as column durability become even more important in high temperature GPC analysis as these columns are not only exposed to harsh organic solvents but are also continuously exposed to extreme temperatures and repetitive temperature cycling. Here we have studied the durability and stability of Tosoh's new TSKgel high temperature GPC columns compared to other commercially available columns for polymer analysis at temperatures above 80 °C.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on a system consisting of an EcoSEC® High Temperature GPC System (HLC-8321 GPC/HT) equipped with RI detector. Separation of unfiltered 200 µL injections occurred over a column bank consisting of one 7.8 mm ID x 30 cm, 13 µm particle size TSKgel GMH$_{HR}$-H(S) HT column (exclusion limit 4 x 10^8 g/mol) (P/N 0018393) (Tosoh Bioscience) or one 7.8 mm ID x 30 cm, 13 µm particle size, commercially available high temperature GPC column. The mobile phase and solvent were 1-chloronaphthalene (Fisher) at a flow rate of 1.0 mL/min. Detetor, pump oven, and column oven were maintained at 220 °C. The polymer samples were dissolved in 1-chloronaphthalene at 250 °C for one hour using the Tosoh sample prep system (P/N 0023801). The final sample concentrations wer approximately 2.0 g/L. Data was processed with the EcoSEC GPC Workstation software.

GPC ELUTION PROFILE FOR A POLYMER BEFORE AND AFTER TEMPERATURE CYCLING OBTAINED USING A COMMERCIALLY AVAILABLE HIGH TEMPERATURE GPC COLUMN

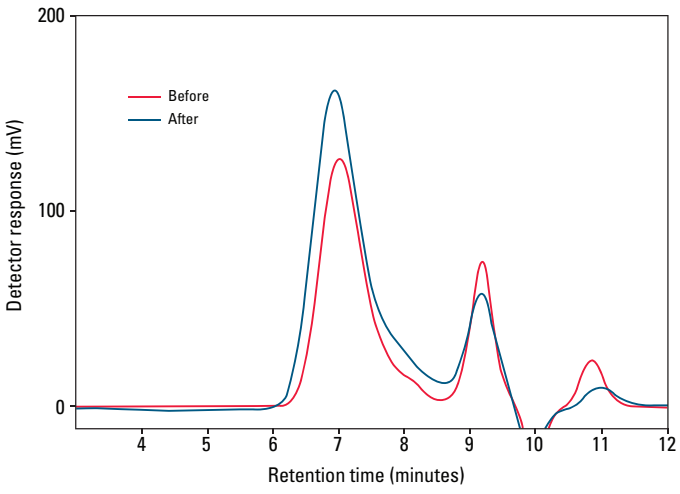

Figure 1

Temperature cycling was performed by flowing 1-chloronaphthalenethrough the EcoSEC High Temperature GPC System at a flow rate of 1.0 mL/min and slowl raising the column oven to 220 °C over nine hours. Samples were injected and molar mass averages were determined after the system reached equilibration at 220 °C. The EcoSEC High Temperature GPC System requires three hours to equilibrate. Temperature cycling times were extended beyond the equilibration time to test the column durability when exposed to extreme temperatures for a prolonged period. Following sample analysis 1-chloronaphthalene remained flowing through the EcoSEC High Temperature GPC System at a flow rate o 1.0 mL/min and the column oven temperature was slowly lowered from 220 °C to room temperature over nine hours. After the EcoSEC High Temperature GPC System was lowered to room temperature the flow was stopped. The entire temperature cycling process was performed multiple times to test column durability.

Molar mass averages were determined for each polymer sample using a calibration curve. A calibration curve for each column set was created for the RI detector at 220 °C using Tosoh polystyrene standards A-500, F-1, F-4, F-10, and F-40. Polystyrene standards were prepared for a final concentraion of 10 g/L.

RESULTS AND DISCUSSION

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 influeces the quality, applicability and selectivity, or resolution, of the GPC column, thus the accuracy of the molar mass averages obtained. A durability and stability study of Tosoh's new TSKgel high temperature GPC columns was performed and the results compared to another commercially available column for polymer analysis at 220 °C.

The weight-, number-, and z-average molar mass values, M_n, M_w, and M_z respectively, obtained for a polymer sample on both a TSKgel GMH$_{HR}$-H(S) HT column and a commercially available high temperature GPC column initially and after multiple temperature cycles are given in Table 1. Little to no variation of the molar mass averages, especially the number- and z-average molar mass values, is seen with the TSKgel GMH$_{HR}$-H(S) HT column, while significant fluctuatio on the other commercially available high temperature GPG column are evident. The repeatability (column accuracy) of the molar mass averages obtained on the TSKgel GMH$_{HR}$-H(S) HT column after temperature fluctuations is an indicatio that when the TSKgel GMH$_{HR}$-H(S) HT column is exposed to extreme temperatures and temperature cycling, column resolution is not compromised. If the resolution of the TSKgel GMH$_{HR}$-H(S) HT column was compromised due to high temperature GPC experimental parameters, as in the other commercially available high temperature GPC column, fluctuations in molar mass averages would be observed between those obtained before and after temperature cycling.

The deterioration of the other commercially available high temperature GPC column is also observed in the GPC elution profiles, Figure 1, as the resolution between the sample and solvent peaks decreases after the column is exposed to temperature cycling. The GPC elution profiles obtained before and afte temperature cycling are slightly shifted for the other commercially available high temperature GPC columns while the GPC elution profile for the TSKgel GMH$_{HR}$-H(S) HT column remain superimposable, Figure 2. 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 th GPC elution profile begins to shift due to a decrease in multipe factors that affect the ability of the columns to separate species varying in hydrodynamic volume.

GPC ELUTION PROFILE FOR A POLYMER BEFORE AND AFTER TEMPERATURE CYCLING OBTAINED USING A TSKgel GMH$_{HR}$-H(S) HT COLUMN

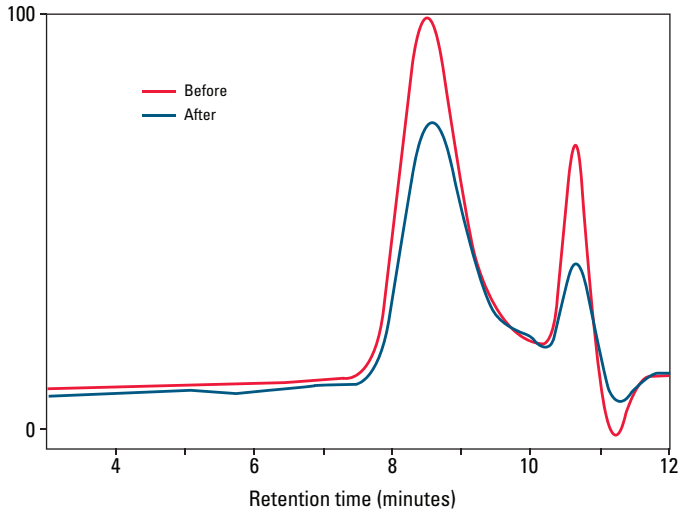

Figure 2

MOLAR MASS AVERAGES AND POLYDISPERSITY FOR A POLYMER BEFORE AND AFTER TEMPERATURE CYCLING OBTAINED USING A TSKgel GMH$_{HR}$-H(S) HT COLUMN AND A COMMERCIALLY AVAILABLE HIGH TEMPERATURE GPC COLUMN

Column	*Mn (g/mol)*	*Mw (g/mol)*	*Mz (g/mol)*	*PDI*
TSKgel GMH$_{HR}$-H(S) HT				
Before	3.9 x 10^3	1.8 x 10^4	3.6 x 10^4	4.6
After	3.6 x 10^3	1.7 x 10^4	3.5 x 10^4	4.7
Commercial column				
Before	6.7 x 10^3	1.9 x 10^4	4.4 x 10^4	2.9
After	4.1 x 10^3	2.4 x 10^4	9.3 x 10^4	5.8

Table 1

In addition to molar mass averages, the polydispersity index, PDI, can also be compared when looking at column performance and durability. In GPC analysis it is often difficult to compare GPC elution profiles and molarass averages obtained for a given polymer under different experimental conditions, i.e. different GPC columns, as GPC columns tend to vary ever so slightly from manufacturer to manufacturer. The molar mass distributions and the PDI values for a given polymer analyzed on a given column set should not change, unless the quality, applicability and selectivity, or resolution, of the column has been compromised. As seen in Table 1 and Figures 3 and 4, the molar mass distribution and PDI values obtained for the polymer samples using the TSKgel GMH$_{HR}$-H(S) HT column remain constant while those obtained using the other commercially available high temperature GPC column change after the columns are exposed to temperature cycling. In general, GPC columns have an expected lifetime that varies depending on analyte and experimental conditions but for high temperature GPC analysis the TSKgel GMH$_{HR}$-H(S) HT column is shown to be more durable than other commercially available high temperature GPC columns.

CONCLUSIONS

Column characteristics such as column durability become even more important in high temperature GPC analysis as these columns are not only exposed to harsh organic solvents but are also continuously exposed to extreme temperatures and repetitive temperature cycling. A decrease in column durability over time will result in superficial broadening of the molar mass range as a result o a decrease in the resolution of a GPC column thus directly impacting the accuracy of the molar mass averages. A durability and stability study of Tosoh's new TSKgel high temperature GPC columns was done and compared to another commercially available column for polymer analysis at 220 °C. The TSKgel GMH$_{HR}$-H(S) HT column was shown to be more durable than the other commercially available high temperature GPC column when exposed to temperature cycling as the molar mass averages and molar mass distribution obtained before and after temperature cycling showed less variation for the TSKgel GMH$_{HR}$-H(S) HT column compared to the other commercially available high temperature GPC column.

OVERLAY OF THE DIFFERENTIAL MOLAR MASS DISTRIBUTION OF A POLYMER BEFORE AND AFTER TEMPERATURE CYCLING OBTAINED USING A COMMERCIALLY AVAILABLE HIGH TEMPERATURE GPC COLUMN

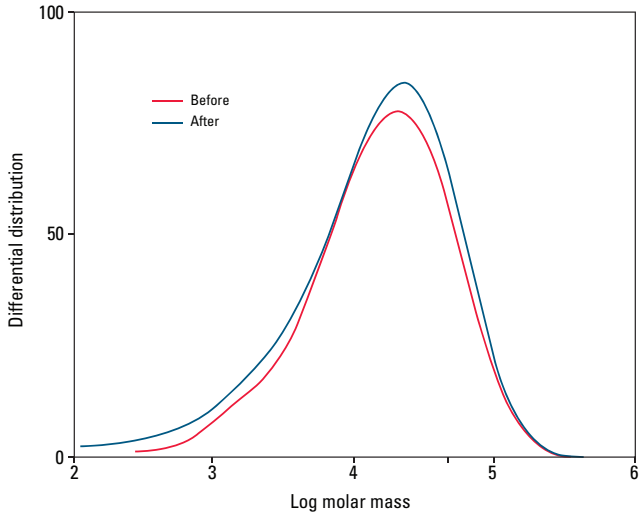

Figure 3

OVERLAY OF THE DIFFERENTIAL MOLAR MASS DISTRIBUTION OF A POLYMER BEFORE AND AFTER TEMPERATURE CYCLING OBTAINED USING A TSKgel GMH$_{HR}$-H(S) HT COLUMN

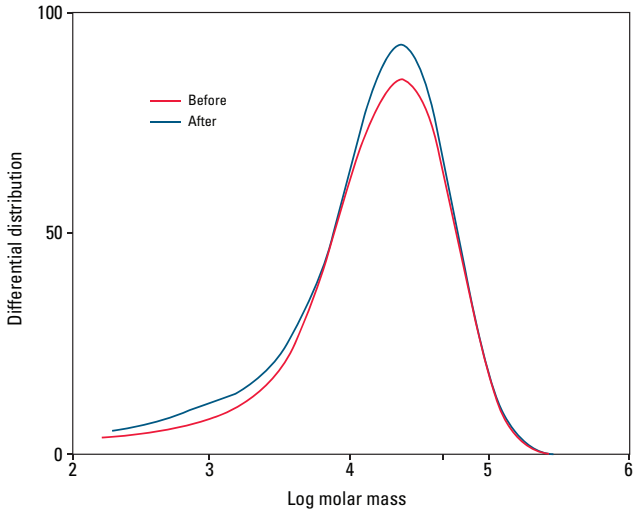

Figure 4

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