Analysis of Polymers for Therapeutics Using the EcoSEC® GPC System

EcoSEC GPC System
APPLICATION NOTE

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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, thus promoting the use of polymers in therapeutics. 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 use of synthetic polymers in therapeutics is continuously growing, thus increasing the need for a method to characterize the molar mass averages and molar mass distributions of these polymers as variations in molar mass averages and molar mass distributions can affect aspects of the therapeutic such as in vitro binding activity and biodegradation.[1] The molar mass averages and molar mass distributions of the polymer being used in therapeutics is critical for designing an effective polymer-based therapeutic and 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 (HLC-8320) 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 particle size TSKgel® SuperHZ4000 (exclusion limit 1 × 10^5 g/mol) (PN 19313), a 4.6 mm ID × 15 cm, 3 µm particle size TSKgel SuperHZ3000 (exclusion limit 6 × 10^4 g/mol) (PN 19312) and a 4.6 mm ID × 15 cm, 3 µm particle size TSKgel SuperHZ2000 (exclusion limit 1 × 10^4 g/mol) (PN 19310) preceeded by the appropriate guard column (PN 19314) (Tosoh Bioscience LLC). The mobile phase and solvent was tetrahydrofuran (THF) (BDH) 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 a twenty-four hour period. For all chromatographic determinations, results are averages of four injections. Data was processed with the EcoSEC GPC Workstation software, version 1.08.

A calibration curve was created for the RI 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 for designing an effective polymer-based therapeutic 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 refractive index detector was used to perform gel permeation chromatography 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, M_n, M_w, and M_z, 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.

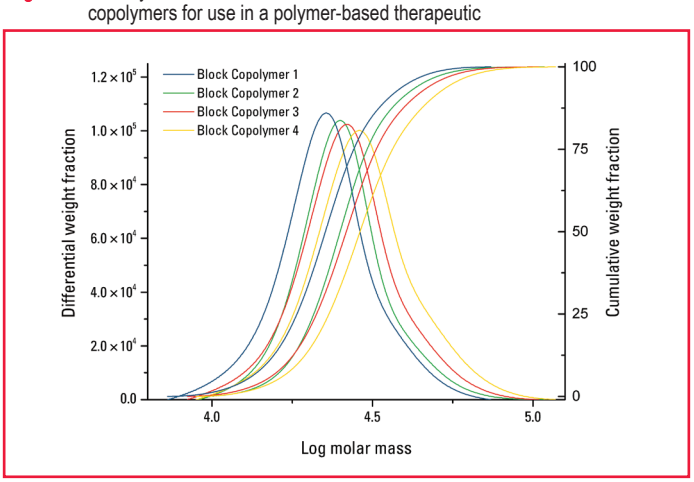
Table 1. Molar mass averages and polydispersity index of four block copolymers for use in a polymer-based therapeutic

Sample	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)	PDI[a]
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 4	2.74×10^4 $\pm 0.01 \times 10^4$	3.10×10^4 $\pm 0.01 \times 10^4$	3.55×10^4 $\pm 0.01 \times 10^4$	1.14 ± 0.01

[a] $PDI = M_w/M_n$ [b] Standard deviations from four injections

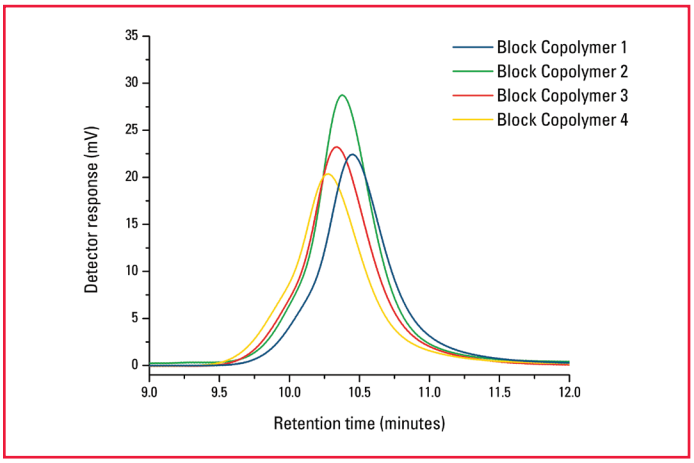
In addition to the molar mass averages, the molar mass distribution can also influence various properties of therapeutics. The molar mass distributions of the four block copolymers are compared in *Figure 1*, thru the differential and cumulative molar mass distributions. The molar mass distributions 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.

Figure 1. Overlay of cumulative and differential molar mass distribution of four block copolymers for use in a polymer-based therapeutic



Information regarding the differences between the four block copolymers for use in a polymer-based therapeutic 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 between the block copolymers, as elution order in GPC is that of an "inversing-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. 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. 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.

Figure 2. GPC elution profile of four block copolymers for use in a polymer-based therapeutic



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 for characterization of polymers intended to be used in a polymer-based therapeutic 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.

References

[1]Duncan, R.; *Nature* Rev., **2003**, 2, 347-360.
[2]Kabanov, A.V.; Okano, T. Challenges in *Polymer Therapeutics. In Polymer Drugs in the Clinical Stage: Advantages and Prospects, Volume 519*, Maeda, H.; Kabanov, A.V.; Kataoka, K., Okano, T. eds.; Academic Press: New York, 2003; pp 1-20.

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