Characterization of a Plastic Alternative via Gel Permeation Chromatography: Polyhydroxybutyrate

EcoSEC GPC System
APPLICATION NOTE

Amandaa K. Brewer, Ph.D, Tosoh Bioscience LLC, King of Prussia, PA 19406

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 non-renewable 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 not made from petroleum but 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 support PHB formation.[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 (HLC-8320) 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 particle size TSKgel® SuperHZM-M column (exclusion limit 4 × 10^6 g/mol) (PN 19662) (Tosoh Bioscience LLC). The mobile phase and solvent were chloroform (Honeywell) 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.

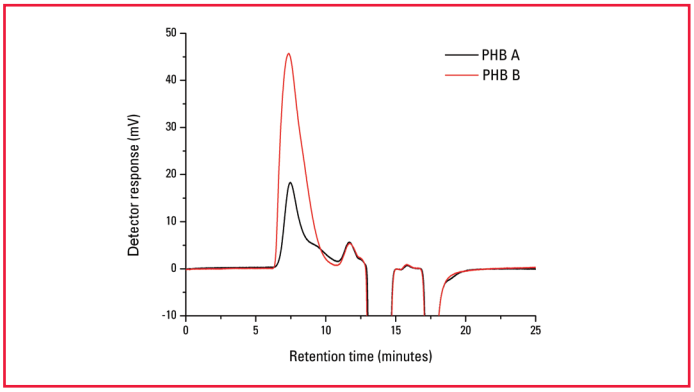
A calibration curve was created for the RI at 35 °C using PStQuick C polystyrene mix standard (PN 21909) (Tosoh Bioscience LLC) ranging in molar mass from 530 to 2.9 × 10^6 g/mol. Calibration curve data for 0.30 mL/min was fitted with a linear function and error values were less than 5%.

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 refractive index 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 commercially available and the homemade 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; 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 column prior to the smaller analytes. In addition to variations in elution time amongst the two samples, the shape of the GPC elution profile shows distinctive differences.

Figure 1. GPC elution profile a commercially available PHB sample (PHB A) and a *homemade* PHB sample (PHB B) as monitored by RI



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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, M_n, M_w, and M_z, 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 *z*-average molar mass values are more comparable to one another. The number-average molar mass 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.

Table 1. Molar mass averages and polydispersity index of a commercially available PHB sample (PHB A) and a homemade PHB sample (PHB B)

Sample	M_n (g/mol)	M_w (g/mol)	M_z (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] $PDI = M_w/M_n$ [b] Standard deviations from four injections

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. 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]

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, which are directly influenced by the GPC elution profiles (retention time and RI detector response), 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. The use of the EcoSEC GPC System for the characterization of polyhydroxybutyrate polymers allowed for the determination of the polystyrene relative molar mass averages and for a comparison of a commercially available PHB polymer with a new homemade PHB polymer. The ability to determine variations in the molar mass averages and molar mass distributions of PHB is essential, as it can affect the thermoplasticity and biodegradability of the plastic.

References

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TOSOH BIOSCIENCE LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Tel: 800-366-4875
email: info.tbl@tosoh.com
www.tosohbioscience.com

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