GPC/SEC Analysis of Biodegradable Polyhydroxyalkanoate on Agilent PLgel 10 µm MIXED-B Columns

Application Note

Materials Testing & Research

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Introduction

Poly-(R)-3-hydrobutyrate (P3HB) is an example of a polyhydroxyalkanoate or PHA, a linear polyester produced naturally by the bacterial fermentation of sugars and lipids under certain environmental conditions. The main advantageous property of P3HB is that it is a biodegradable thermoplastic with physical properties made tuneable by copolymerization with other esters. Potential applications in the medical and pharmaceutical industry include the manufacture of sutures, stents, bone plates, pericardial patches, and other macro devices designed to fulfill a temporary structural role in the body. For biodegradable applications such as these, the molecular weight distribution of the material used is a key component as this controls the rate at which degradation occurs and, therefore, the mechanical strength and longevity of the device within the body.

A sample of P3HB was analyzed by gel permeation chromatography using Agilent PLgel 10 µm MIXED-B columns.

Verified for Agilent
1260 Infinity
GPC/SEC System

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Materials and Methods

The system was calibrated using Agilent polystyrene EasiCal PS-1 standards and, therefore, all molecular weight values quoted are relative to these standards. The sample was prepared as a 0.2% w/v solution by heating to approximately 60 °C for 2 hours. After allowing the solution to cool to room temperature, no insoluble material remained, and so there was no need to filter the solution prior to injection.

Conditions

Column(s):	2 × Agilent PLgel 10 μm MIXED-B, 7.5 × 300 mm (p/n PL1110-6100)
Calibrants:	Agilent EasiCal PS-1 (polystyrene) (p/n PL2010-0501)
Eluent:	Chloroform
Sample concentration:	0.2% w/v
Injection volume:	100 μL
Flow rate:	1.0 mL/min
Temperature:	Ambient
Pressure:	27 bar
Detector:	DRI

Results and Discussion

Figure 1 shows raw data chromatograms for repeat injections of the sample P3HB. The sample clearly elutes within the operating range of the PLgel 10 μm MIXED-B packing, which is the best column selection available.
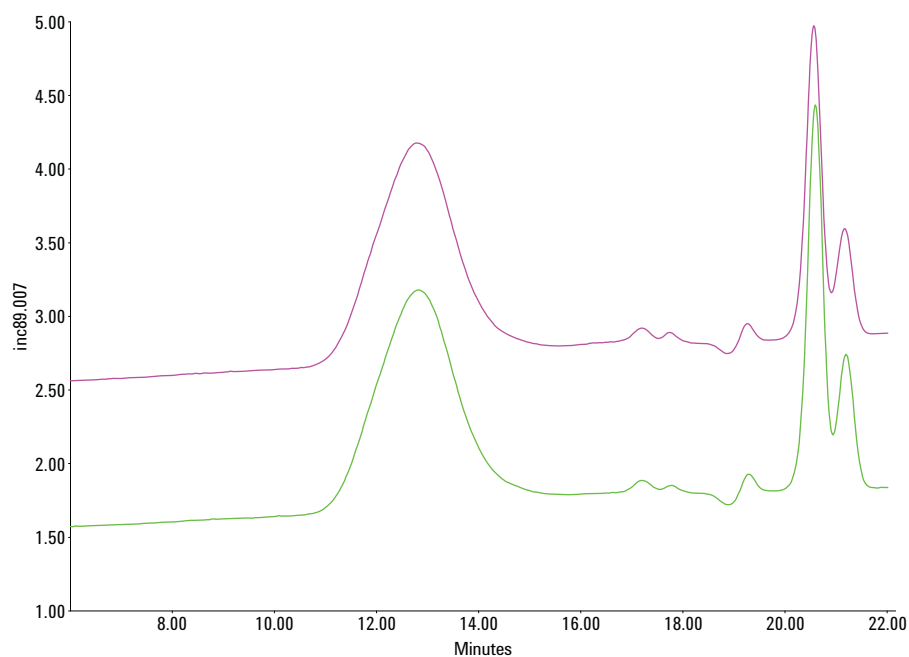

Figure 1. Raw data chromatogram of repeat injections of poly-(R)-3-hydrobutyrate on a 2-column set of Agilent PLgel 10 μm MIXED-B columns.

Figure 2 shows the column calibration curve.

Table 1 shows the molecular weight averages and characteristics calculated for both injections.
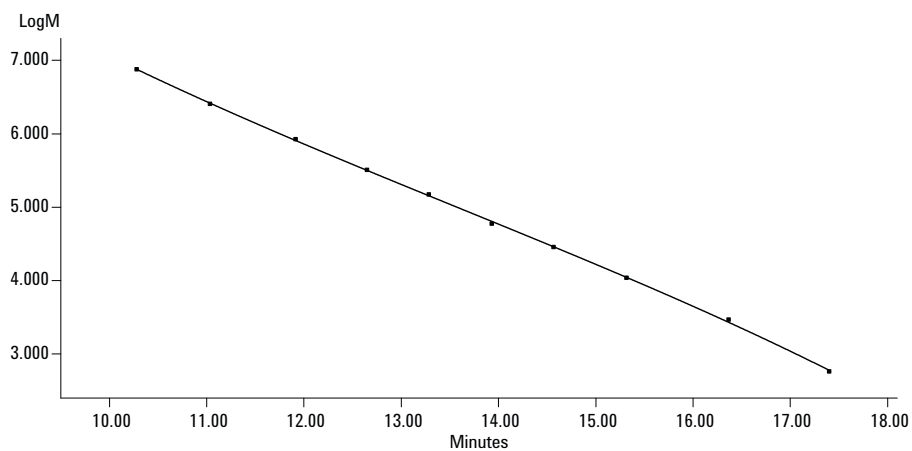

Figure 2. Calibrating the Agilent PLgel column using Agilent EasiCal PS-1 standards.

Table 1. Molecular weight characteristics of 2 injections of poly-(R)-3-hydrobutyrate.

	1st injection	2nd injection
Mz+1	1,284,499	1,311,394
Mz	772,184	790,694
Mw	386,620	395,906
Mp	262,139	267,674
Mn	163,169	167,415
Polydispersity	2.369	2.365
Peak area	32,922	32,849

Figure 3 compares the overlaid molecular weight distribution plots and demonstrates excellent repeatability.

Conclusions

The chromatograms demonstrate the analysis of poly-(R)-3-hydrobutyrate by GPC. The molecular weight averages obtained from 2 repeat injections illustrate the reliability of GPC as a testing method for this biodegradable polymer.
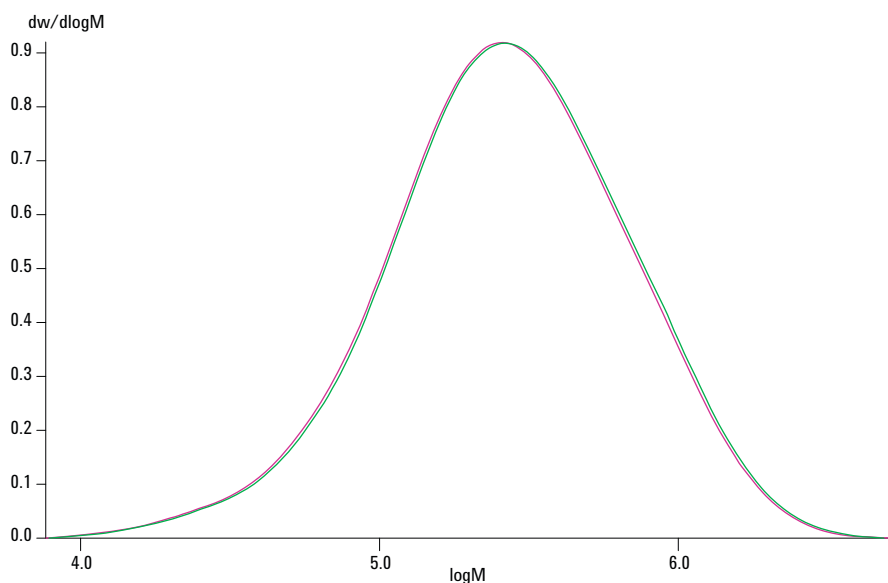
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Figure 3. Overlaid molecular weight distributions of 2 injections of poly-(R)-3-hydroxybutyrate on 2 Agilent PLgel 10 µm MIXED-B columns.

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