MATERIALS ANALYSIS

ACCURATE MOLECULAR WEIGHT ANALYSIS OF POLYHYDROXYBUTYRATE (PHB) A BIO-DERIVED POLYESTER, BY TRIPLE DETECTION GPC/SEC

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INTRODUCTION

Poly-3-hydroxybutyrate (PHB) is a linear polyester of D(-)-3-hydroxybutyric acid which was first discovered in bacteria by Lemoigne in 1925. It is accumulated in intracellular granules by a wide variety of Gram-positive and Gram-negative organisms under conditions of a nutrient limitation other than the carbon source (Dawes and Senior, 1973). PHB is a biodegradable polymer hence used in various applications such as surgical materials and method of drug release.

A major advantage of PHB is its biocompatibility which means it does not cause severe immune response when introduced to soft tissue or its in contact with blood, even after degradation. These are degraded by non-specific lipase and esterases in nature. Determining the Molecular Weight (Mw) of PHB is very important because the physical properties of the end products are directly related to it.

CONVENTIONAL GPC/SEC IS A COMPARATIVE TECHNIQUE

Conventional GPC using a single concentration detector (typically refractive index) is actually a comparative technique. During the analysis the detector tells you how much material elutes from the column at any given retention time. That is then converted into a molecular weight and distribution. If the standards used in the calibration are a different polymer type to the sample being characterized the results are only comparative.

WHAT DOES MULTI-DETECTOR GPC/SEC PROVIDE?

Multi-detector GPC using a concentration detector with a viscometer, or a light scattering detector or both, address the limitations of conventional GPC by providing the following key advantages :

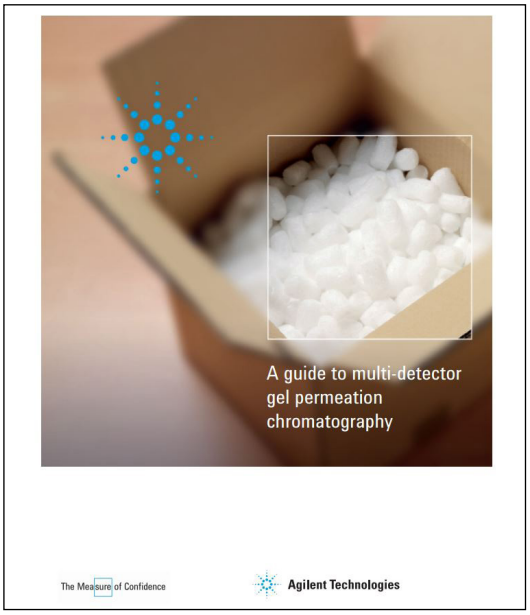
• The calculation of molecular weights that are independent of the chemistry of any standards used in a column calibration.

• The determination of other polymer properties such as conformation and branching that cannot be measured by conventional GPC/SEC.

For further reading, a detailed review of the theory, applications and benefits of Advanced Multi Detector GPC/SEC can be found in the Agilent primer:

'A guide to Multi-Detector Gel Permeation Chromatography' :

http://www.chem.agilent.com/Library/primers/Public/5990-7196EN.pdf



EXPERIMENTAL

Instrumentation
Agilent 1260 Infinity Quaternary Pump (G1311B)
Agilent 1260 Infinity High Performance Autosampler (G1367E)
Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
Agilent 1260 Infinity GPC/SEC Multi-Detector Suite (G7800A)
MDS Viscometer Detector (Option 032)
MDS Light Scattering Detector (Option 033)
MDS Refractive Index Detector (Option 031)

Method for Analysis	
Detectors used	MDS LS, VS, DRI
Mobile phase	Chloroform
Columns	2x PLgel 10µm Mixed-B 7.5 x 300mm (PL1110-6100)
Standards	Polystyrene Medium EasiVials (4ml)
Temperature	50 ˚C (column and detector)
Injection volume	100 µL
Flow Rate	1.0 ml/min
Agilent GPC/SEC software	

INSTRUMENT/DETECTOR/COLUMN CALIBRATION

Instrument calibration was performed with a single well characterized polystyrene standard with known dn/dc, IV, concentration and molecular weight. The column calibration was generated by injection of 3 different pre-weighed EasiVial standard mixtures. The table below shows the PS standards used for conventional calibration.

RT	Mp
13.12	371100
13.62	184900
13.95	113300
14.58	51150
15.15	24600
15.87	10110
16.38	4910
16.95	2590
17.38	1570
17.82	770
18.27	380

A typical chromatogram showing separation of 4 polystyrene standards from one of our pre-prepared EasiVial products is shown in Figure 1. Good resolution of each of the polystyrene standards is achieved.

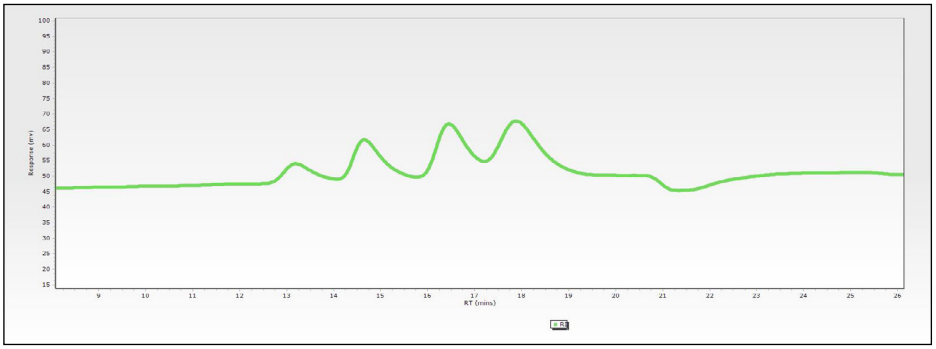


Figure 1: EasiVial (Red)

The conventional calibration curve based on Polystyrene Standards indicates an excellent linear fit across the molecular weight range is shown in Figure 2.

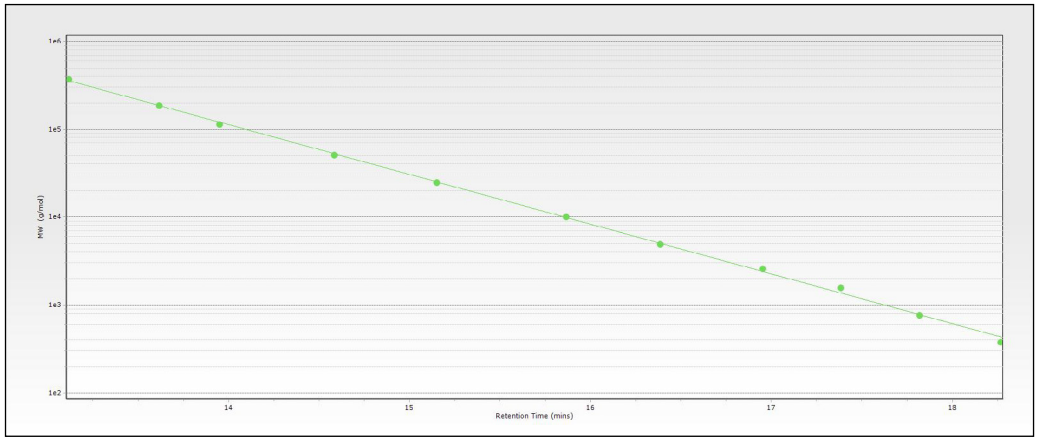


Figure 2: Conventional Calibration

ANALYSIS OF PHB SAMPLES

A molecular weight distribution plot from RI detection (Figure 3) and advanced detector chromatograms of the PHB samples (Figure 4) are shown below. Good responses were obtained from all three detectors, in particular the 90deg LS detector, and Viscometer which gave excellent responses for this material.

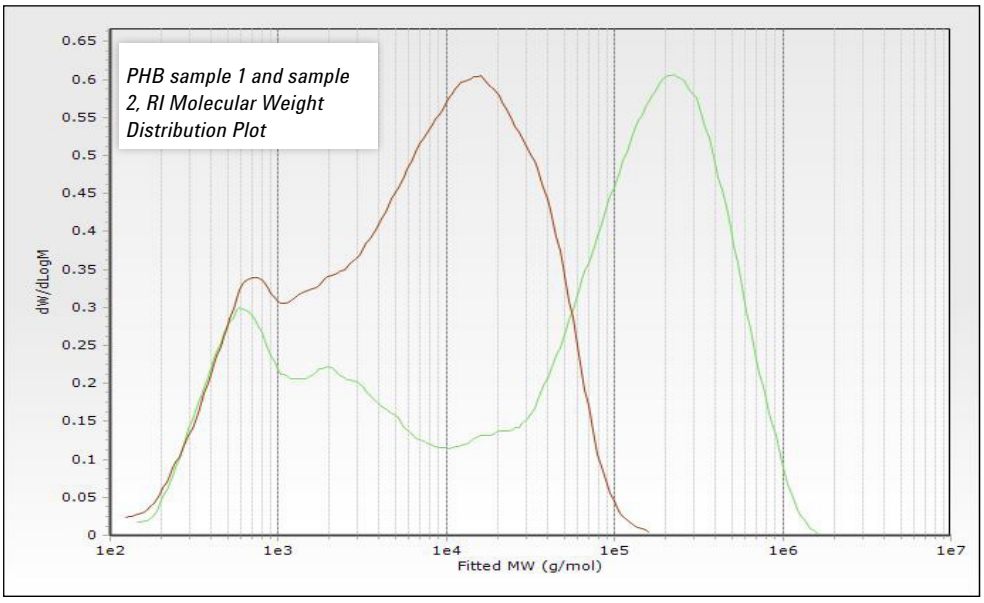
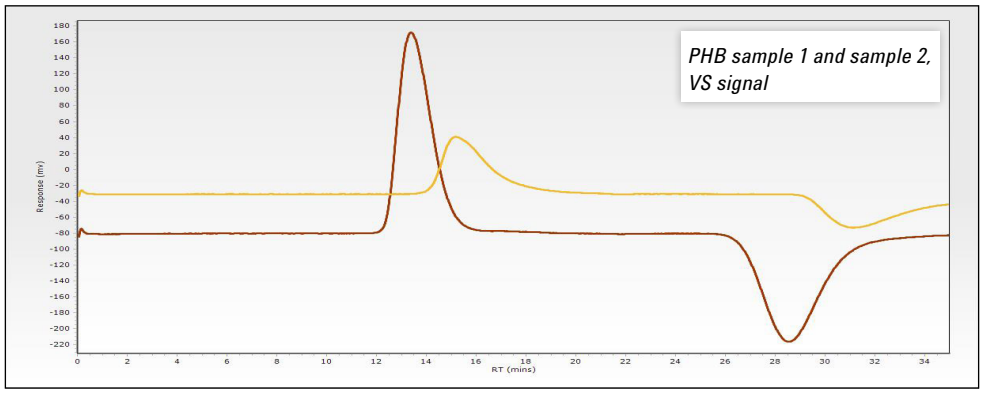


Figure 3: PHB samples Molecular Weight Distribution Plot



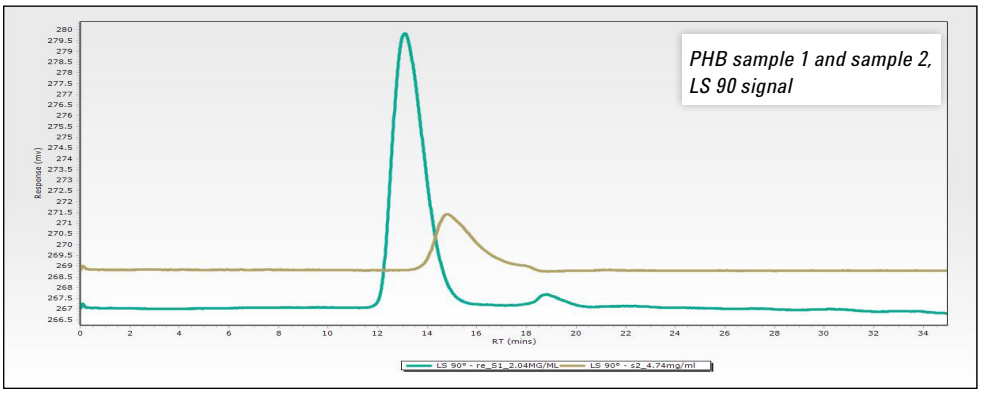


Figure 4: PHB samples Advanced Detector Chromatograms

PHB SAMPLE MOLECULAR WEIGHT AVERAGES

The molecular weight averages of PHB samples were calculated using conventional and triple detection methods and compared below.

	Mol Wt Averages	Conventional GPC	Triple Detection	Expected Mol Wt
PHB Sample 1	Mw	159395	228856	200-300k
	Mn	2447	102313	
	Mp	225519	232564	
PHB Sample 2	Mw	14433	24083	
	Mn	1887	9864	
	Mp	15587	29295	

The expected molecular weight for sample 1 of 200-300k was achieved using advanced detection but grossly underestimated using conventional GPC

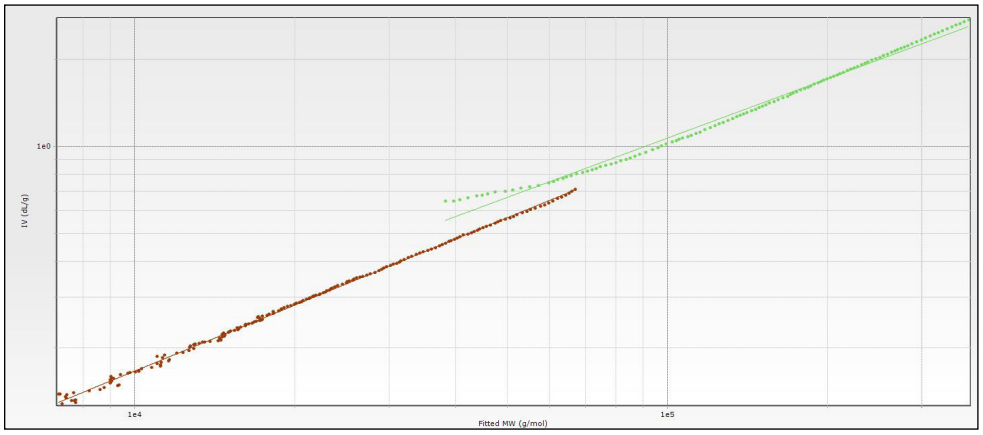


Figure 5: Mark-Houwink Plot of PHB Samples

In Figure 5, we show the Mark-Houwink plots using triple detection for samples 1 and 2. We can clearly see the differences in molecular weight of the two samples, and also from the linear nature of the plots, deduce that both materials are linear in structure.

CONCLUSION

The molecular weight of PHB is considerably understated using a conventional column calibration based on polystyrene standards. By investigating the polymer using advanced detectors such as Viscometry and Light Scattering, we can arrive at the expected true molecular weight values. In addition, by investigating the Mark-Houwink plots provided by the Viscometer we can obtain information about the polymer structure.

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