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Research Paper

Crystallization and its effect on the mechanical properties of a medium chain length polyhydroxyalkanoate

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ABSTRACT

Medium chain length polyhydroxyalkanoates (mcl-PHAs) could play a role in the growing demand for highly elastic and biodegradable materials in the medical field. In this study, a poly (3-hydroxyoctanoate-co-3-hydroxyhexanoate) (P(3HO-co-3HH)) was first fully characterized in terms of molecular weight, microstructural chain parameters and chemical structure by means of gel permeation chromatography (GPC), nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR). As determined by NMR, the synthesized polymer contained 94.3% and 5.7% molar content of 3-hydroxyoctanoate and 3-hydroxyhexanoate, respectively. Since mechanical properties are closely related to thermal history, the effect of crystallization on tensile properties was also investigated in the present study. Three crystallization temperatures were selected (0, 23 and 37 °C), the conclusion reached is that the maximum crystallization rate for this copolymer was achieved at 0 °C. On the other hand, evolution of tensile properties of P(3HO-co-3HH) films stored at room temperature demonstrated that, as crystallization occurred toward the equilibrium state, the polymer underwent a stiffening process. In this sense, secant modulus and tensile strength increased respectively from 8.3 ± 1.0 MPa and 6.4 ± 0.8 MPa after 1 day stored at room temperature to 36.2 ± 3.3 MPa and 16.3 ± 2.1 MPa after 16 weeks.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a group of biodegradable and biocompatible polyesters that are synthesized by numerous microorganisms as intracellular carbon and energy storage compounds (Lee, 1996a, 1996b). To date, more than 100 different monomers have been reported as PHA constituents, providing a set of materials with tunable characteristics in terms of mechanical properties and degradation rates (Steinbüchel and Valentin, 1995). These parameters are greatly affected by the length of pendant groups which extend from the polymer backbones or the distance between the ester linkages (Williams and Martin,

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2002). For example, the most widely studied poly(3-hydroxybutyrate) (P3HB) is a stiff material, presenting a tensile modulus of ~3.5 GPa, tensile strength of ~40 MPa and an elongation at break of ~6%. In contrast, poly(4-hydroxybutyrate) (P4HB) is a highly ductile, flexible material with an elongation at break of around 1000%, a tensile modulus of ~0.15 GPa and a tensile strength of 104 MPa (Saito et al., 1996). Introduction of a comonomer into the polymer backbone is also a valid strategy to tune the final properties of the obtained material (Di Lorenzo et al., 2001). For instance, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HB-co-3HV) containing 20 mol% of hydroxyvalerate, shows an improved flexibility and toughness in comparison to the P3HB homopolymer, having a tensile modulus of ~0.8 GPa, a tensile strength of ~20 MPa and an elongation at break of ~50% (Sudesh et al., 2000).

The (co)polymers presented so far are considered short chain length PHAs as they contain between 3 and 5 carbon atoms in their backbone. Medium chain length PHAs (mcl-PHAs) have 6–14 atoms in their structure, display low crystallinity, low glass transition temperature, low tensile strength and high elongation at break (Rai et al., 2001). Therefore, their use in biomedical applications where elastomeric biomaterials are required (Williams et al., 1999), such as vascular grafts (Shum-Tim et al., 1999), artificial esophagus (Chen and Wu, 2005), tendons and ligaments (Rathbone et al., 2010), etc. is being considered. In this study, a poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate) (P(3HO-co-3HH)) (Fig. 1) was employed as base material and was fully characterized in terms of molecular weight, molecular organization and chemical structure by means of gel permeation chromatography (GPC), nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR).

It is well known that polymers are prone to microstructural rearrangements during storage, these affect the main features of the final product (i.e., crystallinity, mechanical properties, degradation behavior, etc.) (Fernández et al., 2012a, 2012b). Accordingly, in this study the effect of three storage temperatures (0, 23 and 37 °C) on the crystallization behavior of P(3HO-co-3HH) was first studied by differential scanning calorimetry (DSC). Afterwards, the evolution of the mechanical properties of films stored at 23 °C was studied by means of tensile tests and dynamic mechanical analysis (DMA) for a period up to 16 weeks.

2. Materials and methods

2.1. *Synthesis of P(3HO-co-3HH)*

PHA was produced by *P. putida* strain from octanoic acid and glycerol in a fed batch fermentation. The dried biomass was extracted with an organic solvent to obtain the raw polymer.

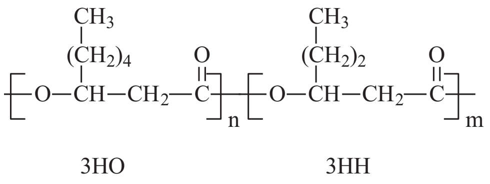
$$\left[-O-\overset{\overset{CH_3}{|}}{\overset{(CH_2)_4}{\overset{|}{CH}}}-CH_2-\overset{\overset{O}{\|}}{C}-\right]_n\left[-O-\overset{\overset{CH_3}{|}}{\overset{(CH_2)_2}{\overset{|}{CH}}}-CH_2-\overset{\overset{O}{\|}}{C}-\right]_m$$

Fig. 1 – Schematic representation of the mcl-PHA employed in this study.

In order to purify the PHA, the raw polymer was mixed with a non-solvent which induced precipitation of the polymer.

2.2. *Characterization of P(3HO-co-3HH)*

Initial characterization of P(3HO-co-3HH) in terms of molecular weight, molecular organization and chemical structure was conducted by means of GPC, NMR and FTIR, respectively.

The molecular weight distribution of the polymer was determined by GPC using a Waters 1515 GPC device equipped with two Styragel columns (10^2–10^4 Å). Chloroform was used as eluent at a flow rate of 1 mL min^{-1} and polystyrene standards (Shodex Standards, SM-105) were used to obtain a primary calibration curve.

Carbon nuclear magnetic resonance (^{13}C NMR) spectrum was recorded in a Bruker Avance DPX 300 at a resonance frequency of 75.5 MHz, using 5 mm O.D. sample tubes. The ^{13}C NMR spectrum of the P(3HO-co-3HH) was obtained at room temperature from a solution of 0.7 mL of deuterated chloroform ($CDCl_3$). Experimental conditions were as follows: 40 mg, inverse gated decoupled sequence; 3 s acquisition time; 4 s delay time; 5.5 μs pulse; spectral width 18,800 Hz and more than 10,000 scans.

Infrared spectrum of the polymer was recorded on a Nicolet AVATAR370 FTIR after dissolving the polymer in chloroform and casting on a KBr pellet. Spectra were taken with a resolution of 2 cm^{-1}and were averaged over 64 scans.

2.3. *Crystallization of P(3HO-co-3HH)*

Crystallization behavior of P(3HO-co-3HH) was studied on a DSC 2920 (TA Instruments). Samples of 5–10 mg were heated from −90 to 100 °C at 20 °C min^{-1}. This first scan was conducted in order to erase the thermal history of the bulk polymer. Then, the sample was cooled down from the melt and maintained isothermally at the selected temperature (0, 23 or 37 °C) for 0, 1, 3, 5, 10 and 24 h. After the isothermal treatment, the sample was quenched within the DSC and a second scan was made from −90 to 100 °C at 20 °C min^{-1}. The second scan was used to determine the glass transition temperature (T_g), melting temperature (T_m) and heat of fusion (ΔH_m) of the sample after the isothermal treatment.

2.4. *Evolution of the mechanical properties at 23 °C*

200–300 μm films for mechanical testing and dynamic mechanical measurements were prepared by pressure melting at 140 °C, followed by water quenching. The amorphous state of the PHA confers it adhesive qualities. Therefore, it could not be detached from the mold just after the quenching from the melt to room temperature (23 °C) and corresponding mechanical characterization was not feasible.

These films were stored at 23 °C for 1, 7, 28 and 112 days. Then, their mechanical properties were determined by tensile tests with an Instron 5565 testing machine at a crosshead displacement rate of 10 mm min^{-1}. Tests were performed at room temperature (21±2 °C) and at human body temperature (37 °C) following ISO 527-3/1995. The mechanical properties reported correspond to average values of at least 5 determinations.