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Polyhydroxyalkanoate-based natural–synthetic hybrid copolymer films: A small-angle neutron scattering study

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Abstract

Polyhydroxyalkanoates have attracted attention as biodegradable alternatives to conventional thermoplastics and as biomaterials. Through modification of their biosynthesis using *Pseudomonas oleovorans*, we have manipulated the material properties of these biopolyesters and produced a natural–synthetic hybrid copolymer of polyhydroxyoctanoate-*block*-diethylene glycol (PHO-*b*-DEG). A mixture of PHO and PHO–DEG were solvent cast from analytical grade chloroform and analysed using small-angle neutron scattering. A scattering pattern, easily distinguished above the background, was displayed by the films with a diffraction ring at $q \sim 0.12\,\text{Å}^{-1}$. This narrow ring of intensity is suggestive of a highly ordered system. Analysis of the diffraction pattern supported this concept and showed a *d*-spacing of approximately 50 Å. In addition, conformation of the hybrid polymer chains can be manipulated to support their self-assembly into ordered microporous films.

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Keywords: Polyhydroxyalkanoates; SANS; Polyethylene glycol

1. Introduction

Under conditions of nutrient stress with an excess of environmental carbon many microorganisms synthesise polyhydroxyalkanoates (PHAs). PHAs function primarily as intracellular reserves of carbon and energy [1,2]. The PHA biosynthetic pathway is highly versatile and has been utilised to synthesise a wide range of PHAs with over 100 different monomeric components [3]. These novel biopolymers have material properties ranging from rigid and highly crystalline to flexible, amorphous and elastomeric [1,4,5]. As a consequence of these material properties and inherent biodegradability, PHAs have attracted considerable attention as environmentally friendly alternatives to the conventional petroleum-based polymers, i.e., as 'bioplastics'. Furthermore, two members: polyhydroxybutyrate (PHB) and polyhydroxyoctanoate (PHO) have demonstrated biocompatibility in mammalian systems, suggesting potential applications as biomaterials for medical implantation devices [6,7].

In an effort to control PHA material properties we have applied the strategic addition of polyethylene glycol (PEG106) to the production of PHO. When compared to the microbial synthesis of PHO, the presence of PEG106 in the fermentation reduced polymer yield and efficiency and modified PHA composition. Manipulation of bioprocessing parameters also resulted in termination of the natural PHA chains with the relatively small synthetic group (Fig. 1). This natural–synthetic hybrid copolymer of PHO-*b*-PEG exhibited similar thermal and crystalline properties to PHO, but showed a molecular weight less than 50% that of its non-hybridised counterpart [8,9].

PHO-*b*-PEG films cast from solvent exhibited little morphological difference from their non-hybridised PHO counterparts. This was not unexpected given the relative

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distribution of molecular masses for the two blocks, approximately 900:1. Small-angle neutron scattering (SANS) was applied to these films to investigate whether the incorporation of the comparatively small hydrophilic PEG group at the end of the hydrophobic PHA chain had any influence on chain organisation.

2. Materials and methods

PHO and PHO-*b*-PEG were produced through the cultivation of *Pseudomonas oleovorans* in a 5 l fermentor (Braun Biostat C, Braun GmbH, Germany) as previously described [9]. Confirmation of PHO chain termination by PEG106 in the hybrid was established using NMR (2-D COSY and HSQC) [8].

2.1. Preparation of PHA films

Polymer films were prepared by dissolving 25 mg of PHO and 100 mg of PHO–PEG in 10 ml of analytical grade chloroform (Sigma–Aldrich, Sydney, Australia), using a biosafety cabinet to prevent any contamination. Samples of solution were then poured onto circular quartz discs and allowed to evaporate with the formation of thin films (approximately 1 mm in thickness). Four films were produced. In a similar fashion, samples of solution were also placed on clean glass slides and solvent allowed to evaporate under moist airflow (92% rH).

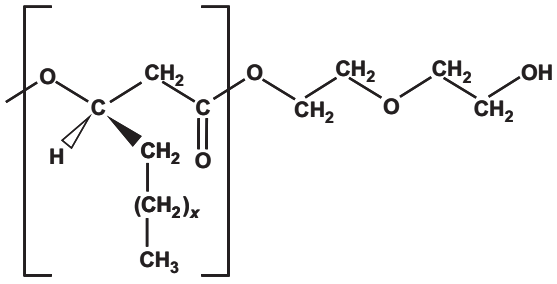

Fig. 1. Chemical formula for natural–synthetic hybrid of PHO–PEG106, $x = 2$, 4 and 6.

2.2. SANS studies

Polymer films on quartz discs were analysed using SANS. Four discs were stacked together to increase scattering intensity. Spectra were collected on the 8 and 30 m instruments at the Centre for Neutron Research (NIST, Gaithersberg, USA). The q-range was $\sim 0.002 < q < 0.25\,\text{Å}^{-1}$ using the two instrument configurations (a low-q and a high-q configuration).

3. Results and discussion

Solvent cast films of PHO–PEG showed no noticeable morphological differences from films cast in a similar fashion using non-hybridised PHO, although significant differences in their respective physiochemical properties were observed [9]. In an attempt to determine whether the small hydrophilic component exhibited any influence on the conformation of the hybrid copolymer chains during film preparation, films were analysed using SANS.

Fig. 2 shows the scattering pattern obtained when the films were analysed for 25 min on the 8 m SANS instrument. The figure provides clear evidence at this low q for sample-dependent scattering. An increase in intensity at higher q was suggestive of a diffraction phenomenon. However, the q-range of the instrument was determined to be unsuitable and consequently the experiment was repeated on the 30 m SANS instrument with an extended q-range; $\sim 0.002 < q < 0.25\,\text{Å}^{-1}$ using the two instrument (a low-q and high-q) configurations.

The two-dimensional SANS pattern for the biopolymer film obtained using the high-q configuration 30 m instruments displayed a narrow ring of intensity (not shown). This pattern suggests a highly ordered system within the film. The one-dimensional SANS profile shown in Fig. 3 is a combination of data obtained from the two instrument configurations. The

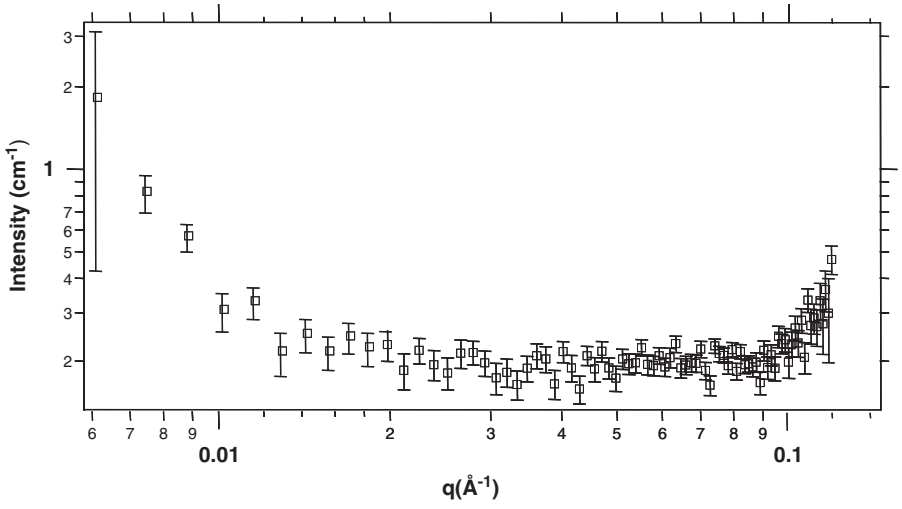

Fig. 2. SANS profile obtained from solvent cast PHO–PEG-based film using low-q configuration.

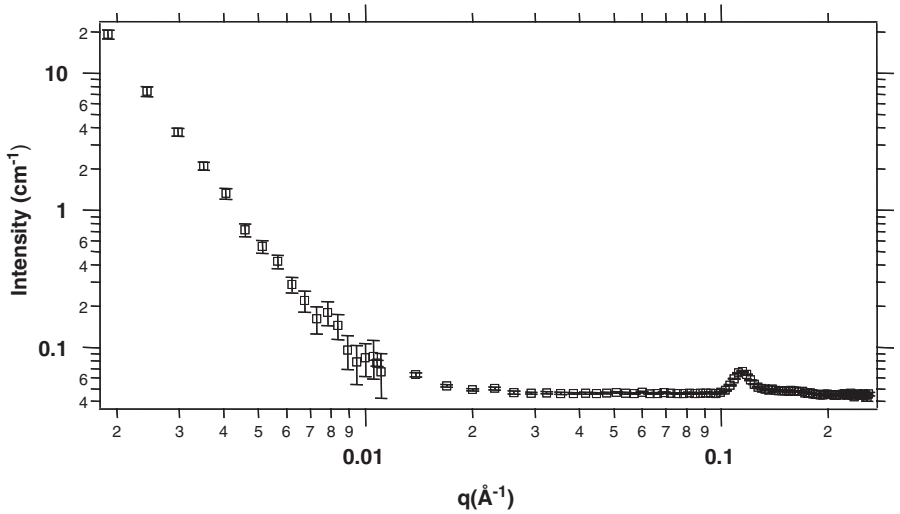

Fig. 3. SANS profile obtained from solvent cast PHO–PEG-based film using both low and high-q configurations, region of overlap is $q \sim 0.01\,\text{Å}^{-1}$.

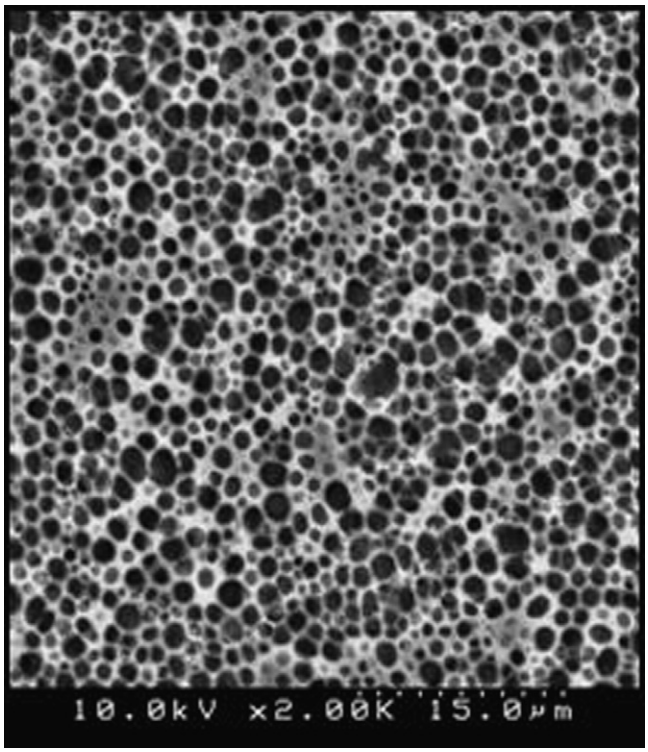

Fig. 4. SEM of microporous film fabricated from PHO-*b*-PEG.

low-q range displays significant sample-dependent scattering above that of the background. A diffraction ring at $q \sim 0.12\,\text{Å}^{-1}$ is clear, supporting the concept of a highly ordered structure with a d-spacing of approximately 50 Å.

One can speculate that the amphiphillic nature of this natural–synthetic diblock copolymer may support some local ordering, however it is unclear how this may result in a diffraction peak of 50 Å. The scattering peak may also be due to lamellar repeats of crystalline regions of the polymer. The nature of this structure is currently under investigation using techniques we have recently developed to biodeuterate the PHA block [10].

A number of synthetic amphiphillic diblock copolymers have been shown to self-assemble into highly ordered microporous films [11]. Such films have a wide range of potential applications, such as a matrix for biomolecules [12]. The exact mechanism for this 'bottom-up' technology remains unclear, but is thought to originate with the formation of aqueous 'breath figures' condensing on the solvent surface [11]. Using similar processing conditions, a microporous film was fabricated using the PHO-*b*-PEG hybrid copolymer (Fig. 4). PHO alone failed to demonstrate any degree of self-organisation.

The production of microporous films from a natural–synthetic hybrid of PHO-*b*-PEG supports the concept of an ordered structure determined by SANS. Further studies are required to clarify the polymer chain conformation in films of these novel hybrids.

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