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LETTER

Preparation of biodegradable poly(3-hydroxybutyrate) (PHB) and poly(ethylene glycol) (PEG) graft copolymer

Cai Zhijiang · Wen Zhihong

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The research on degradable plastics, especially that of biodegradability has become one of the focuses of advanced material researching. Bacterial poly(3-hydroxybutyrate) (PHB) is well known as thermoplastic aliphatic polyester. It has many advantages such as biodegradability, biocompatibility and optical activity [1]. Moreover PHB exhibits thermoplastic behavior and can be processed by traditional processing such as extrusion and injection moulding [2]. As it is a truly biodegradable and excellent biocompatible material, it is suitable for many promising applications: one is as a viable candidate for relieving environment concerns caused by disposal of non-degradable plastic [3]; the other is to provide biomedical material used in biomedical field such as surgical sutures, long term carriers of drugs and tissue engineering [4]. Unfortunately PHB is rather fragile, hydrophobic and for application in which a biodegradable material is required, its degradation time is too long.

In order to obtain overcome its shortcomings, many efforts have been employed, including physical blending and chemical copolymerization. For example, the biosynthesis of copolyester containing 3-hydroxyalkanoates (3HA) units, other than 3-hydroxubutyrate (3HB) units, such as poly(3-hydroxubutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)) has been made [5]. Compared with pure PHB, the copolymers are thermally more stable and exhibit more hydrophilic. The brittleness of PHB could also be greatly improved by the copolymerization. In addition to the biosynthesis way, blending PHB with other polymers is an alternative route to provide material with the desirable properties. These have been numerous reported on blends of PHB/poly (ε-caprolactone) (PCL) [6], PHB/cellulose ester (CE) [7], PHB/poly (lactic acid) (PLA) [8], etc.

However, chemical modification is thought to be a more direct and effective way to improve the inherent properties of PHB. Preliminary studies which used PHB chain segments obtained by the degradation of natural-origin PHB for the synthesis of PHB-containing block copolymers have been reported by Revee et al. [9]. In this case, the living character of the catalyst-polymerization system has provided a route to synthesis of a number of A-B diblock copolymers containing PCL [10] and PLA [11] chain segments with control over the block molecular weights. Work by Yalpani and Marchessault [12] has demonstrated the use of degraded natural-origin PHB for the synthesis of PHB-polysaccharide conjugates.

PEG is a synthetic polymer known to be highly hydrophilic, biocompatible and flexible. In order to satisfy different requirements, especially to biomedical materials, PHB and PEG physical blends have been investigated by our laboratory. The incorporation of low molecular PEG into PHB matrix can improve the hydrophilicity, flexibility and degradation rate. However, its mechanical properties, such as tensile strength and elongation at break, are decreased dramatically.

In this study, PHB and PEG graft copolymer were prepared by two-step method. First, the PEG macromer was synthesized using a literature method [13]; second, the PHB and PEGM were reacted under the ultraviolet (UV) to prepare PHB/PEG graft copolymer. Although graft polymerization onto PHB is a rather difficult due to its high crystallinity and nonactive chemical structure, graft polymerization of PEG onto PHB was successively achieved in this study.

C. Zhijiang (✉) · W. Zhihong
College of Materials Science and Chemical Engineering, Tianjin University of Science and Technology, Tianjin 300072, China
e-mail: caizhijiang@hotmail.com

The poly(3-hydroxybutyrate) (PHB), a white powder sample was kindly provided by Tianjin TianLu Co. Ltd(China), Mw = 430,000 Mw/Mn = 1.49 (obtained by G.P.C. in chloroform at 30 °C). It was purified by precipitation in *n*-hexane from chloroform solution, subsequently precipitated inmethanol from chloroform solution. The sample of poly(ethylene glycol) (PEG) was purchased from Tianjin TianTai Chemical Company (China, Mn = 4,000), was recrystallized from acetone and vacuum dried. Acryloyl chloride was prepared according to a literature procedure [14]. Chloroform, *n*-hexane, methanol, cuprous chloride and anhydrous sodium carbonate were all AR grade and used without further purification PEG (20 g; 1.3 mol) was dissolved in 100 mL 1, 2-dichloroethane, to which 2.0 g (2.0 mol) cuprous chloride, 6.9 g (6.5 mol) anhydrous sodium carbonate and 5.3 mL (6.5 mol) acryloyl chloride were successively added. The reaction proceeded under stirring and nitrogen atmosphere at room temperature for 24 h. As it ended, the product was filtered to remove the solid substances, and the collected liquid was extracted with sodium bicarbonate and then distilled water. Finally the solvent was evaporated and the residual substance was recrystallized with ethanol. The obtained white crystals were just the desired PEG macromer, after being dried under vacuum at room temperature for 24 h.

Chloroform solutions (20 wt/vol%) with different compositions of PEGM/PHB were prepared. The concentrated solutions were poured into TFE molds and irradiated using a 500 W high-pressure UV lamp at room temperature for 2 h. Residual homopolymer and unreacted PEGM were removed by water washing using Soxlet extractor for 24 h. The obtained copolymer gelations were dried in vacuum at room temperature for 48 h. Graft% was calculated from the added weight, i.e.

$$\text{Graft}\% = \frac{W - W_0}{W_0} \times 100 \quad (1)$$

where W_0 and W are weight of PHB and PHB grafted PEG, respectively ^{13}C NMR spectrum of PEGM was recorded on an AC-P400 MHz spectrometer at 400 MHz, using tetramethylsilane as an internal reference and $CDCl_3$ as the solvent FTIR measurements were carried out on a single beam Perkin Elmer Spectra 2000 IR spectrometer under N_2 purging.

A NETZSCH DSC-204 apparatus was used to study the influence of grafting percentage on thermal properties with a thermal program as shown in Fig. 1. In order to measure the glass transition temperature (T_g) of copolymers, the samples were heated from room temperature to 200 °C at 20 °C/min (first run). After holding for 2 min at 200 °C, they were rapidly quenched in liquid nitrogen to –100 °C,

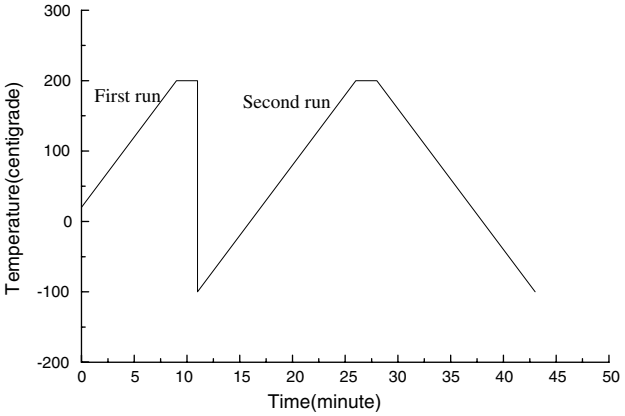

Fig. 1 Thermal program for DSC measurements

followed by heating from –100 °C to 200 °C at 20 °C/min (second run).

The measurement of contact angle was performed at 25 °C in the range of 0.5~20 min by pendant drop method, employing a contact-angle measurement apparatus (type DSA-10, made in KURSS Company, Germany). The static contact angle was measured at contact time t = 30 s. Drops of liquid (1.5~2.0 mm diameter) were prepared with a microsyringe and were dropped onto the surface of polymer films. For each sample, the mean of five separate points was obtained based on the same contact time.

The enzymatic degradation of the PHB/PEG grafting copolymer films was carried out at 37 °C in 0.1 M phosphate buffer (PBS, Oxoid PH: 7.2~7.4) containing lysozyme (Sigma, 0.2% solution in PBS). The dried films were cut into squares and incubated in the reaction solution with shaking, and at various time points the samples were removed, washed in distilled water and allowed to dry in air to constant weight. For each polymer sample, three films were used and the degradation rate was determined by the ratio of the weight loss to the initial weight of samples as shown below.

$$S = \frac{W_0 - W_t}{W_0} \times 100\% \quad (2)$$

Where S is the degradation rate, W_t and W_0 are the weight of samples after dried and the initial weight respectively.

The synthetic route of PHB/PEG grafting copolymer is shown in Fig. 2. Hydroxyl end groups of PEG are reacted with acryloyl chloride to form PEGM. Anhydrous sodium carbonate is used to neutralize acidic HCl molecules released during the reaction, which cuprous chloride, an initiator, is to preclude polymerization of acrylate groups. The introduction of acrylate groups to both chain ends of

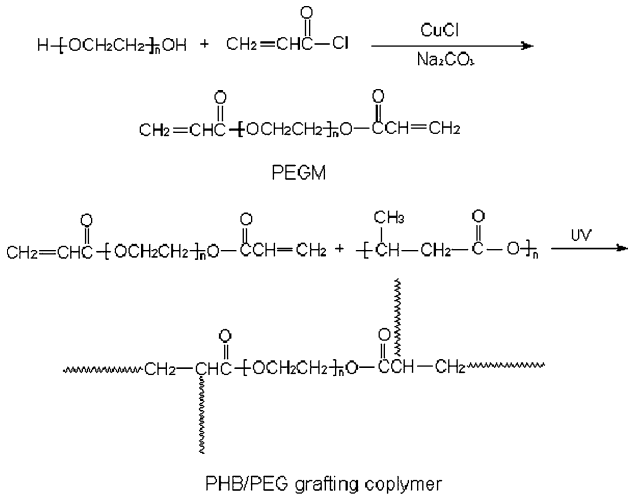

Fig. 2 Synthetic scheme of PHB/PEG graft copolymer by UV-irradiation

PEGM offers the possibility to synthesize PHB/PEG grafting copolymer directly, without adding other multi-functional comonomers. The ^{13}C NMR spectrum of PEGM shown in Fig. 3 clearly indicates the attachment of acrylate groups to both chain ends of PEGM. The peaks at 62.5, 64.1 and 65.1 ppm belong to the methylene units in PEG segment and the peaks at 127.4, 131.6 and 161.4 ppm are the characteristic of acrylate groups.

Preparation of PHB/PEG graft copolymers is performed under the UV-irradiation, owing to the photoactive nature of the acrylate groups. The control of solution concentration seems important to acquire gel films. For this case, the concentration was chosen as 20 wt/vol% and the hydrogel films with smooth surfaces were obtained.

Figure 4 shows the FTIR spectra of PHB/PEG graft copolymer (c), PEGM (b) and pure PEG (a) samples. In Fig. 4b, the peak at 1,720 cm^{-1} is attributed to the newly

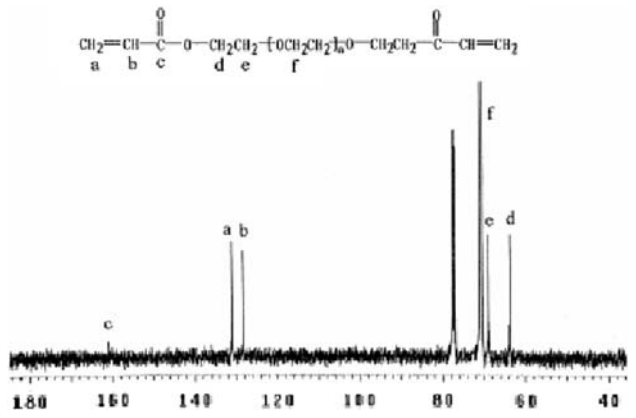

Fig. 3 ^{13}C NMR spectrum of PEGM

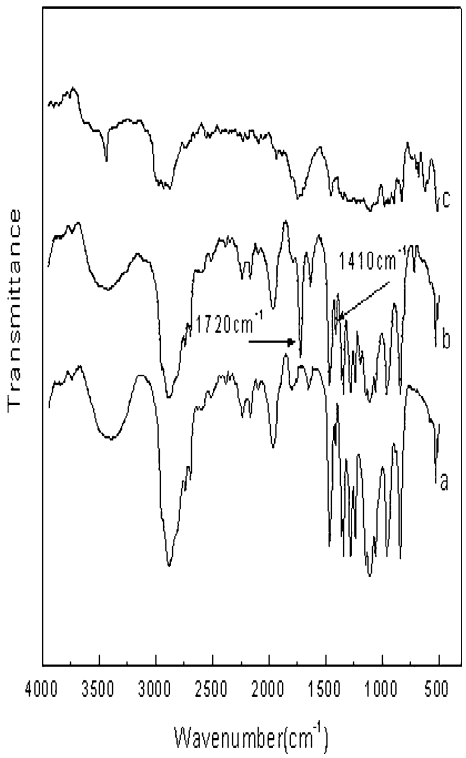

Fig. 4 FTIR spectra of: (**a**) pure PEG; (**b**) PEGM; (**c**) PHB/PEG graft copolymer

formed carbonyl bond in PEGM, due to the reaction between acryloyl chloride and hydroxyl end groups of PEG. The peak position at 1,410 cm^{-1} represents the adsorption of terminal methene in PEGM. All these can confirm that the acryloyl groups have been successfully introduced into PEG chains. Figure 4c is the FTIR spectrum of PHB/PEG grafting copolymers, in which the carbonyl absorption shifts to higher frequency position 1,738 cm^{-1} and the peak also becomes much broader compared with the PEGM, owing to the appearance of carbonyl adsorption of PHB. While the adsorption peak for terminal methene disappears thoroughly. These facts, together with the evidence of PEGM structure observed in Fig. 4b, clearly indicate the formation of PHB/PEG graft copolymers.

The DSC thermograms are summarized in Fig. 5 for PHB and PHB/PEG grafting copolymers with the graft% ranged from 5% to 22%. To eliminate the effect of thermal history, the samples are heated from room temperature to 200 °C at 20 °C/min for the first run, then annealed in Nitrogen after melting for 2 min.

In order to measure the glass transition temperature (T_g) of graft copolymers, the samples are followed by heating to 200 °C at 20 °C/min for the second run after rapidly being quenched in liquid nitrogen to –100 °C. The glass transition temperature (T_g) of pure PHB cannot be detected by a conventional DSC measurement owing to

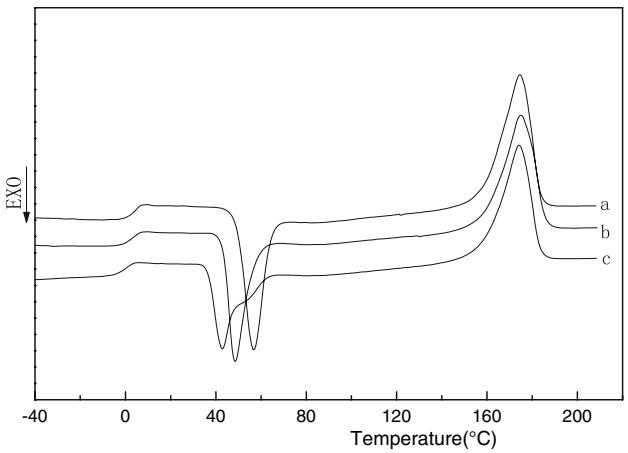

Fig. 5 DSC scanning curves of PHB/PEG graft copolymers (PHB/PEG graft copolymer: graft% = a, 5%; b, 14%; c, 22%)

its high crystallization. To get the glass transition temperature (T_g) of pure PHB, the pure PHB film is experienced DMTA measurement which exhibits a tanδ transition centered around 5 °C corresponding to the T_g of the PHB amorphous region. As for PHB/PEG graft copolymers, the glass transition temperature (T_g) can be detected noticeably by DSC, which is lower than that of pure PHB. Moreover, with the increase of graft percentage, the glass transition temperature is shifted to low temperature, which means the decrease of crystallinity and increase of amorphous region of PHB.

The surface energy of the solid can be estimated by contact angle measurement, together with a theory of intermolecular forces. In this experiment, the drop contour analysis is used for determining the water contact angle. The water contact angle of the PHB/PEG copolymers are shown in Fig 6. It can be seen that the water contact angle decreases with the increase of graft%. For pure PHB the water contact angle value is 64.6°, while with the graft% increased to 14% the water contact angle value lowed to 60.3°. Moreover, the contact angle of the grafted polymers is decrease as the PEG grafting content increase. This indicates that the grafted polymers become more and more hydrophilic and the hydrophilicity of the copolymers are improved. Such a result exhibits that the enhancement of the hydrophilicity is mainly result from introducing of PEG.

The result of the enzymatic degradation of pure PHB and PHB/PEG grafting copolymers using lysozyme are plotted in Fig. 7 as normal weight loss ratio (S) versus elapsed time. PHB and all grafting copolymers show good biodegradability. The grafting copolymers degrade much faster than the pure PHB, and with the graft percent increasing the degradation rate increases too. For Example, the degradation rate of PHB/PEG grafting copolymer with the 14% graft% is twice as high as that of pure PHB. This acceleration of the biodegradation is supposed to be arisen from the lowered crystallinity of PHB. Another possible reason is the hydrophilicity of the PEG, which makes the enzyme easier to attack. Thus, the dissolved the PEG molecules scission by enzyme causes an increase in the surface area that can be attack by enzyme. Therefore, the acceleration of the biodegradation is caused by a combined effect of lower crystallinity and hydrophilicity of PEG as well. It can be concluded that the biodegradability of the PHB can be improved significantly by grafting a hydrophilic biodegradable polymer such as PEG.

Since the crystallinity of PHB affects its degradation rate strongly. How the introduction of PEG into PHB fragment affects the crystallization behavior of PHB is a key factor, which makes us preparation of PHB/PEG graft copolymers with suitable degradation rate. The further study on this respect is still under investigation.

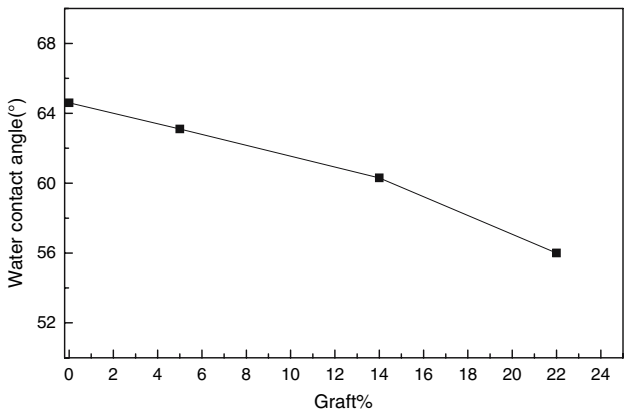

Fig. 6 The water contact angle of the blank PHB and PHB/PEG graft copolymers

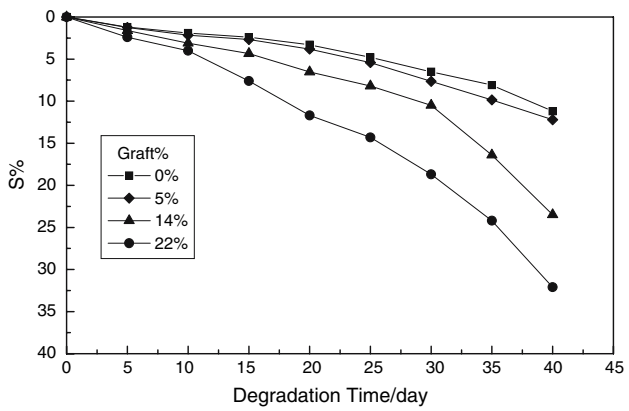

Fig. 7 The weight loss curves of PHB/PEG graft copolymers degradation in lysozyme/PBS

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