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Semi-interpenetrating networks of bacterial poly(3-hydroxybutyrate) with net-poly(ethylene glycol)

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Abstract

Semi-interpenetrating network (IPN) hydrogels based on bacterial poly(3-hydroxybutyrate) (PHB) and net-poly(ethylene glycol) (net-PEG) were prepared by the UV irradiation technique. The swelling and thermal behavior of the hydrogels, as well as their mechanical properties were studied by means of swelling tests, differential scanning calorimetry and tensile experiments. Net-PEG-based hydrogels all show higher equilibrium water contents (EWCs), while a remarkably decreased EWC is observed for the hydrogel containing 75% PHB. The thermal behavior of PHB in the semi-IPNs is not greatly affected by hydrogel composition. However, the crystallinity of PEG segments is noticeably decreased by crosslinking and would further drop with increasing amount of PHB. Incorporation of semi-IPN structure with PHB could significantly improve the mechanical properties of hydrogels when compared with that of pure net-PEG. The PHB/net-PEG (50/50) semi-IPN2, ranking as that possessing the best mechanical properties in the wet state, is expected to be useful as a biomedical material.

Keywords: Semi-interpenetrating network; Poly(3-hydroxybutyrate); Net-poly(ethylene glycol)

1. Introduction

Poly[(*R*)-3-hydroxybutyrate] (PHB) is a naturally occurring, thermoplastic polyester produced by a variety of bacteria [1,2]. It is a truly biodegradable and excellent biocompatible material suitable for two promising applications: one is as a viable candidate for relieving environmental concerns caused by disposal of non-degradable plastics; the other is to provide new-type biomedical products. However, PHB is relatively hard and brittle [3], and its melting behavior is unstable with a narrow thermal processing window [4].

In order to obtain useful new derivatives based on PHB, two main approaches have been extensively studied. One is the biosynthesis of copolyesters containing 3-hydroxyalkanoate (3HA) units, other than 3-hydroxybutyrate (3HB) unit, with a variety of bacteria, such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [poly(3HB-*co*-3HV)] [3] and poly(3-hydroydroxybutyrate-*co*-4-hydroxybutyrate) [(poly(3HB-*co*-4HB)) [5] copolymers. Compared with pure PHB, the copolymers are thermally more stable and exhibit progressively larger processing window. The brittleness of PHB could also be greatly improved by the copolymerization. In addition to the biological way, polymer blending is a simple, well-done, alternative route to modify PHB [6]. Many problems associated with the use of PHB might be alleviated by use of PHB blends. Till now, a lot of PHB blend systems have been studied in order to screen materials with improved properties. However, as far as we know, the research work on PHB interpenetrating network (IPN) has so far remained blank despite excellent performance of the IPN technique employed in other modification fields.

Net-poly(ethylene glycol) (PEG) is a kind of hydrogel which commonly possesses three-dimensional networks and could retain large quantities of water without dissolution. Although hydrogels are similar in the properties of water-swollen state to natural tissues, they are subject to a decrease in mechanical properties. Much attention has been paid to improve the mechanical properties of hydrogels in the swollen state. The main effort focused on introduction of a second component, a hydrophobic or hydrophilic polymer, into the hydrogels to form the so-called IPNs [7–14]. If the introduced component is a linear polymer, the formed network is called a semi-IPN.

It has been reported that PHB and PEG could form miscible blends over the whole composition [15]. The crystallinity of PEG segments has been greatly hindered by the presence of the PHB component. Thus, a combination of

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Table 1
Sample preparation and designations of PHB/net-PEG semi-IPNs

Sample code	PHB (g)	PEGM (g)	1,2-Dicholoroethane (ml)
Net-PEG	0	1.00	5
Semi-IPN1	0.25	0.75	5
Semi-IPN2	0.50	0.50	5
Semi-IPN3	0.75	0.25	5

the linear, crystalline PHB with the flexible net-PEG may produce a new type of hydrogel having desirable mechanical properties. In this paper, PHB/net-PEG semi-IPN networks were synthesized and their thermal and swelling behavior, as well as mechanical properties were characterized by means of differential scanning calorimetry (DSC), swelling and tensile tests.

2. Experimental

2.1. Materials

The PHB sample used in the present paper was kindly supplied by Chengdu Institute of Biology, Academia Sinica, and was prepared via bacteria fermentation using methanol as carbon source by methylotrophic strain 8502-3 (*Hyphomicrobium zaraarzinii* subsp. *Chengduence* subsp. *Nov*). The weight average molecular weight was 2.2×10^5 determined by intrinsic viscosity measurement using the relation $[\eta] = 1.18 \times 10^{-4}\ M_w^{0.78}$ in chloroform at 30°C. PEG ($M_n = 1540$) was recrystallized from acetone and vacuum dried. 1,2-Dichloroethane was purified by distillation over CaH_2. Acryloyl chloride was prepared according to a literature procedure [16]. Cuprous chloride and anhydrous sodium carbonate were both AR grade and used without further purification. All the above chemicals, except those indicated, were all obtained from Shanghai Chemicals Co. Ltd.

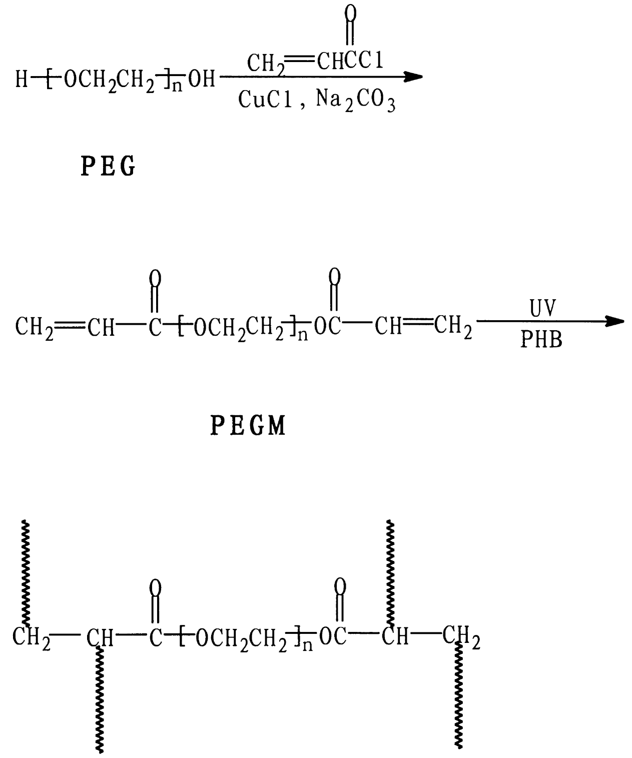

Fig. 1. Synthetic scheme of PHB/net-PEG semi-IPNs.

2.2. Synthesis of diacryloyl PEG macromer (PEGM)

PEG (20 g; 1.3×10^{-2} mol) was dissolved in 100 ml 1,2-dichloroethane, to which 2.0 g (2.0×10^{-2} mol) cuprous chloride, 6.9 g (6.5×10^{-2} mol) anhydrous sodium carbonate and 5.3 ml (6.5×10^{-2} 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, each three times. 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.

2.3. Preparation of PHB/net-PEG semi-IPNs

1,2-Dichloroethane solutions (20 wt/vol%) with different compositions (100/0, 75/25, 50/50 and 25/75 wt/wt) 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. The obtained gelations were dried under vacuum at room temperature for 48 h. Sample preparation and designations of these semi-IPNs are listed in Table 1.

2.4. Characterization

Nuclear Magnetic Resonance (^{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.

Fourier transform infrared (FTIR) spectra of PEGM, net-PEG and semi-IPN2 were recorded on a NICOLET MX-1 IR spectrometer.

Swelling behavior of semi-IPN hydrogels was studied by the general gravimetric method. Dry films were immersed in distilled water and the swollen weight for each sample was measured at a given time. Equilibrium water content (EWC) was calculated according to the following expression:

$$\mathrm{EWC}(\%) = \frac{W - W_d}{W_s} \times 100 \qquad (1)$$

where W_s and W_d denote the weight of swollen and dry hydrogels, respectively.

Thermal behavior was investigated using a Perkin–Elmer

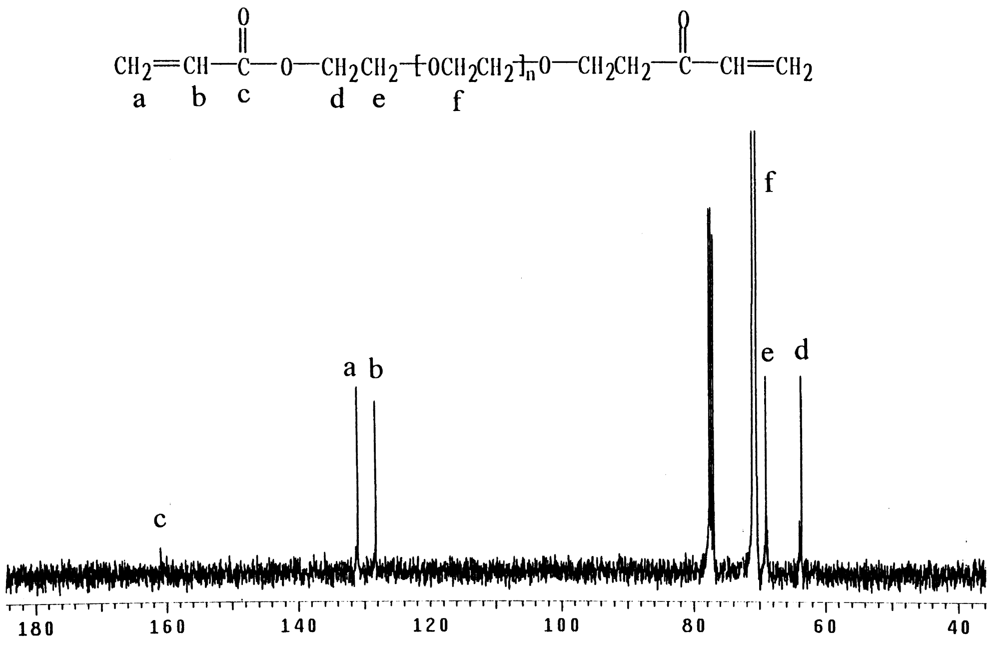

Fig. 2. ^{13}C NMR spectrum of PEGM.

DSC7 apparatus under nitrogen atmosphere. The measurements were carried out by heating samples from 30 to 200°C at a rate of 10°C/min. The melting temperatures (T_m) and apparent enthalpies of fusion (ΔH_f) were determined from DSC endothermal peaks.

Mechanical properties of semi-IPN hydrogels were measured at 23°C on an Instron 4302 machine. The sample films were 0.5 mm thick and tested at a stretching speed of 10 mm/min. From the computer program tensile strength and elongation at break were calculated and the load–extension curve was plotted. Reported values were determined on the average of at least five specimens for each semi-IPN hydrogel.

3. Results and discussion

3.1. Preparation of PHB/net-PEG semi-IPNs

Fig. 1 shows the synthetic route of semi-IPNs composed of PHB and net-PEG. 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, while cuprous chloride, an inhibitor, is to preclude polymerization of acrylate groups. The introduction of acrylate groups to both chain ends of PEGM offers the possibility to synthesize net-PEG directly from the macromer itself, without adding other multi-functional comonomers. The ^{13}C NMR spectrum of PEGM showed in Fig. 2 clearly indicates the attachment of acrylate groups to both chain ends of PEGM.

Preparation of PHB/net-PEG semi-IPNs were performed under the UV irradiation, owing to the photoactive nature of the acrylate groups. The control of solution concentration seems important to acquire smooth and integrated hydrogel films. It has been seen that precipitates of semi-IPNs would be produced from a dilute solution. Thus a concentrated solution is preferable to a dilute one for the formation of integrated semi-IPN networks. For our case, the solution concentration was chosen as 20 wt/vol% and hydrogel films with smooth surfaces were accordingly obtained.

Fig. 3 shows the FTIR spectra of PEGM (a), net-PEG (b) and semi-IPN2 (c) samples. In Fig. 3a, the peak at 1720 cm^{-1} is attributed to the newly-formed carbonyl bond in PEGM, due to the reaction between acryloyl chloride and hydroxyl end groups of PEG. The peak positioned at 1409 cm^{-1} represents the adsorption of terminal methene in PEGM. Both these absorptions will change if crosslinking occurs for PEGM to form net-PEG. The carbonyl absorption would shift to higher frequency position 1740 cm^{-1}, while the adsorption peak for terminal methane disappears thoroughly (Fig. 3b). Fig. 3c is the FTIR spectrum of semi-IPN2, in which a new peak at 1380 cm^{-1} is observed and attributed to the adsorption of methene in PHB. The carbonyl peak of this spectrum also becomes much broader compared with net-PEG, owing to the appearance of carbonyl adsorption of PHB. These facts, together with the evidence of net-PEG structure observed in Fig. 3c, clearly indicate the formation of PHB/net-PEG semi-IPN2.

3.2. Swelling behavior

EWCs of semi-IPN hydrogels are plotted against hydrogel composition in Fig. 4. Photo-crosslinked hydrogels swelled rapidly in water and reached equilibrium within

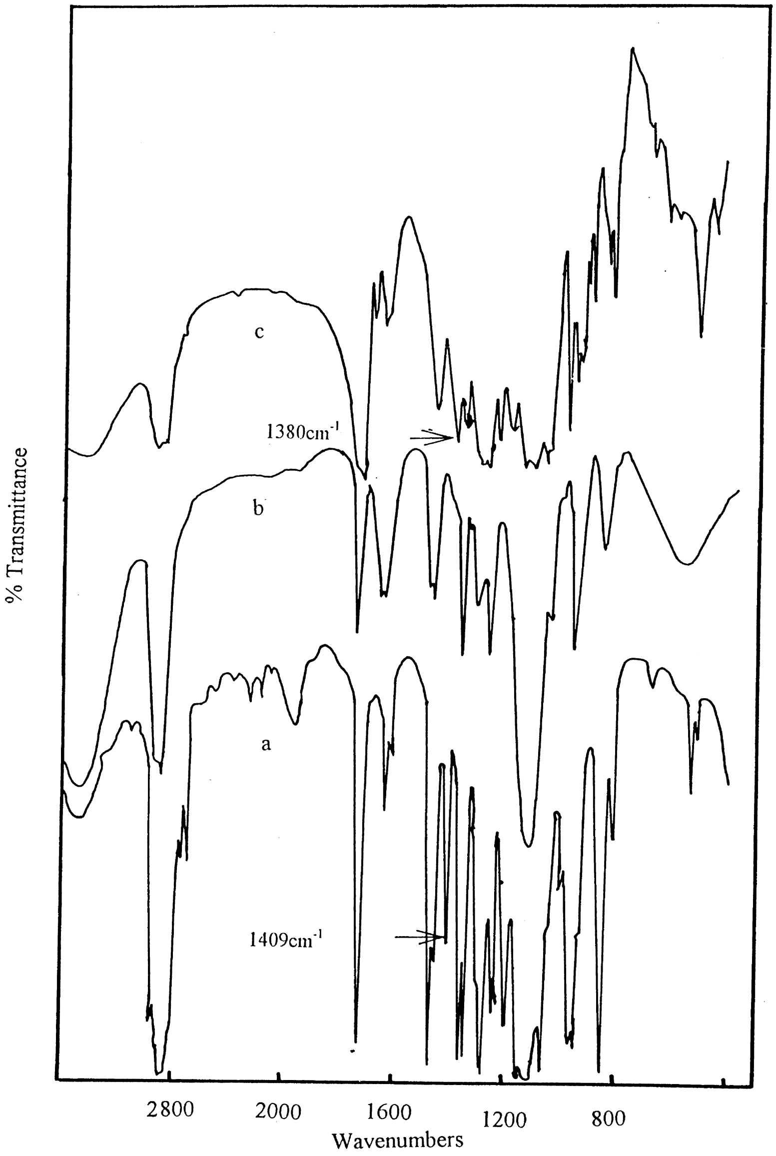

Fig. 3. FTIR spectra of: (a) PEGM; (b) net-PEG; and (c) PHB/net-PEG (50/50) semi-IPN2.

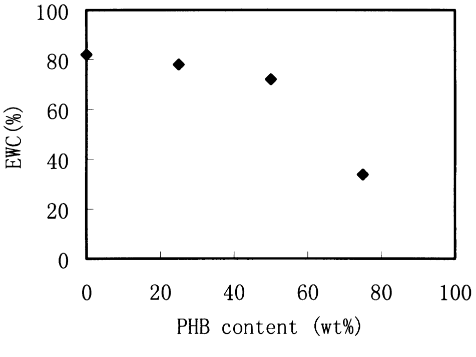

Fig. 4. Equlibrium water content (EWC) of hydrogels as a function of hydrogel composition.

1 h. These observations are almost in agreement with other semi-IPNs [12,13] containing net-PEG.

As shown in Fig. 4, the EWC of semi-IPNs shows a tendency to decrease with increasing amount of PHB. This behavior is opposite to that of β-chitin/net-PEG semi-IPNs [12,13] due to the hydrophilic nature of β-chitin in contrast to hydrophobic PHB. For the case of β-chitin/net-PEG semi-IPNs, aggregates of β-chitin molecules would disentangle when contacting with water, and thereby would not hinder swollen behavior of net-PEG. The observed increase in EWC with β-chitin content may be ascribed to a decreased crosslinking in the hydrogels. However for the PHB/net-PEG semi-IPNs, the crystalline, hydrophobic PHB might form impermeable aggregates of PHB crystals that would hinder swollen behavior of net-PEG. This restriction is observed especially remarkably in PHB-based semi-IPNs. A prominent example for this case is

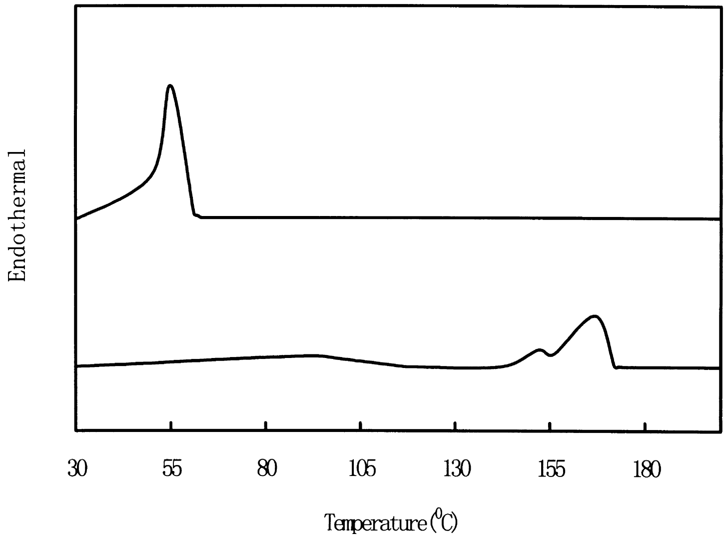

Fig. 5. DSC heating curves of: (a) semi-IPN2 hydrogel; and (b) PEGM.

the semi-IPN3, where PHB sperulites may form continuous crystalline structure over the whole hydrogel and the EWC of this sample is decreased sharply to 33.3%.

3.3. Thermal properties

Fig. 5 shows a typical DSC curve of PHB/net-PEG semi-IPN2 as well as that of linear PEGM. The endothermic peak positioned at about 170°C represents the fusion of PHB phase, whereas the peak below 100°C is attributed to that of PEG segments. Other semi-IPNs shows similar curves to that of semi-IPN2 except for the change in peak positions and intensities.

As shown in Fig. 5, while the linear PEGM shows a sharp melting transition at 55°C, the melting endothermic peak of PEG segments in semi-IPN2 become much broader after crosslinking. If the semi-IPN2 sample has been heat treated beforehand at 180°C for 1 min, its endothermic peak will become sharp again. This fact suggests that thermal history could noticeably influence the thermal behavior of semi-IPNs. From Fig. 5, it is also seen that PHB shows double melting peaks. This double melting could be well explained in terms of melt/recrystallization process [17]. The lower-temperature peak corresponds to the melting of as-formed crystals in the hydrogels, while the higher one results from that of more perfect crystals, produced by recrystallization on the heating run.

Data from DSC heating curves of PEGM and dry hydrogels are listed in Table 2. Whether for PHB or net-PEG, the melting temperature (for PHB, T_m^{PHB} is referred to the higher peak temperature) does not show a obvious change with hydrogel composition. However, T_m^{PEG} for net-PEG of hydrogels is far higher than that of linear PEGM. This behavior is opposite to that of β-chitin/net-PEG semi-IPNs [12,13], in which crosslinking of PEGM leads to a slightly decreased value of T_m^{PEG}. A possible reason for this difference may be due to a lower molecular weight of PEGM ($M_n = 1540$) used to prepare PHB/net-PEG semi-IPNs, thus producing a higher crosslinking density of net-PEG relative to that of β-chitin/net-PEG semi-IPNs (M_n of PEGM is 6000). As we know, the T_m of a crystalline component would shift to higher temperatures if the specimen has previously crystallized when being stretched. A similar case may also occur in the PHB/net-PEG semi-IPNs, where the higher crosslinking density of net-PEG would create stress concentration in the hydrogels and cause a similar stretching effect on PEG segments while they crystallize.

The crystallinity, X_c^{PEG} and X_c^{PEG}, of the PHB phase and net-PEG are obtained according to the following two relations, respectively:

$$X_c^{PHB} = \frac{\Delta H_f^{PHB}}{W^{PHB} \Delta H_{f,0}^{PHG}} \times 100\% \tag{2}$$

Table 2
Data from DSC heating curves of PEGM and dry hydrogels

Sample	ΔH_f^{PHB} (J/g)	T_m^{PHB} (°C)	X_c^{PHB} (%)	ΔH_f^{PEG} (J/g)	T_m^{PEG} (°C)	X_c^{PEG} (%)
PEGM	–	–	–	148.2	55.1	67.7
Net-PEG	–	–	–	53.1	88.3	24.1
Semi-IPN1	18.2	168.9	76.1	23.3	88.5	14.2
Semi-IPN2	36.7	167.6	76.9	9.2	92.3	8.4
Semi-IPN3	58.8	171.5	82.1	0	–	0

Table 3
Mechanical properties of PHB/net-PEG semi-IPNs in the dry and wet states

Sample	In the dry state			In the wet state		
	Initial modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)	Initial modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
Semi-IPN1	22.3	2.5	35.5	10.4	1.4	28.3
Semi-PN2	102.0	6.4	25.4	48.9	3.2	48.5
Semi-PN3	350.6	8.5	3.8	162.2	2.2	4.0

$$X_c^{\mathrm{PEG}} = \frac{\Delta H_f^{\mathrm{PEG}}}{W^{\mathrm{PEG}} \Delta H_{f,0}^{\mathrm{PEG}}} \times 100\% \qquad (3)$$

where $\Delta H_f^{\mathrm{PHB}}$ and $\Delta H_f^{\mathrm{PEG}}$ are the heats of fusion for the PHB phase and net-PEG in the hydrogels, respectively; W^{PHB} and W^{PEG} are the weight fractions of corresponding components in the hydrogels, respectively; and the values of $\Delta H_{f,0}^{\mathrm{PHB}}$ and $\Delta H_{f,0}^{\mathrm{PEG}}$, representing the heats of fusion for 100% crystalline PHB and linear PEG, are taken as 146 and 219 J/g for calculation, respectively. As shown in Table 2, the crystallinity of PHB is not affected noticeably by hydrogel composition and is comparable to that of pure PHB. The crystallinity of net-PEG, however, shows remarkable composition dependence and decrease with increasing amounts of PHB. This fact implies that crystallization of PEG segments is greatly hindered by the presence of the PHB component. Especially in the semi-IPN3, where PHB is the matrix phase, the crystallization of PEG segments is totally suppressed.

As discussed above, crosslinking of PEGM leads to a sharp increase of T_m^{PEG}. Besides this, crosslinking could also result in a drastic decrease of X_c^{PEG} from 67.7% for linear PEGM to 24.1% for pure net-PEG (Table 2). Thus it can be concluded that, the lower value of X_c^{PEG} in semi-IPNs is caused by a combined effect of crosslinking and PHB hindrance as well.

3.4. Mechanical properties

Tensile strength of hydrogels is a very important factor in biomedical applications. In particular, when the hydrogels are in contact with a tissue in the human body, they absorb a large quantity of water and suddenly lose their mechanical strength in the wet state. Many workers have attempted to improve the wet tensile strength. In the case of our present work, the incorporation of hydrophobic PHB into net-PEG was expected to enhance the mechanical properties of hydrogels in the wet state.

Whether in the dry or wet state, the hydrogels all show similar stress–strain curves. The slope of these curves decreases gradually with extension, and at high strain the stress tends to become a plateau. All samples break at the point where the stress reaches a maximum. This fracture behavior is similar to that of elastogel, although brittle PHB component has incorporated into the hydrogels.

Table 3 summarizes the mechanical properties of PHB/net-PEG semi-IPNs in the dry and wet states. Data from pure net-PEG were not obtained owing to its poor strength that precluded measurement under the experimental conditions. As shown in Table 3, the mechanical properties of hydrogels are greatly affected by composition. In the dry state, both modulus and tensile strength of hydrogels increase with increasing amount of PHB. In the wet state, similar results are observed except for the tensile strength of semi-IPN3 hydrogel. These results indicate that PHB could act as a reinforcing agent to improve the mechanical properties of net-PEG hydrogel, even in the wet state.

In addition, a lower crystallinity of net-PEG offers flexibility of the semi-IPN hydrogels in both the dry and wet states. As shown in Table 3, elongations at break for semi-IPN1 and semi-IPN2 range between 25 and 50%. However, this flexibility is greatly undermined in the case of semi-IPN3, where the crystalline PHB becomes the matrix phase of hydrogel. This fact, together with the wet tensile strength of semi-IPN hydrogels, indicates that an excessively higher content of PHB is not favorable to the mechanical properties of hydrogels in the wet state. As a result, the PHB/net-PEG semi-IPN2 ranks as the hydrogel having the best mechanical properties among all samples.

4. Conclusions

Semi-IPN hydrogels based on bacterial PHB and net-PEG were prepared by the UV irradiation technique. The control of solution concentration seems important to acquire smooth and integrated hydrogel films. FTIR measurements confirmed the structures of PEGM and semi-IPNs.

The swelling behavior of hydrogels was studied by the gravimetric method. Net-PEG-based hydrogels (net-PEG, semi-IPN1 and semi-IPN2) all show higher EWCs ranging from 75 to 81%, whereas the PHB-based hydrogel (semi-IPN3) shows a remarkably decreased EWC, due to the hydrophobic nature of PHB component.

Thermal behavior of PHB, including both T_m^{PHB} and its crystallinity, all show minor change with hydrogel compositon. Crosslinking of PEGM, however, would lead to a broader endothermic peak and a higher T_m^{PEG} value relative to those of linear PEGM. In addition to these changes, the crystallinity of net-PEG for semi-IPNs is also greatly decreased below 24.1% in comparison with the value of

67.7% for linear PEGM, due to a combined effect of cross-linking and PHB hindrance.

Mechanical properties of semi-IPNs depend markedly on hydrogel composition. For semi-IPN1 and semi-IPN2, the hydrogels show improved strength and toughness when compared with pure net-PEG, in both the dry and wet states. These improvements are ascribed to a synergistic effect that the crystalline PHB component could improve the strength of hydrogels, while the flexible PEG segments offers roughness to the materials. In the case of semi-IPN3, the crystalline, brittle PHB becomes the matrix phase and dominates the mechanical properties of the hydrogel as a brittle material. Thus the semi-IPN2, ranking as the hydrogel possessing the best mechanical properties, is expected to be useful as a biomedical material.

Acknowledgements

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