

Radiation-Induced Graft Polymerization of Isoprene onto Polyhydroxybutyrate

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ABSTRACT: To improve the tenacity of polyhydroxybutyrate (PHB), isoprene was grafted onto polyhydroxybutyrate by direct radiation. The optimal conditions were determined, at which the grafting process occurred and suitable grafting degree was obtained. The effect of different solvents, monomer concentration and radiation dose on the grafting degree was studied. Some investigations and characterization on the prepared copolymer were made and the possible graft reaction process was explored. Tensile test of the graft polymer was carried out. The results showed that superior tenacity was achieved. The toughening mechanisms of isoprene graft modifying PHB were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

KEY WORDS Polyhydroxybutyrate / Isoprene / Polyisoprene (PIP) / Radiation-Induced Graft Polymerization / Toughen /

Polyhydroxybutyrate (PHB) is one of the naturally occurring polyesters produced by microorganism.¹ The microbial polyesters have attracted industrial attention as environmentally, degradable thermoplastics for a wide range of agricultural, marine, and medical applications. Because of entire biodegradability and good biocompatibility, PHB has many uses in biomedical application, such as drug release, surgical sutures and bone plates. All of these potential uses depend on biocompatibility and slow resorption of microbial polyesters in biological environments (*in vivo*). The ultimate biodegradation product, 3-hydroxybutyric acid, is a normal metabolite in human blood, and PHB shows negligible oral toxicity. However, under the common conditions, PHB is a very brittle plastic, which somewhat limits the application of itself. Many efforts have been taken to enhance its tenacity. A number of PHB/polymer blend systems have been studied, such as PHB/PEO,² PHB/PVAc,³ PHB/PVDF,⁴ PHB/polyepichlorohydrin (PECH),⁵ etc. M. Abbate, *et al.*⁶ studied the tensile properties and impact behavior of PHB/rubber blends. They found that PHB/ethylene propylene rubber (EPR)-*g*-(succinic anhydride) blend enhanced the capability of the material to be plastically deformed. Jin-San Yoon, *et al.*⁷ studied toughening of PHB with polyisoprene (PIP). They grafted poly(vinyl acetate) onto PIP and PHB/PIP-*g*-PVAc had some favorable properties.

Radiation is useful to prepare and modify biomaterials or materials for medical use, because firstly, it does not need any catalyst or initiator, so it is suitable to obtain biomaterials with high purity, and secondly, for

the high energy of radiation, it can prepare polymeric materials under low temperature, so as to restrain the destruction of biomaterials caused by heat.⁸ H. Mitomo *et al.* firstly investigated radiation graft polymerization of PHB and its copolymer poly(hydroxybutyrate-*co*-hydroxyvalerate) (P(HB-HV)).⁹ They successfully grafted methyl methacrylate and 2-hydroxyethyl methacrylate onto PHB and P(HB-HV).

Isoprene was used here as grafting monomer because 1,4-polyisoprene can be used in the field of medicine¹⁰ and polyisoprene is a tough polymer, which can maybe toughen PHB. Isoprene was grafted onto PHB by directly irradiating PHB immersed in isoprene solution. The grafting process was investigated. Mechanical properties of the graft copolymer were determined, which showed that the grafted PHB had superior tenacity to the plain PHB, while at the same time, the tensile strength of the grafted PHB remained nearly the same. The toughening mechanisms were also explored.

EXPERIMENTAL

Materials

Polyhydroxybutyrate (PHB), synthesized *via* bacterial fermentation, was purchased from the Chinese Academy of Science. Isoprene was the product of Fluka Chemika company and was used without further purification.

Graft Polymerization

Direct radiation grafting was used in a nitrogen atmosphere. The glass ampoule containing the monomer solution and PHB was deaerated by bubbling nitrogen,

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sealed and then subjected to ^{60}Co γ -rays at a dose rate of $21.62 \text{ Gy min}^{-1}$ at 25°C . Grafted PHB was extracted in a Soxhlet apparatus with benzene for 72 h to remove any isoprene monomer and adhering homopolymer, and then dried under vacuum at 40°C to constant weight.

Characterization Methods

Thermogravimetry (TGA) of the samples was carried out in a 2050 Thermogravimetric Analyzer at a heating rate of $20^\circ\text{C min}^{-1}$ under nitrogen atmosphere.

The melting temperature (T_m), glass transition temperature (T_g), crystallization temperature (T_c) and enthalpies of melting (ΔH_m) of original PHB and the grafted PHB were studied in a 2910 Modulated DSC apparatus at a heating rate of $10^\circ\text{C min}^{-1}$ under nitrogen atmosphere.

PHB powder was heated from room temperature to 200°C at a heating rate of $10^\circ\text{C min}^{-1}$. The melting temperature (T_m) was determined from the DSC endothermic peaks. The powder was rapidly cooled to -50°C and again, heated to 200°C at the heating rate of $10^\circ\text{C min}^{-1}$. The glass transition temperature (T_g), crystallization temperature (T_c) and enthalpies of melting (ΔH_m) were registered.

^1H NMR spectra of plain PHB and grafted PHB were recorded in CDCl_3 on a dpx300 spectrometer.

Transmission electron microscopy (TEM, H-800, Hitachi) was performed on osmium tetroxide (OsO_4) stained grafted PHB in order to investigate the morphology of the graft product.

Mechanical Properties

Plain PHB and PHB-g-PIP were injected into dumb-like specimens using a CS-183 MMX Mini-Max machine. Tensile test was carried out using an Instron 1122 electronic testing machine (made in England).

A scanning electron microscopy (SEM, S-450, Hitachi) was used to observe the morphology of the section. To observe the fracture events in the grafted PHB, a transmission electron microscopy (TEM) was used. Specimens were prepared from the stress-whitened region caused by tensile testing and the samples were stained with OsO_4 . Both SEM and TEM were performed in order to investigate the nature of fracture of grafted PHB.

RESULTS AND DISCUSSION

Preparation of the Graft Copolymer

To gain proper grafting degree, so as to achieve advisable mechanical properties, an optimal grafting reac-

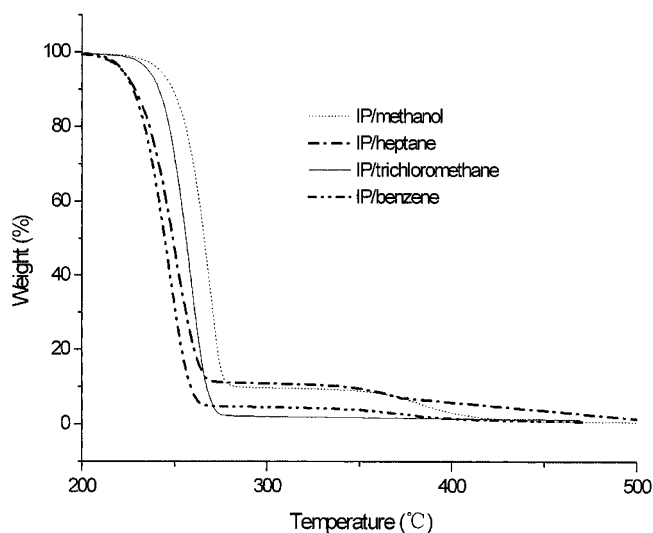


Figure 1. TGA thermograms of grafting copolymers in different solvents.

tion condition is needed. The obvious factors affecting grafting are solvent, monomer concentration and radiation dose.

Effects of Different Solvents. Several solvents such as methanol, heptane, trichloromethane and benzene were chosen. PHB was immersed in isoprene (20 vol% isoprene in each solvent) and the mixture was subjected to ^{60}Co γ -rays for 10 h.

Figure 1 shows TGA thermograms of the grafted copolymers. Because the decomposition temperatures of PHB and PIP are not the same, PHB grafted with isoprene shows two steps in its weight loss curve. The grafting degree of grafted PIP can be calculated by dividing the entire weight loss at the intersection of the steepest tangent line of the first drop to the tangent line of the plateau prior to the second drop.

Grafting degrees in the four solvents are listed in Table I.

Grafting degree was fairly low in benzene, while isoprene cannot be grafted onto PHB in trichloromethane. A much higher grafting degree can be achieved by using methanol or heptane. Heptane is the most favorable solvent in this reaction.

Effects of Monomer Concentration. The effects of various isoprene concentration in methanol on the grafting process were investigated.

Table II lists grafting degree of PHB in isoprene of different concentration.

Except for the case of pure isoprene, the grafting degree did not change much. It is assumed that, in a wide range of isoprene concentration, the affectation of monomer concentration on the grafting degree can be overlooked. While using isoprene without solvent, the grafting degree is dramatically low, which may be due to the volatilization of isoprene when immersing

Table I. Grafting degree of isoprene onto PHB in different solvents

Solvent	Methanol	Heptane	Trichloromethane	Benzene
Grafting degree/%	10.33	10.92	0	3.28

Table II. Grafting degree of isoprene onto PHB with different monomer concentration

Isoprene concentration/vol%	20	40	50	90	100
Grafting degree/%	10.33	9.13	9.24	9.92	4.39

Table III. Grafting degree of isoprene onto PHB in heptane after different radiation time

Radiation time/h	6	10	16	20	24
Grafting degree/%	8.96	13.47	17.88	17.07	17.19

PHB in isoprene, or it can be assumed that solvent is necessary to obtain high grafting degree in this grafting system.

Effects of Radiation Dose. Radiation dose has great influence on the grafting degree. PHB was immersed in isoprene (20 vol% isoprene in heptane) and the mixture was subjected to ^{60}Co γ -rays for different hours. Table III lists the grafting degree calculated from the figure.

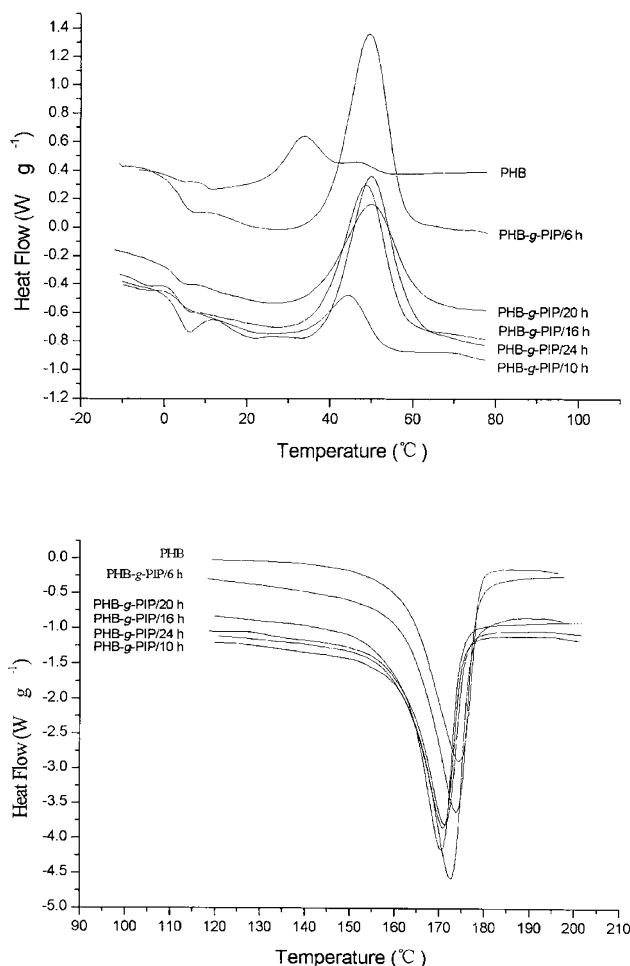
As radiation dose increases, the grafting degree of isoprene onto PHB increases at first. However, this trend does not exit when radiation time is over 16 h. It is supposed that, when the monomer concentration is fixed, there exits a radiation time, at which the grafting degree is the highest.

There are other factors that may affect graft degree, such as PHB concentration in isoprene solution, or relative amount of isoprene monomer to the PHB polymer. To get as high grafting degree as possible, a relative high and fixed amount of isoprene to PHB is adapted. The mass ratio of isoprene to PHB in this paper is 1.5:1. However, this factor should be included and need further research.

Crosslinkage during irradiating must be taken into consideration, for it is often concomitant to the reaction of grafting and affects the degradability of the grafted copolymer. The ability of PHB-g-PIP sample being dissolved in trichloromethane was investigated. The grafted copolymer can all be totally dissolved in hot trichloromethane. That is to say, after being irradiated for 24 h, the gel fraction in the irradiated polymer is nearly zero. It is favorable for PHB to be used as a degradable biomaterial.

Differential Scanning Calorimetry (DSC) Investigation

Typical DSC thermograms of PHB and PHB-g-PIP (20 vol% isoprene in heptane after different times of irradiating) are shown in Figure 2. Table IV lists the thermodynamic parameters obtaining from the curves

**Figure 2.** DSC curves of PHB and PHB-g-PIP.

of the samples, which including melting point (T_m), crystallization temperature (T_c), glass transition temperature (T_g) and enthalpies of melting (ΔH_m) of plain PHB and PHB grafted with isoprene. Melting points of PHB-g-PIP decrease with the introduction of isoprene grafting. Since the T_g of PIP is about -73°C (for *cis*-PIP) or -60°C (for *trans*-PIP),¹¹ it is natural that the T_g of the grafted copolymer decreases with the introduction of PIP, though this trend is fairly slight. Dramatic increase in T_c of the grafted polymers implies change in the crystallization behavior of PHB after irradiated and grafted with isoprene. For the PHB content in the grafted copolymer decreasing, the enthalpies of melting (ΔH_m) are corrected by the weight fraction of both polymers in the grafted samples according to the fol-

Table IV. Melting points, crystallization temperature, glass transition temperature, and enthalpies of plain PHB and PHB grafted with isoprene after the second DSC heating run

Sample	$T_m/^\circ\text{C}$	$T_g/^\circ\text{C}$	$T_c/^\circ\text{C}$	$\Delta H_m/\text{J g}^{-1}$	$(\Delta H_m)_{\text{corr}}/\text{J g}^{-1}$	$X_g/\%$
PHB	174.48	3.93	33.97	74.29	74.29	0
PHB-g-PIP/6 h	173.72	4.02	49.66	96.69	106.20	8.96
PHB-g-PIP/10 h	172.51	3.64	44.78	96.70	111.75	13.47
PHB-g-PIP/16 h	171.17	3.82	49.95	83.14	101.24	17.88
PHB-g-PIP/20 h	170.47	2.50	49.84	69.97	84.37	17.07
PHB-g-PIP/24 h	170.89	3.84	49.01	76.34	92.19	17.19

lowing equation:

$$\begin{aligned} &\text{Corrected enthalpy of melting } (\Delta H_m)_{\text{corr}} \\ &= \Delta H_m / (1 - X_g) \end{aligned}$$

$(\Delta H_m)_{\text{corr}}$ of each grafted sample has large increase compared to that of PHB. It can be deduced that the crystallinity of PHB being irradiated increases.

Analysis of the Radiation Reaction Mechanisms

Figure 3 shows the ^1H NMR spectra of plain PHB and PHB-g-PIP (20 vol% isoprene in heptane after 10 h radiation).

Chemical shift (δ) of the hydrogen on CH_2 and CH group of PIP can be calculated by using Shoolery empirical formula¹²:

$$\begin{aligned} \text{For } \text{CH}_2 : \delta &= 1.25 + \sum \sigma \\ \text{For } \text{CH} : \delta &= 5.25 + Z_{\text{same}} + Z_{\text{cis}} + Z_{\text{trans}} \end{aligned}$$

While σ is the empirical shielding constant of substituent, and Z_{same} , Z_{cis} , Z_{trans} are substitution constant of substituent, which are in the same carbon, in the *cis*-form and *trans*-form in other carbons to hydrogen, respectively.

Hydrogen peaks near 5.14 ppm, 2.04 ppm, 2.01 ppm, and 1.77 ppm are attributed to d', e', f', g' hydrogen atom, respectively.

According to the ^1H NMR spectra of PHB-g-PIP, we can find: after grafting, the number of hydrogen on site a changes from 1 to 0.87, which can be counted by the ratio of the areas under peak a' to peak c'. Similarly, the number of hydrogen on site b changes from 2 to 1.73. It is thought that some of the grafting reaction occur on site a, but, most on site b.

Based on the areas under peaks d' and c', the grafting degree of isoprene onto PHB is found to be 7.33%. If based on the areas under peaks e', f', and c', the grafting degree is only 6.70%. Both values were much less than that obtained from TGA (13.47%). So, during the graft reaction of isoprene onto PHB, branching reaction must have been taken place simultaneously. Graft reaction occurs on the PIP branched chain, and mostly takes place on site e' and f', secondly on site d'. However, because of the complexity of the radiation reaction, there should be several kinds of grafting products.

Morphology of PHB-g-PIP

TEM photographs of the grafted PHB are shown in Figure 4.

From the low magnification TEM photographs, PIP is homodispersed in PHB matrix. The average rubber particle size is nearly 0.25–0.4 μm , while some large particles can reach a diameter of about 0.8 μm . From Figure 4(a) to 4(c), the content of the dark particles increases, which stands for increasing grafting degree along with the extension of radiation time. At high magnification, each rubber particle has its fine structure. The composite rubber particles are formed by both PIP and PHB sub-inclusions, or cell structures.

Mechanical Properties

Figure 5 shows the typical stress–strain curves obtained at room temperature and a strain rate of $2.1 \times 10^{-3} \text{ sec}^{-1}$ for both PHB and PHB-g-PIP.

The corresponding Young's modulus (E) and ultimate parameters such as the stress (σ), the elongation (ϵ) and area under the tensile curve (S) calculated from the stress–strain diagrams are summarized in Table V.

Plain PHB exhibits at room temperature a stress–strain relation typically of a brittle polymer. The material extends linearly to a fracture strain of about 8.2%. However, the grafted PHB shows different properties with plain PHB and with each other. After 6 h radiation, PHB-g-PIP displays the property of ductility. There is an obvious yielding point on the stress–strain curve, and elongation of the grafted PHB can reach as high a value as 17.2%. The value of the area under the stress–strain curve, which stands for tenacity of materials, indicates that the grafted PHB shows much better tenacity than plain PHB. The area of PHB-g-PIP is more than one time larger than that of plain PHB. Naturally, the Young's modulus of grafted PHB is much lower than that of plain PHB because of the introduction of PIP. But, if the radiation time extends, such ductility reduces. Both the elongation and the value of area under the stress–strain curve reduce. However, the Young's modulus of PHB-g-PIP increases gradually, although it is lower than that of PHB. These can be explained

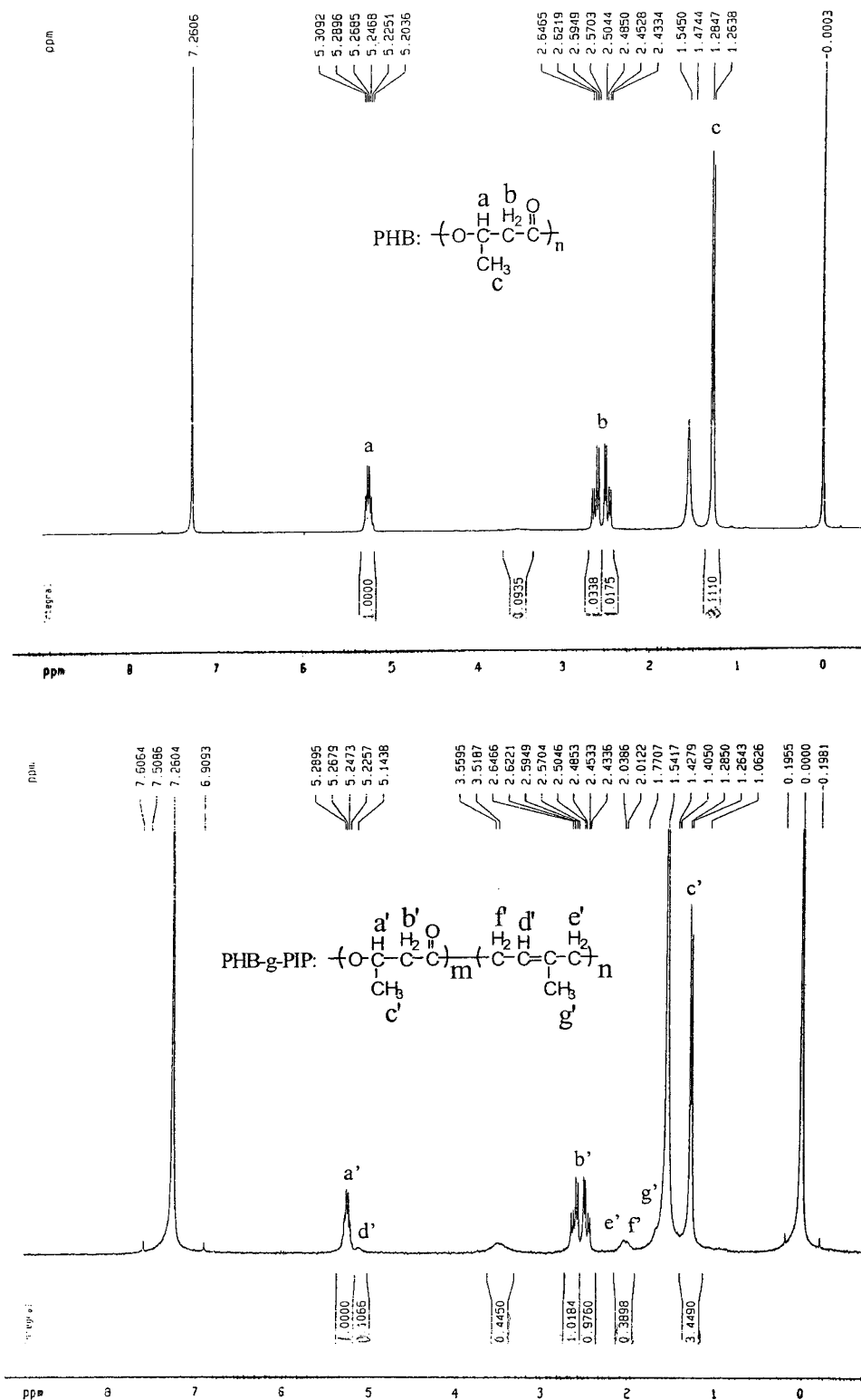


Figure 3. ^1H NMR spectra of plain PHB and PHB-g-PIP.

as high dose radiation results in slight crosslinkage in polymers, which increase rigidity, but, decrease ductility. Stress at break of the grafted PHB shows no obvious differences compared to PHB. However, if simply blending PHB with PIP or other elastomers, it will undoubtedly decrease dramatically. This is due to PHB sub-inclusion within the rubber particles, which maintains the original strength of PHB despite of the exist-

tence of PIP.

Fractographic Analysis and Fracture Mechanism

To interpret the toughen mechanism of PHB grafting with PIP, TEM, and SEM are used to observe the microscopic morphology of the samples after tensile test.

Figure 6 shows typical TEM micrographs from the stress-whiten regions of the grafted PHB fracture sur-

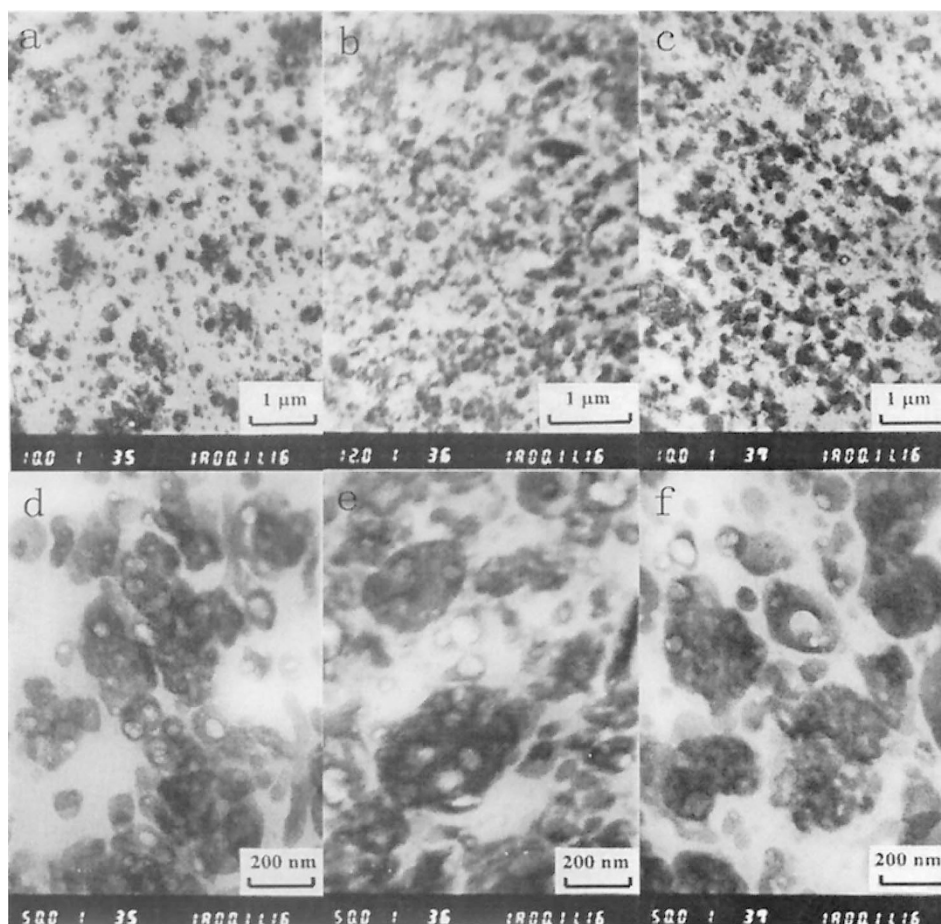


Figure 4. Transmission electron micrographs of PHB-g-PIP (20 vol% isoprene in heptane). Radiation time: (a), (d), 6 h; (b), (e), 10 h; (c), (f), 16 h.

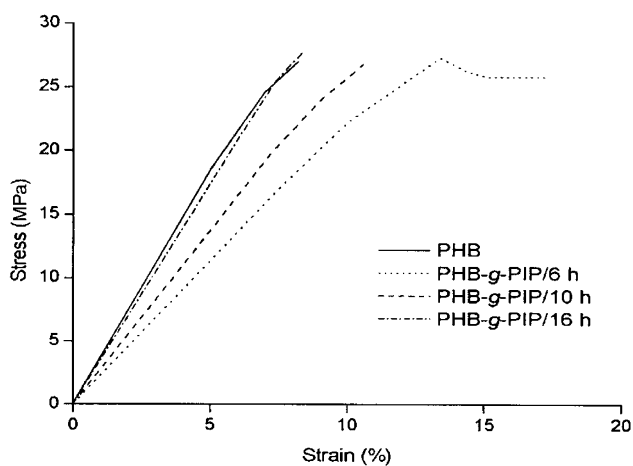


Figure 5. Stress-strain curves at room temperature for PHB and PHB-g-PIP.

faces. In most cases, after tensile test, the spherical rubber particles do not keep their original shapes. However, they change to irregular shapes, as shown in Figure 6a. The rubber particles stretch and tear. By themselves these rubber particles absorb a large amount of energy. What is more, many completely cracked cavities or partially cracked cavities (rubber particles did not tear) can be found within the PHB matrix, as in-

Table V. Mechanical properties of PHB and PHB-g-PIP

Sample	E/GPa	σ/MPa	$\varepsilon/\%$	S
PHB	0.77	27.0	8.2	92.8
PHB-g-PIP/6 h	0.33	26.2	17.2	216.3
PHB-g-PIP/10 h	0.47	26.8	10.6	175.2
PHB-g-PIP/16 h	0.58	27.8	8.4	94.0

dicates in Figure 6b. Occasionally, craze may be observed between two large rubber particles (Figure 6c). However, it seems that small rubber particles are not able to initiate craze.

Figure 7 shows typical SEM photographs of the sections of fractured PHB and grafted PHB samples. The section of PHB sample, which is shown in Figure 7a, indicates that PHB is a very brittle material. However, the photograph of the section of PHB-g-PIP is not the same. The main difference is that there are much more cavities in Figure 7b.

The toughening mechanism of PHB grafted with PIP is as follows: the rubber particles deform and cavitate and the resultant voids form in PHB matrix, which result in marginal improvement of tenacity of PHB.

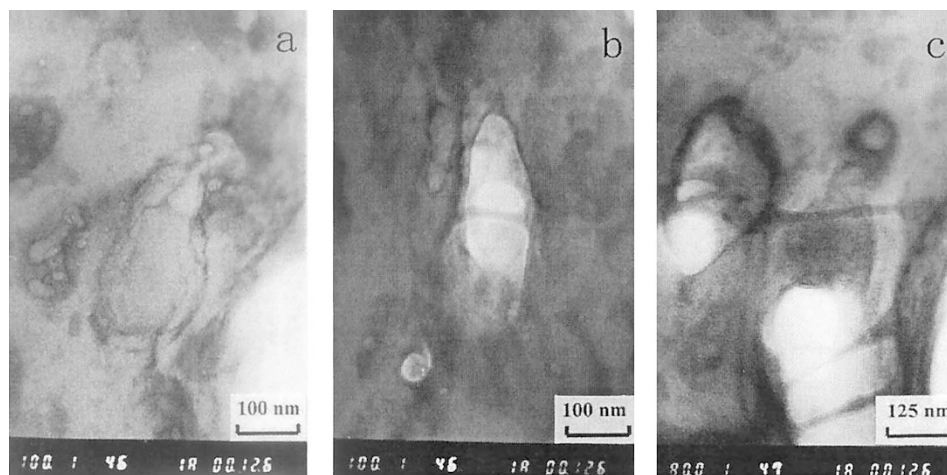


Figure 6. Transmission electron micrographs from stress-whitened regions of PHB-g-PIP fracture surface.

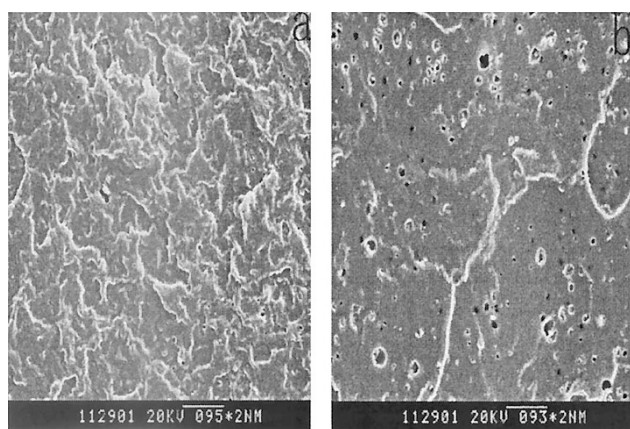


Figure 7. Scanning electron micrographs of PHB and PHB-g-PIP: (a) PHB, (b) PHB-g-PIP.

CONCLUSIONS

Isoprene can be grafted onto PHB by simultaneous irradiation. Solvent and radiation doses have great influence on the grafting degree, while in a wide range of isoprene concentration, the effects of monomer concentration on the grafting degree can be overlooked. When grafting in heptane and with a radiation time of 6 h (dose rate: $21.62 \text{ Gy min}^{-1}$), we can obtain PHB-g-PIP material with most favorable mechanical properties. Transmission electron micrographs show that there exists a cellular-like structure within PHB-g-PIP, and PHB-g-PIP has much better ductility and tenacity than plain PHB. While at the same time, it remains the original tensile strength of PHB. By SEM and TEM, fracture mechanism of grafted PHB is raised. The toughening of grafted PHB lies in the deformation of rubber particles and resultant cavitation. However, since most rubber

particles are rather small, it is assumed that these small particles cannot initiate craze, and only very large particles, small in number, can initiate craze. So, crazing is not the main way of toughening in PHB-g-PIP.

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