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Synthesis and controlled photo- and biodegradabilities of poly[(hydroxybutyrate -*co*-hydroxyvalerate)-*g*-styrene]

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SUMMARY: The graft copolymer, P(HBV-g-St), was synthesized by graft polymerization of styrene (St) onto poly(3-hydroxybutyrate-co-3-hydroxy valerate) [PHBV] under nitrogen atmosphere using benzoyl peroxide (BPO). The structure of poly[(hydroxybutyrate-co-hydroxyvalerate)-g-styrene] [P(HBV-g-St)] was identified by FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectra. The effects of weight ratio of St to PHBV in feed, initiator concentration, reaction time and reaction temperature on the grafting ratio and grafting efficiency were investigated. The thermal decomposition temperature of P(HBV-g-St) was 273℃. The tensile strengths of P(HBV-g-St) before and after photo- or biodegradation were also measured. The photodegradability of P(HBV-g-St) was better than that of PHBV. The film surface of P(HBV-g-St) treated with *Aspergillus nigger* showed extensive grooves and pits as compared with the untreated P(HBV-g-St). It was found that the photo- and biodegradation rates of P(HBV-g-St) and tensile strength of P(HBV-g-St) can be "controlled" by changing the weight ratio of St to PHBV in feed.

Introduction

The photo- and/or biodegradable polymers are attracting much interest in the theoretical and practical standpoints[1-4)], since they can be used as materials for medicine[5-6)] and environmental protection. Among biodegradable polymers, poly(hydroxyalkanoate)s such as homopolymer (PHB) of 3-hydroxy butyrate (HB) and copolymer (PHBV) of HB and hydroxyvalerate (HV) have been studied for their syntheses, properties and applications, because they exhibit higher biodegradability and biocompatibility, and lower toxicity compared with conventional synthetic polymers like polyvinyl chloride (PVC) [7-10)]. However they also have demerits such as poor

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flexibility and relatively high price. Thus, the use of these polymers have been limited to a few exclusive applications such as biomedicine[11].

Among the attempts to improve poor flexibility, the substitution of long chain alkyl group[12], copolymerization[13-16] and graft polymerization[17-20] have been investigated. For instance, the graft radiation-induced grafting of methacrylic acid[17], 2-hydroxyethyl methacrylate [18] onto PHBV were reported. There have been no report, however, in the open literatures about the systematic study on the effects of graft polymerization conditions of St onto PHBV by conventional radical initiator and the photo- and biodegradabilities of P(HBV-g-St).

The aim of this work was to synthesize the photo- and biodegradable graft polymers and to evaluate their photo- and biodegradabilities. In this study, poly [(hydroxybutyrate-co-hydroxyvalerate)-g-styrene] [P(HBV-g-St)] was synthesized by graft copolymerization of St onto PHBV under nitrogen atmosphere by using benzoyl peroxide (BPO). The effects of feed ratio of St to PHBV, initiator concentration, reaction time and reaction temperature were studied on the graft polymerizations. The structure and thermal decomposition of the synthesized P(HBV-g-St) were examined. The photo- and biodegradabilities of the graft polymers were evaluated by Fade-o-Meter and with *Aspergillus nigger*, respectively. The tensile strength and morphology of the graft polymers before and after photo- and biodegradations were also investigated.

Experimental

Materials

PHBV (PHV content: 5 wt%) was used as received from Aldrich Chemical Company. Styrene (St: Aldrich Chem.) was purified by the standard procedure. Benzoyl peroxide (BPO: Hayashi Chem.) was recrystallized from methanol. 1,2-Dichloroethane, methyl ethyl ketone (MEK), and benzene were distilled prior to use.

Graft Copolymerization of St onto PHBV

The graft polymerization was carried out under various experimental conditions as shown in Table 1. A given amount of PHBV was dissolved in 150 mL of 1,2-dichloroethane, and the solution was poured into a three-neck round bottom flask with different weight ratio of BPO as an initiator under nitrogen atmosphere, and the St monomer was then added to the PHBV solution. The solution was stirred for 48 h

TABLE 1. Graft copolymerization conditions used in this study

Condition	Description
Weight ratio of styrene to PHBV	1, 2, 3, 4
Initiator concentration (wt%)[a]	1, 2, 3, 4
Reaction time (h)	24, 36, 48, 72
Reaction temperature (°C)	60, 70, 80

[a] Weight ratio of BPO was based on 10g of PHBV.

at 70℃, and precipitated in excess methanol. After a chosen period of polymerization, the contents was poured into excess methanol with stirring; the precipitate was filtered by using 1G3 glass filter and dried in a vacuum oven until kept at a constant weight. The PS homopolymer was removed by extraction with MEK for 24 h by Soxhlet apparatus. The synthesized P(HBV-g-St) was isolated from the mixtures of nongrafted PHBV with benzene for 24 h by Soxhlet apparatus, and reprecipitated in excess methanol. The grafting ratio and grafting efficiency were estimated as the following equation.[21)]

$$\text{Grafting ratio (\%)} = \frac{\text{weight of polymer in grafts}}{\text{weight of substance}} \times 100$$

$$\text{Grafting efficiency (\%)} = \frac{\text{weight of polymer in grafts}}{\text{total weight of polymer formed}} \times 100$$

Measurements

Thermal Decomposition Temperature. Thermal decompositions of the graft copolymers were examined with a Dupont 951 TGA instrument under N_2 atmosphere at a heating rate of 10℃/min.

Tensile Strength. The tensile strength was measured using a Universal Testing Machine (UTM) [Hounsfield M-serious 500L]. The samples were prepared according to the procedure of ASTM D638-84. The cross-head speed was 5 mm/min., and the initial gauge length was adjusted at 25 mm.

Morphology. The morphologies of the samples before and after photo- and bio-degradations were analyzed by Scanning Electron Microscopy (SEM) instrument [JEOL JSM35-CF]. In the examination of sample surfaces after biodegradation, the sample cultured with *Aspergillus nigger* was gently washed with methanol in order to remove the free debris, and air dried. The samples were coated with gold prior to the installation in the SEM chamber.

Photodegradability 22-24). The photodegradabilities of samples exposed to uv irradiation using Fade-o-Meter (Atlas) at 60℃ with 65% relative humidity were determined by a color difference meter (ND-101 DP). The prepared film specimens were casted from 1,2-dichloroethane solution (ca. 3 wt%) on a nonyellowing urethane-coated hiding paper. The films were slowly dried at room temperature and then kept under vacumm until reached constant weight. The cross-sectional area of the films was 5×10 cm^3, with a thickness of 35 μm. The color difference value (ΔE) of the films after exposing to uv light (280～315 nm) was calculated using the Hunter-Schofield equation.25)

Biodegradability.26) The *Aspergillus nigger* (KCTC 2118) was used as a fungus, cultured in potato dextrus agar (PDA) slant at 30℃ for 48 h, and stored at 4℃. The incubated fungus was then cultured on the sample films in order to evaluate the biodegradability. Media used for the evaluation of biodegradation were as follows: Culture medium consisted of $NaNO_3$ (3.0g), K_2HPO_4 (1.0g), $MgSO_4 \cdot 7H_2O$ (0.5g), KCl (0.5g), $FeSO_4 \cdot 7H_2O$ (0.01g), sucrose (30g), and agar (15g) per liter distilled water.

Results and discussion

Characterization

The structure of the synthesized P(HBV-g-St) was identified by Jasco FT/IR-5300 spectrophotometer. Several characteristic peaks in the IR spectrum of P(HBV-g-St) were appeared at 1710 cm^{-1} (C=O: stretching vibration in PHBV unit), 1620 cm^{-1} (C=C bond; stretching vibration of phenyl ring in PS unit), and 800∼650 cm^{-1} (aromatic =C-H; out of plane vibration), respectively. ^{1}H-NMR (FT-300 MHz Bruker A-3000) spectrum [Fig. 1(B)] of P(HBV-g-St) compared with that of PHBV [Fig. 1(A)] showed several characteristic peaks such as aromatic ring protons at 7.2 ppm, methine protons at 5.3 ppm, methylene protons at 2.5∼2.7 ppm, and methyl protons at 2.2 ppm.

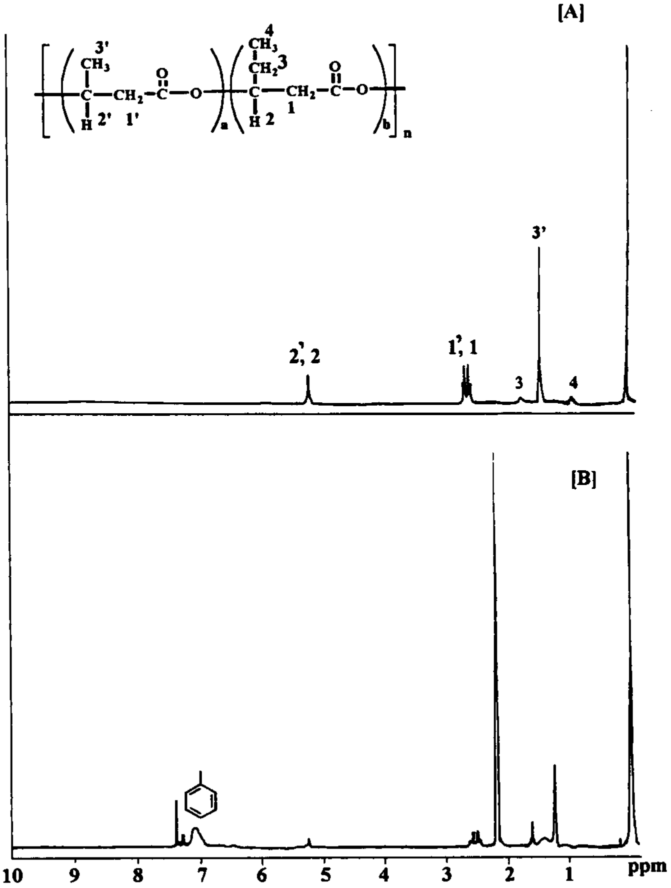


FIG. 1. ^{1}H-NMR spectra of PHBV [A] and P(HBV-g-St) [B] (in $CDCl_3$).

In ^{13}C-NMR spectrum (Bruker A-3000) as shown in Fig. 2, several characteristic peaks of P(HBV-g-St) [Fig. 2(B)] were appeared at 168 ppm and 126~128 ppm due to carbonyl carbons in PHBV and aromatic ring carbons in PS unit, respectively, together with several peaks of alkyl carbons originated from PS and PHBV [Fig. 2(A)].

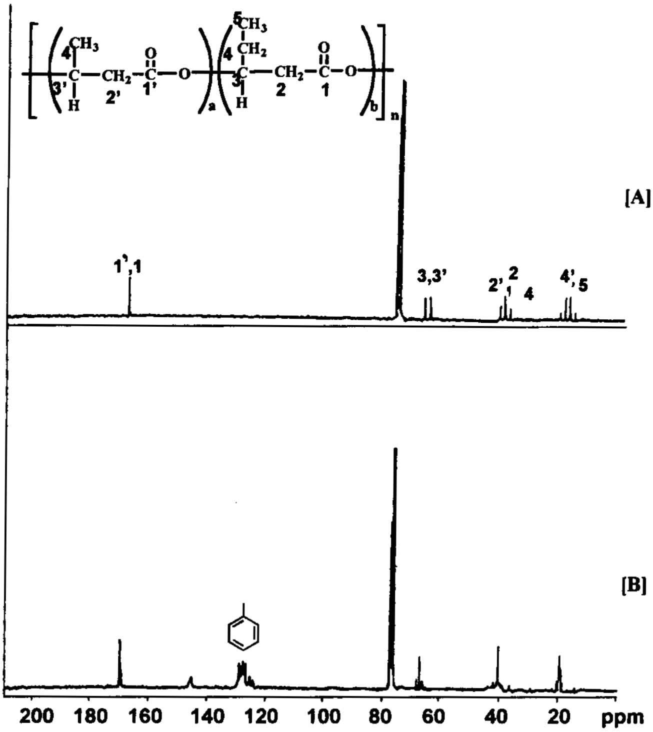


FIG. 2. ^{13}C-NMR spectra of PHBV [A] and P(HBV-g-St) [B] (in $CDCl_3$).

Effect of Reaction Conditions

Monomer Feed Ratio. The graft copolymerizations of St onto PHBV were carried out with the monomer concentrations of 1~4 weight ratios in feed. From Fig. 3, the grafting ratio and the grafting efficiency were increased with increasing weight ratio of St to PHBV up to 2. and thereafter they were decreased. The decreasing phenomena at higher weight ratio mean that PS homopolymer will be formed more readily than a graft polymer, P(HBV-g-St).

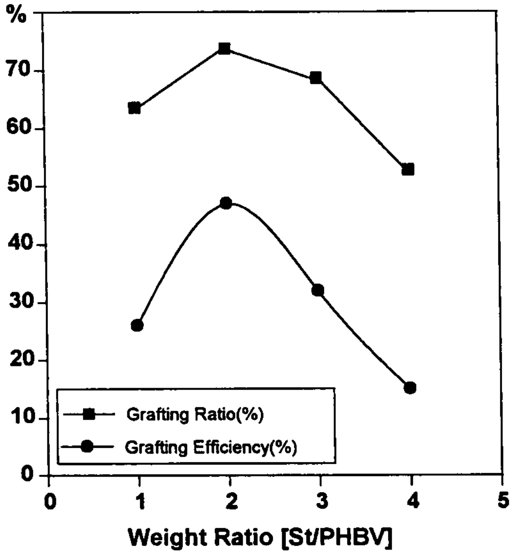


FIG. 3. Effects of weight ratio of St to PHBV in feed on the graft copolymerization : reaction temp., 70℃; reaction time, 48 h; BPO, 2wt% (based on PHBV).

Initiator Concentration. The effects of initiator concentration are shown in Fig. 4. The weight % of BPO was based on the weight of PHBV. The grafting ratio and grafting efficiency increased as increasing of initiator concentration up to 2.0 weight %, but they were decreased at higher initiator concentration. It may be attributed to the reason that PS homopolymer will be formed more readily than P(HBV-g-St) at higher initiator concentrations.

Reaction Time. Fig. 5 shows the effects of reaction time on the graft polymerization. The reaction was carried out at 70℃ with constant concentration of PHBV and BPO.The weight ratio of St to PHBV in feed was fixed at 2.0 together with BPO of 2 weight% based on PHBV. The grafting ratio was linearly increased with increasing reaction time up to 40h and it was leveled off.

The grafting efficiency, however, increased gradually increasing with reaction time. This result for grafting efficiency implies that the growing styryl radicals have more time for graft reaction.

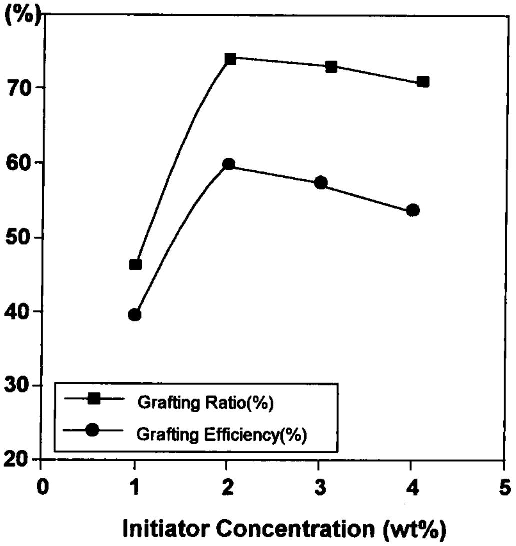


FIG. 4. Effects of initiator concentration on the graft copolymerization : [St]/[PHBV]=2.0 ; reaction temp., 70℃; reaction time, 48 h.

Reaction Temperature. The effects of reaction temperature are shown in Fig. 6. The graft polymerization was carried out with 2 weight% of BPO and 2.0 weight ratio of St to PHBV. The grafting ratio and the grafting efficiency increased with increasing reaction temperature up to 70℃, and they levelled off further raising reaction temperature. This result can be explained by the fact that the formation rate of PS and P(HBV-g-St) is similar at above 70℃.

Thermal Stability

Fig. 7 shows the thermal degradation temperatures of P(HBV-g-St) prepared from the various weight ratios of St to PHBV in feed. Initial decomposition temperature of P(HBV-g-St) was slightly higher than that of PHBV but was lower than that of PS.

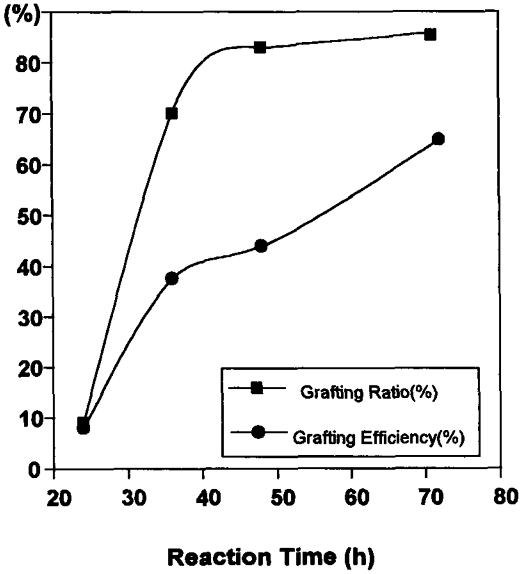


FIG. 5. Effects of reaction time on the graft copolymerization : [St]/[PHBV] =2.0; reaction temp., 70℃; BPO, 2wt% (based on PHBV).

The initial decomposition temperature of P(HBV-g-St) was not changed by the weight ratio of St to PHBV in feed. This result means that the main chain of P(HBV-g-St) is decomposed at around 273℃ although the concentration of St unit in the side chain is different.

Photodegradability and Morphology

The photodegradation was semiquantitatively expressed in terms of color difference (⊿E) with National Beaureau of Standards Unit [27]. Fig. 8 shows the photo-degradation effects on the weight ratios of St to PHBV in feed. The ⊿E value of P(HBV-g-St) is smaller than that of PS but is greater than PHBV. This means that the light resistance of P(HBV-g-St) is better than PS. The ⊿E values of P(HBV-g-St) increased with increasing uv irradiation time up to about 10h regardless of weight ratio of St to PHBV and thereafter they levelled off.

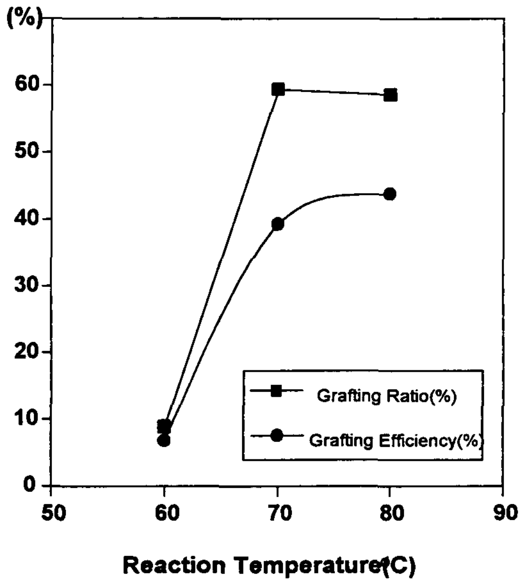


FIG. 6. Effects of reaction temperature on the graft copolymerization : [St]/[PHBV]=2.0 ; reaction time, 48 h; BPO, 2wt% (based on PHBV).

However, the size of ⊿E value of P(HBV-g-St) was dependent on the weight ratio of St to PHBV. Therfore, it may be said that the photodegradation rate of P(HBV-g-St) can be "controlled" by changing the weight ratio of St to PHBV.

After uv irradiation for 100h, the morphology of the film surfaces of P(HBV-g-St) were investigated by SEM. As shown in Fig. 9, P(HBV-g-St) [Fig. 9(B)] showed many pits as compared with untreated P(HBV-g-St) [Fig. 9(A)]. This means that the P(HBV-g-St) films have susceptibility to uv light. The photodegradability of P(HBV-g-St) may be related to the photodegradable property of the PS moiety in P(HBV-g-St).

Biodegradability and Morphology

The graft copolymer film was incubated with *Aspergillus nigger* at 30℃. After 36 days of incubation, the morphology of the film surfaces was investigated by SEM.

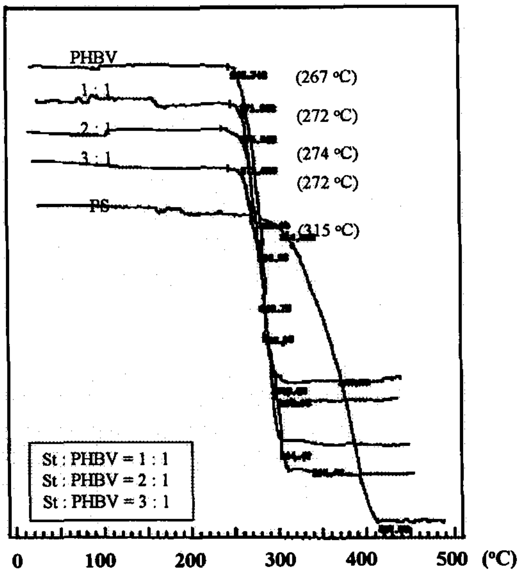


FIG. 7. TGA curves of PHBV and P(HBV-g-St): heating rate, 10℃/min.

The effect of *Aspergillus nigger* on the polymer film is shown in Fig. 10. The film surface of P(HBV-g-St) [Fig. 10(B)] showed extensive grooves and many pits due to biodegradation property as compared with of untreated P(HBV-g-St) film [Fig. 10(A)]. Thus, it is belived that P(HBV-g-St) film has excellent susceptibility to fungal growth. This may be attributed to the biodegradable PHBV moiety in P(HBV-g-St).

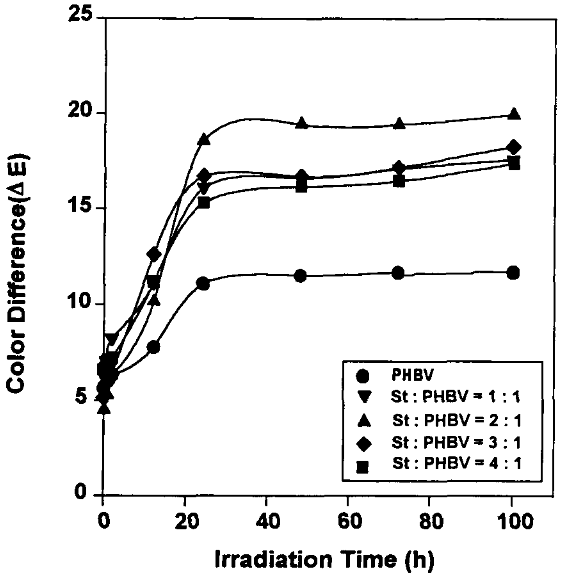
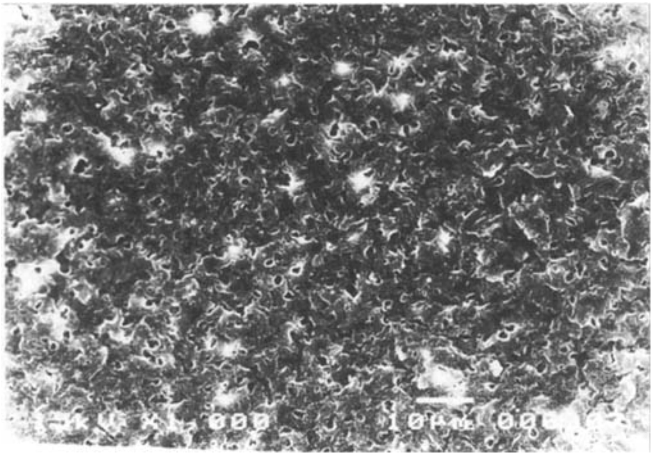


FIG. 8. Plot of color difference (ΔE) vs. irradiation time for PHBV and P(HBV-g-St).

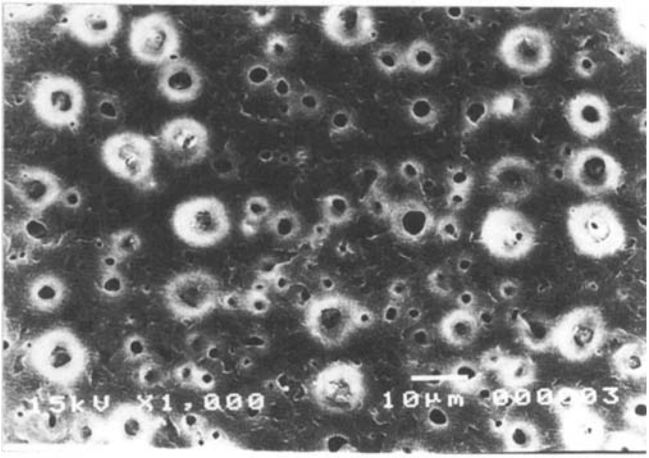
Tensile Strength

The tensile strengths of the samples before and after photo- or bio-degradation are shown in Fig. 11. The tensile strength of P(HBV-g-St) as well as PHBV was significantly decreased by the attack of *Aspergillus nigger*, which proves the biodegradability of the polymers. The tensile strength of the P(HBV-g-St) showed much higher values than that of PHBV, and increased with increasing feed ratio of St to PHBV up to 2.
The values of P(HBV-g-St) after 36 days biodegradation or 100 h of uv irradiation were decreased as compared with untreated P(HBV-g-St).

The tensile strength of the biodegraded P(HBV-g-St) was also increased with increasing weight ratio of St to PHBV up to 2, and thereafter, it decreased. The reult suggests that the tensile strength of P(HBV-g-St) can be "controlled" by changing the weight ratio of St to PHBV in feed.

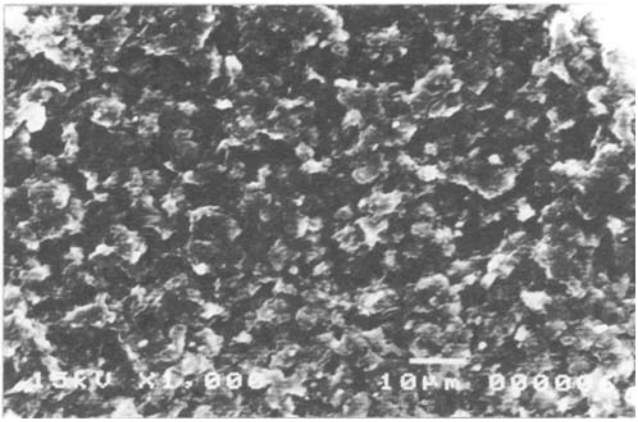


[A] Before UV irradiation (x 1,000)

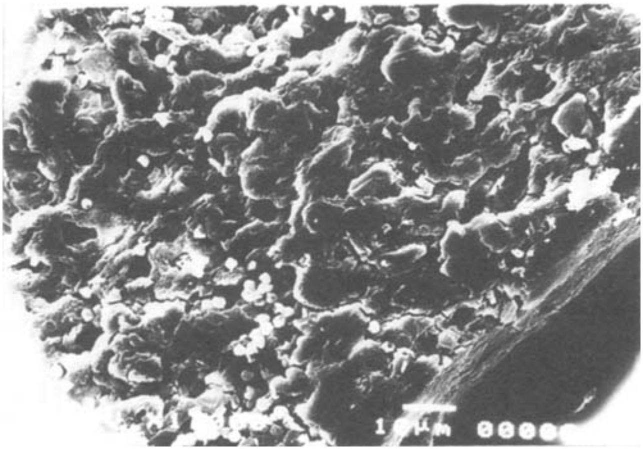


[B] After UV irradiation for 100h (x 1,000)

FIG. 9. Scanning electron micrographs of P(HBV-g-St) before [A] and after [B] photodegradation (× 1000).



[A] Before incubation with *A.niger* (x 1,000)



[B] After incubation with *A.niger* (x 1,000)

FIG. 10. Scanning electron micrographs of P(HBV-g-St) before [A] and after [B] biodegradation (× 1000).

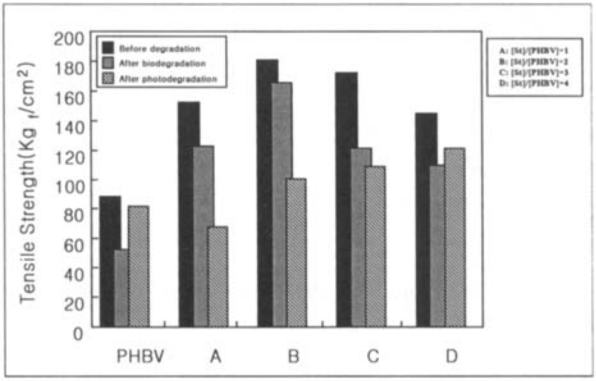


FIG. 11. Tensile strength of PHBV and P(HBV-g-St) before and after photo or biodegradation.

Conclusion

P(HBV-g-St) was synthesized by graft copolymerization of St onto PHBV under nitrogen atmosphere using BPO as an initiator. The effects of weight ratio of St to PHBV in feed, initiator concentration, reaction time, and reaction temperature were studied in the graft copolymerization. The grafting ratios and grafting efficiencies showed the highest values at 2:1 weight ratio of St onto PHBV in feed and 2.0 weight% of initiator, 48 h and 70℃. The thermal decomposition temperature of P(HBV-g-St) was 273℃. The photodegradability of P(HBV-g-St) showed higher values than that of PHBV. The film surfaces of P(HBV-g-St) showed many pits due to the photodegradation property of PS moiety in P(HBV-g-St). In biodegradation test, the film surface of P(HBV-g-St) incubated with *Aspergillus nigger* showed extensive grooves and pits as compared with the untreated P(HBV-g-St). The tensile strengths of P(HBV-g-St) before degradation showed much higher values than that of PHBV, and the tensile strength of P(HBV-g-St) after photo- or bio-degradation was decreased as compared with untreated P(HBV-g-St). It was found that the photodegradation rate and tensile strength of P(HBV-g-St) can be "controlled" by changing the weight ratio of St to PHBV in feed.

Acknowledgements

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