Modification of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Film by Chemical Graft Copolymerization

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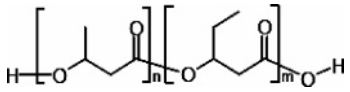
The graft copolymerization of 2-hydroxyethylmethacrylate (HEMA) onto poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) films has been investigated. The graft copolymerization was conducted in aqueous media using benzoyl peroxide (BPO) as chemical initiator. PHBHV films were prepared by solvent casting. Different parameters affecting the graft yield were studied such as monomer concentration, initiator concentration, and reaction time. The extent of grafting has been modulated by the preparation conditions, in particular the concentration of HEMA. However, it is interesting to note that the initiator concentration had only a slight influence on the graft yield. Characterization of the grafted PHBHV films assumed that the graft copolymerization not only occurred on the film surface but also took place into the film bulk. Differential scanning calorimetry showed that crystallinity dramatically decreased with increasing graft yield, indicating that graft copolymerization hindered the crystallization process. Wettability has been obviously improved by grafting a hydrophilic monomer such as HEMA for high graft yield (>130%).

Introduction

Polyhydroxyalkanoates (PHAs) are biosynthesized by a wide range of microorganisms as intracellular energy and carbon storage materials.[1−3] PHAs are produced in large amounts under carbon excess and limitation of, at least, one nutrient essential for cell multiplication. These polymers have attracted great scientific and technological interest due to their thermoplastic properties. PHAs are divided in two groups depending on the number of carbon atoms in the monomer: short chain lengths (scl PHAs) which contain 3−5 carbon atoms and medium chain lengths (mcl PHAs) which contain 6−14 carbon atoms. Poly(3-hydroxybutyric acid) (PHB) and copolymers containing 3-hydroxyvaleric acid (3HV) units (PHBHV) (Scheme 1) are the most common members of the biopolyesters group and commercialized. These bacterial polyesters have interesting characteristics and mechanical properties comparable to synthetically produced degradable polyesters such as poly(lactides).[4] PHB is a polymer with good biocompatibility as evidenced by its lack of toxicity[5] and compatibility[6−8] with tissue and blood.[9] These properties have increased interest in PHB in applications such as drug release[10,11] and biomedical materials including syringe, body part, and blood vessel.[12] The brittleness of PHB limits its potential applications; thus, the thermal and mechanical properties of PHB are favorably changed by copolymerization with 3HV. PHB and copolymers exhibit a number of very interesting properties such as biocompatibility and biodegradability.[13,14]

Consequently, scl PHAs can be considered as polymers with high potential for both environmental and medical applications. However, the intrinsic hydrophobic properties of PHAs restrict their applications as cell colonizing materials. The surfaces of PHB and PHBHV are quite inert and hydrophobic and have no physiological activity. This is unfavorable for adhered cell growth. Therefore, as for many polymer surfaces, the cytocompatibility should be improved by either chemical modification with functional groups or modification of the surface topography. Both parameters play an important role in the interaction between a biomaterial surface and cells, as illustrated by many reports focusing on this topic. Functionalization of the polymer is needed for tissue engineering[15,16] or antibacterial activity of biomaterials.[17,18]

Scheme 1. Chemical Structure of PHBHV, where $n = 88\%$ and $m = 12\%$



Effective chemical modifications include changes in chemical group functionality, surface charge, hydrophilicity, and wettability. Investigation of surface modification can be achieved by means of various chemical or physical processes including graft polymerization using oxygen plasma treatment,[19] UV-induced photografting,[20] γ irradiation,[16,21] and ozone treatment.[17,18]

The purpose of this study is the graft polymerization of 2-hydroxyethylmethacrylate (HEMA) onto PHBHV film using benzoyl peroxide (BPO) as chemical initiator. Introduction of HEMA, and more precisely introduction of hydroxyl groups, should increase the surface hydrophilicity and generate more favorable interaction with cells. Different parameters affecting the graft yield were investigated such as monomer concentration, initiator concentration, reaction time, and thickness of film.

Experimental Section

Materials. The PHBHV (HB = 88%, HV = 12%) copolymer was purchased from Goodfellow (Devon). HEMA and benzoyl peroxide

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(BPO) were supplied by Acros Chemical (Geel, Belgium). Chloroform and ethanol were obtained from Sigma-Aldrich (Ontario, Canada). All solvents and chemicals were used as received except for BPO, which was purified twice by recrystallization in a chloroform/ethanol mixture before use.

Preparation of PHBHV Films. Films of PHBHV were prepared by solvent casting. The PHBHV was dissolved in chloroform in reflux for 2 h to make a 20% w/v (0.2 g mL^{-1}) solution. The mixture was then poured on an automatic film applicator (Sheen Instruments, Surrey, G.B) and cast at 50 mm s^{-1}. Films were dried overnight and then dried in vacuum to remove the residual solvent. Samples were cut in 3.5 × 2.2 cm^2 pieces. An average thickness of 25 μm was obtained using a gap size of 200 μm, 55 μm using 500 μm. and 100 μm using 1 mm.

Graft Copolymerization Procedure. All reactions were heterogeneous and involved PHBHV films. In all cases the PHBHV film was attached with Teflon linkages on a glass slide and placed in a 100 mL round-bottom flask containing an aqueous HEMA monomer solution purged with nitrogen for 30 min. For all experiments, the total volume was 50 mL. The required concentration of BPO was dissolved in 2 mL of acetone and added into the polymerization vessel. The vessel was placed in an oil bath adjusted to the polymerization temperature (80 °C). The reaction was carried out under nitrogen atmosphere. After the reaction time the film was removed from the polymerization vessel and then purified from the unreacted monomer and residual homopolymer poly(hydroxyethylmethacrylate) (PHEMA) by washing it in 100 mL of boiling ethanol for 3 h. The washing ethanol was changed once to completely remove the homopolymer from the film. The film was finally dried to constant weight in vacuum at 40 °C overnight. The efficiency of the extraction method has been tested with a physical blend of PHEMA. After treatment with hot ethanol as described before, free PHEMA is totally removed.

For each concentration, four experiments have been done.

The grafting yield was calculated as a ratio of the increase of mass of the PHBHV film divided by the starting mass of film according the following equation

$$G\% = \frac{M_f - M_i}{M_i} \times 100$$

where M_f is the mass after the grafting and M_i is the initial mass.

In addition, we also express the graft conversion yield of monomer (*GC* %) which is the conversion of monomer (HEMA) to graft copolymer; it is calculated by the following equation

$$GC\% = \frac{M_f - M_i}{M_{HEMAini}} \times 100$$

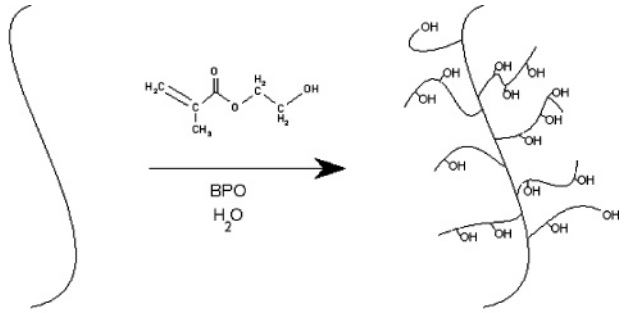
where $M_{HEMAini}$ is the mass of HEMA in the flask before reaction and M_f and M_i are defined above.

Characterization. *Fourier Transform Infrared Spectroscopy.* FTIR spectra were recorded on a spectrometer (Perking-Elmer 1600 Series). The spectra of the film surface were obtained with ATR equipment using diamond crystal.

Measure of Water Contact Angle. For evaluation of wettability, the water contact angle of the grafted PHBHV films was measured at room temperature using a Digidrop apparatus (GBX instruments, Romans, France). A droplet of deionized water (MilliQ water, 2.5 μL) was placed on the surface of a film at room temperature. More than five measurements were carried out for a single sample, and the resulting values are the averaged results.

Scanning Electron Microscopy (SEM). The surface morphologies of the polymer samples, before and after grafting, were observed by scanning electron microscopy (SEM). All observations were carried out with a JEOL 6460LV scanning electron microscopy (JEOL Ltd., Tokyo Japan). The voltage was kept at 15 kV, and the sample was kept at an average distance from the electron gun of about 10 nm. Samples were mounted on aluminum stubs and coated for 120 s at 20 mA with gold/palladium alloy using a sputter coater (Edwards Pirani 501, Ltd., U.K.).

Scheme 2. Synthesis of PHBHV Film-Grafted PHEMA



Size Exclusion Chromatography (SEC). The molecular weights were determined using the size exclusion chromatography (SEC), a refractive index detector, and three columns in series PL gel (Polymer Laboratories, 10 000, 1000 and 500 Å, 5 μm). The mobile phase was chloroform with an eluent flow rate of 1 mL min^{-1}.The sample concentration was 10 mg mL^{-1}, and the injection volume was 50 μL. A calibration curve was generated with polystyrene standards of low polymolecularity purchased from Polysciences (M(g mol^{-1}): 2 656 000, 841 700, 320 000, 148 000, 59 500, 28 500, 10 850, and 2930 and 580).

Differential Scanning Calorimetry (DSC). Differential scanning calorimetry (DSC) measurements were conducted on Mettler Toledo 822 apparatus. DSC was used to study the glass-transition temperature (T_g) and the crystallization behavior of native PHBHV and grafted film. Samples were heated from 25 to 190 °C (first run) with a heating rate of 20 °C min^{-1}. Melting points (T_m) were determined after the first heating run from the maximum of the endothermic peak, and ΔH_m was calculated from the area of the endothermic peak after the first run. Then, the sample was cooled to −45 °C at a cooling rate −80 °C min^{-1} and then heated up again to 190 °C at a heating rate of 20 °C min^{-1} (second run). The glass-transition temperature (T_g) was taken as the midpoint of the transition in the second heating run.

^1H NMR. The ^{1}H NMR spectra were obtained using a Bruker Advance-300 MHz spectrometer in DMSO-d_6.

Thermogravimetric Analysis (TGA). Thermogravimetric analysis (TGA) was conducted with a SETERAM 92 (France) under air atmosphere with a temperature range from 20 to 600 °C at a heating rate of 20 °C min^{-1}.

Results and Discussion

Grafting PHEMA onto PHBHV Films. In order to obtain a simple way for modification of PHAs surface, we proposed using the grafting of vinyl monomer on PHBHV films. The graft copolymerization was carried out with benzoyl peroxide (BPO) as initiator (Scheme 2). BPO has usually been employed as a source of radicals for initiation of vinyl polymerizations and cross-linking both saturated and unsaturated polymers.[22,23] Therefore, initiation was often used in grafting polymerization,[24−28] and it was demonstrated that BPO was efficient to achieve grafting upon other radical initiators such as hydrogen peroxide or 2,2′-azobis(2-methylpropionitrile), AIBN.[29,30] The free-radical fragments from the peroxides can initiate the homopolymerization of HEMA, but it is also speculated that the methine protons of PHBHV, which are the most acidic protons, may be abstracted from the backbone to generate the macroradicals (on the macromolecular chains of PHBV), initiating graft polymerization. Consequently, both homopolymerization and graft polymerization occurred.

In the present study, water was used as the solvent in the polymerization to avoid precipitation of PHEMA, which can

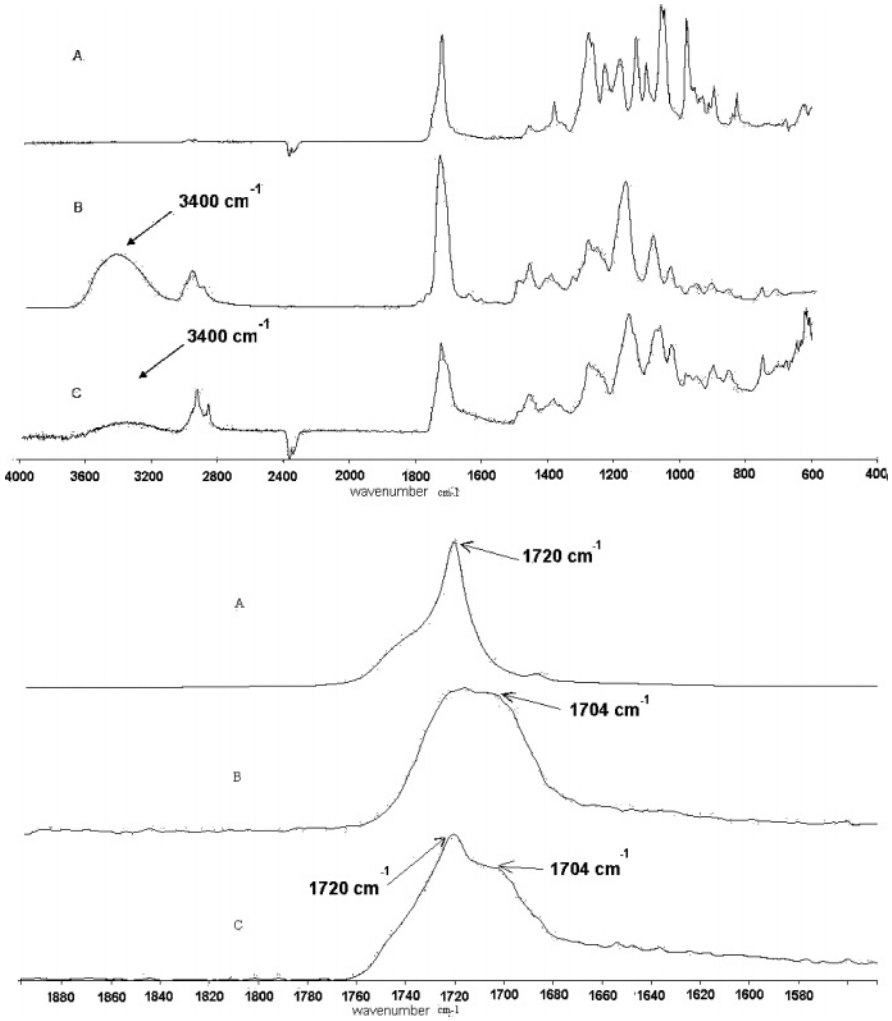


Figure 1. ATR-FTIR spectra: (A) PHBHV copolymer, (B) PHEMA homopolymer, (C) grafted film $G = 170\%$. Film thickness = 55 μm. Reaction time = 4 h. [HEMA] = 0.1 mol L^{-1}. [BPO] = 1.87×10^{-2} mol L^{-1}. The lower figure is an enlargement of the top figure between 1880 and 1580 cm^{-1}.

cause the inability to extract the PHBHV film. Except HEMA, which is water soluble, PHEMA is poorly soluble in water and the other reagents (PHBHV, BPO) are not soluble in water. Consequently, polymerization occurs in heterogeneous conditions. Formation of radicals in polymer chains, which can initiate the grafting reaction, may not be easily achieved due to these heterogeneous conditions. Furthermore, macroradicals formation may be impeded by the high degree of polymer crystallinity. Before processing a complete study of various parameters, preliminary experiments were carried out to demonstrate evidence of PHEMA grafting on PHBHV films. As mentioned before, the reaction gives free PHEMA and graft PHEMA. Since HEMA and PHEMA are soluble in alcohol (ethanol), unreacted HEMA and free PHEMA have been entirely removed by solvent extraction in ethanol after grafting reaction. The first results showed an increase of the mass of the film. The mass increase of the film is evidence that PHEMA is covalently bound to PHBHV. ATR-FTIR results on unmodified and grafted PHBHV films ($G = 170\%$) are shown in Figure 1. The broad absorption at 3400 cm^{-1} was assigned to the stretching vibration of O−H in hydroxyl groups of PHEMA. The carbonyl stretching region is quite different from the spectra of both free PHEMA (Figure 1B) and native PHBHV (Figure 1A). The spectra clearly show an enlargement of the ester carbonyl band attributed to both carbonyls stretching frequencies for a graft yield of 170%. The carbonyl vibration at 1704 cm^{-1} is characteristic of PHEMA, and the crystalline C═O stretching band of PHBHV appears at 1720 cm^{-1}. IR spectra confirm the presence of graft PHEMA.

Evidence for grafting was also achieved by ^{1}H NMR spectroscopy. As PHBHV and PHEMA were soluble in DMSO,[31] the ^{1}H NMR spectrum of grafted film was made in DMSO-d_6. Figure 2 illustrates the ^{1}H NMR spectrum of the grafted films obtained with chemical assignments. As one can see, the spectrum (Figure 2) of the grafted film is an overlay of the spectrum of PHBHV and PHEMA. The presence of signals at 0.7, 1.8, 3.5, 3.9, and 4.7 ppm belonging to PHEMA demonstrate that graft copolymerization was successfully achieved. The chemical shifts at 2.5 and 5.1 ppm are characteristic of the protons of the PHBHV backbone. The signals at 0.9, 1.1, and 1.5 ppm are assigned to the side chains of PHBHV.

As the grafting can be achieved, we studied different parameters of the grafting procedure. The dependence of the initiator concentration was first investigated by setting the monomer concentration to [HEMA] = 0.04 mol L^{-1} and the film thickness to 55 μm.

Influence of the Initiator Concentration. Figure 3 shows the graft yield as a function of the initiator concentration. The initiator concentration used had a minor effect on the graft yield. However, a very slight decrease of the graft yield (35−28%) was observed when the initiator concentration increased. However, the low variation of the values does not allow us to draw conclusions about the influence of PBO concentration on

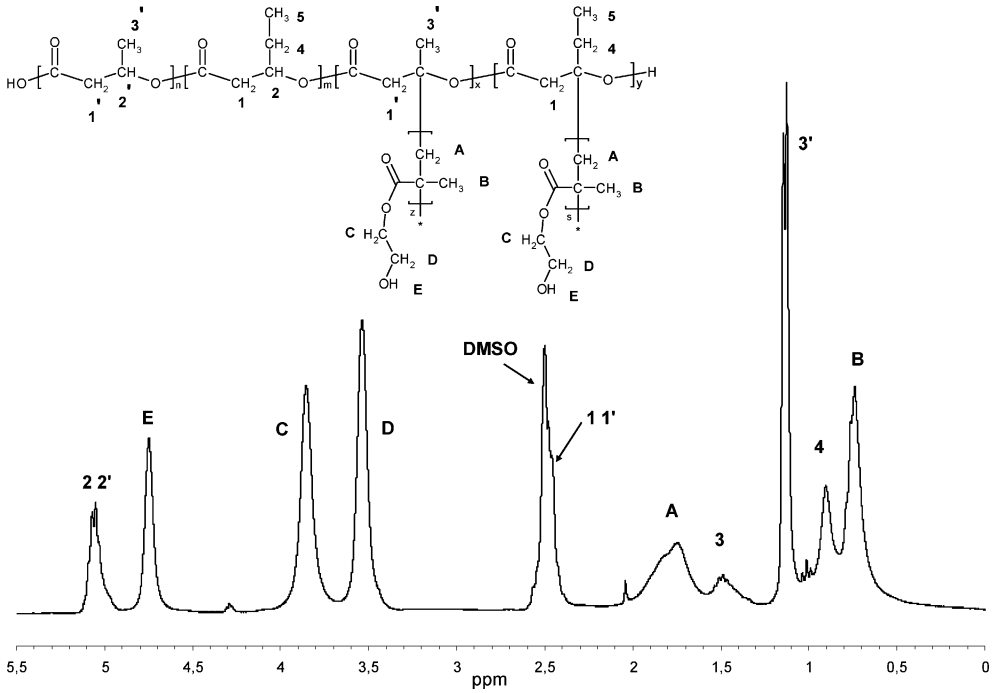


Figure 2. ^{1}H NMR of the grafted film in DMSO-d_6 (*G*raft yield $G = 70\%$).

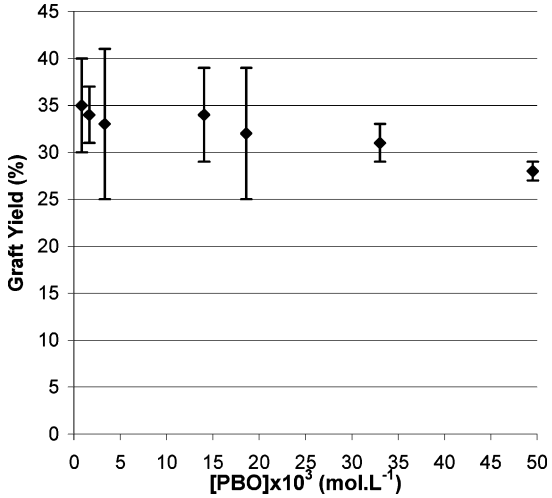


Figure 3. Graft yield as a function of the initiator concentration. Film thickness = 55 μm. [HEMA] = 0.04 mol L^{-1}. Reaction time = 2 h.

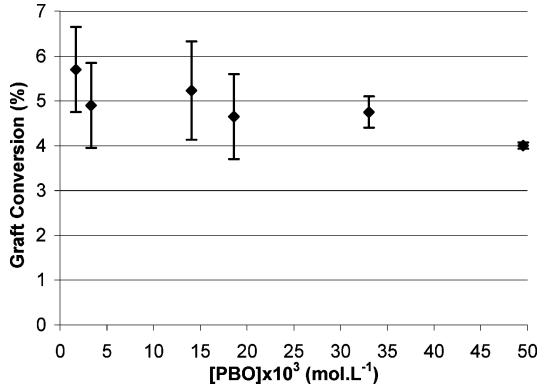


Figure 4. Graft conversion as a function of the initiator concentration. Film thickness = 55 μm. [HEMA] = 0.04 mol.L^{-1}. Reaction time = 2 h.

the grafting process unlike other authors.[25,32] It was generally observed that enhancement of the initiator concentration increases the termination reaction, which should induce a decrease of the graft.

In Figure 4, it is observed that only approximately 5% of the monomer participates in graft polymerization and the remainder is homopolymerized. This trend is generally observed with AIBN, which is well known to be unable to abstract hydrogen from a polymer.[30] BPO was insoluble in solvent reaction (water); these heterogeneous conditions may explain the difficulty of the initiator to abstract a hydrogen atom from PHBHV and migrate into the film. The hypothesis of the macroradical implication in the reaction may be proved by performing a reaction without monomer. The radicals produced by hydrogen abstraction due to an initiation reaction will be involved in secondary reactions which can lead to a degradation of the polymer backbone[21,33,34] or cross-linking.[35] The molecular weight was studied as a function of the initiator concentration. The PHBHV film was stirred with BPO in water at the reaction temperature (80 °C) for 2 h. The film was extracted in ethanol and then dried at 40 °C in vacuum overnight. All samples were soluble in $CHCl_3$, indicating that no cross-linking reaction occurred. Table 1 shows the variation of the molecular weights. As expected M_n and M_w decrease with increasing concentration of the BPO, resulting in degradation of the macromolecular chains. These results suggest that BPO is able to abstract hydrogen from PHBHV even in heterogeneous conditions. The molecular weights are sufficiently high to keep good mechanical properties. Afterward, the concentration of BPO of 1.87×10^{-2} mol L^{-1} was kept.

Table 1. Influence of the Initiator Concentration on the Molecular Weight of the PHBHV[a]

[BPO] (mol L^{-1})	M_n (g mol^{-1})	M_w (g mol^{-1})	$I_p = M_w/M_n$
0	122 200	242 000	1.9
1.41×10^{-2}	72 200	149 300	2.1
1.87×10^{-2}	70 000	136 400	1.9
4.98×10^{-2}	34 000	82 300	2.4

[a] Reaction time = 2 h.

Influence of the Monomer Concentration. As the initiator concentration was fixed (1.87×10^{-2} mol L^{-1}), the effect of the monomer concentration was investigated. As expected, graft

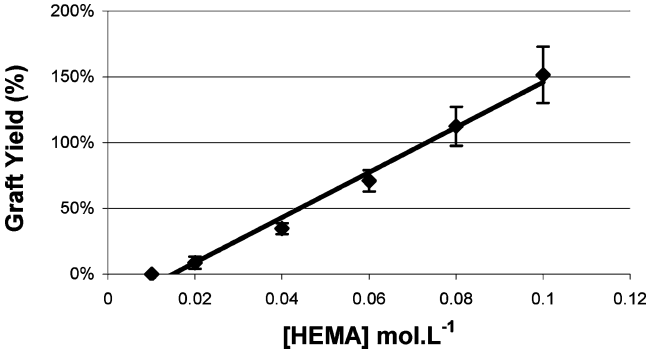


Figure 5. Graft yield as a function of the monomer concentration. Film thickness = 55 μm. [BPO] = 1.87 $\times$ 10^{-2} mol L^{-1}. Reaction time = 2 h.

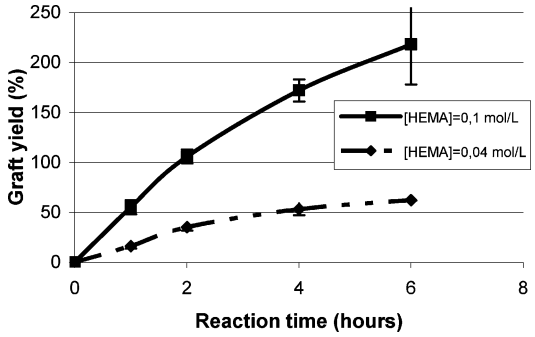


Figure 6. Graft yield as a function of the reaction time. Film thickness = 55 μm. [BPO] = 1.87 $\times$ 10^{-2} mol L^{-1}.

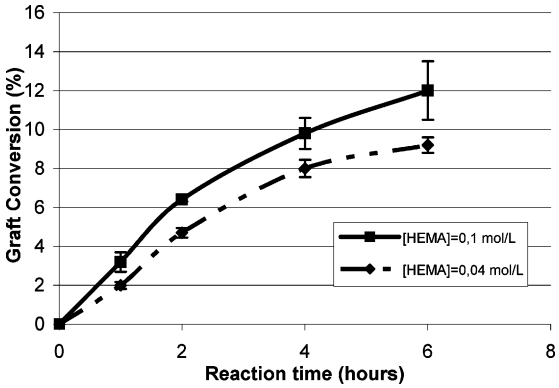


Figure 7. Graft conversion as a function of the reaction time. Film thickness = 55 μm. [BPO] = 1.87 $\times$ 10^{-2} mol L^{-1}.

yield increased with monomer concentration (Figure 5). Previous studies had shown the same dependence[25,30,36] in the grafting of vinyl monomer by radical initiator. Indeed, the increasing monomer concentration increases the probability that the radical of the growing chain reacts with a monomer. When the monomer concentration used is lower than 0.01 mol L^{-1}, no grafting of HEMA is observed. The grafting yield can be modulated from 10% to 150% by variation of monomer concentration from 0.02 to 0.1 mol L^{-1}.

Influence of the Reaction Time. The free-radical reaction is known to be a fast reaction. The graft yield was followed as a function of the reaction time. Figure 6 shows that, as expected, a longer reaction time increased the graft yield. After 6 h, the saturation was not reached for 0.1 mol L^{-1} of HEMA, even if the graft yield is very high. For the lower concentration of monomer, most of the reaction seem to occur within that period. It has to be noted for both concentrations of monomer that even if the graft yield is very high, the *GC*% is quite low as shown in Figure 7. This means that homopolymerization is the predominant reaction. The interesting part of the curves depends on the application. PHBHV should be prevailing, so a 2 h reaction time has been chosen to avoid the grafted yield being above 100%.

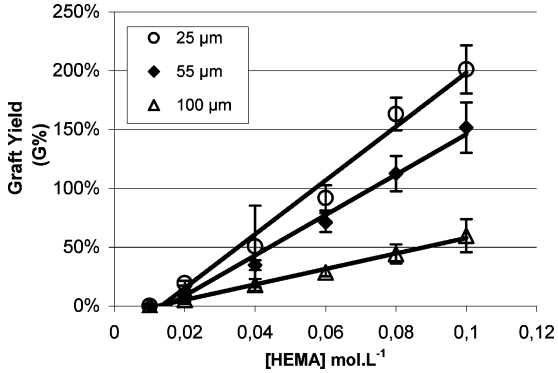


Figure 8. Graft yield (*G*%) as a function of the monomer concentration. Influence on the thickness. [BPO] = 1.87 $\times$ 10^{-2} mol L^{-1}. Reaction time = 2 h.Correlation coefficient: R^2(25 μm) = 0.98, R^2-(55 μm) = 0.99, R^2(100 μm) = 0.99.

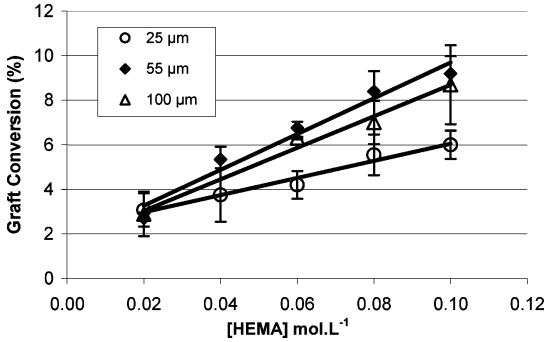


Figure 9. Graft conversion as a function of the monomer concentration. Influence of the thickness. [BPO] = 1.87 $\times$ 10^{-2} mol L^{-1}. Reaction time = 2 h. Correlation coefficient: R^2(25 μm) = 0.96, R^2-(55 μm) = 0.98, R^2(100 μm) = 0.97.

Table 2. Water Contact Angle Measurements[a]

graft yield (%)	water contact angle (deg)
0	77 ± 3
10	74 ± 4
30	64 ± 5
70	55 ± 5
110	49 ± 2
150	37 ± 2

aFilm thickness = 55 mm.

Table 3. Thermal Characteristics of Grafted Filmsms

graft yield (%)	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})
0	2.3	156	65
10	2.9	142	50
30	3.9	143	51
70	2.3	140	37
100	1.6	140	36
130	1.9 and 63	138	22

Influence of the Thickness of the Film. The influence of the thickness of the PHBHV film was then studied. Comparison of the thickness was made by casting 25, 55, and 100 μm films of PHBHV. Graft yield was measured as a function of monomer concentration. Figure 8 shows the graft yield as a function of the monomer concentration with three different thicknesses. The graft yield decreases with the thickness of the film, in good agreement with expected results. On the other hand, the results

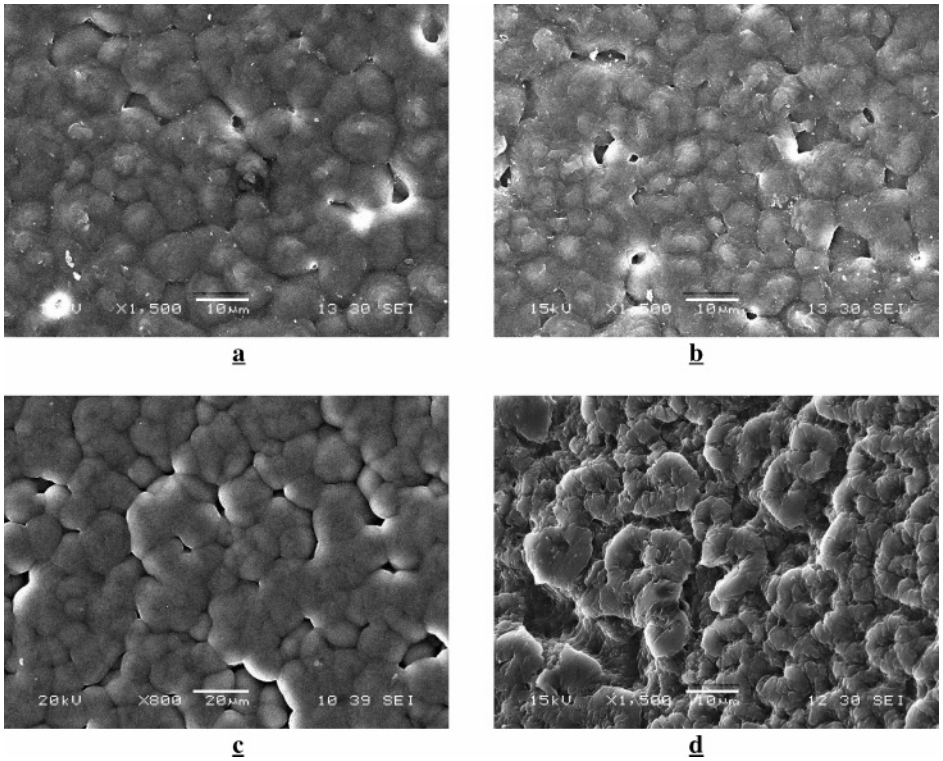


Figure 10. SEM micrographs of (a) unmodified PHBHV, (b) PHBHV-*g*-PHEMA $G = 40\%$, (c) PHBHV-*g*-PHEMA $G = 100\%$, and (d) PHBHV-*g*-PHEMA $G = 300\%$.

Table 4. TGA and DTG Results

polymer	$T_{5\%}$[a] (°C)	T_{max}[b](°C)
PHBHV	281	297
10%	285	302
30%	279	301
70%	262	308
110%	241	315
150%	235	316
PHEMA	228	350

[a] $T_{5\%}$ = temperature when the weight loss is 5%. [b] T_{max} = temperature max of DTG.

Table 5. Weight Composition of Grafted PHBHV Films[a]

graft yield	PHBHV ungrafted	copolymer PHBHV-*g*-PHEMA
10%	50%	50%
32%	28%	72%
60%	23%	77%
120%	16%	84%

[a] Film thickness = 55 μm.

obtained for graft conversion are not compliant with the fact that grafting is supposed to occur at the film surface (Figure 9). The graft conversion shows that more HEMA was grafted on the thicker films. This suggests that the graft reaction did not occur only at the surface of the film, but that the monomer could also diffuse more into the bulk as the film is thicker; then we can suppose that polymerization has taken place deeper. Similar results were obtained with the 55 and 100 μm films. This means that, from a critical thickness, diffusion seems to be limited and polymerization should not occur deeper.

Characterization of the Grafted Film. *SEM.* Evaluation by SEM was investigated to notice any modification of the surface by grafting polymerization. SEM micrographs of native PHBHV and grafted with various graft yields are shown in Figure 10. The surface of native PHBHV prepared by solvent casting is absolutely not smooth. This phenomenon occurred during the chloroform evaporation process. According to the SEM observation, it can be assumed that the roughness promotes diffusion of the monomer and PHEMA chains into the bulk of the film. Consequently, grafting polymerization occurs at the surface but can also occur deeper.

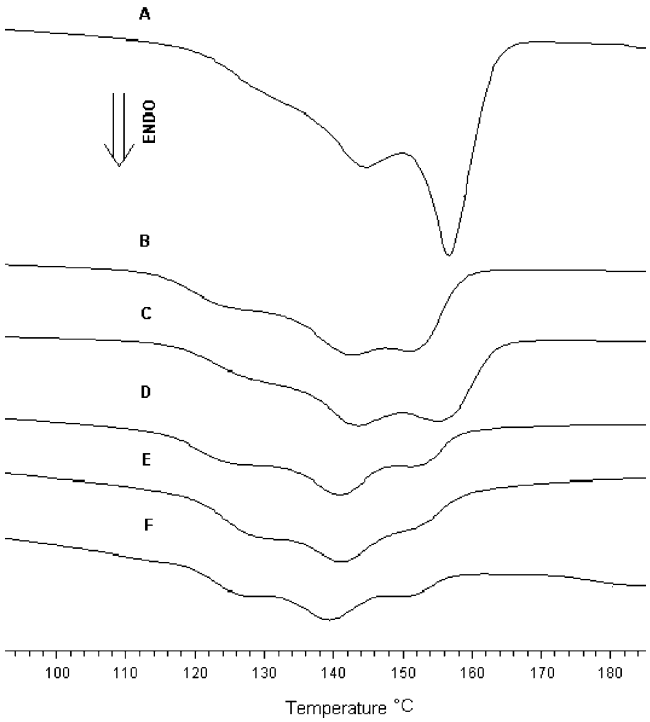


Figure 11. DSC curves recorded during the first heating run: (A) PHBHV; (B) PHBHV-*g*-PHEMA $G = 10\%$; (C) PHBHV-*g*-PHEMA $G = 30\%$; (D) PHBHV-*g*-PHEMA $G = 70\%$; (E) PHBHV-*g*-PHEMA $G = 110\%$; (F) PHBHV-*g*-PHEMA $G = 150\%$.

The surfaces differences of untreated PHBHV samples (Figure 10a), graft films with 40% of graft yield (Figure 10b),

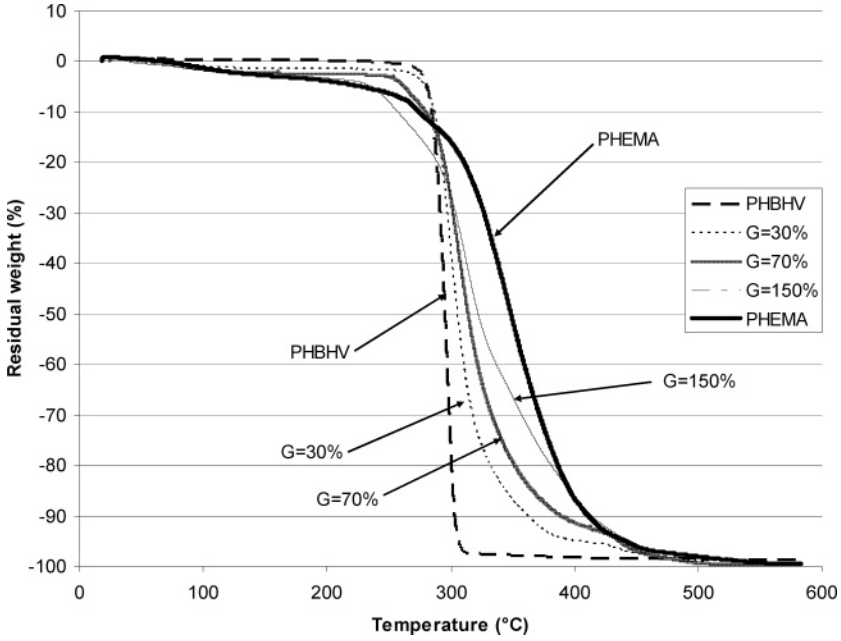


Figure 12. TGA thermograms of PHBHV and grafted PHBHV.

and 100% of graft yield (Figure 10c) are indistinguishable on a microscopic level. These results are in good agreement with the ATR-FTIR results obtained with films of low graft yield that show it was difficult to ascertain the presence of grafted PHEMA chains at the film surface for low weight gain (<100%). Since ATR-FTIR did not penetrate deep into the matrix, the top nanoscale change on the surface is diluted to such an extent that the information is virtually lost. This confirms our above assumption suggesting that monomer could diffuse in the bulk of the film.

For 300% graft yield (Figure 10d), the surface of films as observed by SEM undergoes significant modification as a result of the grafting process. At high graft levels the PHEMA chains are much denser to form independent domains which keep at the surface of the film. Penetration of these chains in the bulk of film is probably hindered. Consequently, the film surface may contain a larger number of grafted chains.

Water Contact Sngle Mesurements. The water contact angles, presented in Table 2, were investigated to subjectively compare the hydrophobicity of native PHBHV and PHEMA grafted PHBHV. The unreacted PHBHV has a contact angle around 75° that is quite in compliance with the literature.[18,19,37] This value shows that the PHBHV unmodified is hydrophobic. The contact angle decreased with the increasing grafting, indicating enhancement of the surface hydrophilicity. This modification is very significant for a graft yield higher than 30%. This result is in good agreement with the assumption of an important quantity of PHEMA which forms their own domains and morphology and covered the surface.

Thermal Properties. Thermal analysis of graft films was performed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC data of unreacted PHBHV and grafted PHBHV with various graft yields are presented in Figure 11 and Table 3. T_g values of the PHBHV in the grafted film were almost independent of the graft yield and remain around 2.5 °C. As we have previously shown that the molecular weight decreased during the graft copolymerization, T_g should slightly decrease as a consequence of the improvement of segmentation mobility. However, the grafting reaction on the macromolecular chain of PHBHV should also generate strong interactions among the chains, resulting in an increasing of T_g. Consequently, the final T_g value resulting for both antagonist phenomena is not dramatically modified. When the grafting of PHEMA becomes prominent with an increase of the HEMA content in the initial mixture, it can be isolated on the DSC curves with a second T_g value of about 63 °C. The T_g at 1.9 °C belongs to PHBHV. The T_g at 63 °C can be attributed to long PHEMA macromolecular chains even if the value is higher compared with pure PHEMA,[38] whose T_g is close to 49 °C. This result can be explained by intensified interactions among the chains and cross-linking reactions. Furthermore, it can be shown that when the graft yield is higher than 130%, the graft film is not entirely soluble in DMSO. The presence of insoluble fraction proves the occurrence of a cross-linking reaction, which confirms the previous hypothesis about T_g values. We have previously shown that no reticulation occurs between radicals produced by removing hydrogen atoms from polymer during the initiation process. However, grafting of HEMA onto PHBHV can involve classical termination by recombination of two growing PHEMA chains leading to reticulation.

DSC melting curves (Figure 11) show that the melting peak of unreacted PHBHV is bimodal, which indicates the presence of imperfect crystals during the drying process. The width of the peak increases with the graft degree. From the data in Table 3 it is found that T_m values and the melting enthalpy of graft films decrease with increasing graft yield. These results show that grafting leads to a decrease of the crystallization ability of PHBHV grafted PHEMA; indeed, PHEMA is an amorphous polymer. Introduction of PHEMA hindered the crystallization of PHBHV by introducing a chain structural irregularity.

Thermogravimetric analysis of PHBHV and the graft copolymers was performed to see the effect of the presence of HEMA on the course of the degradation. TGA thermograms are presented in Figure 12. The initial degradation temperatures from TGA and maximum degradation temperatures from the derivative thermogravimetry (DTG) are reported in Table 4. Degradation of PHEMA and PHEMA-*g*-PHBHV begins around 100 °C, which can be attributed to loss of water. Although PHEMA is first to decompose ($T_{5\%}$ = 228 °C), it is the one which has the best thermal stability (T_{max} = 350 °C). Indeed, it is well known that PHBHV is easily degraded. According to

the results, grafting PHEMA increases the degradation temperature (T_{max}) of the PHBHV, but the improvement is not very considerable. The temperature difference between the unmodified and PHBHV-*g*-PHEMA $G = 150\%$ is only 19 °C.

Copolymer Composition of the Film. PHBHV films were readily soluble in $CHCl_3$ at room temperature. The grafted films did not dissolve in $CHCl_3$ after reaction even at reflux. This change of solubility is a consequence of incorporation of PHEMA onto the PHBHV films. According to previous results, the change of solubility is supplementary proof of formation of a graft copolymer. Extraction with $CHCl_3$ (solvent of PHBHV) of grafted films was used to confirm that a graft copolymer was effective and shows if unmodified PHBHV remains in the graft film. Graft films were extracted from chloroform for 2 h to remove the ungrafted chains of PHBHV from the copolymer. The results (Table 5) show that graft films are composed of two types of macromolecules: unmodified PHBHV chains which are probably located deeply into the film and copolymers resulting from the grafting reaction. For all samples the weight fraction of the copolymer is high and superior to 50%, which proves the grafting reaction is very fast and widespread. Graft copolymers show different solubility of their respective homopolymers as they are insoluble in $CHCl_3$ and ethanol but are soluble in DMSO, which is the common solvent of both homopolymers, indicating that no cross-linking reaction occurs. It has to be noted that over a graft yield of about $G = 130\%$ the copolymer PHBHV-*g*-PHEMA there is an insoluble fraction in DMSO which could cause one to assume that over a certain amount of graft yield cross-linking could take place.

Conclusion

Our study has demonstrated that HEMA can be successfully grafted on PHBHV films by a simple and rapid procedure using PBO as initiator in spite of the inert chemically structure of PHAs and heterogeneous conditions. The results are totally reproducible. The extent of grafting can be modulated by the preparation conditions, particularly the HEMA concentration (rather than the initiator concentration or the reaction time). Results confirmed the occurrence of grafting. However, the question of the mechanism remains unsolved. As one can see, in our conditions the graft polymerization of HEMA on PHBHV is supposed to be conducted by formation of primary radicals on the PHBHV backbone from hydrogen abstraction, which can react with HEMA. However, the hypothesis of a second way of grafting proceeding by formation of a macroradical of the monomer able to attack and bond to the backbone cannot be excluded. An extended study of the copolymer should give more information on the mechanism, but this is not the purpose of this study.

According to the results, it can be assumed that the grafting reaction occurs in the bulk of the film and becomes more prominent at the surface with an increase of the HEMA content in the feed. Consequently, the bulk of graft films appears to be mixed with PHBHV-*g*-PHEMA and free PHBHV. Saturation of the surface appears to be faster with thin films and high HEMA concentration.

Introduction of hydroxyl groups obviously improved the wettability of the graft films. This way of surface modification of PHBHV can be used for other monomer-containing chemical functions. This provides an opportunity to adapt the grafting strategy to a chosen wettability and functionality, therefore potentially improving their ability for cellular interaction.

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