Surface Functionalization of PHBV by HEMA Grafting via UV Treatment: Comparison with Thermal Free Radical Polymerization

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ABSTRACT: The photochemical grafting HEMA onto poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBHV films using benzophenone (BP) or H_2O_2 as initiator was investigated to develop a route of grafting restricted to the surface. The effect of various parameters, such as monomer concentration, initiator, and reaction time, on grafting yield was studied and compared with results obtained when using benzoyl peroxide (BPO) as initiator. The morphology and structure of grafted films were characterized by Fourier Transform Infrared spectroscopy with attenuated reflexion, scanning electron microscope and Energy Dispersive X-ray Analysis. The results show that BP and H_2O_2 are very efficient for the grafting on the surface PHBV. The process is very fast and easy and the results are reproducible in a wide range. Photoinitiation grafted only on the surface in comparison with BPO, where the grafting is located in all the bulk of film. The films grafted by UV in presence of H_2O_2 are totally biodegradable when the graft level was low (less than 10%).
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Key words: PHBHV; hydroxyethyl methacrylate; HEMA; photopolymerization; grafting; UV

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible polymers that are produced by a wide of microorganism.[1–4] These polymers are under study for medical applications because of their biocompatibility and non toxicity to living tissues. Among potential applications, PHAs could be used to make matrices for *in vitro* cells proliferation.[5–9] PHAs are quite inert and hydrophobic, and present no physiological activity, which restricts their applications as cell colonizing materials. Therefore, surface functionalization was required to increase as well as the cellular or bacterial adhesion. Control of the films surface properties, such as wettability, adsorption, chemistry, charge, roughness, and rigidity[10,11] is important when polymeric material is in contact with cells. To trigger the cell-matrix adhesion on polymer surfaces, several surface modification techniques have been recently applied, including alkaline hydrolysis,[12,13] implantation,[14] gamma irradiation,[15] oxygen plasma treatment,[16] UV,[17] or ozone[18] followed by chemical grafting.[19] Surface modification of polymer films offers versatile means for incorporating new functionalities. Furthermore, grafting has been considered generally favored over physical treatments because the chemical grafting methods lead to higher amount of incorporated functional groups and show a superior stability; local motions of polymer segments are hindered.[20] Most of these methods are based on a "grafting-from" process, where radicals are formed along the polymer backbone, followed by a free radical polymerization of vinyl monomers. By careful selection of polymer support and monomer, it is possible to control the hydrophilic/hydrophobic balance of the surface support. We chose 2-hydroxyethyl methacrylate (HEMA) grafted on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBHV films as support materials because PHBHV films possess good chemical stability and mechanical strength, PHEMA is biocompatible,[21] and the formed grafted layers have enough hydrophilicity to modify the hydrophobic/hydrophilic balance of PHBHV.[22]

We have previously reported the grafting of HEMA on PHBHV films via free radical-initiated process using benzoyl peroxide (BPO), as free radical initiator.[22] The results show that grafting proceeds not only on the surface but also in the bulk of polymer, and the chemical and/or physical integrity of the bulk polymer is not well preserved. In the case of PHAs grafted film, biodegradability is modified limiting temporary biomedical or environmental

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applications. Consequently, it is important to develop a chemical modification restricted to the surface.

An alternative method is to use photografting. The UV irradiation mild conditions should restrict the modification to the surface.[23] Several methods for "photografting-from" are now well known, such as the use of benzophenone (BP) as photoinitiator, which after UV exposition and hydrogen abstraction create initiator radicals on the base polymer for heterogeneous graft polymerization.[24–26] Several possibilities were described in the literature: using a BP-monomer mixture, preadsorption of BP onto the polymer surface[27–29] or solvent free photografting technique.[30,31] All these processes have been established as a very selective and efficient approach, but generally no pre-functionalization has been achieved and the grafting was achieved in one step. Ulbricht et al. and Ma et al. have developed a process in two stages with pre-functionalization.[23,32,33] This process in two separate steps should take over better control of surface modification in comparison with free radical method developed previously because it can be considered as a "quasi-living" polymerization via recombination and photocleavage of semipinacol radical.[32,33]

We have chosen to apply this procedure to PHBHV for grafting PHEMA either with BP as photoinitiator or H_2O_2 to create photolabile functions on the polymer surface. Then in a second step, the functionalized films were put in contact with the monomer solution to achieve UV-initiated graft copolymerization. The effects of various parameters, such as initiator, monomer concentration, and reaction time, on graft yield were studied. The morphology of the PHEMA grafts on the film was determined by scanning electron microscopy (SEM) and energy dispersive X-rays analysis (EDX) that was compared with results obtained by free radical polymerization. The effect of PHEMA grafting level on PHBHV biodegradation was also investigated.

EXPERIMENTAL SECTION

Materials

Polymer used in this study was PHBHV with 12% of 3-hydroxyvalerate purchased from Good Fellow. HEMA and BPO were supplied by Acros Chemical. BPO was purified twice recrystallized in a chloroform/ethanol mixture before use.

BP and hydrogen peroxide (H_2O_2) were purchased from Aldrich and used as received. Chloroform, ethanol, anhydrous diethyl ether, chloroacetyl chloride, and triethylamine were obtained from Sigma-Aldrich. All solvents and chemicals were used as received except for HEMA (Aldrich), which was passed through the ready-to-use inhibitor removing column (Aldrich).

Preparation of PHBHV film

The PHBHV was first purified by dissolution in chloroform in reflux for 2 h (20% w/v) and precipitation in ethanol to remove citric ester used as plasticizer. Films were prepared by casting the chloroform solution on a glass plate. Samples were cut in 3.5 × 2.2 cm^2 pieces. Films with an average thickness of 70 μm were obtained for H_2O_2 procedure and 100 μm for BP procedure.

Free radical grafting procedure[22]

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 (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.

Photografting procedures

UV irradiation was carried out with a high-pressure mercury light (Eurolabo, 400W and 250W) equipped with UV light with a wavelength range of 230–500 nm. The distance between the light and the film was 25 cm. All reactions were heterogeneous and involved PHBHV films. Experiments were carried out under an extractor hood. Temperature was kept to 30 ± 2°C.

Surface functionalization and grafting in presence of benzophenone

PHBHV films were soaked in acetone BP solution and dried to constant weight at room temperature to remove acetone. Then, the films with BP adsorbed were put under the UV light. The preirradiated films were immediately lowered into monomer solution purged with argon for 30 min and the UV exposition

was repeated. After photografting polymerization the films were washed in ethanol to remove residual BP, or monomer. The grafted films were dried to constant weight at room temperature.

Surface functionalization and grafting in presence of H_2O_2

The PHBHV film was attached with Teflon linkages on a glass slide to facilitate the manipulation. Films were put into H_2O_2 solution 30% (10 or 20 mL) and placed under the UV light. The preirradiated films were taken from H_2O_2 solution and washed with water to remove excess of H_2O_2 and were immediately lowered into monomer solution purged with argon for 30 min and the UV exposition was repeated. After photografting polymerization the films were washed in ethanol to remove residual monomer and free homopolymer. For both procedures the grafted films were dried to constant weight at room temperature. The graft yield was calculated as a ratio of the increase in weight of the PHBHV film divided by the starting weight of film according the following equation:

$$\%\, G = \frac{W_f - W_i}{W_i} \times 100$$

Where W_f is the weight after the grafting and extraction and W_i is the initial weight.

Characterization

Fourier transformed infrared spectroscopy

FTIR spectra were recorded on a spectrometer (Tensor 27, Bruker). The spectra of the film surface were obtained with ATR equipment using diamond crystal. The ATR-FTIR spectra were recorded at a resolution of 4 cm^{-1} and an accumulation of 32 scans. The spectra were normalized to the intensity of the carbonyl stretching band at 1720 cm^{-1}.

Scanning electron microscopy

The surface morphologies of the polymer samples, before and after grafting, were observed by SEM. All observations were carried out with JEOL 6460LV SEM (JEOL, 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 using a sputter coater (Edwards Pirani 501, U.K). To observe films in cross section, samples have been incorporated into a low viscosity methacrylate resin blend (Metafix, Strauer) described previously.[34] They were polished by a series of grinding (silicon carbide grinding paper P320–P1200) with water as the lubricant. Then, polishing was performed with progressively finer abrasives with two grades of diamond polishing grit suspensions (9 μm and then 3 μm), then alumina (0.05 μm). The polished specimens needed to be sputter-coated with a thin film of conductive material (such as carbon).

Energy dispersive X-rays analysis (EDX)

To assess the localization of the grafted PHEMA onto the PHBHV film, the hydroxyl groups of the PHEMA were esterified with chloroacetyl chloride to observe the element chlorine. A grafted film was immersed in 20 mL of anhydrous diethyl ether containing 2 mL chloroacetyl chloride and 1 mL of triethylamine. The mixture was stirred for 24 h at room temperature. The reacted film was washed in ethanol for 2 h, and dried in vacuum at 40°C. Samples were coated with carbon using a sputter coater. Analysis of the elements was carried out by EDX analysis using an OXFORD INCA 300 system. To determine their distribution, Smart Map acquisition was used. Smart Map performs the simultaneous acquisition of X-ray data from each pixel on the image area. A blank experiment was performed with ungrafted PHBHV and chloroacetyl chloride. Absence of chlorine on the surface of the ungrafted PHBHV showed that the chloroacetyl chloride could only react in presence of PHEMA.

Biodegradation test

The EN 13,432 Biodegradation tests were carried out from Sturm test. The biodegradation tests of PHBHV and grafted PHBHV films were realized in triplicate. Biodegradation was calculated according to the following equation:[35,36]

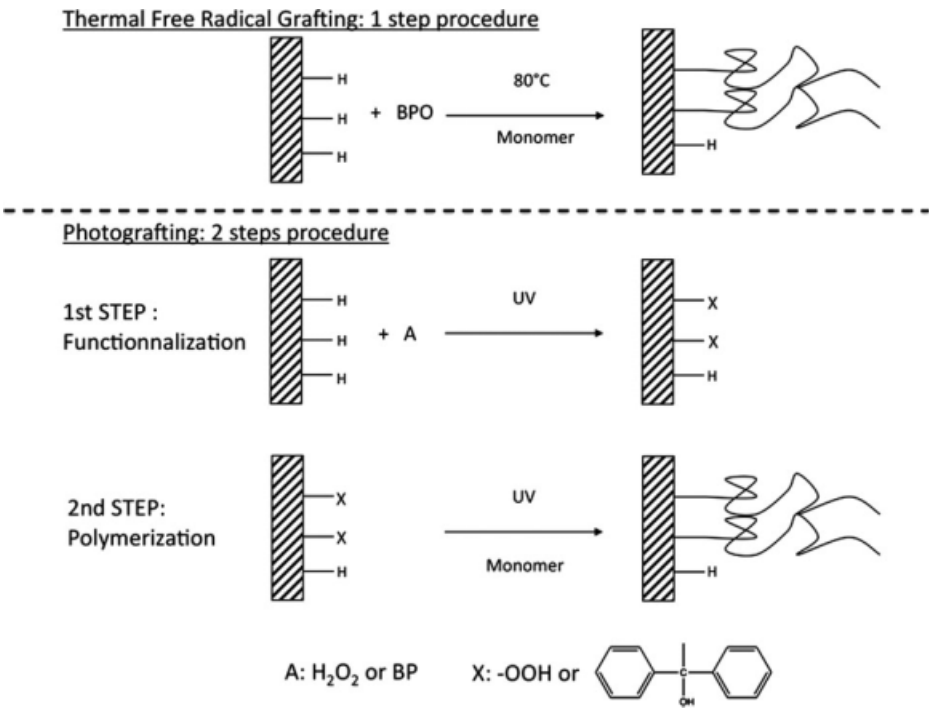
$$\%\ \text{Biodegradation} = \frac{Q(CO_2^{\text{measured}}) - Q(CO_2^{\text{blank}})}{Q(CO_2^{\text{theo}})} \times 100$$

where $Q(CO_2^{\text{measured}})$ was the CO_2 amount accumulated in presence of the material to degrade,

$Q(CO_2^{\text{blank}})$ was the compost CO_2 amount produced without any material to degrade, and $Q(CO_2^{\text{theo}})$ was the theoretical maximum CO_2 amount, which could be produced by the material.

RESULTS AND DISCUSSION

Two routes have been used to introduce graft chains onto the surface of PHBHV films, proceeding by the "grafting-from" method initiated by UV irradiation. Both procedures were carried out by a two-step



Scheme 1 Schematic diagram of the photografting procedure.

method using either BP or H_2O_2 under UV irradiation (scheme 1). The first step is the formation of reactive surface via abstracting hydrogen atoms from the film surface. It is well known that excited BP can abstract hydrogen in the absence of monomer to form surface with semipinacol groups.[23,32,33]

In presence of H_2O_2, hydroperoxide functions were generated directly on the PHBHV film via UV treatment in the presence of oxygen. Several authors have reported that hydroxyl radicals formed under UV irradiation have the ability to abstract hydrogens from the backbone of polymer producing macroradicals, which can react with the monomer to initiate grafting during the second step.[37,38]

In a second step, the surface initiates the graft polymerization under UV irradiation in presence of HEMA monomer. No homopolymerization occurred with BP procedure because of the very short lifetime of semipinacol radicals.[32] However, with hydroperoxide linkages, grafting onto the polymer surface is accompanied by the formation of polymer in the solution surrounding the polymer surface. Reactions were carried out at room temperature to compare with the previous work when BPO was used as thermal initiator at 80°C.[22] This process was carried out by a one step method. All grafting procedures were occurred under heterogeneous conditions. The formation of radicals is not easily achieved, especially because of high cristallinity of PHBHV films and the room temperature used for both photografting processes.

It is known for longer time that the UV irradiation generates radicals on the film surface[17] and/or can also initiate HEMA polymerization. We have demonstrated that 20 min of irradiation (native PHBHV film immersed in a monomer solution 5% v/v) didn't occurred grafting of PHEMA onto PHBHV. Furthermore, it was demonstrated that a maximum irradiation of 30 min didn't cause dramatic degradation of the PHA films. Therefore, the polymerization does not have to exceed 20 min and the total duration of exposure to UV (functionnalization and polymerization) does not have to overtake 30 min. These previous experiments allow to valid grafting process described in scheme 1.

Grafting with H_2O_2

The study was carried out on PHBHV films, with a thickness close to 50 μm. The influence of the functionalization time (first step) on the graft yield was studied. The results are reported in Table I. The graft copolymerization time and the HEMA concentration were maintained identical to the preceding study, which were respectively 20 min and 5% v/v.

The functionalization time (first step) did not affect in a notable way the graft yield. Similar graft yields were obtained with various volumes of H_2O_2 (10 and 20 mL). The solution of H_2O_2 (30% v/v) strongly absorbs photons. In spite of the very broad spectrum of the employed UV light, it is difficult to suppose that photons reach the surface of film to

TABLE I
Influence of the Volume of H_2O_2 and Functionalization Time. [H_2O_2] = 30% (v/v)

	10 mL H_2O_2	20 mL H_2O_2
t (min)	G%	G%
10	9.9 ± 1.6	<5
20	11.3 ± 4.3	9 ± 1.7
30	9.8 ± 3.5	–
40	nd[a]	10.6 ± 4

([HEMA] = 5% v/v, polymerization time = 20 min, film ≈ 90 μm).
[a] degraded film.

abstract hydrogen and to create radicals. It could be more probable that the irradiation created radicals $HO^{\bullet}$, able to abstract hydrogen, thus generating radicals on the surface. This assumption could explain the identical results obtained with different volumes of H_2O_2. The weak graft yield observed for 20 mL of H_2O_2 and 10 min of irradiation can be explained by insufficient time to allow the $HO^{\bullet}$ radicals formed to diffuse to the film. In the case of short time reaction, the volume reduction of the solution on the top of film would make easier the diffusion of the radicals $HO^{\bullet}$. Higher reaction times were not possible to increase the graft yield but induced a more important degradation. Consequently, the first step will be maintained to 10 min with 10 mL of H_2O_2.

To control and modulate the graft yield of PHEMA, the influence of the polymerization time and the monomer concentration were studied. The results obtained are reproducible and show that the percentage of graft yield increased with the polymerization time [Fig. 1(A)]. Beyond 30 min of polymerization, the graft yield reached a maximum value close to 20%; this saturation can be explained by the precipitation of free PHEMA that covers the surface blocking the access to the monomer. When compared to the thermal radical grafting procedure, the graft yield was higher than the photografting initiated by H_2O_2 [Fig. 1(B)], with a lower monomer concentration. We previously demonstrated that very high graft yield could be reached (>100%) with the thermal radical grafting. This could be explained that BPO was very fast and widespread grafting path.

The influence of the monomer concentration [Fig. 2(A)] shows that the graft yield increased with the HEMA concentration as it was previously reported with BPO [Fig. 2(B)]. For graft yields higher than 20%, the films became very deformed and rigid. This modification of the mechanical properties was due to the PHEMA, which is a hard material at ambient temperature ($T_g \approx 80°C$). Monomer concentration led to an increase in viscosity of the monomer solution resulting that the monomer diffused with difficulty in the solution to the surface of polymer. Similar results were reported by Hu et al.[39] who grafted PHEMA onto polypropylene membranes, high HEMA concentration led to a viscous gel formation of PHEMA that was difficult to eliminate from the film. For the optimal monomer concentration of 5% (v/v) the graft yield can be easily modulate by the reaction time in a reproducible way.

Grafting with benzophenone

The photografting in presence of BP was based on a method described by previous studies.[27,32] The protocol generally followed was a direct grafting, where the polymer support, monomer, and BP were introduced together and were reacted in one step. This process was associated with grafting but also with homopolymerization in solution. We chose to use a sequential process (scheme 1) to avoid homopolymerization of the monomer in solution.

To determine the optimal concentration of BP for the functionalization step, several concentrations in BP were studied. Figure 3 displays the effect of BP

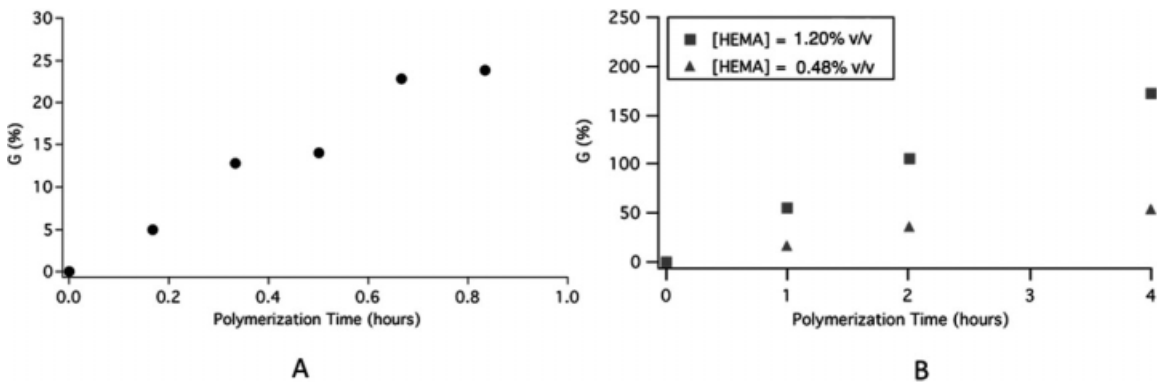


Figure 1 Variation of the graft yield with the polymerization time. (A) photoinitiated in presence of H_2O_2, functionalization time = 10 min, [HEMA] = 5% v/v, HEMA solution V = 20 mL, (B) thermal initiated by BPO, [BPO] = 1.9×10^{-2} mol L^{-1}.

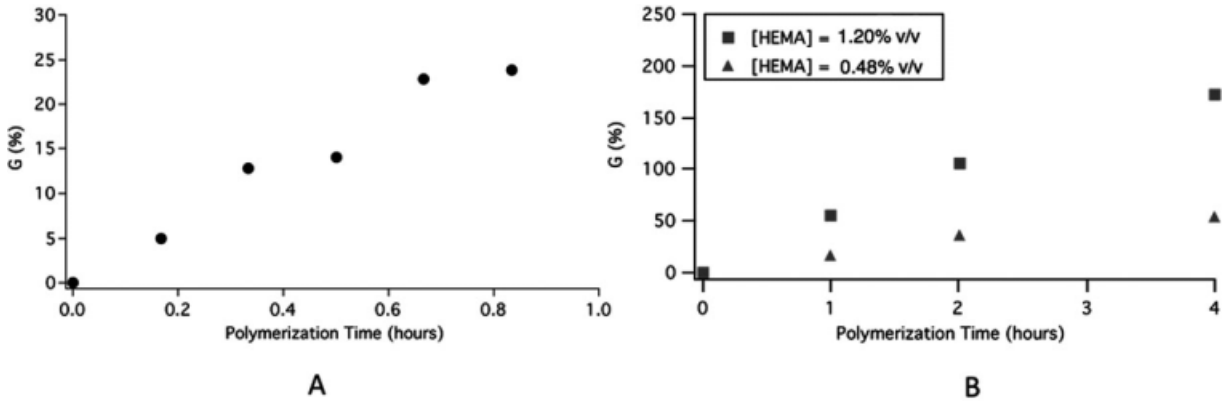


Figure 2 Variation of the graft yield with the HEMA concentration. (A) photoinitiated in presence of H_2O_2, functionalization time = 10 min, polymerization time = 30 min, HEMA solution V = 20 mL, (B) thermal initiated by BPO, [BPO] = 1.9×10^{-2} mol L^{-1}.

concentration and immersion times on graft yield. The results show a similar evolution of the graft yield of two different immersion times indicating a fast BP adsorption. The graft yield increased with the BP concentration and then stabilized around 22.5%. This result was correlated with a larger quantity of BP adsorbed on film and thus it was possible to generate more semipinacol groups.

The influence of the functionalization time on the graft yield was then studied (Fig. 4). The results show that the functionalization time did not affect the graft yield. The reaction of the radicals resulting from the decomposition of the semipinacol groups appears consequently very fast. Therefore, a functionalization time of 5 min was optimal time and kept constant in the rest of the study. Results confirmed the influence of the BP concentration on the graft yield.

The grafting can be controlled in a reproducible way according to the concentration in monomer [Fig. 5(A)]. However it is important to note that for a concentration higher than 10% of HEMA, the films become brittle. When compared to the thermal radical grafting, the graft yield was higher with a lower monomer quantity.

The graft yield increased with the polymerization time [Fig. 5(B)] until reaching saturation after 15 min. Test experiments carried out without preliminary functionalization i.e. with a concentration of BP = 0 mol L^{-1}, show that polymerization led to a mass variation lower than 3%. These results confirm the reaction pathway presented in scheme 1, namely that the grafting onto the PHBHV was initiated by the cleavage of the reactive groups created during the first step.

Comparison of different grafting procedures

The possibility to graft PHEMA, by photochemical way, on films of PHBHV has been previously demonstrated. This method offers the important advantage of working at room temperature. Consequently, the grafted film was not deformed during the grafting procedure that explains the lower graft yield observed for both photografting procedures. However, the major difference between both grafting procedures is based on the initiator radicals formation. In free radical process, initiator radicals formation

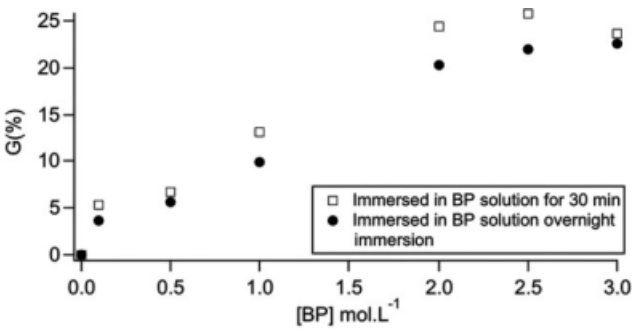


Figure 3 Variation of the graft yield with the benzophenone concentration and immersion time in benzophenone solution. BP solution V = 30 mL. Functionalization time = 5 min. [HEMA] = 5% (v/v). Volume HEMA = 20 mL. Polymerization time = 20 min.

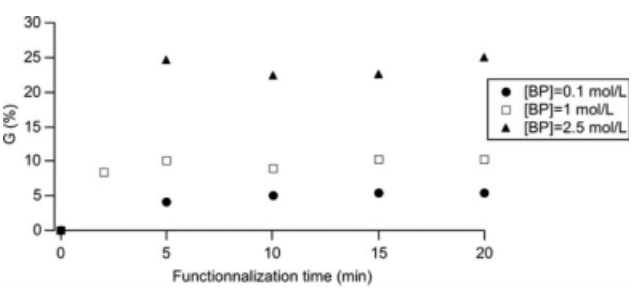


Figure 4 Variation of the graft yield with functionalization time. BP solution = 30 mL. [HEMA] = 5% (v/v). Polymerization time = 20 min.

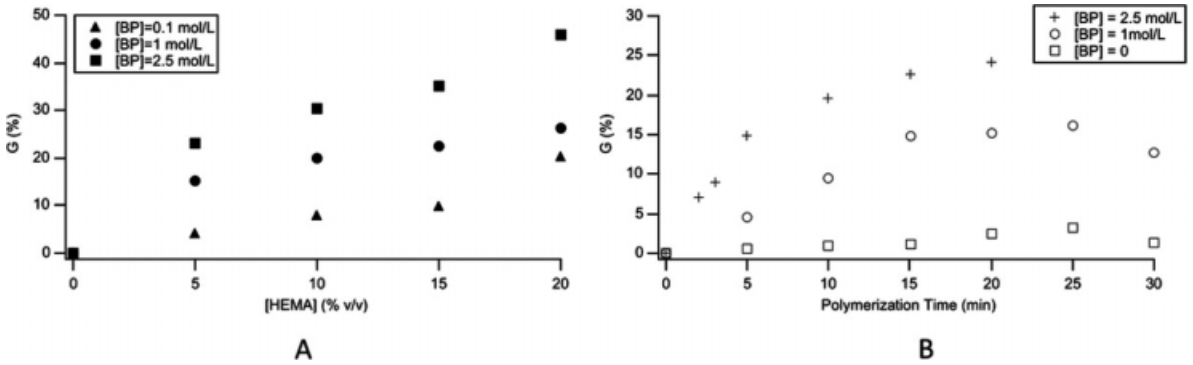


Figure 5 Photografting in presence of BP. (A) Variation of the graft yield with the monomer concentration. (B) Variation of the graft yield with the polymerization time. Functionalization time = 5 min. [HEMA] = 5% (v/v). Volume HEMA = 20 mL.

and polymerization proceed in a single step, whereas the formation of radicals is achieved in a separate step in photochemical way. According to the results it can be assumed that the efficiency of radicals formation is higher in free radical process that explain higher G% with lower monomer concentration. But it is important to note that the aim of this work is to restrict the grafting to the surface of the film not to obtain high graft yields.

Table II summarizes the effect of various experimental parameters on grafting under the different procedures. The results showed that the grafting by hydrogen peroxide depends mainly on the monomer concentration as observed with BPO, whereas with BP, the grafting depends on the BP concentration and monomer concentration. For all processes, the reaction time allows an easier control of the grafting. The most important difference between the both photochemical processes results in the absence of homopolymerization in solution in presence of BP, making this procedure particularly attractive.

Characterization of grafted films

The resulting grafted films were characterized by ATR-FTIR spectroscopy, SEM, and EDX. The characterizations were achieved by comparing systematically grafted films by photografting at room temperature and thermal grafting initiated by BPO.

The occurrence of grafting has been illustrated by ATR-FTIR analysis. Figure 6 showed ATR-FTIR spectra of grafted film with H_2O_2 (G% = 19%) and thermal free radical grafting (G% = 15%). FTIR-ATR spectra of PHBHV grafted and native PHBHV are similar with new absorption bands appeared at 3400 cm^{-1} due to the —O—H stretching vibration gradually enhanced as the grafting degree increased, indicating the presence of PHEMA on PHBHV films. But, from similar graft yields the films prepared by photografting and thermal free radical polymerization showed different profiles in ATR-FTIR spectra. The peak corresponding to the ν(—OH) vibration from the photografted PHEMA was clearly seen. However, in free radical conditions the spectra revealed a small hydroxyl peak. Concerning the carbonyl stretching (Fig. 7), the grafted film via irradiation UV became much broader compared with pure PHBHV, showing the carbonyl of the PHEMA and the PHBHV one, whereas the widening of the carbonyl peak was not visible for free radical grafting. The enlargement of the ester carbonyl band was shown for higher graft yield.[22] This result suggested that under free radical grafting process the polymerization occurred in the surface but also in the bulk of the film, whereas the photografting procedure led to grafted chains principally located on the surface of the film. ATR-FTIR spectra of films grafted with BP were similar to those obtained with H_2O_2 (results not shown). For PHBHV grafted by UV, ATR-FTIR analysis showed that both faces are different. The characteristic of PHEMA was detected on the only face exposed at the UV lamp. The spectrum of the untreated face was similar to the original PHBHV. This indicated that the PHEMA is preferentially located on the surface.

Morphological modifications of the film surfaces were achieved by SEM. SEM micrographies of films surfaces with different graft yield were taken (Fig. 8). As can be seen from Figure 8(A), the native PHBHV film used in this study shows relatively low

TABLE II
Comparison of the Different Graft Procedure

	BPO	H_2O_2	BP
Homopolymerization	yes	yes	no
Influencing graft yield factors			
Initiator amount	no	no	yes
Functionalization time	n/a	no	no
Polymerization time	yes	yes	yes
Monomer concentration	yes	yes	yes

n/a: none applicable, no functionalization was needed.

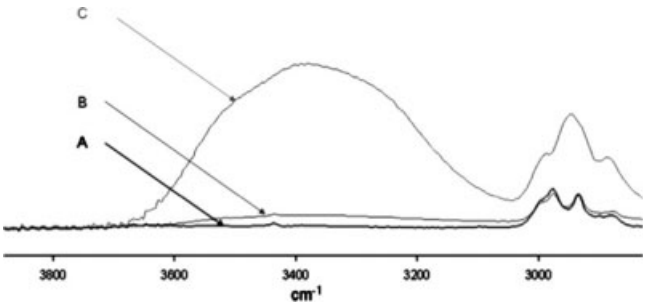


Figure 6 FTIR-ATR spectra enlargement between 3000 and 3800 cm^{-1}. (A) PHBHV unmodified, (B) PHBHV-*g*-PHEMA G = 15% grafted by BPO, (C) PHBHV-*g*-PHEMA G = 19% photografted by H_2O_2.

porosity. Figures 8(B,C) exhibit the surface of free radical grafted films with grafting degree of 40 and 300%, respectively. The difference between micrographies of the PHBHV unmodified and the PHBHV grafted PHEMA G = 40% [Fig. 8(B)] was not notable. This result indicated that the grafting led to short lengths chains or although the grafting could occur as well on the surface of film but also inside film. For a rate of high grafting of G = 300% [Fig. 8(C)], micrography SEM revealed a significant change on the surface. The chains of PHEMA were very dense and led to an increase in the roughness of surface. In the case of grafting in the presence of H_2O_2, roughness appeared at low graft yield [Fig. 8(D)]. However, from a threshold this roughness decreased as the surface was covered with PHEMA [Fig. 8(E)]. The grafting in the presence of BP modified the surface by decreasing the porosity of surface at low graft yield [Fig. 8(F)]. For higher graft yield [Fig. 8(G)], a roughness of surface appeared.

Free radical grafting led to a random distribution of the grafts on the surface. Thus to determine if the PHEMA grafts were distributed in a homogeneous way on the surface of the PHBHV, i.e. if there was any not grafted zone, the PHEMA hydroxyl groups were marked to detect them in microanalysis X (EDX). The hydroxyl groups were esterified in the presence of chloroacetyl chloride to mark the grafts using chlorine atoms, which can be detected by EDX; thus it was possible to locate PHEMA on the surface of material. The yellow pixels revealed the presence of chlorine thus the PHEMA (Fig. 9). The PHBHV film grafted in the presence of BPO with a graft yield of G = 15% grafting present PHEMA as well at surface of film but also in the internal layers of the PHBHV. The density of chlorine present inside film increases with the rate of grafting attesting of the presence of grafts of PHEMA inside film. These results confirm the assumption of a

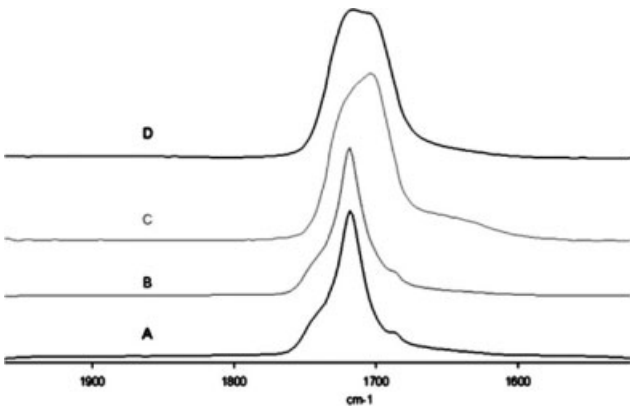


Figure 7 FTIR-ATR spectra enlargement between 1600 and 1900 cm^{-1}. (A) PHBHV unmodified, (B) PHBHV-*g*-PHEMA grafted by BPO G = 15%, (C) PHBHV-*g*-PHEMA photografted by H_2O_2 G = 19%, (D) PHEMA homopolymer.

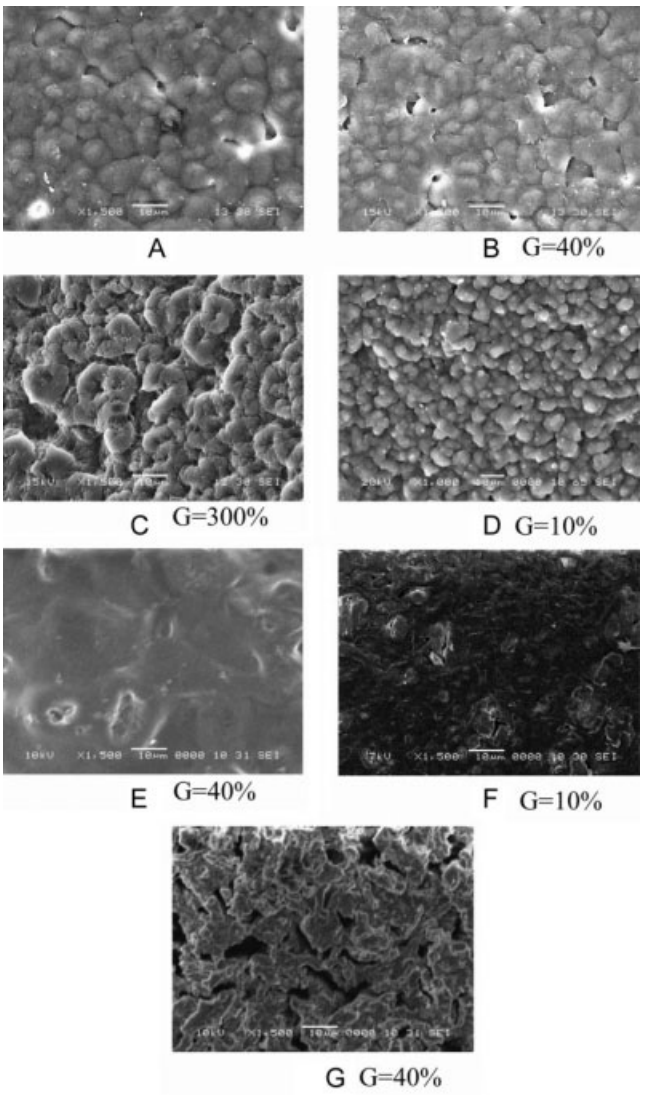


Figure 8 SEM micrographies of (A) PHBHV unmodified PHBHV-*g*-PHEMA grafted by BPO, (B) G = 40%, (C) G = 300% PHBHV-*g*-PHEMA photografted by H_2O_2: (D) G = 10%, (E) G = 40% PHBHV-*g*-PHEMA photografted by BP: (F) G = 10%, (G) G = 40%.

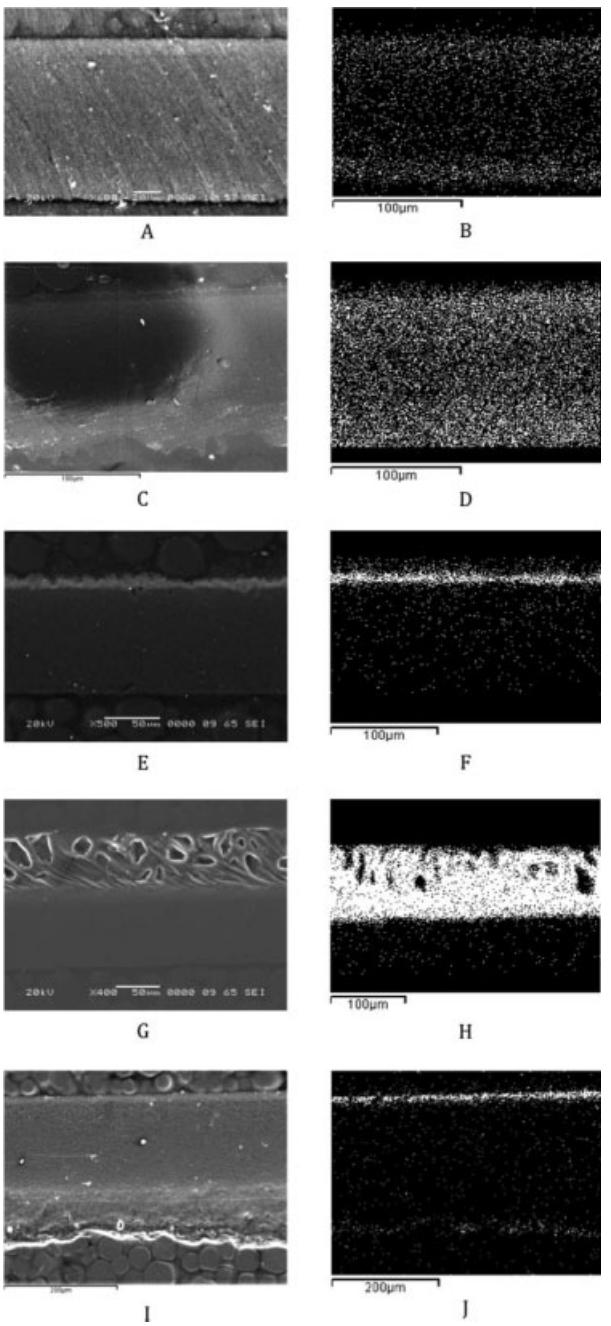


Figure 9 Cross-section of SEM micrographies and the respective EDX maps of PHBHV grafted by BPO (A et B G% = 15%, C et D 60%) et photografted by H_2O_2 (E et F G% = 2%; G et H G% = 48%) et photografted by BP (I et J G% = 49%).

grafting in depth of films of PHBHV. The grafting localization can be explained by the temperature. Indeed, the glass transition of the PHBHV is close to 3°C. However, the thermal grafting was carried out at 80°C, a temperature largely above T_g PHBHV. The temperature increased the chains mobility, which is probably sufficient to facilitate diffusion of reactant, such as monomer and initiator into the film. The grafting in the film bulk can also explain the higher graft yield compared to photografting. Indeed by grafting inside the film the surface of the grafted film could increase that led to graft yield over 150%.

Concerning the grafting by irradiation UV in the presence of H_2O_2 or in the presence of BP, analysis EDX indicated an asymmetrical distribution of chlorine what is in agreement with the presence of grafts PHEMA on only one face, as we had previously suggested from the study by IR. The increase in the graft yield increased the thickness of the layer of grafts without penetrating into the film in opposition to the grafting in the presence of BPO. Thus the zone of localization of the chlorine of PHEMA is extremely dense what probably corresponds to a layer of relatively homogeneous composition made up of grafts PHEMA. The porosity of the grafted zone, which appears very clearly on the Figure 9(G) can be explained with the drying of film after the stage of extraction in ethanol. The density of grafting the films H and J are different but graft yield were similar. It can be explained by thickness different of PHBHV films. The grafted films with BP were thicker than this treated with H_2O_2. This analysis confirms that both photografting procedures led to a grafting only localized on the surface. It can be suppose that the photons cannot reach in-depth film (a part being absorbed by the solution of HEMA). In addition, the temperature of grafting, to the maximum equal to 35°C did not lead to the penetration of the monomer into the film.

Biodegradation of grafted films

PHBHV is a natural polyester, biodegradable in natural environment.[40,41] As a synthetic non-biodegradable polymer was grafted onto PHBHV, biodegradability of the resulting material must be altered and concerns only the polyester skeleton. It was important to study the influence of PHEMA on the biodegradability of grafted PHBHV. The biodegradability was evaluated according the standardize method based on the "Sturm biodegradability test".[35,36] This test evaluates the aerobic biodegradability of organic compounds by measuring inorganic carbon production (CO_2) in sealed vessels. As indicated in Figure 10 biodegradation of grafted film was lower than the native PHBHV. Biodegradation gradually decreased as the grafting degree increased, indicating PHEMA inhibited the biodegradability of modified PHBHV films. However, the films prepared from UV treatment with graft yield lower than 10% were totally biodegradable as native PHBHV. The inhibited effect of PHEMA is more apparent with films grafted by thermal free radical polymerization, which supported the previous results about the internal location of PHEMA that decreased attack by microorganisms present in the compost inoculum.

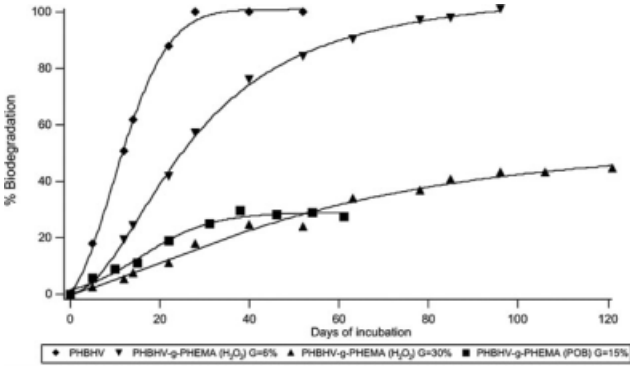


Figure 10 Comparison of the biodegradation of the PHBHV grafted by BPO and by H_2O_2.

CONCLUSION

PHEMA was successfully grafted onto PHBHV films at room temperature by photografting polymerization despite the high degree of crystallinity and nonactive chemical structure of PHBHV. The procedure was very simple and results were reproducible. In this study, PHBHV films were prefunctionalized with BP or H_20_2, and then PHEMA was polymerized from the reactive sites introduced during the functionalization step. Therefore, the grafting degree could be controllable in a wide range. The graft yield was influenced by the reaction time, the monomer concentration, and the concentration of BP. For both procedures, we have demonstrated by SEM and EDX investigations that the location of grafting was restricted to the surface compared with thermal grafting process, where PHEMA are located on the surface but also inside the film. The use of BP with sequential procedure appears more attractive because of no HEMA homopolymerization in solution occurred that makes easier purification. Furthermore, the procedure can be easily transposed to films with higher sizes.

A comparison of the enzymatic degradability demonstrated that the presence of PHEMA affected the degradability of the grafted PHBHV films. The restriction of grafted PHEMA to the surface appears as an important advantage for promoting the degradability of the grafted films. Only low grafting on surface is expected to give a complete degradation.

These photografting copolymers may be useful to regulate cell adhesion. The grafting of various monomers is currently in progress to examine the influence of chemical surface modification on the bacterial adhesion and the enzymatic degradability.

References

1. Anderson, A. J.; Dawes, E. A. Microbiol Rev 1990, 54, 450.
2. Madison, L. L.; Huisman, G. W. Microbiol Mol Biol Rev 1999, 63, 21.
3. Steinbüchel, A.; Lütke-Eversloh, T. Biochem Eng J 2003, 16, 81.
4. Doi, Y. Microbial Polyesters; VCH: New York, 1990.
5. Doyle, C.; Tanner, E. T.; Bonfield, W. Biomaterials 1991, 12, 841.
6. Kunze, C.; Freier, T.; Kramer, S.; Schmitz, K.-P. J Mater Sci Mater Med 2002, 13, 1051.
7. Köse, G. T.; Korkusuz, F.; Korkusuz, P.; Hasirci, V. Tissue Eng 2004, 10, 1234.
8. Chen, L. J.; Wang, M. Biomaterials 2002, 23, 2631.
9. Miller, N. D.; Williams, D. F. Biomaterials 1987, 8, 129.
10. Vitte, J.; Benoliel, A. M.; Pierres, A.; Bongrand, P. Eur Cell Mater 2004, 7, 52.
11. An, Y. H.; Friedman, R. J. J Biomed Mater Res 1998, 43, 338.
12. Rouxhet, L.; Duhoux, F.; Borecky, O.; Legras, R.; Schneider, Y.-J. J Biomater Sci Polym Ed 1998, 9, 1279.
13. Li, J.; Yun, H.; Gong, Y.; Zhao, N.; Zhang, X. J Biomed Mater Res Part A 2005, 75, 985.
14. Zhang, D. M.; Cui, F. Z.; Luo, Z. S.; Lin, Y. B.; Zhao, K.; Chen, G. Q. Surf Coat Technol 2000, 131, 350.
15. Grondahl, L.; Chandler-Temple, A.; Trau, M. Biomacromolecules 2005, 6, 2197.
16. Köse, G. T.; Kenar, H.; Hasirci, N.; Hasirci, V. Biomaterials 2003, 24, 1949.
17. Zhijiang, C.; Guoxiang, C.; Mingming, G.; Zhiyuan, T. J Polym Res 2004, 11, 99.
18. Hu, S.-G.; Jou, C. H.; Yang, M. C. J Appl Polym Sci 2003, 88, 2797.
19. Kim, H. W.; Chung, C. W.; Kim, S. S.; Kim, Y. B.; Rhee, Y. H. Int J Biol Macromol 2002, 30, 129.
20. Yamada, K.; Takeda, S.; Hirata, M. J Appl Polym Sci 2007, 103, 493.
21. Xu, F. J.; Zhao, J. P.; Kang, E. T.; Neoh, K. G.; Li, J. Langmuir 2007, 23, 8585.
22. Lao, H. K.; Renard, E.; Linossier, I.; Langlois, V.; Vallee-Rehel, K. Biomacromolecules 2007, 8, 416.
23. Janorkar, A. V.; Metters, A. T.; Hirt, D. E. Macromolecules 2004, 37, 9151.
24. Tazuke, S.; Kimura, H. Makromol Chem 1978, 179, 2603.
25. Li, Y.; Desimone, J. M.; Poon, C.-D.; Samulski, E. T. J Appl Polym Sci 1997, 64, 883.
26. Xing, C.-M.; Deng, J.-P.; Yang, W.-T. Macromol Chem Phys 2005, 206, 1106.
27. Ke, Y.; Wang, Y.; Ren, L.; Lu, L.; Wu, G.; Xiaofeng, C. J. J Appl Polym Sci 2007, 104, 4088.
28. Yang, Q.; Hu, M.-X.; Dai, Z.-W.; Tian, J.; Xu, Z.-K. Langmuir 2006, 22, 9345.
29. Yang, Q.; Xu, Z.-K.; Dai, Z.-W.; Wang, J.-L.; Ulbricht, M. Chem Mater 2005, 17, 3050.
30. Sun, H.; Wirsen, A.; Albertsson, A.-C. Biomacromolecules 2004, 5, 2275.
31. Wirsen, A.; Sun, H.; Albertsson, A.-C. Biomacromolecules 2005, 6, 2697.
32. Ma, H.; Davis, R. H.; Bowman, C. N. Macromolecules 2000, 33, 331.
33. Ulbricht, M.; Yang, H. Chem Mater 2005, 17, 2622.
34. Fay, F.; Linossier, I.; Langlois, V.; Renard, E.; Vallee-Rehel, K. Biomacromolecules 2006, 7, 851.
35. Yabannavar, A. V.; Bartha, R. Appl Environ Microbiol 1994, 60, 3608.
36. Száraz, L.; Beczner, J. Int Biodeterior Biodegradation 2003, 52, 93.
37. Yuan, X.; Sheng, J.; He, F.; Tang, Y.; Shen, N. J Appl Polym Sci 1998, 69, 1907.
38. Ma, Z.; Gao, C.; Ji, J.; Shen, J. Eur Polym J 2002, 38, 2279.
39. Hu, M.-X.; Yang, Q.; Xu, Z.-K. J Membr Sci 2006, 285, 196.
40. Lee, S. Y. Biotechnol Bioeng 1996, 49, 1.
41. Rosa, D. S.; Filho, R. P.; Chui, Q. S. H.; Calil, M. R.; Guedes, C. G. F. Eur Polym J 2003, 39, 233.