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Biodegradation of γ Irradiated Poly 3-hydroxybutyrate (PHB) Films Blended with Poly(Ethyleneglycol)

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Abstract Poly(3-hydroxybutyrate) (PHB) was evaluated in blends with poly(ethyleneglycol) (PEG) of different weight average molecular weight (Mw = 300, 600, 1,000 and 6,000). Irradiation of the PHB/PEG films was carried out to different levels of irradiation doses (5 and 10 kGy) and the effects were investigated talking into consideration: thermal properties by differential scanning calorimetry (DSC), perforation resistance, water vapor transmission rate and biodegradation in simulated soil. The addition of plasticizer alters thermal stability and crystallinity of the blends. The improvement in perforation resistance due to irradiation was regarded to be a result of the crosslinking effect. Also, biodegradation assays resulted in mass retention improvements with increases in PEG molar masses, PEG concentration and irradiation dose. The irradiation process was shown to hamper the biodegradation mechanism.

Keywords Poly 3-hydroxybutyrate · Biodegradation · Physical–chemical properties · Gamma radiation · Crosslinking

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Introduction

Synthetic polymers are widely used in manufactured products due to their properties and low production costs. However, these polymers are generally resistant to biological degradation when discarded in the environment [1, 2]. Biodegradable products are one of the solutions to plastic waste management and the serious environmental impact generated by them. These are polymers that are initially degraded by the action of microorganisms such as bacteria, fungi and algae [3].

Polyhydroxyalkanoates (PHAs) are a class of biodegradable aliphatic polyesters, which are synthesized by many types of microorganisms [4, 5]. Poly 3-hydroxybutyrate (PHB), one of the most important biodegradable polymers, is well-known for its satisfactory mechanical properties, similar to those of polypropylene (PP). This polymer provides a better carbon dioxide barrier than PE or PP and a reasonable water barrier, better than (polycaprolactone) (Nylon 6.6) [6], as well as good resistance to solubility in water. Poly 3-hydroxybutyrate is biocompatible but has unsatisfactory thermal stability that prevents its larger industrial application. Besides, its degradation depends on environmental microbial activity and the irregularity of the sample surface concerning the presence of fractures and holes that extend the area. It also depends on reactivity, considering the presence of plasticizers and the effects of grafting, crosslinking by irradiation or other polymer surface modifications, all of which prevent the penetration of enzymes [7, 8]. The biodegradation of PHB porous substrates was accelerated by in vitro enzymes and, as reported by Zhijiang [9], a porous configuration was favorable to its degradation. PHB degradation rate is still comparatively slower than those of other degradable polymers probably as a result of relatively poor PHB hydrophilicity [10].

Poly(ethyleneglycol) (PEG) properties, such as high hydrophilicity, biocompatibility and flexibility are adequate for biomaterial compositions. Recently Parra et al. [11] have been blended PHB with PEG in which the incorporation of PEG of low molar mass into the PHB matrix has improved its hydrophilicity, biocompatibility and degradability. However, the effect on the variation of the molar mass (M) of PEG has not been investigated.

Direct irradiation of PHB films by gamma radiation exposure was explored in work with thin films irradiated at 40 kGy. Pronounced degradation occurred at high irradiation doses (40 kGy), with loss of mechanical properties, but no information was reported concerning the biodegradation of the irradiated film [6].

In a previous study we have reported the increase of enzymatic degradation as a result of hydrophilic plasticizer concentration increases [10]. In addition to this, crystallinity, molar mass of the sample and temperature have been regarded as important factors that influence the growth of microorganisms on the polymer surface. Speculations about biodegradable film lifetime control are an important theme of new studies that shall evaluate the surface physical characteristics, surface grafting or crosslinking that modifies the resistance to biodegradation.

The aim of the present study is to examine the biodegradation effects of irradiation on the films when using different weight average molecular weight of PEG. Biodegradation test, mechanical properties (perforation resistance), permeability to water vapor transmission and thermal properties are analyzed under typical conditions of sterilization processes.

Experimental

Materials

Poly 3-hydroxybutyrate (PHB) (lot 67/August 2003), was supplied in powder form from Usina da Pedra PHB of Brazil S. A. (Serrana, SP, Brazil) and had an weight average molecular weight (Mw) of 380,000 g mol^{-1}, with PHB accounting for 99.9% of the dry material.

Poly(ethyleneglycol) (PEG) was supplied by Oxiteno (Mauá, SP, Brazil) and had Mw of 300, 600, 1,000, 1,500 and 6,000 g mol^{-1}. Chloroform was provided by Labsynth Produtos para Laboratório (Diadema, São Paulo, Brazil).

Film Preparation

Films of PHB and PHB/PEG were prepared by casting using the following proportions of PHB/PEG: 100/0, 95/5 and 85/15 (wt/wt%). Each polymer (15% relative to the mass of solvent) was dissolved in chloroform (85%). The

solutions were mixed and stirred thoroughly at 60 ± 1 °C for 10 min. Afterwards the solvent was allowed to evaporate in Pyrex recipients in a saturated atmosphere.

Irradiation of Films

The films were irradiated by a ^{60}Co gamma source, operating at doses of 5 and 10 kGy under a nitrogen gas atmosphere. EMBRARAD provided the source, which had a dose rate of 10 kGy h^{-1}. Dosimetry was performed with Harwell Red Perspex 4034.

Differential Scanning Calorimetry (DSC)

Thermal analysis was performed using a DSC 822e differential scanning calorimeter (Mettler-Toledo, Switzerland) with 10 mg of the sample, under a dynamic atmosphere of nitrogen (N_2), at a heating rate of 10 °C min^{-1}. The PHB was initially heated to 200 °C to eliminate the sample thermal history, and then cooled to room temperature before being immediately reheated to 200 °C. All DSC experiments were done in duplicate and the reported data refers to the second heating [12]. PHB crystallinity in the blends was calculated according to Eq. 1.

$$Xc(\%) = \frac{\Delta H_f \times 100}{\Delta Ho \times w(PHB)} \tag{1}$$

where ΔHf = melting enthalpy of the sample, ΔHo = melting enthalpy of the 100% crystalline PHB which is assumed to be 146 J g^{-1} [12, 13] and w(PHB) = weight fraction of PHB in the sample.

The DSC apparatus was calibrated with Indium (m.p. 156.61 °C; $\Delta H = 28.54$ J g^{-1}) so that crystallinity, melting temperature and crystallization temperature Tm and Tc were obtained.

Perforation Resistance Test

Perforation testing was carried out using a Texture analyzer TA.XT2i from Stable Micro Systems equipped with a cylindrical probe (3 mm diameter) and a cylindrical base of a rigid polystyrene cup (63 mm diameter). The probe was lowered onto a sample of the film at a constant velocity of 1 mm s^{-1}. When in contact with the surface, the strength was recorded until a deformation of 15 mm, or until the rupture of the sample. Three samples were tested following the standard procedures.

Water Vapor Transmission (WVT)

The water vapor transmission (WVT) was determined according to Limmatvapirat et al. [14] in PHB films containing PEG at 5% (PEG 300 or 6,000) and 15% of PEG

(PEG 600, 100, 1,500 or 6,000) (wt/wt%). A stainless steel recipient with 10% in volume of silica gel was closed with a sample of film firmly fixed at the top. The recipient was subsequently placed in a desiccator with distilled water at 25 °C. The relative humidity inside the desiccators was measured as 55% RH, during the test.

The films were removed and weighed each 24 h for 10 days, on a Mettler analytical balance, and the water vapor transmission (WVT) was calculated according to Eq. 2:

$$WVT = \frac{w \times x}{A \Delta Pv} \qquad (2)$$

where WVT is the water vapor transmission (g H2O mm/$cm^{-2)}$, x is the average thickness of the films (0.048×0.012 mm), A is the permeation area (12.57 cm^2), w is the film weight and ΔPv is the vapor pressure difference.

Water Vapor Transmission Rate

The water vapor transmission rate (WVTR) was calculated according to Eq. 3:

$$WVTR = \frac{w \times x}{t \times A} \qquad (3)$$

where *WVTR* is the water vapor transmission rate (g H_2O mm cm^2 h^{-1}). The term x/t was calculated by linear regression from the points of weight gain versus time in a constant rate period. The tests were done in triplicate.

Biodegradation in Simulated Soil

To obtain a simulated soil, we used 23% loamy silt, 23% organic matter (cow manure), 23% sand and 31% distilled water (all wt./wt%). The specimens (2 cm × 2 cm) were weighed and buried in triplicate in simulated soil at room temperature (24 °C) without the presence of light. In this period of time, humidity was maintained with addition of distilled water in each 3 days. Biodegradation was monitored each 7 days for approximately 50 days by measuring mass retention. The buried samples were later recovered, washed with distilled water, and dried at room temperature until there was no further variation in weight. Following weighing, the specimens were buried again in their respective trays.

Optical Microscopy

The behavior of the materials during enzymatic degradation were assessed by optical microscopy (Model XP-500 microscope, Laborana Ltda., São Paulo, SP, Brazil) fitted with a CCD camera (resolution of 330/460 lines). The photographs shown in this work were taken before and after ageing in simulated soil.

Results and Discussion

Differential Scanning Calorimetry (DSC)

The DSC results on melting (Tm) and crystallization (Tc) temperatures of the films, obtained in the program of heating–cooling–reheating, are showed in Table 1. According to this table the peak in the second melting was found to be due to the fusion (Tm) of crystals formed during the crystallization process, which happened in the cooling segment of analytical procedure of DSC. As a consequence to the process, Table 1 show decreases of crystallinity of the PHB film, a fact which has not changed the melting temperature, when compared with PHB powder. The blends having PEG showed a decrease of melting temperature in almost all films and indicate the miscibility of each one, the crystallization temperature remained the same. The addition of PEG of different Mw in the PHB to obtain the films resulted in films of intermediate crystallinity (between PHB powder and processed PHB), except for PEG 6,000. In this particular case we assume that high molar mass PEG induces the crystallization.

Competition between crosslinking and scission reactions, as expected, takes place in the irradiation of polymers. Scission promotes the formation of shorter molecules that crystallize at lower temperatures, increasing the crystallinity degree, while crosslinking reactions impede crystallization. In Fig. 1 the results for PHB/PEG 300 are reported for illustration.

The crystallization of irradiated films with PEG 300 or PEG 6,000 are compared in Table 1: at low PEG content (5%) the observed decrease of crystallization temperature after 10 kGy means the presence of segments, a result of scission with improved mobility. The decrease of crystallinity degree (%) indicates the simultaneous occurrence of crosslinking reactions that impede the crystallization phenomenon.

When comparing PEG 600, 1,000 and 1,500 at high PEG content (15%), the crystallinity increases with irradiation at 5 kGy and decreases at 10 kGy. It means that for high PEG contents, PHB suffers chain scission preferentially and the segments have more mobility to migrate and crystallize on the surface of the crystalline phase. A decrease of melting temperature as a result of the chain scission mechanism was also observed.

The double or multiple melting behaviors sometimes occurs for PHB and its copolymers, and depends on the crystallization conditions [15]. Multiple melting behavior of a polymer is usually attributed to process of partial melting and recrystallization or melting of crystals with different lamellar thickness [16].

Parra et al. [12] have verified that the displacement of the melting temperatures of irradiated films occurs above

Table 1 DSC results of irradiated and non-irradiated PHB/PEG films

PEG concentration (%)	Film PHB/PEG (x) − y kGy	T_2 (°C)	Tc (°C)	Crystallinity (%)
0	PHB powder	172	89	38
	PHB film	171	71	30
5	PHB/PEG 300	157	70	33
	PHB/PEG 300 − 5 kGy	160	74	29
	PHB/PEG 300 − 10 kGy	163	66	23
	PHB/PEG 6,000	162	70	46
	PHB/PEG 6,000 − 5 kGy	166	65	43
	PHB/PEG 6,000 − 10 kGy	165	69	33
15	PHB/PEG 600	172	71	36
	PHB/PEG 600 − 5 kGy	166	73	39
	PHB/PEG 600 − 10 kGy	172	70	37
	PHB/PEG 1,000	163	74	32
	PHB/PEG 1,000 − 5 kGy	126	77	42
	PHB/PEG 1,000 − 10 kGy	127	74	38
	PHB/PEG 1,500	166	70	31
	PHB/PEG 1,500 − 5 kGy	160	76	43
	PHB/PEG 1,500 − 10 kGy	157	75	40
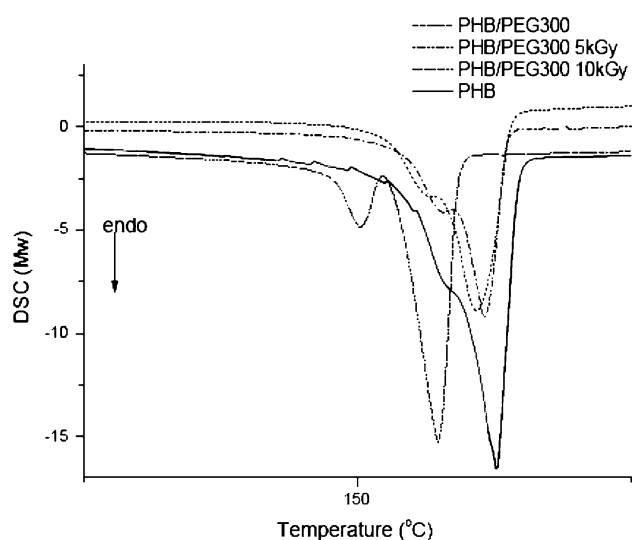
Fig. 1 DSC results of PHB/PEG blends of irradiated and non-irradiated films

10 kGy. The DSC profile of PHB/PEG film irradiated at 40 kGy showed enlarged quasi-double crystallization peaks on cooling. Furthermore in the reheating a second melting peak, separated at about 17 °C from the main melting peak, was observed. At relatively low irradiation dose (below 40 kGy), this peak persists and is attributed to bimodal distribution of crystallite sizes resulting from changes in molar mass due to random scission of long PHB chains, i.e., to the decrease in the molar mass, or to unstable lamellar layers present, generally, on the surface

of the crystals. After irradiation these distinct crystalline structures will become more indistinguishable with the fragmentation of the chains and the double peaks become a single enlarged peak with an increase in dose.

The second melting peak disappears only at high irradiation doses (above 50 kGy) when polymeric crystal lamellae are drastically deformed. This was verified for PHB powder at 300 kGy, as reported by Oliveira et al. [17]. In addition to this, the plasticizer can influence lamellae formation of the polymer. PEG nucleation (crystallizing at lower temperature) reduces the mobility of PHB retarding the lamellae formation. According to Yoshie et al. [18], thinner lamellae have lower melting temperature. Also the crystallization temperature (Tc) has a little shift to lower temperature due to cold crystallization. In the presence of the PEG plasticizers, which quite efficiently reduce the intermolecular interaction in the PHB phase, the mobility of macromolecules necessary for cold crystallization may be noticed at lower temperatures, as observed by Bibers et al. [19].

Irradiation at the level of 5–20 kGy of PHB films containing poly(ethyleneglycol), in agreement with previous work, decreases crystallinity. Oliveira et al. [17] irradiated PHB over the interval from 50 to 300 kGy. They observed that irradiated samples show increases in the degree of crystallinity when compared with non-irradiated samples. Oliveira et al. [17] discussed the results of DSC and X-ray diffraction techniques, reporting that the diffractograms showed that new peaks are not formed by irradiation, that formation of new symmetric orders are absent and that

fragments formed in the radiolysis of the molecules in the amorphous region group promote chemicrystallization. The calculations of crystallite sizes demonstrated that they increase with the irradiation dose, which can be read as a strong sign of predominant chemicrystallization.

It has been argued that, when comparing PHB film with films containing PEG of different Mw, independently of PEG Mw, at high plasticizer content, the crystallinity increases. According to Bibers et al. [19], a certain increase in crystallinity was observed at increased content of plasticizers in PHB as an effect caused by an efficient rise in polymer macromolecular mobility during crystallization, in the presence of these plasticizers. When irradiated, our films showed a decrease in crystallinity owing to the possibility of crosslinking occurring at low levels (10 kGy) of irradiation. Finally, depending on the PEG concentration, the films with 15% of plasticizer did not show notable difference in crystallinity. Otherwise, when irradiated these films with high plasticizer concentration presented an increase in the crystalline phase relative to not-irradiated PHB/PEG in almost all samples, even though some differences were noted, depending on the doses. PEG under radiation produces reactive radicals and in the concentration of 15% are sufficient to react with the PHB macrochain. This infers that PEG-PHB crosslinking competes with degradation at the dose of 5 kGy. For instance after 10 kGy with chain scission degradation remains higher crystalline phase than in the blend not irradiated.

Otherwise, when irradiated these films with high plasticizer concentration presented an increase in the crystalline phase relative to not-irradiated PHB/PEG in almost all samples, even though some differences were noted, depending on the doses. This infers that crosslinking competes with degradation; the first mechanism leads to a decrease of crystallinity, while the second leads to an increase of crystallinity by the improvement of fragment mobility in the crystalline phase.

Perforation Resistance

PHB films perforation resistance are improved in presence of PEG showing the plasticization effect of it in the different molar masses. The irradiation of the films usually contributes to the mechanical property with the crosslink effect obtained thought the combination of the radiation formed radicals. Depending of the radiation level, chain scission reactions compete with the crosslink formation. So perforation resistance enhancement is associated with the irradiation of the films. Irradiation to doses of 5 and 10 kGy improves the mechanical resistance against perforation of the samples, as reported in Table 2. That is due to the crosslinking effect of the radiation process. The decreasing in consequence of higher dose of 10 kGy

reveals the chain scission occurrence corroborating with the former discussion about crystallinity effects. It is important to highlight that a previous study on impact response of composite laminates has brought about impact perforation as the most important damaging stage. Furthermore thickness has proved to have greater influence on impact perforation resistance than in-plane dimensions [20]. After low level irradiation, however, the weight average molecular weight of PEG does not interfere in this physical property.

Water Vapor Transmission Rate (WVTR)

The decrease in WVTR values of the irradiated films is attributed to crosslinking PHB formation, which changes the hydrophilic character of the films. The addition of plasticizer does not alter this property, Table 3. These results are similar those of Rodríguez et al. [15] who analyzed the sorption and diffusion data obtained for water vapor in four different polymers: poly(methylmethacrylate) (PMMA), poly(2-hydroxyethylmethacrylate) (PHEMA), poly(N-vinyl-2-pyrrolidone) (PVP) and poly(acrylonitrile) (PAN) at 35 °C using a gravimetric sorption method. Highest sorption was observed in PVP, followed by PHEMA, PMMA and PAN, with very little water quantity. All polymers exhibited a type III Brunauer, Emmett and Teller (BET) isotherm sorption. The large upturn at high activity for PVP and PHEMA was probably due to plasticization of the polymers by water vapor.

Biodegradation in Simulated Soil

The ageing time of PHB samples plasticized with different concentrations of PEG in simulated soil is shown in Figs. 2, 3 and 4. The irradiated films are included in a specific comparison. Figure 2 shows the pure PHB mass retention as well as the mixture with PEG 1,000 at 15%, with and without irradiation to 5 kGy, during ageing in simulated soil. PHB presented a total mass loss in 21 days and the same behaviour was observed for PHB/PEG 1,000 without irradiation, while the sample irradiated at 5 kGy was mineralized in more time, 42 days.

It is thus possible to infer that irradiation retards film ageing by microorganism digestion. This retardation is attributed to crosslinking in the polymer, which impedes biodegradation. Figure 3 shows the biodegradation behaviour of PHB with different concentrations of PEG 1,000 (5, 10 and 15%).

PHB biodegradation is similar to that of PHB/PEG. Pure PHB was totally biodegraded in 21 days, similar to unirradiated samples of PHB/PEG 1,000. As a consequence of radiation, samples of PHB/PEG 1,000 (5, 10 and 15%), after 10 kGy, were mineralized after 42 days in simulated soil.

Table 2 Results of perforation resistance of irradiated and non-irradiated PHB/PEG films

PEG concentration (%)	Film PHB/PEG $(x) - y$ kGy	Perforation resistance (N)
0	PHB	0.22 ± 0.03
	PHB/PEG 300	0.30 ± 0.02
	PHB/PEG 300 − 5 kGy	0.45 ± 0.07
	PHB/PEG 300 − 10 kGy	0.40 ± 0.03
5	PHB/PEG 6,000	0.19 ± 0.09
	PHB/PEG 6,000 − 5 kGy	0.27 ± 0.04
	PHB/PEG 6,000 − 10 kGy	0.23 ± 0.10
15	PHB/PEG 600	0.30 ± 0.02
	PHB/PEG 600 − 5 kGy	0.48 ± 0.07
	PHB/PEG 600 − 10 kGy	0.42 ± 0.03
	PHB/PEG 1,000	0.37 ± 0.07
	PHB/PEG 1,000 − 5 kGy	0.43 ± 0.03
	PHB/PEG 1,000 − 10 kGy	0.46 ± 0.0 3
	PHB/PEG 1,500	0.22 ± 0.03
	PHB/PEG 1,500 − 5 kGy	0.33 ± 0.05
	PHB/PEG 1,500 − 10 kGy	0.28 ± 0.05
	PHB/PEG 6,000	0.37 ± 0.06
	PHB/PEG 6,000 − 5 kGy	0.48 ± 0.07
	PHB/PEG 6,000 − 10 kGy	0.42 ± 0.03

Table 3 Results of water vapor transmission rate of irradiated and non-irradiated PHB/PEG films

PEG concentration (%)	Film	WVTR 10^{-8} (g mm cm^{-2} h^{-1})	R^2
0	PHB	1.23	0.95
5	PHB/PEG 300	1.33	0.98
	PHB/PEG 300 − 5 kGy	0.086	0.96
	PHB/PEG 300 − 10 kGy	0.058	0.93
	PHB/PEG 6,000	0.480	0.99
	PHB/PEG 6,000 − 5 kGy	0.296	0.99
	PHB/PEG 6,000 − 10 kGy	0.124	0.99
15	PHB/PEG 600	0.490	0.99
	PHB/PEG 600 − 5 kGy	0.044	0.95
	PHB/PEG 600 − 10 kGy	0.030	0.97
	PHB/PEG 1,000	0.498	0.97
	PHB/PEG 1,000 − 5 kGy	0.265	0.96
	PHB/PEG 1,000 − 10 kGy	0.135	0.98
	PHB/PEG 1,500	0.569	0.95
	PHB/PEG 1,500 − 5 kGy	0.328	0.99
	PHB/PEG 1,500 − 10 kGy	0.139	0.99
	PHB/PEG 6,000	0.459	0.95
	PHB/PEG 6,000 − 5 kGy	0.228	0.99
	PHB/PEG 6,000 − 10 kGy	0.039	0.99

In general films with lower PEG concentration should retard biodegradation. In the current case, on the contrary, the test showed that 15% PEG film was more resistant to the attack of the enzymes, which indicates the function of radiation as a surface modifier leading to slower enzymatic degradation.

Figure 4 shows a comparison of the biodegradation behaviors from all the tested samples: pure PHB and with different PEG at 5% (6,000, 1,500, 1,000, 600 and 300), all irradiated to 10 kGy. The irradiated films are all decomposed more slowly. The differences observed per PEG type showed particular biodegradation resistance of the samples of intermediate Mw (PEG 6,000, 1,000 and 1,500). We have attributed it to PHB compatibility and, in this case, to the possibility to participate in crosslinking at the surface level. PEG 300 and 600 plasticizers did not interfere in PHB biodegradation. Possibly, PEG 300 and 600, of lower weight average molecular weight, are more prone to extraction under the wet moiety, while PEG 6,000, with a higher molar mass PEG, and the intermediary molar masses PEG 1,000 and 1,500 easily participate, under irradiation, of crosslink with PHB chains, acting as a surface protective layer to reduce water attack on the polymer in the biodegradation process.

Photomicrography of the samples, before and after 48 days, in simulated soil ageing is shown in Table 4, indicating the progressive degradation of the samples.

Conclusion

We have drawn the conclusion that irradiated films are decomposed by biodegradation more slowly than non-irradiated films and that this retardation is the effect of crosslinking formation in the polymer, which hampers the biodegradation mechanism. The higher molar mass of PEG in the blends the more resistance to attach; the higher the PEG concentration in the films, the more resistant to attack they become.

Irradiation of the films improved the perforation resistance and decreased WVT values of the samples. Crosslinking and changes of the hydrophilic character of the films have been attributed to the effects of irradiation. The evidence of crosslinking reactions in irradiated films with low PEG content is the decrease of crystallization temperature that makes crystallization more difficult. The crystallinity increased with irradiation at 5 kGy and decreased at 10 kGy, but maintained a higher level than that of non-irradiated films. Finally, the irradiation effects on perforation resistance and permeability corroborate the evidence of crosslinking at this level of irradiation.

Irradiated films hamper the enzymatic biodegradation and this leads us to conclude that the function of radiation as a surface modifier leads to slower enzymatic degradation.
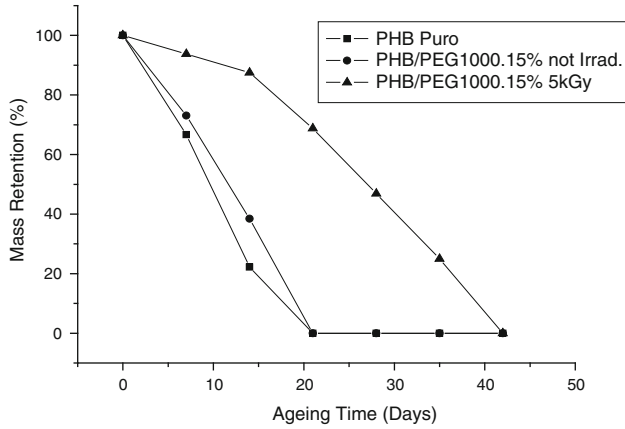
Fig. 2 Mass retention of PHB/PEG 1,000 films of irradiated and non-irradiated to 5 kGy
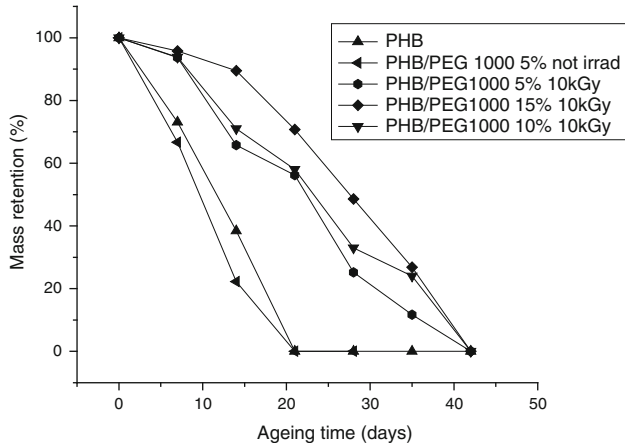

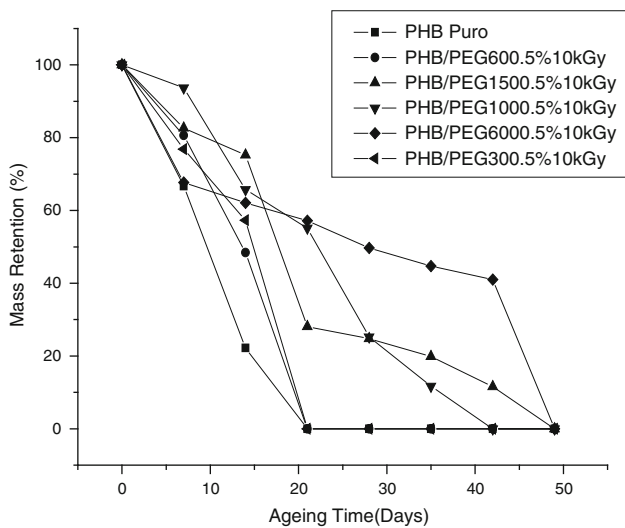**Fig. 3** Mass retention of PHB/PEG 1,000 samples, with different PEG concentrations, irradiated to 10 kGy

Fig. 4 Mass retention of PHB/PEG samples, with different PEG MM, irradiated to 10 kGy

Table 4 Photomicrography of the samples, before and after 48 days in simulated soil ageing

PHB/PEG Formulations	Before Ageing	After Ageing
Pure PHB		
PHB/PEG 6000		
PHB/PEG 1500		
PHB/PEG 1000		
PHB/PEG 600		
PHB/PEG 300		

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