Chemical Degradation of Poly(hydroxybutyrate-*co*-hydroxyvalerate) Microparticles

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Summary: Poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) is a polymer that can be used on preparation of controlled release systems containing actives principles. The release of the compounds encapsulated on those systems can occur by three different forms: diffusion, degradation and matrix polymeric rupture. Therefore, it is interesting to evaluate the behavior of polymeric matrix when it is submitted on a pH change, for example an agricultural soils simulations. The objective of this study is the understanding of the chemical degradation process of microparticles employed with agricultural application. The PHBV microparticles were prepared using o/w simple emulsion technique followed by solvent evaporation, using poly(vinyl alcohol) (PVA) as stabilizing agent. The evaluation of polymeric microparticles, submitted to chemical degradation process in pH 5.6 at room temperature, was determined by conventional characterization techniques such as Scanning Electron Microscopy (SEM), X-ray diffraction (XRD) and Differential Scanning Calorimetry (DSC). By SEM images it is possible to suggest that the chemical degradation process occurred. The indicatives peaks of crystallinity of the PHBV microparticles decrease and occurred the rise of broader peaks relative at appearance of new amorphous regions. The data obtained by thermal analysis shown that microparticles degradation occurred on amorphous and crystallines portions of PHBV.

Keywords: degradation; microparticles; poly(hydroxybutyrate-*co*-hydroxyvalerate); properties evaluation

Introduction

Poly(hydroxyalkanoates) (PHAs) are biodegradable polymers synthesized by microorganisms. These polymers can be degraded through enzymatic or hydrolytic mechanisms. In the hydrolytic degradation (Figure 1), there is an acid or basic hydrolysis of the ester bond. This means that biodegradable polymers can be decomposed in presence of corporal fluids and the resulting products of the degradation can be metabolized and eliminated or absorbed by the organism.[1–5]

Poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV), biodegradable and biocompatible polymer has been used in controlled release systems of bioactive molecules, surgical procedures and on biomedical engineering. The low rate of degradation exhibited by this polymer, in comparison with others biodegradable polymers, makes PHBV suitable for applications reported.[13–14]

As well as the PHBV, biodegradable polymers such as polycaprolactone (PCL) and polylactide (PLA) have been used as matrix of micro and/or nanoparticles, considered non-toxic to live organisms.[8–9,15–19]

Studies in literature report the degradation of biodegradable polyester. Chen and collaborators[15] assess the degradation of

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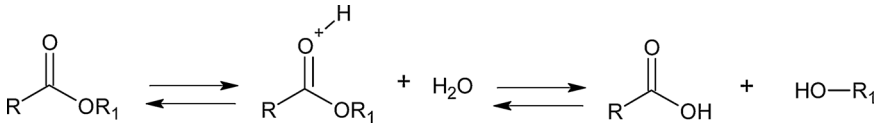

Figure 1.
Scheme of hydrolytic degradation (acid hydrolysis of the ester bond).

the PCL microparticles and compared with the same process realized to films of this polymer. Luo and Netravali[20] studied change on physical and mechanic properties of PHBV films by degradation process in a composting medium.

This study was realized as a way to evaluate the chemical degradation process of the PHBV microparticles, its relationship and influence in the drug release mechanism. Studies that have been realized by the research group focus on agricultural application. Chemical degradation *in vitro* of the PHBV microparticles was realized in acid pH (pH 5.6) (characteristic of the agricultural soils). The changes of morphology, thermal properties as well as crystallinity of the PHBV microparticles before and after degradation process were evaluated.

Experimental Part

Materials

Poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV), average molecular weight, $M_w = 379.160 g.mol^{-1}$, 8.7mol% hydroxyvalerate monomer, was kindly concede from Biocycle (Brazil); poly(vinyl alcohol) (PVA), $M_w = 72.000 g.mol^{-1}$, 88.5mol% hydrolyzed, from VETEC (Brazil); chloroform analytical grade from Synth (Brazil). All the other chemicals were used as received.

Preparation of PHBV Microparticles

PHBV microparticles were obtained by the simple emulsion solvent evaporation method.[6] Typically, 40mL of a PHBV/chloroform solution (5% w/V) was prepared. The solution was then added dropwise into a 200mL of a PVA aqueous solution (2% w/V). The emulsion was formed under mechanical stirring (2000rpm) for 5minutes at room temperature. The chloroform was completely removed by evaporation under mechanical stirring (800rpm) for some hours. The PHBV microparticles were recovered by centrifugation (10000rpm, 15min, 4°C). Microparticles were three times washed with distilled water to remove the PVA residue and each wash followed by centrifugation (10000rpm, 10min, 4°C). The microparticles were dried in a Petri dish at room temperature to obtain a white powder, the PHBV microparticles.

Chemical Degradation Study

In vitro degradation experiments were realized in Curitiba city at room temperature (18 ± 2°C) without temperature control, during the months of June, July and August (winter). For the assay, 200 mg of PHBV microparticles were immersed in phosphate buffer (pH 5.6) to prevent any biological contamination. During the experiment time the buffer medium was changed at every 10 days to preserve it freshly. The samples were kept in the buffer solution during the studied time of 50 days. Then, at each 10 days, the sample was centrifuged, washed with distilled water and dried at room temperature. This study was realized in duplicate.

PHBV Microparticles Characterization

Morphological and of Surface Evaluation

Microparticles based on PHBV, before and after degradation process, had been previously dried at room temperature and were placed on a metallic stub and coated with gold (Balzers sputtering SCD-030) and then observed with a Scanning Electronic

Microscope Jeol JSM 6360 LV with accelerating voltage of 15kV. The SEM images were obtained using specific software, the SEM Control User Interface ® (version 6.01, Jeon Technics, Tokyo, Japan).

Crystallinity Measurements

PHBV microparticles were examined by a Shimadzu XRD-6000X-ray diffractometer using a Cu Kα source ($\lambda = 1.5418$Å) at room temperature. The 2θ was increased from 5° to 50° at a scan rate of $2°min^{-1}$, using a voltage of 40kV and a current of 40mA. This analysis was made to observe changes in the crystallinity of polymer.

Thermal Analysis

The thermal analysis of the samples of PHBV microparticles before and after chemical degradation was made by differential scanning calorimetry (DSC) with a Netzsch DSC 203 F1 MAIA calorimeter. The samples were heated to 200°C, cooled to -40°C and heated to 200°C again under nitrogen stream (20mL. min^{-1}). The heating and cooling rate were $10°C.min^{-1}$ during the thermal analysis. The second heating ramp was used to evaluate the results. Crystallinity percentage (X) was obtained by the means of Equation 1,[21] where ΔHm is melting enthalpy of formulations prepared and ΔHm° is melting enthalpy of a standard sample. In that case, the standard sample used was PHB polymer. Melting enthalpy for PHB polymer is $146\,J.g^{-1}$ according to literature.[22]

$$X = [\Delta Hm]/[\Delta Hm^{\circ}] \times 100 \quad (1)$$

Results and Discussion

Preparation of PHBV Microparticles

The suspensions of the PHBV microparticles were prepared by the simple emulsion method followed by evaporation of the organic solvent using PVA as stabilizing agent, as described in the literature.[6,23] The suspensions were obtained as white liquid. The shape of the microparticles produced using PVA polymer was spherical with smooth and porous surface.

Effects of the Degradation on Microparticles Shape

The morphology and surface of the microparticles changed after degradation experiments. The shape of PHBV microparticles turned in to an irregular shape when degraded for 50 days (Figure 2B).

Also, the surface of the sample degraded by 50 days had shown as lightly loss on rugosity and porosity; properties observed in the PHBV microparticles before occurring the chemical degradation process (Figure 2A). It is possible to observe in the sample after 50 days of degradation the presence of fibers when compared with original sample (Figure 2A).

Crystallinity Measurements

The diffractograms presented (Figure 3) show narrow peaks of crystallinity from

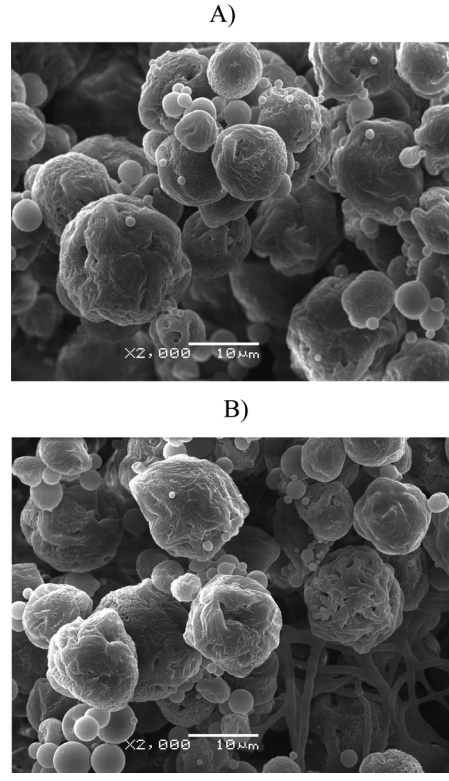

Figure 2.
SEM images of PHBV microparticles: A) before chemical degradation (original) and B) after 50 days of degradation process under pH 5.6 at room temperature ($18 \pm 2°C$).

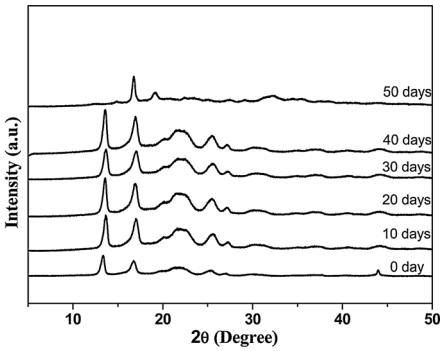

Figure 3.
Diffractograms of PHBV microparticles before and after chemical degradation under pH 5.6 at room temperature ($18 \pm 2°C$).

PHBV and some broader, which indicates that there were amorphous regions in the polymer.

When the profile of diffractogram of the PHBV microparticles before degradation was compared with others samples after acid hydrolytical mechanism (10, 20, 30, 40 and 50 days) (Figure 3) it was possible to observe a loss in the crystallinity on the region of approximately $2\theta = 44°$ in all diffractograms evaluated.

In addition, occurred the appearance of various broader peaks, which can indicates that occurred an increase of new amorphous regions in the polymer. These amorphous halos are represented by 2θ broad range. Analyzing the sample diffractogram of 50 days (Figure 3), it is evident the loss of crystallinity in all 2θ range explored. It was observed that the amorphous halo in approximately 21° disappeared after 50 days, which means that degradation process also occur in amorphous regions. Amorphous and crystalline regions of the 50 days sample were exposed to the buffer medium more significantly in the bulk PHBV microparticles; making this sample so different from the other ones. Therefore, PHBV microparticles suffered chemical degradation in pH 5.6.

The rise of new broader peaks in the range between 28 and 45 ° can be associated with a disorganization of the polymeric matrix during degradation process by acid hydrolysis.

Thermal Analysis

By differential scanning calorimetry (DSC) curves (curves not shown) it is possible to detect that the microparticles studied suffered chemical degradation, by means of acid hydrolysis; based on values obtained for the glass transition temperature (T_g) and melting temperature (T_m) when compared with the T_g and the T_m of formulation before degradation process (Table 1).

Data presented in the Table 1 are regarding at the second heating ramp and, by means of it, it is possible to see that occurred a reduction in the glass transition temperature (T_g); meaning that a low energy is necessary to a polymeric chain movement for the degraded samples in 10, 20, 30, 40 and 50 days when compared with the sample before chemical degradation.

In addition, by melting temperature (T_m) values were observed a decrease in the temperatures of same samples (10, 20, 30, 40 and 50 days), been necessary an amount of low energy to promote the melting of microparticles (ΔH_m) because

Table 1.
Thermal properties of PHBV microparticles before and after degradation.

Samples	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_m (J.g^{-1})	X (%)
0 day	1.3	56.2	165.8	37.0	25.3
10 days	−0.3	65.1	160.9	32.5	22.2
20 days	−0.4	63.4	161.1	30.5	20.9
30 days	−0.1	63.4	161.3	28.0	19.2
40 days	−0.1	65.0	161.3	29.5	20.2
50 days	−0.6	68.0	160.9	25.0	17.1

Note: Study realized in duplicate for each condition ($n = 2$). Heating and cooling rate is 10°C.min^{-1}. Standard deviation was 0.3, approximately.

crystalline region decreased. The values of glass transition temperature (T_g), melting enthalpy (ΔH_m) and crystallinity (X) indicate that chemical degradation occurs at the surface and bulk PHBV microparticles including the amorphous and crystalline regions.[20] Data obtained by differential scanning calorimetry (DSC) are according to the spotted by X-ray diffraction (XRD).

Crystallization temperature (T_c) was increased related with the sample not degraded, considering that it is necessary to liberate bigger latent heat for occuring the formation of local crystallines in the microparticles studied.[21]

Loss of Mass in the Samples

Figure 4 show the loss of mass in the PHBV microparticles samples studied in function of the degradation time (until 50 days), regarding the conditions adopted at the chemical degradation studies. It is evident that there is a chemical degradation of samples studied. The 40 and 50 days samples lost less weight than the 30 day sample (Figure 4).

This observation can be explained by the way as the polymeric chairs, as microparticles, were hydrolyzed. It is possible to suggest that polymeric chairs of the 40 and 50 days samples were reformed at alcohol and organic acid of big carbonic chairs that do not be solved in the buffer medium. So, these products of acid hydrolysis are added at the final weight.

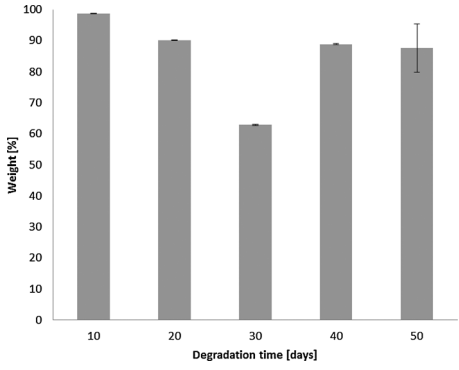

Figure 4.
Loss of mass in the PHBV microparticles samples in function of the degradation time (until 50 days).

Conclusion

Studies of chemical degradation, by means of acid hydrolysis, realized for the PHBV unloaded microparticles, were effective with the evaluation of the results obtained and reasoned by characterization techniques presented in this work. It is possible to say, based on *in vitro* assay, that the hydrolytic degradation begins on the microparticles surface and continue to the bulk PHBV microparticles. By means of this study, it will be possible to evaluate how the release of active principle occurs. Then it will be possible to adopt a kinetic model suitable for the release *in vitro* studies.

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