Available online at www.sciencedirect.com
SCIENCE DIRECT
Polymer Degradation and Stability 91 (2006) 2157–2162
Polymer Degradation and Stability
www.elsevier.com/locate/polydegstab

# Gamma irradiation effects on poly(hydroxybutyrate)

Letícia M. Oliveira [a], Elmo S. Araújo [a,*], Selma M.L. Guedes [b]

[a] *Departamento de Energia Nuclear, Universidade Federal de Pernambuco (UFPE), Av. Prof. Luiz Freire, Cidade Universitária, 1000, 50740-540, Recife-PE, Pernambuco, Brazil*
[b] *Instituto de Pesquisas Energéticas e Nucleares, CNEN, São Paulo, Brazil*

Received 24 November 2005; received in revised form 16 December 2005; accepted 17 January 2006
Available online 10 March 2006

**Abstract**

Brazilian poly(hydroxybutyrate), PHB, as well as its copolymer, poly(hydroxybutyrate-*co*-valerate), P(HB-*co*-HV), containing 6.3 mol% of valerate, were irradiated with γ radiation ($^{60}$Co) at ambient temperature and in the presence of oxygen. The viscosity-average molar mass ($M_v$) was analyzed by the viscosity technique using an Ostwald-type capillary viscometer. The polymers showed a decrease in molar mass with the increase in dose, reflecting the scissions that occurred at random in the main chain. The value $G$ (scissions/100 eV of energy transferred to the system) and the parameter $\alpha$ (scissions per original molecule) were also obtained by the viscosity technique. The melting temperature ($T_m$) was determined by differential scanning calorimetry (DSC) and showed a decrease with increasing irradiation dose. Analyses of DSC also revealed double endothermic peaks, associated with the polymorphic transitions, which became a single peak with increased dose. Thermogravimetry analysis (TGA) revealed small differences between the decomposition temperatures of the irradiated and non-irradiated samples. The degree of crystallinity of PHB samples, on the other hand, which were obtained by the DSC and X-ray diffraction techniques, increased with the irradiation dose. Changes in the lattice parameter of the irradiated samples and in the size of the crystallites were also observed by X-ray diffraction. The samples used in this work did not pass through any purification process and were analyzed in powder form, exactly as they arrived from the factory.


*Keywords:* PHB; γ Radiation; Scissions; Crystallinity

## 1. Introduction

Poly(hydroxybutyrate), PHB, is a linear and isotactic polyester of bacterial origin. PHB can be produced by a number of different bacteria, for example, *Alcaligenes eutrophus, Azotobacter vinelandii, Escherichia coli, Pseudomonas putida*, and others. It is used by bacteria as an energy storage material. Under specific conditions of nitrogen limitation and an excess of carbon food source, which can be a saccharide such as sugar, the bacteria transform the excess of their feeding into intracellular grains of polymer [1–3]. Brazilian PHB is produced by *A. eutrophus* when this bacterium uses the saccharose from sugarcane as a food source.

PHB is degradable in normal environment, either by hydrolytic or enzymatic degradation. It is a thermoplastic and has mechanical properties comparable to traditional polymers, such as polypropylene and polyethylene, and can be formed into films, fibres and sheets. These characteristics make this polymer an object of interest and vast study [2].

However, this polymer presents a high degree of crystallinity which makes it fragile and brittle and therefore unacceptable in a great number of applications. On account of this, other poly(hydroxyalkanoate)s, PHAs, family members have been copolymerized to the PHB with the aim of improving its mechanical properties. After PHB, poly(hydroxyvalerate), PHV, is the most abundantly synthesized PHA in nature, which makes the P(HB-*co*-HV) as one of the most studied copolymers. The copolyesters of PHB and PHV are produced by the same bacteria with the addition of propionic acid to their feedstock [1,2].

* Corresponding author. Tel.: +55 81 2126 7981; fax: +55 81 2126 8250.
*E-mail address:* esa@ufpe.br (E.S. Araújo).

0141-3910/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymdegradstab.2006.01.008

A number of potential applications of the polymers take advantage of their exceptionally mild foreign body response. In the medical and pharmaceutical area the PHA family members have shown to be very promising in their use as release system, drug carriers, repair patches, articular cartilage repair devices, and others [4].

The main method for sterilization of polymers for medical applications is by γ radiation. In this technique, the material is submitted to a dose in the order of 25 kGy, which guarantees great efficiency in sterilization [5]. In this way, it becomes especially necessary to study the chemical and physical effects that are induced by the transference of energy to the polymer. Often, the occurrence of these effects makes the material to become unacceptable for determined applications. A great number of polymeric materials are compatible with the use of γ irradiation as a sterilization mechanism, including polyethylene, polystyrene, and polycarbonate. Poly(tetrafluoroethylene), for example, is not compatible with this type of sterilization because it is extremely sensitive to radiation [6].

Studies of the high energy radiation degradation of non-Brazilian PHB and its copolymers have been undertaken previously, and one of the significant conclusions which can be drawn from these studies is that chain scissions predominate over crosslinking. In addition, the formation of volatile products, such as, carbon monoxide, carbon dioxide and hydrogen, upon the radiolysis of these polymers are observed [7]. So, it is necessary to understand radiation effects on the Brazilian PHB (Biocycle®) with the future objective of making it competitive in the biomaterial markets for implants. No studies about γ irradiation effects on Brazilian PHB are found in the literature.

In this work, the effects of γ radiation on the molecular and physical properties of Brazilian PHB and its copolymer were analyzed. From the determination of molar mass, by means of a viscosity technique, the parameter $G$ (scissions/100 eV) and the parameter $\alpha$, which represents the number of scissions by original molecules, were obtained. Beside the molecular effects, this study also broaches the effects of radiation on thermal properties, from differential scanning calorimetry (DSC) analysis and thermogravimetric analysis. Changes in the crystallinity parameters of the PHB homopolymer were observed by X-ray diffraction.

## 2. Experimental

PHB, year 2003, series 67, was produced and furnished by Copersucar (Cooperative of sugarcane, sugar and alcohol producers from Brazil) and has the trademark Biocycle®. This polymer had a viscosity-average molar mass ($M_v$) of 360,000 g/mol. P(HB-*co*-HV) (series 64), with 6.3 mol% of valerate had $M_v$ of 106,000 g/mol. In this work, results will be presented regarding powder form samples which were analyzed as received from industry.

The samples were irradiated at the Institute of Energy and Nuclear Researches (IPEN-CNEN/SP) in a gammacell-type $^{60}$Co source. The doses varied from 5 to 50 kGy for the viscosity studies. The samples were also irradiated at higher doses of 100 and 300 kGy for the thermal and the X-ray diffraction analyses. The dose rate was 3.6 kGy/h and all the irradiations were carried out at ambient temperature and in the presence of oxygen.

The viscosity of the samples was calculated from the relative viscosity ($\upsilon_{rel} = \upsilon/\upsilon_0 \approx t/t_0$), where $\upsilon$ and $\upsilon_0$ are the cinematic viscosities of the polymer solution and the solvent, and $t$ and $t_0$ are the necessary solution and solvent flow times for the liquid to pass the two viscometer marks, which result in the cinematic viscosity measure. These measures were carried out using an Ostwald-type capillary viscometer immersed in a thermal bath at a temperature of 30 °C.

After obtaining the relative viscosity, the specific viscosity ($\upsilon_{esp} = \upsilon_{rel} - 1$) and the reduced viscosity ($\upsilon_{red} = \upsilon_{rel}/c$), $c$ being the concentration of the solution, were calculated. The intrinsic viscosity was determined by the reduced viscosity extrapolation curve ($[\eta] = \lim_{c-0}\upsilon_{red}$) plotted by the concentration function [8]. To obtain this curve the D-optimized planning was used [9]. After the determination of the intrinsic viscosity, $M_v$ is easily obtained by means of the Mark–Houwink relation [10]:

$$[\eta] = K(M_v)^a, \tag{1}$$

where, the constants $K$ and $a$ were calculated by Akita et al. [10] for the chloroform–PHB system in a bath at 30 °C, being that $K = 1.18 \times 10^{-4}$ dL/g and $a = 0.78$. The ionizing radiation effect on the polymers is expressed in functions of the degree of degradation $G$ (scissions/100 eV) which is calculated from the following relation [11]:

$$10^6/M_v = 10^6/M_{v_0} + 0.0548GD, \tag{2}$$

where, $M_{v_0}$ and $M_v$ being the molar masses before and after irradiation and $D$ the dose in kGy. The $\alpha$ parameter ($\alpha = M_{v_0}/M_v - 1$) is also obtained from viscosity analyses and reflects the number of scissions per original molecule.

The DSC analyses were carried out in Shimadzu DSC-60 type equipment using PHB samples. A unique cycle for each dose was analyzed which varied from 20 °C to 200 °C at a rate of 10 °C/min. The doses studied were 25 and 300 kGy, and 3.6 mg of mass was utilized. The TGA analyses were carried out in a Shimadzu TGA-60 type equipment at a heating rate of 10 °C/min on a non-irradiated sample and on an irradiated sample at 300 kGy.

In the X-ray diffraction the equipment used was a Siemens D-5000 model, Cu tube, voltage 40 kV and current 40 mA. The angle was $2\theta$, scanning of 5 to 35° and continuous acquisition of 0.02° per second. The tablet samples were prepared by the back loading technique. This technique consists of pressing the powder against the opening of an aluminium blade, which is arranged over a smooth and firm surface, in a way that the sample is coplanar with the surface of the blade. The doses studied were 25, 100 and 300 kGy.

## 3. Results and discussions

Fig. 1 shows the inverse of $M_v$ as a function of the irradiation dose for the PHB and P(HB-*co*-HV) samples,

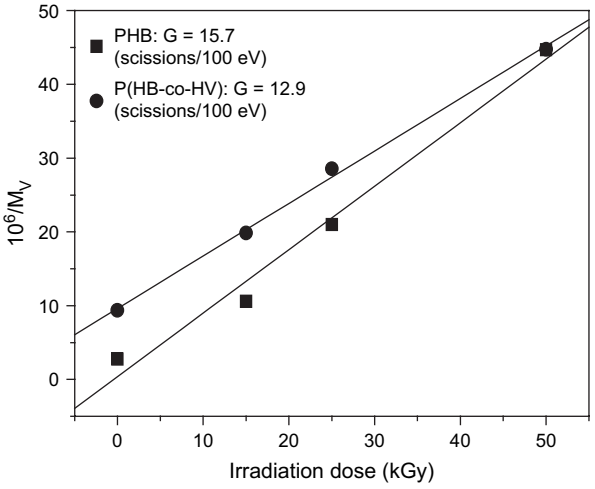


Fig. 1. Reciprocal of $M_v$ as a function of the irradiation dose to PHB and P(HB-*co*-HV) in powder form.

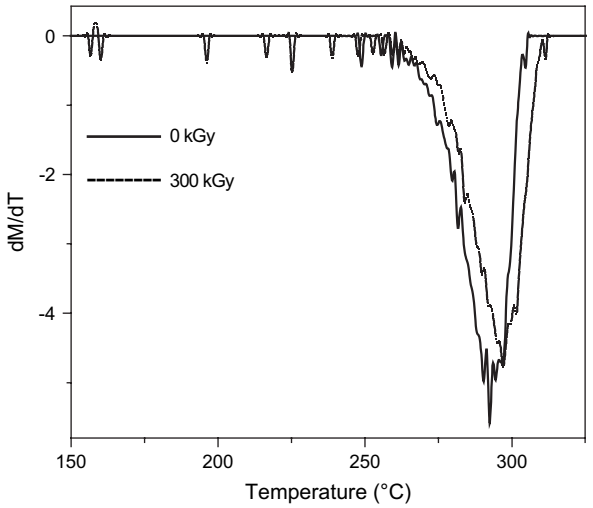


Fig. 3. TGA analysis of PHB at 0 and 300 kGy.

respectively. It is from the slope of the graph of $10^6/M_v \times D$ (kGy), respecting the dose interval in which this curve is linear, that the value of the degree of degradation was obtained. The linear interval guarantees that the scissions in the chain are random and only under this condition Eq. (2) can be used. As shown in Fig. 1, the $G$ values found for the PHB and its copolymer were 15.7 and 12.9 scissions/100 eV, respectively, at a dose interval of 0–50 kGy.

Bibers and Kalnis [12] studied the γ radiation effect on PHB samples in powder form. These authors also used viscosity for determining molar mass, obtaining a degree of degradation of 6.0 scissions/100 eV for PHB samples. Fig. 2 shows the values of the index $\alpha = 7.0$ for PHB and $\alpha = 2.5$ for P(HB-*co*-HV) in the sterilization dose of 25 kGy. Mitomo et al. [13] showed $\alpha$ index as 3 for PHB and 2.6 for P(HB-*co*-HV). These examples indicate that Brazilian PHB degradation parameters are in accordance with those of others PHBs.

From the derivation of the thermal decomposition curve (Fig. 3) the difference between the final decomposition temperatures can be seen. The temperature which registered the total mass loss of the non-irradiated PHB sample was 293 °C. On the other hand, for the thermogram (TGA) of the irradiated sample a total polymer mass loss was registered at 297 °C. It is interesting to note that the irradiated sample shows a slightly higher decomposition temperature than the non-irradiated sample, which means that the scissions that occur due to γ irradiation do not affect the thermal decomposition temperature of the PHB. From the irradiated sample curve it is seen that there is some decomposition at lower temperatures due to decomposition of chains that arise as radiolysis products.

The results in Fig. 4 show the thermograms (DSC) of the PHB after different doses. From this curve the endothermic transitions of crystalline fusion are obtained. It can still be seen that there is a small previous transitions occurrence to $T_m$ (melting temperature) which becomes a single peak with the increase in dose, and which will be called secondary

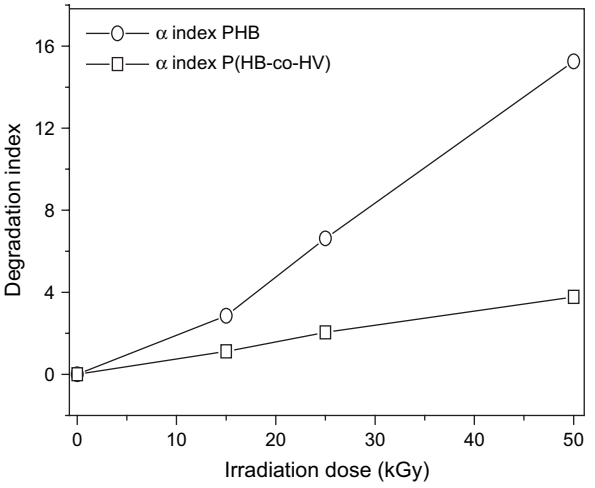


Fig. 2. $\alpha$ Index of the PHB and P(HB-*co*-HV) samples.

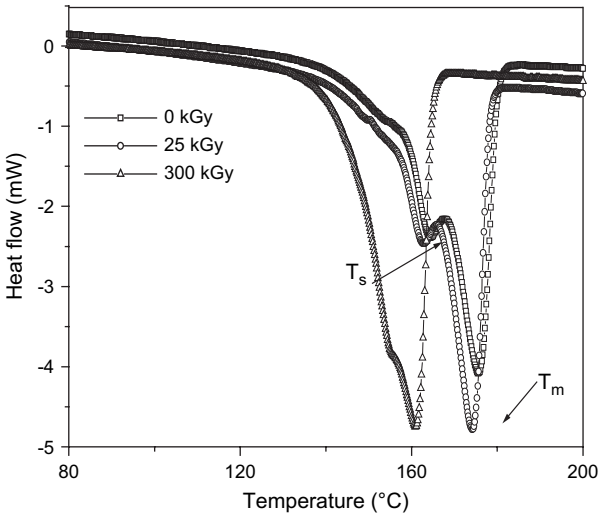


Fig. 4. DSC measures in irradiated and non-irradiated PHB samples.

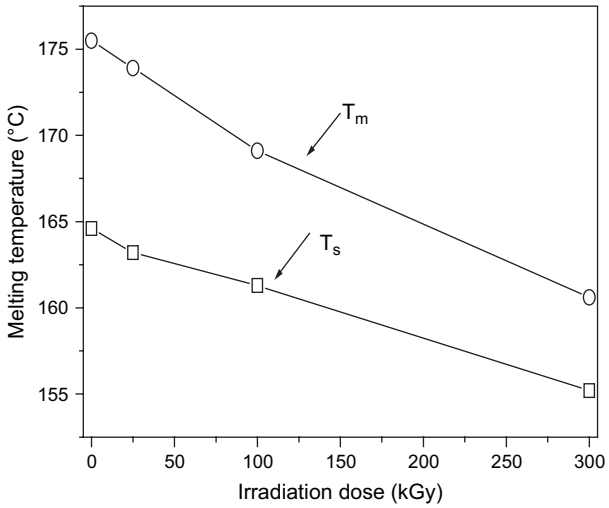


Fig. 5. Reduction of $T_m$ values as a function of the irradiation dose.

melting temperature ($T_s$). Non-oriented isotactic polypropylene iPP also shows double melting peaks which become a single peak with increasing irradiation dose [14].

Multiple melting behaviour of a polymer is usually proposed to link either to the process of partial melting and recrystallization and remelting, or to melting of crystals with different lamellar thickness and/or different crystal structures [15]. The occurrence of double melting peaks could also be related to unstable lamellar layers that are 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 single peak with the increase in dose.

The values of $T_m$ and $T_s$, presented in Fig. 5, reduce with the increase in dose, because the irregularities arising after the radiation give the macromolecules greater mobility, which induces the appearance of a disorder phase at lower temperatures.

The degree of crystallinity was calculated from Eq. (3). The area under the curve, used in the enthalpy calculation was established from an appropriate baseline, drawn in such way that only endothermic peaks were considered [16].

$$\%\chi = \left(\Delta H_{pm}/\Delta H_{pr}\right) \times 100, \tag{3}$$

where, $\Delta H_{pm}$ being the enthalpy to be known and $\Delta H_{pr}$ the enthalpy of PHB reference with 100% of crystallinity ($\Delta H_{pr}$ of PHB = 146 J/g) [13]. Table 1 shows the parameters obtained from the DSC thermograms.

Table 1
$T_m$, $T_s$ values, degree of crystallinity and fusion enthalpy of non-irradiated and irradiated PHB samples

| Dose (kGy) | $T_m$ (°C) | $T_s$ (°C) | %χ | $\Delta H_{pm}$ (J/g) |
|---|---|---|---|---|
| 0 | 176 | 165 | 50 | 73 |
| 25 | 174 | 163 | 56 | 82 |
| 300 | 161 | 155 | 55 | 80 |

The increase in the fusion enthalpy, and consequently in the polymeric crystallinity regarding the dose, also verified in other polymers, such as iPP and polyethylene (PE), is related to the phenomenon known as chemicrystallization. The term means the increase in degree of polymer crystallinity during the radiation exposure as a result of liberation of macromolecular fragments, which were unable to crystallize during the processing. As the glass transition temperature is lower than the ambient temperature, these segments present a lot of mobility and can rearrange themselves in new crystalline structures, probably on the already existing crystals [17].

In Fig. 6 (a) and (b) shown are the diffractograms of the non-irradiated and irradiated PHB samples in doses of 25, 100 and 300 kGy. Comparing these diffractograms, it is verified that there is no formation of new diffraction peaks with the irradiated dose, which indicates that the irradiation dose does not cause the appearance of new crystalline symmetries. The increase in the peak intensity shows that γ irradiation is what provokes the increase in the degree of polymer crystallinity.

The degree of crystallinity was calculated by considering the area under the diffracted peaks, not taking into account the amorphous halo (background amorphous). This method was established by Ruland [18] and allows to calculate the crystallinity of a polymer from Eq. (4):

$$\%C = [I_c/(I_c + KI_a)] \times 100, \tag{4}$$

where, $\%C$ is the crystalline fraction, $I_c$ is the result of the integration of the diffraction peaks, $I_a$, the area under amorphous halo (obtained by the Gaussian approach to all of the diffractograms) and $K$ a constant of characteristic proportionality for each polymer [18]. For PHB this constant was obtained by determining that an $I_c$ function $= f(I_a)$, and the value found was $0.96 \pm 0.03$ [19]. In Table 2 are shown the values of the degree of crystallinity for the non-irradiated and irradiated samples.

These results present an excellent agreement with those found by the DSC technique and reaffirm the increase in crystallinity with the dose of irradiation. Moreover, the increase in the degree of polymer crystallinity due to radiation can also be justified by means of a phenomenon called nucleation. In this phenomenon, the great crystallites or the great orders are destroyed at random by the radiation, followed by the appearance of small and innumerable crystallites [20]. However, if nucleation was a predominant phenomenon, new crystalline symmetries would be observed in the X-ray diffractograms, which does not occur. In this way, it is possible that the chemicrystallization phenomenon be predominate in this case.

New order crystalline will not be formed, but the already existent orders will present a higher number of layers, causing an increase in the degree of crystallinity. The values of the crystallites sizes were obtained from a Sherrer formula [21]:

$$t = 0.9\lambda/(B\cos\theta_B), \tag{5}$$

where, $\lambda$ being the wavelength of the X-ray, in this case 1.541 Å (wavelength of the Cu), $B$ the width of the peaks

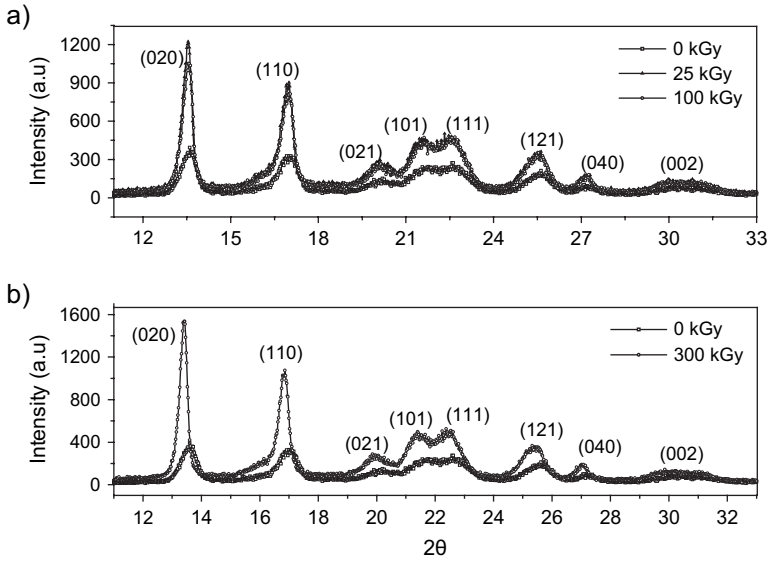


Fig. 6. (a) X-ray diffractogram of non-irradiated and irradiated (25 and 100 kGy) PHB samples. (b) X-ray diffractogram of non-irradiated and irradiated (300 kGy) PHB samples.

(in radians) where the intensity value corresponds to half of the maximum intensity and $\theta_B$ is the angle, in radians, in which the intensity is maximum. For this calculus a Gaussian diffraction peak corresponding to the plane (110) was utilized. Table 3 shows the increase in the thickness of the crystals. If these values increase it is because the fragmented macromolecules rearrange themselves on the surface of the already existent crystals. These macromolecules that present great mobility are liberated from the amorphous region due to scissions in the chain. This way it can be said that there is a predominance of the chemicrystallization phenomenon occurrence.

From the diffractogram (Fig. 6) it is possible to obtain the angle of diffraction for each one of the crystallographic planes (*hkl*). By the Bragg's law (Eq. (6)) it is possible to establish a relationship between the angle of the diffracted beam (obtained from the diffractogram) and the interplanar distance ($d$):

$$n\lambda = 2d\sin\theta, \tag{6}$$

where $n$ is the reflection order (any whole number) and $\lambda$ is the wavelength of the Cu. The magnitude of the distance between two adjacent and parallel planes is a function of both the Miller indexes and the lattice parameters ($a$,$b$,$c$). In this way, for an orthorhombic structure, which is the case of PHB, the relationship between $d$ and the lattice parameters is given by [22]:

$$d = \left(h^2/a^2 + k^2/b^2 + l^2/c^2\right)^{-1/2}. \tag{7}$$

From Eqs. (6) and (7) the PHB lattice parameters, as well as the changes that occurred in these parameters due to $\gamma$ irradiation were established. The parameters $a$, $b$ and $c$ of the unit cell were calculated from the (110), (020) and (021) crystalline planes, respectively, from where the following values were obtained: $a = 5.69$ Å, $b = 13.04$ Å and $c = 5.90$ Å for non-irradiated PHB samples. Fig. 7, however, shows a disarrangement of the diffraction angles of the irradiated samples in relation to the non-irradiated samples. Changes in the diffraction angle imply changes in the interplanar distances, and that, this time they indicate changes in parameters of the irradiated samples lattice. It is expected that parameter $a$, which is found on the carbonyl group axis, where the scissions of the occurrence, experience alterations in value with the increase in irradiation dose. Skrbic [19] and Sato et al. [23] observed changes in value of the lattice parameter $a$ as a consequence of the increase in temperature, but did not detect any changes in the $b$ and $c$ parameters, because the connections along these parameters are stronger. However, $\gamma$ radiation transfers enough energy for the

Table 2
Crystallinity fraction (*%C*) of the PHB at different doses

| Dose (kGy) | *%C* |
|---|---|
| 0 | 51 |
| 25 | 55 |
| 100 | 54 |
| 300 | 54 |

Table 3
Size of the crystallites (in Å) of the PHB at different doses calculated from the plan (110)

| Dose (kGy) | Crystallites size (Å) |
|---|---|
| 0 | 138 |
| 25 | 165 |
| 100 | 158 |
| 300 | 178 |

Table 4
Lattice parameters of the PHB unit cell at different doses

| Dose (kGy) | Lattice parameter (*a*) (110) (Å) | Lattice parameter (*b*) (020) (Å) | Lattice parameter (*c*) (021) (Å) |
|---|---|---|---|
| 0 | 5.69 | 13.04 | 5.91 |
| 25 | 5.72 | 13.10 | 5.94 |
| 100 | 5.71 | 13.08 | 5.93 |
| 300 | 5.76 | 13.15 | 5.98 |

polymeric structure and it also provokes changes in the *b* and *c* parameters.

In Table 4 the parameter values obtained for each one of the doses of irradiation can be seen. These values increase with the irradiation dose, which could be a consequence of the main chain scissions, in this way causing a deflection between molecules, i.e., an expansion of the crystalline chain.

## 4. Conclusions

Gamma radiation causes a great number of scissions in the main chain of PHB and P(HB-*co*-HV). The values *G* (scissions/100 eV) were 15.7 and 12.9 for the PHB and P(HB-*co*-HV), respectively. This way the γ irradiation effect shows a purely destructive character on these polyesters. The DSC thermal analysis shows the occurrence of double endothermic peaks which became a single peak with the increase in dose. These two peaks associated with the melting transition reflect the presence of more than one crystalline order in the polymer. The irradiated samples showed an increase in the degree of crystallinity when compared with the non-irradiated samples. This conclusion pays special attention to the DSC and X-ray diffraction techniques. The diffractograms also show that new peaks are not formed from irradiation, then there does not exist formation of new symmetric orders, because the fragments formed in the radiolysis of the molecules in the amorphous region group themselves together on already existing crystalline arrangements; a phenomenon called chemicrystallization. The calculations referring to the size of the crystallites show that they increase with the irradiation dose, which is a strong sign that chemicrystallization is predominant. By means of these analyses, the values of the chain parameter for the Brazilian PHB and the alteration in its values with the absorbed dose were established.

## Acknowledgements

The authors thank CNPq (Brazilian Research Council) for financial support.

## References

[1] Serafim SL, Lemos PC, Reis MA. Bol Soc Port de Biotecnol 2003;76:15–20.
[2] Sudesh K, Abe H, Doi Y. Prog Polym Sci 2000;25:1503–55.
[3] Ojumu TV. Afr J Biotechnol 2004;3:18–24.
[4] Langer R, Cima LG, Tamada JA. Biomaterials 1990;11:738–45.
[5] Freier T, Kunzen C, Nischan C. Biomaterials 2002;23:2649–57.
[6] Clegg D, Collyer NA, editors. Irradiation effects on polymers. New York: Elsevier Science Publisher; 1991.
[7] Carswell PT, Hill D, ÓDonnell J. Radiat Phys Chem 1995;45:737–44.
[8] Rosen S. Fundamental principles of polymer materials. New York: John Wiley and Sons; 1993.
[9] Barros NB, Pimentel MF. Quím Nova 1995;19:268–77.
[10] Akita S, Einaga Y, Miyaki T. Macromolecules 1976;9:774–8.
[11] Araújo ES, Khoury HJ, Silveira SV. Radiat Phys Chem 1998;53:79–84.
[12] Bibers K, Kalnis M. Mech Compos Mater 1999;35:169–78.
[13] Mitomo H, Watanabe Y, Ishigaki I, Saito T. Polym Degrad Stab 1994;45:11–7.
[14] Stojanovic Z, Popovic K, Galovic S, Milicevic D. Polym Degrad Stab 2005;87:279–86.
[15] Gunaratne LM, Shanks RA, Amarasinghe G. Thermochim Acta 2004;423:127–35.
[16] Kong Y, Hay JN. Eur Polym J 2003;39:1721–7.
[17] Rabello SM, White J. Polímeros: Ciênc Tecnol 1997:47–56.
[18] Ruland W. International Tables of X-ray Crystallography. Birmingham: Kinoch Press; 1974.
[19] Skrbic Z, Divjakovic V. Polymer 1996;37:505–7.
[20] Luo S, Netravali N. J Appl Polym Sci 1998;73:1059–67.
[21] Cullity BD. Elements of X-ray diffraction. New York: Addison-Wesley Publishing Company; 1967.
[22] Callister DW. Ciência e Engenharia de Materiais. Rio de Janeiro: LTC Editora; 2002.
[23] Sato H, Nakamura M, Padermshoke A. Macromolecules 2004;37:3763–9.