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Hydrolysis of Poly(Hydroxybutyrate-*co*-Hydroxyvalerate) Nanoparticles

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ABSTRACT: Poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) is a natural polyester known for its biocompatibility and biodegradability. The hydrolysis of PHBV nanoparticles (90–150 nm) and microparticles (33–58 μm) was investigated. Particles were formulated from preformed polymer(s) by miniemulsification/solvent evaporation technique to obtain nanoparticles or by emulsification/solvent evaporation technique to obtain microparticles. The morphology of the nanoparticles was studied by Field Emission Gun-Scanning Electron Microscopy (FEG-SEM). The kinetics of PHBV degradation was followed by gel permeation chromatography. After storage of PHBV nanoparticles for 25 days at 37 °C, the M_n and M_w of PHBV was reduced up to 85 and 80%, respectively. PHBV nanoparticles stored at 4 °C presented a much lower molecular weight reduction. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Several controlled drug delivery systems are available and can be readily used. However, the use of microparticles and nanoparticles, especially made from biodegradable and biocompatible polymers, appears to be the most interesting way as these systems facilitate the diffusion through biological barriers. Size and morphology of the polymer matrix play an important role in the drug release and pharmacokinetics.[1]

Furthermore, the particle's size and morphology also affect the polymer degradation rate.[2] Hydrolysis and biodegradation are the main mechanisms of polymer degradation. Biodegradation takes place through the action of enzymes and/or chemical deterioration associated with living organisms.[3] Polymer hydrolysis involves the scission of susceptible molecular groups (such as esters) by the reaction with water. The rate and efficiency of hydrolysis mainly depend on parameters as water diffusion in the particles, temperature, pH, and time.[4]

Several polyesters can be degraded by hydrolysis, as poly(L-lactide), poly(ε-caprolactone), and poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV).[5,6] PHBV is a naturally occurring biodegradable and biocompatible polyester, produced as energy storage by bacteria.[7] In addition, PHBV nanoparticles are interesting drug delivery systems.[8–10.] Several authors have studied the biodegradation and hydrolysis of PHBV films or injection-molded pieces.[7,11–15] However, limited information is available on the hydrolysis of PHBV nanoparticles.

In this work, PHBV nanoparticles were produced by the miniemulsification/solvent evaporation technique and PHBV microparticles were produced by the emulsification/solvent evaporation technique. PHBV with two different molecular weights was used to prepare the particles. The effects of PHBV molecular weight, particle size, storage temperature (4 °C and 37 °C), and pH (2.5 and 7.0) on changes of M_n, M_w and the molecular weight distribution of PHBV were studied by gel permeation chromatography (GPC) and the change of morphology by electron microscopy.

EXPERIMENTAL

Materials

PHBV (8.2 HV mol %) with two different molecular weights was used: M_w 255,660 g mol^{-1} and M_w 25,900 g mol^{-1} [referenced in the text as high-molecular weight PHBV (HPHBV) and low molecular weight PHBV (LPHBV), respectively]. HPHBV was kindly supplied by PHB Industrial S.A. Sodium borohydride ($NaBH_4$), chloroform, hexane, and methanol (Nuclear, P.A.), sodium dodecyl sulfate (SDS, Merck), poly(vinyl alcohol) (PVA, Polysciences, 88 mol % hydrolysis degree, M_w 78,000 g mol^{-1}), HPLC-grade chloroform (Merck) were used as received.

PHBV Purification and Molecular Weight Reduction

To purify PHBV, a chloroform solution (5 wt %) was prepared by heating the solution under magnetic stirring. In sequence, the solution was filtered under vacuum and precipitated in hexane. Finally, the precipitated PHBV was dried at 60 °C until no mass variation could be detected.

To prepare the LPHBV, the procedure described by Baran et al.[16] was adopted. PHBV (15 g) was dissolved in chloroform (400 mL) and $NaBH_4$ (130 mg) was dissolved in methanol (44 mL). Then, the solutions were mixed and continuously stirred for 6 h at room temperature. After that the solution was precipitated in cold methanol, filtered under vacuum, and dried at 60 °C until no mass variation could be detected.

Preparation of PHBV Nanoparticles

PHBV nanoparticles were prepared accordingly to the preparation of poly(L-lactide) nanoparticles as described by Musyanovych et al.[5] The organic phase was prepared dissolving PHBV (0.3 g) in chloroform (10 g). To prepare the aqueous phase, sodium dodecyl sulphate (72 mg) was dissolved in water (24 g). The aqueous phase was then added to the organic phase and the macroemulsion was obtained under high stirring rate (1000 rpm) for 60 min. Miniemulsification was performed in an ice bath using sonication for 180 s at 70% amplitude in a pulsed regime (30 s sonication, 10 s pause) using a Branson 450 W sonifier and a ½″ tip. The miniemulsion was then transferred to a round-bottom flask with a wide size neck and kept overnight at 40 °C to complete chloroform evaporation.

Preparation of PHBV Microparticles

Microparticles of PHBV (M-LPHBV and M-HPHBV) were prepared using the emulsification/solvent evaporation technique. The aqueous phase was prepared with PVA (0.6 g) dissolved in water (400 mL) and to the organic phase PHBV (1 g) was dissolved in chloroform (14.8 g). Both phases were mixed and the solvent was removed at ambient temperature by continuous stirring at 700 rpm during 6 h.

PHBV Particles Hydrolysis

The studies on the hydrolysis of PHBV nanoparticles and microparticles were carried out during 50 days. After particle preparation according to the procedures described above, the pH of the particle dispersions was adjusted to either pH 2.5 or 7.0 using solutions of NaOH (1 mol/L) or HCl (0.5 mol/L). In sequence, particle dispersions were stored at pH 2.5 or 7.0 and at two different temperature conditions, 4 and 37 °C. The pH of the dispersions was monitored daily during the storage with the use of a pH meter. Any variation at pH values was corrected with the solutions of NaOH or HCl.

Characterization

GPC was used to determine the molecular weight distributions and averages (M_w and M_n). The measurements were carried out in duplicate on an apparatus consisting of Spectra System P2000 pump, an autosampler Agilent 1100, and Shodex refractive index RI101 detector. Freeze-dried polymer samples were dissolved in chloroform at a concentration of 5 mg mL^{-1}, filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter, and separation was carried with three columns (0.8 × 30 cm, 10 μm) of different porosities (500 Å, 10^4 Å, 10^6 Å) in series from SDV (PSS, Germany) at room temperature and a flow rate of 1 mL min^{-1}. The molecular weights were calculated using polystyrene standards. Thermal transitions were measured by means of differential scanning calorimetry (DSC) using a Perkin Elmer (Jade DSC) calorimeter at a heating rate of 10 °C/min in a nitrogen atmosphere. Samples were first heated from −20 to 200 °C. Then, they were cooled to −20 °C and heated again to 200 °C. The melting temperature and the enthalpy associated with the melting (DH_m) were determined.

The *z*-average diameter and standard deviation (σ) of nanoparticles were determined by dynamic light scattering (Nicomp model 270, PSS Santa Barbara and Malvern—Nanosizer—Nano Series). In the case of microparticles, the average diameter and standard deviation were determined by the procedure described by Leimann et al.[17] Optical observations were carried out with the aid of an optical microscope (Bioval L-2000 A) attached to a digital camera. Images were analyzed with the aid of an image analysis software and about 300 microparticles were counted for each experiment.

The specific area (A_s) of nanoparticles (eq. 1) and microparticles was calculated from the average diameter and average number of particles (N_p) (eq. 2), where A_{PS} (nm^2) is the surface area of one particle, $m_{polymer}$ (g) and $V_{polymer}$ (nm^3) are the mass and volume of polymer used to prepare the particles, respectively, and $V_{particle}$ (nm^3) is the volume of one particle calculated with the average diameter.

$$A_s = \frac{N_P . A_{PS}}{m_{polymer}} \quad (1)$$

$$N_P = \frac{V_{polymer}}{V_{particle}} \quad (2)$$

The morphology of PHBV nanoparticles was characterized by Field Emission Gun-Scanning Electron Microscopy (FEG-SEM; JEOL JSM-6701 F). The samples were prepared by dropping the dispersion without dilution in a stub. After drying at room temperature, the samples were covered with a 60 nm gold layer. The microscope was operated at 15 kV.

RESULTS AND DISCUSSION

When polymer solutions are exposed to high-intensity ultrasonic waves, shearing induced by cavitation may lead to homolytic cleavage of the polymer molecules.[18] To evaluate if the preparation steps of PHBV nanoparticles and microparticles result in the degradation of the polymer, the molecular weight of PHBV was evaluated prior (original polymer) and after each preparation step (stirring, sonication, and solvent evaporation). Nanoparticles and microparticles diameters and surface area results are presented in Table I. The influence of the particle preparation conditions on the evolution of polymer (LPHBV and HPHBV) molecular weight is shown in Figures 1 and 2.

According to the M_n and M_w results shown in Figure 1, both, sonication and solvent evaporation led to a reduction in molecular weight. This effect can be attributed to thermal degradation due to high shear of the system during sonication and it is more pronounced for high-molecular weight PHBV. The hydrodynamic

Table I. Average Diameters, Standard Deviation, and Specific Surface Areas of PHBV Nanoparticles and Microparticles

	Experiment	D_P	σ	A_s ($cm^2\ g^{-1}$)
Nanoparticles	HPHBV	151 nm	65 nm	3.3×10^5
	LPHBV	91 nm	55 nm	5.4×10^5
Microparticles	M-LPHBV	33 μm	16 μm	1.5×10^3
	M-HPHBV	58 μm	55 μm	8.6×10^2

forces associated with the implosion and cavitation processes of the bubbles formed develop sufficient shock wave energy and transient temperature increase for degradation of the polymer molecules.[5,18] The reduction of the molecular weight of HPHBV during solvent evaporation (24 h at 40 °C) is an indication of the high degradation rate of HPHBV nanoparticles. The low-molecular weight PHBV (LPHBV) did not degrade as fast as HPHBV (Figure 1). These results agree with those of Musyanovych et al.[5] who showed that the degradation of poly(L-lactide), poly(D, L-lactide-*co*-glucolide), and poly(ε-caprolactone) with higher molecular weight is affected more by sonication when compared with the low-molecular weight polymers.

In the case of PHBV microparticles (33 and 58 μm) (Figure 2), no effect is seen in the reduction of molecular weight after the particle formation consisting of either HPHBV or LPHBV. This can be explained by the weaker shear forces applied to the system during the preparation of microparticles (700 rpm) and by the considerably smaller surface area of the microparticles compared to the nanoparticles (Table I).

Molecular Weight Evolution of PHBV Nanoparticle Aqueous Dispersions During Storage at 4 °C

The evolution of the molecular weight of PHBV nanoparticles in aqueous dispersions stored at 4 °C was measured to verify if

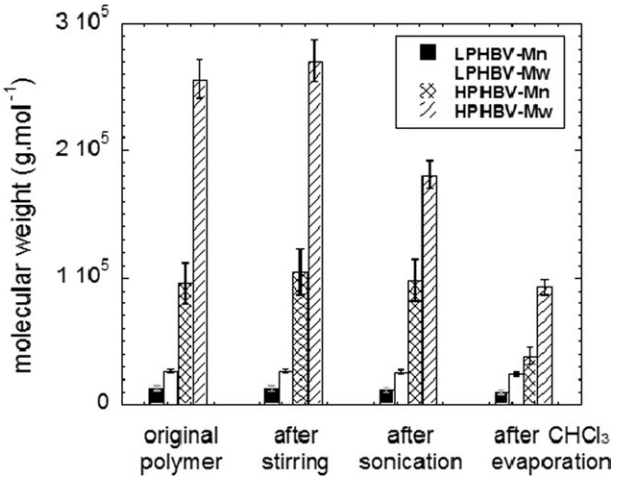

Figure 1. Number and weight average molecular weights of PHBV with two different starting molecular weights (LBHBV and HPHBV) during nanoparticle preparation steps by miniemulsification/solvent evaporation technique.

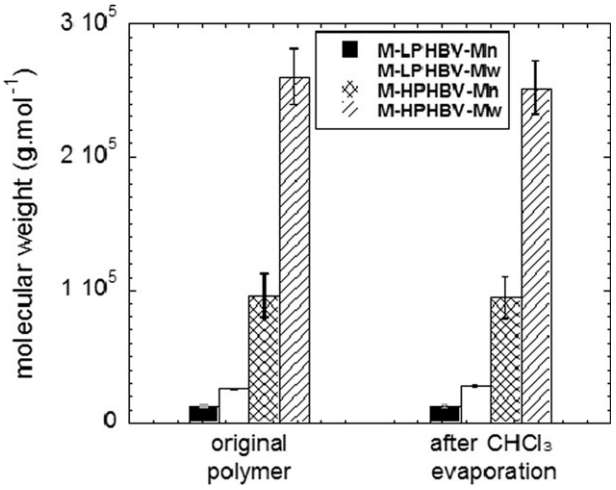

Figure 2. Number and weight average molecular weights of PHBV during microparticle preparation steps by emulsification/solvent evaporation technique.

the polymer would suffer hydrolysis while stored in a fridge. This study was carried out at two different pH values (2.5 and 7.0). Average molecular weights are presented in Table II as a function of storage time.

The decrease of the molecular weight of LPHBV after 50 days of storage at 4 °C and pH 2.5 was negligible [Figure 3(a)]. Therefore, the molecular weight of LPHBV nanoparticles stored at pH 7.0 was not measured. The reduction of the molecular weight of HPHBV nanoparticles, on the other hand, was much more pronounced, especially at pH 2.5 [Table II and Figure 3(b)]. After 6 days of storage at 4 °C and pH 2.5, the M_n decreased to 20% of the initial value, while at pH 7.0 for the same period of time the reduction of M_n up to 54% was detected. As hydrolysis is favored in the acidic medium, the molecular weight decrease is more intense at pH 2.5 than at pH 7.0. The very fast initial molecular weight reduction of HPHBV dispersion agrees with the molecular weight reduction observed during the solvent evaporation (24 h at 40 °C) in the preparation of the HPHBV nanoparticles (Figure 1). For HPHBV, M_n decreased more than M_w, indicating that the degradation leads to changes in the shape of the molecular weight distribution curve at the two pHs [Figure 3(b)]. In Figure 3(b), it may be observed that during hydrolysis the molecular weight distribution was not only dislocated toward lower values, but a second peak of polymer chains with lower molecular weight also appeared, resulting in the increase of the polydispersity index (PI) shown in Table II. This result is an indication that possibly PHBV degradation is not homogenous inside particles.

Effect of Particle Size on the Rate of PHBV Hydrolysis

The effect of the particle size on the PHBV degradation by hydrolysis of nanoparticles and microparticles was evaluated at different temperatures (4 and 37 °C) and pH values (2.5 and 7.0). These conditions were chosen to simulate the storage in a fridge and in the human body, whereas the pH values were chosen to represent different locations in the human body.

Table II. Average Molecular Weight and Polydispersity Index (PI) of PHBV Nanoparticles after Storage at 4°C and pH 2.5 or 7.0

Polymer	pH	Time (days)	M_n (g mol^{-1}) × 10^{-3}	M_w (g mol^{-1}) × 10^{-3}	PI
LPHBV[a]	-	-	9.9 ± 2.5	23.7 ± 1.6	2.4 ± 0.6
LPHBV	2.5	6	9.0 ± 1.8	23.4 ± 1.4	2.6 ± 0.8
LPHBV	2.5	25	7.6 ± 1.7	23.3 ± 1.8	3.1 ± 1.1
LPHBV	2.5	50	7.3 ± 1.3	20.5 ± 1.0	2.8 ± 0.8
HPHBV[a]	-	-	38.2 ± 8.7	92.4 ± 9.3	2.4 ± 1.1
HPHBV	7.0	6	20.7 ± 3.1	76.1 ± 6.1	3.7 ± 2.0
HPHBV	7.0	12	16.5 ± 4.1	66.5 ± 3.3	4.0 ± 0.8
HPHBV	7.0	50	17.4 ± 2.6	56.6 ± 2.8	3.3 ± 1.1
HPHBV	2.5	6	7.4 ± 1.5	71.5 ± 5.0	9.7 ± 3.3
HPHBV	2.5	12	7.0 ± 1.3	41.0 ± 3.7	5.8 ± 2.8
HPHBV	2.5	25	6.2 ± 1.4	36.6 ± 1.8	5.9 ± 1.3
HPHBV	2.5	50	4.2 ± 0.6	34.4 ± 2.4	8.1 ± 4.0

[a]PHBV molecular weight after chloroform evaporation.

The average diameters and specific surface area of PHBV nanoparticles and microparticles are presented in Table I. Both, nanoparticles and microparticles, prepared with the (LPHBV) represented smaller average diameter than the HPHBV. This is expected due to the effect of the polymer molecular weight on the viscosity of the dispersed phase. Figure 4 shows the effect of particle size and temperature on the hydrolytical degradation of PHBV nanoparticles and microparticles at pH 7.0.

PHBV nanoparticles (HPHBV) showed a strong decrease of the molecular weight at both temperatures. The molecular weight of PHBV microparticles (M-LPHBV and M-HPHBV) did not show pronounced changes after 50 days of storage at 4 or 37 °C and pH 7.0, only the value of M_w of M-HPHBV showed a small decrease after 50 days at 37 °C. The considerably faster hydrolysis of the nanoparticles is due to their much larger specific surface area (Table I) that allows a better contact between the aqueous phase and the polymer. The specific area of the M-HPHBV microparticles represents 0.26% of the specific area of HPHBV nanoparticles. In addition, the decrease of the molecular weight of HPHBV nanoparticles was higher when the temperature was set to 37 °C.

To verify morphology changes during the degradation, LPHBV nanoparticles were analyzed by FEG-SEM immediately after preparation and after 25 days of storage at pH 2.5 and 37° C (Figure 5). After 25 days of storage (37 °C and pH 2.5), individual nanoparticles kept their spherical shape and diameter,

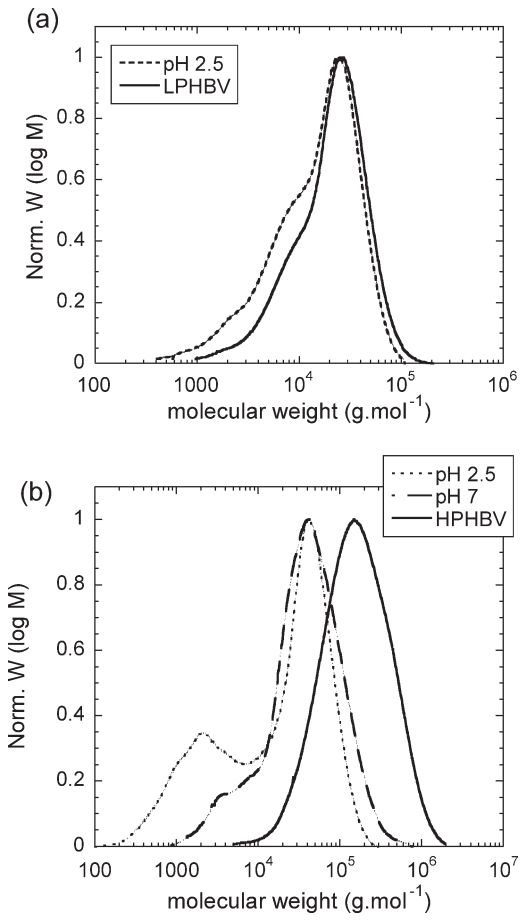

Figure 3. Molecular weight distributions of PHBV nanoparticles before and after 50 days of storage at 4 °C and pH 2.5. (a) LBHBV and (b) HPHBV.

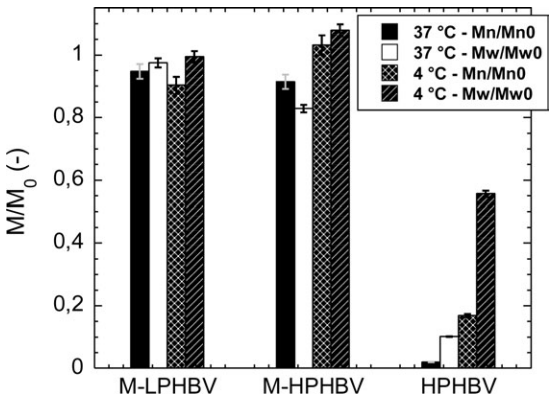

Figure 4. Temperature effect on the hydrolytical degradation of PHBV nanoparticles and microparticles at 4 and 37 °C, pH 7.0 after 50 days of storage.

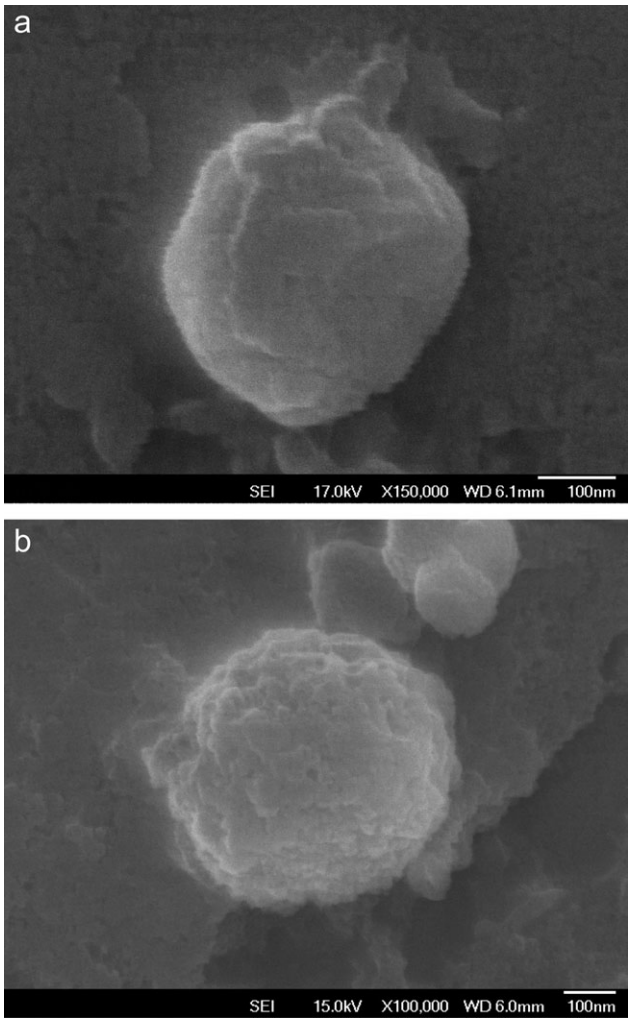

Figure 5. FEG-SEM images of LPHBV nanoparticles (a) after preparation and (b) after 25 days of storage at pH 2.5 and 37 °C.

but the surface became rough indicating material changes due to the hydrolytical degradation. It is worth noting that the particle size did not show important changes over the storage time even though the molecular weight decreased. Li and Vert and Musyanovych et al.[19,5] stated that the interior of polylactide particles degrades faster than the outer surface, as inside the particles, degradation is mainly induced by the water diffusion and production of high carboxylic group's concentration. However, PI values (Table II) increased during PHBV degradation at pH 2.5 when the autocatalytic degradation mechanism was supposed to be suppressed, indicating that another degradation mechanism must be the main one. DSC results of PHBV showed DH_m equal to 74.1 J/g and a melting temperature of 160 °C. Polymer crystallinity (X_c) can be calculated by $X_c = DH^*/DH_{0\ PHB}$ (DH^*: the DH_m of the sample; $DH_{0\ PHB}$: DH_m of a 100% crystalline PHB, which was 164 J/g[20]), resulting in a crystallinity of PHBV of 45.2%. This relatively high crystallinity of PHBV can induce different degradation rates.[14] The amorphous region presenting a much faster degradation rate when compared with the crystalline region. The different degradation rates would lead to an increase of PI and help to explain the bimodal molecular weight distributions shown in Figure 3.

CONCLUSIONS

PHBV nanoparticles and microparticles were prepared with high- and with low-molecular weight PHBV, named respectively, HPHBV with M_w 255,660 g mol^{-1} and LPHBV with M_w 25,900 g mol^{-1}. PHBV nanoparticles showed a capacity for rapid hydrolytical degradation at 37 °C with a pronounced decrease of the molecular weight of HPHBV nanoparticles at both evaluated pH values, 2.5 and 7.0, though being more intense in the former, low pH. When the nanoparticles were kept at 4 °C, instead of 37 °C, the molecular weight reduction was less intense. Furthermore, microparticles of both, LPHBV and HPHBV, that possess a much smaller surface area than the corresponding nanoparticles, did not present a pronounced change in the molecular weight, even at increased temperature. This result indicates that the fast degradation of the PHBV nanoparticles kept at 37 °C is due to their high total superficial area when compared with PHBV microparticles.

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REFERENCES

1. Costa, M. S.; Duarte, A. R. C.; Cardozo, M. M.; Duarte, C. M. M. *Int. J. Pharm.* **2007,** *328*, 72.
2. Kale, G.; Auras, R.; Singh, S. P. *J. Polym. Environ.* **2006,** *14*, 317.
3. Vroman, I.; Tighzert, L. *Materials* **2009,** *2*, 307.
4. Lucas, N.; Bienaime, C.; Belloy, C.; Queneudec, M.; Silvestre, F.; Nava-Saucedo, J.-E. *Chemosphere* **2008,** *73*, 429.
5. Musyanovych, A.; Schmitz-Wienke, J.; Mailänder, V.; Walther, P.; Landfester, K. *Macromol. Biosci.* **2008,** *8*, 127.
6. Priamo, W. L.; de Cezaro, A. M.; Benetti, S. C.; Oliveira, J. V.; Ferreira, S. R. S. *J. Supercrit. Fluids* **2011,** *56*, 137.
7. Pouton, C. W.; Akhtar, S. *Adv. Drug Delivery Rev.* **1996,** *18*, 133.
8. Pich, A.; Schiemenz, N.; Corten, C.; Adler, H.-J. P. *Polymer.* **2006,** 47, 1912.
9. Franceschi, E.; de Cesaro, A. M.; Feiten, M.; Ferreira, S. R. S.; Dariva, C.; Kunita, M. H.; Rubira, A. F.; Muniz, E. C.; Corazza, M. L.; Oliveira, J. V. *J. Supercrit. Fluids* **2008,** *47*, 259.
10. Poletto, F. S.; Fiel, L. A.; Donida, B.; Ré, M. I.; Guterres, S. S.; Pohlmann, A. R. *Colloids Surf. A: Physicochem. Eng. Aspects* **2008,** *324*, 105.
11. Mergaert, J.; Webb, A.; Anderson, C.; Wouters, A.; Swings, J. *Appl. Environ. Microb.* **1993,** *59*, 3233.

12. Jendrossek, D.; Handrick, R. *Annu. Rev. Microbiol.* **2002,** *56*, 403.

13. Scandola, M.; Focarete, M. L.; Adamus, G.; Sikorska, W.; Baranowska, I.; Swierczek, S.; Gnatowski, M.; Kowalczuk, M.; Jedlinski, Z. *Macromolecules* **1997,** *30*, 2568.

14. Li, Z.; Lin, H.; Ishii, N.; Chen, G-Q.; Inoue, Y. *Polym. Degrad. Stab.* **2007,** *92*, 1708.

15. Shah, A. A.; Hasan, F.; Hameed, A.; Ahmed, S. *Biotechnol. Adv.* **2008,** *26*, 246.

16. Baran, E. T.; Özer, N.; Hasirci, V. *J. Microencapsul.* **2002,** *19*, 363.

17. Leimann, F. V.; Gonçalves, O. H.; Machado, R. A. F.; Bolzan, A. *Mater. Sci. Eng. C* **2009,** *29*, 430.

18. Kanwal, F.; Liggat, J. J.; Pethrick, R. A. *Polym. Degrad. Stab.* **2000,** *68*, 445.

19. Li, S.; Vert, M. *Macromolecules* **1994,** *27*, 3107.

20. Barham, P. J.; Keller, A. *J. Polym. Sci. Part B: Polym. Phys.* **1986,** *24*, 69.