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Synthesis of Polyurethane from Poly(3-hydroxybutyrate) and Poly(*p*-dioxanone): Molar Mass Reduction via Sodium Borohydrate

Jonathan Melo Bergamaschi, Eduardo Jorge Pilau, Fábio Cesar Gozzo, Maria Isabel Felisberti*

Summary: Poly(3-hydroxybutyrate) PHB and poly(p-dioxanone) PPD are examples of promising polymers in tissue engineering. The purpose of this work is to synthesize polyurethanes which combine stereoregular and rigid blocks of PHB with stereoregular and flexible blocks of PPD. In order to guarantee stereoregularity, polyols from PHB and PPD were obtained from mass reduction via sodium borohydrate. This route allows us to obtain bifuncional polyols. The polymer molar mass reduction using this strategy was effective, however secondary and non reactive products with diisocyanate like insaturated acid were formed as shown by mass spectroscopy data. The products of mass reduction were characterized by H^1 NMR, FT-IR, DSC and MALDI-TOF/MS. The PU's were synthesized reacting the polyols with hexamethylene diisocyanate (HDI) and they were characterized by FT-IR, H^1 NMR, DSC and XRD.The PU obtained from PHB and PPD polyols presents two crystalline phases, corresponding to both polyester blocks. The introduction of flexible segments of PPD in polyurethane, affected the characteristics of crystalline phase formation of PHB, increasing the melting point of this block in comparison with the pure PHB polyol, probably by increasing the crystallization rate.

Keywords: poly(3-hydroxybutyrate) PHB; poly(*p*-dioxanone) PPD; polyurethane, sodium borohydrate

Introduction

In general aliphatic polyesters are biodegradable and biocompatible. The properties of each polymer, such as degradation speed, mechanical and thermal properties vary with molecular mass[1] and the sequence and structure of their monomers.[2–3] Some strategies such as copolymerization are effectively used to improve or achieve the desired properties.[4–6] Another strategy often used to this purpose, especially in tissue engineering,[7] is the synthesis of functionalized prepolymers for polyurethanes synthesis.[8–9] This last strategy is interesting because it provides the materials with hard and soft segments, which confer specific characteristics useful in sutures, scaffolds for cell growth etc.

PHB, poly (3-hydroxybutyrate), is a semi-crystalline thermoplastic straight chain polyester belonging to the family of polyhydroxyalkanoates. This polymer is a natural material, synthesized from microorganisms subjected to unbalanced nutrient conditions.[10] The homopolymers and its copolymer's biosynthesis, the chemical modifications of these polymers aiming precursors for synthesis of polyurethanes, and the combination with other polymers resulting in blends have been extensively studied in recent decades.[11–12]

Poly (p-dioxanone) (PPD) is a very promising polymer in tissue engineering.

Instituto de Química, Universidade Estadual de Campinas, P.O. Box 6154, 13083-970 Campinas – SP, Brazil
E-mail: misabel@iqm.unicamp.br

However, its applications in the polyurethanes synthesis have been little studied. PPD is a semicrystalline polymer produced by the polymerization of p-dioxanone in the presence of an organometallic of p-dioxanone and an organometallic catalyst.[13–14] PPD degrades by hydrolytic processes at high rates, generally resulting in low molar mass molecules that can be metabolized or bioreabsorbed by the body.[15–16] The presence of an ether bond and an additional $-CH_2-$ in its structure results in a material with high flexibility and good mechanical properties, so that it is widely used in the medical field as a biodegradable suture.[17] Currently, PPD is marketed by Johnson & Johnson™ as a bioreabsorption suture with the trade name of PDS®.[18]

The synthesis of polyurethanes based on PHB and PPD depends on the success of obtaining pre-polymers. There are two main routes for obtaining polyols: 1 - synthesis from monomers, 2 – molecular mass reduction and functionalization of the polymer. The second alternative is particularly useful in obtaining a pre-polymer with high stereoregularity, which is common for polymers such as biosynthesized PHB.

Montoro[19] compared three routes for molar mass reduction of copolymer poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBHV): acid hydrolysis, transesterification with ethylene glycol and reduction via sodium borohydride. After the characterization of low molecular mass polymers, the author concluded that the reduction via sodium borohydride was more efficient. This work supports the reduction of molecular mass and functionalization of PHB and PPD using sodium borohydride, $NaBH_4$. The resulting polyols are used to obtain polyurethanes based on hexamethylene diisocyanate (HDI).

Blends of PHB and PPD[20] were previously studied in our group. These blends are immiscible presenting two amorphous phases and two crystalline phases. Toxicity tests showed that PHB/PPD blends do not present direct or indirect cytotoxicity as a substrate for cellular growth. Therefore, this combination has a potential for biomedical applications.

Experimental Part

Reduction of the Molar Mass of Commercial Polymers Via Sodium Borohydride

PHB (Mn ~ 130,000 g mol^{-1}, melting point of 175 °C) and PPD (Mn ~ 14,000 g mol^{-1}, melting point of 105 °C) were purified by dissolving in chloroform followed by precipitation in a non-solvent, cold ethanol. The proposed methodology to reduce polymers molar mass was based on Baran et al.[21] work. The commercial polymers were solubilized in dry chloroform PA in the proportion of 5% (m/v). $NaBH_4$ was dissolved in methanol at 2% (m/m) and added to the polymer solution. The reaction was carried out under reflux for 40 min in the case of PHB and 20 min for PPD at room temperature. After the reaction, the polyols were precipitated in cold ethanol, filtered, washed with dilute hydrochloric acid and drying with renewal air at 40 °C for 12 h. Finally, the polymers were maintained under vacuum at 40 °C for 4 h.

Polyurethanes Synthesis (PU)

Equimolar ratio of polyols and hexamethylene diisocyanate were used for the polyurethanes synthesis and dibutyl tin dilaurate was used as catalyst (0.05 wt% in relation to polyol). The reaction was carried out in chloroform under reflux and under nitrogen atmosphere. HDI was added slowly to the reaction medium at 0.1 mL.h^{-1} rate, for 3.5 hours. At the end of the reaction n-di-butylamine was added to remove any excess of diisocyanate. The resulting polyurethane was precipitated in petroleum ether, filtered and vacuum oven-dried for air renewal at 40 °C for 12 h. Finally, the PU was dried in the vacuum oven (40 °C) for 4 h. Polyurethanes were synthesized from PHB polyol, called PUHB, and from equimolar mixture of PHB polyol and PPD polyol, called PUHBPD.

Characterization

PHB Polyol e PPD Polyol

The molar mass reduction was monitored by ^{1}H RMN. Polyols were also characterized by MALDI Q-TOF mass spectrometry, infrared spectroscopy, X-ray diffraction and Differential Scanning Calorimetry.

Proton Nuclear Magnetic Resonance (^{1}H NMR)

Proton NMR experiments were carried out with an Inova 500 MHz at 25 °C. The samples were dissolved in deuterated chloroform $CDCl_3$ at 10-20 mg.mL^{-1} concentration. The internal standard was tetramethylsilane TMS. The 45° pulse width, determined from the measurement of the 360° pulse, was 10 ms. The relaxation delay d_1 was set at 3s, from the measurement of the relaxation time T_1, and the number of transients was 64.

Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry (MALDI Q-TOF- MS)

Mass spectrometric measurements were performed using a Waters Synapt HDMS (Waters Corp., Manchester, UK) quadrupole time-of-flight instrument equipped with a 200 Hz solid state Nd:YAG laser. Laser energy of 200–250 a.u. was used. The instrument was operated on V positive mode. The α-ciano-4-hydroxycinamic (α-CHCA) matrix was dissolved in 50% acetonitrile with 0.1% trifluoracetic acid in concentrations equal to 10 mg mL-1, and the solution was mixed with the polymer dissolved in $CHCl_3$. The mixture was dried on the stainless steel target plate.

Fourier Transform Infra Red Spectroscopy (FTIR)

Polyols and PUs were characterized by infra red spectral analysis. An FTIR transmittance spectra of the ground sample was obtained in the 3500–400 cm^{-1} range using an IR spectrometer Bohme MB100. The spectra were taken from thin KBr pellets prepared by compressing an intimate mixture of 1:100 (m/m) material:KBr with 16 accumulation and 1 cm^{-1} resolution. The results of infra-red spectroscopy are favorable of the physical structure.

X-Ray Diffraction (XRD)

Polyols and polyurethanes in a powder form were analyzed on a Shimadzu diffractometer XRD-6000 in the range of $5° < 2\theta < 50°$ at room temperature. Radiation used was obtained from a target of Cu (Kα), with a wavelength of 1.54 Å.

Differential Scanning Calorimetry (DSC)

DSC measurements were performed on a TA Instruments DSC2910 apparatus. Samples of 4-6 mg, sealed in aluminum pans, were first heated to 200 °C, maintained at this temperature for 5 min, thus eliminating the thermal history, cooled to −80 °C, maintained at this temperature for 5 min and finally heated to 200 °C. Cooling and heating scans were performed at the rate of 20 °C/min in an argon atmosphere. DSC curves were normalized with respect to the sample mass.

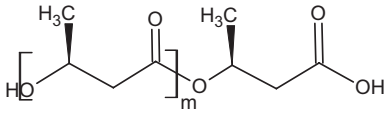
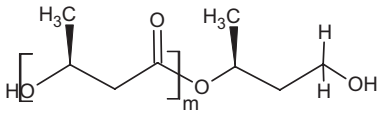
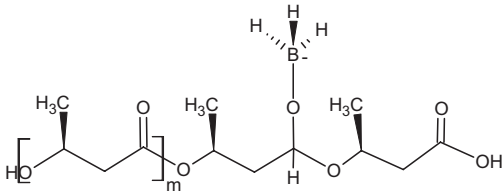
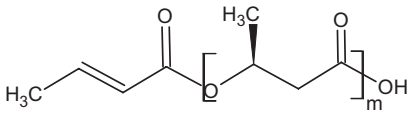
Results and Discussion

Molar Mass Reduction of Starting Polymers

Averages molar masses of polyols were determined by H^1 RMN spectrum by integration of hydroxyl and methyl signs at 2.18 and 1.28–1.27 ppm for PHB polyol, and signals for OH and CH_2 in 3.12 and 4.36, 4.18, 3.81 ppm for the PPD-polyol, respectively. Considering bifunctional molecules, the molecular mass of PHB and PPD are reduced to approximately 550 g mol^{-1} and 500 g mol^{-1}, respectively.

Molar mass reduction by sodium borohydrate for the starting polymer was efficient. Table 1 shows the final polymeric products as verified by mass spectrometry analysis. Main peaks referring to the family of the reduced PHB are labeled ''R'' and differ by 86 Da from each other. The mass corresponding to the mass of the repeating unit of the PHB, $-CH(CH_3)CH_2COO$ (Figure 1) family, labeled ''P'', are con-

Table 1.
Structural formula of species, reduced PHB products and their acronyms.

Product	Acronym ($m = 1,2,3...$)	m/z value examples
	R*m*	R5 = 534 R6 = 620 R7 = 706 R8 = 792 R9 = 878
	P*m*	P5 = 548 P6 = 634 P7 = 720 P8 = 806 P9 = 892
	F*m*	F4 = 546.8 F5 = 632.7 F6 = 718.8 F7 = 804.9 F8 = 890.8
	A*m*	A5 = 603 A6 = 689 A7 = 775 A8 = 861 A9 = 947

stituted by species differing 14 Da from ''R'' peaks, which correspond to terminal hydroxy (OH). Fragments of borohydride substituted at carbonyl groups (F) are presents a mass 12.8 Da higher thean ''R'' peaks. This specie results from the fact that the borohydrate was not completely removed by washing with the dilute HCl solution. An increase in the HCl concentration could result in PHB hydrolysis, generating undesirable products.

The peaks labeled ''A'' are distant by 69 Da from main peaks (R) and correspond to PHB with an unsaturated termination. Identification of these terminations suggests that the reduction reaction using sodium borohydride produces non-reactive diisocyanates that limits the polyurethanes molecular mass growth. Saeed et al.[22] observed the same type of unsaturated termination by submitting PHB to basic transesterification with vegetable oils. Figure 2 shows two proposed mechanisms for PHB reduction via sodium borohydrate. The driving force for the formation of an unsaturate olefinic end group is believed to be due to the intermediate alkoxide anion, which subsequently leads to the formation of an enolate ion generating a stable α-β-unsaturated olefinic end group. A mechanism involving decarboxylation is also probable, however this is not supported by our data.

As expected, the MALDI-Q-TOF mass spectrum for PPD polyol is quite similar, since the two polyesters possess the same chemical reduction mechanism. The visible difference between the two MALDI mass spectra is related to their large mass ranges and oligomers with differences of 102 Da. There are also peaks related to chain terminations whose molecular mass differs from 14 Da to hydroxyl terminals, as shown in the second reaction (Figure 1), 12.8 Da relative to species where boron hydride was bound to carbonyl group and 69 Da, relative to unsaturation acids ending. For reasons of brevity, we do not report the MALDI mass spectrum of PPD polyol.

Figure 3 shows DSC curves corresponding to the second heating scan for PHB and

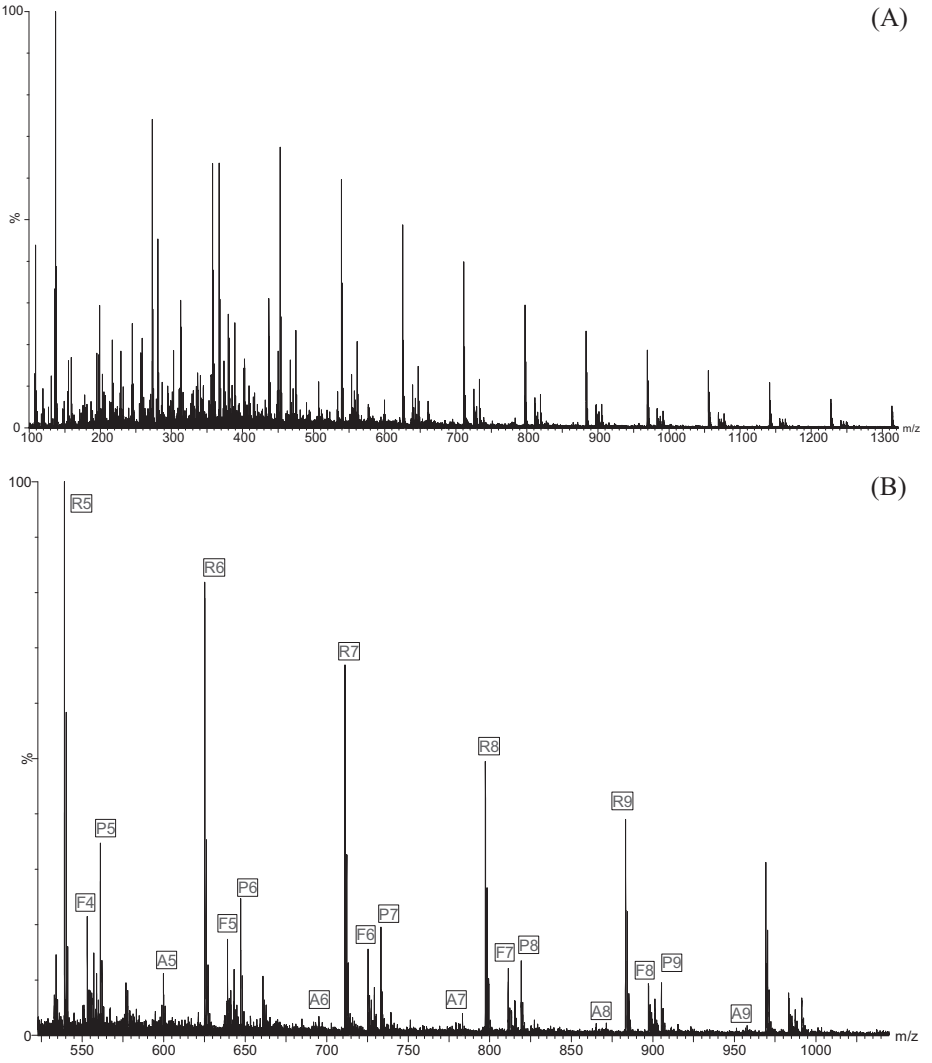


Figure 1.
MALDI-TOF mass spectrum for PHB reduced (A) and expansion between 500–1050 m/z (B).

PPD and for its respective polyols. Melting (Tm), crystallization (Tc) and glass transition (Tg) temperatures, and fusion enthalpy are summarized at Table 2. DSC curve for PHB and PHB polyol, Figure 3(A), shows a glass transition at around 4 °C, an exothermic peak due to crystallization with maximum at 68 °C and an endothermic peak due to fusion with minimum at 171 °C. After molar mass reduction, a significant decrease in glass transition, crystallization and melting temperatures were observed. A similar behavior was also observed for PPD polyol, Figure 3(B), being an expected consequence of molar mass reduction. While molar mass reduction of PHB results in decrease of the melting enthalpy, as a consequence of the degree of crystallinity, no significant differences in the degree of crystallinity for PPD and PPD polyol were observed.

Polyurethanes Synthesis

Figure 4(A) shows infrared spectra for PUHB, PUHBPD and the polyols. The spectrums for the PUs and PHB polyol

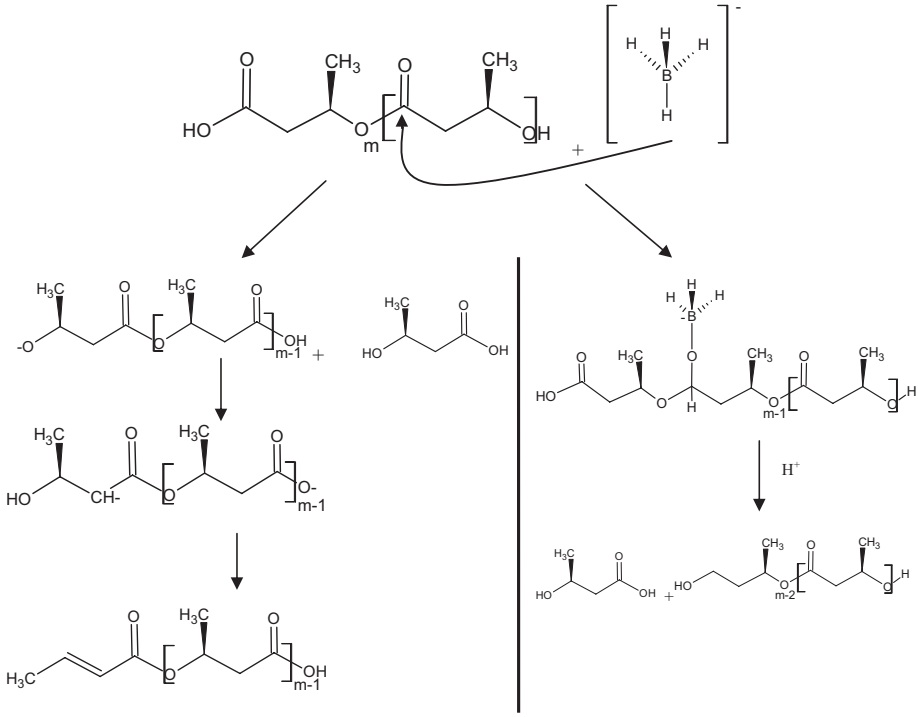


Figure 2.
Proposed mechanisms for PHB reduction via sodium borohydrate.

show bands at 2978-2851, 1720 and 1240-1370 cm^{-1} relative to CH_2 stretching, the aliphatic carbonyl and aliphatic CH deformation bands, respectively. The PPD polyol spectrum shows peaks at wavelengths of 3008-2829 cm^{-1} for CH_2 stretching bands, an intense aliphatic carbonyl C=O band at 1731 cm^{-1} and an aliphatic CH deformation band at 1194–1296 cm^{-1}. Polyurethane spectra do not show free NCO group absorption (2258 cm^{-1}), because they were completely consumed during the reaction. Figure 4(B) corresponds to the spectrum expansion in the wavelength range from 2000 to 1500 cm^{-1}. The PUHBPD spectrum shows a carbonyl

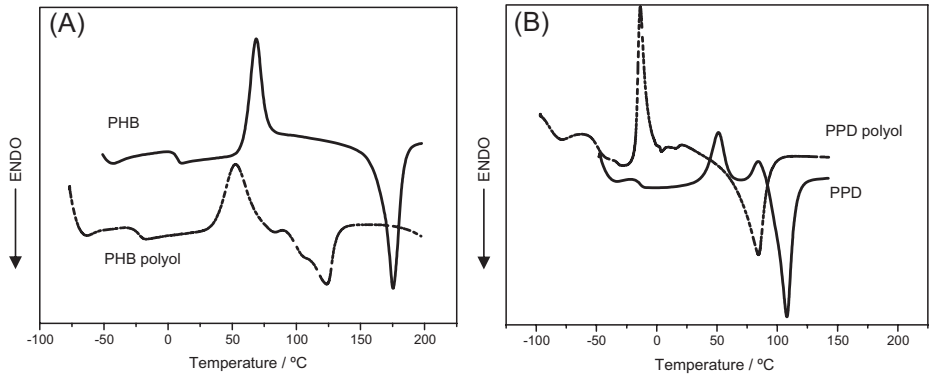


Figure 3.
DSC curves corresponding the second heating for (A) PHB and PHB polyol; and (B) PPD and PPD polyol.

Table 2.
Melting, crystallization, glass transition temperatures and fusion enthalpy for the starting polymers and their respective polyols.

Polymers	Tm/°C	Tc/°C	Tg/°C	ΔHm/J.g^{-1}
PHB	175	68	4	86
PHB polyol	122	49	−24	46
PPD	110	45	−7	98
PPD polyol	84	−14	−44	100

Data obtained from DSC curves from Figure 3.

band corresponding to PHB repeating unit (1720 cm^{-1}), one shoulder corresponding to carbonyl stretch of PPD repeating unit (1731 cm^{-1}) and another shoulder corresponding to carbamate carbonyl (1748 cm^{-1}), Figure 5(B).

H^1 NMR spectra for PUHBPD and PUHB as well as the assignments to the distinct signals are shown in Figures 5 and 6, respectively.

The carbamate structures of polyurethanes can be easily identified in the H^1 NMR spectra. For example, signals around 3.2 ppm and 1.35 ppm (h and I, respectively) are characteristic of the CH_2 sequence of the precursor HDI.

Figure 7 shows the DSC curves for the polyols and polyurethanes. The second heating curve for polyurethane obtained from PHB, the PUHB, presents glass transition and melting at the same temperature range when compared with its prepolymer, PHB-polyol. The cold crystallization for both PHB polyol and PUHB starts at the same temperature, around 30 °C, however the crystallization peak for PHB polyol is broader and more intense. Moreover, there is an apparent decrease in the melting and crystallization enthalpy for PUHB, indicating a lower crystallinity degree, maybe as consequence of the increase of the molar mass and the randomic distribution of the PPD and PHB blocks and of course due to the restrictions to crystallization imposed by the chemical linkages.

The decrease in the crystallinity degree is also due to the introduction of HDI derived segments, which not only represent a defect in its chain structure, but can also influence the dynamics in conformational changes and, therefore, influence the crystallization rate. Despite of polyurethane PUHBPD consists of PHB and PPD polyols, presents a single glass transition at temperatures close to Tg of PHB polyol. This polyurethane still presents an exothermic peak corresponding to cold crystallization of PHB phase around 30 °C, followed by a less intense endothermic peak at the same temperature range of the melting of the PPD polyol and a more intense endothermic peak at temperatures higher than the melting point of the PHB polyol. The crystallinity degree of PHB in the polyurethane is lower in comparison to the value found for the PHB polyol.

The DSC curve for the PUHBPD corresponding to the second heating peak

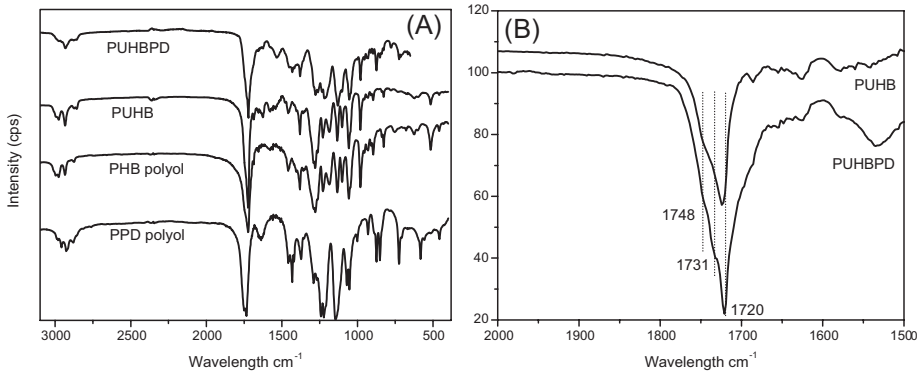


Figure 4.
(A) Infrared spectra for polyurethanes PUHBPD and PUHB, (B) carbonil region of the spectra.

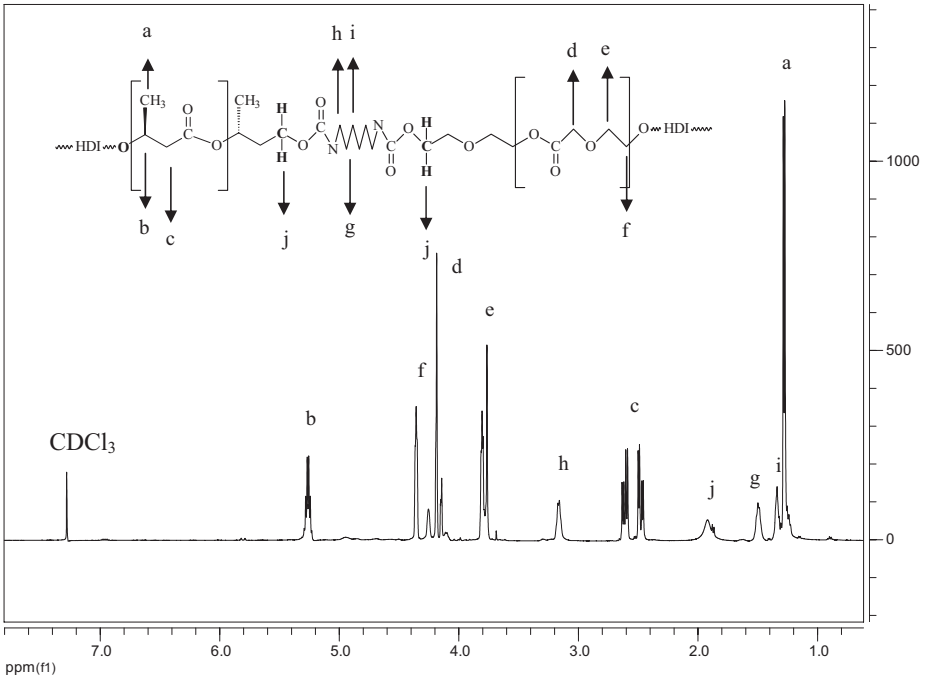


Figure 5.
H^1 NMR spectra for PUHBPD.

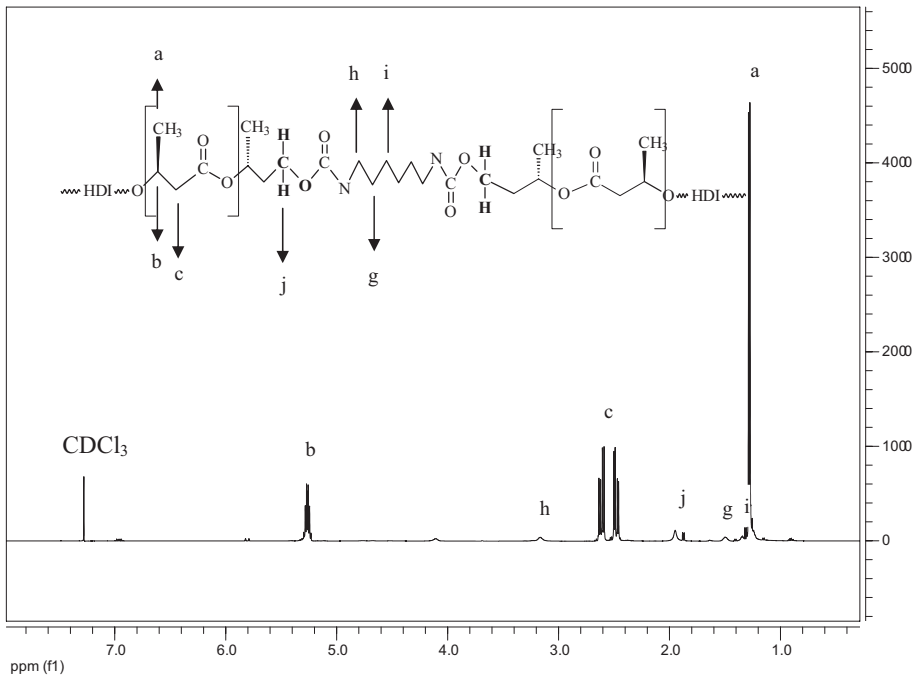


Figure 6.
H^1 de RMN spectrum for PUHB.

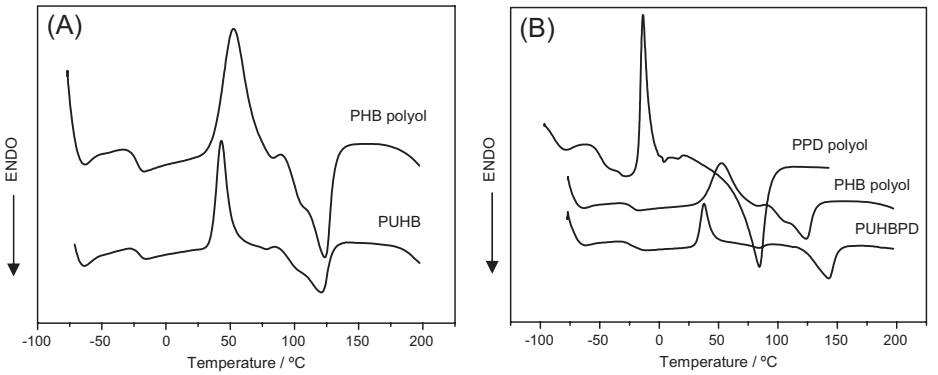


Figure 7.
DSC curves for the second heating for (A) PHB polyol and PUHB; and (B) PPD polyol, PHB polyol and PUHBPD.

does not show cold crystallization for the PPD block. This means that this block crystallizes during the cooling and its crystallization degree is very low. Thus, both blocks, PHB and PPD, have an influence on the crystallization of each other.

Figure 8 shows the X-ray diffraction patterns of the polyols and polyurethanes. The crystal structure of PUHB exhibits no change in the X-ray diffraction profile of PUHB polyol. The crystalline structure consists of an orthorhombic system $P2_1\ 2_1\ 2_1\ \text{-}D^4{}_2$ with $a = 5.76$ Å, $b = 13.20$ Å, $c = 5.96$ Å.[23–24] The PUHBPD diffractogram shows the diffraction patterns characteristic of PHB and PPD polyols unit cell. Therefore, within the PUHBPD polyurethane coexists two crystalline phases and one amorphous miscible phase. The DSC curve for PUHBPD (Figure 7b) shows a crystallization peak with a maximum at 38 °C, a melting peak of low intensity at 84 °C, attributed to crystals formed by PPD blocks melting, and another melting peak with minimum at 148 °C. The X-ray diffraction results suggest that this peak is due

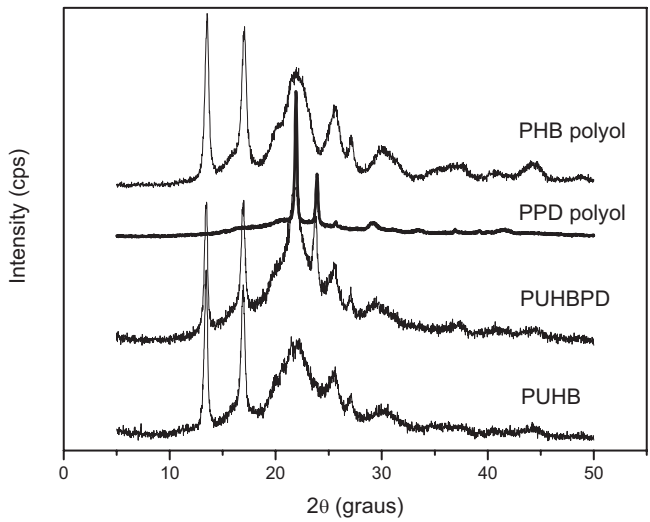


Figure 8.
X ray diffraction patterns: PHB polyol, PPD polyol, PUHBPD and PUHB.

to crystals formed by PHB blocks melting. The shift of the melting point of the PHB block in the polyurethanes to higher temperatures should reflect the morphology of the crystalline phase: The lamella of the PHB crystalline phase in the polyurethane is thicker. This can be due to the introduction of flexible segments of PPD that could change the crystallization kinetics.

Conclusion

The molar mass reduction of polyesters by sodium borohydride inorganic reducer was efficient. This reaction provided polymer functionalization with hydroxyl terminals group without needing pre-polymers synthesis from monomers, preserving the stereoregularity. On the other hand, secondary non reactive terminations products with diisocyanate, such as insaturate acids, which limit the polyurethanes molecular mass growth were also formed. Despite this, polyurethanes constituted by blocks of PHB and PPD have three phases: amorphous miscible, PPD and PHB crystalline. Flexible segments introduced by polyurethane PPD changes the characteristics of PHB crystalline phase, increasing the thickness of the lamellas. However, rigid PHB segments in the PU cause a decrease in the crystallinity of the PPD phase because of the crystallization rate increment.

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[1] K.-K. Yang, X.-L. Wang, H.-X. Huang, *Mat. Chem. Phy.* **2004**, *87*, 218.
[2] P. Degée, P. Dubois, R. Jérôme, *Macromol Chem Phys* **1997**, *198*, 1985.
[3] A. Loefgren, A. C. Albertsson, P. Dubois, R. Jerome, P. Teyssie, *Macromol.* **1994**, *27*, 5556.
[4] H. Shinoda, Y. Asou, A. Suetsugu, K. Tanaka, *Macromol Biosci.* **2003**, 34.
[5] H. L. Chen, J. Z. Bei, S. G. Wang, *Polym Adv Technol.* **2000**, *11*, 180.
[6] N. Bhattarai, H. Y. Kim, D. R. Lee, S. J. Park, *Polym Int* **2003**, *52*, 6.
[7] R. Chandra, R. Rustgi, *Prog Polym Sci*, **1998**, *23*, 1273.
[8] L. Korley, B. Pate, E. Thomas, P. Hammond, *Polymer* **2006**, *47*, 3073.
[9] R. Waletzko, L. Korley, B. Pate, E. Thomas, P. Hammond, *Macrom*, **2009**, *42*, 2041.
[10] C. Galego, R. Rozsa, J. Sanchéz, *Pol. Testing* **2000**, *19*, 485.
[11] F. Coutinho, M. Delpech, *Polímeros: Ciência e Tecnologia*, **1999**, *9*, 1.
[12] L. D. Artavia, C. W. Macosko, *Polyurethane Flexible Foam Formation*, N. C. Hilyard, A. Cunningham, Eds., Chapman and Hall, New York, 1994, 22–55.
[13] H. Nishida, M. Yamashita, T. Endo, Y. Tokiwa, *Macromol*, **2000**, *33*, 6982.
[14] A. P. T. Pezzin, G. O. R. van Ekenstein, E. A. R. Duek, *Polymer*, **2001**, *42*, 8303.
[15] M. A. Sabino, J. Albuerne, A. J. Muller, J. Brisson, R. E. Prud'homme, *Biomacromol*, **2004**, *5*, 358.
[16] M. A. Sabino, G. Ronca, A. J. Muller, *J. Math. Sci.*, **2000**, *35*, 5071.
[17] M. A. Sabino, J. L. Feijoo, A. J. Muller, *Poly. Degrad. Stab.*, **2000**, *73*, 541.
[18] S. Japon, Y. Leterrier, J. A. Manson, *Pol. Eng. and Sc.*, **2000**, *40*, 1942.
[19] S. Montoro, Master Dissertation, Universidade de São Paulo-Lorena, **2005**.
[20] M. Dias, M. C. Antunes, A. R. Santos, M. I. Felisberti, *J. of Mat. Sci. Mat in Medicine* **2008**, *12*, 3535.
[21] E. Baran, N. Ozer, V. Hasirci, *J. Microencapsul.* **2002**, *19*, 363.
[22] K. A. Saeed, F. O. Ayorinde, B. E. Eribo, M. Gordon, L. Collier, *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1951.
[23] J. Cornibert, R. H. Marchessault, *J. Mol. Biol.* **1972**, *71*, 735.
[24] M. Yokouchi, Y. Chatani, Y. H. Tadokoro, K. Teranishi, H. Tani, *Polymer*, **1973**, *14*, 267.

