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Phase behavior of biodegradable amphiphilic poly(L,L-lactide)-*b*-poly(ethylene glycol)-*b*-poly(L,L-lactide)

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Abstract

This study describes the miscibility phase behavior in two series of biodegradable triblock copolymers, poly(L-lactide)-*block*-poly(ethylene glycol)-*block*-poly(L-lactide) (PLLA–PEG–PLLA), prepared from two di-hydroxy-terminated PEG prepolymers (M_n = 4000 or 600 g mol^{-1}) with different lengths of poly(L-lactide) segments (polymerization degree, DP = 1.2–145.6). The prepared block copolymers presented wide range of molecular weights (800–25,000 g mol^{-1}) and compositions (16–80 wt.% of PEG). The copolymer multiphases coexistance and interaction were evaluated by DSC and TGA. The copolymers presented a dual stage thermal degradation and decreased thermal stability compared to PEG homopolymers. In addition, DSC analyses allowed the observation of multiphase separation; the melting temperature, T_m, of PLLA and PEG phases depended on the relative segment lengths and the only observed glass transition temperature (T_g) in copolymers indicated miscibility in the amorphous phase.
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Keywords: Thermal behavior; Block copolymers; PLLA–PEG–PLLA; Biodegradable

1. Introduction

The biomaterials based on poly(L-lactide) (PLLA) has gained clinical attention in recent years due to its acceptance by living organism and have been used to construct three-dimensional scaffold and as suture and drug delivery matrix materials [1–3]. During metabolization in vivo, PLLA is degraded by hydrolysis into lactic acid [4,5], which under aerobic conditions is further metabolized into water and carbon dioxide and finally excreted by the organism [6]. However, the degradation in vivo of this hydrophobic homopolymer occurs predominantly by cellular process, as a result of local inflammatory reaction [7].

Additionally, PLLA is stiff and brittle, by blending with low molecular weight poly(ethylene oxide) (PEO) or poly(ethylene glycol) (PEG), it is possible to improve the elongation at break and tenacity, however, blends of PLLA and PEO lose properties with time due to crystallization and phase separation during aging processes [8–10].

Even though, extensive biocompatibility is one of the most noted advantages of PEO, thus, typically, up to molecular weight of 4000 g mol^{-1} it is 98% excreted by human. The emerging use of PEO in controlled release systems arises from its resistance to protein deposition. The hydrophilic nature of PEO is such that water bonds tightly onto polymer surface by hydrogen linkages, thus, excluding or inhibiting protein adsorption. Many research groups are investigating attachment of PEO chains to therapeutic proteins; PEO chains at the protein surface allow longer circulation time in the organism by avoiding biological events such as endocytosis, phagocytosis, liver uptake and clearance, and other adsorptive processes [11].

Copolymers of L-lactide with hydrophilic monomer such as ethylene glycol have found many medical and pharmaceutical applications due to its amphiphilic nature and biodegradability. The copolymers of poly(ethylene glycol) can act as long-term drug carriers in blood circulation, because it does not stimulate inflammation reaction nor phagocytic uptake by the immune system.

PEG is usually terminated with hydroxyl groups, which provide a ready source for synthetic modification. Diblock PLLA/PEG and triblock PLLA/PEG/PLLA systems have been

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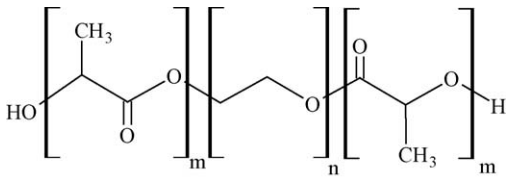

Fig. 1. Chemical structure of the triblock copolymers.

synthesized and characterized with various PLLA contents [12–15]. The free hydroxyl groups of PEG can be readily activated by stannous di-2-ethylhexanoate to function as ring-opening co-initiator for lactide polymerization, forming diblock or triblock copolymers.

The composition, morphology and crystallinity of block copolymers strongly influence its mechanical properties and rate of biodegradation. By carefully adjusting the block copolymer segments sizes through the polymerization parameters, it is possible to modulate the materials characteristics in order to suit a particular application.

This study describes the preparation and the thermal properties evaluation of biodegradable poly(L-lactide)-*b*-poly(ethylene glycol)-*b*-poly(L-lactide) (PLLA–PEG–PLLA) (Fig. 1), prepared from two different PEG prepolymers ($M_n = 4000$ or 600 g mol^{-1}) and various PLLA segment sizes; the PLLA degree of polymerization (DP_{PLLA}) varied from 1.2 up to 145.6.

Each block of the PLLA–PEG–PLLA copolymer would be phase separated due to the asymmetric properties of the constitutive segments, thus the multiphase system behavior, which depends on the chemical composition and the polymer system morphology, will dictate its physical and chemical properties at different temperatures. The crystallization of one block influences the crystalline structure of the other block that is chemically connected to it, as well as the extension of miscibility in the amorphous phase.

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were carried out on homopolymers and block copolymers and provided us important details on polymer morphology and microphases' interactions.

2. Experimental

2.1. Materials

L,L-Lactide [(3*S*)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione], 98% and stannous 2-ethyl-hexanoate ($SnOct_2$), were supplied by Aldrich. Di-hydroxy-terminated poly(ethylene glycol)s, $M_n = 600$ and 4000 g mol^{-1}, were supplied by Oxiteno (Brazil). Chloroform, methanol, hexane, toluene, tetrahydrofuran (THF) and acetone, all analytical grade, were supplied by Synth (Brazil). All materials were used without further purification.

2.2. Synthesis of PLLA–PEG–PLLA copolymers

In a glove box under dry nitrogen atmosphere, pre-weighed amount of poly(ethylene glycol) and stannous 2-ethyl-hexanoate were introduced in a dry tube. The tube was sealed and immersed into silicon oil bath, at 120 °C for 10 min, and then cooled to room temperature. A pre-weighed amount of L,L-lactide (LA), was introduced into the tube under nitrogen atmosphere, and then sealed and immersed in the bath at 120 °C for 22 h. The copolymer thus obtained was purified by dissolution in chloroform and precipitation in methanol (three times). The solid product was dried under reduced pressure at room temperature for 24 h.

2.3. Characterization of the triblock copolymers

2.3.1. NMR spectrometry

^{1}H NMR spectra were measured at 200 MHz in $CDCl_3$ solution using a Bruker AC-200 spectrometer and tetramethylsilane (TMS) was used as the internal reference.

2.3.2. Thermal analyses

DSC runs were performed in a Mettler Toledo 822 equipment under nitrogen flux and samples were sealed in aluminum pans. DSC equipment was previously calibrated with indium ($T_m = 156.6$ °C and $\Delta H_f = 3.26$ kJ mol^{-1}). Each sample of about 10–15 mg was pre-heated to 180 °C and kept at this temperature for 5 min, cooled down at a rate of 20 °C min^{-1} to −65 °C and, after remaining at this temperature during 5 min, was heated up to 250 °C at a rate of 10 °C min^{-1}. TGA–DTA runs were performed in a TGA–DTA simultaneous equipment, model SDT 2960 of TA Instruments, under nitrogen flux. Samples of about 10–15 mg were heated up to 800 °C at a rate of 10 °C min^{-1}.

3. Results and discussion

Two series of triblock copolymers with different lengths of PLLA segments (polymerization degree, DP = 1.2–145.6) were obtained by variation of the relative mass of L-lactide in feed, added to a pre-weighed amount of PEG ($M_n = 4000$ or 600 g mol^{-1}). For composition characterization, ^{1}H NMR spectra were measured at 200 MHz (Fig. 2). ^{1}H NMR spectra in $CDCl_3$ were employed to estimate the LA/EO molar ratio in the copolymer and to identify the specific ester bond due to the copolymer formation. PLLA segments presented the following absorptions: 5.23–5.15δ multiplet, inner methine units; 4.2–4.4δ multiplet, end chain methine units; 1.4–1.6δ inner methyl units. PEG segments presented the following absorptions: 3.65δ singlet, inner methylene units; 4.2–4.4δ multiplet, methylene protons of the acylated PEG end unit. The prepared block copolymers presented wide range of molecular weights (800–25,000 g mol^{-1}) and compositions (16–80 wt.% of PEG) (Table 1).

All samples were characterized by FTIR spectroscopy (not shown here) and the spectra revealed the expected functional groups, for instance, ester C=O groups stretching at 1739 cm^{-1} and C–O groups stretching at 1110 cm^{-1}, along with the noteworthy vanishing of the broad band due to PEG's OH groups stretching at 3500 cm^{-1}. Besides, all samples were submitted to solubility test in water, ethanol, chloroform and tetrahydrofuran and the results were compared to the correspondent homopoly-

Table 1
Characteristics of the triblock copolymers

Copolymer	LA/EO[a] ratio	DP_{PLLA}[b]	M_n[b]	Composition PEG (wt.%)	Physical aspect	T_g (°C)	T_m (°C)
co4000-1	0.15	6.8	5000	80.0	Solid	−48	53
co4000-2	0.35	15.9	6300	63.5	Solid	−46	44
co4000-3	0.79	35.9	9200	43.5	Solid	−44	42
co4000-4	1.27	57.8	12300	32.5	Solid	−46	15, 92
co4000-5	3.20	145.6	25000	16.0	Solid	–	100
co600-1	0.17	1.2	800	75.0	Liquid	–	4
co600-2	0.49	3.3	1100	54.5	Liquid	−44	–
co600-3	0.67	4.6	1300	46.2	Liquid	−46	–
co600-4	1.00	6.8	1600	37.5	Wax	−40	75[c], 95[c]
co600-5	1.3	8.9	1900	31.6	Wax	−39	85[c], 125[c]

[a] Calculated from ^{1}H NMR spectra.
[b] Polymerization degree calculated from ^{1}H NMR spectra and $M_{n(PEG)}$ = 4000 for co4000-*n* and 600 for co600-*n*.
[c] Broad melting peak(s).

mers, PLLA and PEG, soluble only in chloroform and soluble in all four solvents, respectively. All samples presented solubility in chloroform and emulsification in the other three solvents, whereas the increased dispersion degree depended on the emulsion concentration and PLLA segment sizes.

The overall spectroscopic analyses and solubility results allowed us to assume that block copolymers have been prepared.

The two series of block copolymers presented a diversity of physical aspect, from viscous liquid to solid powder. This multiplicity of states allows the copolymers use in diversified medical and pharmaceutical applications. For instance, the viscous liquid can be further modified to become photo curable, and then could be applied as wound curative, while the solid copolymers could find application in micro- and nanoencapsulation of drugs.

TGA was performed to achieve information on the nature of the segmental interactions in the copolymers by measuring the thermostability and the degradation profile. The copolymers presented a dual stage thermal degradation and decreased thermal stability compared to PEG homopolymers (Fig. 3). In the first stage, copolymers degradation proceeded by a PLLA segment chain end "unzipping" mechanism, favored by the six-member cyclic di-lactide formation, and in a second stage at higher temperature, by polymer chain thermal scission. The thermal behavior of amorphous homo- and copolylactide prepared from D,L-lactide has been previously reported and the degradation mechanism has been shown to occur by cyclodepolymerization process [16,17].

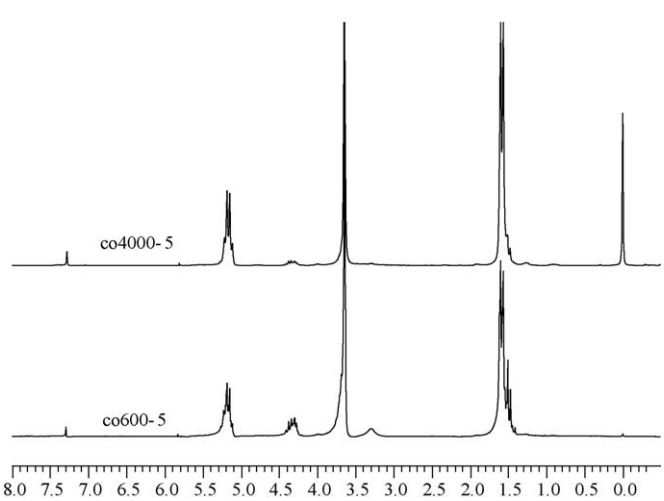

Fig. 2. ^{1}H NMR spectra of copolymers in chloroform solution: (1) PEG600-5 and (2) PEG4000-5.

The thermogravimetric curve of PEG4000, Fig. 3a, shows only one decomposition step at T_i = 370 °C and T_f = 430 °C with a weight lost of approximately 98% and a residue of 2%, suggesting a high purity organic compound. It is well-known that the occurrence of one single degradation step in a narrow temperature range, corroborated by a thin derivative peak (DTG), usually demonstrates one sole degradation reaction, thus confirming the purity certified by the supplier of 98% minimum. Simultaneously, in the DTA curve two endothermic events were observed, one at 60 °C, indicating crystalline melting of PEG and the other, around 410 °C, due to thermal chain scission by endothermic degradation, this later was also observed in the correspondent TG curve.

For the copolymer co4000-1 the TG curve, Fig. 3b, shows dual step decomposition, the first step at T_i = 200 °C with a weight lost of 19% and a second decomposition step, which is the main transition, at T_i = 372 °C with 79% of weight lost, after which an residue of 2% was observed. The DTG curve confirms the two-step degradation process. The DTA curve also confirms these two events at 260 and 420 °C, and shows another third endothermic event around 50 °C, ascribed to the copolymer crystalline melting, also observed by DSC.

For the sample PEG600 the TG curve, Fig. 3c, shows a single degradation step at T_i = 300 °C with approximately 100% of weight lost. The observed profile was confirmed by the DTG curve with a maximum decomposition rate at 400 °C, the same event was observed as an endothermic transition (400 °C) in the correspondent DTA curve.

For the copolymer co600-3, the TG curve, Fig. 3d, presents two-step decomposition at T_i = 80 and 350 °C, respectively. However, both degradation steps were slower compared to those observed for the copolymer co4000-1 (Fig. 3b), as the temperature ranges of co4000-1 degradation were narrower than those of co600-3. The DTG curve confirms the two-step mechanism for

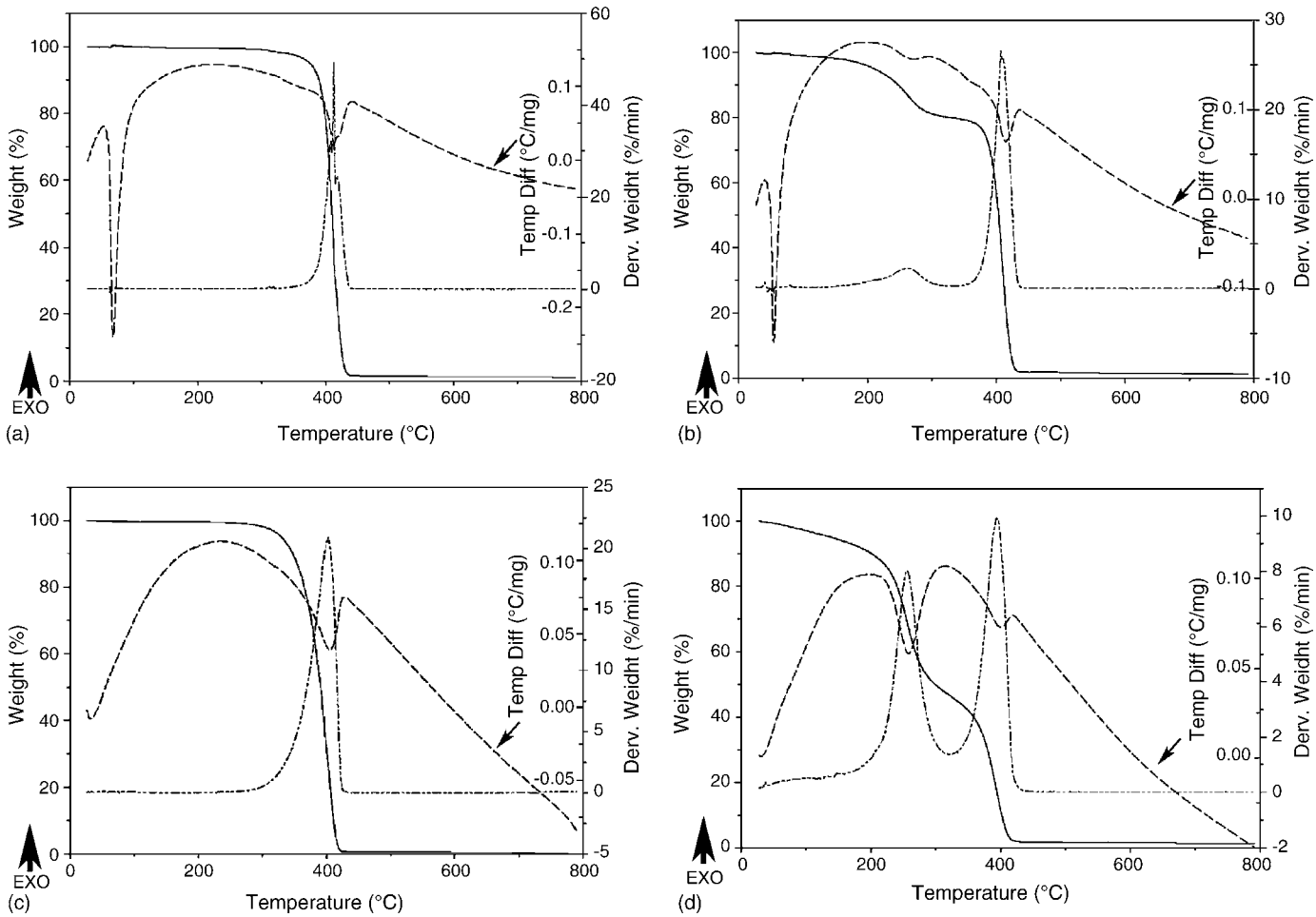

Fig. 3. Simultaneous TGA–DTA analyses: (a) PEG4000, (b) copolymer co4000-1, (c) PEG600 and (d) copolymer co600-3.

copolymers and for co600-3, the maximum of the degradation reactions rate were at 260 and 400 °C, both thermal transitions were again shown as two endothermic events in the DTA curve. These analyses have shown that the co600-3 lost weights are due to thermal scissions of the copolymer chain, correspondent to 56 and 44% at 260 and 400 °C, respectively.

The TG analyses have shown that the copolymers are comprised by two segment types, which thermal stability are very distinct, the PEG segment is more thermally resistant and the degradation temperature observed by DTA is around 400 °C, while the PLLA segment starts to decompose at as low as 260 °C. Additionally, the lost weights are correspondent to the mass percentages of both segments, and the compositions observed by TGA are in accordance to those determined by ^{1}H NMR.

In the present paper, the data from DSC are experimental raw data, as no data conversion was performed in order to quantify the thermal transitions: the glass transition, cold crystallization and crystalline fusion. However, following the valuable suggestions of reviewers, the quantitative thermal analyses of these copolymers will be considered in a future submission, and, as well-established by Wunderlich and co-workers [18–20] and Schick et al. [21–24], among others [25–27], linkage between the thermal behavior and the vibrational molecular motions in the polymeric systems might be characterized, contributing to better understanding of the phase behavior in this class of block copolymers.

DSC analyses (Fig. 4 and Table 1) allowed the observation of multiphase separation, as evidenced by multiple thermal transitions of the block copolymers' constitutive segments: glass transition (T_g), cold crystallization (T_c) and melting temperature (T_m).

The T_m of PLLA segments for PEG600 series decreased with its DP and were observed for DP > 6.8, while the crystalline phase of PEG segments was suppressed, except for co600-1, in which a cold crystallization at $T_c = -35$ °C and a $T_m = 4$ °C were observed. The T_g for co600 series varied from −39 to −46 °C, according to the copolymer PLLA relative concentration. The only observed glass transition temperature (T_g) indicates miscibility in the amorphous phase. The PLLA crystalline phase formation in copolymers was extensively disturbed, its melting peak was hardly observed and the copolymers co600-4 and co600-5 have shown broad melting peaks. This thermal behavior is also indicative of the PEG/PLLA segments miscibility, which would difficult long range segmental motion of PLLA for lamellar crystallization, leading to the formation of small and imperfect crystallites. Such behavior has been frequently observed in semicrystalline segmented copolymers and semicrystalline polymer blends [8–10] with short chain lengths

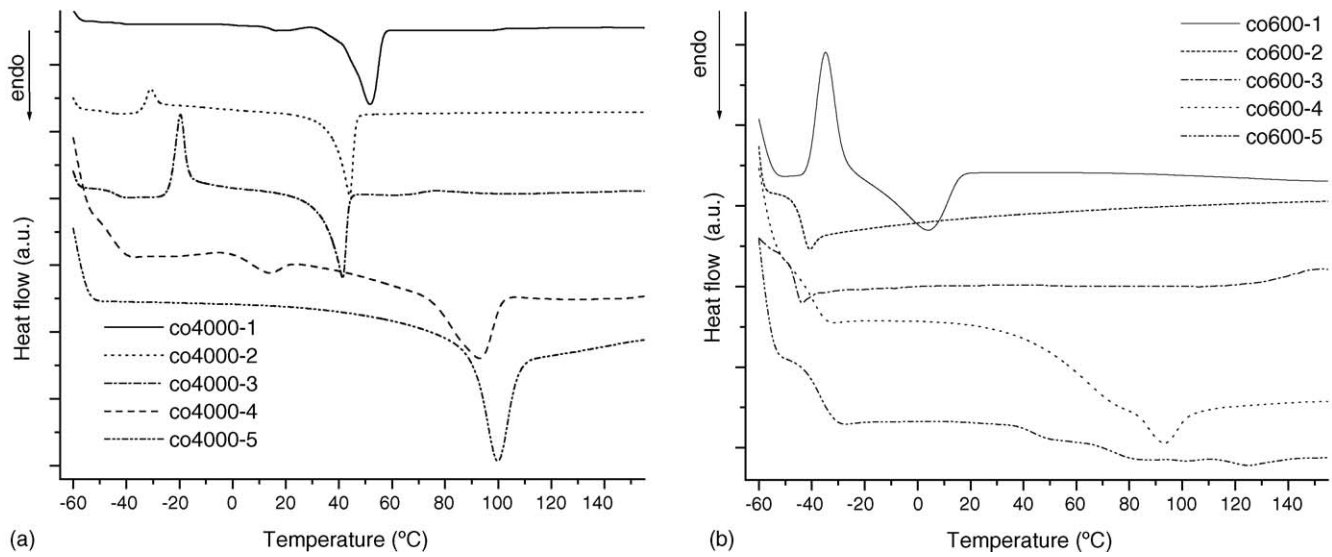

Fig. 4. DSC traces of the two series of triblock copolymers: (a) PEG4000 and (b) PEG600.

[28–31]. Recent work by Teramoto et al. [32] using similar copolyesters of L,L-lactide, ε-caprolactone (CL) and ethylene glycol oligomer (M_n = 400 g mol^{-1}) with weight average molecular weight (M_w) ca. 3000 g mol^{-1}, revealed that all copolymers were amorphous materials having a glass transition temperature (T_g), which increased with increasing LA/CL ratio.

The only observed T_g for co4000 series varied from −44 to −48 °C, and indicates a miscible amorphous phase. An induced cold crystallization (T_c) after T_g, and the correspondent exothermic transition was clearly observed for co4000-2 (T_c = −30 °C) and co4000-3 (T_c = −19 °C), followed by the PEG segments crystalline melting (T_m). The copolymers presented lower T_m compared to PEG homopolymer (around 60 °C), which decreased with increasing LA concentration. The T_m shift in conjunction with the broader temperature range indicates disturbance of the PEG crystalline structure due to segmental interactions with the PLLA fraction, which leads to less perfect and smaller crystallites. The second T_m observed at 92 and 100 °C for co4000-4 and co4000-5, respectively, indicates PLLA segments crystalline melting. Accordingly, the mutual solubilization and the relative segment lengths in copolymers influence the T_m of the PLLA phase. For the co4000-5, PEG segments are completely dissolved in the amorphous phase and no crystalline melting was observed for PEG phase.

The T_m of the PEG's phase was not suppressed in co600-1, co4000-1, co4000-2, co4000-3 and co4000-4, where the relative PLLA segment lengths were short and unable to crystallize, thus forming a homogeneous mixed amorphous phase, with embedded small PEG crystallites.

The thermal behavior observed for these triblock copolymers is very similar to those previously reported for the poly(L-lactide)–poly(ethylene glycol) multiblock copolymers and the PLLA oligomers [28–30]. Their thermal behaviors indicate that only one amorphous phase is present and both the PLLA and PEG blocks can be crystallized, depending on the relative segment sizes. In contrast, diblock copolymers present two melting endothermic peaks (T_m) during heating and two crystallization exothermal peaks (T_c) during cooling [33]. Furthermore, the phase separation between PLLA and PEG blocks takes place due to the crystallization process of PLLA and PEG blocks, since their two blocks are miscible in the amorphous phase.

The triblock copolymers series were able to present wide range of T_m and values as low as 4 °C and as high as 100 °C were observed, which are interesting for pharmaceutical and therapeutic applications, so far as the transition temperature can be used to control the polymer processing and segmental mobility.

4. Conclusions

From the DSC and TG analyses of the two series of PLLA–PEG–PLLA, it was found that up to three phases coexists in copolymers: PEG crystallites, PLLA crystallites and completely miscible amorphous phases. The phase behavior of copolymers depended on its composition and segments' relative sizes, revealed by the transitions temperatures: crystalline melting, T_ms, of PLLA and PEG, and glass transition, T_g.

The copolymers presented a dual stage thermal degradation and decreased thermal stability compared to PEG homopolymers.

The copolymers presented variable degree of miscibility between the component blocks in accordance to the segments' lengths. Although all block copolymers have not shown miscibility in the crystalline phases, the complete miscibility in the amorphous phase was prevalent, and only one T_g was observed for each copolymer. The block structure inhibits the segmental motion of both components and the crystalline phases of PEG600 and PEG4000 segments as well as that of PLLA segment were suppressed, when the blocks presented relatively short length, as a result of the total miscibility in the amorphous phase, which in addition provokes decrease in the T_m of both crystalline phases (PEG and PLLA).

The wide range of T_m observed for the triblock copolymers, from 4 to 100 °C, makes these copolymers promising for a vari-

ety of pharmaceutical applications and temperature controlled therapeutic medications.

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