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Article

Poly(L-Lactide-co-Glycolide) (PLLGA) - Fast Synthesis Method for the Production of Tissue Engineering Scaffolds

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Abstract: Poly(L-lactide) (PLLA) is a biodegradable and biocompatible polyester commonly used in the biomedical field. However, the hydrophobic and crystalline structure of PLLA can difficult cell interactions, offering limitations to some biomedical applications. These limitations can be overcome by random copolymerization of L-lactide (LLA) with glycolide (GA) to obtain poly(L-Lactide-co-Glycolide) (PLLGA) copolymers. Bulk ring-opening polymerization (ROP) of LLA and GA offers versatility to the PLLGAs as regards to their physicochemical properties, hydrophilic/hydrophobic balance, and degradation rates. Thus, this study aims the production of PLLGA via bulk ROP and the investigation of the effect of the different amounts of LLA and GA in the PLLGA copolymers by spectroscopy (FTIR) and thermal analyzes (DSC and TGA) comparing with the PLLA. The applied methodology produced PLLGAs with a rapid copolymerization time (2 h). These PLLGAs presented adequate thermal stability (T_g > 37 °C - physiological temperature and T_{deg} > 200 °C) that, combined with its copolymer structure, makes it a potential biomaterial for application as scaffolds in tissue engineering.

Keywords: bulk ring-opening polymerization; copolymers; biodegradable polymers; biocompatible polymers.

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1. Introduction

Tissue engineering is a field of science that brings together knowledge in the materials science and engineering, chemists, physicians, biologists and related professionals in favor of a single goal: seek solutions to develop biological substitutes capable of regenerating, maintaining or improving the functions of damaged organs or tissues [1]. In this way, the design of materials with desired characteristics or improvement of existent materials properties has been the challenge of engineering scaffolds for tissue regeneration to achieve the interaction between these materials and cells, promoting cell adhesion, proliferation, and differentiation (biocompatibility) [1-2]. To be

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applied in tissue engineering, some desirable scaffolds properties are required, such as biodegradability, appropriate mechanical resistance, scaffold porous architecture, and processability. These properties will define the scaffolds' appropriate application [1].

Among the available biocompatible polymers, copolymers based on lactide (LA) and/or glycolide (GA) (PLGA), exhibit potential biodegradability and biocompatibility with a wide range of physicochemical properties according to the ratio of lactide and glycolide during the polymerization, such as molar weight, crystallinity, hydrophilicity, and degradation properties [3-5]. These characteristics make these polymers attractive and appropriate for a significant number of applications, including the production of medical and pharmaceutical devices, for tissue engineering, controlled drug release, absorbable suture yarns, bone fixation devices, prostheses, among others [1, 6-13]. Most of these PLGA copolymers are applied in areas related to human health [5] since their monomers are endogenous and quickly metabolized via the Krebs cycle. These PLGA copolyesters also have their use approved by the American Food and Drug Administration (U.S. FDA) and the European Medicine Agency (EMA) [14].

Ring-opening polymerization (ROP) of cyclic monomers, as L-lactide (LLA) and glycolide (GA), is the most widely applied method for the synthesis of PLLGA copolymers, since it enables a much higher degree of polymerization due to its precise chemistry control [7, 15-17]. In this way, it produces stereospecific polymer chains, with high molar weight, preferable end-groups, and controlled monomers insertion in the live polymer chain [19]. Copolymerization by ROP can occur in bulk, solution, suspension, or emulsion, under inert atmosphere or in vacuum [6, 15]. The first route (bulk polymerization) is preferred for biomedical applications since it does not use solvents and eliminate the risk of residual toxic organic solvents (such as toluene) in the final product [5, 18]. In practice, ROP of these comonomers requires appropriate catalysts and initiators that, combined with an environmentally friendly reactional condition, minimize the use and/or production of toxic compounds [5, 7-9, 18-26].

The most common catalyst for PLGA polymerization is stannous octoate ($[Sn(Oct)_2]$), which provides high molar weight polymers [7, 9, 15-16, 19, 26-31]. The U.S. FDA approves this catalyst as a food additive [9], and its use for surgical and pharmacological applications [6]. Since it has a high polymerization rate, avoids product racemization, easy purchasing, and high solubility in organic solvents and reactional monomers [22] for PLGA production. The $[Sn(Oct)_2]$ is used to open the lactide and glycolide rings [15] because it is a highly efficient transesterification agent. Its typical bulk reaction times at 100 - 180 °C ranges from a few hours [6-7] to days [32], resulting in copolymers with a random microstructure [20].

Generally, transesterification is thermally sensitive, favoring side reactions with heating [29, 7]. Intermolecular transesterification (back-biting) reactions prevent the formation of block copolymers. Intramolecular transesterification reactions degrade the polymer chains and promote the formation of cyclic oligomers. Therefore, transesterification reactions increase the molar weight distribution [21]. However, $[Sn(Oct)_2]$ should not initiate the ROP in the absence of protic initiators such as alcohol [7, 31]. Initiators affect the ROP conversion rates and the properties of the corresponding polymers (degradation rate, thermal properties, and molar weight). So, water removal is recommended to control the molar weight of the resulting polymer [9]. According to this, 1-dodecanol (lauryl alcohol), which has a hydroxyl group, can act as an initiator, leading to a polymerization growth with a linear structure, that might be combined with bulk ROP, to produce high molar weight polymers [25]. This copolymerization strategy results in polymers with controlled degradation and suitable thermal and mechanical properties [15].

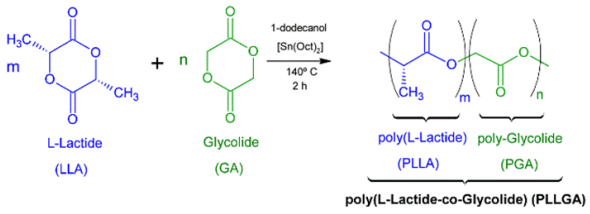


Figure 1. Fast synthesis method for PLLGA production.

Considering the individual characteristics of the PLLA and the PGA, as well as their distinctive properties according to their polymerization routes, the objective of this study is to synthesize PLLA, PGA, and PLLGA by bulk ROP, using $[Sn(Oct)_2]$, as the catalyst, and 1-dodecanol, as the initiator

(Figure 1), in a reduced copolymerization time (2 hours) at 140º C [5-7, 9, 19, 23, 32]. This process may lead to economic advantages (lower energy consumption) and lower side reactions (polymer properties improvement). The effects of the different amounts of LLA and GA in the copolymer chemical properties were evaluated by infrared spectroscopy (FTIR), and thermal properties were evaluated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

2. Materials and Methods

2.1. Materials.

L-lactide (LLA; Purasorb® L) and glycolide (GA; Purasorb® G) monomers were purchased from Purac®. Stannous octoate [Sn(Oct)$_2$] (95%) and 1-Dodecanol (98%) were purchased from Sigma-Aldrich. All reagents were used as received.

2.2. Synthesis of Polymers.

PLLA, PGA, and PLLGA were synthesized by bulk ROP of LLA and GA, using [Sn(Oct)$_2$] and 1-dodecanol. The synthesis of polymers and copolymers were carried following other works [6, 7, 9, 21, 32], in a three-nozzle jacketed glass reactor under inert atmosphere ($N_{2(g)}$ ~ 20 mL/min), adding predetermined amounts of monomers (LLA and/or GA: LLA/GA (w/w) = 70/30 (1), 50/50 (2), 30/70 (3), 100/0 (4), 0/100 (5)), [Sn(Oct)$_2$] (1% w/w) as a catalyst, and 1-dodecanol (0,02% w/w) as initiator at 140 ºC under vigorous mechanical stirring until the monomers were completely melted. After that, the mechanical stirring was carried out for 15 min. The total bulk ROP reaction time was 120 min. The samples produced were cooled at room temperature.

2.3. Polymer Characterization.

Fourier transform infrared (FTIR) spectroscopy analysis was performed using a Thermo Scientific spectrometer (model Nicolet 6700). The samples were analyzed in ATR mode, using a Smart Omni Sampler accessory. The spectra were recorded over a range of 4000 - 675 cm^{-1} at a resolution of 4 cm^{-1}.

DSC measurements were performed in a Mettler Toledo calorimeter (model DSC1), using 40 µL aluminum pans. Approximately 10 mg of samples were weighted. Three dynamical scans were performed for all samples ranging from 25 to 200 ºC at 10 ºC/min, under the nitrogen atmosphere ($N_{2(g)}$ = 50 mL/min). At first, the samples were heated until 200 ºC, remaining at this temperature for 3 min. Then, these samples were cooled until 25 ºC and maintained at this temperature for 3 min. Finally, these samples were heated again until 200 ºC, so, all the thermal transitions were identified in this second heating.

Thermal stability analyses were performed using a Shimadzu TGA-50M thermogravimetric analyzer. Approximately 8 - 10 mg of the samples were scanned from 30 to 500 ºC at a heating heat of 20 ºC/min under an inert atmosphere ($N_{2(g)}$ = 50 mL/min).

All analyses were carried out with unpurified samples of the synthesized polymers.

3. Results and Discussion

3.1. Polymer Characterization.

The chemical structures of PLLA, PGA, and PLLGA copolyesters synthesized here were characterized by FTIR spectroscopy (Figure 2) to assess the extension of the PLLGA copolymerization. Table 1 summarizes the infrared functional groups for PLLA, PGA, and PLLGA.

The FTIR spectra showed the characteristic peaks of LLA and GA homopolymers. These include the absorption that can be assigned to the LLA-LLA groups (~1454 cm^{-1}) in PLLA, referring to the asymmetric bonding of the methyl group (δ_{as} CH_3); and GA-GA bonds (~1423 - 1418 cm^{-1}) in the PGA, relative to the bending of the methylene group (δ CH_2). The PLLGA copolyesters also presented these LLA-LLA and GA-GA absorption bands as well the absorption group at ~1397 cm^{-1} that corresponds to the wagging CH2 (ω CH_2) of LLA-GA bond. These results indicate that the synthesis by bulk ROP for PLLGA occurred successfully [16, 19, 33, 34, 35, 36]. Moreover, the signals at 1189 - 1087 cm^{-1} are also associated with the presence of lactic and glycolic units [33, 34, 36]. In the 1760 - 1725 cm^{-1}, it can be observed the carbonyl stretching (ν C=O) [16, 17, 33-36]. The weak signals observed at 1002 - 800 cm^{-1} can be assigned to the presence of residual oligomers and monomers in the samples since the analyses were performed with unpurified samples.

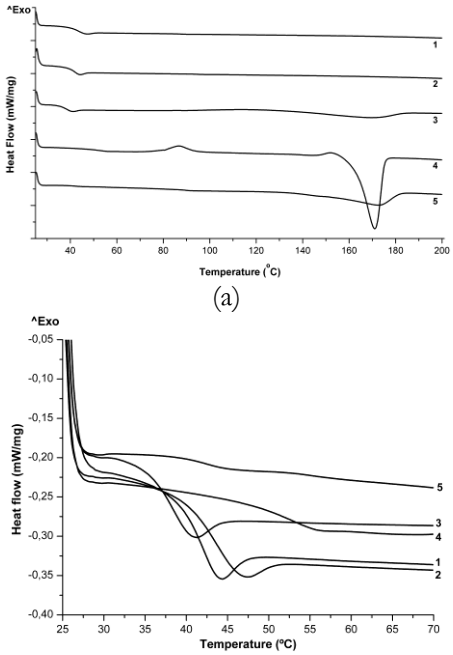


Figure 1. (a) DSC curves obtained during the second heating cycle; (b) Tg for PLLGA 70/30 (1), PLLGA 50/50 (2), PLLGA 30/70 (3), PLLA (4), and PGA (5).

The chain with a growing GA end group has a preference of 3:1 to add another GA unit, while an equivalent chain with an increasing LLA end has a 5:1 choice for the GA according to the literature [8]. Such particularity enables the production of a PLLGA structure with GA blocks separated by single lactide units.

The PLLGA copolymers might have a diversified composition, with the GA being preferably polymerized at low conversions, and the LLA being added in increasing amounts as the GA is progressively consumed in the reaction [8]. This behavior is also observed in the synthesized PLLGA copolymers since the intensity of δ CH_2 (1423 - 1418 cm^{-1}) from GA-GA bonds increases over the intensity of δas CH_3 (~ 1454 cm^{-1}) from LLA-LLA bonds in the PLLGA copolymers according to the increment in the GA content.

DSC curves obtained during the second heating cycle of the samples are presented in Figure 3.

The copolymerization process affects PLLGA thermal phase transition temperatures (i.e., glass transition - T_g, cold crystallization - T_{cc}, and melting - T_m) (Table 2), also influencing their physicochemical properties [39].

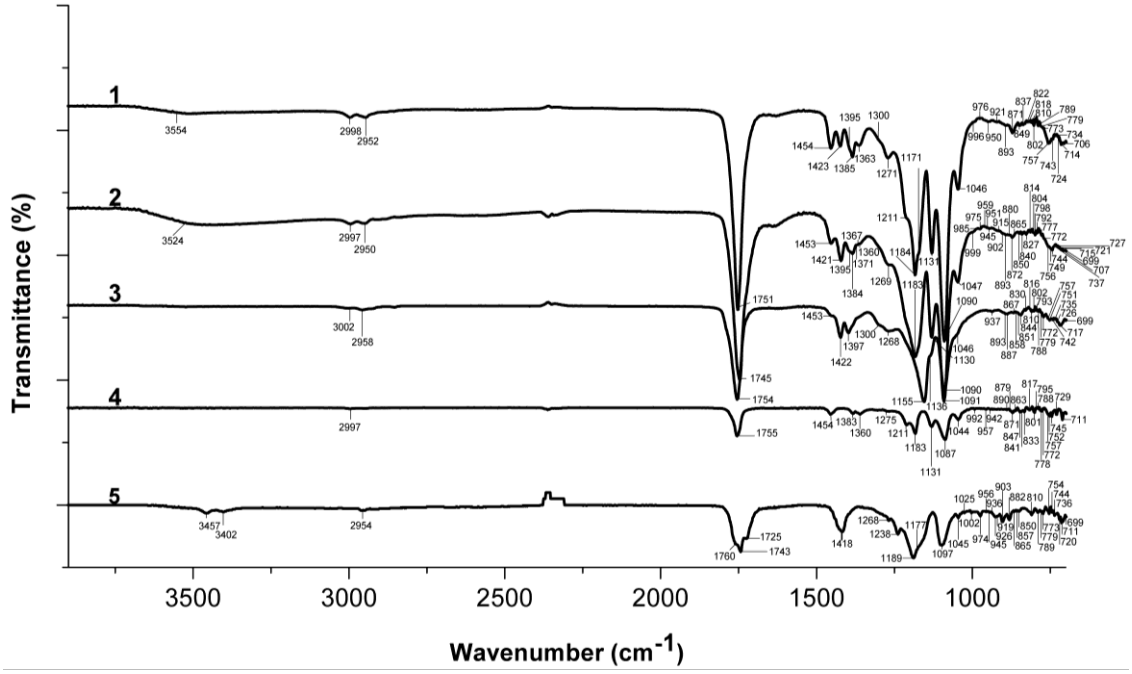


Figure 2. FTIR spectra of PLLGA 70/30 (1), PLLGA 50/50 (2), PLLGA 30/70 (3), PLLA (4), and PGA (5).

Table 1. FTIR infrared absorption band assignments for PLLGA 70/30 (1), PLLGA 50/50 (2), PLLGA 30/70 (3), PLLA (4), and PGA (5); ν, stretching; δ, bending; ω, wagging; τ, twisting; ϱ, rocking; s, symmetric; as antisymmetric, all given in cm^{-1}.

PLLGA 70/30	PLLGA 50/50	PLLGA 30/70	PLLA	PGA	Units	Assignment
3554	3524			3457		ν OH [35]
				3402		
2998	2997	3002	2997		LLA	ν_{as} CH_3 [34, 37]
2950	2952	2958		2954	GA	ν_{as} CH_2 [37, 38]
				1760	LLA and GA	ν C=O [16, 19, 33 - 36]
1751		1754	1755			
	1745			1743		
				1725		
1454	1453	1453	1454		LLA-LLA bonds	δ_{as} CH_3 [16, 19, 34, 35, 37]
1423	1421	1422		1418	GA-GA bonds	δ CH_2 [16, 19, 34, 35 -37]
1395	1395	1397			LLAGA bonds	ω CH_2 [16, 19, 34, 35-37]
1385	1384		1383		LLA	δ_s CH_3 [35, 37]
	1371					
1363	1367	1300	1360		LLA	δ CH [34, 35]
	1360					
1300	1300					
1271	1269	1268	1275	1268	LLA	τ CH_2 [35]
				1238		
1211			1211			
1184	1183		1183	1189	LLA	ν_{as} COC [35, 37]
1171				1177		
		1155				
1131	1130	1136	1131		LLA	ϱ_{as} CH_3 [35, 37]
1090	1090	1091		1097	LLA and GA	ν_s COC [35, 35, 37]
			1087			
1046	1047	1046	1044	1045		ν C-CH_3 [35, 37]
				1025		
				1002	LLA	ϱ CH_3 [35, 37]
996	999		994			
	985					
976	975			974		
950	959		957	956		
	951					
	945		942	945	GA	ν C-COO [37]
		937		936		
921				926		
	915			919		
	902			903		
893	893	893	890			
	880	887		882		
871	872		879			
			871			
		867	863	865		
				857		
	850	851		850		ϱ CH_2 [37]

3.2. Thermal Characterization.

Table 2. DSC results obtained during the second DSC heating cycle for PLLGA copolymers, PLLA, and PGA.

Polymers	T_g (ºC)	T_{cc} (ºC)	T_m (ºC)
(1) PLLGA 70/30	42.8	-	-
(2) PLLGA 50/50	40.7	-	-
(3) PLLGA 30/70	37.6	-	171.0
(4) PLLA	50.0	86.7, 151.8	170.9
(5) PGA	41.1	-	172.6

The T_g of the synthesized PLLGA copolymers is above 37 ºC (physiological temperature), which is coherent with the literature [3, 39]. Chain mobility can be affected by polymer composition, molar weight, inter- and intramolecular forces, degrees of crystallinity, and crosslinking [40], any structural features to reduce the chain mobility can increase its T_g [41]. In this way, random copolymerization of LLA with GA modifies the regularity and the symmetry of the LLA and GA units, producing an amorphous copolymer (PLLGA) [41, 42]. The amorphous structure exerts a significant influence on the PLLGA copolymer properties [41], as evidenced by their T_g ranging from 37 to 50 ºC (Table 2). An increase in the LLA content improved their T_g, as observed here: PLLA (4) > PLLGA 70/30 (1) > PGA (5) > PLLGA 50/50 (2) > PLLGA 30/70 (3) [3-5]. The incorporation of GA into PLLA chains may interrupt the crystallization of PLLA [39], while crystalline regions decrease the chain mobility in the amorphous areas, increasing the T_g [41] (PLLA (4) and PGA (5)).

Amorphous structures can facilitate the hydrolytic degradation of PLLGAs, reducing their crystallinity. This unordered structure facilitates the water and enzyme access for the ester hydrolysis allowing the PLLGAs to be suitable for application as scaffolds [43]. The resistance to hydrolytic degradation of the PLLGAs improves with the LLA content, implying that PLLGAs have tunable properties according to their formulation [8, 37, 43]. However, the T_g values presented in Table 2 (above 37 ºC) suggests a glassy state for the synthesized PLLGAs, which increase the time range for polymer degradation [5]. The rigidity of the polymer chains is intensified with the addition of a methyl group (CH_3) on the LLA unit (in comparison to the GA unity), due to steric hindrance, decreasing the chain flexibility and, thus, increasing the T_g of PLLGAs [5, 9].

T_m is affected by the crystallinity since polymers with a regular and symmetrical structure should facilitate the chain's packaging and crystallization [41]. It can be expected a reduction in the crystallinity and Tm with the comonomer amount in the copolymer formulations [32]. PLLA (4) presents T_g, T_{cc} (exotherm), and T_m (endotherm), as can be seen in Figure 3. The applied temperature used for the bulk ROP methodology in this study (T_g < 140 ºC < T_m) offers the condition for the crystal nuclei to be stable, allowing the crystal growth [41]. During the first heating, sufficient thermal energy is provided for the polymer chain to rearrange in crystalline regions. So, in the cooling, these chains are quenched into a semicrystalline state (cold crystallization). Typical T_m for PLLA ranges from 170 - 180 ºC [15], because of the presence of irregular crystallites, impurities, meso-lactides, and racemization occurrence [41]. A weak T_m shoulder is observed for PLLGA 30/70 (3) at 171 ºC; this might be explained by the LLA segments in this copolymer, which had time to rearrange the polymer chains into low energy structures during the copolymerization [41]. Another observation is about the higher reactivity of GA units, resulting in the formation of crystallizable LLA sequences, which was also reported by Grijpma and Pennings [39].

Korhonen and coworkers [23] produced high molar weight PLLAs at 200 ºC for 1 hour, using [$Sn(oct)_2$] as a catalyst with different co-initiators. They concluded that the presence of co-initiators accelerates the polymerization and increases the molar weight. The PLLGA copolymers with high molar weight synthesized by ROP at 110 ºC for one week revealed that the copolymerization decreases their T_m and heat of fusion but resulting in an apparent T_g [39]. Similar behavior was detected in this investigation, wherein the polymers were synthesized at 140 ºC for 2 hours. These parameters for polymerization reaction are an essential achievement towards a cheaper and rapid procedure to produce polymers due to the lower temperature and reduced reaction time in comparison with the literature [6-7, 9, 19, 23, 39].

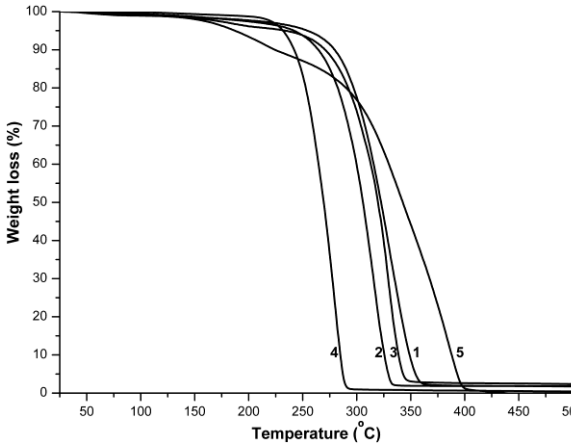
The thermogravimetric analyses of the synthesized polymers are depicted in Figure 4, and the thermal data are presented in Table 3. Thermal degradation process (T_{onset}) starts at 200 ºC, and the mass loss reaches a maximum (T_{max}) at 316, 330,

329, and 280 °C for PLLGA 70/30 (1), PLLGA 50/50 (2), PLLGA 30/70 (3), and PLLA (4), respectively (Table 3). It is already reported that T_{max} for degradation of purified polylactides ranges from 330 - 375 °C [45, 46], with T_{max} ~360 °C, exhibiting low thermal stability at elevated temperatures [45, 46]. Literature reports a relationship between thermal stability with molar weight and polydispersity index. Polymers with higher molar weight and lower polydispersity index present higher thermal stability. In this study, unpurified samples were analyzed, and the presence of residual catalysts, initiators, monomers, or oligomers is reducing the thermal stability of the synthesized polymers [45, 46]. However, the bulk ROP is interesting for tissue engineering applications since a purification stage is avoided due to the absence of solvents, and the desired properties are reached by an easy and low-cost process.

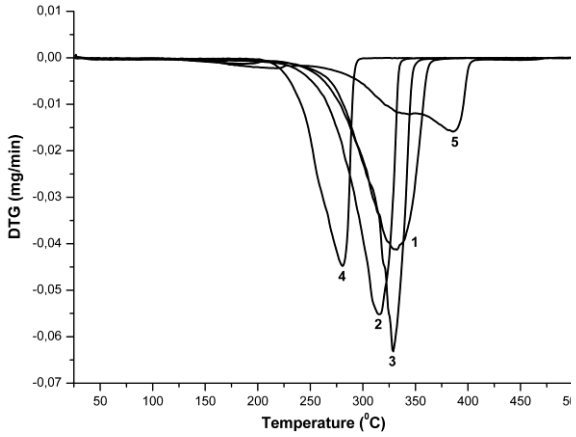
Generally, the primary degradation products for PLLA are CO, CO_2, oligomers, lactide, and acetaldehyde, with non-radical and radical reactions occurring during the degradation [41]. A mechanism for PLLA chain transfer, back-biting (intramolecular transesterification reactions), intermolecular transesterification, and depolymerization in the presence of $[Sn(Oct)_2]$ are suggested as the main thermal decomposition factors [41, 47 - 49] at elevated temperatures (> 240 °C) [49].

For PGA, the primary degradation products would be methyl glycolate, CO_2, formaldehyde, and ketones [50], besides glycolide and cyclic oligomers that are predominantly produced by random chain scission at higher temperatures [41, 50]. Thereby, it is possible to explain that the lower temperature peak (T_{max} = 236 °C) for PGA consists in the presence of glycolide (unreacted monomer), and the higher temperature peak (T_{max} = 386 °C) correspond to the decomposition of cyclic monomers [41]. Thus, as PGA and PLLA have structural similarities, their thermal degradation should follow the same ester degradation mechanism [41, 50].

Additionally, the presence of $[Sn(Oct)_2]$ may influence the polylactides' thermal stability as well as its concentration [22] presumably, because the Sn atoms attached to the polymeric chain end can act as a depolymerization catalyst.



(a)



(b)

Figure 2. (a) Weight loss; and (b) first derivative curves for the synthesized polymers (PLLGA 70/30 (1), PLLGA 50/50 (2), PLLGA 30/70 (3), PLLA (4), and PGA (5)).

The alkoxide anion can attack the carbonyl carbon of the penultimate lactate unit, resulting in the elimination of a LLA unit, also called back-biting depolymerization when this repeatedly occurs [41].

The synthesized PLLGA copolymers started their degradation at temperatures higher than the synthesized homopolymers (> 200 °C), which is in agreement with the observed by Gorrasi et al. [50]. PLLGA has a comparable thermal degradation profile as PLLA and PGA, since they have similar decomposition pathways, with intermediate thermal properties to PLLA and PGA [41].

Also, considering the PLLGA degradation, this copolyester is considered biocompatible since its hydrolysis biodegradation units (glycolic and lactic acid) are biodegradable, being metabolized in the Krebs cycle that produces water and carbon

dioxide (CO_2) as degradation byproducts [7]. In this biodegradation process, the CO_2 can decrease the local pH value, increasing the possibility of cells and tissue necrosis [1]. Hence, the control of the polymerization process and the selection of the polymer composition is essential to avoid problems in scaffold applications for tissue engineering.

Table 3. Thermal data evaluated from TG-DTG.

Polymers	T_{onset}^1 (ºC)	T_{max}^1 (ºC)	T_{offset}^1 (ºC)	T_{onset}^2 (ºC)	T_{max}^2 (ºC)	T_{offset}^2 (ºC)
(1) PLLGA 70/30	209	316	345	-	-	-
(2) PLLGA 50/50	205	330	376	-	-	-
(3) PLLGA 30/70	208	329	360	-	-	-
(4) PLLA	200	280	302	-	-	-
(5) PGA	116	236	244	244	386	411

4. Conclusions

In this work, it was obtained PLLGA copolymers via bulk ROP with a fast copolymerization time (2 h) under controlled conditions (T = 140 ºC, $N_{2(g)}$ ~ 20 mL/min). Using the bulk ROP, it was possible to produce PLLGA copolymers from lactide and glycolide comonomers, with 1-dodecanol as initiator, and $[Sn(Oct)_2]$ as a catalyst. This reactional system reduces undesired side reactions and, consequently, the low-cost process. The infrared spectroscopy of the polymers showed absorption bands that are characteristic of polyesters, indicating that the copolymerization yielded the PLLGA copolymers for the three LLA/GA proportions studied (30/70, 50/50, and 70/30). The DSC curves indicated that the PLLGA 50/50 and 30/70 had no T_m in the temperature range analyzed, whereas the PLLGA 70/30 is semicrystalline, showing a T_m shoulder at 171 ºC. The PLLGA copolymers presented suitable thermal stability that, combined with its amorphous nature, should allow the maintenance of their physicochemical properties and structural integrity during the application as a scaffold, promoting an adequate time for the biodegradability.

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Conflicts of Interest

The authors declare no conflict of interest.

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