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Article

Synthesis and Structural Characterization of Block and Random Low Molecular Weight Copolymers Composed of L-lactic Acid and Isosorbide Succinate Moieties

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Resíduos de succinato de isosorbídeo foram incorporados na cadeia principal de poli(L-lactídeo) (PLLA) visando a obtenção de uma nova classe de polímeros biodegradáveis com propriedades melhoradas. Este artigo descreve a síntese e caracterização de quatro tipos de copolímeros de baixa massa molar. O copolímero **I** foi obtido da mistura dos monômeros L-lactídeo, isosorbídeo e anidrido succínico; **II** do oligo(L-lactídeo) (PLLA), isosorbídeo e anidrido succínico; **III** do oligo(succinato de isosorbídeo) (PIS) e L-lactídeo; e **IV** de reações de transesterificação entre PLLA e PIS. Análises de MALDI-TOFMS e ^{13}C-NMR apresentaram evidências de que cooligomerização foi alcançada em todos os casos. Os dados sugerem que o produto **I** é um cooligômero aleatório e que os produtos **II**-**IV** são cooligômeros em bloco.

Isosorbide succinate moieties were incorporated into poly(L-lactide) (PLLA) backbone in order to obtain a new class of biodegradable polymer with enhanced properties. This paper describes the synthesis and characterization of four types of low molecular weight copolymers. Copolymer **I** was obtained from monomer mixtures of L-lactide, isosorbide, and succinic anhydride; **II** from oligo(L-lactide) (PLLA), isosorbide, and succinic anhydride; **III** from oligo(isosorbide succinate) (PIS) and L-lactide; and **IV** from transesterification reactions between PLLA and PIS. MALDI-TOFMS and ^{13}C-NMR analyses gave evidence that co-oligomerization was successfully attained in all cases. The data suggested that the product **I** is a random co-oligomer and the products **II**-**IV** are block co-oligomers.

Keywords: isosorbide, PLLA, PIS, copolymers, MALDI-TOF

Introduction

The use of biodegradable and biocompatible polymers in biomedical applications has been recognized as desirable, where their non-toxicity is of fundamental importance.[1] The choice of polymers to be employed as biomaterial relies on the general criteria that their mechanical properties and biodegradation time have to be compatible with the application needs.[2]

Most of the biodegradable polymers contain hydrolysable linkages along the polymeric chain, such as polyesters like poly(L-lactide) or poly(L-lactic acid) (PLLA), depending on which starting material was used: L-lactide or L-lactic acid, poly(glycolic acid) (PGA), polycaprolactone (PCL), poly(trimethylene carbonate), and poly(3-hydroxybutyrate) (PHB).[3] Poly(L-lactide) is obtained from L-lactide ((S,S)-3,6-dimethyl-1,4-dioxane-2,5-dione), a cyclic diester of L-lactic acid, a naturally occurring isomer.[2]

A class of biodegradable and bioresorbable polyesters little explored is that derived from 1,4:3,6-dianhydrohexitols and alkyl diacids, *e.g.* poly(isosorbide succinate) (PIS).[4] The 1,4:3,6-dianhydrohexitols, such as isosorbide (1,4:3,6-dianhydro-D-sorbitol or -D-glucitol), are important monomers in view of their non-toxicity and renewable origin.[5,6] Likewise, succinic acid is produced from glucose by fermentation[7] and is readily metabolized by the Krebs cycle.

The incorporation of isosorbide into polyesters backbone has proven to be an advantageous artifice to obtain products of enhanced properties. In special, the inclusion of small quantities of isosorbide into aromatic polyesters affords polymers with higher T_g, opening new windows of applications for these materials.[8,9]

Copolymers can be synthesized through various synthetic routes. Depending on the synthetic method

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employed one can obtain alternating, random, or block copolymer structures.[10] On the other hand, most of the interest on step copolymerization lies on the production of block and random copolymer structures, whose properties can be close or very different to the weighted average of the corresponding homopolymers, respectively.[10]

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) has been used as a very powerful tool on homopolymer and copolymer characterization. From this technique, one can obtain information not only on average molar mass, molar mass distribution and end groups analysis, but also on polymerization mechanisms by analyzing the formation and the reactivity of functional groups as well as determining anomalous structures eventually formed as consequence of side reactions.[11-17]

In this work we investigated four different approaches for the synthesis of biodegradable aliphatic polyesters composed of L-lactic acid (LLA), isosorbide (I), and succinate (S) moieties using copolymerization techniques. SEC, MALDI-TOFMS, ^{13}C NMR spectrometry and WAXD were used to access their structural characterization.

Experimental

Materials

Isosorbide (Aldrich) and L-lactide (Aldrich) were recrystallized, respectively, twice and once from dry ethyl acetate (mp 63.5-63.6 ºC and 92.3-92.7 ºC, respectively). Succinic anhydride (Aldrich) was refluxed with acetic anhydride for 10 min, filtered, and then washed with cold dry ethyl ether[18] (mp 121.0-121.1 ºC). Tin(II) 2-ethylhexanoate (Aldrich; *ca.* 95%; $SnOct_2$) and *p*-toluenesulfonic acid (Aldrich; 98.5%) were used as catalyst without further purification. Chloroform (Synth) and anhydrous methanol (Mallinckrodt) of analytical grade were used as received.

Synthesis

Two samples of PLLA were synthesized in bulk by ring-opening polymerization with different average molar masses. Synthesis of PLLA1 (higher average molar mass): L-lactide (1.000 g; 6.9×10^{-3} mol) and $SnOct_2$ (0.023 g; 5.7×10^{-5} mol) were added to an assay tube, purged with N_2, sealed, and kept in an oil bath at 120 ºC for 35 h. The same procedure was used for PLLA2 (lower average molar mass), except for the amount of $SnOct_2$ (0.0010 g; 2.5×10^{-6} mol). The reaction temperature was chosen based on literature data.[19-21]

PIS was synthesized by step polymerization through azeotropic distillation: isosorbide (14.6 g; 0.1 mol), succinic anhydride (10.0 g; 0.1 mol), *p*-toluenesulfonic acid (0.017 g; 1×10^{-4} mol), and dry chloroform were added to a one-necked round-bottom flask of 250 mL. The reaction was kept in reflux for 28 h, using a modified Dean-Stark apparatus filled with previously dried molecular sieves of 3 Å.

Product **I** was synthesized by copolymerization techniques involving both ring-opening and step copolymerizations from L-lactide, isosorbide, succinic anhydride, and $SnOct_2$; product **II** *via* step copolymerization from PLLA2, isosorbide, and succinic anhydride; product **III** by ring-opening copolymerization from PIS, L-lactide, and $SnOct_2$; product **IV** from copolymerization *via* transesterification reactions between PLLA1 and PIS, in the presence of $SnOct_2$. The general procedure for the four bulk copolymerizations: all reagents were sealed in an assay tube after purging with N_2 and heated in oil bath at different temperature and time. The copolymerization was carried out under reduced pressure only in the synthesis of the product **I**. Table 1 describes the quantities and conditions used for

Table 1. Quantities and conditions used for the syntheses of the products **I**-**IV**, as well as the corresponding yields

Run	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Conditions	Yield / (%)
I[g]	LLA[a]	SA[b]	Isosorbide[c]	$SnOct_2$[d]	150 ºC	43
	1.131 g;	0.196 g;	0.286 g;	3 mg;	10 h -1 atm	
	7.8×10^{-3} mol	2.0×10^{-3} mol	2.0×10^{-3} mol	7.4×10^{-6} mol	24 h -1 mmHg	
II[g]	PLLA2[f]	SA[b]	Isosorbide[c]	—	150 ºC	40
	0.406 g;	0.075 g;	0.108 g;		1 atm	
	$\overline{M}_n = 3.050$ g mol^{-1}	7.5×10^{-4} mol	7.4×10^{-4} mol		32 h	
III[g]	LLA[a]	PIS[e]	—	$SnOct_2$[d]	120 ºC	52
	0.751 g;	0.322 g;		10 mg;	1 atm	
	5.2×10^{-3} mol	$\overline{M}_n = 2.300$ g mol^{-1}		2.5×10^{-5} mol	36 h	
IV[g]	PLLA1[f]	PIS[e]	—	$SnOct_2$[d]	150 ºC	58
	0.500 g;	0.214 g;		0.9 mg;	1 atm	
	$\overline{M}_n = 9.100$ g mol^{-1}	$\overline{M}_n = 2.300$ g mol^{-1}		2.2×10^{-6} mol	16.5 h	

[a]L-lactide; [b]succinic anhydride; [c]1,4:3,6-dianhydro-D-glucitol; [d]tin(II) 2-ethylhexanoate; [e]oligo(isosorbide succinate); [f]oligo(L-lactide) with different average molar masses; [g]oligo(L-lactide-*co*-isosorbide succinate); they contain the same monomer moieties, but were obtained from different ways.

each experiment. The reaction temperature (150 ºC) for the syntheses of **I** and **II** was based on literature values for the production of PIS homopolymer in bulk.[4] The same temperature was also used for the synthesis of **IV** to allow complete melting of the starting materials.

Purification

PLLA1, PLLA2, PIS, and the products **I**-**IV** were purified by dissolving in chloroform (1 volume), precipitating in methanol (10 volumes), and drying the filtrated in a vacuum-oven at 40 ºC for 48 h, except PIS and product **I**, which were vacuum-dried at RT. Table 1 also gives the corresponding yields of these four products. The yields obtained for PLLA1, PLLA2, and PIS were 86%, 64%, and 61%, respectively.

Analysis

Size exclusion chromatography (SEC) was carried out with a Shimadzu HPLC/SEC class-VP system equipped with a Shimadzu RID 10A differential refractive index detector and connected to four Styragel columns (10^2, 10^3, 10^4, and 10^5 Å; Waters). Chloroform was used as mobile phase at a flow rate of 1 mL min^{-1} and low polydispersed polystyrene reference samples (Aldrich/Waters) were used for calibration.

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained in a Bruker DRX 500 (500 and 125 MHz, respectively) or Bruker AC 200E (200 and 50 MHz, respectively). The samples (20-100 mg) were dissolved in $CDCl_3$ (0.5 mL) using TMS as internal reference.

MALDI-TOFMS measurements were performed by using an Ettan MALDI-TOF mass spectrometer (Amersham Biosciences), equipped with a nitrogen laser emitting at 337 nm and operating at an acceleration voltage of 20 kV. The detection of positive ions was carried out in reflection mode and 200 laser shots were summed up *per* full spectrum. Angiotensin, adrenocorticotropic hormone (ACTH), and bovine cytochrome C were used for calibration. Stock solutions were prepared from the samples (10 mg mL^{-1}), the matrix (1,8,9-trihydroxyanthracene; dithranol; 10 mg mL^{-1}), and NaI (5 mg mL^{-1}) in chloroform/tetrahydrofuran (THF) (1:1, v/v). Aliquots of 10 μL of the sample, 25-80 μL of the matrix, and 3-8 μL of NaI were mixed together. 0.5 μL of this mixture was added onto the sample holders (stainless steel; 0.5 μL), and air-dried prior to analyses.

The addition of cationizing agent (*e.g.* NaI salt) in MALDI-TOFMS procedures presents at least one of the following outcomes: (*i*) the signal-to-noise ratio increases, (*ii*) the signals originated by other salts eventually present in the sample are suppressed, and (*iii*) the base-line is improved.[11] The quality of the spectra obtained was significantly improved through the addition of NaI (the corresponding spectra without NaI salt addition are not shown), as well as the best sample-to-matrix-to-cationizing agent ratios.

The diffraction patterns of wide angle X-ray diffraction (WAXD) were recorded in a Rigaku Miniflex powder X-ray diffractometer, using a monochromatic beam with wavelength (λ) of 0.194 nm (FeK_α) or 0.154 nm (CuK_α), voltage of 30 kV, and electric current of 15 mA. The intensities were collected from 2θ range of 3-60° with step scanning mode of 0.02°. The powdered samples were annealed in a vacuum-oven at 90 ºC for 48 h prior to the analyses.

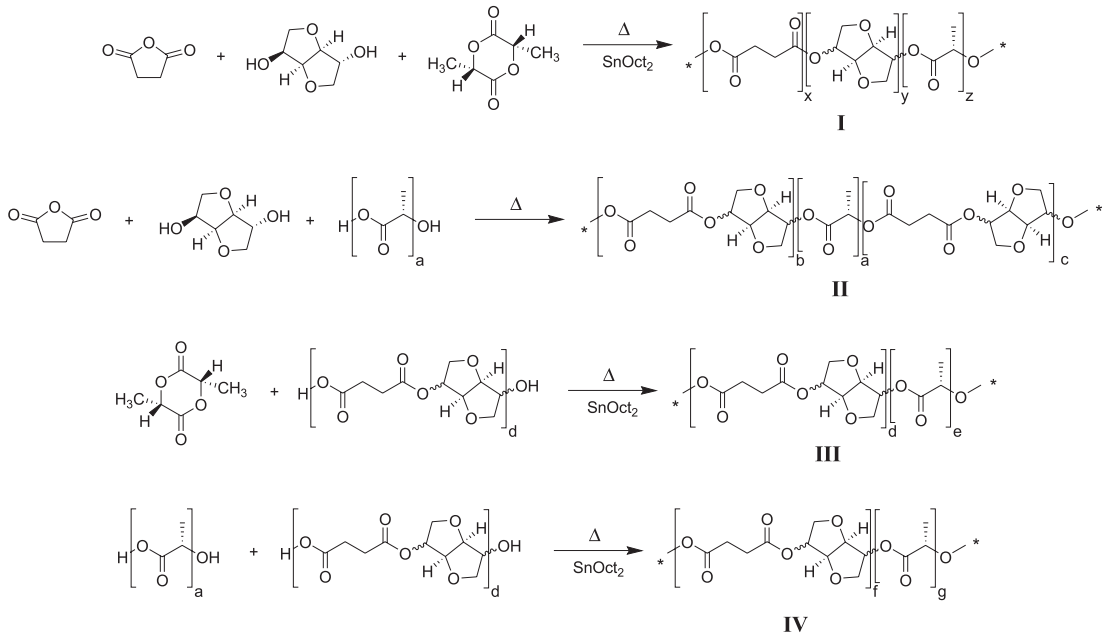
The degrees of crystallinity (χ_c) were obtained from WAXD profiles with curve decompositions following Gauss or Lorentz function fits as follows: the ratio of the area of crystalline peaks to the total area (crystalline peaks plus amorphous halo) was multiplied by 100.

Results and Discussion

PLLA (1 and 2) and PIS were obtained, respectively, in bulk by ring-opening polymerization and by polycondensation *via* azeotropic distillation in order to be used as reagents in subsequent steps. Four products (**I**-**IV**) containing L-lactic acid, isosorbide, and succinate moieties were synthesized from different approaches by means of copolymerization techniques, *i.e.*, using in all cases different feed reagents (see Scheme 1).

Table 2 presents the values of $\overline{M}_n$ and polydispersity index ($\overline{M}_w$ / $\overline{M}_n$) obtained for PLLA (1 and 2), PIS, and **I**-**IV** by SEC and MALDI-TOFMS, for comparison. The $\overline{M}_n$ values obtained by SEC for **I**-**IV**, as well as for PLLA (1 and 2) and PIS, were relatively low, ranging from 4.300 to 5.850 g mol^{-1} and from 2.300 to 9.100 g mol^{-1}, respectively, showing that, in general, co-oligomers and oligomers were attained.

The low average molar mass obtained for PIS is related to the fact that (*i*) isosorbide is a secondary diol and (*ii*) it presents two different hydroxyl groups, *endo* and *exo*, with different reactivities.[4,22] The low average molar masses obtained for PLLA are associated with the relatively low purity of the monomer L-lactide used, which serves to the purpose of this study. Perego *et al.*[21] also obtained PLLA with low average molar masses by using less pure L-lactide. In this work, when very pure L-lactide was used, PLLA with $\overline{M}_w$ up to 347.000 g mol^{-1} was obtained. The approach used for the synthesis of **III** revealed to be the most promising with respect to its average molar mass. Additional



Scheme 1. Proposed chemical structures for the products **I-IV**. See experimental section, where x, y, and z denote the molar fraction of succinate, isosorbide, and L-lactic acid moieties randomly distributed along the co-oligomer chains, respectively. The indices a-g correspond to the number of the respective repeating units in each structure. The proposed structure for **IV** is representing only the overall acylic nucleophilic substitution stemmed from PIS hydroxyl end group attack.

Table 2. Mass compositions determined by ^{1}H NMR and values of $\overline{M}_n$ and polydispersity index ($\overline{M}_w$ / $\overline{M}_n$) obtained by SEC and MALDI-TOFMS, for the sake of comparison

Run	Composition measured / (wt.%)		MALDI-TOFMS		SEC	
	LLA[a]	IS[b]	$\overline{M}_n$ / (g mol^{-1})	$\overline{M}_w$ / $\overline{M}_n$	$\overline{M}_n$ / (g mol^{-1})	$\overline{M}_w$ / $\overline{M}_n$
PLLA1	—	—	—	—	9.100	1.6
PLLA2	—	—	2.200	1.2	3.050	1.3
PIS	—	—	1.750	1.2	2.300	1.4
I[c]	62	38[d]	1.800	1.2	4.300	1.6
II[c]	83	17	2.150	1.1	4.700	1.2
III[c]	61	39	1.900	1.1	4.550	1.4
IV[c]	75	25	2.700	1.2	5.850	1.9

[a]L-lactic acid residues; [b]isosorbide succinate moieties; [c]see Table 1 for definitions; [d]21% (I) and 17% (S); molar composition: 14% (I) and 14% (S).

experiments starting from that approach produced materials with $\overline{M}_w$ ranged from 20.000 to 210.000 g mol^{-1}.

The $\overline{M}_n$ values obtained by MALDI-TOFMS were 42-76% lower than those obtained by SEC. Two possible reasons can explain this difference: (*i*) SEC data calculations were based on polystyrene as reference, which may not reflect correctly the hydrodynamic volumes of the samples and (*ii*) lighter molecules can be preferentially desorbed and ionized,[23] especially in the case of synthetic (co)polymers with polydispersity index higher than 1.2.[23]

Table 2 also presents the mass compositions of the products **I-IV** determined by ^{1}H NMR. The mass compositions ranged from 61-83% (LLA) to 39-17% (IS).

^{13}C NMR spectra (not shown) obtained for the homo-oligomers presented the following chemical shifts in the region of the carbonyl functional group: 169.6 ppm (PLLA2) and 171.4 and 171.1 ppm (PIS), in very good agreement with the data reported in literature.[4,24]

The ^{13}C NMR spectra obtained for **I-IV** in the expanded region of the carbonyl grouping (Figure 1) displayed either additional (**I-III**) or no additional (**IV**) peaks. A qualitative analysis revealed that product **I** exhibited ten additional peaks (171.4, 171.2, 171.1, 170.0, 169.8, 169.7, 169.5, 169.3, 169.2, and 169.0 ppm; Figure 1a), which could emerge from the interactions of the different moiety sequences randomly distributed along the co-oligomer chains, *i.e.*, a random co-oligomer was attained. Similar results were observed by Abe *et al.*[25] when they reported the appearance of six additional peaks in the C=O regions of ^{13}C NMR spectra obtained for random copolymers synthesized

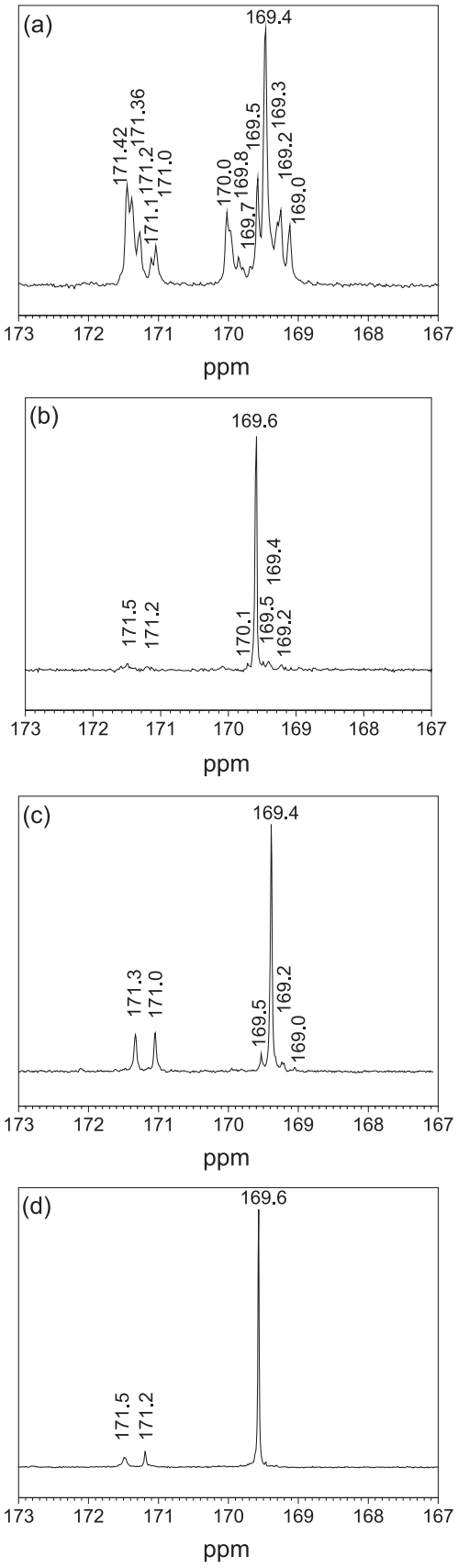


Figure 1. ^{13}C NMR spectra expanded in the carbonyl region obtained for the products (a) **I**, (b) **II**, (c) **III**, and (d) **IV**.

through ring-opening copolymerization from monomer mixtures of (R)-β-butyrolactone and L-lactide.

Conversely, product **IV** displayed no extra peaks (Figure 1d), implying that this could be a block co-oligomer showing lack of random sequences.[8,26] Kricheldorf has obtained copolymers possessing almost block structures from transesterification reactions between two homopolymers.[26]

Although Hiki *et al.*[27] have synthesized copolymers from ring-opening copolymerization of hydroxyl-terminated atactic PHB with L-lactide, the block nature of those products was stated based on the absence of signals due to the alternating HB-LA sequences in the C=O regions of ^{13}C NMR spectra. They also claimed that the detrimental transesterification reactions have not happen during the block copolymerrizations.[27]

Products **II** and **III** showed four (170.1, 169.5, 169.4, 169.2 ppm; Figure 1b) and three (169.5, 169.2, 169.0 ppm; Figure 1c) extra peaks of very weak intensities, respectively. One possible interpretation of these results is that block co-oligomers were obtained, but mild secondary transesterification reactions occurred and shorter blocks (than those that would be formed if transesterification reactions would have not happened) are expected as a result.

In addition to ^{13}C NMR results, MALDI-TOFMS measurements were performed in order to give additional evidence on the co-oligomers formation, as well as on their structures. Figures 2a and b illustrate, respectively, both full and expanded region at mass-to-charge ratio (*m/z*) ranging from 1500 to 2000 of the MALDI-TOF mass spectra obtained for **I** using NaI salt.

Figure 2b exhibits six series of peaks showing the variation of L-lactic acid residues along the series, plus four additional series of peaks exhibiting the variation of succinate and isosorbide moieties. The proposed adduct structures for the product **I** are depicted in Scheme 2.

The end groups shown in Schemes 2-4 are consistent with esterification and transesterification reactions involved in the formation of (co)polyesters,[11,12,14] in addition to sodium-cationized (co)polyester chains terminated with hydroxyl (or carboxyl) and sodium carboxylate end groups formed during MALDI-TOFMS measurements.[11,14]

The calculated *m/z* values for adducts ($[M+2Na-H]^+$, $[M+Na]^+$, or $[M+H]^+$) of the product **I** were obtained by applying equation 1.

$$\text{Adduct } m/z = 100x + 128y + 72z + CE + EG \quad (1)$$

where: x, y, and z correspond to the molar fraction of succinate, isosorbide, and L-lactic acid moieties randomly distributed along the co-oligomer chains, respectively; 100,

128, and 72 refer to the relative molar masses of succinate, isosorbide, and L-lactic acid residues, respectively; CE is the charged species and can assume values of 1, 23, and 46 referring to the relative atomic mass of a proton detached from the matrix, or one or two Na^+ stemmed from NaI salt, respectively; EG is the end group and can assume values of 18, 118, and 146 corresponding to the relative molar masses of hydrogen atom and hydroxyl, hydrogen atom and succinyl residue, and isosorbide residue and hydroxyl, respectively.

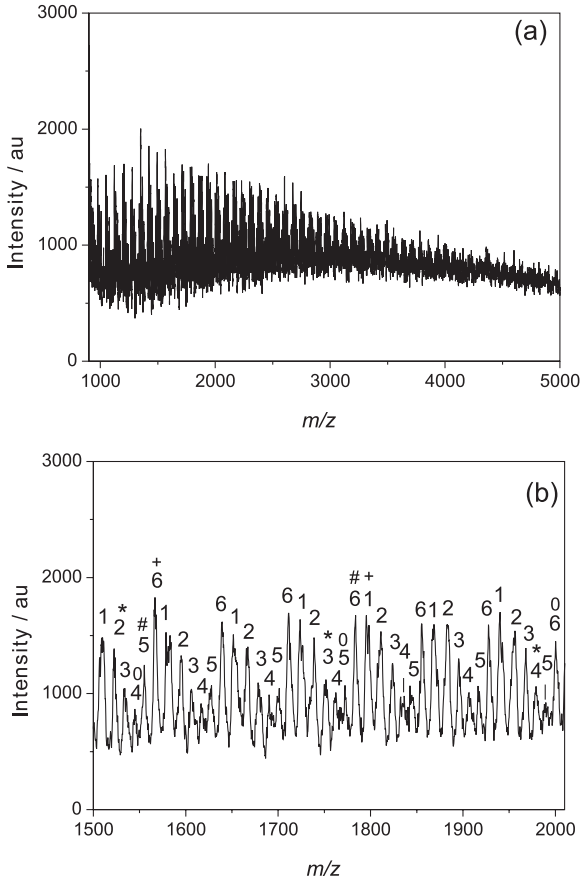


Figure 2. MALDI-TOF mass spectra of the product **I** acquired with NaI in (a) full region and (b) expanded region at *m/z* ranging from 1500 to 2000.

It should be pointed out that artifacts due isobaric effects are ruled out since the relative molar masses of the monomer moieties involved (100 for succinate, 128 for isosorbide and 72 for L-lactic acid) have no multiplicity relationship, enabling sequence analysis from MALDI-TOFMS measurements.

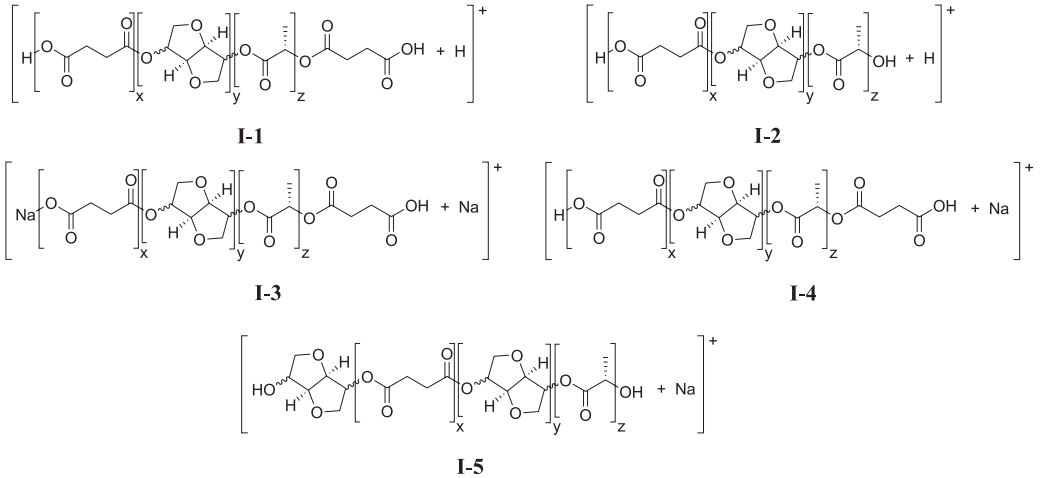
The calculated and experimental *m/z* values obtained for adducts of the product **I** appearing in Figure 2b are presented in Table 3. The calculated and experimental *m/z* values showed a good agreement (Table 3), suggesting that the product **I** is a co-oligomer. The presence of odd number of L-lactic acid moieties (Table 3) implies that intermolecular side transesterification reactions are involved.

Figures 3a and b show, respectively, both full and expanded region at *m/z* ranging from 1500 to 2000 of the MALDI-TOF mass spectra obtained for **II** using NaI. Figure 3b shows five series of peaks and exhibits several series of peaks in common with those shown in Figure 2b for the product **I**. Again, the five series of peaks show the variation of L-lactic acid mers and three extra series of peaks exhibiting the variation of isosorbide succinate repeating units are also depicted.

Scheme 3 illustrates the proposed adduct structures for **II** and the calculated *m/z* values for adducts ($[M+Na]^+$ or $[M+H]^+$) of the product **II** was obtained from equation 2 shown below.

$$\text{Adduct } m/z = 228(b+c) + 72a + CE + EG \quad (2)$$

where: b plus c stand for the total number of isosorbide succinate repeating units in both side blocks, a refers to



Scheme 2. Proposed adduct structures for the product **I**.

Table 3. Calculated and experimental *m/z* values for adducts of the product **I**

Peak series[a]	Adduct structures[b]	Calculated *m/z* values[c] (x,y,z)[d]	Experimental *m/z* values[e]
1	**I-2**	1507 (4,4,8); 1579 (4,4,9); 1651 (4,4,10); 1723 (4,4,11); 1795 (4,4,12); 1867 (4,4,13); 1939 (4,4,14)	1507.0; 1578.7; 1651.5; 1723.8; 1795.6; 1867.3; 1940.2
2	**I-5**	1525 (5,5,3); 1597 (5,5,4); 1669 (5,5,5); 1741 (5,5,6); 1813 (5,5,7); 1885 (5,5,8); 1957 (5,5,9)	1523.7; 1595.0; 1667.3; 1738.9; 1811.2; 1883.0; 1954.8
3	**I-1**	1535 (4,4,7); 1607 (4,4,8); 1679 (4,4,9); 1751 (4,4,10); 1823 (4,4,11); 1895 (4,4,12); 1967 (4,4,13)	1535.3; 1606.0; 1678.2; 1750.0; 1823.9; 1895.4; 1968.0
4	**I-1**	1547 (5,5,4); 1619 (5,5,5); 1691 (5,5,6); 1763 (5,5,7); 1835 (5,5,8);1907 (5,5,9); 1979 (5,5,10)	1545.1; 1617.9; 1690.1; 1762.5; 1836.4; 1906.5; 1979.1
5	**I-2**	1555 (2,2,15); 1627 (2,2,16); 1699 (2,2,17); 1771 (2,2,18); 1843 (2,2,19); 1915 (2,2,20); 1987 (2,2,21)	1555.2; 1627.9; 1699.0; 1772.2; 1842.5; 1916.4; 1988.1
6	**I-2**	1567 (3,3,12); 1639 (3,3,13); 1711 (3,3,14); 1783 (3,3,15); 1855 (3,3,16); 1927 (3,3,17); 1999 (3,3,18)	1567.0; 1639.0; 1711.5; 1783.8; 1855.2; 1927.6; 2000.3
*[f]	**I-1**	1523 (3,3,10); 1751 (4,4,10); 1979 (5,5,10)	1523.7; 1750.0; 1979.1
0[f]	**I-4**	1545 (3,3,10); 1773 (4,4,10); 2001 (5,5,10)	1545.1; 1772.2; 2000.3
+[f]	**I-2**	1567 (3,3,12); 1795 (4,4,12); 2023 (5,5,12)	1566.7; 1795.6; 2023.8[g]
#[f]	**I-3**	1555 (2,2,13); 1783 (3,3,13); 2011 (4,4,13)	1555.2; 1783.8; 2011.5[g]

[a]Peak series shown in Figure 2b for the MALDI-TOF mass spectrum obtained for **I**; [b]adduct structures presented in Scheme 2 for **I**; [c]calculated by applying equation 1; [d]x, y, and z correspond to, respectively, the molar fraction of succinate, isosorbide and L-lactic acid residues randomly distributed over the co-oligomer chains; [e]experimental data; [f]new peak series showing the variation of succinate and isosorbide moieties along those series; [g]values out of the previously established range.

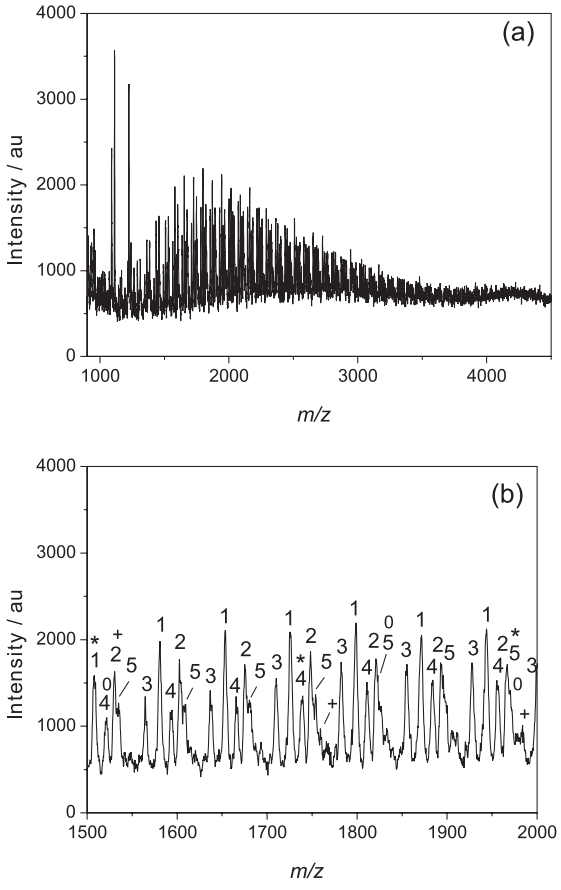


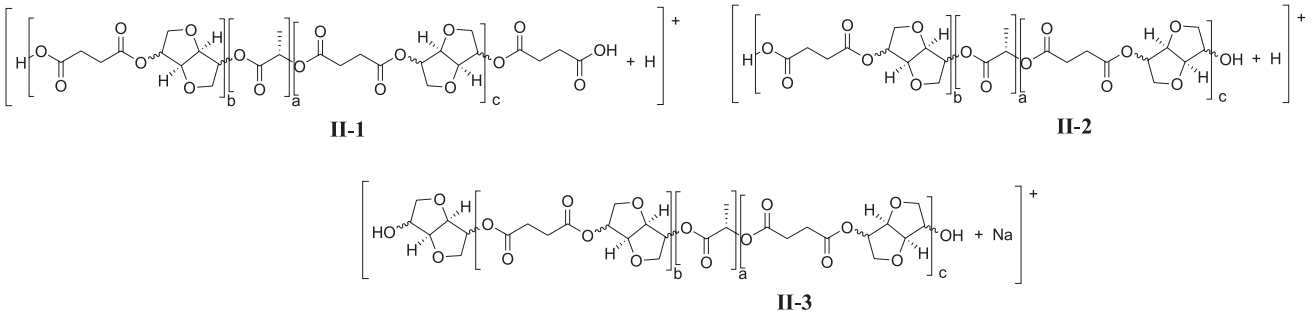
Figure 3. MALDI-TOF mass spectra of the product **II** acquired with NaI in (a) full region and (b) expanded region at *m/z* ranging from 1500 to 2000.

L-lactic acid repeating unit, and 228 correspond to the relative molar mass of isosorbide succinate repeating unit (for the definition of other terms, see equation 1).

As already mentioned, MALDI-TOFMS can be used to obtain sequence information since 228 is neither equal to nor multiple of 72. The calculated and experimental *m/z* values for adducts of the product **II** shown in Figure 3b are summarized in Table 4. The theoretical and experimental *m/z* values are in good agreement (Table 4), implying that **II** is a co-oligomer.

Both full and expanded region at *m/z* ranging from 1500 to 2000 MALDI-TOF mass spectra obtained for **IV** using NaI are shown in Figure 4a and b, respectively. Figure 4b shows three series of peaks exhibiting the variation of L-lactic acid repeating units. In general, these three series of peaks are quite close to the first three displayed in the spectrum obtained for **II** (Figures 4b and 3b), indicating structural similarities.

The MALDI-TOF mass spectra of **III** acquired with NaI for both full and expanded region at *m/z* ranging from 1500 to 2000 are illustrated in Figure 5a and b, respectively. Three series of peaks showing the variation of L-lactic acid mers and four series of peaks exhibiting the variation of isosorbide succinate repeating units along each series are displayed in Figure 5b. Scheme 4 shows the suggested adduct structures for **III**. The calculated *m/z* values for adducts ($[M+Na]^+$ or $[M+H]^+$) of the product **III** were obtained based on equation 2. Table 5 contains

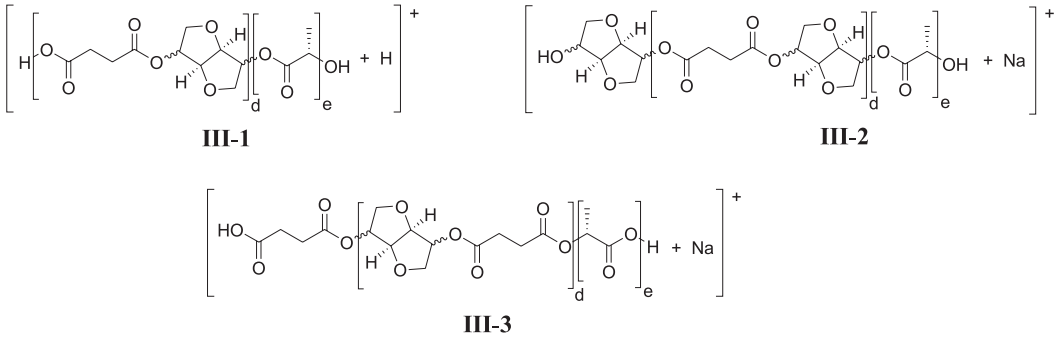


Scheme 3. Proposed adduct structures for the product **II**.

Table 4. Calculated and experimental m/z values for adducts of the product **II**

Peak series[a]	Adduct structures[b]	Calculated *m/z* values[c] (b+c,a)[d]	Experimental *m/z* values[e]
1[f]	**II-1**	1511 (2,13); 1583 (2,14); 1655 (2,15); 1727 (2,16); 1799 (2,17); 1871 (2,18); 1943 (2,19)	1509.3; 1580.8; 1653.6; 1725.0; 1798.8; 1871.6; 1943.8
2[f]	**II-2**	1531 (6,2); 1603 (6,3); 1675 (6,4); 1747 (6,5); 1819 (6,6); 1891 (6,7); 1963 (6,8)	1530.4; 1602.6; 1675.2; 1748.2; 1820.8; 1892.7; 1961.9
3[f]	**II-2**	1567 (3,12); 1639 (3,13); 1711 (3,14); 1783 (3,15); 1855 (3,16); 1927 (3,17); 1999 (3,18)	1564,5; 1636,8; 1710,2; 1782,2; 1854,9; 1927,6; 1999,2
4	**II-3**	1525 (5,3); 1597 (5,4); 1669 (5,5); 1741 (5,6); 1813 (5,7); 1885 (5,8); 1957 (5,9)	1522.9; 1594.3; 1667.2; 1739.6; 1810.9; 1884.0; 1955.3
5	**II-1**	1535 (4,7); 1607 (4,8); 1679 (4,9); 1751 (4,10); 1823 (4,11); 1895 (4,12); 1967 (4,13)	1534.8; 1609.0; 1680.3; 1753.0; 1823.1; 1894.0; 1966.9
*[g]	**II-1**	1511 (2,13); 1739 (3,13); 1967 (4,13)	1509.3; 1739.6; 1966.9
0[g]	**II-1**	1523 (3,10); 1751 (4,10); 1979 (5,10)	1522.9; 1753.0; 1978.2
+[g]	**II-2**	1531 (6,2); 1759 (7,2); 1987 (8,2)	1530.4; 1759.2; 1985.1

[a]Peak series depicted in Figure 3b for the MALDI-TOF mass spectrum obtained for **II**; [b]adduct structures presented in Scheme 3 for **II**; [c]calculated by applying equation 2; [d]both b and c (which were added up) and a refer to the number of isosorbide succinate and L-lactic acid repeating units, respectively; [e]experimental data; [f]those peaks were also observed in the MALDI-TOF mass spectrum obtained for the product **IV** (Figures 3b and 4b); [g]new peak series showing the variation of isosorbide succinate repeating units along those series.



Scheme 4. Proposed adduct structures for the product **III**.

both the calculated and the experimental *m/z* values for adducts of **III**. The co-oligomer nature of the product **III** is evidenced by the good agreement between the calculated and experimental *m/z* values (Table 5).

The presence of odd and even numbers of L-lactic acid repeating units are expected for adducts of **IV**, owing to transesterification reactions involving both PIS and PLLA hydroxyl end groups as nucleophilic agents. On the other hand, odd numbers of L-lactic acid repeating units given in Table 5 for adducts of **III** and shown in Table 4 for adducts of **II** suggest that undesirable side intermolecular transesterification reactions took place. As mentioned, mild secondary transesterification reactions were indicated (by additional peaks of very weak intensities) by ^{13}C NMR qualitative analysis for the products **II** and **III**.

Literature data show the presence of odd and even

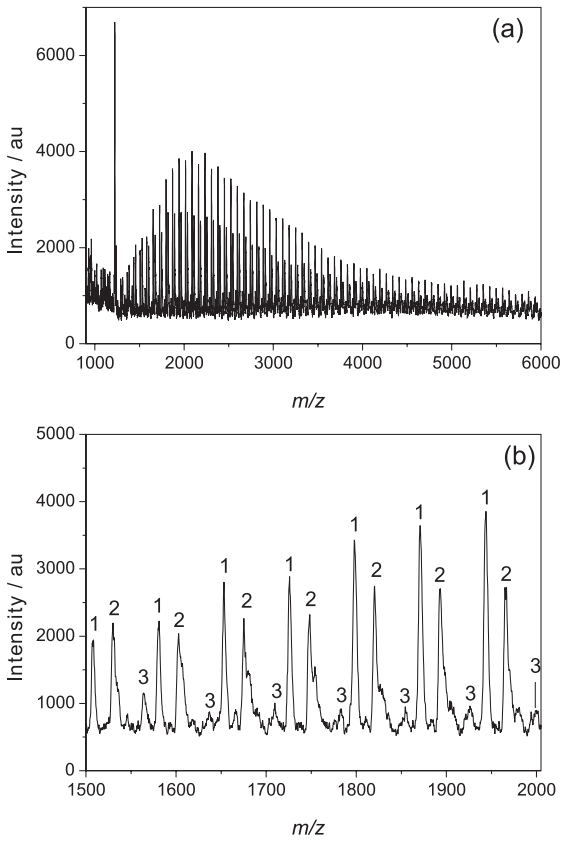


Figure 4. MALDI-TOF mass spectra of the product **IV** acquired with NaI in (a) full region and (b) expanded region at *m/z* ranging from 1500 to 2000.

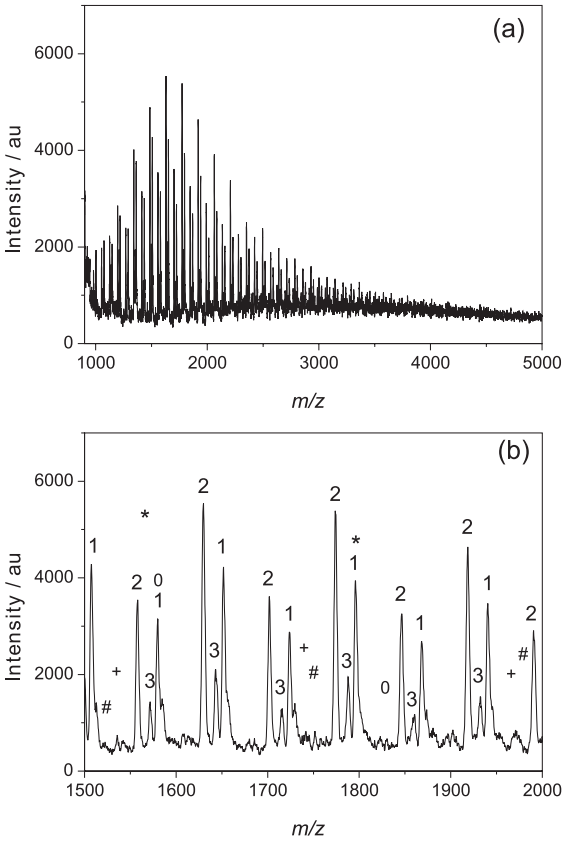


Figure 5. MALDI-TOF mass spectra of the product **III** acquired with NaI in (a) full region and (b) expanded region at *m/z* ranging from 1500 to 2000.

Table 5. Calculated and experimental *m/z* values for adducts of the product **III**

Peak series[a]	Adduct structures[b]	Calculated *m/z* values[c] (d,e)[d]	Experimental *m/z* values[e]
1	**III-1**	1507 (4,8); 1579 (4,9); 1651 (4,10); 1723 (4,11); 1795 (4,12); 1867 (4,13); 1939 (4,14)	1507.5; 1580.1; 1651.8; 1723.8; 1795.8; 1868.1; 1940.4
2	**III-3**	1557 (4,7); 1629 (4,8); 1701 (4,9); 1773 (4,10); 1845 (4,11); 1917 (4,12); 1989 (4,13)	1558.0; 1629.9; 1701.9; 1774.0; 1846.6; 1918.5; 1990.5
3	**III-2**	1573 (3,10); 1645 (3,11); 1717 (3,12); 1789 (3,13); 1861 (3,14); 1933 (3,15)	1571.6; 1643.1; 1715.9; 1787.8; 1860.6; 1932.3
*[f]	**III-1**	1567 (3,12); 1795 (4,12); 2023 (5,12)	1567.1; 1795.8; 2024.0[g]
0[f]	**III-1**	1579 (4,9); 1807 (5,9); 2035 (6,9)	1580.1; 1808.7; 2033.4[g]
+[f]	**III-2**	1513 (4,6); 1741 (5,6); 1969 (6,6)	1512.8; 1741.6; 1968.8
#[f]	**III-2**	1525 (5,3); 1753 (6,3); 1981 (7,3)	1524.6; 1751.5; 1980.1

[a]Peak series shown in Figure 5b for the MALDI-TOF mass spectrum obtained for **III**; [b]adduct structures presented in Scheme 4 for **III**; [c]calculated based on equation 2; [d]d and e refer to the number of isosorbide succinate and L-lactic acid repeating units, respectively; [e]experimental data; [f]new peak series showing the variation of isosorbide succinate repeating units along those series; [g] values out of the previously established range.

numbers of L-lactic acid repeating units obtained by MALDI-TOFMS for poly(L-lactide), even when synthesized at mild conditions.[28-30] Moreover, the obtained products were almost 100% isotactic, *i.e.*, racemization did not take place.[29,30] Lee *et al.*[31] also observed by MALDI-TOFMS odd and even numbers of L-lactic acid mers in their block copolymers synthesized via ring-opening copolymerization of L-lactide with poly(ethylene oxide) or poly(ethylene glycol).

MALDI-TOFMS has been used to obtain sequence information on synthetic copolymer structures.[11,14,15,31,32] For example, a good agreement between experimental and calculated *m/z* data has been used as evidence that copolymer structures were attained.[11,14,15,31,32] In a similar

manner, our work was also based on that assumption, in addition to using supporting techniques.

WAXD was used as supporting technique to determine the degrees of crystallinity of the powdered samples previously annealed. The samples of PIS and **I** were not annealed. The diffraction pattern obtained for PLLA2 is similar to that reported in literature,[33,34] while the diffraction pattern obtained for PIS showed, as expected,[4] only an amorphous halo, characteristic of non-crystalline samples. The diffraction patterns obtained for **II**-**IV** are comparable with that obtained for PLLA2, whilst that obtained for **I** is comparable with that of PIS (see Supplementary Information). This is strong evidence that while **I** is a fully random copolymer, **II**-**IV** preserves blocks of L-lactic acid in its structure.

The degrees of crystallinity (Table 6) were obtained from WAXD profiles with curve decompositions following Gauss or Lorentz function fits (Supplementary Information). They amount to 71% (PLLA2), 0% (PIS), 0% (**I**), 41% (**II**), 40% (**III**), and 52% (**IV**). Shorter blocks and loss in crystallinity are expected as the consequence of undesirable side transesterification reactions. Therefore, the data presented in Table 6 confirms the occurrence of mild secondary transesterification reactions involved during the syntheses of **II** and **III**.

Table 6. Degrees of crystallinity obtained by WAXD for the powdered samples previously annealed (48 h at 90 ºC)

Run	χ_c / (%)[a]
PLLA2	71
PIS	—[b]
I	—[b]
II	41
III	40
IV	52

[a]Degree of crystallinity; [b]observed only an amorphous halo for each sample, which is expected for the scattering patterns produced by non-crystalline samples.

Conclusions

Four types of products (**I**-**IV**) with low average molar masses were synthesized from different approaches *via* copolymerization techniques. The calculated *m/z* values for adducts of **I**-**IV** were in good agreement with the experimental values observed by MALDI-TOFMS, indicating that co-oligomers were obtained.

Although the analysis of ^{13}C NMR data was done in qualitative terms, the findings were consistent with those attained by MALDI-TOFMS. ^{13}C NMR data indicated that mild secondary transesterification reactions took place during the syntheses of **II** and **III** and blocks of shorter size (than those that would be formed if transesterification reactions would have not happened) are expected as a result. MALDI-TOFMS data also suggested that undesirable side intermolecular transesterification reactions were involved during the production of **I**-**III**. The WAXD profiles showed that **I** is completely amorphous and **II**-**IV** are semicrystalline. The degrees of crystallinity obtained by WAXD showed that a mild loss in crystallinity occurred for **II** and **III**, suggesting a shortening of the blocks due to side transesterification reactions, in agreement with ^{13}C NMR data. The data obtained by MALDI-TOFMS together with those by ^{13}C NMR and WAXD gave evidence that random (**I**) and block (**II**-**IV**) co-oligomers were attained. The most promising approach to obtain products with higher average molar masses towards biomaterial application was that used to synthesize the product **III**.

Acknowledgments

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Supplementary Information

Supplementary data are available free of charge at http:jbcs.sbq.org.br, as PDF file.

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Synthesis and Structural Characterization of Block and Random Low Molecular Weight Copolymers Composed of L-lactic Acid and Isosorbide Succinate Moieties

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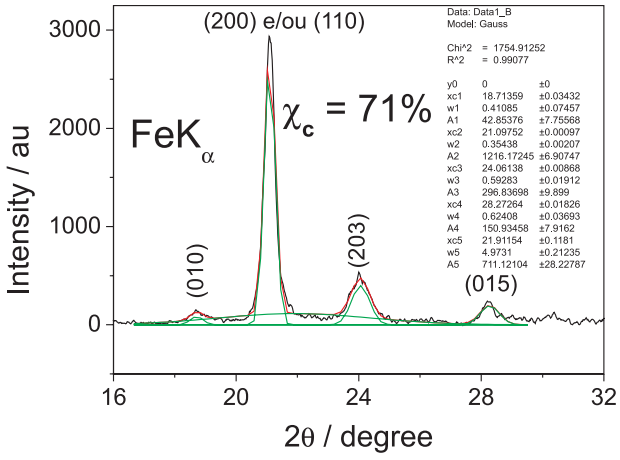

Figure S1. WAXD patterns obtained for powdered samples previously annealed (48 h at 90 ºC) of PLLA2 with curve decompositions following Gauss function fit (R^2 = 0.99). "A5" corresponds to the amorphous halo areas.

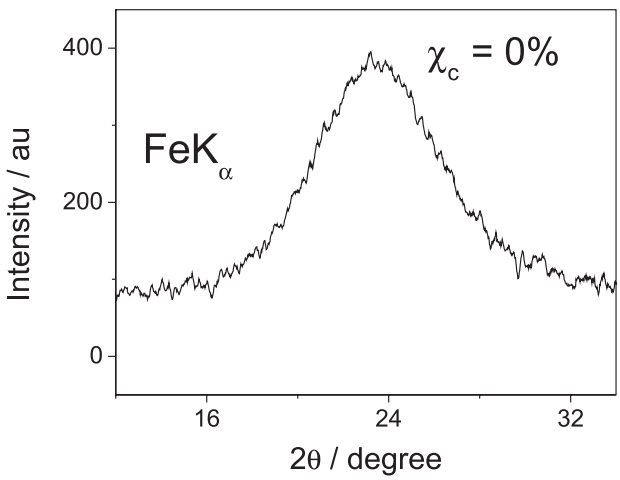

Figure S2. WAXD patterns obtained for powdered samples of PIS.

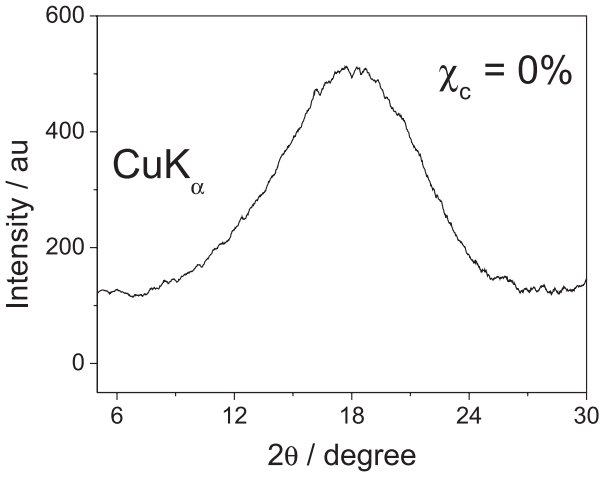

Figure S3. WAXD patterns obtained for powdered samples of **I**.

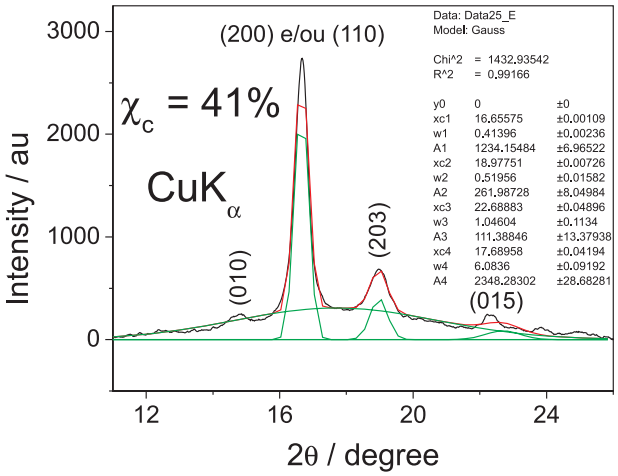

Figure S4. WAXD patterns obtained for powdered samples previously annealed (48 h at 90 ºC) of **II**, with curve decompositions following Gauss function fit (R^2 = 0.99). "A4" corresponds to the amorphous halo areas.

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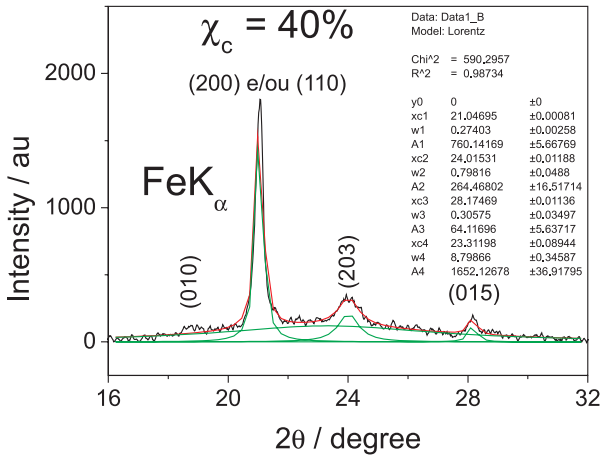

Figure S5. WAXD patterns obtained for powdered samples previously annealed (48 h at 90 ºC) of **III**, with curve decompositions following Lorentz function fit (R^2 = 0.99). "A4" corresponds to the amorphous halo areas.

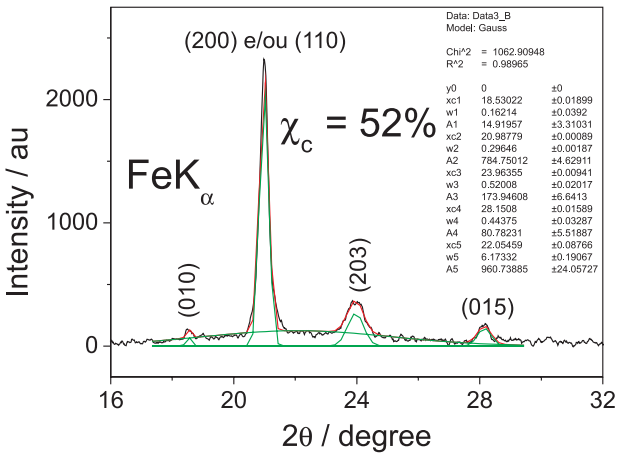

Figure S6. WAXD patterns obtained for powdered samples previously annealed (48 h at 90 ºC) of **IV**, with curve decompositions following Gauss function fit (R^2 = 0.99). "A5" corresponds to the amorphous halo areas.