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ORIGINAL PAPER

Aggregation of polylactide with carboxyl groups at one chain end in the presence of metal cations

Melania Bednarek · Malgorzata Basko · Tadeusz Biedroń · Przemysław Kubisa · Mirosław Pluta

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Abstract Polylactides with one or more carboxyl groups at one chain end were synthesized by cationic polymerization according to activated monomer mechanism and by application of "thiol-yne" click chemistry for subsequent functionalization. End groups of such obtained polylactides were converted into ionic groups by neutralization of polymer solutions with metal oxides, mainly calcium oxide, and the aggregation of individual stereoisomers as well as that of the mixture of poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) was investigated. The extent and progress of the aggregation was followed by viscosity measurements, and aggregated polymers in the solid state were examined by SEM and DSC. Solution viscosity increase was observed upon the aggregation of individual PLA stereoisomers, whereas PLA stereocomplex precipitation occurred in the case of the aggregation of PLLA/PDLA/metal oxide mixture.

Keywords Cationic polymerization · Biocompatible polymers · Polylactide · Aggregation

Introduction

Polylactide (PLA) as a biodegradable, biocompatible polyester has become one of the most intensively studied polymers in the recent years. Its existing and potential applications inspire many research groups to elaborate new methods of polymer synthesis or to improve those already existing, as well as to study the PLA's properties. With respect to biomedical applications, the behavior of polylactide in biological environment may be very important. Many aspects of

M. Bednarek (✉) · M. Basko · T. Biedroń · P. Kubisa · M. Pluta
Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-362 Lodz, Poland
e-mail: bednarek@cbmm.lodz.pl

polymer interactions with organic or inorganic components of biological systems can be considered. Among them, the interaction of PLA chains with metal cations, e.g., Ca^{2+} naturally occurring in biological environment, is worth studying.

PLA synthesized by typically applied methods, i.e., by ring-opening polymerization of cyclic diester-lactide, usually contains carboxyl group at one chain end [1, 2]. We have recently studied the aggregation of other biodegradable polyester—poly(ε-caprolactone) (PCL), containing different numbers of carboxyl groups at one chain end, in the presence of calcium cations [3], and found that the extent of aggregation depended strongly on the number of carboxyl groups (strong synergistic effect) and that PCL chains containing calcium carboxylate groups formed long cylindrical aggregates.

Although the aggregation phenomena of polymers containing ionic groups along their polymer chain have been studied extensively [4, 5], only few papers appeared concerning the aggregation of polymers with an ionic group at one chain end [6–10]. Biocompatible polyesters have not been studied in this respect and our previous article concerning PCL aggregation was the first example. Consequently, we decided to extend our studies to another biocompatible polyester, namely polylactide containing carboxyl groups at its chain end, in the presence of metal cations.

Lactide, in contrast to previously studied caprolactone, contains a chiral center, thus it may exist in two enantiomeric forms. Polymerizations of L- or D-lactide lead to two enantiomeric forms of polymer, namely poly(L-lactide) or poly(D-lactide). It is known that poly(L-lactide) and poly(D-lactide) form stereocomplexes with physical properties different from those of individual stereoisomers [11]. Thus, aggregation of a mixture of enantiomeric polylactides containing ionic groups should involve, in addition to interaction of ionic groups observed earlier for polycaprolactone, also an interaction of two chains of opposite chirality.

The understanding of this combined effect may be interesting from scientific point of view as well as with respect to biomedical applications.

In this article, we describe the results of aggregation studies of PLA containing carboxyl groups at one chain end with consideration of both PLA stereoisomers. We also present methods of the synthesis of polylactide containing one or more carboxyl groups at one chain end. These methods are based on cationic polymerization of cyclic diester-lactide and on post-functionalization by applying "click chemistry" widely used in recent years in the synthesis of functional polymers [12–15]. Cationic polymerization performed in the presence of an initiator containing hydroxyl groups and additional functional group proceeds by activated monomer (AM) mechanism [16] and is a very convenient method of the synthesis of functional polymers.

Two different strategies were applied. The first was one-step synthesis by applying hydroxyacid as an initiator, while the second was two-step strategy—synthesis of PLA with an unsaturated end group with subsequent functionalization by addition of a thiol containing carboxyl groups.

Experimental

Materials

L-Lactide (L-LA, Boehringer Ingelheim, Germany) and D-lactide (D-LA, Purac) were crystallized from dry 2-propanol.

Glycolic acid was dried on vacuum line. Propargyl alcohol (99 %, Aldrich) was stored over molecular sieves and distilled in vacuum before use.

Trifluoromethanesulfonic acid (triflic acid, 98 %, Aldrich), mercaptosuccinic acid (MSA, 99 %, Aldrich), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99 %, Aldrich), CaO (p.p.a., POCh, Poland), Fe_2O_3 (99 %, Aldrich), ZnO (99 %, Aldrich) and cholecalciferol (vitamin D_3, 98 %, Sigma) were used as received.

Dichloromethane (p.p.a., POCh, Poland) and 1,4-dioxane (pure, Chempur, Poland) were dried over calcium hydride and distilled. 1,3-Dichloropropane (99 %, Aldrich), tetrahydrofuran (HPLC grade, Baker) diethyl ether (p.p.a., Chempur, Poland) and acetonitrile (HPLC grade, POCh, Poland) were used as received.

Synthesis of PLA with one carboxyl group at the chain end

A Schlenk tube was charged with L- or D-lactide (2 g, 13.9 mmol) and glycolic acid (9.5 mg, 0.125 mmol), closed with a rubber septum, degassed on the vacuum line and filled with nitrogen. The tube was placed in an oil bath and heated to 105 °C to melt the monomer. Then triflic acid (30 μL of 10 % solution in dichloroethane) was added with a syringe through the rubber septum. The polymerization was conducted at ~105 °C in nitrogen atmosphere with stirring (magnetic stirrer) for about 3 h (after about 2.5 h solidification of polymer was observed, the polymerization vessel was heated for the next 0.5 h). Solid polymer was crushed, washed three times with distilled water to remove catalyst and dried for 5 h at about 40 °C in vacuum. The polymer was analyzed by ^{1}H NMR and MALDI TOF.

Synthesis of PLA with propargyl group at the chain end

L- or D-Lactide (2 g, 13.9 mmol) was placed in a Schlenk tube which was degassed on vacuum line. The tube was filled with nitrogen, next dichloromethane was added with a syringe through the rubber septum followed by the addition of propargyl alcohol (17 μL, 16.5 mg, 0.29 mmol) and triflic acid (8 μL). Polymerization was conducted during about 18 h at room temperature with stirring (magnetic stirrer), then solvent was evaporated, solid polymer was crushed and washed three times with water. The polymer was analyzed by ^{1}H NMR and MALDI TOF. Mn = (^{1}H NMR) ~3,600.

Coupling reaction of propargyl-PLA with mercaptosuccinic acid

0.4 g of propargyl-PLA (Mn ~3,600, ~0.1 mmol of alkyne groups) together with 0.60 g of mercaptosuccinic acid (4 mmol) and ~3 mg of DMPA (~0.012 mmol) were dissolved in 10 mL of THF in a round-bottom flask which was closed with a

rubber septum. The flask was degassed on vacuum line (using a needle) and filled with nitrogen. This cycle was repeated three times. Next the reaction mixture was irradiated with UV lamp (365 nm) for 110 min at room temperature with stirring. Then polymer was precipitated into cold diethyl ether and the precipitate (after decantation of the solvent) was washed twice with Et_2O. The polymer was dried on vacuum line.

Viscosity measurements of PLA-$(COOH)_x$ solutions after addition of CaO

To compare the behavior of solutions of polymers with different numbers of –COOH groups in the presence of calcium cations, 10 wt% solutions of PL-LA-$(COOH)_1$ and PL-LA-$(COOH)_{2.8}$ in 1,2-dichloropropane were prepared and twofold excess of CaO with respect to –COOH groups was added. Thus, 0.1 g of PLLA-$(COOH)_1$ ($\sim$0.027 mmol of –COOH groups) was dissolved in 1 mL of 1,2-dichloropropane and was mixed with 0.003 g of CaO (0.054 mmol). The suspension was vigorously stirred (magnetic stirrer) for about 15 min until the solution became almost clear. Then $\sim$0.3 mL of solution was transferred to the measuring cell of viscosimeter with a pipette, the cell was placed in the instrument and the viscosity was measured periodically without removing the solution from the measuring cell.

Preparation of PLA stereocomplex microparticles and encapsulation of vitamin D_3

10 wt% solutions of PLLA-$(COOH)_1$ and PDLA-$(COOH)_1$ were prepared by dissolving 0.05 g of each polymer ($\sim$0.013 mmol of –COOH groups) in 0.5 mL of 1,2-dichloropropane. Solutions were mixed together with 0.003 g of CaO (0.054 mmol, twofold molar excess with respect to the total number of –COOH groups). The suspension was vigorously stirred (magnetic stirrer) for about 15 min and then was left for about 2 h. After that time two phases were well separated—the precipitate (fraction 1) at the bottom of the flask and the solution above (fraction 2). After decantation, the precipitate was dried in vacuum and analyzed by SEM.

In the attempt of vitamin D_3 encapsulation during PLA stereocomplexation, the same procedure was applied, vitamin D_3 (0.0157 g, 16 wt% with respect to PLAs) was added together with CaO during mixing of PLLA and PDLA solutions. Fraction 1 (precipitate) (0.063 g) and Fraction 2 (solution) (0.096 g) were separated. Both fractions were analyzed by ^{1}H NMR. Fraction 2 contained vitamin D_3 with traces of PLA not involved in stereocomplex. Fraction 1 contained PLA and vitamin D_3 (^{1}H NMR spectrum of precipitate and SEM picture are presented in Fig. 13).

Methods of materials analysis

^{1}H NMR spectra were recorded in $CDCl_3$ using a Bruker DRX500 instrument operating at 500 MHz.

Size-exclusion chromatography (SEC) was performed using an Agilent Pump 1100 Series with Agilent G1379A Degasser and a set of two PL-Gel 5 μ mixed-C

columns. Wyatt Optilab Rex interferometric refractometer and multi-angle laser light scattering (MALLS) Dawn Eos laser photometer (Wyatt Technology Corp., Santa Barbara, CA) were used as detectors. Dichloromethane was used as an eluent at a flow rate of 0.8 mL min^{-1} at room temperature. The system was calibrated according to polystyrene standards.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) measurements were performed with the Voyager Elite (PerSeptive Biosystems, Framingham, MA) time-of-flight instrument equipped with a pulsed N_2 laser (337 nm) and time-delayed extraction source. The accelerating voltage of 20 kV was used. Dithranol was used as a matrix, CF_3COOK as a cationating agent and THF as a solvent.

Viscosity measurements were performed at 25 °C using Brookfield DV-II + viscosimeter with S52 spindle and shear rate (SR) of 10 s^{-1} (which corresponds to 5 RPM spindle rotation).

Scanning electron microscopy (SEM) images were taken using Jeol JSM-5500LV apparatus working in the secondary electron mode with an accelerating voltage of 10 kV.

Differential scanning calorimetry (DSC) analysis was performed under nitrogen at a heating and cooling rate of 10 °C/min on DSC 2920 Modulated TA Instrument. Both temperature and heat flow were calibrated with indium.

Wide angle X-ray scattering (WAXS) analysis was performed by measuring X-ray intensity versus 2θ (from 10° to 40°) in the transmission mode-coupled $\theta/2\theta$. A wide-angle computer controlled goniometer coupled to a sealed-tube source of filtered Cu Kα radiation operating at 30 kV and 50 mA (Philips PW3830) was used. The split system of the diffractometer was adjusted to measure the integral intensity of a given diffraction peak.

Results and discussion

Synthesis of PLA with one carboxyl group at the chain end

Cationic polymerization in which hydroxyacids were used as initiators was successfully used by us earlier for the synthesis of poly(ε-caprolactone) containing different numbers of –COOH groups at one chain end [3]. We tried to apply the same method to the synthesis of functional PLAs. Thus, poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) with one carboxyl group at the chain end were obtained by cationic bulk polymerization performed in the presence of glycolic acid as an initiator as it is shown in Fig. 1.

Cationic polymerization, in contrast to anionic or coordination polymerization, proceeds smoothly in the presence of free carboxyl groups. Bulk polymerization of lactide was performed at 105 °C, i.e., slightly above the monomer melting point (T_m of lactide = 96 °C [17]).

In GPC chromatograms monomodal molecular weight distribution was observed but the determination of true M_n values from GPC analysis is doubtful because of the lack of appropriate standards (in some publications correction factors are used

Fig. 1 Schematic representation of the synthesis of PLA with one carboxyl group at one chain end

but such procedure may be questioned for relatively low molecular weight polymers) [18, 19]. Therefore, for the determination of M_n we relied mainly on ^{1}H NMR analysis which allowed the calculation of molecular weights more reliably by comparison of the intensities of signals corresponding to terminal methine group with those corresponding to methine groups from polymer backbone. On the basis of ^{1}H NMR spectra number average molecular weights M_n were determined as equal to ~3,600 for both PLLA-COOH and for PDLA-COOH.

MALDI TOF analysis (see Fig. 2) showed, however, that only part of macromolecules was initiated with glycolic acid and about half of the whole macromolecules population was initiated with water. Water was probably formed at elevated temperature as a by-product of condensation of hydroxyacid or its higher analogs. Fortunately macromolecules initiated with water have also –COOH and –OH end groups, thus, from the point of view of end-group structure, both series of macromolecules are almost the same, which is important for our studies.

Synthesis of PLA with several carboxyl groups at one chain end

First approach to the synthesis of polylactide with several carboxyl groups at one chain end was based on the same strategy as the synthesis of PLA with one –COOH group. Thus, cationic polymerization was performed in the presence of hydroxy acids with two (malic acid) or three (citric acid) carboxyl groups. Such method was successfully used earlier for polymerization of ε-caprolactone [3]. First experiments

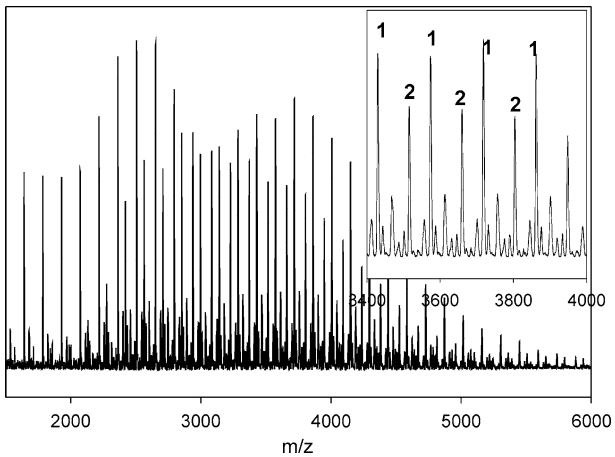

Fig. 2 MALDI TOF spectrum of PDLA-COOH obtained by polymerization initiated with glycolic acid: series *1* corresponds to macromolecules initiated with glycolic acid, series *2* corresponds to macromolecules initiated with water

concerning bulk or solution cationic lactide polymerization showed, however, that it is not possible to obtain fully functionalized polymers by this approach. MALDI TOF analysis of obtained products indicated that significant fraction (over 80 %) of macromolecules was initiated with water. Water was present in the polymerization system as a by-product of the condensation reaction or dehydration product of β-hydroxyacids. Dehydration is competitive to polymerization and was fast enough in comparison with the initiation of polymerization by hydroxyacid to generate a new much more reactive initiating molecule, i.e., water (dehydration was not competitive in the case of the much more reactive monomer—ε-caprolactone). The initiation of polymerization with water resulted in macromolecules containing one –COOH group at the chain end so this method could not lead to PLAs with two or three –COOH groups at the chain end. In this situation another strategy was applied. The PLA functionalization was achieved in two steps. First step was the synthesis of PLA with unsaturated end group to which, in the next step, a thiol containing –COOH groups was attached. We have recently described the results of such functionalization and compared several combinations of PLAs, thiols containing –COOH groups and reaction conditions [20]. The application of thiol-yne coupling by which two thiol molecules could be attached to one PLA chain seemed to be most promising. Thus, functionalized PLA with more than one carboxyl group at the chain end was obtained by the synthesis of PLA with propargyl end group with subsequent thiol-yne coupling with mercaptosuccinic acid. Figure 3 presents the applied strategy.

Efficiencies of both stages were checked by ^{1}H NMR and MALDI TOF analysis. As it was found, in the first step PLA with almost all (over 95 %) macromolecules functionalized with alkyne end groups was obtained. The efficiency of functionalization by thiol-yne coupling was not so good (see Fig. 4) which was in accordance with our earlier observation [20].

In the MALDI spectrum of the product of coupling reaction (Fig. 4) four main series of signals are visible. The assignment of signals is complicated because of the overlapping of signals of macromolecules with different structures. As it can be seen in Fig. 4a, all signals in the groups 1 and 2 are wide, as they contain several signals. Signals 1A and 2A are narrower. The MALDI spectrum shown in Fig. 4b registered in reflector mode displays a splitting of main signals which confirms the presence of different signals under the wide signal. Thus, we concluded that Signal

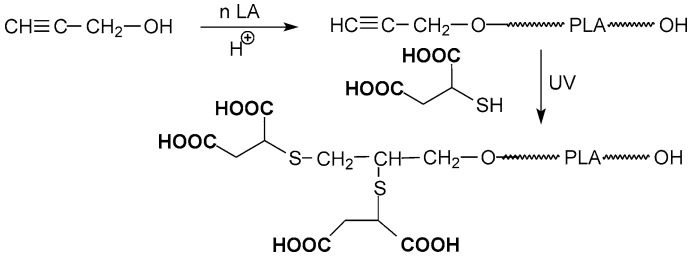

Fig. 3 Schematic representation of the synthesis of PLA with several carboxyl groups at one chain end

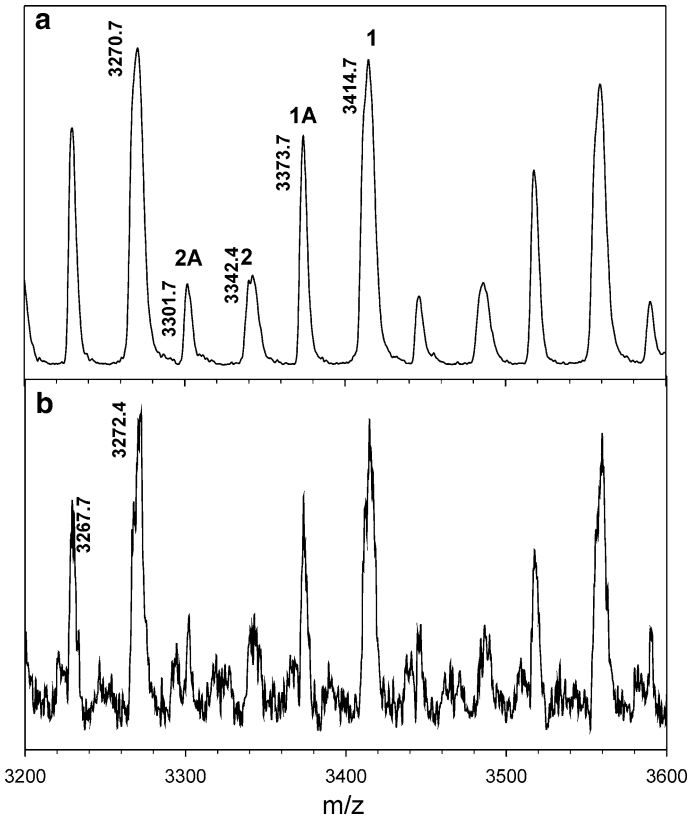

Fig. 4 Fragments of MALDI TOF spectra of the product of coupling reaction of propargyl-PDLA with MSA: **a** registered in linear mode, **b** registered in reflector mode

1 around $m/z \sim 3{,}414$ corresponds to functionalized macromolecules: $M_1 = 21 \times 144.13 + 56 + 150 + 150 + 39 = 3{,}421.7$ and $M_2 = 22 \times 144.13 + 56 + 150 + 39 = 3{,}415.9$ and unfunctionalized macromolecule: $M_3 = 23 \times 144.13 + 56 + 39 = 3{,}410.0$. Signal 1A around $m/z \sim 3{,}373$ corresponds to macromolecules cationated with proton (instead of potassium): $M_4 = 22 \times 144.13 + 56 + 150 + 1 = 3{,}377.9$ and $M_5 = 23 \times 144.13 + 56 + 1 = 3{,}372.0$. Signal 2 around $m/z \sim 3{,}342$ corresponds to macromolecules which underwent transesterification: $M_6 = 41 \times 72.07 + 56 + 150 + 150 + 39 = 3{,}349.9$ and $M_7 = 43 \times 72.07 + 56 + 150 + 39 = 3{,}344.0$ and possibly also unfunctionalized macromolecules: $M_8 = 45 \times 72.07 + 56 + 39 = 3{,}338.2$. Signal 2A around $m/z \sim 3{,}301$ corresponds to transesterified macromolecules cationated with proton: $M_9 = 43 \times 72.07 + 56 + 150 + 1 = 3{,}306.0$ and unfunctionalized ones: $M_{10} = 45 \times 72.07 + 56 + 1 = 3{,}300.2$.

Summarizing the analysis of MALDI TOF spectra recorded for the product of thiol-yne coupling between propargyl-PDLA and MSA (mercaptosuccinic acid), the majority of macromolecules were functionalized with two thiol molecules but

unfunctionalized ones and those functionalized with one thiol molecule were also present.

Thus, MALDI TOF analysis cannot give unequivocal information about the average functionalization efficiency. In this situation, we rather relied on ^{1}H NMR analysis. On the basis of ^{1}H NMR spectrum presented in Fig. 5 the functionalization degree of propargyl-PDLA with MSA was estimated as $\sim$70 % (similar value was found in the case of propargyl-PLLA functionalization), with an assumption that two thiol molecules can be attached to one alkyne group. Calculations were performed by comparison of the intensity of methylene and methine groups in the vicinity of sulfur present in attached thiol group with the intensity of methine end-group –CH(CH_3)–OH or by comparison of the intensity of methylene from residual unreacted propargyl group with the intensity of –CH(CH_3)–OH group.

Thus, we concluded that although the applied method did not yield a uniform product of desired structure, majority of macromolecules indeed contained several carboxyl groups at the chain end. Such products were used for aggregation studies.

Aggregation of PLA with carboxyl groups at the chain end in the presence of metal cations

Aggregation of PLLA-(COOH)$_x$ stereoisomer

Results of earlier studies indicated that polymers containing ionic groups at the chain end tend to form spherical or cylindrical aggregates in solution in nonpolar solvent, in the presence of metal cations [3, 6, 9]. Shapes and sizes of aggregates depend on many factors. The results of our previous work [3] concerning the aggregation of poly(ε-caprolactone) polymers with different numbers of carboxyl groups at one chain end showed that the aggregation tendency depended on the concentration and molecular weight of polymer, the excess of metal cations with

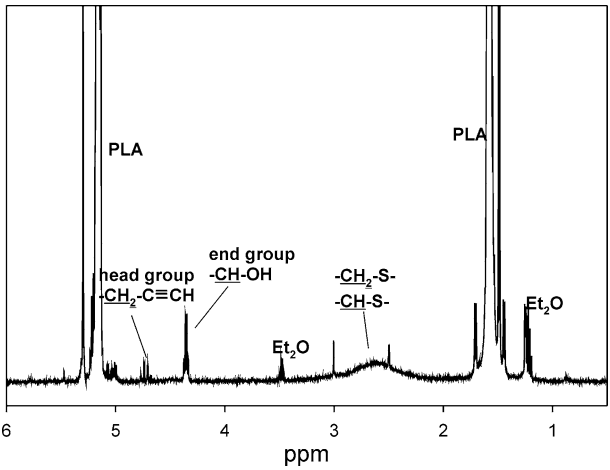

Fig. 5 ^{1}H NMR spectrum of the product of coupling reaction of propargyl-PDLA with MSA

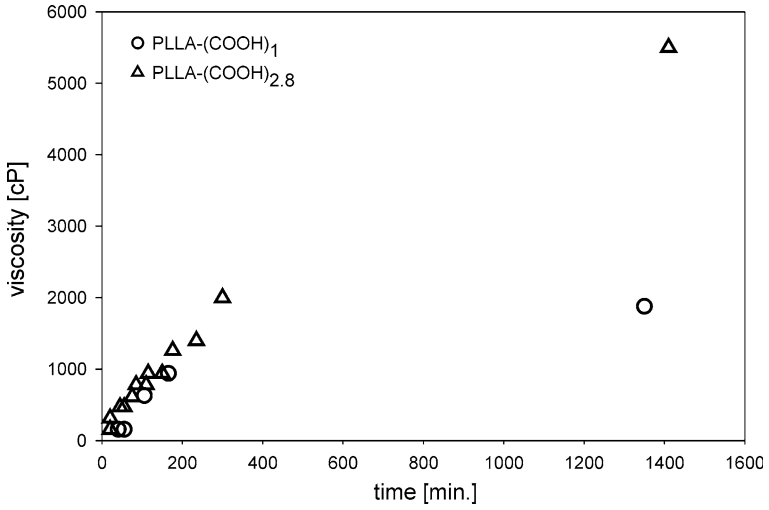

Fig. 6 The evolution of viscosity of 1,3-dichloropropane solutions of PLLA polymers after addition of CaO; [CaO]/[–COOH] = 2

respect to –COOH groups, the size of metal cation, but most of all on the number of ionic groups. The aggregation progress, after addition of metal oxide, manifested itself in gradual increase of solution viscosity (usually from about 100 cP to $10^4 \div 10^6$ cP) up to the formation of stable polymer gels. SEM analysis of dried PCL-$(COOH)_x$ gels formed upon the aggregation indicated the formation of long cylindrical structures.

The aggregation tendency of PLA containing carboxyl groups at the chain end was investigated, similarly to PCL polymers [3], by viscosity measurements. Aggregations of two PLA samples were compared: poly(L-LA) with only one –COOH group at the chain end obtained by cationic polymerization initiated with glycolic acid and poly(L-LA) with more than two –COOH groups on average (PLLA-$(COOH)_{2.8}$) obtained by polymerization initiated by propargyl alcohol and coupled with mercaptosuccinic acid. Figure 6 presents viscosity–time profiles obtained as a result of viscosity measurements performed with 1,2-dichloropropane solutions of those PLLAs after addition of calcium oxide.

The solution viscosities increased, although these increases were not as significant as observed previously for PCL-$(COOH)_x$ polymers and gels which formed in the end were not stable (the gels destroyed upon shaking). In spite of low solution viscosity increases, higher value of viscosity was achieved in the case of PLA with a higher number of carboxyl groups.

Relatively modest increase of viscosity (much lower than in the case of the earlier studied ε-caprolactone polymers) indicated a low tendency to aggregation. That was confirmed by SEM analysis of products after solvent evaporation (Fig. 7).

As it can be seen in Fig. 7, no specific forms can be detected in the SEM images of dried PLLA gels after the aggregation. Rather moderate viscosity increases upon the neutralization of PLLAs solutions with calcium cations and the absence of

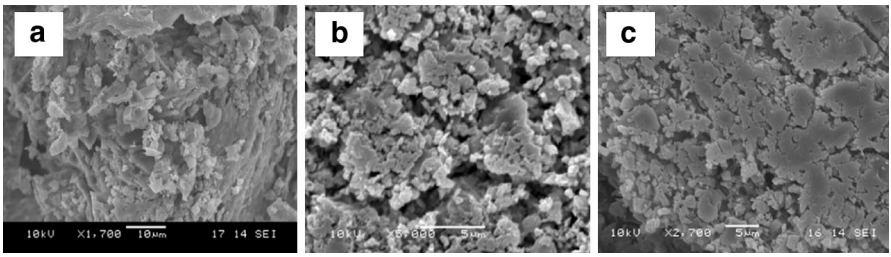

Fig. 7 SEM images of PLLA-$(COOH)_1$/CaO **a** and PLLA-$(COOH)_{2.8}$/CaO, **b** after drying of gels obtained at the end of polymers aggregation in 1,3-dichloropropane solutions; The picture of PLLA-$(COOH)_1$ before aggregation is also shown for comparison (**c**)

detectable forms in SEM pictures of materials obtained after the aggregation indicated a weak tendency of polylactide with carboxyl groups to the aggregation in a solvent of low polarity. It was interesting to find whether this tendency could change when the aggregation was performed in the system of both polylactide stereoisomers.

Aggregation of PLLA-$(COOH)_x$ and PDLA-$(COOH)_x$ mixture

The viscosity changes of mixed, separately prepared solutions of PLLA-COOH and PDLA-COOH after the addition of CaO were followed by Brookfield instrument. It appeared that in this case viscosity of the mixture almost did not change and polymer precipitation from the solution was observed instead. The formed precipitate was PLA stereocomplex. Figure 8 presents the SEM image of the precipitate obtained after the aggregation of both stereoisomers in the presence of calcium oxide.

In the presented SEM picture of the precipitate, among irregular small particles (similar to that of dried gels from the aggregation of one stereoisomer), also uniform microspheres with a diameter of about 1.5–2 μm are present. The phenomenon of microspheres formation was observed sporadically in the case of PLA stereocomplex synthesis from equimolar PLA stereoisomers solutions when specially chosen conditions were applied [21]. We have recently found that regular microspheres were formed in the case of stereocomplexation of PLA with ionic groups at the chain end [22]. Evidently in the system studied by us the addition of calcium oxide to PLA-COOH solutions caused ionization of carboxyl groups. Shapes and sizes of stereocomplex microparticles depend on conditions of their crystallization from a solution. In our study, we did not optimize precipitation conditions, however, we checked the influence of solvent polarity and metal cation on facilitating the ionization of –COOH groups. The appearance of microparticles obtained in chosen solvents using several metal oxides is shown in Fig. 9.

1,3-Dichloropropane seems to favor the formation of spherical particles. We, therefore, chose this solvent for the aggregation in the presence of oxides other than CaO. Metal originated from the introduced oxide remained inside the particles, which could be useful in potential applications of PLA microparticles such as

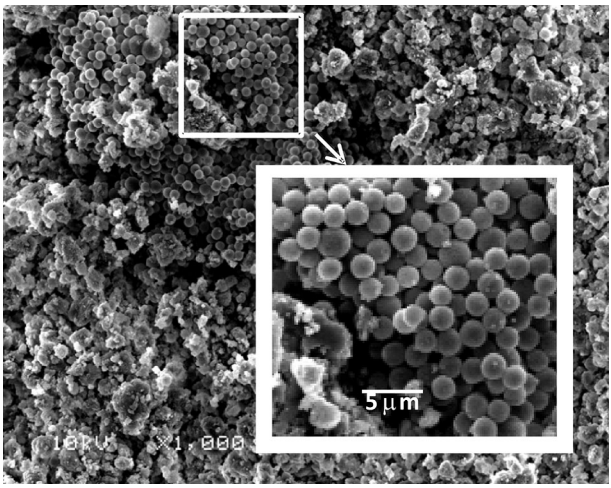

Fig. 8 SEM image of stereocomplex formed of PLLA-COOH, PDLA-COOH and CaO; in the inlet an area in which regular microspheres are present is shown

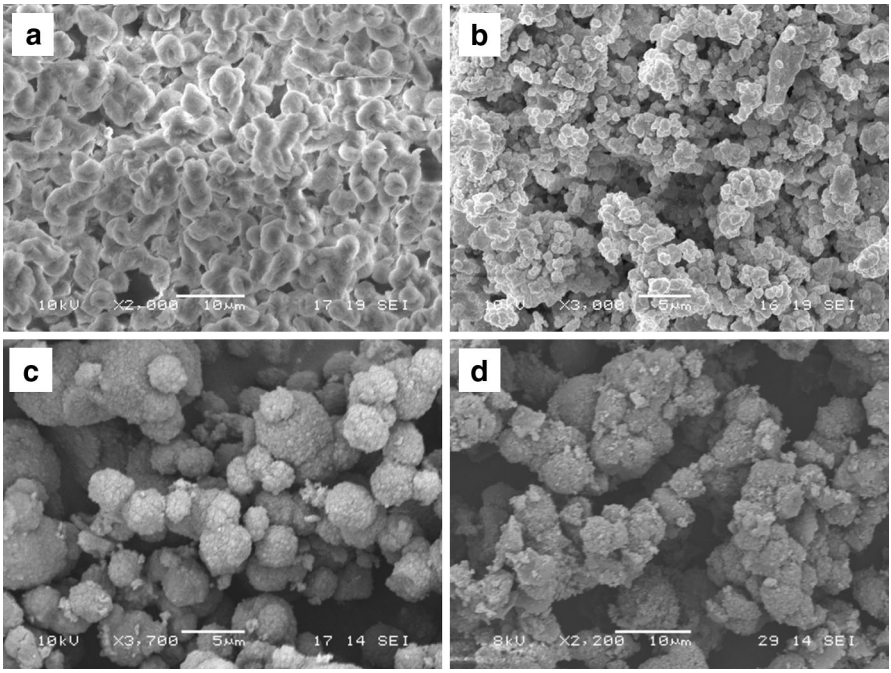

Fig. 9 SEM images of aggregation products of PLLA-COOH/PDLA-COOH performed in the presence of CaO in 1,4-dioxane **a** or in acetonitrile, **b** and performed in the presence of Fe_2O_3, **c** or in the presence of ZnO, **d** in 1,3-dichloropropane

biomedical PLA-based materials or microparticles with iron oxide which display magnetic properties. DSC analysis of the precipitate indeed confirmed that a stereocomplex was formed. In DSC thermograms presented in Fig. 10 melting peaks corresponding to the aggregation product obtained in the system with both PLA-COOH stereoisomers appeared at a significantly higher temperature than peaks corresponding to the aggregation product of PLLA-COOH and to the starting PLLA-COOH (although all melting temperatures are lower than those for high molecular PLA cited in the literature).

The stereocomplex formation upon aggregation of PLLA-COOH/PDLA-COOH/CaO system was also evidenced by WAXS analysis. In WAXS profile (solid line in Fig. 11) the peaks characteristic for PLA stereocomplex [11] can be identified at 2θ equal to 12.0°, 20.9° and 24.1°. WAXS profile obtained for the aggregation product of only one PLA stereoisomer is also shown in Fig. 11 for comparison.

Aggregation with the participation of both stereoisomers was also performed for polylactide with more than one –COOH group at one chain end. Equimolar solutions of PLLA-$(COOH)_{2.8}$ and PDLA-$(COOH)_{2.8}$ in 1,3-dichloropropane were mixed together with adding CaO. Figure 12 presents a SEM image of stereocomplex particles. In the figure, spherical particles of about 2 μm diameter are visible. The lack of very regular microspheres could be explained by the presence of

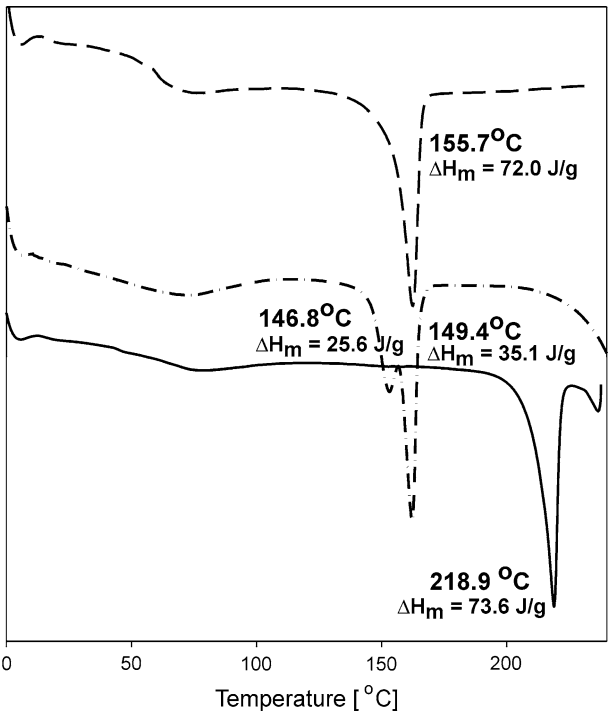

Fig. 10 DSC analysis of the precipitate obtained as result of aggregation in the system with both PLA-COOH stereoisomers (*solid line*); for comparison thermograms of the aggregation product of PLLA-COOH (*dashed line*) and of the starting PLLA-COOH (*dashed-dotted line*) are shown

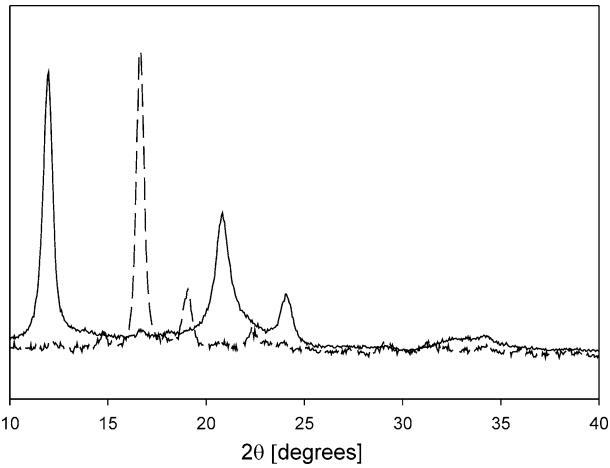

Fig. 11 WAXS profiles of PLLA-COOH/PDLA-COOH/CaO (*solid line*) and PLLA-COOH//CaO (*dashed line*) aggregation products

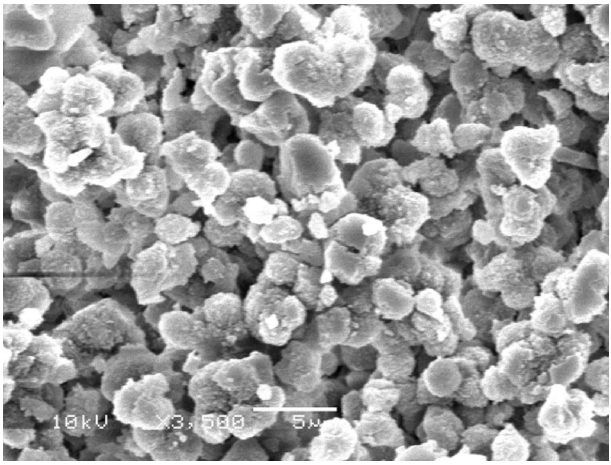

Fig. 12 SEM image of stereocomplex formed of PLLA-$(COOH)_{2.8}$, PDLA-$(COOH)_{2.8}$ and CaO

macromolecules with different functionalities and larger diversity of polymer end groups' interactions with metal cations in the case of PLA with attached thiol molecules.

Phenomena of the formation of small spherical PLA particles with the diameter of a few micrometers may find an application in the encapsulation of active compounds for biomedical purposes. Great efforts are undertaken to develop polymer carriers, including solid nano- and microparticles, for pharmaceutical agents to expand the utility of drugs for various clinical applications. To place a pharmaceutical agent inside the microparticle, the entrapping methods were

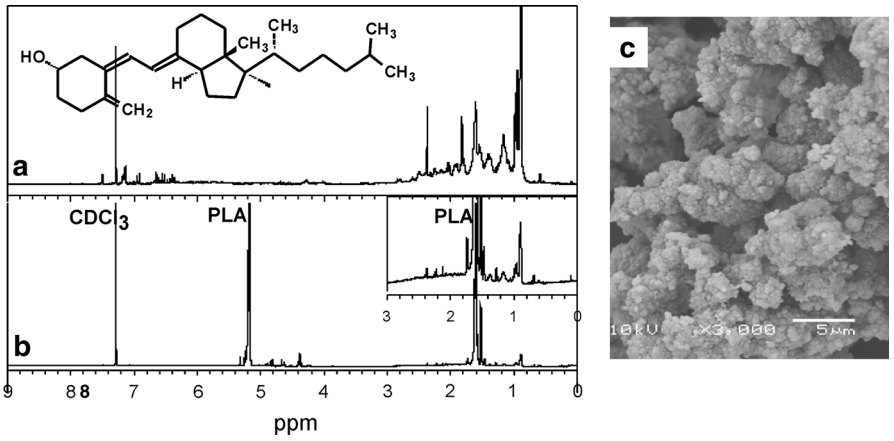

Fig. 13 ^{1}H NMR spectra of vitamin D_3 (**a**), product of aggregation of the PLLA-COOH/PDLA-COOH/CaO/vitamin D_3 system (**b**) and SEM image of this product (**c**)

developed which are based mainly on emulsion techniques like water in oil in water (w/o/w), water in oil in oil (w/o/o) and solid in oil in oil (s/o/o) emulsions [23]. Emulsion/solvent diffusion techniques have also been applied for drugs encapsulation into PLA (PLA copolymers) microspheres [24, 25]. We decided to take an advantage of spontaneous microparticles formation during PLA stereocomplex precipitation for the entrapment of pharmaceutical agents. When microspheres are formed during PLA stereocomplexation, the whole process of emulsification, and therefore the choice of emulsification conditions, can be avoided. Metal cations present in the system can act as a stabilizing agent [25, 26]. Additionally, the presence of metal cations could be advantageous for the pharmaceutical agent assimilation, regarding potential applications. Thus, vitamin D_3 (cholecalciferol) is frequently administered together with Ca^{2+} [27]. First attempts of the PLA stereoisomers aggregation in the presence of several vitamins indicated a loading degree of several percent. Figure 13a, b presents ^{1}H NMR spectra indicating the presence of vitamin D_3 inside particles and Fig. 13c presents a SEM image of the formed microparticles loaded with the vitamin and CaO.

Although the vitamin loading degree is not high (around 4 wt%) it can be quite sufficient in the therapeutic application assuming that the typical supplementing dose per day is around 10 μg applicable in 0.25 g of oil. The work concerning the encapsulation of different pharmaceutical agents during PLA stereocomplexation with a possible support of specific interactions between aggregating system components is in progress.

Conclusions

Poly(lactide) containing carboxyl groups at one chain end undergoes the aggregation in the presence of metal cations in nonpolar solvent. The aggregation

phenomena proceed in different ways depending whether one PLA stereoisomer or a mixture of stereoisomers is used. In the case of one PLA-$(COOH)_x$ stereoisomer, polymer chains undergo self-organization by the interaction of terminal carboxyl groups with metal cations. Such aggregation leads to solution viscosity increase and formation of gels but no defined morphology of aggregates is observed in the dried polymer. The aggregation tendency depends on the number of carboxyl groups.

The aggregation of equimolar mixture of PLA stereoisomers—PLLA-COOH and PDLA-COOH—involves two phenomena, i.e., the interaction of terminal carboxyl groups with metal cations and the interaction between two complementary polylactide (PLLA and PDLA) chains. Such interactions lead to self-organization resulting in the formation of spherical microparticles which contain a metal ion inside. The spontaneous formation of microparticles may find an application in pharmaceutical agent's encapsulation as it was preliminarily shown for vitamin D_3/calcium combination.

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