NITĂ TUDORACHI, AURICA P. CHIRIAC*), RODICA LIPSA
"Petru Poni" Institute of Macromolecular Chemistry
Grigore Ghica Voda Alley, No. 41A, Iasi, Romania

Biodegradable copolymers with succinimide and lactic acid units

Part I. SYNTHESIS POSSIBILITIES

Summary — This paper present the possibility to achieve biodegradable copolymers with succinimide and lactic acid units *via* two different routes of synthesis processes. The first one (variant A) involves *in situ* copolymer synthesis using maleic anhydride, ammonium hydroxide and L(+)-lactic acid and the second (variant B) involves poly(succinimide) synthesis and then its modification with L(+)-lactic acid by mass polycondensation procedure. The prepared copolymers were characterized by IR and ^{1}H NMR spectroscopy, molecular weights and particle size distribution. The synthesized copolymers can be used as possible matrix to achieve some matrix/active principle systems with medical and pharmaceutical applications, especially owing to their biodegradability and biocompatibility.
Keywords: poly(succinimide-*co*-lactic acid), amphiphilic copolymers, synthesis, chemical structure, particles dimensions, molecular weight.

BIODEGRADOWALNE KOPOLIMERY ZAWIERAJĄCE JEDNOSTKI SUKCYNOIMIDU ORAZ JEDNOSTKI KWASU MLEKOWEGO. Cz. I. MOŻLIWOŚCI SYNTEZY
Streszczenie — Przedstawiono dwa różne sposoby otrzymywania kopolimerów zawierających jednostki sukcynoimidu (SI) i kwasu mlekowego (LA) — PSI-*co*-LA. Pierwsza metoda (wariant A) polega na syntezie *in situ* z zastosowaniem bezwodnika maleinowego, amoniaku i LA (schemat A), a wg drugiej (wariant B) wykorzystuje się do tego celu otrzymany wstępnie polisukcynoimid i prowadzi jego polikondensację w masie z LA (schemat B). Uzyskane kopolimery scharakteryzowano metodami spektroskopii IR (rys. 1—3, tabela 1) i NMR (rys. 4) oraz określono ich ciężary cząsteczkowe (metoda GPC, tabela 2) a także wymiary cząstek i rozkład tych wymiarów (rys. 5, tabela 3). Opisane produkty mogą być zastosowane jako biodegradowalne i biokompatybilne nośniki substancji czynnych użytkowanych w medycynie i farmacji.
Słowa kluczowe: amfifilowe kopolimery, sukcynoimid/kwas mlekowy, synteza, budowa chemiczna, wymiary cząstek, ciężar cząsteczkowy.

BIODEGRADABLE POLYMERIC MATERIALS — GENERAL CHARACTERISTICS

Biodegradable polymers have gained importance in pharmaceutical and environmental applications, as well as in packaging. Various features, such as degradation rate, high mechanical properties, biocompatibility, and safety are desired for biodegradable medical materials. Plasticity and reactivity are also required for extension of their utilization. Among them, poly(L-lactide) (PLLA) is one of the most widely utilized classes of biodegradable and bioresorbable polymers in the field of biomedical applications and has been used clinically in wound closure [1, 2] tissue repair and regeneration [3], and/or drug delivery [4]. Biodegradable polymers provide sustained or controlled release of encapsulated drugs and degrade in the body into non-toxic and low-molecular-weight products that can be easily eliminated. Polymeric drug delivery systems have numerous advantages compared to conventional dosage forms, such as improved therapeutic effects and convenience, reduced toxicity. In addition, a wide variety of polymers, processing and manufacturing techniques are explored for incorporation of drug molecules into delivery vehicles of various geometrical shapes. Particularly, sustained release microspheres using biodegradable products such as: poly(lactic acid) (PLA), poly(lactide-*co*-glycolide) (PLGA), and poly(D,L-lactide)-poly(ethylene glycol) (PDLLA-PEG) have been investigated [5].

PLA has good biocompatibility and biodegradability, high mechanical strength, and excellent shaping and molding properties. However, the application scope of

*) Corresponding author; e-mail: achiriac1@yahoo.com

PLA is limited, due to the difficulty of controlled degradation and poor compatibility with soft tissues (due to its high crystallinity) and induction of material defects (based on the lability of melt viscosity). To control the crystallinity and degradation rate, many approaches, for example ring opening polymerization of lactides, chirality's control, copolymerization with other lactones and polyethers have been studied [6—10]. In practice, to decrease PLA crystallinity, synthesis of random copolymers of L-lactide (LA) with depsipeptides consisting of glycolic acid (Glc) and α-amino acids [Lysine (Lys) or aspartic acid (Asp)] having pendant amino or carboxyl groups — poly[(Glc-Lys)-LA] and poly[(Glc-Asp)-LA] — was reported [11, 12].

Poly(glycolide) and its copolymers with lactide, due to their biocompatibility and relative good mechanical properties are usually applied in medical practice as very good materials for biodegradable implants, employed both in bone surgery in the form of screws, plates, surgical nails and in treating injuries of some internal organs in controlled drug release as carriers in the form of microspheres [13, 14]. Also, PLA and its derivatives have been synthesized and investigated as random copolymers, block copolymers and branched polymers [15—20].

Poly(succinimide) (PSI) and sodium poly(aspartate) (PAspNa) have a commercial importance as builders for detergents, scale inhibitors, anticorrosive agents, as they are biodegradable and biocompatible. PSI was synthesized under reduced pressure by thermal polycondensation of L-aspartic acid in dodecane, at 175 °C with phosphorous acid as catalyst [21]. The polycondensation accelerating agents, preferably phosphoric acid, directed to a polymer with raised biodegradability and sufficiently high average molecular weight ($M_w > 60 \cdot 10^3$).

Poly(aspartic acid) (PAS) and its salts can be achieved by reacting maleic anhydride, water and ammonia. Maleic anhydride is hydrolyzed to maleic acid and converted to ammonium salt in the presence of ammonium hydroxide solution. Then, ammonium salt is bulk polymerized at 170 °C, to achieve PSI that is subsequently hydrolyzed to obtain PAS [22]. Poly(aspartic acid) possesses carboxylic acid pendant group in its structural unit and can be further used for various modification purposes. Thus, high water absorbent gels were produced by thermal crosslinking of freeze-dried mixture of partially neutralized poly(aspartic acid) and various amounts of low molecular weight PEG-diepoxide compounds in aqueous medium. These poly(aspartic acid)-based hydrogel materials, possessing inherent biodegradability, potential non-toxicity and biocompatibility are expected to be used as substrates for various biomedical applications and superabsorbent polymers [23].

In order to apply succinimide and lactic acid based copolymers as novel materials, we attempted to synthesize macromolecular chains with mentioned units in their structure. In this study, we describe the preparation of copolymers based on succinimide and L(+)-lactic acid through two different routes of synthesis and the characterization of the synthesized copolymers; we also review the differences in their properties due to the two different synthesis procedures.

EXPERIMENTAL

Materials

L(+)-lactic acid (LA, 90 % aqueous solution) and maleic anhydride (both Fluka), manganese acetate [$(CH_3COO)_2Mn \cdot 4\ H_2O$, Sigma-Aldrich), sodium hydroxide (Oltchim SA Romania), acetone and ammonium hydroxide 25 wt. % aqueous solution (Chemical Company SA, Romania) were used as received.

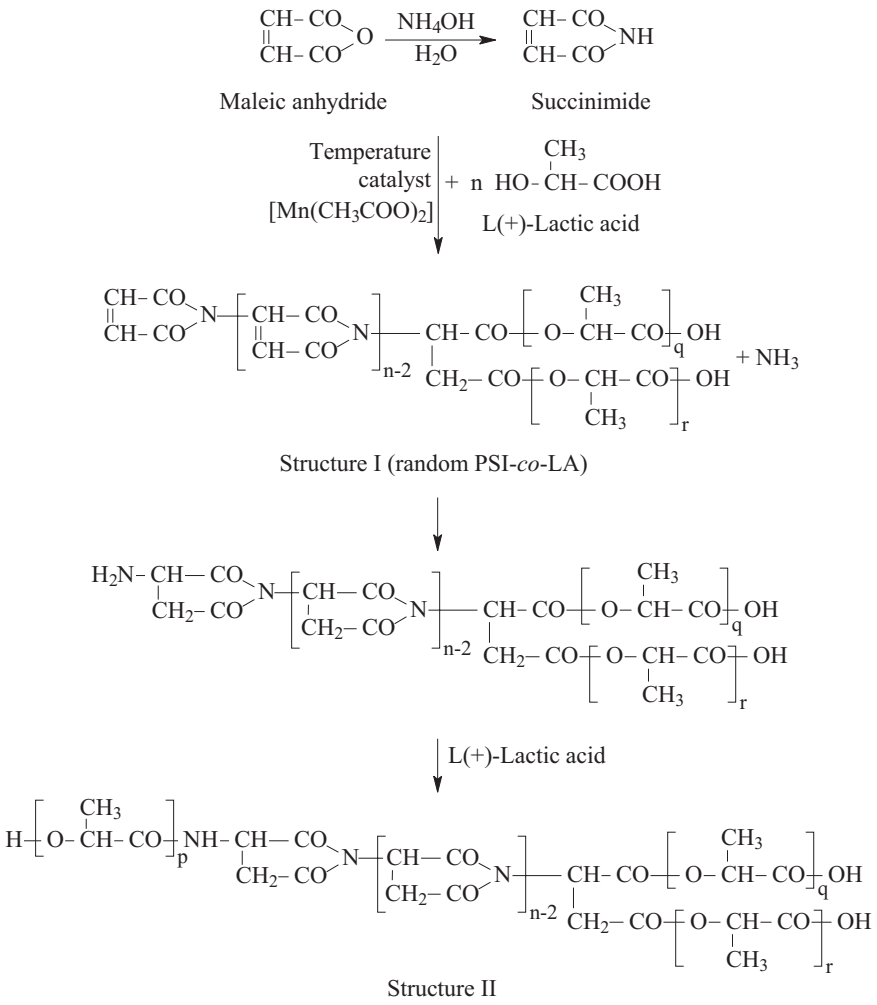
Synthesis

The biodegradable copolymer comprising succinimide and lactic acid units was synthesized using two different procedures. A first one (variant A) involves *in situ* copolymer PSI-*co*-LA synthesis using maleic anhydride, ammonium hydroxide and L(+)-lactic acid while the second one (variant B) involves of poly(succinimide) synthesis and then its modification with L(+)-lactic acid by mass polycondensation procedure.

Variant A — *in situ* synthesis (PSI-*co*-LA)

The synthesis according to the reaction Scheme A illustrates the formation of the succinimide followed by its polycondensing process with L(+)-lactic acid.

Practically, for copolymer synthesis 5.3 g (0.054 moles) maleic anhydride was used, dissolved under stirring in 5.5 ml distilled water, while taking care that the solution temperature didn't exceed 40 °C. After maleic anhydride complete dissolution, 7.75 ml NH_4OH (25 wt. % aqueous solution, 0.055 moles) was gradually added at temperature maintained under 55—60 °C. The obtained solution was cooled at room temperature, then 30 g L(+)-lactic acid (90 wt. % aqueous solution, 0.33 moles) and $(CH_3COO)_2Mn \cdot 4\ H_2O$ as catalyst (0.8 wt. % in proportional to lactic acid) were introduced. Furthermore, the reaction mass was homogenized for 30 minutes by stirring, then was put into a stainless steel autoclave and kept for 5 h at 180 ± 10 °C and *ca.* $3 \cdot 10^5$ Pa (3 atm) pressure. After cooling at room temperature, a viscous yellow-brown solution was obtained. The reaction mass obtained in the autoclave was transferred into reaction vessel (V = 100 ml) equipped with stirrer, heating system and distillation-collecting device. Thermal condition followed at 105—110 °C under vacuum to distil and collect the secondary reaction products. The synthesized copolymer — poly(succinimide-*co*-lactic acid) — presented high viscosity and was dried in vacuum for 48 h at 80 °C, then lyophilized for 48 h at -40 °C and vacuum of 2 torr (266 Pa).



Scheme A. In situ synthesis of PSI-co-LA (variant A)

Variant B — polysuccinimide polycondensation with L(+)-lactic acid

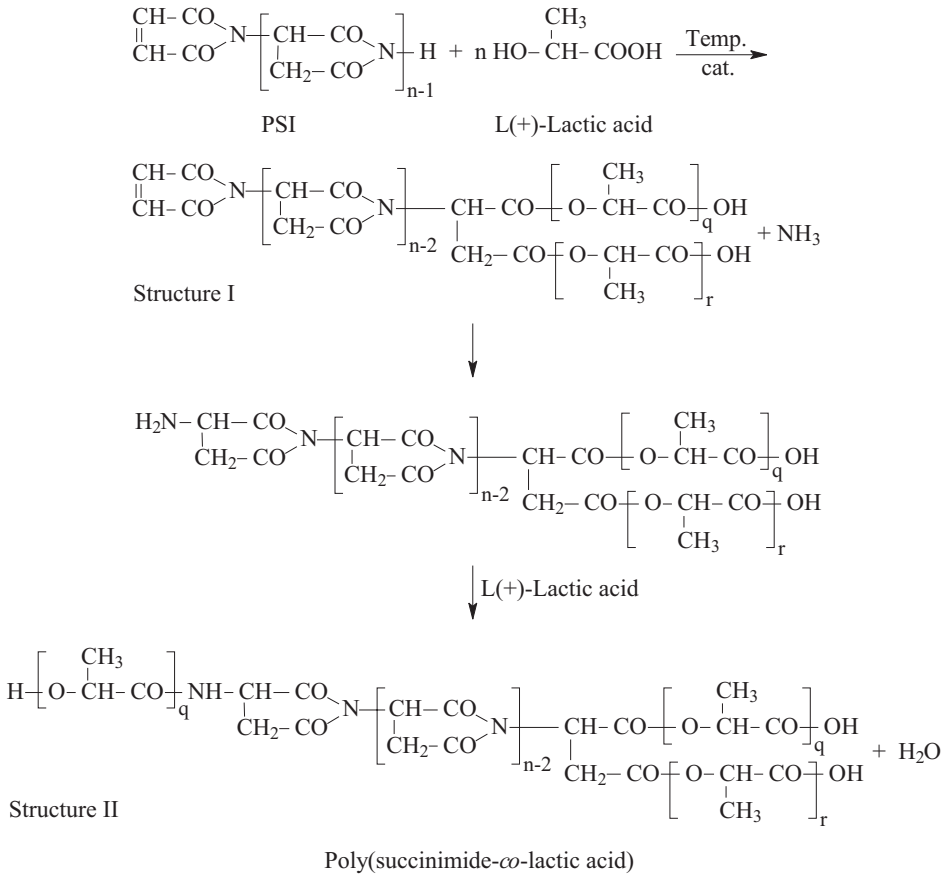
The difference from the previously described variant A is that the copolymer with succinimide and lactic units is synthesized in two stages (see Scheme B).

In the first stage PSI was synthesized from maleic anhydride and ammonia and in the second stage, the prepared PSI was polycondensed with L(+)-lactic acid to get poly(succinimide-*co*-lactic acid) copolymer. In this stage it is possible to make an intermediary product (structure I) through the addition of the NH_3 molecules (obtained as a result of the opening of the succinimide cycle) to the double bond from the final structural units of succinimide. The NH_2 group can also be used to participate at the condensing reaction of lactic acid producing a new macromolecular chain. In the main, at first (PSI synthesis), maleic anhydride 21.2 g (0.216 moles) was dissolved in 22 ml distilled water, then 31 ml NH_4OH (25 wt. %, 0.22 moles) aqueous solution was added. The obtained solution was introduced in the reaction autoclave and, as in variant A, was maintained 5 h at 180 ± 10 °C and under *ca.* $3 \cdot 10^5$ Pa (3 atm) pressure. After autoclave cooling, the resulted reaction mass was introduced in a glass reaction vessel equipped with heating system and vacuum-distillation-collecting device of the secondary reaction products. The reaction mass was maintained for 3 h under reflux, then the temperature was raised to 115—120 °C to distil and collect the secondary products (approximately 28 ml). The resulted PSI was highly viscous and had a red-brown colour.

2.6 g of PSI (0.027 moles) were submitted to mass polycondensation reaction with 15 g L(+)-lactic acid (0.166 moles) in the presence of manganese acetate catalyst $(CH_3COO)_2Mn \cdot 4\ H_2O$ (0.8 wt. % in proportion to the reaction components). The reaction mixture was maintained under reflux for 6 h at 98—100 °C, then the temperature was raised to 115—120 °C and maintained for 3 h. The resulted product was precipitated in acetone, received particles vacuum dried at 80 °C for 48 h and then vacuum (2 tr = 266 Pa) lyophilized for 48 h at -40 °C.

Analytical methods

— Fourier Transform Infrared Spectrometer (FT-IR) Vertex 70 model (Bruker, Germany) for spectra recorded on KBr pellets (5 mg sample/500 mg KBr), was used.



Scheme B. Synthesis of PSI-co-LA by polysuccinimide polycondensation with L(+)-lactic acid (variant B)

— ^{1}H NMR spectra of copolymers were obtained on a Bruker Avance DRX 400 NMR spectrophotometer Rheinstetten (Germany) with 16 scans and 0.1 Hz FID resolution. The samples were dissolved in dimethyl sulfoxide (DMSO) with a concentration of about 5 wt. %. The analyses were carried out at 25 °C and chemical shifts were reported in ppm using tetramethylsilane (TMS) as the internal reference.

— Average molecular weight of the copolymers was determined by gel permeation chromatography (GPC) technique at ambient temperature. The system was equipped with an adjustable flow capacity and constant rate pump LC 1120 and an evaporative mass detector PL-EMD 950 type. It was fitted also with columns PL-gel 5 μm MIXED-D and PL-gel 5 μm MIXED-C packed with styrene/divinylbenzene copolymer. PL-polymer, polystyrene standards 580 and dimethylformamide (DMF) as the mobile phase were employed at a flow rate of 0.7 ml/min (dimethylformamide is a good solvent for both — PSI and copolymers).

— Measurements of the particles dimensions were done with a Mastersizer 2000 system (version 5.31) Malvern Instruments (England) in water, using 0.5 mg copolymer particles previously lyophilized and ultrasonicated in the dispersion unity of the device for 10 seconds. The system device is constituted of an optical bank which uses laser light He-Ne 632 nm/2 mW, a dispersion unity of the sample Hydro 2000A type equipped with stirrer, recirculating pump, ultrasonic and software to record and process results on the computer. The measurements domain is between 0.020—2000 μm.

RESULT AND DISCUSSION

The FT-IR spectra of intermediary products and of synthesized copolymers are presented in Figs. 1—3. The copolymers achieved under the two variants present similar structures, with IR absorption bands situated at close wavelengths (Table 1).

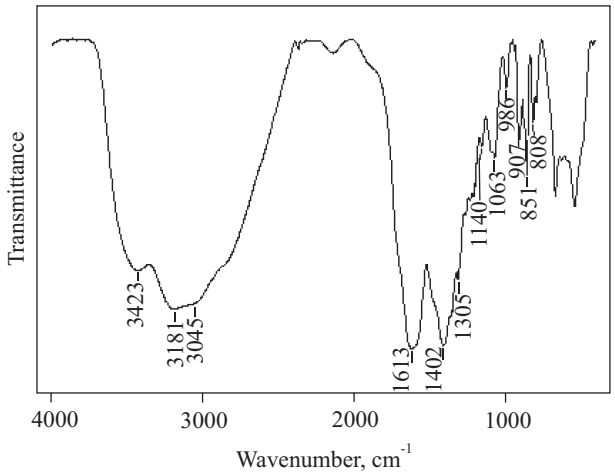


Fig. 1. FT-IR spectrum of polysuccinimide

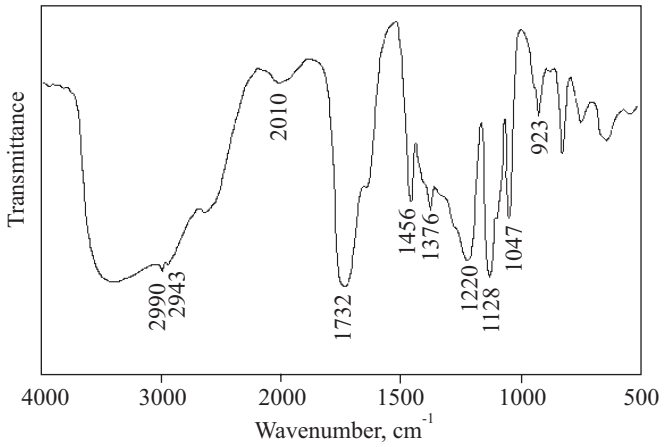


Fig. 2. FT-IR spectrum of L(+)-lactic acid

T a b l e 1. The main FT-IR absorption bands*)

Sample	Functional groups	Wavelengths, cm^{-1}
L(+)-lactic acid	-CH_3 (νCH_3)	2943
	-OH large band (νOH)	3200-3400
	-C-OH secondary alcohols (νC-OH)	1128
	-COOH (νC=O)	1732
PSI	-CO-NH-R secondary amides	1613
PSI-*co*-LA	-COOR (νC=O)	1721 (var. B); 1738 (var. A)
	-CO-NH-R secondary amides (νC-N; δNH amide band II)	1593 (var. B); 1590 (var. A).
	-CH_3 (νCH_3)	2940 (var. B); 2944 (var. A).
	-OH large band (νOH)	3465 (var. B); 3400 (var. A).

*) See Figures 1—3.

Based on other information concerning synthesis of copolymers with similar structures [24], two reaction mechanisms are proposed (see Schemes A and B). By succinimide ring opening and reaction with L(+)-lactic acid two poly(lactic) acid chains are formed (Scheme A). In the presence of manganese acetate catalyst the succinimide reacts through ring-opening polymerization with two hydroxyl groups from different L(+)-lactic acid molecules and eliminating ammonium molecules to give random copolymers. The second possibility can be as follows: NH_3 molecules released by succinimide ring opening (structure I) can be added at -C=C- double bond forming an NH_2 group that takes part at an ammidation reaction with -COOH group from lactic acid, developing a new PLA chain (Scheme B).

The ^{1}H NMR spectra of the copolymers prepared according to mentioned procedures are presented in the Fig. 4. From NMR spectra the ratio between succinimide (SI) and lactic acid (LA) units was determined as r_{SI}/r_{LA} = 51.42/48.58 in case of variant A and r_{SI}/r_{LA} = 52.85/47.15 in case of variant B synthesis.

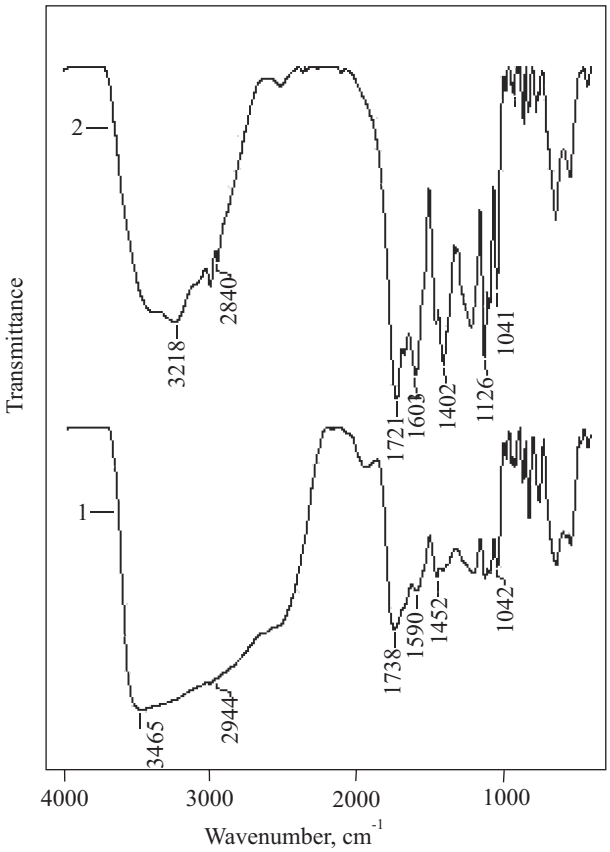


Fig. 3. FT-IR spectra of PSI-co-LA copolymer received according: a) variant A, b) variant B

The average molecular weights of the synthesized copolymers are presented in Table 2.

T a b l e 2. Average molecular weights determined by GPC

Sample	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_w/\overline{M}_n$
PSI-*co*-LA — synthesis variant A	8254	9196	1.114
PSI-*co*-LA — synthesis variant B	12 016	27 061	2.252
PSI	3504	3600	1.027

So, the molecular weight of copolymer from variant B ($\overline{M}_n$ = 12 016), is higher than from variant A ($\overline{M}_n$ = 8254). In the first case, the initial reaction components [maleic anhydride, ammonia and L(+)-lactic acid] were put together from the beginning, while in the second case the reaction components were L(+)-lactic acid and poly(succinimide) prepolymer ($\overline{M}_n$ = 3500), that participated with its molecular weight in the branched copolymer. The conditions of condensation were the same in both cases (180 °C, 3 atm, 5 h). It can be thus concluded that succinimide is less reactive then D,L-lactide [24].

Also, the reduced molecular weights, registered for the copolymers with the succinimide and lactic units synthesized through the above mentioned procedures, compared with the similar data from the literature [25, 26], are justified on the one side through the used catalyst —

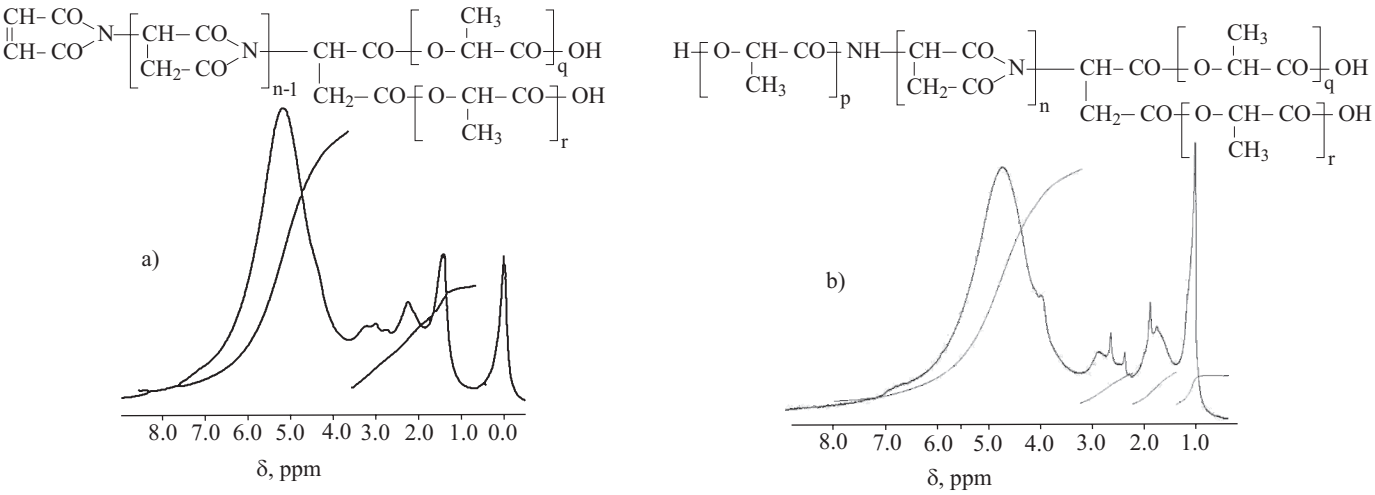


Fig. 4. NMR spectra of PSI-co-LA copolymer received according: a) variant A, b) variant B

T a b l e 3. Particles size distribution

Sample	$d(0.1)$[1], μm	$d(0.5)$, μm	$d(0.9)$, μm	Surface weighted mean[2], μm	Volume weighted mean[3], μm	Uniformity[4]	Span[5]
PSI-*co*-LA (variant A)	0.204	33.23	223.35	0.825	79.28	2.01	6.72
PSI-*co*-LA (variant B)	18.37	64.24	328.32	42.16	121.47	1.37	4.83

[1] $d(0.1)$, $d(0.5)$, $d(0.9)$ — 10 %, 50 % and 90 % of the sample volume are particles with a smaller diameter than specified in the table.

[2] Medium diameter for the equivalent sphere of the same surface with that of the particles, called also media Sauter.

[3] Medium diameter for the volume.

[4] Uniformity — absolute deviation from the median value.

[5] Span — represents the width of the distribution. Span value is calculated with the formula: $[d(0.9) - d(0.1)]/d(0.5)$.

manganese acetate — which presents reduced efficiency comparatively with stannous octoate or tin(II) chloride which are usually utilised, and, on the other side, owing to the reaction conditions favourable for multiple possibilities of competitor reactions as, for example, condensative or block copolymerization.

The prepared copolymers are soluble in water and certain organic solvents (DMF, DMSO, methanol *etc*.).

The results of measurements concerning particles size and dimensional distribution of particles are presented in Table 3 and Fig. 5. We notice that 90 wt. % of the total particle volume PSI-*co*-LA has diameter $d(0.9) < 223.35$ μm (variant A), and $d(0.9) < 328.32$ μm (variant B). For both variants of synthesis there is an important deviation of medium value of particles dimension and particles are heterogeneous in terms of their size (Fig. 5). This fact is also evidenced by the data presented, namely uniformity and span which have enough raised values (Table 3).

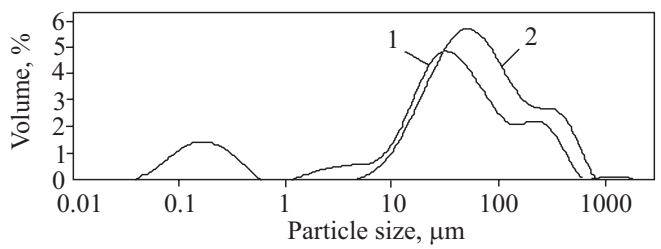


Fig. 5. Particle size distribution of PSI-co-LA copolymer received according: 1 — variant A, 2 — variant B

The results of these measurements are in good agreement with the preparation method. Thus, the copolymer particles dimension in case of the variant A of synthesis, when the macromolecular chains can be regarded more as random and alternating copolymer with succinimide and lactic units obtained through polycondensing procedure, are smaller then those obtained through the variant B, when onto polysuccinimide are grafted lactic acid units.

CONCLUSIONS

This work has proved the possibility to obtain biodegradable copolymers with succinimide and lactic acid units through two different synthesis procedures. In the first case maleic anhydride, ammonia and L(+)-lactic acid were used as reaction components (variant A of synthesis), and in the second case poly(succinimide) was previously synthesized and subject to polycondensation with L(+)-lactic acid (variant B of synthesis). PSI-*co*-LA copolymers obtained through these two methods present similar properties. The characteristics of the synthesized

copolymers suggest their utilization as possible matrix to achieve some matrix/active principle systems with medical and pharmaceutical applications, especially owing to their biodegradability and biocompatibility.

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