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Characterization of star-shaped poly(L-lactide)s by liquid chromatography at critical conditions

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Abstract

A series of linear and star-shaped poly(L-lactide)s (PLA's) have been prepared by living polymerization of L,L-dilactide (LA) and analyzed by liquid chromatography at critical conditions (LC-CC). For the analysis of the PLA's LC-CC conditions have been used corresponding to silica gel as the stationary phase and a mixture of 1,4-dioxane/*n*-hexane (56.25/43.75 by vol%) at 50 °C as the mobile phase. At the critical point of adsorption, a series of linear C_4H_9-PLA-OH's having molar masses (M_n) in the range from 2.3×10^3 to 7.4×10^4, prepared by ring-opening polymerization of LA initiated with $Sn(OC_4H_9)_2$ (THF, 80 °C), showed no dependence of the elution volumes on molar mass. In subsequent experiments, star-shaped PLA's bearing various numbers of PLA-OH arms (R-(PLA-OH)$_x$) have been prepared in a controlled synthesis starting from various polyols (R-(OH)$_x$) containing exclusively primary hydroxyl groups: diethyleneglycol ($x = 2$), trimethylolpropane ($x = 3$), di(trimethylolpropane) ($x = 4$), dipentaerithritol ($x = 6$), and poly(3-ethyl-3-hydroxymethyloxetane) ($\langle x \rangle = 13.4$) and LA monomer. As coinitiator/catalyst tin(II) octoate ($Sn(Oct)_2$) has been used (bulk polymerization, 120 °C). ^{1}H NMR analysis of the resulting star-shaped polymers revealed that all OH-groups in the polyols started growth of the PLA chains. The series of star-shaped PLA's have been analyzed by LC-CC as well as by two-dimensional (2D) chromatography (i.e. LC-CC versus size exclusion chromatography (SEC)) with regard to possible structural imperfections. It has been shown, that the LC-CC elution volumes of the resulting R-(PLA-OH)$_x$ increase with the number of PLA-OH arms, allowing discrimination of the individual R-(PLA-OH)$_x$'s in their mixture. An exponential increase of the retention volume as a function of the number of arms has been found. Eventually, LC-CC measurements of the elution volumes carried out for acetylated star-shaped PLA's (R(PLA-OOCCH$_3$)$_x$) have shown that for the interactions of the R-(PLA-OH)$_x$ macromolecules with the column packing the hydroxyl end-groups are mostly responsible.

Keywords: Liquid chromatography; Polylactides; Liquid chromatography at critical conditions

1. Introduction

Homo-and copolymers of lactides (LA) find a wide range of applications related to their ability to hydrolytical and/or biological degradation [1–4]. First, biomedical applications were developed including bioresorbable surgical sutures [1–3,5], slow release drug delivery systems [6,7], fractured bone fixation [8,9] or tissue engineering [10]. Poly(L-lactide) (PLA) is also being considered as an environmentally friendly commodity thermoplastic and fiber forming material [11–14].

Various practically important properties of PLA such as degradation rate or thermomechanical parameters can be adjusted by the macromolecular architecture (topology), stereochemistry, structure of end-groups, and molar mass [4,15–17]. On the other hand, the macromolecular engineering offers methods for controlled synthesis of PLA composed of macromolecules with tailor-made structure [16–20]. The best method for this purpose is a ring-opening polymerization of the LA monomer (actually cyclic dimer) initiated by covalent metal alkoxides and carboxylates (Eq. (1)) [18,20] although direct polycondensation of lactic acid(s) has also been applied [21,22].

In ring-opening polymerization the architecture of the resulting PLA macromolecule results from the structure of the alkoxide group (RO in Eq. (1)) derived either from the

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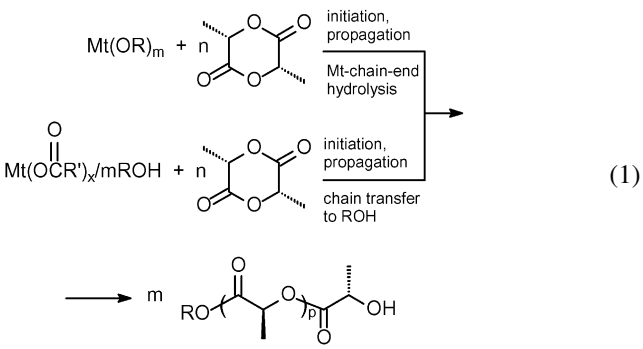
initiator or from the chain transfer agent. For example:



(1)

where, most often, Mt = Zn, Sn(II), Al, Y, Sn(IV); RO stand for alcohol or polyol moiety; $\langle p \rangle = 2n/m - 1$ (for the sake of simplicity intermolecular transesterification is omitted in Scheme 1). For the synthesis of high molar mass linear PLA alkoxide ($Mt(OR)_m$) route is preferred [23] whereas for more sophisticated architectures (for example comb-like or star-shaped) the chain-transfer to alcohol (ROH) approach is more convenient [16,17,19]. Similar results were obtained with primary amines [24,25], including amino dendrimers [26] applied as chain transfer agents. Macromonomers of PLA have also been used for this purpose [27].

In all methods described briefly above PLA's fitted with –OH end-groups are formed. Hydroxyl groups could then be transformed into other end-groups (e.g. the more stable ones) [17,28] or used in constructing more complicated architectures.

Thus prepared set of PLA macromolecules with uniform end-groups could contain a certain fraction of macromolecules differing in end-groups. For example, the LA/$Mt(OR)_x$/R′OH system (in which $[R'OH]_o \gg [Mt(OR)_x]_o$) provides mostly R′OOC-PLA-OH macromolecules and small fraction of ROOC-PLA-OH chains. Similarly, in the LA/$Mt(OOCR'')_x$/R′OH polymerization mixture apart from the desired R′OOC-PLA-OH macromolecules there are also other populations, differing in end-groups, such as HOOC-PLA-OH, R′OOC-PLA-OOCR″, and HOOC-PLA-OOCR″ [24].

Therefore analytical tools are required to determine quantitatively the extent of these structural heterogeneities. Usually, first information allowing a comparison between assumed and practically resulting structures comes from molar mass measurements by size exclusion chromatography (SEC) and/or by osmometry. Then, molar mass distribution traces (also from SEC) could provide the first indication suggesting the presence of some structural imperfections. The structure of end-groups can be determined by NMR or MALDI-TOF mass spectrometry but only for PLA of lower molar masses (typically for M_n below 10^4).

However, the results of SEC, NMR or MALDI-TOF analysis carried out for branched or star-shaped PLA macromolecules can not be interpreted in such a straightforward way as for the linear chains. For example, SEC separates macromolecules according to their hydrodynamic volume, which is different for the same M_n but different topologies. NMR and MALDI-TOF give cumulative mean values of the molar masses and the content of a given end-group per macromolecule. Triple SEC detection can give (from MALLS detector) absolute molar masses but cannot distinguish between macromolecules varied in a number of arms since these are hidden. Only in certain specific instances, when the arms could be detached from the core, measurements of the absolute molar masses and their distribution of the original star-shaped polymer and of arms, after their detachment, could provide an unambiguous information [29]. In the case of star-shaped PLA, where the polyester arms are linked to the R′-$(OH)_x$ core via ester bond this analytical method is not applicable.

Another way to analyze macromolecules having identical molar masses but differing in nature and the number of end-groups is the application of liquid chromatography at critical conditions (LC-CC)). It has been shown theoretically and experimentally that under LC-CC conditions functionality type separations can be accomplished without interference of molar mass effects [30–37]. This technique appears to be promising also for the analysis of star-shaped PLA's.

Thus, in the present paper we describe the synthesis and analysis of PLA stars with various numbers of arms and we eventually show that mixtures of stars with similar molar masses can be separated strictly with regard to the number of arms. Thus structural imperfections can be detected regardless of the molar masses of the species.

2. Experimental part

2.1. Materials

Tin(II) 2-ethylhexanoate (tin octoate ($Sn(Oct)_2$) commercial product (purchased from Aldrich, 95%—according to our determination [38]) was purified by two consecutive high vacuum distillations at 140 °C/3×10^{-3} mbar. Thus purified $Sn(Oct)_2$, stored on the vacuum line, was finally distributed directly into the thin-walled vials or ampoules equipped with breakseals, then sealed off and stored at − 12 °C. Its purity was better than 99.9%

Tin(II) butoxide ($Sn(OBu)_2$) was prepared in the two step synthesis according to Ref. [39]. In the first step $SnCl_2$ was reacted with CH_3OH, in the presence of $(C_2H_5)_3N$ as the HCl scavenger. The precipitated $Sn(OCH_3)_2$ was then refluxed with C_4H_9OH in toluene as a solvent; CH_3OH was removed and the $Sn(OC_4H_9)_2$ formed was finally crystallized from toluene, dried in vacuo, distributed into the thin-walled breakseals, and stored at − 12 °C.

L,L-Dilactide (LA, Boehringer Ingelheim, Germany, ≥99%), crystallized consecutively from dry 2-propanol and toluene, was purified just before use by sublimation in

vacuo (10^{-3} mbar, 85 °C). The purified monomer was distributed into the glass ampoules equipped with breakseals.

Butyl alcohol (BuOH, Aldrich, 99.8%) was dried with Na metal and distributed by distillation into the thin-walled vials.

Acetyl chloride (99.5 + %, Aldrich), commercial product, was degassed and condensed under reduced pressure into the glass ampoule equipped with a breakseal and sealed off after freezing in liquid nitrogen.

Tetrahydrofuran (THF, POCh, Gliwice, Poland, 99%) was kept for several days over KOH pellets, filtered off and refluxed over Na metal. Eventually it was distilled, degassed and stored over liquid Na/K alloy.

Pyridine (99 + %, Aldrich) was kept over KOH for several days, then filtered off and distilled under reduced pressure (80 °C, 1 mbar) from calcium hydride just before use.

1,4-Dioxane (Aldrich, 99.9 + % HPLC grade) and *n*-hexane (Aldrich, 95 + % HPLC grade) were used as received.

Methylene chloride (CH_2Cl_2, POCh, Gliwice, Poland, 99%) was dried with calcium hydride and distilled before use.

Chloroform-*d* and dimethylsulfoxide-d_6 (DMSO-d_6) (Dr Glaser AG, Basel, 99.5 + %D) were dried over calcium hydride and distilled before use.

Di(ethylene glycol) (DEG, Aldrich, 99%) was dried with Na metal and distributed by distillation under reduced pressure (3×10^{-3} mbar, ≈150 °C) into thin-walled vials.

Trimethylolpropane (TMP), di(trimethylolpropane) (DTMP) and dipentaerithritol (DPE) (from Perstorp AB, Sweden) were melted and dried under reduced pressure (10^{-3} mbar, 80 °C) before use.

Poly(3-ethyl-3-hydroxymethyloxetane) (PEHMO) of $M_n = 1430$ (≈13.4-OH groups per macromolecule) was prepared by ring-opening polymerization of 3-ethyl-3-hydroxymethyloxetane initiated with boron trifluoride diethyl etherate. Polymerization procedure and characterization of the resulting PEHMO was described in Ref. [40,41].

2.2. Preparation of linear and star-shaped polylactides

Linear α-butyl-ω-hydroxy-poly(L-lactide)s (Bu-PLA-OH) of various molar masses (M_n) were prepared by LA polymerization initiated by $Sn(OBu)_2$ according to Ref. [23]. M_n were controlled by $144.13([LA]_0 - [LA])/2[Sn(OBu)_2]_0$ ratio. [LA] was determined by means of polarimetry and/or SEC as described more in detail in Ref. [42].

A series of star-shaped, hydroxyl group terminated poly(L-lactide)s (R-(PLA-OH)$_x$) was prepared according to the procedure employing $Sn(Oct)_2$ as coinitiator, elaborated for the linear PLA's [24,38] synthesis. Namely, L,L-dilactide was polymerized with $Sn(Oct)_2$ and the corresponding polyol (bearing 2, 3, 4, 6, and ≈13 primary OH groups) as components of the catalytic initiating system. Polymerizing mixtures were prepared in sealed glass ampoules using standard high vacuum technique. Polymerizations were carried out in bulk at 120 °C. Conversion of LA monomer was monitored by means of ^{1}H NMR of the reacting mixture/chloroform-*d* solutions. Formation of the PLA polymers was monitored by SEC.

A general procedure follows the example described below. $Sn(Oct)_2$ (1 ml of 0.025 mol/l solution in dry THF) and LA (4.98 g (35 mmol)) were transferred under vacuum into breakseals and sealed after freezing in liquid nitrogen. DTMP (0.1822 g (7.26×10^{-1} mmol)) was put into thin-walled vial, melted and dried under vacuum, then sealed after freezing in liquid nitrogen. Breakseals containing $Sn(Oct)_2$/THF solution and LA monomer and tube with immersed DTMP vial were sealed to the reacting (≈10 ml) glass vessel. The breakseals and vial were broken and all components were mixed at room temperature, THF was removed under vacuum and then the reaction vessel was sealed off. The ampoule containing the reacting mixture was placed into a thermostat (120 °C). For analysis the reacting mixture was injected into a SEC apparatus. The analysis of the SEC trace of the crude reacting mixture gave $M_w/M_n = 1.15$ and 99% of LA conversion, vapor pressure osmometry (VPO) $M_n = 8.8 \times 10^3$ g/mol, whereas ^{1}H NMR 8.2×10^3 g/mol (cf. Section 3). The resulting DTMP-(PLA-OH)$_4$) was precipitated into methanol, separated by filtration and washed several times with methanol. The mass of the vacuum dried product was equal to 4.20 g (84% yield). Below are reported ^{1}H NMR data for all the polyols prepared (in brackets are given chemical shifts (δ, in ppm), multiplicities of signals and their relative intensities).

DEG-(PLA-OH)$_2$:

$$O\{C\overset{a}{H}_2\,C\overset{b}{H}_2\,O[C(O)C\overset{f}{H}(C\overset{g}{H}_3)O]_nC(O)C\overset{f'}{H}(C\overset{g'}{H}_3)O\overset{e}{H}\}_2$$

a (3.66, t, 4.3), *e* (2.67, d, 2.0), *f* (5.16, q, 90), *g* (1.58, d, 280), $b + f'$ (4.24–4.39, m, 7.4), g' (1.50, d, 6.3).

TMP-(PLA-OH)$_3$:

$$C\overset{c}{H}_3\,C\overset{b}{H}_2\,C\{C\overset{d}{H}_2\,O - [C(O)C\overset{f}{H}(C\overset{g}{H}_3)O]_nC(O)C\overset{f'}{H}(C\overset{g'}{H}_3)\,O\overset{e}{H}\}_3$$

c (0.86, t, 3.4), *d*(4.05, d of d, 7.2), *e* (2.67, d, 3.0), *f* (5.16, q, 114.5), $b + g$ (1.58, d, 357), f' (4.35, d of q, 3.4), g' (1.49, d, 10.5)

DTMP-(PLA-OH)$_4$:

$$O\{C\overset{a}{H}_2\,C(C\overset{b}{H}_2\,C\overset{c}{H}_3)[C\overset{d}{H}_2\,O - [C(O)C\overset{f}{H}(C\overset{g}{H}_3)O]_nC(O)\,C\overset{f'}{H}(C\overset{g'}{H}_3)O\overset{e}{H}]_2\}_2$$

a (3.22, s, 4.1), *c* (0.82, t, 6.2), *d* (4.02, s, 8.9), *e* (2.67, d, 4.0), *f* (5.16, q, 106.6), $g + b$ (1.58, d, 330), f' (4.35, d of q, 4.0), g' (1.49, d, 12.1).

DPE-(PLA-OH)$_6$:

$$O\{C\overset{a}{H}_2C[C\overset{d}{H}_2O-[C(O)C\overset{f}{H}(C\overset{g}{H}_3)O]_nC(O)C\overset{f'}{H}(C\overset{g'}{H}_3)O\overset{e}{H}]_3\}_2$$

a (3.33, s, 3.9), *d* (4.13, m, 12), *e* (2.67, d, 6.0), *f* (5.16, q, 153.4), *g* (1.58, d, 468), *f′* (4.35, d of q, 6.0), *g′* (1.52, d, 18.9).

PEHMO-(PLA-OH)$_{13}$:

$$[\ldots-OC\overset{b}{H}_2C(CH_2C\overset{a}{H}_3)C\overset{b}{H}_2]_m\{OC\overset{b}{H}_2C(CH_2C\overset{a}{H}_3)C\overset{d}{H}_2O-[C(O)C\overset{f}{H}(C\overset{g}{H}_3)O]_nC(O)C\overset{f'}{H}(C\overset{g'}{H}_3)O\overset{e}{H}\}_{13}$$

a (0.81, s, 40.2), *b* (3.18, m, 52), *e* (2.70, broad s, 13.4), *f* (5.17, q, 76.6), *g* + *g′* (1.58, d, 315), *f′* (4.32, d of q, 13.4).

2.3. Acetylation procedure

R-(PLA-OH)$_x$'s were quantitatively esterified by direct reaction with acetyl chloride carried out at room temperature in dry pyridine used both as a solvent and the HCl scavenger. A general procedure follows the example described below.

DTMP-(PLA-OH)$_4$ of M_n(VPO) = 8.8×10^3 g/mol (1 g, 0.114 mmol) was dissolved in 20 ml of dry pyridine and approx. three molar excess (with regard to the hydroxyl groups) of acetyl chloride (0.12 g, 1.53 mmol) was added. Then the reaction mixture was stirred 8 h at 40 °C. Resulting DTMP-(PLA-OOCCH$_3$)$_4$ was precipitated into a cold methanol, separated by filtration, and washed with water and methanol. Mass of the vacuum dried product and its M_n measured by VPO were equal to 0.96 g (96% yield) and 8.0×10^3 g/mol, respectively.

2.4. Measurements

SEC traces were recorded using a LKB 2150 HPLC pump and set of TSK Gel columns (G 2000 H$_{xL}$ and G 6400 H$_{xL}$) at 20 °C. Wyatt Optilab 903 interferometric refractometer (Wyatt Technology Corp., USA) was applied as detector. Methylene chloride was used as an eluent at flow rate of 0.8 ml/min.The actual number-average molar masses (M_n) of the linear PLA's were determined using a calibration based on PLA standards prepared in our laboratory. The correct M_n values for the linear PLA can also be obtained after multiplying by 0.68 (hydrodynamic volume correction factor, determined by comparing calibration curves for polystyrene and polylactide) the M_n determined with polystyrene standards, at least up to $M_n \leq 2 \times 10^4$ g/mol, for the set of G 2000 H$_{xL}$ and 6000 H$_{xL}$ columns with CH_2Cl_2 as an eluent. M_n higher than 10^4 g/mol were directly determined with a MALLS Dawn F Laser Photometer and using ASTRA v 4.70 program (Wyatt Technology Corp., Santa Barbara, CA). The actual M_n of the deactivated and isolated star shaped PLA's were determined with Knauer Vapor Pressure Osmometer in dry methylene chloride; the M_w/M_n ratios were determined by SEC.

^{1}H NMR spectra were recorded in chloroform-*d* on a Bruker AC200 operating at 200 MHz. Traces of the non-deuterated chloroform were used as internal standards.

2.5. Liquid chromatography at the critical point of adsorption (LC-CC)

The chromatograph for LC-CC measurements consisted of a Waters model 510 pump, a Rheodyne injection valve with a 100 μl loop, two columns: Si-100 and Si-300 (Macherey-Nagel), and Waters 486 tunable UV detector operating at 235 nm. A mixture of 1,4-dioxane and *n*-hexane (56.25:43.75 vol%) was used as mobile phase with flow rate of 0.5 ml/min. The temperature of the columns was set at 50 °C using a Waters column oven.

2.6. Two-dimensional (2D) chromatography

The chromatograph for the first separation step (LC-CC) consisted of a Rheodyne six-port injection valve with a 100 μl injection loop and an electrically driven eight-port injection valve (Valco EHC8 W) to connect LC-CC and SEC chromatographs. In addition, these chromatographs were connected to two storage loops with a volume of 200 μl each. The chromatograph for the second separation step consisted of Waters model 510 pump. The operation of the coupled injection valves was controlled by the software, which was used for data collection and processing (PSS-2D-GPC-Software of Polymer Standard Service, Mainz, Germany). Two columns Si-100 Å and Si-300 Å (Macherey–Nagel) were used in the first run and 1000 Å, 5 μm, high speed column 50 × 20 mm I.D. (PSS-SDV) in the second one. Temperature of columns was set at 50 °C. Mixture of 1,4-dioxane and *n*-hexane (56.25:43.75 vol%) in the first run and THF in the second one were used as mobile phases with flow rates 25 μl min^{-1} and 4 ml min^{-1}, respectively. Concentration of the injected polymer was equal to 40 mg mL^{-1}. Waters 486 tunable UV detector at 235 nm and evaporative light-scattering detector (ELSD) model ELSD 500 of Altech, both after run two were used.

3. Results and discussion

3.1. Synthesis of linear polylactides and their behavior in LC-CC

For a detailed analysis of the star-shaped PLA, which will be discussed later it was necessary to prepare a series of linear polylactide (PLA) samples differing in molar masses. For this purpose we applied the ring-opening polymerization of LA initiated with Sn(OBu)$_2$ (cf. Eq. (1)). As it has recently been shown [23] polymerization in the LA/Sn(OBu)$_2$/THF system is perfectly controlled with respect

to both molar masses of the resulting PLA and their end-groups. The number-average molar mass (M_n) can be calculated from the $144.13([LA]_0 - [LA])/2[Sn(OBu)_2]_0$ ratio (where 144.13 is the LA molar mass). For the covalent metal alkoxide initiated polymerizations the hydroxyl head end-groups are always present. The tail end-groups are transferred directly from the initiator alkoxide group (Eq. (1)).

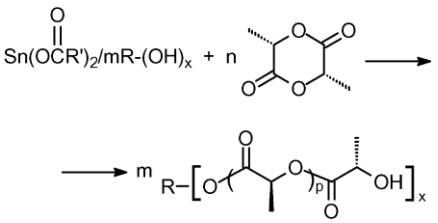
The starting concentrations were as follows: $[LA]_0 = 2.2$ mol l^{-1}, $[Sn(OBu)_2]_0 = 2 \times 10^3$ mol l^{-1}. The polymerization was carried out in THF as solvent at 80 °C. Aliquots of the polymerization mixture were withdrawn after the predetermined times corresponding to the given degrees of the monomer conversion ($\alpha = [LA]_0 - [LA])/[LA]_0$) and related to the assumed M_n's. The predetermined times have been calculated on the basis of kinetic data reported in Ref. [23] Values of the calculated (M_n(calcd)) and determined experimentally (M_n(exp) molar masses of BuPLA-OH obtained at various degrees of monomer consumption are given in Table 1 (entries 1–6).

The prepared series of linear polylactides can be used to find suitable chromatographic conditions for the separation of more complex PLA's. In a first experimental step the critical conditions for PLA must be determined. This was done by measuring the chromatographic behavior of samples of different molar masses in mobile phase of varying composition. Following an approach developed by Schulz et al. [33] for oligomeric PLA, we applied a mixture of 1,4-dioxane (good solvent) and *n*-hexane (poor solvent) as mobile phase. As the stationary phase, we selected a combination of silica gel columns with average pore sizes of 100 and 300 Å (Si-100 and Si-300). The temperature of the LC-CC analysis was set on 50 °C, governing sufficient solubility of the higher molar mass (M_n well above 10^4) poly(L-lactide)s in the eluent. It has been found that at 50 °C critical conditions correspond to a mobile phase composition of 1,4-dioxane/*n*-hexane 56.25:43.75 vol%.

Fig. 1 shows a series of LC-CC traces recorded for Bu-PLA-OH in a wide range of M_n, from 2300 to 74,000 g/mol. Although there is a slight experimental scatter, the elution volumes practically do not depend on the PLA molar masses, as expected for the measurements at the critical point of adsorption.

3.2. Preparation of star-shaped polylactides and their characterization by ^{1}H NMR, SEC and osmometry

Experimental conditions elaborated for the linear PLA were then applied in the LC-CC analysis of the star-shaped PLA's (R-(PLA-OH)$_x$) having various numbers of arms. R-(PLA-OH)$_x$'s were prepared via controlled polymerization of LA initiated by tin octoate ($Sn(Oct)_2$), used in catalytic amounts, and the corresponding polyol, as coinitiator and/or chain transfer agent:



(2)

where $R' = CH(C_2H_5)C_4H_9$, R-(OH)$_x$ stands for polyol (DEG, TMP, DTMP, DPE, PEHMO—see structures

Table 1
Feed compositions of the polymerization mixtures and molar masses (M_n) of the resulting linear and star-shaped polylactides

Entry	Initiating system	$[I]_0$[a] (mol L^{-1})	$[LA]_0$ (mol L^{-1})	α[b]	M_n (calcd)[c]	M_n (exp)	M_w/M_n (SEC)
1.	$Sn(OBu)_2$[d]	1.93×10^{-3}	2.29	0.02	1784	2300[e]	1.59
2.				0.03	2639	3150[e]	1.16
3.				0.05	4349	5160[e]	1.12
4.				0.10	8625	8970[e]	1.12
5.				0.55	47,103	42,900[f]	1.18
6.				0.94	80,446	74,000[f]	1.52
7.	$Sn(Oct)_2$/DEG[g]	0.156			7800	9000[h]/6700[i] (8220[h]/6700[i])[j]	1.11
8.	$Sn(Oct)_2$/TMP[g]	0.165			7400	7800[h]/7500[i] (8400[h]/7200[i])[j]	1.11
9.	$Sn(Oct)_2$/DTMP[g]	0.180	8.4	≈0.99	6900	8800[h]/820[i] (7970[h]/7000[i])[j]	1.15
10.	$Sn(Oct)_2$/DPE[g]	0.168			7400	11,300[h]/11,100[i] (11,400[h]/11,700[i])[j]	1.13
11.	$Sn(Oct)_2$/PEHMO[g]	0.200			7420	8300[h]/8100[i] (7100[h]/7600[i])[j]	1.19

[a] $[I]_0$ stands for starting concentrations of: OBu groups in $Sn(OBu)_2$ or alcohols and polyols.
[b] $\alpha = ([LA]_0 - [LA])/[LA]_0$, as determined by polarimetry and/or from SEC traces of the PLA/LA reacting mixture (RI detection) taking into account refractive index differences of LA and PLA (surface area under the LA signal was multiplied by 0.89).
[c] M_n (calcd) $= 144.13\alpha[LA]_0/[(co)initiator]_0 + M_{ROH}$ (where M_{ROH} stands for molar masses of BuOH or polyols used as coinitiators).
[d] THF as solvent, 80 °C.
[e] Determined using calibration on the linear PLA standards.
[f] Determined with laser light scattering detector.
[g] $[Sn(Oct)_2]_0 = 0.08$ mol/l.
[h] Measured by VPO.
[i] Determined from ^{1}H NMR spectra.
[j] Data for the acetylated PLA (R-(PLA-$OOCCH_3$)$_x$).

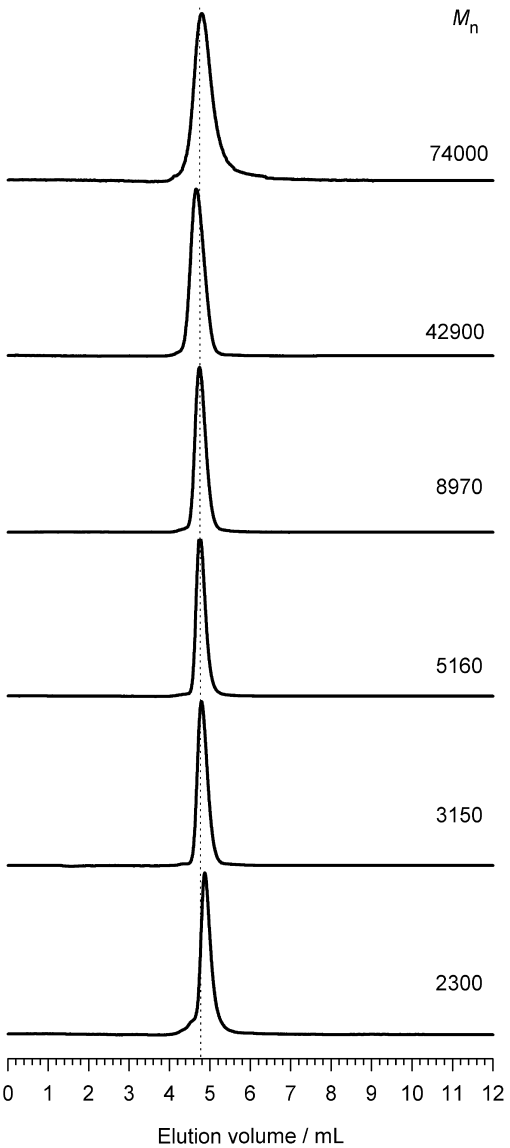
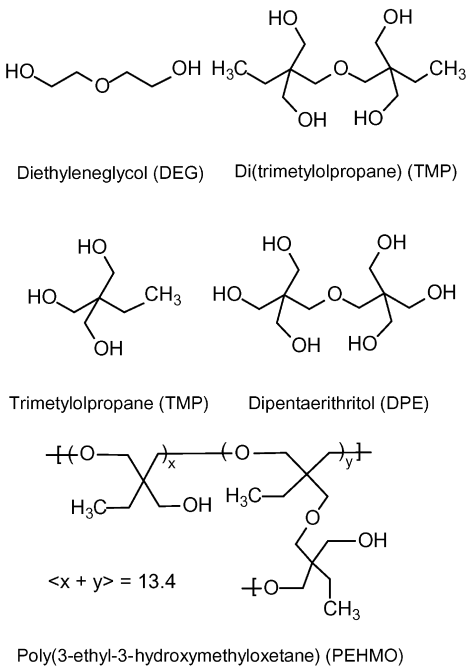


Fig. 1. LC-CC traces of linear polylactides (Bu-PLA-OH) of various molar masses. Mobile phase-1,4-dioxane/*n*-hexane 56.25: 43.75 vol%; 50 °C.

below), $\langle p \rangle = 2n/x - 1$. All polyols were fitted with primary hydroxyl groups, enabling fast coinitiation and chain transfer reactions.

It has been shown in a series of our works published recently [23,24,38,43,44] that the actual initiator in the LA/$Sn(Oct)_2$/ROH system has the tin(II) alkoxide structure and is formed in the carboxylate-alkoxide ligands exchange reaction. Molar masses in this polymerization are controlled by the $144.13 - ([LA]_0[LA])/[R\text{-}(OH)_x]_0$ ratio, providing that the polyol is sufficiently soluble in the polymerization mixture. For example, pentaerithritol (PE) was claimed to be an effective coinitiator/chain transfer agent [17,19,45,46]) in $Sn(Oct)_2$/LA system, but in our hands coinitiation with PE appeared non-quantitative, and thus not allowing synthesis of PLA with predetermined molar masses. Therefore we decided to employ the other tetraol, namely DTMP, of much better solubility. It has also to be stressed



Scheme 1.

that M_n's of star-shaped PLA reported in the present paper were determined by means of osmometry, giving the absolute and real values, in contrast to these obtained by others from SEC measurements based on polystyrene standards and without the necessary correction.

Star-shaped PLA's of $M_n \approx 10^4$ g/mol were prepared according to a procedure described in Section 2. Table 1 reports on the corresponding feed compositions and molar masses of the resulting R-(PLA-OH)$_x$'s (entries 7–11). Generally, there is a good agreement between the assumed molar masses (M_n(calcd)) and determined (M_n(exp)), by means of vapor pressure osmometry and ^{1}H NMR spectroscopy. However, M_n(calcd) are slightly higher than M_n(exp), This effect can be attributed to the incomplete solubility of some polyols in the polymerization medium effecting both coinitiation and transfer—this is particularly pronounced for DPE. Nevertheless, even at high monomer conversion, polydispersity indexes (M_w/M_n) of the star-shaped PLA's, were typically in the range from 1.11 to 1.19. The latter result can be explained, most probably, by a depression of the segmental exchange (intermolecular transesterification) side reactions [42,47] due to the steric hindrance caused by the star-like structure of growing macromolecules. Moreover, linear macromolecules of a given $(M_w/M_n)_{arm}$ located at a core lead to star macromolecules having lower $(M_w/M_n)_{star}$, in comparison with $(M_w/M_n)_{arm}$, as it stems from Eq. (3) [48].

$$(M_w/M_n)_{star} = 1 + \frac{(M_w/M_n)_{arm} - 1}{x} \cdot \frac{(M_{n(star)} - M_{core})^2}{(M_{n(stat)})^2} \quad (3)$$

where x denotes the number of arms in one macromolecule.

Agreement of the assumed and resulting molar masses

does not necessary mean that all hydroxyl groups in a given polyol have been reacted with $Sn(Oct)_2$ and started growth of the PLA chains. This problem can be solved by means of analysis of the ^1H NMR spectrum of the resulting R-(PLA-OH)$_x$. Indeed, the ^1H NMR data reported in the Experimental part show unequivocally that in all of the involved polyols quantitative transformation of the $-CH_2OH$ groups into the $-CH_2O$-PLA-OH macromolecular moieties proceeded. However, size distribution of the individual PLA-OH chains in DTMP-(PLA-OH)$_4$ is still a pending question.

3.3. Analysis of star-shaped polylactides by means of LC-CC

Fig. 2 shows a series of HPLC traces recorded for R-(PLA-OH)$_x$ bearing 1, 2, 3, 4, 6, and 13 PLA-OH arms and with M_n close to 10^4 (linear Bu-PLA-OH and HO-PLA-DEG-PLA-OH can formally be considered as star-shaped PLA's with 1 or 2 PLA-OH arms). Measurements were carried out at 50 °C using the critical compositions that have been established for linear PLA (i.e. 1,4-dioxane/*n*-hexane 56.25: 43.75 vol%).

The samples have similar molar masses and, therefore, cannot be separated by SEC. In LC-CC separation occurs with respect to the heterogeneity of the polymer chain. The critical conditions are optimized regarding the homopolymer chain with a fixed number and type of endgroups. If in other samples the numbers and/or types of endgroups are different, then the chromatographic behaviour of these samples will change. This can be clearly seen for the series of samples shown in Fig. 2.

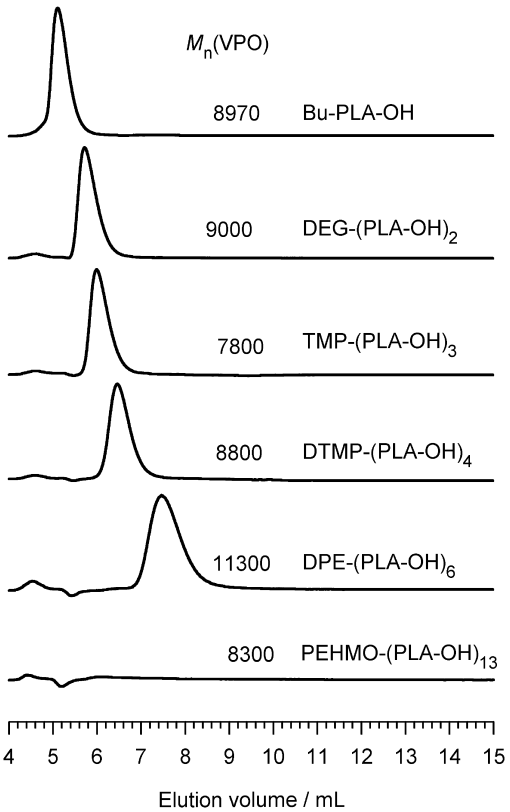


Fig. 2. LC-CC traces of star-shaped polylactides (R-(PLA-OH)$_x$) fitted with various number of PLA arms. Mobile phase-1,4-dioxane/*n*-hexane 56.25:43.75 vol%; 50 °C.

According to Fig. 2 elution volumes increase with the number of PLA-OH arms in R-(PLA-OH)$_x$. The explanation for this behaviour is straightforward: the chromatographic experiments are conducted on a polar stationary phase of silica gel. The interaction between the PLA molecules and the stationary phase occurs mainly through the hydroxy endgroups which are the most polar part of the molecules. The more interacting sites (end groups) the molecules have the stronger will be the interaction with the stationary phase and the larger will be the elution volume.

For polymers bearing 1, 2, 3, 4, and 6 arms this dependence is shown in Fig. 3. The solid line was computed by a 'brut-force' curve-fitting and extrapolated (the dotted line) into the direction of the higher number of arms). Such a behavior results from the increasing strength of the macromolecule—column packing attractive forces increasing with the increasing number of PLA arms fitted with the hydroxyl end-groups. According to the linear free relationship or Martin's rule [49] the capacity factor is supposed to depend on the number of hydroxyl groups exponentially. This means that ln V_R is a linear function of the number of arms. This is indeed the case as can be seen from the insertion in Fig. 3. As can be seen in Fig. 2, the 13-arm PLA is very strongly retained due to the high number of terminal hydroxyl groups.

Differences in the elution volumes are large enough to detect individual species for example in the LC-CC trace of a mixture composed of the 2-, 3-, 4-, and 6-arms R-(PLA-OH)$_x$ all of them having similar molar masses (Fig. 4). First the species with smaller number of the hydroxy end-groups are eluted followed by species having more hydroxy

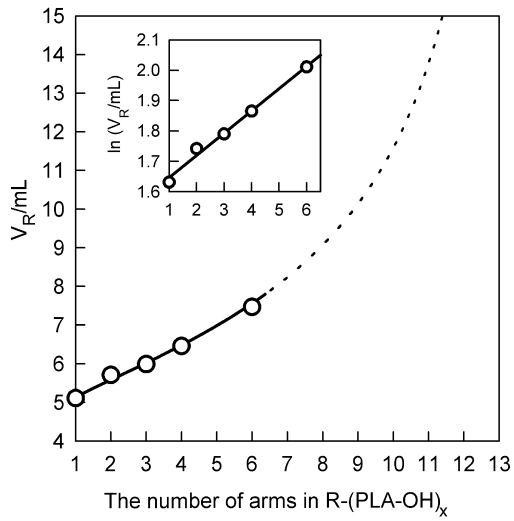


Fig. 3. Dependence of elution volumes measured at the peak maxima in LC-CC traces shown in Fig. 2 on the number of PLA arms in star-shaped polylactides (R-(PLA-OH)$_x$).

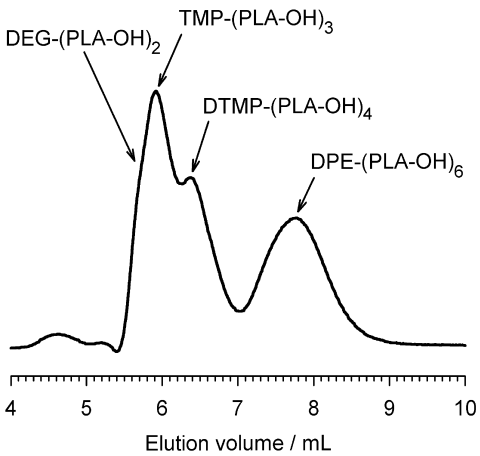


Fig. 4. LC-CC trace of the artificial mixture composed of DEG-(PLA-OH)$_2$, TMP-(PLA-OH)$_3$, DTMP-(PLA-OH)$_4$, DPE-(PLA-OH)$_6$. Mobile phase-1,4-dioxane/*n*-hexane 56.25:43.75 vol%; 50 °C.

endgroups. It is interesting to notice that the 2-arm and the 3-arm nearly co-elute while the 4-arm is more efficiently separated from the 3-arm. A further increase of the number of arms to the 6-arm species results in a near-baseline separation.

For the 13-arms PEHMO-(PLA-OH)$_{13}$ the adsorptive interaction with the stationary phase is so strong that this polymer gives no detectable elution peak and could be eluted from the column only after very long time (Fig. 2, first trace from the bottom). That does not mean, however, that the determination of such highly branched R-(PLA-OH)$_x$ is not possible. It may, however, require to select a different column packing with a lower polarity.

In order to analyse star-shaped PLA's with a large number of arms, these samples can be acetylated to decrease the adsorption on the stationary phase. The analysis of three acetylated polymers (R-(PLA-OOCCH$_3$)$_x$) (Fig. 5) namely TMP-(PLA-OOCCH$_3$)$_3$, DPE-(PLA-OOCCH$_3$)$_6$, and PEHMO-(PLA-OOCCH$_3$)$_{13}$ indicates again that for the interaction with column packing mostly the hydroxyl chain-ends in PLA-OH arms are responsible. The molar masses, determined by VPO and ^{1}H NMR, of R-(PLA-OOCCH$_3$)$_x$ and their R-(PLA-OH)$_x$ counterparts are close one to each other (Table 1), showing that during acetylation procedure a given star-shaped structure remained untouched. Considerably lower LC-CC elution volume dependence on the number of PLA-OOCCH$_3$ arms, when compared to the hydroxyl group terminated polymers, indicates that indeed the interactions of –OH end groups with the column packing is responsible for the success of the applied method. This effect is particularly pronounced for 13-arms PLA (PEHMO-(PLA-OOCCH$_3$)$_{13}$) which elutes very closely to the 3-arm and 6-arm PLA's. For the intact PEHMO-(PLA-OH)$_{13}$ elution could not be monitored at all (Fig. 2). The chromatographic behaviour of the samples as indicated in Fig. 5 is a clear indication for the large polarity differences between the end groups and the polymer chain. The polarity

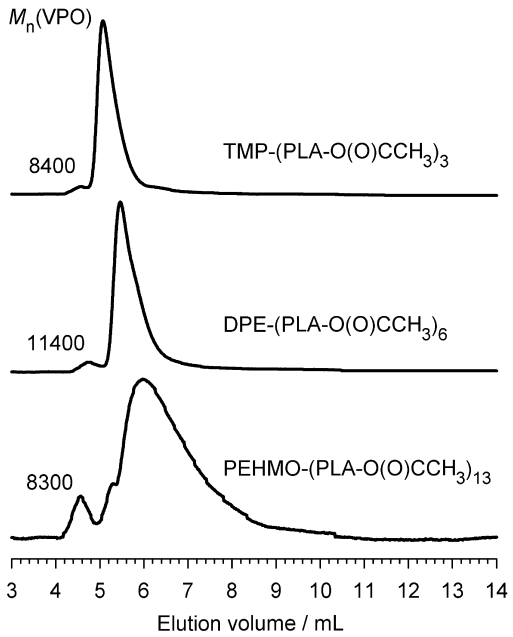


Fig. 5. LC-CC traces of the acetylated star-shaped polylactides (R-(PLA-OOCH$_3$)$_x$) bearing various number of PLA arms. Mobile phase-1,4-dioxane/*n*-hexane 56.25:43.75 vol%; 50 °C.

of the acetyl end groups obviously is very close to the polarity of the macromolecular backbone and, therefore, even large differences in the number of end groups do not manifest themselves chromatographically.

LC-CC is a perfect chromatographic tool to be used as the first dimension in multidimensional chromatographic set-ups.

The combination of LC-CC and SEC in automated two-dimensional chromatography enables the analysis of complex mixtures of polyaliphatic esters of various architectures with regard to functionality and molar mass. A first example of such an analysis has been published recently by us in a preliminary report [50]. The experimental set-up of two-dimensional (2D) chromatography including some applications have been described in Ref. [34]. In brief, the separation according to the endgroups by LC-CC is carried out in the first dimension resulting in fractions that are uniform with regard to chemical composition but polydisperse with regard to molar mass. The fractions from the first dimension are automatically transfered to the second (SEC) dimension where separation occurs with regard to molar mass. Arranging all SEC traces according to the elution volume of the LC-CC a contour diagram is produced that presents the 2D separation in the coordinates composition-molar mass.

As can be seen in the contour diagram (Fig. 6), the complex sample is separated in both dimensions. The separation in the vertical direction corresponds to the functionality type separation. The horizontal direction summarizes the separation with regard to molar mass. The concentration profile is coded by different colours, light colours indicating high concentrations. Thus, the 2D trace of

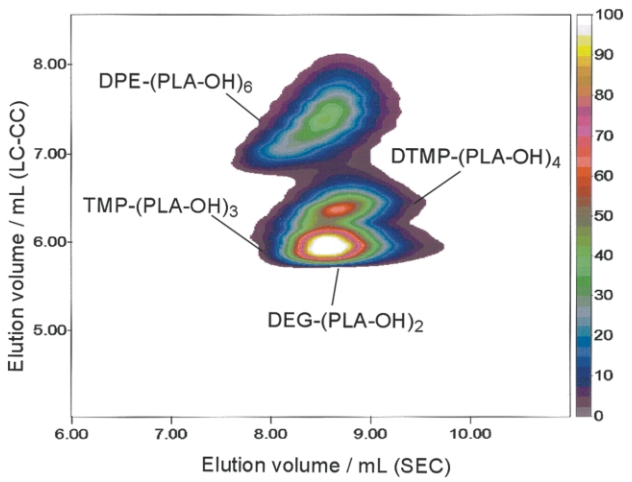


Fig. 6. Two-dimensional LC-CC versus SEC trace of the artificial mixture composed of DEG-(PLA-OH)$_2$, TMP-(PLA-OH)$_3$, DTMP-(PLA-OH)$_4$, DPE-(PLA-OH)$_6$.

a mixture composed of equal masses of DEG-(PLA-OH)$_2$, TMP-(PLA-OH)$_3$, DTMP-(PLA-OH)$_4$, and DPE-(PLA-OH)$_6$ exhibits charcteristic features listed below.

- The 2-arm and the 3-arm PLA could hardly be separated in the LC-CC dimension (cf. Fig. 2).
- The separate contours coming from 2-arm/3-arm, 4-arm, and 6-arm PLA's are clearly seen. The separation power increases exponentially with the increasing number of arms (cf. Fig. 3).
- The higher is the number of the PLA-OH arms the more counter-clockwise are turned the corresponding contours. This behaviour is probably related to the decreasing accessability of the hydroxy end-groups with increasing both the arms number (x) and molar mass of a given R-(PLA-OH)$_x$ fraction. Another possible explanation is that during the polymerization process the statistical differentiation in the PLA-OH arms lengths proceeds and this differentiation is more pronounced for stars with higher x. Then the shorter PLA-OH chains interact with the column packing less effectively.

Acknowledgements

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