Investigation of the influence of impurities on the ring-opening polymerisation of L-Lactide from biogenous feedstock

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Abstract

In order to use a L-lactide monomer that is derived from fermentation processes it is necessary to understand, how the polymerisation process is influenced by impurities which derive from the production process. We have selected a group of likely contaminants and added them at various concentrations to the polymerisation of L-lactide using tin (II)-2-ethylhexanoate as catalyst and 2-methoxyethanol as initiator. The effect of impurities onto the global properties of the polymers such as glass transition temperature, melting point and molecular mass distribution were investigated and NMR and MALDI mass spectrometry were used to identify structural changes within the polymers. Thus, it could be shown that in reference experiments cyclic polymers and linear polymers with different starting groups are formed. Addition of ethanol and sodium carbonate showed the strongest influence on molecular masses as well as polymer structures, which could be elucidated by interpretation of the MALDI mass spectra and NMR data.

Keywords Poly (lactic acid) · Lactide · MALDI mass spectrometry · Biogenous feedstock

Introduction

One of the most heavily used commodity products worldwide are plastics. Plastic products are used in almost each and every part of our lives due to their properties and versatility. Polymers can be used in almost every shape, are light weight, their mechanical properties can be adjusted to a vast scale of requirements and polymers are at the very same time very cost efficient [1]. The production of plastics based on fossil feedstocks is still growing, however it is recently more and more questioned [2, 3]. Therefore, the public's focus is moving towards so called bioplastics. Bioplastics can be divided into two groups, plastics originating from biogenous resources and biodegradable plastics irrespective of their origin. It is not mandatory that these specifications correlate [4, 5].

It is worth saying, that the production and usage of biopolymers is by far not an idea of our age. Already decades ago, biopolymers have been known but have

been displaced by the cheaper and more versatile fossil based products [6, 7]. Biopolymers that recently experience an increasing interest may be cellulose based polymers, starch and starch blends, poly(ϵ -caprolactone), poly(butylene succinate), poly(butylene adipate-co-terephthalate), poly(hydroxy alkanate) and poly(lactic acid) [4]. Although, the properties of these biopolymers differ to the properties of fossil-based large volume polymers, the potential of biopolymers is huge. It is shown that the biodegradability provides a big advantage e.g. in medical applications. Products made from biodegradable plastics can be used in tissue engineering, for implants like screws, plates or anchors, as a fracture fixation or even for drug delivery systems. However, the usage of bioplastics is not limited to niche products in the medical area, but can also be expanded to fibre production and textile industry, the automotive sector or packaging, revealing a huge potential for the future market share [8–10].

The predicted volume of bioplastics has shown high growth rates for years. Market prospects foresee strongly growing production capacities, while the actual growth rate is lagging behind [11]. But still, the successive development of polymerisation plants, property modification of bioplastics and application adaption are leading to a rewarding market section that offers new

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perspectives [2]. Especially two kinds of biopolymers gain special attention: Poly(hydroxy alkanooate) PHA and poly(lactic acid) PLA tend to be among the most promising plastics, due to their versatile properties and broad application areas [8, 11, 12].

There are two basic polymerisation techniques for the synthesis of PLA, polycondensation and ring-opening polymerisation ROP. Numerous publications examine the polymerisation possibilities of direct polycondensation. Ajioka et al. [13] showed in 1995 the applicability of Sn-powder as a catalyst in the direct polycondensation of lactic acid with reaction conditions of 9 h and 150 °C at reduced pressures leading to molecular masses up to 300 000 g mol⁻¹. Kim et al. [14] tested the impact of several Sn and Ti based catalysts via an azeotropic dehydration of lactic acid over 48 to 72 h leading to molecular masses of 33 000 g mol⁻¹, while Nagahata et al. [15] demonstrated that a microwave-assisted direct polycondensation of lactic acid is providing much faster reaction times with suitable molecular masses. The method of direct polycondensation requires quite high reaction temperatures, relatively long reaction times of several days and low pressures to sufficiently remove the condensate and therefore, gaining sufficient molecular masses. Therefore, the molecular masses obtained from direct polycondensation are low compared to ROP. Increasing focus is brought to the synthesis of PLA copolymers from direct polycondensation [16–18].

The more promising polymerisation technique for the synthesis of poly(lactic acid) is the ring-opening polymerisation. The ROP was already studied in 1959 by Kleine et al. [19] who were using Zn and PbO catalysts in a solution and solvent free polymerisation of lactide. Fischer et al. [20] enhanced the ROP investigation by means of SnCl₄ as a catalyst. The polymerisation reaction was conducted in toluene, with reaction times of 20 h leading to promising molecular masses of 80 000–100 000 g mol⁻¹. New catalyst systems are under investigation in recent times. For instance, Myers et al. [21] conducted a metal free approach on ROP of lactides using nucleophilic phosphines as transesterification catalysts. Lohmeijer et. al [22] are investigating guanidine and amidine catalyst systems that show high selectiveness and seem to be well suited for block-copolymers. Still, one of the most common catalyst systems used is Tin (II)-2-ethylhexanoate Sn(Oct)₂ [23–26]. Due to the fact of its FDA approval for low toxicity it is a wide spread catalyst system which produces polymers with high molecular masses, proper yields and reasonable stereoregularity from various lactides and lactones [27, 28]. Karidi et al. [29] show that with simple variations of the initiator used in combination with Sn(Oct)₂, branched polymers of all shapes can be produced. Furthermore,

cyclic PLA is produced with the combination of Sn(Oct)₂ and new initiators by Kricheldorf et al. [27] with molecular masses up to 170 000 g mol⁻¹. Ring-opening polymerisation is a promising technique of producing high molecular mass PLA which is underlined by the predominant amount of research in this field. New trends in PLA synthesis tend to grow in the direction of ring-expansion polymerisation REP, due to advantages in the degradation behaviour of cyclic polymers in comparison to linear polymers [27, 30, 31].

The synthesis route of PLA via ROP is in general leading to polymers with a higher molecular mass than via direct polycondensation, respectively. Therefore, the lactide needs to be of special polymeric grade quality, which purity exceeds the requirements for the food industry [32]. This grade is obtained by the dimerisation of lactic acid and extensive purification steps in lactic acid downstream processing [33]. This downstream processing from the fermentation broth is a complex task, which requires high time and energy consumption [34, 35]. Still the production of lactic acid from fermentation is the most favourable pathway, since racemic pure lactic acid is obtained by the microorganisms utilised [36]. As the fermentation process has to fulfil special conditions for the microorganism's metabolism, the fermentation broth is a highly complex system with a large number of substances that may be present in the product and therefore may also intervene in the polymerisation process [32, 37–39].

The objective of this work was to investigate the impact on the PLA polymerisation process triggered by unwanted substances present in the monomer. These unwanted substances may originate from the fermentation broth or the downstream process of lactic acid. This means that nutritional substances for the microorganisms, metabolic by-products, filtration auxiliaries and the like may act as a contaminant. Therefore, a reference experiment with high monomer purity is established, serving as basis for experiments with doped monomers. The impurities chosen for this study were selected in accordance to literature [32, 34–39].

Materials & methods

L-lactide (purity, 98%) and 2-methoxyethanol (purity, 99,8%) were purchased from Sigma Aldrich. For further purification L-lactide was recrystallised in 2-propanol, dried in vacuo and stored under inert atmosphere. Tin (II)-2-ethylhexanoate (purity, technical grade) was purchased from Alfa Aesar. Ethanol (purity, absolute) and toluene (purity, pa) were purchased from VWR. Toluene was distilled over Na for further purification.

CaCO_3 (purity, 98%), L(+)–aspartic acid (purity, 98 + %) and L(+)–glutamic acid (purity, 99,2%) were purchased from Acros Organics. CaSO_4 (purity, 99%), Na_2SO_4 (purity, pa) and D(+)–glucose (purity, bio-grade) were purchased from Merck. D(+)–sucrose (purity, 99,5%) was purchased from Roth. All other solvents were of reagent grade. If not stated otherwise, all reagents and solvents were used without further purification.

Recrystallised L-lactide was polymerised by the use of Schlenk technique. 1 g of L-lactide was weighed in the glovebox, sealed in a vial and transferred to the prepared round bottom flask. The monomer was added to the reaction vessel with counterflow of inert gas and purged for several minutes. For initiating the ring-opening polymerisation 0.25 mL catalyst solution, (tin (II)-2-ethylhexanoate, 0.2 mol L^{-1} in toluene, 0.05 mmol), and 0.1 mL initiator solution (2-methoxyethanol ME, 0.12 mol L^{-1} in toluene, 0.012 mmol) were added to the L-lactide via a rubber stopper using syringes purged with inert gas. The reaction mixture was stirred and kept at 180 °C for 1 h while solidification occurred. For quenching, the reaction was cooled for several minutes, 5 mL of chloroform were added via the rubber stopper and the reaction mixture was kept boiling under reflux until the formed polymer was fully dissolved. The polymer solution was cooled to room temperature (RT) and the polymer was precipitated by the dropwise addition of 15 mL methanol. The precipitated polymer was vacuum filtrated, washed several times with methanol and dried in a vacuum compartment dryer at 40 °C.

For the study of the influence of impurities on the polymerisation of L-lactide the monomer was doped with different amounts of possible contaminants that may originate from the fermentation process of lactic acid or the down-stream processing previous to polymerisation. The monomer was doped with 0.1 w% (triple determination), 1.0 w% and 1.5 w% respectively. The polymerisation processes were conducted analogous to the reference experiments. For the contamination with ethanol, solutions of 0.21 mol L^{-1} and 2.14 mol L^{-1} in toluene were prepared. The addition of 0.1 mL and 0.15 mL of these solutions account for the addition of 0.1 w%, 1.0 w% and 1.5 w%, respectively.

Differential scanning calorimetry DSC results were obtained using a Mettler Toledo DSC 3+. The experiments were conducted in a Heat-Cool-Heat cycle evaluating the second heating cycle for the investigation of the PLA samples. The measurements were performed from -40 °C to 200 °C with a heating rate of 10 K min^{-1} , with a 3-min isothermal state before and in-between the dynamic sections, at the respective temperature. The nitrogen gas flow was 20 mL min^{-1} .

Size exclusion chromatography SEC was conducted using an Agilent 1200 HPLC equipped with Phenomenex columns, a diode array detector DAD (254 nm) and an evaporative light scattering detector. The polymer samples were dissolved in CHCl_3 at a concentration of approx. 6 mg mL^{-1} . Each sample was doped with 0.5 μL toluene / mL solvent used as an internal standard. The separation took place on a Phenomenex Guard column (SecurityGuard standard), and a series of Phenogel columns (Phenogel 5 μm 10E3 Å, 300 × 4.6 mm; Phenogel 5 μm 10E4 Å, 300 × 4.6 mm; Phenogel 5 μm 10E5 Å, 300 × 4.6 mm). Analyses were carried out in CHCl_3 at 40 °C with a flow rate of 0.35 mL min^{-1} and an injection volume of 10 μL .

Mass spectra were recorded on a Bruker Autoflex III in reflectron mode. The samples were dissolved in chloroform (10 mg mL^{-1}), mixed with *trans*-2-[4-*tert*-butylphenyl-2-methyl-2-propylidene] malonitrile (DCTB; 10 mg mL^{-1} in chloroform) used as matrix and sodium trifluoroacetate (1 mg mL^{-1} in THF) to enable sodium adduct formation in the ratio matrix:sample:salt = 100:10:1. 1 μL of this solution was applied onto the MALDI target.

^1H -NMR and HSQC spectra were recorded on a Bruker Avance III 700 MHz spectrometer. Samples were dissolved in CDCl_3 with a concentration of approximately 1.2 mg mL^{-1} .

Results & discussion

Polymerisation

For the standard value assessment of the poly (lactic acid) synthesis the recrystallised L-lactide was polymerised as stated in the section above. In the following these experiments are conducted as reference experiments. The experiments were performed repeatedly throughout the investigation. During the reaction time the bulk in the round-bottom flask became highly viscous and solid at the end of the polymerisation. A colour change of the reaction mixture from clear to slightly beige could be observed within the reference experiments. After precipitation and filtration, a white powder was obtained.

For the investigation of the impact of possible impurities the following substances were chosen: L(+)–aspartic acid, L(+)–glutamic acid, CaSO_4 , CaCO_3 , D(+)–sucrose, D(+)–glucose, ethanol, and Na_2CO_3 . The syntheses were performed analogous to the standard procedure. The experiments containing the stated quantities of L(+)–aspartic acid, L(+)–glutamic acid, CaSO_4 , and CaCO_3 all showed similar reaction behaviour, leading

to a solid product in the reaction flask. Although the reaction bulks showed light yellow and opaque appearances, white to off-white products were isolated. Also the experiments regarding D(+)-sucrose led to a solid reaction bulk within 1 h. It had a similar yellowish and cloudy appearance, but small dark chunks could be identified. When doping the reference experiment with ethanol also a yellowish and cloudy reaction mixture was obtained. However, the mixture showed a reduced viscosity. Within 1 h reaction time a gel-like product was formed, which was introduced to the workup, leading to an off-white powder of PLA. The syntheses of PLA with D(+)-glucose as a contaminant also showed a significantly reduced viscosity of the reaction bulk. Additionally, the colour of the reaction mixture turned dark brown to black, giving a beige PLA product. The experiments regarding contamination with Na_2CO_3 showed various differences among the concentration variation. Experiments containing 0.1 w% Na_2CO_3 showed reaction properties alike the reference experiments, with light yellow

colouring of the bulk and a white powdery product. When 1.0 w% of Na_2CO_3 was present in the reaction mixture its colour changed to yellow. Sticking to the workup routine no solid polymer could be precipitated. Therefore, additional methanol was added until a precipitate was formed. After filtration an additional oil-like phase was obtained, which was also dried and found to contain PLA. Both samples obtained were investigated. At the presence of 1.5 w% Na_2CO_3 no solid product could be precipitated during the workup. As a consequence, the methanolic solution was removed by the use of a rotary evaporator, leaving a sticky, brown to yellowish paste as a product.

Differential scanning calorimetry

Thermal investigations of the reference experiments indicate an average glass transition temperature T_g of $53\text{ }^\circ\text{C} \pm 3\text{ }^\circ\text{C}$ and an average melting point T_m of

Table 1 Thermal properties T_g and T_m , mean molecular masses M_n and M_w , polydispersity $\bar{D}$ and calculated influences of polymerisation products synthesised. Values stated with 0.1 w% impurity are mean values from 3 repeated experiments. Values stated as reference are mean values from 18 repeated experiments

	Impurity amount	T_g	T_m	Influence on T_m	M_n	M_w	$\bar{D}$	Influence on M_w
	/ w%	/ $^\circ\text{C}$	/ $^\circ\text{C}$	/ %	/ g mol^{-1}	/ g mol^{-1}	-	/ %
Reference	-	54	164	-	20 100	30 900	1.54	-
L(+)-Aspartic acid	0.1	58	166	1.3	21 900	34 700	1.59	12
	1.0	52	164	-0.2	19 400	30 300	1.56	-2
	1.5	53	164	-0.3	19 000	29 900	1.57	-3
L(+)-Glutamic acid	0.1	56	165	0.6	18 500	29 000	1.56	-6
	1.0	49	163	-1.0	10 000	14 800	1.46	-52
	1.5	49	159	-3.0	8 400	11 500	1.36	-63
Na_2CO_3	0.1	55	163	-0.6	19 300	30 700	1.59	-1
	1.0	51	-	-	17 800	22 400	1.26	-45
	1.0	46	-	-	13 100	16 900	1.29	-28
	1.5	26	-	-	3 400	4 000	1.15	-87
D(+)-Glucose	0.1	50	163	-0.9	18 700	30 600	1.63	-1
	1.0	49	160	-2.3	10 000	14 600	1.47	-53
	1.5	47	157	-4.5	9 800	14 500	1.47	-53
D(+)-Sucrose	0.1	52	162	-1.2	17 400	26 700	1.53	-14
	1.0	49	160	-2.5	11 400	17 800	1.56	-42
	1.5	50	161	-1.9	11 300	17 000	1.50	-45
CaSO_4	0.1	51	162	-1.2	18 800	30 700	1.64	-1
	1.0	51	162	-1.4	11 200	17 100	1.53	-45
	1.5	50	161	-2.3	9 700	13 800	1.42	-56
Ethanol	0.1	48	165	0.3	19 300	29 200	1.51	-5
	1.0	46	156	-4.8	9 500	12 400	1.31	-60
	1.5	42	154	-6.4	7 900	9 800	1.24	-68
CaCO_3	0.1	52	163	-0.5	21 700	34 600	1.59	12
	1.0	51	162	-1.6	18 400	27 100	1.48	-12
	1.5	50	162	-1.6	16 100	23 700	1.47	-23

163.6 °C $\pm$ 1.9 °C, respectively. Thermal properties of samples prepared with D(+)-sucrose, CaSO₄, and CaCO₃ indicate only a minor influence. The glass transition temperatures and the melting points of these samples at lower impurity content are within the range of standard deviation of the reference experiments. Increasing impurity contents are leading to a decrease in T_g and T_m of 2–4 °C. The presence of D(+)-sucrose, CaSO₄, and CaCO₃ results in a minor impact on the produced polymer denoting that these impurities have low relevance on the thermal polymer properties. Enhanced influence on the T_g and T_m is seen from experiments doped with L-glutamic acid and D(+)-glucose. At an impurity content of 0.1 w% T_g and T_m show less dependence, but with increasing impurity content T_g and T_m of the samples drop distinctly by 5–7 °C. Further leverage can be identified by the addition of ethanol. The thermal properties are affected strongly by the presence of ethanol in the reaction mixture. The glass transition temperature drops by 12 °C and the melting point is decreased by 10 °C. The investigations concerning Na₂CO₃ do not show an impact on the thermal properties at the amount of 0.1 w%, but with increasing impurity content the glass transition temperature dropped by 28 °C. A melting point could not be detected for samples with 1.0 w% or 1.5 w% Na₂CO₃ respectively. The DSC analysis reveals that Na₂CO₃ is dramatically intervening the polymerisation of L-lactide. No crystalline regions within the synthesised macromolecules are built. The analyses of samples with L(+)-aspartic acid show a different behaviour. T_g and T_m both show an increase at an impurity content of 0.1 w%. Rising amounts of L(+)-aspartic acid depress the thermal properties back into the standard deviation range of the reference experiments. In Table 1 the values for T_g and T_m are given.

The effects of impurities on the melting points are depicted in Fig. 1 in the form of deviation in % relative to the reference experiments. The zero line determines the average melting point of the reference experiments, the grey area describes the standard deviation within the reference experiments.

In Fig. 2 exemplarily the thermal behaviours of CaCO₃, D(+)-glucose, ethanol, and L(+)-aspartic acid are denoted.

All synthesised and investigated polymers show the presence of an exothermic peak in the heating curve. This peak indicates the effect of cold crystallisation, which means that the cooling process was too quick, the crystallisation of the PLA was not sufficient and therefore a crystallisation effect occurs when the polymeric chains become flexible. The cold crystallisation is visualised in Fig. 3, where the thermograms of a reference experiment and a CaSO₄ doped experiment are given. The thermograms also indicate a difference

for stronger contaminated samples, regarding the melting behaviour. With increasing impurity content, an enhanced double melting peak is observed. This holds true for experiments doped with CaCO₃, ethanol, CaSO₄, D(+)-sucrose, D(+)-glucose, and L(+)-glutamic acid. It leads to the assumption that although CaCO₃, CaSO₄, and D(+)-sucrose only have a minor impact on the melting point, there might be an influence on the lamella formation within the crystals [40].

Size exclusion chromatography

The investigations of the reference experiments via size exclusion chromatography indicate a mean number average molecular mass M_n of 20 100 g mol⁻¹ $\pm$ 1 300 g mol⁻¹ and a mean weight average molecular mass M_w of 30 900 g mol⁻¹ $\pm$ 2 800 g mol⁻¹, respectively. The investigations of PLA samples with contaminants showed an influence on both M_n and M_w for all the chosen substances. The contamination with the substances at a level of 0.1 w% led to the result that there is no significant impact on the molecular mass, except for L(+)-aspartic acid and CaCO₃ which cause the M_n and M_w to increase. The changes of M_w at a contamination level of 0.1 w% vary within the standard deviation range of the reference experiments. Therefore, a contamination at this level doesn't seem to cause problems in the polymer formation. Concerning the experiments with higher contamination levels, the interference of the chosen substances is demonstrated severely. The synthesised polymers

Fig. 1 Deviation of the melting point T_m in % relatively to the reference experiments. For samples prepared with 1.0 w% Na₂CO₃ and 1.5 w% Na₂CO₃ no melting point could be measured

Fig. 2 Trend of glass transition temperature T_g and melting point T_m depending on the contamination value; a) CaCO_3 , b) D(+)-glucose, c) ethanol, d) L(+)-aspartic acid

doped with 1.0 w% and 1.5 w% CaCO_3 suffered from a loss in the weight average molecular mass of up to 7 500 Da. The consequences of an impurification on the M_w are even stronger for the monomer pollution with L(+)-glutamic acid, D(+)-glucose, D(+)-sucrose, and CaSO_4 . All of these substances cause a loss in the M_w in the order of approximately 50 to 60%, in this case yielding polymers of only 10 000–17 000 g mol^{-1} . The high impact on the molecular masses is visualised in Fig. 4. In the presence of ethanol, the polymerisation is already highly affected at 1.0 w%. As depicted in Fig. 5 the weight average molecular mass is only reaching a level of 12 400 g mol^{-1} at 1.0 w% pollution and

approx. 10 000 g mol^{-1} at 1.5 w% pollution, respectively. This is a consequence of the fact that ethanol acts as an additional initiator just like 2-methoxyethanol and can therefore start the polymerisation. By calculating the theoretically achievable number average molecular mass M_n , it can be identified that ethanol and 2-methoxyethanol are in competition with each other. When calculating the theoretical achievable molecular mass for the experiments doped with ethanol on the basis of mass conservation by considering the initiator/monomer ratio it can be seen, that the detected molecular masses exceed the theoretically achievable value by the factor of 2 to 3. The calculation is performed with respect to the fact that

Fig. 3 Exemplarily thermogram of a PLA reference experiment and PLA contaminated with 1.0 w% and 1.5 w% CaSO_4

both methoxyethanol and ethanol can trigger a polymer chain growth. When Na_2CO_3 is present in the monomer a dramatic loss of the M_w is emerging in these experiments, revealing a product that can hardly be defined as

a polymer, with a M_w of approximately $4\,000\text{ g mol}^{-1}$ at a contamination value of 1.5 w%. The drastic interferences of possible contaminants from the fermentation broth in the polymer synthesis is depicted in Fig. 4. The deviation in % relative to the reference experiments is given, where the influences are displayed in contrast to each other. Contrary to all other substances used as a contaminant in the L-lactide polymerisation, rising amounts of L(+) -aspartic acid did not cause a significant decrease in the weight average molecular mass of PLA. In fact, a 0.1 w% content is causing an increase of the M_w . The same is true for a 0.1 w% content of CaCO_3 in the reaction mixture. In Fig. 4 this influence is shown, denoting that longer chain lengths result from the presence of 0.1 w% L(+) -aspartic acid and 0.1 w% CaCO_3 . The values determined are given in Table 1.

In Fig. 5 exemplarily the trends in the molecular masses of CaCO_3 , D(+) -glucose, ethanol, and L(+) -aspartic acid are denoted. In Fig. 6 exemplarily SEC curves of PLA samples are given. At a retention time of 31.5 min remaining L-lactide is detected. For the calculation of the molecular mass this area was excluded.

Fig. 4 Deviation of the weight average molecular mass M_w in % relatively to the reference experiments

Fig. 5 Trend of number average molecular mass M_n and weight average molecular mass M_w depending on the contamination value; a) CaCO_3 , b) D(+)-glucose, c) ethanol, d) L(+)-aspartic acid

MALDI mass spectrometry

In contrast to NMR mass spectrometry gives an insight into the various molecular species present in the PLA polymer. Whereas cyclic PLA and linear structures cannot be distinguished by NMR, MALDI MS shows different polymer moieties due to the differences in their end groups as individual series [27, 30, 31].

Even if molecular mass distributions cannot be assessed for large PLAs with high dispersity, it is nevertheless possible to distinguish between smaller and larger molecular mass distributions (see Fig. 7). The analysis of the reference polymer initiated with methoxyethanol given in Fig. 8 shows clearly how complex the formation mechanism of the polymer is. Besides the linear polymer with a methoxyethyl starting group there are cyclic

Fig. 6 Exemplarily illustration of SEC curves of a PLA reference experiment and PLA contaminated with 1.5 w% CaSO_4

Fig. 7 MALDI mass spectra of reference sample, sample with 1.5 w% ethanol, and 1.5 w% Na_2CO_3

molecules and linear molecules with no starting group (H_2O induced) and a methyl starting group. One series that differs from the cyclic species by 60 Da was also found but could not be identified yet.

When ethanol is added it also acts as initiator for the polymerization and a series of molecules with ethyl starting groups is formed. Increasing the ethanol concentration thus leads to a dominance of this initiation mechanism and thus shorter polymer chains (see Fig. 8). When methoxyethanol is substituted with ethanol the same species can be found like in the reference experiment, only the moieties with methoxyethanol starting groups are missing.

The effect of Na_2CO_3 is also clearly visible in the mass spectrum. Only small polymer chains are found, which is in good agreement with NMR data; the moieties identified are methoxy initiated open chains, cyclic polymers, and due to the high alkalinity, the sodium salts of linear chains are also found. To a small extent also molecules ionized with potassium instead of sodium can be found, which is typical for ionization with alkali salts.

Addition of aspartic acid, however, did not produce any difference in the mass spectrum when compared to the reference experiment. Incorporation of aspartic acid into the polymer chain is not observed and it thus remains unclear, why higher molecular masses are obtained.

Fig. 8 MALDI mass spectra of reference sample, sample with 1.5 w% ethanol, and 1.5 w% Na_2CO_3 ; a) cyclic-PLA, b) ME-PLA-H, c) HO-PLA-H, d) Et-O-PLA-H, e) unknown, f) Me-O-PLA-H, g) HO-PLA-Na; Signals between series c and d are likely artefacts

NMR

By investigation of the reference experiments the characteristic signals at 5.16 ppm (quartet, methine group) and 1.57 ppm (doublet, methyl group) of PLA could be found (see Fig. 9). Signals downfield shifted of both, the methine and the methyl group, can be determined to be caused by D-enantiomers and signals shifted upfield originate from shorter polymer chains, caused by the dispersity of the product [41]. Furthermore, methine and methyl end groups of the linear chains within the product are identified at 4.35 ppm (quartet, methin end group) and 1.49 ppm (doublet, methyl end group). Additionally,

the signals from the methoxyethanol starting group can be identified at 4.19 ppm ($\text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-}$), 3.75 ppm ($\text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-}$) and 3.36 ppm ($\text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-}$). The discrepancies of the relative signal areas within methoxyethanol originate from the large number of species in the product, as confirmed by MALDI-MS analyses. Supposedly, an overlapping of signals from different starting groups occurs.

As the MALDI-MS experiment already suggests, no differences in the PLA can be found when polymerisation with aspartic acid is conducted. The NMR spectrum is exactly alike to the reference PLA spectrum.

Fig. 9 Relevant sections of ^1H -NMR spectra of a) reference experiment, b) sample doped with 1.5 w% ethanol and c) sample doped with 1.5 w% Na_2CO_3

When ethanol is added to the reaction it acts as an additional initiator and these end groups were identified at 4.19 ppm (multiplet, $\text{CH}_3\text{-CH}_2\text{-O-}$) and 1.26 ppm (triplet, $\text{CH}_3\text{-CH}_2\text{-O-}$), which was also confirmed by the HSQC spectrum and was further supported by the peak integral ratios. A calculation of the molecular mass for the experiment with 1.5 w% ethanol, which only has ethanol induced linear species, based on the ratio of backbone signals to end group signals is giving a M_n of approx. $5\,000\text{ g mol}^{-1}$ and therefore confirms the SEC results according to the literature [42, 43]. This type of calculation can only be performed when no cyclic molecules are present. In contrast to MALDI-MS traces of the methoxyethanol starting group can be found.

The sample with sodium carbonate shows a distinctly different NMR spectrum as most of the peaks are split apart, which is in accordance with the presence of low molecular mass oligomers [41] (see Fig. 9).

Conclusion

The influence of various compounds, which are considered possible impurities in the manufacture of L-lactide through biogenic processes, on the production of polylactic acid was investigated. Whereas glass transition temperature and melting point stay within rather narrow boundaries, molecular masses vary significantly. It could

be shown that the process is very sensitive to some types of impurities, such as ethanol, which are in competition with methoxyethanol as polymerisation initiator. MALDI mass spectrometry clearly shows the incorporation of the new initiator and explains why higher amounts of these impurities lead to dramatically reduced molecular masses. The biggest difference was observed when sodium carbonate was present in higher concentrations. In this case a work up of the polymer was not possible in the conventional way and all analytical techniques employed showed that the molecular masses of the polymers decreased dramatically. Finally, it was demonstrated that molecular mass differences between SEC measurements and NMR calculations can be explained by the fact that the polymers produced under standard conditions are not strictly linear with methoxyethoxy starting groups but are a mixture of cyclic polymers and linear chains with various starting groups. Therefore, the ratio of starting group to repeat units derived from NMR experiments always leads to erroneous molecular mass values. For linear products the comparison is well suited. Further investigations into the influence of initiators on the tin (II)-2-ethylhexanoate catalysed L-lactide polymerisation will be carried out in order to better understand the formation process of the different polymer moieties.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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