INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Analysis of L-Lactic Acid and Poly (L-Lactic Acid)

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ABSTRACT

Impurities present in L-LA 88 % aqueous solution, the presence of oxalic acid (28 ppm), pyruvic acid (174 ppm), acetic acid (1717 ppm) and succinic acid (388 ppm). Linear PLA oligomers of controlled molecular weight were successfully prepared for the first time using ring opening polymerization (ROP) of the L-lactide. These polymers were terminated by carboxylic acid at one end and hydroxyl at the other. The molecular weights ($\overline{M}_n$) were determined by ^{13}C-NMR technique.

INTRODUCTION

In the dehydropolycondensation of L-lactic acid (L-LA) to poly (L-lactic acid) (PLA), small amounts of impurities are expected to play a major detrimental role. L-LA prepared by fermentation of biomass may contain impurities derived either from the fermentation step itself or from the separation and purification steps after fermentation. Several impurities, for example, methanol, ethanol, acetic acid, pyruvic acid, some dicarboxylic acids like oxalic, fumaric and succinic acids etc, have been reported to occur in the fermentation derived L-lactic acid[1]. The level as well as nature of impurities in the polymerization is believed to have a large effect on the final molecular weight by end capping either the hydroxyl or the carboxylic acid terminal of the growing polymer chain and thereby limiting the chain growth. It has been found that total level of interfering impurities should be less than 100 ppm in order to achieve high molecular weight PLA polymer[2].

This chapter describes the effort to analytically detect and quantitate the level of impurities in the L-LA to be used for all polymerization reactions in the subsequent chapters. The L-LA obtained as an aqueous solution was esterified with ethyl alcohol to form ethyl L-lactate. Both the L-LA aqueous solution and the ethyl lactate were analyzed by gas liquid chromatography (GC), High Performance Liquid Chromatograph (HPLC), LC-MS.

Controlled ring opening polymerization (ROP) of L,L-dilactide (the dilactone of L-LA, alternatively called L-lactide) was also performed with stannous 2-ethylhexanoate (stannous octoate, Sn $(Oct)_2$) catalyst and measured amount of water as co-initiator, to produce linear PLA oligomers of well-defined number average molecular weight with carboxylic acid and hydroxyl end groups. These polymers were desired as model PLA oligomers for any L-LA that would be prepared in the work described in subsequent chapters. The carboxylic end groups were also quantitatively determined using ^{13}C NMR spectroscopic technique, which, in turn, was used to calculate the number average molecular weight.

MATERIALS AND METHODS

Materials: L-lactic acid (L-LA) was obtained from PURAC chemical company, Holland, in the form of 88 % aqueous solution and was used as such, without any purification. Ethanol and benzene were both obtained from S. D. Fine Chemicals, India. Ethanol was purified by distillation and dried on fused calcium chloride followed by magnesium ethoxide before use. Benzene was purified (freed of thiophene) by stirring with 98 % H_2SO_4, washed with sodium bicarbonate and water, followed by drying first over fused calcium chloride and then over sodium metal. THF was dried over fused calcium chloride followed by calcium hydride (CaH_2). Trimethylsilyl chloride (TMSCl), acetone, 98 % H_2SO_4, sodium carbonate, fused calcium chloride and sodium metal were all obtained from S. D. Fine Chemicals, India, and used without purification. CaH_2 was procured from Aldrich Chemicals and was also used without any purification. L,L-dilactide was procured from Aldrich Chemicals and was used after recrystallization from dry THF. Doubled distilled and de-ionized water was used as co-initiator of ROP reactions. Sn (Oct)$_2$ catalyst was procured from Aldrich Chemicals and was freshly distilled before use.

Preparation of ethyl lactate from L-lactic acid: L-lactic acid was azeotropically dried with benzene (thiophene-free) without any catalyst, followed by removal of benzene under reduced pressure of 0.1 mbar at 60 ^{0}C (5 h). The dry, waxy L-lactic acid (10 g) was esterified by refluxing in dry ethanol (50 mol/ mol of L-LA) with a few drops of 98 % H_2SO_4 as catalyst for 20 hours, with intermittent (azeotropic) distilling out of ethanol and by-product water at every 4 hours. After 20 hr the whole reaction mixture was distilled up to dryness, so that all ethanol together. Further fractional distillation at the boiling point of ethanol and under atmospheric pressure removed ethanol and yielded 9.87 g (86 %) pure ethyl lactate.

General procedure for ROP of L-lactide: To 2.20 g (0.015 mol) L-lactide (recrystallized and dried) taken in single-neck, 250 mL volume round bottomed flask, previously passivated with 30 % acetonic solution of TMSCl followed by drying, maintained under inert (argon) atmosphere and fitted with standard joints and, was added Sn (Oct)$_2$ catalyst 0.1214g, 2 mol % of lactide and required amount of de-ionized water was added (0.008mL, 0.03 mol/ mol of lactide) subsequently using a hypodermic syringe. The mixture was stirred at room temperature for 5 h, followed by evacuating and sealing under vacuum and then heating at 120 ^{0}C for 12 h.

Analysis

Gas liquid chromatography (GC): GC-analysis of L-lactic acid was done on Perkin Elmer GC Auto System XL-200 by injecting 0.1, 1 and 10 microliter injection volumes of the L-LA 88 % aqueous solution as such to a Perkin-Elmer BP-20 (polyethylene oxide, terephthalic acid treated) capillary column of 25 meter length by means of on-column injection procedure and comparing with the chromatograms of aqueous solutions of 4, 9 and 19 ppm (that is below 5, 10 and 20 ppm, respectively) concentrations each of methanol, ethanol, acetic acid, and pyruvic acid. The split ratio was 1:60, the detector was FID, the carrier gas was nitrogen with a pressure of 7 psi and the fuel for FID was hydrogen. Oven was isothermal at 50 ^{0}C for 30 min, followed by heating with a ramp rate of 5 ^{0}C/ min from 50 ^{0}C till another 30 min. The injector and detector were kept at 250 and 280 ^{0}C, respectively. Dicarboxylic acids, namely oxalic, fumaric and succinic acid, did not elute through this column.

Ethyl lactate was analyzed under the same conditions, except that the column was Perkin-Elmer BP-21 (polysiloxane) and the injection was done in normal injection mode, where the sample evaporated inside the injector and went into the column in a gaseous form. Ethyl lactate chromatograms (corresponding to 0.1, 1 and 10 micro liter injection volumes) were compared with chromatograms of ethanolic solutions of 4, 9 and 19 ppm (that is below 5, 10 and 20 ppm, respectively) concentrations each of diethyl oxalate and diethyl succinate.

High Performance Liquid Chromatography: Quantification of impurities by HPLC is the process of determining the unknown concentration of a compound in a known solution. It involves injecting a series of known concentrations of the standard compound solution onto the HPLC for detection. The chromatograph of these known concentrations will give a series of peaks that correlate to the concentration of the compound injected.

The HPLC system employed for this work consisted of modular HPLC of WATERS make consisting of: Pumps 3 x 515 HPLC High pressure pumps, Injector: 717-Autosampler, Detectors: 996 PDA [Photodiode Array Detector] 410 RI [Refractive Index Detector] and Millennium Software. The column used for impurity analysis was YMC (polar embedded, C_{18}). The chirality. of 88 % L-LA aqueous solution was determined using MN-chiral 1 column. The chiral purity of 88 % L-LA aqueous solution was obtained as 99, 9 %.

LC-MS: The LC-MS of L-lactic acid was analyzed in methanol solution using API Qstar Pulse.

Molecular weights: Molecular weights (relative, $\bar{M}_n$ and $\bar{M}_w$) and polydispersity ($\bar{M}_w/\bar{M}_n$) were determined with respect to polystyrene standards by size exclusion chromatography on a Thermo Finnigan Spectra Series AS300 machine at 25 ^{0}C by eluting PLA solutions of 10 mg/ mL concentration in $CHCl_3$, with toluene as internal standard, through a series of five μ-Styragel columns of pore sizes 10^5, 10^4, 10^3, 500, and 100 A^0 respectively, and length 30 cm each. $CHCl_3$ was used as the mobile phase (flow rate 1 mL/ min) and a refractive index detector (Spectra Series RI-150) was used for detection of different molecular weight fractions. Molecular weights were calculated with respect to polystyrene calibration. Number average molecular weights and intrinsic viscosities were determined with the help of a KNAUER K-7000 Vapor Pressure Osmometer (25 ^{0}C) and a 3-arm Ubbelohde viscometer (SCHOTT GERATE) at 30 ^{0}C in chloroform.

Quantitative ^{13}C-NMR spectroscopic analysis: For NMR measurements, the samples were dissolved in Chloroform-d in 5 mm dia. NMR tubes at room temperature. The chemical shifts in parts per million (ppm) were reported up field with reference to internal standard chloroform-d at 7.25 ppm. The sample concentration for ^{13}C NMR measurements was 10 % by weight. Proton decoupled ^{13}C NMR spectra with NOE were recorded on a Bruker DRX 500 MHz NMR spectrometer working at 125.577 MHz for carbon-13. $CDCl_3$ served as solvent and TMS as internal standard for all ^{13}C-NMR measurements. Relative peak areas were proportional to the number of carbon atoms. Peak areas were calculated by deconvolution method using WIN-NMR software.

Thermal analysis: Differential scanning calorimetric (DSC) measurements were made on a Perkin-Elmer thermal analyzer model DSC-7 in a nitrogen atmosphere. The measurements were run from –40 to 200 ^{0}C at a heating rate of 10 ^{0}C/ min and a cooling rate of 100 ^{0}C/ min. The glass transition temperature (T_g) and the crystallinity data were recorded from the second and first heating curves, respectively. Crystallinity values for different polymers were calculated from the heat of fusion. By integrating the normalized area of the melting endotherm, determining the heat involved, and rating it to the reference 100 % crystalline polymer (93.6 J/ g)[3], the relative crystallinity of the polymer was assessed. In the present work, the relative degree of crystallinity is referred to as crystallinity, and T_m is the melting temperature.

X-ray analysis: Wide-angle X-ray scattering (WAXS) pattern of the samples was obtained in reflection mode using a Rigaku Dmax 2500 diffractometer and Ni filtered copper radiation. The sample was scanned in the range $2\theta = 10 - 35^0$ and the generator was operated at 40 kV and 150 mA. The FWHM of the 110 peak was determined by peak fitting software available with the Rigaku diffractometer.

RESULTS AND DISCUSSION

Impurity detection and analysis in the L-lactic acid and ethyl L-lactate

L-lactic acid: The total level of impurities in the L-lactic acid 88 % aqueous solution was less than 30 ppm. Only methanol (< 5 ppm), ethanol (< 10 ppm) and acetic acid (< 10 ppm) were found to be present as impurities. Presence of L-lactide was also detected, but its quantification was not attempted because its formation inside the GC column was also possible, since L-LA is a thermally labile material. Some peaks, though with much tailing and disturbed baseline, which eluted after the L-LA remained unidentified. Although pyruvic acid was not found at all in the L-LA sample when injected, it was individually detectable up to the 4-ppm level from its own aqueous solution. So it was thus concluded that pyruvic acid was present in less than 5 ppm level in the L-LA sample and oxalic acid, fumaric acid and succinic acid were found < 5ppm.

HPLC result shows the quantitative estimation of acid impurities present in L-LA 88 % aqueous solution. The quantitative results showed the presence of oxalic acid (28 ppm), pyruvic acid (174 ppm), acetic acid (1717 ppm) and succinic acid (388 ppm). The other acids for example, citric acid, itaconic acid, acotinic acid, propeonic acid, citraconic acid and buatyric acid are observed below the detection limit. The presence of fumaric acid was 0.6 ppm and other impurity are 6.6 ppm. Methanol and ethanol are observed below the detection limit. The purity (percentage) of L-lactic acid is 97.7 %. The chiral purity of L-lactic acid was examined in 1.5 molar solution of sodium hydroxide using at 20 ^{0}C. The specific rotation was obtained as -14.6.

The LC-MS result showed the presence of CH_3COOH---K^+ (100 Da), Lactoyl lactic acid---Na^+ (185 Da), trimer of lactic acid--- Na^+ (257 Da), trimer of lactic acid---K^+ (257 Da), tetramer of lactic acid---Na^+ (329 Da), pentamer of lactic acid---Na^+ (401), hexamer of lactic acid---Na^+ (473 Da), heptamer of lactic acid---Na^+ (563) and cyclic octameer of lactic acid---Na^+ (597 Da).

Ethyl lactate: No impurities peaks were found in the ethyl lactate sample when injected (cut off time of 4 min given for ethanol) and there was only a single peak of ethyl lactate in the chromatogram. Impurities with boiling points less than that of ethanol had presumably been removed at the time of removal of ethanol. However, any impurity with a boiling point higher than ethanol should remain in the sample after esterification. But the fact that no impurities were found implied that the peaks found after the L-LA peak during elution of the L-LA aqueous solution sample might be due to small oligomers of L-LA. The detection level of diethyl oxalate, diethyl fumarate and diethyl succinate was about 4 ppm, under the specified condition of anaysis. Thus it can be inferred that these impurities were present in less than 5 ppm concentration in the ethyl lactate sample.

The GC analysis thus could detect only three impurities, namely methanol, ethanol and acetic acid. Traces of L-lactide that was found could be either due to its presence as an impurity in the sample or its formation during the elution through the heated GC column, or both.

Synthesis and characterization of linear PLA oligomers of controlled number average molecular weight and with both carboxylic and hydroxyl end groups: Synthesis, by ROP of L-lactide: Sn $(Oct)_2$-catalyzed (or, -initiated) ring opening polymerization (ROP) of lactonic monomers, including L-lactide, in presence of alcoholic co-initiators proceeds via a coordination-insertion mechanism of initiation and propagation[4-6], (Scheme-1).

In the present work, L-lactide was thoroughly dried under vacuum and all precautions were taken to avoid in trace of moisture during the reactions. Water was used as the co-initiator, so that carboxylic acid end groups instead of ester end groups as found in the Scheme-1 could be generated at the terminal of each PLLA oligomer chain. The number average molecular weight and degree of polymerization, $\bar{M}_n$ and $\overline{DP}_n$, respectively, of PLA oligomers thus prepared were calculated as:

$$\bar{M}_n = ([M] / [I]) \times M_L \times \text{conversion } \%$$
and
$$\overline{DP}_n = ([M] / [I]) \times \text{conversion } \%$$

Where [M] = moles of monomer, [I] = moles of co-initiator (water) and M_L = molecular weight of L-lactide. Conversion was determined from yield of PLLA oligomer.

Determination of molecular weights of oligomers: The achieved degree of polymerization and number average molecular weight was determined from the ratio of integral of the ester carbonyl carbon to that of the carboxylic acid carbonyl carbon in the ^{13}C-NMR spectrum, as shown in Figure. 2.

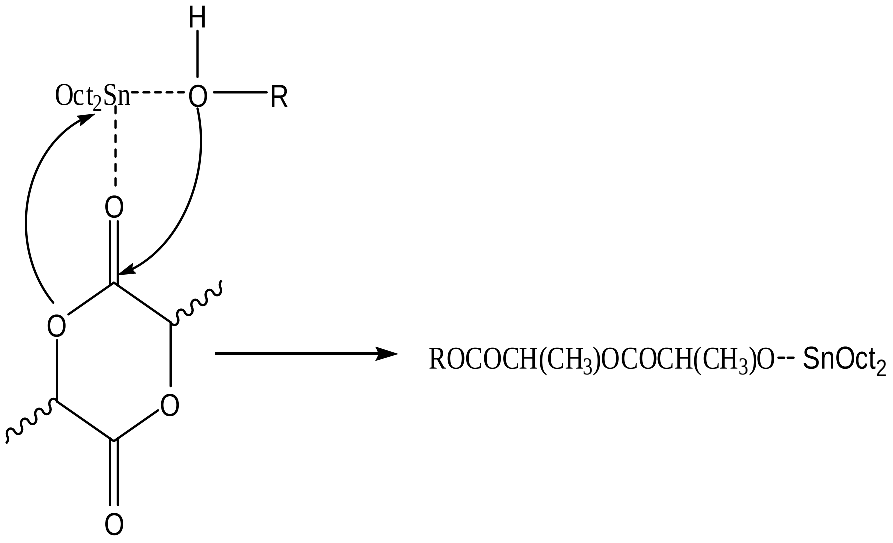

Fig. 1: Coordination-insertion mechanism of ROP of L-lactide

Both theoretically calculated and experimentally obtained values of these parameters are organized in the data shown in Table-1, where samples 1 and 2 are the two PLA oligomers synthesized in this procedure with different [M]/[I] ratios and different Sn $(Oct)_2$ concentrations in proportion to the lactide monomer, to obtain oligomers of different degrees of polymerization and hence different number average molecular weights.

The theoretically calculated and experimentally obtained values of these parameters were found to be in very good agreement, thereby indicating successful use of water as a co-initiator in the ROP of L-lactide.

Thermal characterization (DSC) and powder XRD of oligomers: The thermal characterization data (melting point, T_m, and glass transition temperature, T_g) of the two oligomer samples 1 and 2 are shown in Table-2 as well as in Figure. 3. Both oligomers had similar values of T_m and T_g effect of $\overline{DP}_n$ on T_m is also evident from the result. The powder XRD patterns of the two oligomers are shown in Figure. 4, where it can be found that the two polymers had almost identical patterns, as expected. The crystallinity values of the oligomers calculated from these XRD patterns are shown in Table-2 and they were also found to be similar.

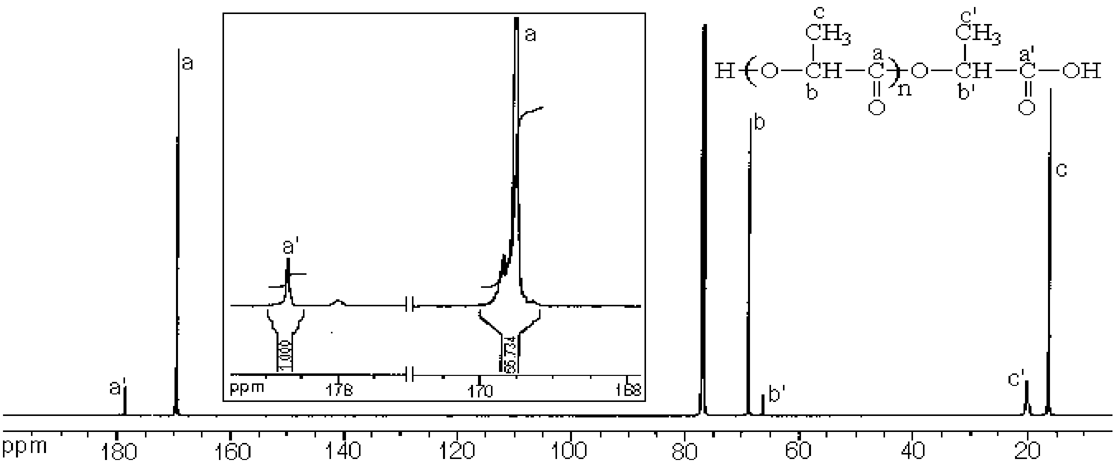

Fig. 2: ^{13}C-NMR spectrum of PLA oligomer 1 synthesized by ROP of L-lactide: inset showing ester carbonyl region (ester as well as carboxylic acid) as enlarged

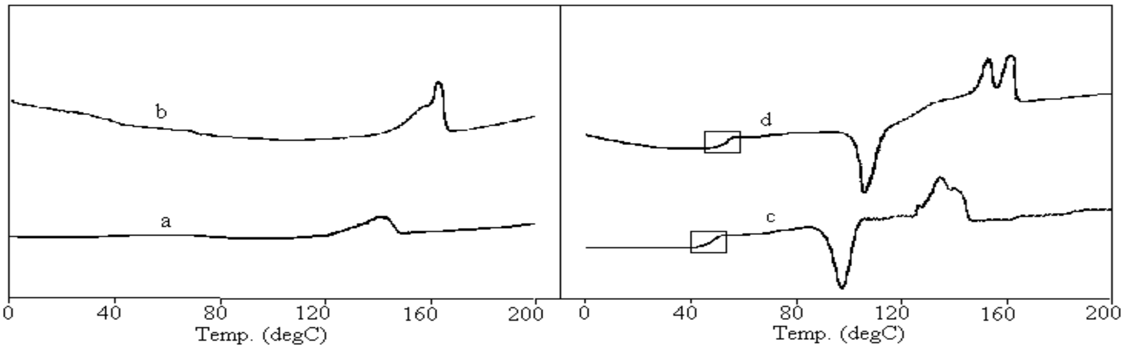

Fig. 3: Thermal characterization (DSC) first and second heating showing Tm and Tg, respectively of PLA oligomers: (a) 1, first heating; (b) 2, first heating; (c) 1, second heating and (d) 2, second heating

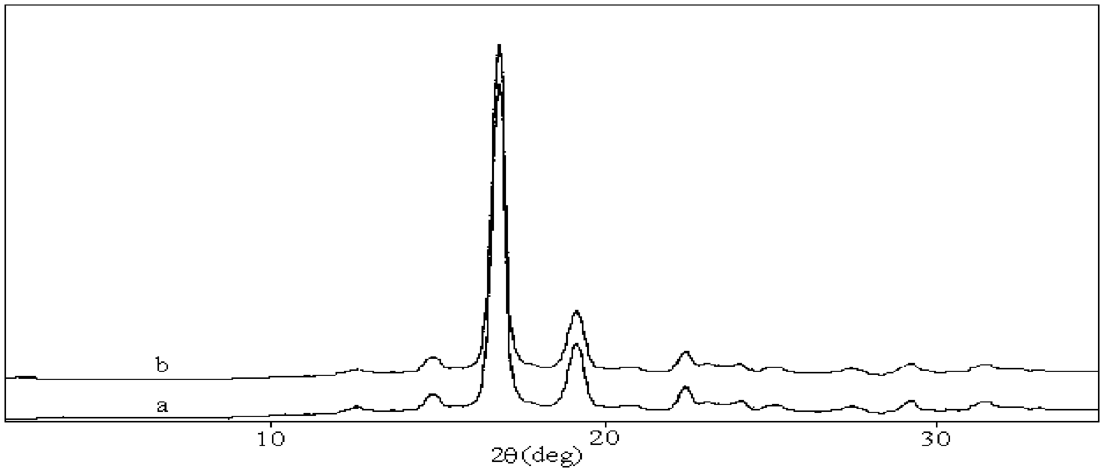

Fig. 4: Powder XRD patterns of PLA oligomers: (a) 1 and (b) 2

Table 1: Number average molecular weights of the PLA oligomers synthesized by ROP of L-lactide with water as co-initiator and Sn $(Oct)_2$ as initiator

PLA sample	[Lactide]/ [Sn(Oct)$_2$]	[Lactide]/ [H_2O]	Yield (%)	$\overline{DP}_{n,\ Calc}$	$\overline{DP}_{n,\ NMR}$	$\overline{M}_{n,\ NMR}$	$\overline{M}_{n,\ VPO}$
1	200	32	86	55	60	4320	4400
2	400	45	87	79	77	5544	5692

Table 2: Thermal characterization and crystallinity values of PLA oligomers synthesized by ROP of L-lactide

PLA samples	T_g (°C)	T_m (°C)	$\Delta H_{melting}$ ($J.g^{-1}$)	% Crystallinity from powder XRD
1	48	141	53.4	85.52
2	51	162	59.7	85.73

CONCLUSION

Impurities present in L-LA 88 % aqueous solution, the presence of oxalic acid (28 ppm), pyruvic acid (174 ppm), acetic acid (1717 ppm) and succinic acid (388 ppm). The other acids for example, citric acid, itaconic acid, acotinic acid, propeonic acid, citraconic acid and buatyric acid are observed below the detection limit. The presence of fumaric acid was 0.6 ppm and other impurities are 6.6 ppm. Methanol and ethanol are observed below the detection limit. The purity (percentage) of L-lactic acid is 97.7 %.

Linear PLA oligomers of controlled molecular weight were successfully prepared for the first time using ring opening polymerization (ROP) of the L-lactide. These polymers were terminated by carboxylic acid at one end and hydroxyl at the other. The molecular weights ($\overline{M}_n$) were determined by ^{13}C-NMR technique.

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