Chapter-5
CHAPTER 5: HOMO AND COPOLYMERIZATION OF ALEURITIC ACID WITH L-LACTIC ACID AND STUDY THE AGGREGATION BEHAVIOR IN DIFFERENT SOLVENTS

5.1. Introduction:
Aliphatic polyesters constitute an important class of polymers because of their biodegradability [1], and biocompatibility [2a, b], that enable their use in drug delivery systems, artificial tissues [3a, b], and commodity materials. Polyesters are commonly produced through either condensation or ring opening polymerization using various catalysts [4-6]. Self-organization of condensation polymer is rare in the literature. Particularly, self-organization of amphiphilic polymers has resulted in assemblies such as micelle, vesicles, fibers, helical, superstructures and macroscopic tubes. These materials have potential application in areas ranging from material science to biological science. Thermo or pH sensitive polymer micelles [7a, b], and vesicles [7c], have been reported in which the nature of the functionality at the corona changes in response to the stimulus. A little attention has been paid to realize an environment-dependent switch from a micelle-type assembly with a lipophilic corona [8]. Here, we report a new class of aliphatic polyester superstructures that exhibit such properties.

Shellac is the only known commercial resin of animal origin. It is an important natural resinous product secreted by an insect (Laccifer lacca), which lives on the sap of some host trees. India is the major shellac producing country in the world. Shellac (Lac) is known to comprise of several hydroxyl acid unit, aleuritic acid and its esters have great importance in industrial domain. It is a valuable starting material for preparation of transparent water-clear adhesive, plasticizers [9]. Aleuritic acid has been used as a raw material for the synthesis of macrocyclic musk like lactones such as ambrettolide, civetone and exaltone. There is only one literature report of poly aleuritic acid, where aleuritic acid has been polymerized thermally and resulted insoluble product [10]. We demonstrate for the first time that the linear homopolymer of aleuritic acid (PAA) is obtained from aleuritic acid (Figure 5.1). The change in the surface of the assembly is the amplified consequence of change in molecular level conformation with each polymer chain due to the presence of 9, 10-hydroxy group in each monomeric unit. These
polymers with such properties could find use in the applications such as carriers for trafficking drugs and as components of smart adhesives. PAA is biocompatible and biodegradable polymer, which could find potential use in biological system.

Block copolymers are often used for a variety of supramolecular assemblies, in which the driving force involves the mutual immiscibility of the block and/or the immiscibility of one of the blocks in the bulk solvent. In case of poly (styrene-co-acrylic acid) block copolymers exhibit several interesting amphiphilic assemblies [11-12]. We aimed to synthesize aliphatic polyester by polycondensation. The hydrophilic 9, 10-hydroxy functionality, the hydrophobic methylene moiety are stitched in the same polymer backbone. The methylene group’s greater than five units in a polymeric chain show zigzag conformation in the polymer molecule [13].

The thermal polymerization of Aleuritic acid leads to insoluble product because both intra and intermolecular condensation are possible leading fast to the formation of fusible ethers, anhydrides, lactones and esters which ultimately become infusible and insoluble three dimensional network structures.

![Aleuritic acid](image)

Figure 5.1: 9, 10, 16-trihydroxy, palmitic acid (aleuritic acid).

These functional polymers can be post modified to crosslink the polymer, or to attach bioactive molecules such as peptides or drugs and have shown potential application in drug delivery systems and scaffold materials. The functional polyesters have tunable mechanical properties with in vivo degradability [14]. Polyesters syntheses have been explored by both chemical synthesis and enzymatic approaches. Several hydroxyl functional polyesters [15-21], and poly (carbonate esters) [22], have been synthesized. Polymers with vicinal diols were prepared by chemical polymerization of L-lactide with protected sugars [23, 24], followed by deprotection [25-27]. Use of monomers, initiators with unsaturated bond enabled the introduction of epoxide groups by post modification reaction with m-chloroperbenzoic acid (m-CPBA), while treatment of allylic side chains
with NMO/OsO₄ resulted in dihydroxylation of side chains [28]. Chemical polymerization of unprotected hydroxyl functional caprolactones [29], and hydroxymethyl substituted 1, 4-dioxan-2-ones [30, 31], resulted in hyper branched structures with comparable molecular weights and degree of branching.

The biodegradable polymers are intensively aliphatic polyester of both natural and synthetic origin. Polyesters can be synthesized by polycondensation of hydroxyl acids or by ring-opening polymerization of cyclic esters (lactones), grafting, chain extension, or transesterification [32]. A wide range of monomers has been used to produce biodegradable polyesters. Their polymerizations can be carried out either in the bulk or in solution. The most useful monomers used for polycondensation are lactic, glycolic, hydroxybutyric acid and hydroxycaproic acids. Polyesters of glycolic and lactic acids are the main group of interest due to their long history of safety.

Lactic acid can be condensed with other hydroxyl acids such as 6-hydroxycaproic acid, glycolic acid, and hydroxybutyric acid or in the presence of diols, diacids, and diamines. Direct condensation usually resulted in low molecular weight copolymers that can then be further linked to yield high-molecular-weight polymers. In the second step, linking molecules such as diisocyanates, bis (amino-ethers), phosgene, phosphate, and anhydrides takes place [33-35].

There is no report available so far, where the 9, 10 secondary vicinal diol is protected and the hydroxy and carboxylic acid groups are free to undergo dehydropolycondensation reaction to produce a linear high molecular weight homopolymer.

Fatty acids are suitable candidates for the preparation of biodegradable polymers [36, 37], as they are natural body components and they are hydrophobic, and thus they may retain an encapsulated drug for longer time periods when used as drug carriers. Aleuritic acid (9, 10, 16-trihydroxy palmitic acid) is common C16 fatty acids with two secondary hydroxyl groups at 9, 10 positions and a primary hydroxyl group in the 16th position. It is produced from resin (Shellac).

The objective of this study is to incorporate aleuritic acid in lactic acid based polymers for the purpose of altering its physical properties. The trifunctionality of aleuritic acid (9, 10, 16-trihydroxy palmitic acid) does not allow forming the linear polymer. Previous study in our laboratory focused on the synthesis aleuritic acid (9, 10, 16-trihydroxy
palmitic acid) homopolymer. The homopolymer synthesized from aleuritic acid by protecting the 9, 10 hydroxyl groups with dimethoxy propane (DMP) to make –COOH and 16th position –OH group free for reaction to make linear polyester \[38\]. These copolymers have pendent hydroxyl groups for aggregation to form micro scale morphologies. Molecular self-assembly of organic molecules has generated a wide variety of objects with nanoscale or micrometer-scale morphologies including micelles \[39, 40\], vesicles \[41, 42\], ribbons \[43\], films \[44\], fibers \[45, 46\], and tubules \[47-48\].

In this present work, we highlight the copolymerization of L-lactic acid (L-LA with protected aleuritic acid in presence of Lewis acid catalyst using dehyropyolcondensation method. The resulted copolymers are pliable, soft, waxy or even viscous liquid copolymers influenced by the aleuritic acid content. The purpose of this study is to investigate the physical properties. In addition, deprotected copolymers focus micelle-like aggregates in various organic solvents and mixed organic solvent at various proportions.

**5.2. Experimental:**

**5.2.1. Materials and Method:** L-lactic acid was obtained from Purac as a 88% (w/w) aqueous solution with impurity, aleuritic acid, tetraphenyltin (Aldrich, USA), p-toluene sulfonic acid (PTSA) (Aldrich, USA), Xylene (S.D Fine Chemicals, India), anisole (Aldrich, USA), sodium sulphate, chloroform and methanol (S.D Fine Chemicals, India), mesitylene (Aldrich, USA), decaline (Aldrich, USA), and diphenyl ether (Fluka, Germany). All solvents were dried by using standard procedures for example toluene by distilling over metallic sodium. All liquids were transferred by syringe under dry argon atmosphere.

**5.2.2. Synthesis of methyl ester of aleuritic acid:** Crude aleuritic acid was converted to methyl ester by using tetraphenyltin (TPT) as a catalyst in dry methanol solution at reflux temperature. The reaction mixture was refluxed for 9 h, during which reaction was monitored using TLC (solvent system; chloroform/ methanol 9/1). The ester was dried using rotavapour and further purified by column chromatography (chloroform/ methanol 9/1). The impurity profile was checked by gas chromatography (Figure 5.2 A and Figure 5.2 B). The ester was recrystallized using ethyl acetate, the crystal was dried under vacuum and the yield was calculated as 90 %, m.p : 71-72 °C , FT-IR (KBr) v \text{ cm}^{-1} : 1740-
1720 cm⁻¹ (COOCH₃). ¹H NMR (500 MHz): δ 3.66 (s, 3H, COOCH₃), 3.64 (t, j = 5.24, 2H, CH₂OH, 3.39 (bs, 2H -CH(OH)-CH(OH)), 2.30(t, j = 7.44,2H,-CH₂-COOCH₃), 1.61-1.31(s, 22H, -CH₂-).

5.2.3. Synthesis of protected aleuritic acid: Pure methyl ester of aleuritic acid (9, 10, 16 tri hydroxy hexadecanoate) (9 gm, 0.028M) was taken in a two-neck flask and equimolar quantity of dimethoxy propane was added, p-toluene sulfonic acid (PTSA) and the toluene were used as catalyst and solvent respectively. The reaction mixture was refluxed under the blanket of inert atmosphere (argon) for 6 h. After the completion of the reaction, the toluene was removed using rotavapour. The reaction mixture was washed several times with deionized water and extracted with chloroform. The chloroform layer was dried over Na₂SO₄ and finally stripped off. Finally, it was vacuum dried to give 8.2 gm (yield- 82%). The structure of protected methyl ester of aleuritic acid (ProAL) (monomer) FT-IR (KBr) ν cm⁻¹: 1740.17cm⁻¹(-COOCH₃), 1377.55 cm⁻¹ and 1368.73 cm⁻¹ (-O-C(CH₃)₂-O-). ¹H NMR (500 MHz) : δ 3.66 (s, 3H, -COOCH₃), 3.64 (t, j = 5.64, 2H, CH₂OH), 3.56 (bs,2H -CH-(O)-CH(O)-), 2.17 (t, j =7.44,2H, -CH₂-COOCH₃), 1.38, (s, 6H, -C-(CH₃)₂-), 1.50-1.31 (s, 22H, -CH₂-). 1.31 (s, 22H, -CH₂-).

5.2.4. Homo polymerization of ProAL: The monomer (ProAL) was taken in three-neck flask and equipped with Dean Stark apparatus, the requisite amount of TPT catalyst was used for the polymerization using various solvents. The characterization of these polymers (PALs) were carried by SEC, DSC, SEM and ¹³C NMR.
5.2.5. Deprotection of homopolymer: The homopolymers were dissolved in chloroform in a single neck flask and equal amount of methanol, catalytic amount of PTSA was added into it. The reaction mixture was stirred at room temperature (25 °C) under inert atmosphere (argon) for 6 h. The resultant polymer was washed with methanol several times and GC confirmed the absence of dimethoxy propane. $^{13}$C CP/MAS NMR, DSC, and TEM confirmed the structure of PAA.

5.3. Characterizations:

5.3.1. FT-IR: IR spectra were recorded as KBr pellets, on Perkin-Elmer Infrared Spectrometer Model 16PC FT-IR, using sodium chloride optics. IR bands are expressed in frequency (cm$^{-1}$).

5.3.2. Size Exclusion Chromatography Molecular weight (SEC): As discussed in Chapter 3.

5.3.3. Differential Scanning Calorimetry (DSC): Differential scanning calorimetry (DSC) measurements were performed on a thermal analyzer in nitrogen atmosphere. The measurements were run from –90 to 200 °C at a heating rate of 10 °C / min and at a cooling rate of 100 °C/min. The glass- transition temperature ($T_g$) and the crystallinity data were recorded from the second and first heating curves, respectively.

5.3.4. Nuclear Magnetic Resonance Spectroscopy (NMR): For NMR measurements, the polymer samples were dissolved in chloroform–d in 5mm diameter. NMR tubes at room temperature. $^1$H NMR spectra were recorded on Bruker DRX 500 MHz with 4 % w/v concentration of solution. The chemical shifts in parts per million (ppm) are 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 Bruker DRX 500 MHz spectrometer working at 125.577 MHz for carbon-13. A digital resolution of 32 K data points/ 18,000 Hz spectral width was used a pulse angle of about 30 along with a relaxation delay of 2s and $10^3$-$10^4$ transients were accumulated. 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 XWIN-PLOT software.
5.3.5. $^{13}$C Cross Polarization /Magic Angle Spinning ($^{13}$C CP/MAS): $^{13}$C CP/MAS NMR spectra were measured with Bruker MSL-300 NMR Spectrometer (75.5 MHz) with CP/MAS accessory at room temperature (25 °C). The sample powder (ca. 200 mg) was placed in a cylindrical ceramic rotor and spun at 3 KHz. Contact time and repetition time were 2ms and 5s respectively. Spectral width and data points were 27kHz and 8K respectively. The $^1$H field strength was 2mT for both the CP and decoupling processes. The number of accumulations were 160-200, $^{13}$C Chemical shifts were calibrated indirectly with reference to the higher field adamantane peak (29.5 ppm relative to tetramethylsilane ((CH$_3$)$_4$Si). The Hartmann-Hann condition was matched using adamantane in each case. The experimental errors for the chemical shifts were within ± 0.1 ppm for broad peaks as described.

5.3.6. Transmission Electron Microscopy (TEM):

5.3.6a. Sample preparation: The sample was dissolved in solvents and mixture of solvents to understand the aggregation behavior. The solutions were collected on 300 mesh carbon coated copper grids. The copper grids were kept overnight on filter paper for drying. TEM imaging was performed using a JEOL 1200EX electron microscope operating at an accelerating voltage of 80 kV. Images were captured using charged couple detector camera and viewed using Gatan Digital Micrograph software.

5.4. Result and Discussion:

Synthesis of poly (aleuritic acid) from aleuritic acid is depicted in Figure 5.3. At the outset, methyl ester of Aleuritic acid was prepared and the hydroxyl groups at 9, 10 position were protected by dimethoxy propane. Protected poly (aleuritic acid) (ProAL) was prepared from protected methyl ester of aleuritic acid by polycondensation method using tetraphenyltin and xylene as catalyst and solvent respectively.

5.4.1. Molecular weight determination: Poly (aleuritic acid) was obtained from protected poly (aleuritic acid) by simple deprotection. At the outset, the effect of polymerization reaction time (5 to 20 h) was studied and shown in Table 5.1. The number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) increased with reaction time to a certain extent (15h) and thereafter declined. The obtained homopolymer in all the cases were soluble in various solvents such as dioxane and dimethylformamide etc. It was observed that at low molecular weight range up to
15,500 Da, the distribution is comparable with the high molecular weight ($\bar{M}_w=120,000$ Da).

The molecular weights were cross checked with light scattering experiments. The highest molecular weight (Mw) was calculated as 98,000. The hike in molecular weight observed may be attributed due to hydrolysis of some protected groups. The hydrolysis of small amount of protected groups do not affects the solubility but micelle structures may be formed which affects the hydrodynamic volume and ultimate high molecular

Figure 5.3: Scheme of polymerization of monomer.

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weights were observed. The low molecular model ester polymer will be prepared and affect of deprotected group on molecular weights will be examined.

5.4.2. Thermal analysis: DSC data of PAL-3 in Figure 5.4A showed two melting points ($T_m$) at -39.0 and -17.0 °C respectively. The $T_m$ value of poly (12-hydroxy stearic acid) has also been observed as -20 °C [49]. These two different melting temperatures of PAL-3 may be due to different crystalline structure in the polymer molecule. DSC analysis of deprotected sample (PAA) in Figure 5.4B showed the values of glass transition $T_g$ and melting transition $T_m$ are -15.50 °C and 75.07 °C respectively.

The second DSC heating curve showed two distinctly difference peaks (melting transitions), of almost similar intensity ($T_{m1} = 43.73 \text{ °C} / \Delta H_f = 14.24 \text{ J/g}$ and $T_{m2} = 70.0 \text{ °C} / \Delta H_f = 29.90 \text{ J/g}$). These two different melting temperatures of PAA may be attributed due to the indication of disturbances in the crystalline growth of the PAA phase. The $T_g$ of PAA was observed in second DSC curve as -29.3 °C.

5.4.3. $^{13}$C Nuclear Magnetic Resonance Spectroscopy: The $^{13}$C NMR spectra of PAL are shown in (Figure 5.5 A) and dept in (Figure 5.5 B). The $^{13}$C NMR spectra show a resonance peak at 173.81 ppm that corresponds to carbonyl carbon. The peak at 107.76 ppm is due to the quaternary carbon atom of the protecting group. The peak at 80.97 ppm is attributed due to 9 and 10 methine groups linked covalently to the oxygen atom of protecting groups. The peak shown at 64.27 ppm is appeared due to primary alcohol group (-CH$_2$OH), which is attached to the end groups of the polymer.

Table 5.1: Effect of reaction time on polymerization reactions of proAL

<table>
<thead>
<tr>
<th>Polymer samples</th>
<th>Time (h)</th>
<th>Yield (%)</th>
<th>$\bar{M}_n$ (GPC)</th>
<th>$\bar{M}_w$ (GPC)</th>
<th>PDI (GPC)</th>
<th>$\bar{M}_w$ (LS)</th>
<th>$T_{m1}$ (°C)</th>
<th>$T_{m2}$ (°C)</th>
<th>$\Delta H_1$ (J/g)</th>
<th>$\Delta H_2$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL-1</td>
<td>5</td>
<td>99.2</td>
<td>2,800</td>
<td>6,400</td>
<td>2.2</td>
<td>nd</td>
<td>-47.0</td>
<td>-3.6</td>
<td>3.2</td>
<td>0.85</td>
</tr>
<tr>
<td>PAL-2</td>
<td>10</td>
<td>99.0</td>
<td>34,100</td>
<td>82,000</td>
<td>2.4</td>
<td>75,400</td>
<td>-41.2</td>
<td>-14.2</td>
<td>2.9</td>
<td>0.52</td>
</tr>
<tr>
<td>PAL-3</td>
<td>15</td>
<td>98.0</td>
<td>46,100</td>
<td>120,000</td>
<td>2.6</td>
<td>98,000</td>
<td>-34.7</td>
<td>-18.7</td>
<td>1.6</td>
<td>6.93</td>
</tr>
<tr>
<td>PAL-4</td>
<td>20</td>
<td>98.0</td>
<td>5500</td>
<td>15,500</td>
<td>2.8</td>
<td>nd</td>
<td>-42.1</td>
<td>-13.2</td>
<td>2.9</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Xylene as a solvent, nd – not determined and temperature of polymerization 145 °C
Figure 5.4A: Differential scanning calorimetry (DSC) first and second heating, thermograms showing melting points (PAL).

Figure 5.4B: Differential Scanning Calorimetry (DSC) first and second heating thermograms showing melting points and glass transition points respectively (PAA).
The peaks at 34.30 ppm, 32.93 ppm, 29.11 ppm, 26.03 ppm and 24.93 ppm are due to the methylene groups present in the polymer backbone. The peak at 27.31 ppm is attributed due to methyl group of the protecting group. The peak at 51.43 ppm (-COOCH₃) is appeared due to CH₃ group attached to ester linkage. The $\bar{M}_n$ calculated from $^{13}$C NMR (quantitative) spectra is 15600 Da. All the assignments confirmed the structure of PAL-3. The end groups of the homopolymers were -COOCH₃ and -CH₂OH.

The $^{13}$C NMR spectrum of PAA is shown in Figure 5.6 A. $^{13}$C CP/MAS NMR spectrum shows the resonance, which corresponds to various groups such as 27.30-34.07 ppm (-CH₂ group), 65.11 ppm (-COOCH₂) and 75.47 ppm (-CHOH—CHOH-) and 174.75 ppm (-COO-) respectively. The linear structure of PAA was confirmed by NMR.

5.4.4. Effect of catalyst concentration on polymerization reaction: Table 5.2 shows the effect of catalyst concentration on polymerization reaction. The catalyst concentration was varied from 0.1- 0.4 wt %. The molecular weight and molecular weight distribution increased up to 0.3 wt % as the catalyst concentration increased and thereafter decreased irrespective of yield. The result shows that the reaction proceeds linearly up to 0.3 wt % catalyst. Lewis acid-catalyzed polycondensation is a reversible equilibrium reaction and aliphatic polyester is known to undergo an irreversible, thermal unzipping, back-biting side reaction, which is also Lewis acid catalyzed, giving rise to reduction of molecular weight and simultaneous formation of cyclic compound, the amount of catalyst used, therefore play a critical role. The mechanism of esterification by tin and tin derivative catalysts in general is well discussed in the literature [50], while the reaction pathway of tetraphenyltin is yet unknown. There is a possibility of alcoholysis one, two, three or all four of the Sn-C linkages of tetraphenyltin in presence of alcohol and protected ester of poly (aleuritic acid) in the reaction system leading to alkoxy-tin or tin carboxylate covalent linkage [51].

Table 5.3 illustrates the effect of reaction temperature on polymerization reaction. The polymerization reactions were carried out in various sets of nonpolar solvents starting from xylene (145 °C), mesitylene (165 °C) to decaline (190 °C). Similarly the reaction was carried out in polar solvents namely anisole (154 °C) and diphenylether (190 °C). The yield of the polymer was comparable either in nonpolar solvent or polar solvents.
Effect of reaction temperature on polymerization reaction: It is important to note that the molecular weight increased with increased in reaction temperature (reflux temperature) of the solvent. DSC data showed two melting temperatures, which increased with increase in reaction temperature. The reaction was carried out at 190 °C (decaline reflux temperature) and yellow hard soluble material was observed. The $^{13}$C CP/MAS NMR of the cross linked polymer is shown Figure 5.6.B which showed resonance at various positions. There are number of reactions occurred in cross linking reaction i.e. etherification as well as small esterification.
Figure 5.6: (A) $^{13}$C CP/MAS (Cross Polarization/ Magic Angle Spinning) N.M.R. (500 MHz) of poly (aleuritic acid) and (B) $^{13}$C CP/MAS of polymer PLA-10.

Table 5.2: Effect of catalyst concentrations on polymerization reactions of proAL

<table>
<thead>
<tr>
<th>Polymer samples</th>
<th>Cat. conc. (wt.%)</th>
<th>Yield (%)</th>
<th>$\bar{M}_n$ (GPC)</th>
<th>$\bar{M}_w$ (GPC)</th>
<th>PDI</th>
<th>$\bar{M}_w$ (LS)</th>
<th>$T_{m1}$ ($^0$C)</th>
<th>$T_{m2}$ ($^0$C)</th>
<th>$\Delta H_1$ (J/g)</th>
<th>$\Delta H_2$ (J/g)</th>
</tr>
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<tbody>
<tr>
<td>PAL-5</td>
<td>0.1</td>
<td>99.2</td>
<td>2,500</td>
<td>5,000</td>
<td>2.0</td>
<td>nd</td>
<td>-38.2</td>
<td>-18.1</td>
<td>2.4</td>
<td>0.36</td>
</tr>
<tr>
<td>PAL-6</td>
<td>0.2</td>
<td>99.0</td>
<td>3,100</td>
<td>6,900</td>
<td>2.2</td>
<td>nd</td>
<td>-38.4</td>
<td>-17.9</td>
<td>2.3</td>
<td>0.29</td>
</tr>
<tr>
<td>PAL-3</td>
<td>0.3</td>
<td>98.0</td>
<td>46,100</td>
<td>120,000</td>
<td>2.6</td>
<td>98,000</td>
<td>-39.0</td>
<td>-17.0</td>
<td>2.2</td>
<td>0.34</td>
</tr>
<tr>
<td>PAL-7</td>
<td>0.4</td>
<td>98.0</td>
<td>8200</td>
<td>24,000</td>
<td>2.9</td>
<td>21,200</td>
<td>-39.4</td>
<td>-16.9</td>
<td>2.1</td>
<td>0.32</td>
</tr>
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</table>

Xylene as a solvent, nd –not determined and temperature of reaction 145 $^0$C.

The products formed were fusible ethers, lactones and small amount of ester group, insoluble product of three-dimensional structures, which were confirmed by solid-state $^{13}$C CP/MAS NMR spectroscopy. The peaks correspond to 81.59 ppm is due to intermolecular reaction result (-CH-OCH-). The peak for two carbons involved in the
intramolecular ether groups appeared at 67.08 ppm. Both the carbons in CH-O-CH structure have the same environment. The peak at 65.93 ppm is due to (-CH-O-CH-) group.

The peak region from 26.5 to 37.2 ppm corresponds to methylene region. All the three hydroxyl and one-carboxylic groups of aleuritic acid capable of entering into reactions both in the form of inter and intra-molecular condensation. Many broad peaks were observed, indicating the absence of selectively in the chemical polymerization. GPC analysis of this polymer was difficult due to its low solubility. DSC analysis of the polymers did not show a melting transition. The polymer obtained in anisole showed \( \bar{M}_n = 821, \bar{M}_w = 2100 \) and dispersity 2.6 respectively. The two observed \( T_m \)s are -40.6 and -14.7 °C. Similar reaction was carried out in diphenylether at 190 °C and low molecular weight polymer was observed.

Table 5.4 showed the comparison results of PAL-3 and PAA. The number average molecular weight ( \( M_n \)), weight average molecular weight ( \( M_w \)) and distribution of PAL-3 are shown in Table 5.4. The melting temperatures \( T_m1 = -39.0 \) and \( T_m2 = -17.0 \) °C were observed in PAL-3. \( T_g \) of PAL-3 was not observed until -90 °C. \( T_g \) may be lower than -90 °C, which is beyond our instrument limitation. PAL-3 was deprotected with mild experimental condition. DSC data showed a single melting temperature at 75.07 °C, which may be attributed due to aggregation of pendent hydroxyl groups and similar crystallite structure. The glass transition temperature (\( T_g \)) was determined as -29.3 °C. The increase in \( T_g \) and \( T_m \) may be attributed due to -OH group interference.

The \( \bar{M}_w = 120,000, \bar{M}_n = 46,100, \) PDI = 2.6 and average DP were determined by size exclusion chromatography (SEC) against polystyrene standards. Hydrolysis of protected poly (aleuritic acid) afforded free hydroxyl groups at, 9 and 10 position of each monomeric unit of the polymer. The homopolymers are linear and soluble in various solvents such as N, N-dimethyl formamide (DMF) and dioxane etc.

5.4.6. TEM analysis: The consequences of 9, 10 hydroxyl groups and aliphatic methylene groups in the main chain of the polymer, the key hydrophilic and hydrophobic functionalities in PAA polymer, within the same monomer of homopolymer should be interesting from an intermolecular phase separation perspective [52].
Table 5.3: Effect of temperature on polymerization reactions of proAL

<table>
<thead>
<tr>
<th>Polymer samples</th>
<th>Solvent</th>
<th>Yield (%)</th>
<th>Temp. (°C)</th>
<th>$\bar{M}_n$ (GPC)</th>
<th>$\bar{M}_w$ (GPC)</th>
<th>$\bar{M}_w$ (LS)</th>
<th>PDI</th>
<th>$T_m$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_1$ (J/g)</th>
<th>$\Delta H_2$ (J/g)</th>
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<td>PAL-8</td>
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<td>145</td>
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<td>6,400</td>
<td>nd</td>
<td>2.2</td>
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<td>13.6</td>
<td>3.2</td>
<td>0.85</td>
</tr>
<tr>
<td>PAL-9</td>
<td>B</td>
<td>98.0</td>
<td>165</td>
<td>17.600</td>
<td>46,000</td>
<td>44,000</td>
<td>2.6</td>
<td>-38.2</td>
<td>17.8</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>PAL-10</td>
<td>C</td>
<td>-</td>
<td>190</td>
<td>-</td>
<td>-</td>
<td>nd</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PAL-11</td>
<td>D</td>
<td>99.0</td>
<td>154</td>
<td>821</td>
<td>2,100</td>
<td>2.6</td>
<td>nd</td>
<td>-40.6</td>
<td>14.7</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>PAL-12</td>
<td>E</td>
<td>-</td>
<td>190</td>
<td>-</td>
<td>-</td>
<td>nd</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Reaction time 5 h, nd-not determined and A= Xylene, B= mesitylene, C=decaline, D= anisole and E= diphenyl ether

Table 5.4: Comparison results of PAA and PAL polymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$\bar{M}_n$ (GPC)</th>
<th>$\bar{M}_w$ (GPC)</th>
<th>$\bar{M}_w$ (LS )</th>
<th>$T_g$ (°C)</th>
<th>$\Delta C_p$ (J/g°C)</th>
<th>$T_m1$ (°C)</th>
<th>$T_m2$ (°C)</th>
<th>$\Delta H_n$ (J/gm)</th>
<th>$\Delta H_n$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL-3</td>
<td>46,100</td>
<td>120,000</td>
<td>98.000</td>
<td>&gt;100</td>
<td>nd</td>
<td>-34.7</td>
<td>-18.77</td>
<td>1.67</td>
<td>6.93</td>
</tr>
<tr>
<td>PAA</td>
<td>nd</td>
<td>Nd</td>
<td>nd</td>
<td>-29.3</td>
<td>0.075</td>
<td>75.07</td>
<td>nd</td>
<td>61.72</td>
<td>nd</td>
</tr>
</tbody>
</table>

nd- not determined

The hydrophilic hydroxyl unit and methylene moiety will be placed on the opposite sides of the polymer backbone in solvents of different polarity. The hydrophobic and the hydrophilic functionalities are stitched together within the same monomer in polymers. Therefore, it may be expected that the spatial distribution of the interior groups of the assembly closely followed the distribution of the functionalities in the corona. Close examination of the normal structure is shown in (Figure 5.7). The core part of the new structure is folded methylene groups, which are dark, and hydroxyl pendant groups are in the periphery. Multiple morphologies of PAA in various solvents such as polar solvents...
and nonpolar solvents and structure property relationship through morphologies were obtained by using TEM.

The polymeric solution in polar solvents was optically clear. Typical TEM photographs for the poly (aleuritic Acid) are displayed in Figure 5.7 and explained the structure of the assemblies. The images obtained from PAA polymer in dioxane and N,N-dimethyl formamide (DMF) are shown in Figure 5.7 (A and B) respectively. The observed solubility characteristic may be attributed to the formation of micelle like structure in dioxane, N, N-dimethyl formamide (DMF), in which the hydrophilic hydroxyl groups are exposed to the bulk solvent and the hydrophobic methylene groups are tucked in the interior of an assembly (Figure 5.7 A and B). Similarly, an inverted micelle-like structure would be expected in a nonpolar solvent, in which the functional group placements are, reversed (Figure 5.7 C). The structural hypothesis also suggests that the hydrophilic hydroxyl groups and hydrophobic methylene moiety will be placed on the opposite sides of the polymer backbone in the solvents of different polarity. The image obtained from polymer exhibit darker core compared to the corona. The darker contrast provided by methylene chains of backbone of PAA are shown in TEM images (Figure 5.7 A and B). For the interaction of the electron beam with material of the polymer, carbon forms a decisive amongst majority of the elements. Further the density of backbone carbon is slightly greater than that of hydroxyl group and hence we except PAA having higher density to appear dark in the TEM image. The TEM picture of pure PAA reflected this in explanation. The image obtained in Figure 5.7 C from toluene solution of polymers was consistent with the expected features. However, nonspherical micelles in solution have been observed only rarely and mostly indirectly.

5.5. Copolymerization of L-lactic acid with aleuritic acid: The homopolymer of protected aleuritic acid was found to be transparent, viscous liquid, which showed a low crystalline melting point and glass transition temperature. By virtue of its low glass transition protected poly (aleuritic acid) could be expected to impart some flexibility to poly (L-lactic acid) (PLA) as a plasticizer. The processing of PLA addresses lot of problems. The copolymers containing protected aleuritic acid is expected to behave as a plasticizer whereas the same copolymer after deprotection might improve the rheological properties due to occurrence of small branching on the PLA chain. Copolymerization of protected
aleuritic acid with L-lactic acid was attempted to improve structure property relation during processing.

Figure 5.7: TEM images of the micelle-like and inverted micelle-like structures formed by PAA polymer (A) Image of normal micelle-like particle from dioxane, (B) Image of micelle-like particle from DMF and (C) Image of inverted micelle-like particle formed by a toluene solution of PAA.

The synthesis of protected aleuritic acid was carried out and characterized. The synthesis of L-lactic acid-protected aleuritic acid copolymers was accomplished by dehydropolycondensation using Lewis acid (tetraphenyltin) as a catalyst and shown in Figure 5.8. 5 mL of L-lactic acid (88% aqueous solution) was taken in a three neck flask and xylene was added (1:1 v/v) proportion. The reactant was refluxed for 6h using Dean Stark apparatus to remove water as an azeotrope and requisite amount of protected aleuritic acid was added into the reaction flask. The reaction mixture was further continued up to 15h. The reaction mixture was cooled and the extra xylene was removed by vacuum distillation. The copolymer of various compositions ranging from 90:10 to
50:50 ratios was prepared accordingly. Reaction scheme for copolymerization is shown in Figure 5.8. The resulting copolymer was dissolved in chloroform in a single neck flask and equal amount of methanol, catalytic amount of PTSA was added into it. The reaction mixture was stirred at room temperature (25 °C) under inert atmosphere (Argon) for 6 h. The deprotected copolymer was dissolved in chilled methanol and filtered using Whatman filter paper. The resulting copolymer was characterized by ¹H NMR, GPC, DSC and aggregation behavior in different solvent was observed by TEM.

Figure 5.8: Reaction scheme of copolymerization.
5.5.1. SEC Analysis: The SEC thermograms of protected copolymer samples are all shown in Figure 9A. The copolymers and homopolymers were prepared by
dehydropolycondensation method using tetraphenyltin as a catalyst and p-xylene as a solvent. Copolymers (COP-1 to COP-5) showed a single peak (Figure 5.9A) whereas COP-2 and COP-3 showed a shoulder peak on them. These results are attributed due to very low molecular weight oligomeric species in equilibrium with each other. The copolymer COP-1 showed $\bar{M}_n$, $\bar{M}_w$ and molecular weight distribution as 7,500, 13,200 and 2.2 respectively.

Table 5.5: Properties of L-lactic acid protected aleuritic acid copolymers

<table>
<thead>
<tr>
<th>Copolymer samples</th>
<th>Feed ratio</th>
<th>Copolymer (comp.)</th>
<th>$\bar{M}_n$ (GPC)</th>
<th>$\bar{M}_w$ (GPC)</th>
<th>PDI</th>
<th>$T_m$ ($^\circ$C)</th>
<th>$\Delta H_f$ (J/g)</th>
<th>$T_g$ ($^\circ$C)</th>
<th>$\Delta C_p$ (j/g*°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>100:0</td>
<td>100:0</td>
<td>900</td>
<td>2,100</td>
<td>2.3</td>
<td>146.0</td>
<td>42.0</td>
<td>44.5</td>
<td>0.46</td>
</tr>
<tr>
<td>COP-1</td>
<td>90:10</td>
<td>85:15</td>
<td>7,500</td>
<td>13,200</td>
<td>1.7</td>
<td>161.4</td>
<td>4.1</td>
<td>10.62</td>
<td>0.50</td>
</tr>
<tr>
<td>COP-2</td>
<td>80:20</td>
<td>75:25</td>
<td>5,700</td>
<td>12,700</td>
<td>2.2</td>
<td>175.5</td>
<td>0.80</td>
<td>-11.5</td>
<td>0.33</td>
</tr>
<tr>
<td>COP-3</td>
<td>70:30</td>
<td>70:30</td>
<td>2,100</td>
<td>6,400</td>
<td>3.0</td>
<td>127.3</td>
<td>5.7</td>
<td>-22.0</td>
<td>0.37</td>
</tr>
<tr>
<td>COP-4</td>
<td>60:40</td>
<td>60:40</td>
<td>1,250</td>
<td>1,750</td>
<td>1.4</td>
<td>148.7</td>
<td>0.41</td>
<td>-30.2</td>
<td>0.44</td>
</tr>
<tr>
<td>COP-5</td>
<td>50:50</td>
<td>50:50</td>
<td>800</td>
<td>2,000</td>
<td>2.5</td>
<td>138.8</td>
<td>1.2</td>
<td>-31.5</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Temperature of polymerization 195°C and time for polymerization 8 hr.

Table 5.6: Properties of L-lactic acid-protected and deprotected aleuritic acid copolymers

<table>
<thead>
<tr>
<th>Copolymer sample.</th>
<th>Feed ratio</th>
<th>$\bar{M}_n$ (GPC)</th>
<th>$\bar{M}_w$ (GPC)</th>
<th>PDI</th>
<th>$T_m$ ($^\circ$C)</th>
<th>$\Delta H_f$ (J/g)</th>
<th>$T_g$ ($^\circ$C)</th>
<th>$\Delta C_p$ (j/g*°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COP-1</td>
<td>90:10</td>
<td>7,500</td>
<td>13,200</td>
<td>1.7</td>
<td>161.4</td>
<td>4.1</td>
<td>10.62</td>
<td>0.50</td>
</tr>
<tr>
<td>COP-2</td>
<td>80:20</td>
<td>5,700</td>
<td>12,700</td>
<td>2.2</td>
<td>175.5</td>
<td>0.80</td>
<td>-11.5</td>
<td>0.33</td>
</tr>
<tr>
<td>DCP-1</td>
<td>90:10</td>
<td>7,500</td>
<td>13,000</td>
<td>1.7</td>
<td>135.6</td>
<td>5.2</td>
<td>34.7</td>
<td>0.43</td>
</tr>
<tr>
<td>DCP-2</td>
<td>80:20</td>
<td>5,700</td>
<td>12,700</td>
<td>2.2</td>
<td>140.0</td>
<td>5.5</td>
<td>40.5</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Figure 5.10: \(^1\)H NMR spectra of copolymers (a) COP-5, (b) COP-4, (c) COP-3, (d) COP-2 and (e) COP-1.
COP-2 showed $\bar{M}_n$, $\bar{M}_w$ and molecular weight distribution as 5,700, 12,700 and 2.2 respectively with a small shoulder peak. Similar observation was obtained in case of COP-3. COP-4 which showed a single peak and the calculated $\bar{M}_n$, $\bar{M}_w$ and molecular weight distribution are 1250, 1750 and 1.4 respectively.

The copolymer COP-5 also showed a single peak and the calculated $\bar{M}_n$, $\bar{M}_w$ and molecular weight distribution are 800, 2000 and 2.5 respectively. The SEC elugrams of deprotected copolymer samples (DCP-1 and DCP-2) are all shown in Figure 5.9B.

5.5.2. Nuclear Magnetic Resonance: The copolymer compositions were determined from peak area in $^1$H NMR spectra and shown in Figure 5.10. Comparison of the peak area in the region $\delta=3.56$ pap due to disubstituated proton contributed by aleuritic acid (9, 10 position) with the area of the proton at $\delta=5.15$ pap due to methane group of the L-lactic acid enables the estimation of the copolymer composition. Samples obtained from mole ratios of protected aleuritic acid: L-lactic acid = 10: 90 to 50: 50 were soluble in CDCl$_3$.

The results of COP-1 to COP-5 along with PLA and protected poly aleuritic acid are shown in Table 5.5. The results of protected copolymers (CAP-1, CAP-2) and deprotected copolymers (DCP-1, DCP-2) are shown in Table 4.6.

5.5.3. Thermal properties: Results of thermal characterization are shown in Table- 5.5 and thermograms are shown in Figure 5.11 A. The glass transition temperature, $T_g$, of the copolymers varied from 31.46 to 10.62 °C. A gradual reduction in the $T_g$ was observed with increase in comonomer incorporation as shown in Table 5.5 and Figure 5.11A thereby indicating increased mobility of the amorphous phase. The crystalline melting point, $T_m$ of PLA phase was also found to be disturbed. The depression of the glass transition temperature was more prominent than $T_m$ of PLA.

Although the absence of $T_g$ characteristic of protected PAA could not be ascertained, yet the absence of a glass transition characteristic of pure homopolymer PLA was, however, sufficient proof of plasticization. Therefore, the lowering of glass transition temperature of PLA by a statistical copolymerization with molar proportions of protected PAA can indeed be called a case of “internal plasticization”.

The copolymers (COP-1 and COP-2) were dissolved in chloroform, equal amount of methanol and catalytic amount of $p$- toluene sulphonic acid (PTSA) was added into it.
The reaction mixture was stirred at room temperature (25 °C) under inert atmosphere (argon) for 6 h. The resultant copolymer was washed with methanol several times and G.C analysis result confirmed the absence of dimethoxy propane. The structures of DCP-1 and DCP-2 were confirmed by \textsuperscript{1}H NMR. Results of thermal characterization of DCP-1 and DCP-2 are shown in Table 5.6 and thermograms are shown in Figure 5.11 B. The protected copolymer COP-1 (waxy mass) and deprotected copolymer DCP-1 (solid powdery mass) showed dramatic increase of $T_g$ values from 10.62 (COP-1) to 34.7 °C (DCP-1) and also affected $T_m$ value. The increase in $T_g$ value may be attributed due to aggregation of hydroxyl groups present at 9 and 10 position of aleuritic acid unit in the copolymer chain. Similarly copolymer COP-2 (highly viscous mass) and after deprotection (DCP-2) also showed increase of $T_g$ value from -11.5 to 40.5 °C and also affected $T_m$ value.

5.5.4. Transmission Electron Microscopy (TEM): The thermal characteristic result showed the aggregation behavior, which was further examined by TEM. Functionalized interfacial organic and polymer layers fabricated from molecular segments with different amphiphilicity can be designed to act as a smart or switchable surface. These surfaces are capable of responding to very suitable changes in the surrounding environment such as pH, surface pressure and temperature, light and solvent quality. In the present system, these deprotected copolymer DCP-1 and DCP-2 aggregate in various solvents and their structures are slightly different from each other. These structures are responsible for controlling physical properties in term of application such as drug delivery and biomimetic materials. The copolymer DCP-1 and DCP-2 used in this study is L-lactic acid and protected aleuritic acid, which was synthesized by dehydropolycondensation and followed deprotection. The polydispersity indices of the copolymers, estimated by gel permeation chromatography were 1.7 and 2.2 respectively.

The consequences of 9, 10 hydroxyl groups of aleuritic acid unit and methylene groups of aleuritic acid and L-lactic acid unit in the main chain of the copolymer, the key hydrophilic and hydrophobic functionalities in copolymer, within the different monomers of copolymers should be interesting from an intermolecular phase separation perspective. The hydrophilic and the hydrophobic will be placed on the opposite sides of the copolymer backbone in solvents of different polarity. The hydrophobic and hydrophilic
functionalities are stitched together within different monomers in copolymers. Figures 5.12A and 5.12 A' showed the morphologies of the aggregates of DCP-1 and DCP-2 copolymers in N, N-dimethylformamide (DMF). DCP-1 gives micelles of low polydispersity whereas DCP-2 shows slightly elongated form. They consist of a hydrophobic units core covered with hydrophilic units forming the corona. Similar observation has been made by Lifeng Zhang et al [39]. Figures 5.13 B and 5.13 B' showed the morphologies of the aggregates from DCP-1 and DCP-2 copolymers in tetrahydrofuran (THF). DCP-1 formed micelles of low polydispersity whereas DCP-2 showed polydispersity. The hydrophobic units’ core are covered with hydrophilic units forming the corona. Figures 5.14 C and 5.14 C' showed the morphologies of the aggregates from DCP-1 and DCP-2 copolymers in dioxane. DCP-1 and DCP-2 give spherical micelles of low polydispersity.

![Figure 5.11 A: Differential Scanning Calorimetry (DSC) second heating thermograms (a) COP-1, (b) COP-2, (c) COP-3, (d) COP-4 and (e) COP-5.](image)

In fact, DCP-2 gives better size of spherical micelles with low polydispersity. These copolymers are not soluble in toluene, whereas PLA is soluble in chloroform. Therefore mixed solvents of toluene and chloroform at various proportions (50:50 and 60:40) were taken and morphologies of these two copolymers (DCP-1 and DCP-2) are shown by
TEM. Figures 5.15 D and 5.15 D’ shows the morphologies of the aggregates in mixed solvents (50:50 ratio of toluene: chloroform). DCP-1 and DCP-2 showed narrow distribution.

Figure 5.11B: Differential Scanning Calorimetry (DSC) of second heating thermograms (a) DCP-1 and (b) DCP-2.

Figure 5.12: TEM images of the micelle-like aggregates in DMF (A) DCP-1 and (A’) DCP-2.
Figure 5.13: TEM images of the micelle-like aggregates in THF (B) DCP-1 and (B') DCP-1.

Figure 5.14: TEM images of the micelle-like aggregates in dioxane (C) DCP-1 and (C') DCP-2.

Figure 5.15: TEM images of the micelle-like aggregates in (50:50) toluene: chloroform (D) DCP-1 and (D') DCP-2.
5.6. Conclusion:
In summery, a new class of value added biodegradable poly (aleuritic acid) from renewable resources (Shellac) are produced by polycondensation. Results show that linear PAA polymer with $\bar{M}_n$~120,000 can be prepared with organotin catalyst. The structure and properties of PAA polymers are determined. Poly (aleuritic acid) s amphiphilic linear homopolymers containing both hydrophilic (hydroxyl pendant groups) and lipophilic functionalities (backbone) in each repeats unit have been synthesized where all the hydroxyl pendant groups aggregate and form new structures. Amphiphilic functions reported here are likely to form the basis for new nanoscale aggregates in solution which could be implications in a broad range of application. A new class of copolymers showed internal plasticization effect when protected aleuritic acid was incorporated in the PLA backbone chain at different molar compositions. These copolymers behaved differently after deprotection of aleuritic acid at 9 and 10 positions. Amphiphilic copolymers containing both hydrophilic and hydrophobic functionalities in
repeat units have been synthesized. These amphiphilic copolymers are soluble in organic and mixed organic solvents and assemble into micelle-like structures. Amphiphilic functions reported here is likely to form the basis for micro scales assembles in solution, which could also have implications in a broad range of applications.

References


