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# Biodegradable Poly-pentadecalactone (PDL) Synthesis via Synergistic Lipase and Microwave Catalysis

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**Abstract** A large number of currently used synthetic biodegradable polymers in biomedical engineering applications are polyesters based materials and thus research on the synthesis, properties, manufacturing and processing of aliphatic polyesters continues to be of great importance. Poly-ω-pentadecalactone (PPDL) a lactone based ring opening polymer has good mechanical properties and the presence of hydrolysable ester linkages along the polymer chain making it desirable as a biodegradable material for diversified biomedical engineering applications. In this paper we report the formation of PPDL using the synergistic effects of lipase and microwave (MW) technology. The effect of reaction time on the PPDL polymer chain growth has been investigated. PPDL have been formed using lipase and MW irradiation at varying reaction time intervals (30-240 mins). Synergistic MW and lipase catalyzed polymerization of PPDL gave a number average molecular weight (Mn) of 24,997 g/mol and a polydispersity index (PDI) of 1.93 in 240 mins as compared to Mn of 8,060 g/mol and PDI of 2.17 using lipase and traditional heating. Thermal characterization of PPDL formed using MW and lipase catalysis showed that MW did not have a detrimental effect on the thermal properties of the polymer obtained.

**Keywords** Microwave, Lipase, Biodegradable Polymer, Poly-pentadecalactone

## 1. Introduction

Biodegradable polymers find various biomedical engineering applications that could be categorized as temporary support device (sutures, bone fixation devices), temporary barrier (artificial skin), drug delivery device (nano, micro particles), tissue engineering scaffold and multifunctional devices (biodegradable drug eluting stents)[1]. Although numerous biodegradable polymers find use in biomedical engineering applications a large number of currently used synthetic biodegradable polymers are polyesters based materials[2]. Thus research on the synthesis, properties, manufacturing and processing of aliphatic polyesters continues to be of great importance. The production of both aliphatic and semi-aliphatic polyesters follows two synthetic strategies (a) either ring-opening polymerization (ROP) of cyclic esters (lactones or cyclic oligoesters) or (b) polycondensation of diacids and diols, hydroxyacids and/or their di-methyl esters[2]. Both of the above methodologies require use of a catalyst or an initiator. In case of biodegradable polymers for pharmaceutical or medical applications, the potential toxicity of the catalyst plays a critical role in the choice of the catalyst used as a residual trace of the catalyst could lead to cytotoxicity[3].This has led to academic and industrial interest to look for a less toxic group of catalysts, initiators and processes for the synthesis and manufacturing of biodegradable polyesters.

Microwave and enzymatic assisted polymer chemistry both individually have been gaining interest in green manufacturing as an alternative energy source and catalyst respectively[4, 5]. Microwave assisted synthesis provides several advantages and benefits such as localized heating, reduced chemical reactions times, increased product yield and increased selectivity[6]. Microwave assisted synthesis has expanded to a variety of biological molecules such as peptides, oligomers and carbohydrates[7]. Comprehensive reviews have covered the use of microwave in organic chemistry[4] and in recent years in polymer chemistry[7]. Enzyme catalyzed polymerizations have been explored intensively since the 1990's. Enzymatic catalysts provide an alternate green catalytic substitute for the toxic metal complexes usually employed in polyester polymer synthesis[5, 8, 9]. The main advantages of enzyme catalyst are 1) high catalytic activity, 2) high efficiency under mild conditions (temperature, solvent), 3) biocompatibility, 4) reusability, and 5) stereo and regioselectivity[5,10]. Enzymatic catalyzed polymerizations serve as an environmental friendly synthetic process and provide an example of green polymeric chemistry[10].

Relatively fewer reports exist on the applications of

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microwave assisted enzymatic reactions, with emphasis on organic small molecule transformations[11-16]. Enhancements in the initial rate of reaction,[17, 18] product yields[15, 19] and enantioselectivity[13] have been reported when using microwave heating as compared to conventional heating. However understanding of this area is poor and often controversial. Leadbeater[12] and co-workers investigated the effect of microwave irradiation on lipase catalyzedtransesterification of methyl acetoacetate in toluene. They reported minor differences between conventional and microwave heating. Rejasse et al.[20] studied the effect of microwave heating on the stability of *Candida antarctica* Lipase B (CALB) and the kinetics of butyl butyrate synthesis. They reported an increase in enzymatic stability in organic medium under microwave field suggesting a possible explanation for an increase in conversion rates under microwave heating[20].

The field of microwave assisted enzymatic polymerizations has been recently receiving attention for exploiting the synergistic benefits of lipase and microwave processed in the field of polymer chemistry[21, 22]. Kerep and Ritter investigated the influence of MW irradiation on lipase catalyzed ring opening polymerization of ε-caprolactone[22]. They reported that MW assisted enzymatic polymerizations had accelerating effects depending on the kind of boiling solvent used[22]. Recently our laboratory investigated the effects of microwave process parameters (power, intensity, MW irradiation time and temperature) on lipase catalyzed polymerization of caprolactone[23]. In this paper we report the formation of poly-ω-pentadecalactone (PPDL) a lactone based ring opening polymer using the synergistic effects of lipase and microwave technology. Good mechanical properties of PPDL and the presence of hydrolysable ester linkages along the polymer chain of PPDL have led to significant interest and consideration of PPDL as a biodegradable material for diversified biomedical engineering applications.

# 2. Materials and Methods

### 2.1. Reagents

The monomer ω-pentadecalactone (PDL)[98%, molecular weight 240.38], Novozyme-435 (*Candida antartica* Lipase B), chloroform ($CHCl_3$), methanol ($CH_3OH$) were purchased from Sigma-Aldrich and used without further purification.

### 2.2. Microwave Assisted enzyme-catalyzed ring-opening Polymerization of PDL

All reactions were carried in solvent-free environment. The PDL monomer (500 mg, 2.08 mmol) and lipase (50 mg of CALB) was used for the reaction. These reagents consisting of an enzyme to monomer ratio of 1/10 wt/wt wereplaced in a 7 mL microwave (MW) reaction vials with temperature monitored using an *in situ* optical probe. The reaction was set in a 'CEM' microwave synthesizer 'Explorer' at constant temperature of 70℃, constant power of 200W and at predetermined reaction time periods of 30 min, 60 min, 120 min and 240 min. The temperature and power setting were determined based on previous protocols established in our laboratory[23]. Control reaction was carried out using enzyme catalyzed polymerization in a traditional oil bath for 240 min. The reactions were terminated by dissolving the residual monomer and polymer in chloroform and separating the insoluble enzyme by filtration using 10-15 mm glass-fritted filters. The polymer was then dissolved in chloroform and precipitated using methanol. The insoluble materials was filtrated and washed three times with 5 mL portions of fresh methanol. The polymer was dried using vacuum oven and subsequently characterized.

### 2.3. Characterization

#### 2.3.1. Nuclear Magnetic Resonance (NMR)

Proton (1H) NMR spectra were obtained on a Bruker Advanced 300 MHz spectrometer at 300 and 75.13 MHz. The chemical shifts in parts per million (ppm) were referenced relative to tetramethylsilane (TMS).

#### 2.3.2. Gel Permeation Chromatography (GPC)

The molecular weights of polymers were measured on a Viscotek GPC max 2001 TDA 360 triple-detector system at a temperature of 35 ℃ .Narrow molecular weight polystyrene standards obtained from Sigma-Aldrich were used to generate the conventional calibration curve. The mobile phase utilized to study these systems was tetrahydrofuran (THF) at a flow rate of 1ml/min.

#### 2.3.3. Thermogravimetric Analysis (TGA)

Thermal analysis was performed on a Perkin-Elmer TGA6/DSC6 system with constant $N_2$ flow. Weight of sample was in the range 8-10 mg. The analyses were performed at a rate of 10℃/ min from room temperature to 600℃

# 3. Results and Discussion

Figure 1 represents the schematic representation of MW assisted Lipase catalysis of poly-ω-pentadecalactone. The reactions were carried out at 70℃ in bulk for up to 4 hours in MW irradiation and a control reaction using traditional oil bath heating.

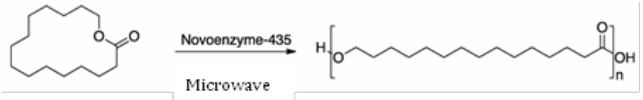


**Figure 1.** Schematic representation of synergistic microwave and novozyme-435 catalyzed polymerization of poly-pentadecalactone from the cyclic monomer ω-pentadecalactone

Figure 2 shows the proton NMR spectra of poly-ω-pentadecalactone at various reaction conditions with novozyme-435 as a catalyst and microwave irradiation along with the control reaction (novozyme-435 catalyzed polymerization of PDL in a traditional heated oil bath). From our 1H NMR spectra we can deduce that ω-polypentadecalactone was formed at all the reaction times. The peaks assignments in 1H-NMR ($CDCl_3$) spectra were: 4.05 (t, J 7 Hz, CHO), 3.64 (t, J 7 Hz, $CH_2OH$), 2.31 (t, J 7 Hz, $CH_2CO$), 1.62 and 1.33 (22H, $CH_2$) ppm. The chemical shifts reported are in good agreement with earlier reports of lipase catalyzed polymerization of ω-pentadecalactone using conventional heating[24].

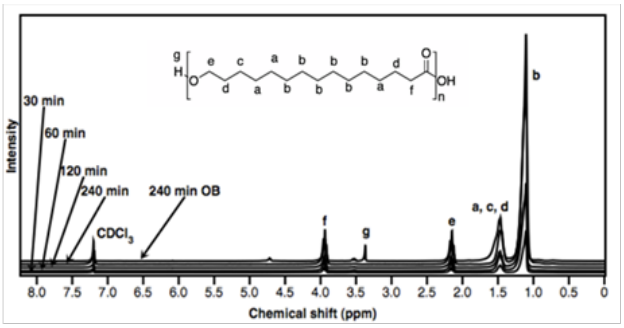


**Figure 2.** 1H NMR spectra of poly-pentadecalactone in $CD_3Cl$ at reaction intervals of 30, 60, 120 and 240 minutes and with conventional heating in an oil bath (240min OB)

**Table 1.** Polymerization of ω-Pentadecalactone in Bulk at 70°C catalyzed by Novozyme-435 under Microwave conditions

| Sample | Mn | Mw | DPavg | PDI | % yield |
|---|---|---|---|---|---|
| PPDL- 30 min | 13,342 | 25,953 | 56 | 1.94 | 62 |
| PPDL- 60 min | 15,066 | 32,670 | 63 | 2.16 | 41 |
| PPDL- 120 min | 19,406 | 39,259 | 81 | 2.02 | 61 |
| PPDL- 240 min | 24,997 | 48,459 | 104 | 1.93 | 56 |
| PPDl- 240 min in oil bath* | 8,060 | 17,555 | 34 | 2.17 | 20 |

*reaction carried out in using lipase catalysis using conventional heating in oil bath, Mn is number average molecular weight, Mw is weight average molecular weight, DPavg is average degree of polymerization and PDI is polydispersity index

Table 1 summarizes the obtained molecular weights, PDI and % yields at different time intervals. Using synergistic lipase and MW irradiation after 240 min (4 hrs) the value ofnumber average molecular weight (Mn) of the polymer obtained was 24,997 g/mol with a polydispersity index (PDI) of 1.93. In contrast the polymer obtained using traditional heating in an oil bath for 240 min gave a Mn of 8,060 g /mol with a PDI of 2.17. This confirms that synergistic effects of MW and lipase catalysis results in a significant (three fold) increase in the Mn obtained as compared to traditional oil bath heating within the same time period. This trend is in agreement with previously reported results on ring opening polymerization (ROP) on caprolactone using synergistic MW and lipase polymerization[23]. Bishht et al[24], have carried out the lipase catalyzed polymerization with lipase catalysis in traditional oil bath and have reported a Mn of 15,300 g/mol (PDI of 4.4) in 24 hrs and Mn of 22,100 g/mol (PDI 3.3) in 72 hrs respectively[24]. Our reported Mn of 24,994 (PDI 1.93) after only 4 hrs is much higher than those even obtained with traditional heating at much higher times of 72 hrs[24]. Figure 3 shows changes of the Mn over time indicating that with increase in the reaction time the Mnvalues went up from 13, 342 g/mol in 30 min to 24,997 g/mol in 240 min (4 hrs).

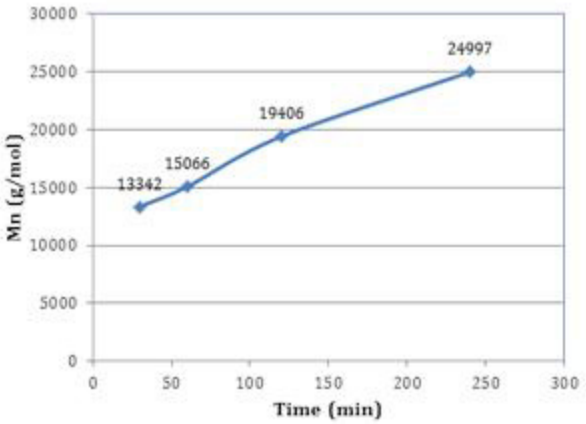


**Figure 3.** Effect of number average molecular weight (Mn) of the PDL formed vs reaction time for microwave assisted Novozyme-435 catalyzed polymerizations

In order to get a better understanding of polymer initiation and prorogation of PDL using synergistic MW and lipase catalysis the PDI and % yield of polymerization were plotted against degree of polymerization (DPavg). Figure 4 shows variations in the PDI vsDPavg which gives an indication on how the dispersity of the length of the polymer chains varies as the polymer chain (PPDL) grows. Similarly figure 5 gives an indication of the changes in % yield (ratio of polymer formed to monomer/low molecular weight oligomer) as the polymer chain grows (DPavg). From figure 4 we can see that PDI increases initially 1.94 (30 min) to 2.16 (60min). During this time the Mn of the polymer rises from 13,342 (DPavg: 55) to 15,066 (DPavg: 63) (figure 3) and % yield drops from 62 to 41 respectively. A mechanism for the lipase-catalyzed ring opening polymerization of lactones is postulated by involving an acyl-enzyme intermediate[25]. The catalytic site of lipase resides in the serine residue and the lipase reaction proceeds via an acyl-enzyme intermediate[5, 25]. The polymerization occurs in two steps i.e ring opening of the lactone to form an oligomer and propagation of these oligomoers to form high molecular weight polymer[5]. Thus during the initial stages with more lactone being ring opened the PDI increases due to which the number of low molecular weight oligomers present decreasing the relative % yield. As time progresses the oligomer and other small polymer chains combine to give high molecular weight polymers. Due to the known chain selectivity of enzymes catalysis the PDI of the polymer decreases. With formation of high molecular weight polymer the % yield increases. This is evident from Figure 4 and Figure 5 where the PDI drops from 2.16 to

1.93 from 60 min to 240 min and subsequently % yield increase from 41 to 56. Similar trends were reported by Bisht et al. while carrying out the lipase catalyzed polymerization using traditional oil bath heating[24].

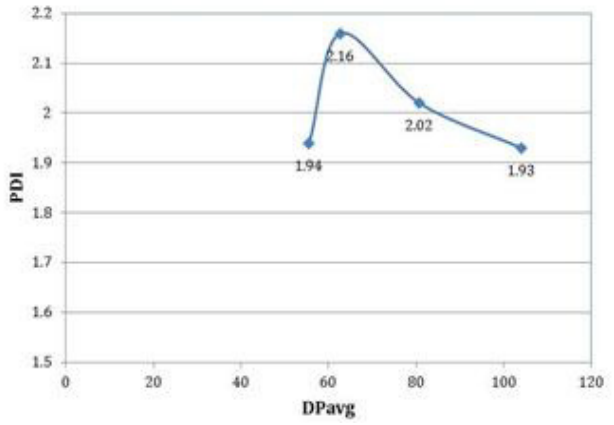


**Figure 4.** Effect of polydispersity index (PDI) of PDL formed vs reaction time for microwave assisted Novozyme-435 catalyzed polymerizations

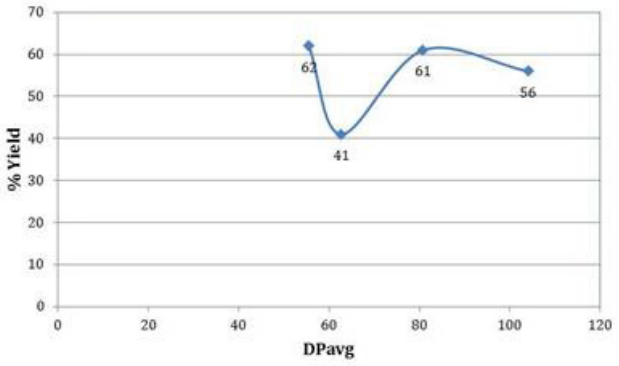


**Figure 5.** Effect of % yield on DPavg for microwave assisted Novozyme-435 catalyzed polymerizations

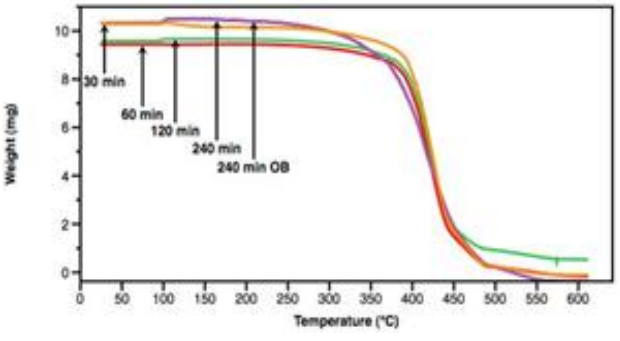


**Figure 6.** TGA curves of Novozyme-435 catalyzed PPDL

Thermal properties of enzyme-catalyzed PPDL in bulk were studied using thermogravimetric analysis (TGA). Figure 6 shows the TGA curves for PPDL synthesized at 30, 60, 120 and 240 minutes along with the control reaction (traditional oil bath heating). The TGA curves shows a weight loss with an onset temperature at above 350℃ and 10% weight loss at 380℃. No solid residue was observed after 500℃. These results are similar to those reported by Letizia-Focareteet al[26], where they have reported the onset temperature of thermal degradation to begin at 350℃ [26]. Thus enzyme catalyzed PPDL using MW irradiation gives similar results to PPDL obtained using conventional heating[26]. This indicates no adverse changes in thermal properties of PPDL formed due to the synergistic MW and lipase heating.

## 4. Summary and Conclusions

In summary we have demonstrated the synergistic MW and lipase catalyzed polymerization of PDL. Synergistic MW and lipase catalyzed polymerization of PPDL gave an average molecular weight (Mn) of 24,997 g/mol and a polydispersity index (PDI) of 1.93 in 240 mins as compared to Mn of 8,060 g/mol and PDI of 2.17 using lipase and traditional heating. Thermal characterization of PPDL formed using MW and lipase catalysis did not have a detrimental effect on the thermal properties of the polymer obtained.

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