Synthesis of poly(ω-pentadecalactone) using Lipase Immobilized onto a Renewable Carrier, Rice husk ash and their Characterization

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ABSTRACT

Rice husk ash is a side-product of rice production; thus, it is a cheap, abundant, and renewable material, and utilized as an enzyme carrier to immobilize Candida antarctica lipase B. In this study, Candida antarctica lipase B immobilized onto rice husk ashes was used to catalyze ring opening polymerization of 16-membered lactone, ω-pentadecalactone. In order to determine the best polymerization conditions for highest molar mass polymer, reactions were proceeded at various temperatures and time periods. The best reaction conditions were obtained as 80°C and 6 hours (M_n= 34255 g mol^{-1}). Molecular structure of this polymer sample was confirmed via proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and Fourier transform infrared spectroscopy (FTIR). Thermal properties were investigated by differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). Consequently, poly(ω-pentadecalactone) was synthesized enzymatically with rapid polymerization tendency and improved thermal properties which were quite close to low-density polyethylene. Successful polymerization results showed that Candida antarctica lipase B immobilized onto rice husk ashes may be a good alternative to commercial immobilized form, Novozyme 435, as a result of its comparable activity and low cost.

KEYWORDS*: Candida antarctica lipase B, Enzymatic ring opening polymerization, Poly(ω-pentadecalactone), Rice husk ash, Immobilization.*

INTRODUCTION

Aliphatic polyesters, such as poly(ω-caprolactone) (PCL), have received enormous interest during the investigations on biodegradable and biocompatible polymers. However, applications of PCL were limited due

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to its relatively low melting point (~60°C)[1]. Poly(ω-pentadecalactone) (PPDL), which is a semi-crystalline polymer, is composed of repeating 14 methylene groups, identical to polyethylene, between the ester group that lead to a higher melting temperature (~100°C)[1–6]. As a result of this fact, it is possible to apply PPDL at higher temperatures than PCL[1,7]. PPDL has excellent thermal and mechanical properties at adequately high molecular weights. Actually, its ductility and tensile strength are comparable to low-density polyethylene (LDPE). Besides, the proximate melting temperature, also the crystallization behaviors and crystal structures of PPDL and LDPE show large similarities[2,3,8–12]. Unlike to LDPE, there exists hydrolysable ester bonds between the repeating 14 methylene groups which makes PPDL a biodegradable material [1,2,5,9–12]. The monomer, ω-pentadecalactone (ω-PDL), has biological origin which can be obtained via modification of fatty acids derived from algae or plant oils[8]. Hence, PPDL is considered as bio-based alternative for polyethylene[2,8]. With these features, PPDL may serve as a good alternative polyester for biomedical applications[1,5,12].

ω-pentadecalactone (ω-PDL) is a commercially available, 16-membered macrolactone that can be easily polymerized via ring opening polymerization (ROP) to obtain high molar mass PPDL[2,5,6,8]. However, ROP mechanism of ω-PDL and other macrolactones differ from lactones with smaller ring sizes such as δ-valerolactone and ε-caprolactone. Small or medium size lactones have ring strain that leads to a large and negative enthalpy of ring opening. On the other hand, polymerization of macrolactones are driven mainly by entropy as a result of low ring strain. Therefore, polymerization of ω-PDL by traditional chemical methods is difficult and result in relatively low molar mass at the end of long polymerization periods[2,3,5,7,13]. Additionally, these chemical catalysts lead to heavy metal impurities in the final product which is undesirable for biomedical applications[1,5,7,14]. However, enzyme catalyzed ROP of ω-PDL and other macrolactones have been shown to be advantageous (mild reaction conditions and high activity for macrolactone polymerization) relative to chemical catalysis. Lipase is the most preferred enzyme for ROP of ω-PDL due to high reactivity of monomer towards lipase catalysis[1,2,4,5,7,13]. Kumar and co-workers achieved significantly higher molar mass PPDL using Novozyme 435 (the commercially available immobilized form of *Candida antarctica* lipase B) catalyzed ROP of ω-PDL. PPDL with number average molar mass of 86400 g mol^{-1} and greater than 90 % yield was synthesized in toluene medium[1,3–5,7]. Also, there exists many other studies that utilize Novozyme 435 for ROP of ω-PDL, but there is no example for an alternative immobilized enzymatic catalyst in literature[6,9,10].

In the present work, ROP of ω-PDL was proceeded via catalysis of *Candida antarctica* lipase B (CALB) immobilized onto rice husk ashes by physical adsorption, that has been obtained previously in our laboratory[15]. Performance of this immobilized lipase was explored to be a more economical alternative catalyst for CALB immobilized onto porous acrylic resin which is known as Novozyme 435. Consequently, the main objective of this study was production of an alternative biopolyester to LDPE by using a new enzymatic catalyst.

MATERIALS AND METHODS

Materials

The monomer ω-pentadecalactone (e≥98%) was obtained from Sigma Aldrich and used as received. Toluene and deuterated chloroform were purchased from Merck. Chloroform and methanol were acquired from Sigma Aldrich. The free form of *Candida antarctica* lipase B (CALB) was purchased from Sigma Aldrich and used as immobilized form (RHA-Im-CALB)[15]. Also, Novozyme 435 was obtained from Sigma Aldrich.

Enzyme Immobilization

CALB was immobilized onto renewable and low-cost silica-based carrier, rice husk ash (RHA). RHA was produced by burning of rice husks which were obtained from a rice producing factory in Turkey. Before immobilization, surface of RHA was modified by using 3-aminopropyltriethoxysilane (3-APTES) and $-NH_2$ groups were introduced to the surface. After that, free CALB (570 mg mL^{-1}) was immobilized onto surface-modified carrier via physical adsorption as a result of weak interactions of functional groups of the carrier and enzyme. Process details and results were given in previous article[15].

Enzymatic Ring Opening Polymerization of ω-pentadecalactone

Polymerization reactions were performed under dry nitrogen in 25-ml 3 neck flasks. Reactions were proceeded at various temperatures (60°C, 80°C, and 90°C) and time periods (0.5 h – 24 h) in 1 g of toluene with a stirring rate of 120 rpm. Calculated amount of immobilized form of CALB (RHA-Im-CALB) and ω-pentadecalactone monomer were introduced to the flask with 20% enzyme concentration (weight ratio of enzyme to monomer). Monomer to toluene ratio was arranged to be 1:2 (w:w). Reactions were terminated by the addition of excess chloroform and after filtration of enzyme from the polymerization medium, chloroform in the filtrate was evaporated in oven at 50°C. Then, the polymer was precipitated in cold methanol and filtrated for purification. Finally, the product was dried in oven at 30°C overnight. Polymer precipitation was not applied to proton nuclear magnetic resonance (^{1}H-NMR) analysis samples.

Instrumental Methods

Proton nuclear magnetic resonance (^{1}H-NMR) analysis was applied on Agilent VNMRS 500 MHz spectrometer at 25°C for the determination of molar mass and monomer conversions. ^{1}H-NMR spectra were recorded in deuterated chloroform ($CDCl_3$) with respect to tetramethylsilane (TMS) standard. Molar mass of polymers were calculated based on the degree of polymerization (DP_n) determined from integral ratios of characteristic peaks of polymer chains ($I_{4.06}$) and chain-ends ($I_{3.65}$) (Equations 1 and 2)[16].

$$DP_n = \frac{I_{4.06} + I_{3.65}}{I_{3.65}} \quad (1)$$

$$M_n = (DP_n) \times 240.38\ g\ mol^{-1} + 241.38\ g\ mol^{-1} \quad (2)$$

Monomer conversion percentages were determined based on the integral ratios of polymer ($I_{4.06}$) and monomer ($I_{4.16}$) characteristic peaks (Equation 3)[16,17].

$$\text{Monomer conversion } (\%)\ \frac{I_{4.06}}{I_{4.06} + I_{4.16}} \times 100 \quad (3)$$

Fourier transform infrared spectroscopy (FTIR) analysis was applied on a Perkin Elmer spectrophotometer in order to define the chemical structure of the polymer samples. Each sample was analyzed by KBr pellet. The spectra were recorded by at least 32 scans with a resolution of 2 cm^{-1}.

Thermal properties were determined by differential scanning calorimetry (DSC) using a Perkin Elmer calorimeter. Under inert nitrogen atmosphere at a 20 ml min^{-1} flow rate 5-10 mg samples were analyzed. Sample scans were carried out between -70 and 200°C at 10°C min^{-1} with heat-cool-heat thermal cycles and melting temperature (T_m), crystallization temperature (T_c), and glass transition temperature (T_g) were measured. Crystallinity percentages were calculated from the ratio of fusion enthalpy (ΔH_f) of the sample to the fusion enthalpy of 100% crystalline polymer ($\Delta H°_f$) (Equation 4)[5].

$$\chi_c = \frac{\Delta H_f}{\Delta H°_f} \times 100 \quad (4)$$

where $\Delta H°_{f\ PPDL} = 233\ J\ g^{-1}$[17].

Thermal gravimetric analysis (TGA) was applied on a Perkin Elmer apparatus for thermal characterization of the samples. The samples (5-10 mg) were heated from 25 to 550°C at a heating rate of 10°C min^{-1} under nitrogen flow.

RESULTS AND DISCUSSION

Enzymatic Ring Opening Polymerization of ω-pentadecalactone and Spectroscopic Characterizations

Previously, ring opening polymerization of ε-CL in toluene medium was performed via RHA-Im-CALB catalysis and PCL was obtained with a number-average molar mass (M_n) of 14000 g mol^{-1}[15]. In this study, RHA-Im-CALB was utilized for synthesis of PPDL.

Figure 1 shows the polymerization results at various reaction temperatures (60, 80, and 90°C). Reactions were proceeded at time periods between 30 minutes and 24 hours. Figure 1 represents both monomer conversions and molar masses which were calculated from ^{1}H-NMR spectra by using the Equations 1-3.

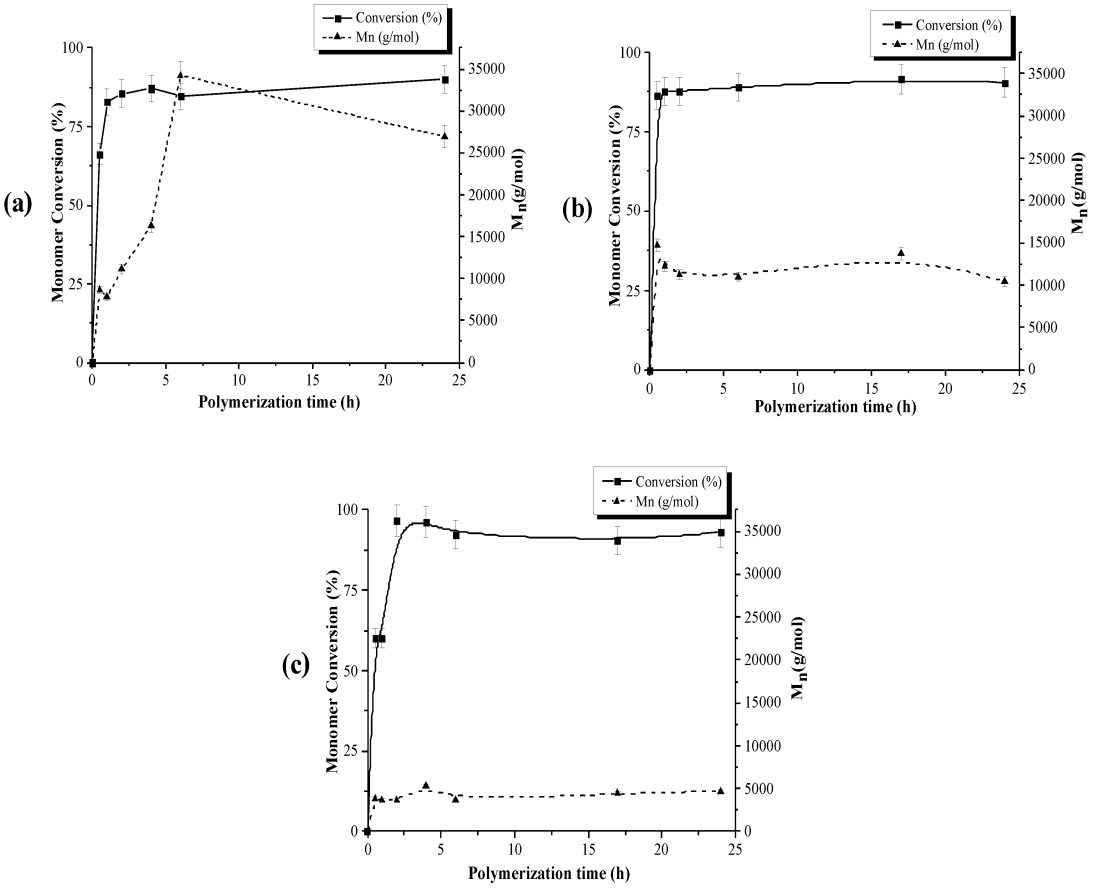

Figure 1. PPDL polymerization results at various reaction temperatures: **(a)** 60°C, **(b)** 80°C, **(c)** 90°C.

As seen from Figure 1, ω-PDL polymerized very rapidly; highest monomer conversions were reached at the end of 1 h (~88%), 4 h (87%), and 2 h (96.6%) reaction periods at 60°C, 80°C, and 90°C, respectively. It has already been known that, lactones with larger rings react faster than the ones with smaller rings, which is a result of higher activity of lipase for macrolactone polymerization[1,4,5,18].

During polymerization, viscosity of the reaction media increases proportional to the molecular weight reached. High viscosity of reaction media may hinder polymerization to go further which may result in lower monomer conversions. In the present study, highest molar mass (M_n= 34255 g mol^{-1}) was obtained at 80°C at the end of 6 hours (Figure 1(b)). At 90°C, ring opening polymerization of ω-PDL resulted in smaller chains of PPDL with low molar masses. This may be a result of decreased enzymatic activity above 80°C. On the other hand, monomer conversion was higher at 90°C (96.6%) compared to other temperatures as a result of low viscosity of reaction media.

In order to compare the performance of home-made immobilized lipase with a commercially available immobilized lipase, polymerizations via same amount of Novozyme 435 were carried out at optimum reaction periods of 60°C, 80°C, and 90°C temperature series in toluene medium, under same inert nitrogen atmosphere. Results were shown in Table 1.

TABLE 1. Comparison of polymerization results obtained via RHA-Im-CALB and Novozyme 435 catalysis.

Reaction Codes	Reaction Conditions	RHA-Im-CALB		Novozyme 435	
		Monomer Conv. (%)[a]	M_n (g mol^{-1})[a]	Monomer Conv. (%)[a]	M_n (g mol^{-1})[a]
PPDL-A	60°C - 17h	91.5	13693	91.8	20270
PPDL-B	80°C - 6h	84.7	34255	98.4	10309
PPDL-C	90°C - 4h	96.2	5276	98.5	12716

[a]Calculated from ^{1}H-NMR spectra.

As seen from Table 1, monomer conversions and molar masses were higher than RHA-Im-CALB catalysis, except PPDL-B reaction conditions. RHA-Im-CALB accomplished better performance than Novozyme 435 at these conditions. This may be resulted from altered stability performances after immobilization of CALB onto different support materials.

Figure 2 represents the ^{1}H-NMR spectrum of PPDL synthesized using RHA-Im-CALB at PPDL-B reaction conditions. Characteristic peaks were assigned to the related protons of ω-PDL and PPDL according to literature as follows: δ 4.06 (OC***H***$_2$), δ 3.65 (HOC***H***$_2$), δ 2.29 (COC***H***$_2$), 1.62 and 1.26 (all other protons) ppm [5,7,19,20]. Additionally, the peaks between δ 4.12-4.16 ppm were related with ω-PDL monomer (d'')[21].

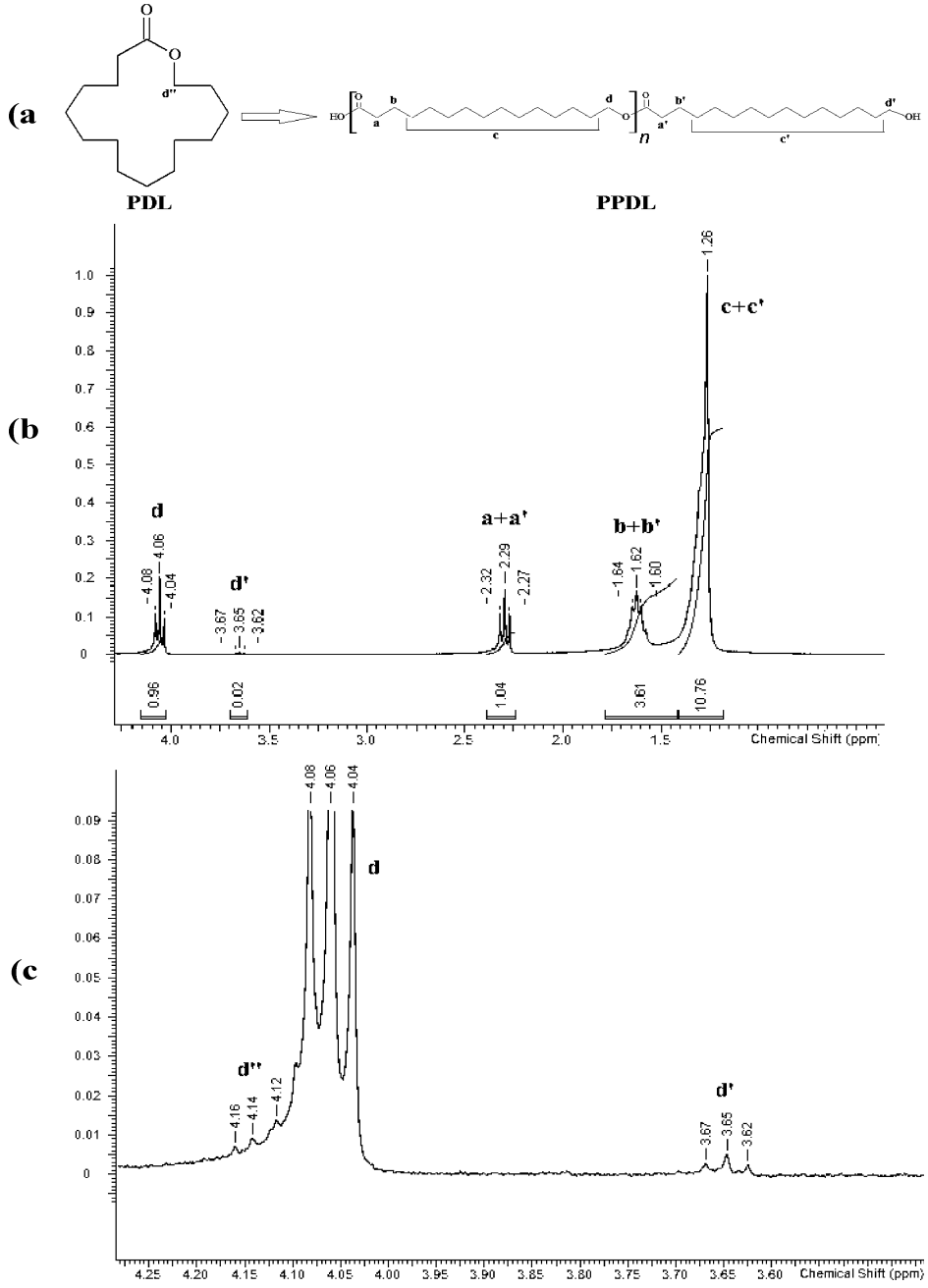

Figure 2. ^{1}H-NMR spectrum of PPDL synthesized using RHA-Im-CALB at PPDL-B reaction conditions: **(a)** ROP of ω-PDL, **(b)** Whole spectrum, **(c)** Zoomed spectrum (4.25-3.60 ppm).

FTIR spectrum (Figure 3) proved that, as a result of higher amount of methylene groups than other groups in the polymer structure, PPDL sample showed intense absorption bands at 2930-2916 cm^{-1} and 2865-2848 cm^{-1} which correspond to the asymmetric and symmetric $–CH_2-$ stretching vibrations, respectively. Also, another intense band was appeared at 1722-1731 cm^{-1} which was related with the –C=O stretching vibrations of ester carbonyl group. The bands at 1471-1463 cm^{-1} were associated with bending vibrations of C-H in methylene group. Absorption bands appeared at 1174-1168 cm^{-1} correspond to C-O stretching vibrations of the ester group. Moreover, bands at 731-720 cm^{-1} were related with the bending vibrations of $–(CH_2)_n-$ for $n \geq 4$[5].

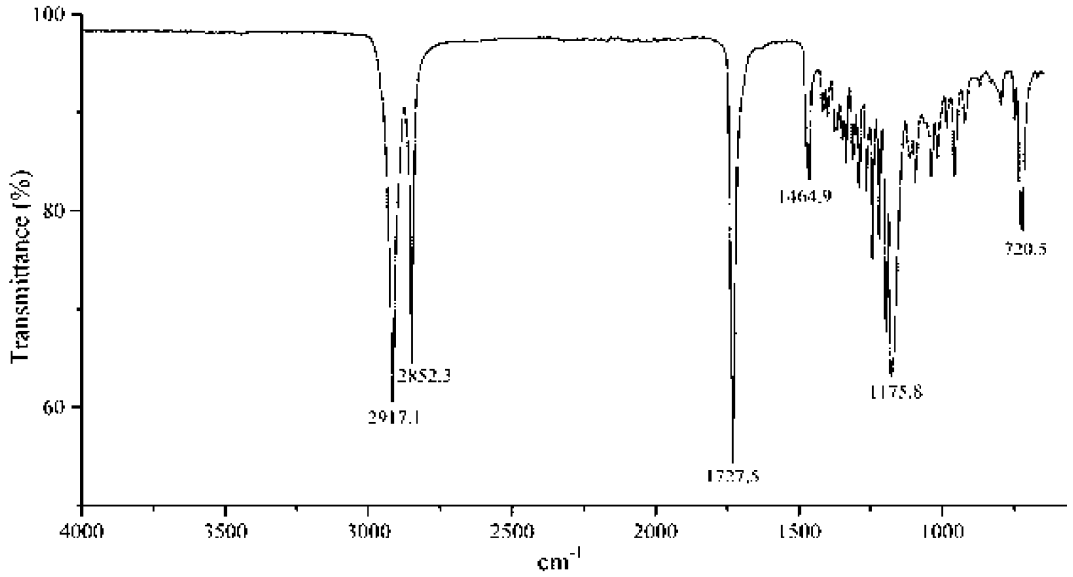

Figure 3: FTIR spectrum of PPDL synthesized using RHA-Im-CALB at PPDL-B reaction conditions.

Thermal Characterization of Poly(ω-pentadecalactone)

DSC and TGA were used to characterize thermal properties of PPDL synthesized via RHA-Im-CALB. DSC curves were shown in Figure 4. A large melting peak at 96.7°C with a melting enthalpy (ΔH_f)of 116.3 J g^{-1} was observed from the second heating scan of PPDL sample which was polymerized at PPDL-B reaction conditions. Additionally, PPDL exhibited an exothermic peak (during cooling) at 78°C (T_c) related with crystallization behavior. Both heating and cooling results were close to literature values[3,5,22]. Crystallinity percentage (χ_c) was calculated from Equation 4[5,7]. PPDL sample showed semi-crystalline structure with 49.9% crystallinity. Glass transition temperature (T_g)

of PPDL was measured as -29.1°C which was compatible with literature[2,3,7]. On the other hand, it is known from literature that, PCL has a very low T_g (around -60 °C), as well as lower T_m(58 °C) [17,22]. Compared with PCL, PPDL may have better ductility with its higher melting and glass transition temperatures.

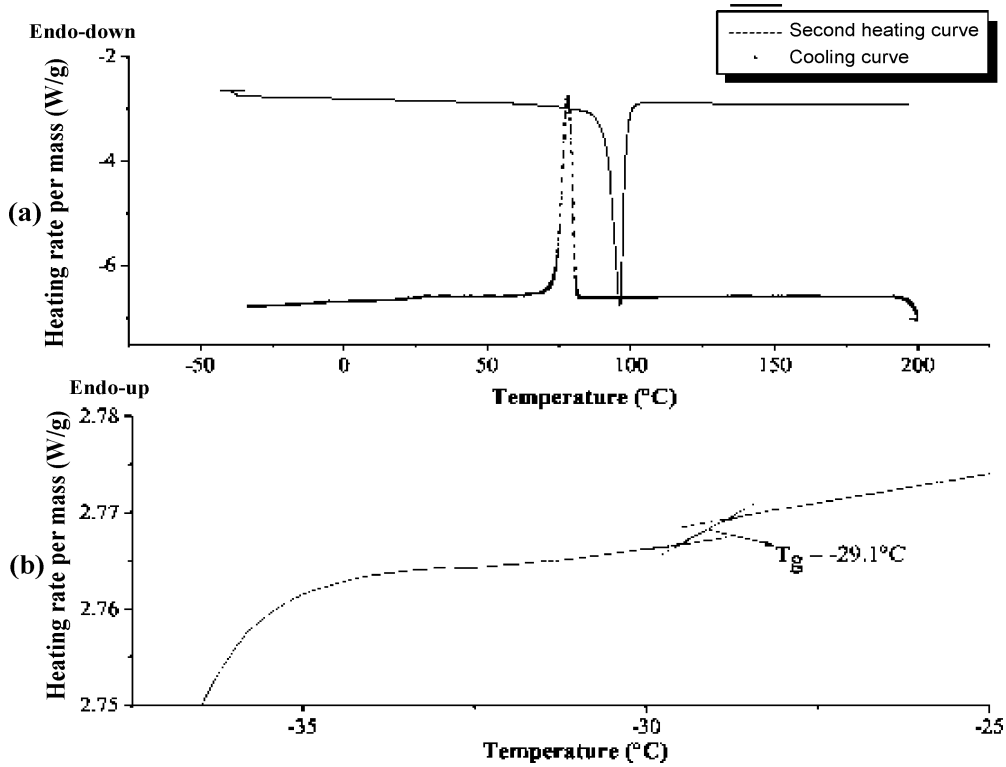

Figure 4. DSC thermograms of PPDL synthesized using RHA-Im-CALB at PPDL-B reaction conditions: (a) Endo-down, (b) Endo-up graphs.

TGA thermograms of PPDL were given in Figure 5. It seems that the weight loss was occurred with a single step. However, the first derivative plot (Figure 5(b)) exhibited a large degradation peak at 428.2°C with a small shoulder at about 475°C. These degradation temperatures were compatible with previous studies[5]. Degradation temperatures of PPDL were higher than PCL when compared with its literature values. PPDL would be a good alternative to PCL, if there is a necessity of using a thermally resistant polymer depending on the application area.

CONCLUSION

In conclusion, poly(ω-pentadecalactone) was successfully synthesized via ring opening polymerization catalyzed by *Candida*

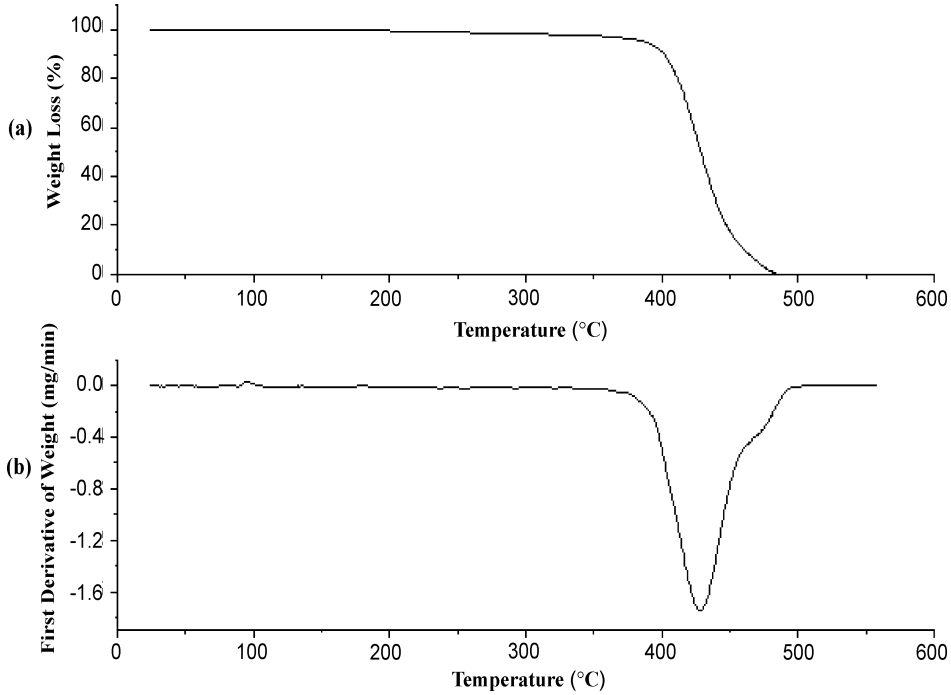

Figure 5. TGA thermograms of PPDL synthesized using RHA-Im-CALB at PPDL-B reaction conditions: **(a)** Weight Loss, **(b)** First derivative of weight graphs.

antarctica lipase B immobilized onto rice husk ashes (RHA-Im-CALB) which is a new immobilized form of CALB and showed a comparable performance with the commercially available immobilized CALB (Novozyme 435). Moreover, rice husk ash is a low-cost, abundant, and renewable enzyme carrier which makes RHA-Im-CALB advantageous over Novozyme 435 that uses acrylic porous resin as immobilization material.

In the present study, effect of temperature and reaction period on molar mass and monomer conversion were investigated. PPDL with highest molar mass was synthesized at 80°C and 6 h (M_n = 34255 g mol^{-1}). This was even higher than the molar mass of polymer sample synthesized via Novozyme 435 catalysis at same conditions (M_n= 10309 g mol^{-1}). The sample with highest molar mass was spectroscopically characterized via both ^{1}H-NMR and FTIR. Characteristic peaks were assigned according to the literature. Also, thermal properties of the same polymer sample were examined via DSC and TGA. The thermal properties of enzymatically synthesized PPDL were close to LDPE and better than PCL. Therefore, PPDL synthesized via RHA-Im-CALB catalysis can be suggested as a good alternative biocompatible and biodegradable biomaterial with improved thermal properties that can be applied in biomedical applications such as drug delivery and tissue engineering.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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