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Effects of different reaction mediums on ring opening polymerization of poly(ϵ -caprolactone) by lipase

Erhan Ozsagioglu*, Banu Iyisan and Yuksel Avcibasi-Guvenilir

Department of Chemical Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, 34469, Maslak-Istanbul, Turkey.

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This work examined the effects of reaction conditions on ring opening polymerization of poly(ϵ -caprolactone) (PCL) by *Candida antarctica* lipase B (Novozym 435) as the catalyst. Different reaction mediums were obtained by changing of temperature, reaction time and solvent types simultaneously. The main purpose of this study was to observe molecular weight distribution, conversion rates, and thermal behaviors of PCL and to find the highest molecular weight in enzymatic reaction conditions. Three different reaction temperatures and solvent types were studied in this work. Reactions were carried out at 40, 60 and 80°C and n-hexane, toluene and diisopropyl ether were used as solvents. One more reaction series were done for toluene solution at 30°C. Moreover, experiments were undertaken at seven different time periods. During all the reactions, monomer, solvent, and enzyme concentrations were not changed. For 1 ml monomer/solvent solution (0.44 M monomer and 0.56 M solvent), 10 mg lipase were used in the experiments. All samples were analyzed by gel permeation chromatography (GPC) and hydrogen nuclear magnetic resonance ($^1\text{H-NMR}$) to obtain molecular weights, conversion rates, and chemical structures. Fourier transform infrared spectroscopy (FTIR) analysis was performed to determine chain structures of PCL samples. Thermal behaviors of samples were analyzed by differential scanning calorimetry (DSC) to examine and compare polymers with each other.

Key words: *Candida antarctica* lipase, enzymatic ring-opening polymerization, polycaprolactone, biopolymers.

INTRODUCTION

Recently, enzymatic polymerization has provided a good alternative for conventional methods due to its mild reaction conditions, its ability to obtain high conversion rates, and its quality of being non-toxic. Among the catalysts, zinc and tin complexes have been widely used as catalysts for the ring-opening polymerization of poly(ϵ -caprolactone) (PCL), but toxicity has been a problem for these activators (Sarazin et al., 2004; Dobrzynski, 2007; Kowalski et al., 2000; Schenck et al., 2002). Moreover,

alcohol based initiators have been used with these metallic catalysts; however, they are not essential for enzymes. The development of lipase catalyzed ring opening polymerization in organic solvents has an important consideration (Marcilla et al., 2006; Kumar et al., 2000). Especially, synthesis of PCL has taken great interest as a result of various kinds of applications of PCL in biomedical area. In these reactions, immobilized *Candida antarctica* lipase (Novozym 435), usually, would be used rather than other kind of lipase enzymes, because it provides higher conversions with different reaction mediums. On the other hand, temperature has an important effect on conversion rates and molecular mass of PCL in reaction medium.

Although, PCL is a synthetic polymer, it is biodegradable and biocompatible. The widespread usage of PCL began with the understanding of its biodegradability. Organic reaction media provides a high

*Corresponding author. E-mail: ozsagioglu@itu.edu.tr

Abbreviations: GPC, Gel permeation chromatography; $^1\text{H-NMR}$, hydrogen nuclear magnetic resonance; FTIR, Fourier transform infrared spectroscopy; PCL, poly(ϵ -caprolactone); DSC, differential scanning calorimetry; M_n , molecular weight PDI Polydispersity Index.

molecular weight and conversion rates for PCL (Peng et al., 2010; Tarvainen et al., 2003; Pastorino et al., 2004).

In this study, we first reported the effects of different reaction media obtained with change in temperature, time and solvent types at the same time. The main purpose of this work was to observe the molecular weight distribution due to various reaction times and to find the conversion rates and the highest molecular weight in enzymatic reaction conditions with distinct mediums. No literature exists on the effect of temperature, reaction time and solvent at the same time on the molecular weight of PCL has been researched. Moreover, to the best of our knowledge, low temperatures such as 40 and 30°C have never been investigated with change in reaction times in different organic solvents. Besides, comparing previous studies of enzymatic synthesis of PCL, the highest molecular weight (M_n) is obtained in this work. Higher M_n enhances mechanical strength and increases degradation times of polymer samples.

All samples were analyzed by gel permeation chromatography (GPC), hydrogen nuclear magnetic resonance ($^1\text{H-NMR}$), Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC).

MATERIALS AND METHODS

C. antarctica lipase B, Novozym 435, (7000 PLU/g), an immobilized enzyme, was purchased from Sigma. ϵ -caprolactone (99%) was provided from Alfa Aesar and stored under dry nitrogen over molecular sieves (3A $^\circ$) to dry over 24 h before use. Organic solvents for reactions were n-hexane (Sigma Aldrich), toluene (Merck) and diisopropyl ether (Merck). Chloroform (Sigma Aldrich) was used to terminate reactions and methanol (Sigma Aldrich) was used to precipitate polymer from reaction medium. Organic solvents for reactions and other chemicals were used without any further purification.

Lipase catalyzed ring opening polymerization of ϵ -caprolactone

All reactions were carried out at the same concentrations for monomer/solvent ratios and enzyme mixture which was for 1 ml of monomer/solvent (0.44 M ϵ -caprolactone and 0.56 M organic solvent) contained 10 mg of lipase. Reaction temperatures were 40, 60, and 80°C and reaction times were 2, 4, 6, 17, 24, 48, and 72 h, respectively. Moreover, one more experimental series were done for toluene solution at 30°C to observe the tendency of the molecular weight distribution at lower temperature. At the end of each reaction time, reaction solution was taken and was terminated by adding an excess of chloroform, and then, the enzymes were removed by filtration. The greatest part of chloroform in the filtrate was removed by evaporation and the polymer in the concentrated solution was precipitated in methanol. Then, the samples were dried at 35°C in an oven.

Characterizations of PCL samples

All samples were analyzed by GPC to obtain molecular weight distribution for samples versus reaction time. GPC was calibrated by polystyrene standards. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1 ml/min at 25°C.

$^1\text{H-NMR}$ spectra were recorded on a Bruker Ultrashield 300 MHz spectrometer using CDCl_3 as solvent and tetramethylsilane as an internal standard. Chemical structure, conversion rates and average number of molecular mass (M_n , g/mol) were determined by $^1\text{H-NMR}$. M_n values were estimated by following formula (Sha et al., 2005):

$$M_{n,\text{NMR}} = \left[\frac{5 \times I_{4.07}}{2 \times I_{3.65}} \right] \times M_{\epsilon\text{-CL}} \quad (1)$$

DSC was used to analyze the change in the thermal transition temperatures of the polymer samples. The device model of DSC is Perkin Elmer Diamond, and all analyses were done from -70 to 220°C as 10°C min $^{-1}$ increasing rate. DSC provides glass transition temperature (T_g , °C) and melting temperature (T_m , °C) for PCL samples. Moreover, the crystallinity values for polymer samples were calculated using the following formula (Harrison et al., 2004).

$$X_c = \left[\frac{\Delta H_m}{\Delta H_m^\circ} \right] \times 100 \quad (2)$$

ΔH_m° value is 139.3 J/g for melting enthalpy of 100% crystalline PCL sample (Peng et al., 2010).

Fourier transform infrared spectroscopy with attenuated total reflectance analysis (FTIR-ATR) was used to define chain structure of polymer samples. FTIR spectra were recorded on a Perkin Elmer Spectrum One series spectrometer between 4000 and 650 cm^{-1} .

RESULTS

Enzymatic polycondensation in diisopropyl ether solvent

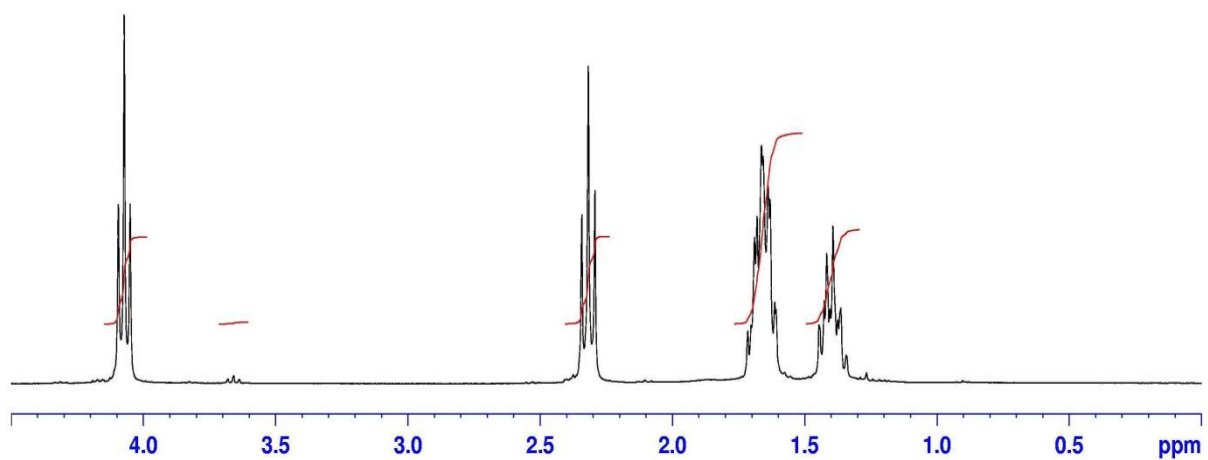
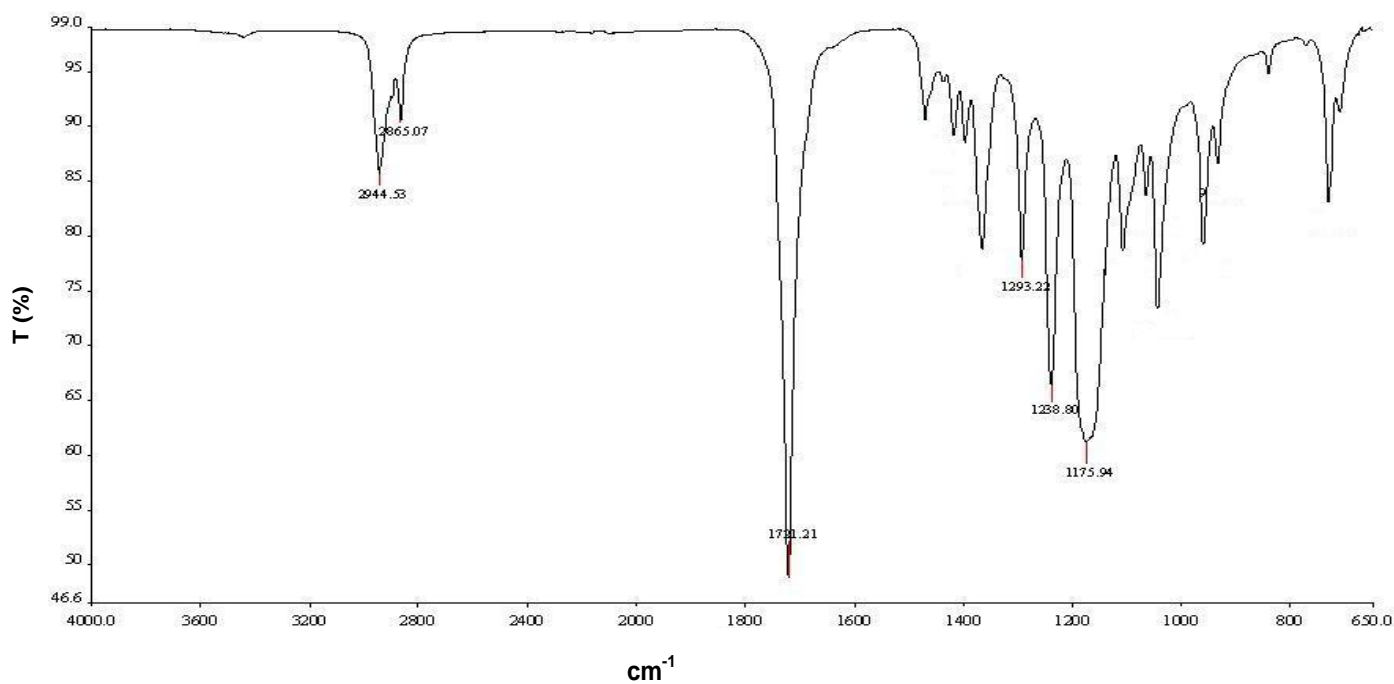
Polycondensation reaction in diisopropyl ether solution reached a maximum point before exhibiting a decrease in molecular mass values for all reaction temperatures. Molecular weight distributions according to reaction time in diisopropyl ether solvent for all temperatures are shown in Table 1.

Maximum molecular mass values were achieved in 24 h at 80°C, in 17 h at 60 and 40°C. The highest M_n (g/mol) and M_w (g/mol) values were obtained at 60°C in 17 h for this experimental series, so, $^1\text{H-NMR}$, FTIR, and DSC analyses were done for the mentioned sample. According to NMR analysis as shown in Figure 1, structural groups in polymer are (δ , ppm): 4.07 (t, CH_2O), 3.65 (t, CH_2OH , end group), 2.32 (t, CH_2CO), 1.6 to 1.7 (m, CH_2); 1.37 (m, CH_2). NMR spectra showed that this sample was a linear homopolymer (Cordova et al., 1998). Moreover, according to Formula 1, M_n values and conversion rate of this sample were estimated to be 13,412 g/mol and 97.9%, respectively. Also, FTIR analysis is as shown in Figure 2 and this spectrum determined the structural chains of PCL; the result of NMR spectra was approved by FTIR.

According to DSC analysis (Figure 3), T_g , T_m and melting enthalpy values were -53.8°C, 68°C and 95.0703

Table 1. Ring opening polymerization of ϵ -caprolactone in diisopropyl ether at 80, 60 and 40°C, respectively.

Temperature	80°C			60°C			40°C		
Reaction time (h)	M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI
72	10,270	14,779	1.4390	9,241	15,487	1.6759	8,217	13,727	1.6706
48	10,616	17,420	1.6410	9,380	14,490	1.5449	9,022	14,140	1.5672
24	12,591	19,854	1.5769	9,474	15,934	1.6819	9,180	13,185	1.4362
17	11,497	19,471	1.6936	12,310	20,150	1.6369	10,687	17,984	1.6829
6	11,370	16,405	1.4428	12,213	18,168	1.4876	10,082	15,145	1.5022
4	11,364	18,898	1.6630	12,149	18,914	1.5569	7,356	12,901	1.7540
2	10,436	17,785	1.7043	10,606	15,748	1.4849	7,028	10,931	1.5553

**Figure 1.** $^1\text{H-NMR}$ spectrum at 60°C in 17 h for diisopropyl ether solvent.**Figure 2.** FTIR spectrum at 60°C in 17 h for diisopropyl ether solvent.

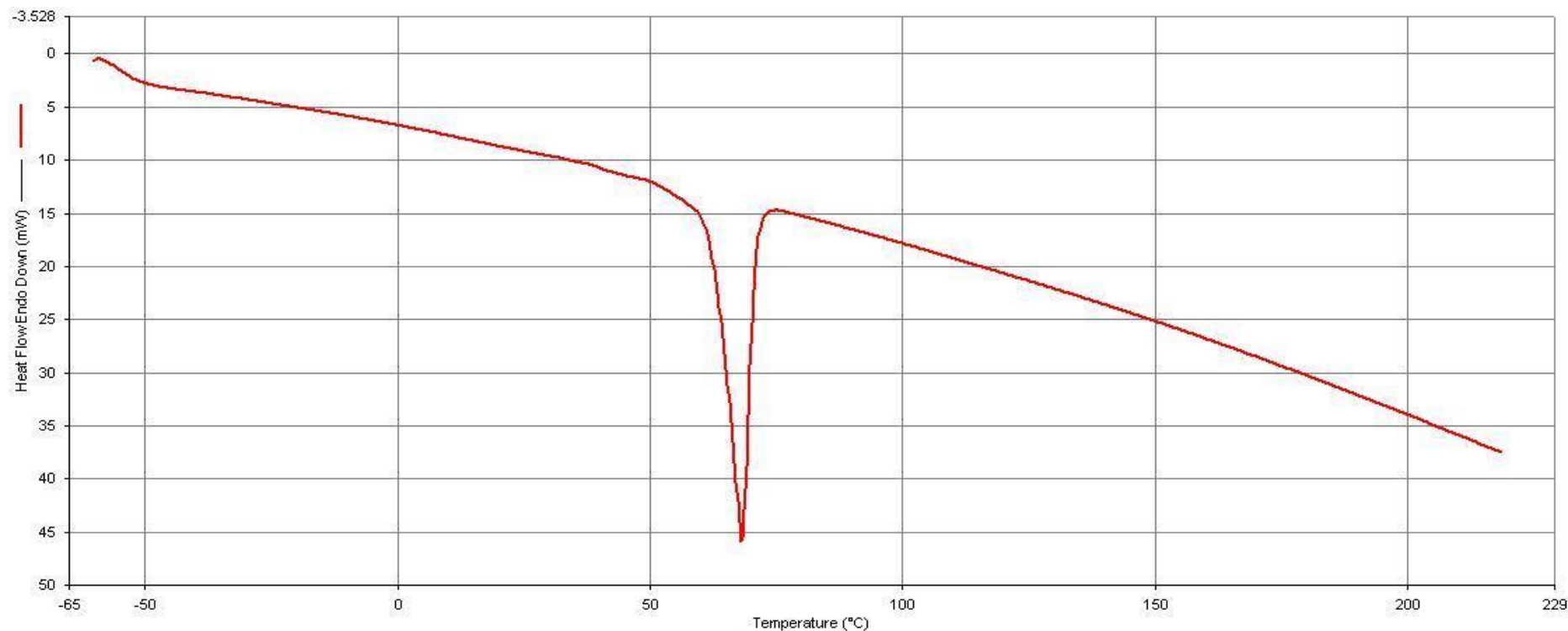


Figure 3. DSC analysis at 60°C in 17 h for diisopropyl ether solvent.

J/g, respectively and crystallinity value was estimated to be 68.3% by Formula 2.

Enzymatic polycondensation in n-hexane solvent

Polymerization reaction was carried out in n-hexane solvent and maximum molecular mass values were reached in lower times for higher reaction temperatures such as 6 h for 60 and 80°C. In Table 2, the results from polycondensation reactions of ϵ -caprolactone

performed in n-hexane were presented. M_n and M_w values were increased continuously from 2 to 72 h at 40°C. Thus, another polymerization reaction was performed in 168 h to obtain molecular mass distribution clearly. According to GPC results, M_n and M_w increased and their values were 10,449 and 17,312 g/mol, respectively.

The highest M_n and M_w were obtained at 80°C in 6 h for n-hexane solvent and this sample was characterized by $^1\text{H-NMR}$, FTIR, and DSC. $^1\text{H-NMR}$ analysis is as shown in Figure 4; signals are (δ , ppm): 4.07 (t, CH_2O), 3.65 (t, CH_2OH , end

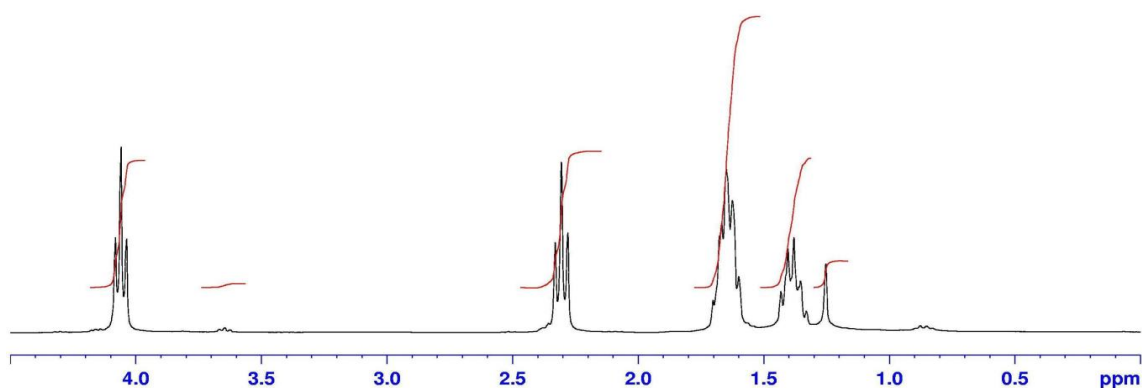
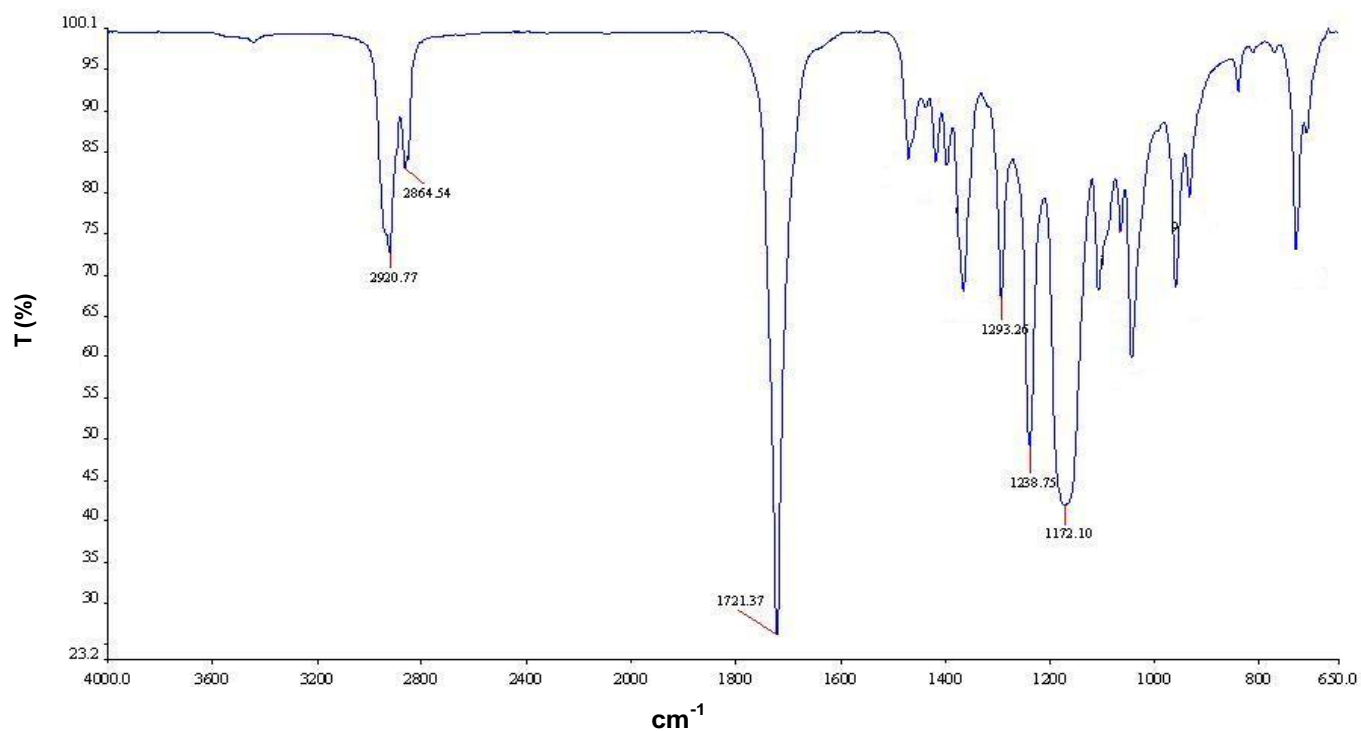
group), 2.32 (t, CH_2CO), 1.6 to 1.7 (m, CH_2); 1.37 (m, CH_2). The signal obtained from NMR determined that polymer structure was a linear homopolymer PCL. M_n was estimated to be 9,512 g/mol by Formula 1 and conversion rates of polymerization reaction were 97.0%.

Furthermore, characterization of polymer chains was determined by FTIR analysis (Figure 5) and results were found same as NMR spectra. DSC analysis is as shown in Figure 6, and T_g and T_m were -57.3 and 65.3°C, respectively; besides, melting enthalpy was 81.6244 J/g, so crystallinity was estimated to be 58.6% by Formula 2.

Table 2. Ring opening polymerization of ϵ -caprolactone in n-hexane at 80, 60 and 40°C, respectively.

Temperature	80°C			60°C			40°C		
Reaction time (h)	M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI
72	9,675	18,051	1.8656	7.236	12.059	1,6665	8.207	13.382	1,6306
48	10,286	17,741	1.7247	7.538	10.949	1,4526	7.326	11.557	1,5775
24	11,335	17,180	1.5156	7.593	10.967	1,4445	7.247	11.613	1,6024
17	12,129	19,723	1.6261	8.064	10.983	1,3619	7.070	11.546	1,6331
6	12,739	21,706	1.7040	10.107	14.605	1,4451	6.690	13.540	2,0240
4	9,504	17,323	1.8227	10.099	15.429	1,5277	6.670	11.855	1,7773
2	8,115	14,244	1.7552	5.545	9.799	1,7670	6.579	11.257	1,7112

* PDI: Polydispersity Index.

**Figure 4.** $^1\text{H-NMR}$ spectrum at 80°C in 6 h for n-hexane solvent.**Figure 5.** FTIR spectrum at 80°C in 6 h for n-hexane solvent.

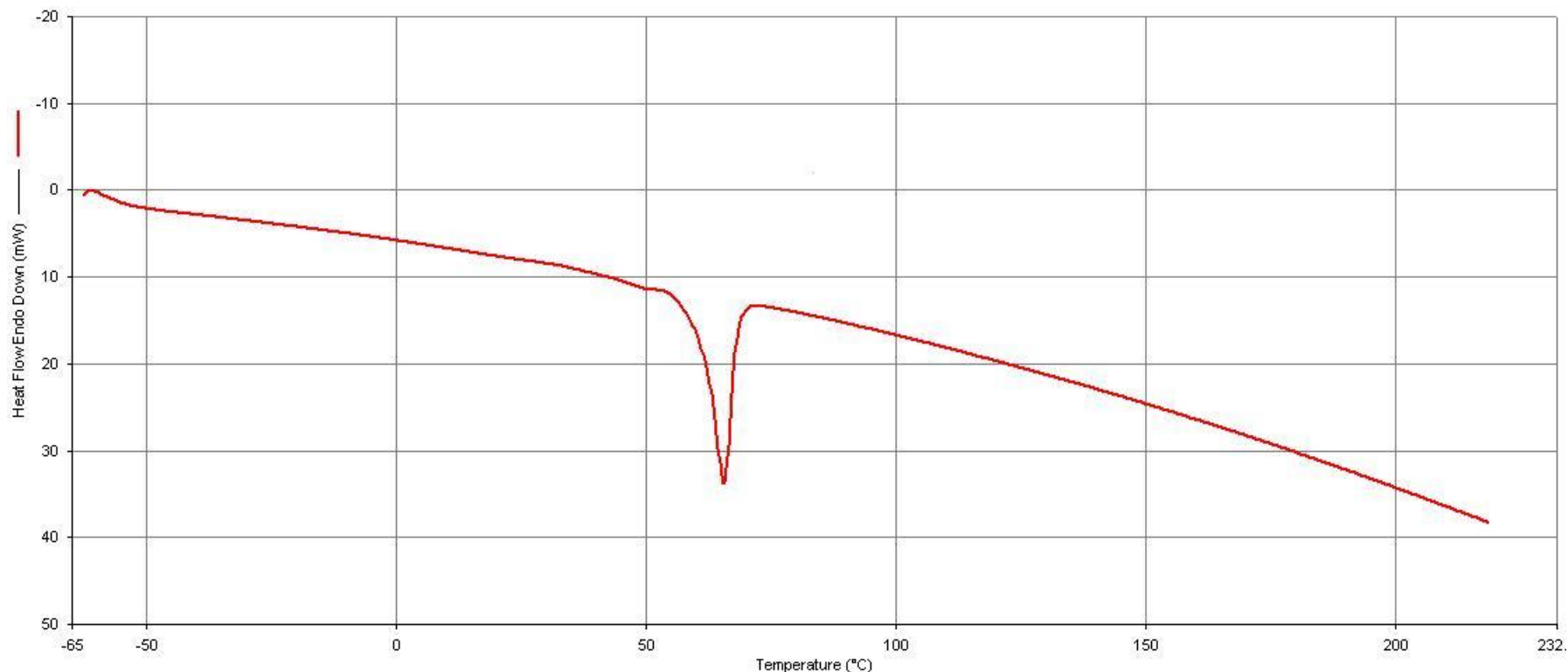


Figure 6. DSC analysis at 80°C in 6 h for n-hexane solvent.

Enzymatic polycondensation in toluene solvent

Polycondensation reactions in toluene solvent had higher molecular mass values than other solvents at all reaction temperatures. At 80°C reaction temperature, 2 h was enough to reach maximum M_n values; on the other hand, 24 h at 60°C and 72 h at 40°C were the maximum points. GPC results for toluene solvent series are as shown in Table 3.

As shown in Table 3, molecular weight distribution at 40°C was increased continuously along 72 h, so one more extra polymerization

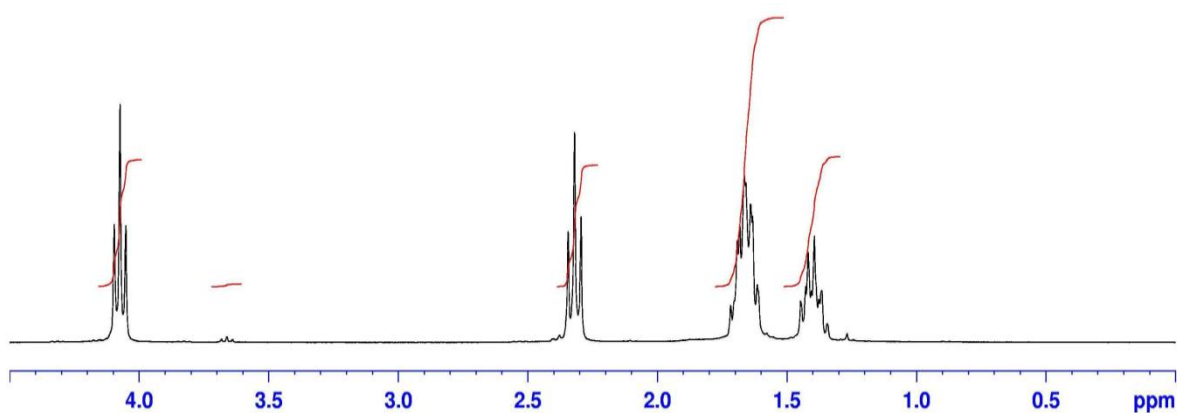
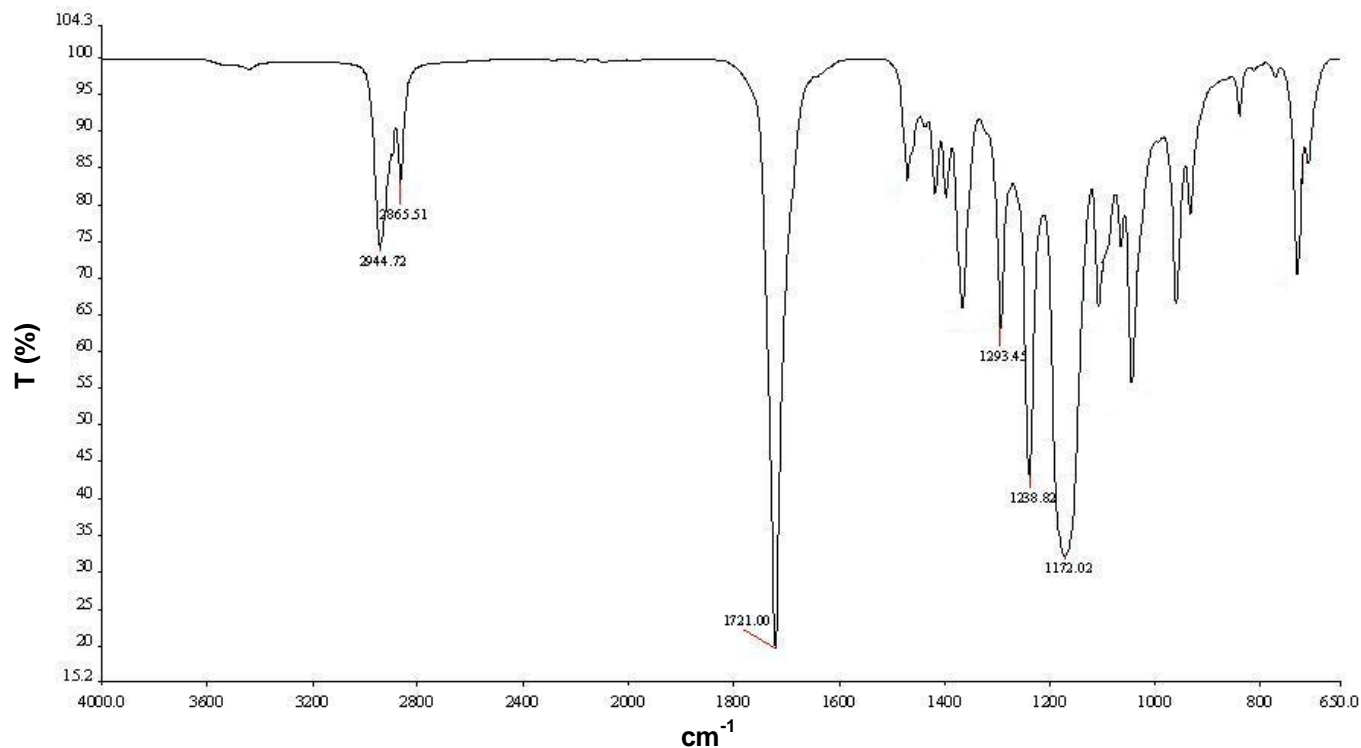
reaction was done in 168 h. However, M_n and M_w values of the experiment in 168 h decreased and GPC results of the mentioned sample were 10.650 g/mol for M_n and 16.498 g/mol for M_w . For all experiments, GPC results showed that the highest M_n and M_w values were reached in toluene solvent at 40°C in 72 h. To our knowledge, enzymatic polymerization of PCL at low temperature has not resulted in such a high molecular weight of PCL in the previous similar studies (Cordova et al., 1998; Kumar et al., 2000; Marcilla et al., 2006). Furthermore, $^1\text{H-NMR}$, FTIR, and DSC analysis were done for the sample

which had the highest M_n and M_w values. NMR spectra is as shown in Figure 7 and structural groups in sample are (δ , ppm): 4.07 (t, CH_2O), 3.65 (t, CH_2OH , end group), 2.32 (t, CH_2CO), 1.6 to 1.7 (m, CH_2); 1.37 (m, CH_2). As shown in Formula 1, M_n value was estimated to be 14,268 g/mol and conversion rate of this polymerization was 98.0%.

Besides, characterization of polymer chains was determined by FTIR (Figure 8) and spectra analysis. DSC analysis is as shown in Figure 9, and T_g and T_m values were -49.3 and 55.8°C, respectively; in addition, melting enthalpy was

Table 3. Ring opening polymerization of ϵ -caprolactone in toluene at 80, 60, and 40°C, respectively.

Temperature	80°C			60°C			40°C		
Reaction time (h)	M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI
72	11,624	19,447	1.6731	12,217	18,281	1.4964	18,114	27,824	1.5361
48	12,033	19,519	1.6220	12,691	19,660	1.5492	13,224	20,864	1.5778
24	12,107	21,433	1.7703	14,236	23,970	1.6838	12,881	21,091	1.6374
17	12,585	20,914	1.6619	13,418	21,606	1.6103	12,500	22,670	1.8136
6	13,399	22,066	1.6469	13,074	22,171	1.6958	12,641	20,993	1.6608
4	13,477	23,513	1.7447	12,803	20,839	1.6277	12,343	21,764	1.7633
2	14,057	21,736	1.5463	8,470	13,034	1.5388	11,219	18,221	1.6241

**Figure 7.** $^1\text{H-NMR}$ spectrum at 40°C in 72 h for toluene solvent.**Figure 8.** FTIR spectrum at 40 °C in 72 h for toluene solvent.

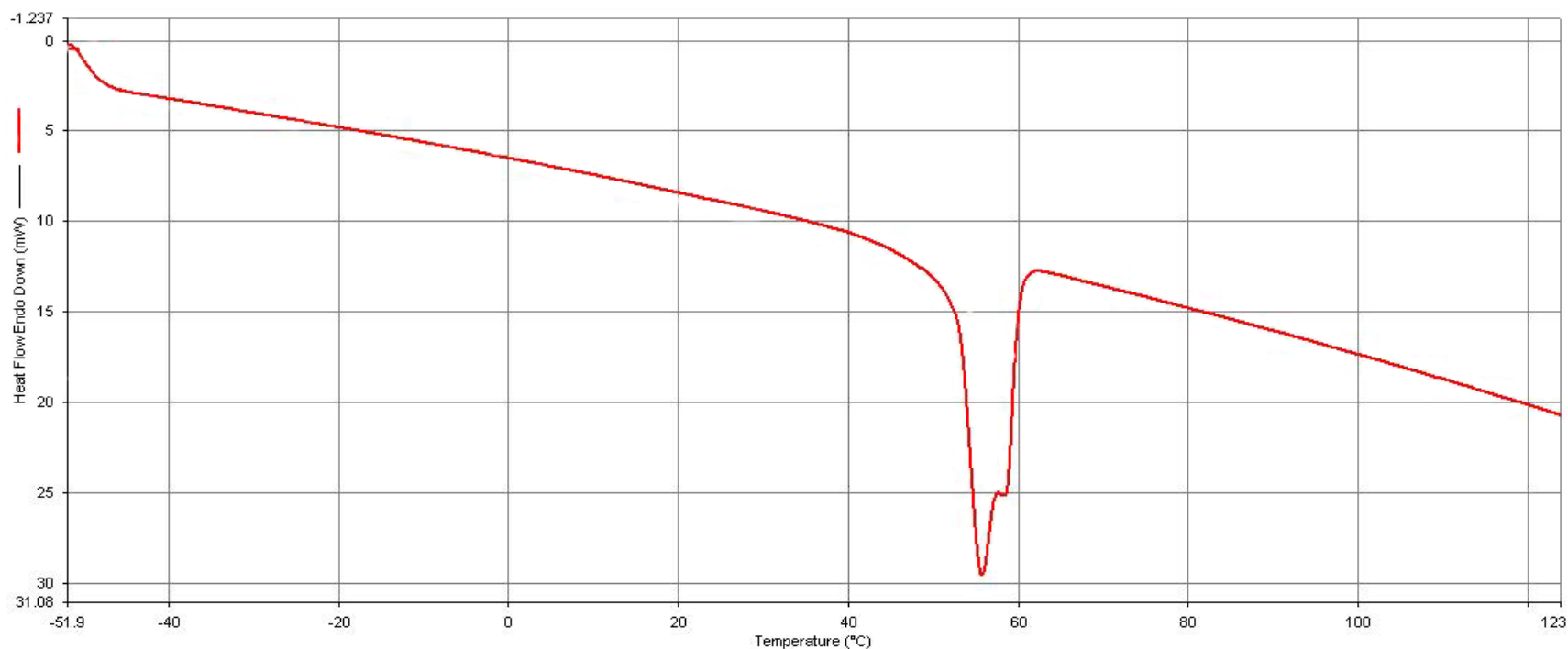


Figure 9. DSC analysis at 40°C in 72 h for toluene solvent.

Table 4. Ring opening polymerization of ϵ -caprolactone in toluene at 30°C.

Reaction time (h)	M_n	M_w	PDI
72	7,388	11,561	1.5648
48	7,530	12,973	1.7229
24	6,549	11,120	1.6981
17	5,575	8,845	1.5865
6	9,025	14,462	1.6024
4	6,647	10,512	1.5816
2	6,799	10,962	1.6124

68.61 J/g, so crystallinity was estimated to be 49.25% by Formula 2.

M_n and M_w values were increased by the descending order of temperature and higher molecular mass values were obtained at 40°C, so more experiments at 30°C were performed to determine the tendency of polymerization reaction. GPC results are as shown in Table 4.

As shown in Table 4, M_n and M_w values were very lower than GPC results of experimental series at 40°C. Moreover, the highest M_n and M_w at 30°C were found as 9.025 and 14.462 g/mol, respectively; these values were lower than the highest M_n and M_w of 40°C. Thus, 40°C is more convenient for polycondensation reaction in

toluene solvent and it shows great effect of temperature on molecular mass values.

DISCUSSION

Molecular mass values in toluene solvent were higher than diisopropyl ether and n-hexane, so ring opening polymerization of ϵ -caprolactone by *C. antarctica* lipase is more stable in toluene. M_n and M_w values in diisopropyl ether reaction medium are higher than those in n-hexane reaction medium because the solubility of diisopropyl ether in water is higher than in n-hexane. Thus, diisopropyl ether solvent removes

confirmed structural groups of PCL as NMR

water molecules easily in reaction medium, so, stability of polymerization reaction is better in diisopropyl ether than in n-hexane. Besides, the experiments with toluene at low temperature (40°C) achieve a very high conversion ratio like 98.0%. To our knowledge, this mentioned conversion ratio has not been reached in previous studies at low temperatures.

The main reason of obtaining higher molecular mass values in toluene is the higher hydrophobicity value of toluene. Higher M_n and M_w values and conversion rates were obtained by hydrophobic reaction medium, because hydrophobic solvents remove water molecules in reaction media. Hydrolysis of polymer chains was prevented, so higher molecular mass values could be reached (Li et al., 2011). Hydrophobicity is not only the main reason for more stable reaction conditions; also, apolarity is a reason for higher molecular mass values (Marcilla et al., 2006). Apolarity value of toluene is higher than n-hexane, thus, higher M_n and M_w values were obtained in toluene solvent.

The tendency of molecular weight distribution of polymer samples results from negative effect of reaction temperature. Besides, thermal degradation occurs on polymer chains (Kumar et al., 2000).

T_g values increase with a rise in reaction time and molecular mass values. Thus, according to DSC analyses, the highest T_g was obtained in toluene solvent at 40°C in 72 h. Moreover, the increase of molecular mass has adverse effect on T_m and crystallinity value of PCL, so, higher M_n and M_w mean lower T_m and crystallinity for polymer sample (Shieh and Yang, 2005). Lowest T_m and crystallinity values were obtained at 40°C in 72 h in this DSC analysis. Due to the increase of the molecular weight of PCL, the amorphous phase in the polymer rises and therefore, T_m decreases.

Enzymatic synthesis of PCL in hydrophobic solvents was carried out to understand more convenient reaction conditions. Higher molecular mass values and conversion rates could be obtained on ring opening polymerization of ϵ -caprolactone with *C. antarctica* lipase enzyme in hydrophobic solvents, since lipase enzyme is more stable and has higher reaction activity. Reactions in toluene were more stable at lower temperatures; however, higher temperatures were needed to obtain increase in the molecular mass and conversion rates in n-hexane solvent. Moreover, it was observed that short reaction time periods seemed enough to raise conversion rates at higher temperatures (97.0% conversion rates at 80°C in n-hexane in 6 h). Increasing molecular mass of PCL results in lower crystallinity, so higher amorphous phase facilitates to get into shape for polymer sample.

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