Effect of ionic liquids on the structural, thermal, and *in vitro* degradation properties of poly(ε-caprolactone) synthesized in the presence of *Candida antarctica* lipase B

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INTRODUCTION

Enzyme catalysis has provided an environmentally friendly, synthetic strategy for a variety of useful polymeric biomaterials, most of which would be difficult to produce using conventional chemical catalysts.[1–5] Much of the difficulty associated with completely removing the residual chemical (metal) catalyst from the final product leads to toxicity problems for biomedical applications. Moreover, enzyme catalysis shows high enantio- and chemoselectivity and can operate under milder conditions than while using typical, metal catalyst which is very important for biomedical applications.[6,7]

Poly(ε-caprolactone) (PCL), a semicrystalline linear resorbable aliphatic polyester, is a widely utilized aliphatic polyester for medical applications, such as polymeric-based drug delivery systems (DDS) or tissue engineering scaffolds due to its good biocompatibility and lack of toxicity. PCL has been successfully synthesized by polycondensation or by ring opening polymerization (ROP). [8,9] ROP gives polyesters with a higher purity and low polydispersity, so it is the preferred way in the controlled release technology in pharmacy.[10]

A number of research topics develop an enzymatic ROP of cyclic monomers in typical organic solvent (e.g., toluene and heptane). Because of the toxicity of their vapour, recent literature reports suggest the possibility of using thermally stable and nonflammable ionic liquids (ILs) as the reaction medium. ILs increasingly attract attention as non-aqueous media for biocatalytic polymer synthesis. ILs can improve the solubility of monomers and/or polymers, provide better enzyme stability, activity and selectivity.[11–15]

In this article, we focus on investigation of the structure and degradation properties of low-molecular weight polyesters synthesized in ILs with respect to their further applications as matrices for DDS (short- and/or middle-term). Polyesters were synthesized in different conditions in two 1-butyl-3-methylimidazolium cation based ILs with two different anions: bis(trifluoromethylsulfonyl)imide and hexafluorophosphate in the presence of *Candida antarctica* lipase B. The kinetics of active substance release from those systems depend not only on the molecular weights of the polymers. Polydispersity index, presence of end-functional groups in the polymer structure, low degree of crystallinity and high purity of the polymers are equally important factors in pharmaceutical fields. The studies on combinations of ILs anion's type and process conditions on the monomer conversion, reaction yield, structure of polymers and their degradation characteristic have been evaluated. To our knowledge, this is the first report in the literature on differences of morphology and degradation properties of biomedical PCLs synthesized in two different ILs in the presence of *Candida antarctica* lipase B.

EXPERIMENTAL

Materials

ε-Caprolactone (CL, 2-oxepanone, 97%, Aldrich, Poznan, Poland) was dried and distilled over CaH_2 at reduced pressure before use. Lipase B from *Candida* (*Pseudozyna*) *antarctica* (CALB) produced by submerged fermentation of a genetically modified *Aspergillus niger* microorganism and adsorbed on a macroporous resin (≥5000 U g^{-1}) were purchased from Sigma (Poznań, Poland). 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (≥ 98%, Aldrich, Poznan, Poland) and 1-butyl-3-methylimidazolium hexafluorophosphate (≥ 98%, Merck, Poland) were used as received. Chloroform and toluene (Avantor, Gliwice, Poland) were used as received. Phosphate buffered saline was purchased from Sigma (Poznan, Poland).

Synthesis of Poly(ε-caprolactone)

Before reaction, monomer (CL, 8.76 mmol), lipase (100 mg) and ILs (1.0 g) were dried under vacuum at room temperature for 2 h. After that, all of these reagents were placed in 10 mL glass ampoules under an argon atmosphere and the reactions were carried out at the required temperature for the appropriate time. After thermostating, the crude products were extracted by extraction with toluene and the enzyme was filtered off. Toluene was removed by evaporation at room temperature under reduced pressure. The final water-soluble monomer was washed away with the mixture of chloroform/distilled water and polymers were dried under vacuum for 4 days.

Spectroscopic Analysis of Polymeric Products

Hydrogen nuclear magnetic resonance (^{1}H NMR), carbon-13 nuclear magnetic resonance (^{13}C NMR), ^{1}H-^{1}H homonuclear correlation (COSY), ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) and ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) spectra of PCLs were obtained on Varian spectrometer with $CDCl_3$ as solvent. Tetramethylsilane was used as the internal standard. Monomer conversion was determined by ^{1}H NMR by the comparison of integrated signal areas of the methylene groups next to the carbonyl group in the CL monomer (4.23 ppm) and the polymer (4.07 ppm). In addition, the degree of polymerization was estimated from the ratio of integral of the signal at 4.07 ppm assigned to the CH_2O groups of PCL main chain to the integral of the signal at 3.66 ppm assigned to the chain-end.

The infrared Fourier transform (FT-IR) spectra were measured from KBr pellets (Perkin Elmer spectrometer, Perkin Elmer, Warsaw, Poland).

Size Exclusion Chromatography Multiangle Laser Light Scatter (SEC/MALLS)

SEC chromatography was carried out on the Agilent 1100 series chromatograph composed of isocratic pump, autosampler, degasser and thermostatic box for columns. Refractive Index (RI) (OPTILAB rex, Wyatt) and MALLS (DAWN EOS, Wyatt Technology Corporation, Santa Barbara, CA) were used as detectors. Two TSK-gel Multipore HXL columns were used for separation. The samples were injected as a solution in methylene chloride. The volume of the injection loop was 100 mL. Methylene chloride was used as a mobile phase at flow rate of 0.8 mL min^{-1}. The calibration of the DAWN EOS was carried out by pure analytical grade toluene and normalization with a polystyrene standard of 30000 g mol^{-1} molecular weights. The measurements were carried out at room temperature. M_n (SEC) values of PCL were calculated by using the correction coefficient; M_n (SEC) = M_n (SEC raw data) × 0.56.[16–18]

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry

The matrix assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) spectra were measured in the linear mode on a Kompact MALDI 4 Kratos analytical spectrometer using a nitrogen gas laser and 2-[(4-hydroxyphenyl)diazenyl] benzoic acid as a matrix.

Thermal Characterization of Ionic Liquids, Enzymes, and Polymers

Thermal properties of the ionic liquids and *Candida antarctica* lipase B were analyzed by thermogravimetric analysis (TGA) on TA Instruments Q50 (New Castle, DE USA). Samples (ca., 10 mg weight) were heated from room temperature to 500 °C at 10 °C min^{-1} under nitrogen flow with a purge rate of 60 mL min^{-1}.

Differential scanning calorimetry (DSC) of the obtained products was conducted on a TA Instruments DSC Q20 (New Castle, DE, USA) under nitrogen atmosphere with a purge rate of 50 mL min^{-1} on 10–20 mg samples placed in aluminum pans. The materials were exposed to successive thermal cycles (heat-cool-heat). Thermal characterization was carried out between 0 °C and 100 °C at 10 °C min^{-1}. First heating removes thermal history and eliminate small residual nuclei that might act as seed crystals.[19] The melting temperature (T_m) and enthalpy of fusion (ΔH_f) were determined from the second heating scan. The crystallization temperature (T_c) were determined from the cooling step. The degree of crystallinity (X_c) was calculated from the peak enthalpies normalized to the actual weight fraction of polymer according to the equation:

Table I. Ring-opening Polymerization of CL in ILs Catalyzed by CALB at 60 and 80 °C for 7 days

Solvent	Temperature (°C)	M_n[a] (Da)	M_w/M_n[b]	M_n[c] (Da)	M_n[d] (Da)	M_w[e] (Da)	Yield (%)	Conversion[f] (%)
[bmim][NTf_2]	60	4500	1.46	4300	2800	3300	90	96
[bmim][PF_6]	60	2400	1.49	2550	1900	2300	93	98
[bmim][NTf_2]	80	4600	1.52	4600	2600	2900	92	97
[bmim][PF_6]	80	3000	1.52	2600	2000	2400	87	96

[a] Determined by SEC using the correction coefficient as follows: $M_{n(SEC)} = M_{n(SEC\ raw\ data)} \times 0.56$.
[b] Molar-mass distribution calculated from SEC chromatogram traces.
[c] Determined by ^{1}H NMR spectroscopy analysis.
[d,e] Determined by MALDI-TOF mass spectrometry.
[f] Determined by ^{1}H NMR by comparison of the integrated signal areas of the methylene group next to the carbonyl group in the monomer (4.23 ppm) and the polymer (4.07 ppm).

$$X_c = [\Delta H_f(T_m)] \times [\Delta H_{f0}(T_{0m})]^{-1}$$

where $\Delta H_f(T_m)$ is the enthalpy of fusion measured at the melting point and $\Delta H_{f0}(T_{0m})$ is a literature value of 139.5 J g^{-1} corresponding to a 100% crystalline PCL.[19,20]

Degradation Assays

The degradation assays were performed in phosphate buffer solution (PBS) at pH 7.4 and 37 °C without stirring on PCL tablets (ca., 100 mg PCL tableted under 8 tones-pressure). The material samples were incubated in glass tubes with 10 mL of PBS solution. The samples were removed at 2 and 4 weeks, washed with distilled water, dried at room temperature and stored for further analyses. The weight loss (W_L) was calculated according to the equation:

$$W_L\% = [(W - W_1)/W] \times 100$$

where W is the weight of dry polymer sample before degradation and W_1 the weight of dry polymer sample after degradation. The experiments have been triplicated.

Morphological Analyses

Observation of polymer tablets before and after degradation (silver plating of each sample before observation) were performed using a LEO 435 VP scanning electron microscope (SEM).

RESULTS AND DISCUSSION

The aim of our research was to obtain low-molecular weight PCLs terminated with functional, hydroxyl groups, which could be subsequently used in pharmacy as drug delivery systems (short- and/or middle-term). The ROP of CL was performed in 1-butyl-3-methylimidazolium cation-based ILs with two different hydrophobic anions: bis(trifluoromethylsulfonyl)imide and hexafluorophosphate (Table I).

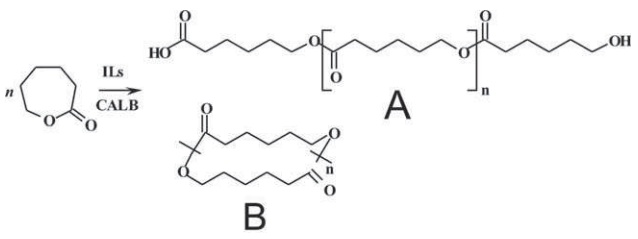

Figure 1. Synthesis of PCL: (A) linear chain of PCL; (B) cyclic PCL.

The polarity values of ILs were found to be close to typical hydrophilic organic solvents and varied in a relatively narrow range of 0.645–0.676.[12] The reactions were carried out with an CALB as a biocatalyst (Figure 1).

Thermal Characteristic of Enzymes and Ionic Liquids

Enzymes (lipases) in non-aqueous media can be active provided that the essential water layer around them is not stripped off. A small amount of water bound to the enzyme is required to maintain the catalytic activity of the enzyme. We indicated that there was~1.0 wt % of free water and ~0.85 wt % of water bound to the enzyme [Figure 2(A)]. The amount of water in the reaction system is crucial for enzyme activity in a hydrophobic media. The thermal characteristic of ILs depicted in Figure 2(B) indicates that the hexafluorophosphate containing ionic

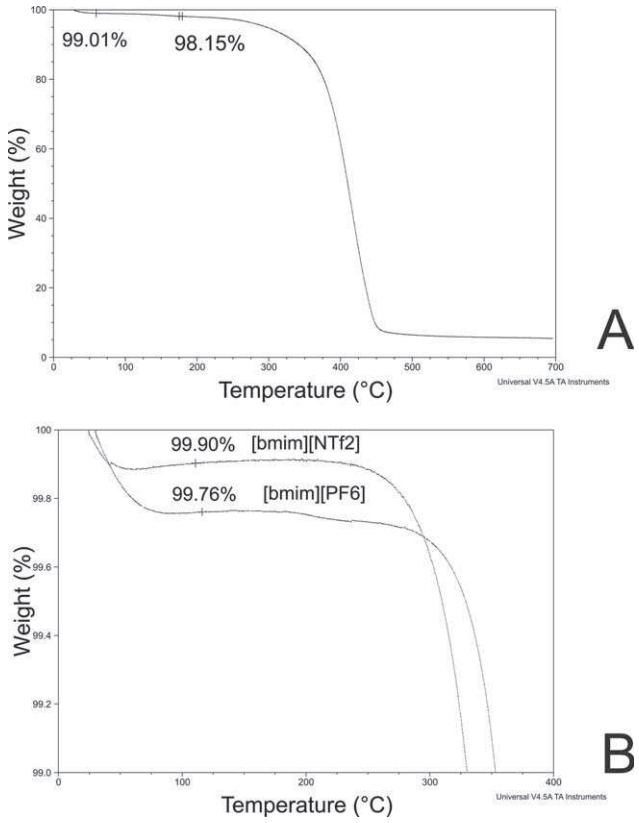

Figure 2. Thermogravimetric analysis (TGA) of CALB (A) and ILs (B).

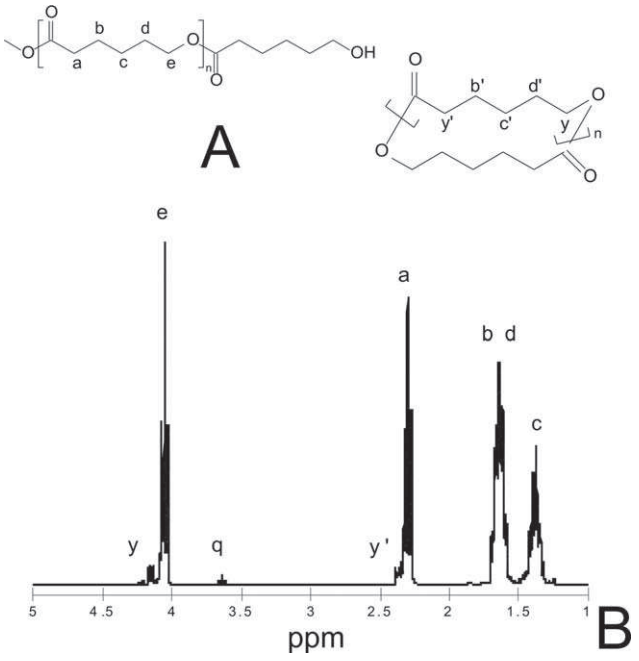

Figure 3. ^{1}H NMR spectrum of synthesized PCL analyzed in $CDCl_3$. The reaction was carried out using CALB and [bmim][NTf_2] as solvent at 60 °C for 7 days. Polymer molecules formulas (A); general spectrum (B).

liquids used in the experiment has lost around 0.24 wt % of water during heating, whereas a weight loss of only around 0.10 wt % was observed for the bis(trifluoromethylsulfonyl)imide.

Analysis and Characterization of the Obtained PCLs

The structure of the synthesized PCLs was confirmed by ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, FT-IR, and MALDI-TOF MS. The molecular weights and molecular weight distributions of the polyesters were determined using MALDI-TOF MS and SEC/MALLS (Table I).

The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) presented in Figure 3 confirmed the structure of PCL due to the presence of the following characteristic signals: δ 1.38 (m, H_c), 1.64 (m, $H_{b,d}$), 2.31 (t, H_a), and 4.07 ppm (t, H_e). There are two chemical shifts, which appear as triplets at 4.07 and 2.31 ppm and two other multiplets at 1.64 and 1.38 pmm assigned to the protons of the methylene groups of the polyester chain. Another feature is the chemical shift for proton from methylene groups next to the end-functional hydroxyl groups, which appears as triplet at 3.66 ppm (q). Chemical shift, which appears as triplets at 4.16 ppm (y) was attributed to the —CH_2O— of the cyclic lactone[21–24] which was likely to be presented as a result of intramolecular transesterification. The triplets at 2.31 (a) and 2.38 ppm (y') also suggested the presence of a diverse mixture of linear polymers and cyclic lactone. The other signals of cyclic PCL at 1.64 and 1.38 ppm were seen as a shoulder of signals b, d, and c. Moreover, the intensity ratio of the end group signal at 3.66 ppm was lower than that of the signal at 4.16 ppm.

The ^{13}C NMR spectrum ($CDCl_3$, 75MHz) confirmed the structure of PCL due to the presence of the following characteristic signals: δ 24.7 (B), 25.7 (C), 28.5 (D), 34.3 (A), 64.3 (E), 173.7 (F), and 62.7 ppm (Q) —CH_2OH, end group (Figure 4). Moreover, the signals at 24.5 (B'), 25.5 (C'), 28.3 (D'), 34.7 (Y'), 63.1 (Y), and 173.5 (F') ppm also suggested the presence of cyclic macromolecules. The chemical shifts from cyclic PCL are offset in ~0.2 ppm with respect to linear PCL.

COSY spectra of PCL are shown in Figure 5. Off-diagonal cross signals for adjacent protons a (2.31 ppm), b (1.64 ppm), c (1.38 ppm), d (1.64 ppm), and e (4.07 ppm) are seen in the 2D correlation chart. Additionally, the couplings between end group protons q (3.66 ppm) and d (1.64 ppm) are present. The protons from —CH_2CO— groups of cyclic lactone at 2.38 ppm (y') and protons from —CH_2O— groups at 4.16 ppm (y) are assigned with the protons b and d at 1.64 ppm, respectively.

HSQC spectra of PCL (Figure 6) and HMBC spectra (Figure 7) provides detailed information about the signals overlapped in ^{1}H- and ^{13}C NMR spectra. Strong correlations at δC/δH 34.3/2.31, 24.7/1.64, 25.7/1.38, 28.5/1.64, 64.3/4.07, and 62.7/3.66 ppm, associated with A-a, B-b, C-c, D-d, E-e, Q-q atoms in linear PCL (Figure 6). Other correlations at δC/δH 34.7/2.38 and 63.1/4.16 ppm were associated with A'-y, Y-y atoms of cyclic PCL.

The ^{1}H NMR spectra of the reaction media used in the experiment have also been analyzed in order to compare the results for the as-synthesized polyesters (Supporting Information Figure S1). The spectra of polyesters synthesized in ILs did not show any proton signals from reaction media. This indicates that the application of appropriate procedures can effectively remove the ILs residues from the polymer matrices. Other authors presented similar results[25,26] and additionally reported that the ^{1}H NMR, ^{13}C NMR, and even ^{19}F NMR spectra of the synthesized polyesters also have not shown any signal attributed to the ILs.[25] A high purity of the final product is mandatory for its potential further applications in medicine and pharmacy (especially as drug delivery systems). In addition, the presence of end-functional hydroxyl groups in PCL chains allows covalent coupling of the therapeutic agents and maintain their activity.

The FT-IR spectrum of the synthesized PCL (Figure 8) shows the characteristic bands near 1727 and 1245 cm^{-1} attributed to C═O and two C—O stretching vibrations. Moreover, the spectrum exhibited the bands at 2938 and 2866 cm^{-1}, assigned to —CH_2— stretching vibration. The band at 3440 cm^{-1} indicated the presence of a hydroxyl end-group, which confirmed the formation of a linear polymer chain.

Figure 9(i) shows the MALDI-TOF mass spectra of the synthesized PCL from ROP of CL using CALB as catalyst. The mass range below 1000 Da (removed from the spectra) was dominated with peaks resulted from matrix-fragments and metal ions.[27] The curve profiles indicated a bimodal distribution. As it's obviously known for broad molecular weight distributions (>1.5), the molecular weight obtained with MALDI-TOF MS is generally lower than that of actual value (Table I).[28] In Figure 9(ii) an expansion of the zone of the MALDI-TOF mass spectra of PCL between 1250 and 1450 *m/z* is shown. The peaks within each individual distribution separated by exactly 114 Da were observed, this value corresponds to the mass of an individual CL repeating unit. The most prominent series of peaks was

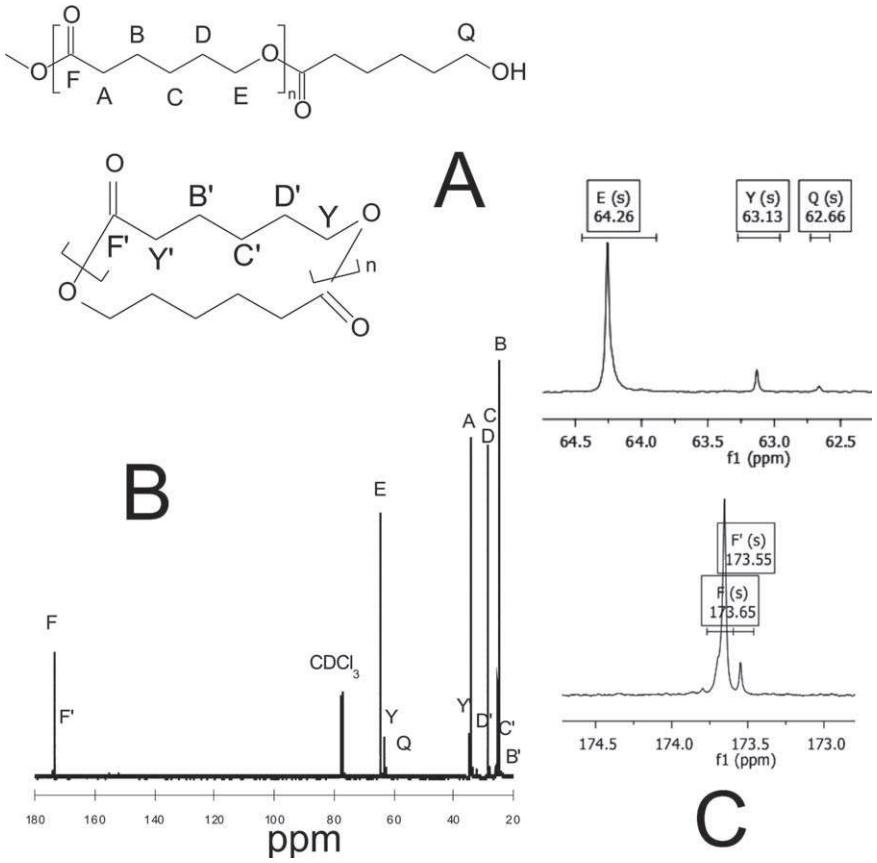

Figure 4. ^{13}C NMR spectrum of synthesized PCL analyzed in $CDCl_3$. The reaction was carried out using CALB and [bmim][NTf_2] as solvent at 60 °C for 7 days. Polymer molecules formulas (A); general spectrum (B); expansion of the domain of interest (C).

assigned to linear PCL chain, terminated with a hydroxyl group and hydrogen atom (residual mass: ca., 41 Da, Na^+ adduct) (A). In addition, the second series of peaks (residual mass: ca., 23 Da, Na^+ adduct) (B) was assigned to cyclic PCLs, doped with sodium ions (the mass was ranging from 1050 to 3000 Da). The cyclic PCLs were formed through intramolecular chain transfer in polymer.[29] Regarding the slow polymerization rate of CL, high M_n values of PCL needs prolonged polymerization time, which significantly induces the formation of low molecular weight products as a result of cyclic species formation during intramolecular transesterification. The content of the macrocyclic products (*MC*) formation is affected by the kind of ILs, temperature and reaction time and was estimated based on the intensity ratio of the peaks for linear and cyclic PCLs. The spectrum also shows the third series of peaks (residual mass: ca., 63 Da, Na^+ adduct) (C), which was assigned to the sodium

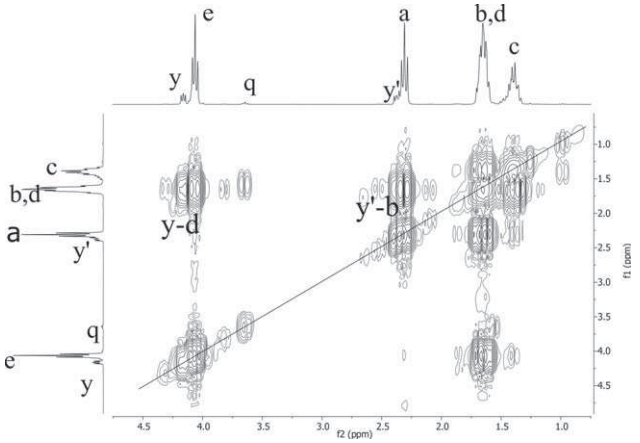

Figure 5. ^1H-^1H COSY spectrum of synthesized PCL analyzed in $CDCl_3$. Reaction was carried out using CALB and [bmim][NTf_2] as solvent at 60 °C for 7 days.

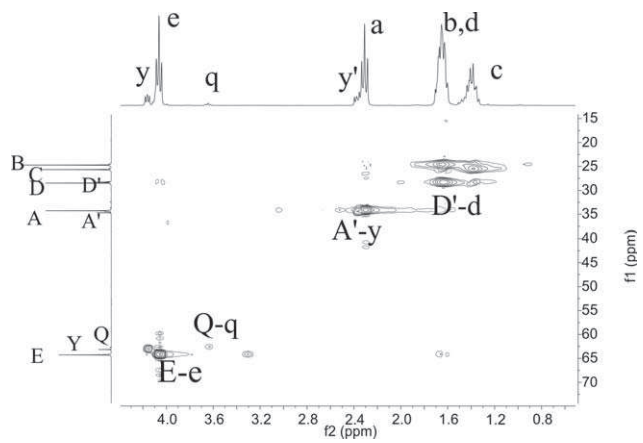

Figure 6. ^1H-^{13}C HSQC spectrum of synthesized PCL analyzed in $CDCl_3$. Reaction was carried out using CALB and [bmim][NTf_2] as solvent at 60 °C for 7 days.

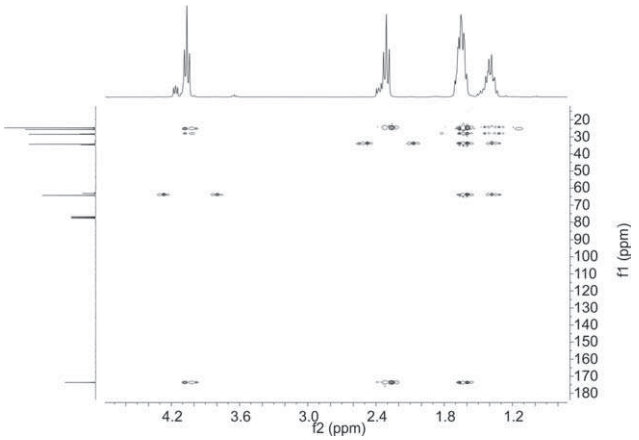

Figure 7. ^{1}H-^{13}C HMBC spectrum of synthesized PCL analyzed in $CDCl_3$. Reaction was carried out using CALB and [bmim][NTf_2] as solvent at 60 °C for 7 days.

salts of carboxyl-PCL and corresponds to a mass spectroscopy artifact.[22,30]

The macrocyclic content of PCLs generally increased following the increase in reaction temperature and reaction time for hydrophobic [bmim][NTf_2] (Figure 10). The highest *MC* content (64%) after 7 days of polymerization at 80 °C was observed. For [bmim][PF_6], however, the *MC* value was not dependent on the reaction time and remained at a similar level ~10–14% at 80 °C.

SEC/MALLS profiles for polymers synthesized in ILs after 7 days of polymerization are shown in Figure 11. The SEC/MALLS traces show that the high molecular weight PCLs (shorter retention time) were the predominant species.[22] In the case of PCL obtained in [bmim][NTf_2] at 60 °C and 80 °C, the number-average molecular weights of the polymers (M_n) and polydispersity indexes (M_w/M_n) were as follow: M_n 4500 Da; $M_w/M_n = 1.46$ and M_n 4600 Da; $M_w/M_n = 1.52$, respectively. For PCL synthesized in [bmim][PF_6] at 60 °C and 80 °C, we found these values as: M_n 2400 Da; $M_w/M_n = 1.49$ and M_n 3000 Da; $M_w/M_n = 1.52$, respectively. The curve profiles show multimodal

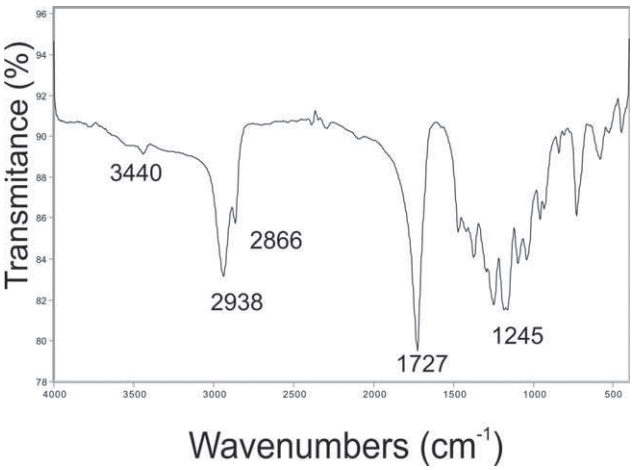

Figure 8. FT-IR spectrum of PCL. Reaction was carried out using CALB and [bmim][NTf_2] as solvent at 60 °C for 7 days.

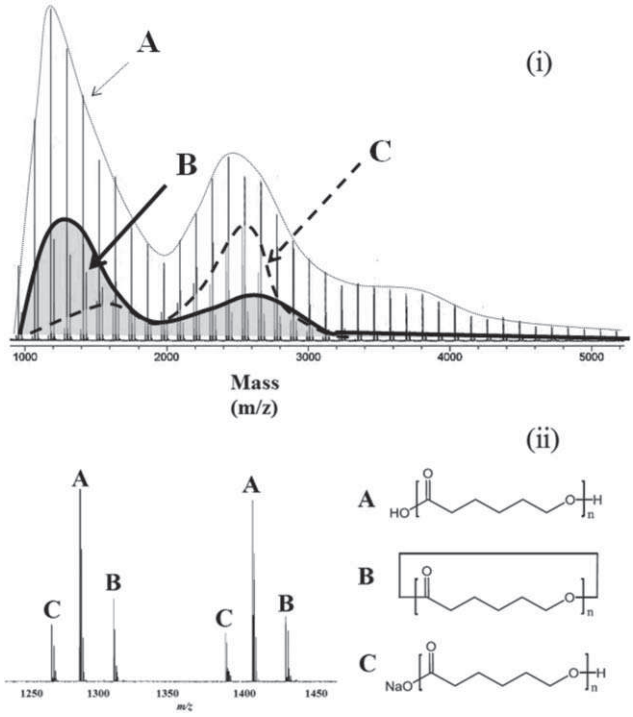

Figure 9. MALDI-TOF mass spectrum of PCL obtained in [bmim][PF_6] for the reaction carried out in 60 °C for 7 days. The full spectrum (*i*) is shown with an expansion (*ii*) of the 1250–1450 *m/z* region. A—linear PCL; B—cyclic PCLs; C—sodium salts of carboxyl-PCLs.

distribution, confirming the presence of different species (either linear or cyclic oligomers) that are not in a fast equilibrium with each other.[27] These results are consistent with those obtained by NMR and MALDI-TOF analysis. Moreover, SEC/MALLS profiles are different for PCLs obtained in two different ILs. It provides information that the reaction medium has an influence on the structure of the synthesized polymers.

The thermal characteristics of the synthesized polymers were evaluated with respect to their further application as matrices in drug delivery systems. The crystallization temperature (T_c), melting temperature (T_m), enthalpy of fusion (ΔH_f) and degree of crystallinity (X_c) were determined by using DSC method.

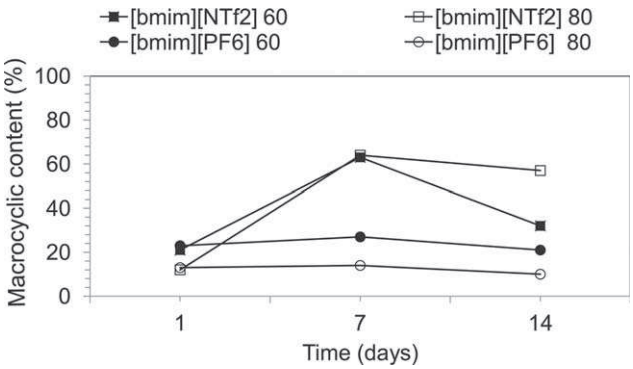

Figure 10. Macrocyclic content (%) of PCLs synthesized in ILs by CALB as a function of the reaction time (up to 14 days) and reaction temperature (60 and 80 °C).

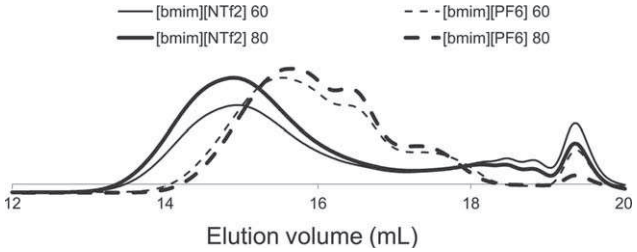

Figure 11. SEC/MALLS profiles for PCLs synthesized in ILs after 7 days of polymerization process.

As it is generally observed, degradation rate of polymers are related to their crystallinity; amorphous domains being degraded first and faster. Thus, we examined the degree of crystallinity of the synthesized PCL: we can see in Table II that PCLs synthesized in [bmim][NTf_2] were characterized by a lower degree of crystallinity (51.4–52.7%) as compared with those obtained in [bmim][PF_6] (55.5–56.5%). The higher polymerization temperatures led to a lower degree of crystallinity for the reactions performed in the presence of [bmim][PF_6]. In addition, we can notice that the degree of crystallinity is closely related with the M_n value: it decreases as the molecular weight increases.

The polymers synthesized in ILs showed bimodal melting peak (Figure 12). These results indicated that the imperfect crystallization existed in the synthesized samples,[31] probably because of the presence of cyclic macromolecules in the structure of the synthesized polymers. Furthermore, these results are well correlated with those obtained from NMR, MALDI-TOF and SEC/MALLS studies, regarding the evidence of multiphase morphology of PCLs. The crystalline imperfections are responsible for the lower values of T_c, ΔH_f, and X_c of the synthesized PCLs in [bmim][NTf_2] compared with [bmim][PF_6].

Effect of Reaction Temperature on Monomer Conversion, Reaction Yield and Polymer Molecular Weight

To examine the effect of the reaction temperature on the CALB activity, 7-days polymerizations were conducted at 60 and 80 °C in two different ILs. The effect of temperature on the monomer conversion, reaction yield and the number-average molecular weight of the polymers were determined in all cases. It was found that the high monomer conversion (96–98%) was achieved in all reactions. Moreover, the yield of the products synthesized in the presence of ILs was also high (87–93%).

The increase in the reaction temperature from 60 to 80 °C results in the highest M_n values (4600 Da) for the reaction conduced in [bmim][NTf_2]. Gorke *et al.* also investigated the influence of the reaction temperature on the ROP of CL using free CALB as catalyst and the [bmim][NTf_2] as the reaction medium. The process was carried out at various temperatures ranging from 25 to 110 °C for 24 h. The authors reported continuing increase of M_n value until it reached a maximum at 90 °C.[15] In this study, we also found that the enzyme activity in [bmim][NTf_2] was better as compared to [bmim][PF_6]. It demonstrates that the hydrophobic ILs with large anions can better protect the lipase from denaturation at a high temperature. Moreover, a lower water content in [bmim][NTf_2] has led to better enzyme stability and activity compared those with [bmim][PF_6]. We found that the number-average molecular weight of PCL synthesized in [bmim][PF_6] is not dependent on the reaction temperature under these conditions. In this case, the M_n value was ~2600 Da, probably due to the rapid inactivation of enzyme under these conditions (within 24 h). Moreover, the molecular weight distribution calculated from SEC chromatogram for the synthesized PCLs in ILs was at the similar level in all cases (1.46–1.52).

Effect of Reaction Time on the ROP of CL

The polymerization of CL was performed at 60 and 80 °C during different times to investigate the effect of the reaction time on M_n and the reaction yield [Figure 13(A,B)]. The reactions were carried out using two different ILs and the reaction time varied from 1 to 14 days.

For [bmim][NTf_2], the M_n value of the synthesized polymers increased together with increasing reaction time achieving maximum (4600 Da) after 7 days at 80 °C. The extending of the reaction time led to decreasing of the M_n value probably due to the thermal deactivation of the enzyme during a long term reaction. However, the reaction yield increased with the increase of the temperature and was the highest after 7 days at 80 °C (92%).

In the case of [bmim][PF_6], there was no significant difference between the M_n values for the reaction conducted during different times and at different temperatures (it was ranged from 2200 to 2600 Da). The increase of the reaction temperature and time resulted in the change of the color of the reaction mixture (from yellow to brown). The reaction yield increased from 76 to 93% within 7 days at 60 °C and was higher compared with the results obtained for [bmim][NTf_2] under the same conditions. At 80 °C the reaction yield was in the range of 77–87% within 14 days of polymerization.

Table II. Thermal Properties of PCLs

Solvent	Temperature (°C)	T_c (°C)[a]	T_m (°C)[a]	ΔH_f (J g^{-1})	X_c (%)
[bmim][NTf_2]	60	21.07 ± 0.1	49.44 ± 0.1	71.80 ± 0.9	51.4 ± 0.8
[bmim][PF_6]	60	28.27 ± 0.1	47.65 ± 0.3	79.80 ± 0.5	56.5 ± 0.5
[bmim][NTf_2]	80	20.58 ± 0.7	44.95 ± 0.1	73.56 ± 0.3	52.7 ± 0.3
[bmim][PF_6]	80	31.03 ± 0.1	47.84 ± 0.1	77.52 ± 0.7	55.5 ± 0.7

[a] Onset temperature.

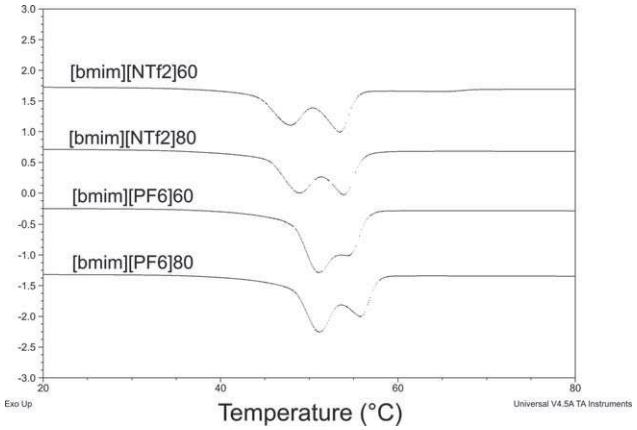

Figure 12. DSC thermograms of PCLs synthesized in Ils. Second scan of heating at a constant rate of 10 °C min^{-1}.

Degradation Assays

Figure 14 presented SEM micrographs (at the same magnification) of tablets of PCL, synthesized in different ILs and at different temperatures for 7 days, before and after degradation test in PBS. We can note an increase of the number and the depth of holes visible on the surface of tablets as a function of the duration of the degradation test. In addition the tablets prepared with PCL synthesized in [bmim][PF_6] appeared clearly less degraded (more regular surface of the tablets, less holes) before and after test (whatever the duration of the degradation test).

These results indicate that hydrolytic degradation of ester bonds occurred mainly by a surface erosion mechanism of the tablet.[32] Moreover, bulk erosion stage is more pronounced with increasing molecular weight of polymer and for PCLs synthesized in [bmim][NTf_2]: mass loss percentage was higher (14–19%) than for PCLs synthesized in [bmim][PF_6] (6–8%) (Figure 15). These results were also consisting with thermal properties of PCLs. Hydrolytic degradation occurs mainly in the polymers amorphous regions (probably dominated by macrocyclic chains in the architecture of PCLs) due the less structural chain packing, which making ester bonds more exposed to attack from water molecules thus the degradation process is faster in [bmim][NTf_2] than in [bmim][PF_6]. This property allows better release of active substance from degradable matrices because the degradation process is initiated first in the amorphous phase.[33] Furthermore we can predict to tune the drug release properties by selecting the polymer synthesis parameters.

CONCLUSIONS

The enzyme-catalyzed ROP of CL in ILs seems to be a promising method of the synthesis of biomedical polyester due to a high purity of the obtained products. During the polymerization of CL in ILs at 60–80 °C polyesters with a high yield (up to 93%) were obtained.

The enzymatic reaction is affected by multiple properties of ILs such as polarity, viscosity and the presence of impurities. In this study, it was determined that the hydrophobic [bmim][NTf_2] with a large anion, less polarity values and water content provides a better enzyme stability and activity compared to [bmim][PF_6]. Moreover, a higher viscosity of [bmim][PF_6] than that of [bmim][NTf_2] may lead to mass transfer limitations and a lower reaction rate resulted in lower M_n of polymer. The high viscosity values of ILs are the result of strong intermolecular forces. However, viscosity of ILs has decreased with a temperature thus this parameter probably has not influenced on enzyme activity. Importantly, during a long-term reaction the small amount of water in the reaction medium can cause degradation of hexafluorophosphate anion resulting in the formation of hydrofluoric acid which can cause enzyme inactivation.

The results reveal that the polymer structure confirms the presence of both, linear and cyclic macromolecules controlling also the thermal properties of the synthesized PCLs and kinetics of polymers degradation. It has been found that the conversion of monomer in ROP of CL hasn't been depended on the kind of ionic liquid used (ca., 100% after 7 days). However, the content of macrocyclic chains in the polymeric product and molecular weight of obtained PCL was depended on the kind of ionic liquid used in the polymerization process. PCLs obtained in [bmim][NTf_2] have been characterized by a higher M_n and *MC* content in comparison to that obtained in [bmim][PF_6]. The macrocyclic chains were amorphous domains in the obtained polymers. Therefore, the PCLs synthesized in [bmim][NTf_2] were degraded faster than PCLs obtained in [bmim][PF_6]. Moreover there is the possibility to control of the PCL

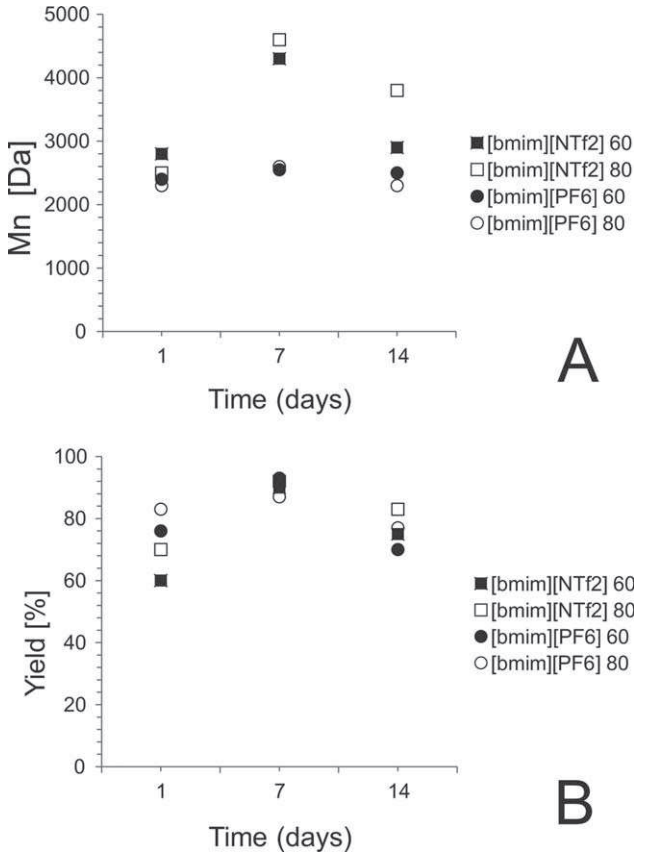

Figure 13. Polymer molecular weight (M_n) (A) and reaction yield (%) (B) as a function of reaction time.

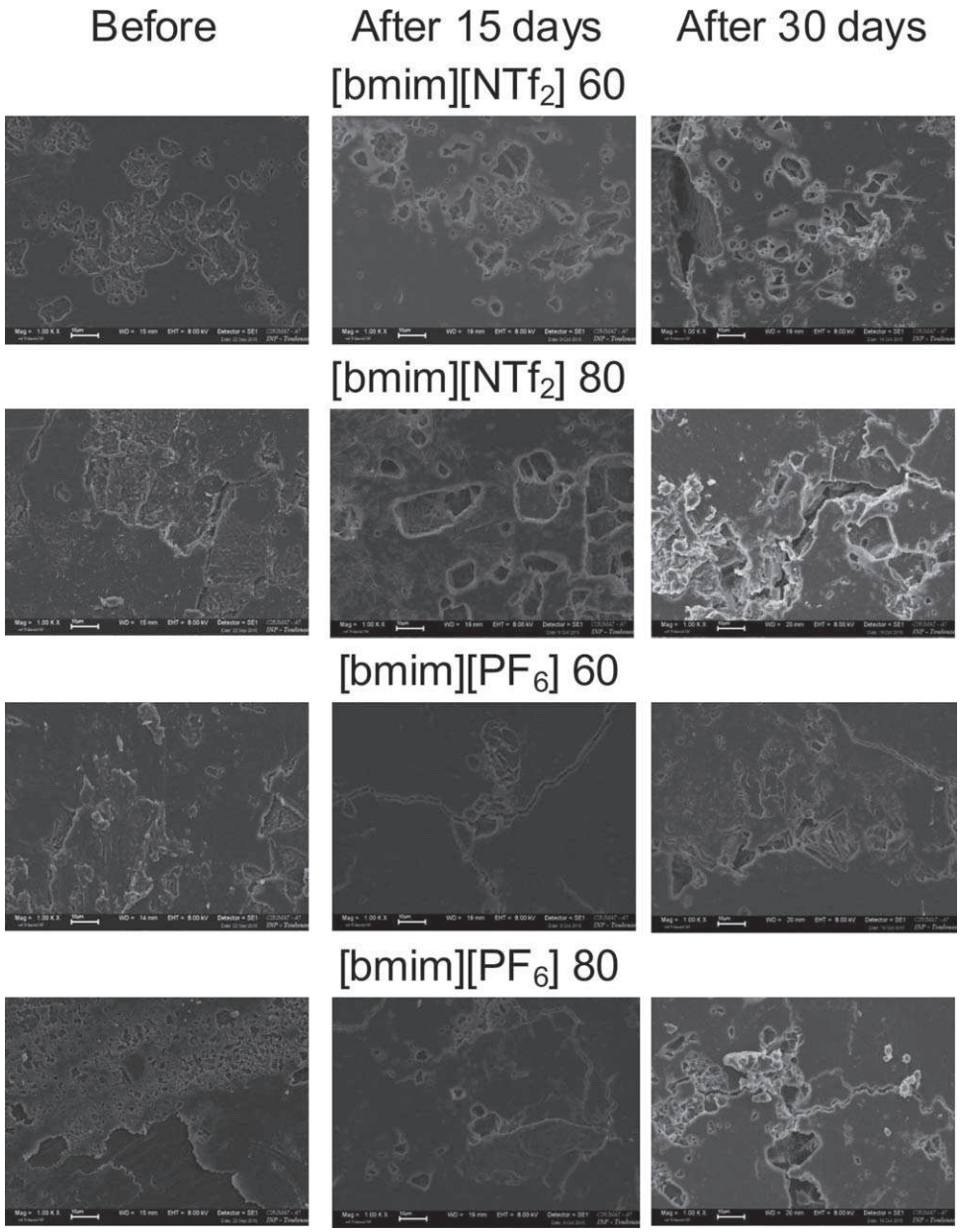

Figure 14. SEM micrographs showing the microstructure of the PCL tablets before and after 2 and 4 weeks of degradation test in PBS solution at 37 °C and pH 7.4 (scale bar corresponds to 10 μm).

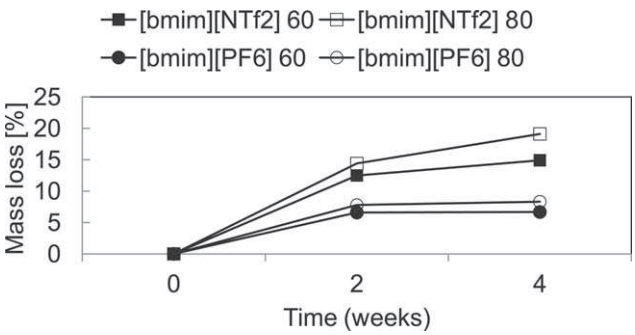

Figure 15. Mass loss of PCLs synthesized in ILs by CALB after different duration of the degradation test in PBS solution at 37 °C and pH 7.4.

degradation and thus drug release by selecting and controlling its synthesis parameters (temperature, time, and ILs type). The obtained results demonstrated that ILs offer new possibilities for solvent engineering in an enzymatic polymerization of biomedical materials.

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