Enzymatic Ring-Opening Polymerization of ε-Caprolactone by *Yarrowia lipolytica* Lipase in Ionic Liquids

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ABSTRACT: *Yarrowia lipolytica* (YLL), *Candida rugosa* (CRL), and porcine pancreatic lipase (PPL) were employed successfully as catalysts in the enzymatic ring-opening polymerization (ROP) of ε-caprolactone in the presence of 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butylpyridinium tetrafluoroborate ([BuPy][BF_4]), 1-butylpyridinium trifluoroacetate ([BuPy][CF_3COO]), 1-ethyl-3-methylimidazolium nitrate ([EMIM][NO_3]) ionic liquids. Poly(ε-caprolactone)s (PCLs) with molecular weights (M_n) in the range of 300–9000 Da were obtained. ^{1}H- and ^{13}C-NMR analyses on PCLs formed by YLL, CRL, and PPL showed asymmetric telechelic α-hydroxy-ω-carboxylic acid end groups. Differences between CP-MAS and MAS spectra are observed and discussed in terms of morphology. MALDI-TOF spectra show the formation of at least seven species. Differential scanning calorimetry (DSC) and Wide Angle X-Ray Scattering (WAXS) results demonstrate the high degree of crystallinity present in all the polyesters. © 2009 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 47: 5792–5805, 2009

Keywords: ionic liquids; lipase; ROP (ring-opening polymerization); Yarrowia lipolytica

INTRODUCTION

Ionic liquids (ILs) have emerged as exceptionally interesting nonaqueous reaction media for enzymatic transformations, and research interest in this area has increased widely during recent years.[1] As the introduction of cleaner technologies is becoming a major concern in both industry and academia, using alternatives to the most damaging solvents has become a high priority. Organic solvents are high on the list of damaging chemicals for two simple reasons: (1) they are used in huge amounts, and (2) they are usually volatile liquids that are difficult to contain. Ionic liquids are considered as a substitute of those volatile organic solvents, not only because of its low vapor pressure and thus being environmentally friendly, more importantly it may show the ability of catalysis as solvent or auxiliary. Moreover, ionic liquids also have many other attractive features, including chemical and thermal stability,

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nonflammability, high ionic conductivity, and a wide electrochemical potential window.[2] The 1-alkyl-3-methylimidazolium ionic liquids are polar solvents. They are miscible with polar solvents like methylene chloride and inmiscible with hexane and usually water (although [BMIM][BF_4] is miscible with water). Polar organic solvents inactivate enzymes, surprisingly ionic liquids do not; this feature extends enzyme-catalyzed reactions to a solvent polarity range that was previously inaccessible. The ability to use solvents with greater polarity increases the solubility of polar substrates, leading to faster reactions and changes in selectivity.[3] Enzymes are usually active in ionic liquids containing BF_4, PF_6, and NTf_2 anions, but not in ionic liquids containing Cl, NO_3, CF_3SO_3, trifluoroacetate, or acetate anions. A possible reason for this difference is the lower hydrogen-bond basicity of the enzyme-compatible anions. The BF_4 anion spreads its negative charge over four fluorine atoms, the PF_6 anion over six atoms, and the NTf_2 anion over five atoms. The lower hydrogen-bond basicity minimizes interference with the internal hydrogen bonds of an enzyme. Consistent with this concept, enzymes are inactive in [BMIM][Cl] (liquid at 65 °C), which has high hydrogen-bond basicity.[3]

The application of lipases in synthetic biotransformations encompasses a wide range of solvolytic reactions of the carboxyl group, such as esterification, transesterification (alcoholysis), perhydrolisis, and aminolysis (amide synthesis). Lipase-catalyzed transesterification to prepare polyesters (replacing the traditional chemical polymerization at >200 °C) has received considerable attention in recent years. *Candida antartica* lipase B has been found to mediate polyester synthesis in the ionic liquids [BMIM][BF_4], [BMIM][PF_6], and [BMIM][NTf_2] at 60 °C, but the molecular weight of the product was rather low compared with a solventless system, perhaps owing to the high viscosity of ionic liquid media.[4] Unique applications of enzymatic polymerizations have been studied by Peng et al.,[5] who reported a novel way for producing high-performance polymers and for functionalization of the carbon nanotubes surface (CNTs) which are used as templates to synthesize regioselective polymers from enzymatic polymerization of phenol in water. For the synthesis of a new biologically functional polymer from a natural resource by an environment-friendly method, Yoshida et al. reported the laccase-catalyzed polymerization of a lignin-based macromonomer, lignocatechol. This reaction was carried out for the first time in ethanol-phosphate buffer solvent system to give crosslinked polymers in good yields.[6] Hepatoma-targeting micelles prepared by self-assembly of galactose-functionalized ribavirin-containing amphiphilic random copolymer as novel drug delivery vehicles was easily synthesized by combining enzymatic transesterification with radical polymerization.[7] Thermosensitive hydrogel made up of poly(*N*-isopropylacrylamide) (PNIPA)-chitosan semi-interpenetrating network (semi-IPN) with ultrarapid responding rate was synthesized and used as a carrier for Horseradish peroxidase (HRP) immobilization; this hydrogel was used as an enzyme-carrier by glutaraldehyde bridge for the polymerization of acrylamide. Polymerization was initiated by a redox system (hydrogen peroxide/acetylacetone (Acac)) and catalyzed by the immobilized enzyme at room temperature.[8]

Uyama and Kobayashi were the first to report the enzymatic ROP of ε-caprolactone in the presence of ionic liquids. They obtained polyesters with molecular weights in the range of 300–4200 Da in 24–168 h using [BMIM][BF_4] and [BMIM][PF_6] ionic liquids and *Candida antartica* lipase.[9] A second report in polyester synthesis was made by Nara et al.,[10] they used diethyl octane-1,8-dicarboxylate and 1,4-butanediol in [BMIM][PF_6] at room temperature and at 60 °C; polymerization was carried out employing *Pseudomonas cepacia* lipase supported on Celite. More recently, Marcilla et al.[11], reported the ROP of ε-caprolactone by *Candida antartica* lipase (Novozyme 435) in [BMIM][BF_4], [BMIM][PF_6], and [BMIM][NTf_2], obtaining polymers with molecular weights in the range 7000–9500 g/mol.

In related reports, Yoshizawa-Fujita et al.[12] reported the lipase-catalyzed polymerization of L-lactide in [BMIM][BF_4] and [BMIM][PF_6] using CAL-B. Molecular weights for PLLA were 55,000 g/mol and below 4000 g/mol, respectively. Fu and Liu[13] reported the synthesis of high molecular weight aliphatic polyesters in 1-alkyl-3-methylimidazolium ionic liquids via two-step polycondensation in a diol/diacid system. Recently we reported the use of *Yarrowia lipolytica* lipase as an efficient catalyst for the ROP of lactones in heptane.[14] Reverse ATRP processes,[15] free-radical polymerizations,[16] microwave-assisted ROP,[17] anionic polymerizations,[18] ring-opening methathesis polymerizations,[19] photopolymetization,[20] frontal polymerization,[21] and radical graft polymerizations[22] in the presence of ionic liquids have been described by various groups.

This work explores the ring-opening polymerization of ε-caprolactone by a low-cost lipase from *Yarrowia lipolytica* in ionic liquids for the first time. High conversions and short reaction times (24 h) are observed. The time dependance of ε-CL conversion and product molecular weight was investigated. The effects of enzyme and monomer concentration, nature and concentration of ionic liquids, and temperature (60, 90, 100, 120, and 150 °C) were evaluated. We found that some ionic liquids accelerated the ROP of ε-caprolactone, and when ionic liquids that contain [BuPy] cation were used, polyesters with narrow polydispersity are formed. This result is uncommon in lipase-catalyzed polymerization literature.

EXPERIMENTAL

Materials

ε-CL (Aldrich Chemicals Co.) was dried over calcium hydride and distilled under reduced pressure before use. *Candida rugosa* lipase Type VII and Porcine pancreatic lipase Type II (25% of protein with a* = 75 units/mg of protein) were obtained from Sigma-Aldrich and used without further purification. Chloroform, toluene, and methanol were obtained from Karal and used as received. Chloroform-*d* (99.8%) was obtained from Sigma-Aldrich. 1-Ethyl-3-methylimidazolium chloride, 1-ethyl-3-methylimidazolium bromide, 1-butyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium bromide, 1-butylpyridinium bromide, and silver (I) oxide were purchased from Fluka and used as received. Tetrafluoroboric acid (48 wt % solution in water), activated charcoal, trifluoroacetic acid, deuterium oxide, 99.9 atom % D, acetone-d_6 99.9 atom % D (1% v/v TMS) were purchased from Sigma-Aldrich and used as received. Lipase production by *Yarrowia lipolytica* (YLL) was carried out according to the literature.[14]

Ionic Liquids Syntheses

Ionic liquids were synthesized according to the literature.[23]

[EMIM]$^+$ [BF$_4$]$^-$: Yield: 95%

^{1}H-NMR (acetone-d_6): δ 1.52 (t, J = 7.23, 3H), 4.00 (s, 3H), 4.33 (q, J = 8.9, 2H), 7.67 (t, J = 1.85), 7.74 (t, J = 1.94), 9.05 (s, 1H); ^{13}C-NMR: δ 15.2, 36.0, 45.1, 122.2, 123.8, 135.9. IR (cm^{-1}): 3162, 3120 ($\upsilon_{C\text{-}H}$) aromatic stretching; 2992, 2954, 2886 ($\upsilon_{C\text{-}H}$) aliphatic stretching; 1634, 1575, 1467 (υ_{ring}) symmetrical stretching; 1340 MeC-H asymmetrical, 1170 ring stretching; 1060, 850 and 756.

[BuPy]$^+$ [CF$_3$COO]$^-$. Yield: 91%

^{1}H-NMR (acetone-d_6): δ 0.93 (t, J = 7.38, H), 1.39 (sextet, J = 7.51, H), 3.34 (s, H), 4.89 (t, J = 7.7, H), 8.26 (t, J = 6.99, H), 8.71 (t, J = 1.2, 1H), 9.42 (d, J = 5.63, 3H). ^{13}C-NMR: δ 13.72, 19.92, 34.19, 62.22, 115.74, 121.68, 129.3, 146.24; 159.33, 159.96, 160.58, 161.21 (due to carboxyl CF_3COO group, $J_{C\text{-}F}$ = 32.4). IR (cm^{-1}): 3138, 3087, 3067 ($\upsilon_{C\text{-}H}$) aromatic stretching and ($\upsilon_{O\text{—}C=O}$); 2968, 2939, 2878 ($\upsilon_{C\text{-}H}$) aliphatic stretching; 1673 ($\upsilon_{C=O}$), 1583, 1490 (υ_{ring}) symmetrical stretching; 1200, 1170 ring stretching, symmetrical; 1125, 830 and 801.

[BuPy]$^+$ [BF$_4$]$^-$. Yield: 92%

^{1}H-NMR (D_2O): δ 0.86 (t, J = 7.29, 7H), 1.26 (sextet, J = 7.27, 6H), 1.92 (quintet, J = 7.4, 5H), 4.54 (t, J = 7.32, 4H), 8.0 (t, J = 7.1, 2H), 8.43 (t, J = 1.32, 1H), 8.74 (d, J = 5.48, 3H). ^{13}C-NMR: δ 12.28, 18.24, 32.19, 61.36, 127.84, 143.8, 145.12. IR (cm^{-1}): 3140, 3096, 3074 ($\upsilon_{C\text{-}H}$) aromatic stretching; 2966, 2939, 2878 ($\upsilon_{C\text{-}H}$) aliphatic stretching; 1636, 1584, 1490 (υ_{ring}) symmetrical stretching; 1431, 1382 MeC-H asymmetrical, 1174 ring stretching, symmetrical; 1062 and 772.

[BMIM]$^+$ [BF$_4$]$^-$. Yield: 96%

^{1}H-NMR (D_2O): δ 0.94 (t, J = 7.74, 3H), 1.14–1.26 (sextet, J = 6.92H), 1.73 (quintet, J = 7.31, 2H), 3.77 (s, 3H), 4.1 (t, J = 7.39, 2H), 7.3 (t, J = 1.9), 7.35 (t, J = 1.9), 8.56 (s, 1H); ^{13}C-NMR: δ 13.4, 19.6, 32.1, 36.2, 49.8, 122.6, 123.9, 136.1. IR (cm^{-1}): 3157, 3120 ($\upsilon_{C\text{-}H}$) aromatic stretching; 2966, 2939, 2876 ($\upsilon_{C\text{-}H}$) aliphatic stretching; 1575, 1467 (υ_{ring}) symmetrical stretching; 1170 ring stretching, symmetrical; 1046, 1034, 1002, 849 and 756.

[EMIM]$^+$[NO$_3$]$^-$. Yield: 93%

^{1}H-NMR (D_2O): δ 1.4 (t, J = 7.8, 3H), 3.8 (s, 3H), 4.1 (q, J = 7.1, 2H), 7.3 (d, J = 2.14, 2H), 8.5 (s, 1H); ^{13}C-NMR: δ 14.01, 35.13, 44.33, 121.4, 122.9, and 132.2. IR (cm^{-1}): 3156, 3110 ($\upsilon_{C\text{-}H}$) aromatic stretching; 2990, 2946 ($\upsilon_{C\text{-}H}$) aliphatic stretching; 1644, 1575 (υ_{ring}) symmetrical stretching; 1360 $Me_{C\text{-}H}$ asymmetrical; 1170 ring stretching, symmetrical; 832 and 758.

Synthesis of Poly(ε-caprolactone)

In a typical experiment 1 mL of ionic liquid, 1.0 g of ε-caprolactone (8.76 mmol), and 0.1 g of *Y. lipolytica* lipase were added to a 10 mL vial previously dried and purged with dry nitrogen. Vials were stoppered with a teflon silicon septum and placed in a thermostated bath at predetermined temperatures (60, 90, 100, 120, and 150 °C) for 24 h. After 24 h, the polymer was extracted by five consecutive extractions with 5 mL toluene, and the enzyme was filtered off. Toluene was removed by evaporation at reduced pressure. Final polymer was crystallized from cold chloroform/methanol and dried under vacuum. Molecular weights and conversions during reaction were monitored by ^{1}H-NMR. The crystallized polymer was analyzed by FT-IR, DSC, WAXS, MALDI-TOF, GPC-MALLS, solution (proton and carbon-13), and carbon-13 Solid-state-NMR. The IL was recovered dissolving it in acetone and passed through an activated carbon and silica gel column to remove impurities according to the literature.[26] NMR data for PCL: ^{1}H-NMR (200 MHz, $CDCl_3$, ppm) δ 4.031 (t, 2H, [CH_2O], 3.613 (t, 2H, [CH_2OH]), 2.363 (t, 2H, [CH_2CO_2H]), 2.28 (t, 2H, [CH_2O_2]), 1.62 (m, 4H, [$(CH_2)_2$]), 1.38 (q, 2H, [CH_2]). ^{13}C-NMR (200 MHz, $CDCl_3$, ppm) δ 177.616 (a), 173.944 (j), 173.754 (g), 64.310 (f), 62.679 (q), 34.373 (k), 34.267 (h), 33.812 (b), 32.401 (p), 28.479 (e), 25.664 (d), 25.444 (m), 24.822 (l), 24.716 (i), 24.488 (c). IR (cm^{-1}): 2945 (υ_{CH}), 1724 ($\upsilon_{C=O}$), 1166 ($\delta_{O-C=O}$).

Synthesis of α-Trifluoroacetate-ω-(trifluoroacetanhydride) PCL by Derivatization of PCL with Trifluoroacetic Anhydride

An excess amount of TFA was added to a solution of PCL in CD_3CN (50 mg/0.75 mL) at ambient temperature. Full derivatization was confirmed by NMR. NMR data for TF-PCL: ^{1}H-NMR (200 MHz, CD_3CN, ppm) δ 4.363 (t, 2H, [CH_2OCOCF_3]), 4.029 (t, 2H, [CH_2O]), 2.681 (t, 2H, [$CH_2CO_2COCF_3$]), 2.283 (t, 2H, [CH_2CO_2]), 1.61 (m, 4H, [$(CH_2)_2$]), 1.388 (q, 2H, [CH_2]).

Characterization

Nuclear Magnetic Resonance and Fourier Transform Infrared

Solution ^{1}H and ^{13}C-NMR spectra were recorded at room temperature on a Varian Gemini 200 (200 MHz). Chloroform-*d* ($CDCl_3$) and CD_3CN were used as solvents. Spectra were referenced to the residual solvent protons at δ 7.27 for $CDCl_3$ and 2.94 for CD_3CN in the ^{1}H-NMR spectrum and the solvent carbon signals at δ 77.23 for $CDCl_3$ and 1.39 for CD_3CN in the ^{13}C-NMR spectrum. Solid-state ^{13}C-NMR spectra were recorded under proton decoupling on Varian Unity *Plus* 300 spectrometer. Approximately 100 mg were packed into 7 mm diameter zirconium rotors, with Kel-F packs. CP-MAS spectra were obtained under Hartmann-Hahn matching conditions and a spinning rate of 4.5 kHz was used. A contact time of 1 ms and a repetition time of 4 s were used. The measurements were made using spin-lock power in radiofrequency units of 60 kHz, and typically 4000 transients were recorded. For MAS spectra, a repetition time of 20 s was used. Chemical shifts were referenced to the upfield peak of adamantane at 29.5 ppm with respect to TMS, as determined on a separate sample.

FT-IR spectra were obtained with the ATR technique on films deposited over a diamond crystal on a Perkin-Elmer 100 spectrometer in the 4000–600 cm^{-1} range with an average of 4 scans at 4 cm^{-1} resolution. Spectra were digitally analyzed using Origin software.

Differential Scanning Calorimetry and Wide Angle X-Ray Scattering

DSC thermograms were obtained in a Mettler-Toledo 820e calorimeter using heating and cooling rates of 10 °C/min and 20 °C/min. The temperature scale was calibrated with high purity standards. The crystallization and melting temperatures (T_c and T_m) are taken from the DSC curves of the cooling and heating scans, respectively, as the peak temperature of the thermal event. The weight fraction degree of crystallinity (X_c) was defined as: $X_c = \Delta H_0^f/\Delta H_0^f$ (ΔT_0^m), where ΔH_0^f is the enthalpy of fusion measured at the melting point and ΔH_0^f (ΔT_0^m) is the enthalpy of fusion of a completely crystalline PCL. A literature value of 139.3 J g^{-1} for ΔH_0^f (ΔT_0^m) was used.[27]

X-ray diffraction patterns were recorded on a Siemens D-500 WAXS diffractometer using a CuKα1 source of 1.5406 Å.

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Analysis

Spectra were recorded in the linear mode by using a Voyager DE-PRO time-of-flight mass spectrometer (Applied Biosystems) equipped with a nitrogen laser emitting at $\lambda = 337$ nm with a 3 ns pulse

width and working in positive-ion mode and delayed extraction. A high acceleration voltage of 20 kV was employed. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix. Samples were dissolved in acetonitrile and mixed with the matrix at a molar ratio of approximately 1:100.

Molecular Weights Measurements by Gel Permeation Chromatography Multi-Angle Laser Light Scattering

Gel permeation chromatography multi-angle laser light scattering (GPC-MALLS) was used to determine molecular weights and molecular weight distributions, M_w/M_n, of polymer samples. The chromatographic set-up used consists of an Alliance HPLC Waters 2695 Separation Module having a vacuum degassing facility on online, an auto sampler, a quaternary pump, a columns thermostat, and a Waters 2414 Differential Refractometer for determining the distribution of molecular weight. A bank of four columns with the following characteristics was used: HSPgel: HR 1.0, HR 2.5, HR 4.0, and HR MB-M (dimensions 150 mm × 6.0 mm) with pore sizes of 50 Å, 500 Å, 1.0E+4 Å, and a mixed bed pore size (100 Å to 1.0E+6 Å) respectively, and particle size 3 and 5 μm. The temperature of the columns was controlled at 33 °C by the thermostat.

RESULTS AND DISCUSSION

Uyama and Kobayashi reported the enzymatic ring-opening polymerization of ε-caprolactone in the presence of [BMIM][BF_4] and [BMIM][PF_6]. In both solvents they reported that *Candida antartica* lipase was efficient for polysterification. They reported conversions of 74 and 97% and molecular weights of 1200 and 4200, respectively, after 168 h.[9]

[BuPy][BF_4], [EMIM][BF_4], [EMIM][NO_3], [BMIM][BF_4], and [BuPy][CF_3COO] were used in the enzymatic ROP of ε-CL. The enzyme activity has been shown by Hofmeister series as an order of the ion effect on protein stability,[28] according to this series [BuPy][BF_4], [EMIM][BF_4], and [BMIM][BF_4] were selected because they combine the same anion with different cation which conducts to differences in the Ils properties. This is the first report of ROP in the presence of [BuPy][BF_4] and [EMIM][NO_3], it has been also reported that [EMIM][NO_3] acts as a denaturing ionic liquid for free enzymes.[29]

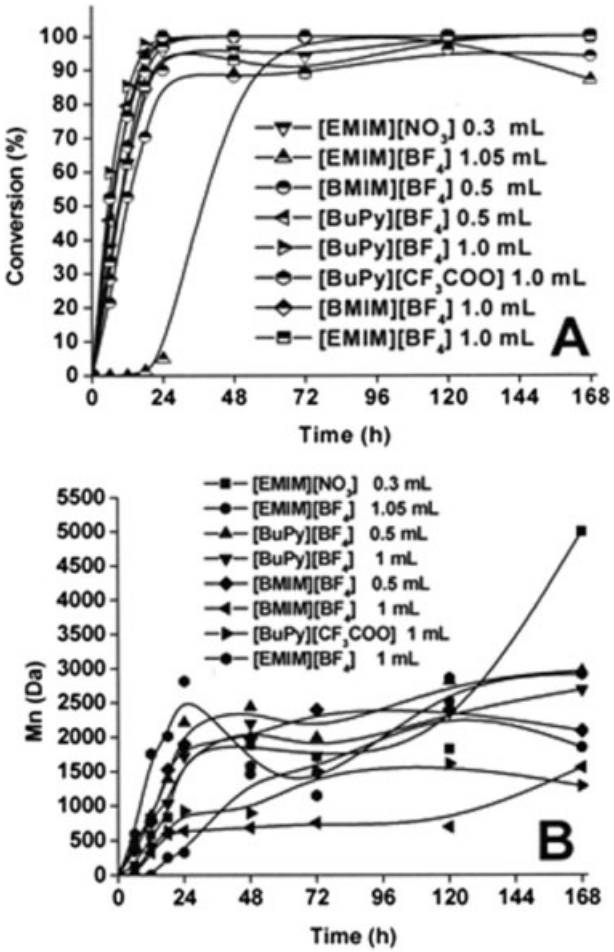


Figure 1. (A) Monomer conversion and (B) Molecular weight as a function of reaction time for the enzyme-catalyzed ε-CL polymerizations at 60 °C with different ionic liquid concentration. R = 3 mmol ε-CL/100 mg YLL.

In this work, *Yarrowia lipolytica* lipase (YLL) with a protein concentration of 0.1568 mg/mL was obtained and tested in the enzymatic ROP of ε-caprolactone in the presence of ionic liquids. The reactions were conducted at 60 °C, in [EMIM][BF_4], [BMIM][BF_4], [BuPy][BF_4], [BuPy][CF_3COO], [EMIM][NO_3] (monomer : ionic liquid 3 mmol: × mL), for 24, 48, 72, 120, and 168 h.

Figure 1(A,B) shows that a change in the ionic liquid cation influences the polymerization rate and the observed molecular weights. It is found that the reaction systems with 1 mL of [BuPy][BF_4], [BMIM][BF_4], and [EMIM][BF_4] are the most suitable for polymerization from the viewpoint of reaction rate (Table 1). The conversion in these systems was similar after 168 h. Hydrogen bonds, act as promoters of highly-

Table 1. Initial Rates for the Enzymatic ROP of 3 mmol of ε-CL/100 mg YLL at 60 °C in Ionic Liquids

Ionic Liquid	Concentration (mL)	[a]Initial Rate (mol L^{-1} s^{-1})
[EMIM][NO_3]	0.3	1.100
[EMIM][BF_4]	1.05	0.262
[BMIM][BF_4]	0.5	2.967
[BuPy][BF_4]	0.5	9.678
[BuPy][BF_4]	1.0	13.163
[BuPy][CF_3COO]	1.0	1.952
[BMIM][BF_4]	1.0	5.565
[EMIM][BF_4]	1.0	5.756

[a] Initial rates were calculated by a curve fitting procedure.

ordered structures in hydrated as well as dehydrated enzymes. Any structural change requires a considerable number of hydrogen bonds to dissociate at the same time (a high enthalpy change is involved), which may contribute significantly to enzyme stability and could also explain hydration-memory and hysteresis effects.[30] Hydrogen bonding could also be the key to understanding the interactions of proteins and ionic liquids. Anions of ionic liquids are capable of interacting with strong hydrogen bonds that maintain the structural integrity of the α-helices and β-sheets, causing the protein to unfold wholly or partially. In that regards the pyridinium cation, which has acidic properties,[31] can easily form stable hydrogen bonds with the enzyme. The ion size could matter, because sterically demanding ions would require many hydrogen bonds to be broken to create few new ones, which could contribute to maintaining stability. In all cases studied here, it was observed that when polymerization is completed, monomer remains soluble in the ionic liquid, and the enzyme tend to segregate to the bottom of the vial. This two-phase system facilitates the polymer extraction and allows the isolation of higher amounts of product (better yields).

Effect of Enzyme Concentration

Through a series of experiments, we have determined that as expected, increasing the ratio of enzyme to initial substrate concentration leads to more rapid attainment of higher molecular weight polyester within shorter reaction times (Fig. 2). However polyesters of the same average molecular weight were obtained from lower enzyme/substrate ratio when the polymerization system contained [EMIM][BF_4] and [BuPy][BF_4] ionic liquids. The source of water for hydrolysis reaction is predominantly the enzyme by itself; if we increase the enzyme concentration will lead to higher water concentration and therefore increased hydrolysis. As a polymer grows, the concentration of reactants decreases and this would lower the rate of reaction. Therefore, there is an overall reduction in polymerization rate as polymer molecular weight and enzyme concentration increase. Figure 2 shows that when the enzyme is in the presence of [BMIM][BF_4], [EMIM][NO_3], and [BuPy][CF_3COO] ionic liquids the increment in the molecular weight is almost null among all the enzyme concentrations. In Table 2, the slopes for each of the ionic liquids studied are shown. The effect of lipase content strongly depended on the nature of anion in the ionic liquids.

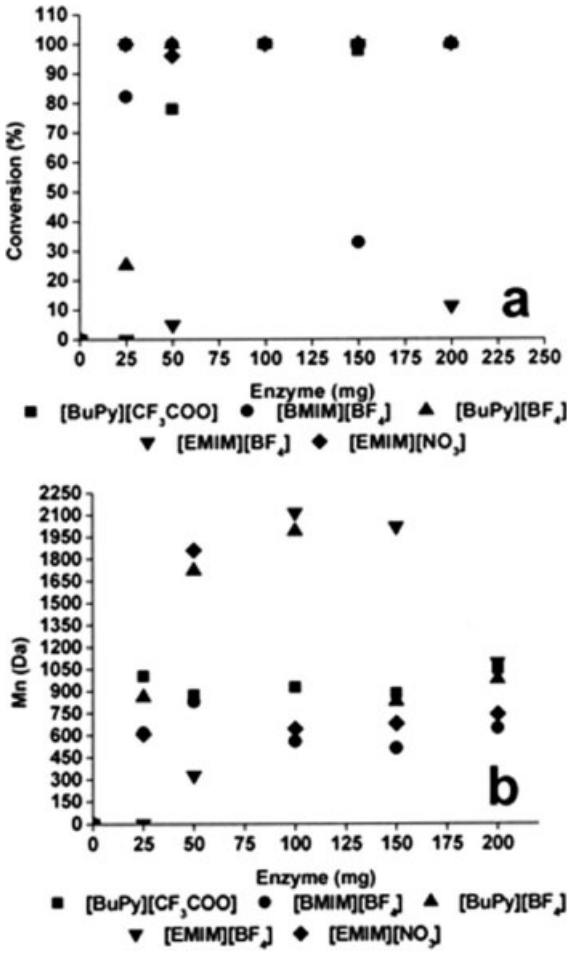


Figure 2. Effect of monomer/initiator ratio on molecular weight (M_n) and conversion of ε-CL, R = 3 mmol ε-CL/x mg YLL, reaction time = 24 h, T = 60 °C, polymerization in presence of ionic liquids.

Table 2. Slopes for the Enzymatic ROP of 3 mmol of ε-CL/x mg YLL at 60 °C in Ionic Liquids

Ionic Liquid	Concentration (mL)	Slopes[a]
[EMIM][NO_3]	0.3	3.3
[EMIM][BF_4]	1.0	1.4
[BMIM][BF_4]	1.0	3.5
[BuPy][BF_4]	1.0	2.9
[BuPy][CF_3COO]	1.0	5.4

[a] Slopes were claculated by a curve fitting procedure.

Effect of Lipase Origin

The effect of various lipases such as *Y. lipolytica, C. rugosa*, and porcine pancreatic in the presence of ionic liquids was investigated. In Figure 3, monomer conversions and molecular weights for PCL synthesized with CRL at 60 °C for 24 h are shown. In Figure 4, plots of conversion and Mn against time are shown for ROP of CL by PPL. In all cases, the variation of the number average molecular weight with time indicates that the molecular weight initially increases and then decreases. The large difference in the polymerization rates observed when CRL (0.536, 0.419, 2.132 $\times$ 10^{-14}, and 0.378 with [BuPy][BF_4], [EMIM][BF_4], [EMIM][NO_3], and [BMIM][BF_4], respectively) and PPL (0.009) are used, compared with those obtained with YLL can be attributed to the widely varying activities of lipases. YLL showed the better catalytic activity compared with the other lipases used in this study. The yields of the isolated polymers were higher for the ones obtained with YLL (90–95%) compared with those obtained with CRL and PPL (40–61%).

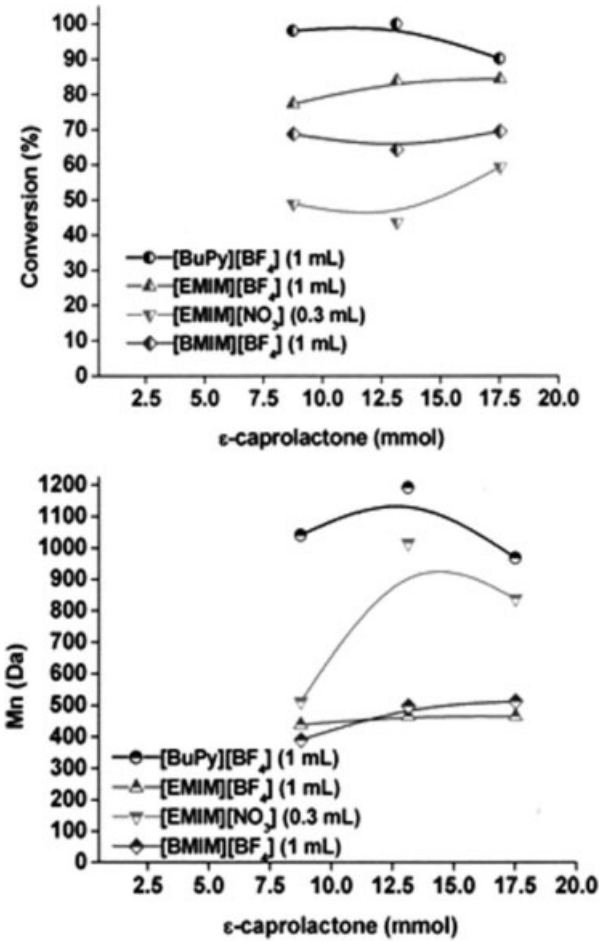


Figure 3. Monomer conversion and molecular weight as a function of monomer concentration for the enzyme-catalyzed ε-CL polymerizations at 60 °C for 24 h with different ionic liquid concentration. R = x mmol ε-CL/100 mg CRL.

Effect of Temperature and Monomer Concentration

A series of experiments were carried out at 90, 100, 120, and 150 °C, using a fixed amount of YLL (100 mg). Figure 5 shows that at 90 °C lower molecular weights are obtained for [EMIM][NO_3]. Monomer conversions after 24 h were of 95, 47, 63, and 96% with [BuPy][CF_3COO], [BMIM][BF_4], [EMIM][NO_3], and [BuPy][BF_4], respectively. The polymerization rates decreased with the increase of temperature in the range 60–90 °C. This can be attributed to the denaturation and deactivation of the enzyme due to the temperature and/or to hydrophilicity of ionic liquids.

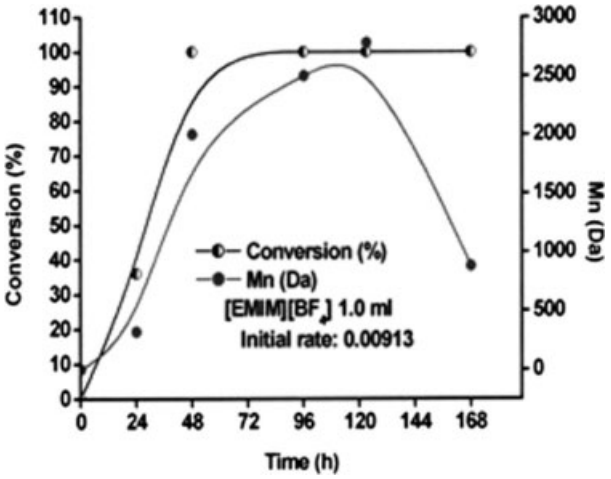


Figure 4. Monomer conversion and molecular weight as a function of time for the enzyme-catalyzed ε-CL polymerizations at 60 °C with 1.0 mL [EMIM][BF_4]. R = 3 mmol ε-CL/100 mg PPL.

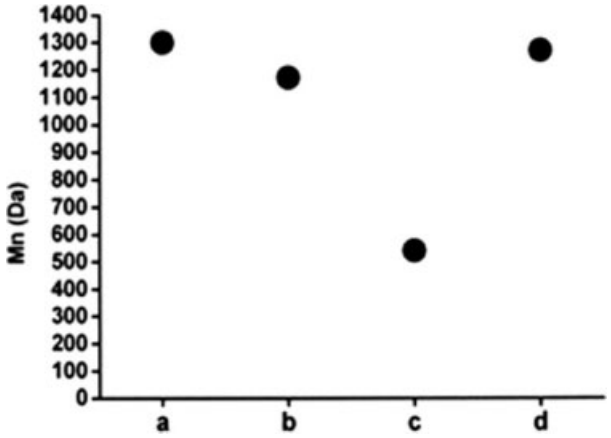


Figure 5. Molecular weights for the enzyme-catalyzed ε-CL polymerizations at 90 °C and 24 h in ionic liquids. R = 43.8 mmol ε-CL/100 mg YLL. (a) 1 mL [BuPy][CF_3COO], (b) 1 mL [BMIM][BF_4], (c) 0.3 mL [EMIM][NO_3] and (d) 1.0 mL [BuPy][BF_4].

The concentration of the monomer in the ionic liquid influences the degree of polymerization (DP) of the obtained polymer and also the amount of cyclic species formed. At low concentrations, mainly linear polymers are obtained. By raising reaction temperature, a sharp increase in the monomer conversion could be observed. When the reaction is carried out for prolonged reaction times at 60 °C, the molecular weights of the obtained polymers decreased as a result of hydrolysis, backbiting and cyclization reactions. At 100 °C and after 16 h, a monomer conversion of 100% was attained for all of the monomer concentrations tried. The molecular weights did not show important increment differences (or are about the same) in all the studied systems, being [BuPy][BF_4] the ionic liquid that bring about the highest molecular weight polymer. At 120 °C, a sharp decrease in reaction time was observed (8 h), being the change in the molecular weights almost null (Fig. 6). At 150 °C a 100% of monomer conversion was achieved after 6 h, the molecular weights were maintained in the range of 1500–3000 Da when [BuPy][CF_3COO], [BMIM][BF_4], and [BuPy][BF_4] ionic liquids were used, in the case of [EMIM][NO_3] we can observe a sharp increase in the molecular weight up to 9,000 Da and then a decrease to 8,000 Da. This behavior was repetitive and can be attributed to depolymerization-degradation reactions as a consequence of temperature and lipase denaturation. The influence of the ionic liquid on the DP of the products was more pronounced at lower tempera-

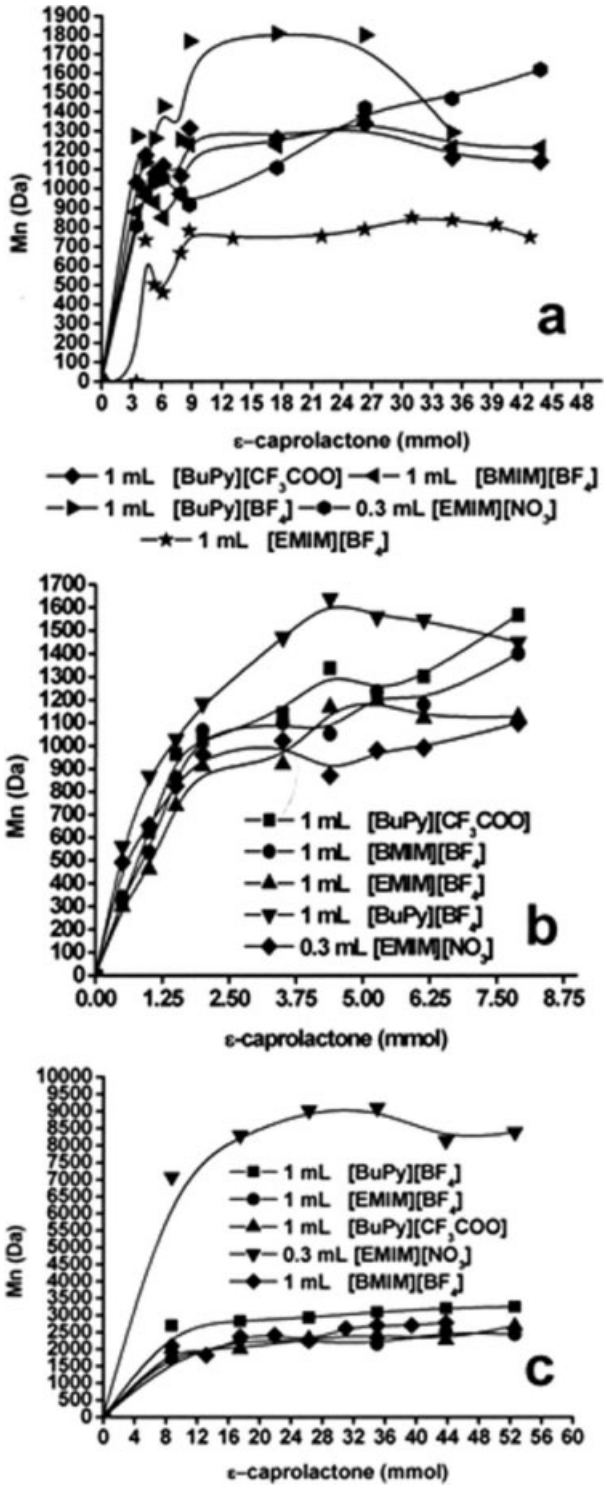


Figure 6. Molecular weights as function of monomer concentration for PCLs obtained by enzymatic ROP with 100 mg YLL in ionic liquids. (a) 100 °C for 16 h, (b) 120 °C for 8 h, and (c) 150 °C for 6 h.

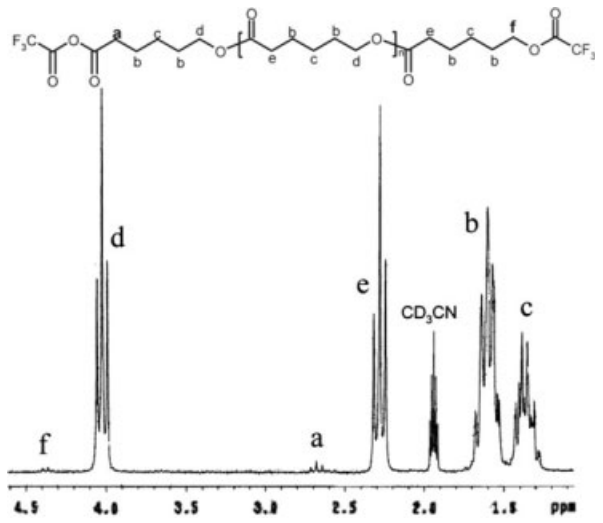


Figure 7. ^{1}H-NMR spectrum of poly(ε-caprolactone) in CD_3CN after derivatization with trifluoroacetic anhydride. M_n(NMR) = 8000 Da. M_n(GPC-MALLS) = 8158. R = 17.52 mmol ε-CL/100 mg YLL, 1 mL [BuPy][BF_4] at 60 °C for 24 h.

tures at which higher molecular weights were obtained with respect to 60 °C.

To determine if polymerizations at 120 and 150 °C were due to enzyme-catalyzed processes as opposed to nonenzyme-mediated reactions, a control was performed where no enzyme was used. In this controls at 150 °C, a monomer conversion of 10% was observed after 24 h, and no polymer could be isolated.

The polymerizations in ionic liquids proceed to molecular weights in the range 2000–9000 Da and polydispersities below 2.5 within 24 h. Here we report a significant improvement in reaction time and molecular weight using free enzymes, compared with the 7 days of reaction time for 4200 Da previously reported,[9] and with the results of Nara et al.,[10] who reported 4300 Da after 7 days with *Pseudomonas cepacia* lipase supported on Celite. Our results are similar to those obtained by Marcilla et al.[11] of 7000–9500 Da using an immobilized enzyme (N435).

Figure 7 shows the ^{1}H-NMR spectra for PCL after being treated with trifluoroacetic anhydride. Observation of only one peak due to the CL end-group carboxylic acid is consistent with the fact that the initiation step is mainly induced by the reaction of serine residue of YLL with CL. Figure 8 shows the GPC-MALLS profiles for the final polymers obtained under the same reaction conditions at 60 °C and in the presence of 1 mL [BuPy][BF_4] for 24 h. We can observe a unimodal distribution for the HA-PCL obtained with YLL with a molecular weight of 8158 and M_w/M_n = 1.6. In the case of PCL obtained with CRL and PPL with molecular weights and polydispersities of 2169, M_w/M_n = 1.54 and 1098, M_w/M_n = 2.56, respectively, a bimodal distribution is observed. These profiles indicate the existence of a mixture of two molecular weight distributions (MWD) that are sufficiently different to be distinguished. The presence of a bimodal molecular weight distribution indicates that there are two different propagating species that are not in fast equilibrium with each other (fast relative to their propagation rates), these two or more different species can be either cyclic species or products obtained by transesterification and/or backbiting reactions. These results are consistent with data obtained by MALDI-TOF, DSC, and solid-state NMR.

Based on these results, we observed that lipases from *C. rugosa* and porcine pancreatic favored the formation of cycles, transesterification reactions and backbiting. The relative intensities of these component molecular weight populations evolve with time. As the higher molecular weight peak decreases in intensity, its maximum shifts to even shorter elution times. A possible explanation for this behavior is that lower molecular weight species of the initially formed PCL is preferentially cleaved to form products with retention times between 16 and 25 min. In other words, the enzyme activated chain segments that are transferred via lipase-catalyzed transacylation are preferentially those formed from PCL substrates that make up

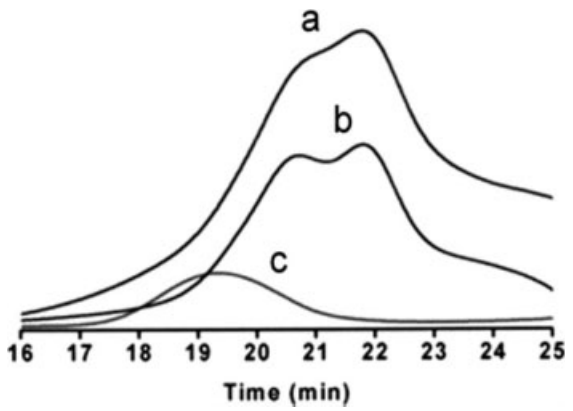


Figure 8. GPC-MALLS profiles for HA-PCLs obtained by enzymatic ROP in 1 mL [BuPy][BF_4] at 60 °C for 24 h. (a) R = 100 mg CRL/13.14 mmol ε-CL. M_n(GPC) = 2169, M_w/M_n = 1.54. (b) R = 100 mg PPL/43.8 mmol ε-CL. M_n(GPC) = 1098, M_w/M_n = 2.56 and (c) R = 100 mg YLL/17.52 mmol ε-CL. M_n(GPC) = 8158, M_w/M_n = 1.6.

Table 3. GPC-MALLS and DSC Data for PCLs Obtained by Enzymatic ROP

Entry	Mn (NMR)	Mn (GPC-MALLS)	Mw/ Mn	Crystallinity (% by DSC)
[a]	8,000	8,158	1.6	74
[b]	1,808	2,340	2.3	64
[c]	837	1,823	1.8	76
[d]	1,377	1,758	1.7	55
[e]	1,158	1,734	2.9	66
[f]	1,465	2,169	1.5	65
[g]	1,739	1,098	2.6	65
[h]	1,283	2,859	1.3	62
[i]	1,172	2,843	1.6	68
[j]	1,300	2,603	1.1	71
[k]	3,250	3,788	2.6	56
[l]	2,699	3,092	2.5	58
[m]	2,426	2,787	2.6	55
[n]	2,693	2,953	3.3	53

[a] 17.52 mmol CL/100 mg YLL/1 mL [BuPy][BF_4]. T = 60 °C. t = 24 h.
[b] 13.14 mmol CL/100 mg YLL/1 mL [BuPy][BF_4]. T = 100 °C. t = 16 h.
[c] 35 mmol CL/100 mg YLL/1 mL [EMIM][BF_4]. T = 100 °C. t = 16 h.
[d] 22 mmol CL/100 mg YLL/1 mL [BMIM][BF_4]. T = 100 °C. t = 16 h.
[e] 35 mmol CL/100 mg YLL/1 mL [BuPy][CF_3COO]. T = 100 °C. t = 16 h.
[f] 13.14 mmol CL/100 mg CRL/1 mL [BuPy][BF_4]. T = 60 °C. t = 24 h.
[g] 43.8 mmol CL/100 mg PPL/ 1 mL [BuPy][BF_4]. T = 60 °C. t = 24 h.
[h] 43.8 mmol CL/100 mg CRL/ 1 mL [BuPy][BF_4]. T = 100 °C. t = 16 h.
[i] 43.8 mmol CL/100 mg YLL/ 0.3 mL [EMIM][NO_3]. T = 90 °C. t = 24 h.
[j] 43.8 mmol CL/100 mg YLL/ 1 mL [BuPy][CF_3COO]. T = 90 °C. t = 24 h.
[k] 52.57 mmol CL/100 mg YLL/1 mL [BuPy][BF_4]. T = 150 °C. t = 6 h.
[l] 35.04 mmol CL/100 mg YLL/1 mL [BMIM][BF_4]. T = 150 °C. t = 6 h.
[m] 52.57 mmol CL/100 mg YLL/1 mL [EMIM][BF_4]. T = 150 °C. t = 6 h.
[n] 52.57 mmol CL/100 mg YLL/1 mL [BuPy][CF_3COO]. T = 150 °C. t = 6 h.

lower molecular weight population. In Table 3 data for GPC-MALLS and thermal properties are shown. For entries H and J, short polymerization times and narrower polydispersities (of 1.3 and 1.1, respectively) were observed. In both cases, [BuPy] cation is present in the ionic liquid. So this observation is probably linked to the effect of the [BuPy] cation on the enzymatic action.[28]

Figure 9(1) shows the ^{1}H-NMR spectrum for PCL obtained with CRL, signals for methylene end groups *b* [—CH_2COOH, δ 2.35] and *q* [—CH_2OH, δ 3.64] are clearly seen. The other peaks in the spectrum are assigned to other methylenes of the [—CO—$(CH_2)_5$—O] repeating unit. Thus, the obtained asymmetric telechelic polymer has carboxylic acid —CO_2H and primary hydroxyl —CH_2OH end groups. Figure 9(2) shows the ^{1}H-NMR spectrum for PCL obtained with PPL. In this spectrum a signal at δ 3.35 (—CH_2—O—CH_2—) is due to a species probably formed by a nucleophilic attack of the enzyme to the methylene in epsilon position of caprolactone.[32] Final polymer shows a bimodal distribution and a broad polydispersity index (2.6) as determined by GPC-MALLS. This observation suggests that parallel reactions are occurring during polymerization.

In the full MALDI-TOF spectrum given in Figure 10, a series of signals dominate, which can be adscribted to the HA-PCL oligomers doped with Na^+ ions. MALDI-TOF spectra show the formation of at least seven species (Scheme 1), the cyclic species are formed as a consequence of backbiting degradation and to the formation of macrocycles which is favored in reactions catalyzed by lipases.[33] This feature is observed for all the lipases studied, the reaction times using lipases to get higher conversions are relatively long (24 h) and

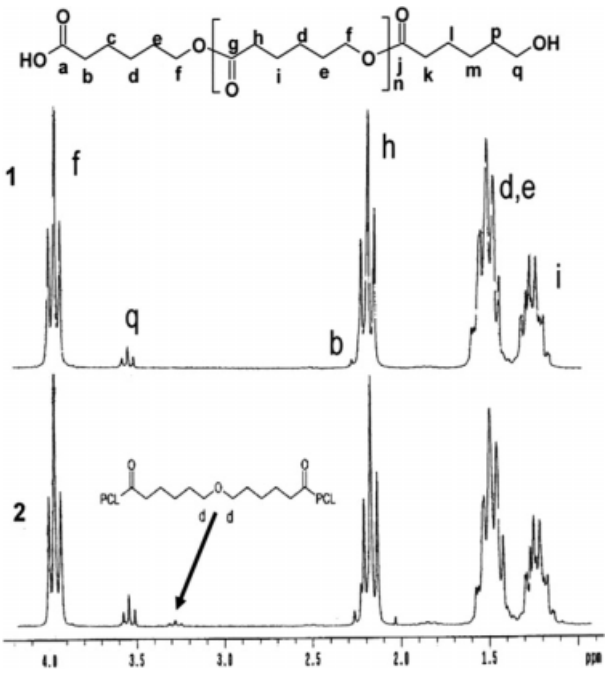


Figure 9. ^{1}H-NMR spectra of poly(ε-caprolactone) in $CDCl_3$ obtained by enzymatic ROP in [BuPy][BF_4] (1 mL) at 60 °C for 24 h. (1) M_n(NMR) = 2340 Da. R = 43.8 mmol ε-CL/100 mg CRL and (2) M_n(NMR) = 1740 Da. R = 43.8 mmol ε-CL/100 mg PPL.

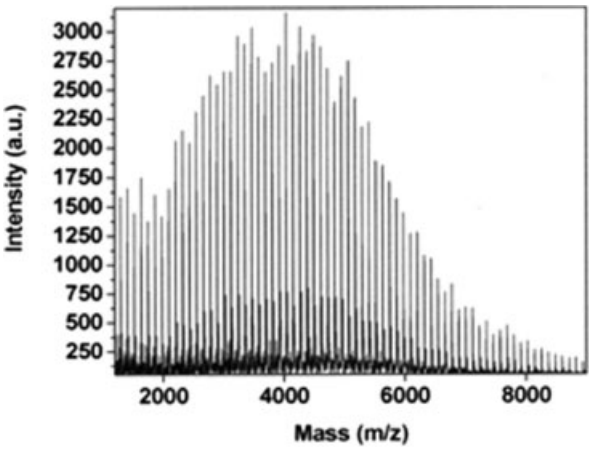
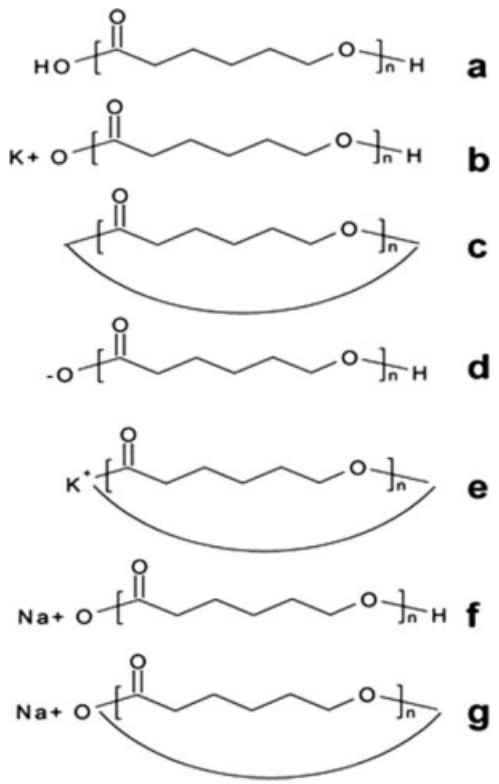


Figure 10. MALDI-TOF spectrum for PCL obtained by enzymatic ROP with 100 mg YLL/ 43.8 mmol ε-CL and 1 mL [EMIM][BF_4] at 60 °C for 24 h. M_n(MALDI) = 2561, M_w/M_n =1.9.

many side reactions occur during this time. In Figure 11, the expanded view for the 900–2600 m/z fragments is shown, it typifies the asymmetric and disperse oligomer distributions of the polyester products obtained under the different reaction conditions. The mass range below 300 was



Scheme 1. Species for PCLs present in MALDI-TOF mass spectra.

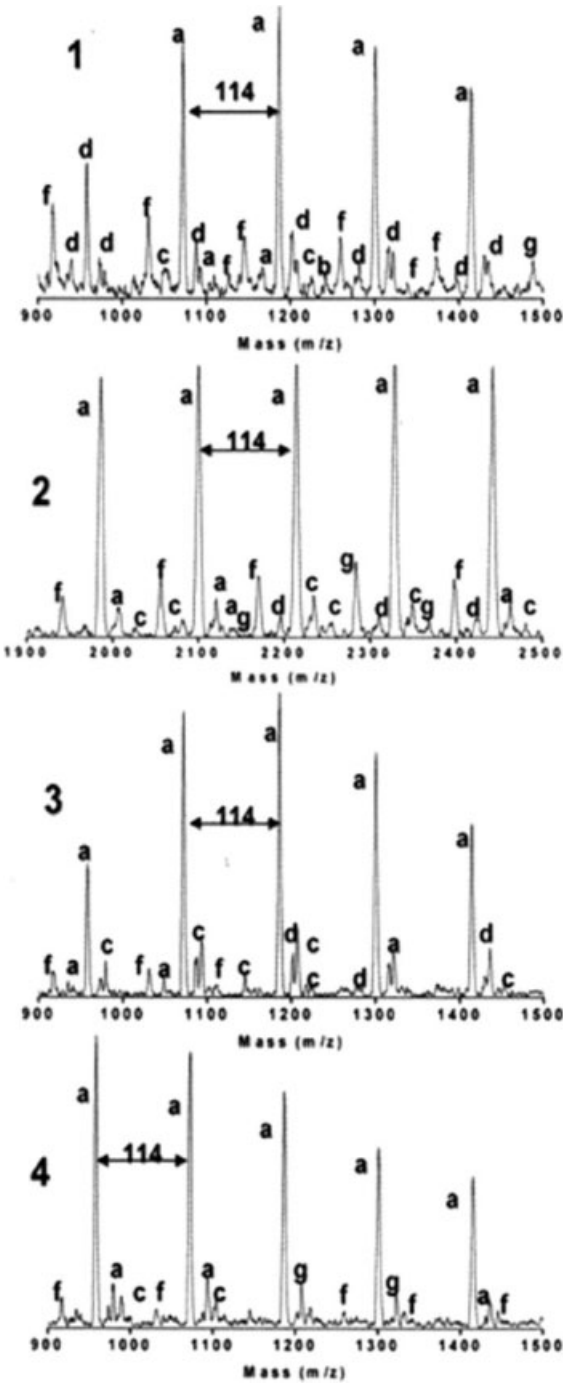


Figure 11. MALDI-TOF spectra of the HA-PCL catalyzed by different lipases at 60 °C for 24 h. expanded view for de 900–2600 m/z fragments. (1) R = 43.8 mmol ε-CL/100 mg YLL/1 mL [BuPy][BF_4] M_n(MALDI)=1754. (2) R = 43.8 mmol ε-CL/100 mg CRL/1 mL [BuPy][BF_4] M_n(MALDI) = 2126. (3) R = 43.8 mmol ε-CL/100 mg PPL/1 mL [BuPy][BF_4] M_n(MALDI) = 1640 and (3) R = 8.76 mmol ε-CL/100 mg YLL/0.3 mL [EMIM][NO_3] M_n(MALDI) = 1569.

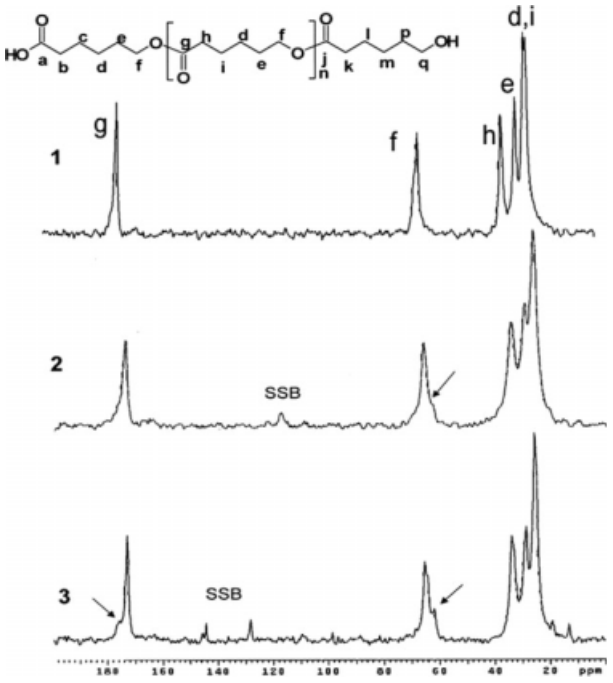


Figure 12. MAS ^{13}C-NMR spectra for poly(ε-caprolactone) obtained by enzymatic ROP in [BuPy][BF_4] (1 mL) at 60 °C for 24 h. (1) M_n(NMR) = 8000 Da. R = 17.52 mmol ε-CL/100 mg YLL. (2) M_n(NMR) = 2340 Da. R = 43.8 mmol ε-CL/100 mg CRL and (3) M_n(NMR) = 1740 Da. R = 43.8 mmol ε-CL/100 mg PPL. SSB = spinning side bands.

dominated by peaks resulting form matrix-fragments and metal ions. The distribution with the highest intensity peaks resulted from Na^+ cationized straight chain oligomers and extends over the mass range of 900–4000. K^+ cationized oligomer peaks show a lower intensity. The frequency of cyclic species formation is affected by the nature of the ionic liquid. The spectra of the polymers obtained in ionic liquids display a lower ratio of cycles to linear polymers in comparison to those obtained in heptane as we reported previously.[14]

In Figure 12, the ^{13}C-NMR MAS spectra for PCL obtained by enzymatic ROP of CL in the presence of 1 mL [BuPy][BF_4] at 60 °C are shown. There are no important differences in the CP-MAS spectra of these PCLs. The carbon resonances identified in the MAS spectra provide information on the more-mobile amorphous component of the polymer. Broader signals are seen in the MAS spectra of PCLs obtained from CRL and PPL biocatalysis, which indicate that these samples have a more amorphous morphology.[14] Important differences for signals due to carbonyl and to the methylene carbon adjacent to oxygen are observed. In the carbonyl zone, a sharp peak due to carboxyl (173.7 ppm) separated from a shoulder at 176 ppm (due to a end-group carboxylic acid functionality, see arrow in Fig. 12) can be seen in the MAS spectra of PCLs obtained from CRL and PPL biocatalysis. This signal is not visible in the spectrum of PCL with M_n = 8000 Da, probably due to its relatively higher molecular weight. Notorious differences are seen in the methylene linked to oxygen zone (carbon *f* of Fig. 12). In particular, a shoulder at about 62.5 ppm is seen in the samples 2 and 3. We have previously reported the observation of al least three peaks in this zone, which indicates the coexistence of various types of phases formed during crystallization.[14] Interestingly, peaks for carbons *d* and *i* are split in the MAS spectrum of PCL with M_n = 8000 Da. This feature is not observed in the CP-MAS spectra (where one peak is observed for the two carbons), and to our knowledge has not been previously reported in the literature for PCLs.

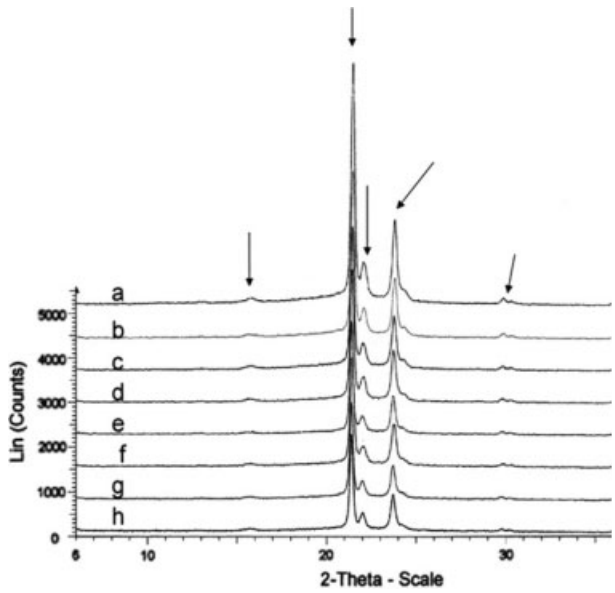


Figure 13. Wide angle X-ray scattering spectra (WAXS) for PCLs obtained by enzymatic ROP with YLL lipase in ionic liquids. (a) 17.52 mmol CL/100 mg YLL/1 mL [BuPy][BF_4]. T = 60 °C. t = 24 h. (b) 13.14 mmol CL/100 mg CRL/1 mL [BuPy][BF_4]. T = 60 °C. t = 24 h. (c) 43.8 mmol CL/100 mg PPL/1 mL [BuPy][BF_4]. T = 60 °C. t = 24 h. (d)43.8 mmol CL/100 mg YLL/0.3 mL [EMIM][NO_3]. T = 90 °C. t = 24 h. (e) 43.8 mmol CL/100 mg YLL/1 mL [BuPy][CF_3COO]. T = 90 °C. t = 24 h. (f) 22 mmol CL/100 mg YLL/1 mL [BMIM][BF_4]. T = 100 °C. t = 16 h. (g) 52.57 mmol CL/100 mg YLL/1 mL [EMIM][BF_4]. T = 150 °C. t = 6 h. (h) 43.8 mmol CL/100 mg CRL/1 mL [BuPy][BF_4]. T = 100 °C. t = 16 h.

Figure 13 illustrates WAXS diffractograms of eight of the synthesized PCLs. These PCLs are semicrystalline materials with a similar degree of crystallinity (DSC) and similar X-ray patterns exhibiting main reflections (marked with arrows) that appear at close but not identical 2θ angles. The degrees of crystallinity derived from by DSC are listed in Table 3. Differences in calorimetric behavior for the PCLs synthesized are appreciable from their corresponding DSC curves. In the second heating the biphasic pattern is better resolved. Observation of two peaks indicates the existence of a multiphase morphology.[14] In all the ATR FT-IR spectra (carbonyl zone) for the PCLs obtained by enzymatic polymerization with YLL, CRL, and PPL lipase at different temperatures and in the presence of different ionic liquids, the observed peak patterns did not reflect differences in polymer morphology.

CONCLUSIONS

High molecular weight aliphatic polyesters were synthesized using a green chemistry route in the presence of ionic liquids. The reaction solvent, monomer concentration, and temperature had profound effects on the polymerization rate, M_n, and polydispersity for YLL, CRL, and PPL catalyzed ε-caprolactone polymerizations. The anion species of the studied ionic liquids showed significant effects on conversion and molecular weight. [BuPy][BF_4] resulted the best ionic liquid for polyester synthesis by YLL and CRL, short polymerization times and narrower polydispersities (1.1 and 1.3) were observed. Polyesters obtained by ROP using ionic liquids proceed significantly faster than those obtained in the presence of organic solvents. This is due to the higher stability that ionic liquids provide to the enzyme at high temperatures. Different crystalline phases present in the polymer can be identified (DSC). Final polymers are asymmetric telechelic α-hydroxy-ω-carboxylic acid poly(ε-caprolactones), as determined by carbon-13 NMR.

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REFERENCES AND NOTES

1. De Diego, T.; Lozano, P.; Gmouh, S.; Vaultier, M.; Iborra, J. L. Biomacromolecules 2005, 6, 1457–164.
2. Malhotra, S. V.; Zhao, H. Phys Chem Liq 2003, 41, 545–557.
3. Park, S.; Kazlauskas, R. J. Curr Opin Biotechnol 2003, 14, 432–437.
4. Sheldon, R. A.; Van Rantwijk, F. Chem Rev 2007, 107, 2757–2785.
5. Peng, Y.; Liu, H.; Zhang, X.; Li, Y.; Liu, S. J Polym Sci Part A: Polym Chem 2009, 47, 1627–1635.
6. Yoshida, T.; Lu, R.; Han, S.; Hattori, K.; Katsuta, T.; Takeda, K.; Sugimoto, K.; Funaoka, M. J Polym Sci Part A: Polym Chem 2009, 47, 824–832.
7. Li, X.; Wu, Q.; Lu, M.; Zhang, F.; Lin, X. J Polym Sci Part A: Polym Chem 2008, 46, 2734–2744.
8. Zhao, Q.; Sun, J.; Ren, H.; Zhou, Q.; Lin, Q. J Polym Sci Part A: Polym Chem 2008, 46, 2222–2232.
9. Kobayashi, S.; Uyama, H.; Takamoto, T. Polym J 2002, 34, 94–96.
10. Nara, S. J.; Harjani, J. R.; Salunkhe, M. M.; Mane, A. T.; Wadgaonkar, P. P. Tetrahedron Lett 2003, 44, 1371–1373.
11. Marcilla, R.; Koning, C. E.; Duxbury, C. J.; Mecerreyes, D.; De Geus, M.; Heise, A. Eur Polym J 2006, 42, 1215–1221.
12. Yoshizawa-Fujita, M.; Saito, C.; Takeoka, Y.; Rikukawa, M. Polym Adv Technol 2008, 19, 1396–1400.
13. Fu, C.; Liu, Z. Polymer 2008, 49, 461–466.
14. Barrera-Rivera, K. A.; Flores-Carreón, A.; Martínez-Richa, A. J Appl Polym Sci 2008, 109, 708–719.
15. Hou, C.; Qu, R.; Ji, C.; Sun, C.; Wang, C. J Polym Sci Part A: Polym Chem 2008, 46, 2701–2707.
16. Woecht, I.; Schmidt-Naake, G.; Beuermann, S.; Buback, M.; García, N. J Polym Sci Part A: Polym Chem 2008, 46, 1460–1469.
17. Liao, L.; Zhang, C.; Gong, S. J Polym Sci Part A: Polym Chem 2007, 45, 5857–5863.
18. Biedroń, T.; Kubisa, P. J Polym Sci Part A: Polym Chem 2007, 45, 4168–4172.
19. Han, H.; Chen, F.; Yu, J.; Dang, J.; Ma, Z.; Zhang, Y.; Xie, M. J Polym Sci Part A: Polym Chem 2007, 45, 3986–3993.
20. Jiménez, Z.; Bounds, C.; Hoyle, C. E.; Lowe, A. B.; Zhou, H.; Pojman, J. A. J Polym Sci Part A: Polym Chem 2007, 45, 3009–3021.

21. Jiménez, Z.; Pojman, J. A. J Polym Sci Part A: Polym Chem 2007, 45, 2745–2754.
22. Ueda, J.; Yamaguchi, H.; Yamauchi, T.; Tsubokawa, N. J Polym Sci Part A: Polym Chem 2007, 45, 1143–1149.
23. Zhao, H.; Malhotra, S. V.; Luo, R. G. Phys Chem Liq 2003, 41, 487–492.
24. Jain, N.; Kumar, A.; Chauhan, S.; Chauhan, S. M. S. Tetrahedron 2005, 64, 1015–1060.
25. Madeira, R. L.; Sheldon, R. A.; Secundo, F.; Van Rantwijk, F.; Carrea, G.; Sorgedrager, M. J. Green Chem 2004, 6, 483–487.
26. Earle, M. J.; Gordon, C. M.; Plechkova, N. V.; Seddon, K. R.; Welton, T. Anal Chem 2007, 79, 758–764.
27. Wang, Y.; Rodriguez Perez, M. A.; Reis, R. L.; Mano, J. F. Macromol Mater Eng 2005, 290, 792–801.
28. Zhao, H. J Mol Catal B 2005, 37, 16–25.
29. Ruiz Toral, A.; De Los Ríos, A. P.; Hernández, F. J.; Janssen, M. H. A.; Schoevaart, R.; Van Rantwijk, F.; Sheldon, R. A. Enzyme Microb Technol 2007, 40, 1095–1099.
30. Van Rantwijk, F.; Secundo, F.; Sheldon, R. A. Green Chem 2006, 8, 282–286.
31. Duan, Z.; Gu, Y.; Zhang, J.; Zhu, L.; Deng, Y. J Mol Catal A 2006, 250, 163–168.
32. Báez, J. E.; Martínez-Richa, A. Polymer 2005, 46, 12118–12129.
33. Cordova, A.; Iverson, T.; Hult, K.; Martinelle, M. Polymer 1998, 39, 6519–6524.