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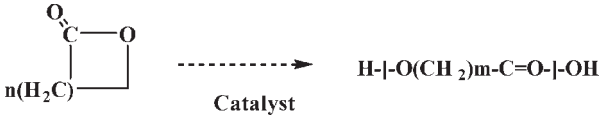
Communication: The polymerization of caprolactone was carried out with enzymatic and non-enzymatic catalysts (see Scheme). Lipases from different sources were tested, as well as an acidic zeolite and a basic free and supported guanidine. The results encouraged the authors to optimize the conditions to obtain polyesters with non-conventional catalysts.



Synthesis of Polycaprolactone Using Free/ Supported Enzymatic and Non-Enzymatic Catalysts

*M. L. Foresti, M. L. Ferreira**

Macromol. Rapid Commun. **2004**, *25*, 2025–2028

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Summary: Polymerization of caprolactone using lipases from *Candida antarctica B*, *Rhizomucor meihei, Candida rugosa*, and *Pseudomonas fluorescens* is highly effective, with 97% conversion into polycaprolactone. Poly(propylene)-supported *Candida rugosa* lipase achieves higher conversion values (85–92%) than free lipase (75%). Acidic and basic non-conventional catalysis with butanol yields 50–85% conversion. Simple UV/visible techniques gave the same results for measuring conversion than other studies. Applications are opened for the non-conventional catalysts.

Mechanism of the polymerization of caprolactone polymerization using a basic catalyst.

Synthesis of Polycaprolactone Using Free/Supported Enzymatic and Non-Enzymatic Catalysts

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Introduction

Some hydrolytic enzymes are stable in organic solvents and they can be used to produce polymers by condensation reactions, which are difficult or impossible to obtain in aqueous media by other methods.[1] Lipases catalyze the polymerization of lactones by ring-opening.[2,3]

The enzymatic polymerization of lactones was reported for the first time in 1993, by the group of Shiro Kobayashi. Lipases polymerized lactones with 4 to 17 atoms in the ring to give the corresponding polyesters.[4] A huge amount of enzyme is needed for the polymerization in organic solvents (20–50% weight enzyme/weight monomer).[5] The role of water in the mechanism is still controversial.

Kobayashi proposed that the enzymatic polymerization proceeds through a mechanism of activated monomer. The slow step is the production of an acyl intermediate at the active site of the enzyme.[6] Mac Donald suggested that the chain propagation is the slow step.[7] Henderson concluded that the initial step was fast in relation to the chain growth when an alcohol is the initiator.[8] The polymerization of caprolactone shares details with the ''immortal'' polymerization. Dong et al. studied lactone polymerization with several lipases from Amano Inc. and they concluded that the highest conversion was obtained for ε-caprolactone and *Pseudomones* lipases.[9] The conversion of monomer and the molecular weight of the product increase at higher reaction temperatures. In the case of ε-caprolactone, lipase from *Pseudomonas fluorescens* supported on Celite or the commercial lipase from *Candida antarctica* (Novozyme 435) showed high activity at lower concentrations than in the case of free lipases (1% weight lipase/weight monomer).

These biodegradable polymers, the polyesters, have multiple applications in medicine, e.g., supports for therapeutic molecule-delivery systems and as nano and microparticles.[10] The most important problem with the use of enzymes is the high cost involved. Although there are other catalysts based on aluminium alkoxides and organolanthanides, the need to purify the product in these cases to generate materials suitable for medical uses and the extra step it entails makes this synthesis route expensive. Alternative catalysts must be found that assure high effectivity with lower costs. No expensive purification steps must be required. Acidic and basic non-conventional catalysts are candidates.

Al-MCM-41, an acidic zeolite, has a uniform structure with a controlled pore of 15 to 100 Å and a high surface area (near 1 000 $m^2 \cdot g^{-1}$). This material can be considered as a group of nanoreactors, where the hexagonal channels of the zeolite isolate the active terminals of propagative polymers and suppress recombination and disproportionation reactions. The molecular weight can be controlled with the molar ratio of initiator/monomer. Because of the acidity of

this zeolite, the lactone monomer can be coordinated and activated. Polymerization of 4 mL of δ-valerolactone proceeds with butanol in the presence of 0.1 g of Al-MCM-41, at 50 °C without solvent, with a molar ratio of 10 to 100 of valerolactone to butanol, giving a conversion higher than 93% at long reaction times (from 200 to 2 500 h).[11]

Several basic catalysts have been tested in the oil transesterification with methanol (amines, amidines, guanidines, and triaminoiminophosphoranes). Unsupported 1,5,7-triazobicyclo[4.4.0]dec-5-ene (TBD) showed the highest activity with 91% conversion into methyl esters at 70 °C with 1 mol-% of catalyst. Biopolymeric-supported TBD showed similar activity. The mechanism proposed (and confirmed) for the transesterification reaction make this compound suitable as a catalyst for the caprolactone reaction.[12] Chitosan-supported TBD gave 12% conversion of oleic acid into ethyl oleate in a solvent-free synthesis at 65 °C after 2 h reaction.[13] This compound, TBD, seemed a suitable alternative catalyst to test in caprolactone polymerization.

The objective of this work was to test the performance of different immobilized lipases and non-conventional catalysts (Al-MCM41 and guanidine TBD) in the polymerization of caprolactone. A low-cost lipase from *Candida rugosa* was tested supported on poly(propylene) by adsorption. Further characterization of the polymer obtained with the more interesting catalysts (using additional techniques) will be the topic of a forthcoming paper.

Experimental Part

Materials

The lipases were generously donated by Amano Inc (USA) and Novo Inc. The following lipases were tested: 1) Lipase AY (Amano Inc.), from *Candida rugosa*, with no more than 4.1% mass loss at 105 °C after 4 h and 33 700 units per gram at pH 7; 2) Poly(propylene)-supported Lipase AY (Amano Inc.) by adsorption from a buffered pH 7 solution; 3) Lipase AK (Amano Inc.) from *Pseudomonas fluorescens* with 30 000 units $\cdot$ g^{-1}; 4) Concentrated solution from Novozyme, source *Candida Antarctica B* NS-40021; 5) Concentrated solution from Novozyme, source *Rhizomucor meihei* NS-40008; 6) Al-MCM 41 with butanol as initiator; and 7) 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) with butanol as initiator. ■ **author: lipase correct instead of lipasa?** ■

All the compounds (caprolactone monomer, isopropyl ether, dichloroethane, ethanol, butanol) were provided by Sigma and they are all HPLC grade.

Method

The experiments were performed (in duplicate) in 10 mL capped vials, with magnetic stirring at a controlled temperature of 65 °C for 5 h, using 0.1 mL caprolactone, 1.5 mL diisopropyl ether, and 0.15 mL of water in all cases. The bath temperature was maintained using a thermostatic bath with recycle using a pump that controls the water flux to the desired level. The experimental conditions were the following:

Free lipase from *Candida rugosa* (CR), 30 000 units $\cdot$ g^{-1} or *Pseudomonas fluorescens* (PF) 30 000 units $\cdot$ g^{-1}: 30 mg lipase. ■ **author: units $\cdot$ g^{-1} okay?** ■

Free lipase from *Candida antarctica B* (CALB), *Rhizomucro meihei* (RM) both 5 000 units $\cdot$ g^{-1}: 0.15 mL Novo solution.

Supported lipase from *Candida rugosa* (CR-PP): 120 mg of immobilized lipase (20–25% weight lipase/total weight). The supported lipase was used in ethyl oleate synthesis at 45 °C with an ethanol/oleic acid molar ratio of 1 to assure activity. Conversion achieved 10%. The catalyst was washed three times with 20 mL of octane and used in caprolactone polymerization. This procedure was done to assure that immobilized lipase is active in ester synthesis, in presence of ethanol.

In case of the acidic zeolite 23.4 mg of Al-MCM41 and 6 μL of butanol were contacted with 0.1 mL of caprolactone. When TBD was used, 24.6 mg of guanidine was contacted with 6 μL of butanol and the same amount of caprolactone. The butanol must be added at 65 °C, otherwise, deactivation is faster than reaction and no precipitate is obtained after 5 h upon ethanol addition. After adding of 1 mL of dichloroethane to the remaining solution at the filtering step (when solids were used) a white power precipitate was obtained after drying. Lipases are soluble in ethanol, therefore, adding 2–3 mL of 0.96% ethanol precipitated the polyester. ■ **author: please check sentence** ■ Ethanol was a better non-solvent for the polymer than dichloroethane.

Results and Discussion

Conversion of Caprolactone using UV/visible Techniques

Using a calibration curve obtained at 210 nm the amount of remaining caprolactone was checked after 5 h. Different amounts (from 20 to 100 μL) of a solution with 100 μL of caprolactone in 1.5 mL of isopropyl ether plus 0.15 mL of water were diluted to 3.5 mL and analyzed at $\lambda = 210$ nm. The calibration curve shows a correlation factor (R^2) of 0.984. Results for the catalysis are presented in Table 1.

Free lipases from *Candida antarctica B*, *Pseudomonas fluorescens*, and PP-supported CR showed the highest conversions. Free CR shows 75% conversion to polycaprolactone (PCL). The order of activity is CALB > CR/PP > PF > Al-MCM41/butanol > CR > guanidine > RM. The lack of needed interfacial activation for CALB and structural facts associated with structural stabilization explains the high activity of CALB. When no ethanol is present, CR or PF shows no strong deactivation. However, CR or PF in solvent-free ethyl oleate synthesis (from oleic acid and ethanol) achieves only approx. 8% of conversion, and up to 18% maximum conversion using different pretreatments.[13] The high activity shown here can be related to the lack of ethanol inhibition and the stabilization of the open form in the case of CR/PP in isopropyl ether as solvent

Table 1. Caprolactone conversion after 5 h at 65 °C. ■ **author: mL correct unit in second column?** ■

Catalyst	Units	Caprolactone conversion[a)]	Solubility of polymer in ethanol[b)]
	mL	%	
CR	515	75	S
CR/PP	600	85–92	S
PF	515	90	S
CALB	430	97	I
RM	430	45	S
Al-MCM 41/butanol	–	85	I
TBD	–	50	I

[a)] Percentage of caprolactone conversion = 100 × (mol caprolactone ($t = 0$) – mol caprolactone ($t = 5$ h))/(mol caprolactone ($t = 0$).

[b)] I = insoluble, S = soluble.

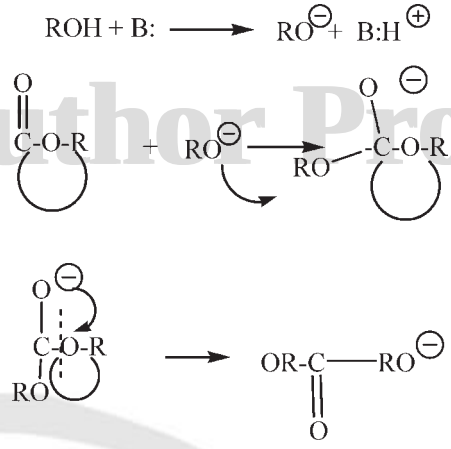

Figure 1. Mechanism of caprolactone polymerization using basic catalyst. B: = TBD.

in the presence of water and with lactone at the active site. Lipase from *Rhizomucor meihei* shows a surprising low conversion, perhaps related to an undesired lactone-associated inhibition reaction, whereas Al-MCM41/butanol displays the same activity as CR/PP. The recovery of mixed solids of zeolite-polycaprolactone encourages us to study the possibility of nanocomposites of zeolite-polycaprolactone using this polymerization procedure. We reproduced the conversion reported in ref.[7] for Al-MCM 41/butanol and in ref.[1–5] for PF/caprolactone or CR/caprolactone using simple UV/visible methods to test conversion. The authors of the references cited above used chromatographic or NMR techniques to determine caprolactone conversion.

Butanol initiates the polymerization upon reaction with guanidine to generate the protonated guanidine and an alkoxide. Figure 1 shows the mechanism for when bases are used for the polyester synthesis. When guanidine was used, the addition of ethanol at the end of the reaction time produces the immediate precipitation of a white powder, but no changes arise in the UV/visible spectrum in the 200–300 nm range, which is assigned to solubilized guanidine. It is possible that the activity in the case of guanidine is only 50% because of deactivation reactions associated with the irreversible hydrogen coordination to the active nitrogen of guanidine. Chitosan-supported TBD using glutaraldehyde with butanol as initiator was an active catalyst in polyester synthesis.[12] This catalyst (100 mg) was as active as TBD in the solvent-free synthesis of ethyl oleate using 10.6 mmol oleic acid and a molar ratio of 1:1 (15% conversion).[13]

Authors of ref.,[9] using ^{13}C NMR spectroscopy, Karl Fischer methods, and chromatographic analysis, clearly demonstrated that conversion is higher if you add water, at percentages as high as 16% initially on enzymatic bulk polymerization (sic) *regulating the initial water content is extremely important for success in obtaining high molecular weight products and a rapid initial polymerization rate* (sic). The addition of water when you use diisopropyl ether is reported to improve conversion. This is explained upon consideration of the enzymatic conformational lability when water is present and the enzymatic polymerization mechanism, which *needs* water at the initial stage (the 43 references of the Shen manuscript[9] are in line with these ideas).

FTIR Characterization of Polycaprolactone

Figure 2 shows the FTIR spectra of the solid obtained with free and supported CR. Polyester-6, the polymer from caprolactone, is partially crystalline with two bands at 1 740 cm^{-1} (from amorphous zones) and at 1 725 cm^{-1} (from crystalline zones). Bands belonging to polyester-6 are also found in the solid recovered when the other free

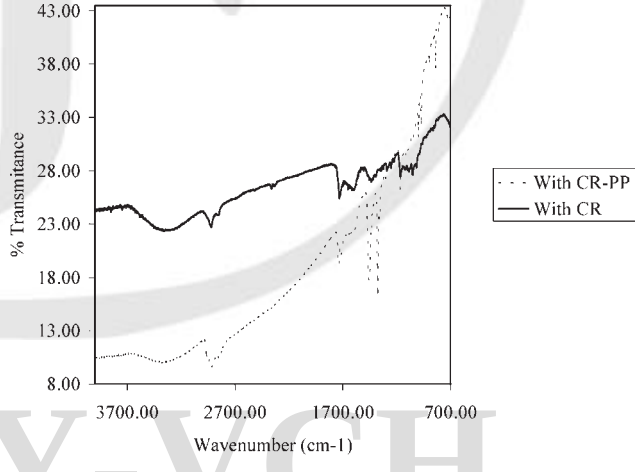

Figure 2. FTIR spectra of polymers produced with free and immobilized *Candida rugosa* lipase.

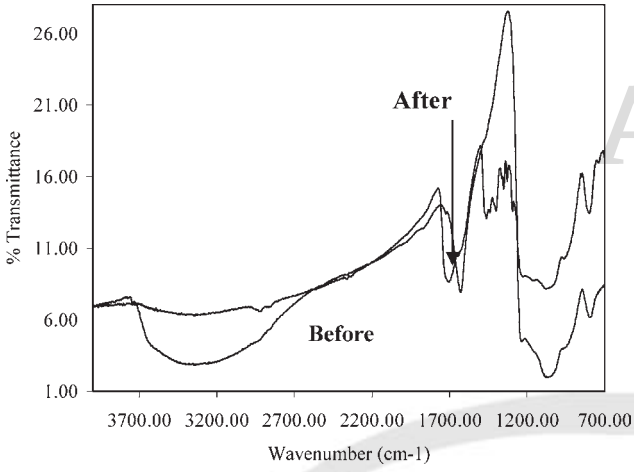

Figure 3. FTIR spectra of Al-MCM 41 before and after caprolactone polymerization.

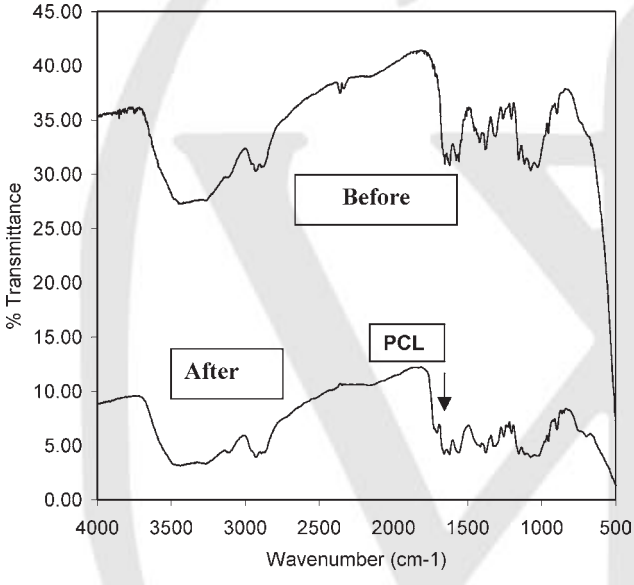

Figure 4. TBD supported on chitosan, before and after reaction.

lipases are used. The UV/visible study showed that lipases remain in solution (do not precipitate) after the addition of ethanol or dichloroethane. Figure 3 shows the FTIR spectra of recovered Al-MCM41 before and after polymerization. Before polymerization several bands appear in the OH region (near 3 500 and 1 650 cm^{-1}) as do structural bands of Si–O–Si (1 100 cm^{-1}). The recovered solid shows bands at 1 730 cm^{-1} (C=O) and in the range of 1 290 to 1 100 cm^{-1}. Several bands arose when the polymer was obtained using TBD at 1 735, 1 428 to 1 473, and from 935 to 1 214 cm^{-1}, characteristic of polycaprolactone.

Using TBD supported on chitosan, the solid recovered showed an additional band at 1 730 cm^{-1}, characteristic of polycaprolactone (see Figure 4). The residue obtained by solvent evacuation showed similar bands.

Conclusion

Al-MCM 41/butanol, guanidine/butanol, and poly(propylene) (PP)-supported CR seem interesting catalysts for polyester synthesis instead of traditional, expensive, lipase catalysts, achieving conversions of up to 90%. TBD is reported for the first time as an active catalyst in polyester synthesis and, as such, can be supported on a suitable support to be reused. Although Al-MCM 41 is covered by polycaprolactone, the polymerization procedure opens the possibility to obtain nanocomposites if an adequate particle size is used. The UV/visible method seems suitable for the analysis of caprolactone conversion when diisopropyl ether is the solvent, especially using heterogeneous catalysts. Although the conversions could be as high as reported in the open literature the time required to obtain them is shorter in our results (5 vs 24 h).

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