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Synthesis of poly(globalide-co-ε-caprolactone) in supercritical carbon dioxide

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ABSTRACT

During the last decades, the development of biodegradable/bioresorbable polymeric materials for biomedical applications has advanced considerably, aiming to improve the patient's life quality through less invasive treatments. In this context, enzymatic ring opening copolymerization of the unsaturated macrolactone globalide (Gl) and the well-known ε-caprolactone (CL), under supercritical conditions, was studied, varying Gl/CL feed ratio (10/90, 25/75, 50/50, 75/25 and 90/10). Poly(globalide-co-ε-caprolactone) (PGlCL) was synthesized using supercritical carbon dioxide ($scCO_2$) and the mixture $scCO_2$+dichloromethane (DCM) as solvents. The synthesized copolymers were evaluated in terms of its final composition (ratio between repeating units), degree of randomness and number average molecular weight (M_n). Final Gl/CL copolymer compositions were determined through ^{1}H-NMR. Differences between Gl/CL feed ratio and Gl/CL copolymer composition were not relevant for the solvents used ($scCO_2$ and $scCO_2$+DCM), i.e., none of the monomers have preferential reaction. PGlCL degree of randomness was determined through ^{13}C-NMR, and the results indicated a random distribution of the repeating units. Regarding the molecular weight, the increase in globalide content enhanced M_n values of the synthesized copolymers, in $scCO_2$ and in $scCO_2$+DCM, due to the higher molecular weight of globalide repeating units, compared to ε-caprolactone units. PGlCL synthesized in $scCO_2$ showed the highest M_n values, reaching up to 25,000 Da. The use of the solvent mixture $scCO_2$+DCM reduced the M_n values due to the dilution of the reaction media. In this way, the decrease in the monomer concentration in the surrounding of the enzyme pellets leads to lower growth of PGlCL chains.

INTRODUCTION

During the last decades, the development of biodegradable/bioresorbable polymeric materials for biomedical applications has advanced considerably, due to the interest in replacing traditional materials used as medical devices. However, the application of polymers in the human body requires a high purity degree of the material, being free of any toxic residue. In this context, the enzymatic synthesis of polymers for biomedical applications is very attractive, since enzymes do not leave toxic residues in the final product, are active under mild conditions of temperature, and provide very specific polymers [1].

The use of supercritical carbon dioxide ($scCO_2$) in substitution to organic solvents is an alternative on enzymatic ring-opening polymerization (e-ROP) reactions. $scCO_2$ is

inexpensive, non-toxic, non-flammable [2], and exhibits transport properties that can accelerate mass transfer in enzymatic reactions [3]. Also, it can be easily separated from the final product by system depressurization and can therefore be reused in the process. In order to improve the system solubilization, it is also possible to use cosolvents, together with $scCO_2$.

The synthesis of poly(ε-caprolactone) (PCL) by e-ROP using organic solvents has been extensively studied [4–6]. PCL is one of the most attractive polymers for medical application, since it is biodegradable and bioresorbable, and presents mechanical properties proper for this application [7]. Some drug delivery devices made from PCL already have FDA approval and trademark [8].

Polyglobalide (PGl) is a biocompatible and non-toxic polymer, produced from the monomer globalide [9]. Globalide is an unsaturated 16-membered macrolactone, which contains a double bond. These double bonds present in globalide structure lead to many possibilities of functionalizing the polymer, making PGl very interesting for application in medical devices, since its biocompatibility and biodegradation may be increased through functionalization.

The synthesis of copolymers composed by globalide (Gl) and ε-caprolactone (CL) units is a versatile alternative for biomedical applications, since the ratio between the monomers may be tuned, and the presence of the double bond enables the functionalization of the copolymer. As consequence, it became possible to manipulate many properties of the final material, such as molecular weight, melting temperature, hydrophilicity, affinity for different human tissues, degree of crystallinity and increase on mechanical resistance for example [10–13].

The present work aimed to study the synthesis of poly(globalide-co-ε-caprolactone) (PGlCL) copolymers by e-ROP, using $scCO_2$ and the mixture $scCO_2$+DCM as solvents. It were studied the effects of the different solvents and the globalide/ε-caprolactone feed ratio on copolymer final composition, repeating units distribution, molecular weight and dispersity.

MATERIAL AND METHODS

Material preparation

Dichloromethane P.A. 99.8% (DCM) and ethanol P.A. 99.8% (EtOH) was purchased from Vetec Química, and used as received. Novozym 435 was kindly donated by Novozymes, Brazil, A/S (commercial lipase B from *Candida antarctica* immobilized on cross-linked polyacrylate beads, esterification activity 42 U/g, measured according to a procedure adapted from literature [14]). Enzymes were dried under vacuum (0.4 bar) and 70 °C, during 16 hours [15] and stored in a desiccator over silica and 4 Å molecular sieves. Globalide was a kind gift of Symrise. ε-caprolactone were purchased from Sigma-Aldrich. Both globalide and ε-caprolactone were dried under vacuum (0.1 bar) and 100 °C, during 24 hours [15] and also stored in a desiccator over silica and 4 Å molecular sieves. Carbon dioxide (99.9% purity) used as solvent was purchased from White Martins S/A, Brazil.

Enzymatic ring-opening polymerization in pressurized solvents

Polymerization experiments were carried out in a high-pressure variable-volume view cell, with a maximum internal volume of 27 mL, with two sapphire windows for visual observation, an absolute pressure transducer (Model LD 301, Smar, USA) and a syringe pump (260HP, Teledyne Isco, Lincoln, NE, USA). The cell contains a movable

piston that allows the control of pressure inside the reactor. The variable-volume reactor allows an accurate control of pressure and temperature during reaction.

For polymerization experiments, the enzyme (Novozym-435), the monomer (globalide and ε-caprolactone) and DCM (when used) were weighed on a precision scale balance and placed inside the reactor, which was immediately closed. CO_2 was loaded into the reactor using the syringe pump, until the desired composition was achieved. The system pressure was increased up to the work pressure. Water from a thermostatic bath was used as heating/cooling fluid. The water flows inside a metallic jacket which surrounds the reactor, keeping the cell at the desired temperature. Once the desired temperature was reached, the reaction time started. The reaction media was kept under constant stirring with the use of a magnetic stirrer and a Teflon^{TM}-coated stirring bar.

To understand the influence of the different globalide to ε-caprolactone proportions on enzymatic synthesis of poly(globalide-co-ε-caprolactone) in $scCO_2$ and $scCO_2$ with cosolvent a series of assays were performed, varying the feed mass ratio of globalide/ε-caprolactone (10/90, 25/75, 50/50, 75/25 and 90/10). These ratios may also be expressed in percentage of globalide relative to the total monomer amount. With the aim of improving the mass transfer (increasing the solubility of the system), DCM was used as cosolvent. DCM has an easy separation from final products due to its low boiling point [16], and promotes good solubilization of globalide and ε-caprolactone [9].

The pressure and temperature conditions of the system were maintained constant at 120 bar and 65 °C, respecting the temperature range of higher enzyme activity [17]. Enzyme content was fixed at 5 wt% (relative to the total monomer amount). For assays which used only $scCO_2$ as solvent, the CO_2:monomers mass ratio was fixed at 1:2. For assays using $scCO_2$, and DCM as cosolvent ($scCO_2$+DCM), DCM:monomers mass ratio was maintained constant at 1:2 for all assays, while the mass ratio CO_2:MIX were 1:2 (where MIX = DCM+monomers).

The reaction conditions were chosen based on previous works [1,18,19], and the reaction time was set at 2 h. After polymerization, the material was purified through solubilization in DCM, followed by the separation of the enzymes and precipitation in cold EtOH. DCM and EtOH were used at the volumetric proportion of 1:6. The polymeric suspension was filtered and dried at room temperature in vacuum, up to constant mass.

Characterization of the copolymer

Gel Permeation Chromatography - GPC: Number average molecular weight (M_n), weight average molecular weight (M_w) and dispersity (Đ) were determined by Gel Permeation Chromatography (GPC). For the analysis, 0.02 g of the copolymer was dissolved in 4 mL of tetrahydrofuran (THF). The obtained solution was filtered through a nylon syringe filter, pore: 0.45 μm, diameter: 33 mm. The molecular weight distributions were obtained using a high-performance liquid chromatography equipment (HPLC, model LC 20-A, Shimadzu) and Shim Pack GPC800 Series columns (GPC 801, GPC 804 e GPC 807), also from Shimadzu. THF was used as eluent with volumetric flow rate of 1 $mL \cdot min^{-1}$ at 40 °C. The calibration was performed using polystyrene standards with molecular weight ranging from 580 to 9.225 x 10^6 $g.mol^{-1}$.

Nuclear Magnetic Resonance - NMR: ^1H NMR and ^{13}C NMR spectroscopy were performed on a Bruker AC-200F NMR, operating at 200 MHz for ^1H NMR and 50 MHz for ^{13}C NMR. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) 0.01% (v/v) (δ=0.00). All samples were solubilized in $CDCl_3$ (δ = 7.27 for ^1H NMR, and δ = 77.0 for ^{13}C NMR).

Poly(globalide-co-ε-caprolactone) ^{1}H NMR ($CDCl_3$ 200 MHz): δ (ppm) 5.49-5.32 (m, C***H***=C***H***), 4.10-4.04 (m, C***H₂***O(C=O)); 2.35-2.26 (m, C***H₂***(C=O)O); 2.07-1.97 (m, C***H₂***(CH=CH)); 1.71-1.62, 1.29 (m, C***H₂***).
Poly(globalide-co-ε-caprolactone) ^{13}C NMR ($CDCl_3$ 50 MHz): δ (ppm) 173.9 (***C***=O), 133.5-125.0 (***C***H=***C***H), 64.1-63.7 (***C***H_2O(C=O)), 34.4-32.0 (***C***H_2(C=O)O), 29.5-23.6 (***C***H_2).

Degree of randomness - R: The degree of randomness of the copolymer was calculated from ^{13}C NMR data through the following equations [20–22]:

$$P_{G/C} = P_{GC} + P_{CG} \quad (1)$$

$$P_{G\ total} = \frac{P_{G/C}}{2} + P_{GG} \quad (2)$$

$$P_{C\ total} = \frac{P_{G/C}}{2} + P_{CC} \quad (3)$$

$$R = \frac{P_{G/C}}{2P_{G\ total}P_{C\ total}} \quad (4)$$

where P_{GG}, P_{CC}, P_{CG} and P_{GC} represents the integral value of the GG, CC, CG and GC diads, respectively, denoting the mol fraction of each diad. $P_{G/C}$ denotes the total integral of the mixed diads (GC and CG) and $P_{G\ total}$ and $P_{C\ total}$ denotes the total mol fraction of G and C units respectively.

RESULTS

Synthesis and composition of PGlCL

The e-ROP reaction of the monomers globalide (Gl) and ε-caprolactone (CL) were carried out using $scCO_2$ and the mixture $scCO_2$ + DCM as solvents. The feed mass ratio of Gl/CL was varied (10/90, 25/75, 50/50, 75/25 and 90/10), while other parameters like pressure, temperature and enzyme content were maintained constant. Table 1 shows ^{1}H NMR spectroscopy data for samples obtained with the use of $scCO_2$ and $scCO_2$ + DCM. Differences between Gl/CL feed ratio and Gl/CL copolymer composition were not relevant for both $scCO_2$ and $scCO_2$+DCM, which means that none of the monomers reacted preferentially. All spectra obtained are in good agreement to the spectra of polyglobalide and poly(ε-caprolactone) reported in literature [9,10,23].

Table 1. ^{1}H NMR data of PGlCL copolymer composition obtained using different solvents ($scCO_2$ and $scCO_2$+DCM) and different globalide/ε-caprolactone feed ratios.

Solvent	Feed Gl/CL (mass ratio)	Feed Gl/CL (mol ratio)	NMR Gl/CL copolymer composition (mol ratio)
$scCO_2$	10/90	5/95	2/98
	50/50	33/67	27/73
	75/25	59/41	62/38
	90/10	81/19	77/23
$scCO_2$ + DCM	10/90	5/95	2/98
	50/50	33/67	37/63
	75/25	59/41	63/37
	90/10	81/19	84/16

The degree of randomness (R) was calculated from Equations (1) to (4), in order to know the distribution of the repeating units in the copolymer chains. For this calculation it were used values determined by ^{13}C NMR diads peak integration of the signal from the carbon next to the carbonyl (***C***H_2(C=O)O), between 32 and 35 ppm. PGlCL synthesized using pure $scCO_2$, with 75/25 Gl/CL feed ratio (mass), was selected for the randomness determination. As shown in Figure 1, signals from Gl-Gl repeating units (GG, 32.6 ppm) can be found next to the signals from CL-CL repeating units (CC, 34.1 ppm) in the spectrum. Gl-CL (GC, 32.0 ppm) and CL-Gl (CG, 34.4 ppm) diads can be found to be adjacent to the Gl-Gl and CL-CL peaks.

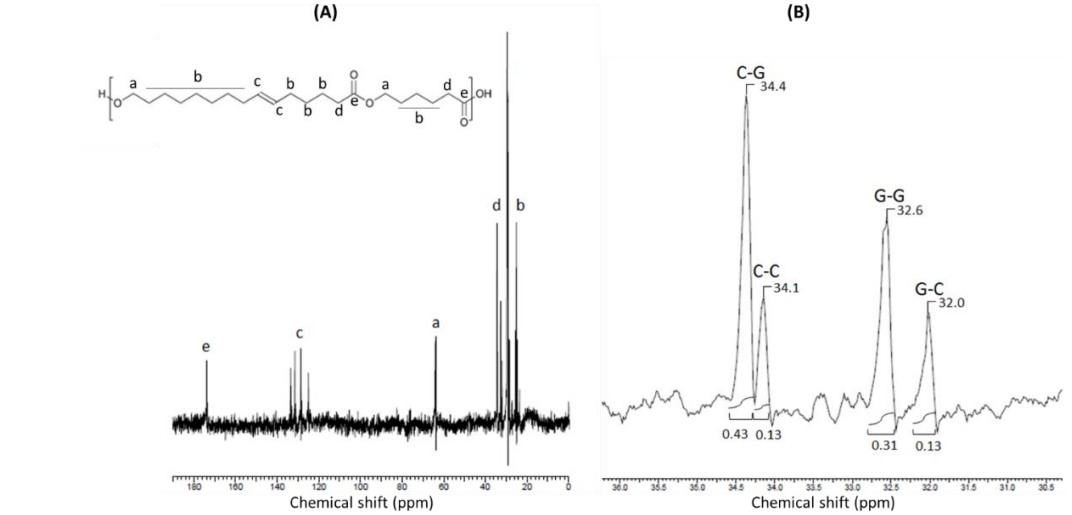

Figure 1: (A) ^{13}C NMR spectrum of PGlCL 75/25 (Gl/CL feed mass ratio). (B) Expanded ***C***H_2(C=O)O region of ^{13}C NMR spectra, with the peaks of each respective diad.

The diad values calculated by integration of the peaks were: GG=0.31, CC=0.13, GC=0.13; CG=0.43, resulting in R=1.1, indicating that PGlCL has a random distribution, which is in accordance with results obtained by different copolymerization studies involving lactones and macrolactones [12,20,24,25]. For R=1, units take a random distribution, and the probability of finding a copolymer unit belongs to Bernoulli statistics. If R < 1, the units tend to cluster in blocks of each units, R = 0 for homopolymers, whereas for R > 1 the sequence length becomes shorter, and finally, R = 2 for alternating copolymers [20,21]. The spectra obtained are in good agreement to spectra presented in literature [20,25].

3.2. Molecular weight and dispersity

Figure 2 shows M_n as function of the feed globalide content (relative to the total monomer amount) for reactions carried out in systems with: and $scCO_2$ + DCM.

For all reaction systems, M_n increased with the increase in globalide content. Globalide is a large molecule, with molecular weight twice higher than ε-caprolactone. Since the ratio between Gl and CL repeating units in the copolymer is in accordance to the monomer feed ratio, it is natural that the higher is the content of repeating units derived from globalide, higher is M_n values.

The systems with $scCO_2$, in comparison to $scCO_2$+DCM systems, provided higher M_n values. Since the experiments were carried on in a reactor with a sapphire window, it was possible to visualize that when e-ROP takes place using $scCO_2$ as solvent, the reaction media stirring was impaired and practically stops during the reaction, due to the formation of large copolymer chains, and consequent increase of the media viscosity. With a poor convection mass transfer, the monomer molecules in contact with the enzyme pellets react with the active sites of the enzymes, generating polymer chains of high molecular weights. The high concentration of monomers surrounding the enzymes enlarges the copolymer chains.

The use of DCM as cosolvent diluted the reaction media and improved convection mass transfer, in comparison to the use of only $scCO_2$. This way, there is a decrease in the monomer concentration in the surrounding of the enzyme pellets, which means smaller grow of the copolymer chains. Córdova et al. [6], Thuretch et al. [26] and Rosso Comim et al. [1] also observed that the monomer concentration has a strong influence on the polymer molecular weight. Many low molecular weight oligomeric chains may be formed in diluted systems, due to the greater difficulty of the monomer molecules to find the enzyme active sites. The formation of low molecular weight cyclic oligomers also occurs more frequently, since intermolecular reactions are not favored by the dilution of the reaction medium.

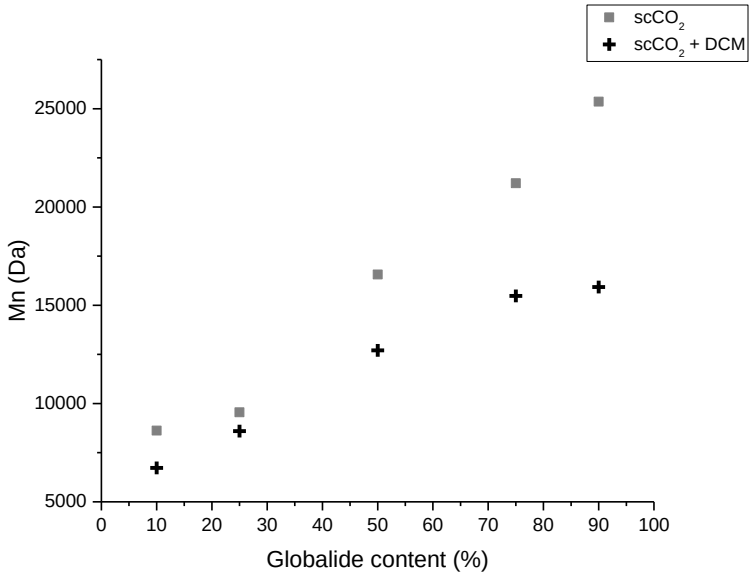

Figure 2. Number average molecular weight (M_n) as function of feed globalide content (relative to total monomer amount) using $scCO_2$ and $scCO_2$+DCM as solvent.

Dispersity (Đ) values varied from 1.3 to 1.6 for samples obtained in $scCO_2$, while for samples obtained by the use of $scCO_2$ + DCM, values varied from 1.3 to 1.7. Đ values are low, and keep practically constant for all systems evaluated, indicating an uniform polymer chain size distribution.

CONCLUSIONS

This work provided important results about the enzymatic synthesis of poly(globalide-co-ε-caprolactone) which was until now poorly reported. Reactions were performed in $scCO_2$ and $scCO_2$+DCM, under different globalide/ε-caprolactone feed ratios. The synthesis of PGlCL by e-ROP was successful, especially when conducted

under the use of $scCO_2$ as solvent, generating random copolymers of M_n up to 25,000 Da and free from toxic residues, being very interesting for future biomedical applications.

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