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Enzymatic ring opening polymerization of ω-pentadecalactone using supercritical carbon dioxide

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ABSTRACT

ω-pentadecalactone, a cyclic macrolactone can be polymerized by enzymatic ring opening polymerization (e-ROP). The resulting polyester, poly(ω-pentadecalactone) has mechanical and biocompatibility properties that make it desirable for biomedical uses, especially for bone and cartilage tissue regeneration. Conventional synthesis of poly(ω-pentadecalactone) involves acid or metal catalysts and organic solvents such as toluene that can leave toxic residuals in obtained material. In this work, e-ROP of ω-pentadecalactone was performed using supercritical carbon dioxide ($scCO_2$) as solvent, a co-solvent and Novozym 435 lipase as catalyst. Different polymerization conditions were tested to obtain high molecular weight polyesters. Reactions conducted using only $scCO_2$ as solvent showed yields around 60 wt% and molecular weights up to 33,000 g/mol. Higher molecular weights were obtained when a co-solvent was used. The results also showed a strong influence of water content on enzyme and monomer concentration in reaction medium over the final polymer molecular weight and reaction yield.

1. Introduction

Over the last years, polymerization of macrolactones has been intensively studied as a new method for the biodegradable polyesters synthesis [1–7]. Ring opening polymerization of macrolactones can be conducted using different catalysts, such as organic, organometallic and enzymatic catalysts. The use of enzymes as biocatalysts in enzymatic ring opening polymerization reactions is a promising technique since that enzymes are considered green catalysts (obtained from animal, plant and microbial sources) and enzymatic reaction generally proceeds using mild reactions conditions with resulting products without toxic metal traces [8–10].

Polyesters derived from macrolactones has been shown effective for biomedical applications due to their good mechanical and biocompatibility properties [11,12]. Among them, poly(ω-pentadecalactone) is a semicrystalline polymer obtained from readily available monomer referred in literature as pentadecanolide or ω-pentadecalactone (ω-PDL) [13]. Crystallization behavior and mechanical properties of poly(ω-pentadecalactone) are similar to that of linear high density polyethylene (HDPE), with the advantage of being degradable due to the presence of hydrolysable ester bonds in the polymer backbone [3].

Despite the use of a green catalyst in e-ROP of ω-pentadecalactone, to our knowledge, all works related in literature reported the use of toluene as the reaction media [2,3,10,14–21]. This can be explained by the fact that, generally, enzymatic polymerization using Novozym 435 is carried out at temperatures from 60 to 80 °C [22], which is higher than the boiling points of most organic solvents, while the boiling point (B_p) of toluene is above 110 °C (under standard ambient temperature and pressure – SATP). Because of the health effects of toluene exposure on the central nervous system [23,24] and cerebral dysfunctions [24,25], along with the difficulty in completely removing the solvent from final polymer, it is of great necessity to find a new "clean" solvent which may be compatible with enzyme and can provide good conditions for e-ROP of ω-pentadecalactone aiming at future biomedical applications.

A candidate solvent to replace toxic organic solvents in polymerization reactions can be supercritical carbon dioxide ($scCO_2$), a low-cost, non-toxic and non-flammable solvent [26]. $scCO_2$ shows transport properties that can accelerate mass transfer in enzymatic reactions [27] and some works point out that the use of supercritical fluids, as $scCO_2$, can improve enzyme activity and enzyme stability after a pre-treatment step before organic reaction [28,29]. Furthermore, CO_2 can be readily separated from the final product, simply

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by system depressurization eliminating completely solvent traces. In this work, along with $scCO_2$ as solvent, the use of two different co-solvents (dichloromethane and chloroform) has been proposed, aiming to improve system solubilization, reducing the required pressures and media viscosity. Both solvents were chosen based on the boiling temperature (B_p = 39–40 °C for dichloromethane and 61–62 °C for chloroform) below the melting temperature (T_m) of poly(ω-pentadecalactone), 97 °C [30]. Moreover, dichloromethane has already been used successfully in the production of biodegradable materials [31–33], chloroform is capable of well solubilizing poly(ω-pentadecalactone), as reported elsewhere [17,19,21] and both solvents have lower cytotoxicity when compared to toluene [34,35], better ecotoxicological data [36] and dermal toxicity results [37].

Though some results have been published about the solubility of ω-pentadecalactone monomer in supercritical carbon dioxide [38], the synthesis of poly(ω-pentadecalactone) using a supercritical fluid has not been reported yet in the open literature. In this context, this work aims to investigate e-ROP of ω-pentadecalactone using $scCO_2$ as solvent and dichloromethane and chloroform as co-solvents in the reaction medium evaluating their influence on final properties of poly(ω-pentadecalactone).

2. Experimental

2.1. Materials

The ω-pentadecalactone monomer was purchased from Sigma-Aldrich (98%), and dried at 100 °C in a vacuum oven (0.1 bar) for 48 h, resulting in a water content of 0.56 ± 0.06 wt%, measured by Karl-Fischer titration method (Mettler Toledo, Model DL 50, Columbus, USA). Before each experiment, dried ω-pentadecalactone (the necessary amount to conduct each reaction) was stored into a sealed vial. The immobilized lipase Novozym 435 (commercial lipase B from *Candida Antarctica*, esterification activity 28 U/g, measured according to a procedure adapted from previous works [39,40]) was kindly donated by Novozymes S/A, Brazil. Carbon dioxide as solvent (99.9%) was purchased from White Martins S/A, Brazil, and the co-solvents chloroform (99.8%, Panreac) and dichloromethane (DCM, 99.8%, Vetec Química) were used as received. Ethanol (99.5%, TecLab) was used without further purification.

2.2. Experimental apparatus

For the enzymatic synthesis of poly(ω-pentadecalactone), a variable-volume view reactor, already described elsewhere and used for polymerization of ε-caprolactone [26] and phase-equilibrium data [37–42] was employed. Briefly, the apparatus used for polymerization reactions consists in a high-pressure variable-volume view cell, with an internal volume of 27 mL. The cell contains a magnetic stirrer bar that provides agitation during reaction, two sapphire windows to allow visual observation of reaction medium, an absolute pressure transducer (Smar, model LD 301) and a movable piston which is permits to control the pressure and volume inside the reactor, manipulated through a syringe pump (ISCO, model 260D) using carbon dioxide as pneumatic fluid. To assess the desired temperatures for polymerizations, the reactor has a metallic jacket and water from a thermostatic bath was used as heating fluid. This kind of apparatus enables the use of $scCO_2$ in different solvent/co-solvent/monomer ratios, evaluating independently the effect of monomer/co-solvent ratio, monomer/co-solvent/$scCO_2$ ratio, water content on enzyme and reaction time, on the final product yielding (wt%), molecular weight (M_n and M_w) and thermal properties of produced poly(ω-pentadecalactone).

2.3. Experimental polymerization procedures

Novozyme 435, monomer ω-pentadecalactone and the co-solvent (when used) were weighed on a precision scale balance (Shimadzu, Model AY220 with 0.0001 g accuracy) and placed inside the reactor, and immediately closed. Then, the reactor was loaded with CO_2 using a syringe pump until desired composition (mass of $scCO_2$ in relation to mass of monomer). Once the desired temperature was reached (fixed at 70 °C, based on previous works [2,3,19,47]), system pressure was increased until attaining of a one-phase system between monomer, $scCO_2$ and co-solvent (when used) (200 bar), then the reaction time was started. After the end of polymerization time, the variable-volume view reactor was slowly depressurized and the synthesized material was collected from the reactor, solubilized in hot chloroform and the enzyme was separated by filtration using a cellulose filter. After filtration, the enzyme was washed again with hot chloroform. Then, the combined filtered phases were precipitated in cold ethanol (volumetric proportion of ethanol to chloroform = 10:1). The precipitate obtained was dried in a convection oven until constant weight and polymerization yield was calculated taking into account the final mass dried polymer in relation to the initial monomer mass used in the reaction.

2.4. Size exclusion chromatography

The produced poly(pentadecalactone) was evaluated in terms of molecular weight using gel permeation chromatography (GPC) in a high performance liquid chromatograph (LC 20A, Shimadzu) equipped with a refraction index detector (RID-10A), a pre-column (PLgel 5 μm MINIMIX-C guard, 50 × 4 mm, Agilent, USA) and a set of two 250 × 4.6 mm columns in series (PLgel 5 μm MiniMIX-C, Agilent, USA). Molecular weights were determined against polystyrene standards in a range from 580 to 3,000,000 g/mol. Chloroform was used as eluent at a flow rate of 0.3 mL/min. Before GPC analysis, polymeric samples were dissolved in hot chloroform (40 °C) overnight and filtered (0.450 μm, Nylon filter).

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements of poly(ω-pentadecalactone) were carried out in a Perkin-Elmer Jade DSC calibrated with zinc and indium, using approximately 9.0 mg of dried polymer. Samples were first heated from −30 °C up to 150 °C at a heating rate of 20 °C/min under nitrogen at 20 mL/min, in order to eliminate their thermal history. Then samples were cooled to −30 °C at a cooling rate of 20 °C/min, maintained at this temperature for 1 min and heated again to 150 °C at a heating rate of 10 °C/min. Melting temperature was determined from the second heating run and the material crystallinity was determined using the fusion enthalpy (ΔH_m) from the second heating run.

3. Results and discussion

3.1. e-ROP of ω-pentadecalactone using $scCO_2$ as solvent

Reactions of ω-pentadecalactone e-ROP using supercritical carbon dioxide as solvent were conducted in the variable-volume view reactor at 200 bar and 70 °C, using Novozym 435 without drying as catalyst (10 wt% in relation to monomer) and different mass ratios between ω-pentadecalactone and $scCO_2$. The mass ratio, reaction yield, molecular weight (M_n and M_w) and dispersities obtained are shown in Table 1 where it can be seen that the reaction yields were in the range of 58 to 64 wt%.

It can also be observed that increasing monomer concentration in the polymerization from 1:2 to 2:1 monomer to solvent mass ratio, the polymer molecular weight (M_w) increased from