



Enzymatically catalyzed degradation of poly (thioether-ester) nanoparticles

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ABSTRACT

Biodegradable synthetic polymeric materials have shown promising results in sustained release of drugs. The knowledge of the in vitro degradation mechanisms of these materials and the evaluation of the parameters that affect it are crucial to make the delivery system more effective. Herein, hydrolytic degradation and enzymatic hydrolysis of poly (thioether-ester) nanoparticles synthesized via thiol-ene polymerization in miniemulsion using α,ω -diene diester monomer from renewable resources were examined. The in vitro degradation was carried out at 37 °C, in an acid solution, containing hydrochloric acid (HCl) (0.01 M, pH 2.8) and phosphate buffer solution (PBS) (0.2 M, pH 7.4), with or without 5% (w/w) of *Candida Antarctica* Lipase B. The change in molar mass, particle size and size distribution, morphology and functional groups during the degradation process were evaluated. The enzymatic degradation of the polymeric nanoparticles was accentuated, showing the hydrolysis of the ester bond with 90% of molar mass reduction in 240 h, while in the acidic solution this reduction was only 37% after 3 months of degradation. In PBS solution, no alteration was observed up to 3 months.

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1. Introduction

Biodegradable synthetic and natural polymeric materials have been used for various purposes, such as wastewater treatment, biotechnology, food and healthcare, bone tissue engineering and temporary implant materials [1,2]. In recent years, these polymers have been extensively studied for controlled drug release, due to their biocompatibility and biodegradability properties. The biodegradability can be defined as the gradual decomposition of the polymer chain in a specific biological activity, with the formation of products that are easily metabolized and eliminated from the human body [3,4] or even degrading into different components, such as carbon dioxide, water and inorganic, by the action of enzymatic processes [5].

Several studies have reported the use of aliphatic polyesters, such as poly (lactic acid), poly (*L*-lactic acid), poly (*D*-lactic acid), a poly (lactic-co-glycolic acid) and polycaprolactone, showing their

degradation potential [6–13]. Factors such as structure, chemical composition, crystallinity, molar mass and dispersity ($\bar{D} = M_w/M_n$) of the polymer, surface area and morphology affect the degradation kinetics and the degradation mechanism by hydrolysis or by enzymatic catalysis. For instance, Leimann et al. (2013) [14] have shown that for polymers as poly(hydroxybutyrate-co-hydroxyvalerate) degradation kinetics are highly dependent on the surface area of the polymer material and, thus, nanoparticles present a quite different degradation behavior from microparticles or bulk materials.

Lipase B is an enzyme belonging to the class of hydrolases, from *Candida Antarctica*, produced by fermentation of the microorganism *Candida sp.* Lipase B is known for being active in a wide range of pH, presents high stability, operates under mild reaction temperatures, is highly specific and when used as a reaction catalyst has the advantages of greater efficiency and lower energy consumption [15,16]. In addition, it has high specificity and regioselectivity, degrading the polymeric material by breaking ester bonds [10,17–19], forming initially oligomers and, after longer degradation times, monomeric units, having as byproducts, in the case of polyesters, carboxylic acids, alcohols and hydroxy acids [20].

The polymerization via thiol-ene addition reactions using

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monomers with α,ω -diene diester bonds, has been recently proven as an effective tool in obtaining biodegradable polymeric materials, allowing the insertion of new functional groups in the polymer chain, such as ether, ester, amide and sulfide bonds resulting from the thiol group, providing degradability characteristics to the material [20–22]. These addition reactions occur following three stages: initiation, propagation and termination. They stand out due to their high yields, for being conducted under mild conditions, usually in water, with low formation of byproducts, presenting a fast polymerization with improved degradability characteristics and to result in a linear semicrystalline poly(thioester-ester) [23–28]. Machado et al. copolymerized the renewable monomer dianhydro-D-glucityl diundec-10-enoate (DGU) with 1,4-butanedithiol through miniemulsion polymerization via thiol-ene addition reaction and formed spherical nanoparticles (200 nm) of a polymer with semi-crystalline structure and molar mass of approximately 20 kDa.

Recent studies involved the degradation of polymers synthesized from the thiol-ene addition reactions. Anseth's group [28] was the first to study the degradation of thiol-acrylate hydrogels proving that it is possible to obtain polymers with specific degradation profiles by varying the thiol:acrylate stoichiometry, which allows a kinetic control of the degradation reaction that occurs by hydrolysis of the ester group, present in the polymer chain. In 2007, Rydholm, Anseth & Bowman [20] evaluated the influence of the distance between the sulfide group and the ester functional group of acrylates, concluding that the decrease in number of carbons and the increase in pH of the degradation medium led to an increase in the ester bond hydrolysis rate by 4 times. In addition, molar mass distribution curves were shifted to lower molar masses when using less crystalline polymers under acidic conditions having a higher rate of degradation in acidic medium.

The study of the degradation of nanoparticles synthesized by these thiol-ene addition reactions using monomers from renewable sources is still rare and a larger number of studies of the *in vitro* degradation behavior is required. The degradability and stability of such particles is an important factor to be taken into account when it comes down to their biomedical applications as nanocarriers. For example, the stability of these nanoparticles in PBS (7.4) and the possibility of enzymatically triggered release towards degradation. In this context, this work aims to evaluate the hydrolytic stability in PBS, sensibility to acidic medium and enzymatic hydrolysis of poly(thioether-ester) nanoparticles obtained by polymerization in miniemulsion via thiol-ene reactions.

2. Experiments

2.1. Materials

Miniemulsion polymerizations were performed using α,ω -diene diester dianhydro-D-glucityl diundec-10-enoate (DGU), synthesized by Machado et al. [25], and 1,4-Butanedithiol ($\text{Bu}(\text{SH})_2$) as monomers; azobisisobutyronitrile (AIBN, 98%, Vetec), which was recrystallized in methanol and dried under vacuum, as initiator; Lutensol AT50 an alkylpolyethylene glycol ether (molar mass 2.8 kDa, BASF) as surfactant. Distilled water was used in all experiments. For nanoparticles degradation assays the following reactants: sodium dihydrogen phosphate (NaH_2PO_4 , P.A 99%, Vetec); sodium hydrogen phosphate (NaHPO_4 , P.A 98%, Vetec); hydrochloric acid (HCl, Dinâmica, P.A. 36.5%); *Candida Antarctica* lipase B free in liquid state (CalB, Novozymes Latin América, Ltda, Araucária, PR, Brasil) previously concentrated; *p*-nitrophenyl palmitate (pNPP) (Sigma-Aldrich, $M_w = 377.52 \text{ g mol}^{-1}$) and sodium carbonate (Na_2CO_3 , Vetec) were used. Tetrahydrofuran (THF, Vetec) was used in the GPC analyses.

3. Methods

3.1. Miniemulsion polymerization

The formulation for the nanoparticles synthesis was optimized by Machado et al. [26]. The aqueous phase was prepared solubilizing 0.15 g of Lutensol AT50 in 5 mL of distilled water under magnetic stirring (150 rpm) for 5 min. For the organic phase, 1 g of DGU monomer and 3.4 mg of AIBN initiator were solubilized under magnetic stirring (150 rpm) for 15 min. The aqueous phase was added to the organic phase under a magnetic stirring (500 rpm) for 15 min, then, 1,4-butanedithiol ($\text{Bu}(\text{SH})_2$) was added to system (1:1 M ratio DGU: $\text{Bu}(\text{SH})_2$) and stirred (200 rpm) for 5 min. Afterwards, the course emulsion was sonicated for 2 min with a Fisher Scientific Sonic Dismembrator (model 500) and a 1/2" tip in ice bath, at 70% of amplitude in pulse regime of 10s, with 5s pause. Finally, the miniemulsions were polymerized in a thermostatic bath at 80 °C for 4 h. Reactions were performed in duplicates. Fig. 1 shows the chemical structure of the polymer.

3.2. Poly(thioether-ester) nanoparticles degradation

In vitro nanoparticles degradation was conducted by adding 1 mL of the latex in three degradation solutions: 2 mL of hydrochloric acid (pH 2.8, 0.01 M) and in 2 mL of the PBS solution (pH 7.4, 0.2 M) with or without CalB 5% (w/w) enzyme. This assays was carried out at 37 °C in a bacteriological oven, model SP-101/42 SPLABOR. Previously, enzyme system was concentrated following the method described by Chiaradia 2015 [16] and the determination of enzymatic activity was performed according to Chiou & Wu (2004) [29], where one unit of enzyme (U) was defined as the amount of enzyme which catalyzed the production of 1 mmol *p*-nitrophenol per minute. The degradation was evaluated until complete degradation of the NPs in enzymatic solution and for the PBS and acidic solution during three months.

3.3. Poly(thioether-ester) nanoparticles characterization

3.3.1. Particle size measurements

The intensity average particle diameter (DP) and dispersity (Pdl) were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS equipment (Malvern Instruments). For the analysis, a drop of the sample, prepared by the miniemulsion polymerization technique, was placed in a glass cuvette and diluted with 1.5 mL of distilled water.

3.3.2. Poly(thioether-ester) nanoparticles morphology

The degradability of the poly(thioether-ester) nanoparticles was estimated by the morphological changes and particle size of the nanoparticles. The samples were diluted to a concentration of 0.05% of solids. Subsequently, a drop of the diluted nanoparticle

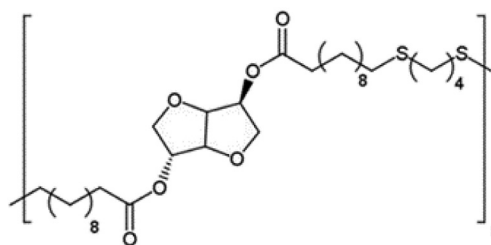


Fig. 1. Structure of the thioether-ester polymer produced in thiol-ene copolymerizations in miniemulsion using α,ω -diene diester dianhydro-D-glucityl diundec-10-enoate and 1,4-Butanedithiol ($\text{Bu}(\text{SH})_2$) as monomers.

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