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Ternary cyclodextrin polyurethanes containing phosphate groups: Synthesis and complexation of ciprofloxacin

Mirna Pereira Moreira [a], George Ricardo Santana Andrade [a], Marcia Valeria Gaspar de Araujo [b], Tatiana Kubota [b], Iara F. Gimenez [a,c,*]

[a] Programa da Pós-graduação em Ciência e Engenharia de Materiais, Av. Marechal Rondon s/n, Cidade Universitária "Prof. José Aloísio de Campos", CEP 491000-000, São Cristóvão, SE, Brazil
[b] CARBOPOL, Laboratório de Síntese de Polímeros Derivados de Carboidratos, Faculdade Pio Décimo, Av. Presidente Tancredo Neves, 5655, Jabotiana, Aracaju, SE, 49095000, Brazil
[c] Programa de Pós-graduação em Química, Av. Marechal Rondon s/n, Cidade Universitária "Prof. José Aloísio de Campos", CEP 491000-000, São Cristóvão, SE, Brazil

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ABSTRACT

Synthesis of ternary polyurethanes (PUs) from hexamethylenediisocyanate, β-cyclodextrin and β-glycerophosphate (acid and calcium salt) was studies varying synthesis parameters such as monomer proportion, heating method (reflux and microwave), and catalyst amount. Favorable conditions were provided by microwave irradiation and use of β-glycerophosphoric acid although the results suggest that it is possible to obtain ternary PUs with the calcium salt. FTIR data indicated the existence of secondary urea linkages. After characterization of ternary PUs by FTIR spectroscopy, XRD and thermal analysis, as well as evidences that the cyclodextrin cavities remained active toward inclusion of guest molecules, the possibility of inclusion of the antibiotic ciprofloxacin was evaluated. Absence of ciprofloxacin melting peak in DSC curves indicated that it is molecularly dispersed within the polymer, possibly included in the cyclodextrin. In vitro release experiments suggested additional non-inclusion interactions, showing also that the use of dialysis membranes may mask the actual release profile.

1. Introduction

Polyurethanes are formed by the reaction of a diisocyanate and a polyol to yield polymers containing the urethane bond (—NH—COO—). Owing to properties such as mechanical flexibility, biocompatibility and nontoxic degradation products, polymers belonging to this class are commonly used in medical applications and devices. Examples include catheters, aortic balloons, total artificial hearts, and mammary implants among others (Burke & Hasirci, 2004). Another crucial characteristic of polyurethanes is the versatility of compositions, resulting from a great variety of commercially available monomers, allowing for rational design of polymers to execute specific functions. In this context biocompatible polyurethanes are often used not only as implants for tissue repair but also as drug delivery systems (Cherng, Hou, Shih, Talsma, & Hennik, 2013). Polyurethanes as drug delivery systems can be loaded with drug molecules through adsorption as well as covalent binding of drug molecules to chain segments. Adsorption can be classified as either physical or chemical depending on the nature of interactions, favored by specific functional groups depending on the drug chemical nature. The presence of ionic moieties in the polymer structure may also be advantageous in some cases. Regarding covalent binding, drug molecules containing hydroxyl groups can react with isocyanates to yield polyurethane chains linked to drug molecules which can be further released through polymer degradation. Combined use as drug delivery systems and implant can also be achieved with polymers, combating device infection in situ (Macocinshi et al., 2014). The introduction of local drug delivery systems based on polyurethane efficiently modulated inflammation, angiogenesis, and fibrosis induced by a microporous polyurethane sample in experimental animals (Macocinshi et al., 2014).

As widely used drug carriers, cyclodextrins have been used as monomers for the preparation of polymers for drug delivery, not only belonging to the polyurethane class (Halpern, Gormley, Keech, & von Recum, 2014) but also including carbonates, esters

* Corresponding author at: Programa da Pós-graduação em Ciência e Engenharia de Materiais, Av. Marechal Rondon s/n, Cidade Universitária "Prof. José Aloísio de Campos", CEP 491000-000, São Cristóvão, SE, Brazil.
E-mail address: gimenez@ufs.br (I.F. Gimenez).

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and polyamidoamines (Tejashri, Amrita, & Darshana, 2013). Those polymers were called nanosponges, since cyclodextrins (CD) are cyclic oligosaccharide molecules exhibiting nanometric cavities with diameters of 0.57 nm, 0.78 nm and 0.95 nm for α-, β-, and γ-CD respectively (Szejtli, 1998). The attractive properties of cyclodextrins include their biocompatibility and ability to form inclusion complexes by the non-covalent inclusion of poorly water soluble drugs into their hydrophobic cavity. This approach may improve the water solubility of the drugs, as well as increasing its stability and promoting controlled release for prolonged periods of time (Tejashri, Amrita, & Darshana, 2013).

The preparation of polyurethanes modified by phosphate groups is appealing for several reasons such as the potential effects of ionic groups in modifying mechanical and thermal behavior of the polymers (Jaudouin, Robin, Lopez-Cuesta, Perrin, & Imbert, 2012; Kakati & George, 1993), and the flame retardancy potential of phosphorus compounds (Velencoso et al., 2011). In terms of biocompatibility, the incorporation of bioactive agents into polyurethane chains have been described through the use of β-glycerophosphate (Beckman, Hollinger, Doll, Guelcher, & Zhang, 2015) and other agents such as dexametasone and citric acid (Zhang, Doll, Beckman, & Hollinger, 2003). In this context, the bioactivity of β-glycerophosphate was also demonstrated as initiator of matrix mineralization in MC3T3-E1 cell cultures (Fratzl-Zelman et al., 1998).

Here we designed novel polyurethanes containing simultaneously β-CD and β-glycerophosphate groups cross-linked by hexamethylenediisocyanate, taking advantage of the potentialities of both polyols: the inclusion capacity of β-CD toward hydrophobic drug molecules and the bioactivity of β-glycerophosphate. This work focuses on the polymer synthesis and characterization, complexation of the antibiotic ciprofloxacin and in-vitro release test. Our aim was to contribute to the development of a ternary polymer with the components cited and to show its capacity of inclusion and release of the antibiotic ciprofloxacin. However, the materials obtained here have potential as component in bone implants delivering therapeutic agents to prevent infections, in an approach already described in several papers (Brooks, Sinclair, Grainger, & Brooks, 2015; Lepretre et al., 2009). Finally, in this work we used microwave technique to synthesize polymers as recently reported as advantageous by Biswas and co-workers (Biswas, Appel, Liu, & Cheng, 2015).

2. Materials and methods

1,6-hexamethylenediisocyanate (HDI), β-cyclodextrin hydrate β-CD, calcium β-glycerophosphate (Ca-Glic), tin octanoate, and ciprofloxacin were purchased from Sigma. Phenolphthalein, KH_2PO_4, K_2HPO_4, Methyl alcohol (PA), dimethylformamide (DMF) were purchased from Vetec and used without further purification.

2.1. Polyurethane synthesis

Prior to polyurethane synthesis, an aqueous solution of Ca-Glic was passed through an ion-exchange column of the strongly acidic resin, Amberlite IR-120(H) (Sigma), as previously reported by Kakati & George (1993) to yield β-glycerophosphoric acid (H-Glic), with improved solubility in DMF used as reaction solvent. Polyurethanes were synthesized in a two-step procedure. First, NCO-terminated pre-polymers were prepared by the reaction of HDI and Ca-Glic (or H-Glic) in DMF (amounts according to Table 1). Reactions were carried out under reflux and also with microwave heating using a CEM MARS digestion system (see Table 1 for conditions). In the second step, a DMF solution of β-CD was slowly added followed by addition of tin octanoate. After the heating time, the polymers were precipitated by the addition of ethyl alcohol, followed by cooling to 5 °C for nearly 12 h, vacuum filtration, washing with acetone (3 × 50 mL) and drying in a vacuum oven at 80 °C for 24 h.

2.2. Evaluation of phenolphthalein inclusion

The procedure adopted here was based on previous works (Makela, Korpela, Puisto, & Lakso, 1988; Mohamed, Wilson, & Headley, 2010). Briefly a 3.75 mmol L^{-1} stock solution of phenolphthalein was prepared in a 94% ethanol:6% water (v/v) mixture. This solution was diluted in a proportion 1:10 with distilled water and the pH adjusted to 10 by the addition of 1 mol L^{-1} Na_2CO_3 aqueous solution. The resulting solution was divided in aliquots of 10 mL and placed in three flasks to which increasing masses of the polymer were added (10, 20, and 30 mg). After sonication for 4 h at 25 °C, the suspensions were centrifuged and the absorbance of the supernatant read at 552 nm. Experiments were performed in triplicate.

2.3. Complexation of ciprofloxacin

Formation of inclusion complexes of ciprofloxacin in the ternary PUs was carried out by kneading, which involves grinding solid components in the presence of minimum amounts of methyl alcohol in an agate mortar for 2 h. Different polymer:drug mass proportions were evaluated (1:2, 1:1, 1:0.5, 1:0.1, and 1:0.01) were also compared with simple physical mixtures, prepared by gently mixing without grinding.

2.4. In vitro release

This study was carried with and without dialysis membranes (MWCO 2000 Da, Sigma) and both the masses of inclusion complexes/free drug and total volumes were properly chosen in order to ensure sink conditions. The samples with 1:0.01 (carrier:drug) mass proportion were chosen for this study for having the least fraction of uncomplexed drug. Solid inclusion complex (0.101 g) was placed in the dialysis bags which were suspended into 500 mL phosphate buffer at pH 7.4 and 25 °C (triplicate, in sealed flasks), under constant stirring (slow rate, 300 rpm). Aliquots were taken at scheduled time intervals, starting at after initial 10 min of contact and the absorbance in UV/visible spectroscopy read at 270 nm. Analogous experiments were carried out with the free drug (the same mass present in the inclusion complex). Experiments without dialysis tubings were also performed.

2.5. Characterization

Samples were characterized by the following techniques: Fourier-Transform Infrared Spectroscopy (FTIR), using a Perkin Elmer Spectrum BX for analysis of KBr discs; Powder x ray diffraction (XRD) using a Rigaku Mini Flex II diffractometer, with Cu κα source (λ = 1.5418 Å), 30 kV, 15 mA in the 10–60° in 2θ; Termogravimetric analysis (TGA) curves were acquired with a TA Instruments SDT 2960, under N_2 flow (100 mL/min) and a heating rate of 10 °C/min in aluminum pans; UV/visible spectra were acquired with a Varian Cary 100 Scan. Solid state ^{13}C NMR data were acquired on a Bruker AC–300 P NMR spectrometer, using a 5 mm Bruker probe. Bloch decay with high power proton decoupling (HPDEC, with a pulse delay of 0.3s); pulse sequences were used to acquire spectra at the spinning rate of 10 kHz.