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Homogeneous hydrolytic degradation of poly(lactic-co-glycolic acid) microspheres: Mathematical modeling

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ABSTRACT

The homogeneous hydrolytic degradation of poly(lactic-co-glycolic acid) (PLGA) microspheres was investigated. A mathematical model was developed that estimates the evolutions of ester bonds concentrations and average molecular weights along the degradation process. The model is based on a detailed kinetic mechanism that includes the hydrolysis of the different types of ester bonds by random chain scission and considers the effect of polymer chemical composition and molecular structure. Novel and published experimental data were used to adjust and validate the model. The experimental work consisted of homogeneous hydrolytic degradation of PLGA microspheres. The predictions are in very good agreement with the experimental results.

1. Introduction

Biodegradable polymer systems have received considerable attention for drug delivery in pharmaceutical and biomedical fields [1,2]. These systems allow to effectively control the drug release within the desired therapeutic range, avoiding consequences of an excess or deficit, which could compromise its effectiveness. Specially, the family of aliphatic polyesters, such as poly(lactide) (PLA), poly(glycolide) (PGA) and their copolymer, poly(lactide-co-glycolide) (PLGA) has been widely employed for these purposes due to their biodegradable and biocompatible properties [3–6]. These polymers are safe for the body and are hydrolyzed to metabolic products [7,8].

The degradation of aliphatic polyesters has been investigated by many authors, both *in vitro* and *in vivo* [9,10]. In particular, PLGA microspheres have been widely studied for drug delivery applications and their degradation is known to be affected by the system preparation method [11,12], by polymer properties such as initial molecular weights, devices morphology and lactide/glycolide ratio of the copolymers [8,13,14], as well as by physical and chemical parameters such as temperature and pH of the external medium [15]. Many studies have also been carried out to evaluate the effect of particle size [16,12], number of carboxylic end groups [17] or enzymes in the external medium [18] on the degradation behavior of poly(α-hydroxy acids). In an aqueous environment, PLGA degrades through bulk erosion. Water penetrates the device and reaches a saturation level very rapidly in comparison with polymer degradation [19]. Water molecules attack ester bonds in the polymer chains through hydrolysis reactions and the chain cleavage produces shorter chains with alcohol (−OH) and carboxylic (−COOH) groups. The carboxylic end groups can act as a catalyst to accelerate the hydrolysis reaction [20]. In addition, the restricted diffusion of degradation products can result in high concentration of carboxylic end groups inside the materials and produce heterogeneous degradation, in which the center of the material is degraded faster than its surface [21,22]. It was shown that large size PLGA devices degraded heterogeneously while small-sized devices such as thin films and microspheres degraded homogeneously [13]. During hydrolysis, the molecular weight of the polymer decreases due to chain scission and when it is small enough, the oligomers can dissolve in the surrounding medium causing the mass loss of the system. Antheunis et al. [23] determined that the critical molecular weight of PLGA oligomers dissolution in PBS buffer at 37 °C was 850 g mol^{-1}. This result suggests that, for this polymer and for the studied conditions, oligomers up to about thirteen monomer units are buffer-soluble. Also, this value corresponds to those reported by Schliecker et al. [24] for PLA.

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Many models have been proposed to describe PLGA degradation, erosion and drug release from the bulk polymer [20]. Typically, polymer degradation is assumed to follow a pseudo first-order kinetics where degradation rate is a function of water, ester bonds or acid catalyst concentrations. The concentrations of the other two species are either ignored or assumed constant [25,26]. In addition, second-order, autocatalytic hydrolysis kinetics for PLA and PLGA have been modeled in several reports [23,27,28,19], where the catalyst and ester bonds concentrations were allowed to vary while the water concentration was assumed constant during degradation. Nishida et al. [28] used moment analysis to predict the change in the average molecular weight of aliphatic polyesters subject to catalysis by carboxylic end groups. Han and Pan [19] developed a model for degradation of bioresorbables polyesters taking into account the autocatalytic hydrolysis reaction and oligomer production and diffusion. The model can predict the evolution of molecular weight distribution in a device as well as the weight loss as a function of time. Antheunis and coworkers [23] proposed a mathematical model based on the autocatalytic kinetics of aliphatic polyesters hydrolysis and it is able to describe the decrease of the average molecular weight and also to reasonably predict the mass loss trend of the polymer phase as a function of degradation time. This model was simplified to develop a basic model that describes the decrease of the number average molecular weight for aliphatic polyesters before mass loss occurs [27].

In the present work, a new mathematical model that simulates the homogeneous degradation of PLGA microspheres is developed. The model takes into account the autocatalytic effect of carboxylic groups and the polymer composition on the polymer degradation rate and it is based on a detailed kinetic mechanism that considers the hydrolysis of the different types of ester bonds in the copolymer by random chain scission. It allows to estimate the evolutions of the ester bonds concentrations and average molecular weights throughout the degradation process and it is also able to predict the mass loss and pH profiles of the system. The model was adjusted and validated with published [29] and new experimental data.

2. Materials and methods

2.1. Materials

PLGA 50/50, Resomer® RG 502H, weight-average molecular weight (M_w) 8650 Da (Boehringer Ingelheim Pharma KG, Ingelheim, Germany), ethyl acetate, tetrahydrofurane (THF), dichloromethane, polyvinyl alcohol (PVA), sodium hydroxide and sodium azide (NaN_3) were provided by Sigma–Aldrich (Argentina) at reagent grade and they were used without further purification. Distilled and deionized water was used to prepare all the solutions.

2.2. Microsphere preparation

Emulsions and PLGA microspheres were prepared based on a method propossed by Sah et al. [30]. The emulsion (O/W) was obtained by dissolving 1 g of PLGA in 4.5 mL of ethyl acetate, this solution was called phase 1. Phase 2 was composed of 15 mL of a PVA aqueous solution (2% w/v). Phase 1 was added dropwise into the mechanically stirred phase 2 using an Ultra-Turrax T25D homogenizer (dispersing element S25N-18G, IKA, Germany) at 3400 rpm. Stirring was increased to 4000 rpm for 15 min to finally obtain the O/W emulsions. Then, water was added to the emulsion and it was magnetically stirred for 50 min until complete evaporation of ethyl acetate from microspheres. Finally, the solid spheres were collected by centrifugation at 2750 rpm for 5 min, lyophilized and stored at −20 °C until further assays.

2.3. Degradation studies

Approximately 65 mg of dry microspheres were placed into a glass vial with sodium azide in d-water (0.02% w/w) as the degradation medium. The vials were orbitally shaken at 50 rpm in an oven with digital temperature control at 37 °C. The degradation medium was removed at different times and the polymer sample was dehydrated under vacuum at room temperature.

During degradation, pH evolution was monitored with an Orion potentiometer. At each time point, four randomly selected samples were centrifuged for 5 min at 3500 rpm to determine the pH of the medium. Then, samples were resuspended with vortexing for 2 min and placed again to continue the degradation test.

2.4. Characterization

2.4.1. Microsphere size distribution

The microsphere suspensions were observed in an optical microscope coupled with a Leica DM 2500M DFC 290HD camera. The particle size distributions were determined by analyzing the images with the Image Pro Plus software.

2.4.2. Mass loss determination

Samples were collected by centrifugation at 3500 rpm for 5 min and then washed with d-water. This step was repeated three times. Then, the remaining mass was dried under vacuum up to constant weight and examined for weight loss. Polymer mass loss was determined gravimetrically using Eq. (1).

$$\%\ Mass\ loss = \frac{w_t}{w_0} \times 100 \quad (1)$$

where w_0 is the initial weight of microspheres and w_t is the weight of the dried microspheres after the incubation period.

2.4.3. Molecular weight of polymer

The average molecular weights of polymer were determined by size exclusion chromatography (SEC). A Waters 1525 pump with four Ultrastyragel® columns (HR 0.5, HR 1, HR 2, HR 3, 7.8 mm × 300 mm, 5 μm) and a Waters 2412 refractive index detector were used with tetrahydrofuran as eluent, with a flow rate of 1.0 mL min^{-1}, at 25 °C. Polystyrene (PS) standards (Shodex SM-105, Showa Denko) were used for calibration.

2.4.4. Morphology studies

The morphology of microparticles was studied by scanning electron microscopy (SEM). Samples were put over an aluminum stub and were then sputter coated with gold under argon atmosphere (SPI Supplies, 12157-AX) and examined using an acceleration voltage of 20 kV, in a JEOL JSM-35C equipped with the image acquisition program JEOL SemAfore.

2.5. Degradation experiments of Blanco et al. [29]

For model adjustment and validation, experiments performed by Blanco et al. [29] were also considered. The experimental work consisted of *in vitro* degradation of PLA, PLGA 50/50 and PLGA 75/25 microspheres (Exps. 1, 2 and 3, respectively) prepared by the spray drying method. For each polymer sample, test tubes of polyethylene containing 40 mg of microspheres and 3 mL of phosphate buffer (1 mM, pH 7.4) were prepared and incubated in darkness at 37 °C. Test tubes were stirred once a day for 1 min in a vortex. At appropriate time intervals, the phosphate buffer was removed and the polymer sample was dried under vacuum. The maximum incubation time was 5 months. The average molecular weights and