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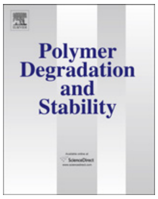

Hydrolytic and enzymatic degradation of a poly(ε-caprolactone) network

I. Castilla-Cortázar[a], J. Más-Estellés[a,*], J.M. Meseguer-Dueñas[a,b], J.L. Escobar Ivirico[a], B. Marí[c], A. Vidaurre[a,b]

[a] Centro de Biomateriales e Ingeniería Tisular, Universitat Politècnica de València, camino de vera s/n, 46022 Valencia, Spain
[b] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Valencia, Spain
[c] Departamento de Física Aplicada-IDF, Universitat Politècnica de Vàlencia, 46022 Valencia, Spain

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ABSTRACT

Long-term hydrolytic and enzymatic degradation profiles of poly(ε-caprolactone) (PCL) networks were obtained. The hydrolytic degradation studies were performed in water and phosphate buffer solution (PBS) for 65 weeks. In this case, the degradation rate of PCL networks was faster than previous results in the literature on linear PCL, reaching a weight loss of around 20% in 60 weeks after immersing the samples either in water or in PBS conditions. The enzymatic degradation rate in Pseudomonas Lipase for 14 weeks was also studied, with the conclusion that the degradation profile of PCL networks is lower than for linear PCL, also reaching a 20% weight loss. The weight lost, degree of swelling, and calorimetric and mechanical properties were obtained as a function of degradation time. Furthermore, the morphological changes in the samples were studied carefully through electron microscopy and crystal size through X-ray diffraction. The changes in some properties over the degradation period such as crystallinity, crystal size and Young's modulus were smaller in the case of enzymatic studies, highlighting differences in the degradation mechanism in the two studies, hydrolytic and enzymatic.

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1. Introduction

The use of non-permanent scaffold materials that, over time, become completely replaced by natural extracellular matrix is central to the tissue engineering approach. The most important role of the use of scaffold *in vivo* is that it should persist in a robust state for sufficient time to allow the formation of new tissue, but also ultimately degrading and becoming replaced by this tissue. For its successful implementation in applications such as surgical sutures, drug delivery systems, and tissue engineering scaffolds, hydrolytic degradation is of crucial importance [1,2].

The terminology used to define synthetic polymer breakdown is not consistent in the literature. In 1992, Vert *et al.* [3] proposed the definitions of *biodegradable*, *bioresorbable*, *bioabsorbable* and *bioerodable*. According to these definitions, poly(ε-caprolactone), PCL, is a semi-crystalline, bioresorbable polymer belonging to the aliphatic polyester family.

The hydrolytic degradation is an autocatalytic process, where the carboxylic groups of the hydroxy acids produced catalyse further hydrolysis [4]. It is widely accepted that hydrolytic degradation of poly(α-hydroxy) esters can proceed via surface or bulk degradation pathways [2]. The diffusion–reaction phenomenon determines the means by which this pathway proceeds. The advantage of surface degradation is the predictability of the process, but surface and bulk degradation are ideal cases to which most polymers cannot be unequivocally assigned [2]. In tissue engineering, surface properties or porosity determine the performance of implantable scaffolds [5].

The enzymatic degradation of PCL polymers has also been studied, especially in the presence of lipase-type enzymes [6–9]. Three kinds of lipase were found to significantly accelerate the degradation of PCL: Rhizopus delemer lipase [6], Rhizopus arrhizus lipase, and Pseudomonas lipase [7,8]. Highly crystalline PCL was reported to totally degrade in 4 days in the presence of Pseudomonas lipase [7,8], in contrast with hydrolytic degradation, which lasts several years.

Accelerated degradation could be achieved using an acidic or basic medium, which would enhance the hydrolysis of polyesters; this would also mimic physiological conditions better than other methods, such as temperature acceleration. The aim of accelerated degradation systems is to accomplish the degradation of polymeric devices within a shorter period of time, thus enabling the study of morphological and chemical changes during degradation in a more acceptable timeframe [4,10,11]. In this way,

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* Corresponding author. Tel.: +34 963877000x88937; fax: +34 963877529.
E-mail address: jmas@fis.upv.es (J. Más-Estellés).

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researchers may anticipate analogous results by altering the rate of reaction whilst maintaining identical principal mechanisms of reaction. However, such data should always be verified against long-term data.

The rate of hydrolytic degradation of ester linkages is affected by a multitude of factors. In general, actions which increase the penetration of water accelerate the rate of hydrolysis (such as using, or blending with, a more hydrophilic polymer). Water absorption is thus a critical factor [12]. Two other important factors are the polymer's glass transition temperature (T_g) and crystallinity, both of which reflect the ability of water to access the polymer chains. A high T_g corresponds to relatively limited molecular motion and low free volume within the polymer network, meaning that less space is available for water molecules to penetrate. Similarly, a high degree of crystallinity limits hydration through the ordered packing of polymer chains. Any action reducing T_g and crystallinity will accelerate hydrolytic degradation.

PCL is one of the most widely studied synthetic polymers and has FDA approval in various devices for medical applications [12]. However, applications of PCL might be limited by the degradation and resorption kinetics of PCL, which are considerably slower than other aliphatic polyesters due to its hydrophobic character and high crystallinity [13]. One way of avoiding crystallinity, and consequently increasing the degradation rate, is to prepare polymer networks. For example, PCL macromers have been synthesized through the reaction of PCL diol with acryloyl chloride and PCL networks were photopolymerised [14]. Thermal, mechanical, and morphological characteristics as well as the degradability of the PCL networks were studied. Bat *et al.* [15] developed a method to obtain form-stable and elastic networks upon gamma irradiation based on high molecular weight (co)polymers of trimethylene carbonate and (ε-caprolactone). The *in vitro* enzymatic erosion behaviour of these hydrophobic networks was studied using aqueous lipase solutions.

A few years ago, our group reported a new strategy for cross-linking and obtaining a hydrophilic polycaprolactone [16]. A PCL macromer was synthesized through the reaction of PCL diol with methacrylic anhydride in order to obtain methacrylate end-capped PCL. PCL networks were prepared by photopolymerisation of the new macromer. Furthermore, the PCL macromer was copolymerized with 2-hydroxyethyl acrylate to improve the water sorption capacity of the system. The synthesis, characterization, and physical properties were described elsewhere [16,17]. Accelerated degradation studies in an alkaline medium have also been reported [18].

In this paper we present an enzymatic and long-term hydrolytic degradation study of these PCL networks, using phosphate buffered solution (PBS) and HPLC-grade water at 37 °C. From these parallel studies we were able to evaluate the effectiveness of the accelerated system [18] when compared with *in vitro* physiological conditions. The degradation process was monitored by determining the weight loss, X-ray diffraction analysis, thermal and mechanical properties, and surface morphology as a function of degradation time.

2. Materials and methods

2.1. Materials

α,ω-dihydroxy terminated polycaprolactone with a molecular weight of 2000 Da and methacrylic anhydride (MA), were supplied by Aldrich. Benzoin (Scharlau, 98% pure) was employed as initiator. Dioxane (Aldrich, 99.8% pure), acetone (Aldrich, 99.5% pure), ethanol (Aldrich, 99.5% pure) and anhydrous ethyl acetate (Aldrich 99.8%) were used as solvents without further purification. Lipase from Pseudomonas (PS) fluorescens (powder, EC 3.1.1.3, 40 units/mg) was purchased from Fluka, Sodium azide (NaN_3) 99% from Aldrich, the buffer solution (phosphate/di-Sodium hydrogen) pH = 7,00 (20 °C) $D = 1.01$ g/cm^3 and HPLC-grade water from Scharlau, and they were used as received.

The synthesis, characterization, and physical properties of the PCL network were published previously [16,17]. In brief, α,ω-dihydroxy terminated PCL was end-capped with methacrylate groups to make a polymerisable macromer. The macromer was obtained by dropping the filtrate into an excess of ethanol, filtrated, recrystallized several times and also purified by column chromatography techniques, using silica gel 60 (70–230 mesh) as the stationary phase and ethyl acetate as the solvent. Finally, the precipitated PCL macromer was dried at 50 °C for 24 h under reduced pressure. PCL networks were prepared by UV polymerization. The PCL macromer was dissolved in dioxane, 35% (w/v) and mixed with benzoin (photoinitiator, 1 wt %). The reaction was carried out under ultraviolet light for 24 h. Low molecular weight substances were extracted by boiling in ethanol for 24 h and then vacuum-dried to constant weight.

2.2. Hydrolytic and enzymatic degradation

In order to assess whether the increased presence of ions influences the process of degradation, hydrolytic degradation at 37.0 ± 0.5 °C and pH = 7.4 was carried out in two different media: phosphate buffered solution (PBS), and HPLC-grade water. The enzymatic degradation assay was carried out in a phosphate buffer saline solution (pH = 7.4 and 37.0 ± 0.5 °C) in the presence of PS lipase, 2.5 units/g of polymer (1 mg/ml), and 0.02% of sodium azide to avoid bacterial proliferation. The hydrolytic solution was changed every two weeks, and the buffer-enzymatic solution was changed twice a week to maintain the enzymatic activity. Specimens, discs of around 5 mm diameter made from films of 0.8 mm thick, were placed in tubes filled with degradation medium; the proportion of the sample to the degradation medium was 1/50 in mass. Three replicates were taken out of the solution at predetermined time intervals, then washed with distilled water and vacuum-dried at room temperature to constant weight.

The degradation process was followed by determining the water absorption and mass loss of the materials. Samples were washed with distilled water and gently wiped with paper. Wet weight was determined in order to evaluate the evolution of the samples' hydrophilicity. The degree of swelling was determined by comparing the wet weight (w_w) at a specific time with the dry weight (w_d) according to Eq. (1)

$$\text{degree of swelling}\left(\%\right) = \frac{w_w - w_d}{w_d} \times 100 \tag{1}$$

The percentage of weight loss was determined after drying the samples in vacuum by comparing dry weight (w_d) at a specific time with the initial weight (w_0) according to Eq. (2)

$$\text{weight loss}\left(\%\right) = \frac{w_0 - w_d}{w_0} \times 100 \tag{2}$$

A balance (Mettler Toledo) with a sensitivity of 0.01 mg was used to weigh the samples.

2.3. Scanning Electron Microscopy (SEM)

To investigate the surface and cross section morphology of dried network samples, SEM pictures of degraded and non-degraded samples were taken using a JEOL JSM-5410 scanning electron microscope.

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