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Lipase mediated enzymatic degradation of polydioxanone in solution

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ABSTRACT

The enzymatic biodegradation of polydioxanone (PDO) in trifluoroethanol (TFE) at various temperatures (25–55 °C) was studied with two different types of lipases, namely immobilized enzyme Novozym 435 and free enzyme porcine pancreas lipase. The biodegradation process was monitored by gel permeation chromatography (GPC). Both enzymes showed the optimum activity at 37 °C and Novozym 435 exhibited better thermal stability over the experimental temperature range. A continuous distribution kinetic model was employed to describe the biodegradation process and the model was used to fit the experimental data satisfactorily and obtain kinetic parameters.

1. Introduction

Synthetic polymers, in the last few decades, have found ever-increased use in various biomedical applications [1]. One such biomedically important synthetic polyester, polydioxanone (PDO) has been approved by the Food and Drug Administration (FDA) as a synthetic absorbable suture (SAS) material for wound closure [2]. Polydioxanone can be synthesized by the ring opening polymerization of p-dioxanone monomers, forming ether-ester bonds, in the presence of heat and catalyst. The excellent flexibility, low inflammatory response and desirable degradation characteristics have resulted in the use of PDO as a suturing material [3,4]. PDO has also been applied in other biomedical applications such as drug delivery, reconstructive surgery, cardiovascular and bone repair applications [4].

One of the important parameters for the successful implementation in tissue engineering applications is the degradation characteristics of the particular polymer. In the case of synthetic absorbable suture materials, studies have indicated that hydrolysis is the primary mechanism of degradation [5]. The effect of various intrinsic and extrinsic factors such as chemical properties and physical form of the polymer, and the pH of medium on the hydrolytic degradation has been investigated. There are some reports on the hydrolytic degradation characteristics of PDO. Sabino et al. [6] studied the hydrolytic degradation of bioabsorbable PDO in a phosphate buffered solution and reported that polymer viscosity and molecular weight decreased with time due to random cleaving of the ester bond. The changes in the tensile property, surface structures and dye diffusion characteristics of the PDO sutures due to hydrolytic degradation have been reported by Lin and co-workers [7]. Ooi and Cameron [8] investigated the micro- and macro-scale deformation of commercially available PDO sutures in the absence as well as presence of extrinsic factors such as hydration and degradation.

In all these reports, researchers have used commercially available PDO sutures and focused mainly on the hydrolytic degradation of the polymeric sutures. However, the enzymatic degradation of PDO in vivo is also a critical issue for the optimal performance of the polymeric biomaterials, especially due to the abundance of a range of potential enzymes in the physiological environment [9]. Among these enzymes, lipases - a subclass of esterases - are ubiquitous in human body fluids and catalyze the hydrolysis of ester bonds in lipids. One of the striking features of the lipases is that these enzymes remain stable and active in organic solvents, possibly due to the presence of a thin layer of water that adheres to the hydrophilic surface of the enzyme, thereby, maintaining the native conformation, which is critical for the enzyme activity [10,11]. Sivalingam et al. [12]. investigated the solvent dependency of the lipase-mediated degradation of poly (ε-caprolactone) (PCL) and demonstrated that the enzyme activity increased with polarity and decreased with viscosity of the solvent. Enzymatic degradation of

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PDO is not well understood. Thus, the study the lipase-mediated enzymatic degradation of PDO solubilized in organic solvent is of interest. In order to determine the degradation of polymers, continuous distribution kinetics has been used. It has been used for polymer degradation with random scission [13], chain end scission [12], polymer crystallization [14] and numerous other applications as summarized in a review [15].

In the present investigation, the kinetics of enzymatic degradation of PDO by lipase in trifluoro-ethanol (TFE) has been studied. The effect of temperature on the lipase-mediated degradation of PDO has also been investigated. The enzymatic degradation has been modeled using continuous distribution kinetics and the rate coefficients as well as enzyme deactivation coefficient have been calculated.

2. Experimental

2.1. Materials

PDO (GPC, number average molecular weight 150,000) was obtained from Sigma Aldrich. Commercial immobilized lipase, Novozym 435 and free lipase enzyme, i.e., porcine pancreas lipase were procured from Sigma. All other chemicals, such as trifluroethanol (TFE) and tetrahydrofuran (THF) were obtained from S.D. Fine Chemicals.

2.2. Degradation experiments

Biodegradation of PDO was carried out in TFE by Novozym 435 and porcine pancrease lipase at four different temperatures, namely 25 °C, 37 °C, 45 °C and 55 °C. Four batches of 15 mL PDO solution (4 g/L) with 1 mg/mL of each lipase were taken in screw-cap culture tubes for each temperature. Reaction vials were kept at constant temperature incubator-shaker to avoid axial gradient of reactant and product. The temperature of the incubator was controlled by a PID controller with a variation of ±1 °C. 500 μL of sample was taken at regular time intervals for analysis in gel permeation chromatography (GPC). Control experiments were carried out in the absence of enzyme and no degradation was observed. Polymer structure was verified before and after degradation by FTIR (Perkin–Elmer).

2.3. GPC analysis

The GPC system (Waters) consisted of an isocratic pump (Water 510), a sample loop (200 ul), three GPC column of varying pore sizes, and an online differential refractive index detector (Water 2410). THF was used as mobile phase at a constant flow rate of 0.9 mL/min. Polystyrene standards were used as a standards for calibration with various molecular weights and converted to universal calibration Mark–Houwink equation given elsewhere [13].

3. Theoretical model

The enzyme degrades the polymer by cleaving at specific chain position and produces oligomers of average molecular weight (x_a). Specific chain scission can be expressed by the following reaction [12]:

$$P(x) \xrightarrow{k_s} P(x - x_a) + Q(x_a) \tag{1}$$

$P(x)$ and $Q(x_a)$ represent the molecular weight of polymer and degraded product, respectively. At any time t, the concentration of polymer and specific product can be denoted as $p(x,t)$ and $q_t(t)$, respectively. Considering$x$ to be a continuous variable, the population balance for the polymer and product can be expressed as following [12].

$$\frac{\partial p(x,t)}{\partial t} = k_s\, a_0(t) \int_x^\infty p(x',t)\delta(x,(x'-x_a))dx' - k_s a_0(t) p(x,t) \tag{2}$$

$$\frac{\partial q(t)}{dt} = \int_{x_a}^\infty k_s a_0(t) p(x',t)\Omega(x_a - x')dx' \tag{3}$$

$a_0(t)$ represents enzyme activity at time t. The stoichiometric kernel,$\Omega(x_a,x')$, determined the product distribution and is represented as $\delta(x_a,x)$ [17]. Degradation rate coefficient, k_s, is reported to be independent of polymer molecular weight [17].

$$\frac{dp^{(i)}}{dt} = k_s a_0(t)(-x_a)^i p^{(0)}(t) - k_s a_0(t) \sum_{t=0}^{i} p^{(i)}(t) \tag{4}$$

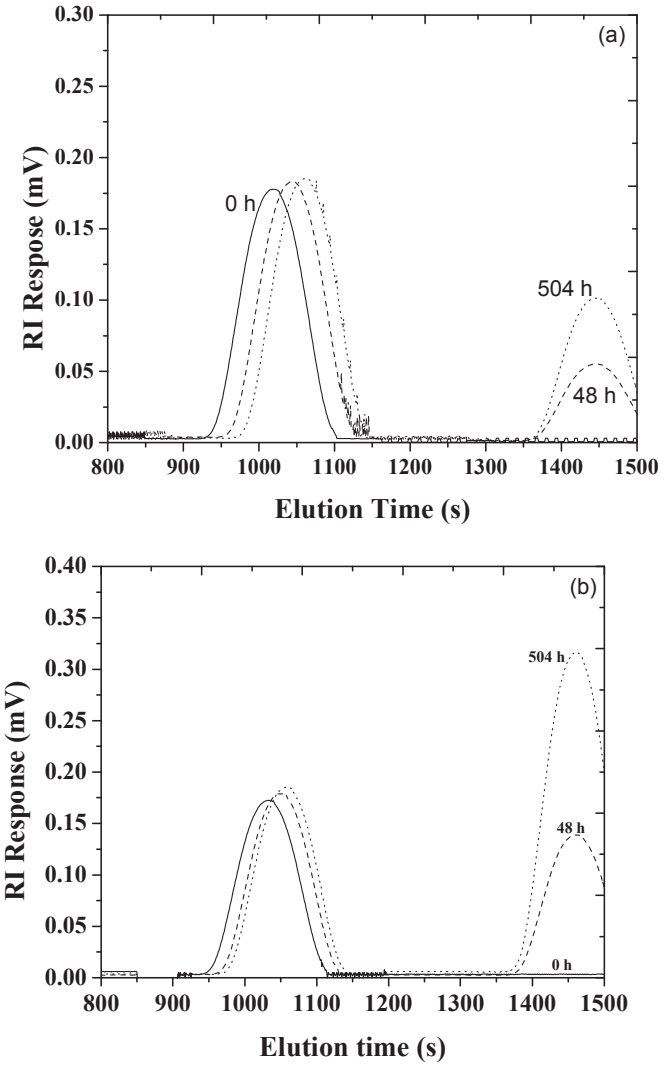

Fig. 1. Gel permeation chromatographs of enzymatic degradation of PDO by (a) Novozym 435 and (b) porcine pancreas lipase. The numbers on the figure indicate reaction time in hours.