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# Polymer Degradation and Stability

# Enzymatic degradation of poly(L-lactide) treated with supercritical carbon dioxide

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## ABSTRACT

To investigate the factors that affect the physical properties of poly (L-lactide) [poly (L-LA)] processed with supercritical carbon dioxide (scCO$_2$), the present work assessed the degradability of poly (L-LA), as well as its thermal and mechanical properties before and after processing. The thermal properties of three types of poly (L-LA) (H100, H440 and REVODE), each having different properties, were examined. Poly (L-LA) films were treated with scCO$_2$ using a high pressure reaction apparatus at 40 °C and 14 MPa for 3 h. The treated samples subsequently underwent enzymatic degradation tests using proteinase K. The poly (L-LA)s processed with scCO$_2$ degraded more slowly compared to polymers not treated with scCO$_2$ during the early stages of degradation. Scanning electron microscopy images of the degraded, scCO$_2$-processed poly (L-LA)s indicated close spacing of the cavities generated by degradation. The melting point ($T_m$) values of all poly (L-LA)s increased with scCO$_2$ processing, which influenced the degradability. Although the degradation of processed poly (L-LA) was slower than that of unprocessed poly (L-LA) in the early stages, the degradability of the treated H440 and REVODE samples was identical to that of specimens without scCO$_2$ processing after 160 h. In addition, the poly (L-LA) treated with scCO$_2$ was found to be degraded by proteinase K at a constant rate. The relationship between degradability and crystallinity was examined, and untreated H100 was observed to rapidly degrade in contact with proteinase K. The crystallinity indicators $X_{c\ DSC}$ and $X_{c\ XRD}$ increased after scCO$_2$ processing, such that the degradability of the treated sample was reduced. In addition, both the $X_{c\ DSC}$ and $X_{c\ XRD}$ values of untreated and treated H100 increased with degradation. Although the degradation curve of the REVODE was similar to that of the H440, the changes in the crystallinity of untreated REVODE were different from the results observed for the H100 and H440. XRD data showed that the diffraction peaks of the untreated H100 and the poly (L-LA)s treated with scCO$_2$, which were more highly crystalline, shifted to smaller angles as the enzymatic degradation progressed. Examination of the mechanical properties indicated increases in tensile strength and elastic modulus and decreases in elongation after scCO$_2$ processing, suggesting that the polymer chains were moved closer together. In conclusion, scCO$_2$ processing appears to uniformly contract polymer chains in both the amorphous and crystalline regions.
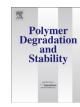
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## 1. Introduction

Biodegradable macromolecules have been used as general-purpose polymers [1—3] and in medical applications [4—6] such as dissolvable sutures and drug delivery systems [7—9]. In addition, polymer processing methods using supercritical carbon dioxide (scCO$_2$) have gained attention with regard to the development of new materials and because the scCO$_2$ is an environmentally friendly ("green") solvent. Supercritical fluids have properties of both a liquid (meaning they can act to dissolve substances) and a gas (meaning they can diffuse). Carbon dioxide, a nontoxic medium with a relatively low critical point ($T_c$ = 31.1 °C, $P_c$ = 7.38 MPa), is

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widely used as a supercritical fluid, and scCO2 extraction technology has been utilized in agricultural [10] and food [11] applications, and as to provide a solvent for dyes [12] and chemical reactions [13]. In the polymer industry, scCO2 has been employed for polymer-processing purposes such as blending [14], additive impregnation [15], polymer foaming [16] and the preparation of microcapsules [17]. In addition, the high affinity of scCO2 toward organic compounds has led to its use in the synthesis of polymers [18−20]. Studies on the preparation of controlled release polymers using scCO2 have reported that *d*-limonene, hinokitiol, *trans*-2-hexenal [21,22], *α*-pinene [23] and essential bark oil from *Thujopsis dolabrata* var. *hondae* [24] can all be incorporated into L-lactide (L-LA) copolymers at high concentrations. The amount of chemical incorporated is dependent on the copolymer and the temperature and pressure applied during scCO2 processing. Previous work has shown that the maximum amount of essential oil is impregnated into these copolymers at 14 MPa [24]. In addition, the amount of chemical incorporated into a copolymer has been found to vary with the solubility of that compound in scCO2.

Many studies have described the changes in the physical properties of biodegradable polymers when processing with scCO2. The results have indicated that the melting temperature of poly (*ε*-caprolactone) decreases at low pressures and passes through a minimum near 10 MPa before leveling off with increasing CO2 pressure at approximately 44 °C [25]. The melting points of poly (3-hydroxybutyrate) and poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) decrease linearly up to 24 MPa during scCO2 processing [25]. Bendaoud et al. reported that scCO2 induces a change in the crystalline structures of starch-based polymers, an effect that is primarily influenced by the processing temperature [26]. Sarikhani et al. reported the solubility of CO2 in poly (lactide) (PLA) melts and the effect of scCO2 pressure on the PLA interfacial tension [27]. Changes in the surface tension and density of a CO2/PLA mixture were examined over the range of 180 to 200 °C and from 6.89 to 20.68 MPa by Mahmood et al. [28]. Sato et al. evaluated the solubility and diffusion coefficient of CO2 in the biodegradable polymers poly (butylene succinate) and poly (butylene succinate-*co*-adipate) between 50 and 180 °C and at pressures up to 20 MPa [29]. These studies have important applications in the polymer industry. However, there have been no investigations to date of the degradability of polymers treated with scCO2.

The effects of scCO2 on the thermal characteristics of polymers, such as the heat of fusion ($-\Delta H_m$), have been reported [30,31], and a previous study found that the crystallinity of poly (L-LA) was increased by scCO2 processing [32]. The present work examined the relationship between the degradability and physical properties of poly (L-LA) treated with scCO2, by forming films with three types of poly (L-LA): Lacea H-100 (referred to as H100), Lacea H-440 (H440) and REVODE 101 (REVODE). These films subsequently underwent enzymatic degradation with proteinase K, and the physical properties of films with or without scCO2 processing were evaluated during this degradation process. The work reported herein also investigated the thermal properties of the degraded poly (L-LA)s by

differential scanning calorimetry (DSC) to determine the effects of scCO2 processing. In addition, changes in morphology were assessed by X-ray diffraction (XRD) and the surfaces of degraded poly (L-LA)s were observed by scanning electron microscopy (SEM).

## 2. Experimental

### 2.1. Materials

The Poly (L-LA) H100 and H440 samples were received as gifts from Mitsui Chemicals, Inc., while the REVODE, a crystalline polymer similar to H100 and H440, was obtained from Zhejiang Hisum Biomaterials. The poly (L-LA) films were prepared by a solvent-cast method using methylene chloride. The physical properties of the films are summarized in Table 1. HPLC grade chloroform was used as the solvent for gel permeation chromatography (GPC). Methylene chloride and chloroform were purchased from Wako Pure Chemical Industries, Ltd. and used without further purification. Carbon dioxide (99.99%) was obtained from Takamatsu Teisan, Inc. Tricine {*N*-[tris(hydroxymethyl)methyl]-glycine} was purchased from Nacalai Tesque, Inc. Proteinase K from *Tritirachium album* (Wako Pure Chemical Industries, Ltd.), in a Tricine buffer at pH 8.0, was used at 37 °C without further purification. Sodium hydroxide for pH adjustment was purchased from Wako Pure Chemical Industries, Ltd. Distilled water was used for the biodegradation tests.

### 2.2. Characterization of polymers

Thermal characteristics (melting point, $T_m$, glass transition point, $T_g$, and heat of fusion, $-\Delta H_m$) were obtained using a Rigaku Thermo Plus 2/DSC8230 differential scanning calorimeter. Samples (5 mg) were heated from room temperature to 200 °C at a rate of 10 °C/min under a nitrogen stream. The $T_m$ and $-\Delta H_m$ values were determined during the initial heating scan and the $T_g$ values were obtained over the second heating period. The extent of crystallinity of each poly (L-LA) sample ($X_{c\ DSC}$) was calculated using the equation [32].

$$Xc.\text{DSC}\ (\%) = \frac{\Delta Hm - \Delta Hc}{\Delta Hm^0} \times 100$$

where $\Delta H_m^0$ is the heat of fusion and $\Delta H_c$ is the heat of crystallization for poly (L-LA). The value of 135 J/g reported by Miyata and Masuko [33] was used for $\Delta H_m^0$, assuming a perfectly crystalline sample. The reported values each represent the average of three measurements.

Crystal structures were investigated with a Rigaku Rint 2100 X-ray diffractometer equipped with a scintillation counter, employing Cu Kα radiation (30 kV, 15 mA) over a scan range of $2\theta = 8$ to $35°$ and at a scan rate of $2°$/min. The degraded poly (L-LA) specimens were each assessed three times and the average values were reported. The percent crystallinity ($X_{c\ XRD}$) of each poly (L-LA) sample was calculated based on the equation [32].

**Table 1**
Properties of poly (L-lactide) films prepared by solvent casting.

| Poly (L-lactide) | $M_n/10^{4a}$ | $M_w/M_n{}^a$ | $T_m{}^b$ [°C] | $-\Delta H_m{}^b$ [J/g] | $T_g{}^b$ [°C] | Tensile strength[c] [MPa] | Elongation[c] [%] | Elastic modulus[c] [MPa] |
|---|---|---|---|---|---|---|---|---|
| H100 | 9.0 | 1.64 | 169 | 27 | 57 | 21 | 321 | 354 |
| H440 | 10.6 | 1.58 | 154 | 15 | 55 | 20 | 285 | 392 |
| REVODE | 10.6 | 1.85 | 148 | 16 | 53 | 15 | 363 | 160 |

[a] Determined by GPC.
[b] Determined by DSC.
[c] Determined by tensile testing.