Preparation and in Vitro Degradation Properties of Polylactide Microcapsules

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Polylactide (PLA) microcapsules with an average diameter of 1.5 μm were prepared by an interfacial deposition technique. The degree of degradation of PLA microcapsules was estimated by determination of the amount of lactic acid as a final product in bulk solution and from the molecular weight distribution of PLA of the microcapsules remaining undegraded by means of gel permeation chromatography using chloroform as the eluent.

The rate of hydrolytic degradation of PLA microcapsules prepared by using poly(D,L-lactide) or poly(L-lactide) was extremely pH-dependent; it was slowest at pH around 5.0 and it increased in both strongly acidic and strongly alkaline solutions. The activation energy of deesterification at pH 7.4 and ionic strength 0.15 was calculated to be 19.9 kcal/mol for poly(D,L-lactide) microcapsules and 20.0 kcal/mol for poly(L-lactide) microcapsules, when the initial weight-average molecular weight was about 100000 for both polymers. These values are comparable to those found for the hydrolysis of alkyl acetates. In buffer solution (pH 9.6), the hydrolytic degradation was enhanced when the ionic strength of the medium was increased.

Chromatographic analysis of the products of hydrolytic degradation suggested that OH or H₂O⁻ attacks the ester bonds located in the crystalline zone, followed by further cleavage of the initial products into fractions with lower molecular weights. Carboxylic esterase promoted the degradation by cleaving the ester bonds located in the crystalline zone directly. Urea and NaSCN accelerated the degradation effectively, while AlCl₃, CaCl₂, and KCl had no or little effect on the degradation rate. Poly(L-lactide) microcapsules were degraded less rapidly than poly(D,L-lactide) microcapsules.

Keywords — poly(D,L-lactide) microcapsules; poly(L-lactide) microcapsules; hydrolytic degradation; carboxylic esterase; activation energy

Polylactide (PLA), a synthetic biodegradable polymer, is used in surgical sutures and implant materials. In this context, many studies have been done on morphological change of the PLA matrix and on the toxicity of and cellular response to the polymer. Recently, the use of PLA as a drug-loaded matrix has been examined in the hope that the matrix will decompose after releasing the drug in a sustained manner over a long period of time in the human body.

Meanwhile, in vitro investigations have suggested that PLA undergoes hydrolysis and that the rate of hydrolysis depends on the initial molecular weight of the polymer. In order to elucidate the mechanism of hydrolysis, the interaction with water molecules and the intra- and intermolecular interactions of the polymer must be investigated from a physicochemical point of view. In the present work, poly(D,L-lactide) and poly(L-lactide) microcapsules of very small size (mean diameter 1.5 μm) were prepared and used to observe the rate of polymer degradation under various environmental conditions at a very high specific surface area of microcapsules in time periods as short as possible.
Experimental

Preparation of Poly(D,L-lactide) and Poly(L-lactide) Microcapsules—Poly(D,L-lactide) and poly(L-lactide) microcapsules containing water were prepared in the following way. Fifty ml of n-hexane was added to 150 ml of 1.5% (w/v) aqueous Pluronic F68 (Asahida Kenka, Japan) solution and the mixture was stirred for 5 min to yield an O/W emulsion. Next, 20 ml of 1.5% (w/v) poly(D,L-lactide) (Polyscience, U.S.A.) or poly(L-lactide) (Mitsui Toatsu, Japan) solution in dichloromethane was added dropwise to the above emulsion, and the system was stirred under reduced pressure for 2 h to allow the organic solvents to evaporate completely. Poly(D,L-lactide) or poly(L-lactide) microcapsules thus prepared were centrifuged at 2000 rpm for 10 min and washed three times with water. The average diameter was determined to be about 1.5 μm from electron micrographs of the microcapsules.

Estimation of Degradation—The degree of degradation of PLA microcapsules was estimated from the rate of decrease in the weight-average molecular weight, $M_w$, of PLA evaluated by gel permeation chromatography (GPC), and from the amount of lactic acid generated as the final product in bulk solution, determined at different degradation periods according to the Barker–Summerson method. In GPC analysis, the eluent was chloroform and the columns were Tosoh 2000H6 (Toyo Soda, Japan) and Shodex AC804 (Shokutosho, Japan). An aliquot of sample suspension was withdrawn from the test suspension at a suitable time and centrifuged at 3000 rpm for 10 min, then the settled microcapsules were extracted into chloroform. The extract was used as the sample of GPC analysis.

Degradation of PLA Microcapsules—In the degradation experiments, PLA microcapsules were dispersed in various solution media at the final concentration of 0.15% (w/v).

Effect of pH of the Medium: Five buffer solutions were used: (1) HCl-trisodium citrate, (2) trisodium citrate–Na$_2$HPO$_4$, (3) KH$_2$PO$_4$–Na$_2$HPO$_4$, (4) KH$_2$PO$_4$–Na$_2$PO$_4$, (5) NaHCO$_3$–Na$_2$CO$_3$. The pH values of the buffers were 1.6, 3.0, 5.0, 7.4, and 9.6, respectively. PLA microcapsules dispersed in these media were kept at 37°C.

Effect of Temperature: PLA microcapsules were dispersed in a phosphate-buffered physiological saline solution and kept at three different temperatures, 21, 37, and 45°C.

Effect of Ionic Strength of the Medium: The ionic strength of the buffer solution (pH 9.6) was adjusted to 0.07, 0.15, 0.30, and 0.45 by adding NaCl. PLA microcapsules were dispersed in each of the solutions and kept at 37°C.

Effects of Neutral Salts: PLA microcapsules were dispersed in trisodium citrate–Na$_2$HPO$_4$ buffer solution (pH 3.0) containing one of the neutral salts at 0.001 M and kept at 37°C. The neutral salts used were AlCl$_3$, CaCl$_2$, KCl, NaCl, and NaSCN.

Effect of Urea: PLA microcapsules were dispersed in trisodium citrate–Na$_2$HPO$_4$ buffer solution (pH 3.0) containing 0.1 M urea and kept at 37°C.

Effect of Esterase: PLA microcapsules suspended in a phosphate buffer solution (pH 7.6, 0.1 M) containing carboxylic esterase (EC 3.1.1.1) were kept at 37°C. The determination of enzyme activity was performed following Kobayashi's method using 0.9 mM p-nitrophenyl butyrate as a substrate. Since the enzyme activity was proportional to the concentration of p-nitrophenol formed from the substrate, the rate of increase in the absorbance at 420 nm during the reaction was measured spectrophotometrically. The initial enzyme activities in the solution were 1.45, 2.22, and 3.96 U/ml.

Results and Discussion

Effect of pH

The decrease of weight-average molecular weight, $M_w$, of PLA in the degradation of poly(D,L-lactide) microcapsules dispersed in each of the buffer solutions is shown in Fig. 1 as a function of immersion time. The observed decreases of $M_w$ indicated that a number of the ester bonds in the polymer are cleaved and that poly(D,L-lactide) microcapsules are degraded significantly faster in the highly alkaline buffer solution (pH 9.6) than in the highly acidic and slightly alkaline buffer solutions. However, no significant change of $M_w$ was observed during the immersion in the slightly acidic buffer solutions. These findings are in accordance with the features of general acid–base catalysis. Since the cleavage of ester bonds takes place by different mechanisms depending on the pH of the medium (acid- and base-catalyzed reactions), and the microcapsule membranes are considered to be composed of both crystalline and noncrystalline zones, the observed differences in the degradation behavior of the microcapsules at different pH values should be interpreted in these terms.

In the highly alkaline solution, the molecular weight distribution reflected a progressive degradation, as shown in Fig. 2. The molecular weight distribution of PLA in poly(D,L-lactide) microcapsules was relatively narrow and the modal molecular weight was about
60000 on day 0. After 6 d, corresponding to the onset of a significant weight loss, the molecular weight distribution became trimodal with two shoulders at the low molecular weight tail of the gel permeation chromatogram; the modal molecular weights of the two fractions were about 12000 and 2500, respectively. On further degradation, the molecular weight distribution continued to change. That is, the fraction of highest molecular weight decreased while the fraction of lowest molecular weight increased and the fraction of middle molecular weight diminished. Similar degradation behavior was also observed in the other buffer solutions in terms of molecular weight distribution after much longer immersion times.

These experimental findings indicate that large quantities of low-molecular-weight PLA are produced by the deesterification reaction via production of polymers of intermediate molecular weights. The depolymerization of PLA molecules constituting the microcapsules may have been accelerated by the production of the intermediate molecular weight fractions, because the decrease of $\overline{M}_w$ was not so rapid until these fractions appeared in the molecular weight distribution. It is suggested that water molecules penetrate first into the amorphous zone of the semicrystalline structure of the material and hydrolysis starts in this zone and then moves gradually into the crystalline zone. In view of this, the fraction with a molecular weight of about 60000 may represent a stable unit of the crystalline zone of PLA microcapsules (see Fig. 2). Consequently, the hydrolytic cleavage of polymer chains of PLA is considered to proceed by an “unzipping” process and not randomly.

Figure 3 shows the weight loss of poly(D,L-lactide) microcapsules during the period of degradation. The weight loss was evaluated from GPC analysis of the undegraded microcapsules. Poly(D,L-lactide) microcapsules in the strongly alkaline medium lost weight rapidly. This suggests that a fraction of low molecular weight species which is soluble in the aqueous medium was generated from the polymer. The fraction is expected to consist of lactic acid as the final product of the degradation.

Figure 4 shows the amount of lactic acid in the bulk solution as a function of degradation period. It appears from the figure that the polymer undergoes “zipping” deesterification and lactic acid is generated at the end region of the polymer. The amount of lactic acid generated is pH-sensitive, and this tendency is quite similar to that observed in Fig. 1 for the change in $\overline{M}_w$. This presumably reflects the number of end groups, which is pH-dependent, in the polymer
matrix being degraded by the unzipping cleavage. Therefore, the degradation of the polymer proceeds hydrolytically by zipping and unzipping processes, and the degradation rate is controlled by the crystallinity of the polymer.

Poly(\(L\)-lactide) microcapsules were degraded less rapidly than poly(\(D,L\)-lactide) microcapsules as shown in Fig. 5, though the dependency of the degradation rate on pH was almost the same for both types of microcapsules. This may be due to the higher crystallinity of poly(\(L\)-lactide).

**Effect of Temperature**

The degradation rate should be affected by temperature. Figure 6 shows the Arrhenius plots for the degradation of poly(\(D,L\)-lactide) and poly(\(L\)-lactide) microcapsules, where \(V=\)
1/80 × (d\(\bar{M}_w\)/dt). Utilizing the Arrhenius equation, activation energy values of 19.9 kcal/mol for poly(D,L-lactide) microcapsules and 20.0 kcal/mol for poly(l-lactide) microcapsules were obtained, which are comparable to the results for the hydrolysis of alkyl acetates. Consequently, the degradation of the polymer can be ascribed to hydrolysis. Although the activation energies for poly(D,L-lactide) and poly(l-lactide) microcapsules were almost identical, the latter was degraded less rapidly than the former. This should be due to the higher crystallinity and hydrophobicity of poly(l-lactide).

**Effect of Ionic Strength**

Figure 7 shows the effect of ionic strength on the rate of decrease in \(\bar{M}_w\) of PLA in the degradation of poly(D,L-lactide) microcapsules suspended in buffer solution of pH 9.6. No significant dependence of the rate on the ionic strength of the bulk solution was detected. However, as indicated in Fig. 8, the amount of lactic acid generated from the polymer increased with time, and the rate of generation increased as the ionic strength of the medium was increased. This may be due to a change in the thickness of the electric double layer on the surface of microcapsules. The surface of PLA microcapsules is negatively charged in alkaline solutions and the thickness of the electric double layer decreases with increase of the ionic strength of the medium. Thus, poly(D,L-lactide) microcapsules are more accessible to OH\(^-\) attack in a medium of higher ionic strength. Poly(l-lactide) microcapsules were degraded less rapidly than poly(D,L-lactide) microcapsules, showing a dependency of the degradation rate on the ionic strength of the medium similar to that for the latter. This is shown in Fig. 9.

**Effect of Neutral Salts and Urea**

Figure 10 shows the effect of neutral salts on the decrease of \(\bar{M}_w\) of PLA in the degradation of poly(D,L-lactide) microcapsules at pH 3.0. The average molecular weight was reduced most significantly when the microcapsules were dispersed in the medium containing NaSCN. The presence of urea in the medium also accelerated the degradation of microcapsules, as shown in Fig. 11. In addition, the degradation of lactic acid from the polymer was enhanced by NaSCN and urea. It is suggested, therefore, that destruction of the water structure on the surface of poly(D,L-lactide) microcapsules promotes the hydrolytic degradation of the polymer.

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Fig. 7. Effect of Ionic Strength of the Medium on the Decrease of Weight-Average Molecular Weight of PLA in the Degradation of Poly(D,L-lactide) Microcapsules Dispersed in a Buffer Solution (pH 9.6)

Ionic strength: ○, 0.07; ●, 0.15; ▲, 0.30; △, 0.45.

Fig. 8. Amounts of Lactic Acid Generated from Poly(D,L-lactide) Microcapsules at Different Ionic Strengths of the Medium

Ionic strength: ○, 0.07; ●, 0.15; ▲, 0.30; △, 0.45.
Effect of Carboxylic Esterase

Figure 12 shows the decrease with time in $M_w$ of PLA in the degradation of poly(D,L-lactide) microcapsules suspended in the phosphate buffer solution (pH 7.6, 0.1 M) containing carboxylic esterase. The rate of $M_w$ decrease increased with increase in the carboxylic esterase concentration. As $M_w$ decreased, the corresponding gel permeation chromatogram became less sharp, showing that the molecular weight distribution of PLA of poly(D,L-lactide) microcapsules became broader at the lower molecular weight tail.

Such a change in the GPC with time was not seen in the simple hydrolytic degradation of poly(D,L-lactide) microcapsules in the absence of the enzyme, (Fig. 2). Hence, carboxylic esterase is supposed to cleave the ester bonds in the polymer in an unzipping mode and
randomly in any region, perhaps after being adsorbed on the surface of poly(D,L-lactide) microcapsules. Carboxylic esterase has a molecular weight of about 96000, and it is impossible for the enzyme to penetrate into the matrix of PLA. This means that the enzyme can hydrolyze only the ester bonds located on the surface of the microcapsules.

**Conclusion**

Poly(D,L-lactide) and poly(L-lactide) microcapsules are degraded through the hydrolysis of ester bonds of the polymer by means of so-called zipping and unzipping processes, and the products of low molecular weight liberated from the polymer may themselves accelerate microcapsule degradation. When the polymers is highly crystallized and has a highly rigid hydration structure on the microcapsule surface, the degradation rate is reduced. Carboxylic esterase accelerated the hydrolytic degradation after being adsorbed on the surface of the microcapsules. The enzymatic hydrolysis proceeds in a manner different from that in alkaline and acidic solutions in the absence of the enzyme, suggesting that deesterification may occur by an unzipping mechanism at any region of the polymer chains.

**References**