Impregnation of Poly(L-lactide-*ran*-cyclic carbonate) Copolymers with Useful Compounds with Supercritical Carbon Dioxide

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ABSTRACT: In this article, we cover the development of L-lactide (L-LA) random copolymers into which useful compounds, such as repellents and antibacterial agents, were impregnated by high concentration. Outstanding controlled release materials were developed with statistical random copolymers of L-LA with cyclic carbonate (CC) [2,2-dimethyltrimethylene carbonate (2,2-DTMC) or tetramethylene carbonate (TEMC)] with tin 2-ethyL-hexanoate as a catalyst at 150°C (2,2-DTMC) or 120°C (TEMC) for 24 h without solvent. The preparation of improved controlled release materials was performed with useful organic compounds with low boiling points and synthetic L-LA random copolymers containing CCs as base materials under supercritical carbon dioxide ($scCO_2$). Low-boiling-point compounds, such as *d*-limonene and hinokitiol, were used. In impregnation experiments with $scCO_2$, the amounts of low-boiling-point compounds increased with increasings L-LA content. The compound content impregnated into poly(L-lactide-*ran*-cyclic carbonate) [poly(L-LA-*ran*-CC)] was greater than that of the experiment with poly(L-lactide-*ran*-ε-caprolactone) previously studied. When the enzymatic degradation of poly(L-LA-*ran*-CC) was performed with proteinase K, copolymers with a greater L-LA content degraded more rapidly than did copolymers with a greater CC content. In a controlled release experiment with poly(L-lactide-*ran*-2,2-dimethyltrimethylene carbonate) (76/24) or poly(L-lactide-*ran*-tetramethylene carbonate) (81/19), the rate of polymer degradation and the rate of impregnated compound release were almost the same. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 1431–1441, 2011

Key words: biodegradable; biomaterials; degradation; polyesters; polycarbonates

INTRODUCTION

The object of this study was to develop an evolutionary material that would enable the controlled release of an insect repellent or antibacterial agent via hydrolysis. The prevention of the breeding and the extermination of food-poisoning bacteria; the inhibition of molds, which breed in walls and furniture and which are visible inside buildings such as houses and museums; and the extermination of insect pests and animals on farms and parks is a serious social problem. Preventive measures include chemical control, extermination, physical inhibition of breeding, and biotic control. Although the effects of chemical control are great, they also require the use of labor. Consequently, we decided to develop a material similar to a drug-delivery systems (DDSs) with high chemical effects but with a reduction in labor requirements. There have been many reports of the controlled release of substances by biodegradable polymers as DDSs.[1–5]

For the production of a DDS, solutions of the polymer and drug are emulsified and mutually distributed; the resulting material can be prepared in microcapsule form. However, because most insect repellents and antibacterial agents are highly volatile organic compounds, this method usually cannot be used for such substances. The methods generally used to impregnate a polymer with a target organic compound are mulling and solvent dissolution. However, there are several problems associated with these methods. Although high-boiling-point compounds can be incorporated into a polymer with the mulling method, compounds with low boiling points that volatilize at the processing temperature cannot be treated in this way. This high-boiling-point compound will be discolored for a high processing

temperature, which melts polymer even if it is able to incorporate it. Solvent dissolution is also subject to problems such as volatilization of the agent at the time of solvent removal and the presence of residual organic solvents.

It is well known that supercritical fluids have some unique properties, such as a liquidlike dissolving power and similar transportation properties to a gas. CO_2, a nontoxic fluid with a relatively low [critical temperature (Tc) = 31C, critical pressure (Pc) = 7.376 MPa], is most widely used in the supercritical fluid field. Supercritical carbon dioxide ($scCO_2$), called *green solvent*, has been used in a variety of industries, such as the food, pharmaceutical, chemical, and plastics industries. Research interests on $scCO_2$ have been widely conducted in many fields, such as in extraction, the separation of small molecules, and the preparation of more complex molecules. Recently, $scCO_2$ has been used for various polymer-processing purposes, such as polymer blending,[6,7] additive impregnation,[8,9] plastic foaming.[10–12] and the preparation of microcapsules.[13–17] In polymer processing, the use of $scCO_2$ has attracted considerable attention. One major advantage of the application of $scCO_2$ to polymers is that the processing conditions and the morphology of polymers are significantly changed by the dissolved CO_2. The presence of CO_2 in a polymer changes its rheological and thermal properties in both the molten and solid states. The dissolution of CO_2 reduces the viscosity of molten polymers[18] and the glass-transition temperature (T_g)[19] and changes the crystallization rate of semicrystalline polymers.[20]

Several researchers have been reported on the solubility of CO_2 into universally applicable polymers. Durill and Griskey[21] studied the solubility of CO_2 and nitrogen (N_2) gases in several polymers, such as low-density polyethylene, high-density polyethylene (HDPE), polypropylene (PP), and polystyrene (PS). Sato and coworkers[22,23] investigated the solubility of CO_2 and N_2 in HDPE, PP, and PS at 100–200°C and at pressures up to 20 MPa. Areerat et al.[24] reported on the solubility and diffusivity of $scCO_2$ in low-density polyethylene, HDPE, PP, PS, and ethylene ethylacrylate copolymer at temperatures from 150 to 200°C and pressures up to 12 MPa. These studies have shown that the solubility of gases in polymers increases as the saturation pressure increases and depends on the chemical structure of the polymer.

We previously attempted to impregnate poly(L-lactide) [poly(L-LA)] and poly(L-lactide-*ran*-ε-caprolactone) [poly(L-LA-*ran*-CL); Fig. 1] with useful organic compounds, such as repellents and antibacterial agents, with $scCO_2$ as the solvent.[25] Poly(L-LA) is known to be a biocompatible biodegradable semicrystalline polymer. To improve these characteristics, such as the hardness and brittleness, L-lactide (L-LA)
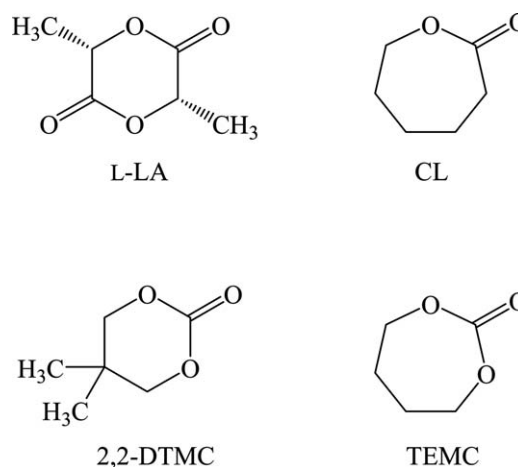

Figure 1 Chemical structures of L-LA, CL, 2,2-DTMC, and TEMC.

copolymers have been synthesized with various lactones, such as CL[26,27] and cyclic carbonate (CC).[28,29] We performed this study to test the impregnation of copolymers of L-LA with CC with *d*-limonene as a repellent and hinokitiol as an antibacterial agent using $scCO_2$. 2,2-Dimethyltrimethylene carbonate (2,2-DTMC) and tetramethylene carbonate (TEMC) as CCs were used to copolymerize with L-LA. (Fig. 1) The degradability of the copolymers and the release of useful compounds into the impregnated copolymers were evaluated by enzymatic degradation with proteinase K.

EXPERIMENTAL

Materials

L-LA [(3S)-*cis*-3,6-dimethyL-1,4-dioxane-2,5-dione] (Aldrich, St. Louis, MO USA) was purified by recrystallization from tetrahydrofuran followed by sublimation at 80°C. 2,2-DTMC, received as a gift from Daicel Chemical Industries, Ltd. (Tokyo, Japan), was purified by recrystallization from diethyl ether followed by sublimation at 100°C. TEMC (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was purified by recrystallization from tetrahydrofuran. Tin 2-ethyL-hexanoate [Sn(oct)$_2$] (Sigma, St. Louis, MO USA) was used as a catalyst without purification. Methanol and chloroform were purchased from Katayama Chemical Industries Co., Ltd. (Tokyo, Japan). Films of the synthetic copolymers were prepared by the solvent-casting method. *d*-Limonene [melting temperature (T_m) = −74.35°C, boiling point = 175.5–176°C] and hinokitiol (T_m = 48–52.5°C, boiling point = 140°C at 10 mmHg) were purchased from Chemical Industries and were used as received. Proteinase K from *Tritirachium album* (Wako Pure Chemical Industries Ltd) was used in *N*-[tris(hydroxymethyl)-methyl] glycine (tricine) buffer (pH 8.0) at 37°C without further purification. Tricine was purchased from
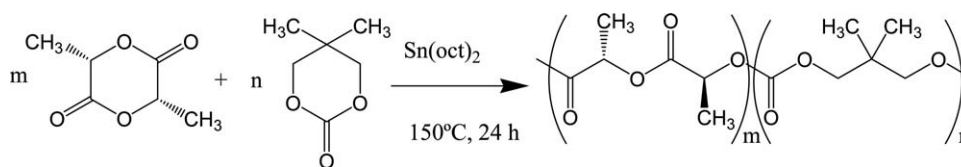
Scheme 1 Copolymerization of l-LA with 2,2-DTMC by Sn(oct)$_2$.

Nacalai Tesque, Inc. (Tokyo, Japan). Ion-exchanged water was used for the degradation tests. Sodium hydroxide for pH adjustment was purchased from Wako Pure Chemical Industries. The dye used for the antimicrobial test was as follows. Methyl orange from Wako Pure Chemical Industries. The *Aeromonas* sp. B-5 strain, which has the ability to decolorize azo dyes, was received from Professor Hayase. L-broth medium was used for the cultivation of *Aeromonas* sp. B-5. The L-broth contained 10.0 g of bacto trypton (Becton, Dickinson, and Co., Franklin Lakes, New Jersey), 5.0 g of yeast extract (Becton, Dickinson, and Co.), and 5.0 g of NaCl (Wako Pure Chemical Industries) per liter.

Characterization of the polymers

The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of the synthetic copolymers were determined by gel permeation chromatography (GPC) with a Hitachi (Tokyo, Japan) D-2520 chromatograph equipped with Shodex GPC K-802.5 and K-804 columns (Showa Denko K.K., Tokyo, Japan) with chloroform as an eluent at 40°C. The flow rate was 1.0 mL/min. The copolymer was detected with a refractive-index detector. The molecular weights were determined with reference to a PS standard.

The thermal characteristics [T_m, T_g, and heat of fusion ($-\Delta H_m$)] were obtained with a Rigaku Thermo Plus 2/DSC8230 (Tokyo, Japan) differential scanning calorimeter. Samples (5 mg) were heated at a rate of 10°C/min from −100 to 200°C in an N$_2$ stream. T_m and $-\Delta H_m$ were determined in the first heating, and T_g was determined in the second heating.

The compositions of L-LA with 2,2-DTMC or TEMC were determined by ^{1}H-NMR spectroscopy with a JEOL (Tokyo, Japan) JNM-ECP 400 MHz spectrometer. The contents of the organic compounds incorporated into the copolymers were also determined by an ^{1}H-NMR (JEOL Ltd., Tokyo, Japan) spectrometer. Chemical shifts were determined relative to residual chloroform at 7.27 ppm.

Synthesis of poly(L-lactide-*ran*-cyclic carbonate) [poly(L-LA-*ran*-CC)]

All polymerizations were conducted under an argon atmosphere with standard Schlenk techniques. CC copolymerized with L-LA was used with 2,2-DTMC (a six-membered compound) or TEMC (a seven-membered compound). The copolymerization of L-LA with 2,2-DTMC was performed with Sn(oct)$_2$ (0.1 mol % of monomer) as a catalyst at 150°C for 24 h (Scheme 1). The resulting mixture was dissolved in chloroform and then poured into excess methanol to precipitate the resulting polymers. The polymer was dried *in vacuo*. In a similar manner, the copolymerization of L-LA with TEMC was performed at 120°C for 24 h (Scheme 2). The molecular weight, composition, and thermal properties of the obtained copolymers were determined by GPC, ^{1}H-NMR spectrometry, and differential scanning calorimetry (DSC), respectively.

Incorporation of useful compounds into poly(L-LA-*ran*-CC) with scCO$_2$

Poly(L-LA-*ran*-CC) was used in the form of a film (thickness = 100 μm). The impregnation of poly(L-LA-*ran*-CC) with *d*-limonene or hinokitiol was carried out in a pressure-resistant container made of stainless steel (0.5 L) with stirring (100 rpm) under scCO$_2$ (40°C, 20 MPa) for 3 h with scCO$_2$ fluid equipment (Akico, Tokyo, Japan). After processing, decompression was gradually carried out over 3 h, and the sample was then removed from the container and weighed. Because large amounts of CO$_2$ were contained in the sample after impregnation, the content of *d*-limonene or hinokitiol was determined by ^{1}H-NMR spectroscopy. The amount present was calculated, and the calculated value was used to evaluate the experimental value.

Degradation and controlled release of poly(L-LA-*ran*-CC)

Proteinase K from *T. album* (activity 25 IU/mg) was used as an enzyme for the degradation of
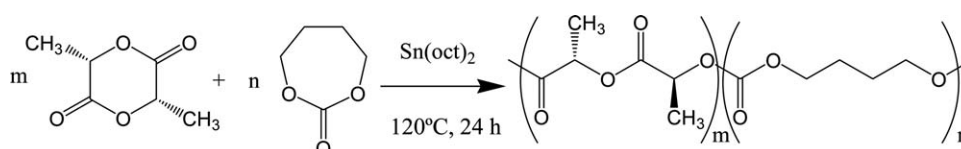
Scheme 2 Copolymerization of l-LA with TEMC by Sn(oct)$_2$.

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TABLE I
Copolymerization of l-LA with 2,2-DTMC

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l-LA/2,2-DTMC feed ratio	Yield (%)	$M_n \times 10^{4\ a}$	$M_w/M_n{}^a$	l-LA/2,2-DTMC composition[b]	T_m (°C)[c]	$-\Delta H_m$ (J/g)[c]	T_g (°C)[c]
10/90	80.5	3.78	1.71	10/90	72.6	10.8	11.4
20/80	84.3	3.12	1.77	21/79	ND	ND	13.5
30/70	82.5	3.08	1.70	30/70	ND	ND	16.8
40/60	84.1	3.16	1.63	41/59	ND	ND	20.5
50/50	91.0	4.52	1.72	51/49	ND	ND	23.6
60/40	83.4	4.86	1.69	58/42	ND	ND	30.9
70/30	82.0	5.28	1.62	76/24	109.9	20.4	38.7
80/20	83.7	4.81	1.61	84/16	130.6	25.8	42.3
90/10	86.9	4.75	1.52	90/10	141.7	31.6	52.0
95/5	87.2	4.96	1.74	98/2	160.9	42.6	53.9
100/0	89.7	3.90	1.64	100/0	167.1	49.0	55.9

Copolymerization was carried out at a monomer/catalyst of 1000 mol/mol at 150°C for 24 h. ND, not detected.
[a] Determined by GPC.
[b] Determined by ^{1}H-NMR spectroscopy.
[c] Determined by DSC.

poly(L-lactide-*ran*-2,2-dimethyltrimethylene carbonate) [poly(L-LA-*ran*-2,2-DTMC)] and poly(L-lactide-*ran*-tetramethylene carbonate) [poly(L-LA-*ran*-TEMC)]. In a 50-mL sample tube, the enzyme was dissolved in tricine buffer at pH 8.0 at 5 IU/mg of polymer. The tube was then placed in a shaking water bath for about 15 min until it reached the degradation temperature of 37°C. A polymer film (ca. 30 mg), prepared by the solvent-casting method, was sealed in a net bag made from polyethylene sheeting (1×1 mm^2 mesh) and placed in a sample tube containing the enzyme and buffer. An enzymatic degradation test was carried out at 37°C with shaking (100 rpm). To maintain the enzymatic activity at the desired level throughout the experiment, the enzyme and buffer solution were replaced every 40 h. After the set incubation period, the samples were thoroughly washed with deionized water and dried *in vacuo* for 3 h. The degradability was evaluated on the basis of the remaining weight of the samples.

A controlled release test was carried out with the same method as used for the enzyme degradation test. Poly(L-LA-*ran*-2,2-DTMC) (76/24) or poly(L-LA-*ran*-TEMC) (81/19) impregnated with *d*-limonene was used as the sample. Poly(L-LA-*ran*-CL) (76/24) containing *d*-limonene was also used for comparison. The *d*-limonene contents of poly(L-LA-*ran*-2,2-DTMC), poly(L-LA-*ran*-TEMC), and poly(L-LA-*ran*-CL) prepared under scCO$_2$ were 5.0, 5.3, and 2.8%, respectively. To evaluate the controlled release properties of the materials during testing, the quantities of the compound remaining in the L-LA random copolymer samples were measured by the ^{1}H-NMR spectrometer.

Poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol was used for the antimicrobial test. The controlled release of hinokitiol from the copolymer was carried out with hydrolysis and degradation with proteinase K. The hinokitiol content of poly(L-LA-*ran*-2,2-DTMC) (91/9) prepared under scCO$_2$ was 10.6%. *Aeromonas* sp. B-5, which has the ability to decolorize azo dyes, was used for the antimicrobial test. The multiplication of *Aeromonas* sp. B-5 was carried out with the common L-broth medium as the nutrition culture medium. Precultivation of *Aeromonas* sp. B-5 was conducted before the antimicrobial test. The preculture solution was dropped into the L-broth medium, which contained dissolved methyl orange and proteinase K and the added copolymer containing hinokitiol, with a sterilized pipette. The quantity of compound remaining in the poly(L-LA-*ran*-2,2-DTMC) sample was measured by ^{1}H-NMR spectroscopy during the test to evaluate the controlled release properties of the material. The turbidity of the cultures was measured at 600 nm as an index of growth with an UV spectrophotometer (Shimadzu Co., Tokyo, Japan). The decolorization of methyl orange was determined by the measurement of the decrease of the supernatant fraction in absorbance at 462 nm. In this experiment, if the controlled release of hinokitiol was effective, multiplication of *Aeromonas* sp. B-5 would be suppressed, and the degree of decolorization of methyl orange dissolved in the culture medium would not decrease.

RESULTS AND DISCUSSION

Synthesis of poly(L-LA-*ran*-2,2-DTMC)

We previously described the impregnation of poly(L-LA-*ran*-CL) with *d*-limonene with scCO$_2$.[25] Random copolymers of L-LA with 2,2-DTMC were synthesized with Sn(oct)$_2$ as a catalyst (0.1 mol % of monomer) to improve the degree of impregnation of poly(L-LA-*ran*-CL). Table I lists the results of

l-LA/TEMC feed ratio	Yield (%)	$M_n \times 10^{4\ a}$	$M_w/M_n{}^a$	l-LA/TEMC composition[b]	T_m (°C)[c]	$-\Delta H_m$ (J/g)[c]	T_g (°C)[c]
10/90	93.3	8.54	1.55	10/90	31.4	19.1	−22.7
20/80	93.6	12.68	1.60	20/80	ND	ND	−16.3
30/70	92.3	7.65	1.52	30/70	ND	ND	−4.7
40/60	94.4	9.48	1.63	40/60	ND	ND	2.6
50/50	92.4	8.37	1.59	50/50	ND	ND	13.6
60/40	95.9	8.35	1.61	60/40	ND	ND	20.2
70/30	98.5	8.00	1.46	70/30	ND	ND	29.0
80/20	92.1	7.14	1.48	81/19	97.9	5.1	41.8
90/10	94.3	4.72	1.43	91/9	134.6	20.5	51.0
100/0	89.7	3.90	1.64	100/0	167.1	49.0	55.9

Copolymerization was carried out at a monomer/catalyst of 1000 mol/mol at 120°C for 24 h. ND, not detected.
[a] Determined by GPC.
[b] Determined by ^{1}H-NMR spectroscopy.
[c] Determined by DSC.

copolymerization at 150°C for 24 h. Synthetic copolymers were obtained in over an 80% yield and with molecular weights over 3.0 × 10^4. Although the molecular weight of the copolymer was low at a lower L-LA feed ratio, the values increased with an increase in the L-LA content. The composition of the synthetic copolymers was almost the same with the feed ratio of L-LA with 2,2-DTMC. T_m, $-\Delta H_m$, and T_g of the poly(L-LA-*ran*-2,2-DTMC) (90/10) were determined to be 141.7°C, 31.6 J/g, and 52.0°C, respectively, on the basis of DSC measurements; this indicated a higher crystalline polymer. These values decreased with the lowering of the L-LA content; copolymers having an L-LA content between 58 and 21 mol % were noncrystalline polymers.

Synthesis of poly(L-LA-*ran*-TEMC)

We carried out the copolymerization of L-LA with TEMC by following the same condition with poly(L-LA-*ran*-2,2-DTMC) synthesis, except that adopted temperature was 120°C instead of 150°C. The results are listed in Table II. The yields obtained were considerably high and were over 90% at all L-LA/TEMC feed ratios. The molecular weight of poly(L-LA-*ran*-TEMC) increased with increasing TEMC content in the TEMC feed ratio range 0–60 mol %. The copolymer was synthesized in a composition ratio identical to the feed ratio of L-LA/TEMC. The structure of TEMC was responsible for the higher yield and molecular weight of poly(L-LA-*ran*-TEMC) compared with that of other copolymers, such as poly(L-LA-*ran*-2,2-DTMC) and poly(L-LA-*ran*-CL). It was thought that poly(L-LA-*ran*-TEMC) could be synthesized with a higher yield and molecular weight because the effect of the distorted seven-membered CC, TEMC, was evident against that of six-membered cyclic compounds such as L-LA, ε-caprolactone (CL), and 2,2-DTMC.

The thermal properties of the copolymer are listed in Table II. T_m, $-\Delta H_m$, and T_g of poly(L-LA-*ran*-TEMC) (91/9) were determined to be 134.6°C, 20.5 J/g, and 51.0°C, respectively, and were lower than those of poly(L-LA-*ran*-2,2-DTMC) at the same composition ratio. We concluded that T_m, T_g, and the crystallinity (indicating $-\Delta H_m$) of poly(L-LA-*ran*-TEMC) decreased compared to that of poly(L-LA-*ran*-2,2-DTMC) because it was easier to form a structure having a greater flexibility from the main polymer chain because of the longer methylene chain between the ester and/or carbonate bonds. Poly(L-LA-*ran*-TEMC) containing L-LA between 20 and 70 mol % was a noncrystalline polymer without a T_m. This result corresponded with the thermal properties of poly(L-LA-*ran*-2,2-DTMC). T_g of the random copolymers of L-LA with TEMC increased with increasing L-LA; this result was identical to that of poly(L-LA-*ran*-2,2-DTMC). The existence of only T_m or T_g for the copolymer of L-LA with TEMC indicated that this copolymer might have been the same as a random copolymer of L-LA with 2,2-DTMC. The tetramethylene carbonate homopolymer (PTEMC; $M_n = 6.51 \times 10^4$, $M_w/M_n = 1.62$) was a high crystalline polymer with a T_m of 54.1°C, a $-\Delta H_m$ of 39.3 J/g, and a T_g of −34.3°C (not shown in Table II). For reference, the ε-caprolactone homopolymer (PCL; $M_n = 6.34 \times 10^4$, $M_w/M_n = 1.70$) gave T_m of 61.4°C, a $-\Delta H_m$ of 98.5 J/g, and a T_g of −61.6°C (data not shown). T_m of PTEMC containing a carbonate bond showed nearly the same value as that of PCL, whereas $-\Delta H_m$ was less than half and T_g was higher than that of PCL (27°C). The differences were attributed to the distinction between the carbonate bond and the ester bond. Repulsion between adjacent carbonate bonds was considered to result in a decrease in the crystallinity ($-\Delta H_m$).

Because the C—O bond is short and has low flexibility, T_g of PTEMC was assumed to be higher than that of PCL. These synthetic copolymers were used as base materials in the impregnation experiments of useful organic compounds with low boiling points with scCO$_2$.
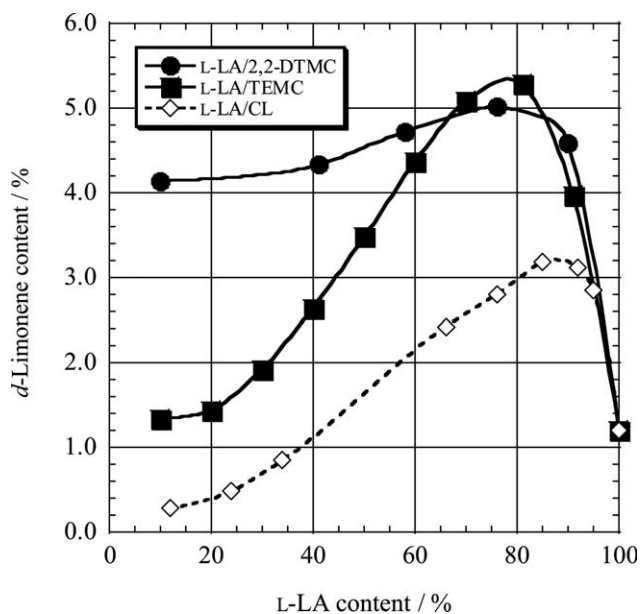
Impregnation of poly(L-LA-*ran*-CC) with *d*-limonene with scCO$_2$

It is well known that various terpene hydrocarbons containing citrus peel and wood have a highly repellent effect on undesirable insects. Impregnation experiments were carried out under scCO$_2$ to incorporate useful compounds into biodegradable polymers. In a previous study, the impregnation of poly(L-LA-*ran*-CL) containing 15 mol % CL with *d*-limonene resulted in a higher content compared with L-LA homopolymer.[25] An increase in the *d*-limonene content is considered to lower the thermal properties, such as T_m, $-\Delta H_m$, and T_g. In this study, impregnation experiments with poly(L-LA-*ran*-CC) were conducted to attempt to increase the content and to evaluate the effect of the polymer structures. 2,2-DTMC and TEMC, which have chemical structures similar to that of CO$_2$, were used for copolymerization with L-LA.

The results of the impregnation of poly(L-LA-*ran*-2,2-DTMC) and poly(L-LA-*ran*-TEMC) with *d*-limonene are listed in Figure 2. The results for this study are indicated by a solid line; those for a previous study[25] using poly(L-LA-*ran*-CL) are indicated by a broken line. Poly(L-LA-*ran*-CL) with 85 mol % L-LA was investigated under scCO$_2$ at 40°C and 20 MPa and yielded a maximum *d*-limonene content of 3.2%.[25] The content of *d*-limonene was further increased by the copolymerization of L-LA with 2,2-DTMC or TEMC, as shown Figure 2.

In the impregnation of poly(L-LA-*ran*-2,2-DTMC), the content of *d*-limonene increased with increasing 2,2-DTMC and poly(L-LA-*ran*-CL). The contents of *d*-limonene were remarkably higher than those of poly(L-LA-*ran*-CL) and reached a maximum with poly(L-LA-*ran*-2,2-DTMC) (76/24) of 5.0%. Because T_m was as low as 50°C or even lower (as shown in Table I), copolymers at 21 to 58 mol % L-LA were dissolved under scCO$_2$ at 40°C and 20 MPa; this resulted in a decrease in the *d*-limonene content. The *d*-limonene content was slightly lower than the maximum content of 5.0%, although the content decreased with increasing 2,2-DTMC. Compared with poly(L-LA-*ran*-CL), it is thought that poly(L-LA-*ran*-2,2-DTMC) is difficult to melt under scCO$_2$ to obtain a higher T_m (122.5°C) and T_g (9.6°C) for the 2,2-DTMC homopolymer compared to a T_m (61.4°C) and T_g (−61.6°C) for PCL.[25]

The *d*-limonene content (5.0%) of the copolymer with 76 mol % L-LA increased 1.6 times compared

Figure 2 Impregnation of an L-LA random copolymer film (100 μm) with *d*-limonene.

to that (3.2%) of poly(L-LA-*ran*-CL) at 85 mol % of L-LA. Because the thermal properties (T_m, $-\Delta H_m$, and T_g) of poly(L-LA-*ran*-2,2-DTMC) were lower than those of poly(L-LA), the content was thought to increase because of plasticization by scCO$_2$. In addition, we considered that the effect of the impregnation of *d*-limonene to soften a copolymer increased because of the presence of a carbonate bond similar to CO$_2$. For these reasons, we presumed that the effect of the impregnation of poly(L-LA-*ran*-2,2-DTMC) with *d*-limonene was greater than that of L-LA homopolymer and poly(L-LA-*ran*-CL) under the same conditions.

The *d*-limonene contents of poly(L-LA-*ran*-TEMC) (81/19) were the highest among the L-LA random copolymers and reached a maximum of 5.3%. The *d*-limonene contents decreased with decreasing L-LA content at less than 81 mol %. The impregnation curve for poly(L-LA-*ran*-TEMC) was similar to that of poly(L-LA-*ran*-CL). The distributions of T_m and T_g of poly(L-LA-*ran*-TEMC) resembled that of poly(L-LA-*ran*-CL).

The common results for the three kinds of L-LA copolymers indicated that the highest value of *d*-limonene content was achieved around 80 mol % L-LA. Although the L-LA homopolymer had a T_m of 167.1°C, $-\Delta H_m$ of 49.0 J/g, and T_g of 55.9°C, the copolymerization of L-LA with other compounds lowered these values and facilitated the softening of the base polymer under scCO$_2$ so that the *d*-limonene content increased. In addition, there was a comparatively higher *d*-limonene content, at 76% or more. The copolymer structure and composition were important factors for increasing the *d*-limonene

TABLE III
Impregnation of Hinokitiol in Poly(l-LA-*ran*-2,2-DTMC) Films Under scCO$_2$

l-LA/2,2-DTMC composition[a]	Concentration (g/L)[b]	scCO$_2$		Time (h)	Content (%)[a]
		Temperature (°C)	Pressure (MPa)		
21/79	4.0	40	20	3	21.7
41/59	4.0	40	20	3	20.9
51/49	4.0	40	20	3	19.1
58/42	4.0	40	20	3	16.2
76/24	4.0	40	20	3	14.1
86/14	4.0	40	20	3	13.0
90/10	4.0	40	20	3	10.6
98/2	4.0	40	20	3	7.9
100/0	4.0	40	20	3	2.4

The impregnation was carried out with poly(l-LA-*ran*-2,2-DTMC) films (100 μm) for 3 h.
[a] Determined by ^{1}H-NMR spectroscopy.
[b] Expressed as the amount (grams) of hinokitiol per volume (0.5 L) of the reactor.

content. Furthermore, the copolymers required crystalline polymers that did not melt under scCO$_2$.

As mentioned previously for the copolymers with 2,2-DTMC, the carbonate bond was an important factor for softening polymers under scCO$_2$. TEMC, which is a CC with a carbonate bond, played an important role in polymer softening and promoting increased *d*-limonene content. Poly(L-LA-*ran*-TEMC) with 81 mol % L-LA raised the *d*-limonene content to 5.3%. The value measured was almost identical to that of poly(L-LA-*ran*-2,2-DTMC) (76/24, 5.0%) and was 1.7 times greater than that of poly(L-LA-*ran*-CL) (85/15, 3.2%). Although TEMC and CL are seven-membered cyclic compounds, their *d*-limonene contents differed greatly because of their distinct carbonate and ester structures.

Impregnation of poly(L-LA-*ran*-2,2-DTMC) with hinokitiol with scCO$_2$

Hinokitiol is an antibacterial agent found in the wood of Japanese cypress trees. The impregnation experiments were performed next with hinokitiol under scCO$_2$. In this study, poly(L-LA-*ran*-2,2-DTMC) was used as the base material because poly(L-LA-*ran*-TEMC) melted easily under scCO$_2$.

The results of the impregnation of poly(L-LA-*ran*-2,2-DTMC) with hinokitiol are listed in Table III. In the impregnation of poly(L-LA) with hinokitiol under scCO$_2$ (40°C, 20 MPa) for 3 h, the hinokitiol content of 2.4% was 2 times greater than that of *d*-limonene (1.2%); this suggested that the solubility of hinokitiol in scCO$_2$ was higher. The hinokitiol content (7.9%) of the copolymer with 98 mol % L-LA increased 2 times compared to that (3.9%)[25] of poly(L-LA-*ran*-CL) (95/5). The hinokitiol content of the copolymer increased with increasing 2,2-DTMC content and reached 14.1% with poly(L-LA-*ran*-2,2-DTMC) (76/24). The hinokitiol contents of the copolymers increased 2 times compared to that of poly(L-LA-*ran*-CL) at the same composition. Furthermore, although the hinokitiol contents of the copolymers increased with increasing 2,2-DTMC content, the copolymers with 58 mol % or less of L-LA were molten under scCO$_2$ at 40°C and 20 MPa. Poly(L-LA-*ran*-2,2-DTMC) with over 76 mol % L-LA was a more highly crystalline polymer with comparatively high $-\Delta H_m$. However, a higher 2,2-DTMC content showed a lower or no $-\Delta H_m$; this indicated a lower degree of crystallinity. The lower degree of crystallinity indicated more free volume inside the copolymer; so, we concluded that the hinokitiol content of the copolymer increased with increasing 2,2-DTMC content. These results indicate that the incorporation of hinokitiol into the copolymers with scCO$_2$ was affected by its compatibility with the copolymers.
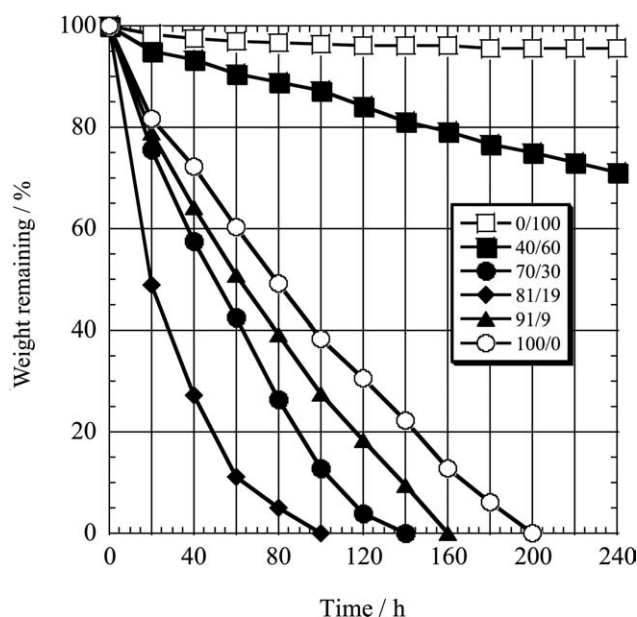
Degradation of poly(L-LA-*ran*-CC)

Enzymatic degradation testing of poly(L-LA-*ran*-CC) was carried out to evaluate its capacity for controlled release. Proteinase K was used as the enzyme for testing enzymatic degradation. Polymer films (ca. 30 mg) prepared by the solvent-casting method were used. The enzyme was dissolved in tricine buffer at pH 8.0 at 5 IU/mg of polymer.

Figure 3 shows the results of the enzymatic degradation test of poly(L-LA-*ran*-2,2-DTMC) with proteinase K. It was previously reported that poly(L-LA) is highly degradable by proteinase K.[25,28–32] In this study, the poly(L-LA) was completely degraded after 200 h. The rate of degradation of poly(L-LA-*ran*-2,2-DTMC) with less than 24 mol % 2,2-DTMC was faster than that of poly(L-LA); the copolymer with 76 mol % L-LA was completely degraded after 80 h. The cause of the higher degradability of
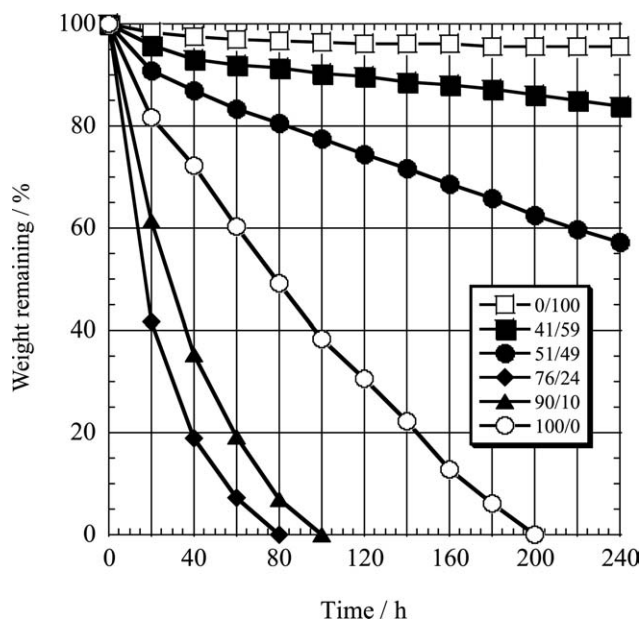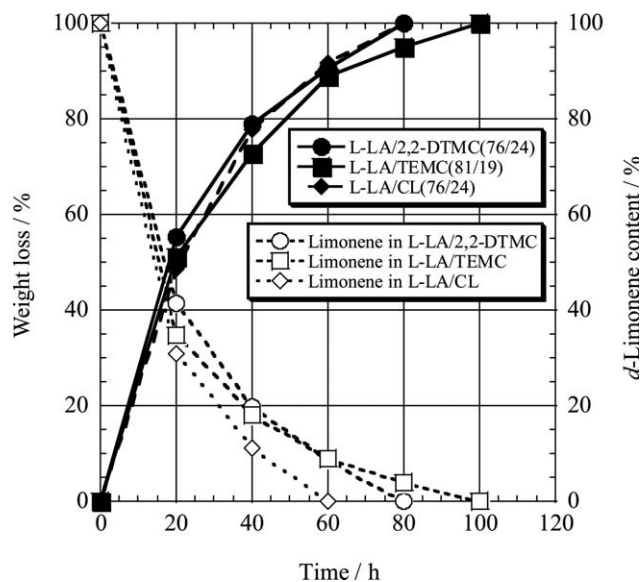
Figure 3 Enzymatic degradation of poly(L-LA-*ran*-2,2-DTMC) by proteinase K in a tricine buffer (pH 8.0).

poly(L-LA-*ran*-2,2-DTMC) versus that of poly(L-LA) was the decrease in the crystallinity by copolymerization with 2,2-DTMC (Table I). In contrast, the degradability of poly(L-LA-*ran*-2,2-DTMC) over 49 mol % 2,2-DTMC decreased compared with that of poly(L-LA). Proteinase K showed a substrate-dependent specific degradability toward poly(L-LA).[30,31] The L-LA unit was also a factor required for the enzymatic degradation of L-LA random copolymers. Thus, the introduction of a small amount of 2,2-

Figure 4 Enzymatic degradation of poly(l-LA-*ran*-TEMC) by proteinase K in a tricine buffer (pH 8.0).

Figure 5 Enzymatic degradation and controlled release of poly(L-LA) random copolymers by proteinase K in a tricine buffer (pH 8.0).

DTMC units into the L-LA sequence made the scaffold susceptible to enzymatic degradation.

The results of the enzymatic degradation test of poly(L-LA-*ran*-TEMC) with proteinase K are shown in Figure 4. As described previously, the rate of degradation of poly(L-LA-*ran*-TEMC) over 70 mol % L-LA was faster than that of poly(L-LA). On the other hand, the one below was slower. The degradability of poly(L-LA-*ran*-TEMC) with 81 mol % L-LA was the highest of those over 70 mol % L-LA; this resulted in complete disappearance after 100 h. The order of degradability of poly(L-LA-*ran*-TEMC) was 81 > 70 > 91 > 100 mol % L-LA. Previous researchers on the enzymatic degradation of L-LA copolymers with proteinase K has reported the highest degradation around 80 mol % L-LA,[28,29] and the results of this study were identical with those previous reports. We assumed that such copolymers have many easily degradable L-LA units and a lower crystallinity than poly(L-LA).

Controlled release from poly(L-LA-ran-CC)

The enzymatic degradation of the films of L-LA random copolymers impregnated with *d*-limonene was carried out, and the quantity of *d*-limonene remaining in the film was measured by ^{1}H-NMR. Poly(L-LA-*ran*-2,2-DTMC) (76/24) and poly(L-LA-*ran*-TEMC) (81/19), which had *d*-limonene contents of 5.0 and 5.3%, respectively, were used in this controlled release test. Poly(L-LA-*ran*-CL) (76/24) containing 2.8% *d*-limonene was used for reference. The results of this controlled release test are shown in Figure 5. The degradation curve of L-LA copolymers versus poly(L-LA-*ran*-CL) is indicated by a solid

line; that of poly(L-LA-*ran*-CL) is indicated by a long-dashed line. The amounts of *d*-limonene for poly(L-LA-*ran*-CC) and poly(L-LA-*ran*-CL) are indicated by a broken line and a dotted line, respectively. The degradability of the L-LA copolymers was evaluated by their percentage weight loss.

Poly(L-LA-*ran*-CL) was completely and easily degraded after 80 h. Although the *d*-limonene contained in the copolymer decreased with the degradation, the release rate of *d*-limonene was faster than the degradation rate of the specific copolymer. That *d*-limonene in the copolymer disappeared completely earlier than the copolymer meant that there was not a sufficient content of *d*-limonene. Moreover, we thought that the diffusion of *d*-limonene from the copolymer also occurred in a gradual hydrolysis without enzyme. Therefore, diffusion and insufficient impregnation (lower *d*-limonene content) were responsible for the high release rate of *d*-limonene.

The degradation curve of poly(L-LA-*ran*-2,2-DTMC) was nearly identical to that of poly(L-LA-*ran*-CL); this resulted in complete disappearance after 80 h. Because the *d*-limonene content of the copolymer was 1.8 times greater than that in poly(L-LA-*ran*-CL), the release rate with degradation for the copolymer was slower than that of poly(L-LA-*ran*-CL). In the experiment for poly(L-LA-*ran*-2,2-DTMC), the degradation curve was symmetrically in accord with the release curve. Because the time when the copolymer was degraded completely was the same as the time until *d*-limonene was released completely, the amount of *d*-limonene was assumed to be maintained until it completely disappeared for the copolymer. On the other hand, although poly(L-LA-*ran*-CL) (76/24) was degraded completely at 80 h, *d*-limonene was 100% released 60 h earlier than the disappearance of the copolymer. According to Kikkawa et al.,[33] in enzyme degradation, the amorphous regions around and under the hexagonal crystal were completely degraded, and crystalline lamellae were clearly observed. When the same composition ratio (76/24) was compared, the *d*-limonene content (5.0%) for poly(L-LA-*ran*-2,2-DTMC) was higher than the content (2.8%) of poly(L-LA-*ran*-CL) (Fig. 2). For the result of the thermal properties, $-\Delta H_m$ of poly(L-LA-*ran*-2,2-DTMC) was 20.4 J/g, in contrast to 27.7 J/g for the poly(L-LA-*ran*-CL) (Table I). We concluded that *d*-limonene was impregnated into the amorphous phase because the crystallites were tightly packed segments. Therefore, the controlled release of poly(L-LA-*ran*-2,2-DTMC) was found to be superior to that of poly(L-LA-*ran*-CL).

Poly(L-LA-*ran*-TEMC) could retain *d*-limonene for a longer time because of a slower rate of degradation compared to that of the other copolymers and the highest content of *d*-limonene. The controlled release of poly(L-LA-*ran*-TEMC) was as excellent as
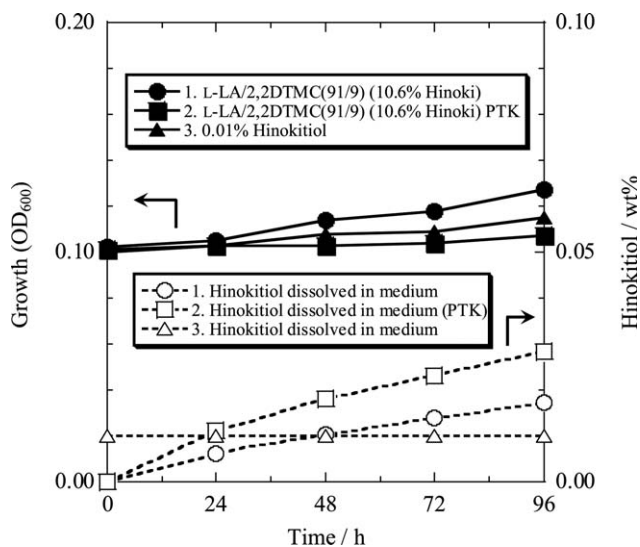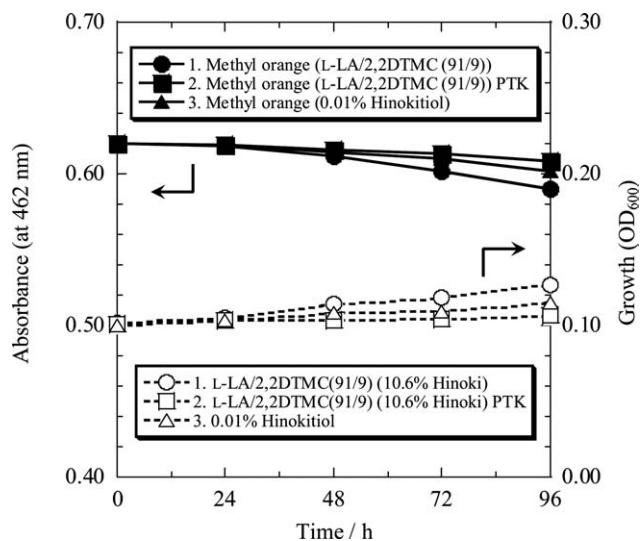

Figure 6 Multiplication curves of the *Aeromonas* sp. B-5 strain for the controlled release test of poly(l-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol.

that of poly(L-LA-*ran*-2,2-DTMC). Consequently, we demonstrated that poly(L-LA-*ran*-CC), which was capable of a higher rate of impregnation, was more suitable as a controlled release material than poly(L-LA-*ran*-CL), as was previously reported.[25]

To evaluate the antimicrobial properties of the hinokitiol released from the copolymer, *Aeromonas* sp. B-5, which has the ability to decolorize azo dyes,[34] was used for the antimicrobial test. Poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol was used for the antimicrobial test. The hinokitiol content of poly(L-LA-*ran*-2,2-DTMC) (91/9) prepared under $scCO_2$ was 10.6%. *Aeromonas* sp. B-5 was precultivated at 37°C for 20 h under shaking in L-broth medium before the antimicrobial test. The L-broth contained 0.01% methyl orange. Four experiments were used for the antimicrobial test, as follows: (1) medium containing poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol (10.6%) without proteinase K, (2) medium containing poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol (10.6%) with proteinase K (1 IU/mg of polymer), (3) medium containing 0.01% hinokitiol, and (4) medium with nothing added. The results of patterns 1–3 are shown in Figures 6 and 7; pattern 4 is not shown.

Figure 6 shows the growth of *Aeromonas* sp. B-5 in the controlled release test of poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol. A sample was taken every 24 h, and the turbidity of the cultures was measured at 600 nm as an index of growth. At each measurement, the concentration of hinokitiol was calculated from the amount obtained by the subtraction of the amount remaining in the copolymer after the test from the total amount contained in the copolymer before the test. Strain B-5 increased remarkably in the L-broth without the

Figure 7 Effect of the decolorization of methyl orange by *Aeromonas* sp. B-5 on the controlled release test of poly(l-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol.

presence of hinokitiol or the copolymer containing hinokitiol (2.169 of OD_{600} (degree of multiplication of a bacillus) after 24 h, not shown in Fig. 6). In addition, methyl orange was well decolorized (no absorbance after 24 h, not shown in Fig. 7). In contrast, the multiplication of strain B-5 was suppressed in the L-broth medium with 0.01% hinokitiol; the degree of growth was only 0.015% after 96 h. In the test with poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol (10.6%), the suppression of strain B-5 was confirmed. Hinokitiol was slowly released in the test without proteinase K, and the hinokitiol concentration was 0.017 wt % after 96 h. Although this amount seemed sufficient to suppress the multiplication of strain B-5, the growth of B-5 was slightly higher than the 0.01% of hinokitiol. In the test with proteinase K, which promoted copolymer degradation, we expected that the concentration of hinokitiol would become high and that more hinokitiol would be released. Actually, the level was 0.028 wt % after 96 h. The degree of growth of B-5 was 0.107 (an increase of only 0.006) in this medium after 96 h; this means that it was suppressed considerably.

A study of decolorizing azo dyes such as methyl orange by *Aeromonas* sp. B-5 has been reported.[34] This study found that it was difficult to decolorize methyl orange through suppression by *Aeromonas* sp. B-5 by the release of hinokitiol. Figure 6 confirms that multiplication of strain B-5 was suppressed. Figure 7 shows the effect of the decolorization of methyl orange by *Aeromonas* sp. B-5 on the controlled release test of poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol. The solid line indicates the absorbance of methyl orange, and the dashed line indicates the growth (OD_{600}) of *Aeromonas* sp. B-5. To determine the degree of decolorization of methyl orange, strain B-5 was cultivated at

37°C under shaking in L-broth medium containing 0.01% methyl orange. After the cells were removed by centrifugation at 11,000 rpm for 10 min, the absorbance of methyl orange in the supernatant fraction was measured at 462 nm with an ultraviolet–visible spectrophotometer. The absorbance of methyl orange was 0.19 after 24 h in the decolorization test with L-broth medium without 0.01% hinokitiol or poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol (10.6%, data not shown). This result indicates that the multiplied *Aeromonas* sp. B-5 decolorized methyl orange. On the other hand, the growth of *Aeromonas* sp. B-5 was suppressed in the L-broth medium containing 0.01% hinokitiol, and the methyl orange was scarcely decolorized, with an absorbance change of 0.62–0.60 after 96 h. A similar result was obtained in the experiment with poly(L-LA-*ran*-2,2-DTMC) (91/9) impregnated with hinokitiol (10.6%). The absorbance of methyl orange decreased from 0.62 to 0.59 by the hydrolysis of poly(L-LA-*ran*-2,2-DTMC) (91/9) containing hinokitiol without proteinase K after 96 h. Furthermore, the least change was a decrease of 0.01 in the enzymatic degradation of the copolymer containing hinokitiol with proteinase K. We concluded that hinokitiol was released from the copolymer by hydrolysis or enzymatic degradation, and the decolorization of methyl orange was affected because of the suppression of *Aeromonas* sp. B-5 by hinokitiol.

CONCLUSIONS

Although it was difficult to incorporate organic compounds with low boiling points into the polymer, such compounds could be included in poly(L-LA-*ran*-CC) with $scCO_2$. Furthermore, poly(L-LA-*ran*-CC) could be impregnated with organic compounds at a higher concentrations than could poly(L-LA-*ran*-CL). The result was the production of an advanced controlled release material.

The copolymerization was carried out at 150°C for L-LA with 2,2-DTMC and at 120°C for L-LA with TEMC with $Sn(oct)_2$ as a catalyst. For the copolymerization of L-LA with 2,2-DTMC, synthetic copolymers were obtained at a yield over 80% and a molecular weight over 3.0×10^4. The composition ratio of the obtained copolymers was almost the same as the feed ratio. In addition, the yields were considerably higher, at over 90% for all L-LA/TEMC feed ratios. The molecular weight of poly(L-LA-*ran*-TEMC) increased with an increase in the TEMC content in the range 0–60 mol % of the TEMC feed ratio. Poly(L-LA-*ran*-TEMC) was obtained at a higher yield and molecular weight than were poly(L-LA-*ran*-2,2-DTMC) and poly(L-LA-*ran*-CL).

To impregnate a higher amount of *d*-limonene, impregnation under $scCO_2$ was carried out with

poly(L-LA-*ran*-2,2-DTMC) and poly(L-LA-*ran*-TEMC) at 40°C and 20 MPa for 3 h. The *d*-limonene contents were remarkably higher than those with poly(L-LA-*ran*-CL) (3.2%) and reached maximums of 5.0% for poly(L-LA-*ran*-2,2-DTMC) (76/24) and 5.3% for poly(L-LA-*ran*-TEMC) (81/19). Consequently, the *d*-limonene content of L-LA random copolymer was increased up to 1.6 times with poly(L-LA-*ran*-2,2-DTMC) and 1.7 times with poly(L-LA-*ran*-TEMC) compared to poly(L-LA-*ran*-CL), respectively.

In the impregnation of poly(L-LA-*ran*-2,2-DTMC) with hinokitiol, the hinokitiol content of the copolymer increased with increasing 2,2-DTMC content, reaching 14.1% with poly(L-LA-*ran*-2,2-DTMC) (76/24) without melt. The hinokitiol contents of the copolymers used were doubled compared to that of poly(L-LA-*ran*-CL) at the same composition; this suggested that the solubility of hinokitiol in scCO2 was higher. We reached a preliminary conclusion that the impregnation of *d*-limonene and hinokitiol in copolymers containing 2,2-DTMC or TEMC increased because of the presence of a carbonate bond similar to CO_2.

In enzymatic degradation with proteinase K, poly(L-LA-*ran*-2,2-DTMC) (76/24) and poly(L-LA-*ran*-TEMC) (81/19) were easily degraded in each copolymer group and disappeared after 80 and 100 h, respectively. The enzymatic degradability of poly(L-LA-*ran*-2,2-DTMC) with proteinase K was higher than that of poly(L-LA-*ran*-TEMC) with respect to the same composition.

The degradability of poly(L-LA-*ran*-2,2-DTMC) (76/24) was the same as that of poly(L-LA-*ran*-CL) (76/24); this resulted in complete disappearance after 80 h. However, the degradability of poly(L-LA-*ran*-TEMC) (81/19) was lower. Because the *d*-limonene content of poly(L-LA-*ran*-2,2-DTMC) was 1.8 times higher than that of poly(L-LA-*ran*-CL), the release rate with the degradation of the copolymer was slower than was that of poly(L-LA-*ran*-CL), although they had the same rate of degradation. Therefore, the controlled release of poly(L-LA-*ran*-2,2-DTMC) was found to be superior to that of poly(L-LA-*ran*-CL). In addition, poly(L-LA-*ran*-TEMC) could be an excellent controlled release material and is capable of being amply impregnated with low-boiling-point compounds.

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