Preparation and Characterization of Novel Biodegradable Optically Active Network Polyesters from Malic Acid

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ABSTRACT: Novel optically active biodegradable network polyesters were prepared from L- and D-malic acid (Ym_L and Ym_D) and glycols of different number of methylene groups ($HO(CH_2)_nOH$, *n*G, $n = 2-6$, 8−10, and 12). Prepolymers were prepared by a melt polycondensation, and the prepolymer films cast from dimethylformamide solution were postpolymerized at 220 °C for various periods of time to form network films. The resultant films were transparent, flexible, and insoluble in organic solvents. The network polyesters obtained were characterized by ^{13}C nuclear magnetic resonance, gel permeation chromatography, infrared absorption spectra, wide-angle X-ray diffraction analysis, density measurement, differential scanning calorimetry, thermomechanical analysis, and scanning electron micrographs. The biodegradation experiments for the network polyester films were carried out in enzymatic solution with *Rhizopus delemar* lipase and in an activated sludge. The degree and rate of biodegradation were estimated by the weight loss of the films. After the incubation for 10 days at 37 °C in *Rh. delemar* lipase buffer solution, the weight loss significantly depended on the number of methylene groups in the glycol component and showed the maximum weight loss of 52 g/m^2 for 4GYm_L (6.8 g/m^2 in the absence of *Rh. delemar* lipase). In the exposure to activated sludge, the network polyester films showed a similar dependence of weight loss on the number of methylene groups as in the case of the enzymatic degradation, while the rate of biodegradation was much slower than that of the enzymatic degradation. The effect of stereochemistry of network polyesters prepared from L-/D-malic acid on the biodegradability was also investigated: the rate of the biodegradation for the network polyesters with L-isomers was slightly higher than that for those with D-isomers.

Introduction

We have studied the synthesis, characterization, and biodegradability of network polyester films with much enhanced resistance to thermal distortion and organic solvents from several polyhydric alcohols and aliphatic dicarboxylic acids of various methylene lengths.[1−4] It was found that their biodegradability behavior is varied depending markedly on their chemical structures, and those prepared from aliphatic dicarboxylic acids with medium methylene length (C_8-C_{12}) and trihydric alcohols such as glycerol[1,2] and 1,1,1-trimethylolethane[3] are degraded thoroughly by a few lipase enzymes. We have now focused on malic acid as the trifunctional monomer because it is obtained from natural resources such as fruit and it is optically active. Water-soluble linear malic acid polymers have been synthesized for biodegradable and bioabsorbable material,[5,6] and it was shown that the main-chain ester bond in poly(α-malic acid) was cleaved randomly and slowly in vitro,[5] but no work on network polyesters from malic acid has been reported in the literature.

In this paper, a series of optically active network polyesters were prepared from L-malic acid and various glycols with different numbers of methylene groups. The effects of the number of methylene groups on the structure and thermal properties as well as biodegradability are investigated. In addition, the effect of stereochemistry of network polyesters prepared from L/D-malic acids on the biodegradability was also examined.

Experimental Section

Monomers. L- and D-malic acids (Ym_L and Ym_D) and glycols (*n*G), with different numbers of methylene groups ($HO(CH_2)_n$-OH, $n = 2-6$, 8−10 and 12), were purchased from Nakarai Tesque (Kyoto, Japan) and used as received.

Preparation of Prepolymers. All prepolymers were prepared by a melt polymerization. A mixture of 20 mmol of Ym_L (or Ym_D) and 10 mmol of glycol was heated at 140 °C for 6−6.5 h in a stream of nitrogen. In a similar manner, the LD-isomer was prepared from a equimolar mixture of Ym_L and Ym_D. The details of polymerization procedures were described elsewhere.[2]

Film Preparation and Postpolymerization. The prepolymer obtained was cast from a ca. 17 wt % dimethylformamide solution at 80 °C on an aluminum plate (about 100 μm thickness). The cast film was postpolymerized at 220 °C for various periods of time in a nitrogen atmosphere. After the postpolymerization, the film was peeled off from the plate and stored in a desiccator over silica gel. The polyester films obtained were flexible, transparent, and insoluble in organic solvents for polyesters such as *sym*-tetrachloroethane/phenol and *m*-cresol. They are denoted using the monomer code: for example, polyester films derived from Ym_L and *n*G are denoted as *n*GYm_L.

Characterization. The Fourier transform infrared (FTIR) spectrum was recorded on a Fuji Electric model FIRIS 25 FTIR spectrometer using a thin film. Carbon-13 nuclear magnetic resonance (^{13}C NMR) measurements were made on a JEOL JNM-EX90A FT-NMR spectrometer at 25 °C in $CDCl_3$. A

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Horiba spectropolarimeter (SEPA-300) was used for a D-line optical rotation measurement with filtered sodium light at 25 °C. Wide-angle X-ray scattering (WAXS) was performed with a Toshiba model ADG-301 X-ray diffractometer with nickel-filtered Cu Kα radiation. Differential scanning calorimetry (DSC) was made on a Perkin-Elmer DSC 7 differential scanning calorimeter with a heating rate of 10 °C/min in a nitrogen atmosphere controlled by a 1020 workstation. To provide the same thermal history, each sample was preheated from room temperature to 100 °C and rapidly cooled to −50 °C. Then the DSC scan was recorded by heating from −50 to 100 °C. Thermomechanical analysis (TMA) was performed in a penetration mode under a pressure of 10 kg/cm^2 and a heating rate of 20 °C/min in a nitrogen atmosphere, using a Seiko model TMA-100 thermomechanical analyzer controlled by a SSC-5200 disk station. The density of the film was measured using a sink and float method in potassium iodide aqueous solution at 30 °C. Gel permeation chromatography (GPC) was performed using a Shimadzu LC-9A system with GPC KF-802 column (Showa Denko) at 40 °C. Tetrahydrofuran was used as the eluent at a flow rate of 1 mL/min. Polystyrene standards with low polydispersities were used for calibration. The surface of the film before and after biodegradation was observed using a Hitachi S-500 scanning electron microscope after coating with gold.

Enzymatic Degradation. The film specimen (20 mm × 20 mm, ca. 120 μm thickness) was placed in a small bottle containing 10 mL of 1/15 mol phosphate buffer solution (pH 7.2) with and without 20 mg of *Rhizopus delemar* lipase (specific activity of 720 unit/mg from Seikagaku Kogyo Co., Ltd.). The bottle was incubated at 37 °C for various periods of time. After incubation the film was washed with water and dried at 80 °C in vacuo. The degree and rate of degradation were estimated from the weight loss expressed as g/m^2, which was calculated by dividing the weight loss by the surface area of the film. The weight loss averaged for two specimens was employed.

Degradation in Activated Sludge. The film specimens were put into a pouch made of 40 mesh nylon net, and the pouch was placed in the aeration tank of the municipal sewage treatment plant in Minamiku, Kyoto (Japan). The plant treatment conditions were the following: pH = 6.6−7.0, temperature = 15.4−19.7 °C, dissolved oxygen = 3.1−8.4 mg/L, mixed liquor suspended solid (MLSS) = 1100−1990 mg/L. At selected time, the film was recovered and was washed with running water and finally dried at 80 °C to constant weight in vacuo. The degree and rate of degradation were estimated from the weight loss using the same procedure as in the case of enzymatic degradation.

Alkali Hydrolysis. Alkali hydrolysis of the film specimen was conducted in a phosphate buffer solution of pH 9 at 37 °C for 5 days. The degree of alkali hydrolysis was also estimated from the weight loss using the same procedure as in the case of enzymatic degradation.

Results and Discussion

Structure of Prepolymers. The structure of prepolymers prepared by a melt polycondensation was determined by ^{13}C NMR spectroscopy. The typical ^{13}C NMR spectra of 10GYm$_{DL}$ prepolymer along with its carbon assignments are depicted in Figure 1. Special attention was paid for carbonyl carbon range as shown in the expanded spectra. The appearance of four carbonyl signals (a−d) represents the presence of all the possible ester types formed by the condensation reaction of Ym$_{DL}$ and 10G (a, b) and by the self-condensation reaction of Ym$_{DL}$ (c, d). The signals at 176.0 (e) and 176.3 (f) ppm are responsible for those of unreacted β-carboxylic acid and α-carboxylic acid on Ym$_{DL}$, respectively.[6]

Degree of Reaction. After casting the prepolymer, it was postpolymerized to form a network. The network

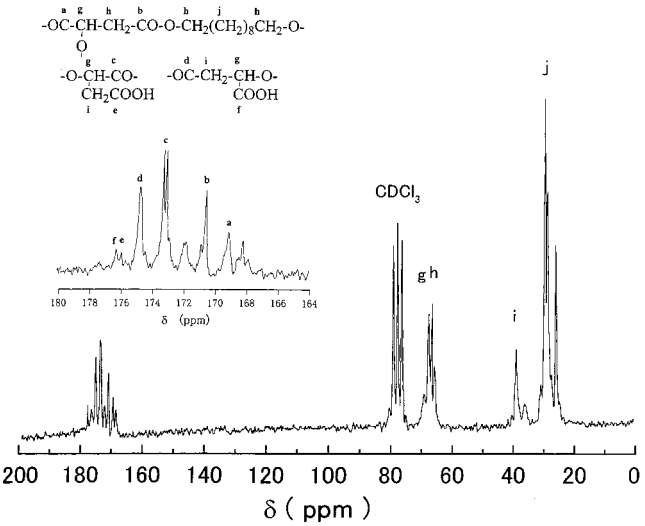

Figure 1. Typical ^{13}C NMR spectra of 10GYm$_{DL}$ prepolymer in $CDCl_3$ at 25 °C.

Table 1. Degree of Reaction (D_R) of *n*GYm$_L$ Films Polymerized at 220 °C for 4 h

polymer code	D_R (%)	polymer code	D_R (%)
2GYm$_L$	79	8GYm$_L$	92
3GYm$_L$	80	9GYm$_L$	94
4GYm$_L$	84	10GYm$_L$	95
5GYm$_L$	88	12GYm$_L$	94
6GYm$_L$	90		

aliphatic polyesters showed infrared absorptions due to hydroxyl group at 3460 cm^{-1} and methylene groups at 2960 cm^{-1}. The absorption at 3460 cm^{-1} decreased with increasing postpolymerization time, while the absorption at 2960 cm^{-1} remained unchanged. Since the postpolymerization proceeds through the reactions between the carboxyl group of malic acid and hydroxyl group of malic acid and glycol, the change of absorption intensity ratio between −OH and ⟩CH$_2$ or ⟩CH groups, A_{OH}/A_{CH_2} , is a measure of the degree of reaction. For 10GYm$_L$, at the beginning of reaction, the ratio of hydroxyl and methylene groups in a monomeric unit, [OH]/[CH$_2$], is 4/14 and varies with the progress of reaction to become (4 − 4)/14 when the network structure of film has completely developed. Thus, the following equation is defined:

$$[OH]/[CH_2] = (4 - y)/14$$

and

$$y = 4 - 14[OH]/[CH_2]$$

Here y is the number of reacted carboxyl groups. The extended general expression for *n*GYm films is

$$y = 4 - (n + 4)[OH]/[CH_2]$$

The degree of reaction (D_R) is calculated as

$$D_R = (y/4) \times 100\ (\%)$$

To obtain the quantitative [OH]/[CH$_2$] ratio in network films, the calibration curve between A_{OH}/A_{CH_2} made by the known diols and alcohols was used.[7]

D_R values of network polyester films from Ym$_L$ are summarized in Table 1. With increasing the length of the methylene chain in the glycol component, D_R

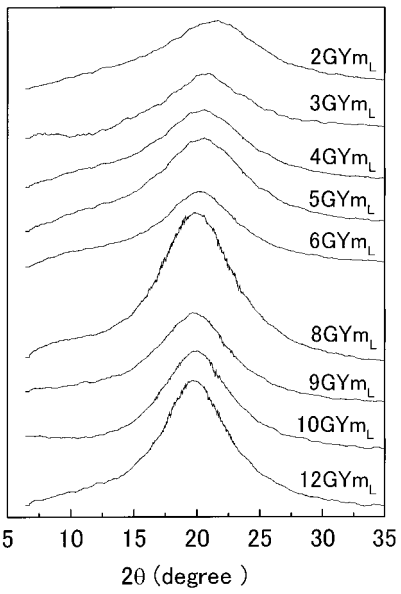

Figure 2. WAXS patterns of *n*GYm$_L$ films.

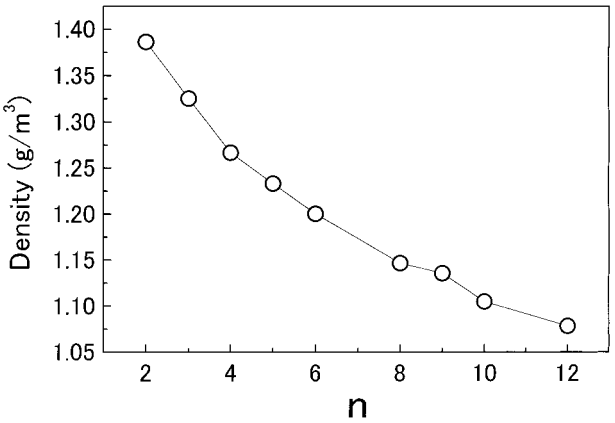

Figure 3. Plots of density vs the number *n* for *n*GYm$_L$ films. The density was measured at 30 °C.

increases gradually and attains a maximum value of 95% for 10GYm$_L$. This is probably due to the longer flexible methylene chain, allowing an increase of the reactivity between the carboxylic group and glycol. The similar dependence of D_R on the number of methylene groups has been observed for network polyesters prepared from aliphatic dicarboxylic acids of various methylene lengths with glycerol (Yg*n*)[1,2] or 1,1,1-trimethylolpropane (Ye*n*).[3] Network polyesters with LD- and D-isomers have almost the same D_R values as the corresponding polyesters with L-isomers.

Structure of Postpolymerized Films. Figure 2 shows WAXS intensity curves of *n*GYm$_L$ films. The distinct single diffraction peaks appears for *n*GYm$_L$ films, which implies some ordered structure of network polyesters. A similar distinct single diffraction peaks were previously reported for Yg*n*[1,2] and Ye*n* films,[3] suggesting some ordered structure owing to the regular network by the symmetric structure of the polyfunctional monomers. *n*GYm$_{DL}$ and *n*GYm$_D$ films also showed a similar distinct single diffraction peak as *n*GYm$_L$ did, suggesting that the network structure of polyester film is hardly affected by the stereochemistry between L- and D-isomers.

Figure 3 shows density versus the number of *n* for *n*GYm$_L$ films. Density decreases gradually with increasing the number of *n*, which would be explained by an increase of the free volume of the network caused by extension of the length between the cross-linked sites. Similar behaviors have been observed for Yg*n*[1,2] and Ye*n* films.[3]

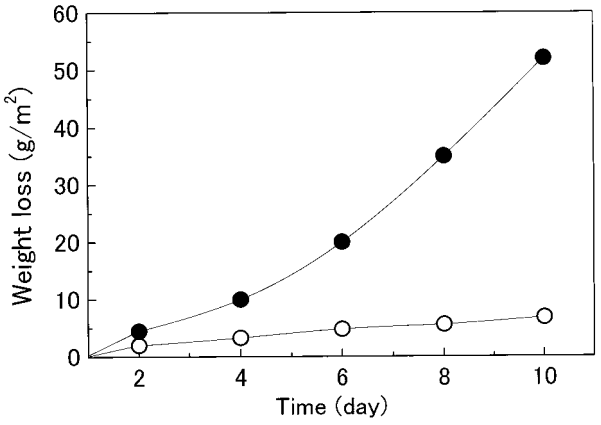

Figure 4. Weight loss of 4GYm$_L$ film against time in a buffer solution with (●) and without (○) *Rh. delemar* lipase at 37 °C.

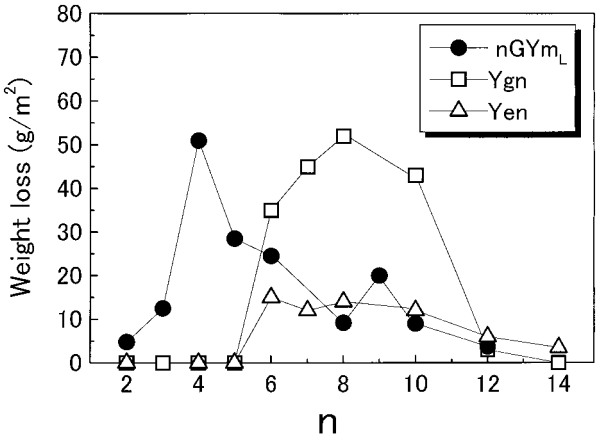

Figure 5. Plots of weight loss vs the number of *n* for *n*GYm$_L$ films (●) degraded in a buffer solution with *Rh. delemar* lipase at 37 °C for 10 days. Replots of those for Yg*n* (□)[2] and Ye*n* (△)[3] films are also shown.

Enzymatic Degradation of Postpolymerized Films. Figure 4 shows the dependence of the weight loss of 4GYm$_L$ film on incubation time in phosphate buffer solution with and without *Rh. delemar* lipase at 37 °C. The phosphate buffer/enzyme solution was replaced every 48 h to keep enzyme activity at desired level throughout the experiment duration. The weight loss due to the buffer solution increases slightly to 6.8 g/m^2 with incubation time, while it increases nonlinearly to 52 g/m^2 under a presence of the lipase, and the film began to fragment after the incubation for 10 days. The surface of 4GYm$_L$ film after the incubation for 10 days was observed by scanning electron micrographs (SEM). It is apparently blemished by *Rh. delemar* lipase, suggesting that enzymatic degradation occurred on the surface of the film. Figure 5 shows the dependence of weight loss for *n*GYm$_L$ films on the number of methylene groups after the incubation in a *Rh. delemar* lipase buffer solution for 10 days at 37 °C. In Figure 5, the weight loss for Yg*n* with incubation time for 2 h and that of Ye*n* with incubation for 2 days are also replotted against the number of methylene groups in aliphatic dicarboxylic acids (HOOC(CH$_2$)$_n$COOH). The weight loss of 2- or 3GYm$_L$ film is considerably small, but it

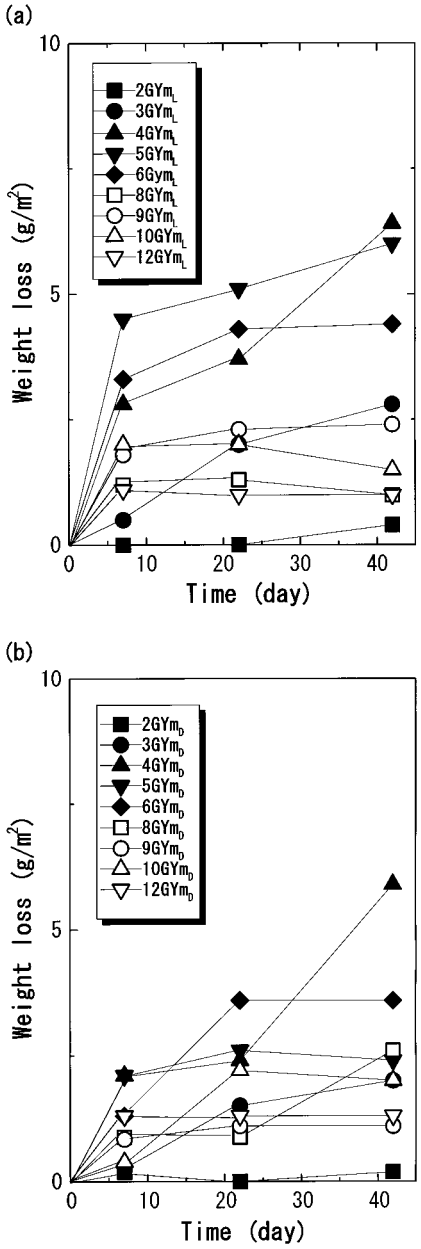

Figure 6. Weight loss of nGYm$_L$ (a) and nGYm$_D$ (b) films against time of exposure to activated sludge.

increases drastically for 4GYm$_L$ (52 g/m^2) and reduces gradually with increasing the number of n. The dependence of weight loss for nGYm films on the number of methylene group is substantially different from that of Ygn and Yen films. The difference may reflect the substrate specificity of *Rh. delemar* lipase for network polyester hydrolysis.

The mode of action of polyester hydrolysis by *Rh. delemar* was studied by identification of hydrolysis products using GPC. For the 4GYm$_L$ sample after degradation, the solution was freeze-dried and the residue was dissolved in tetrahyrofuran and filtered and then subjected to GPC analysis. Some oligomers, 4G and Ym$_L$, components were detected, demonstrating that the 4GYm$_L$ network polyester was degraded completely into monomers.

Degradation of Postpolymerized Films in Activated Sludge. The network polyester films were also exposed to activated sludge at 15−20 °C for various periods of time. The changes of weight loss with immersion time in activated sludge are shown for nGYm$_L$ and nGYm$_D$ films in Figure 6, a and b, respectively. The weight loss increases with immersion time for all the films, indicating that they are biodegraded in activated sludge. The rate of biodegradation is much slower than that of above enzymatic degradation, probably due to the lower immersion temperature (15−20 °C) and/or the lower concentration of extracellular enzymes attacking nGYm$_L$ and nGYm$_D$ films contained in activated sludge. Figure 7 shows the plot of weight loss vs the number of n for nGYm$_L$ and nGYm$_D$ films after the exposure to activated sludge for 42 days. This plot of weight loss for nGYm$_L$ films is in good agreement with that of the enzymatic degradation described above. The effect of stereochemistry between L- and D-isomers on degradation in activated sludge is discussed later. The SEM photographs of the surface of 4GYm$_L$ film before and after the biodegradation in activated sludge for 42 days (weight loss 6.4 g/m^2) are shown in Figure 8. Before exposure to activated sludge, the film surface is smooth. In contrast, the transparent film became opaque and was eroded after exposure to activated sludge, which would be due to the degradation caused by extracellular enzymes contained in activated sludge.

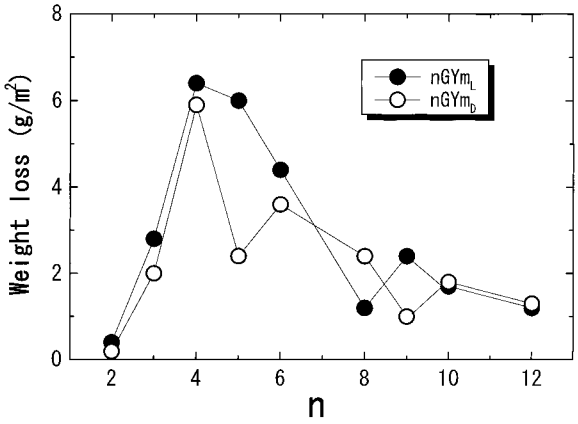

Figure 7. Plots of weight loss vs the number of n for nGYm$_L$ and nGYm$_D$ films after the exposure to activated sludge for 42 days.

The Effect of Stereochemistry on Biodegradability. The effect of stereochemistry on enzymatic degradation is examined for 4GYm series films. Optical rotation was measured for 4GYm series prepolymers because network 4GYm series films postpolymerized at 220 °C for 4 h are insoluble in any organic solvents. Specific optical rotations obtained in 1% tetrahydrofuran solution at 25 °C are −16.9, −0.2, and +16.5 for 4GYm$_L$, 4GYm$_{DL}$, and 4GYm$_D$ prepolymers, respectively, suggesting that optical activity was retained during a melt polycondensation. Figure 9 shows weight loss after the incubation for 10 days in phosphate buffer solution with and without *Rh. delemar* lipase at 37 °C. With increasing D-isomer content, weight loss decreases appreciably in the presence of a lipase, while it is substantially unchanged in the buffer solution without lipase, indicating that *Rh. delemar* lipase degrades network polyester with L-isomers faster than that with D-isomers.

The degradation behaviors of network polyester films with L- and D-isomers in the activated sludge were previously shown in Figures 6 and 7. nGYm$_L$ films exhibit the almost similar degradation time dependence as nGYm$_D$ films do. The weight loss of network polyesters from L-isomers degraded in activated sludge for 42

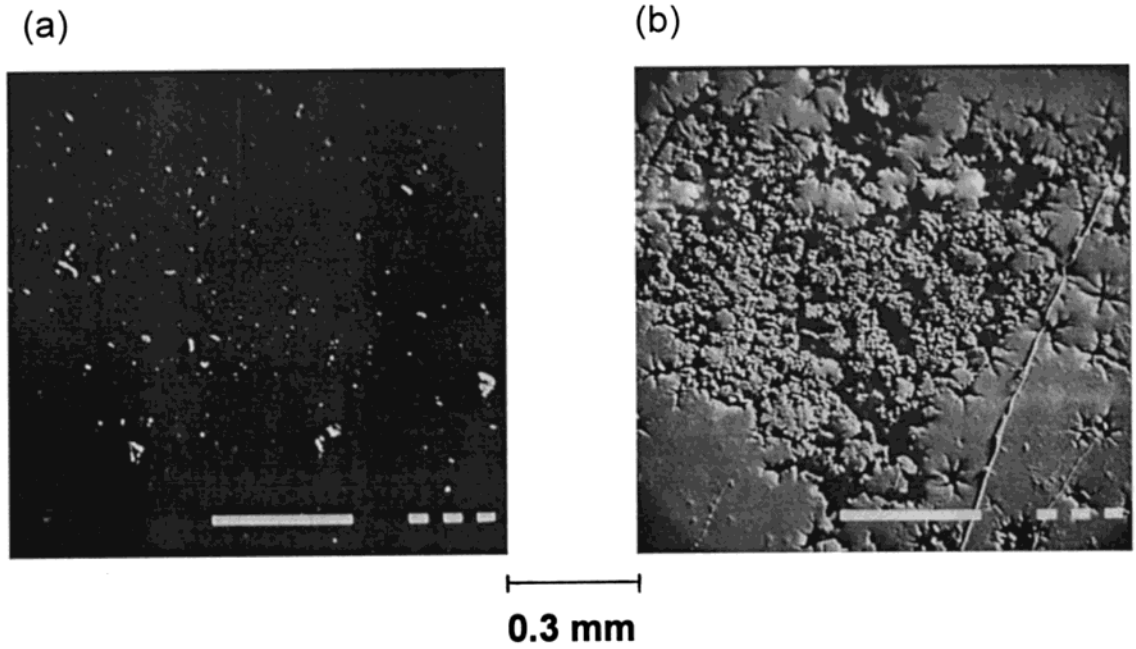

Figure 8. Scanning electron micrographs of the surface of $4GYm_L$ film before (a) and after (b) biodegradation for 42 days of activated sludge exposure.

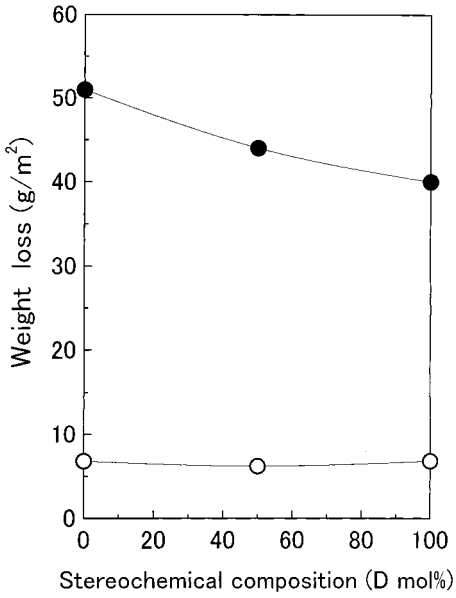

Figure 9. Weight loss of 4GYm stereoisomers degraded in a buffer solution with (●) and without (○) *Rh. delemar* lipase at 37 °C for 10 days.

days was slightly larger than the corresponding polyesters from D-isomers, which is compatible with the results of above enzymatic degradation.

Alkali Hydrolysis. The hydrolysis in highly alkaline buffer solution of pH 9 was carried out to compare with the biodegradation behaviors described above. Figure 10 shows the plot of weight loss of $n\text{GYm}_L$ films degraded for 5 days at 37 °C vs the number of *n*. Weight loss is hardly observed for the films with the longer methylene chain, whereas it increases rapidly with increasing the number *n*. The increase of weight loss would be due to an increase in a concentration of hydrolyzable ester linkage, which is entirely different from the results in biodegradability discussed above.

Thermal Properties of Postpolymerized Films. Figure 11 shows typical DSC scans for $n\text{GYm}_L$ films.

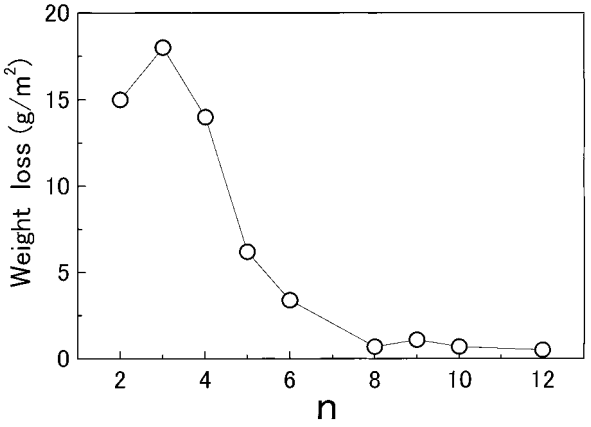

Figure 10. Plots of weight loss vs the number of *n* for $n\text{GYm}_L$ degraded in a buffer solution of pH 9 at 37 °C for 5 days.

Endothermic change due to glass transition (T_g) is observed for all the films. T_g decreases monotonically with increasing the number *n*. T_g is significantly related to the free volume in polymer matrix. The free volume of network (V_f) is estimated from the measured density (ρ) shown in Figure 3 according to the following equation:[8]

$$V_f = 1/\rho - 1.3\ V_w/M$$

where V_w is the summation of group contribution of van der Waals molar volume (cm^3/mol) and *M* is a molecular weight of repeating monomeric unit (g/mol). In this work specific volume of network polyester was employed instead of free volume (V_f) because *M* of $n\text{GYm}_L$ films could not be estimated.[9] Figure 12 shows the relation between T_g and specific volume calculated from the density values shown in Figure 3. It is clearly shown that the decrease of T_g is ascribed to the increase of specific volume. Endothermic melting peak due to the crystallization of the methylene unit in the glycol also appears at 33 °C for $12GYm_L$. However, no peak corresponding to this crystallization was observed in the

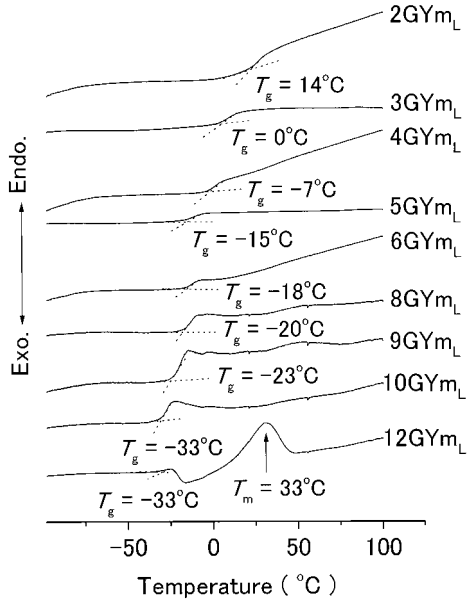

Figure 11. DSC curves of nGYm$_L$ films on the second heating runs; heating rate 10 °C/min.

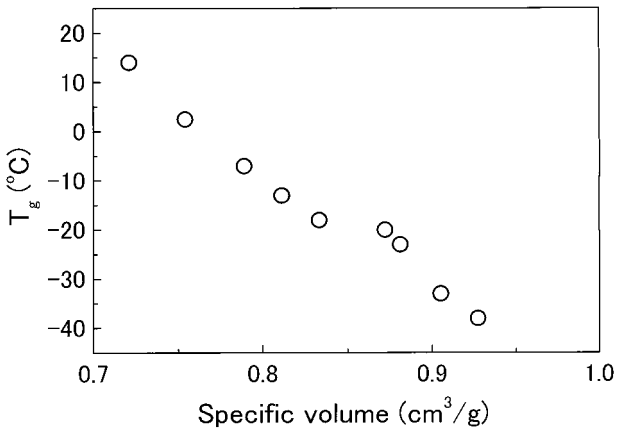

Figure 12. Plots of glass transition temperature vs specific volume for nGYm$_L$ films. The values of glass transition temperature and specific volume were obtained from the densities in Figure 3 and DSC curves in Figure 11, respectively.

WAXS intensity curve shown in Figure 2 because it is measured at room temperature, which is close to the melting transition. Such an endothermic melting peak has been observed for network polyesters from aliphatic dicarboxylic acids with the longer methylene unit and polyhydric alcohol such as glycerol and 1,1,1-trimethylolethane.[2−4]

The heat distortion temperature (T_h), the inflection point of TMA curve, is measured according to the method described previously.[2] The TMA probe penetrated into the film completely in the vicinity of 300 °C due to thermal decomposition. T_h values obtained are plotted against the number n in Figure 13. T_h decreases gradually with increasing the number n, suggesting that increasing the length of the methylene chain in the glycol component enhances the mobility of networks. The plot of T_h vs n corresponds to the depression of T_g with an increase of n shown in Figure 11. The thermal

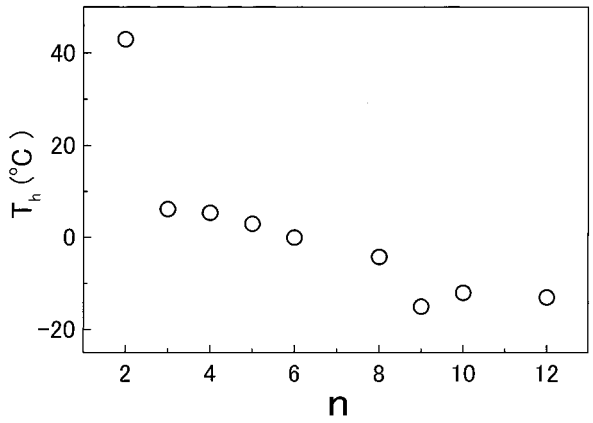

Figure 13. Plots of heat distortion temperature vs the number of n for nGYm$_L$ films.

properties such as T_g and T_h were hardly affected by the stereochemical compositions of network polyester.

Conclusion

Novel biodegradable network polyesters were prepared from malic acid with various glycols with different numbers of methylene groups. It was found that nGYm films are biodegraded both in the buffer solution with *Rh. delemar* lipase and in an activated sludge. After the incubation for 10 days in by *Rh. delemar* lipase buffer solution, the weight loss of nGYm largely depends on the number of n and shows the maximum weight loss for 4GYm. A remarkable dependence of weight loss on methylene chain length also appeared for the biodegradation in activated sludge, which was similar to that of the enzymatic degradation. The stereochemistry between the L- and D-isomer of network polyester films gave rise to the small differences in biodegradation rate: the rate of biodegradation for the network polyester with L-isomer is higher than that with D-isomer.

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(9) The nGYm$_L$ films are formed by the condensation reaction of Ym$_L$ and nG and by the self-condensation reaction of Ym$_L$ as shown in ^{13}C NMR spectra (Figure 1). Thus, the structure of repeating unit nGYm$_L$ films could not be defined clearly.

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