Synthesis, Characterization, and Enzymatic Degradation Studies on Novel Network Aliphatic Polyesters

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ABSTRACT: Network polyesters were prepared from1,1,1-trimethylolethane (Ye), 1,1,1-trimethylolpropane (Yp), 1,2,3,4-butanetetrol (Xe), and D-glucitol (Zs) with aliphatic dicarboxylic acids containing different numbers of methylene groups ($HOOC(CH_2)_{n-2}COOH$, n =4, 6−14, 16, and 20). Prepolymers prepared by melt polycondensation were cast from dimethylformamide solution and postpolymerized at 230 °C for 4 h to form a network. The resultant films were transparent, flexible, and insoluble in organic solvents. The network polyesters obtained were characterized by infrared absorption spectra, wide-angle X-ray diffraction analysis, density measurement, thermomechanical analysis, differential scanning calorimetry, and tensile test. The enzymatic degradation was estimated by the weight loss of the network polyester films in a buffer solution with lipase enzymes at 37 °C. The enzymatic degradation for Ye*n* films was compared with that for the network polyester films of glycerol (Yg) or pentaerithritol (Xp) with various aliphatic dicarboxylic acids reported earlier.[11,12] After the incubation in *Rhizopus delemar* lipase solution for 2 days, the weight loss was hardly observed for Ye4−7, while it increased drastically for Ye8−13 (13−15 g/m^2) and then decreased gradually for Ye14−20. Other lipases of porcine pancreas and *Psedomonas cepacia* hardly degraded Ye*n* films. The degradation rate for 10 series films was decreased as follows:Yg10 >Ye10 > Yp10 = Xe10 > Xp10 > Zs10, corresponding to the increase in the number of hydroxyl groups in the polyhydric alcohols.

Introduction

In recent years much attention has been paid to biodegradable materials both for environmental problems and in biomedical application. Among them synthetic aliphatic polyesters are an important class of biodegradable and hydrolyzable polymers.[1−3] The biodegradable polyesters developed up until now, however, are almost linear polyesters. The incorporation of network structure into the backbone is of interest for new biodegradable materials, since it is expected to give better physical and chemical properties such as resistance to heat distortion and resistance to chemicals.[4−10]

In previous papers, novel network polyesters prepared from aliphatic dicarboxylic acids with glycerol (Yg)[11] or pentaerithritol (Xp)[12] were found to be enzymatically degradable, depending drastically on their chemical structures. Network polyesters from Yg and dicarboxylic acids with medium methylene lengths (C_8−C_{12}) were degraded markedly.[13] Moreover, the degradation rates of Yg series films were much faster than those of Xp series ones. These findings encouraged us to examine the biodegradability of network polyesters with a variety of chemical structures. Up to now no enzymatic degradation of network polyesters has been found in the literature except for a series of our work.[11−13] Very recently Valiente et al.[14] examined the enzyme-catalyzed degradation of network polyesters prepared from poly(1,2-propanediyl fumarate), and they showed that cross-linked chains have not been degraded by *Chromobacterium viscosum* lipase.

In this study, network polyesters were prepared from various polyhydric alcohols and aliphatic dicarboxylic acids with different numbers of methylene groups. The effects of the number of methylene groups and chemical structure of alcohols on physicochemical and thermal properties as well as enzymatic degradation was examined systematically in comparison with previously reported analogues network polyesters prepared from Yg[11]or Xp.[12]

Experimental Section

Monomers. Glycerol (Yg) was distilled under reduced pressure. 1,1,1-Trimethylolethane (Ye), 1,1,1-trimethylolpropane (Yp), pentaerythritol (Xp), 1,2,3,4-butanetetrol [*meso*-erythritol] (Xe), D-glucitol [sorbitol] (Zs), and aliphatic dicarboxylic acids with different numbers of methylene groups ($HOOC(CH_2)_{n-2}COOH$, n = 4, 6−14, 16, and 20) were used as received.

Preparation of Prepolymers. A mixture of dicarboxylic acid and polyhydric alcohols (molar ratio of dicarboxylic acid/polyhydric alcohol: 3/2 for Ye and Yp; 2/1 for Xe; 3/1 for Zs) was heated in a stream of nitrogen at 200 °C for 21−35 min for Ye4−12 and Ye14−20, and for 40−55 min for Ye13, Xe10, and Zs10. In the case of Yp10, a mixture was heated at 180 °C for 40 min and subsequently at 200 °C for 8 min. The details of polymerization procedures were described elsewhere.[12]

Film Preparation and Postpolymerization. The prepolymer obtained was cast from a ca. 17 wt % dimethylformamide solution at 80 °C on an aluminum plate. The cast film was heated at 230 °C for 4 h in a stream of nitrogen. After the aluminum was dissolved off in a 10% HCl solution, a transparent and flexible film was obtained which was insoluble in organic solvents for polyesters such as *sym*-tetrachloro-

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ethane/phenol mixed solvent and *m*-cresol. The polyesters obtained were denoted using the monomer code. For example, the polyesters derived from Ye and *n* are denoted as Ye*n*. In addition, the polyesters from sebacic acid and various polyhydric alcohols are called 10 series films.

Characterization. The Fourier transform infrared spectrum (FTIR) was performed on a Fuji Electric model FIRIS 25 FTIR spectrometer using a thin film. Wide-angle X-ray scattering (WAXS) patterns of the films were measured with a Toshiba model ADG-301 X-ray diffractometer with nickel-filtered Cu Kα radiation. Differential scanning calorimetry (DSC) was performed using a Perkin-Elmer DSC7 controlled by a 1020 TA workstation. It was operated at a heating rate of 5 °C/min in a nitrogen atmosphere. 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 Instruments Model TMA-100 themomechanical analyzer controlled by a SSC-5200 diskstation. The tensile test was performed with a Iwamoto tensile tester at a strain of 100%/min to measure tensile strength, elongation, and Young's modulus. An average value for 5−10 specimens was employed. 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 determined by 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. Poly(ethylene oxide) standards with low polydispersities were used for calibration.

Enzymatic Degradation. The enzymes used in this study are lipases from *Rhizopus delemar* (specific activity of 720 unit/mg from Seikagaku Kogyo Co., Ltd.), *Pseudomonas cepacia* (specific activity of 1,140 unit/mg from Amano Pharmaceutical Co., Ltd.), and porcine pancreas (specific activity of 20 000 units/mg from Sigma Chemical Co., Ltd.). The film specimen (20 mm × 20 mm; about 120 μm thickness) was placed in a vial containing 10 mL of $^1/_{15}$ mol phosphate buffer solution (pH 7.2) with and without 12 000 units of the above-mentioned lipase. The vial was incubated at 37 °C for various periods of time. The phosphate buffer/enzyme solution was replaced every 48 h to keep the enzyme activity at a desired level throughout the experiment duration. After incubation, the film was washed extensively with water and dried at 80 °C in vacuo until a constant weight was reached. The degree of degradation was estimated from the weight loss whose units are g/m^2. The weight loss averaged for two specimens was employed.

Results and Discussion

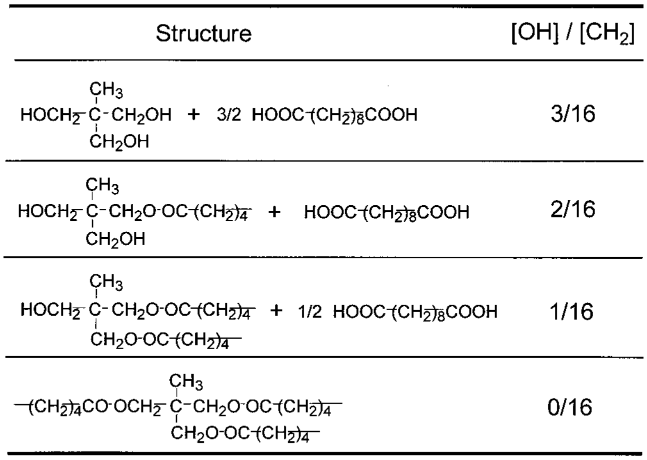

Figure 1. Schematic model of Ye10 network formation.

Table 1. Extent of Reaction (E_R) of Ye*n* Films and 10 Series Films

polymer code	E_R (%)	polymer code	E_R (%)
Ye4	65	Yg10	95
Ye6	85	Yp10	90
Ye7	87	Xe10	88
Ye8	91	Xp10	86
Ye9	91	Zs10	89
Ye10	91		
Ye11	90		
Ye12	89		
Ye13	92		
Ye14	92		
Ye16	93		
Ye20	92		

The network aliphatic polyesters showed infrared absorptions due to the hydroxyl group (−OH) and methylene group (>CH$_2$) at 3460 and 2960 cm^{-1}, respectively. 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 reaction between the hydroxyl group of polyhydric alcohol and carboxyl group of dicarboxylic acids, the change of absorption intensity ratio between −OH and >CH$_2$ groups, A_{OH}/A_{CH_2}, is a measure of the degree of reaction. Figure 1 shows a schematic model structure of monomeric unit and the ratios of [OH]/[CH$_2$] when the network structure of Ye10 film has completely developed. At the beginning of the reaction, the ratio of hydroxyl group and methylene and methyl groups in a monomeric unit, [OH]/[CH$_2$], is 3/16 and varies with the progress of reaction to become (3−3)/16 when the network structure of Ye10 film has completely developed. Thus, the following equations are defined:

$$[OH]/[CH_2] = (3 - y)/16$$

$$y = 3-16[OH]/[CH_2]$$

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

$$y = 3 - (3n/2 + 1)[OH]/[CH_2]$$

The extent of reaction (E_R) is calculated as

$$E_R\ (\%) = (y/3) \times 100$$

Using the procedure applied to Ye*n* described above, E_R values for other series network films were calculated. 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.[6] E_R values of network polyester films are summarized in Table 1. The E_R value for Ye*n* increases with an increase in the number of methylene groups and levels out for Ye8 in which it is about 91%, probably due to the longer flexible methylene chain allowing an increase of the reactivity between Ye and the dicarboxylic acid. This behavior has also been observed for Yg*n* films.[11] E_R's of 10 series films are 86−91%, demonstrating that the postpolymerization reaction is hardly affected by increasing the number of hydroxyl groups on polyhydric alcohols. In the present case, the difference in reactivity between primary and secondary hydroxy groups in Xe and Zs polyols is not measured in the infrared spectrum. Thus it is difficult to distinguish the contribution of these groups in polycondensation.

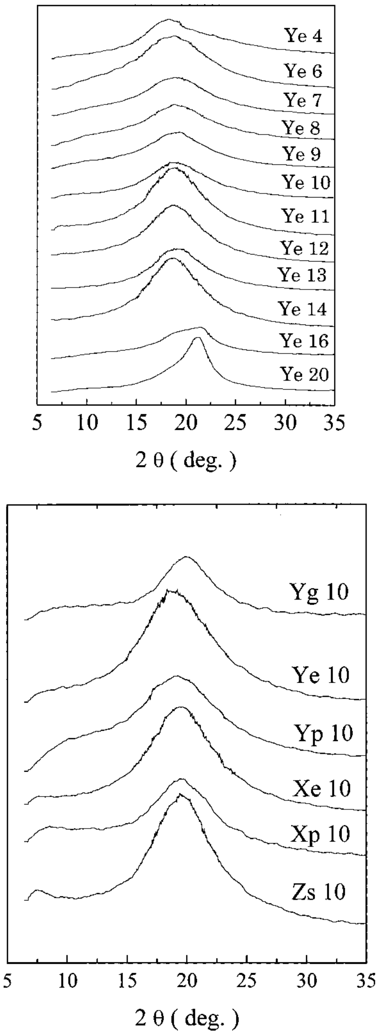

Figure 2. WAXS patterns of Ye*n* films (a) and 10 series films (b).

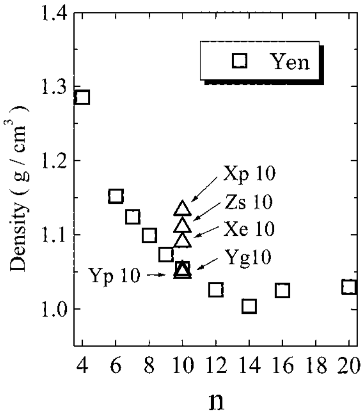

Figure 3. Plots of density vs *n* for Ye*n* films and 10 series films.

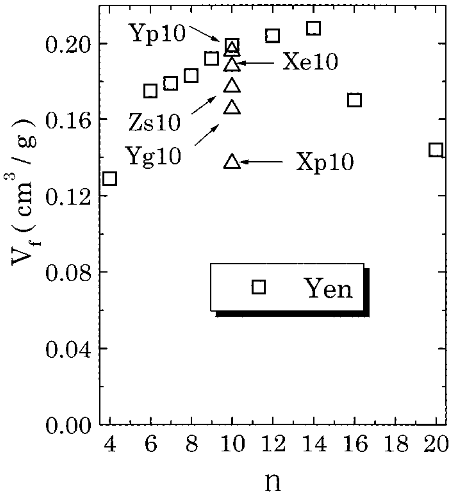

Figure 4. Plots of free volume vs *n* for Ye*n* films and 10 series films.

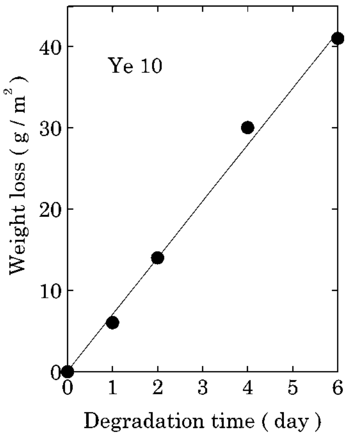

Figure 5. Weight loss of Ye10 film against degradation time in a buffer solution with *R. delemar* lipase at 37 °C.

Figure 2 shows WAXS intensity curves of Ye*n* films (a) and 10 series films (b). A distinct single diffraction peak appears at ca. 18° for both Ye*n* and 10 series films, which also implies the formation of some ordered structure by the regular structure of network polyesters. For Ye16 and Ye20, other diffraction peaks appear at around 21.5°, suggesting the crystallization of films, which is discussed below in relation to the results of DSC scan.

Figure 3 shows the plots of density vs *n* for Ye*n* films and 10 series films. The density decreases gradually with increasing *n*, suggesting an increase of the free volume of the network caused by the extension of the length between the cross-linked sites. Ye10, Yp10, Yg10, Xe10, Xp10, and Zs10 have densities of 1.054, 1.048, 1.052, 1.090, 1.133, and 1.100 g/cm^3, respectively. The highest density of Xp10 would be ascribed to the symmetric structure of Xp monomer. Zs10 shows a relatively higher density irrespective of unsymmetrical structure of Zs monomer, which would be caused by the increased cross-linked density. The free volume of the network (V_f) for both films was estimated from the density (ρ) measured shown in Figure 2 according to[15]

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

where V_w is the summation of the group contribution of the van der Waals molar volume (cm^3/mol) and M is the molecular weight of the repeating monomeric unit (g/mol). V_f values estimated are plotted against n in Figure 4. As expected, V_f increases gradually with increasing n except for the cases of Ye16 and Ye20 which is probably due to the crystallization of the film.

Enzymatic Degradation. Figure 5 shows the plot of the weight loss by the enzymatic degradation for the Ye10 film against degradation time at 37 °C in a buffer solution with *R. delemar* lipase. The weight loss increases almost linearly with degradation time. Blank experiments without the lipase were performed in

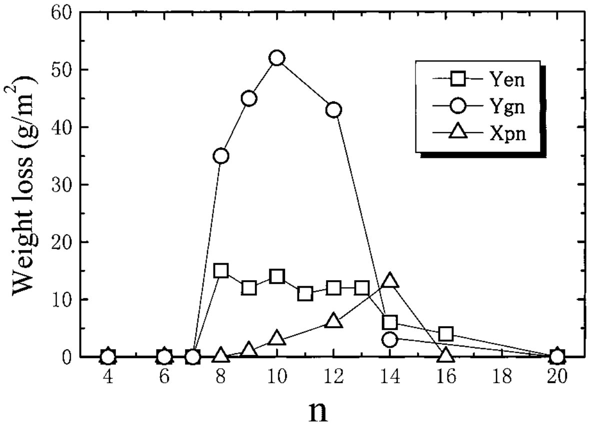

Figure 6. Plots of weight loss vs *n* for Ye*n* films (□) degraded in a buffer solution with *R. delemar* lipase at 37 °C for 2 days. Replots of those for Yg*n* (○) and Xp*n* (△) films are also shown.

Table 2. Weight Loss of 10 Series Films Degraded in a Lipase Buffer Solution at 37 °C for 6 days

polymer code	Yg10[a]	Ye10	Yp10	Xe10	Xp10	Zs10
weight loss (g/m^2)	52	41	6	6	3	1

[a] Weight loss after a 2 h incubation.

parallel in a buffer solution at 37 °C. No weight loss was observed for Ye10 film incubated for 6 days without lipase. Figure 6 shows the number of methylene group dependence of weight loss for Ye*n* (open square plot in the figure) after incubation in a buffer solution with *R. delemar* lipase for 2 days at 37 °C. In that figure, the weight loss for Yg*n* with the incubation time for 2 h and that of Xp*n* with incubation for 6 days[11,12] are also replotted against the number of methylene groups in aliphatic dicarboxylic acid. The films with shorter methylene chains (Ye4–7) show no weight loss, suggesting the difficulty for penetrating lipase into the network film owing to the smaller network size, which is the same as in the case of Yg4–7 and Xp4–8.[12] For Ye*n* films with the number *n* ranging from 8 to 13, weight loss is in the range 10–15 g/m^2 and gradually decreases with increasing *n*. This behavior is essentially the same as in the case of Yg*n* films. On the contrary, *n* dependence of weight loss for Xp*n* films is significantly different from those for Yg*n* and Ye*n* films. This difference is ascribed to the fact that Yg*n* and Ye*n* films are trifunctional network polymers and Xp*n* is a tetrafunctional network polymer. The decrease of the enzymatic degradation rate for the films with a much longer methylene chain is partly related to the crystallite morphology of these samples and the decrease of concentration of enzymatically degradable ester linkages. Both *P. cepacia* lipase and porcine pancreas lipase, which considerably degraded Yg*n* films, hardly degraded Ye*n* films, which would be responsible for the substrate specificity of these lipases. Weight losses of 10 series films after the incubation in a buffer solution with *R. delemar* lipase for 6 days at 37 °C are given in Table 2 to examine the effect of chemical structure of polyhydric alcohols on the enzymatic degradation. An increase of the number of hydroxyl groups on polyhydric alcohols reduces the rate of degradation, which may be ascribed to the increased cross-linking density. The weight loss for Yp10 is much smaller than that for Ye10, probably due to the fact that the more bulky ethyl group on Yp disturbs the degradation of films more than the methyl group on Ye does.

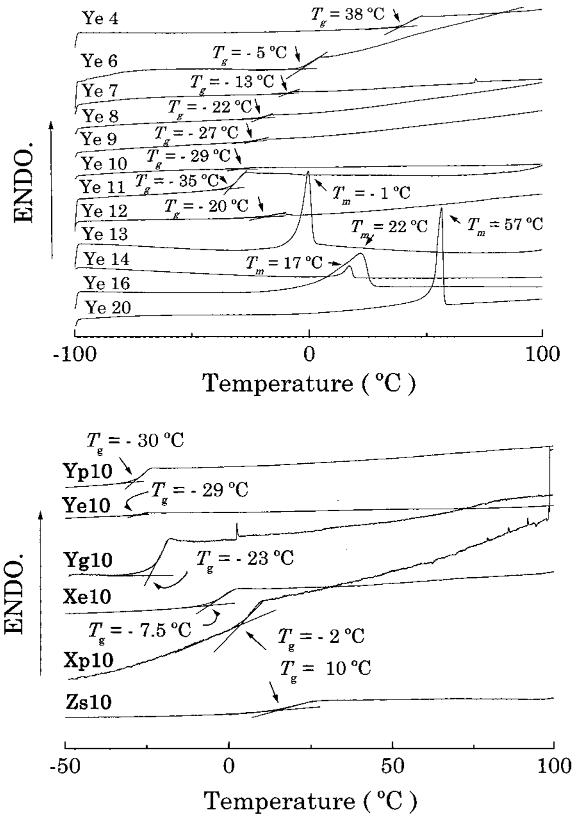

Figure 7. DSC curves of Ye*n* films (a) and 10 series films (b).

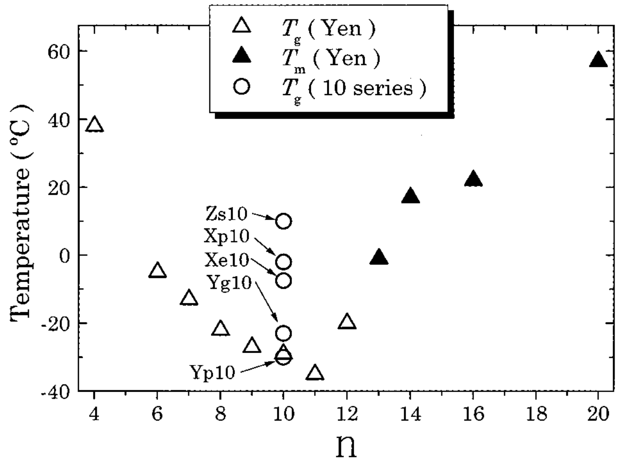

Figure 8. Glass transition temperature and melting temperature of Ye*n* films and 10 series films.

The process of polyester hydrolysis by *R. delemar* was studied through the identification of hydrolysis products using GPC. Ye8 was degraded at 37 °C for 7 days in a phosphate buffer solution with a *R. delemar* lipase. After degradation, the solution was freeze-dried and the residue was dissolved in tetrahydrofuran and filtered, and then the filtrate was subjected to GPC analysis. Ye and 8 components were detected, demonstrating the complete degradation of Ye8 to monomers.

Thermal, Mechanical, and Physicochemical Properties of Postpolymerized Films. Figure 7 shows DSC scans forYe*n* films (a) and 10 series films (b). The endothermic transition due to glass transition (T_g) is measured for both films. T_g values obtained are plotted against *n* in Figure 8, including 10 series films. For Ye*n* films, T_g decreases with increasing *n* and shows

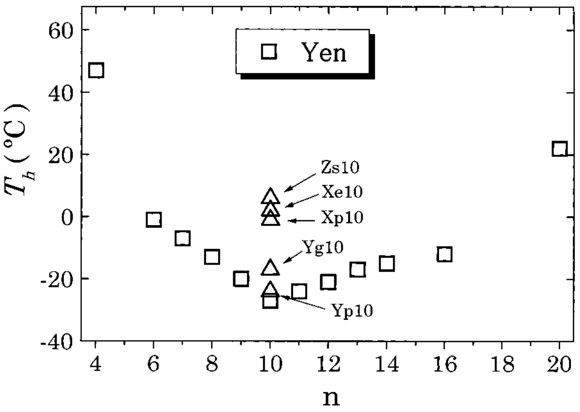

Figure 9. Plots of heat distortion temperature vs *n* for Ye*n* films and 10 series films.

the minimum value of −35 °C for Ye11 and then increases with *n*. The decrease in T_g with increasing *n* corresponds to the increase of free volume shown in Figure 4. For 10 series films, T_g increases with an increase in the number of hydroxyl groups on polyhydric alcohols, suggesting that increased cross-linking density disturbs the mobility of the network molecular chain. The endothermic peak appears for the films with the longer methylene group (Ye13−20), probably due to the melting of the crystal of the methylene unit in the dicarboxylic acids. The melting peaks for Ye16 and Ye20 correspond to the X-ray diffraction peaks at 21.5° in Figure 2a. T_m measured in the DSC scans are also plotted in Figure 8. T_m increases with an increase in the number of methylene groups, which is consistent with the fact that T_m for aliphatic linear polyesters increases with an increase in the number of methylene groups.[16] Similar T_m behavior has been observed for Yg*n* films.[12]

The heat distortion temperature (T_h), the inflection point of the TMA curve, is measured. The TMA probe penetrates into the films completely in the temperature range of 400−500 °C due to thermal decomposition. T_h values of Ye*n* films are plotted against *n* in Figure 9, including those for 10 series films. The plot of T_h vs *n* corresponds well to that of T_g vs *n* shown in Figure 8. The much higher T_h for Ye20 may be due to the crystallinity of the films.

Tensile properties are summarized for both Ye*n* films and 10 series films in Table 3. The number of methylene groups in the dicarboxylic acid hardly affects the tensile properties except for Ye4 and Ye20. The extremely larger Young's modulus for Ye4 would be due to the higher packing density. A higher tensile strength and a higher Young's modulus for Ye20 are significantly related to the crystallinity of the film. The enhancement of tensile strength and Young's modulus for Zs10 in 10 series films may be responsible for the increased cross-linked density.

Table 3. Tensile Properties of Ye*n* Films and 10 Series Films

polymer code	tensile strength (kg/mm^2)	elongation (%)	Young's modulus (kg/mm^2)
Ye4	3	2	169
Ye6	0.3	36	0.9
Ye7	0.3	36	0.9
Ye8	0.3	30	1.0
Ye9	0.2	36	0.7
Ye10	0.2	32	0.7
Ye11	0.2	40	0.7
Ye12	0.2	44	0.6
Ye13	0.2	54	0.5
Ye14	0.2	69	0.4
Ye20	1.5	145	12
Yg10	0.2	29	0.9
Yp10	0.1	30	0.5
Xe10	0.3	39	0.8
Xp10	0.1	22	0.7
Zs10	2	72	15

Conclusions

Novel network polyesters of 1,2,3,4-butanetetrol (Xe), D-glucitol, (Zs), 1,1,1-trimethylolpropane (Yp), and 1,1,1-trimethylolethane (Ye) with aliphatic dicarboxylic acids of different number of methylene group were prepared for biodegradable polyesters. The Ye*n* films of medium methylene chain length (C_8−C_{13}) showed considerable weight loss in a *R. delemar* lipase solution, while *P. Cepacia* lipase and porcine pancreas lipase hardly degraded Ye*n*.

It is noted that the difference of the respective substituents, methyl and ethyl groups in Ye and Yp trifunctional monomers, significantly affects the enzymatic degradability. Weight loss for 10 series films in a *R. delemar* lipase solution was decreased with an increase in the number of hydroxyl group of polyhydric alcohols. A remarkable dependence of enzymatic degradation on methylene chain length was observed for Ye*n* in analogy with Yg*n* previously reported, which was similar to that of triglyceride of fatty acids with different numbers of methylene groups.

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