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Synthesis of New Biodegradable Crosslinked Polyesters for Biomedical Applications and Their *In-Vitro* Degradation

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Two kinds of new aliphatic diols were synthesized by the ring-opening reaction of lactide and glycolide with 1, 4-butanediol, a difunctional initiator, in the presence of stannous octoate. The resulting aliphatic diols were melt-polymerized with D-tartaric acid at 150 °C to produce new crosslinkable polyesters. They were reacted with hexamethylene diisocyanate in THF at 65 °C in a teflon mold for 24 h to prepare sequentially ordered crosslinked polyesters (BD/LT/GL/D-tartarate). Degradation of the prepared yellow crosslinked films was carried out in a buffer solution in order to examine the effect of time, pH, temperature and crosslinking degree on their degradation rate and mechanism. The rate of degradation increased with an increase in pH and temperature, but it decreased with increasing degree of crosslinkage incorporated into the crosslinked polyesters. We also found that the crosslinked polymers were converted into the acidic compounds such as lactic, glycolic, and D-tartaric acids during the degradation.

Introduction

Aliphatic polyesters from cyclic lactones such as lactide, glycolide, ε-caprolactone and so on have attracted much attention in biomedical applications as medical sutures, drug delivery systems, and internal bone fixation, because these polymers have excellent biodegradability and biocompatibility.[1-3] Although metallic devices such as plates, screws, and rods have been widely used in osteoplasty for internal fixation of bone fractures, basically they have two serious problems: one is osteoporosis due to stress protection atrophy and the other is that these nondegradable devices require a second surgical operation for their removal after the bone is healed.[4,5] To overcome these problems, therefore, it has been investigated many studies on biodegradable and bioabsorbable polymeric composites in replacement of metallic devices.[6-8] Especially, biocompatible polyesters from lactide and glycolide have been mainly studied as degradable matrix resins of biomedical composites.[9,10] These thermoplastics, however, also have inherent difficulty in interfacial wetting with reinforcing fibers, which limits optimum stress transfer between fiber and matrix.[7] This seriouly affects hydrolytic stability.[6]

In order to overcome these disadvantages, S. J. Huang *et al.* have examined the possibility of application of crosslinked polymers as matrix materials for composite bone plates.[11] They have focused either on the introduction of crosslinkable unsaturated double bonds into aliphatic polyesters derived from α-hydroxy acids or on their thermal crosslinking that causes morphological change.[12,13]

In this work we report synthesis of new sequentially ordered crosslinked polyesters and their *in-vitro* degradation in a buffer solution. We will also describe the effect of the structural factors on the degradation mechanism.

Experimental

Materials. D,L-Lactide and glycolide from Polyscience were sublimed at 100 °C and 90 °C, respectively, under vacuum prior to use. 1,4-Butanediol was distilled over calcium hydride to remove water. 1,6-Hexamethylene diisocyanate (HMDI) and all the solvents were purified in the usual manner. Stannous octoate (tin(II)-2-ethyl hexanoate), p-toluenesulfonic acid, D-tartaric acid were used without further purification. Buffer solutions were used as received from Micro Essential Laboratory.

Instrumentation. FT-IR spectra were recorded on a Brucker IFS 48 spectrometer. H-NMR spectra were obtained by a Varian Gemini 300 spectrometer. Thermal transition temperatures (T_g) were measured on a Perkin-Elmer 7 series DSC at a heating rate of 10 °C/min under nitrogen atmosphere. Inherent viscosity of crosslinkable polyesters was measured in tetrahydrofuran (THF) solution at 25 °C by using a Cannon-Fenske viscometer.

Synthesis of sequentially ordered oligomeric ester diols. Oligomeric 1,4-butanediol/D,L-lactide (BD/LT) diol (I): Into a 250 mL three-necked round bottom flask equipped with a water-cooled condenser, gas inlet and outlet adapters were placed 81.8 g (0.57 mole) of D,L-lactide, 25.0 g (0.28 mole) of 1,4-butanediol as an initiator, and 0.46 g (0.2 mole% *vs.* D,L-lactide) of stannous octoate as a catalyst. The flask was immersed in a preheated silicone oil bath and maintained at 140+2 °C for 5 h under nitrogen atmosphere. After the reaction was completed, the turbid viscous mixture was dissolved in chloroform and then filtered through a sintered-glass filter to remove the precipitated catalyst. After the solvent was distilled away by using a rotary evaporator, the product was dried *in vacuo* at 50 °C for 24 h. The clear viscous liquid, oligomeric BD/LT diol, was obtained with 85% yield.

Oligomeric 1,4-butanediol/D,L-lactide/glycolide (BD/LT/GL) diol (II): Into a 250 mL two-necked round bottom flask with gas inlet and outlet adapters were added 50 g (0.13 mole) of the synthesized oligomeric BD/LT diol, 32.0 g (0.28 mole) of glycolide, and 0.56 g (0.5 mole% *vs.* glycolide) of stannous octoate. The mixture was heated to 120+2 °C in an oil bath under nitrogen atmosphere. After 12 h the mixture was cooled to room temperature and then dissolved in chloroform. The unreacted glycolide and the catalyst were precipitated in chloroform and then filtered off. After drying the clear viscous liquid, oligomeric BD/LT/GL diol, was obtained with 78% yield.

Preparation of crosslinkable polyesters. Oligomeric BD/LT diol (or BD/LT/GL diol), D-tartaric acid and p-toluenesulfonic acid as a catalyst were added into a 100 mL two-necked round bottom flask equipped with gas inlet and outlet adapters and polymerized at a given temperature for a desired time under nitrogen atmosphere. The polymer was dissolved in THF and then filtered through a sintered-glass filter to remove the insoluble gel. The solution was precipitated into large amount of ethyl ether. The powdered crosslinkable polyesters (III and IV) were dried *in vacuo* at 50 °C for 24 h.

Preparation of crosslinked polyesters. The crosslinkable polyester (10 g) and triethylamine (0.5 mole% *vs.* HMDI) as a catalyst were charged into a 100 mL three-necked flask equipped with a gas inlet adapter, a serum rubber, and a water-cooled condenser with drying tube. The mixture was dissolved with THF (10 mL) to give a 50% solution. After flushing well with nitrogen gas, HMDI was added through a syringe into the solution and then mixed well at room temperature for 5 min. The polymer solution was poured into a Teflon mold (4"X4") and was placed in a preheated oven at 65 °C for 24 h under nitrogen atmosphere. The yellow brittle film, which is the sequentially ordered crosslinked polyester, was obtained after the reaction.

***In-vitro* degradation.** The transparent film 85 μm thick was cut into small pieces with 1×1 cm dimension and they were used for *in-vitro* degradation. The crosslinkable polyester films (III and IV) with no crosslinkage were also employed as control samples.

In-vitro degradation was carried out in three buffer solutions of pH 5.0, 7.0, and 10.0. The buffer solutions were 0.5 M sodium and potassium phosphate solution for pH 5.0, 0.5 M phosphate for pH 7.0, and 0.5 M sodium borate and carbonate for pH 10.0, respectively. Sodium azide (0.02 wt%) was added to each buffer solution to eliminate a possible biological interference in air.

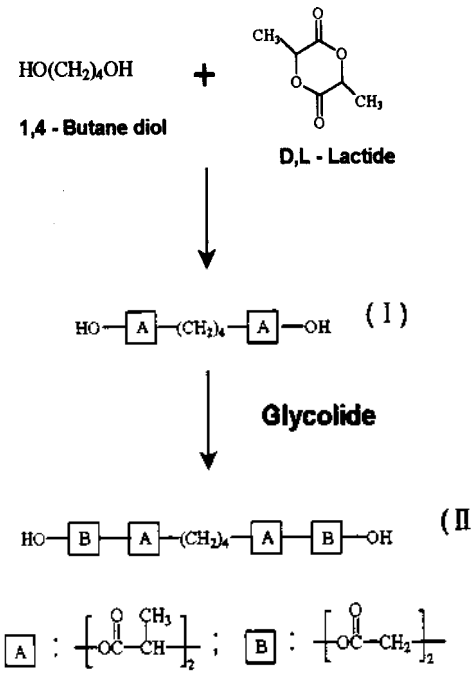
The preweighed film was placed in a borosilicate glass vial containing 15 mL of buffer solution. Degradation was carried out in a thermostat for a desired period. After degradation the buffer solution was filtered through a Whatman filter paper. The degraded film was rinsed thoroughly with distilled water to remove any buffer remaining on the surface, and then dried at 40 °C for 2 days under vacuum. The dried film was weighed to measure mass loss during the degradation. The mass loss was calcuated by the following equation.

$$\text{Mass loss } (\%) = \frac{m_o - m_d}{m_o} \times 100$$

where m_o and m_d are the initial weight of the film and the dried weight after degradation, respectively.[14] pH change of the filterate was checked with a Corning Model 130 pH meter.

Results and Discussion

Synthesis of sequentially ordered oligomeric ester diols. We used 1,4-butanediol as a difunctional initiator for the ring-opening reaction of D,L-lactide to obtain the oligomeric BD/LT diol (I). Subsequently (I) was reacted



Scheme 1. Synthetic scheme of sequentially ordered ester diols.

with glycolide to produce chain extended oligomeric BD/LT/GL diol (II) shown in Scheme 1. IR spectrum of (I) showed a broad absorption band at about 3500 cm^{-1} due to the OH groups at both ends. The absorption band of ester group appeared at 1747 cm^{-1}, which was shifted to lower wavelength than 1767 cm^{-1} of D,L-lactide. This indicates that D,L-lactide was ring-opened by 1,4-butanediol used as an initiator. IR spectrum of (II) was very similar to that of (I).

Figure 1 shows H-NMR spectra of (I) and (II). Methine proton between ester groups in (I) appeared at 5.2 ppm and a new peak of methine proton adjacent to hydroxyl group showed up at 4.4 ppm, which was overlapped with two methylene protons (4.2 ppm) near oxygen in 1,4-butanediol. The secondary hydroxyl groups derived from the ring-opened lactide appeared at 3.0 ppm. Four methyl protons in the middle of 1,4-butanediol also showed a broad peak with multiplet at around 1.5 ppm. The relative integration ratio between the characteristic peaks matched well with the structure of (I), as we expected. This supports that one molecule of lactide is stoichiometrically incorporated into both ends of 1,4-butanediol (Figure 1-I).

As shown in Figure 1-II, the methine proton between ester groups in the ring-opened D,L-lactide appeared at 5.2 ppm. On the other hand, two kinds of methylene protons from glycolide adjacent to ester and to hydroxyl groups were at 4.8 and 4.3 ppm, respectively. The primary hydroxyl group at 3.5 ppm is attributed to the ring-opening reaction of glycolide by BD/LT diol, which is slightly shifted to lower field than 3.0 ppm of (I). Methyl and methylene protons from lactide and 1,4-butanediol still appeared at around 1.5 ppm. From the relative integration ratio of the characteristic proton peaks, we confirmed that (II) was sequentailly ordered diol having one molecule of the ring-opened glycolide moiety at the both ends of (I).

On the basis of the above results, we found that the molecular sequence and/or composition of the new oligomeric aliphatic ester diols could be easily controlled by changing the feed ratio and the order of cyclic lactones such as lactide, glycolide, and so on, if difunctional diol is used as an initiator.

In addition to difunctional diol, even either multifunctional triols or tetraols like glycerol and pentaerythritol could be also used as an initiator for syntheses of molecularly contolled polyesters suitable for biomedical applications in surgical suture, polymeric composites, and drug delivery systems.[15]

Preparation of crosslinkable polyesters. So far

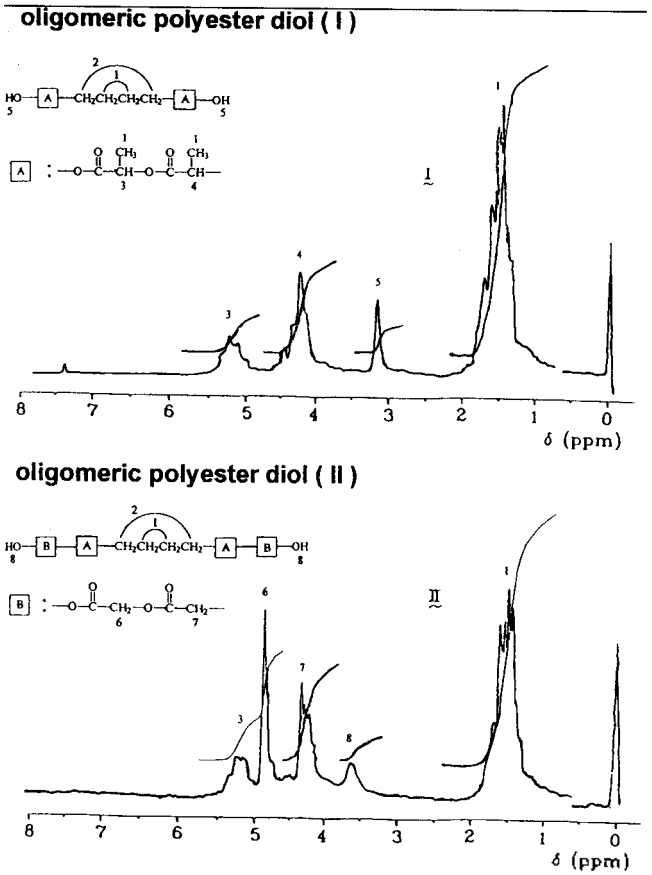


Figure 1. ^1H NMR spectrum of sequentially ordered oligomeric ester diols.

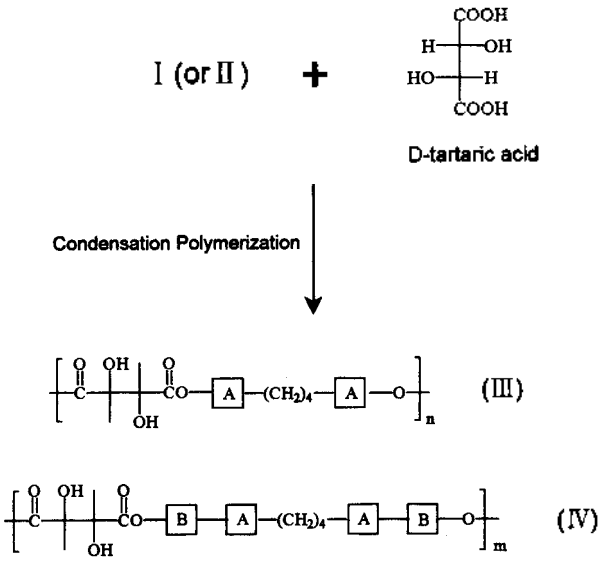
poly(alkylene tartarate)s have been reported as crosslinkable substrates of degradable crosslinked polyesters. They have excellent biodegradability and biocompatibility so that they are being used as matrix resins for controlled releasing drugs.[16]

In this work, therefore, we chose lactide, glycolide, and D-tartaric acid as starting materials to prepare biodegradable and bioabsorbable new crosslinked polymers. Table 1 shows the results of condensation polymerization of sequentially ordered oligomeric ester diols with D-tartaric acid. The resulting crosslinkable polyesters (III and IV) have hydroxyl groups that can act as the crosslinking site (Scheme 2). When (I) was bulk-polymerized with D-tartaric acid at 170 °C, we obtained polymeric gel that is insoluble in most solvents. This might be due to the crosslinking reaction

Table 1. Condensation polymerization of sequentially ordered oligomeric ester diols with D-tartaric acid[a]

EXP.[b] #	DIOL (mole)	D-TT[c] (mole)	Catalyst[d] (mole%)	Polym'n temp (°C)	Polym'n time (hr)	Conv. (%)	Polymer (%) sol	Polymer (%) gel	η_{sp}	T_g (°C)
I-1	0.044	0.04	1.0	170	5	–	x	100	–	
I-2	0.044	0.04	2.0	150	48	48.3	91.3	8.7	0.28	41
I-3[e]	0.044	0.04	2.0	150	24	25.0	95.0	5.0	0.18	
II-1	0.038	0.042	1.0	150	24	68.6	95.2	4.8	0.37	
II-2	0.038	0.042	0.5	150	48	82.4	94.8	5.2	0.43	26

[a] Bulk polymerization. [b] I and II are oligomeric ester diols shown in Scheme 1. [c] D-Tartaric acid. [d] Mole% of p-toluenesulfonic acid versus diol. [e] Solution polymerization in DMF.



Scheme 2. Preparation of sequentially ordered crosslinkable polyesters.

between crosslinkable polyesters produced during polymerization. In case we carried out solution polymerization at lower temperature in order to suppress the crosslinking reaction, the conversion and viscosity of the polymer were so low that it was not desirable to be used as crosslinkable polyester. We, therefore, selected the crosslinkable polyester (III), which was prepared by a bulk polymerization at 150 °C for 48 h, as substrate material of crosslinked polymers for *in-vitro* degradation.

When (II) also was polymerized with D-tartaric acid, the conversion and viscosity of the resulting polymer were much higher than those of the crosslinkable polyester from (I). It is attributable to the difference in the reactivity between the secondary diol (I) and the primary diol (II). High molecular weight polyesters (Exp. II-2 in Table 1) were used to prepare various kinds of crosslinked polymers with different degrees of crosslinkage for *in-vitro* degradation.

Glass transition temperatures (T_g) of the crosslinkable polyester (III) and (IV) were 41 °C (III) and 26 °C (IV), respectively. This results from the lower melting temperature of glycolide than D,L-lactide.[17]

***In-vitro* degradation of crosslinked polyesters.** Table 2 shows the result of preparation of crosslinked polymers with different degrees of crosslinking. Four kinds of sequentially ordered crosslinked polyesters (BD/LT/GL/D-tartarate) were prepared to investigate the influence of time, pH, temperature, and crosslinking degree on the rate and mechanism of degradation in a buffer solution. The mole ratio of NCO/OH means the feed ratio of hexamethylene diisocyanate to crosslinkable polyester, which results in the preparation of the crosslinked polymers with 100 (NCO/OH =1/1) and 50% (NCO/OH=1/2) of crosslinking degree.[18] The crosslinkable polyester (III) and (IV) with no crosslinkage were chosen as comparison samples for the crosslinked polyesters. T_g of the crosslinked polymer was almost constant with the degree of crosslinking. The transparent yellow films were changed into the brownish porous films after degradation.

Table 2. Preparation of crosslinked polymers with different degree of crosslinking for in-vitro degradation

EXP. #	Polyester[a] (g)	HMDI (g)	Mole ratio of NCO/	T_g (°C)
III-A	III(10)	2.32	1/1	50.5
III-B	III(10)	1.16	1/2	50.3
IV-A	IV(10)	3.36	1/1	40.0
IV-B	IV(10)	1.68	1/2	39.0

[a] III and IV are crosslinkable polyesters shown in Scheme 2. [b] Calculated from weight fraction of D-tartarate in crosslinkable polyesters.

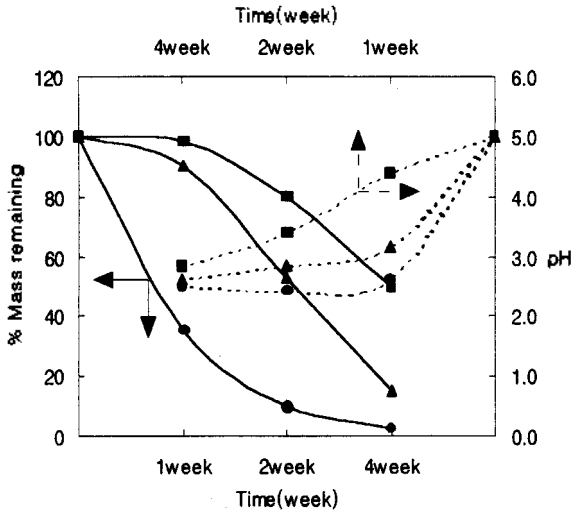


Figure 2. Effect of degree of crosslinking on mass loss (—) and pH change (---) of polyester (III) at pH 5 and 37 °C: ●, no crosslinking; ▲, 50% crosslinking; ■, 100% crosslinking.

Figure 2, 3, and 4 show the mass loss and pH change of the crosslinked polyesters (III-A and B), which were derived from the crosslinkable polyester (III), in buffer solutions of pH 5, 7, and 10 at 37 °C. We observed that the higher the degree of crosslinking the lower the rate of degradation regardless of the change in pH and time. The rate of de-

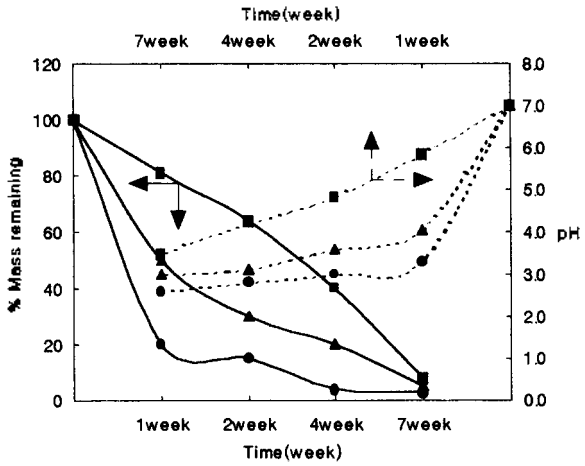


Figure 3. Effect of degree of crosslinking on mass loss (—) and pH change (---) of polyester (III) at pH 7 and 37 °C: ●, no crosslinking; ▲, 50% crosslinking; ■, 100% crosslinking.

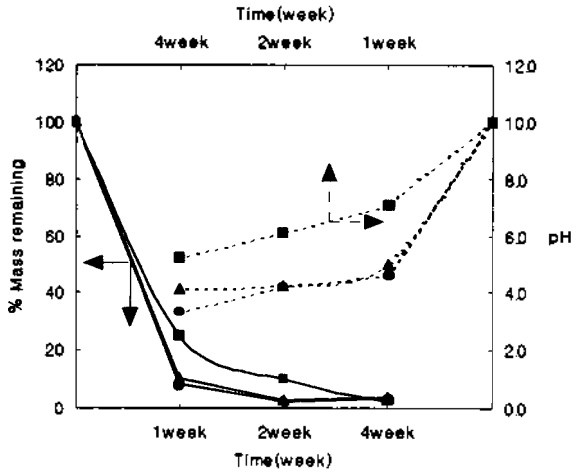


Figure 4. Effect of degree of crosslinking on mass loss (—) and pH change (---) of polyester (III) at pH 10 and 37 °C: ●, no crosslinking; ▲, 50% crosslinking; ■, 100% crosslinking.

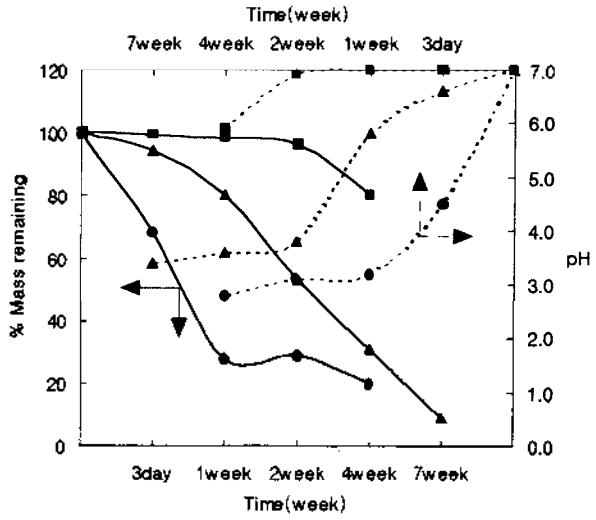


Figure 5. Effect of temperature on mass loss (—) and pH change (---) of polyester (III) with 100% of degree of crosslinking at pH 7: ■, 25 °C; ▲, 37 °C; ●, 50 °C.

gradation of the crosslinked polymer with 100% of crosslinking degree was almost four times slower than that with no crosslinkage. On the other hand, the rate of degradation gradually increased with the increase in pH from 5 to 10. Especially, at pH 10.0 the rate of degradation drastically increased so that over 70% of the samples were degraded after one week (Figure 4). Effect of temperature on rate of degradation at pH 7.0 is shown in Figure 5. The rate of degradation rapidly increased with the increase in temperature from 25 to 50 °C, but at 25 °C the sample was almost not degraded even after 4 weeks.

Figure 6, 7, 8, and 9 show the mass loss and pH change of the crosslinked polyesters (IV-A and B), which were derived from the crosslinkable polyester (IV) in buffer solutions of pH 5, 7, and 10 at 37 °C. Degradation results showed almost the same trend as those of III-A and B except the followings: At pH 5.0 the mass loss (Figure 2) of the crosslinked polymer III-A and B was lower than that of IV-

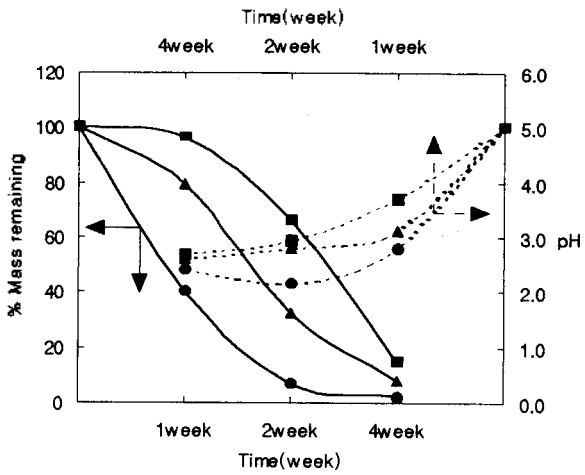


Figure 6. Effect of degree of crosslinking on mass loss (—) and pH change (---) of polyester (IV) at pH 5 and 37 °C: ●, no crosslinking; ▲, 50% crosslinking; ■, 100% crosslinking.

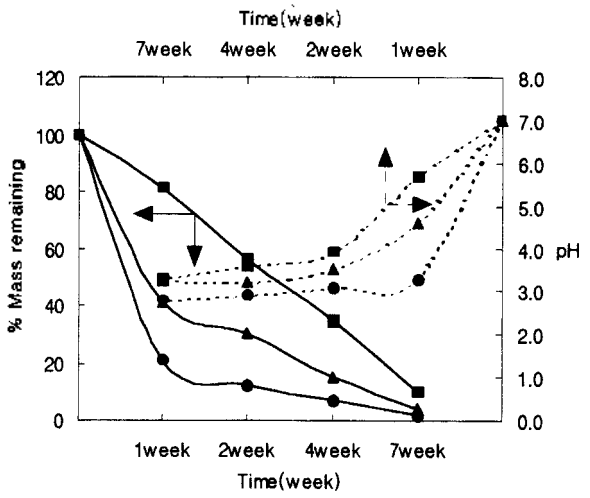


Figure 7. Effect of degree of crosslinking on mass loss (—) and pH change (---) of polyester (IV) at pH 7 and 37 °C: ●, no crosslinking; ▲, 50% crosslinking; ■, 100% crosslinking.

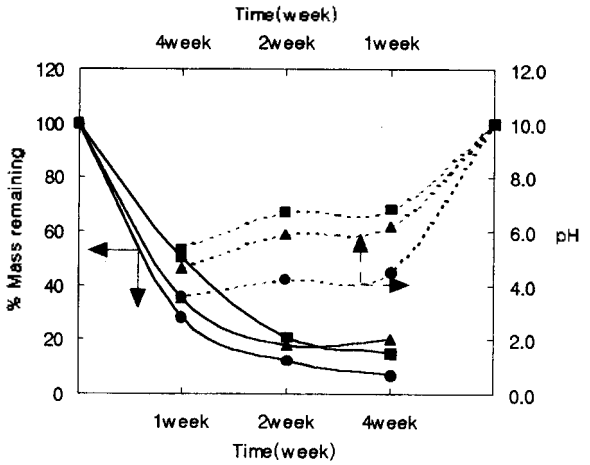


Figure 8. Effect of degree of crosslinking on mass loss (—) and pH change (---) of polyester (IV) at pH 10 and 37 °C: ●, no crosslinking; ▲, 50% crosslinking; ■, 100% crosslinking.

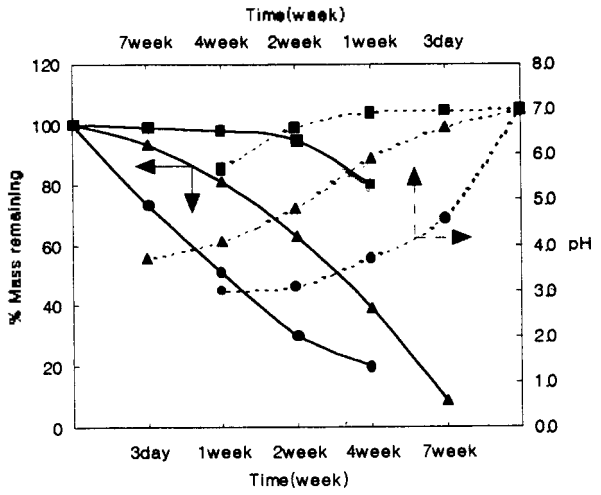


Figure 9. Effect of temperature on mass loss (—) and pH change (---) of polyester (IV) with 100% of degree of crosslinking at pH 7: ■, 25 °C; ▲, 37 °C; ●, 50 °C.

A and B (Figure 6). On the other hand, at pH 10.0 the result showed the opposite phenomenon. This results from the difference of acidity between glycolic and D,L-lactic acid generated during degradation.[19]

Above results are quite consistent with the pH change of the solution after degradation: pH quickly decreased up to 2 weeks and was then leveled off. This strongly suggests that degradation products are some type of acids such as lactic, glycolic, and tartaric acid that was liberated from the crosslinked polyesters. The presence of phosphate or borate buffer would thus convert these acidic products into neutral salts as soon as they are formed, which results in the acceleration of the degradation rate. In other word, if the following reaction occurred during degradation, pH of the degraded solution should remain at the constant value.

$$RCOOH + Na_2HPO_4 \rightarrow RCOO^- Na^+ + NaH^2PO_4 \quad \text{(Reaction 1)}$$
(degradation product) (buffer)

As shown in Figure 2, 3, and 4, pH of the degraded solution of all the samples was always leveled off after 2 weeks regardless of pH of buffer solutions. This means that the concentration of the acidic products, which is generated during degradation, rapidly increases up to two weeks and then decreases. Such an increase of acidity might help enhance the degradation rate. This, however, is not clear yet and we need to examine the influence of pH on the degradation rate in more detail. These results support the mechanism of degradation as described in Reaction 1, which was suggested by C.C. Chu.[20] One important thing is that the introduction of the crosslinkage suppresses the generation of the acidic products during degradation and eventually decreases the degradation rate of the crosslinked polymers.

On the basis of the *in-vitro* degradation results, we found that the change of the degree of crosslinking makes it possible to control the rate of degradation. Although the rate of degradation was much faster than we expected, the new sequentially ordered crosslinked polymers could be suitable either as a matrix resin of biodegradable implants for short term or as a drug carrier in the drug delivery systems.

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