Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Fusibility of Poly(N-carboxy α-Amino Acid Anhydride) Materials Treated under Pressure-Heat Conditions and in Vitro-in Vivo Degradation of Hot-Pressed Materials

Masaharu Asano[a]; Masaru Yoshida[a]; Isao Kaetsu[a]; Hidetoshi Yamanaka[b]; Katsuyuki Nakai[b]; Hisako Yuasa[b]; Keizo Shida[b]; Masanao Oya[c]
[a] Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Takasaki, Gunma, Japan [b] Department of Urology, School of Medicine Gunma University, Maebashi, Gunma, Japan [c] Department of Chemistry, College of Technology Gunma University, Kiryu, Gunma, Japan

To cite this Article Asano, Masaharu , Yoshida, Masaru , Kaetsu, Isao , Yamanaka, Hidetoshi , Nakai, Katsuyuki , Yuasa, Hisako , Shida, Keizo and Oya, Masanao(1984) 'Fusibility of Poly(N-carboxy α-Amino Acid Anhydride) Materials Treated under Pressure-Heat Conditions and in Vitro-in Vivo Degradation of Hot-Pressed Materials', Journal of Macromolecular Science, Part A, 21: 5, 561 — 582
To link to this Article: DOI: 10.1080/00222338408056573
URL: http://dx.doi.org/10.1080/00222338408056573

J. MACROMOL. SCI.–CHEM., A21(5), pp. 561-582 (1984)

Fusibility of Poly(N-carboxy α-Amino Acid Anhydride) Materials Treated under Pressure-Heat Conditions and in Vitro-in Vivo Degradation of Hot-Pressed Materials

MASAHARU ASANO, MASARU YOSHIDA, and ISAO KAETSU

Takasaki Radiation Chemistry Research Establishment
Japan Atomic Energy Research Institute
Takasaki, Gunma 370-12, Japan

HIDETOSHI YAMANAKA, KATSUYUKI NAKAI, HISAKO YUASA, and KEIZO SHIDA

Department of Urology
School of Medicine
Gunma University
Maebashi, Gunma 371, Japan

MASANAO OYA

Department of Chemistry
College of Technology
Gunma University
Kiryu, Gunma 376, Japan

ABSTRACT

Powdered poly(N-carboxy α-amino acid anhydride) materials were treated at temperatures of 50, 100, 150, and 200°C under a pressure of 150 kg/cm^2. A number of hot-pressed materials showed simultaneous fusion and contraction in volume. The fusion temperature of the hot-pressed materials was generally lower than the true melting point of the powdered materials at atmospheric pressure (de-

 0022-233X/84/2105-0561$3.50/0

termined with a Differential Scanning Calorimeter). The hot-pressed materials had high rigidity and transparency. The in vitro-in vivo degradation of hot-pressed materials was also investigated. The homo- and copoly(α-amino acid) materials used in this study were scarcely degraded, though debenzylated terpolymers such as β-benzyl-L-aspartate/aspartic acid/L-leucine and γ-benzyl-L-glutamate/glutamic acid/L-leucine were significantly degraded in both the in vitro and in vivo experiments. It was found that the in vivo degradation profile of hot-pressed terpolymer materials corresponds relatively well to degradation with 0.01% trypsin.

INTRODUCTION

The study of the in vivo degradation of polymer is a prerequisite for the selection of implant materials for different purposes. We are particularly interested in designing a polymeric material with a controlled in vivo degradation to be used as material for a drug delivery system [1]. In this field, one of the important factors is the moldability of the material. We have tried to fuse the biodegradable materials under pressure-heat conditions and found from this trial that the proteins, such as albumins and globulins, fused in the range of about 50 to 110°C under a pressure of 100 kg/cm^2. The hot-pressed proteins were significantly contracted in volume by this treatment and gave high rigidity, hardness, and transparency [2]. The biodegradation of hot-pressed proteins could be controlled by γ-irradiation with a ^{60}Co source. In this case the rate of biodegradation was accelerated in the presence of drugs in drug-protein composite controlled delivery systems [3].

Marck et al. reported that the terpoly(β-benzyl-L-aspartate/L-aspartic acid/L-leucine) materials are degraded when implanted subcutaneously in rats [4]. We also studied the biodegradability of poly(α-amino acid) materials made by polymerization of N-carboxy α-amino acid anhydrides (NCA's) and found that poly(γ-benzyl-L-glutamate) [5] and random copoly(β-benzyl-L-aspartate/γ-methyl-L-glutamate) [6] are degraded in vivo, e.g., a pressed poly(γ-benzyl-L-glutamate) material is degraded up to 9.5% at the 30th implantation day and a pressed copoly(β-benzyl-L-aspartate/γ-methyl-L-glutamate, 50/50) material is degraded up to 36% at the 90th implantation day. In a drug delivery system, testosterone was slowly released from these pressed polymer-drug composites at a constant rate. In this report we describe the biodegradable poly(α-amino acid) materials obtained by polymerization of NCA's and our attempts to fuse the polymer materials under pressure-heat conditions in analogy with that of proteins. The biodegradability of hot-pressed poly(α-amino acid) materials was investigated in both the in vitro and in vivo experiments. Finally, a controlled in vivo degradation was car-

ried out with terpolymers made by partial removal of the benzyl group contained in the materials.

EXPERIMENTAL

Reagents

Pepsin (Poreine stomach mucosa), trypsin (Bovine pancreas), carboxypeptidase A (Bovine pancreas), and α-chymotrypsin (Bovine pancreas) were obtained from Sigma Chemical Co. Thermolysin (Bacterial proteinase) was obtained from Daiwa Kasei Co.

Synthesis and Polymerization of NCA's

From γ-benzyl-L-glutamate, γ-methyl-L-glutamate, β-benzyl-L-aspartate, L-leucine, ε-carbobenzoxy-L-lysine, L-valine, L-alanine, L-isoleucine, DL-alanine, and glycine the corresponding NCA's were prepared by a method using trichloromethyl chloroformate as a phosgenation reagent [7-9].

The homopolymerization of γ-benzyl-L-glutamate NCA, γ-methyl-L-glutamate NCA, β-benzyl-L-aspartate NCA, and L-leucine NCA and random copolymerization of β-benzyl-L-aspartate NCA/γ-methyl-L-glutamate NCA, γ-benzyl-L-glutamate NCA/L-leucine NCA, and β-benzyl-L-aspartate NCA/L-leucine NCA were carried out for 169 h at room temperature (25°C) with 1 mol% triethylamine as an initiator in 100 mL of dichloroethane. The initial amount of monomer was 5 g. On the contrary, the homopolymerization and random copolymerization of NCA's (initial amount of monomer is 5 g) such as ε-carbobenzoxy-L-lysine, L-valine, L-alanine, L-isoleucine, DL-alanine, glycine, ε-carbobenzoxy-L-lysine/L-leucine, ε-carbobenzoxy-L-lysine/β-benzyl-L-aspartate, L-valine/L-leucine, and glycine/L-alanine were carried out for 169 h at room temperature (25°C) with 0.5 mol% n-butylamine as an initiator in 100 mL of acetonitrile. The homopolymers and random copolymers obtained were washed with excess ethanol and then dried in vacuo.

The viscosity in solution of polymer materials (0.25 g/dL) was measured in difluoroacetic acid at 30°C. The inherent viscosity [η] was calculated as reported previously [10] and is listed in Tables 1 and 2. The homopolymers of L-glutamic acid (MW is 1500-2000) and L-aspartic acid (MW is 2500-6000) were purchased from Seikagaku Kogyo Co.

The terpoly(β-benzyl-L-aspartate/aspartic acid/L-leucine) and terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine) were prepared from random copoly(β-benzyl-L-aspartate/L-leucine) and random copoly(γ-benzyl-L-glutamate/L-leucine), respectively, by treatment with a hydrobromic acid/acetic acid mixture (70% HBr solution).

TABLE 1. Fusibility and Appearance of Homopoly(α-Amino Acid) Materials When Heated under a Pressure of 150 kg/cm^2 and Water Content of Hot-Pressed Materials

No.	Homopolymer	η_{sp}/C	Fusibility (°C)				Appearance	W (%)
			50	100	150	200		
1	γ-Benzyl-L-glutamate	0.54	Yes	Yes	Yes	Yes	Hard, transparent	3.6
2	γ-Methyl-L-glutamate	0.93	No	Yes	Yes	Yes	Hard, untransparent	17.0
3	β-Benzyl-L-aspartate	0.20	No	Yes	Yes	Yes	Hard, transparent	0.6
4	ϵ-Carbobenzoxy-L-lysine	0.83	No	No	Yes	Yes	Hard, untransparent	1.4
5	L-Leucine	1.13[a]	No	No	No	Yes	Hard, transparent	0.1
6	L-Valine	0.18						
7	L-Alanine	2.34						
8	L-Isoleucine	0.44						
9	DL-Alanine	0.48	No	No	No	No	-	-
10	L-Glutamic acid	1500-2000[b]						
11	L-Aspartic acid	2500-6000[b]						
12	Glycine	0.26						

[a]Trifluoroacetic acid as a solvent (30°C).
[b]Molecular weight (MW).

TABLE 2. Fusibility and Appearance of Copoly(α-Amino Acid) Materials When Heated under a Pressure of 150 kg/cm^2 and Water Content of Hot-Pressed Materials

No.	Copolymer (composition, %)		η_{sp}/C	Fusibility (°C) 50	100	150	200	Appearance	W (%)
13	β-Benzyl-L-aspartate/	(75/25)	0.30	No	Yes	Yes	Yes	Hard, transparent	7.1
14	γ-methyl-L-glutamate	(50/50)	0.53	No	Yes	Yes	Yes	Hard, untransparent	15.8
15		(25/75)	0.75	No	Yes	Yes	Yes	Hard, transparent	6.8
16	γ-Benzyl-L-glutamate/	(75/25)	1.26	Yes	Yes	Yes	Yes	Hard, transparent	3.2
17	L-Leucine	(50/50)	1.51	No	Yes	Yes	Yes	Hard, transparent	2.2
18		(25/75)	1.55	No	No	Yes	Yes	Hard, transparent	0.8
19	β-Benzyl-L-aspartate/	(75/25)	0.48	No	Yes	Yes	Yes	Hard, transparent	1.6
20	L-leucine	(50/50)	2.61[a]	No	No	Yes	Yes	Hard, transparent	1.0
21		(25/75)	3.62[a]	No	No	Yes	Yes	Hard, untransparent	0.8
22	ε-Carbobenzoxy-L-lysine/ L-leucine	(50/50)	1.11	No	No	Yes	Yes	Hard, untransparent	0.5
23	ε-Carbobenzoxy-L-lysine/ β-benzyl-L-aspartate	(50/50)	0.24	No	No	Yes	Yes	Hard, untransparent	0.6
24	L-Valine/L-leucine	(50/50)	1.10[a]	No	No	No	No	-	-
25	Glycine/L-alanine	(75/25)	0.34						
26		(50/50)	0.36	No	No	No	No	-	-
27		(25/75)	0.75						

[a]Trifluoroacetic acid as a solvent (30°C).

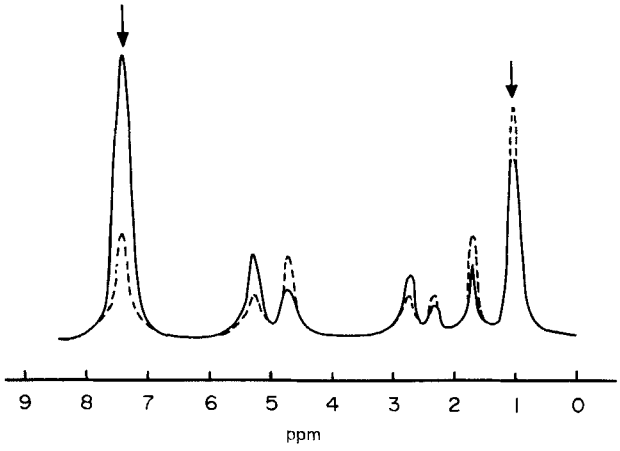


FIG. 1. NMR spectra of copoly(γ-benzyl-L-glutamate/L-leucine) materials and terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine) materials. A copoly(γ-benzyl-L-glutamate/L-leucine, 50/50) material was treated for 30 min at room temperature with 70% HBr solution for partial removal of the benzyl group to obtain a terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine, 11/39/50) material. (—) Before treatment, (---) after treatment.

That is, a mixture of random copolymers (0.01 mol) and 70% HBR solution (10 mL) was treated for 10-40 min at room temperature (25°C) to obtain the terpolymer materials of the required composition. After treatment, the terpolymer materials obtained were washed three times with excess ether, then neutralized with triethylamine because of removal of the HBr salt from terpolymer materials, and dried satisfactorily in vacuo.

The analysis of the polymer composition formed was carried out with a NMR spectrometer, Model JNM-PFT-100, Japan Electron Co. In this case, acetic acid-d_4 was used as a solvent. The NMR spectra of copoly(γ-benzyl-L-glutamate/L-leucine, 50/50) itself and terpoly-(γ-benzyl-L-glutamate/glutamic acid/L-leucine, 11/39/50) prepared by partial removal of the benzyl group contained in the above copolymer are shown in Fig. 1. According to the result of Fig. 1, the intensity ratios of the phenyl proton (7.3 ppm) present in the benzyl group and of the methyl proton (1.0 ppm) present in L-leucine before treatment with 70% HBr solution were first calculated from the area of the peak. Next the phenyl proton/methyl proton ratios after treatment were calculated. Finally, the composition of the partially debenzylated terpolymer was determined from the difference in the two ratios. The compositions of the copolymer and terpolymer analyzed by NMR method are listed in Tables 2 and 3.

TABLE 3. Fusibility and Appearance of Terpoly(α-Amino Acid) Materials When Heated under a Pressure of 150 kg/cm^2

No.	Terpolymer (composition, %)		Fusibility (°C) 50	100	150	200	Appearance
28	β-Benzyl-L-aspartate/ aspartic acid/L-leucine	(37/13/50)	No	Yes	Yes	Yes	Hard, transparent
29		(27/23/50)	No	Yes	Yes	Yes	Hard, transparent
30		(23/27/50)	No	Yes	Yes	Yes	Hard, transparent
31		(19/31/50)	No	Yes	Yes	Yes	Hard, transparent
32	β-Benzyl-L-aspartate/ aspartic acid/L-leucine	(45/30/25)	No	Yes	Yes	Yes	Hard, transparent
33		(33/42/25)	No	Yes	Yes	Yes	Hard, transparent
34		(25/50/25)	No	Yes	Yes	Yes	Hard, transparent
35	γ-Benzyl-L-glutamate/ glutamic acid/L-leucine	(36/14/50)	No	Yes	Yes	Yes	Hard, transparent
36		(25/25/50)	No	Yes	Yes	Yes	Hard, transparent
37		(18/32/50)	No	Yes	Yes	Yes	Hard, transparent
38		(54/46/0)	No	Yes	Yes	Yes	Hard, untransparent
39		(40/60/0)	No	Yes	Yes	Yes	Hard, untransparent

Preparation of Hot-Pressed Poly(α-Amino Acid) Materials

Forty-five mg of poly(α-amino acid) materials was charged into a mold (glass ampule) of columnar form seen in Fig. 2. The mold used in this study has an inside diameter of 4 mm. The piston rod (straight shank) was pushed into a mold under a pressure of 150 kg/cm^2 and then the mold was treated for 30 s at 50, 100, 150, and 200°C, respectively. The poly(α-amino acid) materials apparently fused by the above treatment. Simultaneously, a contraction in volume took place. The hot-pressed polymer materials obtained have high rigidity, hardness, and transparency. The size is, in general, 4 mm in diameter and 3 mm long in columnar form.

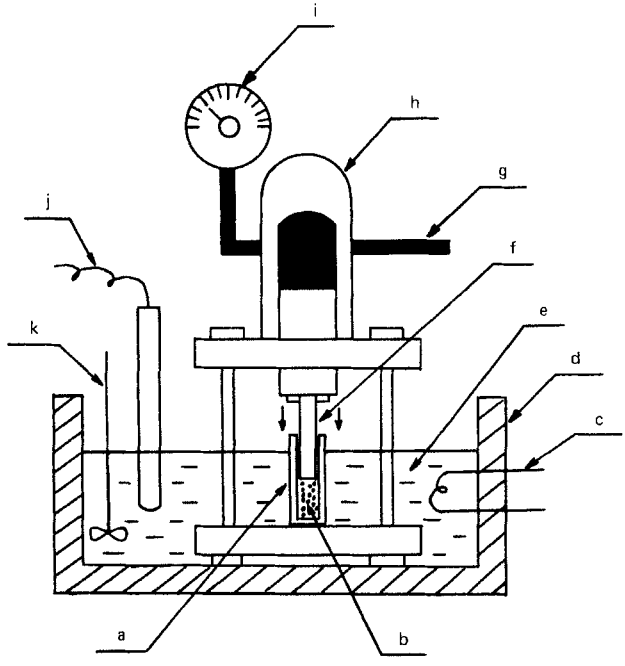


FIG. 2. An apparatus for observation of apparent fusibility of poly(α-amino acid) materials under a pressure of 150 kg/cm^2: (a) glass ampule (4 mm inside diameter), (b) powdered poly(α-amino acid) material, (c) heater, (d) oil bath, (e) silicone oil, (f) straight shank (3.9 mm in diameter), (g) oil pressure, (h) handpress, Model SSP-10 (Shimadzu Seisakusho Co., Ltd), (i) manometer, (j) regulator, (k) stirrer.

Equilibration of Hot-Pressed Poly(α-Amino Acid) Materials in Buffer Solution

The water content (W) was determined from Eq. (1). In this study the hot-pressed polymer materials were immersed for 30 d at 37°C in 0.1 M phosphate buffer solution (pH 7.4).

$$W\ (\%) = \frac{W_w}{W_w + W_d} \times 100 \qquad (1)$$

where W_w is the weight of the water required to saturate the material and W_d is the weight of the dried materials.

Degradation of Hot-Pressed Poly(α-Amino Acid) Materials

In the in vitro experiments, the hot-pressed poly(α-amino acid) materials (45 mg/sample) were immersed in 100 mL of 0.1 M phosphate buffer solution (pH 7.4) containing 0-100 mg of proteolytic enzymes. This mixture was placed in a flask. The test was carried out at 37°C with a shaking incubator (100 times a minute), Model TA-16, Takasaki Kagaku Kikai Co. At fixed time intervals the polymer materials were collected, then dried, and weighed. The in vitro degradation (as a measure of the weight loss of polymer materials) was calculated from

$$\text{Degradation}\ (\%) = \frac{W_0 - W_a}{W_0} \times 100 \qquad (2)$$

where W_0 and W_a are the weights of the dried polymer materials before and after treatment, respectively.

In the experiments in vivo, the hot-pressed poly(α-amino acid) materials were implanted subcutaneously in the back of Wistar rats weighing from 400 to 500 g (3 rats/group). The animals were sacrificed at prescribed time intervals. The polymer materials were collected from sacrificed rats and weighed after drying. The in vivo degradation of hot-pressed polymer materials was calculated from changes in the weight of the materials before and after implantation in a similar manner as in vitro.

Microscopic Observation

The surface structure of hot-pressed poly(α-amino acid) materials in the in vivo experiments was observed with a x-ray microanalyzer, Model JXA-733, Japan Electron Optics Laboratories Co.

Observation of Fusibility of Poly(α-Amino Acid) Materials

The apparent fusibility of poly(α-amino acid) materials was observed at temperatures of 50, 100, 150, and 200°C under a pressure of 150 kg/cm^2, using the apparatus seen in Fig. 2. The apparent fusibility of the materials under the above conditions was defined as the temperature where the fusibility and contraction in volume of the material were simultaneously observed.

RESULTS AND DISCUSSION

Fusibility of Poly(α-Amino Acid) Materials

A photograph of the appearance of a copoly(γ-benzyl-L-glutamate/L-leucine, 50/50) material treated by hot-pressing is shown in Fig. 3. The powdered copolymer showed simultaneous fusion and contraction in volume at 100°C under a pressure of 150 kg/cm^2. The true melting point of powdered copolymer without hot-pressing determined by a Differential Scanning Calorimeter (Model DSC-1B, Perkin-Elmer) was about 220°C at atmospheric pressure. From this experiment it was found that the apparent fusion temperature (in the range of 50 to 100°C) of a hot-pressed copoly(γ-benzyl-L-glutamate/L-leucine, 50/50) material is considerably lower than the melting point (220°C at atmospheric pressure) of powdered copolymer materials. This phenomenon was similarly observed for the other poly(α-amino acid) material systems used in this study. As shown in Fig. 3(b), the hot-pressed polymer materials were very hard and transparent in most of the polymer materials used. This result suggests that poly(α-amino acid) material having high rigidity can be easily prepared by the hot-pressing method.

The fusibility and appearance of homopoly(α-amino acid), copoly(α-amino acid), and terpoly(α-amino acid) materials when heated under a pressure of 150 kg/cm^2 are listed in Tables 1, 2, and 3, respectively. It was found from the results of Table 1 that the homopolymer materials having various protective groups such as γ-benzyl-L-glutamate, γ-methyl-L-glutamate, β-benzyl-L-aspartate, and ϵ-carbobenzoxy-L-lysine apparently fused at 50, 100, 100, and 150°C, respectively, while a homopolymer material of L-leucine having a long side-chain group and no protective group fused at a temperature of 200°C. However, no

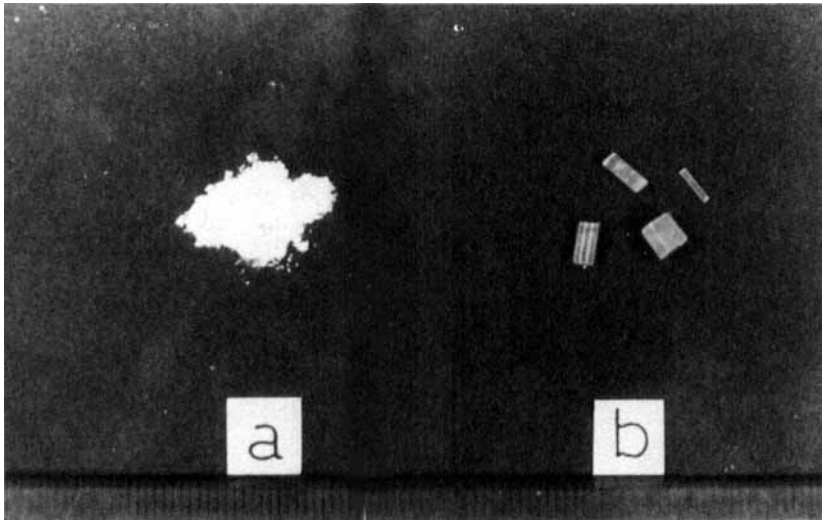


FIG. 3. Appearance of a copoly(γ-benzyl-L-glutamate/L-leucine, 50/50) material treated by hot-pressing. The copolymer material was ground to a powder at -196°C (liquid nitrogen) and then pressed for 30 s at 150 kg/cm^2. The material was photographed with a Nikomat EL, 35-mm film camera (Nippon Kogaku Co., Ltd). (a) Before hot-pressing, (b) after hot-pressing.

other homopolymer materials quite fused when heated under a pressure of 150 kg/cm^2, e.g., homopolymer materials such as poly(L-glutamic acid) and poly(L-aspartic acid) having no protective groups, a homopolymer material such as poly(glysine) having neither protective groups nor side-chain groups, and poly(DL-alanine) having no protective groups and short side-chain groups. From these results it is reasonable to conclude that the fusibility of homopolymer materials is strongly affected by crystallinity and structural factors such as protective groups and side-chain groups. The fusibility and appearance of copolymer materials are listed in Table 2. When a fusible homopolymer of L-leucine and a homopolymer of L-valine having no fusibility were combined, the copoly(L-leucine/L-valine) materials scarcely fused under a pressure of 150 kg/cm^2 (Samples 25-27 in Table 2). In a combination of two fusible homopolymers, the copolymer fused easily in the range of about 50 to 150°C under a pressure of 150 kg/cm^2, e.g., copolymers such as β-benzyl-L-aspartate/γ-methyl-L-glutamate, γ-benzyl-L-glutamate/L-leucine, β-benzyl-L-aspartate/L-leucine, ε-carbobenzoxy-L-lysine/L-leucine, and ε-carbobenzoxy-L-lysine/β-benzyl-L-aspartate. Finally, the fusibility of terpolymer materials such as β-benzyl-L-aspartate/aspartic acid/L-leucine and γ-benzyl-

L-glutamate/glutamic acid/L-leucine when heated under a pressure of 150 kg/cm^2 is listed in Table 3. These terpolymer materials were prepared by partial removal of the benzyl group contained in random copolymer materials such as β-benzyl-L-aspartate/L-leucine and γ-benzyl-L-glutamate/L-leucine. A part of the benzyl groups was converted to carboxyl groups by treatment with 70% HBr solution. That is, a part of the protective groups in copolymer materials was converted randomly into aspartic acid and glutamic acid, respectively. Homopolymeric materials such as poly(aspartic acid) and poly(glutamic acid) do not have fusibility as shown in Table 1. However, the modified terpolymer materials fused in the range of 50 to 100°C under a pressure of 150 kg/cm^2, in spite of the introduction of monomeric segments having no fusibility. We wish to note here that the biodegradation of hot-pressed terpolymer materials can be easily controlled by the introduction of the above segments; in other words, by partial removal of the benzyl group. This result will be discussed in the next sections.

In Vitro Degradation of Hot-Pressed Poly(α-Amino Acid) Materials

The in vitro degradation (enzymatic degradation) or hot-pressed homopoly(α-amino acid) and copoly(α-amino acid) materials is listed in Table 4 as a function of the type of enzyme. The values indicated in Table 4 are the weight loss (in vitro degradation) of the polymer fraction degraded only by proteolytic enzymes. The in vitro degradation of hot-pressed poly(α-amino acid) materials due to the dissolution when immersed in buffer solution containing no enzymes was within 3% in all material systems. It is therefore obvious from the results of Table 4 that the in vitro degradation of hot-pressed homo- and copolymer materials is scarcely dependent on the kind of proteolytic enzyme, and it was found that the extent of degradation in vitro is very low. For example, a homopolymer of L-leucine was not degraded by the proteolytic enzymes used in this study even at the 10th day from the start of the test. A copoly(β-benzyl-L-aspartate/γ-methyl-L-glutamate, 50/50) materials, on the contrary, showed the highest degradation in vitro (5.1%, see Sample 14 in Table 4) when degraded with trypsin. We have already reported the in vivo degradation of the above copolymer materials [6]. The extent of degradation in vivo went up to 36% at the 90th day from implantation. The copolymer material, in this case, was pressed under a pressure of 200 kg/cm^2 after being slurried with dichloroethane. These results indicate that the biodegradation of hot-pressed homo- and copolymer materials requires a period of many months. The water contents of poly(α-amino acid) materials are also listed in Tables 1 and 2. According to these results, the water contents of the materials are very low in all cases except for poly(γ-methyl-L-glutamate) materials and copolymers containing the γ-methyl-L-glutamate structure. Therefore, it is reasonable to con-

TABLE 4. In Vitro Degradation of Hot-Pressed Homopoly(α-Amino Acid) and Copoly(α-Amino Acid) Materials with Proteolytic Enzymes[a]

No.	Poly(α-amino acid) (composition, %)		Enzymatic degradation at 10th day from start of the test (%)				
			Trypsin (pH 7.8)	α-Chymotrypsin (pH 7.8)	Thermolysin (pH 7.8)	Carboxypeptidase A (pH 7.5)	Pepsin (pH 1.8)
1	γ-Benzyl-L-glutamate	(100)	2.1	2.0	1.7	2.2	2.7
2	γ-Methyl-L-glutamate	(100)	2.3	2.6	1.9	2.4	2.5
3	β-Benzyl-L-aspartate	(100)	1.2	1.1	0.7	0.4	0.8
4	ε-Carbobenzoxy-L-lysine	(100)	1.2	0.8	0.9	1.3	1.4
5	L-leucine	(100)	0	0	0	0	0
13	β-Benzyl-L-aspartate/	(75/25)	1.2	1.0	0.8	2.1	1.3
14	γ-methyl-L-glutamate	(50/50)	5.1	3.9	3.2	4.6	4.3
15		(25/75)	1.1	0.8	0.7	0.9	0.9
16	γ-Benzyl-L-glutamate/	(75/25)	2.5	2.2	1.9	2.2	3.4
17	L-leucine	(50/50)	1.3	0.9	0.7	1.3	1.2
18		(25/75)	1.0	1.0	0.5	0.7	0.7
19	β-Benzyl-L-aspartate/	(75/25)	2.7	2.5	2.3	2.4	2.5
20	L-leucine	(50/50)	2.6	2.3	2.2	2.2	2.8
21		(25/75)	2.1	1.9	1.7	1.9	1.8
22	ε-Carbobenzoxy-L-lysine/ L-leucine	(50/50)	0.2	0.1	0	0.2	0.2
23	ε-Carbobenzoxy-L-lysine/ β-benzyl-L-aspartate	(50/50)	0	0.2	0.1	0.2	0.1

[a]The poly(α-amino acid) materials (45 mg) were treated for 30 s at 100°C under a pressure of 150 kg/cm^2. The degradation test was carried out for 10 d at 37°C with 100 mL of buffer solution of various pH's containing 10 mg of enzymes.

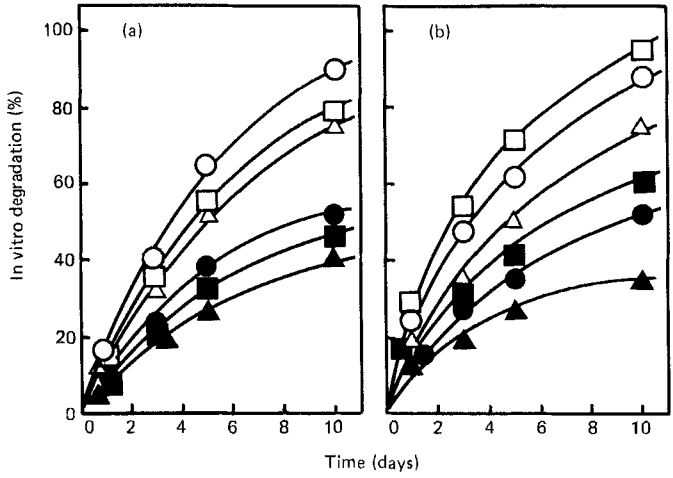


FIG. 4. Effects of five kinds of proteolytic enzyme on the in vitro degradation of terpoly(α-amino acid) materials such as γ-benzyl-L-glutamate/glutamic acid/L-leucine (18/32/50) and β-benzyl-L-aspartate/aspartic acid/L-leucine (23/27/50). The materials (45 mg) were pressed for 30 s at 100°C under a pressure of 150 kg/cm^2. The degradation test was carried out at 37°C with 100 mL of buffer solution of various pH's containing 10 mg of proteolytic enzymes. Terpolymer material: (a) γ-benzyl-L-glutamate/glutamic acid/L-leucine (Sample 37 in Table 3), (b) β-benzyl-L-aspartate/aspartic acid/L-leucine (Sample 30 in Table 3). Proteolytic enzyme: (○) trypsin, pH 7.8; (□) α-chymotrypsin, pH 7.8; (△) carboxypeptidase A, pH 7.5; (●) pepsin, pH 1.8; (■) thermolysin, pH 7.8; (▲) no enzyme (0.1 M phosphate buffer solution only, pH 7.4).

clude that one of the reasons for the low enzymatic reaction of hot-pressed homo- and copolymer materials is due to poor affinity between material and water.

Marck et al. reported the in vivo degradation of terpoly(β-benzyl-L-aspartate/L-aspartic acid/L-leucine) [4]. We have also tried to control the biodegradation of these terpolymer materials which were prepared by partial removal of the benzyl group. The same attempt was made for a terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine) material system. The in vitro degradation of the two terpolymer materials is shown in Figs. 4, 5, 6, and 7. Figure 4 shows the in vitro degradation of hot-pressed terpolymer materials with various proteolytic enzymes. It was found that these terpolymer materials are partly dissolved in buffer solution containing no enzymes. The amounts of dissolved materials were about 38% in terpoly(β-benzyl-L-aspartate/aspartic acid/L-leucine, 45/30/25) and 41% in terpoly(γ-benzyl-L-

glutamate/glutamic acid/L-leucine, 25/25/50), respectively, at the 10th day from the start of the test. Further, it was also found that even after subtraction of the fractions of the water-soluble terpolymer materials described above, the materials degraded rapidly by the action of enzyme. The cause of such a rapid enzymatic degradation of terpolymer material system may be attributed to an increase in the affinity for water. The extent of degradation in vitro of a terpoly(β-benzyl-L-aspartate/aspartic acid/L-leucine, 23/27/50) material decreased in the order of α-chymotrypsin > trypsin > carboxypeptidase A > thermolysin > pepsin, while the order in a terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine, 18/32/50) material was trypsin > α-chymotrypsin > carboxypeptidase A > pepsin > thermolysin. This result indicates that the in vitro degradation of hot-pressed terpolymer materials is significantly affected by the kind of enzyme and by the nature of the material. The effects of the trypsin concentration on the in vitro degradation of terpolymer materials are shown in Fig. 5. The in vitro degradation of the material increased with an increase in tryp-

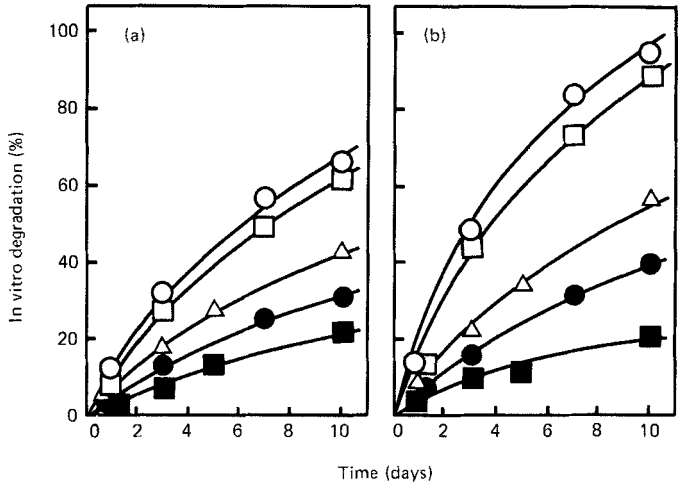


FIG. 5. Effects of trypsin concentration on the in vitro degradation of terpoly(α-amino acid) materials such as γ-benzyl-L-glutamate/glutamic acid/L-leucine (25/25/50) and β-benzyl-L-aspartate/aspartic acid/L-leucine (45/30/25). The degradation test of hot-pressed terpolymer materials (see Fig. 4 for the hot-pressing procedures) was carried out at 37°C with 100 mL of buffer solution (pH 7.8) containing 0-100 mg of trypsin. Terpolymer material: (a) γ-benzyl-L-glutamate/glutamic acid/L-leucine (Sample 36 in Table 3), (b) β-benzyl-L-aspartate/aspartic acid/L-leucine (Sample 32 in Table 3). Trypsin concentration (v/w %): (○) 0.1, (□) 0.05, (△) 0.01, (●) 0.005, (■) 0.

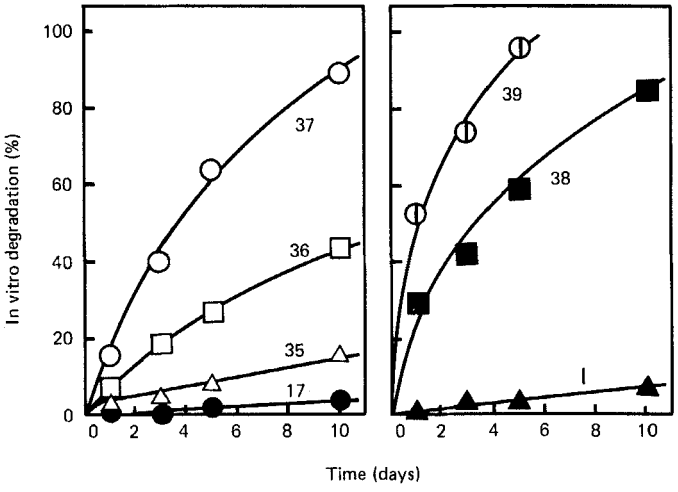


FIG. 6. Effects of the compositions of γ-benzyl-L-glutamate/glutamic acid/L-leucine on the in vitro degradation of terpoly(α-amino acid) materials. The degradation test was carried out at 37°C with 100 mL of 0.1 M phosphate buffer solution (pH 7.4) containing 10 mg of trypsin. The symbols in the figure refer to compositions given in Tables 1, 2, and 3. The hot-pressing procedures were carried out for 30 s at 100°C under a pressure of 150 kg/cm^2. Composition of γ-benzyl-L-glutamate/glutamic acid/L-leucine: (○) 18/32/50, (□) 25/25/50, (△) 36/14/50, (◑) 50/0/50, (●) 40/60/0, (▲) 100/0/0, (■) 54/46/0.

sin concentration. The rate of degradation in vitro of a terpoly(β-benzyl-L-aspartate/aspartic acid/L-leucine, 45/30/25) material, however, was considerably faster than that of a terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine, 25/25/50) material. The effects of the γ-benzyl-L-glutamate/glutamic acid/L-leucine composition on the in vitro degradation of hot-pressed terpolymer materials are shown in Fig. 6. Figure 6 shows that the trypsin degradation increased with an increase of the glutamic acid component in the material. The same tendency was observed in terpoly(β-benzyl-L-aspartate/aspartic acid/L-leucine) material systems as shown in Fig. 7. In this case the degradation in vitro was accelerated by an increase in the aspartic acid component. From these degradation results in vitro, we conclude that the control of the enzymatic degradation of hot-pressed poly(α-amino acid) materials is possible by the partial removal of the benzyl groups contained in the materials.

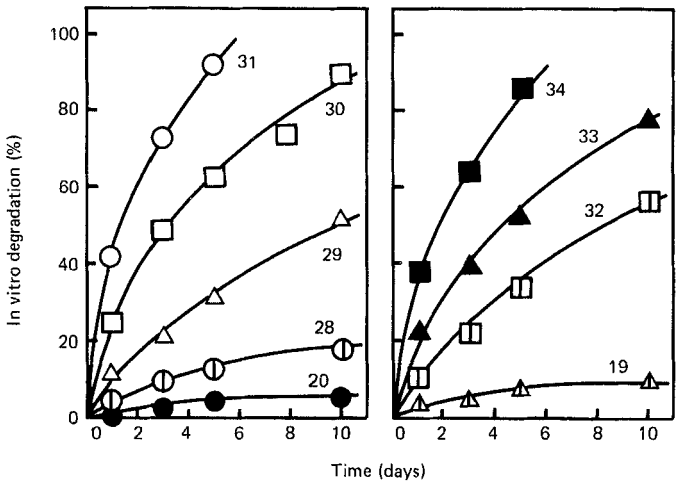


FIG. 7. Effects of the compositions of β-benzyl-L-aspartate/aspartic acid/L-leucine on the in vitro degradation of terpoly(α-amino acid) materials. The degradation test and hot-pressing procedures were the same as those in Fig. 6. The symbols in the figure refer to compositions given in Tables 1, 2, and 3. Composition of β-benzyl-L-aspartate/aspartic acid/L-leucine: (○) 19/31/50, (□) 23/27/50, (△) 27/23/50, (⊕) 37/13/50, (●) 50/0/50, (■) 25/50/25, (▲) 33/42/25, (⊞) 45/30/25, (⧋) 75/0/25.

In Vivo Degradation of Hot-Pressed Poly(α-Amino Acid) Materials

The in vivo experiments were carried out by implanting the hot-pressed poly(α-amino acid) materials subcutaneously in the back of Wistar rats. The in vivo degradation of terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine) materials is shown in Fig. 8. The rate of degradation in vivo was accelerated with an increase in the glutamic acid components. This is in fair agreement with the results of enzymatic (in vitro) degradation in Fig. 5. The in vivo degradation of terpoly(β-benzyl-L-aspartate/aspartic acid/L-leucine) materials was also evaluated and the result is shown in Fig. 9. From the relationship between the in vitro degradation and the in vivo degradation of terpolymer material systems (for example, Fig. 6/Fig. 8 in a terpolymer of γ-benzyl-L-glutamate/glutamic acid/L-leucine and Fig. 7/Fig. 9 in a terpolymer of β-benzyl-L-aspartate/aspartic acid/L-leucine), it was found that the in vivo degradation profile corresponds relatively well with that of 0.01%

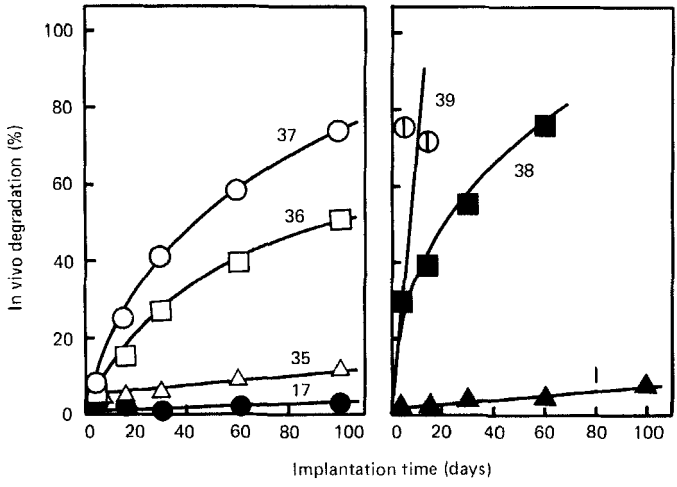


FIG. 8. In vivo degradation of terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine) materials. The materials were implanted subcutaneously in the backs of rats. The symbols in the figure refer to compositions given in Tables 1, 2, and 3. Composition of γ-benzyl-L-glutamate/glutamic acid/L-leucine: (○) 18/32/50, (□) 25/25/50, (△) 36/14/50, (●) 50/0/50, (◑) 40/60/0, (■) 54/46/0, (▲) 100/0/0.

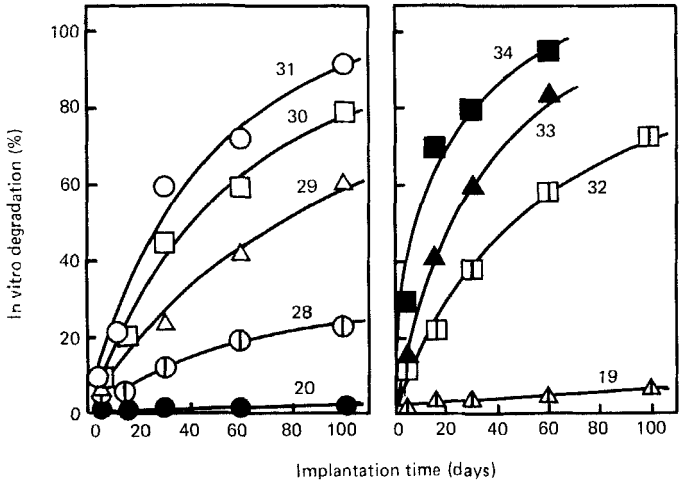


FIG. 9. In vivo degradation of terpoly(β-benzyl-L-aspartate/aspartic acid/L-leucine) materials. The experimental conditions were the same as those in Fig. 8. Composition of β-benzyl-L-aspartate/aspartic acid/L-leucine: (○) 19/31/50, (□) 23/27/50, (△) 27/23/50, (◑) 37/13/50, (●) 50/0/50, (■) 25/50/25, (▲) 33/42/25, (▥) 45/30/25, (◭) 75/0/25.

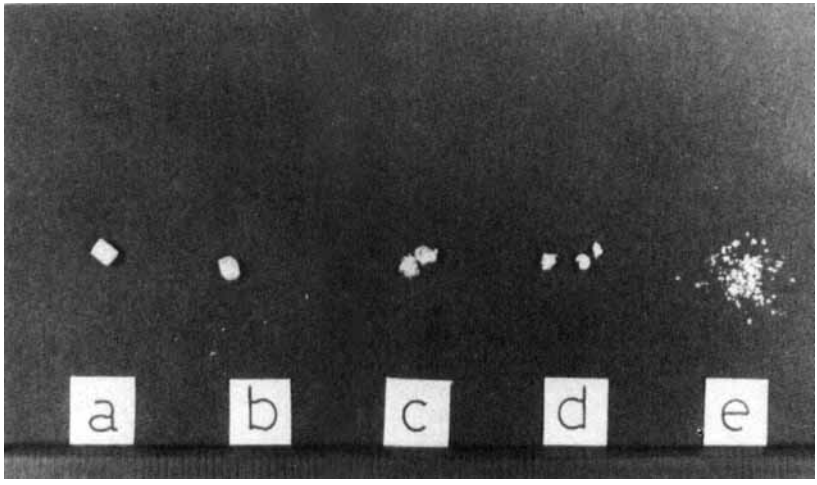
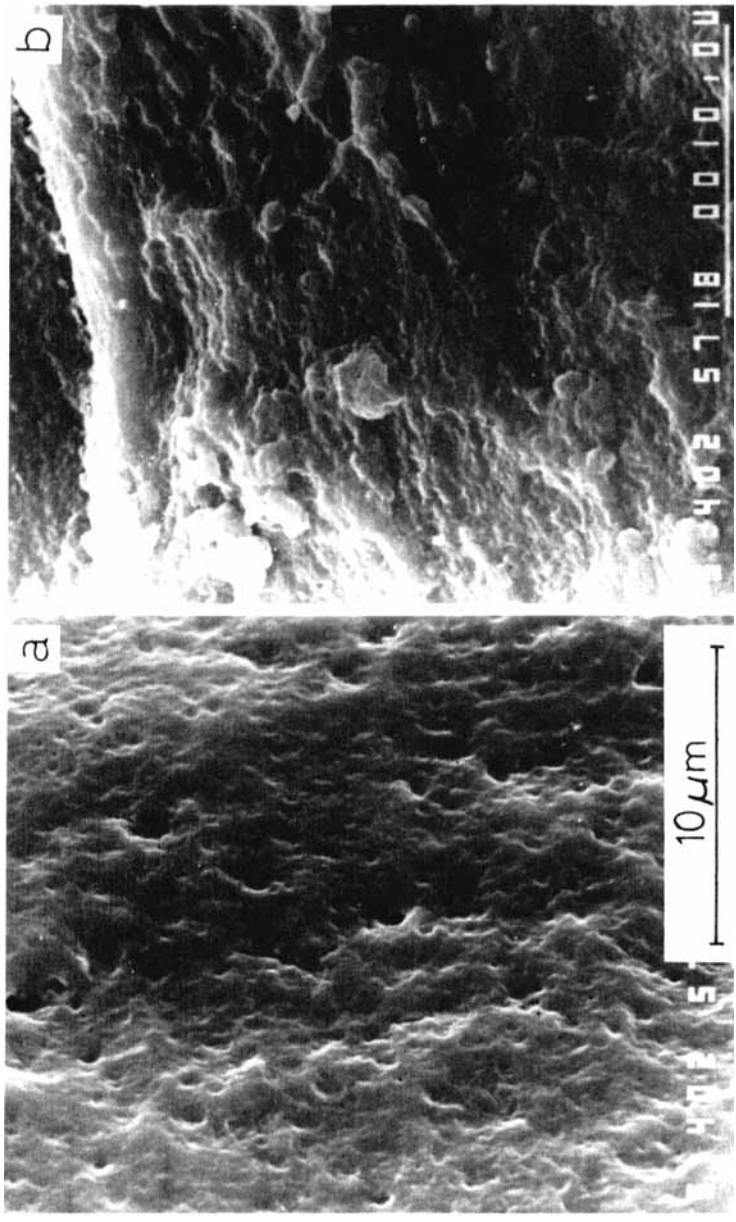


FIG. 10. In vivo degradation sequence of a terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine, 18/32/50) material. The hot-pressed terpolymer material (Sample 37 in Table 3) was implanted subcutaneously in the backs of rats. Implantation time (days): (a) 0, (b) 15, (c) 30, (d) 60, (e) 100.

trypsin. The rate of degradation in vivo was about 10 times slower than that in vitro. The cause of retardation of the in vivo degradation may be related to a lower proteolytic enzyme concentration. However, it is necessary to note here that the in vivo degradation profile of hot-pressed terpolymer materials can be roughly estimated from the profile of the trypsin degradation.

On the other hand, in order to observe the degraded state of hot-pressed terpolymer materials, a terpolymer of γ-benzyl-L-glutamate/glutamic acid/L-leucine (18/32/50) was implanted subcutaneously in the back of rats. The animals were sacrificed at 15, 30, 60, and 100 d from implantation. The implanted materials were collected from sacrificed rats and filmed with a 35-mm film camera. A photograph of the appearance of the implanted materials is shown in Fig. 10. Figure 10 shows that the in vivo degradation of hot-pressed terpolymer materials proceeds from the surface of the materials. The scanning electron microphotographs of these materials are shown in Fig. 11. The photograph shows that the surface of the hot-pressed terpolymer materials has many pores due to the in vivo degradation. This porosity showed an increase with a longer period of implantation. The hot-pressed terpolymer materials resulted in powder at the 100th day from implantation, as shown in Fig. 10(e). This may be due to the gradual increase of porosity resulting in the destruction of the shape of the materials.



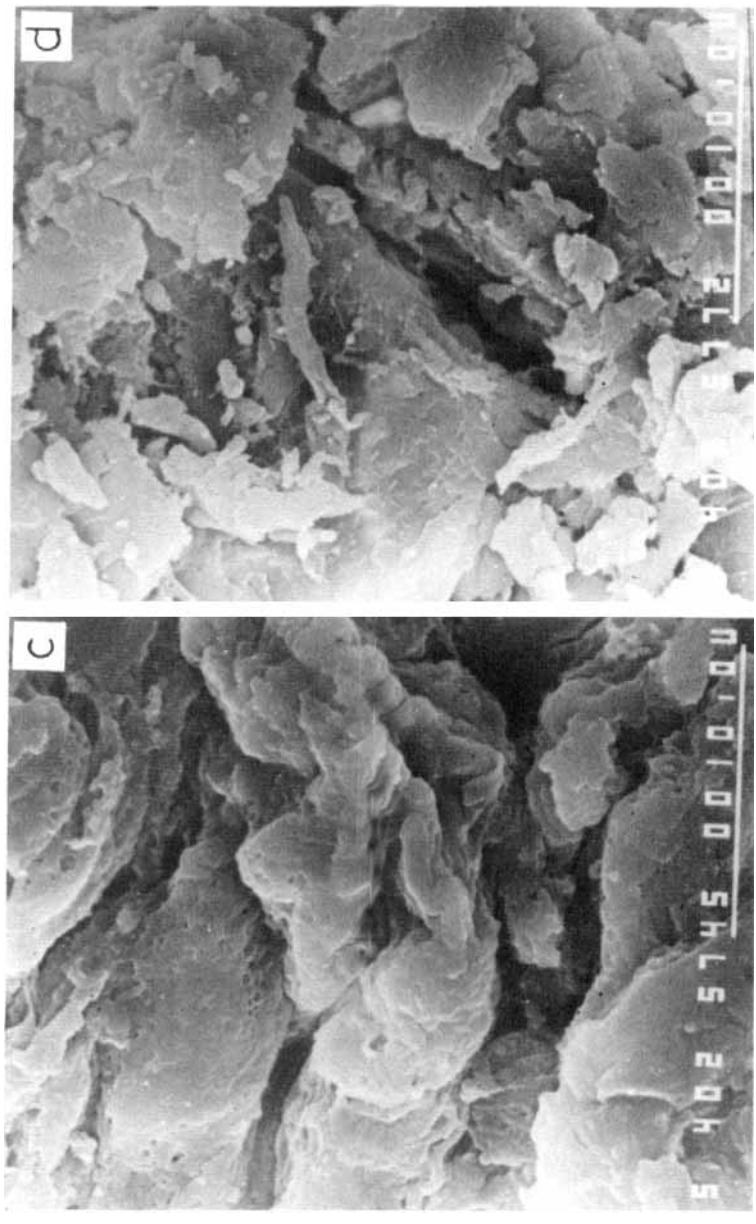


FIG. 11. Scanning electron microphotographs of the surface of a terpoly(γ-benzyl-L-glutamate/glutamic acid/L-leucine, 18/32/50) material. The experimental conditions were the same as those in Fig. 10. Implanatation time (days): (a) 0, (b) 30, (c) 60, (d) 100.

REFERENCES

[1] K. R. Sidman, A. D. Schwop, W. D. Steber, S. E. Rudolph, and S. B. Poulin, J. Membr. Sci., 7, 277 (1980).
[2] M. Asano, M. Yoshida, I. Kaetsu, K. Nakai, H. Yamanaka, and K. Shida, Kobunshi Ronbunshu, 39, 621 (1982).
[3] M. Asano, M. Yoshida, I. Kaetsu, K. Nakai, H. Yamanaka, H. Yuasa, and K. Shida, Ibid., 40, 525 (1983).
[4] K. W. Mark, C. H. R. Wildevuur, W. L. Sederel, A. Bantjes, and J. Feijen, J. Biomed. Mater. Res., 11, 405 (1977).
[5] M. Yoshida, M. Asano, I. Kaetsu, M. Oya, K. Nakai, H. Yamanaka, T. Suzuki, and K. Shida, Kobunshi Ronbunshu, 39, 285 (1982).
[6] M. Asano, M. Yoshida, I. Kaetsu, M. Oya, K. Nakai, H. Yamanaka, H. Yuasa, and K. Shida, Makromol. Chem., 184, 1761 (1983).
[7] M. Oya, K. Uno, and Y. Iwakura, Ibid., 154, 151 (1972).
[8] M. Oya, K. Uno, and Y. Iwakura, in Progress in Polymer Science Japan (S. Onogi and K. Uno, eds.), Kodansha, Tokyo, 1973, p. 70.
[9] M. Oya, R. Katakai, H. Nakai, and Y. Iwakura, Chem. Lett., p. 1143 (1973).
[10] M. Oya, K. Uno, and Y. Iwakura, J. Polym. Sci., Part A-1, 8, 1851 (1970).

Accepted by editor September 30, 1983
Received for publication October 28, 1983