Aliphatic Polyesters. I. The Degradation of Poly(ϵ-caprolactone) *In Vivo*

C. G. PITT, F. I. CHASALOW, Y. M. HIBIONADA, D. M. KLIMAS, and A. SCHINDLER, *Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709*

Synopsis

The degradation of poly(ϵ-caprolactone) in rabbits, rats, and water was studied by measurement of changes in intrinsic viscosity, molecular weight, crystallinity, Young's modulus, and weight. Degradation proceeds by nonenzymatic random hydrolytic cleavage of ester linkages. The process is autocatalytic, and the kinetic relationship $M_n/M_n^0 = \exp(-kt)$ is observed until the M_n has decreased to approximately 5000. Significant weight loss is not observed until this point but, once initiated, the rate of weight loss depends markedly on the particle size. Chain scission is associated with an increase in crystallinity, which partly determines the rate of degradation.

INTRODUCTION

Aliphatic polyesters are one of several classes of polymers which have been shown to undergo degradation and absorption *in vivo*.[1] Polymers of dilactide and diglycolide, better known as poly(lactic acid) and poly(glycolic acid), have been exploited as both bioabsorbable sutures[2,3] and drug delivery systems,[5,6] Poly(ϵ-caprolactone) has been promoted as a soil-degraded container material.[7,8] On the other hand, poly(ethylene terephthalate) is very stable *in vivo*.[9] Presently, there is little information which permits prediction of differences in behavior *in vivo* or estimation of lifetimes. In part, this is because the mechanism by which these polyesters are degraded is not completely understood. There are occasional references to enzymatic processes, but no concrete evidence of their participation.[10,11]

We have previously described the permeability of a number of polylactones and their potential utility for the sustained subdermal delivery of contraceptive agents.[12–14] This paper deals with the rate and mechanism of *in vivo* degradation of one of this class, poly(ϵ-caprolactone).

EXPERIMENTAL

Poly(ϵ-caprolactone) was prepared and characterized by previously reported procedures.[14,15] Films were prepared by molding the polymer in a heated hydraulic press between polypropylene plates at 100–115°C or by solution casting on glass plates using a Boston–Bradley applicator. Tubes and capsules were prepared by melt extrusion at 160°C or by annealing films rolled around a rotating Teflon cylinder *in vacuo*. Both films (2 cm × 1 cm × 0.01 cm) and capsules (~2 cm length, varying wall thickness) were sterilized by γ-irradiation (1.8–2.4 Mrad) or by dipping in Povidine® solution prior to implantation in female New Zealand white rabbits. Implants, up to six per animal, were placed subdermally

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and symmetrically about the dorsal midline. At the specified time intervals, samples were surgically removed, and most or all adhering tissue separated before drying overnight *in vacuo* (capsules were opened to facilitate drying).

Weight loss was determined gravimetrically or by liquid scintillation counting when the polymer was tritium labeled. Gravimetric weights were corrected for tissue remaining after dissolution of the sample. Molecular weights were determined in THF by GPC (Waters Associates) utilizing a set of five μ-Styragel columns of nominal pore sizes of 10^5, 10^4, 10^3, 10^2, and 50 nm, a flow rate of 1 mL/min and a sample size of 1 mg of polymer in 0.5 mL of THF. The GPC traces were evaluated by the universal calibration method[16] using polystyrene standards (Waters Associates and Ventron). The initial elastic moduli were determined with an Instron universal testing machine at a strain rate of 1%/min. Crystallinity was determined by differential scanning calorimetry (Perkin-Elmer DSC-2) at a heating rate of 10°C/min. The evaluation of the DSC traces was based on the reported[17] enthalpy of fusion of 139.5 J/g for 100% crystalline poly(ϵ-caprolactone).

Tritium labeled caprolactone-3,4,5-^{3}H was prepared by Baeyer–Villiger oxidation of cyclohexanone-3,4,5-^{3}H with *m*-chloroperbenzoic acid. Hydrolysis in boiling water for 18 h afforded ω-hydroxycaproic acid with the same specific activity, demonstrating the aqueous stability of the radiolabeled sites.

RESULTS

The changes in the intrinsic viscosity, molecular weight, crystallinity, elastic modulus, and weight as a function of time *in vivo* of a number of samples of poly(ϵ-caprolactone) capsules and films are shown in Tables I and II and Figures 1–3. Capsules were of particular interest because of the use of poly(ϵ-caprolactone) capsules as a subdermal drug delivery reservoir.[13]

Regardless of the initial M_n of the sample, or its geometry, a linear relationship between ln M_n or ln [η] and time was observed until the M_n had decreased to about 5000. At this point curvature in the plot became evident, and there was a significant decrease in the rate of M_n decline (Figs. 1 and 2). No weight loss was observed until this same point, which coincided with a loss of strength to the extent that capsules and films were usually recovered as fragments after this time. This general behavior was observed for all of the samples studied.

An attempt was made to measure the change in the elastic modulus accompanying the molecular weight decrease. The standard dumbbell shape was impractical because implanted samples could not be recovered from the animal without defects, e.g., creases, caused by movement under the skin. Therefore, capsules with a Silastic-covered Teflon insert to provide form stability were used instead. A small increase in the modulus was observed after 4 weeks, but, with one exception, there was no subsequent change. After 48 weeks *in vivo* the capsules were too brittle to be clamped on the Instron and were usually recovered from the animal in fragments.

The probable reason for both the fragmentation of the capsules and the change in the slope of the ln M_n vs. time plots was revealed by measurements of the crystallinity of the samples (Table I, Fig. 3). The crystallinity associated with an initial M_n of 50,000 was 45%. After implantation, there was an initial rapid increase in the crystallinity to approximately 50% within 4 weeks, attributed to

TABLE I
In Vivo Degradation of Poly(ϵ-Caprolactone) Capsules[a] in Rabbit

Time (weeks)	Wt loss (%)	% Crystallinity (SD)	$[\eta]$[b] (dL/g)	GPC			Elastic modulus (kg/cm^2)
				$[\eta]$	$M_n \times 10^{-3}$	M_w/M_n	
0	0	45.3 ± 0.3[c]	0.883	0.944	50.9	1.66	2700 ± 100
4	0.3	52.4 ± 0.4	0.830	—	—	—	3100
4	0.4	49.2 ± 1.9	0.820	—	—	—	3020
8	0.8	54.7 ± 1.1	0.810	—	—	—	3160
8	0.1	53.6 ± 0.2	0.793	—	—	—	3240
16	0.7	57.8 ± 1.0	0.733	0.736	35.9	1.75	3010
16	3.6	56.0 ± 0.8	0.663	0.723	34.4	1.78	3240
32	6.4[d]	59.3 ± 1.1	0.610	0.603	26.7	1.79	4040
32	4.5	56.4 ± 2.8	0.607	0.644	24.5	2.21	3370
48	4.6	63.4	0.490	0.471	16.5	2.11	2960
48	5.7[d]	64.2 ± 0.3	0.472	0.467	17.5	1.94	2850
64	—	66.3 ± 0.0	0.356	0.391	12.9	2.14	e
64	2.6	64.6 ± 0.1	0.364	0.398	13.1	2.15	
80	15.9	69.0 ± 2.0	0.310	0.291	8.8	2.14	
96	12.2	73.8 ± 1.3	0.150	0.222	6.3	2.15	
112	18.1	—	—	0.191	5.3	2.10	
120	7.4	79.5 ± 1.6	0.184	0.174	4.5	2.20	
120	—	—	0.174	0.166	4.2	2.18	
120	7.6	—	0.176	0.181	4.6	2.24	
129	—	—	0.180	0.164	4.5	2.04	
129	27.3	—	0.208	0.163	4.6	1.98	
144							

[a] Capsules melt extruded; dimensions 5 cm × 2.49 mm (od) × 1.96 mm (id); sterilized by γ-irradiation (2.5 Mrads).
[b] Measured in toluene.
[c] Standard deviation, $n = 2$.
[d] Small part of capsule lost during recovery.
[e] Capsules fragmented after this time.

TABLE II
Biodegradation of Tritium Labeled Poly(ϵ-Caprolactone) Capsules[a] in Rabbit

Time (weeks)	Intrinsic viscosity (dL/g)[b]	% Weight change	% Tritium change
0	2.29	—	—
4	2.04	−0.09	−1.1
	2.08	−0.21	+0.6
8	1.93	−0.54	−4.1
	1.90	−0.64	+0.4
16	1.69	0.00	−8.2
	1.67	0.00	−5.3
32	1.24	−0.09	−1.7
	1.21	−0.29	−0.7
57	0.853	−0.48	−6.3
	0.789	−0.84	−4.6
96[c]	0.428	−25.1	−21.1
	0.462	−17.4	−21.9
120	0.254	−3.2	−24.2
	0.264	0.0	−11.1
160	0.169[d]	−1.2	−24.5
	0.181[e]	−50.7[c]	−50.3[c]
200	0.138[f]	+9.3	−22.5
213	0.127[g]	+5.6	−52.0

[a] Capsule dimensions, 2 cm × 1.25 mm (id) × 1.8 mm (od).
[b] Measured in benzene.
[c] Capsules recovered in fragments after 96 weeks and later.
[d] Determined by GPC, M_n = 4800.
[e] M_n = 5200.
[f] M_n = 3500.
[g] M_n = 3200.

annealing of the extruded polymer at the rabbit body temperature of 40°C. Thereafter, there was a consistent but slower increase in crystallinity with chain cleavage to a value of about 80% after 120 weeks (M_n = 4600). This increase in crystallinity must be attributed to crystallization of tie segments made possible by the chain cleavage in the amorphous phase, facilitated by the low glass transition temperature (−60°C) of poly(ϵ-caprolactone).

The final stages of the biodegradation process were examined by implanting tritium labeled poly(ϵ-caprolactone) which had been predegraded under accelerated *in vitro* conditions to an M_n of 3000. Both polymer capsules and powder (≤10 μm) were evaluated, and rat was substituted as the experimental animal to permit the use of enclosed all-glass Roth metabolism cages. Bioerosion of the polymer was monitored by measuring the radioactivity in the urine, feces, expired water, and, at the conclusion of the study, the residual activity at the implant site. The latter was the more accurate measure of bioabsorption. Absorption of the powder was rapid, approximately 50% of the sample radioactivity being excreted within 60 days (Fig. 4); only 9 ± 4% (n = 3) of the original radioactivity remained at the implantation site after 120 days. Absorption of the same polymer implanted as capsules (1 cm × 2.4 mm od × 2.0 mm id) was considerably slower, and could not be efficiently measured by daily collection and assay of excreta because of lower radioactivity levels. However, measurement of residual radioactivity at the implantation sites after 180 days showed 22 ± 1% (n = 2) weight loss.

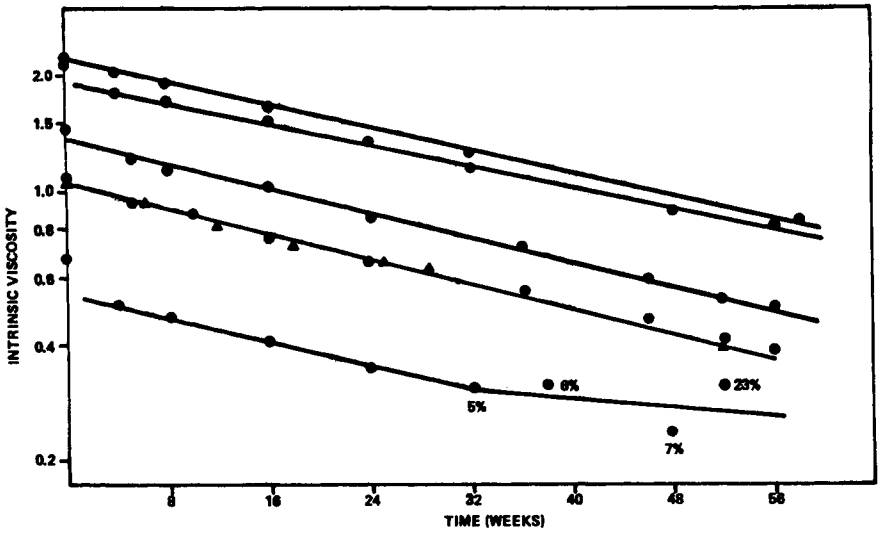

Fig. 1. Semilogarithmic plots of intrinsic viscosities (benzene) of poly(ϵ-caprolactone) capsules and films versus time *in vivo*. Solid data points and triangles indicate capsules and films, respectively. Numbers at individual data points show onset and averaged magnitude of weight loss.

DISCUSSION

The first phase of the biodegradation of poly(ϵ-caprolactone) is consistent with a mechanism which involves random chain scission by hydrolytic cleavage of ester groups. The process must occur in the bulk of the material because, within limits, the rate is independent of geometry despite a tenfold greater surface-to-volume ratio of the films, compared to the capsules. Assuming diffusion of a high molecular weight enzyme into the polymer bulk is kinetically prohibitive, this result excludes enzyme participation in the first phase of polymer degra-

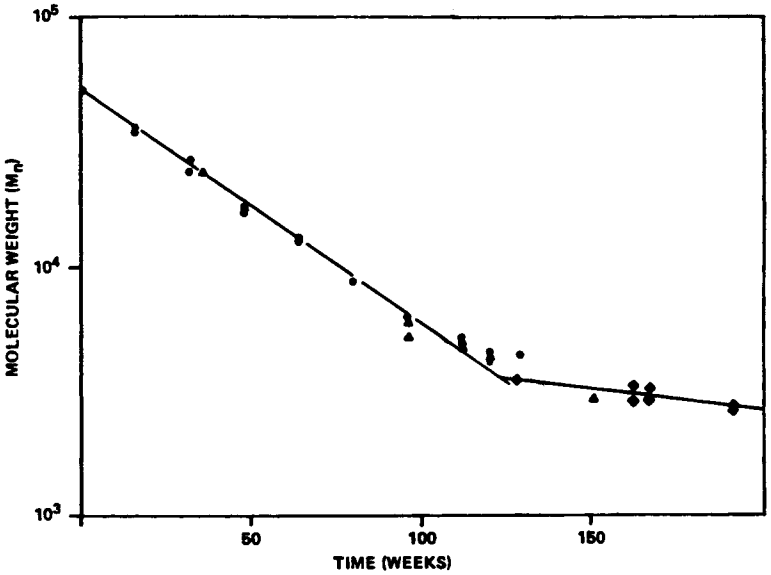

Fig. 2. *In vivo* degradation of poly(ϵ-caprolactone) capsules. (●) PCL—FRL-3 with silastic insert; (▲) PCL—FRL-5B containing norgestrel in sesame oil; (♦) PCL*-1 unfilled.

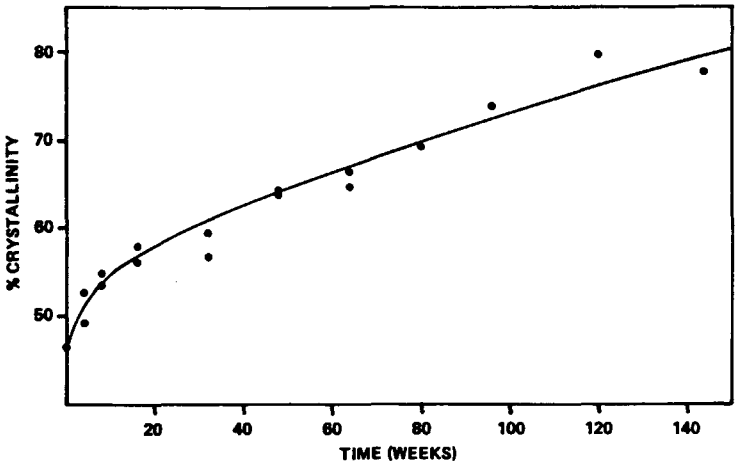

Fig. 3. Change in the crystallinity of poly(ϵ-caprolactone) capsules, initial M_n 50,000, as a function of time in rabbit.

dation. This conclusion is reinforced by the observation that an almost identical rate of chain cleavage was observed in water at 40°C.

The kinetics of chain scission are indicative of an autocatalytic process, in which the carboxylic acid end groups generated by ester hydrolysis participate in the transition state:

$$H_2O + \underset{\underset{OH^+}{\|}}{-C-O-CH_2-} \rightarrow \underset{\underset{OH^+}{\|}}{-C-OH} + HOCH_2-$$

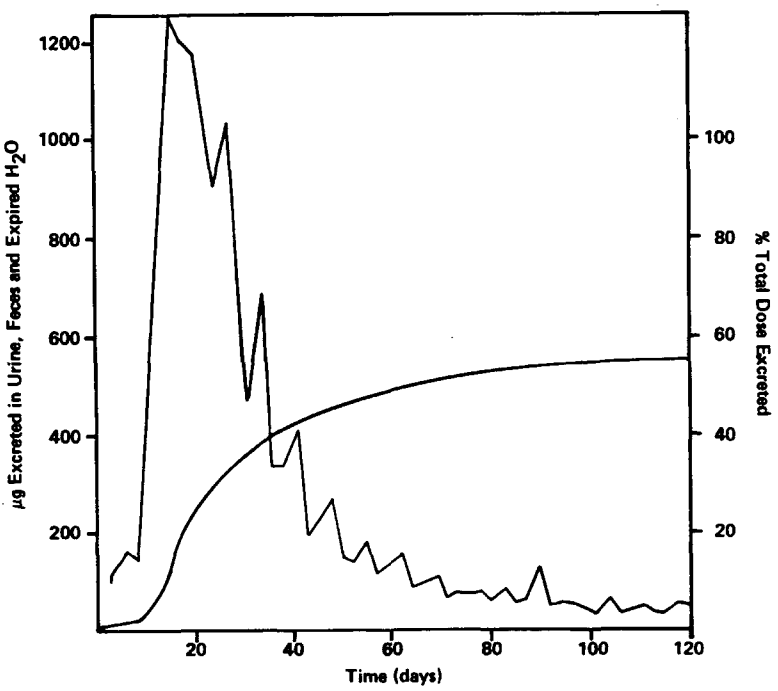

Fig. 4. Daily and cumulative excretion of poly(ϵ-caprolactone) and its metabolites as measured by radioactivity in the urine, feces, and expired water of rat.

The corresponding rate equation is then given by

$$d[\mathrm{COOH}]/dt = k[\mathrm{COOH}][\mathrm{H_2O}][\mathrm{E}] \quad (1)$$

where [COOH], [H_2O], and [E] are the concentrations of carboxyl end groups, water, and ester groups in the polymer, respectively.

While the extent of chain cleavage is small, both [H_2O] and [E] can be assumed constant, and eq. (1) simplifies to a pseudo first order relationship

$$[\mathrm{COOH}] = [\mathrm{COOH}]_0 \exp(k't) \quad (2)$$

or

$$M_n = M_n^0 \exp(-k't) \quad (3)$$

where $k' = k[\mathrm{H_2O}][\mathrm{E}]$. This kinetic expression holds surprisingly well for an extended degradation period, during which M_n decreases to less than 10% of its initial value and the crystallinity increases from 45% to about 80%.

The observation that the kinetic expression (1) incorporates the first power of the carboxylic acid end group concentration is unexpected. The hydrogen ion concentration of a weak acid is proportional to the square root of the acid concentration [eq. (4)]:

$$\mathrm{RCOOH} \rightleftharpoons \mathrm{RCOO^-} + \mathrm{H^+} \quad (4)$$

$$[\mathrm{H^+}] = K_\beta^{1/2} \cdot [\mathrm{RCOOH}]^{1/2} \quad (5)$$

Indeed, Ravens and Ward reported[18] that the hydrolysis of poly(ethylene terephthalate) by water vapor at 150–220°C is proportional to $[\mathrm{COOH}]^{1/2}$. It is possible that, at least in the case of poly(ϵ-caprolactone) and related aliphatic polyesters,[14] the carboxylic acid group participates in the transition state in its undissociated form. This is a reasonable possibility, for the lipophilic polymer bulk cannot be conducive to ionization.

The rate constant k' derived from the initial part of the linear plot in Figure 2 (up to 110 weeks) is 3.07×10^{-3} day^{-1}, i.e., the number of ester groups cleaved per day corresponds to about 0.31% of the carboxylic acid end groups present. The consistency of the data in Figure 2 demonstrates that the value of the rate constant is not affected by the presence of drug and dispersing agent.

The viscosity data for the different polymers presented in Figure 1 cover a range of initial M_n values from about 40,000 to 200,000. The average slope for the five log[η] vs. time plots is $(2.46 \pm 0.07) \times 10^{-3}$ day^{-1}, which can be used to derive the rate constant k' by means of the Mark–Houwink equation:

$$[\eta] = KM_n^a \quad (6)$$

Combining eqs. (3) and (6), one obtains

$$[\eta] = (K/K_0)[\eta]_0 \exp(-ak't) \quad (7)$$

where K_0 and K are the Mark–Houwink constants for poly(ϵ-caprolactone) at times 0 and t, respectively. Provided the shape of the molecular weight distribution does not change appreciably during degradation, the ratio K/K_0 will remain close to unity and, as a good approximation, one obtains

$$[\eta] = [\eta]_0 \exp(-ak't) \quad (8)$$

For poly(ϵ-caprolactone) in benzene at 30°C Koleske and Lundberg[19] have

reported a value of 0.82 for the exponent of the Mark–Houwink equation. Utilizing this value in eq. (8), the value of k' is $(3.00 \pm 0.009) \times 10^{-3}$ day^{-1}. This is in good agreement with the value derived directly from M_n measurements. The small standard deviation demonstrates the molecular weight has little or no effect on the degradation mechanism.

The absence of weight loss from films or capsules of high molecular weight poly(ϵ-caprolactone) is consistent with a random hydrolytic chain scission mechanism. Weight loss reflects the probability that random chain cleavage produces a fragment (monomer or dimer) small enough to diffuse from the material bulk. This probability will become more significant as M_n decreases. A second mechanism of weight loss evidently becomes important when the particle size of the implant is decreased either by fragmentation of the brittle low molecular polymer or when a powdered sample is implanted. Under both conditions the surface-to-volume ratio of the sample increases to such an extent that the contribution of surface reactions becomes significant. Presently, it cannot be decided whether these surface reactions involve a biological mechanism or whether they are of the same nature as the observed bulk degradation.

CONCLUSIONS

Previous studies of poly(lactones) related to poly(ϵ-caprolactone) have often presented conflicting information on the rate of biodegradation. The current results clearly show that measurement of a single property over a limited time period can present a misleading picture. In particular, the molecular weight and the particle size are important in determining the time lag before bioerosion and the rate of bioerosion, respectively.

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