In Vitro Evaluation of Sustained Drug Release from Biodegradable Elastomer

Ryoichi Wada,[1] Suong-Hyu Hyon,[1] Takayuhi Nakamura,[1] and Yoshito Ikada[1,2]

Received November 29, 1990; accepted April 24, 1991

Poly(DL-lactic acid) (PLA), poly(ε-caprolactone) (PCL), and their copolymers (PLA-CL) with various monomer compositions were synthesized, and their properties as matrix for the sustained release of drugs were evaluated. The copolymerization technique produced very soft films which incorporated the drugs without deterioration of the elastic properties. Cisplatin and MD-805 were loaded in the films by casting the polymer solution containing the drugs. Fractions of the drugs released from the PLA-CL films were governed by the initial loading, the film thickness, and the polymer molecular weight. The drug release profiles obeyed the classical Fickian diffusion equation at least in the early stage, but significant hydrolytic degradation of the matrix polymers occurred in the later stage, influencing the kinetics of drug release. The monomer composition of copolymer affected the release profile more strongly than the initial molecular weight of the copolymer.

KEY WORDS: DL-lactide-ε-caprolactone copolymer; biodegradable polymer; cisplatin; MD-805; sustained drug release.

INTRODUCTION

Since the early application of poly(lactic acid) (PLA) for drug delivery (1,2), numerous studies have been performed on the sustained drug delivery systems using PLA, poly (glycolic acid) (PGA), and their copolymers (3–6). We have employed lactic acid oligomers to prepare injectable microspheres incorporating drugs (7,8). The lactic acid oligomers having molecular weights lower than 10,000 were found to be suitable as matrix of injectable microspheres for sustained drug release over a few weeks. However, various formulations other than the microsphere, such as film, sheet, slab, needle, and rod are also suitable in clinical applications. Moreover, soft materials are preferable as implants in the body, because they may not harm the surrounding tissues and can be readily shaped in accordance with the tissue contour. PLA, PGA, and their copolymers are too stiff and rigid at body temperature because of their high glass transition temperature (T_g). In addition, their films are easily torn from the needle hole during suturing. Although the lactic acid oligomers have a lower T_g than high molecular weight PLA, they are brittle and have insufficient mechanical strength.

Pitt and his co-workers have reported that copolymers from ε-caprolactone and lactic acid are biodegradable, rubbery, and relatively high in drug permeability (9). Recently, we have also studied the *in vitro* and *in vivo* degradation of the copolymers and found that no adverse tissue reactions are observed on these copolymers when subcutaneously implanted in dogs for surgical applications (10).

In this work, we study the properties of DL-lactic acid–ε-caprolactone copolymers (PLA-CL) as matrix of sustained-drug release devices to be used in a film or sheet form. Cisplatin and MD-805 were selected for loading in the PLA-CL films. Cisplatin is a potent anticancer agent, but highly toxic with a narrow therapeutic range. Thus, the sustained local release of this drug from the polymeric device implanted near a tumor site may minimize its side effects. MD-805, a highly selective thrombin inhibitor, can inhibit blood coagulation without any effect on platelet functions and prolong the clotting time (11). The sustained release of MD-805 has possible applications not only for implant biomaterials such as biodegradable vascular grafts (12) but also for extracorporeal blood-clotting devices to prevent blood coagulation on their surface during blood circulation. The main purpose of this study was to determine the factors that affect the release profiles of the drugs from PLA-CL films *in vitro*.

EXPERIMENTAL

Materials

PLA, poly(ε-caprolactone), and their copolymers (PLA-CL) with different monomer compositions were synthesized by bulk ring-opening polymerization of DL-lactide and ε-caprolactone at 190°C for 5 hr *in vacuo* using stannous octoate as catalyst (13). The monomers were purified by distillation (ε-caprolactone) and recrystallization (DL-lactide). All the polymerization products were purified by precipitation from the methylene chloride solution in methanol and then dried under a reduced pressure.

Monomer compositions of the copolymers were determined by ^{1}H-NMR spectroscopy on samples dissolved in $CDCl_3$. Molecular weights of polymers were determined by gel permeation chromatography (GPC) using standard polystyrenes for calibration. The dynamic mechanical properties were measured with a torsion pendulum (1 Hz) (Model RD-1100A, Resca Co. Ltd., Tokyo). *cis*-Dichlorodiamine platinum(II) (CDDP) and (2*R*,4*R*)-4-methyl-1-[N^2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginyl]-2-piperidinecarboxylic acid monohydrate (MD-805) were donated by Nippon Kayaku Co. Ltd., Tokyo, and Mitsubishi Kasei Kogyo Co. Ltd., Tokyo, respectively. They were used as received.

Release Test

Films containing a given amount of CDDP or MD-805 were prepared by the solution casting method. Briefly, the polymer and the drug were dissolved in *N,N*-dimethylformamide, and the solution was cast on a glass plate, followed by drying under a reduced pressure at 45°C. The film of 5-mm diameter was weighted, immersed in 10 ml of Tris–NaCl buffer, and stored in a glass vial kept at 37°C in a shaker bath. The whole volume of the buffer medium was pipetted out at predetermined times and the same volume of fresh buffer was added. The concentration of CDDP was

[1] Research Center for Biomedical Engineering, Kyoto University, 53 Kawahara-cho Syogoin, Sakyo-ku, Kyoto 606, Japan.
[2] To whom correspondence should be addressed.

0724-8741/91/1000-1292$06.50/0

Table I. Characteristics of DL-Lactide (LA)–ϵ-caprolactone (CL) Copolymers[a]

Polymer code	CL in monomer feed (mol%)	CL in copolymer (mol%)[b]	Conversion (wt%)	M_w ($\times 10^5$)	M_n ($\times 10^5$)	M_w/M_n
PLA	0	0	98.1	2.20	1.20	1.80
PLA-CL10	10	9	—	2.24	0.96	2.26
PLA-CL15	15	16	91.6	1.37	0.71	1.93
PLA-CL20	20	20	90.7	1.83	0.97	1.88
PLA-CL20[c]	20	—	—	0.78	0.40	1.95
PLA-CL20[d]	20	—	—	0.22	—	—
PLA-CL30	30	30	—	1.40	—	—
PLA-CL40	40	37	83.8	1.55	0.70	2.22
PLA-CL60	60	60	88.1	1.95	0.87	2.25
PLA-CL80	80	79	86.8	2.07	1.01	2.05
PCL	100	100	95.4	2.71	1.40	1.94

[a] Homopolymers and copolymers were obtained by ring-opening polymerization of their monomers at 190°C for 5 hr in the presence of 0.05 wt% stannous octoate.
[b] Determined by ^{1}H-NMR analysis.
[c] At 160°C for 2 hr.
[d] At 160°C for 4 hr.

determined by atomic absorption analysis (14) and the release amount was expressed as a percentage of the initial loading. The released MD-805 was determined by a fluorescence spectrophotometer (Model 650 10-S, Hitachi Co. Ltd., Tokyo) conducted at the excitation wavelength of 325 nm and the emission wavelength of 395 nm. The release curves are presented as the mean of duplicate measurements; the deviation of each datum from the mean value was generally not greater than ±5%.

Lee-White Test

PLA-CL was coated with MD-805 on the inner surface of glass tubes (10 mm in diameter and 25 mm in length). To determine the whole blood clotting time (WBCT) on the surface, human venous blood was collected through a 19-gauge needle into a polypropylene syringe, and then 2 ml of the blood was immediately added to each glass tube coated with PLA-CL20 containing MD-805. The period of time from the start of blood inflow into the syringe to complete clotting in the coated glass tubes was measured as the WBCT. The clot formation was confirmed by the blood fluidity when the tube was tilted. The relative WBCT to that of uncoated glass tube was calculated as follows:

$$\text{relative whole blood clotting time} = \frac{\text{WBCT for each sample}}{\text{WBCT for uncoated glass tube}}$$

In order to determine the minimal concentration of MD-805 to prevent clotting, 0.1 ml of saline containing MD-805 was added to the whole blood by 10^{-12} to 10^{-8} M and the WBCT against the uncoated glass tube was measured.

RESULTS AND DISCUSSION

Polymer Synthesis

Monomer compositions and the weight- and number-average molecular weights (M_w and M_n) of PLA-CL used are given in Table I. The monomer composition of the copolymers found by the NMR analysis was in all cases almost the same as the monomer ratio of the feeds for copolymerization. Therefore, the copolymer samples were named according to the monomer ratio in feed; for instance, the copolymer obtained by the polymerization at a 2 mol% caprolactone in feed is named PLA-CL20. The PLA-CL20 copolymers having different M_w values were obtained under different polymerization conditions, as shown in Table I.

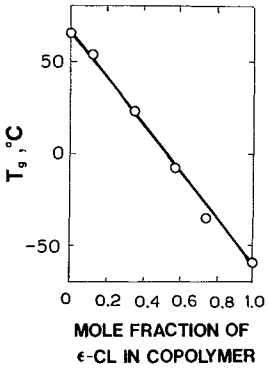

Fig. 1. Glass transition temperatures (T_g) of the PLA-CL copolymers plotted against the composition of copolymers.

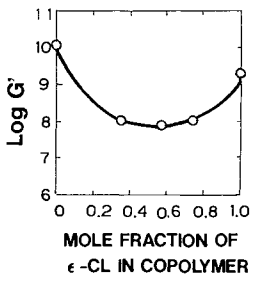

Fig. 2. Dynamic moduli (G') of the PLA-CL copolymers plotted against the composition of copolymers (25°C).

Table II. M_w Decrease for CDDP-Loaded PLA-CL Films During *in Vitro* Hydrolytic Degradation

Polymer code	M_w ($\times 10^5$) after 0 days	7 days	15 days	30 days	60 days
PLA-CL10	2.4	2.3	2.0	1.7	1.3
PLA-CL30	1.4	1.4	1.2	0.63	0.21
PLA-CL40	1.6	1.6	1.3	0.53	0.18

The T_g and the dynamic modulus (G'), determined by the torsion pendulum, are plotted against the copolymer composition in Figs. 1 and 2, respectively. The result shown in Fig. 1 indicates that T_g decreases linearly as the caprolactone concentration in feed becomes higher. This is in good agreement with the result reported by Pitt *et al.* (9). On the other hand, G' has a minimum at the caprolactone concentration of about 50 mol%, as can be seen in Fig. 2. The low G' implies that the PLA-CL copolymers with 30–70 mol% caprolactone are virtually amorphous and rubbery. This may be attributed to destruction of the crystalline structure of respective homopolymers by the copolymerization.

The results of degradation of PLA-CL *in vitro* is given in Table II. It is seen that these copolymers do not undergo any appreciable degradation *in vitro* within a week but exhibit a considerable M_w decrease after 1 month. The higher caprolactone concentration causes enhanced degradation of copolymers at least in this range of caprolactone concentration.

Drug Release

Figure 3 shows the effect of initial drug loading on the CDDP release from the PLA-CL30 film. Apparently, the fraction of CDDP released from the film increases with the increasing loading of CDDP. A similar result was observed for MD-805 loaded in the film of PLA-CL20, as shown in Fig. 4. The effect of film thickness on the release of MD-805 from the same copolymer is shown in Fig. 5. These results clearly demonstrate that the release rate of drugs is controllable by changing the drug loading and the thickness of PLA-CL film. It is expected that the initial M_w of copolymer will also have an effect on the release rate of drug. The result for MD-805 release from the PLA-CL20 film is shown in Fig. 6; the effect of M_w is relatively small, although M_w varied by nearly one order of magnitude.

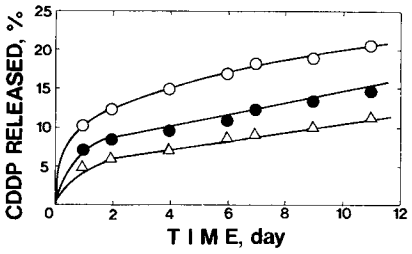

Fig. 3. Effects of initial loading on the release profile of CDDP from PLA-CL30 film: (○) 2.5 wt%, (●) 5.0 wt%, and (△) 10 wt%.

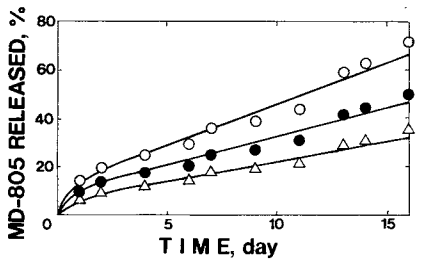

Fig. 4. Effects of initial loading on the release profile of MD-805 from PLA-CL20 film: (○) 2.5 wt%, (●) 5.0 wt%, and (△) 10 wt%.

The diffusion-controlled release of drug from a monolithic film or slab under a sink condition has been described theoretically by Higuchi *et al.* (15–17). If a drug is not completely dissolved in matrix and the remaining solid drug particle is distributed uniformly in the film and small in diameter compared to the average diffusion distance, the following equations are derived (15):

$$M_t/M_\infty = [4D_eC_st(2C_0 - C_s)/C_0^2l^2]^{1/2} \quad (\text{for } C_0 > C_s) \quad (1)$$

$$M_t/M_\infty = [8D_eC_st/C_0l^2]^{1/2} \quad (\text{for } C_0 \gg C_s) \quad (2)$$

where M_t is the cumulative weight of drug released from the unit area of film at time t, $M\infty$ is the total initial drug weight per area, C_s is the drug solubility in the matrix polymer, C_0 is the initial drug concentration, l is the film thickness, and D_e is the effective diffusion coefficient, equal to $D\epsilon/\tau$, where ϵ and τ are the volume fraction and the tortuosity of the polymer matrix, respectively.

In order to study the kinetics of CDDP and MD-805 release from the PLA-CL films, the results shown in Figs. 3 to 5 were processed and are replotted in Fig. 7 as the fraction of drug released ($M_t/M\infty$) versus $(t/C_0l^2)^{1/2}$ to normalize the initial drug loading and the film thickness (18). The excellent linearity of this $M_t - t^{1/2}$ plot implies that the release of the drugs from the PLA-CL films obeys the classical Fickian diffusion. The visual observation on the PLA-CL films loaded with the drugs showed the presence of undissolved drug dispersions in the film, revealing that the drug exceeds its solubility in the film ($C_0 \gg C_s$). In fact, the CDDP solubility in the PLA-CL30 was only 0.32% (w/w) when determined by the partition measurement (9).

The overall Higuchi rate constant (k_H), predicted by Eq. (2) as $(8D_eC_s/C_0l^2)^{1/2}$, is plotted against the copolymer composition in Fig. 8 for MD-805 loaded in PLA-CL films. The release rate constant has a maximum at the caprolactone

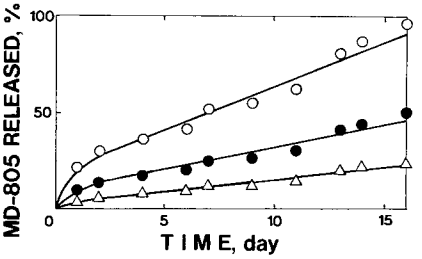

Fig. 5. Effects of film thickness on the release profile of MD-805 from PLA-CL20 film: (○) 110 μm, (●) 230 μm, and (△) 460 μm.

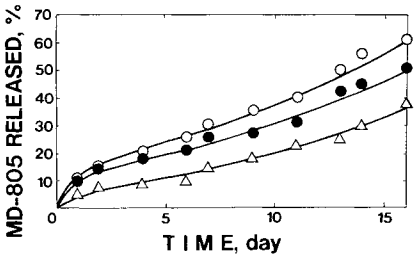

Fig. 6. Effects of initial M_w on the MD-805 release profile from PLA-CL20 film (5% loading and 230-μm thickness): (○) M_w = 22,000, (●) M_w = 78,000, and (△) M_w = 183,000.

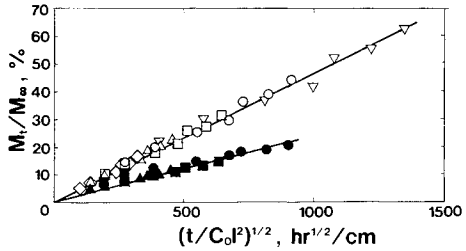

Fig. 7. $(t/C_0l^2)^{1/2}$ – M_t/M_∞ plots for MD-805 (open symbols) and CDDP (filled symbols) loaded in PLA-CL films: (○, ●) 2.5%, (□, ■) 5% (△, ▲), and 10% loading in 230-μm film; (▽) 110 μg and (◇) 460 μm with 5% loading.

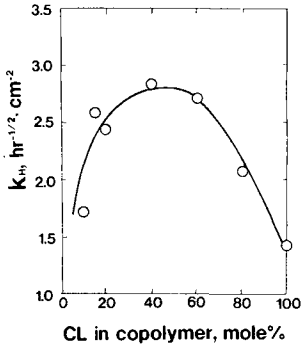

Fig. 8. Effects of copolymer composition on the Higuchi release constant (k_H) for MD-805 loaded in PLA-CL films by 5 wt%.

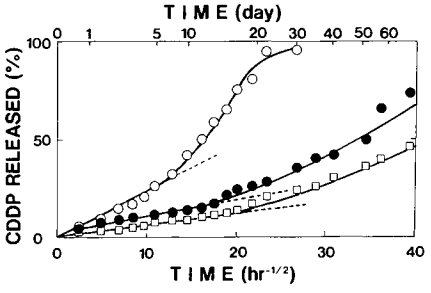

Fig. 9. Higuchi plots for the CDDP release from PLA-CL films with different compositions (5% loading and 230-μm thickness): (□) PLA-CL10, (●) PLA-CL30, and (○) PLA-CL40.

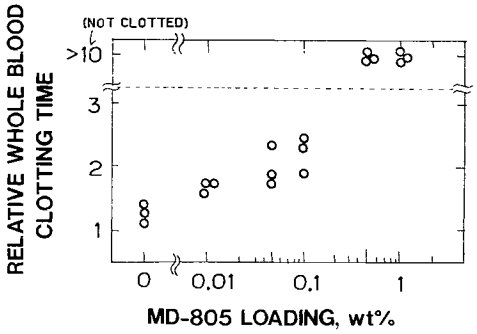

Fig. 10. Clotting times by the Lee-White test for MD-805/PLA-CL20 film coated on glass tube.

fraction of about 50 mol%. As shown in Fig. 1, an increase in caprolactone fraction in copolymer results in a monotonous decrease in T_g. This T_g change must have a considerable effect on the drug permeability through the copolymer film. A further increase in caprolactone fraction beyond 50 mol% will depress the drug diffusion because of the increased crystallinity of PLA-CL, as suggested in Fig. 2. This may explain why there is a maximum in the rate constant of MD-805 release from the PLA-CL film.

The M_t – $t^{1/2}$ plot for CDDP loaded in PLA-CL films by 5 wt% is shown in Fig. 9. The variation in monomer composition among the PLA-CL films is much larger than that in the initial M_w of copolymers. It is interesting to point out that the CDDP release profile fits well on the M_t – $t^{1/2}$ relationship within 10 to 20 days but deviate from the initial slope in the later stage of the release. This may be attributed to the appreciable degradation of the copolymer during the later period of the CDDP release. As the result in Table II suggests, the hydrolytic degradation of the copolymers is negligibly small within 7 days, where the initial release profiles fit well on the straight line in the M_t – $t^{1/2}$ plot. From Fig. 9 and Table II, it is obvious that the polymer degradation is responsible for the enhanced release rates of drug in the later stage. Diffusion coefficients of drug and water are expected to increase when the M_w of the matrix polymer decreases to a significant extent, but erosion of the polymer seems also responsible for the enhanced drug release as the further degradation results in significant mass loss. Pitt *et al.* stated that mass degradation markedly occurred following the significant decrease in M_w of the copolymer (19).

Prevention of Blood Coagulation

The *in vitro* anticoagulant activity of MD-805 released from the PLA-CL coated layer is shown in Fig. 10. The clotting time of human whole blood was 9.93 ± 0.19 min (n = 7) when measured by the Lee-White test without MD-805. The addition of MD-805 at a concentration of 2×10^{-10} M in the whole blood prolonged clotting with WBCT about two times (18.77 ± 0.40 min, n = 3) as long as the control value against the glass tube containing no MD-805. Above 10^{-9} M; MD-805 caused prolonged clotting; WBCT was 10 times longer than the control (the clotting was not observed after 100 min; n = 3). This indicates that, at this MD-805 concen-

tration in the whole blood under a static condition, MD-805 can completely inhibit the thrombin activity required for the whole blood clotting. As demonstrated in Fig. 10, the whole blood in the glass tubes coated with PLA-CL20 containing MD-805 again exhibited a longer WBCT as the concentration of MD-805 incorporated in PLA-CL coatings was higher. On the other hand, clotting was not prolonged when the PLA-CL coating did not contain MD-805. The MD-805 concentration higher than 0.05 wt% in the coating was effective to inhibit whole blood clotting completely. However, these experiments give only the minimum MD-805 concentration that is necessary to prevent the whole blood clotting as a result of MD-805 release toward the outer blood under a static condition. Further studies *in vivo* or *ex vivo* are necessary to determine the minimum MD-805 concentration that is required in the degradable coating to release a sufficient amount of MD-805 near the surface of the coating in contact with the flowing blood.

REFERENCES

1. S. Yolles, D. I. Leafe, and F. J. Meyer. Timed-release depot for anticancer agents. *J. Pharm. Sci.* 64:115–116 (1975).
2. L. R. Beck, D. R. Cowsar, D. H. Lewis, R. J. Coxgrove, C. T. Riddle, S. L. Lowry, and T. Epperly. A new long-acting injectable microcapsule system for the administration of progesterone. *Fertil. Steril.* 31:545—551 (1979).
3. D. L. Wise, T. D. Fellman, J. E. Sanderson, and R. L. Wentworth. Lactic/glycolic acid polymers. In G. Gregoriadis (ed.), *Drug Carriers in Biology and Medicine,* Academic Press, London, 1979, pp. 237–270.
4. L. M. Sanders, J. S. Kent, G. I. McRae, B. H. Vickery, T. R. Tice, and D. H. Lewis. Controlled release of a luteinizing hormone-releasing hormone analogue from poly(D,L-lactide-coglycolide) microsphere. *J. Pharm. Sci.* 75:356–360 (1986).
5. Y. Ogawa, H. Okada, T. Heya, and T. Shimamoto. Controlled release of LHRH agonist, leuprolide acetate, from microcapsules: Serum drug level profiles and pharmacological effects in animals. *J. Pharm. Pharmacol.* 41:439–444 (1988).
6. F. G. Hutchinson and B. J. A. Furr. Biodegradable polymer systems for the sustained release of polypeptides. *J. Control. Release* 13:279–294 (1990).
7. R. Wada, S.-H. Hyon, Y. Ikada, Y. Nakao, H. Yoshikawa, and S. Muranishi. Lactic acid oligomer microspheres containing an anticancer agent for selective lymphatic delivery. I. *In vitro* study. *J. Bioact. Compat. Polym.* 3:126–136 (1988).
8. R. Wada, S.-H. Hyon, and Y. Ikada. Lactic acid oligomer microspheres containing hydrophilic drugs. *J. Pharm. Sci.* 79:919–924 (1990).
9. C. G. Pitt, A. R. Jeffcoat, R. A. Zweidinger, and A. Schindler. Sustained drug delivery systems. I. The permeability of poly(ϵ-caprolactone), poly(DL-lactic acid), and their copolymers. *J. Biomed. Mater. Res.* 13:497–507 (1979).
10. T. Nakamura, S. Hitomi, T. Shimamoto, S.-H. Hyon, Y. Ikada, S. Watanabe, and Y. Shimizu. In A. Pizzoferrato, P. G. Marchetti, A. Ravaglioli, and A. J. C. Lee (eds.), *Biomaterials and Clinical Applications,* Elsevier, Amsterdam, 1987, pp. 759–764.
11. S. Okamoto, A. Hijikata, R. Kikumoto, S. Tonomura, H. Hara, K. Ninomiya, A. Maruyama, M. Sugano, and Y. Tamao. Potent inhibition of thrombin by newly synthesized arginine derivative No. 805. The importance of stereo-structure of its hydrophobic carboxyamide portion. *Biochem. Biophys. Res. Comm.* 101:440–446 (1981).
12. B. van der Lei, P. Nieuwenheis, I. Molenarr, and C. R. H. Wildevuur. Long-term biologic fate of neoarteries regenerated in microporous, compliant, biodegradable, small-caliber vascular grafts in rats. *Surgery* 101:459–467 (1987).
13. A. Schindler, Y. M. Hibionada, and C. G. Pitt. Molecular weight and molecular weight distribution in alcohol-initiated polymerization of ϵ-caprolactone. *J. Polym. Sci. Polym. Chem. Edn.* 20:319–326 (1982).
14. M. F. Para, Jr., and H. C. Harder. Analysis for platinum in biological material by flameless atomic absorption spectrometry. *Clin. Chem.* 23:1245–1249 (1977).
15. T. Higuchi. Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharm. Sci.* 50:874–875 (1961).
16. W. I. Higuchi. Analysis of data on the medicament release from ointments. *J. Pharm. Sci.* 51:802–804 (1962).
17. T. J. Roseman and W. I. Higuchi. Release of medroxyprogesterone acetate from a silicone polymer. *J. Pharm. Sci.* 59:353–357 (1970).
18. C. G. Pitt, M. M. Gratzl, A. R. Jeffcoat, R. Zweidinger, and A. Schindler. Sustained drug delivery systems. II. Factors affecting rates of release from poly(ϵ-caprolactone) and related biodegradable polymers. *J. Pharm. Sci.* 68:1534–1538 (1979).
19. C. G. Pitt, M. M. Gratzl, G. L. Kimmel, J. Surles, and A. Schindler. Aliphatic polyesters. II. The degradation of poly(DL-lactide), poly(ϵ-caprolactone), and their copolymers *in vivo*. *Biomaterials* 2:215–220 (1981).