Controlled Release of a Luteinizing Hormone-Releasing Hormone Analogue from Poly(*d*,*l*-lactide-co-glycolide) Microspheres

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Abstract □ The performance *in vivo* of nafarelin acetate, a potent analogue of luteinizing hormone-releasing hormone, microencapsulated in poly(*d*,*l*-lactide-co-glycolide), was evaluated. The influence of polymer composition and molecular weight on the estrus-suppressing activity of the microspheres in female rats was determined. Compound release was shown to be effected by polymer erosion rather than by diffusion. A triphasic release of compound was observed, which was adjusted by altering the critical parameters of the polymer. A mechanism for the release of the compound was proposed. The primary release phase was compound loss by diffusion from the surface of the microspheres. The secondary phase of subeffective rates of release occurred concomitantly with polymer hydrolysis and a decrease in its molecular weight, although it remained insoluble. Dissolution of low-molecular weight fragments and erosion of the bulk of the polymer then initiated the tertiary phase of release of compound.

Keyphrases □ Microencapsulation—nafarelin acetate in poly(*d*,*l*-lactide-co-glycolide) □ Nafarelin acetate—microencapsulation in poly(*d*,*l*-lactide-co-glycolide) □ Controlled-release formulations—microencapsulation of nafarelin acetate in poly(*d*,*l*-lactide-co-glycolide)

Poly(*d*,*l*-lactide-co-glycolide) and its homopolymers have a long history of use in such products as biodegradable sutures (1) and bone plates (2, 3) and have more recently been used to achieve controlled release of pharmaceuticals. A great deal has been done with narcotic antagonists such as naltrexone (4–7), local anesthetics (8), and steroids (9). A system consisting of norethisterone microencapsulated in poly(*d*,*l*-lactide-co-glycolide) has recently been shown to provide controlled release in clinical studies over 6 months (10). These systems all share a common feature in that the kinetics of compound release are diffusion controlled, through the polymer continuum and/or through channels formed within the device which are achieved by a high loading level of active material.

In this report, the controlled release of nafarelin acetate (I), an analogue of luteinizing hormone-releasing hormone (II) is described. Compound I has high biological potency, ~200 times that of II (11).

(Pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)D-alanine-Leu-Arg-Pro-Gly-NH_2
I

(Pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
II

The system described is controlled by erosion, with the kinetics of compound release determined by parameters of the copolymer.

EXPERIMENTAL SECTION

Materials—Poly(*d*,*l*-lactide-co-glycolide) and microspheres were prepared in-house[1] and used as supplied. Intrinsic viscosity (η_i) which is a measure of relative molecular weight, was determined by measuring the flow times of solutions of the copolymer with concentrations of 1.0–10.0 mg/mL in chloroform through a viscometer[2] at 30°C. Specific viscosities were calculated by:

$$\eta_{sp} = \frac{t_s - t_0}{t_0} \qquad \text{(Eq. 1)}$$

where t_s is the time required for a solution to pass through the viscometer, and t_0 is the time required for the pure solvent to pass through the viscometer. The plot of η_{sp}/C *versus* C was extrapolated back to the ordinate, where the intercept represents the intrinsic viscosity and C is the concentration.

The microsphere-suspending vehicle consisted of 0.5% carboxymethylcellulose[3]–0.8% sodium chloride USP 0.1% polysorbate 80 USP[4], in aqueous solution.

[1]Southern Research Institute.

Table I—Estrus Suppression Activity of I in the Female Rat by Twice-Daily Injection and Continuous Infusion

Total Daily Dose, μg	Percentage of Animals Displaying Complete Estrus Suppression[a]: Twice-Daily Subcutaneous Injection[b]	Continuous Subcutaneous Infusion[c]
0.05	0	0
0.10	50	0
0.20	80	60
0.40	100	100

[a]From 4 d after start of treatment and lasting for the full 14 d of treatment. [b]n = 10. [c]With Alzet minipumps, model 2002, lot 0888; n = 5.

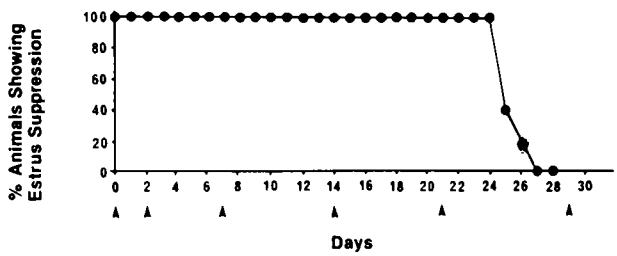

Figure 1—*Biological profile of 50:50 copolymer (η_i = 0.38 dL/g) microspheres containing 1% I injected subcutaneously in female rats at a 300-μg compound dose (n = 30).*

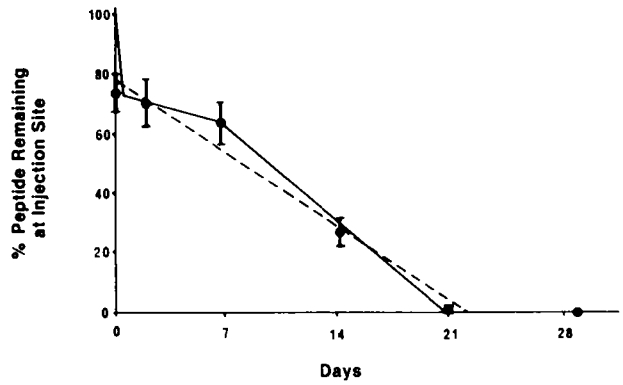

Figure 2—In vivo *release profile of naferelin acetate from 50:50 copolymer microspheres. Key: (——) triphasic kinetics; (- - -), zero-order kinetics.*

[2]Size 75; Cannon-Fenske.
[3]500 cps; Hercules.
[4]Tween 80, ICI Americas, Inc.

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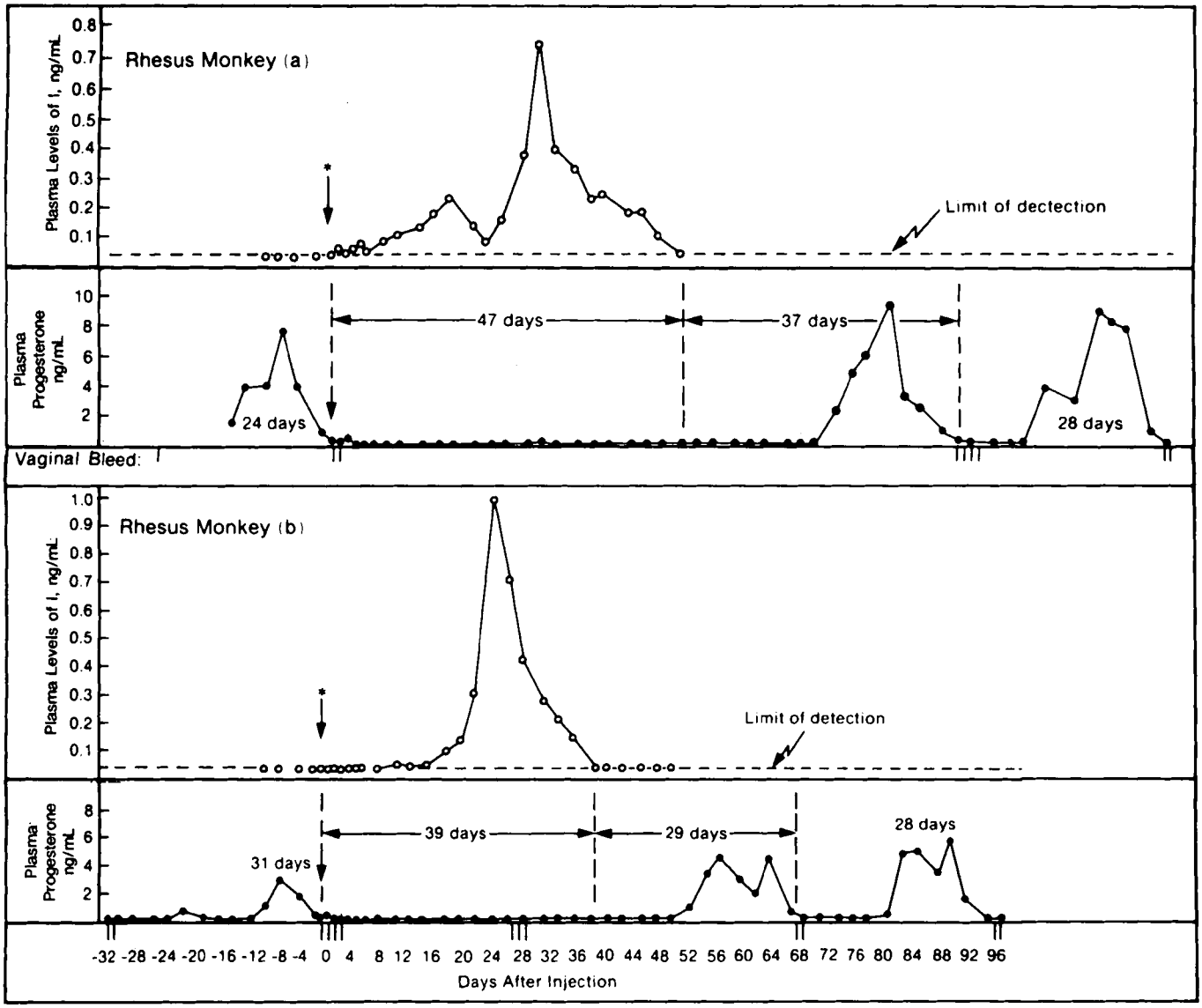

Figure 3—*Plasma levels of nafarelin acetate and progesterone in rhesus monkeys after a single subcutaneous injection of 1.0 mg of nafarelin acetate microencapsulated in 50:50 copolymer. The asterisks represent injection times.*

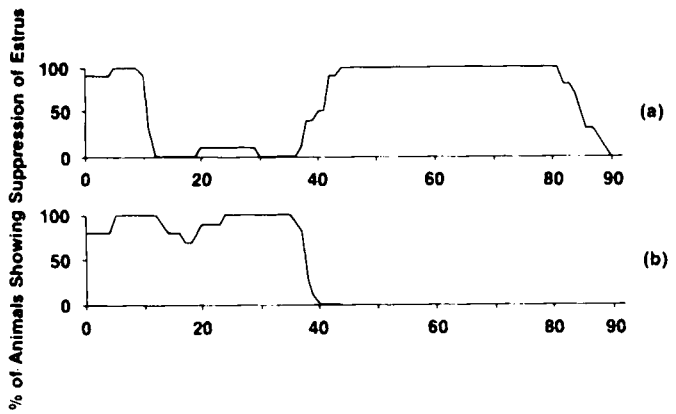

Figure 4—*Estrus suppression profiles provided by (a) 69:31 (η_i = 0.97 dL/g) and by (b) 50:50 (η_i = 1.52 dL/g) copolymer mixtures.*

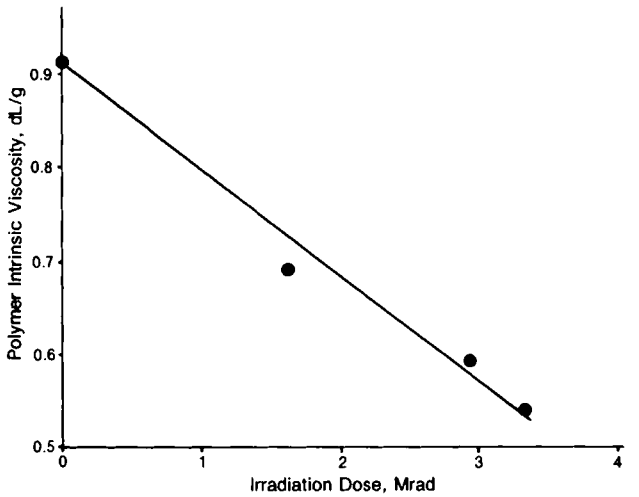

Figure 5—*Effect of gamma-irradiation on intrinsic viscosity of 69:31 copolymer.*

Animals—The rats used were mature females derived from the Sprague–Dawley line (weight, 155–165 g). Adult female rhesus monkeys (*Macaca mulatta*) that had menstrual cycles of predictable length based on prior history were also used.

Microencapsulation of I in Poly(*d,l*-lactide-co-glycolide)—Preparation of microspheres was by phase separation. An aqeous solution of I and a solution of the copolymer in dichloromethane were coemulsified to form a water-in-oil emulsion. A nonsolvent for the copolymer was then added to precipitate out the polymer around the aqueous droplets. The suspension of semi-formed

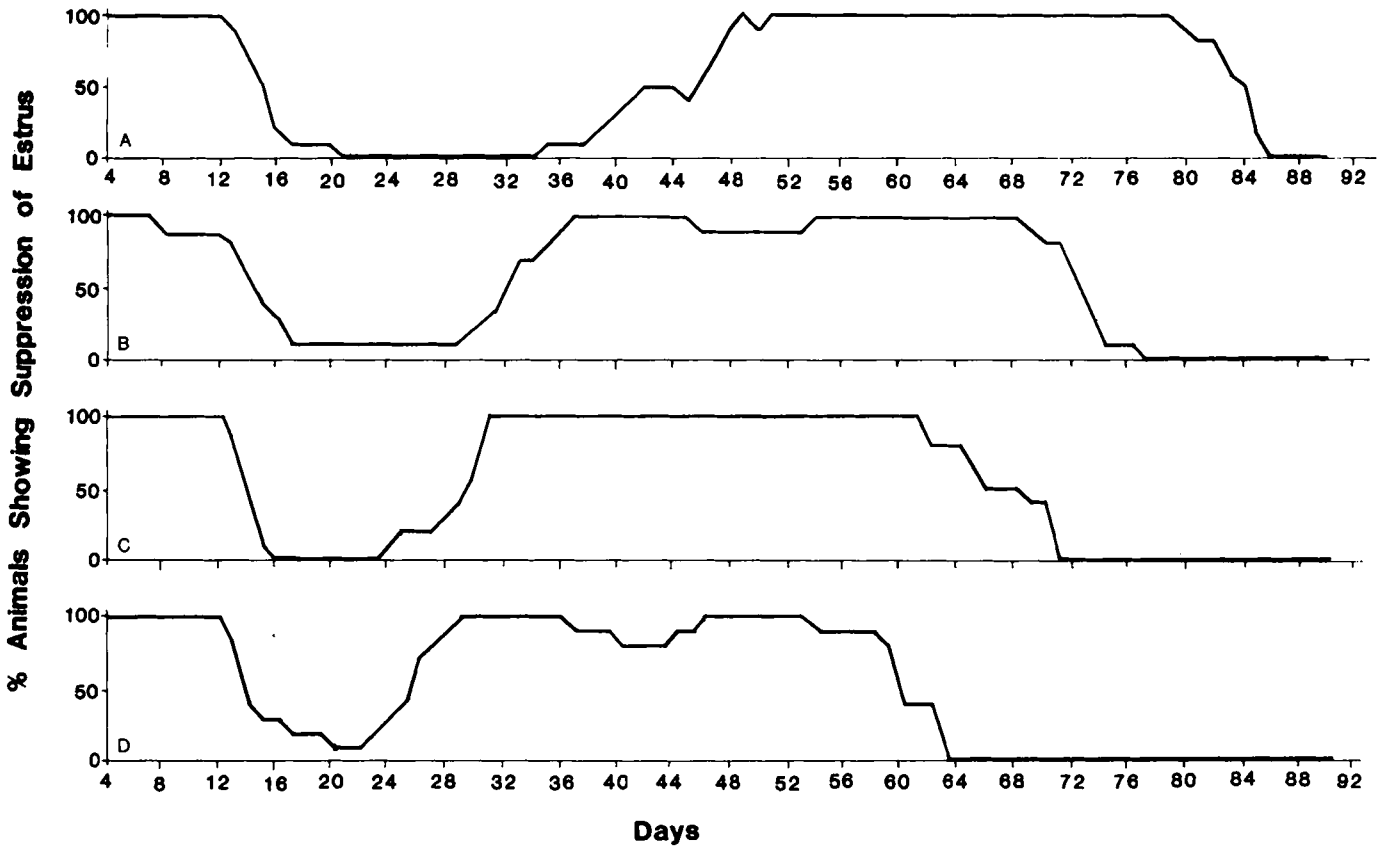

Figure 6—*Effect of irradiation on the biological activity of 69:31 copolymer microspheres (10–40 μm) containing I; n = 10. Key to irradiation: (A) none; (B) 1.6 Mrad; (C) 2.9 Mrad; (D) 3.3 Mrad.*

microspheres was added to a large volume of nonsolvent to cause them to harden and to complete the extraction of dichloromethane. The microspheres were then sieved, washed, and dried. Microspheres were thus prepared to contain ~1% (w/w) I.

Assay of I in Microspheres—A quantity of microspheres containing ~500 μg of I was dissolved in 3.3 mL of acetonitrile–water (9:1), in which both the copolymer and I are sufficiently soluble. Aqueous potassium dihydrogen phosphate (0.184 M) was added to 10.0 mL to precipitate the copolymer, and the suspension was stirred until the precipitate coalesced into a single mass. The supernatant was then assayed by reverse-phase HPLC by using a C_{18} 10-μm column[5], with a mobile phase[6] flow rate of 1.2 mL/min, an injection volume of 100 μL, and a detector wavelength of 225 nm. Compound recovery was 100 ± 1.5%.

Biological Assay—The microspheres were evaluated *in vivo* by using a rat estrus suppression model. It was shown that twice daily injection or continuous subcutaneous administration of ~0.4 μg/d is sufficient to maintain suppression of estrus in this model (Table I). Microspheres containing 300 μg of I were suspended in 0.5 mL of suspension vehicle and administered by a single subcutaneous injection. The animals were monitored for suppression of estrus by daily examination of vaginal smears. This model provides a reliable indication of the duration of compound release, although not of the release rate.

It was not possible to measure plasma levels of I in the rat since the compound is not sufficiently stable in rat plasma *in vitro*. Release kinetics in the rat were, therefore, determined by sequential sacrifice of treated animals, followed by excision of the injection sites and assay of the level of I remaining.

Biological efficacy of I in the female rhesus monkey was evaluated by monitoring menstrual bleeding and by measuring plasma levels of progesterone by RIA (12) after a single subcutaneous injection of microspheres containing 1.0 mg of I on day 1 of the menstrual cycle of the animal. The release profile of the compound was determined by the measurement of plasma levels of I by RIA (13).

Assay of I in Tissue Samples—The tissue samples, *i.e.*, excised injection sites, were frozen for ease of handling and sectioned into thin slices. They were vortexed with 5 mL of acetonitrile–water (9:1) periodically over 4 h. Potassium dihydrogen phosphate (0.184 M; 10 mL) was added, and the suspensions were vortexed over ~4 h. The samples were centrifuged at 1000 × *g* for 20 min and assayed by HPLC as described above. Compound recovery was 83 ± 5%.

[5]μ-Bondapak; Waters Associates.

[6]Acetonitrile–0.175 M aqueous potassium dihydrogen phosphate (30:70).

RESULTS AND DISCUSSION

Injection of rats with microspheres of a low intrinsic viscosity (0.38 dL/g), 50:50 mol% copolymer produced continuous suppression of estrous cyclicity for 24 d, followed by an abrupt resumption of normal estrous cycling (Fig. 1). In this experiment, 30 animals were injected at time zero, and 5 animals were sacrificed at each of the six indicated times. Assay of the excised injection sites indicated a compound release profile (Fig. 2) that exhibited complex kinetic behavior, possibly reflecting a triphasic release mechanism. Overall, release kinetics were quite satisfactory, as indicated by comparison with zero-order kinetics, with exhaustion of the compound from the injection sites at ~22 d. Plasma levels of I in female rhesus monkeys a and b, which received 1 mg of compound in the same 50:50 copolymer, were detectable (*i.e.*, >0.05 ng/mL) for 47 and 39 d, respectively, during which time ovulation was completely inhibited (Fig. 3). Ovulation in these monkeys, as indicated by a rise in plasma progesterone levels, occurred at 19 and 14 d, respectively, after the disappearance of I in the plasma; normal menstrual cycling resumed thereafter.

A high molecular weight 50:50 copolymer (η_i = 1.52 dL/g) and a 69:31 copolymer (η_i = 0.97 dL/g) were screened in the rat estrus suppression model and gave efficacy profiles with degrees of partial or complete discontinuity at an intermediate point of the release cycle (Fig. 4). The data are consistent with the release of compound by homogeneous polymer erosion. A 69:31 copolymer of relatively high intrinsic viscosity gives a clearly triphasic compound release over 90 d (Fig. 4a). It is postulated that the primary phase of compound release is by dissolution from the superficial regions of the microspheres. A secondary, latent period of some 25 d then follows, during which time <0.4 μg/d is released. The copolymer hydrates and hydrolyzes *in situ* and decreases in molecular weight, although it remains water insoluble. There are no grossly observable changes and no release of the compound. The duration of this latent phase is controlled by both the initial molecular weight and by the composition of the polymer, which determines hydrophilicity and the inherent hydrolysis rate of the polymer *in vivo*. When the molecular weight decreases to a point at which there is significant water solubility, the polymer begins to erode homogeneously, and both the compound and polymer degradation products

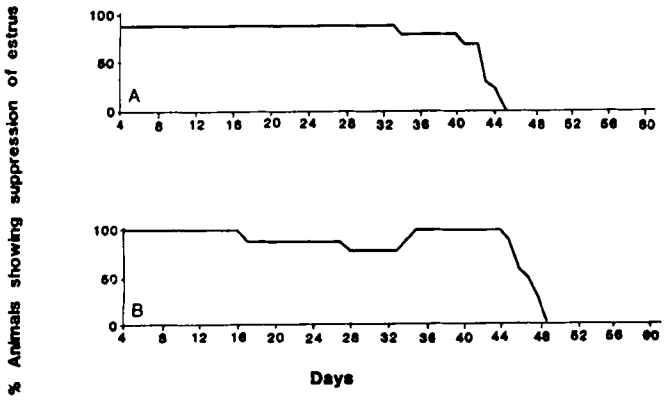

Figure 7—*Effect of size of 55:45 copolymer microspheres on duration of efficacy in female rats; n = 10. Key (A) large, 80–150 μg and (B) small, 30–50 μg.*

diffuse away from the loosening polymer matrix. This phase is diffusion controlled, although an increase in the permeability of the polymer as it erodes tends to compensate for an anticipated decrease in compound release with time. The system providing the plasma profile (Fig. 4a) demonstrates a clear definition of these three phases. When the composition is adjusted to 50:50 mol% *d,l*-lactide-co-glycolide (Fig. 4b) there is partial overlap of the primary and tertiary phases, and when the intrinsic viscosity is then decreased (Fig. 1), there is complete overlap. However, even in the system providing continuous efficacy in the rat (Fig. 1), the data clearly indicate a triphasic compound release, as shown by the release profile (Fig. 2) and by nonlinearity in the plasma profiles (Fig. 3a).

This postulated release mechanism was further supported by a study of the effects of gamma-irradiation on the intrinsic viscosity of a 69:31 copolymer (Fig. 5) and of the performance *in vivo* of the microspheres made from that polymer (Fig. 6). There was a decrease in the intrinsic viscosity of the polymer with an increase in radiation dose and, as would be predicted, a concomitant shortening of the secondary phase and a decrease in the time of onset of the tertiary phase.

The size of the microspheres was shown to have an insignificant influence on the duration of action *in vivo* (Fig. 7). This confirms the homogeneous or bulk nature of hydrolysis of this polymer and indicates that hydration is not a rate-limiting step in polymer degradation.

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