Polyanhydride Microspheres as Drug Carriers. II. Microencapsulation by Solvent Removal

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Synopsis

A new method to prepare polyanhydride microspheres, namely via solvent removal, is presented. Polyanhydrides composed of the following diacids were used: sebacic acid (SA), *bis*(*p*-carboxy-phenoxy) propane (CPP), and dodecanedioic acid (DD). Polymers were characterized by infrared (IR) spectroscopy, X-ray diffraction, viscosity, differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). Drug release was affected by polymer composition, physical properties of the microspheres, and type of drug. The potential for injectable microspheres (size range 1–300 μm) made of copolymer (CPP-SA 50:50), as biodegradable polymer carriers for the controlled release of insulin in treating diabetes mellitus, was assessed. Both 5% and 10% w/w insulin-loaded microspheres were prepared. The 10% loaded microspheres produced the best clinical response, demonstrating five days of urine glucose control and four days of serum glucose control in diabetic rats.

INTRODUCTION

This paper describes a new method for the preparation of bioerodible polyanhydride microspheres. The method is a modification of organic phase precipitation,[1] but it offers a significant advantage: the preparation occurs at room temperature and totally in organic solvents. This latter advantage is particularly important for hydrolytically labile polymers such as polyanhydrides. In previous studies with polyanhydride microspheres, we employed a hot-melt technique to incorporate molecules into the polymers.[2] While that approach has certain advantages,[2] many drugs lose biological activity at high temperature. Therefore, the hot-melt technique can only be used with low melting point polymers.

This fact encouraged us to examine less drastic methods, such as solvent evaporation,[3,4] which has been used extensively in the preparation of bioerodible polymer microspheres.[5–10] However, the presence of an aqueous phase used in this method initiates polymer hydrolysis. It occurred to us that microencapsulation by solvent removal might provide a new approach for capsule formation. In this method, the drug is dispersed or dissolved in a polymer solution of a volatile organic solvent. This mixture is suspended in an organic oil; the organic solvent is extracted into the oil, creating microspheres.

This new method permits the preparation of microspheres from polymers with high melting points. We have now developed polyanhydride microspheres for several new polymers, including ones more hydrophobic than previously used.[2] The following polymers were studied: copolymers *bis* 1,3-(carboxy-

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phenoxy) propane (CPP) copolymerized with sebacic acid (SA) or dodecanedioic acid (DD) with molar ratios of 20 : 80 and 50 : 50.

All the polymers, and the resulting microspheres, were characterized. Polymer molecules were examined by infrared (IR) spectroscopy, X-ray diffraction, differential scanning calorimetry (DSC), and viscometry. The morphology was examined by scanning electron microscopy (SEM). These physical properties were correlated with microsphere performance.

We report here methods developed for microencapsulation and characterization, as well as the results of *in vitro* and *in vivo* release studies using acid orange and insulin.

EXPERIMENTAL

Materials

Sebacic acid and dodecanedioic acid were recrystallized three times from ethanol. *Bis* (*p*-carboxy-phenoxy) propane was synthesized according to the method of Conix[11] and purified by extraction with acetone and ether before use. All solvents were analytical grade. Solid particles of acid orange (Aldrich) and zinc insulin (Eli Lilly), were sieved to a size lower than 50 μm (using U.S. Standard Sieve Series, Newark, Wire Cloth Co., Newark, NJ).

Instrumentation

Thermal analysis of the polymer was determined on a Perkin-Elmer DSC-2 differential scanning calorimeter employing a heating rate of 20°C/min. The molecular weights of the polymers were determined on a Perkin-Elmer GPC system consisting of the series 10 pump and the 3600 Data Station with the LKB 214 rapid spectral detector at 254 nm wavelength. Samples were eluted in chloroform through two PL Gel columns (Polymer Laboratories, 100 Å and 1000 Å pore sizes) in series at a flow rate of 1.5 mL/min. Polystyrene (Polyscience) was used as the calibration standard. Viscosity of polymers was measured in an Ubbelohde Viscometer (Cannon 75) at 23°C using 1.0, 0.5, and 0.25% (w/v) polymer in chloroform solution. Wide-angle X-ray diffraction of polymers in the form of pressed discs (1 mm thick) was recorded on a Phillips X-ray diffractometer using a nickel-filtered CuK source.

Infrared spectroscopy was performed on a Perkin-Elmer Spectrophotometer Model 1430. Polymer samples were film cast onto NaCl plates from solutions of polymer in chloroform.

Polymer Synthesis

Polyanhydrides were synthesized by melt polycondensation.[12] Briefly, recrystallized dicarboxylic acid monomers were converted to the mixed anhydride by reflux in acetic anhydride. Aromatic prepolymers were isolated by crystallization from the concentrated acetic anhydride solution, and purified with dry diethyl ether. Aliphatic prepolymers were recrystallized from toluene and washed with petroleum : diethyl ether (1 : 1 v/v mixture). The prepolymers were then polymerized at 180°C under high vacuum. In a typical polymerization procedure, CPP prepolymer (2.0 g, 5 mmol) was mixed with

sebacic acid prepolymer (1.15 g, 5 mmol) in a glass tube 2 × 20 cm (Kimax) with a side arm equipped with a capillary nitrogen inlet. The tube was immersed in an oil bath at 180°C. After the prepolymers were melted, high vacuum was applied (4–10 μm). The condensation product was collected in an acetone/dry ice trap. The crude polymer was purified by precipitation in dry petroleum ether from methylene chloride solution.

Determination of Polymer Composition

The composition of the copolymer was determined by degrading a weighed sample of copolymer in 1*M* NaOH at 70°C for 24 h. The absorption due to the aromatic diacid (CPP) was measured spectrophotometrically. Extinction coefficients of 24 ± 2 and 50 ± 2 mL/(g · cm) were obtained for the 20 : 80 and 50 : 50 copolymers, respectively.

Solvent Casting

Films of CPP-SA 50 : 50 copolymers for the *in vivo* insulin studies were prepared as follows: solutions of polymer (20% w/v) in methylene chloride and 10% insulin (weight of insulin/weight of polymer) were cast on glass Petri dishes. The dishes were placed on dry ice or stored at −20°C for solvent evaporation. Films 0.8 mm thick were obtained. The films were cut to 1 cm diameter disks.

Calculation of Degree of Crystallinity

The relative degree of crystallinity of the copolymers was calculated from the crystallinity of the homopolymers. The fraction of crystallites estimated from X-ray diffraction is 53% for PCPP, 57% for PSA, and 56% for PDD. The crystallinity was determined by the following equation

$$X_c' = \frac{A_c}{A_a + A_c} \tag{1}$$

were X'_c is the % crystallinity of the homopolymers, A_a is the area under the amorphous hump, and A_c is the area remaining under the crystalline peaks.[13,14] For each copolymer, the degree of crystallinity is defined as:

$$X_c = \frac{\Delta H_{\text{obs}}}{W_a \Delta H_{a,\text{pure}} + W_b \Delta H_{b,\text{pure}}} \tag{2}$$

where X_c is the % crystallinity of the copolymers, ΔH_{obs} is the heat of fusion in cal/g for each copolymer, W_a and W_b are the mole fraction of the monomers in each copolymer and

$$\Delta H_{a,\text{pure}} = \frac{\Delta H_{a,\text{obs}}}{X'_{a,c}} \tag{3}$$

where $X'_{a,c}$ was determined for each homopolymer from Eq. (1). ΔH_{pure} is 52.9, 51.9, and 43.6 cal/g for PSA, PDD, and PCPP, respectively.

In Vitro Degradation Studies

Drug-incorporated microspheres were sieved into different size ranges. Release experiments were conducted in 10–50 mL of phosphate buffer (0.1*N*, pH 7.4) at 37°C in small columns (Bio-Rad polypropylene Economo-column, 10 and 50 mL). Gentamicin Sulphate (Sigma), was added (at a concentration of 0.05 mg/mL) as an antibacterial agent. The buffer solution was changed every hour for the first 24 h, then three times a day for the following days. Toward the end of the experiments, the solvent was changed once every 2–3 days. Each experiment was performed in duplicate. Differences less than 3% were observed. The polymer erosion and drug-release kinetics were followed by measuring the ultraviolet (UV) absorption of the periodically changed buffer solution using a Perkin-Elmer UV/VIS spectrophotometer. Degradation products were measured at 247 nm and acid orange was measured at 490 nm. When drugs showed absorption in both the UV and visible regions, extinction coefficients were measured at appropriate wavelengths and the contribution to the UV absorption was then calculated.

An *in vitro* Bio-Rad assay that measures protein was employed to assess insulin release kinetics. Because the Bio-Rad reagent reacts with very high concentrations of the polymer degradation products, care was taken to dilute the eluant to a point where reaction of breakdown products with the Bio-Rad reagent was negligible. However, to further correct the Bio-Rad assay procedure, a standard curve for the reaction of polymer products with the Bio-Rad reagent was constructed. With the extinction coefficient obtained from this curve, a calculated optical density (OD) was subtracted to account for the presence of breakdown products.

Microsphere Preparation

Several variations of the solvent removal technique were examined. For the PCPP-SA 20-80, MW = 16,000, microspheres were prepared as follows: 1 g polymer was dissolved in 1 mL methylene chloride, Drug or dye was suspended in the solution, mixed, dropped into silicon oil (Dow Corning) containing 1 to 5% of Span 85, and stirred at a known stirring rate. Stirring was done using an overhead stirrer type RZR50, "CAFRAMO," Wiarton Ont. and a three-blade impeller. After 1 h, petroleum ether was introduced and stirring was continued for another hour. The microspheres were isolated by filtration, washed with petroleum ether, dried overnight in a lyophilizer (Labconco, Freeze Dryer 8), sieved (using U.S Standard Sieve Series, Newark, Wire Cloth Co., Newark, NJ), and stored in a freezer.

When trying to apply the same method to PCPP-SA polymers of higher molecular weight or with higher percentages of CPP, the above process results in rod formation rather than microspheres. In this case a different method was used: 2 g polymer were dissolved in 10 mL of methylene chloride, drug was added and the mixture was suspended in silicon oil containing Span 85 and also a known amount of methylene chloride. The amount of methylene chloride depended on the type and molecular weight of the polymer used (e.g., for PCPP-SA 20-80 with a molecular weight of 30,000–40,000, the ratio between the silicon oil and the methylene chloride was 4 : 1 and for PCPP-SA

50 : 50 with MW 40,000 this ratio was 1 : 1). After dropping the polymer solution into the silicon oil, petroleum ether was added and the stirring continued for 2 h. The microspheres were isolated by filtration, washed with petroleum ether, dried overnight in a lyophilizer, and stored in a freezer. All drugs used were sieved to sizes lower than 50μm. Almost no precipitation on the stirrer was observed. The process is reproducible to within 5% with respect to yield and size distribution, if polymers of the same molecular weight were used.

Drug loading for the dye was measured by performing a release study and calculating the cumulative drug that was released throughout the experiment. For measuring insulin loading, the microspheres were dissolved in methylene chloride and the insulin was extracted by addition of water to the organic phase; the eluents were passed through a Perkin-Elmer high performance liquid chromatographic (HPLC) system containing a reversed-phase Vydac peptide/protein, C_{18} column (Rainin) to identify the insulin. A segmental gradient of acetonitrile/water with 0.3% trifluoroacetic acid (all EM Science), progressing from 20% to 80% acetonitrile was used. Insulin was detected at 220 nm. Molecular weights of the polymer of the microspheres, loaded with insulin, were measured immediately after preparation and after 1/2 year storage at $-20°C$ and indicated no significant change.

Surface Morphology of Microspheres

The surface morphology of microspheres was studied, after preparation and after degradation, via stereomicroscopy (Makroskop Wild M420) and scanning electron microscopy (SEM) (ISI Model DS-130). Cross-sections of samples were obtained by embedding the microspheres in mounting media (Histo Prep SO-H-75 Frozen Tissue Embedding Media, Fisher Scientific) and cutting 10 μm sections at $\leq 20°C$ with a microtome (International Equipment Company). Samples for SEM were dried, mounted on metal stubs, and sputter-coated with gold-palladium (Polaron Instrument E5100).

Insulin Release In Vivo

Biological activity of the released insulin was determined *in vivo* by monitoring daily blood and urine glucose levels in polymer-implanted diabetic female Sprague-Dawley rats. Diabetes was previously induced with a tail vein injection of 65 mg/kg of streptozotocin (Upjohn) in pH 4.5, 0.1*M* citrate buffer, the optimal dose for inducing diabetes in these rats.[15] An induction period of 7–10 days after streptozotocin injection was necessary to appreciate the clinical onset of diabetes mellitus. When serum glucose levels reached a minimum of 400 mg/dL, the rats were considered diabetic and were treated with the polyanhydride-insulin microspheres. Daily urine glucose outputs were estimated with Chemstrips UG (Bio-Dynamics). Blood was drawn daily from the tail vein and serum glucose levels were read on a YSI Model 23A glucose analyzer.

During the initial phases of the experiment, microspheres were suspended in physiologic saline and then injected subcutaneously through a standard 18 gauge needle. It became apparent that the partially hydrophobic nature of the microspheres did not allow for adequate suspension in an aqueous solution. Oil

would be better media for suspending the microspheres, but at this stage we did not want to introduce other factors that could influence release rates. Microspheres were therefore implanted through a small (1 cm) surgical incision through the skin of the dorsum of the rat. Microspheres were placed subcutaneously and the skin was closed with 5-0 Ethilon monofilament nylon sutures (Ethicon, Inc.). Sterile technique was employed during all phases of the surgical procedure. Rats were housed in metabolic cages and fed *ad libidum*. Both healthy and diabetic control rats were housed concurrently with the treated rats.

RESULTS AND DISCUSSION

Polymer Characterization

The physical properties of the polymers used in this study are summarized in Table I. These polymers were chosen for three reasons: (1) to investigate the effect of CPP content on microsphere release rates; (2) to investigate the effect of long aliphatic monomers, such as dodecanedioic acid, on microsphere release rates, and; (3) to investigate the effect of changing polymer properties (i.e., viscosity on microsphere performance). Polymers with approximately the same molecular weight were used in order to minimize the number of experimental variables. As seen in Table I, viscosity increased with the fraction of CPP.

Table II summarizes the heat of fusion and IR absorption for the polymers studied. The IR spectra of P(SA) and P(DD) polymers show typical peaks at 1810 and 1740 cm^{-1}. The aromatic P(CPP) polymer absorbs at 1770 and 1710 cm^{-1}. The copolymers P(CPP-SA) and P(CPP-DD) 20 : 80 polymers absorb at 1800 and 1740 cm^{-1}, indicating a low percentage of pure aromatic anhydride bonds. In the P(CPP-SA) and P(CPP-DD) 50 : 50 polymers, three absorption maxima appear at 1800, 1770, and 1730 cm^{-1} which are typical for aliphatic and aromatic polyanhydrides. These results suggest that random copolymers were formed. The heat of fusion for the PCPP, PSA, and PDD homopolymers is high: 23, 30, 29 cal/g, respectively. The heats of fusion for the P(CPP-SA) 20 : 80 and P(CPP-DDP) 20 : 80 polymers are 14.2 and 21 cal/g, respectively.

TABLE I
Physical Properties of Polyanhydrides

Polymer	Molecular weight[a]		Viscosity[b]	Melting point
	Mw	Mn	$[\eta]$ (dL/g)	(°C)
P(CPP-SA) 20 : 80	16200	6300	0.10	72–74
P(CPP-SA) 20 : 80	35000	9200	0.32	72–74
P(CPP-SA) 50 : 50	37200	12900	0.62	138–142
P(CPP-DD) 20 : 80	36500	12100	—	74–76
P(CPP-DD) 20 : 80	28500	10800	0.35	74–76
P(CPP-DD) 50 : 50	37600	11600	—	180–182
P(CPP-DD) 50 : 50	31400	10400	0.42	180–182

[a] Determined by GPC analysis.
[b] Measured at 23°C in chloroform.

TABLE II
Heat of Fusion, Degree of Crystallinity, and IR Characteristics of Polyanhydrides

Polymer	ΔH (cal/g)[d]	% crystallinity	IR[a] (cm^{-1})		
P(SA)	30.20	57.0[b]	1800		1740
P(DD)	29.30	56.0[b]	1810		1740
P(CPP-SA) 20-80	14.20	27.8[c]	1800		1740
P(CPP-SA) 50-50	2.35	4.8[c]	1800	1770	1730
P(CPP-DD) 20-80	21.00	41.7[c]	1810		1740
P(CPP-DD) 50-50	2.70	5.7[c]	1800	1770	1730
P(CPP)	23.00	53.0[b]		1770	1710

[a] Characteristic for anhydride bonds.
[b] Calculated with Eq. (1).
[c] Calculated with Eq. (2).
[d] Determined by DSC.

For the P(CPP-SA) and P(CPP-DD) 50 : 50, the heats of fusion decreased to 2.3 and 2.7 cal/g, respectively. If we assume that the heat of fusion describes only the change from crystalline to amorphous polymer, there is a large decrease in crystallinity when the amount of the CPP in the copolymer increases.

When two monomers—one forming a crystalline homopolymer and another forming an amorphous homopolymer—are copolymerized, the degree of copolymer crystallinity decreases as the second constituent is added to either homopolymer.[16] For the polymers discussed here, both homopolymers are partially crystalline. For each of the homopolymers used (PDD, PSA, PCPP) and copolymers, the relative degree of crystallinity is given in Table II. The degree of crystallinity may play an important role in preventing water diffusion into the polymer bulk and thus preventing bulk erosion. Another important property in preventing bulk erosion is hydrophobicity. In this case the less hydrophobic polymer (20 : 80) has the higher crystallinity, probably due to the crystalline regions of the PSA units. The more hydrophobic polymer (50 : 50), is less crystalline.

Microsphere Preparation

Successful microsphere preparation by solvent removal depends on two factors: the rate of precipitation of the polymer and the rate of methylene chloride diffusion into the silicon oil.

For *low molecular weight* polymers it was possible to obtain a stable suspension for a longer time (without addition of a surface active agent). Since the viscosity of a 27% polymer solution was low, the polymeric solution was efficiently dispersed before precipitation occurred. The removal of methylene chloride was slow, lasting for 2 hours and without any aggregation of microspheres. Petroleum ether was added to accelerate the precipitation. Capsules were sampled at various times during preparation and sectioned. During the first 2 hours, the sphere interior contained a viscous solution. This solution disappeared as the extraction process continued.

The resulting microspheres were large (Fig. 1), but the yield was only 40%. The microspheres were obtained as a free-flowing powder; cross-sections

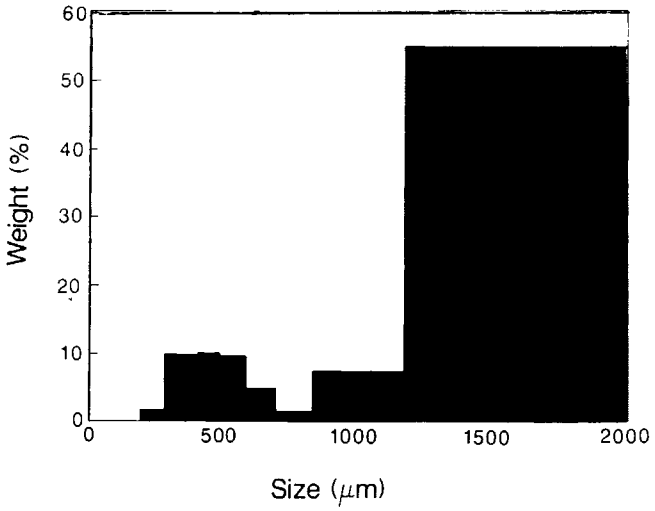


Fig. 1. Size distribution of PCPP-SA 20 : 80 microspheres, (MW = 16,000, stirring rate was 300 rpm).

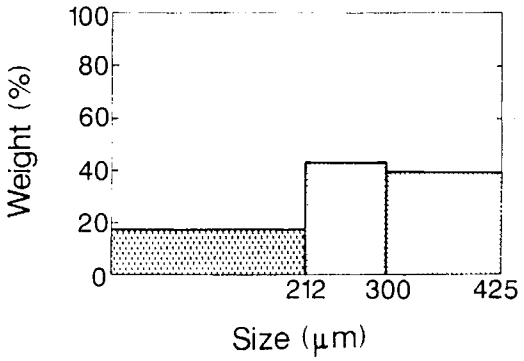
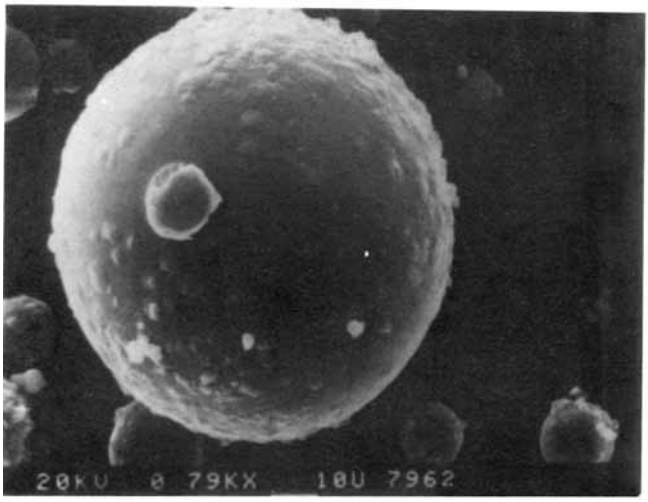


Fig. 2. Size distribution of PCPP-SA 50 : 50 microspheres, (MW = 37000, stirring rate 500 rpm).

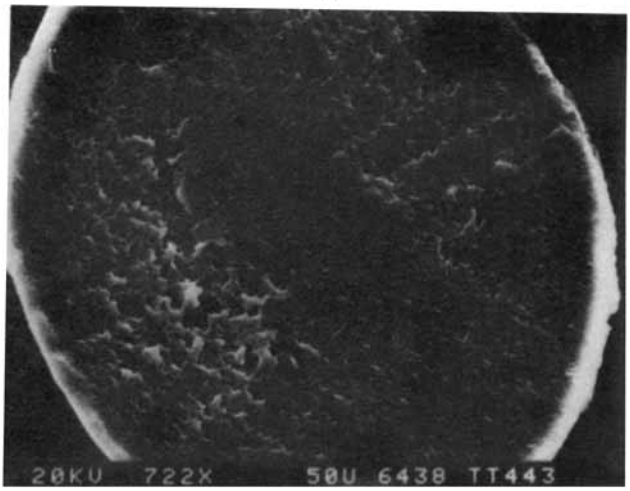
revealed a dense structure with some empty areas due to solvent removal.

For *higher molecular weight* polymers or polymers with a large fraction of CPP, several modifications of the procedure were required: (1) a surface-active agent was required; (2) methylene chloride was added to the oil in order to slow diffusion of solvent from the polymer phase, at least, for the first stage when the suspension is formed; and (3) petroleum ether was added immediately upon getting the suspension of the polymer solution in the oil. Microspheres obtained via this method were smaller (10–425 μm) (Fig. 2), and were obtained as a free-flowing powder with yields greater than 85%.

In all cases, the microspheres' external surface was smooth, with no pores visible to SEM examination [Fig. 3(a)]. SEM examinations [Fig. 3(a)] indicated a rather smooth external face with no visible pores but cross-sections of the P(CPP-SA) 50 : 50 reveal a porous structure with increased density near the external surface (Fig. 3b). P(CPP-DD) 50 : 50 microspheres had a less



(a)

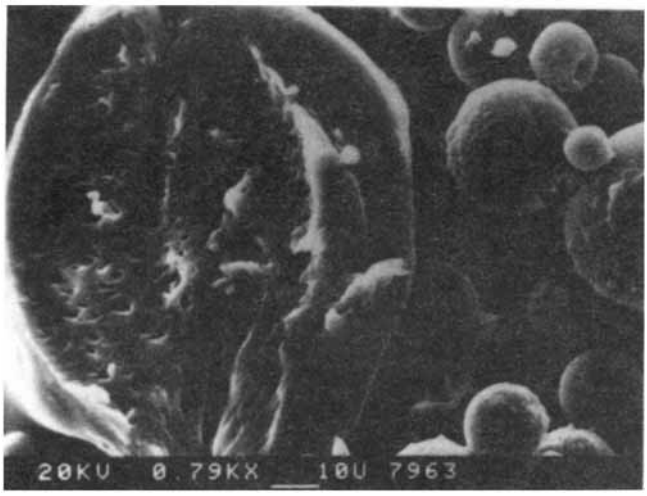


(b)

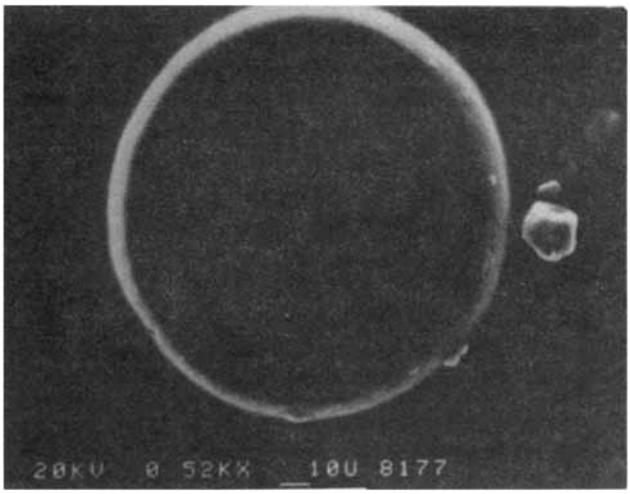
Fig. 3. SEM of polyanhydride microspheres: (a) before degradation, external face; (b) cross-section of PCPP-SA 50 : 50 microspheres; (c) cross-section of PCPP-DD 50 : 50 microspheres; (d) cross section of PCPP-DD 20 : 80 microspheres; (e) after 12 days of degradation in buffer solution.

porous internal face [Fig. 3(c)]. The P(CPP-SA) 20 : 80 and P(CPP-DD) 20 : 80 copolymers both possessed slightly porous cross-sections [Fig. 3(d)]. After degradation, the microspheres lost their integrity, occasionally leaving an empty shell [(Fig. 3(e)]. This occurred both *in vivo* and *in vitro*.

To account for the nature of the microspheres formed, we propose the following explanation: the process for low molecular weight polymers took place slowly and resulted in relatively nonporous microspheres. In contrast, the process for high molecular weight polymers was rapid and resulted in spheres with significant internal porosity. The process of microencapsulation is presumably diffusion-controlled, at least for the first stages where the



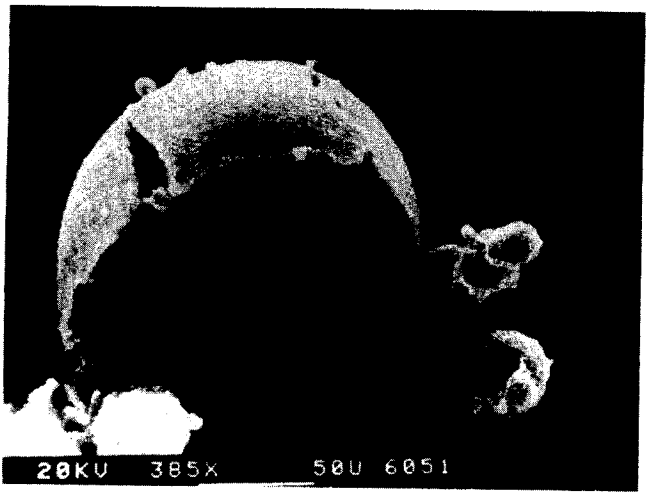
(c)



(d)

Fig. 3. (*Continued from the previous page.*)

difference in concentration of methylene chloride between the two phases is significant. The process can be controlled by increasing the amount of methylene chloride in the oil; this was verified with the high molecular weight polymers where increasing the concentration of the methylene chloride improved the microencapsulation process by yielding microspheres rather than rods. After precipitation begins, the process is more complicated, involving diffusion both in solution and in the already precipitated polymer. The first precipitation occurs in the external area and this layer slows subsequent diffusion of methylene chloride, The removal of the methylene chloride was



(e)

Fig. 3. (*Continued from the previous page.*)

accelerated by addition of petroleum ether, but it was always required to evaporate the traces of methylene chloride entrapped in the microspheres.

Insulin Loading

When insulin-containing microspheres were dissolved in methylene chloride and the insulin was extracted with water, 95% of the insulin was recovered from microspheres for the two loadings that were used (5% and 10% insulin). No change in retention time, as judged by HPLC, was observed for the insulin extracted from the microspheres as compared to free insulin (12.22 min). While other analytical techniques should be employed, this result suggests that no significant reaction between insulin and the polymer occurred.

In Vitro Release Study

A characteristic release curve for P(CPP-SA) 20 : 80 microspheres loaded with 2% acid orange is shown in Figure 4. For convenience, each curve was characterized by a half-time, $T_{1/2}$: the time for 50% of the total substance to the released or degraded. When comparing two different release curves, a smaller $T_{1/2}$ indicates a faster release. Table III summarizes $T_{1/2}$, for acid orange released from different size microspheres. A low molecular weight polymer (MW = 16,000) and the first microencapsulation method were employed. Although the polymer degradation lags behind drug release, the correlation between drug release and polymer erosion is clear.

In Figure 5, release from microspheres prepared with higher molecular weight polymers (35,000–40,000) and a 2% loading of acid orange is shown. Release rate and polymer degradation from the P(CPP-SA) 20 : 80 microspheres was slightly faster than the release and degradation of the P(CPP-SA) 50 : 50 microspheres. The small difference in rate can be partially attributed to the more porous structure of the P(CPP-SA) 50 : 50 microspheres. With the P(CPP-SA) 50 : 50 polymer, there was an initially fast degradation followed by a slower release around 50% degradation (Fig. 6). Figure 7

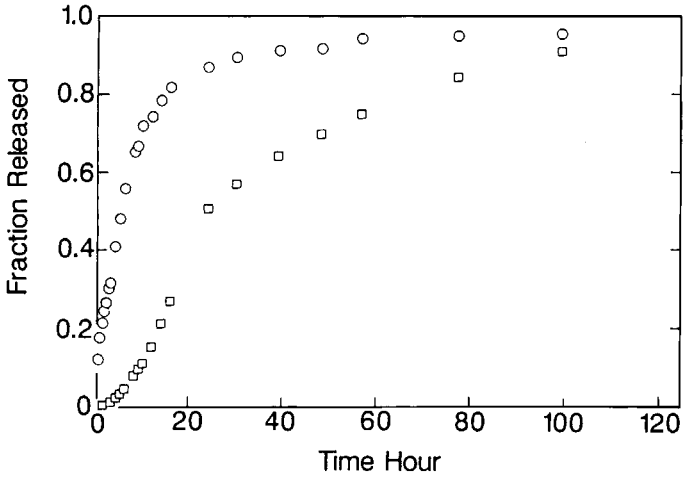


Fig. 4. Release profile of acid orange (2% loading) from PCPP-SA 20:80 microspheres, size 400–500 μm, MW = 16000.

TABLE III
Comparison Between $T_{1/2}$ of Acid Orange and Degradation Product (Polymer Weight Loss), for Different Sizes of Microspheres

Microsphere size (μm)	$T_{1/2}$ (h) acid orange	$T_{1/2}$ (h) polymer
> 1180	33	80
850–1180	20	52
710–850	16	42
600–710	10	40
500–600	5	22
400–500	4	22
300–400	4	20

compares the release from P(CPP-DD) 20:80 and 50:50 copolymers. Again, the release from the P(CPP-DD) 20:80 polymer was faster than for the P(CPP-DD) 50:50 polymer. This time the change was more pronounced, presumably due to the less porous structure of both microspheres.

Release curves for P(CPP-SA) 50:50 and P(CPP-DD) 50:50 polymers are shown in Figure 8; release from the P(CPP-DD) 50:50 is slower. This may be due both to the more hydrophobic nature of the DD monomer and the less porous structure found by SEM [Fig. 3(c)].

Table IV summarizes some of the properties of those polymers and their corresponding microspheres. The P(CPP-SA) 20:80 and the P(CPP-DD) 20:80 are fast degrading polymers due to the rapid hydrolysis of the aliphatic polyanhydride bond.[2,13] They are quite crystalline (Tables II and IV), which may prevent water uptake by the polymer. Therefore, they show a better correlation between polymer degradation and drug release. The second class of polymers was more hydrophobic due to their higher aromatic content: P(CPP-SA) and P(CPP-DD) 50:50. The hydrolysis of these polymers was slower but

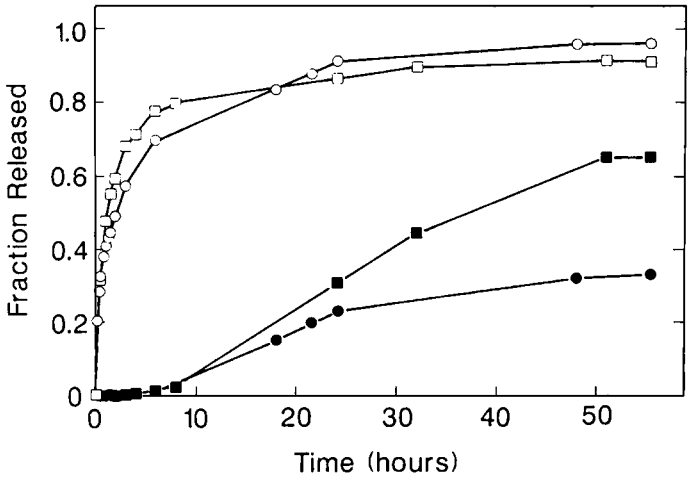


Fig. 5. Acid orange (□) released from (■) PCPP-SA 20 : 80 and acid orange (○) released from (●) PCPP-SA 50 : 50 microspheres, size 300–400 μm.

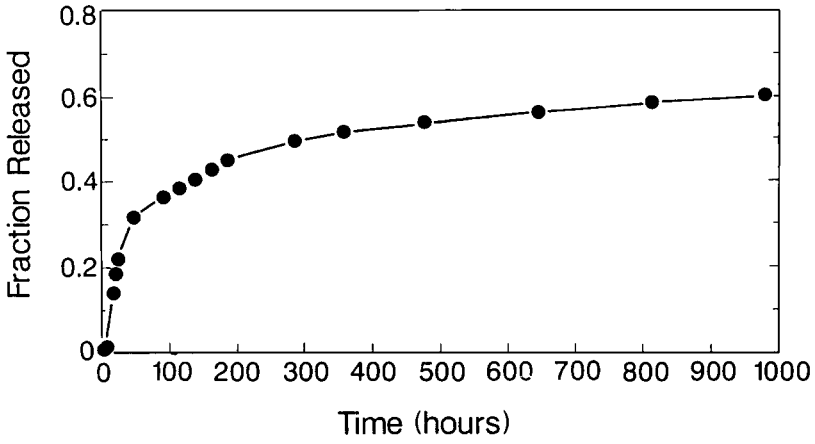


Fig. 6. Release profile of blank PCPP-SA microspheres, size 300–400 μm.

at the same time they were less crystalline. The fact that the structure of the microspheres in this preparation is more porous may contribute to the lack of correlation between polymer and drug released. The situation is complicated since some properties work in opposite directions. For example, for P(CPP-SA) 50 : 50 high hydrophobicity can prevent water uptake by the polymer, on the other hand, low crystallinity and high porosity will accelerate diffusion of solute in the microspheres and result in a poorer correlation between drug release and polymer erosion. When the porosity was lower, as in the P(CPP-DD) polymer, the correlation of drug release and polymer erosion improved.

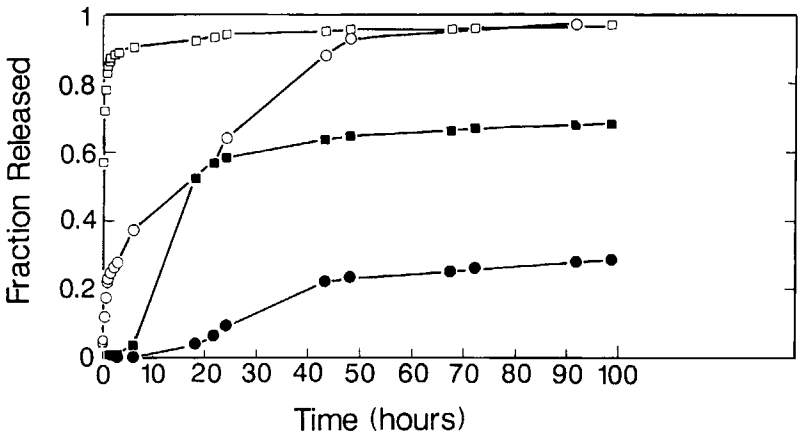


Fig. 7. Acid orange (□) (2% loading) released from (■) PCPP-SA 20:80 and acid orange (○) from (●) PCPP-SA 50:50 microspheres (300–400 μm).

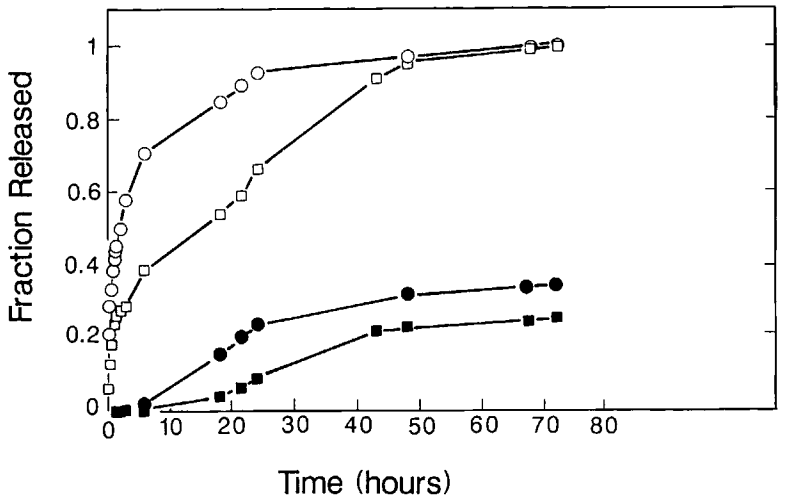


Fig. 8. Acid orange (□) (2% loading) from (■) PCPP-DD 50:50 and acid orange (○) from (●) PCPP-SA 50:50 microspheres (300–400 μm).

TABLE IV
Some Physical Properties of Polyanhydrides

Polymer	Porosity[b]	Crystallinity	Release[a] rate $T_{1/2}$ (h)	Polymer[a] degradation rate $T_{1/2}$ (h)
P(CPP-SA) 20:80 Low MW	−	27.80	4.00	22
P(CPP-SA) 20:80 High MW	±	21.21	1.20	35
P(CPP-SA) 50:50	+	4.80	2.00	> 100
P(CPP-DD) 20:80	±	41.70	0.15	15
P(CPP-DD) 50:50	−	5.70	16.00	> 100

[a] For microspheres loaded with 2% acid orange, size range 200–300 μm.
[b] − = Nonporous [Fig. 3(c)]; + = Very porous [Fig. 3(b)]; ± = Moderately porous [Fig. 3(d)].

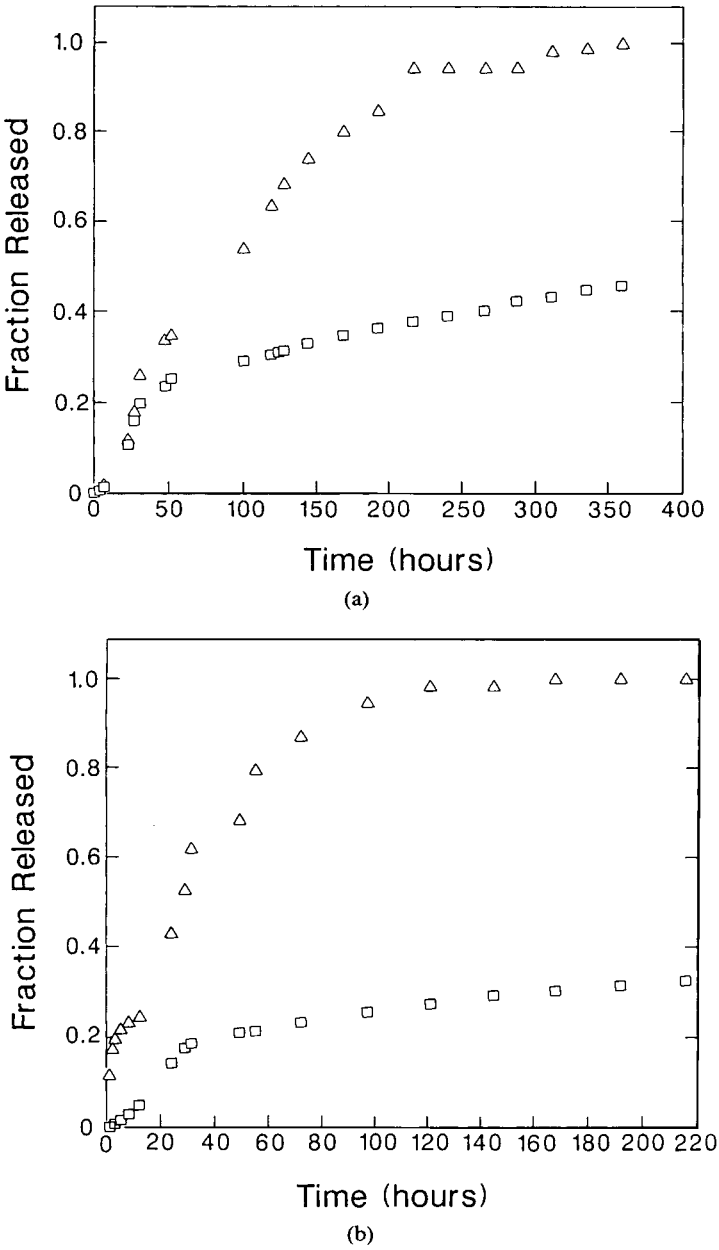


Fig. 9. Insulin (△) released from PCPP-SA 50:50 (□) microspheres: (a) 5% loading; (b) 10% loading.

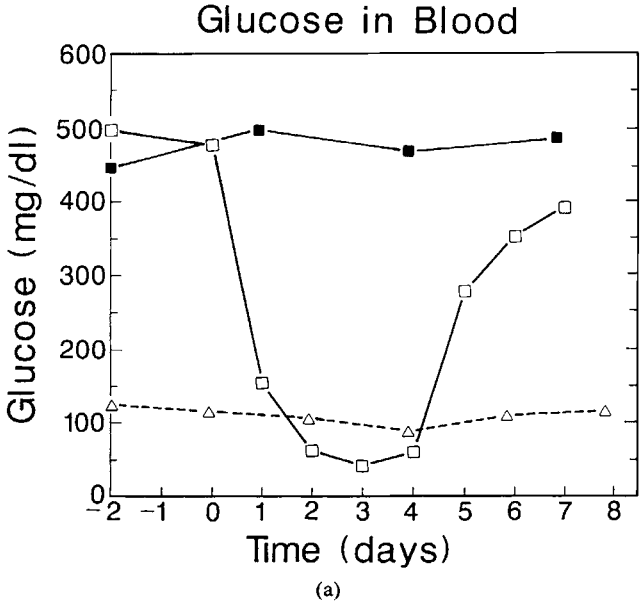
To determine the behavior of a biologically active macromolecule in these microspheres, insulin was chosen as a model substance. *In vitro* insulin release kinetics are shown in Figure 9, for P(CPP-SA) 50:50 microspheres. The microspheres were prepared using the second method and the microsphere size ranged between 50 to 425 μm. The insulin release rate was considerably faster than polymer degradation rates for both 5% and 10% loaded microspheres.

Note that with the 5% loaded microspheres, 59% of the incorporated insulin was released at 90 h, whereas only 28 h were required to release 50% of the insulin from the 10% loaded microspheres. Release of a high molecular weight drug, like insulin, proceeded slowly compared to acid orange. This suggests that diffusion of solute in the microsphere may be important in determining the rate of release.

In Vivo Insulin Study

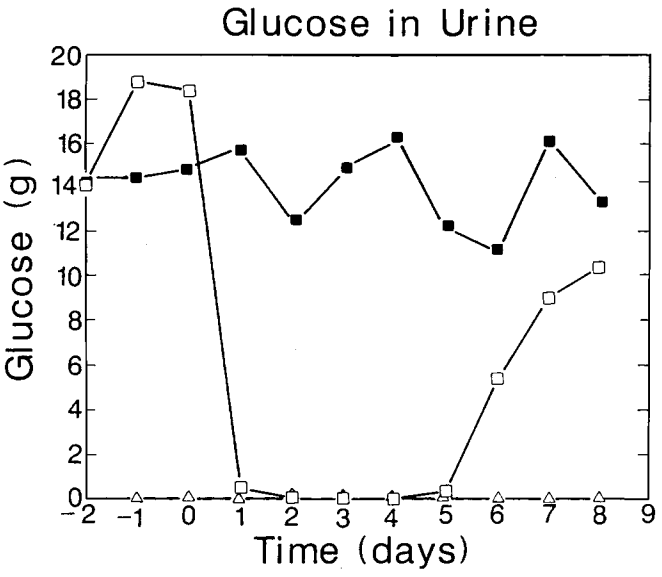
An *in vivo* study with 200 mg of 10% insulin-loaded microspheres demonstrated 5 days of near-zero urine glucose levels and 4 days of serum glucose control. Rats were implanted with microspheres on day 0 (5 rats from each group were used) (see Fig. 10). A 200 mg dose of 5% loaded microspheres gave a 3 day effective decrease in urine glucose and only 1 day of normoglycemia. In a parallel experiment, 100 mg of 10% loaded microspheres showed a 3 day effective lowering of urine glucose and 2 days of serum glucose control. For comparison, urine and blood glucose levels for both diabetic control and healthy control rats are documented. Glucose was never found in the urine of healthy rats (Fig. 10). To compare the microspheres to slabs, a 200 mg polymer film (0.8 mm thick) loaded with 10% insulin was also implanted in the same fashion in diabetic rats. The films gave a 1 day delay in both urine and serum glucose responses. Although urine glucose control was maintained for at least 7 days, only 4 days of near-zero blood glucose control was achieved (Fig. 11).

There is a good correlation between the *in vivo* and *in vitro* study of 10% microspheres. With 10% loaded microspheres, it was possible to obtain a



(a)

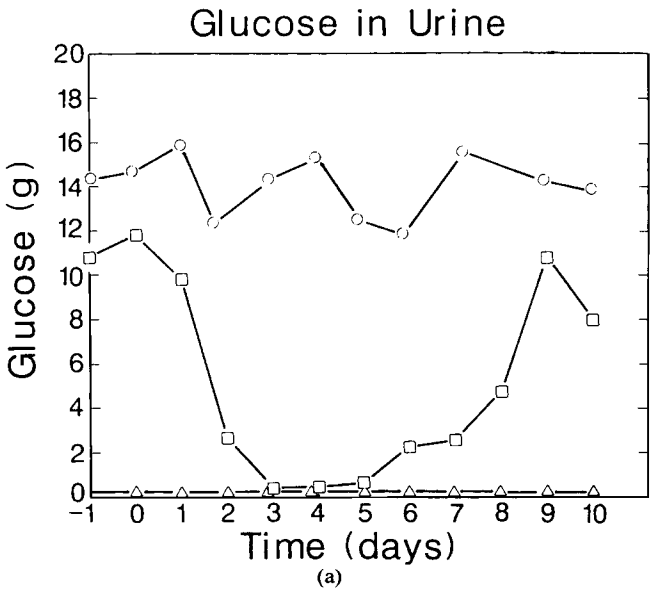
Fig. 10. Blood (a) and urine (b) glucose levels of diabetic rats implanted with polyanhydride microspheres on day 0, size of microspheres was 50–300 μm. (□) Treated; (■) Diabetic; (△) normal.



(b)

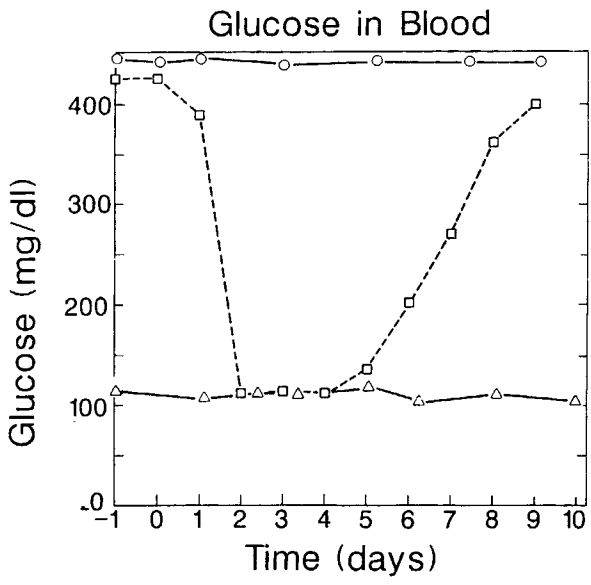
Fig. 10. (*Continued from the previous page.*)

decrease in urine glucose levels to near-zero levels for 5 days, and near normal serum glucose levels for 4 days. The *in vitro* results predicted 5–6 days of release. 5% insulin-loaded microspheres showed only a 3 day response in urine and 1 day of normoglycemia. This attests to the effect not only of the absolute amount of insulin that is available for release, but also to the effect of drug loading on the physical structure of the microspheres. Previous work has



(a)

Fig. 11. Blood (a) and urine (b) glucose levels of diabetic rats implanted with polyanhydride slabs. (○) Diabetic; (△) Healthy; (□) Treated.



(b)

Fig. 11. (*Continued from the previous page.*)

shown that higher drug loading levels lead to an increased porosity of the polymer matrix, which in turn facilitates movement of water into, and insulin molecules out of, the system.[17]

General Comments

The advantage of the method described is that the preparation, carried out in organic solvents, prevents the hydrolytic degradation of the polymer and dissolution of any water-soluble drug.

The microspheres were spherical in shape. The outer surface had a dense structure but the internal structure was slightly porous. Two factors are important: (1) the rate of polymer precipitation in the organic solvent; this can be controlled by the addition of methylene chloride to the organic oil and this stage helps in stabilizing the suspension prior to the removal of the solvent, and (2) the rate of methylene chloride removal, which is faster for the higher molecular weight polymers. The appearance of porous structure inside and a dense structure outside may also be explained because precipitation occurs first in the outer surface of the sphere. Once the external surface crystallizes, the internal core still contains methylene chloride. From that stage on, no decrease in the microsphere size or any shrinking of the structure will occur.

Polymer properties are important in understanding release characteristics. In the present study we examined four types of polymers. If the rate of solute release from these microspheres were determined only by surface erosion of the polymer backbone, it would correlate with the rate of polymer degradation. As shown in Table IV, the rate of polymer degradation is not the only determinant of release. Both degradation of the polymer and diffusion of the

solute must be important. The successful application of these bioerodible microspheres requires a quantitative understanding of the physical properties which influence release rates. With that understanding, polymers can be selected and microspheres can be designed to meet a diverse group of applications.

The microspheres for insulin are limited to short time periods—on the order of one week. Longer-acting polyanhydrides, which could be synthesized by rendering them more hydrophobic and more crystalline,[18] could be used for lengthier durations of action. Nonetheless, a one-week system may be useful in decreasing injection frequency compared to the current daily regimes, as well as reducing potential problems of dose dumping or insulin aggregation that could occur in longer acting (e.g., several months) systems.

This new technique, microencapsulation by solvent removal, can be modified to produce useful microspheres from polymers with different physical properties. In designing a microsphere for a specific purpose (i.e., a given release rate) the properties of the polymer carrier must be considered at two stages: fabrication and release. During fabrication, the rate of solvent removal and the crystallinity of the polymer influence the microstructure of the microsphere. Rapid solvent removal produces greater internal porosity. High polymer crystallinity slows this solvent removal and generates a polarized internal structure (nonporous at the surface and porous in the bulk). During release, the rate of polymer degradation augments the rate of solute release by two mechanisms: (1) directly via erosion and liberation of encapsulated solute and (2) indirectly via generation of internal porosity and alteration of an internal network of water-filled channels.

We propose that the generation of a polarized microstructure during fabrication can be explained by considering the diffusion of organic solvent through a crystalline polymer network. Similarly, the rate of solute release can be modeled as diffusion through an evolving porous microstructure. Changes in the porous microstructure are produced by dissolution of the initially solid solute and by erosion of the polymer backbone.

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