Polyanhydride Microspheres that Display Near-Constant Release of Water-Soluble Model Drug Compounds

Yasuhiko Tabata[1,2] and Robert Langer[1,3]

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A new method to prepare polyanhydride microspheres capable of near-constant sustained release of low molecular weight, water-soluble molecules is presented. The polyanhydrides used were poly(fatty acid dimer) (PFAD), poly(sebacic acid) (PSA), and their copolymers [P(FAD-SA)]. Acid orange 63 (AO), acid red 8 (AR), and *p*-nitroaniline, were used as model release molecules. P(FAD-SA) microspheres containing the molecules with or without gelatin were prepared by a modified solvent evaporation method using a double emulsion. The microspheres were spherical with diameters of 50–125 μm and encapsulated more than 85% of the molecule, irrespective of the compound used. Near-zero-order degradation kinetics were observed for 5 days as judged by sebacic acid (SA) release. Microsphere degradation was pH sensitive, being enhanced at high pH, and became more stable in acidic conditions, irrespective of the incorporation of gelatin in the matrix. For the gelatin-free microspheres, a close correlation of SA release and AO release was observed (2% loading), suggesting a release mechanism that was controlled dominantly by degradation. However, the incorporation of gelatin into the microsphere significantly extended the periods of molecule release from P(FAD-SA) microspheres, although the degradation profile of the microspheres themselves was quite similar to that of gelatin-free microspheres. It is possible that an interaction between FAD monomers and gelatin molecules causes continued release, even after the polymer matrix completely degrades (even after complete degradation, FAD monomers remain because of their poor water solubility). Thermal analysis of polyanhydride microspheres at different degradation stages demonstrated that a crystalline structure was formed between gelatin and the FAD monomers produced with microsphere degradation. This gelatin effect on the extended period of drug release was not observed for microspheres prepared from other polyanhydrides: poly(sebacic acid) and its copolymer of bis(*p*-carboxyphenoxy) propane and sebacic acid. It is therefore likely that the crystalline structure formed between gelatin and FAD monomers may function as a reservoir for water-soluble drugs, leading to an extended period of molecule release from the gelatin-loaded P(FAD-SA) microspheres.

KEY WORDS: water-soluble drug delivery system; controlled release; polyanhydride; biodegradable microspheres; gelatin.

INTRODUCTION

Among different types of polymeric drug delivery systems, degradable systems have the advantage of obviating the need to surgically remove the drug-depleted device (1–3). Potentially, degradable matrix systems also have a number of other advantages in terms of simplicity in design and predictability of release if the release is controlled solely by matrix degradation (4,5). Many classes of polymers have been studied for controlled-delivery applications (6–9), but in most cases, release is augmented by diffusion through the matrix, rendering the release process difficult to control. To maximize control over the release process, it is desirable to have a polymeric system which degrades only from the surface and deters drug permeation. To achieve such heterogeneous degradation, ideally the polymer would have a hydrophobic backbone but contain water-labile linkages.

In considering properties desirable for a degradable drug carrier, we have proposed polyanhydrides. The anhydride linkage is among the most reactive, in comparing the hydrolytic reactivity of different carbon or carbonyl bonds. Due to the hydrolytic sensitivity of the anhydride bond, a change in the chemical structure of the backbone would greatly affect polymer degradation. We have studied drug release and polymer degradation characteristics of a variety of polyanhydrides (10,11). Matrix-type devices of poly[bis(*p*-carboxyphenoxy) alkane anhydride] and its copolymers with sebacic acid displayed near-zero-order erosion. Degradation and release periods from several weeks to several years were possible by changing the polymer composition and the number of methylene groups in the polymer backbone. The release profile of the model drug *p*-nitroaniline closely followed that of polymer degradation, indicating a release mechanism that was dominantly degradation controlled (10).

In addition to matrix-type devices, we have formulated polyanhydrides into microspheres. Many techniques have been used: hot melt (12), solvent extraction (13), solvent removal (14,15), and spray drying techniques (16). However, it has been difficult to prepare polyanhydride microspheres that release water-soluble drugs at a near-constant rate and without an initial burst. In this paper, a new method to prepare polyanhydride microspheres, the modified solvent evaporation method using a double emulsion, has been developed. This method permitted the preparation of microspheres that achieved near-constant release of model drugs without a large initial burst. The polyanhydrides used for microsphere preparation were copolymers of fatty acid dimer (FAD) and sebacic acid (SA) with different molar ratios. We also describe the effect of gelatin incorporation on the extension of drug release from microspheres.

All polymers and resulting microspheres were characterized. Microsphere degradation was examined by infrared (IR) spectroscopy, high-pressure liquid chromatography (HPLC), gel permeation chromatography (GPC), and differential scanning calorimetry (DSC). Morphology was observed by scanning electron microscopy (SEM).

MATERIALS AND METHODS

Materials

Poly(sebacic acid) (PSA; 1), poly(fatty acid dimer) (PFAD; 2), and their copolymers [P(FAD-SA)] with different polymer compositions (3) or the copolymer poly[bis(*p*-

[1] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

[2] Present address: Research Center for Biomedical Engineering, Kyoto University, 53 Kawahara-cho Shogoin, Sakyo-ku, Kyoto 606, Japan.

[3] To whom correspondence should be addressed.

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carboxyphenoxy) propane sebacic acid anhydride] (50/50; M_w = 26,600, M_n = 11,300) [P(CPP-SA)] and poly[bis(*p*-carboxylphenoxy) hexane anhydride] (M_w = 33,700, M_n = 18,400) (PCPH) were kindly supplied from Nova Pharmaceutical Corporation, Baltimore MD (Scheme I). Gelatin, isolated from porcine skin, was obtained from Sigma Chemical Company, St Louis, MO. Poly(vinyl alcohol) (PVA; M_w = 77,000–79,000, 88% hydrolyzed), acid orange 63 (AO; M_w = 832.80), acid red 8 (AR; M_w = 480.43), and *p*-nitroaniline (M_w = 138.13) were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. Other chemical reagents were obtained from Sigma Chemical Company, St Louis, MO, and used without further purification.

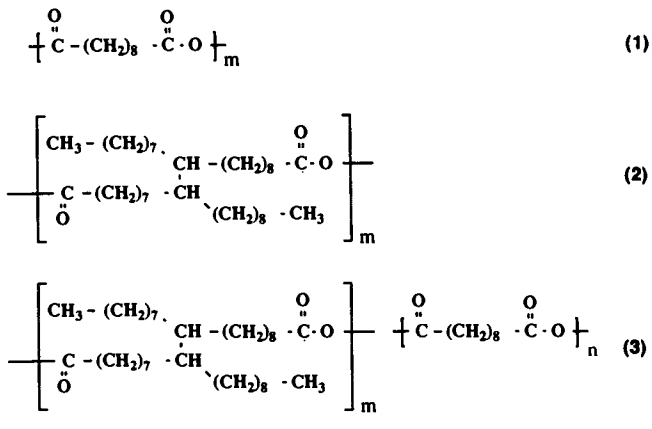
Instrumentation

The molecular weight and polydispersity of polyanhydrides were determined on a Perkin–Elmer GPC system (PE; series 10 pump; 3600 Data Station) with refractive index detection (the LC-25 RI detector). Samples were eluted in chloroform through a Phenogel 5-μm column (Phenomenex, Torrance, CA) at a flow rate of 0.90 mL/min. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA; molecular weight range, 2500 to 500,000) using CHROM 2 and GPC 5 computer programs (Perkin–Elmer).

Thermal analysis of the polymers was determined on a Perkin–Elmer DSC-2 differential scanning calorimeter employing a heating rate of 10°C/min. Infrared (IR) spectroscopy (Perkin–Elmer spectrophotometer Model 1430) was performed on polymer sample films cast onto NaCl plates from solutions of polymer in chloroform.

Microsphere Preparation

Polyanhydride microspheres were prepared by a solvent evaporation method using a double emulsion with a minor modification (17). For standard microsphere preparation, 5 mg AO was dissolved in 100 μL double-distilled water containing 10 mg gelatin (W_1) and poured into 1 mL methylene chloride containing 235 mg polyanhydride (O). The first inner W_1/O emulsion was prepared by agitating for 20 sec at room temperature using a vortex mixer at maximum speed (Vortex Genie, Scientific Inc.), followed by probe sonication (Model VC-250, Sonic & Materials Inc.) at output 4 (50 W) for 30 sec. The first emulsion was poured into 2 mL of aqueous 1% PVA solution saturated with methylene chloride (W_2), followed by vigorous mixing using a vortex mixer for 20 sec. This procedure permitted formation of the double emulsion [$(W_1/O)/W_2$] in which the W_1 phase was homogeneously dispersed in the O phase. The resulting double emulsion was poured into 100 mL 0.1% PVA solution and continuously stirred for 3 hr at room temperature until methylene chloride evaporation was completed. The microspheres were washed several times with double-distilled water by centrifugation (Sorvall Dopont Model RC-5B; 1000*g* for 5 min) and freeze-dried (24 hr; Freeze Dryer, Lab Conc) into powdered microspheres. The microspheres with different compounds and polyanhydrides and gelatin of different loadings were prepared similarly but the total amount of compound, gelatin, and polymer was always 250 mg.



Scheme I

Microsphere shape and size were estimated using a light microscope (Micro Star, American Optical, Buffalo, NY). Photographs of polyanhydride microspheres were taken and the size distribution in several fields was analyzed according to a reference scale.

Degradation Studies

Degradation experiments were conducted on a shaker at 37°C in an air gravity incubator (Imperial Incubator, Lab Line Instrument Inc.). Polyanhydride microspheres (5 mg) were suspended in 2.5 mL 0.1 *M* phosphate-buffered solution (PB; pH 7.4) and the buffer was changed periodically to approximate sink conditions—the concentrations of drug and degradation products were below 10% of saturation values at all times. The degradation kinetics were followed by measuring the UV absorbance of SA in buffer solutions at 210 nm (maximum absorption for SA) in reverse-phase ion-pair high-pressure liquid chromatography with a polymeric C-18 column (Rainin Instrument Company, Inc., Woburn, MA). The mobile phase consisted of acetonitrile in aqueous 0.05 mol/mL tetrabutylammonium phosphate, with a gradient from 15 to 30% acetonitrile.

Molecular weights of polyanhydrides before and after microsphere preparation and during degradation studies were followed by GPC. Morphology and degradation of the microspheres were observed via a scanning electron microscopy (SEM; Cambridge Instruments, 250 Mk) using 3–10 kV. The SEM observation was done on microspheres immediately after preparation and after different periods of degradation. Microspheres for SEM were freeze-dried, mounted on metal stubs with double-sided tape, and coated with gold to a thickness of 200–500 Å.

Release Studies

Release studies of various compounds (AO, AR, and *p*-nitroaniline) from polyanhydride microspheres were performed under the same conditions as the degradation studies. The optical density of the buffer solutions was measured by UV absorbance to determine the amount of different compounds released (for AO, 424 nm; for AR, 508 nm; for *p*-nitroaniline, 380 nm). Every compound absorbs strongly in the visible range and provides minimum interference with the UV analysis of matrix degradation products. A Micro BCA Protein Assay Reagent (Pierce, Rockford, IL) that

measures protein was employed to assess gelatin release kinetics. Because the Micro BCA reagent reacts with very high concentrations of the polymer degradation products, care was taken to dilute buffer solutions to a point where reaction of the degraded products with the Micro BCA reagent was negligible. However, to correct further the Micro BCA assay procedure, a standard curve for the reaction of polymer products with the Micro BCA reagent was prepared. With the extinction coefficient obtained from this curve, a calculated optical density was subtracted to account for the presence of degraded products.

The degradation and release profiles of polyanhydride microspheres at different pH's were investigated according to the same procedure as above. The microspheres were placed in 0.1 *M* phosphate-buffered solution from pH 7.4 to pH 11.0 and in 0.1 *M* KCl/HCl buffer solution (pH 2.0). The degradation and release experiments were done independently in triplicate.

RESULTS

Polymer Characterization

Table I summarizes the physical properties of the polyanhydrides used for microsphere preparation. P(FAD-SA) with approximately the same molecular weights was used to investigate the effect of FAD content on degradation and release profiles. PSA homopolymer becomes more flexible by its copolymerization with FAD.

The heat of fusion and IR absorption of the polymers is summarized in Table II. The identity of polyanhydrides can be confirmed by IR. The doublet occurring between 1670 and 1800 cm^{-1} is characteristic of the carboxylic anhydride. The IR spectra of PSA, PFAD, and P(FAD-SA) copolymers show peaks at 1740 and 1800 cm^{-1} which are typical for aliphatic polyanhydrides. The heat of fusion decreased with the increased amount of FAD in the copolymer. 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 FAD in the copolymer increases. The relative degree of copolymer crystallinity decreased with the increased amount of FAD in the copolymer as calculated from the crystallinity of PSA homopolymer

Table I. Physical Properties of Polyanhydrides

Polymer	Molecular weight[a] M_w	Molecular weight[a] M_n	Polydispersity	Physical properties and appearance
PSA	29,400	16,500	1.78	Fragile
P(FAD-SA)				
8:92	23,700	10,100	2.35	Flexible
25:75	42,900	17,200	2.49	Flexible
25:75	29,000	13,400	2.16	Flexible
25:75	19,700	9,400	2.10	Flexible
25:75	12,300	7,000	1.76	Flexible
44:56	18,000	9,800	1.83	Flexible
PFAD	16,100	7,500	2.15	Clear liquid

[a] Determined by GPC.

Table II. Heat of Fusion, Degree of Crystallinity, and IR Characteristics of Polyanhydrides

Polymer	ΔH (cal/g)[a]	% crystallinity	IR (cm^{-1})[b]	
PSA	28.69	57.0	1800	1740
P(FAD-SA)				
8:92	24.48	52.9	1800	1740
25:75[c]	13.27	35.2	1800	1740
44:56	4.21	14.9	1800	1740
PFAD	—	0.0	1800	1740

[a] Determined by DSC.
[b] Characteristics for anhydride bonds.
[c] M_w, 19,700.

(57%) and the heat of fusion and molar fraction of the monomers in each copolymer according to formulas reported by Mathiowitz *et al.* (18). The degree of crystallinity may play an important role in preventing water diffusion into the polymer matrix, thus preventing bulk erosion. Another important property in preventing bulk erosion is hydrophobicity. In this case, the less hydrophobic polymer has the higher crystallinity, probably due to the crystalline regions of the PSA unit. The more hydrophobic polymer, which has the higher amount of FAD, is less crystalline.

Characteristics of P(FAD-SA) Microspheres

Polyanhydride microspheres, prepared by the modified solvent evaporation method using a double emulsion, were spherical (Fig. 1A). Size distribution measurements showed that more than 85% of the microspheres had diameters ranging from 50 to 125 μm (Fig. 1B).

Microsphere size depended mainly on the mixing rate used in the inner emulsion preparation. Since polyanhydride solutions were transparent and acid orange was colored, it was easy to observe microspheres during different stages of preparation. When the inner emulsion was prepared by vortex mixing, the resulting microspheres were larger with a large inner emulsion. It was found that when prepared by probe sonication, a microfine inner emulsion was formed and the overall microsphere size was much smaller. The recovery of the microspheres was about 85%, irrespective of the type of polyanhydride. The trapping efficiency of dye was 60, 83, 86, and 89% for microspheres with gelatin loadings of 0, 2, 4, and 8%, respectively, irrespective of the compound used.

Degradation Characteristics of P(FAD-SA) Microspheres

The release curves of sebacic acid from different polyanhydride microspheres with loadings of 4% gelatin and 2% AO are shown in Fig. 2A. Sebacic acid was released at a near-constant rate, although an initial lag phase before degradation was observed for the polymer with the lower sebacic acid content. This result may be explained in terms of the higher hydrophobicity of FAD than sebacic acid (SA). It is possible that the hydrophobic nature of the polymer inhibits initial water entrance or that the FAD component slows diffusion of SA from the polymer. In addition, the same profile of sebacic acid release was observed for gelatin-free microspheres, indicating no effect of gelatin on polymer degrada-

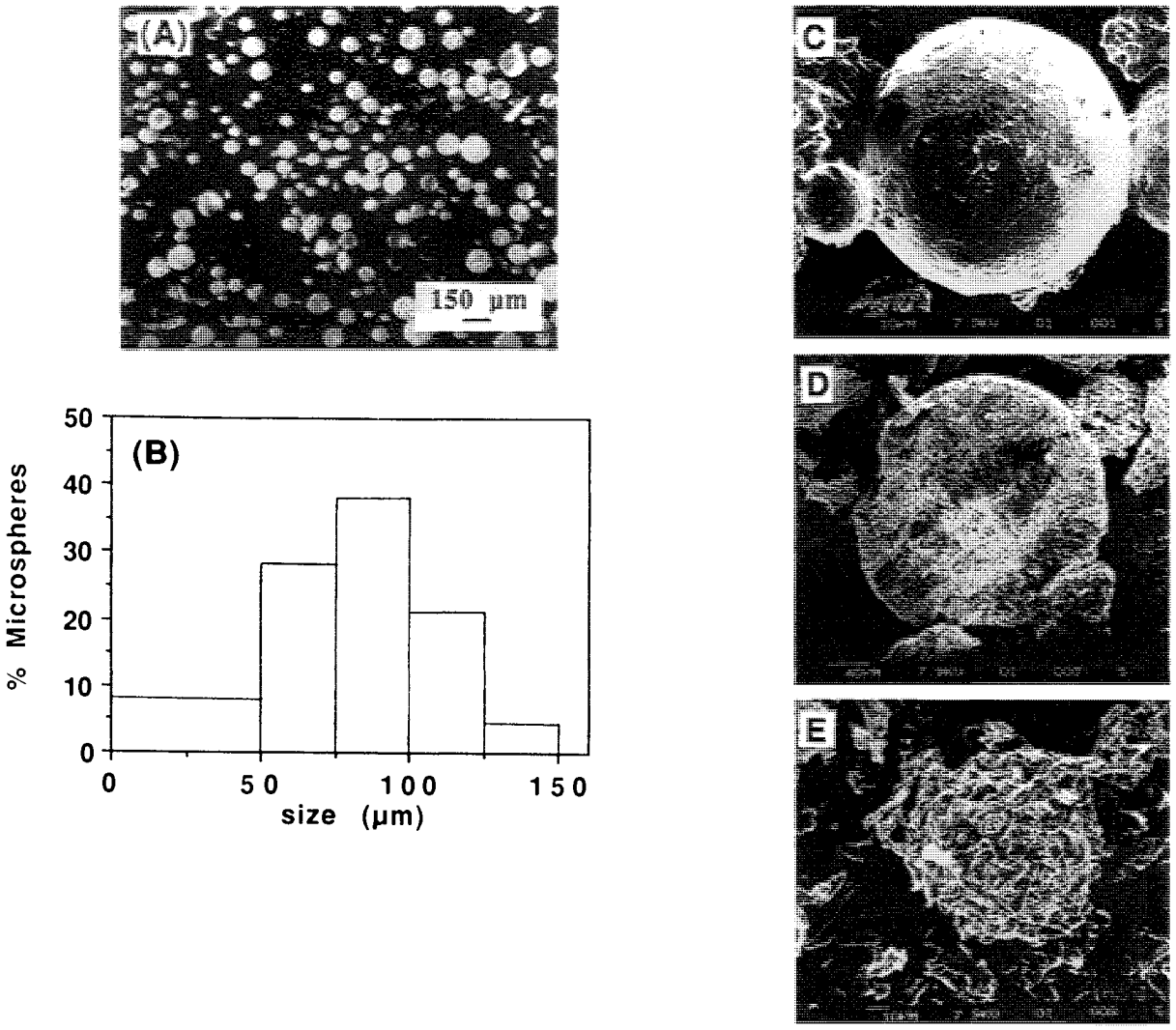


Fig. 1. (A) Micrograph of P(FAD-SA) microspheres by phase-contrast light microscopy. (B) Size distribution analysis of P(FAD-SA) microspheres by light microscopy. Microspheres were prepared by the modified solvent evaporation method and sized with sieves of 150-μm aperture. SEM of P(FAD-SA) 25/75 microspheres at different degradation stages: (C) immediately after preparation, (D) after 44 hr, and (E) after 122 hr in 0.1 *M* phosphate buffer (pH 7.4) at 37°C.

tion itself (data not shown). The erosion rates were pH dependent (Fig. 2B). The erosion rate of P(FAD-SA) microspheres increased significantly from pH 7.4 to 11.0; the rate decreased under acidic conditions.

Figure 3 shows the IR spectra of P(FAD-SA) microspheres at different degradation stages as well as the spectrum for the original copolymer. The relative intensity of the doublet at 1800 and 1740 cm^{-1}, attributed to be carboxylic anhydride bonds, became weaker with degradation time. Instead, a band appeared with time near the 1700-cm^{-1} region, indicating the emergence of carboxylic acid groups due to the hydrolysis of the anhydride linkage. The IR spectrum of microspheres 122 hr after degradation exhibited no anhydride bonds and the spectral pattern was similar to that of FAD monomer, suggesting complete microsphere degradation to remaining FAD monomers. In addition, no change of IR spectrum for the polymers before and after microsphere preparation suggests that no polymer degradation takes place during preparation.

The molecular weight of P(FAD-SA) in microspheres with loadings of 2% AO and 4% gelatin decreases rapidly within the initial 72 hr. This trend was observed irrespective of the type of polyanhydrides and compound/gelatin loading of the microspheres (data not shown). The molecular weight distribution at all times was unimodal and relatively narrow, with no evidence of shoulders corresponding to low or intermediate molecular weight fragments (data not shown). After 127 hr in buffer, the polymer molecular weight decreased from 42,900 to about 600, which corresponded to the molecular weight of FAD. This finding suggests that the microspheres are completely degraded to form FAD monomers that are soluble in chloroform, the solvent used to elute the samples, in addition to water-soluble SA monomers. This complete disappearance of anhydride bonds is also supported by the IR spectral study in Fig. 3. The molecular weight of the original P(FAD-SA) was 42,400 and no loss of polymer molecular weight was observed during microsphere preparation.

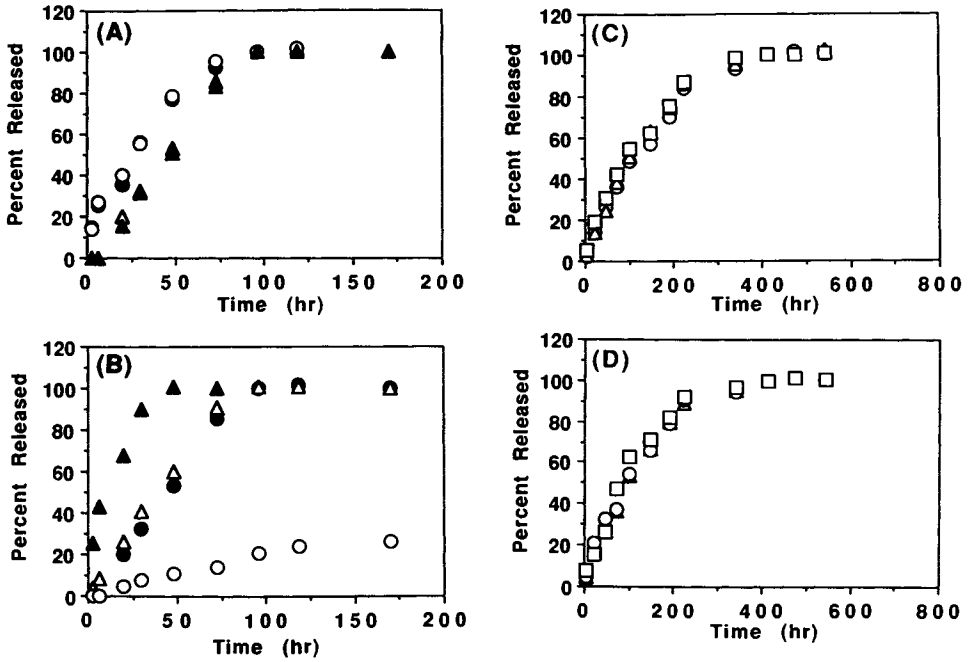


Fig. 2. (A) Degradation profiles of P(FAD-SA) microspheres with different monomer compositions in 0.1 *M*, pH 7.4, phosphate buffer at 37°C: (○) PSA, (●) P(FAD-SA) 8/92, (△) P(FAD-SA) 25/75, and (▲) P(FAD-SA) 44/56 microspheres. (B) pH dependence of degradation rates of P(FAD-SA) 25/75 microspheres in different 0.1 *M* buffers at 37°C: (○) pH 2.0, (●) pH 7.4, (△) pH 8.0, and (▲) pH 11.0. (C) Release of AO (2% loading) from microspheres of P(FAD-SA) 25/75 of different molecular weights in 0.1 *M* phosphate buffer (pH 7.4) at 37°C: (○) M_w = 12,300, (△) M_w = 29,000, and (□) M_w = 42,900 (4% gelatin loading). (D) Release of AO from P(FAD-SA) 25/75 microspheres at different AO loadings in 0.1 *M* phosphate buffer (pH 7.4) at 37°C: (○) 1%, (△) 2%, and (□) 4% AO loadings (M_w = 19,700, 4% gelatin loading).

SEM Observation of P(FAD-SA) Microspheres

Figures 1C, D, and E show scanning electron micrographs of P(FAD-SA) microspheres with loadings of 2% AO and 4% gelatin at different degradation stages. The microspheres were prepared from P(FAD-SA) of molecular weight 42,900 and a 25/75 FAD/sebacic acid molar ratio. Immediately after preparation, the microspheres are spherical and the external surfaces appear smooth without any visible pores (Fig. 1C). The SEM photograph of the microsphere cross section after 44 hr of degradation shows that only the microsphere surface was attacked (Fig. 1D). The same microsphere, after 122 hr of degradation, is shown in Fig. 1E. The spherical shape of the microspheres was no longer observed. A similar profile of microsphere degradation was observed for gelatin-free microspheres. Again, the presence of compound in the microsphere matrix had no effect on the microsphere degradation profile, irrespective of gelatin incorporation.

Release Characteristics of P(FAD-SA) Microspheres

Characteristic release curves for gelatin-loaded microspheres prepared from P(FAD-SA) with different molecular weights are shown in Fig. 2C. AO was released from microspheres at a near-constant rate without any large initial burst. However, no effect of polymer molecular weight on AO release was observed. Figure 2D shows the influence of loading on AO release. The AO loading had no effect on the release profile over the range used in the present study.

In Fig. 4A, AO release from different polyanhydride microspheres is shown. The release rate from the P(FAD-SA) microspheres decreased with increasing amounts of FAD in the copolymer. This may be due to the more hydrophobic nature of the FAD monomer. Microspheres composed of other hydrophobic polymers, such as PCPH, also caused a slower release of AO (Fig. 4A).

The pH effect on the release profile was examined (Fig. 4B). The release rates increased significantly at increased pH while they were reduced under acidic conditions. The pH dependency was similar to that observed for microsphere degradation (Fig. 2B).

Figure 4C shows the release profile of various compounds from P(FAD-SA) microspheres with a 4% gelatin loading. Every compound was released at a near-constant rate without any large initial burst, and no difference in release profile was observed among the different compounds.

Gelatin Effect on Release Characteristics of P(FAD-SA) Microspheres

Figure 4D shows AO and sebacic acid release from P(FAD-SA) microspheres with or without gelatin. For the gelatin-free microspheres, the AO release pattern followed closely that of polymer degradation, and both are almost complete in 100 hr. Interestingly, a much longer release of

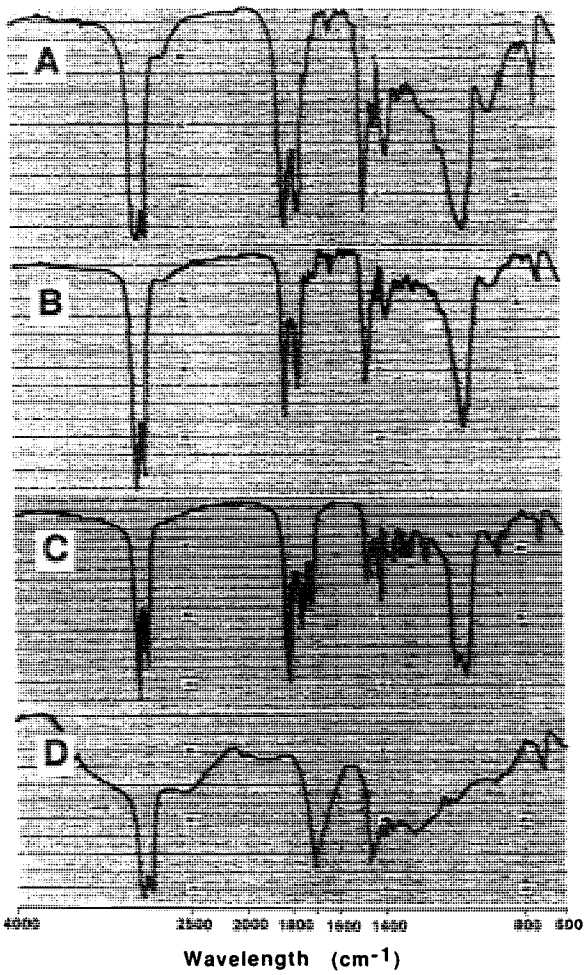


Fig. 3. IR spectra of P(FAD-SA) 25/75 microspheres at different degradation stages: (A) original P(FAD-SA) or P(FAD-SA) microspheres, (B) immediately after preparation, (C) after 40 hr, and (D) after 122 hr in 0.1 *M* phosphate buffer (pH 7.4) at 37°C.

AO was observed from microspheres containing gelatin, although they exhibited a similar sebacic acid release profile to the gelatin-free microspheres.

We used P(CPP-SA) and PSA in place of P(FAD-SA) and investigated the effect of gelatin on AO release from microspheres. However, no significant difference in the release profile of AO was observed between the gelatin-loaded microspheres and the gelatin-free microspheres for the above two polymers (Fig. 4E).

AO release from microspheres containing gelatin of different amounts is shown in Fig. 4F. The period of AO release was significantly extended by increasing the amount of gelatin in the microsphere matrix. Gelatin release from the microspheres was examined (Fig. 4G). AO was released from the microspheres faster than gelatin, irrespective of the amount of gelatin incorporated. Although gelatin release lags behind the AO release, the correlation between the two profiles is clear.

DSC analysis of P(FAD-SA) microspheres was performed to characterize the microspheres' physical state during degradation (Fig. 5). We also monitored melting points of the polymer before and after microsphere preparation. A sharp endotherm was observed for nondegraded original P(FAD-SA) at 60°C, corresponding to the melting of the polymer's crystalline regions (Fig. 5A); the melting phase transition of AO occurred at over 300°C. The heat of fusion was 13.36 and 13.30 cal/g for gelatin-free and 4%-gelatin loaded microspheres, respectively. These values are similar to the heat of fusion for the original polymer, indicating that the crystalline state of the polymer remains unchanged by loading AO and gelatin into the polymer matrix.

The microspheres displayed a sharp endotherm around 59°C before degradation, irrespective of the presence of gelatin in the microsphere matrix. The endotherm became broader and the heat of fusion for the microspheres decreased with degradation time for both types of microspheres. However, a new sharp endotherm at 119°C was observed 122 hr after degradation of microspheres with 4% gelatin, in contrast to gelatin-free microspheres. In addition, no endotherm was observed for gelatin and FAD monomers over a temperature range from −25 to 150°C (data not shown). Degradation studies demonstrate that after 122 hr in buffer, the microspheres were completely degraded to remnants composed of gelatin and FAD monomer in addition to AO. These DSC results suggest the formation of a crystalline structure from the remnants produced with microsphere degradation.

DISCUSSION

A number of degradable polymers have been developed during the past few years for controlled-release drug delivery systems (6–11). Polyanhydrides have been extensively studied as vehicles for the release of bioactive molecules. In particular, the polyanhydride P(CPP-SA) has been used, both experimentally and clinically, for the treatment of neurological disorders and brain tumors (19,20). Polyanhydrides of linear aliphatic diacids are crystalline and brittle (21). Their copolymerization with aromatic diacids results in an increase in polymer flexibility and melting point (18,21). However, the polymer becomes brittle and eventually fragments after exposure to water. This property may cause water-soluble drugs to be released more rapidly than the polymer erodes at high drug loadings. In searching for an optimal carrier for water-soluble compounds, a new aliphatic polyanhydride has been developed. This is a copolymer of fatty acid dimer (FAD), derived from the naturally occurring oleic acid and sebacic acid (SA), P(FAD-SA). Films of P(FAD-SA) were flexible with a low melting point but became soft and left oily, poorly water-soluble FAD monomers after exposure to 0.1 *M* phosphate buffer solution pH 7.4 at 37°C. However, *in vivo* degradation studies showed that the polymer eroded with time and the FAD monomers completely disappeared from the implantation site (22). In addition, the polymer is capable of controlled release of some water-soluble drugs to the rat brain (23).

The current study shows that the solvent evaporation method using a double emulsion can be used for encapsulation of water-soluble drugs. The microencapsulation procedure is reproducible with respect to yield and size distribution; the size of the microspheres can be controlled by the mixing method used in the preparation of the inner emulsion. The yield is high (80–90%). Water-soluble AO was released

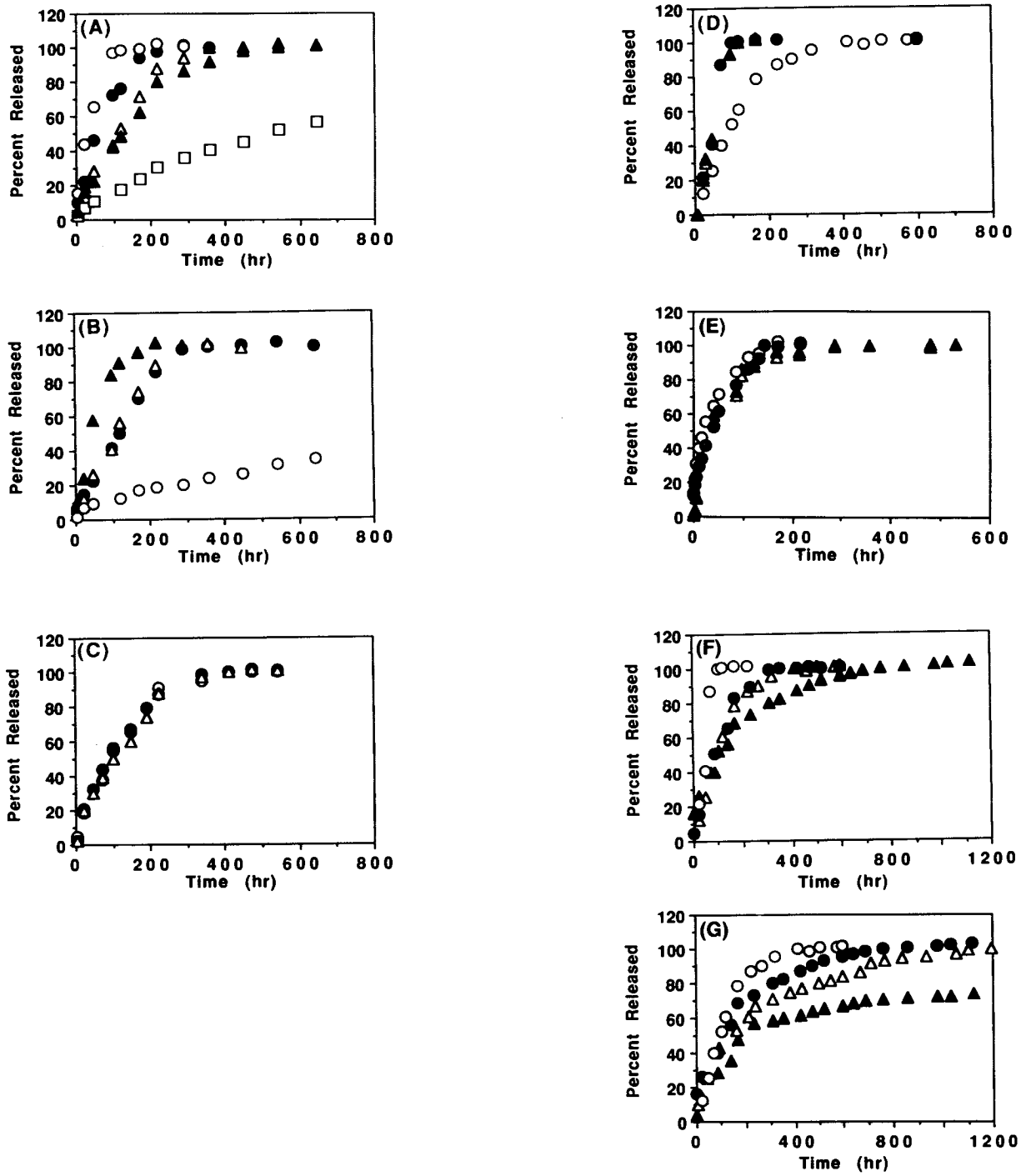


Fig. 4. (A) Release of AO (2% loading) from microspheres of various polyanhydrides in 0.1 *M* phosphate buffer at 37°C: (○) PSA, (●) P(FAD-SA) 8/92, (△) P(FAD-SA) 25/75, (▲) P(FAD-SA) 44/56, and (□) PCPH microspheres. (4% gelatin loading). (B) pH dependence of AO release from P(FAD-SA) 25/75 microspheres in 0.1 *M* phosphate buffer at 37°C: (○) pH 2.0, (●) pH 7.4, (△) pH 8.0, and (▲) pH 11.0 (M_w = 42,900, 2% AO and 4% gelatin loading). (C) Release of various compounds from P(FAD-SA) 25/75 microspheres in 0.1 *M* phosphate buffer (pH 7.4) at 37°C: (○) AO, (●) AR, and (△) *p*-nitroaniline (MW = 42,900, 2% compound loading). (D) AO release (circles) and SA release (triangles) profiles of P(FAD-SA) 25/75 microspheres in 0.1 *M* phosphate buffer (pH 7.4) at 37°C: (○, △) 4% gelatin-loaded and (●, ▲) gelatin-free microspheres (M_w = 42,900, 2% AO loading). (E) Release of AO (2% loading) from PSA (circles) and P(CPP-SA) (triangles) microspheres in 0.1 *M* phosphate buffer (pH 7.4) at 37°C: (○, △) 4% gelatin-loaded and (●, ▲) gelatin-free microspheres. (F) Release of AO (2% loading) from P(FAD-SA) 25/75 microspheres in 0.1 *M* phosphate buffer (pH 7.4) at 37°C: (○) gelatin-free or (●) 2%, (△) 4%, and (▲) 8% gelatin-loaded microspheres (M_w = 42,900). (G) Release of AO (circles) and gelatin (triangles) from P(FAD-SA) 25/75 microspheres in 0.1 *M* phosphate buffer (pH 7.4) at 37°C: (○, △) 4% and (●, ▲) 8% gelatin-loaded microspheres (M_w = 42,900, 2% AO loading).

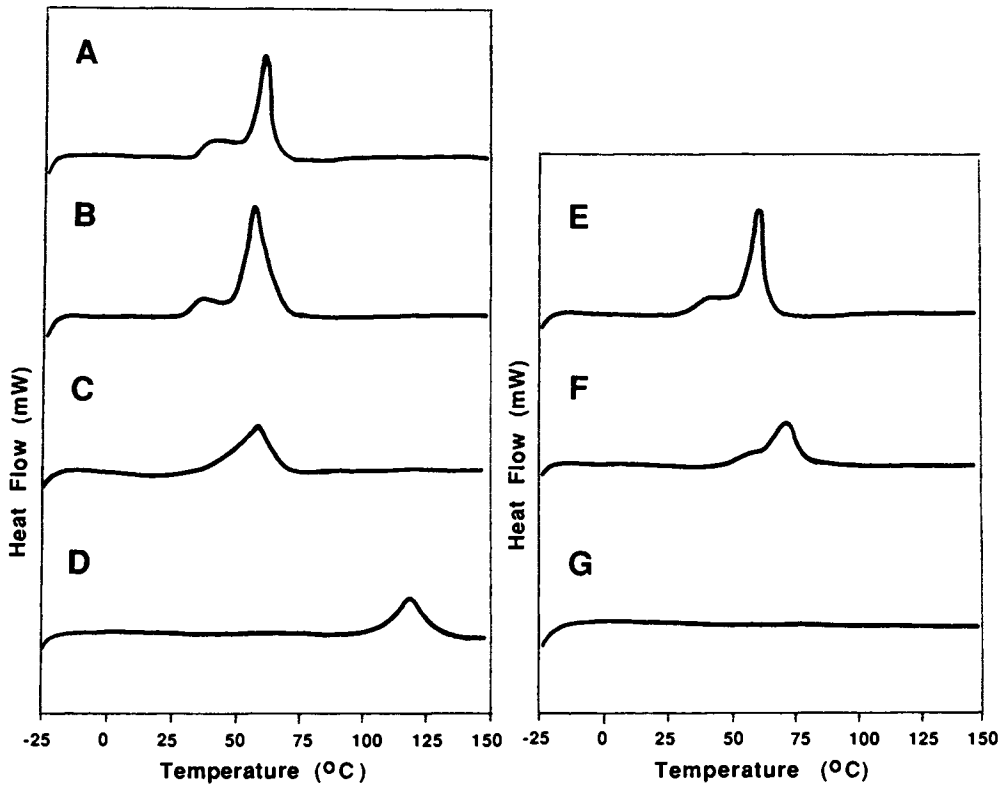


Fig. 5. DSC thermograms of P(FAD-SA) 25/75 microspheres at different degradation stages: (A) original P(FAD-SA) or (B) immediately after preparation, (C) after 27 hr, and (D) after 122 hr of 4% gelatin-loaded microspheres and (E) immediately after preparation, (F) after 27 hr, and (G) after 122 hr of gelatin-free microspheres in 0.1 *M* phosphate buffer (pH 7.4) at 37°C (2% AO loading).

at a near-constant rate without any large initial burst; the release profile was dependent on the type of the polymer which constitutes the microspheres. Release periods of several days to 2 weeks were possible for microspheres of injectable size (<150 μm). A disadvantage of this approach is the requirement of an aqueous phase during the microencapsulation process. Polyanhydrides possess a water-labile linkage. Thus, we tried to minimize the contact time between the polymer and water in order to delay polymer degradation. The GPC study demonstrated no loss of polymer molecular weight for at least 3 hr during microsphere preparation. In addition, the process is performed at a low temperature, which may be important in preventing drug inactivation.

The erosion of polyanhydrides involves a number of steps, one or more of which may be rate controlling. In the first step, water contacts the water labile bond, by either direct access to the polymer surface or imbibition into the polymer matrix interior. Polymer degradation takes place due to bond hydrolysis. The resulting degradation products, either monomers or oligomers, then dissolve. The complete loss of sebacic acid after about 100 hr indicates that during this period water has penetrated the polymer matrix and that the subsequent cleavage of anhydride bonds, followed by dissolution and/or diffusion of degradation products into the surrounding aqueous environment, at least those connected with the water-soluble sebacic acid, has occurred. Moreover, complete disappearance of anhydride bonds was observed 122 hr after microsphere degradation (Fig. 3). Degradation studies showed that the remnants at this time were oily substances soluble in chloroform and their molecular weight was similar to that of FAD monomer. These findings suggest that at around 122 hr the complete cleavage of anhydride bonds in the polymers which constituted the microspheres occurred, leaving oily water-insoluble FAD monomers.

The AO release from gelatin-free P(FAD-SA) microspheres correlated well with SA degradation. However, the incorporation of gelatin in the microsphere matrix extended the release period of water-soluble compounds from microspheres. The release profile of sebacic acid from microspheres containing gelatin was quite similar to that for gelatin-free microspheres (Fig. 4D). Again, no difference in degradation profiles of the microsphere matrix itself was observed between the gelatin-loaded microspheres and the gelatin-free microspheres from the standpoint of anhydride bond cleavage, polymer molecular weight, and microsphere morphology. The gelatin effect in extending release may be due to the interaction between gelatin and other components. We tried to characterize the physical state of gelatin in the microsphere matrix at different stages by DSC. Gelatin did not display any sharp endotherms but showed a glass phase transition at 56°C because of its random coil structure. This phase transition was not influenced by mixing with AO indicating no interaction between AO and gelatin. However, when gelatin was mixed with FAD monomers, a sharp endotherm at 120°C was observed, irrespective of the presence

of AO. The same endotherm pattern can be seen for the degraded microspheres containing gelatin as shown in Fig. 5D. Similar results were obtained for microspheres containing compounds other than AO (data not shown). At this stage of degradation, gelatin and FAD monomer together with unreleased-drug remain in the matrix. These results suggest the formation of a crystalline structure between gelatin and FAD monomers produced via microsphere degradation, irrespective of the type of compound used. However, for P(CPP-SA) and PSA, the gelatin effect in extending AO release from microspheres was not observed. In addition, DSC studies of partially degraded microspheres indicated no interaction between gelatin and the degraded products (data not shown). It is therefore likely that the crystalline structure is specifically formed through a hydrophobic interaction between gelatin and FAD monomers produced during microsphere degradation. The crystalline structure formed may function as a reservoir for water-soluble drugs, leading to the continued release of the drugs even after complete degradation of the polymer matrix.

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