Polyanhydride microspheres: 3. Morphology and characterization of systems made by solvent removal

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The morphological characterization of bioerodible polyanhydride microspheres is described. Microcapsules were prepared by solvent removal with different types of polymers. Polymers and microspheres were characterized by nuclear magnetic resonance spectroscopy (n.m.r.) X-ray powder diffraction, differential scanning calorimetry (d.s.c.) and scanning electron microscopy (SEM). Crystalline polymers like poly(sebacic anhydride) (P(SA)) tended to yield microspheres which had a crenellated and fractured surface, as judged by SEM. Polymers which had lower crystallinity, like carboxyphenoxypropane copolymerized with sebacic acid P(CPP–SA), yielded microspheres with smooth external surfaces and a porous core. Polymer crystallinity remained the same as the blank microspheres when a drug was dispersed as solid particles in the polymer. However, a decrease in polymer crystallinity was observed in cases where a drug formed a solution with the polymer. Understanding morphological and physical properties of these microspheres is essential for developing controllable delivery systems as well as for other applications.

(Keywords: polyanhydrides; microspheres; morphology; drug delivery)

INTRODUCTION

In a previous publication[1] we demonstrated a new method to prepare polyanhydride microspheres. This method, based on solvent removal, offers several significant advantages: the preparation occurs at room temperature and the process is carried out in organic solvents only. The latter advantage was particularly important for hydrolytically labile polymers such as polyanhydrides. In this method, a drug is dispersed or dissolved in a solution of the polymer in a volatile organic solvent. This mixture is then suspended in an organic oil into which the organic solvent is extracted, thus forming microspheres. The biological activity of insulin encapsulated by this method was retained and diabetic rats treated with microspheres composed of P(CPP–SA) 50:50 and containing insulin displayed normoglycaemia for 5 days[1].

This new formulation procedure permits the preparation of microspheres from polymers with different crystallinities and different hydrophobicities. In the current study we have examined the effect of the type of polymer used on microsphere performance, the relation between polymer crystallinity and microsphere morphology, the influence of the microencapsulation process and drug loading on polymer crystallinity, and the effect of drug loading on release rate.

The following polymers were studied: poly(sebacic anhydride) (P(SA)); copolymers of 1,3 bis-(carboxyphenoxypropane) (CPP) with sebacic acid (SA) with molar ratios of 20:80 and 50:50; and copolymers of 1,6 bis-(carboxyphenoxyhexane) (CPH) with sebacic acid (SA) with a molar ratio of 50:50.

All the polymers, and the resulting microspheres, were characterized. Polymers were examined by gel permeation chromatography (g.p.c.), Fourier transform infrared (*FT*i.r.) spectroscopy, X-ray diffraction, and differential scanning calorimetry (d.s.c.); the morphology was examined by scanning electron microscopy (SEM). These physical properties were correlated with microsphere performance. Since one of the potential applications of these systems is for drug delivery, understanding both the physical and chemical properties of these polymers enables a better understanding of their performance.

EXPERIMENTAL

Materials

Sebacic acid and dodecanedioic acid (Aldrich) were recrystallized from ethanol. All solvents were analytical grade or better. Acid orange 8 (Aldrich), methyl red (Fluka AG), myoglobin (Sigma) and *p*-nitroaniline (Aldrich) were available as solid particles. *p*-Carboxybenzoic acid (Aldrich) was crystallized from an acetone/water mixture. Dibromopropane (Aldrich) was used as received.

Instrumentation

Infrared spectroscopy was performed with a Perkin–Elmer Series 1420 spectrophotometer. Polymer molecular

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weight was determined by g.p.c. with a Perkin–Elmer instrument consisting of a Series 10 pump and 3600 Data Station with LKB 214-rapid spectral detector at 254 nm. Polystyrene standards (Polysciences, PA, molecular weight range 500–1 600 000) in chloroform (10 mg ml^{-1}) were used for g.p.c. calibration. The thermal properties of the polymers and microspheres were determined with a Perkin–Elmer DSC-4 differential scanning calorimeter using a heating rate of 20°C min^{-1}. Wide-angle X-ray diffraction of polymers and microspheres in the form of pressed discs (1 mm thick) was recorded on a Rigaku RU300 X-ray diffractometer using a nickel-filtered CuK source. ^1H nuclear magnetic resonance (n.m.r.) spectra were obtained with a Varian 250 MHz n.m.r. spectrometer using deuterated chloroform as a solvent and tetramethylsilane (TMS) as a standard. Morphology of polymers was done on a SEM of the type 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 −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).

METHODS

Polymer synthesis

Poly(anhydrides) were synthesized by melt polycondensation[2]. The following homo and copolymers were prepared: P(SA), P(CPP–SA) 50:50, P(CPP–SA) 20:80, P(CPH–SA) 50:50.

Determination of polymer compositions

Copolymer composition was determined by ^1H n.m.r. spectroscopy by analysing the ratio of the integration peaks at 1.3 ppm (8H sebacic) and those at 6.9–8.2 ppm (8H, CPP or CPH).

Calculation of degree of crystallinity

The relative degree of crystallinity was calculated from X-ray powder diffraction[1,3,4]. For the copolymers P(CPP–SA) 50:50 and P(CPH–SA) 50:50 a combined method of d.s.c. and X-ray diffraction was used[1].

Microencapsulation method

Microspheres were prepared as reported previously[1] by the solvent removal microencapsulation process. Briefly, the polymer was dissolved in methylene chloride, the desired amount of substance (drug) was added and the mixture was suspended in silicone oil containing Span 85 and methylene chloride. After dropping the polymer solution into the silicone oil, petroleum ether was added and the mixture was stirred until sufficient microcapsule hardening was achieved. The microspheres were isolated by filtration, washed with peteroleum ether and dried overnight under vacuum. Drugs (substances) which were insoluble in methylene chloride (myoglobin and acid orange 8) were sieved (using US Standard Sieve Series, Newark, Wire Cloth Co., Newark, NJ) to sizes <50 μm in diameter. *p*-Nitroaniline and methyl red were soluble in the organic solution. Blank microspheres (without drug) were prepared by the same procedure.

Polymer degradation and drug release studies

Two types of release studies were performed. In the first, drug release and polymer degradation products were measured spectrophotometrically[1]. This method monitored the aromatic monomers of the polymers (CPP, CPH). In the second type of release study, weight loss of microspheres during degradation was monitored: weighed samples of microspheres, blank and loaded, were introduced into a buffer solution. After known periods, the microspheres were dried and reweighed. The last method monitors both monomers (SA and CPP). Those results were compared with the release data obtained by spectrophotometry.

Microsphere loading

Experimental loading of microspheres was obtained by summing the total amount of drug released in each release study and dividing by the weight of microspheres used. Expected loading was the initial amount of drug used divided by the initial weight of the polymer and the drug used during the formation of the microspheres.

RESULTS

Polymer characterization

Polymers were characterized by g.p.c. Weight average molecular weights of the various polymers are described in *Table 1*; they range from 20 000 to 55 000. Copolymer composition was verified by ^1H n.m.r. The ^1H n.m.r. of P(CPH–SA) 50:50 is shown in *Figure 1*. The integration ratio between the peaks at 1.3 (8H sebacic) and the peaks at 6.9–8.2 ppm (8H, CPP or CPH), for each of the copolymers was determined (*Table 1*). X-ray diffraction results for pure P(SA) and P(CPP–SA) 50:50 copolymers are shown in *Figure 2*. The crystallinity of the former was 57% and that of the latter was 4%. These polymers, with high and low crystallinity, were chosen to study the effect of crystallinity on microsphere formation.

Table 1 Characterization of polyanhydrides by g.p.c. and ^1H n.m.r.

Polymer	M_w	^1H n.m.r. integration analysis[a]
P(SA)	54 000	—
P(CPP–SA) 20:80	30 800	1:4
P(CPP–SA) 50:50	40 000	1:1
P(CPH–SA) 50:50	20 000	1:1

[a] Integration ratio between peaks at 6.9–8.2 ppm and 1.3 ppm

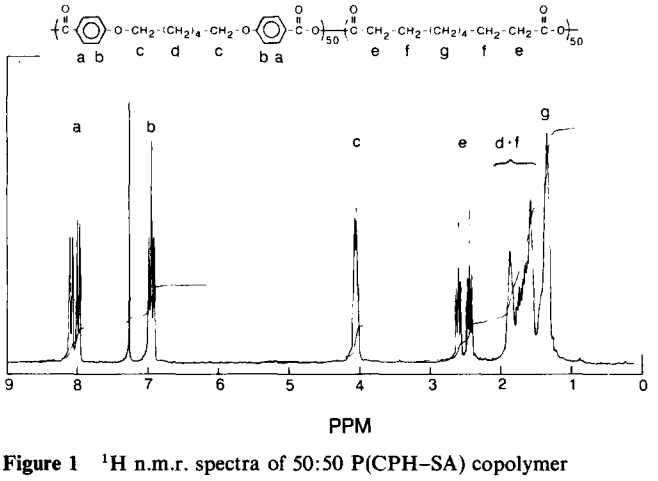

Figure 1 ^1H n.m.r. spectra of 50:50 P(CPH–SA) copolymer

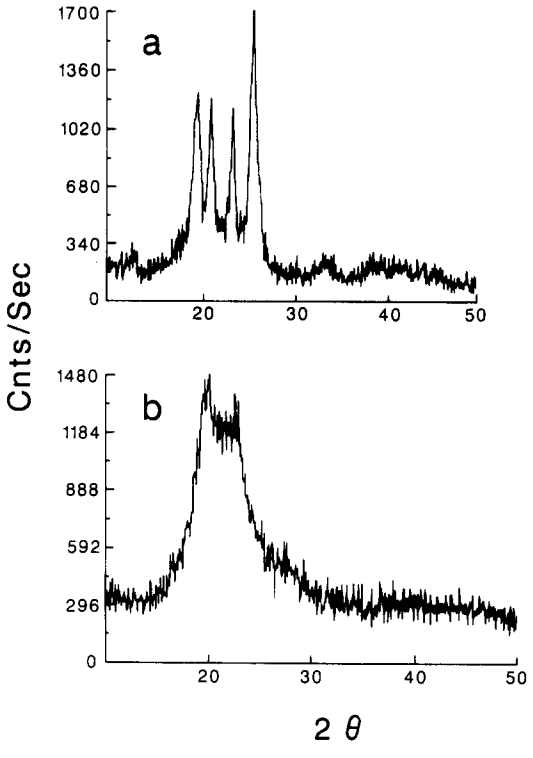

Figure 2 X-ray powder diffraction of polyanhydrides: (a) P(SA); (b) P(CPP–SA) 50:50

Microsphere loading

Microcapsules were prepared by the method described above. Several model substances were chosen to study the influence of the type of drug used on microsphere loading and to investigate the relation between loading and microsphere performance. The substances (drugs) used were acid 8 orange and myoglobin (hydrophilic, water soluble drugs), and *p*-nitroaniline and methyl red (hydrophobic, oil soluble drugs). *Table 2* summarizes the expected and experimental loadings.

The loadings obtained for the hydrophilic drugs (myoglobin and acid orange 8) were slightly higher than expected (*Table 2*). For the hydrophobic dyes (*p*-nitroaniline and methyl red), the experimental loading was much smaller than expected (the amount lost was measured by the absorption in the oil phase and was found to correlate with that lost in the loading).

Morphology of microspheres

Characterization of microspheres by SEM. Scanning electron micrographs of microspheres made from polymer without drug are shown in *Figure 3*. The crystalline P(SA) microspheres display a very rough external structure with fractures extending throughout the entire microsphere (*Figure 3a, e*). The external surfaces of the amorphous P(CPP–SA) 50:50 and P(CPH–SA) 50:50 microspheres were smooth and dense, and the particles were spherical (*Figure 3b, c*). Cross sections of the microspheres reveal a porous structure[1]. A cross section of the P(CPH–SA) 50:50 microspheres displays a very porous structure surrounded by a dense layer near the surface of the sphere (*Figure 3d*). The microspheres made of P(CPP–SA) 20:80 and P(CPP–SA) 50:50 polymers exhibited a less porous internal structure[1], but the external dense layer always appeared. When microspheres containing drugs were prepared, no change was observed in the external surface, for drug loadings up to 16.5%. Above that loading, some crystals appeared on the surface (*Figure 3e, f*).

Table 2 Microsphere (MS) characterization (expected and experimental loading)

Polymer	Drug[a]	Size of MS (μm)	Loading (%)	
			Expected	Experimental
P(SA)	AO	212–300	4.7	7.3
P(SA)	AO	212–300	13.8	16.4
P(SA)	AO	212–300	22.9	37.6
P(SA)	AO	212–300	37.4	71.0
P(SA)	MR	212–300	4.7	0.57
P(SA)	MR	212–300	37.4	5.7
P(CPP–SA) 1:4	MY	300–425	10.0	11.0
P(CPP–SA) 1:4	MY	300–425	10.0	11.0
P(CPP–SA) 1:1	AO	300–425	4.7	5.5
P(CPP–SA) 1:1	AO	300–425	13.8	10.1
P(CPP–SA) 1:1	AO	300–425	22.9	26.0
P(CPP–SA) 1:1	AO	300–425	37.4	34.0
P(CPP–SA) 1:1	*p*NA	300–425	4.7	0.12
P(CPP–SA) 1:1	*p*NA	300–425	13.8	0.20
P(CPP–SA) 1:1	*p*NA	300–425	22.9	3.6
P(CPP–SA) 1:1	*p*NA	300–425	37.4	15.5

[a] AO, acid orange; MR, methyl red; MY, myoglobin; *p*NA, *p*-nitroaniline

Characterization of microspheres by d.s.c. and X-ray diffraction. D.s.c. and X-ray analysis of the polymers, dyes and drug-loaded microspheres were performed to characterize the physical state of the polymers and dyes after microencapsulation. We thus monitored melting points of the dyes and polymer before and after encapsulation. A sharp endotherm was observed for free acid 8 orange, *p*-nitroaniline and methyl red (119.4, 149.8, 181.2°C, respectively), corresponding to the melting phase transitions. The 'blank' microspheres displayed a sharp endotherm at 78°C, corresponding to the melting of the crystalline regions of the polymer (*Figure 4a* and *Table 3*).

The crystallinity of the P(SA) microspheres was lower than the crystallinity of the original polymer (*Table 3*, decrease of 9 cal* g^{-1}). However, the crystallinity is not completely destroyed and the typical powder diffraction of the P(SA) units (*Figure 2*) is still retained. As acid orange is loaded into the system, the crystallinity of the polymer remains almost unchanged. In loaded microspheres (up to 16% loading), no melting corresponding to the acid orange was observed. At 37% loading of acid orange, a defined peak corresponding to the acid orange appeared around 121°C (*Figure 4b*). To study this behaviour further we used X-ray diffraction to examine various microspheres loaded with acid orange. *Figure 5* reveals a well defined diffraction pattern which corresponds to the P(SA) polymer ($19 \leqslant 2\theta \leqslant 31°$, where θ is the diffraction angle) and to the acid orange crystals ($31 \leqslant 2\theta \leqslant 49°$). In addition, the degree of crystallinity of loaded microspheres was 50% while the blank microspheres had 55% crystallinity (*Table 4*).

X-ray diffraction analysis of microspheres loaded with methyl red did not indicate any crystalline diffraction related to the dye, nor did the d.s.c. show any endotherm related to the melting of the dye. However, the melting point of the polymer had slightly decreased compared

* 1 cal ≈ 4.2 J

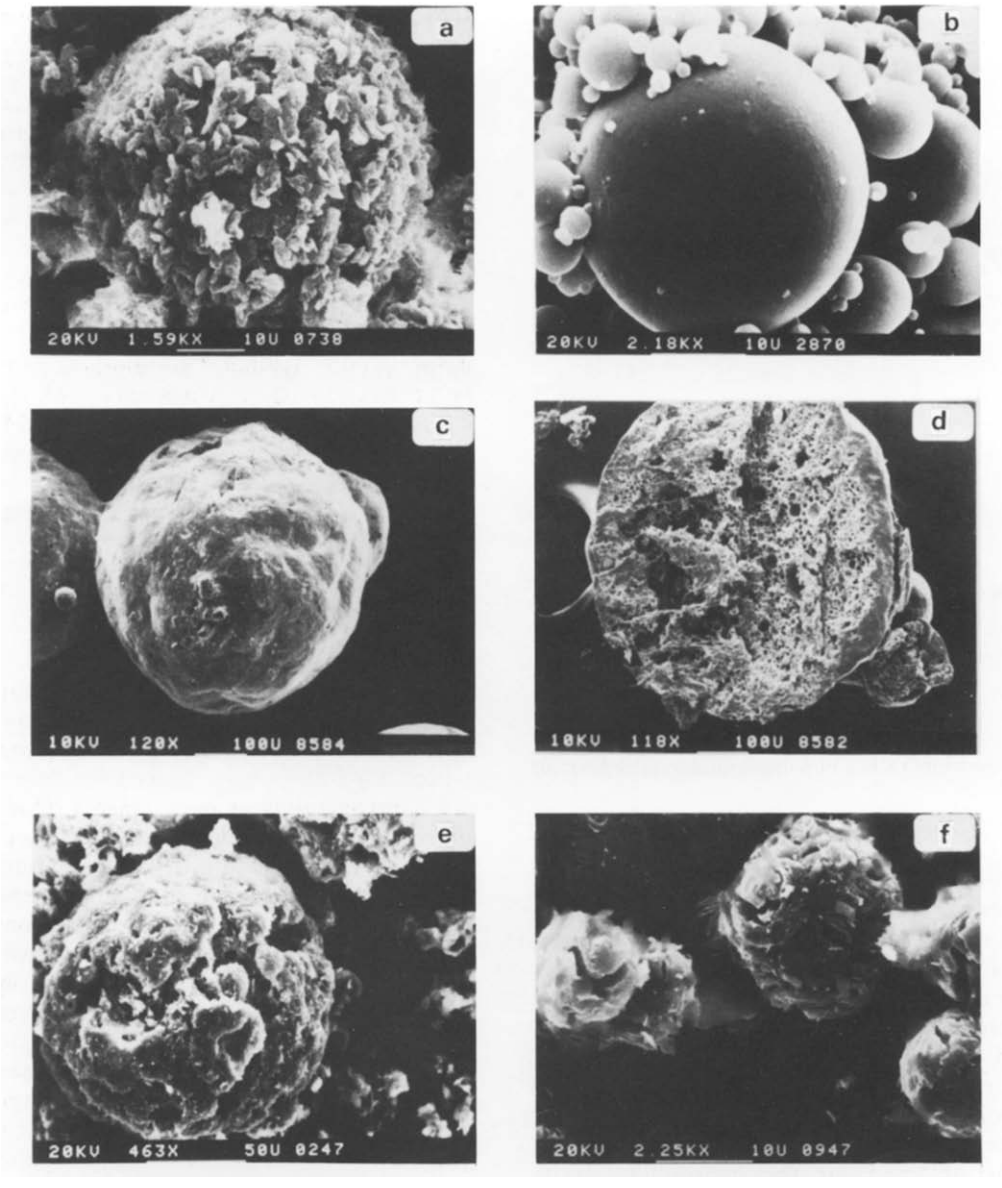

Figure 3 Scanning electron micrographs of polyanhydride microspheres, before degradation: (a) external surface of P(SA) microspheres; (b) external surface of P(CPP–SA) 50:50 microspheres; (c) external surface of P(CPH–SA) microspheres; (d) cross-section of P(CPH–SA) microspheres; (e) external surface of P(SA) microspheres loaded with 30% acid orange; (f) external surface of P(SA) microspheres loaded with 37% methyl red

with blank microspheres. X-ray diffraction displayed the typical diffraction pattern of the pure P(SA), but the crystallinity decreased from 55% to ≈40% (*Table 4*).

The same behaviour was observed for the *p*-nitroaniline-loaded microspheres, i.e. no melting of the dye was observed by d.s.c.. X-ray diffraction indicated that, up to 5% loading, no diffraction of the *p*-nitroaniline was observed, but at 15.5% loading there was diffraction of the crystalline *p*NA. In addition, the crystallinity of the polymer after encapsulation decreased from 50% (for blank microspheres) to 40–47% (for *p*NA microspheres).

Release characteristics

Release studies were performed spectroscopically by following drug release rates and by measuring weight loss of the microspheres during degradation. The studies were performed in two independent experiments. Results for P(SA) microspheres with various loadings of acid orange are shown in *Figures 6* and *7*. The drug was released very quickly. In fact, after 2 h most of the drug was released from each of the microspheres (*Figure 6*). The weight loss measurements, which were used to determine the rate of degradation of the polymers, revealed that the degradation was much slower than the drug release. However, 50% degradation was achieved after 24 h even with the blank microspheres. Microspheres with high drug loading degraded faster than those with low drug loading. Slower release rates were observed for microspheres loaded with the hydrophobic *p*-nitroaniline (*Table 5*). The same correlation of higher loading with faster release rate was obtained. Weight loss measurements (*Figure 8*) indicate a much slower degradation rate of microspheres containing *p*-nitroaniline than of microspheres containing acid orange. In several cases, the blank microspheres degraded almost as quickly as the loaded ones.

The release from P(CPP–SA) 50:50 microspheres loaded with acid orange is shown in *Table 5*. The general

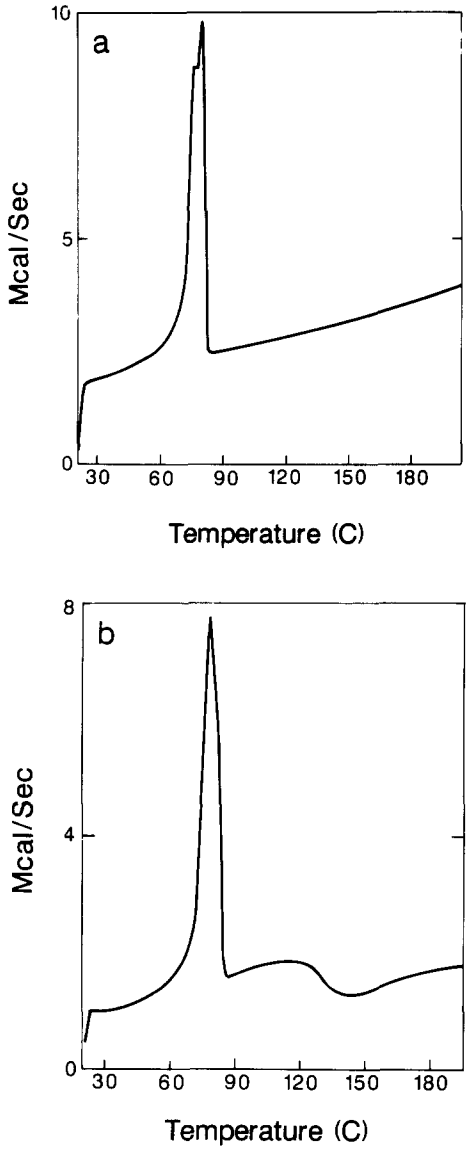

Figure 4 Differential scanning calorimetry of (a) P(SA) microspheres and (b) P(SA) microspheres loaded with 37% acid orange

trend of more highly loaded microspheres displaying faster release rates was again obtained. The polymer degradation rate, although initially high, slowed with time. The weight loss (*Figure 9*) reveals the same degradation behaviour, which is fast up to 25 h and then slower thereafter. Thirty-five hours after degradation, the blank microcapsules lost 50% of their weight, while the 37% loaded microspheres lost 80% of their weight. *Figure 10* compares weight loss and drug release for 5% loaded microspheres. The correlation is quite good since by 30 h most of the drug was released and at this point 57% of the polymer weight was lost.

As a final example of another very hydrophobic polymer, *Figure 11* displays release of acid orange from microspheres made from P(CPH–SA) 50:50. As expected, the release was quite slow even though the microspheres were quite porous (*Figure 3d*), and the polymer degraded at a slower rate than the P(CPP–SA) 50:50 microspheres. By the time the polymer had lost 50% of its weight, almost 90% of the drug had been released.

DISCUSSION

Microsphere loading

The best correlation between the experimental and the expected loadings was obtained for the two hydrophilic drugs, myoglobin and acid orange 8. This is understandable, since microencapsulation took place in an organic solvent, thus preventing the loss of drug (by diffusion) during the encapsulation process. The higher loading that was obtained for the hydrophilic drugs may be explained by the sampling procedure. We used only microspheres between 200 and 300 μm to determine drug loading. Tiny microspheres (5–10 μm) are actually smaller than many of the drug particles (some are as large as 50 μm, see 'Methods') and thus may contain little or no drug. As a result, higher loadings are probably obtained in larger microspheres. The lower loadings obtained with the hydrophobic dyes may be explained by the fact that part of the dye diffused into the oil solution during the

Table 3 Characterization of microspheres by d.s.c.

Polymer	Drug[a]	Loading	T_m (°C)	ΔH (cal g^{-1})	T_m (drug) (°C)
	AO		119.4	9.9	—
	*p*NA		149.88	22.16	—
P(SA)	Pure		78.00	31.00	—
P(SA)	Blank MS		78.25	18.77	—
P(SA)	AO	7.3	80.52	27.06	—
P(SA)	AO	16.4	79.93	27.66	—
P(SA)	AO	37.6	77.50	22.43	121
P(SA)	AO	71.0	76.20	15.86	121
P(SA)	*p*NA	0.12	79.83	22.23	—
P(SA)	*p*NA	0.2	77.93	28.00	—
P(SA)	*p*NA	3.6	76.13	26.16	—
P(SA)	*p*NA	15.5	68.30	20.20	—
P(SA)	MR	0.57	79.89	28.12	—
P(SA)	MR	5.7	79.14	27.99	—
P(SA)	MR	23.0	77.90	26.90	—
P(SA)	MR	37.5	76.39	31.48	—
P(CPP–SA) 50:50	AO	5.5	176.18	0.45	94.7
P(CPP–SA) 50:50	AO	10.1	176.18	0.94	104
P(CPP–SA) 50:50	AO	26.0	176.70	0.87	97

[a] Abbreviations explained in *Table 2*

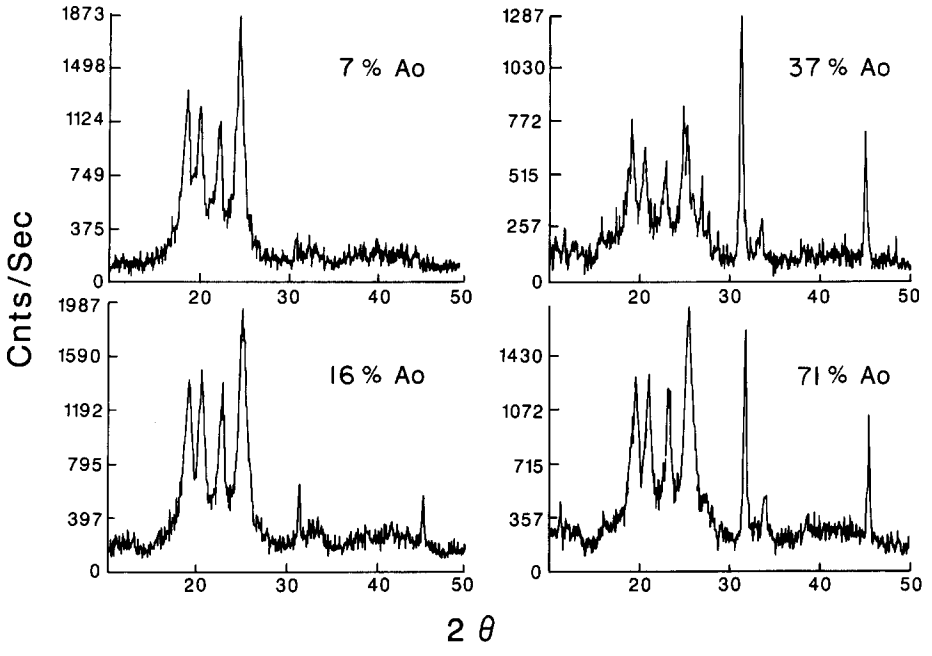

Figure 5 X-ray power diffraction of polyanhydride microspheres for various acid orange loadings

Table 4 Characterization of microspheres by X-ray diffraction

Polymer	Drug[a]	Loading	Crystallinity (%)
P(SA)	Pure		60.3
P(SA)	Blank MS		55.0
P(SA)	AO	7.3	55.6
P(SA)	AO	16.4	57.9
P(SA)	AO	37.6	59.2
P(SA)	AO	71.0	50.0
P(SA)	*p*NA	0.12	35.0
P(SA)	*p*NA	0.2	40.3
P(SA)	*p*NA	3.6	47.0
P(SA)	*p*NA	15.5	45.0
P(SA)	MR	4.76	46.3
P(SA)	MR	9.0	38.0
P(SA)	MR	23.0	45.0
P(SA)	MR	37.5	42.0

[a] Abbreviations explained in *Table 2*

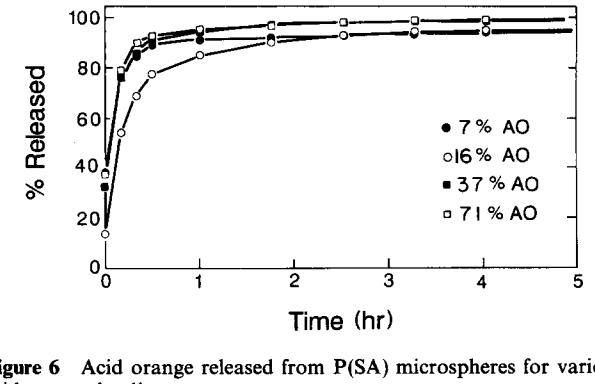

Figure 6 Acid orange released from P(SA) microspheres for various acid orange loadings

encapsulation process. In both crystalline and amorphous polymers, the hydrophobic dyes tended to diffuse into the oil solution during the process, while the hydrophilic dyes were incorporated at loadings up to 100%.

Morphology of microspheres

Characterization by SEM. The dense external structure

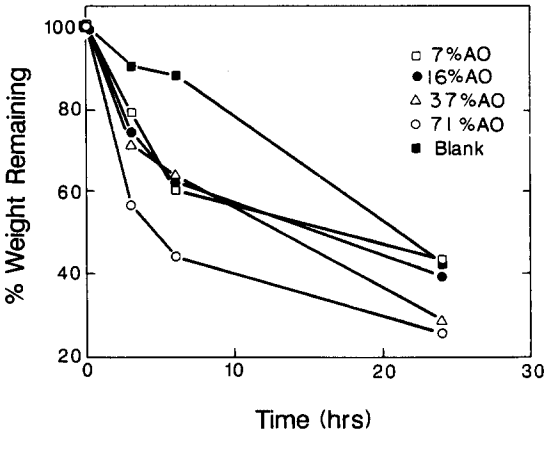

Figure 7 Degradation of P(SA) microspheres measured by weight loss for various acid orange loadings

Table 5 Characterization of release and degradation of microspheres

Polymer	Drug[a]	Loading	$T_{1/2}$ (dye)[b]	$T_{1/2}$ (polymer)[b]
P(SA)	AO	7.3	0.1	—
P(SA)	AO	16.4	0.15	—
P(SA)	AO	37.0	0.06	—
P(SA)	AO	71.0	0.05	—
P(SA)	*p*NA	0.12	4.0	—
P(SA)	*p*NA	0.2	7.9	—
P(SA)	*p*NA	3.6	0.45	—
P(SA)	*p*NA	15.5	0.33	—
P(CPP–SA) 50:50	AO	5.5	1.0	288
P(CPP–SA) 50:50	AO	10.0	1.0	288
P(CPP–SA) 50:50	AO	26.0	0.17	145
P(CPP–SA) 50:50	AO	34.0	0.17	18

[a] Abbreviations explained in *Table 2*

[b] $T_{1/2}$ = time, in hours, for 50% release or degradation of dye or polymer as measured spectroscopically

and the porous core are typical of most of the microspheres produced by this method, especially for the less crystalline polymers. A closer inspection of the physical events occurring while these microspheres were

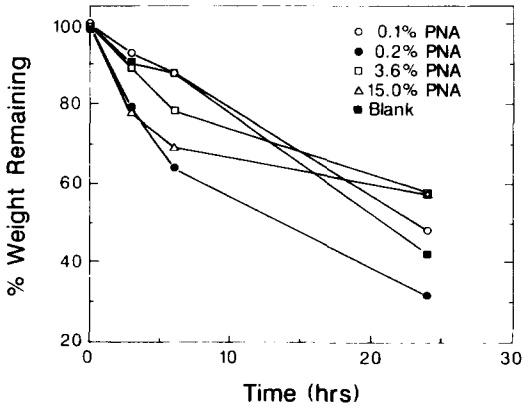

Figure 8 Degradation of P(SA) microspheres measured by weight loss for various *p*NA loadings

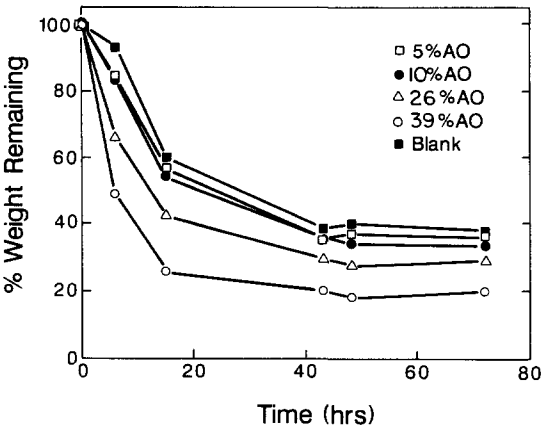

Figure 9 Degradation of P(CPP–SA) 50:50 microspheres measured by weight loss for various acid orange loadings

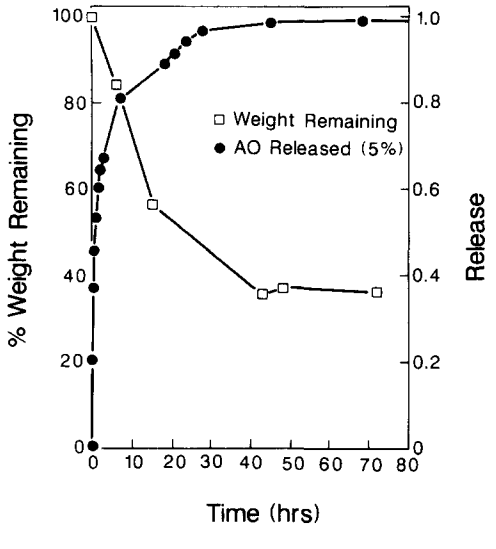

Figure 10 Comparison between weight loss and release rate for P(CPP–SA) 50:50 microspheres

formed may help in understanding their performance. A schematic representation of the process is shown in *Figure 12* and a proposed description of the process taking place is as follows.

The polymer solution is introduced into the oil phase. Then the methylene chloride diffuses quickly into the oil phase. The concentration of the polymer near the wall is high, which explains why precipitation of the outer shell occurs first[1], leaving high concentrations of polymer dissolved in methylene chloride inside the core. This organic solvent can be later removed by adding a non-solvent or applying high vacuum. The process of microencapsulation is, presumably, diffusion controlled, at least during the first stages where the difference in concentration of methylene chloride between the two phases (silicone and polymer solution) is significant. After precipitation begins, the process is more complicated. It involves diffusion of methylene chloride both in the polymer solution and in the already precipitated polymer. The first precipitation occurs in the external area of the forming sphere and this layer slows subsequent diffusion of methylene chloride into the oil phase. This dense external structure and porous internal core is presumably obtained because during precipitation of the microspheres the external surface precipitates first, thus making it difficult for the microsphere to shrink. The remainder of the solvent in the microsphere then diffuses out, leaving a porous structure.

In the more crystalline P(SA) microspheres, no dense external layer was formed, but a quick precipitation of the entire polymer emulsion led to a very porous structure. In other cases in the literature, it was also found that crystalline polyamide polymers tended to yield more porous structures when microcapsules were made by interfacial polymerization[5]. The rate of precipitation may also be a key to understanding the type of

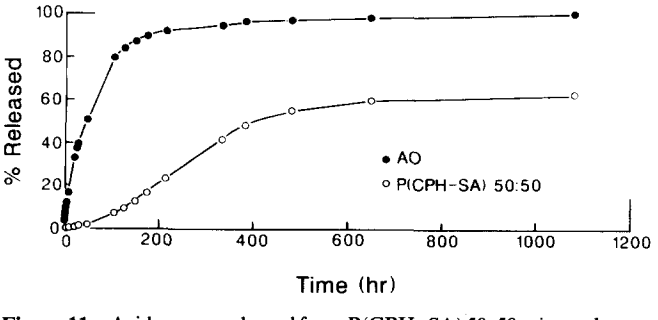

Figure 11 Acid orange released from P(CPH–SA) 50:50 microspheres

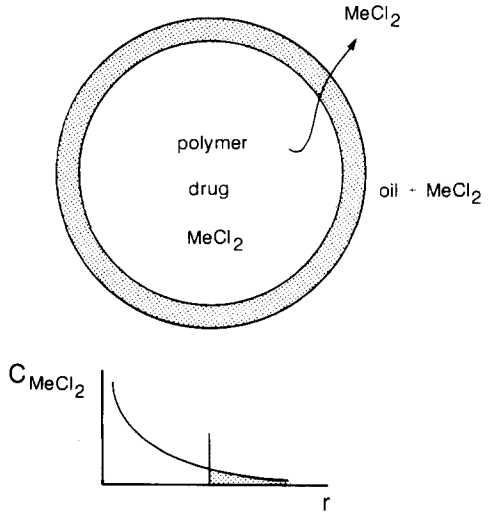

Figure 12 Schematic representation of solvent removal process: r = distance from centre of the microsphere; C_{MeCl_2} = concentration of methylene chloride inside microsphere during solidification process

microspheres which was obtained. The rough surface of the P(SA) capsule may be due to the faster precipitation of P(SA) than of P(CPP–SA) 50:50. Thus precipitation occurred, in the P(SA) microspheres, before a stable emulsion could form. In contrast, in slower precipitating systems (e.g. (P(CPP–SA) 50:50), first an emulsion forms and then precipitation occurs. This 'two-step' process of microencapsulation can be further controlled by using different surface-active compounds to stabilize the emulsion. Note that all experiments were done with the same organic solvents, since different solvents may lead to different external morphologies for the same polymer used.

Characterization of microspheres by d.s.c. and X-ray diffraction. Most of the experiments were done on the more crystalline polymer, P(SA), since it was much easier to measure and follow morphological changes both by d.s.c. and X-ray diffraction. In cases where the drug formed a dispersion in the first stage of microencapsulation, we would expect that, at the end of the process, crystalline drug particles would be dispersed in the polymer matrix. In such a system, d.s.c. would display two endotherms, one relating to the melting point of the drug, the other to the melting point of the polymer. In cases where the drug is dissolved in the organic polymeric solution, solvent removal causes the drug either to dissolve in the polymer or to crystallize out and form a dispersion. In the latter case, d.s.c. would again display two endotherms. In the former case, where the drug forms a solution inside the polymer, no separate event relating to the melting of the drug would occur. However, some changes in the melting of the polymer might occur, provided the concentration of the drug in the polymer phase is high enough to cause changes exceeding the sensitivity of d.s.c. To support our results from d.s.c., we also used X-ray powder diffraction to follow the diffraction of both polymer and drug.

The lower crystallinity of the P(SA) microspheres than of the original polymer may be explained by the fact that the microencapsulation process is so quick that the polymer chains often do not have enough time to crystallize. As acid orange was added crystallinity changed only slightly and no thermal effect was observed until 37% loading. These facts may imply that the dye forms a solution inside the polymer[6] (at loadings $<23\%$). However, the absence of the thermal event at low loadings does not always indicate that this is so. To verify this point we used X-ray diffraction, which indicates that acid orange was dispersed as crystals in the microspheres.

In the case of methyl red, no crystalline diffraction or any endotherm related to the melting of the dye was observed. However, the change in the melting point and the decrease in crystallinity suggest that the drug may be soluble in the polymer. It is quite possible that the dye, being soluble in the amorphous state, hindered the crystallization of the polymer and thus decreased the degree of crystallinity.

In the case of the *p*-nitroaniline-loaded microspheres no melting of the dye was observed by d.s.c., but X-ray diffraction indicated that at high loading there is a typical diffraction of the *p*-nitroaniline and that crystallinity of the polymer after encapsulation was decreased. This suggests that *p*-nitroaniline may be partially dissolved and partially dispersed as crystalline particles in the microspheres (this was also confirmed by SEM, *Figure 3f*).

Similar experiments were performed with P(CPP–SA) 50:50 microspheres loaded with the same substances. Both d.s.c. and X-ray diffraction confirmed that acid orange was dispersed as crystals in the polymer, while the hydrophobic dyes formed solutions inside the polymer.

Release characteristics

The fast release which results in the P(SA) microspheres loaded with acid orange was consistent with their high porosity (as seen by SEM). Microspheres with higher drug loading degraded faster than those with low drug loading. Again, this is to be expected for diffusion-controlled release devices, where water enters the device through the porous structure formed by the dispersed drug. A high drug loading yields a more extensive pore network[7], thereby allowing the water to contact the polymer core and degrade it more quickly. For *p*-nitroaniline, the correlation between the drug release and polymer degradation was better. Since the drug was more hydrophobic and it formed a solution within the polymer, we would expect the drug release to be surface degradation controlled rather than diffusion controlled.

In the release from P(CPP–SA) 50:50 microspheres loaded with acid orange, the polymer degradation rate, although initially high, slowed with time. This phenomenon may be partially attributed to the fact that the degradation products (the CPP monomer) are only slightly soluble in the aqueous solution. The polymer P(CPP–SA) 50:50, as discussed before, is hydrophobic with very low crystallinity. SEM visualization of the morphology of the microspheres indicates a porous structure. When using highly hydrophilic drugs (like acid orange) inside hydrophobic, porous polymers we do not expect to obtain a close correlation between drug release and polymer degradation. In addition, in this particular study, the weight loss study provides the most accurate picture of polymer degradation since it takes into consideration the dissolution of both monomers, while the spectroscopic method only monitors the degradation of the CPP monomer[8]. This is why a better correlation between drug release and polymer degradation was obtained in *Figure 10* than in *Table 5* (where the spectroscopically determined release rates were discussed). As expected, the release from P(CPH–SA) 50:50 was quite slow even though the microspheres were quite porous. The model drug, acid orange, is very hydrophilic and with such a porous system, release should be completed within a couple of hours. The longer release which occurred can be explained by the fact that, in spite of the porous structure, release was controlled by both erosion and diffusion; the hydrophobicity of the polymer is therefore an important determinant of the release process.

SUMMARY

The purpose of this study was to investigate the properties of various poly(anhydride) microspheres made by the solvent removal method. Since one of the advantages of poly(anhydrides) is their ability to display

surface erosion phenomena, especially for hydrophobic polymers fabricated as slabs[9], it was important to determine the morphology of microspheres to predict situations where surface erosion may occur. In a previous study, we have shown that microspheres made by melt microencapsulation may display surface erosion[10]. In the case of solvent removal, we have learned that the crystalline polymers precipitated to form a porous structure. Thus, in spite of the high crystallinity, water penetrated through the pores of the polymer, causing rapid release. The less crystalline polymers, P(CPP–SA) 50:50 and 20:80, precipitated with a more dense structure at the surface, surrounding a porous core. Release rates are controlled by both diffusion and degradation in this type of microsphere. Hydrophobicity of the polymer is an important factor in allowing slow release rates of hydrophilic drugs, and very slow release rates are achieved even in porous microspheres. From a morphological standpoint, certain polymers tend to give denser structures[1] when prepared by the solvent removal method. More work should be done towards achieving a better understanding of this phenomenon. While dense structures are important for surface-eroding systems, many applications could be envisioned for porous microspheres, especially for short time release systems such as oral or topical delivery systems.

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REFERENCES

1 Mathiowitz, E., Saltzman, W. M., Domb, A., Dor, Ph. and Langer, R. *J. Appl. Polym. Sci.* 1988, **35**, 755
2 Domb, A. J. and Langer, R. *J. Polym. Sci. A* 1987, **25**, 3373
3 Young, R. J. 'Introduction to Polymers', Chapman and Hall, London, New York, 1981
4 Kakudo, M. and Kasai, N. 'X-ray Diffraction by Polymers', Elsevier, Amsterdam, 1972
5 Mathiowitz, E. and Cohen, M. D. *J. Membrane Sci.* 1989, **40**, 1
6 Benoit, J. P. and Puisieux, F. In 'Polymeric Nanoparticles and Microspheres' (Eds P. Guiot and P. Couvreur), CRC Press, Boca Raton, Florida, 1986, p. 137
7 Siegel, R. A. and Langer, R. *Pharm. Res.* 1984, **1**, 2
8 Mathiowitz, E., Ron, E., Mathiowitz, G., Amato, C. and Langer, R. *Polymer Prepr.* 1989, **30**, 460
9 Leong, K. W., Brott, B. C. and Langer, R. *J. Biomed. Mat. Res.* 1985, **19**, 941
10 Mathiowitz, E. and Langer, R. *J. Contr. Rel.* 1987, **5**, 13