Preparation and Characterisation of Poly(adipic anhydride) Microspheres for Ocular Drug Delivery

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SYNOPSIS

A novel microsphere-gel formulation was investigated aiming to extend precorneal residence times for ocular drugs. Poly(adipic anhydride) was used for microencapsulation of timolol maleate. A nonaqueous method for the microsphere preparation was required due to the hydrolytical sensitivity of the polymer. Microspheres were prepared with an average diameter of 40 μm. The polymer and the microspheres were characterized before and during degradation using size exclusion chromatography (SEC), differential scanning calorimetry (DSC), x-ray diffraction, infrared spectroscopy (IR), and scanning electron microscopy (SEM). The microspheres had a smooth external surface and a hollow center surrounded by a dense outer shell. Degradation of the microspheres resulted in a constant release of adipic acid, the degradation product, indicating a surface-eroding degradation mechanism. The release of the incorporated substance, timolol maleate, was controlled by the surface erosion of the polymer. The drug release rate profile appeared to be suitable for ocular drug delivery. The incorporation of the microspheres into a gel resulted in an extended release of timolol maleate. This microsphere-gel formulation is expected to result in a higher bioavailability of drug to the eye than standard eye drops. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

In ocular drug delivery, the high rate of tear turnover and the blinking action of the eyelids lead to short precorneal residence times for applied eye drops. Typically, the washout rate reduces the concentration of the drug in a tear film to one-tenth of its starting value in 4–20 min.[1] As a result, only a few percent of the administered drug is absorbed by the eye and the duration of the therapeutic action may be quite short. The classical work of Sieg and Robinson[2] showed that the formulation of the eye drops is decisive for the bioavailability of an ocular drug. The absorbed amount of the model substance, fluorometholone, and its duration in the aqueous humor increased when a suspension was used instead of a solution, and the best result was obtained when the drug was formulated in an ointment. Similar results as with an ointment were obtained when a suspension formulated in a hydrogel was used.[3,4] The combination of particles with a hydrogel thus increases the bioavailability of an ocular drug. A hydrogel may increase the precorneal residence time of a suspension, and the residence time of the hydrogel, therefore, sets the maximal achievable residence time for a given drug. For a given combination of microparticles and a hydrogel, the drug release from the microparticles should extend for a time equal to the precorneal residence time of the hydrogel.

For ocular use *in situ* hydrogels are very attractive because they may be conveniently introduced into the eye as a solution. Salt-free aqueous solutions of deacetylated gellan gum, Gelrite®, form gels when brought into contact with tear fluid.[5] In a patent held by Merck Sharp and Dohme–Chibret, it was reported that fluorescein formulated in a 0.6% (by weight) solution of Gelrite® was still present in the eyes of rabbits 5 h after instillation.[6] Gamma scintigraphic studies of the precorneal drainage of ^{99m}Tc-DTPA in 0.6% (by weight) solution of Gelrite® showed that the drainage rate was one-tenth of that in aqueous solution.[7] This implies that these gels

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remain in the eye considerably longer than ordinary eye drops.

Water-soluble drugs are generally not retained by hydrogels because of their high diffusion coefficients. One way of solving this problem is to incorporate these drugs into polymeric microparticles. The polymers used for this purpose must be biocompatible and provide relevant drug release characteristics. It is, therefore, crucial to choose the most appropriate polymer for the application.

Interest in biodegradable polymers for medical and pharmaceutical applications has accelerated in the recent years. We have contributed in this area with reports on the synthesis and degradation behavior of polycarbonates,[8,9] poly(ether-esters),[10,11] polyesters,[12,13] aliphatic polyanhydrides,[9,14] and different copolymers.[8,9,15-17] These different types of polymer embrace a wide range of degradation rates. They undergo hydrolytic bond cleavage to form water-soluble and preferentially nontoxic degradation products. For drug delivery applications, polyesters[18-25] and polyanhydrides[26-31] have attracted most attention. The difference in degradation mechanism of these polymers is an important factor for their potential as a matrix in drug-release applications. In ideal bulk erosion, material is lost from the entire polymer volume, and the erosion rate, consequently, depends on the total amount of material and generally decreases as the material is depleted. This mechanism corresponds to the description of polyester degradation. It is important to note that the degradation time for the bulk-eroding polymers can be altered by changes in their chemical composition[22] but not by the microsphere size.[21,23]

In contrast, the erosion rate for ideal surface-eroding polymers is directly proportional to the external surface area, and the erosion rate is essentially constant until the polymer is completely eroded. Surface erosion takes place when water penetration is the rate-limiting step and the hydrolysis of chemical bonds is, thus, the fastest process. It has been shown that polyanhydrides can be essentially surface eroding due to their hydrolytically reactive linkage. Langer and his co-workers[27,30,31] have shown that the hydrophobicity and crystallinity of the polyanhydrides are two of the most important factors controlling the type of erosion that takes place. Using aromatic polyanhydride microspheres, Mathiowitz et al.[31] have shown that a continuous drug release can be obtained. For precorneal drug release, the polyanhydrides offer the most attractive properties, as they continuously release the drug in a suitable time range compared, in this context, to the slow eroding poly(lactic-*co*-glycolic) acid materials.[22,23]

The goal of this work was to prepare an ocular drug delivery formulation with a long precorneal residence time and with the ability to release water-soluble drugs over an extended period of time. The aliphatic poly(adipic anhydride), PAA, was investigated as a candidate matrix. A solvent removal method was used for the preparation of PAA-microspheres. The microspheres were characterized with respect to the physical and chemical changes in the polymer matrix as a result of microsphere preparation and *in vitro* degradation. The microspheres were incorporated into a Gelrite® hydrogel to obtain improved ocular control of drug delivery, the hydrogel providing an extended precorneal residence time. The drug-release characteristics of this novel microsphere-gel formulation were evaluated using timolol maleate as model drug.

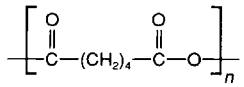
EXPERIMENTAL

Materials

Triethylamine was purchased from Merck–Schuchardt, Germany. Timolol maleate was obtained from Sigma Chemical Co, MO. Span® 80 was purchased from Speciality Chemicals, ICI, Germany. The sesame oil was purchased from Apoteksbolaget, Sweden. (N-*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide, MTBSTFA, 98% was purchased from Fluka Chemie AG. Switzerland. Gelrite® was obtained from Kelco, division of MSD, USA, and bensalkonium chloride from Pharmacia, Sweden. NaCl was purchased from Merck, Germany, and $NaHCO_3$ and $CaCl_2 \cdot 2\ H_2O$ from Sigma Chemical Co. All solvents were of analytical grade and all compounds were used without further purification.

Polymer Synthesis

Poly(adipic anhydride), PAA,



was prepared by ring-opening polymerization of oxepan-2,7-dione[9] with triethyl amine as initiator at room temperature with a reaction time of 1 h. The monomer-to-initiator molar ratio was 250. The polymer was purified by dissolution in methylene chloride and precipitation in petroleum ether. The polymer was isolated by filtration and vacuum dried to constant weight at room temperature.

Preparation

Microsphere

Microspheres were prepared using an oil-in-oil solvent removal technique. 0.5 g poly(adipic anhydride), PAA, and timolol maleate (drug) were dissolved in 4.5 mL methylene chloride. The solution was added dropwise to 50 g sesame oil containing 2% (w) Span® 80 under vigorous stirring. The droplets were decreased by sonication. The emulsion was stirred at 35°C for 1.5 h during the evaporation of methylene chloride. The microspheres were isolated by centrifugation and washed four times with hexane. To minimize the initial burst during the release experiments, the particles were finely washed with water and then vacuum dried.

Microsphere-gel Formulation

A formulation where the PAA microspheres were dispersed into a gel was prepared. The gel was prepared by dissolving 0.7% Gelrite® in distilled water containing 0.01% of the preservative bensalkonium chloride. The dispersion was heated and stirred at 90°C for 15 min and subsequently cooled. PAA (10 mg) microspheres containing 5% timolol maleate were homogeneously dispersed into 0.9 g of the Gelrite® solution. 6.7 g NaCl, 2 g $NaHCO_3$ and 0.08 g $CaCl_2 \cdot 2\ H_2O$ were dissolved in 100 mL water, representing the electrolyte composition of tear fluid. One hundred microliters of this electrolyte was added to the Gelrite® solution to allow gelation on the bottom of the test vials. After 15 min, 20 mL of phosphate buffer (0.14*M*, pH 7.3) was added and the test vials were placed on a shaking board for *in vitro* release studies. Gel formulations containing free timolol maleate in the same amount as in the formulations containing microspheres loaded with timolol maleate were also prepared.

Characterization of the Poly(adipic anhydride) Microspheres

Size

The volume mean diameter (vmd) of the microspheres was determined by laser diffractometry using a Sympatec Helos 12LA (Sympatec GmbH, System–Partikel–Technik, Remlingen, Germany).

Microsphere Loading

The experimental amount of timolol maleate loaded in the microspheres was determined by dissolving a weighed sample of microspheres in methylene chloride. The timolol maleate content was then assayed spectrophotometrically at 295 nm. The theoretical drug loading in the microspheres was calculated by dividing the initial weight of drug used by the initial weight of polymer and drug used for the microspheres.

Morphology

The morphology of the microspheres was examined by scanning electron microscopy (SEM) using a JEOL JSM-5400 scanning microscope. Cross-sections of the samples were obtained by cutting the microspheres with a sharp glass edge. The samples were mounted on metal stubs and sputter coated with gold-palladium (Denton Vacuum Desc II). The sulphur content on the microsphere surface was recorded using a Zeiss DSM 940 (SEM) with a LINK QX 2000 EDX system (energy-dispersive x-ray microanalysis).

Molecular Weight

The molecular weight of the polymer was determined by size exclusion chromatography (SEC). A Waters 6000A pump with five Ultrastyragel® columns (10^5, 10^4, 10^3, 500, 100 Å in pore size) and chloroform as eluent, with a flow rate of 1.0 mL/min, was used at 25°C with a Waters RI 401 refractive index detector. Polystyrene standards with a molecular weight range of 162–411,000 and a narrow molecular weight distribution were used for calibration.

Degradation of the Poly(adipic anhydride) Microspheres

The degradation studies were conducted at 37°C in a shaking water bath. A series of vials, each containing weighed samples of poly(adipic anhydride) microspheres loaded with 5% timolol maleate, were suspended in phosphate buffer (0.14*M*, pH 7.3) under sink conditions. At various times, three vials were removed and the solutions were filtered on preweighed AcetatePlus, supported filters (Micron Separations Inc.). The filters with microspheres were then dried and reweighed. The degraded microspheres were then subjected to different analyses.

Chemical Composition

Changes in the chemical composition within the polymer matrix during the degradation were monitored by infrared spectroscopy (IR) (Perkin–Elmer FT–IR Spectrometer 1725 X) using KBr tablets.

Morphology

Analyses were performed by differential scanning calorimetry (DSC) using a Perkin–Elmer DSC-7 with a heating rate of 10°C/min. The first scan was used to monitor the melting point and the heat of fusion ΔH. The DSC results were combined with x-ray powder diffraction measurements. The relative changes in crystallinity and lamellae segregation were calculated from x-ray data. The x-ray diffraction was recorded on a Philips generator PW 1830 using a nickel filtered CuK_α-source.

Degradation Products

Gas chromatography–mass spectroscopy, GC–MS analysis of the degradation products in the buffer solution was performed according to the method previously reported.[32] Briefly, the buffer solutions were acidified with sulphuric acid and an internal standard was added. The samples were extracted with diethyl ether and the solution was subsequently evaporated to dryness. To the dry residues, 20 mL of the derivatisation reagent, MTBSTFA, was added and the solutions were diluted with 100 mL iso-octane. The GC–MS equipment used was a Perkin–Elmer 8500 with a WCOT fused-silica CP-Sil-19 CB capillary column (25 × 0.32 mm i.d.) connected to a Perkin–Elmer ITD mass spectrometer. Helium was used as carrier gas.

In Vitro Release of Timolol Maleate

The release of timolol maleate from the microspheres alone, the microsphere-gel formulation and the free timolol maleate-gel formulation was studied under sink conditions in a 0.14*M*, pH 7.3 phosphate-buffered aqueous medium at 25, 37, and 60°C using a shaking water bath. Samples were periodically collected, the dispersion was centrifuged and the buffer was analyzed spectrophotometrically for timolol maleate content at 294 nm using a Hewlett–Packard 8451A diode array spectrophotometer. Each series of experiments was performed in duplicate or triplicate.

RESULTS AND DISCUSSION

In this study, the aliphatic polyanhydride, poly(adipic-anhydride), was investigated as a possible matrix candidate for an ocular drug delivery microsphere-gel formulation. Microspheres containing 5% timolol maleate were prepared using a solvent removal technique. After characterization, the microspheres were exposed to hydrolysis. The polymer degradation and the drug release from the microspheres were studied. It was found that the time scale for release of the drug from these microspheres seemed to be suitable for the application. As mentioned in the introduction, the *in situ* hydrogels are very attractive for ocular drug delivery because they remain in the eye for a considerable time and they are easily dropped into the eye. Because water-soluble drugs are generally not retained by a hydrogel for a sufficient length of time, a formulation consisting of a combination of the poly(adipic anhydride) microspheres and a hydrogel was the emphasis of this study.

Preparation and Characterization of the Poly(adipic anhydride) Microspheres

Aliphatic polyanhydrides degrade in aqueous solutions in a few hours,[9] and it was, therefore, preferable to prepare the PAA microspheres in organic solvents. An oil-in-oil solvent-removal technique was applied. In this study, methylene chloride was used as the volatile organic phase as it is a good solvent for the poly(adipic anhydride). The drug, timolol maleate, was to some extent soluble in methylene chloride so that it could be dissolved together with the polymer at a low loading. However, to achieve a higher loading, the drug was suspended as particles. This polymer/drug mixture was emulsified in an organic oil phase, sesame oil. Microspheres were formed after extraction of the methylene chloride into the oil.

The microspheres achieved had a relatively broad size distribution; 80% of the microspheres had a volume distribution ranging from 15 to 80 μm, with an average of 40 μm. The microspheres are visualized in Figure 1(a). The size of the microspheres is important for the degradation time. Tamada et al.[30] have shown a surface area dependence for the erosion of aromatic polyanhydrides. They used discs of the same diameter but of different thicknesses. Their results showed that the erosion rates for the different discs were nearly identical but that the discs that were twice as thick took nearly twice as long to erode. The size of the microspheres is also very important when they are used in suspensions for eye treatment, as microspheres that are too small have a short precorneal residence time, while microspheres that are too large cause irritation. Incorporation of the spheres in a gel diminishes this problem.

Microsphere Loading

The timolol maleate incorporated is water soluble. The oil-in-oil extraction technique is, therefore,

most appropriate for the microsphere encapsulation, as the tendency for the hydrophilic drug to diffuse out from the methylene chloride-polymer droplets to the surrounding organic oil phase is very low. The theoretical and experimental drug loadings are summarized in Table I. Three different loadings were studied and, for the lowest loading, 5%, the entrapment efficiency was 94.4%. For the 10 and 20% loadings the entrapment efficiency was much less, probably because of the limited solubility of the drug in methylene chloride. Instead, a high concentration of crystallized drug was found on the surface of the spheres. Figure 2(a) and 2(b) reveals that most of these drug crystals were lost in the final aqueous washing. The surface of the 5% loaded microspheres was not visibly affected by the aqueous washing.

Table I Timolol Maleate Loading in the Poly(adipic anhydride) Microspheres

Theoretical Loading (%)	Experimental Loading (%)	Entrapment Efficiency (%)
5.4	5.1	94.4
9.5	5.3	55.8
20.1	7.4	36.8

Morphology

Scanning electron micrographs of the 5%-loaded PAA-microspheres are shown in Figure 1(a). The microspheres were spherical in shape and displayed an external surface partly covered with small particles. The number of cracks and pores visible on the surface was shown to increase with increasing drug loading as is evident in Figure 1(a) for 5% loading and in Figure 2(b) for 20% loading. This increased amount of pores in the microspheres with high loading is probably due to the loss of loose particles, most likely drug crystals, in the final aqueous washing step, as discussed above. The small particles on the surface of the 5% loaded microspheres were exposed to an elemental analysis (EDX), which revealed a larger amount of sulphur in the smallest particles. Because sulphur is a component of the incorporated drug, it seems probable that these particles are crystallized timolol maleate embedded in the surface and that the larger particles are merely fragments of precipitated polymer. The SEM examination also revealed that most of the microspheres had a hole in the sphere wall. This hole is thought to be a result of rapid precipitation of the entire polymer emulsion. As described by Mathiowitz et al.,[31,33] the solvent-removal technique allows the volatile solvent in which the polymer and drug is dissolved to be slightly soluble in the organic oil phase. As a result, the methylene chloride in this case diffuses into the oil as soon as the polymer-drug solution is introduced. A rapid precipitation of the polymer occurs and leaves some solvent entrapped inside the spheres. As shown in Figure 1(b), a dense outer shell was created, leaving a hollow core inside the spheres where methylene chloride was trapped. This entrapped methylene chloride finely broke out of the spheres, leaving a hole in the wall. It is important that the retained polymer matrix was dense, because this is a vital property for the drug release mechanism.

Molecular Weight

The microsphere preparation evidently led to a decrease in the molecular weight of the polymer. The

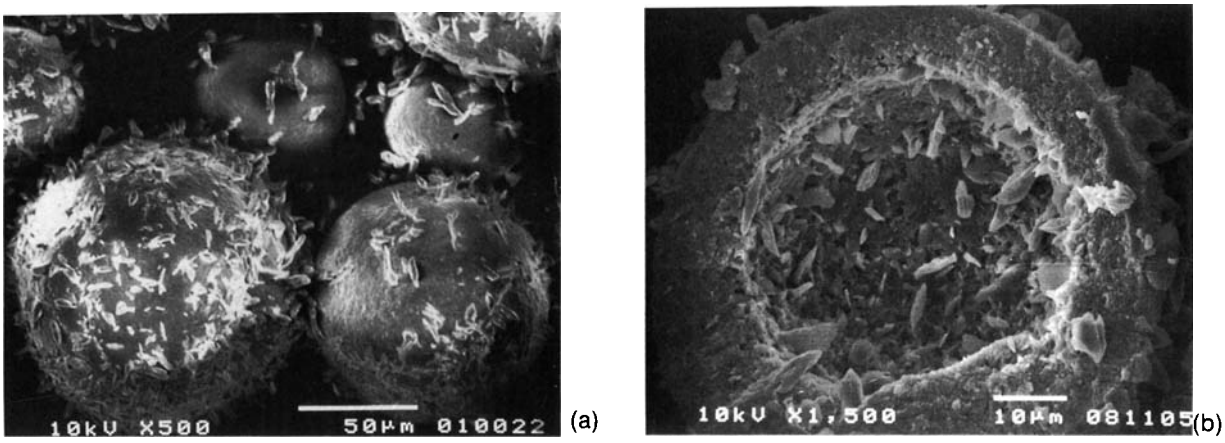


Figure 1 SEM micrographs of 5% loaded poly(adipic anhydride) microspheres: (a) external surface; (b) cross-section of a PAA microsphere.

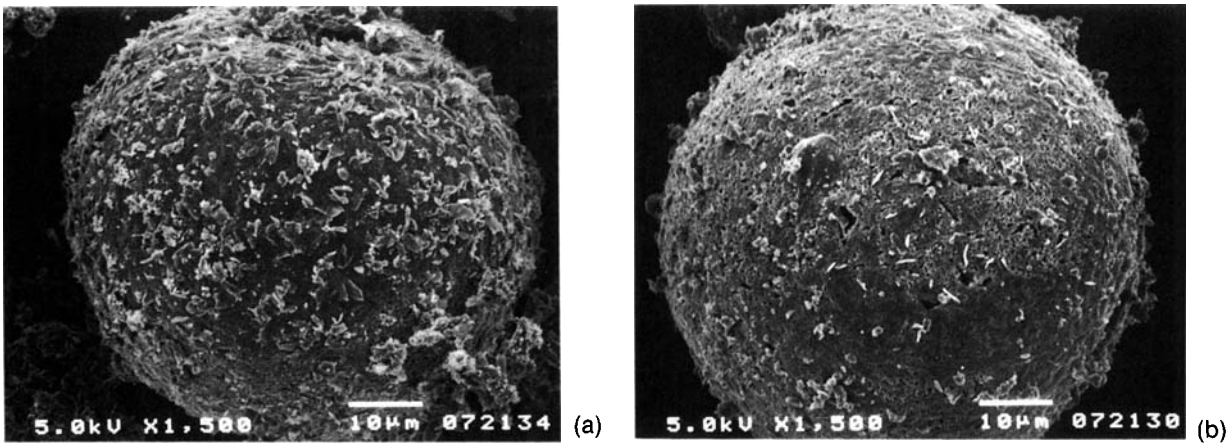


Figure 2 SEM micrographs of 20% loaded poly(adipic anhydride) microspheres: (a) before the aqueous washing step; (b) after the aqueous washing step.

weight average showed a greater decrease than the number average molecular weight, indicating that there was a cleavage of the longest polymer chains. This is also evident in the narrow molecular weight distribution of the polymer after the microencapsulation (Table II). It is possible that this degradation is due to the final aqueous washing step after the microsphere preparation.

Degradation of the Poly(adipic anhydride) Microspheres

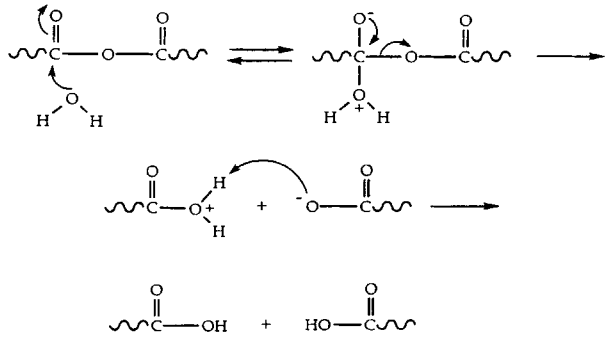
Scheme 1 shows how carboxylic end groups are formed during the hydrolysis of the PAA. Using IR-spectroscopy it was possible to follow the progress of formation of the carboxylic acid end groups. The spectra of the original polymer and of the microspheres at different degradation stages were studied. The spectrum for the original polymer showed the characteristic doublet at 1800 and 1740 cm^{-1} attributed to the carboxylic anhydride bonds [Fig. 3(a)]. In the IR spectrum for the microspheres, a weak peak corresponding to carboxylic acid groups was observed near 1700 cm^{-1}, [Figure 3(b)]. The acid end groups originated from the chain cleavage also recorded in the SEC analysis. The degradation was probably due to the final aqueous washing step in the encapsulation process. Figure 3(c) shows the carbonyl peaks corresponding to the microspheres after 0.5 h degradation in a phosphate buffer. The peak related to the carboxylic acid end groups formed is here already dominating over the anhydride peak. The spectrum for further degraded microspheres was identical to that for adipic acid (data not shown), which is the most probable degradation product according to Scheme 1. Table III summarizes the IR spectral data.

Table II Molecular Weight

Sample	M_n (g/mol)	M_w (g/mol)	M_w/M_n
PAA	1300	3500	2.7
PAA-microspheres	550	1200	2.2

Morphology

The original polymer, drug-loaded microspheres and degraded microspheres were subjected to DSC and x-ray analyses to characterize the physical state of the polymer during the preparation and degradation processes. In the DSC thermogram (Fig. 4) a sharp endotherm was observed for the poly(adipic anhydride) (a) at 76°C corresponding to the melting of the crystalline regions of the polymer. For the PAA microspheres (b), the melting endotherm was split into two, a sharp peak at 69°C and a small peak at



Scheme 1 Hydrolysis of polyanhydrides to carboxylic acids.

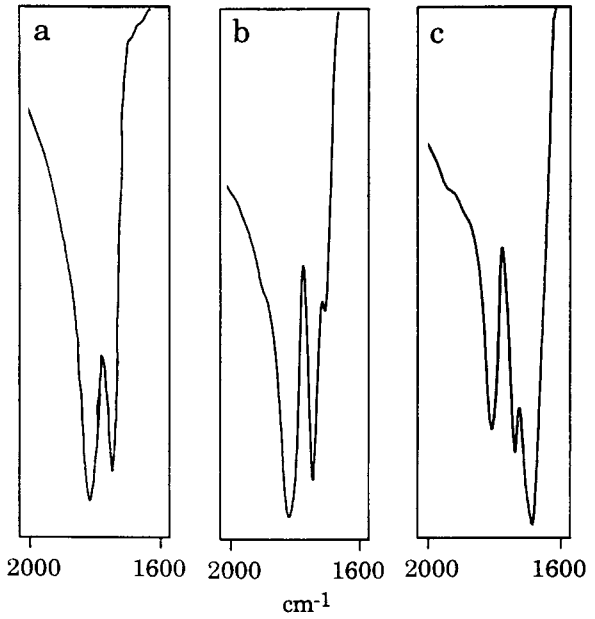


Figure 3 IR spectrum of (a) poly(adipic anhydride); (b) PAA microspheres; (c) PAA microspheres degraded for 0.5 h in pH 7.3 phosphate buffer at 37°C.

90°C. By means of x-ray powder diffraction, a lamellar segregation in the polymer due to the microsphere formation was detected and, as a consequence of the different lamellar thicknesses more than one melting peak was observed. It was also established by x-ray diffraction that the crystallinity of the microspheres was 6% lower than the crystallinity of the original polymer. This decrease in crystallinity may be explained by the assumption that the polymer chains do not have sufficient time to crystallize when the polymer precipitates during microsphere preparation. After degradation of the microspheres in buffer solution for half an hour (c) three peaks were observed in the DSC thermogram. A minor endothermic peak was observed at 62°C followed by two slightly overlapping peaks at 127°C and 147°C, the latter being substantially stronger than the former. As large amounts of degradation product are set free due to the erosion of the polymer, it seems likely that these peaks originate from adipic acid

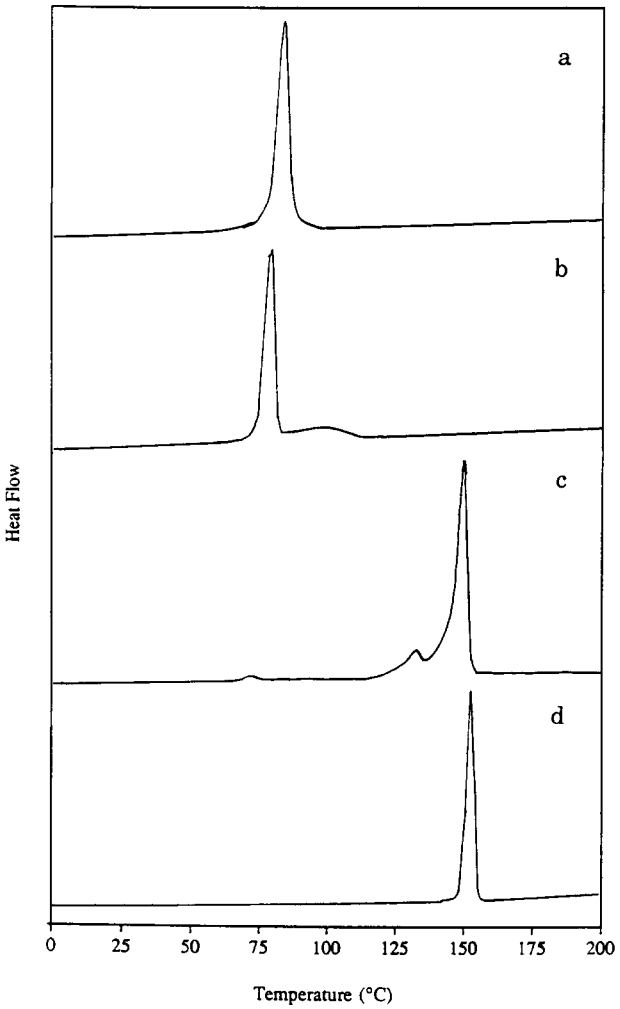


Figure 4 DSC thermograms of (a) poly(adipic anhydride), PAA; (b) PAA microspheres; (c) PAA microspheres degraded for 0.5 h in pH 7.3 phosphate buffer at 37°C; (d) adipic acid.

crystals. This hypothesis is supported by two facts. First, adipic acid (d) showed a clear melting peak at 153°C, which correlates very well with the endotherm for the degraded microspheres. Second, a pronounced increase in the heat of fusion was recorded for the degraded microspheres, which directly reflects an increase in crystallinity (Table III). Ti-

Table III Polymer Characteristics from IR and DSC Measurements

Sample	IR (cm^{-1})			T_m (°C)	ΔH (J/g)
PAA	1816	1743		76	133
PAA-microspheres	1814	1744	1725 (w)	69	129
PAA-microspheres degraded 0.5 h	1814	1745	1694 (s)	147	183

molol maleate was not detected in the temperature range studied, and the thermogram for empty microspheres was identical to that for the loaded microspheres.

The morphology changes during the degradation were also monitored by SEM-micrographs (Fig. 5). During the first hour in a phosphate buffer, the surface including the surface of the inner cavity became progressively more rough. It seemed that the amorphous parts disappeared, first leaving crystal-like outgrowths emerging from the matrix. After 2 h, most of the spheres had collapsed and the remaining crystalline regions had formed a fine network.

Degradation Mechanism

GC–MS analysis was used to investigate the degradation products from the polymer in the degradation medium. A good correlation was achieved when the weight loss of degraded samples was compared with the rate of production of the degradation product, adipic acid. Figure 6 shows that the degradation rate was approximately constant throughout the process, implying that the degradation product, adipic acid, was set free as soon as it was formed. This indicates that the PAA-microspheres were surface eroded, rather than bulk eroded. The assumption that this degradation is a surface phenomenon is linked to the theory that the acidic degradation products inhibit further hydrolysis of the anhydride bonds in the bulk.[34] The dense shell-matrix of the PAA microspheres, referred to in connection with the SEM examination, hinders water diffusion into the bulk, and makes the degradation on the surface the most dominant. Langer and his co-workers[27,30,31] have extensively studied poly(sebacic anhydride) and the copolymer of 1,3-bis-(carboxyphenoxypropane) and sebacic acid. By varying the ratio of the aliphatic to the aromatic monomers in the copolymer, they have shown that the hydrophobicity and the crystallinity of the polymer are two of the most important factors controlling the type of erosion taking place. Both high

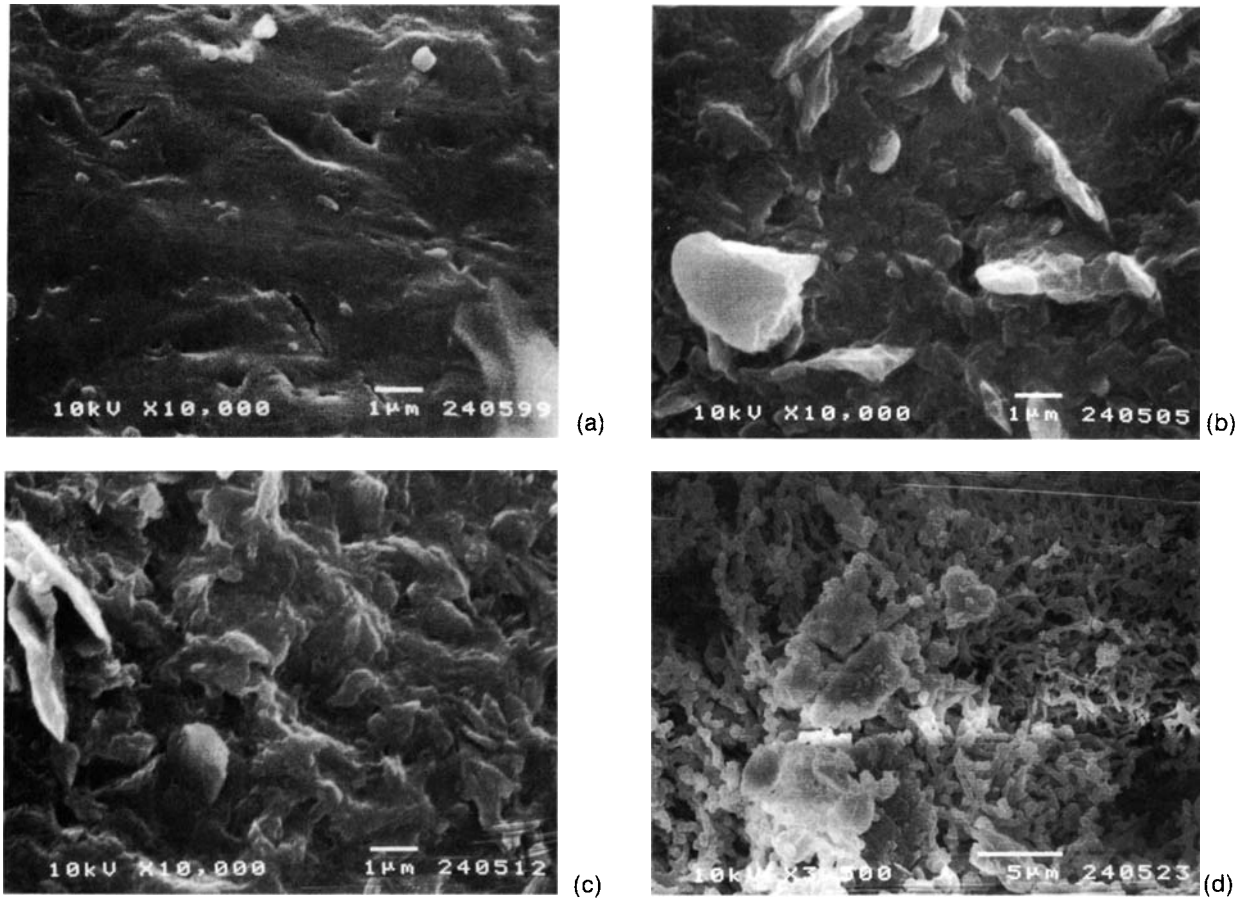


Figure 5 SEM micrographs showing the external surface of the PAA microspheres at different degradation stages, (a) immediately after preparation, (b) after 0.5 h, (c) after 1 h, and (d) after 2 h of degradation in pH 7.3 phosphate buffer at 37°C.

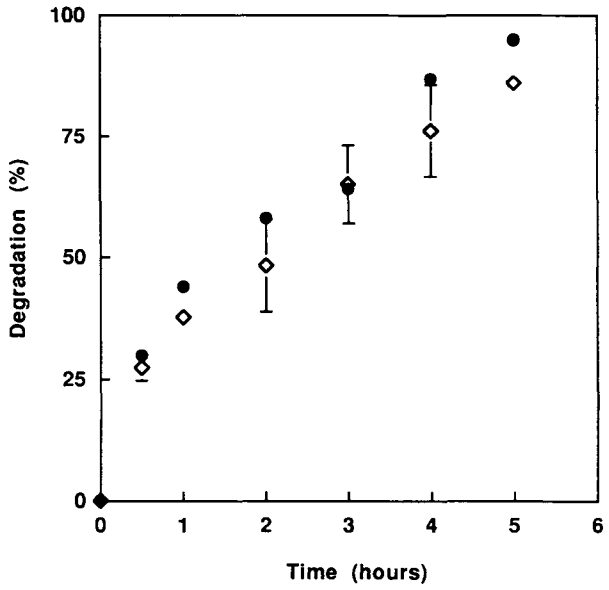


Figure 6 Degradation of PAA microspheres in phosphate buffer at 37°C measured as (●) percentage of maximum released adipic acid and as (◇) percentage weight loss.

hydrophobicity and high crystallinity prevent water diffusion into the polymer bulk and, thus, prevent bulk erosion. The aliphatic PAA exhibits a hydrophilic backbone that usually facilitates water uptake but it still seems to be surface eroded. This deviation may be explained by the high crystallinity and the noticeable high reactivity of the anhydride linkage of the PAA, which prevent bulk erosion. Furthermore, and important for an *in vivo* application, the degradation product of the polymer, adipic acid, was not expected to have any adverse effects in the precorneal area.[35] After systemic absorption, the adipic acid is expected to be metabolized by the normal β-oxidation of fatty acids.[36]

In Vitro Release of Timolol Maleate

Microspheres

The release of timolol maleate from 5% loaded microspheres was studied at 25, 37, and 60°C (Fig. 7). The drug release was strongly temperature dependent. At 25°C most of the drug was released in 24 h, whereas at 37°C and 60°C the release was complete after 7 and 2 h, respectively. The release rates were consistent with the hydrolytic sensitivity of PAA seen in the degradation studies. During the degradation of the microspheres, the buffer capacity was exceeded due to adipic acid formation. The decrease in pH could, therefore, be expected to correlate with the formation of adipic acid, i.e., to the degradation rate. By measuring the pH and the concentration of timolol maleate, it was found that the correspondence between the drug release rate and the rate of formation of adipic acid was greater at higher temperatures (data not shown). The degradation and release rates at 60°C were rapid and simultaneous. The drug release at 37°C was the most interesting for the intended application, and the rate of formation of adipic acid at 37°C was, therefore, more carefully measured using GC–MS. As shown in Figure 8, the release of timolol closely followed the formation of adipic acid, i.e., the polymer erosion. These results indicate that poly(adipic anhydride) microspheres loaded with timolol maleate form an erosion-controlled drug-release system. A dominantly erosion-controlled release mechanism was also observed in drug release experiments by Leong et al.[27] from copolymer compositions with a hydrophobic backbone. Characteristic for the surface-eroding polyanhydrides also recognized here is the approximately constant release rate. Bulk-eroding polyesters, such as poly(lactic-*co*-glycolic) acid, often reveals an S-shaped release profile at low loading.[23–25] However, it should be noted that, at 25°C, diffusion of the drug seemed to be involved to some extent, as the release rate was slightly faster than the decrease in pH.

Microsphere-Gel Formulation

The release of timolol maleate from this microsphere-gel formulation was compared to the release

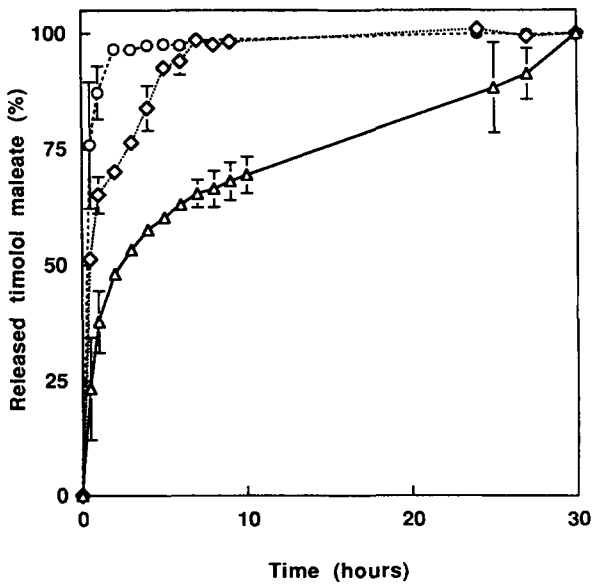


Figure 7 Timolol maleate release from PAA microspheres in pH 7.3 phosphate buffer at different temperatures: (△) 25°C; (◇) 37°C; (○) 60°C.

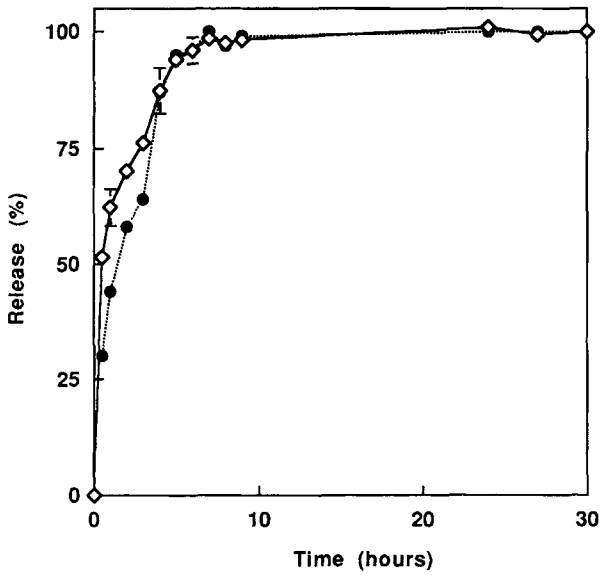


Figure 8 Degradation profile of PAA microspheres and their drug release in pH 7.3 phosphate buffer at 37°C: (◇) timolol maleate, (●) adipic acid.

of timolol maleate from PAA-microspheres and from the gel (Fig. 9). The fastest release was displayed when free timolol maleate had been incorporated into Gelrite®. In 1 h, 83% timolol maleate was released, whereas only 48% timolol maleate was released from the microspheres in the same time when they were incorporated in the Gelrite® gel. The gel retained the water-soluble drug to some extent due to restricted diffusion. This observation was in agreement with previous results.[5,37] However, when the drug was encapsulated in the PAA-matrix, the release from the gel was instead predominantly controlled by the polymer degradation rate. The encapsulation of the timolol maleate thus extended the time for release. The lower rate of release observed at the initial stage of the release for the microspheres in the gel than for the microspheres alone was probably caused by a lower convection around the microspheres in the gel. The release of timolol maleate from PAA microspheres alone resulted in 68% timolol maleate release in 1 h, i.e., 20% more than for the microsphere-gel formulation. A disadvantage of the use of particle-suspensions in the eye might be drainage of the particles, leading to an incomplete drug release. The major advantage of using a gel formulation containing microspheres is the improved precorneal retention of the microspheres during administration.[3]

It was shown that the incorporation of timolol maleate into PAA microspheres is a possible way of extending the presence of the drug in the gel formulation. Provided that the gel remains in the eye during the time for drug release, this system may result in a precorneal residence time of several hours. As a result, a higher bioavailability of timolol maleate is expected for this novel formulation particularly in relation to conventional eye-drop solution.

CONCLUSION

The results confirmed that the aliphatic poly(adipic anhydride), PAA, is a suitable degradable matrix for precorneal drug release. Poly(adipic anhydride) microspheres with an average diameter of 40 μm and loaded with 5% timolol maleate were prepared using the nonaqueous solvent removal technique. Changes in the morphology, such as lamellae segregation and a decrease in crystallinity, were found in the polymer after the microsphere preparation. Those changes were probably caused by fast precipitation of the microspheres. In the SEM examination, it was seen that the interior of the spheres were hollow. However, the polymer matrix retained a dense structure, which is vital for the surface-eroding mechanism. Degradation of the PAA-microspheres in a phosphate buffer led to a substantial increase in crystallinity of the matrix and gave rise to the formation of adipic acid as degradation product. The rate of production of the degradation product was approximately constant, suggesting that these mi-

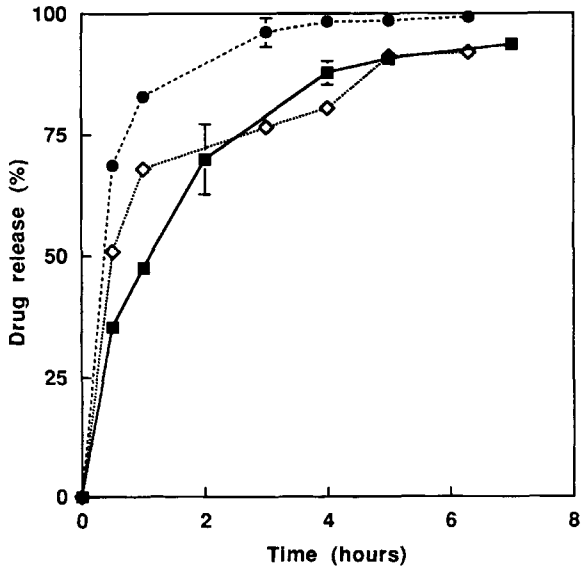


Figure 9 Comparison between timolol maleate release from (●) Gelrite®, (◇) PAA microspheres, (■) PAA microspheres incorporated into Gelrite® in pH 7.3 phosphate buffer at 37°C.

crospheres displayed surface erosion. The release of the incorporated drug, timolol maleate, was predominantly controlled by the erosion of the polymer. The timolol maleate was completely released from the microspheres after 7 h at 37°C. The PAA-microsphere system itself provided a suitable release profile for ocular drug delivery. However, the initial drug release rate was decreased to some extent when the PAA-microspheres were incorporated into an *in situ* gelling polysaccharide, Gelrite®. The expected improved ocular bioavailability of this novel microsphere-gel drug delivery formulation remains to be compared with that of ordinary eye drops.

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