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Preparation of biodegradable poly(lactic-co-glycolic) acid microspheres and their in vitro release of timolol maleate

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Summary

Poly(lactic-co-glycolic) acid, PLG, 50:50 microspheres containing homogeneously dispersed timolol maleate were prepared in an oil-in-oil solvent evaporation method. By varying the PLG concentration in the microsphere preparation, different particle sizes were obtained. A constant drug loading of about 2% (w/w) independently of PLG and timolol maleate concentration was obtained. As the drug loading was low and the particles exhibited a slow release of drug, it is suggested that the release of drug was due to polymer degradation rather than to pore diffusion. Timolol release was initiated with a small burst. After a lag time, a second period of extensive and rapid release of drug was observed. This secondary burst was accompanied by the collapse of the polymer matrix. The release from microspheres with polyethylene glycol (PEG) incorporated showed no secondary burst. After the secondary burst there was very little, if any, further release of drug. The presence of detergent in the release medium resulted in a smooth and relatively fast release rate from the particles. In this case no secondary burst was detected.

Introduction

In pharmaceuticals, in order to achieve effectively an optimal medical effect, it is important to be able to control the concentration of drug and the timing of the medicament at the site of action. Some drugs, e.g., anti-infectives and peptides, may require a prolonged duration of action in order to fulfil their intended effect. To obtain an appropriate dosing of these drugs, one may design formulations which provide their control.

One approach to the development of such systems involves the use of a biodegradable polymer matrix in which the drug is incorporated. This system may be designed as an implant or as a dispersed system such as microspheres depending on the therapeutic situation. Biodegradable polymers may be defined as synthetic or natural polymers, which are degradable in vivo, either enzymatically or non-enzymatically, to produce biocompatible or non-toxic by-products. An important family of biodegradable polymers are the polyesters of lactic acid, glycolic acid and poly(lactic-co-glycolic) acid, PLG. The polymers are from the beginning water-insoluble but during the degradation process hydrolytic backbone cleavage occurs and the polymers are decom-

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posed into small water-soluble non-toxic molecules.

The release characteristics from the PLG matrix are influenced by several factors, the degradation rate being one example. By varying the molecular weight or the ratio of glycolic acid in the polymer the degradation rate can be varied.

A lower molecular weight results in a higher rate of degradation, since low molecular weight polyesters are accompanied by an elevated amount of carboxylic end groups, which leads to an enhanced degree of hydrophilicity and, therefore, an increased degradation rate (Wise et al., 1987; Hutchinson and Furr, 1990).

By increasing the proportion of glycolic acid in the copolymer PLG, the crystallinity can be reduced to complete amorphousness. The P(D,L)LG copolymers are amorphous for glycolic acid content between 0 and 70%. The incorporation of this more hydrophilic component facilitates the penetration of water into the matrix and consequently increases the degradation rates (Gilding and Reed, 1979).

For partly crystalline polymers, an increase in crystallinity is observed during the process of degradation, since degradation occurs in two stages. Initially water diffuses into the amorphous regions and hydrolytic degradation occurs; the second stage is due to hydrolytic degradation in the crystalline domains. Even for intrinsically amorphous and semi-crystalline polymers Vert et al. (1991) observed progressive crystallization during degradation, leading to crystallized oligomeric degradation products, which appeared much more resistant to degradation than the initial amorphous material.

If the degradation of the polymer were to occur only at the surface of the microsphere, and if the drug were to be uniformly distributed throughout the whole matrix, there would be a one to one relationship between the drug release and the weight loss of the polymer. Among others, Heya et al. (1991a) have shown that although the drug release rate was correlated to the erosion of the polymer, the drug release rate was much more rapid than the weight loss of the polymer, suggesting that the drug was able to diffuse through pores as a result of the bulk degradation in the polymer matrix. It was much more prominent in microspheres prepared with a PLG of low molecular weight, probably due to the higher hydrophilicity. It has been shown that for thick polymer samples the bulk degradation proceeds heterogeneously and occurs more rapidly in the centre than at the surface (Li et al., 1990a,b).

Drug loading also affects the release mechanism and release rate. In the case when the drug content is very low, and the drug is loaded as small (solid) crystals or phase separated in the polymer, the crystals are finely dispersed in the polymer matrix with no contact between each other. Solvent and/or drug molecules must diffuse through the polymer matrix to allow drug release, which consequently leads to slow release. In practice, release follows the degradation of PLG. In microspheres with considerably higher drug loading, all the drug entrapped in the polymer forms a network, where the majority of the crystals are in contact with each other and the drug is released because of dissolution-diffusion, the polymer matrix acting as a drug reservoir only.

Most of the experiments reported on drug release from PLG systems have been performed at rather high drug loading ratios, at which the drug is released via pore diffusion rather than by the erosion process. It was the purpose of this work to determine whether the release of timolol maleate from poly(lactic-co-glycolic) acid (PLG) microspheres could be controlled by particle erosion, which would be expected at low loading ratios. The effect of different conditions for preparation of microspheres on the drug release kinetics was also investigated. We have further studied the effect on drug release due to the presence of surfactant in the release medium.

Materials and Methods

Materials

The polymer used was poly(DL-lactic acid-co-glycolic acid) 50:50, inherent viscosity 0.2, purchased from Boehringer Ingelheim. Timolol maleate salt was obtained from Sigma. Span 80

and Tween 80 were obtained from Speciality Chemicals, ICI. The sesame oil was purchased from Apoteksbolaget, Stockholm, Sweden. *n*-Hexane and acetonitrile were obtained from Merck. Polyethylene glycol (PEG, M_w 6000) was from Farbwerke Hoechst AG. All compounds were used without further purification.

Methods

Microsphere preparation

Microspheres were prepared using a solvent evaporation method. An oil-in-oil system based on the method described by Hyon and Ikada (1991) for microspheres comprising polylactic acid and a water-soluble drug was adopted for PLG. 0.25–1.0 g PLG was dissolved in 4.5 ml acetonitrile. This solution was mixed with a second solution containing a known amount (0.025 g) of timolol dissolved in 0.5 g distilled water. These two solutions were carefully mixed. A few preparations were made without water. The PLG solution was added dropwise to 50 g sesame oil containing 2% (w) Span 80 under vigorous stirring. To decrease the particle size sonication was carried out. The emulsion was stirred at 50°C for 1 h or at 35°C for 2 h during evaporation of acetonitrile and water. The microspheres were washed three to four times with hexane. To avoid aggregation and to reduce the initial burst during the release experiments the particles were finally washed with water containing 0.1% Tween 80 and then vacuum dried. In some cases the microspheres were sieved during this last washing process.

Microscopy studies

The Nicon Microscope Optiphot, used to evaluate the size of the particles and to follow the degradation of the particles, was equipped with a polaroid camera. The size was determined as the average size of the particles in the micrograph. In some preparations the size was verified with a Sympatec Helos Vectra particle size analyser.

Determination of timolol content in the microspheres

A weighed amount of microspheres was dissolved in acetonitrile. The timolol content was then assayed spectrophotometrically at 295 nm (Perkin-Elmer, lambda 2, UV/Vis spectrophotometer).

In vitro release studies

The release of timolol from various microsphere systems was studied under sink conditions by using a pH 7.3 phosphate-buffered aqueous medium at 23°C. Each batch of particles was divided into glass tubes, where they were suspended in the buffer solution. The dispersion was stirred with magnetic bars. The tubes were periodically collected, the dispersion was centrifuged and the buffer was analysed for timolol content. One release experiment was performed in phosphate buffer containing 0.1% Tween 80.

Results and Discussion

Microsphere preparation

Temperature

During preparation the drug matrix had a tendency to be sticky, leading to aggregation of the microspheres. This was probably due to the high solvent evaporation temperature, 50°C. The T_g for the polymer is 40–55°C according to the manufacturer. In later studies the temperature was lowered below T_g resulting in somewhat less aggregation.

Another possible way to reduce the stickiness would be to modify the polymer thermal properties to some extent. It is possible to shift the T_g to higher temperatures by its conversion to salt (Wada et al., 1991).

Emulsifying effect and addition of water

By sonication of the emulsion before solvent evaporation, the droplet size was reduced, resulting in a smaller size of the microspheres obtained (cf. Fig. 1a with b and Fig. 1c with d, respectively).

Inclusion of water in the particles had a dramatic influence on the particle size. By using water in the particle preparation, the particle diameter became significantly larger than without water (cf. Fig. 1a with c and Fig. 1b with d, respectively. This large particle size can be ex-

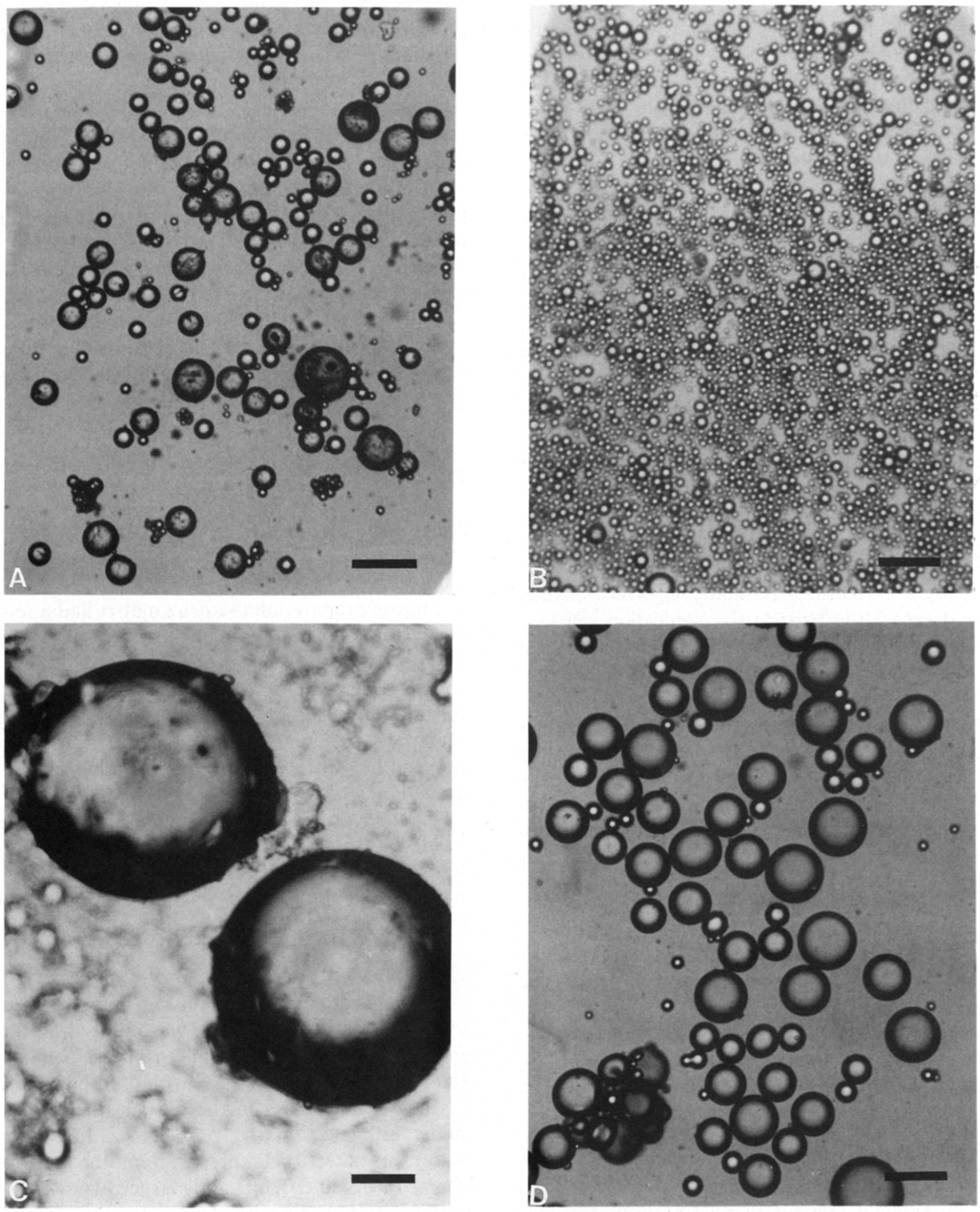

Fig. 1. Effect of emulsification and inclusion of water. Processing: (A) without sonication, without water; (B) with sonication, without water; (C) without sonication, with water; (D) with sonication, with water. Bar, 100 μm.

plained by a higher surface tension for the emulsion droplets, since a larger energy is required to increase the surface area when the surface tension is higher.

PLG concentration in the dispersion phase

The preparations discussed below are summarized in Table 1. In order to observe the effect of the PLG level, three different concentrations (5, 10 and 20% (w/v)) of PLG were used in the preparation of the microspheres. It was found that an increase in the PLG concentration resulted in a larger particle diameter (Fig. 2). Assuming that the emulsion droplets are not affected by the PLG concentration and that the matrix of the prepared microspheres are of the same density independent of the PLG concentration it is expected that the particle diameter will vary as 1, 1.3 and 1.6 as the PLG content in the droplets varies as 1, 2 and 4 respectively. Experimentally it was found for PLG concentrations 5, 10 and 20% (w/v) that the average particle diameter for the corresponding microspheres was 90, 130 and 160 μm, respectively (Table 1) which is in agreement with the theoretically expected values.

Drug loading

As the PLG concentration in the dispersion phase in the emulsion was varied (5, 10 and 20% (w/v)) (Table 1), the timolol concentration was kept constant (0.5% (w/v)). Thus, the theoretical timolol content in the particles decreased with increasing PLG concentrations (9.4, 5.0, and 2.6% (w/v)). However, the actual timolol content did not decrease as might have been expected, but was found to remain constant at about 2% (w/w). This might be due to ion interaction between amino groups on the timolol and carboxy end groups on the PLG. Nevertheless, the drug yield (actual timolol content $\times$ 100/theoretical timolol content) was increased with an increasing PLG concentration.

Drug release

We observed a triphasic release behavior of timolol in our study (see Fig. 3). Initially, a burst was observed due to release of drug located near the microsphere surface. Then there was a period of slow release when the degradation medium diffused into the polymer matrix, degradation occured and the drug diffused out of the microspheres. The third phase, a secondary burst, oc-

TABLE 1

Summary of experimental data

PLG concentration in solution (w/v%)	Timolol concentration in solution (w/v%)	Timolol content in spheres (w/w%)	Theoretical timolol content (w/w%)	Average diameter (μm)	Drug yield (%)	Drug release (% of included drug) $t \approx 5$ weeks
5	0.5	2.3	9.4	91	24	40.3
5	0.5	2.2	9.4	9	24	36.2
5 [b]	0.5	2.2	9.5	68	21	39.8
5 [b,c]	0.5	2.3	9.1	10–120	23	37.7
10	0.5	2.0	5.0	98	39	26.0
10 [a]	0.5	2.2	4.9	140	42	31.3
10 [a]	0.5	2.1	5.0	5– 20	41	34.0
10 [b]	0.5	1.8	4.8	230	36	24.8
10 [b,c]	0.5	3.8	4.8	50–210	75	32.0
10 + 10% PEG	0.5	2.0	4.8	40– 80	42	28.0
20	0.5	2.2	2.6	161	84	33.7
20 [b,c]	0.5	2.4	2.3	50–600	100	32.0 [d]

[a] Sonicated after preparation. [b] Produced at 35°C. [c] Released in 0.1% Tween solution. [d] $t \approx 2$ weeks.

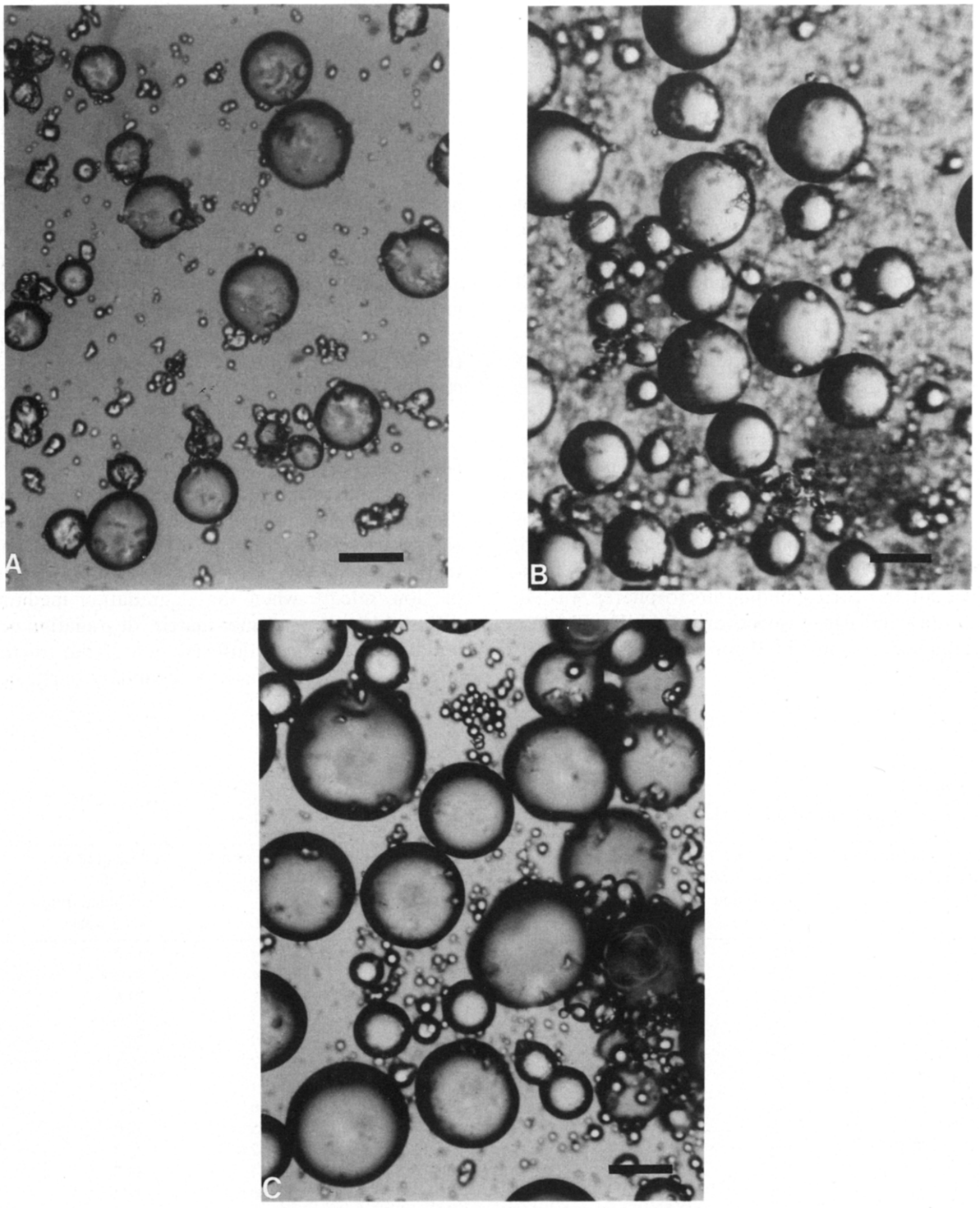

Fig. 2. Micrographs from (A) 5% PLG, (B) 10% PLG, (C) 20% PLG. Bar, 100 μm.

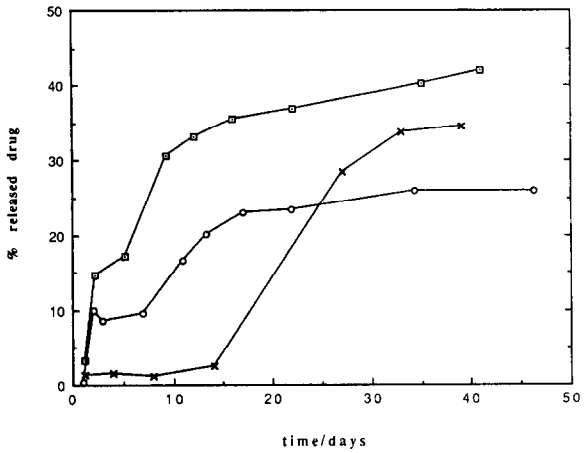

Fig. 3. Timolol released from PLG particles showing triphasic behavior and the effect of PLG concentration in the preparation: (□) 5% PLG, (○) 10% PLG, (×) 20% PLG.

curred when the matrix had become more water soluble. The erosion occurred throughout the entire matrix, which became very loose and the microspheres collapsed. This triphasic phenomenon has also been observed elsewhere (Sanders et al., 1984; Spenlehauer et al., 1988).

Initial burst

An initial burst is often observed due to release of drug from the surface or release of drug located near the microsphere surface. In our study the initial burst due to timolol crystals on the surface was reduced by washing the microspheres before the release studies.

Secondary burst

For the particles containing 20% PLG, drug release started immediately with a very small burst for a few hours which corresponded to about 3% of the initial drug loading. Slow release was then observed for 2 weeks, probably following the erosion of the particles. After 14 days a massive release of timolol was observed. That a collapse of the particles really occurred during the late stages of the secondary burst was verified

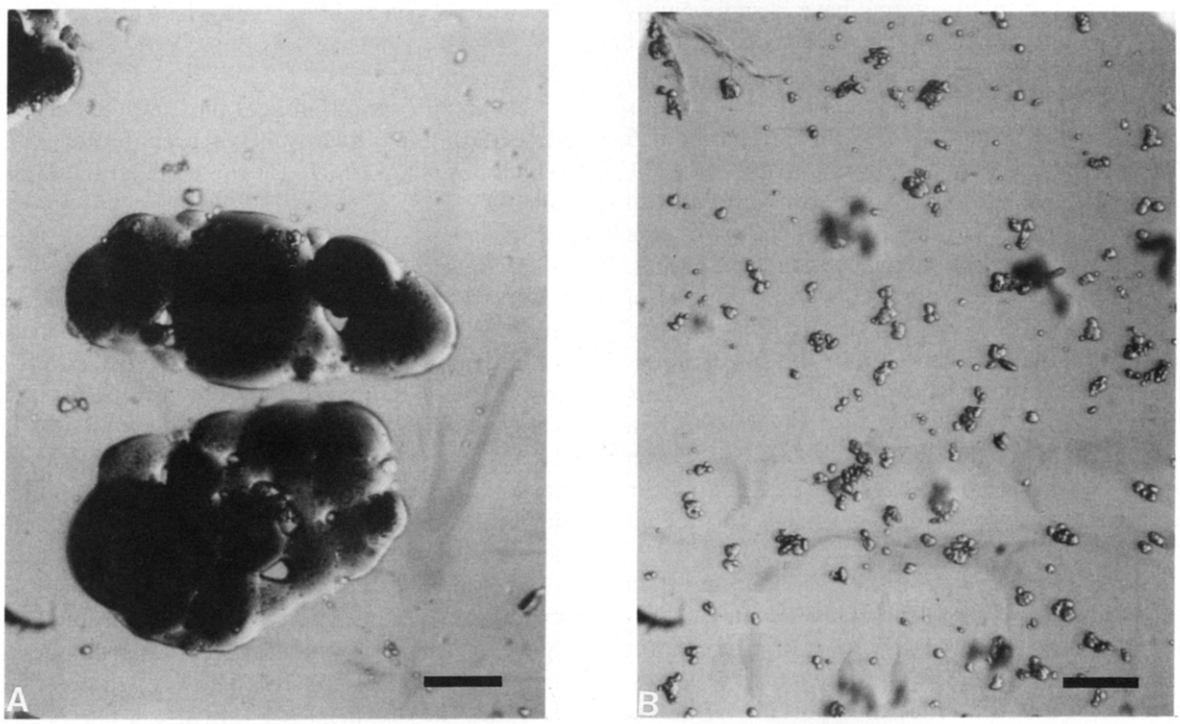

Fig. 4. Degradation of microspheres at the onset of collapse. The micrographs show the microspheres just before (A) and after (B) collapse. Bar, 100 μm.

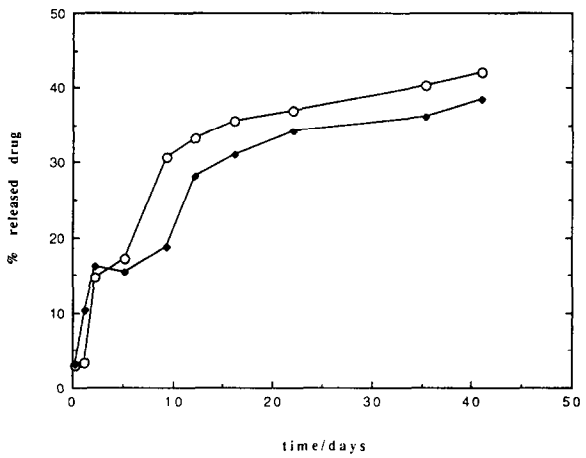

Fig. 5. Timolol released from 5% PLG particles of different sizes: (♦) smaller than 100 mm; (○) larger than 100 μm.

by subsequent microscopy studies during the drug release process. The main stages of the process can be followed in the micrographs where Fig. 2c shows the undegraded microspheres and Fig. 4a illustrates the particles after degradation for 29 days. Here the microspheres have a significantly different appearance in the microscope; after 1 additional day the particles collapsed (Fig. 4b). For 10% PLG particles this enhanced release is detected after about 10 days and after about 5 days or earlier for the 5% PLG particles (Fig. 3). These effects could be attributed to variations in the size/porosity of the particles affecting the time before the onset of collapse of microspheres. However, from the experiments with particles of different sizes, obtained by sieving (Table 1 and Fig. 5), it can be concluded that the size is unimportant when the release is studied over a long period of time, which has also been shown in previous studies (Cha and Pitt, 1988). The reason for the difference in release kinetics due to the variation in PLG concentration needs further investigation.

The size of the microspheres is important in the case where drug is incorporated on the surface or when the initial release is of interest, but as the bulk erosion of the polymer matrix begins, the surface area becomes unimportant (Baker et al., 1988; Cha and Pitt, 1988; Visscher et al., 1988).

Final stage

After the second burst, when only about 35% of the drug has been released, very little, if any, release of the drug is observed. Since crystallization has been observed in other studies during the degradation process (Vert et al., 1991) it is possible that drug might have been entrapped within this crystalline structure. In the study by Vert et al. (1991), it was shown that when the integrity of the specimens was lost, the residual crystalline matter formed during degradation appeared very resistant; therefore, this phenomenon might provide an explanation as to why complete release does not occur.

Timolol contains amino groups that can interact with the carboxy end groups of the PLG matrix. Such ion interaction might also provide an explanation for our observation that only about 35% of the incorporated timolol was released. It has been reported that a basic drug can be retained in the microsphere as a result of the interaction between the hydrolysed carboxy end groups of PLG and the cationic residues of the basic drug (Cha and Pitt, 1988; Heya et al., 1991b).

Temperature

No significant differences in drug release were detected among microspheres prepared at 35°C, which lies below the T_g for the polymer (cf. Figs 3 and 6).

Additive

The readily soluble additive, polyethylene glycol (PEG), was included to enhance the release rate. This was carried out in order to provide a system with a greater content of water-soluble substances in the PLG matrix. This should increase the extent of water transport into the matrix which can result in two effects: it can facilitate the initial stage of hydrolysis and offer less resistance to further diffusional release of timolol. Theoretically, the prepared particles are capable of acquiring a PEG content of 10%, however, the experimental yield was not determined. It is expected that the amount of PEG included in the particles will affect the release rate. An enhanced rate of release was not observed when PEG was added, however, the re-

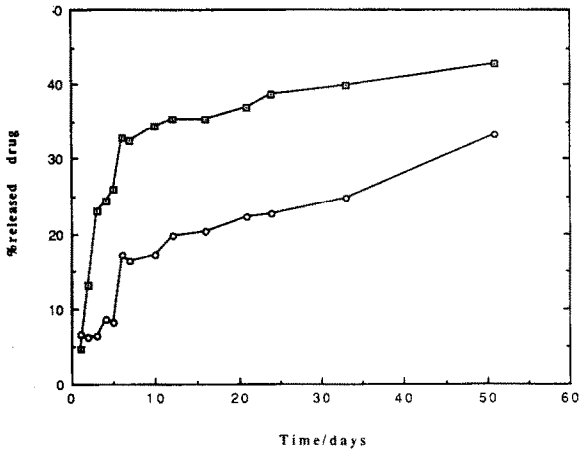

Fig. 6. Timolol released from PLG particles produced below T_g: (□) 5% PLG; (○) 10% PLG.

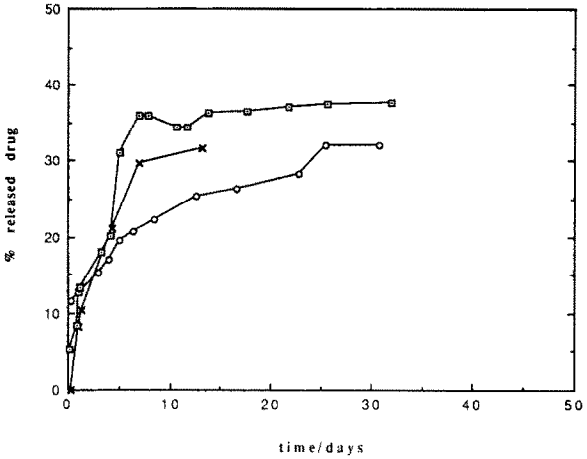

Fig. 8. PLG particles produced below T_g: (□) 5% PLG; (○) 10% PLG; (×) 20% PLG. Timolol release was analyzed in phosphate buffer containing Tween 80.

lease rate became relatively constant in contrast to the triphasic behaviour observed in the absence of PEG (Fig. 7). The microscopy studies revealed that the microspheres were undergoing gradual collapse throughout the release process.

Sonication

Some particles were sonicated after preparation in order to disperse the microspheres in the release medium. It may be concluded that the particle surface was slightly disrupted, however, no significant effect on the rate of release was observed (Fig. 7).

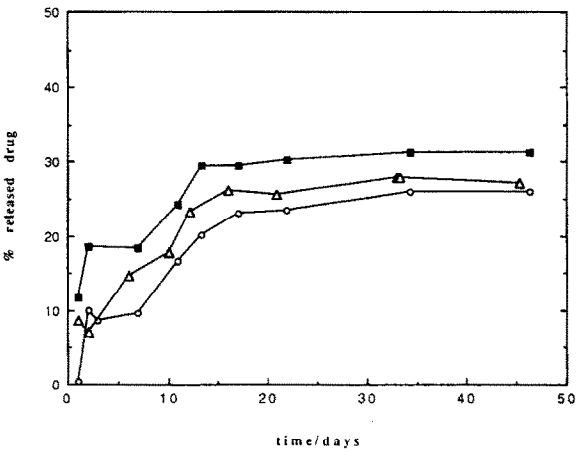

Fig. 7. Timolol released from 10% PLG particles: (○) 10% PLG; (△) 10% PLG + PEG; (■) 10% PLG, sonicated.

Release medium

Microspheres were prepared at 35°C as described above. Analysis of drug release was carried out in phosphate buffer containing 0.1% Tween 80. The release profiles (Fig. 8) were smoother than that from microspheres degraded in the absence of surfactant and represented a faster release rate. No secondary burst was detected, a finding which is consistent with the results of microscopy in which very early collapse of the microspheres was demonstrated. The detergent adsorbs on the microsphere surface, resulting in the surface being changed from hydrophobic to hydrophilic in nature. The reversal of surface polarity enhances the degree of wetting of the microspheres. Furthermore, the detergent acts as a solubiliser of hydrophobic polymer fragments. These two effects lead to the more rapid collapse of the particles. Since biological systems contain surface-active substances, such an effect should be taken into account when in vitro and in vivo results from degradation studies of PLG are compared.

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