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Effect of Solvent Composition During Preparation on the Characteristics of Enoxacin Microparticles

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Abstract

We have studied the effect of the solvent system during preparation on the morphology, encapsulation efficiency, and release characteristics of enoxacin microparticles intended for localized delivery to the bone for the treatment of bone infections.

Microparticles of enoxacin were formulated using poly(glycolic acid–co–DL–lactic acid) (PGLA) of different viscosity grades by the solvent–evaporation technique. Microparticles prepared with pure dichloromethane had smoother surfaces and less tendency to aggregate than microparticles prepared with dichloromethane–acetone solvent mixtures, which had porous surfaces.

Approximately 65% of the microparticles prepared with pure dichloromethane were $< 125\,\mu$m in diameter compared with 16% (approx.) of microparticles prepared with dichloromethane–acetone mixtures. Increasing the proportion of acetone from dichloromethane–acetone, 10 : 0, to dichloromethane–acetone, 1 : 1, resulted in an increase in encapsulation efficiency from 25 to 37%, and an increase in the yield of microparticles harvested from 39 to 51%. Although a further increase in the amount of acetone to dichloromethane–acetone, 1 : 9, had no significant effect on the yield, aggregation, or fraction of microparticles below 125 μm in diameter, the encapsulation efficiency increased to 56%. Approximately 55% of enoxacin was released in 24 h for microparticles prepared with dichloromethane–acetone, 1 : 9, compared with 100% release in 10 h and 2 h for microparticles of the same size range prepared with dichloromethane–acetone, 1 : 1, and dichloromethane–acetone, 10 : 0, respectively.

The results suggest that the composition of the dichloromethane–acetone solvent system significantly influences the encapsulation efficiency and the rate of release of enoxacin from microparticles. This is important for the formulation of sustained-release enoxacin microparticles for the localized treatment of osteomyelitis.

Fluoroquinolones are antimicrobial agents with a high potency against several Gram-negative and Gram-positive pathogens (Gotoh et al 1995; Soboh et al 1995). They are the drugs of choice for the treatment of osteomyelitis, because they have activity against the commonly involved bacterial pathogens and can penetrate bone and furnish adequate levels (Gentry & Rodriguez-Gomez 1990, 1991; Lew & Waldvogel 1995).

The treatment of osteomyelitis with a biodegradable, parenteral controlled-release ciprofloxacin system was reported by Overbeck et al (1995). Localized delivery of an antibiotic to the infected site by use of biodegradable controlled-release drug-delivery systems administered close to the site of bone infection, will enable the infection to be exposed to the antibiotic for a prolonged period of time. Polylactic and polyglycolic acids, including their copolymers, are the biodegradable polymers most studied for the controlled release of biologically active agents, because the monomers are intermediates in mammalian energy metabolism. These polymers are histocompatible and have been established by Food and Drug Administration licensure and years of use as absorbable sutures for medical application (Morris et al 1994). The use of these polyesters also obviates the need for surgical removal and makes them suitable for encapsulation of bioactive agents for parenteral drug delivery.

The method commonly used for the encapsulation of bioactive materials in the preparation of microparticles is the solvent–evaporation techni-

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que (Bodmeier & McGinity 1987, 1988; Jeffrey et al 1991, 1993; Conti et al 1992; Sansdrap & Moes 1993; Song et al 1997). Although the procedure is very easy to accomplish, several factors influence the type of microparticle formed and the release kinetics. Factors that influence the type of microparticle obtained include the phase volume of the emulsion, drug solubility, type of organic solvent, polymer–solvent–non–solvent interactions, temperature, rates of solvent diffusion, type and concentration of emulsifier, polymer composition, viscosity or molecular weight, and drug loading (Bodmeier & McGinity 1987, 1988 ; Jeffrey et al 1991, 1993 ; Conti et al 1992; Sansdrap & Moes 1993; Song et al 1997).

Different solvent systems have been used for the preparation of microparticles by the solvent–evaporation technique (Conti et al 1992). In the preparation of an oil-in-water (o/w) emulsion, the immiscibility of the organic phase with the aqueous phase is essential because water-miscible organic solvents such as acetone or DMSO do not form microdroplets upon emulsification but form irregular agglomerates (Bodmeier & McGinity 1988). The encapsulation efficiency during solvent–evaporation procedures for preparation of o/w emulsions is greatest for drugs that are insoluble in the aqueous medium (Bodmeier & McGinity 1987). Different solvent mixtures have been used to increase encapsulation efficiency, notably dichloromethane–acetone or dichloromethane-DMSO mixtures, because of the higher rate of polymer precipitation (Bodmeier & McGinity 1988; Conti et al 1992). However, no detailed study has been undertaken to determine the effect of solvent composition during preparation on the surface morphology and release rates of the microparticles formed. The purpose of this study was to find the optimum dichloromethane–acetone solvent mixture for preparation of enoxacin microparticles with the lowest release rate which could be applied for localized treatment of osteomyelitis.

Materials and Methods

Materials

Poly(glycolic acid–co–DL–lactic acid) (PGLA), composition 50 : 50, of various viscosity grades were supplied by Birmingham Polymers (Birmingham, AL). Poly(vinyl alcohol) (PVA) (MW 30 000–70 000) and enoxacin were obtained from Sigma (St Louis, MO). Dichloromethane (HPLC grade), methanol (reagent grade), and acetone (regent grade) were supplied by Fisher Scientific (Norcross, GA). Water was distilled and demineralized. All materials were used as supplied.

Preparation of microparticles

Microparticles were prepared by the solvent–evaporation technique. Poly(vinyl alcohol) was dissolved in water to furnish a 1% w/v solution. PGLA (500 mg) was dissolved in dichloromethane, acetone or dichloromethane–acetone mixtures (10 mL), and enoxacin (300 mg) was dispersed in the PGLA solution. Afterwards, the drug-polymer dispersion was added to the PVA solution (25 mL) with stirring at 800 rev min^{-1} with a Lightnin Mixer (General Signal, NY). After 10 min the stir rate was reduced to 400 rev min^{-1} and the organic solvent was evaporated overnight. The microparticles were collected by filtration, washed gently with a methanol–water mixture, and dried in air for two days. All products were sieve-sized by use of a combination of 40, 60, and 120 mesh US-standard sieves. Fractions between 40 and 60 mesh (250–425 μm), between 60 and 120 mesh (125–250 μm), and from 120 to 0 mesh (< 125 μm) were collected and used for further studies. The effects on the kinetics of enoxacin release of the composition of the organic solvent during preparation, and of the viscosity of PGLA, were investigated.

Morphology of microparticles

The surface morphology, shape and size of enoxacin microparticles were determined by means of a Jeol JSM-840 scanning electron microscope (SEM; Jeol, Japan). Before analysis, microparticles were coated with gold-palladium in a sputter-coating apparatus. The internal structure of the microparticles was revealed by dispersing the microparticles in glue and then cutting the dried matrix with a razor blade. The cross-section was coated with gold palladium and observed by SEM.

Determination of encapsulation efficiency

Enoxacin microparticles (10 mg) were dispersed in dichloromethane (5 mL) which dissolved the polymer but not the drug. Enoxacin was extracted several times with an aqueous solution (pH 11) and analysed spectrophotometrically. The amount of the drug in the microparticles was determined by use of an UV-1201 spectrophotometer (Shimadzu, MD, USA) at 265 nm. The encapsulation efficiency was determined as the ratio of the amount measured to the initial amount of enoxacin added during preparation.

In-vitro release of enoxacin from microparticles

The equivalent of 10 mg of the microparticles or the free drug was used in all studies. Enoxacin microparticles or the free drug were placed in distilled demineralized water (900 mL) in dissolution beakers (USP Apparatus 2), and stirred at 100 rev min^{-1} by means of a VK 7000 dissolution-testing station (VanKel Industries, Carry, NC) interfaced with a VK 8000 sampling station (VanKel) and a UV-1201 spectrophotometer. The dissolution system was programmed to collect samples automatically through full-flow filters (VanKel) at specified times from different beakers and circulate them through an UV-1201 spectrophotometer for automatic measurement of absorbance at 265 nm and recording on a computer. Each sample was automatically returned to its original beaker after each measurement. The volume of the dissolution medium in the beakers was maintained at 900 mL throughout the dissolution studies. Concentrations of dissolved enoxacin were calculated from a standard curve.

Results and Discussion

The solvent compositions used for the preparation of microparticles are shown in Table 1. It was possible to prepare microparticles with dichloromethane, but not acetone, as the only organic phase. A similar observation was made by other investigators (Bodmeier & McGinity 1988; Conti et al 1992). The addition of volumes of dichloromethane as low as 1 mL, to 9 mL acetone resulted in the formation of microparticles. The microparticles prepared with dichloromethane as the only organic phase are spherical and discrete (Figure 1A). The surfaces of such microparticles are smooth and virtually free from pores. They had the least tendency to aggregate, as indicated by the sieve-size distribution (Table 2). Approximately 65% of the microparticles were $< 125\ \mu$m in diameter compared with 16% (approx.) for microparticles prepared with dichloromethane–acetone mixtures. Use of the solvent mixture dichloromethane–acetone, 1 : 1, for the preparation resulted in slightly larger microparticles with pores on the surface (Figure 1B). Further increasing the proportion of acetone, to dichloromethane–acetone, 1 : 9, did not seem to increase the size of microparticles or the number of surface pores (Figure 1C). However, the use of PGLA of lower viscosity, i.e. 0·70 and 0·19 dl g^{-1} resulted in microparticles with distorted surfaces compared with those prepared with PGLA 1·32 dl g^{-1} when the solvent composition was dichloromethane–acetone (1 : 9) (Figures 1D, E). It is apparent from Figure 1 that the composition of the organic solvent during preparation influences the size and external morphology of microparticles produced. The differences between the surface morphology and sizes of the microparticles could be explained by the solubility and miscibility of the organic solvent system with the aqueous phase. Dichloromethane is immiscible with water, but slightly (1·96%) soluble in water (Horvath 1982). This immiscibility enables microdroplets to be formed in the presence of an emulsifying agent. The volume of the aqueous phase used for the preparation, 25 mL (compared with 10 mL of dichloromethane), is too small to enable significant diffusion of dichloromethane into the aqueous phase. Therefore, the lowest rate of polymer precipitation occurred when dichloromethane was the only organic phase used for the preparation of the microparticles. Consequently, smooth microparticles were formed with virtually no pores on the surface. Reducing the amount of dichloromethane from 10 mL to 5 mL coupled with the addition of 5 mL acetone, results in a rapid precipitation of the polymer. Acetone is miscible with water, and the rapid extraction of acetone from the microdroplet into water leads to the formation of a number of pores on the surface of the microparticles. Further reduction of the amount of dichloromethane to 1 mL, with addition of 9 mL acetone increases the rate of polymer precipitation because of massive efflux of acetone from the microdroplets. It was possible to form micro-

Table 1. Solvent composition used for the preparation of enoxacin microparticles.

Solvent system	Dichloromethane (mL)	Acetone (mL)
1	10	0
2	5	5
3	1	9
4	0	1

Table 2. Particle-size distribution of microparticles prepared with organic solvents of different composition.

Dichloromethane–acetone (mL)	$< 125\ \mu$m (%)	125–250 μm (%)	250–425 μm (%)
10 : 0	64·3 ± 2·9	20·0 ± 2·5	15·7 ± 2·6
1 : 1	16·2 ± 2·4	45·3 ± 1·1	38·5 ± 1·3
1 : 9	16·8 ± 1·5	37·7 ± 0·8	45·5 ± 0·7

Data are means ± s.e.m. (n = 3 or 4).

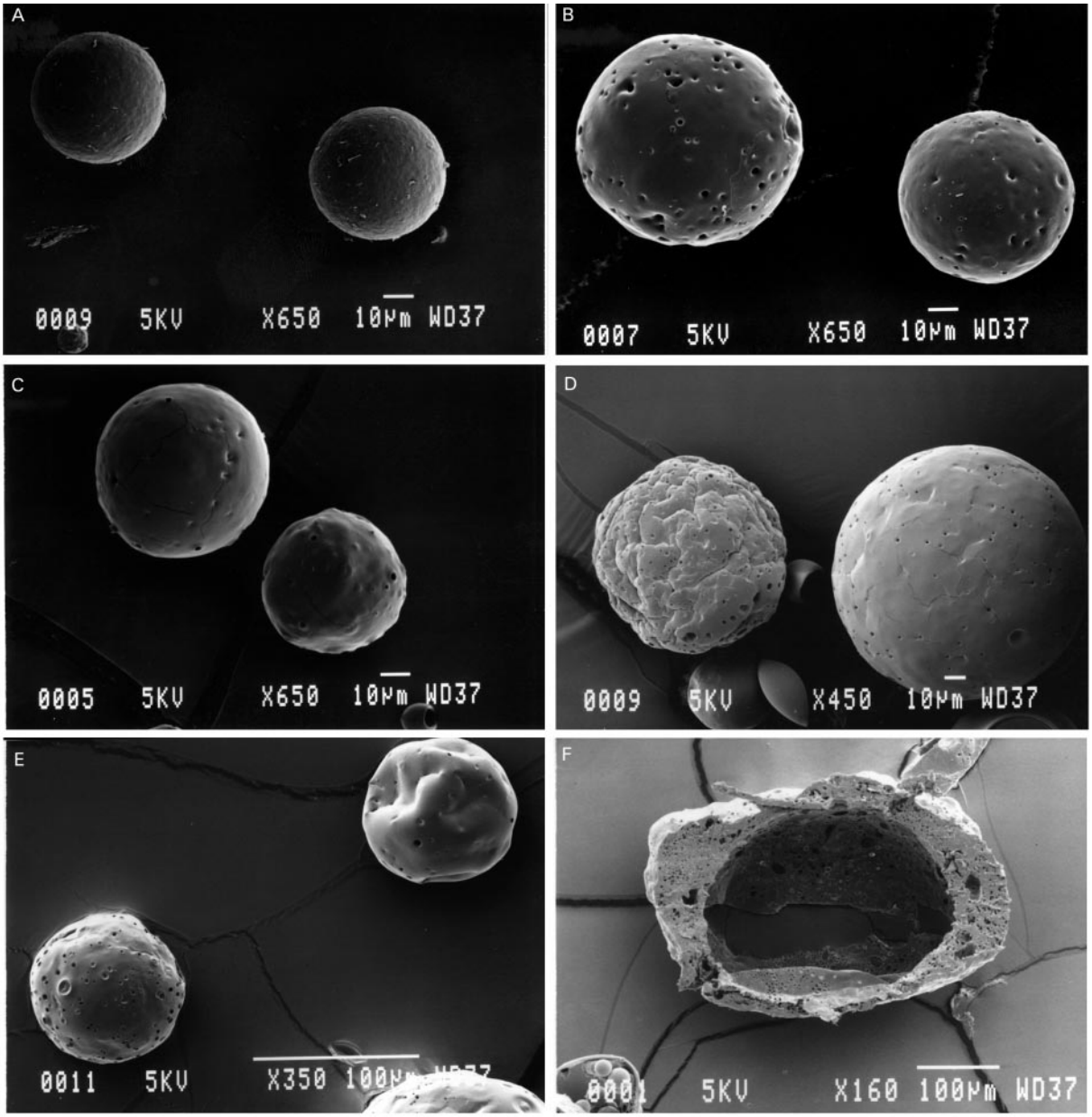

Figure 1. Scanning electron micrographs showing the effect of dichloromethane–acetone composition (mL) during preparation on the morphology of enoxacin microparticles collected in the sieve size range 250–425 μm. A. 10 : 0, B. 5 : 5, C. 1 : 9, D. 1 : 9, E. 1 : 9, F. transverse section of C. Figures A, B, C and F were prepared with PGLA of viscosity grade 1·32 dl g^{-1}. Figures D and E were prepared with PGLA of viscosity grade 0·70 and 0·19 dl g^{-1} respectively.

particles with dichloromethane–acetone, 1 : 9, because microdroplets were formed immediately after the dispersion of the polymer solution and drug was added to the aqueous phase, which was being stirred at 800 rev min^{-1}. Any attempt to reduce the dichloromethane composition below 1 mL, with addition of acetone to give 10 mL solvent mixture, did not result in the formation of microparticles.

Scanning electron microscopic examination of microparticle cross-sections shows that the microparticles are hollow with walls consisting of polymer films and pores (Figure 1F). It is not clear whether or not the drug was lost to the other half during sectioning. Furthermore, the section of the microparticle prepared with dichloromethane-acetone, 1 : 9, indicates that the thickness of the microparticle wall is not uniform, suggesting that microparticles are not highly spherical (Figure 1F).

The efficiency of encapsulation by the microparticles was dependent on the fraction of acetone in the solvent mixture during preparation (Table 3). It has been reported that the efficiency of encapsulation by microparticles prepared with dichloromethane was lower than by those prepared with a

Table 3. Efficiency of encapsulation by microparticles prepared with organic solvents of different composition.

Dichloromethane–acetone (mL)	Encapsulation efficiency (%)
10 : 0	24·9 ± 1·9
1 : 1	37·4 ± 1·2
1 : 9	55·7 ± 0·9

Data are means ± s.e.m. (n = 3 or 4).

Table 4. Yield of microparticles prepared with organic solvents of different composition.

Dichloromethane–acetone (mL)	Yield (%)
10 : 0	38·9 ± 2·8
1 : 1	51·2 ± 2·7
1 : 9	54·8 ± 2·4

Data are means ± s.e.m. (n = 3 or 4).

dichloromethane–acetone mixture (Bodmeier & McGinity 1988). In the current experiment, an increase in acetone composition at a constant internal phase volume from dichloromethane–acetone, 10 : 0, to dichloromethane–acetone, 1 : 9, resulted in an increase in encapsulation efficiency from 25% to 56% (Table 3) and an increase in the yield of microparticles (Table 4). However, no significant difference can be seen between the yield of microparticles prepared with dichloromethane-acetone, 1 : 1, and dichloromethane-acetone, 1 : 9.

The effect of organic solvent composition during preparation on the release of enoxacin from microparticles is shown in Figure 2. The higher the proportion of acetone during preparation, the slower the release of enoxacin from the microparticles. Approximately 55% of the encapsulated enoxacin was released in 24 h when the proportion of dichloromethane to acetone was 1 : 9. In contrast, microparticles prepared with dichloromethane–acetone 1 : 1 were completely depleted of enoxacin in 10 h. In the absence of acetone during preparation, the rate of enoxacin release was much higher; release was complete in 2 h. It is clearly apparent from Figure 2 that the inclusion of acetone in the organic phase during preparation plays a role in the rate of enoxacin release. The effect of solvent composition during preparation on the size and porosity of the microparticles produced does not seem to contribute significantly to these differences in release rates. The SEM pictures in Figure 1 indicate that microparticles prepared with dichloromethane as the only organic phase resulted in the formation of smooth particles without pores. It would be expected that the rate of release from such microparticles would be lower than that from porous microparticles. Although the mechanism by which increasing amounts of acetone in dichloromethane–acetone mixtures reduce the rate of release of enoxacin is not clearly understood, it is reasonable to suggest that the rate of enoxacin release from the microparticles depends on the rate of polymer precipitation during preparation, because polymer solvent composition has been shown to determine the rate of polymer precipitation (Bodmeier & McGinity 1988). Although microparticles prepared with dichloromethane–acetone mixtures have pores on their surfaces, the transverse section (Figure 1F) shows that the pores are discontinuous, and as such do not play a major role in the kinetics of drug release. Consequently, the release of enoxacin from microparticles prepared with dichloromethane–acetone mixtures occurs through a polymeric phase and pores on the surface rather than through pores only.

The effects of particle size and viscosity grade of the PGLA polymer used for the preparation of microparticles are depicted in Figures 3 and 4 , respectively. The viscosity grade or the particle size of a microparticle is known to influence the rate of drug release from microparticles (Fong et al 1986). Particle size did not significantly influence the rate of release of enoxacin because microparticles in the sieve-size range 250–425 μm were usually aggregates of smaller microparticles which deaggregated during preparation for SEM examination (Figure 1). Particle size influences the rate of release of drug from microparticles, predominantly because it determines the total surface area exposed to the release medium. The insignificant difference

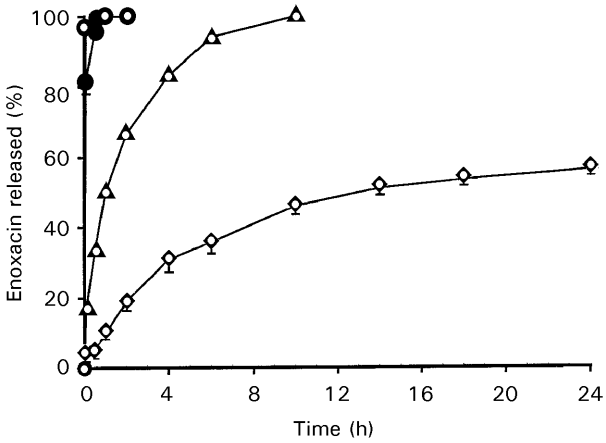

Figure 2. Effect of dichloromethane–acetone composition during preparation on the release of enoxacin from microparticles of sieve-size fraction 250–425 μm prepared with PGLA of viscosity grade 1·32 dl g^{-1}. ● Free drug, ○ dichloromethane–acetone 10 : 0, ▼ dichloromethane–acetone 1 : 1, ◇ dichloromethane–acetone 1 : 9.

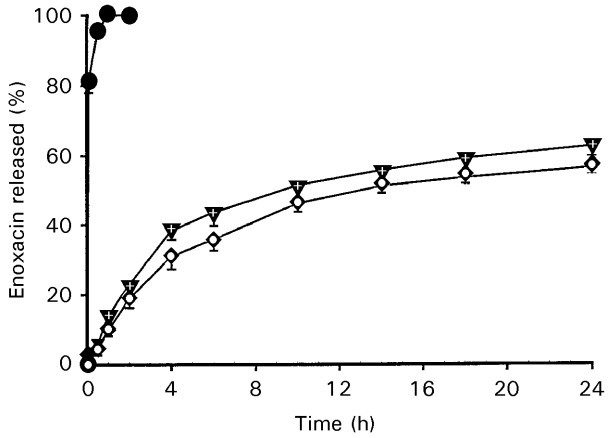

Figure 3. Effect of particle size on the release of enoxacin from microparticles prepared with PGLA of viscosity grade 1·32 dl g^{-1} and dichloromethane–acetone, 1 : 9. ● Free drug, ▼ 125–250 μm, ◇ 250–425 μm.

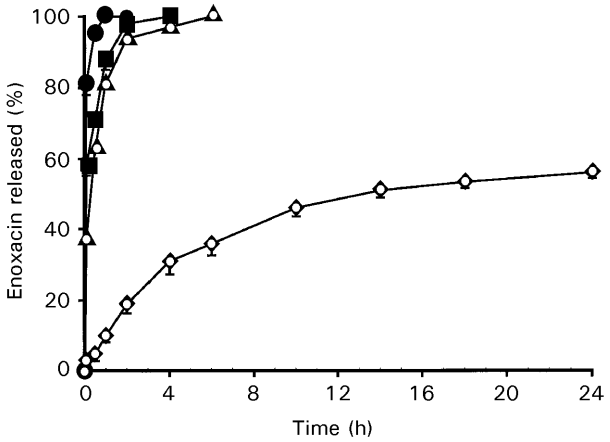

Figure 4. Effect of PGLA viscosity grade on the release of enoxacin from microparticles of sieve-size fraction 250–425 μm prepared with dichloromethane–acetone, 1 : 9. ● Free drug, ■ 0·19 dl g^{-1}, ▼ 0·70 dl g^{-1}, ◇ 1·32 dl g^{-1}.

between release rates could be attributed to deaggregation of the microparticles of the large sieve-size fraction in the release medium. Figure 4 shows that the higher the viscosity grade of the polymer used for the preparation, the lower the rate of release of enoxacin. The permeability of a polymer decreases with increasing molecular weight, leading to a reduced rate of drug release (Fong et al 1986). It has been observed that the glass transition temperature (T_g) of poly-DL-lactide increases with increasing polymer molecular weight and that the rate of release of theophylline decreased as the polymer molecular weight was increased (Omelczuk & McGinity 1992). The T_g of PGLA decreases significantly during the first hour of immersion in water (Shah et al 1992). Progesterone release from poly-DL-lactic acid microspheres of different molecular weight has been observed to occur above the respective T_g of the polymers after immersion in water (Aso et al 1994). The significant differences between the rates of release could, therefore, be attributed to differences between the T_g of different viscosity-grade polymers upon immersion in water.

The kinetics of enoxacin release from PGLA microparticles prepared by the solvent–evaporation procedure procedure do not fit a first-order, matrix or biphasic model, and seem to be very complex. It has been reported that cephalexin microspheres prepared by simple coacervation by non-solvent addition using poly(L-lactic acid) followed second-order kinetics (Owusu-Ababio & Rogers 1996a). In comparison, the release of ciprofloxacin from similar microparticles prepared by a simple coacervation procedure was biphasic with an initial matrix-type release followed by a first-order release (Owusu-Ababio & Rogers 1996b). Mechanisms responsible for the release of drugs from PGLA polymers include glass, the transition temperature, extent of hydration, and surface or bulk erosion which are driven by the hydrolytic chain scission of the polyester chain (Shah et al 1992).

Further optimization of this system is possible by careful selection of similar polymers with high glass transition temperatures, for example poly-DL-lactic acid, use of copolymers containing a high fraction of lactic acid, an increase in the viscosity grade of the polymer, or a reduction in drug loading.

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