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In vitro and in vivo release of ciprofloxacin from PLGA 50:50 implants

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Abstract

Poly(lactides-co-glycolides) [PLGA] are widely investigated biodegradable polymers and are extensively used in several biomaterials applications as well as drug delivery systems. These polymers degrade by bulk hydrolysis of ester bonds and break down into their constituent monomers, lactic and glycolic acids which are excreted from the body. The purpose of this investigation was to develop and characterize a biodegradable, implantable delivery system containing ciprofloxacin hydrochloride (HCl) for the localized treatment of osteomyelitis and to study the extent of drug penetration from the site of implantation into the bone. Osteomyelitis is an inflammatory bone disease caused by pyogenic bacteria and involves the medullary cavity, cortex and periosteum. The advantages of localized biodegradable therapy include high, local antibiotic concentration at the site of infection, as well as, obviation of the need for removal of the implant after treatment. PLGA 50:50 implants were compressed from microcapsules prepared by nonsolvent-induced phase-separation using two solvent–nonsolvent systems, viz., methylene chloride–hexane (non-polar) and acetone–phosphate buffer (polar). In vitro dissolution studies were performed to study the effect of manufacturing procedure, drug loading and pH on the release of ciprofloxacin HCl. The extent of penetration of the drug from the site of implantation was studied using a rabbit model. The results of in vitro studies illustrated that drug release from implants made by the nonpolar method was more rapid as compared to implants made by the polar method. The release of ciprofloxacin HCl from the implants was biphasic at $\leq$20% w/w drug loading, and monophasic at drug loading levels $\geq$35% w/w. In vivo studies indicated that PLGA 50:50 implants were almost completely resorbed within five to six weeks. Sustained drug levels, greater than the minimum inhibitory concentration (MIC) of ciprofloxacin, up to 70 mm from the site of implantation, were detected for a period of six weeks.

Keywords: Poly(lactides-co-glycolides); Microcapsules; Implants; Drug-release; Ciprofloxacin

1. Introduction

Biodegradable polymers have become increasingly popular as carriers in the design of drug delivery systems since the mid-1970s. As these polymers degrade in the body to small molecular weight compounds that are either metabolized or excreted, they obviate the need for removal of the carrier after the device is exhausted. Poly(lactide-co-glycolides) [PLGA] are widely investigated biodegradable poly-

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mers and copolymers. Because the biocompatibility of these polymers is well established, they have been extensively used in biomaterial applications such as tracheal replacement, ligament reconstruction, surgical dressings and dental repairs, and as carriers in drug delivery systems [1]. The PLGA polymers are esters of α-hydroxy acetic acid and breakdown by bulk hydrolysis of ester bonds to their constituent monomers, lactic and glycolic acids, which are eliminated from the body through Kreb's cycle. These polymers can be fabricated as microcapsules, implants or films.

The purpose of this study was to develop and characterize a biodegradable, implantable delivery system containing ciprofloxacin hydrochloride (HCl) for the localized treatment of osteomyelitis. In addition, the extent of penetration of ciprofloxacin from the site of implantation in the bone using an in vivo rabbit model was also studied. Osteomyelitis, either acute or chronic, is an inflammatory bone disease caused by pyogenic bacteria and involves the medullary cavity, cortex and periosteum. One of the most common causes of the disease is post-operative sepsis following orthopaedic procedures. The current therapy includes surgical debridement of the infected region followed by prolonged administration of oral and parenteral antibiotics for a period of four to six weeks [2]. Some of the disadvantages of prolonged parenteral therapy are patient discomfort, high cost of treatment and the development of systemic toxicity. Prolonged oral antibiotic therapy may also be associated with patient compliance problems. In addition, because osteomyelitis results in bone necrosis and destruction resulting in limited vascularity to the site of infection, systemic therapy may fail to produce therapeutic tissue concentrations of the antibiotic. To overcome some of these problems associated with the treatment of osteomyelitis, localized drug therapy using nonbiodegradable polymethyl methacrylate (PMMA) bone cement implants was introduced in the 1970s.

Since 1973, spherical, nonbiodegradable PMMA bone cement implants containing antibiotics have been extensively used for prophylaxis and treatment of bone infections [3]. The advantages of local antibiotic therapy include high, local, tissue, drug concentration, while simultaneously minimizing high, potentially toxic, systemic drug levels. However, previous studies in this laboratory have shown that the in vitro release of tobramycin from PMMA spherical beads is incomplete and poorly controlled [4]. Another disadvantage of the nonbiodegradable carriers is that a second surgery is required for removal of the implants. Therefore, studies were undertaken to develop a biodegradable delivery system using PLGA copolymers as carriers. Ciprofloxacin was selected because it is a relatively newer antibiotic with a broad spectrum of activity, few reported toxic side effects and good bone penetration properties. Fig. 1 shows the chemical structure of ciprofloxacin.

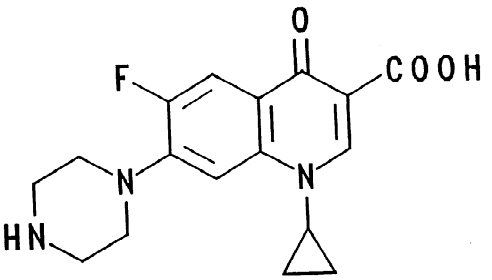

Fig. 1. Chemical structure of ciprofloxacin.

2. Materials and methods

2.1. Materials

Ciprofloxacin HCl, sodium chloride, sodium citrate, sodium hydroxide and sodium borate were purchased from Sigma Chemicals Co., St. Louis, MO. The polymer, PLGA 50:50 was obtained from Medisorb Technologies International L. P., Cincinnati, OH. Methylene chloride, acetone, hexanes, hydrochloric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate and potassium hydroxide were obtained from Fisher Scientific, Fairlawn, NJ.

2.2. Preparation of microcapsules

Microcapsules were prepared by nonsolvent-induced phase-separation using two solvent–nonsolvent systems, namely, methylene chloride–hexane (nonpolar) and acetone–Sorenson's phosphate buffer

(polar). Varying proportions of drug and polymer were used to obtain microcapsules theoretically containing 10%, 20%, 35% and 50% w/w drug loading. Placebo microcapsules were prepared by omitting the drug from the nonsolvent. The precise procedures for both microencapsulation methods used are described below.

2.3. Nonpolar procedure

A 2.5% w/v solution of the polymer in methylene chloride was added drop-wise at a rate of $\leq$5 ml min^{-1} to a 20-fold excess of the nonsolvent, hexane, while stirring the system with a magnetic stirrer at 700–800 rpm. To prepare drug-loaded microcapsules, a known amount of the drug was suspended in the nonsolvent. The microcapsules were equilibriated to harden for two to three hours. The final product was then filtered over vacuum, air-dried at ambient room temperature and sieved to remove any aggregates.

2.4. Polar procedure

This method was innovative in that it involved the use of an aqueous nonsolvent, thereby minimizing the use of organic solvents in the preparation of PLGA 50:50 microcapsules. A 2.5% w/v solution of the polymer in acetone was added drop-wise to a 20-fold excess of the nonsolvent, Sorenson's phosphate buffer, pH 7.4, containing the suspended drug. To minimize the loss of drug due to dissolution, the buffer was previously presaturated with ciprofloxacin hydrochloride. The microcapsules were prepared by a procedure identical to that described for the nonpolar procedure above, equilibrated to harden for three to four hours, filtered over vacuum, dried in a vacuum oven at room temperature for three to five hours, and sieved to remove any aggregates.

2.5. Assay of drug content of microcapsules prepared by polar and nonpolar methods

Triplicate samples (50 mg) of ciprofloxacin-containing microcapsules were suspended in 100 ml 0.1M HCl and agitated over a magnetic stirrer for 12–14 hours to completely extract all the drug. The microcapsules were then filtered, the filtrate appropriately diluted with 0.1M HCl and analyzed spectrophotometrically at 277 nm.

2.6. Preparation of implants

Implants (0.45×0.51 cm) were prepared by compression of 100 mg of either placebo or drug-loaded microcapsules in a Carver hydraulic press at a pressure of one metric ton, using a die-and-punch apparatus.

2.7. In vitro drug-release studies

The influence of manufacturing procedure, drug loading and pH on the release of ciprofloxacin HCl from PLGA 50:50 implants was investigated. Differential, drug-release studies were performed by immersing one implant in 20 ml Sorenson's phosphate buffer, pH 7.4 (except for the effect of pH of the dissolution medium on drug release). To evaluate the effect of pH of the dissolution medium on the release of ciprofloxacin HCl, differential drug release studies were performed in buffers having an ionic strength of 0.1 at the following pH's: pH 4.5 and 6.0 (citrate buffer), pH 7.4 (phosphate buffer) and pH 9.4 (borate buffer). For studies at each condition, triplicate samples of implants were maintained at 37°C in a shaking water bath. At various time intervals, the implants were transferred into fresh 20 ml buffer, and the amount of drug released was determined by a direct spectrophotometric assay at 277 nm using a Shimadzu UV-VIS spectrophotometer, Model 160.

2.8. In vivo drug release and extent of penetration into bone

Implants compressed from microcapsules made by polar procedure were implanted into the right distil femur of 15 New Zealand white rabbits using a modification of the method described by Wei [5]. A 4 mm diameter cavity was made longitudinally from the intercondylar notch to a depth of 15 mm. Two cylindrical implants (4×5 mm each) containing 50 mg ciprofloxacin each, were inserted into the cavity. The animals were sacrificed at three days, two, four, five and six weeks. After sacrifice, the right femur was divided into 5 mm thick transverse sections at 5, 20, 40 and 70 mm proximal to the intercondylar

notch. The bone was then pulverized in 2–4 ml sterile normal saline, centrifuged and drug levels determined by HPLC after extraction. The proprietary HPLC procedure was developed at Medtox Laboratories using a C-18 column for the separation of ciprofloxacin, detection being done using a fixed wavelength (Medtox Laboratories, St. Paul).

3. Results and discussion

3.1. Assay of drug content of microcapsules prepared by polar and nonpolar methods

The recovery of ciprofloxacin HCl was greater than 90% at all drug loading levels from microcapsules prepared by both polar and nonpolar procedures. Ciprofloxacin HCl has an absorption maximum at 277 nm in 0.1 M HCl, and the molar absorptivity, calculated from the standard curve was 4.1×10^4 L $mol^{-1}cm^{-1}$ The coefficients of variation for inter-day variability at high (10 μg ml^{-1}), medium (5 μg ml^{-1}), and low (2 μg ml^{-1}) concentrations were 2.81%, 2.82% and 5.69%.

3.2. Preparation and weight variation of implants

The implants (0.515×0.495 cm) were prepared by compressing 100 mg of microcapsules prepared either by the nonpolar or polar methods. The mean weight of implants was greater than 98 mg, the small batch-to-batch coefficient of variation (<3%) was indicative of the reproducibility of the method.

3.3. In vitro drug release studies

3.3.1. Effect of manufacturing procedure on the release of ciprofloxacin

The influence of manufacturing procedure on drug release from PLGA 50:50 implants containing 20% w/w drug is shown in Fig. 2. A more rapid drug release was obtained from implants prepared by the nonpolar method. As has been reported earlier [6], these implants degraded faster than those prepared by the polar procedure, and therefore, were more porous, resulting in more rapid drug release. Implants prepared by the polar procedure swelled to a lesser extent because of a denser, more closely packed matrix, resulting in a slower uptake of the dissolution medium, and slower drug release.

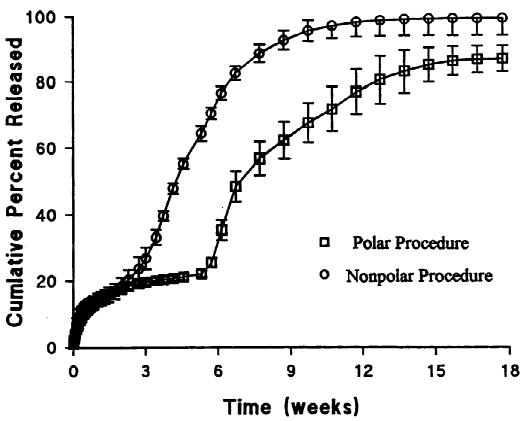

Fig. 2. Effect of manufacturing procedure on the release of ciprofloxacin HCl from PLGA 50:50 implants at pH 7.4.

Drug release from implants prepared by both polar and nonpolar procedures was biphasic indicating that ciprofloxacin HCl was released initially due to diffusion, followed by matrix erosion. Hutchinson et al., [7–9] and Shah et al., [10] also reported similar biphasic profiles for the release of water-soluble peptides from PLGA polymers. To describe this biphasic drug release profile from polymers undergoing homogenous degradation, Heller et al. [11], modified the square-root-time model [12] to account for the progressively increasing porosity of such matrices. This model characterizes drug release due to a combination of diffusion and erosion processes and is described by Eq. (1).

$$dM_t/dt = A/2[(2P_0e^{kt}C_0)/t]^{1/2} \quad (1)$$

where M_t is the drug released at time t, P_0 is the initial permeability of the polymeric matrix, A is the surface of both sides of the slab, k is the first-order rate constant for the change in permeability, and C_0 is the initial concentration of drug in the polymer. The release of ciprofloxacin HCl from PLGA 50:50 implants conforms to the shape exhibited by the theoretical curve described by this equation. When compared with the cumulative amount of lactic acid monomer produced due to degradation of the polymeric matrix [6] (polymer degradation has been discussed in another publication), the second phase

of drug release coincided with the appearance of lactic acid monomer further confirming that ciprofloxacin HCl was now being released due to matrix erosion (Fig. 3A).

3.3.2. Effect of drug loading ratio on the release of ciprofloxacin HCl

Prolonged release of ciprofloxacin HCl was obtained at all drug loading levels with ≈70% to 80% of the drug being released in seven weeks for implants prepared by both manufacturing procedures (Fig. 4A,B). Sustained release of nafarelin, from PLGA 50:50 microspheres, for a duration of seven weeks at drug loading levels of 2.3 to 9.2% has been observed by Sanders et al., [13]. However, Sampath

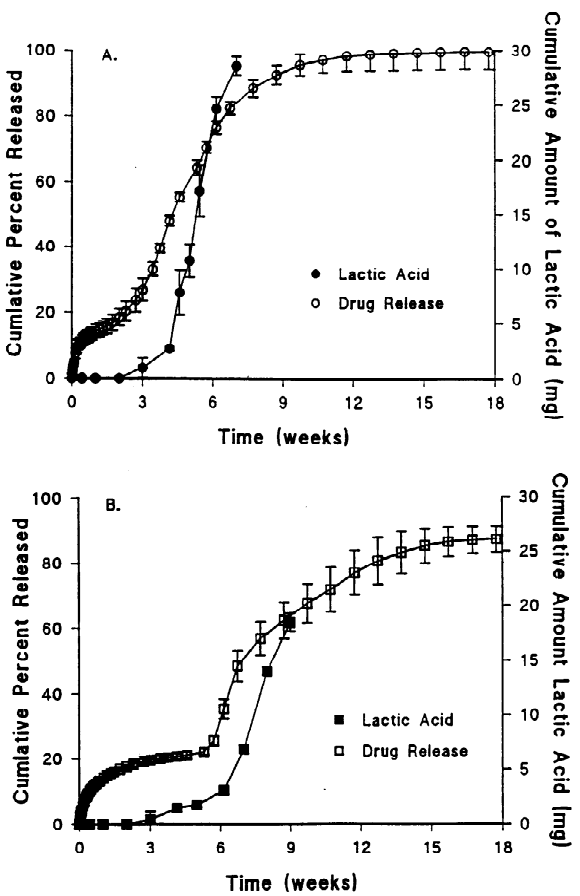

Fig. 3. Correlation between drug release profile and appearance of lactic acid due to degradation for PLGA 50:50 implants prepared by nonpolar (A) and polar (B) methods.

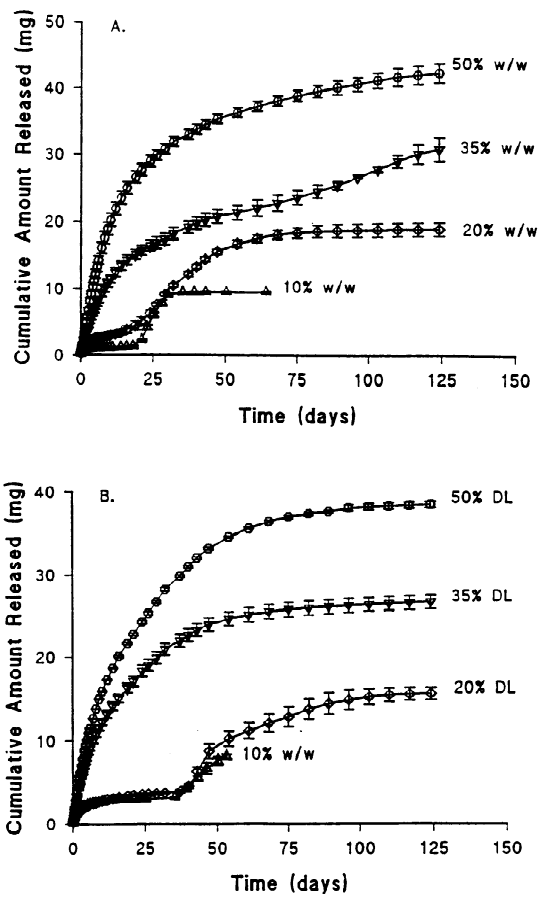

Fig. 4. Effect of drug loading on the cumulative amount of ciprofloxacin HCl released from PLGA 50:50 implants prepared by nonpolar (A) and polar (B) methods.

[14] reported that greater than 80% of gentamicin sulphate was released within a week from implants containing 2% w/w drug. This indicates that the physicochemical properties of the drug, in addition to polymer properties, also influence drug release from PLGA matrices. The cumulative amount of ciprofloxacin HCl released increased with an increase in drug loading for implants prepared by both methods (Fig. 4A,B). Drug release was biphasic for 10% and 20% w/w drug loadings, comprising of an initial diffusive phase and a second erosion phase, but monophasic for the higher two loading levels of 35 and 50% w/w, being described by the square-root-time relationship ($r^2 \geq 0.92$) for implants made by both procedures (Table 1). Hutchinson et al., [7]

Table 1
Influence of manufacturing procedure on the dissolution parameters for square-root-time model for the release of ciprofloxacin hydrochloride from PLGA 50:50 implants

Method	Drug Loading (% w/w)	r^2	Slope	Intercept
Nonpolar	35	0.978	6.44	1.91
	50	0.925	10.2	6.13
Polar	35	0.938	6.46	4.35
	50	0.951	7.54	2.78

have suggested that parameters such as drug loading and device geometry control the initial diffusive phase of drug release, whereas the second erosion phase is dependent on the intrinsic properties of the polymer influencing degradation such as the rate of water uptake, monomer ratio and molecular weight. When these two phases do not overlap a biphasic release is obtained as observed at 10 and 20% w/w drug loadings. However, if the initial diffusive phase overlaps with the erosion phase, continuous release, as seen with drug loading ratios of 35% and 50% w/w, is obtained.

3.3.3. Effect of pH of the drug-release medium on the release of ciprofloxacin HCl

PLGA 50:50 implants containing 20% w/w drug were used for these studies. For implants made by the nonpolar procedure, no difference as a function of pH was observed in the rate and extent of release of ciprofloxacin HCl in the initial diffusive phase, however, in the erosion phase, the rate of drug release increased in acidic media (pH 4.5 and 6.0), as compared to that in neutral and basic media (Fig. 5A). The increased rate of drug release in acidic media may be due to the increased solubility of ciprofloxacin HCl in the dissolution medium (ionization scheme shown in Eq. (2)). Similarly, an increased drug release was expected in basic media due to higher solubility of the anionic form of ciprofloxacin at this pH compared to that near the pI.

The general ionization scheme of ciprofloxacin for the anionic, zwitterionic and cationic forms is given below. The structure of ciprofloxacin is shown in Fig. 1.

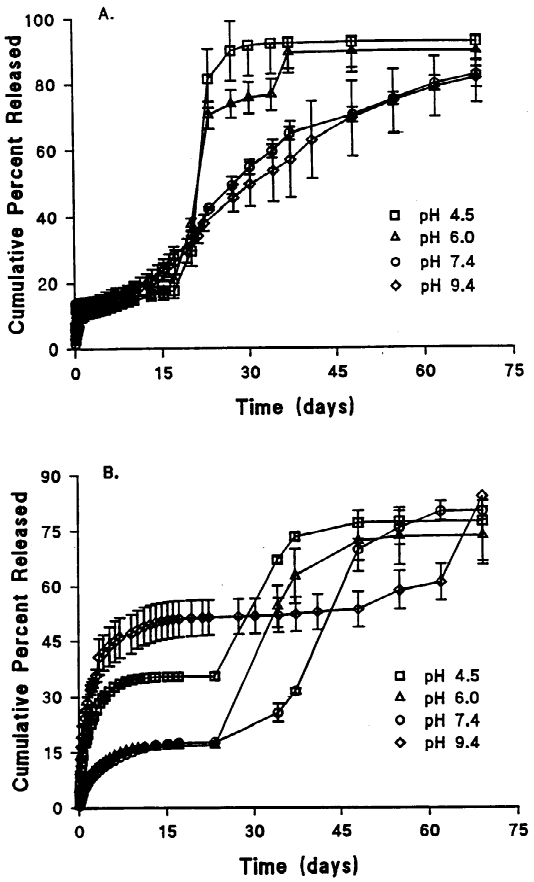

Fig. 5. Effect of pH of the drug-release medium on the release of ciprofloxacin HCl from PLGA 50:50 implants prepared by nonpolar (A) and polar (B) methods.

$$\underset{\text{(acid media)}}{{}^{+}Ao} \leftrightarrow \underset{\substack{\text{(neutral media)}\\ \text{pI} = 7.4}}{{}^{+}A^{-}} \leftrightarrow \underset{\text{(basic media)}}{{}^{-}Ao} \quad (2)$$

From this scheme it is evident that at pH values below the isoelectric point the amine species is ionized, at pH values around the isoelectric pH (pI = 7.4) the drug exhibits zwitterionic properties, and at pH values above the isoelectric point the carboxyl group is ionized. Based on these ionization properties, the solubility of ciprofloxacin will increase in an acidic environment, decrease to a

minimum around the pI, and then increase again above the pI.

The lower rate of drug release during the erosion phase in basic media (borate buffer, pH 9.4), compared to that in acidic media, may be explained by the ionization reactions which occur in the diffusion layer. At surfaces within the implant where drug is dissolving, there will be a high concentration of ciprofloxacin HCl. Dissolution of this species will result in microenvironmental regions which are acidic (the pH of a 1% solution of ciprofloxacin HCl is 3.6) [15]. According to the diffusion layer model, ions from the basic buffer in the bulk solution will diffuse into the diffusion layer and react with the hydronium ions released as a result of the dissolution and subsequent ionization of the hydrochloride salt of the drug. The net result will be a shift in pH toward the pI and a decrease in drug solubility. This explanation is consistent with the ionization scheme for this drug and with the general concepts of mass transfer with chemical reaction [16]. A decrease in dissolution rate as a function of pH has also been demonstrated for several amphoteric β-lactam antibiotics [17].

Since polymer erosion is affected by medium pH as has been discussed in another publication [6], in addition to drug solubility, matrix erosion was also expected to affect drug release. However, based on the results of drug release studies, it appears that the aqueous solubility of ciprofloxacin plays a dominant role in the mechanism of release from PLGA 50:50 matrices.

Fig. 5B shows the effect of pH of the dissolution medium on drug release from implants prepared by the polar method. The presence of superficial and unentrapped drug may be the reason for increased drug release in the diffusion phase in acidic and basic media. Since increased drug release in the diffusion phase is seen both in acidic (pH = 4.5) and basic (pH = 9.4) media, it appears that the unentrapped drug is in its crystalline salt form, and based on the ionization scheme discussed above increased solubility would be observed in both acidic and basic media.

3.3.4. In vivo drug release and extent of penetration

Table 2 shows the local bone concentrations at various distances from the site of implantation. Animals sacrificed at 3 days had average drug levels of 9.9 $\mu g\ g^{-1}$ at 20 mm from the site of implantation, with one animal showing detectable drug (2.5 $\mu g\ g^{-1}$) at 70 mm. At two weeks, levels averaged 1.1×10^{3} $\mu g\ g^{-1}$ at 20 mm and 50.7 $\mu g\ g^{-1}$ at 70 mm from the site of implantation. By week six, average levels of 1.8×10^{3} $\mu g\ g^{-1}$ at 5 mm, 1.6×10^{3} $\mu g\ g^{-1}$ at 20 mm, 58.6 $\mu g\ g^{-1}$ at 40 mm and 10.7 $\mu g\ g^{-1}$ at 70 mm from the site of implantation were obtained. The results of this study are preliminary and the standard deviations (S.D.) are high due to the small number of animals used. The serum minimum inhibitory concentrations (MICs) of ciprofloxacin against S. aureus, S. epidermidis, P. aerogin-

Table 2
Bone concentration of ciprofloxacin hydrochloride at various distances from the site of implantation

Time of Sacrifice	Average ($\pm$S.D.[a]) Ciprofloxacin Concentration in the Bone ($\mu g\ g^{-1}$)			
(weeks)	5 mm	20 mm	40 mm	70 mm
0.43[b] (3 days)	142.4	9.9	16.83	2.5
2	497.3±299.0	$1.1\times10^{3}\pm1.7\times10^{3}$	23.4±3.8	50.7±66.2
4	373.8±244.9	159.5±64.0	160.5±199.6	12.2±3.6
5[c]	855.6	1.4×10^{3}	2.1×10^{3}	26.8
6	$1.8\times10^{3}\pm1.2\times10^{3}$	$1.6\times10^{3}\pm402.3$	58.6±32.6	10.7±5.0

[a] $n=3$.
[b] 1 animal died.
[c] 2 animals died.

osa and P. mirabilis are 1–2 μg ml^{-1} [15]. And, of some importance is the observation that in this in vivo model sustained tissue levels greater than 1 μg g^{-1} of drug, both adjacent to and distil from the site of implantation, were observed. These results are comparable to literature reports for local delivery systems. For example, in the investigations of Wei et al. [5], for the release of an aminoglycoside, dideoxykanamycin B, using lactic acid oligomer as the drug carrier in rabbits with osteomyelitis, drug concentrations of 2.6 μg g^{-1} in the bone marrow, within 5 cm from the implantation site were observed after six weeks. Drug levels exceeding the MIC (1.56 μg g^{-1}) were also found in the cortical and cancellous bone surrounding the implant. Mean serum concentrations were always less than 1.3 μg ml^{-1}.

PLGA (52:48) microcapsules containing ampicillin have also been used for the localized prophylaxis and treatment of osteomyelitis [18]. Local treatment, immediately following infection was as effective as parenteral ampicillin. When treatment was delayed for a week, only four out of eight dogs treated with local, microencapsulated ampicillin developed osteomyelitis, as compared with 6 out 8 dogs treated with parenteral antibiotics. Sterile bone cultures were obtained for all the animals if surgical debridement preceded local ampicillin. These investigators concluded that localized therapy with antibiotic-containing PLGA microcapsules would be potentially useful in the prevention and treatment of the disease. Using locally inserted PLGA 50:50 implants Sampath [14] has also observed relatively high local gentamicin sulphate concentrations of up to 33 μg g^{-1} in the bone and 13 μg g^{-1} in the soft tissue at the times of sacrifice (six to eight weeks), in dog tibiae infected with osteomyelitis using *S. aureus*.

4. Conclusions

Two methods, using aqueous and organic nonsolvents, were developed for the preparation of PLGA 50:50 microcapsules by nonsolvent-induced phase separation. The polar method was innovative in that it is consistent with the current trends of minimizing the use of organic solvents in the preparation drug delivery systems. The results of in vitro release of ciprofloxacin HCl from PLGA 50:50 implants illustrated that drug release from implants made by the nonpolar method was more rapid as compared to implants made by the polar method. The release of ciprofloxacin HCl from the implants was biphasic at $\leq$20% w/w drug loading, and occurring due to initial diffusion followed by matrix erosion, the release was monophasic at drug loading levels $\geq$35% w/w. Finally, a more rapid drug release in the erosion phase was observed from PLGA 50:50 implants in acidic media as compared with neutral and basic media.

Results of the preliminary in vivo studies indicated that local delivery of ciprofloxacin using PLGA 50:50 as carrier may be useful in the treatment of deep skeletal infections. PLGA 50:50 implants were almost completely resorbed within five to six weeks [6]. Sustained drug levels, greater than the MIC of ciprofloxacin, up to 70 mm from the site of implantation, were detected for a period of six weeks. Both in vitro and in vivo studies indicated that a prolonged release of ciprofloxacin was observed for a period $\geq$six weeks. Further studies, using larger number of animals and animals infected with osteomyelitis, are required to confirm the effectiveness of localized PLGA implants for the treatment of this disease.

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