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HYDROXYAPATITE–CIPROFLOXACIN MINIPELLETS FOR BONE-IMPLANT DELIVERY: PREPARATION, CHARACTERIZATION, *IN-VITRO* DRUG ADSORPTION AND DISSOLUTION STUDIES

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ABSTRACT

The present study was aimed to prepare, characterize, evaluate in-vitro drug adsorption and dissolution studies of hydroxyapatite (HAp)-ciprofloxacin minipellets for bone-implant delivery to treat various bacterial bone infections. Ciprofloxacin loaded hydroxyapatite powders were synthesized by precipitation technique varying different experimental conditions like drug amount added and addition rate of orthophosphoric acid as one of the starting material in the synthesis process. HAp-ciprofloxacin minipellets (2 mm X 2 mm X 1 mm) were prepared by compressing synthesized HAp-ciprofloxacin powders. Highest drug loading was observed 76.64 ± 0.47 % w/w with incorporation efficiency of 87.43 ± 0.18 % w/w. Characterization of this drug delivery was done by P-XRD and FT-IR spectroscopy. Even at the highest drug loading (76.64 ± 0.47 % w/w), ciprofloxacin was present in a non-crystalline state. The in-vitro ciprofloxacin adsorption by HAp powder was achieved maximum after 24 hours at various temperatures and found to follow Freundlich isotherm. The in-vitro ciprofloxacin release from various hydroxyapatite-ciprofloxacin minipellets was slow and sustained for several weeks. The drug release pattern from these minipellets was correlated well with Higuchi model. This proposed methodology provides advantage of producing high drug loading (% w/w) in the HAp-system to get effective drug concentration at the diseased bone site for a prolong period as well as to reduce the implant size with facilities of small surgery and decreased hospitalization period.

Key words: *Hydroxyapatite; Minipellets; Bone-implant; Drug delivery; Ciprofloxacin*

INTRODUCTION

During last few decades, an impressive progress has been recorded in terms of developing bone drug delivery.[1-2] In the treatment of bacterial bone infections like osteomyelitis prolonged systemic antibiotic therapy is usually used.[3] But, the localized delivery of antibiotics from an implantable drug delivery system offers considerable advantages by producing effective drug concentration at the diseased site, where only small fraction of any given dose actually reaches with the limitation of systemic exposure of antibiotics over the traditional methods of therapy.[4-8] Fluroquinolones are considered the drug of choice for bone infections mainly osteomyelitis because of their favorable penetration and anti-bactericidal effect on all the probable pathogens.[9-10] Ciprofloxacin is currently the most widely used fluroquinolones for bacterial bone infections, since the minimum inhibitory concentration (MIC) is low (0.25 – 2 μg/ml) for most of the bacterial pathogens that cause osteomyelitis, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Proteus mirabilis*.[11] In fact, the MIC of ciprofloxacin for *Staphylococcus aureus* is 0.25-1 μg/ml, which is most frequently found in infected bone of a patient with osteomyelitis.[12]

A number of biomaterials have tried and also investigated as carrier for transport and sustained release of antibiotics

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as local bone drug delivery.[13-19] Among all implantable delivery systems described in the literature, hydroxyapatite (HAp) is the most promising carrier for drug delivery in various bone infections due to having a chemical composition very close to the inorganic mineral phase of natural bone.[20] Its chemical formula is $Ca_{10}(PO_4)_6(OH)_2$ and molecular weight is 1004.62. Actually, the hydroxyl ion (OH^-) can be replaced by F^-, Cl^-, CO_3^{2-} and other ions in the collagen fiber matrix.[21] It has excellent biocompatibility, osteoconductivity and ostheophilic nature.[22-27] Therefore, it would be acceptable candidate for long-term use.[28] Its porous character also offers excellent binding affinity for a variety of pharmacological agents like antibiotics, hormones, enzymes, steroids, anticancer agents, vaccines and other agents.[29-37] Again, this porous characteristic of HAp has ability to promote much faster tissue growth into available pores.[38] These properties of HAp have promoted intensive efforts by drug delivery research groups to investigate HAp-based drug delivery systems, which contribute most promising therapeutic category in many clinical applications. Synthetic HAp is known to be similar to naturally occurring HAp on the basis of crystallographic and chemical studies.[39-40] Different techniques have been employed to produce HAp synthetically. The most popular and widely researched technique is precipitation technique.[41-43] A number of approaches on the development of hydroxyapatite HAp-based antibiotic bone delivery have already been investigated and shown promise in the treatment of bone infections, like osteomyelitis mainly.[44-49] An investigation on the HAp-ciprofloxacin delivery system was performed by Pham et al. based on precipitation technique and spray drying.[50] But, the ciprofloxacin loading into the HAp-system was not satisfactory (0.25% to 2% w/w only) in the study. They designed cylindrical implants of size, 5 mm X 8 mm by compressing agglomerated HAp-based microspheres loaded with ciprofloxacin. These implants showed sustained release rate of ciprofloxacin for few days only. But, study of HAp-antibiotic delivery systems with high drug load and sustained release of drug for several weeks is very few. The effective antibiotic therapy for various bone infections need to irrigate the infected site of bone tissues by therapeutic level of antibiotic concentration for several weeks.[6]

In the present investigation, we have aimed to develop various HAp-ciprofloxacin minipellets for bone-implant delivery with high amount of drug loading (% w/w) by precipitation technique and also to provide more sustained antibiotic release for several weeks, which may enable clinicians to achieve effective antibiotic therapy to treat various bacterial bone infections. The increased amount of antibiotic loading into HAp-system will help to reduce the size of implant size. This may lead facilities like small surgery and decreased hospitalization period in comparison formulation developed with low antibiotic load. In this study, we made an attempt to synthesize high drug loaded HAp-ciprofloxacin powders by precipitation technique varying different experimental conditions like drug amount added and addition rate of orthophosphoric acid as one of the starting material in the synthesis process. The addition rate of orthophosphoric acid in the synthesis process was slower (1 ml/min and 0.5 ml/min) in comparison with previously reported literatures by using the precipitation technique where, the addition of orthophosphoric acid in the synthesis process was done at a time,[50] made this present work a novel of its kind. Therefore, the objectives of this investigation were to synthesize high amount of drug loaded HAp-ciprofloxacin powders by precipitation techniques, prepare HAp-ciprofloxacin bone-implantable minipellets of small size (2 mm X 2 mm X 1 mm) by compressing synthesized various HAp-ciprofloxacin powders, characterize the drug delivery system and evaluate the *in-vitro* drug release characteristics from such an implantable delivery system. We also aim to have an insight into the *in-vitro* adsorption study of ciprofloxacin by synthesized HAp to reveal the extent and mechanism of material-drug (HAp-ciprofloxacin) interaction that is not a common thing with other previous investigations.

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl was gifted from Dr. Reddy's Laboratories (Hyderabad, India). Calcium hydroxide

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(Qualigens fine chemicals, Mumbai, India), orthophosphoric acid (Qualigens fine chemicals, Mumbai, India) were used. All other chemicals were of analytical grade.

Synthesis of HAp-ciprofloxacin powders

The HAp–ciprofloxacin powders were prepared by precipitation method. In brief, 50ml of aqueous suspension of 0.5 M calcium hydroxide [$Ca(OH)_2$] was prepared and vigorously stirred for 10 min. The 50 ml of 0.3 M orthophosphoric acid [H_3PO_4] was slowly added into the $Ca(OH)_2$ suspension. Then drug was added to this and carefully adjusted the pH (10.5) by 1 M ammonium hydroxide [NH_4OH] solution. The suspensions were well stirred (600 rpm) using magnetic stirrer for 30 mins and aged for overnight at room temperature (as shown in Figure 1.). Precipitates were subjected vacuum filtering using Buchner funnel, repeatedly washed with deionized water and filtered again. The precipitates were dried at room temperature for 48 hours. Dried lamps of powders were ground by clean pestles and mortars. Deionized water was used to obtain solutions.

Various ciprofloxacin loaded HAp powders were synthesized under various experimental conditions, listed in Table 1.

Table 1: Experimental conditions to synthesize various HAp-ciprofloxacin powders by using precipitation technique.

Formulation codes	Drug amount added in the synthesis process	Addition rate of orthophosphoric acid in the synthesis process
F-HCip/1	2 gm	0.5 ml/min
F-HCip/2	2.5 gm	1 ml/min
F-HCip/3	2.5 gm	0.5 ml/min

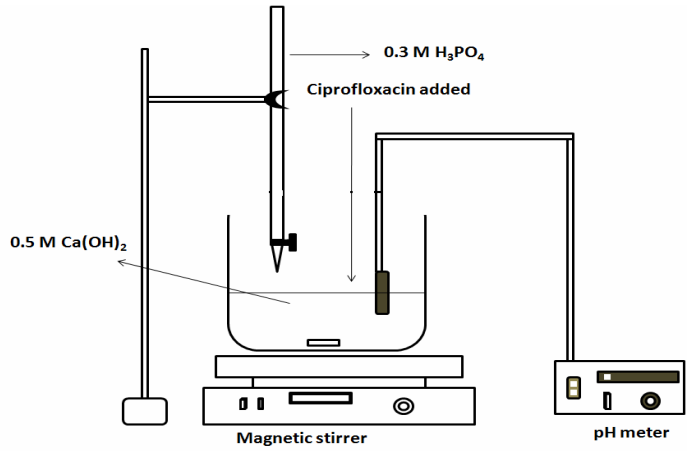

Figure 1: Schematic drawing of the synthesis apparatus for HAp-antibiotic powders using precipitation technique.

Determination of drug loading (% w/w) and drug incorporation efficiency (% w/w)

Filtrates of the suspensions of HAp-ciprofloxacin powders, which were obtained after washing by deionized water, were taken and analyzed to determine the drug loadings (% w/w) and the drug incorporation efficiencies (% w/w). Absorbance values were measured from filtrate dilutions of different formulations at the maximum wavelength (λ_{max}) of these concentrations were measured using a UV-VIS spectrophotometer (Thermo Spectronic UV-1). Maximum wavelength (λ_{max}) obtained by scanning all samples from 200 to 400 nm and this was 274 nm.

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Thus, drug incorporated in this system after drug loading were calculated easily for different formulations.

$$\text{Drug loading (\% w/w)} = \frac{\text{Drug incorporated (a)}}{\text{Dry powder weight after loading (b) - (a)}} \times 100 \quad \text{.....(1)}$$

Drug incorporation efficiency (% w/w)

$$= \frac{\text{Drug incorporated}}{\text{Amount of drug added in the synthesis process}} \times 100 \quad \text{.....(2)}$$

Manufacturing of various HAp-ciprofloxacin minipellets

Ciprofloxacin loaded HAp powders were compressed by a pressure of 25kg/cm^2 (2 Tons) using a hydraulic press and minipellets of size, 2 mm X 2 mm X 1 mm were prepared.

Characterization of HAp-ciprofloxacin delivery

Powder X-Ray diffraction

Samples were exposed to CuKα radiation (35 KV x 30 mA) in a wide-angle X-ray diffractomter (SEIFERT X-Ray Diffractometer, XRD 3000 P, RICH SEIFERT & CO. Gmb H & Co KG. D-2070, Ahrensburg). The instrument was operated in the step-scan mode in increments of 0.050° 2θ. The angular range was 5^0 to 40^0 2θ, and counts were accumulated for 1 second at each step.

Fourier transform-infrared (FT-IR) spectroscop

Samples were reduced to powder and analyzed as KBr pellets by using a Fourier transform – infrared (FT-IR) spectroscope (Perkin Elmer Spectrum RX I).

In-vitro drug adsorption study

Blank HAp powders were prepared via, precipitation technique using the same method of preparation of HAp–ciprofloxacin powders except the addition of ciprofloxacin. The addition rate of orthophosphoric acid to the synthesis process was 0.5 ml/min.

Determination of adsorption equilibrium time of ciprofloxacin by HAp

100 mg synthesized blank HAp powders was placed to a screw cap bottle containing 10 ml of a specified concentration, 1359.43 μM/lit of ciprofloxacin solution. The bottles were kept in water baths at constant temperatures of 8°C, 35°C and 45°C. At 0.5 hr, 1 hr, 2 hrs, 4 hrs, 12 hrs, 24 hrs and 48 hrs of incubation, solutions were filtered (Whatman® filter) to separate ciprofloxacin adsorbed HAp powders. The drug concentrations in the supernatants were measured spectrophotometrically (Thermo Spectronic UV-1) at λmax = 274 nm. The amounts of ciprofloxacin adsorbed on the HAp were calculated from the differences in the powder free antibiotic solutions (control) and equilibrium concentrations after adsorption per unit mass of HAp powders.

Determination of extent and mechanism of ciprofloxacin adsorption by HAp

100 mg synthesized blank HAp powder was placed to a screw cap bottle containing 10 ml of a specified concentration ranging from 27.19 μM/lit to 1359.43 μM/lit of ciprofloxacin solution. HAp powder free ciprofloxacin solutions of various concentrations were prepared also. The bottles were kept in water baths for 2 days at constant temperatures of 8°C, 35°C and 45°C. Then solutions were filtered (Whatman® filter) to separate ciprofloxacin adsorbed HAp powders. The drug concentrations in the supernatants were measured spectrophotometrically (Thermo Spectronic UV-1) as stated above.

In-vitro dissolution studies

Samples containing 5 minipellets of each formulations were placed in Falcon tubes containing 5 ml of pH 7.4 phosphate buffer saline (PBS) at 37 ± 0.5 °C. Elution fluids were replaced by fresh buffer at regular intervals. Removed

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elution fluids were collected for determination of ciprofloxacin concentrations by using a UV-VIS spectrophotometer (Thermo Spectronic UV-1) at the maximum wavelength (λ_{max}). Maximum wavelength (λ_{max}) obtained by scanning all samples from 200 to 400 nm and this was 271 nm.

RESULTS AND DISCUSSION

Various ciprofloxacin loaded HAp powders were achieved by precipitation technique. Precipitation technique was chosen to synthesize these powders in contrast to other techniques, because relatively large amount of HAp can be produced at reasonable cost. Furthermore, the only byproduct of the reaction is water [10 $Ca(OH)_2$ + 6 H_3PO_4 → $Ca_{10}(PO_4)_6(OH)_2$ + 18 H_2O] and the reaction involves no foreign elements.[43]

High drug loadings (% w/w) and also high drug incorporation efficiencies (% w/w) were observed in case of all three formulations (Table 2) in contrast to previously reported literatures by using the precipitation technique to synthesize ciprofloxacin loaded HAp system.[50] Increases in drug loading (% w/w) and also in drug incorporation efficiency (% w/w) were observed in case of formulation F-HCip/3 (76.64 ± 0.47 % w/w and 87.43 ± 0.18 % w/w respectively).

Table 2: Drug loadings (% w/w) and drug incorporation efficiencies (% w/w) of various HAp-ciprofloxacin powders by using precipitation technique. (Mean ± SE, n=3).

Formulation codes	Drug loadings (% w/w)	Drug incorporation efficiencies (% w/w)
F-HCip/1	64.14 ± 0.58	86.56 ± 0.66
F-HCip/2	75.15 ± 0.55	85.64 ± 0.14
F-HCip/3	76.64 ± 0.47	87.43 ± 0.18

Powder X-ray diffraction profiles of blank HAp powders and ciprofloxacin loaded powders (F-HCip/3) are shown in Figure 2. The blank HAp powders exhibited typical diffraction pattern of HAp, which occurs between 2θ, 26-32°.[28] A comparison between powder X-ray diffraction patterns HAp-ciprofloxacin powders and blank HAp powders did not show any major differences in the diffraction patterns indicating that the drugs were present in a non-crystalline form in HAp system even at a drug loading of 76.64 ± 0.47 % w/w. This reveals that drugs (here ciprofloxacin) in the HAp system was amorphous or in solid solution.

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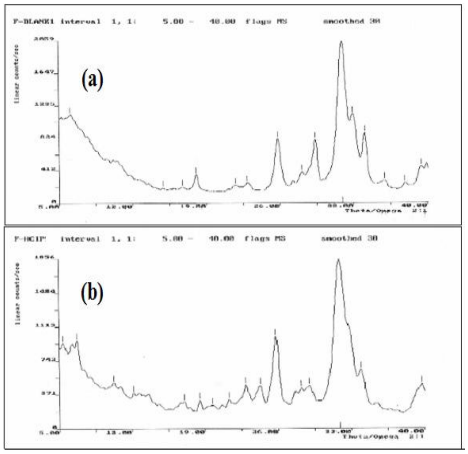

Figure 2: Powder X-ray diffraction profile of Hap blank powders (a) and HAp-ciprofloxacin powders (F-HCip/3) (b).

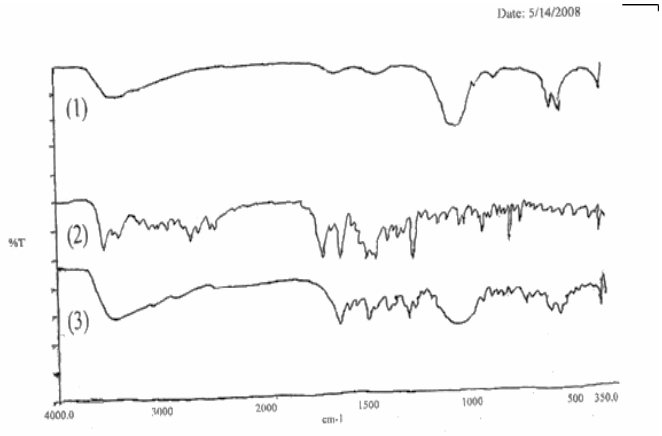

Figure 3: FT-IR spectra of HAp blank powders (1), ciprofloxacin (2) and HAp-ciprofloxacin powders (F-HCip/3) (3).

Fourier transform- infrared (FT-IR) spectra of HAp blank powders, ciprofloxacin and HAp-ciprofloxacin powders (F-HCip/3) are represented in Figure 3. Synthesized HAp blank powder and standard ciprofloxacin samples were used for comparison. The FT-IR spectrum of synthesized HAp blank powder shows the characteristic peaks, namely the PO_4 narrow peak at 963 cm^{-1}, the PO_4 peak at 1039 cm^{-1}, the PO_4 peaks at 604 cm^{-1} and 564 cm^{-1}, and the O-H peak at 668 cm^{-1}, as expected.[51] The incorporation of ciprofloxacin into the HAp powders lead to emergence of characteristic peak (1709 cm^{-1} for COO^- and 1625 cm^{-1} for C=O in both HAp-ciprofloxacin powders and minipellets).[52] In the FT-IR spectra of HAp-ciprofloxacin powders, the characteristic peaks of ciprofloxacin were same as in the standard samples or very slightly shifting of these peaks occurred. This confirms the presence of ciprofloxacin in the HAp-system without or with very minute interaction.

The *in-vitro* adsorption study of ciprofloxacin by synthesized HAp using precipitation technique was done to determine adsorption equilibrium time and to reveal the extent and mechanism of material-drug (HAp-ciprofloxacin) interaction. The amount of ciprofloxacin adsorbed on the synthesized HAp powders from 1359.43 µ mol/lit aqueous solution of ciprofloxacin at various time intervals showed that the maximum adsorption values were achieved in 24 hours (i.e. the adsorption equilibrium time of ciprofloxacin adsorption by synthesized HAp powders) and remains almost the same up to 48 hours in case of 8ºC, 35ºC and 45ºC temperature (Figure 4). The adsorption of ciprofloxacin by HAp powders is very well described mathematically by Freundlich adsorption isotherm (Figure 5), defined by the relationship:[53]

$$x/m = K_F . C_{Eq}^{1/n} \quad \text{..... (3)}$$

where x/m is the amount of solute adsorbed per unit weight of adsorbent, C_{Eq} is the residual liquid phase concentration at equilibrium, K_F and n are the Freundlich adsorption isotherm constants. This equation linearizes in natural logarithmic form:

$$\ln x/m = \ln K_F + 1/n \ln C_{Eq} \quad \text{.....(4)}$$

for parameter evaluation.[53-54]

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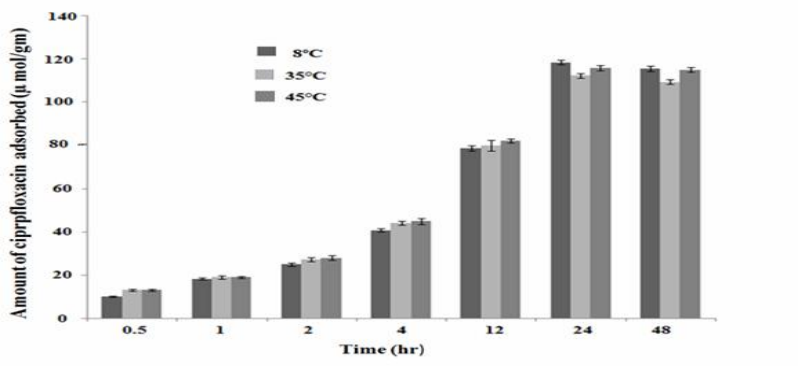

Figure 4: Adsorption of ciprofloxacin on HAp with time from 1359.43 µ M/lit aqueous solution of ciprofloxacin at 8ºC, 35ºC and 45ºC.

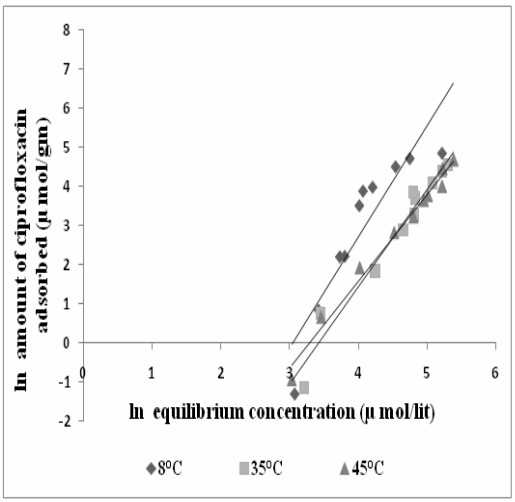

Figure 5: Freundlich adsorption isotherm of ciprofloxacin by HAp powders: natural logarithm of the amount of ciprofloxacin adsorbed per gram HAp powders (µM/gm) vs. natural logarithm of the ciprofloxacin equilibrium concentration (µM/lit)

Table 3: Apparent Freundlich adsorption isotherm constants of ciprofloxacin adsorption by HAp powders from aqueous solution

Temperature (°C)	Slope (1/n)	Intercept (ln K_F)	n	K_F	R^2
8°C	2.8307	- 8.6294	0.3533	0.00017	0.8325
37°C	2.4900	- 8.5500	0.4016	0.00019	0.9598
45°C	2.2190	- 7.3076	0.4507	0.00067	0.9848

The Freundlich adsorption isotherm constants (n and K_F) were calculated from slopes and intercepts of trend lines at 8°C, 37°C and 45°C, where K_F and *n* are Freundlich adsorption isotherm constants related to sorption capacity and sorption intensity of the adsorbent. K_F can be defined as the adsorption or distribution coefficient and represents the quantity of drug adsorbed onto HAp for a unit equilibrium concentration. The *in-vitro* adsorption study of ciprofloxacin by synthesized HAp was done at different temperatures to know the effect of temperature on extent of

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material-drug (HAp-ciprofloxacin) interaction. When the data from the adsorption isotherm at different temperatures (8°C, 37°C and 45°C) were plotted by linearized form of Freundlich adsorption isotherm equation (Equation no.-4), good-fit straight line resulted, as seen by the high R^2 values (Table 3). The differences in calculated values of Freundlich adsorption isotherm constants (K_F and n) at different temperatures were observed. K_F values were found to be increased with the increasing temperature, indicating that the adsorption is an endothermic process (Table 3). This may be a result of increase in the mobility of the drug with increasing temperature.[55] An increasing number of molecules may also acquire sufficient energy to undergo an interaction with active sites at the surface. Furthermore, increasing temperature may produce a swelling effect within the internal structure of the HAp enabling large drug to penetrate further.[56]

All of this HAp-ciprofloxacin minipellets slowly released the drug incorporated and sustained for several weeks. The drug release pattern from these minipellets was shown in Figure 6 and this indicate the release rate of ciprofloxacin from these minipellets depended on the amount drug load (% w/w) as expected, increasing drug loaded minipellets increased the extent of ciprofloxacin released, where as lowering drug loaded minipellets increased the rate of ciprofloxacin released from HAp-ciprofloxacin minipellets. So, the pattern of ciprofloxacin release from high amount of drug loaded HAp-ciprofloxacin minipellets may enable to achieve and maintain a therapeutic drug concentration in the infected site of bone tissues.

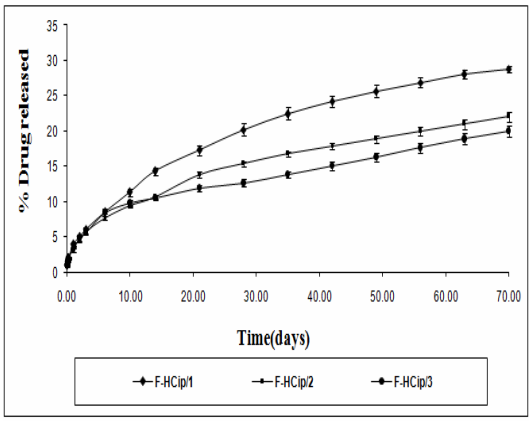

Figure 6: *In-vitro* drug release profile from various HAp-ciprofloxacin minipellets up to 10 weeks (70 days). (Mean±SE, n=3)

In order to predict and correlate the release behavior of ciprofloxacin from different HAp-ciprofloxacin minipellets, it is necessary to fit into a suitable mathematical model. The *in-vitro* ciprofloxacin release data from various composites were evaluated kinetically various mathematical models like zero order, first order, Higuchi, Hixon-Crowell and Korsmeyer-Peppas model equations.[57-60]

Zero-order kinetics: $F = K_O t$, where F represents the fraction of drug released in time t, and K_O is the apparent release rate constant or zero-order release constant.

First-order kinetics: $\ln (1-F) = - K_1 t$, where F represents the fraction of drug released in time t, and K_1 is the first – order release constant.

Higuchi Model: $F = K_H t^{½}$, where F represents the fraction of drug released in time t, and K_H is the Higuchi dissolution constant.

Hixon – Crowell model: $Wo^{1/3} – Wt^{1/3} = Ks t$, where Wo is the initial amount of drug in the composites, Wt is the remaining amount of drug in the composites at the t, and K_S is a constant incorporating the surface volume relation. Dividing the above equation by $Wo^{1/3}$ and simplifying:

$(1-F)^{1/3} = 1 – K_E t$, where F= 1- (Wt/Wo) and F represents the drug dissolved fraction at time t, and K_E is the release constant.

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Korsmeyer – Peppas Model: $F = K_P t^n$, where F represents the fraction of drug released in time t, K_P is the rate constant and n is the diffusional exponent, this indicates the drug release mechanism.

The results of the curve fitting into these above mentioned mathematical models indicate the release behavior of ciprofloxacin from various HAp-ciprofloxacin minipellets The changes in morphology of the HAp-ciprofloxacin minipellets (F-HCip/1) before and after 10 weeks of *in-vitro* dissolution study are shown in Figure 8 (a, b). Many additional pores were visible on the surface of the minipellets after *in-vitro* dissolution studies for 10 weeks (Table 4). When the release rate and their respective correlation coefficients of these HAp-ciprofloxacin minipellets were compared, it was found to follow the Higuchi model over a period of 21 days (Figure 7) and the release of ciprofloxacin from these minipellets followed by a matrix diffusion controlled mechanism as described by Higuchi.[61]

and the surface of the minipellets were rough than before the study. All these observations from the *in-vitro* dissolution results and the SEM images were collectively indicating additional pathways for penetration of the release medium into the HAp matrix

Table 4: Results of curve fitting of the *in-vitro* ciprofloxacin release data for different HAp–ciprofloxacin minipellets (up to 21 days).

Formulation codes		F-HCip/1	F-HCip/2	F-HCip/3
Zero order	K_o	0.0055	0.0041	0.0040
	R^2	0.9239	0.9247	0.9614
First order	K_1	0.0060	0.0041	0.0044
	R^2	0.9327	0.9647	0.9290
Higuchi Model	K_H	0.0281	0.0194	0.0210
	R^2	0.9959	0.9962	0.9920
Hixon-Crowell	K_E	0.0034	0.0017	0.0018
Model	R^2	0.7869	0.8605	0.7657
Korsmeyer-Peppas	K_P	0.0294	0.0215	0.0249
Model	n	0.4870	0.4240	0.4135
	R^2	0.9966	0.9687	0.9664

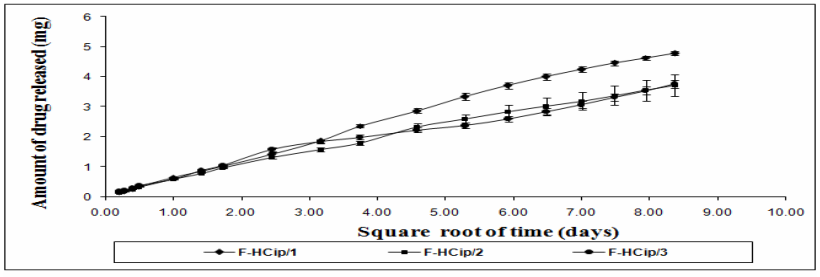

Figure 7: *In-vitro* released amounts of drug from various HAp-ciprofloxacin minipellets vs. square root of time ($t^{1/2}$).

(Mean±SE, n=3)

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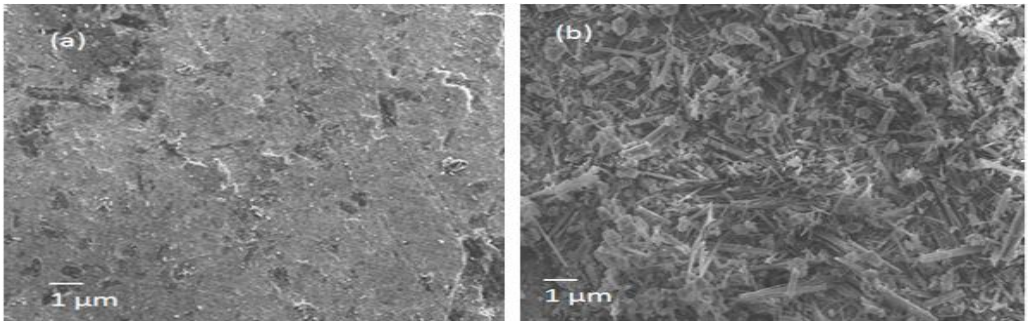

Figure 8: SEM images of HAp-ciprofloxacin minipellets surface (F-HCip/1) before (a) and after 10 weeks (b) of *in-vitro* drug dissolution studies.

CONCLUSION

In conclusion, the proposed methodology may enable the development of HAp-ciprofloxacin minipellets as bone-implant for local therapy in various bone infections display several advantages. First, high amount of drug loaded HAp-antibiotic minipellets can be possible to develop and also to get effective therapeutic level of antibiotics in the diseased site of the bone tissues. Secondly, prolonged release of antibiotics at the infected site achieves elevated local antibiotic concentrations while minimizing any risk of systemic toxicity. Thirdly, the high amount of antibiotic loaded minipellets will help to reduce the implant size with facilities of small surgery and decreased hospitalization period. Fifthly, the osteoconductive and ostheophilic property of the HAp carriers may fill the bone defects to promote bone regeneration in the dead space of the diseased site eliminating the need of second surgical procedure for their removal. Sixth, this type of HAp-based bone delivery can also be developed for the treatment of osteoporosis, osseous tumors etc. in which local drug delivery is effective with the need to fill defects in the skeleton. Seventh, depending on the specificity of illness, various pharmacological agents like anticancer agents, growth factors, proteins, enzymes, etc., can be loaded using this methodology to provide local release in the infected site. In order to evaluate the effectiveness of the HAp-ciprofloxacin minipellets as bone-implant delivery for use in the treatment of bone infections, further studies including *in-vivo* experiments, are required.

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