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Synthesis and Characterization of Hydroxyapatite-Ciprofloxacin Delivery Systems by Precipitation and Spray Drying Technique

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ABSTRACT This investigation synthesized and characterized hydroxyapatite (HAP) microspheres, agglomerated microspheres, and implants containing ciprofloxacin. This delivery system is to be used as an implantable drug delivery system for the treatment of bone infections. The HAP microspheres were made by chemical precipitation followed by a spray-drying technique. Agglomerated microspheres were prepared by a wet granulation process using a granulator. Implants were prepared by direct compression of the granules on a Carver press. Ciprofloxacin was analyzed by high-performance liquid chromatography. Characterization of the HAP microspheres include particle size, size distribution, physical state of the drug in the microsphere, and microstructure of the drug delivery system before and after in vitro release. The particle size, porosity, and morphology of the microspheres were dependent on viscosity and concentration of the slurry as well as the atomization pressure used during spray drying. Even at the highest drug load (2% wt/wt), the drug was present in a noncrystalline state. The drug release from the agglomerated microspheres was quick and almost complete within 1 hour. However, compressing the same amount of agglomerated microspheres into an implant greatly reduced the rate of ciprofloxacin release. Only 12% (wt/wt) of the drug was released from the implant within 1 hour. The in vitro release of ciprofloxacin from these implants follows a diffusion-controlled mechanism. This method provides a unique way of producing various shapes and drug loads of HAP microspheres that can be easily manufactured on a commercial scale.

Key Words: Hydroxyapatite, Calcium, Microspheres, Agglomerated microspheres, Ciprofloxacin, Implant, Precipitation, and Spray drying.

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INTRODUCTION

In the treatment of chronic osteomyelitis, conventional antibiotic therapy alone does not always yield satisfactory results. Although the dose of antibiotics administered systemically is high, therapeutically effective drug concentration is not always achieved at the site of infection since bones are moderately perfused organs and the blood supply in infected bone tissues is, therefore, often reduced. As a result, interest in targeted local delivery of antibiotics has risen [1] . The advantage of this approach is that an effective drug concentration (or dose) is attained at the site of infection, while the systemic drug concentration is very low. A review of the various implantable drug delivery systems developed for the treatment of osteomyelitis has also been published [2-3]. Among all implantable delivery systems described in the pharmaceutical literature, HAP, which is the major component of bone has the added advantage of being biocompatible, being bioresorbable, and

having high binding affinity for a variety of molecules [4].

A number of calcium-based delivery systems containing antibiotics have already been investigated and have shown promise in the treatment of osteomyelitis [5-7]. Self-setting apatite cement with a phase of HAP is one of the potential drug delivery carriers with advantages related to its dissolution properties and the possibility of shaping it into different shapes and sizes [8,9]. In this approach, the antibiotic solution is mixed with the powder phase homogeneously. A setting time is required for hardening in situ. The disadvantage of this method is that the drug delivery system has to be prepared with the proper drug load in the surgical room to achieve definite shape of the implant by injection molding. After hardening of the cement, the device is no longer flexible. A so-called 1 stage operation has also been developed by Itokazu et al. to simplify the process of incorporating drugs into HAP systems [10]. Preshaped porous HAP blocks were mixed with an antibiotic solution and centrifuged or decompressed in a vacuum. The antibiotic was thus impregnated into the porous HAP. It was then ready for implantation. The major drawback of all the above described HAP systems is that it may not be possible to produce these drug delivery systems at a commercial scale with variable drug concentrations. Finally, the preparation of the delivery system during surgery introduces an additional variable that can increase the potential for risk and the level of inconsistency.

Therefore, the objectives of this investigation were to (1) synthesize HAP microspheres containing ciprofloxacin by a precipitation and spray-drying method that can be easily commercialized, (2) prepare cylindrical implants by compression of agglomerated microspheres, (3) characterize the drug delivery system, and (4) evaluate the in vitro release characteristics and mechanism of release of the drug from such a delivery system.

MATERIALS AND METHODS

Calcium hydroxide, phosphoric acid, perchloric acid, citric acid, dibasic sodium phosphate, acetonitrile, methanol, ammonium hydroxide, water (high-performance liquid chromatography [HPLC] grade) (Fisher Scientific, Fairlawn, NJ), and ciprofloxacin (Bayer Pharmaceutical, West Haven, CT) were used as received.

Preparation of HAP Microspheres

The patented process to synthesize the microspheres has been described in detail elsewhere [11] . It is illustrated in Figure 1 [12] . Calcium hydroxide and phosphoric acid were used as starting materials. A precursor gel containing a calcium-to-phosphorous ratio of 10:6 and ciprofloxacin was prepared carefully by adjusting the pH to 10 by ammonium hydroxide. The suspension was mixed well using a magnetic stirrer (600 rpm) at 25ºC. The viscosity of the gel was adjusted up to 2-3 cst by adding water to the suspension. After adjusting the viscosity of the gel, a spray-drying process was applied using Berkley Advanced Biomaterials' pilot production spray dryer to form the HAP microspheres with 3-dimensional architectures through optimization of the process parameters. Atomization was carried out at pressures of 1, 3, and 5 $kg.cm^2$. The atomized liquid drops were dried by a coaxial flow of air maintained at a flow rate of 1 $m^3.min^{-1}$. The inlet temperature for spraying was 25ºC and the outlet spraying temperature was 100ºC. The spray setting parameters (viscosity, pressure, and temperature) were important for controlling the morphology of the microspheres.

Preparation of HAP Implants

To improve the compaction properties of the microspheres, agglomerated HAP microspheres were prepared prior to compression. The HAP microspheres containing ciprofloxacin were mixed with the proper amount of water to create a paste that was flowable in an extruder under a pressure of 80 g/cm^2 at room temperature. A granulator was used to extrude the agglomerated HAP microspheres. The granules were air-dried for at

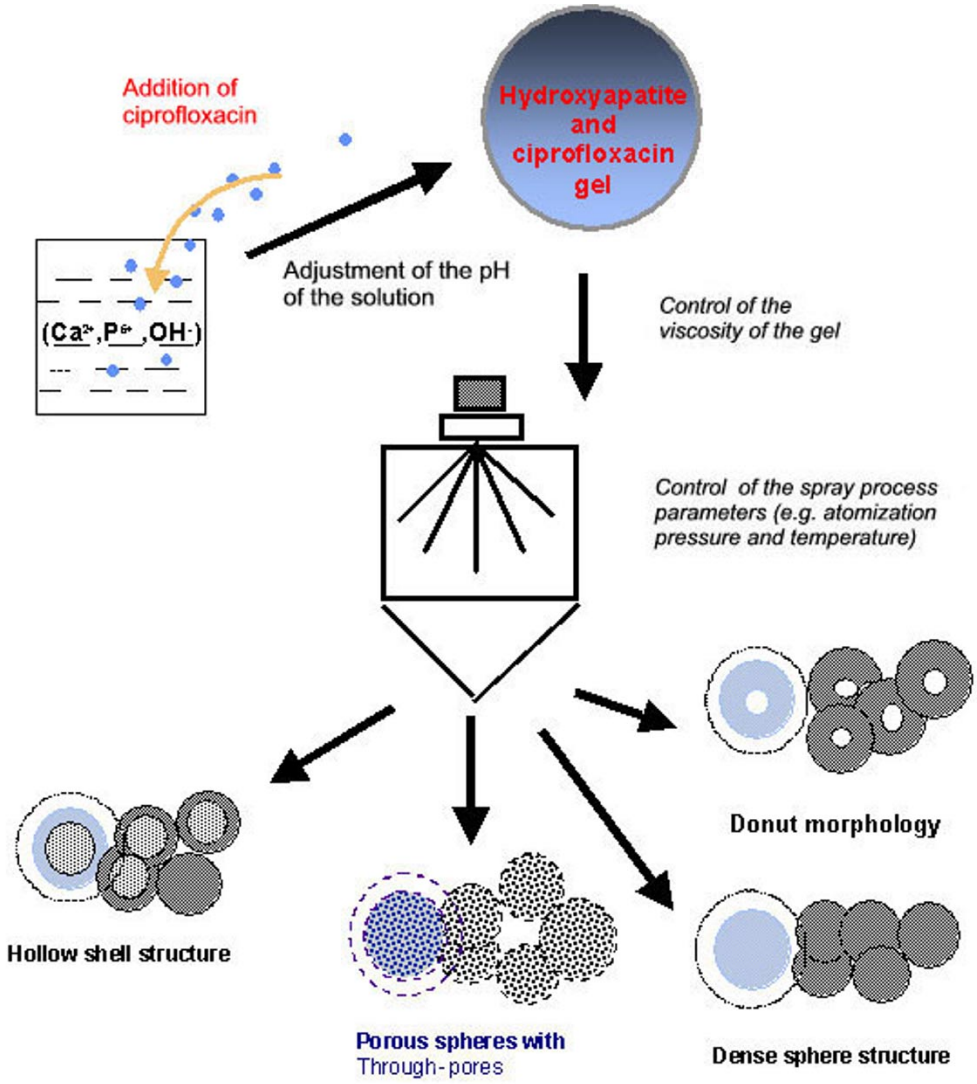

Figure 1 - Flow chart for the synthesis of hydroxyapatite microspheres containing ciprofloxacin.

least 12 hours. Two sizes of sieves were used to produce the granules ranging from 0.4 to 0.6 mm and from 0.6 to 1.4 mm, respectively. No additives were used in this process. Cylindrical implants (5x8 mm) were made by compressing the agglomerated microspheres in a Carver press (Carver Inc., Wabash, IN) with 900 psi [6.2×10^6 pascal] pressure and a 10-second dwell time) using a specially designed die and punch assembly (Natoli, Saint Charles, MO).

Powder X-Ray Diffraction

Samples were exposed to CuKα radiation (45 kVx40 mA) in a wide-angle X-ray diffractometer (model D5005, Siemens, Madison, WI). The instrument was operated in the step-scan mode in increments of 0.05º 2θ. The angular range was 5º to 40º 2θ, and counts were accumulated for 1 second at each step.

Differential Scanning Calorimeter

A differential scanning calorimeter (DSC) (model DSC-50, Shimadzu, Kyoto, Japan) was connected to a thermal analysis operating system (TA-50WS, Shimadzu, Kyoto, Japan). The sample to be analyzed (3-5 mg) by DSC was crimped nonhermetically in an aluminum pan and heated from 30ºC to 200ºC at a rate of 10ºC/minute under nitrogen purge.

In Vitro Release of Ciprofloxacin

In vitro release of the drug from the agglomerated microspheres and implants was determined by 2 independent methods. For a short-term in vitro release, USP Dissolution Apparatus 1 with 500 mL of dissolution medium (Sorensen's phosphate buffer, pH 7.4 at 37ºC) was used. For long-term in vitro release studies, the method described by Cohen et al. was slightly modified and used in this study [13]. A 50-mL Erlenmeyer flask with a ground-glass stopper, containing 40 mL of the release medium was shaken in a horizontal shaker bath maintained at 37 ± 1ºC at 80 rpm. At definite time intervals, 0.5 mL of the release medium was collected and replaced with 0.5 mL of fresh buffer. The ciprofloxacin concentration was measured by liquid chromatography (LC). The weight of the implant used in the in vitro release study ranged from 0.2 g to 1 g. The in vitro release profiles of the drug from both microspheres and implants were then evaluated and compared.

HPLC Analysis of Ciprofloxacin

The HPLC system consisted of a pump (model LC-600) programmed by a system controller (model SCL-6B), an ultraviolet-visible spectrophotometric detector (model SPD-6AV), and a recorder (model CR501) from Shimadzu (Tokyo, Japan). The separation was carried out using a Sphrisorb C18 pH stable column (Phase Separations, Norwalk, CT), 15 cm long. The mobile phase consisted of citrate buffer:acetonitrile:methanol (85:10:5 vol/vol/vol) with an apparent pH adjusted to 2.4 with perchloric acid. The flow rate was maintained at 1.5 $mL.min^{-1}$, and the column effluent was monitored at 280 nm. Phenacetin was used as the internal standard. Relative standard deviations for within-day and day-to-day precision were within 5%. The standard curves were linear over a concentration range of 0.1 to 10 $\mu g.mL^{-1}$.

Drug Incorporation Efficiency

Weighed amounts of the agglomerated microspheres (10 mg) were dissolved in 2 mL of 0.1 N HCl and sonnicated for 1 minute. The volume and pH of the solution were adjusted to 50 mL and 2.4, respectively, with mobile phase and perchloric acid. The final solution was filtered through a syringe filter, and 20 µL was analyzed by LC. The incorporation efficiency was determined as follows: Incorporation efficiency (%) = (Experimentally determined ciprofloxacin amount in the microspheres/Theoretical amount added) x 100.

RESULTS AND DISCUSSION

HAP microspheres with different morphologies can be achieved by this process, which has been extensively studied and reported elsewhere [12]. Morphologies of these spray-dried materials include donut shapes, solid spheres, and hollow spheres. Only HAP solid spheres were investigated in this study. The surface topography, size, and size distribution of the spray-dried products were obtained by SEM characterization.

HAP microspheres containing ciprofloxacin were prepared by precipitation and spray drying. The theoretical drug load in the microspheres ranged from 0.5% to 2% (wt/wt). The particle size and size distribution of these microspheres was determined by using a particle size analyzer (HELOS ParticleSize Analyzer, Sympatec GMBH, Hamburg, Germany). The size distribution of HAP microspheres containing ciprofloxacin is shown in Figure 2. The average diameter was found to be 6.23 ± 0.05 µm. The surface morphology of the ciprofloxacin-loaded HAP microspheres is shown in Figure 3 (A) and the agglomerated microspheres in Figure 3 (B). The microstructure of both the microparticulate systems was found to be spherical

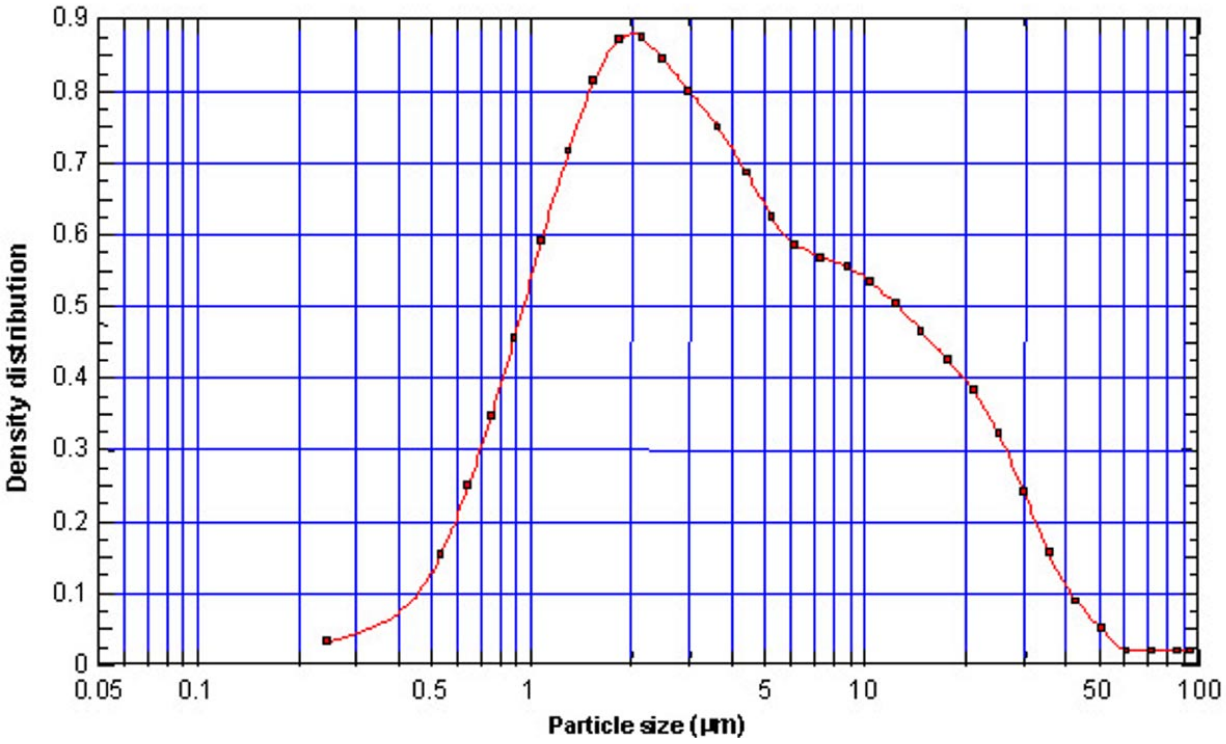

Figure 2 - Size distribution of the hydroxyapatite microspheres containing ciprofloxacin measured by the particle size analyzer.

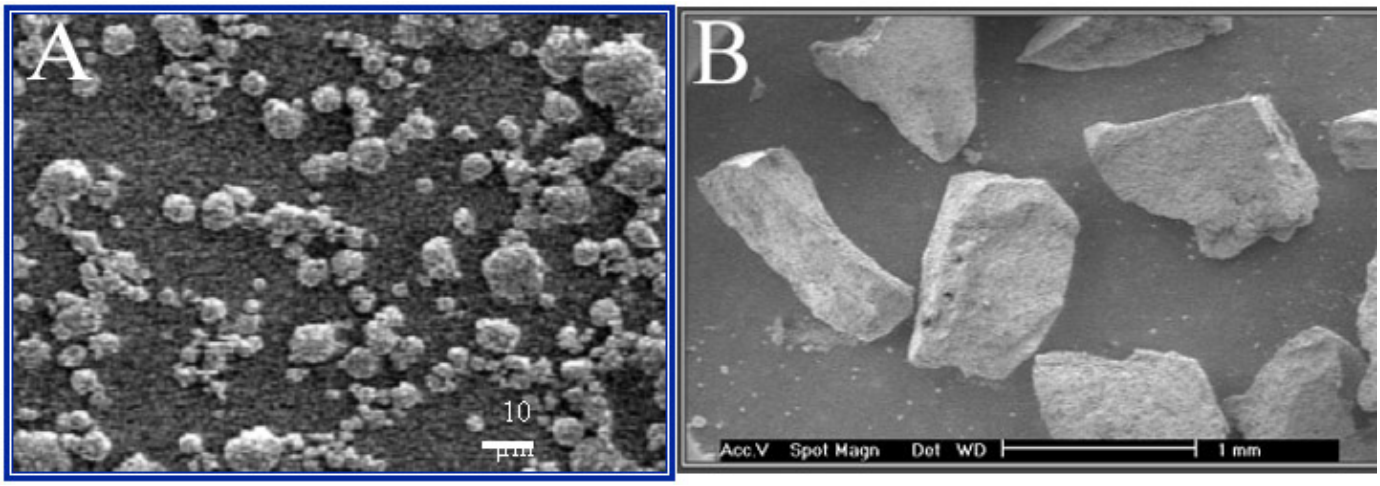

Figure 3 - Scanning electron micrograph of (A) hydroxyapatite microspheres containing ciprofloxacin (at x1000) and (B) agglomerated hydroxyapatite microspheres prepared by the wet granulation process (at x25).

with rough surfaces. Since HAP implants were made by a compression process, the spray-dried microspheres were agglomerated into larger particles to achieve improved flow and compression properties. The agglomerated microspheres were used for the in vitro release studies as well as in fabricating the implants. Powder X-ray diffraction patterns of HAP microspheres containing 2% (wt/wt) ciprofloxacin are shown in Figure 4. A comparison between the diffraction patterns of

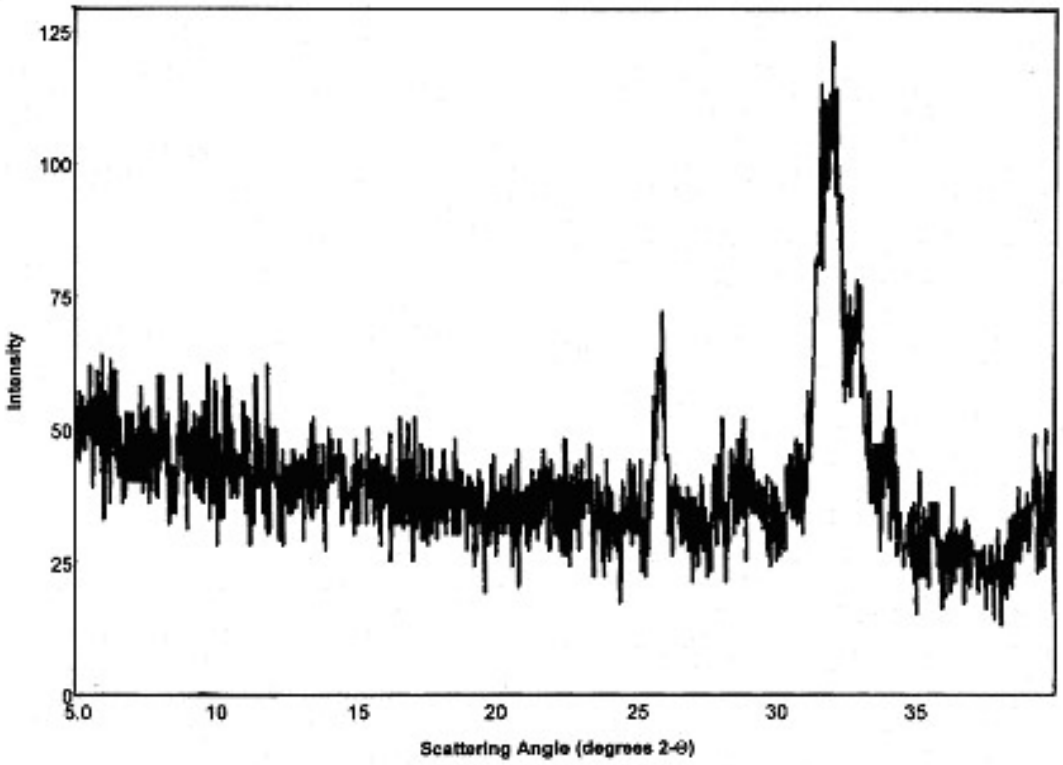

Figure 4 - X-ray diffraction pattern of hydroxyapatite microspheres containing 2% (wt/wt) ciprofloxacin.

ciprofloxacin-loaded and -unloaded HAP microspheres did not show any defferences in the diffraction patterns indicating that the drug is present in a noncrystalline form in the microspheres even at a concentration of 2% (wt/wt). The crystalline nature of the drug and its crystal habit have already been reported elsewhere [14]. DSC was also used to study the physical state of the drug in the microspheres. No melting endotherm was recorded in the DSC curves of HAP microspheres containing 2% (wt/wt) of ciprofloxacin, which also indicates that the drug is present in a noncrystalline state in the microspheres. The incorporation efficiency of HAP microspheres was evaluated for 3 different drug-loaded microspheres. The ciprofloxacin content was determined by HPLC, and the experimental measurement was compared to the nominal (theoretical) drug load. Three nominal drug loads (0.5%, 1%, and 2% wt/wt) were investigated in this study. The experimentally determined ciprofloxacin amount in each of the microspheres was 0.25% ± 0.002%, 0.53% ± 0.02%, and 1.19% ± 0.004% (wt/wt), respectively. Therefore, the calculated incorporation efficiency was within the range of 50% to 60% wt/wt.

The ciprofloxacin release profiles from agglomerated microspheres and implants containing the same amount of drug are plotted in Figure 5.

The results of this study indicate that drug release from the HAP microspheres is fast and complete within 1 hour. Compressing agglomerated microspheres into implants can sustain the release of drug over a prolonged period. The effect of the drug load on the release characteristics and the mechanism of release of ciprofloxacin from this implantable drug delivery system were then evaluated. Figure 6 is a plot of the in vitro release of ciprofloxacin from 3 different drug-loaded implants.

As expected, an increase in drug load increased the rate and extent of ciprofloxacin release. The amount of drug released was then plotted against the square

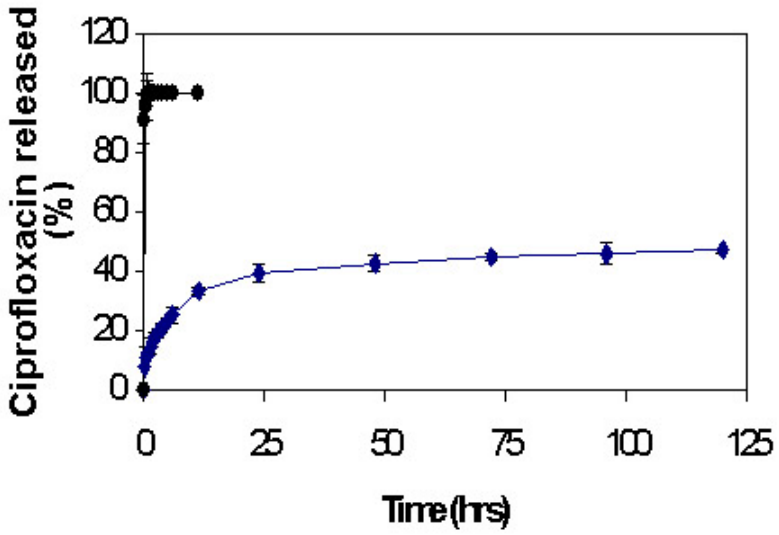

Figure 5 - In vitro release of ciprofloxacin from agglomerated microspheres and implants containing similar drug load versus time: (◆) implant, (●) agglomerated microspheres.

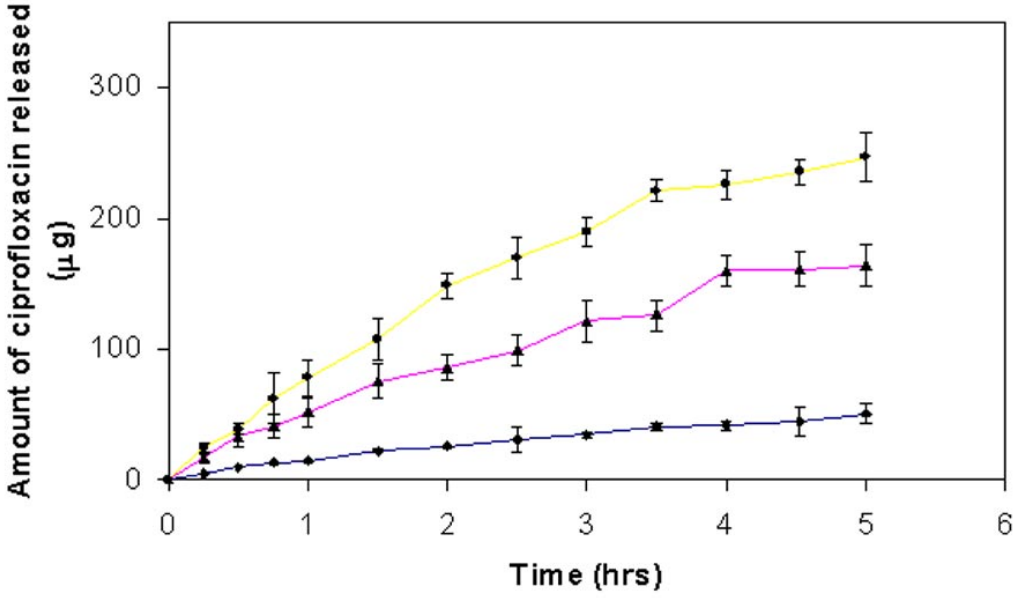

Figure 6 - Effect of drug load on the in vitro release of ciprofloxacin from hydroxyapatite implants: (◆) 0.5% (wt/wt), (▲) 1% (wt/wt), and (●) 2% (wt/wt) nominal drug load in the implant.

root of time. The results are depicted in Figure 7. A linear relationship was evident in the case of the 3 different drug-loaded implants. Results of this study further suggested that the release of ciprofloxacin from these implants followed a matrix diffusion controlled mechanism as described by Higuchi [15]. The slope of the lines in Figure 7, which is a measure of the rate of drug release from the delivery system, was then determined. The slopes for the 0.5%, 1%, and 2% (wt/wt) ciprofloxacin implants were 23.6, 81.4, and 126.1 ($\mu g.hr^{-1/2}$), respectively.The changes in morphology of the agglomerated microspheres after in vitro release studies are shown in Figure 8. Many additional

pores are visible on the surface of the implant after in vitro release, indicating an additional pathway for penetration of the release medium into the matrix.

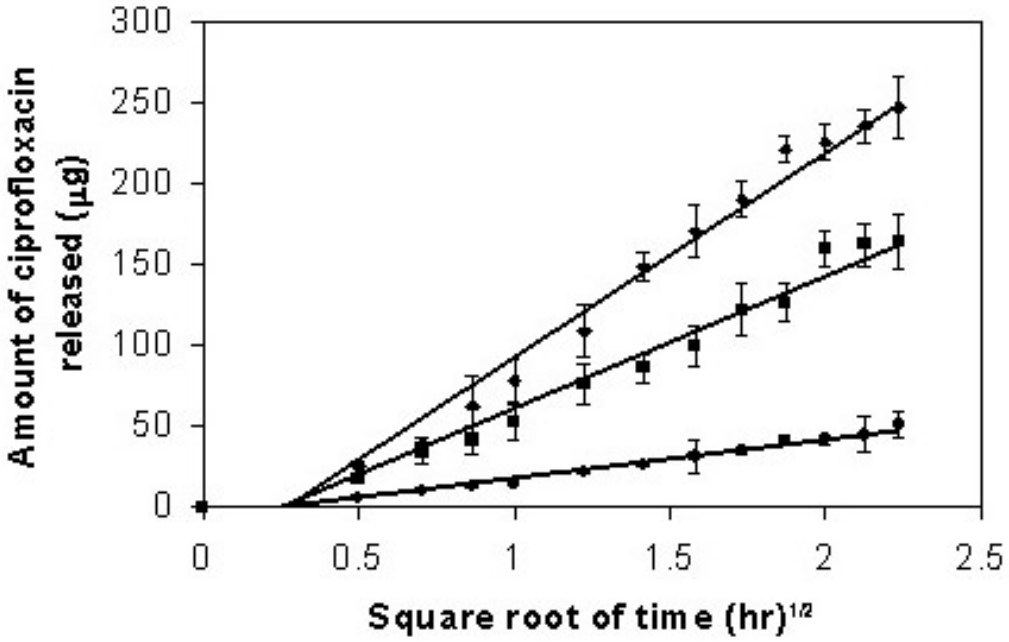

Figure 7 - In vitro release of ciprofloxacin from hydroxyapatite implants versus square root of time: (●) 0.5% (wt/wt), (■) 1% (wt/wt), and (◆) 2% (wt/wt) theoretical drug load in the implant.

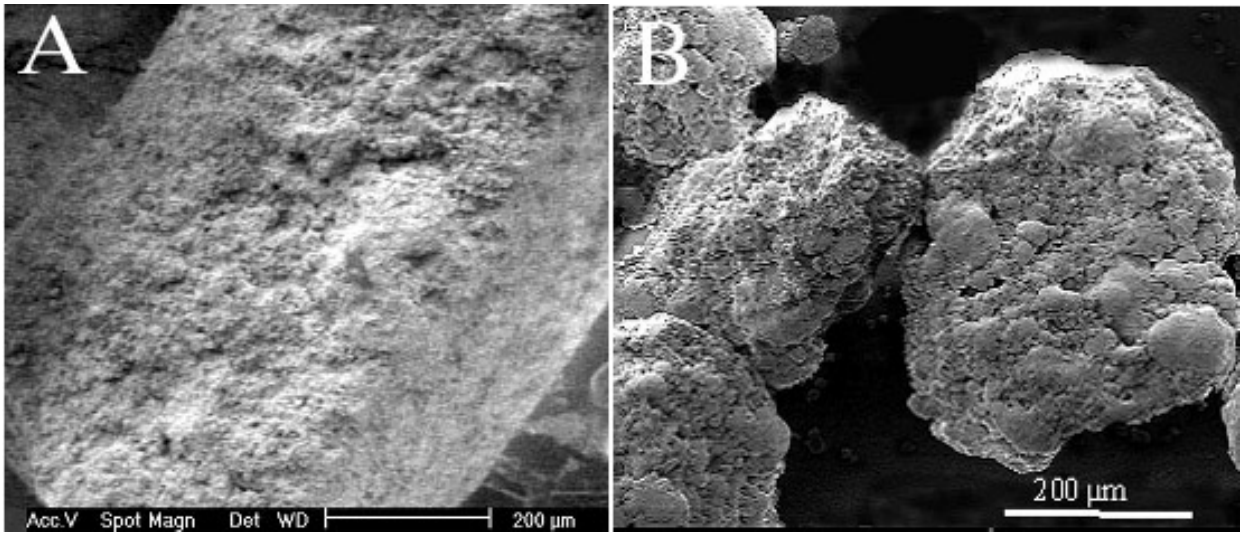

Figure 8 - Scanning electron micrographs of hydroxyapatite agglomerated microspheres containing ciprofloxacin: (A) before in vitro release (at x250), and (B) after 5 hours in vitro release (at x250).

CONCLUSION

HAP microspheres containing ciprofloxacin with controlled morphology were synthesized and characterized. The synthesis method involved chemical precipitation followed by a spray-drying step. The concentration of the slurry, atomization pressures, and temperature determined the size and morphology of the spray-dried powder. DSC and X-ray results revealed that the drug in the HAP

microspheres was amorphous or in solid solution. A wet granulation process was used to prepare agglomerated microspheres. The in vitro release of ciprofloxacin from the agglomerated microspheres was fast and complete within 1 hour. On the other hand, implants have a lower release rate that can be sustained for several days. The drug release patterns from these implants correlated well with Higuchi's square-root model. One of the unique advantages of this method as compared to already-reported HAP drug delivery systems is the ease of manufacturing the microspheres and the implants with variable concentrations and shapes. A report on the in vivo evaluation of this drug delivery system will be issued in the future.

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