

Research Article

A Processing Approach to Tuning the Drug Delivery Characteristics of Calcium Polyphosphate Matrices

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Abstract Amorphous calcium polyphosphate (CPP) has potential as an implantable drug delivery matrix by virtue of a low temperature gelling protocol that has been shown to eliminate burst release and extend drug release time from the matrix. However, a greater understanding of this material's interaction with aqueous environments is needed to more fully exploit this application. Variations in aqueous exposure were assessed using as-made amorphous CPP as well as CPP processed using established low temperature protocols. Solid-state ³¹P-NMR along with thermal and X-ray diffraction analyses were used to track resulting structural changes. Exposure to aqueous environments caused a reduction in CPP chain length that was dependant on gel time and mode of exposure. Significantly, increased gel times or water availability further resulted in crystallization events upon drying, except in the presence of a buffered solution. In general, drug elution studies showed an increase in the burst release of vancomycin from CPP disks gelled for extended periods, with matrix-water interactions appearing to be most influential during the drug loading stage. Overall, this study shows that CPP drug delivery matrices can be produced with tailored properties by closely controlling CPP-water interactions during processing.

Keywords calcium phosphate; drug delivery; antibiotics; structural characterization

1 Introduction

Chronic bone infections (osteomyelitis) typically result in significant bone loss and are often very difficult to eradicate through systemic treatments due to disruption of the local circulation. Localized drug delivery strategies are therefore essential. To this end, recent efforts have sought to develop degradable, potentially osteopromotive matrices from calcium polyphosphates (CPP), a unique class of calcium phosphates typified by a phosphate chain

structure $\{[\text{Ca}(\text{PO}_3)_2]_n\}$ that has demonstrated utility in bone-interfacing applications [3]. A low temperature gelling and drying protocol (designated G1) exploiting the hygroscopic nature of the amorphous phase was developed that enabled antibiotic loading with a corresponding reduction in burst release and extended release overall compared to the ungelled matrix [1,2,7]. A subsequent G2 protocol involving comminution of G1 disks followed by compaction, regelling and drying was found to completely eliminate the burst release and further extend the release of vancomycin (VCM) from these matrices [5]. While it is clear that drug release properties from these matrices are dependant at least in part on the processing conditions, particularly CPP interaction with the aqueous environment that yields the characteristic gelling response, the impact of resulting structural changes on these release properties remains poorly understood. Such interactions would also influence subsequent matrix degradation during elution. Here, variations in aqueous exposure (limited water, excess water, and TRIS buffered saline) will be assessed with respect to changes in matrix structure and subsequent drug release properties.

2 Materials and methods

CPP particulates and disks were used for structural characterization and elution studies, respectively. Starting CPP powder ($< 45 \mu\text{m}$) was synthesized using the method described by Pilliar et al. [6]. Matrix loading to produce G1 disks was achieved using the process developed by Dion et al. [1]. Here, 2.25 g of CPP powder was mixed with 903 μL of water (blanks) or a 0.125 mg/ μL solution of vancomycin, such that all loaded disks contained 7.5 mg of the therapeutic. The resulting hand-mixed paste was packed into polyvinylsiloxane molds, then placed in a humidity chamber at 37° C and 100% relative humidity for

Sample group	% Q ⁰	% Q ¹	% Q ²	Average CL	Total mass loss (%)	Primary exothermic peak (°C)
Raw CPP	0	3	97	67	0	667.5
G1	0	5	95	40	10	566.6
G2	1	8	91	25	12	535.8
1 : 1 wet gel	6	14	80	13	28	476.4

Table 1: Summary of chain length and thermal analysis data for raw, G1 and G2 powders and a 1 : 1 gel.

the designated gel time period prior to drying at 37° C for 24 hours. G2 disks were produced by the method of Petrone et al. [5]. Briefly, G1 disks (loaded or unloaded) were ground in a planetary ball mill and then sieved to obtain a G1 powder having a particle size of less than 45 μm . Approximately, 150 mg of G1 powder was placed into a punch-die system (8 mm DIA \times 2 mm) and compacted for 5 minutes at 113 MPa, after which the punches were removed and the dies containing the disks were placed in a humidity chamber at 37° C and 100% relative humidity for a predetermined time. The disks were subsequently dried at 37° C for 24 hours prior to removal from the die. To further explore the interaction of CPP and water, a constant amount of as-made CPP powder ($< 45 \mu\text{m}$) was mixed with varying amounts of water leading to different CPP : H₂O mass ratios (1 : 1 to 1 : 5) in sealed scintillation vials. After 1 hour at 37° C, the covers were removed and the vials were placed in an oven at 60° C to dry for 48 hours. In addition, a viscous gel was made by incubating equal amounts of amorphous CPP powder and water (1 : 1) at 37° C for 24 hours.

Single pulse excitation experiments were performed on as-made amorphous CPP, G1 powder, G2 powder, and a wet 1 : 1 gel by ³¹P MAS Solid State NMR using a Bruker Avance spectrometer (400 MHz, 9.4 T, 4 mm rotor) having a spinning rate of 8000 Hz. The integrated peak areas (%) for each phosphorus species (Q⁰, Q¹, Q²) present were subsequently used to calculate the average phosphate chain length (CL) using the following equation adapted from Kulaev et al. [4]:

$$\text{CL} = 2(Q^1 + Q^2)/Q^1.$$

Additional structural information for these CPP systems was obtained by thermal analysis and X-ray diffraction. Simultaneous differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) (Netzsch Luxx 409 PC) were performed using a heating rate of 5° C/min or 20° C/min from room temperature up to a minimum of 700° C. Powder samples were placed in covered Pt crucibles and weighed using the instrument's internal balance. A constant flow of N₂ gas was blown over all samples for the duration of the experiment at a rate of 30 mL/min to maintain an inert environment. X-ray diffraction patterns were collected using a JD2000 spectrometer with a Philips

diffractometer and then analyzed using Match! Version 1.3. All samples were scanned from $2\theta = 5$ to 70° at 0.05 increment with a dwell time of 5 seconds.

An elution protocol developed and reported on by Dion et al. was used to evaluate the release of drug from the CPP disks for all elution studies [2]. Briefly, disks were placed in separate cornea viewing chambers filled with 15 mL of 0.1 M Tris buffered saline (TBS), incubated at 37° C and gently agitated at 90 rpm on a horizontally rotating plate. At designated time points, 7 mL of the media was removed from the chamber and replaced with 7 mL of fresh, 37° C, TBS buffer. VCM levels in the media were analyzed by UV-Vis spectrophotometry at $\lambda = 282 \text{ nm}$.

3 Results and discussion

Not surprisingly, a reduction in chain length (c.a. 67 to 13) was observed with increasing water exposure, (Table 1), with established gelling protocols (5 hours G1, 5 hours G2) retaining more aspects of the long-chain structure. This increasing exposure (increasing water availability) resulted in increased water uptake, with a corresponding mass loss upon heating, as well as a shift and eventual loss of the characteristic exothermic crystallization peak for CPP and the development of several endothermic peaks (figure not shown). Up to three levels of water interaction with the CPP were suggested by TGA traces with “weakly bound” or trapped water driven off just above 100° C, followed by additional mass loss between 200 and 400° C. These discrete mass losses became decidedly less distinct with increasing water availability (1 : 2 to 1 : 5). Only the G2 samples displayed the possible presence of a third, more strongly bound form of water that is released during an exothermic event at 460° C. Overall, the observations support a 3-step gelling process involving initial wetting of the CPP solid, water infiltration into and around chains of CPP and lysis of the phosphate chains, the kinetics of which are dependent on CPP surface area, CPP : water, and the state of the water (gas or liquid).

Sufficient exposure to water (gelling) either through longer exposure times (G1) or an increased CPP : water ratio (1 : 1 or higher) resulted in crystallization events upon drying, with evidence of the starting calcium phosphate monobasic monohydrate powder (PDF#72-1137) at 1 : 4

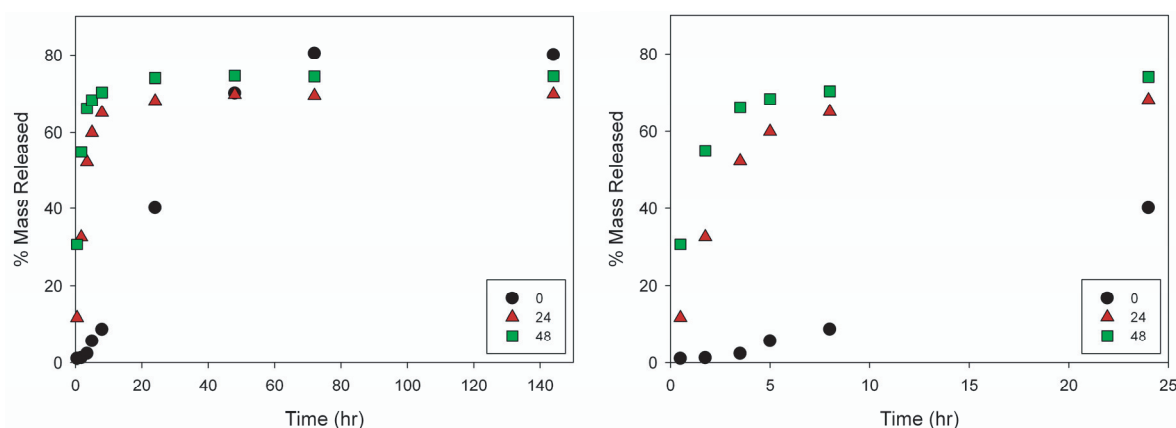


Figure 1: Release profiles for G2 disks with a drug-loading gel time (G1) of 0, 24, or 48 hours; the first 24 hours of elution are highlighted (right).

ratios or higher. Notably, these crystallization events were suppressed when a higher ion strength solution such as 0.1 M TBS was present, even with significant exposure (1 : 150, 10 days).

The impact of water exposure as a function of “gel” time for CPP disks processed using established protocols (G1 drug loading + G2) was evident in VCM release profiles. An extended gelling time during loading (24 and 48 hours, with post compaction or G2 gel time of 5 hours) resulted in a significant increase in the burst release compared to disks that did not undergo a G1 gelling step (Figure 1). This burst release is attributed to an increase in the diffusion rate of VCM out of the CPP disks when the chains are shorter as well a corresponding loss of further “gelling” ability in the elution media. All disks that showed elimination of burst behavior also demonstrated swelling behavior, typically between 4 and 8 hours of elution. This swelling may cause a change in the diffusion properties of the drug out of the material. It is also likely that crystallization events resulted in some phase separation of VCM from the matrix.

These elution studies further indicated a greater impact of water interactions at the drug loading stage (G1), with a strong relationship between G1 loading gel time (not the total gel time G1 + G2) and the drug release properties. This observation is consistent with the greater water availability—and hence a greater drive for chain lysis—during this initial gelling stage compared to the G2 step, where aqueous exposure for the dense powder compacts is in the form of atmospheric water only. Still, a need for some “gelling” during drug loading in order to promote drug entrapment or interaction with the matrix is suggested by greater retention of VCM in the 24 to 72 hour period when a 2 hour G1 gel time is used compared to no prescribed gelling during loading (data not shown). Additionally, molecular level drug interactions with the CPP matrix, considered minimal with a weakly polar

VCM molecule, will likely impact the gelling process and subsequent release characteristics.

4 Conclusions

Exposure of amorphous CPP to water results in an uptake of water that ultimately leads to hydrolysis of the polyphosphate chain and the formation of shorter chained species. Significantly, increased gel times or water availability further resulted in crystallization events upon drying, except in the presence of higher ionic strength solutions. The extent of any structural transformation was dependent on the amount of water available during exposure, the exposure time, and the nature of the solution. CPP interactions with water during drug loading (G1) rather than overall exposure to water as required for processing (G1 + G2) had a much greater impact on drug release behavior. Overall, this study shows that CPP drug delivery matrices can be produced with tailored properties by closely controlling CPP-water interactions during processing.

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