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Calcium polyphosphate precipitation – A strategy to tune the chain length of the glass and control the subsequent release of vancomycin

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HIGHLIGHTS

- We present a systematic preparation of CPP from aqueous sodium polyphosphate.
- Precipitation significantly increased CPP chain length compared to furnace protocol.
- Precipitation did not reduce early stage release of vancomycin from CPP.
- This study revealed valuable mechanistic data on the precipitation of CPP.

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ABSTRACT

Using a multi-variable design approach this study sought to determine whether calcium polyphosphate (CPP) could be precipitated with a significantly higher chain length than achieved with conventional melt-derived glass and subsequently enhance the performance of the CPP-based drug delivery matrix. Manipulating aqueous sodium polyphosphate concentration, order of reactant addition, and Ca/P molar ratio at mix of reactants across a minimum of two levels was found to significantly influence the chain length, Ca/P ratio, and residual sodium of the resulting CPP precipitates. The various interactions of these three variables were also found to have a significant impact on the aforementioned properties of the precipitates and we successfully fabricated a precipitate with a 6-fold increase in chain length over that achieved by conventional melting. Despite not seeing a significant improvement in drug release properties, our systematic preparation of calcium polyphosphate from aqueous sodium polyphosphate solutions yielded valuable mechanistic data on this interesting fabrication strategy.

1. Introduction

Calcium phosphate materials have been widely studied for use as biomedical implant materials [1]. They have gained recognition in pharmaceutical formulations due to a chemistry that allows them to readily absorb different chemical species on their surfaces, and a favorable *in situ* biocompatibility and bioactivity [2,3]. Condensed calcium polyphosphates (CPP[1]) possessing a lower Ca/P ratio of 0.5 are relatively new to the field of biomedical engineering and may offer an advantage in this regard while possessing some potentially unique drug delivery attributes.

CPP consists of phosphate units connected in a linear chain-like structure by a phosphoanhydride bond, with calcium ions serving as network modifiers between these chains [4,5]. Several studies have previously confirmed the osteoconductivity, biocompatibility and degradable nature of CPP [5–13] and it has been studied for use in cartilage and bone repair, as well as for angiogenesis [9,14–18]. In the literature, CPP is most commonly fabricated in the furnace [6,19]. Unfortunately, only a few studies have examined the chain length of this "melt-derived" CPP as a function of furnace conditions and the results have proven inconclusive [20–22].

Earlier reports by Masson et al. [23] and Sinyaev et al. [24] revealed that CPP could be precipitated starting with a monovalent polyphosphate in accordance with the following aqueous reaction: $2MPO_3 + CaCl_2 \rightarrow Ca(PO_3)_2 + 2MCl$, where M is a monovalent cation. Such a precipitation strategy may be able to

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[1] CPP: Calcium Polyphosphate.

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take advantage of the pre-existing chain structure of the starting polyphosphate reactant. Sodium polyphosphate ($NaPO_3$ or NaPP[2]) glass, for example, has been reported to have a chain length much greater than that of the standard melt-derived CPP glass [25]. However, the extent to which the greater chain length of NaPP can be converted to that of CPP has not been previously pursued. Precipitation studies of other calcium phosphates have indicated that a number of factors including solution pH, temperature, the Ca/P ratio, ionic strength and concentration of the reagents may influence the nature of the final precipitate [1,26–31]. The primary objective of the study was therefore to determine through multivariable analysis the impact of three key fabrication variables, namely aqueous NaPP concentration, order of reactant addition, and Ca/P molar mix ratio of reactants, on the chemistry and chain length of the resulting CPP precipitate. We hypothesized that our precipitation strategy could be optimized to create a CPP with significantly greater chain length than that achieved through furnace fabrication.

In addition, our recent development of a low-temperature protocol for inclusion of thermally labile biological agents in amorphous CPP has further supported the potential of this material for local drug delivery [32–35]. Dion et al. [32], for example, found that increasing CPP glass exposure time to humidity from 5 h to 24 h resulted in a small reduction in average chain length, and a subsequent increase in the therapeutic burst release coupled with a decrease in the duration of sustained antibiotic release *in vitro*. Our corollary argument is that an increase in chain length resulting from this alternative precipitation protocol could enhance the sustained delivery of therapeutic agents from the CPP matrices. Our secondary objective then was to compare the *in vitro* elution of an antibiotic from the CPP glass matrices achieved using either the furnace or the new precipitation protocol.

2. Materials & methods

2.1. CPP fabrication

Our melt-derived CPP glass was produced following the protocol developed by Pilliar et al. [6]. In short, calcium monobasic monohydrate was calcined at 500 °C for 10 h to produce CPP via a condensation reaction in accordance with the reaction: $nCa(H_2PO_4)_2{}^*H_2O \rightarrow [Ca(PO_3)_2]_n + 3nH_2O$. The resulting powder was then melted at 1100 °C for 2 h and quenched in distilled water. After washing the resulting amorphous CPP frit in 100% ethanol, the frit was milled and sieved to obtain <45 μm fraction of particles.

NaPP was obtained by heating sodium phosphate monobasic monohydrate in a Pt crucible at 800 °C for 20 h in a protocol similar to Strauss et al. [36]. The melt was then rapidly quenched on a copper plate and cooled to room temperature. Aqueous solutions of this NaPP were next mixed with 10% (w/v) aqueous solutions of $CaCl_2{}^*2H_2O$ in order to form a CPP precipitate. The precipitation set-up was similar to that of the Stirred Tank model analyzed by Benet et al. [30] and that which is commonly employed in industry. In accordance with this model the reactant(s) are added near the blade of the continuously rotating mixer impeller within a rigidly contained beaker. The solution was maintained at 20 °C and stirred at a continuous speed of 190 rpm to closely resemble prior precipitation studies [24,37,38]. From available literature on phosphate precipitation [1,26–31] and supported by our own preliminary, single variable analysis of precipitation variables we anticipated that design variables which influence feed blending, such as the bulk and local concentrations of chemical species may be of potential significance in our system. Three variables which may influence the bulk and local concentrations of the chemical species include: reactant order (variable A), Ca/P Molar Mix Ratio of Reactants (variable B), and NaPP concentration (variable C). The defined levels for each of these three variables are shown in Table 1.

After no further precipitation from solution was observed, the precipitate was rinsed three times with cold distilled water in order to remove as much of the co-precipitated chlorides as possible [24] and stored in ethanol [39] for a minimum of 30 min. Precipitates were subsequently freeze dried for a minimum of 48 h until change in weight loss was less than 1% of total sample weight.

The goal in fabricating CPP glass from the NaPP was to achieve a greater chain length than the melt-derived CPP but still maintain its basic chemistry. For example, a precipitate with a Ca/P ratio close to 0.5 and as low a residual sodium (Na) content as possible was considered to have met our design criteria. All precipitates were therefore assessed for chain length, Ca/P ratio, and residual sodium content.

2.2. Precipitate characterization

The composition of our CPP was confirmed after dissolution in 6 N HCl followed by assessment with ICP-OES (PerkinElmer Optima8000). Calcium, phosphorus and sodium were measured at wavelengths of 317.933 nm, 213.617 nm, and 589.592 nm, respectively.

To determine chain length of the soluble NaPP, acid-base titration was used to obtain the number-average value in accordance with Mehrotra [25]. Here, 10% (w/v) NaPP solutions were mixed until all material was dissolved before diluting by a further factor of 2. Using 0.1 M HCl, the pH of the NaPP solution was dropped below 4.5. Next, 1.0 M NaOH was added in 2.5 μL increments until a pH above 9.5 was achieved. Chain length (n) was determined using the volume necessary to go from a pH at the early inflection point (a pH of ~4.5) to the latter inflection point (a pH of ~9.0) Mehrotra [25]: n = [2000*weight of polymer dissolved in solution]/[Molecular Weight*volume of NaOH between inflections].

Chain lengths (n) of the CPP precipitated samples were analyzed by liquid-state ^{31}P NMR experiments. Samples were dissolved in 200 mM aqueous solutions of disodium ethylenediaminetetraacetate dehydrate (Na_2-EDTA) and analyzed using a Bruker AV500 MHz NMR spectrometer. Spectra are reported using an 85% solution of H_3PO_4 in H_2O as a reference.

Line shape simulations were then performed using DMFit 2010 Software for each phosphorus species (Q^1, Q^2, Q^3), where Q^1 is a chain end group, Q^2 is an internal phosphate group, and Q^3 is a branched phosphate group. The integrated peak areas (%) for each species were subsequently entered into the following equation adapted from Kulaev et al. [40]: $n = 2^*[Q^1 + Q^2 + Q^3]/[Q^1 - Q^3]$.

The precipitates were also characterized with X-ray Diffraction (XRD) and Differential Scanning Calorimetry/Thermogravametric analysis (DSC/TG) systems to further elucidate their structural nature. A Bruker D8 Advanced XRD system complete with a high speed LynxEye™ detector and DIFFRAC^{plus} software compared XRD profiles of the precipitates to the available powder diffraction files of crystalline CPP. In addition, samples were heated in a Netzsch Luxx 409 PC DSC/TG unit from 20 °C to 1100 °C at a rate of 10 K/min in a Pt pan. The resulting thermal profiles were assessed for weight loss upon heating with a Proteus Analysis 5.2.0 software.

2.3. Drug loading and in vitro elution study

Based on the multi-variable analysis, one of the precipitate conditions that best met and one that most poorly met our design criteria were further evaluated against the melt-derived CPP for

[2] NaPP: Sodium Polyphosphate.