EFFECT OF ORGANOPHOSPHATES ON THE FORMATION OF MAGNESIUM-FREE WHITLOCKITE

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Abstract: Objective of the present study was to synthesize Mg-free whitlockite (WH) particles using organophosphates with different alkyl chain lengths via a solvothermal reaction. The effects of the organophosphates and their Ca/P molar ratio on the formation of WH were investigated. With increasing alkyl chain length of the organophosphate and increasing Ca/P molar ratio, the dominant phase changed from WH to hydroxyapatite (HA). This result suggests that the organophosphates exhibited different hydrolysis rates. With all of the organophosphates, WHs were obtained only at a Ca/P molar ratio of 0.25. In addition, an analysis of the local structure using 1H and 31P nuclear magnetic resonance spectroscopy indicated that the WH contained protonated phosphate (HPO42−) and trace amount of HA subphase.

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INTRODUCTION

Although β-tricalcium phosphate (β-TCP; β-Ca3(PO4)2) is recognized as a synthetic bone graft substitute, the structurally similar mineral magnesium whitlockite (Mg-WH; Ca9Mg3(HPO4)(PO4)3) has not been investigated in this application. Recent study by Hang et al. demonstrated that Mg-WH is an osteoconducive material with resorbability intermediate between those of hydroxyapatite (HA) and β-TCP.1

The terms β-TCP and Mg-WH have been used interchangeably because the X-ray diffraction (XRD) patterns of Mg-WH and β-TCP are similar to each other. However, structural differences exist between them: β-TCP has Ca2+ ions and vacancies in its Ca(4) sites, whereas Mg-WH has H+ and Mg2+ ions in its Ca(4) and Ca(5) sites, respectively. Misinterpretation of the XRD patterns for Mg-WH and β-TCP is one of the reasons why Mg-WH has not been studied as a synthetic bone graft substitute.

Mg-WH was previously predicted to precipitate from aqueous solutions only in the presence of Mg2+ ions.2 However, several studies have demonstrated that Mg-free whitlockite (WH) (i.e., H-substituted β-TCP) can be synthesized by precipitation from an aqueous solution.3-5 Inspired by Zhu’s study,6 we investigated the formation mechanism of WH in a binary mixed solvent of N,N-dimethylformamide (DMF) and ethylene glycol (EG) using triethyl phosphate (TEP).7 The alkyl chain length of the organophosphate affects its hydrolysis rate and influences the subsequently obtained final product, but we did not focus on this effect in our previous study.8

The aim of the present study is to build on our previous study by investigating the effects of organophosphates and their Ca/P molar ratio on the formation of WH in binary DMF-EG solvents. For this purpose, three different organophosphates; trimethyl phosphate (TMP), TEP, and tributyl phosphate (TBP), are used to synthesize WH. The local structure of the WH is then analyzed by solid-state nuclear magnetic resonance (NMR) spectroscopy.

MATERIALS AND METHODS

Synthesis method

Calcium chloride (CaCl2, Cat. No. 793639), TMP (Cat. No. 241024), TEP (Cat. No. 538728), TBP (Cat. No. 538728), DMF (Cat. No. 227056), and EG (Cat. No. 324558) were purchased from Sigma-Aldrich, Japan, and were used without further purification.

CaCl2 (1.2 mmol) was dissolved in 30 mL of DMF-EG mixed solvents with a volume ratio of 50:50 (vol.%). TMP, TEP, or TBP was added to the CaCl2 solution at Ca/P ratios of 0.25, 0.50, and 1.50 under continuous magnetic stirring. The resulting solution was transferred into a 50 mL polytetrafluoroethylene (PTFE) autoclave (HU-50, SAN-Al Kagaku, Japan) at a filling ratio of 60 vol% and reacted under a pressure of 0.2 MPa at 200 °C for 12 h in an oven. The precipitate was separated by centrifugation, washed twice with acetone and then ultrapure water, and subsequently dried in an oven at 80 °C for 24 h.

Characterization

The crystalline phases were characterized using an X-ray diffractometer (RINT Ultima+, Rigaku...
The effects of organophosphates and their Ca/P molar ratio on the formation of WH in DMF–EG binary solvent mixtures were investigated. Figure 1 shows XRD patterns for the final products synthesized using (a) TMP, (b) TEP, and (c) TBP and at Ca/P molar ratios of 0.25, 0.50, and 1.50. In the case of TMP, single-phase WH (ICDD PDF 00-009-0169) was obtained at Ca/P molar ratios of 0.25 and 0.50, whereas a biphase of HA (00-009-0432) and WH was formed at a Ca/P molar ratio of 1.50. When TEP was used, single-phase WH was formed at Ca/P molar ratios of 0.25 and 0.50; however, single-phase HA was formed at a Ca/P molar ratio of 1.50. When TBP was used, single-phase WH was formed at a Ca/P molar ratio of 0.25, whereas a biphase of WH and HA and single-phase HA was obtained at Ca/P molar ratios of 0.50 and 1.50, respectively. The lattice constants of HA formed from TMP, TEP, and TBP at Ca/P molar ratio of 0.25 were α = 9.473 ± 0.003 and c = 6.855 ± 0.003 for TMP, α = 9.482 ± 0.004 and c = 6.851 ± 0.004 for TEP, and α = 9.421 ± 0.005 and c = 6.881 ± 0.005 for TBP. The crystallite size of WH calculated by XRD patterns was 286 ± 31 Å for TMP, 298 ± 17 Å for TEP, and 451 ± 33 Å for TBP.

The WH powders synthesized at a Ca/P molar ratio of 0.25 were subjected to FT-IR analysis (Fig. 2). The FT-IR spectra show signals of PO₄³⁻ groups (ν₃: ~1030, ~1080 cm⁻¹, ν₁: ~960 cm⁻¹, ν₂: ~545, ~605 cm⁻¹), HPO₄²⁻ groups (~870 cm⁻¹), and adsorbed water (~3400 cm⁻¹).

The N₂ adsorption–desorption curves for the WH powders synthesized at a Ca/P molar ratio of 0.25 show IUPAC type II isotherms (data not shown). The BET SSA for WH samples prepared using TMP, TEP,
and TBP was 39.3, 44.6, and 47.0 m²·g⁻¹, respectively.

The particle morphologies of WH synthesized at a Ca/P molar ratio of 0.25 were observed with SEM (Fig. 3). WH were composed of 100 to 200 nm spherical particles; however, there was no obvious difference in the particles size among organophosphates. In addition, elemental analysis of HA synthesized with TEP at a Ca/P molar ratio of 1.50 showed the presence of Cl⁻ ions together with Ca²⁺ and PO₄³⁻ ions (data not shown).

The local structure of WH powders synthesized using TMP, TEP, and TBP at a Ca/P molar ratio of 0.25 was analyzed by ¹H and ³¹P MAS-NMR. Figure 4a shows ¹H MAS-NMR spectra of WH. The peak at -0 ppm is assigned to lattice OH⁻ in HA, and that at -5.4 ppm is attributable to adsorbed H₂O molecules.¹³ The shoulder peak at -10.8 ppm is assigned to protonated phosphate (HPO₄²⁻) in the WH structure.¹⁴ Unassignable peaks at ~0.8, ~2.4, ~7.4, and ~12.1 ppm were also observed. The peak at ~12.1 ppm is similar to the chemical shift of HPO₄²⁻ in the calcium hydrogen phosphate dihydrate (DCPD) lattice. However, because this signal for DCPD should be weak, the assignment to HPO₄²⁻ in the DCPD lattice was dismissed.

Figure 4b displays the broad ³¹P MAS-NMR spectra of WH, which attribute to PO₄³⁻ ions in WH. The ¹H MAS-NMR peak at ~0 ppm (lattice OH⁻ in HA) should correspond to the ³¹P MAS-NMR peak at ~3 ppm (PO₄³⁻ in HA).¹³ However, it was not distinct due to overlap with the broad peak of WH. On the basis of the ³¹P MAS-NMR study of Mg-WH by McCubbin et al.,¹⁴ the broad peaks were deconvoluted into three peaks centered at 2.0, 1.0, and 0 ppm, however; the deconvoluted peaks did not fit accurately.

## DISCUSSION

The present study investigated the effects of organophosphates and their Ca/P molar ratio on the formation of WH in binary DMF–EG solvents. According to a study by Baradaran et al.,¹⁵ DMF decomposes to dimethylamine ((CH₃)₂-NH²⁺) and formic acid as following Eq. (1). Subsequently, the formic acid decomposes to carbon monoxide (CO) and water as following Eq. (2). It was noted that water (H₂O⁺) molecule adsorbed onto the chemical reagents triggered the reaction in Eq. (1) and subsequently water formed by decomposition of the formic acid in Eq. (2) provoked the reaction of Eq. (1).

\[
\text{HCO-} \text{N(NH}_2\text{)}_2 + \text{H}_2\text{O}^+ \rightarrow \text{HCOOH} + (\text{CH}_3)_2\text{-NH}^2+ \quad (1)
\]

\[
\text{HCOOH} \rightarrow \text{CO} + \text{H}_2\text{O} \quad (2)
\]

Amorphous-precursor-mediated WH formation via a solid–solid phase transformation has been demonstrated in our previous study.¹¹ The nucleation of the amorphous precursor was dominated by the slow hydrolysis of organophosphates, which retarded reaching critical supersaturation and subsequently hindered fast nucleation. In the first step of the reaction, the organophosphates (TMP, TEP, and TBP) are gradually hydrolyzed to supply free phosphate ions, as shown in Eqs. (3)–(5):¹⁶

\[
\text{PO(CH}_3\text{O)}_3 + 3\text{H}_2\text{O} \rightarrow \text{H}_3\text{PO}_4 + 3\text{CH}_3\text{OH} \quad (3)
\]

\[
\text{PO(C}_2\text{H}_5\text{O)}_3 + 3\text{H}_2\text{O} \rightarrow \text{H}_3\text{PO}_4 + 3\text{C}_2\text{H}_5\text{OH} \quad (4)
\]
PO(C₂H₅O)₃ + 3H₂O → H₃PO₄ + 3C₂H₅OH  \text{(5)}

where H₂O is formed by Eq. (2). The PO₄³⁻ ion concentration gradually increases until it reaches the point of critical supersaturation. Because the hydrolysis of organophosphates is slow, reaching critical supersaturation takes several hours. For example, in a previous study,¹¹ the reaction with TEP required 3 h to reach supersaturation. The hydrolysis of TEP is faster than that of TEP because TEP has shorter alkyl chains than TEP, as reported by Westheimer et al.¹¹ Also, Fig. 1 clearly shows that the hydrolysis rate for the organophosphates decreases in the order TMP > TEP > TBP.

Second, nucleation occurs and amorphous precursors are formed, resulting in a substantial decrease in the concentration of free ions in the solution, impairing further nucleation. The amorphous precursor transformed into WH. This phase transformation from the amorphous phase could be explained by a “solid-solid phase transformation” proposed by Tao et al.⁷ They reported amorphous-precursor mediated formation of WH in an EG solvent. The interfacial energy between WH crystal and amorphous precursor was lower compared to that between WH crystal and solution. Hence, the free energy barrier between WH crystal and amorphous precursor was far lower than that between WH crystal and solution. The transformation of amorphous precursor to WH was therefore thermodynamically preferred. In the present study, it was assumed that the interfacial energy between the WH crystals and amorphous precursor was lower than that between the WH crystals and the DMF–EG binary solvents. Hence, the free energy barrier between the WH crystals and the amorphous precursor was far lower than that between the WH crystals and the DMF–EG binary solvents. Accordingly, the transformation of the amorphous precursor to WH was thermodynamically favorable.

The nominal concentration (i.e., the Ca/P molar ratio) influenced the concentration of critical supersaturation and the subsequently formed product. To form β-TCP with stoichiometric Ca/P molar ratio of 1.50, 0.8 mmol of nominal amount of organophosphate is sufficient for 1.2 mmol of CaCl₂, according to following reaction given in Eqs. (6)–(7). It was noted that WH was treated as β-TCP in Eqs. (6)–(8) for simplification. Indeed, the yield in the present study coincided with the theoretical yield of WH (0.12 g) calculated by Eqs. (6)–(8).

3CaCl₂ + 2PO(CH₃O)₃ + 6H₂O → Ca₃(PO₄)₂ + 6H⁺ + 6Cl⁻ + 6CH₃OH  \text{(6)}

3CaCl₂ + 2PO(C₂H₅O)₃ + 6H₂O → Ca₃(PO₄)₂ + 6H⁺ + 6Cl⁻ + 6C₂H₅OH  \text{(7)}

3CaCl₂ + 2PO(C₆H₁₂O₂)₃ + 6H₂O → Ca₃(PO₄)₂ + 6H⁺ + 6Cl⁻ + 6C₆H₁₂O₂H  \text{(8)}

Nevertheless, when the nominal amount of organophosphate (0.8 mmol), i.e., Ca/P molar ratio of 1.50, was subjected to the reaction, the major product was HA irrespective of which type organophosphate was used. It was considered that the nucleation of amorphous precursor was provoked at last when organophosphate supplied 0.8 mmol of free phosphate ion, as the stepwise hydrolysis of organophosphate was slow. In the case of low amount of organophosphate (0.8 mmol), i.e., Ca/P = 1.50, the release of free phosphate ion by the hydrolysis of organophosphate might not attain to 0.8 mmol. Accordingly, organophosphate concentrations that led to the release of more than 0.8 mmol of phosphate ions enabled the formation of WH, whereas those that led to the release of less than 0.8 mmol of phosphate ions resulted in the formation of HA. Indeed, 0.72 mmol of free phosphate ion would give rise to HA with stoichiometric Ca/P molar ratio of 1.67. It was also implied that the chemical composition of amorphous precursor with higher Ca/P ratio than 1.67 had different
interfacial energy compared to that with a Ca/P ratio than 1.50; therefore, resulting products varied at different Ca/P ratios.

Moreover, it was suggested that HA formed at a Ca/P molar ratio of 1.50 was chlorine-substituted HA. The lattice constants of HA were close to those of chlorine-substituted HA (a = 9.4991 Å and c = 6.8636 Å) reported by Hughes et al.18 compared with stoichiometric HA (a = 9.3983 Å and c = 6.8677 Å).19 The presence of Cl− ions by elemental analysis of HA synthesized with TEP at a Ca/P molar ratio of 1.50 is further evidence that the chlorine-substituted HA was formed. Overall, the schematic illustration showing relationship between the formed products and the Ca/P molar ratio using TMP, TEP, and TBP is shown in Fig. 5.

The differences in the hydrolysis rates of the various organophosphates also affected the crystallite size of WH. Although there was no obvious difference of the particles size of WH synthesized at a Ca/P molar ratio of 0.25 (Fig. 3), the crystallite size of them increased with increase of the alkyl chain lengths. The difference of the crystallite size of WH is explained by LaMer model of nucleation.20 If the hydrolysis rate of organophosphate decreases with increase of the alkyl chain lengths, the nucleation and amorphous precursor transformation into WH retard with increase of the alkyl chain lengths. Hereby, the resulting crystallite size of WH would increase with increase of the alkyl chain lengths.

In a recent study, Stähli et al.21 revealed that the Ca(4) sites of WH were partially substituted by H+ ions, accompanied by the inversion of P(1)O4 tetrahedra. Upon substitution of H+ ions at the Ca(4) sites, the presence of the protonated phosphate (HPO42−) was confirmed in both the FT-IR spectra (Fig. 2) and the 1H MAS-NMR spectra (Fig. 4a). The 1H MAS-NMR spectra (Fig. 4a) also showed that trace amount of HA was contained in WH, which was not detected in the XRD patterns (Fig. 1) or FT-IR spectra (Fig. 2) because of its low concentrations. In addition, the peak assignable to HA increased in intensity with increasing alkyl chain length of the organophosphates. The results imply that, when the hydrolysis rate of organophosphate decreases, the amorphous precursor becomes difficult to transform into WH.

The P(1) and P(2) environments are affected by the WH caused by partial substitution of the Ca(4) sites by H+ ions, consistent with the report of Stähli et al.21

CONCLUSIONS

The objective of the present study was to synthesize WH particles using organophosphates with different alkyl chain lengths via a solvothermal reaction. The effects of organophosphates and their Ca/P molar ratio on the formation of WH were investigated. With increasing alkyl chain length of the organophosphate and increasing Ca/P molar ratio (decreasing P), the dominant phase changed from WH to HA, suggesting that the hydrolysis rate of the organophosphates differed. WHs were formed only at a Ca/P molar ratio of 0.25 irrespective of which organophosphate was used. Moreover, 1H and 31P MAS-NMR spectra showed that the WH contained protonated phosphate (HPO42−) and trace amount of HA subphase.

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