AMORPHOUS-PRECURSOR MEDIATED FORMATION OF MAGNESIUM-FREE WHITLOCKITE

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Keywords: amorphous precursor, whitlockite, β-tricalcium phosphate, solvothermal reaction, bone substitute

Abstract: Calcium phosphates (CPs) were widely used as synthetic bone graft substitutes. However, the control of particle size and morphology of CP materials have not been achieved substantially, even though these factors would influence the physicochemical properties. Recent studies revealed that a uniform nanostructured Mg-free whitlockite (WH), which was structurally similar to a high temperature polymorph of β-tricalcium phosphate, was synthesized by precipitation in organic solvents of N,N-dimethylformamide (DMF) and ethylene glycol (EG). The present study therefore aims to synthesize WH particles in the DMF-EG binary solvents by a solvothermal reaction to investigate the formation mechanism of the WH particles. The nucleation of the amorphous precursor, which occurred at 3 h, was dominated by the slow hydrolysis of organophosphate, which retarded reaching critical supersaturation and hindered the fast nucleation. The investigation on the effect of reaction time on WH formation revealed that the amorphous-precursor mediated WH formation via a solid-solid phase transformation occurred between 4 and 9 h of reaction time.

INTRODUCTION

In the past few decades, calcium phosphate (CP) ceramics; hydroxyapatite (HA; Ca₁₀(PO₄)₆(OH)₂) and β-tricalcium phosphate (β-TCP; Ca₆(PO₄)₂), are broadly recognized as synthetic bone graft substitutes due to their chemical similarity to the bone composition. In particular, β-TCP is of paramount interest as it could be resorbed by osteoclasts in vivo and get replaced by native bone tissue. Whitlockite (Mg-WH; Ca₁₃Mg₆(HPO₄)₂(PO₄)₁₂) is a natural occurring mineral. Mg-WH was reported to occur in dental calculi of human tooth enamel, human articular cartilage, hyper-mineralized osteocyte lacunae in human alveolar bone, calcification in uremic artery.

As X-ray diffraction patterns of β-TCP and Mg-WH are similar to one another, the terms β-TCP and Mg-WH have been used interchangeably. However, there are structural differences between them; β-TCP has Ca and vacancy in Ca(4) site, while Mg-WH has H and Mg in Ca(4) and Ca(5) site, respectively.

In conventional β-TCP manufacturing (i.e., solid-state or wet-chemical methods), a calcining process is always requisite as β-TCP is a high temperature polymorph of CaP in the range from ~700 to 1125°C. The calcination process leads to the sintering of particles, which results in increased particle size and decreased specific surface area. Therefore, it is difficult to control the particle size and morphology of β-TCP.

Meanwhile, it was believed that Mg-WH could be precipitated from aqueous solutions only in the presence of Mg ion. However, several studies demonstrated that Mg-free whitlockite (WH), in other words, hydrogen-substituted β-TCP, has been synthesized by precipitation in aqueous solutions under autoclave conditions and in organic solvents such as ethylene glycol mixed solution of N,N-dimethylformamide (DMF) and ethylene glycol (EG). A recent study reported by Stähli et al. for the first time elucidated that the structure of WH was fitted more precisely to whitlockite structure model than β-TCP, where Ca(4) site was partly substituted by H along with the inversion of P104 tetrahedra.

Brushite cement comprising β-TCP and monoclinic phosphate monohydrate (Ca₃(PO₄)₂·H₂O) is clinically applied for bone graft substitute. The β-TCP particles with the controlled morphology and particle size would allow a high packing density in the brushite cement, which would give rise to high mechanical property. Zhu et al. reported the nanostructured WH particles (~120 nm) with size-controlled morphology. Therefore, the WH particles could be replaced with β-TCP particles in the brushite cement system. However, the formation mechanism of the WH particles in the DMF-EG binary solvents was not reported in detail.

The aim of the present study is to build on the work of Zhu et al. to investigate the formation mechanism of the WH particles in the DMF-EG binary solvents. For that purpose, several experimental parameters would be assessed to examine the effects...
of solvent ratio, reaction time, reaction temperature, and Ca/P molar ratio on the WH formation.

MATERIALS AND METHODS

Synthesis method

Calcium chloride (CaCl₂), triethyl phosphate (TEP), N,N-dimethylformamide (DMF), and ethylene glycol (EG) were purchased from Sigma Aldrich, Japan, and were used without further purification.

A typical reaction used to synthesize the WH particles was inspired by Zhu et al. with some modifications. Briefly, CaCl₂ was dissolved in 30 mL of mixed solvents of DMF and EG with a volume ratio of 50:50 (vol.%). Triethyl phosphate was added to the CaCl₂ solution while continually magnetic stirring. The resulting solution was transferred into 50 mL of polytetrafluoroethylene (PTFE) autoclave (HU-50, SAN-AI Kagaku Co., Ltd., Japan) at a filling ratio of 60 vol.% and reacted at 200 °C for 12 h in an oven. The vapor pressure in the solvothermal reaction was calculated by Antoine equation. The pressure with DMF:EG = 50:50 was approximate 0.2 MPa at 200 °C. It was noted that the reaction temperature was one set by oven; therefore, there might be a difference between set temperature and exact reaction temperature. The precipitate was separated by centrifugation, washed twice with acetone, followed by ultrapure water, and then dried in an oven at 80 °C for 24 h.

Experimental parameters

Several experimental parameters were assessed to examine the effects of solvent ratio (DMF:EG ratio from 0:100 to 100:0), reaction time (from 3 to 24 h), reaction temperature (from 160 to 220 °C), and Ca/P molar ratio (from 0.22 to 2.22). In the experiments assessing the parameter of reaction time, the precipitates of reaction time for 4 to 7 h were washed with acetone or with acetonitrile, followed by ultrapure water to examine the effect of washing process on the formed products. The parameters at each experiment were indicated in bold in Table 1.

In addition to the above experiments, following two experiments were also conducted. The effect of residual ions after nucleation on the final products was investigated. The reaction solution with DMF:EG = 50:50 and Ca/P molar ratio of 0.72 was subjected to the reaction at 200 °C for 5 h followed by 7 h at 200 °C with and without solvent exchange. The solvent was exchanged after the removal of the reacted solvent by centrifuging. Also, the effects of washing and drying process on the final products were examined. The dried samples after the reaction at 200 °C for 5 h which were washed with acetone or with acetonitrile, followed by ultrapure water were subjected to the further reaction with DMF:EG = 50:50 at 200 °C for 24 h.

Characterization

Crystalline phase was measured using an X-ray diffractometer (XRD; RINT Ultima+, Rigaku Corp., Japan), equipped with CuKa radiation source, k = 1.5418 Å, 40 kV, and 30 mA. The crystalline phase was identified with respect to the ICDD PDF reference patterns. Fourier-transform infrared (FT-IR) spectra were measured by a KBr method using a FT-IR spectrometer (FT/IR-6100, JASCO Corporation, Japan) with a resolution of 4 cm⁻¹. A scanning electron microscope (SEM; JSM-6610LA, JEOL, Japan) was used to observe the surface morphology of the WH particles at an accelerating voltage of 15 kV. The samples were gold-sputtered coated prior to the observation. Specific surface area (SSA) of the WH was determined by a Brunauer-Emmett-Teller (BET) surface area analyzer (BELSORP-max, Micromeritics, Japan) using a N₂ adsorption method. The samples were pre-dried at 100 °C for 2 h under vacuum. Thermal stability of WH was characterized by a thermogravimetry - differential thermal analysis (TG-DTA, TG-DTA2000SA, Bruker, Japan). To compensate the result of TG-DTA, WH calcined at 450, 700, 1000, and 1200 °C were analyzed by XRD.

For the dissolution test, 0.05 mol L⁻¹ tris(hydroxymethyl)aminomethane-hydrochloric acid (Tris-HCl) buffer at pH 7.3 was prepared, which was

| TABLE 1 | Experimental parameters examining the effects of solvent ratio, reaction time, reaction temperature, and Ca/P molar ratio on the final product. The parameters at each experiment were indicated in bold. |
|----------|---------------------------------------------------------------|
|          | Ca (mmol) | P (mmol) | Ca/P molar ratio | DMF:EG volume ratio | Reaction temperature (°C) | Reaction time (h) |
| Standard experiment | 1.20 | 2.48 | 0.72 | 50:50 | 200 | 12 |
| Solvent ratio | 1.20 | 2.48 | 0.72 | 0:100, 10:90, 20:80, 30:70, 50:50, 70:30, 80:20, 90:10, 100:0 | 200 | 9 (20:80, 30:70, 50:50, 70:30) |
| Reaction time | 1.20 | 2.48 | 0.72 | 50:50 | 200 | 3, 4, 5, 6, 7, 9, 12, 18, 24 |
| Reaction temperature | 1.20 | 2.48 | 0.72 | 50:50 | 160, 180, 200, 220 | 12 |
| Ca/P molar ratio | 1.20 | 0.54–5.41 | 0.22, 0.44, 0.59, 0.72, 0.74, 2.22 | 50:50 | 200 | 12 |

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used to mimic a physiological pH environment. Calcium ions released from WH were measured using a calcium ion-selective electrode (Horiba, Ltd., Japan), according to a JIS T0330-3. Ten milligrams of WH were added to 100 mL of 0.05 mol L\(^{-1}\) Tris-HCl buffer at pH 7.3 and 25 ± 2 °C with stirring at 430 ± 15 rate per minute. For comparison, a synthesized β-TCP by a conventional solid-state reaction\(^{24}\) was also subjected to the dissolution test. The dissolved Ca\(^{2+}\) ion of WH and β-TCP was calculated as a percent of total Ca\(^{2+}\) ions using the chemical compositions of Ca\(_{20.2}(HPO_{4})_{1.1}(PO_{4})_{12.4}\) for WH\(^{20}\) and Ca\((PO_{4})_{2}\) for stoichiometric β-TCP.

RESULTS

The effect of solvent ratio of DMF:EG on the final products was examined. Figure 1 shows XRD patterns of the formed powders synthesized with various DMF:EG ratios at 200 °C for 12 h. A high crystalline HA (ICDD-PDF #00-09-0432) was formed without the DMF (= 0:100). At 10:90, the low crystalline HA with an unidentified strong peak at 20 = 8.8° was obtained. In the range from 20:80 to 70:30, a single phase WH (ICDD-PDF #00-09-0169) was formed, whereas the low crystalline HA was formed at 80:20 and 90:10. Without the EG (= 100:0), α-calcium pyrophosphate (α-CPP, ICDD-PDF #00-09-0345) was obtained. The formed powders with 20:80 at 200 °C for 9 h was WH and amorphous phase (Fig. S1a). WH was obtained at 30:70 and 50:50, while HA was formed at 70:30. The formation range of WH was between 20:80 and 70:30 at 200 °C for 24 h (Fig. S1b).

Figures 2a and b show XRD patterns exhibiting the effects of reaction time and washing process on the final products with DMF:EG = 50:50 at 200 °C. The small amount of viscous precipitate formed at 3 h dissolved after washed with ultrapure water, implied that it was a water-soluble gel. The samples of reaction time for 4 to 7 h were washed with acetone or with acetone, followed by ultrapure water. The formed product at 4 h was an unidentified phase, which was the amorphous phase with the unidentified strong peaks at 20 = 6.2 and 8.4° similar to the peak at DMF:EG = 10:90 in Fig. 1 (Fig. 2a). After additional washing with ultrapure water, the unidentified phase converted to HA and the strong peaks at 20 = 6.2 and 8.4° disappeared and decreased, respectively (Fig.2b). The result suggested that the unidentified phase was attributed to water-soluble amorphous phase and was hydrolyzed to form HA. The amorphous phase with the unidentified strong peak at 20 = 8.6° and weak WH peaks were formed at 5 and 6 h of reaction time after washing with acetone. Additional washing with ultrapure water led to the formation of amorphous precursor phase which had a similar pattern to an amorphous calcium phosphate (ACP)\(^{16}\).

The FT-IR spectra of the formed powders at 5 h of reaction time washed with acetone or with acetone, followed by ultrapure water showed no spectral difference between them, except for a distinct split of v3 (PO\(_4\))\(^{3-}\) group, ~1000 cm\(^{-1}\)\(^{25}\) in the formed powder washed with acetone (Fig. S2). The XRD pattern at 7 h after washing with acetone was assigned to WH and amorphous phase with the unidentified strong peak at 20 = 8.7°, while after additional washing with ultrapure water, the unidentified strong peak disappeared. The results implied the transformation of amorphous phase to WH between 4 h and 7 h, a small amount of the formed WH was washed out after washed with ultrapure water, together with the unidentified strong peak. At 9 h of reaction time, the amorphous phase disappeared so that the WH single phase was formed when washed with acetone, followed by ultrapure water (Fig. 2c). Further reaction time did not change the crystal phase and crystallinity.

The effect of residual ions after nucleation on the final products was investigated. The reaction solution with DMF:EG = 50:50 was subjected to reaction at 200 °C for 5 h, and subsequent 7 h with and without solvent exchange. A single phase WH was formed regardless of the solvent exchange (Fig. S3a). The results indicated that the amorphous phase, which was formed after 5 h of reaction time, transformed to WH within 7 h of additional reaction time irrespective of the solvent with or without calcium and phosphate ions. Besides, the effects of washing and drying process on the final products were examined. The dried samples after the reaction with DMF:EG = 50:50 at 200 °C for 5 h, which were washed with acetone or with acetone and, followed by ultrapure water, were subjected to additional reaction with DMF:EG = 50:50 at 200 °C for 24 h. The final

![FIGURE 1](image-url) XRD patterns of the formed powders synthesized with various DMF:EG ratios at 200 °C for 12 h. (a) 4 = 60° and (b) 25 = 35°, ○: unidentified peak.
products were biphase of WH and HA, while the amount of WH washed with acetone was higher than that with acetone and ultrapure water (Fig. S3b).

The reaction time of WH formation was plotted as a function of DMF content, together with the final products (Fig. 3). The WH phase was formed in the range of 20 to 70 vol.% of DMF at 200 °C between 12 and 24 h, whereas its formation was in the range of 30 to 50 vol.% of DMF at 200 °C for 9 h. It was noted that the reaction was performed at the nominal Ca/P molar ratio of 0.72, and therefore the results might alter if a different nominal Ca/P molar ratio was used.

The effect of reaction temperature on the final products was examined with DMF:EG = 50:50 for 12 h (Fig. 4a). No precipitation was obtained at 160 °C. The XRD pattern at 180 °C was assigned to the low crystalline HA including the unidentified phase, which was a similar diffraction pattern to DMF:EG = 10:90 in Fig. 1 (Fig. S4). At more than 200 °C, the WH single phase was obtained.

The Ca/P molar ratio of reaction solution also affected the final products (Fig. 4b). WH was formed between Ca/P = 0.22 and 0.72, while biphase of WH

FIGURE 2 XRD patterns of the formed powders synthesized for varying reaction time at 200 °C. Reaction time from 4 to 7 h and washed (a) with acetone and (b) with acetone, followed by ultrapure water (2θ = 4 – 60° and 20 – 35°), and (c) reaction time from 9 to 24 h washed with acetone, followed by ultrapure water. ▲: WH (#00-09-0169), ●: HA (#00-09-0432), ○: unidentified peaks

FIGURE 3 The reaction time of WH production plotted as a function of DMF content, together with the final products, where WH could be formed in a shaded area. All the samples were synthesized with Ca/P molar ratio of 0.72 at 200 °C.
and HA and HA single phase were formed at Ca/P = 0.74 and 2.22, respectively.

The N$_2$ adsorption–desorption showed IUPAC type II isotherms (Fig. S5). The BET SSA of WH was as follows: 32.0 m$^2$g$^{-1}$ for DMF:EG = 20:80, 30.2 m$^2$g$^{-1}$ for 30:70, 35.2 m$^2$g$^{-1}$ for 50:50, and 71.5 m$^2$g$^{-1}$ for 70:30.

The WH powder synthesized with DMF:EG = 50:50 at 200 °C for 12 h was subjected to FT-IR (Fig. 5a) and dissolution test together with β-TCP synthesized via the conventional solid-state reaction (Fig. 5b). The FT-IR spectrum showed PO$_4^{3-}$ groups ($v_3$: 1200, 1080 cm$^{-1}$, $v_4$: 970 cm$^{-1}$, $v_6$: 545, 605 cm$^{-1}$), HPO$_4^{2-}$ group (~870 cm$^{-1}$), and adsorbed water (~1600 and ~3400 cm$^{-1}$). The dissolved Ca$^{2+}$ ion was normalized as a percent of total Ca$^{2+}$ ions in WH and β-TCP. The percent of dissolved Ca$^{2+}$ ion of WH and β-TCP was plotted as a function of time up to 180 min, showing that the dissolution of WH was twice higher compared to β-TCP.

FIGURE 4 XRD patterns of the formed powders synthesized with DMF:EG = 50:50 for 12 h (a) at various reaction temperature and (b) with various Ca/P molar ratio at 200 °C. No precipitate was obtained at 160 °C. •: HA (#00-09-0432), ○: unidentified peak

FIGURE 5 (a) FT-IR spectrum and (b) dissolution of the WH powder synthesized with DMF:EG = 50:50 at 200 °C for 12 h. The percent of dissolved Ca$^{2+}$ ion from the WH and β-TCP synthesized by the conventional solid-state reaction was calculated as a percent of total Ca$^{2+}$ ions in WH and β-TCP.
Figure 6 shows the surface morphology of WH particles synthesized with (a) DMF:EG = 20:80, (b) 30:70, (c) 50:50, and (d) 70:30 at 200 °C for 12 h.

Thermal stability of WH was characterized by the TG-DTA (Fig. 7a). The DTA curve of WH showed exothermic peaks at (a) 667 °C and (b) 1124 °C, while the TG curve exhibited weight loss at 667 °C and no weight loss at 1124 °C. The WH powder was subjected to calcina-
tion at 450, 700, 1000, and 1200 °C and following XRD analysis to clarify the crystal phase of WH (Fig. 7b). The WH phase remained after calcination at 450 °C. The WH phase was decomposed to β-TCP and α-CPP after calcination at 700 °C and the α-CPP phase was transformed to β-CPP (#00-09-0346) after calcination at 1000 °C, which was a similar trend as reported by Stähli et al.20 After calcination at 1200 °C, α-TCP (#00-09-0348), β-TCP, and β-CPP were obtained. As the results, the exothermic peaks at 667 °C and 1124 °C was due to the decomposition of WH to β-TCP and α-CPP and the transformation from β-TCP to α-TCP, respectively.

DISCUSSION

Inspired by the first report of Zhu et al.19, we replicated the synthesis of WH in the DMF-EG binary solvents. The formation mechanism of WH was investigated by assessing the several experimental parameters: solvent ratio, reaction time, reaction temperature, and Ca/P molar ratio.

Investigation on the reaction time showed that nucleation occurred at 3 h and the WH formation proceeded via the transformation of the amorphous precursor phase between 4 and 9 h (Figs. 2a-c). The presence of HPO$_4^{2-}$ group (∼870 cm$^{-1}$) in WH was also confirmed (Fig. 5a), as previously observed in WH.12,19,20 The precipitation of particles in the DMF-EG binary solvents could be interpreted by the LaMer model20 as follows (Fig. 8). In the first step, the ion concentration increases gradually until it reaches the point of critical supersaturation. The TEP is hydrolyzed gradually to supply free phosphate ions as given in Eq. (1).21

$$\text{PO(C}_2\text{H}_3\text{O})_3 + 3\text{H}_2\text{O} \rightarrow \text{H}_3\text{PO}_4 + 3\text{C}_2\text{H}_3\text{OH} \quad \text{Eq. (1)}$$

where water resulted from the adsorption onto the chemical reagents. As its hydrolysis is slow, it takes several hours to reach critical supersaturation. In the second step, nucleation occurs, which results in significant depletion of the concentration of free ions in the solution, impairing further nucleation. From the results of Figs. 2a and b, the nucleation of amorphous precursor phase is presumed to occur at about 3 h. In the third step, the amorphous precursors eventually transformed (crystalized) to WH at the same time. According to Ostwald’s rule, the first formed phase is the closest in free energy to the mother solution; therefore, the least stable phase, i.e., amorphous precursors, transformed to a phase with increasing stability, i.e., WH. Figures 2a and b pronouncedly showed that the transformation (crystallization) of amorphous precursor phase to WH phase started at 4 h and completed at 9 h. This phenomenon could be explained by a “solid-solid phase transformation” from the amorphous phase proposed by Tao et al.16 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 as the interfacial energy between

![FIGURE 8](image_url) Schematic illustration showing the formation mechanism of WH by the LaMer model.
WH crystal and amorphous precursor phase was lower than that between WH crystal and DMF-EG binary solvents, the free energy barrier would behave the same manner. As a result, the transformation of amorphous precursor phase to WH would be possible.

Interestingly, the weak WH and unidentified strong peaks formed at 5 and 6 h after washing with acetone disappeared after additional washing with ultrapure water (Figs. 2a and b). The results indicated that as the small amount of WH formed on the amorphous precursor surface at the early stage of transformation between 4 and 7 h was washed out with ultrapure water, together with unidentified phase observed in Fig. 2a.

On the contrary, there were some discrepancies between the present study and Zhu’s report. WH was formed within 4 h of reaction time at above 160 °C in the Zhu’s report, while it was formed at 9 h at above 200 °C in the present study (Figs. 2c and 4a). This discrepancy may be due to difference of organophosphate used for the solvothermal reaction. The hydrolysis of trimethyl phosphate (TMP) used in the Zhu’s report was faster than that of TEP as TMP has shorter alkyl chains than TEP. As a result, this difference caused the discrepancy in reaction time and temperature of WH.

A broad range of the nominal Ca/P molar ratio from 0.22 to 0.72 led to the formation of WH, while higher Ca/P molar ratio of 2.22 resulted in the formation of HA (Fig. 4b). The previous report demonstrated that Ca/P molar ratios of WH were 1.48 ± 0.01, 1.51 ± 0.02, 1.443 ± 0.003, which were relatively close to 1.50 of stoichiometric β-TCP. To form β-TCP with stoichiometric Ca/P molar ratio of 1.50, 0.8 mmol of nominal amount of TEP is sufficient for 1.2 mmol of CaCl₂, according to following reaction given in Eq. (2). It was noted that WH was treated as β-TCP in Eq. (2) for simplification. Nevertheless, when the nominal amount of TEP (0.8 mmol), i.e., Ca/P molar ratio between 0.74 and 2.22, was subjected to the reaction, the final product was HA.

\[
\begin{align*}
3\text{CaCl}_2 + 2\text{PO(C}_2\text{H}_5\text{O})_3 + 6\text{H}_2\text{O} & \rightarrow \text{Ca}_3(\text{PO}_4)_2 + 6\text{H}^+ + 6\text{Cl}^- + 6\text{C}_2\text{H}_5\text{OH} \\
& \quad \text{Eq. (2)}
\end{align*}
\]

Taking into account the Ca/P molar ratio of the amorphous precursor (1.47 ± 0.05) in the Tao’s report, the amorphous precursor shared the similar chemical composition with WH. Thereby, it was considered that the first nucleation of amorphous precursor was provoked when TEP supplied 0.8 mmol of free phosphate ion, as the stepwise hydrolysis of TEP was slow. In the case of small amount of TEP (0.54 or 1.62 mmol, i.e., Ca/P = 2.22 or 0.74), the release of free phosphate ion by the hydrolysis of TEP might not attain to 0.8 mmol. In general, the hydrolysis of TEP will proceed fast in the acidic solution. According to a study by Baradaran et al., DMF decomposed to dimethylamine ((CH₃)₂-NH²⁺) above 100 °C as following Eq. (3). It was noted that water (H₂O⁻) molecule adsorbed onto the chemical reagents.

\[
\begin{align*}
\text{HCO-} (\text{NH}_3)_2 + \text{H}_2\text{O} & \rightarrow \text{HCOOH} + (\text{CH}_3)_2\text{-NH}²⁺ \\
& \quad \text{Eq. (3)}
\end{align*}
\]

As the decomposition of DMF led to basic solution in DMF-EG binary solvents with DMF-EG = 50:50, the hydrolysis of TEP was slow. Moreover, the solvent exchange after 5 h did not affect the formation of WH (Fig. S3a), suggesting that after the nucleation, residual phosphate ions or TEP did not involve the transformation of amorphous precursor to WH. As such, the retarded nucleation due to the slow hydrolysis of TEP was probably the key factor for this solvothermal reaction.

The solvent ratio of DMF:EG affected the WH formation, which enabled in the range of 20:80 to 70:30 (Fig. 1). The previous study by Zhu et al. demonstrated that 1:1 (= 50:50) of DMF-water binary solvents and 1:1:1 (= 33:33:33) DMF-water-EG ternary solvents led to the formation of HA, but did not state about DMF:EG ratio in the binary solvents. As given in Eq. (3), the decomposition of DMF to dimethylamine: (CH₃)₂-NH²⁺ provided basic solution. The DMF-EG solvents with the low DMF ratio, i.e., 0:100 and 10:90, might become acidic as EG was acidic. In such acidic solution, TEP was hydrolyzed faster and the interfacial energy between WH crystal and amorphous precursor phase changed. Accordingly, the transformation of amorphous precursor to HA was thermodynamically preferred. In the high DMF ratio between 80:20 and 100:0, pH was too basic to hydrolyze TEP. As the results, the interfacial energy between WH crystal and amorphous precursor phase preferred thermodynamically to form HA or α-CPP. In addition, when the amorphous precursor of 5 h of reaction washed with acetone and, followed by ultrapure water, which was subjected to additional 24 h of reaction, led to lower amount of WH as compared to washed with only acetone (Fig. S3b). The results implied that the washing with ultrapure water made it less likely to transform to WH, which was consistent with the result that water in DMF-water-EG ternary solvents hindered the WH formation.

The N₂ adsorption-desorption showed different results compared with the result by Zhu et al. The present WH powder had no mesopores but macropores (Fig. S5). The interpace among the particles due to particle agglomeration led to macropores. The distribution of macropore varied by the degree of particle agglomeration. The BET SSA of the present WHs (30.2 - 71.5 m²·g⁻¹) were significantly lower than the result by Zhu et al. (98.86 m²·g⁻¹). This difference could be explained by the particle size of the synthesized WH; ~200 nm for the present WH and ~120 nm for WH of Zhu et al. The Zhu’s WH particles were composed of smaller particles than the
present WH particles. Based on the assumption that a nanosized particle is spherical shape, the SSA (s) is expressed as Eq. (4):

\[ s = 6 \rho^{-1} d^{-1} \quad \text{Eq. (4)} \]

where, \( \rho \) and \( d \) are particle density and particle diameter, respectively. Since SSA increased with decreasing particle size, the smaller particle size of Zhu’s WH\(^{16} \) had the higher SSA compared with WH of the present study. The difference of the particle size of WH was also explained by LaMer plot (Fig. 8). If the hydrolysis of TMP in the Zhu’s report\(^{16} \) was faster than that of the present study, the nucleation occurred fast and subsequently the fast transformation of amorphous precursor to WH. Hereby, the resulting particle size of WH would be smaller than that of the present study.

The SSA of WH for DMF:EG = 70:30 was significantly higher than that of 20:80, 30:70, and 50:50. The particle size of WH also seemed slightly smaller than the others (Fig. 6). Besides, the crystallinity of WH for DMF:EG = 70:30 was lower than that of the others (Fig. 1). It was considered the higher amount of DMF might suppress the crystal growth.

The dissolution of WH was twice higher than \( \beta \)-TCP (Fig. 5b). Stähli et al.\(^{20} \) revealed that the Ca atom in WH synthesized in EG was missing in Ca(4) site, resulting in calcium-deficient WH. The calcium-deficient HA had a higher dissolution property than stoichiometric HA,\(^{16} \) while Mg-substituted \( \beta \)-TCP had lower dissolution characteristic as compared to pure \( \beta \)-TCP.\(^{31} \) Therefore, with WH of the present study being calcium-deficient WH, WH would be have a higher dissolution of WH than \( \beta \)-TCP.

Regarding the thermal stability of WH, the presence of HPO\(_4\)\(^2-\) group in the WH structure (Fig. 5a) was also corroborated by the decomposition of WH to calcium pyrophosphate (Fig. 7). As HPO\(_4\)\(^2-\) group was thermally unstable, it decomposed to pyrophosphate group as following Eq. (5), which was consistent with previous study.\(^{32} \)

\[ 2\text{HPO}_4^{2-} \rightarrow \text{P}_2\text{O}_7^{4+} + \text{H}_2\text{O} \quad \text{Eq. (5)} \]

In addition, the \( \beta \)-TCP phase was still observed even after calcination at 1200 \(^\circ\)C (Fig. 7b), although the \( \beta \) to \( \alpha \) transformation of TCP was detected at 1124 \(^\circ\)C (Fig. 7a). According to the study by Torres et al.,\(^{33} \) \( \beta \)-TCP comprising \( \beta \)-CPP impurity led to the \( \alpha \) to \( \beta \) reverse transformation of TCP after the heat treatment at 1250 \(^\circ\)C followed by slow cooling under the rates of 5 and 13 \(^\circ\)C/min. Similarly, the present result might be caused by the \( \alpha \) to \( \beta \) reverse transformation of TCP.

**CONCLUSIONS**

The present study investigated the formation mechanism of WH by the solvothermal reaction in the DMF-EG binary solvents. The nucleation of the amorphous precursor occurred at 3 h of reaction time was dominated by the slow hydrolysis of TEP, which retarded reaching critical supersaturation and hindered the fast nucleation. The investigation on the effect of reaction time on WH formation revealed that the amorphous-precursor mediated WH formation via the solid-solid phase transformation occurred between 4 and 9 h of reaction time. Moreover, the nominal TEP concentration influenced the concentration of critical supersaturation and subsequent WH formation; TEP concentration releasing more than 0.8 mmol of phosphate ions, which enabled the WH formation. Also, the broad range of DMF:EG ratio from 20:80 to 70:30 permitted the WH formation. This WH manufacturing mechanism, which controlled the interfacial energy would provide a novel strategy of materials synthesis.

**APPENDIX**

Supporting information related to this article can be found on Mendeley Data at http://dx.doi.org/10.17632/s4w3w67rxd.1.

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