



Novel *in-situ* synthesis and characterization of nanostructured magnesium substituted β -tricalcium phosphate (β -TCMP)

Donghyun Lee^{a,c}, Charles Sfeir^b, Prashant N. Kumta^{b,c,*}

^a Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, USA

^b Department of Oral Biology, University of Pittsburgh, Pittsburgh, PA 15260, USA

^c Department of Mechanical Engineering and Materials Science, Chemical Engineering and Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261, USA

ARTICLE INFO

Article history:

Received 11 April 2008

Accepted 17 May 2008

Available online 3 June 2008

Keywords:

Nano-materials

Brushite

TCMP

Calcium Phosphate

ABSTRACT

Tricalcium phosphate (TCP, $\text{Ca}_3(\text{PO}_4)_2$) in its pure form cannot be synthesized under physiological conditions in normal aqueous solutions due to phase instability, resulting in its transformation to hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) in the presence of water. However, substituting magnesium in lieu of calcium is known to stabilize TCP, preventing its conversion to hydroxyapatite. There are several methods known for synthesizing magnesium substituted tricalcium phosphate (TCMP). In the present study, a novel *in-situ* method has been developed to synthesize β -TCMP using magnesium substituted brushite as a precursor. Substitution of 50% of calcium by magnesium results in the formation of semi-spherical nanocrystalline particles (~100 nm) of brushite. Boiling the nanocrystalline brushite powder in aqueous condition for only 30 min results in the generation of rosette shaped nanocrystals (~80 nm) of β -TCMP that emerge from the original brushite spheres. The β -TCMP particles exhibit a specific surface area of ~200 m²/g. Details about the synthesis procedure and the possible mechanisms involved in the formation of β -TCMP from Mg-substituted brushite is further discussed.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Calcium phosphate ceramics such as hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP) are considered good candidate substitute materials for bone implant applications [1]. The synthesis of calcium phosphates are usually based on an acid–base reaction between several calcium orthophosphate combinations conducted *in-situ* in an aqueous medium [2]. The pH dependant solubilities of calcium phosphates play an important role in determining which calcium phosphate phase is stabilized [2,3]. β -TCP is known to be unstable and highly soluble in water compared to hydroxyapatite. It is also known to transform to HA in the presence of water. Therefore, due to its instability in water, β -TCP cannot be synthesized using aqueous conditions particularly, under physiological conditions ($T=37^\circ\text{C}$, $\text{pH}=7.5$, $P=1\text{ atm}$) [4–9]. Even β -TCP powders synthesized using conventional solid state methods followed by sintering to form β -TCP disks are known to transform to HA when soaked in water [10]. This hydrolytic instability makes it incredibly challenging to study the interaction of β -TCP with various types of cells. Moreover, these instabilities also prevent its direct use for gene and protein delivery applications involving its synthesis in the presence of

cells and macromolecules such as DNA and proteins. These studies are therefore very critical for the design of the much needed smart materials for various biotechnological applications such as tissue engineering, gene and drug delivery. However, by substituting magnesium into the β -TCP lattice, the structure can be stabilized rendering the phase more resistant to dissolution, hydrolysis and transformation to form HA in the presence of water under physiological conditions [4,5,10,11]. In addition, magnesium enables β -TCP to be synthesized under normal aqueous conditions. Hence, it is not surprising that magnesium substituted β -tricalcium phosphate (β -TCMP) is found in normal dental caries [4].

Thus far, β -TCMP has been generally synthesized by precipitation and hydrolysis methods published by R. Z. Legeros [4]. Legeros reported that β -TCMP can be precipitated by adding a solution containing Ca^{2+} and Mg^{2+} drop-wise into another solution containing NaH_2PO_4 , maintained at $95\text{--}100^\circ\text{C}$, followed by digestion for an additional 5 h [4]. The phosphate can also be synthesized by the hydrolysis method. This procedure consists of using monetite as the starting precursor. Monetite crystals are suspended in solution containing Ca^{2+} and Mg^{2+} ions, which is maintained at $95\text{--}100^\circ\text{C}$ followed by a reflux step for 8 h [4]. In both approaches, the ability to synthesize a stable form of β -TCP is largely dictated by the ability of Mg^{2+} ions to diffuse into the phosphate lattice enabling the transformation. It would therefore be useful to develop approaches where in the Mg^{2+} is already incorporated into the parent structure. Such an approach would help control the amount of Mg^{2+} incorporated into the phosphate lattice while also directly resulting in a

* Corresponding author. Department of Mechanical Engineering and Materials Science, Chemical Engineering and Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261, USA.

E-mail address: pkumta@pitt.edu (P.N. Kumta).

Table 1

Chemical analysis (ICP-OES) result obtained from Galbraith Laboratories, INC (Knoxville, TN) for magnesium substituted brushite and β -TCMP boiled for 8 h

		Mg-substituted brushite	β -TCMP boiled for 8 h
Weight %	Ca	19.70%	25.59%
	Mg	5.31%	5.15%
	P	18.22%	19.31
Mole fraction	Ca	0.836	1.671
	Mg	0.371	0.743
	P	1	2

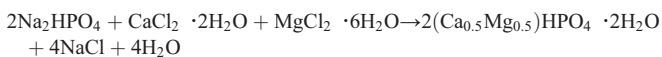
stabilized TCP phase. Additionally, this would have a significant impact on the kinetics of the transformation reaction leading to the formation of a stable form of β -TCP. The present manuscript reports on the synthesis of a novel approach utilizing the above concept.

In this study therefore, β -TCMP is synthesized by an *in-situ* growth mechanism in which the magnesium substituted brushite is used as the precursor to initiate the formation of β -TCMP. Brushite, instead of monelite, has been selected as the precursor material due to the ease of synthesizing brushite. Both magnesium substituted brushite and β -TCMP synthesized by this *in-situ* method have been characterized by X-ray diffraction, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy and Brunauer–Emmett–Teller (BET) analyses. Some of the initial results contributing to this in-depth study have also been briefly reviewed in our previous publication [12]. More detailed and in-depth analyses are however provided in this publication.

2. Experimental procedures

2.1. Synthesis of magnesium substituted brushite

Magnesium substituted brushite has been synthesized following the addition of Mg according to the stoichiometry of $Mg/(Ca + Mg) = 0.5$ ratio in order to react with Na_2HPO_4 according to the chemical reaction given below. This ratio of total Mg substitution for Ca (50% Mg) was selected to study the transformation of brushite to β -TCMP since this was also the ratio used in the methods originally reported by Legeros [4].



Specifically, the steps involved in the approach can be described as follows: 0.025 mol of $CaCl_2 \cdot 2H_2O$ (ACS reagent grade, ACROS) and 0.025 mol of $MgCl_2 \cdot 6H_2O$ (99%, ACROS) were simultaneously dissolved in 100 ml of de-ionized water (NANO pure, M Ω cm 18.2). 0.05 mol of Na_2HPO_4 (ACS reagent grade, anhydrous, ACROS) was also dissolved in 100 ml of de-ionized water. Both solutions were stirred until the salts

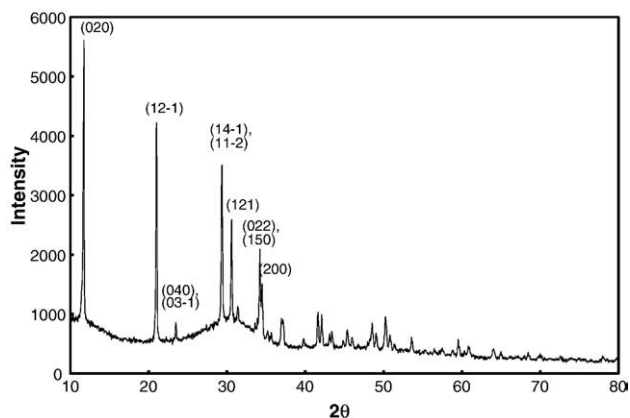


Fig. 1. X-ray diffraction pattern obtained from the as-prepared magnesium substituted brushite.

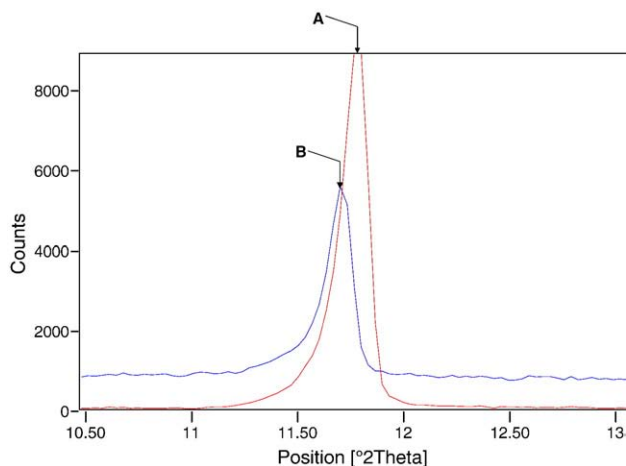


Fig. 2. X-ray diffraction showing peak shifts ($2\theta \sim 0.09^\circ$) corresponding to the (020) direction after Mg is substituted indicative of lattice expansion. A. Unsubstituted brushite, B. Mg-substituted brushite.

are completely dissolved. 50% $Mg/(Ca + Mg)$ solution is then added to the Na_2HPO_4 solution at an addition rate of ~ 4 ml/s, while continuously stirring, using the Fisherbrand Variable-Flow Chemical Transfer Pump. The precipitate is then centrifuged, washed, and dried at $60^\circ C$ in a drying-oven overnight.

2.2. Synthesis of β -TCMP

β -TCMP is synthesized using a novel *in-situ* growth technique. The magnesium substituted brushite powder is used as a precursor for synthesizing the β -TCMP phase. The magnesium substituted brushite powder synthesized following the method described above is dispersed in 200 ml of de-ionized water, and then transferred to a vial connected to a condenser. The powder is then boiled for 8 h, 4 h, 1 h, 30 min and 15 min, respectively to determine the minimum time required for synthesizing β -TCMP. The powder is then collected each time after boiling and washed with de-ionized water. The collected powder is then dried at $60^\circ C$ in a drying-oven overnight.

2.3. Characterization methods

The powders were analyzed using the X-ray diffraction (Philips X'pert Pro diffractometer) with $CuK\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$) from 2θ value of 10° to 80° . Step size (2θ) and measured time per step (s) for Mg-substituted brushite and β -TCMP were 0.033° and 90.17 s, and

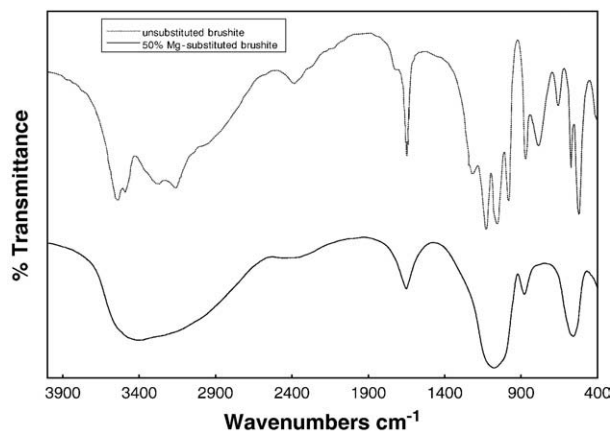


Fig. 3. FTIR spectra of Mg-substituted brushite and unsubstituted brushite. Broadening of the pattern of the Mg-substituted brushite compared to unsubstituted brushite indicates the significant distortion of Ca–P related bonds.

Table 2
Main infrared vibrational modes observed in brushite [13]

IR brushite wavenumbers (cm ⁻¹)	IR brushite vibration modes
3541–3480	O–H stretching of lattice water molecules
3282–3166	
2930	(P)O–H stretching
2385	Combination H–O–H bending and rotation of residual free water
1600–1720 (Broad)	
1649 (thin)	H–O–H bending of lattice water molecules
1208	P–O–H in-plane bending
1135	P–O stretching
1065	P–O stretching
987	
877	P–O(H) stretching
795	P–O–H out-of-plane bending
661	Water liberations
576	O–P–O(H) bending mode
528	Water translation
408	
370	
363	
337	Lattice modes

0.033° and 1000.13 s, respectively. Fourier transform infrared spectroscopy (FTIR, Mattson, Galaxy series FTIR 5000) also has been conducted using the KBr pellet method. To measure the specific surface area of the synthesized calcium phosphate powders, multi-point (5) Brunauer–Emmett–Teller (BET) analysis is conducted (N₂ adsorption, Quantachrome Inst., NOVA-2000, Syosset, NY). In order to analyze the morphology and microstructure of the synthesized calcium phosphate powders, scanning electron microscopy (Philips

XL30 FEG ESEM) has been used. Samples were sputter coated with palladium (0.05 nm thickness) since calcium phosphate powders are electrically non-conductive. Chemical analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES) was also conducted by Galbraith Laboratories, INC. (Knoxville, TN). The accuracy of the technique is reported to be ± 10 wt.%. Thermal analysis has been conducted using CAHN TG-171 (Thermo Cahn, Madison, WI), and Lindberg box furnace [Lindberg/blue, Riverside, MI], to study the thermal stability and the chemical nature of the unsubstituted brushite and magnesium substituted brushite. Samples have been thermally analyzed by heating to 900 °C in stagnant air atmosphere using a step size of 5 °C/min.

3. Results

3.1. Characterization of magnesium substituted brushite

The synthesized magnesium substituted brushite powder was chemically analyzed by ICP-OES method, by Galbraith Laboratories, INC. (Knoxville, TN). Results of the detailed analysis are given in Table 1. Based on these results shown, the formula of the brushite phase can be analyzed to be Ca_{0.836}Mg_{0.371}HPO₄·2O. This gives a Mg/(Ca+Mg) ratio of 0.307 which is less than the nominal ratio (based upon the amount of Mg in the precursor solution) of (Mg)/(Ca+Mg)=0.5 used for synthesizing the Mg-substituted brushite. One possible explanation for the lower amount of Mg incorporated in the brushite lattice compared to the nominal composition present in the precursor solution is because this is probably the maximum amount of solubility of Mg²⁺ in the brushite lattice needed to preserve its structural integrity. Therefore, only limited

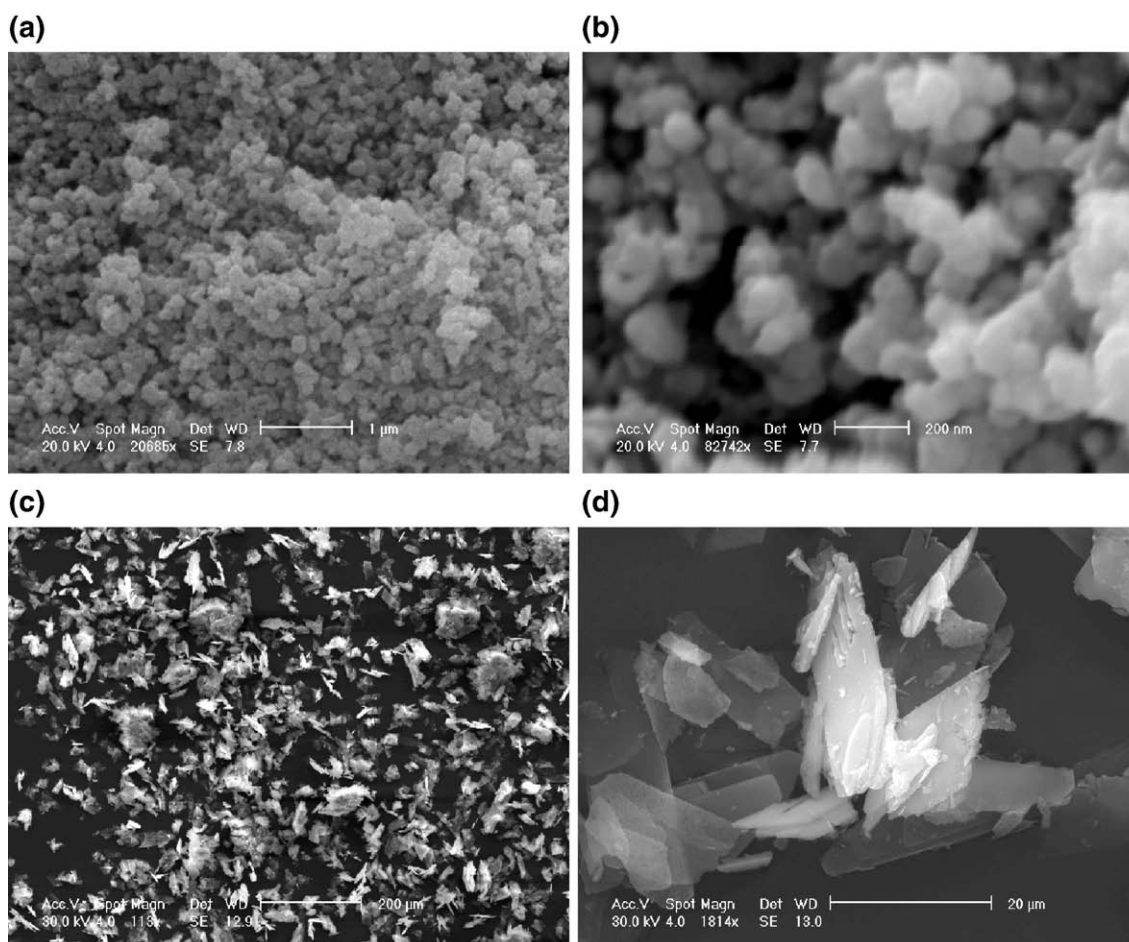


Fig. 4. SEM images showing the morphology of (a) and (b); as-prepared magnesium substituted brushite powder and (c) and (d); as-prepared pure unsubstituted brushite. Note: The spherical morphology of the magnesium substituted brushite particles and platelet morphology of unsubstituted brushite.

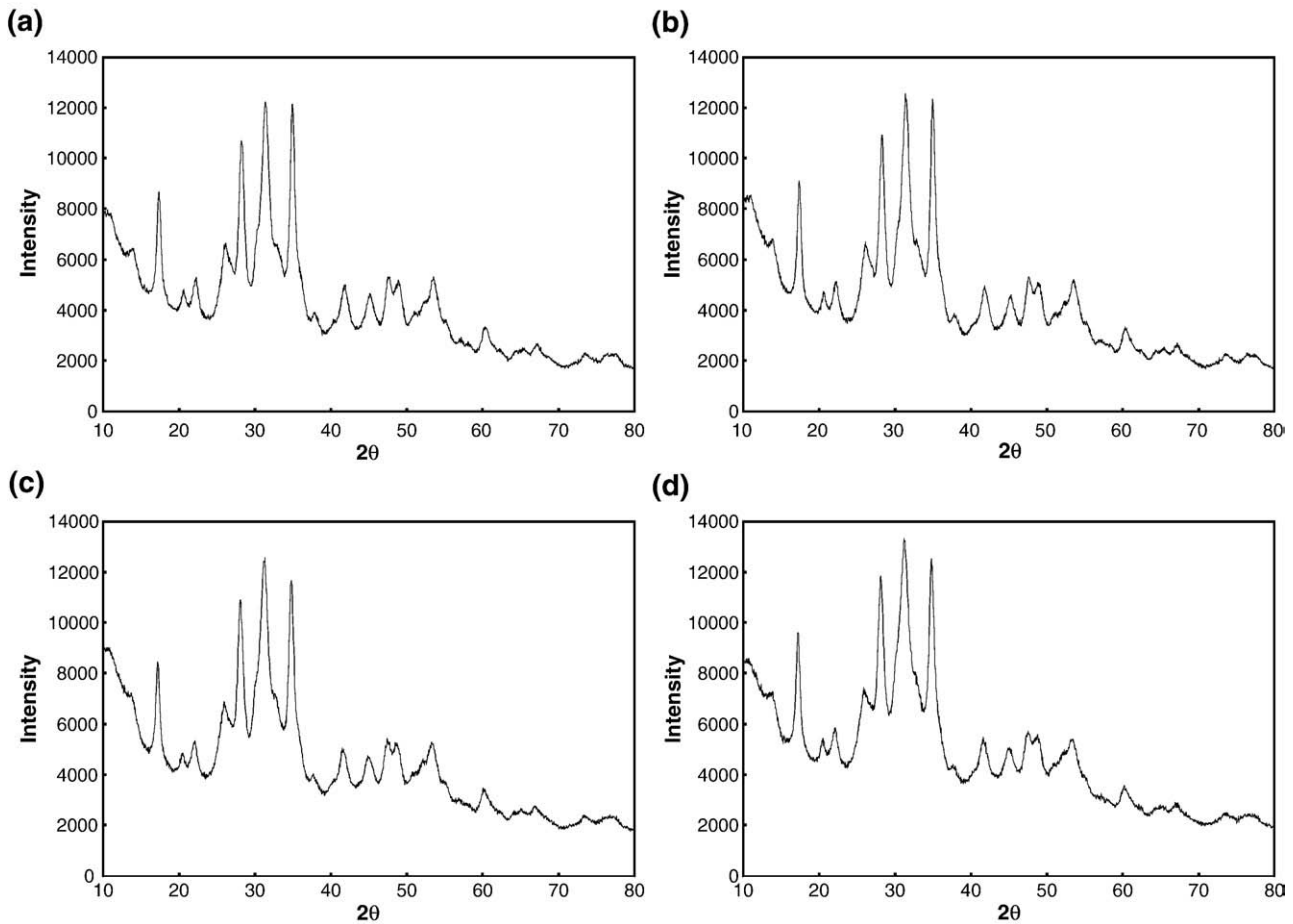


Fig. 5. X-ray diffraction patterns obtained on magnesium substituted brushite boiled for (a) 8 h, (b) 4 h (c) 1 h, (d) 30 min. Note: The pattern corresponds to poorly crystalline β -TCMP. No brushite or monetite phase is observed to remain.

amount of Mg can be incorporated into the actual brushite lattice. Nevertheless, the $(Ca+Mg)/P$ ratio of magnesium substituted brushite is 1.207, which is 17% higher than the ratio observed in normal brushite ($Ca/P=1$). This indicates that there is excess magnesium in the solid product compared to the typical brushite composition, which is not completely incorporated into the brushite lattice. This excess Mg probably remains either as an amorphous phase or as a different crystalline phase, or is present as excess magnesium in the interstitial sites of the brushite lattice. The XRD pattern (Fig. 1) indicates peaks corresponding to the brushite phase overlapped over a broad amor-

phous background suggesting the possible presence of an amorphous phase. It is also observed in Fig. 1 that the strong preferred orientation characteristic of brushite along the (020) direction is reduced significantly. Therefore, it appears that there is significant presence of defects or disorder in the brushite phase causing it to lose its characteristic platelet morphology due to a lowering of its preferential lattice directionality. Peak shift to lower angles of $2\theta=0.09^\circ$ is also observed in the (020) direction compared to the unsubstituted brushite, which indicates lattice expansion in this direction caused by magnesium substitution (Fig. 2). This observed peak shift in the XRD pattern is a clear indication of the incorporation of Mg into the brushite phase causing an

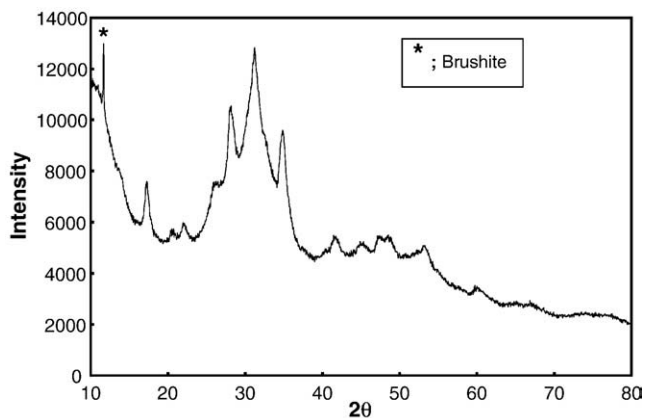


Fig. 6. X-ray diffraction pattern obtained on magnesium substituted brushite boiled for 15 min. Note: The pattern yields poorly crystalline β -TCMP although the presence of untransformed brushite is seen.

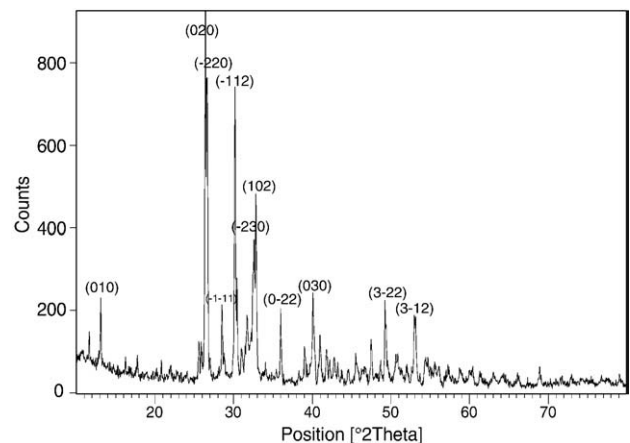


Fig. 7. Unsubstituted brushite boiled for 1 h. Note complete transformation to monetite.

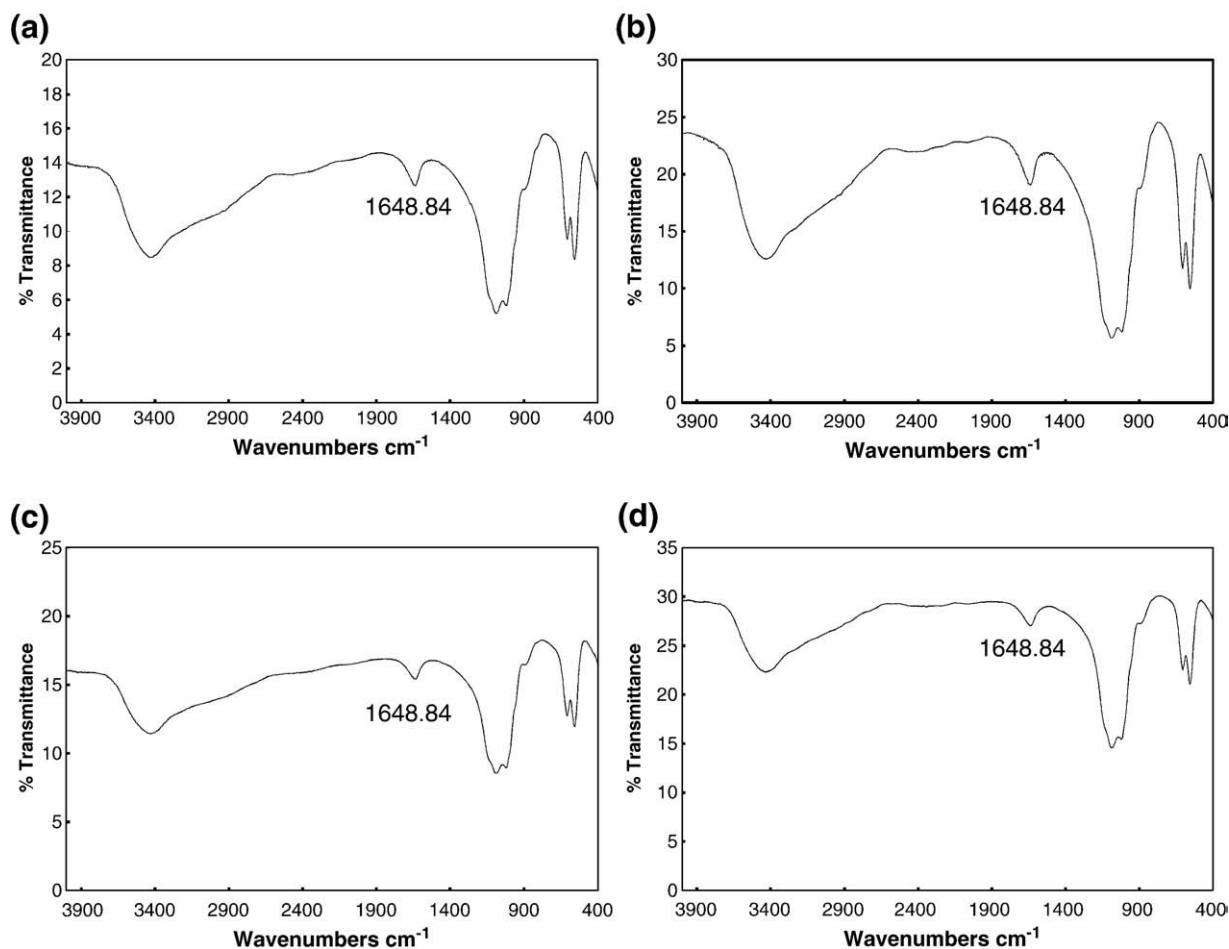


Fig. 8. FTIR spectra of magnesium substituted brushite boiled for (a) 8 h, (b) 4 h, (c) 1 h, (d) 30 min. Note: There is an absorption at $\sim 1649 \text{ cm}^{-1}$ which indicates the presence of H–O–H bending.

expansion of the lattice and increase in the lattice parameter. This could be considered rather unusual since Mg^{2+} is smaller in size compared to Ca^{2+} . Hence it is possible that the Mg^{2+} is probably occupying an interstitial site rather than an actual lattice site causing disorder and loss in the preferred orientation.

As can be seen in Fig. 3, the FTIR results of magnesium substituted brushite indicate peaks characteristic of pure brushite. However, the representative peaks are considerably broad probably suggesting a distortion of the structure considerably altering the specific geometric linkage of the Ca–P related bonds due to the presence of excess

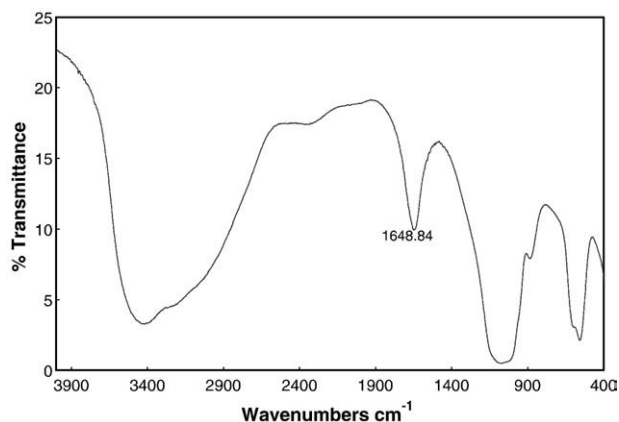


Fig. 9. FTIR spectra collected on magnesium substituted brushite boiled for 15 min. Note: The spectra resembles the pattern of magnesium substituted brushite (see Fig. 3).

magnesium corroborating the XRD result. This result also is in agreement with the chemical analysis and XRD results. The complete list of the primary vibration modes expected in brushite is given in Table 2.

The specific surface area of pure unsubstituted brushite synthesized in water has been measured to be $18.63 \text{ m}^2/\text{g}$. The specific surface area of magnesium substituted brushite measured by BET analysis however is $28.17 \text{ m}^2/\text{g}$. The slight increase in specific surface area can be explained on the basis that magnesium substituted brushite has a smaller particle size compared to that of pure unsubstituted brushite as discussed below.

Results of the SEM analysis are shown in Fig. 4. The magnesium substituted brushite results in a drastic change in morphology of brushite from the characteristic platelet morphology that is normally seen for pure brushite exhibiting the monoclinic crystal structure (Fig. 4(c), (d)). The introduction of excess magnesium appears to destabilize the equilibrium platelet morphology transforming the platelets, resulting in the formation of nanosized semi-spherical particles (slightly flattened from one side) $\sim 100 \text{ nm}$ in size (Fig. 4(a), (b)) which could also result in a more heterogeneous non textured structure. This change in morphology and particle size is in agreement with the X-ray diffraction pattern, indicating loss of the preferential

Table 3

Specific surface area of the starting precursor (Mg-substituted brushite) and β -TCMP obtained after boiling the precursor for different time periods

Materials	Mg-substituted brushite (precursor)	15 min boiled β -TCMP	30 min boiled β -TCMP	1 h boiled β -TCMP	4 h boiled β -TCMP	8 h boiled β -TCMP
Surface Area(m^2/g)	28.17	138.31	179.37	204.87	192.46	171.72

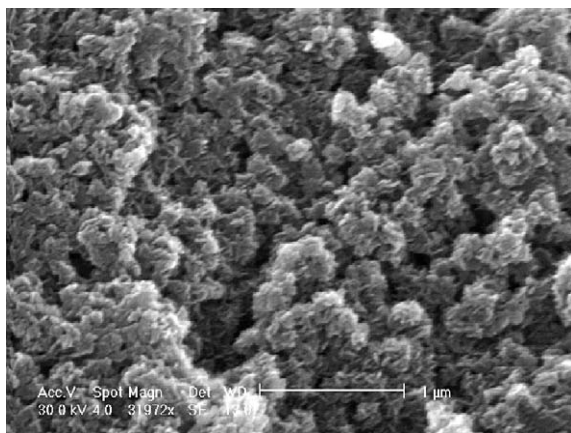


Fig. 10. SEM images showing the morphology of β -TCMP obtained by boiling Mg-substituted brushite for 15 min. Note the platelets growing from the spherical brushite sphere.

orientation in the (020) direction of pure brushite. This explains why the magnesium substituted brushite exhibits a much higher surface area compared to pure unsubstituted brushite, which validates the hypothesis proposed above.

3.2. Characterization of β -TCMP

Chemical analysis (ICP-OES) on β -TCMP obtained by boiling magnesium substituted brushite for 8 h conducted by Galbraith Laboratories, INC (Knoxville, TN) yields a chemical formula,

$\text{Ca}_{1.671}\text{Mg}_{0.743}(\text{PO}_4)_2$, which correspond to $(\text{Ca,Mg})_{2.414}(\text{PO}_4)_2$. The β -TCMPs obtained after boiling for different time periods are also expected to yield similar composition. The structural formula derived from chemical analysis implies that the β -TCMP powder has a lower Ca and/or Mg than the stoichiometric β -TCP composition possibly, due to the presence of vacancies (\square) or the possible retention of hydrogen ions (H) chemically bonded in brushite. We speculate the presence of vacancies while the retention of hydrogen is also plausible since the β -TCMP phase is derived from brushite which contains bonded hydrogen. While the present manuscript is not focused on identifying the transformation pathways, it can be speculated that the transformation of the Mg-substituted brushite phase to TCMP is induced by a combination of complex diffusion induced shear mechanisms accompanied by a chemical induced hydrolysis, dissolution and re-precipitation pathways. The brushite phase could thus undergo hydrolysis to yield an apatite type phase that releases the alkaline-earth hydroxides to form the TCMP phase containing either vacancies in the alkaline-earth lattice sites or retain the bonded hydrogen already present in the brushite lattice. However, more detailed study will be required to validate the exact transformation mechanisms, which is not the goal of the present study and will be the focus of on-going studies that will be published subsequently. Thus, if we denote the presence of vacancies or hydrogen ions by X, the as-boiled β -TCMP can be expressed by the chemical formula, $\text{Ca}_{1.671}\text{Mg}_{0.743}\text{X}_{0.586}(\text{PO}_4)_2$ (X=H and/or \square (\square ; vacancy)).

To determine the minimum boiling time required for magnesium substituted brushite to transform into β -TCMP, beginning from 8 h, the boiling time was reduced down to 15 min. It was found that after 30 min of boiling, magnesium substituted brushite completely transforms to β -TCMP. Thus XRD patterns of all the powders obtained

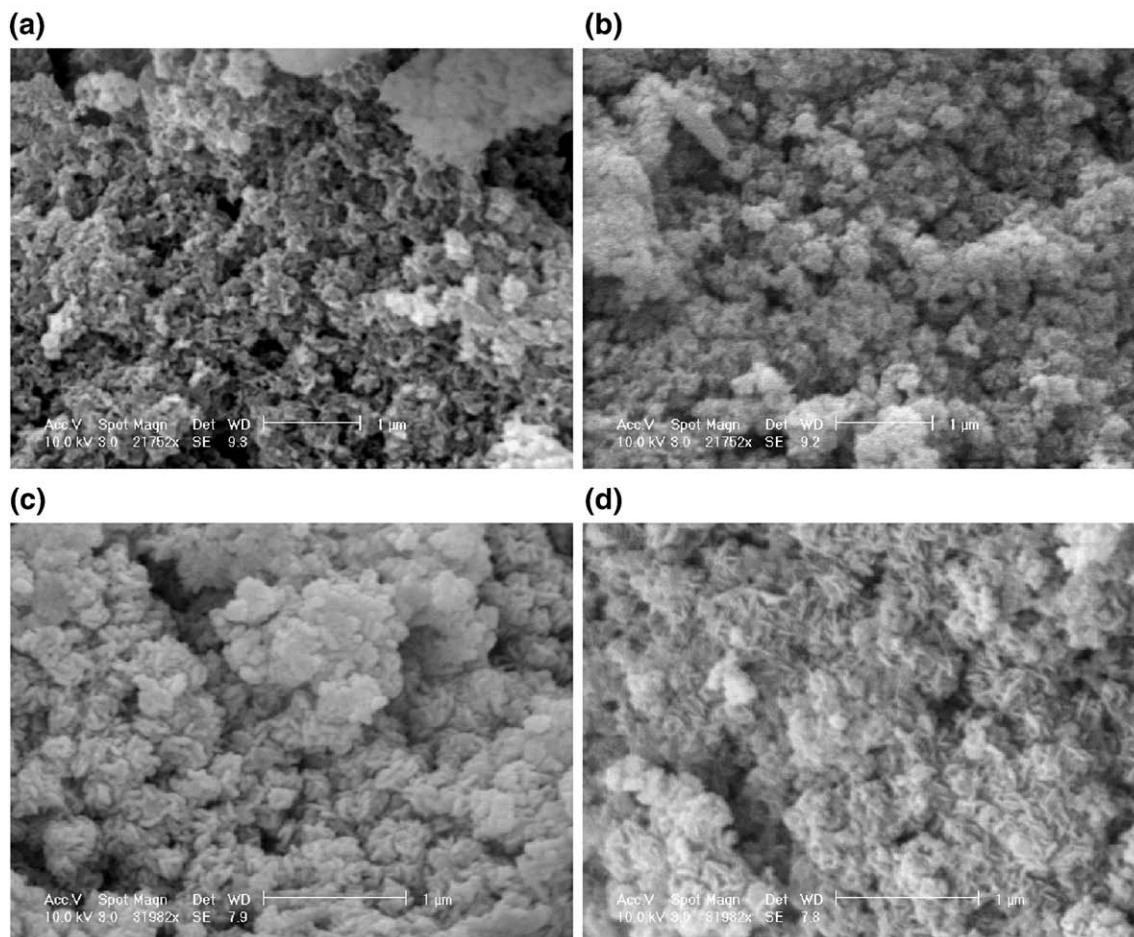


Fig. 11. SEM images showing the morphology of β -TCMP obtained by boiling Mg-substituted brushite for (a) 30 min, (b) 1 h, (c) 4 h, (d) 8 h. Note the platelets growing from the original spherical brushite sphere.

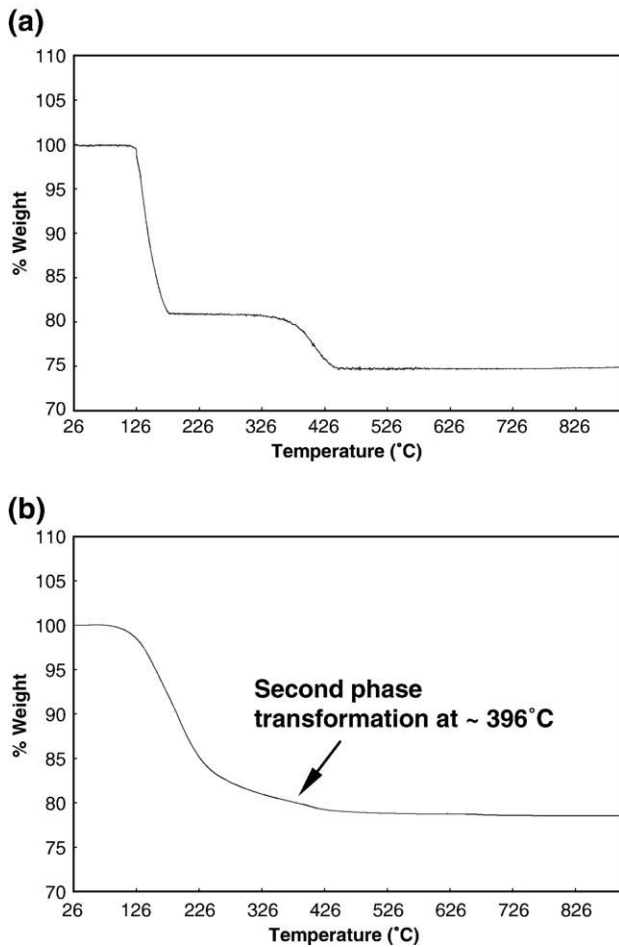


Fig. 12. (a) Thermal analysis of unsubstituted brushite; First phase transformation occurs at $\sim 125^\circ\text{C}$, and second phase transformation occurs significantly at $\sim 350^\circ\text{C}$. (b) Thermal analysis of Mg-substituted brushite; First phase transformation occurs at $\sim 100^\circ\text{C}$, and second phase transformation occurs at $\sim 396^\circ\text{C}$, however, not significantly enough.

after boiling for 30 min, 1 h, 4 h, 8 h exhibit identical patterns (Fig. 5) indicating that a boiling time of only 30 min is sufficient to cause the transformation of brushite to β -TCMP. Boiling the Mg-substituted brushite for only 15 min however, was not sufficient to completely transform it to β -TCMP. As can be seen in Fig. 6, the major characteristic peak of brushite $2\theta = 11.69^\circ$ still remain, although the majority of the peaks correspond to β -TCMP phase suggesting that it is the major phase. On the other hand it should be noted that unsubstituted brushite, when boiled for 1 h, completely transforms to monetite indicating the strong role of Mg in stabilizing the TCP phase (Fig. 7).

FTIR analysis shows the presence of the absorption at 1648.84 cm^{-1} in Fig. 8, which indicates the presence of H–O–H bending in the structure (Table 2). This shows the presence of residual H_2O that remain during the transformation of brushite to β -TCMP resulting in the hydrated form of β -TCMP. Thus the β -TCMP phase can be represented as $\text{Ca}_{1.671}\text{Mg}_{0.743}\text{X}_{0.586}(\text{PO}_4)_2 \cdot \chi\text{H}_2\text{O}$ ($\text{X} = \text{H}$ and/or \square). As shown, boiling the Mg-substituted brushite above 30 min results in the β -TCMP phase that exhibits identical FTIR plot (Fig. 8). On the other hand, the IR spectra of β -TCMP obtained after boiling magnesium substituted brushite for 15 min however shows a slightly different pattern as shown in Fig. 9. The spectra resembles that of the magnesium substituted brushite (Fig. 3) indicating the incomplete transformation of brushite to β -TCMP.

To evaluate the specific surface area of the transformed β -TCMP, BET analysis was conducted on the transformed β -TCMP powders. The results are summarized in Table 3. As shown in Table 3, there is a

tremendous increase in specific surface area with values in the range of $200\text{ m}^2/\text{g}$, which is a significant increase in comparison to the specific surface area of the Mg-substituted brushite precursor, which yielded $28.17\text{ m}^2/\text{g}$. However, β -TCMP, obtained after boiling Mg-substituted brushite for only 15 min, has a slightly lower specific surface area compared to the β -TCMP powder obtained after boiling magnesium substituted brushite for longer periods of time.

The SEM analysis clearly provides the explanation for the significant increase in the measured surface area (Figs. 10 and 11). It appears that the solution induced thermal incubation treatment results in the evolution of the rosette shaped morphology of β -TCMP from the spherical shaped Mg-substituted brushite particles. Thus from each of the spherical particles of magnesium substituted brushite, dendritic platelets appear to grow out, corresponding to the TCP phase, which likely results in a drastic increase in surface area. The size of each platelet is in the range of $\sim 80\text{ nm}$. The β -TCMP phase obtained after boiling for the period in excess of 30 min results in almost no change in the morphology and crystallite size. Therefore, it can be inferred that the fully grown β -TCMP crystal size is in the range of $\sim 80\text{ nm}$. Compared to the β -TCMPs obtained after boiling for longer periods of time, the β -TCMP derived from boiling for 15 min indicates no big difference in the size of the platelets (Fig. 10). This suggests that it is possible that the powder obtained after boiling for only 15 min has regions of the original untransformed nanospherical Mg-substituted brushite phase. Thus the surface area of these partially transformed β -TCMP powders obtained

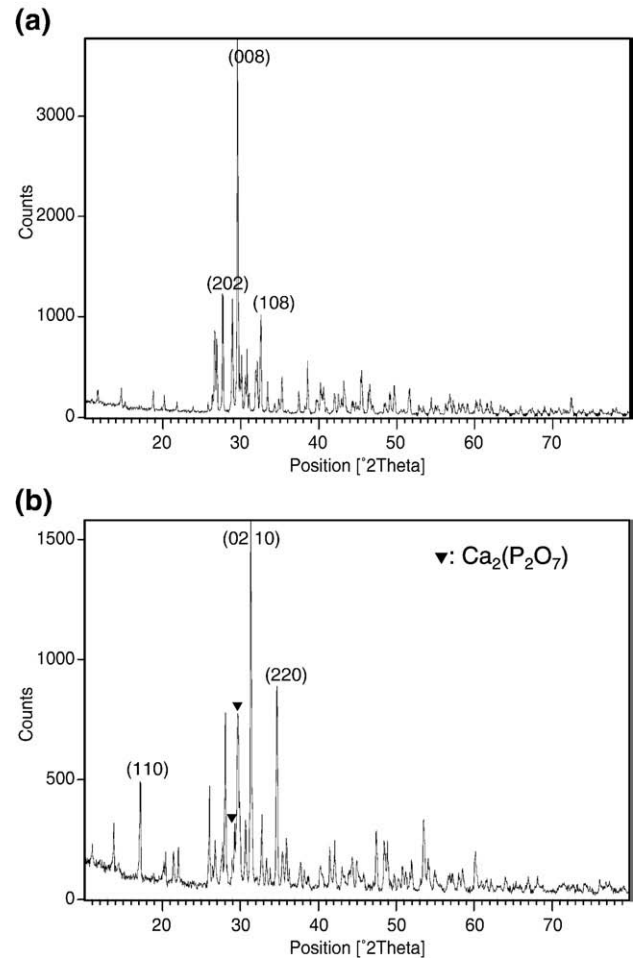


Fig. 13. Thermally heat treated (a) unsubstituted brushite and (b) magnesium substituted brushite. (a) unsubstituted brushite completely transforms to $\text{Ca}_2(\text{P}_2\text{O}_7)$ whereas (b) magnesium substituted brushite transforms to TCP mixed with small amount of $\text{Ca}_2(\text{P}_2\text{O}_7)$.

after 15 min of boiling are lower ($138.31 \text{ m}^2/\text{g}$) than completely transformed β -TCMP powders ($\sim 200 \text{ m}^2/\text{g}$).

3.3. Thermal analysis of brushite

To study the thermal stability and the chemical nature of the magnesium incorporated into each of the two calcium phosphate structures, both magnesium substituted brushite and unsubstituted brushite have been thermally analyzed up to $900 \text{ }^\circ\text{C}$ (step size $5 \text{ }^\circ\text{C}/\text{min}$ in an air atmosphere). TG analysis results of unsubstituted brushite and magnesium substituted brushite are given in Fig. 12. The unsubstituted brushite exhibits first a phase transformation at $\sim 125 \text{ }^\circ\text{C}$, while the magnesium substituted brushite exhibits this transition at $\sim 100 \text{ }^\circ\text{C}$. Unsubstituted brushite also exhibits a significant second phase transformation occurring at $\sim 350 \text{ }^\circ\text{C}$, while the magnesium substituted brushite exhibits second phase transformation which is not very significant (at $\sim 396 \text{ }^\circ\text{C}$). XRD analysis of the magnesium substituted brushite powder after thermal analysis indicated transformation to β -TCMP mixed with small amount of the pyrophosphate phase $\text{Ca}_2(\text{P}_2\text{O}_7)$, whereas unsubstituted brushite transformed completely to pure $\text{Ca}_2(\text{P}_2\text{O}_7)$ without exhibiting the presence of any β -TCP type phase. (Fig. 13). These transformation results also validate the incorporation of Mg into the brushite lattice. To analyze the phase formed as a result of the first phase transformation at $\sim 100 \text{ }^\circ\text{C}$, unsubstituted brushite and magnesium substituted brushite were both heat treated up to $225 \text{ }^\circ\text{C}$ in air with a

step size of $5 \text{ }^\circ\text{C}/\text{min}$. Upon heat treatment, unsubstituted brushite completely transformed to monetite by dehydration of the brushite phase (Fig. 14-a). However, magnesium substituted brushite transformed to an amorphous phase mixed with small amount of monetite (Fig. 14-b). These results indicate that the magnesium substituted brushite is essentially comprised of a mixed phase of small amount of unsubstituted brushite and magnesium substituted brushite. However, even this small amount of brushite phase seems to contain some magnesium, since the unsubstituted brushite transforms to monetite when stored in the shelf for more than 6 months, whereas the magnesium substituted brushite continues to exhibit the brushite phase even after storage for more than 1 year. This is also seen by the peak shift in the XRD pattern collected on magnesium substituted brushite (Fig. 2) as discussed earlier. The remarkable stability of the Mg-substituted brushite therefore, indicates that the substituted phase is likely to be a mixture of brushite phase containing a smaller and a larger amount of magnesium substituted into the brushite lattice contributing to the formation of an amorphous phase and the crystalline brushite phase upon thermal treatment to $225 \text{ }^\circ\text{C}$. It will be useful to determine the two different levels of magnesium substitutions in the brushite lattice causing these two transformations which would be useful in determining the mechanisms of Mg incorporation and its role in conversion of brushite to β -TCMP. These studies form part of the future work.

4. Discussion

The discussion will focus on two aspects of the study conducted. First is the shorter transformation time needed for inducing the brushite to β -TCMP transformation compared to the conventional methods, and the second, is the actual transformation mechanism.

Compared to the conventional approach for synthesizing β -TCMP, the process time has been reduced significantly from 8–5 h to only 30 min. The reason for this reduction in time can be explained by the reduced diffusion time. In the conventional methods, brushite and monetite, which are dispersed in the solution containing Mg^{2+} ion tend to transform to β -TCMP after prolonged period of reaction time. This is because Mg^{2+} ions first need to diffuse into the brushite or monetite lattice to trigger the transformation of brushite to TCP, which is a kinetically slower process due to ion diffusion from the solution phase into the solid phase. However, with the magnesium already substituted into the brushite lattice, the kinetic barrier of diffusion of Mg into the brushite lattice is now significantly reduced, thus resulting in a shorter brushite to β -TCMP transformation time as demonstrated by our results. Even in the 15 min of boiling of brushite, only small amount of brushite remains untransformed. The actual time of boiling required for transformation can thus be inferred to be between 15 min and 30 min. Further study may be required to determine the exact time required for the formation of β -TCMP. Nevertheless, the generation of Mg-substituted brushite significantly reduces the time required for initiating the diffusion induced transformation of β -TCMP.

Thermal analysis study has revealed that there is $\text{Ca}_2(\text{P}_2\text{O}_7)$ phase formed upon heat treatment of unsubstituted brushite up to $900 \text{ }^\circ\text{C}$ (Fig. 13). However, all of the powders obtained by boiling magnesium substituted brushite indicate absence of $\text{Ca}_2(\text{P}_2\text{O}_7)$ or monetite phases, which is the resultant phase obtained by thermal treatment of the unsubstituted brushite phase or even boiling the unsubstituted brushite for 1 h (Figs. 5 and 7). This shows that upon boiling, magnesium substituted brushite transforms completely to β -TCMP, and even the small amount of existing brushite phase remaining after boiling for a short time of 15 min is likely to be Mg enriched since there is no formation of monetite upon storage as is the case with the unsubstituted brushite.

Although further in-depth study is required to determine the exact mechanism of transformation of Mg-substituted brushite to β -TCMP,

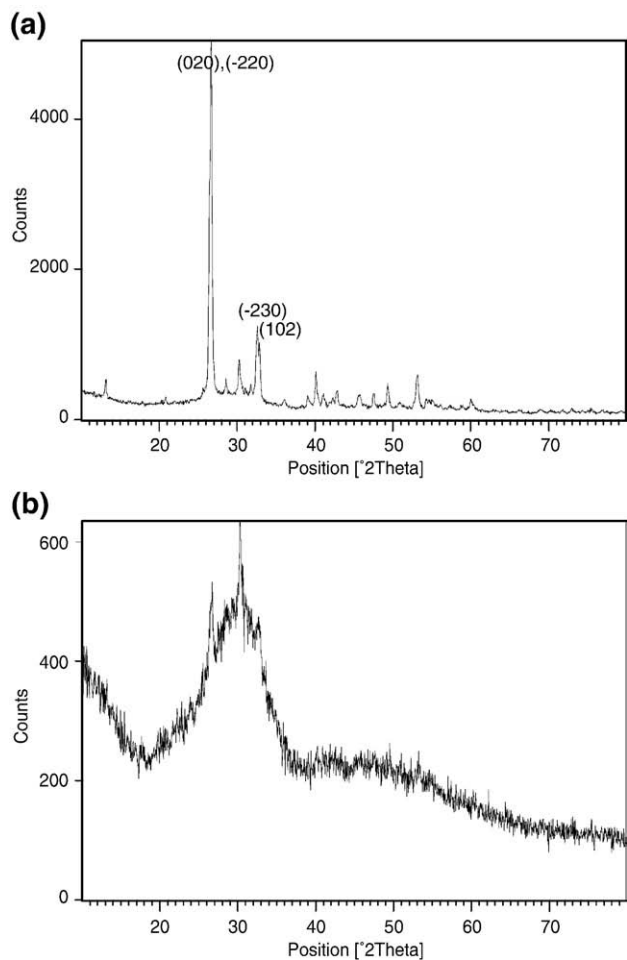
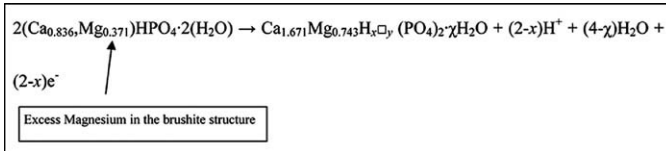


Fig. 14. (a) Unsubstituted brushite heat treated to $225 \text{ }^\circ\text{C}$ (step size = $5 \text{ }^\circ\text{C}/\text{min}$) transforms to monetite, whereas (b) magnesium substituted brushite transforms to amorphous phase mixed with small amount of monetite upon heat treatment to $225 \text{ }^\circ\text{C}$ (step size = $5 \text{ }^\circ\text{C}/\text{min}$).

one can justifiably speculate the mechanisms involved in the transformation based upon the data gathered above. It has been shown that the ratio of Ca:Mg:P has not changed due to the transformation of brushite to β -TCMP. For brushite, Ca:Mg:P=0.836:0.371:1, and for β -TCMP, Ca:Mg:P=1.671:0.743:2=0.8355:0.3715:1, indicating that the ratio is invariant. This suggests that there is excess magnesium remaining in the Mg-substituted brushite structure, but less magnesium compared to stoichiometric β -TCP remaining in the β -TCMP structure. Therefore, we can postulate that proton extraction from the brushite structure is the primary step responsible for the actual process of transformation. However, since there is less Ca^{2+} and Mg^{2+} in the β -TCMP structure than required, we can conclude that there is retention of protons in the structure and/or vacancies. Therefore, we can hypothesize the β -TCMP transformation reaction to be as follows;



where X=H and $y = \square$ (\square ; vacancy) and $x/2 + y = 0.586$ (x ; H^+ content in β -TCMP, y ; \square content in β -TCMP).

5. Conclusions

Magnesium is usually substituted into β -TCP to allow the synthesis of β -TCMP under aqueous conditions. The synthesis of magnesium substituted tricalcium phosphate has been studied by the method of *in-situ* growth process. Magnesium substituted brushite was used as the precursor, which upon boiling results in the formation of β -TCMP. Using the method described above, 30 min of boiling was the

minimum time required to synthesize β -TCMP. Compared to the other processes, the boiling time has been reduced significantly. The β -TCMP synthesized in this manner is very likely to be the hydrated form, which can be represented as $\text{Ca}_{1.671} \text{Mg}_{0.743} \text{X}_{0.586} (\text{PO}_4)_2 \cdot \chi \text{H}_2\text{O}$ ($\text{X}=\text{H}$ and/or \square). The synthesis of β -TCMP using this novel approach clearly broadens the applicability of β -TCMP in biological systems which were limited by the prolonged periods of boiling required in the conventional approaches used for synthesizing β -TCMP. Furthermore, the approaches results in nanosized (~ 100 nm) brushite semi-spheres and 80 nm sized platelets of β -TCMP exhibiting a specific surface area as high as $205 \text{ m}^2/\text{g}$.

Acknowledgments

This work was supported by NSF-NIRT (CTS-0210238), DURINT, ONR (N00014-0110715) NIH-NIBIB (1R01EB002706) and NIH-NIDCR (5R03DE015905).

References

- [1] M. Winter, P. Griss, K. de Groot, H. Tagai, G. Heimke, H.J.A.V. Dijk, *Biomaterials* 2 (1981) 159.
- [2] U. Gbureck, O. Grolms, J.E. Barralet, L.M. Grover, R. Thull, *Biomaterials* 24 (23) (2003) 4123.
- [3] L.C. Chow, S. Takagi, K. Ishikawa, in: W.E. Brown, E. Constanz (Eds.), *Hydroxyapatite and Related Materials*, CRC Press, Boca Ration, FL, 1994, p. 127.
- [4] R.Z. Legeros, *Calcium Phosphates in Oral Biology and Medicine*, Karger, Basel, 1991.
- [5] S.H. Kwon, Y.K. Jun, S.H. Hong, H.E. Kim, *J. Eur. Ceram. Soc.* 23 (7) (2003) 1039.
- [6] K. De Groot, *Biomaterials* 1 (1) (1980) 47.
- [7] T.R. Narayanan Kutty, *Ind. J. Chem.* 11 (7) (1973) 695.
- [8] H.C.W. Skinner, J.S. Kittelberger, R.A. Beebe, *J. Phys. Chem.* 79 (19) (1975) 2017.
- [9] H. Newesely, *J. oral rehab.* 4 (1) (1977) 97.
- [10] F.H. Lin, C.J. Liao, K.S. Chen, J.S. Sun, C.P. Lin, *Biomaterials* 22 (2001) 2981.
- [11] G. Daculsi, R.Z. LeGeros, A. Jean, B. Kerebel, *J. Dent. Res.* 66 (8) (1987) 1356.
- [12] P.N. Kumta, C. Sfeir, D. Lee, D. Olton, D. Choi, *Acta Biomater.* 1 (2005) 65.
- [13] L. Tortet, R. Gavarri, G. Nihoul, A.J. Dianoux, *J. Solid State Chem.* 132 (1997) 6.