Hydroxyapatite Prepared by Co-Precipitation with Calcium Carbonate: Effects of Digested Calcium Carbonate and Phosphate Sources

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Received: 12 May 2016; Accepted: 10 August 2016

Abstract

The Hydroxyapatite (HAP) was prepared by co-precipitation of calcium carbonate ($CaCO_3$) with various orthophosphate sources such as phosphoric acid (H_3PO_4), ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) under ambient conditions. $CaCO_3$ was digested by 20% HNO_3 to produce calcium cation which reacted with the orthophosphate ion to form hydroxyapatite. All XRD patterns closely matched with the HAP reference data. There was no change in the sharpness of the diffraction peaks and little or no change in the peak intensity was observed when compared to various phosphate sources (H_3PO_4, $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$). The FTIR spectrum showed without the characteristic bands of B-type CO_3^{2-} substitution at 1421-1473 cm^{-1} (v3) and 874 cm^{-1} (v2). Moreover, the FTIR spectrum showed without the characteristic peak of A-type CO_3^{2-}substitution at 1540 cm^{-1}. The precipitation of HAP from digested $CaCO_3$ solution occurred at 3 hours, while the reaction from non-digested $CaCO_3$ solution occurred at 18 hours. The digestion of $CaCO_3$ reduced the preparation time of HAP and also decreased the CO_3^{2-}substitution in HAP.

Keywords: Hydroxyapatite, Calcium carbonate, Co-precipitation

Introduction

Hydroxyapatite (HAP), whose chemical formula is $Ca_{10}(PO_4)_6(OH)_2$, is one of the most biocompatible materials due to its similarity with mineral constituents found in hard tissues such as teeth and bones. It is a very attractive material for biomedical applications, such as a bone substitute material in orthopedics and dentistry due to its excellent biocompatibility, bioactivity, and osteoconduction properties. In addition, HAP has a wider scope of applications in diverse fields like chromatography, solid state ionics, catalysts, drug delivery systems and fuel cells. It has a promising application as a chemical gas sensor (Mahabole, Aiyer, Ramakrishna, Sreedhar, & Khairnar, 2005). Various processes have been employed to prepare HAP powders, including chemical co-precipitation (Inthong, Tunkasiri, Eitssayeam, Pengpat, & Rujijanagul, 2013), the sol-gel process, ultrasonication technique, microwave assisted technique (Waheeda, Sultana, Jamila, & Hussainb, 2013), ultrasonic spray-pyrolysis (An et al., 2007), hydrothermal syntheses (Kim & Ohtsuki, 2016), emulsion processing (Sonoda, Furuzono, Walsh, Sato, & Tanaka, 2002) and mechanochemical method (Nasiri-Tabrizi, Honarmandi, Ebrahimi-Kahrizsangi, & Honarmandi, 2009). Among these processes, chemical co-precipitation is the most promising method because of its simplicity in experimental operations, low operating temperature, and high yields of pure products (Kong, Ma, & Boey, 2002). For example, Bouyer, Gitzhofer, and Boulos. (2000) prepared nanometer size HAP crystals through a wet chemical precipitation method at different synthesis temperatures and with various reactant addition rates. This work shows that shape, size, and specific surface area of the HAP nanoparticles are very sensitive to the reaction temperature and also to the reactant addition rate. The measured pH at the end of synthesis, which is strongly linked with the reactant addition rate, is a key parameter which can be used to determine the purity of the synthesized HAP nanocrystals and also for the stabilization (dispersion) of the suspension. Bezzi et al. (2003) prepared HAP by a sol-gel method, using $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$ as raw materials, and EDTA and urea as aids. The above studies showed a trend to superfine in the development of HAP suspension polymerization dispersants. Kong et al. (2002) prepared nanosized hydoxyapatite powders by a co-precipitation process which used calcium nitrate and phosphoric acid as starting materials. The average grain size of the HAP powders were found to be 50-70 nm and showed no change when calcined at temperatures up to 600°C for 4 hours. Dense HAP ceramics were also obtained by sintering the synthesized HAP powders at temperatures from 1000°C to 1200°C with a final average grain size of 2 to 7 µm correspondingly. Piantone, Bodénan, Derie, and Depelsenaire. (2003) examined the reaction of phosphate with various mineral species and described the usual sequence of events when calcium ions react in solution with phosphate: the first mineral species to precipitate was $CaHPO_4.2H_2O$ (brushite), followed by $Ca_3(PO_4)_2$ (whitlockite), $Ca_4H(PO_4)_3.5H_2O$ (octacalcium phosphate), and, finally, $Ca_5(PO_4)_3OH$ (hydroxylapatite). The theoretical phase diagram describing the stable minerals in pH vs. total CO_3^{2-} coordinates showed that when the CO_2 gas partial pressure was near atmospheric pressure there was a narrow pH range between 6 and 9.5 where calcite could be converted into apatite. Verwilghen et al. (2009) synthesized HAP starting from $CaCO_3$ and monoammoniumphosphate in stoichiometric amounts. The novel aspect concerned the reaction conditions, which were simply mixing the reactants in water at ambient pressure and a temperature of 60°C. The reaction was continued for 72 hours. The $CaCO_3$ solid phase slowly produced CO_2 gas and buffered the solution at a pH value of 8.5 where HAP precipitated. Besides, the synthesis of calcium HAP starting from $CaCO_3$ and different orthophosphate sources, including orthophosphoric acid, potassium, sodium, and ammonium dihydrogen orthophosphates was investigated under ambient conditions. The reaction started with $CaCO_3$ dissolution in an acid medium, followed by rapid precipitation of calcium cations with orthophosphate species to form calcium phosphate. The reaction was continued for 48 hours under ambient conditions (25°C, atmospheric pressure). Orthophosphoric acid was the most interesting orthophosphate source since it was the most efficient for the dissolution of $CaCO_3$ and was the only reactant leading to complete consumption by precipitation of the starting orthophosphate species within 48 hours (Minh, Lyczko, Sebei, Nzihou, & Sharrock, 2012). However, investigation focusing on the acid digestion of $CaCO_3$ before HAP formation has not yet to be published. In this study, the $CaCO_3$ was first digested with acid to form as Ca^{2+} ions. The digested $CaCO_3$ was used to prepare HAP powder with various orthophosphate sources. The synthesized HAP powders were characterized by XRD, SEM, and FTIR measurement. The HAP powder was also prepared from non-digested $CaCO_3$ by the same method for comparison.

Materials and Methods

Synthesis and characterization of hydroxyapatite

A fine powder of calcium carbonate ($CaCO_3$, 99%, Carlo Erba) was used as calcium source. Orthophosphoric acid (H_3PO_4, 85%, Ajax Finechem), ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$, 98%, Ajax Finechem), and diammonium hydrogen orthophosphate ($(NH_4)_2HPO_4$, 98%, Ajax Finechem) were used as orthophosphate sources without further modification. $CaCO_3$ (10 g) was digested by 40 mL of the 20% (w/v) HNO_3 (69.5%, Carlo Erba). The solution mixture was topped up with deionized water to 50 ml. Ammonium hydroxide (NH_4OH, 30%, Carlo Erba) was pre-added to a digested $CaCO_3$ solution to raise the pH level to 8-12 prior to the precipitation of HAP. 50 ml of a suspension containing $CaCO_3$ was introduced into a 250 ml Erlenmeyer flask. The typical phosphorus sources, H_3PO_4, $NH_4H_2PO_4$, and $(NH_4)_2HPO_4$, were dissolved separately in 50 ml of deionized water at a molar ratio of Ca/P 1.7 and each solution was dripped into the alkaline $CaCO_3$ solution, then stirred at 50°C under ambient pressure. The reaction was continued for 3 hours. The white solids collected by filtration were oven dried at 600°C for 4 hours to yield a fine powder. The product was then rinsed with distilled water to dissolve any unreacted phosphate. The crystal phase of the synthesized powder was examined by an X-ray powder diffraction method using an X-ray diffractometer (Phillips Panalytical X'Pert Pro MPD). The diffraction patterns were collected over a 2θ range from 10 to 80° with a scan speed of 0.5°/min. A Fourier transform infrared (FT-IR, Shimadzu FTIR-8900) analysis using KBr method was also done. Microstructures of HAP obtained were examined by scanning electron microscopy (SEM, LEO 1450 VP).

Results and Discussion

HAP was prepared by co-precipitation of non-digested $CaCO_3$ and digested $CaCO_3$ with various phosphorus sources (H_3PO_4, $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$). The possible chemical reactions for the co-precipitation of non-digested $CaCO_3$ may be expressed in Eq. (1)-(3) as:

$$10CaCO_3+6H_3PO_4+6NH_3+2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2+3(NH_4)_2CO_3+7H_2CO_3 \quad (1)$$

$$10CaCO_3+ 6NH_4H_2PO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2+ 3(NH_4)_2CO_3+ 7H_2CO_3 \quad (2)$$

$$10CaCO_3 +6(NH_4)_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)_2CO_3 + 4H_2CO_3 \quad (3)$$

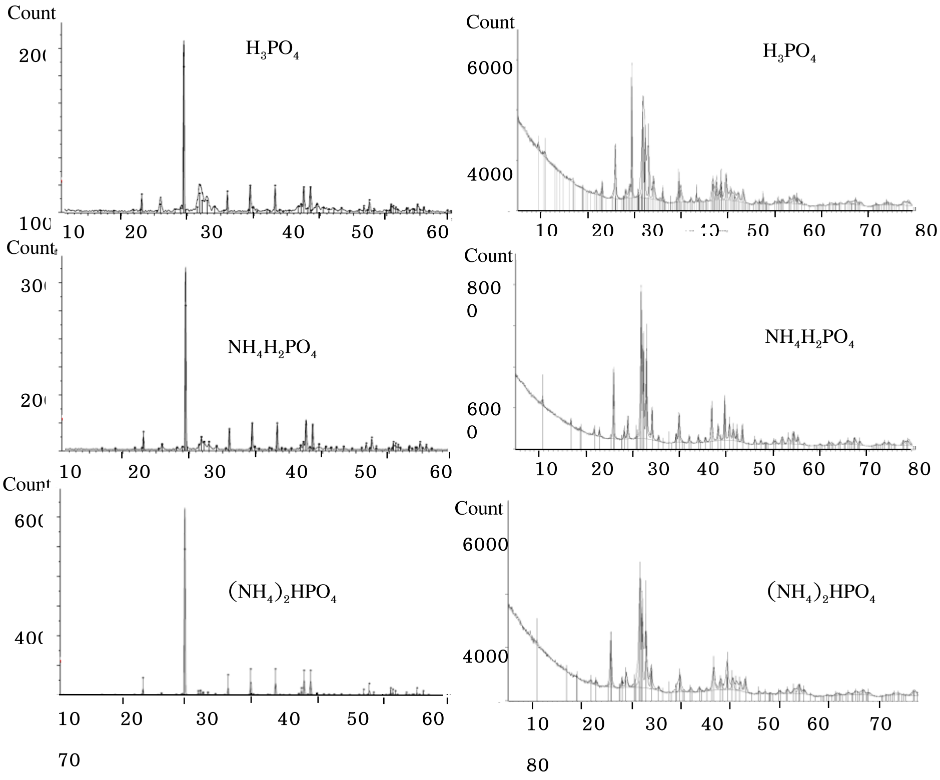
The chemical reactions between digested $CaCO_3$ and phosphate can be expressed in Eq. (4)-(5). The hydrogen ions from the acid solution reacted with $CaCO_3$ and formed Ca^{2+} ions and CO_2, reducing CO_3^{2-} substitution in HAP (Minh et al., 2012).

$$2H^+ +CaCO_3 \rightarrow Ca^{2+} + H_2O + CO_2 \quad (4)$$

$$Ca^{2+} + \text{orthophosphates} \rightarrow \text{calcium orthophosphates} \quad (5)$$

The resulting products were examined by XRD. The resulting spectra may be seen in Figure 1. To compare the source of calcium between non-digested $CaCO_3$ and the digested $CaCO_3$ in the HAP synthesis, the XRD results showed that HAP was synthesized by the non-digested $CaCO_3$ with a reaction time of 18 hours. When Verwilghen et al. (2009) synthesized HAP starting from non-digested

$CaCO_3$ and monoammoniumphosphate at ambient pressure and 60°C, HAP was synthesized in 72 hours. The reaction of non-digested $CaCO_3$ showed the XRD peak at about 29.7° indicating that carbonate was detected. As seen in Figure 1(a), the XRD pattern showed the existence of $CaCO_3$. For 18 hours period of reaction time, the process was gotten the better result. However, HAP was synthesized within 3 hours by the digested $CaCO_3$ when reacted with various phosphorus sources (H_3PO_4, $NH_4H_2PO_4$, and $(NH_4)_2HPO_4$) without any remaining CO_3^{2-}.



(a) (b)

Figure 1 XRD patterns of HAP syntheses using H_3PO_4, $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$ as orthophosphate sources reacted with the non-digested $CaCO_3$ at solution pH 9 for (a) 3 h and (b) 18 h.

As seen in Figure 1, the identification of HAP was primarily based on the two most prominent peaks at 26° and at 32-34°. The presence of HAP's characteristic diffraction peaks near 25°, 33°, 40°, and 50° confirmed that the synthesized material was HAP (Piantone et al., 2003). All XRD patterns matched with HAP reference data and showed little change in 2θ and sharpness of the peaks for the various phosphorus sources (H_3PO_4, $NH_4H_2PO_4$, and $(NH_4)_2HPO_4$). From the results, the appropriate time of reaction for non-digested $CaCO_3$ was 18 hours. Therefore, the digestion of $CaCO_3$ reduced the preparation time of HAP and decreased the presence of CO_3^{2-} in HAP. Minh et al. (2012) described a reaction pathway of the co-precipitation of HAP in acidic conditions in Figure 2. First, $CaCO_3$ powder was digested in acid solution to form soluble calcium cations. These cations were then precipitated with orthophosphate anions to form mono-calcium phosphate monohydrate ($Ca(H_2PO_4)_2.H_2O$) and dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$) or brushite as the first intermediates. $Ca(H_2PO_4)_2.H_2O$ evolved to $CaHPO_4.2H_2O$ by the reaction with more calcium cations. $CaHPO_4.2H_2O$ was then transformed into dicalcium phosphate anhydrous ($CaHPO_4$) as another intermediate and finally $Ca_5(PO_4)_3OH$. In acidic condition, the presence of carbonic gas or (bi) carbonate anions in the reaction mixture led to the formation of B-type calcium carbonate-apatite (CAP) in parallel with the formation of calcium hydroxyapatite as the final product.

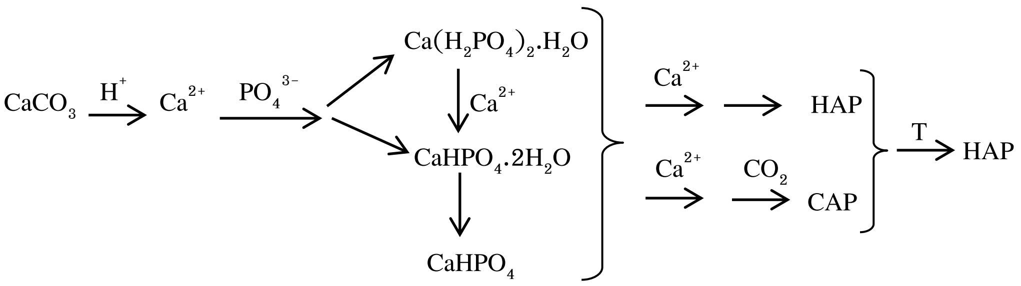


Figure 2 Reaction pathway in the HAP synthesis (Minh et al., 2012).

The FT-IR spectra of synthesized HAP by $CaCO_3$ digestion are shown in Figure 3-5. The spectra indicated absorption bands at 563, 604 and 1032 cm^{-1}, corresponding to PO_4^{3-} ions, and a broad band at around 3500 cm^{-1} and 640 cm^{-1}, corresponding to a PO_4^{3-} group which confirmed the formation of the characteristic HAP structure containing a hydroxyl group. The apatite lattice can be substituted by CO_3^{2-} ions, known respectively as A-type and B-type substitutes. The FTIR spectrum showed without the characteristic bands of B-type CO_3^{2-}substitution at 874 cm^{-1}(v2) and 1421-1473 cm^{-1} (v3). Furthermore, there was no characteristic peak of A-type CO_3^{2-}substitution at 1540 cm^{-1}. The results showed that the preparation of HAP from $CaCO_3$ digestion decreased the CO_3^{2-} substitution in HAP (Hui, Meena, Singh, Agarawal, & Prakash, 2010).

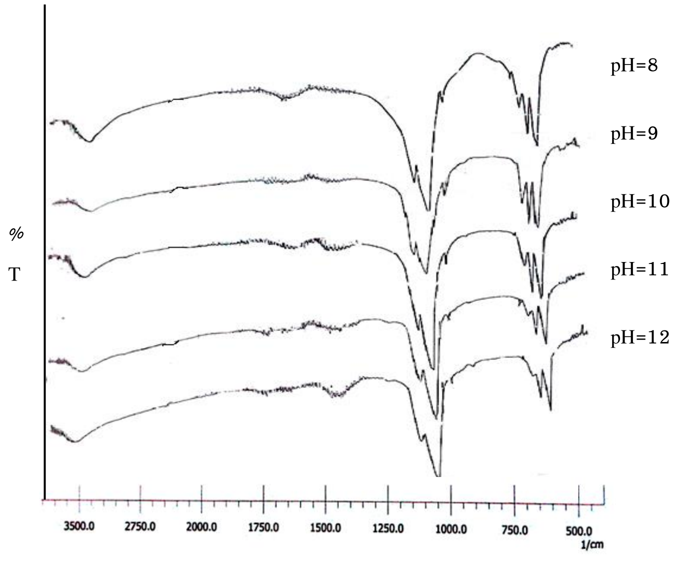


Figure 3 Infrared spectra of HAP prepared by co-precipitation of H_3PO_4 with digested $CaCO_3$.

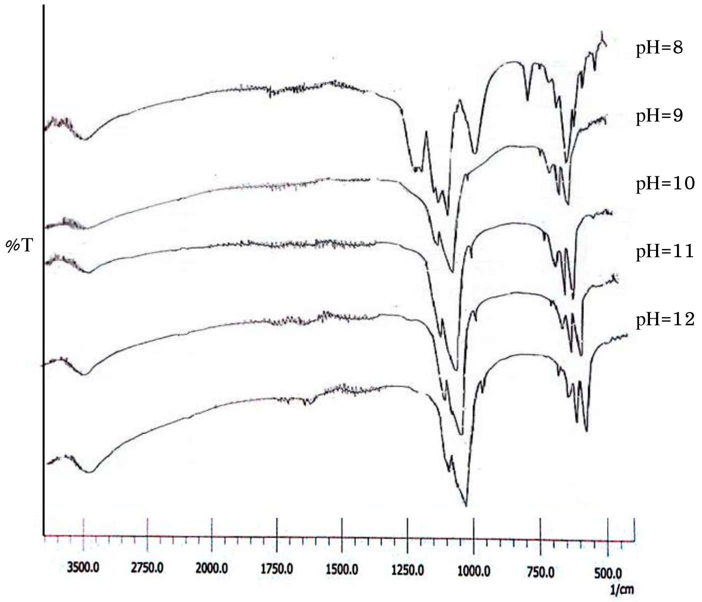


Figure 4 Infrared spectra of HAP prepared by co-precipitation of $NH_4H_2PO_4$ with digested $CaCO_3$.

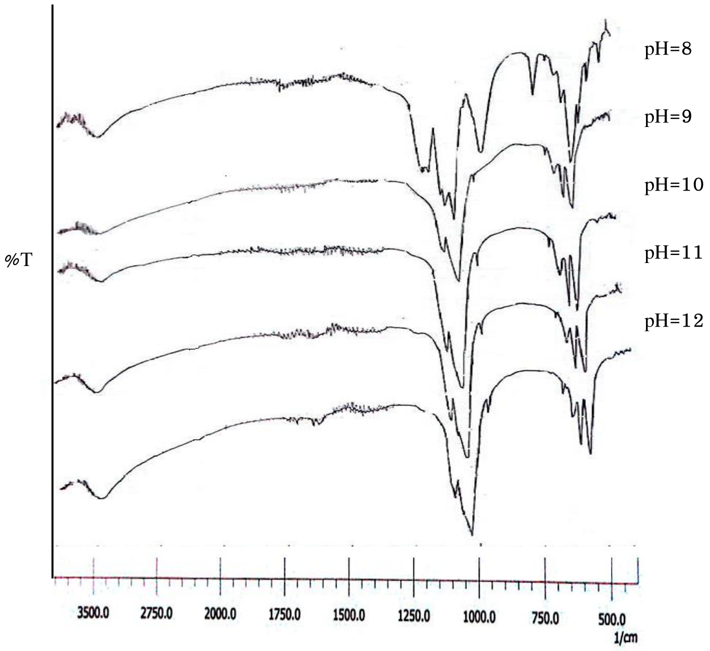
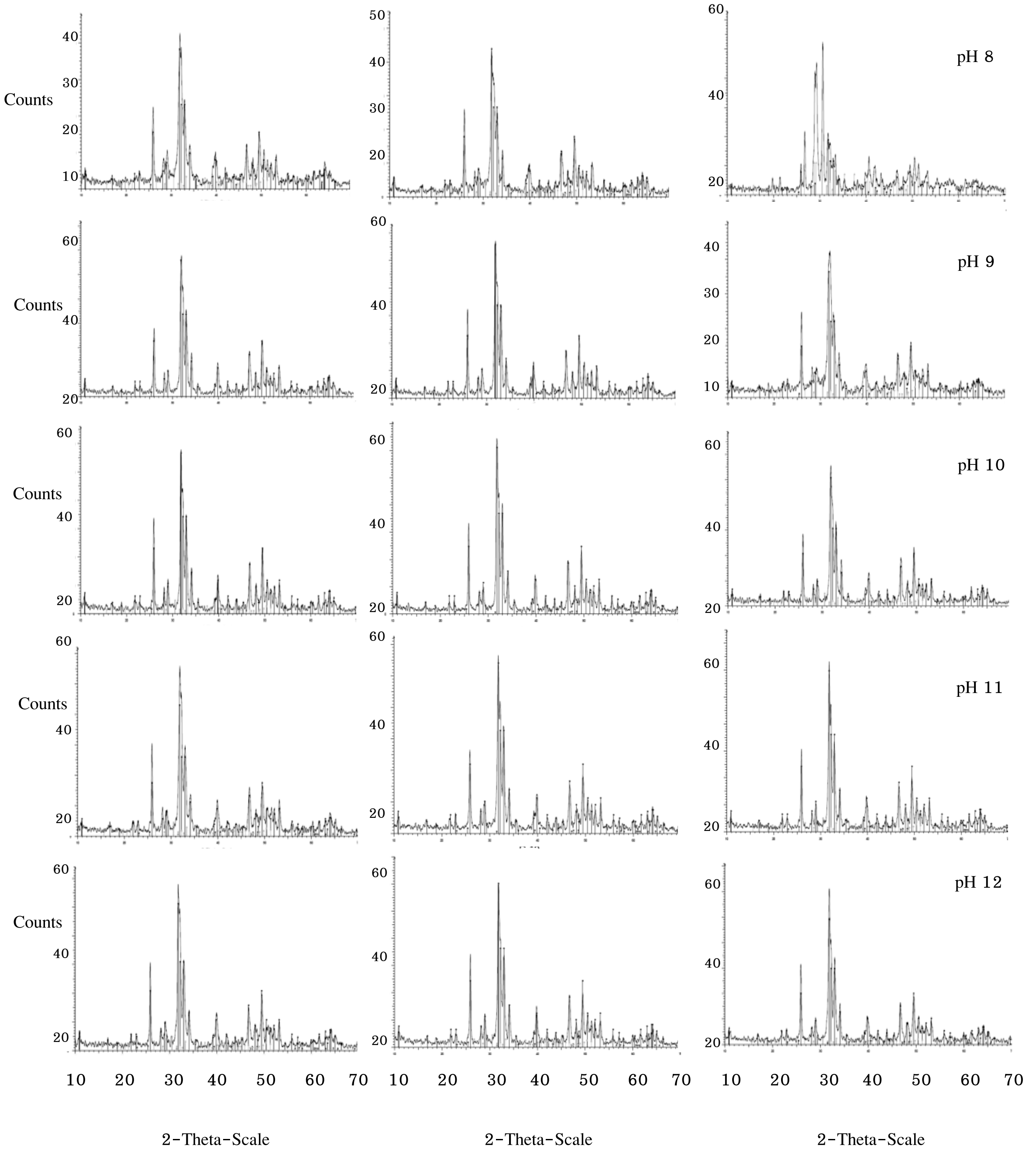


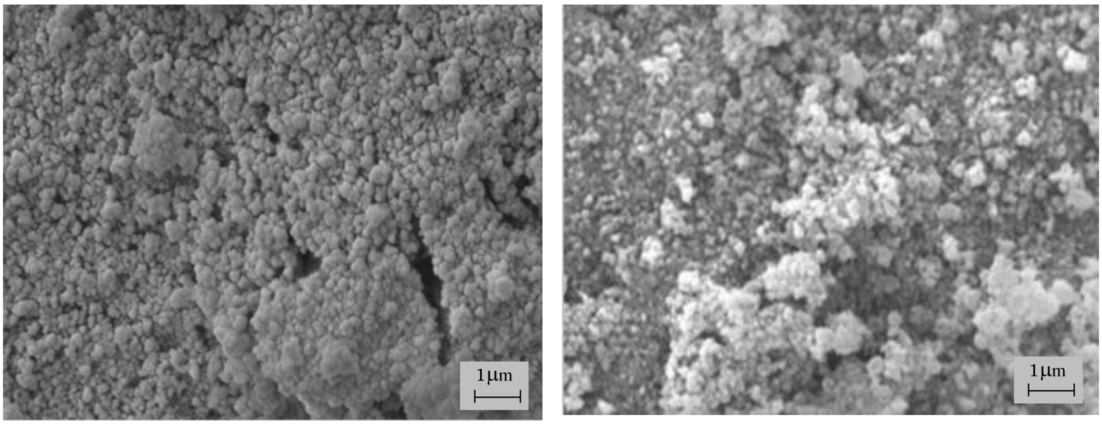
Figure 5 Infrared spectra of HAP prepared by co-precipitation of $(NH_4)_2HPO_4$ with digested $CaCO_3$.



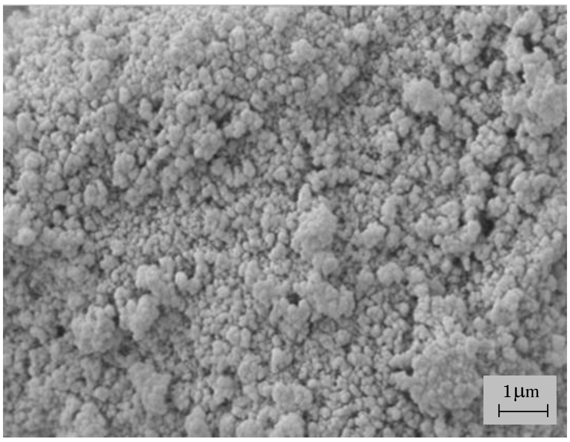
(a) (b) (c)

Figure 6 XRD patterns of HAP in the syntheses using H_3PO_4 (a), $NH_4H_2PO_4$ (b) and $(NH_4)_2HPO_4$ (c) as orthophosphate sources reacted with the digested $CaCO_3$ at pH 8-12 for 3 h.

The precipitation of HAP from digested $CaCO_3$ was observed at pH 8-12. The FTIR spectra (Figure 3-5) of all samples have shown bands corresponding to HAP structure. Similarly, the results from XRD patterns (Figure 6) have shown that the samples underwent a pure apatite phase and the sharp peaks confirm that they were well crystallized. It indicated that the different selections of pH value (pH 8-12) were successful to prepare HAP by digested $CaCO_3$. According to the study of the precipitation of calcium phosphates as a function of the concentration of the calcium ions, phosphate ions and the pH at constant ionic force by Recillas et al. (2012) found that at pH = 6 there is no precipitation, and at a pH higher than 7.4 it is feasible that HAP and TCP will precipitate. One must recall the following facts: $CaCO_3$ was unstable under acidic pH conditions, but reaction with phosphoric acid produces poorly soluble monetite coatings which block further reaction. Furthermore, HAP was formed at basic pH levels, where $CaCO_3$ was stable and no reaction was expected unless the system was heated under pressure such as takes place in the hydrothermal process. At ambient pressure, hydrogenphosphate groups were first absorbed on the carbonate surface, and then were deprotonated to form calcium orthophosphate, releasing the carbonate ions into the solution (Verwilghen et al., 2009).



H_3PO_4 $NH_4H_2PO_4$



$(NH_4)_2 HPO_4$

Figure 7 SEM analysis of synthetic HAP using H_3PO_4, $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$ as orthophosphate sources reacted with the digested $CaCO_3$ at pH 9 for 3 h.

The synthetic HAP using H_3PO_4, $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$ as orthophosphate sources reacted with the digested $CaCO_3$ at pH 9 obtained after 3 hours of reaction was analyzed by SEM. Figure 7 depicted a SEM micrograph of HAP powder, that's morphology was characterized by agglomerated nanoparticles with sizes smaller than 1 μm. The shapes of which were almost the same and the size was always between 0.5μm and 2 μm, built up from fine particles about 100-300 nm in size. Individual fine particles with spherical and semi-spherical shapes were observed. The agglomerates were irregular ovular shapes and spherical shapes.

Conclusion

In this study, $CaCO_3$ was digested before co-precipitation with the various phosphorus sources (H_3PO_4, $NH_4H_2PO_4$, and $(NH_4)_2HPO_4$). This new synthetic pathway produced HAP without the creation of CO_3^{2-}. The Ca^{2+} ions released by the digestion of $CaCO_3$ were then used as reactants in the formation of HAP. It is noteworthy that Ca^{2+} ions barely appear in the HAP structure. The conclusion of the experiment with prevented the presence of CO_3^{2-} in HAP. The effect of the reagents, such as H_3PO_4, $NH_4H_2PO_4$, and $(NH_4)_2HPO_4$, indicated that all of the phosphates could be used successfully in the production of HAP. The results of testing various pH levels indicate that a pH of 8-12 is required in the successful production of HAP using the digested $CaCO_3$.

Acknowledgement

The authors wish to thank Thepsatri Rajabhat for provision of the laboratory facilities which enabled this work to be carried out and also Bureau of the Budget for financial support.

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