Preparation of hydroxyapatite by aqueous precipitation from calcium carbonate and phosphoric acid

Byeong Woo Lee
Department of Ocean Advanced Materials Convergence Engineering, Korea Maritime and Ocean University, Busan, Korea

E-mail: bwlee@kmou.ac.kr

Abstract. Hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, HAp) is the representative biological mineral which is hard to attain the solution equilibrium and difficult to produce by solution synthesis. HAp finds new application fields to remove harmful elements from polluted wastewater in industrial scales. The low-cost manufacturing process should be developed using abundant available CaCO$_3$ materials. Calcium carbonate (CaCO$_3$), as an economically feasible and abundant precursor, has been used in calcium source for the HAp synthesis. The synthesis was conducted by reacting CaCO$_3$ with phosphoric acid (H$_3$PO$_4$) in aqueous solution with aid of citric acid and sodium hydroxide under moderate synthesis conditions. Compositionally pure HAp has been prepared. The process offered a relatively fast, convenient and economical route for the synthesis of HAp.

1. Introduction

Calcium phosphates have proved attractive for biological applications. Among the calcium phosphates, significant attention has been given to hydroxyapatite (HAp, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) due to its prominent bioactivity and biocompatibility. HAp is quite close to inorganic part of a human bone and has been widely utilized in the biomedical field, as implant and restoration materials, and in drug delivery systems [1,2]. Since HAp is a multifunctional material, HAp finds new applications including the fields of heterogeneous catalysis and toxic material fixation with use of improved adsorption or catalytic decomposition activity against heavy metals, bacteria, viruses and other biohazardous substances [3-6]. HAp or chemically modified HAp are host to many substitutions by cations, anions and anionic radicals that resemble its original constituents in size and charge. This material stabilizes metals by chemically binding them into new stable phosphate phases and other relatively insoluble phases [6]. For example, minor to major amounts of Cd, Co, Cu, Sr, Hg, Fe, Mn, Pb, and U are known to replace calcium. However, it is important to develop a low-cost synthesis procedure for implying HAp at industrial scale for the waste water treatment. Mass production of HAp with using various kinds of economical biogenic sources such as seashells and eggshells is necessary. These shells are composed of 95% calcium carbonate (CaCO$_3$), and the remainder is thermally decomposable organic matter and other compounds [7]. CaCO$_3$ is abundantly available in nature as the cheapest calcium source. Various processes have been developed for the synthesis of HAp using CaCO$_3$ from low-cost starting materials.

In the present study, CaCO$_3$ has been used as a calcium precursor by assuming that the abundant biogenic materials were used.
Various wet chemical techniques were developed for the synthesis of HAp using different calcium and phosphorus containing starting materials. Chemical precipitation from aqueous solution is the most common synthesis route to prepare HAp and the process offers advantageous features such as a simple, environmentally friendly and low-cost synthesis [8-12]. Using the precipitation process, relatively large amount of HAp can be produced without expensive and sometimes harmful organic solvents.

In order to reduce or eliminate the generation of byproducts, a wet chemical process using calcium hydroxide and phosphoric acid as starting materials was developed [8, 9].

\[ 10 \text{Ca(OH)}_2 + 6 \text{H}_3\text{PO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 8 \text{H}_2\text{O} \]  \hspace{1cm} (1)

According to Eq. (1), the use of Ca(OH)\(_2\) and H\(_3\)PO\(_4\) can generate HAp avoiding the generation of byproducts except water (H\(_2\)O). Since Ca(OH)\(_2\) is obtained from CaCO\(_3\) by heating and hydration steps, Ca(OH)\(_2\) costs more than CaCO\(_3\) [10]. In this view, the use of CaCO\(_3\) and H\(_3\)PO\(_4\) as starting reactants for the synthesis under moderate conditions is cost effective. The reaction byproduct is CO\(_2\) gas, which leaves the reaction mixture in gaseous form. The expected reaction is as follows:

\[ 10 \text{CaCO}_3 + 6 \text{H}_3\text{PO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 10 \text{CO}_2 + 8 \text{H}_2\text{O} \]  \hspace{1cm} (2)

In this study, the one-step synthesis of HAp from aqueous solutions without Ca(OH)\(_2\) production process has been proposed based on the Eq. (2). Calcium carbonate (CaCO\(_3\)) has been used in HAp synthesis via simple precipitation conditions. Previously, several studies have been employed to produce HAp from calcium carbonate sources using phosphate solutions [11, 12]. However, attaining the solution equilibrium is very difficult to achieve. The reaction times to form single phase HAp was too long from tens of hours up to days, which makes them unfeasible for the economical production. In the present study, the effects of additional reactants on the phase formation of HAp from CaCO\(_3\)-H\(_3\)PO\(_4\) via aqueous precipitation was investigated. Unlike other precipitation processes with a long, time-consuming process, citric acid was used to resolve some unreacted calcium carbonate to increase the reaction speed in mixed solution and pH was controlled to above 6 using NaOH to form HAp phase conveniently.

2. Materials and methods

| Sample | Reaction Composition | Reaction (Solution) pH |
|--------|----------------------|------------------------|
| Hap-0  | 0.5 CaCO\(_3\) + 0.3 H\(_3\)PO\(_4\) | 6.2                    |
| Hap-1  | 0.5 CaCO\(_3\) + 0.3 H\(_3\)PO\(_4\) + NaOH | 9.0                    |
| Hap-2  | 0.5 CaCO\(_3\) + 0.3 H\(_3\)PO\(_4\) + Citric acid (0.15 mole) + NaOH (→ lower pH) | 6.7                    |
| Hap-3  | 0.5 CaCO\(_3\) + 0.3 H\(_3\)PO\(_4\) + Citric acid (0.15 mole) + NaOH (→ higher pH) | 8.9                    |

In the study, experiments were done using distilled water as solvent at room temperature. HAp (Ca/P=1.67) nanoparticles were prepared by a one-step wet precipitation route under controlled synthesis conditions. Ca/P molar ratio, 1.67, of initial reagents was adjusted in order to synthesis HAp powders. Calcium carbonate powder (98%, Junsei) and orthophosphoric acid (85 wt% in water, Junsei) were used as received. Citric acid (99%, C\(_6\)H\(_8\)O\(_7\)·H\(_2\)O, Junsei) was used as an additional chemical to resolve some remaining CaCO\(_3\). Reaction pH was controlled by adding sodium hydroxide (97%, NaOH, Dae Jung). Undiluted H\(_3\)PO\(_4\) (0.3 mole) was added dropwise onto a slurry containing CaCO\(_3\) (0.5 mole, 50 g in 200 ml water (2.5 M)) in 20 min and, subsequently, citric acid (0.15 mole) and sodium hydroxide were added. Then the mixtures were ball milled for 6 h at 250 revolutions per minute. Table 1 shows the prepared samples from different reactants and reaction pH. The resultant precipitates were washed and filtered with distilled water and dried at 60°C. Unlike the other
preparative processes for the hydroxyapatite, no long maturing process was involved. The overall precipitation process is shown in Figure 1.

The prepared powders were calcined at different temperatures ranging from 500°C to 900°C to study phase developments. The properties for the powders were characterized using X-ray diffraction (XRD, Cu-Kα radiation, λ = 1.5418 Å). The particle sizes of the synthesized powders were also calculated by the XRD peak broadening according to the Scherrer’s equation (Dcal (particle size) = 0.89 λ/(B cos θ): where λ is the wavelength, B is the full width at half maxima (FWHM) in radians and θ is the diffraction angle).

![Figure 1. HAp powder synthesis procedure by precipitation from CaCO₃-H₃PO₄ with citric acid and sodium hydroxide.](image)

3. Results and discussion

In the present study, HAp (Ca/P=1.67) powders were prepared by a new one-step approach of the aqueous precipitation method with use of citric acid and sodium hydroxide. Without citric acid and sodium hydroxide, the autogenerated reaction pH was as low as 6.2 and the prepared powder has been shown with various phases such as unreacted calcium carbonate, calcium hydroxide and DCPD (Dicalcium phosphate dehydrate, Ca/P=1, CaHPO₄·2H₂O) as shown in Figure 2. DCPD is prone to be produced in low pH condition < pH ~6 in calcium phosphate aqueous chemistry [13]. As heat-teat temperature increase from 500°C to 900°C, desirable HAp phase was formed, but the impurities of β-TCP and free CaO were also found. Insufficient HAp synthesis condition led to formation of detectable β-TCP and free CaO according to the Eq. (3).

\[
\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \text{ (HAp)} \rightarrow 3 \text{Ca}_3(\text{PO}_4)_2 (\beta\text{-TCP }) + \text{CaO} + \text{H}_2\text{O} \quad (3)
\]

The effect of both citric acid and sodium hydroxide on the preparation of phase pure HAp is presented in Figure 3. When the powder was prepared without citric acid, a large amount of undesirable calcium carbonate which is unreacted was also found with HAp peaks (HAp-1). Single phase HAp was difficult to obtain even at relatively higher pH 9.0 without the aid of citric acid. In the case of HAp-2, 0.15 mole of citric acid was added to HAp-1 composition to resolve unreacted calcium carbonate.
carbonate at relatively lower pH condition of 6.7. The low pH resulting from less amount of sodium hydroxide addition led to the extra DCPD phase formation as shown in Figure 1 for HAP-0. For HAP-3, however, a sufficient amount of sodium hydroxide was added to achieve pH 8.9, leading to the single HAP phase formation. In addition, the broad and weak HAP peaks indicate poor crystallinity and/or nanometer scale crystallites of the HAP powders synthesized. The prepared powders are approximately 20 nm in particulate size calculated by the Scherrer’s equation. The most distinct (002) reflections (near 2θ 26°) in the XRD patterns were taken into calculation.

Figure 2. X-ray diffraction patterns of as-prepared and heat-treated powders from CaCO₃-H₃PO₄ without citric acid and sodium hydroxide (autogenerated pH 6.2, HAP-0).

Figure 3. X-ray diffraction patterns of as-prepared powders from CaCO₃-H₃PO₄ under different reaction conditions with or without citric acid (HAP-1, HAP-2 and HAP-3).

Figure 4 shows the XRD patterns of the synthesized HAP powders heat-treated at 500°C and 900°C from the composition HAP-3 with both citric acid and sodium hydroxide at relatively higher pH 8.9. HAP was the only phase without any trace of impurity or secondary phases. These results confirmed that the starting reactants and pH played a significant role in obtaining pure HAP. The overall peaks of HAP become more distinct as the heat-treatment (calcination) temperature increases, and the narrow peak width at 900°C implies increased crystallinity and particulate size. The particulate size increased
from 25 to 70 nm when the calcination temperature increased from 500 to 900°C, respectively. Since the crystallinity and the crystallites size of HAp can be tailored by the calcination step, the prepared HAp having different powder properties can find also various applications. It has been demonstrated that amorphous or low crystallinity HAp crystals have a better biocompatibility or an enhanced activity of removing harmful elements rather than well crystallized HAp crystallites [14,15].

In most chemical precipitation processes for calcium phosphates, the powder properties depend heavily on the processing parameters during the precipitation reaction due to the slow reaction rate and the unstable nature of the precursor solution. The morphology, phase, and crystallinity of precipitated powders were closely related to precursor concentration, precursor dropping rate, reaction pH, and maturing (aging) time in solutions. For example, to prepare single HAp phase particles, normally very slow precursor dropping rates such as a few drops per minute and a very long aging time over 24 h were essential at low aqueous precursor concentrations of less than 0.6 M Ca²⁺ [16,17]. However, in this study, nanometer scale HAp powders were conveniently prepared in a fast and simple manner: faster precursor dropping rate at higher precursor concentration of 2.5 M Ca²⁺. Therefore, it can be proposed that the new one-step process in this study is economically feasible of HAp preparation in large quantities.

![Figure 4. X-ray diffraction patterns of as-prepared and heat-treated powders from CaCO₃-H₃PO₄ with citric acid and sodium hydroxide under higher pH 8.9 (HAp-3).](image)

### 4. Conclusions
Nanometer-sized HAp powders were conveniently prepared in a fast and simple manner from calcium carbonate and phosphoric acid with aid of additional reactants, citric acid and sodium hydroxide. The effect of both citric acid and sodium hydroxide on the preparation of phase pure HAp has been investigated. The pure HAp powders (HAp-3) were synthesized with citric acid (0.15 mole) and sodium hydroxide (pH 8.9). In the case of HAp-3, phase pure HAp without any noticeable secondary phase can be synthesized. It was also shown that, as the calcination temperature increased from 500°C to 900°C, the overall peaks of HAp become more distinct. The narrower peak width and higher peak height at 900°C suggest the increased crystallinity and particulate size. The new one-step approach of the aqueous precipitation method adopted in this study using calcium carbonate offered a relatively simple, convenient and economical route to synthesize HAp without long ripening time used in most wet precipitation studies.

### Acknowledgement
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (Ministry of Science and ICT) (No. 2017M2B2A9073125).
References

[1] Zhou H and Lee J 2011 Acta. Biomater. 7 2769 (https://doi.org/10.1016/j.actbio.2011.03.019)

[2] Orlovskii V, Komlev V and Barinov S 2002 Inorg. Mater. 38 973 (https://doi.org/10.1023/A:102058580572)

[3] Vukomanovic M, Zunic V, Otonicar M, Repnik U, Turk B, Skapin S and Suvorov D 2012 J. Mater. Chem. 22 10571 (https://doi.org/10.1039/C2JM00136E)

[4] Fihi A, Len C, Varma R S and Solhy A 2017 Coord. Chem. Rev. 347 48 (https://doi.org/10.1016/j.ccr.2017.06.009)

[5] Takagi O, Kuramoto N, Ozawa M and Suzuki S 2004 Ceram. Int. 30 139 (https://doi.org/10.1016/S0272-8842(03)00061-0)

[6] Jeanjean J, Rouchaud J C and Tran L 1995 J. Radioanal. Nucl. Chem. Lett. 201 529 (https://doi.org/10.1007/BF02162730)

[7] Hamester M R, Balzer P S and Becker D 2012 Mater. Res. 15(2) 204 (https://doi.org/10.1016/S1516-1439201200500014)

[8] Akao M, Aoki H and Kato K 1981 J. Mater. Sci. 16 809 (https://doi.org/10.1007/BF00552220)

[9] Abidi S and Murtaza Q 2014 J. Mater. Sci. Techno. 30 307 (https://doi.org/10.1016/j.jmst.2013.10.011)

[10] Cahyaningrum S E, Herdyastuty N, Devina B and Supangat D 2018 IOP Conf. Series: Mater. Sci. Engine. 299 012039 (https://doi.org/10.1088/1757-899X/299/1/012039)

[11] Minh D P, Rio S, Sharrock P, Sebei H, Lyczko N, Tran N D, Raii M and Nzihou A 2014 J. Mater. Sci. 49(12) 4261 (https://doi.org/10.1007/s10853-014-8121-7)

[12] Verwilghen C, Chkir M, Rio S, Nzihou A and Sharrock P 2009 Mater. Sci. Engin. C 29(3) 771 (https://doi.org/10.1016/j.msec.2008.07.007)

[13] Mansour S F, El-dek S I, Ahmed M A, Abd-Elwahab S M and Ahmed M K 2016 Appl. Nanosci. 6 991 (https://doi.org/10.1007/s13204-015-0509-4)

[14] Müller W E G, Tolba E, Schröder H C, Muñoz-Espi R, Diehl-Seifert B and Wang X 2016 Acta Biomater. 31 358 (https://doi.org/10.1016/j.actbio.2015.11.060)

[15] Vermeul V R, Szecsody J E, Fritz B G, Williams M D, Moore R C and Fruchter J S 2014 Groundwater Monitor. Remed. 1 (https://doi.org/10.1111/gwmr.12055)

[16] Sokolova M, Kreiebergs I, Zalite V and Berzina-Cimdinaa L 2012 Eurasia Conf. Chem. Sci. Proc. p 1

[17] Bang L T and Othman R 2014 Ceramics-Silikaty 58 157 (http://www.ceramics-silikaty.cz/2014/pdf/2014_02_157.pdf)