Synthesis of Hydroxyapatite using Precipitated Calcium Carbonate (PCC) from Limestones

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Abstract. Hydroxyapatite (HAp) is a material that widely applied in bone and teeth implant due to its biocompatibility and bioactivity. This material can be prepared from PCC by precipitation method using CaO and H₃PO₄ in ethanol. In this work, variations of phosphoric acid amount and aging time were investigated. The synthesized HAp was characterized by FT-IR, AAS, UV-Vis Spectrophotometer, PSA, SEM, and powder XRD. The results showed that the high concentration of calcium in PCC gives better yields in which PCC obtained from carbonation method has higher yield than that of caustic soda method. The determination of optimum phosphoric acid addition based on targeted Ca/P ratio (1.67) from HAp was obtained on the addition of 0.127 mol phosphoric acid with Ca/P ratio of 1.66. The aging time gave significant effect to the particle size of synthesized HAp. The smallest particle size was obtained in aging time for 48 hours as high as 49.25 µm. FTIR spectra of the synthesized HAp show the presence of hydroxyl (-OH) group at 3438.8 cm⁻¹, PO₄³⁻ at 557.39 and 1035.7 cm⁻¹, and CaO at 1413.72 cm⁻¹. The synthesized HAp forms agglomeration solid based on the SEM analysis. The powder XRD data shows three highest peaks at 2θ i.e. 27.8296; 31.1037; and 34.3578 which corresponds to β-TCP (tricalcium phosphate) in accordance with JCPDS no.09-0169. The characteristic 2θ peak of hydroxyapatite with low intensity is observed from the synthesized HAp refer to the JCPDS data no. 09-0432.

1. Introduction

Bone and teeth are the important role in the human’s body. The injury of their tissues may cause disabled structure and deficiency of body’s function. The efforts to repair the tissue’s structure can be conducted by addition and substitution of tissue so called implant [1,2]. The materials widely applied for bone and dental implant such as metal, ceramic, polymer, composite, and carbon [3].

Ceramic is one of material that capable of implant due to its high compatibility and inert. There are two type of ceramic, they are bioactive and non-reactive ceramic. The bioactive ceramic often utilized for implant is hydroxyapatite due to its high suitability in the body and bioactive [3]. Hydroxyapatite (HAp) is the mineral apatite (Ca₁₀(PO₄)₆(OH)₂) which have Ca/P ratio 1.67. 65% of its mineral fraction in the human’s bone comprised of hydroxyapatite [4]. This mineral can be applied for bone reconstruction and teeth implant [5].

The production of HAp requires sufficient high calcium source such as egg-shell [6], gypsum [2], blood clams [7], mammal’s bone, clamshells, and coral [8]. However, these materials are not easily found in the nature.
The calcium source in the huge amount can be obtained from limestone. Lailiyah, et.al[8] reported the natural limestone which located at Tuban Regency, East Java, Indonesia has the high purity and calcium fraction until 98%. To synthesize HAp, the limestone treated to be Precipitated Calcium Carbonate (PCC) by caustic soda and carbonation method. PCC is the derived product from limestone which re-carbonated by producing CaCO$_3$[9].

This paper reports the synthesis hydroxyapatite by wet-precipitation method using variation of Ca amount, phosphate amount, and aging time. The advantages of this method include easier manufacturing in low temperature [10], cost-effective, and simple [6]. Wahdah, et. al. had successfully synthesized HAp from cow-bone by wet-precipitation at 60°C in ethanol media and H$_3$PO$_4$ as phosphate source [11].

2. Materials and Methods
2.1. Materials and Measurements
The chemicals used in this work include ethanol (96%), H$_3$PO$_4$ (85%), ammonium molybdate tetrahydrate, ammonium metavanadate, HNO$_3$ (65%), HCl (37%), and distilled water. To calculate the Ca/P ratio, the P amount was determined by UV Vis spectrophotometry whereas the Ca amount was measured using atomic adsorption spectroscopy. FTIR, powder XRD and SEM were also used to characterized the synthesized HAp.

2.2. Preparation of Precipitated Calcium Carbonate (PCC)
Precipitated Calcium Carbonate (PCC) was obtained from caustic soda and carbonation method and both were calcined at 900°C for 20 mins, cooled down to room temperature and then grinded using mortar. In caustic soda method, the limestone is firstly calcined at high temperature into CaO and then added with water to form Ca(OH)$_2$. The PCC was obtained by reacting the Ca(OH)$_2$ solution with sodium carbonate solution [12,13]. Unlike in caustic soda method, the PCC synthesized using carbonation method was obtained by adding CO$_2$ gas into the Ca(OH)$_2$ solution. The precipitated was formed when at pH 7 [14].

2.3. Synthesis of Hydroxyapatite (HAp)
2.3.1. Effect of Phosphate Amount
Two different solution are prepared in a beaker glass by dissolving 10 g of CaO in 50 ml ethanol (solution A) and phosphoric acid (0.0871; 0.1071; 0.1270 moles) with 50 ml ethanol (solution B), heating the solution might be needed to dissolve the mixture. Solution B is then inserted into an intravenous bottle to be passed through in a line into solution A. Solution B is added into solution A, then mixed and heated at 60°C. After solution B was run out, the mixture was continued to be heated at 60°C for 1 h. The mixture was then allowed to stand at room temperature for 24 hrs. After that, the mixture was stirred and heated again at 60°C for 30 min which eventually filtered off. The precipitate was dried in an oven at 100°C for 1 h, then heated in a furnace at 800°C for 5 hrs [11]. The dry solid that was obtained as HAp was weighed and characterized.

2.3.2. Effect of Aging Time
Procedure 2.2.1 was repeated using phosphoric acid amount (mol) that gives the highest yield and done in a variety of aging time (12 and 48 hrs). The synthesized HAp obtained from the highest yield in this procedure was characterised using FT-IR, powder-XRD, and SEM.

3. Result and Discussion
3.1. Preparation of PCC
PCC synthesized by caustic soda method has Ca amount of 31.5%, on the other hand, the PCC obtained from carbonation process has Ca amount of 34.1%. As presented in Table 1, the Ca amount influenced the yield. The highest yield was obtained by carbonation method which was 79.4%. This may due to higher Ca amount in the PCC from carbonation than in PCC from caustic soda method.
Based on this result, the PCC from carbonation was used as the starting material on the synthesis of hydroxyapatite. The hydroxyapatite was synthesized by mixing calcined CaO and phosphoric acid (H₃PO₄) as phosphate source in ethanol media. The synthesized HAp result was represented in Figure 1.

Table 1. Preparation of PCC

|                  | Caustic soda | Carbonation |
|------------------|--------------|-------------|
| Ca amount (%)    | 31.5         | 34.1        |
| HAp Mass (g)     | 13.7         | 14.2        |
| Yield (%)        | 76.4         | 79.4        |

Figure 1. HAp (calcined at 800°C) synthesized from PCC obtained using carbonation method

3.2 Effect of Phosphate Amount

Synthesis of hydroxyapatite from PCC was conducted by adding various mole of H₃PO₄ as the source of phosphate. This mol variation of phosphate was aimed to investigate the exact ratio of Ca/P and hydroxyapatite. Petit, et. al. reported that hydroxyapatite had Ca/P ratio as high as 1.67 [4]. The Ca/P ratio of synthesized HAp obtained from various amount of phosphate is given in Table 2.

Table 2. Ca/P ratio in various H₃PO₄ amount

| H₃PO₄ amount (mole) | Ca amount (%) | P amount (%) | Ca/P ratio |
|---------------------|---------------|--------------|------------|
| 0.0871              | 52.74         | 21.29        | 2.48       |
| 0.1071              | 42.67         | 23.27        | 1.83       |
| 0.1271              | 41.77         | 25.22        | 1.66       |

From several variations of H₃PO₄, the Ca/P ratio which has nearest value to 1.67 was obtained at 0.1271 mol. The increase of Ca/P ratio can enhance the strength of HAp. This H₃PO₄ mol that give the best Ca/P ratio was used in the aging time experiments.

3.3 Effect of Aging Time

The effect of aging time toward the particle size of HAp was conducted in various aging time of 12, 24, and 48 hours. The synthesized HAp was characterized by Particle Size Analyzer (PSA) to determine the particle distribution on each aging time. The particle size of synthesized HAp is shown in Table 3 and their particle distribution is displayed in Figure 2.

Based on the Table 3, it is found that the aging time influences the particle size of the hydroxyapatite. The smallest particle size was obtained at aging time 48 hours. Longer aging time may result in a second precipitation phase. The precipitate size was smoother in a lower temperature. However, it may cause roughing of particle which was the small particle tend to dissolved and the large particle getting bigger [15].
Table 3. Particle size of synthesized HAp in various aging time

| Aging time (hours) | Diameter (µm) 10% | Diameter (µm) 50% | Diameter (µm) 90% |
|--------------------|-------------------|-------------------|-------------------|
| 12                 | 15.36             | 63.53             | 117.41            |
| 24                 | 15.71             | 40.67             | 111.03            |
| 48                 | 11.93             | 27.48             | 49.25             |

Figure 2. Particle distribution of the synthesized HAp in various aging time (top = 12 h, middle = 24 h, bottom = 48 h).
3.4 Characterisation of the Synthesized HAp

The synthesized HAp was characterized with FTIR, SEM, and powder XRD. The IR spectra of both PCC and synthesized HAp is presented in Figure 3 for comparison within a range of 4000-450 cm$^{-1}$. The IR spectra of PCC indicates the carbonate group of CaCO$_3$ polymorph calcite at 2981, 2871, 2515, 1799, 1421, 875, and 711 cm$^{-1}$. Moreover, the polymorph vaterite appeared at 2362 cm$^{-1}$. The FT-IR spectra of synthesized HAp in various aging time showed the stretching vibration of PO$_4^{3-}$ at 1031.63 and 972.06 cm$^{-1}$. The band at 3452.34 cm$^{-1}$ assigned to the hydroxyl (-OH) group whereas the band at 1413.72 cm$^{-1}$ is correspond to Ca-O. The comparison between FTIR spectra of the synthesized HAp and the standard and the reference hydroxyapatite is presented in Table 4. The synthesized HAp shows some similarities with the standard and reference HAp spectra including the presence of PO$_4^{3-}$, CaO, and OH bands.

![Figure 3. FTIR spectra of PCC (left) and synthesized HAp (right) in various aging time (black = 12 hrs, red = 24 hrs, blue 48 hrs).](image)

**Table 4.** Comparison of FT-IR spectra between the synthesized HAp with the standard and the reference HAp

| Functional Groups | Wavenumber (cm$^{-1}$) | 12 h | 24 h | 48 h | Standard HAp | Reference HAp [16] |
|-------------------|------------------------|------|------|------|--------------|-------------------|
| PO$_4^{3-}$       |                        | 611.4| 611.4| 611.4| -            | -                 |
|                   |                        | -    | -    | -    | 960.5        | 960               |
|                   |                        | 975.9| 972.1| 974.0| 1035.7       | 1035.7            |
|                   |                        | 1041.5| 1400.2| 1035.7| 1035.7       | 1000-1150         |
|                   |                        | 1118.6| 1647.2| 1128.3| 1128.3       | 1639.5            |
|                   |                        | 1417.6| 1400.2| 1413.7| 1413.7       | 1400-1700         |
|                   |                        | 1635.5| 1647.1| 1510.2| 1510.2       | 1510.2            |
|                   |                        | 1645.2| 1645.2|     |              |                   |
| CaO               |                        | 3492.9| 3483.2| 3452.3| 3438.8       | -                 |
| OH                |                        |      |      |      |              |                   |

Characterization of PCC and the synthesized HAp using SEM was conducted to study and to compare the size and morphology before and after the synthesis. The SEM images of PCC and synthesized HAp is shown in Figure 4. The PCC has a dominant cubic structure and known to be in the calcite polymorph [17]. In contrast, the synthesized hydroxyapatite is agglomerated in a smooth morphology, which is similar to that of reported by Hui in 2010 [18].

In order to investigate the crystallinity, the synthesized HAp was analyzed using powder-XRD which then compare it with the JCPDS data based on the 20 value. The powder spectra of PCC and the synthesized HAp is presented in Figure 5. The powder XRD pattern of PCC has 5 identical 20 peaks with the JCPDS data of CaCO$_3$ no. 05-0586 which correspond to calcite polymorph. Unlike the PCC,
the powder XRD pattern of the synthesized HAp has different characteristic 2θ peaks compared to the JCPDS data of HAp no 09-0432. Instead, it has close similarity to the JCPDS of β-TCP (tricalcium phosphate). There was, however, 2θ peaks that correspond to the hydroxyapatite in lower intensity than in the JCPDS data of HAp which indicate the presence of HAp in a small amount. This result reveals that the bulk sample of the synthesized HAp is not pure and contains β-TCP hence intensive purification is needed for further investigation. The impurity occurred may be due to the solution pH was not suitable for synthesis of HAp, in which in this work the pH effect was not investigated yet. According to Kannan, et.al. [19], the pure HAp can be synthesized at pH 10. At pH 9, it may produce the mixture of β-TCP and HAp, whereas in pH 8, only β-TCP is obtained.

4. Conclusion

Synthesis of HAp was conducted from PCC as starting material which obtained from limestone by precipitation method. The high concentration of calcium in PCC gives better yields in which PCC obtained from carbonation method has higher yield than that of caustic soda method. The determination of optimum phosphoric acid addition based on targeted Ca/P ratio (1.67) from HAp was obtained on the addition of 0.1271 mol phosphoric acid with Ca/P ratio of 1.66. The aging time gave significant effect to the particle size of synthesised HAp. The smallest particle size was obtained in aging time for 48 hours as high as 49.25 µm. However, the synthesized HAp obtained using this method was agglomerated in a smooth morphology and based on powder XRD pattern, the product not pure and contained the mixture of HAp and β-TCP.
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