Synthesis of hydroxyapatite nanoparticles from egg shells by sol-gel method

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Abstract. Hydroxyapatite, [Ca10(PO4)6(OH)2] (HAp) is widely used in medical fields especially as a bone and teeth substitute. Hydroxyapatite nanoparticles have been successfully synthesized from egg shells as a source of calcium by using sol-gel method. The egg shells were calcined, hydrated (slaking) and undergone carbonation to form Precipitated Calcium Carbonate (PCC). Then the PCC was added (NH4)2HPO4 to form HAp with variation the mole ratio Ca and P (1.57 ; 1.67 and 1.77), aging time (24, 48, and 72 hr) and under basic condition pH (9, 10 and 11). The formation of hydroxyapatite biomaterial was characterized using XRD, FTIR, SEM-EDX. The XRD patterns showed that the products were hydroxyapatite crystals. The best result was obtained at 24 hr aging time, pH 9 with hexagonal structure of hydroxyapatite. Particle size of HAp was 35-54 nm and the morphology of hydroxyapatite observed using SEM, it showed that the uniform crystal of hydroxyapatite.

1. Introduction
Hydroxyapatite, Ca10(PO4)6(OH)2, is a compound with hexagonal structure and white solids. Hydroxyapatite is known as one of the ingredients with bioactive, biocompatible and osteoconductive properties that chemically resemble for bone and also for tooth mineral components [1, 2]. Therefore, hydroxyapatite is widely used in the field of biomedical as filler and coating on bone and dental implants. Hydroxyapatite is also widely used as a catalyst and adsorbent because their structure is porous and also has heat resistance [2, 4].

Synthetic hydroxyapatite can be prepared using basic ingredients of calcium from chemicals such as Ca(NO3)2 [4], Ca(OH)2 [5], CaCO3 or from natural-made materials such as limestone [6] and bioanorganic materials such as bone [7, 8], cockle shells[2] shellfish [9,13], coral or egg shell [5, 6, 14, 16]. In this study the synthesis of hydroxyapatite was done using the eggshell as base material. The selection of egg shell as a source of calcium is due to abundant availability of egg shells in Indonesia.

In the previous work, we have synthesized hydroxyapatite from the cockle shells’ PCC using hydrothermal method [2]. Precipitated Calcium Carbonate (PCC) is a calcium carbonate compound (CaCO3) that can be processed from the material through a series of chemical reactions. PCC particles’ is homogeneous in the same size with micro-scale particle [10,11]. The common methods used to produce synthetic nanoparticle HAp including the precipitation [18], hydrothermal [2,16], mechanochemical [17] and sol-gel [8,12]. Sol-gel method offers some advantage in the molecular-level of the calcium mixing andphosphorusprecursors,itiscapable to improve the results of the chemical homogeneity HAp to a significant extent, rather than conventional techniques [8].

Agrawal et al. [8] used the sol–gel process in Hap synthesized with Ca(NO3)2.4H2O as calcium source and P2O5 as phosphate sources. Ca/P ratio of 1.67 with the aging time variation of 10-15 hr. Anuar et al.
performed hydroxyapatite preparation using sol gel method of Ca(NO$_3$)$_2$, 4H$_2$O as a source of calcium and P$_2$O$_5$ as a phosphate source with a Ca/P mole ratio of 1.67. some variations used are made with the speed of 100-500 rpm and temperature of 600-800 °C. In the present work, the synthesis of a nanoparticle HAp powder via sol-gel method was prepared using the egg shells’ PCC. To the best our knowledge, there are no reports about the synthesis HAp from the egg shells’ PCC using sol-gel method.

2. Materials and methods

2.1 Materials preparation

The egg shells was collected from Pekanbaru, Riau; diammonium hydrogen phosphate (NH$_4$)$_2$HPO$_4$, Merck); 2M HNO$_3$; ammonium hydroxide (NH$_4$OH) 65% (Merck); CO$_2$ and aquadest. The egg shells samples were washed and cleaned, then sun dried for two days followed by drying oven at 110°C for 2 hr. Dried and cleaned egg shells were then crushed and grounded with blender into egg shells powder form.

![Figure 1. Chicken egg shells.](image)

2.2 The formation of precipitated calcium carbonate (PCC)

Azis et al. [2] reported about the procedure for PCC formation from eggshells using the carbonation modification method. The PCC results are then used as the raw material for the synthesis of hydroxyapatite using the sol-gel method. PCC has a low solubility in water, so in this study PCC was dissolved using 0.3 M HNO$_3$. The formation of PCC can be represented by the following reactions [10, 11]:

**Calcination:**

\[
2\text{CaCO}_3 + \text{Heat} \rightarrow 2\text{CaO} + 2\text{CO}_2
\]

**Hydration:**

\[
\text{CaO} + 2\text{HNO}_3 \rightarrow 2\text{Ca(NO}_3)_2 + \text{H}_2\text{O}
\]

\[
\text{Ca(NO}_3)_2 + 2\text{NH}_4\text{O}_3 \rightarrow \text{Ca(OH)}_2 + 2\text{NH}_4\text{NO}_3
\]

**Precipitation:**

\[
\text{Ca(OH)}_2 + \text{CO}_2 \rightarrow \text{CaCO}_3 + \text{H}_2\text{O}
\]

2.3 Synthesis of hydroxyapatite

Vijayalaksmi and Rajeswari [19] procedures were used for synthesis of hydroxyapatite by sol-gel method. The egg shells’ PCC and (NH$_4$)$_2$HPO$_4$ were mixed by varying aging time (24, 48, 72 h), mol ratio of Ca and P (1.57; 1.67 and 1.77) and pH (9, 10, 11). PCC was dissolved in 0.3M HNO$_3$, while (NH$_4$)$_2$HPO$_4$ was dissolved with aquadest. Furthermore, PCC solution and (NH$_4$)$_2$HPO$_4$ solution were mixed in beaker glass. The solution was stirred for 3 h at a stirring speed of 300 rpm until the gel formed. Then, the aging process was done according to the adjusted variable. After the aging process, the formed gel is dried in an oven at 80 °C for 24 hr. The obtained solids were cleanly washed by distilled water to separate the hydroxyapatite from the rest of the reactants and dried out for 3 h in the oven. Finally, the obtained solids were calcinated for 1 hour at 500 °C.
The chemical reaction is shown as follow:

\[
10\text{Ca(NO}_3\text{)}_2\cdot 4\text{H}_2\text{O} + 6(\text{NH}_4\text{)}_2\text{HPO}_4 + 8 \text{NH}_2\text{OH} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 20\text{NH}_4\text{NO}_3 + 20 \text{H}_2\text{O}
\]

2.4 PCC and HAp characterization

The mineralogy of PCC and HAp were characterized using X-ray diffraction (X’Pert Powder DY 3688) with Cu Kα radiation. The surface morphology was probed using scanning electron microscopy (SEM) linked to energy dispersive X-ray micro analysis (EDX) (JEOL JED 2300). The Fourier Transform Infrared Spectroscopy (FTIR, Perkin Elmer Spectrometer Frontier) was used to analysis bonding structure in the samples. The surface area of the hydroxyapatite were characterized using BET measurements, Surface Area Analyzer (SAA, Quantachrome NovaWin2).

3. Results and discussion

The synthesis of Precipitated Calcium Carbonate (PCC) was acquired through the modification of the carbonation method. The comparison of XRD pattern among of obtained PCC and the standard calcium carbonate (ICDD – 01-083-1762 (rhombohedral calcite)) are shown in Figure 2 (a-b). The characteristic peak with the highest intensity of the obtained PCC (Figure 2a) matches with the pattern of calcium carbonate standard and the pattern also clearly shows the PCC is the only phase.

![Figure 2. XRD diffractograms of product: (a) PCC of egg shells, (b) calcite, Calcium Carbonate standard (ICDD 01-083-1762).](image)

At the synthesis stage of HAp from PCC egg shell by sol-gel method, a very similar pattern of FTIR spectra was obtained for all synthesized compounds and show PO$_4^{3-}$ groups at 1025-1029 cm$^{-1}$ and no ammonia or NH$_3$ group uptake at 3500 cm$^{-1}$ wave numbers. The result of characterization with FTIR spectroscopy varied mole ratio Ca/P (1.57; 1.67; 1.77), pH 9 with aging time 24 h rare depicted in Figure 3.
Figure 3. FTIR spectrum of hydroxyapatite at varied mole ratio Ca/P (1.57; 1.67; 1.77), pH 9, aging time 24 hr.

X-ray diffraction analysis of synthetic hydroxyapatite showed a spectral pattern similar to the standard hydroxyapatite spectrum of ICDD 01-089 at 2θ value: 25.8°, 31.9°, 32.2°, 32.9°, 33.96°, 39.74°. Figure 4. shows some hydroxyapatite diffractogram of the synthesis result at 24 hr aging time, compared to ICDD standard.
Hydroxyapatite made with the ratio of reactant of Ca / P 1.77 aging time of 24 h (Figure 4c) produces X-ray diffraction patterns very similar to the ICDD standard (Fig.4d) and there are no other crystalline phases are detected other than phase. Analysis using SEM-EDX was performed to find out the hydroxyapatite morphology of the synthesis. The results of characterization shown that the hydroxyapatite morphology in the form of agglomerates, as shown in Figure 5.

![Figure 5. SEM image of hydroxyapatite compound of egg shells’ PCC at varied molar ratio Ca/P at aging time 24 h (a)1.77 (b)1.67](image)

From the surface area analysis with BET, the hydroxyapatite surface area is about 8.968 m²/g, at 24 hr aging time and the mole ratio of Ca / P is 1.77. The mean grain size of the crystallites of HA particles was obtained using the Scherrer’s equation [20], with calculated crystal size of 35-54 nm.

4. Conclusion

Hydroxyapatite nanoparticle was successfully synthesized from egg shell via formation Precipitated Calcium Carbonate (PCC) followed with sol-gel process. The results from the analysis of compound proved that hydroxyapatite synthesis of egg shells through the formation of PCC produced the excellent result. Synthesis HAp at 24 h aging time and mole ratio of Ca/P is 1.77 achieved hydroxyapatite with hexagonal crystal structures. Furthermore, the X-ray diffraction patterns and characterization with FTIR are matched with standard HAp. The dried Hap particles powder was very pure with the specific surface area of 8.968 m²/g.

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