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Synthesis and characterization of calcium precursor for hydroxyapatite synthesis from blood clam shell (*Anadara antiquata*) using planetary ball mill process

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Abstract. Calcium precursor for synthesizing of hydroxyapatite can be obtained from natural material like *A. antiquata* (blood clamshell). Calcium synthesis was carried out through ball mill and calcinations process with high purity residues. Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Analysis (EDX), X-Ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR) were used for characterization. Analysis for blood clam shell powders revealed a reduction of average size from 217 μ m to 76 μ m and size of powder crystal is 29,2nm. This powders contained calcium element about 49,67% as the effectiveness of calcination and ball mill process. In morphology, powders have fine needles-like shape but still in agglomerate that can be reduced with extended ball mill process. This powders had a reduction of weight powders from 35,5% to 17,7% and dominated Ca(OH)₂ that could be used as calcium precursor in synthesishydroxyapatite.

Keywords: calcium, hydroxyapatite, blood clamshell, ball mill

1. Introduction

Hydroxyapatite (HA) is the calcium-phosphate material used to coated for an orthopedic metal implant or bone replacement material which had similarity with bone tissue constituent in the form of apatite calcium nanocrystal sized 10-60 nm in length and 2-6 nm in width [1]. HA characteristic depends on the techniques and calcium precursor sources[4] to produce phase-pure and thermal-stable HA[5]. Calcium precursor provided in living thing materials such as egg shells [6]; fish scales, corals, snail shells and Moluccas shells [7]. One of the potential calcium sources is blood clamshell (*Anadara antiquata*) that lived in mud area like mangrove. Giri et al. [8] reported Indonesia has mangrove area approximately 3,112,989 ha (22,6% of globalarea).



Synthesize hydroxyapatite made out of from various calcium sources such as calcium carbonate (CaCO_3)[6], calcium oxide (CaO)[2], calcium hydroxide ($\text{Ca}(\text{OH})_2$)[9-11], calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), and calcium chloride (CaCl_2) [11]. Ball mill as a mechanochemical method might be done to extraction coarse contained calcium precursor[13] In this research, synthesis of calcium precurs or was carried out with ball mill process combine with the calcination process to obtain nanocrystal of calcium precursors.

2. Experimental

2.1. Preparation of Blood Clam Shell Powders

Blood clam shell (*A. antiquata*) was collected from Pantai Air Manis, Padang, West Sumatra. First, clam shells cleaned, steamed for 30 minutes, air dried and crushed manually with hammers to obtain coarse powders. Furthermore, ball mill (Pulverisette 6 Classic Line Fritsch Planetary Mono Mill) process was conducted with 200 rpm for 15 minutes, four times replications and uses the agate-like ball. The powders were sieved with the vibrating-sieve machine (Retsch) based on standard ASTM E11 with the number of sieve 35, 60, 120 and 230 for 10 minutes and used amplitude 80. Calcination process conducted with oven/stove (Nabertherm P320) until temperature 800°C for 10 hours. The final treatment, hydrochloric acid treatment, was done to acquire high castability of nanocrystalline (1-100 nm). Characterization and measurement were did for dried $\text{Ca}(\text{OH})_2$.

2.2. Analysis

Characterization conducted for four step resultants including first ball mill (phase 1 powders), first calcination (phase 2 powders), a second ball mill (phase 3 powders) and second calcination (phase 4 powders). Morphology assessment and size distribution assayed clam shell powders with SEM (Hitachi S 3400) 5 kV, objective aperture 4, work distance 5 mm and magnification 20000x. Chemical composition examination was carried out with EDX (Horiba) connected with SEM 15 kV, objective departure 2 and dead time 20-30%. Mineral, crystallography structure and crystal size assessment used to XRD (PANalyticalX'Pert Pro X-ray Diffractometer). Functional group examination in clam shell was investigated with Fourier Transform Infrared Spectroscopy or FTIR.

3. Results and Discussion

3.1 Powders size distribution and morphology

SEM assessment revealed the effect of ball mill and calcination process in the synthesis of calcium compound from the clamshell. The results yielded powders from ball mill following calcination process have the finest size and more uniform size distribution (Phase 4) than the previous powders. Ball mill may becaused reduction of powders size[14]. Figure1 displays morphology of clam shell powders from ball mill and calcination process. The resultant powders of ball mill process formed in agglomerate that contain protein and unnecessary organic compound for synthesis hydroxyapatite. The attractive force was formed for finer powders in agglomerates. Calcination 800°C process could be removed organic compound and increased cristanility as the effect of high temperature [15] so clarified grain boundary. Material cristanility will affect biological response when implantation. Wang *et al.*[16] reported hydroxyapatite with high cristanility resulted in reduction solubility rate that inhibited bone formation. Agglomeration caused by calcination process and also discovered by Wu *et al.*[12] in synthesis hydroxyapatite from oyster shell *Crassostrea gigas* the effect of sintering from high-temperature calcination 1000°C . Particle agglomeration will seem vivid because of high-temperature sintering. Nasiri-Tabrizi *et al.*[14] reported ball mill process effectively decreased agglomerate formation, thus may reduction crystal size continuous followed by milling time additional through collision effect and increasing the lattice strain. The parameter of ball mill process has a pivotal role in the result of powders.

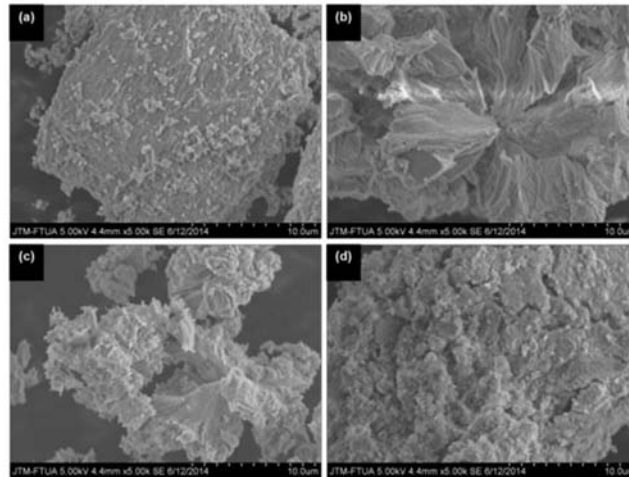


Figure 1. The clam shell morphology resulted with SEM in magnification 5000x. (a) phase 1 powders (lump and coarse surface texture), (b) phase 2 powders (sheet and agglomerate), (c) phase 3 powders (needle), and (d) phase 4 powders (agglomerate and crack)

Grain size arrangement in clamshell powders influenced by a ball mill and calcination process. The rough powder from the first step had grain size of 217 μ m. Calcination process effect agglomeration with resulted increasing of powders size of 340 μ m and 100 μ m corresponded to phase 2 and 3 powders respectively. When ball mill applied to powders, reduction grain size occurred in phase 4 powders become finer in size of 76 μ m. Its means, ball mill effectiveness to reduce of powder size. The final step of hydrochloric acid treatment showed clam shell powders had nanoparticle 100-1000 nm. However, still discovered fine particle (100-2500 nm) and formed agglomerate as resultant of calcination process. Figure 1 shows irregular particle with unevenly distributed particle size. Similar condition informed by Ho *et al.*[2] in egg shell. Weight measurement revealed 35,5% and 17% of lost weight based on the result of first and second calcination respectively. This is effective of calcination in reduction organic compound within powders.

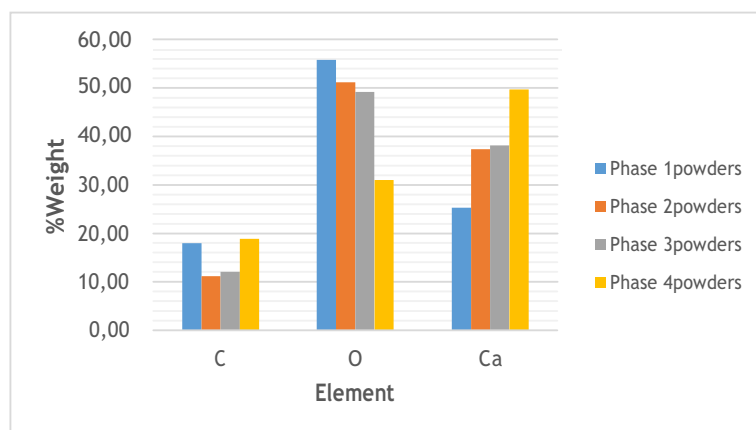


Figure 2.Weight percentage chart for a contained element in clam shell powders; phase 1 powders, phase 2 powders, phase 3 powders, and phase 4 powders.

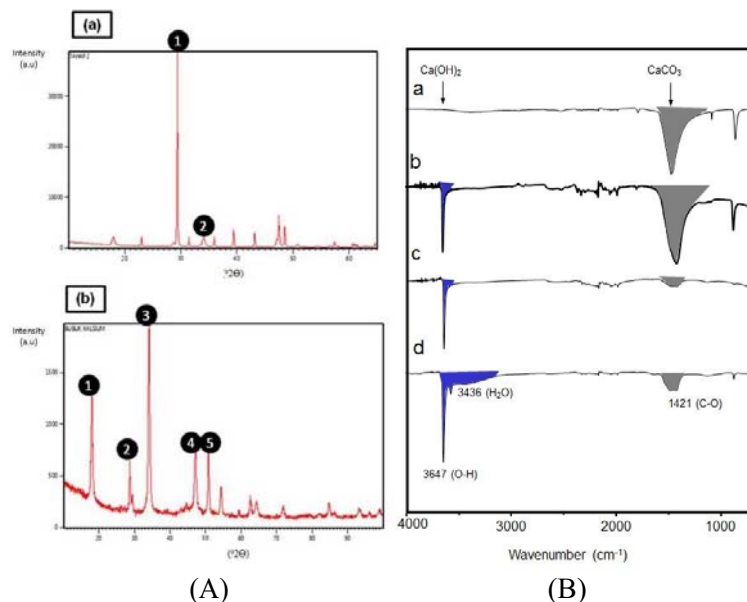


Figure 3. (A) Diffractogram from clamshell powders by XRD at; (a) first calcination and (b) second calcination; and (B) Spectrum of assessment result from FTIR; (a) phase 1 powders, (b) phase 2 powders, (c) phase 3 powders, (d) phase 4 powder

3.2 The chemical composition of powders

Analysis of weight percentage curve revealed contained an element in clamshell powders, and ball mill process is effectiveness heating in carbon (C), oxygen (O) and calcium (Ca). Figure 2 yielded changing element percentage value of C and O indicated decreased organic compound contents. However, increased C element percentage may occur in the last step due to C element contents in carbon tape kept at holder because of finer powders. Increased of Ca element from 25,30% to 49,67% shows the effectiveness of the calcination process to results clam shell powders with high calcium contents as precursor hydroxyapatite. Previous research revealed calcium contents in crabs and eggshell is 37,96% and 37,6% in respectively[17,18].

3.3 Crystal of powders

The crystallographic identification of phases of the sample by XRD incorporated in the database in *International Centre for Diffraction Data (ICDD)*. Calcination process effects decreased powders crystal size from 68,8 nm (calcination I) to 29,2 nm (calcination II) as shown in table 1. These combination process with ball mill has been removed organic compound in powders and decreased powders size. Akramet al. [5] reported calcination process is conducted to remove the organic component and to eliminate the pathogen. Mandal et al. [13] reported the most efficient milling to obtain the finest crystal on nanometer-size depend on decreased ball size, increased number of ball, and critical speed (60%).

Table 1. Comparison of resulted data XRD from clam shell powders in vary calcination process

| Comprise Parameters | Calcination I Process | Calcination II Process |
|------------------------|--------------------------------|--------------------------------|
| Compound | <i>Calcium Carbonate</i> (*82) | <i>Calcium Hydroxide</i> (*90) |
| Crystal Size[τ] | 68,8 nm | 29,2 nm |

*Score in *list* (confidence rate)

Figure 3(A) shows there was found the highest intensity peaks of CaCO_3 at 2θ angle of $29,39^\circ$ corresponding to a Miller indices of (104) before calcination and after calcinations was observed the highest intensity peak of Ca(OH)_2 at 2θ angle of $34,13^\circ$ corresponding to a Miller indices of (101) [9]. Also, CaO contents were observed at low-intensity peak. Based on the XRD result, decomposition of CaCO_3 to Ca(OH)_2 has been done caused by high-temperature calcination at 800°C between ball mill process and removed organic compound within powders. It means there is the effectiveness of heating between of ball mill process in forming Ca(OH)_2 . These transformation has been reported at a temperature range between 700°C - 1000°C [6].

Ho *et al.*[2] found calcium compound as precursor hydroxyapatite from eggshell powders such as CaCO_3 (450°C) and CaO (900°C) contents. Khiri *et al.*[4] observed calcium precursor in the form high purity of CaO from shell powders of *A. granosa*. Goloshchapovet al. [10] observed the eggshell dominated consist of CaCO_3 and this decomposition is CaO at 900°C that can be used as calcium precursor in synthesizing of HA.

3.4 Functional Group of Powders

Functional group assessment of Ca(OH)_2 and CaCO_3 compound with FTIR in standard spectrum revealed the results in figure 3(B). The C-O functional group present with characteristic peaks at 1421 cm^{-1} which corresponded to the CaCO_3 while the O-H functional group present with characteristic peaks at 3647 cm^{-1} which corresponded to the Ca(OH)_2 . The characteristic band at 3436 cm^{-1} is related to the presence of H_2O in the last FTIR sample as hydrochloric acid treatment. The sharp peak of Ca(OH)_2 in the last sample appropriate with the XRD result in figure3(A).

4. Conclusions

Powders of blood clam shell by ball mill and calcinations had finer and uniform size with the final size of $76\ \mu\text{m}$ with high of calcium content with the percentage in 49,67% and the smallest of powders crystal size of 29,2nm. Reduction of powders size affected by collision during ball mill and heating of calcinations that removes the organic component. Ball mill and calcinations process in short time effectiveness in the synthesis of calcium precursor for hydroxyapatite in the form of Ca(OH)_2 based on demonstrated data by XRD and FTIR.

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