**Geloina coaxans** shell as calcium source on synthesis hydroxyapatite

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**Abstract.** *Geloina coaxans* shell (GCS) is one of mollusc shell mainly composed by calcium carbonate. In this work, calcium carbonate has been converted to calcium oxide by calcination at 1000°C for 12 hours. The calcined of *geloina coaxans* shell were treated with HNO₃ to produce Ca(NO₃)₂ as calcium source on synthesis hydroxyapatite. Orthophosphoric acid (H₃PO₄) was used as phosphate donor. Reaction of Ca/P has been done by precipitation method at molar ratio of precursors of 1.67 and pH adjusted at 10 using NH₄OH. The XRD result revealed that hydroxyapatite can be prepared at 3 M of HNO₃ and stirring time for 240 minutes. Specific band of hydroxyapatite such as PO₄ and OH observed using FTIR instrument. Analysis of crystal size using Schererr equation proved nanosize of powder hydroxyapatite can be produced.

1. **Introduction**

Hydroxyapatite is one of calcium phosphate salt which have many application in several fields. Similarity of hydroxyapatite structure with inorganic component of teeth and bone will be the reasons to produce hydroxyapatite. Properties of hydroxyapatite or [Ca₁₀(PO₄)₆(OH)₂] such as biocompatible, bioactive, osteoconductive and non toxic caused many applications in biomedics or ortopedics. Hydroxyapatite also can be used as catalyst [1,2] and adsorbent for removing the trace metal.

Hydroxyapatite have been produced by several sources of calcium carbonate. Usability of waste to obtain hydroxyapatite is become a way to reduce the enviromental problem. Waste kina (*Evechinus chloroticus*) shell converted to hydroxyapatite using pyrolisis method and produced hydroxyapatite with porous structure [3]. Hydroxyapatite also have been produced using *Mussel* shell with microwave method [4]. *Macoma balthica* was also a potensial material to prepare nano hydroxyapatite [5].

The objective of this work is to produce hydroxyapatite using waste of *Geloina coaxans* shell. *Geloina coaxans* shell (GCS) is one of mollusc shell with high calcium carbonate. It is potential to convert the hydroxyapatite material. *Geloina coaxans* shell as precursor is advantages due to low cost material. *Geloina coaxans* shell and H₃PO₄ are used as starting materials on synthesis hydroxyapatite.HNO₃ is used to produce Ca(NO₃)₂. Variation of HNO₃ and stirring time will be done to learn the crystalinity of hydroxyapatite sample. Synthesis is processed by precipitation method with ratio precursor 1.67 and pH is 10. Precipitation is one of simple and rapid method to produce hydroxyapatite. The obtained Powder is then characterized using XRD (X-ray Diffraction) technique to analyze mineral phase of hydroxyapatite synthesized and FTIR (Fourier Tranform Infra Red) instrument was then used to observe functional group of sample hydroxyapatite.
2. Experimental section
Geloina coaxans shell (GCS) were collected and washed to remove the impurities. The GCS were dried in sunlight and continued using oven for 2 hours at 100 °C. Sampel GCS was calcined at 1000 °C for 12 hours. The powder obtained was CaO. The calcined GCS was treated using HNO₃ to produce Ca(NO₃)₂ in various concentration of HNO₃. H₂PO₄ solution was added drop wise to Ca(NO₃)₂ solution. Stirring rate was adjusted at 300 rpm and pH was 10 using NH₄OH. The obtained suspension was kept for 12 hours at room temperature. next step is the obtained suspension was filtered and dried for 2 hours at 105 °C. The Preparation of the powder was calcined for 1 hour at 900 °C and characterized using XRD and FTIR. Variation of HNO₃ and stirring time were chosen as reaction parameter of synthesis.

3. Results and discussion

3.1 Concentration of HNO₃
Synthesis hydroxyapatite has been done using waste of Geloina coaxans shell as calcium source and H₃PO₄ as phosphate precursor. The reaction adjusted using ratio of precursor 1.67 at pH was 10 by precipitation method. Several reaction parameters such as concentration of HNO₃ and stirring time selected to learn crystalinity of hydroxyapatite synthesized. The XRD and FTIR analysis were done to examine the crystalline structure and functional group of hydroxyapatite synthesized. The result of various concentrations of HNO₃ is shown in Figure 1.

![Figure 1. XRD pattern of hydroxyapatite in various concentration of HNO₃ (a) 3 M ; (b) 5 M](image)

Based on Figure 1 Both of sample hydroxyapatite shown crystalline phase of hydroxyapatite. The specific peak of hydroxyapatite observed in both of produced hydroxyapatite. The highest intensity of XRD pattern appeared at 2θ =31.7°. Hydroxyapatite peaks also can be seen at 2θ =32.8°; 33.9°. These results are similar compared than JCPDS file of hydroxyapatite No 09-0432. Secondary phase of calcium phosphate also observed at 2θ =30.5°; 2θ =21.67° and 2θ =22.75° with minor intensity (α-β TCP). Hydroxyapatite synthesized using 3 M of HNO₃ have higher intensity and proved better crystallinity of hydroxyapatite compared than 5M of HNO₃. The XRD patterns of sample correspond to crystal orientation plane of hydroxyapatite [211]. Percentage of hydroxyapatite obtained was 71,39% and 65,06% for 3 and 5 M of HNO₃ respectively. The result of hydroxyapatite pattern, noted it shown
that the concentration of HNO₃ influence crystalinity obtained, although both of sample prepared had similar diffraction patterns. Previous research, pure hydroxyapatite also produced using 3 M of Ca(NO₃)₂ and calcined at 900 °C specific peak appeared at 2θ = 31.8° by sol-gel method. Reaction of synthesis divided in several steps

\[
\begin{align*}
\text{CaCO}_3 & \rightarrow \text{CaO} + \text{CO}_2 \\
\text{CaO} + 2\text{HNO}_3 & \rightarrow \text{Ca(NO}_3)_2 + \text{H}_2\text{O} \\
10\text{Ca(NO}_3)_2 + 6\text{H}_3\text{PO}_4 + 20\text{NH}_4\text{OH} & \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 20\text{NH}_4\text{NO}_3 + 18\text{H}_2\text{O}
\end{align*}
\]

Debby Schereer equation also be used to estimate crystalite size of sample hydroxyapatite. Schererr equation can be seen below

\[
L = \frac{0.89 \lambda}{\beta \cos \theta}
\]

L= crystal size; \( \lambda = \) Cu K \( \alpha \) (0,154060); \( \beta \)=FWHM

The crystal size of hydroxyapatite at stirring time for 60 minutes and 3 M of HNO₃ was 37.48 nm. Hydroxyapatite nano-wire and nano-rod also produced with crystal size was 12 - 20 nm[4]. Analysis FTIR was also done to observe functional group of hydroxyapatite synthesized. FTIR spectra of hydroxyapatite at stirring time for 60 minutes and 3 M of HNO₃ is shown in Figure 2.

![Figure 2. FTIR spectra of hydroxyapatite at stirring time for 60 minutes and 3M of HNO₃](image)

| Table 1. FTIR spectra of hydroxyapatite synthesized at stirring time for 60 minutes and 3M of HNO₃ |
|-----------------------------------------------|-----------------|------------------|
| Functional groups    | Wavenumber (cm⁻¹) | Chen et al (2015) | Sasikumar et al (2006) |
|----------------------|-------------------|-------------------|------------------------|
| v O-H                | 3571              | 3567              | 3571                   |
| v PO₄                | 1025              | 1037              | 1045                   |
| v PO₄                | 568               | 567               | 571                    |
| v C-O                | 1410              | 1462              | 1550                   |

The FTIR spectra of sample hydroxyapatite shown characteristic vibration of phosphate in two vibrational bands at 1025 cm⁻¹ (\( v_3 \)) and 568 cm⁻¹ (\( v_4 \)). Hydroxyl group (OH) of hydroxyapatite also assigned at 3571 cm⁻¹ that revealed formation of hydroxyapatite phase. From the Previous research, it
also observed that OH at 3567 and 3571 cm\(^{-1}\) respectively [6,7]. The band appeared at 1410 cm\(^{-1}\) corresponded to functional of CO\(_3\)\(^2\). 

3.2 Stirring time

The XRD pattern of hydroxyapatite on variation of stirring time for 60; (b)120; (c)240; and (d) 360 minutes, it was depicted in Figure 3.

![Figure 3](image)

Figure 3 XRD pattern of hydroxyapatite in various stirring time (a) 60; (b)120; (c)240 and (d) 360 minutes

The stirring time influenced the crystalinity and purity of hydroxyapatite synthesized. The characteristic peak of powder hydroxyapatite appeared at 2\(\theta\) = 31.7\(^o\), additional peak of hydroxyapatite also observed at 2\(\theta\) = 32.9 \(^o\). These are suitable with JCPDS of hydroxyapatite file No 09-0432. The secondary phase of calcium phosphate salt also observed at 2\(\theta\) = 21.7 \(^o\) with minor intensity, the mineral was TCP-tricalcium phosphate. The XRD pattern shown, the highest intensity of hydroxyapatite peak have been obtained at stirring time for 240 minutes, the percentage of hydroxyapatite prepared was 96 \%. Analysis crystal size using Schereer equation, the crystal size of hydroxyapatite was 53.24 nm. Synthesis hydroxyapatite using Ca(OH) and H\(_3\)PO\(_4\) as precursors also fabricated hydroxyapatite nanopowder with crystalsize was 26 nm. The percentage of hydroxyapatite obtained was 86\% at 2\(\theta\) = 31.7 \(^o\) for stirring time 240 minutes[8], this result indicated that type of calcium as precursor also influence hydroxyapatite. The powder of the obtained hydroxyapatite was also analyzed using FTIR analysis. Specific band of hydroxyapatite can be seen on Figure 4 and Table 2.
Figure 4. FTIR spectra of hydroxyapatite at stirring time for 240 minutes and 3M of HNO$_3$

Table 2. FTIR spectra of hydroxyapatite obtained at stirring time 240 minutes and 3M of HNO$_3$

| Functional group | Results | Wu et al (2013) | Shavandi et al (2014) |
|------------------|---------|----------------|----------------------|
| v O-H            | 3571    | 3580           | 3570                 |
| v PO$_4^-$       | 1035    | 1031           | 1100                 |
| v PO$_4^3-$      | 569     | 554            | 565                  |
| v CO$_3^-$       | 1465    | -              | 1478                 |

Vibrasi of phosphate or PO$_4^3-$ ($v_4$) appeared at 569; 613 cm$^{-1}$ while PO$_4^3-$ ($v_3$) observed at 1024 and 10535 cm$^{-1}$ respectively. This group also observed at 1037 ; 960 dan 567 cm$^{-1}$ using abalone shell as precursor [5] and at 603 dan 565 cm$^{-1}$ [6]. OH stretching also assigned at 3571 cm$^{-1}$. Usability of microwave method to Mussel shell as calcium source also obtained OH stretching at 3570 cm$^{-1}$ [6], 3580 cm$^{-1}$ [9].

4. Conclusion
The hydroxyapatite can be synthesized by precipitation method using Geloina coaxans shell as calcium source. Usability Geloina coaxans shell as precursor is advantages due to low cost material. The XRD result indicated that pure phase and high crystallinity of hydroxyapatite powder can be obtained. The result of FTIR also proved that specific band of hydroxyapatite appeared in characteristic band of hydroxyapatite.

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