**Effect of Temperature and H3PO4 Flowrate on The Synthesis of Nano-hydroxyapatite Based on Rice Field Snail Shells *(Pila ampullacea)* by Ultrasonic Assisted Precipitation Method**

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**Abstract.** *Nano-hydroxyapatite with the chemical formula Ca10(PO4)6(OH)2 is a calcium phosphate compound that is currently widely developed because it is biocompatible with human bones and teeth. One of the raw materials for the synthesis of nano-hydroxyapatite that meets the criteria is the shell of the rice snail (Pila ampullacea), with a very high calcium content. Based on previous studies, the synthesis of nano-hydroxyapatite with perfect particle size has not been obtained. In this study, we synthesized nano-hydroxyapatite using the Ultrasound Assisted Precipitation method. CaO from rice field snail shells reacted with H3PO4 precursor to form precipitated hydroxyapatite nanoparticles. This study aims to determine the effect of variation in temperature and synthesis time on the characteristics of nHAp from the rice field snail shell using FTIR and XRD analysis. The research variables of nano-hydroxyapatite with variations in temperature and synthesis time are 60oC, 80oC and flowrates of 0,25 mL/min, 0,5 mL/min, 1 mL/min, 2 mL/min, and 3 mL/min. From the results of the FTIR analysis, it was found that the synthesis of nano-hydroxyapatite has been successfully synthesized with the best conditions for obtaining nano-hydroxyapatite crystals at a temperature of 80°C. The comparison of crystallinity based on the results of the XRD test found that the synthesis temperature of 80°C has a higher crystallinity.*

# INTRODUCTION

Nano-hydroxyapatite, Ca10(PO4)6(OH)2 is the most common phosphate apatite, which has been extensively studied both experimentally and theoretically due to its considerable industrial, technological, and biotechnological interest. Hydroxyapatite possesses two different phases: hexagonal and monoclinic, the former hexagonal phase is the most common existent form and plays an important role in bone formation [1]. Nano-hydroxyapatite has a monoclinic or hexagonal crystal structure with a Ca/P ratio of 1.67. The specialty of nano-hydroxyapatite is the ability to interact with living bone tissue, forming strong bonds with bone without causing toxicity or inflammation [2]. The specialty of nano-hydroxyapatite is the ability to interact with living bone tissue, forming strong bonds with bone without causing toxicity or inflammation [3]. Nano-hydroxyapatite has been widely studied and applied in the fields of orthopedics, dentistry, and medicine. The use of nano-hydroxyapatite as a bone regeneration material in the orthopedic field for bone treatment, both repairing fractured bones and fractures [4]. In addition, nano-hydroxyapatite is used as a basic material for making implants because it is one of the constituents of hard tissue in the bones of the human body [5].

The good characteristics of nano-hydroxyapatite cause its use in dentistry to be quite extensive, such as bone tissue reconstruction, tooth building, soft tissue engineering, and treatment of periodontal defects, dental implant coatings, and filler restoration materials [7]. Nano-hydroxyapatite (nHAp) is also used as a tooth building material in dental caries or cavities. The most common oral health problem is dental caries. Dental caries can be found in the patient's oral cavity, regardless of age, social status, and gender [8]. The results of the Basic Health Research by the Health Research and Development Agency in 2018 stated that the largest proportion of oral health problems in Indonesia was dental caries, 88.8% [6]. Nano-hydroxyapatite of various morphologies and surface properties can be used as a drug carrier for the delivery of various molecular drugs (drug delivery) due to its customizable size, highly active surface, unique physical properties and chemical properties, ease of modification, non-toxicity, biocompatibility, and non-inflammation [9].

One of the raw materials for nano-hydroxyapatite synthesis that meets the criteria is the shell of the rice snail *(Pila ampullacea).* The shell contains 95-99% CaCO3 compound, while the rest is silica and other organic materials. The very high calcium content of the shell of the rice field snail shells *(Pila ampullacea)* has the potential to be utilized as a more valuable material for industrial and medical purposes [10]. The existence of rice snail shells is easy to find, and the number continues to increase in Indonesia. In addition, the utilization of rice snail shells is still not optimal, and often, only the meat is used for culinary businesses [11].

In this study, the nano-hydroxyapatite was synthesized using the ultrasonic wave-assisted precipitation method so that the ultrasonic waves could be distributed more evenly, and then the reaction of hydroxyapatite could occur uniformly. The various temperatures and H3PO4 flowrate were set on the synthesis of hydroxyapatite from rice field snail shells *(Pila ampullacea)*. The synthesis temperature variations used are 60°C and 80°C, while the H3PO4 flowrate variations in the synthesis are 1 mL/min, 2 mL/min, and 3 mL/min. The characteristic of the synthesized hydroxyapatite particle was obtained using FTIR (Fourier Transform Infra-Red) and XRD (X-Ray Diffraction) analysis.

# MATERIALS AND METHODS

# MATERIALS

The material used in this research was rice field snail shell (*pila ampullacea*), Phosphoric Acid Precursor (H3PO4) (Merck, Kenilworth, NJ, USA) Grade: ACS, ISO, Reag. Ph Eur 85%, nitrogen gas, and distilled water.

# METHODS

**Pre-Treatment Process**

The rice snail shells were washed until cleaned to remove the impurities from the shells. The clean shells were dried under the sun for 2 days to remove the water content. The dried shells were crushed and ground to a powder using mortar and pestle then sieved to obtain the uniform size of powder. The shell powder was calcined at 1000°C for 8 hours in the muffle furnace to remove the organic component and convert the calcium carbonate content into calcium oxide according to the reaction,

CaCO3 CaO + CO2

**Hydroxyapatite Synthesis Process by Precipitation Method**

Figure 1 shows the schematic of the ultrasonic-assisted precipitation method apparatus. Calcined CaO powder was added with boiling distilled water in the reactor with the temperature set to 60℃ and 80℃. Moreover, the power of the ultrasonic bath was set to 100%. H3PO4 solution (1.3 M, 100 mL) was added to the reactor using a dosing pump with various flowrate of 0,25 mL/min, 0,5 mL/min, 1 mL/min, 2 mL/min, and 3 mL/min. The reaction occurred in the reactor as follows,

CaO + H2O Ca(OH)2

10 Ca(OH)2 + 6 H3PO4 Ca10(PO4)6(OH)2 + 16 H2O

The precipitation results were washed with distilled water and dried in an oven at 110℃ for 3 hours, then sintered at 800℃ for 6 hours in the muffle furnace to obtain the hydroxyapatite powder.

Diagram of a chemical reaction

Description automatically generated

**FIGURE 1.** Schematic of Ultrasound-Assisted Precipitation Method Synthesis Apparatus

**Characterization of Hydroxyapatite Powder**

The characteristic of hydroxyapatite was determined from FTIR and XRD analysis. The FTIR analysis was conducted using Agilent Cary 630, with a wavenumber from 650 - 4000 cm-1 to determine the functional group of hydroxyapatites. The XRD analysis was carried out on a Philips X'pert MPD type diffractometer with configuration of CuKa radiation at 40kV, 30 mA, to obtain the crystallinity phase of hydroxyapatite powder.

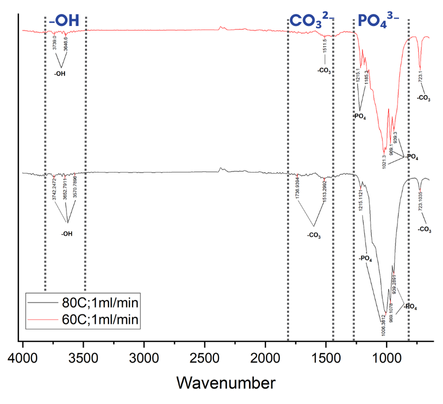
# Result and discussion

The functional group of hydroxyapatite powder synthesized from rice field snail shells was identified using FTIR spectroscopy analysis. **Figure 2** shows the FTIR spectrum analysis of hydroxyapatite powder at various temperature conditions and flowrates of H3PO4 solution in the hydroxyapatite synthesis process. **Figure 2(a)** shows the presence of hydroxyl groups (-OH) and phosphate groups (PO₄³⁻). The FTIR spectrum detected the presence of hydroxyl groups (-OH) at the range of 3300–3850 cm⁻1 wavelength, also the presence of phosphate group at 1000-1150 cm⁻1 wavelength. This result shows that the hydroxyapatite material has been successfully synthesized. Moreover, the various flowrate of H3PO4 solution from 0.25 mL/min to 3 mL/min did not affect the result significantly because the molar reactant of Ca(OH)2 and H3PO4 at various flowrate were adjusted to the reaction stoichiometrically.

A diagram of a graph

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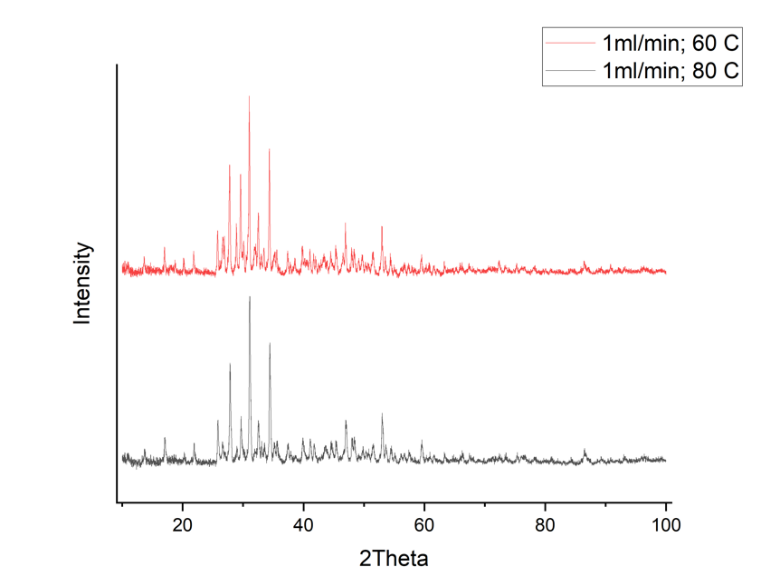
**(a)**



**(b)**

**FIGURE 2.** FTIR result of nano-hydroxyapatite synthesized at 80°C with flowrates of 0,25 mL/min, 0,5 mL/min, 1 mL/min, 2 mL/min, and 3 mL/min (a), flowrates of 1 mL/min synthesized at 60°C and 80°C (b)

**Figure 2(b)** shows the FTIR spectrum of hydroxyapatite at various temperature conditions during the synthesis process. The presence of carbonate groups in the samples can be observed further in **Figure 2 (b)**. The graphs show the comparison of nano-hydroxyapatite synthesized at the same flowrate but at a different temperature. The peaks indicating the presence of carbonate groups are stronger at 60°C compared to the ones at 80°C, this means that the nano-hydroxyapatite synthesized at 80°C has less contamination because of the higher temperature condition in the synthesis process.



**FIGURE 3.** XRD test comparison of nano-hydroxyapatite synthesized at 60 °C and 80°C with flowrates of 1mL/min.

**Figure 3** shows the XRD peak pattern of the hydroxyapatite powder with a flowrate of H3PO4 of 1mL/min on various temperature synthesis (60°C and 80°C). It shows that there is no significant difference in the XRD peak pattern between 60°C and 80°C. The main peaks of hydroxyapatite for both temperature conditions are at 2θ = 32.55°, 35.24°, and 34.59°, which means similar to the hydroxyapatite standard (JCPDS 09-0432). Moreover, from the XRD peak pattern results, the crystallinity degree of the hydroxyapatite powder could be determined from the ratio of crystalline peaks area to the total peaks area, which could be obtained using OriginPro software. The % crystallinity was calculated from the following equation.

**TABLE 1.** Crystallinity of Nano-hydroxyapatite Powder

|  |  |  |
| --- | --- | --- |
| Variables | | Crystallinity |
| Temperature | Flowrate |
| 80°C | 0,25 mL/min | 43% |
| 1 mL/min | 84% |
| 3 mL/min | 67% |
| 60°C | 1 mL/min | 76% |

From the crystallinity percentage calculated, which is shown in **Table 1**, the highest crystallinity is 84% with the variable of 1 mL/min flowrate and 80°C synthesis temperature. The lowest crystallinity is 43% from the variable of 0,25 mL/min flowrate and 80°C synthesis temperature. This result shows that the crystallinity increases with the flowrate of H3PO4, but with extremely high flowrate, the crystallinity also decreases. The result shows that to obtain good crystallinity, using extremely low or extremely high flowrates is not ideal. Meanwhile, comparing the crystallinity we obtained using the same flowrate but different temperatures, the one with higher temperature (80°C synthesis temperature) has a higher crystallinity percentage, which means that using higher temperatures would help the particles to form more uniformly, resulting in higher crystallinity. According to the research by Siswanto (2020), which states that the crystallinity of human bone is in the range of 69% to 87%, it is only the hydroxyapatite synthesized with 1 mL/min phosphoric acid flowrate that is within the range, proving that using extremely low or extremely high flowrates were not ideal for nano-hydroxyapatite synthesis.

# CONCLUSION

Nano-hydroxyapatite was synthesized from the rice field snail shells with high calcium contents, using the ultrasound-assisted precipitation method at various temperatures and flowrate. Based on the results of the FTIR analysis, the hydroxyl group (-OH) was detected in the range of wave numbers 3300-3850 cm-1 and the phosphate group at wave numbers 1000-1150 cm-1, which means that the nano-hydroxyapatite synthesis has been successful. The best condition to obtain nano-hydroxyapatite crystals is at 80oC, this means that the nano-hydroxyapatite synthesized at 80°C has less contamination because of the higher temperature. In the comparison of crystallinity based on XRD test results using various temperatures, it was found that the synthesis temperature of 80°C had a higher percentage of crystallinity, which means that using a higher temperature will help particles to form more uniformly. Meanwhile, for the H3PO4 flowrate, using a moderate flowrate is proven to be more effective, with the crystallinity rate of the extremely low or extremely high flowrate being lower than the moderate flowrate used.

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# References

[1] Z. Yuan, S. Li, J. Liu, X. Kong, and T. Gao, “Structural, electronic, dynamical and thermodynamic properties of Ca10(PO4)6(OH)2 and Sr10(PO4)6(OH)2: First-principles study,” *Int. J. Hydrogen Energy*, vol. 43, no. 29, pp. 13639–13648, 2018, doi: 10.1016/j.ijhydene.2018.03.096.

[2] A. R. Noviyanti, H. Haryono, R. Pandu, and D. R. Eddy, “Cangkang Telur Ayam sebagai Sumber Kalsium dalam Pembuatan Hidroksiapatit untuk Aplikasi Graft Tulang,” *Chim. Nat. Acta*, vol. 5, no. 3, p. 107, 2017, doi: 10.24198/cna.v5.n3.16057.

[3] L. M. Cursaru *et al.*, “Hydroxyapatite from Natural Sources for Medical Applications,” *Materials (Basel).*, vol. 15, no. 15, 2022, doi: 10.3390/ma15155091.

[4] I. A. Suci and Y. D. Ngapa, “Sintesis dan Karakterisasi Hidroksiapatit dari Cangkang Kerang Ale-Ale Menggunakan Metode Presipitasi Double Stirring,” *Cakra Kim.*, vol. 8, no. 2, pp. 73–81, 2020.

[5] A. F. Akbar, F. Q. ’Aini, B. Nugroho, and S. E. Cahyaningrum, “SINTESIS DAN KARAKTERISASI HIDROKSIAPATIT TULANG IKAN BAUNG (Hemibagrus nemurus sp.) SEBAGAI KANDIDAT IMPLAN TULANG,” *J. Kim. Ris.*, vol. 6, no. 2, p. 93, 2021, doi: 10.20473/jkr.v6i2.30695.

[6] Riskesdas Kementrian Kesehatan RI, “Laporan Riskesdas 2018 Nasional.pdf,” *Lembaga Penerbit Balitbangkes*. 2018.

[7] M. Mozartha, “Hidroksiapatit Dan Aplikasinya Di Bidang Kedokteran Gigi,” *Cakradonya Dent J*, vol. 7(2), no. 2, pp. 807–868, 2015.

[8] R. Amalina, D. Monica, A. Feranisa, F. Y. Syafaat, M. Sari, and Y. Yusuf, “PEMBUATAN GEL HIDROKSIAPATIT CANGKANG KERANG-SIMPING (Amusium pleuronectes) DAN PENGARUHNYA SETELAH APLIKASI DI LESI WHITE-SPOT EMAIL GIGI DEVELOPMENT OF HYDROXYAPATITE ASIAN MOON SCALLOP (Amusium pleuronectes) GEL AND ITS EFFECT AFTER APPLICATION ON TOOT,” *Cakradonya Dent. J.*, vol. 13, no. 2, pp. 81–87, 2021, [Online]. Available: http://www.jurnal.unsyiah.ac.id/CDJ

[9] S. Mondal, S. V. Dorozhkin, and U. Pal, “Recent progress on fabrication and drug delivery applications of nanostructured hydroxyapatite,” *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology*, vol. 10, no. 4, pp. 1–32, 2018, doi: 10.1002/wnan.1504.

[10] H. Delvita, D. Djamas, and Ramli, “KARAKTERISTIK KALSIUM KARBONAT (CaCO 3 ) DALAM CANGKANG KEONG SAWAH (Pila ampullacea) YANG TERDAPAT DI KABUPATEN PASAMAN,” *Pillar Phys.*, vol. 6, pp. 17–24, 2015.

[11] Dewi Sriwahyuni, “Penggunaan Cangkang Keong Sawah,” 2020.