

Study of Hydroxyapatite from White Mussel Shells Using Nitric Acid Solvent with the Sol-Gel Method

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Article Information	ABSTRACT
Article History Received : June 19, 2025 Revised : July 07, 2025 Published : July 18, 2025 Keywords: <i>Hydroxyapatite;</i> <i>Mussel Shells;</i> <i>pH;</i> <i>Sol-Gel;</i> <i>Stirring Temperature</i>	Mussel shells contain high calcium levels, making them suitable for hydroxyapatite (HAp) synthesis. HAp is a bioceramic widely applied in bone implant materials. This study synthesized HAp from white mussel shells (<i>Corbula faba Hinds</i>) using nitric acid via the sol-gel method. The effects of pH (8–12) and stirring temperature (30°C–90°C) on product yield and crystallinity were evaluated. X-ray fluorescence (XRF) was used to analyze elemental composition, while X-ray diffraction (XRD) characterized the crystalline phase. Results showed that increasing pH and temperature generally enhanced both yield and crystallinity. Optimal conditions were observed at pH 10 and higher temperatures, demonstrating the effective conversion of mussel shells into hydroxyapatite. These findings support the potential of marine biowaste as a sustainable raw material for biomedical applications.

INTRODUCTION

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is one of the biomaterials widely used in the medical field, especially as a bone and dental implant material, due to its bioactive and biocompatible properties, as well as its structure that resembles the natural mineral composition of human bone (Sari & Purwaningsih, 2022). The demand for hydroxyapatite in Indonesia continues to increase, yet most of it is still imported from countries such as Japan, the United States, and Switzerland. This dependence on imported products has made hydroxyapatite very expensive in the domestic market, reaching around 1.5 million rupiah for just 5 milligrams, thereby motivating the search for cheaper and more accessible local sources of hydroxyapatite.

Hydroxyapatite is the most stable calcium phosphate compound. Several types of calcium phosphate can be formed during the reaction between calcium and phosphate, depending on the Ca/P ratio (Malau & Adinugraha, 2020). These compounds generally have a crystalline structure and are classified into four main phases: dicalcium phosphate (CaHPO_4), octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$), tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), and hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) (Edi Cahyaningrum et al., 2021).

One potential local calcium source is the waste from white mussel shells (*Corbula faba Hinds*), which contains a high level of calcium carbonate (CaCO_3), approximately 93.49% (Andyana et al., 2021). Various previous studies have shown that seashells can be successfully synthesized into hydroxyapatite using methods such as sol-gel and hydrothermal techniques. The sol-gel method was chosen in this study due to its advantages in producing hydroxyapatite with high crystallinity, good purity, and controlled particle size at relatively low reaction temperatures (Edi Cahyaningrum et al., 2021). The pH and reaction temperature are known to significantly

influence the synthesis outcomes of hydroxyapatite, including product yield, crystallinity, and purity (Lee et al., 2020).

Although previous studies have demonstrated the potential of seashell waste as a raw material, systematic studies focusing on optimizing pH and temperature specifically for white mussel shells via the sol-gel method are still limited. The lack of comprehensive data on these parameters makes it difficult to achieve consistent quality standards required for biomedical applications. Therefore, there is a need for research that not only synthesizes hydroxyapatite from local waste but also fine-tunes the synthesis conditions to enhance yield, crystallinity, and purity.

This study aims to synthesize hydroxyapatite from white mussel shells using nitric acid as a solvent through the sol-gel method, as well as to examine the effect of pH and reaction temperature variations on the yield, crystallinity, and hydroxyapatite content produced. The significance of this research lies in its effort to develop a more economical and environmentally friendly local biomaterial, while also supporting the reduction of dependence on imported hydroxyapatite in Indonesia.

RESEARCH METHODS

The equipment used in this study includes a series of hydroxyapatite synthesis apparatus shown in Figure 1, consisting of a beaker glass, magnetic stirrer, stand, clamp, burette, and thermometer, as well as other tools such as a water bath and furnace. The materials utilized were white mussel shell (*Corbula faba Hinds*) waste, 65% nitric acid (HNO_3), diammonium hydrogen phosphate $((\text{NH}_4)_2\text{HPO}_4)$, ammonium hydroxide (NH_4OH), and distilled water.

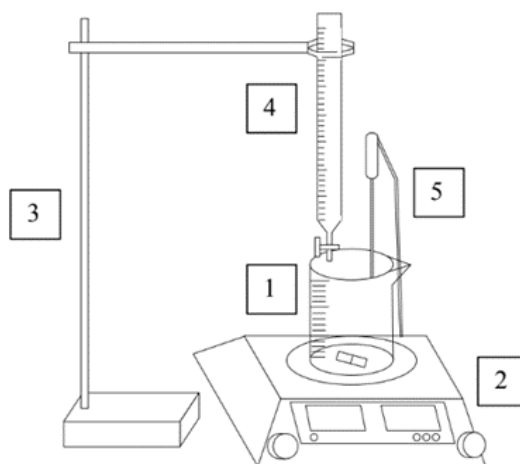


Figure 1. Hydroxyapatite Synthesis Experimental Apparatus Setup

Preparation of Raw Material

The mussel shells were first washed thoroughly to remove adhering impurities and organic matter, then dried in an oven at 110°C for 24 hours. The dried shells were crushed using a mortar and pestle and sieved to achieve a uniform particle size below 100 mesh. The powder was then calcined in a furnace at 900°C for 3 hours to convert calcium carbonate (CaCO_3) to calcium oxide (CaO). The composition of the calcined mussel shell powder was analyzed using X-Ray Fluorescence (XRF) spectroscopy with a 0–100% scan range, calibrated using certified reference materials.

Experimental Design

The method employed for synthesis was the sol-gel method. A factorial design was applied, combining five pH levels (8, 9, 10, 11, 12) and five reaction temperatures (30°C , 45°C , 60°C , 75°C , 90°C), resulting in 25 experimental conditions. The independent variables were pH and temperature, while the dependent variables included hydroxyapatite yield, crystallinity, and

purity. These pH and temperature ranges were selected based on preliminary experiments and prior studies that identified their significant role in hydroxyapatite formation (Lee et al., 2020).

Synthesis Procedure

For each condition, 5.6 grams of CaO powder were dissolved in 20 mL of 65% nitric acid, followed by the addition of distilled water to a final volume of 200 mL to form calcium nitrate solution. Separately, 7.9 grams of $(\text{NH}_4)_2\text{HPO}_4$ were dissolved in 200 mL of distilled water. The phosphate solution was added slowly to the calcium nitrate solution under continuous stirring at 300 rpm. The pH was gradually adjusted to the target level (8, 9, 10, 11, or 12) using NH_4OH solution. The mixture was stirred for 1 hour at the designated temperature, maintained using a water bath with $\pm 1^\circ\text{C}$ accuracy.

After stirring, the solution was kept in the water bath for an additional hour to promote gel formation, then aged at room temperature for 24 hours to ensure complete precipitation. The precipitate was separated by filtration and washed repeatedly with distilled water until the washings reached neutral pH. The product was dried in an oven at 110°C for 24 hours and then calcined in a furnace at 800°C for 3 hours to enhance crystallinity.

Characterization

X-Ray Diffraction (XRD) analysis (model [insert model here]) was conducted to determine the phase composition and crystallinity of the product. The XRD scan was performed from 10° to 80° 2θ using $\text{Cu-K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$), at 40 kV and 30 mA, with a scanning speed of $2^\circ/\text{min}$. Crystallite size was estimated using the Scherrer equation where applicable.

RESULTS AND DISCUSSION

The white mussel shell powder was analyzed using the X-Ray Fluorescence (XRF) method to determine the CaO content. The analysis results are presented in Table 1 below.

Table 1. Compound Composition of White Mussel Shell Powder

Compound	Composition
CaO	95,76%
Fe_2O_3	1,56%
Al_2O_3	1%
SrO	0,42%
SO_3	0,34%
Eu_2O_3	0,22%
K_2O	0,21%
BaO	0,2%

Based on Table 1, the white mussel shell powder contains various oxide compounds, with calcium oxide (CaO) being the most dominant at 95.76%. The high CaO concentration indicates that the calcination process effectively removed organic matter and converted calcium carbonate (CaCO_3) into CaO. This conversion is essential, as CaO serves as a precursor in the formation of hydroxyapatite (HAp), a key material in biomedical applications. The predominance of CaO supports the use of white mussel shells as a promising raw material for HAp synthesis. Recent advancements in hydroxyapatite research have emphasized improving the material's purity, crystallinity, and biocompatibility to meet clinical performance requirements, particularly in bone and dental implant applications (Meilianti, 2017). Natural calcium sources such as seashells, animal bones, and eggshells are increasingly explored due to their abundance, renewability, and environmental benefits (Hutabarat et al., 2019). Utilizing these biowastes not only lowers production costs but also promotes sustainable material development by minimizing environmental impact. The calcium content in these natural sources plays a critical role in determining the calcium-to-phosphorus (Ca/P) ratio during synthesis. This ratio significantly

influences the resulting calcium phosphate phase and its stability (Noviyanti et al., 2017). A deviation from the ideal Ca/P ratio of approximately 1.67 can lead to the formation of undesirable phases, such as tricalcium phosphate or dicalcium phosphate, which may compromise material performance. Conversely, a stoichiometric ratio promotes the formation of stable, bioactive hydroxyapatite with superior mechanical integrity and biological integration (Leni & Hariyani, 2021). Therefore, the high CaO content in white mussel shells not only verifies their suitability but also supports current efforts in developing eco-friendly and clinically effective biomaterials.

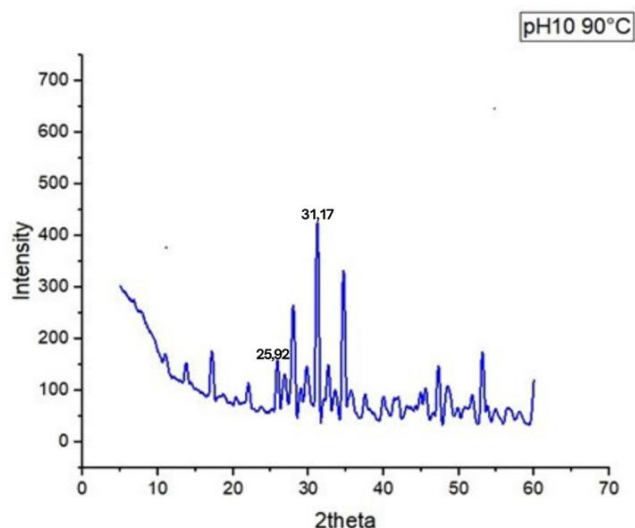


Figure 2. XRD Analysis Results of Hydroxyapatite at 90°C Reaction Temperature and pH 10

The purity of hydroxyapatite can be determined using the Match software with the JCPDS (Joint Committee on Powder Diffraction Standards) database. The XRD test results for the sample at a reaction temperature of 90°C and pH 10 first appeared at an angle of 25.92°, with the highest hydroxyapatite peak observed at 31.17°. Compared to the JCPDS standard 24-0033, which has 2θ values at 25.878°, 31.737°, and 32.863°, it can be concluded that the sample at 90°C and pH 10 has formed hydroxyapatite (HA).

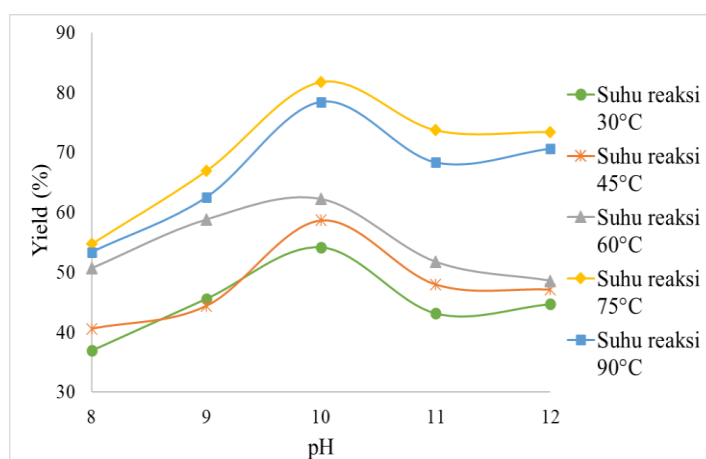


Figure 3. Effect of pH on Hydroxyapatite Yield (%)

The effect of pH on product yield is illustrated in Figure 3. The yield increased progressively from pH 8 to 10 and then declined at pH 11 and 12. Similarly, the influence of reaction temperature showed a rise in yield between 30°C and 75°C, with the maximum yield of 81.74% achieved at 75°C and pH 10. However, a decrease in yield was observed at 90°C.

The increased yield from pH 8 to 10 can be attributed to improved solubility and availability of calcium and phosphate ions in the solution, which facilitates nucleation and crystal growth of hydroxyapatite (Prayoga et al., 2021). At pH levels beyond 10, excess hydroxide ions may induce competing reactions, such as the formation of calcium hydroxide or other unwanted phases, thereby reducing hydroxyapatite formation. High alkalinity also disrupts ionic balance and decreases supersaturation—an essential condition for effective precipitation.

In terms of temperature, the increase in yield up to 75°C is due to the enhancement of molecular kinetics. Elevated temperatures improve ion mobility and reaction rates, enabling more efficient bonding between Ca^{2+} and PO_4^{3-} ions (Purnama et al., 2023). However, at 90°C, the solubility of calcium phosphate increases, causing the ions to remain in solution rather than precipitate, which decreases product yield (Malau, 2020; Jamarun & Septiani, 2021). Excessive heat can also lead to the formation of amorphous or unstable calcium phosphate phases, unsuitable for biomedical use. Thus, optimal yield is achieved under conditions that maintain ion supersaturation and minimize side-phase formation (Sumarto, 2021; Siswoyo et al., 2020). Maintaining optimal pH and temperature is therefore essential to ensure both the quantity and quality of hydroxyapatite, especially in applications that demand high crystallinity and phase purity (Sinulingga & Sirait, 2021).

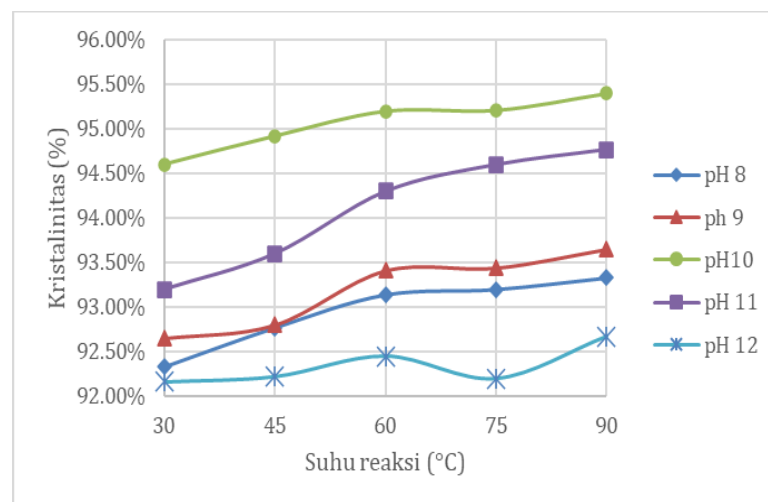


Figure 4. The Relationship Between Reaction Temperature (°C) and Hydroxyapatite Crystallinity (%)

Based on Figure 4, the crystallinity of hydroxyapatite increases with rising reaction temperature, reaching a maximum value of 95.40% at pH 10 and 90°C. This trend indicates a positive correlation between temperature and the degree of crystallinity under the tested conditions. Additionally, the highest crystallinity was consistently observed at pH 10, suggesting that pH also plays a significant role in crystal structure development.

Higher reaction temperatures promote greater ion mobility and accelerate reaction kinetics, facilitating the orderly arrangement of Ca^{2+} and PO_4^{3-} ions into a stable crystal lattice (Hariani et al., 2020). At elevated temperatures, the solubility of hydroxyapatite itself tends to decrease, which favors crystal growth by enabling precipitation to proceed more completely (Akbar, 2021; Anggresani et al., 2019). These conditions lead to the formation of larger, more ordered, and structurally robust crystals. In contrast, lower temperatures often result in calcium-deficient or amorphous hydroxyapatite phases that are mechanically and chemically unstable (Haruda et al., 2021).

High crystallinity is essential because it directly influences the mechanical strength, thermal stability, and biological performance of hydroxyapatite in clinical applications (Wijanarko & Irfa'i, 2023). Therefore, optimizing temperature is not only important for phase control but also for ensuring material functionality in biomedical contexts.

Similarly, pH affects crystallinity by controlling the chemical environment during nucleation. A basic condition around pH 10 promotes the formation of stoichiometric hydroxyapatite, which is more ordered and biocompatible (Putri, 2021). In contrast, low pH introduces excess H^+ ions that may lead to the formation of calcium-deficient hydroxyapatite (CdHA), while extremely high pH levels can trigger unwanted phase formation, reducing overall crystallinity and material purity (Wardiana & Cahyaningrum, 2019).

CONCLUSION

Based on the results of the study, white mussel shells have shown strong potential as a raw material for hydroxyapatite synthesis due to their high calcium content. The synthesis process using the sol-gel method produced hydroxyapatite with optimal yield and crystallinity under controlled pH and temperature conditions. These results indicate that biowaste such as mussel shells can be effectively converted into high-quality hydroxyapatite that meets biomedical standards. Scientifically, this study contributes to the development of sustainable biomaterials by demonstrating the feasibility of using natural calcium sources in place of synthetic precursors. The resulting hydroxyapatite holds promising potential for medical applications, particularly in bone repair and implant materials, due to its compatibility with physiological conditions and structural similarity to natural bone.

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