A Comparative Study of Hydroxyapatite Synthesis from Crab shells (Portunus pelagicus) in Various Concentrations of H3PO4 and KH2PO4 Solutions for Bone Graft Applications

Eva Oktavia Ningrum1, a), Villia Lidzati Kamilah1), Suprapto Suprapto1), Achmad Dwitama Karisma1), Tobing Gumelar1), Vita Fatichah Rizqiyah Febriani1), Ummu Zahroh Ma’mun1), Maharani Sugito Rosanti1), Haykal Nur Fajri Ramadhan1), M Aldi Nugroho1), Silma Elvaretta Aska1), Dimas Gilang Venanto1), Sinung Widiyanto2), Imam Safari Azhar3)

1Department of Industrial Chemical Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, 60111, Indonesia

2Department of Marine Engineering, Universitas Hang Tuah, Surabaya 60111, Indonesia

3Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60312, Indonesia

a) Corresponding author: [eva-oktavia@chem-eng.its.ac.id](mailto:eva-oktavia@chem-eng.its.ac.id)

**Abstract.** This study investigates the comparative effects of H₃PO₄ and KH₂PO₄ concentrations on hydroxyapatite (HAp) synthesis from calcined crab shells (Portunus pelagicus). X-ray fluorescence (XRF) analysis revealed that both precursors produced high calcium content, reaching 93.09% with H₃PO₄ and 93.11% with KH₂PO₄, compared to 90.55% in untreated shells. Fourier Transform Infrared (FTIR) spectroscopy identified characteristic functional groups (OH⁻, PO₄³⁻, and CO₃²⁻), confirming HAp formation. X-ray diffraction (XRD) analysis demonstrated that HAp synthesized with KH₂PO₄ exhibited higher crystallinity (up to 99.06% at 0.8 M) compared to H₃PO₄, which showed decreasing crystallinity with increasing concentration. Crystal size analysis indicated nano-sized crystals for both precursors, with H₃PO₄ yielding smaller crystals, enhancing bioactivity and resorbability for medical applications. Energy-dispersive X-ray spectroscopy (EDX) analysis showed that the Ca/P ratio decreased with increasing precursor concentration, with H₃PO₄ at 1 M achieving a ratio closest to the standard of 1.67. These findings highlight the potential of H₃PO₄ and KH₂PO₄ in producing high-quality HAp suitable for biomedical use, with distinct differences in crystallinity and nano-scale morphology.

**Keywords**: *Bone Grafts, Hydroxyapatite,* H₃PO₄*,* KH2PO4

# INTRODUCTION

Tooth extraction can significantly alter the dimensions of the alveolar ridge and initiate remodeling of the alveolar bone. Most bone volume loss occurs within the first 3 to 6 months post-extraction, with bone width reduction ranging from approximately 29% to 63%. Consequently, various procedures, such as tooth grafting and Guided Bone Regeneration (GBR), are recommended to maintain alveolar ridge dimensions. Materials utilized in tooth grafting must exhibit osseointegration properties, osteogenesis, osteoconduction, and osteoinduction. The primary bone graft materials include autografts, allografts, and xenografts. Autogenous bone grafts are the gold standard due to their osteoconductive, osteoinductive, and osteogenic capabilities. Nevertheless, autografts are characterized by rapid and unpredictable resorption patterns [1]. Most commercial bone graft materials are in granule form, lacking dimensional stability, and unsuitable for addressing extensive or vertical defects/atrophy. Although block bone grafts have been proposed, they are difficult to shape and are prone to fragility. Consequently, current bone graft materials often need to meet the criteria for osseointegration, osteogenesis, osteoinduction, and osteoconduction.

Hydroxyapatite (HAp) is a calcium phosphate compound widely used in biomedical applications due to its similarity to the mineral component of human bone [2] Hydroxyapatite extracted from crab shells, following calcination, contains 91.96±5.07% calcium, rendering it a potential source of HAp [3,4]. Its applications range from bone graft materials to coatings for dental implants, driven by its excellent biocompatibility, osteoconductivity, and bioactivity. The quality of HAp is influenced by several parameters, including synthesis temperature, precursor type, precursor concentration, solute concentration, pH levels, and calcination processes. While most research focuses on optimizing synthesis temperature and calcination conditions, there is a growing need to understand the role of precursors in achieving medical-grade HAp, particularly nano-hydroxyapatite (nano-HAp), which has enhanced bioactivity and resorbability.

Previous studies have demonstrated that the synthesis temperature significantly affects HAp crystallinity, particle size, and purity. For instance, calcination temperatures above 1000°C are often used to achieve high crystallinity, which is essential for bone graft applications as it impacts the material's degradation rate and osteoinductive properties [5]. However, achieving nano-sized crystals with uniform morphology remains challenging, as higher temperatures can promote crystal growth, leading to larger particle sizes. Similarly, solute concentrations, such as calcium and phosphate sources, influence the Ca/P ratio, a critical factor in determining the bioactivity and stability of HAp in physiological environments [6,7]. Despite extensive investigations into these factors, precursor selection and its concentration are underexplored. Precursors, such as phosphoric acid (H₃PO₄) and potassium dihydrogen phosphate (KH₂PO₄), play a pivotal role in facilitating nucleation and crystal growth during synthesis. Prior research has shown that precursors influence the formation of functional groups, crystallinity, and particle size, all of which are crucial for biomedical applications. For example, acidic precursors like H₃PO₄ tend to produce smaller crystal sizes due to their influence on pH and ionic interaction during synthesis [8,9].

However, studies comparing the effects of different precursor types and concentrations on nano-HAp remain limited. This research aims is investigating the effects of varying concentrations of H₃PO₄ and KH₂PO₄ on the synthesis of nano-HAp derived from crab shell waste. The choice of H₃PO₄ and KH₂PO₄ stems from their established role in providing phosphate ions essential for HAp formation and their ability to modulate the pH and reaction kinetics during synthesis [10–12]. Unlike previous studies, which primarily focus on temperature optimization, this study emphasizes precursor selection and concentration, assessing their impact on key parameters such as crystallinity, particle size, and functional group formation. Furthermore, while much of the existing research evaluates HAp for general biomedical applications, this study specifically considers the stringent requirements for bone graft materials, including crystallinity (62%-87% as per FDA standards) and nano-sized particles for enhanced bioactivity [13]. By addressing the interplay between precursor concentration and these critical parameters, this research provides new insights into optimizing HAp for bone graft applications, offering a sustainable and efficient approach to utilizing marine-derived resources like crab shells.

This work highlights in understanding precursor-driven synthesis of nano-HAp and its implications for meeting medical-grade standards. It builds on previous reports by comparing the efficacy of H₃PO₄ and KH₂PO₄, analyzing their distinct effects on HAp quality, and proposing optimal conditions for achieving high-performance biomaterials suitable for clinical applications.

# EXPERIMENTAL

## Materials

The materials utilized included waste shells from crab shells (Portunus pelagicus), 85% ortho-phosphoric acid (H₃PO₄), and analytical grade monopotassium phosphate (KH₂PO₄) from Merck. These were the calcium (Ca) and phosphorus (P) precursor compounds for hydroxyapatite synthesis.

## Pre-Treatment of Raw Materials

The pre-treatment of raw materials began with thoroughly washing the crab shells (P. pelagicus) to remove any impurities. This washing process continued until the water ran clear. Subsequently, the shells were dried at room temperature to eliminate moisture. The final step in the pre-treatment involved crushing the shells to reduce their size, thereby facilitating the calcination process.

## Calcination Process of Crab shells (P. pelagicus)

During the calcination process, the crushed crab shells (P. pelagicus) were placed in a furnace and heated to 1000°C for 5 h. This process eliminated organic components and converted calcium carbonate (CaCO₃) into calcium oxide (CaO).

## Hydroxyapatite Synthesis by Precipitation Method

The synthesis process began with the preparation of precursor solutions of H₃PO₄ and KH₂PO₄ at concentrations of 0.5 M, 0.8 M, and 1 M in 100 mL each. Subsequently, 5.164 g of CaO obtained from calcination were dissolved in 500 mL of boiling distilled water to form a Ca(OH)₂ solution. This solution was then combined with 100 mL of the prepared precursor solution and added dropwise into a bath sonicator while heated. The mixture was stirred with a magnetic stirrer for 2 h to achieve homogeneity, followed by filtration to collect a white precipitate. The precipitate underwent three rinses with distilled water to remove impurities. After filtration, the residue was dried at 110°C for 1 h and sintered at 800°C for 4 h in a furnace.

# RESULT AND DISCUSSION

## XRF Analysis

During the calcination of the crab shells (P. pelagicus) at 1000°C., the weight of the crab shells (P. pelagicus) decreased due to the removal of shell filler elements, as evidenced by a reduction in weight from 42.83 g before calcination to 25.7 g of CaO powder. After calcination, the chemical composition of the samples was measured to determine the calcium content of the reactant as shown by **Table 1**.

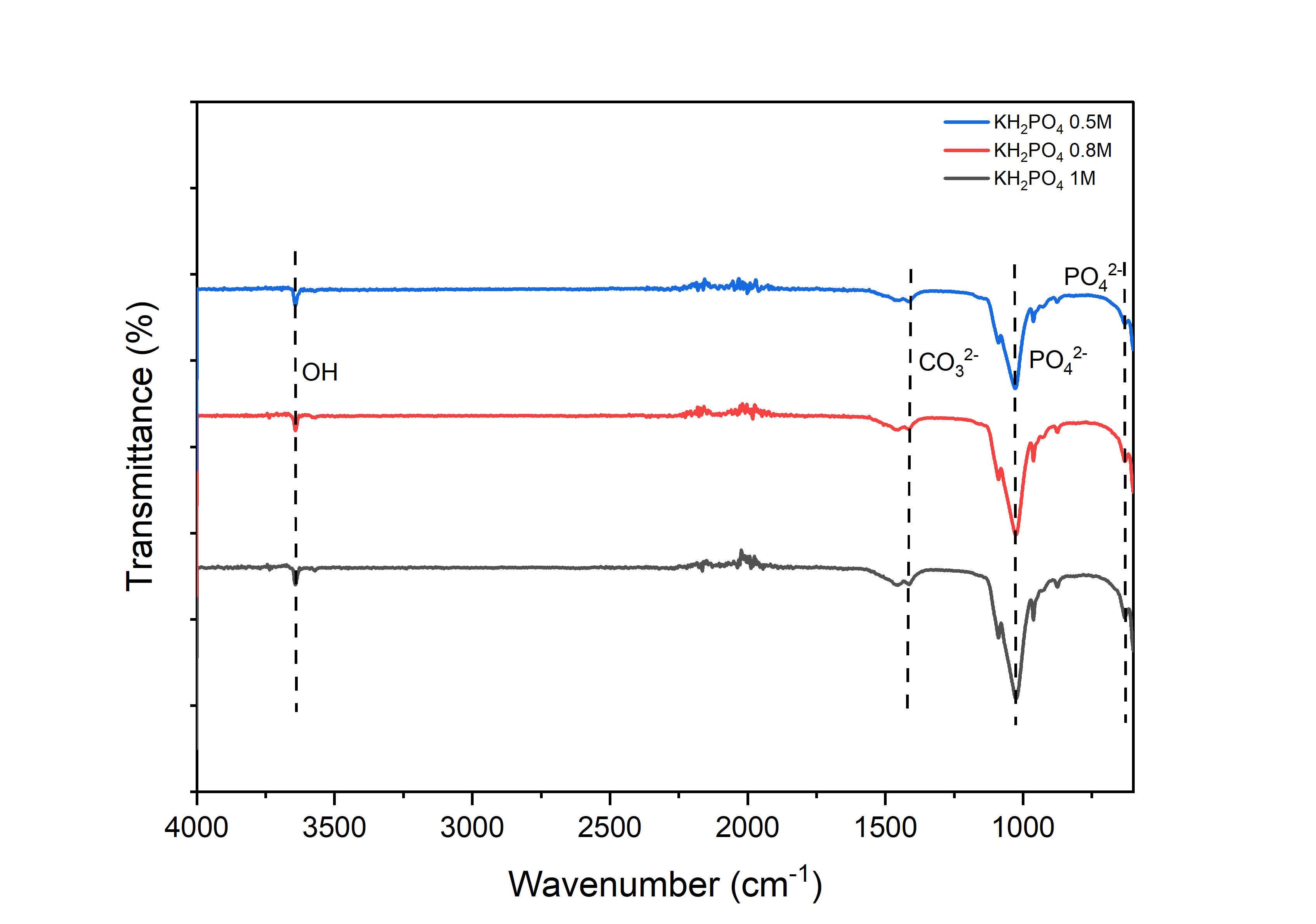
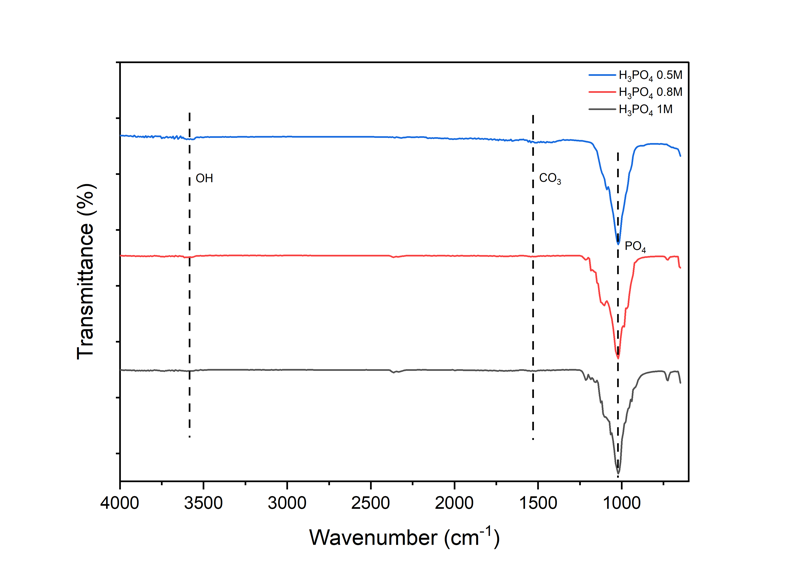
**TABLE 1.** XRF Analysis of calcined crab shells (P. pelagicus) at 1000°C

|  |  |  |
| --- | --- | --- |
| **Component** | **Sample crab shells 1** | **Sample crab shells 2** |
| Ca | 93.09 | 93.11 |
| Cu | 2.04 | 0.24 |
| Sr | 1.96 | 2.4 |
| P | 1.8 | 1.8 |
| S | 0.23 | 0.24 |
| Fe | 0.14 | 0.13 |
| Mn | 0.11 | 0.12 |

Based on the XRF analysis results presented in Table 1, the calcium content in crab shells (P. pelagicus) is notably high, reaching 93.09% and 93.11%, respectively. In comparison, untreated crab shells (P. pelagicus) typically contain 90.55% calcium, indicating that synthesized crab shells (P. pelagicus) are a promising calcium source for hydroxyapatite production [5], [6], [16,17].

## FTIR Analysis

After synthesizing hydroxyapatite (HAp) from crab shells, it was characterized using Fourier Transform Infrared (FTIR) spectroscopy to identify its functional groups. The FTIR spectrum in **Figure 1** shows absorption peaks corresponding to CO₃²⁻, PO₄³⁻, and OH⁻ groups in the synthesized HAp sample.



1. (b)

**FIGURE 1.** FTIR Spectrum of Hydroxyapatite Synthesis with (a) H₃PO₄ solution (b) KH2PO4 solution

The FTIR spectra of hydroxyapatite samples synthesized with varying concentrations of H₃PO₄ and KH2PO4 are presented in **Table 2.**

**TABLE 2.** Functional Groups of Hydroxyapatite Synthesis with H₃PO₄ and KH2PO4 Solutions

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Functional Group** | **H3PO4 Concentration** | | |  | | **KH2PO4 Concentration** | | |
| **0.5 M** | **0.8 M** | **1 M** | | **0.5 M** | | **0.8 M** | **1 M** |
| OH- | 3652.58 cm-1 | 3652.79 cm-1 | 3675.15 cm-1 | | 3641.96 cm-1 | | 3641,98 cm-1 | 3641,74cm-1 |
| CO32- | 1558.02 cm-1 | 1654.93 cm-1 | 1543.11 cm-1 | | 1558.02 cm-1 | | 1654.93 cm-1 | 1543.11 cm-1 |
| PO43- | 998.99 cm-1  872.18 cm-1 | 984.01 cm-1  879.65 cm-1 | 976.56 cm-1  849.89 cm-1 | | 998.99 cm-1  872.18 cm-1 | | 1028.74 cm-1  984.01 cm-1 | 1021.29 cm-1  976.56 cm-1 |

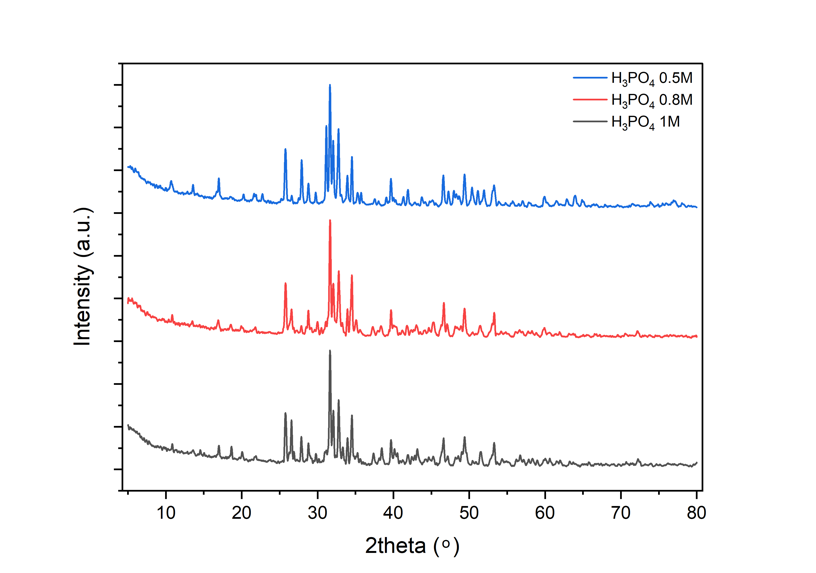
According to **Table 2**, The FTIR results indicate the presence of OH⁻, PO₄³⁻, and CO₃²⁻ functional groups at each concentration of H₃PO₄ and KH₂PO₄. The OH⁻ bonds are observed at wavenumbers between 3200 cm⁻¹ and 3600 cm⁻¹. The PO₄³⁻ functional group appears in the wavenumber range of 1100-550 cm⁻¹, while the CO₃²⁻ functional group shows absorption peaks in the range of 1700-1400 cm⁻¹ [9], [10], [11], [12]. The hydroxyl group arises from hydrogen bonding with the H-O-H functional group vibration [22]. The reduced intensity peaks of the hydroxyl group indicate water loss due to calcination or increased sintering temperature of hydroxyapatite. Additionally, carbonate (CO₃²⁻) functional groups are present in all crab shell hydroxyapatites, formed due to atmospheric CO₂ during synthesis. Although carbonate compounds are considered impurities in hydroxyapatite, their presence does not interfere with its function since human bones naturally contain 4-6% carbonate [23]. The presence of OH⁻, PO₄³⁻, and CO₃²⁻ functional groups in KH₂PO₄ and H₃PO₄ suggests the presence of hydroxyapatite in these compounds. This result is consistent with previous research by Ganachari et al. [24] and Panda et al. [25]. The presence of hydroxyl (OH) and carbonate (CO₃) absorption peaks in the IR spectra of H₃PO₄ has been reported previous studies investigating the synthesis of hydroxyapatite (HAp). the stretching vibrations of CO2−3 ions, confrming the elimination of carbon impurities due to the calcination of HAp sample at higher temperature.

From the FTIR results for both KH₂PO₄ and H₃PO₄ precursors at various concentrations, it is observed that there is no significant shift in the wave number of the functional groups. This indicates that the chemical bonding of the phosphate groups remains stable across the tested concentrations, suggesting that the increase or decrease in the concentration of these acids does not lead to substantial changes in the molecular structure of the phosphates. This indicates that variations in the concentrations of H₃PO₄ and KH₂PO₄ precursors do not significantly affect the molecular structure of the hydroxyapatite during synthesis.

## XRD Analysis

The hydroxyapatite (HAp) samples were analyzed using X-ray Diffraction (XRD) to assess their crystallinity following the addition of varying amounts of KH₂PO₄ and H₃PO₄, comparing them against the JCPDS database for HAp standards, as illustrated in **Figure 3.**

A graph of different colored lines

Description automatically generated

1. (b)

**FIGURE 3.** XRD Analysis of Hydroxyapatite Synthesis with (a) KH2PO4 Solution (b) H₃PO₄ Solution

According to de Wolf (JCPDS 09-0432), the characteristic peaks of hydroxyapatite appear at 2θ angles of 31.77°, 32.19°, and 32.90°, respectively. **In Figure 3**, the X-ray Diffraction (XRD) analysis results using KH₂PO₄ at 1000°C with synthesis concentrations of 0.5 M, 0.8 M, and 1 M show the main peaks at diffraction angles 2θ as follows: 31.74321°, 31.69424°, and 31.79219°. Conversely, testing with H₃PO₄ shows the main peaks at diffraction angles 2θ as follows: 31.62°, 32.06°, 32.73°, and 40.13° for 0.5 M concentration; 31.64°, 32.08°, 32.80°, and 40.09° for 0.8 M concentration; and 31.60°, 32.06°, 32.78°, and 40.01° for 1 M concentration. Both KH₂PO₄ and H₃PO₄ demonstrate that atoms are arranged regularly in a crystalline CaO form, albeit with slightly different peak positions [26], resembling the pattern of commercial hydroxyapatite characterization from JCPDS data (Card No. 09-432).

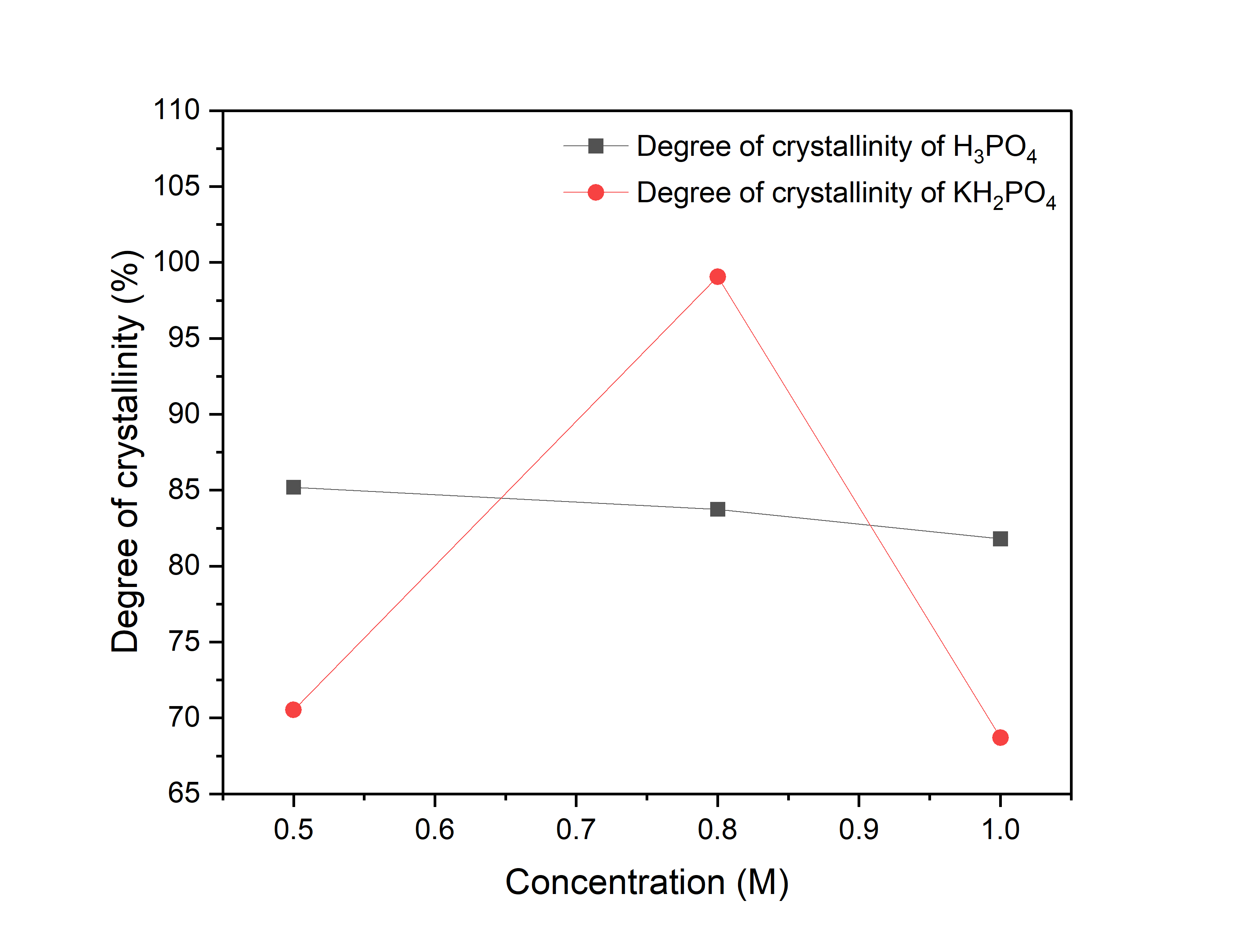
The crystallinity of hydroxyapatite is evaluated based on the intensity and Full Width at Half Maximum (FWHM) of the diffraction peaks. Higher peak intensity does not necessarily indicate higher crystallinity; instead, the crystallinity region of hydroxyapatite is characterized by the high intensity and width of the diffraction peaks. The FWHM values of the diffraction peaks significantly affect this characteristic. The calculation of hydroxyapatite crystallinity was performed using Origin Graphing Analysis software. The degree of crystallinity is further determined by comparing the crystalline area fraction with the total area fraction (crystalline and amorphous) [26]. Therefore, using both KH₂PO₄ and H₃PO₄ results in hydroxyapatite with similar crystalline structures despite minor differences in diffraction peak positions and intensities.

## XRD Analysis

The synthesized hydroxyapatite (HAp) was analyzed using X-ray diffraction (XRD) data analysis by comparing the crystalline area fraction to the total area fraction (crystalline plus amorphous). The results of the crystallinity analysis of HAp synthesized with H₃PO₄ and KH₂PO₄ are depicted in **Figure 4**.

**TABLE 4.** % Crystallinity of Hydroxyapatite Synthesis with H₃PO₄ and KH2PO4 Solutions

|  |  |  |
| --- | --- | --- |
| **Concentration** | **HAp synthesis with H₃PO₄ solution** | **HAp synthesis with KH2PO4 solution** |
| 0.5 | 85.2 % | 70.53 % |
| 0.8 | 83.75 % | 99.06 % |
| 1 | 81.79 % | 68.70 % |



**FIGURE 4.** Effect of H₃PO₄ and KH2PO4 Concentrations on the Crystallinity of Crab Shell Hydroxyapatite

Based on X-ray Diffraction (XRD) analysis, it is observed that increasing concentrations of H₃PO₄ lead to a decrease in the crystallinity degree of hydroxyapatite (HAp), specifically to 85.2% at 0.5 M, 83.75% at 0.8 M, and 81.79% at 1 M. Conversely, for KH₂PO₄, the highest crystallinity of 99.06% is achieved at a concentration of 0.8 M, indicating enhanced crystallinity with longer stirring times. The increase in KH₂PO₄ concentration contributes to the improved crystallinity of Ca₁₀(PO₄)₆(OH)₂ [27]. This high crystallinity is attributed to faster nucleation and crystal growth processes due to the high supersaturation of the synthesis solution. The decrease in crystallinity with H₃PO₄ is linked to its direct effect on the synthesis's calcium (Ca) content [26]. Lower pH values during synthesis, associated with increased H₃PO₄ concentrations, further contribute to reduced crystallinity [28].

According to FDA standards, the minimum required crystallinity degree for medical-grade hydroxyapatite is 62% [29], while native human bone exhibits crystallinity ranging from 69% to 87% [30]. Thus, the crystallinity of the synthesized samples meets the criteria for native bone. Moreover, crystallinity significantly affects osteoinduction (bone growth stimulation). Higher crystallinity values correlate with lower degradation rates, which typically enhance osteoinductive potential [19], [31]. Therefore, despite KH₂PO₄ demonstrating more significant variability than H₃PO₄ at specific concentrations, both samples exhibit crystallinity levels consistent with native bone.

**TABLE 5.** Hydroxyapatite Crystal Size (nm)

|  |  |  |
| --- | --- | --- |
| **Concentration** | **HAp synthesis with H₃PO₄ solution** | **HAp synthesis with KH2PO4 solution** |
| 0.5 | 58.34 | 70.53 |
| 0.8 | 48.87 | 49.41 |
| 1 | 41.67 | 61.78 |

According to the analysis results presented in **Table 5**, the crystal size of hydroxyapatite (HAp) decreases with increasing concentration of H₃PO₄. It was noted that in the hexagonal phase, the crystal size of HAp decreases with decreasing pH, which slows down the formation of HAp at lower pH levels [32]. The smallest recorded crystal size of HAp in this study is 41.46 nm. Nano-sized HAp crystals are preferred in medical applications due to their higher resorbability and bioactivity than micron-sized crystals [33]. Similarly, using KH₂PO₄, the crystal size ranges from 44.62 to 49.37 nm, also falling within the nano range. Nano-sized HAp crystals offer enhanced resorbability and bioactivity due to their larger contact surface area, facilitating greater chemical reactivity during HAp formation [33]. The crystal size inversely correlates with the Full Width at Half Maximum (FWHM) value; a smaller FWHM indicates a larger crystal size. The breadth of X-ray diffraction peaks, governed by the Scherrer formula, widens for crystals with particle sizes below 100 nm, enabling the average particle size estimation.

Analysis results in **Table 5**, the crystal size of hydroxyapatite (HAp) decreases with increasing concentration of H₃PO₄.  Stated that in the hexagonal phase, the crystal size of HAp decreases with decreasing pH, as the formation of HAp slows down at lower pH. The smallest recorded crystal size of HAp is 41.46 nm. In medical applications, nano-sized HAp crystals exhibit higher resorbability and bioactivity than micron-sized ones [33]. Meanwhile, with KH₂PO₄, the crystal size ranges from 44.62 to 49.37 nm, also in the nano range. Nano-sized HAp crystals exhibit higher resorbability and bioactivity. Nano-sized materials have a larger contact surface area, facilitating chemical reactivity in HAp formation. The crystal size is inversely proportional to the FWHM value; a small FWHM value indicates a larger crystal size. The breadth of X-ray diffraction peaks is influenced by crystal size, as indicated by the Scherrer formula. The diffraction curve profile widens for crystals with particle sizes less than 100 nm. The peak width of diffraction can be used to estimate the average particle size.

In conclusion, nano-sized HAp crystals formed using both H₃PO₄ and KH₂PO₄ solutions, which are advantageous for medical applications owing to their superior resorbability and bioactivity. However, crystals synthesized with H₃PO₄ tend to be smaller compared to those with KH₂PO₄, indicating that H₃PO₄ is more effective in producing nano-sized crystals. This smaller size potentially enhances the reactivity and performance of HAp in medical contexts.

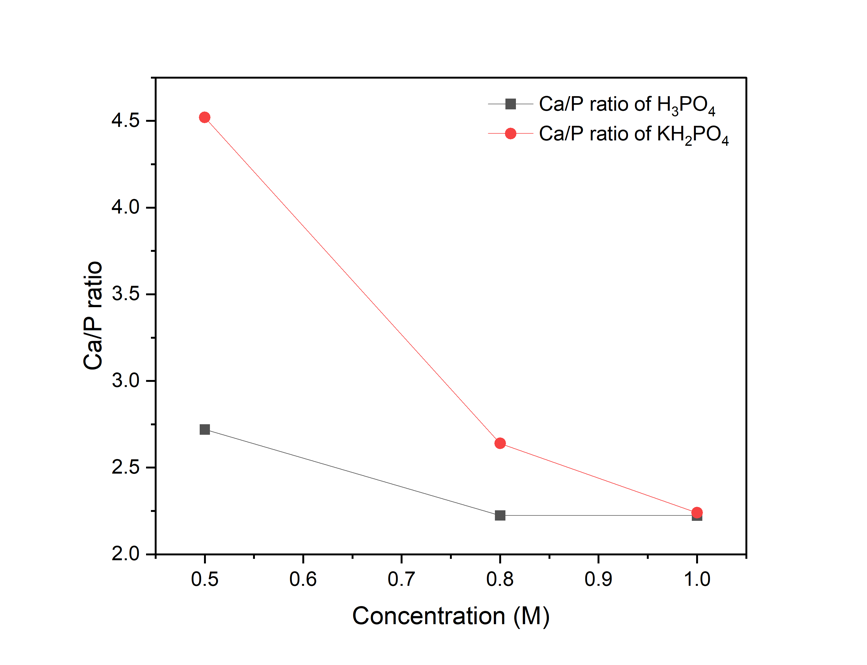
## EDX Analysis

Hydroxyapatite (HAp) synthesis results were analyzed using energy-dispersive X-ray spectroscopy (EDX) to determine the Ca/P ratio, which will be compared against FDA and ISO 1375:2015 standards. The EDX analysis results are presented in **Figure 5.**

**TABLE 6.** Ca/P Ratios of Hydroxyapatite Synthesis with H₃PO₄ and KH2PO4 Solutions

|  |  |  |
| --- | --- | --- |
| **Concentration** | **HAp synthesis with H₃PO₄ solution** | **HAp synthesis with KH2PO4 solution** |
| 0.5 | 2.72 | 4.52 |
| 0.8 | 2.224 | 2.64 |
| 1 | 2.223 | 2.24 |

As shown in **Table 6**, the Ca/P ratio results from EDX analysis of hydroxyapatite samples synthesized with varying concentrations of KH₂PO₄ and H₃PO₄ are illustrated in **Figure 5**.



**FIGURE 5.** Effect of H₃PO₄ and KH2PO4 Concentrations on the Ca/P Ratio of Crab Shell Hydroxyapatite

**Figure 5** indicates that the Ca/P ratio of the synthesized hydroxyapatite sample decreases with increasing concentrations of H₃PO₄ and KH₂PO₄ used in the synthesis process. This reduction is attributed to higher concentrations of H₃PO₄ and KH₂PO₄, which increase phosphate interaction with calcium, thereby reducing the relative amount of calcium reacted compared to phosphate. Moreover, higher concentrations of H₃PO₄ and KH₂PO₄ lower the pH, resulting in higher concentrations of H⁺ ions in the reaction solution [4]. This increase in HPO₄²⁻, formed from H⁺ and PO₄³⁻, further decreases the available calcium [34,35]. Among all variables, the optimal concentration appears to be 1 M H₃PO₄, which exhibits the lowest Ca/P ratio approaching the standard value of 1.67.

# CONCLUSION

Based on XRF analysis, crab shells (P. pelagicus) synthesized with KH₂PO₄ and H₃PO₄ exhibit elevated calcium levels, positioning them as promising sources for hydroxyapatite (HAp) production. Both treatments yield comparable calcium content, surpassing that of untreated crab shells. FTIR analysis reveals the characteristic absorption peaks corresponding to hydroxide (OH⁻), phosphate (PO₄³⁻), and carbonate (CO₃²⁻) groups in the synthesized hydroxyapatite samples. These functional groups are crucial for understanding the structural and chemical composition of the material. XRD analysis reveals that both treatments produce crystalline structures akin to commercial HAp, with KH₂PO₄ achieving higher crystallinity at 0.8 M concentration. However, higher concentrations of H₃PO₄ lead to decreased crystallinity. The crystal size of HAp is smaller with H₃PO₄, enhancing its effectiveness for medical applications due to increased resorbability and bioactivity. The Ca/P ratio declines with higher concentrations of both reagents, with 1 M H₃PO₄ demonstrating the optimal ratio closest to the standard value. In conclusion, both KH₂PO₄ and H₃PO₄ prove effective in generating nano-sized HAp crystals suitable for medical use.

## ACKNOWLEDGMENTS

The work was supported by Research, Innovation, and Entrepreneurship Proyek Higher Education for Technology and Innovation (HETI) ADB loan number 4110-INO 2023, Institut Teknologi Sepuluh Nopember, International Collaboration Research (ICR) Scheme under contact number 0007/01.PKS/PPKHETI/ITS/2023.

# REFERENCES

1. P.T. Sudheesh Kumar, S. Hashimi, S. Saifzadeh, S. Ivanovski, C. Vaquette, Additively manufactured biphasic construct loaded with BMP-2 for vertical bone regeneration: A pilot study in rabbit, Materials Science and Engineering: C 92 (2018) 554–564. https://doi.org/10.1016/j.msec.2018.06.071.

2. S. Mondal, T.P. Nguyen, V.H. Pham, G. Hoang, P. Manivasagan, M.H. Kim, S.Y. Nam, J. Oh, Hydroxyapatite nano bioceramics optimized 3D printed poly lactic acid scaffold for bone tissue engineering application, Ceram Int 46 (2020) 3443–3455. https://doi.org/10.1016/j.ceramint.2019.10.057.

3. F. Dewi, D. Fransiska, N. Dharmayanti, D. Aulia, Pengaruh Suhu Sintering pada Sintesis Hidroksiapatit dari Tepung CaO Cangkang Rajungan (Portunus sp.), 2023. http://ojs.umrah.ac.id/index.php/marinade.

4. E.O. Ningrum, I.S. Azhar, A.H. Tiwikrama, Blue Crab Shell Waste Valorization as A Source of Hydroxyapatite for Biocompatible Dental Implant Coating Fabrication, Rasayan Journal of Chemistry 17 (2024) 381–390. https://doi.org/10.31788/RJC.2024.1728696.

5. M. Trzaskowska, V. Vivcharenko, A. Przekora, The impact of hydroxyapatite sintering temperature on its microstructural, mechanical, and biological properties, Int J Mol Sci 24 (2023) 5083.

6. K. Benataya, M. Lakrat, O. Hammani, M. Aaddouz, Y. Ait Yassine, H.A. Abuelizz, A. Zarrouk, K. Karrouchi, E. Mejdoubi, Synthesis of High-Purity Hydroxyapatite and Phosphoric Acid Derived from Moroccan Natural Phosphate Rocks by Minimizing Cation Content Using Dissolution–Precipitation Technique, Molecules 29 (2024) 3854.

7. N. V Zhirenkina, M.A. Mashkovtsev, N. V Obabkov, A.S. Kosykh, S. V Bujnachev, A. V Ponomarev, The effect of pH of precipitation and the ratio of Ca/P on the properties of hydroxyapatite particles, in: AIP Conf Proc, 2019: p. 20077.

8. F.L. Muntean, I. Olariu, D. Marian, T. Olariu, E.L. Petrescu, T. Olariu, G.A. Drăghici, Hydroxyapatite from Mollusk Shells: Characteristics, Production, and Potential Applications in Dentistry, Dent J (Basel) 12 (2024) 409.

9. K.L. da Silva, D. Hortkoff, M.W. Favoreto, M. Rezende, J.M. Nadal, A. Armas-Vega, A.D. Loguercio, P.V. Farago, Preparation and Characterization of a Novel Hydroxyapatite-Capsaicin Composite Intended for the In-Office Dental Bleaching Use, Journal of Composites Science 8 (2024) 496.

10. D. Pham Minh, S. Rio, P. Sharrock, H. Sebei, N. Lyczko, N.D. Tran, M. Raii, A. Nzihou, Hydroxyapatite starting from calcium carbonate and orthophosphoric acid: synthesis, characterization, and applications, J Mater Sci 49 (2014) 4261–4269.

11. H.A. Batista, F.N. Silva, H.M. Lisboa, A.C.F.M. Costa, Modeling and optimization of combustion synthesis for hydroxyapatite production, Ceram Int 46 (2020) 11638–11646.

12. A. Yelten-Yilmaz, S. Yilmaz, Wet chemical precipitation synthesis of hydroxyapatite (HA) powders, Ceram Int 44 (2018) 9703–9710.

13. Guidance for Industry Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Rockville, 1997.

14. E.O. Ningrum, E.L. Pratiwi, I.L. Shaffitri, A.F.P. Putra, A.D. Karisma, S. Suprapto, Production of bone implant filaments from blue crab shells (Portunus pelagicus) in various synthesis conditions and blending ratios of hydroxyapatite (HAp)-polycaprolactone (PCL), IOP Conf Ser Earth Environ Sci 963 (2022) 012021. https://doi.org/10.1088/1755-1315/963/1/012021.

15. S. Rujitanapanich, P. Kumpapan, P. Wanjanoi, Synthesis of Hydroxyapatite from Oyster Shell via Precipitation, Energy Procedia 56 (2014) 112–117. https://doi.org/10.1016/j.egypro.2014.07.138.

16. D.E. Wiyono, S.A. Siregar, U. Zahroh Ma’mun, S. Rosanti, E.O. Ningrum, Prosiding Seminar Nasional Teknik Kimia “Kejuangan” Sintesis dan Karakterisasi Nano-Hidroksiapatit dari Cangkang Rajungan Sebagai Material Pembuatan Filament 3D Printing dengan Kombinasi Poly(caprolactone), (2023).

17. N.G. Romadhona, N.P. Syafira, T. Gumelar, V.F. Rizqiyah, E.O. Ningrum, Prosiding Seminar Nasional Teknik Kimia “Kejuangan” Sintesis dan Karakterisasi Hidroksiapatit Cangkang Rajungan dengan Variasi Suhu Kalsinasi dan Konsentasi KH2PO4 menggunakan Metode Presipitasi Sebagai Sediaan Biomaterial Implan Tulang, (2023).

18. I. Raya, E. Mayasari, A. Yahya, M. Syahrul, A.I. Latunra, Shynthesis and Characterizations of Calcium Hydroxyapatite Derived from Crabs Shells (*Portunus pelagicus*) and Its Potency in Safeguard against to Dental Demineralizations, Int J Biomater 2015 (2015) 1–8. https://doi.org/10.1155/2015/469176.

19. A. Sobczyk-Guzenda, P. Boniecka, A. Laska-Lesniewicz, M. Makowka, H. Szymanowski, Micro- and Nanoparticulate Hydroxyapatite Powders as Fillers in Polyacrylate Bone Cement—A Comparative Study, Materials 13 (2020) 2736. https://doi.org/10.3390/ma13122736.

20. M.T. Hooi, S.W. Phang, H.Y. Yow, E. David, N.X. Kim, H.L. Choo, FTIR spectroscopy characterization and critical comparison of poly(vinyl)alcohol and natural hydroxyapatite derived from fish bone composite for bone-scaffold, J Phys Conf Ser 2120 (2021) 012004. https://doi.org/10.1088/1742-6596/2120/1/012004.

21. R. Utami, D. Gustiono, M.D. Effendi, S. Roseno, H.D. Fahyuan, M.Z. Nasri, Synthesis and characterization of hydroxyapatite bioceramics from shells of serai snail and mangrove crab in Tanjung Jabung beach: effect of milling process, IOP Conf Ser Mater Sci Eng 1173 (2021) 012028. https://doi.org/10.1088/1757-899X/1173/1/012028.

22. B. Chudhuri, D. Bhadra, S. Dash, G. Sardar, K. Pramanik, B.K. Chaudhuri, Hydroxyapatite and Hydroxyapatite-Chitosan Composite from Crab Shell, J Biomater Tissue Eng 3 (2013) 653–657. https://doi.org/10.1166/jbt.2013.1126.

23. S.-C. Wu, H.-C. Hsu, S.-K. Hsu, Y.-C. Chang, W.-F. Ho, Synthesis of hydroxyapatite from eggshell powders through ball milling and heat treatment, Journal of Asian Ceramic Societies 4 (2016) 85–90. https://doi.org/10.1016/j.jascer.2015.12.002.

24. S. V Ganachari, A.A. Bevinakatti, J.S. Yaradoddi, N.R. Banapurmath, A.M. Hunashyal, A.S. Shettar, Rapid synthesis, characterization, and studies of hydroxyapatite nanoparticles, Adv Mater Sci Res. 2016; 1 (1): 9-13. 10 Adv Mater Sci Res 2016 Volume 1 Issue 1 (2016).

25. M. Panda, S. Joshi, O. Annalakshmi, B. Venkatraman, Optically stimulated luminescence properties of chicken eggshell derived hydroxyapatite for dosimetry applications, J Radioanal Nucl Chem (2024) 1–10.

26. G.M. Poralan, J.E. Gambe, E.M. Alcantara, R.M. Vequizo, X-ray diffraction and infrared spectroscopy analyses on the crystallinity of engineered biological hydroxyapatite for medical application, IOP Conf Ser Mater Sci Eng 79 (2015) 012028. https://doi.org/10.1088/1757-899X/79/1/012028.

27. E. Oktavia Ningrum, I. Safari Azhar, W. Ciptonugroho, S. Sabar, S. Suprapto, A. Dwitama Karisma, M. Josef Kridanto Kamadjaja, T. Anggi Margaretha, N. Ulayya Khoirummata’Addunya, S. Widiyanto, A Polycaprolactone-Hydroxyapatite (PCL/HAp) Scaffold, Prepared from Blue Crab Shell (Portunus Pelagicus) Waste, for Bone Substitution Applications, ChemistrySelect 9 (2024). https://doi.org/10.1002/slct.202303971.

28. M. Hermassi, C. Valderrama, J. Dosta, J.L. Cortina, N.H. Batis, Evaluation of hydroxyapatite crystallization in a batch reactor for the valorization of alkaline phosphate concentrates from wastewater treatment plants using calcium chloride, Chemical Engineering Journal 267 (2015) 142–152. https://doi.org/10.1016/j.cej.2014.12.079.

29. N. Fatimah, B. Utami, Sintesis dan Analisis Spektra IR, Difraktogram XRD, SEM pada Material Katalis Berbahan Ni/zeolit Alam Teraktivasi dengan Metode Impregnasi, JC-T (Journal Cis-Trans): Jurnal Kimia Dan Terapannya 1 (2017) 35–39. https://doi.org/10.17977/um026v1i12017p035.

30. Siswanto, D. Hikmawati, N. Benecdita, S. Nurmala, Synthesis of Hydroxyapatite Based on Nano Coral Using Precipitation Method for Bone Substitution, J Phys Conf Ser 1445 (2020) 012015. https://doi.org/10.1088/1742-6596/1445/1/012015.

31. M.T. Islam, R.M. Felfel, E.A. Abou Neel, D.M. Grant, I. Ahmed, K.M.Z. Hossain, Bioactive calcium phosphate–based glasses and ceramics and their biomedical applications: A review, J Tissue Eng 8 (2017) 204173141771917. https://doi.org/10.1177/2041731417719170.

32. D. Sánchez-Campos, M.I. Reyes Valderrama, S. López-Ortíz, D. Salado-Leza, M.E. Fernández-García, D. Mendoza-Anaya, E. Salinas-Rodríguez, V. Rodríguez-Lugo, Modulated Monoclinic Hydroxyapatite: The Effect of pH in the Microwave Assisted Method, Minerals 11 (2021) 314. https://doi.org/10.3390/min11030314.

33. Y. Rizkayanti, Y. Yusuf, Optimization of the temperature synthesis of hydroxyapatite from indonesian crab shells, 2019. https://www.researchgate.net/publication/330986497.

34. I.-H. Lee, J.-A. Lee, J.-H. Lee, Y.-W. Heo, J.-J. Kim, Effects of pH and reaction temperature on hydroxyapatite powders synthesized by precipitation, Journal of the Korean Ceramic Society 57 (2020) 56–64. https://doi.org/10.1007/s43207-019-00004-0.

35. V. Rodríguez-Lugo, T.V.K. Karthik, D. Mendoza-Anaya, E. Rubio-Rosas, L.S. Villaseñor Cerón, M.I. Reyes-Valderrama, E. Salinas-Rodríguez, Wet chemical synthesis of nanocrystalline hydroxyapatite flakes: effect of pH and sintering temperature on structural and morphological properties, R Soc Open Sci 5 (2018) 180962. https://doi.org/10.1098/rsos.180962.