Optimization of Hydroxyapatite Synthesis from Crab Shells (Portunus Pelagicus) via Ultrasonic Assisted Precipitation

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**Abstract.** This study focused on the effect of the concentration of H3PO4 and titration volume on the functional group characteristics and morphology of hydroxyapatite. Hydroxyapatite was synthesized using various concentrations of H₃PO₄ and titration volumes. The report demonstrated that the concentration of H3PO4 and the titration volume significantly affect mass change during synthesis. Increased H3PO4 concentrations and larger titration volumes were correlated with higher hydroxyapatite mass. Fourier-transform infrared (FTIR) spectroscopy consistently identified phosphate (PO43-) and hydroxyl (OH) groups, confirming successful synthesis. Energy-dispersive X-ray (EDX) analysis showed a decreased Ca/P ratio with increasing H3PO4 concentrations and titration volumes.

**Keywords**: *Dental Implants, Hydroxyapatite, Titration Volume*

# INTRODUCTION

Oral diseases, including tooth loss, represent a significant yet often overlooked global public health issue. Tooth loss is a crucial indicator of oral health deterioration, predominantly resulting from dental caries and periodontal disease. Over time, these conditions can lead to complete tooth loss, severely impacting oral health and overall quality of life[1]. The World Health Organization's (WHO) Global Oral Health Status Report of 2022 estimates that oral diseases affect 3.5 billion people worldwide, with three out of four affected individuals residing in middle-income countries. The global prevalence of total tooth loss among individuals aged 20 years or older is approximately 7%, rising to 23% among those aged 60[2]. Several treatment options are available to address tooth loss, including fixed dentures[3], removable dentures[4], and dental implants[5]. The primary differences between dentures and dental implants are their structure, functionality, longevity, and maintenance requirements. Implants are anchored into the jawbone, mimicking natural tooth roots, while dentures rest on the gums. Dental implants offer superior stability for biting, chewing, and speaking, whereas dentures may feel less natural and can impose limitations on chewing. Implants are designed to last a lifetime and help prevent bone loss, while dentures typically need replacement every five to seven years and may contribute to bone loss. Although implants involve higher initial costs, they are considered a long-term investment. Dentures are initially less expensive but incur additional costs over time. Choosing between implants and dentures depends on oral health, bone density, budget, and personal preference[6]. Dental implants are favored for their superior user comfort and aesthetic appeal, driving demand for advanced biomedical materials and health research[7].

One of the primary materials used in dental implants is hydroxyapatite. Hydroxyapatite, a member of the mineral apatite family, contains hydroxide and has the chemical formula Ca10(PO4)6(OH)2[8][9]. Its advantages include a chemical structure similar to bone and teeth, bioactivity, and the ability to act as a barrier against releasing metal ions from body tissues, making it an ideal biomaterial for dental implants[10]. Hydroxyapatite (HAp) benefits include its biocompatibility, good osteoconductivity, non-toxicity, non-immunogenicity, and ability to integrate with bone[11]. Hydroxyapatite can be synthesized from various calcium-containing materials, such as crab shells (Portunus pelagicus) [12][13]. Previous research indicates that crab shells (P. pelagicus), post-calcination, contain 91.96 ± 5.07% calcium, highlighting their potential as a raw material for hydroxyapatite. Utilizing crab shells (P. pelagicus) for hydroxyapatite production reduces waste and aligns with the Sustainable Development Goals (SDGs) by promoting green chemistry and technology through waste recycling[14].

Hydroxyapatite exhibits excellent biocompatibility and bioactivity due to its chemical composition, closely resembling human bone tissue[15][16]. It supports bone regeneration and remineralization, facilitating the optimal absorption of essential phosphate and calcium ions into the bones [17]. The most effective synthesis of HAp is achieved through ultrasonic precipitation using a water bath sonicator. This method offers several advantages, including simple chemical reactions, high particle size uniformity, economic feasibility, and ease of scalability, making it ideal for large-scale production [18]. Additionally, this method allows hydroxyapatite production in large quantities and with high purity[19]. The synthesis process involves reacting calcium hydroxide with other reactants to form hydroxyapatite (Ca10(PO4)6(OH)2). Factors such as temperature, precursor or reactant concentration, solute concentration, and titration rate significantly influence the quality of the resulting hydroxyapatite[20]. While much research has focused on the general synthesis of hydroxyapatite, few studies have explored the specific effects of varying concentrations and volumes of phosphoric acid (H₃PO₄) on the properties of the resulting HAp. Previous studies have primarily investigated the impact of synthesis parameters such as temperature and reactant type on the morphology and crystallinity of HAp. For instance, studies have shown that a higher concentration of H₃PO₄ leads to smaller particle sizes, while variations in titration rates affect the stoichiometric balance of calcium and phosphate, directly influencing the Ca/P ratio and bioactivity of HAp [21].

This research addresses the effects of different H₃PO₄ concentrations and titration volumes on the functional group characteristics, Ca/P ratio, color, and mass of the synthesized hydroxyapatite. The aim is to produce hydroxyapatite that adheres to medical standards, specifically for use as a biomaterial in dental implants. By providing new insights into the synthesis process, this study contributes to advancing the field of biomaterials, helping to optimize HAp production for clinical applications. Furthermore, this research enhances the understanding of how slight modifications in synthesis conditions can improve the quality and effectiveness of hydroxyapatite for medical use, ultimately benefiting the scientific community's exploration of biocompatible materials in regenerative medicine.

# Materials and Method

The materials utilized were crab shells (P. pelagicus) as a source of calcium oxide (CaO) and 85% orthophosphoric acid (H₃PO₄) as a precursor and source of phosphate. The synthesis of crab shell-derived hydroxyapatite began with the pretreatment of raw materials. This involved thoroughly washing the crab shells (P. pelagicus) to eliminate contaminants, continuing until the wash water ran clear. Subsequently, the crab shells (P. pelagicus) were dried in an oven at 80°C to remove moisture content. The final step in the pretreatment process was crushing the dried crab shells (P. pelagicus) to facilitate subsequent combustion. The calcination stage involved heating the crushed crab shells (P. pelagicus) in a furnace at 1000°C for 5 h to eliminate organic components and convert calcium carbonate into calcium oxide. The reaction occurring during the calcination process was as follows:

(1)

The synthesis of hydroxyapatite via the precipitation method started with the preparation of H₃PO₄ solutions at concentrations of 1.25 M and 1.4 M. This was achieved by dissolving 8.44 mL and 9.45 mL of 85% H₃PO₄ into distilled water to make a total volume of 100 mL. Next, 5.164 g of calcined CaO were dissolved in 500 mL of boiling distilled water to form a Ca(OH)₂ solution. The Ca(OH)₂ solution was then reacted with the previously prepared 100 mL H₃PO₄ solution by slowly adding the H₃PO₄ solution using a dosing pump in a sonicator water bath. Titration volume of H₃PO₄ solution precursor of 25, 50, 75, and 100 mL were applied during this process. The chemical reaction occurring during the formation of hydroxyapatite was as follows:

(2)

(3)

Upon completion of the reaction, the resulting solution was stirred using a magnetic stirrer at 80°C for 2 h to ensure homogeneity. The homogeneous solution was filtered through filter paper to obtain a white precipitate. This precipitate was washed three times with distilled water to remove any impurities. The washed precipitate was then sintered at 800°C for 4 h. The sintering process aimed to transform the hydroxyapatite constituents from amorphous to crystalline, thereby enhancing the material's structural integrity and suitability for biomedical applications, such as dental implants.

# Result and Discussion

## Hydroxyapatite Yield

The hydroxyapatite product was subsequently weighed to determine the final yield, utilizing the H3PO4 concentration and titration volume variables outlined in the research methodology. The yield of the hydroxyapatite synthesis are presented in **Figure 1.**

A graph of a number of points

Description automatically generated with medium confidence

**FIGURE 1**. Yield of Hydroxyapatite Synthesis in Various Titration Volumes

**Figure 1** illustrates that a lower titration volume results in a reduced mass of hydroxyapatite. The observed decrease in hydroxyapatite mass at lower titration volumes is explained by the limited availability of phosphate ions, which are crucial for the hydroxyapatite formation reaction. The stoichiometry of the reaction between calcium and phosphate requires a precise balance, and reduced phosphate availability disrupts this balance, yielding less hydroxyapatite. Conversely, as titration volumes increase, more phosphate is introduced, resulting in a higher reaction completion and increased product yield. This aligns with previous findings that emphasize the importance of reactant ratios in optimizing yield during hydroxyapatite synthesis [22]. Higher H3PO4 concentrations directly enhance hydroxyapatite formation due to an increased number of reactive phosphate ions. This is supported by studies showing that elevated precursor concentrations lead to improved nucleation and crystal growth, resulting in higher material output [23][24]. The relationship between phosphate ion availability and hydroxyapatite mass underscores the importance of titration and concentration adjustments for scalability in biomedical applications.

**FTIR Analysis**

The results of the hydroxyapatite synthesis include a characteristic test aimed at identifying the presence of functional groups within the material. **Figure 2** below illustrates the PO43- and OH- absorption peaks in the crab shell-derived hydroxyapatite product.

**A comparison of a graph

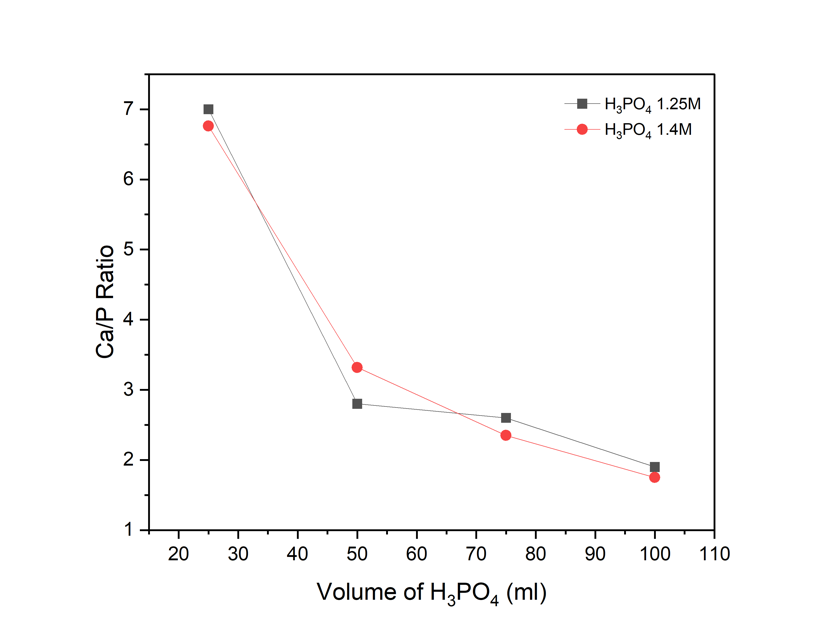
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**FIGURE 2.** Functional Group of Hydroxyapatite Synthesis with (a)1.25 M of H3PO4 and (b) 1.4 M of H3PO4 of Various Titration Volumes

Based on the FTIR analysis, **Figure 2** shows the presence of characteristic hydroxyapatite functional groups hydroxyl (OH-) and phosphate (PO43-) in crab shell-derived hydroxyapatite synthesized with H3PO4 concentrations of 1.25 M and 1.4 M and titration volumes of 25, 50, 75, and 100 mL. The hydroxyl (OH-) functional group is observed in the 3600-3700 cm-1 wave number range, attributed to hydrogen bonding and H-O-H functional group vibrations. The phosphate (PO43-) functional group is detected within the 900-1100 cm-1 range. This finding is consistent with previous research [25], indicating that the phosphate functional group appears in 600-420 cm-1, 990-950 cm-1, 1100-1020 cm-1, and 2200-1990 cm-1. The presence of the phosphate group (PO43- ) confirms the successful reaction between calcium and phosphate during the hydroxyapatite synthesis [26].

## EDX Analysis

Ca/P ratio analysis was conducted on the synthesized hydroxyapatite using EDX analysis. This analysis aimed to determine the Ca/P ratio of hydroxyapatite according to the standards set by the FDA and ISO 1375 in 2015, which is 1.67. The results of the EDX analysis are depicted in **Figure 3.**

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**FIGURE 3.** Comparative Variations Concentration H3PO4 and Volume Titration on the EDX Analysis

**Figure 3** indicates that higher concentrations of H3PO4 result in a lower Ca/P ratio [20]. This is because increased H3PO4 concentration leads to more phosphate reacting with calcium, thus reducing the relative amount of calcium compared to phosphate and consequently decreasing the Ca/P ratio[27]. Additionally, increasing the volume of H3PO4 can lower the pH, thereby increasing the concentration of H+ ions in the H3PO4 reactant, which consists of H+ and PO43-. This heightened concentration of H+ ions significantly reduces Ca content, further lowering the Ca/P ratio[28].

# Conclusion

Based on the research findings, the concentration of H3PO4 and the titration volume significantly influence the mass change. Higher concentrations of H3PO4 and larger titration volumes leads to an increased hydroxyapatite mass. FTIR analysis of all concentration consistently identifies phosphate (PO43-) and hydroxyl (OH-) groups within the hydroxyapatite structure, confirming successful synthesis. Furthermore, EDX analysis also showed a decrease in the Ca/P ratio with increasing concentrations of H3PO4 and titration volumes during hydroxyapatite synthesis.

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