Synthesis of Hydroxyapatite Nanoparticles Using Eggshells and Two Different Phosphate Sources

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ABSTRACT

Hydroxyapatite, the primary bone substitute, has gained increased interest of researchers. The primary purpose of this study was to synthesize hydroxyapatite nanoparticles through the utilization of eggshell waste and two distinct phosphate sources ((NH₄)₂HPO₄ and Na₃PO₄.12H₂O). The chemical precipitation approach was chosen for this study, then the final products were named HAP-1 and HAP-2. Following this, the synthesized particles were investigated using XRD, FTIR, and SEM methods. The crystallite size of the synthesized materials was also determined by employing the Debye-Scherrer equation. The synthesis of the HAP granules was confirmed through XRD analysis, and the crystallite sizes of HAP-1 and HAP-2 were calculated to be 37.79 nm and 28.7 nm, respectively. The SEM results also indicates that the powders had a spherical-like morphology, but were highly agglomerated due to their nanoscale dimensions.

Keywords: Biomaterials, Chemical synthesis, Eggshell, Hydroxyapatite.

1. INTRODUCTION

Hydroxyapatites, bio ceramics belonging to the calcium phosphate family, with a hexagonal structure [1], pH in the range of 4 to 12 [2] and chemical composition similar to that of natural bone, dentin, and enamel have gained significant interest in the field of dentistry and clinical applications, including implant fabrication and bone replacement [1], [3]–[4], hydroxyapatites are ideal composition for forming a stable bond with living tissues that is resistant to dissolution, rejection, irritation, and breakage [1], [3], [4], [6]–[10].

The density of hydroxyapatite ranges from 3.14 to 3.16 g/cm³ [1]. Hydroxyapatite’s natural composition is Ca₁₀(PO₄)₆(OH)₂; however, the most frequently employed formula is Ca₁₀(P₂O₆)(OH)₂, indicating the presence of two entities in unit crystal structure [11]. The stoichiometric ratio of Ca to P is 1.67, which is a crucial parameter in HAP synthesis [3], [12].

A multitude of natural calcium sources, including bovine bones [13]–[16], fish bones [17], corals [6], [7], [12], [18]–[23], marine shells [24], and eggshells [18]–[20], [22]–[24], have been employed in the synthesis of hydroxyapatite by numerous researchers. Eggshell comprises approximately 11% of the egg’s total weight and is composed of 1% magnesium carbonate, 94% calcium carbonate, 1% calcium phosphate, and 4% organic materials [4]. About 250,000 tons of eggshell waste are generated annually only by the culinary industry on a global scale. Despite the utilization of eggshells as fertilizers due to their high calcium and nitrogen content, the majority of them are discarded. Approximately 80% of them are incinerated, a process in which carbon dioxide is generated [25]. The synthesis of hydroxyapatite from eggshell is not only economical but also ecologically sustainable. In addition, eggshell exhibits greater body biocompatibility throughout the implantation process [10].

Numerous methods are applied in the synthesis of Hydroxyapatite nanoparticles, including solid-state, acid-base, precipitation, sol-gel, hydrothermal, mechanochemical, plasma techniques, layer hydrolysis of calcium phosphate combustion, and other wet chemistry methods [3], [5], [21]. Wet chemistry and precipitation are extensively employed in the production of HAP powders due to their cost-effectiveness, straightforward methodology, and ability to precisely control particle size, morphology, and chemical composition [1], [26].

In this study, chemical precipitation was utilized as a method to synthesize the hydroxyapatite and subsequently, XRD, FTIR, and SEM were chosen as methods to investigate the powders that were synthesized.
2. Materials and Methods

2.1. Materials

Hydrogen Peroxide 35% (Pasha), Di ammonium Hydrogen Phosphate (Merck Code 101126), Trisodium phosphate dodecahydrate (Merch Code 106572), Acetic Acid 100% (Merch 100056), eggshell waste.

2.2. Characterization Method

X-ray diffraction is applied to investigate whether the hydroxyapatite is synthesized or not. It was carried out using PHILIPS-binary instrument and X-ray generated from Cu-Kα with the wavelength of 1.54056 Å. The angle of diffraction (2θ) varied from 6 to 120.

In order to calculate the crystallite size Debye-Sherrer equation \( t = \frac{K \lambda}{B \cos \theta} \) was applied, in which it stands for crystallite size, \( K \) is a numerical factor which refers to the crystallite-shape (0.9), \( \lambda \) is the wavelength of X-ray (1.54056 Å) and \( B \) is the width of half maximum of diffraction peak and \( \theta \) is the Bragg angle.

Fourier Transform Infrared Spectroscopy (FTIR) using instrument model Equinox 55 made by BRUKER company in the range of 400–4000 cm\(^{-1}\) was used in order to detect the chemical bonds of the synthesized powder.

Microstructure characterization was done using Scanning Electron Microscopy with VGA/TESCAN instrument.

2.3. Synthesis Process

Eggshells were boiled in a solution of hydrogen peroxide to eliminate any organic compounds, and then they were dried at 100 °C for 10 h. In the subsequent step, the eggshells were ground to a size lower than 63 μ. To synthesize HAP, the precipitation process was selected as the synthesis method. In order to carry out this approach, it is needed to first obtain a solution of calcium source and then another solution of phosphate source separately. Acetic acid was utilized as a suitable solvent for calcium carbonate (CaCO\(_3\)) because CaCO\(_3\), the primary component of eggshells, is insoluble in water, while Calcium Acetate- the product of the reaction that took place between calcium carbonate and acetic acid- is soluble in water.

In order to synthesize calcium acetate, firstly, the weights of raw materials were calculated using stochiometric ratio. Then, acetic acid solution more that stochiometric ratio were gradually added to the suspension of eggshells in deionized water boiling and stirring on magnetic heater-stirrer. The container then covered by an aluminum foil to avoid evaporation of acetic acid until the reaction was completed and a transparent liquid was produced. In the next step, the solution was centrifuged, and precipitations were removed. The separated solution was then boiled until the total solvent was evaporated, and then the residue was dried at 100 °C for 12 h. The dried product then was grinded to be used in the next step of synthesizing hydroxyapatite.
Synthesis of Hydroxyapatite Nanoparticles Using Eggshells

For the first sample, which is named HAP-1, Diammonium hydrogen phosphate \((\text{NH}_4)_2\text{HPO}_4\) was used as a phosphate source. Based on the stochiometric ratio of \(\text{Ca}/\text{P} = 1.67\), the weight of calcium acetate produced in the previous step and the weight of Diammonium hydrogen phosphate were calculated, and then the solution of each precursor was prepared in deionized water. In the next step, the solution of di ammonium hydrogen phosphate was gradually added to the solution of calcium acetate stirring on the magnetic stirrer. Precipitation resulted from this reaction was centrifuged, filtered, dried at 100 °C for 12 h and finally ground to less than 63 μ.

The same procedure was applied for Trisodium phosphate dodecahydrate \((\text{Na}_3\text{PO}_4.12\text{H}_2\text{O})\) and the resulted precipitation from reaction between Trisodium phosphate dodecahydrate and calcium acetate was named HAP-2. Finally, after 12 h of drying at 100 °C, these products were ground to be characterized by applying various methods.

### 3. Result and Discussion

#### 3.1. XRD

The X-ray diffraction images of synthesized HAP-1 and HAP-2 are shown in Fig. 1. The peaks observed in the XRD patterns indicate that the synthesis of hydroxyapatite nanoparticles was accomplished successfully, in accordance with standard card ICDD number 96-101-1243.

The pH of HAP-1 is approximately 5.6 in the acidic range, whereas HAP-2 is approximately 11 in the alkaline range. Due to the \(\text{Na}^+\) ion from the precursor and the ability to absorb more \(\text{CO}_2\) from the environment, HAP-2 particles have a higher pH. An increased pH value may also lead to increased carbonate ion absorption [27], [28]. Additionally, the crystallite sizes of the hydroxyapatites that were synthesized were determined to be 37.79 nm and 28.7 nm, respectively, using the Debay-Sherrer equation. Furthermore, the widespread peaks observed in the XRD patterns indicate that the powders synthesized are nanocrystalline.

#### 3.2. FTIR

The FTIR patterns of HAP-1 and HAP-2 are depicted in Figs. 2a and 2b, respectively. The bending modes \((\nu_{4a}\) and \(\nu_{4b}\)) of the phosphate group are represented by the bands at 601.98 cm\(^{-1}\) and 565.64 cm\(^{-1}\) in Fig. 2a, and the bands at 603.11 cm\(^{-1}\) and 566.84 cm\(^{-1}\) in Fig. 2b. Furthermore, the bands observed at approximately 1034 cm\(^{-1}\) correspond to the stretching mode \((\nu_{3a})\) of the phosphate group as it appears in the crystal structure of HAP. The hydroxyl group stretching mode \((\nu_s)\) is attributed to the bands near 3434 cm\(^{-1}\) in the crystals of both HAP-1 and HAP-2. The presence of weak bands at approximately 874 cm\(^{-1}\) in both figures may also indicate the existence of a trace quantity of \(\text{HPO}_2\)\(^{2-}\), which can occasionally exist as a structural defect in the HAP crystal. Moreover, both spectra exhibit weak bands at approximately 1631 cm\(^{-1}\), which is attributed to the bending mode of absorbed water molecules. This band is typical of the majority of FTIR spectra [2].

The FTIR spectrum of HAP-2 (Fig. 2b) exhibits an additional band at 1466.54 cm\(^{-1}\), which represents the stretching mode of the carbonate group in the crystal structure of HAP-2, according to Koutsopoulos [2]. The HAP in alkali range tends to absorb more \(\text{CO}_2\) from the ambient, which leads to incorporation of carbonate ions \((\text{CO}_2\)\(^{2-}\)) in the HAP-2 structure [27], [28]. Carbonate ions are frequently substituted for hydroxyl or phosphate groups in HAP crystals; the former is referred to as A-type CHAP, while the latter is called B-type. Additionally, mixed AB-type is documented in bone minerals. This band proves that HAP-2 is classified as an A-type CHAP [2], [29].

#### 3.3. SEM

Figs. 3a and 3b show the scanning electron microscopy pictures of HAP-1 and HAP-2, respectively. As it is shown the morphology of the synthesized powders are quasi-spherical which is highly agglomerated due to their small particle size in the nanometer range.
4. CONCLUSION

HAP nanoparticles were successfully synthesized using eggshell as calcium source and the used phosphate sources. The particles and crystallite size of produced powder from Na₃PO₄·12H₂O were smaller than that made from (NH₄)₂HPO₄. The pH of HAP-2 was higher than HA-1 and in alkaline range due to the presence of Na⁺ in the structure while HAP-1 was in acidic range. The higher pH led to absorption of more carbonate ions in the lattice and resulted in an extra band of carbonate in the FTIR spectra which is replaced with the phosphate group of HAP-2 structure. The quasi-spherical particles of synthesized HAP tended to be agglomerated due to their nano size.

REFERENCES


