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Synthesis and Characterization of Nano-Hydroxyapatite Particles by Using Co-Precipitation Method for Bone Scaffold

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ABSTRACT

Hydroxyapatite has become an important material for bone scaffold fabrication. Various method has been investigated in developing nano-hydroxyapatite that mimicking the native bone properties. Co-precipitation approach is the best synthesis method due to capability in producing hydroxyapatite with controlled size and morphology. However, to determine the parameters in producing good hydroxyapatite has become a challenge. Therefore, the aim of this study is to synthesis the nano-hydroxyapatite with right size and composition via co-precipitation method. In this study, calcium nitrate tetrahydrate and ammonium phosphate dibasic were mixed for 3 hours at pH11 before left ageing for 24 hours. The morphology of the hydroxyapatite nanoparticle was in rod-like look as observed by Transmission Electron Microscopy (TEM). The average diameter of the hydroxyapatite nanoparticle was 8.49 ± 0.36 nm, whereas the average length was 37.46 ± 1.86 nm. Energy Dispersive X-ray Spectroscopy (EDX) analysis yield the result of Ca/P ratio of 1.43 which is close to the stoichiometry of hydroxyapatite, 1.67. Produced nanoparticle confirmed co-precipitation method is the best choice in synthesizing nano size hydroxyapatite that suitable for bone tissue engineering application

INTRODUCTION

Bone is a complex composite material consisting of 50-70% inorganic materials, primarily hydroxyapatite, 20-40% organic components, predominantly type I collagen, 5-10% water, and approximately 3% lipids (Christy et al., 2020; Collins et al., 2021). Therefore, hydroxyapatite has become an important component in many biomedical applications such as dental and bone implants. Hydroxyapatite ($Ca_{10}(PO_4)6(OH)_2$) is a popular calcium phosphate in biomedical fields due to its non-toxicity, bioactivity, biocompatibility, non-inflammatory and non-immunogenicity, biodegradability, osteoconductivity, and stability under physiological conditions (Salahuddin, Ibrahim & El-Kemary, 2023). Hydroxyapatite is commonly used as a bioactive coating for dental and orthopedic implants due to its chemical and structural similarities to bone and teeth, as well as its high osteoconductivity and inductivity (Zhou & Lee, 2011; Ielo et al., 2022).

Numerous hydroxyapatite nanostructured materials of various forms have been developed utilizing diverse processes to meet the needs of biomedical applications. The preparation processes have a considerable impact on the physicochemical properties, chemical content, structure, morphology, size, and crystallinity of synthetic hydroxyapatite materials (Biedrzycka et al., 2021; Salahuddin et al., 2022). The synthesis techniques of hydroxyapatite can be divided into four main groups: dry method, wet method, high-temperature processes, and synthesis methods based on biogenic sources or bioinspired approaches, as shown in Figure 1. The wet technique is a popular method for yielding hydroxyapatite due to its low cost, production of water as the only residue, and crystallinity similar to bone tissue, making it biocompatible (Rosa et al., 2022).

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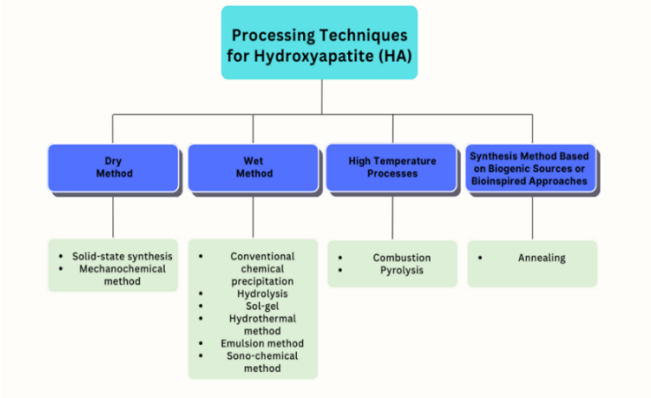


Fig.1 Processing techniques for hydroxyapatite

The co-precipitation method is prevalent because it allows for the development of large amounts of hydroxyapatite at a low cost (Santos et al., 2004; Pu'ad et al., 2020). Chemical precipitation usually requires multiple procedures. First, calcium- and phosphate-containing reagents, such as calcium hydroxide or calcium nitrate as the Ca^{2+} source and orthophosphoric acid or diammonium hydrogen phosphate as the PO_4^{3-} source, were combined according to the molar ratio of hydroxyapatite. The pH of the combination was then adjusted, usually to an alkaline level, while the temperature was kept between room temperature and the boiling point of water (Catros et al., 2010; Huang et al., 2011; Pu'ad et al., 2020). The solution was then agitated to allow aging, after which the precipitates were washed, filtered, and dried before being crushed into powder (Sadat-Shojai et al., 2013; Pu'ad et al., 2020).

Researchers have explored the possibility of synthesizing nano-hydroxyapatite (nHA), which is identical to bone hydroxyapatite. Nano-hydroxyapatite with a particle size of 1-100 nm has favorable features such as a larger surface area to enhance osteoblast function, close contact with surrounding tissues, strong biological activity, and the ability to address the issue of low biodegradability (Shahrezaie et al., 2017; Kubasiewicz-Ross et al., 2017; Mo et al., 2023). The smaller particle size of nano-hydroxyapatite contributed to superior mechanical performance and degradation rates, improving the scaffold's overall efficiency (Rodovalho et al., 2024). The precipitation method is one of the most common ways to synthesize nano-hydroxyapatite. The co-precipitation process produced needle-shaped nano-hydroxyapatite compared to other synthetic methods (Zhang & Gonsalves, 1997; Shokrzadeh et al., 2023).

This procedure involved the reaction of calcium and phosphate sources in an aqueous solution, which resulted in the synthesis of hydroxyapatite. Typically, the reagents were introduced drop by drop, with continuous stirring and mild conditions, to ensure that the molar Ca/P ratio remained at approximately 1.67 (Sadat-Shojai et al., 2013; Fiume et al., 2021). This approach proved beneficial since it was simple and inexpensive, allowing for the manufacture of hydroxyapatite with controlled particle size and morphology. Mobasherpour et al. (2007) used calcium nitrate tetrahydrate (Ca(NO3)2.4H2O) and diammonium hydrogen phosphate ((NH4)2HPO4) as starting materials to synthesize HA by chemical precipitation. The pH was adjusted by adding an ammonia solution to a calcium nitrate solution, followed by gradually adding a diammonium hydrogen phosphate solution, bringing the total pH to 11. The powder was synthesized by centrifuging and calcining the fluid for an hour, resulting in the successful synthesis of spherical-like hydroxyapatite with diameters of 8-20 nm (Mobasherpour et al., 2007).

Therefore, the chemical precipitation approach necessitated specific processing parameters for the production of hydroxyapatite. One important parameter was that the chemical precursor must be selected based on the HA molar ratio. However, an imbalanced molar ratio in the chemical precursor could lead to the formation of an alternative compound. Secondly, the challenge was to find the ideal pH for hydroxyapatite formation (Pu'ad et al., 2020). Therefore, the purpose of this study was to investigate the formation of hydroxyapatite particle slurry using the co-precipitation method. TEM and EDX were used for comprehensive analyses. The Ca/P ratio of the synthesized hydroxyapatite was determined using EDX analysis, whereas the average diameter and length of the generated particles were measured using TEM analysis.

MATERIALS AND METHOD

This section discussed the materials and methodology used to produce hydroxyapatite nanoparticles

Materials

Chemicals and reagents were purchased from the following manufacturers. Ammonium phosphate dibasic and calcium nitrate tetrahydrate were purchased from Sigma-Aldrich. Sodium hydroxide was purchased from Merck.

Co-Precipitation of Hydroxyapatite

The co-precipitation method was used to create the hydroxyapatite slurry. Fig. 2 below shows the procedures involved in the co-precipitation of hydroxyapatite. First, 2.36 g of calcium nitrate tetrahydrate was dissolved in 10 ml of deionized water, and the pH was maintained at 11. Next, 0.79 g of ammonium phosphate dibasic was dissolved in 10 ml of deionized water, and the pH was regulated between 11 and 12. This solution was introduced dropwise into the calcium nitrate tetrahydrate solution while continuously stirring, and the pH was maintained at 11 by adding sodium hydroxide. The solution was stirred for 3 hours to obtain a homogeneous solution. After three hours of stirring, the solution was permitted to age overnight. Following that, the generated solution was withdrawn from the flask, and the precipitate was washed three times with deionized water to produce a clear hydroxyapatite slurry.

Characterization of Hydroxyapatite

Transmission Electron Microscopy (HR-TEM, HT7700, Hitachi, Japan) was utilized to measure hydroxyapatite particle size in length and diameter. TEM is a useful technique for examining hydroxyapatite, a key mineral component in bone tissue. In this study, TEM was used to investigate hydroxyapatite and offered valuable information about its structure and morphology. It revealed structural characteristics at the nanometer scale, indicating that hydroxyapatite met the requirements for biological usage. The length and diameter of hydroxyapatite were determined using TEM images. The HA elemental analysis was performed using Energy Dispersive X-ray Spectroscopy (EDX). EDX provides qualitative and semi-quantitative information about the elemental composition of

HA. EDX was performed to determine the Ca/P ratio of HA generated.

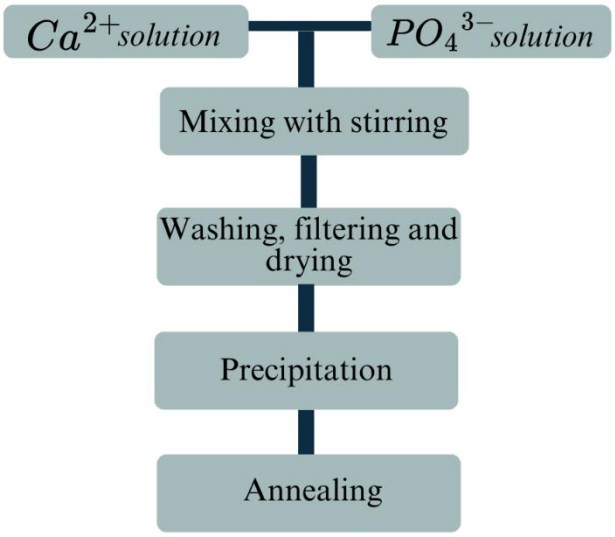


Fig. 2 Co-precipitation procedure

RESULTS AND DISCUSSION

This section deliberated on the morphology and characterization of the produced hydroxyapatite nanoparticles, with the first section including the TEM results followed by the EDX analysis.

Characterization of Hydroxyapatite

TEM image analysis

The hydroxyapatite slurry was observed under a TEM microscope to analyze the length and diameter of hydroxyapatite nanoparticles. Fig. 3 below shows the hydroxyapatite particles obtained through TEM. According to the image below, hydroxyapatite particles exhibited a rod-like structure, consistent with findings from Huang et al. (2003) using the co-precipitation method. From the image, analysis of the length and diameter of hydroxyapatite was recorded in Figure 4 (a) and (b).

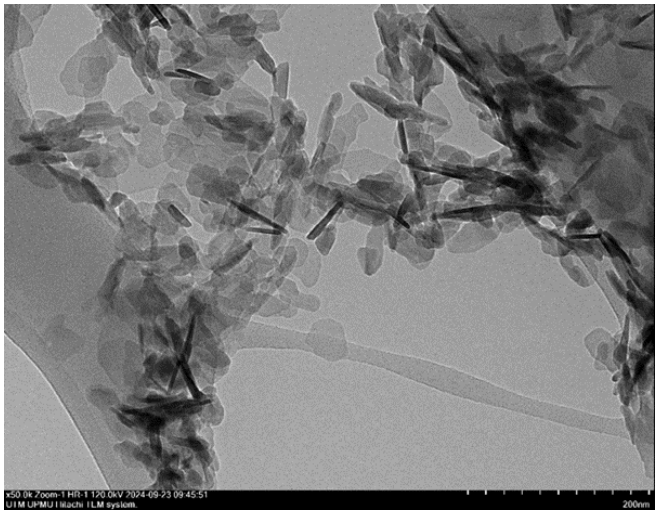
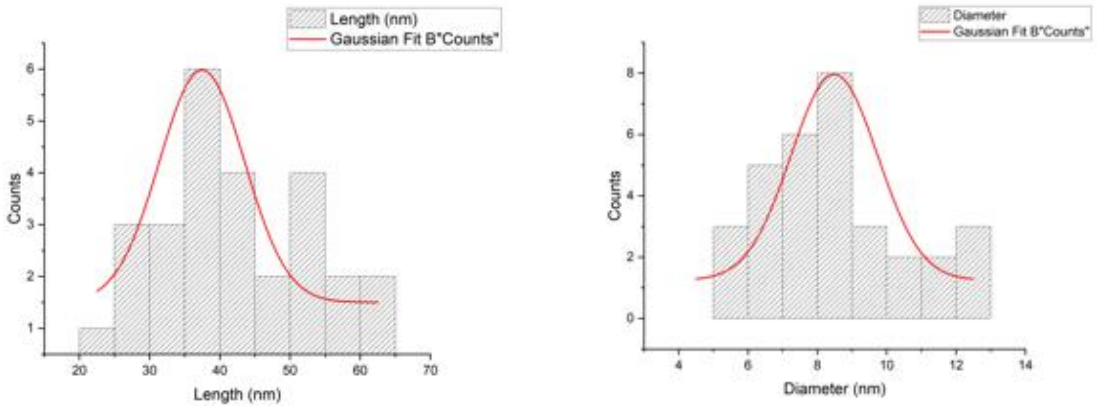


Fig. 3 TEM micrographs of hydroxyapatite nanoparticles

Fig. 4(a) depicted the Gaussian distribution of hydroxyapatite (HA) nanoparticles, which had an average particle length of 37.46 ± 1.86 nm. This low standard deviation indicated a high level of consistency in particle length across the sample. Notably, the reported maximum length, 60 to 70 nm, was within the indicated HA nanoparticle range of 60-100 nm, as stated by Huang et al. (2003). This meant that, while the majority of particles had lengths near the mean, a minority exceeded the expected range, indicating considerable variability in particle elongation within the sample. The average diameter, according to the research, was 8.49 ± 0.36 nm. Individual particle diameters ranged from 3 to 13 nm, which was in close agreement with the known diameter standards for HA particles, which were generally rod-shaped. The limited variability in diameter measurements suggested homogeneity in particle thickness, and this range supported the notion that the HA particles in this sample retained their distinctive rod-like morphology. The produced particles were consistent in size and shape with hydroxyapatite nanoparticles commonly utilized in biomedical applications. This was supported by the fact that,



(a) (b)

Fig. 4 (a)Gaussian distribution on HA nanoparticles length (b)Gaussian distribution on HA nanoparticles diameter

overall, the length and diameter distributions matched well with accepted standards for HA nanoparticles. This uniformity affected characteristics like compatibility and bioactivity, making it essential for medical applications needing regulated particle size and shape.

Energy Dispersive X-ray Spectroscopy (EDX) analysis

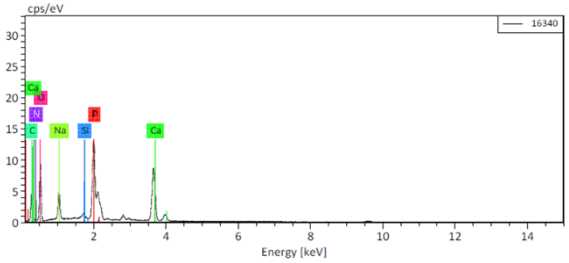


Fig. 5 EDX spectra of hydroxyapatite nanoparticles

The elemental composition of the hydroxyapatite (HA) nanoparticles was determined using Energy Dispersive X-ray Spectroscopy (EDX), and the resulting spectra were shown in Figure 5. EDX detected the presence of essential elements, identifying separate peaks for calcium (Ca), phosphorus (P), carbon (C), oxygen (O), nitrogen (N), and sodium (Na). Calcium and phosphorus were particularly important because they constituted the basic components of HA nanoparticles. The presence of Ca and P peaks in the EDX spectra confirmed the effective production of HA particles with the desired composition. Table 1 presented quantitative data from the EDX examination, which demonstrated that the Ca/P ratio was calculated to be 1.43. This ratio was slightly lower than the optimal Ca/P molar ratio of 1.67 for stoichiometric hydroxyapatite (Sadat-Shojai et al., 2013), but it was close enough to meet the compositional requirements for applications such as bone scaffold technology.

Table 1 Ca/P ratio for hydroxyapatite nanoparticles

Elements	Atomic (%)	Ca/P ratio
Calcium (Ca)	8.79	1.43
Phosphorus (P)	5.98	

CONCLUSION

This study wrapped the methods used to produce nano-hydroxyapatite by using the co-precipitation method, and the results demonstrated that the size of the particles was 37.46 ± 1.86 nm in length and 8.49 ± 0.36 nm in diameter. The produced nano-hydroxyapatite was more suitable for bone scaffold uses compared to hydroxyapatite since the produced diameter aided in biological activity in the tissue engineering field. It was recommended that future work involve incorporating the particles into the scaffold and assessing the in-vitro biocompatibility with the produced nano-hydroxyapatite particles. A more comprehensive evaluation should be included to construct better bone scaffolds with impressive performance.

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