ORIGINAL ARTICLE

Synthesis and Characterization of Eggshell Based Hydroxyapatite Nanoparticles: X-ray Diffraction and Spectroscopy Analysis

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ABSTRACT

Introduction: Hydroxyapatite is a bio ceramic that has chemical and physical similarities to the minerals of bones and teeth. Hydroxyapatite is a biocompatible material utilized in some medical application such as bone repair, tissue regeneration and reinforcement material due to its good mechanical and biological properties. It can be extracted from natural materials such as coral and eggshell. The content of $CaCO_3$ (calcium carbonate) in chicken eggshells reaches 94% which makes it a potential candidate for producing hydroxyapatite. Utilization of egg shells can also contribute to the reduction of agricultural waste. **Methods:** Hydroxyapatite nanoparticles were synthesized from eggshell by hydrothermal method then processed by ball-milling to form nano particle size. Hydroxyapatite crystal structure identified by X-ray Diffraction (XRD) and the functional group of hydroxyapatite was confirmed by Fourier-transform infrared spectroscopy (FTIR). **Results:** Eggshell Based Hydroxyapatite Nanoparticles can be extracted by hydrothermal method in 243,4 nm particle size measured by PSA analysis. XRD analysis showed crystalline phase identified the hydroxyapatite with small impurities such as silica. FTIR analysis confirmed the synthesized hydroxyapatite with eight main peak of wave number identified the functional groups of hydroxyapatite. **Conclusion:** Eggshell Based Hydroxyapatite Nanoparticles was good candidate of biomaterial for reinforcement material or other application, but other strategy needed to eliminate the impurities especially for tissue regeneration material.

Keywords: Hydroxyapatite; Eggshell; Analysis; Characterization

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INTRODUCTION

Calcium phosphate (CaP) is the primary mineral of bones and teeth. Hydroxyapatite, one of calcium phosphate, is the most similar to the mineral in bone. It has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. It has CaP with thermodynamically stable crystalline phase and has a 1.67 Ca/P ratio (1). Hydroxyapatite crystals are known to have biocompatible properties and are the largest building blocks of enamel and dentin (2). Hydroxyapatite can help new bone growth without involving fibrous tissue. It also can replace bone damaged by tumors, congenital diseases and as bone implants. It is currently the most widely used material in biomedical applications because it has stable chemical properties, and has a composition and crystal structure similar to human bone (3). In dentistry, hydroxyapatite has been used in various fields such as repair of periodontal defects, augmentation of edentulous ridges, manufacture of implants, pulp capping materials, fillers to strengthen GIC and composite resins, materials for the treatment of early carious lesions, and as a remineralizing agent in toothpaste (4,5). The breadth of application is inseparable from its extraordinary properties such as biocompatibility, osteoconductive, as well as bioactivity, non-toxicity, and non-inflammatory properties (6,7).

Hydroxyapatite can be obtained from chemicals such as $Ca(NO_3)_2$, $Ca(OH)_2$, $CaCO_3$, or naturally occurring materials such as limestone (8), as well as natural materials such as bones, shells, starfish, coral, seaweed, and egg shells (9). Chicken egg hells are a good source of calcium, especially chicken egg shells are easier to obtain than other calcium sources. The content of $CaCO_3$ (calcium carbonate) in chicken egg shells reaches 94 – 97% so that it can be used as an ingredient for Hydroxyapatite synthesis (9).

The egg shell is composed of three primary layers: the cuticle, which is the outermost layer surrounding the eggshell; the testa, which is the next layer; and the mammillary layer, which is the innermost layer. The outer skin membrane and the inner skin membrane are two skin membranes that are located beneath the mammary layer. About 95% of the testa layer is composed of calcium carbonate, 3.3% of it is organic protein, and 1.6% of it is water. Calcium carbonate, in the form of calcite, constitutes 94–97% of egg shells' chemical components, with 3–4.5% organic content. Small amounts of additional elements have also been found, including MgO (0.83%), SO_3 (0.66%), P_2O_5, Al_2O_3, K_2O, SiO_2, Cl_2O_3, and SrO (0.43%) (10).

By generating 67,201,200 kg of egg shells, Indonesia was the eighth-largest egg-producing nation in the world in 2017. The majority of the waste produced by chicken egg shells is dumped in landfills, which frequently result in environmental issues. Waste from chickens is decomposed into ammonia, hydrogen sulfide, and amines, all of which have a strong odor. Eggshell waste contains diseases like Escherichia coli (E. coli) and Salmonella in addition to offensive odors and attracting rodents, flies, and insects (10). Therefore, egg shells can be used as an alternative biomaterial due to the widely available waste. Hydroxyapatite from egg shells can be processed by a simple and inexpensive method.

There are several methods of making hydroxyapatite crystals, such as precipitation methods, hydrothermal, mechanochemical, biomimetic deposition, sol-gel method, and electrodeposition method. The end result can be solid ceramics, powders, ceramic coatings, or porous ceramics (1). Materials based on apatite that are extremely crystalline and doped can be created using the hydrothermal process. The production of Hydroxyapatite is accomplished using this process, which also offers high quality and purity. Additionally, a very excellent form of the Hydroxyapatite structure is produced by this process. This approach has a number of benefits, including affordability, speedy reaction times, ease of use, and high-quality hydroxyapatite production. It is simple to generate pure and homogeneous Hydroxyapatite crystals with a distinctive shape using this technology, which is also connected to a low energy dissolving process, at relatively low temperatures (8).

The hydrothermal method is the most widely used method of Hydroxyapatite synthesis from eggshells because it has proven to be the most convenient method to use. A novel technique for creating practical materials is to synthesize hydroxyapatite from eggshell in phosphate solution at high temperature. This technique uses $Ca(OH)_2$ and $CaHPO_4.2H_2O$ as starting materials to hydrothermally produce single crystals of fine hydroxyapatite (10).

MATERIALS AND METHODS

Hydroxyapatite was prepared by the hydrothermal method describes in previous study (5). The material were the chicken egg shells from Golden Comet chicken which was collected from Serang, Banten, Indonesia. About 500 grams of chicken egg shells washed with water to remove contaminants. The egg shells were crushed using a planetary ball mill untill 100 mesh. Calcination at 1000°C for 5 hours to convert $CaCO_3$ to CaO. The synthesis was carried out at a temperature of 230 °C. CaO and $(NH_4)_2HPO_4$ (diamonium hydrogen phosphate) were added to 50 ml distilled water with a mole ratio of Ca/P = 1.67. The mixture was then heated for 48 hours at 230 °C in a autoclave. The resulting hydroxyapatite was filtered, rinsed with distilled water to a pH of 7, and dried at 110 °C for two hours in order to eliminate NH_4OH. Particle size was seen using the Particle Size Analyzer (DelsaTM Nano Zeta Potential and Submicron Particle Size Analyzer - Beckman-Coulter). To identify and characterize hydroxyapatite as the main phase produced, stuctures and purities X-ray powder diffraction (Rigaku miniflex 600 Cu Kalpha radiation) was used. The identification of the functional group of hydroxyapatite was confirmed by Fourier Transform Infrared (Thermo Scientific Nicolet iS10.

RESULTS

PSA Analysis

Based on the data obtained from the test results, the average of grain size of the synthesized hydroxyapatite was 243.4±5 nm. The distribution of particle size on the hydroxyapatite grains was presented in figure 1.

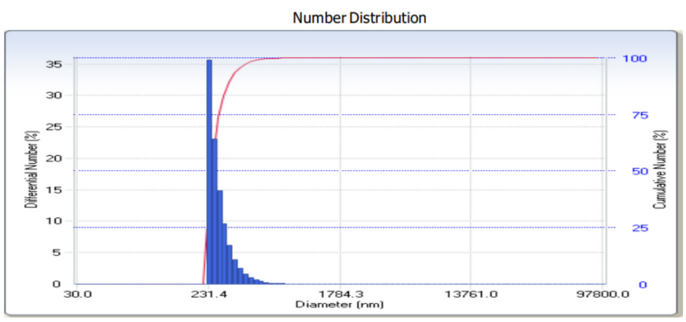

Figure 1 : Number Distribution of eggshell based hydroxyapatite particles.

XRD Analysis

Based on the results of the XRD (figure 2) test carried out on the synthesized hydroxyapatite (98,5%), it is known that the hydroxyapatite synthesis process has been successfully carried out. However, at the end of the synthesis it was still visible that there were impurities in the form of silica (1,5%).

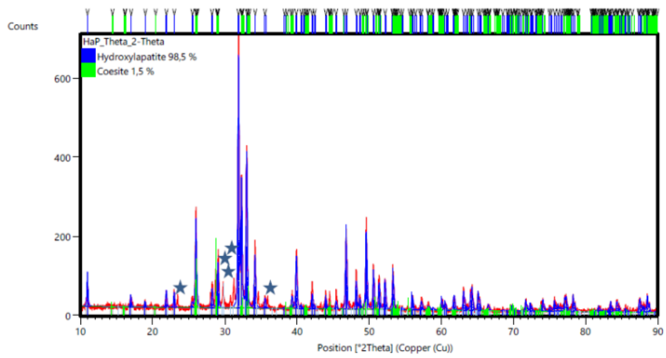

Figure 2 : Analitical XRD pattern of eggshell based hydroxyapatite.

FTIR Analysis

Based on the literature, hydroxyapatite has 9 main peaks namely at 472; 566; 603; 633; 960; 1035; 1092; 1403 and 3570 cm^{-1}. Meanwhile, the test results show that there are 8 main peaks, namely at 474.60; 571.86; 602.08 and 633.63; cm^{-1} (typical group absorption of hydroxyapatite (fingerprint)); 962.22 and 1046.34 cm^{-1} (absorption of aromatic phosphate groups); 2002.68 cm^{-1} (cyanad group absorption) and 3572.35 cm^{-1} (-OH group absorption). The FTIR spectra was presented in Figure 3.

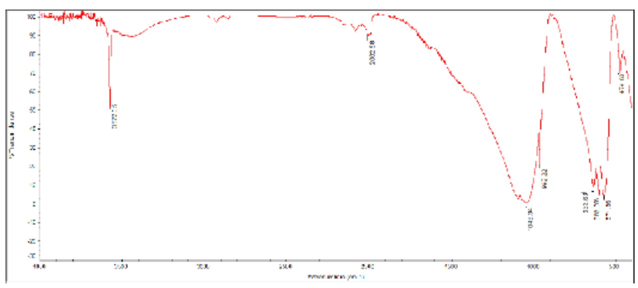

Figure 3 : FTIR spectra of eggshell based hydroxyapatite.

DISCUSSION

This particle size is an ideal size as a candidate for filler material in composites. One of the functions of the filler is to function as a reinforcing material in various applications, such as as an ingredient in the manufacture of scaffolds in tissue regeneration, bone grafts and as reinforcement in dental restoration materals (11-13).

The XRD patterns of hydroxyapatite are shown in figure 2. It was noted that hydroxyapatite had its main peak at around 2θ $\sim 31^0$. However, at the end of the synthesis it was still visible that there were impurities in the fiorm of silica (1,5%). which came rom the egg shell which was used as the starting material for the synthesis. In addition, there are still peaks that cannot be identified and are not compounds of the elements Ca, Si, O, H, and P as well as compounds of the elements period 1 – 4 as shown on the XRD graph with the sign "*" (figure 2).

Spectroscopy analysis was conducted to confirm the functional groups of eggshell based hydroxyapatite. The P-O bond from the PO43- was stretched at 571, 962, and 1046 cm-1, as seen in the spectra. Bending of the P–O bond of hydroxyapatite was observed at 602 cm^{-1}, the O–H stretching and bending appeared at 3572 and 633 cm^{-1}, respectively. The functional groups detected on eggshell based hydroxyapatite are shown in Table 1. Additionally, the development of a band at 3572 cm^{-1}, possibly caused by the appearance of HO hydroxyls, showed the creation of $Ca(OH)_2$.

CONCLUSION

Bases on the study conducted, it can be concluded that hydroxyapatite can be produced from chicken egg shells with high purity. Calcination results produce hydroxyapatite with high crystallinity. Therefore, the use of hydroxyapatite from eggshells as a medical application is a potential that should be developed

ACKNOWLEDGMENT

Thank you to the Faculty of Dentistry. Universitas Brawijaya, Malang, Indonesia.

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