SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE EXTRACTED FROM THREE TYPES OF EGGSHELLS BY PRECIPITATION METHOD

Thi Thi Aye[1], Nyo Nyo Myint[2], Thein Aye[3], San San Thwin[4]

Abstract

In this research, hydroxyapatite (HAp) was synthesized from three types' eggshells (hen eggshells, quail eggshells, and duck eggshells) by using method of precipitation. Eggshells are a rich source of calcium carbonate, which corresponds to about 94% of the shell. Calcium carbonate ($CaCO_3$) from eggshells and orthophosphoric acid (H_3PO_4) were used as precursor to produce hydroxyapatite (HAp). The major constituent calcium carbonate ($CaCO_3$) three types' eggshells powder were heated temperature at 1000°C for 3h to obtain calcium oxide (CaO). The crystal structures, elemental concentrations of HAp powder were analyzed by X-ray diffraction (XRD), and energy dispersive X-ray fluorescence (EDXRF) techniques. And then, crystal structures, crystallite sizes of HAp powder were calculated from XRD data results. The ratio values of the calcium (Ca) to phosphorous (P) were calculated from the EDXRF results. Finally, the analysis of HAp was discussed from the calculation of all above result data. The hydroxyapatite product from waste eggshells (hen eggshells, quail eggshells, and duck eggshells) can reduce environmental pollutions. Therefore, the HAp powder was prepared in this study. The obtained hydroxyapatite powder has great potential in biomedical applications.

Keywords – hydroxyapatite, hen eggshells, quail eggshells, duck eggshells, precipitation method, XRD, EDXRF

Introduction

Over the past 30-40 years, one of the most significant developments in orthopaedics has been the use of bioceramic material for bone replacement, reconstruction and repair. Bioceramics are biocompatible ceramic materials, and commonly include bioglass, calcium phosphates (such as hydroxyapatite (HAp), β-tricalcium phosphate (β-TCP)), and biphasic calcium phosphate. Calcium phosphate ceramics has great importance in the field of tissue engineering for the biological applications [1]. One of the most common apatite used as bioceramic in medicine and dentistry is hydroxyapatite (HAp) due to its bioactivity and osteoconductive properties [2]. HAp implants have been used clinically in the form of powders, granules, cements, dense and porous blocks, biphasics, coatings, and as various composites [3]. The advantage of using HAp as a bioceramic or biomaterial compared to other bioceramics, such as Bioglass or A-W glass-ceramic, is its chemical similarity to the inorganic component of bone and tooth. Presently hydroxyapatite has received much more interest as an implant material with applications in dentistry and orthopedics [4]. Hydroxyapatite (HAp) is a main component of bone mineral but in some cases carbonate-apatite is a main hard tissue component, as in dental enamel [5]. Other applications of HAP include femoral plugs in total hip replacement and HAP coating on metal components for cementless fixation [6]. Hydroxyapatite, a naturally occurring form of calcium phosphate, is the main mineral component of bones and teeth. Chemically hydroxyapatite is $Ca_5(PO_4)_3OH$ but often written as $Ca_{10}(PO_4)_6(OH)_2$ [7]. Natural hydroxyapatite is usually extracted from biological sources or wastes such as mammalian bone (e.g. bovine, camel, and

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horse), marine or aquatic sources (e.g. fish bone and fish scale), shell sources (e.g. cockle, clam, eggshell, and seashell), plants and algae and also from mineral sources (e.g. limestone) [8]. There are numerous methods have been reported for the preparation of hydroxyapatite from eggshell such as solid state reaction, hydrothermal reaction, co-precipitation reaction, sol-gel synthesis, mechano-chemical synthesis etc [9]. One of them is the sol-gel precipitation method. A simple sol-gel precipitation method can be used to prepare Nano hydroxyapatite from eggshells [10]. The main objectives of this study are to synthesis the natural HAp from shell sources (hen eggshell, duck eggshells, and quail eggshells) using precipitation method.

Materials and Methods

Materials

In this research, calcium oxide (CaO) extracted from hen eggshells, quail eggshells and duck eggshells. Hen eggshells, quail eggshells and duck eggshells were collected from Yenanchaung area in Magway Division. The needed phosphate was extracted from orthosphoric acid (H_3PO_4). Phosphoric acid, distilled water as a solvent, ammonium hydroxide (NH_4OH) as pH controller and filter paper were purchased from Academy Chemical Shop, Babetan Township in Yangon.

Preparation of three types' eggshells (hen eggshells, quail eggshells, and duck eggshells) powder

In this research, hen eggshells, quail eggshells and duck eggshells were used as a starting material for calcium oxide (CaO). Hen eggshells, quail eggshells and duck eggs were collected form Yenanchaung area in the Magway Division. In physics department laboratory at Yenanchaung University, hen eggshells, quail eggshells and duck eggs were washed with distilled water to clean. Firstly, the cleaned hen eggshells were cooked with distilled water until temperature at 100℃ has been reached for 30 minutes. When temperature at 100℃ has been reached, hen eggshells pot was put a few minute to cool. A few minute later, the shell membranes were removed from hen eggshells. Hen eggshells are cleaned with distilled water. The cleaned hen eggshells were obtained. The obtained hen eggshells were crushed with the use of laboratory motor and pestle. The hen eggshell powder was obtained. The major constituent calcium carbonate ($CaCO_3$) hen eggshell powder was heated in Protherm furnace (physics department laboratory in Yenanchaung University) at 1000 °C temperature for 3 hour to obtain calcium oxide (CaO). Finally, white calcium oxide (CaO) hen eggshell powder was obtained.

Precipitation Synthesis

Hydroxyapatite powder is extracted from hen eggshells, quail eggshells, and duck eggshell were synthesized by precipitation method. The precipitation method is simplicity of the process and cost effectiveness. The principle of this method is the reaction between calcium hydroxide and phosphoric acid with a molar ratio of Ca/P= 1.67. The calcined (CaO) powder of heneggshells, quail eggshells, and duck eggshells were mixed with distilled water to form a 1.67M $Ca(OH)_2$ solution. H_3PO_4 solution with concentration 1 M were added to these 1.67M $Ca(OH)_2$ solution by drop wise through a burette and stirred. The pH of the mixtures were measured by using pH papers; if the pH was less than 9 then the NH_4OH should be added by drop wise. The mixtures were stirred for 2hr and heated at 60°C and then filtered with filter papers. After that the mixtures were allowed to precipitation at room temperature for 24 hours. The aging solution is filtered and the precipitate is washed with distilled water. The filtration result was dried in a furnace at 100°C. After the drying process was completed, the obtained results were heated at 900 °C for 3 hours. Finally, white powder was obtained. The procedure of hydroxyapatite powder from hen eggshells, quail eggshells, and duck eggshells by precipitation method are shown in Figures 1, 2, and 3. The flow chart of sample preparation is shown in figure 4.

Sample Powder Characterization

The obtained powder after the precipitation method were analyzed by X-ray Diffraction (XRD), and Energy Dispersive X-ray fluorescence (EDXRF) techniques. X-ray Diffraction (XRD) to identify crystal structure, crystalline size and lattice constants. Energy Dispersive X-ray fluorescence (EDXRF) technique was used to analyze the elemental concentrations of the hydroxyapatite (HAp) powder and the ratio of the value of calcium (Ca) to phosphorous (P).

Figure 1. The sample preparation of hydroxyapatite powder from hen eggshells by precipitation method

Figure 2. The sample preparation of hydroxyapatite powder from quail eggshells by precipitation method

Figure 3. The sample preparation of hydroxyapatite powder from duck eggshells by precipitation method

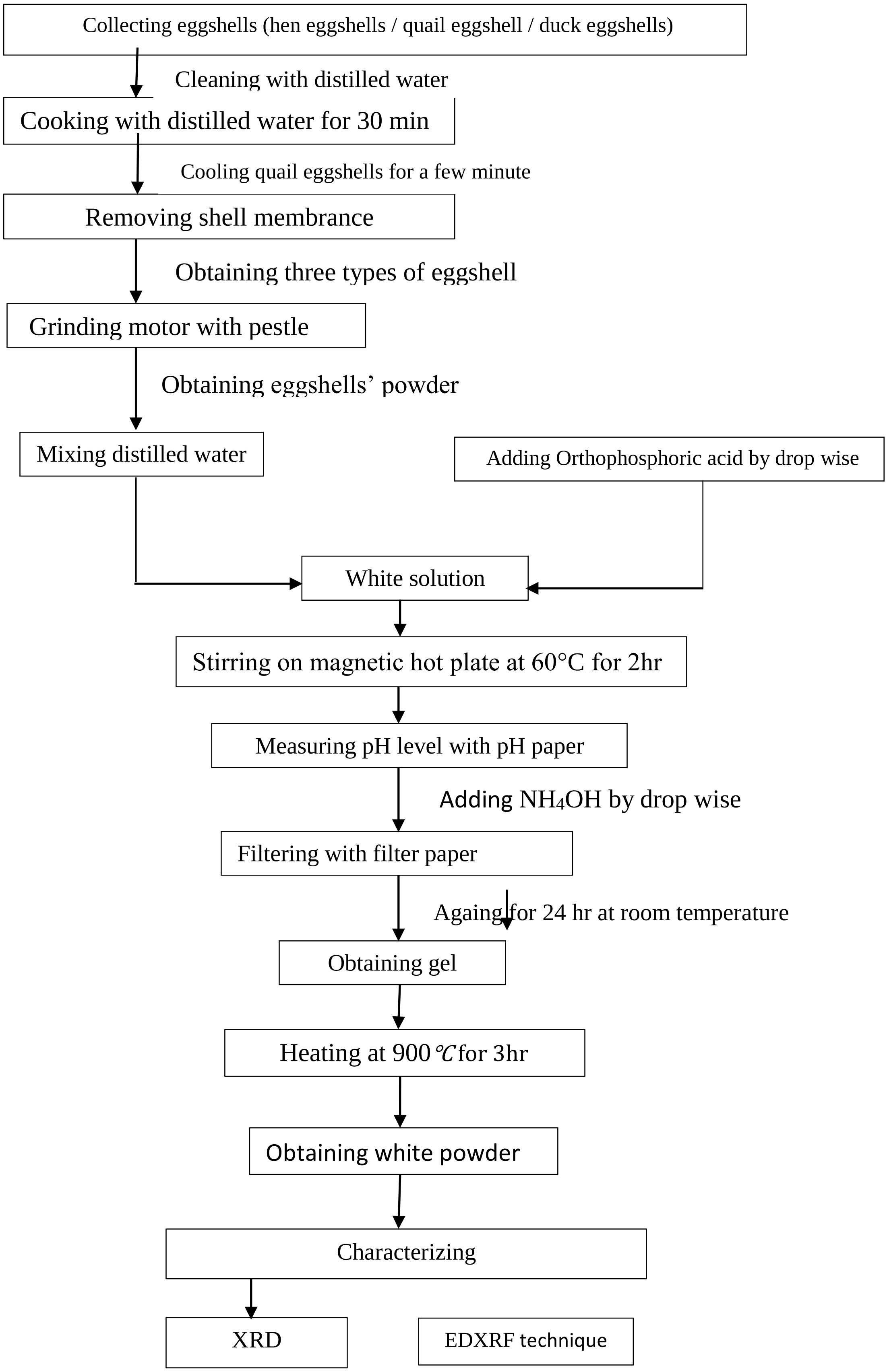

Figure 4. The flow chart of the HAp sample preparation from hen eggshells, quail shells, and duck eggshells

Results and Discussions

Characterization of HAp powder from hen eggshells, quail eggshells, and duck eggshells by (X-ray Diffraction XRD analysis)

XRD analysis is used to confirm crystal structures, crystalline sizes and lattice constants of the HAp powder from three type eggshells after the precipitation method. The powder of XRD data results similar to the HAp pattern (IJDD 00-001-1008) with a chemical reaction equal to $Ca_{10}(PO_4)_6(OH)_2$ were found. The XRD patterns of the HAp powder of hen eggshells, quail eggshells, and duck eggshells at 900℃ for 3hr are depicted in Figure 5.

The lattice constant values of hen eggshells, quail eggshells and duck eggshells are a = 10.37030 (Å), b = 10.37030 (Å), c = 37.08500 (Å), alpha (α) = 90°, beta (β) = 90°, gamma (γ) = 120°, a = 9.41800(Å), b = 9.41800(Å), c = 6.88400(Å), alpha (α) = 90, beta (β) = 90°, gamma (γ) = 120°, a = 10.43800, b= 10.43800, c = 37.15000, alpha (α) = 90°, beta (β) = 90°, gamma (γ) = 120°. So, the crystal structure of hydroxyapatite powder from hen eggshells, quail eggshells and duck eggshells were hexagonal structure ($a=b\neq c$, $\alpha= \beta\neq\gamma$). The crystallite sizes for three strongest peaks of HAp powder from hen eggshells, quail eggshells, and duck eggshells were estimated by the Scherrer's equation (1). The average crystalline size of hen eggshells, quail eggshells, and duck eggshells were shown in Table (1).

$$D= \frac{k\lambda}{\beta\cos\theta} \qquad (1)$$

Where, D = The crystalline size of the particle

β = The full width of the peak at half maximum intensity located at 2θ

λ = The wavelength of X-ray radiation

k = Scherrer's constant (k=0.94)

Energy Dispersive X-ray Fluorescence (EDXRF) analysis

Hydroxyapatite (HAp) powder from hen eggshells, quail eggshells, and duck eggshells were synthesized by EDXRF technique to confirm the elemental concentrations and the ratio of Ca/P. Figures 6 is shown the EDXRF graph of the HAp samples at 900℃ for 3hr. Table 4 was shown the elemental concentrations of HAp powder from hen eggshells, quail eggshells, and duck eggshells. The ratio of the values of calcium (Ca) to phosphorous (P) of hen eggshells, quail eggshells, and duck eggshells are 2.46, 2.87, and 2.72 from EDXRF data results. They also contain three minor elements namely these are Sr, Fe, and Zn found in the HAp powder from hen eggshells, quail eggshells, and duck eggshells.

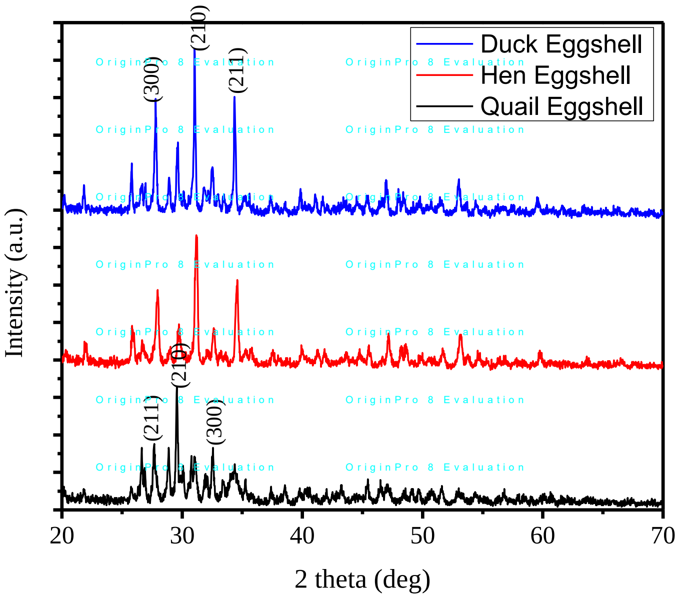

Figure 5. The XRD pattern of HAp powder from hen eggshells, quail eggshells, and duck eggshells at 900°C for 3h

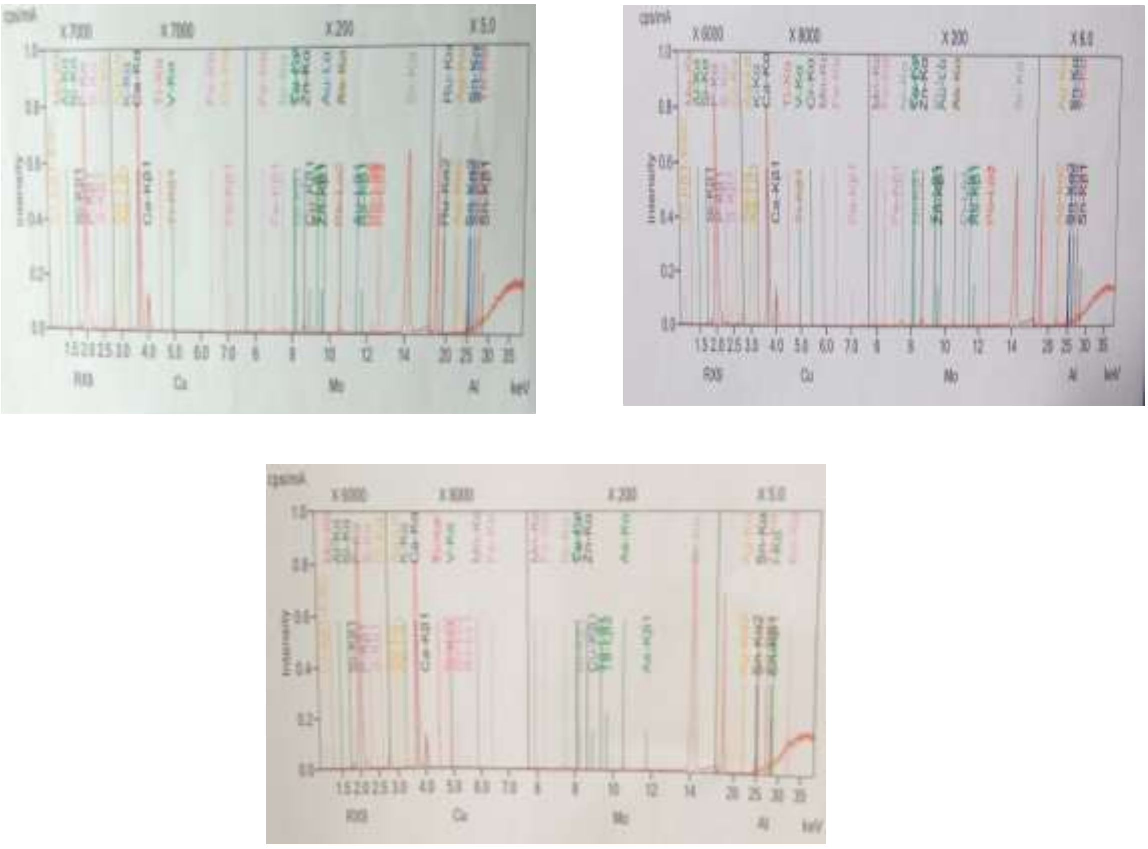

Figure 6. EDX line spectrum of HAp Sample from hen eggshells, quail eggshells and duck eggshells

Table 1. The average crystalline size for three strongest peaks of HAp sample from hen eggshells, quail eggshells, and duck eggshells at 900℃ for 3hr

Sample	Peak no	(hkl) plane	2θ (degree)	θ (radian)	FWHM (radian)	Crystalline size D(nm)	Average crystalline size
HAp 1	1	210	31.1933	15.59665	0.261	33.006022	32.380577
	2	211	34.5668	17.2834	0.2629	33.053392	
	3	300	27.9658	13.9829	0.2751	31.082316	
HAp 2	1	210	29.5856	14.7928	0.1565	54.835836	47.717258
	2	211	27.6984	13.8492	0.1989	42.965323	
	3	300	32.5538	16.2769	0.1906	45.350614	
HAp 3	1	210	31.0681	15.53405	0.1287	66.914921	57.942318
	2	211	34.3924	17.1962	0.1339	64.866571	
	3	300	27.8091	13.90455	0.2033	42.045462	

Table 4. Quantitative EDXRF results of HAp Sample from hen eggshells, quail eggshells, duck eggshells at 900℃ for 3hr

	Hen eggshells	quail eggshells	duck eggshells
Element	**Weight %**	**Weight %**	**Weight %**
Ca	**8.70**	**9.11**	**9.37**
P	**3.54**	**3.17**	**3.45**
Sr	**0.0136**	**0.0120**	**0.0190**
Fe	**0.0032**	**0.0012**	**0.0009**
Zn	**0.0021**	**0.0019**	**0.0002**

Conclusion

This research has been reported hydroxyapatite with calcium precursor from hen eggshells, quail eggshells, and duck eggshells and phosphate from H_3PO_4 were successfully synthesized by precipitation method. The average crystalline sizes were determined by using XRD results data. The mean crystalline sizes of HAp from three types of eggshells were 32.380577 nm, 47.7172582 nm, and 57.9423187 nm at 900℃ for 3hr. The lattice constant values of hen eggshells, quail eggshells and duck eggshells are a = 10.37030 (Å), b = 10.37030 (Å), and c = 37.08500 (Å), alpha (α) = 90°, beta (β) = 90°, gamma (γ) = 60°, and a = 9.41800(Å), b = 9.41800(Å), c = 6.88400(Å), alpha (α) = 90, beta (β) = 90°, gamma (γ) = 60°, and a = 10.43800, b= 10.43800, c = 37.15000, alpha (α) = 90°, beta (β) = 90°, gamma (γ) = 60°. So, the crystal structures of hydroxyapatite powder from hen eggshells, quail eggshells and duck eggshells were hexagonal structure ($a=b\neq c$). The ratio of the values of calcium (Ca) to phosphorous (P) of hen eggshells, quail eggshells, and duck eggshells are 2.46, 2.87, and 2.72 from EDXRF data results. Some of the impurities that may arise in the process of synthesis of hydroxyapatite based on natural sources are related to carbonate groups. The obtained HAp can be used for many biomedical applications (hard tissue replacement in medical and dental therapy). Thus, the aim of this research is to extract hydroxyapatite from shell sources (hen eggshell, duck eggshells, and quail eggshells) using a combination of chemical precipitation and calcination methods, which will also reduce the environmental impacts caused by the improper disposal of the wastes.

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