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Synthesis and Characterization of Nano Hydroxyapatite from Waste Egg Shells for Orthopedic and Dental Applications

S.S. Raza[*1], *M.U. Manzoor*[1], *S.Ali*[1], *F.Hussain*[1], *T.Ahmad*[1], *F.Riaz*[1]

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Abstract

Hydroxyapatite is widely used in orthopedic and dentistry as a bio-coating of implants for improvement in its osteointegration with bone tissue. Hydroxyapatite powder was synthesized by a precipitation technique using hen's eggshells. The synthesis technique involved the calcination of eggshell powder to obtain calcium oxide and then its titration at a controlled flow rate with phosphoric acid. Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray diffraction (XRD) investigations were carried out to confirm the formation of hydroxyapatite powder. Scanning Electron Microscopy (SEM) demonstrated the shape and structure of the as-synthesized nano hydroxyapatite powder. The obtained results correlate with the literature. It was observed that the hydroxyapatite powder formed was highly crystalline and all the peaks corresponded with hydroxyapatite powder based on the standard XRD pattern of Hydroxyapatite powder (JCDPS card no. 09-432). It was clear from the small peaks that some Tri-Calcium Phosphate (TCP) was also formed along with the hydroxyapatite. Hydroxyapatite powder formed was subjected to thermal treatment and resulted in improved crystallinity and refined grain size. It was noted that higher surface area biogenic hydroxyapatite powder with highly interconnected nanoparticles will be useful in bone morphogenesis during bone surgery.

***Keywords**: Hydroxyapatite, Calcination, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray diffraction Spectroscopy (XRD), Scanning Electron Microscopy (SEM)*

1. Introduction:

Biomaterials are the materials that interact with the living cells with in the body. These are the major materials of a concern of the current research era. They have a wide variety of applications in human body because of their excellent biocompatibility and bioactivity [1-3] along with improved mechanical properties. They are used as an implant material, bone fixation plates and screws, scaffolds and dental cement as a filling material. Main types of biomaterials are metals, composites, polymers and ceramics [4, 5]. Among the many ceramics based biomaterials, calcium phosphate has the properties that are very much similar to natural bone. Four main type of calcium phosphate are hydroxyapatite (HA), tri-calcium phosphate (TCP), calcium deficient hydroxyapatite (CDHA) and biphasic calcium phosphate (BCP) [5, 6]. Based on the properties of bioactivity, ion release and mechanical strength, hydroxyapatite and tri-calcium phosphate are mainly used. Osteoconductive and osteoinductive features make HA a promising material for bone regeneration application [7].

The synthetic hydroxyapatite [is an alternate to natural bone because of its non-toxicity and non-

[1] *Institute of Metallurgy and Materials Engineering (IMME), University of the Punjab, Lahore, Pakistan*
*** Corresponding Author:** (s_shahzadraza@yahoo.com)

inflammatory features [8]. The stoichiometric Ca/P ratio of HA should be of 1.67. Sol-gel process, wet chemical technique and hydrothermal method are the noble techniques for the production of hydroxyapatite powder [9, 10]. A lot of research has been conducted and geared up in improving the properties of hydroxyapatite alongside its cost-cutting production techniques in bulk quantity. Natural materials or inorganic sources are the main raw materials used for the synthesis of synthetic hydroxyapatite [11, 12]. Many researchers have studied cost effective natural organic sources as calcium salts, coral, eggshell, fish bone, seashells, plants for the synthesis of hydroxyapatite [13-17]. Among them, chicken eggshell is of common interest in this research because of its readily availability at no cost, rich in calcium [18] and can produce hydroxyapatite powder that is capable of promoting greater bone reformation [19]. The characteristics of hydroxyapatite like phase composition, particle size, crystallinity, mechanical properties and thermal stability [20] are very much dependent on the synthesis conditions that includes raw materials used, concentration, pH, reagents and processing temperature [21]. Due to these synthesis condition, different morphologies of hydroxyapatite like rod shape, needle shape, spherical, flower shape were obtained [1]. One of the important processing parameter is pH value to control the morphologies of hydroxyapatite particles.

Different researchers have used various techniques, specially addition of strong alkali to maintain the pH of the solution. Goh et al. [1] washed, dried and crushed hen's eggshells and then the obtained calcium carbonate ($CaCO_3$) powder is calcined at 900°C to obtain calcium oxide (CaO) powder. The obtained (CaO) powder is stirred with distilled water. Phosphoric acid (H_3PO_4) was added slowly and to neutralize the acid, ammonium hydroxide (NH_4OH) was added to maintain the pH of the solution. The obtained mixture was microwaved, washed and filtered to obtain hydroxyapatite. Agbabiaka et al. [22] used sodium hydroxide to maintain the pH of the solution before the addition of the phosphoric acid. They start with three stage heating of eggshells to obtain calcium oxide (CaO) powder followed by addition of distilled water and vigorous stirring. Phosphoric acid was added and the solution was filtered to obtain hydroxyapatite powder. Pu'ad et al. [23] used a complicated lengthy process to synthesize hydroxyapatite from eggshells. Crushed calcium carbonate was calcined at 900°C and then mixed with distilled water. The solution is treated with phosphoric acid to obtain hydroxyapatite. The obtained solution was aged for 24 hours and stirred and aged again for same 24 hours. The solution was then filtered and dried and then calcined at different temperatures. Ramesh et al. [24] used di-calcium hydrogen phosphate dehydrate (DCPD) for homogeneous mixing to treat with 700°C calcined calcium hydroxide instead of phosphoric acid followed by wet attrition, drying and heat treatment.

In this research work, simple and cost cutting technique was used to synthesize hydroxyapatite powder. No additional alkali solution was used to maintain the pH of the solution only controlled and drop wise addition of precursor solution technique was used.

2. Materials & Method:

Phosphoric or Ortho- Phosphoric acid having a density of 1.88 g/cm^3 was used. Phosphoric acid is an agent which binds with divalent ions such as Fe^{++}, Mg^{++} and Ca^{++}. It is also a constituent of the bone. The eggshells have about 11% of the total weight of the egg and the chemical composition of eggshell is shown in table 1.

Table 1: Chemical Composition of eggshell

Component	Percentage
Calcium carbonate	94
Calcium phosphate	1
Magnesium carbonate	1
Organic matters	4

The high concentration of calcium carbonate i.e. 94% was the reason for the choice of hens' eggshells as a Precursor. First of all, the egg shells were collected and their surfaces were mechanically cleaned. The inner membrane of eggshells was

taken off. The eggshells were cleaned by brushing their outer surfaces to remove the dirt adhered. Liquid soap was used for this purpose. The eggshells were then washed with distilled water. This process was repeated until it was assured that no residue or detergent was left on eggshells. The eggshells were then dried and crushed into powder. The powder of eggshells was subjected to calcination for removal of organic matter and transformation to calcium oxide, the main precursor. Powder was placed in a muffle furnace for a two stage thermal treatment. The first stage consists of heating egg shells to 350°C for a continuous period for about 2 hours and all organic residues were destroyed. The second stage consisted of heating the sample further to about 900°C for a continuous time of 4 hours. At 900°C temperature the calcium carbonate of eggshells transformed into calcium oxide by releasing carbon dioxide (CO_2) as shown in the equation (1).

$$CaCO_3 \quad CaO + CO_2 \qquad (1)$$

The white eggshell powder turned brownish black due to burning of organic matter such as membranes and other surface impurities etc. In muffle furnace white crystalline agglomerates were formed as end result in crucible. The powder was filtered and thoroughly washed with double distilled water and filtered again and dried at 80°C for 3 hours. The dried clean powder of calcium oxide was stored in an air tight container for future use. 280 mg of calcined calcium oxide was dispersed in 50ml of distilled water to obtain 1 molar solution. The CaO was converted into $Ca(OH)_2$. The chemical reaction is shown in equation (2)

$$CaO + H_2O \xrightarrow{Exo} Ca(OH)_2 \qquad (2)$$

The solution was placed in a sonicator for 1 hour for homogenous dissolution. The stock solution of Phosphoric Acid was calculated to be 14.615 M based on a density of 1.685 g/mL, a formula weight of 98 g/mol, and a concentration of 85% w/w. To make a 0.06 M solution, 0.411 mL of stock solution was slowly added to 25 mL deionized water. Adjusted the final volume of solution to 100 mL with deionized water 50ml of this 0.06 M solution was taken out to titrate with 50ml of 0.1 molar $Ca(OH)_2$. The acid was added drop wise to the suspension at room temperature. Initially, the pH of the solution was found to be 12. The pH was brought to 9.5 and then maintained to 8.5 - 9.5 by setting the appropriate flow rate of acid as 1.1 ml/min. After completion of addition of phosphoric acid, precipitates were formed. The whole process was carried out in vigorous agitation with the help of a magnetic stirrer. The reaction occurred is shown in equation (3).

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + H_2O \qquad (3)$$

The precipitates formed were subjected to aging treatment for 24 hr. For aging treatment the precipitates were kept at room temperature in flask. During the aging period the precipitates grow. The values of pH measured in equal intervals of total time of 45 minutes with flow rate of 1.1 ml/min approximately is shown in figure 1.

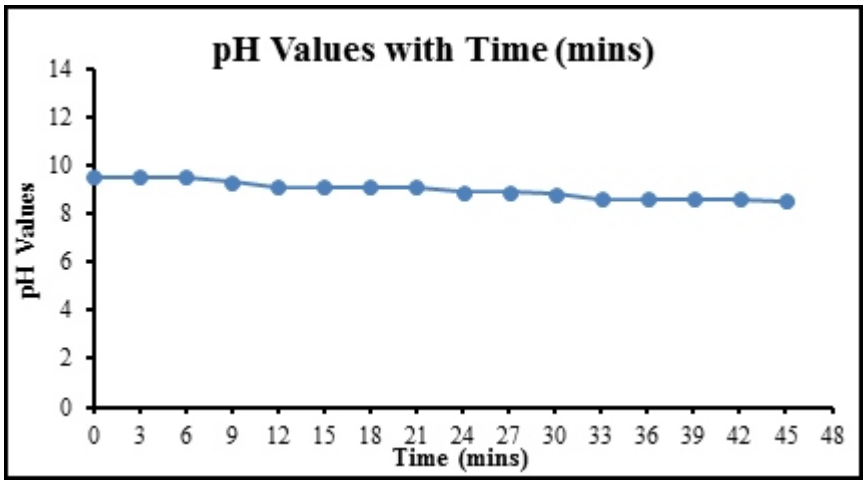

Figure 1: Graph between pH values and time

Figure 2 shows the flow sheet of the whole process.

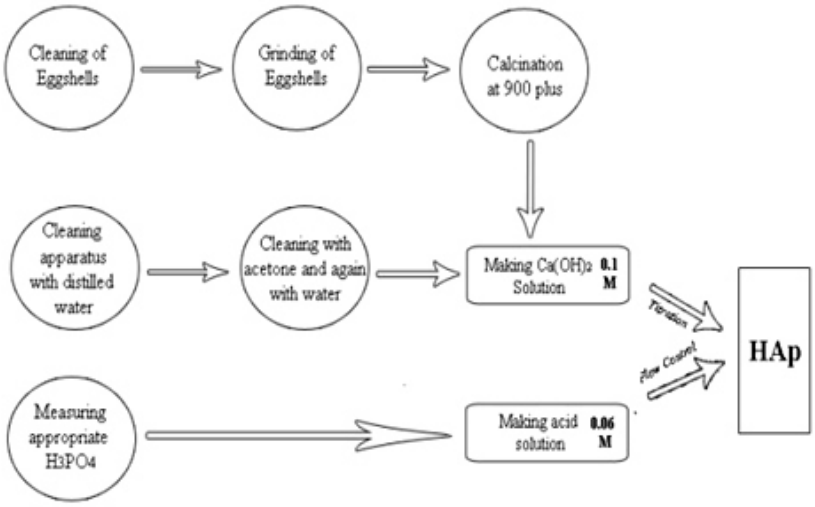

Figure 2: Flow sheet of the whole process

Afterwards the suspension was centrifuged for 1h at 600 rpm. The precipitates were dried for 2 hours in an oven at 95°C for the removal of moisture. The product was further subjected to sintering at 900°C for 4 hours. Fourier Transformed Infrared Spectroscopy (FTIR) technique was used for the characterization of calcium and phosphorus based biomaterial. The wavelength range used for FTIR of HA was 4000-400 cm^{-1}. The pallet used for sample preparation was of potassium bromide (Alkyl Halide). The most characteristic chemical groups in the FTIR spectrum for synthesized Hydroxyapatite powder are PO_4^{-3} OH^-, CO_3^{-2}, as well as HPO_4^{-2} that characterize non-stoichiometric Hydroxyapatite powder. Pam Analytical X'pert Pro available at (IRCBM), COMSATS was used to carry out the XRD anaylsis of the product with Cu Ká. The Voltage setup was 35kV and a current of 30 mA was used. The range of angle was kept 20°-80° with 0.02° stepping. The morphology of the obtained powder was examined using scanning electron microscope, SEM (Hitachi S-3400 N) with an accelerating voltage of 15 kV.

3. Results and Discussion:

The FTIR spectra of as synthesized hydroxyapatite is shown figure 3.

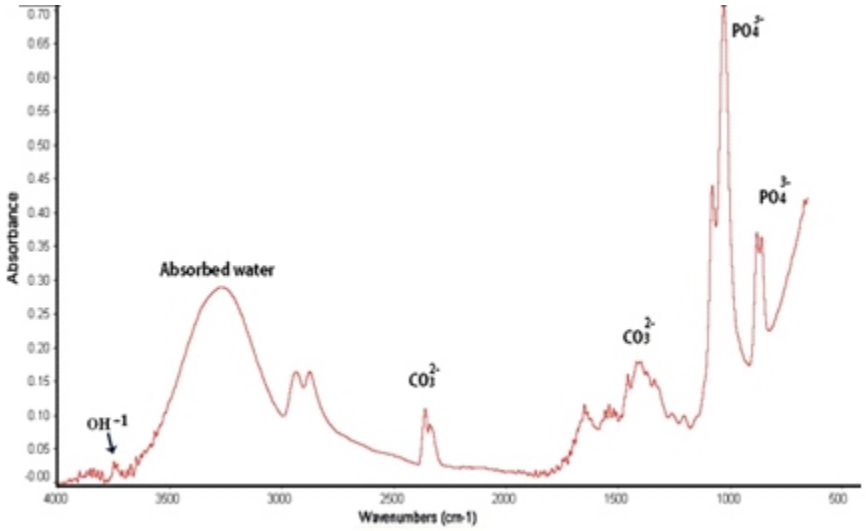

Figure 3: FTIR spectra of as synthesized Hydroxyapatite

The characteristic peak of phosphate group (PO_4^{-3}) forms intensive Infra-Red absorption bands at 600 and 800 cm^{-1} and at 1000 1100 cm^{-1} indicated asymmetric bending vibrations, symmetric stretching, and asymmetric stretching of phosphate group (PO_4^{-3}), respectively. Absorbed water band is relatively wide, from 3600 to 3000 cm^{-1} and this corresponds to the stretching mode of H_2O [25]. For OH^- strong peaks can be seen at 3720-3600 cm^{-1} showing O-H stretching vibrations [26]. CO_3^{-2} group forms intensive peaks between 1360 and 1530 cm^{-1} associated with out-of-plane bending mode and asymmetric stretching, respectively. Similar bands were also observed in previous studies [25, 27, 28]. The XRD pattern of as synthesized hydroxyapatite is shown in Figure 4.

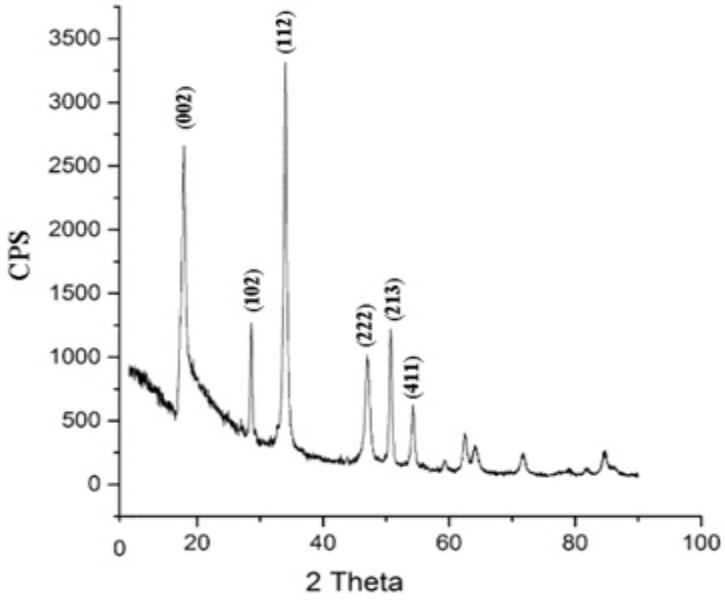

Figure 4: XRD pattern of synthesized Hydroxyapatite powder with 0.02 stepping

It can be seen from the figure 4 that sharp and well defined peaks are observed from as synthesized hydroxyapatite. The hydroxyapatite powder formed was highly crystalline and all the peaks corresponded with hydroxyapatite powder based on the Standard XRD pattern of Hydroxyapatite powder (JCDPS card no. 09-432). It was clear from the small peaks that some tri-calcium phosphate was also formed along with the hydroxyapatite. The peaks were also compared from a number of literary sources and all peaks formed in were corresponding to those present in the literature [29]. The scanning electron microscopy of as sintered hydroxyapatite is shown in figure 5.

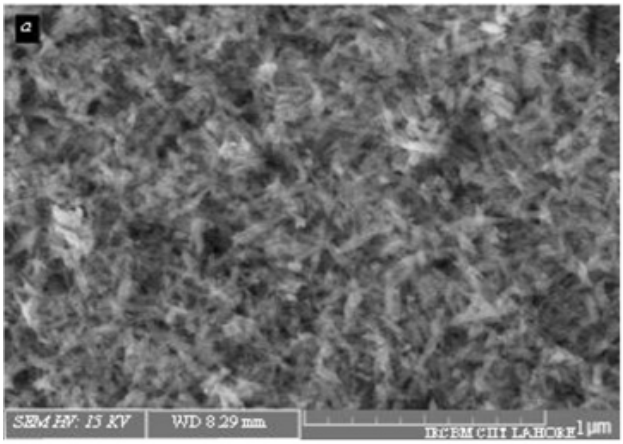

Figure 5: Scanning Electron Microscopy of as sintered hydroxyapatite

A typical flower-like morphology, comprising of petal-like flakes having an average size of 100 200 nm in width are visible. A similar flower- like morphology has been reported in the literature for microwave irradiation eggshells-derived HA [24, 25]. Different morphology is seen in the SEM micrograph of the eggshell calcined at 900°C as shown in Figure 6 as compared to as synthesized.

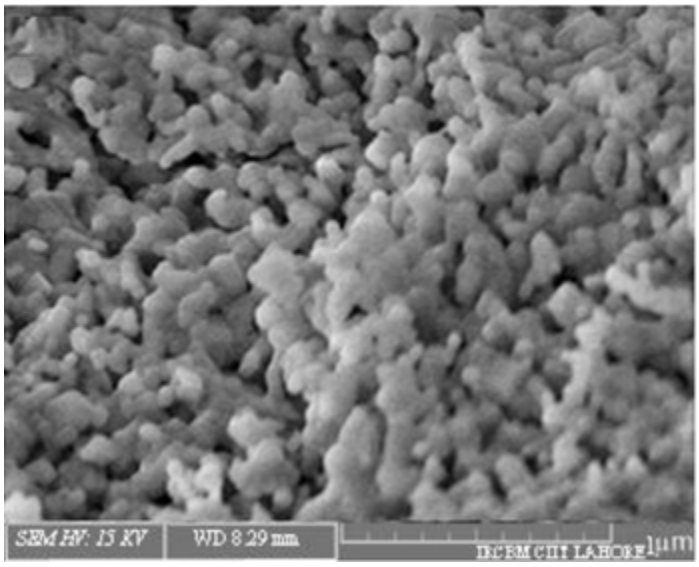

Figure 6: Scanning Electron Microscopy of Hydroxyapatite powder calcined at 900°C

The nano hydroxyapatite powder formed was subjected to thermal treatment in a control environment. The thermal treatment resulted in improved crystallinity and refined grain size.

4. Conclusions:

Hydroxyapatite powder was successfully synthesized using hen's eggshells and titrated with Ortho-phosphoric acid as precursors. The pH factor during the process was maintained by controlling the dropwise flow of phosphoric acid solution and completely eliminating the use of external alkaline solution. The synthesized hydroxyapatite powder was highly crystalline as confirmed by the peaks of XRD and also correlated with the literature. Scanning electron microscopy also confirmed the crystallinity of the as synthesized powder prior to calcination. The synthesis process used was quick and takes lesser time as compared to those where pH is controlled by using other solutions.

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