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PROSPECT OF HYDROXYAPATITE POWDER PRODUCED FROM WASTE POULTRY EGG-SHELLS FOR BIOMEDICAL APPLICATIONS

Alafara A. BABA[1]*, Adeola A. WOMILOJU[1]*, Fatimah A. ABUBAKAR[2], Abdullah S. IBRAHIM[1], Anoka A. NJAN[3], Folahan A. ADEKOLA[1], Abdul G. F. ALABI[4]

[1]Department of Industrial Chemistry, University of Ilorin, P.M.B. 1515, Ilorin-240003, Nigeria.
[2]Department of Biochemistry, University of Ilorin, P.M.B. 1515, Ilorin-240003, Nigeria.
[3]Department of Pharmacology and Theurapeutic, University of Ilorin, P.M.B. 1515, Ilorin-240003, Nigeria.
[4]Department of Materials Science and Engineering, Kwara State University, Malete, Nigeria.

*Correspondences:
E-mail:alafara@unilorin.edu.ng(A.A.Baba),Tel:+2348035010302; womiadeola2205@gmail.com

Abstract

Till date, the synthesized calcium phosphate compounds such as hydroxyapatite (HAp) have generated a great deal of interest because of their wide array of medical applications in orthopedics, plastic and dental surgeries. With these defined uses, considerable efforts should continuously be directed towards developing artificial bone and teeth that do not cause damage in healthy tissue and made available as demanded. With every day records, where several million tons of egg-shells are generated as bio-waste across the world; therefore, for conversion of *waste-to-wealth,* this study examines the prospect of HAp powder, successfully prepared using calcined waste poultry egg-shells as a calcium precursor via chemical precipitation method using phosphoric acid as a phosphorous source. At optimal conditions, the pure HAp crystal produced with safe *Acute* and *Sub-chronic* toxicity tests gave Ca/P ratio of 1.66 (against conventional Ca/P ratio of 1.67) was well characterized for use in defined oral and orthopaedic treatments.

Keywords: Hydroxyapatite powder, Waste Poultry egg-shells; Toxicity tests; Ca/P ratio; Orthopaedics uses.

Introduction

Egg-shell is a waste material, with several million tons generated daily across the world. Adequate utilization of this type of waste material for value added products reduces environmental problems and promotes sustainable development [1]. Egg-shells is composed majorly of calcium carbonate and serve as a source of calcium for synthesis of pure hydroxyapatite powder (HAp). HAp synthesized from egg-shells demonstrates superior sinterability, good resemblance with human hard tissues, higher bone formation and an environmentally friendly source for HAp preparation [1, 2].

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is an inorganic bioactive material and a major constituent of hard tissues belonging to a class of phosphate. HAps are attractive materials due to its excellent biocompatibility, bioactivity, bioresorbility, osteoconductivity and osteointegration properties in biomedical applications such as dentistry, healing damaged bones, for implant and scaffold and as a drug delivery agent [3].

A variety of synthesis techniques includes the wet chemical precipitation, sol-gel method, hydrothermal and mechano-chemical method has been developed to synthesize HAp from different precursors with variations in the parameters to produce Hap with significant purity and high level of solubility and crystallinity [1-4].

The most widely used of these techniques for Hap synthesis is the chemical precipitation which requires low-cost raw materials with possibility of controlling process parameters such as particle size, distribution, shape and agglomeration. Based on these considerations, in the present study we aim to synthesis Hap by chemical precipitation method from egg-shells a common waste using phosphoric acid as a phosphorous source for possible biomedical applications.

Methodology

Synthesis of CaO from Eggshell

Waste eggshells collected were immediately stored in the refrigerator after their surfaces had been cleaned. They were repetitively washed with distilled water and their membranes peeled off, boiled in water for 15 minutes to remove dirts and organic residue. The eggshells were dried at room temperature to remove the water and moisture content of the shells. Thereafter, cleaned and dried eggshells sample was crushed with the aid of a table top hammer mill and they were pulverized

into fine particles with the aid of acetone-rinsed mortar and pestle. These fine powders were placed in the muffle furnace and calcination in air atmosphere at 700°C for 2 hours each [5]. The thermal treatment ensured the burning out of organic materials was burnt out and its transformation into calcium oxide according to the following stiochiometry:

$$CaCO_{3\,(s)} \rightarrow CaO_{(s)} + CO_{2(g)} \uparrow \qquad (1)$$

Synthesis of HAp powder

A measured amount of calcined eggshell now calcium oxide (CaO) was dispersed in 250ml doubly distilled water which was then vigorously stirred to react and form a suspension of $Ca(OH)_2$. The beaker was covered in order to avoid possible contamination via contact with atmosphere. The stoichiometric amount was decided on in accordance with the quantity of calcium present in the calcined eggshell. In this reaction, the CaO transformed into $Ca(OH)_2$ as shown by the following equation:

$$CaO_{(s)} + H_2O_{\,(l)} \rightarrow Ca(OH)_{2\,(l)} \qquad (2)$$

Under rigorous stirring, 6.82ml of orthophosphoric acid was dissolved in 100ml of distilled water (1M) and then added drop wise to the $Ca(OH)_2$ solution at a rate of 1.5ml/min. The reactant was stirred continuously at room temperature for some hours to aid the maturation stage. After completion of mixing, the Eggshell-HAp solution/suspension was aged for 72 hours which caused precipitation hardening during which the pH was monitored and maintained at 11 with the addition of 1M NH_4OH that helped stabilise the pH of the super saturation. After the ageing process, the solutions were filtered and washed with distilled water, dried in the oven at 100°C for 2 hours and the dried samples was calcination at 1000°C to obtain eggshell hydroxyapatite (EHAp) crystalline powder [6]. The resulting powders were analysed using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) analyses respectively.

Animals

Albino rats (150- 200g) obtained from small animal holding unit of Department of Biochemistry, University of Ilorin, Nigeria, were used for the study. The animals were housed in cages at room temperature and moisture, under naturally illumated enviroment 12: 12 h dark/ lightcycle. They were fed on standard diet and had free asscess to water. The rats were used in accordance with NIH Guide for the care and use of Laboratory Animals [7].

Acute toxicity study HAp powder

The LD_{50} of the sample was tested to determine the safety of the powder according to the guideline set by Organization for Economic Cooperation and Development NO 423 [8]. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats per group and adminstered 10, 100, 100 mg/kg body weight of the dissoved powder orally. The animals were observed for first 4 h and 24 h for sign of toxicity and mortality. The results of this phase informed the choice of doses for the second phase, in which 2000, 3000, and 4000 mg/kg bw were administered to another set of three rats per group. The rats were also observed for sign of toxicity such as pawlicking, salivation, stretching of the entire body, weakness respiratorydistress, coma and dealth for 72 h.

Results and Discussions

Characterization Studies

The waste eggshells as analyzed by Energy Dispersive X-ray Fluorescence (EDXRF), are composed of 61.28% Ca, 0.79% S, 0.36 P and 0.30% Si. The mineralogical examination by X-ray Diffraction (XRD) is composed only of calcite, $CaCO_3$ {96-900-7688}.

Quantitative analysis by EDS of synthesized HAp using waste eggshells

The EDS spectrum of the synthesized HAp powders after heat treatment and reaction with ortho-phosphoric acid is summarized in Fig 1. The Ca/P stoichiometric ratios are also included, contents of elements present in the hydroxyapatite was confirmed from the EDS analysis.

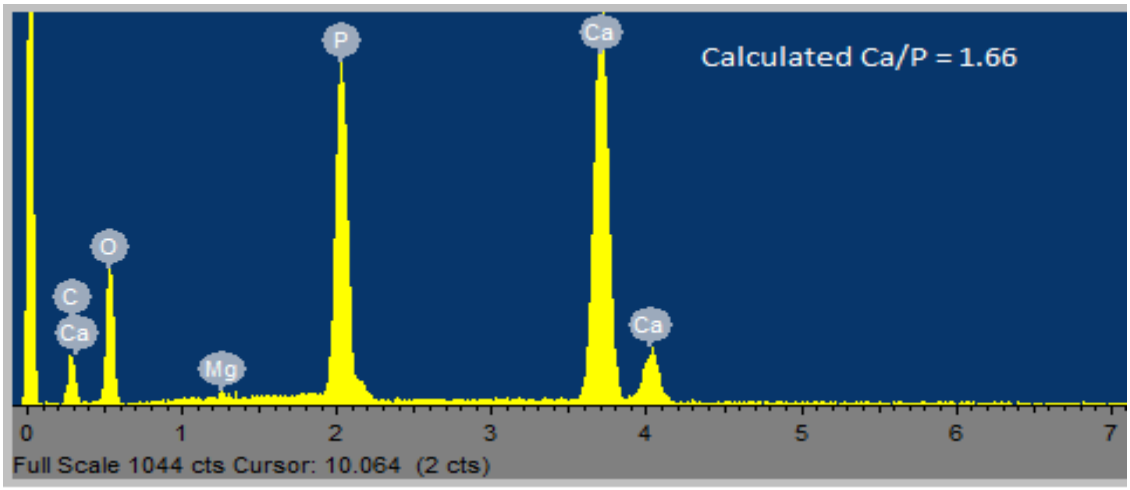

Figure 1: EDS spectra of the HAp product with **Ca/P ratio 1.66** prepared using eggshells calcinated at 800°C after heat treatment by 1M H_3PO_4

From fig. 1, the Ca/P ratio of the HAp synthesized using the egg-shells calcined temperatures calculated at 800°C was found to be 1.66. However, the Ca/P ratio synthesized at lower temperatures such as 600°C and 700°C increases. It is evident that the Ca/P obtained in Fig 1 is apparently the closest to the stoichiometric ratio of 1.67 amenable for biomedical applications [5, 9].

Acute toxicity test

There was no mortality observed in rats after oral adminstration of the HAp powder at doses as high as 4000 mg/kg signifying that the oral LD50 was 4000 mg/kg. Thus, the experimental doses used were within safe margine. The result show that HAp pòwder has negligible toxicity, as shown in the LD_{50} value of 4000 mg/kg [10].

Conclusions

This study presents a chemical method to produce pure, stoichiometric and stable HAp powder using eggshell with orthophosphoric acid solution under precipitation conditions. The proposed method for the synthesis of hydroxyapatite powders led to obtaining a product with a high degree of crystallinity and purity. The EDS patterns reveal that hydroxyapatite is major phase presented in synthesized powders, with the ratio of Ca/P was found to be 1.65 and 1.66 at 700°C and 800°C respectively which is an acceptable range for biomedical applications. According to the biochemical aspect of this study, there was no obvious alteration in the functional indices of liver and kidney which compared favourably with the control values following the administration of the powder. The research thus, shows eggshell as a possible recycling material for producing HAp powder, which can also help in future waste management and keeping environment clean.

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