

SYNTHESIS OF HYDROXYAPATITE FROM WASTE EGG-SHELL BY PRECIPITATION METHOD

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ABSTRACT

In this study, precipitation method using orthophosphoric acid and incubated egg-shell as raw materials was used to prepare Hydroxyapatite (HAp) powder. The raw egg-shells powder consisting primarily: Monohydrocalcite : $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ (29-0306), Ikaite : $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ (37 - 0416) and Hydromagnesite : $\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 4\text{H}_2\text{O}$ (25 - 0513) were calcined to produce lime (CaO) and then reacted with distilled water to form calcium-hydroxide ($\text{Ca}(\text{OH})_2$) which was used as precursors. The effect of calcinations at different temperatures (ranging from 300 - 600°C) and particle sizes (ranging from 0.09 - 0.6 mm) were studied on the waste egg-shells. The as-heated powders and processed powders were characterized by photomicrographic analysis, X-ray powder diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS). The influence of temperature on calcined egg-shell powder was investigated. Increasing temperature of the processed powder decreased the Ca/P ratio of the HAp powder and thus changed the surface morphology of the samples. At optimal conditions, calculated stoichiometric ratio of Ca/P of the synthesized HAp powder (74 - 0565) of 1.65 closed to the theoretical value of Ca/P ratio (1.67) and amenable to biomedical applications, was obtained.

Keywords: Hydroxyapatite; Egg-shell; Precipitation Method, Ca/P Stoichiometric Ratio.

INTRODUCTION

Hydroxyapatite (HAp), is a naturally occurring mineral form of calcium apatite with the formula $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ which is usually written as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. It is comprised of two unit crystal cell entities. HAp is a calcium phosphate ceramic, chemically similar to bone mineral and therefore considered as one of the few materials capable of direct-bonding osteogenesis (Hench and Wilson, 1993). HAp is the main inorganic constituent of bones in humans and it is one of the most versatile materials used for implantation purpose due to its similarity to natural bone material (Hui *et al.*, 2010). It has thus gained considerable attraction due to its excellent osteoconductive and osteointegration properties, allowing synthetic HAp to be used widely in clinical surgery and biomedical applications (Bingol and Duncan, 2012).

Many studies have indicated that HAp ceramics show no toxicity, inflammatory and pyrogenic responses. It has excellent fibrous tissue formation between implant and bone, and has

the ability to bond directly to the host bone (Agrawal *et al.*, 2011). Dense sintered HAp has many bone replacement applications and is used for repairing bone defects in dental and orthopaedic sites, immediate tooth replacement, augmentation of alveolar ridges, pulp capping materials and maxilla facial reconstruction (Shiny *et al.*, 2000). HAp can be used as bone scaffolds and luminescence materials and it also has many important applications in drug delivery and in biomedical engineering (Ming-Guo, 2012). Although many other compounds exist with similar or even identical chemical make-up, the body responds much differently to them. HAp has also been studied for other non-medical applications, for example, coating of hydroxyapatite is often applied to metallic implants, especially stainless steel and titanium alloy to improve the surface properties, as well as packing media for column chromatography, gas sensor and catalysts. (Gergely *et al.*, 2010; Weng, 2009).

Synthetic HAp has been produced using several methods by many researchers under different

experimental conditions. Viable techniques include precipitation method, sol-gel approach, hydrothermal method, multiple emulsion technique and biomimetic deposition technique using different calcium and phosphorus precursors including egg-shell as calcium source (Agrawal *et al.*, 2011; Kimura, 2007; Earl *et al.*, 2006; Afshar *et al.*, 2003; Tas, 2000; Chai and Ben-Nissan, 1999).

The precipitation technique was adopted in this study, owing to rapid synthesis and cost effective HAp powder formation at low temperature and pressure as compared to other techniques. This study was designed to produce HAp using waste egg-shells collected from Tanke area, Ilorin South L.G.A. of Kwara State, Nigeria. Egg-shells are good precursors for HAp synthesis due to high calcium carbonate (94%), calcium phosphate (1%), organic matter (4%) and magnesium carbonate (1%) content and readily available for use as a biomedical material (Rivera *et al.*, 1999).

MATERIALS AND EXPERIMENTAL PROCEDURE

Collection and Sample Processing

Incubated egg-shells of hen collected in bulk were sourced from Tanke area, Ilorin South L.G.A. of Kwara State, Nigeria. They were mechanically cleaned with distilled water and air-dried at room temperature. They were then crushed with mortar and pestle and pulverized into three different particle sizes: 0.09 mm, 0.3 mm and 0.6 mm, respectively. The egg-shell sample was analyzed using X-ray diffraction (Philips PW 1800 XRD at CERD, OAU Ile-Ife, Nigeria); photomicrographic analysis (at the Department of Geology and Mineral Sciences, University of Ilorin, Ilorin-Nigeria), scanning electron microscopy (SEM) and energy dispersive spectroscopy (at electron microscope unit of the University of Cape-Town, South-Africa, using FEI Nova-nano SEM 230 coupled with EDS X-mas detector made by FEI, Eindhoven, Holland), respectively.

Physicochemical Studies

Moisture Content Determination (MC):

One (1) gram of the crushed egg-shell sample was weighed and put into the crucible. The whole lot

was put inside the Gallenkamp, model DV-160 oven BS for about 5 hours at 105°C. After heating for about 5 hours, the sample was withdrawn from the oven and was quickly transferred into the dessicator in order to prevent further moisture absorption from the atmosphere. Later the cooled product was then reweighed. The difference in mass which was the moisture was then determined (Baba *et al.*, 2005).

Loss of Mass on Ignition (LOMOI):

The weighed crucible and oven-dried egg-shell sample was put inside the muffle furnace and roasted at high temperature of about 500 - 600°C for about 2 hours. After roasting, the sample was withdrawn from the furnace and allowed to cool for about an hour. Later, the cooled product was then reweighed (Baba *et al.*, 2005).

pH Determination:

Ten (10) gram of the crushed egg-shell powder was put inside a conical flask. This was followed by the addition of 25 ml distilled water and the mixture was stirred vigorously for about 5 minutes and then allowed to equilibrate. The pH of the supernatant solution was then determined using, HANNA pHep[®] pH meter. The pH measurement was monitored daily for over a period of one week (Baba *et al.*, 2005).

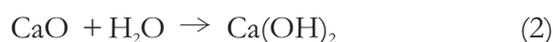
Calcination of Egg-shell Sample

Ten (10) gram of pulverized egg-shell sample (0.09 mm particle size) was accurately weighed into 4 labeled crucibles. The crucibles containing the sample were then subjected to thermal treatment in the muffle furnace, each for 2 hours at the temperatures of 300°C, 400°C, 500°C and 600°C, respectively (Hui *et al.*, 2010). At each of these temperatures, egg-shells identified as calcite phase apparently transformed into calcium oxide (CaO) by freeing carbon dioxide (CO₂). Thus, the combustion of CaCO₃ to CaO is consistent with the following reaction (Rivera *et al.* 1999):



Synthesis of Hydroxyapatite Powders

The CaO was hydrolysed to obtain Ca(OH)₂ solution as follows:



100 ml of the 0.5 M $\text{Ca}(\text{OH})_2$ solution was accurately measured and stirred intensely on a hot plate equipped with magnetic stirrer for 1 hour at a temperature of 90°C after which 100 ml of the 0.3 M ortho-phosphoric acid solution was added dropwise to the $\text{Ca}(\text{OH})_2$ solution for 1 hour at the same temperature (90°C). The resulting mixture was allowed to cool before ageing. The mixture was then covered and left to age for 48 hours during which the pH was monitored and maintained at pH of 10. This was achieved using 1 M NH_4OH (Afshar, 2003). After the ageing process, the solution was filtered and consequently oven dried at 60°C for about 2 hour. This was followed by thermal treatment of the residue at a temperature of 700°C for another 2 hours in the muffle furnace to obtain HAp crystalline powder.

The overall reaction for this procedure is consistent with the following stoichiometry:



The experiment was repeated using waste egg-shells calcined at other temperatures: 400, 500 and 600°C , respectively. The resulting powder (carbon-coated specimens) was analysed by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) analyses, respectively in order to examine the morphology and Ca/P stoichiometric ratio.

RESULTS AND DISCUSSION

Characterization Studies

Photomicrographic Analysis

Figure 1 shows the photomicrography of pulverized waste egg-shell sample.

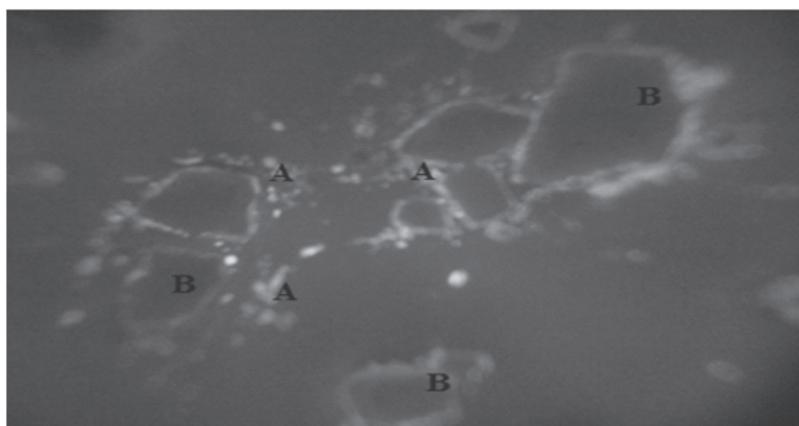


Fig. 1: Photomicrography of Pulverized Waste Egg-shells Sample (0.09 mm)

A = CaCO_3 . B = mixture of organic matter and magnesium carbonate. From the photomicrographic analysis of the pulverized waste egg-shells, shining golden particles can be observed (A). This may be due to CaCO_3 which is the dominant compound (94%) in egg-shells. The dark brown portion (B), may perhaps correspond

to mixture of organic matter (4%) and magnesium carbonate (1%), respectively (Schwetman and Cornell, 1991).

Morphological Purity by XRD

The XRD result of the pulverized waste egg-shell sample is shown in Figure 2.

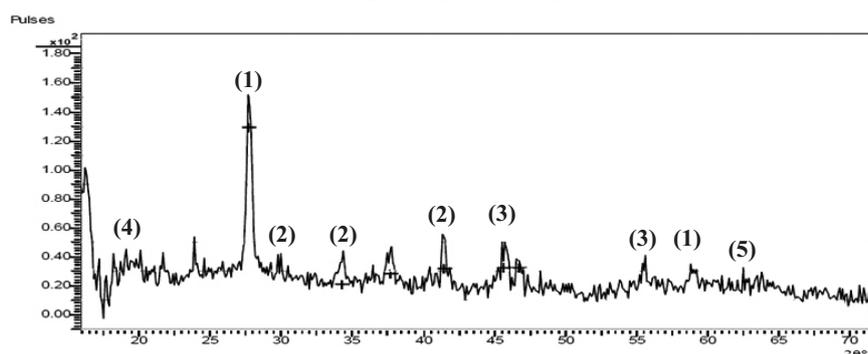


Fig. 2: XRD Spectra of 0.09 mm Pulverized Waste Egg-shell Sample.

The result of morphological purity by X-ray diffraction (XRD) with corresponding Joint Committee of Powder Diffraction Standard (JCPDS) file numbers for peak attributions written in curl brackets, showed that the egg-shells under study consist predominantly of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ (Monohydrocalcite) - {29-0306}, indicated as (1) on the spectra and $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ (Ikaite) - {37-

0416} (2). Other compounds detected include $\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 4\text{H}_2\text{O}$ (Hydromagnesite) - {25-0513} (3); $\text{MgCO}_3 \cdot 5\text{H}_2\text{O}$ (Lansfordite) - {35-0680} (4) and Ca (Calcium) - {23-0430} (5).

Structural Morphology by SEM

The SEM micrograph of the pulverized egg-shell sample is presented in Figure 3.

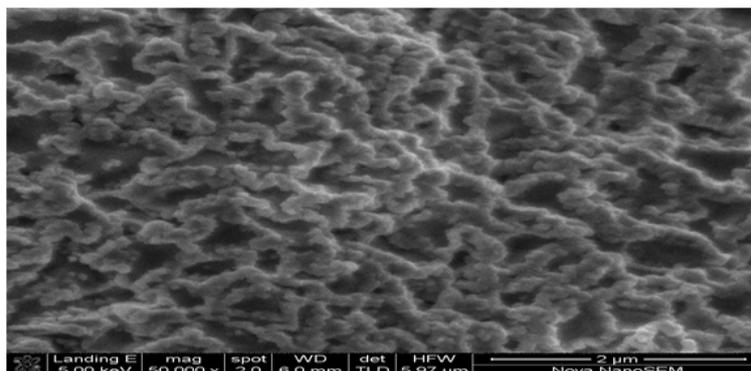


Fig. 3: SEM Micrograph of the Pulverized Waste Egg-shell Sample.

Analysis of the SEM micrograph of the pulverized waste egg-shell sample in Figure 3 above shows irregular uniform pattern and thus, the powder appears to form thread-like particles of indistinguishable morphology.

Quantitative Analysis by EDS.

The quantitative examination of the pulverized waste egg-shells by EDS is shown in Figure 4.

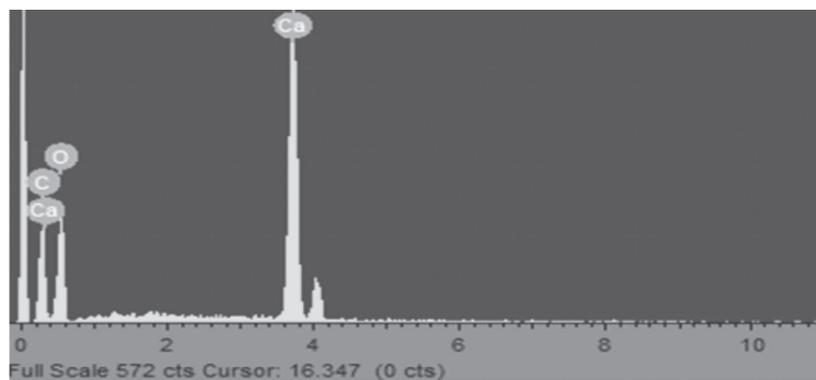


Fig. 4. EDS Spectra of Waste Egg-shell Sample

Analysis of the raw waste egg-shells with respect to the EDS spectra, as shown in Figure 4, indicated that only calcium, oxygen and carbon are the significant elements present in the sample. The mean elemental composition as examined by EDS is in the following proportions: Ca = 21.88%, O = 45.09% and C = 32.92%

Physicochemical Studies.

Result of Moisture Content and Loss of Mass on Ignition

The result of moisture content of the raw/pulverized raw egg-shell is calculated to be 1.1%. Apparently, the percentage mass loss on ignition was 3.8%. This may account for the presence of organic matter and other impurities present in the pulverized egg-shell sample.

pH Determination

The pH of the supernatant pulverized waste egg-shell solution was measured to be 8.5. This indicates that the surface morphology of the pulverized egg-shell solution is slightly basic.

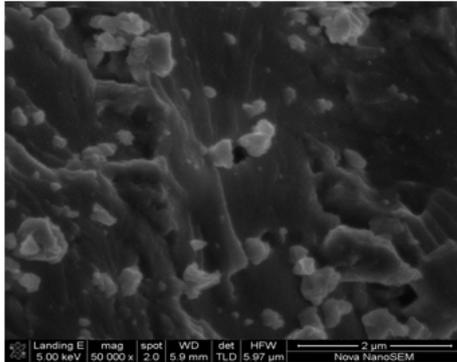


Fig. 5a. SEM Micrograph of Egg-shell Calcined at 300°C

The calcined powder at 300°C (Fig. 5a) appeared to be dense with no specific morphological structure. Thus, there exist some powder particles of irregular sizes and shapes present on the dense surface. However, Figures 5b, 5c and 5d

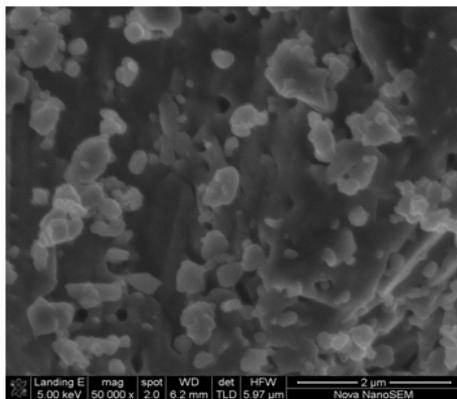


Fig 5c. SEM micrograph of egg-shell calcined at 500°C

At 500°C (Fig. 5c), a dense surface morphology was observed unto which several crystals were deposited on the surface. The SEM image in Figure 5d (treated at 500°C) is similar to the surface morphology depicted in Figure 5c, showing densification behaviour. However, the observed powder particles deposited on the dense surface

Synthetic Analysis of Calcined Egg-Shells Structural Morphology by SEM

The SEM micrographs of the pulverized waste egg-shells sample calcined at different temperatures are reported thus:

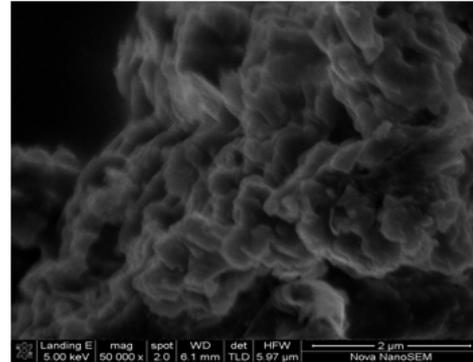


Fig. 5b. SEM Micrograph of Egg-shell Calcined at 400°C

correspond to pulverized egg-shells calcined at 400, 500 and 600°C, respectively. Fig. 5b (treated at 400°C) revealed a cloudy dense surface morphology of the particle with voids being found in between the cloudy particle.

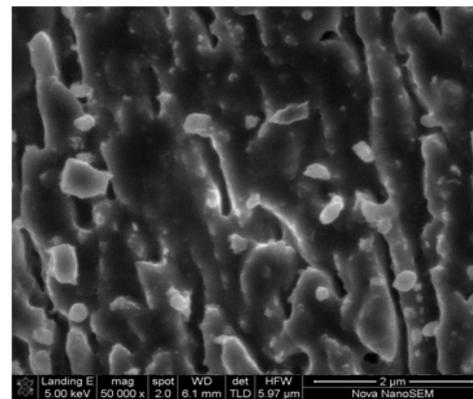


Fig 5c. SEM micrograph of egg-shell calcined at 600°C

appears to be of different sizes and shapes.

Quantitative Analysis by EDS

Figures 6(a-d) show the EDS spectra for the calcined egg-shell sample at temperatures of 300, 400, 500 and 600°C, respectively.

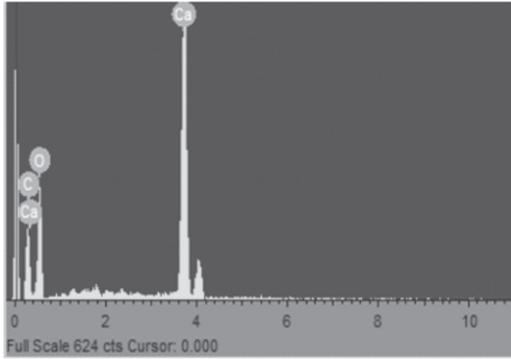


Fig 6a. EDS Spectra of Egg-shells Calcined at 300°C

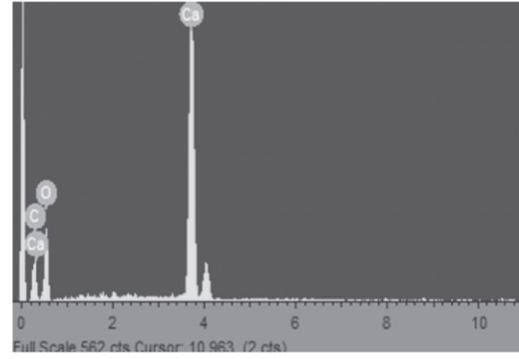


Fig 6b. EDS Spectra of Egg-shells Calcined at 400°C

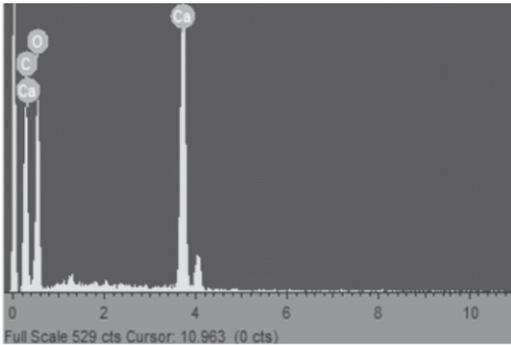


Fig 6c: EDS Spectra of Egg-shell Calcined at 500°C

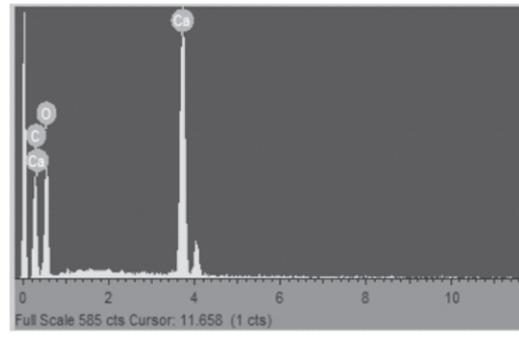


Fig 6d: EDS Spectra of Egg-shell Calcined at 600°C

With respect to the EDS spectra, only calcium, carbon and oxygen are present in all the calcined egg-shell powder. The compositions of these elements at various temperatures are depicted as follows:

(i) 300°C:- **C** (25.76%); **O** (48.08%); **Ca** (26.16%)

(ii) 400°C:- **C** (16.04%); **O** (47.09%); **Ca** (36.02%)

(iii) 500°C:- **C** (15.84%); **O** (46.53%); **Ca** (37.63%)

(iv) 600°C:- **C** (15.04%); **O** (46.09%); **Ca** (38.87%)

These results affirmed that increase in the decomposition temperature of the calcined pulverized egg-shells led to decrease in percentage carbon and oxygen which is due to its conversion to carbon dioxide during heating. Although, the mass of calcium content remained constant, while the percentage increases for all of the calcinated pulverized powder, for better crystallinity prior to further treatment with ortho-phosphoric acid. It is

important to note that the MgCO_3 in the raw egg-shells (Fig. 2) occurred in traces and for this reason (after heat treatment), the component is not significant and was not detected by the EDS machine.

Analysis of Synthesized HAp Structural Morphology by SEM

The SEM results of these investigations were depicted in Figures 7a-d. Figure 7a corresponds to the SEM micrograph of synthetic HAp from egg-shells calcined at 300°C and appropriately treated with ortho-phosphoric acid. The micrograph showed aggregated, agglomerated powder particles with different sizes. For that of 400°C, the morphology revealed a poorly crystalline, dense pattern as shown in Figure 7b.

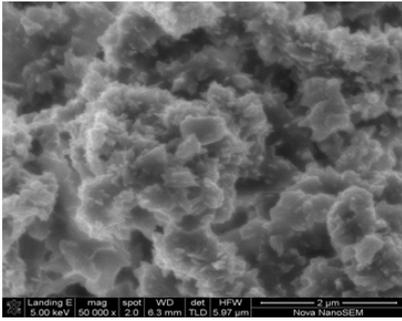


Fig.7a. Synthetic HAP from Egg-shells calcined at 300°C

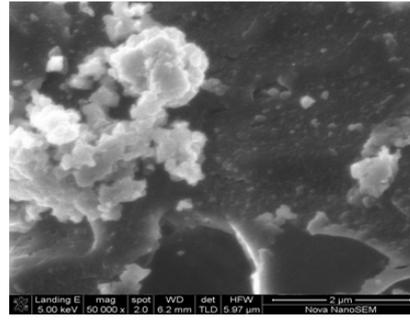


Fig.7b. Synthetic HAP from Egg-shells calcined at 400°C

Figures 7c and 7d show SEM micrographs of HAP synthesis at 500°C and 600°C, respectively.

They both showed fine agglomerates which were of irregular, oval and spherical-pellet-like shapes.

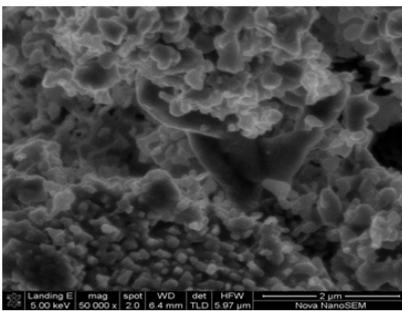


Fig. 7c. Synthetic HAP from Egg-shell calcined at 500°C

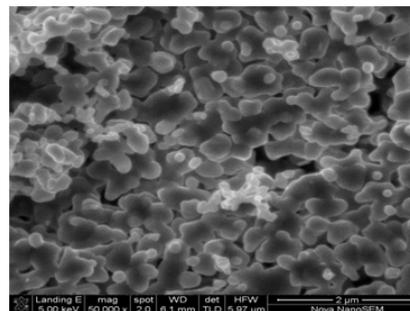


Fig.7d. Synthetic HAP from Egg-shells calcined at 600°C

Quantitative Analysis by EDS

The EDS spectra of the synthesized HAP powders after heat treatment and reaction with

ortho-phosphoric acid are presented in Figures 8(a-d). The calculated Ca/P stoichiometric ratios are also included in each case.

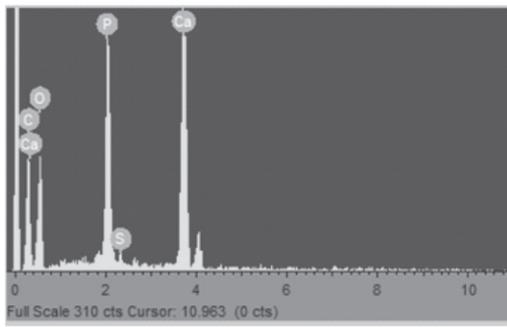


Fig. 8a. EDS Spectra of Hap Synthesis at 700°C using Egg-shell Calcined at 300°C

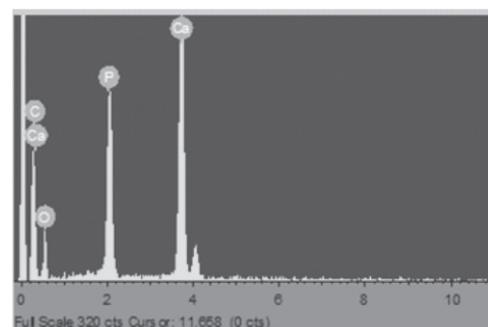


Fig. 8b. EDS Spectra of Hap Synthesis at 700°C using Egg-shell Calcined at 400°C

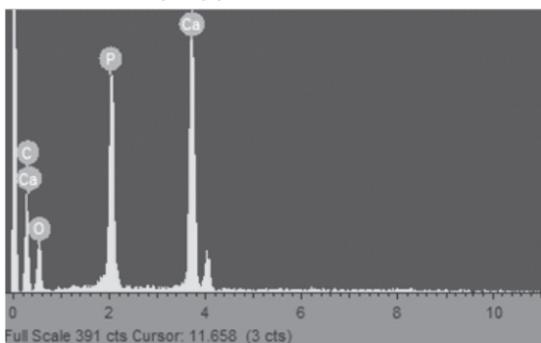


Fig. 8c. EDS Spectra of Hap Synthesis at 700°C using Egg-shell Calcined at 500°C

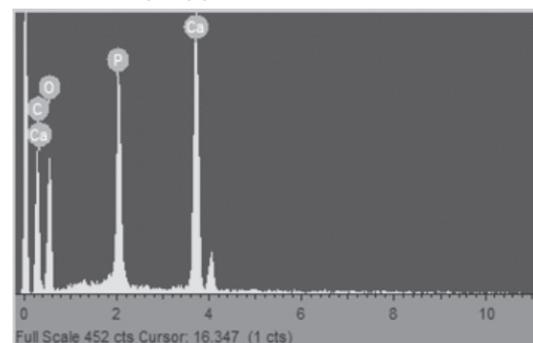


Fig. 8d. EDS Spectra of Hap Synthesis at 700°C using Egg-shell Calcined at 600°C

Figure 8a corresponds to the EDS spectra of HAp synthesized using egg-shells calcined at 300°C. The Ca/P ratio with respect to the EDS data at optimal conditions (pH of 10, 0.3 M H₃PO₄, re-treated temperature 700°C) was calculated to be 1.80. However, Figure 8b, 8c and 8d correspond to the HAp powders synthesized using egg-shells calcined at 400°C, 500°C and 600°C, respectively. The Ca/P ratio of the HAp synthesized using the egg-shells calcined at these temperatures was calculated to be 1.72, 1.69 and 1.65, respectively. Evidently, the Ca/P stoichiometric ratio decreases as the calcination temperature increases. The Ca/P obtained in Figure 8d (1.65) is apparently close to the stoichiometric ratio of 1.67 required for biomedical applications. Finally, analysis of the synthesized HAp powder examined by XRD confirmed the presence of HAp with JCPDS File number 74-0565.

CONCLUSION

In this study, waste egg-shell was used successfully to produce HAp using low temperature treatment by reaction with 0.3 M H₃PO₄ at pH of 10. The results, after detailed characterization showed that calculated Ca/P stoichiometric ratios ranging from 1.69 - 1.65 at optimal conditions (500°C-600°C) affirmed the possibility of producing HAp that may be found suitable for biomedical and non-medical applications.

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