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Synthesis and Characterization of Hydroxyapatite from Chicken Eggshells for Dental Applications

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Abstract: *The study involves the synthesis and characterization of hydroxyapatite (HAp) that can be used for the coating of dental implants or replacement of other body tissues from eggshells. Raw chicken eggshells were grinded into fine particles less than 250 µm and calcined at 900 °C for 2 hours to transform the calcium carbonate from the eggshells to calcium oxide and to eliminate the organic phases or residue present. 0.6 M phosphoric acid was added to the calcium oxide in water. The final solution was left to age at various intervals: 0, 6, 16 and 24 hours to form hydroxyapatite. The synthesized hydroxyapatite particles were oven dried at 100 °C for 2 hours and sintered at 1200 °C. In general, the XRD patterns correspond to the characteristic peaks of HAp but some differences were observed. The identification of HAp is primarily based on the two most prominent peaks at 26° and at 32–34°. The presence of HAp's characteristic diffraction peaks near 25°, 33°, 40°, and 50° confirmed that the synthesized material was HAp. Typical absorption bands corresponding to carbonated HAp was observed by different vibrational modes of the phosphate PO_4^{3-}, hydroxyl OH^- and carbonate CO_3 groups. The peaks at 567.8, 608.9, 1115.57 and 1007.9 cm^{-1} are attributed to vibrations of tetrahedral phosphate group in HAp. The peak extending from 3500 to 3642 cm^{-1} is due to the stretching vibration of OH^- group of HAp. The Ca/P ratio, which is close to stoichiometric HAp (1.67), was observed at the aging time of 24 hours.*

Keywords: *Hydroxyapatite, Eggshells, Dental Implants, Characterization.*

Introduction

Human body and parts including the tooth, degrade especially at the old age. Tooth damage can be caused by accidents, injuries, defects, diseases or decay. Dentistry is a field of medical science concerned with the prevention, diagnosis, treatment of diseases of the teeth and adjacent tissues, as well as the restoration of missing dental and maxillofacial structures. Every dental restorative procedure requires the use of materials. Dental implants also considered as an artificial tooth root, are biocompatible metal anchors surgically positioned in the jaw bone (in other words, surgically traumatized bone) underneath the gums to support an artificial crown where natural teeth are missing. Dental implants are the most natural looking tooth replacement option and do not slip like dentures. Implants may be the right choice for a person missing one, multiple, or even all of their teeth (Oshida *et al.*, 2010)

Hydroxyapatite (HAp) is the most useful inorganic biomaterial used for biomedical applications (Agrawal *et al.*, 2014). It is a naturally occurring ceramic mineral of calcium phosphate in the apatite family with chemical formula $Ca_{10}(PO_4)_6(OH)_2$. It is bioactive, osteophilic in nature

making it suitable for use in the human body particularly for dental systems (Ben-Nissan, 2003). The human bone contains 70 % hydroxyapatite and 30 % other organic elements (largely collagen) (Prabakaran *et al.*, 2005). Teeth enamel is mainly made up of the mineral hydroxyapatite. It is the hardest tissue in the human body because it contains almost no water. Structurally, enamel covers the entire anatomic crown of the tooth above the gum and protects the dentin (Tillberg *et al.*, 2008; Vaderhobli, 2011). According to Raihana *et al.*, (2008), Hydroxyapatite ceramic has a very good bioactivity with many proven medical applications in the form of porous, dense and granular bodies and it is mostly applied in coating form.

An eggshell consists of a three-layered structure, namely the cuticle, the spongeous layer and the lamellar layer. The cuticle layer represents the outermost surface and it consists of a number of proteins. Spongeous and lamellar layers form a matrix constituted by protein fibers bonded to calcite calcium carbonate crystals $CaCO_3$ in the proportion of 1:50. The eggshell represents about 11 % of the total weight of egg and is composed of 94 % calcium carbonate, 1 % calcium phosphate, 4 % organic matter and 1 % magnesium carbonate (Eric *et al.*, 1999). The presence of high amounts of calcium carbonate makes eggshell a suitable calcium source as a starting material for production of hydroxyapatite for commercial use.

Eggshells are a form of agricultural waste. Tons of eggs are produced yearly. In Africa alone, three million tonnes of eggs were being produced as at 2012. Nigeria is the largest egg producer in Africa recording an average annual growth rate of four percent between 2000 and 2012 when the output reached 640,000 tonnes (FAO, 2017). Using the data above, we can deduce that at least 330,000 tonnes of eggshells are waste in Africa and 70,400 tonnes of eggshells are left as waste yearly in Nigeria alone. This is quite a heavy amount of waste material coming from eggs alone. These wastes are mostly not disposed properly and can lead to adverse effect on human health, air borne diseases and environment: pollution (land, air and water).

Experimental Procedure

Extraction and Synthesis of Hydroxyapatite from Egg Shell

The eggshells were thoroughly cleaned using distilled water and dried in an oven for 30 minutes. The eggshells were grounded using a mortar and pestle and then made into fine powder using a blender. The resulting powder were screened using a 60 mesh sieve to ensure uniform particle size (0-250 microns), and finally the powdered eggshells were calcined at 900 ℃ for 2 hours converting the calcium carbonate into calcium oxide with the evolution of carbon dioxide as represented in Equation (1);

$$\boldsymbol{CaCO_3 \underset{yields}{\Longrightarrow} CaO + CO_2;\ T = (900)\ ℃} \qquad \textbf{(1)}$$

Hydroxyapatite was synthesized using the procedure adapted from Adeogun *et al.*, 2017. After the heat treatment, weighed amount of the calcium oxide were dispersed in distilled water and stirred for 30 minutes with a magnetic stirrer producing calcium hydroxide as represented in Equation (2);

$$\boldsymbol{CaO + H_2O \underset{yields}{\Longrightarrow} + Ca(OH)_2} \qquad \textbf{(2)}$$

200mL of 0.6M phosphoric acid solution was added to calcium hydroxide suspension at the rate of 1 $mLmin^{-1}$ in order to achieve the stoichiometric calcium to phosphorous ratio, which is 1.667. The reactants (calcium hydroxide and phosphoric acid) were stirred for 2 hours and left to age for 12 hours and some for 24 hours to view the effect of aging time on the morphology. Ammonium hydroxide (NH_4OH) was added in order to raise pH of the solution prior to the precipitation of HAp. The solution was kept at pH of 9 and another at pH of 10. The pH is measured with a pH checker and addition of Ammonium hydroxide was stopped when desired pH was reached.

$$\mathbf{10}Ca(OH)_2 + \mathbf{6}H_3PO_4 \underset{yields}{\Longrightarrow} Ca_{10}(PO)_4(OH)_2 + \mathbf{18}(H)_2O \quad \textbf{(3)}$$

Finally, the synthesized hydroxyapatite was separated by filtration. The final products were washed thoroughly to get rid of any excessive acid and base ion. It was then dried for 1 hour at 100 ℃. The synthesized hydroxyapatite powder was sintered at 1200 ℃ for 2 hours (Kamalanathan *et al.*, 2014). The crystallographic structure of the synthesized powder was examined by X-ray diffractometer (XRD). An XRD operated at 40 kV and a 30 mA Cu target was used. The 2θ scanning range was performed from 2 - 70^0 continuous scan and a scan speed of 8°/min with pre-set time of 0.15 sec.

Fourier Transform-Infrared Spectroscopy (FTIR) Analysis was used to determine the organic and inorganic functional group present in the synthesized hydroxyapatite. The measurements were carried out in the transmission mode in the mid-infrared range with wave numbers from 400 cm^{-1} to 4000 cm^{-1} using Schimadzu's UV-2550, JAPAN.

Results and Discussion

Effect of Calcining Time and Temperature

Figures 1 show sample of the eggshells before and after calcination. There was a color change from light brown of the eggshells powder in fig 1 (a) to black ash in fig 1 (b) which is as a result of the calcination of raw eggshells. This occurred after putting the eggshell powder in the furnace to heat for some hours. The black color can be attributed to the carbon released during the reaction process. This shows incomplete calcination. On further heating for 2 hours at 900 ^{0}C the resultant powder was whitish as shown in Figure 1(c) indicating the presence of calcium oxide and complete calcination. This shows that carbon dioxide (CO_2) and other organic matter present in the eggshells have been eliminated.

a b c

Figure 1: Images showing Grinded Raw Eggshells (a), Semi Calcined Eggshells (b), Completely calcined Eggshells to Calcium Oxide (c)

Physiochemical Characterization (X-ray Diffraction (XRD))

Figures 2 to 5 Show XRD patterns of HAp samples obtained at various aging times.

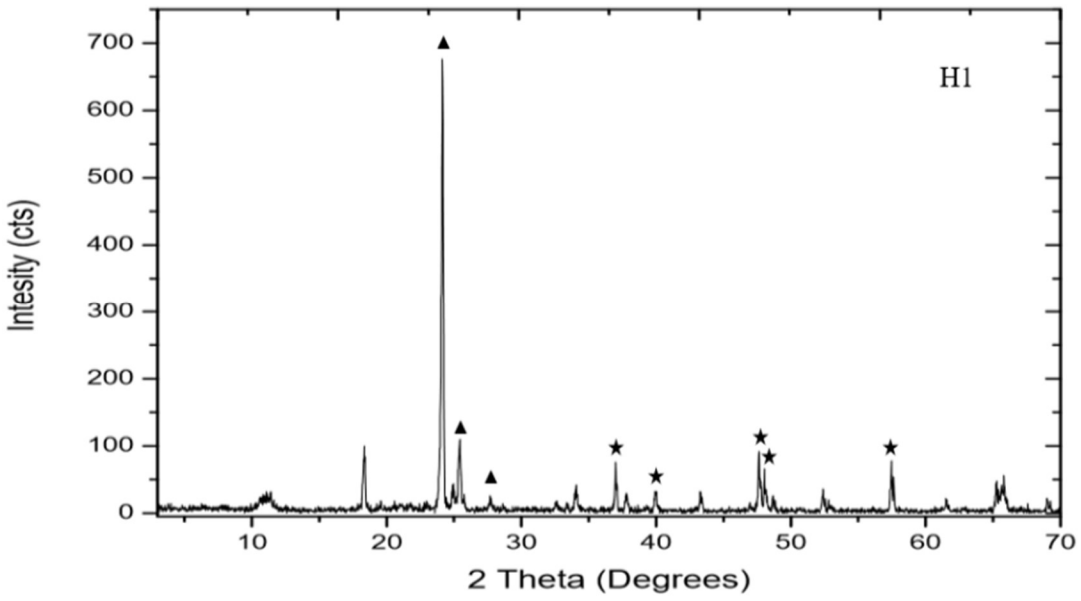

Figure 2: XRD patterns of Hydroxyapatite obtained after aging for 0 hours

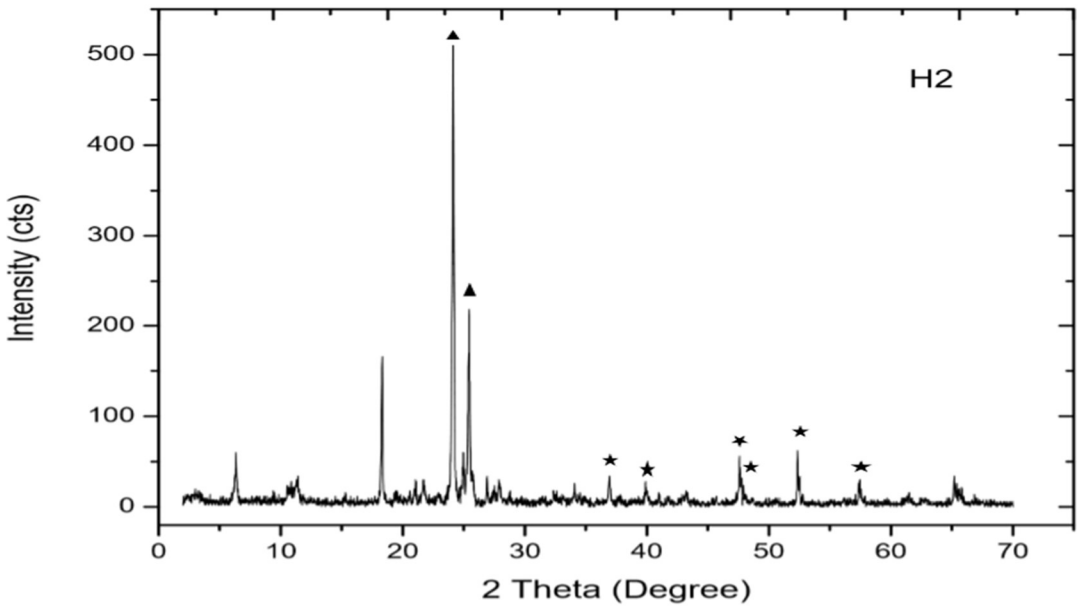

Figure 3: XRD patterns of Hydroxyapatite obtained after aging for 6 hours

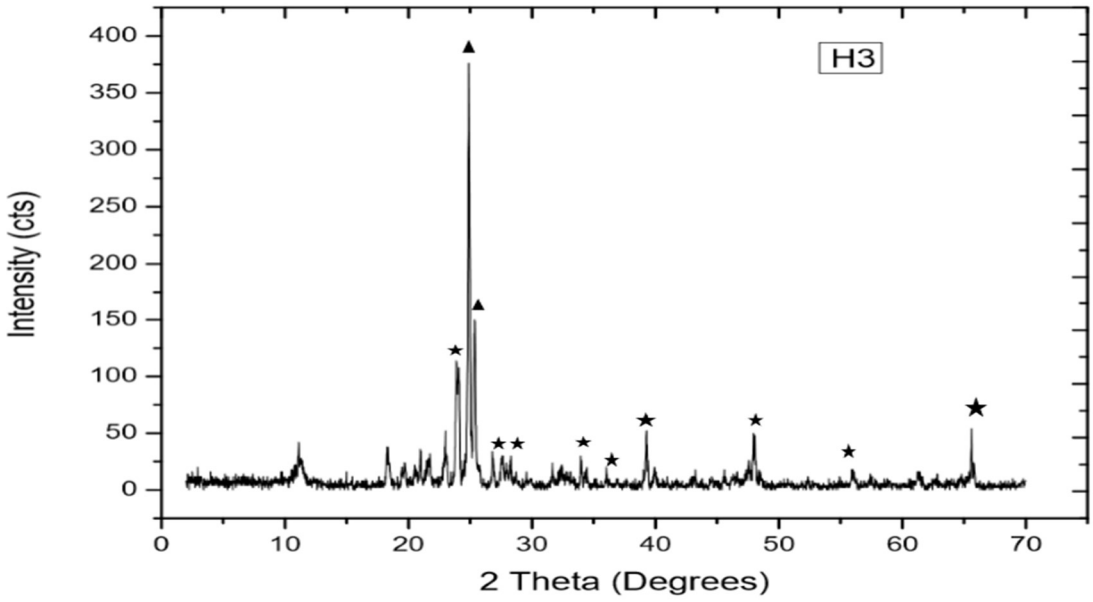

Figure 4: XRD patterns of Hydroxyapatite obtained after aging for 16 hours

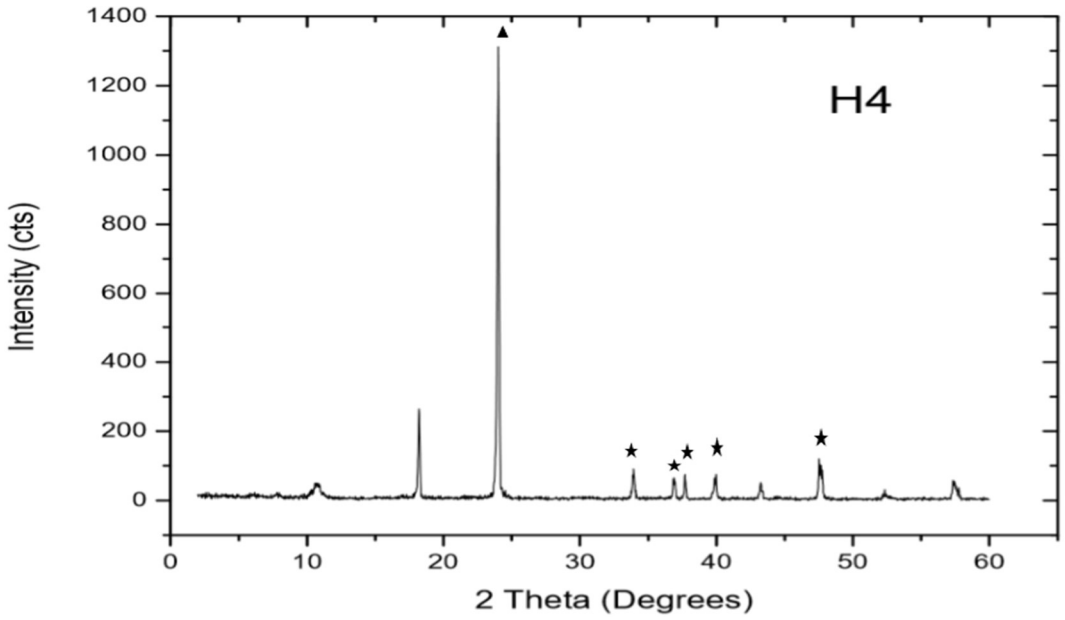

Figure 5: XRD patterns of Hydroxyapatite obtained after aging for 24 hours

In general, the XRD patterns correspond to the characteristic peaks of HAp but some differences were observed. The identification of HAp is primarily based on the two most prominent peaks at 26° and at 32–34°. The presence of HAp's characteristic diffraction peaks near 25°, 33°, 40°, and 50° confirmed that the synthesized material was HAp (Piantone *et al*., 2003). As seen in Figures 2 - 5, in this case however, it was observed that the most prominent peak was at 26° only for all the four samples at different aging times. This can be attributed to the phosphate source used for the synthesis and the presence of β- Tricalcium phosphate (TCP: $Ca_3(PO_4)_2$) owing to incomplete reaction and some side reactions that occurred during synthesis. There was substantial increase in peak height and decrease in peak width thus indicating an increase in crystallinity and crystallite size (Kamalanathan *et al.*, 2014).

The FTIR absorption spectra of the synthesized HAp were obtained for the analysis of functional group present in the samples as shown in Figures 6 to 8. Typical absorption bands corresponding to carbonated HAp was observed by different vibrational modes of the phosphate PO_4^{3-}, hydroxyl OH^- and carbonate CO_3 groups. The peaks at 567.8, 608.9, 1115.57 and 1007.9 cm^{-1} are attributed to vibrations of tetrahedral phosphate group in HAp, the peak extending from 3500 to 3642 cm^{-1} is due to the stretching vibration of OH^- group of HAp while the bands at 1638 and 2550 cm^{-1} can be attributed to adsorbed water molecules (Adeogun et al., 2017).

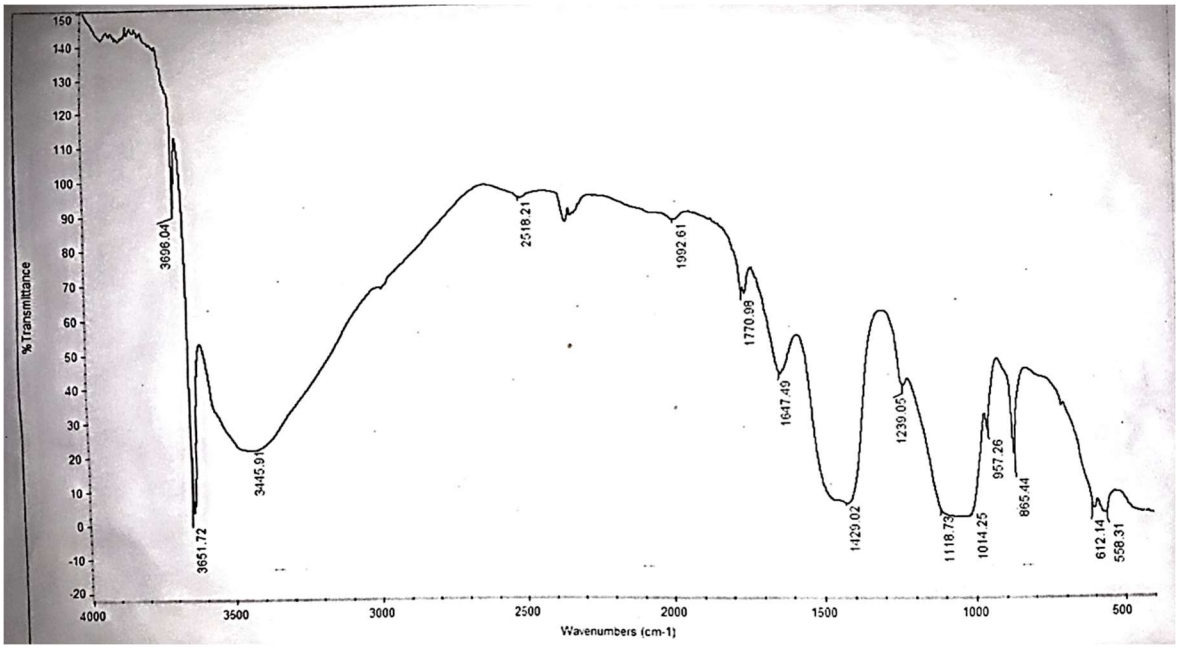

Figure 6: FTIR for HAp left to age for 0 hours

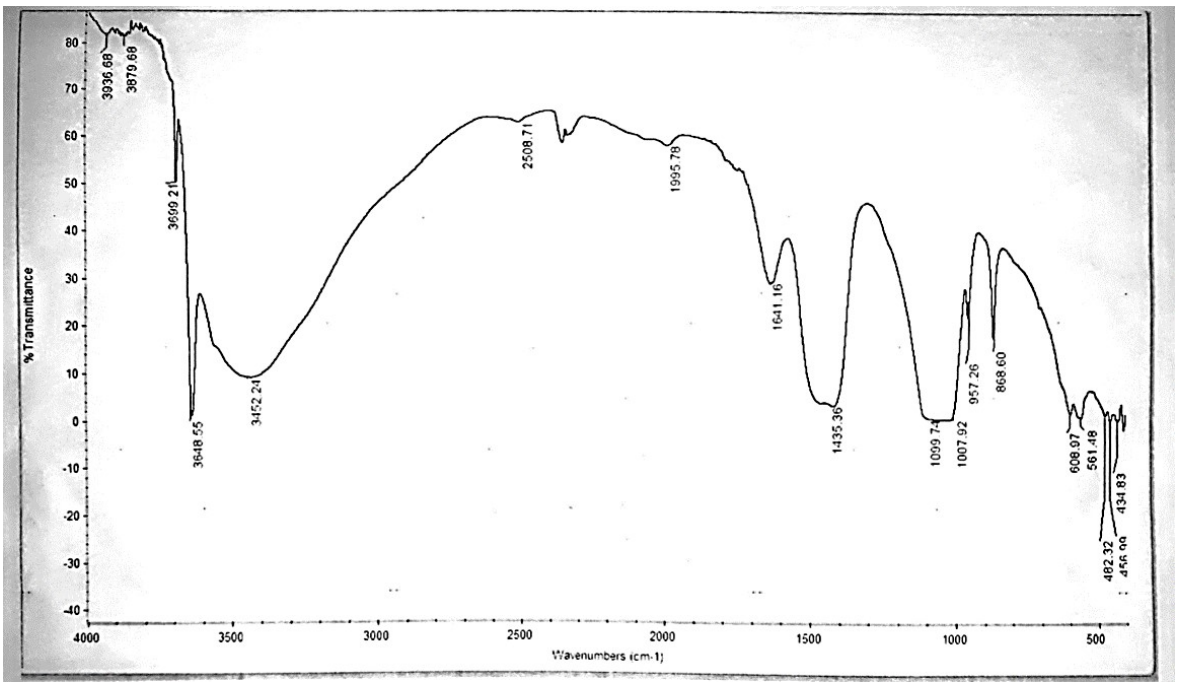

Figure 7: FTIR for HAp left to age for 6 hours

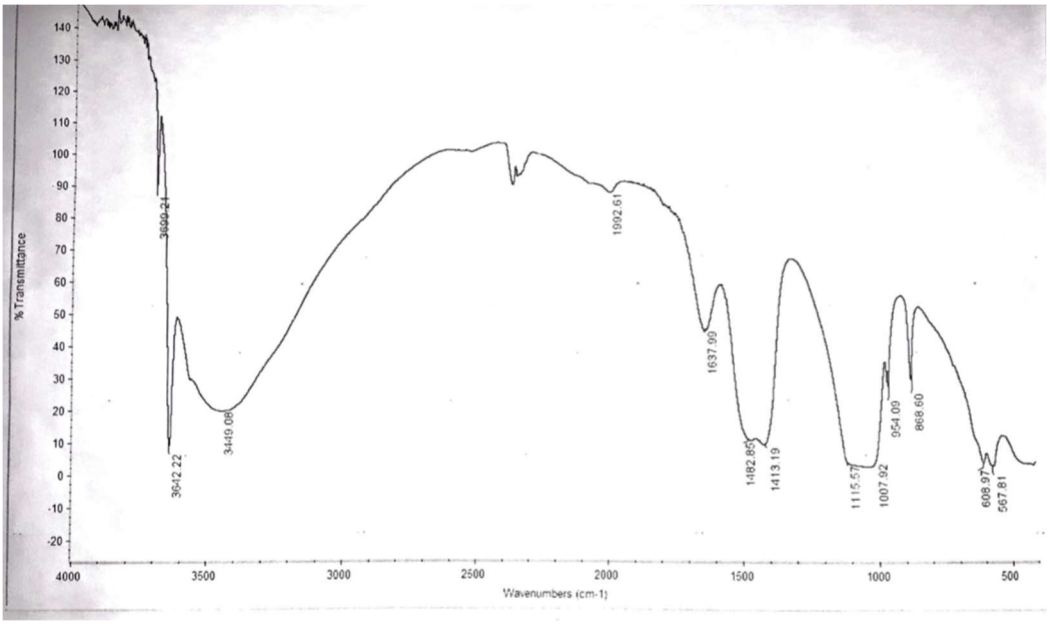

Figure 8: FTIR for HAp left to age for 24 hours

Ca/P composition analysis

Table 1 shows the Ca/P ratio for various aging times gotten from the composition analysis. The Ca/P ratio, which is close to stoichiometric HAp (1.67), was observed at the aging time of 24 hours. At lower aging times; 0, 6 hours, unreacted CaO was observed. At longer aging times, β-TCP phase was observed (Sang-Jin *et al.*, 2007).

Table 1: Ca/P ratio of hydroxyapatite obtained from various aging times

Aging time (Hours)	Ca/P ratio	Observed phases
0	1.956132	HAp + CaO
6	1.821426	HAp + CaO
16	1.698138	HAp + β-TCP
24	1.681597	HAp + β-TCP

Conclusion

Hydroxyapatite can be successfully synthesized from raw eggshells by the wet chemical precipitation process. Observations show that calcining at 900 °C for 4 hours converts the eggshells to calcium oxide. Optimum aging time for the synthesis of hydroxyapatite by addition of phosphoric acid to a solution of calcium oxide in water was found to be 24 hours. The reaction occurred at a pH above 8 to stabilize the precipitated HAp. The Ca/P ratio which is close to stoichiometric HAp (1.67), was observed at the aging time of 24 hours. However, β-Tri Calcium Phosphate ($Ca_3(PO_4)_2$) was found to have formed at various intervals owing to incomplete transformation of calcium oxide to HAp.

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