

# Enrichment of a Nigerian Calcite Ore for Hydroxyapatite Production

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## Abstract

In this study, Hydroxyapatite (HAp) powders have been prepared from Calcite ore of Nigerian origin using diammonium hydrogen phosphate as the Phosphorus source via hydrothermal method. The HAp powders formed is used to determine the chemical structure and nature of HAp crystals at different reaction temperature and time of operation were characterized by X-ray Fluorescence (XRF), X-ray Diffraction (XRD), Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-ray (EDS) and Fourier Transform Infra-red (FT-IR) techniques. Using a set of experimental conditions, different morphology, crystallinity and stoichiometric ratios were obtained. At optimal conditions (1M (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 85°C, 6 hours reaction time), a rectangular, rod-like and layered HAp crystal formed yielded Ca/P molar ratio of 1.66 which is close to the stoichiometric HAp ratio of 1.67 required for biomedical applications.

**Keywords:** Hydroxyapatite; Calcite ore; Nigeria; Hydrothermal method; Ca/P stoichiometric ratio.

## 1. INTRODUCTION

Hydroxyapatite (HAp), an important inorganic biomaterial has attracted the attention of researchers in recent years [1]. Thus, considerable efforts have been geared towards developing relatively cheap and accessible artificial bones and teeth that would not cause damage to healthy tissues. Consequently, synthetic ceramic materials based on calcium phosphate (CaP) particularly in the composition of tricalcium phosphate TCP – Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> and Hydroxyapatite (HAp – Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>] have extensively been studied and clinically used [2]. HAp has widely been used in biomedical and dental applications due to its similarity to main

mineral components of hard tissues of human body such as bone, dental enamel and dentin and also its biocompatibility, bioactivity and low solubility in moist media [3]. All these functions are possible due to its osteoconductive and osteointegration distinct properties exhibited by HAp powders [4].

Several methods have been utilized for the production of HAp under different experimental conditions by many researchers. Some of these techniques include precipitation, multiple emulsion, hydrothermal, electrodeposition, sol-gel and biomimetic deposition methods. [1,2, 4-9, 11]. Of these aforementioned techniques,

hydrothermal method, due to its ease of crystal surface control, higher colloidal stability, low cost and time saving considerations is often preferred [3, 8]. This approach utilizes single or heterogeneous phase reaction at elevated temperature ( $T > 25^{\circ}\text{C}$ ) and pressure ( $P > 100\text{kPa}$ ) for compound crystallinity in aqueous solutions where the Ca/P stoichiometric ratio of the precipitate formed improves with increasing reaction temperature [3-5, 8, 10]. Despite many works on HAp synthesis from various precursors such as calcium sulfate hemihydrate [4], egg-shell [7, 10], dead snail shells [12], animal bones [13] among others, little or no effort has been directed towards the use of Calcite ore found in abundance across the globe.

In Nigeria for example, vast deposits of Calcite ore occurs in the Benue trough, Sokoto, and Borno (Chad) Basins. However, the ore at Mfamosing, near Calabar (Cross River State) is the purest deposit in Nigeria. It is about 50 meters thick at the quarry site [14]. Other important locations of Calcite ore deposit with

their approximate proven reserves in Nigeria are summarized in Table 1.

Till date, it is important to note that Calcite ore is widely used as crushed stone or aggregate for general building purposes, road beds and railway lines, production of asbestos, glass and ceramics in Nigeria [14]. Thus, it is a worthwhile venture to upgrade its uses for producing high grade HAp. Although, Ewekoro ore (Ogun State) is not the most abundant as compared to those of Nkalagu (Ebonyi State), but due to its ease of accessibility and minimal protocols in obtaining the ore, Ewekoro Calcite ore was chosen for this study. To the best of our knowledge, there is practically limited data in this area of research in Nigeria [7]. Therefore, utilization from this abundant ore deposit for producing high grade HAp would no doubt add to country's economic viability, growth and development. Hence, the aim of this investigation was geared towards producing high HAp powder that would be amenable in biomedical applications via diammonium hydrogen phosphate medium by hydrothermal method.

Table 1: Locations of Calcite ore (Limestone) deposits in Nigeria with their proven reserves (million tonnes) [13]

| State       | Location         | Estimated reserves (million tonnes) |
|-------------|------------------|-------------------------------------|
| Ogun        | Ewekoro          | 35                                  |
|             | Shagamu          | 10                                  |
|             | Ibeshe           | -                                   |
| Cross River | Mfamosing        | 30                                  |
|             | Odukpani, Obubua | -                                   |
| Ebonyi      | Nkalagu          | 174                                 |
| Enugu       | Nkanu            | 110                                 |
|             | Odomoki          | 54                                  |
|             | Ngbo             | 2                                   |
| Gombe       | Ashaka           | -                                   |
| Edo         | Akoko-Edo        | 10                                  |
| Imo         | Umu-Obon         | 101                                 |
| Sokoto      | Kalambaina       | 101.6                               |
| Benue       | Yandeu           | 70                                  |
|             | Igumale          | 110                                 |

- not available.

## 2. MATERIALS AND METHODS

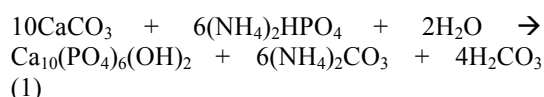
Calcite ore used for this study was kindly supplied by Lafarge Cement Industry, Ewekoro, Ewekoro Local Government Area, Ogun State, Nigeria. It was then crushed with acetone-rinsed

mortar and pestle and pulverized into four different particle sizes: 0.09 mm, 0.112 mm, 0.25 mm and 0.3 mm. The particle size 0.09 mm (owing to its large specific surface area) unless otherwise stated was used for all experiments in

this study. Commercial grade Calcite ore ( $\text{CaCO}_3$ ) was used as starting material to synthesize the HAp powder. Diammonium hydrogen phosphate  $(\text{NH}_4)_2\text{HPO}_4$ , BDH product with a molecular weight of 132.06 g/mol was used in preparing the aqueous reactant solutions.

## 2.1 Procedures

HAp powder was synthesized at the following temperatures: 25°C, 55°C and 85°C to monitor the reaction kinetics. The reaction conversion was achieved according to the following reaction (equation 1):



At a set of experimental condition, 40 ml of 33.015 g of  $(\text{NH}_4)_2\text{HPO}_4$  in 250 ml glass reactor was mixed with 4 g of ore at prescribed temperature. The resulting solution was intensely stirred on a Bibby Stuart hot plate equipped with magnetic stirrer for different reaction times from 30 minutes to 360 minutes for 25°C, 55°C and 85°C, respectively. At the end of each reaction, and for different time and temperature, the solid product formed was thoroughly washed with de-ionized water and was then filtered to eliminate any water soluble impurities. The resulting solid

was then air dried and oven-dried at 75°C for 1 hour [4]. The solids were characterized by X-ray diffraction (The EMPYREAN X-ray Diffractometer), Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (FEI, Nova-nano SEM 230 coupled with EDS X-mas detector by FEI, Eindhoven, Holland) for material purity and morphological studies, respectively. The Energy Dispersive X-ray fluorescence (EDXRF: The MINI PAL 4) and Fourier Transform Infra red (BUCK M530 FT-IR) were employed for elemental and functional group analyses, respectively. (*Hint: HAp powder formed at 25°C and 55°C during 2 and 4 hours treatments were not considered in this study due to their poor crystallinity*). Prior to the HAp powder preparations, the physicochemical analysis of the raw calcite ore such as the moisture content, loss of mass on ignition and pH were carried out as detailed elsewhere [15, 18].

## 3. RESULTS AND DISCUSSION

### 3.1 Characterization studies

The chemical composition of the Ewekoro Calcite ore characterized by EDXRF technique which is only semi-quantitative showed that CaO (84.43%),  $\text{Al}_2\text{O}_3$  (3.6%),  $\text{SiO}_2$  (5.9%) and  $\text{Fe}_2\text{O}_3$  (3.8%) constituted the major elemental species.

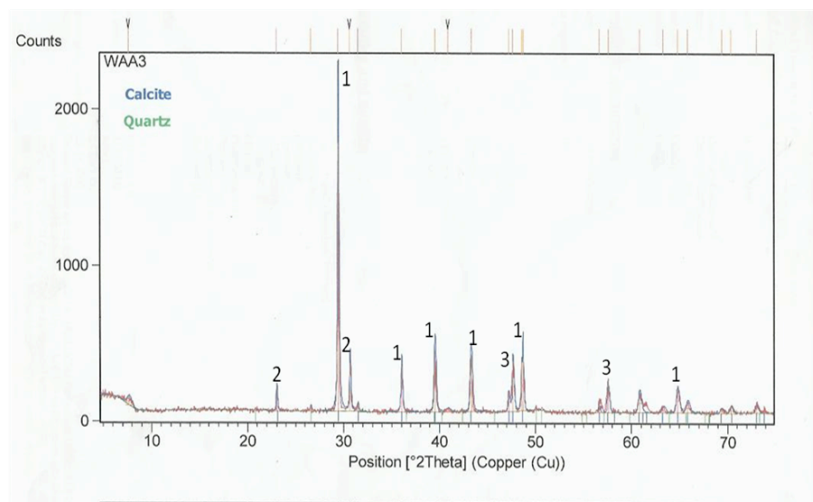


Fig.1 XRD pattern of Ewekoro (Nigeria) Calcite ore with important compounds detected. The Joint Committee on Powder Diffraction Standard file numbers (JCPDS) numbers are put in curl brackets: (1)  $\text{CaCO}_3$  {85-1108}, (h k l = 1 0 4); (2)  $\text{SiO}_2$  {75-8320}, (h k l = 0 1 1); (3) Mixtures of impurities e.g.  $\text{Al}_2\text{O}_3$ ,  $\text{MnO}_2$ ,  $\text{Fe}_2\text{O}_3$ , etc.

Other compounds detected and found occurring from low to trace levels ( $\leq 1\%$ ) are  $\text{TiO}_2$  (0.15%),  $\text{V}_2\text{O}_5$  (0.031%),  $\text{Mn}_2\text{O}_3$  (0.054%),  $\text{Y}_2\text{O}_3$  (0.66%),  $\text{In}_2\text{O}_3$  (0.1%),  $\text{Eu}_2\text{O}_3$  (0.01%)  $\text{Yb}_2\text{O}_3$  (0.01%) and  $\text{Lu}_2\text{O}_3$  (0.07%). The X-ray results are shown in Fig. 1.

The result of the morphological purity by X-ray Diffraction (XRD) shows that the raw calcite ore consists primarily of Calcium carbonate ( $\text{CaCO}_3$ ) and  $\alpha$ -quartz ( $\text{SiO}_2$ ).

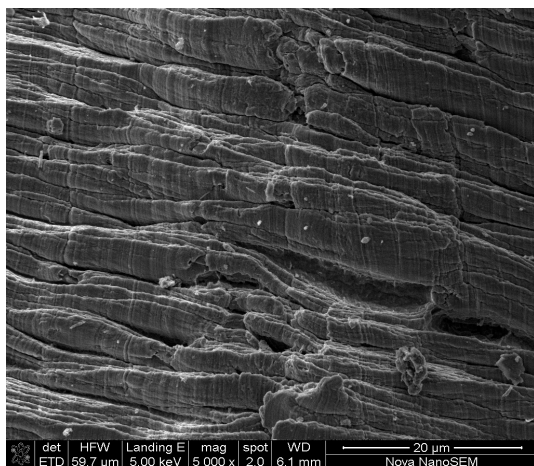


Fig. 2: SEM micrograph of raw calcite ore

The structural micrograph by SEM of raw calcite ore shown in Fig. 2 gave irregular, uniform layered rod-like pattern and rocky morphology. However, the results of the physico-chemical analyses performed on the pulverized calcite ore showed that:

- (i) The moisture content of the ore is  $2.01 \pm 0.03\%$ . The moisture content enables us to find out the state of dryness of the ore and to make appropriate corrections in the analytical data [15, 16];

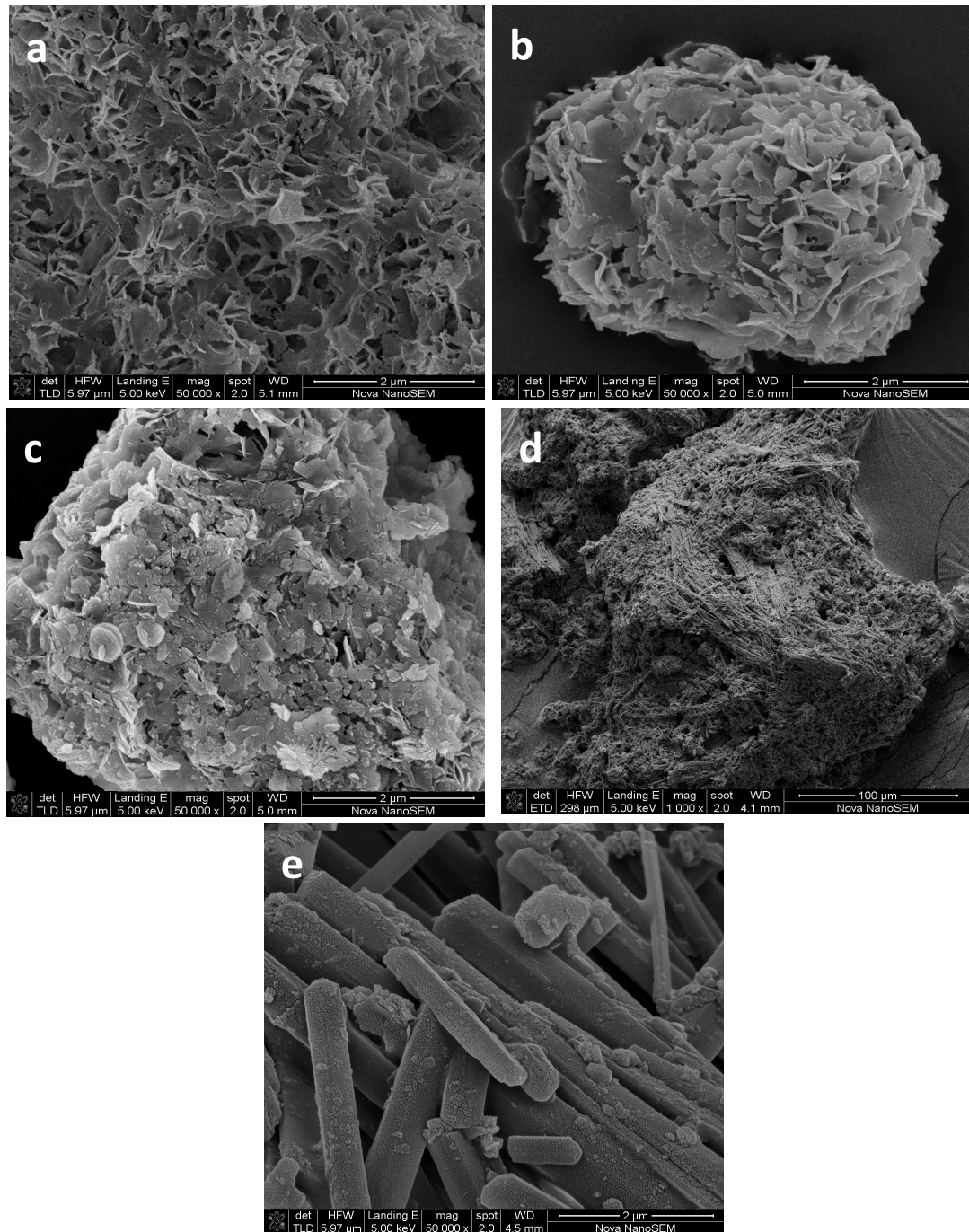
- (ii) The loss of mass on ignition is  $1.02 \pm 0.011\%$ . This value may account for possible presence of organic/decayed matters present in the ore under study [15].
- (iii) The pH of the calcite ore-water suspension was 8.24. This value indicates the surface morphology of the calcite ore – water solution to be slightly basic.

### 3.2 Hydroxyapatite analyses

#### 3.2.1 HAp powder structural morphology at different temperatures

The SEM micrographic structures of the synthesized HAp powders at different temperatures with diammonium hydrogen phosphate are summarized in Fig. 3 (a-e).

Fig. 3(a) corresponds to the SEM micrograph of synthetic HAp powder obtained during treatment at  $25^\circ\text{C}$  for 6 hours showing a loosely packed agglomerated plate-like structure. This apparently indicates gradual formation of HAp. At  $55^\circ\text{C}$  for 6 hours (Fig. 3b), the formation gave spherical and condensed crystal structure. The structure formed revealed agglomerated powder with voids present in between the agglomerates. An increase in the reaction temperature reveals a more spherical and condensed crystal particles of the synthetic HAp produced at  $85^\circ\text{C}$  for 1 hour (Fig 3c). During 4 hours treatment at  $85^\circ\text{C}$ , the image in Fig 3(d) shows that the HAp powder granule is a soft, microporous, flood-like (erosion) morphological structure. Increasing reaction time up to 6 hours at  $85^\circ\text{C}$  revealed rectangular, rod-like and clean layered crystals of synthetic HAp product powder formed (Fig 3e). The product of the HAp formed at this optimal condition is comparable with some literature results [11, 19-23].



**Fig. 3:** SEM micrograph of HAp product obtained under hydrothermal conditions after reaction with 1M  $(\text{NH}_4)_2\text{HPO}_4$  solution for (a) 6 hours at 25°C, (b) 6 hours at 55°C, (c) 1 hour at 85°C, (d) 4 hours at 85°C, (e) 6 hours at 85°C.

### 3.2.2 Quantitative Analysis of HAp powder formed by EDS

The EDS spectra of the synthesized HAp powders at different temperatures (25<sup>0</sup>C - 85<sup>0</sup>C)

by diammonium hydrogen phosphate for the previously reported morphological structures {Figs. 3(a-e)} are presented in Fig 4(a-e).

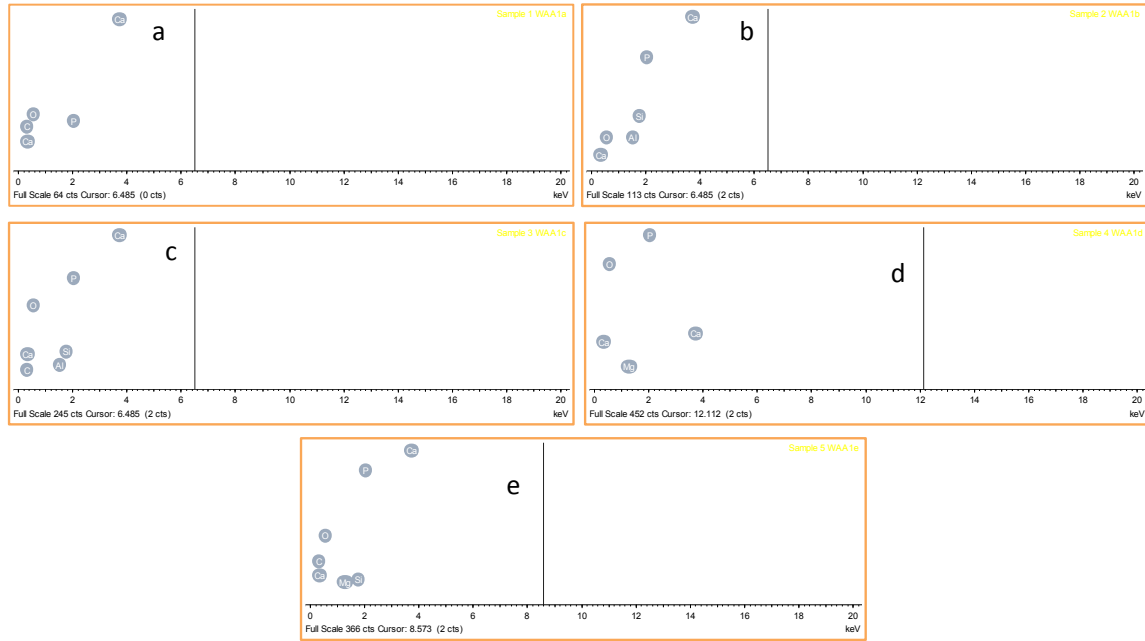


Fig 4. EDS spectra of HAp product obtained under hydrothermal conditions after reaction with 1M (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> solution for (a) 6 hours at 25<sup>0</sup>C, (b) 6 hours at 55<sup>0</sup>C, (c) 1 hour at 85<sup>0</sup>C, (d) 4 hours at 85<sup>0</sup>C, (e) 6 hours at 85<sup>0</sup>C.

Table 2: Elemental composition of the synthesized HAp formed at different reaction time and temperature (Extracted from Figs. 4a-e).

| Elements (%) | 6 hours at 25 <sup>0</sup> C | 6 hours at 55 <sup>0</sup> C | 1 hour at 85 <sup>0</sup> C | 4 hours at 85 <sup>0</sup> C | 6 hours at 85 <sup>0</sup> C |
|--------------|------------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|
| Ca           | 26.5                         | 40.52                        | 32.67                       | 22.82                        | 22.58                        |
| P            | 6.4                          | 15.94                        | 15.45                       | 12.52                        | 13.59                        |
| Si           | -                            | 3.2                          | 1.29                        | -                            | 1.8                          |
| Mg           | -                            | -                            | -                           | 2.71                         | 1.19                         |
| O            | 42.62                        | 33.91                        | 40.66                       | 62.05                        | 45.68                        |
| C            | 24.48                        | -                            | 8.81                        | -                            | 15.16                        |
| Al           | -                            | 2.0                          | 1.12                        | -                            | -                            |

- Not detected

It is important to note that at higher reaction temperature (85<sup>0</sup>C), the Ca/P ratio decreases with increasing reaction times of 1 hour, 4 hours and 6 hours operations as depicted from Fig 4(c-e) with values of 2.11, 1.82 and 1.66, respectively. This

shows that there is a drastic reduction in the Ca/P stoichiometric ratio of the HAp powder crystal. The decrease in the Ca/P ratio could be due to the nature of the crystal formed with increasing reaction time, which apparently favors the HAp

formation. Hence, the Ca/P ratio obtained in Fig. 4e is apparently very close to the stoichiometric ratio of 1.67 required of HAp powder amenable for biomedical applications. Further work is ongoing to assess the powder crystallinity and type at higher reaction times.

### 3.2.3 HAp functional group characterization by FT-IR

The FT-IR analysis was performed using Bulk M530 FT-IR Spectrometer. The FT-IR spectra were recorded and the spectra of the synthesized HAp powder obtained at different reaction temperatures for various reaction times are summarized thus (Fig 5).

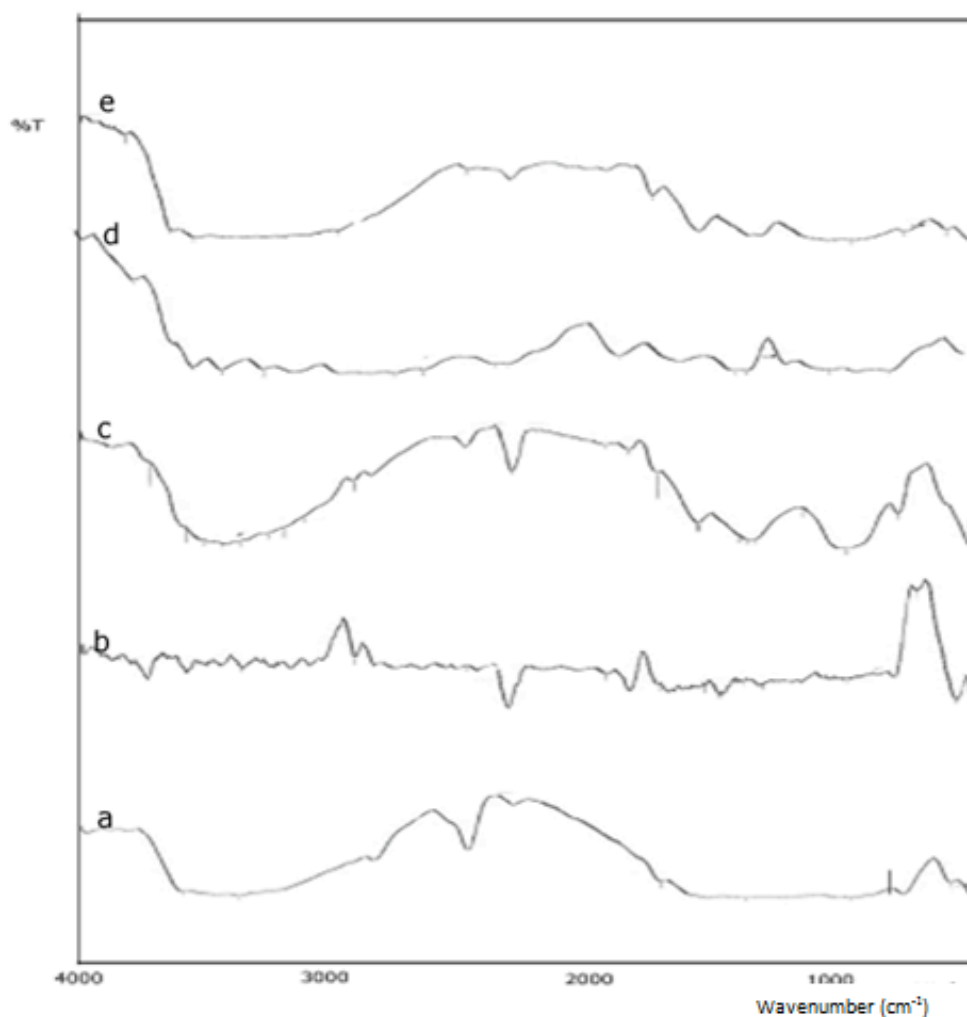


Fig 5: FT-IR spectra of HAp synthesized under hydrothermal conditions after reaction with 1M  $(\text{NH}_4)_2\text{HPO}_4$  for (a) 6 hours at 25<sup>0</sup>C, (b) 6 hours at 55<sup>0</sup>C, (c) 1 hour at 85<sup>0</sup>C, (d) 4 hours at 85<sup>0</sup>C, (e) 6 hours at 85<sup>0</sup>C.

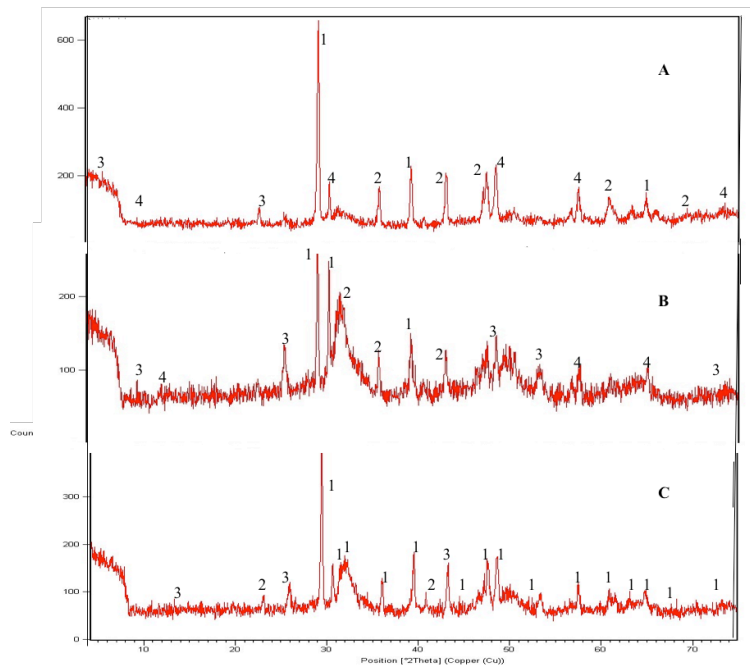
### 3.2.4 XRD Analysis

The XRD analysis was performed on the selected HAp crystals formed at different temperatures and contact times by EMPYREAN X-ray Diffractometer. The XRD spectra of the synthesized HAp powder at 25°C, 55°C and 85°C during 6 hour reactions is summarized in Figs. 18A, B and C, respectively. It is important to note that the HAp crystal formed during reaction at 25°C for 6 hours (A) and 55°C for 6 hours (B) were dominated by some impurities including

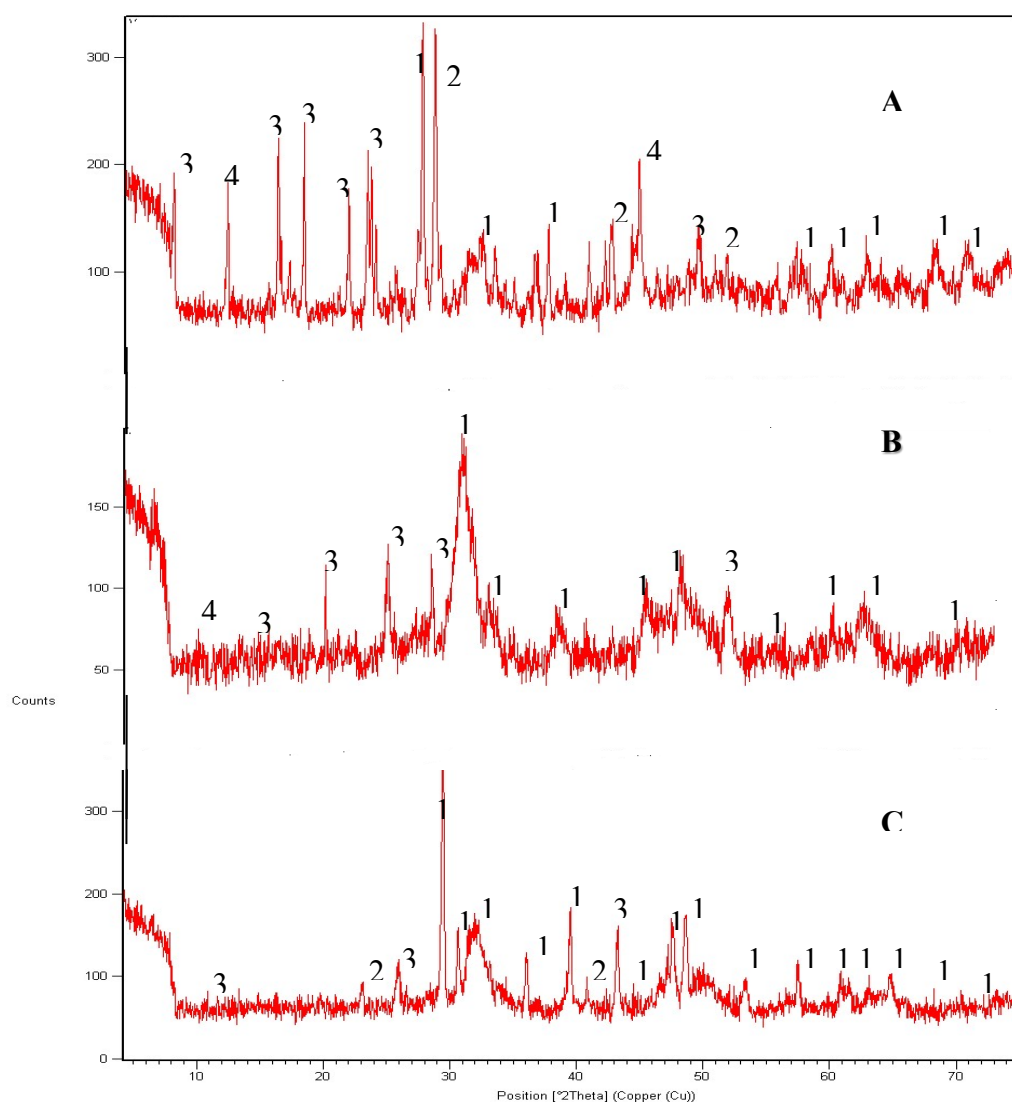
presence of silica was obtained in each case. However, during reaction time of 6 hours at higher reaction temperature (85°C) (C), the impurities were recorded at low to trace levels. Thus, higher reaction temperature and time seems favourable for the formation of HAp powder. Furthermore, the extent of impurities removal from the HAp crystal formed at optimal temperature at different reaction times of 1, 4 and 6 hours is summarized in Fig. 7A, B and C, respectively.

Table 3: FT-IR spectra of the synthesized HAp at various reaction temperature and time (Extracted from Fig. 5).

| Assignments                                   | Observed Vibrational Frequencies (cm <sup>-1</sup> ) |                 |                |                 |                 |
|-----------------------------------------------|------------------------------------------------------|-----------------|----------------|-----------------|-----------------|
|                                               | 6 hours at 25°C                                      | 6 hours at 55°C | 1 hour at 85°C | 4 hours at 85°C | 6 hours at 85°C |
| OH <sup>-</sup> stretch                       | 3603                                                 | 3521            | 3593           | 3539            | 3551            |
| PO <sub>4</sub> <sup>3-</sup> stretch $\nu_1$ | 961                                                  | 962             |                |                 | 962             |
| PO <sub>4</sub> <sup>3-</sup> bend $\nu_3$    | 1073                                                 | 1075            | 1074           | 1071            | 1072            |
| PO <sub>4</sub> <sup>3-</sup> bend $\nu_4$    | 607                                                  |                 |                |                 |                 |
| CO <sub>3</sub> <sup>2-</sup> group           | 1474                                                 | 1470            | 1483           | 1475            | 1454            |
| Structural OH <sup>-</sup>                    |                                                      | 3596            | 3454           | 3430            |                 |



**Fig. 6:** The XRD patterns of synthesized HAp powders at 25°C (A), 55°C (B) and 85°C (C) for 6 hours with important compounds detected. The Joint Committee on Powder Diffraction Standard file numbers (JCPDS) numbers for peaks assignment are put in curl bracket: **1** Hydroxyapatite - HAp {70- 0794}; **2** Ankerite - C<sub>2</sub>CaFe<sub>0.6</sub>Mg<sub>0.4</sub>O<sub>6</sub> {41-0586}, (h k l = 1 0 4); **3** Silica SiO<sub>2</sub>{74-3486},(h k l = 0 1 1) ; **4** Magnesium Calcium carbonate - (Mg<sub>0.03</sub>Ca<sub>0.97</sub>)(CO<sub>3</sub>) {89-1304}.



**Fig. 7:** The XRD pattern of synthesized HAp at optimal temperature and reaction times of 1 hour (A), 4 hour (B) and 6 hours (C) with other compounds detected. The JCPDS File numbers are put in curl brackets: **1** Hydroxyapatite - HAp {70-0794}; **2** Dolomite -  $\text{C}_2\text{CaMgO}_6$  {75-3706} (h k l = 1 0 4); **3** Silica -  $\text{SiO}_2$  {70-2535} (h k l = 0 1 1); **4** Calcium carbonate -  $\text{CaCO}_3$  {01-0837} (h k l = 1 0 4).

### 3.3 CONCLUSIONS

HAp powders have been synthesized successfully from Calcite ore of Nigerian origin using diammonium hydrogen phosphate as the Phosphorus source via hydrothermal method. Detailed characterization aimed at determining the chemical structure of the HAp crystals

formed at different reaction temperature and time of operations were examined by EDS, SEM, FT-IR and XRD techniques. At optimal conditions (1M  $(\text{NH}_4)_2\text{HPO}_4$ , 85°C, 6 hours reaction time), a rectangular, rod-like and clean layered HAp crystal formed was confirmed by the EDS

analysis which yielded a Ca/P ratio of 1.66 close to the stoichiometric ratio of 1.67. It is then assumed that this product could find application in biomedical applications; and testing of this product for possible industrial uses in orthopedics medicine is on in our laboratory.

### Acknowledgement

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