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# OPTIMIZATION OF PREPARATION CONDITION FOR HYDROXYAPATITE FROM CATFISH BONES

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## Abstract

*Production of CFHAP at optimized experimental conditions of temperature and time was carried out using the central composite design (CCD) under response surface methodology (RSM). The production temperature ranged from 300 °C to 1000 °C and time from 1 to 2 h respectively. With this Design Expert Software, a total of thirteen (13) runs were used to design the experimental matrix of which the software specify. The resultant calcined material for each run was weighed to determine the percentage yield while the adsorption capacity was determined by using a standard method, Methylene Blue Number method (MBN). The determined MBN was used to estimate the pore size. Catfish bone calcined at 300 °C for 1 h revealed the highest methylene blue number which is an indication of largest pore size. Characterization of the raw catfish bones and the run with the highest MBN (optimized CFHAP) was done to determine the morphology, functional groups, elemental composition, surface area, pore size and the pore volume using scanning electron spectroscopy (SEM), Fourier Transform Infrared spectroscopy (FTIR), Energy Dispersive Spectroscopy (EDS) and Brunauer-Emmet-Teller (BET) surface area analyzer respectively. From the study, it was conclude that natural hydroxyapatite can be produced at optimized condition from catfish bones at the temperature of 300 $^0$C in 1 h.*

## 1.0 Introduction

Apatite is the name given to a group of crystals of the general chemical formula $M_{10}(XO_4)_6 Y_2$. Where M = $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$, $Na^{+}$, $Pb^{2+}$, $La^{3+}$ and many rare earth elements; $XO_4$ = $PO_4^{3-}$, $VO_4^{3-}$, $SiO_4^{4-}$, $CO_3^{2-}$; Y = $OH^-$, $Cl^-$, $F^-$, $CO^{2-}$ and other various anions. Hydroxyapatite with the chemical formula $Ca_{10}(PO_4)_6 OH_2$ belongs to the apatite family. There are two types of hydroxyapatite, natural and synthetic hydroxyapatite. Natural hydroxyapatites are the

type of apatite prepared from animal bones and also produced from phosphate rich rocks (28). Hydroxyapatite is the main inorganic component of living hard tissue such as bone and teeth [2, 17, 20 & 23]. Various methods have been employed for the preparation of HAP from natural sources such as coral or bone; thermal decomposition, subcritical water process and alkaline hydrolysis [1, 9, 21, 22 &26]. Synthetic apatite is prepared by chemically reacting a hydroxide source (calcium hydroxide) with a phosphate source (phosphoric acid) [24]. Hydroxyapatite is an ideal material for long term containment of contaminant because of its high sorption capacity, low water solubility, high stability under reducing and oxidizing conditions [30]. It can also be considered as a good supporting material owing to its transparency and excellent adsorption properties towards different aqueous and air pollutants [8, 13,14,24]. Researchers have used hydroxyapatite to remove different types of heavy metals plumbs [5,13,16,31]. HAP has had a wide acceptance in medicine and dentistry [2] and due to its chemical structural similarity to bone minerals, hydroxyapatite had been successfully used for bone substituents. Hydroxyapatite is not only bio compatible, non toxic, but also bioactive i.e it has got the ability to form a direct bond with living tissues [1, 9, 19]. Hydroxyapatite can be use in chromatography as a filling material for columns, [8, 18]. Optimization is a procedure for developing the best possible product in its class [3]. Techniques involving choice of process combinations for optimization without due consideration of relevant experimental designs is scientifically unreliable and irreproducible. Mathematical modeling of which response surface methodology (RSM) is one, provides a precise map leading to successful optimization. Central composite design is one of the response surface design widely used in practice in optimization process. This is because of its efficiency with respect to the number of runs required.

## 2.0 Materials and Methods

The catfish bone used in this research work for the production of hydroxyapatite at optimized condition were obtained from Favour farm along ring road, Osogbo, Osun State, Nigeria. The catfish bones was transported to Chemistry Laboratory, LAUTECH Ogbomoso and kept at $4^0$C in the refrigerator prior to the time of use.

### 2.1 Production of HAP from catfish bones at optimized condition

Catfish bones stored in the refrigerator were allowed to thaw. The catfish bones were then washed in flowing stream of water and then soaked in 30% hydrogen peroxide for a day to remove all residual organic matter after which it was rinsed with distilled water, air-dried for 48 h and then crushed into smaller pieces. These crushed catfish bones were then heated in an oven at $105^o$C for 3 h and kept in descicators to prevent adsorption of moisture from the atmospehere [28]. The Central Composite Design (CCD) under Response Surface Methodology (RSM) embedded in the design expert software (6.0.8) was employed to investigate the effect of heat treatment (300 – 1000 $^0$C) and time (1 – 2 h) on the adsorption properties of the adsorbent produced. Catfish bones of 10 g inside crucible was charged into the furnace and heated at the temperature and the time as suggested by the software (CCD under RSM in design expert) for each run. The calcined catfish bones were collected for each run and allowed to cool. The resultant product (calcined catfish bones) is termed hydroxyapatite [3, 6, 28]. The two (2) responses under the investigation were the percentage yield and pore size. The resulting calcined material for each run was weighed to determine the percentage yield and also, the adsorption capacity was estimated by using a standard method, Methylene Blue Number method (MBN). The estimated MBN was used to determine the pore size.

### 2.2 Percentage yield

The cooled calcined materials obtained for each run were weighed to determine the yield of each run at different calcine temperature and time (11). The percentage yield for each material was determined using equation (1)

$$\text{Percentage yield } \% = \left(\frac{W_F}{W_1} X100\right) \quad (1)$$

Where $W_1$ = initial weight before calcination and $W_F$ = final weight after calcinations.

### 2.3 Estimation of HAP pore size

A standard method, Methylene Blue Number was used to estimate the pore size of the catfish bones hydroxyapatite produced for each runs. It was assumed that the largest pore size will adsorb most (11). The methylene blue number is defined as the maximum amount of dye adsorbed. Using this method, 1 g of hydroxyapatite (adsorbent) was mixed with 10 ml of 100mg$L^{-1}$ methylene blue solution for 24 h at room temperature. The concentration of methylene blue left in the solution after adsorption onto HAP was analyzed for each run of experiment by using UV/vis spectrophotometer at 645nm, then the amount of methylene blue adsorbed for each solution was calculated using equation 2.

$$Q(mg/g) = \frac{(C_o - C_e) \times V}{M} \quad (2)$$

where $C_0$ (mg $L^{-1}$) is the initial concentration of methylene blue solution, t = 0 is the starting time. $C_e$(mg$L^{-1}$) is the concentration of the methylene blue solution at equilibrium time.
V(L) is the volume of the solution treated. M(g) is the mass of the adsorbent.

### 2.4 Characterization study of HAP Produced from catfish bones at Optimized Condition

Characterization of the optimized hydroxyapatite produced was done using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Brunauer – Emmett – Teller (BET) and Energy Dispersive X-ray Spectroscopy (EDX).

## 3.0 Results and discussion

### 3.1 Effect of Calcine Temperature and Time on Percentage Yield of CFHAP

The result of the percentage yield (%) obtained after calcination of 10 g of catfish bones for each run at the suggested temperature and time by the software are shown in Table 1. The range of percentage yield of the thirteen runs is between 63.09 and 96.42%.The hydroxyapatite calcined at 115.00 for 1.50 h have the highest % yield (96.42%). The percentage yield obtained for the HAP investigated in this study is in agreement with the result obtained for other living hard tissues like swine bone [7,16, 29]. However, the percentage yield may not directly relate to the efficiency of carbon in removing the targeted adsorbate but may be significant to their commercial utilization [4].

### 3.2 Effect of Calcine Temperature and Time on MBN

The results of the pore size as indicated by the Methylene Blue Number (MBN) of the HAP produced at the varied conditions of temperature and time as given by the software are presented in Table 1. The MBN ranged between 6.4 and 9.7 mg/g for the thirteen runs. The HAP produced at $300^0$C in 1h has the highest pore size with MBN of 9.7 mg/g. Therefore, it is observed that the HAP with the highest MBN adsorbed most. This might be because of its largest pore size. From the result, it could be deduced that the adsorbent produced at $300^0$C in 1 h adsorbed most owing to its largest surface area and largest total pore size. The result is in agreement with the reported observation of Agnieszka *et al.,* 2009 and Shyamsundar, 2009 respectively. Therefore, the HAP produced at $300^0$C in 1 h is the optimized HAP produced.

Table 1: Results of Responses from Experimental Data

| | Temperature ($^{o}$C) | Time (h) | Percentage Yield (%) | MBN mg/g |
|---|---|---|---|---|
| 1 | 650.00 | 1.50 | 74.86 | 6.5 |
| 2 | 1000.00 | 1.50 | 63.09 | 7.4 |
| 3 | 650.00 | 1.50 | 74.86 | 6.4 |
| 4 | 650.00 | 2.21 | 70.71 | 7.9 |
| 5 | 300.00 | 2.00 | 72.26 | 9.3 |
| 6 | 650.00 | 0.79 | 72.69 | 8.0 |
| 7 | 1000.00 | 2.00 | 63.09 | 7.4 |
| 8 | 650.00 | 1.50 | 74.86 | 6.4 |
| 9 | 300.00 | 1.00 | 82.04 | 9.7* |
| 10 | 650.00 | 1.50 | 74.85 | 6.4 |
| 11 | 155.00 | 1.50 | 96.42* | 6.4 |
| 12 | 650 | 1.50 | 74.86 | 6.5 |
| 13 | 1000.00 | 1.00 | 64.10 | 7.6 |

### 3.3 Characterization of HAP Produced at Optimized Condition.

Characterization of HAP produced at optimized conditions was done to investigate the proof of formation of hydroxyapatite.

### 3.4 Scanning Electron Microscope (SEM)

Figures 1 a and b revealed the micrograph and the microstructure of catfish bones before calcination and after calcination at optimized conditions respectively. Figure a revealed an irregularity in powder shapes with little agglomerate and presence of quite smaller pores. This is in agreement with the observation of Barakat *et al*., 2009 and Fernare and Adjiane-Zafour, 2013 respectively. From figure b, examination after calcination confirmed the presence of more wider open pores in the powder with no agglomerate structure. After calcination of the catfish bones, the pores became wider (larger) in which the HAP containment property stem from. The microstructure as revealed by the SEM is in agreement with BET surface area analysis results.

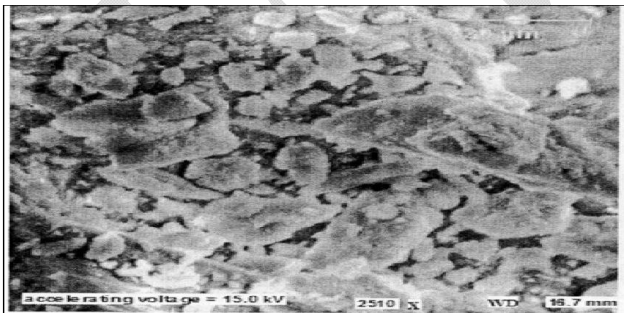



Fig.1a: SEM image of raw catfish bones

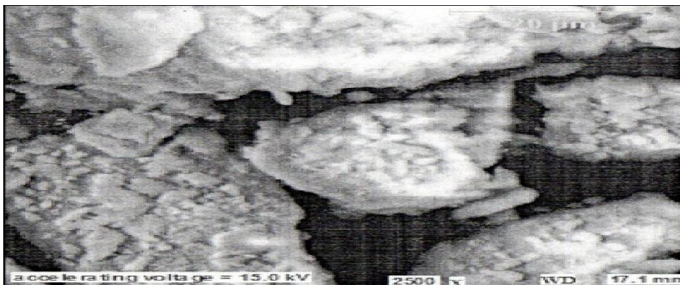



Fig. 1b: SEM image of optimized HAP

### 3.5 Surface area analysis by BET analyzer

The specific surface area, pore volume, pore radius of the raw catfish bones and HAP at optimized condition were determined by BET Analyzer. The results were determined using BET plots as shown in Fig. 2a and 2b, these are presented in Table 2. From the result, calcination has influenced the physical

properties of the catfish bones. The total specific surface areas increased from 190$m^2$/g to 359 $m^2$/g. The total pore volume increased from 119 $cm^3$/g to 146 $cm^3$/g and the pore sizes enlarged from 210 nm to293 nm.

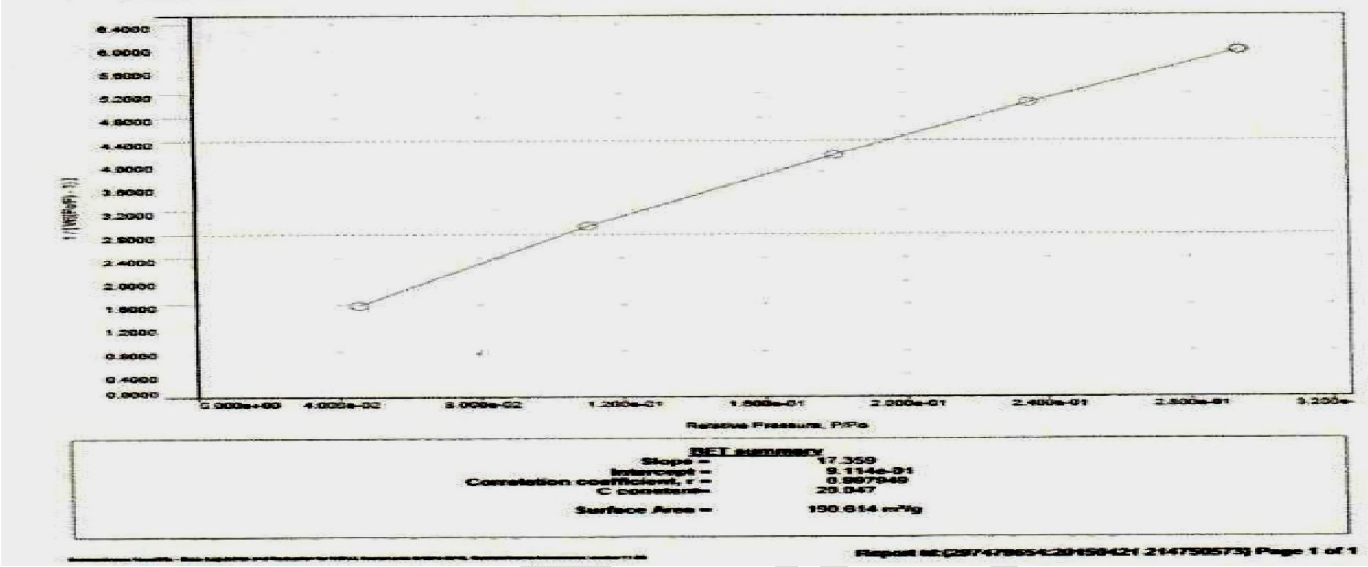
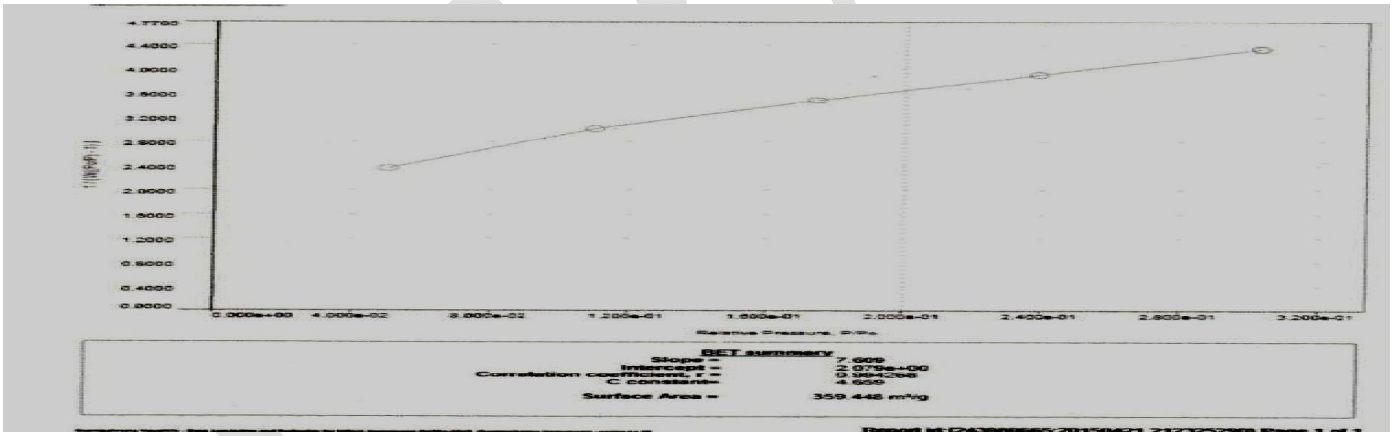



Fig. 2a: BET plot of the surface area of catfish bones before calcination



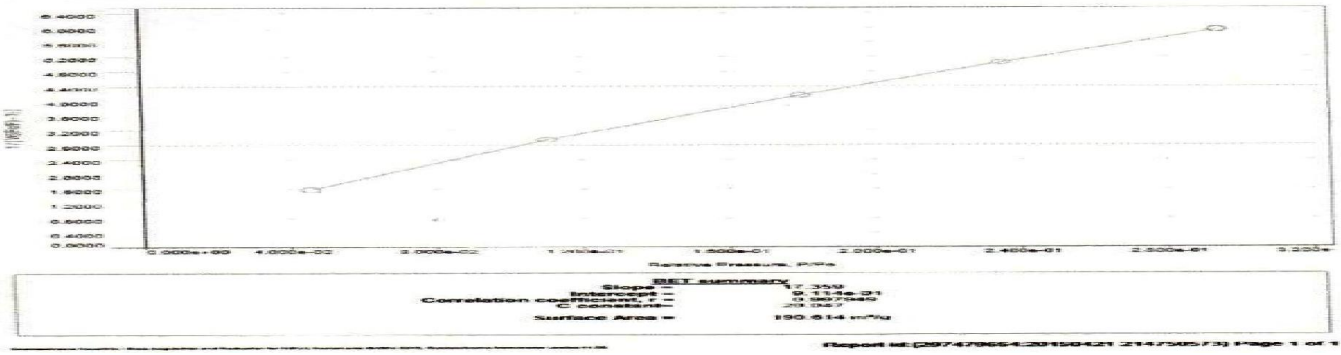



Fig. 2b: BET plot of the surface area of catfish bones before calcnation

Table 2: The BET Result of catfish bones and optimized HAP

| Parameters | Before Calcination | After calcination (HAP) |
|---|---|---|
| Surface area ($m^2/g$) | 190.00 | 359.00 |
| Pore volume ($cm^3/g$) | 119.00 | 146.00 |
| Pore radius (nm) | 210.00 | 293.00 |

It could be deduced from the result that catfish bones calcined at optimized condition (HAP) is of larger surface area, pore volume and pore size when compared to the catfish bones before calcination, the adsorption property stem from this.

### 3.6 FTIR characterization of catfish bones and HAP produced at optimized condition

The main functional groups present in the catfish bones were revealed by spectrum in Fig.3a. The sharp vibration observed at 3431.36$cm^{-1}$ indicate the presence of O-H groups [27]. The 2941.44$cm^{-1}$ bending vibration found depicts the presence of hydrocarbon (C – H) group. The medium and sharp peak of a phosphorus function in the form of hydrogen bonding to phosphorous (phosphine) was absorbed at a wavelength of 2353.16 $cm^{-1}$. The sharp peak obtained at 1649.143 $cm^{-1}$ was attributed to alkene (C=C) and (C=O). The absorption peak at 1438.90 $cm^{-1}$ showed the presence of sulfate (S=O) and the vibration at 1037.70$cm^{-1}$ is due to presence $PO_4^{3-}$ [12]. Present the results of side by side

The FTIR of the catfish bones calcined at optimized condition (HAP) is shown in Fig. 3b. Some bonds are found shifted, including are -OH at 3431.3 $cm^{-}$shifted to 3437.15 $cm^{-1}$. The medium and sharp peak of P–H shifted from 2353.16$cm^{-1}$ to 2358.92 and 1649.14 $cm^{-1}$ (C–H) peak shifted to 1633.71$cm^{-1}$ in the catfish bones after calcinations (HAP). The peak at 2941.44$cm^{-1}$(C-H) group disappeared while other peaks at 775.38 $cm^{-1}$ and 3776.62 $cm^{-1}$ appeared. The stretch at 775.38$cm^{-1}$ attributed to sulfur function esters. The peak found at the wave length 3776.6$cm^{-1}$ is due to the presence of water molecules. The peak at 1041.56 $cm^{-1}$ in the HAP indicate the presence of $PO_4^{3-}$. However, the presence of main functional group of hydroxyapatite -OH and $PO_4^{3-}$ in calcined bones indicated that HAP was produced.

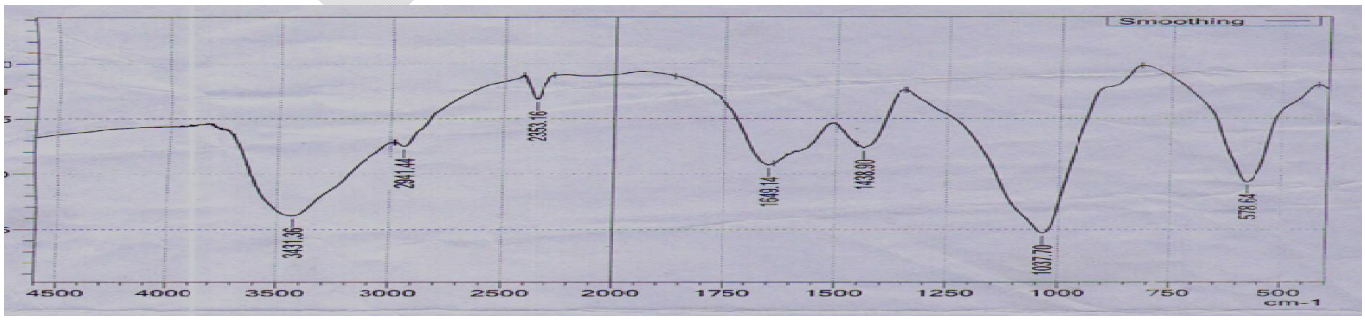


Figure 3a: FTIR of catfish bones spectra before calcined

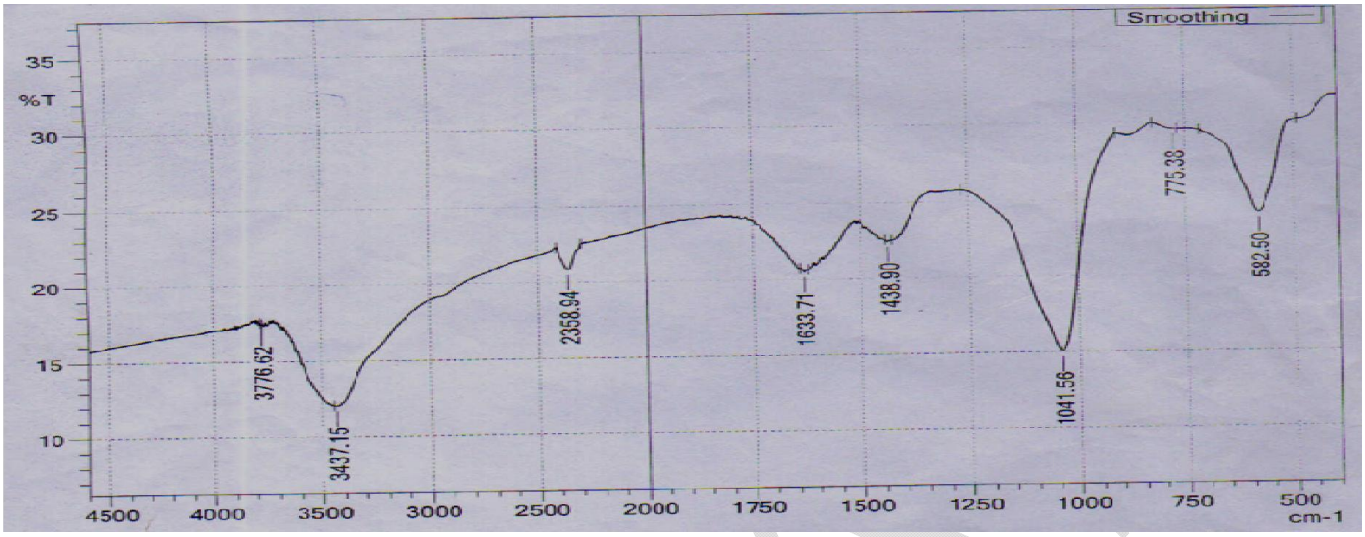



Fig. 3b: FTIR spectra of optimized CFHAP

**3.7 Quantitative analysis of catfish bones and optimized HAP by Energy Dispersion Spectroscopy (EDX)**

Analysis of the catfish bones before calcination and after calcination with respect to EDX of catfish bones before calcination indicated that as zinc, magnesium and barium. The mean element composition as examined by EDX for the catfish bones before calcination and after calcinations recorded calcium to be 38%, only calcium, oxygen and phosphorus are the significant elements present in the sample and after the calcination of the catfish bones (HAP), calcium, oxygen and phosphorus were also revealed as major significant elements present with some minor component such 40.8%; oxygen 16%, 14%; phosporous 42%, 40%; magnesium 2.5%, 2.0% and barium 1.0%, 1.7% respectively. These were shown in Table 3**.**

Table 3: Elemental Composition of catfish bones Before and After Calcination

| Sample | Elemental composition (%) | | | | | |
|---|---|---|---|---|---|---|
| | Ca | O | P | Zn | Mg | Ba |
| Catfish bones before Calcination | 38 | 16 | 42 | 0.0 | 2.5 | 1.0 |
| Catfish bones after Calcination (HAP) | 40.8 | 14 | 40 | 1.2 | 2.0 | 1.7 |

**4.0 Conclusion**

Conclusively, catfish bones were found to be a good raw material for the producing of natural hydroxyapatite and the optimized condition for the production can be attain at the calcine temperature of 300 $^0$C for 1 h.

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