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## Evaluation of Physicochemical Properties of Nano-Sized Hydroxyapatite Particles Synthesized from Cowbones

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### **Abstract:**

Pure and white Hydroxyapatite  $Ca_{10}(PO_4)_6(OH)_2$  powder was synthesized from animal bone (Cowbone) using thermal decomposition method. The physicochemical properties of the synthesized hydroxyapatite were evaluated using X-ray diffraction analysis and Fourier Transform Infrared spectroscopy. The X-ray diffraction results revealed crystalline phase of hydroxyapatite while Scanning Electron Microscope analysis revealed the microscopic structure and nano size of the hydroxyapatite particle. Fourier Transform Infrared spectroscopy was used to identify functional groups in the material and did not indicate any organic components of the bone. Thermo gravimetric analysis confirmed the thermal stability of the hydroxyapatite. The in-vivo cytotoxicity test using Wistar Albino Rats did not indicate any toxic effects of the material at acute level. The research has not only shown economic potentiality of producing nano hydroxyapatite from this waste but offers a means of impacting positively on the environment.

**Keywords:** Thermal, synthesis, Albino Rats, Hydroxyapatite, microscope

### **1. Introduction**

Hydroxyapatite  $Ca_{10}(PO_4)_6(OH)_2$  (HA) is an inorganic component of natural bone and is very biocompatible and therefore used extensively in developing biomaterials (Bahrololoomet *al*, 2009). Synthetic hydroxyapatite is a popular bone replacement material because it has similar crystal structure to native bone apatite and this resemblance is the origin of the excellent biocompatibility that HA exhibits with tissues enabling it to be incorporated into the body in the remodeling of the bone (Cox, 2014). In the last two decades, the use of bio-waste materials to produce HA powders using various synthetic techniques has been widely studied using such materials as eggshells, animal bone and cereals (Teerawat, 2015). The process of the preparation of HA from these materials is an interesting procedure where economic and environmental benefits are obtained through waste recovery, moreover, HA produced from biowastes such as animal bone may be less likely to be rejected by living organs due to physicochemical similarity to human bone (Sobczak *et al*, 2009; Ozawa and Suzuki, 2002). Several investigations have also been carried out to determine how critical properties of HA can be effectively controlled by varying the processing parameters taking into consideration of wide variety of methods for the preparation of Nano particles of hydroxyapatite (Shojal, 2013). The research aimed at synthesizing hydroxyapatite at nano level from this biowaste (cowbone) in order to contribute to the clinical demand of the material because hydroxyapatite in nano size has become a more preferred material as orthopedic and dental implants compared to hydroxyapatite in micro scale.

### **2. Materials and Methods**

#### **2.1. Sample Collection and Preparation**

Cowbone (femur) samples were collected from two different abattoirs, Kawo and Tudun Wada all within Kaduna metropolis, Nigeria and the bone samples collected were labeled K-Bone and KB-Bone respectively. The adhering meats on the Cowbone samples (femur; KB- BONE (403.25 g). K-BONE (451.65 g)) collected were first removed and scrapped mechanically using knife, then boiled separately in a cooker for 1 hr. 30 mins to remove blood, remaining fat and adhering meat tissue (Mucaloet *al*, 2004). Cowbone samples were deproteinized externally by washing with 1M HCl solution and finally followed with 1M NaOH solution to ensure complete removal of any remaining proteins. The samples were then thoroughly washed with distilled water and dried in oven at 160 °C for 6 hrs., after which the bone samples were observed

to have turned black with reduced weights of 288.0 g and 360.65 g for KB-BONE and K-BONE respectively. The dried bone was broken into pieces using hand grater and later ground using pestle and mortar into smaller particle size. The ground bones were later sieved to obtain particle size less than 450  $\mu\text{m}$  (KB-BONE 104.47 g; K-BONE 124.8 g) (Nasser *et al.*, 2009).

### 3. Synthesis of Hydroxyapatite Using Thermal Decomposition Method

#### 3.1. Synthesis of Nano Sized Hydroxyapatite from Cow Bone Samples (Soheila *et al.*, 2017)

The prepared samples of cowbone were used to synthesize nano sized hydroxyapatite using Thermal Decomposition method. Twenty grams (20g) of the black bone powder was measured using analytical balance in four open aluminium crucibles each and then heated in furnace (furnace SXL, model no; 1006) at 900°C for 5 hours and was allowed to cooled inside the furnace overnight (Nasser *et al.*, (2009) and Bahrololomet *et al.*, 2009). The nano-sized hydroxyapatite was obtained after heating the black bone sample at 900°C for five hours as white solid powder. The white solid powder of nano hydroxyapatite was further ground using pestle and mortar into fine white powder

### 4. Characterization of Synthesized Ha

#### 4.1. Physical Characterization

Conductivity was determined using conductivity meter, Jenway, England, with model No. DDS.307; refractive index was measured using Refractometer, Bellingam and Stanley England, with Model No. 909271 while the colour of the synthesized HA was determine using visual comparison

#### 4.2. In-Vivo Cytotoxicity Test

Lorke's method of in-vivo cytotoxicity test was used to investigate the toxicity effects of the hydroxyapatite material using ninety (90) Wistar Albino Rats. In the first phase, different dose concentrations of 10 mg/kg, 100 mg/kg, 1000 mg/kg of the synthesized HA were administered while in the second phase 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the synthesized HA material were also administered. The different concentrations were orally given to the Rats using syringe depending upon their respective weights and were evaluated after 24 hrs. (Lorke, 1983).

#### 4.3. Chemical Characterization

Scanning Electron Microscope, phenomenon prox model, with model no. 4.5.3 was used to study the grain size and surface morphology of HA; Panalytical Empyrean Model X-ray diffractometer was used to determined size of the crystal particles, phase composition and degree of crystallinity; Thermogravimetric analyser, Perkin Elmer TGA, with model No. 400) was used to evaluate the thermal stability and percentage weight loss of HA. Fourier Transform Infrared spectrophotometer, FTIR machine MB with model number 3000 was used to characterize the functional groups in the material and the spectra was acquired over a range of 400-4000  $\text{cm}^{-1}$ . The nano particle size was calculated using Scherrer's formula,

$$D = \frac{0.9\lambda}{b \cos \theta}$$

Where

$\lambda$  = 0.154 nm for Cu

b = FWHM (Full width Half Maximum)

$\theta$  = Diffraction angle

D = particle size in nano meter

### 5. Results and Discussion

#### 5.1. Synthesis and Physical Characterization

The results of synthesis and evaluation of physicochemical properties HA is presented in Table I.A total of eighty-four grams (84 g) of the nano HA particles was synthesized from 160 g of powdered bone sample used representing about 53% yield. The colour of the HA synthesized is white which is in agreement with the report of Jeong (2012) on the colour of pure hydroxyapatite. The conductivity values range between 1.21 -1.05. while refractive index average value ranged between 1.668-1.657 these values observed agreed with those reported by Gittingset *et al.*, (2009). Hydroxyapatite in nano size derived from animal bone is considered economically viable because of availability and uncomplicated procedure. It was also observed from this study that some nano size hydroxyapatite synthesized from Bone-KCM could be classified as mesoporous materials (materials with pore sizes between 2-50nm) and these materials have recently been seen to be very promising in fields of separation science, optics, drug delivery and tissue engineering. In tissue engineering, mesoporous materials have also been shown to be attractive candidates as scaffold materials especially for bone regeneration, because high surface area scaffolds have been shown to enhance cell adhesion and cell proliferation

S/N	Bone Sample	Quantity of hydroxyapatite Synthesized (g)	Colour	Refractive Index	Conductivity (ms/cm)
1	K-BONE	54	White	1.668	1.21
2	KB-BONE	30	White	1.657	1.05

Table 1: Results of Synthesis and Evaluation of Physicochemical Parameters of HA

## 6. Chemical Characterization

### 6.1. SEM Analysis

SEM images of nano hydroxyapatite synthesized from Cowbone (Fig1-2) showing that it contains a range of particles in agglomerates with spherical, hexagonal and irregular shapes and which conforms to the microstructure characteristic of nano particles. Bahrololomet *al.*, (2009) characterized hydroxyapatite from bovine bone ash and reported that its SEM images has spherical, hexagonal and irregular shapes which is consistent with the present study. The nanosize particles have the tendency to agglomerates thereby leaving pores in between them and because of this they are referred to as porous material which is beneficial as they would permit the circulation of body fluid throughout the coating when it is used as biomaterial implant (Huiet *al.*, 2010). The SEM images also did not show any amorphous organic material which indicated that the organic component of the bone was eliminated after heating the bone at 400°C. and a similar observation was reported by Holden *et al.*, (2005).

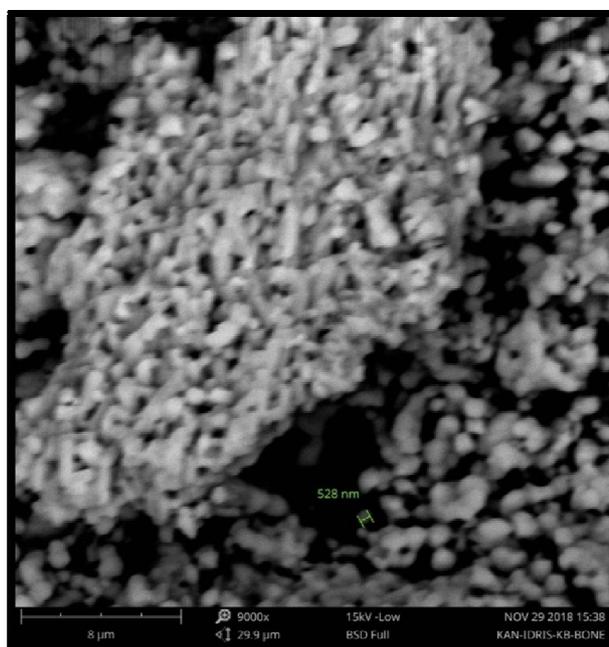


Figure 1: SEM Image for Hydroxyapatite Synthesized from Cowbone (KB-BONE) (9000x)

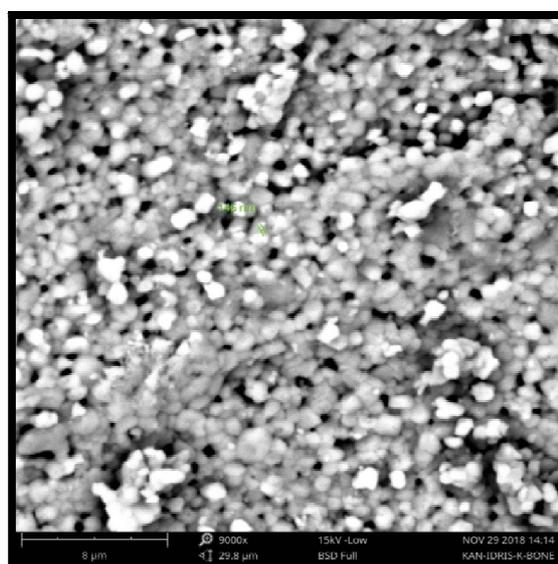


Figure 2: SEM Image for Hydroxyapatite Synthesized from Cowbone (K-BONE) (9000x)

### 6.2. FTIR Analysis

The study FTIR spectra (Fig 3-4) for the synthesized HA from samples of Cowbone revealed vibrations due to OH-groups at  $3857.8\text{ cm}^{-1}$ ,  $3570.5$  and  $3738.5\text{ cm}^{-1}$  and these values are similar to those reported by Huiet *et al.* (2010) who detected OH-groups in FTIR spectra at  $3751.1\text{ cm}^{-1}$  and Nasser *et al.* (2009) detected OH- peak with O-H vibration at  $3569\text{ cm}^{-1}$  while Bahrololomet *et al.*, (2009) detected vibrations due to OH- at  $3571.9\text{ cm}^{-1}$  and  $34536\text{ cm}^{-1}$ .

The vibrations at  $875.9\text{ cm}^{-1}$ ,  $1412.7\text{ cm}^{-1}$  and  $1457.4\text{ cm}^{-1}$  are corresponding to vibration mode due to  $\text{CO}_3^{2-}$  ion and this agrees with Brahimet *et al.*, (2014) who observed  $\text{CO}_3^{2-}$  at  $876\text{ cm}^{-1}$  and  $1456\text{ cm}^{-1}$  while Salma *et al.*, (2010) detected absorption bands of  $1482\text{ cm}^{-1}$ ,  $1424$  and  $875\text{ cm}^{-1}$  ascribed to  $\text{CO}_3^{2-}$  ion. The main characteristic broad bands of  $\text{PO}_4^{2-}$  stretching were observed at  $1021.3\text{ cm}^{-1}$  and  $1088.4\text{ cm}^{-1}$  and the results is in agreement with Tanaka *et al.*, (2003) who detected characteristic peaks due to  $\text{PO}_4^{2-}$  stretching vibrations at  $1051\text{ cm}^{-1}$ . The amide peaks representing the proteinous part of the bone usually at  $1559\text{ cm}^{-1}$  and  $1636\text{ cm}^{-1}$  were not seen in the FTIR spectra. This confirmed the elimination of collagen after heating the bone samples above  $400^\circ\text{C}$ . Figueiredo *et al.* (2017) in the study of microstructure of hydroxyapatite derived from human and animal bone observed that all absorptions bands originated by collagen, precisely those at  $1548$  and  $1634\text{ cm}^{-1}$  disappeared after calcination at  $600^\circ\text{C}$ , which suggests that the organic component of the bone has been removed as observed in the study.

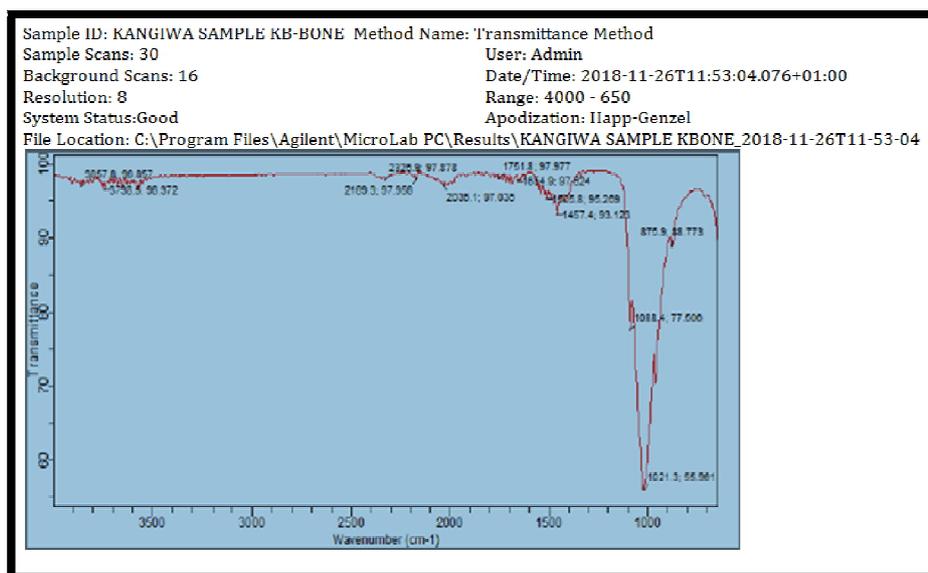


Figure 3: FTIR Result for Hydroxyapatite Synthesized from Cowbone (KB-BONE)

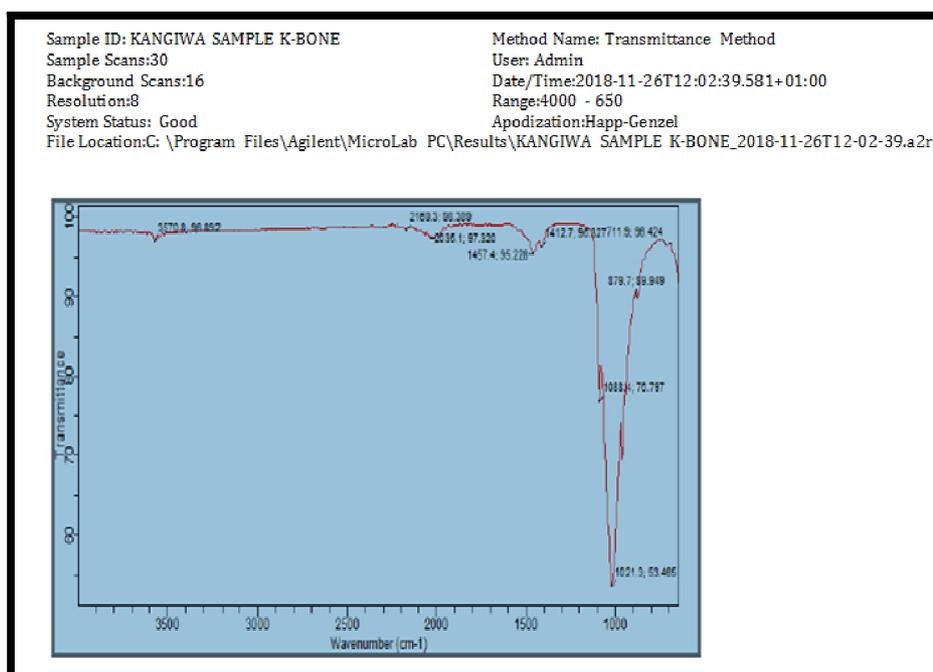


Figure 4: FTIR Result for Hydroxyapatite Synthesized from Cowbone (K-BONE)

### 6.3. TGA Analysis

The TGA analysis (Fig 7 and 8) shows that there was minor weight loss below  $280^\circ\text{C}$  and major weight loss between  $230 - 500^\circ\text{C}$  for the samples analyzed representing 6 % minor weight loss and 74 % major weight loss for HA



SAMPLE	DIFFRACTION IN DEGREE (2θ)	B (FWHM) IN DEGREE	B (FWHM) in Radian (10 <sup>-3</sup> )	PARTICLE SIZE IN nm
KB-BONE	21.9624	0.1023	1.78	79 nm
	23.0620	0.3070	5.36	54 nm
	61.8119	0.3070	5.36	50 nm
	69.8954	0.3070	5.36	27 nm
	72.3802	0.1023	1.78	79 nm
K-BONE	21.9612	0.2047	3.57	39nm
	26.0593	0.1279	2.23	103nm
	31.9387	0.1791	3.12	52 nm
	34.2320	0.1791	3.12	47nm
	40.6401	0.1791	3.12	65 nm

Table 2: Particle Size for Nano-Sized Hydroxyapatite Obtained from Cowbone

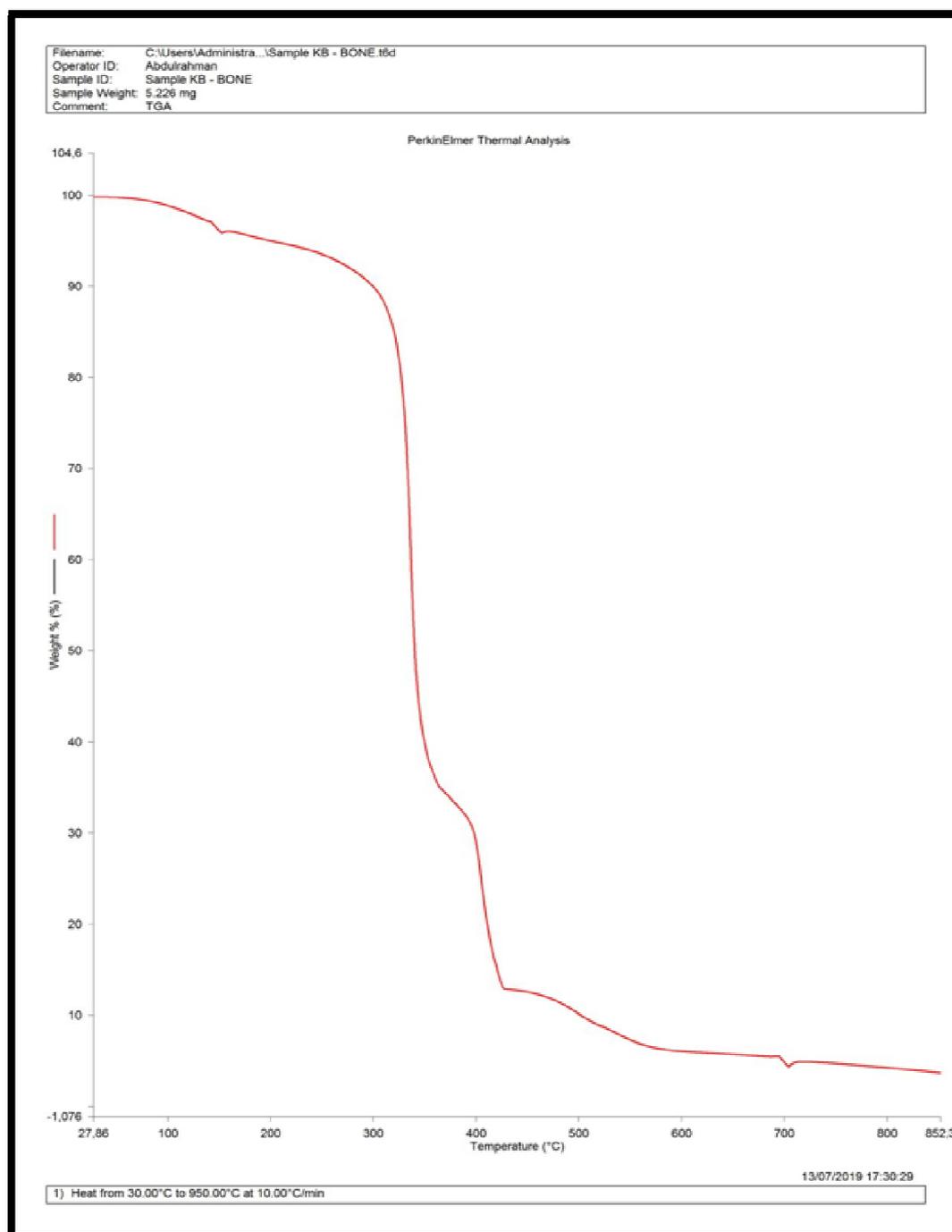


Figure 7: TGA Results for Hydroxyapatite Synthesized from Cowbone (KB-BONE)

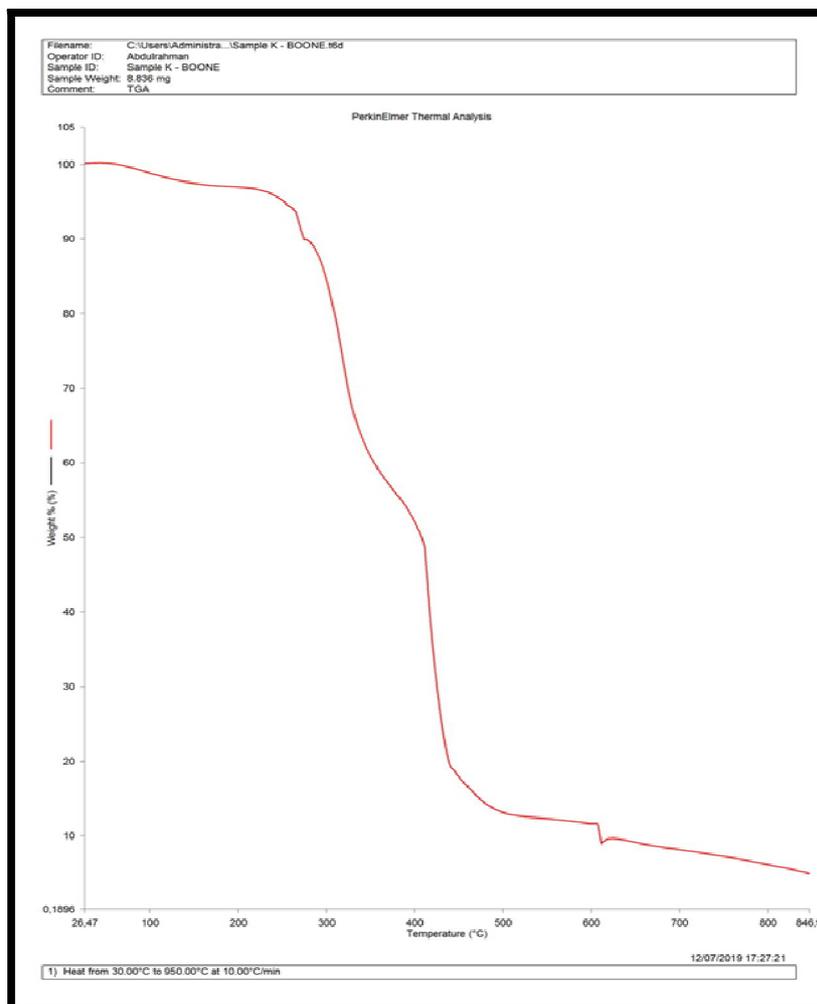


Figure 8: TGA Results for Hydroxyapatite Synthesized from Cowbone(K-BONE)

6.5. In-vivo Cytotoxicity Test

The results from in-vivo cytotoxicity test in both Phase 1 and phase 11 presented in Table 3 did not indicate any toxicity effects arising from different dose concentrations of the synthesized material administered orally to the Albino Rats after 24 hrs. evaluation. The results of the acute toxicity test in this study are similar to the results of chronic toxicity investigation of nano-HA using Wistar rats reported by Remiya and Mohanan (2017).

Sample	Different Dose Concentrations Administered					
	Phase I (mg/kg)			Phase II (mg/kg)		
	10	100	1000	1600	2900	5000
K-BONE	0/3	0/3	0/3	0/1	0/1	0/1
KB-BONE	0/3	0/3	0/3	0/1	0/1	0/1

Table 3: Results of In-Vivo Cytotoxicity Test of Synthesized Nano-HA Using Wistar Albino Rats

KEY: Numerator Is the Number of Deaths of Animal Recorded and Denominator Is the Number of Animals Used

7. Conclusion

Nano sized Hydroxyapatite (HA) powder was synthesized from cowbone samples using thermal decomposition method. The physical characterization indicates pure and white product in powdered form while in chemical characterization, FTIR and XRD indicated the product as having major phase as hydroxyapatite, crystalline and in nano size. SEM analysis revealed the nano grain size of the material in nano metric region while TGA indicated thermal stability of the material between 700 °C and 900 °C. In-vivo cytotoxicity test using Wistar Albino Rats revealed no toxic effects of the material. The study showed the economic viability of using this natural source in producing HA material which will contribute in meeting the clinical demand of the HA for orthopedic applications in an environmentally friendly process.

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## 9. References

- i. Bahrololoom, M.E., Javidi, M. Javapour, S. and Ma J. (2009): Characterization of natural hydroxyapatite extracted from bovine cortical bone ash. *Journal of Ceramic Processing Research*. 10(2): 129-138.
- ii. Brahim, C.I., Khalid, Y., Ahmed, Y. and Asmae, M. (2014): A Novel Method to synthesize nano crystalline hydroxyapatite: characterization with X-ray diffraction and infrared spectroscopy. *Journal of Applied Chemistry*. 7(3): 407-112.
- iii. COX S.C. (2014); Synthesis Methods of hydroxyapatite. *Researchgate*. 1: 1-9.
- iv. Dan Nicole, U., Nicole, A., Rodica, M.F., Elena, U.S. and Cristiana, Z.R. (2016): Synthesis Method for Obtaining Hydroxyapatite Nano Powders by Chemical Precipitation. *Journal of Recent Researches in Communication, Automation, Signal Processing, Nanotechnology, Astronomy and Nuclear Physics*. 2:346-353.
- v. Enamul Hogue M., Nurulk, S., Leng-Chuam, Y. and Ansari, M.N.M (2014): Synthesis and Characterization of Hydroxyapatite bioceramic. *International Journal of Scientific Engineering and Technology*. 3(5): 458-462.binson
- vi. Figueiredo M., Fernando A., Martins G. Freitas J, Judas F and Figueiredo M (2010): Effect of the calcinations temperature on the composition and microstructure of hydroxyapatite from human and animal bone, *Journal of Ceramic International* 36: 2383-2393
- vii. Gittings, J.P, Bowen, C.R., Dent, A.C.E., Turner I.G., Baster F.R. and Chaudhuri, J.B. (2009).Electrical Characterisation of Hydroxyapatite-based bio-ceramics *Acta Biomaterialia* 5: 7843-754.
- viii. Holden, J.L., Phahey, P.P. and Clement, J.G. (2005): An X-ray Study of the Paracrystalline nature of bone apatite. *Journal of Forensic Science International*.74:29-45.
- ix. Jeong, K.I. (2012): Experimental study of osteo-integration and stability of intentionally exposed hydroxyapatite coating implants'. *Journal of the Korean Maxillofacial Reconstructive Surgery* 34(1) 12-16.
- x. Loo, S.C.J., Moore, T., Bank, B. and Alexis, F. (2010): Biomedical Application of hydroxyapatite Nano particles. *Journal of Current Pharmaceutical Biotechnology* (11) : 333-342
- xi. Lorke D (1983): A new Approach to Practical Acute toxicity testing, *Journal of Toxicology* 54:275-287
- xii. Masud R, Naznin A, Shaifur R, Hossen M.J. and Sikder MA (2017): Extraction of hydroxyapatite from bovine and human corticole bone by thermal decomposition and effect of Gamma Radiation: A comparative study, *International Journal of Complementary & alternative medicine* 8(3): 263-272
- xiii. Mucola, M.R., Foster, D.L., Wieggo, S. and Kirbu, J. (2004): The novel use of waster animal bone from New Zealand agricultural sources as a feed back for framing plasma sprayed hydroxyapatite coatings on biomedical implants materials. *JABFM* 2: 65-119
- xiv. Nasser, A.M.B., Myung, S.K., Omran, A.M. Fahemem, A. S. and Hak, Y.K. (2009): Extraction of Pure Natural Hydroxyapatite from the bovine bones bio-wastes by three different methods. *Journal of Materials Processing Technology*.209: 3408-3415.
- xv. Ozawa, M. and Suzuki, S. (2002): Micro structural development of natural hydroxyapatite originated from fish bone waste through heat treatment. *Journal.American.Ceramics. Society*.85(3), 1315-1317.
- xvi. Remiya N.S and Mohanan R.V (2017); Investigation of chronic toxicity of hydroxyapatite nano particles administered orally for one year in Wistar rats, *Journal of Materials Science and Engineering*, Vol 76(1):518-527
- xvii. Salma K., Liga, B.C. and Nataliya, B. (2010): Calcium phosphate bioceramics prepared from wet chemically precipitated powders, *Journal of Processing and Applications of Ceramics*. 4(1) 45-51,
- xviii. Sandeep, S., Deepak, Mehta and Agarwal, R.D. (2012): Characterization and thermal analysis of hydroxyapatite bioceramic powder synthesized by sol-gel technique: *International Journal of Advanced Science Research and Technology* 2(3):281-282.
- xix. Shoja, M.S. (2013); Synthesis Methods for Nano-sizedhydroxylapatite with diverse structures, *ACTA Biomaterialia* Vol. 9(8) pp. 7591-7621.
- xx. Sobczak, A., Kida, A. Kawaski, Z. and Wzoreck, Z. (2009). Evaluation of the biomedical properties of hydroxyapatite obtained from bone waste. *Polis Journal of Chemical Technology* 11(1): 37-43.
- xxi. Soheila, S.R., Omid, G., Ahmad, M and Mohammed, H.F. (2017): A comparative study of physicochemical properties of hydroxyapatite powders derived from natural and synthetic sources. *Russian Journal of Non-ferrous Metals*.58 (3): 276-286.
- xxii. Teerawat, L. (2015): Synthesis of hydroxyapatite from biogenic wastes, *Engineering Journal* 42(3): 269-275.