

# Studies of Cadmium on Hydroxyapatite Nanocrystals

\*<sup>1</sup>V. Ochigbo, <sup>1</sup>M.O. Ochigbo-Ejembi, <sup>2</sup>V.O. Ajibola, <sup>2</sup>E.B. Agbaji, <sup>3</sup>A. Giwa,
<sup>4</sup>P. Manivasakan, <sup>4</sup>V. Rajendran

 <sup>1</sup>National Research Institute for Chemical Technology (NARICT), Zaria-Nigeria
 <sup>2</sup>Department of Chemistry, Ahmadu Bello University, Zaria-Nigeria
 <sup>3</sup>Department of Textile Science and Technology, Ahmadu Bello University, Zaria-Nigeria
 <sup>4</sup>Centre for Nano Science and Technology, K.S.Rangasamy College of Technology, Tiruchengode-637 215,Tamil Nadu India.
 \*Corresponding author: ochigbovic@gmail.com

# ABSTRACT

Nanocrystalline bioactive hydroxyapatite (HA) ceramic powder was synthesized at physiological conditions of temperature and pH using the wet precipitation (WT) and hydrothermal (HT) techniques. Toxicity of Cadmium ion (Cd<sup>2+</sup>) on the hydroxyapatite was studied. Calculated amounts of Cadmium nitrate and chloride solutions were used as the dopants on the hydroxyapatite powder. The resultantpowders obtained were analysed using Particle size Analyser (PSA), energy dispersive X-ray fluorescence (XRF/EDX), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM). The results indicated that irrespective of the techniques employed in the synthesis of the hydroxyapatite and the source of Cd<sup>2+</sup> dopants, HA obtained from both techniques gave crystalline powders and  $Cd^{2+}$  exchanged well with the Calcium ion ( $Ca^{2+}$ ) of the hydroxyapatite via adsorption and dissolution ion exchange mechanism depicting its toxic potentials. The crystallinity decreases in the order: hydrothermal > wet precipitation techniques and Cadmium nitrate (CdN)> Cadmium chloride (CdC) sources. Hydroxyapatite (HA) obtained by wet precipitation doped with cadmium from nitrate salt is more toxic than those of hydrothermal and chloride salts. The quest to know the effect of cadmium on the structural properties of hydroxyapatite when used as an artificial bone or as bone filler/implants necessitated this research.

Keywords: Cadmium, dopants, hydrothermal, hydroxyapatite, nanocrystals, wet precipitation.

# INTRODUCTION

Hydroxyapatite, with the chemical formula  $Ca_{10}(PO_4)_6(OH)_2$  is a naturally occurring mineral in the inorganic component of human bone and tooth enamel in a nano range. The constituent elements of HA are primarily calcium and phosphorus, with hydroxide ions that are eliminated at elevated temperatures and a stoichiometric Ca/P ratio of 1.667. Hydroxyapatite is the most researched calcium phosphate biomaterial. This biomaterial is widely used to repair, fill, extend and reconstruct damaged bone tissue [1, 2]. The apatite (HA) structure is so tolerant to ionic substitutions that Ca<sup>2+</sup> in the crystals can be replaced by various divalent cations including Mg<sup>2+</sup>,  $Cd^{2+}$ ,  $Sr^{2+}$ ,  $Pb^{2+}$  and  $Ba^{2+}$  [3-6]. Cadmium ion( $Cd^{2+}$ ) shows a negative effect on bone tissues. However, its mechanism is not fully understood. One of the possible reactions involves interaction between cadmium and calcium, which results in calcuria and leads to a reduction in calcium absorption from intestines. Nevertheless, it has been reported that a relationship exists between the increased content of cadmium in the body and a decreased level of mineral density of the bones, increased bone resorption and decreased levels of the parathyroid hormone. This metal influences trace elements that affect bone tissue, mainly due to the interaction among zinc, iron, and copper [7]. Cadmium accumulated in bones can affect the activity of bone cells which inturn directly increase the loss of mineral elements in the bones, as it is known to activate osteoclasts and inhibits osteoblasts [8]. The toxic activity of cadmium is not only limited to bone tissue quantity reduction but also to its quality deterioration. Cadmium exposure also results in a decreased level of types I and V collagen in the bones and leads to their increased solubility by damaging the intermolecular cross-links [9]. A change in the collagen structure decreases its endurance, thus increasing susceptibility to deformation and fractures.

This study is aimed at investigating the toxicity of Cadmium ion  $(Cd^{2+})$  from two sources of cadmium salts at series of concentrations on the structural properties of hydroxyapatite synthesized by wet precipitation and hydrothermal techniques at physiological conditions.

#### MATERIALS AND METHODS

Analar grade calcium nitrate tetra hydrate ( $Ca(NO_3)_2.4H_2O$ ), Diammonium hydrogen phosphate (( $NH_4$ )<sub>2</sub>HPO<sub>4</sub>) and liquid ammonia ( $NH_3$ ) and nitric acid ( $HNO_3$ ) from Merck chemical were used.

Pure and doped nanocrystalline HA powders were synthesized through a wet precipitation and hydrothermal processing methods. The preparation conducted was according to the method reported [10] but with some modifications. In wet precipitation synthesis, the aging was done at room temperature for 24 h. The white precipitate obtained was centrifuged, filtered under mild suction, washed repeatedly with distilled deionised water and then dried in an oven at 80 °C for 24 h. While in the hydrothermal, aging was done in an autoclave for 24 h at 150 °C. Then, suspension was allowed to cool naturally followed by centrifugation, filtering under mild suction and washing with distilled deionised water and dried in an oven at 80 °C for 24 h. The HA crystals obtained with these methods were ground with a mortar and pestle and calcined at 300 °C in a muffle furnace under static atmosphere for 3 h. In order to study the effect cadmium on the HA, the synthesized nanocrystalline HA powder doped with cadmium, 0.5 g of HA was added to an 25 ml aliquot of 0.2, 0.4 and 0.6 M cadmium nitrate and chloride solution respectively followed by overnight continuous shaking of suspension at 37 °C in an electrical incubator shaker. After achieving saturation ion exchange equilibrium, the suspension was centrifuged, filtered under mild suction with little washing and then dried using air oven at 80 °C overnight. The pure and doped HA were then characterized.

#### **Characterization**

The particle size distribution was determined with a sub micrometer particle size analyzer (Nanophox, Sympatec, Clausthal-Zellerfeld, Germany) according to the dynamic light scattering technique. Elemental compositions of the prepared HA and doped HA samples were ascertained using X-ray fluorescence (XRF) spectrometer (EDX-720; Shimadzu, Kyoto, Japan). The presence of functional groups was confirmed through Fourier transform infrared spectrometer in the range of 4000–400 cm<sup>-1</sup> using KBr as a reference. The crystalline nature of HA and doped HA powders were characterized through X-ray diffraction analysis using an X-ray diffractometer (X' PertPro, PANalytical, Almelo, The Netherlands) with CuK $\alpha$  as the line source ( $\lambda$ =1.5418 Å) operated at 40 kV with 2 $\Theta$  value varying from 10° to 80° and the average crystallite size was estimated from the Debye- Scherrer approximation and the powder surface morphology was done using the scanning electron microscope (SEM) (JEOL JSM-6390LV, Japan) at 20 kV with a magnification of 10,000x at 1nm scale.

# **RESULTS AND DISCUSSION**

Fig.1 shows the particle size distribution of hydroxyapatite prepared by hydrothermal and wet precipitation techniques. The average particle size distribution of the hydroxyapatite nanoparticles is 26 nm and 29 nm (Fig.1), for the wet precipitation and hydrothermal methods respectively. Expectedly, the hydroxyapatite obtained by wet precipitation has smaller particle size considering the particle size distribution than the hydrothermal method which by virtue of its higher temperature of aging should produce larger crystals since a hydrothermal treatment temperature can assist apatite crystal growth as high temperature can provide a highly active surface for the small apatite precipitate particles to bind to each other and grow into larger crystals than the wet precipitation where aging was done at ambient temperature [11,12]. However, considering the almost similar results obtained for the two methods, it indicates that the aging time was the same. Aging time of the initial hydroxyapatite precipitate significantly affects the growth of the Nano apatite crystals [13].



Fig.1. Particle size distribution of HA synthesized via Hydrothermal (HT) and Wet precipitation (WT)

XRF/EDX results for the synthesized hydroxyapatite before and after doping with cadmium from chloride and nitrate salts are tabulated in Table 1. The presence of calcium, phosphorus and the metal ion dopants ( $Cd^{2+}$ ) were all confirmed. The Ca/P and (Ca+Cd)/P ratios were estimated for wet precipitation and hydrothermal techniques indicated that, irrespective of the method of synthesis used in the preparation of HA, the amount of Ca<sup>2+</sup> and M-dopant exchangeability increases as the concentration of the metal dopant increases.

hudrowyopatita and admium donad IIA										
Mathada	ante and	Caulmul	n dopec				W	itatian T	1 :	
Methods		Hydrotne	rmai Tec	nniques (	HI)		wet precip	ntation 1	ecnniques	(w1)
Weight% / Parameters	Ca	Р	Ca/P	Cd	(Ca+Cd)/P	Ca	Р	Ca/P	Cd	(Ca+Cd)/P
НА	57.305	34.521	1.66	-	-	57.344	34.754	1.65	-	-
0.2% CdC	59.424	35.583	1.67	1.637	1.716	58.244	34.463	1.69	2.828	1.772
HA 0.4% CdC HA	58.642	33.702	1.74	4.242	1.866	57.462	32.649	1.76	6.641	1.963
0.6% CdC HA	57.728	32.800	1.76	4.833	1.907	56.278	31.617	1.78	7.565	2.019
0.2% CdN HA	58.562	34.652	1.69	2.011	1.748	57.652	33.519	1.72	4.675	1.859
0.4% CdN	57.641	33.318	1.73	4.290	1.859	56.461	32.263	1.75	7.524	1.983
0.6% CdN HA	56.994	32.568	1.75	6.626	1.953	55.944	31.607	1.77	8.306	2.033

Table 1. Table showing the XRE/EDX results of hydrothermally and wet precipitated

CdC – Cadmium Chloride, CdN – Cadmium Nitrate, HA – Hydroxyapatite

The Ca<sup>2+</sup>/M-dopant exchangeability revealed that the chloride component of the metal (Cd) exchanges with Ca<sup>2+</sup> more than their nitrate components. This may be attributed to the higher reactivity of chloride than nitrates. This resultcompares well with those reported byGomez-Moraleset al[14]. These suggests that the reaction of CaHA with Cd<sup>2+</sup> takes place through two steps resembling the reaction of MgCaHA with Ca<sup>2+</sup> previously reported by Bigiet al[15]. This discrepancy observed in the ion exchangeability between  $Cd^{2+}$ -  $Ca^{2+}$  with respect to the salts of the metal ion may be attributed to ionic radius, electro negativity and hydration energy. The ionic radius of  $Cd^{2+}(0.97A^{\circ})$  is almost the same as that of  $Ca^{2+}(0.99A^{\circ})$ . However, because of chloride ion being more electronegative than the nitrate ion,  $Cd^{2+}$  ion from nitrate salt seems difficult to allow for exchange with  $Ca^{2+}$  ion when compared to  $Cd^{2+}$  from chloride ion [16,17].

The FT-IR characterization was carried out on the samplesto study the spectra characteristics ofpure and doped hydroxyapatites (Figs.2 and 3) which indicate the chemical bonding in the pure and doped HA powders. The presence of  $(PO_4)^{3-}$  is attested to by the P-O stretching mode appearing between 1000-1500cm<sup>-1</sup> (V<sub>3</sub>) and 962cm<sup>-1</sup>(V<sub>1</sub>); the O-P-O bending modes observed at 630, 567cm<sup>-1</sup> (V<sub>4</sub>) and 468cm<sup>-1</sup> (V<sub>2</sub>) [18].



Fig.2: FT-IR spectra of hydrothermally synthesised pure and doped hydroxyapatite (a) Cd doped HA with cadmium nitrate as a doping source (b) Cd doped HA with cadmium chlorideas a doping source and (c) pure-HA.

Besides, those typical bands of HA, two bands at 1640 cm<sup>-1</sup> and around 3440 cm<sup>-1</sup> are due to adsorbed molecular water. The two bands at 3568 and 633cm<sup>-1</sup> are assigned to the stretching and bending mode of OH groups respectively. In addition, the shoulder at 870cm<sup>-1</sup> is assigned to (HPO<sub>4</sub>)<sup>2</sup> groups formed upon reaction of some surface (PO<sub>4</sub>)<sup>3-</sup> with water. Whereas, two peaks around 1423 and 1455 cm<sup>-1</sup> indicate the presence of either carbonate or ammonium groups which resulted due to interaction with atmospheric air in case of carbonated or a residue of ammonia solution that was not completely removed from the suspension during washing with distilled water [19, 20] which might have entered the medium during synthesis of HA, as the pH was maintained using ammonium solution.Bands around 1630 and 3425 cm<sup>-1</sup>corresponding to the bending vibration of the H-O-H adsorbed in the HA crystals decreases while other characteristic peaks of HA were unchanged irrespective of the dopants.



Fig.3: FT-IR spectra of wet precipitated pure and doped hydroxyapatite: (d) Cd doped HA with cadmium nitrate as a doping source (e) Cd doped HA with cadmium chloride as a doping source and (f) pure-HA.

This observation is more pronounced in the wet precipitated than hydrothermally prepared HA which has sharper peaks. OH<sup>-</sup> peaks are broader in the wet precipitated than hydrothermally prepared HA; this also applies to the Chloride (Cl<sup>-</sup>) and the nitrate (NO<sub>3</sub><sup>-</sup>) salts. The decrease of the peak intensities upon introduction of the metal ion (Cd<sup>2+</sup>) dopants may be ascribed to the dehydroxylation which might accompany the formation procedure at different metal dopants [21,22]. The intensities of the P-OH band are changed by the reaction with cadmium (Cd<sup>2+</sup>), the band at 3568cm<sup>-1</sup>grows, the band at 633cm<sup>-1</sup> reduces after the doping. Similar change of the surface P-OH bands was also observed in CdCaHAsolid solutions [14]. This verifies that the surface phase formed on the particles by the reaction with Cd<sup>2+</sup> is CdCaHA.



Fig.4: XRD pattern of pure and doped hydroxyapatite, (a) HA obtained by HT with cadmium doping using cadmium nitrate source, (b) HA obtained by HT with cadmium doping using cadmium chloride source, (c)pure-HA obtained by HT, (d) HA obtained by WT with cadmium doping using cadmium nitrate source, (e) HA obtained WT with cadmium doping using cadmium chloride source and (f) pure-HA obtained by WT.

Fig.4 shows the XRD pattern of hydroxyapatite obtained by wet precipitation and hydrothermal methods, doped with Cadmium (Cd<sup>2+</sup>) from their chloride and nitrate salts respectively. It was observed that the XRD pattern of the doped hydroxyapatite are similar to that of the pure hydroxyapatite. The diffraction peaks of the pure HA obtained by wet precipitation and hydrothermal found to be consistent with the international Centre for diffraction [23] files for Calcium phosphate. The predominant HA phase were confirmed with JCPDS file number 00-009-0432. These data confirm the major phase as hydroxyapatite particles. The diffraction peaks for the doped HA were similar on introduction of the metal dopants. The intensities of the XRD pattern shows that all Cd-HA peaks corresponds to the peaks of pure HA irrespective of the metal salts (Cl<sup>-</sup> or NO<sub>3</sub><sup>-</sup>); The diffraction pattern of (Cd<sup>2+</sup>) ions from nitrate (NO<sub>3</sub><sup>-</sup>) salt displays well defined and sharper peaks in agreement with high degree of crystallinity than the (Cd<sup>2+</sup>) ions from chloride (Cl<sup>-</sup>) salts, which exhibited broader diffraction peaks in agreement with reduced crystallinity. Peak sharpening and intensities enhancements suggest that there is an increase in crystallite size and a concomitant increase in crystallinity. Also, the peak broadening

is more evident for  $CdCl_2$  than  $Cd(NO_3)_2$ , suggesting a greater difficulty for CaHA to host  $Cl^-$  ion than  $NO_3^-$  ion. These results support the ion exchange mechanism [24].

	<b>5</b> I I	2	1			
S.No.	Anotita	Wet precipitation	n Technique (WT)	Hydrothermal Technique (HT)		
	Apatte	Crystallite size D (nm)	Degree of crystallinity (Xc)	Crystallite size D (nm)	Degree of crystallinity (Xc)	
1	HA	5.0316	0.5374	5.0812	1.8136	
2	CdC-HA	2.1877	0.0423	3.8236	0.2268	
3	CdN-HA	5.1044	0.5374	7.6471	1.8136	

Table 2: The crystallite size (D) and degree of crystallinity (Xc) of doped hydroxyapatite (HA) obtained by wet precipitation and hydrothermal techniques

**HA** =Hydroxyapatite, **CdC**– **HA**= HA doped with cadmium chloride, **CdN**–**HA** = HA doped with cadmium nitrate

The degree of crystallinity and crystallite size of the powders were evaluated from the XRD pattern using the using the Derby Scherer equation presented in Table 2 indicated that the degree of crystallinityand crystallite sizeboth depended upon the sources of the metal dopants and the synthetic routes for the HA. Thus, both the crystallinityand crystallite size increases with the addition of the metal ion (Cd<sup>2+</sup>) dopant except for cadmium (Cd<sup>2+</sup>) ion dopant from the chloride salt where both crystallinity and crystallite size decreases than for the pure HA. This observed reduction in size is in agreement with the smaller ionic radius of Cd<sup>2+</sup> (0.97A°) with respect to that of Ca<sup>2+</sup> (0.99A°) and greater reactivity of chloride ion compared to nitrate ion. However, this difference in ionic radii appears too small to justify the observed reduction in size; instead, the reduction may be attributed to the nature of the cation-oxygen interaction [16,25].

Table 3 shows the calculated lattice parameters of the pure and doped HA. It is well known that the Ca<sup>2+</sup> ion in HA can be easily ion exchanged with many other metal ions[11]. The ionic radius of Cd<sup>2+</sup> (0.973A°) being smaller compared to that of Ca<sup>2+</sup> (0.99A°), it is expected that Ca<sup>2+</sup> can be easily substitute in HA crystal lattice. These possible changes in the lattice parameters were calculated according toYfantis*et al*[26] and tabulated in Table 3 using the space group of HA as  $\rho 6^3/m$  (Hexagonal, slightly non-stoichiometric). The latticesparameters (a and c)

of the pure hydrothermally and wet precipitated HA were calculated as (0.943 and 0.686nm) and (0.942 and 0.687nm) respectively.

Table 3: The lattice parameters of optimized hydrothermally and wet precipitated hydroxyapatite and doped HA

S/No.	Apatite	Hydrot (H	thermal IT)	Wet precipitation (WT)		
		a (nm)	c (nm)	a (nm)	c (nm)	
1 2 3 4	HA <sub>Theoretical</sub> HA <sub>Exptal</sub> CdC-HA CdN-HA	0.942 0.943 0.943 0.942	0.688 0.686 0.686 0.684	0.942 0.942 0.942 0.941	0.688 0.686 0.686 0.685	

HA = Hydroxyapatite, CdC - HA = HA doped with cadmium chloride, CdN - HA = HA doped

with cadmium nitrate

Doping with  $Cd^{2+}$  ion from both the Chloride and Nitrate salts as observed from Table 3, shows that the lattice parameters (a and c) were almost the same to those of the pure hydroxyapatites (HA) though vary slightly to different degrees. This difference in lattice parameters as observed may be attributed to the smaller ionic radius of  $Cd^{2+}$  with respect to  $Ca^{2+}$  [27]. This change in lattice parameters of CdHA, demonstrated that structurally incorporation of  $Cd^{2+}$  into HA crystals might just be a surface effect [26,27]. The slight decrease in lattice parameters by  $Cd^{2+}$  further demonstrates isomorphorus substitution in hydroxyapatite.

Fig.5 shows the SEM images (monographs) of the hydrothermally and wet precipitated HA doped with cadmium respectively from their chloride and nitrate salts. The pure hydroxyapatite showed a well distinct and uniformly distributed rod like morphology for the hydrothermally prepared HA and a small rod like or platy morphology for the HA prepared by the wet precipitation method. As seen from the monographs of HA for HTCdC, HTCdN, WTCdC and WTCdN, there significant morphological change on doping the HA with Cd<sup>2+</sup>ion, except for the slight change seen from the nitrate salts of the Cd<sup>2+</sup>ion dopant when compared to the chloride component. This may be attributed to the better reactivity of the Cl<sup>-</sup> than the NO<sub>3</sub><sup>-</sup> ions. These results are in agreement to those reported byGomez-Morales *et al* [14].





Fig. 5: SEM images of pure and doped hydroxyapatite (a) HA HT CdN(b) HA HT CdC(c) HA HT (d) HA WT CdN(e) HA WT CdC(f) HA WT

## CONCLUSION

Hydroxyapatitenano-crystals prepared by wet precipitation and hydrothermal methods were successfully doped with  $Cd^{2+}$  ions from the chloride and nitrate salts. XRF/EDX analysis confirmed the presence of this metal ion in the powders after doping irrespective of the source of the metal dopants though the amount of the metal ion found in hydroxyapatite from hydrothermal technique was less than those found in hydroxyapatite from the wet precipitation method. This could be attributed to the nature of reaction of HA with the metal solution. While it is proposed that in hydrothermally prepared HA ion exchange with the metal solution follows adsorption/diffusion mechanism, wet precipitated HA follows dissolution and precipitation mechanism. FT-IR confirms the characteristic peaks of hydroxyapatite, with the only noticeable change as decrease in the peak intensity of the H-O-H bands; P-OH around 3568 cm<sup>-1</sup> grows and those around 633cm<sup>-1</sup> decreases upon doping with Cd<sup>2+</sup>. Crystallize sizeand degree of crystallinity obtained from XRD pattern using Debye Scherrer equation indicated decrease in both (Xc) and (D) with addition of the metal dopant owing to smaller ionic radius of Cd<sup>2+</sup> (0.97A°) compared to a Ca<sup>2+</sup> (0.99A°). Furthermore, in consideration of sources of the metal

dopants  $Cd^{2+}$ , the ions from the nitrate salt is adsorbed more compared to those from the chloride salt irrespective of the method or the synthetic route for the HA used. This suggests  $Cd^{2+}$  ion from the nitrate salt which has greater adsorption rate onto HA is more toxic to the bone/body system than the  $Cd^{2+}$  ion from the chloride salt with less adsorption rate onto HA. This discrepancy in the adsorption rate could be attributed to the reactivity of these anions.

Toxicologically, HA prepared by the hydrothermalis preferred to those prepared from wet precipitation since; it adsorbs less of the metal dopant from solution which invariably suggests that it will adsorb less amount of such metal from the total burden of metal present in the bone when used as an artificial bone or as bone filler/implants. The wet precipitation method is recommended for the synthesis regarding quantity of product as it gives large quantity of the powder than the hydrothermal method.

## Acknowledgements

One of these authors (Dr. V. Ochigbo) is thankful to CNST, KSRCT and its management for providing facilities to carry out the research work under research academic fellowship.

### REFERENCES

- Aili, W, Dong, L., Hengbo, Y., Huixiong, W., Yuji, W., Min, R. Tingshun, J. Xiaonong, C. & Yiqing, X. (2007). Size-controlled synthesis of Hydroxyapatite nanorods by chemical precipitation in the presence of organic modifier. *Journal of Material Science and Engineering*, C 27, 865-869.
- [2]. Ajibola, V.O., Thamos, S.A. (1997). Transformation of Amorphous Calcium Phosphate to Hydroxyapatite in the presence of some ions. *Bulletin of Chemical Society Ethiopia*, 11(1), 19-24.
- [3]. Akesson, A., Bjellerup, P. & Lundh, T. (2006). Cadmium-induced effects on bone in a population-based study of woman. *Environmental Health Perspectives*, 114, 830–834.
- [4]. Beale, A. M., Grandjean, D., Kornatowski, J., Glatzel, P., De Groot, F.M.F., Weckhuysen,
  B. M., 2006. Unusual coordination behavior of Cr<sup>3+</sup> in Microporus alumina-phosphate. *The Journal of Physical Chemistry B*; 110, 716-722.
- [5]. Bigi, A., Ripamonti, A., Cojazzi, G., Pizzuto, G., Roveri, N. & Koch, M. H. (1991). Structural analysis of turkey tendon collagen upon removal of the inorganic phase. *Int J BiolMacromol*, 13, 110-114.

- [6]. Boucetta, C., Kacimu, M. & Ensuqueb, A. (2009). Oxidative dehydrogenation of propane over chromium –lead calcium-hydroglpitite. *Applied Catalysis A; General*, 356, 201-210.
- [7]. Brown, P. W. B., Constants., 1994. *Hydroxyapatite and Related Materials*, CRC press, Inc., Bonca Raton, p. 3.
- [8]. Caroline, E., Victoria, E., Gnanam, F. D. (2002). Synthesis and characterization of biphasic calcium phosphate. *Trends in Biomaterials and Artificial Organs*, 16: 12.
- [9]. Cheng, Z. H, Yasukawa, A., Kandori, K. & Ishikawa, T. (1998). Fine particles: synthesis, characterization and mechanisms of growth Faraday Transaction. *Journal of Chemistry Society*, 94, 1501.
- [10]. Crosman, A., Gelbard, G., Poncelet, G., Parvillescu, V. I. (2004).Oxidativedehydrogenation of propane over chromium-loaded calcium-hydroixyapatite.Applied catalysis A: General, 23, 264.
- [11]. Elliott, J.C. (1994). Structure and Chemistry of the Apatites and Other Calcium Orthophosphates Elsevier, Amsterdam, p. 111.
- [12]. Feng, W., Mu-sen, L., Yu-peng, L. & Sheng-song, G. (2005). Synthesis and microstructure of hydroxyapatite powders in stimulated body fluid. *Journal of Materials Science*, 40, 2073 6.
- [13]. Galicka, A., Brzóska, M. M. & Średzińska, K. (2004). Effect of cadmium on collagen content and solubility in rat bone. *Acta Biochimica Polonica*, 51, 825–829.
- [14]. Gomez-Morales, J., Torrent-Burgues, J., Boix, T., Fraile, J. & Rodriguez-Clemente, R., (2001). Precipitation of stoichiometric hydroxyapatite by a continuous method. *Crystal Research Technology*, 36:15.
- [15]. Inikori, G.O. (1992). Synthesis and Isomorphous Substitution Reactions of some hydroxyapatite. *PhD Thesis* submitted to Ahmadu Bello University, Zaria Nigeria.
- [16]. JCPDS (2001). Hydroxylapatite, Joint Committee for Powder Diffraction Standards, International Committee for Diffraction Data.
- [17]. Salemi, H., Behnamghader, A., Afshar, A., Ardeshir, C. M. & Forati, T. (2007). Calcium Phosphate Formation on Alkaline-Treated Titanium by Biomimetic Synthesis, *Proceedings* of the 2007 WSEAS Int. Conference on Cellular & Molecular Biology - Biophysics & Bioengineering, Greece, pp. 126-129.

- [18]. Samira, M. S., Khairi, M. T., Abdelsattar, M. S., Lolfi, I. A. S. & Faten, A. M. (2012). Synthesis and characterization of hydroxyapatite contain Chromium. *Journal of Biophysical Chemistry*, 3(4), 278-282.
- [19]. Syed Sibte, A. A. & Qasim M. (2013). Synthesis and Characterization of Nano hydroxyapatite powder using wet chemical precipitation. U.P.B Sci. Bull., Series B, Vol.75, Iss.3
- [20]. Watanabe, T., Kawachig, G., Kamitakahara, M., Kikuta, K. & Ohtsuki, C. (2009). Formation of needle-like hydroxyapatite by hydrothermal treatment of CaHPO<sub>4</sub>.2H<sub>2</sub>O combined with β-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. *Journal of the Ceramic Society of Japan*, 117 (1366): 759-764.
- [21]. Wilson, A. K. & Bhattacharyya, M. H. (1997). Effects of cadmium on bone: an in vivo model for the early response. *Toxicology and Applied Pharmacology*, 145, 68–73.
- [22]. Yasukawa, A., Ouchi, S., Kandori, K. & Ishikawa, T. (1996). Preparation and characterization of magnesium-calcium hydroxyapatite. *Journal Material Chemistry*. 6, 1401-1405.
- [23]. Yasukawa A, Yokoyama T., Kandori, K. & Ishikawa T. (2006). Reaction of calcium hydroxyapatite with Cd2+ and Pb2+ ions. *Colloids and Surfaces A*, AphysicochemicalEngineering aspects 299: 203-208.
- [24]. Yasukawa, A., Higashijima, M., Kandori, K. & Ishikawa T. (2005). Preparation and characterization of cadmium – calcium hydroxyapatite solid solution particles. *Colloids* and Surface A, 268: 111.
- [25]. Yasushi, T. & Hironori, A. (1990). Removal of coexisting Pb<sup>2+</sup>, Cu<sup>2+</sup>and Cd<sup>2+</sup>ions from water by addition of hydoxyapatite powder. *Journal of Chemical Engineering of Japan*, 23 (1), 75 79.
- [26]. Yfantis, C. D., Yfantis, D. K., Depountis, S., Anastassopoulou, J. & Theophanides, T. (2008). Academic Environment of Biomaterials Science and Engineering at the School of Chemical Engineering of NTUA, 5th WSEAS /IASME International Conference on Engineering Education (EE'08), Greece, ISSN: 1790-2769, pp. 27-32.
- [27]. Zhang, X. & Vecchio, K. S. (2007). Hydrothermal synthesis of hydroxyapatite rods. Journal of Crystal Growth, 308, 133 – 140.

http://www.unn.edu.ng/nigerian-research-journal-of-chemical-sciences/