IN VITRO PREPARATIONS AND STUDIES ON THE SUBSTITUTION OF CADMIUM AND LEAD IONS ON BIOMIMETIC HYDROXYAPATITE NANOCRYSTALS

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DECLARATION

I declare that the work in the thesis entitled "*IN-VITRO* PREPARATIONS AND STUDIES ON THE SUBSTITUTION OF CADMIUM AND LEAD IONS ON BIOMIMETIC HYDROXYAPATITE NANOCRYSTALS" has been performed by me in the Department of Chemistry, Ahmadu Bello University, Zaria and Centre for Nano Science and Technology, College of Technology, Karuveppampatti Sennimalai-Gounder Rangasamy (K.S.R) Educational Institution Tiruchengode, Tamil Nadu India, under the supervision of Prof. V.O. Ajibola, Prof (Mrs) E.B. Agbaji, Dr. A Giwa and Prof. V. Rajendran**.** The information derived from the literature has been duly acknowledged and a list of references provided. No part of this work was previously presented for another degree or diploma at any other university.

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Victor, OCHIGBO Date

CERTIFICATION

This Thesis entitled "*IN-VITRO* PREPARATIONS AND STUDIES ON THE SUBSTITUTION OF CADMIUM AND LEAD IONS ON BIOMIMETIC HYDROXYAPATITE NANOCRYSTALS" by Victor, OCHIGBO meets the regulation governing the award of the degree of Doctor of Philosophy (PhD) Analytical Chemistry of the Ahmadu Bello University, Zaria and is approved for itscontribution to knowledge and literary presentation.

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(Dean, School of Postgraduate Studies)

DEDICATION

This work is dedicated to my wife and Son (Adole Jnr).

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LIST OF ABBREVIATION

ACP	Amorphous Calcium Phosphate
BET	Brunauer-Emmett Teller
BMD	Bone Mineral Density
Ca-HA	Calcium Hydroxyapatite
CdCaHA	Cadmium Calcium Hydroxyapatite
Cd-HA	Cadmium Hydroxyapatite
DD	Double Distilled
D_{hkl}	Crystallite Size
DLS	Dynamic Light Scattering
DTA	Differential Thermal Analysis
EDX	Energy Dispersive X-ray
FT-IR	Fourier Transform Infrared Spectroscopy
HA	Hydroxyapatite
HT	Hydrothermal Techniques
ICDD	International Centre of Diffraction Data
IQ	Intelligence Quotient
JCPDS	(Joint Committee on Powder Diffraction Standards)
NIST	National Institute of Science Technology
NP	Nanoparticles
PbCaHA	Lead Calcium Hydroxyapatite
Pb-HA	Lead Hydroxyapatite
PS	Pore Size

PSA	Particle Size Analyser
PSD	Particle Size Distribution
PVA	Poly Vinyl Alcohol
PV	Pore Volume
RBCs	Red Blood Cells
SAED	Selected Area Electron Diffraction
SBF	Simulated Body Fluid
SEM	Scanning Electron Microscope
SSA	Specific Surface Area
TEM	Transmission Electron Microscope
TG	Thermogravimetry
TTCP	Tetra Calcium Phosphate
β-TCP	Beta Tri-Calcium Phosphate
α-TCP	Alpha Tri-Calcium Phosphate
WT	Wet Precipitation Techniques
Xc	Degree of Crystallinity
XRD	X-ray Diffraction
XRF	X-ray Fluorescence

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ABSTRACT

The hydrothermal and wet precipitation methods are successful routes to synthesize high purity hydroxyapatite (HA) as well as Cadmium and Lead doped hydroxyapatites at physiological conditions. In this research, $Ca(NO_3)_2.4H_2O$ and $NH_4H_2PO_4$ were chosen as the precursor materials used to synthesize HA. HA was first synthesized via optimization of the wet precipitation method under vigorous stirring using various precursor concentrations of 0.5, 1.0, 1.5 and 2.0M. The prepared HA powders were calcined at different temperatures of 300, 500, 800 and 1100°C for 3 hours and were characterized using XRF/EDX, FTIR and XRD. The FTIR bands were distinct, narrower with increased intensities when the precursor concentration and calcination temperature increases. XRF/EDX indicates an increase in Ca/P ratio as calcination temperature increased from 300°C to 500°C and at a particular calcination temperature, an increase in precursor concentration showed an irregular increase in the Ca/P ratio, an indication of a weak effect of precursor concentration on Ca/P ratio. XRD showed that as the precursor concentration is increased, with respect to the calcination temperature, peak intensities increased. These observations of peak intensities and degree of crystallinity are reflected with a corresponding increase in crystallite size (D) with increase in both the precursor concentration and calcination temperature. The result showed that the HA particles were all in nano regime for all the precursor concentrations, although, the 0.5M precursor concentration and calcination temperature of 300°C gave the HA with greater similarity to the natural bone apatite as regards the Ca/P ratio. Based on the optimum conditions (0.5M and 300 °C) investigated at the first stage of the synthesis, further synthesis was carried out using the wet precipitation and hydrothermal methods at physiological conditions of pH and temperature (7.4 and 37 °C). The as-prepared powders were characterized via XRF/EDX, FTIR, XRD, SEM, PSA, BET, TEM and TG/DTA. The result obtained indicated particle nano-size particle with sizes of 26 nm ± 5% and 29 nm ± 5% respectively for the wet

precipitation and hydrothermal methods and these were found to be thermally stable up to 1200^{o}C. SEM images showed a non-uniform particle with various degrees of agglomeration that were observed to be highest for the hydrothermally prepared HA compared to the wet precipitated HA. The synthesized HA samples by both methods were then tested for their bioactivity via *in-vitro* analysis by immersion of the HA in a simulated body fluid (SBF) solution for 21 days. The pH of the SBF was constantly monitored for ion exchange and the percentage weight loss of the HA were estimated suggesting the biodegradation of HA in SBF. After the incubation period, the HA powder were then characterized via XRF/EDX, FTIR, XRD and SEM and the result indicated high bioactivity of the HA powders. Based on the good response of bioactivity for these HA's, they were subsequently doped with cadmium (Ca^{2+}) and lead (Pb^{2+}) ions from chloride and nitrate sources respectively and further characterized using XRF/EDX, FTIR, XRD and SEM. The result particularly the XRD studies indicated that while (Ca^{2+}) ion was adsorption onto the hydroxyapatite (HA), (Pb^{2+}) ion was incorporated into the hydroxyapatite (HA), to different degrees depending on the HA synthetic route. The effect of lead (Pb^{2+}) on the HA produced a slight increase in the lattice parameters of the apatite structure resulting in a slight shift of the XRD patterns for Pb-HA samples when compared to the Cadmium (Cd^{2+}) ions' effect. This change on lattice parameters is due the larger ionic radius of Pb^{2+} (1.30A^{o}) than Cd^{2+} (0.97A^{o}) with respect to Ca^{2+} (0.99A^{o}). Toxicologically, HA prepared via hydrothermal method is preferred to those prepared from the wet precipitation because the HA from the hydrothermal method adsorbs less of the metal dopant from solution suggesting that it will adsorb less amount of such metal from the total burden of it present in the bone when it is used as artificial bone or bone filler/implants. However, when quantity of HA is the research problem, the wet precipitation (WT) method is recommended as it produced a larger quantity of the powder than the hydrothermal method. This quantity increased as precursor concentration is increased. The

wet precipitation method is also recommended for use because it is less complicated and less expensive than the hydrothermal method.