

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

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NIGERIA**

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*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

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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 its contribution to knowledge and literary presentation.

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## **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
$\beta$ -TCP	Beta Tri-Calcium Phosphate
$\alpha$ -TCP	Alpha Tri-Calcium Phosphate
WT	Wet Precipitation Techniques
Xc	Degree of Crystallinity
XRD	X-ray Diffraction
XRF	X-ray Fluorescence

## TABLE OF CONTENTS

<b>TITLE</b>	<b>Pages</b>
Title page	ii
Declaration	iii
Certification	iv
Dedication	v
Acknowledgment	vi
List of Abbreviation	viii
Table of Contents	x
List of Figures	xvi
List of Tables	xx
List of Schemes	xxii
List of Appendices	xxiii
Abstract	xxiv
<b>CHAPTER ONE</b>	
<b>1.0 INTRODUCTION</b>	<b>1</b>
1.1 <b>Nanomaterials</b>	<b>1</b>
1.2 <b>Types of Nano-materials</b>	<b>2</b>
1.3 <b>Apatite</b>	<b>4</b>
1.4 <b>Statement of the Problem</b>	<b>8</b>
1.5 <b>Justification of the Research</b>	<b>9</b>
1.6 <b>Aim and Objectives</b>	<b>11</b>
1.6.1 Aim	11
1.6.2 Objectives	11

## CHAPTER TWO

<b>2.0</b>	<b>Literature review</b>	<b>13</b>
<b>2.1</b>	<b>Biomaterials</b>	<b>13</b>
<b>2.2</b>	<b>Types of Synthetic Biomaterials</b>	<b>15</b>
2.2.1	Metallic biomaterials	15
2.2.2	Ceramic and Glass Biomaterials	15
2.2.3	Polymeric biomaterials	19
<b>2.3</b>	<b>Bio-ceramics/ Calcium Phosphate Ceramics</b>	<b>19</b>
2.3.1	Biological Apatite	22
2.3.2	Calcium Hydroxyapatite	23
2.3.2.1	Hydroxyapatite and its Synthesis	24
2.3.2.2	Preparation Methods of Hydroxyapatite	29
2.3.2.3	Ion Exchange in Calcium Hydroxyapatite	33
<b>2.4</b>	<b>Bones</b>	<b>35</b>
2.4.1	Interaction of Cadmium and Bone	36
2.4.2	Interaction of Lead and Bone	39
2.4.2.1	Bone Lead as a Cumulative Exposure Marker	44
2.4.2.2	Bone Lead as a Retroactive Exposure Marker	45
2.4.2.3	Bone Lead as a Risk Factor for Disease	46

## CHAPTER THREE

<b>3.0</b>	<b>Materials and Methods</b>	<b>47</b>
<b>3.1</b>	<b>Reagents</b>	<b>47</b>
<b>3.2</b>	<b>Experimental Method</b>	<b>48</b>
3.2.1	Preparation of Solutions for the Synthesis of Hydroxyapatite (HA)	48

3.2.1.1	Procedures for Hydrothermal (HT) Method	48
3.2.1.2	Procedures for Wet Precipitation (WT) Method	49
3.2.2	<b>Preparation of Simulated Body Fluid (SBF) for Bioactivity Study</b>	<b>52</b>
3.2.2.1	Procedures for the Bioactivity Study of Hydroxyapatite (HA)	53
3.3.3	<b>Doping of Hydroxyapatite (HA)</b>	<b>56</b>
3.3.3.1	Preparation of Cadmium ( $\text{Cd}^{2+}$ ) and Lead ( $\text{Pb}^{2+}$ ) Ions Solutions for Doping of Hydroxyapatite (HA)	56
3.3.3.2	Procedures for Doping of Hydroxyapatite (HA)	56
3.4	<b>Instrumentation</b>	<b>58</b>
3.4.1	Particle Size Analyzer (PSA)	58
3.4.2	Brunauer-Emmett Teller (BET)	58
3.4.3	X-Ray Fluorescence Spectrometry (XRF/EDX)	59
3.4.4	Fourier Transform Infrared (FT-IR) Spectroscopy	60
3.4.5	X-Ray Diffraction (XRD)	61
3.4.6	Scanning Electron Microscopy (SEM)	62
3.4.7	Transmission Electron Microscopy (TEM)	62
3.4.8	Thermogravimetric/Differential Thermal Analysis (TG/DTA)	63

## CHAPTER FOUR

4.0	<b>RESULTS</b>	<b>64</b>
4.1	<b>Results of the Characterization for Optimization of Wet Precipitation (WT) Method for the Synthesis of Hydroxyapatite (HA)</b>	<b>64</b>
4.1.1	FTIR Studies	64
4.1.2	Elemental Composition Studies	65
4.1.3	XRD Studies	65
4.2	<b>Characterization of Hydroxyapatite (HA) Obtained by Optimized Hydrothermal (HT) and Wet Precipitation (WT) Reactions</b>	<b>81</b>
4.2.1	FTIR Studies	81
4.2.2	Elemental Composition Studies	81
4.2.3	Particle Size Analysis	82
4.2.4	Surface area Analysis	82
4.2.5	Structural Studies	82
4.2.6	SEM Morphological Studies	83
4.2.7	TEM Morphological Studies	84
4.2.8	Thermal Measurements	84
4.3	<b>Results of Characterization of the Bioactivity Study of Hydroxyapatite (HA) Obtained via Optimized Hydrothermal (HT) and Wet Precipitation (WT) Reactions</b>	<b>95</b>
4.3.1	FTIR Studies	95
4.3.2	Elemental Composition Studies	95
4.3.3	Structural Studies	95

4.3.4	SEM Morphological Studies	96
4.3.5	pH / Biodegradation Studies	96
<b>4.4</b>	<b>Results of Characterization for Doping of Hydroxyapatite (HA) Obtained via Optimized Hydrothermal (HT) and Wet Precipitation (WT) Methods with Cadmium (Cd<sup>2+</sup>) and Lead (Pb<sup>2+</sup>) ions from their Chloride and Nitrate Salts</b>	<b>104</b>
4.4.1	FTIR Studies	104
4.4.2	Elemental Composition Studies	105
4.4.3	Structural Studies	105
4.4.4	SEM Morphological Studies	106
CHAPTER FIVE		
5.0	<b>DISCUSSION</b>	<b>130</b>
5.1	<b>Optimization of Wet Precipitation (WT) Method for the Synthesis of Hydroxyapatite (HA)</b>	<b>130</b>
5.2	<b>Characterization of Hydroxyapatite (HA) Obtained by Optimized Hydrothermal (HT) and Wet Precipitation (WT) Reactions</b>	<b>133</b>
5.3	<b>Bioactivity Study of the Synthesized Hydroxyapatite (HA)</b>	<b>142</b>
5.4	<b>Doping of Hydroxyapatite (HA) with Cadmium (Cd<sup>2+</sup>) and Lead (Pb<sup>2+</sup>) ions</b>	<b>146</b>
CHAPTER SIX		
6.0	<b>SUMMARY, CONCLUSION AND RECOMMENDATIONS</b>	<b>154</b>
6.1	<b>Summary</b>	<b>154</b>
6.2	<b>Conclusion</b>	<b>160</b>

<b>6.3 Recommendation</b>	163
<b>References</b>	164
<b>Appendices</b>	193

## LIST OF FIGURES

Fig. 2.1	Calcium Hydroxyapatite Structure	24
Fig. 4.1	FTIR spectra of Hydroxyapatite (HA) Synthesized via Wet Precipitation Method using Different Initial Precursor Concentrations and Calcinated at 300°C.	68
Fig. 4.2	FTIR spectra of Hydroxyapatite (HA) Synthesized via Wet Precipitation Method using Different Initial Precursor Concentrations and Calcinated at 500°C.	69
Fig. 4.3	FTIR Spectra of HA Synthesized via Wet Precipitation Method using Different Initial Precursor Concentrations and Calcinated at 800°C.	70
Fig. 4.4	FTIR spectra of Hydroxyapatite (HA) Synthesized via Wet Precipitation Method using Different Initial Precursor Concentrations and Calcinated at 1100°C.	71
Fig. 4.5	FTIR Spectra of Hydroxyapatite (HA) Synthesized using Initial Precursor Concentration of 0.5M and Calcinated at Different Temperatures.	72
Fig. 4.6	FTIR Spectra of Hydroxyapatite (HA) Synthesized using Initial Precursor Concentration of 1.0M and Calcinated at Different Temperatures.	73
Fig. 4.7	FTIR spectra of hydroxyapatite (HA) Synthesized using Initial Precursor Concentration of 1.5M and Calcinated at Different Temperatures.	74
Fig. 4.8	FTIR Spectra of Hydroxyapatite (HA) Synthesized using Initial Precursor concentration of 2.0M and Calcinated at Different Temperatures.	75
Fig. 4.9	XRD Pattern of Hydroxyapatite (HA) Synthesized via Wet Precipitation Method using Different Initial Precursor Concentrations and Calcinated at 300 °C.	78



Fig. 4.10	XRD pattern of Hydroxyapatite (HA) Synthesized via Wet Precipitation Method using Different Initial Precursor Concentrations and Calcinated at 500 °C.	79
Fig. 4.11	FTIR bands of Hydroxyapatite (HA) Synthesized via Optimized Hydrothermal and Wet Precipitation Methods.	86
Fig. 4.12	Particle Size Distribution of Hydroxyapatite (HA) Synthesized via Optimized Hydrothermal and Wet Precipitation Methods.	88
Fig. 4.13	XRD Pattern of Hydroxyapatite (HA) Synthesized via Optimized Hydrothermal and Wet Precipitation Methods.	91
Fig. 4.14	SEM Image of Hydroxyapatite (HA) Synthesized via Optimized Hydrothermal Method	92
Fig. 4.15	SEM Image of Hydroxyapatite (HA) Synthesized via Optimized Wet Precipitation Method	92
Fig.4.16	TEM image of Hydroxyapatite (HA) Synthesized via Optimized Hydrothermal Method	93
Fig. 4.17	TEM image of Hydroxyapatite (HA) Synthesized via Optimized Wet Precipitation Method	93
Fig. 4.18	TG/DTA Curve of Hydroxyapatite (HA) Synthesized via Optimized Hydrothermal Method	94
Fig. 4.19	TG/DTA Curve of Hydroxyapatite (HA) Synthesized via Optimized Wet Precipitation Method	94
Fig. 4.20	FTIR Bands of Hydroxyapatite (HA) Synthesized via Hydrothermal and Wet Precipitation Methods Incubated in SBF for 21 days	97
Fig. 4.21	XRD Pattern of Hydroxyapatite (HA) Synthesized via Hydrothermal and Wet Precipitation Methods Incubated in SBF for 21days	99
Fig. 4.22	SEM Image of Hydrothermally prepared Hydroxyapatite (HA) incubated in SBF for 14 days	100
Fig. 4.23	SEM Image of Hydrothermally Prepared Hydroxyapatite (HA) Incubated in SBF for 21 days	100

Fig. 4.24	SEM Image of Wet Precipitated Hydroxyapatite (HA) Incubated in SBF for 14 days	101
Fig. 4.25	SEM image of Wet Precipitated Hydroxyapatite (HA) Incubated in SBF for 21 days	101
Fig. 4.26	Plot of Variation of pH with time for Incubation of Hydroxyapatite (HA) in SBF	102
Fig. 4.27	FTIR Bands of Hydroxyapatite (HA) Synthesized via Hydrothermal and Wet Precipitation Methods Doped with Cadmium Chloride	107
Fig. 4.28	FTIR Bands of Hydroxyapatite (HA) Synthesized via Hydrothermal and Wet Precipitation Methods Doped with Cadmium Nitrate	108
Fig. 4.29	FTIR Bands of Hydroxyapatite (HA) Synthesized via Hydrothermal and Wet Precipitation Methods Doped with Lead Chloride	109
Fig. 4.30	FTIR Bands of Hydroxyapatite (HA) Synthesized via Hydrothermal and Wet Precipitation Methods Doped with Lead Nitrate	110
Fig. 4.31	FTIR Bands of Hydrothermally Synthesized Hydroxyapatite (HA HT) and (HA HT) Doped with Cadmium Chloride and Nitrate	111
Fig. 4.32	FTIR Bands of Wet Precipitated Hydroxyapatite (HA WT) and (HA WT) Doped with Cadmium Chloride and Nitrate	112
Fig. 4.33	FTIR Bands of Hydrothermally Synthesized Hydroxyapatite (HA HT) and (HA HT) Doped with Lead Chloride and Nitrate	113
Fig. 4.34	FTIR Bands of Wet Precipitated Hydroxyapatite (HA WT) and (HA WT) Doped with Lead Chloride and Nitrate	114
Fig. 4.35	FTIR Bands of Hydrothermally Synthesized Hydroxyapatite (HA HT) Doped with Cadmium and Lead Chloride	115
Fig. 4.36	FTIR Bands of Wet Precipitated Hydroxyapatite (HA WT) Doped with Cadmium and Lead Chloride	116
Fig. 4.37	FTIR Bands of Hydrothermally Synthesized Hydroxyapatite (HA HT) Doped with Cadmium and Lead Nitrate	117
Fig. 4.38	FTIR Bands of Wet Precipitated Hydroxyapatite (HA HT)	

	Doped with Cadmium and Lead Nitrate	118
Fig. 4.39	XRD Patterns of Hydrothermally Prepared HA, Doped with Cadmium and Lead ions from the Chloride and Nitrate Salts	122
Fig. 4.40	XRD Patterns of Wet Precipitated HA, Doped with Cadmium and Lead ions from the Chloride and Nitrate Salts	123
Fig. 4.41	SEM Image of Hydrothermally Prepared HA Doped with Cadmium Chloride	126
Fig. 4.42	SEM Image of Hydrothermally Prepared HA Doped with Cadmium Nitrate	126
Fig. 4.43	SEM Image of Hydrothermally Prepared HA Doped with Lead Chloride	127
Fig. 4.44	SEM Image of Hydrothermally Prepared HA Doped with Lead Nitrate	127
Fig. 4.45	SEM Image of Wet Precipitated HA Doped with Cadmium Chloride	128
Fig. 4.46	SEM Image of Wet Precipitated HA Doped with Cadmium Nitrate	128
Fig. 4.47	SEM Image of Wet Precipitated HA Doped with Lead Chloride	129
Fig. 4.48	SEM Image of Wet Precipitated HA Doped with Lead Nitrate	129

## LIST OF TABLES

Table 2.1	Biomaterials and their Properties.	13
Table 2.2	Bio-Ceramics and their Applications.	17
Table 2.3	Materials for use in the Body	18
Table 2.4	Composition of Biological Apatite and Hydroxyapatite.	22
Table 2.5	Properties of Biological Apatite and HA.	23
Table 2.6	Comparisons of Different Methods for Preparation of HA.	32
Table 3.1	List of Chemicals/Reagents with their Sources and Form used	48
Table 3.2	List of Reagents used for Preparing Simulated Body Fluid (SBF).	54
Table 4.1	FTIR Absorption in Band Positions of Hydroxyapatite (HA) as Reference	76
Table 4.2	Elemental Compositions and Ca/P ratio of Hydroxyapatite (HA) Obtained at Optimized Wet Precipitation Method	77
Table 4.3	The Crystallite Size (D) and Degree of Crystallinity (Xc) of Hydroxyapatite (HA) obtained at Optimized Wet Precipitation Method	80
Table 4.4	Elemental Compositions and Ca/P ratio of Hydroxyapatite (HA) Obtained at Optimized Wet Precipitation and Hydrothermal Methods	87
Table 4.5	Specific Surface Area (SSA), Pore Size (PS), Pore Volume (PV) and Average Particle Size (D <sub>A</sub> ) of Hydroxyapatite (HA) before and after Calcinations from BET Analysis	89
Table 4.6	XRD in Peak Positions of Hydroxyapatite (HA) as Reference	90
Table 4.7	The Elemental Compositions and Ca/P ratio of HA Synthesized via Optimized Hydrothermal and Wet Precipitation before and after Incubation in SBF	98
Table 4.8	The Weight and Percent Weight loss of Optimized Hydrothermally and Wet Precipitated HA before and after Incubation in SBF	103
Table 4.9	The XRF/EDX Results of Optimized Hydrothermally and Wet Precipitated Hydroxyapatite (HA) and Cadmium Doped HA	119
Table 4.10	The XRF/EDX Results of Optimized Hydrothermally and Wet precipitated Hydroxyapatite (HA) and Lead Doped HA	120

Table 4.11	The XRD Peaks of Hydroxyapatite (HA) obtained by Optimized Hydrothermal and Wet Precipitation Methods and ‘Cd and Pb’ Doped HA	123
Table 4.12	The Crystallite Size (D) and Degree of Crystallinity (Xc) of Doped Hydroxyapatite (HA) obtained by Optimized Wet Precipitation and Hydrothermal methods	124
Table 4.13	The Lattice Parameters of Optimized Hydrothermally and Wet precipitated Hydroxyapatite (HA) and Doped HA	125

## LIST OF SCHEMES

Scheme 1.1	The Hierarchical Structure of a typical Bone at various length scales	5
Scheme 2.1	Lead Metabolism Model	43
Scheme 3.1	Preparation of HA Nanoparticles via Conventional Chemical Precipitation	51
Scheme 3.2	Flow Chart for the Synthesis of HA via Hydrothermal and Wet Precipitation Method	52
Scheme 3.3	Flow Chart for HA Bioactivity Study	56
Scheme 3.4	Flow Chart for the Doping of Hydroxyapatite (HA)	58

## LIST OF APPENDICES

<b>Appendix I:</b> JCPDS 09-0432 – Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	193
<b>Appendix II:</b> JCPDS 24-0033 – Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	193
<b>Appendix III:</b> JCPDS 74-0563 – Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	194
<b>Appendix IV:</b> JCPDS74-0566– Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	194
<b>Appendix V:</b> JCPDS 73-1731– Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	195
<b>Appendix VI:</b> JCPDS73-0294 – Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	195
<b>Appendix VII:</b> JCPDS72-0293 – Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	196
<b>Appendix VIII:</b> JCPDS72-1243– Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	196
<b>Appendix IX:</b> JCPDS 76-0694 – Hydroxyapatite, Joint Committee for Powder Diffraction Studies, International Committee for Diffraction Data (ICDD)	197

## 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,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $\text{NH}_4\text{H}_2\text{PO}_4$  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  $26 \text{ nm} \pm 5\%$  and  $29 \text{ nm} \pm 5\%$  respectively for the wet



precipitation and hydrothermal methods and these were found to be thermally stable up to 1200°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 ( $\text{Ca}^{2+}$ ) and lead ( $\text{Pb}^{2+}$ ) 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 ( $\text{Ca}^{2+}$ ) ion was adsorption onto the hydroxyapatite (HA), ( $\text{Pb}^{2+}$ ) ion was incorporated into the hydroxyapatite (HA), to different degrees depending on the HA synthetic route. The effect of lead ( $\text{Pb}^{2+}$ ) 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 ( $\text{Cd}^{2+}$ ) ions' effect. This change on lattice parameters is due the larger ionic radius of  $\text{Pb}^{2+}$  ( $1.30\text{\AA}$ ) than  $\text{Cd}^{2+}$  ( $0.97\text{\AA}$ ) with respect to  $\text{Ca}^{2+}$  ( $0.99\text{\AA}$ ). 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.