

Original Research Article

**Microstructure and In Vitro Bioactivity of Metal Substituted
Hydroxyapatite**

Abstract:

Pure Hydroxyapatite (HAp) nanoparticles doped with Mn^{2+} and Fe^{3+} ions were synthesized using wet chemical method (WCM). Samples were characterized by different spectroscopic techniques such as XRD, FTIR, and ESR. The measurements revealed that a typical HAp powder pattern was obtained. Comparing with pure stoichiometric HAp, both Mn^{+2} substituted HAp (Mn-HAp) and Fe^{+3} substituted HAp (Fe-HAp) did not demonstrate significant structure deviation. Since ion exchange mechanism was applied for the preparation process, the morphology and particle size were not significantly affected. The FTIR absorption spectra of the doped samples are presented as absorption bands characterizing Mn^{+2} , and Fe^{+3} which occupy different crystalline sites. The obtained data agrees well with that obtained from XRD the crystal field parameters and degree of crystallinity for sites of these ions in the HAp matrix can be estimated. The addition of iron and manganese ions into hydroxyapatite results in improvement of the biological performance of the implant. Iron and manganese substituted hydroxyapatite has shown superior biological performance compared to its stoichiometric counterparts in vitro. The addition of such ions also enhances the magnetic properties of hydroxyapatite for the use in hyperthermia medical applications.

Keywords: Hydroxyapatite, bioactivity, crystallinity, hyperthermia

1. Introduction

Development of new materials is considered to be one of the most important objectives of biomaterials science for bone substitution. Hydroxyapatite (HAp) $\{Ca_{10}(PO_4)_6(OH)_2\}$ is one of the important biominerals of interest in the broad group of calcium phosphate based bioceramics and the main inorganic constituent of bone and teeth. HAp is mainly used as bone and dental replacement material [1-3]. Hydroxapatite is reported to be fully non-resorbable when tested in vivo.

The major advantage of hydroxyapatite phase of calcium phosphate materials is that the nano size (10–100 nm) hydroxyapatite crystalline arrays along the collagen fibers inside the bone is able to bond directly with the hard tissue and can provide perfect lattice matching between the fracture bone

38 surfaces. Moreover, AH exhibits osteoconductive behavior, is stable toward
39 bioresorption, and has no adverse effects on the human organism [1–5, 10].

40 Hydroxyapatite (HAp) is the main inorganic phase of human bones and teeth
41 [1-3]. Its structure is nearly similar to natural bone material which consists of
42 nearly 72 wt% apatitic materials, respectively. Calcium to phosphate ratio
43 (Ca/P) in the building structure of HAp is 1.67. This ratio is very close to that
44 Ca/P of natural bone [4-5]. Hydroxyapatites have a wide range of applications
45 both in medicine and dentistry due to the some characteristic properties [6-7].
46 The most important characteristic properties of HAp are good bioactivity and
47 biocompatibility. The biocompatibility property comes from the non toxic
48 effects on human tissues [8-11]. Implants coated with HAp promote a direct
49 physiochemical bond with bone, which leads to more rapid implant fixation and
50 the development of a stable bone biomaterial interface [12].

51 The main disadvantage of HAp is that they cannot be applicable to use for
52 heavy load bearing applications due to its low mechanical properties in wet
53 environments. In addition, HA can be dissolved directly during its setting in
54 living tissues. The problems associated with rapid dissolution, fatigue failure
55 and the creation of particulate debris [13] can be solved by addition some types
56 of reinforced agent such as iron oxide and Mn oxides to HA. In addition, these
57 agency can play good role in enhancing the magnetic properties of the product.

58 Magnetic-nanoparticles are used in bio-applications are usually made
59 from biocompatible materials such as magnetite (Fe_3O_4) for which susceptibility
60 is large. These particles have to be integrates into structure of hydroxyapatite
61 thus it has the potential to achieve localized tumor heating without any side
62 effects i.e hyperthermia effects. One of the main biomedicine sciences is the
63 hyperthermia application, consists of targeting magnetic nanoparticles to tumor
64 tissue followed by application of an external alternating magnetic field. The
65 temperature in tumor tissue is increased to above 43°C , which causes necrosis of
66 cancer cells, but does not damage surrounding normal tissue [14]. So, as an
67 trial, Magnetic hydroxyapatite containing iron or Mn or both have to prepare
68 and study, thus form a composite material [5-17].

69 Nanoparticles are incorporated into different materials such as polymers,
70 noble metals, metal oxides and silica. Many researchers have developed
71 different magnetic nanoparticles of ferromagnetic bioglass ceramics which
72 provide magnetic properties for hyperthermia purposes [18].

73 It was reported that insertion of the spinel ferrite MnFe_2O_4 through a wet
74 chemistry process resulted in nanoparticles having a core-shell structure (in
75 which the core was made up of the ferrite and the shell of HAp. Synthetic HAp
76 has been successfully used in hard tissue surgery, as it is capable of undergoing
77 bonding osteogenesis and is relatively insoluble *in vivo* [19].

78 Mn^{2+} containing HAp and tri calcium phosphate (TCP) were studied
79 previously [20-21]. The motivation for the addition of Mn^{2+} ions to HAp was

due to the fact that divalent Mn^{2+} has been linked to the activation of integrins which are defined as a family of receptors which mediate cellular interactions with extracellular matrix and cell surface ligands. In presence of Mn^{2+} ions, cell adhesion is promoted because the ligand affinity of integrin increases.

The aim of this study is to construct a new **species** of magnetic hydroxyapatites. These types are having a good degree of magnetic properties and compatibility properties. These types of hydroxyapatites contain Fe^{+3} and Mn^{+2} ions into the structure of HAp aiming to limit formation of magnetic secondary phase and able to be manipulated in situ by magnetic forces. These new types of magnetic hydroxyapatites are good candidate to be used in hyperthermia applications. In fact, the use of magnetic stimulation in the field of tumor treatment is one of the modern trends which have received increased attention in scientific circles.

Materials and Methods

2.1. Preparation hydroxyapatite

Analytical grades of calcium nitrate $Ca(NO_3)_2 \cdot 4H_2O$ (Merck) and diammonium hydrogen Phosphate $(NH_4)_2HPO_4$ (Sigma-Aldrich) with Ca/P molar ratio 1.67 were used to produce pure HA was produced using the following chemical reaction:



$MnCl_2 \cdot 4H_2O$ (Sigma-Aldrich) and $FeCl_3 \cdot 6H_2O$ (Merck) were used as sources of, Mn^{+2} and Fe^{+3} respectively. The different types of hydroxyapatites were presented in table 1.

Table 1 : Chemical formulas and symbols for prepared samples.

	Chemical Formula	Symbol
	$Ca_{10}(PO_4)_6(OH)_2$	HAp
Group (A)	$Ca_{9.2}Fe_{0.8}(PO_4)_6(OH)_2$	Fe _{0.8} - HAp
	$Ca_{8.4}Fe_{1.6}(PO_4)_6(OH)_2$	Fe _{1.6} - HAp
	$Ca_{7.6}Fe_{2.4}(PO_4)_6(OH)_2$	Fe _{2.4} - HAp
	$Ca_{6.8}Fe_{3.2}(PO_4)_6(OH)_2$	Fe _{3.2} - HAp
Group (B)	$Ca_{9.2}Mn_{0.8}(PO_4)_6(OH)_2$	Mn _{0.8} - HAp
	$Ca_{8.4}Mn_{1.6}(PO_4)_6(OH)_2$	Mn _{1.6} - HAp
	$Ca_{7.6}Mn_{2.4}(PO_4)_6(OH)_2$	Mn _{2.4} - HAp
	$Ca_{6.8}Mn_{3.2}(PO_4)_6(OH)_2$	Mn _{3.2} - HAp

To produce a pure hydroxyapatite sample, a calculated amount of calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) was dissolved in 1L distilled water with vigorous stirring (1 h) to form 0.5 M/L. then, Calculated amount of diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$) was also dissolved in 1L distilled water with vigorous stirring (1 h) to form 0.5 M/L and added drop wise to the calcium nitrate, the pH of the solution was adjusted to 9 using ammonia solution. The two solutions were mixed to obtain a Ca/P molar ration of 1.667 at a controlled constant pH of about 10-11. The mixture was continuously stirred about 1hr at temperature 80 °C, until a white transparent gel was obtained. The gel was cooled and incubating at 37 °C for 24 h until a white precipitate was produced. The precipitate was decanted and filtrated under vacuum by using sintered Buchner funnel with continued washing by distilled water to remove ammonia solution and then dried in oven at 90 °C. The powdered sample was sintered at 10 °C/min at temperatures 500, 600, 700, 800 and 900 °C for 1 h then placed in air-cooling to ambient temperature. The sintered product was crushed using agate mortar and pestle to obtain resultant powder.

2.2. FTIR measurements

Fourier transform infrared absorption signals of the studied HAp samples were measured at room temperature (20 °C) in the wavelength range 4000-400 cm^{-1} using a computerized recording FTIR spectrometer (Mattson5000, USA). Fine powdered samples were mixed with KBr in the ratio 1:100 for quantitative analysis and the weighed mixtures were subjected to a load of 5t/ cm^2 in an evocable i.e to produce clear homogenous discs. Then, the IR absorption spectra were immediately measured after preparing the discs to avoid moisture attack.

2.3. X-ray diffraction analysis

The structure of dried and calcined samples was assessed using an X-ray powder diffractometer (a Philips PW1390 X-ray diffractometer) with Cu $\text{K}\alpha$ target (Ni filter), wavelength (λ) = 1.54 Å. C/S= 1×10^3 , KV = 40, time constant (T.C) = 2 and mA = 25 was used. The Bragg's angle (2θ) in the range of 4–70°, step size = 0.02 and step time 0.4s at room temperature. Crystallographic identification of the phases of synthesized apatites was accomplished by comparing the experimental XRD patterns to standards complied by the Joint Committee on Powder Diffraction Standards (JCPDS).

The size of the crystallites responsible for the Bragg reflection of the (002) and (300) planes were determined using Scherer equation:

$$d = \frac{K \lambda}{\beta \cos \theta} \quad (2)$$

where d is the crystalline diameter in nm, β is the peak width at half-maximum peak height in radians, λ is the x-ray wavelength, typically 1.54 Å, and θ is the Bragg angle.

2.4. Differential Scanning Calorimetry and Thermogravimetric Analysis

DSC/TG analysis was carried out using a NETZSCH STA 409C/CD instrument. Known mass (≈ 30 mg) of crushed samples was placed into an aluminum pan then sealed with a crimped lid and heated from 25 °C to 1000 °C at a rate of 5 °C/min with argon as carrier gas at a flow rate of 30 cm³/min.

3. Results and Discussion:

Calcium phosphate ceramics, particularly those with Ca/P ratios between 1.5 and 1.67, i.e., tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) and stoichiometric calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), respectively, have long been used as prime candidate biomaterials to restore, reconstruct, and replace human bone tissues [22-26]. This is because of their close chemical similarity to minerals found in calcified tissues, such as bone, tooth enamel and dentine, in both humans and vertebrates. Hydroxyapatite (HAp) is one of the most important calcium phosphates and has received wide attention as bone substitutes over the past few decades[22, 27-29].

Figure 1 shows the thermogram of the synthesized pure hydroxyapatite material. The figure reveals four distinct stages of decomposition. The first stage that occurs between 35 and 110 °C is due to loss of adsorbed water with the value about (5.5% $\approx$ 0.0956 mg). The second stage for weight loss that occurs between 110 and 220 °C is assigned to the decomposition of ammonium (5.43% $\approx$ 0.094 mg). The third stage is due to the dehydration of $\text{Ca}(\text{OH})_2$ into CaO also contributes the total weight loss in this stage is 7.9% $\approx$ 0.13 mg. The fourth weight loss that occurs between 300 and 700 °C is due to the decomposition of nitrate is about (4.37% $\approx$ 0.075 mg). This temperature range for nitrate decomposition was also reported by Liu et al [30-31]. From the thermogram

presented in figure 1, it can **deuce** that temperature between 500- 900°C is a convenient for sintering process.

Figure 2 shows the XRD patterns of heat treated samples at 700°C (Plot A) and at 900 °C (Plot B) for 1 h. It can be noted from this figure that amorphous hump is limited and it can't be detected. This indicates that the prepared sample has crystalline structure and the measured degree of crystallinity is 41.4%. On the other hand, the crystalline-structure peaks become sharper in the sample sintered at, 900 °C resulting in crystallinity of 55.56%. Increasing degree of crystallinity to 55.56% with increasing temperature, may lead to conclude that any initial amorphous matrix present in sample is well crystallized by the effect of heat treatment. The angular location and intensity in fig 2 of

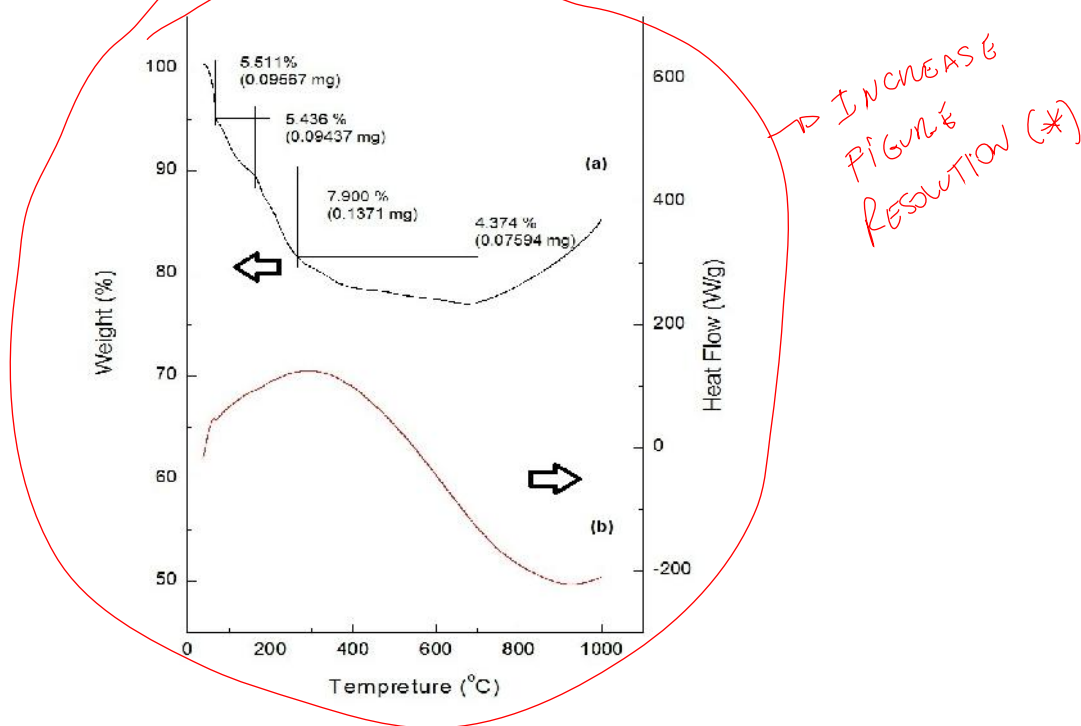
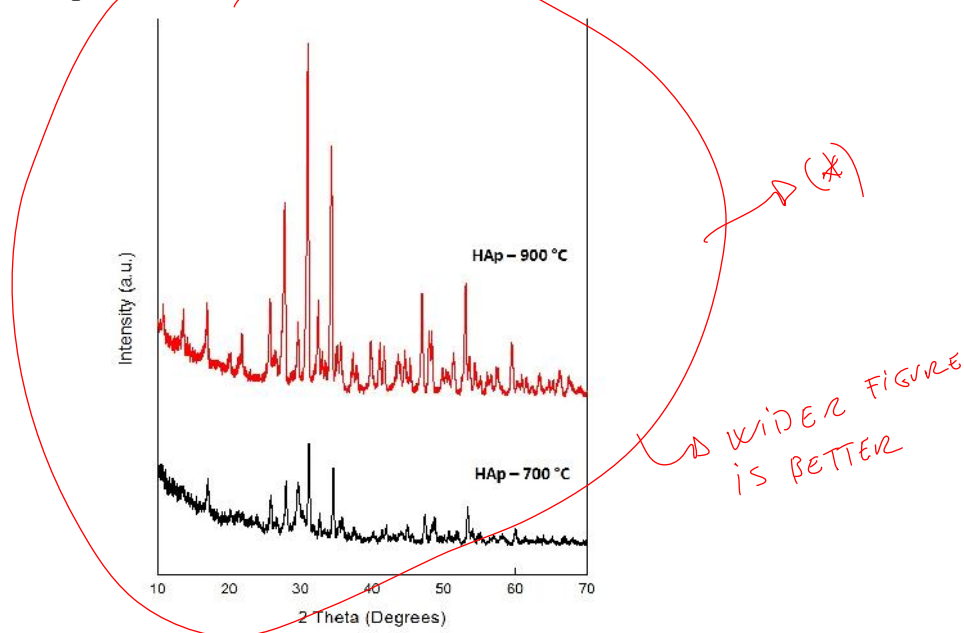


Figure 1: DSC and TGA curves of synthesized pure hydroxyapatite material

nearly all the peaks in sample sintered at 700 °C closely match the standard JCPDF database (70-0364), which indicates that the major crystalline phases are tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ (JCPDS 70-0364). Besides, some extra XRD peaks can be observed in figure 2 that match the standard of HAp $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (JCPDS 72-1243) crystalline apatite phases. Then both hydroxyapatite (HAp) and calcium phosphate crystalline phases could be

189 formed by the effect of the heat treatment process. These phases are considered
190 the essential phases required for bone construction.



191

192 *Figure 2: XRD patterns of heat treated hydroxyapatite samples at*
193 *700°C (Plot A) and at 900 °C (Plot B) for 2 h.*

194 FTIR spectroscopic Analysis

195 FTIR of the as-prepared derived at 100 °C HAp is shown in figure 3a.
196 The figure reveals the amorphous nature of the materials since there are no
197 characteristic sharp peaks in the spectrum. This gel is then dried and fired at
198 temperature ranges from ≈ 500 to 900 °C which helps it to convert slowly to
199 crystalline hydroxyapatite phase with Ca/P ratio ≈ 1.67 [32]. All hydroxyapatite
200 characteristic bands are shown clearly in figures 3(b-f).

201 Figure 3a shows an FTIR spectrum of the material derived at 100 °C. The
202 broad envelope between 2700 and 3700 cm^{-1} is attributed to the OH stretch of
203 $\text{Ca}(\text{OH})_2$, and HAp, and NH stretch of NH_4^+ . The peak at 1763 cm^{-1} is assigned
204 to N=O stretch of NO_3^- . The bending mode of H_2O is observed at 1631 cm^{-1} .
205 The intense peak at 1382 cm^{-1} is assigned to N–O stretch of NO_3^- . The PO_4^{2-}
206 stretching mode is positioned at 1031 cm^{-1} . The other bending modes of PO_4^{2-}
207 are observe at 603 and 566 cm^{-1} . The sharp peak at 825 cm^{-1} is also assigned to
208 NO_3^- bending mode.

209 For sample sintered at 500 to 900 °C, the broad envelope observed in the
210 above spectrum between 2700 and 3700 cm^{-1} indicates the complete loss of

water, dehydration of $\text{Ca}(\text{OH})_2$, and decomposition of NH_4^+ . The peaks in the lower energy region include peaks due to PO_4^{2-} mode at 985, 1027, 1062 and 579 cm^{-1} , and that due to amorphous calcium phosphate at 1165, 1124, 757 and 579 cm^{-1} (common in PO_4^{2-} mode). Further, there is no peak corresponding to NO_3^- in this spectrum. FTIR spectrum of the sample sintered at temperature greater than 1000 $^\circ\text{C}$ does not show any peak corresponding to that of HAp. This further confirms the XRD findings that the HAp is completely transformed to a well crystallized HAp. A similar observation was reported by for the samples calcined at 1000 $^\circ\text{C}$ [33-38]. These assignments were summarized in the following table.

Table 2: Assignments for pure HAp calcined at different temperatures

Assignments	Wavenumber (cm^{-1})
OH stretch and NH stretch of NH_4^+	2700-3700
N=O stretch of NO_3^-	1760 and 830
Bending mode of H_2O	1630
N-O stretch of NO_3^-	1380
$\text{P}_2\text{O}_7^{4-}$ stretching modes	1160, 1120 and 750
PO_4^{3-} stretching modes	1060 and 1030
PO_4^{3-} bending modes	1032, 1060 and 560
Common for $\text{P}_2\text{O}_7^{4-}$ and PO_4^{3-}	580

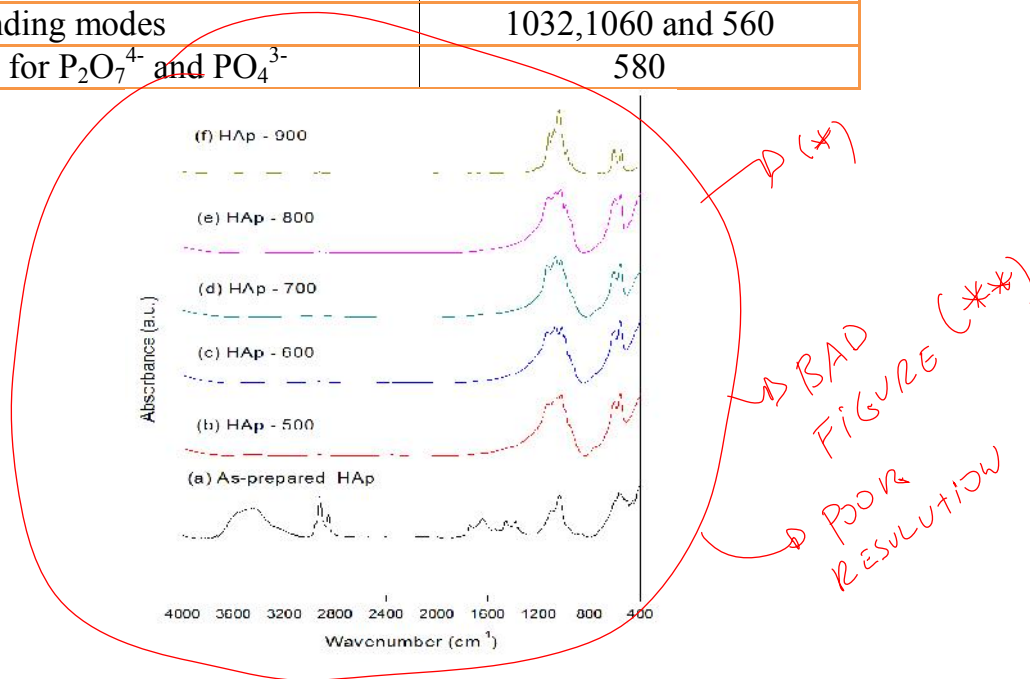


Figure 3: FTIR of pure as-prepared hydroxyapatite (HAp) and heat treated at different temperatures.

The asymmetric stretching (ν_3) mode of PO_4^{3-} ions were detected at 1040, and 1092 cm^{-1} and bending mode (ν_4) at 601, 575 and 561 cm^{-1} [39]. Symmetrical stretching modes of PO_4^{3-} ions ν_1 and ν_2 were also found at around

960 and 470cm^{-1} , respectively [40]. Stretching mode of the hydroxyl group (OH^-) were detected at around 3570 and 1630 cm^{-1} respectively [41].

3.3 FTIR spectra of hydroxyapatite doped with Fe.

Figure 4.1 (b-e) show hydroxyapatites doped with different concentrations of Fe atoms at the expense of calcium atoms with the stoichiometric ratio of $(\text{Ca}+\text{metal})/\text{P}=1.667$. The figure reveals some few structural changes that may be attributed to incorporation of metal ions in hydroxyapatite structure. In Fig. 4.2(b-e), the peak in wavenumber range 500 to 700 cm^{-1} are now merged and becomes a broad peak rather than splitting in pure. There is an increase in the intensity of the peak at 720 cm^{-1} and the envelope in the ranges of 800 to 1300 cm^{-1} to become broader, this may also be attributed to bonding of Fe to HA structure. The sharpness of vibrational peaks at 972 cm^{-1} , 1160 cm^{-1} in hydroxyapatite decreases and the peaks become broader and less intense. The absence of the sharp vibrational peaks at 940cm^{-1} , 1110 cm^{-1} , and 1178 cm^{-1} may also be seen. Additional bands of low intensities are visible at 818 cm^{-1} and 833 cm^{-1} . These bands can be assigned to ν_1 and ν_3 vibrations of Fe_3O_4 groups.

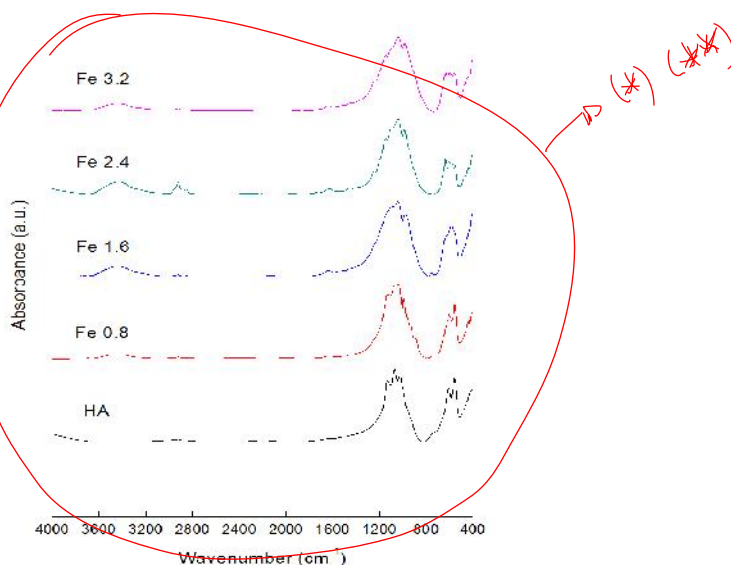


Figure 4.1: FTIR of (a) pure hydroxyapatite HAp and (b-e) Hydroxyapatite doped with concentrations of Fe atoms.

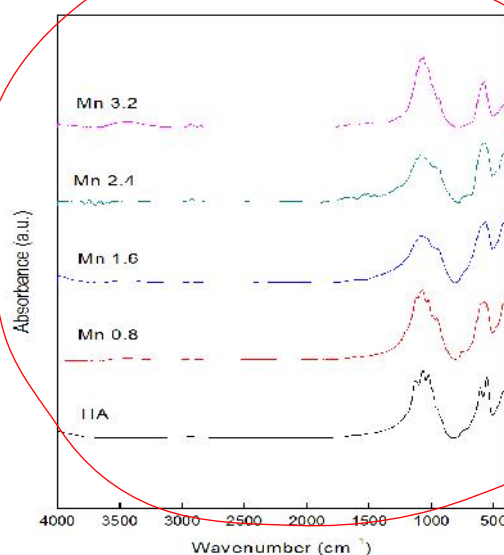


Fig. 4.2 FTIR of (a) pure hydroxyapatite HAp and (b-e) Hydroxyapatite doped with concentrations of Mn atoms.

Clearly the characteristic band positions observed for both of the synthesized samples are in good agreement with that of pure HAp. This observation supported the formation of the expected cation-substituted HAp within the present experimental protocol.

3.4 XRD of the pure hydroxyapatite

Fig. 5 shows XRD for pure HAp. The majority of peaks are indexed to the hexagonal lattice of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ crystal while other peaks are indexed for β -calcium phosphate ($\beta\text{-Ca}_3(\text{PO}_4)_2$). The wide and high peaks reveal that the pure HAp has a very small size (nanoparticles), also excellent crystal quality and no impurities were detected by X-ray. The d-spacing, intensities and lattice parameters for the hexagonal HAp are compared with JCPDS Card (data file No. 72-1243) standard for HAp. The obtained lattice parameters are ($a=9.432$ and $c=6.881$) and the d values of the strongest three lines are 2.81, 2.72 and 2.78.

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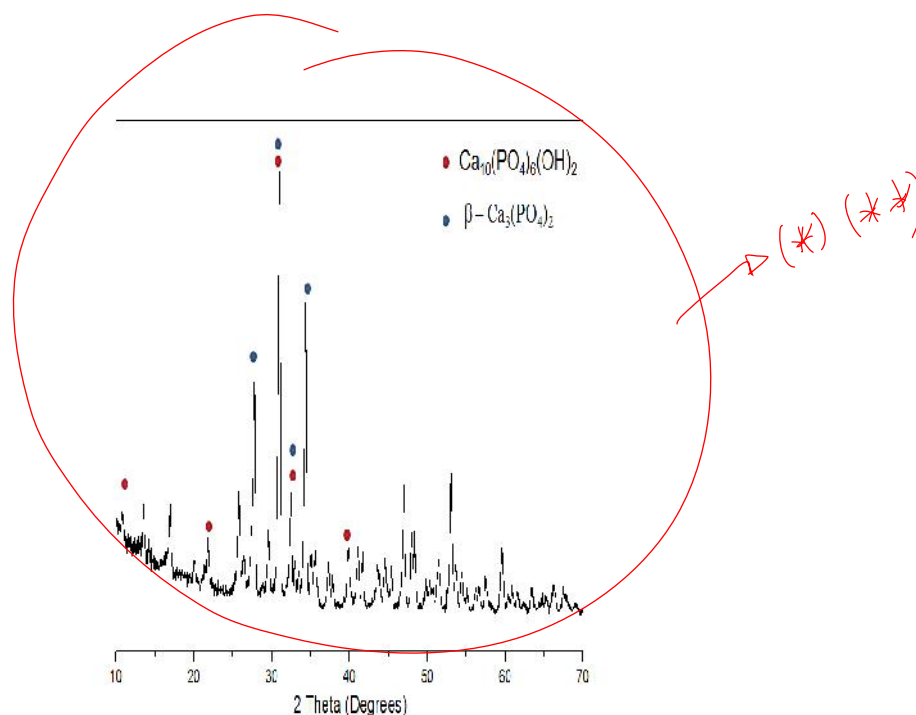


Figure 5: XRD of pure hydroxyapatite.

3.5 XRD of pure HAp and Fe doped hydroxyapatites

Figure 6 shows XRD patterns of all samples HAp and hydroxyapatite doped with concentrations of Fe atoms. The peak positions are not clearly changed, but the peaks intensity changed and thus the degree of crystallization is changed gradually. Clearly a number of prominent peaks for apatite phase were in the XRD pattern. However, the observed intensity and d-spacing values for both the samples are in excellent agreement with the JCPDS standard data for HAp.

The amount of Fe added to the basic hydroxyapatite changes the basic structural unit and lowers the degree of crystallinity by the formation of newly crystalline phases. These phases are magnetite (Fe_3O_4) iron oxide, ICSD file no. 89-0951, cubic with lattice parameters ($a=8.491$) in case of samples (b-e), and calcium iron phosphate $\text{Ca}_9\text{FeH}(\text{PO}_4)_7$ ICSD file no. 89-0513 cubic phase with lattice parameters ($a=10.33\text{\AA}$ and $c=37.125\text{\AA}$).

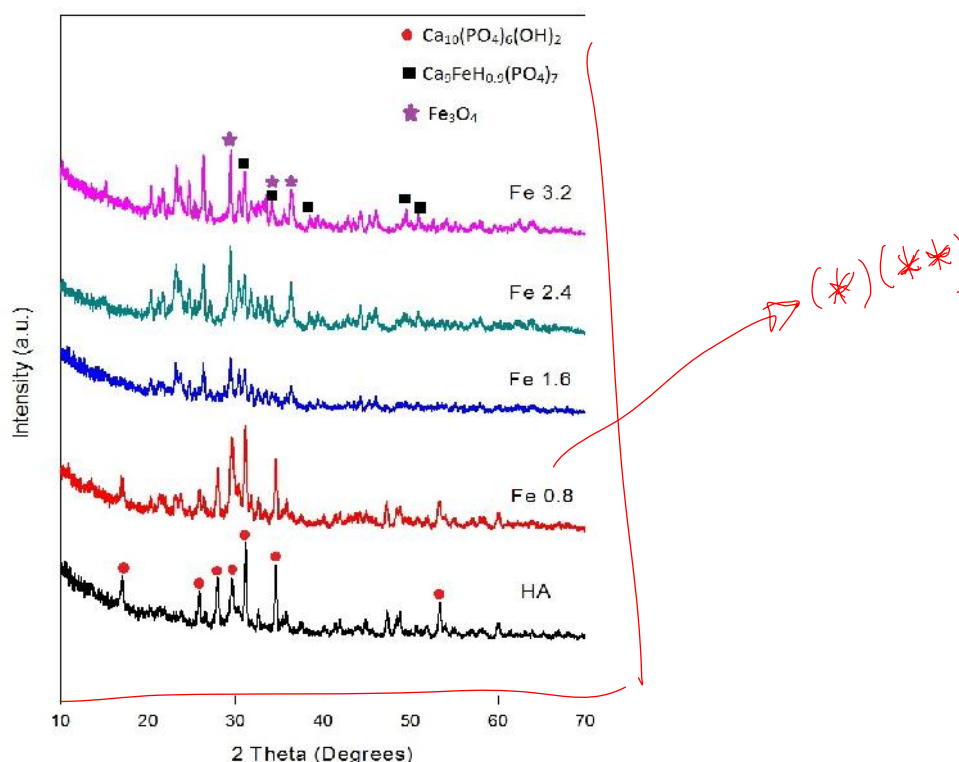


Fig.6. XRD of (a) pure hydroxyapatite HAp and (b-e) Hydroxyapatite doped with concentrations of Fe atoms.

3.6 XRD of pure HAp and Mn doped hydroxyapatites

The XRD spectrum of the Mn-doped apatite [Fig. 7 (b-e)] shows the presence of amorphous phase which supports the observed FTIR data. The reason of this nature is the temperature effect. It is well established that the degree of crystallinity increases with the increase of sintering temperature resulting several distinct peaks.

XRD patterns of all samples HAp, Hydroxyapatite doped with concentrations of Mn atoms showed that peaks present agree well with ICSD file no. 72-1243 which is hexagonal hydroxyapatite and the patterns displayed extra peaks.

The amount of manganese added distorted the structure unit of HAp and lower the degree of crystallinity to some extent and formation of a newly crystalline phases. These phases are manganese oxide (β - Mn_3O_4) ICSD file no. 89-4887, orthorhombic one with lattice parameters ($a=9.55$, $b=9.79$, and $c=3.02$), and manganese phosphate ($\text{Ca}_9\text{MnH}(\text{PO}_4)_7$) ICSD file no. 70-0009.

Also, it can be noted that lines corresponding to crystalline phases of other calcium-phosphate-based compounds are also present.

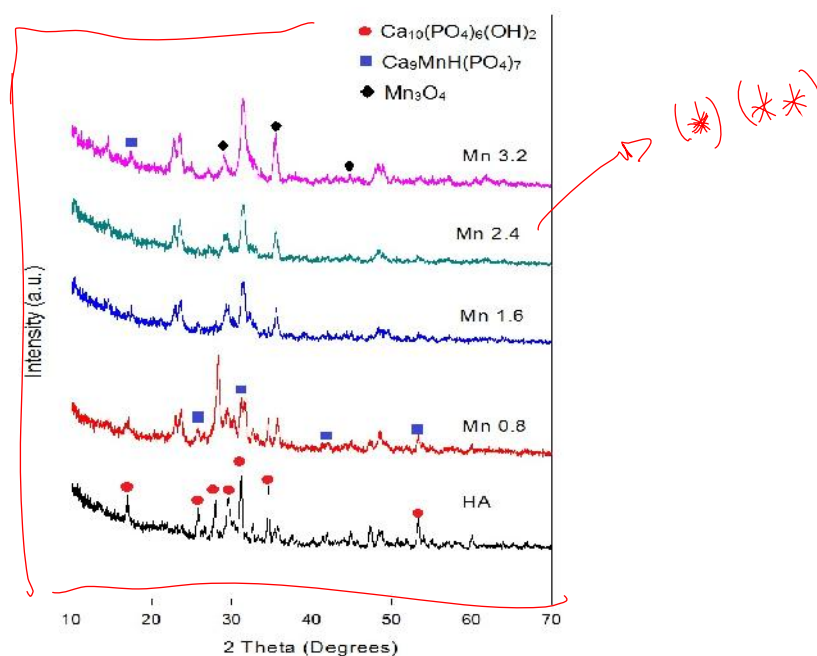


Fig.7. XRD of (a) pure hydroxyapatite HAp and (b-e) Hydroxyapatite doped with concentrations of Mn atoms.

The crystallite size, crystallinity index and lattice parameters of doped and calcined (at 700°C) samples were calculated as described previously. The calculated values are tabulated in Table 3.

The lattice parameters and cell volume values of the Fe-HAp and Mn-HAp did not significantly change as compared to those of pure HAp.

Analysis an increase of the a axis and a decrease of c axis was detected as expected in the case of Ca substitution with ion species having a lower radius. This observation confirmed the formation of Fe-HAp and Mn-HAp substituted apatite of hexagonal structure and conclusively proved that a variety of substitutions of both cationic and anionic is possible in hydroxyapatite structure without any significant modification of its hexagonal system.

Table 3: Crystallite size, degree of crystallinity and lattice parameters for pure HAp and HAp doped with Fe atoms.

Symbol	Crystallite size	Degree of crystallinity	Lattice parameters	
HAp	37.63768	41.42 %	a=9.420 Å	c=6.881 Å
Fe _{0.8} - HAp	41.41286	39.88 %	a=9.392 Å	c=6.870 Å
Fe _{1.6} - HAp	34.46734	46.44 %	a=9.434 Å	c=6.869 Å
Fe _{2.4} - HAp	34.46303	46.86 %	a=9.441 Å	c=6.873 Å
Fe _{3.2} - HAp	31.8164	46.84 %	a=9.443 Å	c=6.872 Å
Mn _{0.8} - HAp	31.85732	48.21 %	a=9.392 Å	c=6.870 Å
Mn _{1.6} - HAp	34.49934	40.03 %	a=9.444 Å	c=6.869 Å
Mn _{2.4} - HAp	(No Peak at 25.8)	38.97 %	a=9.421 Å	c=6.853 Å
Mn _{3.2} - HAp	(No Peak at 25.8)	26.92 %	a=9.423 Å	c=6.862 Å

However for both cases lower values of crystallite size and crystallinity were observed as substitution significantly reduces the crystallite size as well as crystallinity. Possibly, the changes in cell volume for the latter case would be due to the substitution of more cation.

3.7 Electron paramagnetic resonanace

It is well known that the total magnetic field of each ion is established from the spin-spin interaction of neighboring paramagnetic ions [42]. The spin-spin interaction caused by small magnetic fields and this play an important role to alter the total magnetic field of each ion. So, the energy levels of the unpaired electrons to be shifted which led to a variation of energies resulting in a broadened EPR signal.

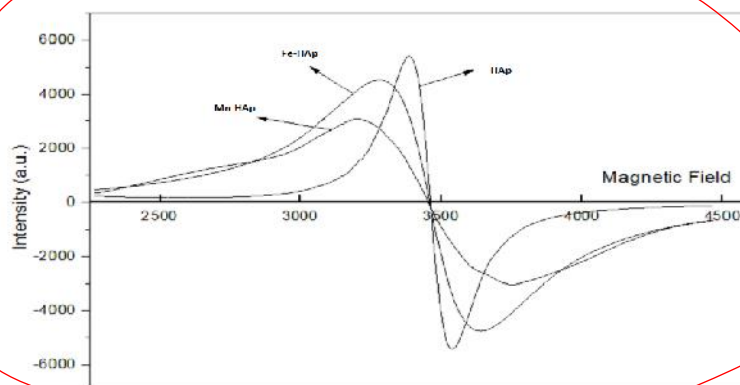


Fig.8. EPR of pure hydroxyapatite, Fe-doped and Mn-doped hydroxyapatites

In Fe-HAp sample, the broad feature in the g of 2.0 regions is attributed to separation of ferric oxide phases with different content in iron cations (Fe^{2+} and Fe^{3+}), and their separation results presence of surface oxyhydroxides and Fe-O-Fe clusters [43]. The presence of high concentration of Fe^{3+} caused electron spin-spin interactions between neighboring Fe^{3+} nuclei which yielded the broad signal. The poor crystallinity with sample Fe-HAp that results from the Fe-phosphates and Fe-oxyhydroxides would be expected to have a high concentration of neighboring Fe^{3+} ions. These agree well with results of XRD and FTIR results. The rhombic crystal field symmetry assigned to Fe-HAp suggested that Fe was occupying the rhombic Ca(2) site and not the axial Ca(1) site. However, charge compensation from Fe^{3+} substituting for Ca^{+2} could cause asymmetry distortion and lead to a rhombic environment in the Ca(1) site.

The number and position of EPR transitions for isolated Fe^{3+} ions in a site of well-defined symmetry observable in a powder spectrum depends sensitively on the local ligand-field symmetry of the sites and possible magnetic interactions between them. In the presence of a rhombic distortion of the ligand-field the g values of the three doublets of the $S = 5/2$ system change as a zero-field splitting parameters depending of its value it becomes possible to observe ESR signal arising from the $\pm 3/2$ and $\pm 5/2$ doublet of the excited states. Iron (III) rhombic crystal field symmetry may indicate substitution into either of the Ca sites in Fe-HAp.

Manganese (IV) may be the cause of the low field X-band hyperfine patterns. Manganese (IV) would have originated from Mn^{+2} oxidation during Mn-HAp synthesis. However, the pink color of the Mn-SHA materials indicated that Mn+2 was the dominant oxidation state in the g-value 2 [44].

The hyperfine lines of the Mn-HAp were barely detectable. Minor spectral differences were observed between the Mn-HAp and Fe-HAp showed a marked decrease in peak intensity relative to standard HAp.

The large decrease of the Mn-HAp g 2.01 peak indicated that nonstructural Mn phase(s) such as poorly crystalline Mn-oxyhydroxide and Mn-phosphate phase(s) associated with the SHA crystallites [45]. Poorly crystalline Mn phase(s) also explain the broad g-value 2.01 peak in Mn-HAp that was superimposed on the hyperfine pattern because Mn^{+2} would be closely associated with one another leading to spin-spin interactions.

The central sharp peak observed in Mn-HAp and was possibly from a small concentration of an inorganic or organic contaminant in the synthesis reagents. The high intensity of the Mn^{+2} peaks Mn-HAp dominated the spectrum and masked any contamination peak. Spin-spin exchange interactions because of Mn and Cu atoms were the cause of an increase of signal broadening [46]. The entire Fe-HAp spectrum was superimposed on the broad signal that suggested the presence of sites with strong spin-spin interactions caused by poorly crystalline Fe-oxyhydroxide or Fe-phosphate phase(s).

The crystalline structure has been determined using X-ray and neutron diffraction, electron diffraction patterns of HA single crystal are not readily obtained. Its single crystal pattern of the (001) zone is especially valuable because it reveals the features of its apatite structure.

Conclusion:

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Nano-sized particles of pure HAp and various Fe and Mn atoms doped HAp powders have been successfully prepared via a wet chemical method. Heating the hydroxyapatite thermally results in increasing the crystallinity of prepared samples. Doping of Fe into HAp powders has greatly influenced the structure in a trend that the crystallinity were decreased with Fe and Mn atoms substitution. Good agreement with FTIR results were obtained where the splitting of band in wavenumber range $500-700\text{ cm}^{-1}$ is decreased up on addition of different metal ions in substitution of Calcium ions. The data obtained from XRD for different powder samples were found to be in a good agreement with that obtained from FTIR. The magnetic properties from ESR results played a vital role to enhance the bioactivity and physiochemical properties of the apatite.

References:

1. Nudelman, F., et al., *The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors*. Nature materials, 2010. 9(12): p. 1004-1009.

- 406 2. Zhou, H. and J. Lee, *Nanoscale hydroxyapatite particles for bone tissue engineering*. Acta
407 Biomaterialia, 2011. **7**(7): p. 2769-2781.
- 408 3. Swetha, M., et al., *Biocomposites containing natural polymers and hydroxyapatite for bone*
409 *tissue engineering*. International journal of biological macromolecules, 2010. **47**(1): p. 1-4.
- 410 4. LeGeros, R.Z., et al., *Fundamentals of hydroxyapatite and related calcium phosphates*.
411 Advanced Biomaterials: Fundamentals, Processing, and Applications, 2010: p. 19-52.
- 412 5. Gergely, G., et al., *Preparation and characterization of hydroxyapatite from eggshell*.
413 Ceramics International, 2010. **36**(2): p. 803-806.
- 414 6. Uskoković, V. and D.P. Uskoković, *Nanosized hydroxyapatite and other calcium phosphates:*
415 *chemistry of formation and application as drug and gene delivery agents*. Journal of
416 Biomedical Materials Research Part B: Applied Biomaterials, 2011. **96**(1): p. 152-191.
- 417 7. Sadat-Shojai, M., et al., *Hydroxyapatite nanorods as novel fillers for improving the properties*
418 *of dental adhesives: synthesis and application*. dental materials, 2010. **26**(5): p. 471-482.
- 419 8. Li, M., et al., *In situ synthesis and biocompatibility of nano hydroxyapatite on pristine and*
420 *chitosan functionalized graphene oxide*. Journal of Materials Chemistry B, 2013. **1**(4): p. 475-
421 484.
- 422 9. Chen, Y., et al., *In vitro biocompatibility and osteoblast differentiation of an injectable*
423 *chitosan/nano-hydroxyapatite/collagen scaffold*. Journal of Nanomaterials, 2012. **2012**: p. 3.
- 424 10. Cheng, L., et al., *Osteoinduction of hydroxyapatite/ β -tricalcium phosphate bioceramics in*
425 *mice with a fractured fibula*. Acta biomaterialia, 2010. **6**(4): p. 1569-1574.
- 426 11. Ripamonti, U., L.C. Roden, and L.F. Renton, *Osteoinductive hydroxyapatite-coated titanium*
427 *implants*. Biomaterials, 2012. **33**(15): p. 3813-3823.
- 428 12. Maxian, S.H., J.P. Zawadsky, and M.G. Dunn, *In vitro evaluation of amorphous calcium*
429 *phosphate and poorly crystallized hydroxyapatite coatings on titanium implants*. Journal of
430 biomedical materials research, 1993. **27**(1): p. 111-117.
- 431 13. Kane, R.J. and R.K. Roeder, *Effects of hydroxyapatite reinforcement on the architecture and*
432 *mechanical properties of freeze-dried collagen scaffolds*. Journal of the mechanical behavior
433 of biomedical materials, 2012. **7**: p. 41-49.
- 434 14. Neuwelt, E.A., et al., *The potential of ferumoxytol nanoparticle magnetic resonance imaging,*
435 *perfusion, and angiography in central nervous system malignancy: a pilot study*.
436 Neurosurgery, 2007. **60**(4): p. 601-612.
- 437 15. Reddy, L.H., et al., *Magnetic nanoparticles: design and characterization, toxicity and*
438 *biocompatibility, pharmaceutical and biomedical applications*. Chemical reviews, 2012.
439 **112**(11): p. 5818-5878.
- 440 16. Ge, F., et al., *Effective removal of heavy metal ions Cd^{2+} , Zn^{2+} , Pb^{2+} , Cu^{2+} from aqueous*
441 *solution by polymer-modified magnetic nanoparticles*. Journal of hazardous materials, 2012.
442 **211**: p. 366-372.
- 443 17. Lu, A.H., E.e.L. Salabas, and F. Schüth, *Magnetic nanoparticles: synthesis, protection,*
444 *functionalization, and application*. Angewandte Chemie International Edition, 2007. **46**(8): p.
445 1222-1244.
- 446 18. Pankhurst, Q.A., et al., *Applications of magnetic nanoparticles in biomedicine*. Journal of
447 physics D: Applied physics, 2003. **36**(13): p. R167.
- 448 19. Wei, G. and P.X. Ma, *Structure and properties of nano-hydroxyapatite/polymer composite*
449 *scaffolds for bone tissue engineering*. Biomaterials, 2004. **25**(19): p. 4749-4757.
- 450 20. Pereira Moreira, M., et al., *Simultaneous Insertion of Mg^{2+} , Sr^{2+} and Mn^{2+} Ions into*
451 *Hydroxyapatite Structure*. Key Engineering Materials, 2012. **493**: p. 20-26.
- 452 21. Mayer, I., et al., *Crystal structure and EPR study of Mn-doped β -tricalcium phosphate*.
453 Materials Research Bulletin, 2008. **43**(2): p. 447-452.
- 454 22. Campoccia, D., L. Montanaro, and C.R. Arciola, *A review of the biomaterials technologies for*
455 *infection-resistant surfaces*. Biomaterials, 2013. **34**(34): p. 8533-8554.

- 456 23. Chen, Q., et al., *Bonding strength of glass-ceramic trabecular-like coatings to ceramic*
457 *substrates for prosthetic applications*. Materials Science and Engineering: C, 2013. **33**(3): p.
458 1530-1538.
- 459 24. Garmendia, N., B. Olalde, and I. Obieta, *16 - Biomedical applications of ceramic*
460 *nanocomposites*, in *Ceramic Nanocomposites*, R. Banerjee and I. Manna, Editors. 2013,
461 Woodhead Publishing. p. 530-547.
- 462 25. Goh, Y.-F., et al., *In vitro study of nano-sized zinc doped bioactive glass*. Materials Chemistry
463 and Physics, 2013. **137**(3): p. 1031-1038.
- 464 26. Jones, J.R., *Review of bioactive glass: From Hench to hybrids*. Acta Biomaterialia, 2013. **9**(1):
465 p. 4457-4486.
- 466 27. *A History of Biomaterials*, in *Biomaterials Science (Third Edition)*, B.D.R.S.H.J.S.E. Lemons,
467 Editor. 2013, Academic Press. p. xli-lilii.
- 468 28. *Chapter 20 - Dental Implants*, in *Phillips' Science of Dental Materials (Twelfth Edition)*,
469 K.J.A.S.R. Rawls, Editor. 2013, Content Repository Only!: St. Louis. p. 499-518.
- 470 29. Bose, S. and A. Bandyopadhyay, *Chapter 1 - Introduction to Biomaterials*, in *Characterization*
471 *of Biomaterials*, A.B. Bose, Editor. 2013, Academic Press: Oxford. p. 1-9.
- 472 30. Liu, X., et al., *Mechanical properties of bioactive glass (13-93) scaffolds fabricated by robotic*
473 *deposition for structural bone repair*. Acta Biomaterialia, 2013. **9**(6): p. 7025-7034.
- 474 31. Liu, Y., J. Lim, and S.-H. Teoh, *Review: Development of clinically relevant scaffolds for*
475 *vascularised bone tissue engineering*. Biotechnology Advances, 2013. **31**(5): p. 688-705.
- 476 32. Shadanbaz, S. and G.J. Dias, *Calcium phosphate coatings on magnesium alloys for biomedical*
477 *applications: a review*. Acta biomaterialia, 2012. **8**(1): p. 20-30.
- 478 33. Bellucci, D., et al., *A new hydroxyapatite-based biocomposite for bone replacement*. Materials
479 Science and Engineering: C, 2013. **33**(3): p. 1091-1101.
- 480 34. Grasso, S., et al., *Low temperature spark plasma sintering of 45S5 Bioglass®*. Journal of Non-
481 Crystalline Solids, 2013. **362**(0): p. 25-29.
- 482 35. Li, H.C., et al., *Effect of various additives on microstructure, mechanical properties, and in*
483 *vitro bioactivity of sodium oxide-calcium oxide-silica-phosphorus pentoxide glass-ceramics*.
484 Journal of Colloid and Interface Science, 2013. **405**(0): p. 296-304.
- 485 36. Scalera, F., et al., *Influence of the calcination temperature on morphological and mechanical*
486 *properties of highly porous hydroxyapatite scaffolds*. Ceramics International, 2013. **39**(5): p.
487 4839-4846.
- 488 37. Sprio, S., et al., *Synthesis and mechanical behavior of 6-tricalcium phosphate/titania*
489 *composites addressed to regeneration of long bone segments*. Journal of the Mechanical
490 Behavior of Biomedical Materials, 2013. **17**(0): p. 1-10.
- 491 38. Wu, Q., et al., *Effects of microwave sintering on the properties of porous hydroxyapatite*
492 *scaffolds*. Ceramics International, 2013. **39**(3): p. 2389-2395.
- 493 39. Chang, C., et al., *Fabrication and properties of chitin/hydroxyapatite hybrid hydrogels as*
494 *scaffold nano-materials*. Carbohydrate polymers, 2013. **91**(1): p. 7-13.
- 495 40. Shen, Y., et al., *Synthesis of strontium substituted hydroxyapatite whiskers used as bioactive*
496 *and mechanical reinforcement material*. Materials Letters, 2012. **70**: p. 76-79.
- 497 41. Rehman, I. and W. Bonfield, *Characterization of hydroxyapatite and carbonated apatite by*
498 *photo acoustic FTIR spectroscopy*. Journal of Materials Science: Materials in Medicine, 1997.
499 **8**(1): p. 1-4.
- 500 42. Desrosiers, M. and D.A. Schauer, *Electron paramagnetic resonance (EPR) biodosimetry*.
501 Nuclear Instruments and Methods in Physics Research Section B: beam interactions with
502 materials and atoms, 2001. **184**(1): p. 219-228.
- 503 43. Jiang, M., et al., *Fe 2+/Fe 3+ substitution in hydroxyapatite: Theory and experiment*. Physical
504 Review B, 2002. **66**(22): p. 224107.

- 505 44. Oliveira, L., A. Rossi, and O. Baffa, *A comparative thermoluminescence and electron spin*
506 *resonance study of synthetic carbonated A-type hydroxyapatite*. Applied Radiation and
507 Isotopes, 2012. **70**(3): p. 533-537.
- 508 45. Ravindranadh, K., et al., *Optical and structural Properties of undoped and Mn²⁺*
509 *doped Ca-Li Hydroxyapatite Nanopowders using Mechanochemical Synthesis*. Journal of
510 Luminescence, 2014.
- 511 46. Chaikina, M., et al., *Mechanochemical Synthesis of Hydroxyapatite and Its Modifications:*
512 *Composition, Structure, and Properties*. Russian Physics Journal, 2014. **56**(10): p. 1176-1182.