Fabrication and characterization of highly crystalline Hydroxyapatite used for bone joining, replacement and reconstruction

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Abstract

This work focuses on studying the effect of sintering temperature ,pressing pressure and sintering time (soaking time) on phases generated, porosity and fracture strength of sintered HA powder compact at a range of (800-1200)˚C , (38.378-153.513)Mpa and (1-5)hr respectively, and evaluation of sintered HA powder compact in synthetic body fluids(SBF), to indicate the biological bioactivity . Both Ca/P ratio and crystallinity were increased after calcination, where the Ca/P ratio raised from 1.7 to 1.9 and the height of Hydroxyapatite peak intensity was also increased .An important Secondary phases were appeared especially the $Ca_2P_2O_7$ phase. X-ray diffraction patterns and electrical microscopic pictures of polished surfaces of the Hydroxyapatite compact after sintering had revealed the process of densification and crystallization of Hydroxyapatite . The increase of temperature leads to grain growth ,while surface cracking and other defects became lower i.e. porosity and surface voids .The maximum content of Hydroxyapatite and $Ca_2P_2O_7$ were found seen at pressing pressure of (76.756)Mpa. Fracture strength was reached its maximum amount at(1200) ºC, (115.134)Mpa and after (3)hrs of soaking time.Radiographic X-ray pictures ,XRD test and electron microscopy photographs of sintered samples before and after incubation in vitro shows an alteration in sample morphology i.e. (surface erosion) and a decrease in sample diameter and the utilization of β-$Ca_2P_2O_7$ indicates the chemical reactivity of prepared Hydroxyapatite powder compact .

Key words: Bioceramics, preparation of Hydroxyapatite, Bioactive Hydroxyapatite, Reinforcement of Hydroxyapatite

1.Introduction

The biomaterials industry has witnessed enormous growth rates in both materials research and clinical applications. Common orthopedic surgeries now demand a large quantity of bioactive bone substitutes with a variety of osteogenic responses. Many ceramics and glasses are bioactive. In particular , in the calcium phosphate system , Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] and tricalcium phosphate (TCP) [$Ca_{10}(PO_4)_2$] are excellent bioactive materials suitable for hard tissue prosthetics[1]. Hydroxyapatite is chemically similar to the mineral component of bones and hard tissues in mammals. It is one of few minerals that are classed as bioactive, meaning that it will support bone ingrowth and osteointegration when used in orthopedic, dental and maxillofacial applications[2]. Synthetic Hydroxyapatite is frequently used as reference material in biomineralization and biomaterials studies. The composition, physiological properties, crystal size and morphology of synthetic apatite are extremely sensitive to preparative conditions[3]. This work focuses on studying the effect of sintering temperature ,pressing pressure and sintering time (soaking time) on phases generated, porosity and fracture strength of sintered HA powder compact on biological property in vitro implantation.

2.Experimental Work

2.1 Preparation of HA powder :

A suspension of (27)gm of $Ca(OH)_2$ was dissolved in 1000ml of distilled water and vigorously stirred , then a solution of (24.6)gm of H_3PO_4 was diluted in 1000ml of distilled water was slowly added as dropwise over 1 to 2hrs under a condition of pH=8 to produce a gelatinous precipitate. The reaction mixture was aged at room temperature for a week. The resulting slurry was cleared by removing floated liquid and then washed with distilled water followed by clearing from floating liquid and then washed with ethanol and the floated liquid was also cleared. The resulting slurry was dried at 80ºC in an oven over night; the resulting material was re-milled and calcined at 800ºC for 3hrs. The finely ground powder was mixed with 2% PVA and 250ml warm distilled water and mixed for 15 mins, and then dried in an oven at 80ºC and then the re-milled powder was sieved to(<106μm) [4,5].

2.2 Preparation of HA samples

Pressing was done by a hydraulic pressing machine with capacity of (384.31)Mpa in a tool steel die of (2.55)cm in diameter with pressing pressure of (38.378,76.756,115.134,153.135) Mpa. Sintering of samples were done at different temperatures of (800, 900,1000,1100,1200)ºC and at different soaking times (1,2,3,4,5)hrs. with a heating rate of 5ºC/min[6].

2.3 Inspection of sintered samples

2.3.1 Powder evaluation

X-ray diffraction inspection was performed by using Philips diffractmeter pw 1840 with a Cu Kα radiator tube and Ni filter and the diffraction angle (2Θ) was between (20-60) º. Ca/P ratio of the HA powder was evaluated before and after calcinations by using atomic absorption. Electron microscopic examination was used to evaluate the particle shape .Figure (1) shows the particle shape of HA powder which obviously seems irregular and semicircular with a wide distribution .And Particle size distribution evaluation was carried out in a laser diffraction particle size analyzer named “SHIMADZU SALD-2101”, and results revealed the mean value of diameter was (87.821)μm and the median value was (123.483)μm. Apparent and real densities were measured by the conventional methods i.e. cup cone and pecnometer respectively.

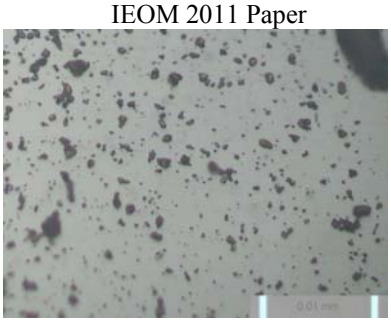


Figure (1) shows the particle shape of fabricated HA powder at a magnification of (X270)

2.3.2Physical properties of sintered samples :

Open porosities of sintered HA compact powder was measured, using the standard Archimede's method [7].

Fracture strength test was carried out by using the diameterial compression disc test (Brazilian test) where the disc was placed between two surfaces and a load was applied in the rate of (0.5)mm/min , and the force obtained at fracture was recorded , The following equation was applied to calculate the fracture strength of material :-

$$\sigma_f = \frac{2p}{\pi dt} \qquad (1)$$

σ_f :fracture strength (N/mm^2).
P : applied load (N).
d :sample diameter (mm).
t :sample thickness (mm).

2.4 Biocompatibility test :

2.4.1 Preparation of synthetic body fluid (SBF):

SBF is known to be a metastable buffer solution, and even a small, undesired variance in both of the preparation steps and the storage temperatures may drastically affect the phase purity and high-temperature stability of the prepared HA, as well as the kinetics of the precipitation processes.Merck(Darmstadt,Germany)grade,NaCl(99.5%),$NaHCO_3$(99.5%),KCl(99.0%),$Na_2HPO_4.2H_2O$(99.5%),$MgCl_2.6H_2O$(98%),Na_2SO_4,($CH_2OH)_3CNH_2$(99.2%),$CaCl_2.2H_2O$(99%) and HCl(37vol%,Carlo-Erba,Italy) were used in the preparation of the synthetic body fluids .SBF solutions were prepared by dissolving appropriate quantities of the above chemicals in de-ionized water . Reagents were added, one by one after each one was completely dissolved in 700ml of water, in the order given in table (1). A total of 40ml of 1M HCl solution was consumed for pH adjustments during the preparation of SBF solutions .The 15ml aliquot of this amount was added just before the addition of the 6^{th} reagent, i.e., $CaCl_2.2H_2O$. The second portion of the HCl solution was used in the reminder of the titration process. Following the addition of the 8^{th} reagent (tris(hydroxymethyl)aminomethane), this solution was then titrated with 1M HCl to the pH of 7.4 .During the titration process ,the solution was also continuously diluted with consecutive additions of de-ionized water to make the final volume equal to 1 [8] .

Table (1) explain the chemical composition of SBF solutions[8]

Order	Reagent	Amount (gpl)
1	NaCl	6.547
2	$NaHCO_3$	2.268
3	KCl	0.373
4	$Na_2HPO_4.2H_2O$	0.178
5	$MgCl_2.6H_2O$	0.305
6	$CaCl_2.2H_2O$	0.368
7	Na_2SO_4	0.071
8	$(CH_2OH)_3CNH_2$	6.057

2.4.2 Test method :-

The implanted sample was washed in pure ethanol ,rinsed with distilled water and air-dried before the immersion in SBF. The selected sample was immersed in SBF for a month .XRD photography was taken for each sample at the time of immersing every (10) days for a month to see the changes on each sample . XRD test and electron microscopy photographs of sintered samples before and after incubation in vitro was taken to show the alteration in sample morphology

3.Resullts and discussion

3.1 The role of drying and calcination on fabricated hydroxyapatite powder:

Both Ca/P ratio and crystallinity were increased after calcination, where the Ca/P ratio was increased from 1.7 to 1.9 and the height of (HA) peak intensity was also increased. Secondary phases were appeared i.e.(β-$Ca_3(PO_4)_2$,$Ca_2P_2O_7$,β-$Ca_2P_2O_7$,$Ca_4P_2O_9$) , according to the X-ray diffraction patterns as explained in appendix (1a,b).The existence of secondary phases such as $Ca_2P_2O_7$ and $Ca_3(PO_4)_2$ associated with (HA) will

enhance the biological activity of (HA) .The increase in Ca/P ratio is due to the appropriate pH and calcination temperature which leads to more easily increase of Ca/P ratio up to about 1.9 .Such increases in Ca/P ratio may be related to ease of release of $(PO_4)^{3-}$ ions from the solid phase to the liquid phase at high pH (8) and high temperature of calcination (800) ºc [9] .

3.2 X-ray diffraction observation:

HA phase was the major phase at almost X-ray patterns reflections, also $Ca_2P_2O_7$ is observed at many X-ray diffraction patterns. Other secondary phases indicated were α-$Ca_2P_2O_7$, β-$Ca_2P_2O_7$, γ-$Ca_2P_2O_7$, δ- CaP_2O_6,$Ca(PO_3)_2$,$Ca_3(PO_4)_2$,β-(CaP_2O_6) and $Ca_4P_2O_9$. It can be seen in appendix (2 a-e) that intensity peak heights of HA vary with the increase of temperature , and it reaches the maximum height at 900ºc , then decreases at 1000ºc to increase again at 1100ºc and decreases again at 1200ºc. The decrease of the peak heights is because of the decomposition and the formation of different phases .The increase of temperature leads to grain growth at which the maximum grain size can be observed at 1200ºc. X-ray diffraction patterns in appendix (3a-d) revealed that with increasing pressing pressure, the peak heights of (HA) is increased, then it decreases after pressure amount of (76.756)Mpa. Depending on heights of peak intensities of X-ray diffractions it is obvious that the maximum content of (HA) and $Ca_2P_2O_7$ is observed at a pressing pressure of (76.756)Mpa , then it decreases with further increase in pressure amount .This may be attributed to the fact that , with increasing pressing pressure ,the particles will become much closer ,bridging during sintering will, therefore, be easier [10].While much increase in pressing pressure might decrease the permeability between particles and cause gas capturing which leads to blowing and distorting the bridges between particles at the earliest stages of sintering which perhaps affect the formation and distribution of the generated phases[10]. Also results had revealed that peak heights of X-ray diffraction patterns in appendix (4a-e) of (HA) is varying with increasing the time of sintering (soaking time), at which the maximum peak height was at a soaking time of (1)hr .After (3)hrs both peak heights and crystallization decrease to increase again after (4hrs) and more secondary phases were formed. After (5)hrs peak heights of (HA) and crystallization decrease again. The decrease in crystallization of (HA) may be related to the formation of α-$Ca_2P_2O_7$ which is clearly indicated by X-ray patterns after (3)hrs and (5)hrs of soaking.

3.3 Effect of sintering temperature, pressing pressure and soaking time on porosity and fracture strength:-

Porosity is decreased with increasing temperature as shown in table (2) due to the reduction in pore volume and the total volume of the sintered compact powder[11], while the (OH) as a vapor phase released from (HA) could represent the major reason that caused the remaining of micro pores.The maximum porosity was 47.058% at 900ºc and the minimum porosity was 12.57% at 1200ºc.

Table (2) the values of porosity and fracture strength at different sintering temperatures.

No.	tering temperature ˚C	Porosity %	acture strength N/mm^2
1	800	47.058	2.28
2	900	49.645	1.37
3	1000	44.217	1.15
4	1100	40.839	3.817
5	1200	12.57	6.57

At low temperatures fracture strength is relatively high as temperature increases the fracture strength decreases and it reaches its minimum amount (1.15)N/mm^2 at (1000)◦c as shown in table (2) i.e. fracture at this regime is brittle and occurs by extension of inherent flaws[11], at all intermediate temperature regime , the strength falls because the formation of low strength phases (β-$Ca_2P_2O_7$,γ-$Ca_2P_2O_7$ and $Ca_3(PO_4)_2$), and the fracture occurs from flaws now produced by limited plastic flow[11]. At high temperatures regime where the strength raises again with temperature increase until it reaches its maximum amount (6.57)N/mm^2 at 1200ºc due to the increase in mechanical bonding[12] and fracture at this regime is accompanied by extensive plastic deformation[3]. Porosity is decreased with increasing pressing pressure ,the maximum porosities was (17.766)% at pressing pressure of (38.378)Mpa , and the minimum porosities was (4.022)% at pressing pressure of (153.315)Mpa , and as shown in table (3),that decrease in porosities value is due to the increase in densification and packing between particles.

Table (3) the values of porosity and fracture strength at different pressing pressure.

No.	essing pressure Mpa	Porosity %	acture strength N/mm^2
1	38.378	17.766	3.46
2	76.756	9.58	10.08
3	115.134	7.096	17.13
4	153.315	4.022	23.34

Fracture strength is increased with increasing the pressing pressure, which may be related to the suitable inter-particles distance which provides a good bridging during the sintering process. Also micro-cracks interaction with the residual strain field around line tension creates a process zone ahead of the crack tip[10] causing an increase in fracture strength. The maximum fracture strength was (23.43) N/mm^2 at pressing pressure of (153.315)Mpa ,and the minimum fracture strength was(3.46)N/mm^2 at pressing pressure of (38.37)Mpa as shown table (3). Open porosity was varying with soaking time as shown in table (4).

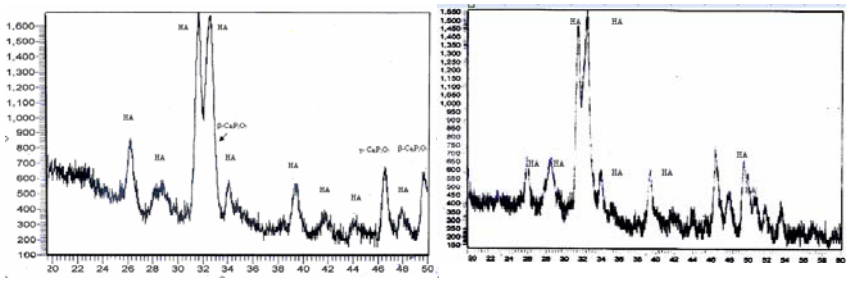
Table (4) the values of porosity and fracture strength at different sintering time (soaking time).

No.	soaking time hrs	Porosity %	acture strength N/mm^2
1	1	11.17	12.28
2	2	19.895	12.228
3	3	9.146	15.526
4	4	17.368	3.105
5	5	5.389	12.07

Porosity was increased as the time of soaking increases after (2)hrs. This may be due to atoms which bond to each other forming small necks during sintering .So this kind of pores could be seen during necking and before densification at which it reaches its maximum amount. After (2)hrs of soaking ,the decrease in open porosity may be related to the good densification .It may also be due to the grain boundary mechanism of sintering which happens at the end of sintering at which atoms move on grain boundary , trying to fill up the residual holes (pores) , increasing the densification and decreasing the porosity[10]. Porosity then start increasing after (3)hrs of soaking due to the disappearance of ($Ca_2P_2O_7$,Dx=3.09) that results in decreasing densification , while the appearance of this phase ($Ca_2P_2O_7$) again at (5)hrs results in the increase of density and the decrease in both porosity. The maximum open porosity was (19.895)% after (2hrs), and the minimum open porosity was (5.389)% after (5)hrs as shown in table (4). Fracture strength increases with the increase of soaking time and because of the formation of strengthening phases $Ca_2P_2O_7$ and α-$Ca_2P_2O_7$, except after (3)hrs of soaking where the formation of β-(CaP_2O_6) results in a sharp decrease of fracture strength, followed by a second increase at (5)hrs of soaking time. The maximum fracture strength was (15.526)N/mm^2 after (3)hrs and the minimum fracture strength was (3.105) N/mm^2 at(4hrs) as shown in table (4).

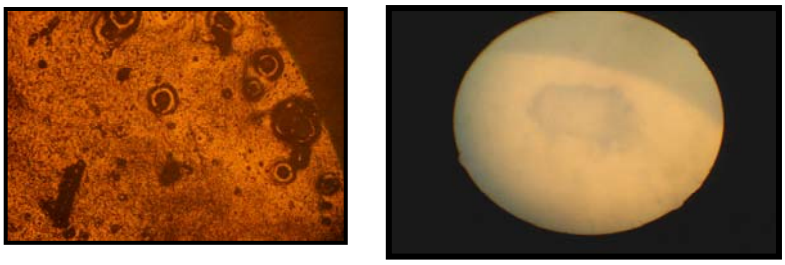
3.4 Biological evaluation:

Sintered Hydroxapatite (HA) powder compact was incubated for 30 days in a simulated body fluid (SBF) .Upon incubation in vitro in (SBF), an apatite layer forms on the surface which is considered essential for the nucleation of biological apatite ,the promotion of protein adsorption and cell adhesion , and ultimately ,the creation of a strong bond with the surrounding tissue. An x-ray diffraction (XRD) test and optical microscopic observation were done for the incubated sintered sample at sintering conditions of (1100˚C, 3hrs, 76Mpa).The XRD in fig. (2b) reveals the absence of β-$Ca_2P_2O_7$ phase compared to the original XRD patterns of the same sample before incubation in fig.(2a).This may be related to the faster release of Ca^{+3} ions than from HA phase to the (SBF) environment. The peaks of HA phase had also changed and became less in height in some positions. This may related for two reasons , first ;because of Ca^{+3} ion release to the surrounded (SBF) environment , at which the calcium amount was 37.3% and phosphorous was 19.6% and after incubation, these amounts became 13.6% and 3.6% respectively , second ; the formation and precipitation of a new HA phase on the surface of the incubated sample , generated from the surrounding environment of (SBF). An optical microscopy photograph of the sintered sample before and after incubation in fig. (3a, b) shows a direct visualized erosion of the incubated sample. It can be seen from the radiographic x-ray pictures in figure (4) that sample colors fainted and the diameter decreased about (0.15) mm. It can be said that there was a change in the topography of the incubated sample , which may be related first, to the general erosion because of Ca^{+3} ion release and second a precipitation of a large , irregular, smooth HA on surface from in vitro (SBF) environment incubation on to dense , polycrystalline HA[13].



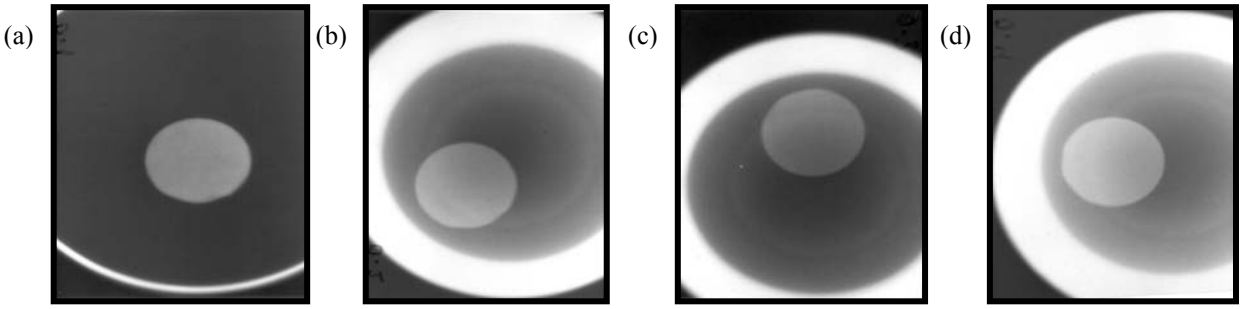
(a) (b)

Figure (2) shows the XRD diffraction patterns of the implanted sample (a) before incubation in SBF (b) after



(a) (b)

Figure (3) shows the optical microscopy photographs of the sintered sample **(a)** before incubation in SBF **(b)**after incubation in SBF.



(a) (b) (c) (d)

Figure (4) shows the radiographic pictures of the implanted samples during the period of incubation .(a) 1st day of incubation , (b) After 10 days of incubation(c) After 20 days of incubation , (d) After 30 days of incubation .

4.Conclusions:-

- At 1000◦c, a critical point exists in fracture strength due to the formation of many phases.
- 1200◦c, is preferred to get high fracture strength, where the fracture strength was (6.57) N/mm^2.
- A pressing pressure of (153.513)Mpa is preferred to get high fracture strength where it reaches (23.34)N/mm^2 .
- At soaking time of (3)hrs and (4)hrs, a change in porosity and fracture strength occurs due to the change in the formed phases.
- Soaking time of (3)hrs, is preferred to get high fracture strength where it reaches (15.526) N/mm^2.

Acknowledgment

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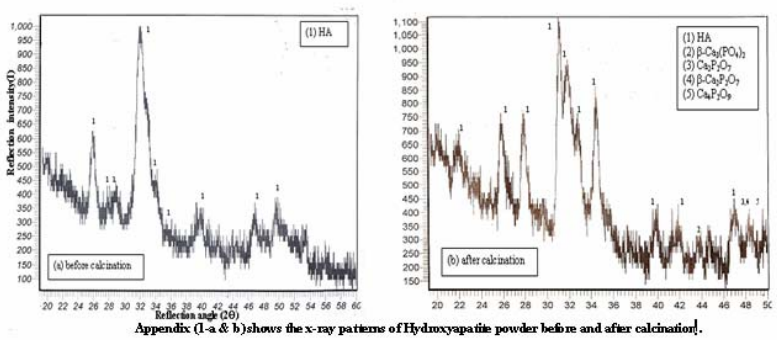
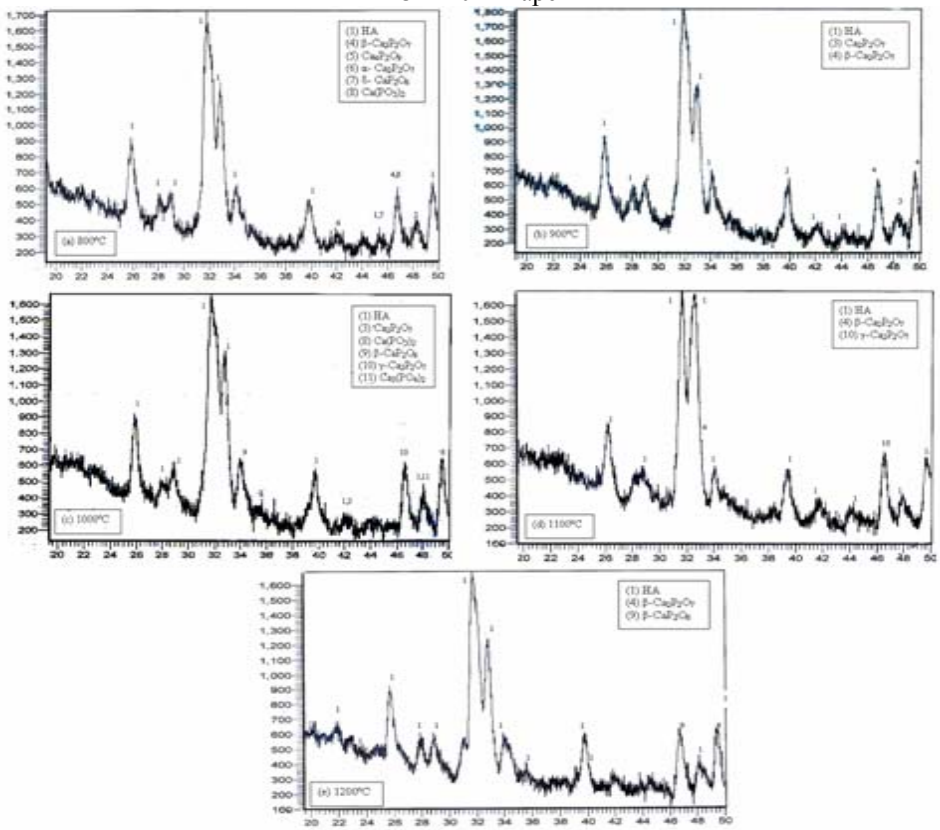
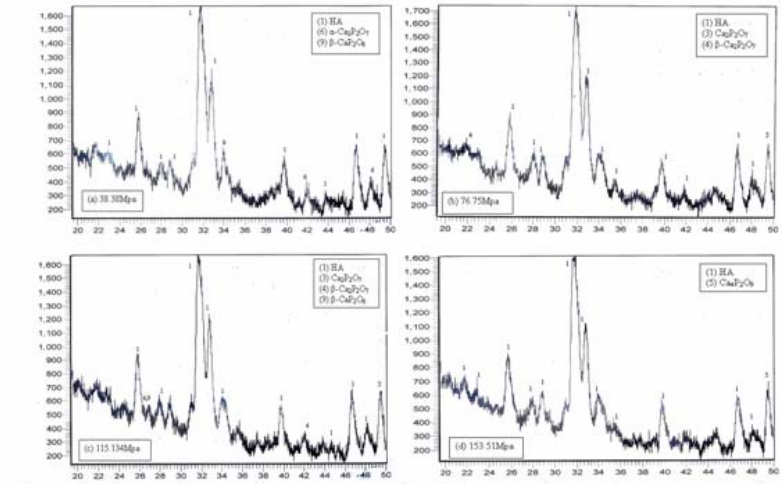


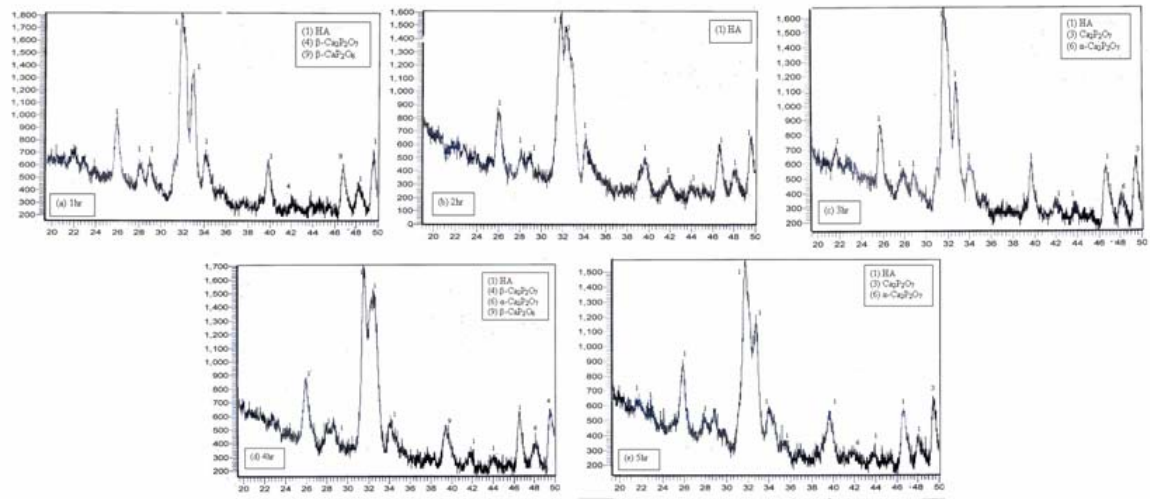
Appendix (1-a & b)shows the x-ray patterns of Hydroxyapatite powder before and after calcination.



Appendix (2-a to e) shows x-ray patterns for Hydroxyapatite powder compact samples at temperature range (800-1200)° C .



Appendix (3-a to d) shows the x-ray patterns for Hydroxyapatite powder compact samples at pressing pressure range (38.38-153.5)Mpa .



Appendix (4-a to e)shows the x-ray patterns of Hydroxyapatite powder compact samples at sintering time (soaking time) range (1-5)hr .