Chemical Characteristics of Hydroxyapatite from Oyster Shell by Thermo-Chemical Process

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ABSTRACT: Oyster shell is basically made of calcium carbonate. An attempt is made to convert calcium carbonate of oyster shell to hydroxyapatite (HA), a substitute material for bone and teeth in orthopedic and dentistry field due to their chemical and biological similarity to human hard tissue. In the present work, hydroxyapatite (HA) was successfully synthesized by wet precipitation method using Ca $(NO_3)_2$, and $(NH_4)_2HPO_4$, and NH_4OH as starting materials. The oyster shell was found to decompose within 1000^0C to all the carbonate phases. The calcined oyster shells were then treated with acids followed by different chemicals in ammoniacal media maintaining proper stoichiometry to produce fine hydroxyapatite (HA) as filter cake with a Ca/P molar ratio of 1.67. The dried HA powder was extremely pure. Different characterization techniques were adopted both for calcined oyster shell and HA by scanning electron microscopy (SEM), X-ray diffractometer (XRD), fourier transform infrared spectroscopy (FTIR) and thermo gravimetric analyzer (TGA). The absorption bands corresponding to phosphate and hydroxyl functional group which are characteristics of hydroxyapatite were confirmed by FTIR. Besides its effectiveness in bone substitution, the prepared HA holds great potential in the field of dental application.

KEYWORDS: Oyster shell; Hydroxyapatite; Wet precipitation; Sintering; Calcination.

I. INTRODUCTION

Materials which are used for the repair and reconstruction of diseased or damaged parts of the musco-skeletal system are defined as biomaterials such as hydroxyapatite (HA), bioglass, biopolymer, and Ca-phosphates etc (Burg *et al.,* 2000). A lot of research has been carried out to find out the substitution to support the bone in the medical field. Presently steel as support to bones is used but it can react with body fluids [T = 37^0C and pH = 7.4] when kept for many years, so it requires substitute. Next comes to the inert materials like bioglass but due to formation of fibrous tissue of variable thickness they can lead to tumor. Now-a-days ceramics specially the bio ceramics are the better alternatives since they have high corrosion resistance (Urist and Johnson, 1941), better compressive strength, relatively low density and low weight. Porous bio-active ceramics such as hydroxyapatite, Ca-phosphates are attractive for bone regeneration and reconstruction due to their bone bonding ability and good growth property (Sevitt, 1981). Among them hydroxyapatite is taken as the best alternative as it contains same chemical nature and similar crystallographic structure with bone excellent biocompatibility (McKibbin *et al.,* 1970).

Hydroxyapatite (HA) $Ca_{10} (PO_4)_6(OH)_2$ is a synthetic biomaterial. Due to its chemical and structural similarity with the mineral phase of bone and teeth, HA is widely used for hard tissues repair. As a result, this inorganic phosphate has been studied extensively for medical applications in the form of powders, composites or even coatings (Weng *et al.,* 2000; Cheng *et al.,* 2003; Riman *et al.,* 2002; Huang *et al.,* 2000; Weng *et al.,* 2003; Weng *et al.,* 2002; Choi *et al.,* 2004; Weng and Baptista, 1998; Cheng *et al.,* 2003; Cheng *et al.,* 2001; Weng *et al.,* 2002). It is also observed that dense sintered HA has many bone replacement applications and is used for repairing bone defects in dental and orthopedic sites, immediate tooth replacement, augmentation of alveolar ridges, pulp capping material and maxillo facial reconstruction, etc (Shiny *et al.,* 2000). For substituting or repairing the bone, the designed material must has the ability to create a bond with the host living bone (Kokubo *et al.,* 2003). Hence, it is always desirable to include a high degree of crystallinity and chemical stability among the desirable properties of an ideal hydroxyapatite (Jha *et al.,* 1997; Tsui *et al.,* 1998).

Furthermore, HA has also been studied for other non-medical applications, for example, as packing media for column chromatography, gas sensors, catalysts, etc. (Cheng *et al.,* 2003; Weng and Baptista, 1997). However low strength and toughness restrict monolithic HA applications to those that require little or no load-bearing parts (Liu *et al.,* 2001). Due to its diverse applications, the materials properties accordingly need to be tailored for real world application. Hence researchers have tried to customize its properties such as bioactivity, mechanical strength, solubility and sinterability by controlling its composition, morphology and particle size (Cheng *et al.,* 2003; Cheng *et al.,* 2001).

Natural bioceramics such as skeletons of marine organisms like corals, sea urchins, oyster shells, etc. have been used to prepare HA powder (Lemos *et al.,* 2006). Among them oyster shells are abundant in Bangladesh. Most of the oyster shells are discarded with no further use once the flesh is stripped off; except that a small amount is used for art creation. As a result, oyster shell piles are common in areas of oyster production with no further utilization.

Composition, structure, size, shape and morphology of hydroxyapatite are determined by the method of preparation. Several methods have been reported for the synthesis of HA include precipitation technique (Santos *et al.,* 2004; Manuel *et al.,* 2002; Manuel *et al.,* 2003), sol-gel approach (Chai and Ben-Nissan, 1999), hydrothermal technique (Manafi and Joughehdoust, 2009), multiple emulsion technique (Kimura, 2007), biomimetic deposition technique (Tas, 2000; Thamaraiselvi *et al.,* 2006), electrodeposition technique (Shikhanzadeh, 1998) etc.

The most popular and widely researched technique for synthesis of HA is precipitation technique. This technique is also called as wet precipitation or chemical precipitation or aqueous precipitation. This technique is chosen widely to synthesize HA in contrast to other techniques. Because, relatively large amount of HA can be produced by precipitation technique in absence of organic solvents at a reasonable cost (Santos *et al.,* 2004).

In the present work, hydroxyapatite (HA) was successfully synthesized from oyster shell by wet precipitation method using Ca $(NO_3)_2$, $(NH_4)_2HPO_4$, and NH_4OH as starting materials. The oyster shell was found to decompose within 1000^0C to all the carbonate phases. The calcined oyster shells were then treated with acids followed by different chemicals in ammoniacal media maintaining proper stoichiometry to produce fine hydroxyapatite (HA) as filter cake with a Ca/P molar ratio of 1.67. The dried HA powder was extremely pure. The final product is characterized by X-ray diffraction, scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR) and thermo gravimetric analysis (TGA).

II. MATERIALS AND METHODS

Materials
Specimens of oyster shells were collected from Saint Martin (island) in Chittagong division, Bangladesh. Shells were washed with tap water and rinsed in distilled water to remove the mud, sand and other impurities. The cleaned shells were dried in direct sunlight for 2 days. The cleaned and dried oyster shells were ground in mortar and then sieved.

Synthesis of hydroxyapatite
Dry and cleaned oyster shell was calcined in an electrical muffle furnace (KSL 1100X) at $800^°C$ so that all organic matters and proteins escaped out. Oyster shell was transformed into calcium oxide by releasing carbon 1000^0C at 4 hours. CaO obtained from oyster shell was then converted into calcium nitrate $[Ca(NO_3)_2]$ in concentrated nitric acid under constant stirring. To synthesize HA powder 0.5 M $(NH_4)_2HPO_4$ solution was prepared and added with continuous stirring to a solution of 0.5 M $Ca(NO_3)_2.4H_2O$ to maintain 1.67 Ca/P ratio and vigorously stirred for 30 minutes. At the same time pH was observed and then adjusted 10.5 by adding drop wise 1 M ammonium hydroxide $[NH_4OH]$ solution. The suspension was well stirred (1000 rpm) using magnetic stirrer for 2 hour and aged for overnight with ice bath. A white precipitate was formed. The precipitates were subjected vacuum filtrating using buchner funnel, repeatedly washed to obtain pH 7 with deionized water and filtered again. The precipitates were dried at 80^0C for 48 hours. Dried lumps of powders were ground by clean pestles and mortars.

Preparation of HA pellet
HA powder and 4% Poly Vinyle Alcohol as a binder were mixed homogeneously by using mortar pestle for half an hour. It was then dried at 100°C for 24 hour in a conventional drier. After drying HA powder was again pestled for the purpose of homogeneity.

The pellets were prepared using a universal testing machine (UTM) (FS - 300kN, Testometric, England) at 25°C. The cylindrical die was used which made of carbon steel and had an opening of 10 mm in diameter. The die was rinsed with acetone and wiped clean using tissue paper before each use. HA powder was loaded in amounts 0.8 gm into the die and then compressed at a rate of 2 mm/min until a desired pelletizing pressure was reached. The pressure was released after 10 second and the piston was removed. The pellets were removed from the die by removal of the backstop and pushed out by applying pressure on the pellet by the same arrangement used for pelletization at a compression rate of 2 mm/min. The pelletizing pressure used in this work was 16 kN.

Sintering of prepared pellet

The prepared tablets were dried for 24 hours. Thus, in one batch at least 4 samples were placed in the alumina

furnace plate. Sintering of the tablet samples was performed at various temperatures and times into the pre programmed furnace. At various temperatures with constant time i. e at isochronous condition tablet samples were sintered at $935°C$, $985°C$ and $1035°C$ for 2 hour. At various times with constant temperature i.e at isothermal condition tablet samples were sintered for 1 hour, 2 hour and 5 hour at $985°C$. The heating rate was relatively slower and there was a holding period of two hours at 600ºC for binder removal. The cooling rate from the peak temperature was relatively faster. The sintering temperature and time were varied from 935ºC to $1035°C$ for 1 to 5 hour respectively in the present work. The heating and cooling rate were fixed and their effects were not investigated.

Characterization

The weight loss and thermal stability of samples were also evaluated from the thermogravimetric analysis data using thermo gravimetric analyzer (Model: SEIKO EXTAR TG/ DTA 6300, SEIKO, JAPAN). The X-ray powder diffraction (XRD) analysis of the sample was done by X-ray diffractometer (Model: D8 advance, Bruker AXS, Germany). The functional groups present in newly synthesized products were ascertained by fourier transform infrared spectroscopy (FTIR). Morphology of the hydroxyapatite powders was studied using scanning electron microscopy (SEM) on JEOL JSM-6490LA (The microscope has a high resolution of 3.0 nm).

III. RESULTS AND DISCUSSION

DTA/TGA analysis

TGA/DTA and DTG analysis was carried out by TG/DTA 6300, SII (Nano Technology Japan) and the system controlled by Exstar 6300 controller. Experiments have been performed using simultaneous TGA-DTA analysis by heating the sample at 20^0C/min in the temperature range 0^0C and 1000^0C in nitrogen atmosphere. DTA/TGA analysis of oyster shell in the Figure 1 (A) showed the weight loss at temperature between 150^0C and 200^0C. This is due to the physically water absorption. Over a wide range of temperature from $300–500^0$C, the weight loss is due to the decomposition of $MgCO_3$ combined with the combustion of hydrocarbons. The weight loss along with endothermic peak at $700–800^0$C indicates the decomposition of $CaCO_3$ following the reaction: $CaCO_3 \rightarrow CaO+ CO_2\uparrow$. So it is confirmed from the thermal analysis that oyster shell mainly contains $CaCO_3$ along with small amount of $MgCO_3$ and other organic impurities.
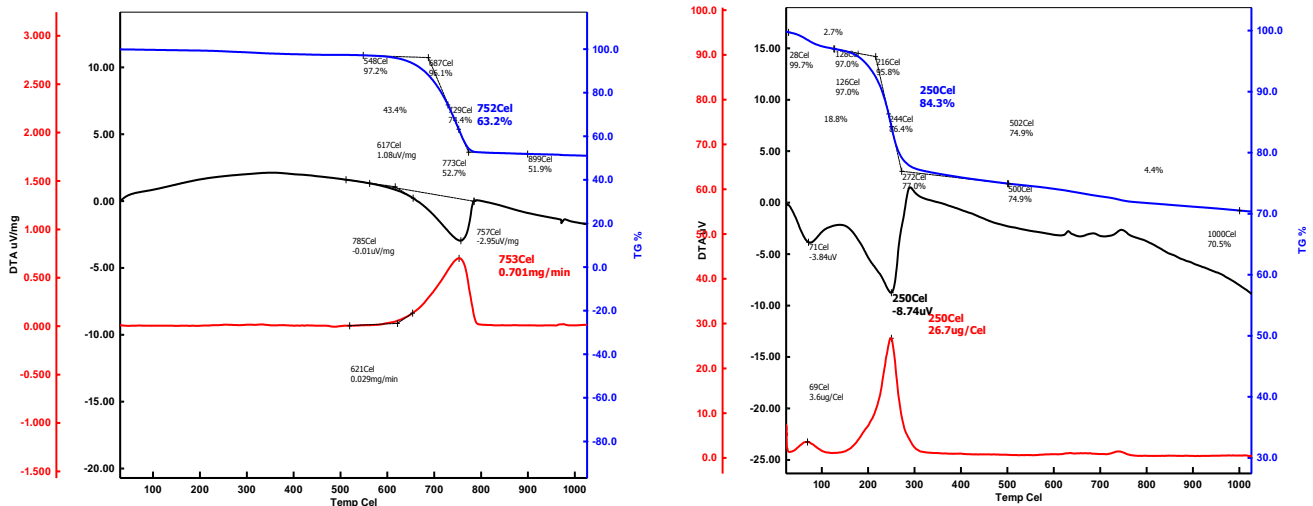
Figure 1(A). TGA/DTA analysis of as dried oyster shell   **Figure 1(B).** TG, DTA and DTG curve for HA powder

Figure 1(B), shows the TGA, DTA and DTG curves for unsintered HA powder. The top one is the TG, the bottom one is the DTG and the middle one is the DTA curve for HA powder. The TG curve shows the two stage degradation. The first stage, continuing up to about 128℃, was related to the loss of the absorbed and bound water. The mass loss of this stage was about 2.7%. Following a thermal stability zone at a range 128-216℃, the second stage was probably associated with the structural degradation on the main chain. In this stage, a more significant weight loss (18.8%) occurs above 216℃. DTA curve shows two endothermic peaks at 71℃ and 250℃. The DTG curve depicts one endothermic peak at 250℃ where the maximum degradation rate was 26.7 μg/℃. With increasing temperature from 272^0C to 1000°C no peak has been observed, except a weight loss of 4.4% is observed at the TGA curve in the temperature range which is assumed to be resulted from gradual dehydroxylation in hydroxyapatite powder.

X-ray diffraction

Figure 2 (A) shows the X-ray diffraction pattern of raw oyster shell calcined at 800^0C. Inspection of the figure it reveals the presence of $CaCO_3$ (88.6%). The mineral phase present in the as-collected oyster shell was identified as calcite; other crystalline species CaO (11.4%) was detected. The sharp peaks of the diffractogram in the Figure 2 (B) confirmed the presence of CaO obtained at 1000^0C which has a porous appearance, brown color and fragile consistence; again no other species was found. It is obvious that the observed change in the weight corresponds to the loss of gaseous CO_2. As the data reported by Gil-Lim et al that oyster shell is entirely composed of $CaCO_3$ (approximately 96%) and other minerals of trivial amount (Yoon et al., 2003). Figure 2 (C), shows the XRD pattern of HA powder. HA peaks are much more defined which is probably due to an increase of size. The merged peaks reveal the amorphous nature of phosphate compound. The highest peak was found at 31.90^0 with the intensity of about 615 counts. As this pattern shows amorphous nature of the phosphate compounds, micro crystals were not present or present in very small amount. XRD pattern shows the presence of 100% HA. As previously mentioned, the Ca/P ratio for HA samples is a basic parameter in determing its properties and thermal stability. The results show clearly that only the HA phase was formed due to the Ca/P ratio which was maintained at 1.67.

Further effect of sintering temperature on the formation of HA can be shown in the Figure 2 (D). The Figure elicits the XRD patterns of HA heated at various temperatures such as 935^0C, 985^0C, and 1035^0C. It can be seen that spectrum [b] is more sharpen than spectrum [a] but spectrum [c] is less sharpen than spectrum [b] as well as spectrum [a]. The irregular pattern as shown in the figure indicates the presences of secondary phase on HA phase and there exist two phases-hexagonal and rhombohedral. The hexagonal phase was hydroxyapatite $[Ca_{9.868} (PO_4)_{5.586}(OH)_{4.006}]$, 39.1%. The rhombohedral phase was whitlockite $[Ca_{18}Mg_2H_2(PO_4)_{14}]$, 60.9%. It can be proposed that with the temperature

increase, Ca^{2+} is released from HA structure which results in HA structure being deficient in terms of Ca^{2+} and the Ca/P ratio changes to the characteristic value for whitlockite.

Diffractograms by XRD analysis of the HA obtained by sintering at different time are shown in Figure 2 (E). From the XRD patterns of the sample it can be shown that, with increased sintering time, irregular spectrum were also observed. Similar composition of whitlokite (60.9%) and HA (39.1%) throughout the sintering time was obtained. It can be concluded from the phase analysis of calcined HA that with the increase in temperature the whitlockite phase was increased. Hydroxyapatite phase is prominent at low temperature and whitlockite phase is prominent at high temperature.
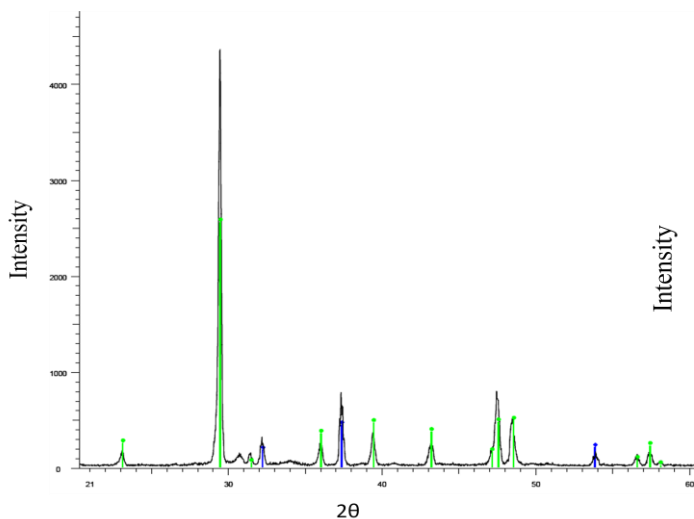
Figure 2 (A). XRD patterns of oyster shell
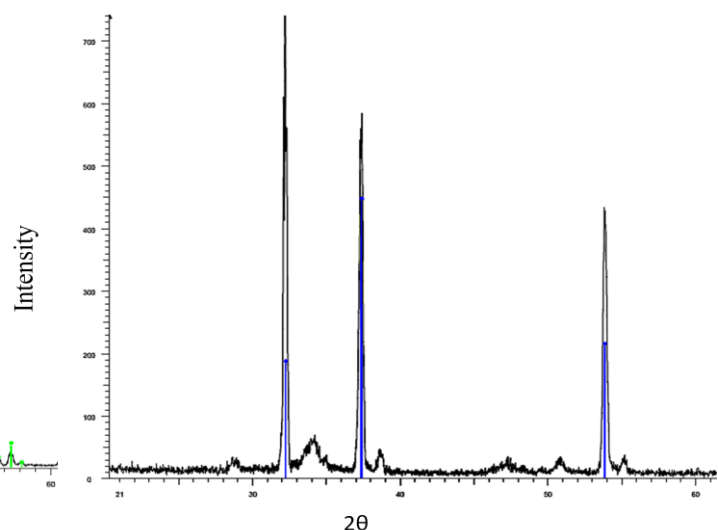
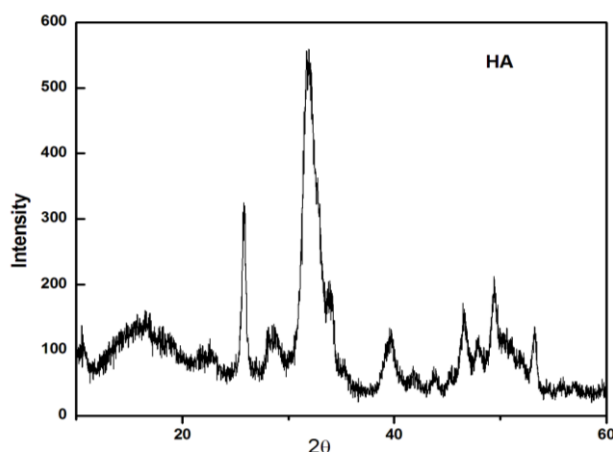**Figure 2 (B.)** XRD patterns of oyster shell (1000^0C for 4 hrs.)
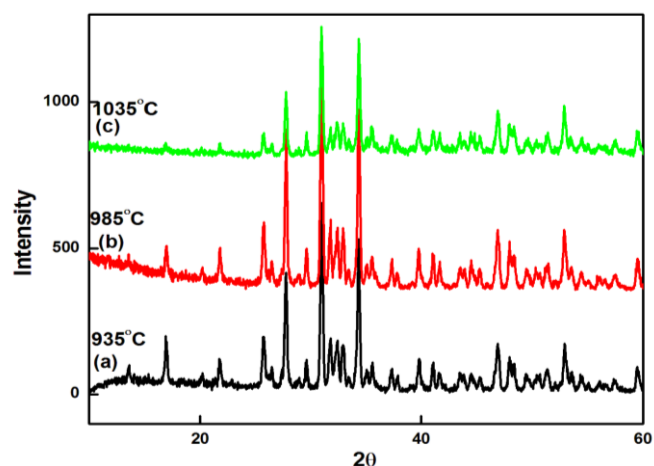

Figure 2(C). XRD pattern of HA powder

Figure 2 (D). XRD patterns of the sintered samples produced at different temperatures for 2 hour [a].935^0C, [b].$985℃$ and [c].$1035℃$ (black)
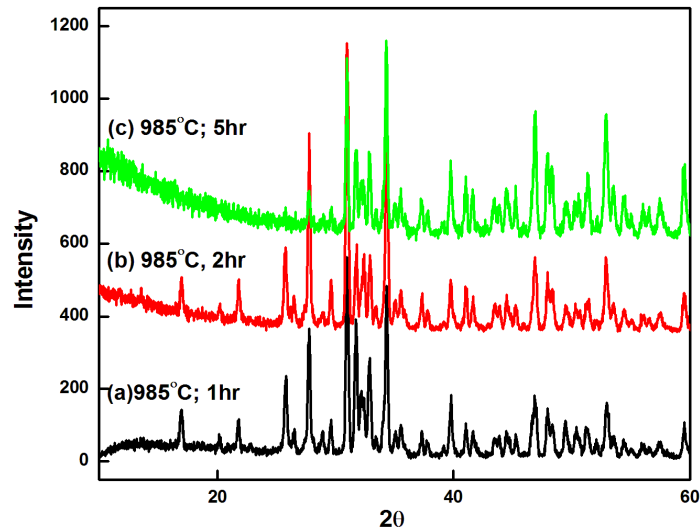
Figure 2 (E).XRD pattern of sintered sample at isothermal condition, 985℃ for 1hour (a), 2 hours (b) and 5 hours (c)

FTIR (Fourier Transform Infrared Spectroscopy) analysis

HA was also characterized by FTIR which is shown in the Figure 3. Fourier transform infrared (FTIR) spectroscopy was employed to characterize the different functional groups of HA powder. The figure shows the FTIR spectrum of HA. The spectrum was recorded in the range of (4000–400) cm^{-1}. The representative FTIR spectrum shows all characteristic absorption peaks of HA. The phosphate ions, PO_4^{3-} are the principal molecular components of HA giving to the IR absorbance in the 550-1200 cm^{-1} region. The characteristic peaks at 825cm^{-1} and 1080cm^{-1} correspond to the stretching vibration of PO_4^{3-} and at 565 cm^{-1} to the fundamental bending of PO_4^{3-} . As other major components, OH$^-$ ions are identified by observation of the broad band from about 3700cm^{-1} to 2500 cm^{-1}. The peak of this band at 3432cm^{-1} is a typical assignment of the stretching mode of OH$^-$ ions. In addition, the two distinct bands are observed at 3153cm^{-1} and 1640cm^{-1}, which arises from the stretching mode and bending mode of HO$^-$ molecules, respectively.
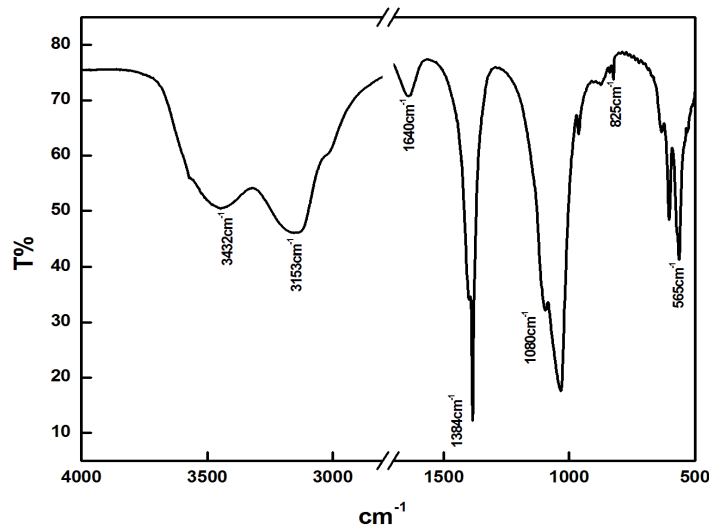

Figure 3. FTIR analysis of HA powder

SEM analysis

The morphologies of as synthesized HA powders are shown in Figures. 4 (A) - 4 (C). From the Figure 4 (A) synthesized HA powders are almost regular and round in shape; with a little agglomerated structure whereas sintered HA powders are agglomerated almost irregular in shape. It can be proposed that calcination treatment of the raw oyster shell has a significant effect on sintering temperatures. HA (Ca/P = 1.67) samples show grains more rounded and with coalescence markedly reduced in respect to other newly produced HA treated at different sintering temperatures as shown in the figures 4 (B).

The crystal size of HA powder at higher sintering temperatures (935 °C to 1035 °C) was increased. The formation of these microstructures of HA in the thermal process can be attributed to the tendency of particles to crystallize and agglomerate at high temperatures. The particles had irregular shapes, including small spheres, agglomerated together in some parts. It can be concluded that higher temperature decreases the probability of grain growth and the densification can be improved.
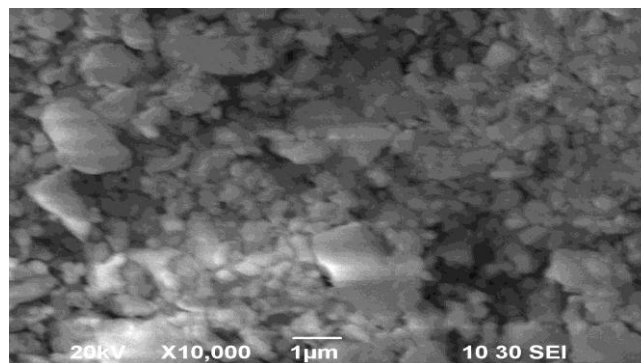

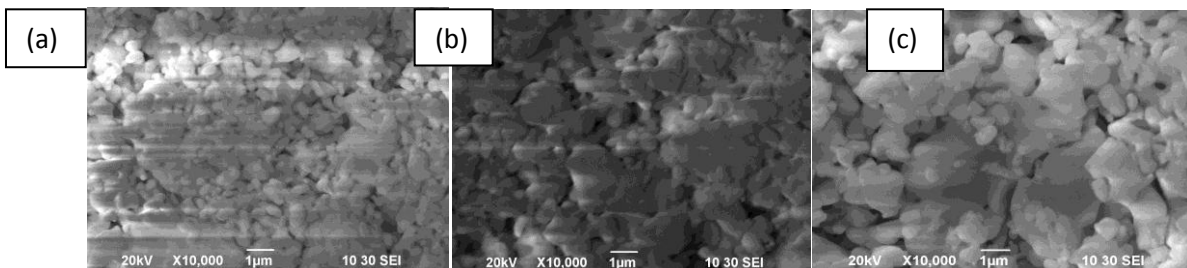**Figure 4 (A)**. SEM image of Hydroxyapatite before sintering

Figure 4 (B). SEM image of hydroxyapatite after sintering (b) at 935℃ for 2 hr; (c) at 985℃ for 2 hr.; (d) at 1035℃ for 2 hr
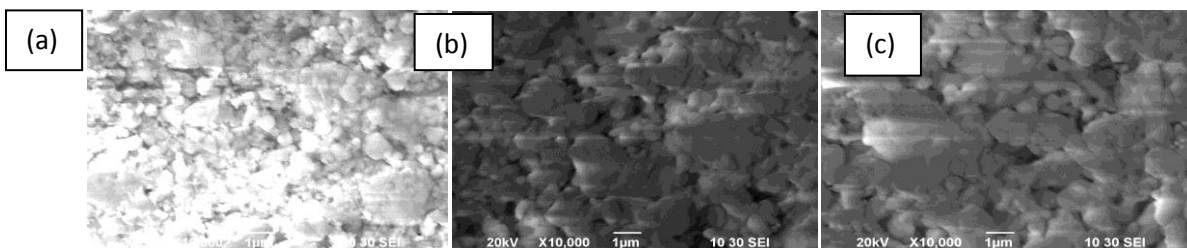

Figure 4 (C). SEM image of hydroxyapatite after sintering (e) at 985℃ for 1 hr; (f) at 985℃ for 2 hrs; (g) at 985℃ 5 hrs

IV. CONCLUSION

The present work is based on the utilization of biological waste (oyster shell) to produce hydroxyapatite for bio-medical applications. The oyster shell seems to be a promising source of calcium for preparing hydroxyapatite with excellent properties essential for hard tissue replacement by wet precipitation method. XRD analysis indicated phase purity and crystallinity of hydroxyapatite powder. It showed that at higher sintering temperature secondary phase of HA was formed and HA phase was prominent at lower temperature. TG/DTA result showed that the main constituent of oyster shell is calcium carbonate ($CaCO_3$) and also showed the thermal stability of HA powder. The prepared HA from oyster shell might be more useful for the treatment of oral bone defects in comparison with conventional HA, and more effective as a bone replacement material to promote bone formation.

ACKNOWLEDGEMENT

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