

Characterisation of natural hydroxyapatite extracted from bovine cortical bone ash

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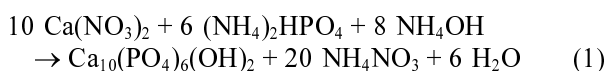
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Hydroxyapatite powder was prepared by burning bone and heat treating the bone ash obtained at 600-1100 °C in an air furnace. The black ash was converted to a white powder after heat treatment. X-ray diffraction analysis and Fourier transform infra-red spectroscopy indicated that the white powder was hydroxyapatite and did not contain any organic components of the bone. Furthermore, phase transformation of the resulting hydroxyapatite to other calcium phosphate phases did not occur up to 1100 °C. X-ray fluorescence analyses revealed that calcium and phosphorous were the main elements and magnesium and sodium were present as minor impurities. The results of the energy dispersive X-ray analysis showed that the Ca/P ratio of this natural hydroxyapatite varies between 1.46 and 2.01. The resulting material was found to be thermally stable up to 1100 °C. The density of natural hydroxyapatite heat treated at 800 °C was measured to be 3.187 cm³.

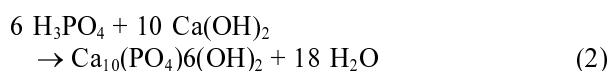
Key words: Bone ash, Natural hydroxyapatite, Heat treatment, Characterisation.

Introduction

Hydroxyapatite, [Ca₁₀(PO₄)₆(OH)₂], has been used to manufacture biomaterials for hard tissue repair and replacement as it is the inorganic constituent of natural bone and is very biocompatible [1, 2]. It is the most commonly used calcium phosphate in orthopaedics as it is osteoconductive [3]. Its production has been well reported [4-7]. There are different methods for synthesis of hydroxyapatite which include precipitation [8, 9], hydrolysis [10, 11], and hydrothermal synthesis [12, 13]. Among these methods, the precipitation technique seems to be the most commonly used. One of the precipitation processes by which hydroxyapatite can be produced is the reaction of calcium nitrate with diammonium hydrogen phosphate as follows:



The above process can be polluting to some extent because of the by-products of the reaction shown in Equation (1) which include ammonium nitrate. Another precipitation method for producing hydroxyapatite is the reaction between phosphoric acid and calcium hydroxide:



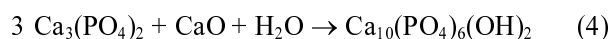
As seen, the by-product of the above reaction is water and therefore this process is non-polluting. This process is also simpler than the process shown in Equation (1)

and is a suitable method for the industrial production of hydroxyapatite [4]. In general, these precipitation methods require highly controlled parameters such as composition and purity of the starting materials, pH and temperature of the solutions prepared for producing high quality hydroxyapatite. Another important parameter to be considered is the stoichiometric Ca/P ratio.

An alternative method for the preparation of hydroxyapatite can be its extraction from natural resources. In fact, some researchers have attempted to synthesise hydroxyapatite from biological materials. Corals [14], eggshells [15, 16] and ostrich eggshells [17] have also been utilized to produce hydroxyapatite or used as a bone substitute. The main constituent of an eggshell is calcium carbonate (94%) and calcium phosphate (1%). It also has 4% organic compounds and 1% magnesium carbonate. By heating eggshells at 900 °C for 2 hours, Rivera *et al.* [15] converted the calcium carbonate content of eggshells into calcium oxide via the following equation:



Then they added the calcium oxide obtained from eggshells to a tricalcium phosphate solution and heated the mixture to 1050 °C for 3 hours to produce hydroxyapatite, according to:



Krishna *et al.* modified the above process by using a microwave oven to produce nanocrystalline hydroxyapatite from eggshells [16]. A group of Korean scientists synthesized calcium phosphate powders from cuttlefish bone [18]. Their process was partly similar to the work reported by Rivera *et al.* [15] and produced CaO by burning cuttlefish bone. Another process for preparation

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of hydroxyapatite and other calcium phosphate materials for biomedical applications can be their extraction from bone, since hydroxyapatite is the main inorganic constituent of natural bone. Hydrothermal synthesis of hydroxyapatite from bovine bone has been reported by Jinawath *et al.* [19]. In their extensive studies, a group of New Zealand researchers have used bone to produce orthopaedic implants [20]. Through a process of boiling, solvent treatment and deproteination, they converted bovine bone to an implant material. An alternative process in producing hydroxyapatite from bovine bone can be its extraction from bone ash. Two advantages of this process are the removal of all organic components of bone and also it prevents the possibility of transmission of dangerous diseases. When a piece of bone is burned fully, its organic components are removed. The remaining ash contains the inorganic constituents of the bone. Consequently, bone ash can be used to extract hydroxyapatite. Assuming that the remaining ash can be converted fully to hydroxyapatite, about 1.6 kg compact bone should yield 1 kg hydroxyapatite. Such a process would certainly be an economic method for producing hydroxyapatite to be used as a biomaterial in orthopaedic and dental implants.

The aim of the present research was to investigate the possibility of producing hydroxyapatite from bone ash and also to evaluate heat treatment and thermal conditions carried out on bone ash in order to get hydroxyapatite powder. In order to determine the phase purity and thermal stability of the resulted material various chemical and physical characterisation techniques were employed.

Experimental Procedure

Extraction process

Different types of bones from different animals were used as the starting materials for this process. Bovine femur bone, sheep femur bone, sheep skull flat bone, chicken femur and plaice vertebrae were chosen to compare the efficiency of the process for these different types of bone, i.e. the amount of powder obtained per unit weight of bone. This was to find out which type of bone is the best to use regarding the amount of bone ash produced after burning bone. It was found that the highest amount of bone ash powder per unit weight of bone was obtained for bovine femur bone. Bovine tibia, humerus and ulna were also tested and the results were similar to that obtained for bovine femur. Hence, bovine femur, tibia, humerus and ulna were chosen as the main starting biological material. The spongy bones were discarded and the cortical bone was de-fleshed. The bone marrow and all pieces of meat and fat were removed. By using a gas torch and applying direct flame to the cleaned bone, organic components were burned. The product of this thermal process contained some char due to burning of organic components. To remove the remaining char, the black powder (bone ash) was placed in an air furnace at different temperatures between 600 °C to 1100 °C for

3 hours and finally it was cooled inside the furnace. Following this process, the black ash turned into a white granular powder.

Characterisation

Chemical characterisation (FTIR, XRF, XRD and EDX)

Fourier transform infrared (FTIR) spectroscopy is a powerful analytical technique for the characterisation of biomaterials. Thus, to distinguish the presence of organic species and also the degree of probable dehydroxylation of hydroxyapatite during heat treatment, FTIR analysis was performed in the present investigation by using a Shimadzu 8300 Fourier transform infrared spectrometer. The measurements were carried out in the transmission mode in the mid-infrared range with wave numbers from 400 cm^{-1} to 4500 cm^{-1} . To record the FTIR spectrum of each sample, 2 mg of the powder was mixed and pressed with 300 mg of KBr to get a pellet for FTIR analysis. A Philips, PW 2400 X-ray fluorescence spectrometer was used to obtain elemental chemical composition of the extracted powder. In order to prepare samples for XRF analysis, the powder was poured into a special die and compacted in a pressing machine with 8 tonnes load to prepare a disk shaped specimen 30 mm in diameter and 5 mm in thickness. The phase composition and purity of the materials investigated were determined by X-ray diffraction (XRD). A Bruker, D8 Advance X-ray diffractometer with $\text{Cu K}\alpha$ radiation was used for this study. Powder X-ray diffraction spectra were taken at 40 kV and 40 mA and recorded from 5 to 100 degrees for 2θ at a scanning speed of 2.5 degrees/minute and a step size of 0.02 degrees. The resulting patterns were studied quantitatively by Rietveld analysis which used the fundamental parameter procedure implemented in TOPAS R-version 2.1. For semi-quantitative analysis and to study the Ca/P ratio in the extracted material, a JEOL, JSM-5410LV scanning electron microscope (SEM) equipped with an Oxford energy dispersive X-ray (EDX) analyser working at 15 kV accelerating voltage was used. Ten measurements were performed and it was assumed that the peak height is proportional to the mole fraction of an element.

Physical characterisation

The density of the powder was measured using a Pycnometer (Micrometrics, AccuPyc 1330). The particle size of the heat treated bone ash was in the range of a few millimetres scale. To make the particles smaller, it was milled for 1 to 4 hours to get a fine powder. A planetary ball mill system (Fritsch, Pulverisette 5), consisting of a tungsten carbide bowl and balls, was used to prepare fine particles after heat treating. The feed ratio was 30 g powder to 300 g ball (1 to 10 weight ratio). The speed was fixed at 250 rpm and the milling time set at 1 to 4 hours with pause and reverse mode. A laser particle size analyser (Fritsch Particle Sizer, "analysette 22") was used to determine the particle size distribution of the fine powder. A Cambridge Camscan scanning electron

microscope (SEM) was used to study the size and morphology of the powder. To evaluate the thermal stability of the extracted material, differential thermal analysis (DTA) and thermogravimetric analysis (TGA) were performed using a Netzsch STA 449C thermal analyser by changing the temperature at a rate of $10\text{ K}\cdot\text{minute}^{-1}$ from room temperature up to $1100\text{ }^{\circ}\text{C}$ in air. The weight of the sample for thermal analysis was 100 mg and $\alpha\text{-Al}_2\text{O}_3$ was used as a crucible and a reference sample.

Results and Discussion

Chemical characterization

FTIR analysis

The FTIR spectrum of the black powder (bone ash) is presented in Fig. 1. It shows a series of bands in the mid-infrared region; a strong band at 1043 cm^{-1} , another band at 1456 cm^{-1} with a shoulder at 1417 cm^{-1} and also a small sharp band at 667 cm^{-1} . Comparison of infrared spectra obtained from analysis of bone and bone ash can give an indication of the removal of organic components after burning bone. Different types of chemical bonds which are present in various components of bone give characteristic infrared absorption bands. Changes in the environment of molecules cause shifts in the intensities and positions of their corresponding absorption bands. The infrared spectrum of bone has been recently reported by Boskey and Camacho and shows the presence of the major inorganic species, phosphate and carbonate groups (from hydroxyapatite), and also the organic components such as amide functional groups from the protein constituents of bone, i.e. collagen [21]. Although they recorded infrared absorbance between 800 cm^{-1} and 1800 cm^{-1} , nevertheless, comparing their results for bone with the FTIR spectrum obtained for bone ash in the present study show clearly that all organic components of bone have disappeared after burning. The bands associated with the amide groups of proteins (1250 cm^{-1} , 1560 cm^{-1} and 1650 cm^{-1}) which are observed on the spectrum of bone do not exist on the FTIR spectrum of bone ash prepared in this investigation. On the other hand, the bands for phosphate and carbonate groups in bone ash occur at the same wavenumbers as those reported for bone. This comparison shows that the

procedure of burning bone adopted here is sufficient to remove all organic components of bone. Furthermore, there are no absorption bands related to C-H bonds in the FTIR spectrum of the bone ash. This indicates that heat treating of the bone ash results in total removal of the organic materials of bone [22]. Therefore, all bands observed in the FTIR of Fig. 1 are associated with the inorganic components of bone which were present in the bone ash. These bands can be divided into three main categories associated with phosphate, carbonate and hydroxyl groups. One strong and relatively broad band at 1043 cm^{-1} , two relatively strong and sharp bands at 567 cm^{-1} and 603 cm^{-1} and another band at 962 cm^{-1} which appear on the FTIR spectrum of Fig. 1 are due to the phosphate group. Tanaka, *et al.* have also observed two bands at 603 and 1051 cm^{-1} due to the stretching vibrations of the phosphate group [1]. The bands which appear at 873 cm^{-1} , 1417 cm^{-1} and 1456 cm^{-1} are associated with the carbonate group. The carbonate ion can competitively substitute at two sites in the apatite structure, the hydroxyl and the phosphate ion positions, giving A-type and B-type carbonated apatite respectively. These two types of substitution can occur simultaneously, resulting in a mixed AB-type substitution which constitutes bone mineral. Thus, the peak position of carbonate ions in FTIR spectra depends on whether the carbonate ion is substituted for the hydroxyl ion or the phosphate ion in the hydroxyapatite lattice. There is also a relatively broad band at 3436 cm^{-1} which is attributed to the hydroxyl group.

The FTIR spectra of samples of bone ash which were heat treated at 600 , 700 , 800 , and $1100\text{ }^{\circ}\text{C}$ are presented in Fig. 2 (a, b, c and d). They provide a number of spectral details indicating some changes have occurred. The band at 2341 cm^{-1} has disappeared after heat treating bone ash which might be due to the elimination of char in the bone ash in the form of carbon dioxide gas release. This is in agreement with the change of colour from black bone ash to a white powder after heat treating. The broad band at 3436 cm^{-1} observed in the spectrum of Fig. 1 has almost disappeared and been replaced by a small peak at 3571 cm^{-1} in the spectra of Fig. 2, which is due to the carboxyl stretching. Barinov *et al.* have recently investigated the effect of sintering temperature on the FTIR of carbonated hydroxyapatite and reported that the band at 3570 cm^{-1} due to the hydroxyl group stretch mode disappeared after sintering hydroxyapatite at $1100\text{ }^{\circ}\text{C}$ and $1500\text{ }^{\circ}\text{C}$ [23]. It was found here that as the heat treatment temperature increased up to $1100\text{ }^{\circ}\text{C}$, the band at 634.5 cm^{-1} which originates from libration of the hydroxyl ion is very sensitive to temperature and disappears, while the band at 3571.9 cm^{-1} which corresponds to the stretching vibration bands of the hydroxyl ion is more stable and becomes broader. This is due to the dehydroxylation of hydroxyapatite which may occur at temperatures above $850\text{ }^{\circ}\text{C}$. The results of these FTIR analyses show that heat treating of the bone ash above $800\text{ }^{\circ}\text{C}$ can cause dehydroxylation of the natural hydroxya-

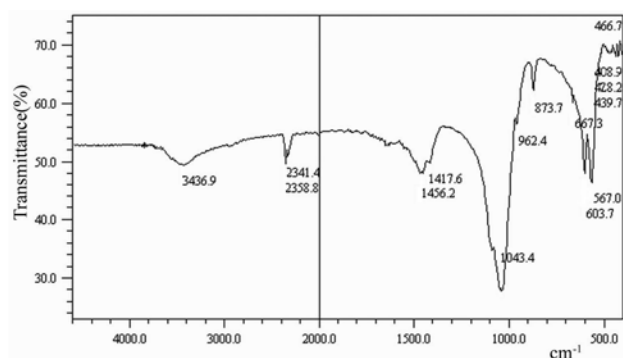


Fig. 1. The FTIR spectrum of the bone ash.

determining the composition of the hydroxyapatite in their calcified tissues.

XRD analysis

Fig. 3 shows the XRD patterns of the black powder produced after burning bone, i.e. the bone ash (a) and its heat treated forms produced at 600 °C (b), 700 °C (c), 800 °C (d) and 1100 °C (e). These diffraction patterns show a gradual increase in the degree of sharpness of peaks with increasing heat treatment temperature, indicating the extent of crystallinity of the NHA produced at various temperatures. The diffraction pattern (Fig. 3(a)) of the bone ash is very broad which indicates the presence of

small crystals of hydroxyapatite. Other diffraction patterns of Fig. 3(b)-(e) show patterns which are narrow and sharp compared with the pattern of the bone ash before heating. The resulting sharpening of the XRD pattern of the heat treated bone ash could be due to a change of the crystal size of the powder. Similar observations have been reported by Shipman *et al.* [25] and they explained the results of their XRD patterns in terms of alteration in crystal size. They found that there was a gradual increase in hydroxyapatite crystal size associated with an increase in the heat treatment temperature [25]. The black powder, produced in this study, began recrystallization at about 600 °C without decomposing to any other compound of the calcium phosphate family. Peaks which would indicate the thermal decomposition of hydroxyapatite into α -tricalcium phosphate and tetracalcium phosphate were not observed at any temperature up to 800 °C. Sharp clear reflections observed after heat treating at 800 °C correspond to hydroxyapatite and this confirms the phase purity and high crystallinity of hydroxyapatite produced after heat treating the bone ash at this temperature. Lee *et al.* also reported fully-crystallized hydroxyapatite at 900 °C when they synthesized hydroxyapatite from calcined cuttlefish bone [18]. Fig. 3(e) shows the appearance of a low intensity peak at 2θ of 31.1° corresponding to β -tricalcium phosphate. Keeping the powder at 1100 °C for 3 hours resulted in this peak with low intensity whereas heating the bone ash at temperatures lower than 1100 °C did not give any indication of the presence of β -tricalcium phosphate as an impurity phase. As mentioned, the crystallite size influences peak broadening. It should be noted that XRD analysis only provides a spatial average of crystallite size estimates. Thus, changes in peak broadening represent changes to the crystallite size distribution. There are some chemical factors which affect the crystallite size. These include denaturing of the bone matrix during burning through release of water, in which the mineral crystals recrystallise, and removal of the collagen fibril networks which influence the crystallite size of the bone ash, as seen in Fig. 3(a).

The results of the quantitative analysis which have been computed by the use of a Rietveld analysis are presented in Fig. 4 and the calculated crystallographic parameters

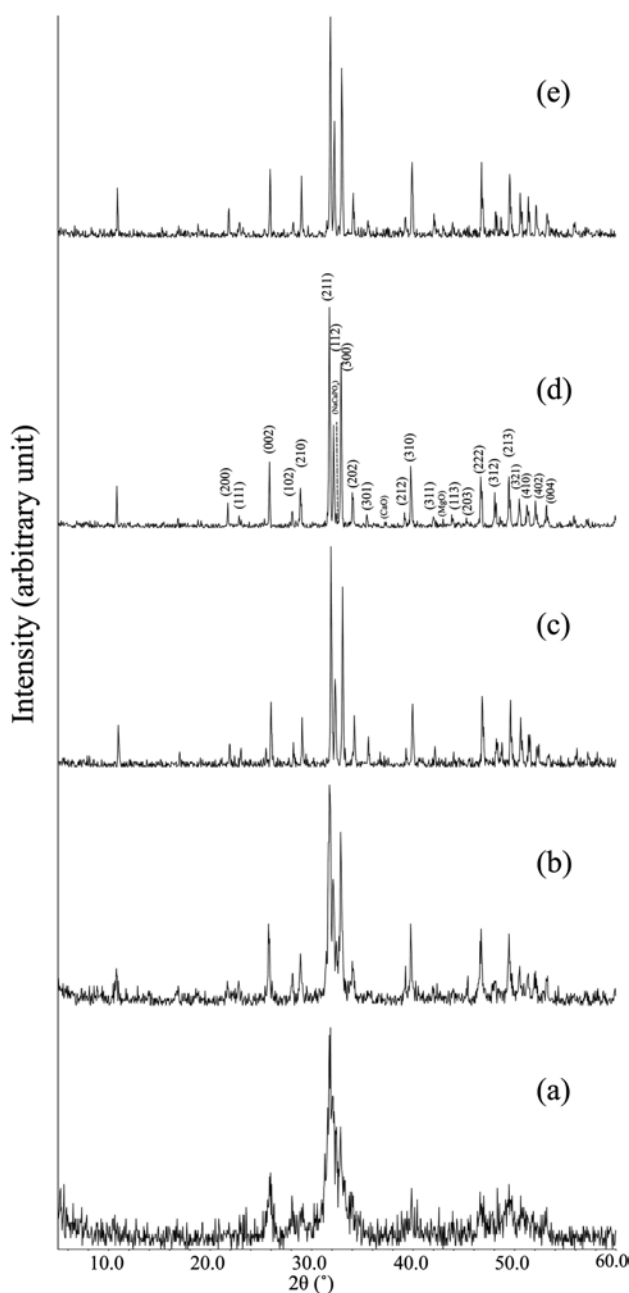


Fig. 3. XRD patterns of bone ash (a) and its heat treated forms produced at different temperatures; 600 °C (b), 700 °C (c), 800 °C (d) and 1100 °C.

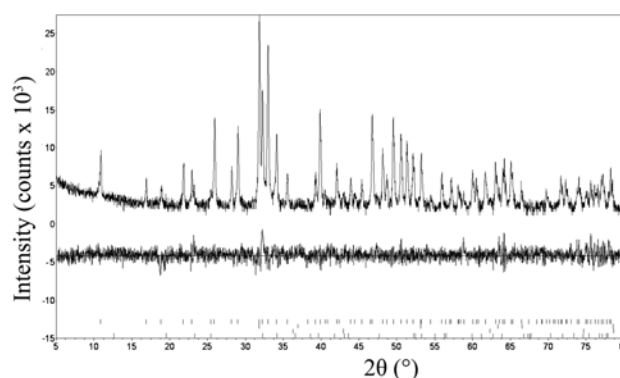


Fig. 4. Quantitative analysis of the XRD pattern of NHA.

Table 3. Crystallographic parameters of NHA resulted from quantitative analysis

Lattice parameters (Å)			Cell volume (Å ³)	Crystallite size (nm)	Crystal density (g/cm ³)	R _{Bragg} (%)
a	c	c/a				
9.4084	6.8756	0.7308	527.1	133	3.145	9.5

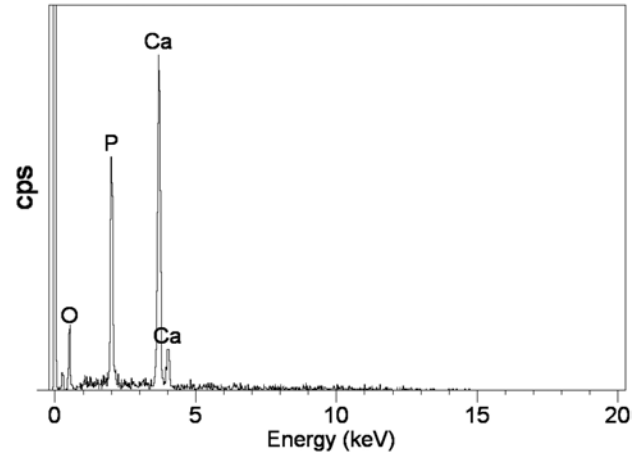
Table 4. Chemical composition of NHA from quantitative analysis of XRD spectra

Component	2θ value (°)	Content (wt.%)
NaCaPO ₄	33.7	2
CaO	37.4	1
MgO	42.8	1
hydroxyapatite	31.8	96

are shown in Table 3. The average crystallite size of the NHA heat treated at 800 °C was determined to be 133 nm which is very close to the value of the crystal size of synthetic hydroxyapatite sintered at 900 °C as reported by Madhavi *et al.* [26]. These results confirm the previous discussion regarding the effect of the heat treatment temperature on the crystal size of hydroxyapatite. The unit cell parameters found here are in good agreement with the results reported by other researchers for synthetic hydroxyapatite [6, 26]. The chemical composition of the NHA from quantitative analysis of the XRD spectra is shown in Table 4. These results indicate that this NHA contains some NaCaPO₄, CaO and MgO as impurities. These are in good agreement with the results of the XRF analysis (Table 1 and 2), where Na and Mg were detected as the minor impurity elements. The content of CaO was found to be 1 wt.% (Table 4). It should be appreciated that low concentrations of CaO is advantageous because the presence of CaO decreases the biocompatibility of hydroxyapatite [22]. The study of Joschek *et al.* on the characterisation of natural hydroxyapatite, Endobon, indicated the presence of MgO, CaO, Ca₄O (PO₄)₂ and NaCaPO₄ as impurity phases [24]. The concentration of Ca₄O (PO₄)₂ in their product was very low, 0.09 %. However, Ca₃ (PO₄)₂ and Ca₄O (PO₄)₂ as the impurity phases in their product could be formed during the sintering process from non-stoichiometric hydroxyapatite of the bovine bone. In other words, when Endobon is heated at temperatures higher than 1200 °C tricalcium phosphate and tetra calcium phosphate are formed due to phase transformations of hydroxyapatite at high sintering temperatures. On the other hand, these two phases are not present in the NHA extracted from bone ash in this study because the optimum heat treating temperature which was found here (800 °C) is lower than the phase transformation temperature of hydroxyapatite to other calcium phosphate phases. Thus, they were detected in the XRF results and XRD spectra of this study as minor impurity phases.

Study of Ca/P ratio with SEM-EDX

The results of the SEM-EDX analysis (Fig. 5) showed

**Fig. 5.** The SEM-EDX analysis of NHA powder.

that the Ca/P ratio of NHA investigated in this study varies between 1.46 and 2.01 with an average of 1.67. The values of Ca/P ratio reported by Lee *et al.* [18] during synthesis of hydroxyapatite from cuttlefish bone and phosphoric acid were 1.70 and 1.64 for two different mixing ratios of the calcined cuttlefish bone to phosphoric acid. The Ca/P ratio found in the present investigation agrees with the above values reported by Lee *et al.* The essential difference between synthetic and natural hydroxyapatite is that the latter showed a higher Ca/P ratio than the synthetic material, while the former was closer to the stoichiometric hydroxyapatite [22, 24]. It should be noted that there is a technical problem with the semi-quantitative analysis of Ca/P ratio of hydroxyapatite by the use of SEM-EDX. In other words, during the analysis at a fixed current density and increasing irradiation time, the X-ray intensity of phosphorus decreases more rapidly than that of calcium leading to an increase of the Ca/P ratio at a high electron irradiation dose. Thus, in this study, the accelerating voltage was set at 15 kV and the analysis was performed at a low irradiation time to overcome the above mentioned problem.

Physical Characterisation

Density and Particle Size

The density of hydroxyapatite heat treated at 800 °C for 3 hours was found to be 3.187 g/cm³. This result is in good agreement with the calculated value (3.145 g/cm³) from the crystallographic parameters of NHA, shown in Table 3. The particle size of the resulting hydroxyapatite powder obtained after heat treating bone ash can determine the effectiveness of the powder for a particular application. Thus, the powder obtained after heat treatment was milled for 1 to 4 hours with increments of 1 hour. Then the

particle size distribution of the fine powders was measured. The results for different times of milling from 1 hour to 4 hours are illustrated in Fig. 6(a)-(d). The statistical data for the particle size distributions, extracted from Fig. 6, and also the values of specific surface area (SSA) are presented in Table 5. The particle size distributions were bimodal, regardless of the milling times. The first modes (M_1) associated with smaller particles were centred at 1.07 μm , 1.27 μm , 1.41 μm and 1.64 μm for 1 hour, 2 hours, 3 hours and 4 hours of milling, respectively. The second mode (M_2) for larger particles occurred at 16.32 μm , 16.94 μm , 17.2 μm and 17.73 μm for the same times of milling. As seen in Fig. 6(a), the percentage of particles of the first mode (M_1) is much higher than the percentage of particles of the second mode (M_2) for 1 hour milling and consequently the modes are not of the same size for this time of milling. When the powder was milled for longer than 1 hour, the percentage of M_1 decreased while the percentage of M_2 increased and eventually they became of the same size with 4 hours milling time (Fig. 6(b) to Fig. 6(d)). The ratios of (M_1/M_2) have been calculated (Table 5) to be 2.9, 1.4, 1.2 and 1.0 for 1 hour, 2, 3 and 4 hours, respectively. These results indicate that smaller size particles were obtained after 1 hour milling. When the milling time was increased to 2, 3 and 4 hours, the size of particles also increased. In fact, the arithmetic mean diameter of particles produced after 1 hour milling

was 6.66 μm , while the arithmetic mean diameters of particles obtained after milling for 2, 3 and 4 hours were 9.29, 9.34 and 9.33 μm , respectively. The increase in particle size for the powders milled for more than 1 hour might be due to the agglomeration of particles after milling the powder for a long time, i.e. longer than 1 hour. Thus, it appears that 1 hour milling is enough to get small size particles of hydroxyapatite after heat treating the bone ash. As mentioned earlier, the desirable particle size of hydroxyapatite powder depends on its specific application. For example, Fernandez *et al.* have used hydroxyapatite powders with a large particle size (32 μm)

Table 5. Statistical data for particle size distribution of heated (at 800 °C) NHA powder after milling and the values of the specific surface area

Specimen	(a)	(b)	(c)	(d)
Milling Time (h)	1	2	3	4
Arithmetic Mean Diameter (μm)	6.66	9.29	9.34	9.33
Standard Deviation (μm)	2.58	3.05	3.06	3.05
Particle Size of Mode 1 (μm)	1.07	1.27	1.41	1.64
Particle Size of Mode 2 (μm)	16.32	16.94	17.20	17.73
Percent of mode 1 (% M_1)	53	36	34	33
Percent of mode 2 (% M_2)	18	25	27	32
% M_1 /% M_2 Ratio	2.90	1.40	1.20	1.00
Particles Smaller than 5 μm (%)	72.60	58.80	56.30	53.50
Particles Smaller than 1 μm (%)	40.00	20.00	20.00	20.00
Specific Surface area (m^2/g)	1.50	1.10	1.03	0.97

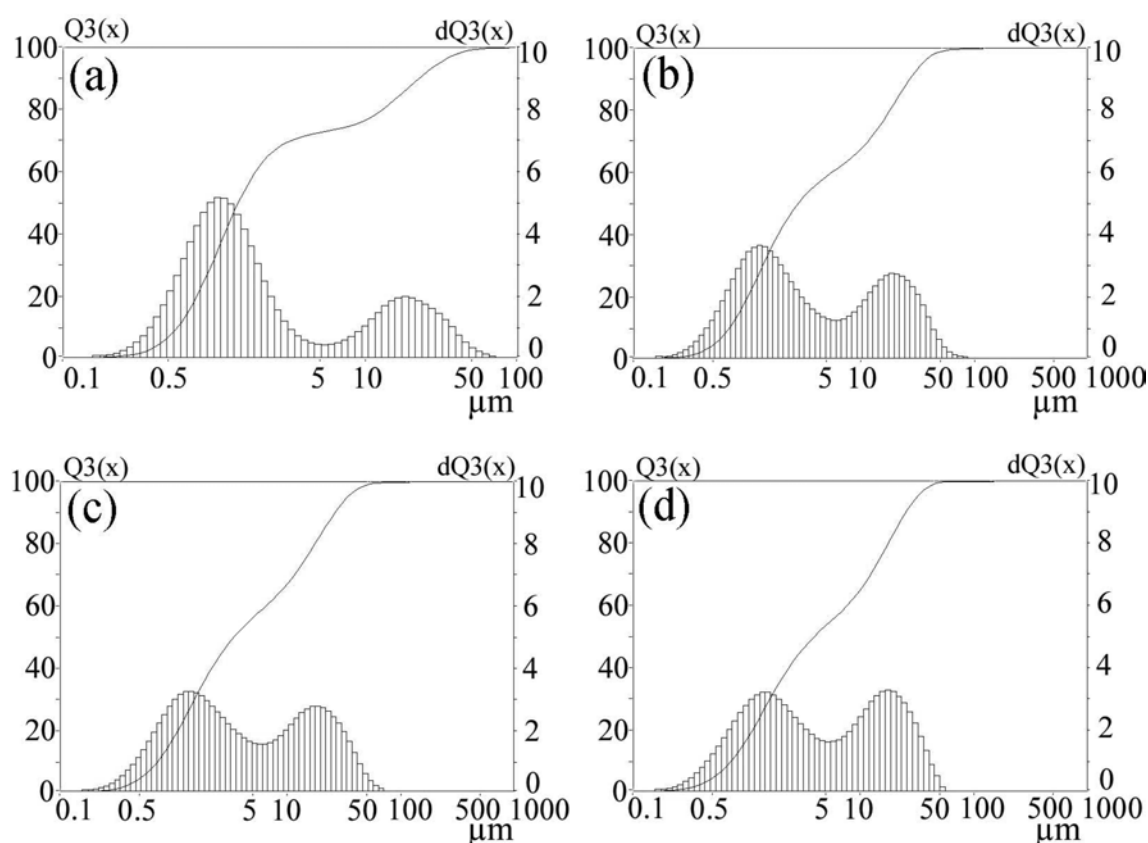


Fig. 6. Particle size distribution of the heat treated (at 800 °C) NHA powder milled for different times; (a) 1 hour, (b) 2 hours, (c) 3 hours and (d) 4 hours.

to coat Ti-6Al-4V substrates by utilizing the high-velocity oxy-fuel (HVOF) process [27]. It has also been reported that for plasma spray coating of hydroxyapatite, only particles between 150 μm and 200 μm are suitable [28]. On the other hand, very small particles (nano-sized) of hydroxyapatite can have a very large surface area and consequently nanocrystalline hydroxyapatite is very active and mimics the hydroxyapatite crystals in natural bone. Utilization of hydroxyapatite nanoparticles in drug delivery devices is another application of small particles of hydroxyapatite. To make these devices, it is important to control the particle size of hydroxyapatite nanoparticles during its synthesis. The influence of synthesis parameters on the particle size of nanostructured hydroxyapatite for targeted and controlled release of therapeutic agents has recently been studied by Loo *et al.* [29]. Medium size particles (submicrometre size to a few micrometres) of hydroxyapatite can be used for electrophoretic deposition onto metallic substrates [30]. Ma *et al.* have reported electrophoretic deposition of 0.3 μm particles of hydroxyapatite to make a bioactive porous hydroxyapatite scaffold [31]. They also used submicrometre particles of hydroxyapatite for electrophoretic coatings on carbon rods [32] and titanium substrates [33]. The aim of the present study was to produce NHA from bone ash and subsequently use it for electrophoretic deposition. As seen in Table 5, the heat treated hydroxyapatite which was milled for 1 hour had a particle size distribution suitable for electrophoretic deposition. 72.6% of its particles were smaller than 5 μm and 40% of its particles had submicrometre size, i.e. less than 1 μm . The specific surface area of the powder milled for 1 hour was 1.5 m^2/g . The data in Table 5 indicate that the heat treated hydroxyapatite powder which was milled for 1 hour showed the best results. There is a considerable change of the parameters shown in Table 5 for the powder milled for 1 hour compared with those which were milled for more than 1 hour. Thus, it seems that milling for more than 1 hour causes agglomeration of the powder.

Morphology

To confirm the influence of milling on the particle size and morphology, microstructures of the heat treated hydroxyapatite powders before milling and also after 1 hour milling were investigated and the SEM micrographs are given in Fig. 7. These SEM images gave insight into the hydroxyapatite structure with respect to particle size and shape. Fig. 7(a) which was for the bone ash heated at 800 $^{\circ}\text{C}$ before milling shows a wide range of particle sizes and shapes. The particles had irregular shapes with edges and corners, rather than being spherical, and most of them were in the range of 1 or 2 mm in dimensions. This irregular shape of the particles and also their large sizes might be due to grinding the burnt bone during the production of the bone ash. It should be noted that Fig. 7(a) does not show the presence of any amorphous organic material indicating that the organic

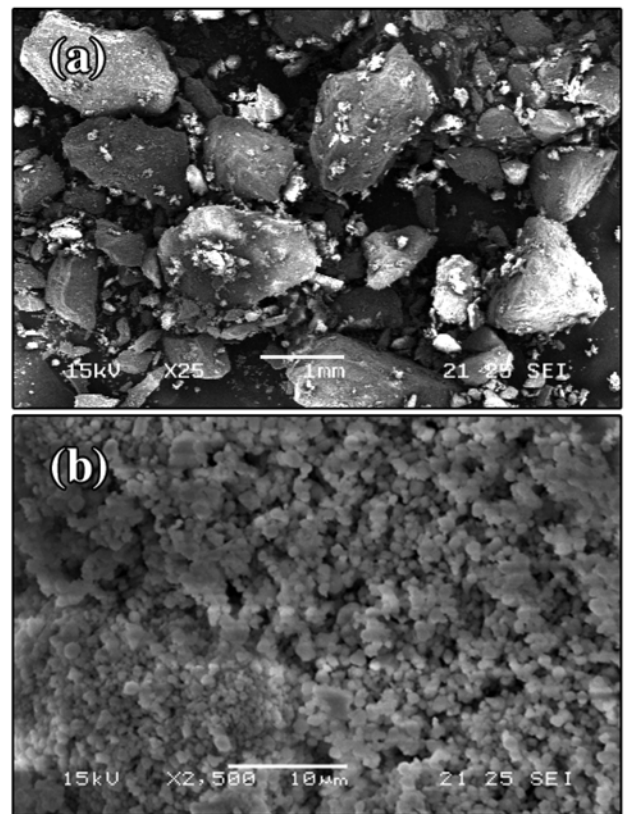


Fig. 7. The SEM micrographs of the heat treated (at 800 $^{\circ}\text{C}$) powder: (a) before milling and (b) after milling for 1 hour.

component of the bone has been completely removed. This is expected since combustion of the organic portion of the bone tissue takes place at about 400 $^{\circ}\text{C}$. The remaining char which gave a black colour to the bone ash was eliminated completely during heating and consequently a white powder was obtained at 800 $^{\circ}\text{C}$ which was hydroxyapatite, as characterized in this study. When bone is heated gradually from 200 $^{\circ}\text{C}$ to 1600 $^{\circ}\text{C}$, macroscopical (i.e. colour) changes and also microstructural changes occur which include recrystallisation of the bone mineral. Holden *et al.* [34] attempted SEM observations of heat treated human bone and reported that the organic components of the bone tissue are eliminated at 400 $^{\circ}\text{C}$. This is followed by recrystallisation of the bone mineral at 600 $^{\circ}\text{C}$ which produces a range of crystal morphologies, including spherical, hexagonal, platelets and irregular shapes. They found that these different morphologies depended on the temperature and duration of heating and also on the age of the deceased person from whom the bone had been obtained [34]. Accordingly, the morphology of the hydroxyapatite produced from bone ash in the present study also might be influenced by the type of animal, the age of the animal and also the type of nutrition of that particular animal from which the bone for this study was obtained. Although at an early stage of this investigation bones of different animals (chicken, plaice, sheep and calf) were used to produce bone ash, bovine bone which yielded the highest amount of bone

ash after burning was chosen as the best starting natural source to get hydroxyapatite. Unfortunately, there was no control on the age and nutrition of the calves from which the bone was obtained. Thus, further study is necessary to look at the influence of these biological factors on the morphology of hydroxyapatite. Changes in colour, morphology and also recrystallisation of bone minerals due to heating have also been reported by other researchers [25, 35]. Fig. 7(b) shows the SEM micrograph of the powder heated at 800 °C for 3 hours, and then milled for 1 hour. As seen, the particles had irregular shapes, including small spheres, agglomerated together in some parts. The sizes of individual particles were in the range of submicrometre to a few micrometres which are in good agreement with the results of the particle size distributions, presented in Fig. 6 and Table 5. Thus, the arithmetic mean diameters of the particles given in Table 5 should be considered as “agglomerate size rather than particle size. According to the data shown in Table 5 about 73% of the particles of the heat treated bone ash after 1 hour milling were less than 5 µm. Observation of Fig. 7(b) supports these data on particle size distribution.

DTA-TGA

The results of differential thermal analysis (DTA) and thermogravimetric analysis (TGA) of NHA are shown in Fig. 8. A small weight loss was detected by TGA and a small endothermic transition was observed in the DTA at low temperatures (less than 200 °C). The endothermic reaction at low temperatures is attributed to evaporation of adsorbed water [22-24]. Hu *et al.* investigated the thermal analysis of coral, as a part of their study in order to produce hydroxyapatite from coral, and reported that thermal analysis of coral shows three regions of weight loss [36]. The endothermic loss at 50-140 °C corresponds to the evaporation of absorbed water. They attributed the exothermic losses at 150-450 °C to the removal of organic compounds in coral and the endothermic loss at 600-750 °C to the decomposition of calcium carbonate to calcium oxide [36]. Olsen *et al.* [37] have also suggested that heating of bones results in weight losses at temperatures lower than 225 °C (due to water evaporation), at temperatures

between 225 °C and 500 °C (caused by the combustion of the organic components of bone) and at temperatures higher than 500 °C (as a result of the decomposition of structural carbonate by release of CO₂ gas). Clearly, the exothermic losses at 150-450 °C, observed by Hu *et al.* [36] which corresponded to the removal of organic compounds in coral, or at 225-500 °C, reported by Olsen *et al.* [37] due to the removal of organic components of bone, was not observed in the DTA-TGA curves of the present study because the organic components of bone were removed completely during heating the bone to produce bone ash as confirmed by the previous discussion of FTIR analysis. Further weight loss at high temperatures (800-1100 °C) can be due to partial dehydroxylation of hydroxyapatite [24]. This dehydroxylation was also confirmed by the results of FTIR study, as seen in Fig. 2(c) and Fig. 2(d). Thus, the results of DTA-TGA in this study indicated that the NHA produced here is stable up to 1100 °C. It should be mentioned that when the bone ash was heated at 1100 °C for 3 hours, a peak with low intensity attributed to β -tricalcium phosphate just began to appear as discussed previously (Fig. 3). Haberkorn *et al.* [22] reported an exothermic transition due to the decomposition of CaCO₃ in the DTA curve of natural hydroxyapatite extracted from pig bone. They showed that by heat treating the material at 700 °C and at higher temperatures the concentration of carbonate groups decreased and as a result one peak corresponding to CaO appear in their XRD pattern. Also, their results showed that for the sample heat treated at 800 °C the concentration of carbonate groups and also CaO is very low, ~2% and 0.1%, respectively. Dissociation of calcium carbonate at temperatures between 400 and 600 °C in air has been reported by some researchers [24, 36]. Krishna *et al.* [16] also observed a 45% weight loss between 50 and 830 °C and a sharp endothermic peak at 830 °C in their DTA, during their study of hydroxyapatite synthesis from eggshell, and explained their results in terms of decomposition of calcium carbonate to calcium oxide. There is no exothermic reaction related to carbonate decomposition in the DTA curve of the natural hydroxyapatite investigated in this study. However, the presence of carbonate groups in NHA was detected by FTIR analysis. During heat treatment of the bone ash at 800 °C for 3 hours, carbonate groups were decomposed and their concentrations were decreased which resulted in the formation of 1 % CaO in the XRD spectra. The results of this DTA-TGA study indicated that NHA produced here had sufficient thermal stability up to 1100 °C.

Conclusions

This study showed that bovine bone can be used as a natural source for production of hydroxyapatite. The process requires burning bone to remove its organic components to get bone ash which can subsequently be heat treated to produce hydroxyapatite acceptable for use

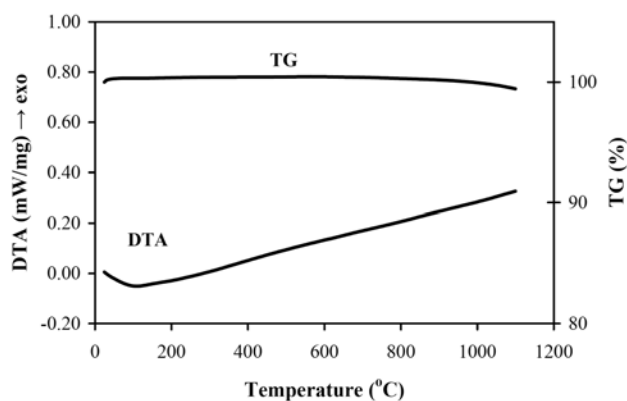


Fig. 8. The DTA-TG curves of NHA.

in orthopaedic and dental applications. The results of this study showed that the optimum heat treatment temperature to prevent phase transformation of hydroxyapatite prepared from bone ash is 800 °C. In addition, heating the bone ash at temperatures up to 1100 °C indicated the fact that this natural hydroxyapatite (NHA) is stable at temperatures lower than 1100 °C. The size of NHA particles can be changed by milling in order to get a particle size suitable for a specific application.

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