

# Synthesis of Hydroxyapatite through Ultrasound and Calcination Techniques

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**Abstract.** There is a growing demand for hydroxyapatite (HA) especially in medical applications, production of HA which is totally green is however a challenge. In this research, HA was produced from biowaste through ultrasound followed by calcination techniques. Pre-treatment of the biowaste was effectively achieved through the help of ultrasound. After calcination at 950°C, the obtained HA was characterized through Thermogravimetric (TGA) analysis, X-ray diffraction analysis (XRD) and Fourier transform infrared spectroscopy (FTIR). Spectrum of the produced HA was compared with standard HA index. The spectrum is in agreement with the standard HA as confirmed through FTIR, XRD and TGA result. Furthermore, morphological study of the HA through Field emission scanning electron microscope (FESEM) shows almost uniform spherical shape for the HA as expected. Based on the results obtained herein, combining ultrasound with calcination can help to produce pure HA with potential medical applications without the use of any organic solvent.

## 1. Introduction

Biomaterials based on inorganic calcium orthophosphate are currently being investigated due to their broad range potential application in the field of medicine especially as bone replacements materials. Hydroxyapatite (HA), is in the forefront of these investigations [1, 2]. Specifically, HA has been reported to exhibit highly desirable bioactive, nontoxic, noninflammatory, biocompatible, nonimmunogenic and properties. These properties make HA an important candidate for good osteointegration [3-5]. There have been several reports on the production of HA either from synthetic or natural sources. It was however reported that most of the synthetic techniques are either time consuming, complicated or biologically unsafe leading to safety issues as well as high cost of the produced HA [6, 7].

To overcome this challenge, HA is being extracted from natural sources with potential benefits such as economic feasibility, possibility for large scale production and environmental friendliness [8]. Extraction of HA from biowastes is often achieved through ordinary calcination. Other methods which may be used are subcritical extraction and alkaline hydrothermal processes [6]. However, most of the reported researches often use chemical solvents for fat removal, defleshing/cleaning of the waste materials before extraction. On the other hand, it has also been reported that unlike natural bones, the synthetic approach often eliminate carbonate groups from the obtained HA which may negatively influence the biological features of the apatite obtained [6].

Although research on HA synthesis from natural sources and biowastes date back to about 4 decades ago [10], as much as we know the potential of ultrasound technique have not been exploited. Interestingly, ultrasound method has been perceived to be suitable for technically feasible, easy and environment benign treatment of biowaste before extracting HA from them. Ultrasound technique is



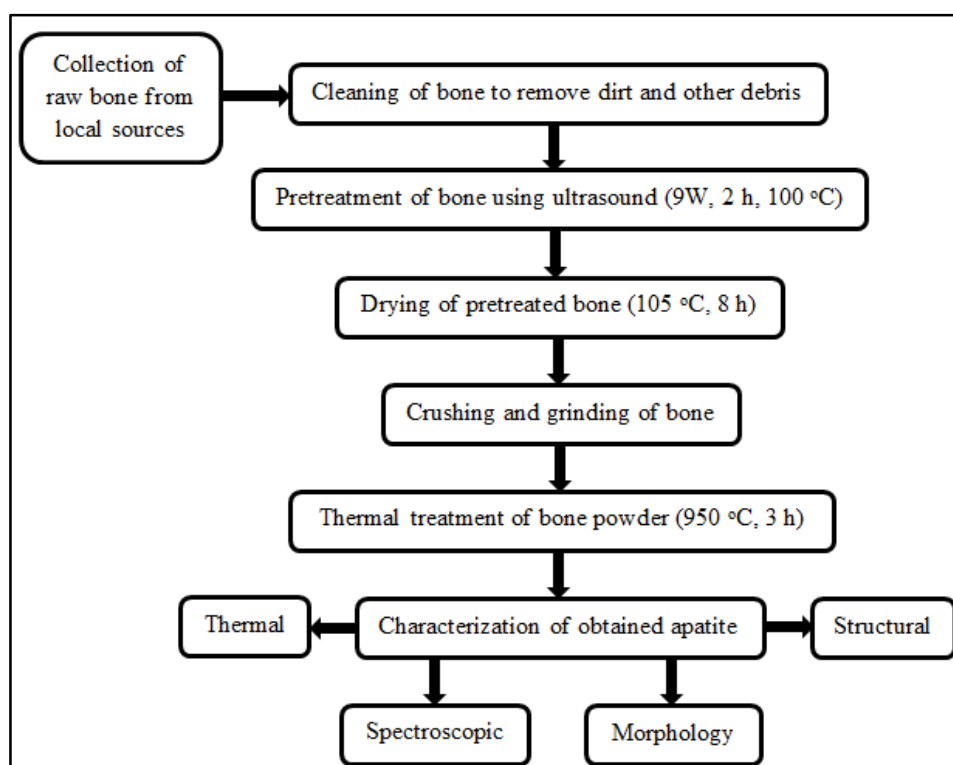
well established in separation technology but has been recently observed to exhibit certain features necessary for biowaste treatment [11, 12].

Some potential benefits of ultrasound include better purification with very little or no alkali concentrations, property enhancement, faster reaction rate and economic feasibility [12]. Combining ultrasound treatment with calcination process is therefore thought to be a great idea for an organic solvent free extraction of HA from biowastes. The aim of this research was to combine the potential of ultrasound with calcination process to synthesize environment benign HA from biowaste namely cow bone and to investigate the properties of the produced HA.

## 2. Materials and Methods

### 2.1. Bone Preparation and production of hydroxyapatite

The femur of an about 3 years old cow was collected from Jaya Gading, Kuantan, Malaysia. After initial drying in air for 48 h, the bones were oven dried for 8 h at 60 °C. After this, an Ultrasonic Bath operating at power of 9 W was used to agitate the bones in order to remove fats, flesh, and other adhering materials from the bone. This was continued for 2 h at 100 °C in a water medium ensuring a ratio of 1:50 (w/v) for bone and water respectively. An aluminium sponge in 40 °C distilled water was used to wipe the ultrasound treated bones and then left to dry normally for 24 h after which they were oven dried at 105 °C for 8 h. Grinding was carried out with the help of a Germany Retsch ultra centrifugal mill (ZM 200) after which the bone powder was sieved to obtain a particle size of about 450 µm. The HA was then obtained through thermal treatment of the bone powder via calcination process. This was carried out at 950 °C in a laboratory furnace for 3 h. The obtained material was stored for further analysis whereas an untreated sample of the bone powder was also stored for comparison. The flow chart of the experimental procedure is shown in Figure 1.



**Figure 1.** Flow chart of experimental procedure

### 2.2. Thermogravimetric analysis

Thermogravimetric analysis was conducted on the samples using a TA analyser (TGA Q500 V6.4, Germany). About  $5 \pm 2$  mg samples were placed in a platinum crucible and analysis was carried out

under nitrogen atmosphere at a flow rate of 40 mL min<sup>-1</sup>. This was conducted from room temperature till 1000 °C at 10 °C min<sup>-1</sup>.

### 2.3. Fourier Transform Infrared (FTIR) spectroscopy

Functionality of the samples was investigated through FTIR analysis. This was used to study differences in functional groups of the obtained material and the raw bone powder through their spectra analysis. It was also used to identify possible differences between the produced HA and standard HA. The KBr method was used to obtain IR spectra at a wavelength range of 400-4000 cm<sup>-1</sup>.

### 2.4. Field emission scanning electron microscopy

Surface morphology of the samples was done through FESEM analysis. FESEM analysis was conducted with a ZEISS, EVO 50 scanning electron microscope. To prevent electrical discharge, the samples were first dried to remove moisture and then sputter coated with platinum prior to FESEM examination.

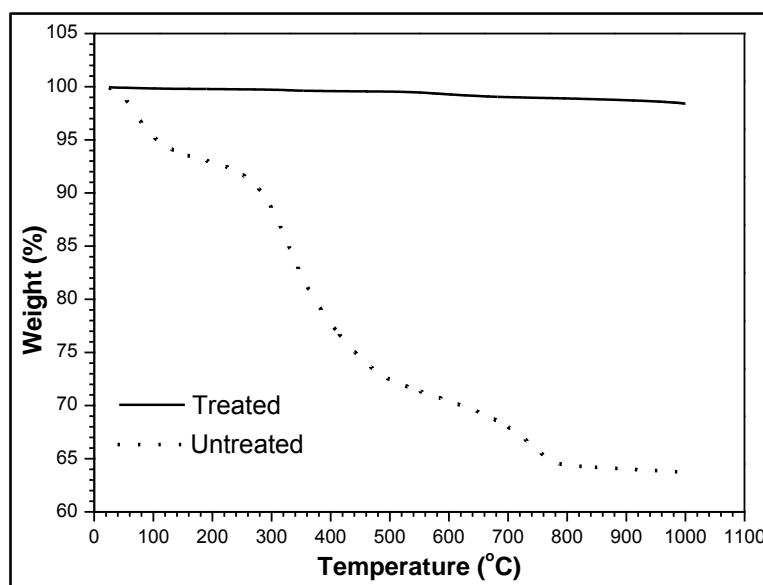
### 2.5. X-ray diffraction analysis

X-ray diffraction analysis (XRD) was conducted using an X-ray diffractometer at 1deg min<sup>-1</sup> over a range of 3-60° at sampling step of 0.02°. For phase identification, spectrum of the obtained material was validated against the XRD spectrum for standard HA (ICCD standard HA, DB card number 01-074-9761).

## 3. Results and Discussion

### 3.1. Thermo gravimetric analysis (TGA)

Figure 2 illustrates the TGA spectra for the untreated and treated bone powder. As can be seen from the Figure for the untreated sample, a weight loss occurred from about 50-200 °C which is due to release of adsorbed surface moisture. This stage of weight loss was not significant in the spectrum of the treated sample indicating that the sample is totally dry. Around 250-500 °C the spectrum of the untreated sample showed another stage of weight loss which is attributed to the burning of organic materials present in the raw bone powder. This also was not significant in the spectrum of the treated samples which indicates that organic components have been removed from the treated material. Generally for both samples, a weight loss occurred from 600-800 °C which may be associated with endothermic dissociation of trace  $\text{CO}_3^{2-}$ .

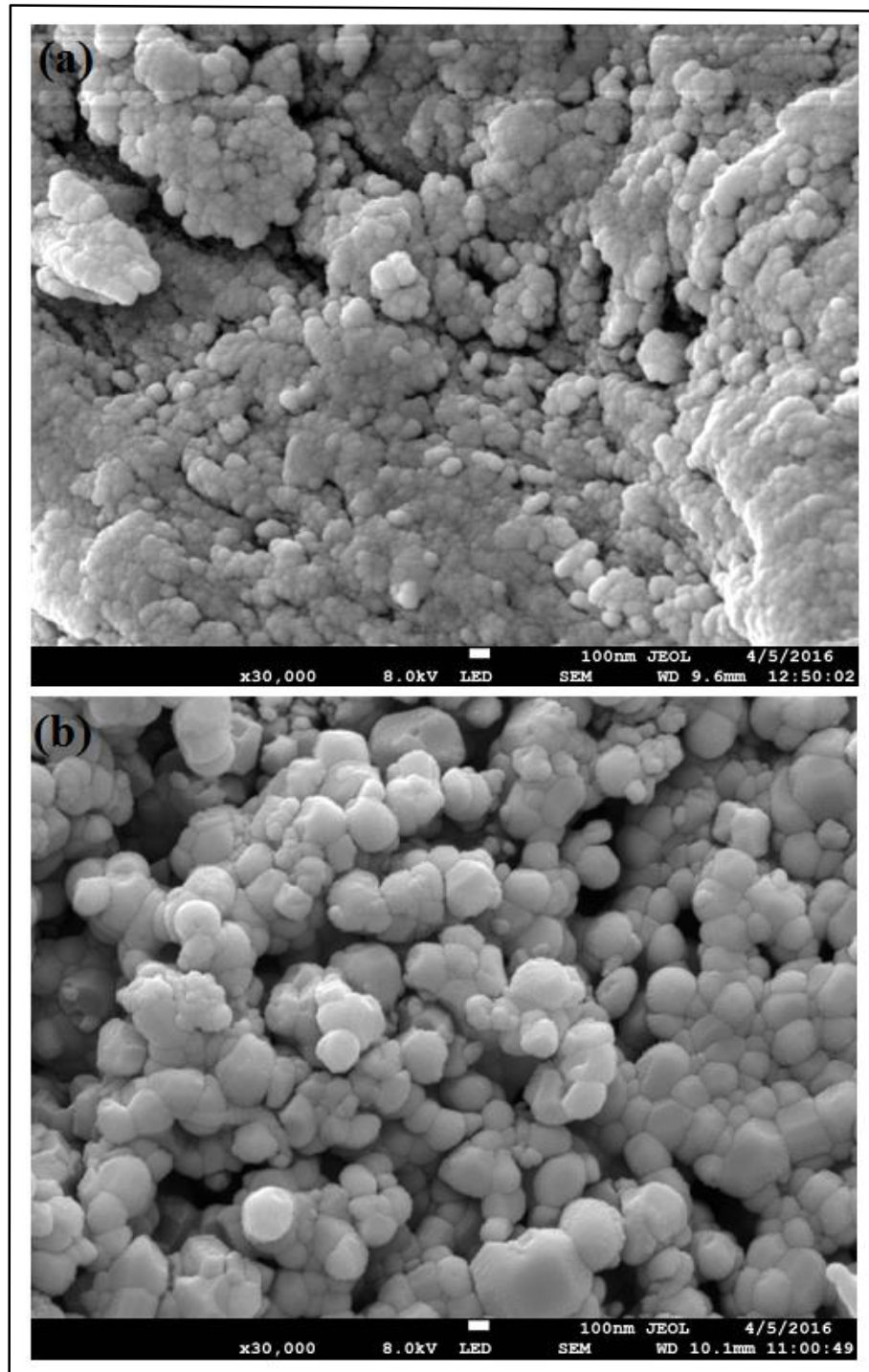


**Figure 2.** TGA spectra for untreated and ultrasound/calcination treated cow bone powder

It was reported in literature that decomposition of  $\text{CO}_3^{2-}$  often take place around 400-600 °C in air and 500-890 °C under nitrogen atmosphere [13]. This aligns with the result obtained in this study and it indicates that the synthesized material HA exhibits certain percent of  $\text{CO}_3^{2-}$  ions. It is worthy of note that on the overall, there was no much pronounced weight loss due to endothermic and exothermic reaction in spectrum of the treated sample. This is an indication of good thermal stability of the obtained material as expected of chemical HA. The overall weight loss for the two samples after 800 °C is 35.5% and 1.0% for the untreated and treated samples respectively. From literature review, it was reported that bone generally consists of about 30-40% organic components and about 60-70% inorganic components [14, 15]. Therefore, the higher amount of weight loss recorded for the untreated sample can be attributed to the presence of larger percent of organic constituents in the untreated bone powder compared with the treated sample.

### 3.2. *Field emission scanning electron microscopy*

The FESEM images of the untreated and treated samples are shown in Figure 3. It can be seen from Figure 3 that the produced HA reveals a spherical morphological shape which is nearly uniform and with better dispersion than the untreated sample. HA have been reported to exhibit different shapes with respect to the synthetic method. The spherical shape obtained herein is therefore acceptable [20, 21]. It is interesting to note that the treated sample exhibits reasonable interconnectivity such as those found in natural bone apatites. In addition, it shows great increase in size compared with the untreated sample. This is because HA particles tend to crystallize and grow up at higher temperature as reported in literature [6, 9].



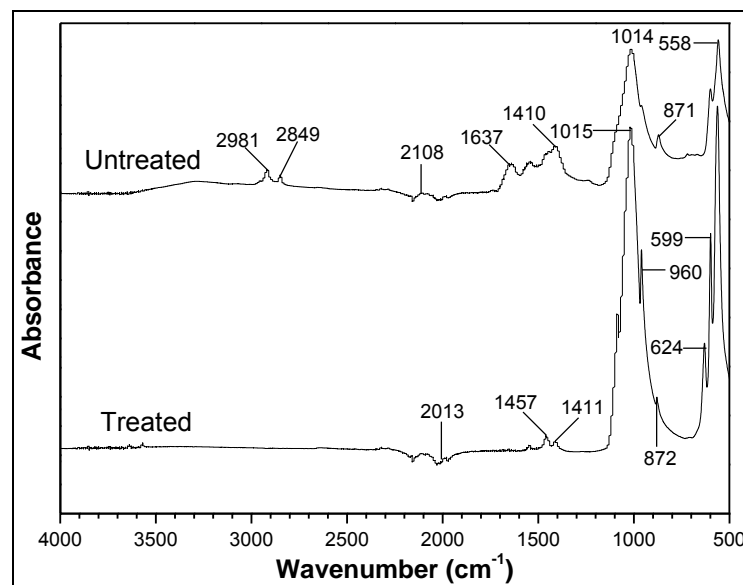
**Figure 3.** FESEM images for (a) untreated and (b) ultrasound/calcination treated cow bone powder

### 3.3. Fourier transforms infrared spectroscopy analysis

The FTIR spectra of the untreated and treated samples are illustrated in Figure 4. The band at higher wavelength from 3200-3500  $\text{cm}^{-1}$  is an attribute of  $-\text{OH}$  stretching vibration of bonded hydroxyl groups [11]. This functional group can also be seen at 1637  $\text{cm}^{-1}$  in the spectrum of the untreated sample. This indicates the presence of adsorbed surface water on the untreated bone powder [9, 18], as supported by the TGA result of this study. Also, this peak may be attributed to  $-\text{OH}$  groups from the HA structure [19]. At 2840-2930  $\text{cm}^{-1}$  there is a low intense peak which is characteristic of stretching vibrations from C-H bonds [11]. Presence of this band in the spectrum of the untreated sample indicates the presence of organic materials in the untreated bone powder as indicated by the

TGA results. However, this band was not present in the spectrum of the treated sample. This confirms the full removal of organic materials from the bone powder after thermal treatment [19]. The double split peaks which can be seen at  $1457\text{ cm}^{-1}$  and  $1411\text{ cm}^{-1}$  are characteristics of asymmetric  $\text{CO}_3^{2-}$  stretching and this shows that  $\text{CO}_3^{2-}$  was substituted into the lattice of the obtained product. This conforms to the results obtained through TGA and XRD analysis of this study. It is also in agreement with previous research work [18]. The peaks at  $1015\text{ cm}^{-1}$  and  $960\text{ cm}^{-1}$  are attributed to stretching vibration of phosphate  $\text{PO}_4^{3-}$  ions whereas the peak around  $550\text{-}650\text{ cm}^{-1}$  are attributed to phosphate deformational vibrations [7, 9]. The peak at  $872\text{ cm}^{-1}$  further indicates the ionic substitution of  $\text{CO}_3^{2-}$  into the obtained product. This is typical of type –A carbonate substitution, and suggests the presence of surface labile  $\text{CO}_3^{2-}$  in the material obtained after treatment [9].

Calcium phosphates including HA exhibits a compositional structure which have been reported to be very similar to natural bones [17, 20]. Based on the observations reported herein, it can be concluded that the obtained material is structurally similar to HA and can therefore be exploited for bone replacement purposes.



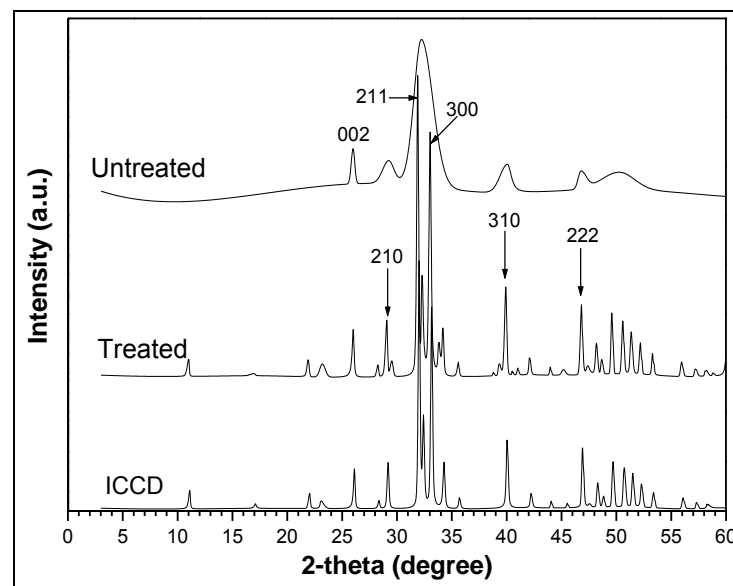
**Figure 4.** FTIR spectra for untreated and ultrasound/calcination treated cow bone powder

### 3.4. X-ray diffraction analysis

Figure 5 illustrates the XRD diffractograms for the untreated and treated samples as well as a standard XRD spectrum for HA (ICCD standard HA, DB card number 01-074-9761) in order to validate the synthesized product. As can be seen in Figure 5, XRD pattern of the treated sample is very intense which suggests the crystalline nature of the produced material [16]. The characteristic peaks of standard HA can be seen in the spectrum of the produced material. This indicates that ultrasound treatment followed by calcination was effective to remove collagen and other organic components from the raw bone powder without causing undesired disruption to the molecular skeletal of the obtained HA [21]. Based on the close resemblance of the peaks at  $2\theta$  regions 26, 29, 32-34, 40 and 47-54 with standard HA, we can infer that the obtained product is hydroxyapatite (HA) [22].

On the contrary, spectrum of the untreated sample as shown in Figure 5 can be seen to be broader and short of some peaks from the standard HA spectra. This is attributed to the presence of fibrous collagen in the untreated sample which might lead to undesired dispersion of the X-ray radiations [6].





**Figure 5.** XRD spectra for untreated, ultrasound/calcination treated cow bone powder and standard HA

#### 4. Conclusion

Hydroxyapatite (HA) was extracted from biowaste namely cow bone through a combination of ultrasound treatment in ordinary water medium and calcination process at 950 °C. Treatment with ultrasound was suitable for proper cleaning of the raw cow bone. The HA obtained after calcination at 950 °C for 3 h revealed highly desirable properties in term of morphology, chemical structure and compositional constituents. Results of XRD and FTIR are both consistent and revealed that chemical features of the produced HA conform to the standard HA. Also, there is good agreement in the main phase of the synthesized HA and standard HA as confirmed through all the characterization techniques employed herein. Based on the TGA and FTIR results, it is evident that HA obtained through ultrasound and calcination processes can help to avoid the total elimination of  $\text{CO}_3^{2-}$  from the HA which will help to enhance the biological properties of the HA. More importantly, the total avoidance of organic solvent through the techniques employed herein can be a way forward on how to reduce the amount of chemicals associated with conventional HA synthesis. Moreover, use of biowaste for HA synthesis can offer both economic and environmental benefits and the HA obtained exhibits desired features for medical application.

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