



Facile Extraction of Natural Hydroxyapatite (HA) from Biowaste and Its Characterizations

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Abstract

Natural Hydroxyapatite (HA) with chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is a major mineral storage form of calcium and phosphorous in bone and it has been applied in many fields. In the present study, HA was extracted from biowaste; namely the buffalo bone by alkaline hydrolysis process followed by calcination at 900°C with holding time 6 hrs. in muffle furnace. The isolated material was analyzed by X-ray Diffraction (XRD), Fourier Transform Infra-Red Spectroscopy (FTIR) and Field Emission Scanning Electron Microscopy (FESEM) etc. The SEM image suggested the isolated material had rod shaped and agglomerated. FTIR study indicated the phosphate and hydroxide were the major functional groups along with carbonate present in it. Further, XRD analysis inferred that the isolated material was only HA phased. The specific surface area of the isolated HA was determined by methylene blue adsorption method and it was found to be $141.12 \text{ m}^2/\text{g}$ and pH point of zero charge (pH_{PZC}) of the extracted biomaterial was determined to be 6.8 by pH the drift method. Thus the pure HA powder was synthesized by an environmentally friendly, cost effective method and may be applicable in many fields including waste water treatment and biomedicine etc.

Keywords: Natural hydroxyapatite, waste bone, calcination, characterizations, cost effective

Introduction

Hydroxyapatite (HA) is the primary mineral component of bone and teeth (Kantharia et al., 2014) and the main factor responsible for their hardness and strength (Ibrahim et al., 2020). This bio-inspired material is of great interest in many fields because of its remarkable structure and inherent properties. It has been used in the replacement of bone tissue reconstruction of skull defects, tissue engineering, cultivated bone synthesis, biosensor, and removal of heavy elements from polluted water and as drug carrier etc (Fiume et al., 2021)(Ma et al., 2010). Hence the synthesis of HA, with its various morphologies and textures, has encouraged many academic and industrial researchers for numerous applications. Some of the important synthetic methods for the preparation of HA are dry methods, wet methods (co-precipitation, sol gel, emulsion etc), energy input methods etc (Malla et al., 2020). Many of the above mentioned synthesis processes are complicated, costly, environmentally hazardous or biologically unsafe processes. Therefore, recently natural hydroxyapatite has been extracted by normal calcination of some bio-wastes namely bovine bones (Rana et al., 2017) and bones of pig (Lim et al., 2014) etc as such extraction process of HA from bio-waste which is economically and environmentally preferable (Barakat et al., 2009). Hence, in the present study alkaline hydrolysis followed by calcination process was used for the extraction of HA from waste buffalo bones as biowaste.

Material and methods

Extraction of HA

The extraction of HA from Femur bone of buffalo was carried out by alkaline hydrolysis followed by calcination process as describe elsewhere. In short femur part of bone were taken from slaughter house, in Kathmandu. It was cleaned with water and then several times with acetone to remove fats and other impurities. The clean

dried bones were grinded and sieved with 150 micrometer pore size. The bone powder was then hydrolyzed by 4M NaOH with 1:40 (Solid: Liquid) at 250°C for 4-5 hours. The neutral deproteinized and defatted bone powder was then calcined at 900°C for 5 hours in a muffle furnace to obtain white powder of HA (Barakat et al., 2009, Parajuli et al., 2022). The extraction process photographs are summarized in figure 1 below.

Structural Characterizations

For the determination of different the functional groups present in the prepared HA, FT-IR analysis was carried out in the Central Department of Chemistry, Kirtipur, Kathmandu. Morphology of the isolated HA was investigated by using JEOL JSM-6301F microscope with accelerating voltage of 10 kV in Japan. The phase identification of the isolated material was carried out by X-ray Diffractometer with monochromatic $\text{Cu K}\alpha$ radiation at Nepal Academy of Science and Technology (NAST). The samples were scanned at 2θ from 20 to 80° .

Determination of pH_{PZC}

For the determination of pH_{PZC} , the pH drift method was applied. In this method with the help of a glass electrode and pH meter, the pH of 0.01 M NaCl was adjusted from 2 to 9 by adding either HCl or NaOH and then required amount of HA was added to 50 ml of the different pH solutions. The whole contents were stirred for 24 hrs and the final pH of the different solutions was again measured after filtration. The differences between initial pH ($\text{pH}_{\text{initial}}$) and final pH (pH_{final}) were calculated for all pH solutions. The cross point between the curve and the straight line originating from the zero difference in pH parallel to x-axis gave the value of pH_{PZC} (Fiol & Villaescusa, 2009).

Determination of specific surface area of HA

Spectrophotometric determination of specific surface area from Methylene Blue adsorption method was applied (Methodology, 1998). The determination of specific surface area, the maximum absorbance wavelength (λ_{max}) as well as calibration plot of methylene blue solution were carried out. In this method, small amount of HA as adsorbent is added to the varying concentrations of methylene blue solution. The solutions are kept in mechanical shaker for 24 hours. The resultant supernatant solution is pipetted out and the absorbance is taken at 665 nm wavelength. With the help of calibration curve, the linearized Langmuir adsorption isotherm plot for methylene blue is plotted. The specific surface area is generally calculated by the following equation which is given as:

Where, S_{MB} is the specific surface area in $10^{-3} \text{ km}^2\text{kg}^{-1}$, N_g is the number of molecules of methylene blue adsorbed at monolayer of adsorbent in kg, a_{MB} is the occupied surface area of one molecule of methylene blue N is Avogadro's Number, M is the molecular weight of the methylene blue and N gives mmol/g which is equivalent to Q_m of the Langmuir equation (eq-2).

$$\frac{C_e}{Q_e} = \frac{1}{Q_m \cdot b} + \frac{C_e}{Q_m} \quad (\text{eq-2})$$

where C_e is the equilibrium concentration of adsorbent in mg/L, Q_e is the equilibrium mass of adsorbent in mg/g, b is the Langmuir binding constant (L/mg), and Q_m is the maximum mass of adsorbed metal ion in mg/g (Karmacharya et al., 2016).

Results and discussion

Characterizations of HA

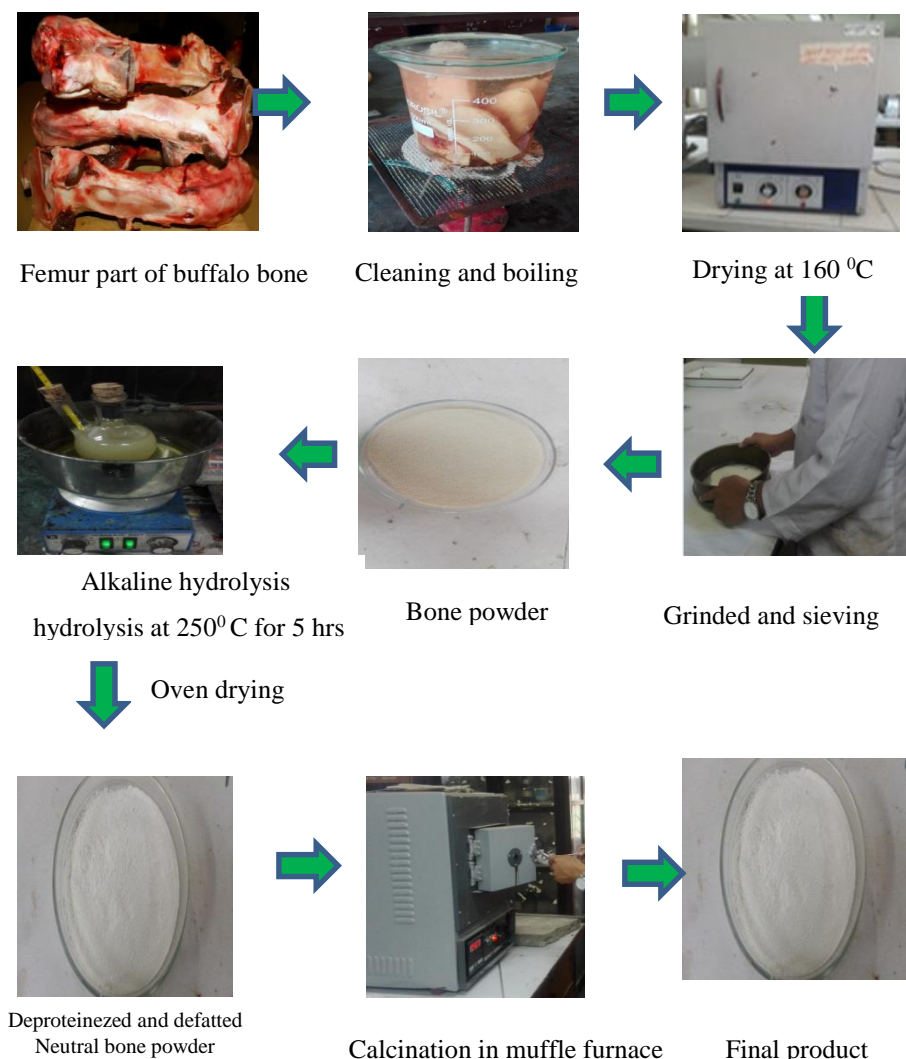


Figure 1: Photographs of procedure for synthesis of HA from waste buffalo bone

$$S_{MB} = \frac{N_g \times a_{MB} \times N \times 10^{-20}}{M} \quad (\text{eq-1})$$

The white powder prepared in lab by the alkaline hydrolysis process followed by calcination of waste bone was characterized via different scientific tools such as FTIR, SEM, XRD studies along with the determination of pH_{pzc} and specific surface area of the extracted HA.

with the reported SEM images and found to be good agreement

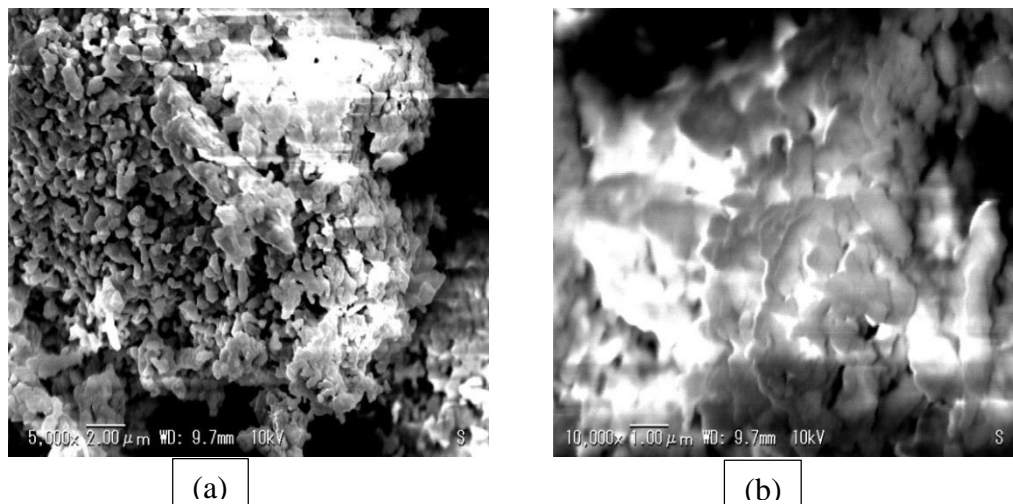


Figure 3: SEM images of *sample* (HA) (a) Low magnification (b) high magnification

FTIR Study

Figure 2 represents the FTIR analysis of the alkali treated raw bone powder, and alkali treated and then calcinated at 900°C bone powder (HA). The presence of PO_4^{3-} , CO_3^{2-} and OH^- absorption bands in both samples are seen but these bands are more clearly visible in calcined bone powder as the calcination process destroys the cross linked structure present in raw bone (Khoo et al., 2015). Broad absorption band around 3350 cm^{-1} of the raw bone powder is due to absorb water molecule along with OH^- group present in it. This broad absorption peak seems to be removed in (HA) sample due to release of absorbed water molecule at high temp however the small peak around 3584 and the 632 cm^{-1} are still present in calcinated sample due to vibration of OH^- group present in the sample (Adenan et al., 2018). The bands appeared in the range of $962\text{--}1105\text{ cm}^{-1}$ indicated the phosphate (PO_4^{3-}) groups stretching and 470 cm^{-1} showed the bending of phosphate groups (Bahrololoom et al., 2009). Similarly the organic impurities that are seen in raw bone powder are off in the calcinated sample. Further, the intensity of duplet peaks at $1400\text{--}1600\text{ cm}^{-1}$ range in raw bone char were decreased as calcination removed the CO_3^{2-} present in the raw sample (Markovic, 2004). As the required absorption bands for HA are clearly present the calcined sample, further characterizations were carried out only for the calcined sample (HA).

SEM analysis

The morphology of the isolated particles (HA) by using the SEM is shown in figure 3.

From the result of SEM, it was found that the morphology of obtained HA particles had rod shaped with alveolate agglomerate which might be due to the Oswald ripening. It was shown that the powder form of HA particles contained mixture of particles with different sizes and aggregation which induced the porous agglomerate structure. The obtained SEM images was compared

with those image (Shavandi et al., 2015).

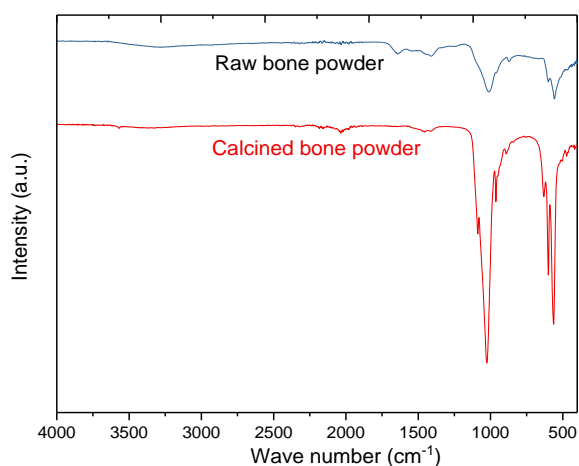


Figure 2: FTIR spectra of raw bone powder sample and calcined sample (HA)

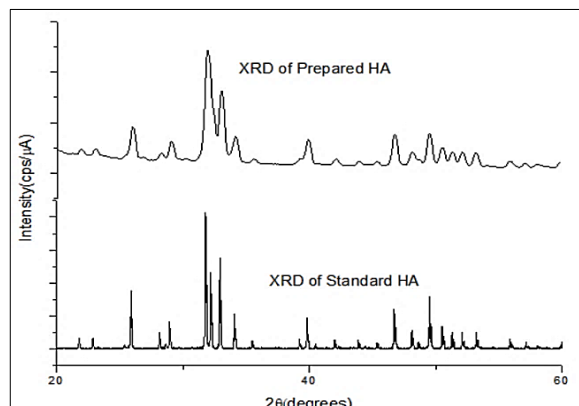


Figure 4: Comparing XRD of prepared hydroxyapatite with standard one

XRD analysis

The XRD of sample (HA) is nearly matched with the standard XRD of hydroxyapatite [JCPDS card No. 09-0432] and is presented in the Figure 4. The broad diffraction bands at $2\theta = 26, 32, 33, 34, 40, 47$ and 49 in XRD analysis showed the strong evidence for the formation of HA as there are similarity of peaks of sample with standard HA and proved that the formed compound is indeed only HA phase (Adenan et al., 2018).

pH point of zero charge (pH_{pzc}) determination

The point of zero charge defines the condition of the solution (in particular, the pH value) for which the surface of the material possess no charge. In fact it is often regarded as a characteristic parameter for a given surface in a given aqueous solution Thus, depending on the pH, their surfaces might be positively or negatively charged, which has a direct influence regarding the interactions of electrostatic ions. At $pH > pH_{pzc}$, the material surface becomes negatively charged, and on the other hand, surface becomes negatively charged values of $pH < pH_{pzc}$ (Parajuli et al., 2020).

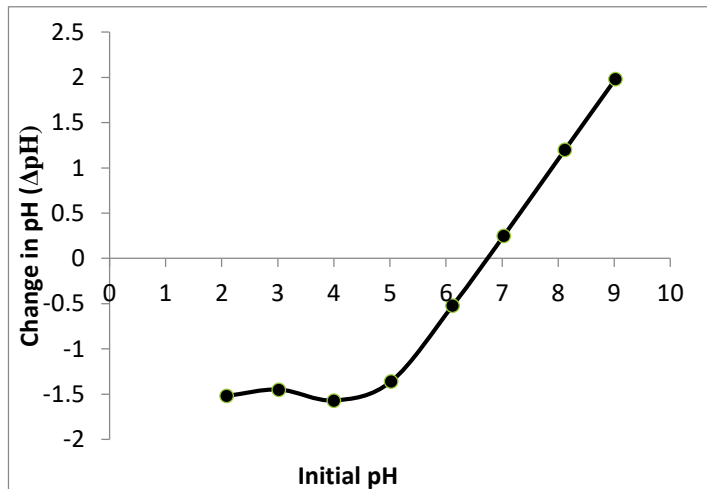


Figure 5: pH_{ZPC} plot of HA by pH drift method

Determination of specific surface area

In this study, pH_{ZPC} of HA was found to be about 6.8 which is obtained by plotting change in pH versus initial pH and shown in figure 6. The results obtained in this study is slightly higher than reported in the literature data (Skwarek et al., 2017). Moreover the pH_{pzc} value obtained was in accordance with the standard value where pH_{pzc} value for HA was found to be in the range of 6.4-8.5 which depends upon the condition of preparation, washing and storage of apatite materials (Bell et al., 1973).

The maximum absorbance of methylene blue was obtained at 665 nm which is in trend with the previous reported value (Methodology, 1998). With the help of calibration curve, the linearized Langmuir adsorption isotherm plot (figure. 6) for methylene blue as adsorbate, the specific surface area of Hydroxyapatite was evaluated by applying the eq-1 and the specific surface area of the adsorbent material was found to be $141.1 \text{ m}^2/\text{g}$. It was reported that the Langmuir surface area of HA with

average size 13.22 nm of about $167.11 \text{ m}^2/\text{g}$ which was synthesized from fish bone scales (Kongsri et al., 2013).

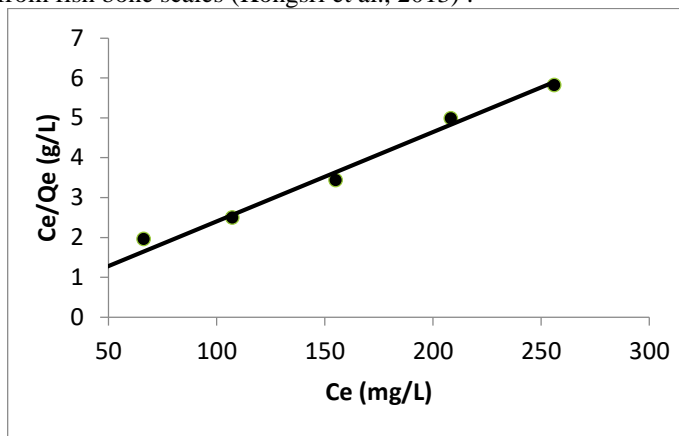


Figure 6: Langmuir adsorption isotherm of methylene blue onto HA

Conclusions

In present work hydroxyapatite was prepared from buffalo bone by alkaline hydrolysis method followed by calcination at 900°C . The HA thus prepared was then characterized by various spectrophotometric methods such as FT-IR, SEM, XRD as well as determination of point of zero charge by pH drift method and specific surface area of the isolated HA by methylene blue adsorption method. The broad diffraction bands at $2\theta = 26, 32, 33, 34, 40, 47$ and 49 degree in XRD analysis (based on ICDD 9-432) showed the strong evidence for the formation of HA. The morphology of prepared hydroxyapatite revealed that the hydroxyapatite contained rod shape with porous agglomerate structure from SEM analysis. The point of zero charge of hydroxyapatite was found to be 6.8 by pH drift method and specific surface area was found to be $141.1 \text{ m}^2/\text{g}$ by methylene blue adsorption method. Thus natural HA can be isolated from waste buffalo bone by simple alkaline hydrolysis of the followed by calcination process and it may applicable in many fields including waste water treatment and biomedicine etc.

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