# A Comparative Study on Hydroxyapatite Derived from Bovine Bones and Synthetic Sources

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**Abstract**

Hydroxyapatite (HA), a mineral component of bones and teeth, has been widely studied for various medical applications. The purpose of this research is to compare the HA from diverse bovine sources and chemical synthetic in the respectively physical and chemical powder properties such as grain size, morphology, crystallinity, phase stability and chemical functional groups. Bovine HA (B-HA) were extracted from the fresh femur bones of adult bovine, calf and bovine bone bio-waste. Synthesized HA (S-HA) were prepared by chemical precipitation method with the pH 6.0 and 12.0 of mother liquor. All of HA samples then were calcined at 800 °C. The TEM observation illustrated that particle shapes and sizes of HA differed depending on their bovine sources. In addition, XRD and FT-IR results implied that pure HA have been successfully obtained in B-HA group while S-HA with high pH value of 12.0 occurred the phrase transformation after thermal treatment.

**Keywords**

Hydroxyapatite • Chemical reaction • Bovine bone

## 1 Introduction

HA is an abundant mineral part of human hard tissue with its chemical formula of $Ca_5(PO_4)_3(OH)_2$ and molar ratio Ca/P of 1.67 [1, 2]. HA can directly bond with native bone without formation of collagen interface layer, which usually appear in cases of many bio-inert material implantations [3]. Due to these properties, HA has been used for decades as an alternative biomaterial for bone grafting [4, 5]. In fact, HA can either be synthesized from the chemical reactions or derived from the natural sources [6]. The laboratory synthesized HA can be obtained by using precipitation [7], ultrasonic [8], spray drying [9], sol-gel [10], multiple emulsion [11] and microwave assistant [12]. It is worth noting that the structural and the mechanical properties of synthetic HA can be modified by varying the processing method. However, these synthesis procedures might be complicated because of dependence on many factors such as the starting chemicals, concentration of the initial solution, pH maintenance and synthetic method. About the natural HA, it can be directly taken from common bio-waste sources such as eggshell [13], porcine [14], bovine [15], fish bone [16] by the one of the following methods as thermal decomposition [17], subcritical water process [18] or alkaline hydrolysis [19]. Besides, HA extracted from animal bone could be a non-stoichiometric material due to the presence of other calcium phosphate (CaP) phases or the trace ions such as Na, Mg, and Zn [20, 21].

Therefore, the manufacturing stage of HA from diverse sources and its final powder properties have been investigated as crucial factors to produce the desired biomaterials. There was some evidence that demonstrated the powder properties of nature HA corresponding to the different strains of animals. However, the factors influencing animal physique such as age have not been considered. Consequently, the primary purpose of this research is to compare the HA from different bovine sources in the respective physical and chemical properties such as grain size, morphology, crystallinity, phase stability, and chemical functional groups. The natural HA powders were obtained from fresh femur bones of adult bovine (HA-A), calf (HA-C) and bovine bones bio-waste (HA-W). In addition, the synthetic HA group with influencing factor of varying pH value at 6.0 and 12.0 were also prepared for the comparison."

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## 2 Materials and Methods

### 2.1 Materials

Calcium hydroxide ($Ca(OH)_2$, 98%) and phosphoric acid ($H_3PO_4$, 85–87%) was purchased from GuangDong GuangHua Sci-Tech Co., Ltd, China. The fresh femur bones of adult bovine (2–3 years old) and calf (10–12 months old) were collected from a slaughterhouse while the bovine bone bio-waste was from a local animal boneyard

### 2.2 Methods

**Hydroxyapatite synthesized by chemical reaction.** S-HA samples were obtained from chemical substances through a 4-step process included stirring, pH adjustment, microwave irradiation and calcination. Firstly, $Ca(OH)_2$ was mixed with $H_3PO_4$ at 1.67 of Ca/P ratio to produce a precursor precipitation. Throughout the mixing process for 2 h, the pH of the system was maintained at pH 6.0 and 12.0. After that, these mixtures were placed in a micro-wave at the power of 750 W for 25 min and then calcined at 800 °C for 3 h under ambient condition.

**Hydroxyapatite derived from bovine bone.** HA was extracted from bovine bone by the same method in the previous research [22]. Briefly, the fresh femur bones were washed and went to the 5-h boiling process in ambient condition and then 2-h boiling process under pressure. The boiling procedure was repeated three times. After that, they were calcined at 800°C for 3 h. The HA obtained from femur of adult bovine, calf, and waste bone was labeled as HA-A, HA-C, HA-W in turn.

**Characterization.** Firstly, the morphology and particle size of HA samples were analyzed by transmission electron microscopy (TEM) observation. Then, the crystal structure of each sample was characterized by X-ray diffractometer (XRD). Data were collected over the diffraction angles (2θ) from 20° to 80° with scanning speed of 2°/min. Fourier transform infrared (FT-IR) spectra of the samples were obtained with wave number from 4000 to 400 $cm^{-1}$.

## 3 Results and Discussion

In the Fig. 1, the TEM images show the size and morphology of HA particles with the magnification of 50.0 k times. HA at pH 6.0 and 12.0 had non-uniform shape distribution with early high agglomeration by forming neck between HA particles, bonding them together. It was, therefore, difficult to distinguish separate grain and the size of S-HA particles could not temporarily be estimated. In contrast with S-HA group, HA particles in B-HA groups had uniform spherical shape with their diameters varied according to the different bovine sources. In specific, the particle diameters of HA-A, HA-C and HA-W were approximately 200, 100, and 170 nm, respectively.

From these results, they suggested that S-HA would be easy to achieve the interconnection in microstructure under the thermal treatment. About the B-HA, the bovine sources

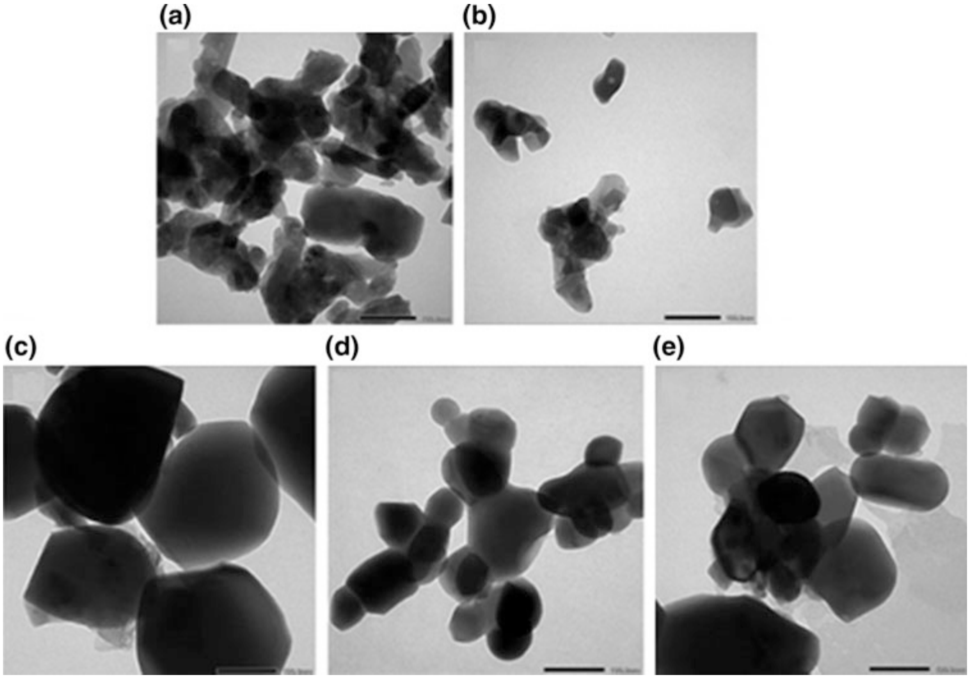

**Fig. 1** TEM images of HA with **a** pH 6.0, **b** pH 12.0, **c** HA-D, **d** HA-C and **e** HA-W at the magnification of 50.0 k times with scale bar of 100.0 nm

clearly had an impact on the calcination behavior such as the grain sizes of HA particles, which required for further investigations. Besides, the uniform spherical shape of HA particles, along with particle size, could be one of the important effect on their interactions with live cells [23].

The successful synthesis of HA from chemical reaction and bovine bone was confirmed by XRD and FT-IR analysis. In Fig. 2, the XRD patterns of all HA samples calcined at 800 °C exhibited in sharp diffraction peaks, indicating a high crystallinity, and well matched with standard peaks of HA (JCDS File No.9-432) [24]. Briefly, the peaks of pure HA at diffraction angles as 2θ of 21°, 25°, 28°, 29°, 31°, 32°, 35°, 36°, 37°, 39°, 40°, 42°, 43°, 45°, 46°, 48°, and 49° were fully showed in the spectra in both S-HA and B-HA. However, those of HA with pH 12.0 appeared strange peaks at the diffraction angle 2θ of 29.2°, 43°, 47.3°, and 48.3°, which suggested the generation of tri-calcium phosphate (TCP) as by-product after the calcination.

In Fig. 3, B-HA presents the FT-IR spectra of chemical functional groups in HA structure, which consists of hydroxyl ($OH^-$) and phosphate ($PO_4^{3-}$) band. For the ($OH^-$) band, two peaks at 3572 cm$^{-1}$ and 631 cm$^{-1}$ represented for hydroxyl stretching mode and vibrational mode (v1), respectively. Next, for the $PO_4^{3-}$ group consists of four vibrational modes v1, v2, v3, and v4. In detail, a peak at 954 cm$^{-1}$ represented for v1 vibration, a weak peak at 471 cm$^{-1}$ represented for v2 vibration, two peaks at 1087 and 1046 cm$^{-1}$ represented for v3 vibration, and a set of peaks at 638, 599, and 574 cm$^{-1}$ represented for v4 vibration. In addition, the carbonate ($CO_3^{2-}$) group can also be seen in the infrared spectrum. For v2 and v3 vibrations of $CO_3^{2-}$ group, a peak at 875 cm$^{-1}$ indicated for v2 vibration and two peaks at 1650 and 1300 cm$^{-1}$ indicated for v3 vibration [25]. The peak for v4 vibration of this group had very low intensity and hence it was not able to be seen.

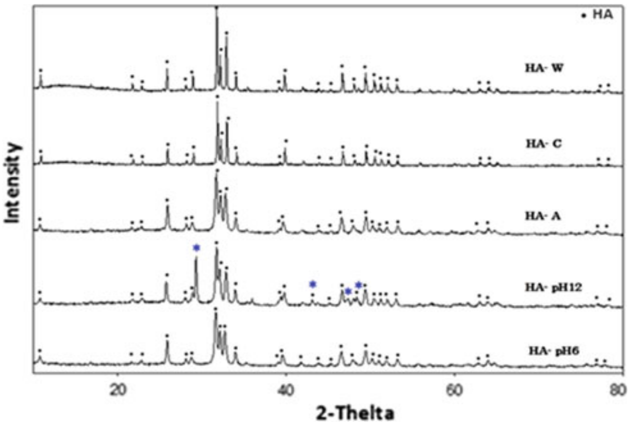

**Fig. 2** XRD results of all HA samples. Blue asterisks indicate not HA peaks at the 2θ angles of 29.2°, 43°, 47.3°, and 48.3°

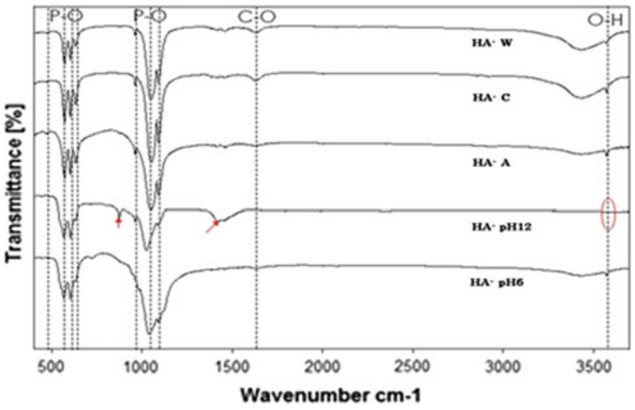

**Fig. 3** FTIR results of all HA samples. The red ellipse indicates the lack of hydroxyl band in the spectrum of HA with pH 12.0

Beside the peaks of the main structure of HA but lack of ($OH^-$) peak at 3572 cm$^{-1}$ as indicated by the red ellipse, the spectrum of HA with pH 12.0 noticeably presented the bands of $PO_4^{3-}$ stretch ν1 at 960 cm$^{-1}$ and $PO_4^{3-}$ bend ν3 at 1122 cm$^{-1}$ corresponding to TCP [26].

From the XRD and the FT-IR results, HA derived from bovine bones had no signs of HA phase decomposition into secondary phases such as α-TCP, β-TCP, TTCP or CaO after calcination. In opposition to S-HA, although the temperature of 800 °C had been carefully chosen to transform the precursor precipitation into HA and prevent the appearances of other calcium phosphate (CaP) phases, these results indicated that decomposition into other CaP phrases in S-HA group has still considerably occurred. Thus, this was not in agreement with some reports those had mentioned the decomposition of HA into secondary phases proceeded above 1100 °C [27], thereby suggesting that HA synthesized with high pH is thermodynamically less stable than bovine HA.

## 4 Conclusion

In this study, the different routes of HA was a critical factor influencing grain size, formation of microstructure and phase stability. Under the heat treatment, TEM images illustrated that S-HA group was accessible to achieve the microstructure by the grain interconnection while HA particles in B-HA group varied their uniform spherical shape according to the different bovine sources. XRD and FI-IR demonstrated that B-HA was beneficial in producing thermally stable phase of stoichiometric HA whereas the phrase decomposition took place in S-HA after calcination below 1000 °C. Thus, these primary results in this study suggest that the unique characterizations of natural and synthetic HA must be significantly considered to achieve the desired HA properties and the suitable stability sources for biomedical applications.

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**Conflict of Interest** The authors declare that they have no conflict of interest.

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