

소 뼈로부터 추출한 천연 hydroxyapatite bioceramic의 물리화학적 특성

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Physiochemical Characterization of Natural Hydroxyapatite Bioceramic Extracted from Bovine Bones

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1. Introduction

The synthesized hydroxyapatite (HAp) does not have the same biological properties of natural apatite present in bones. To enhance its biological properties, substitution of some foreign ions/groups is highly desirable [1]. The carbonate substitution is of particular importance because the mineral phase of bone, dentin and enamel mainly consists of carbonated hydroxyapatite (CHAp), containing 4 - 6 wt% carbonate [2]. Extraction of natural hydroxyapatite from bio-wastes by calcination process eliminates the carbonated hydroxyapatite which affecting the biological properties of the obtained apatite. In the present study natural apatites have been extracted from the bovine bones by subcritical water extraction and alkaline hydrothermal processes as new proposed methods as a trial to save the carbonate present in the natural HAp. Also, the conventional thermal method was applied to evaluate the apatites obtained by the proposed methods. The first method is the hydrothermal alkaline hydrolysis; this process was exploited to hydrolyze collagen like protein (i.e. keratin) in an autoclave [3] or by microwaves [4]. In this study, it has been applied to hydrolyze collagen in an autoclave. Subcritical water or pressurized low polarity water (PLPW) is a promising extraction and fractionation technique that uses hot liquid water under pressure. Increasing the water temperature up the normal boiling point causes changes in solvent polarity, surface tension, and viscosity [5]. Hence, the PLPW at various temperatures has been utilized to plucking the collagen out the bovine bones leaving the desired natural apatite. The third process is direct heating of the bovine bone to remove the collagen which can be thermally eliminated [6].

2. Materials and methods

Cylindrical hydrothermal stainless steel reactor was used in this process. The grinded bones were added to deionized water at solid to liquid ratio of 1:40. The reactor was tightly sealed and heated in silicon oil bath at 250 °C for 1 h. The reactor was cooled by quenching in big amount

of cold water. Thus obtained solid product was filtered, washed by distilled water and dried at 80 °C for 30 min. For the second proposed method, sodium hydroxide was used in the alkaline hydrothermal hydrolysis of collagen. The same aforementioned hydrothermal stainless steel reactor was exploited in this process. In the thermal decomposition process 1 gm sample was heated in the furnace to 850 °C for 1 h.

3. Results and discussion

Fig. 1 shows the FT IR spectra of pure hydroxyapatite, the raw pulverized and the treated bones by the three proposed methods. As shown from this figure, there are distinct differences between the spectra of the raw bovine bone and the treated ones. For more extensive study, the spectra were carefully analyzed to investigate the collagen bands. The collagen as a protein can be detected by FT-IR in three main amide regions amide I, II and III [7]. The amide I band, with characteristic frequencies in the range from 1600 to 1700 cm^{-1} , is mainly associated with the stretching vibrations of the carbonyl groups (C=O bond) along the polypeptide backbone and is a sensitive marker of the peptide secondary structure. It is known that there are typical bands at 1630 - 1640, 1650 - 1660 and 1680 - 1700 cm^{-1} in the amide I region of the protein. Fig.2.A shows the spectra corresponding to amide I, we have estimated the second derivative for the obtained spectra in Fig.2. to clarify the bands. As shown in these figures, some peaks appear in the spectra of the raw bone within the aforementioned corresponding ranges for amide I. Amide II bands appear within the range of 1500-1600 cm^{-1} , for this amide protein have distinct peaks at 1521, 1533, 1539, 1559 and 1576 cm^{-1} . Similarly, all the peaks corresponding to the amide II clearly appeared in the raw bone spectra. In the amide III band of protein (within range of 1200 - 1360 cm^{-1}) there are bands at 1229 - 1235, 1243 - 1253 and 1270 - 1300 cm^{-1} . As in the case of Amides I and II, all the peaks assigned at the aforementioned ranges. From the aforementioned detailed analysis of the IR spectra, it might be concluded that the proposed extraction methodologies and the traditional one can be invoked to remove the collagen from the bovine bone. Presence of carbonate group in the carbonated hydroxyapatite is either at the phosphate tetrahedron (B-type) or at the hydroxyl site (A-type). Biological apatites have both types; however, B-type is more abundant. As shown in Fig. 3.A the intensity of the OH stretching band is very small in the spectra of bovine bone treated by the subcritical water (Sub), moderate for the alkaline hydrothermal one and high in the spectra of calcined bovine bone (Cal). Fig. 3.B shows the bands of the carbonate $\nu_2 \text{CO}_3$ and $\nu_2 \text{CO}_3$ for both types. As shown in this figure, the amount of carbonate group varies according to the treatment process. Fig.4 shows the XRD results obtained. As shown in the result, all the peaks corresponding to the standard hydroxyapatite are obvious in the spectra of the treated bones which emphasis that the proposed treatment processes have not affected the molecular skeleton of the biological hydroxyapatite. EDX analysis for the obtained apatite was performed and the results are shown in Fig. 5. As shown in the figure, the Ca/P ratio for the apatite obtained by the subcritical water and alkaline hydrothermal methods were 1.56 and 1.86, respectively. Variation of these values than the standard HAp value might be due to implication of the carbonate group in the apatites obtained by those methods. Fig.6 shows the TEM results. As shown from this figure, the calcination process produced good atomic arrangement apatite (Fig.6 Cal. B) since the atoms are arranged in parallel planes. The atoms in the apatite obtained by the subcritical water extraction process have relatively good arrangements;

however, the particle size is small compared with the other processes (Fig.6. Sub. A). In a case of the alkaline process, the atoms have not uniform arrangement as shown in the selected area electron diffraction pattern for this apatite.

4. Conclusion

The proposed and the traditional processes can be exploited to extract natural biological apatite bioceramic from the bovine bone bio waste. Subcritical water extraction has the advantage of producing carbonated hydroxyapatite which is preferred in the biomedical applications than the ordinary hydroxyapatite. The alkaline hydrothermal process also relatively maintains the carbonate ion present in the biological apatite but less than the subcritical water method. The thermal process almost produces hydroxyapatite carbonate-free. The apatite obtained by the thermal process has higher particle size and good atomic arrangement than the proposed processes. The alkaline hydrothermal process produces relatively better shape nanoparticles (nanorods) than the other two processes.

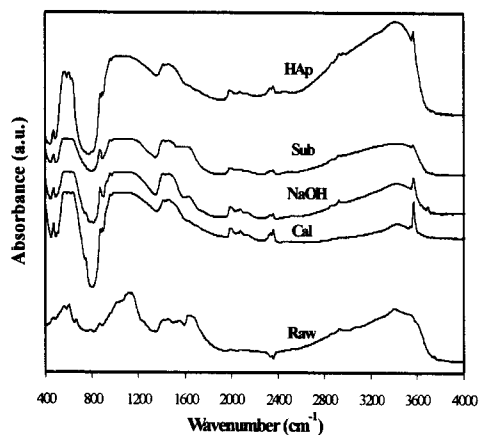


Fig. 1 FT-IR for the pure HAp, and the treated bones

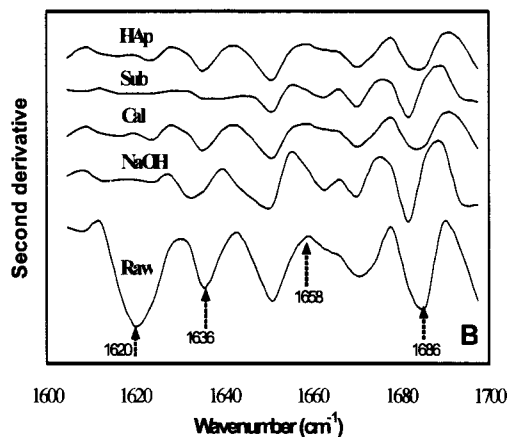


Fig. 2 Second derivative of FT-IR spectra within the region of Amide I.

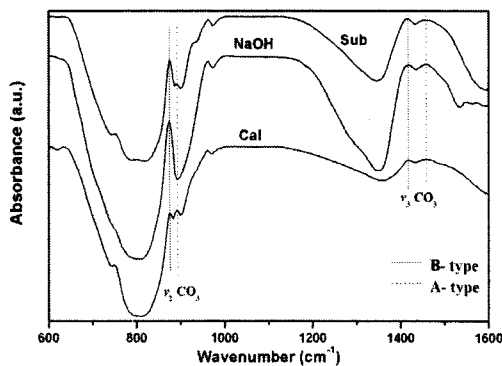
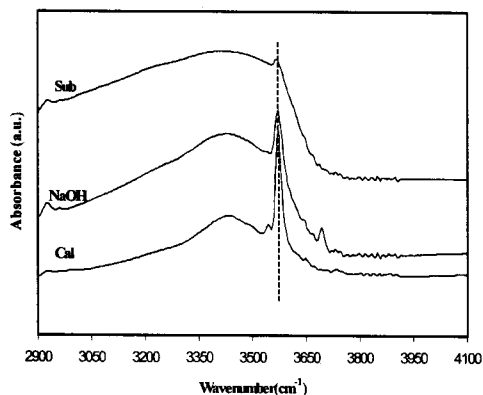


Fig.3 IR spectra of the apatites obtained, (left) OH stretching; (right) v_2 CO_3 and v_3 CO_3 modes

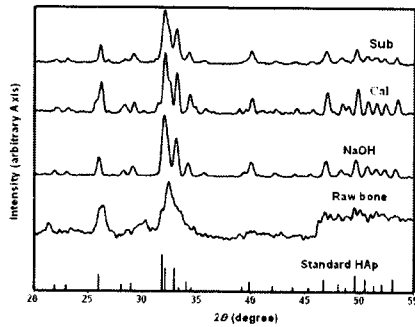


Fig.4. XRD for the raw and treated bones

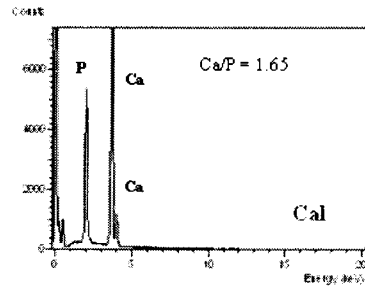


Fig.5 EDX result for calcined bones

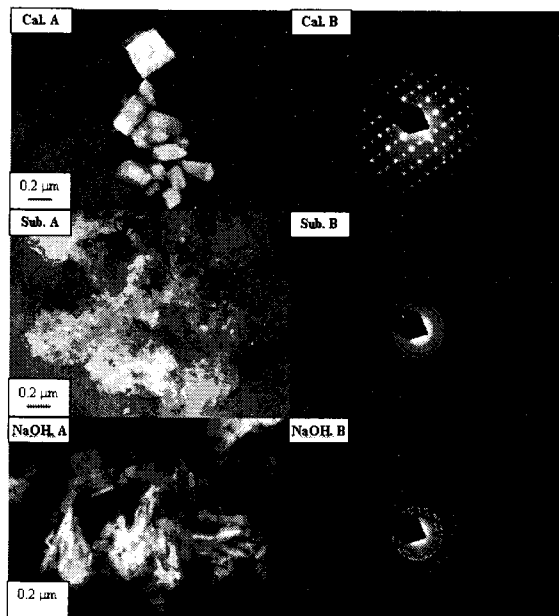


Fig.6 TEM micrographs for the obtained apatites.

5. Acknowledgement

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6. References

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