

EFFECT OF SONOCHEMICAL TECHNIQUE ON THE MORPHOLOGY AND CRYSTALLINITY OF HYDROXYAPATITE NANOPARTICLES

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ABSTRACT

Hydroxyapatite (HA) is the main mineral component of bones and teeth, and for this reason its synthetic analogous has been used with excellent clinical results as bone substitute. The biological apatite is typified as a poorly crystalline carbonate apatite (CHA) formed by nanosized particles with a great superficial area. This work aimed to study the influence of ultrasound technique on the morphology and crystallinity of nanoHA synthesized by chemical precipitation method. The nanoHA particles were obtained from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ raw solutions and a powerful ultrasonic sound (UP400S) was used at different conditions during the synthesis. X-ray Diffraction, Infrared spectroscopy, Chemical Analysis and Scanning Electron Microscopy were used to characterize the synthesized nanoHA particles. The results indicated that HA nanoparticles with nanorods morphology were obtained in all conditions. By Scherrer's formula, the crystallite size was determined and it was found that the increasing in ultrasound amplitude enhances the nanoparticles crystallinity.

Key-words: nanoparticles, hydroxyapatite, sonochemical technique

INTRODUCTION

The human bone tissue is a composite of crystals of nanodimensioned hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA) immersed in a collagen matrix. Typically, 70 % of the bone matrix contains nanocrystalline HA which is 20-80 nm long and 2-5 nm thick dispersed in a soft hydrogel template rich in collagen and non-collagenous

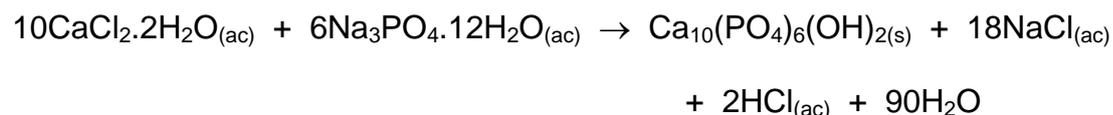
proteins. It is well recognized that synthetic HA has been demonstrated excellent biocompatibility and bioactivity properties with respect to bone cells and tissues, due to its similarity in chemical composition with the hard tissue of the human body^(1,2). Furthermore, nanosized hydroxyapatite mimics the properties of natural bone with better faithfulness because of its major correspondence in morphology, crystallinity and superficial area. Different researches have illustrated that nanohydroxyapatite increased osteoblast adhesion on nano grained materials in comparison with the microsized analogous^(3,4). Moreover the long-term functions and *in vitro* osteoblast proliferation were also enhanced on ceramics with grain sizes less than 100 nm^(4,5).

HA powders can be synthesized by various methods such as sol-gel, hydrothermal, mechano-chemical, spray drying, combustion synthesis and precipitation⁽⁶⁾. The precipitation is the most studied method for producing HA powders. This method is economic, in general produces HA particles with a large particle size distribution and agglomerates. Ultrasonication is an effective way of breaking up aggregates and of reducing the size of nanoparticles. It has been well recognized that the ultrasonic irradiation causes cavitation in an aqueous medium where the formation, growth and collapse of microbubbles occur⁽⁷⁾. This can stimulate the reactivity of chemical species involved, resulting in the acceleration of the heterogeneous reactions between liquid and solid reactants effectively.

In this work, the influence of ultrasound technique, using a powerful ultrasonic sound, on the morphology and crystallinity of nanoHA synthesized by chemical precipitation method was studied.

MATERIALS AND METHODS

HA powders were prepared by wet precipitation method at room temperature using the reagents, CaCl₂.2H₂O (EMSURE/Merck, Germany, p.a.) and Na₃PO₄.12H₂O (B'Herzog, Brazil, p.a.) according to the following reaction:



The calcium precursor was dropped into the phosphate solution while it was being irradiated with powerful ultrasonic sound (UP400S) supplied by Hielscher Ultrasound Technology, Germany which has a frequency of 24 kHz and 400 w of

power. A reaction using the same reagents and mechanical stirring without the assistance of ultrasonic irradiation was carried out for comparison.

In order to evaluate the effect of ultrasonic parameters on the nanoHA particles, different sonication amplitudes were tested. Specifically, wave amplitudes were 20, 60 and 100 % while the pulse was maintained at 0.5. The samples were named as SI, SII and SIII, respectively. Also it was used mechanical stirring as a conventional methodology of synthesis.

The powders were characterized by chemical analysis to determine the calcium/phosphorous ratio. The calcium (Ca) was determined by complexometric titration with EDTA while the phosphorous was determined by the spectrophotometric technique through the reaction with ammonium molybdate in the presence of suitable reducing agents to form a blue coloured complex. Moreover, the samples were analyzed by infrared spectroscopy (Perkin Elmer Spectrum One). About 10 mg of each sample was blended with KBr for IR spectroscopy (Sigma-Aldrich) and then pressed into translucent pellets for the measured. Spectra between 4000–400 cm^{-1} range were obtained from co-addition of 30 scans.

The phase composition and crystallinity of the synthesized HA nanoparticles were determined by X-ray diffraction (XRD) using Philips X'Pert Diffractometer, Netherlands, with Cu K α radiation $\lambda = 1.5406 \text{ \AA}$ generated at 40 mA and 40 kV. Data were collected in the 2θ interval from 20 to 60° with step size of 0.05° and count time of 2 s. The crystalline (particle) size of the powders was calculated from the XRD data using the Scherrer's equation (A):

$$X_s = \frac{0.9\lambda}{FWHM \cos\theta} \quad (\text{A})$$

where X_s is the crystalline size (nm); λ the wavelength of monochromatic X-ray beam; FWHM is the full width at half maximum for the diffraction peak under consideration; and θ the diffraction angle⁽⁸⁾.

The fraction of crystallinity X_c of the nanoHA particles was determined⁽⁹⁾ from the equation (B) where β is the FWHM:

$$X_c = \left(\frac{0.24}{\beta} \right)^3 \quad (\text{B})$$

The size and morphology of synthesized HA nanoparticles were examined by Field Emission Scanning Electron Microscopy (FESEM) Quanta FEG, 450.

RESULTS AND DISCUSSION

The chemical analysis reveals that the Ca/P ratio was near to 1.67 for the four samples which corresponding to the stoichiometric HA. Fig. 1 shows the infrared spectra of synthesized HA, which are featured by phosphate absorption bands. The triply degenerated asymmetric stretching and bending vibrations of PO_4^{3-} were at 1030 and 570 cm^{-1} , respectively. Peaks of CO_3^{2-} (1420 and 860 cm^{-1}) associated with some carbonate ions incorporated into the HA are detected, which is a result from the atmospheric CO_2 dissolved in the solutions used for the preparation of the HA nanoparticles. The wide band centred at 3400 approximately and the peak at 1640 cm^{-1} correspond to the remaining water. In the SIII sample, it was detected a sharp vibration band derived from hydroxyl ions at 3560 cm^{-1} as a shoulder of the broad absorption band originated from the absorbed H_2O .

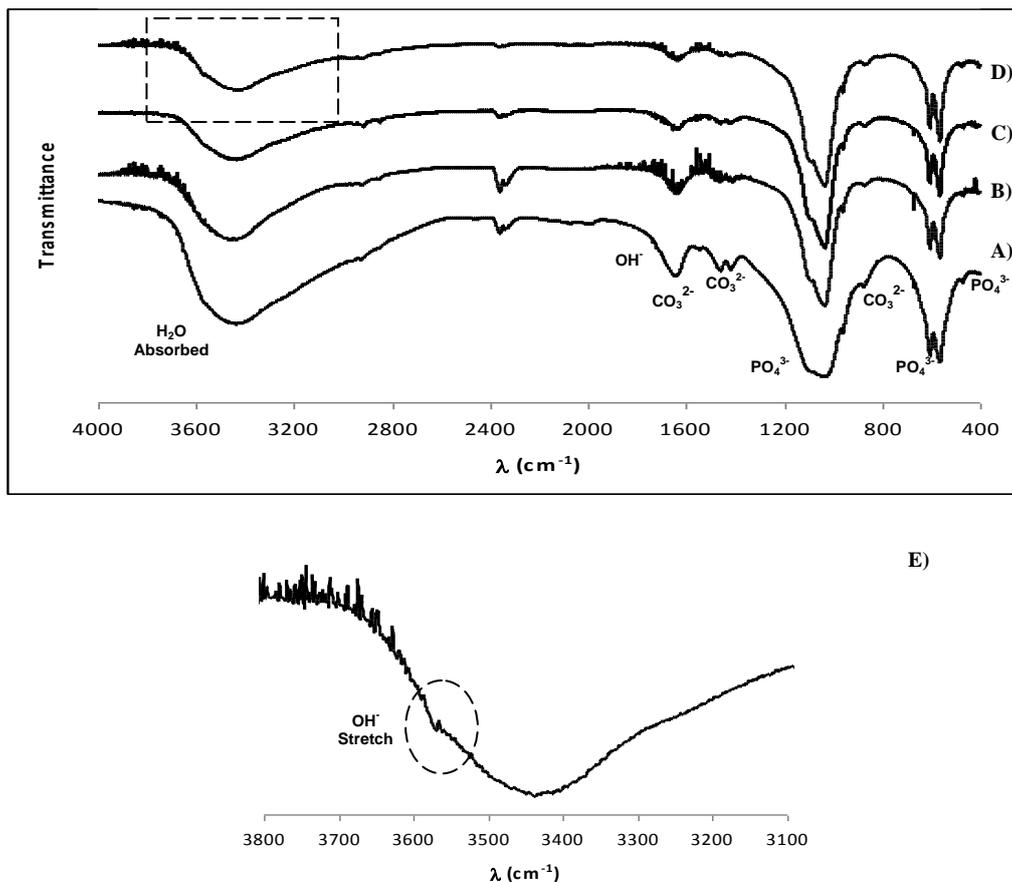


Fig. 1. FTIR spectra of synthesized HA. a) Mechanical stirring, b) SI, c) SII, d) SIII, e) Magnification of the FTIR spectrum of the SIII sample.

The samples obtained under ultrasonic sound appeared to be more crystalline when compared with the HA prepared by mechanical stirring (Fig. 2). As observed in Fig. 2, the application of ultrasonic irradiation promoted an increase of the intensity in the XRD peaks (see the (0 0 2) reflection peak at around $2\theta = 25.9^\circ$). Also, the amplitude variation of ultrasonic irradiation induced an increase in the intensity of XRD peaks, as shown in Fig. 2 (SIII). Moreover, the effect of ultrasonic irradiation provoked a better resolution of the peaks at the XRD patterns.

These results indicated that all peaks are attributed to stoichiometric HA and no other calcium phosphate peaks were detected. A high consistency between the data with JCPDS No. 09-0432 was observed.

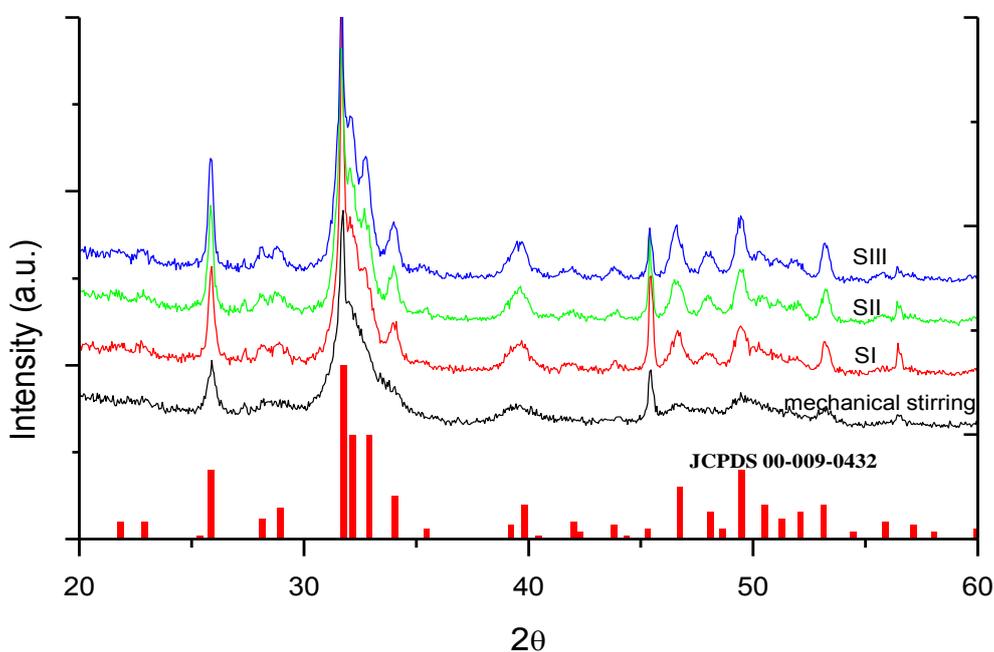


Fig. 2. XRD patterns of SI, SII, SIII samples and the mechanical stirring sample is used for comparison.

The calculated crystallite sizes and fraction of crystallinity for the samples are presented in Table 1. The crystalline size X_s of synthesized nanoHA powders was calculated using the equation (A). The (0 0 2) reflection peak from the XRD patterns was used to calculate the average nanoHA crystalline size from the equation (A). The calculation was done so that a comparison could be made of the powders produced by the different ultrasonic treatments and the powder without the assistance of ultrasonic irradiation. The fraction of crystallinity was calculated using the equation (B).

Table 1. NanoHA crystalline size and fraction of crystallinity obtained from XRD patterns.

Samples	crystallite size (nm \pm 5 %)	fraction of crystallinity
SI	33	0.9
SII	41	1.8
SIII	41	1.8
mechanical stirring	28	0.5

As expected from the XRD patterns the ultrasonic irradiation increase the crystallite size and the fraction of crystallinity. No differences for these parameters were found between the samples SII and SIII.

The size and morphology of the HA nanoparticles obtained under ultrasonic irradiation are presented in Fig. 3. From the FESEM images, it is observed that the all powders presented rod-like nanosized particles, which seem as agglomerates. The formation of nanorods is favored in conditions such as medium concentration of hydroxide ($9.5 \geq \text{pH} \geq 7.75$) and low temperature⁽¹⁰⁾ (room temperature).

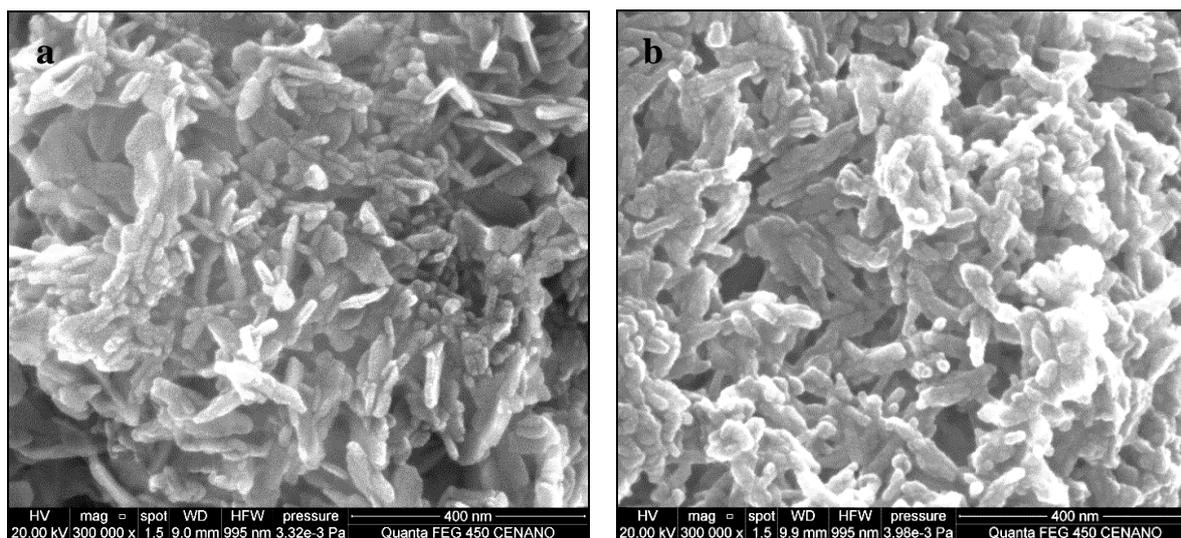


Fig. 3. FESEM micrograph of samples: a) SI; b) SII.

The nanoparticle dimensions from the FESEM micrographs are presented in Table 2. It is noted that no significant differences were detected between the dimensions of samples with and without ultrasonic irradiation. However, the dimensions of SI, SII and SIII samples increased with the ultrasonic wave amplitude variation.

Table 2. Dimensions of the synthesized HA nanoparticles.

samples	large (nm)	Width (nm)
SI	69 ± 22	20 ± 4
SII	79 ± 28	23 ± 4
SIII	71 ± 22	23 ± 4
Mechanical stirring	77 ± 25	22 ± 4

CONCLUSIONS

HA nanoparticles were successfully synthesized by chemical precipitation method using ultrasonic irradiation. The chemical analysis results revealed that Ca/P ratio of the obtained powders corresponded with a stoichiometric HA. No other calcium phosphate phase different to the stoichiometric HA were detected at the XRD patterns. As the ultrasonic irradiation amplitude was increased, the crystallinity and size of the HA particles increased as evidenced by the XRD and SEM results, respectively. The nanoHA obtained by this method can be used as a biomaterial with enhanced attributes for biomedical applications.

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