

Structural Properties of Hydroxyapatite with Particle Size Less Than 10 Nanometers.

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Abstract. Hydroxyapatite, HA, with crystal size smaller than 10 nm was synthesized by dropwise addition of calcium nitrate and ammonium phosphate under controlled conditions. Powder was composed by agglomerates of nano-particles with specific surface area higher than 200 m²/g. HA samples had a main pore size distribution centered at 13-15 nm with a secondary mode centered at 3-5 nm. High resolution TEM revealed that individual particles had nearly spherical shapes and were mainly composed of a crystalline core and an amorphous external component. EXAFS measurements confirmed that the short range order of those HA nanocrystals was similar to that of HA sintered at high temperature. HA powder with small particle size incorporates in its structure more water and carbonate ions than HA prepared in normal conditions.

Introduction

Efforts have been made in the past years to improve mechanical properties and bioactivity of hydroxyapatite and other calcium phosphates. Several directions have been tested such as treatment of HA surface, HA association with inorganic and organic compounds and adsorption of biomolecule [1-3]. Recently studies concerning the properties of bioceramics with very small crystal size were also carried out as a complementary direction to improve the biological response of hydroxyapatite. In this direction, some questions immediately arise: what is the lower limit for HA crystal size, what is the structure of these nano-crystals and how HA chemical activity, crystallinity, dissolution rate and sintering are affected by crystal size. In this work we adjusted the synthesis procedure in order to produce materials with very small particle size in the order of 10 nm or less.

Materials and Methods

Hydroxyapatite powder were prepared by dropwise addition of calcium nitrate and ammonium phosphate under controlled conditions of temperature, pH, stirring velocity, reagent concentration, addition rate and aging time. The precipitate was separated by filtration, repeatedly washed with deionized water and dried at 37 °C (sample 134b) or lyophilized (sample HA126 and HA134a). The elemental concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP). Fourier transform infrared spectroscopy (FTIR) was used to investigate the vibrational modes of PO₄³⁻, OH⁻, CO₃²⁻ groups. Mass spectrometry (MS) measured the carbonate and water associated to the HA structure. The degree of crystallinity of HA structure and the formation of secondary mineral phases were analyzed by X-ray powder diffraction using a conventional X-ray source and synchrotron radiation at the Brazilian Synchrotron Light Laboratory (LNLS/Campinas/Brazil). Conventional XRD patterns were collected with a high resolution Rigaku Rota Flex, using a flat-plate Bragg-Brentano geometry, and graphite monochromated CuK α

radiation. Structural refinement was performed using the Rietveld technique from Fullprof software. Particle short range order was investigated by spectroscopy of X-ray absorption (EXAFS) from synchrotron radiation (LNLS/Campinas/Brazil). Particle morphology was determined by high resolution transmission microscopy (HRTEM) at LNLS/ Campinas/Brazil. The pore size distribution was characterized by BET isotherms.

Results and Discussion

The FTIR spectrum of HA with small particle size had broader phosphate bands than normal HA in the 1000 cm^{-1} region and an absence of OH^{-1} bands at 3740 cm^{-1} and 630 cm^{-1} was observed. This may be associated with the high content of water and carbonate incorporated into HA structure as was also verified by mass spectroscopy. This last technique showed that the smaller the crystal size, the more effective is the incorporation of CO_3^{2-} and H_2O groups into PO_4^{3-} sites and c axis. The incorporation of these impurities into HA structure probably perturbs the OH^{-1} and the PO_4^{3-} vibrational modes.

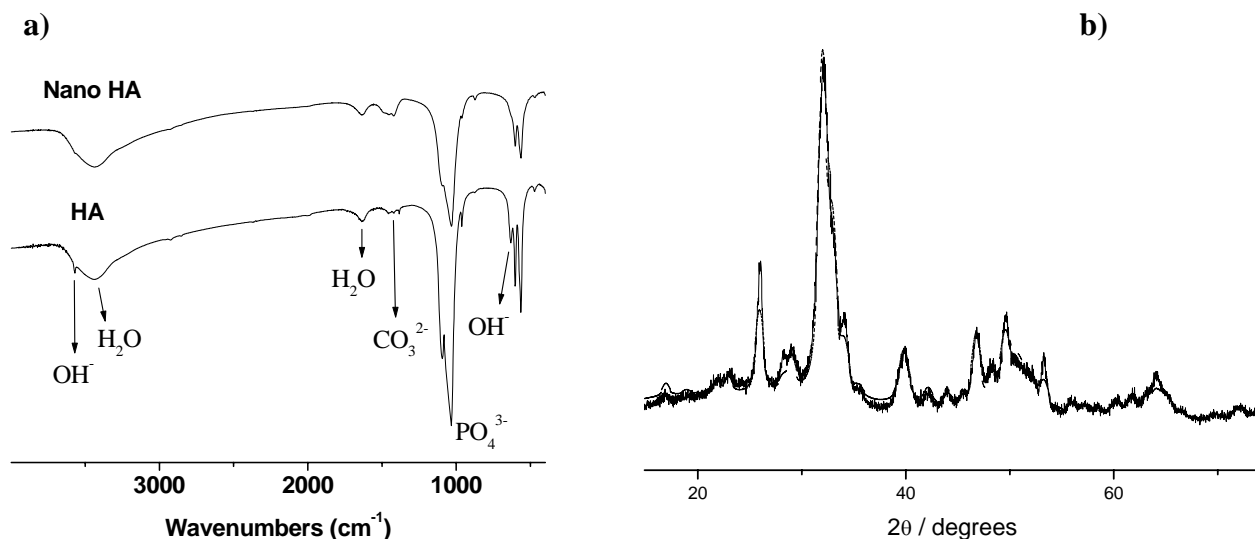


Fig. 1: a) FTIR pattern of HA powder synthesized under normal conditions and that of HA with small crystal size; b) XRD pattern of nano HA (continuous line) and Rietveld fitting of experimental data (dotted curve).

The Rietveld refinement of the X-ray diffraction pattern revealed that no other crystalline phase besides HA was formed in sample HA126 and HA134, which were synthesized in similar conditions. However, both samples had amorphous contribution that varied from 18% (sample HA126) to 38 % (sample 134) as shown in Fig. 2. This amorphous phase was transformed into crystalline HA when sample was dried at $37\text{ }^{\circ}\text{C}$, indicating that non-crystalline phase has similar stoichiometry as HA. Following the XRD results, the ordered phase of these samples had a mean crystallite size of 7.0 and 9.5 nm, respectively. The HA crystals had pronounced needle shape with the largest and smallest dimensions oriented along the 002 and the 300 directions of HA, respectively. The average maximum strain decreased with the increase of the crystallite size.

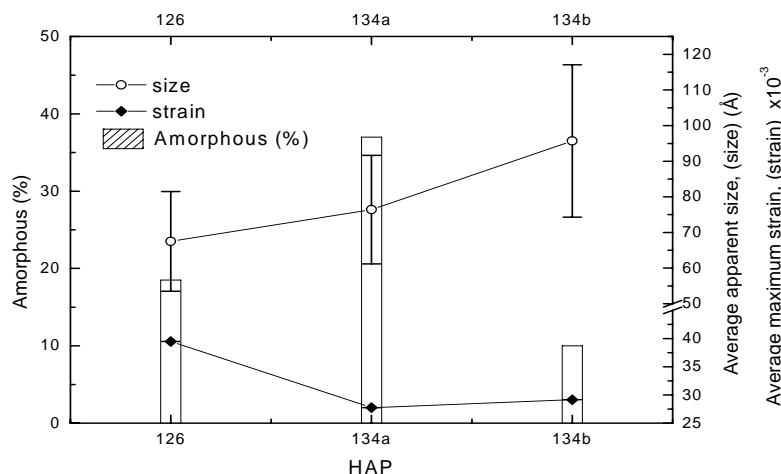


Fig. 2: Crystal size, amorphous content and strain of two HA samples (HA126 and HA134) synthesized in similar conditions. Sample HA134b was dried at 37 °C and HA134a was lyophilized.

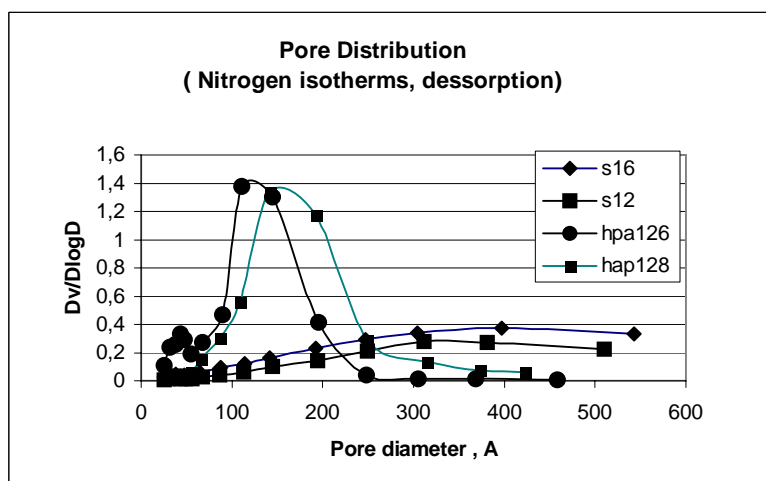


Fig. 3: Pore diameter distribution of normal HA (S16 and S12) and a HA with small crystal size (HA126 and HA 128).

The HA with small crystals (HA126 and HA 128) has a different pore size distribution from that of the HA synthesized in normal conditions (HA samples HA 12 and HA16) as shown in Fig.3. It has a sharp mode centered at a pore diameter of about 13- 15 nm. However, small modes at 3-5 nm were also present, suggesting the existence of crystals smaller than 5 nm. Those pores should be associated with very small particles not sensitive to XRD. The nitrogen isotherms also showed that the specific surface area is larger than 200m²/g, which corresponds to 4 times the values usually reported for normal HA.

The EXAFS analyses showed that local order of nano HA was similar to that of HA with high degree of crystallinity and confirmed that 30% of the HA sample was disordered. However, both crystalline and amorphous phases have the same local structure. These results reinforced the hypothesis that nano-particles were constituted of a crystalline core and an external amorphous shell. This idea was confirmed by HR-TEM analyses. The HR-TEM analyses showed that HA particles are nano-agglomerates of crystals as showed Fig. 4a. Each single particle have a nearly spherical shape with a diameter of 3- 6 nm, Fig.4b. These sizes correspond to 3–6 times the HA unit cell parameters along a/b directions, or 4-9 times that along c direction.

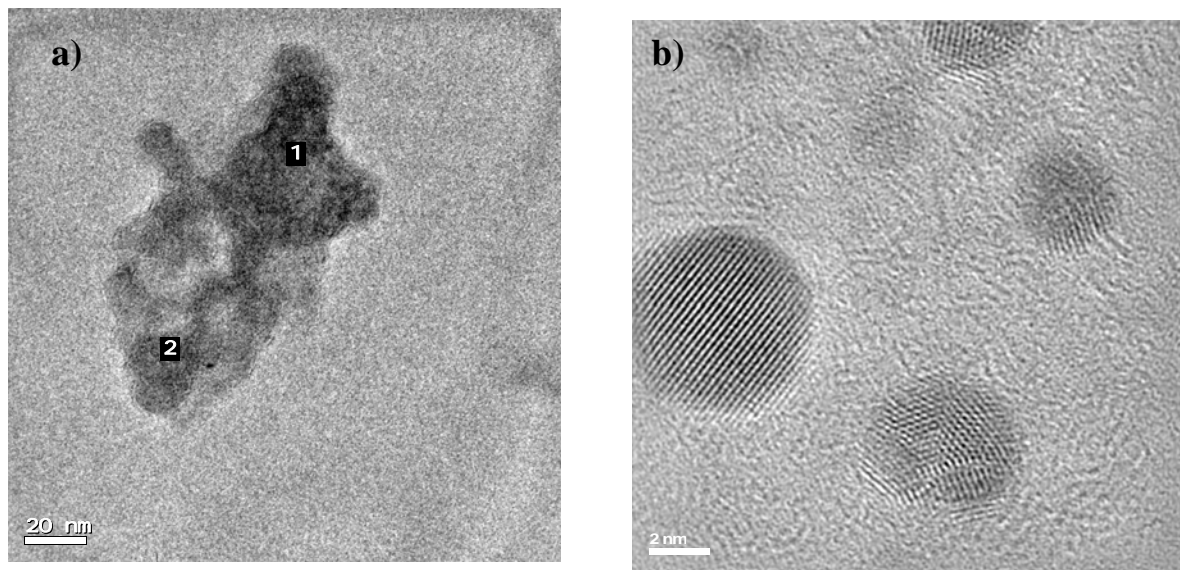


Fig.4 TEM images of sample HA126: a) agglomerate of crystals and b) isolated particles.

Conclusions

Hydroxyapatite powder with crystal size smaller than 10 nm was synthesized under controlled conditions. Sample characterization showed that the short and long range order of HA structure were stable even when particle size was on the order of a few unit cell parameters.

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