

## Nanocrystalline Hydroxyapatite-Bisphosphonate Composites

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The direct synthesis of hydroxyapatite–1,8-octan-bisphosphonic acid (HAp–BISPH) nanocrystals has been carried out in presence of increasing amounts of BISPH in solution, by hydrothermal method at 120 °C for 15 h. XRD, IR, NMR-MAS (<sup>31</sup>P, <sup>1</sup>H and <sup>13</sup>C), TEM, AFM, TGA and chemical analysis were used to characterize the structure, morphology and composition of the products. X-ray powder diffraction patterns show that the incorporation of bisphosphonate moieties induces a significant loss of the material crystallinity and a clear decrease of the crystallite size. TEM and AFM images show that the precipitated apatite particles prepared in the presence of this bisphosphonic acid are nanosized. The IR and NMR-MAS <sup>1</sup>H spectroscopy show that the BISPH can replace the OH- groups of the apatitic structure.

**Keywords:** Calcium hydroxyapatite, Bisphosphonate, Composite.

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### 1. INTRODUCTION

Calcium hydroxyapatite, Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub> designated as CaHAp, is widely studied due to its excellent biocompatibility, bioactivity and similarity in chemical composition with human bone tissues [1]. The functionalization of CaHAp with various organic substances has been the subject of many studies that give novel applications to this material. There are many studies on the interaction between hydroxyapatite and bisphosphonates such as alendronate [2] zoledronate [3], risedronate [4] and clodronate[5]. Bisphosphonates are an important class of compounds that have a high bone resorption and are used in the treatment of a variety of bone diseases, such as metastatic bone disease, Paget's disease, and osteoporosis [6]. Herein we report the synthesis of calcium hydroxyapatite (CaHAp) by hydrothermal method, in the presence of different amounts of 1,8-octan-bisphosphonic acid (BISPH). The structure, chemical composition and surface property of the new composites CaHAp-BISPH were investigated and discussed based on the results obtained.

### 2. SYNTHESIS

CaHAp is synthesized by adding a 0.75 M calcium nitrate solution (11 ml) and 0.25 M diammonium hydrogen phosphate solution (20 ml) into a flask containing a dilute solution of ammonium hydroxide in order to keep the pH above 10 during precipitation. The obtained solution is treated in autoclave (V = 50 ml) under hydrothermal conditions at 120°C, for 15 h. The products are filtered, washed with double distilled water, and dried overnight at 100°C. The hybrid phases are designed by CaHAp-BISPH(x) where x is the molar ratio with  $x = n(\text{bisphosphonic acid})/n(\text{apatite})$ . They are obtained following a similar procedure except that an amount of bisphosphonic acid is added to the phosphate solution.

### 3. RESULTS AND DISCUSSION

#### 3.1 X-ray diffraction

For all synthesized samples, the X-ray diffraction pattern, reported in Fig.1, indicates the presence of hydroxyapatite as the unique crystalline phase (JCPDS card No. 9-432). This structure was conserved after BISPH incorporation. However, the crystallinity of the prepared hydroxyapatite is strongly affected. In fact, the powder diffraction patterns of the products obtained in the presence of different amounts of BISPH in solution display an increasing broadening of the diffraction lines with increasing BISPH content. The line broadening of the two reflections (002) and (310) was used to evaluate the crystallite size (D) of the obtained material. The values of D were calculated using the Debye-Scherrer method [7]. These values indicate that the precipitated apatite particles prepared by hydrothermal method consists of nanosized crystallites. Both D<sub>002</sub> and D<sub>310</sub> decrease on increasing the organic moieties.

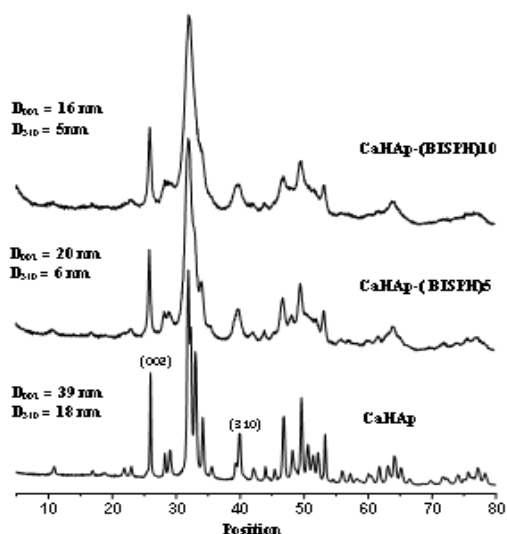


Fig. 1 – XRD of CaHAp prepared in the presence of different BISPH amounts

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### 3.2 IR spectroscopy

The spectrum of pure hydroxyapatite, CaHAp, displays the absorption bands characteristic of  $(\text{PO}_4)^{3-}$  modes at  $963\text{ cm}^{-1}$  ( $\nu_1$ ),  $473\text{ cm}^{-1}$  ( $\nu_2$ ),  $1022\text{-}1089\text{ cm}^{-1}$  ( $\nu_3$ ) and  $562\text{-}600\text{ cm}^{-1}$  ( $\nu_4$ ) (Fig. 2). The absorption bands characterizing the stretching and the libration modes of  $\text{OH}^-$  groups are shown, respectively, at  $3570\text{ cm}^{-1}$  ( $\nu_s$ ) and  $630\text{ cm}^{-1}$  ( $\nu_l$ ). After reaction with BISPH acid, the IR spectra display the presence of new absorption bands.

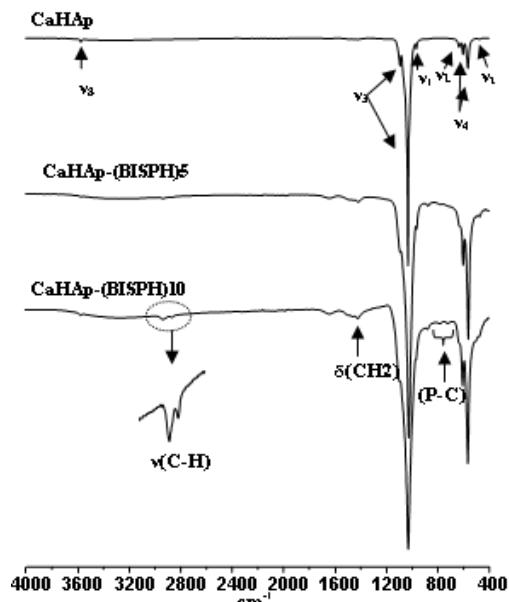


Fig. 2 – IR of CaHAp prepared in the presence of different BISPH amounts

These new bands were obtained at  $717$  and  $786\text{ cm}^{-1}$  and they are attributed to  $\nu(\text{P-C})$ . Moreover, very weak bands near  $3000\text{-}3200$  and  $1466\text{ cm}^{-1}$  were seen; they are attributed respectively to the C-H and the  $\text{CH}_2$  vibration mode of octane group of BISPH acid. This can be explained by the formation of new CaHAp-BISPH hybrid compounds.

### 3.3 NMR spectroscopy

The  $^{31}\text{P}$  CP-MAS NMR spectrum of the CaHAp, given in Fig. 3, shows an intense isotropic signal around  $2.6\text{ ppm}$  characteristic of the apatitic phase ( $\text{PO}_4$ ). After treatment, the broadening of this signal is related to the heterogeneity of phosphorus environment. Also, the  $^{31}\text{P}$  NMR MAS spectra of modified phases show the presence of new signals around  $27$  and  $34\text{ ppm}$  with a low intensity characteristic of phosphonate group ( $\text{PO}_3$ ).

The  $^{13}\text{C}$  CP-MAS NMR spectra of grafted CaHAp material, reported in Fig. 3, present a large signal in the range  $20\text{-}30\text{ ppm}$ , attributed to the carbons of octane group.

For all phases, the  $^1\text{H}$  CP/MAS NMR spectrum exhibits two signals at  $0$  and  $5.2\text{ ppm}$  attributed respectively to hydroxyl groups of the hydroxyapatite and water molecules adsorbed on the surface (Fig. 3). For treated hydroxyapatite, the spectrum present a new signal at  $2.6\text{ ppm}$  associated to the protons of  $-\text{CH}_2$  groups of 1,8-octan-bisphosphonic acid. A decrease in

signal intensity of hydroxyl groups of the hydroxyapatite is observed and is explained by the replacement of these groups by BISPH.

### 3.4 Elemental analysis

The results of the chemical analysis of the synthesized materials are summarized in Table 1. The chemical analysis of phosphorus and carbon element reveals that the weight percentage of the latter increases progressively, with the increase of BISPH amounts.

Table 1 – Chemical analysis (% weights $\pm$ 0.05) of the modified apatites.

Samples	% Ca	% P	% C
CaHAp	37,32	17,06	-
CaHAp-(BISPH)5	35,62	17,38	2,05
CaHAp-(BISPH)10	34,05	18,56	4,64

### 3.5 Thermal analysis

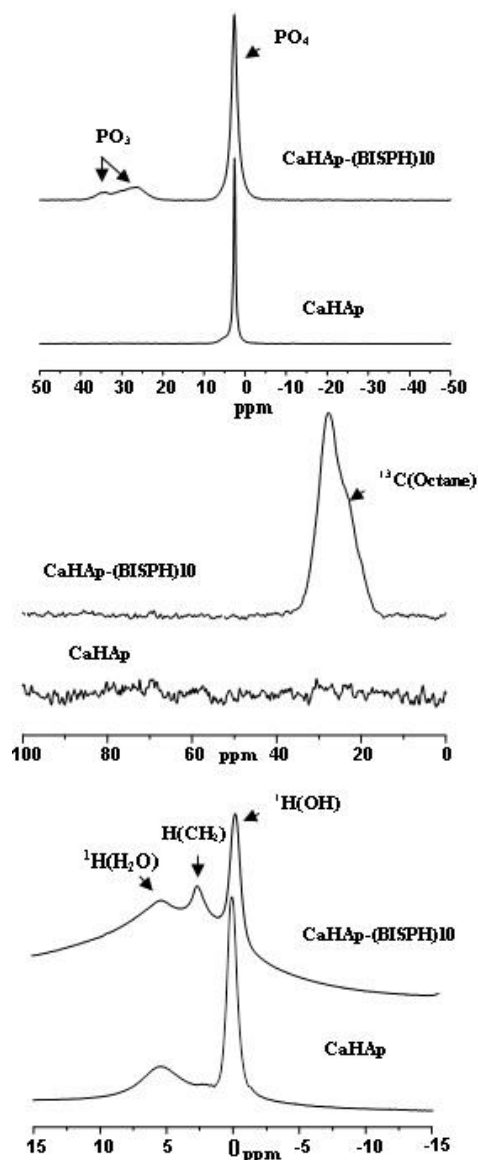


Fig. 3 – MAS NMR  $^{31}\text{P}$ (a),  $^{13}\text{C}$ (b) and  $^1\text{H}$ (c) of CaHAp and CaHAp-BISPH

The thermo gravimetric scans (TG) of CaHAp-BISPH samples, reported in Fig. 4, displays a first thermal process between 50 °C and 200 °C, due to water loss, and a second one between 200 °C and 600 °C, due to the degradation of the organic material. The weight loss associated to this second process allows to evaluate the relative amount of BISPH acid in the CaHAp-BISPH composites hybrids. The relative amount of BISPH acid increases with increasing of its concentration in the start. This result is in agreement with the increase of the percentage of carbon determined by chemical analysis. The differential thermal analysis curves (DTA) of the CaHAp-BISPH samples displays an exothermal effect observed in the temperature range 250–550 °C with a peak top at 330°C. This peak, which is absent in the DTA plot of non-modified CaHAp, corresponds to the combustion of the organic material (octane group). Furthermore, the intensity of these peak increases with increasing BISPH amount.

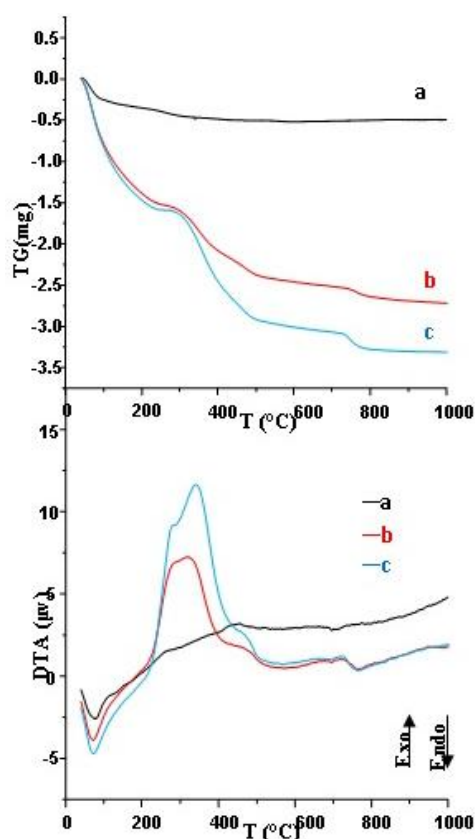


Fig. 4 – TG and DTA of CaHAp (a), CaHAp(BISPH)5 (b) and CaHAp(BISPH)10 (c)

### 3.6 TEM and AFM observations

From the photomicrographs of TEM (Fig. 5), it can be seen that the size of precipitated apatite particles, prepared with and without BISPH acid, is on the nanometer scale. CaHAp is constituted of rod-shaped crystals, with mean dimensions up to about 40–10 nm. In comparison, CaHAp-BISPH are observed shorter in good agreement with the decrease of crystallite size demonstrated by XRD

The AFM images, reported in Fig. 5, show that the size of apatitic particles is on the nanometer scale in agreement with the results of TEM. The surface of the CaHAp is highly rough. However, after treatment of hydroxyapatite, the surface loses its roughness and becomes almost smooth with a more or less compact structure consisting of fine crystallites condensed.

### 4. CONCLUSION

Composite CaHAp–(BISPH) nanocrystals, with different 1,8-octan-bisphosphonic acid content, can be prepared by hydrothermal method. The presence of the BISPH acid in the reaction solution does not change the apatitic structure, but reduces the crystallinity and the crystallite sizes. TEM and AFM images confirm the reduction of crystallite sizes and indicate the presence of nanosized crystallites. According to NMR spectroscopy, the decrease in signal intensity of hydroxyl groups (OH) of suggests that the replacement of these groups by BISPH.

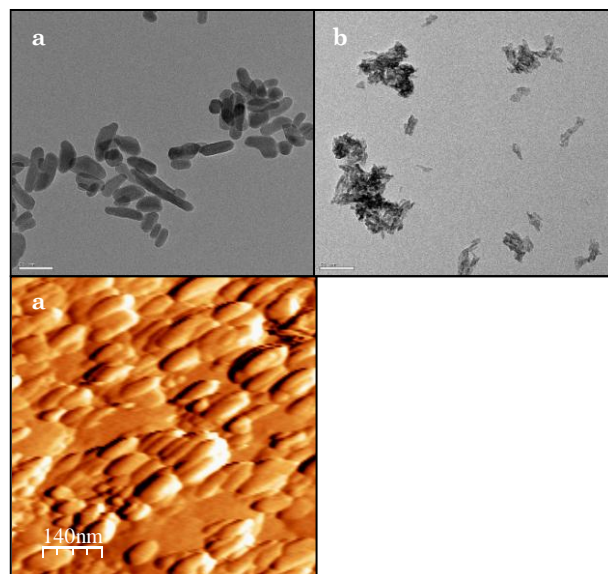


Fig. 5 – TEM and AFM images of CaHAp (a) and CaHAp(BISPH)10 (b)

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