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Synthesis of hybrid compounds apatite–alendronate by reactive milling and effects on the structure and morphology of the apatite phase

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Abstract

The preparation of apatite–alendronate hybrid materials by reactive milling is proposed in this work. Calcium phosphate compounds of various compositions have been associated to bisphosphonates and found suitable for local application with release kinetics of the drug compatible with the inhibition of bone resorption. Hybrid compounds have been obtained by reactive milling. The compositions used were: AP(X-100), Alendronate(X) where $X=7$ and $X=15$. An interaction between the hydroxyl group of the apatite and the amine group of alendronate can be identified with FTIR and enables to confirm the formation of the hybrids. The incorporation of the alendronate hinders the growing of the apatite crystals resulting in smaller coherent domains of diffraction for the apatite phase.

Keywords: D. Apatite; Alendronate; Hybrids; Reactive milling

1. Introduction

Hydroxyapatite (HAp) functions as a bioactive material that directly regulates the behavior of both normal and transformed cells [1]. For example, HAp has been shown to enhance normal bone formation and to alter growth and expression profiles of bone metastatic tumors [2]. Also, HAp can bypass a host foreign body response system and integrate with the surrounding tissues, unlike other artificial materials. The versatile apatite structure accept numerous substitutions such as K^+, Na^+, Mg^{2+}, OH^- or CO_3^{2+} (replacing to the Ca^{2+}, PO_4^{3-} and/or OH^- ions). These ions and groups may play a role on the reactivity, stability and properties of the apatite [3]. Also, hydroxyapatite has the ability to form strong chemical bonds with natural bone and a variety of molecules [4,5]. Bisphosphonates (Bps), which possess a strong affinity to hydroxyapatite under physiological conditions [6] are between those molecules with a great medical potential.

Bisphosphonates are synthetic pyrophosphate analogs, which have a P–C–P bridge and two phosphonic acid groups bonded to the same central carbon [7–9]. There are also two sides chains in the structure normally referred as $R1$ and $R2$. The biological activity and affinity to bone are defined by this structure. It is generally accepted than the $R1$ side chain determines the binding to bone mineral and the cellular effects depend on the $R2$ side chain [10–12]. The bisphosphonates are widely used drugs for the treatments in bone disorders, such as osteoporosis, Paget's desease or hypercalcemia [10].

The Bps are currently administered orally, but their absorption is very poor (about 1% of administered dose) and only the 20% of the absorbed compound is incorporated into bone. Consequently, other administration routes such as nasal delivery, subcutaneous or intramuscular injection have been developed [13,14]. The main goal for these drugs is their in situ administration, avoiding in this way the side effects associated with conventional systemic

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therapy. Calcium phosphate compounds of various compositions have been associated to Bps and found suitable for local application with release kinetics of the drug compatible with the inhibition of bone resorption [15].

In this paper, we proposed the preparation of HAp–Bp hybrid materials by reactive milling and to study the effects of the incorporation of alendronate on the structure of different apatites. Apatites with different features have been prepared by a co-precipitation route with both mechanical and ultrasonic stirring. Then hybrids compounds were obtained by reactive milling.

2. Materials and methods

Apatites were synthesized by a coprecipitation method assisted either by mechanical stirring or ultrasound stirring. The hydroxyapatite was prepared from two aqueous solutions; The first solution was prepared with 8 mL of H_3PO_4 (85% J.T.-Baker)+235 mL deionized water, which it was added dropwise to a second aqueous solution, prepared with 14.75 g $Ca(OH)_2$ (Sigma-Aldrich)+200 mL deionized water. Powders were washed with distiller water and dried at 100 °C during 24 h. While the synthesis assisted by mechanical stirring was maintained for 20 h (sample identified as Ap1), the synthesis assisted by ultrasound stirring was maintained for 0.5 h at ~35 °C (sample Ap2). Hybrid compounds were obtained by reactive milling in a RETCH MM400 mill with a setting of vibrational frequency of 30 Hz. Powders of apatite and alendronate (Sigma-Aldrich) were mixed for 45 min. The compositions used were: $AP_{(X-100)}$, $Alendronate_{(X)}$ where $X=7$ and $X=15$ and it refers to the percentage of alendronate. The names for the hybrid compounds are Ap1–A7 Ap2–A7 for the hybrids with 7% of alendronate, and Ap1–A15 Ap2–A15 with a 15% of alendronate. The apatites were also subjected to the same process free of bisphosphonate and were identified as Ap1-0 and Ap2-0.

The apatites and hybrid compounds were analysed in a Bruker D8 Advance with CuK_α ($\lambda=1.54$ Å) radiation, and with a diffraction angle (2θ) range between 5° to 60°, and the structural analysis was performed by FullPROF suite. A Perkin–Elmer Spectrum One apparatus was utilized for registering the ATR–IR spectra, in order to know the absorption bands that correspond to the apatite and alendronate, as well as to prove the interaction between both. The specific surface area of the apatites Ap powders was evaluated by the Brunaer–Emmett–Teller method in a Bell Prep II-Belsorp II, Bell Japan Inc. Thermal properties were studied in a TGA instrument Q500 with a heating rate to 10 °C min^{-1} and a maximum temperature of 800 °C. The morphology and microstructure was analized by a Field Emission Scanning Electron Microscopy SEM model JEOL JSM-670IF.

3. Results and discussion

The X-ray diffractions patterns obtained from Ap1 (Fig. 1(a)) and Ap2 (Fig. 1(b)) show the characteristic peaks

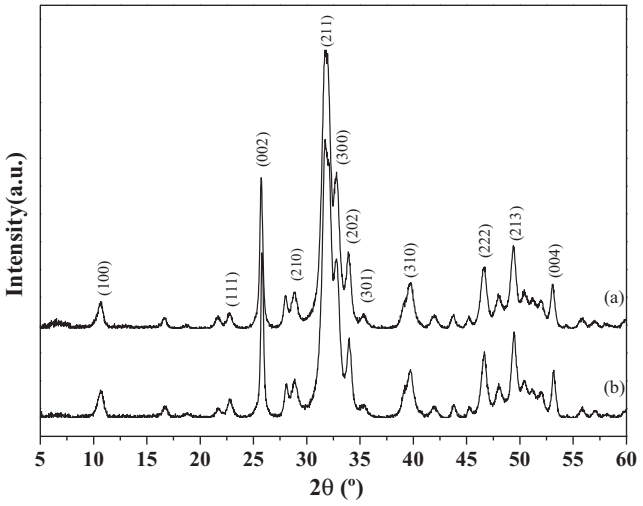

Fig. 1. X-ray diffractograms of apatite obtained by co-precipitation rute: (a) Ap1 and (b) Ap2.

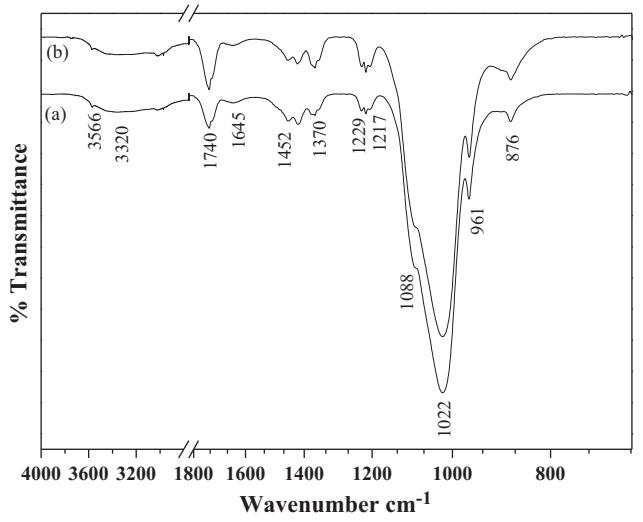

Fig. 2. ATR–IR spectra from apatites obtained by co-precipitation route: (a) Ap1 and (b) Ap2.

of a hydroxyapatite phase according to the crystallographic letter JCDS-9-0432, no secondary phases could be detected. ATR–IR spectra obtained (Fig. 2(a) and (b)), are similar and they show the characteristic absorption bands of hydroxyapatite; the bands of the PO_4^{3-} group are observed about at 1088 cm^{-1} and 1022 cm^{-} in both spectra Ap1 and Ap2. They arise from a triply degenerate stretching vibration mode v_3 [16–19]. The bands about at 961 cm^{-1} for both, Ap1 and Ap2 spectra, belong to the vibration mode v_1 [16–19]. The broad bands about at 3566, 3320 and 1645 cm^{-1} for Ap1 and Ap2 spectra is due to adsorbed water [16,18,19]. The bands about at 1452, 1422 and 876 cm^{-1} for Ap1 and Ap2, which indicate the presence of CO_3^{-2}, substituting to PO_4^{3-} in the apatite lattice [16,19,20], indicating that both synthetized apatites are carbonated apatites of the β-type [21]. The bands located between 1229–1217 cm^{-1} that correspond to HPO_4^{2+}, these