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Hydrothermal synthesis and thermal evolution of carbonate-fluorhydroxyapatite scaffold from cuttlefish bones

Emilija Tkalčec [a,*], Jasminka Popović [b], Sebastijan Orlić [a], Stjepan Milardović [a], Hrvoje Ivanković [a]

[a] Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, Zagreb, Croatia
[b] Division of Materials Physics, Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia

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ABSTRACT

Phase composition, crystal structure and morphology of carbonated fluor/hydroxyapatite synthesized hydrothermally from aragonitic cuttlefish bones were studied by powder X-ray diffraction (PXRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) combined with energy dispersive X-ray spectroscopy (EDS). The product of synthesis has been characterized as carbonated fluor/hydroxyapatite with carbonate incorporated inside channel (A-type) and substituted for the PO_4^{3-} group (B-type). The vibration band at 874 cm^{-1} assigned to bending (ν_2) mode undoubtedly confirmed carbonate substituted for PO_4^{3-} group, while the band at 880 cm^{-1} was attributed to A-type carbonate substitution. The additional sharp and intense band at 865 cm^{-1} considered as "non-apatitic" carbonate substitution is not assigned with certainty so far. Evolution of CO_2 from tetrahedral (PO_4^{3-}) sites with the increase in heat-treatment temperature is evident by the changes in tetrahedral bond lengths and angles, as obtained by the Rietveld structure refinement. Also, changes in the isotropic temperature parameters for the *2a* site point to A-type carbonate incorporation as well.

1. Introduction

Hydroxyapatite HA, $Ca_{10}(PO_4)_6(OH)_2$ has been shown to be chemically and crystallographically very similar, though not identical, to biologically formed hydroxyapatite [1–5]. The latter is nanosized carbonated hydroxyapatite (CHA), with incorporation of 3–6 wt.% of carbonate ions in its structure [6,7]. The carbonate ion can be incorporated inside channel (A-type) or can substitute for phosphate group (B-type). B-type CHA that most closely resembles the mineral phase of biological apatites has been extensively investigated; recent experimental and theoretical publications address the controversial subjects of the carbonate substitution, crystallographic structure and hydroxyl content of the bioapatites [8–17]. It is generally agreed that the planar carbonate ion substitutes for the phosphate tetrahedron in the B-type carbonated hydroxyapatite (CHA) and carbonated fluorapatite (CFA) structures, however, the question of the exact arrangement of the planar carbonate ion substituting for the tetrahedral phosphate has not been completely resolved yet [18]. This has become a major issue because detailed crystallographic structure properties are related to lattice modification caused by the carbonate substitution, the fractional atomic coordinates and the orientation of the carbonate group, i.e., parameters that consequently determine the physical properties of the materials. HA has a high dissolution rate in the biological system [19], poor corrosion resistance in an acid environment and poor chemical stability at high temperature [20], which has restricted its wider applications in the field of orthopedics and dentistry. It was suggested that fluorine-substituted hydroxyapatite (FHA), $Ca_{10}(PO_4)_6(OH)_{2-2x}F_{2x}$ where F replaces OH only partially, has better thermal and chemical stabilities than hydroxyapatite [21].

It is generally accepted that HA belongs to hexagonal crystal structure, space group $P6_3/m$ [1] with unit cell dimensions $a = 9.432$ Å and $c = 6.881$ Å, although very pure end member of HA crystallizes in monoclinic space $P2_1/b$ [6]. Fluorapatite, FA, belongs to the same space group as HA differing only in substitution of fluoride for the hydroxyl groups in HA structure. Since F^- is smaller than OH^-, the substitution results in a contraction of the *a*-axis dimension to 9.368 Å, but with no significant change in the *c*-axis length ($a = 9.368$ Å, $c = 6.884$ Å; PDF# 15-0876). That phenomenon could be explained by considering the crystal structure of HA with randomly oriented OH^- groups, which brings about a certain degree of disorder to the crystal structure. Once the OH^- groups were partially substituted by the F^- ions, the hydrogen of the OH^- groups was bound to the nearby F^- ions, producing a quite well ordered apatite structure, which caused an increase of the thermal and chemical stabilities of the HA matrix. However, it has been reported that if all of the OH^- groups in HA are replaced by F^- to form fluorapatite (FA), the resulting material is not osteoconductive [21]. Therefore, only a certain amount of F^- ions should be substituted for the OH^- groups forming fluorhydroxyapatite (FHA). Various methods have been developed in an attempt to tailor the fluorine content of FHA to achieve the best biological properties; such as precipitation [20], sol–gel [22], hydrolysis [23], mechanochemical [24] and

* Corresponding author. Tel.: +385 1 4597 219; fax: +385 14597 250.
E-mail address: etkalcec@fkit.hr (E. Tkalčec).

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hydrothermal [25]. Hydrothermal conversion of biogenic aragonite to apatite has been successfully obtained using corals [26], cuttlefish bones [27–29], and nacres [30,31]. However, in that case carbonate hydroxyapatite (CHA), or carbonate fluorapatite (CFA) is formed. The formation of carbonated fluorhydroxyapatite should not be excluded too.

Cuttlefish bone (*Sepia officinalis*) was utilized as a source of biogenic calcium carbonate to prepare calcium phosphate scaffolds, mainly by several hydrothermal approaches. The aim of the scaffold-based bone tissue engineering is to repair and/or regenerate bone defects by using a scaffold as a platform for carrying cells or therapeutic agents to the site of interest. An ideal scaffold aims to mimic the mechanical and biochemical properties of the native tissue. In order to effectively achieve these properties a scaffold should have a suitable architecture favoring the flow of nutrients for cell growth. It should also have osteoconductive properties, supporting cells through a suitable surface chemistry [32,33]. To the best of our knowledge, Rocha et al. [27–29] were the first authors who transformed cuttlefish bones hydrothermally into hydroxyapatite tissue scaffolds retaining the cuttlebone architecture, noting that the channel sizes of the cuttlebone samples investigated were beneficial for bone in growth (~100 × 200 μm). A range of other benefits including good machinability were also recognized, indicating rather promising results. Kannan et al. [34] studied the transformation of aragonitic cuttlefish bones into fluorinated hydroxyapatite scaffolds with different levels of fluorine substitution (46% and 85%) on the OH^- sites via hydrothermal transformation at 200 °C. Based on the Fourier transform infrared (FTIR) analysis, the authors declared nanosized AB-type, carbonated crystallites with unit cell parameters at 1200 °C typical for FA lattice, experiencing contraction along the *a*-axis rather than the *c*-axis.

One of the most outstanding features of nanocrystalline apatites of biological or synthetic origin is the presence of "non-apatitic" environments of the mineral ions [35]. These environments have been observed in some apatites [36,37], but not yet studied in species derived from cuttlefish bones. In this work we synthesized nanosized carbonated fluor/hydroxyapatite by hydrothermal treatment of cuttlefish bones, *S. officinalis*, from the Adriatic Sea. Compositional, structural and microstructural evolution of hydrothermally prepared fluor/hydroxyapatite during additional heat-treatment has been investigated. Changes of vibration bands for A- and B-substitution and "non-apatitic" carbonate with respect to applied heat treatment were monitored. Special attention was given to correlate the FTIR spectra and their characteristics with structural changes of crystalline phase induced by expelling carbonate, since the properties of the synthetic material are controlled by their crystallographic structure.

2. Materials and methods

2.1. Materials

The starting materials, pieces of native cuttlefish bones, *S. officinalis*, from the Adriatic Sea, were heated at 350 °C for 3 h, to remove the organic part of the shells. For hydrothermal treatment only pieces of cuttlefish bone from internal lamellae spacing were used, since by the pre-treatment at 350 °C the aragonite in external wall (dorsal shield) partially transforms into calcite, which further transforms into HA more difficult than aragonite [38]. Small pieces of bones (about 2 cm^3) were treated with the required volume of an aqueous solution of 0.6 M $NH_4H_2PO_4$ to set the Ca/P molar ratio to 1.67 and fluorine was introduced via NH_4F solution to achieve stoichiometric level of substitution. Cuttlefish bone and the solutions were sealed in Teflon lined stainless steel pressure vessel and heated at 200 °C in step of 20 °C and dwelled for 24 h in an electric furnace. The pressure inside the reactor was self-generated by water vapor. The converted sample assigned as HT-25 was washed with boiling water and dried at 110 °C. Grinding of sample was accomplished with an agate mortar and pestle, to produce a fine powder that was used in thermal analysis, X-ray diffraction, and FTIR experiments.

2.2. Methods

Thermal behavior of the hydrothermally prepared sample was characterized by differential thermal analysis (DTA, Netzsch STA 409 analyzer). About 50 mg of the sample was placed in Pt crucible and heated at the rate of 10 K min^{-1} in a synthetic air flow of 30 cm^3 min^{-1} with α-alumina as a reference. In order to establish the structural evolution of phosphate phase with temperature, hydrothermally prepared and washed sample (HT-25) was heated in DTA apparatus up to 600 °C, 790 °C, 950 °C, 1100 °C and 1300 °C, respectively, and then cooled down with the cooling rate of the furnace. Sample codes: FA-600, FA-790, FA-950, FA-1100 and FA-1300 will be used for thermally treated specimens in further discussion to make a distinction between hydrothermally prepared origin and additionally heat-treated specimens.

The samples were studied by X-ray diffraction (XRD) analysis using a Shimadzu XRD 6000 diffractometer with CuKα radiation. Data were collected over a 2θ range between 10 and 110° 2θ in a step scan mode with steps of 0.02° and counting time of 4 s per step. For the purpose of unit cell parameter determination, as well as for quantitative phase analysis, silicon (99.999 wt.%, Aldrich) was added as an internal standard. Changes of crystal structure synthesized origin upon heating were followed by Rietveld structure refinement approach [39]. Topas 2.1 software [40] was used for the data evaluation. Data of Perdikatsis [41] were applied as a starting model for the refinement of fluorapatite. Polynomial model was used to describe the background. Diffraction profiles were described by Pseudo–Voigt function. During the refinement a zero shift, scale factor, half-width parameters (U, V, W), asymmetry parameters and peak shape parameters were simultaneously refined. Structural parameters, atomic coordinates and B_{iso} for two Ca atoms (*4f* and *6h*), one P atom (*6h*), two mirror plane oxygen atoms O1 and O2 (*6h*), and one out of plane oxygen atom O3 (*12i*) were also refined. Infrared (IR) spectra of the samples were acquired using a Bruker Vertex 70 Fourier transform infrared (FTIR) spectrometer in ATR (attenuated total reflectance) mode. The samples were pressed on a diamond and the absorbance data were collected between 400 and 4000 cm^{-1} with spectral resolution of 1 cm^{-1} and 64 scans.

The sample morphology and microanalysis of phases have been examined by scanning electron microscopy (SEM, TESCAN VEGA TS5136LS) equipped with an energy dispersive X-ray spectrometer (Oxford INCA X-sight).

3. Results

3.1. Morphology and microstructure

The morphology of the hydrothermally prepared sample HT-25 is shown in Fig. 1. The hydrothermally transformed material (HT-25) perfectly preserved the morphology of the initial aragonitic cuttlefish bone, while its composition and structure completely changed. The lamellar matrix of cuttlefish bone consists of many horizontal thin sheets (lamellae) supported by transversal pillars that form chambers sealed from each other [38].

SEM image (Fig. 1a) shows that the chamber-like architecture of aragonitic cuttlefish bone is retained during the hydrothermal transformation into fluorapatite. Pillars are uniformly covered with nanosized particles of apatite (Fig. 1b). The higher resolution of image (Fig. 1c) indicates the existence of many uniform, "dandelion-like" microspheres assembled with radially oriented nanorods with an average diameter of about 200 nm and an average length of about 0.4 μm. SEM images of sintered scaffold at 1100 °C with overall structure (Fig. 1d), a pillar covered with sintered features (Fig. 1e) and its higher resolution image (Fig. 1f) exhibited porous and interconnected structure of the scaffold.