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Peroxide-doped apatites: Preparation and effect of synthesis parameters

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ABSTRACT

This contribution focused on the *preparation* of peroxide-doped calcium phosphate apatites — in view of potential uses as bioactive bioceramics with antimicrobial functions, and on their *main physico-chemical characteristics*. Two synthesis routes were investigated. First, the hydrolysis of β-TCP in the presence of H_2O_2 was followed. However, only elevated concentrations in H_2O_2 in the medium or temperatures around 150 °C allowed us to reach the complete β-TCP-to-apatite hydrolysis process, and the obtained samples exhibited a high crystallinity state with no non-apatitic chemical environments. The second protocol tested consisted in the direct apatite precipitation in the presence of H_2O_2 in the medium (at room temperature). This protocol led to single-phased nanocrystalline apatites, and our data indicate that part of the apatitic OH^- ions were substituted by oxygenated species, and typically by peroxide ions (quantified). Physico-chemical modifications in the form of an improvement in crystallinity state, an increase in unit cell volume, and the presence of additional Raman bands were noticed and discussed.

1. Introduction

Calcium phosphates are major candidates for the design of bioceramic-based systems intended to bone regeneration (self-supported scaffolds, injectable bone cements, and implant coatings). In particular, calcium phosphate apatite compounds and predominantly nanocrystalline ones occupy a very peculiar position since they mimic the physico-chemical characteristics of bone mineral [1].

In addition to bare apatitic systems, a great deal of studies have also been devoted to explore associations between apatite crystals and a variety of (bio)molecules or drugs, such as antibiotics, anticancer drugs, growth factors, and vitamins [2], so as to convey additional properties to be exploited on a localized level at the site of implantation. Beside such hybrid organic/inorganic materials, it is however also possible to modify the biological and/or physico-chemical behavior of apatite systems by adequate ion substitutions. For example, the "doping" of calcium phosphate apatites by magnesium or strontium ions that has been reported (e.g. [3,4], either throughout the lattice or more specifically on the surface of the nanocrystals) is aimed at favoring osteoblast cell activity while limiting osteoclast resorptive functions, which may then be used for locally treating osteoporotic sites. The incorporation of silicate ions in phosphate crystallographic sites has also been shown to improve bone regeneration processes [5]. In another context, the substitution of calcium by lanthanide ions such as europium or terbium ions was also shown to convey luminescence properties to apatite particles that can then be exploited for other (not bone-related) applications such as cancer diagnosis [6–8].

The substitution of hydroxide OH^- ions (located in the so-called "apatitic channels" of the apatite lattice) by other anions has, in contrast, received globally less attention to this date, except for the particular case of A-type carbonation. The substitution of apatitic OH^- ions by oxygenated species, and especially by peroxide anions (i.e. species containing at least a O – O covalent bond), could however be seen as a way to modify apatite features that may find new applications in medicine, possibly for bone site asepsis after surgery. Indeed such oxygenated species are characterized by oxidizing properties that are naturally used in vivo (formation of Reactive Oxygenated Intermediates, ROI [9–11]) for fighting against infections. For instance, phagocytes locally produce superoxide (O_2^{2-}) ions to fight against bacteria [12,13]. The generation of ROI is also the mode of action of antibiotics such as fluoroquinolones [14]. This explains the use of peroxide-containing materials as antibacterial agents [13,15–17]. It should also be noted that natural pathways of elimination of such oxygenated species also exist in vivo, so as to regulate their overall activity. This regulation implies in particular specific enzymes such as superoxide dismutases and peroxydases (e.g. catalase), which ensure antioxidizing effects. Despite these statements, the development of oxygenated apatites as "reactive" implantable bioceramics has not received much development in medicine to date.

Although "oxygenated" apatites have not been overly investigated as compared to other substituted apatites, some past studies have however reported the possibility for apatitic channels to incorporate oxygenated species such as H_2O_2 or O_2 [18] or molecular ions including O_2^{2-} (the peroxide ion) and superoxide O_2^- [19]. Furthermore, the incorporation of such species in the apatite lattice was found to lead to specific modifications as compared to hydroxyapatite [18,20–22], and in particular a decrease in the FTIR intensity of OH^- bands and the appearance of additional Raman bands due to the presence of

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peroxide O_2^{2-} ions (in particular at 750 cm^{-1} due to the symmetrical vibration of the O_2^{2-} ion). Unit cell parameters were also found to be altered upon apatite "oxygenation", although varying results were reported probably due to different synthesis protocols resulting in different oxygenated species and apatite compositions [18,20].

Taking into account the above statements, the present contribution aims at determining with further details the physico-chemistry of oxygenated apatites which could find future applications for example in bone infection treatments. In this original contribution, the preparation of peroxide-doped apatites was examined, and the impact of synthesis conditions on their physico-chemical characteristics – which we followed by several complementary techniques (XRD, FTIR, Raman, chemical analyses, and SEM) – was explored. Two different synthesis routes were considered in the presence of H_2O_2 in the reaction medium: 1) the hydrolysis of beta tricalcium phosphate (β-TCP) into apatite (a type of protocol initiated in early studies [18,23]), and 2) the direct apatite precipitation at room temperature from aqueous calcium and phosphate solutions.

2. Materials and methods

2.1. Preparation of peroxide-doped apatites

Two types of synthesis protocols were used in this work, either based on the hydrolysis of β-TCP (β-$Ca_3(PO_4)_2$) or on direct precipitation. In both cases, hydrogen peroxide/water media were used.

In the first case, 500 mg of β-TCP (previously synthesized from calcining amorphous tricalcium phosphate at 900 °C for 24 h [24]) was mixed with 30 mL of hydrogen peroxide H_2O_2 (stock solution at 110 vol. oxygen, equivalent to 9.82 mol/L H_2O_2) in deionized water in a closed container, and then heated to 100 °C for 24 h. After cooling, the compounds obtained for varying starting amounts of H_2O_2 were filtered on Büchner funnel and freeze-dried prior to analysis. Table 1 reports the concentrations in hydrogen peroxide that were tested in this work.

In the second protocol, direct precipitations were carried out in a way similar to the previously reported preparation of nanocrystalline biomimetic apatites [25] but in the presence of increasing amounts of H_2O_2 in the medium. A solution «A» containing 0.3 M of calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) was prepared in a mixture of H_2O_2/H_2O in variable proportions (see Table 2). A solution «B» of di-ammonium hydrogenphosphate $(NH_4)_2HPO_4$, 0.6 M, was prepared in parallel in the same H_2O_2/H_2O medium. A volume of solution «A» was then poured into the doubled volume of solution «B», under stirring. The precipitation medium was then left to mature for 3 days at room temperature before filtration on Büchner funnel and freeze-drying.

2.2. Physico-chemical characterization

The nature and crystallographic structural features of the crystalline phases obtained in this work were investigated by X-ray diffraction (XRD) using a Seifert XRD 3000 TT diffractometer (Cu Kα1Kα2 radiation). The counting time was of 60 s for every step of 0.04°, typically in the 2θ range 20–70°. Unit cell parameters and volume were evaluated by XRD data treatment thanks to the JANA 2006 software, considering the systems as single-phased apatites ($P6_3/m$ space group).

Table 1
Initial proportions of H_2O_2 tested for the β-TCP synthesis route.

Sample reference	Vol.% of H_2O_2 stock solution (at 110 vol. oxygen) in the medium	Initial molar concentration in H_2O_2 in the medium (M)
H0%	0%	0
H5%	5%	0.49
H10%	10%	0.98
H25%	25%	2.46
H50%	50%	4.91
H75%	75%	7.37

Table 2
Initial proportions of H_2O_2 tested for the direct precipitation synthesis route.

Sample reference	Vol.% of H_2O_2 stock solution (at 110 vol. oxygen) in the medium	Molar concentration in H_2O_2 in the medium (M)
3d-10% H_2O_2	10%	0.98
3d-25% H_2O_2	25%	2.46
3d-50% H_2O_2	50%	4.91

Complementary analyses were run by Fourier transform infrared (FTIR) spectroscopy. Spectra were recorded on a Nicolet 5700 spectrometer in the range 400–4000 cm^{-1} (64 scans, resolution of 4 cm^{-1}). Spectral deconvolutions were carried out as previously described [25], after subtraction of a linear baseline, in the 400–800 cm^{-1} wavenumber range corresponding to the $\nu_2\nu_4(PO_4)$ and $\nu(OH)$ vibration modes of phosphate and hydroxide ions respectively, using the ORIGIN 8.1 software.

Raman spectra were recorded on a Horiba Jobin-Yvon LabRAM HR 800 spectrometer, typically in the range 100–4000 cm^{-1} using a $\lambda = 532$ nm laser. The acquisition was realized with an exposure time of 180 s and 6 accumulations for each point with an optical objective of ×100 and a diaphragm opening of 100 μm.

Scanning Electron Microscopy (SEM) analyses were performed on a LEO 435 VP microscope operated at 10–15 kV.

The chemical composition of the samples obtained was drawn from the measurement of ionic contents. The determination of the calcium and orthophosphate (PO_4^{3-} and HPO_4^{2-} ions) contents were obtained, after dissolution of the samples in perchloric acid, by way of EDTA complexometry and spectrophotometry (using the phospho–vanado–molybdenic complex, with $\lambda = 460$ nm) [26], respectively. Peroxide titration was performed by manganimetry titration using potassium permanganate $KMnO_4$ [27], exploiting the redox couple MnO_4^- (purple)/Mn^{2+} (colorless) and the reaction:

$$2MnO_4^- + 5H_2O_2 + 6H^+ \rightarrow 2Mn^{2+} + 5O_2 + 8H_2O. \quad (1)$$

For this titration, powder apatite samples were first dissolved in concentrated perchloric acid ($HClO_4$), and the obtained solution was then titrated by addition of a solution of $KMnO_4$ (0.005 M). The equivalence was then reached when the purple coloration of the permanganate ion persisted in the reacting medium.

3. Results and discussion

As mentioned above, two types of protocols were investigated in this work. The general goal was to obtain single-phased peroxide-doped apatites allowing varying amounts of peroxide doping. Also, the search for rather low crystallinity states and/or small constitutive crystals was an appealing secondary objective in view of obtaining potentially reactive apatite compounds [25] in view of future medical applications.

3.1. Synthesis by hydrolysis of β-TCP in H_2O_2/H_2O media

In a first step, we investigated the possible hydrolysis of β-TCP into apatite by heating in aqueous medium (mixtures of H_2O_2 and H_2O in pre-selected proportions), at a temperature of 100 °C for 24 h. Fig. 1 reports the XRD patterns obtained for 0, 25% and 75% (in volume) of H_2O_2 (stock solution at 110 vol. oxygen) in H_2O. In this figure, the characteristic patterns of the initial β-TCP compound (corresponding to JCPDS card No. 09-169) as well as of a reference stoichiometric hydroxyapatite HA (JCPDS card No. 09-432) were also added for comparative purposes.

Results indicated (see Fig. 1) that the sample treated at 100 °C for 24 h in the absence of H_2O_2 (sample H0%) was still composed of β-TCP, thus witnessing the absence of hydrolysis into apatite in these conditions. In contrast, increasing proportions of H_2O_2 in the reacting medium were found to allow a progression of the TCP-to-apatite