The role of the counter-ions present in syntheses on the thermal stabilization of strontium and/or calcium apatites

Leila Melo da Silva [a], Daniela dos Santos Menezes [a], Luis Eduardo Almeida [a], Karine Anselme [b], Joseph Dentzer [b], Euler Araujo dos Santos [a],*

[a] Laboratório de Biomateriais – P²CEM, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, São Cristóvão, 49100-000 Sergipe, Brazil
[b] Institut de Science des Matériaux de Mulhouse (IS2M), CNRS UMR7361, Université de Haute-Alsace, 15, rue Jean Starcky, BP 2488, 68057 Mulhouse, France

ARTICLE INFO

ABSTRACT

The goal of this work was to study the thermal stabilization of calcium apatites in which the Ca^{2+} ions were substituted for Sr^{2+} in increasing concentrations via ionic co-substitutions. Two distinct standard syntheses were proposed for comparative purposes: one using counter-ions that were not easily incorporated into the apatite structure (NH_4^+/NO_3^-) and one using counter-ions that can be easily incorporated into the structure (Na^+/Cl^-). After calcination, only the apatites synthesized in the presence of NH_4^+/NO_3^- presented phase transformation. In contrast, the apatites synthesized in the presence of Na^+/Cl^- formed a solid solution after calcination, with Na^+, Ca^{2+}, Sr^{2+} and Cl^- sharing the same apatite lattice. Wavelength dispersive X-ray fluorescence spectroscopy (WDXRF), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and temperature-programmed desorption (TPD) techniques showed that the counter-ions present during the syntheses that are associated with CO_3^{2-} play an important role in the thermal stabilization of the apatites.

1. Introduction

The extracellular matrix of bone tissue is composed of an inorganic phase consisting of a mineralized carbonated calcium apatite and an organic phase formed mainly by type I collagen. This apatite is commonly called biological apatite and is generally organized in the form of calcium-deficient nanosized crystals containing anionic and cationic substitutes, such as Na^+, Mg^{2+}, Sr^{2+}, K^+, Cl^-, F^-, Zn^{2+}, Mn^{2+}, CO_3^{2-}, HPO_4^{2-} and SiO_4^{4-} [1–3]. In the biological environment, apatite precipitation onto type I collagen fibers occurs in the presence of blood plasma, which contains several of these ions. All of these ions have important roles in the maintenance of bone metabolism [4,5]. In fact, efforts to develop and improve the biomedical applications of synthetic apatite targeting are now focused on the ability to incorporate these ionic groups in the apatite structure [6,7]. With an adequate chemical design, synthetic apatites can be used to control the behavior of cells, improve the biocompatibility of existing materials, and allow proteins and growth factors to exhibit normal physiological activity.

The first generation of so-called "smart bioceramics" was based on the association of proteins with ceramics (via absorption or adsorption) with the goal of stimulating cells via integrins using specific amino acid sequences. Several studies have demonstrated how specific amino acid sequences and other organic molecules can drive intracellular cascades, leading to the control of cell behavior [8–11]. However, the commercialization of protein-containing bioceramic devices is not easily achieved due to their high cost and difficulties in handling, structural stabilization, and achievement of the purity necessary for their use in biomedical domains.

However, it has been demonstrated that when inserted in bioceramics, i.e., apatites and bioglasses, some ions can induce responses similar to those induced by specific proteins and growth factors. Additionally, under certain conditions, these ions can be adequately stabilized in the structures without encountering the problems of storage, transport temperature, high cost, etc. Thus, the doping of bioceramics with functional ions such as Mg^{2+}, Sr^{2+}, Mn^{2+} and Zn^{2+} is a promising alternative for the development of the new generation of smart bioceramics [4,12–14].

In this sense, Sr^{2+} ions have been frequently considered to serve as doping elements capable of controlling osteoblast activity and, therefore, the biological performance of apatitic ceramic systems when implanted into the body [15–18]. Sr^{2+} is found in biological calcifications and is associated with a decrease in bone resorption and an increase in the proliferation and differentiation of osteoblasts [19,20]. Studies have reported an increase in alkaline phosphatase, type-I collagen and osteocalcin levels in the presence

of Sr^{2+} ions as well as a decrease in bone resorption due to the inhibition of osteoclast-resorbing activity and differentiation.

Despite the benefits of Sr^{2+} in apatitic structures, its presence directly affects crystallinity by introducing defects that can induce phase transformation at high temperatures. The new phases of the apatites after thermal treatments are generally less biocompatible than the original apatite; indeed, this is the case with α-tricalcium phosphate (α-TCP) and β-tricalcium phosphate (β-TCP). Some phases are even toxic to cells, as is the case with calcium oxide (CaO) [21]. Most ceramic devices made for implantation into the body require heat treatment during manufacturing. This necessitates the identification of new strategies for stabilizing these ions in the apatite structure during thermal treatment.

Several studies have demonstrated that, conversely to the structural instability caused by the presence of a single doping element, co-substitution with multiple elements with different sizes and charges can stabilize the apatite structure, preventing phase transformation at high temperatures [22–24]. Several theories attempt to explain how co-substitution can stabilize apatite structure. The most accepted hypothesis is that areas of stress in the crystal lattice caused by the insertion of a substituent can be compensated for by the insertion of other ions of different sizes and charges. Some studies have shown that ions easily found during the precipitation of apatites in aqueous media, such as Na^+, Cl^- and CO_3^{2-}, can thermally stabilize the apatitic phase in association with other doping elements [25,26].

The purpose of this work is to study how the replacement of Ca^{2+} with Sr^{2+} in a calcium apatite may be affected by the presence of other elements of different charges and sizes, specifically the counter-ion pairs Na^+/Cl^- and NH_4^+/NO_3^-, which are generally present in standard syntheses. Additionally, this study investigates how these counter-ions can stabilize the apatite structure.

2. Materials and methods

2.1. Synthesis in the presence of Na^+/Cl^-

Calcium and/or strontium apatites were obtained using the wet precipitation method [4,24,27] via the reaction of phosphoric acid with calcium and/or strontium chlorides. The proportions of Ca^{2+} and Sr^{2+} used in the syntheses are presented in Table 1.

A phosphoric acid solution (0.334 mol/L) was dripped into a calcium and/or strontium chloride solution (0.220 mol/L) under stirring at 60 °C without atmospheric control. The pH was maintained at 10.0 by adding sodium hydroxide solution (0.1 mol/L) during the synthesis. The mixture was incubated for 24 h at 60 °C under stirring. The precipitate was filtered, washed in distilled water until the supernatant was neutral (pH ≈ 7) and air-dried at 100 °C for 24 h. Next, the precipitate was ground in an agate mortar and sieved through a 106 μm mesh. All reagents were at least 98% pure and were purchased from Sigma–Aldrich. Three independent syntheses were performed to evaluate the reproducibility of the analyses used in this work.

Table 1
Nomenclature for the apatites synthesized in the presence of Na^+/Cl^- and NH_4^+/NO_3^-.

$Sr^{2+}/(Sr^{2+}+Ca^{2+})$ ratio	Sample nomenclature	
	Na^+/Cl^-	NH_4^+/NO_3^-
0.0	Sr-HA$_{Cl}$ – 0%	Sr-HA$_N$ – 0%
0.2	Sr-HA$_{Cl}$ – 20%	Sr-HA$_N$ – 20%
0.4	Sr-HA$_{Cl}$ – 40%	Sr-HA$_N$ – 40%
0.6	Sr-HA$_{Cl}$ – 60%	Sr-HA$_N$ – 60%
0.8	Sr-HA$_{Cl}$ – 80%	Sr-HA$_N$ – 80%
1.0	Sr-HA$_{Cl}$ – 100%	Sr-HA$_N$ – 100%

2.2. Synthesis in the presence of NH_4^+/NO_3^-

To understand the effect of the counter-ions Na^+ and Cl^- on the thermal stabilization of the calcium and/or strontium apatites, we have proposed a control synthesis in which Na^+ and Cl^- were exchanged for counter-ions that are not easily inserted in the apatite lattice. In this sense, the same procedure described in Section 2.1 was employed; however, the chlorides were replaced by nitrates. All nitrates are soluble and can be eliminated from the precipitated phase by filtration. In addition, the pH was maintained at 10.0 using NH_4OH instead NaOH. NH_4^+ is not easily inserted in the apatite lattice and can be completely eliminated from the apatites by thermal treatment at relatively low temperatures.

2.3. Composition of the apatites

2.3.1. Elemental analyses

The presence of the elements Ca, Sr, P, Na and Cl and their concentrations in the obtained apatites were measured by wavelength dispersive X-ray fluorescence spectroscopy (WDXRF) in a Bruker spectrometer (S8-Tiger). The analyses were performed with tablets produced by uniaxial pressing of the original apatite powders without the addition of boric acid. The molar percent (mol%) of each element was calculated and normalized to the total quantity of elements detected in each sample. Three samples from three different syntheses were analyzed.

2.3.2. Evolution of PO_4^{3-}, CO_3^{2-} and OH^- groups in the apatitic structures

All syntheses were conducted without atmospheric control, resulting in the inherent insertion of CO_2 in the apatite lattice as CO_3^{2-}. Because CO_3^{2-} is able to replace either PO_4^{3-} or OH^- groups in apatite lattices, infrared spectroscopy was proposed as a means to understand the evolution of these groups in the synthesized materials. The PO_4^{3-} and OH^- groups were observed using Fourier transform infrared spectroscopy (FTIR). The changes in the CO_3^{2-} groups were followed using attenuated total reflectance (ATR) to estimate the quantity of CO_3^{2-} in A- and B-type substitutions. The analyses were conducted in absorbance mode from 4000 to 400 cm^{-1} (step size of 4 cm^{-1}) using a Perkin-Elmer (Spectrum BX) spectrometer.

2.4. Phase transformations

The obtained apatite powders were calcined at 1000 °C for 2 h with a heating rate of approximately 3 °C/min. The thermal stability of these apatites and the phase transformation during calcination were accessed by X-ray diffraction (XRD). The analyses were performed from 10° to 60° (step size of 0.02° and fixed time of 2 s per point) using a Shimadzu (XRD 6000) powder diffractometer (CuK$_\alpha$ λ = 1.5405 Å; 60 kV, 55 mA, Ni filter; scintillation counter).

2.5. CO_3^{2-} release from apatites

The amount of CO_3^{2-} ions released from the apatites was estimated by temperature-programmed desorption (TPD). The assays were performed in a vacuum device associated with a line-of-sight detection quadrupole mass spectrometer. The gases released from the samples during heating were analyzed by mass spectrometry. The typical frequency for data acquisition was two mass spectra per minute, and the mass range used was 1–100 mass units. The signals derived from CO_2 (m/z = 44) and CO (m/z = 28) generated during the heating of 50 mg of apatite powder up to 950 °C (heating rate of 3 °C/min) were monitored. The temperatures of maximum desorption were measured from the CO and CO_2 desorption curves. The