SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF NIOBIUM-DOPED HYDROXYAPATITE CERAMICS

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Abstract

Hydroxyapatite (HAp) ceramic materials are considered as one of the most promising implant materials in bone surgery and in dentistry. They exhibit unique biocompatibility, bioactivity, and osteoconductivity, which are the most desirable biomaterial features. However, HAp itself is brittle, has low strength, high degree of crystallinity and low solubility at physiological pH. Doping synthetic HAp with metal ions plays an important role in improving its structural and physico-chemical properties. HAp doped with niobium ions has not been widely investigated so far. However, the results of studies available in the literature show that the synthesized $CaO-P_2O_5-Nb_2O_5$ compounds still show good biocompatibility, very low cytotoxicity and, additionally, they stimulate osteoblast proliferation. Therefore, this study is dedicated to the niobium-doped HAp ceramics obtained by two methods: mechano-chemical synthesis and sol-gel method. Bioceramics chemical composition, morphology, and structure were characterized by means of scanning electron microscopy imaging, BET method, X-ray diffraction, Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. The results indicate that nanoceramics of non-stoichiometric HAp with a clear Ca deficiency on the nanograin surface were obtained. Moreover, it has been observed that the presence of Nb dopants and the synthesis method directly affect unit cell parameters, crystallinity degree, crystallites size, porosity and distribution of niobium in the grain structure. Mechano-chemical synthesis has allowed effective niobium incorporation into the HAp structure, leading to the quite homogeneous Nb distribution in the grain volume. Whereas, Nb-doping by sol-gel method has led to dopants location mainly on the grain surface.

Keywords: *structure, nanoceramics, mechano-chemical synthesis, sol-gel method, hydroxyapatite, niobium*

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Introduction

Apatites are the most important and most abundant components of hard human tissues. For that reason, these materials have been objects of intensive research for the last decades. In this group hydroxyapatite (HAp) ceramics is the most promising material because its chemical composition is similar to that of a natural bone. Furthermore, it exhibits biocompatibility, bioactivity and osteoconductivity, which are one of the most desirable biomaterial characteristics [1,2]. For these reasons, HAp is widely used in medical applications, for example as bone graft substitutes, in drug delivery systems and hypodermic devices. In addition, hydroxyapatite thin layers on metal implants significantly improve biological properties of these systems [3].

Depending on the HAp application, different properties of this material are required. These properties are strongly related to the structure, morphology and chemical composition of the obtained material. Currently, many methods of hydroxyapatite synthesis are used to precisely define the properties of the obtained ceramics. However, a versatile method of synthesis is of interest as it gives the possibility to in-build other ions, organic elements or drugs into the HAp structure in an easy manner and at low temperature. The methods of HAp synthesis can be divided into: i) dry methods – in which the material is a result of a solid state reaction of precursors and ii) wet methods – in which reaction occurs in mixtures of solutions of precursors [4]. Among the wet methods the precipitation should be mentioned as the most frequently used and the sol-gel technique. The latter one is becoming more and more popular because it allows nanocrystalline materials to be created and chemically modified (by, for example, doping with ions) in a relatively easy way [5,6].

The main disadvantages of hydroxyapatite are brittleness and low strength. Other drawbacks include design limitations, a high degree of crystallinity which results in low solubility in physiological pH and non-biodegradability. Thus, a substantial effort has been devoted to improve synthetic HAp structural and physico-chemical properties. One of the strategies, still used, is doping HAp with ions [7]. Various substitutions are used, namely cationic (e.g. using magnesium, strontium, zinc), anionic (e.g. using carbonates, fluorides, silicates) and multi-ionic. For instance, strontium doped HAp exhibits greater hardness and compressive strength. On the other hand, magnesium-doped HAp shows a slight deterioration of these properties but the improved fracture toughness [8]. HAp doped with niobium ions has not been extensively investigated up to now. The results found in the literature [9-11] show that synthesized $CaO-P_2O_5-Nb_2O_5$ compounds exhibit good biocompatibility, very low cytotoxicity when compared to calcium-phosphate doped with other metals and they can enhance human osteoblast function. Moreover, they have promising mechanical parameters. For these reasons, Nb-doped HAp has the potential to be used as a biomaterial in bone tissue repair [9] and as a buffering layer between metal implants and natural tissue.

In this paper, we compare structural and morphological properties of pure hydroxyapatite and niobium-doped hydroxyapatite prepared via a dry method – the mechano-chemical synthesis and a wet method – the sol-gel technique.

Materials and Methods

Synthesis

In this paper, hydroxyapatite powders were synthesized by two methods: the mechano-chemical synthesis and the sol-gel method. In this way, undoped and Nb-doped powders were obtained. The amount of dopant was set at 10 mol% of phosphorus.

In the mechano-chemical synthesis, stoichiometric amounts (i.e. molar ratios Ca/P and Ca/(P+Nb) of 1.67) of calcium hydroxide ($Ca(OH)_2$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and niobium pentoxide (Nb_2O_5) were used as precursors. First, the powders were quickly mixed and ground in an agate mortar. Next, they were milled in the planetary ball mill using rotation speed of 400 rpm for 16 h (including an hour of break after each hour of milling). Lastly, the samples were ground in a mortar to obtain fine powders. The studied samples are referred to as: MS-HAp (undoped material) and MS-Nb-HAp (niobium-doped material).

In the sol-gel method ethanol solutions of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), phosphorus pentoxide (P_2O_5) and niobium (V) chloride ($NbCl_5$) were used as precursors. The amounts of substrates were chosen to obtain a stoichiometric material (i.e. molar ratios Ca/P and Ca/(P+Nb) of 1.67). To obtain the solutions, a calcium-containing precursor was dissolved in a beaker containing 50 ml of ethanol and phosphorus- and niobium-containing precursors (or only phosphorus in the case of an undoped sample) were dissolved together in a separate beaker containing 50 ml of ethanol. The solutions were mixed and stirred at room temperature. Gelation of the mixtures occurred in a water bath at 60°C. Gel aging and drying at 80°C lasted for 24 h. The dried gels were sintered at 700°C with heating and cooling speed rates of 10°C/min and then ground in an agate mortar. These powders are referred to as: SG-HAp (undoped material) and SG-Nb-HAp (niobium-doped material).

Characterization

The morphology and composition of powders were analyzed under FEI Quanta FEG 250 scanning electron microscope (SEM) with energy dispersive spectroscopy (EDX) system. EDX results were averaged from three (or more) 1200 μm x 1000 μm area analyses, the acquisition time for each analysis was set at 50 s. Then, a molar ratio of Ca/(P+Nb) was calculated (for stoichiometric hydroxyapatite this ratio equals 1.667 [1]). For SEM imaging the samples were gold coated by sputtering, to avoid charging.

The surface area was investigated by adsorption of nitrogen at 77 K (Quantachrome Autosorb IQ-Chemisorption & Physisorption Gas Sorption Analyzer) according to the BET method. Prior to the analysis the powders were degassed for 3 h at 150°C.

The X-ray diffraction (XRD) of the powders was performed using Philips X'Pert-MPD X-ray Diffraction System operating with CuKα radiation in the 2θ range of 20-55° and with scan step size of 0.02°. Based on the XRD pattern, the samples phase compositions were determined and the crystallites size, lattice parameters as well as unit cell volumes were estimated.

The investigation of functional groups was done by Fourier transform infrared (FTIR) spectroscopy using PerkinElmer FT-IR/FIR Spectrometer Frontier in attenuated total reflectance (ATR) mode in the range of 550-4000 cm^{-1} with scan step size of 1 cm^{-1} and resolution of 2 cm^{-1}.

The surface composition was analyzed by X-ray photoelectron spectroscopy (XPS) using Omicron XPS operating with Mg anode, achromatic radiation, scan step size of 0.05 eV and pass energy of 50 eV. The effects associated with the charge loading of samples were removed by calibrating to the carbon C1s band (284.5 eV) [12,13]. The elemental composition analysis and decomposition of the obtained bands were carried out using the CasaXPS software [14].

Results and Discussions

The SEM images of obtained powders are shown in FIG. 1 and the results of their detailed analysis and EDX measurements are presented in TABLE 1. As it can be observed, both the undoped powders are composed of sphere-like nanograins approximately 100 nm (MS-HAp) and 50-200 nm (SG-HAp) in diameter. Additionally, in the SG-HAp sample, particles with morphology of whiskers - measuring about 200 nm in length - are also observed. This suggests the possible presence of two different calcium-phosphate phases in the compound, with one of them of the Ca/P molar ratio evidently lower than 1.5. The doped MS-Nb-HAp sample also consists of sphere-like nanograins (diameters of approximately 100-300 nm), but these grains form a rather solid porous structure (with the expanded surface and complex porosity). The SG-Nb-HAp sample has a solid structure that is highly porous and the pore size distribution is narrow, with the mean micropores' size of about 100-250 nm in diameter. These results show that the addition of niobium affects the morphology of hydroxyapatite materials, in general changing it from granular to a more solid one and increasing the sample porosity. However, the details of the morphology are strongly dependent on the synthesis procedure.

Only in the case of the SG-Nb-Hap sample the EDX analysis detected the trace amounts of chlorine - the residual substrate used for nobium doping, no other elements or impurities were registered apart from the elements related to the assumed material composition (P, Ca, Nb and O). Additionally, it was revealed that the relation between the main elements of synthesized powders differs from the target one (Ca/(P+Nb) = 1.667). The results also indicate that doping through the mechano-chemical synthesis is more efficient than through the sol-gel process. The obtained molar ratio of Nb/P was 0.08 and 0.04 for the samples of MS-Nb-HAp and SG-Nb-HAp, respectively.

To check the SEM visible morphology changes induced by doping, the N_2 adsorption isotherms of prepared powders were collected and used to calculate the BET surface area. The results are shown in FIG. 2 and TABLE 2. The undoped powders had a relatively small BET surface area. Doping with niobium increased the BET surface area to 31.02 m^2/g for the Hap sample prepared via the mechano-chemical synthesis (with the value six times higher than for the undoped material) and to 10.35 m^2/g for the sample synthesized using the sol-gel method (the increase by only 15% in respect to the undoped material). These results, like the SEM results, suggest that doping affects the powders morphology, but in the case of the mechano-chemically synthesized powders, it causes a greater surface development than when using the sol-gel method.

The XRD patterns of the powders are shown in FIG. 3. The diffraction peaks are mainly indexed to the hexagonal HAp phase (PDF#74-0566), confirming the synthesis of the non-stoichiometric form of hydroxyapatite (see TABLE 1). The hexagonal unit cell parameters derived from the XRD patterns analysis and unit cell volumes are presented in TABLE 3. Only in the SG-HAp sample additional peaks corresponding to the presence of other calcium-phosphate phase, with a lower Ca/P molar ratio (PDF#70-2065), were detected (peaks marked by X in FIG. 3). This result perfectly correlated with the grains shaped as nanowhiskers observed in the SEM images (see FIG. 1c).

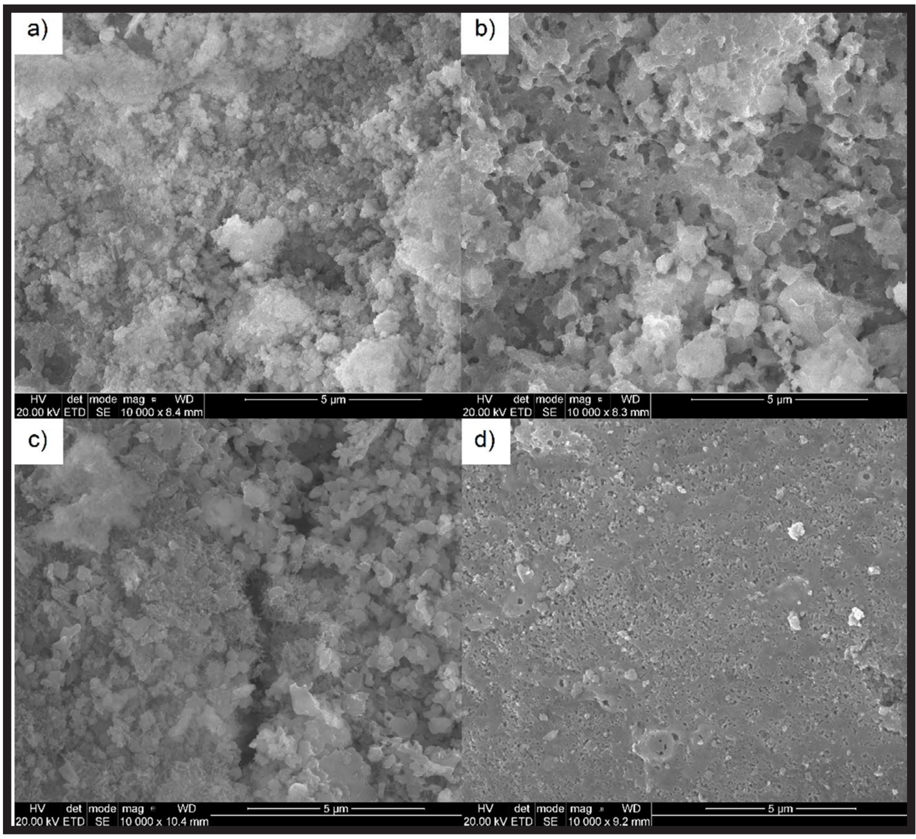

FIG. 1. SEM images of synthesized powders:
a) MS-HAp,
b) MS-Nb-HAp,
c) SG-HAp,
d) SG-Nb-HAp.

TABLE 1. SEM imaging and EDX analysis results.

Sample	Size [nm]	Ca/(P+Nb) molar ratio	Nb/P molar ratio
MS-HAp	**Nanograins** 100	1.53 ± 0.08	-
MS-Nb-HAp	**Nanograins** 100-300	1.77 ± 0.09	0.08
SG-HAp	**Nanograins** 50-200 **Nanowhiskers** 200	1.52 ± 0.08	-
SG-Nb-HAp	**Pores** 100-250	1.68 ± 0.09	0.04

TABLE 2. BET surface areas of synthesized powders.

Sample	BET surface area [m²/g]
MS-HAp	5.36
MS-Nb-HAp	31.02
SG-HAp	8.97
SG-Nb-HAp	10.35

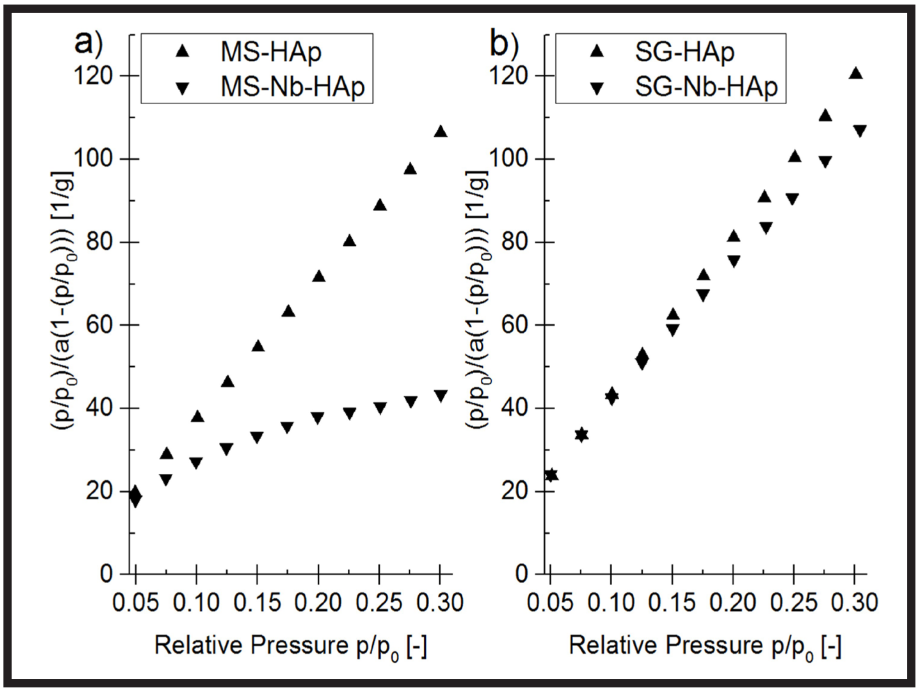

FIG. 2. N_2 adsorption isotherms, used for BET analysis, of powders synthesized through:
a) mechano-chemical synthesis,
b) sol-gel method.

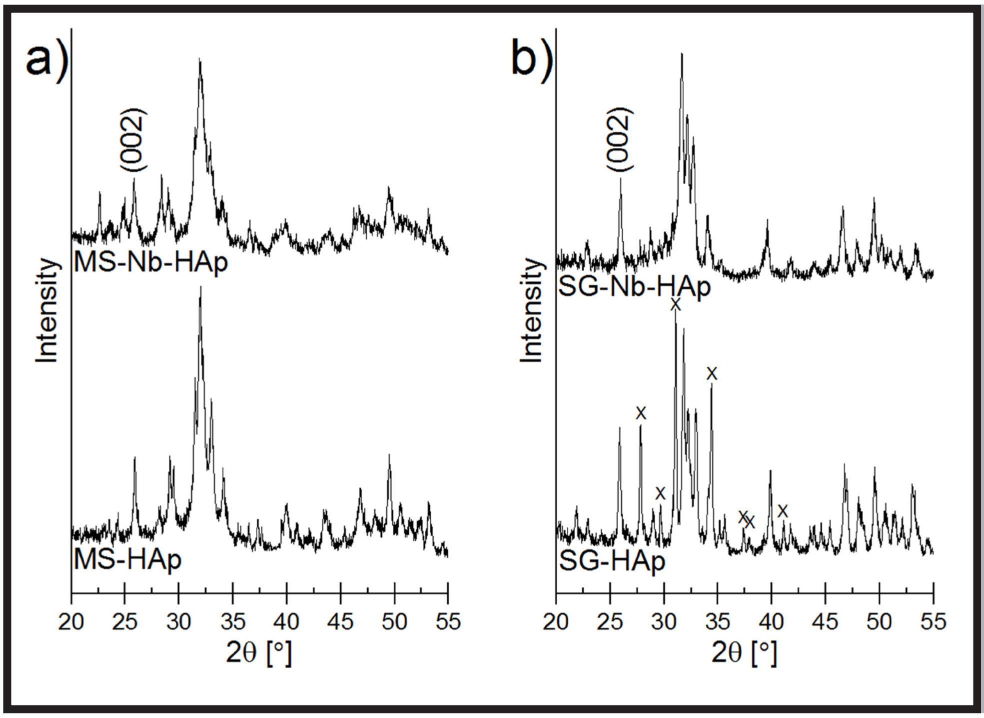

FIG. 3. XRD patterns of powders synthesized through:
a) mechano-chemical synthesis,
b) sol-gel method.
X – the second phase with lower Ca/P molar ratio.

TABLE 3. Crystallite sizes, lattice parameters and unit cell volumes of prepared powders obtained by XRD patterns analysis.

Sample	Crystallite size [nm]	Lattice parameters [Å] (hexagonal)	Unit cell volume [Å^3]
MS-HAp	24.90 ± 0.55	a = b = 9.40(1) c = 6.89(1)	526.85
MS-Nb-HAp	18.80 ± 0.33	a = b = 9.41(1) c = 6.90(1)	528.99
SG-HAp	33.11 ± 0.92	a = b = 9.43(1) c = 6.89(1)	529.34
SG-Nb-HAp	26.12 ± 0.60	a = b = 9.48(1) c = 6.86(1)	533.31

In the XRD measurements, the most visible effects of Nb doping were the crystallinity degree decrease, broadening of diffraction peaks (independently on the sample preparation procedure) and the disappearance of the additional phase observed in the material prepared by sol-gel method. The peaks broadening effect indicates the decrease in the mean crystallite size. The size was determined by the Sherrer formula and the results obtained for all the samples are presented in TABLE 3. The calculations were performed using (002) peak parameters (peak marked in FIG. 3). It should be emphasized that crystallite sizes in the undoped HAp were in the range of 25-35 nm and doping actually reduced this value to 18-27 nm. Nevertheless, the unit cell volume of the samples increased due to doping. Moreover, in the XRD pattern of the Nb-doped samples additional peak at 22.8° appeared (with a higher relative intensity in the MS sample), which can indicate that Nb was effectively incorporated into the hexagonal HAp structure (PDF#72-1484 – hexagonal niobium oxide phase).

The FTIR spectra of obtained powders are shown in FIG. 4. In all the samples, the characteristic peaks of HAp were observed. The intense band appearing at roughly 1020 cm^{-1} and the less intense band at 940 cm^{-1} were assigned to the asymmetric (v_3) and symmetric (v_1) stretching modes in the $(PO_4)^{3-}$ anions, respectively [15]. Moreover, in the FTIR spectra of undoped samples and the MS-Nb-HAp v_3 the vibration mode of $P(OH)_2$ groups located at approximately 1000 cm^{-1} and a very weak and broad absorption band of the $(HPO_4)^{2-}$ groups located at 740 cm^{-1} was also noted [15]. The presence of $(HPO_4)^{2-}$ anions serves as an indirect confirmation of the non-stoichiometricity of the obtained hydroxyapatite materials. The broad bands at around 3220 cm^{-1} and 2850 cm^{-1} are associated with the vibration modes of H-O-H bonds of water adsorbed on the powders and the stretching modes of N-H bonds in the ammonium groups, respectively [15]. Both bands are visible for the mechano-chemically synthesized materials (FIG. 4a). The N-H vibration bands are non-existent in the SG materials (FIG. 4c) because none of the substrates used in their synthesis contained ammonia or its compounds. Moreover, in the spectra of MS materials, the presence of a band located at about 3600 cm^{-1}, attributed to the stretching vibration of the OH^- functional groups inbuilt in the HAp lattice [15], should be underlined. In addition, the carbonate bands located at approximately 1440 cm^{-1} and 860 cm^{-1} are observed in the FTIR spectra [15]. They might come from the atmosphere carbon compounds which combined with the HAp structure during synthesis. This effect is much more pronounced in the case of mechano-chemical synthesis. The detailed analysis revealed that CO_3^{2-} ions introduced into the structure of HAp mainly substituted the PO_4^{3-} ions. This phenomenon may additionally improve the biological activity of HAp, as the carbonated HAp is essential for bonding with natural bone [16].

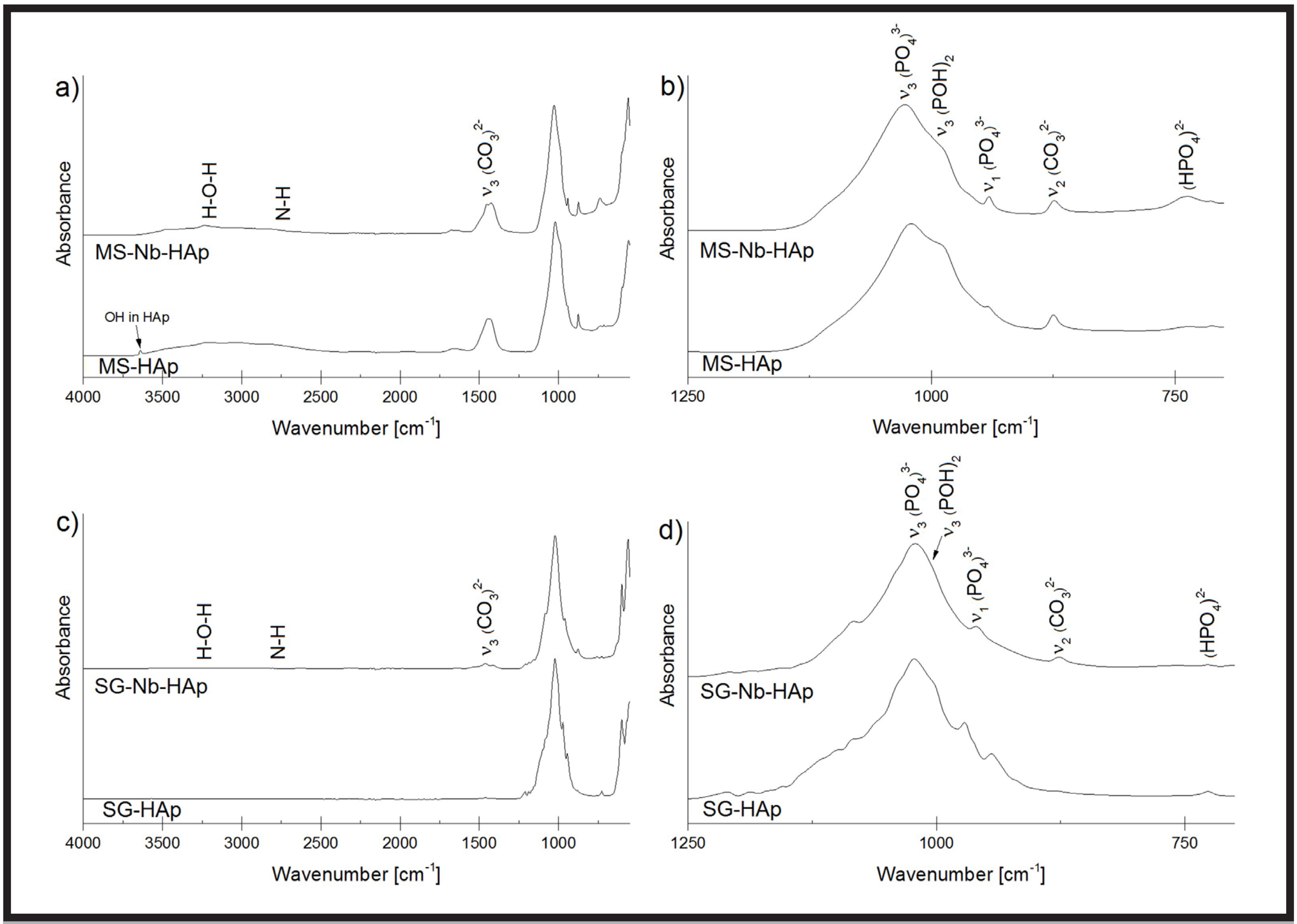

FIG. 4. FTIR spectra of powders synthesized through: a)-b) mechano-chemical synthesis and c)-d) sol-gel method.

TABLE 4. XPS quantitative analysis results.

Sample	Ca/(P+Nb) molar ratio	Nb/P molar ratio	Nb^{+5}/ (Nb^{+5}+Nb^{+4}) [%]
MS-HAp	1.06 ± 0.1	-	-
MS-Nb-HAp	0.96 ± 0.1	0.02	68(10)
SG-HAp	1.05 ± 0.1	-	-
SG-Nb-HAp	1.29 ± 0.1	0.07	80(5)

Using the XPS spectra, a quantitative analysis was carried out, based on the decomposition of Ca2p, P2p, and Nb3d bands. The results of this analysis presented in TABLE 4 show that the surface of the grains of all the samples exhibits calcium deficiency, independently from the method of synthesis and Nb presence. This Ca deficiency equals 30% in the case of the undoped powders and after Nb-doping it increases to 45% and decreases to 23% for the materials prepared by the MS and the SG method, respectively. At the same time, in the MS-Nb-HAp sample the pronounced decrease in Nb surface concentration is noted, whereas in the SG-Nb-HAp sample this concentration increases with respect to volume value (compare Nb/P data presented in TABLES 1 and 4). The detailed Nb3d line decomposition unveiled the presence of two niobium valency states, Nb^{+5} [17] and Nb^{+4} [18], as shown in FIG. 5.

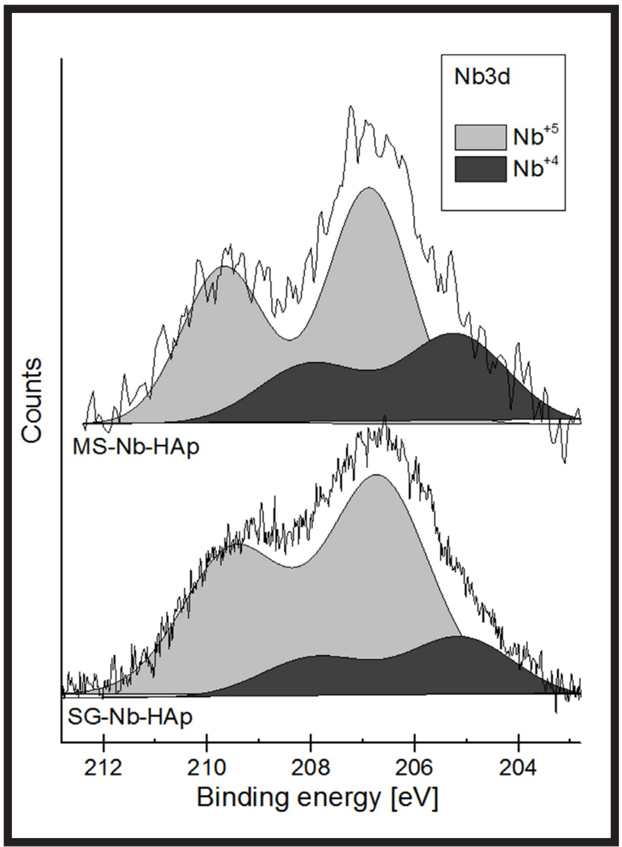

FIG. 5. Nb 3d XPS spectra decomposition performed for Nb-doped nanoceramics.

However, in either sample the Nb^{+5} state dominates and its contribution is almost the same in terms of uncertainty range (about 70% and 80% in the MS-Nb-HAp and the SG-Nb-HAp, respectively, see TABLE 4). These results confirm that the mechano-chemical synthesis allows effective niobium incorporation into the HAp structure (in the nanograins core the Nb/P molar ratio reaches nominal value, i.e. ~10%). Whereas, Nb doping by the sol-gel technique resulted in dopants location on the sample surface rather than in the sample volume. The additional XPS spectra analysis on the O1s band confirms that the dominant calcium-phosphate phase on the sample surface is the phase with the Ca/(P+Nb) molar ratio of approximately 1.0.

Conclusions

In the paper, the results of the structural characterization of the niobium-doped HAp ceramics obtained by either the mechano-chemical synthesis or the sol-gel method, are presented and compared. The results showed that nanoceramics of non-stoichiometric HAp with the surface Ca deficiency were obtained. However, in the sol-gel technique the presence of two crystalline calcium-phosphate phases - one hexagonal HAp phase and another one with Ca/P molar ratio lower than 1.5 - were detected. The Nb-doping process, independently from the synthesis method, caused the changes in the unit cell parameters, manifested in the increase in the volume of the unit cell, the decrease in the crystallinity degree and the average crystallites size and the slight increase in the material active surface area. The detailed analysis demonstrated that niobium (mainly in forms of Nb^{5+} species) was more effectively incorporated into the hydroxyapatite structure by the mechano-chemical method than by the sol-gel approach. This is evidenced by a different distribution of niobium in the grain structure. The mechano-chemical synthesis led to obtaining a quite homogeneous Nb distribution in the volume of the grains, whereas the sol-gel method resulted in the dopants location dominant on their surface. In terms of bioactivity, the nanoceramics obtained and presented in this work reveal attractive and varied morphological and structural properties. Therefore, they may find a potential application as a biomaterial in bone tissue repair or as a buffer layer prepared by the sol-gel method. Further studies related to the use of other synthesis procedures and techniques as well as other dopants concentrations are in progress.

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