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INFLUENCE OF DIFFERENT CALCIUM AND PHOSPHORUS SOURCES IN THE SYNTHESIS OF NANOHYDROXYAPATITE POWDER

BY

ANA-MARIA BARGAN, SIMONA ILISEI, GABRIELA CIOBANU[*]
and CONSTANTIN LUCA

“Gheorghe Asachi” Technical University of Iaşi,
Faculty of Chemical Engineering and Environmental Protection

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Abstract. Due to the high similarity to the inorganic material of bone and tooth, also due to its excellent biocompatibility and bioactivity, hydroxyapatite (HA) is one of the most investigated materials for biomedical applications as bone substitute and implants. The main purpose of the research in this study is to compare two type of stoichiometric hydroxyapatite performed by chemical precipitation method using different source of calcium and phosphorus. The synthesized samples were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM) techniques.

Key words: hydroxyapatite, chemical precipitation method.

1. Introduction

Synthetic hydroxyapatite with chemical formula $Ca_{10}(PO_4)_6(OH)_2$ (HA) and molar rapport Ca/P = 1.67 (Monma *et al.*, 1987) is a calcium phosphate with an apatitic structure occurs naturally in the human body and characterized by very good biocompatibility and bioactivity. So many kinds of industrial applications

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[*] Corresponding author; *e-mail*: gciobanu03@yahoo.co.uk

such as catalyst support, liquid-chromatographic columns, lighting materials, powder carriers, chemical sensors, ion conductors, retardant of cancer cells and drug delivery agent, etc. (Kim & Park, 2005; Wang *et al*., 2006), require synthetic HA have corresponding properties such as mechanical strength, adsorption capability and so on. Those corresponding properties usually depend on the particle size, particle size distribution, morphology and surface area, etc. (Bose & Saha, 2003). All these properties are dependent on the method of synthesis.

Various synthesis routes including wet chemical synthesis (such as precipitation method, sol-gel technique, hydrothermal and hydrolysis) (Ferraz *et al*., 2004) used different source of calcium and phosphorus as starting materials. Our effort was focused on preparing HA powder by two common precipitation methods, developed by Rathje (Rathje, 1939) and Hayek - Newesely (LeGeros *et al*., 1993). In this study we investigate the correlation between the precipitation conditions (starting materials) and properties of the samples (the crystallinity and morphology of the materials).

2. Experimental

2.1. Materials

In this study we have synthesized two samples of hydroxyapatite, HA-I sample by Rathje method and HA-II sample by Hayek - Newesely method. The materials used in the synthesis of nanocrystalline powders are present in Table 1. All chemicals were supplied by Sigma-Aldrich (Germany).

Table 1

Materials Used in the Synthesis of HA-I and HA-II Samples

Sample	Materials	Chemical formula
HA-I	Calcium hydroxide	$Ca(OH)_2$
	Orthophosphoric acid	H_3PO_4
	Sodium hydroxide	$NaOH$
	Ethanol	C_2H_5OH
HA-II	Calcium nitrate tetrahydrate	$Ca(NO_3)_2 \cdot 4H_2O$
	Diammonium phosphate	$(NH_4)_2HPO_4$
	Ammonium hydroxide	NH_4OH
	Ethanol	C_2H_5OH

2.2. Methods

Synthesis of HA-I powder: Hydroxyapatite powder, HA-I sample, was synthesized by Rathje method, who consisted of drop-wise addition of phosphoric acid (0.1 M) to a stirring suspension of calcium hydroxide (0.167 M) according to the reaction given bellow:

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O \qquad (1)$$

During synthesis, pH value is adjusted to 10 with NaOH solution (10 M), the temperature is maintaining at 60ºC and the time of mixing is 5 h. The Ca/P molar ratio was maintaining at 1.67, specifically to the production of pure and stoichiometric hydroxyapatite. After maturation period, 24 h, the precipitate was washing with water to remove residual impurities, and then with ethanol to remove water and improve the dispersability. The filtrates are dried in vacuum dryer for 24 h at 90ºC and calcined at 800ºC for 2 h. The fine green powder obtained has been characterized by XRD and SEM techniques.

Synthesis of HA-II powder: Hydroxyapatite powder, HA-II sample, was synthesized by Hayek and Newesely method that consisted of reaction between calcium nitrate and ammonium phosphate with added ammonium hydroxide according to the reaction given bellow:

$$\begin{gathered}10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow \\ Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O\end{gathered} \qquad (2)$$

Synthesis of HA-II powder occurs like the obtaining of HA-I sample, with the same parameters. After calcinations, the white powder obtained has been characterized by XRD and SEM techniques.

Samples characterization: Structural characterization of the samples was done using X-ray diffraction. XRD pattern was taken using a X'PERT PRO MRD diffractometer with Cu-Kα radiation ($\lambda = 1.5418$ A). Data were collected in the 2θ range of 20-80º. The research technique is based on fact that X-ray diffraction of each crystalline material is unique and recognizable, and the result of XRD analysis can identify the crystalline structure. The sample in the form of powder is subject to a diffraction beam of monochromatic X-rays, and that faced with the obtained diffraction shown in the literature. The scanning electron microscopy (SEM) was used to observe the morphology of samples, using a QUANTA 200 3D Dual Beam scanning electron microscope.

3. Results and Discussions

3.1. Chemical Structure of Hydroxyapatite

With the general formula $Ca_{10}(PO_4)_6X_2$ where X is typically OH^- (hydroxyapatite, HA) or F^- (fluorapatite, FAp), apatite are thermodynamically the mainly stable phases among the calcium phosphates and, therefore, can be considered as the probable final product in many reactions (Mathai *et al.*, 2001). Due to the small size of crystals (needle-like crystals of approximately 5-20 nm width by 60 nm length), apatite can be regarded as nanocrystalline materials (Pramanik *et al.*, 2007).

Hydroxyapatite is part of the composition of bioactive and bioresorbable ceramics, and is used in bone reconstruction, in particular for the fabrication of implants which densely fuse with bone (for example in skull restorations after operations or trauma), tooth-root implants, biological tooth fillings, cure of diseases of the periodontal (tissue around teeth), maxillofacial reconstruction, grafting and stabilizing skull bone, joint reconstruction, for the endoprosthesis of hearing aids, cosmetic eye prostheses, etc. (Hulbert *et al.*, 1987).

The physical, chemical and biological properties of HA were showed to be controlled by its crystal structure and composition (Feng *et al.*, 2005). To improve this property and to enhance bioactivity of HA we need to obtain substituted hydroxyapatite. The HA structure (Fig. 1) have a good flexibility in accepting anion/cation substitutions in its network. Anion substitution reaction, PO_4^{3-} groups by HPO_4^{2-} and CO_3^{2-}, OH^- by F^- modify the surface properties and the thermal stability of the material (Shimoda *et al.*, 1990). The substitution of Ca^{2+} with divalent metal ions such as: Sr^{2+}, Mg^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+} and Ba^{2+} (O'Donnell *et al.*, 2008; Qi *et al.*, 2008; Tamm *et al.*, 2006; Yasukawa *et al.*, 2012) and by trivalent cation (Cr^{3+}, Al^{3+} and Fe^{3+}) and lately with rare heart ions (Ce^{3+}) (Feng *et al.*, 2005), affects important characteristics of HA, such as crystallinity, solubility, thermal stability, metabolizability, etc. (Neuman *et al.*, 2006).

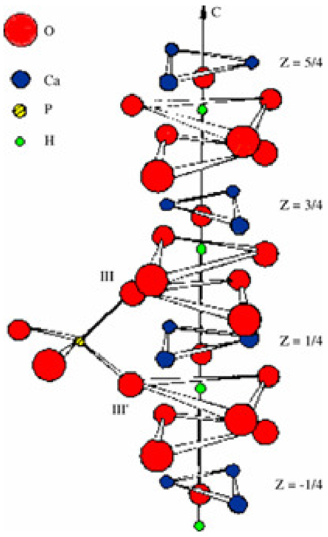

Fig. 1 − The structure of calcium hydroxyapatite (Bagambisa *et al.*, 1994).

3.2. Samples Characterization

X-ray diffraction: In order to investigate the influence of different type of calcium and phosphorus sources used in this work, on nanocrystallization and phase transformation in hydroxyapatite powder, we compared the XRD pattern of HA-I and HA-II samples. XRD patterns characteristic of HA-I and HA-II calcined powders obtained in this work are shown in the Fig. 2.

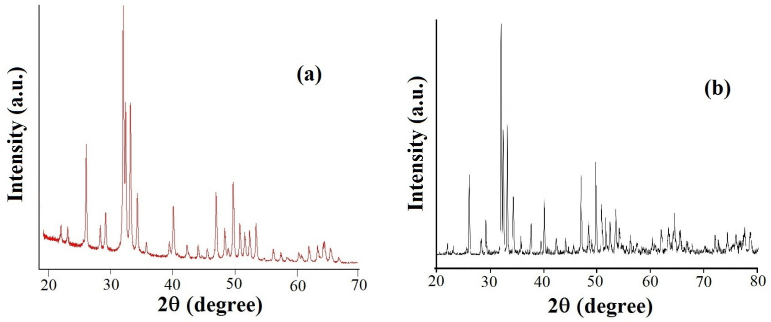

Fig. 2 – XRD pattern of HA-I (*a*) and HA-II (*b*) samples.

All XRD patterns shows diffraction lines characteristic of the hydroxyapatite, present in standards and in literature. The major phase, as expected, is hydroxyapatite, which is confirmed by comparing data obtained with the JCPDS Data Card 09-0432 (for hydroxyapatite). Going into details, it can be seen that XRD diffractogram of the HA-I calcined sample contain the characteristic diffraction peaks of hydroxyapatite but their width indicate that the material contain a little amorphous phase.

So, we can conclude that XRD diffractogram of the two samples indicate that the HA-I sample has the crystalline-amorphous structure and HA-II sample has the higher crystalline structure.

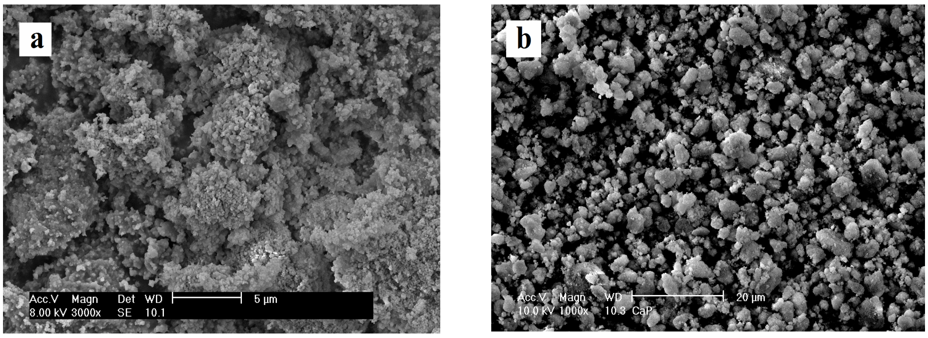

Fig. 3 – SEM images of the hydroxyapatite powders: HA-I (*a*) and HA-II (*b*) samples.

Morphological analysis: SEM images of the sample calcined, HA-I and HA-II, are shown in Fig. 3. In the calcined hydroxyapatite powders the particles are agglomerated in spherical-like shape aggregates with an average diameter varying between 60 and 90 nm. It can be seen that surface porosity was more visible in the HA-I sample than HA-II sample, with a distribution of very fine particles, who cold welded together make the large agglomerates. There are many micropores with spherical shape who can allow the use of the material in application from biomedical field, such as carrier drug in the body (Zhu *et al.*, 2004), as bone substitutes for grafting in bone replacement (Heise *et al.*, 1990).

4. Conclusions

Method based on precipitation reaction was successfully used to obtain nanocrystalline powders of hydroxyapatite using the following materials: calcium hydroxide ($Ca(OH)_2$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) as calcium source, and orthophosphoric acid (H_3PO_4) and diammonium phosphate $(NH_4)_2HPO_4$) as phosphorus source. XRD pattern and SEM images confirms that the obtained powders are stoichiometric hydroxyapatite, and reveal that HA-I sample has lower crystallinity, but higher surface porosity, compared with HA-II sample.

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INFLUENŢA UNOR SURSE DIFERITE DE CALCIU ŞI FOSFOR ÎN SINTEZA PULBERILOR NANOCRISTALINE DE HIDROXIAPATITĂ

(Rezumat)

Hidroxiapatita (HA) este unul dintre biomaterialele cele mai investigate pentru aplicaţii biomedicale găsindu-şi aplicare sub formă de implanturi şi substituenţi osoşi. Aceasta se datorează similitudinii ridicate cu materialul anorganic din oase şi dinţi, precum şi datorită biocompatibilităţii şi bioactivităţii sale excelente. Scopul principal al cercetărilor în acest studiu este de a compara două tipuri de hidroxiapatită stoechiometrică obţinute prin metoda precipitării chimice folosind diferite surse de calciu şi fosfor. Probele sintetizate au fost caracterizate prin tehnici precum microscopie electronică cu baleiaj (SEM) şi difracţie de raze X (DRX), studiindu-se astfel cristalinitatea şi morfologia materialelor obţinute.