PREPARATION AND CHARACTERIZATION OF HYDROXYAPATITE NANOPOWDERS DOPED WITH SILVER IONS

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Silver doped hydroxyapatite was prepared by the precipitation method, using the precursor of hydroxyapatite, $CaCl_2$-0.5M and (NH_4) $2HPO_4$-0.3M; silver sulfate (Ag_2SO4) is added to the precursors in order to bring the ions of silver (Ag at 10%) in the structure of hydroxyapatite. The morphology, structure and composition of prepared samples were characterized by X-ray diffraction, the complex thermal analysis, electron microscopy coupled with energy dispersion X-ray spectroscopy and transmission electron microscopy. Nanometric particles of silver doped hydroxyapatite ions were obtained by replacing calcium ions in its structure. Adding silver to hydroxyapatite as a functional group can play an important role in attaching other macromolecules to the surface of biomaterials and also may present special antibacterial properties.

Keywords: silver doped hydroxyapatite, nanoparticles, morphology, structure

1. Introduction

Nanotechnology is enabling technology that deals with nano-meter sized objects. It is expected that nanotechnology will be developed at several levels: materials, devices and systems. Living organisms are built of cells that are typically 10 μm across. However, the cell parts are much smaller and are in the sub-micron size domain. Even smaller are the proteins with a typical size of just 5 nm, which is comparable with the dimensions of smallest manmade nanoparticles. This simple size comparison gives an idea of using nanoparticles as very small probes that would allow us to spy at the cellular machinery without introducing too much interference. Understanding of biological processes on the nanoscale level is a strong driving force behind development of nanotechnology [1].

In recent years, these materials have emerged as important players in modern medicine, with applications ranging from contrast agents in medical

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imaging to carriers for gene delivery into individual cells. Nanoparticles have a number of properties that distinguish them from bulk materials simply by virtue of their size, such as chemical reactivity, energy absorption, and biological mobility [2]. Nanomaterials have unique physicochemical properties, such as ultra small size, large surface area to mass ratio, and high reactivity, which are different from bulk materials of the same composition. These properties can be used to overcome some of the limitations found in traditional therapeutic and diagnostic agents [3]. Nanoparticles may have different effects on human health relative to bulk material from which they are produced. Increase in biological activity of nanoparticles can be beneficial, detrimental or both [4].

A list of some of the applications of nanomaterials to biology or medicine is given below: fluorescent biological labels, drug and gene delivery, bio detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, tumour destruction via heating (hyperthermia), separation and purification of biological molecules and cells, MRI contrast enhancement, phagokinetic studies [1]. Nanoparticles have made a tremendous impact in the treatment of various types of cancer, as evidenced by the numerous nanoparticle-based drugs and delivery systems that are in clinical use [2].

One of the most studied biomaterial for its extraordinary biocompatibility, bioactivity, and osteoconductivity is the hydroxyapatite (HAp), a bioceramic material from the family of apatites with the general formula $Ca_{10}(PO_4)_6(OH)_2$. Being the main inorganic constituent in human bones and teeth, HAp is widely used in medical applications such as implants, coatings, prostheses. Due to the high demand for new biocompatible and antibacterial materials, the scientists thought of immobilizing antibacterial metals in the matrix of biomaterials [5]. Microorganism adhesions on implant surfaces represent an initial crucial step in infections [6].

Recent study suggests that nHAp can inhibit cancer cell proliferation and have a potential application in cancer treatment [7]. Miriam Miranda reported the antimicrobial activity for HAp/nAg powder again Gram-positive and Gram-negative bacterias as well as yeast [8].

Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells. Additionally, silver has been used in water and air filtration to eliminate microorganisms [4]. The silver nanoparticles/hydroxyapatite composites are promising candidate materials for detection of protein [9]. Drug delivery to the central nervous system remains a challenge in developing effective treatments for neurodegenerative diseases [2].

Some cations that can be substituted into hydroxyapatite include zinc, magnesium, silver, strontium, lanthanum, cadmium, lead, copper, and iron. The HAp properties, including morphology, lattice parameters, stability, mechanical

properties and magnetic properties are affected by the ions incorporation, by dopind [10].

Several studies have been focused on demonstrating the antimicrobial properties of the silver doped hydroxyapatite [11, 12, 13, 14, 15].

The aim of this study is to prepare nano silver doped hydroxyapatite powder that may have a large potential demand in the field of biomedical engineering.

2. Materials and methods

For the preparation of silver doped hydroxyapatite the method used was wet precipitation. The precursor solutions used for the preparation of the hydroxyapatite were $CaCl_2$-0.5M (Sigma–Aldrich) and $(NH_4)_2HPO_4$-0.3M (Sigma–Aldrich). Silver was reacted in form of silver sulfate (Ag_2SO_4) (Sigma–Aldrich) dissolved in HNO_3. The Ca/Ag ratio used in the synthesis was 1/ 0.05. Salt solutions were mixed vigorous stirring at room temperature for 5 hours. The precipitate obtained was washed many times with deionised water, up to pH 8. The resulting material was dried at 80°C for 72h and then thermal treated at 450°C, for 2 hours.

The samples obtained were analyzed by X-ray diffractometry (XRD) using a SHIMATZU XRD 6000 diffractometer, with CuKα (λ=1.5405 Å) radiation, scanning speed 20/min., in 2θ = 20 - 60 grd range. To study the powder obtained, through complex thermal analysis was used a TGA/SDTA 851e Mettler Toledo instrument. The dry powder was heated up to 700^{o}C, with a temperature growing rate of 10^{o}C/minute, using pure alumina as reference. Scanning electron microscopy (SEM) study was performed on a Quanta Inspect F type microscope equipped with an energy dispersive X-ray attachment. The Transmission Electron Microscopy (TEM) analyzes was carried out with TecnaiTM G2 F30 S-TWIN High Resolution Transmission Electron Microscope (HRTEM), equipped with STEM – HAADF detector, EDX and EELS, with the following characteristics: acceleration voltage of 300 KV obtained from a Shottky Field emitter with a high maximum beam current > 100nA, high probe current 0.6 nA in a 1 nm spot, 15 nA in a 10 nm spot, small energy spread 0.8 eV and with a spot drift of 1 nm / minute; TEM point resolution of 2 Å and line resolution of 1 Å.

3. Results and Discussion

Substitutions can occur in hydroxyapatite for the calcium ions, the phosphate groups or the hydroxyl groups. It is assumed that cations substitute into the lattice at calcium sites. Although there is a large quantity of experimental data regarding cation substitution in HAp there is a limited understanding of the

mechanisms of substitution or the exact locations of the substituted ions in the HA structure. [10].

The XRD pattern, presented in Figure 1a, for unsubstituted powder, thermal treated at 450°C, for 2 hours, shows the characteristic peaks of hydroxyapatite ($Ca_5(PO_4)_3(OH)$ as single phase (ASTM no. 24-0033). The X-ray diffraction performed on the powder substituted with silver ions is shown in Figure 1b. Apart from the characteristic Bragg reflections of hydroxyapatite can be noticed the diffraction peaks of silver oxide (JCPDS 42-0874). The XRD results of these samples demonstrate that the synthesized silver modified HAp powder is polycrystalline. The presence of Ag_2O phase in your sample proved that not all silver enters into the lattice.

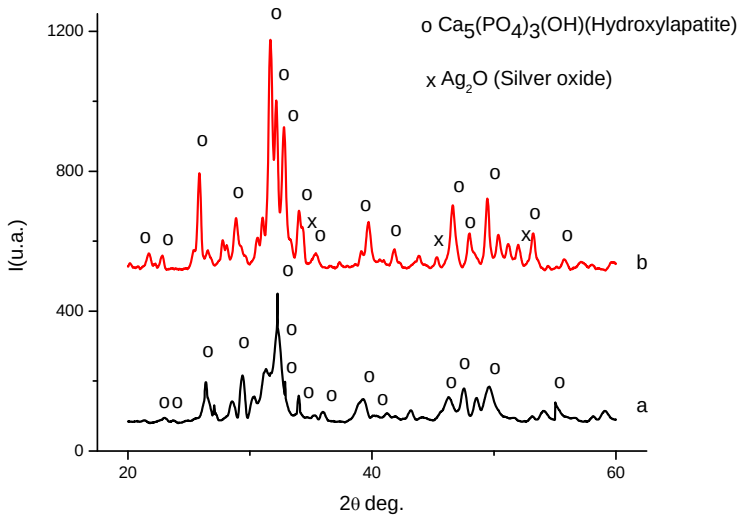


Fig. 1. The XRD patterns for the hydroxyapatite powder (a) and silver doped hydroxyapatite (b), annealed at 450°C/ 2h

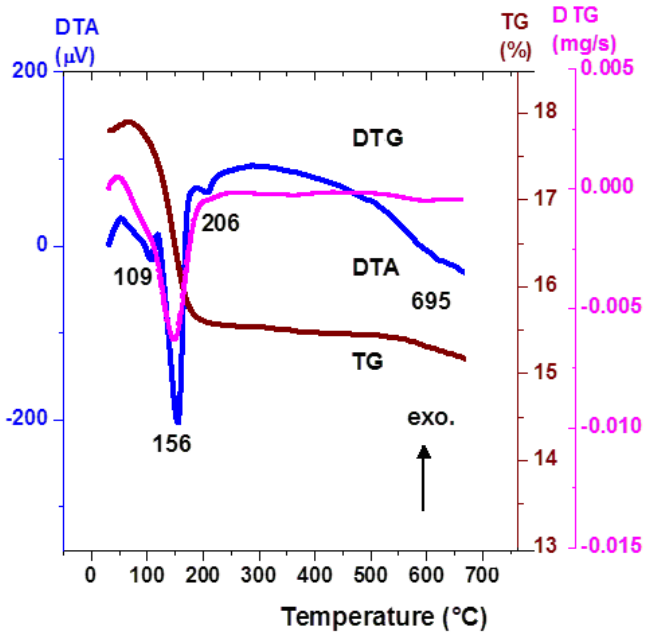


Fig. 2. Complex thermal analysis of silver doped hydroxyapatite obtained by precipitation method

DTA-TGA curves of silver doped hydroxyapatite ions are shown in Fig. 2. It can observe the existence of four endothermic peaks (109°C, 156°C, 206°C and 695°C), with weight loss. A significant weight loss (10.2%) occurred in the temperature range 100°C to 200° C, this corresponds to water evaporation loss after dehydration silver doped hydroxyapatite. In the temperature range 200°C and 575 °C continue mass loss of 1,36%. By the heat treatment temperature (450°C) do not notice other important processes in terms of thermal behavior of silver doped hydroxyapatite.

The size and morphology of the powder of silver doped hydroxyapatite was analyzed with a scanning electron microscope and the images are presented in Figure 3. The SEM investigation indicates that the powder of hydroxyapatite substituted with silver ions presents particles which form agglomerates. The average size of such powders is of the order of nanometers.

Also the TEM micrographs, shown in Figures 4a and 4b, indicate the polyhedral shapes and nanometric sizes of silver doped hydroxyapatite. The particles showed high agglomeration tendency. The agglomerates are formed from particles with different geometries varrying from spherical to needle like-shape and having size under 100 nm.

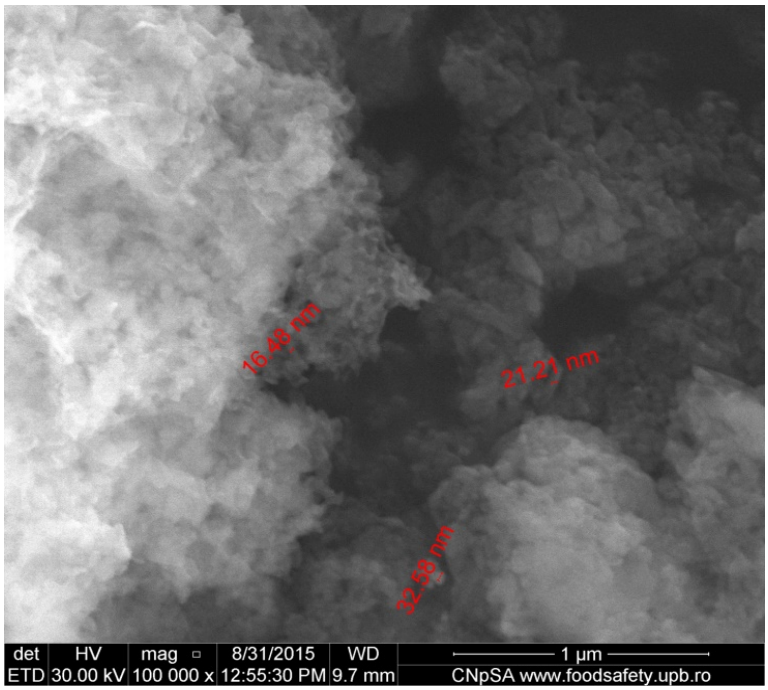
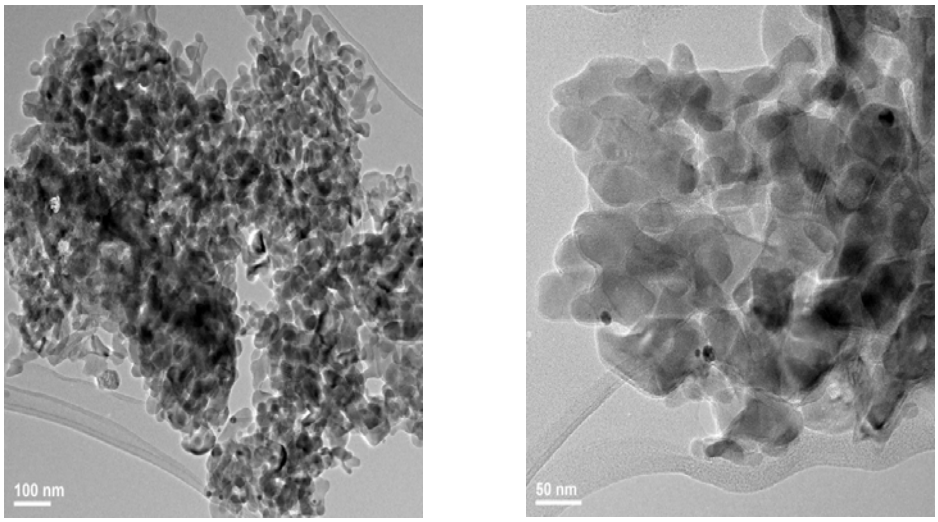


Fig. 3. Scanning Electron Microscopy images of the silver doped hydroxyapatite at a 100 000 x magnification

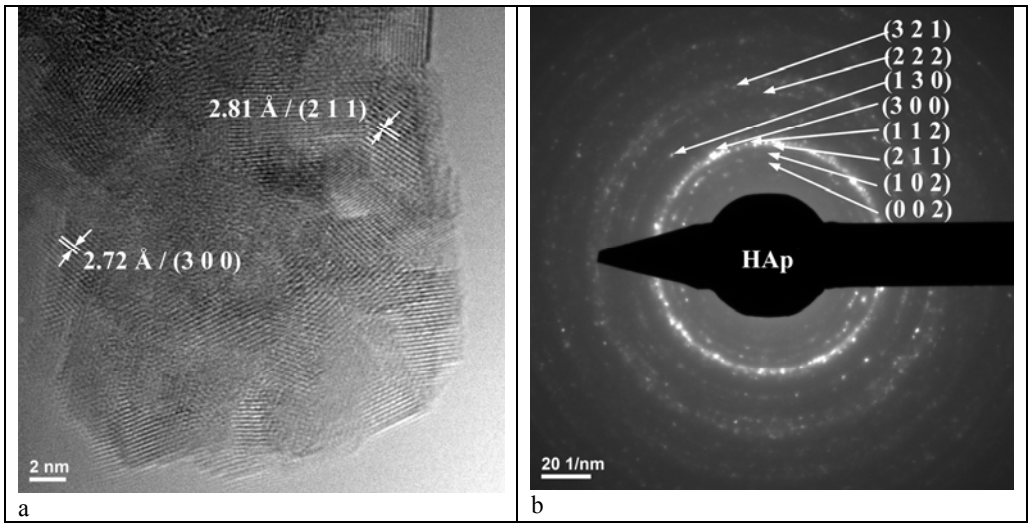
The High-Resolution Transmission Electron Microscopy and Selected Area Electron Diffraction images indicate additional information regarding mineral composition of the material.

The HRTEM analysis show the presence of the characteristic atomic planes in the crystal corresponding to the crystallographic plane distance of hydroxyapatite. HRTEM images obtained on silver doped hydroxyapatite show clear lattice fringes of polycrystalline hydroxyapatite of d = 2.81 Å corresponding to the (211) and d = 2.72 Å corresponding to the (300) crystallographic planes of hexagonal hydroxyapatite (JCPDS 79-5683) (Figure 5a) [15].



a b

Fig. 4. TEM image of the synthesized hydroxyapatite modified with silver ions (a,b)



a b

Fig. 5. High-Resolution Transmission Electron Microscopy image (a) and Image Selected Area Electron Diffraction (b) for silver doped hydroxyapatite

The SAED of silver doped hydroxyapatite presented in Figure 5b proves the presence of $Ca_5(PO_4)_3(OH)$ as single crystalline phase with the most intense planes (002), (102), (211), (112), (300), (130), (222) and (321) as identified from the region presented in the bright field images. Also, the HRTEM and SAED data, are in good concordance with XRD data.

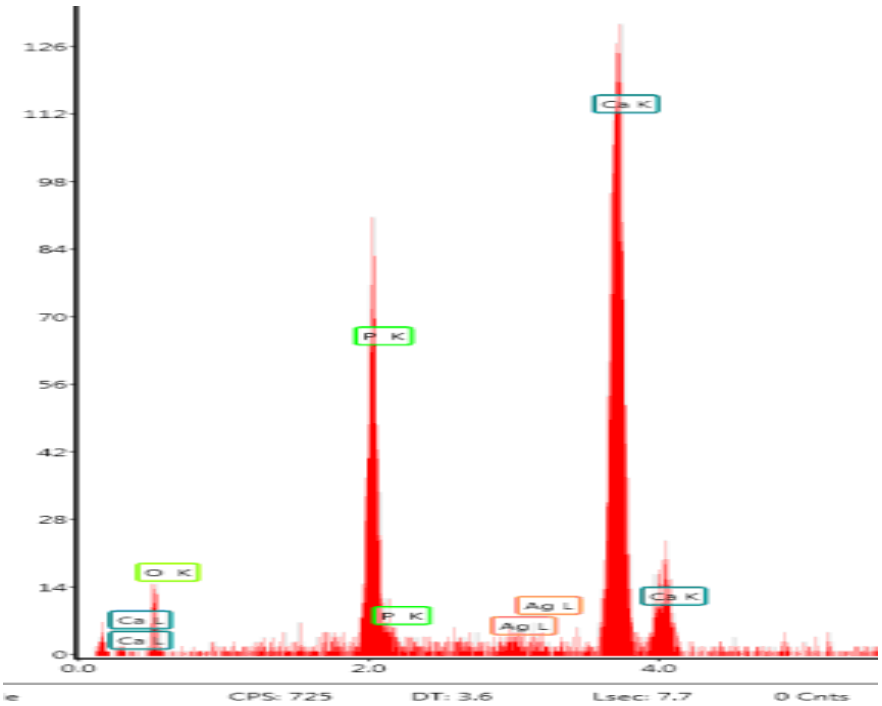


Fig. 6. The energy dispersive X-ray analysis of silver doped hydroxyapatite

In our analysis by energy-dispersive spectroscopy of silver doped hydroxyapatite we confirmed the presence of hydroxyapatite elemental components (calcium, phosphor, oxygen) and elemental silver signal (Fig. 6).

6. Conclusions

Silver modified hydroxyapatite was prepared by wet precipitation method. The SEM and TEM investigation of obtained powder showed the formation of nanoparticles of silver modified hydroxyapatite with tendency to form agglomerates. The future research will be focused to bind organic and inorganic complex groups to the silver modified hydroxyapatite nanopowders, which will be useful to apply this kind of materials for the cancer treatment.

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