Materials Science and Engineering C 47 (2015) 333–338

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Structural and dielectric properties of yttrium-substituted hydroxyapatites

Omer Kaygili [a,*], Sergey V. Dorozhkin [b], Tankut Ates [a], N. Canan Gursoy [c], Serhat Keser [d], Fahrettin Yakuphanoglu [a], A. Birkan Selçuk [e]

[a] Department of Physics, Faculty of Science, Firat University, 23119 Elazig, Turkey
[b] Kudrinskaja sq. 1-155, 123242 Moscow, Russia
[c] Department of Microbiology and Clinic Microbiology, Inonu University, 44280 Malatya, Turkey
[d] Department of Chemistry, Faculty of Science, Firat University, 23119 Elazig, Turkey
[e] Technology Department, Saraykoy Nuclear Research and Training Centre, 06983 Ankara, Turkey

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 2 October 2014
Accepted 10 November 2014
Available online 11 November 2014

Keywords:
Hydroxyapatite
Yttrium (Y)
X-ray diffraction (XRD)
Antimicrobial activity
Dielectric properties

ABSTRACT

Hydroxyapatite (HAp) samples doped with 0, 2 and 4 at.% of yttrium (Y) were characterized using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy attached with energy dispersive X-ray (EDX) spectroscopy, antimicrobial activity tests and dielectric studies. The hydroxyl groups observed in FTIR spectra confirmed the formation of HAp phase in the studied samples. The crystallite size, crystallinity degree and lattice parameters of the samples were changed with Y content. The volume of the unit cell was gradually decreased with the addition of Y. Undoped and Y-containing HAp samples were screened to determine their in vitro antimicrobial activities against the standard strains. It was found that no samples have any antimicrobial effect. The relative dielectric permittivity and dielectric loss are affected by Y content. While the alternating current conductivity increases with increasing frequency, it decreases with increasing Y content.

1. Introduction

Due to the great similarity with the inorganic components of human bones and teeth, calcium orthophosphates and calcium orthophosphate-based materials have a great interest for biomedical applications. Among them, hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is one of the most known implant materials used in several clinical applications (i.e., orthopedics, dentistry, neurosurgery and plastic surgery) due to its superior biological responses (e.g., non-toxicity, high biocompatibility and osteoconductivity) in the physiological environments [1–6]. The stoichiometric HAp has hexagonal crystal structure with the lattice parameters $a = b = 0.9418$ nm, $c = 0.6884$ nm and the unit cell volume of $V = 0.5288$ nm^3 [7–9].

However, both the composition and properties of the chemically pure HAp do not fully correspond to those of bones and teeth. Therefore, doping of HAp with various ions has been used to improve its properties. Metal ions such as Ag^+, Mg^{2+}, Zn^{2+}, Sr^{2+}, Al^{3+}, Ce^{3+}, La^{3+}, Bi^{3+}, Y^{3+} and Eu^{3+} can substitute Ca^{2+} ions in the HAp structure [10–22]. These ionic substitutions affect the crystallinity, lattice parameters and morphology of HAp. Since the physical, chemical and biological properties of HAp directly linked with its crystal structure and composition, the ionic substitutions provide the possibilities to control the characteristic properties of HAp [22–26].

Yttrium (Y) has been extremely used in medical applications. Some applications given in the literature can be summarized as follows: Y^{3+} is used in the treatment of hepatocellular carcinoma [27,28]. Thomas et al. [29] reported that ^{90}Y-containing HAp could serve as an alternative therapy for chronic synovitis because of bleeding disorders. Zhang et al. [30] reported that Y^{3+} promoted the adipocyte transdifferentiation of primary mouse osteoblasts. Liu et al. [31] reported that Y-containing HAp synthesized by hydrothermal method accelerates the human periodontal fibroblast growth and restricts slightly the oral bacterial growth. The work of Nathanael et al. [32] revealed that the mechanical performance of the Y-doped HAp nanorod reinforced high molecular weight polyethylene (HMWPE) composites was higher than those of the pure HAp nanorod reinforced HMWPE composites.

The dielectric and electric properties of a biomaterial synthesized for bone substituting or bone repairing applications have a great importance because bone is a dielectric material. Additionally, the electromagnetic fields have been used for bone healing applications, and the effects of the electrical stimulation have been reported [33–39]. Many authors have investigated and determined these properties for HAps and calcium phosphate based samples [40–44].

Even though, in the literature, there are some investigations related to Y-containing HAps, the investigation of their antimicrobial and

* Corresponding author.
E-mail address: okaygili@firat.edu.tr (O. Kaygili).

http://dx.doi.org/10.1016/j.msec.2014.11.039
0928-4931/

dielectric properties is new in comparison to the earlier studies. In present work, we synthesized the pure- and Y-doped HAp samples using the precipitation method and investigated the effects of the addition of Y on the crystal structure, phase composition, crystallinity, chemical composition, morphology, dielectric properties and antimicrobial activity of HAp.

2. Materials and method

2.1. Synthesis of the samples

The yttrium doped HAp samples were synthesized for the various molar ratios of Y/(Ca + Y):0, 0.02 and 0.04, and were named as Y1, Y2 and Y3, respectively. The (Ca + Y)/P molar ratios were adjusted to 1.67. Diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, Merck) was dissolved in the distilled water using a magnetic stirrer and heated to temperature of 90 °C. Meanwhile, calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, Merck) and yttrium(III) nitrate hexahydrate ($Y(NO_3)_3 \cdot 6H_2O$, Sigma-Aldrich) were also dissolved separately in the distilled water and poured in one flask and then as-prepared solution was added drop by drop to $(NH_4)_2HPO_4$ solution. During the synthesis, the pH of the mixture was adjusted and kept at ~10 with ammonium hydroxide (NH_4OH, Sigma-Aldrich). The reaction was performed at 90 °C for 6 h. Afterwards, the suspension was filtrated and the precipitates were washed out by distilled water and dried in an oven at 110 °C for 22 h. The obtained powders were calcined at 700 °C for 2 h.

2.2. Characterization of the samples

2.2.1. X-ray diffraction (XRD) measurements

X-ray diffraction (XRD) analyses were performed on a Bruker D8 Advance diffractometer using a CuKα radiation with wavelength of λ-0.15406 nm produced at 40 kV and 40 mA, and the XRD data were collected over the 2θ range of 20°–55° at every 0.02° for the scan speed of 2° min^{-1}. The crystalline phases were identified by reference to the Joint Committee on Powder Diffraction Standards (JCPDS) files.

The percentage of the formation of the hydroxyapatite phase was estimated in the 2θ range of 20°–55° using the following relation:

$$\%HAp\ phase = \frac{Area\ under\ peaks\ \ belonging\ to\ HAp\ phase}{Total\ Area\ under\ all\ peaks} \times 100\%. \quad (1)$$

Similarly, the percentage of the beta tricalcium phosphate (β-TCP) was computed. The lattice parameters (*a* and *c*) were calculated with the relation belonging to hexagonal structure [45]:

$$\frac{1}{d^2} = \frac{4}{3}\left(\frac{h^2 + hk + k^2}{a^2}\right) + \frac{l^2}{c^2} \quad (2)$$

where *d* is the distance for two adjacent planes, and *h*, *k* and *l* are the Miller indices. The volume of the hexagonal unit cell was calculated by the following relation [45]:

$$V = 0.866 a^2 c \quad (3)$$

and Scherrer equation can be used to determine the crystallite size (*D*) [45]:

$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad (4)$$

where β is the full width at half maximum (FWHM) in radian and θ is the diffraction angle in degree. The crystallite size of the samples was evaluated for the perpendicular crystal planes of (002) and (300) as D_{002} and D_{300}, respectively. The crystallinity degree (X_C) was calculated by the following relation [46]:

$$X_C \approx 1 - \frac{V_{112/300}}{I_{300}} \quad (5)$$

where $V_{112/300}$ is the intensity of the hollow between (112) and (300) crystal planes, and I_{300} is the intensity of the (300) plane.

2.2.2. Fourier transform infrared (FTIR) analysis

Fourier transform infrared (FTIR) spectra were collected by a PerkinElmer Spectrum One spectrometer in the region 450–4000 cm^{-1} using KBr pellets with a spectral resolution of 4 cm^{-1}.

2.2.3. Microstructural observations

The microstructure and elemental compositions of the samples were investigated using a scanning electron microscope (SEM, ZEISS EVO 50) equipped with an energy dispersive X-ray (EDX, Oxford Instruments Inca Energy 350) spectrometer operated at 10 kV. The as-synthesized HAp samples were uniaxially compacted into disks, with a diameter of 13 mm and a thickness of 2 mm, using a MTI 24T Desktop Hydraulic Pressing Machine under pressure of 10 MPa. All the samples were coated with a conductive layer of gold for 20 s using a Denton Desk V coater, and then the microstructures were observed.

2.2.4. Antimicrobial activity tests

Antimicrobial activities of the pure and Y-containing HAp samples were determined by using agar dilution procedure recommended by the Clinical and Laboratory Standards Institute [47,48]. Minimal inhibitory concentrations for each compound were investigated against standard bacterial strains; *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were obtained from American Type Culture Collection (Rockville, MD.) and the fungal strains *Candida albicans* and *Candida tropicalis* were obtained from the Department of Microbiology, Faculty of Medicine, Ege University (Turkey). The inoculum was prepared by making a direct broth of isolated colonies selected from an 18- to 24-hour blood agar plate. Bacterial strains were subcultured on Muller Hinton Broth (HiMedia Laboratories Pvt. Ltd., Mumbai—India) and fungal strains were also on RPMI 1640 Broth (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Their turbidities matched that of a McFarland no. 0.5 turbidity standard, and the absorbance should be 0.08 to 0.13 at 625 nm for this standard [49]. Since the pure and Y-containing HAp samples have limited solubility, the stock solution of all compounds was prepared in dimethyl sulfoxide (DMSO) at 800 μg/ml concentration. All of the dilutions were done with distilled water. 1 ml of this stock solution was mixed with 9 ml of Muller Hinton Agar (MHA), sterilized using an autoclave and cooled until 50 °C. Then MHAs containing the HAp samples were waited until they solidify. By making serial twofold dilutions, the concentrations of the tested compounds were 800, 400, 200, 100, 50, 25, 12.5 and 6.25 μg/ml. Ampicillin and ciprofloxacin were used as antibacterial standard drugs, while fluconazole were used as antifungal standard drugs whose minimum inhibitory concentration (MIC) values are provided. A loopful (0.01 ml) of the standardized inoculum of the bacteria and yeasts (10^6 CFU/ml) was spread over the surface of agar plates. All the inoculated plates were incubated at 35 °C and results were evaluated after 16–20 h of incubation for bacteria and 48 h for yeasts. The lowest concentration of the compounds that prevented visible growth was considered as the minimal inhibitory concentration (MIC).

2.2.5. Dielectric studies

To make the dielectric measurements, all the samples were grinded and uniaxially compacted into disks, with a diameter of 13 mm and a thickness of 2 mm under pressure of 10 MPa. The dielectric measurements were performed using a HIOKI 3532-50 LCR HiTESTER at room temperature. Using Eqs. (6), (7) and (8), the relative permittivity (ε'),