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IOP Conf. Series: Materials Science and Engineering **597** (2019) 012004 doi:10.1088/1757-899X/597/1/012004

Microwave synthesis and study of physicochemical properties of hydroxyapatite modified with silver and zinc ions

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Abstract. The authors obtained two-phase samples of silver- and zinc-modified hydroxyapatite (AgHA, ZnHA) by liquid-phase deposition under microwave exposure; the main phase for all samples was hydroxyapatite $Ca_5(PO_4)_3(OH)$ with hexagonal syngony. The parameters of the elementary lattice of HA were studied via X-ray phase analysis and confirmed the formation of solid substitutional solutions while modifying HA with silver and zinc ions. Solubility has been estimated at 20 ºC for a 0.9% NaCl solution; specific surface area and porosity have been estimated for ZnHA powders obtained in presence of aminoacids. Surface morphology and dispersiveness have been studied for AgHA and ZnHA powders. Hammett method helped to establish the presence of acidic and basic centers on the surface of ZnHA and AgHA. Koch biotesting showed HA, AgHA, and ZnHA acting as inhibitors thus affecting *E. coli* tested samples. HA, AgHA, and ZnHA powders can be recommended for medical purposes.

1. Introduction

Creating biomaterials for recovering human bone tissue is currently an apparent problem. For this purpose, materials based on hydroxyapatite (HA) are widely used not only in surgery, dentistry, and injury treatment, but also in neurosurgery, cosmetology, and pharmaceutical industry. Hydroxyapatite with a composition $Ca_{10}(PO_4)_6(OH)_2$ is an inorganic component of bone and dental tissues; has certain chemical, biological, and mechanical properties [1, 2]. Mineral constituent of a bone includes HA crystals, which may contain various elements (e.g., potassium, zinc, silver, magnesium, fluorine, copper, etc.). Idealized implants, ceramics, and cements made of HA should have structure, composition, and morphology identical to human dental tissue [3-5].

Among a large number of cation- and anion-modified hydroxyapatite samples, HA containing silver (AgHA) and zinc ions (ZnHA) are of great interest to date. The ions are chosen because of their biological role and specific functions they perform in a human body [6, 7].

The purpose of the research project is to study the effect of Ag^+ and Zn^{2+} on the phase composition and on physicochemical and biological properties of hydroxyapatite powders obtained via microwave synthesis.

Synthesis of silver- and zinc-modified hydroxyapatite powders with initial ion content from 0.1 to 0.9 mol.% was carried out by liquid-phase deposition from aqueous solutions in a microwave field.

Physical, chemical, structural, surface, and biological properties for the obtained hydroxyapatite powders have been studied using a number of methods.

Phase composition for the isolated AgHA and ZnHA samples was determined by X-ray phase analysis using a Shimadzu XRD 6000 diffractometer, PCPDFWIN and JSPDS PDF 4+ databases, and POWDER CELL 2.4 full-profile analysis software.

Surface morphology for AgHA and ZnHA powders has been examined by SEM. Powder dispersiveness has been estimated using microphotography by a secant method in Adobe Photoshop CS5, as well as using specific surface area and pycnometric specific gravity of the powders.

Complexiметric titration has been employed to determine solubility of AgHA and ZnHA powders in 0.9% NaCl solution.

BET-method allowed the authors to estimate the specific surface area value for ZnHA. The acidic and basic properties of the latter were detected by Hammett method. These properties are studied in comparison with non-modified HA.

Zinc-modified HA powders underwent Koch biotesting.

The HA crystal structure, whose elementary lattice is shown in Figure 1, allows for substitution of certain elements with the other ones. Certain atoms can take different positions within HA structure both fully and partially, thus leading to diversity in the composition of natural phosphates with apatite structure. The ability of atoms or ions to enter the structure of another substance is pre-determined by the individual properties of these atoms or ions (i.e., size, charge, or electron structure) and by the peculiarities of a crystal lattice for substances forming solid solutions. The difference in atomic size for different constituents must be insignificant and should not exceed 8-15%.

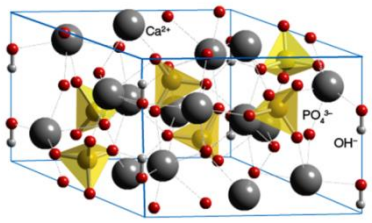


Figure 1. Elementary cell of hydroxyapatite crystal structure.

For a cation substitution, it is important to consider the charge and size of ions, as well as related electrochemical potential (φ). These values for Ca^{2+}, Ag^{+}, and Zn^{2+} cations are provided below.

Ion	Ca^{2+}	Ag^{+}	Zn^{2+}
Ionic radius r_i, nm	0.106	0.113	0.083
Potential $\varphi \cdot 10^{28}$, C^2/m	2.426	2.265	3.096

Silver and zinc ions can substitute calcium ions within a crystal lattice in an isomorphic manner on the basis of their ionic radius value. This results in the formation of mixed crystal of variable composition, otherwise known as substitutional solid solutions.

Cation substitutions within HA $Ca_{10}(PO_4)_6(OH)_2$ cause the changes in the parameters of crystal lattice an in the degree of crystallinity, which significantly affects HA solubility under physiological conditions and its chemical and biological properties [6]. However, this does not induce significant changes in the lattice symmetry.

Zinc is a microelement necessary to sustain many biochemical processes. Zinc takes part in osteogenesis and in collagen synthesis [6]. It is also involved in cell fission, in pancreatic gland insulin synthesis, and is an essential element for skin regeneration, for hair and nail growth, and for sebaceous gland secretion process. Additionally, it contributes to the vitamin E absorption and helps to maintain its normal concentration in blood. Zinc plays a key role in alcohol processing.

The antiseptic properties of silver have been known since ancient times whereas its biological function in a human body requires is not fully understood. It is the ability of silver ions to bring harm to pathogenic microorganisms that is currently used in medicine to cure numerous diseases including those attacking ENT-organs, bronchial tube, lungs, mouth cavity, digestive tract, and skin.

2. Experimental

Liquid phase synthesis of HA(1), AgHA(2), and ZnHA(3) powders was carried out using a stoichiometric ratio Ca/P = 1.67((Ca+M)/P=1.67) and the following reaction equations:

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH = Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O; \quad (1)$$

$$(10–0.5x)Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + xAgNO_3 + 8NH_4OH = Ca_{(10–0,5x)}Ag_x(PO_4)_6(OH)_2 + 6H_2O + 20NH_4NO_3; \quad (2)$$

$$(10–x)Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + xZn(NO_3)_2 + 8NH_4OH = Ca_{(10–x)}Zn_x(PO_4)_6(OH)_2 + 6H_2O + 20NH_4NO_3; \quad (3)$$

where x = 0.1; 0.3; 0.5; 0.7; 0.9 (mol.%).

Aqueous solution of calcium nitrate was mixed with a solution of ammonium hydrophosphate, whose concentration made 0.5 M and 0.3 M, respectively. The synthesis of AgHA and ZnHA required introducing sample weights of silver or zinc into a solution of calcium nitrate. A pH value of 10-11 in a solution of reactants was reached with an aqueous solution of ammonia (25%, ρ = 0.9 g/ml). The reaction mixture underwent microwave exposure with 110 W during 40 minutes and then allowed to stand at room temperature during 48 hours. The precipitate was then filtered, rinsed with a diluted solution of ethanol, and dried until constant weight (~ 15 h) at 90 ºC. After that, the sample was ignited during 4 hours at 800 °C [8].

3. Experimental results and discussion

To determine the phase composition for the obtained HA, AgHA, and ZnHA samples with different content of Zn^{2+} and Ag^{+} ions, the authors carried out X-ray phase analysis using PCPDFWIN database and POWDER CELL 2.4 full-profile analysis software.

X-ray patterns for AgHA and ZnHA samples show that all the powders are twophase with hexagonal syngony hydroxyapatite $Ca_5(PO_4)_3(OH)$ being the main phase. As the silver and zinc ions content increases in the initial solution (x values), there is a regular decrease in the amount of the main phase and an increase in the one for the second phase, that is, for β-tricalciumphosphate $Ca_3(PO_4)_2$ (Tables 1 and 2).

Table 1. The results of X-ray phase and X-ray diffraction analyses for HA and ZnHA powders.

Sample x (Zn^{2+}), mol.%	Phase	wt.%	Elementary cell parameters		
			a, Å	c, Å	V, Å^3
HA	$Ca_5(PO_4)_3(OH)$	100.0	9.3690	6.8321	519.36
ZnHA 0.1	$Ca_5(PO_4)_3(OH)$	100.0	9.3750	6.8375	520.45
ZnHA	$Ca_5(PO_4)_3(OH)$	86.0	9.4601	6.8791	494.78
0.3	$Ca_3(PO_4)_2$	14.0	–	–	–
ZnHA	$Ca_5(PO_4)_3(OH)$	85.0	9.4127	6.8714	527.24
0.5	$Ca_3(PO_4)_2$	15.0	–	–	–
ZnHA	$Ca_5(PO_4)_3(OH)$	70.0	9.4132	6.8712	527.28
0.7	$Ca_3(PO_4)_2$	26.0	–	–	–
ZnHA	$Ca_5(PO_4)_3(OH)$	41.0	9.2063	6.7408	494.78
0.9	$Ca_3(PO_4)_2$	59.0	–	–	–

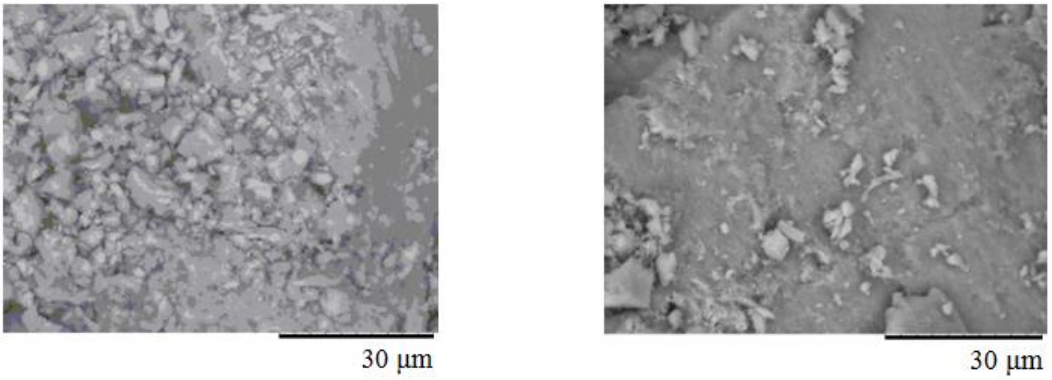
Table 2. The results of X-ray phase and X-ray diffraction analyses for AgHA powders.

Sample	Phase	wt.%	Elementary cell parameters

x (Ag^+), mol.%			a, Å	c, Å	V, $Å^3$
AgHA	$Ca_5(PO_4)_3(OH)$	98.0	9.3289	6.8248	593.95
0.1	$Ca_3(PO_4)_2$	2.0	–	–	–
AgHA	$Ca_5(PO_4)_3(OH)$	87.0	9.4144	6.8762	609.44
0.3	$Ca_3(PO_4)_2$	10.0	–	–	–
	$CaAgPO_4$	3.0	–	–	–
AgHA	$Ca_5(PO_4)_3(OH)$	86	9.4438	6.8942	614.86
0.5	$Ca_3(PO_4)_2$	14	–	–	–

Changing the parameters of HA elementary cell (Tables 1 and 2) for ZnHA and AgHA samples implicitly confirms that Zn^{2+} and Ag^{2+} ions are present in HA crystal lattice and thus ZnHA and AgHA can be considered as HA-based substitutional solid solutions.

The surface morphology for ZnHA (x = 0.1) and AgHA (x = 0.1) and the dispersivness were determined via micrographs (Figure 2) obtained with X-Max ShiftED 300 scanning electron microscope.



a) b)

Figure 2. Micrographs of the ignited powders' surface: a) ZnHA (x = 0.1); b) AgHA (x = 0.1).

The ZnHA and AgHA powders are agglomerates of various sizes: in ZnHA samples, 70% of the particles have a size of up to 5 μm; AgHA samples are dominated by larger agglomerates (7–20 μm). The SEM results show that the surface morphology of the powders is almost identical, while their dispersiveness differs.

Table 3. Solubility values for HA, ZnHA, and AgHA powders ignited at 800 ºC (20 ºC, 0.9% wt.%, NaCl solution; ammonia buffer, Eriochrome Black T indicator).

Content x (Zn^{2+}/Ag^+), mol.%	Solubility $C_M^{2+}\cdot10^3$, mol / l		
	ZnHA	AgHA	HA
0	–	–	1.6 ± 0.08
0.1	0.50 ± 0.08	1.19 ± 0.06	–
0.3	0.69 ± 0.04	1.67 ± 003	–
0.5	0.45 ± 0.04	1.44 ± 0.09	–
0.7	0.38 ± 0.04	–	–
0.9	0.22 ± 0.04	–	–

To estimate the solubility of HA, ZnHA, and AgHA samples, the authors determined the total concentration of Ca^{2+} and Zn^{2+} (Ag^+) ions in the physiological solution (ω (NaCl) = 0.9%) where the

samples were kept for 3 days and stirred to reach complete saturation of the solution compared with a solid phase.

Complexiметric titration of calcium ions (together with zinc and silver ions) in the presence of Eriochrome Black T indicator helped to identify the solubility values (C_M^{2+}, mol / l; at 20 ºC) for HA, ZnHA, and AgHA obtained by liquid-phase microwave synthesis and ignited at 800 ºC.

The obtained solubility values are given in Table 3. It is noted that an increase in the content of Zn^{2+} and Ag^{+} ions while synthesizing hydroxyapatite slightly reduces the solubility in a physiological solution compared with non-modified HA.

The porosity of calcium phosphate ceramics is a very important characteristic. The porosity of natural bone tissue is not a constant value, therefore, the methods for obtaining HA can be different depending on the potential application area of HA-based bioceramics [9, 10]. It is known that applying combustible organic additives in obtaining synthetic HA contributes to an increase in its porosity. So, in order to observe changes in HA porosity, the authors employed aminoacids (glycine and aspartic acid) and zinc salts containing the same aminoacids (Table 4, samples 3-6).

The specific surface area (SSA), volume, and pore size of HA-based powders were measured via adsorption with subsequent degassing under ~ 0.1 Pa at 200 °C for two hours on a TriStar 3020 gas-solid analyzer (BET method).

Applying aminoacids and their zinc salts does not contribute to an increase in porosity, therefore, it can be recommended only when synthesizing hydroxyapatite with low porosity. However, to obtain a highly porous HA, it is preferable to select other combustible additives.

Table 4. The results of determining the specific surface area and porosity of HA and ZnHA.

	Sample	S, m^2/g	Total pore volume, cm^3/g	Average pore size, nm
1	HA	15	0.037	10.0
2	ZnHA (x = 0.5)	21	0.046	8.8
3	ZnHA with HGly	18	0.039	8.6
4	ZnHA c H_2Asp	18	0.038	8.4
5	HA with $ZnGly_2$	20	0.041	8.2
6	HA with ZnAsp	20	0.041	8.2

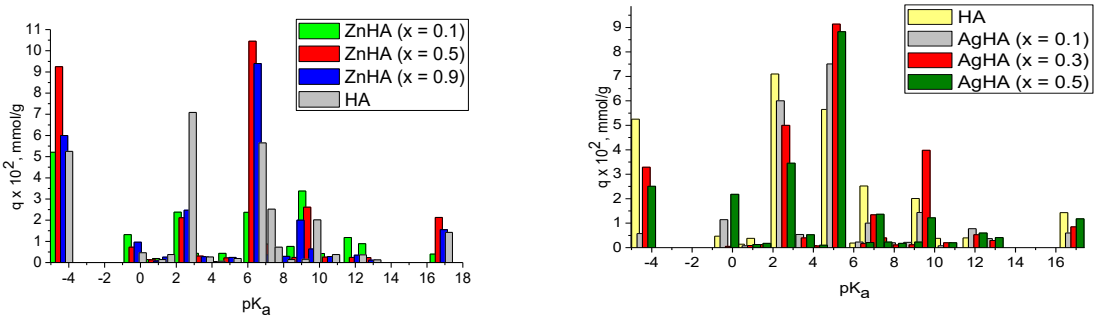
A Koch biotesting of ZnHA samples (x = 0.3 and 0.7) was carried out with reference to their effect on *E. coli* bacteria (*Escherichia coli*, ATCC 25922) while cultivating the latter in standard *LB* nutrient medium. Sterilization conditions of the samples (25 min, 5 atm, 112 ºC) do not decompose hydroxyapatite according to the literature data.

The results of biotesting (Table 5) show that HA and ZnHA powders inhibit the growth of *E. coli* under the cultivation conditions specified above, that is, they suspend the development of *Escherichia coli*, but do not completely decompose it. The ZnHA sample at x = 0.7 showed a more than twofold decrease in the number of bacteria compared with the ZnHA sample at x = 0.3. The inhibitory effect of HA sample is even more evident.

Table 5. The test sample count under different experimental conditions (significance value = 95%).

	Sample	Count (*E. coli*), cfu/ml
1	Reference sample	$(2.10 \pm 0.28)10^8$
2	HA	$(3.60 \pm 0.85)10^7$
3	ZnHA (x = 0.3)	$(9.70 \pm 0.65)10^7$
4	ZnHA (x = 0.7)	$(4.40 \pm 0.94)10^7$

Both acidic (Ca^{2+}, Ag^{+}, and Zn^{2+}), and basic (PO_4^{3-}, OH^{-}) Lewis centers might be present pn the surface of HA powders. Brønsted acid-base centers are possible as well. Therefore, the estimation of the relative distribution of acid-base centers on the surface of HA powders was carried out by the Hammett method. The results demonstrate that an increase in the content of the doping ion (Zn^{2+}, Ag^{+}) in HA changes the quantitative ratio of acid-base centers (Figure 3).



a) b)

Figure 3. Histograms of the acid-base centers distribution on the surface of ZnHA powder (a) and AgHA powder (b).

4. Conclusions

1. Samples were obtained by liquid phase deposition within a microwave field at atmospheric pressure with different initial content of zinc and silver ions (from 0.1 to 0.9 mol.%). Hydroxyapatite $Ca_5(PO_4)_3(OH)$ with hexagonal syngony served as the main phase for all the samples.
2. According to XRD, the elemental composition of the powders obtained shows that the molar ratio of the elements (Ca + M) / P is 1.57-1.67 for ZnHA; 1.65-1.67 for AgHA, while the Ca / P elements ratio for bone tissue ranges from 1.5 to 2.0.
3. It has been established that ZnHA and AgHA powders are poorly soluble at 20 ºC in NaCl. An increase in the content of Zn^{2+} и Ag^{+} ions leads to a slight decrease in the solubility of powders compared with non-modified HA.
4. The surface morphology, specific surface area, porosity, and dispersiveness of ZnHA powders do not undergo notable change when applying aminoacids and their zinc salts in the course of microwave synthesis.
5. The surface of ZnHA and AgHA powders is characterized by the presence of acidic (Ca^{2+}, Ag^{+}, and Zn^{2+} ions), basic (PO_4^{3-} and OH^{-} ions) Lewis centers, and Brønsted acid-base centers. Their ratio changes with increasing the content of biogenic Zn^{2+} и Ag^{+} ions in HA.
6. Biotesting of ZnHA powders showed its inhibitory effect on the growth of *E. coli* bacteria.
7. Phase composition, low solubility in physiological solution, and morphological characteristics of ZnHA and AgHA powders are similar to those of non-modified hydroxyapatite and allow the authors to recommend these powders for medical purposes.

Acknowledgements

This research is supported by the Russian Foundation for Basic Research (No. 17-03-00698\18).

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