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COMPOSITE MATERIALS BASED ON ZINC SULFIDE AND ZINC OXIDE: STRUCTURAL AND BIOCIDAL PROPERTIES

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Introduction

Development of multifunctional nanostructured materials for medicine, such as orthopedics and dentistry, remains a topical subject of medical science. The widespread use of drugs with antimicrobial action has led to the formation of microorganism resistance against wide range of antibiotics. One of the approaches to dissolving this problem around the world is an active search for inorganic and organic substances and their modification by inorganic bioactive ions for the initiation of a controlled reaction in the tissues and provision of antimicrobial activity [1,2]. Complex organic compounds, modified by transition elements, play an important role in the synthesis of materials, catalysis, photochemistry, biological systems, exhibit antimicrobial properties comprising therapeutic agents [3].

Biological apatite, that is a part of the bone tissue, includes a number of the inorganic ions such as Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Na^{+}, K^{+}, Fe^{3+}. Established the fact of increasing biological activity of apatite as a result of the inclusion of their composition of small number of zinc, magnesium, silver ions[4]. A number of recent studies in the field of medical science is devoted to the zinc sulfide (ZnS) and zinc oxide (ZnO). In a significant number microelement zinc found in the bone tissue and promotes bone density and prevents bone loss [5], improves proteins adhesion and the antimicrobial activity [6]. Researchers attention also attracted zinc sulfide nanoparticles in connection with application in optoelectronics, systems of molecular recognition and use their luminescent and fluorescent properties in probes creation [7]. It is known that ZnO-based materials have a pronounced biocompatibility, are characterized by high limit strength, absolute mechanical hardness, as well as the ability to withstand the harsh operating conditions. Recently [8] it was shown the presence of high antibacterial properties for the synthesized composite zinc sulfide with Alginate (ZnS+Alginate) toward the same row of gram-positive and gram-negative microorganisms studied in this work.

The aim of this work is the study of structural and biocidal properties of composite material based on zinc oxide and zinc sulfide (ZnS-ZnO) and its complex with an organic substance - sodium alginate (ZnS-ZnO-Alg) for use in biomedical purpose.

Materials and methods

The next chemicals were used: Sodium alginate (low viscosity, E407, China), zinc nitrate $Zn(NO_3)_2$, thiourea $CS(NH_2)_2$ (Sinopharm Chemical Reagent Co., Ltd), 25 mas.% solution of ammonia. All reagents were analytically grade.

Synthesis of composite materials

For the synthesis of ZnS-ZnO composite 50 ml 0.2M solution zinc nitrate was added to the 50 ml 0.2M thiourea $CS(NH_2)_2$ solution and stirred in a shaker for 60 minutes. The formation of the compound took place when added to a mixture of 25 mas.% solution of ammonia with the subsequent heating at 80 ºC for 30 minutes. Synthesis of the metalorganic complex of ZnS-ZnO-Alg was performed by above mentioned procedure, but to the thiourea solution was previously added 1 ml of 3 mas.% solution of sodium alginate under ultrasonic mixing. As a result of chemical reactions were obtained ZnS-ZnS and ZnO-ZnO-Alg as suspensions, which were thoroughly washed with deionizied water and centrifuged to obtain the product in form of hydrogel with moisture degree about 90%. For the next research composites were dried or lyophilized.

Antibacterial test

Study of antibacterial activity of the ZnS-ZnO and ZnS-ZnO-Alg particles was carried out with the use of nutrient mediums: Muller Hinton (Obolensk, Russia), meat-pepton nutrient (MPN) (Makhachkala, Russia). As the reference cultures were used *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *S. aureus* ATSS 29213, *S. aureus* ATSS-6538, *C albicans* ATCC 885-653, which were obtained from the laboratory of general microbiology with Museum of microorganisms (Mechnikov IMI of Ukrainian Medical Sciences Academy).

Microbial inoculum was prepared in a MPN, which was used as a growing medium. The microorganisms were cultivated at 37º C and left overnight (late exponential stage of grows). Bacterial concentration corresponded to 0,5 units of optical density according Mc Farland scale was achieved using device Densi-La-Meter (PLIVA-Lachema, Czech Republic, wave length 540 nm) according to the instructions to the device and methodology [9,10].

Determination of the minimum bactericidal concentration (MBC) was carried out by a modified serial diluted method in liquid nutrient broth followed plating on solid nutrient medium. MBC was defined as the endpoint, where no bacterial grows can be detected. Because the experimental compounds are sparingly soluble, several suspensions of each samples with different concentrations of solid nanocomposite in MPN were prepared in glass tubes for experiment. To the control sample (nanocomposite free MPN) as well as to the experimental suspensions were added 0.1 ml of microbial inoculum (so that the initial numbers of *E. coli, S. aureus and C albicans* in inoculums were $\approx 10^5$ CFU/ ml). The test tubes were incubated at 37ºC in the thermostat during the 24 hours. Then from all dillutions 0.1 ml aliquots were placed in Petri dishes, over layered with Muller-Hinton agar and incubated at 37 º C for 24 hours. Concentration of the

nanocomposite in the tube, which provided absence of bacterial growth, was identified as MBC. Minimal inhibitory concentration (MIC), which is defined as endpoint where no visible turbidity can be detected with respect to the control, was not determined because of the high degree of turbidity of experimental suspensions.

Also, the study of the sensitivity of the above listed microorganisms to experimental samples was carried out by the method of diffusion in Agar in the modification of the wells. Suspensions of ZnS-ZnS and ZnO-ZnO-Alg in MPN with concentration of 10 mg/ml and 5 mg/ml was prepared for determination of the antibacterial and fungicidal actions respectively.

Integral index of antibacterial activity (A) expected according to the methodology [10] by the following formula:

$A = \sqrt{\left(\frac{a_1 \cdot D_1}{25}\right)^2 + \cdots + \left(\frac{a_n \cdot D_n}{25}\right)^2}$, where:

a_n – the proportion of patients with selected pathogenic microorganism in a particular disease, (range 0-1);

D_n – the average value of the diameter of zones of inhition growth of the studied test strains of microorganisms;

A – integral index of the drug antimicrobial activity;

25 –constant.

Ranges of the efficiency indicator:

1.0-1.5 – the drug shows a weak antimicrobial activity;

1,5-2,5-drug shows a mean antimicrobial activity;

more than 2.5 – drug shows strong antimicrobial activity.

Statistical processing of data was carried out using the program Exel (VS Office 2003) with geometric mean and the possibility of discrepancies (p) indicators.

Structural characteristics

The crystallinity and structure of precipitates were examined using an X-ray diffractometer DRON 3 (Burevestnik, www.bourevestnik.ru) connected to a computer-aided system for the experiment control and data processing. The Ni-filtered CuKα radiation (wavelength 0.154 nm) with a conventional Bragg-Brentano θ 2θ geometry was used. The current and the voltage of the X-ray tube were 20 mA and 40 kV respectively. The samples were scanned in the continuous mode at a rate of 2.0º / min in 2θ range of 10º to 60º. All experimental data was processed by means of the program package DifWin 1 (Etalon TC, www.specord.ru). Identification of crystal phases was done using a JCPDS card catalog (Joint Committee on Powder Diffraction Standards, www.icdd.com).

Microelement composition

The elemental composition of synthesized samples was studied by an X ray fluorescence (XRF) analysis using ElvaX Light SDD spectrometer (www.elvatech.com). It is capable of identifying elements from Na (Z = 11) to U (Z = 92). A rhodium anode tube is used to obtain an X ray radiation. The voltage of the X ray tube was 12 kV. The current was selected automatically to provide a sufficient load of simultaneously registered characteristic photons of ~50 000 counts. The registration time was 30 s.

Results and discussion

The study was carried out on ZnS-ZnO-Alg and ZnS-ZnO synthesized by the "wet chemistry". The samples were thoroughly washed and dried at a temperature of 37ºC. It was fine powder of light yellow color, which is planned to include to the composition of the hydroxyapatite (HA) based material for filling damage bone tissue. Solubility product (SP) of zinc sulfide by different information sources is from $7x10^{-27}$ to $1{,}1x10^{-21}$ [12]. At the same time SP of the synthetic HA is $1x10^{-64}$, biological apatite- $2{,}87x10^{-36}$. Thus, the solubility of ZnS is considerably higher compared with the HA. The latter means that in the physiological conditions under enzymes action the ZnS-ZnO and ZnS-ZnO-Alg additions have to dissolve much faster than the basic material and perform antimicrobial function during the postoperative period.

X-ray structural analysis (Fig. 1) indicate that in the composite material, synthesized both in the presence of sodium alginate (ZnS-ZnO-Alg) and without sodium alginate adding (ZnS-ZnO) exist two phases: ZnS and ZnO.

Formed during the synthesis ZnS crystals have the cubic phase of sphalerite type (JCPDS 5-566) with average crystal size 23nm. ZnO crystals have hexagonal phase (JCPDS 80-75) with a average size about 35nm. Synthesized in the presence of sodium alginate ZnS and ZnO crystals substantially reduced and their average sizes are about 10 nm and 12 nm, respectively.

The value of the parameter *a* for the synthesized ZnS corresponds to a standard size of the crystal lattice (about 0.54 nm). ZnO crystals in composite highlighted the increasing parameter *a* as compared with the standard: 0.54 nm against 0.32 nm (Table1) . Microelement composition of samples, determined using the method of FRA, shown in Fig. 2. Based on RFA calculations show that ZnS-ZnO sample contains up to 50 wt. % zinc oxide. Zinc oxide content in the ZnS-ZnO-Alg is about 25 wt.%. (Table. 1).Thus, it was found that the synthesis of composite in the presence of sodium alginate reduces the size of the crystallites and increases the content of ZnS phase for 25w.% compared with ZnO phase. Thiourea obviously influences the forming of ZnO crystalline phase by increasing the parameter *a* of its crystal lattice.

The aim of the microbiology test was to probe the antibacterial activity of the experimental substances toward the next types of bacteria: *E.coli* (Gram-negative), *S.aureus* (Gram-positive) and *C.albicans* (fungi).

The minimum bactericidal concentration was determined by above described method placing the liquid from each tube with a sample on a Mueller-Hinton solid culture medium.

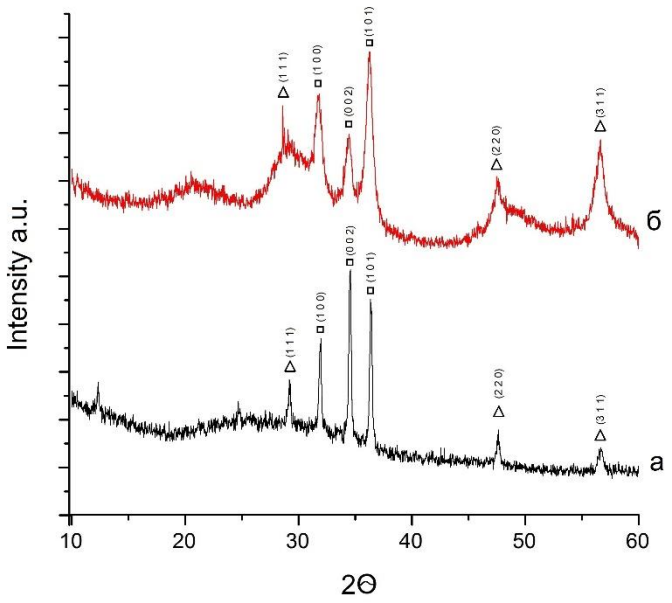

Fig. 1. X-ray diffraction spectra of the dried at 37 ºC а) ZnS- ZnO, б) ZnS- ZnO-Alg . Miller Indices shows compounds plane: Δ-ZnS; □ – ZnO.

Table 1. Structural characteristics of ZnO and ZnS consisting experimental composites

Sample	Phase	Content in the composite ,%	Miller index	Crystallite size, nm	Lattice parameters, nm	
					a	*c*
ZnS-ZnO-Alg	ZnS (cubic, sphalerite)	75	(1 1 1)	13.902	0.539	-
			(2 2 0)	7.941	0.541	-
			(3 1 1)	8.514	0.538	-
	ZnO (hexagonal)	25	(0 0 2)	13.07	0.563	0.519
			(1 1 0)	10.792		
ZnS-ZnO	ZnS (cubic, sphalerite)	50	(1 1 1)	22.911	0.529	-
			(2 2 0)	31.011	0.539	-
			(3 1 1)	14.856	0.545	-
	ZnO (hexagonal)	50	(0 0 2)	36.96	0.56	0.518
			(1 1 0)	32.088		

The solid sabstance fraction has remained at the bottom of the tube in the form of insoluble residue. MBC of ZnS-ZnO-Alg samples against all studied microorganism strains was 1,25 mg/ml. MBC of ZnS- ZnO samples ranged from 5 mg/ml for *C. albicans* to 12.5 mg/ml for *E. coli*. It is possible that due to small sample solubility in experimental conditions and small ion diffusion of the active substance there was no full contact with the whole bacterial cell volume. As ZnS-ZnO-Alg samples differe from ZnS- ZnO samples by smaller crystallite size and greater solubility, they exhibit a marked antimicrobial effect.

At the same time in direct contact of the entire surface of the sample with bacterial cells under condition of the modifying method of diffusion into agar, both types of samples showed high antimicrobial activity. The results are presented in Table. 2.

To determine the integral antimicrobial activity of two types of experimental samples against all studied test strains, integral index (*A*) was calculated. The applied vector theory allowed to present *A* as a vector in n-dimensional coordinates space by a zone of growth inhibition for each investigated test microorganism.

The results showed that A value for ZnS- ZnO and ZnS- ZnO-Alg is 1.57 and 1.9 respectively. Thus, according to the guidelines for a range of efficiency index both types of samples belong to those exhibiting the average antimicrobial activity.

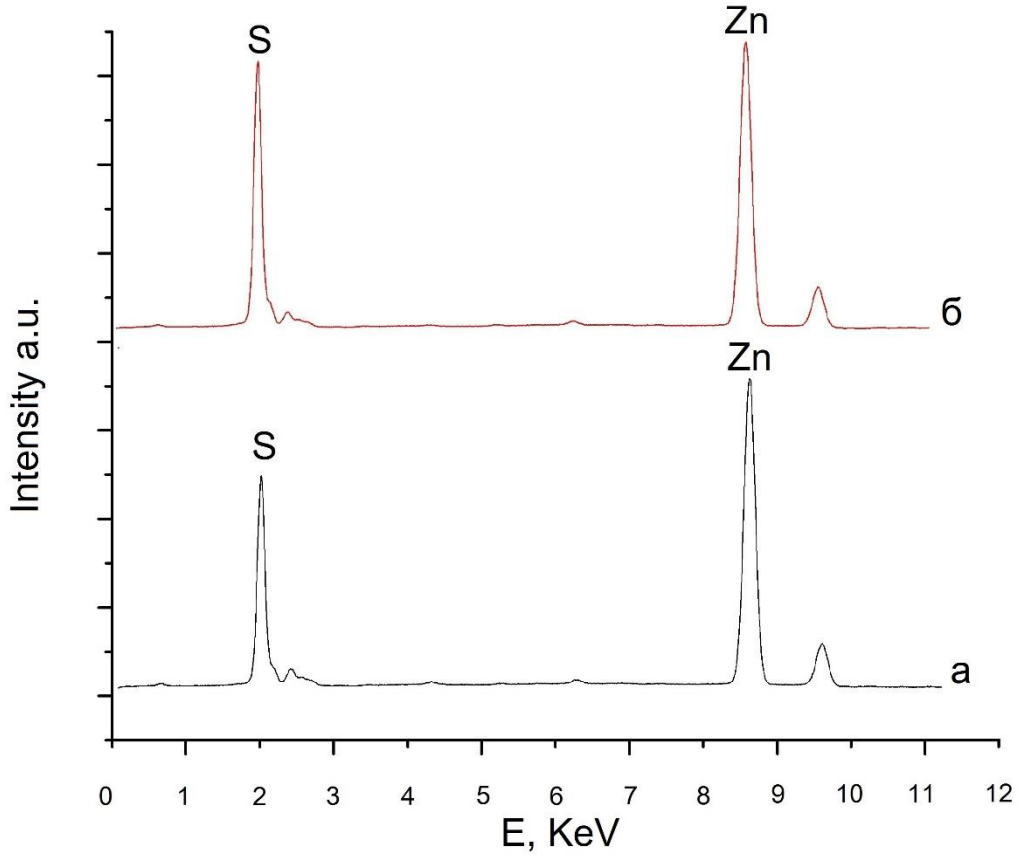

Fig. 2. Microelement composition of samples: a) ZnS- ZnO, b) ZnS- ZnO-Alg, by X-ray fluorescence analysis

Table 2. Biocidal ability of zinc compounds, M±m

Microorganism test- strains	MBC (mg/ml) by serial dilutions method		Zone of growth inhibition (mm) by modifying agar diffusion method	
	ZnS-Alg	ZnS	ZnS-Alg	ZnS
S. aureus ATCC 29213	1,25±0,4	11,25±0,6	–	–
S. aureus ATCC 25923	–	–	20,0±1,33	16,0±1,0
S. aureus ATCC 6538-P	1,25±0,2	12,5±0,8	20,5±1,0	15,5±0,66
E. coli ATCC 25922	1,25±0,4	12,5±1,2	27, 0±0,66	24,0±2,0
C. albicans ATCC 885-653	1,25±0,3	5,0±0,4	25,0±1,33	22,0±1,77

Note: $p \leq 0,05$

Scientific sources give two major mechanisms of antimicrobial action ZnO: a) toxic effects of zinc ions in the cell membrane of bacteria; b) toxicity ROS (reactive oxygen spices), formed with the participation of ZnO and ZnS, on components of the bacterial cell. Antibacterial activity is the result of the formation such ROS, as hydrogen peroxide (H_2O_2), peroxide anion (O^{2-}), hydroxyl radicals (OH^-). These particles damage cellular components such as DNA, lipids and proteins [13]. Positively charged zinc ions also can directly interact with negatively charged components of the bacterial wall [14]. According to TEM, FE-SEM and AFM analyzes action of the ZnO containing composites appears in violation of the integrity of the cell membrane, which leads to damage of membrane proteins and lipid layer [15]. Analysis of the experimental data showed that higher antimicrobial activity have examples of great content ZnS. Antimicrobial properties of zinc compuonds nanoparticles are provided, firstly, their high reactivity, defined by size (less 100 nm) [16]. Also during ZnS dissolving is formed sulfide anion in which the sulfur atom has an unshared electron pair, thus given anion can form a donor-acceptor atoms ties with the functional groups of the bacterial cell wall components, disrupting their metabolism.

Conclusion

By the applied method of synthesis the composites ZnS- ZnO and ZnS-ZnO-Alg have been obtained. X-ray structural analysis of samples proved the presence of ZnO and ZnS phase with defined structure: ZnS has a cubic crystal structure type sphalerite (JCPDS 5-566) with average crystallite size of 23 nm and ZnO - hexagonal structure (JCPDS 80-75) with an average size of about 35 nm. The introduction of sodium alginate to the reaction mixture during synthesis reduces the size of ZnS and ZnO crystallites to 10 nm and 12 nm, respectively. In the ZnS-ZnO-Alg samples, synthesized in presence of sodium alginate, the ZnS phase content increased for 25wt.% compared with the ZnO phase, which was confirmed by X-ray fluorescence analysis. Microbiological studies have shown the presence of antimicrobial activity of samples against Gram-positive bacteria *S. aureus*, Gram-negative *E. coli* and fungi *C. albicans.* The estimated values for the integral antimicrobial activity, calculated by the vector theory, are for ZnS-ZnO and ZnS-ZnO-Alg 1,57 and 1,9 respectively. It means that both types of samples have average antimicrobial activity.

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Introduction. The widespread use of drugs with antimicrobial action has led to the formation of microorganism resistance against wide range of antibiotics. One of the approaches to dissolving this problem is the substances modification by inorganic bioactive ions in oder to initiate a controlled reaction in the bone tissues and provision of antimicrobial activity. It is known that ZnO-based materials have a pronounced biocompatibility, they are characterized by high limit strength, absolute mechanical hardness, as well as the ability to withstand the harsh operating conditions. The aim of this work is the study of structural and biocidal properties of composite material based on zinc oxide and zinc sulfide (ZnS-ZnO) and its complex with an organic substance - sodium alginate (ZnS-ZnO-Alg) for use in biomedical purpose. **Materials and methods.** For the synthesis of ZnS-ZnO composite 50 ml 0.2M solution zinc nitrate was added to the 50 ml 0.2M thiourea CS $(NH_2)_2$ solution and stirred in a shaker for 60 minutes. The formation of the compound took place when added to a mixture of 25 mas.% solution of ammonia with the subsequent heating at 80 ^oC for 30 minutes. Synthesis of the metalorganic complex of ZnS-ZnO-Alg was performed by above mentioned procedure, but to the thiourea solution was previously added 1 ml of 3 mas.%

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