Synthesis and antimicrobical activity of composite oxides nanoparticles based on ZnO

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Abstract. It should be noted that zinc oxide nanoparticles have increased antibacterial activity. ZnO/Me composite nanoparticles have a more pronounced antimicrobial effect in comparison with monometallic nanoparticles obtained by similar conditions. In present work ZnO/Ag and ZnO/CuO composite nanoparticles were obtained by the electrical explosion of Zn and Cu or Zn and Ag wires in oxygen containing atmosphere. Composite nanoparticles were characterized by transmission electron microscopy, XRD analysis and micro electrophoresis. Antibacterial activity of composite nanoparticles against Gram-positive E.coli and Gram-negative MRSA was studied. The as-synthesized nanoparticles demonstrated excellent bactericidal effect due to their unique properties including not only components additive effect but also synergetic one.

1. Introduction

Nanoparticle metal oxides represent a new class of materials that are increasingly being developed for use in research and medical applications. These metal oxides are interesting not only for a wide range of physical and chemical properties, but also for antibacterial activity [1]. And the problem of formation of stable strains of microorganisms contributes to the development of directions related to the development of nanomaterials, which can become a new alternative to the already known antibiotics [2]. Throughout the world, there is one complex problem that modern bacterial-infection therapy faces: the emergence of drug-resistant microorganisms, such as methicillin-resistant Staphylococcus aureus (MRSA) [3]. And according to preliminary experimental data, zinc oxide-based composite nanoparticles influence the clinical strain of MRSA. This makes it possible to develop promising areas of research based on zinc oxide composite materials. And the development of these new composite nanoparticles will allow to obtain materials with increased functional properties and antibacterial activity. Thus, for example, nanocomposites based on metal oxides are used to create new effective antimicrobial agents [4, 5]. Zinc oxide (ZnO) is an important metal oxide used for various biological applications [6, 7]. Such composite nanomaterials based on zinc oxide with antimicrobial activity are of great interest for various biomedical applications.

2. Materials and methods

Composite nanoparticles (Zn_xMe_{1-x})O, where Me – Cu, Ag were obtained by a electric explosion of two intertwined wires in a buffer gas containing argon and oxygen. The oxygen content was 20 vol. %. The buffer gas pressure for all samples was 3·10^5 Pa.
2.1. The morphology of nanoparticles
Composite nanoparticles morphology and particle size distribution were studied by the transmission electron microscopy (TEM) method using the electron microscope JEM-2100 (JEOL, Japan) with the integrated energy-dispersive analysis system X-Max (Oxford Instruments, GB). The phase composition of the samples was studied by X-ray analysis (XRD) on CuKα radiation in the scanning mode in the range of 20 angles from ~25 to ~75°, with a pitch of 0.02 using a XRD Shimadzu 6000 diffractometer (Shimadzu, Japan). The zeta potential of nanoparticles was measured in deionized water at 25 °C and pH 7.2 using the Zetasizer Nano ZSP (Malvern Instruments Ltd, GB).

2.2. Determination of the minimum inhibitory concentration
The nanoparticles antibacterial activity was determined using Gram-positive clinical strain of methicillin-resistant Staphylococcus aureus MRSA ATCC 43300 and a Gram-negative Escherichia coli ATCC 25922. The minimum inhibitory concentrations (MIC) values were determined by 96-well plate microdilution method. In a typical procedure, 100 µl of Mueller Hinton broth, 20 µl of bacterial suspension (10^8 CFU/ml) and 50 µl of aqueous dispersions of nanoparticles were added to each well. The plates were then incubated at 37 °C for 24 hours. After incubation, optical density (OD) was measured at 620 nm using the Multiskan FC microplate spectrophotometer (Thermo Fisher Scientific, USA).

To determine the bacterial growth dynamics in the phosphate buffered saline (PBS) in the presence of nanoparticles a suspension method was used. The 300 μg sample weights were added to 30 ml PBS. After that, 300 µl bacterial suspensions with the concentration of 10^7 CFU/ml was added and incubated at room temperature under stirring on a PE-6600 shaker (Ecroskhim, Russia). The 50 aliquots µl were sampled from each container after 1, 3, 6, 9 and 24 hours and distributed on Petri dishes with Mueller Hinton agar. The number of colony-forming units (CFU) was calculated after 24 hours incubation at 37 °C. Microbial suspensions without nanoparticles in sterile PBS were used as controls.

3. Results and discussion

3.1. Production of nanoparticles
As a result of joint electrical explosion of two intertwined Zn/Ag, Zn/Cu wires (EEIW) in the oxygen-containing atmosphere, nanoparticles and their agglomerates of various size are formed. For a sample (Zn_{0.55}Cu_{0.45})O the maximum quantity of particles has the number average particle size of particles in the range of 70-100 nm, for a sample (Zn_{0.55}Ag_{0.45})O more than 40% of all particles have the size in the range of 20-50 nm. At electrical explosion of couple of Zn/Cu metals particles, spherical and close to spherical shape, are formed. At the same time copper and zinc on particles are distributed not evenly, division of components (figure 1(a)) is observed. The sample received at dispersion of Zn and Ag (figure 1(b)) delays, represents particles of irregular shape. At the same time the form Janus particles, where one of parts is enriched with Zn, other Ag is characteristic of many particles and there is a clear boundary of division of components.

3.2. Analysis of composite nanoparticles
According to the X-ray analysis data, at joint electro-pulse dispersion of Zn/Cu metals in an oxygen-containing atmosphere (figure 2(a)) a mixture of ZnO phases (PDF card 01-075-0576), CuO (PDF card 00-002-1040) and Cu_2O (PDF card 01-078-5772) is formed. The main peaks on the diffractogram of the sample obtained at dispersion of Zn/Ag pair (figure 2(b)), peaks correspond to ZnO (PDF card 01-075-0576) and Ag (PDF card 01-071-3762). Thus, under the experimental conditions silver does not react with oxygen.

All particles have a positive zeta potential in water, measured at 25 °C and at physiological pH values. At the same time, the zeta potential of ZnO–CuO nanoparticles at pH 7 is approximately +40 mV, ZnO–Ag(O) nanoparticles - +28 mV. The point of zero charge (IEP) of nanoparticles is in the pH 9-9.7 range.
At electrical explosion in the oxygen-containing atmosphere of Zn, Cu, Ag wires nanoparticles are formed, having the following characteristics: at dispersion of Zn the faceted particles of ZnO and their agglomerates are formed, the majority of which have the average particle size in the range of 20-70 nm). More than 80 % of the particles are in the 20-100 nm range. The electrical rupture of a Cu conductor results in the formation of spherical nanoparticles, 45 % of which are in the 70-100 nm range. The particles contain CuO and Cu₂O, as well as a low intensity reflex characteristic of Cu. Silver under these conditions does not react with oxygen and as a result of electrical explosion of Ag wire spherical silver nanoparticles are formed, 77 % of which are in the range of 0.2-0.7 nm.

All particles have a positive zeta potential in water, measured at 25 °C and at physiological pH values. The zeta potential of (Zn₀.₅₅Cu₀.₄₅)O nanoparticles at pH 7 is approximately +40 mV, (Zn₀.₅₅Ag₀.₄₅)O nanoparticles - +28 mV. The point of zero charge (IEP) of nanoparticles is in the pH 9-9.7 range. Thus, under conditions of combined electro-pulse dispersion of zinc and the second metal in an oxygen-containing atmosphere, composite nanoparticles consisting of zinc oxide, second metal oxide, or non-responsive metal with oxygen can be obtained.
3.3. Antibacterial activity

In the study of antimicrobial activity of composite nanoparticles, it was found that the nanoparticles under study most effectively inhibit the growth of MRSA (clinical strain) ATCC 43300 compared with particles that were obtained by dispersing single conductors in the same conditions. The minimum inhibitory concentration for \((Zn_{x}Me_{1-x})O\) nanoparticles is several times lower than for \(Me(O)\) nanoparticles (figure 3). Preliminary growth analysis data show that ZnO-based nanoparticles have a much higher antibacterial effect on Staphylococcus aureus. But composite nanoparticles also affect E.coli ATCC 25922, but are not as pronounced. The data obtained suggest that nanoparticles containing ZnO can potentially be used as a new promising bacteriostatic agent and used in the development of derivative agents to control the spread and infection of various strains of bacteria. At the same time, the results of the study of the dynamics of microorganism reduction during the incubation of MRSA with composite nanoparticles \((Zn_{0.55}Cu_{0.45})O\) and \((Zn_{0.55}Ag_{0.45})O\) in PBS show that after 1 hour of incubation, the complete suppression of bacterial activity occurs (figure 3(a), (i)). And single nanoparticles of metals (ZnO, AgO, CuO) showed bactericidal action only after 3 hours of incubation (figure 3(c), (e), (g)). And on E.coli, complete suppression of microorganisms occurs after 3 hours of incubation with nanoparticles Ag(O), \((Zn_{0.55}Ag_{0.45})O\), CuO (figure 3(d), (f), (j)) and after 1 hour at \((Zn_{0.55}Cu_{0.45})O\) (figure 3(b)).
Figure 3. MIC of \((Zn_{x}Me_{1-x})O\) nanoparticles.

4. Conclusions
Composite nanoparticles the received electrical explosion of two intertwined \(Zn/Ag, Zn/Cu\) wires in the oxygen-containing atmosphere can turn out in the form of nanoparticles of various structure, such as mix of oxides or as in case of joint electric explosion of \(Zn\) and \(Cu\) of wires, mix of oxides and spinels. The synthesized composite nanoparticles have the greatest antimicrobial activity. They show bactericidal actions on Gram-positive MRSA and on Gram-negative \(E.coli\), than the nanoparticles
received in similar conditions at electro pulse dispersion of single wires of the corresponding metals, including silver in the oxygen-containing atmosphere. The presented results, at more in-depth study of the biological action of the received nanoparticles, can find application for development of new antibacterial agents and the strategy of fight against pathogenic microorganisms.

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**References**
[1] Jones N, Ray B, Ranjit K T and Manna A C 2008 *FEMS Microbiol. Lett.* **279**(1) 71–6
[2] Alswat A A, Ahmad M B, Saleh T A, Hussein M Z B and Ibrahim N A 2016 *Mater. Sci. Eng.*** 68, 505–11
[3] Koch G, Yepes A, Förstner K U, Wermser C, Stengel S T, Modamio J and Lopez D 2014 *Cell*** **158**(5) 1060–71
[4] Kaushik M, Niranjan R, Thangam R, Madhan B, Pandiyarasan V, Ramachandran C and Venkatasubbu G D 2019 *Appl. Surf. Sci.* **479** 1169–77
[5] Azam A, Ahmed A S, Oves M, Khan M S, Habib S S and Memic A 2012 *Int. J. Nanomedicine*** 7 6003
[6] Arias C A and Murray B E 2009 *N. Engl. Med.* **360**(5) 439–43
[7] Balouiri M, Sadiki M and Ibnsouda S K 2016 *J. P. A.* **6**(2) 71–9