ZnO NANOPARTICLES OBTAINED BY PULSED LASER ABLATION AS AN ANTIBACTERIAL AGENT

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Abstract

Water dispersion of zinc oxide nanoparticles was obtained by pulsed laser ablation. Atibacterial activity of the dispersion to E.coli was studied. It was shown that ZnO nanoparticles exhibit stronger bacteriecidal effect than silver nanoparticles. An attempt for understanding of a mechanism of antibacterial effect of ZnO nanoparticles was made. It was found that zinc (II) ions and hydrogen peroxide are not responsible for zinc oxide nanoparticles antibacterial activity. It was suggested that ZnO nanoparticles obtained by PLA method are a promising antibacterial agent for using different fields.

Keywords: zinc oxide; nanoparticles dispersion; pulsed laser ablation (PLA); antibacterial activity

1. INTRODUCTION

Zinc oxide is known as an inorganic antibacterial agent and exhibits strong both antibacterial and antifungal activity [1-5]. It was also found [6] that a contact with ZnO slurry increases the sensitiveness of bacteria to some antibiotics. Moreover, ZnO can be used in water at pH values from 7 to 8 [7], and it is suitable for application in washing, food industry, and for drinking water treatment.

ZnO bactericidal effect increases with the decreasing of particle size [5]. But the point is that the smaller the particles – the lower the stability of their dispersion. That is why new methods for enhancing stability of ZnO dispersions are needed [8]. It was also shown, that ZnO activity strongly depends not only on its particle size, but on their composition and defective structure [4, 5]. Thus, the method of ZnO nanoparticles preparation plays a crucial role in their activity. Moreover, the main problems of using nanoparticles and their dispersions in biology and medicine are purity of the nanoparticles, and biocompatibility of the solvent. Thus, development of new methods for producing stable dispersions of "pure" nanoparticles in acceptable liquids seems to be a very important task.

Pulsed laser ablation (PLA) is a method based on the ejection of small amounts of a material from the solid target surface by its interaction with short, intense laser pulses [9]. Using this technique in our laboratory the stable dispersions of pure zinc oxide nanoparticles were obtained in water in the absence of surfactants and stabilizing agents [10]. ZnO nanoparticles obtained were characterized. The particles size was found to be in the range of 5-100 nm with the average size of 10 nm, and BET surface area was of 19.3 m2/g [10]. In the present work antibacterial activity of pure ZnO nanoparticles dispersion obtained by pulsed laser ablation method was studied. An attempt to understand the mechanism of antibacterial effect of zinc oxide was made.

2. EXPERIMENTAL

2.1. Synthesis

ZnO nanoparticles dispersions were obtained by pulsed laser ablation method (PLA) as described in the previous work [10]. The fundamental harmonic of Nd:YAG laser (LS-2132UTF, LOTIS TII, Belarus) was used for irradiation (1064 nm, 7 ns, 200 mJ, 15 Hz). Metallic zinc target (99.9%) was immersed in a glass (50 ml) with the solvent (distilled water). Concentration of the dispersions was calculated using measuring of Zn target weight loss. In order to stimulate oxidation process all the dispersions after preparation were aerated by air (15 minutes, gas flow rate of 1 l/min). Metal nanoparticles water dispersions were obtained from metallic targets (Au, Pt, Ag) using the same technique.

2.2. Characterization

Spectral properties of the dispersion were studied using Spectrophotometer Cary100 (Varian, USA). Absorbance was investigated in the region of 200-900 nm. BET surface area of ZnO powder obtained by drying of the dispersion was measured using TriStar II 3020 gas adsorption analyzer (Micromeritics, USA). pH (23°C) was measured using pH-meter pH-150MI ("Izmeritelnaya Technika" Ltd., Russia). Concentration of zinc (II) ions was measured by standard addition method using stripping voltammetry on P-8nano (LLC "Elins", Russia) potentiostat-galvanostat with three electrodes cell (Ag/AgCl with 1 M KCl, Pt plate, and cylindrical glassy carbon)

2.3. Antibacterial activity experiment

For antibacterial experiments E.coli, a Gram negative bacterium, was selected as the target organism. The culture E.coli B-6954 was obtained from Russian Collection of Microorganisms. All materials were sterilized in an autoclave before the experiments. Meat-peptone broth was used for culturing the bacteria at 37°C on an orbital shaker at a rotation speed of 200 rpm. The growth time was 24 hours. 1 ml of every liquid sample was added to 4 ml of a daily culture E.coli with initial concentration of 4.0×10^2 or 3.0×10^5 CFU (colony forming units) per ml. 0.1 ml of obtained mixtures was deposited on individual Petri dishes with Endo agar. Then the samples were incubated for 24 h at 37°C, and the amount of grown colonies was counted.

2.4. Comparative samples

Supernatant of ZnO water dispersion was obtained via 21,000 rpm centrifugation for 60 min using Allegra 64R (Beckman Coulter, USA). $ZnCl_2$ and H_2O_2 obtained from Sigma-Aldrich were ACS reagent grade, and were used without additional purification. Concentrations of zinc-containing samples were specified for zinc (II) ions.

3. RESULTS AND DISCUSSION

Figure 1 represents photos of dispersions obtained by laser ablation of metallic zinc target in water. Absorbance spectra for them are presented on Figure 2. Dispersion after PLA is of a brown color (Figure 1a), and shows absorbance peak at about 250 nm that belongs to plasmon of metallic zinc (Figure 2, curve 1). After keeping in air this peak decreases, and a small shoulder at 300-350 nm appears (Figure 2, curve 2). This shoulder transforms into a band of exciton absorbance of zinc oxide after air bubbling. Aeration leads to a change of the color of the dispersion up to grey (Figure 1b), and results in complete disappearance of the plasmon peak of zinc, and formation of absorption edge of a semiconductor – ZnO (Figure 2, curve 3). As it was confirmed by XRD analysis, nanoparticles obtained contain only one phase – hexagonal ZnO (wurtzite), and, as it was mentioned before, TEM has shown that the average particle size is about 10 nm [10]. Thus, ablation process forms metallic zinc nanoparticles in water, and they start oxidation after contact with air. Additional aeration of the dispersion results in complete oxidation to ZnO.
Antibacterial activity of ZnO water dispersion was tested along with distilled water parallel test: distilled water was irradiated by laser under the same conditions in order to exclude probable influence of other species in water on the results. Moreover, the dispersions of noble metals nanoparticles (Au, Pt, Ag) obtained by laser ablation were tested to compare results. The data obtained is presented in Table 1.
As it is seen from the results, ZnO water dispersion showed 100% bactericidal effect under the experimental conditions. Even though silver nanoparticles are known as a strong antibacterial agent, their activity was found to be lower than it of ZnO nanoparticles.
Many studies of possible antibacterial mechanism of ZnO nanoparticles have been done recently. There are several main ideas on this question. One of the most popular versions is that reactive oxygen species (ROS), including hydrogen peroxide, which are formed on ZnO surface, are responsible for its bactericidal effect [4-7]. Another popular version is the interaction of bacteria with zinc (II) ions, obtained during ZnO

dissolving [11]. Authors of [12] suggested that ZnO nanoparticles can penetrate cell membranes of bacteria, and mechanically damage them, or even may accumulate inside the cells. As it can be seen, the question of the mechanism of ZnO nanoparticles antibacterial activity is still under discussion. The point is that nanoparticles properties strongly depend on the methods of their preparation. Thus, there might not be a uniform mechanism for different ZnO particles.

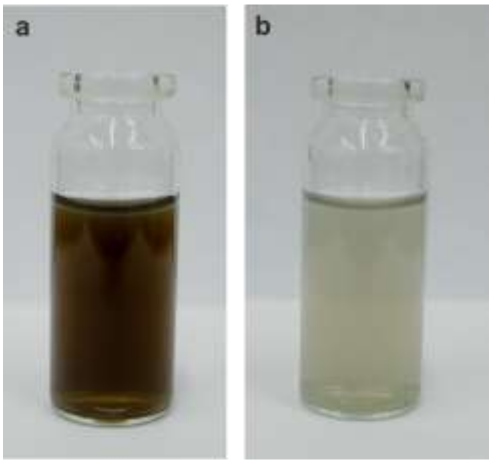

Fig. 1 Photographs of zinc oxide nanoparticles dispersions (0.2 g/l) in water after preparation (a) and after aeration (b).

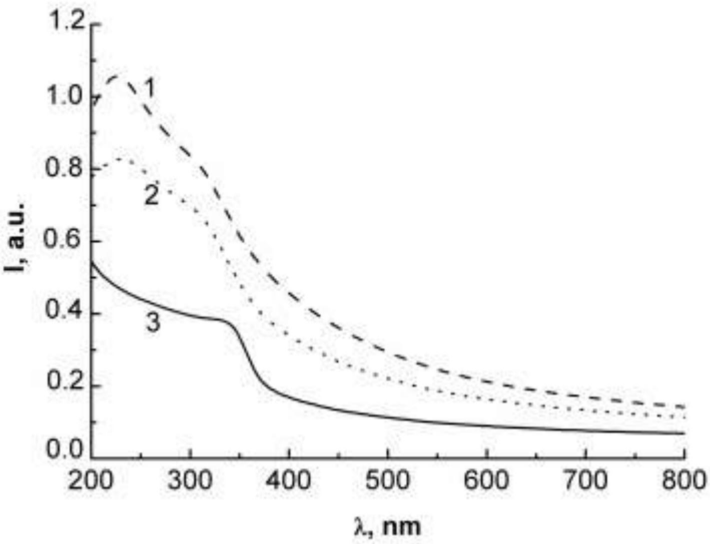

Fig. 2 Absorbance spectra of ZnO nanoparticles dispersion (0.2 g/l) in water: (1) after preparation; (2) after keeping in air for 15 min; (3) after aeration for 15 min.

In order to better understand the mechanism of antibacterial activity of ZnO nanoparticles obtained by PLA an experiment was conducted as follows. Firstly, a supernatant of ZnO water dispersion was obtained by centrifugation. The concentration of zinc (II) ions in ZnO water dispersion and in its supernatant was measured by standard addition method using stripping voltammetry. The amount of 6.9(±0.3)×10^{-4} M of Zn^{2+} was detected in ZnO dispersion, and 7.4(±0.3)×10^{-4} M of Zn^{2+} was found in its supernatant. Thus, the content of zinc ions is the same in the dispersion and in the solvent after deletion of the nanoparticles.

Hence, if Zn^{2+} ions are the main reason of ZnO dispersion antibacterial activity, it would be detected by using supernatant instead of nanoparticles dispersion.

Table 1 Data on E. Coli concentration before and after incubation

Sample	Initial concentration of E.coli, CFU/ml	Concentration of E.coli after 24 h, CFU/ml
Distilled water	5.8×10^2	3.6×10^3
ZnO dispersion (50 mg/l)	5.6×10^2	0
Au dispersion (50 mg/l)	3.0×10^2	3.2×10^3
Pt dispersion (50 mg/l)	3.1×10^2	3.7×10^3
Ag dispersion (50 mg/l)	2.7×10^2	10^1

At the same time zinc (II) chloride solution with concentration of 50 mg/l (by Zn) was used for comparison. Excessive concentration of Zn^{2+} was believed to show unambiguously whether zinc ions were responsible for ZnO bactericidal effect.
Secondly, according to [6, 7], the formation of hydrogen peroxide is one of the primary factors in the antibacterial mechanism of ZnO powder slurry. In the work [13] it was found that the bulk concentration of H_2O_2 in 1 g/l ZnO powder dispersion was about 0.5 mg/l. The authors [6] shown that the killing effect of ZnO powder slurry was greater than that of hydrogen peroxide solution with corresponding concentration. That is why they also used 0.31 g/l (0.0155 M) solution of H_2O_2 in their experiment. In present study the solution of hydrogen peroxide with the same concentration was used to compare its antibacterial effectiveness with it of other samples.
Antibacterial experiment in this case was performed using the same protocol but with more concentrated E.coli culture. The results obtained are presented in Table 2. It may be noticed that pH values were almost the same for supernatant, zinc chloride, and hydrogen peroxide solutions. And it was a little more basic for distilled water and ZnO nanoparticles dispersion.

Table 2 Data on E. Coli concentration before and after incubation

Sample	pH	The number of E.coli in the co-culturing, CFU/ml		Tendency
		5 min	24 h	
Distilled water	8.36	$3.1\cdot10^5 \pm0.78$	$8\cdot10^5\pm1.24$	↑
ZnO (50 mg/l)	8.15	$2.8\cdot10^5 \pm0.51$	$3\cdot10^4\pm0.90$	↓
Supernatant	6.82	$3.1\cdot10^5 \pm0.38$	$5\cdot10^5 \pm0.80$	–
$ZnCl_2$ (50 mg/l)	6.75	$3.2\cdot10^5 \pm0.70$	$1\cdot10^5\pm0.97$	–
H_2O_2 (0.0155 M)	6.80	$3.0\cdot10^5 \pm0.50$	$4\cdot10^5\pm1.13$	–

For the experiment with higher concentration of bacteria it should take more time to inhibit their growth or kill them. But it is seen that even in this case ZnO leads to the decrease of the concentration of E.coli. Zinc chloride solution and supernatant did not decrease the concentration of bacteria. Thus, antibacterial activity of zinc oxide nanoparticles dispersion is not based on zinc (II) ions reacting with bacteria. Also it should be mentioned that hydrogen peroxide in the exceed concentration did not show bactericidal effect under experimental conditions. This fact denotes that ZnO nanoparticles activity is not due to H_2O_2 formation.

4. CONCLUSION

In the present work ZnO water dispersion was obtained by pulsed laser ablation and its antibacterial activity to E.coli was studied. It was found that as-prepared ZnO nanoparticles exhibit stronger bactericidal effect than silver nanoparticles. The investigation of the mechanism of ZnO antibacterial activity has shown that both Zn^{2+} and hydrogen peroxide were not responsible for bacteria growth inhibition. Thus, the antibacterial action of ZnO nanoparticles is based on another factor(s). This question requires more detailed investigation in future. But at this stage we suggest that stable anti-bacterial dispersion of ZnO nanoparticles obtained by pulsed laser ablation can be used in different fields, such as cleaning, purification, disinfection and others.

ACKNOWLEDGEMENTS

This study was supported by the Tomsk State University Academic D.I. Mendeleev Fund Program (research grant # 8.2.57.2015) and the Program of Increasing the International Competitiveness of the Tomsk State University for 2013-2020 years.

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