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ANTIMICROBIAL PROPERTIES OF SEMICONDUCTIVE OXIDE NANOPARTICLES. FROM FUNDAMENTAL TO APPLICATION[1]

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ABSTRACT

A problem of modern society is the spread of diseases worldwide. Infection control and maintaining a high level of hygiene by applying antimicrobial coatings (including medical products, packaging materials, membrane filter/water treatment or filters in air conditioning) are of the greatest importance. Hospitals, pharmaceutical production, food factories must be thoroughly disinfected in order to destroy pathogenic microbes. Microbial contamination of water is a major threat to public health. With the emergence of organisms resistant to many antimicrobial agents there is an increased demand for improved disinfection methods.

Recently, the confluence of nanotechnology and biology has led to metals and metal oxides under the form of nanoparticles as potential antimicrobial agents. Nanoparticles have unique and well defined physical and chemical properties which can be manipulated suitably for desired applications. The applications of nanoparticles as antimicrobials is gaining relevance in prophylaxis and therapeutics, in medical devices, food industry and textile fabrics.

This work focuses on the properties of ZnO nanoparticles doped with different concentrations of silver. The mechanism of action of nanoparticles as bactericidal will be highlighted in this study. Specific applications of the investigated nanoparticles are presented.

KEYWORDS: semiconductive oxide nanoparticles, antimicrobial properties

1. Introduction

Increasing health and hygiene requirements have increased interest in obtaining functional semiconductor oxide nanomaterials which have non-toxic and bioactive properties, are cost-effective and antimicrobial and provide UV protection. A large number of materials which were considered to be safe develop toxicity at nano size ranges which is mainly related to the increased specific surface area and high reactivity of nano size materials [1]. In this context, in recent years an increasing interest in the synthesis of new materials with improved efficiency has been manifested. Among these materials, semiconductor oxide nanoparticles (ZnO, TiO_2, SnO_2, CuO, etc.) present a great potential [2]. Polymer nanocomposites containing semiconductive zinc oxide nanoparticles have attracted a great interest due to their unique chemical and physical properties and important biological applications especially duet o their bactericidal effect [3]. The semiconductor oxides have an antibacterial activity of bactericidal and bacteriostatic type, as a result of the photocatalytic effect in the presence of UV radiation. Due to this property, these oxides have a very high potential for numerous applications in various antiseptic fields [2, 4]: in food industry for the processing of fresh food, which can not be pasteurized; in pharmaceutical industry for the production of antiseptics (bandages, burn); in textile industry for removal of microorganisms from raw materials; in medical clinical laboratories, medical wards with a higher risk

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factor for infection with pathogenic microorganisms; antiseptic filter air conditioning, where microorganisms can grow and become very harmful to human health, potable water, etc. Antimicrobial efficiency of biocidal materials varies greatly between different types of microorganisms. Disinfection resistance varies in the order of Gram-negative bacteria, Gram-positive bacteria. To interpret the effect of antimicrobial metal oxide semiconductor nanoparticles a number of mechanisms have been proposed. The antimicrobial and antioxidative activity of ZnO nanoparticles in suspension or applied on the fabric or other materials, is based on a number of mechanisms, most of which are known [7]: interruption membrane/cell wall transfer of electrons; the penetration of ions into the cell, which prevents DNA replication and affects the structure and permeability of cell membranes; generation of reactive oxygen species (ROS) [5-10]. The combination of ZnO with silver is advantageous for several reasons. On the one hand, silver is a well-known antibacterial material, furthermore it might improve the photocatalytic action of the ZnO. This report concerns the solvothermal preparation of zinc oxide and silver- doped zinc oxide nanoparticles for antimicrobial applications. This manuscript reports studies on Ag/ZnO nanoparticles with high antibacterial activities prepared by a chemical procedure successfully prepared. The resulting materials were investigated using electron microscopy (SEM), optical absorption (UV–VIS). Also, the antimicrobial activity of the as prepared materials was measured using the paper disc method.

2. Experimental details

2.1. Materials and microorganisms

Silver nitrate ($AgNO_3$), acetic acid (CH_3COOH), zinc oxide (ZnO) nanoparticles were purchased from Sigma–Aldrich and used as received.

Antimicrobial susceptibility was performed on isolated bacteria from urine culture Gram-negative bacteria, *Escherichia coli (E. coli)* and the Gram-positive bacteria, *Staphylococcus aureus (S. Aureus)* on Mueller-Hinton agar.

2.2. Synthesis

ZnO was dopped with different amounts of $AgNO_3$ (0.1, 5 and 15at%). The commercial nanoparticles are spherically shaped with a diameter smaller than 50 nm. Isopropanol was used as solvent for the preparation of all the solutions. An amount of 0.3 g ZnO nanoparticles was dispersed in 100mL isopropanol and 0.1 at%, 5 at% or 15 at% $AgNO_3$ was added in these ZnO dispersions under magnetic stirring for 2 h at room temperature in dark condition. The resulting nanocomposite precipitates were washed several times with ethanol and dried at 70°C.

2.3. Characterization of Ag/ZnO nanoparticles

The crystalline structures of the obtained nanoparticles were identified by X-ray diffraction patterns using a DRON-3 diffractometer system (Burevestnik, USSR) with CoKα radiation, λ=1.789 Å. SEM images were obtained with a Quanta 200 scanning electron microscope operating at 15kV and X-ray Energy Dispersive Spectrometer (EDS-FEI). Specimens were prepared by dispersing the samples by sonication in 2-propanol and by depositing a few drops of the suspensions on carbon-coated grids.

2.4. Antimicrobial testing

The antimicrobial activity of Ag/ZnO composites with respect to simple ZnO was investigated by using the paper disc method on Mueller-Hinton agar without blood against the Gram-negative bacteria, *Escherichia coli (E. coli)* and the Gram-positive bacteria, *Staphylococcus Aureus (S. Aureus)* on Mueller-Hinton agar with blood. For this, it has been used sterilized paper disc of 6 mm in diameter impregnated with 10 µl (5mg/1mL) solution of the composite. In each sterilized culture dish, 0.2mL fresh broth cultured for 24h was added followed by 20mL melted nutrient agar medium at approximately 50^{o}C. The dishes were then cooled down to room temperature before being ready for use. The samples were then gently pressed against the medium plate to have good contact with the inoculated agar, then turned flat. The samples stayed in a constant temperature incubator at 37 ± 1^{o}C for 24 h, before the inhibition zones were measured.

The method for antimicrobial testing was standardized by correlation of zone diameters with minimal inhibitory concentration determined in broth.

3. Results and discussions

3.1. XRD analysis

Figure 1 shows the X-ray diffraction patterns of the ZnO and Ag/ ZnO nanoparticles examined in the form of powder. Diffraction lines exhibited three additional peaks at 2θ = 37.3^o, 40.3^o and 42.6^o, which were assigned to the (1 0 0), (0 0 2), (1 0 1) planes of specifically hexagonal zinc oxide nanoparticles. No characteristic peaks of silver phases were observed in Figure 1, indicating that the samples are single crystalline phase. These data revealed the successful formation of Ag/ZnO.

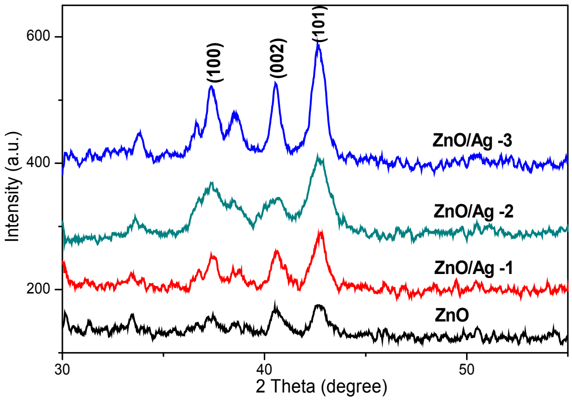

***Fig. 1.** XRD patterns for ZnO and Ag/ZnO nanoparticles with different concentration of Ag*

3.2. Surface morphologies

Figure 2 shows SEM images of undoped and ZnO nanoparticles doped with different concentrations of Ag (0.1, 5, 15 at%). These images indicate the existence of agglomerates. With the increase of dopant concentration, there is a decreased tendency of these agglomerations (Figure 2d). EDS spectra indicate the presence of the Ag dopant, with the other chemical elements from the composition of the samples (Zn and O). The peaks of carbon (C) and iron (Fe) come from the substrate of samples used to perform the SEM analysis. The obtained nanopaticles contain a smaller amount of silver as compared to the concentration of Ag^+ ions in solution. At the same time, the EDS results confirm the increase of the amount of silver in the obtained nanoparticles with the increasing concentration of the dopant in solution.

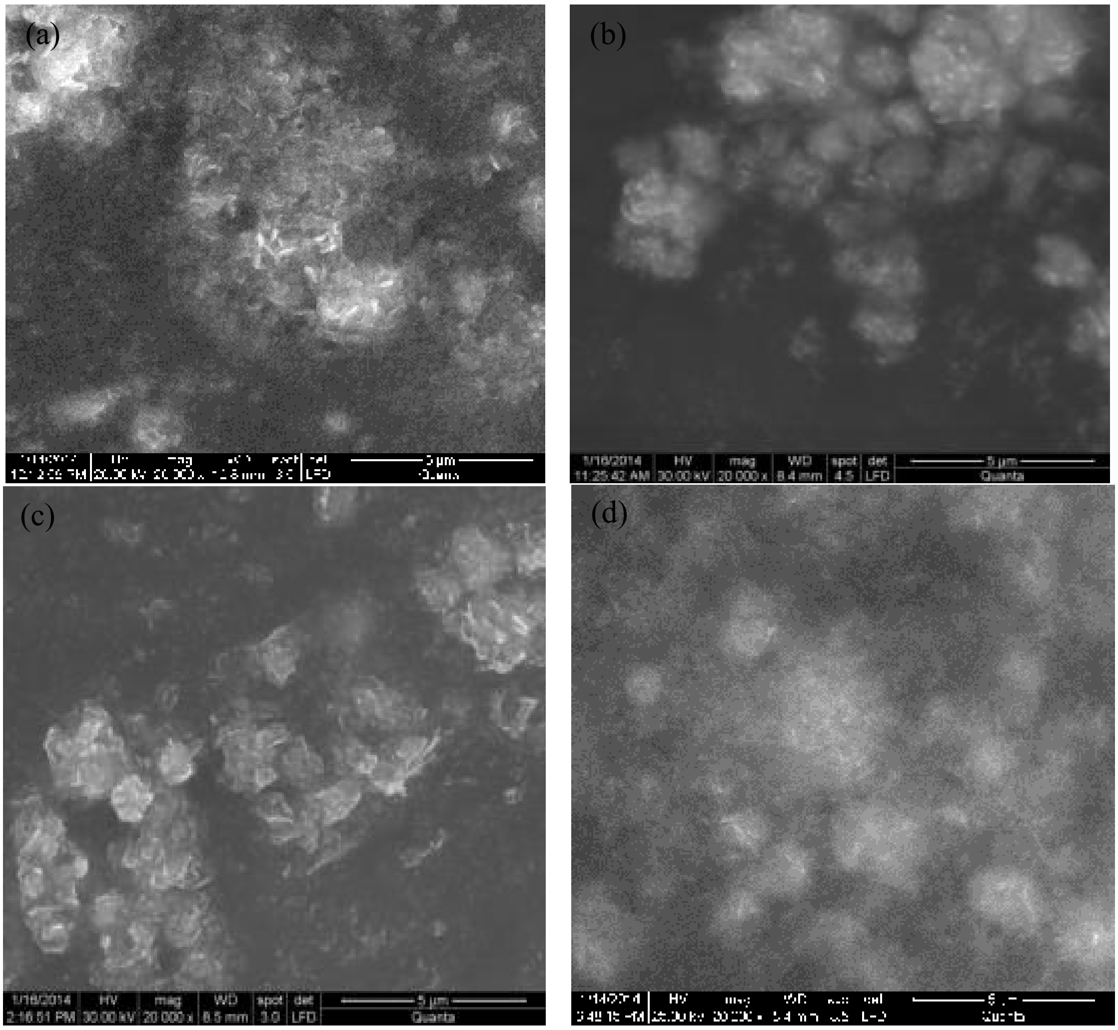

***Fig. 2.** SEM images for a) ZnO nanoparticles; b-d) Ag/ZnO nanoparticles with different concentration of Ag*

3.3. Antimicrobial activities

In Figure 4 it is presented the antimicrobial activity of ZnO (1) and Ag/ZnO (2-5) nanoparticles (6-9) tested by the disc and well diffusion agar methods. The presence of an inhibition zone clearly indicated the antibacterial effect of these nanoparticles. The size of inhibition zone was different according to the type of bacteria, and the concentrations of Ag doped ZnO nanoparticles.

According to the results, it can be concluded that ZnO nanoparticles are effective antibacterial agents both on Gram-positive and Gram-negative bacteria. The same results were confirmed in the study of Zhongbing *et al.* (2008) in which Gram-negative membrane and Gram-positive membrane disorganization was approved by transmission electron microscopy of bacteria ultrathin sections [11]. Makhluf *et al.* (2005) attributed the antibacterial behavior of MgO to several mechanisms, namely generation of reactive oxygen species (ROS), interruption of the membrane/cell wall by transfer of electrons; ion penetration into the cell, generated in ZnO using chemiluminescence residues, prevents the replication of DNA and affects the structure and permeability of the cell membrane [12].

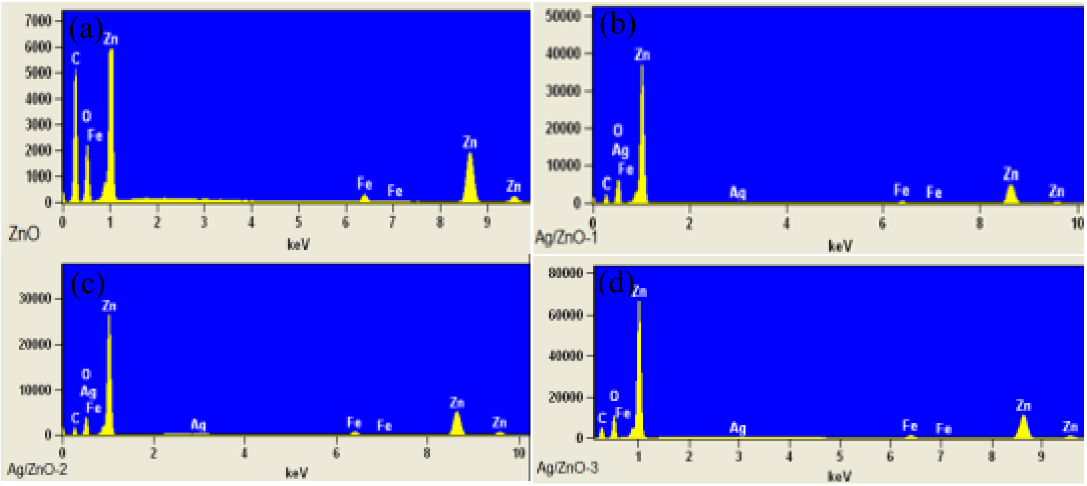

Fig. 3. *EDX spectrum for a) ZnO nanoparticles; b-d) Ag/ZnO nanoparticles with different concentration of Ag*

Sawai *et al.* (1996b) measured the active oxygen species, the H_2O_2 produced in ZnO residue and the concentration of H_2O_2 produced was directly proportional to the concentration of ZnO particles [13]. H_2O_2 was also detected by Yamamoto *et al.* (2004) [14]. Stoimenov *et al.* (2002) suggest that there could be electrostatic interactions between the involved area and bacteria [15].

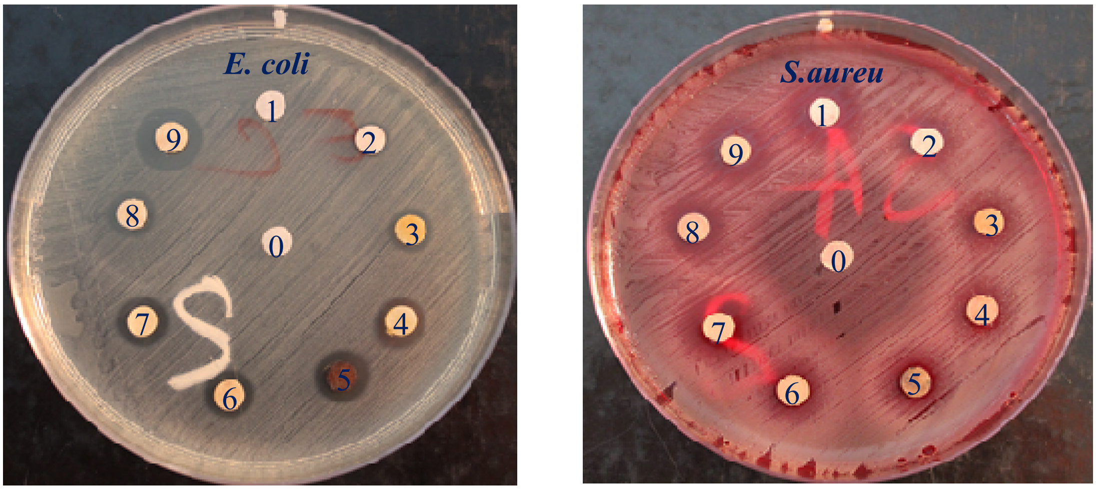

Fig. 4. *Zone of inhibition for ZnO (1), Ag/ZnO (commercial ZnO nanoparticles) (2-5), Ag:ZnO (ZnO nanoparticles obtained of synthesis) (6-9)*

Karunakaran C. *et al.* (2011) obtained Ag-doped ZnO nanoparticles with an average size of 50 nm, which leads to an increase in the contact surface and thus to an increase of their antimicrobial activity. The experimental evidence suggests that the dominant mechanisms of bacterial activity of ZnO is based on the production of reactive oxygen species (ROS - in particular in the presence of ultraviolet light), which chemically interact with the bacterial cell. [5-10]. Under light irradiation ZnO nanoparticles (Eg = 3.37 eV) generated electron–hole pairs. The hole (h^+) reacted with OH^- on the surface of NPs, generating hydroxyl radicals ($OH^•$), superoxide anion (O^{2-}) and perhydroxyl radicals ($HO_2^•$). These highly active free radicals damaged the cells of microorganism as a result of decomposition and complete destruction [16-17].

On the other hand silver ions disrupt the DNA replication and cell division. Both antimicrobial agents seem to compromise the integrity of bacterial membrane because of chemical interactions.

The results of the antimicrobial tests confirm that the optical and photo-catalytical behaviour of the hybrid metal (Ag)/semiconductor (ZnO) composite nanoparticles is consistent with the increase of the photo-catalytic activity of Ag/ZnO composite nanoparticle with respect to intrinsic ZnO nanoparticles [18]. This recommends Ag-doped ZnO nanoparticles composite for potential application in medical devices, food industry and textile fabrics.

4. Conclusions

ZnO and ZnO/Ag nanoparticles were prepared. The XRD measurement confirms that the sol-gel derived ZnO and silver-doped ZnO nanoparticles consist of Wurtzite-type nanocrystallites with different crystalline orientation, but (101) is the dominating peak. Increasing dopant concentration, respectively Ag, leads to a decrease in the size of nanoparticles that increase the specific surface area but also decrease the agglomeration.

Antimicrobial activity was recorded with respect to the increased amount of dopant.

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