Influence of size scale and morphology on antibacterial properties of ZnO nanoparticles

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ABSTRACT

Nanoparticle metal oxides represent a new class of important materials that are increasingly being developed for use in research and health-related applications. Although the *in vitro* antibacterial activity and efficacy of bulk zinc oxide material have been investigated, knowledge about the antibacterial activity of ZnO nanoparticles is very deficient. In this work we have synthesized ZnO nanoparticles with different size and morphologies using two different kinds of procedure: hydrothermal synthesis and mechanochemical processing. Characterization of the prepared nanopowders was preformed using a XRD, FE SEM, HR TEM technique and Malvern's Master Sizer instrument for particle size distribution. The antibacterial behavior of ZnO nanoparticles were tested to gram-negative and grm-positive bacterial cultures, *Escherihia coli (E. coli)* and *Staphylococus aureus (S. aureus)*. In all examined samples, ZnO nanoparticles demonstrated a significant bacteriostatic activity.

EXPERIMENTAL

Hydrothermal synthesis of ZnO nanoparticles

ZnO nanostructures were synthesized through a low temperature hydrothermal process using a zinc acetate dihydrate $Zn(CH_3\ COO)_2{\cdot}2H_2O$ and natrium hydroxide NaOH as precursors. In a typical procedure 0,5 M solution of NaOH was added drop-wise into a 0,2 mM water solution of $Zn(CH_3\ COO)_2{\cdot}2H_2O$ with 5wt% of PVP at 60 °C under constant stringing of 2000 rpm. About 1,5 l of as-prepared suspension was thermally treated in 2 l *Parr* stainless steel stirred reactor under non equilibrium condition up to 120 °C at a constant heating rate of 2 °C/min, under constant stirring of 400 rpm. Reaction time was 72 h. pH value of the reaction solution was 11 and maintained constant through hydrothermal process.

In the second sets of experiments instead of PVP as a stabilizing agent we used PVA in the same mass concentration of 5 wt%. All the other reaction parameters such as temperature, time of reaction, molar ratio of Zn^{2+}: OH^-=3:1 were retained the same. After hydrothermal treatment, the autoclave was quenched down to room temperature. The precipitate was washed with distilled water to remove all possible impurities.

Mechanochemical processing of ZnO nanoparticles

The mixture of $ZnCl_2$ and $H_2C_2O_4{\cdot}2H_2O$ powders with a molar ratio of $[HC_2O_4]^-$:Zn^{2+}=2:1 was sealed in an agate vial with alumina balls (∅ 8 mm). Milling was performed in a planetary ball mill Retsch PM4, using the ball-to-powder mass ratio 10:1. Mechanochemical process was carried out for 4 h at 180 rpm applying reversal mode of milling. The as-milled powder was then thermally treated at 450 °C in air for 1 h.

Indicator strains and culture conditions

The microorganisms used in this study were Gram-positive bacteria *Staphylococcus aureus* ATCC 25922 and Gram-negative bacteria *Escherichia coli* ATCC 25923. The growth of microorganisms for investigation was carried out in a Trypton Soy broth or agar, supplemented with 0.6% v/v yeast extract (TSYB or TSYA - Institute of Immunology and Virology, Torlak, Belgrade). A potassium hydrogen phosphate buffer solution (pH 7.0) was used as a liquid medium for quantitative testing of the antibacterial activity of the samples. For inoculums preparation, the microorganisms were cultivated in TSBY at 37 °C and left overnight (late exponential stage of growth). The obtained numbers of *S. aureus* and *E. coli* in inoculums were 2.1·107 CFU/mL and 4.1·107 CFU/mL, respectively.

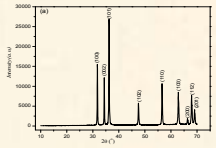

Fig 1. XRD patterns of ZnO powders

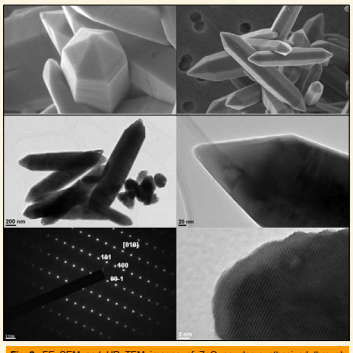

Fig 2. FE SEM and HR TEM images of ZnO powder synthesized through hydrothermal process in the presence of 5 wt% of PVP

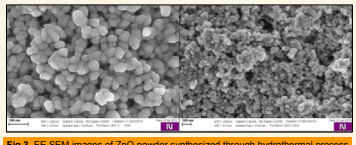

Fig 3. FE SEM images of ZnO powder synthesized through hydrothermal process in the presence of 5 wt% of PVA

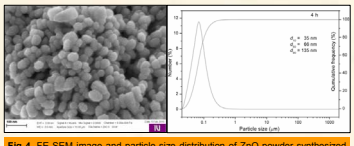

Fig 4. FE SEM image and particle size distribution of ZnO powder synthesized via mechanochemical processing in the presence of oxalic acid

RESULTS

The typical XRD pattern of the ZnO powder is shown in Fig. 1. All of the diffraction patterns can be indexed as a hexagonal wurtzite ZnO structure which are consistent with the values in the standard card (JCPDS 36-145). No diffraction patterns from any other impurities were detected, which confirms that the synthesized powder was pure ZnO hexagonal phase for all synthesized ZnO samples apart from synthesis procedure.

The morphologies and structural characterization of the ZnO samples synthesized using both, hydrothermal and mechanochemical processing are examined using FE SEM and HR TEM techniques. Morphology of the ZnO powders synthesized via hydrothermal process is presented in Fig 2. It is found that the ZnO powder crystals is composed of well-defined hexagonal prism rods with hexagonal like pyramid structures on each side of the rod. The most of the rods have quite uniform diameters of about 150 nm and lengths of 1-2 μm. The TEM, HRTEM and SAED Fig. 2 analysis was used to estimate the crystal structure, morphology and crystallites size of the ZnO synthesized by hydrothermal procedure. The TEM and HRTEM micrographs are reveal a single-crystal nature of the investigated sample.

Fig. 3 present FE SEM images of the ZnO powder synthesized hydrothermayl using a 5 wt% of PVA as a stabilizing agent. Morphology of the particles is entirely different form the ZnO sample hydrothermally synthesized in the presence of the same mass concentration of PVP. This fact can be explained with a different nature of the PVP and PVA stabilizing agents. As it can be seen form the Fig. 3 all of the particles are spherical in shape, uniform and well dispersed. Average particle diameter is around 30 nm.

Fig. 4 shows morphology of mechanochemicaly synthesized ZnO nanopowder using a oxalic acid as a stabilizing agent. All of the particles are uniform, spherically shaped, with average particle diameter around 60 nm. Agglomeration of the ZnO particles is not dominant

According to the results, all samples showed noticeably antibacterial activity. Sample B showed lowest percent of viable cells reduction for *Staphylococcus aureus*, but highest for *Escherichia coli*, after first hour of incubation. After second hour of incubation there are no detectable viable cells of both strains in presence of B sample. Samples A and C showed similar percent of reduction of viable cells for *S. aureus* after first and second hour of incubation, and for *E. coli*, after second hour of incubation.

Considering the results of antibacterial activity of ZnO powders depending on particle morphology no significant changes in the antibacterial activity were not obtained. These particulars can be explained with the fact that differences in particles dimensions and forms are not of critical importance for bacteriostatic properties. At larger differences in dimensions of ZnO particles for one or two order of magnitude it can be expected an important difference of antimicrobial activities of ZnO particles.

Bacteria Culture / Sample		*Staphylococcus aureus*				*Escherichia coli*			
		1 h		2h		1h		2h	
		CFU/ml	*R(%)*	CFU/ml	*R (%)*	CFU/ml	*R(%)*	CFU/ml	*R (%)*
Control	O	$2.1x10^5$	-	$1.2x10^5$	-	$4.1x10^5$	-	$2.4x10^5$	-
ZnO mechanochemical	A	$1.5x10^2$	***99.93***	$1.2x10^2$	***99,90***	$2.5x10^4$	***93.90***	no growth	***100.00***
ZnO hydrothermal PVA	B	$1.2x10^2$	***99.94***	$3.0x10^2$	***99,75***	$1.6x10^4$	***96.10***	$1.1x10^3$	***99.54***
ZnO hydrothermal PVP	C	$2.9x10^3$	***98.62***	no growth	***100.00***	$1.5x10^3$	***99.63***	no growth	***100.00***

Table 1. Results of antimicrobial test for ZnO nanopowders on *S. aureus* and *E. coli* bacterial cultures.

CONCLUSION

In this study we have demonstrated antibacterial activity of ZnO nanoparticles prepared using a hydrothermal and mechanochemically processing. One of the objectives of this work was to examine in which way particle size and different types of morphology will have an effect on bacteriostatic properties of ZnO particles. FE SEM and HR TEM analysis showed different morphology of sinthesized ZnO nanoparticles: hexagonal prisms with length of 1-2 μm and diameters around 150 nm, and spherically shaped particles with diameters around 30 and 60 nm.

Apart from different forms of prepared ZnO nanoparticles, antimicrobial tests showed impressive antibacterial properties, above 99 % microbial cells reduction, toward gram positive bacteria *S. Aureus* and gram negative bacteria *E. Colli*. No significant change in antibacterial activity depending on the morphology and size of ZnO nanoparticles was observed.