ANTIMICROBIAL PROPERTIES OF ZnO SUBMICROMETER PARTICLES

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Abstract

Zinc oxide is widely used in the manufacture of a vast number of consumer goods and industrial applications. The employ of nano and sub-micrometer zinc oxide materials has recently been introduced as a more economic and more effective alternative to bulk zinc oxide. Furthermore zinc oxide is being used as antibacterial material since metal oxides are recognized as possessing antibacterial properties and are regarded as safe materials to human beings and animals. The present work aims to present our study on the antibacterial properties of zinc oxide particles prepared by a polyol thermal method. Spherical submicrometer ZnO particles were prepared and their antibacterial properties were tested in aqueous suspensions of *E. coli* bacteria. For ZnO concentrations higher than 50 ppm a inhibition of the growth rate of bacteria higher than 95 % is achieved.

Keywords:

Zinc oxide, submicrometer particles, antibacterial, *E. Coli*.

1. INTRODUCTION

Zinc oxide is widely used in the manufacture of a vast number of consumer goods from rubber or paints to body lotions [1] among many others. Zinc oxide submicrometer and nanoparticles of different shapes and sized (the possibilities include among much others nanorods, nanospirals and nanoflowers [1]) have been introduced due to environmental and economic concerns related to the industrial use of zinc oxide

Although silver nanoparticles are the most used nanomaterials for antibacterial purposes, concerns on the use of silver nanomaterials are being raised more frequently [2] and therefore the search for alternative materials is of the upmost importance. Zinc oxide, and in general other metal oxides such as TiO_2, MgO and CaO, is interesting materials to use as antibacterial agents as they are not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals [3] and are less expensive than the silver nanoparticles based antibacterial products. Although a large number of methods can be found in the literature for the preparation of zinc oxide materials [1] the polyol thermal method [4] offers good solvents properties and wide operating-temperature range for preparing metallic oxide compouns. This operating temperature range may permit hydrous reactions that cannot be conducted in aqueous solution under atmospheric pressure.

This work aims to study the antibacterial properties of zinc oxide particles prepared by the polyol hydrothermal method using diethyleneglycol as solvent. The characteristics and stability of the nanoparticles will be followed by DLS and the antibacterial properties will be accessed by tests in aqueous suspensions of E. coli bacteria.

2. EXPERIMENTAL

All chemicals were reagent grade and used without further purification. Zinc acetate (sigma-aldrich), diethyleneglycol (Fluka) were used for the preparation of the submicrometer zinc oxide particles. Ultrapure water (Millipore, MilliQ) was used in the preparation of all aqueous solutions and in the cleaning of the glassware. Nurtrient Broth (NB) used was constituted by peptones (15 g/L), yeast extract (3 g/L), D(+)-glucose (1g/L) and sodium chloride (6 g/L) all reagents from Fluka.

ZnO nanoparticles were prepared by a polyol thermal method [4]. Briefly, 4 g of zinc acetate was dissolved in 200 mL of diethyleneglycol. The mixture was heated, with stirring, from 120 ºC to 180 ºC in a liquid paraffin bath. At 173 ºC the first sign of precipitation was observed and a color change was observed. After cooling to room temperature, the solids were separated from the supernatant by centrifugation and washed with alcohol several times. The powders were then dried in air at 60 °C.

Nanoparticles were characterized by dynamic light scattering (DLS) technique (AvidNano W130i) according to DLS standard operating procedure in quartz cuvette. Scanning Electron Microscopy – SEM- (FEI Quanta 400 FEG ESEM / EDAX Genesis X4M) was used to analyze the shape and size of zinc oxide particles.

The antibacterial activity of ZnO suspensions was evaluated against *E. coli* (LMG 8223). The concentration of *E. coli* (Gram negative) was adjusted by measuring the absorbance at 600 nm according to the procedure described in the literature [5]. In the present work, the concentration of bacteria was in the range of 1.5-3.0×10^8 CFU/mL. In 50 mL tubes, 10 mL of NB, 10 mL of ZnO test solution and 20 μL of bacteria inoculum were added. Then the tubes were placed at 37 ± 2 ºC for a 24 hour incubation period at the end of which the absorvance of the suspensions was measured at 600 nm. Water was used as a control of bacterial growth in absence of ZnO particles. A blank solution having ZnO particles suspension and in the absence of inoculum was also used in order to evaluate possible changes in the optical properties of the particles suspensions. The inhibition of bacterial growth was calculated relatively to the control. All tests were performed in duplicate.

3. RESULTS AND DISCUSSION

The size and stability of the zinc oxide submicrometer particles was evaluated by DLS preparing a aqueous solution of 1 mg ZnO submicrometer particles in 10 mL of purified water. Analysis of DLS data showed that the size of the ZnO particles were of around 200 nm.

Table 1 Evaluation of time stability of ZnO particles.

	Synthesis #1	Synthesis #2
as prepared	219 nm	212 nm
after 48h	238 nm	249 nm
after 5 days	283 nm	310 nm
after 7 days	295 nm	345 nm
after 10 days	331 nm	350 nm
after 1 month	433 nm	415 nm

The good reproducibility of the method can be verified by comparison of the size of the particles in two different syntheses and by the stability behavior of the particles prepared in those two batches.

The evaluation of the DLS data displays a small change in nanoparticles size with time showing that the aqueous dispersions of ZnO suffer slight agglomeration or the presence of a ripening effect. The use of ethanolic suspensions increases the stability of the particle dispersions.

In order to confirm DLS information and to obtain morphologic information SEM images of the ZnO particle were obtained and analyzed. Fig. 1 displays a SEM

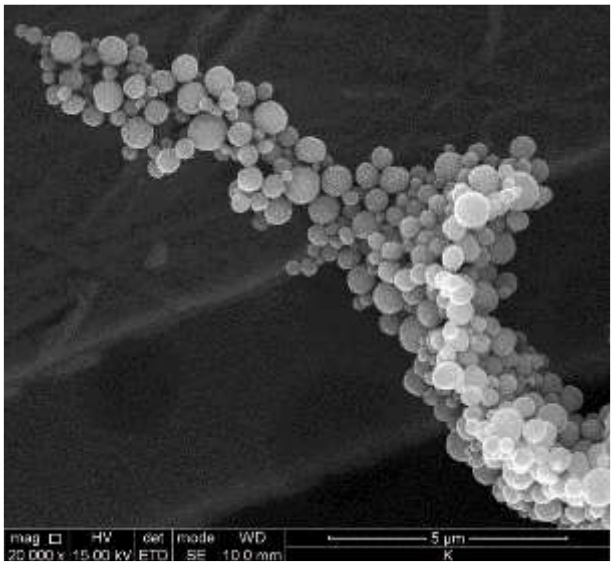

Fig. 1 SEM image of ZnO particles prepared by the diethylene glycol thermal method.

SEM images clearly show the existence of spherical ZnO particles however this image also reveals that the size distribution is not very narrow as can be observed by the analysis of the size frequency distribution plot that is represented in Fig. 2.

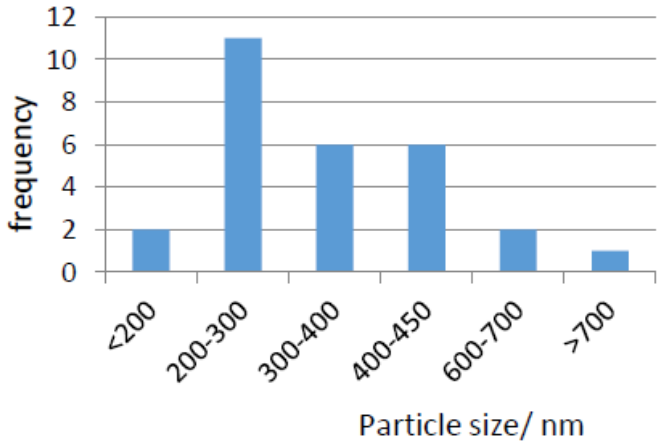

Fig. 2 Frequency distribution of the size of ZnO particles extracted from the analysis of SEM images.

In order to evaluate the effect of the ZnO submicrometer particles on the inhibition of growth in bacteria suspensions, different amounts of particles suspensions were put in contact with a constant amount of *E.*

Coli inoculum. After the incubation period the absorvance of the suspensions was measured at 600 nm and the inhibition rate was evaluated using the following equation 1.

$$Inhibition\ rate\ (\%) = \frac{A_{control} - A_{ZnO\ suspension}}{A_{control}} x100 \quad (1)$$

Plot in Fig. 3 shows the effect of increasing concentration of ZnO submicrometer particles in the inhibition of the bacteria growth rate. Concentration of ZnO suspensions was varied between 5 and 100 ppm.

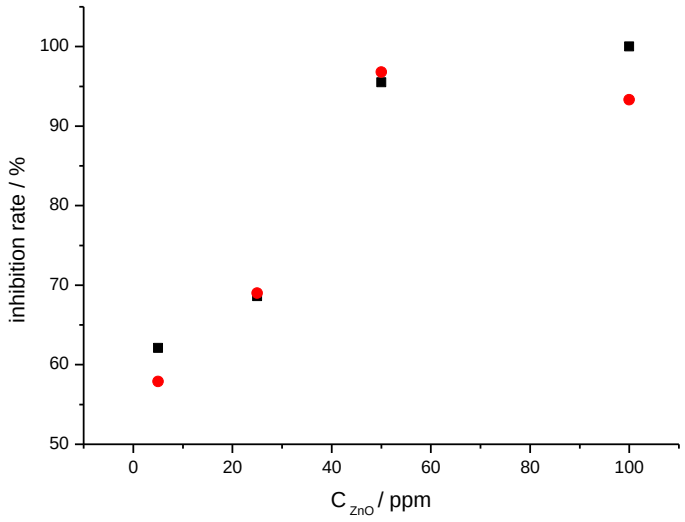

Fig. 3 *E. coli* growth inhibition rate in presence ofthe ZnO suspensions (red and black data -comparison of the results of the duplicate essays).

For the lowest ZnO concentration (5 ppm) a net reduction of bacteria population is observed however the rate of inhibition is of around 60 %. Increasing the concentration of ZnO suspensions an increase on the inhibition rate is observed reaching values of 95 % inhibition rate at a concentration level of 50 ppm.

4. CONCLUSION

Using diethylene glycol as solvent for the preparation of ZnO particles allowed the preparation of submicrometer spherical particles with size of the order of 200 nm. The method proved to be reproducible and the aqueous dispersions of ZnO particles present a limited change in size of the particles with time. DLS results were confirmed by the analysis of the SEM images of the ZnO particles prepared in this work.

Incubation of *E. coli* suspensions in the presence of different amount of ZnO particles shows a clear reduction of the growth rate of the bacteria. For suspensions with ZnO concentration higher than 50 ppm the inhibition rate is close to 100%.

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