IN VITRO ANTIBACTERIAL ACTIVITY OF ZnO NANOPARTICLES PREPARED USING SODIUM DODECYL SULFATE AS STABILIZING AGENT

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Abstract. ZnO nanoparticles (NPs) were synthesized by cost effective reverse micelle method by using Sodium dodecyl sulfate as stabilizing agent. The particles having different size in the range 40 to100 nanometer were prepared by varying the calcination temperature. The prepared particles were characterized by scanning electron microscopy Transmission electron microscopy. The antibacterial activities of ZnO nanoparticles were tested against gram positive strains, like *Staphylococcus aureus*, *Streptococcus haemolyticus* and *Bacillus cereus* and gram negative species (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi*, *Vibrio cholerae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes*) using the disc diffusion method. All samples showed antibacterial activity to at least 9 of the tested organisms. The present study reveals the effectiveness of ZnO nanoparticles as antibacterial agent both against gram positive and gram negative bacteria. It is found that the activity increases with the decrease in particle size.

Key words: ZnO nanoparticles, antibacterial activity, pathogenic bacteria, disc diffusion method.

INTRODUCTION

In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, the undesirable side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobial substitutions from various sources such as nanomaterials. Newly developed nanocomposites with bactericidal properties occupy considerable attention in recent years, not only

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because of their impact on human health and safety, but also because of the possibility of extended lifetime of materials used in everyday life. Many heavy metals and metal oxides either in their free state, or in compounds at very low concentrations, are toxic to microbes. These inorganic materials kill bacteria through various mechanisms, such as by binding to intracellular proteins and inactivating them, generation of reactive oxygen species and via directs damage to cell walls [1]. Because of their electronic structure, semiconductors such as titanium oxide (TiO_2) and zinc oxide (ZnO) have been applied to a variety of environmental processes such as remediation of organic contaminants and destruction of microorganisms [3, 7, 10, 14]. Nano ZnO can be used as an antibacterial agent. It is useful against both gram positive and gram negative bacteria. It can also act as an antifungal agent. Nano ZnO after making composites with polymers retains considerable amounts of antibacterial activity. Therefore we can make polymer products which have antibacterial activity. Nano ZnO has also applications in cosmetics. ZnO is a major component of calamine lotion. Nano ZnO which has particle size below 30 nm is transparent in nature. They can be used for preparing transparent sunscreens. Nano ZnO coated cotton fabrics have UV blocking property. It also improves the strength and air permeability of the fabric. The usage of pushpanjan, probably Zinc oxide, as a salve for eyes and open wounds, is mentioned in the Indian medical text the Charaka Samhita, thought to date from 500 BC or before. A ZnO nanoparticle is an active ingredient for dermatological applications in creams, lotions and ointments because of its antibacterial properties [14]. It is reported that the anticancer effects of ZnO nanoparticles on human brain tumor U87 and cervical cancer Hela also have promising activity, which varies with the changes in the structure and the size [5]. In the light of these, the present work was to investigate the antibacterial activity of zinc oxide nanoparticles with different size.

MATERIALS AND METHODS

SYNTHESIS OF ZnO NANOPARTICLES

The ZnO nanoparticles were prepared by reverse micelle method. In a typical experiment, first solution was prepared by dissolving 8.636 g $ZnSO_4$, 3.603 g CH_3COOH, and 40 mg SDS as surfactant in 1 dm^3 of water. The second solution was prepared by 0.09 M NaOH pellets and 25 mL 70% ethanol in dm^3 of water. Then the first solution was added to the second solution with continuous stirring. The obtained precipitate $Zn(OH)_2$ was filtered by using Whatman filter (grade-41) and air dried. The white solid product was washed with ethanol six times and with water ten times to remove impurities. Then dried precipitate was transferred to

silica crucible and ignited at 200 °C, 550 °C, 750 °C, and 900 °C for one hour. The obtained powders were characterized using TEM and SEM. Scanning electron micrographs of ZnO nanoparticles were obtained by (Philips XL 30). The samples were coated with gold prior to scanning. Transmission electron micrographs were taken in a JEOL JEM – 3010 TEM microscope at an accelerating voltage of 100 kV.

TEST BACTERIA

A total of eleven bacterial species were tested in the present study. The gram positive strains were *Staphylococcus aureus* (MTCC 7405), *Streptococcus haemolyticus* (clinical strain) and *Bacillus cereus* (MTCC 430) and gram negative species were *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 2405), *Proteus vulgaris* (MTCC 779), *Pseudomonas aeruginosa* (MTCC 2453), *Salmonella typhi* (MTCC 531), *Serratia marcescens* (MTCC 97), *Proteus rettgeri* (clinical strain), *Vibrio cholerae* (clinical strain) and *Enterobacter aerogenes* (MTCC111). The clinical strains were originally isolated from clinical materials collected from patients and identified using standard biochemical tests. The bacterial strains were maintained on nutrient agar slants at 4 °C.

CULTURE MEDIA AND INOCULUMS PREPARATION

Nutrient agar/broth (Himedia, India) was used as the bacterial culture medium in the bacterial assays. Loops full of all the bacterial cultures were inoculated in the 50 mL of sterile nutrient agar (NA) in 100 mL conical flask at 37 ºC for 72 hrs.

DISC DIFFUSION METHOD

The samples obtained were screened for their antibacterial activity *in vitro* by the disc diffusion method using various bacterial strains [6, 9]. The paper discs (6 mm diameter, Whatman No. 1 filter paper) containing 1.0, 2.0, 5.0, 10.0 mg/mL ZnO powder were placed aseptically on the agar surface with the help of a sterile forceps and paper discs were pressed slightly with the forceps to make complete contact with the surface of the medium. The plates were kept at room temperature for half an hour and subsequently incubated at 37 ºC and observed for zone of inhibition after 24 hours. The inhibition zone around each disc was measured in millimeters and the assay was carried out three times for each extract. The results were recorded by measuring the zone of growth inhibition surrounding the disc.

RESULTS AND DISCUSSION

SCANNING ELECTRON MICROSCOPY

The scanning electron micrographs of nanocrystalline ZnO powders are shown in Figure 1(a–c). As seen in the figure, the powder calcinated at 900 °C (sample B) has a hexagonal structure and the particles have sizes in the range of a few hundred nanometers. As we decrease the calcination temperature, it can be seen that the size decreases and the shape changes from hexagonal to almost spherical. At 550 °C (sample A) the size is around 40 nm and at 200 °C (sample C) the size is around 20 nm. But below 500 °C the material will not get converted into ZnO completely [8].

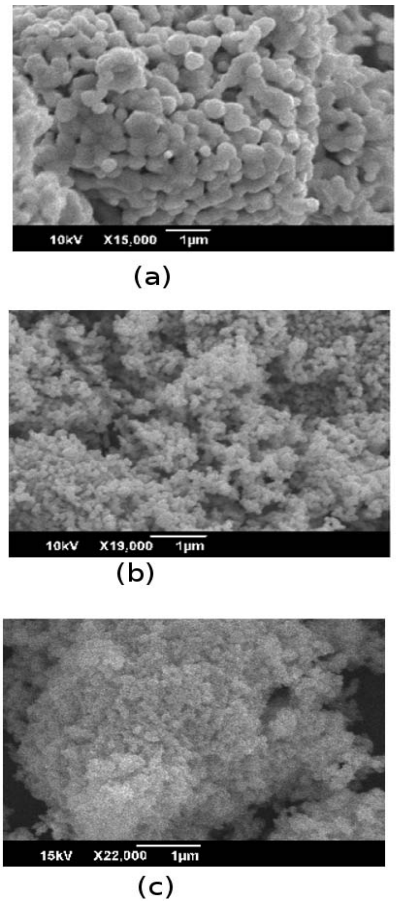

Fig. 1. SEM micrographs of nano ZnO: (a) at 900 °C; (b) at 550 °C; (c) at 200 °C.

TRANSMISSION ELECTRON MICROSCOPY

The formation of the metal oxide nanoparticles was proved by transmission electron microscopy. Figure 2 shows TE images recorded from drop coated films of the ZnO nanoparticles prepared by the reverse micelle method. The particles are mono disperse in nature with an average size of 40–60 nm. The nanoparticles are predominantly in spherical shape.

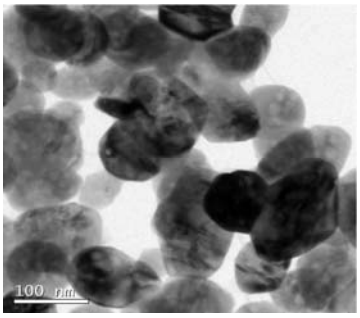

Fig. 2. TEM image of ZnO at 900 °C.

THE ANTIBACTERIAL STUDIES

The antibacterial activity of ZnO samples was studied against both gram positive (*Staphylococcus aureus*, *Streptococcus haemolyticus*, and *Bacillus cereus*) and gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi*, *Vibrio cholerae*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*). The results of antibacterial screening of ZnO nanoparticles are shown in Table 1. The result disclosed the inhibitory actions of samples. All samples showed antibacterial activity to at least 9 of the tested organisms. Among the various gram positive bacteria used, the sample A showed maximum activity (zone of inhibition 16 mm) against *Bacillus cereus* (Fig. 3(a)) whereas it showed moderate activity against *Streptococcus haemolyticus* (zone of inhibition 15 mm) and *Staphylococcus aureus* (Fig. 3(b)).

Similarly among 9 gram negative bacteria tested, again sample C showed maximum activity (zone of inhibition 17 mm) against *Enterobacter aerogenes* and *Salmonella typhi* (Fig. 3(c)). Sample A has appeared to be the most effective sample. In the case of gram negative bacteria *Klebsiella pneumoniae*, sample B exhibited more inhibition of 10 mm. Sample A has particle sizes in the range 40 nm, while sample B has particle sizes in the range 100–150 nm. The difference in size is the reason for the difference in activity of the samples. Sample C even though has smaller particle size than A and B, it was not converted completely into ZnO from $Zn(OH)_2$. This was the reason why sample C shows lower activity compared to other samples. In the case of nano ZnO there exists an inverse relationship between particle size and antibacterial activity.

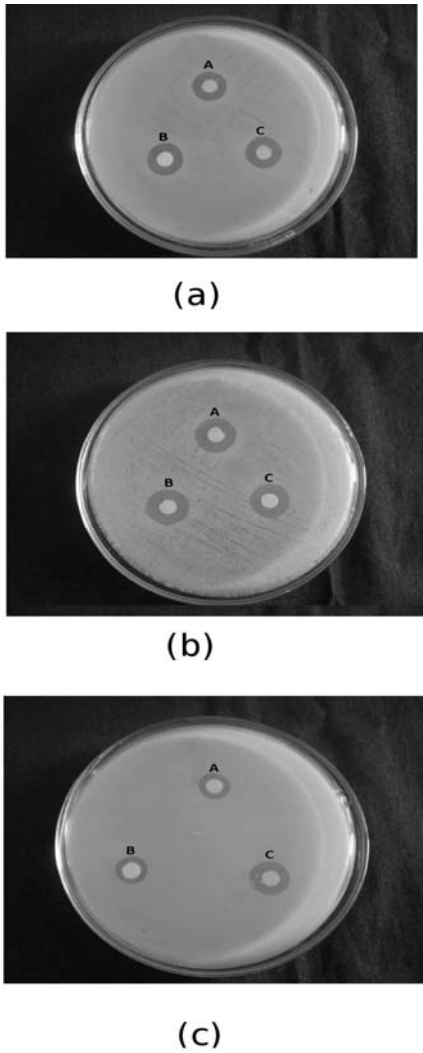

Fig. 3.: Shows the enhancing of antibacterial activity with corresponding decrease in the particle sixe. (a). Antibacterial activity showing against *Bacillus cereus* of nano ZnO at 900 °C (A), at 550 °C (B), at 200 °C (C). (b) *Staphylococcus aureus* of nano ZnO at 900 °C (A), at 550 °C (B), at 200 °C (C). (c) *Salmonella typhi* of nano ZnO at 900 °C (A), at 550 °C (B), at 200 °C (C).

The present study demonstrated that ZnO samples have an antibacterial effect on three gram positive and 9 gram negative bacteria. ZnO appears to strongly resist microorganisms. There are some reports on the considerable antibacterial activity of CaO, MgO and ZnO, which is attributed to the generation of reactive O_2 species on the surface of the oxides, studied by a conductometric method. The nanostructures of ZnO have increased the antibacterial effect.

Table 1

Antibacterial effect of nano ZnO (A) at 900 °C, (B) at 550 °C and (C) at 200 °C

Bacteria	Zone of inhibition of materials (mm)		
	A	B	C
Bacillus cereus	16	13	12
Staphylococcus aureus	13	12	10
Streptococcus haemolyticus	15	13	11
Enterobacter aerogenes	15	14	17
Salmonella typhi	13	15	17
Pseudomonas aeruginosa	14	13	11
Vibrio cholerae	13	11	10
Proteus vulgaris	14	12	9
Escherichia coli	15	12	10
Klebsiella pneumoniae	9.1	10	7.3
Serratia marcesens	16	14	13

Particles exhibit both qualitatively and quantitatively better antibacterial properties than bulk ZnO with a particle size of 2 μm. The results clearly demonstrate that nanosized ZnO is a more effective antimicrobial agent than bulk ZnO. The presence of an inhibition zone clearly indicates that the mechanism of the biocidal action of ZnO involves disrupting the membrane. The high rate of generation of surface oxygen species from ZnO leads to the death of the bacteria [2]. Interestingly, the size of the inhibition zone increased significantly with decreasing size of the ZnO particles. It is believed that cell death is caused by the decomposition of the cell wall followed by the subsequent decomposition of the cell membrane. The damage to the cell membrane leads directly to the leakage of minerals, proteins and genetic materials causing cell death. The results of this study may be applicable to medical devices that are coated with nanoparticles against microbes. The enhanced bioactivity of smaller particle is attributed to the higher surface area to volume ratio. ZnO nanoparticles were found to be more abrasive than bulk ZnO and thus contribute to the greater mechanical damage of the cell membrane and the enhanced bactericidal effect of ZnO nanoparticles. ZnO nanoparticles can enhance the antibacterial activity of antibiotics. The previous reports demonstrated that the generation of H_2O_2 from ZnO leads to the penetration of particles into the cell membrane of bacteria, to the formation of injuries and finally the death of bacterium has occurred [15]. The antimicrobial effect of ZnO nanoparticles against food borne pathogen may lead to the proficient application in food packaging and preservation process [12]. The ability of the antimicrobial agent to rupture bacterial cells is tested by the disk diffusion method. The presence of an inhibition zone clearly indicates that the mechanism of the biocidal action of ZnO involves disrupting the membrane. A rapid and uncontrolled multiplication of pathogenic microbes can seriously compromise health and hygienic living standards. Antimicrobial garments may also be useful for individuals coming into

contact with patients, such as visitors, nurses, doctors and other health care workers. Toxicity studies have shown that zinc ions do not cause any damage to the DNA of human cells [4, 11, 13]. They showed that on contact with bacteria, the cytotoxic behavior of ZnO nanoparticles ruptures the lipid bilayer of bacterium resulting in leakage of cytoplasmic contents. The antimicrobial properties of cotton fabrics finished with ZnO nanoparticles against a variety of bacterial strains.

CONCLUSION

In conclusion, ZnO nanoparticles exhibit the antibacterial activity and this antibacterial activity increases with the decreasing size.

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