CHAPTER 6

Antibacterial Behavior of ZnO Nanostructures
6.1 Introduction

Nanotechnology has attracted global attention because nanoparticles have properties unique from their bulk equivalents. Nano-sized particles of ZnO have more pronounced antimicrobial activities than large particles, since the small size (less than 100 nm) and high surface-to-volume ratio of nanoparticles allow for better interaction with bacteria. Recent studies have shown that these nanoparticles have selective toxicity to bacteria but exhibit minimal effects on human cells. Nanoparticles of Ag, CuO and ZnO are being used industrially for several purposes including amendments to textiles, cosmetics, sprays, plastics and paints [1]. A common feature of these three nanoparticles is their antimicrobial activity [2-8]. The antimicrobial activity of nanoparticles largely has been studied with human pathogenic bacteria, mainly *Escherichia coli* and *Staphylococcus aureus*. Nanoparticles of Ag, CuO and ZnO are reported to attack bacterial membranes. Short exposure of *Escherichia coli* cells to nano-Ag destabilizes the outer membrane, collapses the plasma membrane potential and decreases Adenosine triphosphate (ATP) [9]. Exposure of *Escherichia coli* to nano-ZnO also causes loss in membrane integrity [6]. Toxicity of nanoparticles of CuO and ZnO are connected with cell membrane damage [10]. Nanoparticles action may be due in part to their release of free ions. Heavy metal ions have diverse effects on bacteria cell function. Zn also is an essential element for cells; levels of Zn above the essential threshold level inhibit bacterial enzymes including dehydrogenase [11]. Additionally, loss of membrane potential is associated with inhibition by Zn ions at cytochrome c oxidase in Rhodobacter sphaeroides. The aim of this study [12] was to evaluate the antimicrobial activity of nano-Ag, nano-CuO and nano-ZnO using a biosensor constructed in Pseudomonas putida KT2440. This pseudomonad is beneficial in the environment because of its bioremediation potential and it is a strong root colonizer [13-15]. The biosensor was constructed to emit light from luxAB genes under the control of a promoter containing a single heavy metal binding domain. Because the luciferase encoded by luxAB requires Flavin mononucleotide (FMNH₂) as a substrate, expression from this promoter permits light output dependent on the energy status of the cells [16]. Size and, thus, aggregation of nanoparticles are important in nanotoxicity. For nano-ZnO, particles of 8 nm in size were more toxic to *S. aureus* than those that were reported to be larger (50-70 nm).
Antibacterial agents are of relevance to a number of industrial sectors including environmental, food, synthetic textiles, packaging, healthcare, medical care, as well as construction and decoration. The studies investigated the antibacterial activity of ZnO particles against *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, and *Staphylococcus aureus* etc. and the main conclusions of these studies can be summarized as:

- ZnO particles are effective for inhibiting both Gram-positive and Gram-negative bacteria. They even have antibacterial activity against spores that are high-temperature and high pressure resistant [17-20].

- Smaller ZnO particles have a better antibacterial activity [21,22].

- The antibacterial activity depends on the surface area and concentration, while the crystalline structure and particle shape have little effect. The higher the concentration and the larger the surface area, the better the antibacterial activity [23].

- High temperature treatment of ZnO particles has a significant effect on their antibacterial activity. Treatment at a higher temperature leads to a lower activity [19].

- The mechanisms of the antibacterial activity of ZnO particles are not well understood although [24,25] proposed that the generation of hydrogen peroxide be a main factor of the antibacterial activity, while [26] indicated that the binding of the particles on the bacteria surface due to the electrostatic forces could be a mechanism.

- However, a few studies have suggested that the primary cause of the antibacterial function might be from the disruption of cell membrane activity [27]. Another possibility could be the induction of intercellular reactive oxygen species, including hydrogen peroxide (H$_2$O$_2$), a strong oxidizing agent harmful to bacterial cells [28,29]. It has also been reported that ZnO can be activated by UV and visible light to generate highly reactive oxygen species such as OH$^-$, H$_2$O$_2$, and O$_2$.$^2$. 

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178
In this chapter we report study on antibacterial behavior of ZnO nanostructures [undoped and Mn doped (with Mn content: 5 mol%, 10 mol% and 15 mol%) ZnO nanorods/nanoparticles] against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcese*us, *Proteus vulgaris*.

### 6.2 Bacterial Cultures and Evaluation of Antibacterial Activities

#### 6.2.1 Master Solution

To make dispersed undoped and Mn doped (with Mn content: 5 mol%, 10 mol% and 15 mol%) ZnO nanorods/nanoparticles for the antibacterial test, we took a preset amount of dry undoped and Mn doped ZnO nanorods/nanoparticles mixed with distilled water in a glass beaker separately with the aid of stirrer. Once the samples were dispersed in water, the beaker was placed in an ultrasonicator. The reason for the use of sonication was to breakdown the agglomerate. After 30 minutes of sonication the so called dispersed undoped and Mn doped ZnO nanorods/nanoparticles were produced which had concentration of 5 mg/ml. Similar procedure was followed to obtain disperse undoped and Mn doped ZnO nanorods/nanoparticles in methanol medium.

#### 6.2.2 Culture and Inoculums

Standard bacterial cultures *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcese*us, *Proteus vulgaris* were procured as from B. R. D school of Biosciences, S. P. University, Gujarat.

The bacterial cultures were streaked to check the purity and a single colony was inoculated in 5 ml Luria Bertani broth, incubated overnight at 30°C, 150 rpm. We determined the optical densities of the actively growing cultures by measuring absorbance at 600 nm.
6.2.3 Agar Diffusion Method

The anti-bacterial/microbial activity was studied using gel diffusion method [30]. 100 μl of actively growing target culture was affixed with 5 ml of 1% top agar vortexed and pour onto the agar plate, allowed to solidify. The solidified plate was bored to form 5 wells of 4 mm diameter using cork borer. The wells were then inoculated with 100 μl aliquots of 5 mg/ml dispersed undoped and Mn doped ZnO nanorods/nanoparticles. The nanorods/nanoparticles were allowed to diffuse into the agar followed by incubating the plates at 30°C for 48 hrs. Upon incubation the zone of clearance around the wells were measured with ruler and evaluated with respect to water/methanol media respectively.

6.3 Results and Discussion

6.3.1 Evaluation of Antibacterial Properties

The figures 6.1 to figures 6.6 shows agar diffusion test of undoped and Mn doped ZnO nanorods/nanoparticles (with Mn content: 5 mol%, 10 mol% and 15 mol%) dispersed in water and methanol respectively and are showing the inhibition zone around the cavity.
Figure 6.1 (a) Evaluation of antibacterial action for *Staphylococcus aureus* dispersed in water medium

Figure 6.1 (b) Evaluation of antibacterial action for *Staphylococcus aureus* dispersed in methanol medium
Figure 6.2 (a) Evaluation of antibacterial action for *Escherichia coli* dispersed in water medium

Figure 6.2 (b) Evaluation of antibacterial action for *Escherichia coli* dispersed in methanol medium
Figure 6.3 (a) Evaluation of antibacterial action for *Bacillus subtilis* dispersed in water medium

Figure 6.3 (b) Evaluation of antibacterial action for *Bacillus subtilis* dispersed in methanol medium
Figure 6.4 (a) Evaluation of antibacterial action for *Pseudomonas aeruginosa* dispersed in water medium

Figure 6.4 (b) Evaluation of antibacterial action for *Pseudomonas aeruginosa* dispersed in methanol medium
Figure 6.5 (a) Evaluation of antibacterial action for *Serratia marcescens* dispersed in water medium

Figure 6.5 (b) Evaluation of antibacterial action for *Serratia marcescens* dispersed in methanol medium
Figure 6.6 (a) Evaluation of antibacterial action for *Proteus vulgaris* dispersed in water medium

Figure 6.6 (b) Evaluation of antibacterial action for *Proteus vulgaris* dispersed in methanol medium
The results of the quantitative antibacterial assessment by agar diffusion is shown in Table 6.1 and it is observed that the size of the inhibition zone is more significant for all samples dispersed in methanol medium than the water medium therefore we can conclude that the antibacterial activity by the test microorganism are more dominating in samples dispersed in methanol. No antibacterial activity was found against the *Escherichia coli* for the samples dispersed in water medium. The
Bacillus subtilis exhibits the strongest antibacterial activity in both media compared to the other test microorganisms. The reason for the difference in the antibacterial activity for different test microorganisms may be due to the difference in structure and thickness of the membrane cell wall. The antibacterial activity depends on the surface area and concentration, while the crystalline structure and particle shape have little effect. The presence of an inhibition zone clearly indicates that the mechanism of the biocidal action of ZnO involves disrupting the membrane. We could also observe that doping of the ZnO nanoparticles does not result in appreciable enhancement of the activity.
6.4 Conclusion

Antibacterial potential of both ZnO nanoparticles as a function of water and methanol media were tested against six different bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Proteus vulgaris*. The test was performed by Agar diffusion method. From the study, both types of nanostructures (i.e nanorods and nanoparticles) were observed to have strong antibacterial potential against *Bacillus subtilis* and weak antibacterial potential against *Escherichia coli*. Doping of Mn in ZnO nanoparticles did not result in appreciable enhancement of the antibacterial activity.
6.5 References


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