CHAPTER 5

APPLICATIONS OF ZINC OXIDE NANOSTRUCTURES

(ZnO-Ns)

5.1 Introduction

The nanoparticle synthesis of controlled size, its distribution, shape and surface state is recognized to be of prime importance as their properties are essential for successful application. Among all nanomaterials, nanoparticles of metal oxides are very attractive as their unique characteristics make them the most diverse class of materials with properties covering almost all aspects of solid-state physics, materials science and catalysis. Indeed, the crystal chemistry of metal oxides, i.e. the nature of the bonding, varies from highly ionic to covalent or metallic.

Nanoscale Zinc oxide \(^1\text{-}^6\) is an inorganic compound appearing invisible when added to other materials like ointments and fungal powders. Its transition from micro to nano form increases the material surface and hence improves different properties. Zinc Oxide have found a good citation since few decades and found a broad spectrum for applications as cold, rashes, sunscreen lotions; in paints and coatings; in LED’s, transparent thin film coatings and various others, because of their various characteristic properties
evolved at nano stage. A colloidal system (solid, liquid or gas) consists of two different phases namely a dispersed phase (or internal phase) with particle diameter of 5 – 200 nm and a continuous phase (or dispersion phase). Colloidal science plays a vital role in the field of biological science along with our environment. The Nanoscale Zinc Oxide colloidal science has opened an important paradigm for study as far as its anti-bacterial, anti-fungal, photo-catalytic activity and UV filtering properties are concerned. The anti-microbial effect and photo-catalytic degradation by Zinc oxide nanostructures have been presented below:

5.2 Anti-microbial Activity of ZnO Nanostructures (Ns)

5.2.1 Introduction

Due to diversified uses of Metal Oxide Nanoparticles (MONPs) in research, industrial and health related applications, metal oxide nanoparticles are increasingly being developed through inexpensive and user friendly approaches. Hence, in other words, the effect of MONPs with the pathogenic microbes is an evolving field of research. Out of these, Zinc oxide Nanoparticles (ZnO-Nps) are useful as antimicrobial agents against microbes of therapeutic significance when blended with medicines, ointments and personal care products. Due to specific compatibility towards both aqueous and organic solvents, ZnO-Nps are allowing for incorporation into most material processes, therefore, ZnO-Nps are also considered as bacteriostatic and fungistatic against microbes of industrial importance when included into materials, such as surface coatings (paints), wallpapers, textile fibers, plastics and ceramics. The advertisements seen by us for antibacterial refrigerators and bathroom
tiles are some of the best examples. There are several examples of highly significant antimicrobial activity of ZnO-Nps against medically and industrially important Gram-positive and Gram-negative bacteria, ascomycetes and protozoans 7-20.

The antimicrobial potentiality of ZnO-Nps against different disease causing pathogenic microbial strains has been already evaluated using qualitative and quantitative assays. The applications of ZnO-Nps for Staphylococcus aureus (cellulitis) 33 [Seil et al., 2011], Salmonella typhimurium (typhoid fever) 22 [Kumar et al., 2011], Candida albicans (candidiasis) 17 [Lipovsky et al., 2011], Listeria monocytogenes (septicaemia) 30 [Arabi et al., 2012], Campylobacter jejuni (Guillain-Barré syndrome) 25 [Xie et al., 2011] and Pseudomonas aeruginosa (bacteremia) 21 [Feris et al., 2010] are well documented. Besides, ZnO-Nps could potentially be used as an effective antimicrobial agent to protect agricultural and food safety from foodborne pathogens especially Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens 31 [Jiang et al., 2009], Salmonella enteritidis 32 [Jin et al., 2009] and Botrytis cinerea, Penicillium expansum 19 [He et al., 2011] by disintegrating the cell membrane and increasing the membrane permeability and oxidative stress to lyse these food-borne bacteria 25,32 [Jin et al., 2009; Xie et al., 2011]. These points suggested that the application of ZnO-Nps as antimicrobial agents in medicine and food systems may be effective for microbial growth inhibition.

Plausibly, the antibacterial activity of ZnO-Nps is dependent on its size, morphology and architecture of nanoparticulate 34
[Yamamoto, 2001]. The enhanced surface area of ZnO-Nps allows for increased interaction with microbes which permits using a smaller amount of ZnO-Nps for the same or improved biostatic behavior. Moreover, the microbicidal property of these nanoparticles suggested that these particles were more diffusible in the growth medium which in turn allowed greater interaction between microbial cells and nanoparticle. From the previous reports, it is considered that ZnO-Nps-cell surface interaction affects the cell morphology as well as cell membrane permeability, consequently the entry of ZnO-Nps induces oxidative stress in bacterial cells resulting in the inhibition of cell growth and eventually cell death.

All of the above information regarding ZnO-Nps suggests that these nanoparticulates have a potential application as a pathogen growth inhibitor and may have future applications in the development of derivative agents to control the spread and infection of a variety of microbial strains.

From the literature, the studies revealed that the nanoscaled ZnO particles were more toxic to all bacterial species than other nanoparticles of different inorganic oxides like aluminium oxide, silicon dioxide, etc.\textsuperscript{21-30}

The Gram +ve bacteria, \textit{Bacillus subtilis} is a rod shaped bacteria which is commonly known as normal gut commensal in humans. Due to the emergence of anti-biotic resistant drugs, alternate medications are under primary considerations. A noteworthy experimentation was concerned with anti-bacterial activity of therapeutically viable Gram +ve bacteria, \textit{Bacillus subtilis} and it was found that reported ZnO-NBs have become the
promising entities for terminating the growth of these bacterias. The anti-bacterial activity of synthesized ZnO-NBs was tested against Gram +ve bacteria, *B. subtilis* (MTCC 121). Though *B. subtilis* is not as much harmful as other pathogenic bacterias, but it is an easy model for doing the clinical studies and checking the anti-bacterial activity as the mechanism for attacking on the Gram +ve bacterial colonies are generally same $^{31-42}$.

5.2.2 Anti-bacterial activity of ZnO-NBs

The anti-microbial activities were studied by Disc diffusion method with the help of nutrient agar along with suspended microbes, by dipping the pad into the culture. After streaking the agar plate, these are incubated for 24 hrs. Antibiotic discs were kept on agar surfaces and the anti-bacterial activities were noted from the inhibition zones (IZ) results and finally the calculation of diameters of IZ of stains were carried out. The ZnO Nano-bunches (ZnO-NBs) fabricated at different temperature ranges were taken and their colloidal suspensions were tested for their activity against *Bacillus subtilis*. For this purpose the filter paper disc technique was exploited. Prior to this, the ZnO-NBs were attempted with bactericidal activity by disc diffusion method for *Bacillus subtilis*. The cultures used were kept over night at 37 °C on nutrient agar. On preceding the process, the cultures were centrifuged at moderate rpm range and the pellets were diluted in NSS to get the count more than 100 cfu/ ml. The bacterial culture suspension was extended on the plates of Nutrient agar homogeneously and 8 mm discs of Hi-media Pvt Ltd make were
Figure 5.1  Antimicrobial activities of ZnO-NBs prepared at different temperature ranges against Gram-positive (*Bacillus subtilis*)

made sterile for ZnO-NBs. These plates were further kept at 37 °C for another day. Standard anti-biotic discs of 0.03 mg each and doxycycline were utilized and ZnO-NBs media of similar concentrations were set as control. Later the resulting inhibition zones (IZ), in mm of bacterial growth were calculated for the determination of anti-bacterial activities.

The influence of metal oxide nano-particulates (MO-Nps) with the pathogenic bacterias is an evolving area for research.
Consequently, after fruitful characterization results, the peanut shaped ZnO nano-bunches fabricated at different temperature range were tested for their bactericidal performances. The different proportions of ZnO nano-bunches exhibited significantly anti-bacterial activity against the Gram +ve bacteria, *Bacillus subtilis*, responsible for producing the heat stable toxin amylopsin related to food borne illness. The concentration dependent anti-bacterial activity shown in terms of zone of inhibition was visible on NA plates (Figure 5.1). At highest concentration, the growth of bacterial strains, *Bacillus subtilis* was stop down appreciably (Table 5.1).

Table 5.1  Anti-bacteril activity of Peanut-shaped ZnO-NBs prepared at different temperature ranges; TEMP-I and TEMP-II, against *B. subtilis* (MTCC 121)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>Zone of Inhibition (mm) <em>B. subtilis</em> (MTCC 121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut-shaped ZnO-NBs (40±3°C) TEMP-I</td>
<td>250</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>20</td>
</tr>
<tr>
<td>Peanut-shaped ZnO-NBs (60±3°C) TEMP-II</td>
<td>250</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>22</td>
</tr>
<tr>
<td>ZnO Powder</td>
<td>250</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>17</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>30 µg/disc</td>
<td>33</td>
</tr>
</tbody>
</table>
At 500 µg/ml of Peanut shaped ZnO nano-bunches, maximum zone of inhibition (IZ) i.e. 22 mm was recorded for *B. subtilis* (MTCC 121) by ZnO-NBs fabricated at TEMP-II. After that the maximum zone of inhibition (IZ) of about 20 mm was recorded for *B. subtilis* (MTCC 121) by Peanut-shaped ZnO nano-bunches fabricated at TEMP-I. The initial readings also postulate that the anti-bacterial performances of ZnO-NBs may be dependent of size & morphology of peanut-shaped ZnO-NBs.

The bactericidal properties of these peanut shaped ZnO-NBs recommend that these nano-bunches were more diffusible in the growth culture medium which allows the superior interferences among bacterial cells and that of nano-bunches. Similar anti-bactericidal impacts of some other ZnO nano-particles fabricated from other techniques are also known. The stains of Gram+ bacteria, *Bacillus subtilis*, were most vulnerable to the ZnO nano-particulates. This might be due to presences of some proteins and polysaccharides on surface of cell wall of the bacteria. Therefore the Peanut-shaped ZnO-NBs action on *B. subtilis* changes the cell wall morphology as well as its permeability, ultimately leading to the bacterial growth by its death.

### 5.2.3 Conclusion

In addition to all, the synthesized peanut-shaped ZnO-NBs also showed significant antimicrobial activity suggesting that peanut-shaped ZnO-NBs can be better agents to control the spreading of bacterial infections. Though *B. subtilis* is not as much harmful as other pathogenic bacterias, but it is an easy model for doing the clinical studies and checking the anti-bacterial activity, as the mechanism
for attacking on the Gram +ve bacterial colonies are same. Therefore, we can postulate that our synthesized peanut-shaped ZnO-NBs may be externally used in a variety of therapeutic formulations as it is treated for potential interventions in extrinsic skin problems like aging, sunburn, etc.

5.3  **Photo-catalytic Activity of ZnO Nanostructures (Ns)**

5.3.1  **Introduction**

With the emergence of industrialization, technological advancements and different techniques used for agricultural productivity may directly or indirectly leading to cause harmful results in the environment. The different types of dangerous compounds eluting from the drugs, dyes, surfactants (used in shampoos, rinses, etc.), pharmaceutical and many other chemical/gas industries; insecticide, fungicides, herbicides, etc from agri-practices; all may resulting into the huge contamination of water bodies, soil, air, etc. Hence, for the sake of mankind, flora and fauna, it is necessary that all the harmful and poisonous contaminants going into different water bodies (viz. surface water, underground water, etc), could be checked and sound initiatives should be taken to purify the different water resources, used for the betterment of life. *Photocatalysis* means the use of photon energy in the catalytic reactions (like photosynthesis in plants). Since few years, photo-catalytic processes involving semiconductor ZnO Nanostructures under UV light illumination have been shown to be potentially beneficial and helpful in the treatment of various hazardous pollutants. Different studies have proved this with different pollutants like dyes, drugs,
surfactants, pesticides, herbicides, insecticides and fungicides that can be completely mineralized in the presence of ZnO Nanostructures.

5.3.2 Mechanism

As far as the mechanism of photo-catalytic degradation is concerned, it is comparable with the mechanism of chlorophyll in photosynthesis. On the exposure of light/photon energy (where $h\nu > 3.2$ eV) on the ZnO Nanostructures; free electrons ($e^-$) find its position at Conduction state, leaving behind holes ($h^+$) at the Valence state. These further leads to the oxidation-reduction reactions between the pollutants and ZnO Nanostructures, both present in the water solution and hence the photo-catalysis of pollutants (viz. drug, dye, surfactant, etc) occurs.

\[
\text{ZnO}_{(Ns)} + h\nu \rightarrow e^- + h^+ \quad \text{Eq. (5.1)}
\]

\[
\text{O}_2 + e^- \rightarrow \text{O}_2^- \quad \text{Eq. (5.2)}
\]

\[
\text{H}_2\text{O} + h^+ \rightarrow \text{OH} + \text{H}^+ \quad \text{Eq. (5.3)}
\]

It is the oxidation reactions which are responsible for the photo-catalytic degradation of harmful drugs, dyes, etc. Both the free electrons ($e^-$) as well as the free holes ($h^+$), interact with Oxygen and Water molecules, to form radicals of superoxides’ and hydroxyl groups respectively. A good amount of O$_2$ vacancies can catch semi-conductor electrons leading to oxidation of organic substances. On the other hand the hydroxyl groups increases with the decrease in semi-conductor electron densities. This overall increases the photo-catalytic activity of ZnO Nanostructures.
Previous studies support the degradation of diverse pollutants by ZnO Nanostructures efficiently \(^{43-73}\). Y Lv et al. synthesized ZnO Nanostructures and studied its photo-catalytic activity in degradation of rhodamine B (RhB) and expedient recycling of the synthesized ZnO-Ns provide it potential in waste water treatment. R. Saravanan et al. showed the photo-catalytic activity of the synthesized ZnO Nanorods for the degradation of methylene blue and methyl orange \(^{72}\). R. Ullah and J. Dutta reported that Mn-doped ZnO (ZnO:Mn\(^{2+}\)) was more effective for the photo-catalytic degradation of Methylene Blue than the undoped ZnO upon its visible light exposure \(^{62}\). C. Hariharan et al. showed that the listed aromatic & aliphatic chloro-compounds (listed water contaminants) can reduce the used ZnO Nanstructures, they reported an effective procedure to identify and degrade these contaminants \(^{63}\). Our study is also based on the mechanism exploited by the scientists earlier, using ZnO Nanostructures for photo-catalytic activity.

5.3.3 Photo-catalytic Degradation of Acid Red 183 in the presence of ZnO Nano-whiskers (ZNWs)

Under UV light source, the photocatalytic activity of the ZnO Nano-whiskers (ZNWs) was investigated by studying the degradation of Acid Red 183 as a function of irradiation time.

5.3.3.1 Procedure

Stock solution of the dye derivative containing desired concentration was prepared in double distilled water. An immersion well photochemical reactor made of Pyrex glass equipped with a magnetic stirring bar, water circulating jacket and an opening for supply of atmospheric oxygen was
used. The aqueous solution of the dye (250 mL) with 0.3 mM concentration was taken into the photoreactor and required amount (2 gL\(^{-1}\)) of photocatalyst was added for irradiation experiment. The solution was stirred bubbled with atmospheric oxygen for at least 15 minutes in the dark to allow equilibration of the system so that the loss of compound due to adsorption can be taken into account. The zero time reading was obtained from blank solution kept in the dark but otherwise treated similarly to the irradiated solution. The suspension was continuously purged with atmospheric oxygen throughout each experiment. Irradiation was carried out using a 125 W medium pressure mercury lamp. The light intensity falling on the solution was measured using a UV-light intensity detector (Lutron UV-340) and was found to be 1.74-1.78 m Wcm\(^{-2}\). IR radiation and short wavelength UV radiation were eliminated by a water circulating Pyrex glass jacket. Samples (10 mL) were collected before and at regular intervals during the irradiation and analyzed after centrifugation.

5.3.3.2 Characterization

The photo-decolourization of acid red 183 was monitored by measuring the absorbance at their \(\lambda_{\text{max}}\) as a function of irradiation time using UV spectroscopic analysis technique (Shimadzu UV-Vis 1601).
5.3.3.3 Experimental

The different types of experimental conditions have been placed to draw three sets of results. The three types of experimental condition along with their graph are as:

5.3.3.3.1 Experimental conditions – 1

Reaction vessel : immersion well photochemical reactor made of Pyrex glass, light source: 125 W medium pressure mercury lamp (1.74 - 1.78 mWcm$^{-2}$), absorbance was followed at 494 nm, photocatalyst : ZnO Nano-whiskers (ZNWs), Acid red 183 (0.30 mM), volume (250 mL), continuous stirring and air purging, irradiation time: 150 min.

5.3.3.3.2 Experimental condition - 2

Reaction vessel : immersion well photochemical reactor made of Pyrex glass, light source: 125 W medium pressure mercury lamp (1.74-1.78 mWcm$^{-2}$), absorbance was followed at 494 nm, photocatalyst: ZnO Nano-whiskers (ZNWs), Acid red 183 (0.30 mM), volume (250 mL), continuous stirring and air purging, irradiation time: 150 min.
5.3.3.3.3 Experimental condition – 3

Reaction vessel: immersion well photochemical reactor made of Pyrex glass. Acid red 183 (0.30 mM), volume (250 mL), photocatalyst: ZnO Nano-whiskers (ZNWs) (1 gL⁻¹), Sachtleben Hombikat UV100 (1 gL⁻¹), Millennium PC500 (1 gL⁻¹), light source: 125 W medium pressure mercury lamp (1.74-1.78 mWcm⁻²), continuous stirring and air purging, irradiation time: 150 min.
5.3.3.2 **Result and Discussions**

An aqueous solution of dye derivative, acid red 183 (0.3 mM, 250 mL) on irradiation with a 125 W medium pressure mercury lamp in the presence of ZnO Nano-whiskers (ZNWs) (2 gL$^{-1}$) with constant stirring and bubbling of atmospheric oxygen lead to the decolourization of the dye. Experimental condition – 1 (Figure 5.2) shows the change in absorbance at different time interval on irradiation of Acid red 183 in the presence of ZNWs where the absorption intensity decreases with increasing irradiation time.
Experimental condition – 2 (Figure 5.3) shows the change in concentration of Acid Red 183 in the presence and absence of ZnO as a function of irradiation time. [The concentration of dye was calculated by standard calibration curve obtained from the absorbance of the dye at different known concentration]. It could be seen from the figure that 68% decolourization takes place after 150 min of irradiation in the presence of ZnO Nano-whiskers. Blank experiment was carried out by irradiating the aqueous solution of the compound in the absence of photocatalyst, where no observable decrease in the dye concentration could be seen. Experimental condition – 3 (Figure 5.4) shows the comparison of
decolourization rate of Acid red 183 in the presence of different types of photo-catalysts viz. ZnO Nano-whiskers (ZNWs), UV100, PC500. It was found that the synthesized ZNWs shows the maximum decolourization rate of acid red 183.

5.3.3.3 Conclusion

The photo-degradation of Acid Red 183 by using the photo-catalyst, ZnO Nano-whiskers (ZNWs) showed good results of absorption and 68% of decolouration takes place after 150 min of irradiation in the presence of ZnO Nano-whiskers experiments. Finally it was also observed that the decolourization rate of Acid red 183 by ZNWs was maximum, when compared with other photo-catalysts.

The versatile nature of ZnO Nano-whiskers (ZNWs) leads to its explorations in different applications. Whether it may be anti-microbial activity shown by nano-scaled ZnO or it may be the functions of sensing various biological molecules, chemicals, gaseous, etc; whether it may be photo-catalytic activity by ZnO against different pollutants or it may be anti-cancerous activity or the functioning as solar cells; so on and so forth. Its huge applications perspective proved its vast requirement worldwide. Furthermore, enhancement of its quality characteristic with improvement in its properties further leads to its better usage. Since few decades, with the advent of nanotechnology, the applications of nano-scaled ZnO have been mounting rapidly. But still there is a big room left for various scientific groups worldwide, in order to present more efficient and innovative technologies based on ZnO Nanostructures.
5.4 References


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