ORIGINAL ARTICLE

Anti-microbial activity of cobalt doped zinc oxide nanoparticles: Targeting water borne bacteria

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Band gap;
Water borne bacteria;
Antibacterial activity

Abstract Zinc oxide (ZnO) nanoparticles were chemically synthesized with cobalt doping and characterized through UV–visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) techniques. Cobalt doped ZnO nanoparticles were found to be crystalline having a single phase as confirmed by XRD and SEM. It has been observed that the increase in the percentage of Co from 0% to 5% in ZnO, increases the crystallite size from 20.5 to 25.7 nm and accordingly its band gap varies from 3.22 to 3.30 eV. After treatment morphology of materials was changed from rod to spherical shaped. Further these nanomaterials were applied as a bactericidal agent to control water borne bacterial pathogen. Cobalt doping on zinc oxide and exposure of sunlight enhanced the antibacterial activity against water borne bacterial isolate at 50 \( \text{lg} \) concentration. Interestingly, most effective bactericidal results were found against Escherichia coli and Vibrio cholerae.

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1. Introduction

Clean water is a basic necessity for human life. Fresh and clean water availability is equally important for other animals and agriculture farming. Naturally water is recycling but not sufficient for all population and not consumable according to standard parameter. Presently, most of the natural water reservoirs are significantly contaminated with pathogenic bacteria. Globally, approximate 700 million peoples are facing huge scarcity of water, and this problem is increasing day by day, predicting nearly about 1.8 billion people face water scarcity.
problem in 2025 [1]. Approximately 12 million people die annually due to water borne disease (WHO 2012) [2] and most of the diseases occurring in developing countries are through the consumption of contaminated water [3]. Presently, water disinfection is carried out through different techniques such as chlorination, UV treatment and ozonisation, etc. but these disinfection routes have limitations on large scale implementation [4–7]. Advanced nanotechnology with extraordinary efforts creates new opportunity to defeat poverty, remove malnourishment and disease free environment and intensification of human power. Nano material applications open the door to improve the waste treatment methods including decontamination of the finest contaminants from water due to reactive surface of smart materials, induce coating on pollutant further restrain the toxicity of compounds and kill the pathogens. Therefore, there is a notable application of such type of antibacterial nanomaterials in water purification system developments [8] like membrane filtration preparation [9]. Combination of antimicrobial and photocatalytic activity of nanomaterials makes them more functional with attached membranes, multiple treatment task in a single step process to overcome the fouling effect significantly [8]. Membrane attached nanomaterials are too important to prevent the loss of nanomaterials and less effective to human health and ecosystems [9,10].

A number of synthesis methods are available for the preparation of pure and doped ZnO nanomaterials, like hydrothermal, hydrolysis, sol–gel, vapor condensation, spray pyrolysis and organic precursor flame decomposition [11–16]. The Sol–gel method is widely applied for innovative ceramics material preparation and crystalline materials of ores for metallurgical treatment and other value-added materials. The Wet-chemical method is also good for production of huge amount of nanoparticles at low cost rate [17,18]. Numbers of metals and compounds have been used as antimicrobial agents to control water borne pathogenic microorganisms [8]. In general, silver, gold and zinc nanomaterials exhibited important biological activities and structural properties [19–21]. Bacterial population is naturally capable of developing resistance against antibiotics and metals and spreads more potential and creates health problems, drug resistance in bacteria leading to ineffectiveness of medicine for treatment of a number of diseases [22]. Consequently, there has been cumulative attention in the development of stable antimicrobial metal nanomaterials for human welfare [23]. Nanomaterial based antimicrobial medicines are something more functional and stable in harsh conditions [24]. Nowadays, ZnO is more focussed by researchers due to its antibacterial activity and stability during rough and tough processing and safe materials for human and ecosystem [25,26]. ZnO nanoparticles were added in wallpaper of hospital to overcome the microbial load on walls and prevent nosocomial infection [27]. Mechanistically the antimicrobial activity of ZnO has enhanced due to the presence of water molecules on its surface, these aqueous suspensions of ZnO and water generate free radicals of hydroxyl and oxygen species which is responsible for remarkable oxidative stress in treated bacterial cells. ZnO NPs act as a potential antibacterial agent from the series of other metal oxides like Al2O3, SiO2 and TiO2, to control Escherichia coli, B. subtilis and Pseudomonas sp. Perishable agricultural product and food borne organism also control the application of ZnO NP [28]. Recently, many complexes and nanomaterials of Co(II) showing antimicrobial activity have been synthesized [29–31]. But, there are significant results over antibacterial activity of cobalt doped ZnO while both zinc and cobalt are the essential elements for human and animal health in trace amount and

![Figure 1](image_url)  
**Figure 1**  
Tauc plot of nanomaterials of (a) pure ZnO (b) 1% of Co doped ZnO (c) 3% of Co doped ZnO and (d) 5% of Co doped ZnO.
additionally photocatalytic active material. We have, therefore, been motivated to take up this task, to evaluate the antimicrobial activity of cobalt doped ZnO NP against water borne bacterial pathogens.

2. Methods

2.1. Synthesis and characterization techniques

High purity chemicals \( \text{ZnCl}_2 \cdot 2\text{H}_2\text{O} \) and \( \text{CoCl}_2 \cdot 6\text{H}_2\text{O} \) (purchased from Thomas Baker Ltd., India) and liquid ammonia (Qualigens Fine Chemicals) were used as preparatory materials for \( \text{Zn}_{1-x}\text{Co}_x\text{O} \) \((x = 0, 0.01, 0.02, 0.03, 0.04 \text{ and } 0.05) \) series. All the chemicals were used without any treatment. Initially 100 ml of distilled water was mixed with citric acid until pH becomes 1.5 of the solution on magnetic stirring, further stoichiometric quantities of \( \text{ZnCl}_2 \cdot 2\text{H}_2\text{O} \) and \( \text{CoCl}_2 \cdot 6\text{H}_2\text{O} \) were mixed properly forming a solution, furthermore 15 ml ethylene glycol was mixed in the above reaction and stirred for 30 min. After that drop wise addition of liquid ammonia (15 mol/L) and stirring were continued for next 30 min; as a final point a gel appeared further which was washed with distilled water. Reaction materials were dried in oven at 100 °C for 48 h and further heated at 400 °C for 3 h for proper calcination of

Figure 2  X-ray diffraction pattern of 0% pure ZnO, 1% of Co doped ZnO, 3% of Co doped ZnO and 5% of Co doped ZnO.

Figure 3  Doping analysis of ZnO and Co doped ZnO nanoparticles by scanning electron microscope coupled with energy dispersive X-ray spectroscopy, here, pure ZnO (A and a), 1% Co doped ZnO (B and b), 3% Co doped ZnO (C and c), 5% Co doped ZnO (D and d).
These materials were further examined by different techniques. Initially, UV–visible absorbance spectra were observed (Perkin Elmer), structure and size were determined by XRD (Rigaku) using Cu-K\(\alpha\) radiations (\(\lambda = 0.15406\) nm) and scanning electron microscopy (JEOL) at varying ranges of magnification and voltages. The composition of materials was determined by energy dispersive X-ray spectroscopy (EDS, Inca Oxford) coupled with SEM.

### 2.2. Determination of antimicrobial activity

Antimicrobial activities of the synthesized pure ZnO and cobalt doped ZnO nanomaterials were performed against water borne Gram-negative (Shigella dysenteriae, Salmonella typhi, Vibrio cholerae and E. coli). The antibacterial activity of pure ZnO and Co doped ZnO was done by modified nanoparticle diffusion method [33]. Specific cultures of microorganisms were subculture on nutrient broth at 35 ± 2°C on a shaker incubator at 150 rpm. Each bacterial isolate culture was uniformly spread onto the separately on plates by sterile spreader. Bacterial culture lawn on nutrient agar plate was prepared by spreading the 100 µl liquid culture which has approximately 10⁶ CFU/ml from every tested bacterial microorganism on media containing plates. Plates were left standing for 15 min to let the culture get absorbed. The 8 mm size wells were punched into the agar with the head of sterile micropipette tips, further each well was sealed from the bottom by 10 µl of molten agar to avoid diffusion or leakage of loaded sample in glass surface of petri plate. Nanomaterial’s suspension in double distilled water (50 µg/100 µL) was poured into

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sunlight exposure</td>
<td></td>
</tr>
<tr>
<td>0% Co doped ZnO</td>
<td>13 ± 0.5 15 ± 0.6 17 ± 0.5 18 ± 0.4</td>
</tr>
<tr>
<td>1% Co doped ZnO</td>
<td>13 ± 0.7 15 ± 1.0 17 ± 0.5 18 ± 1.0</td>
</tr>
<tr>
<td>2% Co doped ZnO</td>
<td>14 ± 0.5 16 ± 0.8 18 ± 1.2 19 ± 0.5</td>
</tr>
<tr>
<td>3% Co doped ZnO</td>
<td>15 ± 1.0 17 ± 1.3 19 ± 1.5 20 ± 0.8</td>
</tr>
<tr>
<td>4% Co doped ZnO</td>
<td>16 ± 1.5 18 ± 1.0 20 ± 0.8 21 ± 0.7</td>
</tr>
<tr>
<td>5% Co doped ZnO</td>
<td>17 ± 1.2 20 ± 0.6 21 ± 1.2 22 ± 1.5</td>
</tr>
<tr>
<td>2 h sunlight exposure</td>
<td></td>
</tr>
<tr>
<td>0% Co doped ZnO</td>
<td>15 ± 1.0 17 ± 0.5 18 ± 1.2 19 ± 1.0</td>
</tr>
<tr>
<td>1% Co doped ZnO</td>
<td>16 ± 0.5 17 ± 1.6 18 ± 1.0 20 ± 0.5</td>
</tr>
<tr>
<td>2% Co doped ZnO</td>
<td>17 ± 0.5 18 ± 0.5 20 ± 0.5 20 ± 0.8</td>
</tr>
<tr>
<td>3% Co doped ZnO</td>
<td>17 ± 1.0 19 ± 0.4 21 ± 1.2 21 ± 1.4</td>
</tr>
<tr>
<td>4% Co doped ZnO</td>
<td>18 ± 0.5 19 ± 1.5 22 ± 0.5 23 ± 1.2</td>
</tr>
<tr>
<td>5% Co doped ZnO</td>
<td>18 ± 1.3 21 ± 1.0 23 ± 1.0 24 ± 0.5</td>
</tr>
</tbody>
</table>
each well of plate. After incubation at 35 ± 2°C for 24 h appeared zone of inhibition in form of bacterial growth retardation was measured. Distilled water was used as a blank or negative control and standard antibiotic (tetracycline) used as a positive control. Separately, triplicate set of nanomaterial loaded well plate was exposed 2 h in sunlight and further incubated at optimum temperature.

### Table 2: Minimum bactericidal concentration (MBC) of Co doped ZnO nanomaterials in sunlight exposed and unexposed condition.

<table>
<thead>
<tr>
<th>Organism</th>
<th>MBC (µg/ml) of ZnO with Co doped (%) in sunlight unexposed /exposed condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. dysenteriae</td>
<td>&gt;80/ &gt;80/ &gt;75/ &gt;70/ &gt;65/ &gt;60/ &gt;50/ &gt;50/ &gt;40/ &gt;40/ &gt;30/ &gt;30</td>
</tr>
<tr>
<td>S. typhi</td>
<td>&gt;75/ &gt;70/ &gt;65/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;50/ &gt;50/ &gt;40/ &gt;40/ &gt;30/ &gt;30</td>
</tr>
<tr>
<td>V. cholera</td>
<td>&gt;75/ &gt;70/ &gt;65/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;50/ &gt;50/ &gt;40/ &gt;40/ &gt;30/ &gt;30</td>
</tr>
<tr>
<td>E. coli</td>
<td>&gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60/ &gt;60</td>
</tr>
</tbody>
</table>

2.3. Determination of bacterial growth pattern in presence of nanomaterial

Minimum inhibitory concentration means smallest concentration of compounds or substance that declines the organism growth in specific media. In our case pure and Co doped ZnO nanomaterial applied as a growth inhibitor of microorganisms further determined based on batch culture methods with containing different concentration (10–100 µg/ml) of pure and cobalt doped zinc oxide nanoparticles in specific media of each bacterial culture. Sterile eppendorf containing from 10 to 100 µg of nanomaterials in 100 µL double distilled water were sonicated for 30 min to prevent aggregation of the nanoparticles and then mixed with specific media of each bacterial. Further incubated in an orbital shaker at 150 rpm and 35 ± 2°C, shaking incubation minimize clumping and bottom settlement of the nanoparticles during incubation of bacteria. Bacterial growth was determined by absorption or optical density measured at 600 nm by using simple spectrophotometer (Double Beam UV–VIS spectrophotometer UV5704SS, Electronic Corporation, India). In these experiments a positive control has nanomaterials in medium without inoculum while negative control has inoculated media without nanomaterials. The optical density values of positive controls media were deducted from the main experimental values (flask of

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**Figure 4** Antibacterial pattern in absence (A) and presence (B) of sunlight exposure (1 h), in the form of zone of inhibition by pure ZnO and Co doped ZnO against water borne bacterial isolate of S. dysenteriae, S. typhi, V. cholera and E. coli when grown without sunlight exposure.
inoculated media with amended nanoparticles). All the setup of experimental flasks was conceded in triplicate.

3. Results and discussion

The X-ray diffraction measurements of the ZnO and Co-doped ZnO material samples annealed at 400 °C, showed wurtzite like structure and further reaffirmed from the ICDD card No. 80-0075 (Fig. 2). The crystal dimension of ZnO declines with an increased amount of ε Co²⁺. Scanning electron microscope images established ZnO grains have a spherical morphology with atypical width of 55 nm for pure ZnO, while 20 nm for 5% Co-doped ZnO [34]. The band gap energy was calculated using the Tauc relation [35]

\[ a \frac{h\nu}{E_g} = A (\frac{h\nu}{E_g})^n \]

where \( A \) is a constant, \( a \) is the absorption coefficient and \( n = \frac{1}{2} \) direct for band gap semiconductor which shows intensification of dopant conc. The band gap was determined from Fig. 1 as 3.22, 3.24, 3.26 and 3.30 eV for the pure and 1%, 3%, and 5% cobalt doped ZnO, respectively (Fig. 1). Our experimental analysis of SEM and EDX showed Co doped with ZnO nanomaterials as indicated in Fig. 3. Dielectric constant was influenced from varying concentrations of dopant. A decrease in dielectric constant was observed due to leaping in charge frequency of transporters between doping agent and core metal ions [34].

In our study, antimicrobial patterns of pure ZnO nanoparticles were highest against *E. coli* (18 ± 0.4 mm) and lowest against *S. dysenteriae* (13 ± 0.4) (Table 1). However, for cobalt doped zinc oxide nanoparticles, antibacterial activity was enhanced significantly (30%, 33%, 23% and 22%) against *S. dysenteriae*, *S. typhi*, *V. cholerae* and *E. coli*, respectively. Photo-catalytical exposure (2 h) of ZnO nanomaterials showed enhanced (20%, 23%, 28% and 26%) activity against *S. dysenteriae*, *S. typhi*, *V. cholerae* and *E. coli*, respectively. However, only zinc oxide nanoparticle activity without doping of cobalt was increased photo-catalytically from unexposed plate 15%, 13%, 6% and 5% of *S. dysenteriae*, *S. typhi*, *V. cholerae* and *E. coli*, respectively (Table 1). Furthermore, we checked the bacterial *S. dysenteriae*, *S. typhi*, *V. cholerae* and *E. coli* growth patterns in the presence of cobalt doped and undoped zinc oxide nanomaterials. Growth of bacteria in the presence of 100 µg/ml of different percentages of Co doped zinc oxide nanoparticles and pure ZnO amended in nutrient broth medium, here (A) *S. dysenteriae* (B) *S. typhi* (C) *V. cholera* (D) *E. coli* depicted in (Fig. 5).

![Figure 5](image-url) Growth pattern of bacteria in presence of 100 µg/ml of different percentages of Co doped zinc oxide nanoparticles and pure ZnO amended in nutrient broth medium, here (A) *S. dysenteriae* (B) *S. typhi* (C) *V. cholera* (D) *E. coli*. 
of nanomaterials have considered extensively for pathogenic microbes such as *E. coli* and *S. aureus* as standard test strains [36]. Zhang et al. [37] have earlier reported the ZnO bactericidal property in *E. coli*, *S. typhi*, *B. subtilis* and *S. aureus*. The mechanistically bactericidal behavior of ZnO and Co doped ZnO might be due to chemical interactions between nanomaterials and membrane proteins and formation of free radicals from lipid bi-layer in the presence of ZnO particles [23,38,39]. In comparison, Vijayaraghavan et al. [40] have already reported the varying values of MIC of ZnO nanoparticles against the Gram-negative bacteria. On testing cobalt doped ZnO, a significant change in the bactericidal property was observed. Minimum bactericidal concentration was determined by the standard broth dilution method. MBC for pure ZnO nanoparticles was found to be 80–60 μg/ml while for 1% to 5% Co doped ZnO, values of MBC lie between 80 and 50 μg/ml in non-photolytic condition while under light exposure (2 h) its MBC significantly changes from 50 to 30 μg/ml in case of *E. coli* and similar (enhanced activity) results were observed in other bacteria as shown in Table 2. In addition, at varying nanoparticle concentrations or different type nanomaterials of same concentration was amended in growing media to perform bacterial growth curves experiment. All bacterial strains’ growth pattern is shown in Fig. 5A–D. The enhanced biocidal activity of ZnO nano particles was previously reported by Vijayaraghavan et al. [40]. ZnO nanoparticle surface roughness was responsible for disorganization of both cell wall and cell membrane of *E. coli*. The team of Applerot et al. [41] has also identified the antimicrobial property of ZnO nanoparticles due to the formation of OH and ·O2 species in bacterial cell. Accumulation of ZnO inside the *E. coli* cells leading to membrane damage and cell death was reported by Bruyner et al. [42]. Other hypothesis included ZnO nanoparticles binding to the bacterial surface by electrostatic forces which directly disrupt the membrane [43]. Jin et al. [44] have analysed the antibacterial activity of zinc oxide quantum dots and suggested, the unique and acceptable mechanism of oxidative stress generation by ZnO nanoparticles to disrupt the cell membrane. Our results (Figs. 4 and 5) showed that cobalt doping in ZnO nanoparticles proportionally increases the bactericidal activity because particle size decreases from 25.7 to 20.5 nm with an increase in cobalt concentration from 0% to 5%. The surface to volume ratio increases with the decrease in the particle size. Therefore, its bactericidal activity increases due to enhanced binding forces and generation of free radicals in cell. In Fig. 5, we have observed growth pattern of water borne bacteria in the absence and presence of ZnO and cobalt doped ZnO nanoparticles. In the absence of ZnO nanoparticles, the optical density recorded was maximum which indicated thrive growth of strain in nutrient broth medium while in the presence of ZnO and cobalt doped ZnO nanoparticles, it decreases with an increase in concentration of cobalt in ZnO in nutrient broth medium. Similar result has been observed with different water borne bacterial isolate [20–23].

4. Conclusions

Chemical synthesis of Co-doped ZnO nanoparticles was successfully achieved with crystalline and wurtzite like structure. Further, focused on applied part of synthesized cobalt doped ZnO nanomaterials as an effective and potent antibacterial agent against water borne bacteria in presence and absence of sunlight. Furthermore, application of cobalt doped ZnO NP can be recommended as a potential water decontamination agent to protect water borne bacterial pathogens. The future studies will probably witness important novel developments in applied nanoresearch regarding antimicrobial agent.

Authors’ contributions

MO carried out the experimental design and analysis and drafted the manuscript. MA carried out the synthesis characterization of nanoparticles and drafted the manuscript. MSK and ASA analysed the data and provided technical support. AA and IMII read and approved the final manuscript. All authors read and approved the final manuscript.

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