



## Evaluation of Antimicrobial Potential of Cadmium Sulphide Nanoparticles against Bacterial Pathogens

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Accepted on: 24-11-2013; Finalized on: 31-01-2014.

### ABSTRACT

Emerging infectious diseases and the increase in incidence of drug resistance among pathogenic bacteria have made the search for new antimicrobials inevitable. In the current situation, one of the promising and novel therapeutic agents could be the nanoparticles. In this study, antimicrobial susceptibility testing of CdS nanoparticles (pure and 1% Cu doped) was done against *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumoniae*. The antimicrobial activity of the nanoparticles was assessed by well diffusion method. The tested concentration range of CdS nanoparticles was 1mg/ml to 100mg/ml and activity was determined by measuring the zone of inhibition. After the preliminary antimicrobial qualitative test, the MIC and MBC was determined. Cu doped CdS nanoparticles were more effective with MIC range 0.078-0.52 mg/ml as compared to pure CdS nanoparticles with range 0.15-0.83 mg/ml. The highest activity was observed against *Staphylococcus aureus* with MIC 0.078 mg/ml and *E. coli* was least susceptible with MIC 0.83 mg/ml. Scanning electron microscopy (SEM) study was also used to determine the effect of the nanoparticles on morphological changes of test microorganism.

**Keywords:** CdS nanoparticles (nps), Doping, MBC, MIC, SEM

### INTRODUCTION

Pathogenic bacteria remain a major health concern, which are responsible for causing a large number of hospitalizations and deaths each year.<sup>1</sup> Although we have current treatments such as antibiotics, bacteria are gaining resistance to these therapeutics at an alarming rate.<sup>2</sup> Therefore, the development of new antimicrobial compounds or the modification of those available is a high priority area of research.<sup>3</sup> A nanoparticle (10<sup>9</sup>m) is defined as a small object that behaves as a whole unit in terms of its transport and properties.

Nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and its unique chemical and physical properties.<sup>4</sup> There are reports on antimicrobial activity of nanoparticles such as Ag, Au, MgO, CuO, Al, TiO<sub>2</sub>, etc. which are effective against different drug resistant bacterial, viral and fungal strains.<sup>5</sup> In addition to Ag, Au etc. nanoparticles attention has also been focused on the synthesis of Cd, Zn, Fe nanoparticles.<sup>6</sup> CdS has been studied due to its potential technological applications in environmental sensors and biological sensors.<sup>7</sup> CdS nanoparticles (hexagonal) have increased photocatalytic activity for degradation of methylene blue and better hydrogen production activity for water photolysis.<sup>8</sup>

Doping is the process of adding controlled impurities to a semiconductor material which enhances its electrical conductivity and physical and chemical properties.<sup>9</sup> There have been significant changes in physical, chemical, and biological properties of host material on doping depending on types of dopant and its concentration. Ag/Cu Doped TiO<sub>2</sub> nanoparticles are used as effective

antimicrobial agents.<sup>10</sup> Mn and Co doped ZnO nanoparticles have enhanced antibacterial activity than pure ZnO nanoparticles.<sup>11</sup>

Cu doped CdS nanoparticles are crystalline in nature and their particle size decreases with the increase in doping concentration of Cu.<sup>12</sup> There is one report about the antibacterial activity of CdS nanoparticles on *E. coli*.<sup>13</sup> Cu and Cu doped nanoparticles have antibacterial activity against the *E. coli* and also inhibits the adhesion of bacteria on biofilm development.<sup>14</sup> So in the present study we will explore the antimicrobial potential of CdS nanoparticles (pure and Cu doped) against a vast range of pathogenic bacteria.

### MATERIALS AND METHODS

#### Materials

#### Microbial culture

Five bacterial strains, namely *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhimurium* (MTCC 98), *Pseudomonas aeruginosa* (MTCC 741) were procured from IMTECH Chandigarh, India. Clinical isolates of all these microbial culture were procured from PGIMER, Chandigarh and IGMC, Shimla, India. All isolates were maintained by sub-culturing once in a month on nutrient agar and stored at 4°C.

#### Nanoparticles

CdS nanoparticles (pure and 1% Cu doped) with an average size of 2-10 nm, characterized by SEM, TEM, XRD and UV-Vis. Spectroscopy were obtained from Dept. of Physics, Chitkara University, Chandigarh.



## Chemicals

Chemicals and media used were purchased from HIMEDIA.

## Methodology

### Nanoparticles preparation

A stock suspension was prepared by suspending the nps in methanol to yield a final concentration of 100 mg/ml. This stock solution was then sonicated for 30 minutes by repeating cycle after every 5 minutes. Every assay was done within 1-2 hour of sonication. The suspension was kept at 4°C and subjected to vigorous vortex mixing before assay.

### Inoculum Preparation

Inoculum was prepared using standard protocol M7-A7-CLSI with suitable modifications. In which bacterial cultures were grown on nutrient agar plates and maintained on the nutrient agar slants at 4°C.<sup>15</sup> 4-5 well isolated colonies of the same morphological type were selected from an agar plate culture and transferred into a tube containing 5-6 ml of nutrient broth medium. The broth culture was incubated at 37°C for 24 hrs. The turbidity of inoculum was compared with 0.5 McFarland standards, containing  $1-2 \times 10^8$  cfu/ml.

### Antimicrobial Activity of CdS nanoparticles (pure and 1% Cu doped)

#### Well Diffusion Assay

The antimicrobial activity of CdS nps was evaluated by agar well diffusion assay.<sup>16</sup> Muller Hinton Agar (MHA) plates were prepared for testing antibacterial activity. The prepared inoculum of bacteria was spread on plates (100µl). Wells were made and filled with 30µl of CdS nps in a concentration of 100mg/ml, 50mg/ml and 10 mg/ml each.<sup>17</sup> Ciprofloxacin was used as positive control. Methanol was used as negative control. MHA plates were incubated at 37°C for 24 hours. After incubation the zone of inhibition was measured using Hi antibiotic zone scale. The experiment was carried out in triplicates and standard deviation calculated.

#### MIC (Minimum Inhibitory Concentration)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of any anti-microbial agent that inhibits the growth of the test organism. MIC of CdS nps (pure and 1% Cu doped) were performed using broth micro dilution method.<sup>15</sup> Bacterial strains were grown overnight on nutrient agar plates at 37°C. In this test, double-strength Muller-Hinton broth was used, 4X strength nps solutions were prepared in methanol and then serial dilutions were made in the concentration range of 10mg/ml-0.039mg/ml. The test bacteria were at a concentration of  $2 \times 10^8$  cfu/ml. In a 96 well microtitre plate, 100 µl of double-strength MHB, 50µl (each) of the nps dilutions and 50µl of bacterial suspensions were mixed and incubated at 37°C for 24 hours. The final volume in each well was 200 µl. The 10<sup>th</sup> well was taken

as a positive control containing inoculum and antibiotic. 11<sup>th</sup> well was taken as negative control containing methanol and inoculum. After 24 hours plates were observed for the results. The lowest concentration showing visual inhibition of growth was considered as the MIC of the nps for the respective organism. The experiment was carried out in triplicates and standard deviation calculated.

#### Minimum Bactericidal Concentration (MBC)

10 µl of suspension from each well of micro titre plate was inoculated on nutrient agar plate and incubated at 37 °C for 24 hours. The same was repeated for control wells (positive and negative) containing MHA, inoculum and antibiotic. The nps concentration causing bactericidal effect was selected based on absence of colonies on the agar plates. The experiment was carried out in triplicates, mean was taken and standard deviation calculated.

#### Scanning electron microscopy

Deleterious effect of nanoparticle on surface of bacteria was determined by performing SEM of the nanoparticle treated bacteria. Small agar pieces were cut out from the zone of inhibition and they were fixed in 3 % (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.2) for one hour and then washed three times in sodium phosphate buffer. The pieces were then post-fixed in 1 % (w/v) osmium tetroxide for an hr and then washed three times in the sodium phosphate buffer. They were dehydrated in a graded alcohol series. The last stages of dehydration were performed with propylene oxide.<sup>18</sup> The specimens were dried and were mounted onto stubs using double-sided carbon tape. They were examined in a FEI Quanta 250 Scanning Electron Microscope.

## RESULTS

CdS nps (pure and 1% Cu doped) were studied for antimicrobial effect against five different pathogenic bacteria (*S. aureus*, *E. coli*, *K. pneumoniae*, *S. typhimurium* and *P. aeruginosa*).

#### Antimicrobial assay of CdS nps (pure and 1% Cu doped) Well Diffusion Assay

Different concentration of CdS nps 100mg/ml, 50mg/ml and 10mg/ml were used for antimicrobial assay. All the bacterial species were found to be susceptible for both pure CdS and 1% Cu doped CdS nps. Cu doped CdS nps were more effective as compared to pure CdS nps. CdS nps (pure and 1% Cu doped) showed maximum activity against *S. aureus* showing inhibition zone diameter 32 mm and 36 mm respectively at lowest concentration i.e. 10 mg/ml. Table 1 shows the inhibition zone diameter of CdS nps (pure and 1% Cu doped) against bacteria and figure 1 shows the antibacterial activity of CdS nps (pure and 1% Cu doped) against *S. aureus* (Clinical Isolate).



**Table 1:** Inhibition zone diameter of pure CdS and 1% Cu doped CdS nps against bacteria

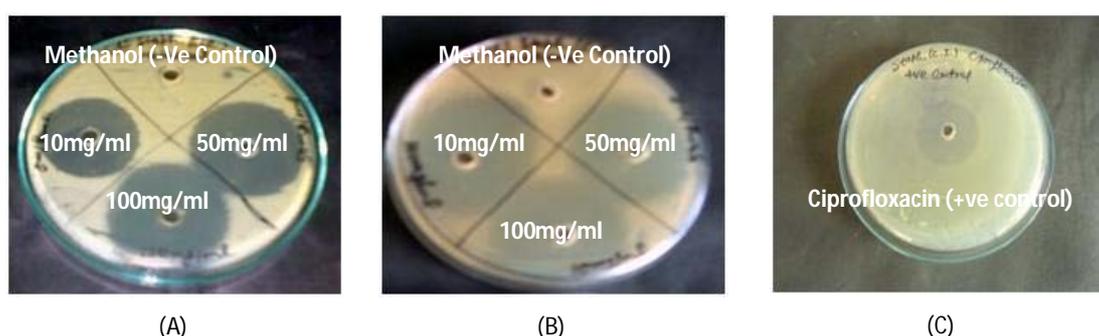
Microbes	*IZD(mm) for Pure CdS nps ± SD Conc. mg/ml.			*IZD(mm) for 1% Cu doped CdS nps ± SD Conc. mg/ml.		
	100	50	10	100	50	10
<i>S. aureus</i> (Std.)	38.3 ± 0.57	35.6 ± 0.57	32 ± 0	40.3 ± 0.57	37.6 ± 0.57	36 ± 0
<i>S. aureus</i> (C.I.)	35.3 ± 0.57	32.3 ± 0.57	30 ± 0	38.6 ± 0.57	36 ± 1	33 ± 1
<i>K. pneumoniae</i> (Std.)	35 ± 0	33 ± 0	29.6 ± 0.57	39.6 ± 0.57	36 ± 0	33.3 ± 0.57
<i>K. pneumoniae</i> (C.I.)	33 ± 0	30.6 ± 0.57	26.6 ± 0.57	34.6 ± 0.57	32.6 ± 0.57	30 ± 0
<i>P. aeruginosa</i> (Std.)	32.3 ± 0.57	28.6 ± 0.57	24.6 ± 1.53	36.6 ± 0.57	32.6 ± 0.57	30 ± 0
<i>P. aeruginosa</i> (C.I.)	31.3 ± 0.57	28 ± 0	25.6 ± 0.57	33.3 ± 0.57	29.3 ± 0.57	23.6 ± 1.52
<i>S. typhimurium</i> (Std.)	35.3 ± 0.57	32 ± 0	29 ± 1	40 ± 0	38.3 ± 0.57	35.3 ± 0.57
<i>S. typhimurium</i> (C.I.)	29 ± 0	26.6 ± 0.57	24 ± 1	39.6 ± 0.57	37 ± 0	34.6 ± 0.57
<i>E. coli</i> (Std.)	35.3 ± 0.57	33 ± 0	30.3 ± 1.15	40 ± 1	36.3 ± 0.57	33 ± 1
<i>E. coli</i> (C.I.)	31 ± 1	28 ± 0	25.6 ± 0.557	34 ± 1	30.3 ± 0.57	27.3 ± 1.15

\*IZD = Inhibition Zone Diameter, Std = Standard Strain C.I. = Clinical Isolate

**Table 2:** MIC and MBC of pure CdS and 1% Cu doped CdS nps against bacteria

Microbes	Pure CdS		1% Cu doped CdS	
	MIC(mg/ml)	MBC(mg/ml)	MIC(mg/ml)	MBC(mg/ml)
<i>S. aureus</i> (Std.)	0.20 ± 0.09	3.33 ± 1.44	0.078 ± 0	2.5 ± 0
<i>S. aureus</i> (C.I.)	0.63 ± 0	10 ± 0	0.31 ± 0	5 ± 0
<i>K. pneumoniae</i> (Std.)	0.31 ± 0	5 ± 0	0.15 ± 0	2.5 ± 0
<i>K. pneumoniae</i> (C.I.)	0.63 ± 0	10 ± 0	0.31 ± 0	8.33 ± 2.88
<i>P. aeruginosa</i> (Std.)	0.26 ± 0.09	3.33 ± 1.44	0.26 ± 0.09	2.5 ± 0
<i>P. aeruginosa</i> (C.I.)	0.63 ± 0	10 ± 0	0.31 ± 0	5 ± 0
<i>S. typhimurium</i> (Std.)	0.15 ± 0	5 ± 0	0.15 ± 0	5 ± 0
<i>S. typhimurium</i> (C.I.)	0.31 ± 0	10 ± 0	0.31 ± 0	10 ± 0
<i>E. coli</i> (Std.)	0.31 ± 0	5 ± 0	0.31 ± 0	5 ± 0
<i>E. coli</i> (C.I.)	0.83 ± 0.36	10 ± 0	0.52 ± 0.36	10 ± 0

Std = Standard strain, C.I. = Clinical, MIC = Minimum inhibitory concentration, MBC = Minimum bactericidal concentration



**Figure 1:** Antibacterial activity of CdS nanoparticles at different concentrations against *S. aureus*. A – Pure CdS nps; B- Cu doped CdS nps; C- +ve control.

#### MIC (Minimum Inhibition Concentration) and MBC (Minimum bactericidal Concentration) of CdS nps (pure and 1% Cu doped)

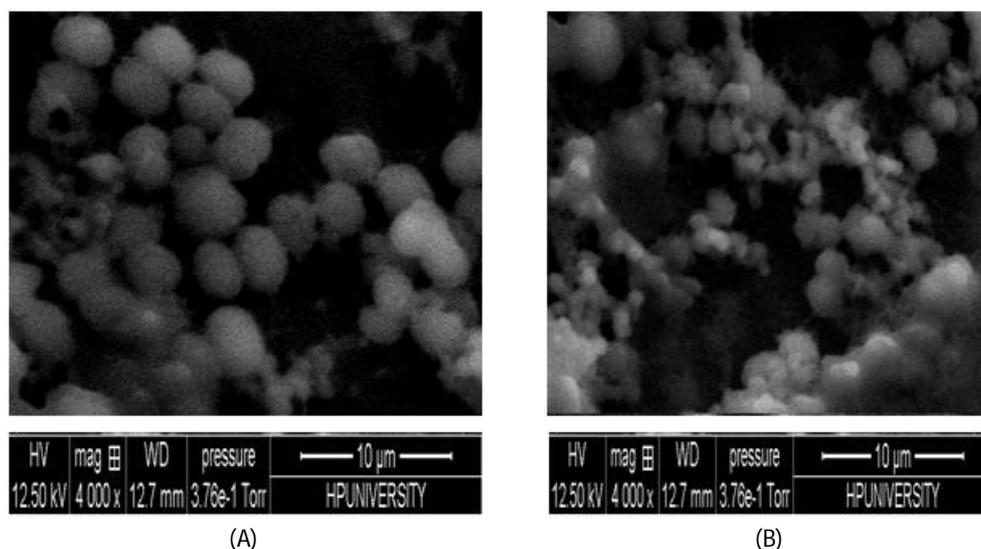
The results of MIC and MBC correlates well with those of well diffusion assay which is a qualitative test. There is a significant difference in the MIC values of pure CdS nps (0.15-0.83 mg/ml) and 1% Cu doped CdS nanoparticles

(0.078-0.52 mg/ml) as shown in table 2. The lowest MIC of CdS nanoparticles and 1% Cu doped CdS nanoparticles was reported against *S. typhimurium* (Std.) and *S. aureus* (Std.) respectively. Table 2 shows the MIC and MBC of CdS nanoparticles against different bacteria.

### Scanning electron microscopy

Deleterious effect of nanoparticle on surface of bacteria was determined by performing SEM of the nanoparticle treated bacteria. SEM study shows that the nanoparticles

treated cells appeared to be shrinking and damaged as compared to control. Figure 2 shows effect of 1% Cu doped CdS nanoparticles on morphology of *S. aureus*.



**Figure 2:** Effect of Cu doped CdS nanoparticles on morphology of *S. aureus*. A) Control B) Nanoparticles treated *S. aureus*

### DISCUSSION

In this study, antimicrobial susceptibility testing of CdS nanoparticles (pure and Cu doped) was done against bacteria *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumoniae*. Cu doped CdS nanoparticles were more effective with MIC range 0.078-0.52mg/ml. as compared to pure CdS nanoparticles with MIC range 0.15-0.83mg/ml. This indicates the fact that dopant was interfering with the active principle as the MIC values are smaller in Cu doped CdS nps as compared to pure CdS nps for different groups of bacteria. The highest activity was observed against *Staphylococcus aureus* with MIC 0.078 mg/ml. The bacteria least susceptible was *E. coli* with MIC 0.83mg/ml. The antimicrobial study also showed that standard isolates were more susceptible than clinical isolates. The effect is more pronounced in case of Gram positive bacteria as compared to Gram negative bacteria.

CdS nanoparticles showed broad spectrum activity. We speculate and reported the antimicrobial activity of CdS nanoparticles over a vast range of bacteria as compared to work done previously. There is one report about the antibacterial activity of CdS nanoparticles on *E. coli*.<sup>13</sup>

The multidrug resistance is one of the most alarming problems these days. The clinical isolates used in this study were drug resistant bacteria. The results from the present study are compared with the existing literature. Silver nanoparticles have been evaluated for their antimicrobial activities against a wide range of pathogenic organisms.<sup>19</sup> The highest sensitivity was observed against Methicillin resistant *Staphylococcus aureus* (MRSA) followed by Methicillin resistant *Staphylococcus epidermidis* (MRSE) and *Streptococcus pyogenes*. A

moderate antimicrobial activity was observed in case of the gram negative pathogens *Salmonella typhi* and *Klebsiella pneumoniae*.<sup>20</sup> Humberto *et al.*<sup>21</sup> reported the antibacterial activity of silver nanoparticles against drug-resistant bacteria (*P. aeruginosa*) and the MIC obtained for *P. aeruginosa* was 83.3µg/ml. While the MIC obtained *i.e.* 0.26 mg/ml in the present study is higher than that of Ag nanoparticles. The MIC obtained above indicated that silver nanoparticles have a bactericidal rather than bacteriostatic effect on bacteria.<sup>21</sup> According to Ansari *et al.*,<sup>22</sup> the MIC of silver nanoparticles towards drug resistant *S. aureus* was 12.5 µg/ml which is the lowest value obtained amongst the various other studies related to silver nanoparticles which suggests that silver nanoparticles exhibit excellent bacteriostatic and bactericidal effect against drug resistant *S. aureus*. The ZnO nanoparticles showed antimicrobial activity against Gram positive and Gram negative bacteria. *S. aureus* was inhibited by ZnO nanoparticles at 0.5mg/ml.<sup>23</sup> which is higher than the value of MIC of nanoparticles used in the present study *i.e.* 0.078 mg/ml. Thati *et al.*<sup>24</sup> reported the antibacterial effect of ZnO nanoparticles against drug-resistant *S. aureus*.

There are several mechanisms that have been proposed to explain the antibacterial activity of nps like deactivation of cellular enzymes and DNA by coordinating with the electron donating groups, pits formation in bacterial cell walls, leading to increased permeability and eventually the cell death etc. It is believed that the high affinity of Ag towards sulphur and phosphorus is the key element of the antimicrobial effect.<sup>25,26</sup> The generation of hydrogen peroxide from the surface of ZnO is considered as an effective means for the inhibition of bacterial growth.<sup>27</sup> One of the possible

mechanism for ZnO antibacterial activity is the release of Zn<sup>2+</sup> ions which can damage cell membrane and interact with intracellular contents.<sup>28</sup> The antibacterial activity also depends on the surface area and concentration of nps, while the crystalline structure and particle shape have little effect. Although the exact mechanism of action of CdS nps is not known but it may be any of the above mentioned mechanisms, through which CdS nps shows antimicrobial action. To observe morphological alteration after the cell was treated with nps we used scanning electron microscope. When the bacterial cells treated with nanoparticles were compared with untreated cells (Figures 2). The nps treated cells appeared to be shrinking and damaged as compared to control.

## CONCLUSION

CdS nps (pure and 1% Cu doped) showed broad spectrum antibacterial potential. Enhancement in antimicrobial effect on doping has been observed.

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**Source of Support:** Nil, **Conflict of Interest:** None.

