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Archives of Physics Research, 2013, 4 (5):32-39
(http://scholarsresearchlibrary.com/archive.html)

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ISSN : 0976-0970
CODEN (USA): APRRC7

Structural, thermal and antimicrobial property of CdSe nanoparticles synthesized by chemical route

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ABSTRACT

Cadmium selenide (CdSe) nanoparticles have been synthesized at three different temperatures (Room temperature (RT), 60°C and 80°C) by chemical route using cadmium acetate, sodium selenosulphate as cadmium and selenium precursors respectively. Triethanolamine and ammonia were used as capping and pH controlling agent. The particle size and crystalline structure of the synthesized nanoparticles were determined by transmission electron microscope (TEM). The analysis of TEM images indicated that CdSe nanoparticles possess cubic structure with particle radius is below 60nm. Thermal stability of CdSe nanoparticles are determined by thermogravimeteric analysis indicating weight loss region is between 650ºC-800ºC. The antimicrobial properties of as synthesized nanoparticles at different temperatures were investigated using gram-positive (Staphylococcus aureus, Bacillus subtilis) and gram-negative bacteria (Pseudomonas aeruginosa, Serratia marcescens and Proteus vulgaris) as test organism. The bactericidal effect of CdSe nanoparticles dispersed in acetone medium were determined by measuring the diameter of inhibition zone in gel diffusion tests. Bacterial sensitivity to nanoparticles was found to vary depending on the microbial species. Gel diffusion test revealed greater effectiveness of the cadmium selenide nanoparticles with Staphylococcus aureus and Bacillus subtilis compared to other test organisms. Bacillus subtilis exhibited maximum susceptibility to CdSe nanoparticles synthesized below 80°C. Further, we studied minimum inhibitory concentration of CdSe nanoparticles against the test cultures which is described in the paper.

Keywords: CdSe nanoparticles; chemical route; structural property; thermal property; antimicrobial activity

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INTRODUCTION

During the last decade significant interest has arosed in the research on synthesis of Cadmium selenide (CdSe) nanoparticles for biological, biomedical and pharmaceutical applications due to their known antimicrobial properties which appear to be dependent on the composition, coating, size of nanoparticles and the environmental conditions under which experiments are conducted, including exposure to light [1-3]. Various methods were reported to synthesize nanoparticles such as laser ablation [4], microwave assisted method [5], solvothermal method [6], sonochemical method [7], the non-organometallic precursor method [8] and the organometallic precursor method [9]. Non aqueous route or organometallic precursor route is generally not preferred owing to its unstable, expensive,

limited production and toxic nature. Thus there is a need to develop aqueous method in order to provide rapid, safe and scalable production of nanoparticles for practical use.

The Cadmium ion exhibits broad-spectrum biocidal activity towards many different bacteria, fungi, and viruses [10-12]. CdSe nanoparticles have superior fluorescent properties, currently used as effective alternates or complementary tools to conventional fluorescent dyes in advanced biosensors [13], cell imaging [14], and in vivo animal tracking [15] because of their great photostability, bright photoluminescence, narrow emission, and broad ultraviolet (UV) excitation. Also, fluorescent detection plays an important role in both studies of complex microbial populations and the identification of bacteria.

The bactericidal effect of transition metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution [16]. Three possible mechanisms through which nanocrystals could pass through bacterial cell walls and membranes are nonspecific diffusion, nonspecific membrane damage, and specific uptake. Recently, a number of nanoparticles (Ag, ZnO, TiO_2, cadmium telluride (CdTe)) with antimicrobial activities have been reported recently against both gram-positive and gram-negative bacteria [17-26] with few reports available on CdSe nanoparticles.

The reports on CdSe quantum dots showed that they are more toxic to *Pseudomonas aeruginosa* than cadmium salts due to the release of dissolved heavy metals [27]. It has been demonstrated that antibody-quantum dot conjugates exhibit stronger antibacterial effects in comparison to bare quantum dots (QD) [28]. The study on evaluation of the toxicity of a series of QD compositions, namely CdSe, CdTe against luminous bacterium (*Photobacterium phosphoreum*) as a microbial sensing element has been investigated [11]. At present, mechanism of interaction between nanoparticles and microorganisms is still not clear although photogeneration and formation of reactive oxygen species (ROS), which damage membrane has been proposed to be a key mechanism for the antimicrobial activity of QD. The phototoxicity generated by sunlight and high intensity lamps cause the direct release of metal ions (e.g., Cadmium ion) [29-30].

The objective of present work was to compare the bactericidal effect of CdSe nanoparticles synthesized chemically at different temperatures using various microbial strains. Such a comparative study would reveal strain specificities and would eventually lead to better utilization of nanoparticles for specific application. The antimicrobial and minimal inhibitory concentration (MIC) of CdSe nanoparticles was determined by agar cup diffusion assays.

MATERIALS AND METHODS

2.1 Preparation of CdSe nanoparticles

The chemicals used for the preparation of CdSe nanoparticles were analytical grade cadmium acetate dihydrate [$(CH_3COO)_2Cd.2H_2O$] (99%), selenium powder [Se] (99.5%), Triethanolamine (TEA) [$N(CH_2CH_2OH)_3$] and sodium sulphite [Na_2SO_3] (98%). Luria Bertani Broth (LB) purchased from Himedia, India**.** Standard bacterial cultures: *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescens, Proteus vulgaris* and standard fungal cultures: *Candida albicans, Fusarium oxysporum and Aspergillus niger* were procured from B. R. D school of Biosciences, S. P. University, Gujarat.

The method used for synthesizing CdSe nanoparticles at different temperatures is described in literature [31].

Different characterization techniques like Energy dispersive analysis of X-rays (EDAX) (Model-Philips EDAX XL-30 electron microscope), X-ray diffraction (XRD) (Model-Philips Xpert MPD, Powder diffractogram), Transmission electron microscope (TEM) (Model- Tecnai 20, Philips, Holland) and Thermogravimeteric analysis (TGA) (Perkin Elmer pyris 1) were used to study the synthesized CdSe nanoparticles.

The EDAX and XRD results of CdSe nanoparticles synthesized at different temperatures are described in literature [31].

2.2 *Evaluation of antibacterial activity of the CdSe nanoparticles*

The antibacterial activity of CdSe nanoparticles was measured initially by agar gel diffusion method followed by estimation of minimum inhibitory concentration (MIC). The petriplates overlaid with the test microrganisms (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescens, Proteus vulgaris, Candida albicans, Fusarium oxysporum and Aspergillus niger*) in which bored wells (4mm) were filled with 100 µl of 8.40 mg/ml dispersed solution of CdSe nanoparticles (16 mg of CdSe nanoparticles chemically synthesized at RT, 60°C and 80°C dissolved in 3 ml of acetone) and incubated at 37°C for 24h. Upon incubation, the zone of inhibition around the wells were measured and evaluated with respect to solvent control.

To evaluate the minimum inhibitory concentration (MIC), 5.33mg/ml CdSe nanoparticles chemically synthesized at room temperature **(**RT), 100 µl of actively growing test culture was added to nutrient broth supplemented with varying concentrations (5330 µg/ml to 53.3 µg/ml) of CdSe nanoparticles. Control tubes were incubated at 37°C for 24h. The turbidity of the tubes was measured using spectrophometer (Spectronic, Ahmedabad, India) at 600nm.

RESULTS AND DISCUSSION

1.1 Transmission electron microscope (TEM)

Figure 1(a)-(c) illustrates the typical particle size images of the CdSe nanoparticles synthesized at (RT), 60°C and 80°C. TEM reveals that CdSe nanoparticles have good size distribution and nearly spherical morphology. The radius of particles are lying between 21nm to 50nm at RT, 30nm to 50nm at 60°C and 27nm to 60nm at 80°C.

The electron diffraction patterns for CdSe nanoparticles synthesized at RT, 60°C and 80°C are shown in Figure 2(a)-(c). Presence of rings in diffraction patterns confirms that the synthesized CdSe nanoparticles using chemical method are polycrystalline in nature. Knowing electron beam wavelength λ, values of interplanar spacing (d) corresponding to all rings have been calculated from the following equations and are shown in Table 1.

$$\lambda = \sqrt{\frac{1.5}{V}}\ nm \qquad (1)$$

$$d = \frac{2\lambda L}{Ring\ diameter}\ \text{Å} \qquad (2)$$

where V is the accelerating potential of the electron beam (=200kV) and L is the distance between photographic film and the specimen which was kept 460mm during measurement.

From Table 1, it is clear that the measured lattice spacing of the lattice plane matches well with the zinc-blende structure of CdSe as given in JCPDS file (No. 19–0191). This confirms that the synthesized nanoparticles possess the cubic structure.

Table 1. Selected area electron diffraction pattern (SAED) analysis for CdSe nanoparticles synthesized at different temperatures

Conditions	Ring No.	Diameter of Ring (mm)	Calculated d_{hkl} (Å)	Standard d_{hkl} (Å)	*(h k l)*	N
RT	1	11	2.290	2.149	(2 2 0)	8
	2	18	1.399	1.394	(3 3 1)	19
	3	22	1.145	1.169	(5 1 1)	27
60°C	1	7	3.599	3.51	(1 1 1)	3
	2	11	2.290	2.14	(2 2 0)	8
	3	14	1.799	1.83	(3 1 1)	11
	4	20	1.259	1.24	(4 2 2)	24
80°C	1	12	2.099	2.149	(2 2 0)	8
	2	20	1.259	1.240	(4 2 2)	24
	3	32	1.095	1.074	(4 4 0)	32

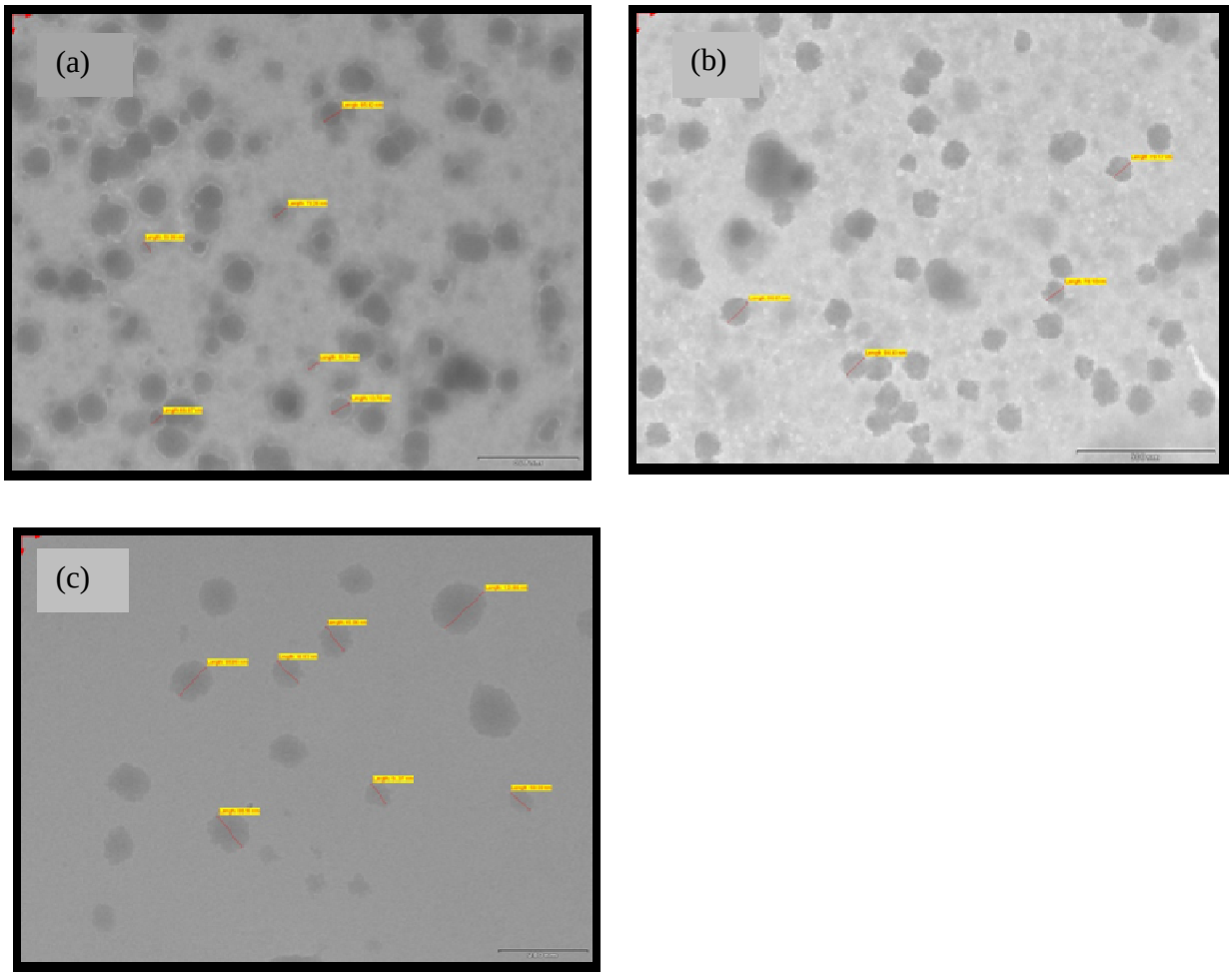
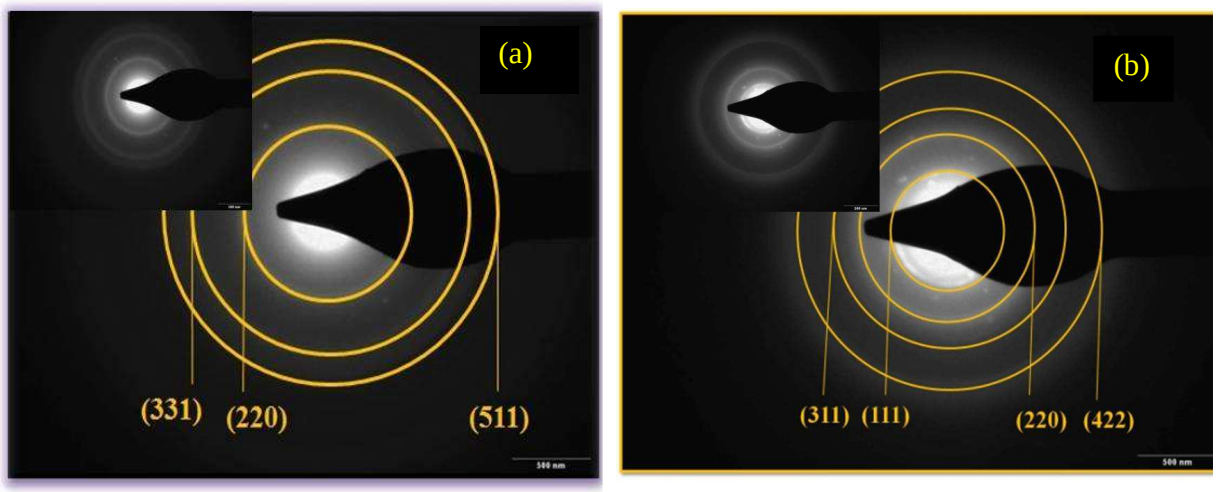


Figure 1 TEM images of CdSe nanoparticles synthesized at (a) RT, (b) 60°C and (c) 80°C



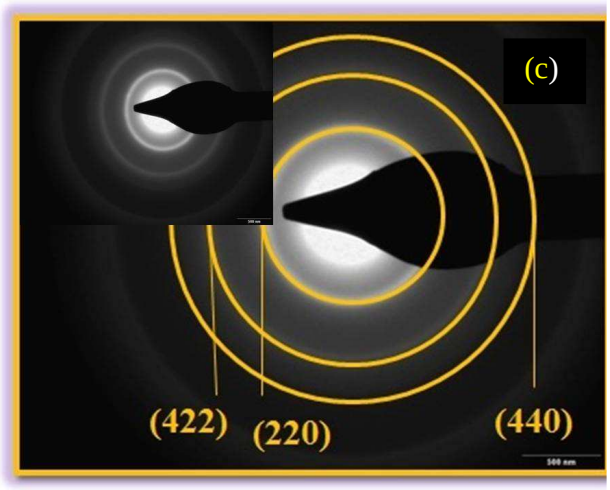


Figure 2 SAED pattern of CdSe nanoparticles synthesized at (a) RT, (b) 60°C and (c) 80°C.

3.2 Thermogravimetric analysis (TGA)

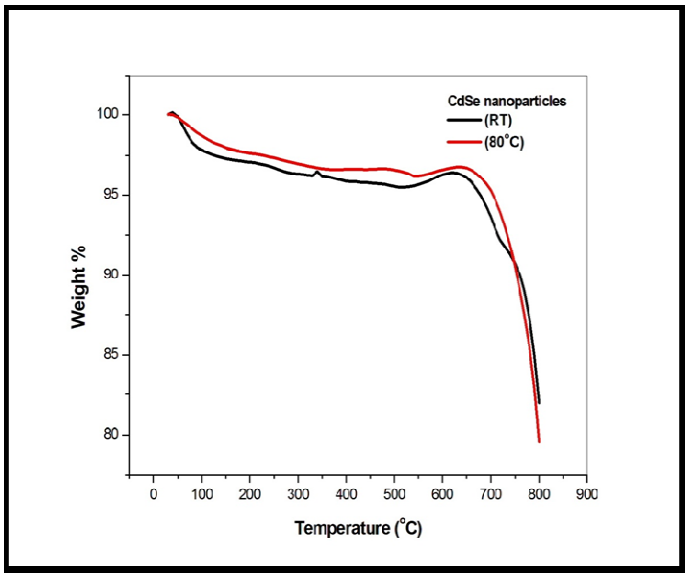


Figure 3 TGA curve of CdSe nanoparticles in air atmosphere

Figure 3 shows TGA curves of CdSe nanoparticles synthesized at RT and 80°C in air atmosphere. It was observed that both thermograms were stable upto 600°C and material start decomposing at nearly 650ºC-800ºC. It was found weight loss of sample in this region is less which is around 14%-17% indicating good stability of material. Thermal activation energy of as-synthesized samples were calculated within the region of weight loss using Broido equation

$$lnln\left(\frac{1}{y}\right) = \frac{E}{RT} + constant \qquad (3)$$

where y is the fraction of the number of initial molecules not yet decomposed, E is the activation energy and R is the gas constant. The calculated activation energies have values 1.541eV and 0.549eV at RT & 80°C which indicate that

energy decreases with increase of temperature thereby suggesting that CdSe nanoparticles synthesized at higher temperature require less amount of energy for their decomposition.

3.3 Evaluation of antibacterial properties

The antibacterial properties of the 8.40mg/ml CdSe nanoparticles were tested against gram-positive and gram-negative bacteria. Figure 4(a)-(d) shows antibacterial test results of CdSe nanoparticles synthesized at three different temperatures (A-RT, B-60°C, C-80°C) dispersed in acetone media by gel diffusion method.

It was found that the size of the inhibition zone was higher against *Bacillus subtilis and Staphylococcus aureus* at RT and 60°C (Table 2). No antibacterial activity was found against all test cultures (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescen and Proteus vulgaris*) in case of sample synthesized at 80°C, suggesting that the antibacterial activity was higher for the samples synthesized at lower temperature. Both *Serratia marcescens* and *Proteus vulgaris* exhibited moderate sensitivity to CdSe nanoparticles synthesized at 60°C and no antibacterial activity was found at RT. Among all, *Bacillus subtilis* exhibited maximum susceptibility, while *Pseudomonas aeruginosa* was found to be least susceptible to CdSe nanoparticles. Increasing the synthesizing temperature of CdSe nanoparticles resulted in significant reduction of antibacterial activity of CdSe may be due to increase in particle size of CdSe nanoparticles. The difference of the sizes of zone of inhibition between the CdSe nanoparticles synthesized at different temperatures could be correlated to the difference in nanoparticles diffusion tendency in cells due to the difference in their sizes producing different amount of reactive oxygen species (ROS).

The MIC, defined as the lowest concentration of material that inhibits the growth of an organism [32], was determined for CdSe nanoparticles synthesized at RT. A lower MIC corresponds to a higher antibacterial potency. The MIC tests were performed against different bacteria (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescens, Proteus vulgaris) and fungi (Candida albicans, Fusarium oxysporum and Aspergillus niger*) on agar plates treated with different concentrations of CdSe nanoparticles (5330µg/ml-53.3µg/ml) synthesized at RT. The CdSe nanoparticles did not inhibit *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Aspergillus niger*. The MIC results of CdSe nanoparticles chemically synthesized at RT exhibited significant growth inhibition of *Staphylococcus aureus, Bacillus subtilis, Serratia marcescens and Candida albicans* with MIC listed in Table 2. The antibacterial concentration range defined in our study is different with the reported range, which was 10 to 40nM for bare CdSe and 2 to 10nM for core shell nanoparticles [10].

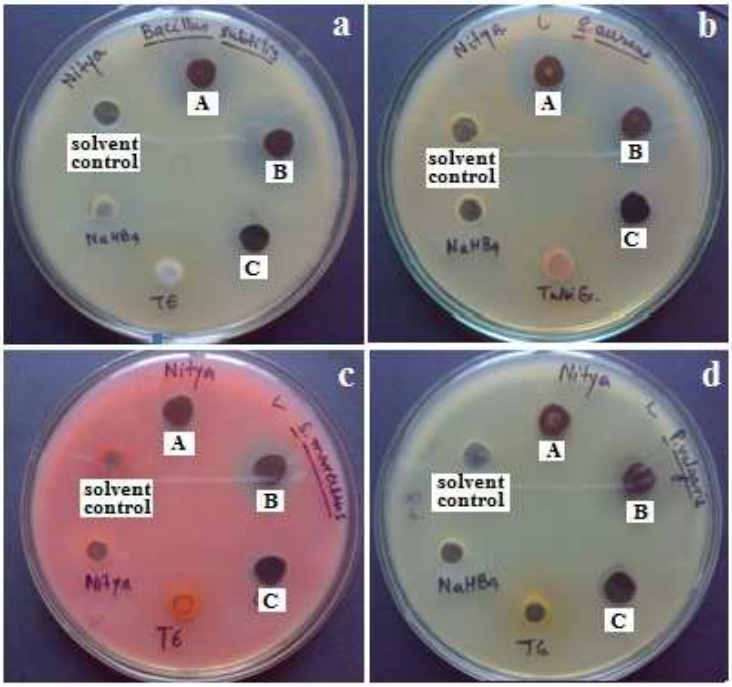


Figure 4 Anti-bacterial activity of CdSe nanoparticles (NPs) against (a) Bacillus subtilis ATCC 6051, (b) Staphylococcus aureus MTCC 87, (c) Serratia marcesens and (d) Proteus vulgaris. (where, A stands for NPs synthesized at RT, B stands for NPs synthesized at 60°C and C stands for NPs synthesized at 80°C; NPs dispersed in acetone).

From above study, it is clear that CdSe nanoparticles possess good antibacterial potential particularly against gram-positive as compared to gram negative bacteria. The difference in the sensitivity between the gram-positive and gram-negative bacteria can be attributed due to differences in their cell membrane structure which interferes with quantum dots binding, leading to ROS generation or the direct oxidation of cell lipids and proteins [33]. CdSe nanoparticles may have an antibacterial mechanism similar to that of reported CdTe quantum dots [26]. However, in future, there will remain a need that more studies in depth should be carried out in order to understand how CdSe nanoparticles react with different test cultures to cause the antibacterial effects. CdSe nanoparticles can be considered as a novel antimicrobial agent only if the toxicity of these nanoparticles against human cell lines is significantly lower than the one observed for several microbial strains. Toxicity studies of human cell lines have not been studied and should be needed to explore in future work.

Table 2. Antimicrobial activity of CdSe nanoparticles against bacterial and fungal strains

Test organism	Inhibition Zone (mm)			MIC* (µg ml^{-1})
	RT	60°C	80°C	(RT)
Staphylococcus aureus MTCC 87	12.5	16.5	ND	5330
Bacillus subtilis ATCC 6051	22	21	ND	266.5
Serratia marcescens	ND	15	ND	266.5
Proteus vulgaris	ND	12	ND	
Candida albicans				5330
Fusarium oxysporum				266.5

**MIC means minimum inhibitory concentration, ND means not detected*

CONCLUSION

In summary, the synthesis of CdSe nanoparticles at different temperature was carried out by a chemical route in the presence of TEA as a complexing agent. TEM confirmed that the synthesized particles are in nanometer range having spherical shape morphology. The selected area electron diffraction patterns indicated that the synthesized nanoparticles are crystalline in nature and belong to face centered cubic structure. TGA study indicated the stability of material upto 600^{o}C. Bioactivity of CdSe nanoparticles synthesized at different temperatures was studied by antimicrobial and MIC test using a standard microbial method. The enhanced activity of CdSe nanoparticles synthesized at RT and 60°C for *Staphylococcus aureus* and *Bacillus subtilis* compared to others is attributed to the difference in cell wall structure between gram negative and gram positive microorganisms. Bactericidal effects of CdSe nanoparticles become less effective at higher temperature (80°C). In short, the synthesized nanoparticles can be considered as antimicrobial agents since inhibition of growth of bacterial and yeast strains were observed.

Acknowledgements
Authors are thankful to SICART, Vallabh Vidyanagar for EDAX, XRD and TEM analysis of CdSe nanoparticles. Thank to Dr. J Varghese (ERDA), Vadodara for TGA study.

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