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Biosynthesis of semiconductor nanoparticles by using sulfur reducing bacteria *Serratia nematodiphila*

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Abstract. The synthesis of semiconductor nanoparticles is a growing research area due to the prospective applications for the development of novel technologies. In this paper we have reported the biosynthesis of Cadmium sulfide nanoparticles (CdSNPs) by reduction of cadmium sulphate solution, using the bacteria of *Serratia nematodiphila*. The process for the synthesis of CdS nanoparticles is fast, novel and ecofriently. Formation of the CdS nanoparticles was confirmed by surface Plasmon spectra using UV-Vis spectrophotometer and absorbance strong peak at 420 nm. The morphology of crystalline phase of nanoparticles was determined from Scanning Electron Microscopy (SEM), Energy Dispersive X-ray spectroscopy and X-ray diffraction (XRD) spectra. The average size of CdS nanoparticles was in the range of 12 nm and the observed morphology was spherical. The results indicated that the proteins, which contain amine groups, played a reducing and controlling responsibility during the formation of CdS nanoparticles in the colloidal solution. Antibacterial activity against some bacteria such as Bacillus subtilis, Klebsiella planticola. CdS nanoparticles exhibiting good bactericidal activity.

Keywords: biosynthesis; cadmium sulfide; semiconductor nanoparticles; SEM; UV-vis spectrophotometer; antibacterial activity

1. Introduction

Semiconductor nanocrystals or colloidal quantum dots (QD's) have concerned to a great extent interest in both essential research and technical applications since their interesting and novel electronic and optical properties (Yang *et al.* 2005, Sanghi *et al.* 2009). Among these, cadmium sulfide has been expansively considered due to its budding technological applications in field effect transistors, solar cells, photovoltaic, light emitting diodes, photo catalysis, photoluminescence, infrared photo detector, environmental sensors and biological sensors Alivisatos 1996, Kolvin *et al.* 1994, Prasad *et al.* 2010). The preparation of CdS nanoparticle has been carried out using various methods, such as polymer template-guided synthesis (Sherman *et al.* 2004) hydrothermal and solvothermal methods (Nie *et al.* 2003, Sanghi *et al.* 2009) chemical and biological methods. The synthesis of nanoparticle different methods, including using biological methods as capping agents, has been pursued. At the present, it is still complicated to

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expect how change various parameter of nanoparticle synthesis will have effect on the physical properties of the resulting material (Whitling *et al.* 2000, Rozamond *et al.* 2004). Microorganism has the endogenous capacity to grace fully regulate synthesis of inorganic material. Synthesis of CdS nanoparticle using *Clostridium thermoaceticum,* then may will precipitate CdS at the cell surface with in the medium from $CdCl_2$ in the occurrence of cysteine hydrochloride in the nutrient source. The majority almost certainly; cysteine acts as the source of sulfide (Cunningham 1993). *Klebsiella pneumoniae* exposed to Cd^{2+} in the nutrient source were create to shape 20 – 200 nm CdS on the cell outer layer (Holmes *et al.* 1997, Bai *et al.* 2009). Microbial resistance against heavy metal ions has been demoralized for biological crystal recovery improvement passing through reduction of the metal ions or formation of metal sulfides (Stephen *et al.* 1999, He *et al.* 2007).

2. Materials and methods

2.1 Isolation and identification

The sulphur reducing bacteria were isolated from chemical company effluent collected from Vellore. The collection of samples was inoculated in a specific mineral medium. The isolated organism was maintained for sulphur reducing medium. The organisms were cultivated in 1liter of specific mineral medium containing 1.5g of sodium sulphate, 0.5g of K_2HPO_4, 3.5 of sodium lactate,1.0g of Beef extract, 2.0g of peptone, 0.1g of Calciumchloride, 0.392g of Ferrous ammonium sulphate, 2.0g of magnesium sulphate, 0.1g of Sodium ascorbate (Jones *et al.* 1971). The organism was incubated at 30°C to 35°C. The isolates were morphologically and microbiologically characterized as *Serratia nematodiphila*. In this study *Serratia nematodiphila* (strain CAA) was used to synthesis the Cadmium sulfide nanoparticle and it maintain at Microbial Type Culture Collection & Gene bank (MTCC), Chandigarh.

2.2 Extracellular thesis of CdS nanoparticle

Serratia nematodiphila (CAA) was inoculated into flaks containing sterile nutrient broth and the flask was incubated at 35°C for 24 hours in 180 rpm. After the incubation period the culture was centrifuged at 10,000 rpm and bacterial supernatant was mixed with 1 mM Cadmium sulphate. The pH of the solution was adjusted to 7.0 to 7.5 and then the resultant solutions were kept in rotary shaker (180 rpm) till the change in the color, of the solution was observed.

2.3 Characterization of CdS nanoparticle

The bio reduction of CdS ions in aqueous solution was monitored by UV-Vis spectra of the solution between 300 nm – 600 nm using Perkin – Elmer spectrophotometer. The nanoparticles were scanned the infrared in the region of 4000 – 400 cm^{-1} Fourier Transform Infrared spectrometer (Thermo Nicolet Model – 6700). The CdS nanoparticle suspension was air-dried on the specimen grid and observed with a JEOL JEM-1010 Scanning Electron Microscope. The crystalline phases of the products were determined by X-ray powder Diffractometer (Seifert – 3000p). The Energy Dispersive X-ray analysis, the CdS nanoparticle was dried on a carbon coated copper grid and performed on a HITACHI SU6600 model.

2.4 Antibacterial activity of CdS nanoparticle

The CdS nanoparticle synthesized using *S.nematodiphila* was tested for antimicrobial activity by agar well diffusion method against pathogenic microbes for *B.subtilis,* (3053) *K.planticola* (2727). The pure cultures of bacteria were subcultured on nutrient broth. Each strain was swapped homogeneously onto the individual plates using sterile cotton swabs. Wells of 10mm diameter were through on Muller Hinton agar using gel puncture. Different concentration of CdS nanoparticle 50 µl, 100 µl and 150 µl was poured on each well. After 24hours incubation the various levels of zone of inhibition was measured. Three replicates of experiments were carried out.

3. Result and discussion

3.1 Isolation and identification of microorganism

In this study used the strain was isolated from chemical company waste water. The isolates were morphologically and biochemical characterized as S.*nematodiphila..S. nematodiphila* produce red pigment. The accession number for *S. nematodiphila* (JQ701743) was gram positive, rod shaped and non motile bacteria to identify and maintain at Microbial Type Culture Collection & Gene bank (MTCC), Chandigarh.

3.2 Visual identification

The aqueous cadmium sulphate ions were reduced by using the extracellular supernatant of *S. nematodiphila* Fig. 1(a). A 24 hrs incubation of $CdSO_4$ with *S. nematodiphila* biomass, the formation of white color reveals the synthesis of CdS nanoparticle, after 24 hrs the precipitation of CdS nanoparticles on the absorbance bottom of the conical flask indicates the CdS nanoparticles

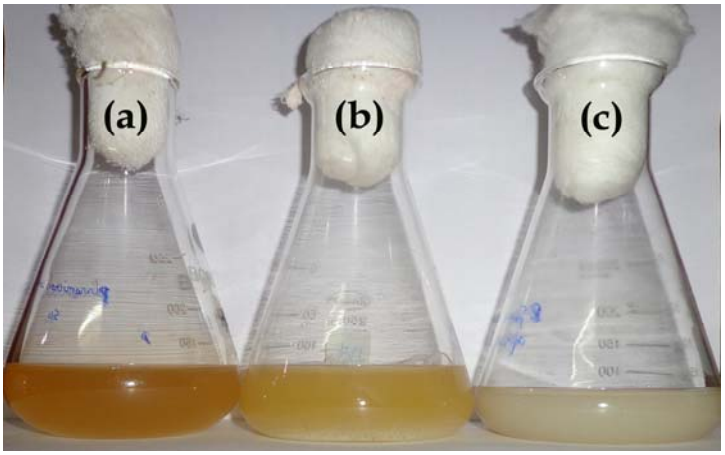


Fig. 1 Picture of conical flasks containing the Extracellular synthesis of the *S. nematodiphila* biomass (flask a), 1mM cadmium sulphate mixed with biomass at the beginning of reaction showing no color change (flask b), and after 1 day reaction (flask c)

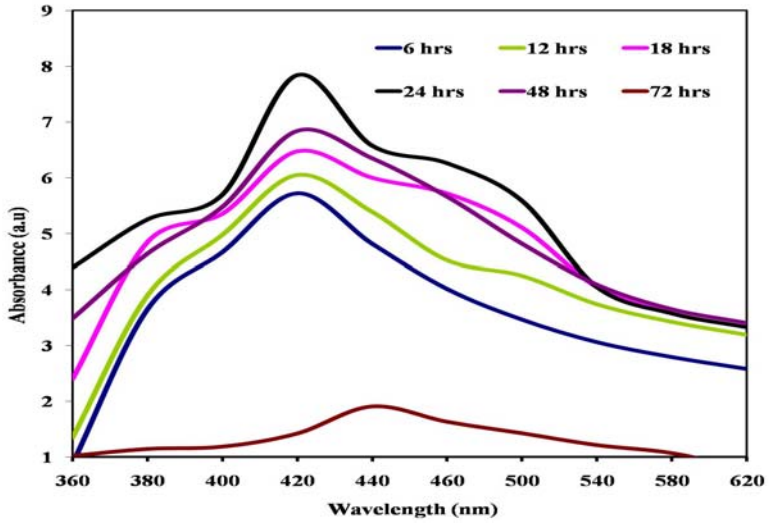


Fig. 2 Absorption spectrum of CdS nanoparticles synthesized by the culture supernatant of *S.nematodiphila* (420 nm)

synthesis process was completed. The decreased absorbance of CdS nanoparticles in UV-vis spectroscopy at 48 hrs, are also suggest that the CdS nanoparticles synthesis process was completed at 24 hrs. The appearance of white color was due to the excitation of surface Plasmon vibrations. In *S.nematodiphila* after 6 hrs incubation the color of $CdSO_4$ treated flask was changed in yellow to white in color precipitation in settle down in the flask Fig. 1(b). After 24 hrs, the color changed white in color indicates the reduction was completed CdS nanoparticles Fig. 1(c). Previously, Rozamond and Sweeney (2004) have reported the synthesis of CdS nanoparticles using *E.coli.*

3.3 UV-Spectrophotometer

The biosynthesis of CdS nanoparticles by reduction of aqueous metal ions during revelation of *S.nematodiphila* supernatant can be easily monitored by using UV-visible spectrophotometer. UV-visible spectra of CdS nanoparticles were measured at different time intervals from 6 hrs, 12 hrs, 18 hrs, 24 hrs, 48 hrs and 72 hrs. UV – Vis spectrum a broad peak was observed between 420 nm to 430 nm at 24 hrs incubation to the presence of CdS nanoparticles. The strong peak corresponding to the surface Plasmon resonance occurs at 420 nm is formation of CdS nanoparticles in the quantum size regime (Fig. 2). UV-visibly indicates the structure of CdS nanoparticles is very stable and could be stored for a long time period with no corrosion in ambient conditions. The similar peak was observed for nanoparticle synthesized by using *K. pneumoniae* Mousavi *et al.* (2012).

3.4 FTIR

The wave number (cm^{-1}) or occurrence of peak assigned to the kind of vibration, intensity and functional groups of the CdS nanoparticles synthesized using *S.nematodiphila* is shown in figure.

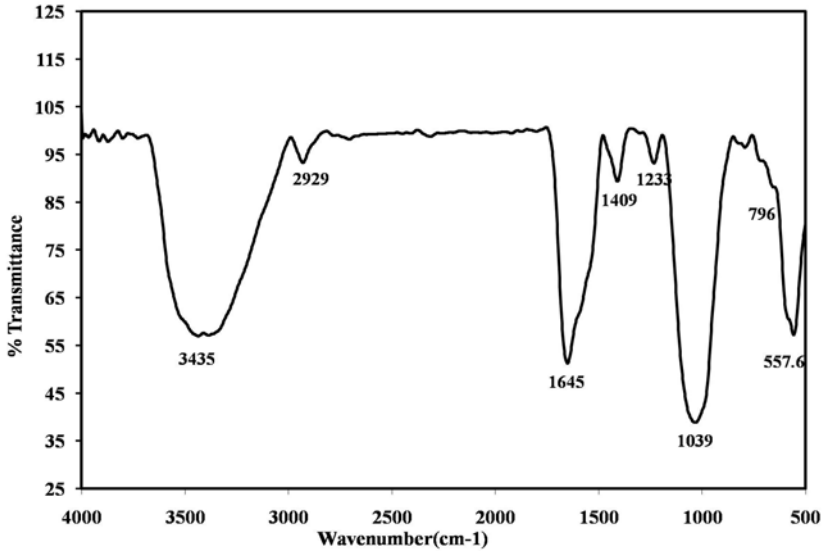


Fig. 3 FTIR spectra recorded from powder of CdS nanoparticles synthesized using *S. nematodiphila*

A number of vibration bands can be seen in the region 4000 – 500 cm^{-1} (Fig. 3). The absorption peaks located at around 3435 cm^{-1} can be assigned to the N-H stretching vibrations due to the amide linkages of proteins and amino acid residues in polypeptides respectively (Bai *et al.* 2009). The weaker band at 2929 cm^{-1} correspond to the C-H stretching vibrations alkanes groups respectively. The strong peak at 1645 cm^{-1} due to the N-H stretching vibrations of the amide I and amide II of proteins, respectively (Huayue Zhu 2009). The small band at 1409 cm^{-1} reveals the aromatic groups respectively. The peaks at 1233 cm^{-1} and 1039 cm^{-1} can be assigned to the C-N bending vibrations of aliphatic amines respectively. The band seen at 796 cm^{-1} and 557 cm^{-1} and are identified as alkyl halides and arises due to C –Cl stretching. The evidence suggests that the biological molecules can possibly carry out the function for the formation and stabilization of the CdS nanoparticles in the aqueous medium. It is earlier reported that proteins can bind to CdS nanoparticles either through free amine groups or cysteine residues in the protein (Sanghi *et al.* 2009).

3.5 XRD

The XRD pattern of CdS nanoparticles obtained. There are three intense peaks in the whole spectrum of 2Θ values ranging from 20 to 70. A comparison of our XRD spectrum with the standard confirmed that the CdS particles formed in the present study in the form of nanocrystals, as evident from the peaks at 2Θ values of 26.3º, 44.4 º and 52.0 º integrated intensity values of (111), (220) and (311) for cubic CdS phase respectively (Fig. 4). These agree with those reported standard (JCPDS co). The broadening of Bragg's peak indicates the formation of CdS nanoparticles. The mean particle diameter CdS nanoparticle was calculated from the XRD pattern according to the line width of the (220), refraction peak using following the Scherrer equation

$$\mathbf{D = 0.94\ \lambda / \beta\ (Cos\ \theta)}$$

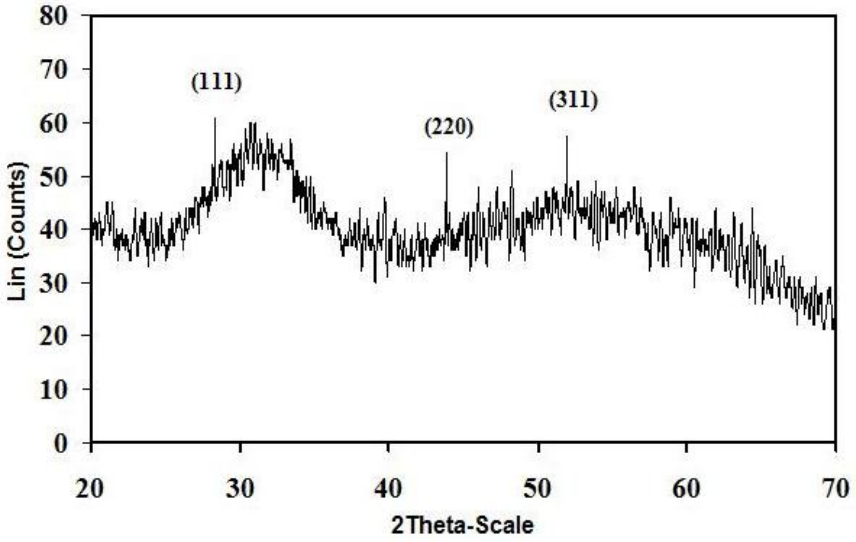


Fig. 4 XRD pattern of synthesized CdS nanoparticles by using *S. nematodiphila*

Where β is the full width at half maximum (FWHM), λ is the X-ray wavelength and θ is the reference peak width at angle, D is the average crystallite area size perpendicular to the sparkly planes. It reveals that the capping agents play an important character in affecting the crystal field bend, stability breaking and a special role on the modification of crystal segment of CdS nanoparticles during synthesis.

3.6 Scanning electron microscope and Energy dispersive x-ray image of CdS nanoparticles

Scanning Electron Microscope analysis was used to know the shape of CdS nanoparticles. SEM images of CdS nanoparticles viewed at different magnification like 10,000X, 20,000X (Scale bar 500 nm) (Fig. 5(a).The images confirmed the formation of few nanoparticles capped with the biomolecules present in the biomass supernatant. The SEM images shows the synthesized CdS nanoparticles are predominantly spherical in shape with aggregates (Fig. 5(a)). Andeani *et al.* (2011) suggest that the protein molecules perform as a surface coating molecules which keep away from the internal agglomeration of the particle and indicating stabilization of nanoparticles. The particle size is found to be around 12 nm by *S. nematodiphila* respectively. Similarly, Hosseinian *et al.* (2010) obtained that spherical shape of synthesis of CdS nanoparticles by chemical method. The Energy Dispersive X-ray analysis (EDX) reveals strong signal in the Cd and S and confirms the presence of CdS nanoparticles Fig. 5(b). Semiconductor CdS nanocrystals generally show a representative visual absorption peak approximately at 3.1keV due to the surface Plasmon resonance. The synthesized CdS nanoparticles were stable in solution more than the period of two months in room temperature. Similar result could be observed with biological synthesis of CdS nanoparticles (Pandian *et al.* 2011).

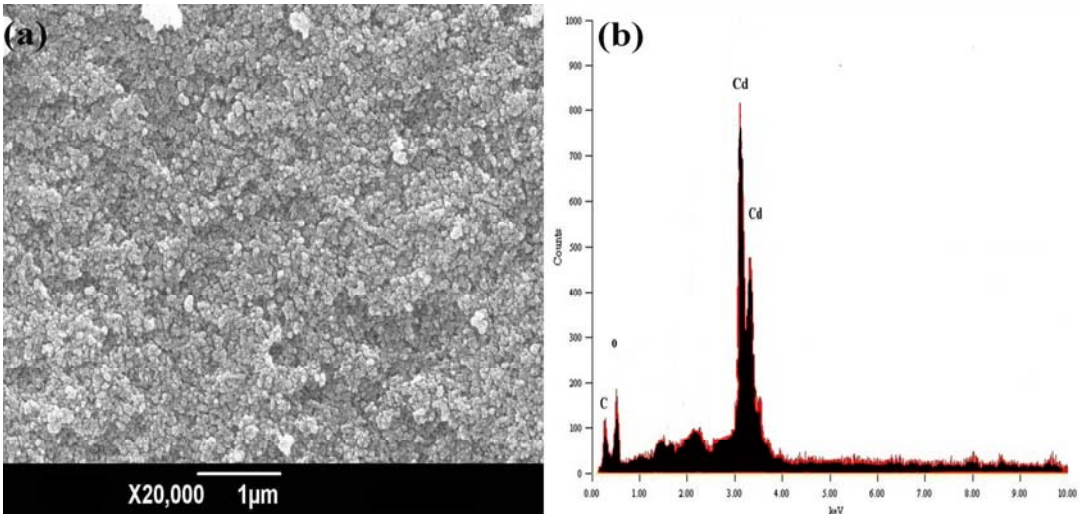
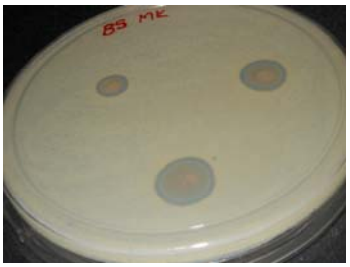


Fig. 5 SEM micrograph of CdS nanoparticles formed after reaction of culture supernatant with 1mM cadmium sulphate for 24hrs (b) Energy Dispersive X-ray of CdS nanoparticle

Table 1 shows zone formation against various pathogenic bacteria

S. No	Pathogenic bacteria	Concentration Of Cds nanoparticles	Zone of inhibition (mm in diameter)	ZOI in Agar well plate
1	*Bacillus subtilis*	50 µl	23 ±0.198	
		100 µl	39 ±0.260	
		200µl	54 ±0.296	
2	*Klebsiella planticola*	50 µl	20±0.253	
		100 µl	35±0.306	
		200µl	51±0.322	

± Standard deviation

3.7 Antimicrobial activity

The antimicrobial activity of CdS nanoparticles was investigated against pathogenic bacteria, viz., *B.subtilis* and *K.planticola* using well diffusion method (Table 1). The highest antimicrobial activity was experimental against gram positive bacteria *B.subtilis* (54±0.29). The activity was

limited against *K.planticola* (51±0.322). The CdS nanoparticles also showed the good activity against gram positive and gram negative bacteria. The structure of the cell wall difference between gram positive and gram negative bacteria. The gram positive bacteria formation of the cell wall is collected of deep layer of membrane, consisting of linear polysaccharide chains and the gram negative bacteria possess the slender layer of membrane. Shukla and Russel (2012) reported that nanoparticles discharge the ions, which react with the thiol groups the proteins present on the bacterial cell membrane. Such proteins outcropping during the bacterial cell surface, allowing the carry of nutrients through the cell membrane.

4. Conclusions

In this study, eco-friendly production of CdS nanoparticles was carried out using inactivated supernatant of *S.nematodiphila.* Since, the culture supernatant is playing important role for controlling the size and shape of the nanoparticles. These nanoparticles are found to be stable in water for more than three months that can be attributed to surface binding of bacteria with the reduced materials. The morphological (SEM with EDS) and structural (XRD) including spectroscopic techniques UV-vis and FTIR studies that the bacteria might have played an important role in the stabilization of CdS nanoparticles. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other semiconductor materials.

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