

## Biosynthesis, Purification and Characterization of Cadmium Sulfide Nanoparticles Using Enterobacteriaceae and their Application

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The area of nanotechnology encompasses the synthesis of nanoscale materials, the understanding and the utilization of their physicochemical and optoelectronic properties, and the organization of nanoscale structures into predefined superstructures. The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. Nanotechnology has recently emerged as an elementary division of science and technology that investigates and regulates the interaction at cell level between synthetic and biological materials with the help of nanoparticles. A wide range clean, nontoxic and eco-friendly synthesis of nanoparticles is an important aspect of current nanotechnology. Microbial synthesis of nanoparticles is a Green chemistry approach that interconnects nanotechnology and microbial biotechnology. Microorganisms play an important role in the eco-friendly synthesis of metal nanoparticles. This study illustrates the synthesis of CdS nanoparticles using the bacteria of *Enterobacteriaceae* (*Escherichia coli* PTCC 1533 and *Klebsiella pneumonia* PTCC 1053) after 96 h of incubation at room temperature (30°C) and pH 9. The morphology of the samples was analyzed using Scanning electron microscopy (SEM). The size of CdS nanoparticles in aqueous solution has been calculated using UV-Vis spectroscopy, XRD, FTIR, EDS and SEM measurements. The nanoparticles are found to be polydisperse in the size range 5–200 nm.

**Keywords:** Nanotechnology, CdS Nanoparticles, Microorganisms, Eco-friendly, Enterobacteriaceae.

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### 1. INTRODUCTION

Nanotechnology is foreseen to significantly influence science, economy and everyday life in the 21st century and also to become one of the driving forces of the next industrial revolution.

Different fields of this novel technology comprise the production, characterization and manipulation of nanoscale structures. Besides the rather established chemical and physical production procedures [1-16], numerous organisms have been found to synthesize nanoparticles.

Although ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction techniques have been used successfully to produce nanoparticles, they remain expensive and involve the use of hazardous chemicals. Therefore, there is a growing concern to develop environment-friendly and sustainable methods. Since the synthesis of nanoparticles of different compositions, sizes, shapes and controlled dispersity is an important aspect of nanotechnology and new cost effective procedures are being developed.

However, despite the stability, biological nanoparticles are not monodispersed and the rate of synthesis is slow. To overcome these problems, several factors such as microbial cultivation methods and the extraction techniques have to be optimized and the combinatorial approaches such as photobiological methods may be used. Cellular, biochemical and molecular mechanisms that mediate the synthesis of biological nanoparticles

should be studied in detail to increase the rate of synthesis and improve the properties of nanoparticles [17].

Biologic production systems are of special interest due to their effectiveness and flexibility. Regarding morphology and metabolic pathways, microbial cells are highly organized units that are capable of synthesizing reproducible particles with well-defined size and structure. Furthermore, biogenic nanoparticles often exhibit water-soluble and biocompatible properties which are essential for many applications.

The unique optical, chemical, photo-electrochemical, and electronic properties of (metal and semiconductor) nanoparticles are size dependent and differ from the properties of the corresponding bulk material [18-19].

Recent developments in the organization of nanoscale structures into predefined superstructures ensure that nanotechnology will play an increasingly crucial role in many key technologies of the new millennium. It is gaining importance in areas such as catalysis, optics, biomedical sciences, mechanics, magnetics, and energy science. The synthesis of nanomaterials over a range of chemical composition and high monodispersity is still challenging in material science.

It is well known that many organisms can provide inorganic materials either intra- or extracellularly. Hence, such microorganisms are recently found as possible eco-friendly nanofactories [20-21].

Interactions between metals and microbes have been exploited for various biological applications in the fields of bioremediation, biomineralization, bioleaching, and biocorrosion and the microbial synthesis of nano-

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particles has been emerged as a promising field of research as nanobiotechnology interconnecting biotechnology and nanotechnology [22].

Microbial detoxification can be made either by extracellular biomineralization, biosorption, complexation or precipitation or intracellular bioaccumulation. Extracellular production of metal nanoparticles has more commercial applications in various fields. Since the polydispersity is the major concern, it is important to optimize the conditions for monodispersity in a biological process. In case of intracellular production, the accumulated particles are of particular dimension and with less polydispersity [17,23].

Biosynthesis of nanoparticles is accomplished using microorganism which grabs target ions from their solutions, and then accumulates the reduced metal in its element form through enzymes generated by microbial cell activities. It can be categorized into intracellular and extracellular synthesis according to the place where nanoparticles are formed. The intracellular method consists of transporting ions into the microbial cell to form the nanoparticles in the presence of enzymes. The extracellular synthesis of nanoparticle involves trapping the metal ions on the surface of the cells and reducing ions in the presence of enzymes[24-26].

Living organisms have the endogenous ability to exquisitely regulate synthesis of inorganic materials such as amorphous silica (diatoms), magnetite (magnetotactic bacteria), gypsum, and calcium carbonate layers (S-layer bacteria) and minerals such as calcite into functional superstructures. Because of this ability to precisely direct the shape and crystallinity of a developing inorganic material, there is great interest in exploiting living organisms such as bacteria and fungi for inorganic materials synthesis.

*Klebsiella pneumoniae* exposed to Cd<sup>2+</sup> ions in the growth medium were found to form 20–200 nm CdS on the cell surface. Intracellular CdS nanocrystals, composed of a wurtzite crystal phase, are formed when *Escherichia coli* is incubated with cadmium chloride and sodium sulfide. Nanocrystal formation varies dramatically depending on the growth phase of the cells and increases about 20-fold in *E. coli* grown in the stationary phase as compared with that grown in the late logarithmic phase [27-29].

Among these, CdS has been extensively studied due to its potential technological applications in field effect transistors, solar cells, photovoltaic, light emitting diodes, photocatalysis, photoluminescence, infrared photodetector, environmental sensors and biological sensors. The preparation of CdS nanoparticles has been carried out using various methods such as microwave heating, microemulsion synthesis, chemical synthesis, photoetching and ultrasonic irradiation[30-49].

Nanoparticles can be utilized as fluorophores in fluorescence in situ hybridization (FISH). QDs (CdS NPs) attached to a specific oligonucleotide probe or immunoglobulin G(IgG) have been used to successfully detect the human Y chromosome, and to locate cancer markers in cellular imaging in cancer studies. To further facilitate in vivo real-time imaging, biocompatible and soluble QDs can be prepared by encapsulating them in a n-poly (ethyleneglycol) phosphatidylethanolamine (PEG-PE) phospholipid blockcopolymer micelle. A simi-

lar application of QD-based tracers was reported for targeting and imaging cancer cells in vivo. QDs can be further conjugated with specific antibodies to detect pathogenic microorganisms[50-52].

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Bacterial strains

The present study uses Bacterial strains of *Enterobacteriaceae* (*Escherichia coli* PTCC 1533 and *Klebsiella pneumoniae* PTCC 1053) as a potential producer for green synthesis of CdS nanoparticles. The strains were prepared of IROST. Bacterial strains were inoculated in BHI broth under sterile conditions and incubated at 37°C. The strains inoculated in Nutrient Agar, EMB Agar and Endo Mediums. For identification of strains, biochemical tests were conducted.

### 2.2 Biological synthesis of CdS nanoparticles

In this study, bacterial suspension with optical density of 3 MC Farland, which corresponds to  $9 \times 10^8$  CFU/ml was used. Suspension was inoculated in Nutrient broth and incubated at 37°C for 24h for growing of bacteria. After the incubation period, the suspension were centrifuged at 5000 rpm for 15 min at room temperature and resulting supernatants were extracted for further processing. The obtained supernatants were washed with phosphate buffer saline (pH7.0) for 3 times. 1mM solution of CdCl<sub>2</sub> (for *E. coli*) or CdSO<sub>4</sub> (for *K. pneumoniae*) was prepared using deionized water. 35 ml of the solution was added to supernatants and resulting solution was kept for incubation in a shaker at 220 rpm and room temperature for 30 min. Then, 35 ml of 1mM Na<sub>2</sub>S solution was slowly added to the solution. The samples were then incubated at room temperature with end-over-end rotation for 10 min[53-56].

### 2.3 Effect of pH and Temperature on nanoparticles synthesis

To find the conditions under which maximum amount of nanoparticles were synthesized, 1mM concentration of CdCl<sub>2</sub> or CdSO<sub>4</sub> and Na<sub>2</sub>S were added to the solution containing biomass and incubated in different pH conditions (5–11) and different temperatures. The pH of the incubation mixtures was adjusted using 1M HCl and 1M NaOH solutions. The optimum condition for synthesis of nanoparticles is temperature of 30°C and pH of 9.

### 2.4 Effect of CdCl<sub>2</sub> or CdSo<sub>4</sub> and Na<sub>2</sub>S on the nanoparticles synthesis

In this research it was also studied what CdCl<sub>2</sub> or CdSo<sub>4</sub> and Na<sub>2</sub>S concentrations yield the maximum fluorescence. The study was carried out by adding different concentrations to the solution ranging from 1 to 10mM at pH 9.0 and temperature 30°C.

### 2.5 Synthesis of CdS nanoparticles at various growth phases and time period

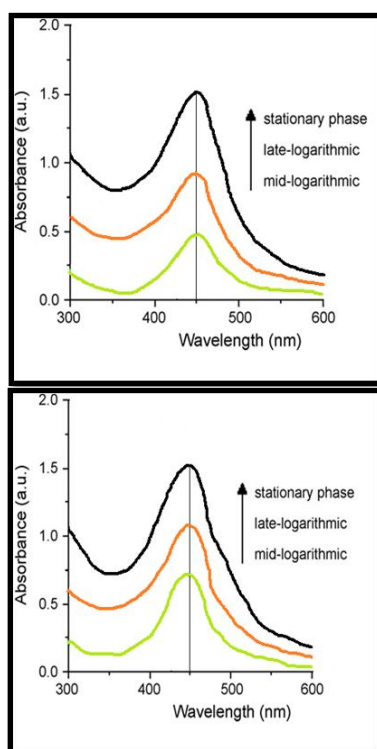
To find the effect of growth phase of the organism on CdS nanoparticles production, Enterobacteriaceae was inoculated into nutrient broth of four different flasks.

The flasks were allowed to grow at various growth stages (lag phase, log phase, late log phase and stationary phase). After that the biomass was incubated with cadmium chloride or cadmium sulfate and sodium sulfide solution. The effect of time over the growth was evaluated by collecting the samples at every 1 h up to 120 h. Maximum amount of nanoparticles synthesized by bacterial strains was achieved in stationary phase.

### 3. RESULTS

#### 3.1 UV-Visible spectrophotometer

For measuring the amount of UV-Visible absorption by synthesized CdS nanoparticles, samples were washed twice with 50mM phosphate buffered saline (pH 7.0). Then, ultrasonic disruption of cells was performed using an ultrasonic processor (Retsch, UR1) over three 45 S periods with 10 s intervals between periods. The sonicated samples were then filtered using a 0.22 $\mu$ m filter to eliminate cell-debris and other pollutants. The filtered solutions were then used for characterization of CdS nanoparticles. The dried particles were dispersed in de-ionized water and were measured using a UV-Visible spectrophotometer (CARY,100Conc, UV Pharma spec 1700 with a resolution of 0.72 nm and optical path length of 1 cm) in the wavelength range of 300-600 nm (see Fig. 1). The maximum absorption was at 400-450 nm in UV-Visible spectroscopy.

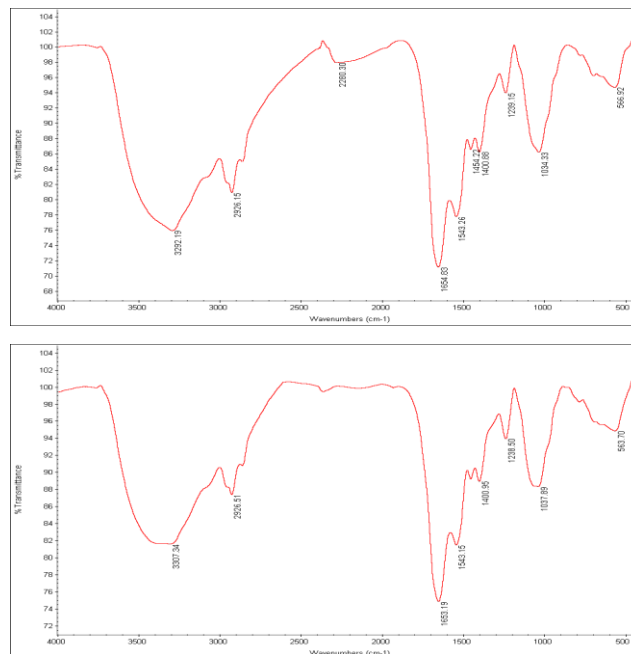


**Fig. 1** – Absorbance vs. wavelength spectrum of colloids of CdS nanoparticles at 30°, pH 9, stationary phase for *Escherichia coli* (a) and *Klebsiella pneumoniae* (b). The maximum absorption was at 450 nm

#### 3.2 FT-IR and XRD analysis

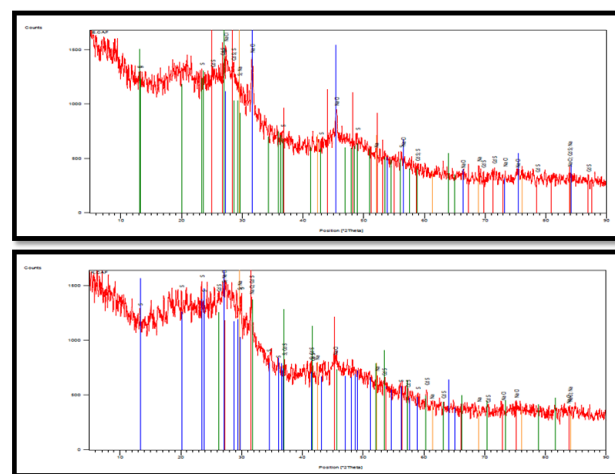
Purification of CdS nanoparticles was carried out according to the method previously described. For FT-IR

and XRD analysis, samples were dried. Freezing-drying method was used for this step. First, the samples were frozen at -20°C for 24 h and then dried at -37°C temperature for 10h with Freeze-drier system (CHRist, ALPHA 1-4LD). As shown in Fig. 2, the obtained dried sample was subjected to FT-IR spectrum using Fourier Transform IR spectrophotometer (NEXUS, Germany).



**Fig. 2** – FT-IR spectrum of pure CdS NPs. Cadmium sulfid nanoparticles were synthesized by *Enterobacteriaceae: Escherichia coli* (top) and *Klebsiella pneumoniae* (bottom)

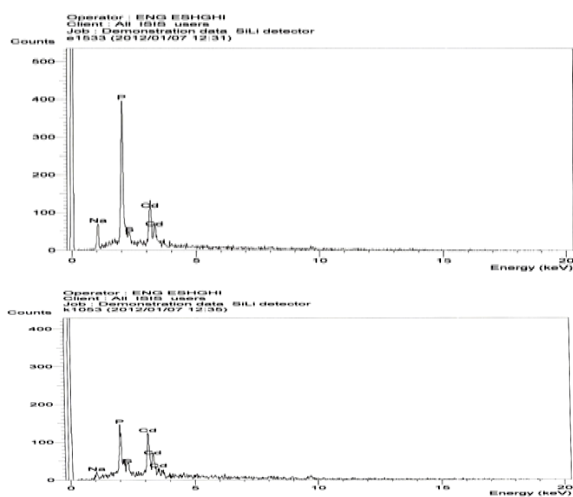
The phase formation and purity of CdS nanoparticles were checked by recording the powder XRD patterns (see Fig. 3) using an XDL 3000 powder X-ray diffractometer (SEIFERT, 3003 PTS). The X-ray diffracted intensities were recorded from 10° to 80° 2 $\theta$  angles. FTIR studies revealed that amino groups bound to particles account for the stability of NPs. Also FTIR studies established the existence of protein as the stabilizing and capping agent.



**Fig. 3** – Indexed X-ray diffraction patterns of n-CdS at room temperature, pH 9: *Escherichia coli* (top) and *Klebsiella pneumoniae* (bottom)

### 3.3 EDS (Energy Dispersive Spectroscopy)

In order to determine the elemental composition of the synthesized nanoparticles, EDS spectrum was recorded. In the recorded EDS spectrum, strong signals showed the presence of Cd and S. This confirms that the nanoparticles are made of CdS alone. EDS spectrum was recorded based on the micrographs measurements focusing on clusters of the nanoparticles. Resulting EDS spectrum from purified and dried CdS nanoparticle was shown in (see Fig. 4). This figure also shows the signals from Cd and S elements from other metals. In the analysis of CdS nanoparticles by energy dispersive spectroscopy (EDS) (LEO 440i, OXFORD), the presence of elemental CdS signal was confirmed. The CdS nanocrystallites display an optical absorption band peaking at 3-4 keV, which is the typical absorption of metallic CdS nanocrystallites due to the surface plasmon resonance.



**Fig. 4** – Purified and dried sample was subjected to EDS analysis which showed the presence of especially Cd and S from other metals (upper: *Escherichia coli* and lower: *Klebsiella pneumoniae*)

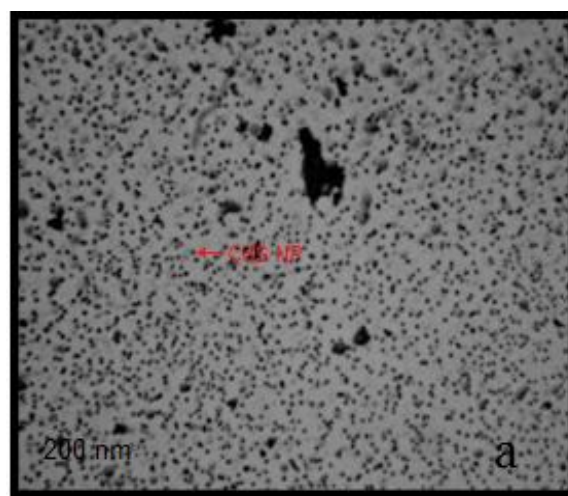
### 3.4 Scanning Electron Microscopy (SEM)

Electron micrographs of crystal morphologies and extracellular organization of CdS nanoparticles found in *Enterobacteriaceae*. For scanning electron microscopy (SEM) analysis, samples of the biologically synthesized nanoparticles were prepared on gold-coated SEM grids. In order to confirm the synthesis of nanoparticles, images were recorded using SEM (LEO 440i, OXFORD). These images showed (see Fig. 5) that the synthesized nanoparticles were spherical in shape and had uniform morphology. The size of the particles ranged between 5 and 200nm and the shape of the particles were found to be almost spherical.

## 4. DISCUSSION

In the last decades, nanotechnologies and biotechnologies, regarded apart from each other, are foreseen as the future technologies with the most perspectives, meeting the challenges of the 21st century. The combination of these two scientific fields founded the new

interdisciplinary science nanobiotechnology [1]. Increasing awareness towards green chemistry and biological processes has led to a desire to develop an environment-friendly approach for the synthesis of non-toxic nanoparticles. Unlike other processes in physical and chemical methods, which involve hazardous chemicals, microbial biosynthesis of nanoparticles is cost-effective and eco-friendly approach. Therefore, microbial synthesis of nanoparticles has been emerged as an important branch of nanobiotechnology. Future research on microbes-mediated biological synthesis of nanoparticles with unique optoelectronics, physicochemical and electronic properties are of great importance for applications in the areas of chemistry, electronics, medicine and agriculture [17]. Metallic and inorganic nanoparticles exhibit unique properties in terms of particle aggregation, photoemission, electrical and heat conductivity, and catalytic activity. These properties have recently been applied in different biological studies including bio-molecule detection, sample separation, purification and concentration, substrate coding, and signal transduction and amplification. In their application to environmental research, these nanoparticles further enhance the detection sensitivity of microbial monitoring, and the degradation and recovery efficiency of chemicals. Nevertheless, these nanoparticles exhibit potential threats to human health owing to their cytotoxicity. These health-related effects should be carefully addressed before the massive contamination of the environment by these nanoparticles [50]. In this study, the ability of *Enterobacteriaceae* in synthesizing Cadmium sulfide nanoparticles has been investigated. It was found out that the exposure of bacterial cells to Cadmium Sulfide ions, results in formation of Cadmium Sulfide nanoparticles either intracellularly or extracellularly. The synthesized nanoparticles were characterized and the effect of various conditions on the synthesis were analyzed. The synthesized Cadmium Sulfide nanoparticles were characterized using UV-Visible spectroscopy, scanning electron microscopy (SEM), XRD and FTIR analysis and energy-dispersive spectroscopy (EDS).



**Fig. 5** – SEM images of n-CdS synthesized using (a: *Escherichia coli* and b: *Klebsiella pneumoniae*) (scale bar at 200 nm).

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