Intracellular biosynthesis of cadmium sulfide nanoparticles

Intracellular biosynthesis of Cadmium Sulfide nanoparticles using culture supernatant of *E s c her i c h ia c o li*

Abstract

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 new costeffective procedures are being developed. 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.

Here an attempt was made to biologically synthesize fluorescent cadmium sulfide nanoparticles. The present study uses *Esc h er ic h i a c o l i* PTCC 1330 as a potential producer for the green synthesis of CdS nanoparticles. Biologically synthesized nanoparticles were characterized and confirmed after 24 h of incubation at room temperature using electron microscopy, XRD, EDS and FTIR. The size distribution of the nanoparticles was found to be 5–200nm followed by which the consequence of time, growth of the organism, pH, concentration of CdCl2 and Na2S on the synthesis of nanoparticles were checked. Enhanced synthesis and fluorescence emission of CdS nanoparticles were achieved at pH 9.

Keywords: Nanotechnology; Biological synthesis; Silver nanoparticles; *Esc h er i c h i a c o l i* ; Eco-friendly

1. Introduction

Bionanotechnology has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for synthesis of nanomaterials. Nanoparticles are clusters of atoms in the size range of 1–100 nm. “ Nano” is a Greek word synonymous to dwarf meaning extremely small. The use of nanoparticles is gaining impetus in the present century as they posses defined chemical, optical and mechanical properties. The metallic nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains[1-2].

Nanoparticles exhibit completely new or improved properties compared to larger particles of the bulk material and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles. Nanoparticles present a higher surface area to volume ratio with decrease in the size of the particles[1-2].

As the specific surface area of nanoparticles is increased, their biological effectivene can also increase on the account of a rise in surface energy. Nanoparticles have a wide range of applications, as in combating microbes, biolabelling, and in the treatment of cancer. Size control during synthesis of particles is an important criterion in the arena of silver nanoparticle biosynthesis. Depending on the size of the nanoparticles, their applications branch out. Although AgNPs are synthesized both intra- and extra-cellularly, the latter method of biosynthesis

of nanoparticles is highly advantageous because of ease of control over the environment, large-scale synthesis and easy downstream processing steps. It is well known that the electronic and optical properties of metal nanoparticles are heavily size- and shape-dependent. Controlling the size, shape and surrounding media of metal nanoparticles are important as many of their intrinsic properties are determined by these parameters[3-8].

This study illustrates the synthesis of CdS nanoparticles using the bacterium *Esc h er i c h i a c o l i* PTCC 1330. The morphology of the samples was analyzed using Transmission electron microscopy (TEM) and the particles formed were characterized to be nanoparticles. The size of CdS nanoparticles in aqueous solution has been calculated using UV–Vis spectroscopy, XRD, EDS, FTIR and TEM measurements. The nanoparticles are found to be polydisperse nanocubes in the size range 5–200 nm[3].

2. Materialsand methods

2. 1. Mate ri als, bac t eri a l str a in

The test strain was: *Esc h er ic h i a c o l i* PTCC 1330, The strain was prepared of IROST, CdCl2 was purchased from Hi Media laboratories, India and Na2S was purchased from Merck, Germany. All other chemicals used are of analytical grade.

2. 2. Preparation of s u p ernatants

Muller-Hinton broth (MHB) was prepared, sterilized, and inoculated with a fresh batch of test strain. The culture flasks were incubated for 24 h at 30ºC

for bacteria. After the incubation period, the cultures were centrifuged at 12, 000 rpm and their supernatants were used for further experiments.

2. 3. Synthe s is of Cadmi u m Sulfide n a noparti c les

The obtained supernatants were washed with phosphate buffer saline (pH 7. 0) for 3 times. 1mM solution of CdCl2 (for *E. c o l i*) 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 Na2S solution was slowly added to the solution. The samples were then incubated at room temperature with end-over-en rotation for 10 min[9-10].

2. 4. Purif ic ation of na n oparti c les

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. 22µm filter to eliminate cell-debris and other pollutants. The filtered solutions were then used for characterization of CdS nanoparticles.

2. 5. Effe c t o f growth pa r ameters on CdS nanoparti c les pro d uction

2 . 5 . 1 E ff ect o f C d C l 2 a n d N a 2 S c o n c e n t r a tio n o n s y n t h es i s a n d p a r ti c l e S i zes

To obtain the optimum concentration of CdCl2 and Na2S that yields the maximum synthesis of nanoparticles and particle-size distribution, CdCl2 and Na2S, at concentrations ranging from 1 to 10mM, was added to the supernatant at pH 9. 0 and temperature 30ºC.

2 . 5 . 2 E ff ect o f t em p e r a t ure an d p H o n n an o p a r ti c l e sy n t h es i s a n d p a r ti c l e si zes

To obtain optimum conditions for maximum synthesis of nanoparticles and particle-size distribution, the obtained the optimum concentration of CdCl2 and Na2S was added to the supernatant and incubated at various temperatures (25–30 ºC) and pH conditions(5–11). 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 30ºC and pH of 9.

2. 6. Synthe s is of CdS nanoparti c les at various g rowth phas e s and time p eriod

To find the effect of growth phase of the organism on CdS nanoparticles production, *Esc h e r i c h i a c o l i* 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 strain was achieved in stationary phase.

3. Resultsand discussion

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water-treatment. The development of techniques for the controlled synthesis of nanoparticles of well defined size, shape and composition, to be used in the biomedical field and areas such as optics and electronics, has become a big challenge. Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology.

One of the most exciting research areas in modern nano-science and technology is the interaction between inorganic molecules and biological structures. It is well established now that many organisms can produce inorganic materials either on intra- or extra-cellular level. In order to meet the growing demand of nanoparticles, eco-friendly methods for nanomaterials synthesis need to be developed which are free of using toxic chemicals in the synthesis protocol[11-13].

In addition to gold and silver nanoparticles, semiconductors such as CdS, ZnS, and PbS have been greatly focused on. Development of protocols for the synthesis of such semiconductors (the so-called quantum dots) is growing. These luminescent quantum dots are emerging as a new class of

materials for biological detection and cell imaging, based on the conjugation of semiconducting quantum dots and biorecognition molecules. Fluorescent nanoparticles (CdS, CdSe, CdTe and etc.) can be used for conjugation of biomolecules instead of organic fluoroprobes such as peptides, antibodies and nucleic acids. Moreover, different electronic catalytic and optical behaviour of CdS and NPs have been investigated before. Also, CdS nanoparticles are used for cancer diagnosis and treatment. CdS is immensely used in field effect transistors, solar cells, light emitting diodes, photocatalysis, photoluminescence, infrared photodetector, environmental sensors and biological sensors [14-18].

3. 1. Characteriza t ion of synthesi z ed Cadmium Sulfide nan o parti c les

3. 1. 1. UV-Visible s p ec tr ophotometer

Purified CdS nanoparticles were dried at 30 ºC for 4 h. The dried particles were dispersed in deionized water and were measured using a UV–Visible spectrophotometer (CARY, 100Conc, UV Pharma spec1700 with a resolution of 0. 72 nm and optical path length of 1 cm) in the wavelength range of 300-600 nm (Fig. 1). The maximum absorption was at 400-450 nm in UV-Visible spectroscopy.

3. 1. 2. FT-IR and XRD a nalysis

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 -20ºC for 24 h and then dried at -37ºC temperature for 10h with Freeze-drier system(CHRist, ALPHA

1-4LD). The obtained dried sample was subjected to FT-IR spectrum (Fig. 3) using Fourier Transform IR spectrophotometer (NEXUS, Germany). The phase formation and purity of CdS nanoparticles were checked by recording the powder XRD patterns (Fig. 2) using an XDL 3000 powder X-ray diffractometer(SEIFERT, 3003 PTS). The X-ray diffracted intensities were recorded from 10â-¦ to 80â-¦ 2θ 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.

3. 1. 3. EDS (Energy Di s persive S p e c troscopy)

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 (Fig. 4). 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 (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.

3. 1. 4. Transmission El ec tron M i cro s copy (TE M)

Transmission electron microscopy (TEM) (model EM 208 Philips) was used to determine the morphology and shape of nanoparticles. Purified CdS nanoparticles from extra-cellular culture supernatant using centrifugation was characterized by TEM. TEM revealed the average size of particles as 100 nm. TEM images show that they are relatively uniform in diameter and have spherical shape. The different fractions obtained on a continuous sucrose gradient were analyzed. (Fig. 5) shows a representative TEM image recorded from the drop-coated film of the CdS nanoparticles synthesized by treating the CdCl2 and Na2S solution with culture supernatants of *E. c o l i* PTCC 1330. The particle size histogram of CdS particles (Fig. 5) shows that the particles range in size from

5 to 200 nm and possess an average size of 75. 5 nm.

In addition, the TEM image shows at least two different areas, one with higher contrast due to the CdS nanoparticles and other with lower contrast probably due to other micro (or even nano) crystals originating from insoluble Cd, S salts.

24 h (particles at higher resolution shown by scale bar of a: 100 nm, b: 200nm).

4. Conclusions

Bacterial strain of *Es c h er ic h i a c o l i* PTCC 1330, studied in the present research, can be used in order to biologycal synthesizer of Cadmium Sulfide

Nanoparticles under special conditions of Time, pH, Temperature, grow of the organism, concentration of CdCl2 and Na2S.

The synthesis of nanoparticle circles around enzyme phytochelatin synthase, which exists in surface of *E. c o l i* . This enzyme has been previously used for in vitro synthesis CdS nanoparticle under special conditions . The enzyme catalyzes the reaction of transpeptidation of *c* -Glu-Cys dipeptide from a GSH molecule to a second molecule of GSH. Thus, phytochelatin synthase enzyme may be involved in the synthesis of CdS nanoparticles.

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