Potent antibacterial activity of nano CdO synthesized via microemulsion scheme

M. Shukla 1, S. Kumari 1, S. Shukla 2, R.K. Shukla 3

1Department of Chemistry, Agra College, Agra-282002, India
2Department of Botany, RBS College, Agra-282004, India
3Department of Chemistry, RBS College, Agra-282004, India

Received 16 Sept 2011, Revised 8 Mar 2012, Accepted 8 mar 2012
* Corresponding Author: Email: vermasuman03@yahoo.co.in

Abstract
The antibacterial activities of different concentration of the CdO nanoparticles were tested by treating Escherichia coli (Gram negative) cultures with CdO nanoparticles. Study indicates that cadmium oxide nanoparticles show effective antibacterial activity toward the gram-negative bacterium E. coli. Cadmium oxide nanoparticles have been successfully synthesized from CTAB/n-butyl alcohol/cyclohexane/water as water-in-oil microemulsion. A rational mechanism of synthesis based on fusion, aggregation, and coalescence of microemulsion droplets is proposed for the selective formation of different morphologies. X-ray powder diffraction and Scanning electron microscopy with EDAX analysis have been used to characterize the cadmium oxide nanoparticles.

Keywords: Escherichia coli, antibacterial agent, optical density, reverse micelle, scanning electron microscopy.

1. Introduction
Antimicrobial modification to prevent the growth of detrimental microorganisms is a highly desired objective. Microbial infestation can result in serious infection [1-2]. Hence, there is a significant interest in the development of antimicrobial materials and surfaces for applications in the health, biomedical, food and personal-hygiene industry. The nanomaterials, based on the metal ions, exhibit broad-spectrum biocidal activity towards different bacteria, fungi, and viruses [2]. Nanomaterials are known to deactivate cellular enzymes and DNA by coordinating to electron donating groups such as thiols, carboxylates, amides, imidazoles, indoles, hydroxyls, and so forth. They cause pits in bacterial cell walls, leading to increased permeability and cell death [3]. Antibacterial agents, used in textile industry, are divided into two parts: the organic and inorganic matters. The organic antibacterial materials have been used as insecticides and bactericide for many years. Unfortunately, high temperatures in manufacturing process reduce their antibacterial properties. However inorganic antibacterial agents show excellent resistance against the bacterial and thermal stability [4]. Nano-structured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical
applications [5-7]. At present, the use of nano-structured materials is becoming more widespread and a major advantage over either organic or inorganic nanoparticles offers many possibilities of applications in the areas of physics, chemistry, pharmacy, surface coating agents, textile sizing, agriculture, biochemistry and so on [5-10]. It has been demonstrated that specially formulated metal oxide nanoparticles have good antibacterial activity, and antimicrobial formulations comprising nanoparticles could be effective bactericidal materials [11]. Over the past few decades, inorganic nanoparticles, whose structure exhibit significantly novel and improved physical, chemical, and biological properties and functionality due to their nano scale size, have elicited much interest. Nano-materials are called “a wonder of modern medicine”. It is stated that antibiotics kill perhaps a half dozen different disease-causing organisms but nano-materials can kill some 650 cells [12]. Resistant strains fail to develop if we apply nanoparticle-based formulations in their culture media. In laboratory tests with nanoparticles, the bacteria, viruses, and fungi are killed within minutes of contact.

To date there are many methodologies available for synthesizing metal oxide nanoparticles such as hydrothermal, solvothermal and microwaves assisted methods. Microemulsion has been successfully employed to obtain relatively small particles (i.e. High surface area) with well controlled properties [13]. However, water-in-oil (w/o) microemulsion or reversed micelle techniques is most recognized method due to several advantages, for instance, soft chemistry, no extreme pressure or temperature control requirements, ease of handling and no special or expensive requirements. Uniform and monophasic nanostructures can be obtained by controlling microemulsion parameters (solvent, surfactant, co-surfactant and water to surfactant ratio) [14-15]. The microemulsion (ME) is thermodynamically stable dispersion of nanometer-sized water droplet in a continuous oil medium. The stability of these drops is attributed to the presence of adsorbed surfactant molecules at the oil-water interface of the drop. The drops undergo Brownian motion, thus colliding with each other and occasionally coalescing. Reactants predissolved in water drops undergo chemical reaction in this process. Subsequently, the insoluble reaction product nucleates to form a nanoparticle inside the drops. The water-pool of w/o microemulsion act as nonreactors to form the nanoparticles and the surrounding surfactant layer limits their growth and protect them from aggregation [16]. Ethayaraja et al. [17] give the combined approach of experiments and Monte Carlo simulation to understand the mechanism of microemulsion synthesis of nanoparticles.

This study aimed to investigate the potent long lasting antibacterial activity of nano-CdO toward the gram-negative bacterium E. coli, synthesized via microemulsion scheme.

2. Experimental

2.1 Materials

Cetyl tri-methyl ammonium bromide (CTAB), CdCl$_2$.6H$_2$O, CdO, cyclo-hexane, ascorbic acid, sodium citrate tribasic dehydrate, ammonium sulphate and ethanol were purchased from Sigma and Aldrich. All the chemicals used were of molecular grade. Deionized water was used throughout the experiment. Gram-negative bacterium E. coli was used for the present experiment. Nutrient Broth (sigma) was used in growing and maintaining the bacterial cultures. CdO nanoparticles, with the particle-size of 45 nm, were used throughout the experiment. The particles were suspended in sterile water and sonicated for 15 min before use.

2.2 Microemulsion synthesis of CdO nanoparticles

To prepare the microemulsion cyclohexane(oil), CTAB(surfactant), n-butanol(co-surfactant) were used as oil phase. Surfactant and co-surfactant were mixed by magnetically stirring until the mixture became transparent and the mole ratio of the surfactant to co-surfactant was 1:4. The microemulsion was divided into two parts one containing CdCl$_2$.6H$_2$O and other containing the precipitating agent, NaOH. Both the microemulsions were mixed together with continuous stirring drop by drop until the pH~9.0 at room temperature and agitated vigorously using magnetic stirrer. After mechanical agitation for about 72 hours, the product was collected by centrifugation at 20,000 rpm and washed with deionized water and then alcohol. The as-prepared precursor was dried in air and then in oven at 70°C for 1 hour. Finally, the precursor was sintered at 720°C for 1 hour in muffle furnace.
2.3 Antibacterial Properties

Bacterial susceptibility to nanoparticles

To examine the susceptibility of E. coli to nano CdO, three estimation methods were used with three tiles repetition. Bacterial growth in the presence of nano CdO in liquid medium. In the first method, the bacteria were grown in nutrient broth (NB). To start the growth, 2 mL of the overnight-cultured E. coli stock was added to 100 mL NB, containing 0.12% glucose with and without 0.01, 0.5 and 1% nano CdO, separately. The bacteria were aerobically cultured at 30ºC for 24 hours. Optical density (OD) measurements were taken at 600 nm to monitor the bacterial concentration.

Bacterial killing in the presence of nano CdO in liquid medium

In the second method, the culture solution was centrifuged and the cells were washed and re-suspended in distilled water, reaching a final concentration of 6.3 log CFU/ml in each of the sample flasks and incubated at 4ºC. The final concentration of the E.coli suspensions was made in 100 ml distilled water. Different amounts of nano CdO (0.01, 0.5 and 0.1%) were then separately added to the bacterial suspensions to keep in contact with the bacterial cells and shaken at 40ºC for 48 hours. Optical density (OD) was measured to obtain the results. Aliquots of 0.1 ml of the growth mixtures (water +bacterial cells + nanoparticles) were sampled every two hour. The number of resulting bacterial cells was noted after every two hours of incubation. Bacterial number was determined by measuring the optical density (OD) at 600 nm. The OD values were converted into the E. coli concentration as log CFU/ml [18].

Bactericidal effect of nano CdO on E.coli by well diffusion method

In the third method bacteria were grown on nutrient agar plates. Approximately 105 CFU were applied to the plates. Three wells were made with the help of well borer. Different concentration of CdO nanoparticles (0.01%, 0.5% and 1%) were added to the three walls. The plats were incubated at 300 C for 48 hours.

3. Results and discussion

3.1 X-Ray Diffraction

X-Ray Diffraction pattern shown in Fig 1 (X-Pert, PRO XRD system, Netherland) reveals crystalline nature of the samples.

![XRD pattern for CdO nanoparticles](image)

**Fig 1.** XRD pattern for CdO nanoparticles

The average crystalline size was 40 nm obtained from the FWHM of peak corresponding to 2θ=32.986º, calculated by Debye-Scherrer formula [19] which is given by:

\[ L = \frac{K\lambda}{\beta \cos \theta} \]
where L is the average size of crystalline assuming to be cubic three dimensional, K=0.94 particle diameter. The λ (1.541 Å) is wavelength of X-ray, β is full width at half maximum (FWHM) of the diffraction peak and θ is diffraction angle of the diffraction. FWHM is calculated by the Warren’s formula $B^2 = (B_M^2 - B_S^2)$ where $B_M$ is the full width at half maxima of the sample and $B_S$ the full width at half maximum of the standard quratz.

3.1 SEM-EDAX analysis
The morphology of the cadmium oxide nanoparticles was investigated with a scanning electron microscope (SEM, LEO-0430, Cambridge) at room temperature, mounted directly onto sample stub and coated with gold film (~200 nm) under reduced pressure (0.133 Pa).

![Fig 2. Scanning electron micrograph of CdO nanoparticles](image1)

The scanning electron micrograph (Fig 2) and EDAX (Fig 3) analysis revealed that the crystallites of cadmium oxide nanoparticles is spherical having diameter 40 nm and no characteristic peaks of impurities or other precursor compounds are observed.

3.3 Reaction mechanism in formation CdO nanoparticles in microemulsion
During the preparation of nanoparticles, the following reactions might occur:
Reaction (1) and (2) take place in the microemulsion droplets. In the present case, microemulsion was used as the reaction media to prepare CdO nanoparticles. The micro droplets in the microemulsion are in spherical shape with different diameter from 10-100 nanometer and their sizes can be adjusted artificially. Due to the controllable water pool, microemulsion serves as excellent media for preparation of wide variety of colloidal nanoparticles. Moreover, microemulsion provides the easy control over the nanoparticle size and shape. The mechanism of the reaction in microemulsion can be divided into three steps: I- solublization and mixing of reactants: in this way two different reactants in different micelles (nuclei) exchange with each other and the reaction takes place. Due to the coalescence of the reverse micelles, the exchange of the material in water droplets occurs, which causes a reaction between the cores of reversed micelles. Since the diameter of the water droplet is constant, nuclei in different water cores cannot exchange each other. It is a precondition to accomplish the potential reaction that the reactants exchange via colliding between two water nuclei[20-21], II-contact of different reactants: instant dimmer is formed while the droplets collide each other, III-Reaction, nucleation and growth of dimmer: this instant dimer provides water channel for the two droplets to exchange reactants at this moment. The formation of instant dimer changes the shape of surfactant layer, in very short time due to high energy of the system. Just during this uninterrupted combination and separation, the reaction takes place and then molecules of metal oxide engender. Some of the molecules aggregate as nucleus that accelerates the reaction as a catalyst. More and more resultant molecules adhere on the nucleus to form a particle of nanosize.

3.3 Antibacterial activity
During the recent analysis the antibacterial activities of different concentrations of nano-CdO were investigated to find out the best concentration that can have the most effective antibacterial property against the E. coli culture. Good growth inhibition results were observed when the bacterial cells were incubated with CdO nanoparticles during the liquid cultures.

**Effect of nano CdO on the growth of E. coli in liquid medium**
In the first study, we investigated the effect of different concentrations of nanoparticles in liquid culture of E. coli. The optical density of the medium was investigated as the number of bacteria after contact with the nanoparticles. Fig. 4 shows the effect of different concentrations of nano-CdO on the growth of E. coli.

![Fig. 4. E.coli concentration dependence upon different concentrations of CdO in the culture medium](chart)
As demonstrated in this figure, 0.01% nano-CdO did not have antibacterial effect while, 0.5 and 1% nano-CdO was highly efficient in inhibiting the E.coli growth as compared to control group. This figure shows that the presence of 0.5% nano-CdO caused a 1.5 times decrease and 1% nano CdO caused a 2.5 times decrease in the optical density of bacterial cultures as compared to control experiment.

**Bactericidal effect of nano CdO on E.coli in liquid medium**

The second study, estimation of the number of viable E. coli cells in contact with 1% CdO was carried out in water at 4ºC for different contact time intervals. Our result showed the reduction of E. coli cells, upon the addition of 1% nano CdO to the bacterial culture. Fig. 5 represents the number of viable E. coli cells in contact with 1% CdO, suspended in water at 4 ºC for different contact times. After the E. coli were suspended in water alongwith 1% nano CdO, it showed complete bacterial killing after 36 hours of their contact with 1% nano-CdO. Fig. 5 shows that administration of nano CdO to the bacterial cultures killed most of the bacteria in 2 days. These results demonstrate that nano CdO have a high antibacterial efficiency against E. coli.

![Graph showing bacterial killing kinetics](image)

**Fig. 5**: Killing kinetics of 1%CdO on the E. coli culture.

**Bactericidal effect of nano CdO on E.coli by well diffusion method**

Our data of third study, well diffusion method is in accordance with the above two estimation methods. After 48 hours the plates were visualized. Fig. 6 shows that the zone of inhibition was absent, 1.2 mm and 3.1mm around 0.01%, 0.5% and 1% CdO nanoparticle well, respectively.

![Image showing well diffusion](image)

**Fig. 6**: Bactericidal effect of nano CdO on E.coli by well diffusion method
The present data demonstrate that a formation made with the biologically stabilized nano CdO can be useful in the treatment of infectious disease caused by E. coli. Our data is in accordance with the previous studies, dealing with the antibacterial effects of nanomaterials [22-24]. The above data demonstrate that CdO nanoparticles can be useful in the treatment of infectious diseases caused by E.coli. Several investigators have suggested the possible mechanism involving the interaction of nano materials with the biological macromolecules. It is believed by Zhang and Chen that microorganisms carry a positive charge. This creates an “electromagnetic” attraction between the microbes and treated surface. Once the contact is made, the microbe is oxidized and dead instantly [23]. Wang et al. concluded in their studies that in the aqueous system, both nanoparticles and bacteria tended to aggregate, and the nanoparticle toxicities were mainly attributed to the ions dissolved in the solutions [25]. Russell and Hugo concluded in their studies that a strong binding of nanoparticles to the outer membrane of E.coli causes the inhibition of active transport, dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and protein synthesis, leads to cell lysis [26-27]. Generally it is believed that nanomaterials release ions, which react with the thiol groups (-SH) of the proteins present on the bacterial cell surface. Such proteins protrude through the bacterial cell membrane, allowing the transport of nutrients through the cell wall. Nano materials inactivate the proteins, decreasing the membrane permeability and eventually causing the cellular death [23].

Conclusion

It is concluded that the CdO nanoparticles shows effective antibacterial activity towards the gram negative bacterium E.coli. Water-in-oil microemulsion, or reverse micelles, offered versatile and attractive approach for uniform nanoparticle formation since they provide good control over particle shape and size. This technique has its own advantage and is subjected to wider use in preparing nanoparticles.

Acknowledgement

Thanks are due to financial assistance provided by UGC, New Delhi and IIT Guwahati, for analysis.

References


