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Der Pharma Chemica, 2013, 5(5):301-306
(<http://derpharmachemica.com/archive.html>)



ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis and characterization of nanocrystalline CdFe_2O_4 and its antibacterial activity against *Escherichia coli*

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ABSTRACT

Nanocrystalline CdFe_2O_4 of were synthesized by sol-gel citrate method. Structural properties, antimicrobial activity were investigated. An assay shows the broad spectrum antimicrobial activity of nanoparticles against Gram negative pathogenic strain. The existence of CdFe_2O_4 structure of ferrite was confirmed by X-Ray diffraction (XRD) and Fourier Transform infrared (FTIR). Result indicated that nanosized particles greatly influence the antimicrobial activity of the sample

Keywords: CdFe_2O_4 nanoparticles, antimicrobial activity, FTIR, XRD

INTRODUCTION

The emergence of infectious diseases in general poses a serious threat to public health worldwide, especially with the emergence of antibiotic-resistant bacterial strains. Generally, both Gram-positive and Gram-negative bacterial strains are thought to present a major public health problem. Over the years, antibiotics have been used to control infections resulting from both community and hospital environments [1–3]. Current advances in the field of nanobiotechnology, particularly the ability to prepare metal oxide nanomaterials of specific size and shape, are likely to lead to the development of new antibacterial agents. The functional activities of nanoparticles are influenced largely by the particle size. Therefore, nanoparticles have received great attention due to their unique physical, chemical, and effective biological properties in various fields, including medicine. The Properties of nanoparticles can easily be altered by reducing or changing their size, especially when the manipulations are done at the nanometer scale [4–7]. In addition, nanoparticles with smaller particle size have been reported to show good antimicrobial activity [8]. Antimicrobial activity of nanoparticles has largely been studied with human pathogenic bacteria such as *Escherichia coli* [12-13]. Bactericidal activity of such nanoparticles in part depends on (1) size, (2) stability, and (3) concentration in the growth medium. While growing in medium amended with nanoparticles, the bacterial population growth can be inhibited by specific nanoparticle interactions [7]. 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 [9-11].

In recent years, nanotechnology has been flourishing. The development of Nano materials is a foundation for the development of nanotechnology. Nanobiotechnology is highly interdisciplinary by nature and requires close collaboration between biologists, physical scientists and engineers [25] Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefit [26] Nanoparticles exhibit completely new or improved properties based on specific

characteristics such as size, distribution and morphology. As specific surface area of nanoparticles is increased, their biological effectiveness can increase in surface energy. Nano materials refer to materials with special properties, whose geometric dimension reaches Nano scale. Among Nano- materials, great importance has been attached to Nano oxide [14-16]. Nano-structured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, specially in biological and pharmaceutical applications [17-19]. In recent years, nanotechnology has been flourishing the development of Nano materials is a foundation for the development of nanotechnology. Nano materials refer to materials with special properties, whose geometric dimension reaches Nano scale. Among Nano-materials, great importance has been attached to Nano oxide [14-16]. Nano-structured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, specially in biological and pharmaceutical applications [17-19]. The aim of this paper is to explain theoretically the reason of nano- CdFe_2O_4 formation when using the sol-gel process that gives a single phase at both lower sintering temperatures [20]. This study aimed to investigate the potent long lasting antibacterial activity of nano- CdFe_2O_4 toward the gram-negative bacterium *E. coli*, synthesized via sol-gel citrate method.

MATERIALS AND METHODS

All chemicals and solvents were analytical grade and purchased from commercial sources.

Synthesis of CdFe_2O_4 nanoparticles

The CdFe_2O_4 nanoparticles prepared by using sol-gel citrate method. The stoichiometry mixture of Cadmium nitrate, Ferric nitrate magnetically stirred with citric acid and ethanol at 80° C for 3hrs to get homogeneous and transferent solution. The solution was further heated at about 130° for 12 hrs in pressure vessel to form the gel precursor. The prepared product was subjected to 3hrs heat treatment at 350°C in muffle furnace and then milled to a fine powder. The dried powder then calcinated in range of 350°-650°C in order to improve the crystallinity of material

Method for antimicrobial activity

Bacterial susceptibility to nanoparticles

To examine the susceptibility of *E. coli* to nano CdFe_2O_4 , three estimation methods were used. Bacterial growth in the presence of nano CdFe_2O_4 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 0.5 and 1% nano CdFe_2O_4 , separately. The bacteria were aerobically cultured at 30°C for 24 hours. Optical density (OD) measurements were taken at 600 nm to monitor the bacterial concentration.

Bacterial killing in the presence of nano CdFe_2O_4 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 4°C. The final concentration of the *E. coli* suspensions was made in 100 ml distilled water. Different amounts of nano CdFe_2O_4 (0.5 and 0.1%) were then separately added to the bacterial suspensions to keep in contact with the bacterial cells and shaken at 40°C 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 [24].

*Bactericidal effect of nano CdFe_2O_4 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. Different concentration of CdFe_2O_4 nanoparticles (, 0.5% and 1%) were added to the disc. The plats were incubated at 300° C for 4 hours.

RESULTS AND DISCUSSION

Morphological analysis

1. XRD

X-Ray diffraction pattern shows in Fig 1. (X-pert, PRO XRD System, Punjab) reveals crystalline nature of sample.

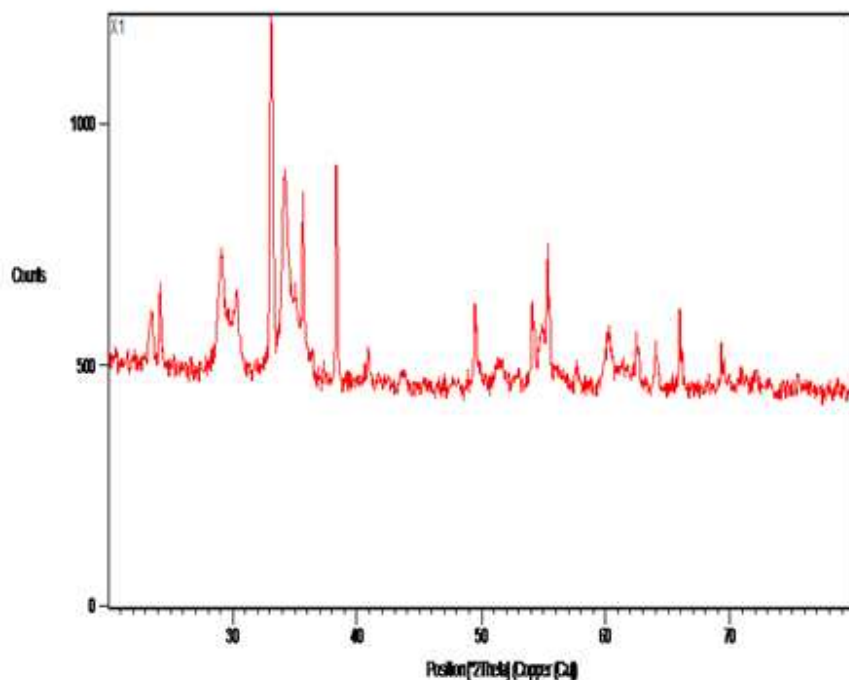


Fig 1.XRD pattern for CdFe₂O₄ Calcinated at 550°C

The average crystalline size was 30nm obtained from FWHM of peak corresponding to 2θ calculated by Debye – Scherrer formula which is given by, $L = k \lambda / \beta \cos\theta$ Where, L is the average size of crystal, K= 0.9 particle diameter, the λ (0.154 Å) is wavelength of X-Ray, β is full width at half maximum (FWHM) of the diffraction peak and 2θ is the diffraction angle of diffraction. FWHM is calculated by warren’s formula $B^2 = (B_m^2 - B_s^2)$ where B_m is full width at half maximum of the sample and B_s the full width at half maximum of standard quartz.

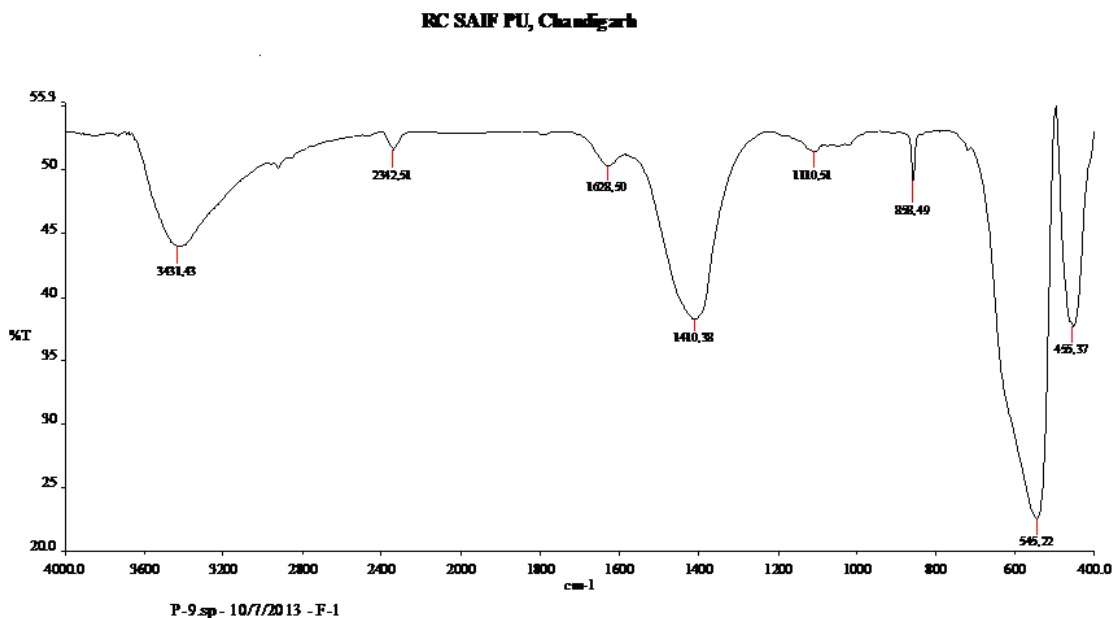


Fig 2. FTIR spectrum of CdFe₂O₄ nanoparticle calcinated at 550°C

2. FTIR

FTIR spectra were recorded in range of 400-4000 cm^{-1} **Fig 2.** Shows the FTIR spectra of CdFe_2O_4 sample calcinated at 550°C. It is observed that most intense band of spectrum, splitted in 1410 cm^{-1} and 455 cm^{-1} , due to stretching of Cd-O-Fe bond of tetrahedral building units forming the structure. The wide absorption in the high energy region of spectrum, centered at 3431 cm^{-1} , resulting from -OH stretching is associated to the vibration of water molecule coordinated to ferrite structure that is a broad band at 3431 cm^{-1} that assigned to absorption of H_2O from the atmosphere or OH group of alcohol. Very small band at 2342 cm^{-1} is due to adsorbed or atmospheric CO_2 . The characteristics small band at 1628 cm^{-1} is assigned to both asymmetric and symmetric C-O respectively. Two small peaks at 455 and 545 cm^{-1} that are characteristics of poorly crystalline $\alpha\text{-Fe}_2\text{O}_3$.

Antimicrobial properties

During the recent analysis the antibacterial activities of different concentrations of nano- CdFe_2O_4 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 CdFe_2O_4 nanoparticles during the liquid cultures.

Effect of nano CdFe_2O_4 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. 3 shows the effect of different concentrations of nano- CdFe_2O_4 on the growth of E. coli. **Fig. 3.**

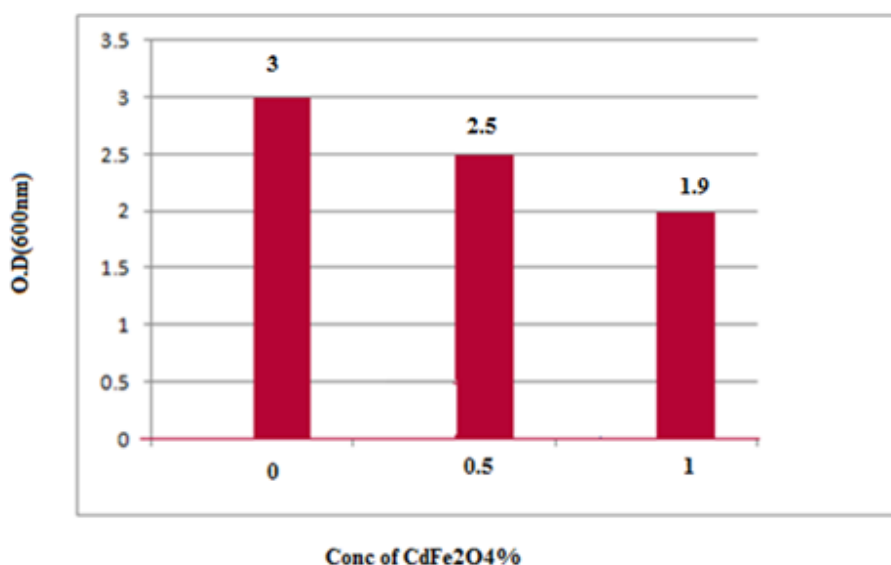


Fig 3. E.Coli concentration dependence upon different concentration of CdFe_2O_4 in culture medium

As demonstrated in this figure, 0.5% nano- CdFe_2O_4 did not have antibacterial effect while, 1% nano- CdFe_2O_4 was highly efficient in inhibiting the E.coli growth as compared to control group. This figure shows that the presence of 0.5% nano- CdFe_2O_4 caused a 0.5 times decrease and 1% nano CdFe_2O_4 caused a 0.6 times decrease in the optical density of bacterial cultures as compared to control experiment.

Bactericidal effect of nano CdFe_2O_4 on E.coli in liquid medium

The second study, estimation of the number of viable E. coli cells in contact with 1% CdFe_2O_4 was carried out in water at 4°C for different contact time intervals. Our result showed the reduction of E. coli cells, upon the addition of 1% nano CdFe_2O_4 to the bacterial culture. Fig. 4 represents the number of viable E. coli cells in contact with 1% CdFe_2O_4 , suspended in water at 4 °C for different contact times. After the E. coli were suspended in water along with 1% nano CdFe_2O_4 , it showed complete bacterial killing after 36 hours of their contact with 1% nano- CdFe_2O_4 . Fig. 4 shows that administration of nano CdFe_2O_4 to the bacterial cultures killed most of the bacteria in 2 days. These results demonstrate that nano CdFe_2O_4 have a high antibacterial efficiency against E. coli.

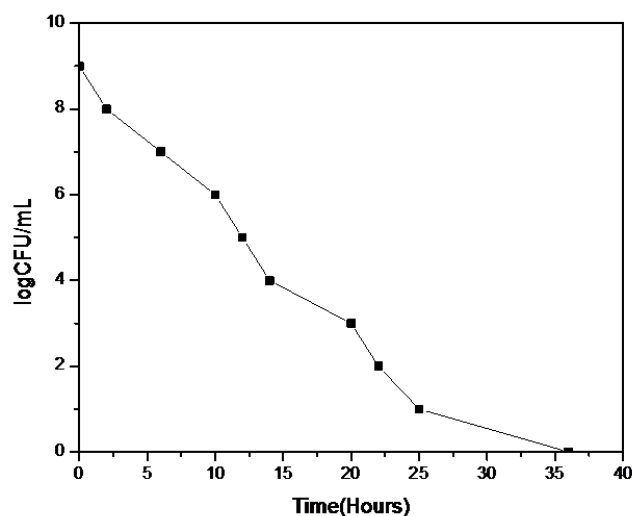


Fig. 4: Killing kinetics of 1% CdFe₂O₄ on the E. coli culture

Bactericidal effect of nano CdFe₂O₄ on E.coli by Disc diffusion method

Our data of third study, Disc diffusion method is in accordance with the above two estimation methods. After 4 hours the plates were visualized. Fig. 5 shows that the zone of inhibition was absent, 12 mm, 15mm around 0.5% and 1% CdFe₂O₄ nanoparticle well, respectively.

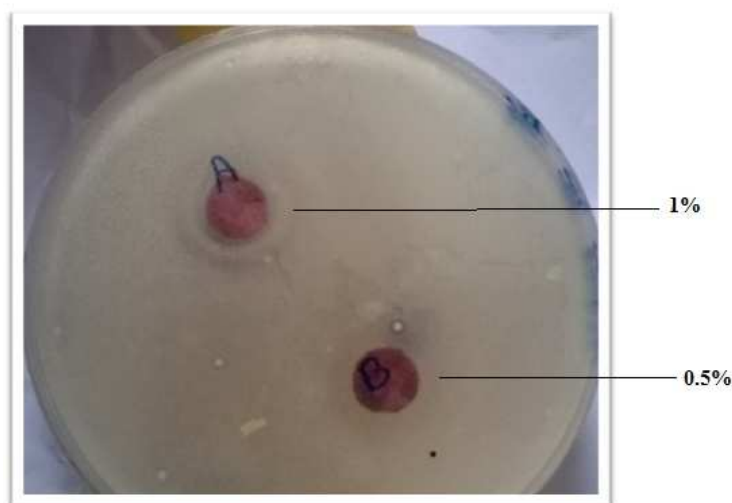


Fig 5. Bactericidal effect of nano CdFe₂O₄ on E.coli by Disc diffusion method

The present data demonstrate that a formation made with the biologically stabilized nano CdFe₂O₄ 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 [21-23]. The above data demonstrate that CdFe₂O₄ nanoparticles can be useful in the treatment of infectious diseases caused by E.coli.

CONCLUSION

It is concluded that the CdFe₂O₄ nanoparticles shows effective antibacterial activity towards the gram negative bacterium E.coli. Offered versatile and attractive approach for uniform nanoparticle formation since they provide good control over particle shape and size. Nanosized particles greatly influence the antimicrobial activity of the sample This technique has its own advantage and is subjected to wider use in preparing nanoparticles.

Acknowledgement

The author is thankful to Nanotechnology Laboratory, Shri Shivaji Science College, Amravati for providing research facilities and encouragement.

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