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ANTIFUNGAL EFFECTS OF CADMIUM SULPHIDE NANOPARTICLES SYNTHESIZED THROUGH WET CHEMICAL PRECIPITATION METHOD

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ABSTRACT

It was investigated that the cadmium sulphide nanoparticles causes lethal cellular toxicity on three different pathogenic fungal species namely Candida albicans, Candida parapsilosis and Candida trophicalis respectively. It was found that cadmium sulphide nanoparticles, whose size ranges between 35nm and 65nm were synthesized through wet chemical precipitation method. This was further characterized by X-Ray Diffraction analysis and Fourier Transformed Infra Red Spectroscopy. Different concentrations of cadmium sulphide nanoparticles, such a way, 250µg/ml 500µg/ml and 1mg/ml were taken and subjected to antimicrobial test, whose inhibitory zone varies for each candida species. Also, it was predicted that 1mg/ml nanoparticle concentration was responsible for significant fungicidal effects which produces a maximum zone of inhibition of 17mm diameter against Candia trophicalis.

Keywords: Cadmium nanoparticles, Antibiotics, Antimicrobial activity, Pathogenic strains

1. INTRODUCTION

The wet chemical method is used for the fabrication of materials typically a metal oxide starting either from a chemical solution or colloidal particles to produce an integrated network particle. Typical precursors are metal alkaloxide and metal chloride which undergoes hydrolysis is polycondensate reactions to form a colloid, a system composed of solid particles (size ranging 1 nm to 1µm) dispersed in a solvent.

$$Cd\ (CH_3COO)_2.2H_2O + Na_2S.H2O \rightarrow CdS + 2\ Na\ CH_3COO$$

Connecting the metal centers with oxy (M-OM) or hydroxy (M-OH-M) bridges, therefore generating metal – oxo or metal hydroxy polymers in solution. The drying process serves to remove the liquid phase thus forming a porous material and then a thermal treatment may be performed in order to form further polycondensation and hence mechanical properties.

Cadmium sulphide is a chemical compound with formula CdS. Cadmium sulphide is yellow in color and is a semiconductor (2.42eV). It exists in nature as two different minerals, greenokite and hawleyite. Cadmium sulphide is a direct band gap semiconductor (gap 2.42 eV) and has many applications for example in light detectors. It forms thermally stable pigments and with addition of eg. Cd Te.Hgs color ranging from deep red to yellow is formed.

CdS have been extensively studied due to their potential applications such as field effect transistors, light emitting diodes, photo catalysis and biological sensors. Many synthetic methods are been employed to prepare CdS nanoparticles including soft chemical reaction, solid state reaction, sol gel process, microwave heating, photo etching and reverse micelle. In this investigation, we have used wet chemical precipitation method to produce CdS nanoparticles of small sizes using chemical reaction.

In this study, the antimicrobial properties of cadmium nanoparticles synthesized rendering maximum inhibitory effects against three major pathogenic strains Candida albicans, Candida trophicalis, and Candida parapsilosis was discussed.

2. MATERIALS AND METHODS

2.1 Synthesis of Cadmium sulphide nanoparticles

2.1.1 Wet Chemical Precipitation method

In the present work, a direct precipitation method was employed to synthesize nano-sized CdS particles using Cd $(CH_3COO)_{2.2H2O}$ was taken as a precursor material and Na_2S was added in the ratio of 1:1 ratio. Initially 6.633 g of Cd $(CH_3COO)_{2.2H2O}$ was stirred with 50 ml of ethanol at room temperature for 2 hours. Dissolve 1.951g of Na_2S with 50 ml of ethanol in

another beaker and its added dropwise to the above prepared precursor contained using adding appropriate amount of NaOH in the solution. The mixed solution was stirred at room temperature for 2 hrs to get the orange precipitate in the bottom of the solution and the pH is maintained using appropriate method of NaOH in the solution to form the precipitate instead of gel formation. After getting the precipitate, the solution was centrifuged for 10 to 15 min at 1500 rpm and washed with ionized water to separate the solute particles from the solution.

The resultant powder was dried at 80˚C for 4 hrs and then the sample was kept in the controlled atmosphere to reduce the contamination and no other impurities incorporate into the material from the surroundings. Hence the dried sample was grained using the acetone as binder and then the sample was allowed to annealed at 250˚C for 3 hrs to relive the stress and reduce the aggregation of the particle. Hence the crystalline properties of the material could be increased where the material was subjected to high temperature sintering. Finally fine grained homogenously distributed orange CdS particles occurred without any agglomeration.

CdS nanoparticles were prepared in the presence of an organic solution of thioglycerol by dissolving of cadmium acetate and sodium sulfide. Thioglycerol stabilizes the particle surfaces formed during the reaction. Argon gas has been used for deoxygenating reaction vessel. The reaction time was about 48 minutes. Thioglycerol solution in ethanol was used with concentrations of 0.01 M and 0.02 M at a fixed precursor concentration of 0.02 M and also by varying TG concentration from 0.001M to 0.007 M at a fixed precursor concentration of 0.005 M at pH= 5. The precipitate was air dried to get powder of CdS nanoparticles [1].

Tri-n-octylphosphine oxide (TOPO) was dried and degassed by heating to about 100 °C. The temperature was then stabilized at 280 °C under inert atmosphere. Cd[S(Ox]2 (1) (0·52 g, 1 mmol) dispersed in 15 cm3 of tri-n-octylphosphine (TOP) was rapidly injected into the hot TOPO (20 g) at 280 °C. After sudden fall in temperature to 240°C the temperature was stabilized at 280 °C and continued for 30 min. After cooling to 70 °C an excess of methanol was added to give a flocculent precipitate. The solid was separated by centrifugation and the precipitate was washed several times with methanol to remove the excess TOPO. The precipitate was evaporated under vacuum to get cadmium sulphide nanoparticles [2].

Cd(CH3COO)2·2H2O (5 mmoles, 1.33 g) and *o*-diaminobenzene, o-dab, (10 mmoles, 1.08 g) were dissolved in 50 mL water. It is stirred for two hours and the resulted suspension was added thioacetamide (10 mmols, 0.75 g). The mixture was stirred again for four hours. An

orange precipitate was formed which was isolated by vacuum filtration, washed with water and then with methanol and dried. [3]The Cd2+/thiourea precursors solution was prepared by mixing CdCl2. 2.5H2O (40 mg, 0.175 mmol) and thiourea in 14 mL of ultrapure water. 20 mL of an aqueous solution of MPA were added and the pH of the mixture was adjusted to 10 by adding 1 M NaOH. The typical molar ratio of Cd2+/thiourea/MPA was 1/1.7/2.3 in our experiments. The solution was deaerated with nitrogen-bubbling for 30 min.It is then transferred into a Teflon-lined stainless steel autoclave with a volume of 125 mL. The autoclave was maintained at 100 °C and then cooled down to room temperature [4]

2.2 Characterization studies on Cadmium sulphide nanoparticles

Electron-diffraction patterns and high-resolution lattice images obtained with transmission electron microscopy (TEM) indicated that the CdS nanoparticles were crystalline with *d*spacings corresponding to the zinc blende structure (*d*spacings, 0.336, 0.206, 0.176, 0.133, 0.118 nm)[5]. Absorption, fluorescence spectroscopy, and transmission electron microscopy were employed for characterization, which revealed that the prepared CdS nanoparticles had a well-resolved cubic structure and were monodisperse in size. It was also found that the CdS nanoparticles were dispersed in solution as single entities and showed a very good resistance against oxidation for months, according to their polymer shell. The particle size was controllable in the range between 2 and 4 nm by adjusting the polymer concentration and choice of the solvent [6].

The particles are characterized using XRD, UV {visible, photoluminescence and Raman spectroscopy.TheXRD peaks are found to be very broad indicating very ¯ne size of the grains of the sample. The XRD pattern exhibits prominent, broad peaks at 2µ values of 26.60, 44.30 and 52.03± and a shoulder at »31.5± which could be indexed to scattering from 1 1 1, 2 2 0, 3 1 1 and 2 0 0 planes respectively of cubic CdS .The spectrum exhibits a well-defined absorption feature (peak) at »480 nm which is considerably blue-shifted relative to the peak absorption of bulk CdS indicating quantum size effect. The photoluminescence (PL) spectra of nanoparticles of CdS for different excitation wavelengths of 300, 240 and 230 nm (energies 4.1, 5.1 and5.4 eV).The Raman spectrum of nanoparticles of CdS is in the range 180-400 cm^{-1}. The spectrum exhibits a strong but broadpeak at 302 cm^{-1} corresponding to the LO phonon mode [7].

X-ray diffraction patterns of CdS nanoparticles are characteristic of the hexagonal phase and the 'a' and 'c' values were found to be 4.124 and 6.686Å.The XRD peaks are very broad indicating the presence of very fine grains of particles. The XRD pattern exhibits broad peaks of 26.778, 43.928 and 51.678^0. The size of the particle increases as the concentration of the capping agent increases [8].

2.3 Antimicrobial Test

2.3.1 Test organisms used for the analysis

Clinical isolates of **C. *albicans* MTCC 183, *C. parapsilosis* MTCC 2509, *C. tropicalis* MTCC 184 we**re collected from KMCH, Coimbatore (India). Each test strain was inoculated in Mueller Hinton liquid medium (broth) and incubated in a temperature controlled shaker (120 rpm) at 30°C overnight. A reference broad spectrum antibiotic flucanazole which was used for the analysis were purchased from Sigma Aldrich, Bangalore, India and Merck Limited, Mumbai, India.

2.3.2 Well diffusion assay (Eloff, 1998)

Potato Dextrose Agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 h growing fungal cultures (Candida albicans, Candida trophicalis, *and* Candida parapsilosis) were swabbed on it. Five wells (A, B, C, D, E of 10mm diameter) were made by using cork borer. Different concentrations (250µg/ml, 500µg/ml, 1mg/ml of nanoparticle solutions were prepared and loaded into the wells A, B and C respectively. A reference antibiotic flucanazole 1mg/ml and a negative control DMSO were loaded in the wells D and E respectively. The plates were then incubated at 37ºC for 24h. After incubation the inhibition diameter was measured.

3. RESULTS &DISCUSSION

3.1 X- RAY DIFFRACTION ANALYSIS

The scintillation detector present in the instrument moves through the required angle at specific counts and scans the sample with a start angle at 10^0 and a stop angle at 70^0.The output was obtained in the form of a graph with 2θ on x- axis and intensity on y-axis. The obtained graph containing different peaks corresponding to different planes of the crystal was compared with the standard data in JCPDS tool. From the result obtained, the average size of the nanoparticle was calculated using Scherer's formula,

$$D = 0.9\lambda / \beta \cos\theta$$

Where,

λ is wavelength of copper Kα line (1.5406Å), θ is diffraction angle, β is full width at half maximum of peak (FWHM), and D is the average particle size. Depending upon the characteristic peaks obtained in (Fig.1), the X-RD pattern defines the distribution of the size of the particle as 66.5 nm, 50.15 nm, 50.94 nm, 51.96 nm, 34.9 nm, 35.93 nm, 54.41 nm, 36.91 nm, 56.62 nm, 39.55 nm and 39.95 nm, respectively.

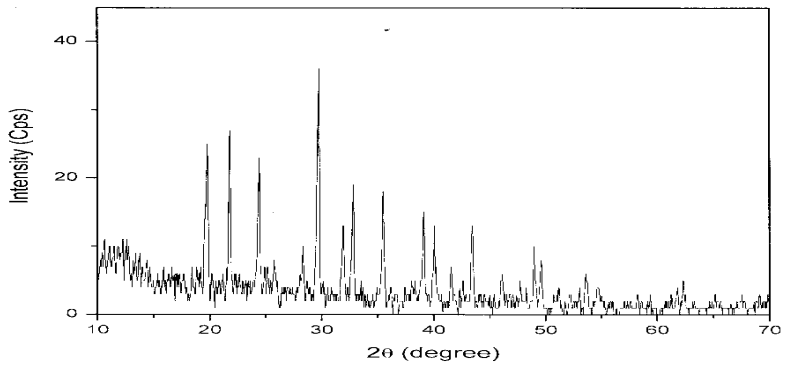

Fig 1. X-RD pattern for CdS nanoparticles

The XRD analysis was performed for CdS nanoparticles and its characteristic peaks were observed.

3.3 FTIR ANALYSIS

The FTIR spectrum of CdS is shown in (Fig.2). In the higher energy region the peak at 3411cm^{-1} is assigned to O-H stretching of absorbed water on the surface of CdS. The presence of water is confirmed by its bending vibration at 1558 cm^{-1}. The peak at 1410 cm^{-1} is assigned to bending vibration of methanol used in the process.The weak peaks due to C-H stretching are observed at about 621cm^{-1}. Upon verification with the present database, the spectrum matches with that of CdS.

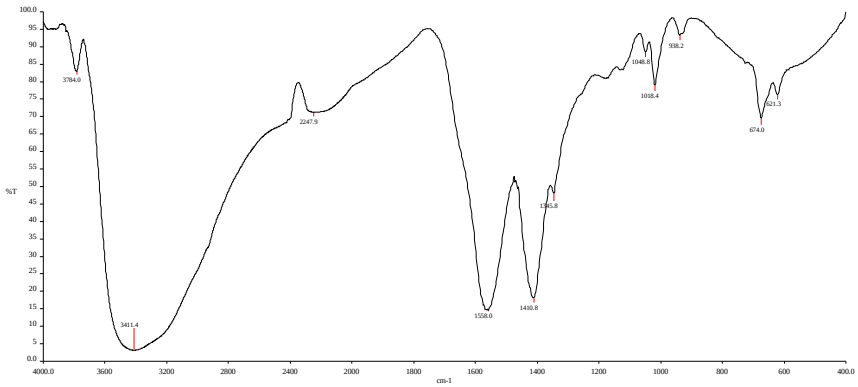

Fig 2. FTIR spectrum for CdS nanoparticles

3.4. ANTIFUNGAL ACTIVITY

A characteriestic fungicidal activity was observed for 1mg/ml concentration of cadmium sulphide nanoparticels agianst C.albicans (Fig.3), which was found to be equal to the effect produced by the reference antibiotic flucanazole, whose zone of inhibition was found to be 17mm in diameter. But, for C.parapsilosis (Fig.4) and C.tropicalis (Fig.5), the maximum inhibitory effect were 9mm and 10mm diameter which was found to be higher than the flucanazole producing the zone of inhibition 2mm and 9mm diameter respectively. Besides, 1mg/ml DMSO (Dimethyl sulfoxide) was used as a negative contro (Table.1).

It was suspected that the nanoparticles reacts with the sulphuhydryl groups and the peripheral proteins present in the outer membrane complex of the fungal species causes the permeability of the Cadmium sulphide nanoparticles to move into cells which leads to cellular toxicity(9). It was believed that the active transport mechanism, periplasmic enzyme and dehydrogenase activity was inhibited and eventually the inhibition of DNA, RNA and protein synthesis leads to lysis of the cells (10, 11).

Table.1

Pathogenic fungal strains	**Zone of inhibition (mm)**				**Negative control**
	Flucanazole 1mg/ml	**CdS 250µg/ml**	**CdS 500µg/ml**	**CdS 1mg/ml**	**DMSO 1mg/ml**
C.tropicalis MTCC 184	±15	±12	±14	±17	-
C.albicans MTCC 183	±2	±3	±4	±9	-
***C. parapsilosis* MTCC 2509**	±9	±3	±6	±10	-

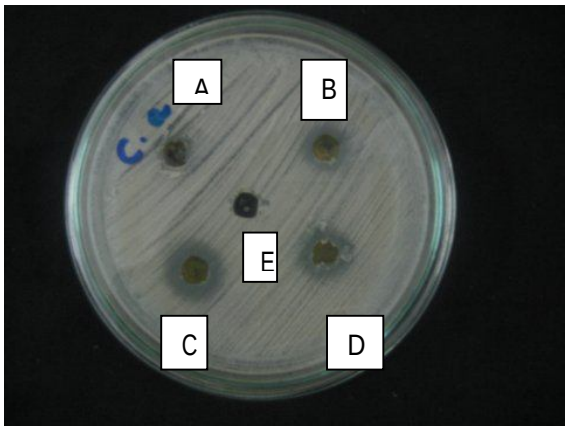

Fig.3 Candida albicans

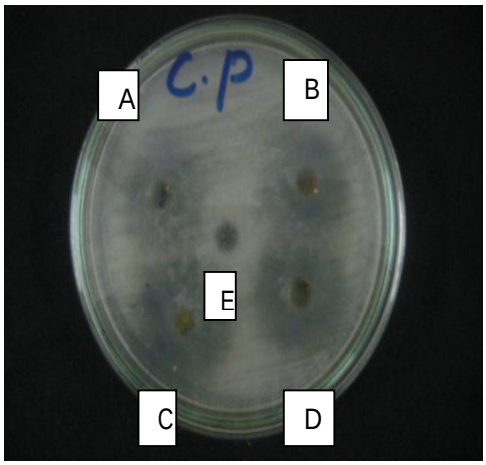

Fig.4 Candida parapsilosis

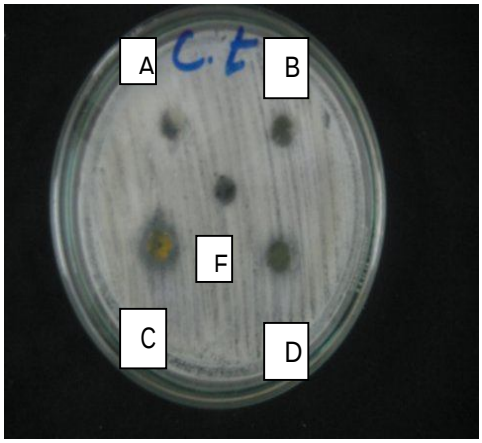

Fig.5 Candida trophicalis

A-250µg/ml CdS, B-500µg/ml CdS, C- 1mg/ml CdS,D-1mg/ml flucanazole, E-1mg/ml DMSO

4. CONCLUSION

It was found that 1mg/ml concentration of cadmium sulphide nanoparticles was required for producing lethal cellular toxicity effects. It was concluded that the sulfuhydryl groups create pores over the cell membrane, which initiates the permeability of cadmium nanoparticles to move into the cell, thereby interfering with the synthesis of DNA resulted in the inhibition of protein synthesis and subsequently causes cell lysis.

5. REFERENCES

1. Jamali SH, Saievar-Iranizad E, Farjami Shayesteh S. Synthesis, optical and structural characterization of CdS nanoparticles. IJNN, 2007; 3(1):57-63.
2. Karuppasamy K, harkesh BS, shailendra KK. Synthesis and characterization of CdS and CdSe nanoparticles prepared from novel intramolecularly stabilized single-source precursors. J. Chem. Sci., 2009; 121(3): 293–296.
3. Dumbrava A, Badea C, Prodan G, Ciupina V. Synthesis and characterization of cadmium sulfide obtained at room temperature. Chalcogenide Letters, 2010; 7(2**):** 11 – 118.
4. Aboulaich A, Billaud D, Abyan M, Balan L, Gaumet JJ. One-Pot Noninjection Route to CdS Quantum Dots via Hydrothermal Synthesis. Appl. Mater. Interfaces,2012:4(1): 2561−2569.
5. Limin Qi , Cölfen H , Antonietti M. Synthesis and Characterization of CdS Nanoparticles Stabilized by Double-Hydrophilic Block Copolymers. Nano Lett, 2001; 1 (2): 61–65.
6. Rajeev R P, Abdul Khadar M. Characterization of chemically synthesized CdS nanoparticles. Journal of Physics, 2005; 65(2): 801-805.

7. Karuppasamy K, Harkesh B S, Shailendra K K. Synthesis and characterization of CdS and CdSe nanoparticles prepared from novel intramolecularly stabilized single-source precursors. J. Chem. Sci. 2009; 121(3): 293–296.
8. Manickathai K, KasiViswanathan S, Alagar M, Synthesis and Characterization of CdS and CdO nanoparticles. Indian Journal of Pure and Applied Physics.2008; 46(3): 561-564.
9. Vigneshwaran N, Kumar S, Kathe AA, Varadarajan PV, Prasad V. Functional finishing of cotton fabrics using zinc oxide-soluble starch nanocomposites. Nanotechnol.2006; 17(5): 5087–95.
10. Rezaei-Zarchi, S., Javed, A., Ghani, M.J., Soufian, S., Firouzabadi, F.B., Moghaddam, A.B. and Mirjalili, S.H., Comparative study of antibacterial activities of TiO2 and CdO nanoparticles aginst the pathogenic strain of Escherichia coli. Iran J. Pathology2010:5(11): 83-89.
11. Wang, Z., Lee, Y. H., Wu, B., Horst, A., Kang, Y., Tang, Y. J. and Chen D. R., Anti-microbial activities of aerosolized transition metal oxide nanoparticles. Chemosphere, 2010:8(4): 5-529.

Abbreviations

X-RD	X-ray diffraction
SEM	Scanning Electron Microscopy
FTIR	Fourier transforms Infra Red spectroscopy
CdS	Cadmium Sulphide