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Synthesis of CdO NPs for antimicrobial activity

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Abstract: In this study, (50 - 110) nm cadmium oxide (CdO) nanoparticles were synthesized by chemical method. The optical, structural and topographical properties of the synthesized nanoparticles were investigated by using UV-VIS absorption, Transmission electron microscopy TEM, atomic force microscopy AFM, and x-ray diffraction XRD. The bacterial resistance represents a problem and the outlook for the use of antibiotics in the future is still uncertain. Therefore, it must be taken measures to reduce this problem. Antibacterial activity of the Cadmium oxide nanoparticles were investigated against several pathogenic bacteria, including Klebsiella pneumoniae; Acinetobacter baumannii; Pseudomonas aeruginosa and Staphylococcus aureus by using well diffusion method ,the results showed that Cadmium nanoparticles had inhibitory effect against all pathogenic bacteria with inhibition zone (18 ,18 ,14 and 17 mm) for S. aureus, K. pneumonia, A. baumannii and P.aeruginosa respectively. CdO nanoparticles had inhibitory effect against S. aureus (22 mm) ; K. pneumonia (18mm) and A. baumannii (14mm).

Keywords: Nanoparticles , XRD antimicrobial activity, CdO, TEM.

1 Introduction

Nanotechnology is applied to various fields such as biological, physical, chemical, and engineering sciences where novel techniques are being developed to probe and manipulate single atoms and molecules. Among all Nanoparticles the metallic one have applications in diverse areas such as cosmetics, coating, electronics, packaging and biotechnology[1].

The extensive use of chemotherapeutic antibacterial agents has generated the selective pressure to encourage the escalating rates in antibiotics resistance [2]. Emergence of new resistant bacterial strains to current antibiotics has become a serious public health issue, which raised the need to develop new bactericidal materials [3].

In this regard, synthesis or extraction of compounds such as nanoparticles with antimicrobial properties is essential, and has potentially promising applications in the fight against the ever-growing number of antibiotic resistant pathogenic bacteria which pose a continuous threat to human and animal health [4].

2 Experimental works

Re-distilled water was used throughout the experiment. In a typical procedure, 1.5 g of Cd(NO3)2.H2O (BDH Chemicals Ltd Pool England) was dissolved in 50 mL of PVP (Sigma Aldrich USA) 1 WT. %. The solution was added into a round-bottom flask with stirring. The color of the mixture was blue. About 15 ml of NaOH (1M) was rapidly added to the mixture, and a nanopowder suspension was formed . The suspension was kept at 75 °C for 1 h. A large amount of black precipitate was produced. After cooling to room temperature, the particles were separated by centrifugation and were washed with distilled water to remove any contaminations. The particles were then dried in an oven at 80 °C.

The structure of CdO nanoparticles drop casted on glass substrate was investigated by means of X-ray diffractometer (XRD-6000, Shimadzu, X-ray, diffractometer) with Cukα radiation at a wavelength of (λ = 0.154056 nm). The optical absorption of colloidal CdO NPs was measured using a spectrophotometer (Cary, 100 Conc plus, UV-Vis-NIR, Split- beam Optics, Dual detectors). The shape and size of the CdO nanoparticles were investigated by using atomic force microscopy TEM (Angstrom AA 3000) Isolates of Klebsiella pneumoniae; Acinetobacter baumannii; Pseudomonas aeruginosa and Staphylococcus aureus were obtained from Department of Biology/College of Science/Al-Mustansiriyah University/Baghdad / Iraq.

Antibacterial activity of CdO nanoparticles were screened for their antibacterial effect against pathogenic bacteria by

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well diffusion method. Plates were prepared by spreading approximately 105 cfu/ml culture broth of each indicator bacterial isolates on nutrient agar surface. The agar plates were left for about 15 min before aseptically dispensing the 50µl of CdO into the agar wells already bored in the agar plates. The plates were then incubated at 37 °C for 18 - 24 h. Zones of inhibition were measured and recorded in millimeter diameter [5].

The Antimicrobial susceptibility of pathogenic isolates was done by using Kirby-Bauer disc diffusion technique on Mueller Hinton agar [Oxoid, England] using overnight culture at a 0.5 McFarland standard followed by incubation at 35 oC for 18 h. following Clinical and Laboratory Standards Institute (CLSI) guidelines with commercially available antimicrobial discs (Bioanalyse/Turkey).Isolates were tested against the following antimicrobial agents: ceftazidime , cefoxitin , ciprofloxacin ,cefepime and cefotaxime.

To determine combined effects between antibiotics and cadmium nanoparticles, as described by Roy et al.[2010] with modification, each standard paper disc of antibiotics (mention above) was further impregnated with cadmium nanoparticles solution. A single colony of pathogenic bacterial isolates were grown over night in Muller-Hinton broth medium at 37Co. The inoculums were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard and were applied to the plates along with the standard and prepared disks containing of cadmium nanoparticles. After incubation at 37Co for 24 hour, the zones of inhibition were measured.

3 Results and Discussion

The XRD diffraction patterns of synthesized CdO nanoparticles (CdO NPS) films and deposited on glass substrate as shown in Figure1.

The XRD patterns for CdO film which presented in Figure 1 have peaks of cubic face-centered CdO corresponding to (111), (200) ,(220) and (311) and planes which have been compared with standard X-ray diffraction data file (JCPDS file No. 75-0594) [6]. In the present investigation, the films exhibit a preferential orientation along the (200) diffraction plane which were grown CdO thin films on glass substrate by the chemical method.

The intensity and the value of full width at half maximum FWHM of main diffraction peaks, indicating the formation of smaller particles. No diffraction peaks related to other phases were observed in the XRD spectra of CdO thin film. The mean crystallites size D of (111 plane was determined using the follow ing Debye–Scherrer formula (XRD line broadening) [7] and listed in Table 1., (1) where λ is the wavelength of xray, θ is the diffraction angle and β is the FWHM.

The sharp XRD peaks indicate that the particles were of polycrystalline structure, and that the nanostructure grew with a random orientation [21]. The microstrain (ε) and the dislogation density (σ) can be calculated by using the following relations [19] ,see Table (1):

$$\gamma = \frac{\beta \cos\theta}{4} \quad (2)$$

$$\sigma = \frac{1}{D^2} \quad (3)$$

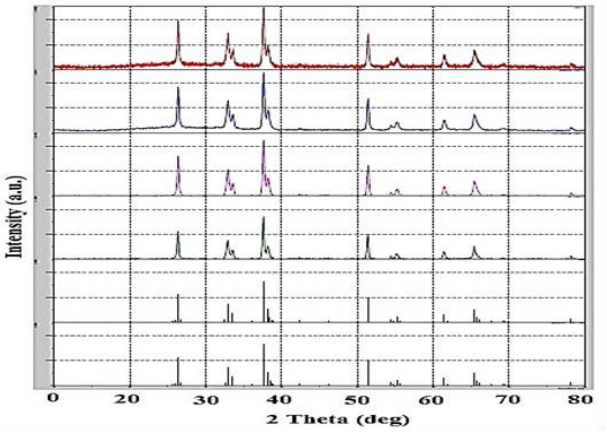


Fig. 1: *XRD patterns of CdO thin film.*

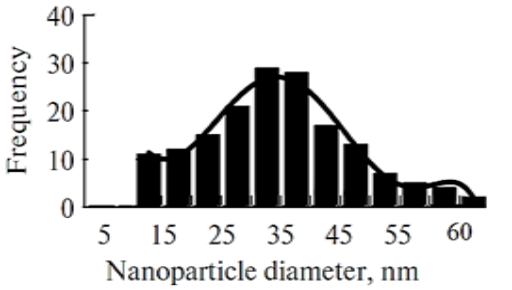
Table 1. : Powder X-ray diffraction data of CdO**.**

2 Theta (deg)	(deg) β	(hkl) planes	D (nm)	ε x 10^{-4}	δ x10^{14} lines . m^{-2}
33.57	0.33	(111)	24.91	16.11	13.90
38.92	0.06	(200)	126.46	0.62	2.73
55.25	0.42	(220)	21.34	21.95	16.23
65.78	0.28	(311)	33.77	8.76	10.25

The TEM images for CdO nanoparticles is shown in Fig. 2, The TEM micrographs confirm the formation of well-defined CdO nanoparticles having a diameter of approximately 32 nm to 41 nm) which prepared by chemical method.

The Transmittance spectrum is taken by Cary 100 Conc plus UV-Vis Spectro-photometer 350 nm to 1100 nm. The UV –Vis spectra is very important because it is provide the details related with the optical band. The optical transmittance of the CdO thin film at 15 min deposit time was around 20% at wavelength 350 nm then increases sharply to 60% at wavelength 1100 nm as shown in figure 3,a. Also it is observed that the optical transmittance spectra shift towards shorter wavelength as particle size decrease due to increase in optical energy band gap.

Figure 3,b shows that graph between $(\alpha h\nu)^2$ versus photon energy (hv) gives the value of direct band gap .The extrapolation of the straight line to $(\alpha h\nu)^2 = 0$, gives the value of band gap . From the UV spectra shows the absorbance



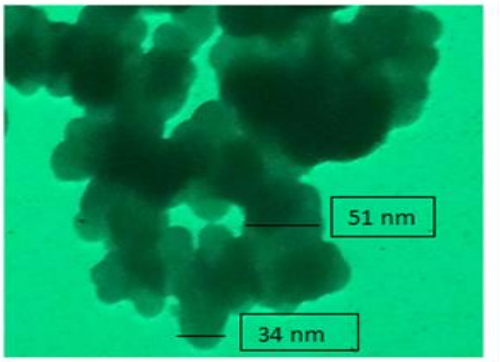


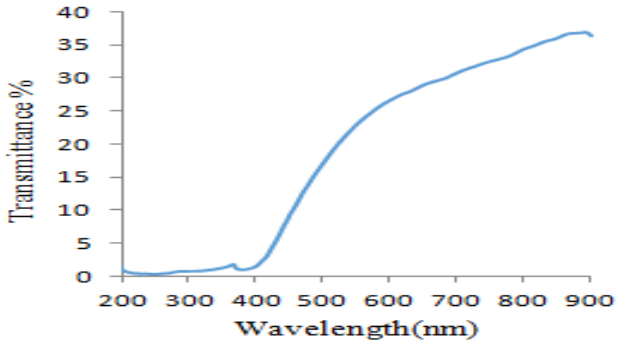
Fig. 2: TEM images of CdO nanoparticles and their size distribution histograms.

decreases with increasing wavelength and the energy gap increase from 2.3 eV to 2.45 eV from bulk to the thin film via quantum size effect.

Nanoparticles have many applications in medicine as antibiotics only eliminate a small number of pathogenic bacteria while the nanoparticles eliminate different type of pathogens (Hernandez-Sierra etal.2008)

The most inhibition effect has emerged on cadmium and copper against pathogenic bacteria. Cadmium nanoparticles had inhibitory effect against all pathogenic bacteria with inhibition zone (18, 18, 14 and 17 mm) for S. aurous, K. pneumonia, A. baumannii and P.aeruginosa respectively. Copper nanoparticles had inhibitory effect against S. aureus (22 mm); K. pneumonia (18mm) and A. baumannii (14mm), while cobalt and Fe nanoparticles had inhibitory effect against P.aeruginosa only (Table 2).

In investigation of nanoparticles effect on pathogenic bacteria we get that cadmium has most inhibitory effect on bacteria and the effect of cadmium nanoparticle on S. aureus bacteria than A. baumannii and P. aeruginosa, this result was agree with result of Salehi et al.[9]. Cadmium Oxide nanoparticles have been applied by many scholars up to now. In the same way, we have used Cadmium Oxide nanoparticles to confront bacteria which are pathogenic [10].



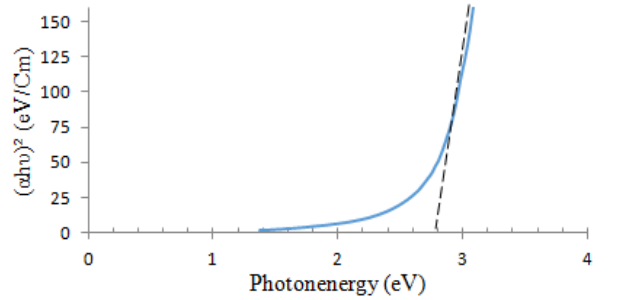


Fig.3: Transmittance Spectra of CdO thin film and $(\alpha h\nu)^2$ versus photon energy gap of CdO thin film continuously.

Table 2: Antibacterial activity of CdO Np_s against pathogenic bacteria.

Inhibition Zone of CdO NPs in (mm)	Bacteria
18	*S. aureus*
18	*K. pneumonia*
14	*A. baumannii*
17	*P.aeruginosa*

Sundrarajan et al. [11] study the antibacterial properties of Magnesium nanoparticles on gram-positive Staphylococcus aureus and gram-negative Escherichia coli and they results showed that the inhibition zone diameter against gram-positive bacteria was bigger than that of the gram-negative

Table 3. Combined effect between Antibiotics and cadmium nanoparticles against pathogenic bacteria.

FOX+ cds	FOX	CAZ+ cds	CAZ	CTX+cds	CTX	CPM+cds	CPM	CIP + cds	CIP	Bacterial isolates
R	R	R	R	S	S	R	R	S	S	S.aureus
R	R	S	S	R	R	R	R	30mm	17mm	K. pneumonia
R	R	R	R	R	R	R	R	R	R	A. baumannii
R	R	R	R	R	R	R	R	R	R	P.aeruginosa

R=resistant, S=Sensitive, cds = cadmium nanoparticles, CAZ= ceftazidime ,FOX=cefoxitin , CIP=ciprofloxacin, CPM=cefepime , CTX= cefotaxime

bacteria.Barzegari et al., [12] studies the effect of Titanium oxide on Staphylococcus and concluded that this nanoparticle has a good antibacterial effect on this gram-positive bacterium.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 [13].

The effect of cadmium nanoparticles combined with different antibiotics was investigated against pathogenic bacteria using disk diffusion method. The diameter of inhibition zones (mm) around the different antibiotic discs included (ceftazidime ,cefoxitin , ciprofloxacin ,cefepime and cefotaxime) with and without cadmium nanopatrticles against bacterial isolates were measured. The antibacterial activities of some antibiotics like ciprofloxacin have been increased in the presence of cadmium nanopatrticles against K. pneumoniae isolate while others didn't affect (Table 2).

There is great need of agents to kill bacteria due to the antibacterial agents resistance [Suchitra et al., 14]. Combined use of antibiotic – nanoparticle conjugates towards decreasing antibiotics resistance currently observed for specific bacteria and conventional antibiotics [15]. Roy et al.[16] suggested the mechanisms involving the interaction of nanomaterials with biological molecules and believed that bacteria carry a negative charge while metal oxides carry a positive charge, this cause attraction between bacteria and treated surface leads to oxidizing of microbe and finaly dead.

Antibiotic molecules had many active groups such as amino groups and hydroxyl, which reacts simply with nanoparticles by chelation, causing synergistic effect [Rajawat and Quresh, 12].

The combination of metal nanoparticles and antibiotics could increase the antibiotics' efficacy against resistant bacteria. In addition, nanoparticle–antibiotic conjugates lower the amount of both agents in the dosage, which reduces harmfulness and increases antimicrobial properties. Additionally, due to this conjugation, the antibiotics concentrations were increased at the location of antibiotic–microbe contact and thus accelerate the binding between antibiotics and microbes [Fayaz et al.16].

4 Conclusions

This work has clearly presented how CdO thin film was prepared by chemical method (simple, cost, and quick method for the synthesis of CdO nanostructure).The behavior of the film as illustrated in the figures shows that the film is a visible transmitting thin film with a good crystallize.Also,the results show that cadmium and copper nanoparticles have inhibitory effect against pathogenic bacterial isolates .

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