Research Article

Journal of Environmental Nanotechnology

Antibacterial Applications of Un-doped and Doped CdO Nanocrystalline Thin Films

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Received: 18.12.2018 Accepted: 10.01.2019 Published: 30-06-2019
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ABSTRACT

Nanocrystalline thin films of un-doped and Al-doped cadmium oxide were synthesized using the Chemical bath deposition method and were annealed at 500 ˚C. The films were characterized to study their structural, optical and compositional properties. Antibacterial activity of CdO films was assayed by using Agar well-diffusion technique. The antibacterial activity of un-doped and Al-doped CdO solutions was analyzed using the gram-positive bacterium, *Bacillus cereus* and the gram-negative bacterium, *Vibrio cholera*. The present study has revealed that the diameter of the zone of inhibition is found to be more for gram-negative bacteria than gram-positive for both un-doped CdO and Al-doped CdO.

Keywords: Ammonium hydroxide; Cadmium chloride; *Bacillus cereus*; *Vibrio cholera*.

1. INTRODUCTION

A number of species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, leprosy and plague. The most common deadly bacterial diseases are respiratory infections, with tuberculosis killing about 2 million people per year. In developed countries, antibiotics are used to treat bacterial contamination and are also used in farming. In industry, bacteria are important in sewage treatment, breakdown of oil spills, production of cheese and yogurt through fermentation and the recovery of gold, palladium, copper and other metals in the mining sector.

Gram stain, also called Gram's method, is a method of staining used to discriminate bacterial type into two large groups, namely gram-positive and gram-negative. A Zone of Inhibition test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic variance and to technologically ensure the potential of solids and textiles to inhibit microbial growth. A bacterial strain of significance is grown in pure culture, which is spread over the face of a sterile agar plate. The antimicrobial agent is applied to the center of the agar plate. If the antimicrobial agent percolates from the object into the agar and then puts forth a growth-inhibiting effect, then a clear zone called the zone of inhibition appears around the test product. The size of the inhibition zone is usually associated to the level of antimicrobial activity present in the sample or product – a larger zone of inhibition typically means that the antimicrobial activity is more potent and powerful.

Bacterial strains of gram-positive *Bacillus cereus* and gram-negative *Vibrio cholera* were used in this study. *Vibrio cholera* is a type of gram-negative bacterium, having a curved-rod shape, several kinds of which can cause food-borne infection, usually associated with eating under-cooked seafood. *Bacillus cereus* is a gram-positive, rod-shaped, aerobic, facultatively anaerobic and beta-hemolytic bacterium commonly found in soil and food.

2. EXPERIMENT

In the present work, cadmium oxide (CdO) thin films were prepared on glass substrates by Sol-gel chemical bath deposition technique. In a conical flask, 0.1 M of cadmium chloride ($CdCl_2.2½H_2O$) was dissolved in 250 ml of de-ionized water. The solution was continuously stirred by a magnetic stirrer for 1 h to get a clear homogeneous solution. Ammonium hydroxide ($NH_3.H_2O$) was added with this solution drop-wise till the pH reached a value of 12. The solution was taken in small beakers and the glass substrates were immersed into beakers for 24 h. The glass slides were dried in a hot air oven and were annealed to 500 °C; thus, the un-doped CdO thin film was prepared. The same procedure was continued to dope aluminum to CdO. Anhydrous aluminum chloride ($AlCl_3$) was added to pure CdO precursor in the beaker (Ramiz Ahmed Al-Ansari, 2016) for different concentrations. The obtained results for the lesser concentrations of 1% and 2% of Al doping exhibit similar results for XRD analysis; hence, 3 wt. % and 5 wt. % concentrations were optimized. This solution was stirred using a magnetic stirrer for 2 h and the glass substrates were dipped into the beaker for 24 h.

Molecular grade chemicals were used for the experiment. Throughout the experiment, de-ionized water was used. Gram-positive bacterium, Bacillus cereus, and Gram-negative bacterium, Vibrio cholerae, were used for the present experiment. Nutrient broth (Sigma) was used for growing and maintaining the bacterial cultures. CdO nano-thin films, 3 wt. % Al-doped CdO films, and 5 wt. % Al-doped CdO films were used throughout the procedure. The films were suspended in ethanol individually and used.

3. X-RAY DIFFRACTION OF PURE CdO THIN FILMS

The structural properties of CdO nanoparticles were investigated using an X-ray diffraction analysis. The X-ray diffraction patterns were used to identify the structure and to calculate the particle size of synthesized cadmium oxide nanoparticles. The lattice parameter was calculated from the equation,

$$a \text{ (in A}^{\circ}) = d\sqrt{h^2 + k^2 + l^2} \qquad (1)$$

where, ‘d’ is the spacing between adjacent (hkl) planes and ‘a’ is the lattice constant. The sharp peak values were used to calculate the lattice parameter and grain size.

The calculated lattice constant for the dominant peaks of CdO nanocrystalline thin films is averaged to a 4.011 A$^{\circ}$ which is close to the reported value of 4.695 A$^{\circ}$.

The information on the strain **ε** as well as the crystalline size (D) were calculated from the full-width at half-maximum (FWHM) of the prominent XRD peaks using the Scherer-Bragg’s relation,

$$D = k\lambda/\beta \cos\theta \qquad (2)$$

where, **λ** is the wavelength of the X-ray, β is the full-width at half-maximum of the corresponding peak of the XRD pattern.

Dislocation density and Micro-strain

Dislocation density δ and micro-strain ε were calculated using the equations (3) and (4):

$$\delta = 1/D^2 \text{ in lines / m}^2 \qquad (3)$$

$$\varepsilon = \lambda/D \sin\theta - \beta/\tan\theta \qquad (4)$$

The microstructural parameters like grain size, dislocation density and strain for pure CdO nanocrystalline thin films are listed in Table 1, from which it was evident that the average grain size reached a maximum of 38.76 nm.

4. MORPHOLOGICAL ANALYSIS

The morphology and microstructure of CdO products were examined by SEM images (Fig. 4). The figure reveals the structure of the CdO nanoparticles, which were grown in pure and doped thin films on the glass substrates. It can be seen that all the substrates were completely surrounded by CdO nanoparticles.

5. RESULTS AND DISCUSSION

The existence of multiple diffraction peaks of (011), (111), (200), (220), (311) and (222) planes specify the polycrystalline nature of the CdO compound with cubic structure (Ngamnit Wongcharoen *et al.* 2012). The obtained XRD patterns are shown in Fig. 1.

The XRD peaks indicated the good crystalline nature of synthesized CdO. All CdO thin films were having good adhesion to the substrate and were transparent, having better uniformity, free from pinholes, and were stable for a long period when kept in the atmosphere (Gbadebo Taofeek Yusuf *et al.* 2016). The XRD pattern exposed diffraction peaks approximately at 33°, 55.5° and 68° of 2θ values, indicating the hkl values as (111), (220) and (222) which corresponded to polycrystalline structure having the characteristic peaks of the face-centered cubic structure of CdO (JCPDS Card No. 05-0640, 73-2245 and 78-065) (Gbadebo Taofeek Yusuf *et al.* 2016, Ngamnit Wongcharoen *et al.* 2012, Saha *et al.* 2007).

For Al-doped CdO thin films with different concentrations of Al, it was capable of providing the cubic crystalline structure with polycrystalline nature in the same annealing temperature of 500 °C. The film did not stick to the substrate properly, formed as aggregates on the surface, thus producing numerous peaks in an amorphous fashion (Sivakumar *et al.* 2012). This performance can be related to the presence of Al-Cd compounds in the amorphous phase with an increasing doped CdO grain size (Ngamnit Wongcharoen *et al.* 2012).

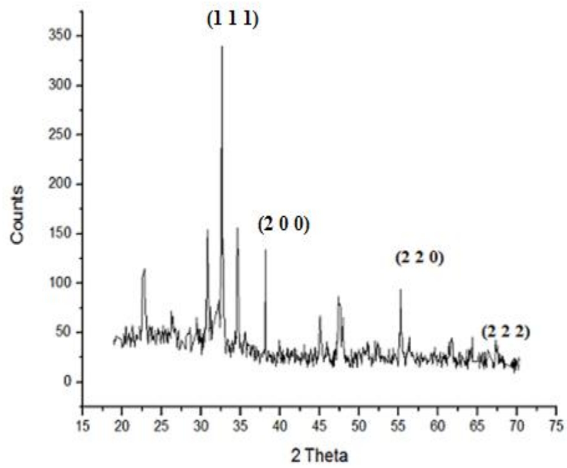

Fig. 1: XRD spectrum of CdO thin film

Doping CdO films with Al caused a small reduction in the intensity of all peaks and especially of the (220) plane. The doped CdO films were having (111) planes as preferred orientations. A similar observation was also reported by Saha *et al.* 2007.

It was noticeable from the figure that the diffraction peak from (1 1 1) emerged as the strongest orientation for the doped film (Saha *et al.* 2007). The variation of the intensity of (1 1 1) peak in the Al-doped CdO films was not uniform when Al concentration was increased uniformly from 3 to 5 wt. % in the solution. This behavior implied that the variation of the crystallite size of the preferential orientation was not uniform due to the increase of aluminum concentration in the solution. The grain size of the crystallites changed randomly for a regular rise of aluminum concentration.

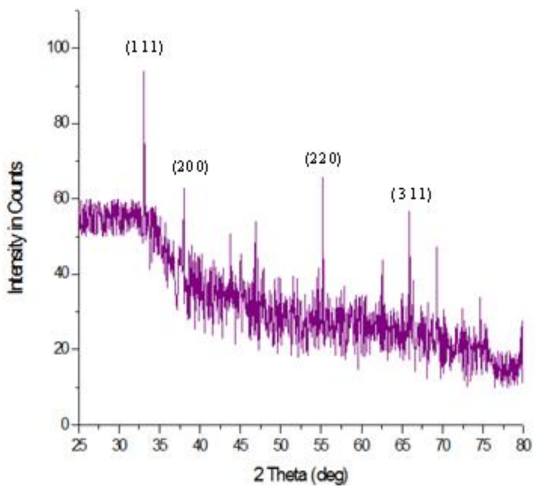

Fig. 2: 3 wt. % Al:CdO thin film

The intensity of all peaks rapidly decreased and full-width at half-maximum (FWHM) decreased for the films with high Al content (5%). The lattice parameter increased from 4.032 Å to 4.076 Å, when the Al percentage increased from 3 to 5%.

Al was chosen in this work as a dopant to organize and improve the properties of CdO thin films. Al^{3+} ion has three valence electrons and the ionic radius of Al^{3+} ion (0.68 Å) was slightly smaller than that of Cd^{2+} ions (0.95 Å). Thus, it was expected that Al^{3+} ions doping in CdO will lead to enhancement in electrical conductivity by increasing electron concentration (Gupta *et al.* 2008). Since Al^{3+} has a lesser ionic radius than that of Cd, there would be a significant difference in its electrical property (Zheng *et al.* 2010). This is attainable by a shift in the optical band gap along with the enhancement in the transparency of CdO films. The effect of doping on the physical properties of CdO thin films was reported by Zheng *et al*. The hkl and d values found for the Al-doped CdO film deposited at different concentrations (for an annealing temperature of 500 °C) were agreeing well with the values found in JCPDS cards 05-0640 and 78-0653. The calculated value of lattice parameters, approximately for Al-doped CdO film with 3 wt. % was, 'a' was 4.032A°. For the increased concentration of Al doping (5 wt. %), 'a' was 4.076A°, which was is in very good agreement with the reported value of a = 4.695A°.

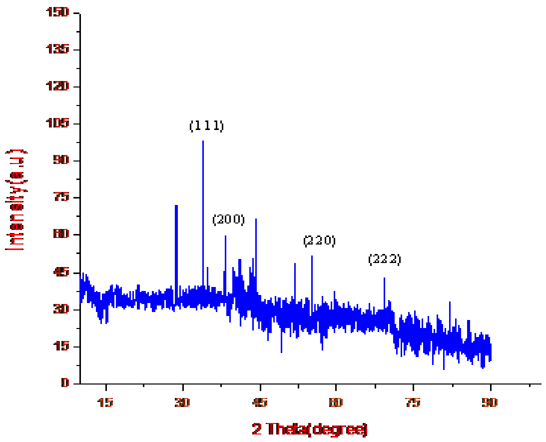

Fig. 3: 5 wt. % Al:CdO thin film

Table 1. The structural parameters of pure CdO nanocrystalline thin films

Film	$2\Theta_{deg}$	FWHM radians	Lattice strain	Dislocation density$\times10^{14}$ (δ) lines/m^2	Micro strain (ε)	Average crystalline size (D) nm
Pure CdO	15.2526	0.3444	0.0225	17.1319	9.0365	38.76
	18.3321	0.1476	0.0080	03.1414	8.7565	
	22.6727	0.1968	0.0087	05.5651	6.8558	
	26.3868	0.5904	0.0223	49.9450	4.2314	
	29.0478	0.1476	0.0051	03.1105	5.5734	
Al:CdO 3 wt. %	5.7385	0.5063	0.0882	37.18024	20.67552	28.148
	18.2243	0.5196	0.0285	38.91642	6.48847	
	31.8419	0.1299	0.0041	2.401467	5.160884	
	66.5304	0.9092	0.0133	110.1068	1.422715	
	84.4461	0.2598	0.0029	8.524695	4.87948	
Al:CdO 5 wt. %	27.4841	0.3897	0.0141	21.714637	1.50669	45.624
	31.8421	0.1299	0.0041	2.401467	5.16084	
	45.5578	0.0974	0.0021	1.323002	3.738737	
	56.7029	0.2598	0.0045	9.205038	2.762732	
	66.3474	0.3897	0.0057	20.235832	2.219410	

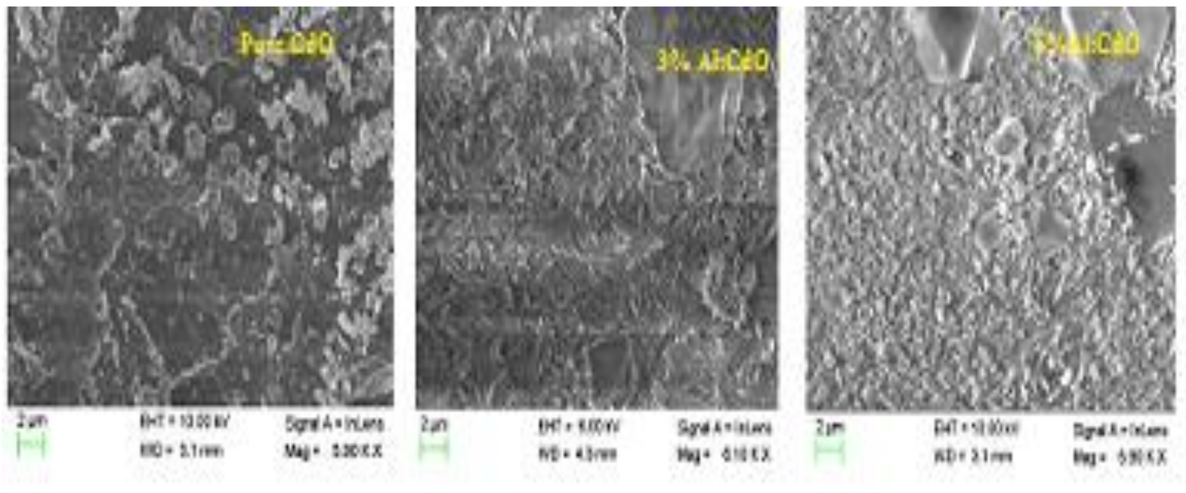

Fig. 4: SEM images of un-doped and Al-doped CdO thin films

A careful observation of pure CdO surface has shown grains-like mounts with no well-defined boundaries. It can be seen that grains clump together and hence do not display homogeneous distribution for un-doped CdO samples; similar observations were reported earlier (Aswani *et al.* 2014). One can clearly observe a rough surface with small grains. No pinholes or cracks were observed. The surface roughness was too high due to the changing grain size distribution on the surface of the film (Sukru Karatas *et al.* 2012). Average grain size could not be calculated due to clumping (Colak *et al.* 2013).

The particle size was in a few nanometer ranges and it exhibited a needle-like structure (Aswani *et al.* 2014) for Al-doped CdO nanopowder.

It was observed that the doping of Al has stimulated an obvious change in grain size. Furthermore, the influence of incorporating Al on the surface morphology of the samples can be seen (Gupta *et al.* 2008). The thin films with 3% and 5% doping concentrations were uniform and homogeneous, with few defects. The change in particle size can be attributed to the difference in the radius of ions between Cd and Al (Lokhande, 2004). The aluminum-doped films have shown smoother surfaces compared to cadmium oxide films.

6. BACTERICIDAL EFFECT ON GRAM-POSITIVE (*Bacillus cereus*) BACTERIA

Antibacterial activity of CdO films was assayed by using the Agar well-diffusion technique. The gram-positive bacterium, *Bacillus cereus*, was grown on nutrient agar plates. Three different plates were used. Wells were made on the plates with the help of a well borer. The CdO nano-thin films were dissolved in ethanol and prepared to the form of a solution and un-doped, 3 wt. % Al-doped and 5 wt. % Al-doped CdO were added to the three wells. Likewise, the solutions of un-doped precursor solution were taken on another plate.

In the first disc, which is impregnated with gram-positive bacterium (*Bacillus cereus*), un-doped CdO and pure distilled water as the control solution, were taken in 2 wells. The discs were incubated for 24 hours at 37 $^{\circ}$C. After 24 h, the plates were visualized. Bindhu *et al.* (2016) reported that the un-doped cadmium oxide nanoparticles inhibited the gram-positive bacteria *Bacillus cereus* to a diameter of 13 mm (Bindhu *et al.* 2016). For the un-doped cadmium oxide, the diameter of the zone of inhibition was found to be 28 mm (Fig. 2), which is comparatively an improved result to Bindhu *et al.* (2016). From the figure, it was also observed that there is no zone of inhibition around the control solution, the distilled water.

The second disc displayed the inhibition zone of Al-doped CdO solution. Fig. 3 reveals the level of inhibition effects against *Bacillus cereus*, which gives the Zone of Inhibitory action (ZOI) as 33 mm for 3 wt. % Al doping and 35 mm for 5 wt. % Al doping. It was obvious that the present study is the first work, in which the authors have used Al dopant in 3 wt. % and 5 wt. %, and we have achieved the maximum value of antibacterial effect, identified so for.

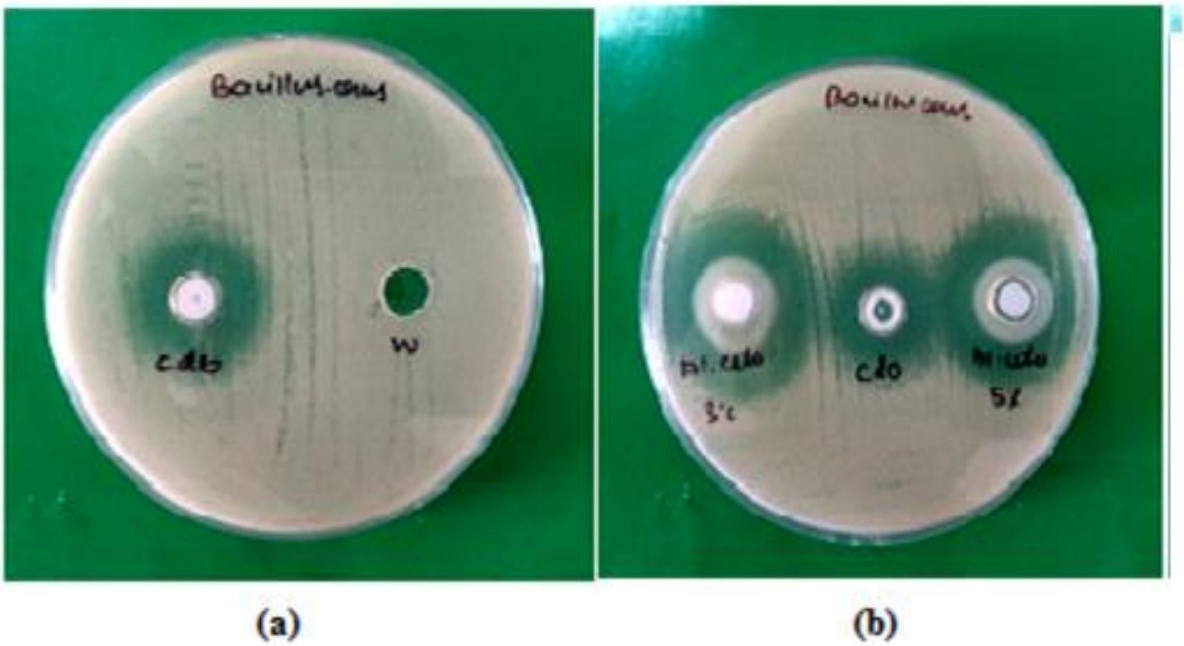

Fig. 5: Bactericidal effect of (a) un-doped CdO (b) Al-doped CdO on *Bacillus cereus* by Well-diffusion method

The present data demonstrated that a formation made with the organically stabilized nano-CdO can be useful in the treatment of infectious disease caused by *Bacillus cereus*. It was implicit that the microorganisms bear a positive charge (Zhang *et al.* 2009) which generates an "electromagnetic" attraction between the bacteria and the considered surface. Zhang H *et al.* realized that once the contact is made, the microorganism will be oxidized and becomes lifeless immediately. Russell and Hugo concluded in their studies that 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, leading to cell lysis (Russell *et al.* 1994). 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 extend beyond the bacterial cell membrane, permitting the transfer of nutrients through the cell wall. Nanomaterials diminish the proteins, lessening the membrane permeability and eventually causing cellular death (Zhang *et al.* 2009). Wang *et al.* (2010) accomplished in their work that in the aqueous system both nanoparticles and bacteria tended to combine, and the nanoparticle toxicities were mainly ascribed to the ions dissolved in the solutions.

7. BACTERICIDAL EFFECT ON GRAM-NEGATIVE (*Vibrio cholerae*) BACTERIA

The antibacterial activity of un-doped and doped CdO solutions was analyzed using the gram-negative bacterium *Vibrio cholera*. This bacterium has not been taken much for analysis for the antimicrobial assay. Our work is the first attempt to examine the two different doping percentages of Al. The un-doped CdO solution was assayed in the bacteria-packed disc, which inhibited a zone diameter of 33 mm (Fig. 2 a). 3 wt. % Al-doped CdO solution furnished a Zone of Inhibition (ZOI) to the gram-negative bacterium *Vibrio cholera* for about 42 mm. The ZOI by 5 wt. % Al-doped CdO solution appeared to be 47 mm (Fig. 2 b), which is the greatest value attained among the reviewed survey.

Ravichandran *et al.* demonstrated that the maximum ZOI action, emerged in the entire test when compared with un-doped and La-doped CdO. The antibacterial effect boosts with the increase in La doping concentration may be due to: (i) the generation of Reactive Oxygen Species (ROS) (ii) the release of Cd+ ions and (iii) the size of the nanoparticles (Ravichandran *et al.* 2016). In 2015, Ravichandran *et al.* explained the creation of ROS (Ravichandran *et al.* 2015) as follows:

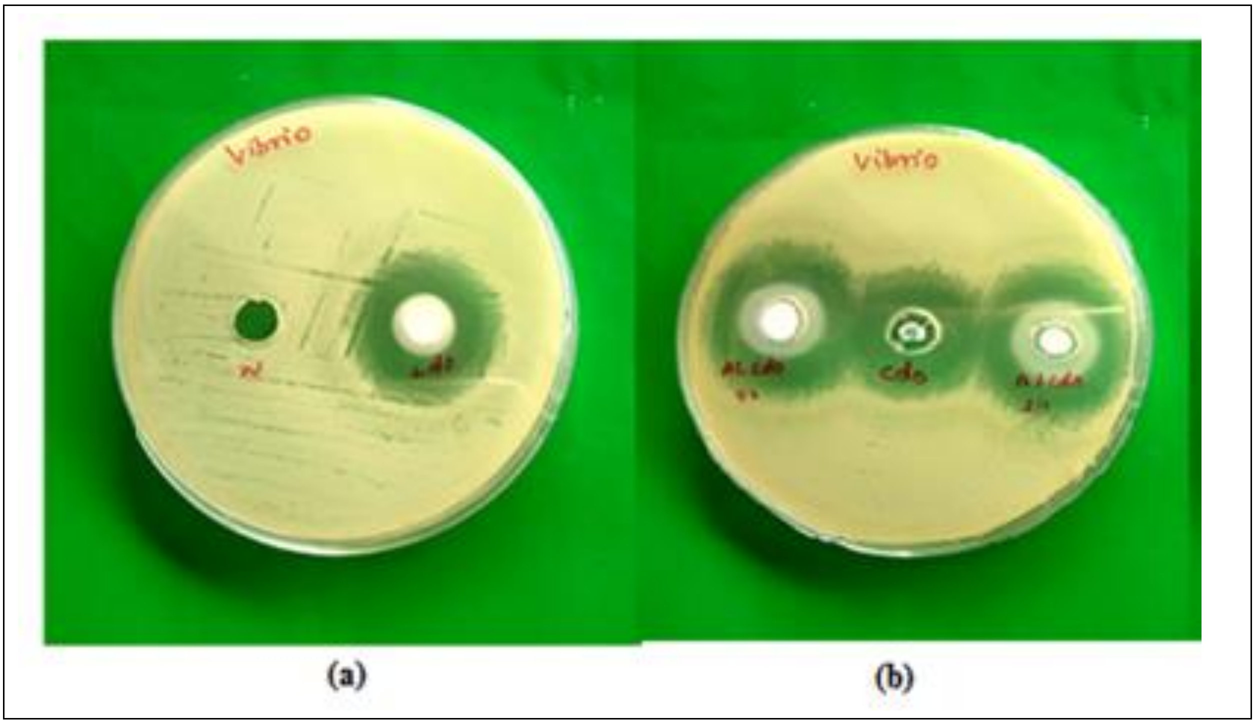

Fig. 6: Bactericidal effect of un-doped and doped CdO on *Vibrio cholerae* by Well-diffusion method

$$CdO + h\upsilon \rightarrow h^+ + e^-$$

$$h^+ + H_2O \rightarrow OH^- + H^+$$

$$O_2 + e^- \rightarrow O_2^-$$

$$O_2^- + H^+ \rightarrow HO_2^-$$

$$H^+ + HO_2 + e^- \rightarrow H_2O_2$$

$$H_2O_2 + O_2 \rightarrow OH^- + O_2 + OH$$

The results revealed that AgNPs synthesized from *C. bonplandianum* demonstrated effective antibacterial activity in gram-negative than in gram-positive bacteria. It can be suggested that gram-negative strains of bacteria *E. coli* and *P. aeruginosa* with thin cell wall is more susceptible to cell wall damage compared to gram-positive strain bacterium *S. aureus* with a thick cell wall.

The toxic hydrogen peroxide released damages the structure of the bacteria cell membrane and depresses the activity of some enzymes, which cause it to die eventually (Karthik *et al.* 2014). The basic mechanism of antibacterial action of the material states that the production of reactive oxygen species on the surface of these nanoparticles in light causes oxidative stress in a bacterial cell and leads to its death. The ROS contains the most reactive hydroxyl radicals (OH) and less-toxic superoxide anion radical (O_2-). This damages the DNA, cell membrane, etc., leading the cell to death. This is attributed to the electrostatic attraction between the negatively charged bacteria and the positively charged nanoparticles (Abdulrahman Syedahamed Haja Hameed *et al.* 2013).

Table 2. Zone of Inhibition

Bacterium	ZOI in mm by Un-doped CdO	ZOI in mm by 3 wt. % Al-doped CdO	ZOI in mm by 5 wt. % Al-doped CdO
GRAM-POSITIVE (*Bacillus cereus*)	28	33	35
GRAM-NEGATIVE (*Vibrio cholera*)	33	42	47

8. CONCLUSION

Nanocrystalline thin films of un-doped and Al-doped cadmium oxide were synthesized. The structural and morphological properties of CdO nanoparticles were investigated using an X-ray diffraction analysis and Scanning electron microscopy, respectively.

In the present study, the diameter of the zone of inhibition was found to be more for gram-negative bacteria than gram-positive bacteria for both un-doped CdO and Al-doped CdO.

The values of zone of inhibition were given in Table 1. No citation was found on these particular doping percentages of Al to CdO films; this work was the first of its kind to demonstrate the result of maximum inhibition zones of both gram-positive and gram-negative bacteria.

It was found that there was an increase in the inhibition value for Al-doped CdO when compared with other CdO films. With the increase in doping percentage, the inhibition zone diameter enlarged. The cadmium oxide nano-thin films can kill the gram-negative bacterium, *Vibrio cholerae* than the gram-positive bacteria, *Bacillus cereus*, comparatively.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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