*Nanochem Res 1(2): 222-228, Summer and Autumn 2016*

RESEARCH PAPER

# Synthesis, Characterization and *in Vitro* Antibacterial Activities of CdO Nanoparticle and Nano-sheet Mixed-ligand of Cadmium(II) Complex

***Arezoo Kamali [1], Zohreh Rashidi Ranjbar [1] * and Elham Rezvan Nejad [2]***

[1] *Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, Iran*

[2] *Graduate University and Advanced Technology of Kerman, Kerman, Iran*

**ARTICLE INFO**

***Article History:***
*Received 19 February 2016*
*Accepted 9 May 2016*
*Published 1 July 2016*

***Keywords:***
*Antibacterial*
*CdO*
*Mixed-ligand complex*
*Sonochemistery*
*Thermolysis*

**ABSTRACT**

Here, we report the synthesis of a Schiff-base mixed-ligand complex of cadmium(II) in bulk and nano-scales *via* the precipitation and sonochemical methods, respectively. The complex formula is $[Cd(3\text{-bpdh})(3\text{-bpdb})Cl_2]_n$ (1), where the ligands are 3-bpdh = 2,5-bis(3-pyridyl)-3,4-diaza-2,4-hexadiene and 3-bpdb = 1,4-bis(3-pyridyl)-2,3-diaza-1,3-butadiene. The structure of mixed-ligand complex (1) was characterized by IR, $^1$H NMR and elemental analyses. Cadmium(II) oxide nanoparticles were prepared by direct thermolysis from nanosheet of complex (1). The cadmium(II) oxide structure was characterized by X-ray Diffraction (XRD) and Energy Dispersive X-ray analyses (EDAX). Size, morphology and structural dispersion of all obtained nanostructures were characterized by Scanning Electron Microscopy (SEM). The Schiff-base ligands, bulk and nano-scales of complex (1) and cadmium(II) oxide nanoparticles were analyzed for antibacterial activities against *Bacillus alvei* (bacteria causing the honey bee European foulbrood disease). The Minimum Inhibitory Concentrations (MIC) has been shown moderate antibacterial activities compared with some other standard drugs. Known antibiotics like penicillin and SXT (Trimethoprim/sulfamethoxazole) were used as positive control.

**How to cite this article**
*Kamali A, Rashidi Ranjbar Z, Rezvan Nejad E. Synthesis, Characterization and in Vitro Antibacterial Activities of CdO Nanoparticle and Nano-sheet Mixed-ligand of Cadmium(II) Complex. Nanochem Res, 2016; 1(2): 222-228.*
DOI: 10.7508/ncr.2016.02.009

## INTRODUCTION

Microbial infestation can result in serious infections [1-2]. Antimicrobial compounds could prevent the growth of detrimental microorganisms. So, antimicrobial compound modification is a highly desired objective. Accordingly, there is a significant interest to develop and modify the antimicrobial compounds for biomedical, food and personal-hygiene industry.

Nanoparticles increase chemical activity due to their large surface to volume ratio. Therefore, nanoparticles with antimicrobial properties have the best efficiency [3]. The study on nanomaterial as great antibacterial agents is due to the increase in resistance strains of most bacteria against some potent antibiotics [4]. Antimicrobial compounds 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 [5]. Also, nanomaterials release ions to react by the thiol groups (-SH) of the proteins 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 these proteins and decrease the membrane permeability [5].

* Corresponding Author Email: *zoh.rashidi@uk.ac.ir*

It has been demonstrated that specially formulated metal oxide has good antibacterial activity [6]. In recent years, some researchers have examined the antibacterial properties of cadmium oxide nano structures [7,8]. Cadmium(II) oxide nano structures have been synthesized *via* various physical and chemical methods such as: microwave plasma [9], solid-vapor deposition [10], sol-gel [11], mechanochemical [12], microemulsion [13], solvothermal methods [14].

In this research, Antibacterial activity of cadmium complexes on *Bacillus alvei* bacteria is examined. *Paenibacillus alvei* is an aerobic, Gram-positive, and endospore-forming bacterium, which shows swarming activity on solidified culture media [15]. *P. alvei*, Brevibacillus laterosporus, Enterococcus faecalis, and Achromobacter Eurydice occur as secondary invaders of honeybees during outbreaks of European foulbrood [16]. In addition, *P. alvei* is described as a causative agent of human infections [17].

American beekeepers have used the antibiotic for prevention of European foulbrood disease (EFB) since the 1950s. The use of antibiotics has increased antibiotic resistance. The use of new, commercial antibiotics can create strains that are unaffected by a suite of compounds [18] and can further affect the beekeeping industry since many antibiotics leave residues in hive products [19].

Therefore, in this article, the synthesis and characterization of the mixed ligand complex of cadmium(II) in bulk and nano-scales are investigated. Also, the preparation and characterization of cadmium(II) oxide are reported. Ultrasonic waves are used to prepare some different complexes in nano-scale as previously reported in the literature [20,21]. Moreover, the antibacterial activities of the Schiff-base ligands, bulk complex, cadmium(II) mixed-ligand nano-sheet and cadmium(II) oxide nanoparticles on *Bacilluse alvei* are investigated.

## EXPERIMENTAL

### *Materials and Physical Techniques*

All reagents for the synthesis and analysis were commercially available and used as received. The antibacterial activities were evaluated using *Bacillus alvei* bacteria that causes the honey bee European foulbrood disease.

An ultrasonic bath (type; DT510H, 50-60 HZ 230 W) was used for the ultrasonic irradiation. Melting points were measured on an Elemental Engineering Ltd.-IA9200 apparatus. FTIR spectra were recorded using Brucker FT-IR Tensor 27spectrophotometers. $^1$H NMR spectra were measured by a BRUKER DRX-500 AVANCE spectrometer at 500 MHz. The samples were characterized by a scanning electron microscope (SEM) (Company KYKY and model EM3200) with gold coating.

**Synthesis of 2,5-bis(3-pyridyl)-3,4-diaza-2,4-hexa-diene (3-bpdh) ligand.** 1.53 ml methyl 3-pyridyl ketone was dissolved in ethanol (25 ml), followed by dropwise addition of 1.53 ml hydrazine monohydrate solution in ethanol (25 ml). After the addition of two drops of formic acid, the mixture was stirred at room temperature for 24 h. The solvent was removed under vacuum, and upon removal of the solvent, bright yellow crystalline solid was obtained [22].

IR (υ, cm$^{-1}$): 702(s), 814(s), 1018(s), 1075(w), 1120(w), 1366(s), 1412(s), 1603(s), 1699(w), 2969(w) and 3446(w).

**Synthesis of 1,4-bis(3-pyridyl)-2,3-diaza-1,3-buta-diene (3-bpdb) ligand.** 25 ml ethanolic solution of hydrazine monohydrate (1.53 ml) was added dropwise to a solution of pyridine-3-carbaldehyde (5 ml) dissolved in ethanol (25 ml). Two drops of formic acid were added and the mixture was stirred at room temperature for 24 h. The yellow solid formed was filtered [23].

IR (υ, cm$^{-1}$): 522(w), 660(w), 846(w), 1344(w), 1405(w), 1511(br), 1550(w), 1604(s), 1650(s), 3045(s) and 3067(s).

**Synthesis of $[Cd(3\text{-}bpdh)(3\text{-}bpdb)(Cl)_2]_n$ (1), in bulk form.** The bulk form of complex (1), $[Cd(3\text{-}bpdh)(3\text{-}bpdb)\ Cl_2]_n$, as yellowish powder was obtained from adding an ethanolic solution of two Schiff-base ligands, 3-bpdb (0.105 g, 0.5 mmol) and 3-bpdh (0.12 g, 0.5 mmol), to an ethanolic solution of $CdCl_2$ (0.154 g, 0.50 mmol) and $NaClO_4$ (0.061 g, 0.50 mmol). IR (υ, cm$^{-1}$): 1362(s), 1425(w), 1589(w), 1616(s), 2922(w), 3067(w), 3426(w). $^1$H NMR (δ, ppm): 2.21 (s, $3H_{Me}$), 7.47 (s, $1H_{imine}$), 7.52-7.64 (m, $2H_{Py}$), 8.01-8.40 (m, $2H_{Py}$), 8.81-9.24 (m, $4H_{Py}$). For $C_{26}H_{24}N_8ClCd$ Anal. Calcd., %: C, 28.91; H, 3.15; N, 10.38. Found, %: C, 28.50; H, 3.08; N, 10.29.

**Synthesis of $[Cd(3\text{-}bpdh)(3\text{-}bpdb)(Cl)_2]_n$ (1), as nano-sheet.** We demonstrate the use of sonochemistry to prepare nanometer-scale of complex (1). The method affords nano-sheet of complex (1) with a narrow size distribution. In this method, 10 ml solution of $CdCl_2$ (0.154 g, 0.50 mmol) and $NaClO_4$ (0.061 g, 0.50 mmol) was

poured into round-bottom flask and placed in an ultrasonic bath. Then, 5 ml solution of 3-bpdb (0.105 g, 0.5 mmol) and 3-bpdh (0.12 g, 0.5 mmol) poured dropwise into this solution for one hour. The yellowish powder was filtered, and dried in ambient atmosphere.

IR (υ, $cm^{-1}$): 1365(s), 1474(s), 1484(w), 1613(s), 2923(w), 3071(w), 3423(w).

**Synthesis of cadmium(II) oxide nanoparticles by direct thermolysis.** As expected, CdO nanoparticles with sizes of 28±3 nm were generated *via* athermolysis reaction of complex (1) nano-sheet, in air atmosphere at 700 °C for two hours.

### *In Vitro Antibacterial Assay*

The antibacterial nature of all samples was studied by "Well Diffusion method". Sterilization and preparation of the media are the initial steps in this test. All glasswares and equipment used in the experimental part are sterilized and incubated at 125 °C for 1 h. The Inhibition Zone Diameter (IZD, mm) was recorded for each compound at 100 µg $ml^{-1}$. Powdered samples were taken and dissolved in DMSO and mixed well until they are dissolved in the solvent. In order to clarify the effect of DMSO in the biological screening, blank studies were carried out, and no activity was observed against any bacterial strains in pure DMSO. The macro-dilution broth susceptibility assay was used for the evaluation of Minimal Inhibitory Concentration (MIC). 12 Test tubes are required for macro-dilution method. By including 1 ml nutrient broth in each test tube, and then adding 1 ml extract with concentration 100 mg $ml^{-1}$ in the first tube, we made serial dilution of this extract from first tube to the last one. Bacterial suspension was prepared to match the turbidity of 0.5 Mcfarland turbidity standards. Then, 1 ml of bacterial suspension was added to each test tube. After incubation at 37 °C for 24 h, the last tube was determined as the minimal inhibitory concentration (MIC) without turbidity.

## RESULTS AND DISCUSSION

Elemental analysis and spectroscopy data show that complex (1), $[Cd(3\text{-bpdh})(3\text{-bpdb})Cl_2]_n$, has been synthesized. Two Schiff-base ligands are coordinated to cadmium(II) by their nitrogen atoms of pyridine groups (Fig. 1). The IR spectra of the free Schiff base ligands and corresponding complexes show several bands in the 400-4000 $cm^{-1}$ region. The IR spectra of complex (1) in bulk and nano scales are approximately the same as shown in Fig. 2. The weak

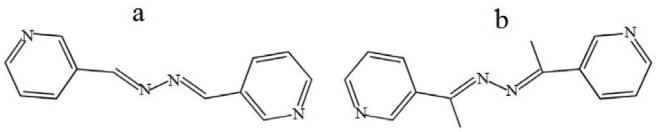


**Fig. 1.** Two Schiff-base ligands; a)3-bpdb and b)3-bpdh.

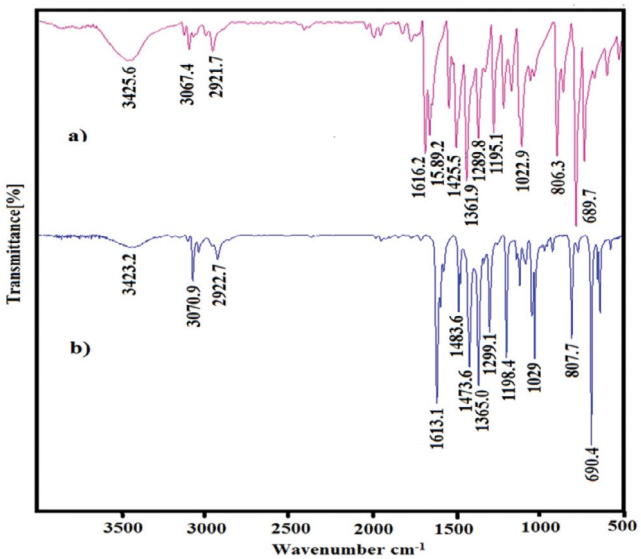


**Fig. 2.** The IR spectra of complex (1) at (a) bulk form and (b) nano-scale.

broad band at around 3423 $cm^{-1}$ and the relatively weak absorption bands at around 2922-3070 $cm^{-1}$ are due to the O-H (water molecules) and C-H (aromatic rings) modes, respectively. The variable intensity absorption bands in the frequency range 1300-1600 $cm^{-1}$ correspond to ring vibrations of the "py" moiety of the ligands. The ν(C=N) bands appearing at 1603 $cm^{-1}$ in 3-bpdh and 1604 $cm^{-1}$ in 3-bpdb are shifted to higher frequencies by about 12 $cm^{-1}$ in the corresponding complex, indicating that the ligands are coordinated to the metal ions through the nitrogen atoms of the pyridyl groups not to the azomethine groups (Fig. 2). $^1H$ NMR spectrum in DMSO-$d_6$ shows some characteristic signals at 7.52 to 9.24 ppm corresponding to protons of the aromatic rings. Two single peaks at δ = 2.21 and 4.74 ppm correspond to methyl and imine groups of 3-bpdh and 3-bpdh molecules, respectively.

The size, structural morphology and dispersion of nanosheet complex (1) and CdO nanoparticles were investigated by Scanning Electron Microscopy (SEM). As the SEM images in Fig. 3 show, complex (1) has good morphology as nano-sheet with the average thickness of about 130 nm. Thickness and structural dispersion were estimated by using measurement software. The obtained data were used to sketch a histogram plot (Fig. 7a).

Fig. 4 shows CdO nanoparticles with good dispersion in size and morphology. The average size (27±2 nm) of the particles and structural dispersion was estimated from the shown histogram plot in Fig. 7b. As the Energy-dispersive X-ray spectroscopy (EDAX) data show cadmium and oxygen atoms are elementary components in the structure of CdO nanoparticles with the highest percentage (Fig. 5).

Fig. 6 shows the XRD pattern of CdO nanoparticles and the diffraction intensities recorded from 10° to 80°, in 2θ angles. The observed pattern matches with the reference of JCPDS File with No = 73-2245. The lattice plan with (111) miller indices has the highest intensity in 2theta rang of 32.98°. Also, the average size of the nanoparticles estimated using the Debye-Scherrer equation was found to be 40 nm. The Debye-Scherrer equation is "D = kλ/βcosθ" where

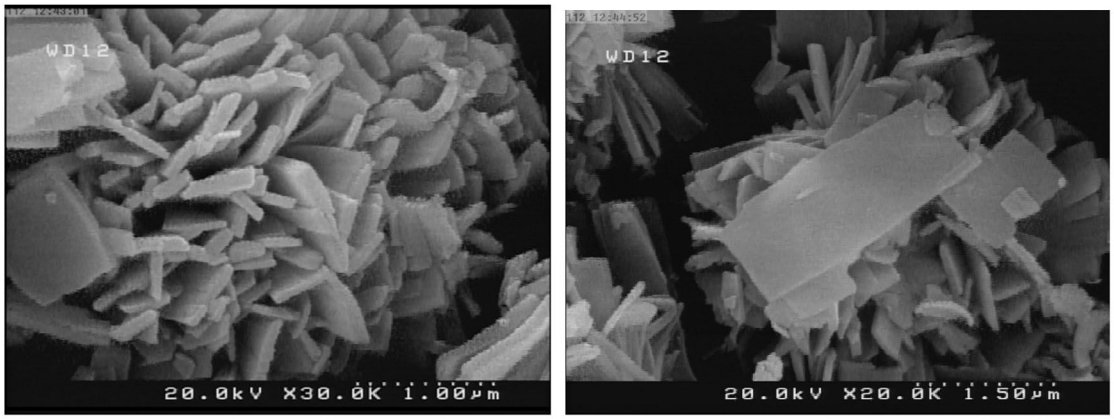


**Fig. 3.** The SEM image of nano-sheet complex (1), $[Cd\ (3\text{-bpdh})(3\text{-bpdb})\ Cl_2]_n$.

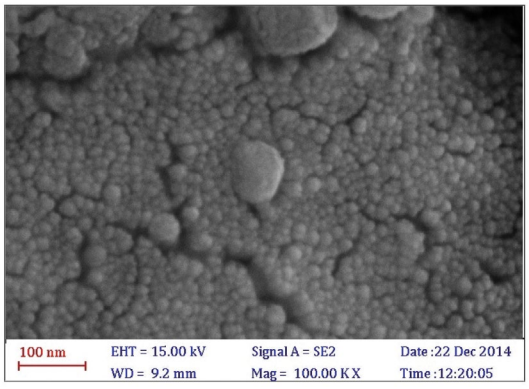


**Fig. 4.** The SEM image of CdO nanoparticles.

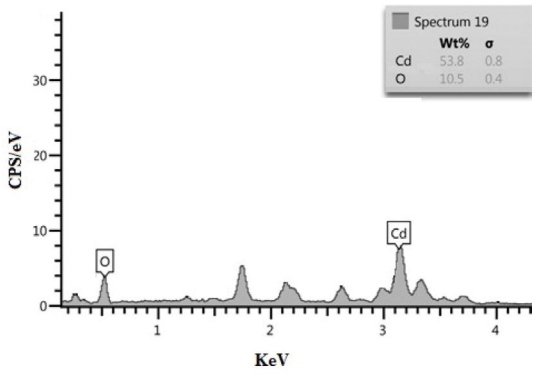


**Fig. 5.** EDAX analysis data of CdO nanoparticles.

D = diameter of nanoparticles, k = 0.94, λ (used wavelength of X rays) = $1.5406 \times 10^{-10}$, β = full width at half-maximum, θ = Bragg angle.

*In vitro* antibacterial activities were tested by the well-known diffusion method using Nutrient agar. The Inhibition Zone Diameter (IZD, mm) antibacterial activities and Minimal Inhibitory Concentrations (MIC, μg $ml^{-1}$) data of the schiff base ligands, the bulk and nano forms of complex (1), CdO nanoparticles, Penicillin and Trimethoprim/ sulfamethoxazole (SXT) are shown in Table 1. Penicillin and SXT were selected as the standard compounds. The *Bacillus alvei* (RITCC 2384), causing the honey bee European foulbrood disease, was used as investigated organism. Based on the data reported in Table 1, two Schiff-base ligands have lower anti-bacterial activity compared with the bulk and nano forms of complex (1) and two standards; Penicillin and SXT (30 mg/ disk). The more activity of the bulk and nano forms of complex (1) compared with ligands is due to positive charge of central cadmium(II) ions. The nitrogen atoms of Schiff-base ligands ($L_1$, $L_2$) are coordinated to cadmium(II) ions. The

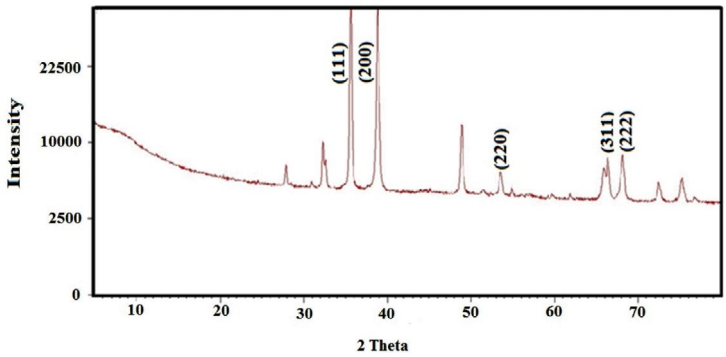


**Fig. 6.** XRD pattern of CdOnanopaticles.

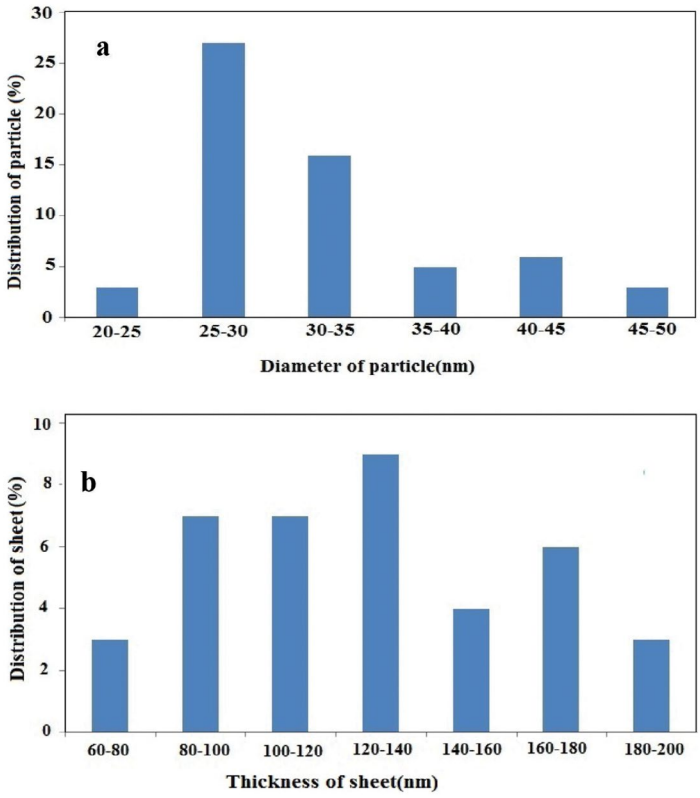


**Fig. 7.** Histogram plot of a) CdO nanoparticles b) complex (1) nano-sheets.

**Table 1.** Inhibition Zone Diameter (IZD, mm) and Minimal Inhibitory Concentrations (MIC, μg $ml^{-1}$) against Growth of Bacteria

| Samples | $L_1$ | $L_2$ | Complex 1 bulk form | Complex 1 nano-sheets | CdO nano-particles | Penicillin | SXT |
|---|---|---|---|---|---|---|---|
| IZD (mm) | 12 | 16 | 18 | 24 | 12 | 33 | 26 |
| MIC (mg $ml^{-1}$) | 12.50 | 6.25 | 3.12 | 28.50 | 0.028 | 12.50 | 6.25 |

coordination causes the pi electron delocalization in over the whole chelate moiety and increases the lipophilic nature of complex. So, the complex (1) would be stronger in penetrating through the lipid layers of bacteria membranes and acts as a better anti-microbial agent. Also, the nano-sheet of complex (1) has good activity against *Bacillus alvei* bacteria compared with bulk form; this is probably due to the diminished particle size. The MIC values show all compounds are effective on *Bacillus alvei* bacteria (Table 1).

Antibacterial effect of either natural compounds or synthetic agents is based on the nature of the agents and their quality of affecting and working on targeted organisms. Understanding the mechanism (s) of action in different anti-microbial agents is an important prerequisite to understand mechanisms of resistance. In fact, in many cases, an elucidation of resistance mechanisms has enhanced understanding of specific mechanisms of action. In the present investigation, although mechanism of action is not clear, it can be assumed that the chelation theory [24] can be used to explain the increased activity of the metal complexes as observed in earlier studies with analogous palladium complexes [25]. However, some previous studies have shown that the macrocyclic ligands activity decreases upon coordination [26].

In conclusion, complex (1) was prepared in bulk and nano scales. Complex (1) as nano-sheet structure was also obtained using ultrasonic irradiation in methanol solution. Both of them are characterized by IR and $^1$H NMR spectroscopies. Calcination of nano structure of Complex (1) at 700 $^o$C produces nanoparticles of CdO. These nanoparticles are characterized by XRD, SEM and EDAX. In addition, experimental investigation shows that these compounds have antibacterial activity against *Bacillus alvei*. Results also show that the bulk and nano mixed-ligand of complex (1) have stronger antibacterial effect against *Bacillus alvei* bacteria compared with Schiff base ligands and cadmium(II) oxide. The biological properties of two cadmium complexes are more significant with respect to the free ligand. The complexation processes reduce the polarity and increase the lipophilic nature of $Cd^{2+}$ and π-electron delocalization in the ligand structure. This achievement can be explained by the Overtone's concept and Tweedy's chelation theory [27,28].

So, the permeation was increased through the lipid layer of the cell membrane of microorganism and the antibacterial activity of the cadmium complex was improved. Also, MIC data show that the CdO nanoparticles have potent antibacterial activities and would be appeared as a good biologically active product compared with free ligands and two scales of complexes. We propose that the small size (≈30 nm) and spherical morphology of these particles might be the reason for the improvement of the antibacterial activity. These parameters (size and shape) might increase supporting the diffusion into cell membranes.

## ACKNOWLEDGEMENTS

The authors acknowledge financial support by Shahid Bahonar University of Kerman.

## REFERENCES

[1] Jones GL, Muller C, O'Reilly M, Stickler D. Effect of triclosan on the development of bacterial biofilms by urinary tract pathogens on urinary catheters. J. Antimicrob. Chemother. 2006; 57 (2): 266-72.

[2] Greenberg CB, Steffek C. Bio-adhesion to thin films in relation to cleaning. Thin Solid Films. 2005; 484 (1): 324-7.

[3] Gyssens IC. Antibiotic policy. Int. J. Antimicrob. Agents. 2011; 38: 11-20.

[4] Shivaji S, Madhu S, Singh S. Extracellular synthesis of antibacterial silver nanoparticles using psychrophilic bacteria. Process Biochem. 2011; 46 (9): 1800-7.

[5] Holt KB, Bard AJ. Interaction of silver(I) ions with the respiratory chain of Escherichia coli: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag+. J. Biochem. 2005; 44 (39): 13214-23.

[6] Shukla M, Kumari S, Shukla S, Shukla R. Potent antibacterial activity of nano CdO synthesized via microemulsion scheme. J Mater Environ Sci. 2012; 3(4): 678-85.

[7] Rezaei-Zarchi S, Javed A, Javeed Ghani M, Soufian S, Barzegari Firouzabadi F, Bayanduri Moghaddam A, et al. Comparative study of antimicrobial activities of $TiO_2$ and CdO nanoparticles against the pathogenic strain of

Escherichia coli. Iran J Pathol. 2010; 5 (2): 83-9.
[8] Hossain ST, Mukherjee SK. CdO nanoparticle toxicity on growth, morphology, and cell division in Escherichia coli. Langmuir. 2012; 28 (48): 16614-22.
[9] Jong Hun K, Yong Cheol H, Han Sup U. Direct Synthesis and Characterization of CdO Nano-Cubes. Jpn. J. Appl. Phys. 2007; 46 (7R): 4351.
[10] Zaien M, Omar K, Hassan Z. Growth of nanostructured CdO by solid-vapor deposition. Int. J. Phys. Sci. 2011; 6 (17): 4176-80.
[11] Rashidzadeh M, editor Antibacterial properties Of CdO Nano-Cubes synthesized via Microwave Method. Adv Mat Res. 2014; 839: 294.
[12] Yang H, Qiu G, Zhang X, Tang A, Yang W. Preparation of CdO nanoparticles by mechanochemical reaction. J. Nanopart. Res. 2004; 6 (5): 539-42.
[13] Liu Y, Yin C, Wang W, Zhan Y, Wang G. Synthesis of cadmium oxide nanowires by calcining precursors prepared in a novel inverse microemulsion. J. Mater. Sci. Lett. 2002; 21 (2): 137-9.
[14] Ghosh M, Rao C. Solvothermal synthesis of CdO and CuO nanocrystals. Chem. Phys. Lett. 2004; 393 (4): 493-7.
[15] Kim Y-G, Lee J-H, Cho MH, Lee J. Indole and 3-indolylacetonitrile inhibit spore maturation in Paenibacillus alvei. BMC Microbiol. 2011; 11 (1): 1.
[16] Forsgren E. European foulbrood in honey bees. J Invertebr Pathol. 2010; 103: S5-S9.
[17] Reboli A, Bryan C, Farrar W. Bacteremia and infection of a hip prosthesis caused by Bacillus alvei. J. Clin. Microbiol. 1989; 27 (6): 1395-6.
[18] Kochansky J, Knox D, Feldlaufer M, Pettis J. Screening alternative antibiotics against oxytetracycline-susceptible and-resistant Paenibacillus larvae. Apidologie. 2001; 32 (3): 215-22.
[19] Feldlaufer MF, Pettis JS, Kochansky JP, Kramer M. Residue levels in honey after colony treatment with the antibiotic tylosin. Am Bee J. 2004; 144: 143.
[20] Shirazi FS, Akhbari K. Solid-state thermal conversion of a nanoporous metal-organic framework to a nonporous coordination polymer. RSC Adv. 2015; 5 (63): 50778-82.
[21] Shirazi FS, Akhbari K. Sonochemical procedures; the main synthetic method for synthesis of coinage metal ion supramolecular polymer nano structures. Ultrason. Sonochem. 2016; 31: 51-61.
[22] Dong Y-B, Smith MD, Layland RC, zur Loye H-C. A novel noninterpenetrating polycyclohexane network: a new inorganic/organic coordination polymer structural motif generated by self-assembly of "T-shaped" moieties. Chem. Mater. 2000; 12 (4): 1156-61.
[23] Kesslen EC, Euler WB. Synthesis and characterization of pyridine end-capped oligoazines. Tetrahedron Lett. 1995; 36 (27): 4725-8.
[24] Choudhary A, Singh R. Synthetic, spectroscopic and toxicological aspects of newely designed tetraazamacrocyclic complexes of copper(II). Rasāyan J Chem. 2008; 1 (1): 15-21.
[25] Roy TG, Hazari SK, Barua KK, Kim DI, Park YC, Tiekink ER. Syntheses, characterization and anti-microbial activities of palladium(II) and palladium(IV) complexes of 3, 10-C-meso-Me8 [14] diene (L1) and its reduced isomeric anes (LA, LB and LC). Crystal and molecular structure of $[PdL1][Pd(SCN)_4]$. Appl. Organomet. Chem. 2008; 22 (11): 637-46.
[26] Roy TG, Hazari SK, Dey BK, Meah HA, Rahman MS, Kim DI, et al. Synthesis, electrolytic behaviour and antimicrobial activities of cadmium complexes of isomers of 3, 10-C-meso-3, 5, 7, 7, 10, 12, 14, 14-octamethyl-1, 4, 8, 11-tetraazacyclotetradecane. J. Coord. Chem. 2007; 60 (14): 1567-78.
[27] Tweedy B. Plant extracts with metal ions as potential antimicrobial agents. Phytopathology. 1964; 55: 910-4.
[28] Searl JW, Smith R, Wyard S. Electron Spin Resonance Absorption for Polycrystalline Substances: II. Proc. Phys. Soc. 1961; 78 (6): 1174.