RESEARCH PAPER

Synthesis, characterization, and antibacterial activities of Ag_2O nanoparticles and silver (I) nanorod compound

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ABSTRACT

In this work, we synthesized a new compound of silver(I) with a Schiff-base ligand, 2,5-bis(3-pyridyl)-3,4-diaza-2,4-hexadiene (L=3-bpdh), $[Ag(3\text{-bpdh})(NO_3)]_n$ (1). The compound was characterized by infra-red (IR) spectroscopy. The nanorods of this compound were synthesized by sonochemistery method and characterized by IR spectroscopy and scanning electron microscopy (SEM). The nanoparticles of silver(I) oxide were obtained by direct thermolysis at 700°C on air atmosphere and characterized by X-ray diffraction, SEM, and energy dispersive X-ray analyses. The Schiff-base ligand, bulk and nano-forms of compound (1) and silver (I) oxide nanoparticles were screened for antibacterial activities against two Gram-positive (*Staphylococcus aureus, Enterococcus faecalis*) and two Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria. The results revealed that all compounds exhibit antimicrobial activities. The compound 1 in nano-scale and silver(I) oxide nanorods have a stronger antibacterial effect in comparison with Schiff base ligand and bulk form of the compound 1.

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INTRODUCTION

Silver nanoparticles (AgNPs) belong to a special group of materials with many applications in various fields such as dentistry, clothing, photography, catalysis, mirrors, optics, electronics, and food industry. Due to such potentials many methods of synthesis of AgNPs have been developed. Such methods should control the size of AgNPs. Efficient synthesis of small particles without bulking was favorable [1, 2]. The most important methods for the synthesis of AgNPs are follows: chemical reduction [3, 4], optical reduction [5], micelles [6], gamma irradiation [7], sol-gel [8] and biosynthetic methods [9–11]. When the size of silver particles decreases to nano-scale, their antibacterial efficacy increases because of their larger total surface area per unit volume [12, 13].

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It is generally believed that heavy metals react with proteins by combining the thiol (SH) groups, leading to the inactivation of proteins [14]. Recent, microbiological and chemical experiments implied that interaction of silver ions with thiol groups played an essential role in bacterial inactivation [15]. However, the antimicrobial effects of silver nanoparticles (Ag-NPs) were not fully investigated.

The aim of this work is synthesis, characterization, and study the antibacterial activities of silver(I) complex $[Ag(3\text{-bpdh})(NO_3)]_n$ in nano-scale as well as bulk. The antibacterial activities of the Schiff base ligand and silver(I) oxide nanoparticles are also investigated against two Gram-positive and two Gram-negative bacteria.

MATERIALS AND METHOD

Materials and physical techniques

All reagents for the synthesis and analysis were

commercially available and used as received. 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. IR spectra were recorded using Brucker FT-IR Tensor 27 spectrophotometer. X-ray powder diffraction (XRD) measurements were performed using a Philips X'pert diffractometer with monochromatized Cu k_α radiation. The samples were characterized with a scanning electron microscope (SEM) (Company KYKY and model EM3200) with gold coating.

Synthesis of the Schiff base ligand

1.53 mL methyl 3-pyridyl ketone was dissolved in ethanol (25mL), followed by dropwise addition of 1.53 mL hydrazine monohydrate solution in ethanol (25 mL). After addition of two drops of formic acid, the mixture was stirred at ambient temperature for 24 h. The solvent was removed under vacuum, and upon removal of the solvent, bright yellow crystalline solid was obtained [16].

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). ^{1}H-NMR (DMSO, δ): 9.1(s, 2H); 8.6 (d, 2H); 8.3 (d,2H); 7.5 (d.d, 2H) ppm

Synthesis of silver(I) complex $[Ag(3\text{-}bpdh)(NO_3)]_n$

2,5-Bis(3-pyridyl)-3,4-diaza-2,4-hexadiene (L=3-bpdh) (0.12 g, 0.5 mmol) was dissolved in methanol (10 mL) and poured dropwise into prepared alcoholic solution of silver(I) nitrate (0.085 g, 0.50 mmol) and sodium perchlorate (0.061 g, 0.50 mmol). After this addition, a light-yellow precipitate was obtained.

Synthesis of silver(I) $[Ag(3\text{-}bpdh)(NO_3)]_n$(1) nanorods by sonochemical method

10 ml of an alcoholic solution containing silver(I) nitrate (0.085g, 0.50 mmol) and sodium perchlorate (0.061 g, 0.50 mmol) in a round-bottom flask was placed into ultrasonic bath, then 5 ml solution of 3-bpdh (0.12g, 0.50 mmol) poured dropwise into this solution for 1 h. The nano-structure product was filtered, and then dried.

Synthesis of silver(I)oxide by direct calcination

Ag_2O were prepared by heating compound **1** in an electrical furnace at 700 °C for 2 h.

Antibacterial activity test

Assessment of antibacterial effect of the components was carried out by using well diffusion method [17, 18]. It was determined against the two Gram-positive bacteria: *Staphylococcus aureus* PTCC1112, *Enterococcus faecalis* (isolated from clinical samples, Afzalipour Hospital in Kerman, Iran) and also two Gram-negative bacteria: *Escherichia coli* PTCC 1330 and *Pseudomonas aeruginosa* ATTC 27853.

In order to test the antimicrobial activity, the samples were dissolved in HNO_3 (70%). Then, after adding 0.1 molar sodium bicarbonate to the solution, the pH value increased to a neutral range of 7-8. Media Agar (20 mL) was poured into each 15 cm Petri dish. Growth was adjusted to a turbidity equivalent to a 0.5 McFarland standard. 0.01 μL of suspension containing approximately 10^8 bacteria/mL was placed over Agar in Petri dishes and dispersed. Then, wells were cut and 50 µl of the compound was added. The plates were then incubated at 37°C for 24-48 h. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Two standard antibiotics [Penicillin (10 mg) and Trimethoprim/Sulfamethoxazole (SXT)] were used as the positive controls.

The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by macro dilution assay (NCCLS, 2008). The cultures were prepared in 24 h and 72 h broth cultures of microorganisms, respectively. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms and the MBC was defined as the lowest concentration of compound to kill the microorganisms. Serial dilutions ranging from 0.195– 50 mg/ml were prepared in medium.

RESULTS AND DISCUSSION

IR Spectroscopy

Elemental analysis and spectroscopy data show complex (**1**), $[Ag(3\text{-}bpdh)(NO_3)]_n$, has been synthesized. The Schiff-base ligand was attached to silver (I) by the nitrogen atoms of pyridine groups (Fig. 1). The IR spectra of compound **1** in bulk form and nanorod are the same. The IR spectrum of compound **1** (Fig.2) in nanoparticle show a weak broad band at around 3400 cm^{-1} and the relatively weak absorption bands at around 3038–3072 cm^{-1}, due to the O–H (water molecules) and C–H modes (aromatic rings), respectively. The variable intensity absorption bands in the frequency range 1300–1613 cm^{-1} correspond to ring vibrations of the "py" moiety of the ligands.

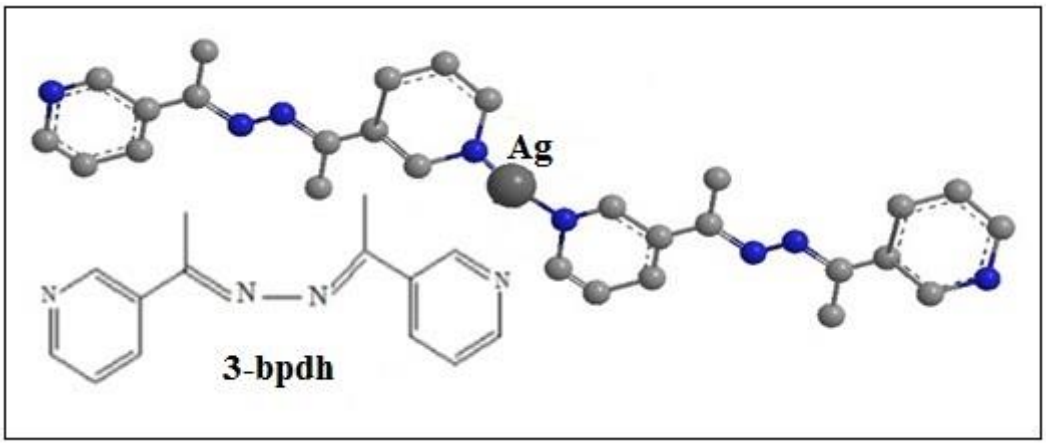

Fig.1. The structures of Schiff-base ligand (3-bpdh) and complex (1), $[Ag(3\text{-bpdh})(NO_3)]_n$. The color of atoms: carbon=gray, silver= dark gray, nitrogen=dark blue.

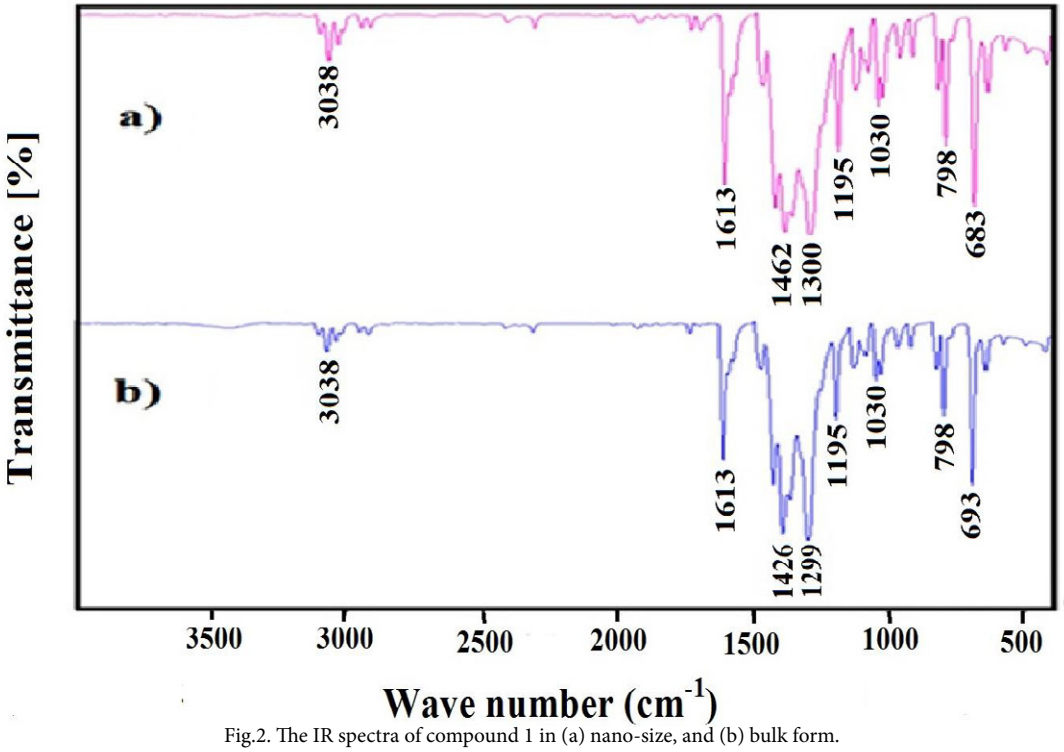

Fig.2. The IR spectra of compound 1 in (a) nano-size, and (b) bulk form.

SEM-EDAX analysis

The morphology, structure and size of the compound **1** were investigated by Scanning Electron Microscopy (Fig.3). Fig. 3 clearly indicates the nanorod morphology for the particles, prepared by sonochemical process, with the average diameter of about 53 nm. Structural dispersion was estimated by using measurement software. The obtained data were used to sketch a histogram plot (Fig. 5a).

silver(I) oxide was obtained with thermolysis in air atmosphere at 700˚C for 2 h. The morphology and structure of the silver(I)oxide were investigated by Scanning Electron Microscopy (Fig. 4). From the histogram plot in Fig. 5b, the average size of 155 nm was estimated for the particles and structural dispersions.

The energy-dispersive X-ray (EDAX) spectroscopy of Ag_2O nanoparticles shows the

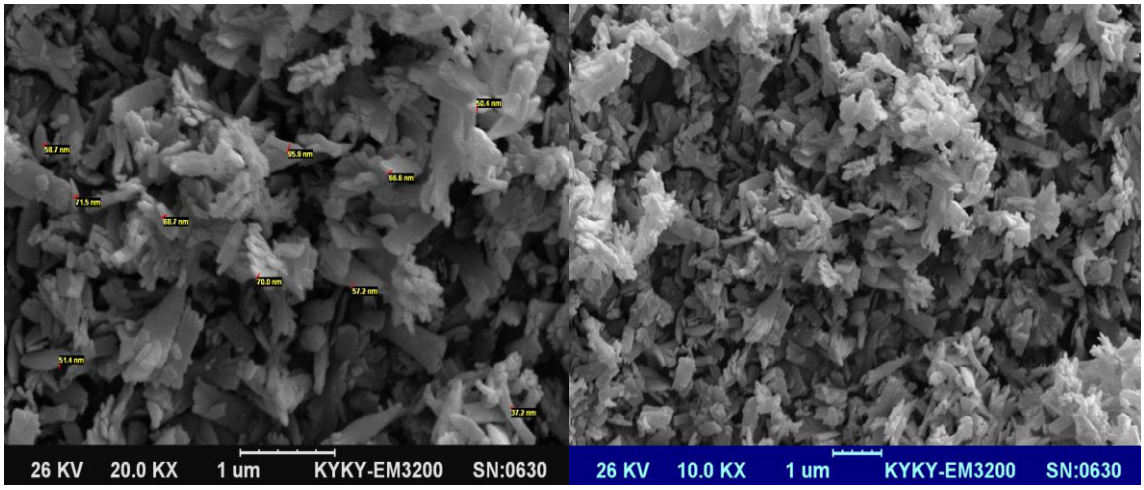

Fig.3. The SEM image of nano-rod complex (1), $[Ag\ (3\text{-bpdh})(NO_3)]_n$

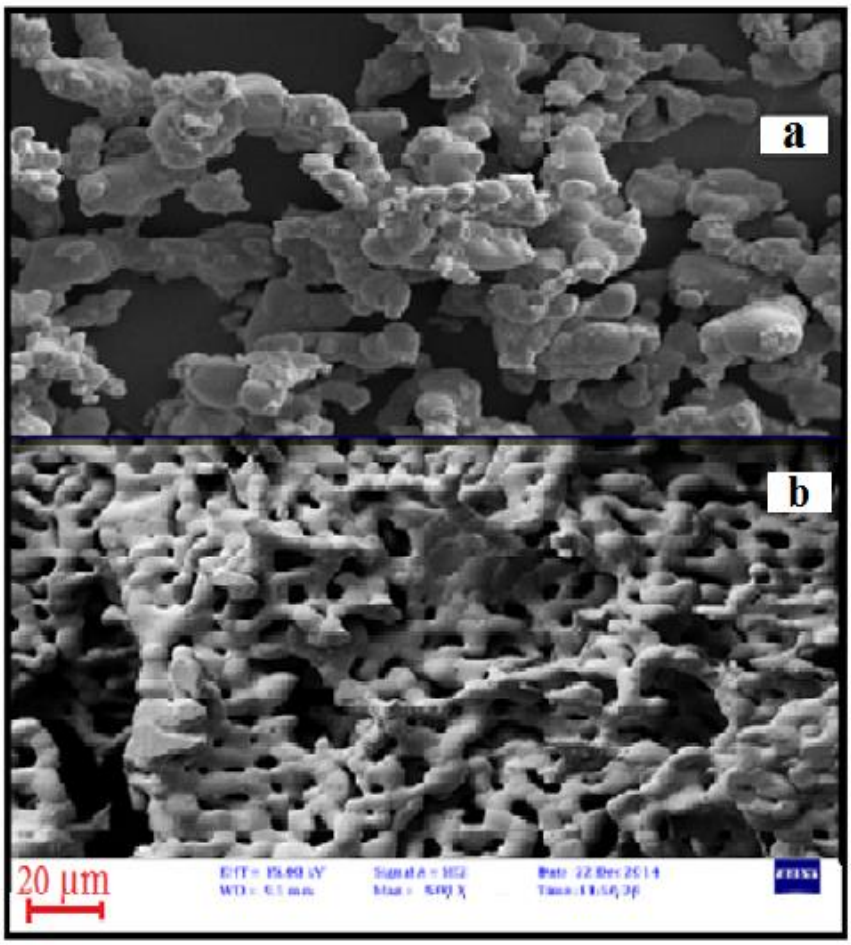

Fig.4. The SEM image of silver(I) oxide nanoparticles prepared by calcination of compound 1
a)1 Mm b)20 Mm scale bar

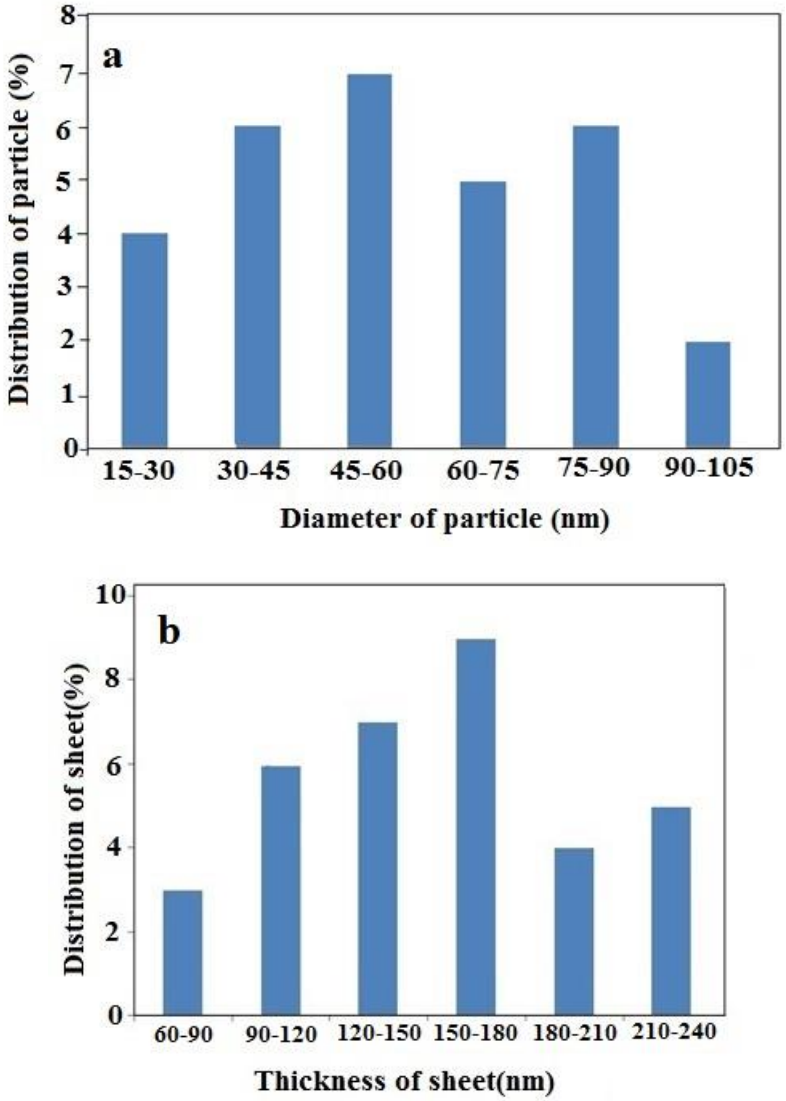

Fig. 5. Histogram plot of a) complex (1) nanorod, and b) Ag_2O nanoparticles

presence of silver and oxygen as the only elementary components (Fig.6).

X-ray diffraction

Fig. 7a shows the XRD pattern of Ag_2O nanoparticles that is the residue from the calcination of compound **1**. The diffraction intensities were recorded 2θ angles from 10˚ to 80˚. Four additional broad bands are observed at 2θ = 37˚ (111), 48˚ (200), 68˚ (220) and 77˚ (311) planes of silver(I) oxide.

The average size (24.4 nm) of Ag_2O nanoparticles can be estimated using the Scherrer equation from the line broadening of the (111) reflection. $D=k\lambda/\beta\cos\theta$, where D=thickness of the nanocrystal, $k_{(Constant)}= 0.94$, λ= wavelength of X-rays, β=width at half maxima of (111) reflection at 2θ Bragg's angle.

IR spectroscopy of silver(I) oxide

Fig. 7b shows the IR spectrum of silver(I) oxide. The absorption bands at 520 cm^{-1} shows Ag–O bond, and the absorption bands at 3430 and 1638 cm^{-1} are attributed to the ν(OH) stretching and bending vibrations, respectively, indicating the presence of physisorbed water molecules linked to Ag_2O nanoparticles [19].

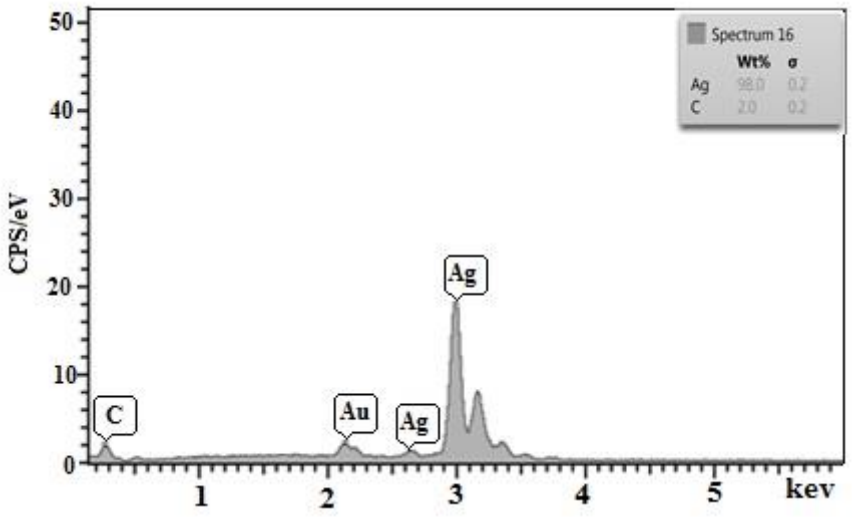

Fig.6. Energy-dispersive X-ray analysis of Ag_2O nanoparticles produced by calcination of compound 1

a

b

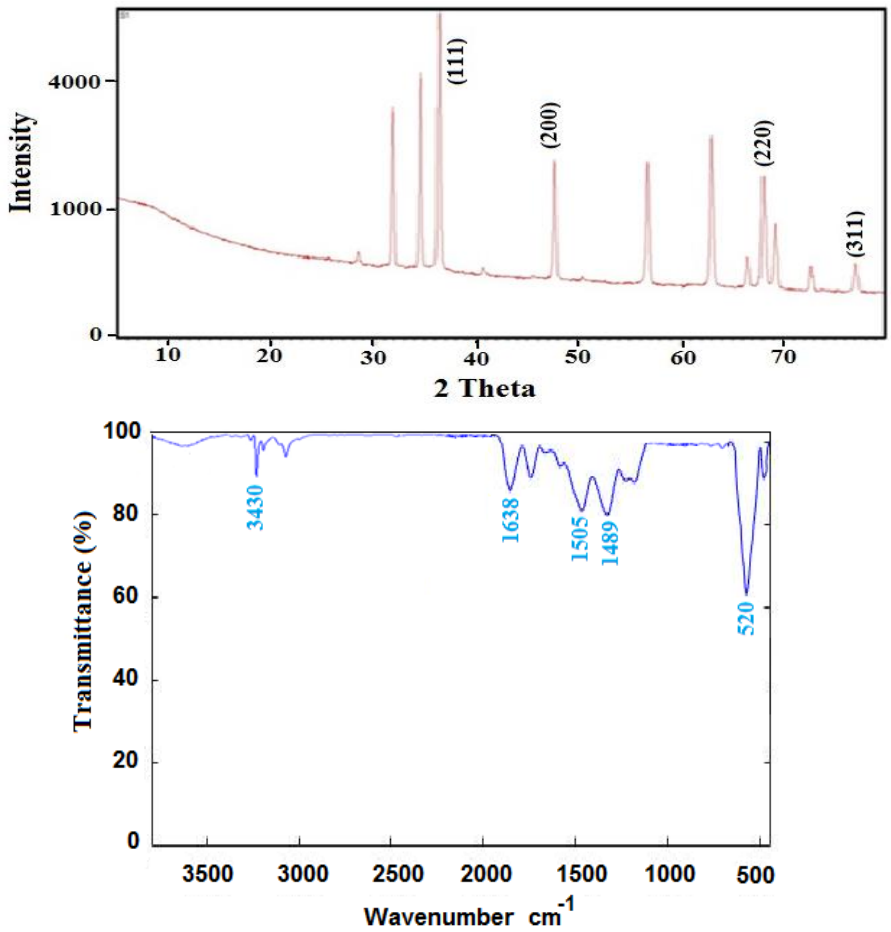

Fig. 7 a) XRD pattern and b) IR spectrum of Ag_2O nanoparticles

Table 1. Inhibition zone diameter (IZ) of samples (50 mg/mL) against microorganisms; antibacterial activities.

Microorganisms	Inhibition Zone Diameter (mm)					
	L	compound **1** in bulk form	compound **1** in nano-scale	Ag_2O	Penicillin	SXT
***Staphylococcus aureus* PTCC1112**	11	14	17	18	30	36
***Escherichia coli* PTCC1330**	10	12	17	18	-	40
Enterococcus faecalis*	-	9	10	17	-	40
***Pseudomonas aeruginosa* ATCC27853**	9	12	14	14	-	33

*isolated from clinical samples

Table 2. In vitro antimicrobial activity of the compounds, minimal inhibitory concentrations (MIC, mg/ml).

Microorganisms	MIC (mg/mL)			
	L	compound **1** in bulk form	compound **1** in nano-scale	Ag_2O
***Staphylococcus aureus* PTCC1112**	12.5	3.12	6.25	6.25
***Escherichia coli* PTCC1330**	25	12.5	3.12	25
Enterococcus faecalis*	-	25	3.12	1.56
***Pseudomonas aeruginosa* ATCC27853**	12.5	3.12	1.56	3.12

*isolated from clinical samples

Antibacterial activity

Table 1 shows the antibacterial activity results of Schiff bases ligand, bulk and nanorod complex and silver (I) oxide nanoparticles evaluated by well diffusion method against *Staphylococcus aureus*, *Enterococcus faecalis*and *Escherichia coli* and *Pseudomonas aeruginosa*. We found that the ligand shows lower antibacterial activity compared to the other compounds (bulk complex, nano-rod complex and silver (I) oxide nanoparticles). It seems that the nano-rod complex was more effective than the bulk complex against all tested bacteria; this is probably due to the diminished size of particles in these complexes. The MIC values for the compounds were in the range of 0.195-50 mg/ml. The results show that the compounds were effective on bacteria (Table 2). According to the results, the complex was more effective against bacteria. It is probably due to the positive charge of central atom shared with donor atoms of ligand and pi electron delocalization in over the whole chelate moiety, leading to the lipophilic nature and positive charge of complex. This property makes it stronger in penetrating through the lipid layers of microbial membranes and therefore, a better anti-bacterial agent.

CONCLUSION

The Schiff base ligand (L), 2,5-bis(3-pyridyl)-3,4-diaza-2,4-hexadiene (3-bpdh), the bulk and nanorod complex, and silver(I) oxide nanoparticles were synthesized and characterized by spectroscopic methods such as IR, SEM, XRD and EDAX. In addition, experimental investigations showed that these compounds have an antibacterial activity against *E. coli, S. aureus, Enterococcus faecalis* and *P. aeruginosa*. Results showed that compound **1** in nano-scale and silver(I) oxide nanorods have stronger antibacterial effects against *E. coli, S. aureus,* and *P. aeruginosa* bacteria in comparison with Schiff base ligand, and that nanorod silver(I) particles have a good antibacterial activity in comparison with bulk complex.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

REFERENCES

1. Matei A, Cernica I, Cadar O, Roman C, Schiopu V. Synthesis and characterization of ZnO – polymer nanocomposites. International Journal of Material Forming. 2008;1(S1):767-70.
2. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances. 2009;27(1):76-83.
3. Ghaffari-Moghaddam M, Eslahi H. Synthesis, characterization and antibacterial properties of a novel nanocomposite

based on polyaniline/polyvinyl alcohol/Ag. Arabian Journal of Chemistry. 2014;7(5):846-55.
4. Lee SM, Song KC, Lee BS. Antibacterial activity of silver nanoparticles prepared by a chemical reduction method. Korean Journal of Chemical Engineering. 2010;27(2):688-92.
5. Khanna PK, Singh N, Charan S, Subbarao VVVS, Gokhale R, Mulik UP. Synthesis and characterization of Ag/PVA nanocomposite by chemical reduction method. Materials Chemistry and Physics. 2005;93(1):117-21.
6. Noritomi H, Miyagawa S, Igari N, Saito H, Kato S. Application of Reverse Micelles of Alkyl Glucosides to Synthesis of Silver Nanoparticles. Advances in Nanoparticles. 2013;02(04):344-9.
7. Hettiarachchi MA, Wickramarachchi PASR. Synthesis of chitosan stabilized silver nanoparticles using gamma ray irradiation and characterization. Journal of Science of the University of Kelaniya Sri Lanka. 2012;6(0):65.
8. Raffi M, Akhter JI, Hasan MM. Effect of annealing temperature on Ag nano-composite synthesized by sol–gel. Materials Chemistry and Physics. 2006;99(2-3):405-9.
9. Ghaffari-Moghaddam M, Hadi-Dabanlou R. Plant mediated green synthesis and antibacterial activity of silver nanoparticles using Crataegus douglasii fruit extract. Journal of Industrial and Engineering Chemistry. 2014;20(2):739-44.
10. Ghaffari-Moghaddam M, Hadi-Dabanlou R, Khajeh M, Rakhshanipour M, Shameli K. Green synthesis of silver nanoparticles using plant extracts. Korean Journal of Chemical Engineering. 2014;31(4):548-57.
11. Tripathy A, Raichur AM, Chandrasekaran N, Prathna TC, Mukherjee A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of Azadirachta indica (Neem) leaves. Journal of Nanoparticle Research. 2009;12(1):237-46.
12. Qian X-F, Yin J, Huang J-C, Yang Y-F, Guo X-X, Zhu Z-K. The preparation and characterization of PVA/Ag2S nanocomposite. Materials Chemistry and Physics. 2001;68(1-3):95-7.
13. Rong MZ, Zhang MQ, Zheng YX, Zeng HM, Friedrich K. Improvement of tensile properties of nano-SiO 2 /PP composites in relation to percolation mechanism. Polymer. 2001;42(7):3301-4.
14. Campbell PN. Principles of biochemistry second edition. Biochemical Education. 1993;21(2):114.
15. Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterialaction of silver ions. Letters in Applied Microbiology. 1997;25(4):279-83.
16. 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. Chemistry of Materials. 2000;12(4):1156-61.
17. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology. 1966;45(4_ts):493-6.
18. Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G. Antimicrobial activity of some of the south-Indian spices against serotypes of Escherichia coli, Salmonella, Listeria monocytogenes and Aeromonas hydrophila. Brazilian Journal of Microbiology. 2006;37(2):153-8.
19. Esmaeili E, Salavati-Niasari M, Mohandes F, Davar F, Seyghalkar H. Modified single-phase hematite nanoparticles via a facile approach for large-scale synthesis. Chemical Engineering Journal. 2011;170(1):278-85.