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Spectroscopic Studies, Electrochemical Behavior and Docking Simulation of Bimetallic Copper-Zinc Compound

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Abstract
A new complex with the formula $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ **(a)** that opd =ortho-phenylenediamine was synthesized. The complex **(a)** was characterized using cyclic voltammetry (CV), spectroscopic methods (IR, UV-Vis) and SEM method. FT-IR show the thiocyanate ligand is located in the form of a bridge. The 1-2-phenylenediamine ligand is also bonded to metal ions through the non-bonding electron pairs of nitrogen atoms through bidentate ligand. The studies obtained from the UV-Vis spectrum confirm n → π* and π→π* intra-ligand transitions. The images obtained with the help of scanning electron microscope (SEM) showed that the particles have a uniform morphology. The average size diameter obtained from the Debye-Scherrer equation of nanocrystal complex **(a)** was approximately 73 nm. Finally, the antibacterial activity of the complex was investigated. The antibacterial activity of complex **(a)** against gram-negative bacteria is greater than that against gram-positive bacteria.

Keywords
Ortho-Phenylenediamine; Copper-Zinc Compound, Antimicrobial Activity.

1. INTRODUCTION

Bacteria, which are called bacterium in the singular and are considered the main group in the body of prokaryotes. Bacteria have many of the characteristics and features of a living organism and are among the most important and diverse microorganisms. Since there has often been a connection between bacteria and pathogens, they have usually been studied and examined from the perspective of pathogenicity [1-7]. However, it should be noted that due to their important function in the ecosystem, they have a prominent and beneficial role in creating order and harmony between the cycles of living organisms and a small number of them cause diseases in humans and animals and, in general, life on earth would be disrupted without them [8-11]. These unicellular prokaryotes reproduce by binary fission. The nucleus and nuclear membrane are absent in these unicellular organisms and their chromosome is a double-stranded and circular DNA. An antimicrobial agent is a compound that kills or inhibits the growth of microorganisms. Antimicrobial drugs are classified according to the type of microorganism they act against. For example, antibiotics act against bacteria and antifungals act against fungi. The main category of antimicrobial compounds are compounds called disinfectants, which act completely non-selectively and destroy all microbial agents. However, there are other antimicrobial drugs that have selective toxicity. That is, the drug is harmless to the host and harmful to the pathogen [12-15]. Antibacterial compounds are used to eliminate bacterial infections. The toxicity of these compounds to humans and other animals is very small. However, long-term use of antibacterial compounds reduces the number of microbial fluxes in the body, which may have negative effects on health [16-20]. In this work, we use an aromatic diamine compound as an important ligand. O-phenylenediamine (OPD) is a white compound that is used as a ligand in the synthesis of coordination compounds [21, 23]. $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ (a) was synthesized and characterized using cyclic voltammetry (CV), spectroscopic methods (IR, UV-Vis) and SEM. By comparing the obtained results, the antimicrobial activity of opd ligand was confirmed and also the antibacterial activity of ligand increased with the

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formation of nano-complex. The evaluation of antimicrobial effects of the metal complexe (a) against two gram-positive and two gram-negative bacteria strains was examined by standard methods.

2. EXPERIMENTAL

2.1. Materials and apparatus

All chemicals were utilized without additional purification, and the necessary reagents were prepared immediately prior to the commencement of the experiments and analyses. Infrared spectra (400-4000 cm^{-1}) were obtained as 1% dispersions in KBr pellets with a Shimadzu-470 Plus spectrometer. Cyclic voltammogram was obtained by use of a SAMA500 device. Electronic absorption spectra were obtained on a Cary Bio 300 spectrometer at room temperature and in a DMF solution.

2.2. Synthesis of $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ (a)

To prepare the cationic part, (0.85 g, 0.5 mmol) of copper (II) chloride and (0.1 g, 1 mmol) of orthophenylenediamine ligand were dissolved in ethanol solution. Then the copper (II) chloride solution was poured into a round-bottomed flask and the orthophenylenediamine ligand solution was added dropwise while stirring and refluxed at 60°C for 5 hours.

To prepare the anionic part, (0.14 g, 0.5 mmol) zinc (II) nitrate, (0.16 g, 2 mmol) sodium thiocyanate and (0.05 g, 0.5 mmol) orthophenylenediamine were dissolved in a minimum ethanol solution. Then, the beaker of zinc (II) nitrate solution was poured into the round bottom flask, and the orthophenylenediamine ligand solution and sodium thiocyanate solution were added dropwise with stirring, respectively. Afterward, the final suspension was thermally treated in an auto-clave for 48 h at 120 ˚C and the product was isolated and washed with ethanol and DMF many times. Finally, the obtained product was dried at 100 ˚C in the oven overnight [24] .

3. RESULTS AND DISCUSSION

3.1. Characterizations of $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ (a)

FT-IR spectrum of complex **(a)** show in Fig. 1. The N-H stretching of the orthophenylenediamine ligand is observed in the region of 3152-3342 cm^{-1} and the C=C stretching absorptions of the aromatic ring of this ligand are observed in a doublet at 1450-1620 cm^{-1}. A strong absorption peak at 755 cm^{-1} is related to the stretching vibration of the C=S bond of the thiocyanate ligand and indicates that the thiocyanate ligand is attached to the zinc metal from the nitrogen atom as a terminal ligand. The absorption at 2150 cm^{-1} also indicates that the thiocyanate ligand is also located in a bridged form [25-27].

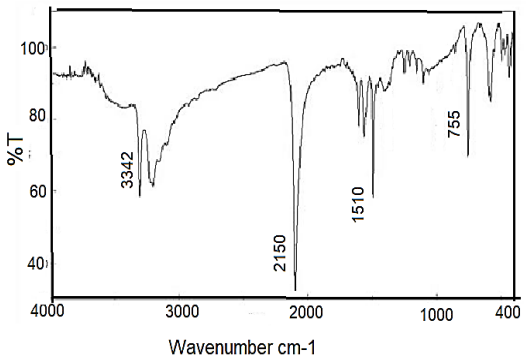

Fig. 1. FT-IR spectrum of **(a)**

3.2. Electronic absorption spectra of $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ (a)

In the electronic spectrum of complex (a), absorption bands are observed at 425 nm, which are attributed to d-d transitions of the Cu^{2+} ion. [28, 29]. The Zn^{2+} ion has no d-d electron transitions due to the d^{10} electronic configuration. Since the d orbitals are completely filled, no absorption bands are observed in the UV-vis. The absorption bands in the 290 and 225 nm regions are assigned to intraligand transitions, (n→π*) and (π→π* of the orthophenylenediamine ligand and the thiocyanate ligand. (Fig. 2).

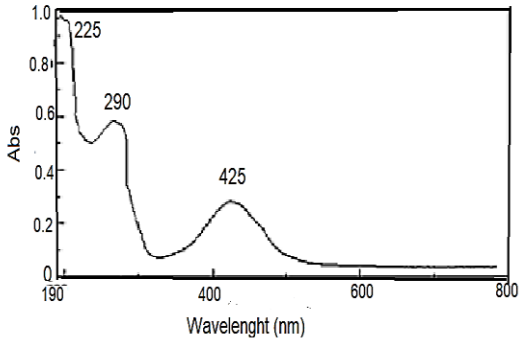

Fig. 2. Electronic absorption spectra of $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ **(a)**

3.3. Cyclic Voltavmetery of $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ (a)

Cyclic voltammogram of the complex **(a)** was recorded at −2.0 to 2.5 V vs. Ag/AgCl using different switching potentials at varying scan rates. The cyclic voltammogram of the complex was obtained at 25°C in DMF solution containing 0.1M TBAH as supporting electrolyte with scan speed of 500 mvs-1 (Fig. 3). The voltammogram of the complex shows a quasi-reversible wave in the potential Ec= -0.3 v related to the reduction of the ligand opd and the quasi-reversible wave potential Ec= 0.7v assigned to the reductionof Cu (II) ion [30].

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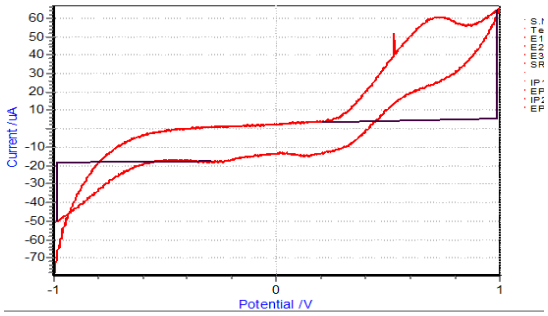

Fig. 3. Cyclic voltammogram of $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ **(a)**

3.4. scanning electron microscopic of $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ (a)

The SEM picture show the shape of the nanoparticles and the surface morphology of the nanocrystal complex as well, due to the distortion of the SEM image, the diameter of the nanoparticles is overestimated [31]. The average size diameter obtained from the Debye-Scherrer equation of nanocrystal complex **(a)** was approximately 73nm.

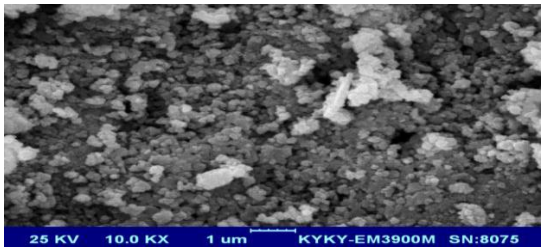

Fig. 4. SEM images of nanocrystal complex **(a)**

3.5. Investigation of the antimicrobial activity of complex (a)

The Minimum Bactericidal Concentration (MBC), Minimum inhibitory concentration (MIC) and Inhibition Zone (IZ) parameters of the complex (a), was screened against two gram-positive standard strain (*staphylococCusaureu* and *nterococCus faecalis)* and also two gram-negative standard strain (*EscherichiaColi* and *Pseudomonas eruginos*). Table 1, show Antimicrobial activity results of the disk diffusion test.

Table 1. Antimicrobial activity results of the disk diffusion test.

	$[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$	Gentamycin	Amikacin
EscherichiaColi	14.7	19.2	
Pseudomonas eruginos	15.7	20.1	
staphylococCusaureu	12.5		18.8
nterococCus faecalis	14		19.2

Minimum growth inhibitory concentration (MIC, mg/mL) of the complexes prepared on the bacteria under study (Table 2).

Table 2. Minimum growth inhibitory concentration (MIC, mg/mL)

	$[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$
EscherichiaColi	0.028
Pseudomonas eruginos	0.020
staphylococCusaureu	0.084
nterococCus faecalis	0.038

Antimicrobial activity results of the Minimum Bactericidal Concentration (MBC) test show in Table 3.

Table 3. Minimum Bactericidal Concentration ((MBC, mg/mL)) test.

	$Cu(opd)_2(H_2O)(\mu\text{-})[(SCN)Zn(opd)(SCN)_3$ هیدروترمال
Escherichia Coli	0.018
Pseudomonas eruginos	0.016
StaphylococCusaureu	0.084
nterococCus faecalis	0.058

The antibacterial activity against gram-negative bacteria is greater than that against gram-positive bacteria. This difference is probably due to the difference in the structure of the bacterial cell wall. Gram-positive and negative bacteria have similar internal structures, but the structure of their outer membrane is different. Gram-positive bacteria are composed of a thick peptidoglycan layer that includes teichoic acid and lipoteichoic acid, but gram-negative bacteria have a thin peptidoglycan layer that allows complexes to penetrate more easily. Another reason for better antibacterial property of the compounds compared with that of the free ligand is described through the Tweedy's chelation theory [20].

3.6. Molecular docking results

Molecular docking of the compounds was performed using MOE software. The structure of complexe (a) were calculated using GUASS VIEW 6 software and after optimization of the structure by GUASSIAN 9 software, they were used in molecular docking calculations. The structures of Escherichia coli and Staphylococcus aureus bacteria were taken from www.RCSB.org. The active sites in the bacteria were identified by the software and docking was performed at the same active hub position and finally the best position was identified. In these docking studies, the

AMBER 10H force field was used. In each docking, the structure with the lowest docking energy was used. The results of molecular docking of the complex ($[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3)]$ with two bacteria, Escherichia coli and Staphylococcus aureus, are given in the Table 4.

Table 4. Molecular docking results for complex (a) against two bacteria.

Dock Name	Binding energy (kcal/mol)	RMSD
Zn-Cu @ Escherichia coli	-30.4612	2.3
Zn-Cu@ Staphylococcus aureus	-21.5476	2.37

Based on the data in the table above, the complex $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3)]$ can form an acceptable bond with Escherichia coli bacteria. The active sites of Staphylococcus and Escherichia coli aureus bacteria for binding are shown in Fig. 5.

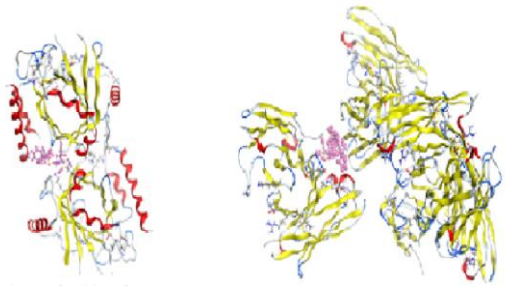

Fig. 5. A) complex (a) against Staphylococcus aureus; B) complex (a) against Escherichia coli.

Fig. 6 show the binding site of the complex $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3)]$ with Staphylococcus aureus and Escherichia coli bacteria, respectively.

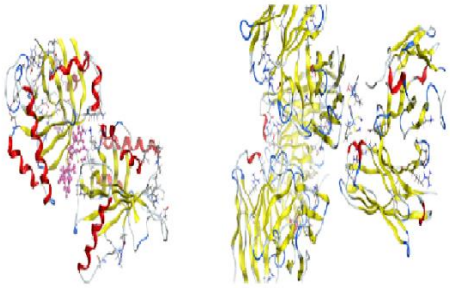

Fig. 6. A) Complex (a) to StaphylococcusCusaureu; B) Complex (a) to Escherichia coli.

Fig. 7 show the binding sites of the complex $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3)]$ with Staphylococcus aureus and Escherichia coli bacteria.

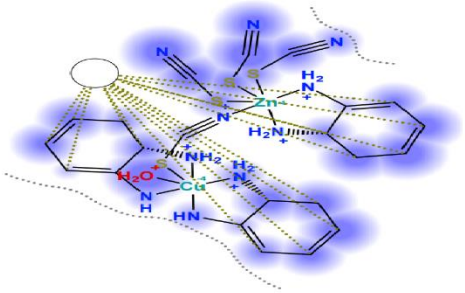

Fig. 7. Binding sites of the complex (a).

Fig. 8. show the amino acids involved in the complex $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3)]$ in the docking position with the bacterium Escherichia coli (Fig. 8 A), and amino acids involved in the complex in the docking position with the bacterium Staphylococcus aureus (Fig. 8 B).

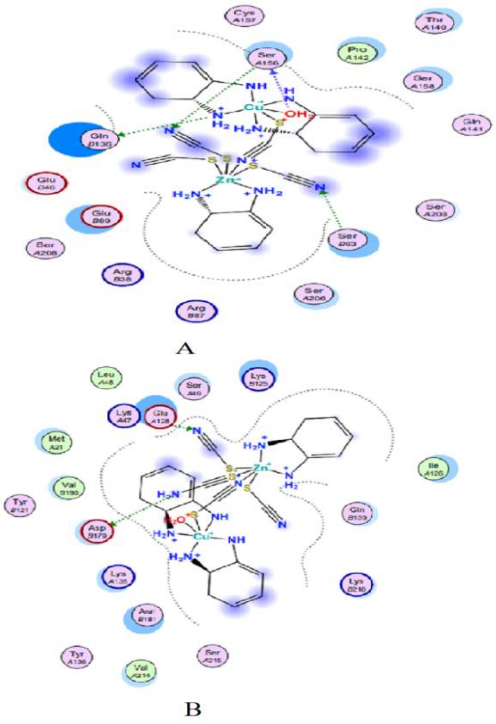

Fig. 8. Amino acids involved in the complex **(a)** in the docking position with the bacterium Escherichia coli (Fig. 8 A) and Staphylococcus aureus (Fig. 8 B).

4. CONCLUSIONS

This study suggested a nano- complex via solvothermal reaction. This compound characterized using cyclic voltammetry (CV), spectroscopic methods (IR, UV-Vis) and SEM method. Cyclic voltammetry (CV) results showed the presence of metal ions and electroactive ligands. The results of diffraction pattern analysis (XRD) showed that the sample particles converted into nanomaterials have appropriate crystallinity. The structure of the complex was studied and

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identified by molecular docking, which shows that the complex has acceptable stability and that the metal and ligand bind together under appropriate conditions. The antibacterial activity of complex 1 is greater against gram-negative bacteria than gram-positive bacteria.

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چکیده

یک کمپلکس جدید با فرمول **(a)** $[Cu(opd)_2(H_2O)(\mu\text{-}SCN)Zn(opd)(SCN)_3]$ که در opd=ortho-phenylenediamine سنتز شد. کمپلکس **(a)** با استفاده از روش های ولتامتری چرخه ای (CV)، روش های طیف سنجی (IR، UV-Vis) و تکنیک SEM شناسایی شد. FT-IR نشان می دهد که لیگاند تیوسیانات به شکل پل قرار گرفته است. لیگاند ۱-۲-فنیلن دی آمین نیز از طریق جفت الکترون های غیرپیوندی اتم های نیتروژن بصورت دودندانه به یون های فلزی اتصال دارد. مطالعات به دست آمده از طیف UV-Vis انتقالات درون لیگاندی $n \rightarrow \pi^*$ و $\pi \rightarrow \pi^*$ را تایید می کند. تصاویر به دست آمده با کمک میکروسکوپ الکترونی روبشی (SEM) نشان داد که ذرات مورفولوژی یکنواخت دارند. اندازه متوسط ذرات توسط معادله دبای- شرر، ۷۳ نانومتر محاسبه شد. در نهایت فعالیت ضد باکتریایی کمپلکس **(a)** بررسی شد و مشخص گردید که فعالیت ضد باکتریایی این کمپلکس در برابر باکتری های گرم منفی بیشتر از باکتری های گرم مثبت است.

کلید واژه ها

ارتوفنیلن دی آمین؛ ترکیب دو فلزی مس ــ روی؛ فعالیت ضد باکتری.

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