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Nanocomposite for Enhancement the Biological Activity of Cu(II)-complex from New Cefotaxime Derivative

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ABSTRACT

A novel cefotaxime derivative, Schiff base ligand, is used to create copper complex. Molar conductivity, elemental study, Infrared, uv-vis spectra, magnetic moment, and thermal behavior are used to describe the complex. The compound molar ratio is discovered to be one metal to one ligand. The infrared bands show that the Schiff base binds copper ion as tridentate via carboxylate, acetylacetone carbonyl and imine group. The magnetic moment, uv-vis transitions, and ESR spectrum, on the other hand, show that the Cu^{II}-complex structure is octahedral. Thermal analysis also reveals the presence of water and methyl alcohol in the compound. Moreover, Sol-gel process is utilized for silica nanoparticles preparation. While, the complex is loaded on silica nanoparticles after surface modification. Whereas, the CuII-complex and nano-complex are tested for Gram (+) (*B. subtilis* and *S. aureus*) and Gram (-) (*E. coli* and *P. aeruginosa*) microbes. The biological results exposed that the synthesized complex has high potential to overcome the resistance of microbes.

1. Introduction

Applications of inorganic chemistry in medicine may be seen in the creation of metal complexes, which are given a great deal of attention in the management of various illnesses including chemotherapeutic and antibacterial [1, 2]. Metals are used in the realm of medication development for a variety of reasons. Generally, during a period of clinical medication usage, bacteria frequently begin to adapt to antibiotics. Metal complexes become a different source of antibacterial agents as a result [3]. Additionally, the insufficiency of pharmacokinetic properties in ineffectual chemotherapeutic drugs has prompted the development of metal base drugs and their use as a technique for drug carrier by altering drug features like the effectiveness of distribution and adsorption to get around these limitations and enhance lyophilic and lyophobic ability and permeation of medical products [4].

Cisplatin is regarded as one of the earliest efficient metal complexes with the ability to cure various malignancies and has demonstrated a high efficient technique in some tumor situation, prompting and opening a feasible area for the study of new metal medicines for prospective therapeutic uses [5].

There are various complexes that have received significant attention due to the disastrous consequences and lack of sensitivity displayed by other platinum medical products, for example; silver compounds have been utilized for the cure of infections as well as in dental care and stents, while gold complexes are used to treat joint inflammation [6]. Furthermore, copper (II) complexes exhibit high antitumor activity and anti - bacterial efficiency [7].

Because the chemical composition of cephalosporin medicines have a β-lactam moiety, they are classified as beta-lactam drugs and thus are extensively used during clinical therapy for prevention and infectious diseases. Streptococcus and Staphylococcus microbes can cause inflammation when they enter torn skin and tissue and lead to diseases that can be cured with a first-generation cephalosporin antibiotic that has strong action against a gram-positive bacterium [8]. The later generations of cephalosporins, on the other hand, are widespread antibiotics with more action towards gram-negative microbes than the initial generation [9]. Among the most significant aspects of cephalosporin medicines is that, due to the unique construction of lactam antibiotics, they may be employed in place of penicillin antimicrobial drugs to prevent penicillin allergies in some cases [10].

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In this investigation, copper complex formulation and nanotechnology are intended to reduce bacterial resistance and increase antibiotic effectiveness. Elemental study, spectroscopy, magnetic moment and thermal behavior are used to describe the complex. Moreover, Cu-complex and nano-complex are tested for Gram (-) and Gram (+) microbes.

2. Experimental

2.1 Material and methods

High purity chemicals ($Cu(NO_3)_2.6H_2O$, methanol, acetylacetone, cefotaxime, tetraethylorthosilane (TEOS), aminopropyltriethoxysilane (APTS), ethanol, ammonium hydroxide, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and DMSO) are utilized without additional purification. CHNS VARIO EL-III (Germany) monitors the CHN% elemental study whereas Adwa instrument (AD8000) measures conductivity. Sherwood balance is used to compute the magnetic moment by determining the molar susceptibility. For spectroscopic research, infrared spectrophotometer (Bruker) and UV-visible spectrophotometer (Evolution series) are employed. While, the X-band EMX analyzer is used to record the ESR spectra (Bruker). Additionally, TGA examination is carried out using a Shimadzu 60 thermal instrument with a temperature elevation of 20°C/min over 28 to 800 °C in nitrogen. Whereas, Transmission electron microscopy is used for detect nanoparticles size and shape.

2.2 Syntheses

2.2.1 Schiff Base preparation

The Schiff base is created by combining acetylacetone and cefotaxime for 8 hours in methanol (Figure 1).

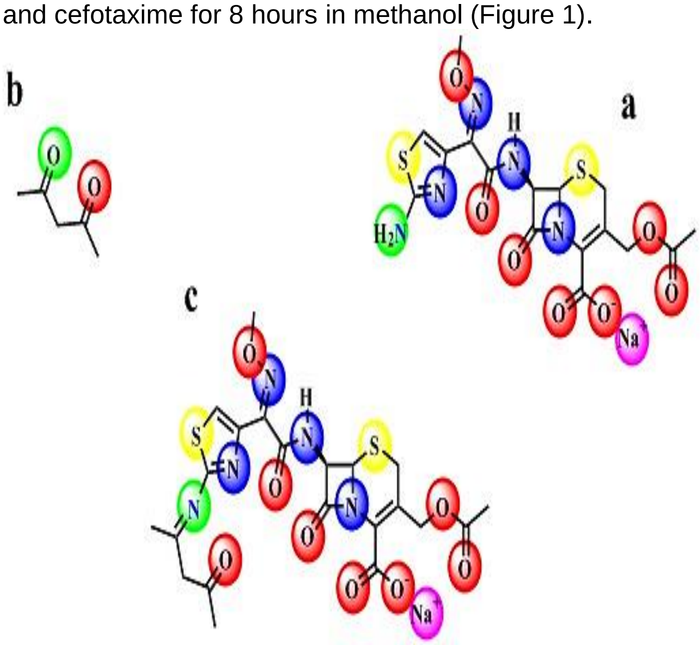
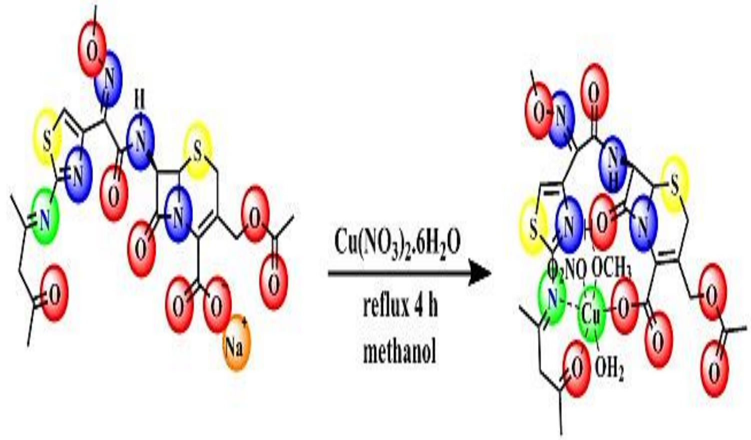


Figure 1. Schiff base preparation reagents: a. cefotaxime, b. acetylacetone and c. Schiff base.

2.2.2 Copper complex synthesis

The Schiff base is subjected to a four-hour reflux reaction with a methanol solution of $Cu(NO_3)_2.6H_2O$ (Scheme 1). The copper complex is created and precipitated. The precipitate is filtered, washed several times with water and CH_3OH, and then allowed to dry.



Scheme 1. Cu^{II}-complex preparation.

2.2.3 Preparation of complex-SiNps

Sol-gel method is utilized for the preparation of silica nanoparticles. Ethanol and distilled water are mixed then TEOS is added to the mixture and the mixture pH is adjusted at 11 by NH_4OH. The mixture put under stirring for 2 hours, then nanoparticles is separated and dried at 50 °C (Figure 2). Nanoparticles are modified by mixing SiNPs with APTS in toluene and reflux at 95 °C for 4h [11].

Schiff base is loaded on the SiNPs surface by using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride. Modified SiNPs is dispersed in methanol, then Schiff base and EDC is added and stirred for 2 hours [12]. The product is separated, washed and dried at 50 °C.

Copper salt is added to the Schiff base-SiNPs and stirred for 24 hours. Nano-complex is isolated, washed and dried at 50 oC. Figure 3 displays the change on the IR spectra of modified SiNPs, Schiff base-SiNPs and complex-SiNPs, which proves that ligand is labeled on nanoparticles and nano-complex is prepared.

2.4 Antimicrobial Study

By using an altered disc diffusion approach *in vitro*, the biological impact of the copper complex is assessed against four different bacterial species: Gram (+) (B. subtilis and S. aureus) and Gram (-) (E. coli and P. aeruginosa) microbes. A paper disc is loaded with Cu complex dissolved in DMSO and 0.1ml of bacteria has been distributed on plate. Set the disc on the dish and is infected for 24-48 hours. The substance disperses from the disc onto the plate and after killing the germs surrounding the disc, the clean zone is evaluated [13].

3. Results and Discussion

According to elemental study in Table 1, the copper complex is created via a metal-Schiff base reaction in a 1:1 ratio. Normal solvents (methanol, DMF, ethanol and acetone) cannot dissolve complex; only DMSO does. The produced compound has low conductivity value indicates that it is not charged in its solution.

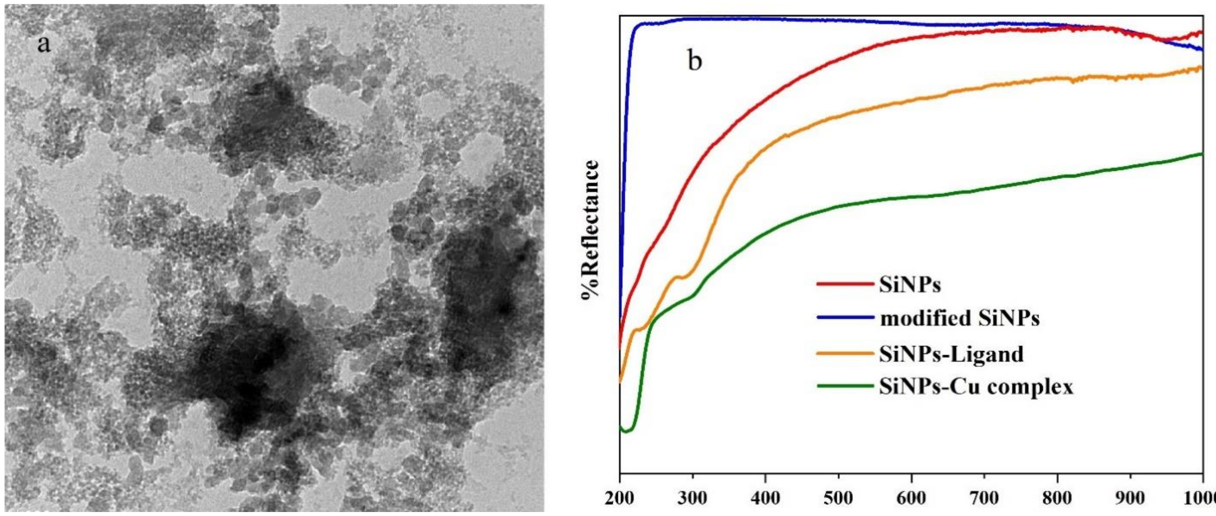


Figure 2. a. nanoparticle TEM image and b. uv-vis spectra for the compounds.

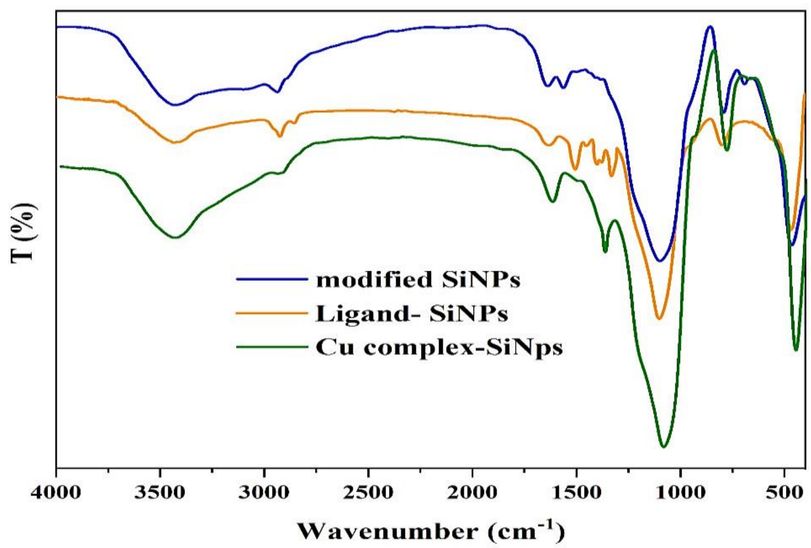


Figure 3. FTIR of modified SiNPs, Schiff base-SiNPs and complex-SiNPs.

Table 1. Elemental study and conductivity of Cu-complex

Formula	M.wt	colour	M. P.	Elemental analysis				Conductivity $ohm^{-1}cm^{-1}mol^{-1}$
				C%	H%	N%	M%	
				Found (Calc.)	Found (Calc.)	Found (Calc.)	Found (Calc.)	
Copper II complex $[Cu^{II} L^{-}(NO_3^{-})(H_2O)(CH_3OH)](H_2O)$	730.18	green	>250	35.88 (36.19)	3.90 (4.14)	11.73 (11.51)	8.28 (8.70)	7.69

3.1 FTIR

The vibration result from copper complex (Figure 4) is summarized from infrared spectrum and displayed in Table 2. The appearance of v(NH), v(OH) and v (H_2O) bands in complex spectrum are a broad and observed between 3060 $cm^{-1}$and 3700 cm^{-1} . The acetylacetone carbonyl and imine group are detected at 1520 and 1620 cm^{-1}, respectively. Meanwhile, they are found respectively in Schiff base at 1530 and 1606 cm^{-1} [14-16]. The carboxylate vibrations [$v(_{COO})_{asym}$ and $v(_{COO})_{sym}$] and are shifted in complex to lower wavenumbers and observed at 1570 and 1355 cm^{-1} respectively. The difference between the vibration bands of carboxylate proves the mono-dentate nature of carboxylate to bind copper ion [17]. Furthermore, Cu-N and Cu-O interactions are displayed at 588 and 454 cm^{-1}, respectively.

Table 2. Cu-complex and ligand FTIR data

Compound	IR (cm^{-1})							
	ν(C=O) β-lact. (ester)	ν(C=N)	ν(C=O)*	ν $(COO)_{asy}$	$ν(COO)_{sym}$	Δν	ν(M-O)	ν(M-N)
Schiff base ligand $L^{-}Na^{+}.(H_2O)(CH_3OH)$	1742 (1662)	1606	1530	1580	1377	- - -	- - - -	- - - -
Copper II complex $[Cu^{II}L^{-}(NO_3^{-})(H_2O)(CH_3OH)](H_2O)$	1740 (1662)	1620	1520	1570	1355	215	588	454

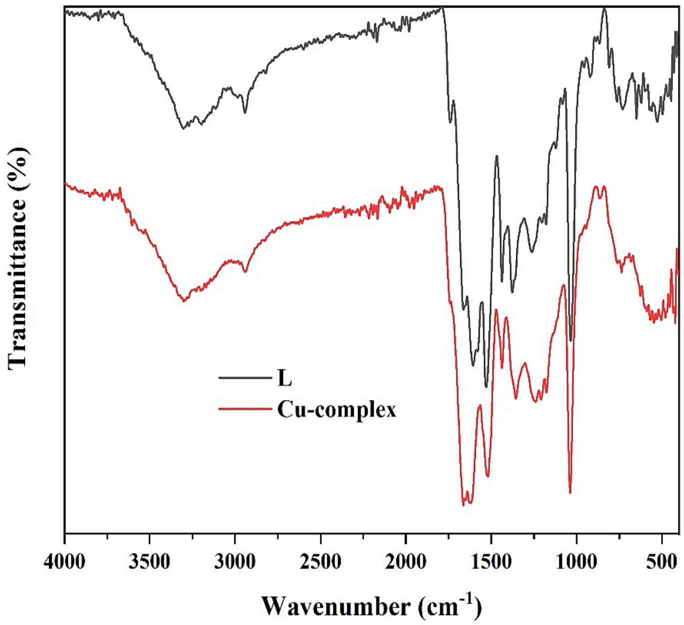


Figure 4. Cu-complex and ligand IR spectra

3.3 UV-Vis spectra

The spectra of ligand and Cu^{II}-complex transitions are observed between 200 and 1000 nm by Evolution™ 200 UV-vis spectrophotometer. The transitions π - π* and n - π* are identified at 236 and 245 nm, respectively, whereas, these transitions in complex show a red shift and are respectively identified at 245 and 308 nm (**Table 3**). Meanwhile, the copper complex spectrum displays two bands in the visible region at (772 nm, 12953 cm^{-1}) and (939 nm, 10650 cm^{-1}), which can be assigned to $d_{xy} \rightarrow d_{x^2-y^2} + d_{xz}$, $d_{yz} \rightarrow d_{x^2-y^2}$ and $d_{z^2} \rightarrow d_{x^2-y^2}$, respectively (**Figure 5**). These bands confirm that the complex is distorted by Jahn-Teller effect and the complex has D_{4h} configuration [18]. As well, the magnetic moment is identified as 2.25 BM, which indicates that the stereostructure around copper is an octahedral [19].

Table 3. Electronic spectra values and magnetic moment

Compound	Peak		assignment	μ_{eff}	Proposed structure
	nm	cm^{-1}			
Schiff base ligand	230 299	43478 33445	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	- - -	- - - -
Copper II complex $[Cu^{II}L(NO_3^{-})(H_2O)_2(CH_3OH)]$	245 308 772 939	40816 32468 12953 10650	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ $d_{xy} \rightarrow d_{x^2-y^2}$ + d_{xz} , $d_{yz} \rightarrow d_{x^2-y^2}$ $d_{z^2} \rightarrow d_{x^2-y^2}$	2.25	O_h

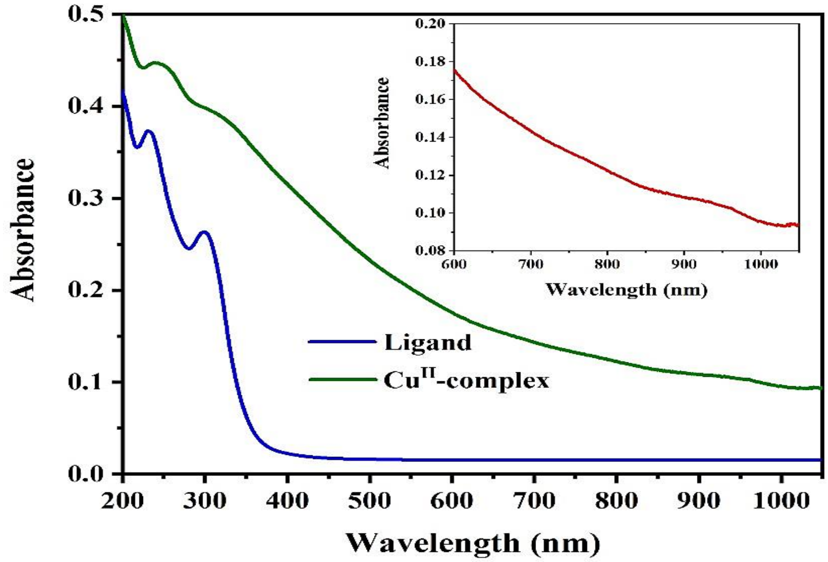


Figure 5. Electronic spectrum of Cu-complex and ligand

3.4 ESR spectrum

The spectrum obtained from ESR spectrometer can be used to identify the coordination of complex. **Figure 6** exhibits the ESR spectrum of Cu complex. The complex spectrum provides the following g-values: $g_{||}$ = 2.07779 and $g_{\perp}$ = 2.16869. Consequently, the configuration of copper complex is clarified to be compressed octahedral coordination due to the observed g-values from the spectrum, where $g_{\perp} > g_{||}$. Besides, the unpaired electron will be in d_{z^2} ground state [20, 21].

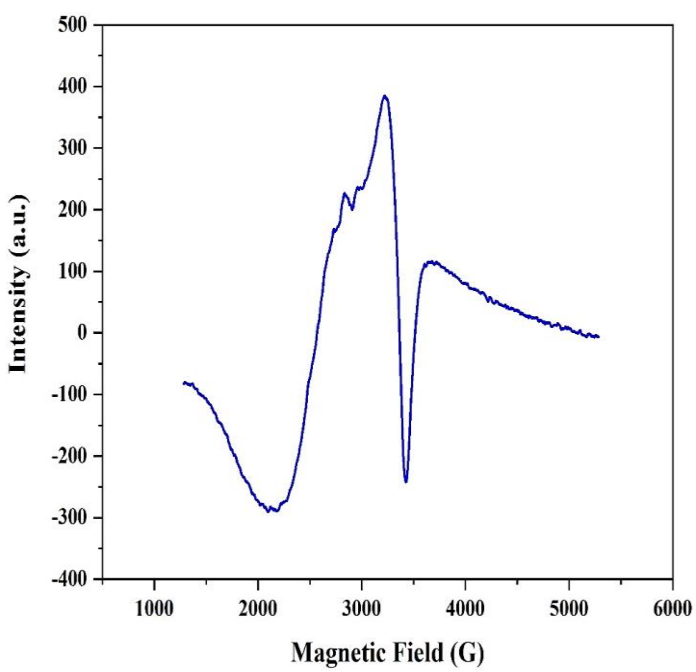


Figure 6. Cu-complex ESR spectrum

3.5 Thermal analysis

The thermal behavior of Cu-Schiff base complex is displayed in **Figure 7**. Water and methanol molecules are liberated in the first step with weight loss of 6.77 % (Calcd. = 6.86 %) at 10-105 ºC (**Scheme 2**). Whereas, the second stage exhibits a weight loss of 79.03 % (Calcd. = 78.96 %) due to the removing of coordinate H_2O and nitrate group, besides, complete decomposition of the Schiff base between 105 ºC and 800 ºC (**Table 4**). Furthermore, the remaining of complex is observed as CuO (Calcd. = 10.89 %) and 2C (Calcd. = 3.29 %).

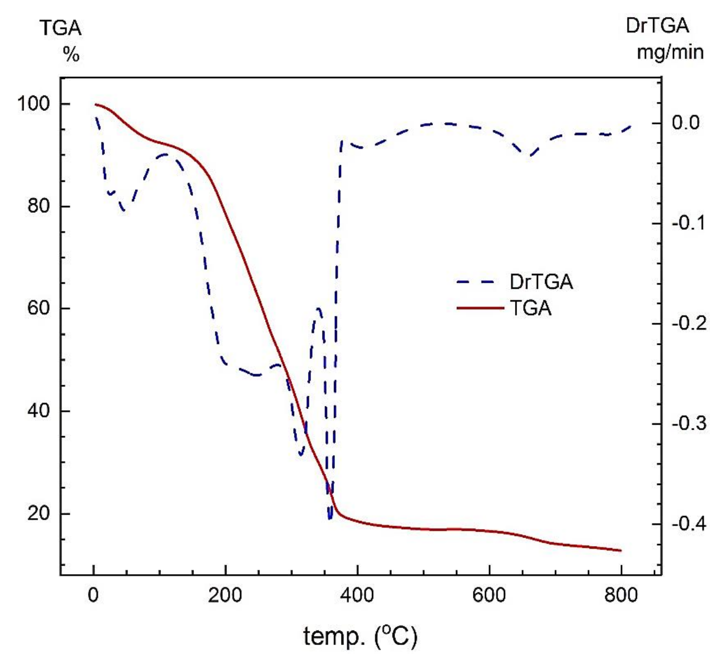
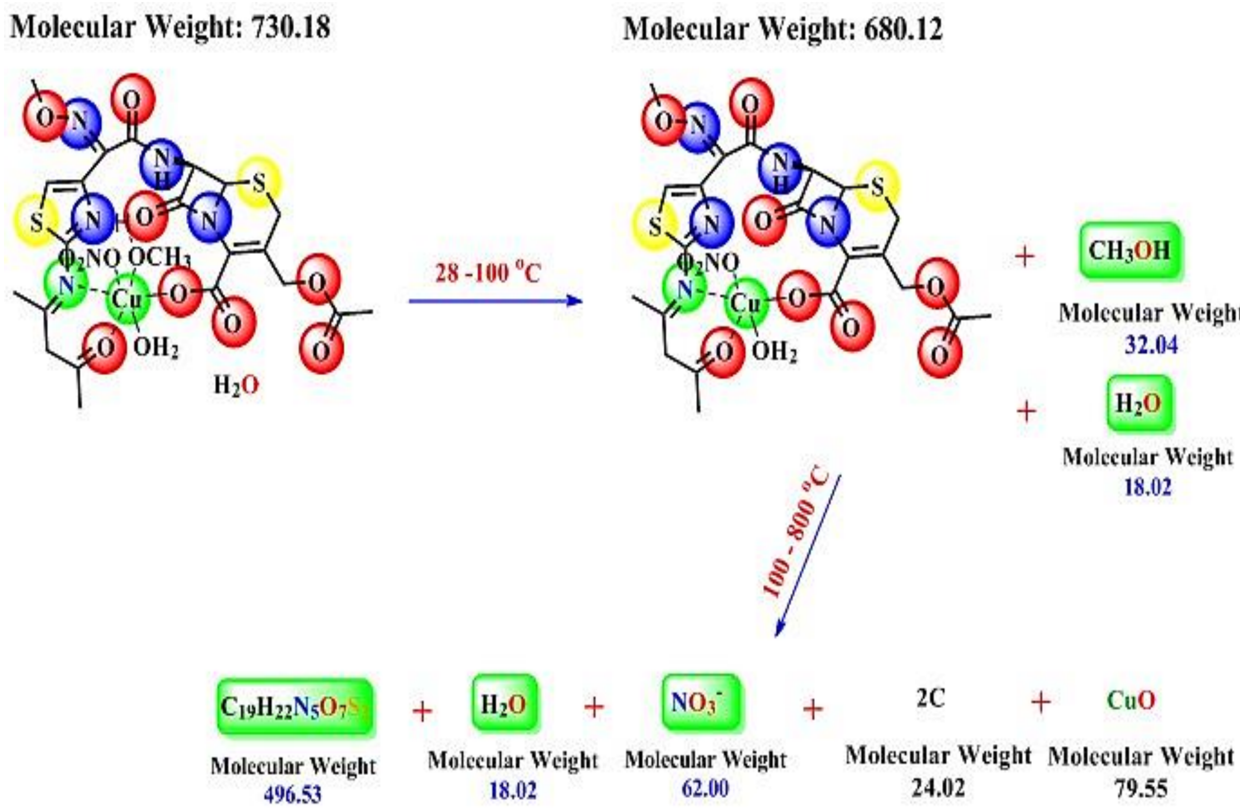


Figure 7. Thermal analysis of copper Cu-complex

Table 4. Thermal analysis data

Compound	Steps	Temp. Range °C	Mass loss %		Decomposition Process	Residue
			Found	Calcd.		Found (Calcd. %)
Copper II complex $[Cu^{II} L^{-}(NO_3^{-})(H_2O)(CH_3OH)](H_2O)$	I	10-105	6.77	6.86	$CH_3OH + H_2O$	CuO + 2C 14.20 (14.18)
	II	105-800	79.03	78.96	$H_2O + C_{19}H_{22}N_5O_7S_2 + NO_3^{-}$	



Scheme 2. Suggested Cu-complex decomposition

3.6 Antimicrobial

The biological impact of the copper complex and nano-complex are measured and compared with its ligand against four different bacterial species via disc diffusion approach: Gram (+) (*B. subtilis* and *S. aureus*) and Gram (-) (*E. coli* and *P. aeruginosa*) microbes (**Figure 8**).

The Schiff base display no activity against the target microbes, while Cu-compound does not effect on *S. aureus*. The observed clear zone on *B. subtilis* plate indicates that copper complex has less potential to decline bacteria than nanocomposite. While, the nano complex is highly active and displays large inhibition zone against *S. aureus*. Moreover, the nano-complex shows high inhibition activity against *E. coli*. Besides, the highest activity of Cu-complex is observed against *P. aeruginosa*.

It is clear that:

1. the bacteria display high resistance against the ligand.
2. copper complex has activity against all microbes except *S. aureus.*
3. complex-SiNPs shows the greatest inhibition with *B. subtilis, S. aureus* and *E. coli.*
4. *P. aeruginosa* is greatly influenced by Cu-compound
5. the activity of compounds can clarify as:
   Complex-SiNPs > copper complex > Schiff base

The data shown in **Table 5** supports the idea that a molecule's activity depends on how its atoms are arranged in space, which affects how reactive the compound interacts with the protein sites of microorganisms [22]. Moreover, it proves the impact of metal-complexes to overcome the resistance of microbes [23].

Table 5. Antimicrobial measurements

Sample	Inhibition zone diameter (mm / mg)			
	gram positive bacteria		gram negative bacteria	
	B. subtilis	S. aureus	E. coli	P. aeruginosa
Schiff base	Inactive	Inactive	Inactive	Inactive
$[Cu^{II} L^{-}(NO_3^{-})(H_2O)(CH_3OH)](H_2O)$	9 ±0.0	Inactive	10.33 ±0.58	10.33±0.58
Complex-SiNPs	11.33 ±0.58	10.33±0.58	11.00 ±1.73	9.67±0.58

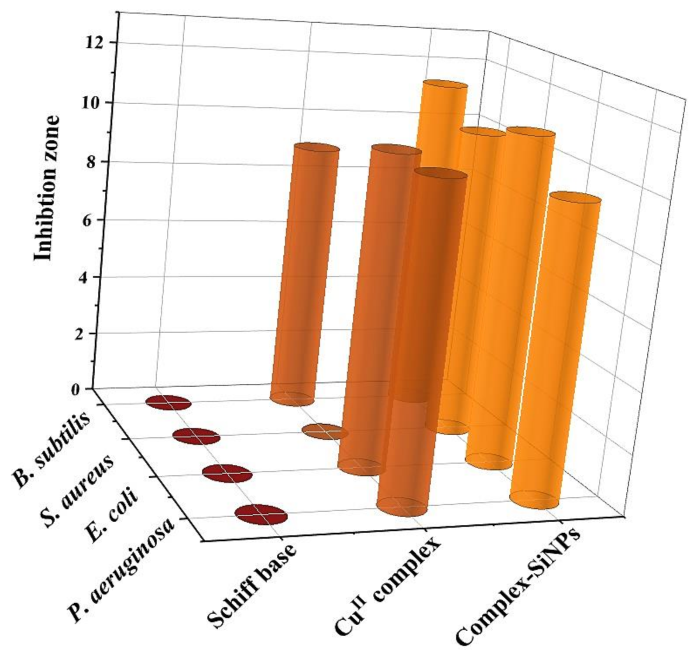


Figure 8. Inhibition zone of the compounds

Conclusion

New Cu^{II}-complex drug from novel cefotaxime derivative and Cu-complex-SiNPs are prepared and described. The obtained complex has an octahedral structure and thermal stability. The biological impact of the copper complex and nano-complex are measured and compared with its ligand against microorganisms: *E. coli* and *P. aeruginosa* as G^-, *S. aureus* and *B. subtilis* as G^+. The biological results exposed that the synthesized complex has high potential to overcome the resistance of microbes. Besides, the nano-complex displays high activity and greatest inhibition zone.

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