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Electrochemical and Microbial Behaviour of Cu(II)-Complexes N, N'-Diimine and Heterocyclic Ligand in Non Aqueous Solvents

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ABSRACT

Indeed, the rise of antimicrobial resistance is a significant global concern. The discovery of new active compounds against novel targets is crucial to combat this issue. Copper complexes have been found to often demonstrate enhanced biological activity compared to the parent ligand alone. The use of copper complexes in the form of drugs can modify their pharmacological and toxicological properties. Low molecular weight copper complexes (Cu^{2+}) have been proven beneficial against several diseases such as tuberculosis, rheumatoid arthritis, gastric ulcers, and cancers. Here in this the biological activity of copper complexes with 2.9-dimethyl 1,10-phenanthroline (dmp) and 4-NOHA as a secondary ligand were synthesized and characterized by and as a primary ligand .The copper(II) complexes are redox active and its electrochemical behavior are studied by cyclic voltammetry technique, cyclic voltammograms were recorded in dimethyl sulfoxide (DMSO) and dimethylformamide (DMF).

Key words: *Copper ,toxilogy, cytotoxicity antimicrobilal ,biological activity, electrochemical behaviour*

1. INTRODUCTION

According to a recent review article [1], indole derivatives have various biological activities, such as antiviral, anti-inflammatory, anticancer, anti-HIV, antioxidant, antimicrobial, antitubercular, antidiabetic, antimalarial, anticholinesterase activities, etc. Indole is also a common scaffold in many synthetic drug molecules, such as 1,10-phenanthroline and prodigiosin.

M. Mustafa Cetin and coworker, study a series of 1, 10-phenanthroline and Prodigiosin derivatives consisting of their copper(I) complexes have been synthesized and characterized. Their biological activities were tested in vitro on six different cell lines (including the normal cell line). To obtain additional parallel validations of the experimental data, some in silico modeling studies were carried out with mTOR and HDAC1 enzymes, which are very crucial drug targets, to discover novel and potent drugs for breast cancer and related brain metastases disease.

The scientific community is struggling to keep up with the pace at which bacterial infections are evading antibiotics through the development of multidrug resistance. As of July 2019, there were 42 antibiotic drug candidates in clinical trials[1]. While this may sound encouraging, the context is important: In 2018 there were over 1100 medicines and vaccines in clinical trials as cancer treatments. Furthermore, almost 75% of the antimicrobials under clinical development are simply derivatives of already known and used antibiotics, meaning that they will likely be prone to existing resistance mechanisms. To make things worse, only one of the remaining 11 entirely new compounds is effective against the notoriously more resilient Gram-negative strains. Commercial development of new antibiotics is unlikely to refill the antibiotic pipeline in the near future, with the number of pharmaceutical companies actively researching new antibiotics still shrinking every year due to the unfavorable return on investment in the field.[2,3]

A recurrent trend in the antibiotic chemical landscape is that antibacterial compounds are rarely "drug-like", *i.e.* they do not conform to the physicochemical and reactive functionality guidelines developed to direct the discovery of new therapies.

According to a recent paper, the authors synthesized and characterized a new copper(II) complex with 4-hydroxynicotinic acid and 2,9-dimethyl-1,10-phenanthroline as ligands. They found that the complex has a distorted octahedral geometry, with two 4-

hydroxynicotinate anions acting as bidentate ligands and two 2,9-dimethyl-1,10-phenanthroline molecules acting as bidentate ligands. The complex exhibited good thermal stability and fluorescence properties [4].

The authors also performed cyclic voltammetry of the complex in acetonitrile solution with 0.1 M tetra-n-butylammonium hexafluorophosphate as the supporting electrolyte. They observed two reversible redox couples at 0.17 and 0.54 V, corresponding to the Cu(II)/Cu(I) and phenanthroline/phenanthroline radical cation processes, respectively. They also studied the effect of scan rate and concentration on the electrochemical behavior of the complex.[5]

2. METHODS AND MATERIALS

2.1 REAGENT

All chemicals and reagents used analytical grade(A.R.) and used without any further purification. Copper (II) nitrate trihydrate, 4-hydroxynicotinic acid, and aromatic dines (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma Aldrich Chemicals Pvt. Ltd. ;and were used as such. Analytical grade DMSO and DMF were procured from E-Merck India Ltd. Ethyl alcohol was purchased from Bengal Chemicals, India.

2.2 SYNTHESIS OF COMPLEXES

The present mixed ligand copper(II) complexes were prepared by mixing of equal amounts (3.33 mM) of ethanolic solution of the first diimine ligand (2,9-dimethyl-1,10-phen) and second ligand (4-OHNA) with the same ratio of copper nitrate salt $Cu(NO_3)_2$ and this mixture were refluxed for one hour(1h). The obtained complexes were filtered and washed several times with ethanol to get complexes in pure form. The complexes then dried in desiccators over anhydrous calcium chloride($CaCl_2$) to remove moisture contents. The find yield ranged from 80-90 percent. The dried complexes were characterized and subjected to further elemental , spectroscopic and electrochemical analysis. The obtained complexes were found to soluble in non aqueous solvent DMSO and DMF. All melting points, colour, C, H, N, magnetic susceptibility, proposed formulae of the complexes given the Table 1.

3. INSTRUMENTATION

3.1 Cyclic voltammetry: In detail of the instrumentation of cyclic voltammetry were reported in my previous research article (10b)

3.2 Antibacterial activity testing

The metal salt, ligand and complexes were subjected to in vitro, for their antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi belonging to yeasts *Candida* spp.(*Candida parapsilosis* & *Candida albicans)* such as ***Escherichia coli* (*E. coli*), Neisseria gonorrhoeae** and ***Klebsiella pneumoniae* (*K. pneumoniae*);** Gram positive bacterial strains such as ***Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes* (*S. pyogenes*)** bacterium by disc diffusion method. In this method, activity of the test compounds was expressed by measuring the diameter of zone of inhibition. The plates were observed for zones of inhibition after one day i.e. 24 h, and incubation at 37-28 °C. The diameters of the zone of inhibition produced by the complexes were compared with a standard antibiotic drug **Gentamycin, Amikacin** and antifungal drug **Ketoconazole**, also known by its brand name Nizoral, is a versatile medication used to treat various fungal infections.

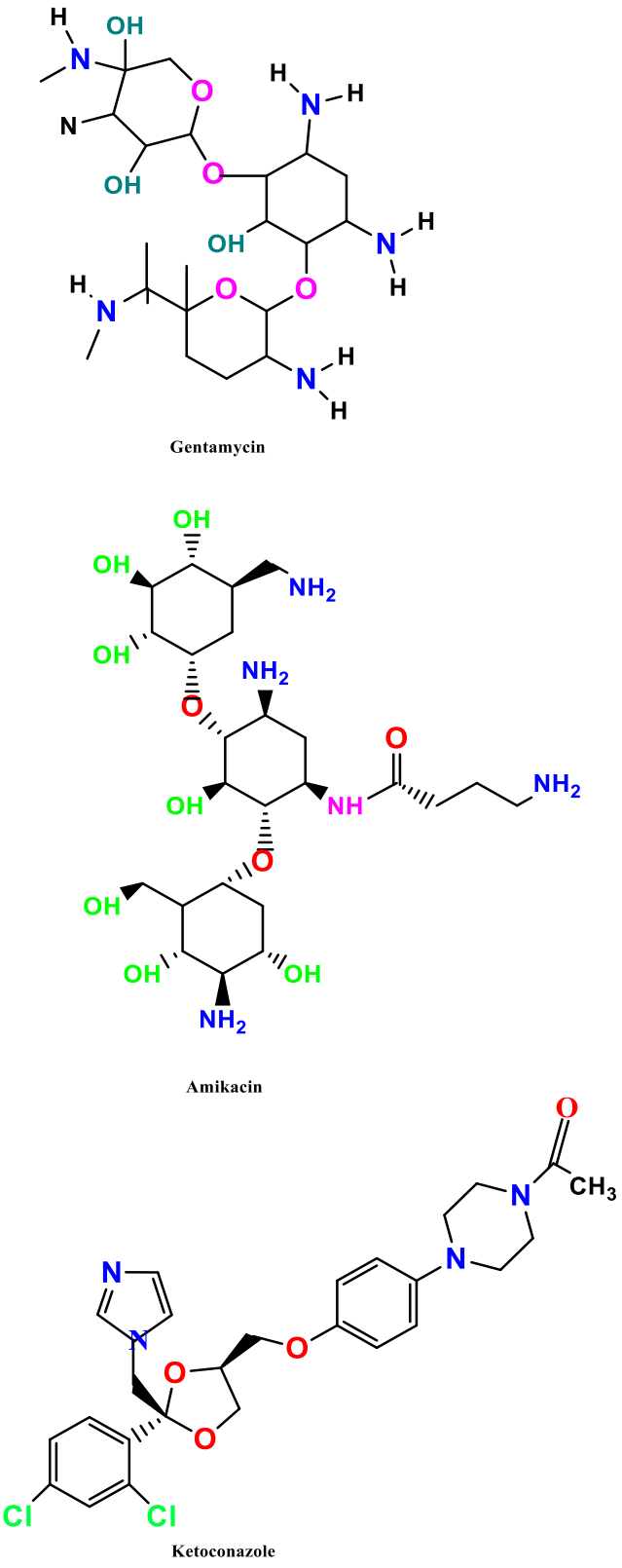


Figure 1: Structure of antibiotic and antifungal drug

3.3 MEDIA PREPARTION AND STERLIZATION

Mueller Hinton culture media:This is a common medium used in microbiology for antibiotic susceptibility testing. The steps you've mentioned are quite accurate:

- **Weighing and Dissolving**: 38g of the culture medium is suspended in 1L of distilled water. The medium is then dissolved by stirring with a sterilized glass rod.

- **Sterilization**: The mixture is covered tightly with aluminium foil and autoclaved for 20-22 minutes at 121°C. This step is crucial to kill any existing microorganisms and to sterilize the medium.

- **Cooling**: After autoclaving, the agar is allowed to cool while still maintaining it in a molten stage. This is important to prevent premature solidification of the medium.

- **Pouring**: Once the Petri dishes are dried, the freshly prepared and cooled Mueller–Hinton agar is spread on the surface of the dishes.

This process ensures that the medium is sterile and ready for the growth of microorganisms for further testing. [6]

3.4 Inoculation of test plates

A small volume, about 0.1 mL of the bacterial suspensions were inoculated onto the dried surface of Muller–Hinton agar plate and streaked (swabbed) by the sterile cotton swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60 °C each time to ensure an even distribution of inoculums and the rim of the agar was swabbed. The lid was left ajar for 5–15 min, to allow for any excess surface moisture to be absorbed before applying the samples on the respective well.

3.5 Sample injection and incubation

Anti-bactericidal activities of each reagent and synthesized complexes were evaluated by the disc diffusion method. Agar were prepared by using a sterilized cork borer with 6 mm diameter, 4 mm deep and about 2.5 cm apart to minimize overlapping of zones. Then holes of 6 mm diameter were punched carefully using a sterile cork borer. The metal complex and , solvents (DMSO & DMF) were carefully injected to the respective disc in duplicate. The reference antibiotic agent disc (gentamycin) was dispensed via sterile pair of forceps onto the surface of the inoculated agar plate and pressed down to ensure complete contact with the agar surface. It was allowed to diffuse for about 40 min before incubation and then the plates were incubated at 37 °C for 24 h. After 24 h incubation, the antibacterial activity was evaluated by measuring the diameter of inhibition zones in millimetre. The test was carried out in duplicate and the results were recorded as mean ± standard deviation.

We are followed as the preparation method W.H. Mahmond etal. And his coworkers [1]. A filter disk (5 mm) was transferred into 250 ml. flasks containing 20 ml of working volume of tested solution (100 g/ml). All flasks were autoclaved for 20 min at 121 ^{0}C. LB agar media surfaces were inoculated with two investigated bacteria (gram positive and gram negative) and two strains of fungi then, transferred to a saturated disk with a tested solution in the centre of Petri dish (agar plates). Finally, all these Petri dishes were incubated at 25 ^{o}C for 48 h. where clear or inhibition zones were detected around each disk. Control flask of the experiment was designed to perform under the same condition described previously for each microorganism but with dimethyl sulfoxide (DMSO) solution only and by subtracting the diameter of inhibition zone resulting with dimethyl sulfoxide from that obtained in each case, so antibacterial activity could be calculated [4]. All experiments were performed as triplicate and data plotted were the mean value.

4. RESULT AND DISCUSSION

4.1 Elemental analysis

The elemental analysis (Cu, C, H, N %) supporting the structure formulae of mixed ligand complexes, Table 1 and Fig. 2.

4.2 Spectral studies

Electronic: The UV-Visible spectra of all these complexes were recorded in non-aqueous medium (DMSO and DMF) solutions with a Perkin-Elmer-Lambda 35 spectrophotometer. The assignment for the electronic spectra are given in Table 2 and fig.3.

The electronic spectrum of copper (II) complexes that you described shows a single broad absorption band of high intensity at the range 679 nm- 800 nm, which belongs to the 2Rh->2T2g transition. This indicates that the copper (II) ion has a d9 configuration

and is in a distorted octahedral geometry, with four ligands in the equatorial plane and two ligands in the axial positions. The distortion is caused by the Jahn-Teller effect, which lowers the symmetry and splits the d orbitals further. The strong band at 450nm [Cu(dmp)2],460 nm and 453 nm in DMSO and DMF, respectively, could be attributed to a LMCT transition from the oxygen atom of the solvent to the copper (II) ion.

Infrared Spectroscopy: The FT-IR spectra were performed on Perkin-Elmer 577 FT-IR spectrometer from KBr pellets in the range 4000-400cm^{-1}. Infrared spectroscopy can be used as a good analytical tool follow the complexation of the transition metal ion by the organic ligands. Generally, 4-OHNA as a bidentate natured ligand after a base hydrolysis reaction followed by keto-enol tautomerization then coordinate with metal ion through two O atoms. Coordination of the copper (II) with functional groups of the mixed ligands aromatic diimines and 4-OHNA are detailed in Table-2 and Figure 5. From the data of all these complexes are similar and exhibits the strong characteristics bands at 1559 cm^{-1}, assigned to V_{asym} (COO-) and bands at 1381 cm^{-1}, assigned to V_{asym} (COO-) stretching frequency for mixed ligand complexe. The sepration (V) values (Table 2) of asymmetric and symmetric frequency of carboxylate ion fall in the borderline of mono and bidentate mode of coordination of carboxylic group of 4-OHNA. The bands at 3451 cm^{-1} assigned to presence of water molecule. Bands at 1278 cm^{-1}, assigned to V_{NO} of unidentate ONO_2- . -898 cm^{-1} (Table 2) assigned to C=N stretching vibrations due to coordination of 1,10-phen and 2,9-dimethyl 1,10-phenanthroline ligands to copper (II) ion[7,8 & 9].

These spectroscopic results supporting the proposed structure of mixed ligand copper complex is distorted square-pyramidal , in this complex copper is coordinated by two oxygen atom of one by carboxylate O & another from 4-OHNA anion ligand and two coordinating of nitrogen of diamine ligand.

4.3 Antimicrobial activity

The antibacterial activities the of aromatic diimines, 4-OHNA ligands, copper nitrate and and mixed ligand complexes against **Bacillus subtilis, Staphylococcus aureus, Neisseria gonorrhoeae, *Klebsiella pneumoniae* (*K. pneumoniae* and Escherichia** coli are presented in Table 3 and Figure 4. The 4-OHNA ligand has no activity at all towards **Bacillus subtilis** and **Escherichia coli (Fig. 4 & 5)**. This is attributed to its very resourceful nutritional capability, adaptability to various hydrocarbon rings, and the possession of pump mechanism which ejects metal complexes as soon as they enter the cell [10&12]. In addition, **Bacillus subtilis, Staphylococcus aureus and Escherichia coli** and sensitive to all the complexes, and an inhibitory zone range of 10/0-20.0 mm (Table 2a &2b). In all cases, the metal complexes are more active than the 4-OHNA ligand expectedly due to chelation, which reduced the polarity of the metal storm, mainly because of partial sharing of its positive charge with donor groups of the ligand and possible. electron delocalisation on the aromatic rings. This increased the lipophilic character, favoring its permeation into the bacterial membrane, causing the death of the organism [11]. A look at the antibiotic, gentamycin & amikacin, activities (6.0 – 9.0 mm) against the various bacterial isolates relative to the metal complexes (10.0 – 20.0 mm) showed that the activities of the former are much lower, with optimum activity being about half of metal complexes against all the bacterial organisms.

When the antimicrobial activity of metal complexes is investigated, the following principal factors should be considered: (i) the chelate effect of the ligands; (ii) the nature of the N-donor ligands; (iii) the total charge of the complex; (iv) the existence and the nature of the ion neutralizing the ionic complex and (v) the nuclearity of the metal centre in the complex. This is probably one of the reasons for the diverse antibacterial activity shown by the complexes pebbles the nature of the metal ion coordinated to 4-OHNA ligand may have a significant role to this diversity. In general, all the complexes exhibit better inhibition than free 4-OHNA against ***Bacillus subtilis, Staphylococcus aureus, Neisseria gonorrhoeae, Klebsiella pneumoniae and Escherichia coli*** (Table 6). More specifically, all the complexes 1 and 2 show the best inhibition among all the complexes in this study and it is one and halt to twenty times more active than free ligand diamine and 4-OHNA against all the microorganism used, indicating that the coordination of the 4-OHNA ligand to copper(II) ion has enhanced its antimicrobial activity complexes . On the other hand, the rest complexes present higher antimicrobial activity to diamine & 4-OHNA against the five microorganisms.[13]

The preliminary fungitoxicity screening of the 4-OHNA and aromatic diamine and mixed-ligand complexes were performed against the *Candida parapsilosis* in vitro by the diffusion technique [1],4-OHNA and all the metal complexes showed no fungal growth inhibition[10-13]. (Fig. 4).

4.4 Electrochemical behaviour

The copper(II) complexes are redox active and cyclic voltammograms were recorded in DMSO,DMF &ethyl alcohol exhibits a reversible oxidation process (Fig. 5) Table at +0.48 V assigned to the Cu++ /Cu+ redox couple. Controlled potential coulometry shows the involvement of one electron per molecule for this reduction process. The redox couple Cu++ /Cu+ showing the following features that anodic to cathodic peak current ratio is equal to 1.0 indicating simple electron process. The shapes for oxidation and reduction of cyclic voltammetry also suggested that the electrode process is diffusion controlled, clearly indicating that Cu^{2+} is coordinated to two dmp molecule.In addition to these plot between peak current Ip_c or Ip_a was proportional to square root of scan rate giving a straight line passing through origin showing as would anticipitd diffusion controlled process fig. 6.The value of ΔEp progressively increases from with increasing scan rate for 2,9-dimethyl-1,10-phenanthroline, a change of reversible to quasi-

reversible redox processes were observed.The Copper mixed ligand complex easily goes to redox process shows .Ligand-based reduction processes are poorly defind[5].

Table 1: Formula, formula, elemental analyses and room temperature magnetic moments (μ_{eff}) of the mixed-ligand copper (II) complexes.

Com. No.	Formula	Empirical Formula	Mol. Weight	Colour	M.P. (°C)	Elemental Analyses, % Cal. (Found)					μ_{eff} RT (B.M.)
							Cu	C	H	N	
1	[Cu(4-OHNA)(dmp)(ONO_3)](H_2O)	$CuC_{20}H_{17}N_4O_6CI$	508	Green	175 ±1	Cal.	12.50	47.24	3.46	11.02	1.78
						Obs.	(12.58)	(47.24)	(3.52)	(11.03)	

Table 2: UV-Visible is spectral data for copper(II) mixed ligand complexes

Complex	Colour of solid compound	Colour	λmax (nm) .£=,$Lmol^{-1}$ cm^{-1})	Assignment of bonds
[$Cu(dmp)_2$	Green	C_2H_5OH		
		Blueish green	450(1387)	CT
		DMSO		
[Cu(4-OHNA)(dmp)(ONO_3)](H_2O)	Green	Green	742(58)	d-d
			456(1389)	CT
		DMF		
		Yellowish Green	770(70)	d-d
			453(1385)	CT

Table 3a : Antimicrobial activity data of the ligands and their mixed ligand complexes

Complex	Zone of inhibition, diameter in mm					*Candida parapsilosis* Fungi
	Gram+ve Bacteria		Gram-ve Bacteria			
	Bacillus subtilis(A)	Staphylococcus Aureus(B)	Neisseria gonorrhoeae(C)	Escherichia coli (D)	*Klebsiella pneumoniae* (E)	
4-OHNA	0.0	2.7	2.8	0.0	2.9	0.0
Diimine(dmp)	5.2	5.6	17	7.1	16	7.3
$CuNO_3$	5.3	6.0	0.0	8.0	0.0	1.0
[$Cu(dmp)_2$]	11	13	15	17	19	
[Cu(4-OHNA)(dmp)(ONO_3)](H_2O) 4	17	18	20	19	19	0.0
Gentamycin	6	9.2	7	6	8	0.0
Amikacin	6	9.0	7	6	6	0.0
Ketokonazole	-	-	-	-	-	9.0

Table 3b: Antimicrobial activity data of the ligands and their mixed ligand complexes

Complex	Zone of inhibition, diameter in mm					*Candida albicans (Fungi)*
	Gram+ve Bacteria		Gram-ve Bacteria			
	Bacillus subtilis (A)	Staphylococcus Aureus (B)	Neisseria gonorrhoeae (C)	Escherichia coli (D)	*Klebsiella pneumoniae* (E)	
4-OHNA	0.0	2.7	2.8	0.0	2.9	0.0
Diimine(dmp)	5.3	5.5	18	7.1	17	7.4
$CuNO_3$	5.3	6.0	0.0	8.0	0.0	1.1
[$Cu(dmp)_2$]	11	13	15	17	19	
[Cu(4-OHNA)(dmp)(ONO_3)](H_2O) 4	17	18	20	19	20	0.0

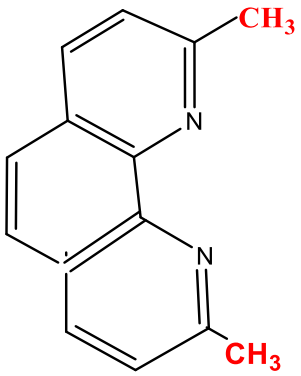
Gentamycin	6	9.2	7.5	7	8	0.0
Amikacin	6	9.0	7.6	6	6	0.0
Ketokonazole	-	-	-	-	-	9.1

Table 4: Cyclic voltammetry data for 1mM mixed- ligand Cu(II) complexes in ehtnol.

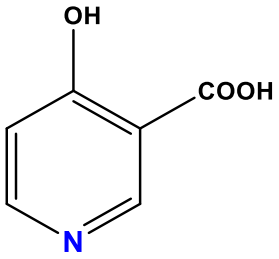
Scan Rate (mVs^{-1})	Epc (mV)	Epa (mV)	E^0(mV)	ΔEp(mV)Δ	Ipa/Ipc
[$Cu(dmp)_2$] in C_2H_5OH/0.2M $NaClO_4$					
25	460	520	490	60	1.0
50	460	530	495	70	1.0
100	450	530	490	80	1.0
200	450	540	495	90	1.0
300	440	545	492.5	105	1.0
[$Cu(dmp)_2$] in C_2H_5OH/0.1 M TBAP					
25	620	680	650	60	1.0
50	620	680	650	60	1.0
100	610	690	650	80	1.0
200	600	700	650	100	1.0
300	595	730	662.2	135	1.0

Table 5: Cyclic voltammetry data for 1mM mixed- ligand Cu(II) complexes.

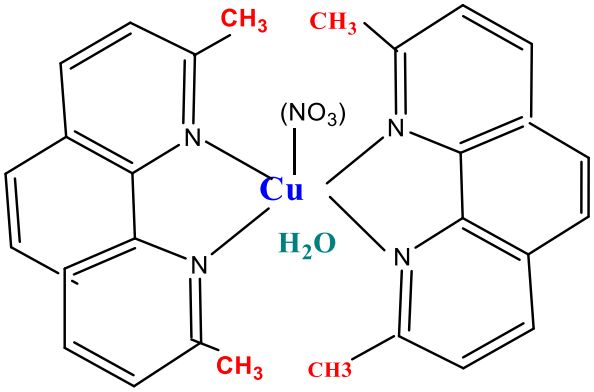
Scan Rate (mVs^{-1})	Epc (mV)	Epa (mV)	Epa' (mV)	E^0(mV)	ΔEp(mV)Δ	Ipa/Ipc	Ipa'
[Cu(4-OHNA)(dmp)(ONO_2)](H_2O)] in DMF/0.2M $NaClO_4$							
25	260	441	632	350	180	-	1.1
50	253	445	646	349	192	-	1.5
100	246	451	659	348	204	-	2.0
200	240	469	670	355	230	-	2.5
300	-	-	671	-	-	-	-
[Cu(4-OHNA)(dmp)(ONO_2)](H_2O)] 0.2M in DMSO $NaClO_4$							
25	271	358	602	314	86	0.2	1.1
50	264	359	609	311	94	0.1	1.5
100	252	361	622	306	108	0.1	2.0
200	242	367	633	304	124	0.1	2.4
300	236	373	639	304	136	-	-



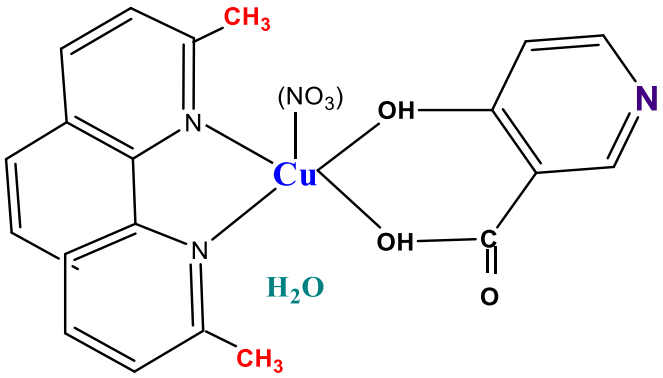
2,9-dimethyl-1,10 phenanthroline(dmp)
Primary ligand



4-OHNA
Secondary ligand

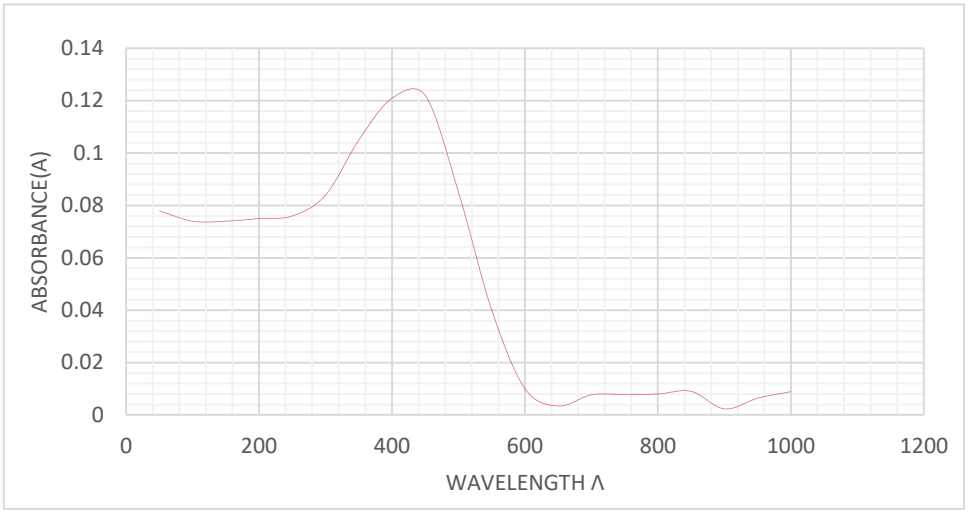


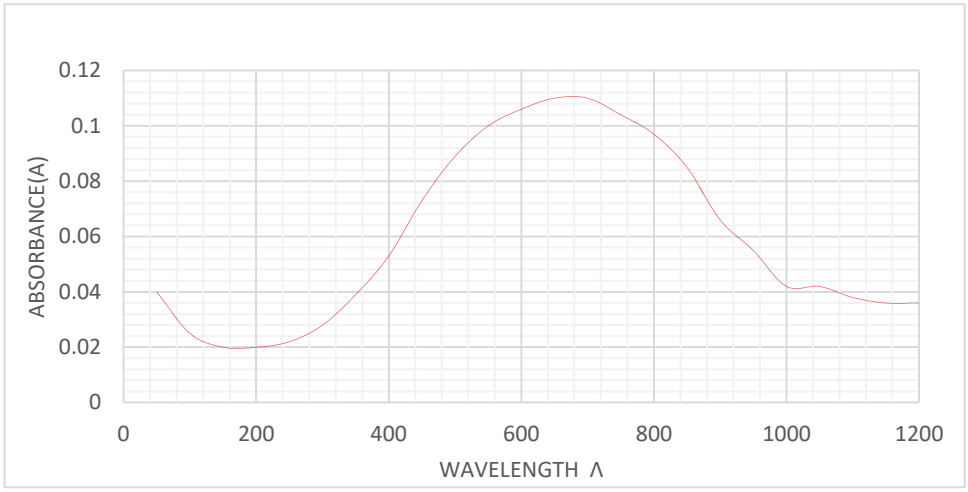
$Cu(dmp)_2$



[Cu(4-OHNA)(dmp)(ONO2)](H2O)]

Figure 2: Structure of ligands and complexes.





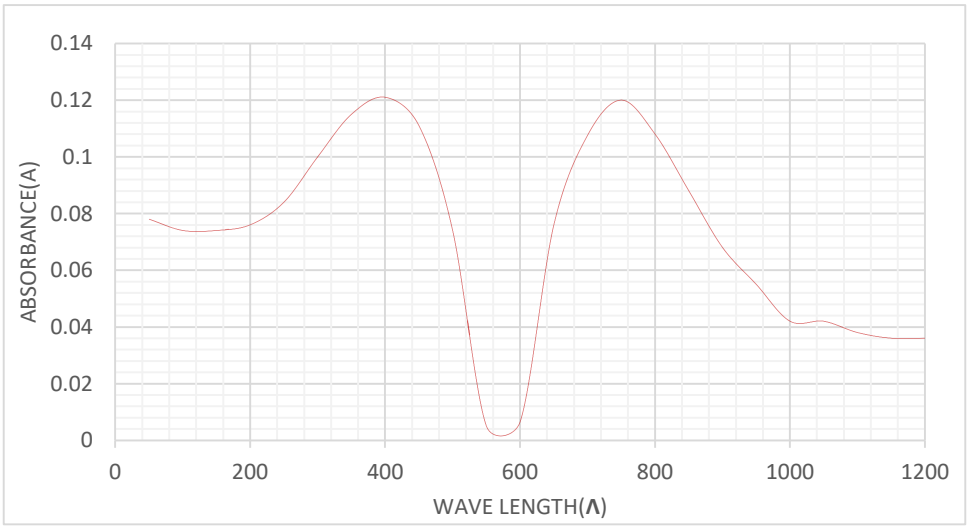


Figure2: UV-visible spectra of $Cu(dmp)_2$ in C2H5OH and $Cu(4\text{-}OHNA)(dmp)(ONO_2)](H_2O)]$ in DMSO and DMF[respectively.

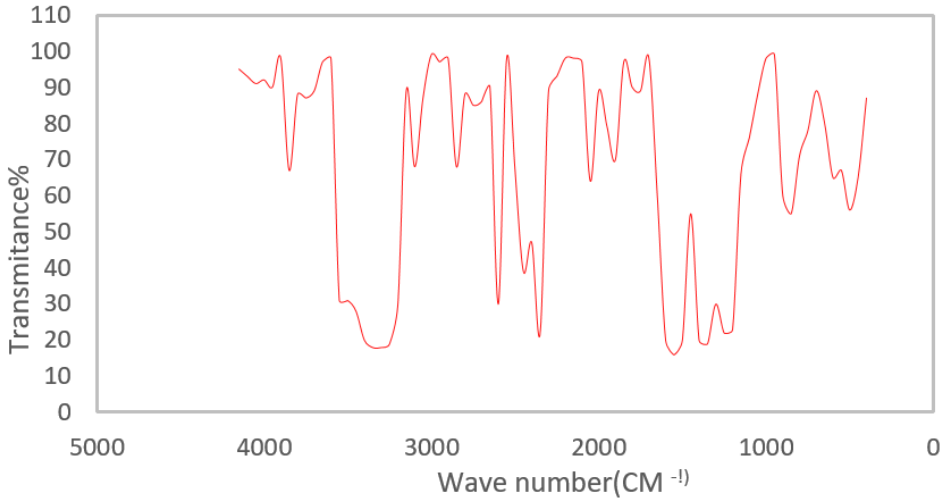


Figure 3: IR spectra of copper mixed ligand complex in DMSO

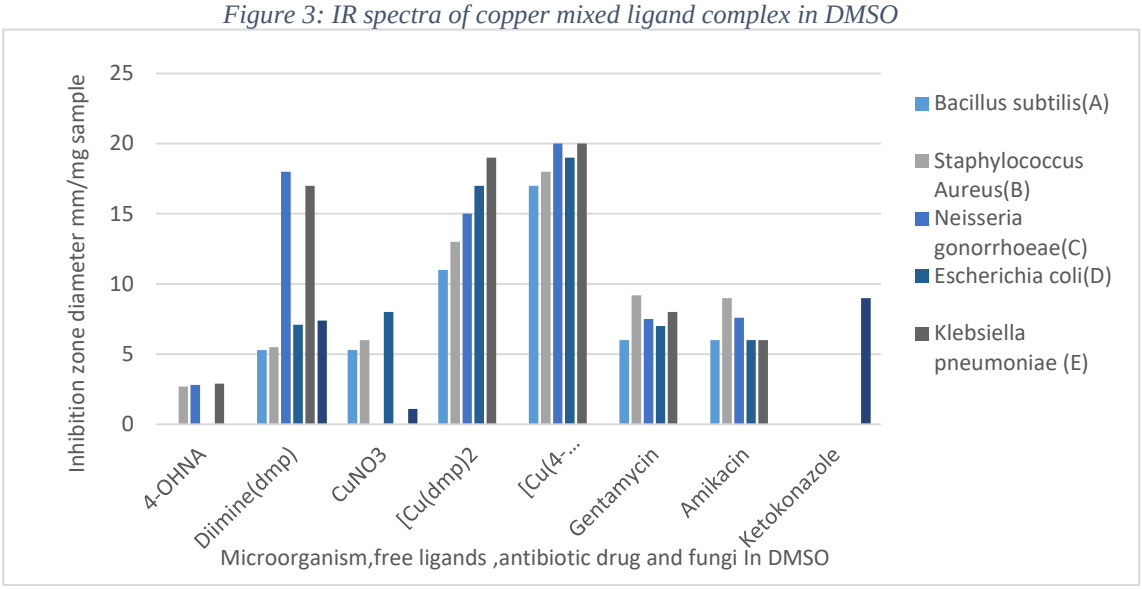


Figure 4:Biological activity of free ligand, copper nitrate, copper complexes, antibiotic drug and fungal in DMSO.

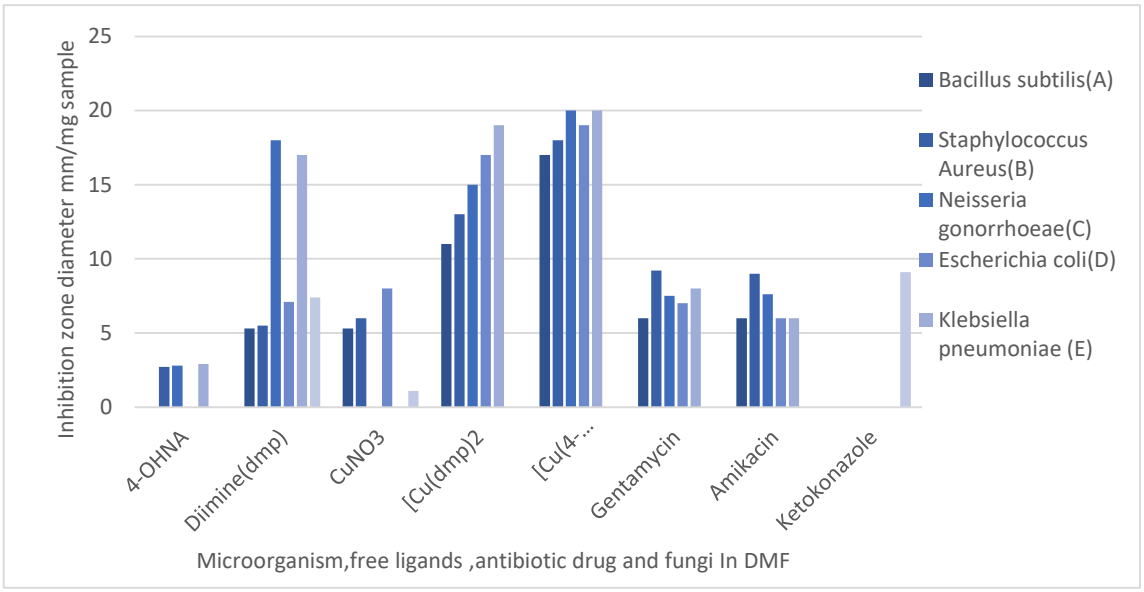


Figure 5: Biological activity free ligand, metal salt, copper complexes, antibiotic drug and antifungal in DMF.

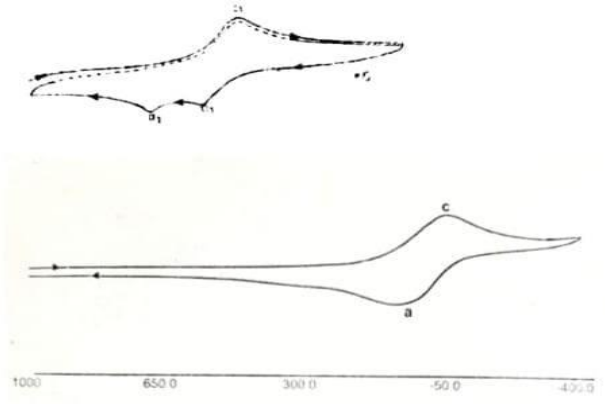


Figure 5: Cyclic voltammogram of $Cu(dmp)_2$ and$[Cu(4\text{-}OHNA)(dmp)(ONO_2)](H2O)]$

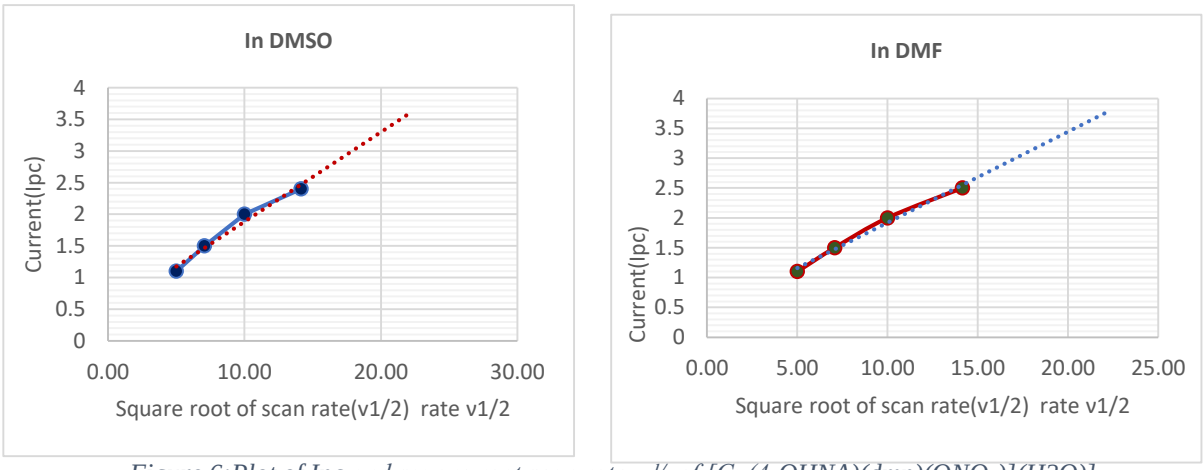


Figure 6:Plot of Ipc and square root scan rate v ½ of $[Cu(4\text{-}OHNA)(dmp)(ONO_2)](H2O)]$.

CONCLUSION*:*

The biological activities of copper mixed ligand complex are enhanced as compared to the metal salt and free ligand. The complexation of secondary 4-OHNA seems to play a significant role in enhancing the antimicrobial activity of the Cu^{+2} ion coordinated to the primary ligand diamine complex . The differences in the antimicrobial activities of the complexes could indeed be due to the participation of the secondary ligand in the coordination of the Cu^{+2}. The antimicrobial activity of complexes are more as compared to metal salt and free ligand. The activity are slight deferrer in both solvent . The electrochemical behavior of these complexes showing a quasi-reversible one-electron transfer process without any chemical complications in DMSO and DMF is also noteworthy. The redox couple shifted to more positive in TBAP supporting electrolyte.

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