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Electrochemical and Microbial Behaviour of Some Copper(II) Complexes in Non Aqueous Solvents

Dr. Mala Kumari
Department of Chemistry, S.C. College, Ballia, U.P., India.
E-mail:kumrimala310@gmail.com

Abstract

Copper is one of the most fascinating elements for various biochemical applications. Copper compounds show vast array of biological actions, including anti-inflammatory, anti-proliferative, biocidal and other. It also offers a selection of radioisotopes, suitable for nuclear imaging and radiotherapy. Quick progress in nanotechnology opened new possibilities for design of copper-based drugs and medical materials. In current time copper has not found many uses in medicine, but number of ongoing research, as well as preclinical and clinical studies, will most likely lead to many innovative applications of copper in upcoming time. Abrupt in nanotechnology yawning new chances for design of copper-based drugs and medical materials. In recent time, copper has not found many uses in medicine, but number of ongoing research, as well as preclinical and clinical studies, will most likely lead to many novel applications of copper in the near future.

In The paper some copper (II) complexes were prepared Using Primary ligand e.g. 2,2'-bipyridine(2,2'-bipy),1,10-phenanthroline(phen), and 5,5' -dimethyl-1,10-phenanthroline secondary ligand 4-hydroxynicotinic acid (OHNA) and characterized by elemental analysis and spectroscopic technique. The electrochemical behaviour of these complexes was recorded by using cyclic voltametric techniques.

Key words: Drug, Electrochemical behaviour Microorganism, Fungitoxicity, Chemotherapeutic, Analgesic, Anti-inflammatory and Antimicrobial activity.

Introduction: Metal-based drugs are a research area of increasing interest for inorganic, pharmaceutical and medical chemistry and have concentrated much attention as an approach to new drug development [1] &[2]. As far as we know, the selection of appropriate metal ions and organic ligands is the key in the

construction of complexes. Metal ions, especially their radii and coordination geometry, determine the extending direction and coordination modes of the organic ligands, which is important for the structure of the complexes, Among the metal complexes, those of 1, 10-phenanthroline have attracted line 1, 10-phenanthroline as chelating nitrogen donor ligands is among the most efficient chelators for transition and post-transition metal ions with which it form stable complexes in solution. The presence of aromatic and/or hetero aromatic groups into the structure of nitrogen donors gives these ligands with additional properties. For instance, poly nitrogen donors containing aromatic and/or hetero aromatic groups conjugate the ligational ability with the photo physical properties typical of these groups and, accordingly, they have been widely used as chemo sensors for metal ions in solution, since their coordination may affect the properties of the photosensitive group giving rise to an optical response. It is well known been also reported that some mixed ligand complexes of 1, 10-phenanthroline (phen)e.g. [Cu(phen)] have an antitumor activity where it inhibited DNA or RNA polymerase activities. Microbial infections often produce pain and inflammation. Chemotherapeutic, analgesic and anti-inflammatory drugs are prescribed simultaneously in normal practice. The compound possessing all three activities are known for some Pyridine and phenol derivatives [4]. These compounds are able to block cartilage destruction during the inflammatory process and thus are a promising class of anti-inflammatory compounds.Early reports have shown that carboxylate compounds and nitrogen heterocyclic ligands have been successfully employed in the generation of many novel structures .

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.

Copper has an established history as an antimicrobial agent, with its efficacy and mechanism of action against microorganisms well-studied. Copper ions released from surfaces can lead to RNA degradation and membrane disruption

of enveloped viruses. Copper nanoparticles (CuNPs) have been developed for antimicrobial performance due to their larger surface-area-to-volume ratio, resulting in increased toxicity compared to the metal.

Copper salts and copper pyrithionate complexes have been shown to be effective in combating antibiotic resistance due to their inhibitory action against metallo-beta-lactamases. Copper complexes with bis(thiosemicarbazone) ligands have shown antibacterial activity against a range of medically significant pathogens.

The use of copper complexes in the form of drugs can modify their pharmacological and toxicological properties. Low molecular weight copper complexes (Cu2+) have been proven beneficial against several diseases such as tuberculosis, rheumatoid arthritis, gastric ulcers, and cancers.

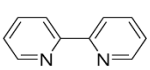
However, it's important to note that while copper complexes show promise in combating antimicrobial resistance, further research is needed to fully understand their mechanisms of action and potential side effect[3 a-c]

Copper deficiency can inhibit angiogenesis, thus preventing the growth of tumor cells or an inflammation to spread. In this connection copper in control levels can be potentially used as a strategy in the cancer therapy or against neurodegenerative diseases [68]. The role of copper in angiogenesis processes is not yet understood, and further investigation is needed. In order to heightened biological activity, it has been a common practice to synthesize Cu(II) complexes of biologically active ligands. Thus complexes of thiosemicarbazones have been extensively studied in efforts to synthesize efficient anticancer drugs Such complexes were also found to inhibit enzymatic activity and induce cell apoptosis [2a] such activity is usually correlated with anticancer drug validity[2b].

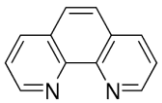
In this paper reported the synthesis of three mixed ligand copper (II) complexes containing aromatic diimines as a primary ligand while 4-hydroxynicotimic acid (4-OHNA) as secondary ligand (Scheme 1). These complexes formulated as: [Cu(4-OHNA)(bipy)H_2O]$NO_3$1; [Cu(4-OHNA)(5,5′-Me_2bipy)(ONO_2)]H_2O, 4.

The complexes were procured by traditional room temperature synthesis and were structurally characterized by elemental analysis, molar conductance, spectral FT-IR, UV-Visible) and room temperature magnetic measurements. The electrochemical behaviour of these complexes are studied by cyclic voltametric technique, in non-aqueous solvent like dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) solvent containing 0.2M sodium perchlorate ($NaClO_4$) and 0.1M tetra butyl ammonium perchlorate (TBAP) as supporting electrolyte at a Platinum working electrode.) Antimicrobial activity (in vitro) have been study after complexation.

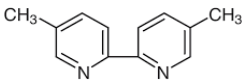
Scheme 1, Ligands used for the synthesis of mixed -ligand complexes



2,2'-bipyridine

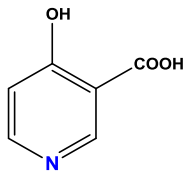


1,10-phenanthroline



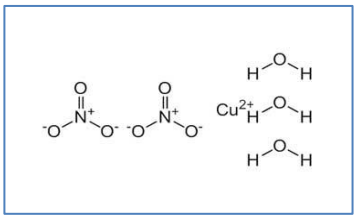
.5,5'-dimethyl-2.2'-bipyrine

Figure 1: Primary ligand



4-hydroxynicotinic acid

Figure 2: Secondary ligand



Copper nitrate trihydrate

Figure 3: Metal salt

2. METHODS AND MATERIALS

2.1. REAGENT

All chemicals and reagents used for the synthesis were commercially available and used without further purification. Copper (II) nitrate trihydrate, 4-chloronicotinic acid, and aromatic dines (2,2′-bipyridine, 5.5′-dimethy1-2,2′=bipyridine and 1,10-phenanthroline) were purchased from Sigma Aldrich Chemicals Pvt. Ltd. And were used as such. Analytical grade DMSO and DMF were procured from EMerck 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 dimine ligand (2,2-bipy/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 . The find yield ranged from 80-90 percent. The dried complexes were subjected to further elemental and spectroscopic analysis. The obtained complexes were found to soluble in non aqueous solvent DMSO and DMF.

3. INSTRUMENTATION

3.1 Cyclic voltammetry:All the experimental analysis were performed as described in [5].

3. 2. ANTIBACTERAL ACTIVITY TESTING

The metal salt, ligand and complexes were evaluated for in vitro antibacterial activities against strains of the Gram-negative bacterial strains 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**.

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 PLATE

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[7].

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 given by 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 [7]. Allexperiments were performed as triplicate and data plotted were the mean value.

3.6. DATA VALUE AND VALIDATION

As mentioned earlier, these three complexes were synthesized by traditional or solvent evaporation method. All these complexes (1-3) were obtained by mixing of copper nitrate $Cu(NO_3)_2.3H_2O$: 4-OHNA : aromatic diimines in 1 : 1 : M ration into 1 : 2 water ethanol mixture under basic condition. In these complexes the secondary ligand 4-chloronicotinic acid subjected to be a base hydrolysis reaction followed by keto- enol tautomerization (Scheme 2) Figure 4.

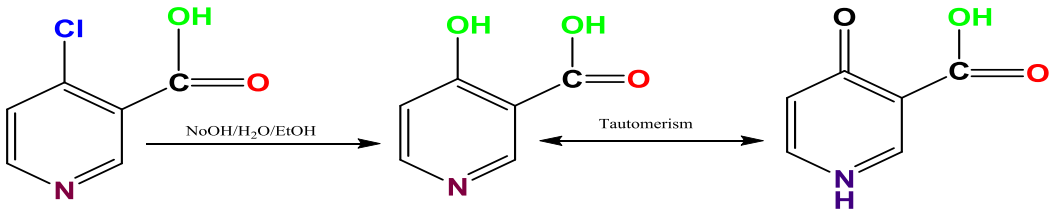


***Figure 4**:Sheme 2 hydrolysis of 4-chloronicotic acid.*

3.7. RESULT AND DISCUSSION: The elemental analysis (Cu, C, H, N %) supporting the structure formulae of complexes 1, 2 & 3; Table 1 Figure 5.

3.8. 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 1586 cm^{-1}, 1585 cm^{-1} (Figure 1, complex 1) assigned to V_{asym} (COO-) and bands at 1386 cm^{-1}, 1385 cm^{-1}, 1408 cm^{-1} and 1381 cm^{-1} assigned to V_{asym} (COO-) stretching frequency for all these complexes. 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

3369 cm cm^{-1}, 3450 cm^{-1}, 3451 cm^{-1} assigned to presence of water molecule. The bands in complexes 1 and 3 at 320 cm^{-1} and 918 cm^{-1} assigned to presence of coordinated water molecule (Table 2). Bands at 1483 cm^{-1}, 1265 cm^{-1}, 1503 cm^{-1} and 1278 cm^{-1} (Figure 6) assigned to V_{NO} of unidentate ONO_2- . the medium intensity band is at 1393 cm^{-1} assigned to C=C in cpmplex 2, and bands at 840 cm^{-1}-898 cm^{-1} (Table 2) assigned to C=N stretching vibrations due to coordination of 2,2'-bipy, 5,5'-Me_2-bipy, and 1,10-phen ligands to copper (II) ion[8]&[10].

3.9. ELECTRONIC ABSORPTION SPECTRA

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 4 and figure 7. The electronic spectrum of copper (II) complexes give a single broad absorption of high intensity is observed at the range 679 nm- 800 nm which belong to ^2Rh->$^2T_{2g}$ thus the shape of complex may be distorted octahedral. A strong band at 460 nm and 453 nm in DMSO and DMF.

3.10. CYCLIC VOLTAMETRIC

The electrochemical behaviour of all these complexes1,2&3 were carried out by using cyclic voltammetry (CV) technique (BASI EPSILON MODEL, USA) in non-aqueous medium (DMSO and DMF) containing 0.2 M $NaClO_4$ and TBAP as supporting electrolyte. CV of all these complexes set out in Tables 5,6,7 and 8 and Figure 8 & 9 . All these complexes 1, 2, and 3 exhibiting a diffusion controlled, quasi-reversible reduction couple attributed to $Cu^{2+/+}$ change with formal potential values given in Tables 4 and 5. The peak current ratio (Ipa/Ipc) is 1.0 for complex 2 and less than 1.0 for complex 1&3 in DMSO and DMF respectively, for all these complexes .The plot of Ipc vs square root of the scan rate ($V^{1/2}$) correspond to gives a straight line without any intercept. Controlled potential coulometry experiments at -130 mV in DMSO containing 0.2 M $NaClO_4$ for I have showed that one electron per molecule involved in the redox process. On the basis of present data showed the process in quasi-reversible,

one electron transfer without any chemical complications in DMSO and DMF, respectively EC mechanism [14].

STRUCTURE INTERPRETATION

On the basis of above results and observations proposed the structure of all these complexes are given in Figure 5. The 4-OHNA and aromatic diimines coordinate through O and N atoms to copper (II) ion. Consequently, the structures proposed square pyramidal geometry.

ANTIMICROBIAL ACTIVITY

The antibacterial activities of the 4-OHNA and aromatic diimines ligands 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 10. The 4-OHNA ligand has no activity at all towards **Bacillus subtilis** and **Escherichia coli (Figure 7)**. 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 [11]. 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 4). 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, favouring its permeation into the bacterial membrane, causing the death of the organism [12]. A look at the antibiotic, gentamycin, 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 3 show the best inhibition among all the complexes in this study and it is one and halt to twenty times more active than 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. On the other hand, the rest complexes present higher antimicrobial activity to 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 albicans* in vitro by the diffusion technique [1],4-OHNA and all the metal complexes showed no fungal growth inhibition][11-13]. (Figure 10).

TABLE 1: Formula, formula, elemental analysis of the mixed-ligand Cu(II) complexes 1-3.

Complex No.	Formula	Empirical Formula	Mol. Weight	Colour	M.P. (°C)	Elemental Analyses, % Cal. (Found)			
						Cu	C	H	N
1	[Cu(4-OHNA)(bipy)(H_2O] (NO_3)	$CuC_{16}H_{13}N_4O_6$ CI	456	Blue	215±1	13.92 (11.81)	49.57 (49.64)	2.85 (3.00)	12.28 (12.23)
2	Cu(4-OHNA)(5,5′-Me_2bipy)(ONO_2)(H_2O) 2	$CuC_{18}H_{17}N_4O_6$ CI	484	Light sky blue	205±1	13.92 (11.81)	49.58 (49.64)	2.85	12.28
3	Cu(4-OHNA)(phen))(H_2o)](NO_3)-2	$CuC_{18}H_{13}N_4O_6$ CI	480	Blue	299±1				

TABLE 2 : FT-IR spectral data of mixed ligand copper (II) complexes (1-4).

Complex	**$\nu_{asy(COO-)}$**	**$\nu_{sym(COO-)}$**	**$\nu_{asy(COO-)}$ $\nu_{syM(COO-)}$=$\Delta\nu$**	**v(O-H)**	**H_2O stretching Coordinated**	**v(N-O)**	**Pyridine ring stretch**	**v(C=C)**	**v(C=N)**
[Cu(4-OHNA)(bipy)H_2O]NO_3 1	1586	1386	200	3069	920	1483	1023	-	840
[Cu(4-OHNA)(5,5′-Me_2bipy)(ONO_2)] H_2O, 2	1585	1385	200	3450	-	1265	1022	1393	844
[Cu(4-OHNA)(bhen)(H_2O) 3	1612	1408	203	3451	918	1501	1023	-	846

TABLE 3: UV-Visible is spectral data for Cu(II) complexes 1-3.

Complex	Colour of solid compound	DMSO		DMF		Assi. of bonds
		Colour	..max (nm) (..,$Lmol^{-1}$ cm^{-1})	Colour	..max (nm) (..,$Lmol^{-1}$ cm^{-1})	
[Cu(4-OHNA)(bipy)(H_2O][(NO_3) 1	Dark Blue	Blue	682 (63)	Blue	675 (140)	d-d
[Cu(4-OHNA)(5,5′-Me_2bipy)(ONO_2](H_2O) 2	Sky Blue	Blue	670 (30)	Blue	669 (60)	d-d
[Cu(4-OHNA)(phen)(H_2o)](NO_3) 3.	Blue	Blue	695 (71)	Blue	687 (170)	d-d

TABLE 4: Antimicrobial activity data of the ligands and their mixed ligand complexes.

Complex	Zone of inhibition, diameter in mm					Candida Albicans
	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	5.4	5.7	7.2	7.4	16	7.3
1	18	17	14	20	14	0.0
2	12	11	13	11	12	0.0
3	10	10	12	10	11	0.0
Gentamycin	6	9	7	6	8	0.0
Ketokonazole	-	-	-	-	-	9.0

Table 5: Cyclic voltammetry data for 1mM mixed- ligand Cu(II) complexes in Dimethyl sulfoxide(DMSO) containing 0.2M $NaClO_4$.

Complex	Scan Rate (mVs^{-1})	Epc (mV)	Epa (mV)	E^0(mV)	ΔEp(mV)Δ	Ipa/Ipc
[Cu(4-OHNA)(bipy)(H_2o)](NO_3)-1	25	-90	12	-39	102	0.9
	50	-94	20	-37	114	0-9
	100	-99	27	-36	126	0.9
	200	-104	32	-36	138	0.9
	300	-106	32	-37	138	0.9
	400	-108	32	-38	140	0.9
	500	-108	32	-38	140	0.9
[Cu(4-OHNA)(phen)(H_2o)](NO_3)-2	25	-31	115	42	146	1.4

	50	-32	116	42	148	1.4
	100	-34	118	42	152	1.3
	200	-34	118	42	152	1.3
	300	-34	120	43	154	1.2
	400	-34	120	43	154	1.2
	500	-36	120	42	156	1.2
[Cu(4-OHNA)(5,5′-Me_2bipy) (ONO_2)] (H_2O)-3	25	32	136	84	104	0.8
	50	32	138	53	106	0.7
	100	32	140	54	108	0.7
	200	30	140	55	110	0.6
	300	30	140	55	110	0.6
	400	30	140	85	110	0.5
	500	30	142	56	112	0.4

TABLE 6: Cyclic voltammetry data for 1mM mixed- ligand Cu(II) complexes in Dimethyl sulfoxide(DMSO) containing 0.1M TBAP.

Complex	**Scan Rate (mVs^{-1})**	**Epc (mV)**	**Epa (mV)**	**E^0(mV)**	**ΔEp(mV)Δ**	**Ipa/Ipc**
[Cu(4-OHNA)(bipy))(H_2o)](NO_3)-1	25	30	135	52.5	105	0.9
	50	28	138		110	0-9
	100	25	137	55	112	0.9
	200	29	144	57.5	115	0.9
	300	25	145	60	120	0.9
	400	25	147	61	122	0.9
	500	20	147	63.5	127	0.9
[Cu(4-OHNA)(phen)	25	31	148	58.5	117	1.4

)(H_2O)](NO_3)-2						
	50	28	150	61	122	1.4
	100	25	152	63.5	127	1.3
	200	20	155	65	135	1.3
	300	18	160	71	145	1.2
	400	15	162	73.5	147	1.1
	500	15	165	75	150	1.1
Cu(4-ONHA)(5,5′-Me_2bipy)(ONO_2)](H_2O)-3	25	40	162	61	122	0.8
	50	38	165	63.5	123	0.7
	100	35	167	66	122	0.6
	200	30	170	70	124	0.6
	300	25	175	75	135	0.5
	400	20	178	79	140	0.5
	500	18	180	81	142	0.5

TABLE 7: Cyclic voltammetry data for 1mM mixed-ligand Cu(II) complexes in Dimethylformamide(DMF) containing 0.2M $NaClO_4$.

Complex	Scan Rate (mVs^{-1})	Epc (mV)	Epa (mV)	E^0(mV)	ΔEp(mV)Δ	Ipa/Ipc
[Cu(4-ONHA)(bipy)(H_2O)](NO_3)-1	25	-86	40	-23	126	0.5
	50	-88	40	-24	128	0.5
	100	-89	41	-24	128	0.6
	200	-89	41	-24	130	0.6
	300	-89	43	-23	132	0.6
	400	-90	44	-23	134	0.6

	500	-92	46	-23	138	0.6
[Cu(4-ONHA)(phen)(H_2O)](NO_3)-2	25	-57	21	-18	78	0.3
	50	-58	24	-17	82	0.3
	100	-58	28	-15	86	0.4
	200	-62	34	-14	96	0.5
	300	-64	36	-14	100	0.6
	400	-64	36	-14	100	0.6
	500	-64	36	-14	100	0.6
[Cu(4-ONHA)(5,5′-Me_2bipy)(ONO_2)](H_2O)-3	25	-78	12	-33	90	0.8
	50	-80	14	-33	94	0.8
	100	-80	16	-32	96	0.8
	200	-80	18	-31	98	0.8
	300	-80	20	-30	100	0.8
	400	-80	22	-29	102	0.8
	500	-80	24	-28	104	0.8

TABLE 8: Cyclic voltammetry data for 1mM mixed-ligand Cu(II) complexes in Dimethylformamide (DMF) containing 0.1M TBAP.

Complex	Scan Rate (mVs^{-1})	Epc (mV)	Epa (mV)	E^0(mV)	ΔEp(mV)Δ	Ipa/Ipc
[Cu(4-ONHA)(bipy)(H_2O)](NO_3)-1	25	25	85	30	60	0.8
	50	22	95	32.5	73	0.8
	100	20	103	41.5	83	0.88

	200	18	110	46	92	0.89
	300	15	115	50	100	0.90
	400	10	120	55	110	0.90
	500	10	125	57.5	115	0.90
[Cu(4-ONHA)(phen)(H_2O)](NO_3)-2	25	30	100	35	70	0.99
	50	28	105	37.5	75	0.99
	100	25	105	40	80	1.0
	200	23	125	42.5	85	1.0
	300	20	138	47.5	95	1.0
	400	18	160	57.5	115	1.0
	500	15	170	60	120	1.0
[Cu(4-ONHA)(5,5′-Me_2bipy)(ONO_2)](H_2O)-3	25	40	130	45	90	0.8
	50	45	139	47	94	0.8
	100	40	142	48	96	0.88
	200	38	146	49	98	0.88
	300	35	150	50	100	0.90
	400	30	163	55	110	0.95
	500	28	170	60	115	0.95

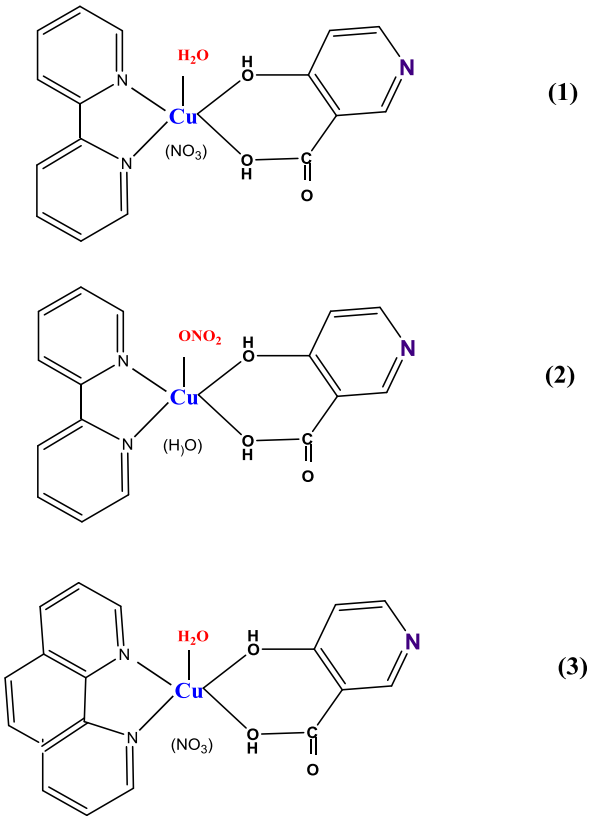


Figure 5: **Structures of Cu (4-OHNA)(bipy) (H2o)](NO3)- (1), [Cu(4-OHNA) (phen))(H2O)](NO3)-(2) & Cu(4-ONHA)(5,5′-Me2bipy)(ONO2)](H2O)- (3)**

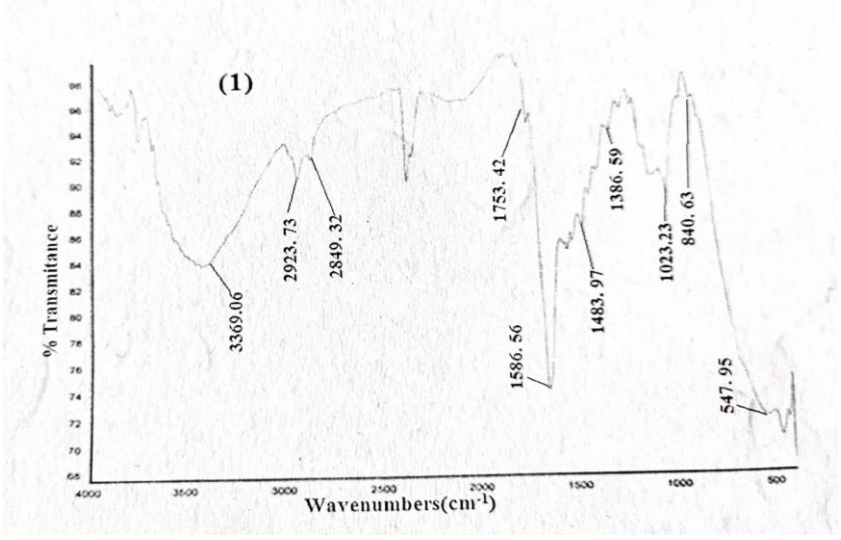


Figure 6: **Infrared(IR) spectra of copper(II)-mixed ligand complex, [Cu(4-ONHA)(bipy)(H2O)](NO3**

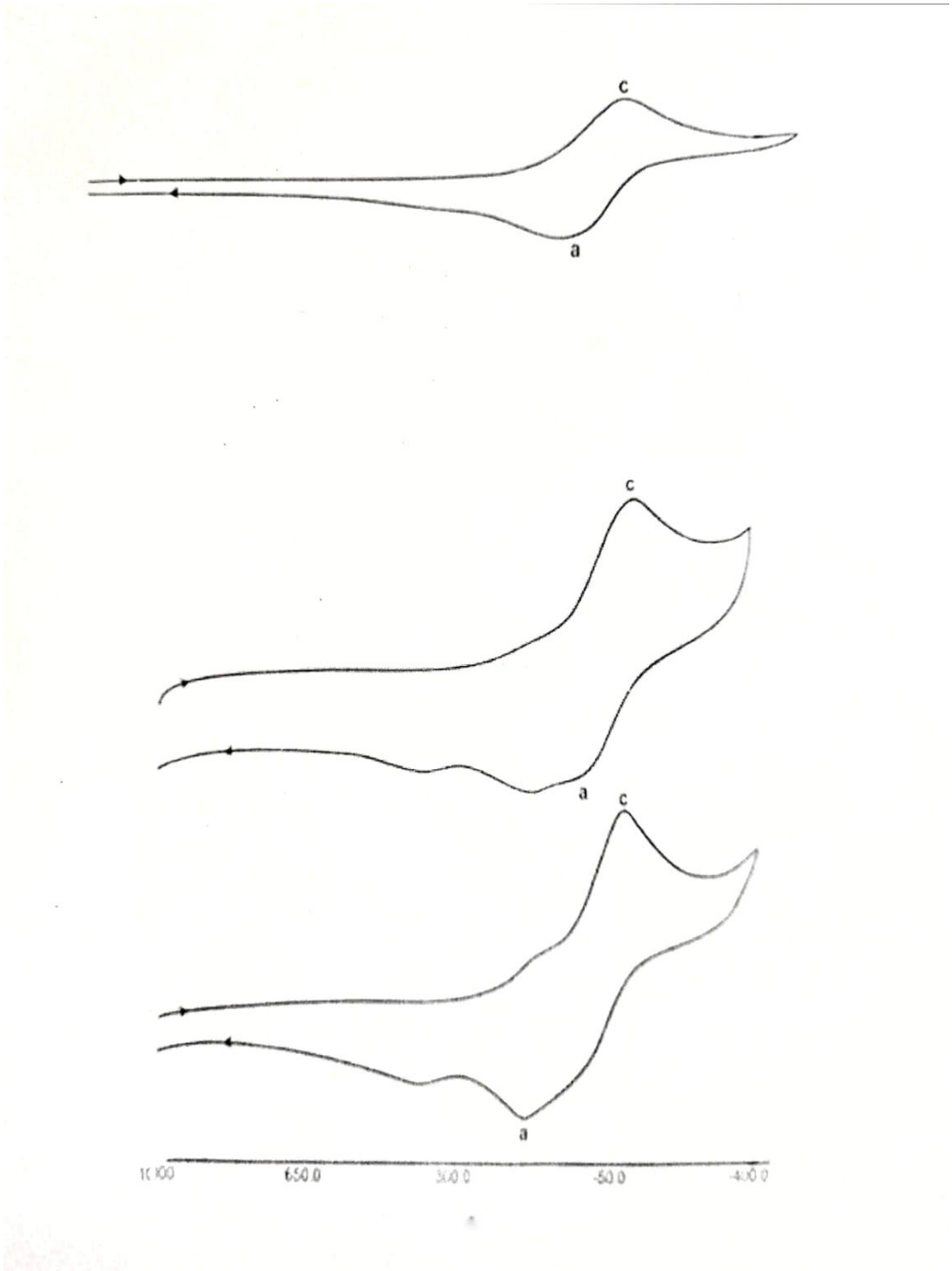


Figure 8:**Cyclic voltammogram of complex-1,in dimethyl sulfoxide (DMSO)complex-2 & complex-3 in dimethyl formamide(DMF).**

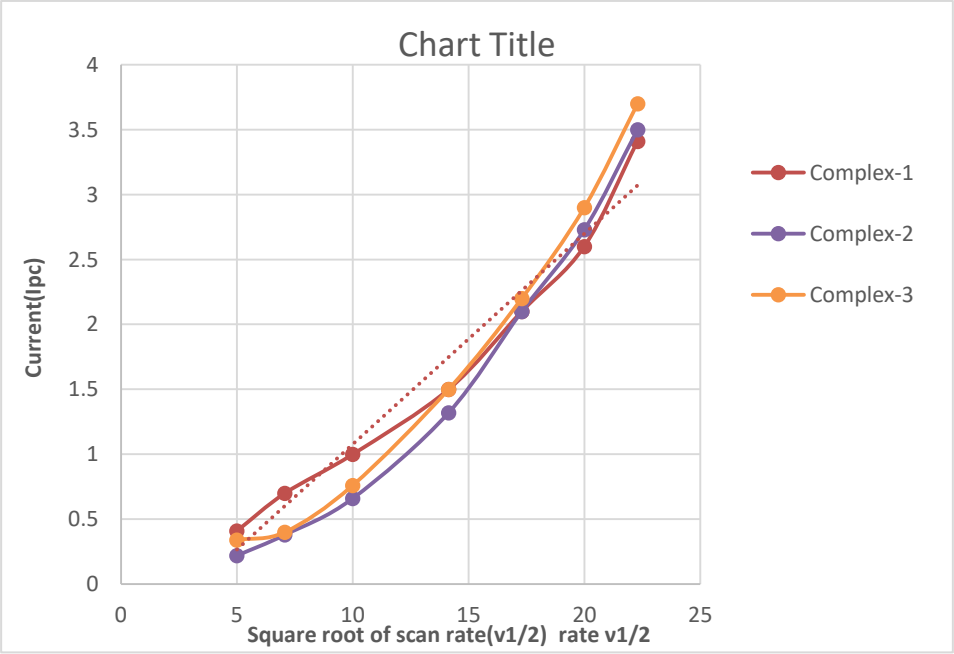


Figure 9: **Plot of cathodic peak current(Ipc) and square root scan rate v ½**

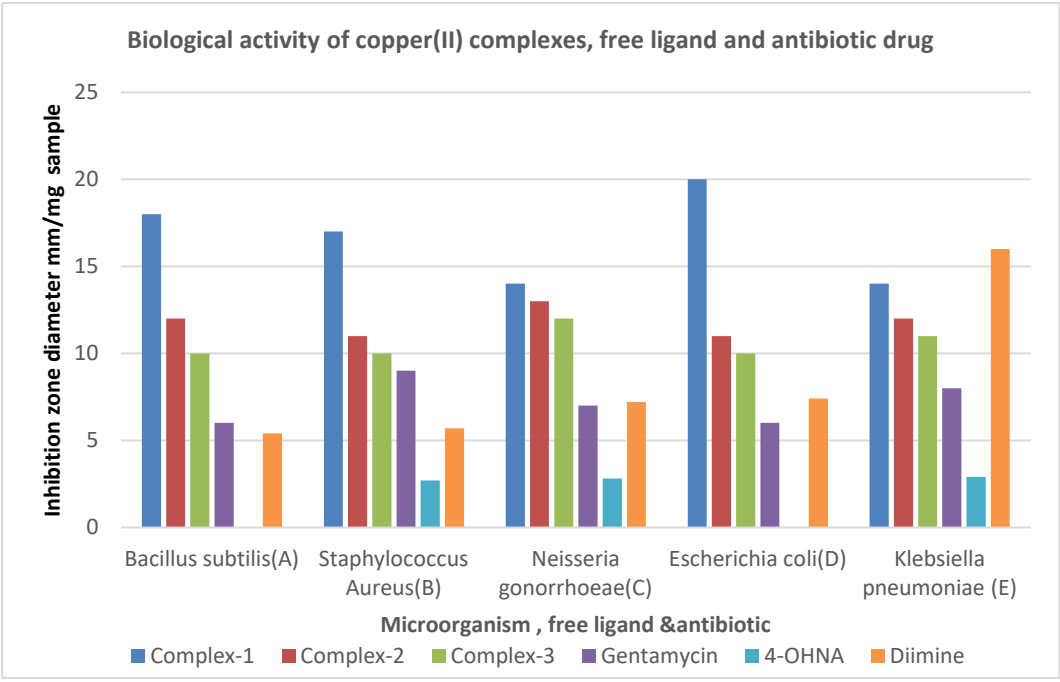
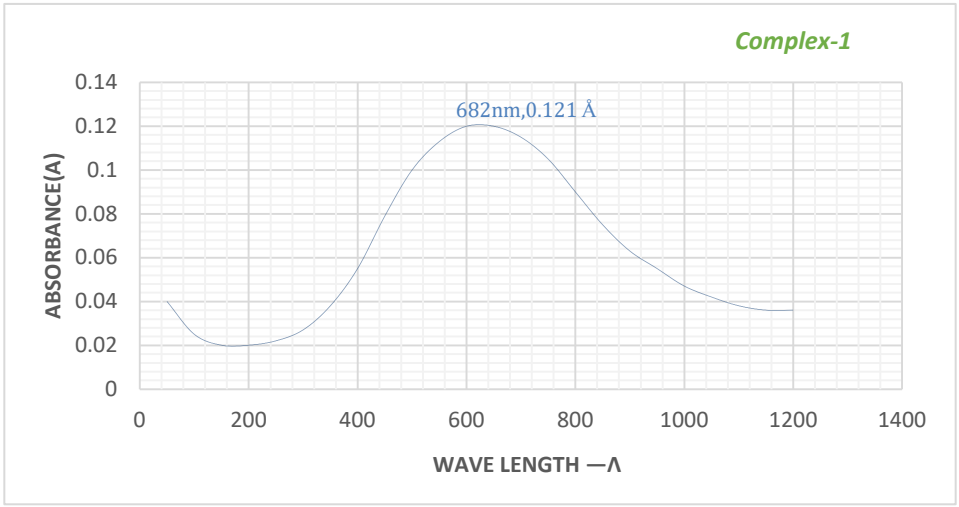
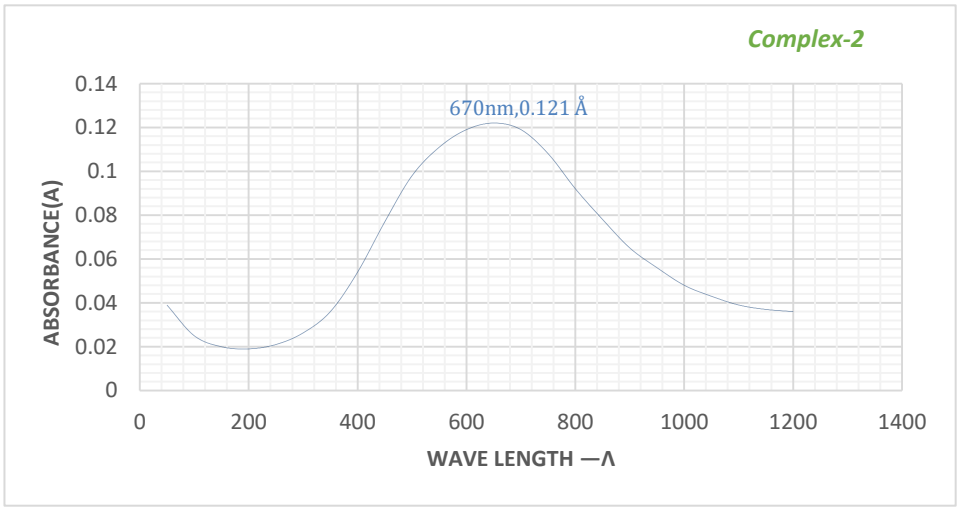


Figure 10: **Biological activity of copper complexes (1), (2) & (3), Free ligand 4-OHNA & diimine and antibiotic drug gentamycin, 4-OHNA.**


Complex-1
682nm,0.121 Å
ABSORBANCE(A)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
200
400
600
800
1000
1200
1400
WAVE LENGTH —Λ


Complex-2
670nm,0.121 Å
ABSORBANCE(A)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
200
400
600
800
1000
1200
1400
WAVE LENGTH —Λ

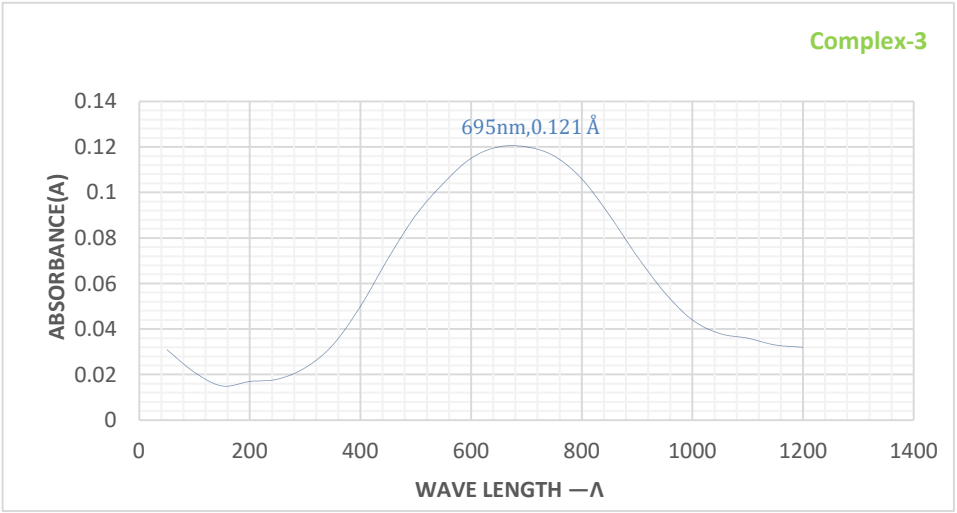


Figure 7:**UV-Visible electronic spectra of Cu(4-OHNA)(bipy))(H2o)](NO3)- (1), [Cu(4-OHNA)(phen))(H2O)](NO3)-(2) & Cu(4-ONHA)(5,5'-Me2bipy)(ONO2)](H2O)- (3):**

CONCLUSION:

The biological activities of copper complexes such as Cu(4-OHNA)(bipy)(H2O)](NO3)-(1), Cu(4-OHNA)(phen)(H2O)-(2) & Cu(4-ONHA)(5,5'-Me2bipy)(ONO2)](H2O)- (3) are enhanced compared to the free ligand. The complexation of secondary 4-OHNA seems to play a significant role in enhancing the biological activity of the Cu+2 ion coordinated to the primary ligand imine. The differences in the biological activities of the complexes could indeed be due to the participation of the secondary ligand in the coordination of the copper ion. 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. In my opinion this research could potentially contribute to the modelling & development of new drugs.

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