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Spectroscopic, Electrochemical, Catechol oxidase and catalase-like Activities of New Copper(II) Tweezers of Benzimidazole Incorporating Amino Acid Moieties

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Two mixed ligand complexes, viz., $[Cu(Bz_{OH})(Gly)]ClO_4$ 1 and $[Cu(Bz_{OH})(Glu)]ClO_4$ 2, where Bz_{OH} = 1-benzimidazolylethanol, Gly = Glycine, and Glu = L-glutamic acid were synthesized Their structures and properties were characterized by elemental analysis, IR, Raman, UV–vis, and ESR spectroscopy. Electrochemical measurements, including cyclic voltammetry and electrical molar conductivity were also performed. Spectral features point to square-planar CuN_2O_2 coordination geometries for both complexes. Spectroscopic and electrochemical studies were performed in order to correlate structural features of the complexes with their catecholase and catalase-like activities. The electrochemical behavior for the oxidation of catechol was studied using cyclic voltammetry at a carbon paste electrode modified by complex 1. The increasing in both anodic and cathodic peak current is due the presence of the complex as a structural mimics of *catecholase*, which acts as a redox mediator or electron shuttle which catalyze the oxidation and reduction of catechol at the surface of carbon paste modified electrode. Complex 1 showed high catalase activity with IC_{50} = 2.0 mM. These studies showed that this complex was found to be promising candidates as durable electron-transfer catalyst being close to the efficiency of the mimicking enzymes displaying either catalase or tyrosinase activity to serve for complete reactive oxygen species (ROS) detoxification with respect to peroxides. The nuclease activity of complex 1 was also assessed by its ability to cleave bacterial plasmid DNA in the absence of any external additives

Keywords: Enzyme mimicking; Copper(II) complexes; characterization; Catecholase, Catalase;, electron-transfer catalysts

1. INTRODUCTION

Copper(II) ion plays an important role in biological systems, supramolecular chemistry and various enzymatic reactions, such as catechol oxidase [1-4], superoxide dismutase [5-12] and catalase like activity [13-17]. Catechol oxidase, is a type III copper protein containing a active site specializing in two-electron oxidation of a broad range of catechols to the highly reactive o-quinones that auto-polymerize to produce melanin, which in turn guards damaged tissues against pathogens and insects among many of its protective functions [18]. It is a general aim of modern catalysis to develop substances that are able to act as highly active and, more importantly, highly selective catalysts throughout the field of chemical synthesis. In order to achieve this goal various approaches may be tried. One of the most promising ways is attempting to mimic Nature's most efficient catalysts, the enzymes [19]. Catalase, which is the sensible protective enzymes, plays an important role in the conversion of hydrogen peroxide to less harmful dioxygen and water ($2H_2O_2 \rightarrow 2H_2O + O_2$) [20]. Several studies have been carried out on metal complexes to act as catalase [21-24]. The synthesis and study of structural models can lead the understanding of fundamental mechanistic aspects of enzymes as well as the development of structure reactivity relationship. The major task in designing these model compounds depends on the proper choice of ligands as well as the steriochemical environment surrounding the metal centers. Ternary complexes play an important role in biological process like as an exemplified by many instances in which enzymes are known to be activated by metal ions [25,26]. Imidazole nitrogen donor atoms of histidyl residues are the most common binding sites in various metaloenzymes [27]. Therefore, ligands containing imidazole or benzimidazole rings can potentially mimic the binding sites and catalytic activities of the enzymes. The ligand 2-hydroxyethylbenzimidazole (Bz_{OH}) is a bidentate NO donor ligand with donor groups suitably placed for forming two 5-membered chelate rings. Amino acids are the structural unit of proteins. These are essential constituents of all living cells and contain one or more amino and carboxylic groups and have good coordination sites for the metal complexation. The present work stems from our interests to develop this chemistry further by synthesizing new ternary copper((II) complexes of a-amino acid (glycine or L-glutamic acid) and N,O-donor heterocyclic base (Bz_{OH}). This amino acid with its terminal $–C(=O)-NH_2$ group has the potential to form significant hydrogen bonding interactions with the double stranded DNA and could show good DNA-binding propensity. Herein, we report the synthesis of two mixed ligand complexes, viz., $[Cu(Bz_{OH})(Gly)]ClO_4$ **1** and $[Cu(Bz_{OH})(Glu)]ClO_4$ **2**, where Bz_{OH} = 1-benzimidazolylethanol, Gly = Glycine, and Glu = L-glutamic acid have been synthesize and characterized. The catecholase and catalase-like activities of both complexes were investigated. Furthermore, complex **1** exhibits hydrolytic cleavage of the bacterial plasmid DNA in the absence of any external additives.

2. EXPERIMENTAL

2.1. Materials and instrumentations

Caution! Perchlorate salts being potentially explosive, only small quantity was handled with care.

All chemical used were of analytical grad. Microwave reactions were performed with a Millstone Organic Synthesis Unit (MicroSYNTH with touch control terminal) The IR spectra were recorded using Alpha-Atunated FT-IR Spectrophotometer Bruker in the range of 400–4000 cm^{-1}. The electronic absorption spectra were obtained in DMF solution with a UV-Lambda 25 Perkin Elmer. NMR spectrum was recorded on Bruker spectrometers. ^{1}H NMR spectra were measured on a JEOL EX-400 instrument using TMS as an internal standard. Magnetic moments were measured by Gouy's method at room temperature. ESR measurements of the polycrystalline samples at room temperature were made on Varian E9 X-band spectrometer using a quartz Dewar vessel. All spectra were calibrated with DPPH (g = 2.0027). The specific conductance of the complexes were measured using freshly prepared (10^{-3} M) solutions in DMF at room temperature, using YSI Model 32 conductance meter. The copper content was determined by using atomic absorption technique, after destruction of the complexes with concentrated H_2SO_4/H_2O_2 mixture.

2.2. Syntheses

2.2.1. Microwave synthesis of 1-benzimidazolylethanol Bz_{OH}

A mixture of o-phenylenediamine (10.8 g, 0.1 mol) and lactic acid (20 ml) were heated at 100 ^{o}C by using microwaves irradiation at 500 W for 20 min. The resulting solution was poured into ice water (500 ml) and basified by sodium carbonate. The precipitated was filtered off, wash by water and dried. The product was recrystallized using ethanol/water to give Bz_{OH} as a whitish brown crystals. Yielded 15.9 g (97 %), m.p.; ^{1}H-NMR, (DMSO-d_6); □ 1.5 (d, J = 6.6, 3H, -CH_3), 4.6 (s, 1H, OH); 5.7 (d, J = 6.6, 1H, CH); 7.1 (m, 2H, H-Ar), 7.5 (m, 2H, H-Ar); 11.6 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6); □ 22.81 (q, CH_3), 63.54 (d, CH), 111.07, 118.27, 120.72, 121.36, 158.40.

*2.2.2. Synthesis of mixed complexes **1** and **2***

The mixed ligand complexes were prepared by a general synthetic method in which an aqueous solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (0.37 g, 1.0 mmol) was initially reacted with an aqueous solution of glycine (0.083 g, 1.1 mmol) or L-glutamic acid (0.161 g, 1.1 mmol) treated with NaOH (0.040 g, 1.0 mmol), followed by slow addition of a methanolic solution of the 2-hydroxyethylbenzimidazole (Bz_{OH}) [0.162 g, 1.0 mmol)]. The reaction mixture was stirred at 40 ^{o}C for 2 h and filtered. The filtrate on slow evaporation yielded 0.327 mg (82%) of **1** as greenish blue crystals and 0.362 mg (79%) of **2** as blue crystals.

2.3. Electrochemistry:

The cyclic voltammetry (CV) of copper(II) complexes **1** and **2** were obtained with Auto lab potentiostat PGSTAT 302 (Eco Chemie, Utrecht, The Netherlands) driven by the General purpose Electrochemical Systems data processing software (GPES, software version 4.9, Eco Chemie).

Electrochemical cell with three electrodes was used, platinum wire electrodes were used as working and counter electrodes and SCE as reference electrode. The electrochemical experiments were preformed in Britton–Robinson (BR) buffer (pH = 7) as a supporting electrolyte.

*2.3.1. Preparation of Unmodified and modified Carbon Paste Electrodes (CPE) by complexes **1** and **2***

Unmodified carbon paste electrode was prepared by mixing 65% graphite powder and 35% paraffin wax. Paraffin wax was heated till melting and then, mixed very well with graphite powder to produce a homogeneous paste. The resulted paste was then packed into the end of an insulin syringe (i.d.: 2mm). External electrical contact was established by forcing a copper wire down the syringe. Carbon paste modified electrodes was prepared by hand mixing 60% graphite powder and 30% paraffin wax and 10 % of complexes **1** and **2**. The resulted paste will be packed into the end of an insulin syringe. External electrical contact was established by forcing a copper wire down the syringe. The surface of the electrode was polished with a piece of weighting paper and then rinsed with distilled water thoroughly.

2.4. Catalase activity

The catalase-like activity of complexes **1** and **2** in DMSO was determined by colorimetric method using catalase assay kit purchased from Biodiagnostic Co., Cairo, Egypt, according to the method described by Aebi [29]. Briefly, catalases-like activities of different concentrations of both complexes (0.5 - 10.0 mM) were done by reacting with known quantity of H_2O_2. The reaction was stopped after exactly one minute. In the presence of peroxidase (HRP) remaining H_2O_2 reacted with 2,3 Dichloro-2,hudroxybenzenesulfonic acid (DHBS) and 4-aminophenazone (AAP) to form chromophore with a color intensity inversely proportional to the catalase activity of the model complex.

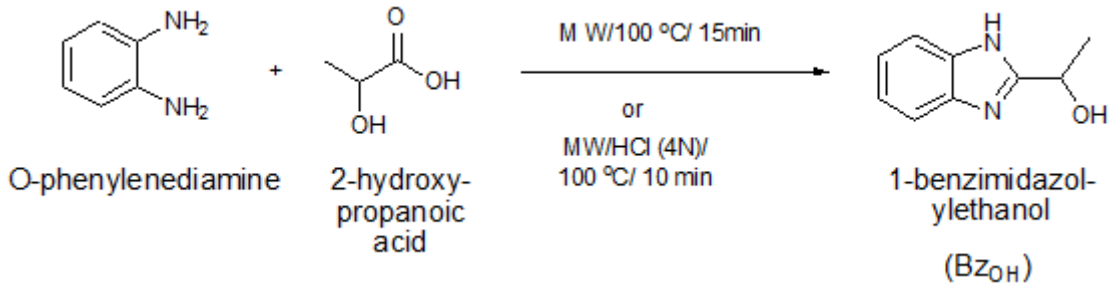
2.5. Nuclease-like activity assay

A mixture contained 4 µl of supercoiled pGEM-T easy plasmid vector (Promega, MD. Co.), 6 µl of TE (Tris 10 mM-EDTA 1.0 mM) buffer pH. 8.0, 10 µl of the Glycine complex at concentration (10.0 mM, 8.0 mM, 6.0 mM, 4.0 mM,2.0mM, 1.0 mM and 0.5 mM) or 10 ul DMSO were prepared. After incubation at 37°C for one hour time, 4 µl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in H_2O) were added to each tube and the solution was loaded onto a 1% agarose gel. The electrophoresis was carried out for ~one hour at 100 V in TAE buffer (Tris, Acetate, EDTA buffer pH 8.0). Gels were stained with ethidium bromide then destained in water prior to being photographed under UV light.

3. RESULTS AND DISCUSSIONS

*3.1. Characterization of the ligand BzOH and copper(II) complexes **1** and **2***

The syntheses of the ligand 1-benzimidazolylethanol (Bz_{OH}) was carried in microwave at 500 W and 120 ^{o}C for 10–15 min (Scheme 1).



Scheme 1. Microwave-assisted synthesis of 2-hydroxyethylbenzimidazole

The ligand was characterized by different techniques such as elemental analysis, ^{1}H-^{13}C NMR, and FT-IR, as well as Raman spectroscopes (Tables 1 and 2). The obtained Bz_{OH} - behaves as a bidentate ligand. Recently there has been a considerable interest in the mixed chelation because it occurs commonly in biological fluids, which contain millions of potential ligands which are likely to compete for metal ions, found *in vivo* [30,31].

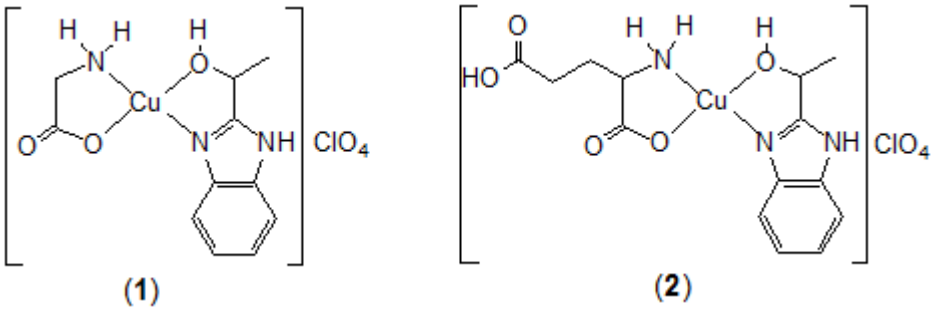
Table 1. Molecular formulae, elemental analyses, and physical properties of the ligand BzOH and copper(II) complexes **1** and **2**

Compound	Found (Calc.)%					λ_M^*
	%C	%H	%N	%Cl	%Cu	
Bz_{OH}	66.58 (66.65)	6.27 (6.21)	17.33 (17.27)			—
$[Cu(Bz_{OH})(Gly)]ClO_4$ (1)	33.75 (33.09)	3.63 (3.53)	10.61 (10.52)	9.05 (8.88)	15.94 (15.92)	84.2
$[Cu(Bz_{OH})(Glu)]ClO_4$ (2)	35.33 (35.68)	3.67 (3.85)	9.08 (8.92)	7.57 (7.52)	13.43 (13.48)	82.6

*In DMF, 1 mmol dm^{-3} solution at 25 ^{o}C; in S cm^{2} mol^{-1}

The preparation of the two mixed ligand complexes **1** and **2** are mainly involved the equimolar interaction of copper(II) perchlorate hexahydrate and sodium salt of amino acids (Na-AA) as a primary ligand, followed by the reaction with metahnolic solution of 1-benzimidazolylethanol (Bz_{OH}) as a secondary ligand. The chemical analysis and some physical properties of the isolated pure complexes are listed in Table 1. The analytical results demonstrate that all the prepared mixed ligand copper(II)

complexes have 1:1:1(Copper / Bz_{OH} / Gly or Glu) stoichiometry (Scheme 2). The pure solid chelates are blue. They are soluble in DMSO. The reported microcrystalline complexes are non-hygroscopic in nature and stable as solids or in solution under the atmospheric conditions. The structures and properties were also characterized by FT-IR, UV–vis, and ESR spectroscopy. Electrochemical measurements including cyclic voltammetry and electrical molar conductivity, and magnetic moment measurements were also studied. The complexes are formulated as $[Cu(Bz_{OH})(AA)]ClO_4$ {where BzOH = 2(α-hydroxyethyl)benzimidazole and AA^- = Glycinate (**1**) and L-glutamate (**2**). The molar conductivities of copper(II) complexes **1** and **2** in DMF solutions are 84.2 and 82.65 Scm^2mol^{-}, respectively corresponding to a 1:1 type of electrolytic nature of the complexes (65-90 Scm^2mol^{-1}) [32] giving $[Cu(Bz_{OH})(AA)]^+$ in solution. These observations demonstrate that, in solution the perchlorate anion does not participate in coordination. The electronic spectral and magnetic susceptibility measurements were used for assigning the stereochemistry of each complex. Electronic spectra indicate square planar geometry for both complexes. This was also corroborated by the effective magnetic moment of the complexes.



Scheme 2. The proposed structures of mixed ligand copper (II) complexes 1 and 2

*3.2. FT-IR and Raman spectra of the ligand 1-benzimidazolylethanol and its copper(II) complexes **1** and **2***

The solid-state vibrational properties of the ligand Bz_{OH} and the obtained mixed complexes **1** and **2** were examined by FT-IR and Raman spectroscopes (Table 2). The characteristic *ν*(O–H) and *ν* (N–H) vibration frequencies of the ligand exhibits strong bands at 3450 and 3441 cm^{-1}, respectively in its IR spectrum, caused by doubly intramolecular hydrogen bonding between the hydroxyl hydrogen atom and NH nitrogen atom [33,35]. The (C=N) asymmetric stretching frequency is expected to appear at *ca.* 1326 cm^{-1}. The Raman spectrum of the ligand is just a characteristic of the strength of the vibration bond as IR absorption bands. The comparison of the IR spectra of the ligands BzOH, Gly, L-Glutamic acid, and their mixed copper(II) complexes **1** and **2** indicated that these ligands acted as bidentate ligands. The observed shifts in the stretching frequencies of □(C=N) are indicative of the formation of these complexes. As a result of coordination, the stretching frequencies of the C=N bond in the imidazole unit of the benzimidazole rings shows opposite change. A downward shift (5-10 cm^{-1}) of ν(C=N) in the IR spectra of the complexes as compared to their values for the free ligand,

suggesting coordination through the pyridine nitrogens of the benzimidazole rings [36]. Generally, the IR spectra of the free ligands show a broad band around 3450 cm^{-1} which can be attributed to NH stretching vibration of benzimidazole moiety.

Table 2. IR and Raman wavenumbers (cm^{-1}) and tentative assignement of the most important bands in the ligand Bz_{OH} and its mixed complexes **1** and **2**

Bz_{OH}		$[Cu(Bz_{OH})(Gly)]ClO_4$ (1)		$[Cu(Bz_{OH})(Glu)]ClO_4$ (2)		Tentative assignments
IR	Raman	IR	Raman	IR	Raman	
3450	–	3462	–	3459	3466	ν_{OH}
3442	-	3441	3204	3441	3454	$\nu_{NH\ (BzOH)}$
-			3162		3167	$\nu_{NH\ (NH2)}$
3045	3071	3057	3071	3059	3071	ν_{CH}
-		1402	1411	1392	1401	$\nu_{s(COOH)}$
-		1615	1619	1609	1615	$\nu_{as(COOH)}$
1427	1443	1431	1425	1429	1426	δ_{NH}
1369	1374	1380	1390–	1382	1398	δ_{CH}
1326	1318	1315	1315	1316	1334	ν_{CN}
-	-	1082	1088	1078	1986	δ_{ClO4}
829	817	893	825	892	874	ν_{ring}
–	–	537	569	541	557	ν_{Cu-O}
-	-	428	454	427	482	ν_{Cu-N}

The position of this band remains at nearly the same frequency in the spectra of the metal complexes suggesting the non-coordination of this group [37,38]. The stretching band of □(O-H) showed high shifts in both complexes. This due to their involvement in the coordination to the copper(II) centers. The $\nu_s(COO^-)$ bands shifts towards lower wave numbers while the $\nu_{as}(COO^-)$ frequencies shift towards higher wave numbers compared to the positions of these bands in the IR spectrum of the free amino acid. The Δ values [$\nu_{as}(COO^-)$ - $\nu_s(COO^-)$] are about 213–316 cm^{-1}. The free amino acids exhibit $\nu_s(NH_2)$ around 3120 cm^{-1} [39-42]. The band is shifted to lower frequency region ($\Delta\nu$=20-80 cm^{-1}) in the spectra of the complexes, indicating the involvement of the amino nitrogen in coordination.. This behavior suggests the coordination of the amino acid through the amino and carboxylate groups in a bidentate fashion [43]. Owing to the heavy mass of the central atom and the relatively low order of the coordinated bonds, the corresponding stretching frequency appears in the low frequency region. So the conclusive evidence of the coordination of the mixed ligands with the copper ions was shown by the appearance new bands at ~ 428 and 537-541 cm^{-1} assigned to the ν(Cu—N) [44,45] and ν(Cu—O) [37] vibrations, respectively. These bands were absent in the spectrum of the ligand itself, thus confirming participation of the O and N atoms in the coordination.

Both complexes show strong bands at 1078 and 1082, respectively, supporting the presence of uncoordinated perchlorate ion [46], which was also confirmed by conductivmetry data

3.3. Electronic spectra

The electronic absorption spectra of the present copper(II) complexes **1** and **2** in DMF show a lower energy bands at 14577 cm^{-1} in the case of **1**, 15213 for **2**, 19577 for (**1**), and 19898 cm^{-1} for **2** are assigned to $^2B_{1g} \longrightarrow {}^2B_{2g}$ and $^2B_{1g} \longrightarrow {}^2A_{1g}$ spin allowed transitions respectively [47,48] The band at 27570 for **1** and 28341 cm^{-1} for **2** can be attributed to ligand metal charge transfer transition. Another band observed at high energy region 30322 for **1** and 32411cm^{-1} for **2** is due to the symmetry forbidden charge transfer transition [49]. From these observations, one can conclude that the copper(II) complexes 1 and 2 have a square planar geometry [37, 50,51].

3.4. E. S. R. spectra and magnetic moment studies

The room temperature magnetic moments and details of the polycrystalline e.s.r. spectra of the studied copper(II) complexes are listed in Table 3. The data in Table 3 display that the observed magnetic moments of the reported copper(II) complexes **1** and **2** are 1.87 and 2.05 BM respectively and corresponding to one unpaired electron in a consistence with the mononuclear monomeric structure of these complexes. This result is in accord with the fact that the spin-orbit coupling for copper(II) complexes is positive and the magnetically diluted copper(II) ion should exhibit magnetic moments very close to the spin-only value, as expected for a simple S=1/2 paramagnetic with dx^2-y^2 based ground state [52,53]

Table 3. Magnetic moment values and ESR spectral data of complexes **1** and

Complex	$_{\|}\mathbf{g}$	$_{\perp}\mathbf{g}$	$_{av}\mathbf{g}$	G	μ_{eff} (BM)
1	2.257	2.053	2.123	4.849	1.87
2	2.322	2.073	2.159	4.41	2.05

These normal magnetic moments excluded any significant interaction between neighboring copper(II) ions in the polycrystalline state [52,53]. This finding is further confirmed from the clear resolution of the e.s.r. spectra which give G-values 4.849 and 4.411> 4.0 [54]. The EPR spectra of the synthesized copper(II) complexes were recorded as polycrystalline sample on X-Band at frequency 9.1 GHz under the magnetic field strength 3100 G scan rate 1000 recorded at room temperature. The analysis of spectra give $g_{\|}$ = 2.257 and 2.322 and $g_{\perp}$ = 2.053 and 2.073 for complexes **1** and **2** respectively. In the square planar geometry the unpaired electron lies in the $dx^2 - y^2$ orbital giving $^2B_{1g}$ as the ground state with $g_{\|} > g_{\perp}$. The observed g values of both complexes **1** and **2** are characteristic of a square planar geometry. The trend $g_{\|} > g_{\perp} > g_e$ (2.0023) observed for these complexes show that the unpaired electron is localized in dx^2–y^2 orbital of the copper(II) ions. The g values are related by the

expression: G = ($g_{||}$ - 2)/($g_{\perp}$-2) which measures the exchange interaction between the copper centers in the polycrystalline solid. The G values in the range excluded any exchange interaction between the copper(II) centers for these complexes. However, these spectral features together with the position of the d–d absorption point to a square-planar structure where the equatorial plane is determined by two coordinated nitrogen atoms and two ionic covalent coordinate oxygen donors. Thus, based on of spectral studies the reported complexes were assigned to have four coordinated square planar geometry.

3.5. Electrochemical studies

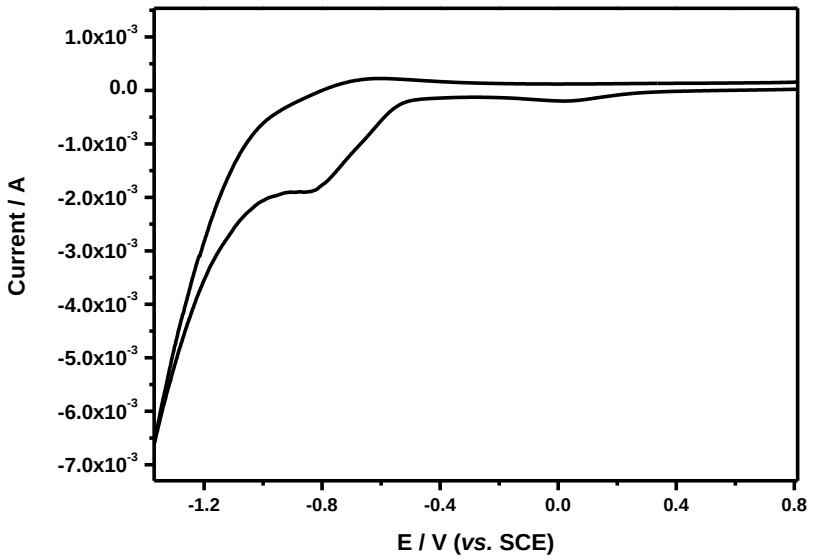


Figure 1. Cyclic voltammogram for complex **1** in BR buffer (pH 7.0) at 50 mV/s.

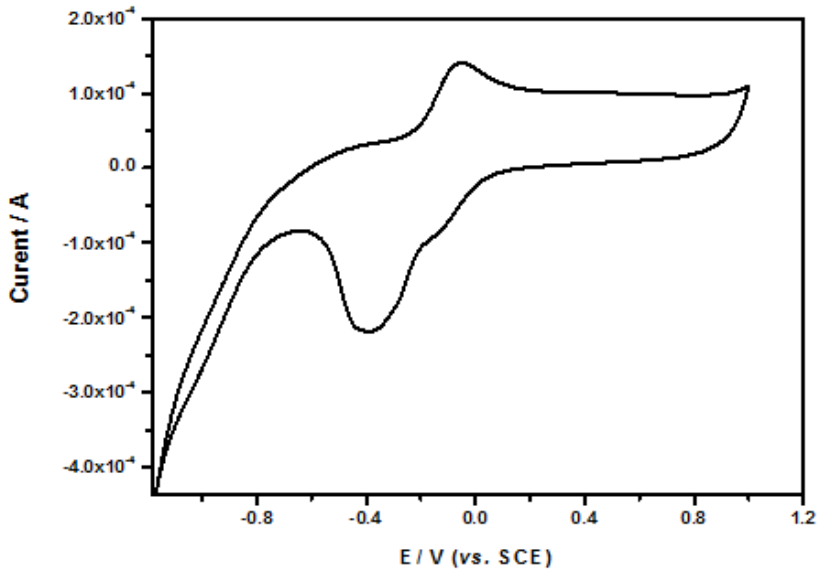


Figure 2. Cyclic voltammogram of copper(II) complex **2** in BR buffer (pH 7.0) at 50 mV/s.

Table 4. The electrochemical data of copper(II) complexes **1** and **2** (scan rate 50 V/s).

Compound	In the absence of DNA				In the presence of DNA			
	E_{pa} (V)	E_{pc} (V)	ΔE^a (V)	$E_{1/2}{}^b$ (V)	E_{pa} (V)	E_{pc} (V)	ΔE (V)	$E_{1/2}$ (V)
(1)	-0.69	-0.83	0.14	-0.76	-0.63	-0.85	0.22	-0.535
(2)	-0.37	-0.05	0.32	-0.105	-0.4	-0.02	0.38	-0.21

[a]$\Delta E = E_{pa} - E_{pc}$ and [b]$E_{1/2} = 1/2\ (E_{pa} + E_{pc})$.

The electrochemical properties of copper(II) complexes **1** and **2** were studied by cyclic voltammetry in DMSO. The voltammogram of complex **1** (Figure 1) showed a well defined redox process corresponding to the formation of Cu(II)/Cu(I) at -0.83 V and -0.69 V (*vs*. SCE) for the cathodic and anodic peak currents, respectively. The peak separation potential $\Delta Ep = (Epa - Epc)$, between the anodic and cathodic peaks is 140 mV (Table 2). This indicates a quasi-reversible redox process assignable to Cu(II)/Cu(I) couple [55] and the cathodic to anodic peak currents ($Ip_a/Ip_c \sim 1$) corresponding to a simple one electron process. The electrochemical behavior of copper(II) complex **2** was also studied and showed a well defined redox couple at -0.39 V and -0.05 V for the cathodic and anodic peak current, respectively (Figure 2). The peak separation potential $\Delta E = (Epa - Epc)$, between the anodic and cathodic peaks is 320 mV (Table 4). This large peak separation potential suggests that reaction is quasi-reversible [52] behavior with a slow electron transfer.

3.6. Electrochemical oxidation of catechol to o-quinone

The electrochemical behavior of the prepared electrodes was examined in a potential range from -1.0 to +1.3 V (vs. SCE) using Britton-Robinson buffer at pH 7 using $1x10^{-3}$ M catechol. Figure 3 showed the cyclic voltammogram for 1×10^{-3} M of catechol at a bare carbon paste electrode (CPE) and carbon paste electrode modified by complex **1**. In a bare CPE a redox couple was observed with anodic and cathodic peak at about +0.49 and -0.07 V (vs. SCE), respectively. The redox peaks due to the oxidation and reduction of Cu(I)/Cu(II) couple [56,57]. At carbon paste electrode modified by complex **1** a well defined redox system was appeared in the presence of 1×10^{-3} M catechol. The oxidation peak appeared at + 0.67 V and the reduction peak appeared at -0.13 V (vs. SCE) with a much higher peak current value. A shift in both cathodic and anodic peaks was observed, the anodic peak shifted to more positive value, while the cathodic peak shifted to more negative value and this may be due to the interaction with catechol [56]. This means that the electrode containing copper(II) complex **1** which acts as a biomimetic enzyme presents electro-catalytic activity to the oxidation of catechol to *o*-quinone. The increase of current peaks indicates that a higher amount of complex **1** was oxidized and reduced. The shift in potentials suggest a diffusion controlled redox process. The effect of potential scan rate on the peak current of catechol at a carbon paste electrode modified by complex **1** was studied. By increasing the scan rate values from 25 to 100 mV/s, both the oxidation and reduction peak currents increased (Figure 4). By plotting the relation between the anodic and cathodic peak currents

with the square root of scan rate values in the range of 25 to 100 mVs^{-1}, (Figure 5), a linear relation was observed, this indicated that the electrode reaction was diffusion controlled process [58].

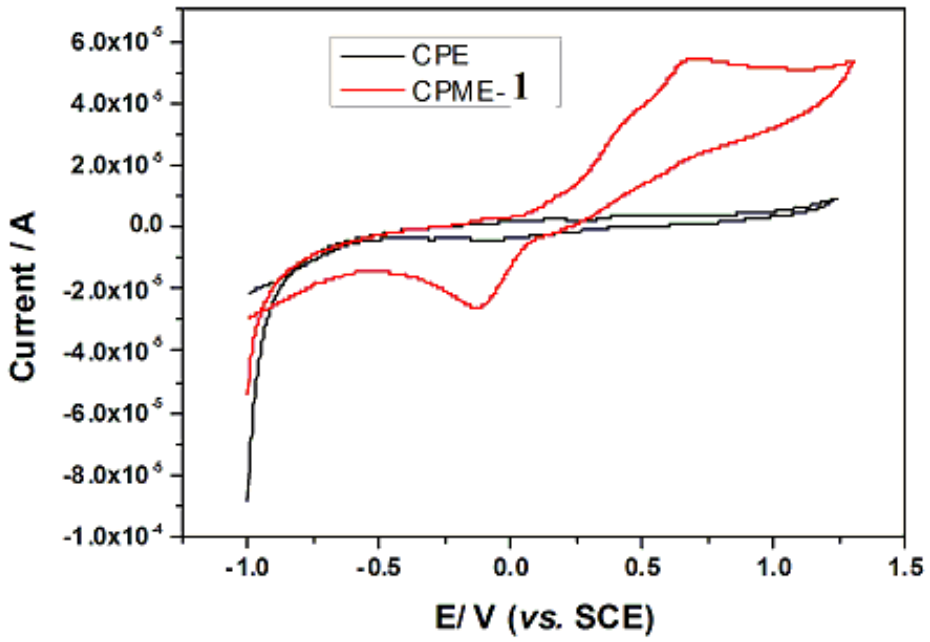


Figure 3. Cyclic voltammetry for $1x10^{-3}$ M catechol at CPE modified by copper(II) complex **1**

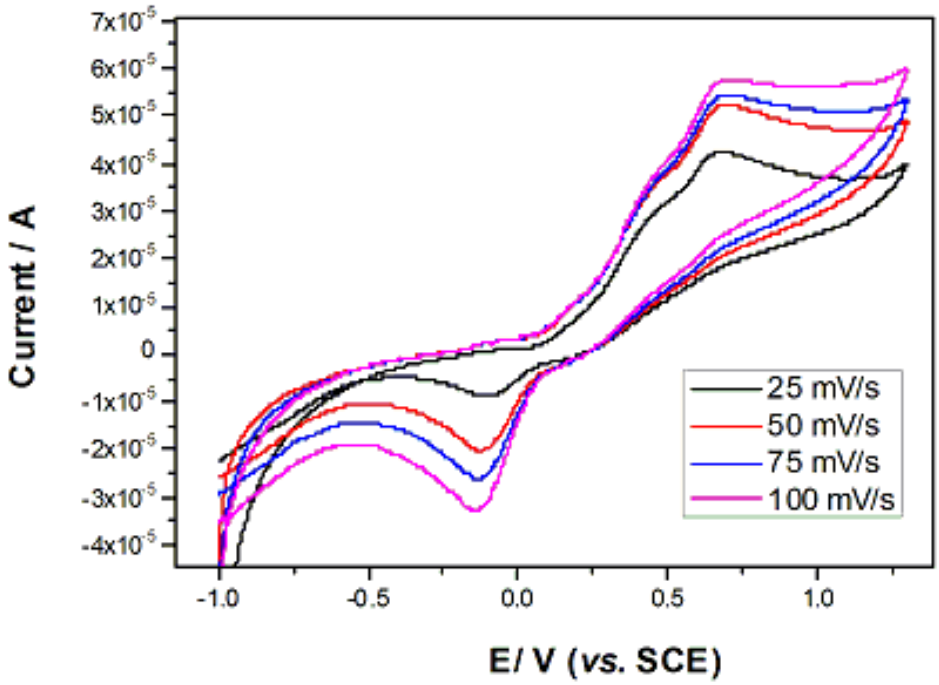


Figure 4. Effect of scan rate on the peak current of catechol

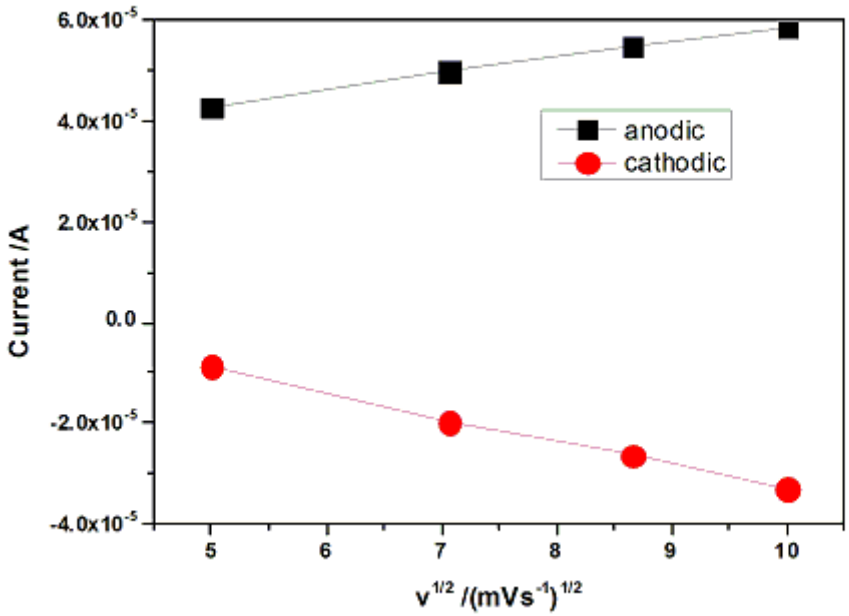


Figure 5. The peak current vs. the square root of scan rate.

At the carbon paste electrode modified by copper(II) complex **1** by increasing the scan rate values, both the oxidation and reduction peaks increased. The peak currents for anodic and cathodic peaks in presence of carbon paste electrode modified by complex **1** are much higher than that in a bare carbon paste electrode.

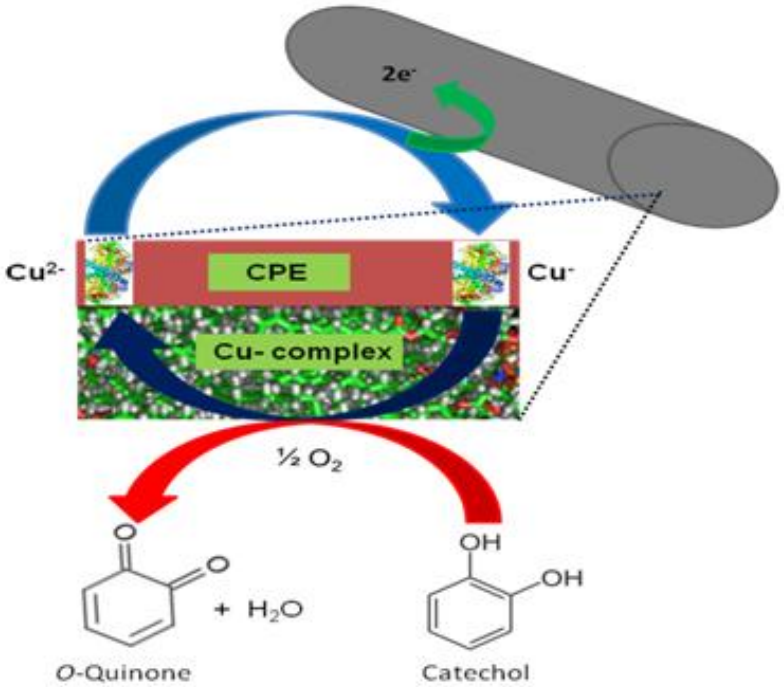


Figure 6. The oxidation of catechol catalyzed by Cu- complexes mimic catechol oxidase

The increasing in both anodic and cathodic peak current due the presence of complex **1** as a mimic of catecholase which acts as a redox mediator or electron shuttle which catalysis oxidation and reduction of catechol at the surface of carbon paste modified electrode [59-61]. The mechanism of catalytic reactions can be illustrated in Figure 6.

3.7. Catalase like activities

The catalytic activity studies of copper(II) complexes **1** and **2** in DMSO towards the disproportionation of hydrogen peroxide were also performed. Experiments were repeated several times to ensure consistency of the results. The studies showed that, only complex **1** is catalytically active. Where at a concentration of 10.0 mM, the activity was equivalent to 875.40 U/L (Figure 7), at 8.0 mM, the activity was 703.88 U/L, at 6.0 mM, the activity was 590.61 U/L, at 4.0 mM, the activity was 512.9 U/L, at 2.0 mM, the activity was 420.7 U/L, and at 1.0 mM, the activity was 181.22 U/L. While at 0.5 mM, there is no significant difference with DMSO (were 58.25 U/L and 42.06 U/L respectively). These results showed that complex **1** has high catalase activity with IC_{50} = 2.0 mM. Since the benzimidazole ring has strong pi donating ability. It should be noted that heterocyclic base alone could not catalyze the disproportionation of H_2O_2. When the coordinate copper(II) ion is present in structure, the catalytic reactivity greatly enhances. Because, in the catalytic process the electron transfer.

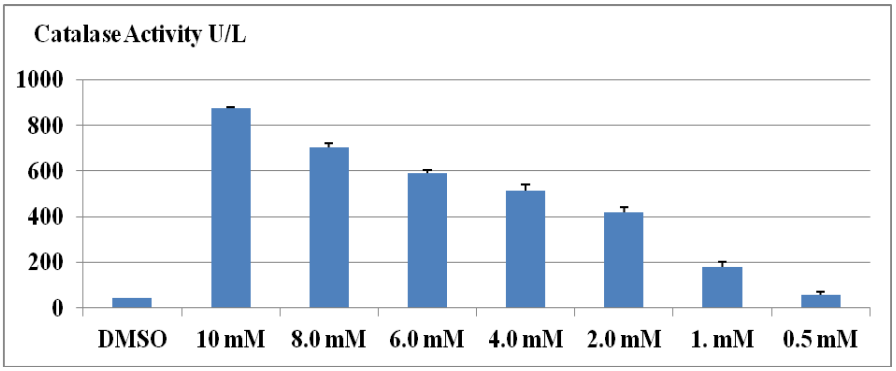


Figure 7. Catalase activity of complex **1**. DMSO was used as a negative control. The data were represented as Catalse activity U/L induced by the complex at a concentration of 10.0, 8.0 and 6.0., 4.0 mM, 2.0 mM, 1.0 mM and 0.5 mM respectively.

3.8. Nuclease like-activity

The nuclease-like activity of copper(II) complex **1** was investigated was investigated under aerobic conditions at room temperature using bacterial plasmid and in the absence of any external additives. The experimental observations demonstrated that complex **1** has promising ability toward the cleavage of the plasmid DNA.

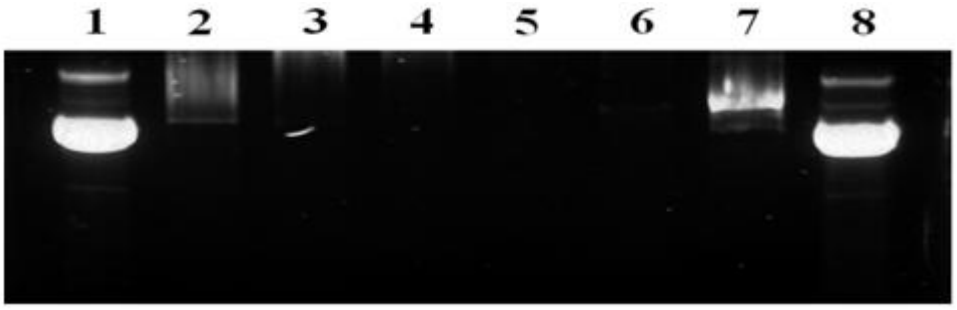


Figure 8. Assay of DNase mimic activity of copper(II) complex **1** after incubation for one hour at 37 C°: (1) is plasmid with buffer and DMSO, (2) is plasmid with buffer and complex **1** at a concentration of 10 mM, (3) is plasmid with buffer and complex **1** at a concentration of 8 mM, (4) is plasmid with buffer and complex **1** at a concentration of 6 mM, (5) is plasmid with buffer and complex **1** at a concentration of 4 mM, (6) is plasmid with buffer and complex **1** at a concentration of 2.0 mM, (7) is plasmid with buffer and complex **1** at a concentration of 1.0 mM, (8) is plasmid with buffer and complex **1** at a concentration of 0.5 mM.

The nuclease-like activity was correlated with the concentration of the examined complex. The obtained results (Figure 8) showed that the examined complex at concentrations of 1.0-10.0 mM are capable to cleavage DNA, while at at concentration of 0.5 mM was failed to cleavage DNA and was similar to that of DMSO alone.

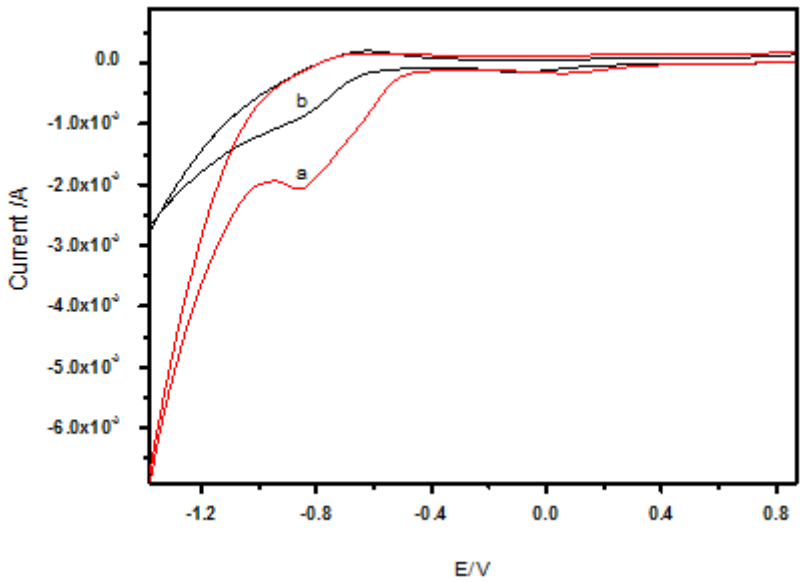


Figure 9. Cyclic voltammograms o copper(II) complex **1** (a) in the absence (solid line) and (b) in presence (dashed line) of DNA in BR buffer (pH 7.0) at 50 mV/s.

The cyclic voltammetry was also used to study the interaction of copper(II) complex **1** with bacterial plasmid DNA due the similarity between various redox chemical and biological processes [62]. A strong DNA binding usually results in a decrease in current due to the diffusion of the complex-adduct formed. Upon the addition of DNA to copper(II) complex **1**, the cathodic and anodic

peak currents were decreased (Figure 9). Where the cathodic and anodic peak potentials were shifted to more positive value in the anodic peak and to more negative value for the cathodic peak (Table 4). The decrease in peak currents can be attributed to the diffusion of the copper(II) complex **1** bound to the large, slowly diffusing DNA molecule [63] and the resulted peak current due the equilibrium of free and DNA-bound complex **1** to the electrode surface. Moreover, the obvious positive shifts of peak potentials also indicate that this interaction mode may be intercalation between copper(II) complex **1** and DNA [64]. This shift in potentials and decrease in current suggest that both complexes binds to DNA. Due to the labile coordination sphere, copper(II) complexes have attracted special attention as endonuclease mimics. Different mechanisms for DNA cleavage have been assumed including hydrolytic or oxidative pathways. Hydrolytic cleavage directly breaks the phosphodiester bond but does not result in sugar damage [65,66]. The phosphatodiester backbone of DNA is very stable and resistive to hydrolytic cleavage. It has been shown that copper(II) complexes of linear or macrocyclic polyamines, aminoglycosides and histidine show good nuclease activity [67]. Copper(II) complex **1** exhibits appreciate nuclease activity in the absence of any added external reductant. This is a valuable feature for an application as chemotherapeutic agents in anticancer treatments.

4. CONCLUSION

Herein, we reported the synthesis and characterization of two new mixed ligand complexes of copper(II) with amino acids such as glycine or L-glutamine as primary ligands and 2-hydroxymethylbenzimidazole as a secondary ligand. The IR, Raman, electronic, and ESR spectra of the complexes $[Cu(Bz_{OH})(Gly)]ClO_4$ **1** and $[Cu(Bz_{OH})(Glu)]ClO_4$ **2**, could be recorded and adequately interpreted on the basis of its peculiar structural characteristics. A squar–pyramidal geometry is proposed for both complexes Complex **1** shows an important superoxide dismutase and catalase-like activity suggesting that this type of complexes may constitute a new and interesting basis for the future search of new and more potent SOD-mimetic drugs. The interactions between copper(II) complex **1** and bacterial plasmid DNA had also been investigated using gel electrophoresis assay and cyclic voltammetry

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