Research Article

Synthesis, Spectroscopy, Thermal Analysis, Electrochemistry and Superoxide Scavenging Activity of a New Bimetallic Copper(II) Complex

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A new bimetallic copper(II) complex has been synthesized with ligand obtained by the condensation of salicylaldehyde and the amine derived from reduction of nitration product of benzil. The ligand was characterized by ^1H NMR and mass spectra, and the binuclear Copper(II) complex was characterized by vibrational and electronic spectra, EPR spectra, and magnetic moment measurement. Thermogravimetric analysis study and electrochemical study of the complex were also done. The complex was found to show superoxide dismutase activity.

1. Introduction

Copper is a biologically important metal found in a number of enzymes such as—superoxide dismutase, tyrosinase, B-hydroxylases, monoaminoxidase, and ascorbic acid oxidase [1–3]. Complexes of copper in oxidation state +2 were found to show significant antioxidant and anti-free radical activity also [4, 5]. Bimetallic copper complexes are potential models for a number of important biological systems containing couple sites [6] and have been studied extensively [7–12]. Bimetallic complexes of copper with Schiff bases have been shown to be useful chemical probe of DNA and have found its importance in various biochemical and biomedical applications [13, 14]. Synthesis of a new copper(II) complex with multidentate Schiff base ligand also contributes in the development of coordination chemistry [15–17].

Superoxide dismutase (SOD) is an enzyme which protects cells from the toxic effect of superoxide [18]. The three main types of SOD are—Cu-Zn-SOD, Mn-SOD, and Fe-SOD of which the first one is found in mammals [19]. Deficiency or imbalance of SOD in human body leads to many diseases and disorders such as diabetes, ischemia, cataract, Parkinson's disease, and cancers [20, 21]. Such disorders could be treated

by supplementation of antioxidant enzymes but administration of these enzymes through oral or intraperitoneal routes is severely restricted due to their rapid degradation and short lifetime in biological systems [22]. Small metal complexes having good superoxide scavenging activity should be good candidate in this respect.

A good number of copper complexes have been reported to mimic SOD activity which includes copper(II) complexes with Schiff base ligands derived from various aldehydes and ketones [23], imidazole bridged copper(II) complexes [24], planar copper(II) complex on addition of a base such as N-methyl imidazole or pyridine [25], curcumin complexes of copper(II) [26], and so forth. It is also reported that the copper(II) complexes with Schiff base ligands of salicylaldehyde semicarbazone have SOD activity which could be tuned by heterocyclic bases pyridine and N-methyl imidazole [27]. There are recent reports on copper complexes with SOD activity having pyridine and pyrimidine derivatives [28–30].

In this paper, we report the synthesis of a new ligand by the condensation of salicylaldehyde and the amine derived from reduction of nitration product of benzil. The ligand was characterized by ^1H NMR and mass spectra. Binuclear copper (II) complex of the ligand was synthesized, and its FTIR

spectra, electronic spectra, EPR spectra, thermogravimetric (TGA) analysis study, and electrochemical study results are reported.

2. Experimental

All the chemicals and solvents used for the synthesis are reagent grade. Benzil, ethylenediamine, and salicylaldehyde were purchased from Merck. The FTIR spectra were recorded using KBr discs on a Perkin-Elmer spectrum RXI FTIR system. The NMR spectra were recorded in Bruker Ultra shield 300 spectrophotometer. The electronic spectra in the range 200–1000 nm were obtained in acetonitrile on a UV-1800 SHIMADZU spectrophotometer. Thermogravimetric measurements were carried out on a METTLER TOLEDO TGA/DSC instrument. Magnetic moment measurements were recorded at room temperature by the Gouys method using Cambridge Magnetic Balance. CHI 600B Electrochemical Analyzer (USA) with a three electrode cell assembly was used for electrochemical studies. The electrodes were cleaned as per reported procedure [31]. The SOD activity of the copper complex has been studied by the method of NBT reduction using $\text{KO}_2^{\bullet}$ as the source of superoxide radical [32].

2.1. Synthesis and Characterization of Schiff Base Ligand (L). 1 g benzil was taken in 10 mL of 1:1 conc. HNO_3 : H_2SO_4 mixture and refluxed for 6 hours. A yellow product was obtained which was filtered and washed many times with distilled water. After drying, 0.5 g of the product was dissolved in 10 mL methanol. A freshly prepared solution of SnCl_2 was added to this solution drop wise till the color became dark brown. The solvent was evaporated and the product was washed many times with distilled water and dried. The product was then dissolved in methanol and made basic by adding NaOCH_3 . Salicylaldehyde was added dropwise under stirring till the dark brown color of the solution became light brown. The solvent was evaporated under vacuum and product was washed many times with distilled water and dried. The product was further washed with n-hexane to remove any unreacted aldehyde present. The synthetic path for the ligand has been shown in Scheme 1.

Yield: 65%. Elemental analysis: C 73.72% (calc. 73.46), H 4.52 (calc. 4.40), N 8.35% (calc. 8.16). ESI-MS m/z (rel. int. %) 687 (M^+). FTIR (KBr pallet, cm^{-1}): 2852 ($\nu_{\text{C-H}}$ of C_6H_5); 924.5 cm^{-1} , 880 cm^{-1} , 841 cm^{-1} , 817 cm^{-1} , 765 cm^{-1} (C-H out of plan vibration for C_6H_5); 1352 ($\nu_{\text{C-O}}$); 1680 ($\nu_{\text{C=O}}$); 1597 ($\nu_{\text{C=N}}$); 1381 ($\nu_{\text{C-N}}$); 3430 ($\nu_{\text{O-H}}$ alcoholic). ^1H NMR (300 MHz) CDCl_3 : 8.876 (s, HC=N), 8.393 (d, Ar-H), 8.418 (s, HC=N), 7.819 (d, Ar-H), 7.587 (m, Ar-H), 7.543 (m, Ar-H), 7.31 (m, Ar-H), 4.823 (s, $-\text{OH}$) (Figure 1).

2.2. Synthesis of the Complex. 0.1 mol of L was dissolved in 10 mL of methanol, and 0.2 mol of Cu-acetate was added in small portions with continuous stirring followed by further stirring for 3 hours. Dark green product was obtained which was filtered and washed with distilled water. The compound was recrystallized from CH_3CN .

FIGURE 1: ^1H NMR spectra of L in CDCl_3 .

FIGURE 2: UV/Visible spectra of $\text{Cu(II)}_2 \cdot \text{L} \cdot 2\text{H}_2\text{O}$ in DMSO.

3. Results and Discussion

3.1. Characterisation of the Bimetallic Copper(II) Complex

3.1.1. UV/Visible and FTIR Spectroscopy. The uv/visible spectra of L showed two well-defined peaks at 213 nm and 323 nm, due to $\pi - \pi^*$ and $n - \pi^*$, respectively, together with a shoulder at 400 nm. Figure 2 shows the electronic spectra of the copper(II) complex in DMSO. When L formed complex with Cu(II), the peaks at 213 nm and 323 nm disappeared and the shoulder became a well-defined peak at 380 nm. Binding to Cu(II) ion redistributed the electron densities of L and hence the first two peaks disappeared. The 380 nm peak should be due to ligand to metal charge transfer. Another peak at 700 nm was observed due to $d \rightarrow d$ transition originating at Cu(II).

SCHEME 1

FTIR spectra for the complex synthesized showed peaks at 2861 cm^{-1} ($\nu_{\text{C-H}}$ of C_6H_5); 772.8 cm^{-1} , 718.9 cm^{-1} (C-H out of plan vibration for C_6H_5); 1616.5 cm^{-1} ($\nu_{\text{C=O}}$); 1538.1 cm^{-1} ($\nu_{\text{C=N}}$); 1391 cm^{-1} ($\nu_{\text{C-N}}$); 1349.3 cm^{-1} ($\nu_{\text{C-N}}$); 3430.9 cm^{-1} ($\nu_{\text{O-H}}$ coordinated H_2O). The stretching frequency due to $\nu_{\text{C=O}}$ has decreased to 1616.5 cm^{-1} in the complex from 1680 cm^{-1} in the ligand. This indicates possibility of formation of H bonding between coordinated water molecule and the carbonyl groups.

3.1.2. Magnetic Moment Measurements. The magnetic moment value was measured to be 2.199 BM which was much higher than the single electron value of 1.74 BM. This magnetic moment value can be explained by considering that the complex formed is a bimetallic Cu(II) one. Because of exchanged couple phenomena, the observed magnetic moment is smaller than the total magnetic moment due to two single Cu(II) ions, that is, $1.74\text{ BM} \times 2 = 3.48\text{ BM}$.

3.1.3. EPR Studies of the Complex. The X-band EPR spectra of the complex was recorded as the polycrystalline samples at room temperature (Figure 3). The g_{iso} value and geometric parameter G , that is, the measurement of exchange interaction between the copper centers were evaluated by using the following expression [31]:

$$g_{\text{iso}} = \frac{(g_{\parallel} + 2g_{\perp})}{3}, \quad (1)$$

$$G = \frac{(g_{\parallel} - 2.0023)}{(g_{\perp} - 2.0023)} = \frac{4K_{\parallel}^2 \Delta E_{xz}}{k_{\perp}^2 \Delta E_{xy}}. \quad (2)$$

The calculated value of g tensor parameter was $g_{\parallel} = 2.24$ and $g_{\perp} = 2.11$. Hence $g_{\parallel} > g_{\perp} > 2.0023$ which reveals that $d_{x^2-y^2}$ is the ground state [30]. The value of G was calculated to be 2.207 which means G is less than 4 indicating effective interaction between the copper centers [32].

3.1.4. Thermogravimetric Analysis. Thermogravimetric (TG) weight loss curves and the corresponding differential thermogravimetric (DTG) curves for the complex are shown in Figure 4. The complex showed two well-defined steps at 160°C and 290°C together with another steps at 350°C . The loss in weight in the first step is 4.02% which should be due to the two co-ordinated water molecules (calculated weight loss was 4.28%). The second, and third weight losses are 65.95% and 6.58%, respectively, totaling 72.53%. This large weight drop can be explained by considering that the residue is a 1 : 1 mixture of Cu_2O and CuO (calculated weight loss 72.51%).

Based on various spectroscopic and magnetic studies together with the TG analysis results, the structure of the complex has been confirmed to the one shown in Scheme 2.

3.1.5. Electrochemical Study of the Complex. Figure 5 shows the cyclic voltammogram of $\text{Cu(II)} \cdot \text{L} \cdot 2\text{H}_2\text{O}$ in CH_3CN using platinum disc as working electrode and Ag-AgCl as the reference. The cyclic voltammetric profile is of quasi reversible one with the redox potential value $+0.105\text{ V} \pm 0.005\text{ V}$ with peak potential difference $\Delta E = 0.228\text{ V}$. This redox potential is due to $\text{Cu(II)}/\text{Cu(I)}$ redox couple. The ratio of cathodic to anodic current is found to be 0.949. The redox potential value was further confirmed by square wave voltammogram (not shown). Observation of only one redox couple in the cyclic voltammogram or square wave voltammogram confirms that both the Cu(II) ions are in identical coordination environment.

The plot of cathodic and anodic currents versus square root of scan rate was found to be linear. This linearity in redox currents against square root of scan rate indicates the redox process is diffusion controlled. Double potential step chronocoulometry was done for the complex in DMSO. The plot of charge (Q) versus square root of time ($t^{1/2}$) was gradual, and no sharp decrease was observed. This confirms

FIGURE 3: X-band EPR spectra of $\text{Cu(II)}_2\cdot\text{L}\cdot 2\text{H}_2\text{O}$ in solid state.

FIGURE 4: TGA curve of $\text{Cu(II)}_2\cdot\text{L}\cdot 2\text{H}_2\text{O}$.

that the complex was not adsorbed onto the electrode surface, and the redox process is diffusion controlled.

3.1.6. Superoxide Dismutase (SOD) Activity of the Complex. The SOD activity of the copper complex has been studied by the method of NBT reduction using $\text{KO}_2^{\cdot-}$ as the source of superoxide radical [32]. The blue color developed due to the formation of dye was measured immediately at 560 nm against an appropriate blank. One unit of SOD activity (IC_{50} value) was defined as the test substance required for 50% inhibition of NBT reduction by the superoxide anion [27]. A linear relation was obtained between the concentration of the copper complex and the inhibition of the superoxide ion. The 100% of superoxide activity corresponds to an assay performed in the absence of complex. In order to determine the concentration of the complex required to yield 50% inhibition of the reaction, we plotted the percentage of inhibition against the metal complex concentration. A linear relation was obtained between the concentration of the copper complex and the inhibition of the superoxide ion and from this plot IC_{50} value could be determined. The obtained value was 0.150 mM which was lower than the IC_{50} value reported [25, 27]. The IC_{50} value of the native enzyme is 0.72 μM .

FIGURE 5: Cyclic voltammogram of $\text{Cu(II)}_2\cdot\text{L}\cdot 2\text{H}_2\text{O}$ in DMSO. Working electrode: Pt disc, reference electrode: Ag-AgCl, supporting electrolyte: TBAP.

SCHEME 2

4. Conclusions

A new Schiff base ligand system obtained from benzil by firstly introducing nitro groups which were reduced to amine and secondly allowing condensation between the amine and salicylaldehyde has been synthesized and characterized. The ligand binds to two Cu(II) ions giving a new bimetallic Cu(II) complex which shows efficient superoxide dismutase activity.

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