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Journal of Chemical and Pharmaceutical Research, 2015, 7(2):102-108

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, characterization, DFT/TDDFT calculation and superoxide dismutase activity of copper(II) complex with ligand derived from benzil and cysteine

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ABSTRACT

*The condensation product of benzil and amino acid cystein has been isolated and used to form mononuclear complex with Copper(II) (Cu(II)L). The ligand (**L**) has been characterised by – UV/visible, FT-IR, ^{1}H and ^{13}C NMR, ESI Mass spectroscopy etc. The Cu(II)L complex has been characterised by various spectroscopic techniques - UV/visible, FT-IR, ESI Mass, EPR and Thermo Gravimetric Analysis. The structure of Cu(II)L has been optimised by DFT calculation. TDDFT calculation has been performed to predict UV/Visible spectrum of Cu(II)L which is identical to the experimental one. Cyclic voltammogram showed quasi reversible behaviour with redox potential value -0.680 V at Platinum working electrode with Ag-AgCl (1 M NaCl) as reference electrode. Cu(II)L showed superoxide dismutase activity with IC_{50} value $7.8x10^{-6}$ M.*

Key words: Benzil, Cysteine, Copper(II), IC_{50}, DFT, TDDFT

INTRODUCTION

Schiff bases prepared by condensing carbonyl compounds and amines with the elimination of water, is an important class of ligands for coordination complexes. Schiff bases have been used for the synthesis of a large variety of metal complexes including copper having applications in different fields such as – structural elucidation, bioinorganic, catalysis, luminescence etc. [1-9]. The synthesis of mononuclear copper(II) Schiff base complexes are of interest because of their magnetic properties [10-12], catalytic activity [13], chemical structure [11,14-16], DFT calculation [17,18] etc.. Different amino acids or their derivatives such as glycine [19-22], phenylglycine [23], valine [24], tyrosine [25], alanine [26], histidine [27] etc. have been used to synthesise Schiff base ligands for metal complexes. Schiff base ligands based on cysteine and benzil has been recently reported for in situ synthesis of oxovanadium(IV) complex [28].

Superoxide dismutase (SOD) is an enzyme involved in protecting biological cells from the toxic effects of superoxides [29]. Based on the metal ions present in the active sites, SODs have been divided into – Cu-Zn-SOD, Mn-SOD and Fe-SOD out of which the first one is found in mammals [30]. Deficient level of SOD concentration in human body is one of the reasons behind diseases and disorders like - diabetes, ischemia, cataract, Parkinson's disease, cancers etc [31,32]. Supplementation of antioxidant enzymes should be a part of the treatment but administration of these enzymes through oral or intraperitonial routes is severely restricted due to their rapid degradation and short life time in biological systems [33]. Small metal complexes having good superoxide scavenging activity are potential candidate in this regard.

Wide range of applicability of DFT methods to obtain the structures of molecular complexes is well documented. Moreover, TDDFT methods are successful in explaining the electronic properties of complexes [34]. Herein we

have used DFT method to obtain the structure of the Cu^{2+} ions-ligand complex. The TDDFT method is employed to obtain the electronic spectra (UV-Visible) of the complexes.

In this paper, we report the synthesis and characterisation of the Schiff's base ligand (**L**), derived by condensation of benzil with amino acid cysteine, and its Cu(II) complex. The complex has been characterised by various spectroscopic techniques and its superoxide dismutase activity is also reported.

EXPERIMENTAL SECTION

Chemicals and Instrumentation

All the chemicals were purchased from Loba Chemie. The UV/Visible spectra were recorded on a UV-1800 SHIMADZU spectrophotometer. FTIR spectra were recorded using KBr pallet (4000-400 cm^{-1}) on a PERKIN ELMER spectrum RXI FTIR system. ^{1}H NMR spectra were recorded in a Bruker Ultrashield 300 MHz spectrometer using D_2O as solvent. EPR spectra were recorded in Bruker EMX spectrophotometer (centre field 4000.000 G, sweep width 8000.000 G, Resolution 1024 points, Microwave frequency 9.877 G Hz, Power 0.188 mW). Thermo gravimetric analysis (TGA) studies were done on PERKIN ELMER TGA 4000 analyser. Magnetic susceptibility measurements were done at room temperature by the Gouy method using Cambridge Magnetic Balance. CHI 600B Electrochemical Analyzer with a three electrode cell assembly was used for electrochemical studies. Electrochemical experiments were carried out under a blanket of Nitrogen gas after passing the gas through the solution for 10 minutes. The reference electrode was Ag/AgCl (3 M NaCl) and $NaNO_3$ (0.1 M) was the supporting electrolyte. In the Square Wave Voltammetry (SWV) experiments, the square wave amplitude was 25 mV, the frequency was 15 Hz and the potential height for base stair case wave front was 4 mV. The working electrode was cleaned as reported[35].

Synthesis and characterization of Ligand (L, $C_{20}H_{16}N_2O_4S_2$)

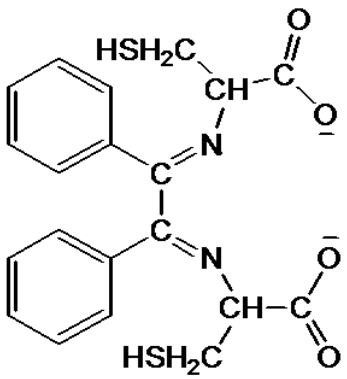
The ligand has been reported for in situ formation of complexes with V=O [28], but was not reported as isolated product. We have prepared and isolated the ligand for the first time. 2.1 g (0.01 mol) benzil and 2.42 g (0.02 mol) cysteine were taken in 20 mL 1:2 (v/v) CH_3OH:H_2O. 5 drops of 30% NaOH was added and stirred well. The mixture was refluxed for 1 hour. Brown coloured product was obtained which was filtered, washed with water and dried. Yield: 60%.

Elemental analysis: C = 57.50%, N = 6.69%, H = 4.36%, S = 15.33%. The calculated values are C = 57.55, N = 6.71%, H = 4.31% and S =15.34%. The theoretical calculation is based on the formula $C_{20}H_{16}N_2O_4S_2$ based on the structure shown in Scheme 1.

FT-IR analysis (KBr, cm^{-1}): 3400 (ν_{C-H}), 2500 (ν_{S-H}), 1400 ($\nu_{COO, symm}$), 1586 ($\nu_{C=N}$), 1602 ($\nu_{C=O}$).

ESI-MS analysis: Molecular ion peak was observed at m/z 415.66, calculated molecular mass of **L** is 414.504.

^{1}HNMR analysis (DMSO-d_6, ppm): 7.264 to 7.546 (Ar-H); 2.18 (-CH_2-S-); 1.65 (–SH). ^{13}C NMR: 173.55 (C=O); 126.91 to 141.36 (C_6H_5); 76.57 and 77.43 (C-N-), 47.03 and 48.17 (-C-S-).



Scheme 1: Structure of L

Synthesis of Cu^{2+} complex of L

0.250 g of $CuSO_4.5H_2O$ (0.001 mol) and 0.414 g (0.001 mol) of **L** were dissolved in 20 mL 1:1 (v/v) MeOH:H_2O and stirred for 1 hour at room temperature. Blue coloured precipitate was obtained which was filtered, washed with water and dried.

RESULTS AND DISCUSSION

Characterisation of Copper(II) complex (Cu(II)L)

Elemental analysis of Cu(II)L

The percentage of C, H and N were found to be: C = 50.7% (50.2%); H = 3.30% (3.37); N = 5.95% (5.86%); S = 13.48% (13.39%); Cu =13.33% (13.28%). The values within brackets are theoretically calculated values based on 1:1 composition between Cu(II) and **L**. The good agreement between the experimental values and the theoretically calculated ones supports the complex as Cu(II)L.

Electronic and FT-IR spectra of Cu(II)L

UV/Visible spectra of Cu(II)L (10^{-3} M) was recorded in CH_3OH. Absorption peak at λ_{max} value 630 nm was observed (Figure 1). This symmetric absorption peak is characteristic for square planar Cu(II) complex due to d_z2 to d_x2-$_y2$ transition[36].

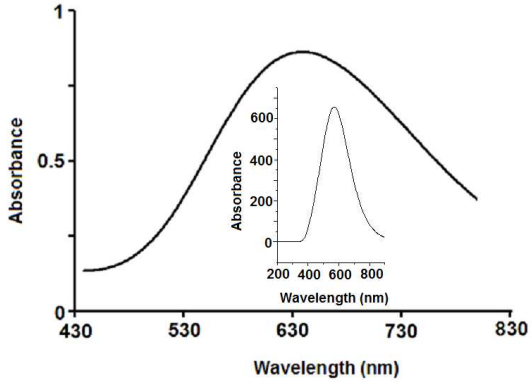


Fig. 1: UV/Visible spectrum of Cu(II)L in CH_3OH. Inset: TDDFT predicted UV/Visible spectrum of the Cu(II)L complex in CH_3OH

FTIR spectra of Cu(II)L$(H_2O)_2.2H_2O$ was recorded in KBr pallet. Peaks were observed at 721.2 cm^{-1}, 750.31 cm^{-1} due to ν_{C-H} out of plane vibration of C_6H_5; 400.32 cm^{-1} due to ν_{C-N}; 1602.85 cm^{-1} due to $\nu_{C=O}$ and 2852.72 cm^{-1} due to ν_{C-H} of C_6H_5.

ESI-Mass spectroscopic analysis of Cu(II)L

The ESIMS spectra of the synthesised copper complex showed M/z peak at 479.19 which corresponds to the formula Cu(II)L$(H_2O)_2.2H_2O$. The calculated molecular mass based on this formula is 478.05.

EPR spectral analysis of Cu(II)L

Figure 2 shows the X–band EPR spectrum of the complex as polycrystalline sample at room temperature. The calculated value of g tensor parameter was $g_{\parallel} = 2.26$ and $g_{\perp} = 2.09$. Hence $g_{\parallel} > g_{\perp} > 2.003$ which reveals that the unpaired electron of Cu(II) belongs to $d_{x^2-y^2}$[32]. The g_{iso} value was calculated to be 2.144.

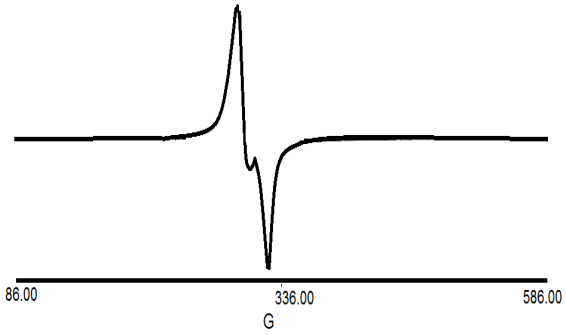


Fig. 2: The X–band EPR spectra of Cu(II)L recorded as the polycrystalline samples at room temperature

Thermo gravimetric analysis (TGA) of Cu(II)L

The complex was subjected to thermo gravimetric analysis and only one weight loss has been observed from *ca.* 250 ^oC to *ca.* 500 ^oC. Figure 3 shows the TGA curve for the Cu(II)L complex and the total weight loss is found to be %. The theoretically calculated complete weight loss of the ligand with the formation of CuO is %. Hence, the TGA experiment confirms the formation of the Cu(II)L complex.

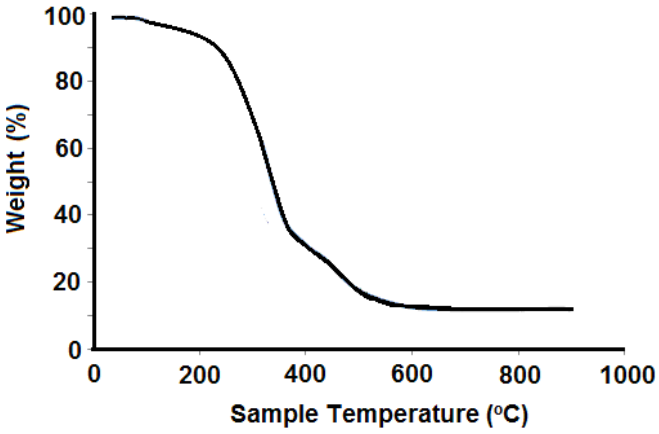


Fig. 3: TGA curve of Cu(II)L

Cyclic voltametric studies of Cu(II)L

Cyclic voltammogram of Cu(II)L was recorded in CH_3CN using Platinum as working electrode and Ag-AgCl (1 M NaCl) as the reference with scan rate 0.100 Vs^{-1} (Fig. 4). A quasi reversible cyclic voltammogram was observed with redox potential value -0.680 V. The separation in peak potential value is found to be 0.430 V.

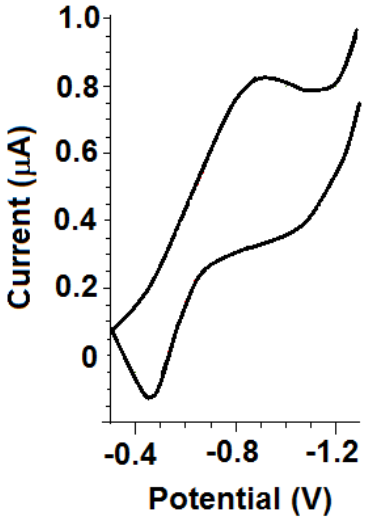


Fig. 4: Cyclic voltammogram of Cu(II)L in CH_3CN. Working electrode Pt disc, Reference electrode Ag-AgCl (1 M NaCl) and supporting electrolyte 0.1 M $NaNO_3$

DFT structure optimisation and TDDFT calculation for UV/visible spectral assignment of Cu(II)L

In present day density functional theory (DFT) has become an effective tool for determining structure, electronic properties of molecules, vibrational frequencies, atomization energies, ionization energies etc. [38-41]. Since we could not develop the crystal for X-ray analysis, we analyzed the complex formation between Cu^{2+} and the ligand with the help of DFT. The geometry is optimized using 6-31+G(d)[42] basis set, with Becke three-parameter exchange and Lee, Yang and Parr correlation functional, B3LYP [43]; the optimized structure is shown in Figure 4. Few important structural parameters are presented in table 1.

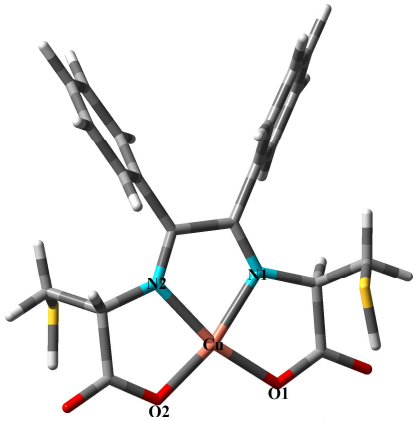
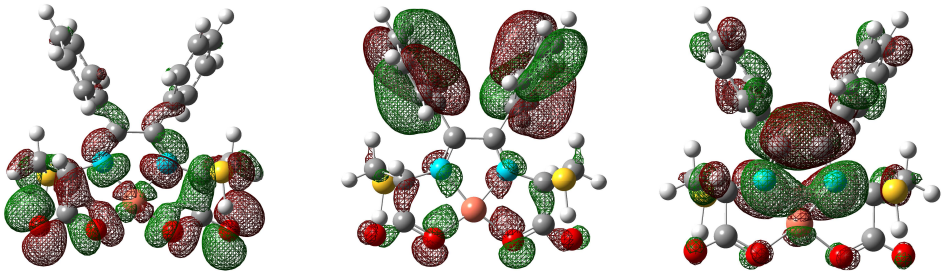


Fig. 5: DFT optimised structure of Cu(II)L

Time dependent DFT (TDDFT) calculations were performed on the optimized structure of the compounds to assign the observed electronic transitions in the UV/Visible spectrum [44] implemented in Gaussian09 [45]. To incorporate the effect of solvent phase, TDDFT calculations were carried out at the same level of theory in methanol using PCM model [46].



HOMO **HOMO-10** **LUMO**

Fig. 6: The HOMO and LUMO of Cu(II)L complex associated with electronic transitions

Table 1: Structural parameters of the Cu(II)L complex obtained at B3LYP/6-31+G(d) level of theory

Cu-N1	1.97
Cu-N2	1.97
Cu-O1	1.91
Cu-O2	1.91
∠N1CuN2	80.7
∠N1CuO1	84.9
∠O1CuO2	109.7

TDDFT calculations shows that for the UV-visible spectra of the complex comprise of two main peaks which are very close to each other (Figure 1, Inset); the peak at 594nm is due to HOMO→LUMO and 590nm correspond to HOMO-10→LUMO transition. The orbitals associated with the transitions are shown in Figure 5.

Superoxide dismutase (SOD) activity of the Cu(II)L complex

The SOD activity of Cu(II)L has been studied by the method of Nitrobluetetrazolium (NBT, Structure included in SI) reduction with $KO_2^{\cdot-}$ used as the source of superoxide radical[47]. Formation of formazon dye develops blue color which was measured

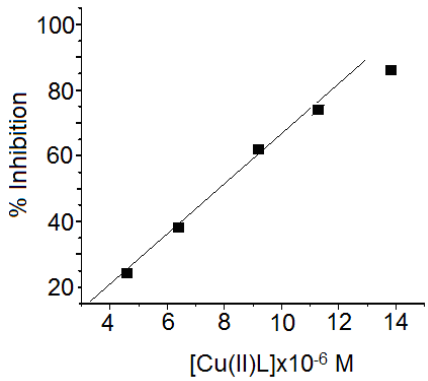


Fig. 7: Plot of the percentage inhibition of superoxide formation against the Cu(II)L concentration

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[48]. The 100% of superoxide activity corresponds to an assay performed in the absence of the 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 concentration (Figure 6). A linear relation was obtained and the obtained IC_{50} value was $7.8x10^{-6}$ M (the IC_{50} value of the native enzyme is $9.5x10^{-9}$ M). This IC_{50} value is smaller than many reported ones e.g. IC_{50} values are found in the range $3.0x10^{-5}$ M to $3.7x10^{-5}$ M for Cu(II) complexes of amino acids[49]; $9.9x10^{-5}$ M to $2.4x10^{-4}$ M for the Cu(II) complexes with simple dipeptides [50]. It has been proposed that only complexes with IC_{50} values below $20x10^{-6}$ M may become clinically interesting. Therefore, Cu(II)L fulfils this requirement and appears as an interesting possibility for further investigations in the field of SOD-mimetic drugs.

CONCLUSION

The condensation product of benzil and cysteine has been isolated and characterised for the first time and used as ligand for the synthesis of mononuclear copper(II) complex. The copper(II) complex showed characteristic UV/visible, FTIR, Mass and EPR spectral spectra. Thermogravimetric analysis curve showed one step temperature dependent weight loss for the complex. Cyclic voltammogram showed a quasi reversible electrochemical behaviour. DFT calculation was done to view probable structure of the complex. TDDFT calculations provided theoretical UV/Visible spectrum which matched with the experimental one. The complex showed SOD activity with IC_{50} = $7.8x10^{-6}$ M.

Acknowledgement
DST, New Delhi and UGC, New Delhi are thanked for financial support under FIST and SAP respectively.

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