3.1 Introduction:

Copper is the third most abundant metallic element in the human body following iron and zinc and it is important in all other forms of life too [1]. The biologically important metal Cu is found in a number of enzymes such as – Cu-Zn superoxide dismutase, tryosinase, B - hydroxylases, monoaminoxidase, ascorbic acid oxidase etc [2-4]. It is found that Cu (II) complexes show significant antioxidant and anti free radical activity [5,6]. Bimetallic Cu complexes are potential models for a number of important biological systems containing couple sites [7] and have been studied extensively [8-13]. Metal complexes of Schiff bases have played a central role in the development of coordination chemistry. Transition metal Schiff base complexes are applicable in areas, such as electrochemistry, catalysis, bioinorganic, metallic deactivators, separation technique and environmental chemistry. Schiff base metal complexes are important in asymmetric catalytic system [14] as they can often mimic the biological sites. Because of the versatility of the steric and electronic properties which can be fine-tuned by choosing the appropriate amine precursors and ring substituents, metal complexes of schiff bases derived from aromatic carbonyl compounds have received a great deal of attention in connection with asymmetric catalysis and metalloprotein modelling [15-20]. Studies on new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists [21, 22]. Again studies have shown that amino acid Schiff bases and their metal complexes have antibacterial [23, 24] anticancer and antiviral effects and could also inhibit superoxide anion radicals [25]. Bimetallic complexes of copper (II) with Schiff bases have been shown to be useful chemical probe of DNA and have found its importance in various biochemical and biomedical applications [26, 27]. Synthesis of a new copper (II) complex with multidentate Schiff base ligand contributes in the development of coordination chemistry [28-30].

Superoxide dismutase (SOD) is an anti oxidant enzyme known to protect cells from toxic effects of superoxide ion (O$_2^-$) by its dismutation into dioxygen and hydrogen peroxide in biological systems [31]. Three basic types of SOD’s are known, viz. Cu-Zn-SOD, Mn-SOD and Fe-SOD of which Cu-Zn-SOD is found in higher organisms, especially mammals [32]. It has been suggested that superoxide and related
free radical may contribute significantly to sustain chronic inflammation by promoting connective tissue degradation. A number of studies, which have done during recent years, have indicated the imbalance or deficiency of such antioxidant enzymes which can lead to several disorders including diabetes, ischemia cataract, Parkinson’s disease, cancers and many others [33-34]. Such disorders could be treated by supplementation of antioxidant enzymes 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 [35]. Small metal complexes having good superoxide scavenging activity should be good candidate in this respect.

The role of the Zn (II) ion in CuZnSOD appears to be primarily structural. There is no evidence that water, anions, or other potential ligands can bind to Zn, so it is highly unlikely that superoxide could interact with that site. Moreover, removal of Zn does not significantly diminish the SOD activity of the enzyme. Low molecular weight metal conjugates of SOD provide advantages over the natural enzymes in respect of their ability in crossing the cell membranes, offering no immunogenicity, possessing longer life-time of the active forms, possibility of oral administration and comparative low costs [36]. Since Cu site is the site of primary interaction of superoxide with the protein, many cuprous complexes have been synthesised and tested for SOD -like activity and most of them appeared to be very efficient. The ligands most frequently used in modelling binuclear Cu centers in SOD enzyme include the ligands like imidazole, benzimidazole, pyrazoles and pyridine, respectively [37].

A number of imidazolate-bridged dicopper complexes have been prepared and characterized by several workers [38]. There is also report that the Cu (II) complexes with Schiff base ligands of salicylaldehyde semicarbazone has SOD activity which could be tuned by heterocyclic bases pyridine and N-methyl imidazole [39]. S. Dutta et al. reported one copper-oxaprazinate [Cu2 (oxa)4 ]2DMSO complex having good SOD activity with IC₅₀ value 10.1µM [40]. Again S. Dutta et al. investigated the SOD mimicking potential of three pyridazolate bridged copper complexes and out of those three the lowest IC₅₀ value (3.8x10⁻⁷M) was found for [Cu2 {bis3,6 pyrazol-1-yl) pyridazine}Cl4OH]Cl [41]. Atanu Barik et al. reported two stoichiometrically different copper (II) complexes of curcumin (stoichiometry, 1:1 and 1:2 for copper:curcumin ) as superoxide dismutase mimic having IC₅₀ value 6.7µM and 68µM respectively [42].
There are recent reports on Cu complexes with SOD activity having pyridine and pyrimidine derivatives [43, 39].

In this chapter, we focus on 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 FTIR, $^1$H NMR and Mass spectra. Binuclear Copper (II) complex of the ligand was synthesized and its FTIR spectra, electronic spectra, EPR spectra, thermogravimetric analysis (TGA) study and electrochemical study results are reported. The complex was tested for superoxide scavenging ability by the NBT method.

3.2 Experimental:

Benzil was purchased from Loba chemie while ethylenediamine, salicylaldehyde and copper acetate were purchased from Merck. All solvents were purified by standard method.

3.2.1 Synthesis:

3.2.1.1 Synthesis and Characterization of Schiff Base Ligand (L):

1 g benzil (0.0047mol) was taken in 10 mL of 1 : 1 (v/v) conc. HNO$_3$ : H$_2$SO$_4$ 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 colour 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 (2mL) was added drop wise under stirring till the dark brown colour of the solution became light brown. The solvent was evaporated under vacuum and the 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 3.1 below.
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 cm⁻¹ (ᵦ C–H of C₆H₅); 924.5 cm⁻¹, 880 cm⁻¹, 841 cm⁻¹, 817 cm⁻¹, 765 cm⁻¹ (C–H out of plan vibration for C₆H₅); 1352 cm⁻¹ (ᵦ C=O); 1680 cm⁻¹ (ᵦ C=O);1601 (ᵦ C=N ); 1354 cm⁻¹ (ᵦ C=N ); 3440 cm⁻¹ (ᵦ O–H alcoholic).

¹H NMR (300 MHz) CDCl₃ δ in ppm: 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, –OH) (Fig. 3.3).
Fig. 3.1: ESIMS spectrum of L

Fig. 3.2: FTIR spectrum of L in KBr
3.2.1.2 Synthesis of the Complex:

0.0687 g (0.1 mol) of L was dissolved in 10 mL of methanol, and 0.04 g (0.2 mol) of Cu (CH$_3$COO)$_2$.H$_2$O 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$_3$CN.

Fig. 3.3: $^1$H NMR spectrum of L in CDCl$_3$
3.3 Results and Discussion:

3.3.1 Characterisation of the Binuclear Copper (II) Complex:

3.3.1.1 UV/Vis Spectroscopy study:

The UV/vis spectrum of L showed two well-defined peaks at 213 nm and 323 nm, due to $\pi - \pi^*$ and $n - \pi^*$ transition respectively, together with a shoulder at 400 nm. Fig.3.4 shows the UV/vis spectrum of the copper (II) complex in CH$_3$CN. In the complex the peaks at 213 nm and 323 nm were found 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) ion.

![UV/Vis spectrum](image)

**Fig.3.4:** UV/Vis spectrum of Cu (II)$_2$.L.$2\text{H}_2\text{O}$ in CH$_3$CN.
3.3.1.2 FTIR Spectroscopic analysis of the complex:

FTIR spectrum for the complex synthesized (Fig.3.5) showed peaks at 2861 cm\(^{-1}\) (\(\nu_{\text{C-H}}\) of \(\text{C}_6\text{H}_5\)); 772.8 cm\(^{-1}\), 718.9 cm\(^{-1}\)(C–H out of plan vibration for \(\text{C}_6\text{H}_5\)); 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\(_2\)O). 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.

![FTIR spectrum of Cu (II)\(_2\)L.2H\(_2\)O in KBr](image)

**Fig.3. 5:** FTIR spectrum of Cu (II)\(_2\)L.2H\(_2\)O in KBr

3.3.1.3 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 binuclear 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, \(1.74 \text{ BM} \times 2 = 3.48 \text{ BM}\).

### 3.3.1.4 EPR studies of the complex:

The X – band EPR spectra of the complex was recorded as the polycrystalline sample at room temperature (Fig. 3.6). The \(g_{\text{iso}}\) value and geometric parameter \(G\) i.e. the measurement of exchange interaction between the copper centers were evaluated by using the expression [44]

\[
g_{\text{iso}} = \frac{\left( g_{\|} + 2g_{\perp} \right)}{3}
\]

\[
G = \frac{\left( g_{\|} - 2.0023 \right)}{\left( g_{\perp} - 2.0023 \right)} = \frac{4K_{||}^2 \Delta E_{xx}}{k_{\perp}^2 \Delta E_{xy}}
\]

The calculated value of \(g\) tensor parameter were \(g_{\|} = 2.24\) and \(g_{\perp} = 2.11\). Hence \(g_{\|} > g_{\perp} > 2.0023\) which reveals that \(d_{x^2-y^2}\) is in the ground state [45]. The value of \(G\) was calculated to be 2.207 which mean \(G\) is less than 4 indicating effective interaction between the Cu centers [46].
3.3.1.5 Thermo gravimetric Analysis (TGA) study:

Thermo gravimetric (TG) weight loss curves and the corresponding differential thermo gravimetric (DTG) curves for the complex are shown in Fig. 3.7. 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 because the calculated weight loss for this step is 4.28%. The second and third weight losses are 65.95% and 6.58%, respectively, totalling 72.53%. This large weight drop can be explained by considering that the residue is a 1:1 mixture.

Fig.3.6: X-band EPR spectrum of Cu (II) 2.L.2H2O in solid state
of Cu$_2$O and CuO. The total calculated weight loss for this has been 72.51%.

Based on various spectroscopic and magnetic studies together with the TGA results, the structure of the complex has been confirmed to the one shown in Scheme 3.2.

![TGA curve of Cu(II)$_2$.L.2H$_2$O.](image)

**Scheme 3.2** Probable Structure of Cu(II)$_2$.L.2H$_2$O

![Scheme 3.2 Probable Structure of Cu(II)$_2$.L.2H$_2$O](image)
3.3.1.6 Electrochemical Study of the Complex:

Figure 3.8 shows the cyclic voltammogram of Cu (II).L.2H2O in CH3CN 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 Cu(II)/Cu(I) redox couple. The ratio of cathodic to anodic current is found to be 0.949. 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 that the redox process is diffusion controlled. In case of adsorption of the complex onto the electrode surface plot of currents against scan rate would be linear.

Fig.3.8: Cyclic voltammogram of Cu (II).L.2H2O in CH3CN. Working electrode: Pt disc, Reference electrode: Ag-AgCl, Supporting electrolyte: TBAP
Double potential step chronocoulometry was done for the complex in CH\textsubscript{3}CN has been shown in Fig.3.10. The plot of charge ($Q$) versus square root of time ($t_{1/2}$) was gradual, and no sharp decrease was observed. This confirms that the complex was not adsorbed onto the electrode surface, and the redox process is diffusion controlled.

**Fig.3. 9:** Plot of peak currents vs. square root of scan rate
3.4 Superoxide Dismutase (SOD) Activity of the complex:

The SOD activity of the copper complex has been studied by the method of NBT reduction using KO$_2^-$ as the source of superoxide radical [47]. The blue colour developed due to the formation of formazon dye (MF$^+$) 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 [39]. 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 concentration and from this plot the IC\textsubscript{50} value could be determined. The obtained value was 0.150 mM which was lower than the IC\textsubscript{50} value reported [43, 39]. The IC\textsubscript{50} value of the native SOD enzyme is 0.72 \(\mu\text{M} [40] \text{.}

![Graph showing inhibition of NBT reduction as a function of [Cu (II)\(_2\).L.2H\(_2\)O]](image)

**Fig.3. 9:** Percentage of inhibition of NBT reduction as a function of [Cu (II)\(_2\).L.2H\(_2\)O]
3.5 Conclusion:

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.
3.6 References:

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