APPENDIX I

List of Research Papers


DNA interactions of mixed ligand copper(II) complexes with sulphur containing ligands

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Mixed ligand copper (II) complexes having the composition [Cu(thiosemicarbazide)(ortho phenanthroline)(dmso)](I), [Cu(thiosemicarbazide)(dmso)](II), [Cu(thiosemicarbazide)(C3H5)](III), [Cu(phen)(C3H5)](IV), [Cu(phen)(thiosemicarbazide)(C3H5)](V) and [Cu(dmso)(thiosemicarbazide)(C3H5)](VI) (where thiosemicarbazide, phen = ortho phenanthroline and dmso = dimethyl sulphoxide) have been synthesized and characterized on the basis of elemental analysis, conductivity measurements, magnetic susceptibility data, electronic, IR and ESR spectroscopy. Electrochemical behaviour of these complexes has been investigated by cyclic voltammetry. All complexes undergo quasi-reversible one-electron electrochemical reduction (CuII/CuI) in the potential range 0.17-0.50 V against Ag/AgCl reference electrode. The 

E1/2 values of mixed ligand complexes are less than the parent complexes, presumably due to the increase in ligand number and size of the complex. The binding studies of copper complexes with CT-DNA have been investigated using absorption spectroscopy.

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Of the sulphur donor ligands, thiosemicarbazide or/and thiosemicarbazones possess a wide spectrum of medicinal properties including activity against influenza, protozoa, certain kinds of tumour, bacteria and have been suggested as possible pesticides, fungicides. Their activity has frequently been thought to be due to their ability to chelate metals. From the survey of existing literature, it appears that metal complexes of thiosemicarbazides have played a vital role in the development of coordination chemistry. It is well known from the literature that the compounds containing >C=S moiety have a strong ability to form metal complexes and exhibit a wide range of biological activity. Thiosemicarbazides are biologically active pharmacophores, besides having good complexing ability and their activity enhanced on complexation with metal ions. Thiosemicarbazides usually act as chelating ligands for transition metal ions by bonding through the sulphur and azomethine nitrogen atom; although in some cases they behave as mono dentate ligands where bond through sulphur only.

Although copper(II) complexes of thiosemicarbazide are reported in the literature, spectral and magnetic properties of mixed ligand copper(II) complexes with such sulphur containing ligands are not reported so far. In recent years, there has been considerable interest in metal-DNA interactions. In continuation of ongoing research, we report herein the synthesis, characterization, spectral properties and DNA interactions of copper(II) mixed ligand complex with thiosemicarbazide or dimethyl sulphoxide.

Materials and Methods

Most of the reagents used were of analytical grade and were used without further purification. Thiosemicarbazide (Thomas Baker); Copper (II) chloride dihydrate; ortho phenanthroline (S.D. Fine); dimethyl sulphoxide (Merck) were used for preparation of complexes. Dimethyl sulphoxide was also used for spectral studies. Conductivity measurements were made in dimethyl formamide (DMF). CT-DNA used was from Gienie, Bangalore.

Infra red spectra (4000-270 cm⁻¹) in KBr disc were recorded on Nicolet protege 460 FT-IR spectrophotometer. Electronic spectra were recorded on T-Comp 8500 spectrophotometer. Molar conductance measurements were made on freshly prepared complex solution (10⁻³ M) in dimethyl formamide at 30 ± 2°C with a CC601 model (Century Co.) direct reading conductivity bridge. Magnetic measurements were made on a magnetic susceptibility balance (Sherwood Scientific, Cambridge, UK). High puri-
hydrated copper sulphate was used as a standard. Voltammetric measurements were performed on a Bio-Analytical System (BAS) CV-27 assembly in conjunction with a X-Y recorder. Voltammetry measurements were made with degassed (N₂-bubbling for 5 minutes) solution of the complex (10⁻³ M) in dimethyl sulfoxide (DMSO) containing tetraethyl-ammonium bromide (0.1 M) as supporting electrolyte. The three-electrode system consisted of a glassy carbon (working), platinum wire (auxiliary) and Ag/AgCl (reference). ESR spectra were recorded on Varian E-112 X-band spectrophotometer at 1.17 K in DMSO solution.

Preparation of complexes

The complexes (I-VI), were prepared by mixing CuCl₂·2H₂O with the required amount of ligand to yield \([\text{Cu(tsc)}Cl]_2\) (I), \([\text{Cu(tsc)}Cl]_2\) (II), \([\text{Cu(phen)}Cl]_2\) (III), \([\text{Cu(dmso)}Cl]_2\) (IV) \([\text{Cu(phen)}(\text{tsc})Cl]_2\) (V) and \([\text{Cu(dmso)}(\text{tsc})Cl]_2\) (VI) (where tsc = thiosemicarbazide, phen = ortho phenanthroline and dmso = dimethyl sulphoxide). Complexes I-IV were prepared according literature reports while the complexes V and VI were prepared by the following method (Scheme 1).

**Synthesis of \([\text{Cu(phen)}(\text{tsc})Cl]_2\) (V) and \([\text{Cu(dmso)}(\text{tsc})Cl]_2\) (VI)**

To a stirred ethanolic solution of thiosemicarbazide (0.001 mol) added (Cu(phen)Cl₂) (III) (0.001 mol) and stirred for about 30 minutes. The brown precipitate which was formed was filtered and dried under vacuo. \([\text{Cu(dmso)}(\text{tsc})Cl]_2\) (VI) was prepared similarly with complex IV and refluxing for 30 min over hot water bath.

**DNA binding experiments**

The interaction of the complexes with DNA was carried out in tris-buffer. Solution of calf thymus-DNA (CT-DNA) in (0.5 mM NaCl/5 mM Tris-HCl; pH = 7.0) buffer gave absorbance ratio at 260 nm and 280 nm of 1.89 indicating that the DNA was sufficiently free of proteins. The DNA concentration per nucleotide was determined by absorption coefficient (6600 dm⁻³ mol⁻¹ cm⁻¹) at 260 nm. Stock solutions stored at -1°C were used after no more than four days.

The electronic spectra of copper(II) complexes were monitored in the absence and presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration (4.95 x 10⁻⁵ M) and varying the nucleic acid concentration (0.19 - 10.4 M). Absorption values were recorded after each successive addition of DNA solution. The intrinsic binding constant (Kₐ) was determined according to the following equation:

\[
\frac{[\text{DNA}]}{[\text{DNA}](e_\lambda - e_0)} = \frac{[\text{DNA}]}{[\text{DNA}](e_0 - e_\lambda) + 1/K_\lambda} (e_\lambda - e_0)
\]

where \(e_\lambda, e_0\) and \(e_\lambda\) correspond to apparent, bound and free metal complexes extinction coefficients respectively. A plot of \([\text{DNA}]/(e_\lambda - e_0)\) versus \([\text{DNA}]/(e_0 - e_\lambda)\) gave a slope of \(1/(e_0 - e_\lambda)\) and intercept equal to \(1/K_\lambda(e_\lambda - e_0)\); \(K_\lambda\) is the ratio of the slope to the Y-intercept.

**Results and Discussion**

The complexes are stable at room temperature, non-hygroscopic and insoluble in water, partially soluble in methanol and ethanol and readily soluble in DMF and DMSO. The color, analytical data, molar conductivity and magnetic moment data are presented in Table 1. The molar conductivity data indicate that these complexes are non-electrolytes. The room temperature magnetic moments lie in the range 1.70-1.97 BM indicating the presence of one unpaired electron as expected for copper (II) complexes.

Electronic spectral data of copper complexes recorded in dimethyl sulfoxide show high intensity bands due to intra-ligand and charge transfer transitions. However, the spectra have low intensity broad structured bands in the 14245-10.045 cm⁻¹ range as expected for five coordinate complexes.

The infrared spectrum of thiosemicarbazide shows medium bands at 3360 cm⁻¹ and 3248 cm⁻¹, which are respectively assigned to asymmetric and symmetric stretching vibrations of terminal NH₂ group. These bands shift towards lower frequency in the complexes (3338-3342 and 3235-3249 cm⁻¹ respectively) suggesting the participation of terminal NH₂ group in coordination. A strong band observed at 1160 cm⁻¹ is
assigned to C=S stretching vibration. This band shifts to lower frequency in the complexes (1139-1143 cm\(^{-1}\)) indicating the involvement of thioketo sulphur in complex formation. The non-ligand bands observed in far IR region also support the involvement of terminal NH\(_3\) group and thioketo sulphur in coordination. The IR spectrum of DMSO displays strong S=O absorption band at 1050 cm\(^{-1}\), which in the complexes is observed at lower frequency (1025 cm\(^{-1}\)). This implies that the DMSO binds with copper through oxygen.

The ESR spectra of the copper(II) complexes were recorded in DMSO solution at LNT and analyzed by Kneubuhl’s method\(^1\). The calculated average g-factor lies in the range 2.1 - 0.04 are in agreement with an orbitally non-degenerate ground state.

The typical spectrum of [Cu(tsc)Cl\(_2\)] is given in Fig. 1. The spectrum exhibits orthorhombic ESR spectra and corresponding g-values are rather consistent for the rhombic system with \(g_1 > g_2 > g_3\). If the value of the parameter \(R\) defined by the ratio \(g_2 R / g_2 R\) is greater than one\(^1\), then the \(d_{yz}\) ground state, while for a \(d_{z^2}\) ground state, \(R\) is expected to be less than one\(^1\). The \(R\) values in the present study support a five coordinated square pyramidal geometry for complexes I, IV and VI and trigonal bipyramidal geometry for complexes II, III and V. A pentacoordinated structure is thus, suggested for these complexes. The two configurations, square pyramidal and trigonal bipyramidal, are characterized by the ground states \(d_{yz}\) and \(d_{z^2}\) respectively\(^1\). The ESR spectra of copper(II) complexes provide a very good basis to distinguish between these two configurations.

On the basis of electronic and ESR spectral data, pentacoordinate structure is suggested for complexes...
DNA interaction studies

The interactions of the complexes with CT DNA were investigated using absorption spectra. The electronic spectra of [Cuphen(tsc)Cl] in the absence and presence of CT DNA are given in Fig. 3. All complexes have a large separation (130-610 nm) between anodic and cathodic peaks, indicating the quasi-reversible character.

Cyclic voltammetric studies

The electrochemical behavior of complexes was investigated by cyclic voltammetry at glassy carbon working electrode (Table 2). The cathodic peak current function values were found to be independent of scan rate. Repeated scans as well as different scan rates indicate that the complexes do not undergo dissociation in DMF medium. The reduction [Cu(II)/Cu(I)] couple for the complexes was observed in the potential range 0.170-0.360 V versus Ag/AgCl reference electrode. The E_{1/2} values are comparable to the E_{1/2} values of Cu(II) complexes of sulphur containing ligands.

All complexes exhibit quasi-reversible behavior as indicated by non-equivalent current intensity of cathodic and anodic peaks. The difference $E_{pa} = E_{pa} - E_{pc}$ indicates quasi-reversible character of the complexes. All complexes have a large separation (130-610 mV) between anodic and cathodic peaks, indicating the quasi-reversible character.
In the presence of increasing amounts of CT-DNA, the [Cu(phenHtse)Chl] complex showed an increase in intensity (hyperchromicity (31±0.5%) and bathochromic shifts (maximum (3±1) nm) for their red shift absorption peak maxima. The value of $K_b$ evaluated for the complex using Eq. 1 is found to be $8\times10^6 M^{-1}$ (see Fig. 3 Inset). On addition of CT-DNA to the copper(II) complexes, there is an increase in molar absorptivity as well as a significant shift in the wavelength attributed to hyperchromism and bathochromic shift, which suggests that the complex is bound to CT-DNA strongly. During these titrations, the change in peak intensity (absorption spectra) is analogous to similar observations made earlier for other mixed ligand complexes$^{20}$. Since the binding constants (10$^6 M^{-1}$) are high, the complexes may be regarded as efficient intercalator of DNA.

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References