

Interaction of calf thymus DNA with new asymmetric copper(II) *N,N*-ethane bridged N_2S_2 macrocycle

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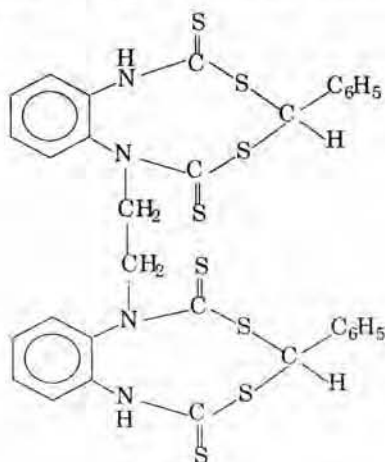
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Novel asymmetric metalated Cu(II), Ni(II) double decker *N,N*-ethane bridged N_2S_2 macrocycles have been synthesized. Structure elucidation of the complexes has been done on the basis of elemental analysis, IR, UV-vis, EPR, conductance measurements, ^1H NMR and ^{13}C NMR spectroscopy. All the spectroscopic data indicate that the metal ions attain square-planar geometry and are ionic in nature. The kinetic studies have been carried out with calf thymus DNA (CT-DNA) to ascertain the metal-DNA interaction by spectrophotometric method at the λ_{max} (446 nm) of Cu(II) complex at $30 \pm 1^\circ C$. On interaction with CT-DNA, the absorption spectra exhibit a shift in wavelength, and also a steep decrease in absorbance which clearly indicates strong binding of CT-DNA with the Cu(II) complex. The rate constants (k_{obs}) have been calculated under pseudo-first-order conditions and the plot of k_{obs} versus [DNA] gives a straight line. The electrochemistry of DNA-metal complex interaction has been carried out in $H_2O/DMSO$ (95:5) solution at different scan rates. The shift in formal potential (E^0) after the interaction with CT-DNA clearly indicates the binding of Cu(II) complex to CT-DNA. Besides this, the ratio of anodic to cathodic peak currents I_{pa}/I_{pc} is ~ 0.8 for the free Cu(II) complex while for DNA bound metal complex, the ratio decreases to (0.5) suggesting that CT-DNA is bound strongly to the Cu(II) complex.

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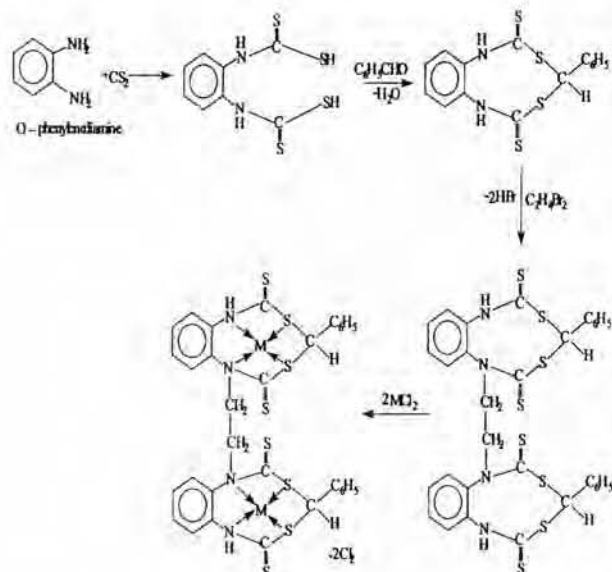
DNA-metal complex interaction has become a subject of intense research¹⁻⁷. This interaction is essentially noncovalent⁸⁻⁹, either by intercalation, groove binding or external electrostatic binding. The binding of DNA to metal complex is closely related to the structure of the complex and the macrocyclic framework forms a suitable platform for holding the metal ions through nitrogen, sulphur and oxygen donor atoms. N_2S_2 heteroatom macrocycle has

enabled efficient synthesis of new chiral dinuclear species linked through an ethylene moiety as shown below:



Cancer is derived from numerous tissues with multiple etiologies. Thus, the therapy for curing cancer must be diverse, as the disease itself. The chemotherapy is widely used for treating cancer¹⁰⁻¹². Majority of the chemotherapeutic drugs are DNA targeted, e.g., cisplatin or its analogue intercalate into the DNA helix¹³. Much emphasis must be laid on the molecular design of the chemotherapeutic drugs¹⁴⁻¹⁵ so that they work on the specific target on a particular tumour type. In this regard, chiral complexes can prove as more efficient and are regiospecific promising drugs.

The interaction of copper(II) complex (Scheme 1) with CT-DNA has been studied by UV-vis spectroscopy and cyclic voltammetry, as the changes in absorbance and redox potential values are directly related to the structure of the complex. The nickel(II) complexes have been synthesized only for structure elucidation.



Materials and Methods

All experiments involving the interaction of the copper(II) complex with CT-DNA were carried out in aqueous solution with varying concentration of CT-DNA (10×10^{-3} , 12×10^{-3} , 14×10^{-3} , 16×10^{-3} and 18×10^{-3} mol dm⁻³), carbon disulfide (S.D. Fine-Chem Ltd.), 1,2-dibromoethane (Merck), CuCl₂, NiCl₂ [hydrated], benzaldehyde (BDH) and *o*-phenylene-diamine (Fluka), were used as received. The CT-DNA concentration was determined by absorption spectrophotometry. The stock solution of CT-DNA was prepared by dissolving in *tris*-HCl buffer at pH 7 and dialysing exhaustively against the same buffer for 48 h. The solution gave a ratio of $>>1.8$ at A_{260}/A_{280} indicating that CT-DNA was free from protein¹⁶. Microanalyses of the complexes were obtained on Carlo Erba Analyser model 1106. IR spectra (200–4000 cm⁻¹) were recorded on a Carl-Zeiss Specord M-80 spectrophotometer in nujol mulls. The electronic spectra were recorded on a Systronics 119 spectrophotometer (Esp-300). The NMR spectra were recorded on an amx-500 instrument. Cyclic voltammetry (CV) measurements were carried out on CH instrument electrochemical analyzer. High purity aqueous H₂O/DMSO (95:5) was employed for CV studies with 0.4 M KNO₃ as the supporting electrolyte. A three-electrode configuration was used comprising a Pt disk working electrode, Pt wire counter electrode and Ag/AgCl as the reference electrode. To study the redox properties of the

copper(II) complex with CT-DNA, we have investigated the cyclic voltammogram of the free copper(II) complex and the DNA bound copper(II) complex in H₂O/DMSO (95:5). Kinetic experiments were performed under pseudo-first order conditions using Systronics 119 spectrophotometer. The k_{obs} values were obtained by linear least squares regression method. Experiments were carried out at room temperature $30 \pm 1^\circ\text{C}$. Viscosity measurements were carried out using Ostwald's viscometer at 25°C . Flow time was measured with a digital stop-watch. Each sample was measured four times and an average flow was calculated. Data were presented as (η/η_0) versus binding ratio ($[\text{Cu}]/[\text{DNA}]$)¹⁷ where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solution ($t > 100$ s) corrected for the flow time of buffer alone (t_0), $\eta = t/t_0$ ¹⁸.

Synthesis of C₁₅H₁₂N₂S₄ [L]

Carbon disulfide (6.1 cm³, 0.1 mol) was added dropwise with constant stirring to a solution of *o*-phenylenediamine (5.4 g, 0.05 mol) in absolute EtOH (50 cm³) maintained at the temperature of less than 5°C . The reaction mixture was stirred constantly for 1 h till a brown precipitate was obtained, which was filtered, washed thoroughly with hexane and dried *in vacuo*. The solid brown product (5.2 g, 0.02 mol) was dissolved in MeOH (25 cm³) and benzaldehyde (2.12 cm³, 0.02 mol) was added. The reaction was boiled to reflux for *ca.* 10 h. A dark brown precipitate was obtained. It was filtered, washed with ether and dried *in vacuo*.

Synthesis of C₃₂H₂₆N₄S₈ [L]

To the solution of C₁₅H₁₂N₂S₄ (3.48 g, 10 mmol) was added 1,2-dibromoethane (0.43 cm³, 5 mmol) in 2:1 molar ratio. The reaction mixture was then boiled to reflux for *ca.* 7 h. A black precipitate was obtained, which was filtered, washed thoroughly with ether and dried *in vacuo*.

Synthesis of C₃₂H₂₆N₄S₈Cu₂Cl₄

To a solution of C₃₂H₂₆N₄S₈ (0.720 g, 1 mmol) in MeOH was added CuCl₂ hydrated (0.342 g, 2 mmol) in 1:2 molar ratio. The reaction mixture was boiled to reflux for *ca.* 8 h. and a brown precipitate was obtained, washed thoroughly with hexane and dried *in vacuo*.

Similar method was adopted for the synthesis of nickel(II) complex. Physical and analytical data are shown in Table 1.

Table 1 — Colour, M.pt., yield, elemental analysis of the ligands and the complexes

Compound	Colour	M. pt. °C	Yield %	% Found/(Calcd.)		
				C	H	N
1 C ₈ H ₈ N ₂ S ₄	brown	80-83	80	36.9 (36.7)	3.1 (2.9)	10.7 (10.8)
2 C ₁₅ H ₁₂ N ₂ S ₄ [L]	brown	260-265	72	41.8 (41.9)	3.8 (3.9)	9.7 (9.8)
3 C ₃₂ H ₂₆ N ₄ S ₈ [L']	black	245 (d)	59	53.5 (53.2)	3.8 (3.6)	8.0 (7.8)
4 C ₃₂ H ₂₆ N ₄ S ₈ Cu ₂ Cl ₄	brown	260 (d)	54	39.2 (38.9)	2.7 (2.6)	5.8 (5.7)
5 C ₃₂ H ₂₆ N ₄ S ₈ Ni ₂ Cl ₄	red	250 (d)	51	39.2 (38.9)	2.7 (2.6)	5.8 (5.7)

d = decomposes

Results and Discussion

IR spectra

The ligands exhibit thione-thiol tautomerism, since they contain a thioamide $\nu(\text{HN-C}=\text{S})$ functional group¹⁹⁻²⁰. However, the absence of $\nu(\text{SH})$ band and the presence of $\nu(\text{NH})$ band indicates that the ligand [L] in the solid state remains in the thio form. This contention is further supported by ¹H NMR, which does not show any signal due to presence of S-H protons. The $\nu(\text{C-S})$ and $\nu(\text{C}=\text{S})$ bands appear at 739-757 and 1067-1084 cm⁻¹, respectively. The $\nu(\text{C-N})$ band appears at 1365 cm⁻¹ but in our ligand L' there is a shift of 34 cm⁻¹ and a change in the intensity of the $\nu(\text{C-N})$ band which supports the formation of the ligand with 1,2-dibromoethane. This is further confirmed by the appearance of new band at 2840 cm⁻¹ due to CH₂ group. The IR spectra of the complexes exhibit shift in $\nu(\text{N-H})$, $\nu(\text{C-S})$ bands indicating the coordination of the metal through nitrogen and sulphur atoms. This is also evidenced by far-IR spectra which reveals $\nu(\text{M-N})$ and $\nu(\text{M-S})$ bands at 422-430 cm⁻¹ and 353-355 cm⁻¹ region, respectively²¹⁻²².

Electronic absorption spectra

The absorption spectrum of [L'] recorded in MeOH exhibits bands at 224, 244 and 304 nm. These bands are attributed to $\pi-\pi^*$ and $n-\pi^*$ transitions respectively (Fig. 1).

The absorption spectrum of the copper(II) complex in DMSO reveal characteristic bands of MLCT transitions at 378 and 392 nm. A strong absorption maxima appears at 446 nm assigned to $d-d$ transition characteristic of square-planar d^9 copper complexes involving (d_{xz} , $d_{yz} \rightarrow d_{x^2-y^2}$) orbitals (Fig. 2)²³. These transitions are usually made up of three spin-allowed

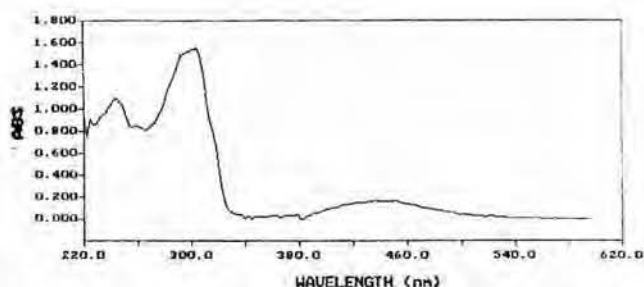
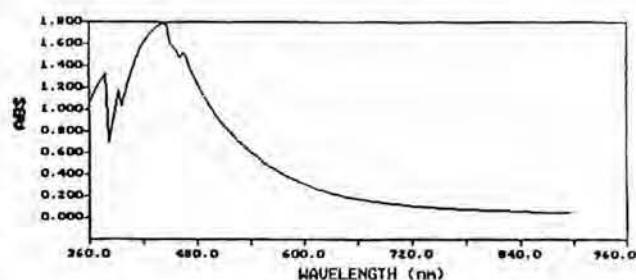
Fig. 1 — Electronic spectrum of the ligand C₃₂H₂₆N₄S₈.

Fig. 2 — Electronic spectrum of the Copper (II) Complex.

transitions namely: $^2A_{1g} \leftarrow ^2B_{1g}$, $^2B_{2g} \leftarrow ^2B_{1g}$ and $^2E_g \leftarrow ^2B_{1g}$. Two of these transitions are indicated by weak shoulder or humps in the absorption spectrum.

The nickel(II) complex exhibits a broad band at 452 nm assigned to $^1A_{1g} \rightarrow ^1B_{1g}$ transition typical of low-spin square-planar geometry²⁴.

EPR spectra

The room temperature EPR spectrum recorded for the copper(II) complex reveals a signal for $g_{||}$ and $g_{\perp}$ at 2.20 and 2.07, respectively for square-planar geometry of the copper(II) complex²⁵. The presence of $g_{||} > g_{\perp}$ in the EPR spectrum of the copper(II) complex also supports the square-planar geometry²⁶.

Table 2 — ^1H NMR data (ppm)

Compound	Phenyl	CH_2	NH	CH
1 $\text{C}_{15}\text{H}_{12}\text{N}_2\text{S}_4$ [L]	7.1-7.2 (m)	—	5.0 (s)	5.6 (d)
2 $\text{C}_{32}\text{H}_{26}\text{N}_4\text{S}_8$ [L']	7.2-8.2 (m)	3.6 (s)	4.6(d)	5.2 (s)
3 $\text{C}_{32}\text{H}_{26}\text{N}_4\text{S}_8\text{Ni}_2\text{Cl}_4$	7.2-8.2 (m)	2.8 (s)	3.6 (s)	4.7 (s)

Table 3 — ^{13}C NMR data (ppm)

Compound	Ar carbon	CS_2	S-C-S	CH_2
1 $\text{C}_{15}\text{H}_{12}\text{N}_2\text{S}_4$ [L]	118-136	170.8	48.9-49.9	—
2 $\text{C}_{32}\text{H}_{26}\text{N}_4\text{S}_8$ [L']	119-126	168	47.5-48.8	38-40
3 $\text{C}_{32}\text{H}_{26}\text{N}_4\text{S}_8\text{Ni}_2\text{Cl}_4$	115-125	171	39.3-41.5	36-38

Table 4 — ^1H NMR data of $\text{C}_{32}\text{H}_{26}\text{N}_4\text{S}_8$ and correlation with 2D COSY- NMR spectra(ppm)

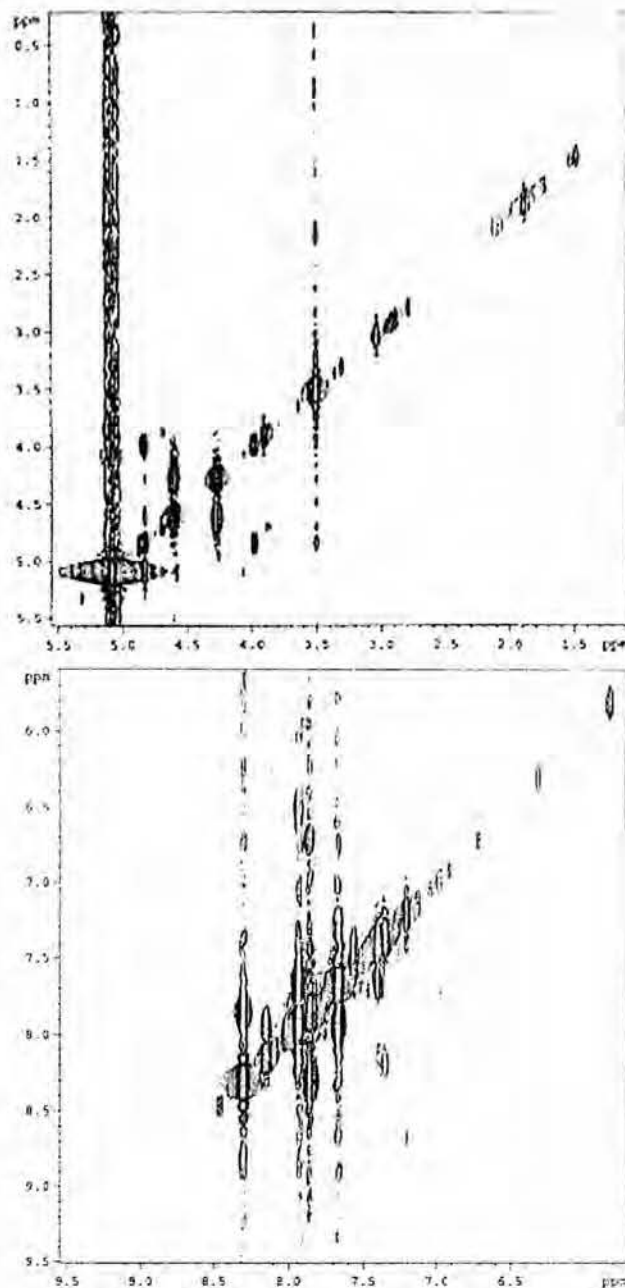
	Proton	^1H NMR	2D COSY correlations
1	Phenyl	7.1-7.2	7.0-8.3
2	$-\text{CH}_2-$	3.6	3.5
3	$-\text{NH}-$	4.6	4.7
4	$-\text{CH}-$	5.2	5.1

NMR studies

To have a better understanding regarding the structure of the ligand [L'] and nickel(II) complex, ^1H , ^{13}C NMR and 2D COSY NMR (Fig. 3) studies were carried out and are given in the (Tables 2, 3 and 4). Peak assignments were made on the basis of the peak integration and multiplicity. The ^1H NMR spectra of the ligand exhibits peaks at 7.2-8.2 p.p.m. assigned to the phenyl protons. The signal due to the CH_2 protons was observed at 3.2 p.p.m.²⁷ The NH and CH group show signals at 4.6 and 5.2 p.p.m, respectively. On complexation with nickel(II), there was a shift in NH, CH and CH_2 proton signals indicating the coordination of the metal through nitrogen and sulphur atoms.

Cyclic voltammetry

The cyclic voltammetry is an important tool to measure the formal electrode potential of electron transfer reactions. Recently, its application to study metallointercalation and coordination of metal ion to DNA has given insight to this subject and also cyclic voltammetry complements the other spectroscopic methods of investigation such as UV-vis to understand the nature of DNA binding in metal

Fig. 3 — 2D-COSY NMR of ligand $\text{C}_{32}\text{H}_{26}\text{N}_4\text{S}_8$.

complexes²⁸⁻²⁹. The cyclic voltammogram of the copper(II) complex at a scan rate 0.1 V s^{-1} reveals a quasi-reversible well separated redox wave due to one electron transfer reaction attributed to the Cu(II)/Cu(I) couple with $E_{1/2}$ values -0.498 V and -0.532 V respectively (Fig. 4). The profile of the voltammogram obtained at variable scan rates is almost similar (Fig. 5) which reveals the quasi-

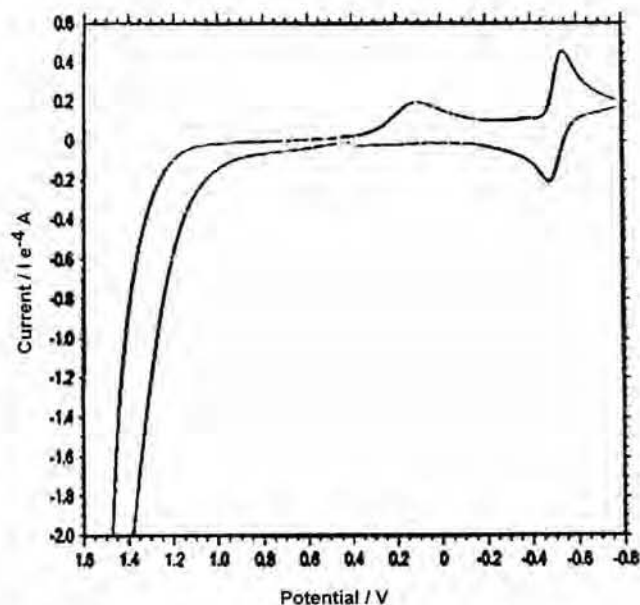


Fig. 4 — Cyclic voltammogram of the copper (II) complex at a scan rate of 0.1 Vs⁻¹ (Initial E(V) = 0.4, High E(V) = 1.5, low E(V) = -0.8, sensitivity (A/V) = 2e-5)

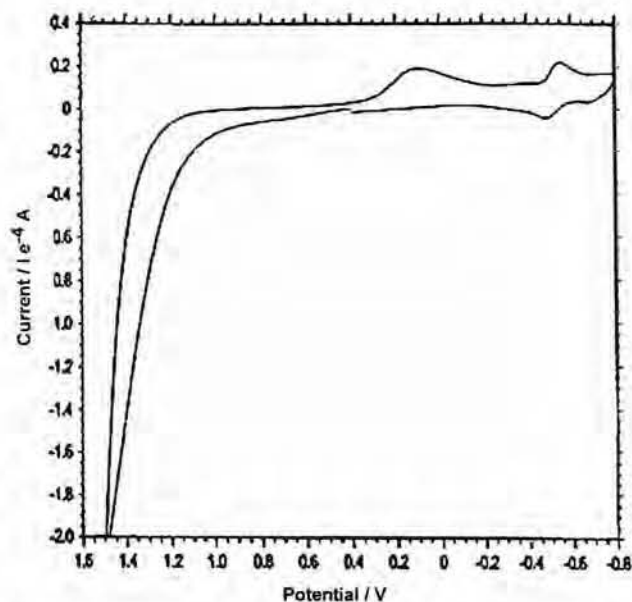


Fig. 6 — Cyclic voltammogram of the copper (II) complex after the addition of CT-DNA at a scan rate of 0.1 Vs⁻¹ (Initial E(V) = 0.4, High E(V) = 1.5, low E(V) = -0.8, sensitivity (A/V) = 2e-5)

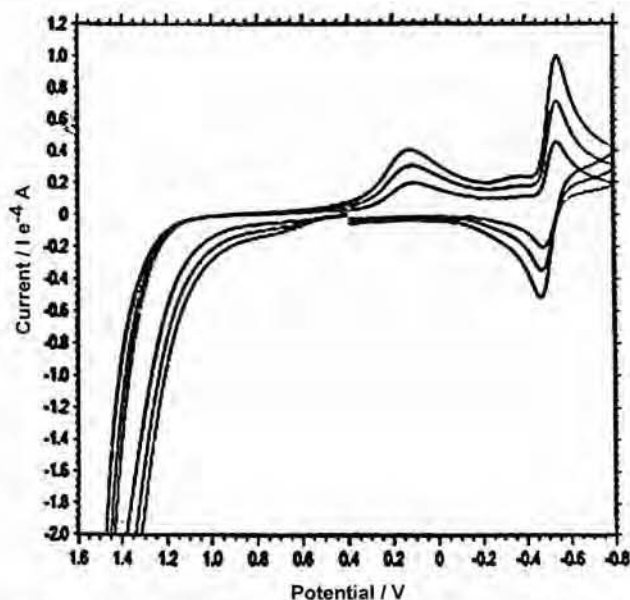
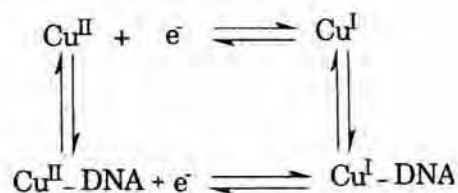


Fig. 5 — Cyclic voltammogram of the copper (II) complex at a scan rate of 0.1, 0.2 and 0.3 Vs⁻¹ (Initial E(V) = 0.4, High E(V) = 1.5, low E(V) = -0.8, sensitivity (A/V) = 2e-5)

reversibility of the process. For a reversible wave, E_p is independent of the scan rate and i_p (as well as the current at any point of the wave) is proportional to the $v^{1/2}$ ³⁰. The limiting peak potential separation ΔE_p is equal to 63 mv, which is in good agreement with Nernstian value for one electron transfer couple (59

mv). On addition of CT-DNA, the complex experiences a shift in $E_{1/2}$ values as well as E_p values of 9 mv and 13 mV at the scan rate of 0.1 V s⁻¹ (Fig. 6). The ratio of anodic to cathodic peak currents I_{pa}/I_{pc} is 0.8 in the free copper(II) complex while on addition of CT-DNA the ratio (I_{pa}/I_{pc}) decreases (0.5), suggesting that CT-DNA moiety is bound strongly to the complex. Moreover, there is decrease in the voltammetric peak currents upon the addition of CT-DNA due to the diffusion of equilibrium mixture of the free and DNA bound metal complex to the electrode surface²⁹. The changes in formal potential of free copper(II) complex and the CT-DNA bound complex reveal the strong intercalation of the complex with the DNA helix and also suggest the stabilization of Cu(II) over Cu(I) as depicted in a square redox scheme shown as:



Kinetic studies

The binding of the copper(II) complex to CT-DNA has been characterized through the absorption changes spectrophotometrically. The absorption

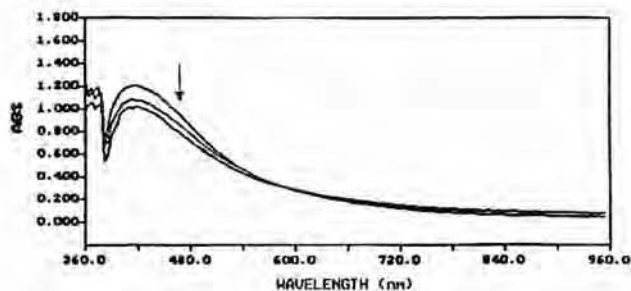
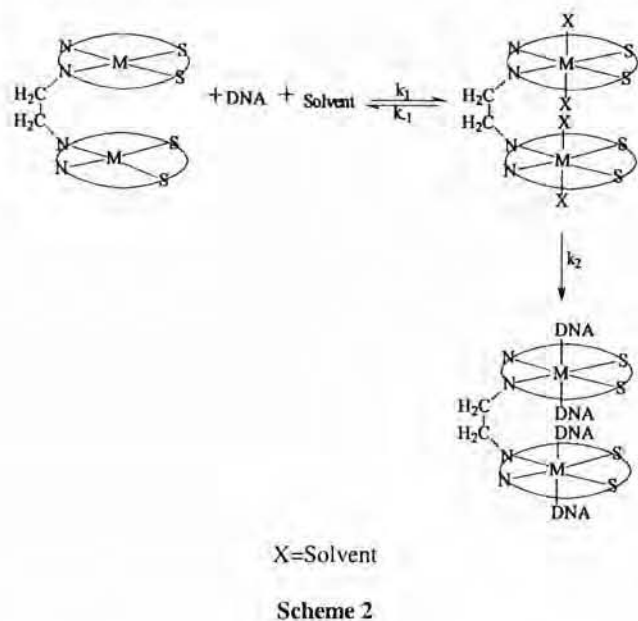


Fig. 7 — Absorption spectra of copper(II) complex showing hypochromicity after addition of CT-DNA.

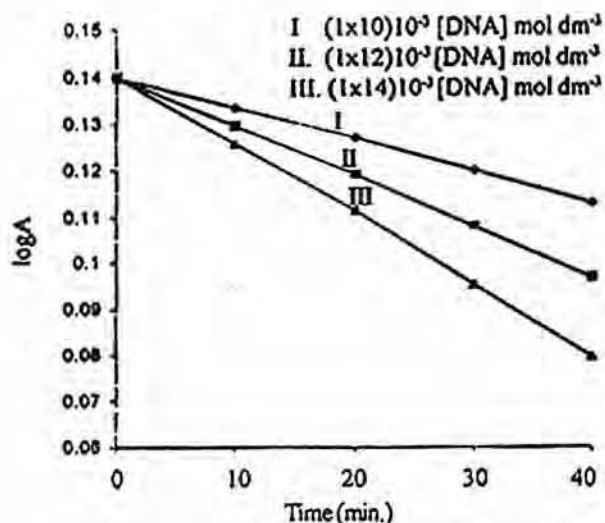


Fig. 8 — Plot of $\log A$ versus time for $C_{32}H_{26}N_4S_8Cu_2Cl_4$ at varying concentration of CT-DNA (10 – 18×10^{-3} mol dm^{-3}).

spectrum of the copper(II) complex reveals a band at 446 nm, λ_{max} of the copper(II) complex in $H_2O/DMSO$ (95:5) at $30 \pm 1^\circ C$.

Kinetic experiments were performed at the λ_{max} (446 nm) of the copper(II) complex at a fixed concentration (10^{-3} mol dm^{-3}) with varying concentration of CT-DNA (10×10^{-3} to 18×10^{-3} mol dm^{-3}). On addition of CT-DNA to the copper(II) complex, there is decrease in molar absorptivity as well as significant shift in λ_{max} (40 nm). The decrease in absorption intensity and significant shift in wavelength is attributed to hypochromism and blue shift, which clearly suggests that the complex is bound to CT-DNA strongly³¹ (Fig. 7). On varying the concentration of CT-DNA, there is pronounced decrease in the intensity, which indicates the predominant factor for such binding is concentration. Thus, as the concentration of the CT-DNA increases, the extent of the metal complex-DNA interaction also increases. The rate constants k_{obs} values were obtained by plotting $\log A$ versus time and are shown graphically in (Fig. 8) which clearly indicates pseudo-first order kinetics with respect to [DNA]. The following mechanistic pathway has been proposed (Scheme 2).

If the proposed mechanism is correct, then this rate law Eq. (1) holds good,

$$k_{obs} = k_1 k_2 [DNA] / (k_{-1} + k_2) \quad \dots (1)$$

The rate law according to the relation (1) should give a straight line for k_{obs} versus [DNA] (Fig. 9). Our results are in accordance with Eq.(1) and give a linear plot with slope equal to $k_1 k_2 / k_{-1} + k_2$ showing pseudo-first order dependence for interaction of copper(II) complex with CT-DNA.

Viscosity measurements

Hydrodynamic measurements that are sensitive to length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data³²⁻³³. For further clarification of the interaction between dinuclear complex $C_{32}H_{26}N_4S_8Cu_2Cl_4$ and DNA, viscosity measurements were carried out. A classical intercalation model results in lengthening of the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to increase in DNA viscosity. In contrast, a partial or non-classical intercalation of

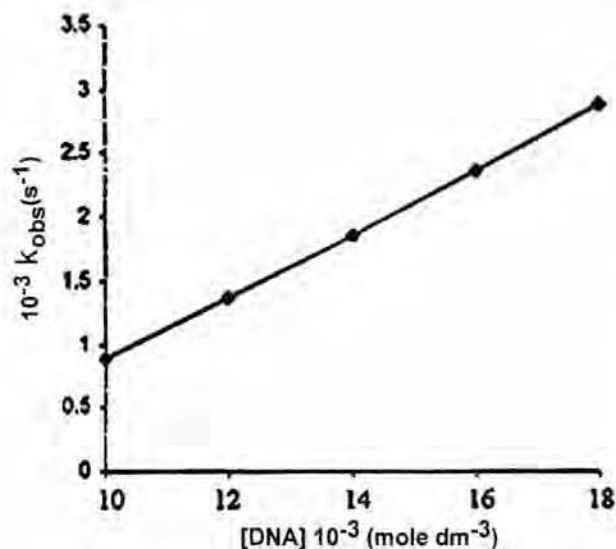


Fig. 9 — Plot of K_{obs} versus concentration of CT-DNA for $C_{32}H_{26}N_4S_8Cu_2Cl_4$ [fixed concentration of complex (1×10^{-3} mol dm⁻³)].

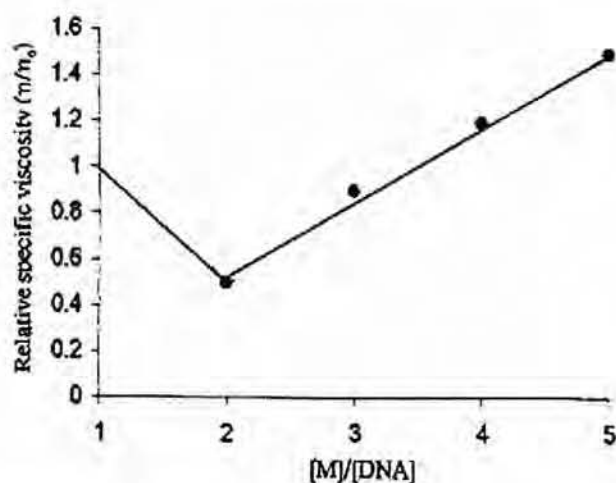


Fig. 10 — Effect of increasing amount of $C_{32}H_{26}N_4S_8Cu_2Cl_4$ on the relative viscosity of CT-DNA at $29 \pm 0.1^\circ C$.

ligand could bend or kink the DNA helix, reducing its effective length and concomitantly its viscosity. The effects of the dinuclear complex $C_{32}H_{26}N_4S_8Cu_2Cl_4$ on the viscosity of CT-DNA are shown in (Fig. 10). The viscosity of DNA (8.3×10^{-6} M) increases upon addition of increasing concentrations (1.6×10^{-5} , 2.5×10^{-5} , 3.3×10^{-5} , 4.1×10^{-5} M) of the dinuclear Cu(II) complex. The results show that the complex intercalates into DNA base pairs.

Conclusion

The compounds can have potential applications in cancer. The interaction of complex $C_{32}H_{26}N_4S_8Cu_2Cl_4$ with calf thymus DNA exhibits strong intercalative binding mode, probably due to dimer, which may result in π - π stacking.

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