CHAPTER VI

New Homodi- and Heterotrinuclear Metal Complexes of Schiff Base Compartmental Ligand: Interaction Studies of Copper Complexes with Calf-Thymus DNA
CHAPTER VI

Experimental

All starting materials were commercially available and used as purchased. Benzaldehyde, Ethanolamine, Diethylxoxalate, MnCl$_2$.4H$_2$O, CoCl$_2$.6H$_2$O, NiCl$_2$.6H$_2$O and CuCl$_2$.2H$_2$O were obtained from E. Merck. Phenylhydrazine was received from s. d. fine Chem. Ltd. SnCl$_4$ was purchased from Lancaster. Microanalysis (CHN) was performed using a Carlo Erba Analyzer Model 1108. Molar conductances of the solutions (10$^{-3}$ M) were measured at room temperature on a Digisun Electronic Conductivity Bridge. IR spectra were obtained on an Interspec 2020 FTIR spectrometer as nujol mull. Electronic spectra were recorded on a Systronics 119 spectrophotometer (ESP-300). Solid state EPR spectra of the copper complexes were recorded on a Varian E 112 X-band spectrometer at liquid nitrogen temperature. The NMR spectra were obtained on a Bruker DRX–300 spectrometer in CD$_2$Cl$_2$ and CDCl$_3$.

Synthesis of ligand [LH$_2$] [C$_{32}$H$_{32}$N$_6$O$_2$]

The ligand LH$_2$ was prepared by employing a three step synthetic method (Scheme 1) To a solution of benzaldehyde (5.01 ml, 50 mmol) in MeOH (25 ml) was added phenylhydrazine (4.92 ml, 50 mmol) dropwise with constant stirring, which immediately precipitated to give a solid yellow Schiff base. The product was filtered, washed with hexane and dried in vacuo. To the solution of Schiff base (3.91 g, 20 mmol) in MeOH (50 ml) was added diethylxoxalate (1.32 ml, 10 mmol) in 2:1 molar ratio. This solution was
refluxed for ca. 1 hour and cooled at room temperature, then conc. HCl (5 ml) was added dropwise with constant stirring. The resulting mixture was refluxed for ca. 6 hour and later the volume of the solution was reduced to half on rotatory evaporator. This solution was kept aside for some time while green crystalline solid product appeared which was filtered and dried in vacuo. The product separated above (13.33 g, 2 mmol) was dissolved in MeOH (25 ml) and ethanolamine (0.32 ml, 4 mmol) was added in 1:2 molar ratio. The resultant mixture was set aside till orange solid product LH₂ separated out on evaporation. It was thoroughly washed with MeOH and dried under vacuum.

**Synthesis of [LCu''₂Cl₂] [C₃₂H₃₀N₆O₂Cu₂Cl₂]**

To a solution of the ligand LH₂ (0.53 g, 1 mmol) in dichloromethane (25 ml) was added CuCl₂·2H₂O (0.34 g, 2 mmol) in MeOH in 1:2 molar ratio. The coloured product was isolated by slow evaporation of the resultant mixture, filtered, washed with hexane and dried in vacuo.

**Synthesis of [LMn''₂Cl₂] [C₃₂H₃₀N₆O₂Mn₂Cl₂]**

Ligand LH₂ (0.53 g, 1 mmol) in dichloromethane (25 ml) and MnCl₂·4H₂O (0.32 g, 2 mmol) in MeOH were added together. The resulting mixture was set aside for slow evaporation. A green solid product obtained, was filtered off, washed with hexane and dried in vacuo.
Scheme 5
**Scheme 6a**

*Synthesis of [LCo$^{	ext{II}}_2$Cl$_2$] $[\text{C}_{32}\text{H}_{30}\text{N}_6\text{O}_2\text{Co}_2\text{Cl}_2]$*

To a methanolic solution of CoCl$_2$·6H$_2$O (0.47 g, 2 mmol) was added ligand LH$_2$ (0.53 g, 1 mmol) in dichloromethane (25 ml). The reaction mixture was left for sometime for slow evaporation, a brown solid product was collected, washed thoroughly with hexane and dried in vacuo.

*Synthesis of [LNi$^{	ext{II}}_2$Cl$_2$] $[\text{C}_{32}\text{H}_{30}\text{N}_6\text{O}_2\text{Ni}_2\text{Cl}_2]$*

To a solution of the ligand LH$_2$ (0.53 g, 1 mmol) in dichloromethane (25 ml) was added NiCl$_2$·6H$_2$O (0.47 g, 2 mmol) in MeOH in 1:2 molar ratio. The reddish brown coloured product was isolated by slow evaporation of the resultant mixture, filtered, washed with hexane and dried in vacuo.
Synthesis of \([\text{LCu}^{II}_2\text{Sn}^{IV}\text{Cl}_6]\) \([\text{C}_{32}\text{H}_{30}\text{N}_6\text{O}_2\text{Cu}_2\text{SnCl}_6]\)

To a solution of complex \(\text{LCu}^{II}_2\text{Cl}_2\) (0.72 g, 1 mmol) in dichloromethane (25 ml) was added \(\text{SnCl}_4\) solution (0.11 ml, 1 mmol) in \(\text{CCl}_4\) (5 ml). The resulting solution was kept aside for 4 hour until dark purple solid was separated. The product was isolated, washed thoroughly with hexane and dried in vacuo.

Synthesis of the complex \([\text{LNi}^{II}_2\text{Sn}^{IV}\text{Cl}_6]\) \([\text{C}_{32}\text{H}_{30}\text{N}_6\text{O}_2\text{Ni}_2\text{SnCl}_6]\)

\(\text{LNi}^{II}_2\text{Cl}_2\) (0.71 g, 1 mmol) complex was dissolved in dichloromethane was allowed to react with \(\text{SnCl}_4\) (0.11 ml, 1 mmol) in \(\text{CCl}_4\) (5 ml). On slow evaporation dark brown solid was obtained. The product was isolated, washed thoroughly with hexane and dried in vacuo.

\[
\begin{align*}
\text{LM}^{II}_2\text{Cl}_2 + \text{SnCl}_4 &\rightarrow \text{LM}^{II}_2\text{SnCl}_6,
\end{align*}
\]

\(M=\text{Cu}^{II}, \text{Ni}^{II}\)

Scheme 6b
Results and discussion

Schiff base compartmental ligand abbreviated LH₂ was synthesized by a three step synthetic route represented in Scheme 5, which contains two donor set N₂ and N₂O₂. The dinuclear complexes LCu"₂Cl₂, LMn"₂Cl₂, LCo"₂Cl₂, LNi"₂Cl₂ and trinuclear complexes 1.Cu"₂Sn⁴⁺Cl₆, LNi"₂Sn⁴⁺Cl₆ were synthesized and characterized by various physico-chemical methods. Their proposed structures are shown in Scheme 6 a,b. In the ligand LH₂, diazine N-N nitrogen atoms do not participate in bond formation due to the formation of a three membered ring, which will be unstable. Thus, only terminal nitrogen atoms of diazine participate in complexation with two chloride ions in inner sphere showing a square planar environment around the metal ion. This is also authenticated by elemental analysis and conductance data, which show that the complex is covalent in nature. However, in N₂O₂ cavity of ligand LH₂, two Schiff base nitrogens and oxygen atoms are involved in coordination with the second metal ion by substitution of 2Cl⁻ as 2HCl. The second metal ion in the N₂O₂ environment exhibit rhombic geometry.

Furthermore, homodinuclear complexes were further metallated with SnCl₄ to achieve new hetero trinuclear complexes of the type [LM"₂Sn⁴⁺Cl₆] where M= Cu(II) and Ni(II).

Both the complexes are air stable, soluble in CH₂Cl₂, DMF and DMSO (Table 14).

IR spectra

The IR spectrum of the ligand LH₂ reveals two characteristic bands at ca. 1651 cm⁻¹ and 1688 cm⁻¹ attributed to azomethine ν(C=N) groups [276]. In the complexes LCu"₂Cl₂,
LMn$^{II}$Cl$_2$, LCo$^{II}$Cl$_2$ and LNi$^{II}$Cl$_2$ the peak at 1651 cm$^{-1}$ remains unaltered indicating that the two azomethine groups of N–N diazine linkage are not involved in complex formation. However, the band at 1688 cm$^{-1}$ was shifted to lower frequency (70-80 cm$^{-1}$) exhibiting the coordination of azomethine nitrogen atom of N$_2$O$_2$ cavity to the metal ions. Coupled with this observation, the free ligand (LH$_2$) also shows peaks at 1025 cm$^{-1}$ and 1509 cm$^{-1}$ assigned to $\nu$(N–N) and $\nu$(C–N) groups, respectively [277]. The shift in $\nu$(N–N) and $\nu$(C–N) bands to lower frequency by ca. 18-27 cm$^{-1}$ support the involvement of two nitrogen atoms of diazine linkage in complexation. The $\nu$(O–H) band at 3305 cm$^{-1}$ in the free ligand [278] is absent in the spectra of complexes suggesting deprotonation of alcoholic group in coordination. This is further confirmed by the appearance of $\nu$(M–N), $\nu$(M–Cl) and $\nu$(M–O) absorptions at 530-537 cm$^{-1}$, 360-365 cm$^{-1}$ and 475-482 cm$^{-1}$, respectively in the far IR region (Table 15). In addition, complexes LCu$^{II}$Sn$^{IV}$Cl$_6$ and LNi$^{II}$Sn$^{IV}$Cl$_6$ show one distinct $\nu$(Sn–O) band at 465-470 cm$^{-1}$ confirming the formation of trinuclear complexes [279].

Electronic spectra

The absorption spectrum of the ligand LH$_2$ recorded at room temperature in dichloromethane exhibits one sharp band at 380 nm assigned to $n$$\rightarrow$$\pi^*$ transition associated with the azomethine chromophore. The absorption spectrum of complex LCu$^{II}$Cl$_2$ shows a broad band at 532 nm attributed to $^2$B$_{1g}$$\rightarrow$$^2$A$_{1g}$ transition, [280] which is well within the range of 500-555 nm expected for square planar and rhombic Cu(II)
complexes. Both square planar and rhombic geometries exhibit d-d absorption bands in same region, therefore it is difficult to distinguish these absorption bands [281]. The tetrahedral complexes also exhibit a narrow band below 100 nm. However, no such bands are observed in the present complexes, thus ruling out tetrahedral or pseudo tetrahedral geometry. The complex LNi\textsuperscript{II}\textsubscript{2}Cl\textsubscript{2} has diamagnetic behaviour and its electronic spectrum shows a shoulder at 497 nm ascribed to $^1A_{1g} \rightarrow ^1B_{1g}$ transition supporting the square planar geometry around Ni(II) ion [228]. The weak bands at 470 nm and 446 nm in complexes LMn\textsuperscript{II}2Cl\textsubscript{2} and LCo\textsuperscript{II}2Cl\textsubscript{2}, respectively, also support the square planar geometry of the metal ions [201].

**NMR studies**

\textsuperscript{1}H and \textsuperscript{13}C NMR spectra of the ligand (LH\textsubscript{2}), complexes LNi\textsuperscript{II}2Cl\textsubscript{2} and LNi\textsuperscript{II}2Sn\textsuperscript{IV}Cl\textsubscript{6} recorded in CD\textsubscript{2}Cl\textsubscript{2} and CDCl\textsubscript{3} exhibits characteristic signals observed at 6.8-7.2 ppm and 7.9-8.2 ppm [282] assigned to aromatic protons and azomethine groups, respectively, suggesting the nonparticipation of azomethine group present in the complex formation. Additionally, \textsuperscript{1}H NMR spectrum of the ligand LH\textsubscript{2} reveals two signals at 3.5 ppm and 6.2 ppm attributed to CH\textsubscript{2}O group [232] and OH group respectively. On complexation, the resonance of CH\textsubscript{2}O group shifted slightly downfield relative to the free ligand, supporting the coordination of CH\textsubscript{2}O group to the metal center in the N\textsubscript{2}O\textsubscript{2} tetradentate Schiff base cavity. However, the signal observed at 6.2 ppm ascribed to OH group [283] completely
disappears which confirms the coordination of the metal to oxygen atom by the elimination of two HCl molecules (Table 16).

The complex LNi\textsuperscript{II}_2Sn\textsuperscript{IV}Cl\textsubscript{6} show well-resolved \textsuperscript{13}C NMR spectrum characterized by chemical shifts significantly different from those observed in the free ligand and complex LNi\textsuperscript{II}_2Cl\textsubscript{2}. The peak at 125 ppm is attributed to C=N which is slightly high field in comparison to free ligand indicating the coordination of C=N group to metal center in complex LNi\textsuperscript{II}_2Cl\textsubscript{2}. The \textsuperscript{13}C NMR spectrum of complex LNi\textsuperscript{II}_2Sn\textsuperscript{IV}Cl\textsubscript{6} exhibits signals at 77.0 ppm and 126 ppm ascribed to N−CH\textsubscript{2}−CH\textsubscript{2}−O and Ar−C group, respectively (Table 17).

Cyclic voltammetry studies

The electrochemical methods are complementary tools over the previously used methods of studying interaction of complexes towards CT DNA. The cyclic voltammograms (CV) of complexes LCu\textsuperscript{II}_2Cl\textsubscript{2} and LCu\textsuperscript{II}_2Sn\textsuperscript{IV}Cl\textsubscript{6} were obtained in H\textsubscript{2}O/DMSO (95:5) solution, at scan rate 0.2 V s\textsuperscript{-1} over a potential range from 1.6 to −1.2 V. In the absence of CT DNA, the anodic peak potential (E\textsubscript{pa}) of complex LCu\textsuperscript{II}_2Cl\textsubscript{2} appeared at −0.48 V and the cathodic (E\textsubscript{pc}) at −0.58 V (Figure 87 a). The CV of complex LCu\textsuperscript{II}_2Cl\textsubscript{2} reveals a one electron quasireversible wave attributed to the redox couple Cu(II)/Cu(I) with the formal electrode potential, \(E^0 = −0.53 \text{ V}\), the ratio of anodic peak current to cathodic peak current (I\textsubscript{pa}/I\textsubscript{pc}) is 0.47 and \(\Delta E_p = ± 0.10 \text{ V}\) which is larger than the Nernstian value observed for the one electron transfer couple. On addition of CT DNA, the complex
Figure 87. Cyclic voltammogram (scan rate 0.2 Vs⁻¹, DMSO, 25⁰C, pH 7.5) of (a) complex \( \text{LCu}^{II}_2\text{Cl}_2 \) alone and (b) complex \( \text{LCu}^{II}_2\text{Cl}_2 \) in presence of CT DNA. \([\text{LCu}^{II}_2\text{Cl}_2]\) 1x10⁻³ M, \([\text{DNA}]\) 6 x 10⁻³ M
LCu$^{II}_2$Cl$_2$ shows a shift in $E_{pc}$ (-0.58 V), $E_{pa}$(-0.47 V) and $\Delta E_p$ (0.10 V) values indicating strong binding of dinuclear complex with CT DNA (Figure 87 b). The decrease in ratio of anodic to cathodic peak currents from 0.47 to 0.33 signify that adsorption of Cu(I) is enhanced in the presence of CT DNA [147]. Further, the shift in $E^0$ value and increase in peak height potentials suggest that both Cu(II) and Cu(I) form of complex L$\text{Cu}^{II}_2\text{Cl}_2$ bind to CT DNA [210]. The ratio of equilibrium constants $K_+/K_{2+}$ for the binding of the Cu(II) and Cu(I) forms of complex L$\text{Cu}^{II}_2\text{Cl}_2$ to CT DNA have also been calculated using the following equation (Scheme 7)

$$E_b^0 - E_f^0 = 0.059 \log \left(\frac{K_+}{K_{2+}}\right)$$

suggesting that both Cu(II) and Cu(I) forms interact with DNA to the same extent [233].

![Scheme 7](image)

The cyclic voltammogram of complex L$\text{Cu}^{II}_2\text{Sn}^{IV}\text{Cl}_6$ in absence of CT DNA shows $\Delta E_p$ = 0.10 V (Figure 88 a.) In addition to changes in the formal potential, the voltammetric peak currents decrease upon addition of CT DNA to the complex (Figure 88 b).
Figure 88. Cyclic voltammogram (scan rate 0.2 Vs⁻¹, DMSO, 25°C, pH 7.5) of (a) complex \( \text{LCu}^{II}_{2\text{Sn}^{IV}\text{Cl}_6} \) (b) complex \( \text{LCu}^{II}_{2\text{Sn}^{IV}\text{Cl}_6} \) in presence of CT DNA at different time intervals. \([\text{LCu}^{II}_{2\text{Sn}^{IV}\text{Cl}_6}] 1 \times 10^{-3} \text{ M}, [\text{DNA}] 6 \times 10^{-3} \text{ M}\)
The decrease in current may be due to the diffusion of an equilibrium mixture of free and DNA-bound metal complex to the electrode surface [284]. The cyclic voltammogram is not affected by tin atom, as tin atom is not readily accessible to the electrode surface in this range [285].

Absorption spectral features of DNA binding

Electronic absorption spectroscopy is universally employed to determine the binding of complexes with DNA. Figure 89 shows the absorption spectra of complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) and \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) (1.6 \( \times \) 10\(^5\) M) in the presence of increasing amounts of CT DNA (1.6 - 6.4 \( \times \) 10\(^5\) M). In the UV region, complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) shows an intense absorption band at 256 nm attributed to ligand to metal charge transfer (LMCT) band. The interaction of complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) with CT DNA results small perturbation in LMCT band with red shift of 2 nm which is probably due to the binding of the complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) to the DNA bases through covalent bond formation at the vacant position of the coordination sphere of the metal ion [214, 218]. On the other hand, complex \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) displays two well resolved bands at 252 nm and 325 nm attributed to LMCT bands. Upon the addition of CT DNA, complex \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) exhibits hyperchromism in both the absorption bands. There is also an appreciable blue shift of 5 nm in one of the LMCT bands, from 325-320 nm. The increase in absorbance and blue shift of 5 nm in this region suggests that complex \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) strongly binds to CT DNA. Such findings in the light of previous
Figure 89. Absorption spectral traces of (a) complex LCu"Cl₂ (b) complex LCu"Sn"Cl₆ in Tris HCl buffer (0.01 M, pH 7.5) upon addition of CT DNA. Inset: Plots of [DNA] / εₐ εₐ vs. [DNA] for the titration of CT DNA with complexes; ■: experimental data points, full lines: linear fitting of the data. [Complex] 1.6 x 10⁻³ M, [DNA] 1.6-6.4 x 10⁻³ M
reports [215, 240, 286] support the view that complex $\text{LCu}^{II}_2\text{Sn}^{IV}\text{Cl}_6$ possess three metal centers, two Cu(II) and one Sn(IV) atom having different specificity at the molecular level. Complex $\text{LCu}^{II}_2\text{Sn}^{IV}\text{Cl}_6$ has better prospectus as an antitumour agent than complex $\text{LCu}^{II}_2\text{Cl}_2$ as it contains one Sn(IV) atom, a hard Lewis acid which additionally binds electrostatically to the phosphate backbone of the DNA helix [110] while the Cu(II) center may preferentially coordinate with the $N_7$ position of guanine, thus perturb the hydrogen bonding between the base pairs and results in destabilization of DNA [287]. In fact, Sn(IV) phosphate binding has been detected both in solution and solid state [99, 288].

Quantitative parameter binding constant $K_b$ has also been calculated to compare the binding affinity of complexes $\text{LCu}^{II}_2\text{Cl}_2$ and $\text{LCu}^{II}_2\text{Sn}^{IV}\text{Cl}_6$ towards CT DNA. The $K_b$ values for complexes $\text{LCu}^{II}_2\text{Cl}_2$ and $\text{LCu}^{II}_2\text{Sn}^{IV}\text{Cl}_6$ are $3.2 \times 10^3 \text{ M}^{-1}$ and $9.6 \times 10^3 \text{ M}^{-1}$, respectively. The $K_b$ value of complex $\text{LCu}^{II}_2\text{Sn}^{IV}\text{Cl}_6$ is higher than that of complex $\text{LCu}^{II}_2\text{Cl}_2$ but is lower than those observed for classical intercalators (EB-DNA $1.4 \times 10^6$ in 25 mM tris-HCl/ 40 mM NaCl buffer pH = 7.9, $3.0 \times 10^6$ in 5 mM Tris-HCl/ 50 mM NaCl buffer, pH = 7.2). This is an indicative of the binding of the complexes with CT DNA but with an affinity less than the classical intercalators.

**Fluorescence studies**

No luminescence was observed for the complexes $\text{LCu}^{II}_2\text{Cl}_2$ and $\text{LCu}^{II}_2\text{Sn}^{IV}\text{Cl}_6$ upon excitation at the $n \rightarrow \pi^*$ band either in DMSO or in the presence of CT DNA.
Figure 90. Emission spectra of EthBr bound to DNA in the presence of (a) complex $LCu^{II}_2Cl_2$ (b) complex $LCu^{II}_2Sn^{IV}Cl_6$ in Tris-HCl buffer $[EthBr] = 2 \times 10^{-5} M$, $[DNA] = 1 \times 10^{-4} M$, $[complex] = 0.8 \times 10^{-5} M$, $\lambda_{ex} = 510 nm$, $\lambda_{em} = 610 nm$
Hence competitive binding studies using EthBr bound to DNA was carried out for these complexes. The quenching extent of fluorescence of EthBr bound to DNA is utilized to determine the extent of binding of complexes and DNA [289]. Binding of the complex results in the displacement of bound EthBr molecule with the reduction of emission intensity due to fluorescence quenching of free EthBr by water [290].

The emission spectra of EthBr bound to DNA in presence and absence of complex LCu^{II}_2Cl_2 and LCu^{II}_2Sn^{IV}Cl_6 are given in Figure 90. The addition of the complex LCu^{II}_2Sn^{IV}Cl_6 to CT DNA pretreated with EthBr causes appreciable reduction in the emission intensity, indicating that the displacement of the EthBr fluorophore by the complex LCu^{II}_2Sn^{IV}Cl_6 results in a decrease of the binding of the ethidium to the DNA [171, 291]. However, slight decrease in emission intensity is observed for the complex LCu^{II}_2Cl_2 indicating low binding affinity of complex LCu^{II}_2Cl_2 with CT DNA in comparison to complex LCu^{II}_2Sn^{IV}Cl_6.

The fluorescence-quenching curves of EthBr bound to DNA by the complex LCu^{II}_2Cl_2 and LCu^{II}_2Sn^{IV}Cl_6 are shown in Figure 91. The Stern-Volmer quenching constant K values for complexes LCu^{II}_2Sn^{IV}Cl_6 and LCu^{II}_2Cl_2 are 0.45 and 0.10, respectively, [290] suggesting that complex LCu^{II}_2Sn^{IV}Cl_6 interacts with CT DNA more strongly in comparison to complex LCu^{II}_2Cl_2 which is consistent with the absorption studies results.
Figure 91. Fluorescence quenching curve of DNA bound EthBr by complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) (●) complex \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) (■) \([\text{EthBr}] = 2 \times 10^{-5} M, [\text{DNA}] = 1 \times 10^{-4} M, [\text{complex}] = 0-8 \times 10^{-3} M\).

Viscosity studies

To further clarify the binding modes of the complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) and \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) with DNA, viscosity measurements were carried out. There is a marked effect of complexes \( \text{LCu}^{II}_{2}\text{Cl}_2 \) and \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) on the viscosity of CT DNA as depicted in Figure 92. With an increasing amount of complex \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \), the relative viscosity of DNA decreases while the increasing amount of complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) further reduces the effective length of DNA which supports that both the complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) and \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) bind through partial non classical intercalation mode but with different affinity. However, complex \( \text{LCu}^{II}_{2}\text{Sn}^{IV}\text{Cl}_6 \) shows strong binding in comparison to complex \( \text{LCu}^{II}_{2}\text{Cl}_2 \) presumably due to electrostatic interaction with DNA [237].
Conclusions

The new homodinuclear complexes LCu$^{II}_2$Cl$_2$, LMn$^{II}_2$Cl$_2$, LCo$^{II}_2$Cl$_2$, LN$^{II}_2$Cl$_2$ and heterotrinuclear complexes LCu$^{II}_2$Sn$^{IV}_2$Cl$_6$, LN$^{II}_2$Sn$^{IV}_2$Cl$_6$ were synthesized from compartmental Schiff base ligand containing N$_2$ and N$_2$O$_2$ donor sets in different environments. The interaction studies of complex LCu$^{II}_2$Cl$_2$ and LCu$^{II}_2$Sn$^{IV}_2$Cl$_6$ with CT DNA were carried out employing absorption studies, cyclic voltammetry, fluorescence studies and viscosity measurements. Analysis of the results suggests that heterotrinuclear complex LCu$^{II}_2$Sn$^{IV}_2$Cl$_6$ binds to CT DNA mainly by electrostatic interaction that is different from the binding mode of homodinuclear complex LCu$^{II}_2$Cl$_2$. The $K_b$ values of
complex LCu$^{II}_2$Cl$_2$ and LCu$^{II}_2$Sn$^{IV}$Cl$_6$ is $3.2 \times 10^3$ M$^{-1}$ and $9.6 \times 10^3$ M$^{-1}$, respectively, which indicate that the complex LCu$^{II}_2$Sn$^{IV}$Cl$_6$ binds more avidly to CT DNA than complex LCu$^{II}_2$Cl$_2$ which is quite evident as complex LCu$^{II}_2$Sn$^{IV}$Cl$_6$ possess three metal centers. The two ‘copper’ centers may be associated covalently or by non-covalent interactions, however the ‘tin’ metal ion, a hard Lewis acid binds electrostatically to neutralize the dinegative charge of phosphate sugar and brings structural and conformational changes in DNA helix. These studies suggest that complex LCu$^{II}_2$Sn$^{IV}$Cl$_6$ having tin modulation has a better prospectus as an antitumour agent than the complex LCu$^{II}_2$Cl$_2$ which may have activity only due to copper metal ions.
Table 14. Physical and analytical data of the ligand and complexes

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<tr>
<th>Complexes</th>
<th>Colour</th>
<th>M.p. (°C)</th>
<th>Yield (%)</th>
<th>C</th>
<th>Found (calcd) (%)</th>
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<tbody>
<tr>
<td>LH₂</td>
<td>Orange</td>
<td>120</td>
<td>75</td>
<td>71.9 (72.1)</td>
<td>5.9 (6.0)</td>
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<tr>
<td>LCu^{II}_{2}Cl₂</td>
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<td>98</td>
<td>70</td>
<td>52.5 (52.8)</td>
<td>4.1 (4.1)</td>
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<tr>
<td>LMn^{II}_{2}Cl₂</td>
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<td>100</td>
<td>68</td>
<td>54.2 (54.1)</td>
<td>4.2 (4.3)</td>
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<tr>
<td>LCo^{II}_{2}Cl₂</td>
<td>Brown</td>
<td>200</td>
<td>66</td>
<td>53.4 (53.6)</td>
<td>4.0 (4.1)</td>
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<td>LNí^{II}_{2}Cl₂</td>
<td>Reddish brown</td>
<td>110</td>
<td>68</td>
<td>53.4 (53.5)</td>
<td>4.3 (4.2)</td>
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<tr>
<td>LCu^{II}_{2}Sn^{IV}Cl₆</td>
<td>Violet</td>
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<td>60</td>
<td>39.0 (39.0)</td>
<td>3.1 (3.0)</td>
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<tr>
<td>LNí^{II}_{2}Sn^{IV}Cl₆</td>
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<td>150</td>
<td>63</td>
<td>39.3 (39.4)</td>
<td>3.0 (3.0)</td>
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Table 15. IR data for ligand and complexes (cm⁻¹)

<table>
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<th>Groups</th>
<th>LH₂</th>
<th>LCu^{II}_2Cl₂</th>
<th>LMn^{II}_2Cl₂</th>
<th>LCo^{II}_2Cl₂</th>
<th>LNi^{II}_2Cl₂</th>
<th>LCu^{II}_2Sn^{IV}Cl₆</th>
<th>LNi^{II}_2Sn^{IV}Cl₆</th>
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<tbody>
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<td>ν(C=Ω) (N₂O₂ donor set)</td>
<td>1688</td>
<td>1608</td>
<td>1610</td>
<td>1612</td>
<td>1611</td>
<td>1615</td>
<td>1618</td>
</tr>
<tr>
<td>ν(N–N)</td>
<td>1025</td>
<td>1007</td>
<td>1005</td>
<td>1000</td>
<td>1002</td>
<td>1004</td>
<td>1006</td>
</tr>
<tr>
<td>ν(C–N)</td>
<td>1509</td>
<td>1490</td>
<td>1488</td>
<td>1489</td>
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<td>1484</td>
<td>1482</td>
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### Table 16. $^1$H NMR data of Ligand LH$_2$ and Complexes LN$^{II}_2$Cl$_2$ and LN$^{II}_2$Sn$^{IV}$Cl$_6$ (ppm)

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### Table 17. $^{13}$C NMR data of Ligand LH$_2$ and Complexes LN$^{II}_2$Cl$_2$ and LN$^{II}_2$Sn$^{IV}$Cl$_6$ (ppm)

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CHAPTER VII

Template Synthesis of Novel Carboxamide Dinuclear Copper (II) Complex: Spectral Characterization and Reactivity towards Calf-Thymus DNA
CHAPTER VII

Experimental

All reagents were of the best commercial grade and were used without further purification. Phthalic anhydride, CuCl$_2$.2H$_2$O, NiCl$_2$.6H$_2$O were procured from E. Merck and 1,8-diamino-3,6-diazaoctane from Fluka. Microanalyses were performed using Carlo Erba Analyzer Model 1108. Copper and nickel contents were determined on GBC 932 Plus atomic absorption spectrophotometer. Molar conductances were measured at room temperature on a Digisun Electronic conductivity Bridge. Fourier-transform IR (FTIR) spectra were recorded on an Interspec 2020 FTIR spectrometer, as KBr pellets. UV/vis spectra were recorded on a USB 2000 Ocean Optics spectrometer and the data were reported as $\lambda_{\text{max}}$/nm. The solid state EPR spectrum of the copper complex was acquired on a Varian E 112 spectrometer using X-band frequency (9.1GHz) at LNT. ESI-MS spectra were recorded on Micromass Quattro II triple quadrupole mass spectrometer. The NMR spectra were obtained on a Bruker DRX-300 spectrometer.
Synthesis of Bis [aqua 1,8-(1,2-dicarboxamido benzene)-3,6-diazoctane copper (II)]
tetrachloride [MacCu₂(BC)]Cl₄ [C₂₈H₄₄N₈O₆Cu₂Cl₄]
1,8-diamino-3,6-diazoctane (2.98 ml, 20 mmol) was added dropwise to the ethanolic
solution of CuCl₂2H₂O (3.40 g, 20 mmol) in a 1:1 molar ratio. The resulting deep blue
solution, was stirred at 25 °C for 30 minute and subsequently phthalic anhydride (2.90 g,
20 mmol) dissolved in 25 ml ethanol was added to it. The reaction mixture was refluxed
for ca. 1 hour and a light blue solid product was obtained. The product was filtered,
washed with ethanol and dried in vacuo.

Synthesis of Bis [aqua 1,8-(1,2-dicarboxamido benzene)-3,6-diazoctane nickel (II)]
tetrachloride [MacNi₂(BC)]Cl₄ [C₂₈H₄₄N₈O₆Ni₂Cl₄]
To the ethanolic solution of NiCl₂6H₂O (4.74 g, 20 mmol) was added dropwise 1,8-
diamino-3,6-diazoctane (2.98 ml, 20 mmol) in a 1:1 molar ratio. The resulting purple
solution, was stirred at 25 °C for 20 minutes and subsequently phthalic anhydride (2.90 g,
20 mmol) dissolved in 20 ml ethanol was added to it. The reaction mixture turned green,
which was refluxed for ca. 4 hours and a light green solid product was obtained. The
product was filtered, washed with ethanol and dried in vacuo.
1,8-Diamino-3,6-diazaoctane + 2M Cl₂ (hydrated) + 2 Phthalic anhydride

- 2H₂O EtOH

\[ \text{M} = \text{Cu(II), Ni(II)} \]

Scheme 8
Results and discussion

The synthetic procedure for the complexes is illustrated in Scheme 8. CHN analysis and spectral data (UV/vis, IR, EPR, $^1$H, $^{13}$C NMR, and atomic absorption) were consistent with the proposed formulation of the dinuclear complexes. The new complexes [MacCu$_2$(BC)]Cl$_4$ and [MacNi$_2$(BC)]Cl$_4$ are air and moisture stable solids with solubility in water and DMSO, respectively but they are insoluble in methanol and diethyl ether. The molar conductance value of [MacCu$_2$(BC)]Cl$_4$ and [MacNi$_2$(BC)]Cl$_4$ in water and DMSO, respectively indicates that these complexes are 1:4 electrolytes (Table 18). Analytical data revealed that the complexes contain two metal centers and a water molecule in the coordination sphere. These indications were further supported by atomic absorption data and IR spectral bands. DNA binding studies were performed with the Cu(II) complex and analogous Ni(II) complex was synthesized only for NMR studies.

IR Spectral studies

The IR spectra of the complexes [MacCu$_2$(BC)]Cl$_4$ and [MacNi$_2$(BC)]Cl$_4$ showed characteristic bands in the region 3150-3268 cm$^{-1}$ ascribed to $\nu$(N–H) of carboxamide moiety [292]. A medium intensity sharp band was observed in the region 1704-1713 cm$^{-1}$ assigned to $\nu$(C=O) of carboxamide linkage [293]. Previous reports on carboxamide ligands also support these assignments [294-295] and confirm the non-involvement of $>\text{C}=\text{O}$ group in coordination with the metal ion. Additionally, signals at $\sim$1394, $\sim$2877 and $\sim$2933 cm$^{-1}$ were attributed to $\nu$(C–N), $\nu$(CH$_2$) and aromatic $\nu$(CH)
vibrations, respectively. A broad band in the range 3300-3500 cm\(^{-1}\), assigned to \(\nu(\text{O-H})\) of the water molecule present in the lattice or coordinated to metal ion was observed in the complexes [215]. The far IR spectra of the complexes \([\text{MacCu}_2(\text{BC})]\text{Cl}_4\) and \([\text{MacNi}_2(\text{BC})]\text{Cl}_4\) exhibited absorptions at 416 and 433 cm\(^{-1}\), ascribed to \(\nu(\text{Cu-N})\) and \(\nu(\text{Ni-N})\), respectively (Table 19).

**Absorption spectral studies**

The absorption spectra of the complexes \([\text{MacCu}_2(\text{BC})]\text{Cl}_4\) and \([\text{MacNi}_2(\text{BC})]\text{Cl}_4\) (1x10\(^{-3}\) M) were recorded at 25\(^{\circ}\)C in H\(_2\)O and DMSO, respectively in 200-800 nm as depicted in Figure 93. The solution spectra of the complexes were observed as three distinct bands. Complex \([\text{MacCu}_2(\text{BC})]\text{Cl}_4\) displayed a weak broad band around 619 nm due to d-d transition of the cupric ion. This band was assigned to \(d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}\) transition characteristic of square pyramidal geometry of Cu(II) complexes [296]. The diamagnetic Ni(II) complex \([\text{MacNi}_2(\text{BC})]\text{Cl}_4\) showed a d-d band at 576 nm, assigned to \(^3\text{B}_1(\text{F}) \rightarrow ^3\text{E}(\text{F})\) transition. The \(\lambda_{\text{max}}\) value was consistent with a pentacoordinate environment around the Ni(II) ion [297]. In addition, complex \([\text{MacCu}_2(\text{BC})]\text{Cl}_4\) and \([\text{MacNi}_2(\text{BC})]\text{Cl}_4\) displayed two strong bands in the UV region one at 245 nm attributed to intraligand charge transfer transitions and another at 268 nm assigned to ligand to metal charge transfer transition.
Figure 93. Absorption spectra of [MacCu₂(BC)]Cl₄ (1x10⁻¹ M) in H₂O (curve a) [MacNi₂(BC)]Cl₄ (1x10⁻³ M) in DMSO (curve b) in the spectral range 220-800 nm.

NMR spectral studies

The complex [MacNi₂(BC)]Cl₄ was characterized by ¹H and ¹³C NMR spectroscopy in DMSO-d₆ (Figure 94). The ¹H NMR spectrum of the complex [MacNi₂(BC)]Cl₄ showed signals for aliphatic and aromatic protons with chemical shift values in accordance with the proposed structure. The ¹H NMR spectrum revealed characteristic signal at 8.1 ppm of the amide –NH proton [298]. Multiplets in the range of 7.8-7.4 ppm arise due to aromatic protons. Signals due to CH₂ protons (adjacent to amide –NH proton) were observed from 4.2-3.1 ppm [299]. Furthermore, resonances at 3.0 and 2.9-2.6 ppm were assigned to (NCH₂) and NCH₂CH₂ protons of presence of 1,8-diamino-3,6-diazaoctane [228].
Figure 94. \(^1\)H NMR spectrum of complex [MacNi\(_3\)(BC)]Cl\(_4\) in DMSO-\(d_6\)

The \(^{13}\)C NMR spectrum of complex [MacNi\(_3\)(BC)]Cl\(_4\) exhibited a characteristic signal at 167.8 ppm due to >C=O functional group of amide linkage (Figure 95) [300]. Additionally multiplets in the region 122-134 ppm were ascribed to aromatic carbons. Signals in the range 35.3-40.3 ppm were assigned to N–C–C–N carbon atoms [148]. Thus \(^1\)H and \(^{13}\)C NMR data are in well agreement with the proposed structure (Table 20).
The X-band electron paramagnetic resonance spectrum of complex [MacCu₂(BC)]Cl₄ was recorded at frequency of 9.1GHz under the magnetic field strength 3000 ± 1000 gauss using TCNE as field marker at LNT. Generally, the spectral parameters reflect the usual increase of the equatorial ligand field in four coordinate complexes vs. the five coordinate counterparts. The complex [MacCu₂(BC)]Cl₄ showed an anisotropic spectrum with different $g_{||}$ and $g_{\perp}$ values. The EPR spectrum of complex [MacCu₂(BC)]Cl₄
exhibited \( g_\| = 2.20 \), \( g_\perp = 2.04 \) values and \( g_{av} = 2.09 \) computed from the formula \( g_{av}^2 = g_\|^2 + 2g_\perp^2 / 3 \). The values \( g_\| \) and \( g_\perp \) were in good agreement with an essentially \( d_{x^2-y^2} \) copper (II) ground state and were anticipated for square pyramidal geometry [301]. The order \( g_\| > g_\perp > g_e \) (2.0023) further confirm that the ground state of copper (II) is predominantly \( d_{x^2-y^2} \). The g values are also related to the axial symmetry parameter, G, by the expression \( G = (g_\| - 2)/(g_\perp - 2) \). The G value measures the extent of the exchange interaction between two copper centers in the polycrystalline solids. If \( G < 4 \), considerable exchange interaction occurs, whereas if \( G > 4 \) exchange interaction is negligible. In the present case \( G = 4.56 \) which confirm that the two Cu(II) centers are too far apart to interact with each other [302].

**DNA binding studies**

Metal complexes generally bind to DNA both through covalent as well as non covalent modes. When the present dinuclear Cu(II) complex interacts with DNA, the two labile water molecules in the complex are replaced by a nucleophiles on DNA, usually a nitrogenous base such as guanine N7, leading to a strong covalent binding of the complex with DNA [303]. In addition, incorporation of more than one Cu(II) center in a single complex produces enhanced electrostatic interactions to the anionic DNA phosphate backbone and facilitates its binding to DNA [304]. The mode and propensity of binding of the complex [MacCu2(BC)]Cl4 to calf thymus DNA has been detected by absorption
titration, fluorescence spectroscopy, circular dichroism studies, cyclic voltammetry and viscosity measurement.

**Absorption studies**

Electronic absorption spectroscopy is universally employed to determine the binding of complexes with the DNA helix. Any interaction between the complex and DNA is expected to perturb the ligand centered spectral transitions of the complex [33]. The absorption spectrum of complex [MacCu$_2$(BC)]Cl$_4$, exhibited bands around 245, 268 and 619 nm. On the incremental addition of CT DNA (0.8 - 4.1 x $10^{-4}$ M) to the complex [MacCu$_2$(BC)]Cl$_4$ (1.6 x $10^{-4}$ M), a considerable increase in molar absorptivity (Figure 96)

![Figure 96](image)

**Figure 96. Hyperchromism of complex [MacCu$_2$(BC)]Cl$_4$ upon addition of CT DNA at 245 nm**
accompanied by a red shift of 7 and 12 nm, respectively of the $\pi \rightarrow \pi^*$ absorption bands was observed. The hyperchromic and hypochromic effects are the spectral features of DNA binding concerning its double helix structure [236]. Therefore, the observed hyperchromic changes in the UV spectrum of the complex [MacCu$_2$(BC)]Cl$_4$ suggest strong binding of CT DNA, probably due to covalent bonding (Figure 97).

![Figure 97. UV spectral traces of complex [MacCu$_2$(BC)]Cl$_4$, in Tris-HCl buffer (0.01 M, pH 7.2) upon addition of CT DNA. Inset: Plots of [DNA]/ $\varepsilon_{578}$ vs. [DNA] for the titration of CT DNA with complex [MacCu$_2$(BC)]Cl$_4$, and linear fitting of the data. Concentration of [MacCu$_2$(BC)]Cl$_4$ = 1.6 x 10$^{-4}$ M, [DNA] = 0.8 - 4.1 x 10$^{-4}$ M]
Structurally, complex \([\text{MacCu}_2(\text{BC})]\text{Cl}_4\) provides two water molecules directly attached to the metal ion, which can be replaced by a nucleophile in DNA, usually a nitrogenous base such as guanine \(N_7\) leading to a strong binding of the complex \([\text{MacCu}_2(\text{BC})]\text{Cl}_4\) [31, 250, 305]. The present ligand system lack extended \(\pi\)-systems thus ruling out the possibility of any intercalative interactions but have the potential to bind to DNA covalently. Furthermore, appreciable changes are observed also in the ligand field (LF) band of the complex \([\text{MacCu}_2(\text{BC})]\text{Cl}_4\). Intensity of the LF band at 619 nm was found to decrease (hypochromism) with the addition of CT DNA as shown in Figure 98.

\[\text{Figure 98. Absorption spectral traces of complex } [\text{MacCu}_2(\text{BC})] \text{Cl}_4 \text{, in Tris HCl buffer } (0.01 \text{ M, pH 7.2}) \text{ upon addition of CT DNA in the LF band. Concentration of } [\text{MacCu}_2(\text{BC})] \text{Cl}_4 = 1.6 \times 10^{-4} \text{ M, [DNA]} = 0.8 - 4.1 \times 10^{-4} \text{ M} \]
These results further support that the complex [MacCu₂(BC)]Cl₄ is coordinated presumably to a DNA base such as guanine N₇ [161, 173]. Additionally, a strong hydrophobic interaction between the macrocyclic moiety and the hydrophobic DNA interior is plausible [171]. It is interesting that the complex [MacCu₂(BC)]Cl₄ may sterically clash with the DNA surface, and the coordinated –NH– and carbonyl oxygens of complex [MacCu₂(BC)]Cl₄ may involve in hydrogen bonding with the N₇/O₆ sites of the intrastrand guanine bases. Therefore, it is reasonable to assess that complex [MacCu₂(BC)]Cl₄ binds covalently to CT DNA. Nevertheless, hydrogen-bonding interactions cannot be ruled out. Our results are consistent with a number of earlier reports on DNA interaction [132, 237].

To enable quantitative estimation of DNA binding affinity of complex [MacCu₂(BC)]Cl₄, the intrinsic binding constant K_b was determined using eq (1) by concomitant recording of the UV traces with increasing concentration of DNA. The intrinsic binding constant K_b for complex [MacCu₂(BC)]Cl₄ was determined as K_b = 1.95 x 10⁴ M⁻¹ which is of lower magnitude than those of classical intercalator (EB-DNA 1.4 x 10⁶ M⁻¹ in 25 mM tris-HCl/ 40 mM NaCl buffer pH = 7.9, 3.0 x 10⁶ M⁻¹ in 5 mM Tris-HCl/ 50 mM NaCl buffer, pH = 7.2).
Emission spectral studies

As the complex [MacCu₂(BC)]Cl₄ is non-emissive both in the presence and absence of CT DNA, competitive ethidium bromide (EthBr) binding studies were undertaken to gain support for the mode of binding of the complex [MacCu₂(BC)]Cl₄ with DNA. The molecular fluorophore EthBr emits intense fluorescence in the presence of CT DNA due to its strong intercalation between the adjacent DNA base pairs and stabilization of its excited state [251]. The study involves addition of the complexes to DNA pretreated with EthBr and then measurement of emission intensities of DNA-bound EthBr. The extent of quenching of fluorescence of EthBr bound to DNA would reflect the extent of binding of complexes to DNA [289].

Two mechanisms have been proposed to account for the quenching of EthBr emission, the replacement of the molecular fluorophores (if complexes binds to DNA more strongly than EthBr) and/or by electron transfer. The non replacement-based quenching has been correlated with DNA-mediated electron transfer from the excited EthBr to an acceptor (e.g. cupric ion, Cu²⁺) [161]. The emission intensity of DNA-bound EthBr is quenched considerably on addition of complex [MacCu₂(BC)]Cl₄ (Figure 99). As complex [MacCu₂(BC)]Cl₄ binds to DNA via covalent bonding, it cannot displace the strongly DNA-bound EthBr. So the observed quenching is obviously due to the facile intramolecular photo induced electron transfer from the excited EthBr to complex [MacCu₂(BC)]Cl₄ bound to DNA (E₁/₂ 140mV) [306]. Perhaps, it is possible that the low energy ligand based π* orbitals in complex [MacCu₂(BC)]Cl₄ facilitate the photo induced
electron transfer. Besides, the more rigid and shorter A-like conformation of DNA, is likely stabilized by the complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$ through hydrophobic interaction in DNA grooves is also not suitable for intercalative interaction by EthBr leading to its destabilization of the excited state of bound EthBr [169].

**Figure 99.** Emission spectra of EthBr bound to DNA in the presence of complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$ in Tris-HCl buffer. $[\text{EthBr}] = 2 \times 10^{-5} \text{ M}$, $[\text{DNA}] = 0.3 \times 10^{-4} \text{ M}$, $[\text{complex}] = 0 - 1.2 \times 10^{-4} \text{ M}$. Arrow shows the intensity change upon increasing concentration of the complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$

The fluorescence quenching curve of EthBr bound to DNA by the complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$ is shown in Figure 100. The quenching plot illustrate that the quenching of EthBr bound to DNA by the complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$ is in good
agreement with the linear Stern-Volmer equation. The value of quenching constant $K$ for the complex $[\text{MacCu}_2(BC)]\text{Cl}_4$ estimated using eq. (2) is 0.16, suggesting the strong interaction of the complex $[\text{MacCu}_2(BC)]\text{Cl}_4$ with DNA, which is consistent with the above absorption spectral results.

![Graph](image)

**Figure 100.** Fluorescence quenching curve of DNA bound EthBr by complex $[\text{MacCu}_2(BC)]\text{Cl}_4$. [EthBr] = $2 \times 10^{-5} \text{ M}$, [DNA] = $0.3 \times 10^{-4} \text{ M}$, [complex] = $0 - 1.2 \times 10^{-4} \text{ M}$

**Circular dichroic spectral studies**

The circular dichroic spectrum of calf thymus DNA exhibited a band at 278 nm due to base stacking and at 248 nm, due to right handed helicity (Figure 101, curve a). These bands are quite sensitive to the mode of DNA interaction with complexes [307]. On interaction with metal complexes, the changes in the CD spectrum of DNA may correspond to the changes in DNA structure [308]. Simple groove binders and
electrostatic interaction of complexes show less or no perturbation on the base-stacking and helicity bands, while intercalators enhance the intensities of both the bands stabilizing the right-handed B conformation of CT DNA as observed for classical intercalator [309].

Figure 101. CD spectra of CT DNA alone (curve a) (1 x 10^{-4} \text{M}, \text{Tris HCl buffer, 25}^\circ\text{C, pH 7.2}) CT DNA in presence of complex [MacCu_{2}(BC)]Cl_{4} (1 x 10^{-4} \text{M}) (curve b)

Upon addition of complex [MacCu_{2}(BC)]Cl_{4}, the CD spectrum of DNA undergoes changes in both the positive and negative bands (Figure 101, curve b) with slight shift in the band positions. In the presence of complex [MacCu_{2}(BC)]Cl_{4}, the positive band
showed remarkable decrease in molar ellipticity revealing significant Cu(II) complex-CT DNA interaction [218]. The extended π systems reduce the helical twist angle of the DNA base pairs and increase the intensity of the base-stacking band [310-311]. Thus the decrease in the stacking band on addition of complex [MacCu₂(BC)]Cl₄ rules out the intercalation mode of binding. Furthermore, decrease in the intensity of DNA helicity band indicates the hydrophobic interaction of –NH– groups with DNA. Such spectral changes are characteristic of B to A conformational change [254]. These CD spectral results support that the complex [MacCu₂(BC)]Cl₄ interact with CT DNA, and that the binding event induces certain conformational changes in DNA.

**Viscometry studies**

To support the DNA binding mode, viscosity measurements complex [MacCu₂(BC)]Cl₄ bound to DNA has been undertaken. The changes in the specific relative viscosity of DNA on addition of increasing concentrations of complex [MacCu₂(BC)]Cl₄ are shown in Figure 102. The decrease in relative viscosity of DNA observed for the complex [MacCu₂(BC)]Cl₄ suggest covalent binding of the complex [MacCu₂(BC)]Cl₄ with CT DNA, which produced bends or kinks in the DNA and thus reduced its effective length and concomitantly its viscosity [161]. These result suggests that complex [MacCu₂(BC)]Cl₄ may bind to DNA covalently.
Figure 102. Effects of increasing amount of complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$ on the relative viscosity of CT DNA at $29.00 \pm 0.01^\circ$C. $[\text{DNA}] = 4 \times 10^{-4} \, M$, pH 7.2

Redox studies

Cyclic voltammetry has been employed to study the interaction of the complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$ with CT DNA with a view to further explore the DNA binding modes assessed from the absorption, emission and viscometric studies. The cyclic voltammogram of the complex $[\text{MacCu}_2(\text{BC})]\text{Cl}_4$ in the absence of CT DNA (Figure 103, curve a) reveal a non-Nernstian but a fairly reversible/quasi-reversible one electron redox process involving the Cu(II)/Cu(I) couple, as judged from the peak potential separation ($\Delta E_p$) and formal potential ($E_{1/2}$) of 110mV (59mV for one electron transfer process) and 230mV, respectively. At different scan rates, the cyclic voltammogram did not show any major changes.
Figure 103. Cyclic voltammogram (scan rate 0.1 Vs^{-1}, H_{2}O, 25^{0}C, pH 7.2) of complex [MacCu_{2}(BC)]Cl_{4} alone (curve a) and complex [MacCu_{2}(BC)]Cl_{4} in presence of CT DNA. (curve b). Concentration of [MacCu_{2}(BC)]Cl_{4} 1 \times 10^{-3} M, [DNA] 6 \times 10^{-3} M

Keeping all the parameters constant (T= 25^{0}C, scan rate 0.1 Vs^{-1}, potential range 0.60 to -0.10 V), complex [MacCu_{2}(BC)]Cl_{4} experienced a significant reduction in cathodic peak current on addition of DNA (Figure 103, curve b) which was due to the slow diffusion of an equilibrium mixture of the free and DNA-bound complex [MacCu_{2}(BC)]Cl_{4} to the electrode surface [250]. Further, the observed shift (90 mV) in \(E_{1/2}\) value to more negative potential suggest that both Cu(II) and Cu(I) forms of the complex [MacCu_{2}(BC)]Cl_{4} bind to DNA but with Cu(II) displaying higher DNA binding affinity than Cu(I) form [303].
This is illustrated by the ratio of the equilibrium constants \( \frac{K_+}{K_{2+}} \) for the binding of Cu(I) and Cu(II) species to DNA. The value has been estimated from the net shift in \( E_{1/2} \) on the addition of DNA assuming reversible electron transfer using the following equation:

\[
E_b^0 - E_f^0 = 0.0591 \log \left( \frac{K_+}{K_{2+}} \right)
\]

where \( E_b^0 \) and \( E_f^0 \) are the formal potentials of the Cu(II)/Cu(I) couple in the bound and free forms, respectively, and \( K_+ \) and \( K_{2+} \) the corresponding binding constants for the binding of the 1+ and 2+ species to DNA, respectively (Scheme 9). The \( K_+/K_{2+} \) value for complex [MacCu_2(BC)]Cl_4 is 0.03, which is less than unity suggesting preferential stabilization of Cu(II) form over Cu(I) form on binding to DNA [210, 233].
Conclusion

The spectroscopic and analytical data presented above clearly indicate that the dinuclear Cu(II)/Ni(II) complexes resulting from the reaction between 1,8-diamino-3,6-diazaoctane, CuCl$_2$.4H$_2$O/NiCl$_2$.6H$_2$O and phthalic anhydride contain two benzene-1,2 dicarboxamide units closed by (CH$_2$)$_2$-NH−(CH$_2$)$_2$-NH−(CH$_2$)$_2$ links. The dinuclear Cu(II) complex is highly water soluble and exhibit square pyramidal geometry with two water molecules coordinated to metal ion. The biological target of this complex is unknown, however, we have shown here that the complex [MacCu$_2$(BC)]Cl$_4$ is able to bind to CT DNA under physiological pH 7.2. Supported by the various DNA binding experiments, it is likely that complex [MacCu$_2$(BC)]Cl$_4$ interacts covalently with DNA, by replacement of the coordinated H$_2$O molecules with DNA bases. In addition, the presence of coordinated −NH− groups facilitates hydrogen-bonding interactions with DNA. Therefore, the DNA binding studies of complex [MacCu$_2$(BC)]Cl$_4$ could provide information good enough for the rational design of new hydro soluble DNA binding agent. The final scope of this work is to better understand the interaction of water-soluble copper complex with DNA in view of its potential application in chemotherapy.
Table 18. Physical and analytical data of the complexes

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<td>Green</td>
<td>77</td>
<td>210</td>
<td>325(^b)</td>
<td>39.6 (39.7)</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.2 (5.2)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) in H₂O
\(^b\) in DMSO
Table 19. IR data of complexes [MacCu\textsubscript{2} (BC)]Cl\textsubscript{4} and [MacNi\textsubscript{2} (BC)]Cl\textsubscript{4} (cm\textsuperscript{-1})

<table>
<thead>
<tr>
<th>Functional Groups</th>
<th>[MacCu\textsubscript{2} (BC)]Cl\textsubscript{4}</th>
<th>[MacNi\textsubscript{2} (BC)]Cl\textsubscript{4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\nu(\text{N-H})) (of carboxamide moiety)</td>
<td>3152</td>
<td>3263</td>
</tr>
<tr>
<td>(\nu(\text{C=O}))</td>
<td>1713</td>
<td>1704</td>
</tr>
<tr>
<td>(\nu(\text{C-N}))</td>
<td>1398</td>
<td>1394</td>
</tr>
<tr>
<td>(\nu(\text{CH}_2))</td>
<td>2877</td>
<td>2880</td>
</tr>
<tr>
<td>aromatic (\nu(\text{CH}))</td>
<td>2933</td>
<td>2952</td>
</tr>
<tr>
<td>(\nu(\text{O-H}))</td>
<td>3450</td>
<td>3385</td>
</tr>
<tr>
<td>(\nu(\text{M-N}))</td>
<td>416</td>
<td>433</td>
</tr>
</tbody>
</table>
Table 20. NMR data of the complex [MacNi₂ (BC)]Cl₄ (ppm)

<table>
<thead>
<tr>
<th>Groups</th>
<th>¹H</th>
<th>¹³C</th>
</tr>
</thead>
<tbody>
<tr>
<td>—NH— of amide</td>
<td>8.1</td>
<td>—</td>
</tr>
<tr>
<td>Ar protons</td>
<td>7.8-7.4</td>
<td>—</td>
</tr>
<tr>
<td>—CH₂ (adjacent to amide —NH)</td>
<td>4.2-3.1</td>
<td>—</td>
</tr>
<tr>
<td>—NCH₂</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>—NCH₂CH₂</td>
<td>2.9-2.6</td>
<td>—</td>
</tr>
<tr>
<td>&gt;C=O</td>
<td>—</td>
<td>167.8</td>
</tr>
<tr>
<td>Ar—C=</td>
<td>—</td>
<td>122-134</td>
</tr>
<tr>
<td>N—C—C—N</td>
<td>—</td>
<td>35.3-40.3</td>
</tr>
</tbody>
</table>