Synthesis and Biophysical Studies of Bis-macrocyclic cobalt/copper (II) Complexes having Pyridine Spacer with CT DNA and 5’ GMP
CHAPTER V

Experimental

Reagent grade Chemicals and solvents were used without further purification. CoCl₂,2H₂O, NiCl₂,6H₂O, CuCl₂,2H₂O, Tris-base (Merck), 1,8-diamino-3,6-diazaoctane (Fluka), formaldehyde, perchloric acid (BDH) and 2,6-diaminopyridine (Lancaster) were used as received. Calf thymus DNA (CT DNA) and guanosine-5’-monophosphate disodium salt (5’GMP) were purchased from Sigma chemical Co. and Fluka, respectively.

Molar conductances $\lambda_M$ (in $\Omega^{-1}$cm$^2$ mol$^{-1}$): at 25 °C, Digisun electronic conductivity bridge. UV/Vis Spectra: USB 2000 Ocean Optics spectrometer; H₂O solns. $\lambda_{\text{max}}$ in nm. IR Spectra: KBr pellets, Interspec 2020 FT-IR spectrometer; in cm$^{-1}$. $^{13}$C and $^1$H NMR Spectra: at 75 MHz and 300 MHz respectively; Bruker-DRX-300 spectrometer; δ in ppm. EPR Spectra: Varian-E-112 spectrometer; at the X-band frequency (9.1GHz) at liq. N₂ temp. Elemental analyses: Carlo Erba analyzer model 1108. Electrospray mass spectra; Micromass Quattro II triple quadrupol mass spectrometer.

All the experiments involving interaction of the complex with CT DNA were performed in twice distilled buffer containing tris(hydroxymethyl)-aminomethane (Tris, 0.01 M) and adjusted to pH 7.5 with hydrochloric acid. Solution of 5’GMP was prepared in double distilled water. Absorption spectral titration experiments at constant concentration of the complexes [6.6 x 10$^{-5}$ M] while varying the CT DNA concentration and 5’GMP were
performed on USB 2000 Ocean Optics spectrometer and UV-1700 PharmaSpec UV-Vis spectrophotometer (Shimadzu) respectively. Cyclic voltammetric studies were performed on a CH Instrument Electrochemical analyzer in a single compartmental cell with 0.4 M KNO₃ as a supporting electrolyte. A three-electrode configuration was used comprising of a Pt wire as auxiliary electrode, platinum micro cylinder as working electrode and Ag/AgCl as the reference electrode. Electrochemical measurements were made under a dinitrogen atmosphere. All electrochemical data were collected at 25°C and are uncorrected for junction potentials.

**Synthesis of 2,6-bis[1,3,6,9,12-pentaazacyclotridecane pyridine cobalt(II)] perchlorate** \[C_{21}H_{43}N_{11}Co_2\] (ClO₄)₄

To a stirred methanol solution of CoCl₂.2H₂O (2.37 g, 10 mmol) were slowly added 1,8-diamino-3,6-diazaoctane (1.49 mL, 10 mmol), 37% formaldehyde (3.03 mL, 20 mmol) and 2,6- diaminopyridine (0.54 g, 5 mmol). The resulting mixture was refluxed for ca. 24 h until a dark reddish brown solution appeared. This solution was cooled and filtered under vacuum. Excess perchloric acid in methanol was added to the filtrate and left to stand over night. Reddish brown solid product was filtered off, thoroughly washed with methanol and dried in vacuo over fused CaCl₂.
Synthesis of 2,6-bis[1,3,6,9,12-pentaazacyclotridecane pyridine nickel(II)] perchlorate [C_{21}H_{43}N_{11}Ni_2] (ClO_4)_4

This dark brown coloured complex was synthesized by a procedure similar to that described for [C_{21}H_{43}N_{11}Co_2] (ClO_4)_4 using NiCl_2·6H_2O (2.37 g, 10 mmol).

Synthesis of 2,6-bis[1,3,6,9,12-pentaazacyclotridecane pyridine copper(II)] perchlorate [C_{21}H_{43}N_{11}Cu_2] (ClO_4)_4

This light brownish complex was obtained by the method analogous to that for [C_{21}H_{43}N_{11}Co_2] (ClO_4)_4 using CuCl_2·2H_2O (1.70 g, 10 mmol).

Results and discussion

The new bis-macrocyclic complexes [C_{21}H_{43}N_{11}Co_2](ClO_4)_4, [C_{21}H_{43}N_{11}Ni_2](ClO_4)_4 and [C_{21}H_{43}N_{11}Cu_2](ClO_4)_4 have been synthesized by the template condensation reaction of 1,8-diamino-3,6-diazaoctane, formaldehyde and 2,6-diaminopyridine with Co(II), Ni(II) and Cu(II) chlorides, respectively. These complexes were isolated by the addition of perchloric acid in methanol as depicted in Scheme 3. These complexes are stable at room temperature and are soluble in polar solvents such as water, DMF and DMSO but insoluble in methanol and diethylether. The values of molar conductance measured in water indicate that the complexes are 1:4 electrolytes. Analytical data were consistent with the proposed formulation of the bis-macrocyclic complexes. DNA binding studies were performed with [C_{21}H_{43}N_{11}Co_2](ClO_4)_4 and [C_{21}H_{43}N_{11}Cu_2](ClO_4)_4 complexes. The analogous nickel complex was synthesized only for NMR studies. The important properties and physical data of the complexes are given in Table 9.
Scheme 3. Synthetic route for the bis-macrocyclic complexes $[C_{21}H_{43}N_{11}Co_2](ClO_4)_4$, $[C_{21}H_{43}N_{11}Ni_2](ClO_4)_4$, and $[C_{21}H_{43}N_{11}Cu_2](ClO_4)_4$.

Infrared spectra

The solid-state IR spectra of the bis-macrocyclic complexes $[C_{21}H_{43}N_{11}Co_2](ClO_4)_4$, $[C_{21}H_{43}N_{11}Ni_2](ClO_4)_4$, and $[C_{21}H_{43}N_{11}Cu_2](ClO_4)_4$ exhibit peak at 1558-1650 cm$^{-1}$ and a sharp peak at 1462-1505 cm$^{-1}$ attributed to the azomethine $\nu(C=\text{N})$ and $\nu(C=\text{C})$ stretching modes, [255,256] respectively indicating the presence of pyridine spacer moiety in the complexes. The absence of characteristic $\nu(NH_2)$ vibration [257] at 3400
cm$^{-1}$ supports the deprotonation of the amine groups and the formation of the macrocyclic framework via condensation with formaldehyde. The bands observed in the range 1538-1550 cm$^{-1}$ corresponding to $\nu$(C—N) confirm the macrocyclic structure of complexes. Additionally, broad bands at 3200-3250 cm$^{-1}$ were ascribed to the stretching vibration of $\nu$(NH) of the (NH—CH$_2$—CH$_2$—NH) linkage [258]. Other diagnostic bands of medium intensity at 2769-2781 cm$^{-1}$ and 1300-1400 cm$^{-1}$ were assigned to C—H stretching and bending vibrational modes, respectively [259]. The bands in the region 480—513 cm$^{-1}$ were ascribed to the $\nu$(M—N) stretching vibrations. The spectra also display strong absorption bands near 1000 cm$^{-1}$ (antisymmetric stretch) and sharp bands at 625—633 cm$^{-1}$ (antisymmetric bend) due to the uncoordinated ClO$_4^-$ anions [260-262] (Table 10).

**NMR spectral studies**

To further elucidate the structure of the bis-macroyclic complexes, the diamagnetic complex [C$_2$H$_{43}$N$_{11}$Ni$_2$](ClO$_4$)$_4$ was characterized by $^1$H and $^{13}$C NMR spectroscopy, showing characteristic aliphatic and aromatic signals with chemical shifts in accordance with the proposed structure (Table 11). The aromatic region of the spectrum of [C$_2$H$_{43}$N$_{11}$Ni$_2$](ClO$_4$)$_4$ was consistent with the presence of a doublet at 7.69-8.02 ppm and a well-resolved triplet at 6.97-7.31 ppm due to equivalent and non-equivalent hydrogens from the pyridine spacer [263]. Comparison of $^1$H NMR spectra for free 2,6-diaminopyridine with [C$_2$H$_{43}$N$_{11}$Ni$_2$](ClO$_4$)$_4$ also evidences that condensation of 2,6-diaminopyridine has occurred and confirmed the formation of the bis-macrocycle. The $^1$H NMR spectrum of the free 2,6-diaminopyridine displays primary amine proton signals at
5.29 and 5.59-5.61 ppm which disappear in \([C_{21}H_{43}N_{11}Ni_2](ClO_4)_4\) with simultaneous emergence of a new prominent peak at 2.58 ppm corresponding to the -CH\(_2\)-N-CH\(_2\)-linkage protons [264]. A series of non-overlapping multiplets in the high field region at 3.12-3.88 ppm and 4.86 ppm were attributed to the -(CH\(_2\))\(_2\)-NH-(CH\(_2\))\(_2\)-NH- chain of the macrocyclic framework [265, 266].

The \(^{13}\text{C}\) NMR spectrum reveals signals in the region 32.6-39.04, 45.58-48.77 and 51.31-55.73 ppm representing - CH\(_2\) - carbons of macrocyclic backbone [267]. Peaks at 134.23 and 120.12 ppm appear due to the aromatic ring carbons. This pattern of \(^{13}\text{C}\) NMR resonances was consistent with \(^1\text{H}\) NMR data and agrees well with the proposed bis-architecture of the complexes (Table 12).

**Mass spectroscopy**

ESI MS mass spectroscopy provides the key evidence for the formation of the macrocyclic complexes. The ESI MS spectrum of the \([C_{21}H_{43}N_{11}Ni_2](ClO_4)_4\) complex shows peaks at m/z 868, 385, 223 and 141 corresponding to the molecular ions of the formulations \([M-ClO_4^- + 3H^+]^+, [M-2ClO_4^- + 2H^+]^{2+}, [M-3ClO_4^- + H^+]^{3+}\) and \([M-4ClO_4^-]^{4+}\) respectively, resulting from the stepwise loss of the perchlorate anions (Table 13). The mass spectrum also shows some prominent peaks representing successive fragments of the complex molecule. The appearance of peaks at m/z 522, 161, 121, 305 and 100 are ascribed to the species \([C_{13}H_{23}N_5Cl_2O_8Ni], [C_{12}H_{23}N_5Cl_2O_6Ni - 2ClO_4^-]^{2+}, [C_6H_{20}N_5Cl_2O_8Ni - 2ClO_4^-]^{2+}, [C_6H_{16}N_4Cl_2O_8Ni - ClO_4^-]^{1+}\) and \([C_6H_{16}N_4Cl_2O_8Ni - 2ClO_4^-]^{2+}\) respectively. The peaks occurring at m/z 149 and 77 corresponding to 1,8-
diamino-3,6-diazaoctane and pyridine group, respectively, further confirm this fragmentation pattern.

**Electronic absorption Spectra**

The UV/Vis absorption spectra of \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_{2}](\text{ClO}_{4})_{4}\), \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Ni}_{2}](\text{ClO}_{4})_{4}\) and \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_{2}](\text{ClO}_{4})_{4}\) complexes (1x10⁻³ M) were recorded at 25 °C in H₂O. In the UV region the complexes display two main transitions i) an intense band at 325-340 nm, attributed to LMCT transitions and ii) a strong band at 230-250 nm ascribed to intraligand charge transfer transitions [268]. The visible absorption spectra of complex \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_{2}](\text{ClO}_{4})_{4}\) was characterized by an absorption band at 425 nm due to the metal d-d electronic transition attributed to \(^{1}A_{1g} \rightarrow ^{1}B_{1g}\) transition [269] consistent with a square planar geometry around the cobalt metal ion. Similarly, complex \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Ni}_{2}](\text{ClO}_{4})_{4}\) displayed a shoulder at 415 nm [229] assigned to the \(^{1}A_{1g} \rightarrow ^{1}A_{2g}\) transition of low spin Ni(II) in a square planar environment. Complex \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_{2}](\text{ClO}_{4})_{4}\) shows a distinct absorption band at 585 nm due to ligand field d-d transitions assigned to \(^{2}B_{1g} \rightarrow ^{2}A_{2g}\) transitions, again in agreement with the square planar geometry of Cu(II) ion [270].

**EPR spectral studies**

The solid state X-band EPR spectra of the complex \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_{2}](\text{ClO}_{4})_{4}\) was acquired at a frequency of 9.1 GHz under the magnetic field strength 3,000±1,000 guass using tetracyanoethylene (TCNE) as field marker at LNT. The complex shows an anisotropic spectrum with \(g_{||} = 2.049\) and \(g_{\perp} = 2.01\) and \(g_{av} = 2.02\) computed from the expression \(g_{av} = \frac{g_{||} + 2g_{\perp}}{3}\).
These parameters are in good agreement to the values reported for other related square planar Cu(II) systems \[271\] and are typical of axially symmetrical d\(^9\) Cu(II) complexes. The trend \(g_\parallel > g_\perp > 2\) reveals that the unpaired electron is present in the \(d_y^2\) orbital \[272\]. For a Cu(II) complex, \(g_\parallel\) is a parameter sensitive enough to indicate covalence. For a covalent complex, \(g_\parallel < 2.3\) and for an ionic environment, \(g_\parallel = 2.3\) or more. In the present complex \(g_\parallel < 2.3\) indicates an appreciable metal-ligand covalent character \[273\]. The G factor defined as \(G = (g_\parallel - 2)/(g_\perp - 2)\) indicative of exchange interactions between the Cu(II) sites equal to four suggests negligible exchange interactions.

**Biophysical studies with CT DNA and 5’GMP**

DNA binding is the critical step for many cytotoxic compounds as it is the primary pharmacological target of antitumor drugs. Metal complexes are particularly attractive systems to study as the ligating organic molecules are held firmly in place by the metal ions, giving specific shape and surface features to the complex that can be exploited to affect the DNA-metallocomplex interaction. The mode and propensity of binding of \([C_{21}H_{43}N_{11}Co_2](ClO_4)_4\) and \([C_{21}H_{43}N_{11}Cu_2](ClO_4)_4\) complexes to CT DNA has been evaluated by the absorption titration, luminescence titration, cyclic voltammetry and viscosity measurements.

**Absorption titration**

The potential CT DNA binding ability of complexes \([C_{21}H_{43}N_{11}Co_2](ClO_4)_4\) and \([C_{21}H_{43}N_{11}Cu_2](ClO_4)_4\) were studied by UV/Vis spectroscopy by following the intensity
changes of the intraligand transition bands. Figure 50a-b displays the absorption spectra of $[\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_{2}](\text{ClO}_4)_4$ and $[\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_{2}](\text{ClO}_4)_4$ in the absence and presence of CT DNA respectively. On addition of CT DNA to the aqueous solution of $[\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_{2}](\text{ClO}_4)_4$ and $[\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_{2}](\text{ClO}_4)_4$ there is an incremental increase in absorbance of IL transitions. A moderate bathochromic shift (4-5 nm) in UV region was also observed for $[\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_{2}](\text{ClO}_4)_4$ and $[\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_{2}](\text{ClO}_4)_4$. Hyperchromicity with bathochromic shift is a feature of DNA binding mode either through covalent coordinate linkage to N7 nucleobase of DNA [116, 274] or electrostatic interaction to the major/minor groove of DNA helix [275].

Figure 50a. UV spectral traces of $[\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_{2}](\text{ClO}_4)_4$ complex in Tris-HCl buffer (0.01M, pH 7.2) upon addition of CT DNA. Inset: Plots of $[\text{DNA}] / \varepsilon_{r-r}$ versus $[\text{DNA}]$; ($\bullet$), experimental data points; full lines, linear fitting of the data. $[\text{complex}] = 6.6 \times 10^{-3}$ M.
Figure 50b. *UV spectral traces of [C$_{21}$H$_{43}$N$_{11}$Cu$_2$](ClO$_4$)$_4$ complex in Tris HCl buffer (0.01M, pH 7.2) upon addition of CT DNA.* Inset: Plots of [DNA]/$e_e$-$e_f$ versus [DNA]; (■), experimental data points; full lines, linear fitting of the data; [complex] = 6.6 x 10$^{-3}$ M.

There is every possibility for both binding modes due to the molecular structure of the complexes—strong Lewis acid metal centres separated at an optimum distance, which favours coordination to the N7 of guanine nucleobase while electrostatic interaction could be due to interaction of positively charged metal ions with negatively charged oxygens of the phosphate backbone of the DNA helix. Since no significant changes were observed in the LF bands covalent/coordinate linkage to the N7 of nucleobase is strongly ruled out. This is also evidenced by the absorption titration with 5′GMP. Intercalation which leads to hypochromic spectral feature [276] is again ruled out in complexes [C$_{21}$H$_{43}$N$_{11}$Co$_2$](ClO$_4$)$_4$ and [C$_{21}$H$_{43}$N$_{11}$Cu$_2$](ClO$_4$)$_4$. Therefore it is reasonable to assume that the complexes bind electrostatically to DNA. The cationic core of
[C21H43Ni1Co2](ClO4)4 and [C21H43Ni1Cu2](ClO4)4 could exert an enhanced electrostatic attraction due to an increase in the positive charge by the incorporation of more than one metal centers in a single molecule [277]. The higher the positive charge a dinuclear species possesses, the more cellular uptake is observed [278]. This feature along with high binding affinity of [C21H43Ni1Co2](ClO4)4 and [C21H43Ni1Cu2](ClO4)4 may facilitate access to DNA target in a tumor cell. Besides this, divalent cations can repel and displace other counterions, leading to strong attraction with the proximal phosphate groups. The result is DNA bending by electrostatic collapse around a divalent cation [279]. Such general affinity would induce structurally ill-defined DNA aggregates [280]. Recently Farrell et al. [281] have reported a phosphate backbone-binding mode for a polynuclear Platinum (II) complex that has planar arrays of hydrogen bond donors leading to association with the DNA phosphate backbone. It is obvious that the coordinated –NH-groups of the present macrocyclic complexes may also sterically clash with the DNA surface and involve in the hydrogen bonding with the phosphate oxygens. Additionally hydrogen bonding, in which the –NH– of the ligand serves as a hydrogen bond donor would be expected to create partial negative charge on the ligand nitrogen atoms. This charge effect would make the ligand a better σ-donor to the metal, rendering the coordination of metal ion to the nucleobase difficult. In order to compare quantitatively the binding strength the intrinsic binding constant K_b for [C21H43Ni1Co2](ClO4)4 and [C21H43Ni1Cu2](ClO4)4 were calculated to be 1.64x10^5 M\(^{-1}\) and 2.05x10^5 M\(^{-1}\) respectively. These values are significantly lower than typical intercalators (EthBr-DNA ~ 10^6 M\(^{-1}\)).
Interaction with 5’ GMP

To corroborate our results of UV/Vis titrations with CT DNA, interaction studies of [C21H43NiCo2](ClO4)4 and [C21H43NiCu2](ClO4)4 with 5’GMP in double distilled water were carried out (Figure 51a–b). The interaction between the metal complex and 5’GMP is expected to perturb the ligand field transitions of the metal complex due to coordinate/covalent linkage either to N7 or O6 of guanine base [282]. However upon addition of 5’GMP, there is substantial increase in the absorption of IL bands in the UV region while small changes were observed at the absorption maxima due to LMCT bands. No shifts in the visible absorption bands were observed which implies that there is no change of the coordination environment of the metal ions [283]. Coordination to the N7 and O6 positions of 5’GMP are thus ruled out, in view of the steric hindrance by the macrocyclic rings. These results reveal that the complexes interact with 5’GMP exclusively through hydrogen bonding of –NH- groups to base nitrogen atom or phosphate oxygen atom or by electrostatic interactions of the metal ions utilizing the phosphate oxygen atoms of the nucleotide.

Fluorescence spectroscopic studies

As the complexes [C21H43NiCo2](ClO4)4 and [C21H43NiCu2](ClO4)4 are non-emissive both in presence and absence of DNA, competitive DNA binding experiments with the proven DNA intercalator, ethidium bromide provided additional information about the DNA binding properties of the bis- macrocyclic complexes [C21H43NiCo2](ClO4)4 and
Figure 51a. UV spectral traces \([C_2iH_{43}Ni_{11}Co_2](ClO_4)_4\) complex in double distilled water upon the addition of 5'GMP.

Figure 51b. UV spectral traces \([C_2iH_{43}Ni_{11}Cu_2](ClO_4)_4\) complex in double distilled water upon the addition of 5'GMP.
[C_{21}H_{43}N_{11}Cu_{2}](ClO_4)_4. EthBr shows reduced fluorescence intensity in a buffer medium due to the quenching by solvent molecules and an increase in fluorescence intensity when bound to DNA, because of its strong intercalation between the DNA base pairs and stabilization of its excited state [284]. Addition of Complexes [C_{21}H_{43}N_{11}Co_{2}](ClO_4)_4 and [C_{21}H_{43}N_{11}Cu_{2}](ClO_4)_4 to EthBr-DNA system decreases the emission intensity at 590 nm indicating that complexes have a good affinity for DNA (Figure 52a-b). The extent of fluorescence quenching reflects the extent of binding of complexes to DNA [285]. Two mechanisms have been proposed to account for the quenching viz; the displacement of ethidium bromide from DNA and electron transfer from excited ethidium to an acceptor (e.g; cupric ion, Cu^{2+}) [286]. As complex [C_{21}H_{43}N_{11}Co_{2}](ClO_4)_4 and [C_{21}H_{43}N_{11}Cu_{2}](ClO_4)_4 bind to DNA via surface binding, displacement of strongly bound EthBr cannot take place. Instead the observed quenching is due to the facile intramolecular photoinduced electron transfer from the excited EthBr to complex bound to DNA [287]. The quenching extent by [C_{21}H_{43}N_{11}Cu_{2}](ClO_4)_4 is larger than [C_{21}H_{43}N_{11}Co_{2}](ClO_4)_4. This is expected, as the reduction of DNA-bound complex [C_{21}H_{43}N_{11}Cu_{2}](ClO_4)_4 is easier than [C_{21}H_{43}N_{11}Co_{2}](ClO_4)_4, (E^0 = -445 mV and -415 mV for [C_{21}H_{43}N_{11}Co_{2}](ClO_4)_4 and [C_{21}H_{43}N_{11}Cu_{2}](ClO_4)_4, respectively). Moreover, it is possible that the low energy ligand based π* orbitals could facilitate the photoinduced electron transfer.
Figure 52a. Emission spectra of EthBr bound to CT DNA in the presence of 
$[C_2H_4N_2O_2Cl_2]$ and in Tris-HCl buffer. Inset: Plots of $I/I_0$ vs [complex]/[DNA]; 
[DNA] = 3.3x10^{-5}M; $\lambda_{ex}$ = 510nm.

Figure 52b. Emission spectra of EthBr bound to CT DNA in the presence of 
$[C_2H_4N_2O_2Cl_2]$ and in Tris-HCl buffer. Inset: Plots of $I/I_0$ vs [complex]/[DNA]; 
[DNA] = 3.3x10^{-5}M; $\lambda_{ex}$ = 510nm.
The emission spectra of the EthBr-bound DNA in the absence and presence of complex
\([\text{C}_2\text{H}_{43}\text{N}_{11}\text{Co}_2](\text{ClO}_4)_4\) and \([\text{C}_2\text{H}_{43}\text{N}_{11}\text{Cu}_2](\text{ClO}_4)_4\) along with the titration plots (inset)
are given in Figure 52a-b. The plots depict that the quenching of EthBr bound to DNA by
the metal complex is in good agreement with the linear Stern-Volmer equation, implying
that the complex competes with EthBr in binding to DNA.

In the plot of \(I_0/I\) vs \(r\), the Stern-Volmer quenching constant \(K_v\) is given by the ratio of
the slope to the intercept. The \(K_v\) value for the complex \([\text{C}_2\text{H}_{43}\text{N}_{11}\text{Co}_2](\text{ClO}_4)_4\) and
\([\text{C}_2\text{H}_{43}\text{N}_{11}\text{Cu}_2](\text{ClO}_4)_4\) as estimated by using equation 2 were found to be 0.07 and 0.44
respectively.

**Viscosity studies**

To explore further the mode of binding to DNA, the viscosity of the CT DNA solution
were measured by varying the concentration of the added metal complexes.
Hydrodynamic method, such as determination of viscosity, which is exquisitely sensitive
to the change in length of DNA, is the most effective means for studying the binding
mode of complexes to DNA in solution especially in absence of crystallographic data
[288]. Electrostatic interactions typically cause less pronounced or no change in the DNA
solution viscosity, while partial intercalation induces static bends in DNA double helix,
reducing its effective length and concomitantly its viscosity [126].

The variation of the relative specific viscosity with addition of the increasing
concentration of the complex \([\text{C}_2\text{H}_{43}\text{N}_{11}\text{Co}_2](\text{ClO}_4)_4\) and \([\text{C}_2\text{H}_{43}\text{N}_{11}\text{Cu}_2](\text{ClO}_4)_4\) is given
in the Figure 53a-b. The viscosity of DNA decreases steadily with increasing
concentration of the metal complex, indicating that the DNA becomes more compact because of its interaction with metal complex. These experimental results support the idea that the complex $[\text{C}_2\text{H}_4\text{N}_1\text{Co}_2](\text{ClO}_4)_4$ and $[\text{C}_2\text{H}_4\text{N}_1\text{Cu}_2](\text{ClO}_4)_4$ bind to DNA by simple electrostatic interactions. In addition to this hydrogen bonding interactions of the coordinated $-\text{NH}-$ groups with the base pairs leads to the bending of the DNA chain resulting decrease in the DNA viscosity [221]. These observations are consistent with the results from UV/Vis and luminescence titrations.

Cyclic Voltammetry

Cyclic Voltammetry is employed to study the interaction of metal complexes with DNA owing to the resemblance between electrochemical and biological reactions. Based on the shift of the formal potentials in the cyclic voltammograms, the relative binding affinities and binding modes of the metal complexes with DNA can be deduced. The redox behavior of the bis-macrocyclic complex $[\text{C}_2\text{H}_4\text{N}_1\text{Co}_2](\text{ClO}_4)_4$ and $[\text{C}_2\text{H}_4\text{N}_1\text{Cu}_2](\text{ClO}_4)_4$ in absence and presence of CT DNA has been studied by means of cyclic voltammetry in H$_2$O/DMSO (95:5) over a sweep range of 1.6 to $-0.8$ V at a scan rate of 0.3 V S$^{-1}$. The CV of bis-macrocyclic complex $[\text{C}_2\text{H}_4\text{N}_1\text{Co}_2](\text{ClO}_4)_4$ (Figure 54) in the absence of CT DNA features a non-Nernstian one quasi-reversible redox wave at cathodic peak potential $E_{pc}$ of $-0.430$ V and and anodic peak potential of $-0.401$ V, attributed to the Co(II)/(I) couple. The formal electrode potential $E_{1/2}$ estimated as the
Figure 53a. Effect of increasing amount of \([C_{21}H_{43}N_{11}Co_2](ClO_4)_4\) complex on the relative viscosity of CT DNA at 29 ± 0.1 °C. [DNA] = 4x10^{-3} M.

Figure 53b. Effect of increasing amount of \([C_{21}H_{43}N_{11}Cu_2](ClO_4)_4\) complex on the relative viscosity of CT DNA at 29 ± 0.1 °C. [DNA] = 4x10^{-3} M.
average of the anodic and cathodic peak potentials is $-0.415 \text{ V} (-415 \text{ mV})$. The ratio of anodic and cathodic peak currents, $I_{pa}/I_{pc} \sim 0.5$ and $\Delta E_p = 0.30 \text{ V} (30 \text{ mV})$ (Nernstian value = 59 mV) imply a one electron transfer process of Co(II)/Co(I). The voltammograms obtained at various scan rates does not show any marked change. In presence of CT DNA at the same concentration of metal complex, there is a considerable shift in the formal potential $E_{1/2} = -0.445 \text{ V}$. The cathodic peak potential shifts to negative side (-0.490 V), while the anodic peak potential shifts to the positive side (-0.400 V). The ratio $I_{pa}/I_{pc}$ decreases (0.43) for the CT DNA bound complex $[C_{21}H_{43}N_{11}Co_2](ClO_4)_4$ suggesting that the complex binds strongly to CT DNA by electrostatic association [289]. Cyclic Voltammogram of the complex $[C_{21}H_{43}N_{11}Cu_2](ClO_4)_4$ (Figure 55) shows a well-defined redox process corresponding to the formation of the Cu(II)/Cu(I) couple with formal

![Figure 54](image_url)
Figure 55. Cyclic voltammograms at a scan rate 0.2 V/s in H$_2$O; curve a [C$_{21}$H$_{43}$N$_{11}$Cu$_2$]ClO$_4$ complex alone; curve b [C$_{21}$H$_{43}$N$_{11}$Cu$_2$]ClO$_4$ complex in presence of CT DNA.

potential $E_{1/2}$ = -0.375 V. The couple is found to be quasi-reversible with $\Delta E_p = 70$ mV and the ratio of the anodic to cathodic peak currents ($I_{pa}/I_{pc}$ = 0.23) correspond to the simple one electron process. The large peak width for the one electron couple Cu(II)/Cu(I) indicates the structural reorganization of the coordination sphere during the electron transfer [290]. Upon the addition of CT DNA the formal electrode potential $E^0$ value (-0.415 V) shifts towards more negative side.

The apparent reduction in peak currents of [C$_{21}$H$_{43}$N$_{11}$Co$_2$]ClO$_4$ and [C$_{21}$H$_{43}$N$_{11}$Cu$_2$]ClO$_4$ upon the addition of CT DNA is attributed to the diffusion of the complexes bound to large, slowly diffusing DNA molecules. These results are comparable with the behaviour of other reported mononuclear and their corresponding
polynuclear complexes [291]. The occurrence of a single redox wave implies that the two copper (II) centers in \([C_2\text{H}_4\text{N}_{11}\text{Cu}_2](\text{ClO}_4)_4\) simultaneously exchange electrons with the electrode and that the coulombic interactions between the metal centers are negligible [292]. Further the shifts in \(E_{1/2}\) to more negative values support the binding of the complexes to DNA surface with significant contributions from electrostatic forces of attraction [293].
Table 9. Physical and analytical data of the complexes \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_2]\)(\text{ClO}_4)_4, \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Ni}_2]\)(\text{ClO}_4)_4\) and \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_2]\)(\text{ClO}_4)_4.\)

<table>
<thead>
<tr>
<th>Complexes</th>
<th>M.p</th>
<th>Colour</th>
<th>Yield</th>
<th>(\lambda_m^a)</th>
<th>Found (calcd) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td>(%)</td>
<td>(\Omega^{-1} \text{cm}^2 \text{mol}^{-1})</td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>([\text{C}<em>{21}\text{H}</em>{43}\text{N}_{11}\text{Co}_2])(\text{ClO}_4)_4</td>
<td>230</td>
<td>Reddish brown</td>
<td>65.0</td>
<td>443</td>
<td>26.32 (26.12)</td>
</tr>
<tr>
<td>([\text{C}<em>{21}\text{H}</em>{43}\text{N}_{11}\text{Ni}_2])(\text{ClO}_4)_4</td>
<td>140</td>
<td>Dark brown</td>
<td>62.0</td>
<td>332</td>
<td>26.22 (26.14)</td>
</tr>
<tr>
<td>([\text{C}<em>{21}\text{H}</em>{43}\text{N}_{11}\text{Cu}_2])(\text{ClO}_4)_4</td>
<td>190</td>
<td>Light brown</td>
<td>60.0</td>
<td>307</td>
<td>25.54 (25.88)</td>
</tr>
</tbody>
</table>

\(a\) - Water
Table 10. IR data of the complexes \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Co}_2](\text{ClO}_4)_4\), \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Ni}_2](\text{ClO}_4)_4\) and \([\text{C}_{21}\text{H}_{43}\text{N}_{11}\text{Cu}_2](\text{ClO}_4)_4\) (cm\(^{-1}\)).

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\nu(\text{NH}))</th>
<th>(\nu(\text{CH}_2))</th>
<th>(\nu(\text{C}==\text{N})_{\text{py}})</th>
<th>(\nu(\text{C}==\text{C})_{\text{py}})</th>
<th>(\nu(\text{ClO}_4^-))</th>
<th>(\nu(\text{M}--\text{N})).</th>
</tr>
</thead>
<tbody>
<tr>
<td>(<a href="%5Ctext%7BClO%7D_4">\text{C}<em>{21}\text{H}</em>{43}\text{N}_{11}\text{Co}_2</a>_4)</td>
<td>3247</td>
<td>2781</td>
<td>1558</td>
<td>1480</td>
<td>1079, 633</td>
<td>513</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BClO%7D_4">\text{C}<em>{21}\text{H}</em>{43}\text{N}_{11}\text{Ni}_2</a>_4)</td>
<td>3250</td>
<td>2769</td>
<td>1650</td>
<td>1505</td>
<td>1085, 625</td>
<td>480</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BClO%7D_4">\text{C}<em>{21}\text{H}</em>{43}\text{N}_{11}\text{Cu}_2</a>_4)</td>
<td>3200</td>
<td>2775</td>
<td>1591</td>
<td>1462</td>
<td>1034, 621</td>
<td>505</td>
</tr>
</tbody>
</table>
Table 11. $^1$H NMR data of $[C_{21}H_{43}N_{11}Ni_2](ClO_4)_4$ complex (ppm).

<table>
<thead>
<tr>
<th>Complex</th>
<th>$-\text{CH}_2-N-\text{CH}_2-$</th>
<th>$-\text{CH}_2-\text{CH}_2-$</th>
<th>$-\text{NH}-$</th>
<th>$\text{Py-H}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$<a href="ClO_4">C_{21}H_{43}N_{11}Ni_2</a>_4$</td>
<td>2.58</td>
<td>3.12-3.88</td>
<td>4.86</td>
<td>7.69-8.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.97-7.31</td>
</tr>
</tbody>
</table>

Table 12. $^{13}$C NMR data of $[C_{21}H_{43}N_{11}Ni_2](ClO_4)_4$ complex (ppm).

<table>
<thead>
<tr>
<th>Complex</th>
<th>$-\text{CH}_2-N-\text{CH}_2-$</th>
<th>$-\text{CH}_2-\text{CH}_2-$</th>
<th>$\text{Py-H}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$<a href="ClO_4">C_{21}H_{43}N_{11}Ni_2</a>_4$</td>
<td>32.6-39.04</td>
<td>51.31-55.73</td>
<td>134.23, 120.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45.58-48.77</td>
<td></td>
</tr>
</tbody>
</table>
Table 13. ESI MS mass spectral data of [C$_{21}$H$_{43}$N$_{11}$Ni$_2$](ClO$_4$)$_4$ complex (m/z).

<table>
<thead>
<tr>
<th>Complex</th>
<th>[M-ClO$_4$' + 3H']$^+$</th>
<th>[M-2ClO$_4$' + 2H']$^{2+}$</th>
<th>[M-3ClO$_4$' + H']$^{3+}$</th>
<th>[M-4ClO$_4$']$^{4+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="ClO$_4$">C$<em>{21}$H$</em>{43}$N$_{11}$Ni$_2$</a>$_4$</td>
<td>868</td>
<td>385</td>
<td>223</td>
<td>141</td>
</tr>
</tbody>
</table>