Synthesis and Spectroscopic Investigations on Nickel(II) and Cobalt(III) Complexes of Dicyandiamide with 2-Alkoxyethanols (Alkoxy = Methoxy, Ethoxy or Butoxy); DNA Binding Interactions on Nickel(II) nitrate Complexes

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Ni(II) and Co(III) complexes of dicyandiamide in alkoxyethanols (alkoxy = methoxy, ethoxy or butoxy) were synthesized and characterized by elemental analysis, magnetic moment, conductance, IR, UV and ^{1}H NMR spectral studies. In the ^{1}H NMR spectra appearance of new multiplet peaks at 3.25-3.50 ppm assignable to CH_3-O-CH_2-CH_2-O- for complex [Ni(II)(1-amidino-O-methoxyethylurea)$_2$](NO$_3$)$_2$ and 3.30-3.45 ppm due to CH_3-CH_2-O-CH_2-CH_2-O- for complex [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$](NO$_3$)$_2$, respectively suggests the addition of alkoxyethanols in complexation which is also supported by IR studies. The electronic spectral studies on Ni(II) and Co(III) complexes indicate square planar and octahedral structures, respectively. Fluorescence, UV absorption as well as thermal denaturation studies revealed that [Ni(II)(1-amidino-O-methoxyethylurea)$_2$](NO$_3$)$_2$ and [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$](NO$_3$)$_2$ complexes are avid binder to calf thymus DNA (CT-DNA).

Key Words: NMR, Alkoxyethanols, Dicyandiamide, Square planar, Octahedral, DNA Binding.

INTRODUCTION

Transition metal complexes having the ability to bind and nick double stranded DNA under physiological conditions are of current interests for various applications in nucleic acid chemistry, *viz.*, as diagnostic agents in medicinal applications and for genomic research[1-6]. Although hydrogen-bonding interactions have long been considered to be of importance in biological systems, it is only recently that the transition-metal complexes containing ligands with versatile hydrogen-bonding capabilities have been used to bind DNA bases and other anions and to construct a network of coordinated complexes connected through intermolecular interactions[7].

In continuation of our earlier investigations on metal complexes of nitrile group ligands in different alcohols[8-10] and with the aim of obtaining an improved insight into the chemical and structural factors that govern the formation of complexes we have extended our investigations to a newly synthesized Ni(II) and Co(III) complexes of 1-amidino-O-2-alkoxyethylureas (where alkoxy = methoxy, ethoxy or butoxy).

The DNA interactions of the complexes have been analyzed by means of quenching the fluorescence of the DNA-ethidium bromide (EB) systems, absorption spectral titration and DNA thermal denaturation.

EXPERIMENTAL

IR spectra were recorded on KBr disks on a Shimadzu FT-IR-8400S. Electronic spectra were recorded on a Perkin Elmer UV-vis Lambda 35 spectrophotometer. The fluorescence spectra were recorded by Perkin Elmer LS55 fluorescence spectro-photometer. The room temperature magnetic moments (μ_{eff}) were measured using Sherwood scientific magnetic susceptibility balance (MSB). Conductivity measurements were measured by Eutech instruments con 510 conductivity. ^{1}H NMR spectra were recorded using Bruker Avance 400 (400 MHz) NMR spectrometer. Microanalyses were performed on a C, H, N Perkin Elmer 240 ℃ elemental analyzer. Calf thymus (CT) DNA and *tris*-HCl molecular biological grade were purchased from Merck (India), ethidium bromide (EB) was obtained from Sigma and all other chemicals were of spectroscopic grade. DNA concentration was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm. Solution of calf thymus DNA in 5 mM, *tris*-HCl (pH 7.2) buffer gave ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} *ca.* 1.9 indicating that the DNA was sufficiently free of protein[11].

DNA absorption experiments were carried out by varying the DNA concentration and maintaining the complex concentration constant. Absorbance values were recorded after each successive addition of DNA solution. The intrinsic binding constant (K_b) was determined according to the following equation[12].

$$[DNA]/(\epsilon_A - \epsilon_F) = [DNA]/(\epsilon_B - \epsilon_F) + 1/K_b(\epsilon_B - \epsilon_F)$$

where ϵ_A, ϵ_F, ϵ_B correspond to apparent, free and bound metal complexes extinction coefficient, respectively. In the plot [DNA]/(ϵ_A - ϵ_F) *versus* [DNA], K_b is then given by the ratio of the slope to intercept.

DNA melting experiments were carried out by monitoring the absorption intensity of CT-DNA at 260 nm at various temperature both in the absence and presence of the complexes. Measurements were carried out using a Perkin-Elmer Lamda 35 spectrophotometer equipped with a Peltier temperature controlling programmer (PTP6). The fluorescence spectral method using ethidium bromide as reference was used to determine the relative binding properties of the complexes to CT-DNA in 5 mM *tris*-HCl. Fluorescence intensities of ethidium bromide at 601 nm with an excitation wavelength of 510 nm were measured at different complex concentrations. The apparent binding constant (K_{app}) was calculated using the equation[13].

$$K_{EB}[EB] = K_{app}[complex]$$

where the complex concentration was the value at a 50 % reduction of the fluorescence intensity of ethidium bromide (EB) and $K_{EB} = 1 \times 10^7$ M^{-1}, [EB] = 1.3 μM.

Preparation of complexes: (Table-1) For the complexes *bis*(1-amidino-O-2-alkoxyethylurea)nickel(II) nitrate (where alkoxy = methoxy, ethoxy or butoxy) were prepared by refluxing nickel(II) nitrate hexahydrate (0.01 mol) and dicyandiamide (0.02 mol) in 2-methoxyethanol, 2-ethoxyethanol or 2-butoxyethanol on a steam bath for 3 h. The yellow complexes were filtered off immediately and washed several times with acetone and dried in air.

The golden yellow compounds of *bis*(1-amidino-O-2-alkoxyethylurea)nickel(II) sulphate (where alkoxy = methoxy or ethoxy) were prepared by refluxing nickel perchlorate hexahydrate (0.01 mol) and dicyandiamide (0.02 mol) in 2-methoxy-ethanol or 2-ethoxyethanol on a steam bath for 3 h and evaporated to a minimum volume. After keeping overnight in a refrigerator, aqueous solution of ammonium sulphate was added to the golden yellow solution for immediate precipitation of the nickel(II) complexes. The yellow complexes were filtered off immediately, washed thoroughly with distilled water, acetone and dried in air.

Tris(1-amidino-O-2-alkoxyethylurea)cobalt(III) nitrate (where alkoxy = methoxy or ethoxy) were prepared by refluxing cobalt(II)nitrate hexahydrate (0.01 mol) and dicyandiamide (0.03 mol) in 2-methoxyethanol or 2-ethoxyethanol on a steam bath for 1 h. After refluxing 1 h the unreacted cobalt(II) compound was filtered off and the dark chocolate coloured filtrate was evaporated to maximum concentration and kept it overnight at room temperature. The orange coloured crystals were filtered off and washed rapidly with acetone and dried in air.

RESULTS AND DISCUSSION

The IR spectra of complexes show absence of band at *ca*. 2165 cm^{-1} which corresponds to ν(C≡N) indicating that nitrile group was completely absent in the complexes and new bands at *ca*. 970 and 1247cm^{-1} characteristic of ν_s(C-O-C) and ν_a(C-O-C) were observed[10,14]. Lowering of ν(C=N) band at *ca*. 1550 cm^{-1} which was also supported by lowering of ν(C-N) band in the complexes indicates azomethine nitrogen is coordinated to the metal[15-16]. The increased value of ν(C=N) *ca*. 1681 cm^{-1} in all the complexes, is presumably due to a change in the C=N bond order on coordination through the nitrogen atom, facilitated by transfer of electron density from C-O-C moiety. The electron density on the N=C-O-C fragment of the 1-amidino-O-2-alkoxyethylurea group is more delocalized in the complexes and the bond order of >C=O group is raised[8,9,14,17,18]. The band appear at *ca*. 490 cm^{-1} has been assigned to ν(M-N). A very strong absorption band at *ca*. 1384 cm^{-1} in the IR spectra of complexes is due to ionic nitrate[19,20]. Bands around 619 (s), 1118 (vs) and 975 (vw) cm^{-1} have been assigned for tetrahedral sulfate ion in the sulfato complexes[10,21] of nickel.

The electronic spectrum of Ni(II) nitrate complexes in DMF and DMSO exhibit a broad band at *ca*. 23310 cm^{-1} and a shoulder around 19607 cm^{-1} corresponding to $^1A_{1g} \rightarrow {}^1B_{1g}$[19] and $^1A_{1g} \rightarrow {}^1A_{2g}$[22] transitions suggesting the presence of square-planar geometry. The electronic spectra of Co(III) complexes show an absorption

TABLE-1
ANALYTICAL, MOLAR CONDUCTANCE AND ELECTRONIC SPECTRAL DATA OF NICKEL(II) AND COBALT(III) COMPLEXES

Compound	Complex	Colour	m.p. (°C)	Analytical found/calcd (%)				Conductance at 25 °C conc. (mm) Λ_M (Ω^{-1} cm^2 mo1^{-1})				Electronic spectra			μ_{eff} (BM)	Yield (%)
				C	H	N	Ni	Conc.	Water	DMF	MeOH	Sol.	λ_{max} (cm^{-1})	$\in$		
DCDA		Colour less	210	23.00 (22.54)	4.80 (4.79)	66.70 (66.60)										
[Ni(AEtOMeUH)$_2$](NO$_3$)$_2$	1	Orange yellow	210	24.01 (23.87)	4.78 (4.77)	27.82 (27.85)	11.83 (11.67)	2 1		123 130	146 160	DMF DMSO	23310 19607(sh) 23228 19646 (sh)	45 30 40 20	Diamag.	90
[Ni(AEtO EtUH)$_2$](NO$_3$)$_2$	2	Orange yellow	225	26.80 (27.13)	5.85 (5.87)	26.91 (26.38)	11.07 (11.05)	2 1		126 134	140 158	DMF DMSO Pyridine	23121 19230 (sh) 23307 19805 (sh) 23668 19342 (sh)	40 27	Diamag.	80
[Ni(AEtOBut UH)$_2$](NO$_3$)$_2$	3	Orange Yellow	222	32.80 (32.72)	6.12 (6.13)	23.87 (23.86)	10.15 (10.00)	2 1		124 136	150 165	DMF DMSO	23212 23980 19607 (sh)	78 55	Diamag.	80
[Ni(AEtO MeUH)$_2$]SO$_4$	4	Light Yellow	220	25.25 (25.27)	5.07 (5.05)	23.05 (23.59)	12.40 (12.36)					Solid	23228		Diamag.	60
[Ni(AEtO EtUH)$_2$]SO$_4$	5	Light yellow	225	28.70 (28.65)	5.63 (5.57)	22.12 (22.28)	11.75 (11.65)					Solid	23668		Diamag.	40
[Co(AEtO MeUH)$_2$](NO$_3$)$_2$	6	Golden yellow	195	24.85 (24.82)	4.92 (4.96)	29.00 (28.96)	8.21 (8.13)	2 1	360 380	122 150		DMF Solid	20811 20790	84	Diamag.	75
[Co(AEtO EtUH)$_2$](NO$_3$)$_2$	7	Orange yellow	185	28.20 (28.16)	5.48 (5.47)	27.32 (27.37)	7.70 (7.69)	2	362 380	125 160		DMF Solid	20790 21413	81	Diamag.	65

band at *ca.* 20811 cm^{-1} in DMF corresponding to $^1T_{2g} \rightarrow \, ^1E_g$ transition suggested that the Co(III) complexes exhibit low spin octahedral geometry[23]. The absorption spectra of the complexes in solid state is much higher energy compared to that solution which suggest that presence of strong intermolecular interaction in the solids. The shift in band positions in DMF and DMSO may be attributed to axial ligation of the solvent molecules. The highly intense absorption band at *ca.* 28000 cm^{-1} in the cobalt (III) complexes is due to charge transfer band. The molar conductance values of Ni(II) nitrate complexes in DMF or MeOH in the range 127-145 Ω^{-1} cm^2 mol^{-1} indicate that these complexes are bi-univalent electrolytes[17,24] in these solvents and that of the Co(III) complexes are tri-univalent electrolytes[19,24] in water at 25 ºC giving molar conductance value *ca.* 360 Ω^{-1} cm^2 mol^{-1}. Magnetic moment measurement indicates that all the complexes were diamagnetic.

^{1}H NMR spectra of dicyandiamide and the nickel(II) complexes showed (Fig. 1) a singlet at 2.5 ppm corresponding to the NH$_2$ group, which indicates that NH$_2$ group of dicyandiamide does not take part in complexation[25]. More intense peak at 2.5 ppm in the complexes supports the formation of *bis* (ligand) nature of nickel(II) complexes. The NH group of ligand showed a sharp singlet at 6.559 ppm but after complexation with the nickel(II) ion it showed a weak broad signal at 5.74 ppm corresponding to the decrease in electron density on NH proton[26]. In the nickel(II) complexes there is a signal at 7.45 ppm which is due to NH proton of amidine part adjacent to the alcoholic group after coordination. A weak singlet at 10.32 ppm is due to NH proton[27,28]. The medium singlet peak at 4.14 and 4.15ppm are[28,29] corresponding to OCH$_3$ and OCH$_2$CH$_3$ of [Ni(II)(1-amidino-O-2-methoxyethylurea)$_2$] (NO$_3$)$_2$ and [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$](NO$_3$)$_2$ complexes. Another interesting observation from the ^{1}H NMR spectra is the appearance of new multiplet peaks at 3.25-3.50 ppm assignable to CH$_3$-O-CH$_2$-CH$_2$-O- for [Ni(II)(1-amidino-O-2-methoxyethylurea)$_2$](NO$_3$)$_2$ and at 3.30-3.45 ppm due to CH$_3$-CH$_2$-O-CH$_2$-CH$_2$-O- for [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$](NO$_3$)$_2$[30,31], respectively which indicates that addition of alkoxyethanols take place in complexation which is also supported by IR studies.
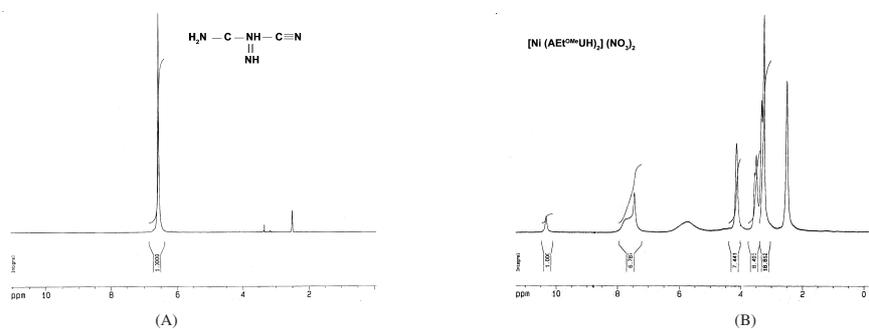

Fig. 1.   NMR spectra of (A) dicyandiamide and (B) Ni(II)(1-amidino-O-2-methoxyethyl-
         urea)$_2$](NO$_3$)$_2$ recorded in DMSO-d_6

It is pertinent to mention here that the structures of *bis*(N-alkylamidino-O-methylurea)copper(II) cation[32] and *bis*(N-alkylamidino-O-alkylurea)copper(II) nitrates and tetrafluoroborates[33] consist of [CuL$_2$]$^{2+}$ cation showing their interactions of N-H donors and N-H group with anions. Based on our experimental findings, the structures of [Ni(II)(1-amidino-O-2-methoxyethylurea)$_2$](NO$_3$)$_2$, [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$](NO$_3$)$_2$ (Fig. 2) and Ni(II)(1-amidino-O-2-butoxyethylurea)$_2$-(NO$_3$)$_2$ have been purposed having hydrogen bonding interactions of complex ion with nitrate ions.
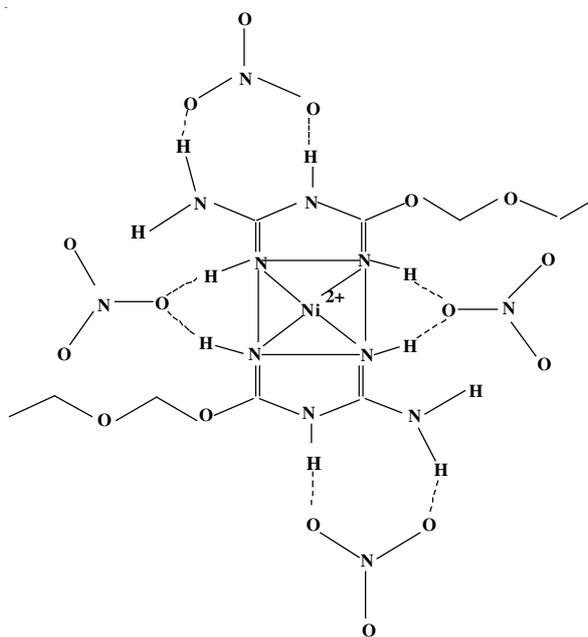

Fig. 2.   Proposed structure of [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$]$^{2+}$ cation showing the hydrogen bonded nitrate ions

DNA interaction studies: The absorption spectra of [Ni(II)(1-amidino-O-2-methoxyethylurea)$_2$](NO$_3$)$_2$ (Fig. 3) and [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$](NO$_3$)$_2$ complexes in the presence of increasing CT-DNA concentration show that as the concentration of DNA increased the band *ca.* 280 nm exhibit hypochromism. The spectra indicate that there are some interactions between the complex and DNA. The values of K_b for [Ni(II)(1-amidino-O-2-methoxyethylurea)$_2$](NO$_3$)$_2$ and [Ni(II)(1-amidino-O-2-ethoxyethylurea)$_2$](NO$_3$)$_2$ are 1.8×10^4 and 1.6×10^4 M^{-1}, respectively.

Ethidium bromide emits intense fluorescent light when it is intercalated between adjacent DNA base pairs. The addition of a second DNA binding molecule can quench the DNA-EB adduct emission by either replacing the ethidium bromide (EB) and or by accepting the excited state electron of the EB through a photoelectron transfer mechanism[34,35]. Fig. 4 shows the emission spectra of EB bound to DNA
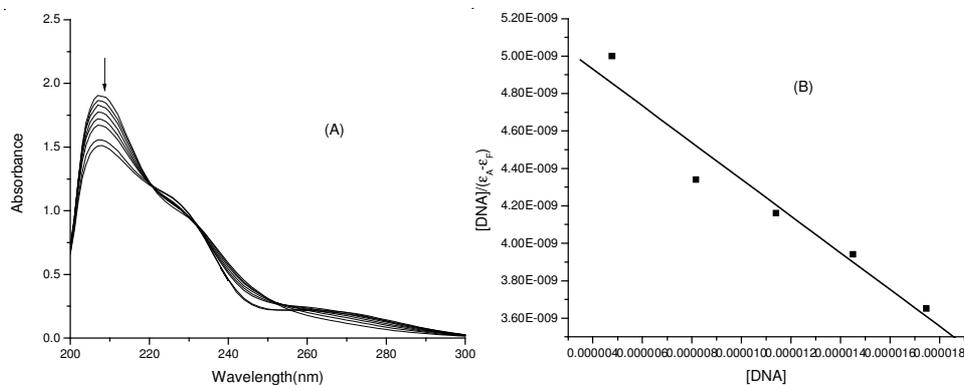
Fig. 3. (A) Absorption spectra of Ni(II)(1-amidino-O-2-methoxyethylurea)₂](NO₃)₂ (50
μM)in the absence (top curve) and presence (subsequent curves) of CT-DNA. Ar-
row shows the absorbance changes upon CT-DNA concentration. (B) Plot of [DNA]/
(ϵ_A - ϵ_A) *versus* [DNA] for CT-DNA with Ni(II)(1-amidino-O-2-
methoxyethylurea)₂](NO₃)₂

both in the absence and presence of the complexes with varying concentrations.
Addition of complex to DNA that has been previously treated with EB causes an
appreciable reduction in emission intensity, thus indicating that the complex binds
to DNA. The association constant (K_{app} × 10^{-6} M^{-1}) values of complex [Ni(II)(1-
amidino-O-2-methoxyethylurea)₂](NO₃)₂ and [Ni(II)(1-amidino-O-2-ethoxy-
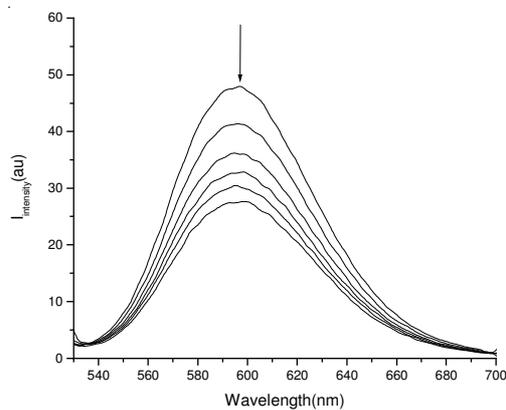ethylurea)₂](NO₃)₂ are 5.0 and 4.9, respectively.

Fig. 4. Emission spectra of ethidium bromide bound CT-DNA in the absence (top curve)
and presence (subsequent curves) of increasing concentration of [Ni(II)(1-amidino-
2-ethoxyethylurea)₂](NO₃)₂. The arrow shows the changes in intensity at increasing
concentrations of the complex

Thermal behaviour of DNA in the presence of metal complex can give insight
into their conformational changes when temperature is raised and information about

the interaction strength of the complex with DNA. The double stranded DNA tends to gradually dissociate to single strands on increase in the solution temperature and generates a hyperchromic effect on the absorption spectra of DNA bases (λ_{max} = 260 nm). In order to identify this transition process, the melting temperature (T_m), which is defined as the temperature where half of the total base pairs gets non-bonded, is a valuable parameter. Interaction of natural or synthesized organic and metallointercalations generally results in a considerable increase in melting temperature[36].

Experimentally the DNA melting temperature is found as 57 ºC and get minor increases to *ca*. 64 ºC ($\Delta T_m = 7$ ºC) with increasing addition of complex concentration (Fig. 5) suggesting DNA binding mode is primarily electrostatic or groove binding nature of the complexes.
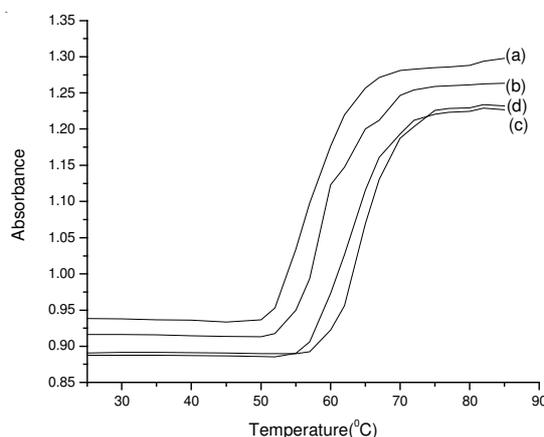

Fig. 5.   Thermal denaturation profiles of CT-DNA before (a) and after (b) 1.17×10^{-5} M, (c) 1.92×10^{-5} M and (d) 2.65×10^{-5} M addition of increasing amount of [Ni(II)(1-amidino-2-ethoxyethylurea)$_2$](NO$_3$)$_2$

Conclusion

^{1}H NMR studies on nickel(II) complexes [Ni(II)(1-amidino-O-2-alkoxyethylurea)$_2$] (NO$_3$)$_2$, where alkoxy = methoxy or ethoxy show that addition of alkoxyethanols take place in these complexes which is also supported by IR spectra. From the magnetic moment value, elemental analysis and electronic spectra, these nickel(II) complexes were found to have square planar geometry around the central metal ion and cobalt(III) complexes exhibit low spin octahedral geometry. Based on absorption, fluorescence as well as thermal denaturation data, nickle(II) nitrate complexes bind to DNA by electrostatic or groove binding.

ACKNOWLEDGEMENTS

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