Synthesis, Characterization, DNA Binding And Cleavage Studies of Cu(II) and Ni(II) Complexes Containing Mixed Ligands

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Abstract:- **The DNA interactions of newly synthesized mixed ligand complexes of the type $[Cu(L^1L^2L^3)]$ $(PF_6)_2$complex (1)and $[Ni(L^1L^2L^3)]$ $(PF_6)_2$complex (2)(where L^1 = 1, 10-phenanthroline and L^2 = 1*H*-benzimidazole-2-thiol and L^3 = 4*H*-1,2,4-triazol-4-amine) have been evaluated by absorption spectral studies. The results of binding constant for complexes(1) and (2) are 3.1×10^5 M^{-1} and 5.4×10^5 M^{-1} respectively, indicate that both the complexes intercalate with CT-DNA. The relative nuclease activity of these complexes were studied by gel electrophoresis using pUC 19 DNA.**

Key words: Cu(II) and Ni(II) complexes, DNA binding, viscosity measurements, thermal denaturation and cleavage studies.

I. INTRODUCTION

The study of mixed ligand-complex formation is relevant in the field of analytical chemistry,where the use of mixed ligand complexes allows the development of methods with increasedselectivity, sensitivity and has also great importance in the field of biological and environmentalchemistry.[1]

The coordination chemistry of Schiff bases as multi dentate ligands gained much importancefor more than two decades because of their use as models in biological system.[2-5] Transitionmetal complexes of 1, 10-phenanthroline (phen) or their modified variants are widely employed in several research areas including bioinorganic and biomedical chemistry.[6-13]Schiff bases are considered as a very important class of organic compounds which have wide applications in many biological aspects. Some Schiff bases were reported to possess antibacterial, antifungal and antitumor activities.[14] Due to their multiple implications,the transition metal complexes with Schiff bases, as ligands, are of paramount scientific interest.Schiff bases with donors (N, O) have structure similarities with natural biological systems and due to the presence of imine group (the -N=CH-), are utilized in elucidating the mechanism of transformation and rasemination reaction in biological systems.[15] Schiff base complexeshave been used as drugs. Moreover, it is well known that some drug activities, whenadministered as metal complexes, are being increased, and several Schiff base complexeshave also been shown to inhibit tumor growth. The effect of the presence of methyl substituentin the phenyl rings of aromatic Schiff bases on their antimicrobial activity has been reported.[16]

In view of this, we have synthesized new complexes of Cu(II), and Ni(II) containing ligands 1,10-phenanthroline(L^1), 1*H*-benzimidazole-2-thiol(L^2) and 4*H*-1,2,4-triazol-4-amine (L^3).

II. EXPERIMENTAL

All reagents and solvents were of AR grade, solvents were purified and used. $CuCl_2$, $NiCl_2$, ammoniumhexafluorophosphate(NH_4PF_6), Dimethylsulphoxide(DMSO) and Tris-HCl buffer were purchased from qualigens (Mumbai, India). Calf thymus Deoxyribonucleic acid(CT-DNA) and plasmid University of California 19 Deoxyribonucleic acid(pUC19 DNA) were purchased from Bangalore Genei, Bangalore, India.

Procurement of ligands

The ligands 1,10-phenanthroline (L^1), 1*H*-benzimidazole-2-thiol(L^2) and 4*H*-1,2,4-triazol-4-amine (L^3) were purchased from Hi-media chemicals, Mumbai.

Synthesis of $[Cu(L^1L^2L^3)]$ $(PF_6)_2$[Complex (1)].

To the solution of 1,10-phenanthroline(L^1) in ethanol a solution of $CuCl_2$(0.241 g, 1 mmol) prepared in ethanol was added and followed by the addition of ethanolic

solutionof 1*H*-benzimidazole-2-thiol(L^2) and a solution of 4*H*-1,2,4-triazol-4-amine(L^3) in ethanol in 1:1:1:1 (M:L^1:L^2:L^3) molar ratio. The solution was refluxed on a water bath for 1h. A green colouredprecipitate was obtained onaddition of solution of ammoniumhexafluorophosphateto the cold filtrate. Then it was separated by filtration and dried under vacuum and recrystallizedfrom ethyl acetate. Yield of the complex 74%. Analysis:$C_{21}H_{18}N_8SP_2F_{12}Cu$: Calc. (%); C 32.78, H 2.32, N 14.52, Fe 8.23. Found(%): C 32.81, H 2.35, N 14.58, Cu 8.27.

Synthesis of [Ni($L^1L^2L^3$)] $(PF_6)_2$[Complex (2)].

Refluxing of a ethanolic solution of mixture prepared by mixing of nickel chloride (0.238 g, 1 mmol) in the solution of 1,10-phenanthroline (L^1) in ethanol, a solution of 1*H*-benzimidazole-2-thiol (L^2) and ethanolic solution of 4*H*-1,2,4-triazol-4-amine (L^3) in 1:1:1:1 (M:L^1:L^2:L^3) molar ratio about 1h yielded a cream white(light yellow) coloured solution. The solution was cooled and ammoniumhexafluorophosphatein ethanol was added to get the precipitate which was filtered, dried in vacuumand recrystallizedin ethyl acetate. Yield 78%. Analysis:$C_{21}H_{18}N_8SP_2F_{12}Ni$: Calc. (%); C 33.01, H 2.32, N 14.61, Fe 7.65. Found(%): C 33.04, H 2.37, N 14.68, Fe 7.69.^{1}H NMR, δppm(DMSO, 400 MHz), (TMS):10.0(s, 2H), 8.62(s, 2H($_{1,8}$)), 7.97(s, 2H($_{15,18}$)), 7.55(d, 4H($_{7,2,16,17}$)), 7.22(d, 4H($_{3,4,5,6}$)), 5.34(s, 4H($_{9,10,13,14}$))

The proposed structure of the complexes is given in Fig.1.

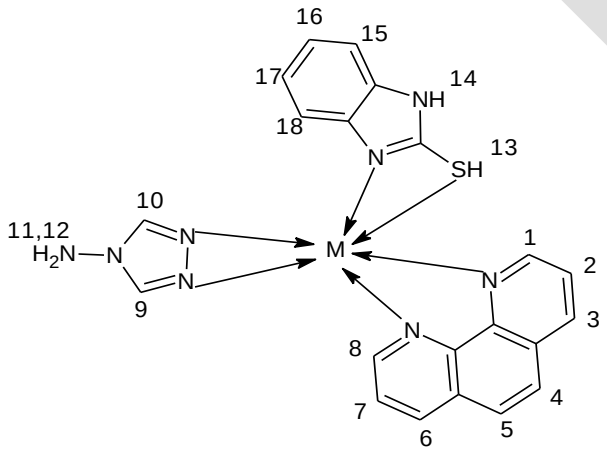

Fig.1. The proposed structure of the complexes where M = Cu^{2+} or Ni^{2+}

III. RESULTS AND DISCUSSIONS

Characterization of complexes

The analytical data of the complexes is given in experimental section. The elemental analysis data agree with the theoretical values within the limit of experimental error. These complexes are soluble in DMF, DMSO and in buffer(pH 7.2) solution. The observed conductivity measurement of 10^{-3}M solution values in the DMF fall in the region 160-170Ω^{-1} cm^2 $mole^{-1}$ indicate that uni-bivalent nature of the complexes.

IR spectra

The IR spectra of ligandand the complexeswere recorded in the range of 4000-400 cm^{-1} on KBr pellets. The spectra of the ligand 1*H*-benzimidazole-2-thiol (L^2) show bands at 1622 cm^{-1} assigned due to νC=N aromatic hydrocarbon, 3100 cm^{-1} assigned to νC-H group and 3200 cm^{-1} assigned to νO-H group. The spectra of all the complexes show a peak in the range 1625 cm^{-1} to 1690 cm^{-1} for νC=N group are shifted slightly indicating that the coordination taken place through nitrogen atom. The band due to νS-H appeared around 2940 cm^{-1}. The position of an S-H band of uncoordinated ligandfound to be shifted by 10-20 cm^{-1} after the formation of complexes. Besides, the complexes show new bands at 400-430 cm^{-1} are assigned to ν(M-N) bands.[17] In addition, the IR spectrum of the PF_6 salts of each complex showed a strong band in the range 725 cm^{-1} to 750 cm^{-1} ascribed to the counter anion.[18]

^{1}H NMR spectra

The ^{1}H NMR spectra of the uncoordinated ligandas well as their complexes were recorded in DMSO-d_6. The results are tabulated in Table 7.2. The NH and SH proton signals in the free ligandappeared at δ12.5 and the position of the peak at 7.1ppmis due to the benzimidazolering protons. In the case of complex (2), the resonance at δ10.0 is due to the protons of –NH group. The signal at 8.62ppmassigned to two adjacent protons of nitrogen atoms of L^1. The peaks due to H_{15} and H_{18} of benzimidazolemoiety appeared at δ7.97 and a signal at 7.55ppmare due to the resonance of H_2, H_7, H_{16}, and H_{17} protons. The doublet at 7.22ppm is ascribed to H_3, H_4, H_5 and H_6 proton signals. A signal obtained at 5.34ppmis due to the proton of –SH group.

Absorption Spectral Studies

Absorption titrationis used to monitor the interaction of the complexes **(1)**and**(2)** with CT-DNA. The absorption spectra of the complexes **(1)**and**(2)** in the absence and presence of CT-DNA are given in Fig. 2 and 3 respectively. Figure 2 depicts well resolved band at 302 nmfor complex **(1)** andFig. 3 depicts well resolved band at 253 nmfor complex **(2)** with increasing the DNA concentration (0-200 μM). The result shows that the absorbance(hypochromism) decreased by the successive

addition of CT-DNA to the complex solution. The hypochromismobserved for the bands of complex **(1)** and **(2)** are accompanied by a small bathochromic shift were 1, and 2 nmin Fig. 2 and 3 respectively. The hypochromicand bathochromic shifts are observed for the complexes suggest that binding is intercalativemode. In order to compare quantitatively, the DNA binding strengths of these complexes, the intrinsic DNA binding constants $K_b$are determinedfrom the decay of the absorbance302 nmfor complex **(1)** and253 nmfor complex **(2)** with increasing concentrations of DNA. The observed $K_b$values of complex **(1)**and**(2)** are equal to the classical intercalatorsbound to CT-DNA. The K_b values of complex **(1) and (2)**are 3.1 x 10^5 M^{-1} and 5.4 x 10^5 M^{-1} respectively. The K_b values for complex **(1)** and **(2)**are calculated as per the procedure.[19]So, it is obvious that the present complexes are involved in intercalative interactions with CT-DNA.

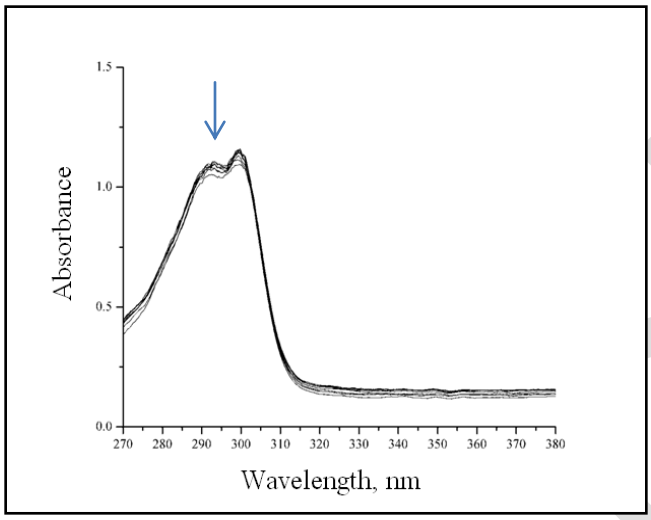

Fig. 2FIG.2. Absorption spectra of complex **(1)** in *Tris*-HCl buffer upon addition of DNA. [Cu] = 0.5μM, [DNA] = 0-200 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.

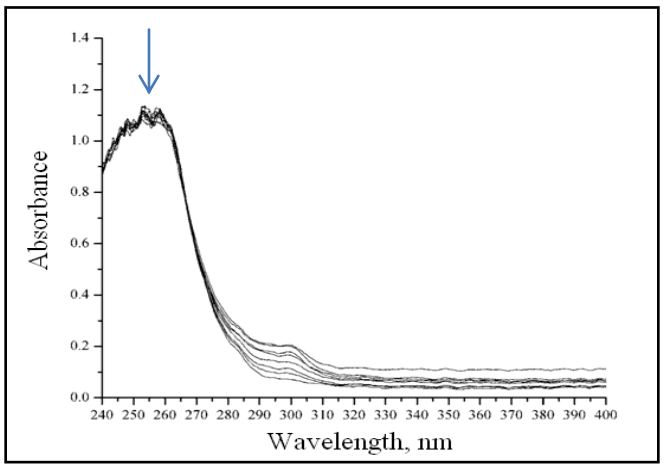

Fig. 3 Absorption spectra of complex **(2)** in *Tris*-HCl buffer upon addition of DNA. [Ni] = 0.5μM, [DNA] = 0-200 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.

Viscosity measurements

The DNA binding modes of the complexes were further investigated by viscosity measurement, which is sensitive to the increase in length of DNA and is regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data.[20] To understand the nature of DNA binding of mixed ligand Cu(II) and Ni(II) complexes, viscosity measurements were carried out onCT-DNA by varying the concentration of the added complex. Representative plots of relative viscosity (η/η_0) vs. [Complex] /DNA is shown in Fig. 4. As can be seen, there is positive change in viscosity with increasing additions of the concentration of the complexes to DNA. These results suggested that, both the complexes intercalated between two adjacent base pairs of DNA through a classical intercalation mode.

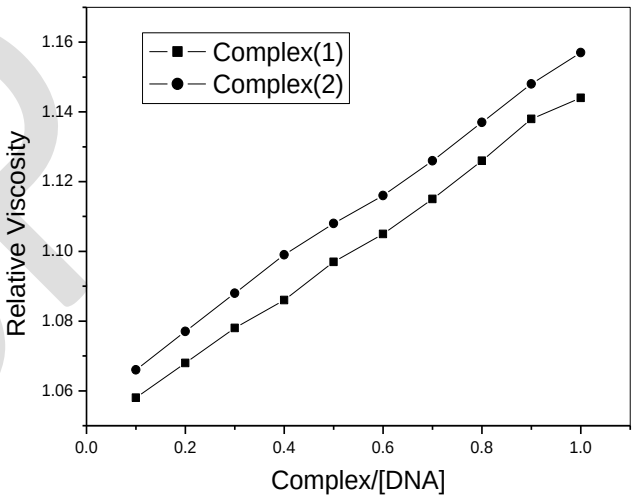

Fig.4. Plot of relative viscosity Vs [complex] /[DNA]. Effect of complex(1), complex(2),on the viscosity of CT-DNA at 25 (±0.1)°C, [Complex] = 0-100 μM, DNA= 50 μM.

Thermal denaturation studies

Thermal behaviors of DNA in the presence of complexes can give insight into their conformational changes when the temperature is raised, and offer information about the interaction strength of complexes with DNA. It is well known that when the temperature in the solution increases, the double-stranded DNA gradually dissociates to single strands, and generates a hypochromic effect on the absorption spectra of DNA bases (λmax = 260 nm). In order to identify this transition process, the melting temperature *Tm* is usually introduced, which is defined as the temperature where half of the total basepairs is bounded. According to the literature, the intercalation of natural or synthesized organic and metallointercalators generally results in a considerable increase in melting

temperature(*Tm*). As shown in Fig. 5, the *Tm*DNA was found to be 55 ± 1ºC under experimental conditions. Under the same set of conditions, addition of complex (**1**) and (**2**) increased *Tm*(±1◦C) by 1ºC and 2ºC respectively, which indicates that these compounds stabilize the double helix of DNA. The increase in *Tm*of the latter is comparable to that of classical intercalators.[21] So from the above data it is concluded that the new Cu(II) and Ni(II) mixed ligand complexes act as a new class of DNA intercalators.

The observations made during the absorptiontitration, viscosity measurements and thermal denaturation experiments are comparablewith those reported earlier for variousmetallo-intercalators, thus suggesting that the complexes **(1)** and **(2)** bound to DNA by intercalation.[22-40]

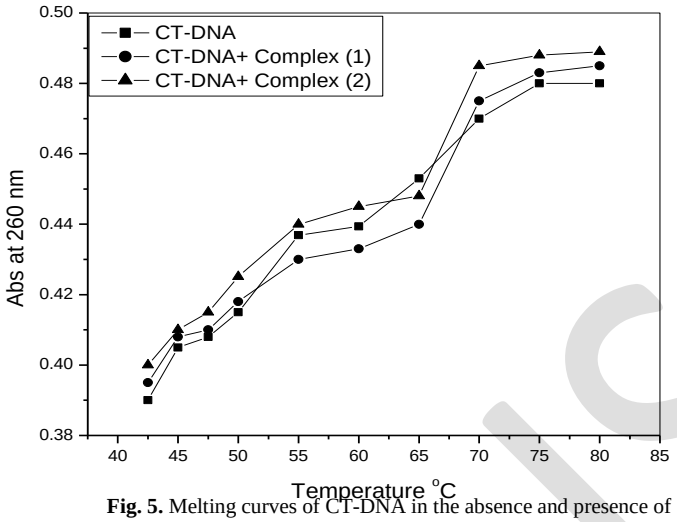

Fig. 5. Melting curves of CT-DNA in the absence and presence of complexes.

DNA cleavage studies

In order to determine the ability of complexes **(1)** and **(2)** for DNA sission,the complexes were incubated at different concentrations with supercoiled pUC19 DNA for 1 hour in 50 mM Tris-HCl/50 mM NaCl buffer (pH 7.2) using hydrogen peroxide (H_2O_2) activation. Control experiments using H_2O_2 do not show any apparent cleavage of DNA(Fig. 6, lane 1). At the concentration of 40 μM and 80 μM in Fig. 6, complex **(1)** is able to convert 85 % and 95 % of the initial SC (Form I) to NC (Form II) (lane 2 and 3) and the complex **(2)** is able to convert 75 % (40 μM) and 80% (80 μM) of the initial SC (Form I) to NC (Form II) (lane 4 and 5).

Fig. 6 Agarose gel electrophoresis of supercoiled pUC 19 DNA (0.5μg) by the Complex **(1)**and **(2)** in a buffer containing 50 mM*Tris*-HCI and 50 mMNaCI at 37°C. Lane 1, DNA alone; Lane 2, DNA+20 μM of complex **(1)**; Lane 3, DNA+40 μM of complex **(1)**; Lane 4, DNA+40 μM of complex **(2);**Lane 5, DNA+80 μM of complex **(2)**. Forms I and II are supercoiled and nicked circular forms of DNA respectively.

CONCLUSION

The experimental results obtained by absorption spectra, viscosity and thermal denaturation studies indicate that the new metal complexes viz, $[Cu(L^1L^2L^3)]$ $(PF_6)_2$ [Complex **(1)**] and $[Ni(L^1L^2L^3)]$ $(PF_6)_2$ [Complex **(2)**] bound to the double stranded DNA with binding constant K_b= 3.1 × 10^4 M^{-1} for complex **(1)** and K_b= 5.4 × 10^4 M^{-1} for complex **(2)** respectively. The viscosity of the solution of the DNA bound to the complexes increased with increase in concentration of the complexes suggesting the intercalation of all the complexes with DNA. Thermal denaturation experiments also revealed the intercalation of all the complexes with DNA. Further all the complexes have been investigated for their cleavage activity. The results show that all the complexes are exhibiting fairly good nuclease activity.

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