Synthesis, Characterization, DNA Binding and Photo Cleavage Studies of Mixed Lig and Nickel(II) Complexes Containing 5-Methyl-1,3,4-Thiadiazole with 1,10 Phenanthroline and 2'2 Bipyridine

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Abstract

The two new mixed ligand Ni(II) complexes of the type $[Ni(Phen)_2(L)](PF_6)_2$ (1), and $[Ni(bpy)2(L)](PF_6)_2$ (2) (where, L= 5-methyl-1,3,4-thiadiazole-2-thiole phen=1,10-phenanthroline, bpy=bipyridine) were synthesized and structurally characterized. Elemental analysis, magnetic and spectroscopic data suggested octahedral geometry for both the complexes. Binding of these complexes with CT-DNA were analyzed by absorption spectra, viscosity and thermal denaturation studies. Detailed analysis revealed that the metal complexes intercalates into the DNA base stack as intercalator with binding constant $K_b = 1.7x10^4 M^{-1}$ for complex (1) and $K_b = 1.5x10^4 M^{-1}$ for Complex (2) respectively. The photocleavage activities of the complexes were studied with supercoiled (SC) pUC19 DNA by using gel electrophoresis, and the results show that complexes have potent nuclease activity.

Key Words: *Mixed Ligand; CT-DNA; Viscosity; Photocleavage; Electrophoresis*

I. INTRODUCTION

The discovery of cis platin as an anticancer agent explore the interaction of transition metal complexes with DNA has been a subject of intense research in the field of bioinorganic chemistry[1,2]. But the use of cisplatin to cure variety of cancers is still limited by dose-limiting side effects [3] and inherited or acquired resistance phenomena, only partially amended by employment of new platinum drugs [4,5]. These drawbacks of platinum drugs stimulated the researchers to develop alternative strategies, based on different metals, with improved pharmacological properties and aimed at different targets [6]. The interaction of transition metal complexes with DNA through either hydrolytic or oxidative pathway has gained much attention in recent days. Because the transition metal complexes having unique electronic and spectroscopic signatures which offer a multitude of coordination geometries and mechanism of cytotoxic action which is related to DNA binding affinity [7] and can also vary accordingly as the biological activity is strongly dependent on structure–activity relationship. Besides this, metal complexes also utilize or create open coordination positions for DNA binding and hydrolysis generates reactive oxygen-containing species or other radicals for DNA oxidation [8,9].

Ligands in the metal complexes play a major role in their binding to DNA [10]. 2,2-bipyridine and 1,10-phenanthroline are strong bidentate ligands which form stable chelates with many transition metals. These ligands have a starring role in the field of chemistry and molecular recognition due to their usefulness in medicine and in the industry [11]. Metal complexes of S-, N-, and O-chelating ligands have attracted considerable attention because of their interesting physico-chemical properties, pronounced biological activities and their use as models for metalloenzyme active sites[12]. Thiadiazole is an important class of S, N-containing heterocycles and has been reported to be biologically compound [13] The therapeutic and diagnostic properties of transition metal complexes have attracted considerable attention leading to their application in many areas of modern medicine [14] Nickel is an essential element present in several enzymes and plays a vital role in physiological processes as a cofactor in the absorptionof iron from the intestine[15]. With the biological importance of nickel, it is important to study their complexes with bioactive ligands to understand functions of the complexes and to find new bioactive compounds [16,17].

Complexes of nickel with nitrogen-based heterocyclic ligands have been widely investigated owing to their potential applications as functional

solid materials, photosensitization reactions, bioinorganic chemistry, medicine, catalysis and a variety of biological activities, such as antimalarial, antibacterial, antitumoral and antiviral activities [18]. Ni(II) complexes, which strongly bind and cleave DNA, exhibit prominent anticancer activities and regulate apoptosis [19]. Various mixed-ligand metal complexes were found to be particularly useful because of their potential to bind DNA via a multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electro- chemical, and photochemical reactivities [20]. Hence the efficient enhancement of DNA binding and cleavage activity of mixed ligand metal complexes and in continuation of our research work [21-23] stimulate us to design, synthesized and characterized new Nickel(II) complexes of mixed ligands,1,10 - phenanthraline/2,2-bypyridine and 5-methyl-1,3,4-thiadiazol-2-thiol to evaluate and understand the factors on the DNA-binding and cleavage properties. Furthermore, the DNA binding properties of the complexes have been investigated by thermal denaturation, UV-absorption, viscosity and as well as its cleavage activity towards DNA has been studied by gel electrophoresis.

II. EXPERIMENTAL

All chemicals used in present work were of analytical reagent grade purchased commercially. Commercial solvents were distilled and then used for the preparation of ligand and its complexes. 2,2-bipyridine, 1,10-phenanthroline $NiCl_2.6H_2O$, *Tris*-HCl and ammoniumhexaflurophosphate (NH_4PF_6) were purchased from Merck (India), calf thymus (ds) DNA and super coiled (SC) pUC19 DNA were purchased from Bangalore Genie (India), Agarose (molecularbiology grade) and ethidium bromide were purchased from Himedia. *Tris*–HCl buffer solution used for binding andcleavage studies was prepared using deionised double distilled water.

A. Physical measurements

Micro analyses (C, H, and N) were performed in Carlo-Erba 1106-model 240 Perkin-Elmer analyzer. Melting points were determined in open capillaries and are uncorrected IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets. 1H-NMR spectra were recorded on a Bruker AC-P500 spectrometer (300 MHz) at 25◦C in $CDCl_3$ with TMS as the internal reference. UV visible absorption spectra were recorded using Shimadzu 1650 PC model UV spectrophotometer at room temperature. Viscosity measurements were carried out on Brookfield viscometer at room temperature. Thermal denaturation studies were carried out with aPerkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier teyrrcontrolling programmer.

B. DNA binding and cleavage experiments

The concentration of CT DNA was measured by using its known extinction coefficient at 260 nm (6,600 M^{-1} cm^{-1}) [24] . The absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}) for CT DNA were measured to check its purity. The ratio A_{260} /A_{280} was found to be 1.84, indicating that CT-DNA was satisfactorily free from protein [25]. Buffer [50 mM tris(hydroxymethyl)aminomethane, pH 7.2, 50 mM NaCl] was used for the absorption, viscosity and thermal denaturation and cyclic voltammetric experiments.

Absorption titration experiments were carried out by varying the DNA concentration (0-100 μM) and maintaining the complex concentration constant (30 μM). Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 min). The observed values for the complexes were then fit in to Eq.1 to obtain the intrinsic binding constant, K_b [26].

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_a-\varepsilon_f) \quad [1]$$

Where ε_a, ε_f and ε_b are the apparent, free and bound metal complex extinction coefficients, respectively. a plot of [DNA]/ (ε_b-ε_f) versus [DNA] gave a slope of $1/(\varepsilon_b-\varepsilon_f)$ and a y intercept equal to $1/K_b(\varepsilon_b-\varepsilon_f)$, where K_b is the ratio of the slope to the y intercept.

Viscosity measurements were carried out at 25±1 °C using semimicro dilution capillary viscometer at room temperature. Measurement of DNA viscosity is regarded as the least ambiguous and the most critical test of a DNA binding model in solution and affords stronger arguments for an interactive DNA binding mode [27,28]. The DNA viscosity is enhanced significantly due to complete or partial intercalation of drugs in to DNA base stacking but it is slightly disturbed by electrostatic or covalent binding of molecules [29]. To understand the nature of DNA binding of the mixed ligand metal complexes, viscosity measurements were carried out on CT DNA by varying the concentration of the added complexes. Flow times were measured, using a digital stopwatch, at least three times and were accepted if they agreed within 0.1s. Reduced specific viscosity was calculated according to Cohen and Eisenberg [30]. Plots of $(\eta/\eta_o)^{1/3}$ (η and η_o are the reduced specific viscosities of DNA in the presence and absence of the drug) vs. [drug]/[DNA] were constructed. Data were presented as (η/η_o) vs binding ratio, where η is the viscosity of DNA in the presence of complex and $\eta_o$is the viscosity of DNA alone.

Thermal denaturation experiments were carried out by monitoring the absorption of CT DNA at 260 nm at various temperatures in the presence (5-10 μM) and the absence of each complex. The melting temperature (T_m, the temperature at which

50% of double stranded DNA becomes single stranded) and the curve width (σ_T, the temperature range between which 10 and 90% of the absorption increases occurred) were calculated as reported

Electrophoresis through agarose is the standard method used to separate, identify or purify DNA fragments.Using this technique, bands containing as little as 1-10 ng of DNA can be detected by direct examination of the agarose gel (stained with ethidium bromide) in the UV light. When an electric field is applied across the gel, DNA, which is negatively charged at neutral pH, migrates toward the anode. The intact supercoiled (Form I) DNA migrates faster than the single nicked (Form II) in the gel. This technique has been employed to identify the product/s of the DNA photocleavage, which was carried out in this work.

III. PREPARATION

A. Synthesis of ligand

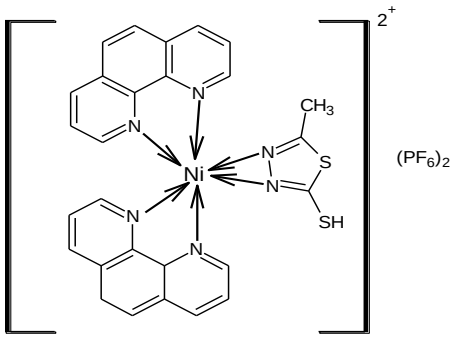
Ligand 1,10-phenanthroline, bipyridine and 5-methyl-1,3,4-thiadiazole-2-thiole (L) was purchased from Sigma Aldrich (Bangalore).

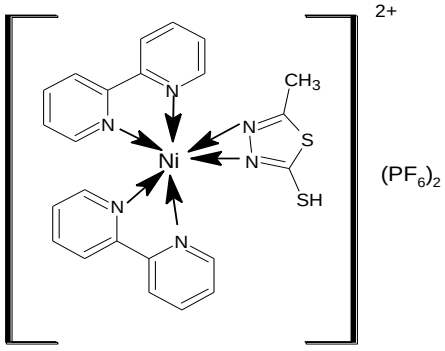
B. Synthesis of Complexes

The complexes $[Ni(phen)_2Cl_2]$ / $[Ni(bpy)2Cl_2]$ were prepared by literature method [31]. Solution containing $[Ni(phen)_2Cl_2]$ / $[Ni(bpy)2Cl_2]$ (0.49 g, 1 mmol) and 5-methyl-1,3,4-thiadiazole-2-thiole (L) (0.1322g, 1mmol) (50 ml) in ethanol was refluxed for 1 hr with stirring and further stirred for 4-5 hr under nitrogen. Then it was filtered and the crude complex was precipitated upon addition of saturated ethanolic solution of ammonium hexaflurophosphate. The complex was filtered and recrystallized (acetone-ether).

Anal. Calc. for $C_{27}H_{20}N_6S_2NiP_2F_{12}$: C, 38.61; H, 2.38; N, 10.00; Ni,6.98 ; Found: C,38.61 ; H,2.39 ; N,10.01 ; Ni,6.24, μ_{eff} = 2.98 ±0.02 BM.

Anal. Calc. for $C_{23}H_{20}N_6S_2NiP_2F_{12}$: C, 38.85; H, 2.52; N, 10.60; Ni, 7.41. Found: C,38.85 ; H,2.53 ; N,10.61 ;Ni, 7.42;μ_{eff} =2.92 ±0.02 BM





Scheme 1. *Structure of $[Ni(phen)_2(L)](PF_6)_2 .2H_2O$ [1] :* **Scheme. 2.** *Structure of $[Ni(bpy)_2(L)](PF_6)_2 .2H_2O$ [2]*

IV. RESULT AND DISCUSSION

A. Characterization of Ni(II) complexes

The elemental analytical data, IR and magnetic moment data of the new complexes are agreed with the theoretical values within the limit of experimental error and confirmed the formula of the complexes were $[Ni(phen)2(L_2)](PF_6)_2$ and $[Ni(bpy)2(L_2)](PF_6)_2$. They are insoluble in water, but soluble in DMF, DMSO and in buffer (pH 7.2) solution. Magnetic moment of Ni(II) complexes lie in the range 2.91–3.12BM at room temperature. These values are in tune with a high spin configuration and show the presence of an octahedral environment around the Ni(II) ion in all the complexes [32,33].

a. IR-Spectra

The ligand 5-methyl-1,3,4-thiadiazole-2-thiol shows absorption bands at 2868 and 1590 cm−1 due to ν(SH) and ν(C=N), respectively. The ν(C=N) band shifted to 1580 cm−1 for complexes (**1**) and (**2**), respectively. This indicate that the nitrogen atom is involved in coordination to the Ni(II) ion. Besides, the complexes show low frequency in the region 410–445cm−1 are assigned to (M-N) bands [34-38]. In addition the IR spectrum of the PF_6 salt of each complex showed a strong band in the region 843–847 cm−1 ascribable to the counter anion and this band was absent for the corresponding chloride salts [39].

b. UV-visible spectra

The absorption spectra of the ligand L_2 and these complex with ligand L_2 were recorded in DMSO solvent in the range of 200-800nm. The UV-visible spectra of this ligand 5-methyl-1,3,4-thiadiazole-2-thiole (L_2) was characterized by prominent bands at 300nm due to intraligand transition π- π* transition. Spectra of complexes 1 and

2 showed four bands in the region 225nm,270nm,294nm,450nm and 245nm,296nm,307nm,530nm respectively.

Spectra of complexes have bands lower than 400nm due to intramolecular π- π* and n- π* transitions for aromatic ring. Bands at 450nm and 530nm are spectral features of octahedral complexes. These observations suggest an octahedral geometry of Ni(II).[40].

B. DNA binding Studies

1. Absorption spectra

In the presence of increasing concentrations of calf thymus DNA, the intra ligand and metal-to-ligand (MLCT) transitions of the complex were significantly perturbed, indicating interaction of the complex with DNA. Fig.1 and Fig.2 shows the absorption spectra of the complex in the presence of increasing amounts of CT-DNA at room temperature. With increasing CT-DNA concentration, 4-6 nm red shifts were observed for the complexes at MLCT band. The percentage hypochromicity of the MLCT band upon binding to DNA was observed to be 34% for complex (**1**) 33% for (**2**) respectively. In order to further investigate the binding strength of the complex, the intrinsic binding constant K_b of the complex with CT-DNA was determined by monitoring the changes of absorbance in the MLCT band with increasing concentration of DNA. This values are comparable is larger than those of so-called DNA-intercalative Ru(II) complexes ($1.1x10^4$ - $4.8 x10^4$ M^{-1}) [41,42] for $Ru(bpy)2(dppz)]^{2+}$ ($>10^6$) [43]. The observed K_b values for the complexes are in the order of $1.7x10^4$ (1) > $1.5x10^4$ **(2)** respectively. These spectral characteristics uggest that the complexes interact with DNA, most likely through intercalative mode.

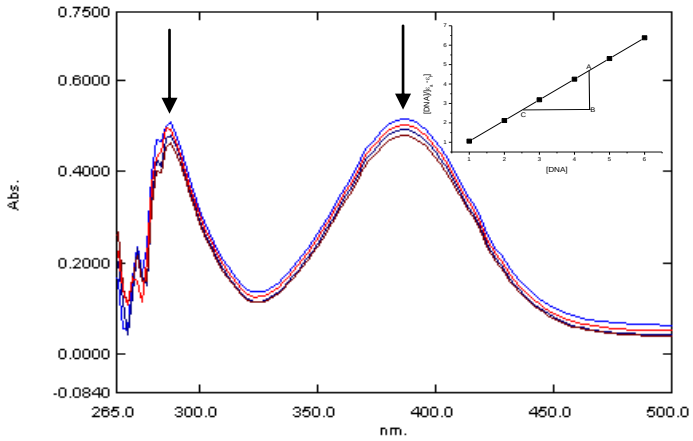


Fig.1. Absorption spectra of complex in Tris-HCL buffer upon addition of CT DNA. [Ni]=0.5 μM, [DNA]=0.1μM. Arrow shows the absorbance changing upon the increase of DNA concentration.

The inner plot of [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with Co(III) complex

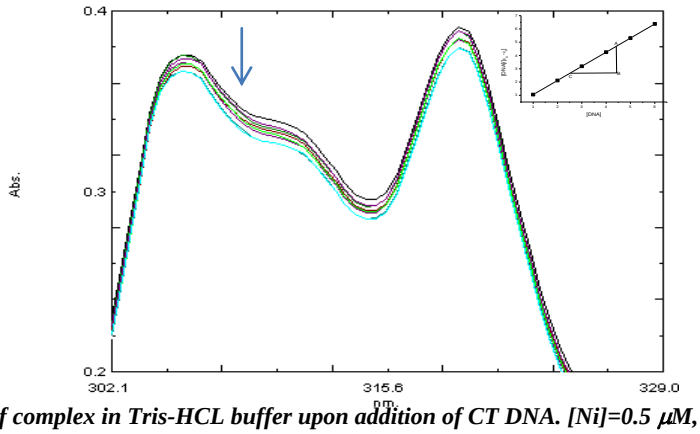


Fig.2. Absorption spectra of complex in Tris-HCL buffer upon addition of CT DNA. [Ni]=0.5 μM, [DNA]=0.1μM. Arrow shows the absorbance changing upon the increase of DNA concentration.

The inner plot of [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with Co(III) complex

2. Viscosity measurements

To further explore the binding of complexes to CTDNA, viscosity measurements were carried out on CT-DNA by varying the concentration of the added complexes. Viscosity measurements that are sensitive to length change of DNA are regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data [44]. It is popularly accepted that a classical intercalation mode results in lengthening the

DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial and/or nonclassical intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity [45,46]. Fig.3. shows the effects of complexes together with Ethidium bromide on the viscosity of rod-like DNA. Ethidium bromide is a known DNA classical intercalator and increases the relative specific viscosity by lengthening of the DNA double helix through the intercalation mode [47]. Upon increasing the containing of complexes the relative viscosity of DNA increases steadily, similar to the behavior of the ethidium bromide. The increased degree of viscosity, which may depend on the affinity for DNA. The viscosity results show that the complexes intercalate between the base pairs of DNA.

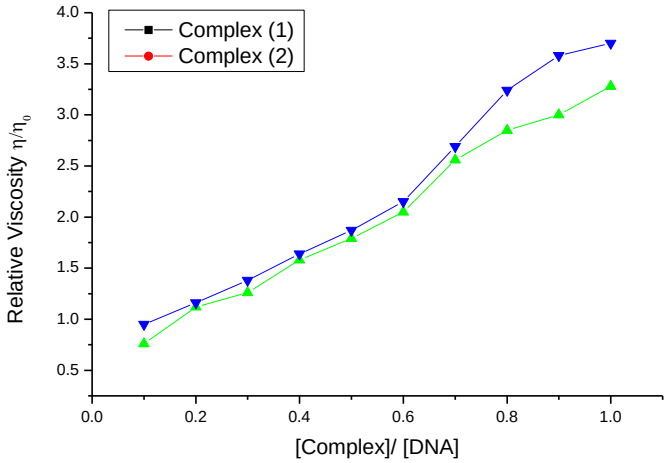


Fig.3. *Effect of increasing amounts of the complex* ***[Ni(phen)$_2$(L$_2$)](PF$_6$)$_2$ .2H$_2$O]*** *[---▼---] and* ***[Ni(bpy)$_2$(L$_2$)](PF$_6$)$_2$ .2H$_2$O*** *] [----▲-----] on the relative viscosities of CT-DNA at 25 (±0.1) ℃.*

3. Thermal denaturation studies

According to the literature [48-50], the intercalation of natural or synthesized organics and metallointercalators generally results in a considerable increase in melting temperature (*Tm*). The DNA melting experiment for the new complexes revealed that *Tm* of CT-DNA increase in the presence of the complexes under our experimental conditions. The observed melting temperatures in the absence of complexes were 68-71.6 ± 0.5 °C, whereas in the presence of complexes were 80-81.8 ± 0.5 °C, respectively. The moderate increases in *Tm* of the Ni(II) complexes are comparable to those observed for classical intercalators [48-50], and lend strong support for intercalation into the helix.

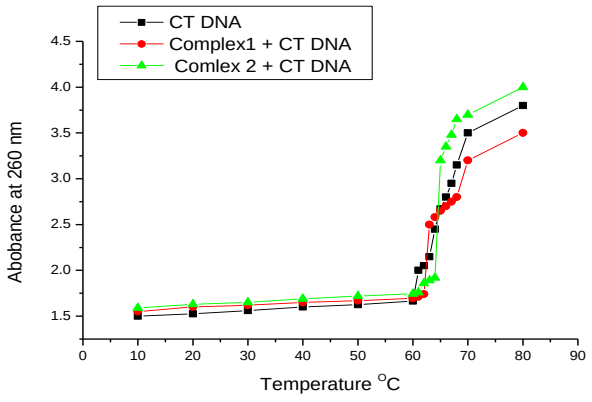


Fig. 4. *Melting curves of CT-DNA in the absence and presence of complexes*

C. DNA Cleavage Studies

The photocleavage of super coiled (SC) pUC19 DNA (0.1 µL(0.2 µg)) to its nicked circular (NC) form was determined by agarose gel electrophoresis in Tris-HCl buffer(50 mM, pH 7.2) containing NaCl (50 mM).The cleavage reactions mixture containing, 0.53 µM, 1.06µM and 2.12 µM complexes in 20µl buffer were photo irradiated using monochromatic UV or visiblelight. The samples were then incubated for 1 hour at 37° C followed by

addition to the loading buffer containing 25% bromophenolblue, 0.25% xylene cyanol, 30% glycerol (3 µL) and finally loaded on 0.8% agarose gel containing 1.0 µg/mL ethidium bromide. Electrophoresis was carried out at 50 V for 2 hours in Tris-borate EDTA (TBE) buffer. Bands were visualized by UV light and photographed to determine the extent of DNA cleavage from the intensities of the bands using syngene Gel Documentation System. The wavelength used for the photo-induced DNA cleavage experiments were 365 nm.

DNA is converted from Form I to Forms II and III due to the activities of the complexes. We can observe the fastest migration in the case of SC form (Form I) if only one strand is cleaved. The super coils relax to convert into a slower-moving form (Form II) if only one strand is cleaved. A linear form (Form III) is produced, when both strands are cleaved, which migrates between Forms I and II. In the absence of the complex (lane 1) Fig. 5 & 6 DNA remains in the super coiled (SC) form. Incubation with different concentration of complex the plasmid DNA is converted from Form I to Forms II at concentration of $0.17x10^{-3}$, $0.34x10^{-3}$ and $0.51x10^{-3}$ µg for lane 2, 3, 4 respectively. When the complex concentration increased, the intensity of the circular supercoiled DNA (Form-I) decreases while that for the nicked (Form-II) apparently increases. Both the complexes exhibit nuclease activity. At the concentration of$0.17x10^{-3}$, $0.34x10^{-3}$ (lanes 2 and 3) complexes (**1**) and (**2**) show lesser cleavage activity whereas at higher concentration of $0.51x10^{-3}$ µg (lanes 4) shows higher cleavage activity. From these experimental results, we infer that the complex (**1**) containing 5-methyl-1, 3, 4-thiadiazole-2-thiole (L_2) and phenanthroline ligand shows more cleavage activity than the complexes (2) containing 5-methyl-1,3,4-thiadiazole-2-thiole and bypyridine ligands. The cleavage activity observed for the complexes is in the order $Ni(bpy)_2(L_2)$ <$Ni(phen)_2(L_2)$ (Fig.5 & 6).

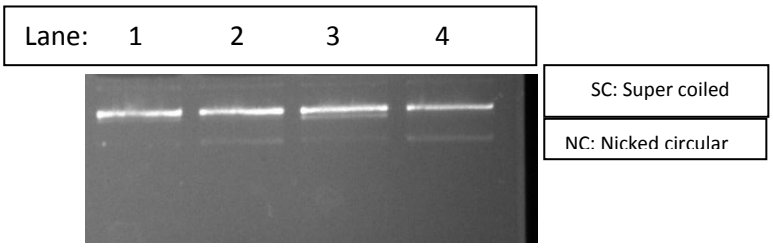


Fig.5. DNA cleavage activity of the complex 2. Lane 1: Control, Lane 2-4: complex (2) with increasing concentration $0.17*10^{-3}$, $0.34*10^{-3}$ and $0.51*10^{-3}$ µg,

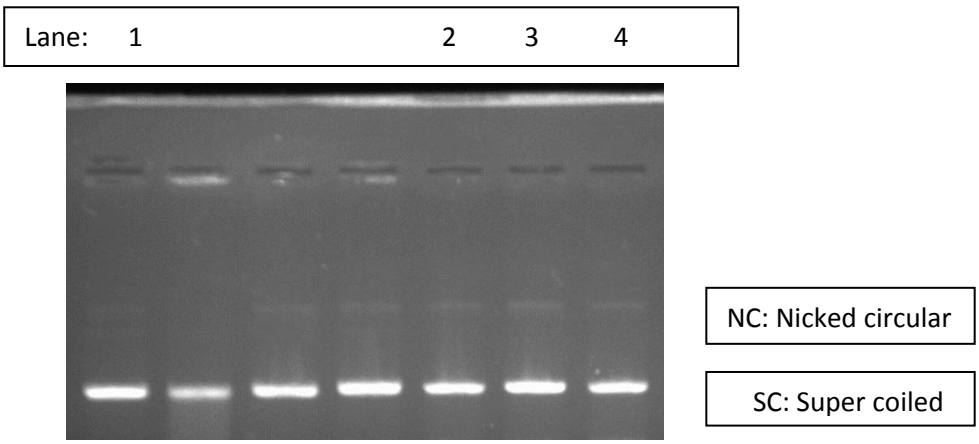


Fig.6. Lane 1: Control, Lane 2-4: complex (1) with increasing concentration $0.53*10^{-3}$, $1.06*10^{-3}$ and $2.12*10^{-3}$ µmol.

CONCLUSION

In this paper, two new mixed ligand complexes of Ni(II) has been synthesized and characterized. Moreover, the DNA-binding properties of the mixed ligand complexes of Ni(II) were investigated by electronic absorption, thermal denaturation, and viscosity measurements. The DNA photocleavage studies were monitored by Gel Electrophoressisby using pUC19 DNA. The experimental results indicate that the mixed ligand complexes of Ni(II) can bind to CT-DNA by partial intercalation mode. Information obtained from our study will be helpful to understand the mechanism of interactions of thiadiazole alongwith 1,10

phenanthroline/2'2 bipyridine and their complexes with nucleic acids and should be useful in the development of potential probes of DNA structure and conformation.

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