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Research Article

Studying DNA Interactions of Ni(II) Complexes of Thiosemicarbazone Containing Vic-Dioxime Ligands

Tiyosemikarbazon İçeren Vis-Dioksim Ligandının Ni(II) Kompleksinin DNA Etkileşimlerinin İncelenmesi

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Abstract

Vic-dioxime ligands and their metal complexes are very important for their biological activities and thoroughly studied. In addition, several thiosemicarbazone derivatives were synthesized and their potentials as anti-tumor agents were reported. As well as this, Nickel (II) complexes, among the other metal complexes, have an important proportion in DNA interaction studies. The compounds which have all these features (vic-dioxime, thiosemicarbazone group and Ni(II)) are not evaluated for their interactions with DNA.

DNA interactions of Ni(II) complex of thiosemicarbazone containing vic-dioxime ligand will be studied by using UV titration, floresans, and agaroz gel electrophoresis methods for the first time.

Keywords: DNA, Intercalation, Nickel (II) complex, Thiosemicarbazone, Vic-dioxime

Öz

Vis-dioksim ligantları biyolojik aktiviteleri açısından çok önemlidir ve metal kompleksleri yoğun bir şekilde çalışılmıştır. Ayrıca birçok tiyosemikarbazon türevi de sentezlenmiş ve potansiyel anti-tümör ajanları olarak rapor edilmiştir. DNA'ya bağlanma çalışmalarında kullanılan çeşitli metal kompleksleri arasında Nikel (II) kompleksleri de yer almaktadır. Bütün bu özellikleri (vis-dioksim, tiyosemikarbazon grubu ve Ni(II)) bir arada bulunduran bileşikler literatürde DNA'ya bağlanması açısından daha evvel hiç değerlendirilmemiştir.

Tiyosemikarbazon içeren vis-dioksim ligandının Ni(II) kompleksinin DNA ile etkileşimleri UV titrasyon yöntemi, flouresans, ve agaroz jel elektroforez yöntemleri ile ilk defa çalışılmıştır.

Anahtar Kelimeler: DNA, İnterkalasyon, Nikel(II) kompleksi, Tiyosemikarbazon, Vis-dioksim

1. Introduction

Vicinal-dioxime complexes are an important group of coordination compounds from different aspects. These classes of compounds are employed as the model compounds for examining the structure of vitamin B12, which have important roles in biological mechanisms (Chakravorty 1974). In addition, thiosemicarbazones and their metal complexes possess a range of biological applications; antitumor, antiviral, antibacterial, and antifungal activities have been studied (West et al. 1991). DNA binding behaviors of some Ni(II) complexes containing thiosemicarbazones were studied in a number of articles (Pelosi 2010, Prabhakaran et al. 2012).

Several cationic metal complexes possessing planar aromatic ligands may bind to DNA by intercalation, which involves stacking of the planar aromatic ligand between the adjacent base pairs of the DNA duplex (Coban et al. 2016, Coban et al. 2013, Fu et al. 2014, Medici et al. 2014, Patel, M. et al. 2014, Shobha Devi et al. 2014). Such metallointercalators tend to be strongly mutagenic, and some have shown promising chemotherapeutic activity, which correlates well with DNA-binding affinity (Medici et al. 2014, Patel et al. 2014, Qin et al. 2015, Shahabadi and Nemati 2014).

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In this study, two previously synthesized (Babahan et al. 2014) Ni(II) complexes of planar non-aromatic thiosemicarbazone containing vic-dioxime ligands were investigated as DNA binders. They possess a large planar surface which was expected to intercalate into DNA base pairs even though non-aromatic structure.

2. Material and Methods

All reagents and solvents were of commercial origin and used without further purification unless otherwise noted. Solutions of calf thymus DNA (CT-DNA; purchased from Sigma) in 50 mM ammonium acetate (pH 7.5) had a UV–Vis absorbance ratio of 1.8–1.9: 1 at 260 and 280 nm (A260/A280 = 1.9), indicating that the DNA was sufficiently free of (Coban and Yildiz 2014). The concentration of DNA was determined spectrophotometrically using a molar absorptivity of 6,600 M^{-1} cm^{-1} (260 nm) (Liu et al. 2011). Double-distilled water was used to prepare buffers. Stock solution of CT-DNA was stored at 4 °C and used within 4 days. The compounds and ligands were prepared by literature methods (Babahan et al. 2014). All solutions of the Ni(II) complexes were prepared by dissolving a weighed amount (2-3 mg) in 0.5 mL DMSO for solubility reasons and were then diluted (up to 150 times without precipitation) with 50 mM ammonium acetate (pH 7.5) to the required concentration.

2.1. Absorption and Emission Titrations

UV–Vis spectra were recorded with a Varian Cary 100 spectrophotometer and emission spectra were recorded with a PerkinElmer LS 55 spectrofluorophotometer at room temperature.

For the absorption and emission titrations, ligands and Ni(II) complexes (about 1 mmol) were dissolved in a minimum amount of DMSO (0.5 mL), and were then diluted in buffer (5 mM ammonium acetate, pH 7.5) to a final concentration of 20 μM. Titrations were performed in a 10-mm stoppered quartz cell by using a fixed concentration of the complex (20 μM), to which the CT-DNA stock solution was added in increments of 1 μL to a DNA-to-nickel concentration ratio of 6:1. Analysis was performed by means of a UV–Vis or fluorescence spectrophotometer by recording the spectrum after each addition of DNA. Ligands and Ni(II) complex–DNA solutions were incubated for 10 min each time before the spectra were recorded. A control solution of 20 μM Ni (II) complex in the same buffer was also treated in the same manner. Cell compartments were thermostated at 25 ± 0.1°C.

For emission intensity measurements, the excitation wavelength was fixed and the emission range was adjusted before the measurements. Ammonium acetate (5 mM), pH 7.5 buffer was used as a blank to make preliminary adjustments. All measurements were performed with a 5-nm entrance slit and a 5-nm exit slit. The complexes were excited at 380 nm; the emission spectra were monitored between 600 and 800 nm.

2.2. Competitive Studies

The competitive behavior of each Ni(II) complexes with ethidium bromide (EB) was investigated by fluorescence spectroscopy in order to examine whether the Ni(II) complexes are able to displace EB from the DNA–EB complex.

DNA was pretreated with EB at a DNA-to-EB concentration ratio of 50:1 for 30 min at 27 °C to prepare the initial DNA-EB complex. The intercalating effect of the Ni(II) complexes with the DNA-EB complex was studied by adding a certain amount of a solution of the Ni(II) complex in increments to the solution of the DNA-EB complex. The influence of each addition of Ni(II) complex to the solution of the DNA-EB complex was obtained by recording the change in the fluorescence spectrum at 640 nm. To study the competitive binding of the Ni(II) complexes with EB, EB was excited at 453 nm in the presence of DNA alone as well as in the presence of the Ni(II) complexes.

2.3. DNAse Activity by Gel Electrophoresis

Gel electrophoresis experiments were performed using pBR322 negatively supercoiled plasmid DNA and 1 % agarose gels together with a tris(hydroxymethyl) aminomethane-borate-EDTA running buffer solution. Reaction mixtures (10 mL) containing 0.1 mg pBR322 together with different amounts of **K1** and **K2** (0, 50, 500 μM) in 50 mM ammonium acetate buffer, pH 7.5 was adjusted at 0 °C, were then incubated at 36 °C for 1 h in the dark. Prior to the samples being loaded onto the gel, 2.5 mL of 0.25 % bromophenol blue loading buffer and sucrose in water (40 % w/v) was added to the reaction mixtures. Gels were obtained at room temperature by using a Thermo midi horizontal agarose gel electrophoresis system and applying a potential of 35 V for 4 h. The resulting gels were stained with EB solution (0.5 mg mL^{-1}) for 45 min, after which they were soaked in water for a further 20 min. Gels were visualized under UV light and photographed.

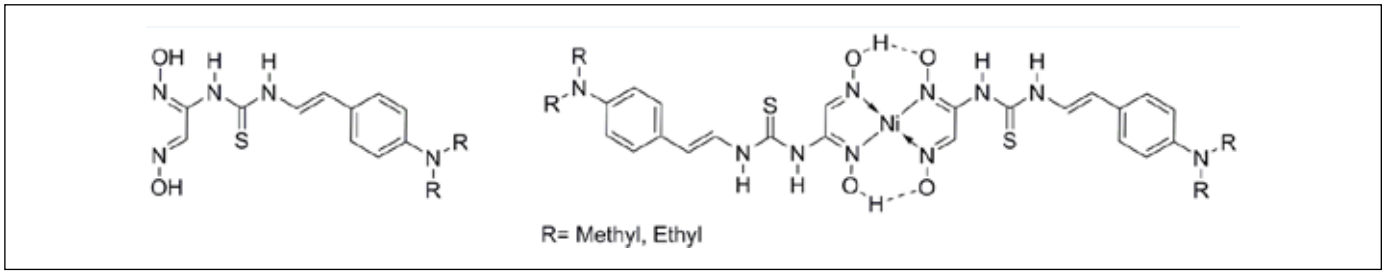

Figure 1. Molecular structure of the ligand $\mathbf{LH_2}$ and complexes $\mathbf{Ni(LH)_2}$.

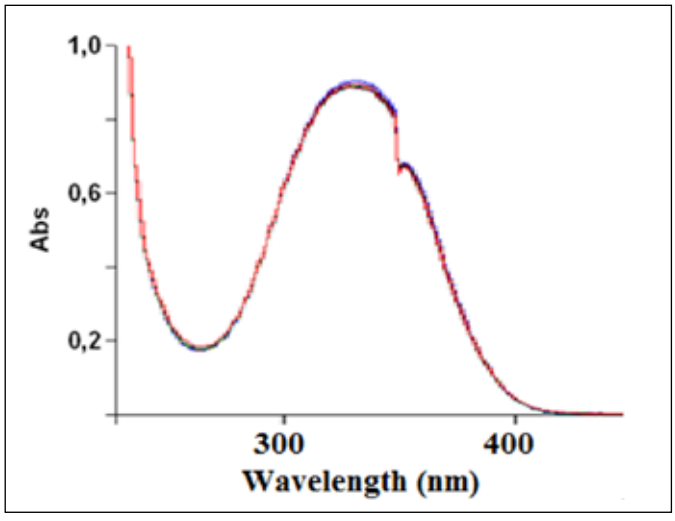

Figure 2. The absorption spectra of the ligand in the absence and presence of CT-DNA ([**L**]= 20 μM; [DNA]= 0 – 20 μM).

3. Results and Discussion

The ligands and complexes were synthesized by the synthetic procedure (Babahan et al. 2014) and the structures were confirmed by CHN analyses, ESI-MS and ^{1}H NMR spectroscopy. These ligands were synthesized by the first reaction between thiosemicarbazide and related aldehydes to yield the thiosemicarbazones which then reacted with anti-chloroglyoxime to produce (2E)-2-[4-(dialkylamino) benzylidene]-N-[(1Z,2E)-N-hydroxy-2-(hydroxyimino) ethanimidoyl]hydrazine carbothioamide in the presence of alcohol. These ligands ($\mathbf{LH_2}$) have the thione form (H-N-C=S) in the solid state and nickel complexes were prepared by the reaction of the ligand with $NiCl_2.xH_2O$ in alcohol. According to the FTIR spectrum a hydroxyl group disappears and intramolecular hydrogen bonds form upon Ni(II) complexation ([Ni($\mathbf{LH}$)$_2$]) which involves the C=N bond of the vic-dioximes groups, with N,N' coordinated with the Ni(II) ion Ni-S bond is not formed (Fig. 1) (Babahan et al. 2014).

3.1. Electronic Absorption Titration

Electronic spectra are a useful way to investigate the interactions of complexes with DNA. A complex bound to DNA through intercalation usually results in hypochromism and red shift (bathochromism) owing to the intercalation mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The extent of the hypochromism in the visible metal-to-ligand charge transfer band is commonly consistent with the strength of the intercalative interaction while the red shift is associated with the decrease in the energy gap between the highest and the lowest molecular orbitals (HUMO and LUMO) after binding of the complex to DNA (Barton et al. 1984). On the other hand, if the interaction is only electrostatic or grove binding then hyperchromism and blue shift occurs in the spectra (Arjmand and Aziz 2009, Xu et al. 2008).

The absorption spectra of the ligand in the absence and presence of CT-DNA (at a constant concentration, 20 μM) are shown in Fig. 2. The observed very slight hypochromism (approximately 2 %) and no red shift, indicate the interaction is very weak.

On the other hand, absorption spectra of **K1** and **K2** in the absence and presence of CT-DNA (at a constant concentration of complexes, 20 μM Ni(II)) are shown in Fig. 3. The observed hypochromism increases with increasing DNA concentration, indicating an intercalative binding mode, however, again no red shift in the metal-to-ligand charge transfer band of the complexes was observed and this also suggests that the binding mode of both **K1** and **K2** was not a classical intercalation (Arjmand and Aziz 2009, González-Ruiz et al. 2011, Sirajuddin et al. 2013). When the amount of DNA was increased, the decreases in the intensities of the π-π* transitions were 9 % for **K1** at a DNA-to-Ni (II) concentration ratio of 1.05, and 11.1 % for **K2** at a DNA-to-Ni(II) concentration ratio of 0.95. To quantitatively compare the DNA-binding affinities of

the two complexes, the intrinsic binding constants K_b of the two complexes with regard to DNA were obtained by monitoring the changes of the π-π* absorbance at 348 and 349 nm for **K1** and **K2**, respectively, according to the following equation:

$$[DNA]/(\varepsilon_A - \varepsilon_f) = [DNA]/(\varepsilon_B - \varepsilon_f) + 1/K_b(\varepsilon_B - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of the nucleic acid in base pairs, ε_A is the apparent absorption coefficient obtained by calculating A_{obs}/[Ni], and ε_f and ε_B are the absorption coefficients of the free and the fully bound platinum complex, respectively. In the [DNA]/(ε_A - ε_f) versus [DNA] plot, K_b is given by the ratio of the slope to the intercept. The values of the intrinsic binding constants K_b for complexes **K1** and **K2** were derived to be 5.1 (± 0.3) x 10^5 M^{-1} and 7.1 (± 0.6) x 10^5 M^{-1}, respectively.

3.2. Emission Spectra

In the absence of DNA, complexes **K1** and **K2** can emit weak luminescence in ammonium acetate buffer at ambient temperature, with the maximum at 680 and 780 nm, respectively. On addition of CT-DNA, the emission intensity increases slightly (Fig. 4). This implies that the complexes can interact with CT-DNA and are protected by DNA efficiently, since the hydrophobic environment inside the DNA helix reduces the accessibility of solvent water molecules to the complex and the mobility of the complex is restricted at the binding site, leading to a decrease of the vibrational modes of relaxation.

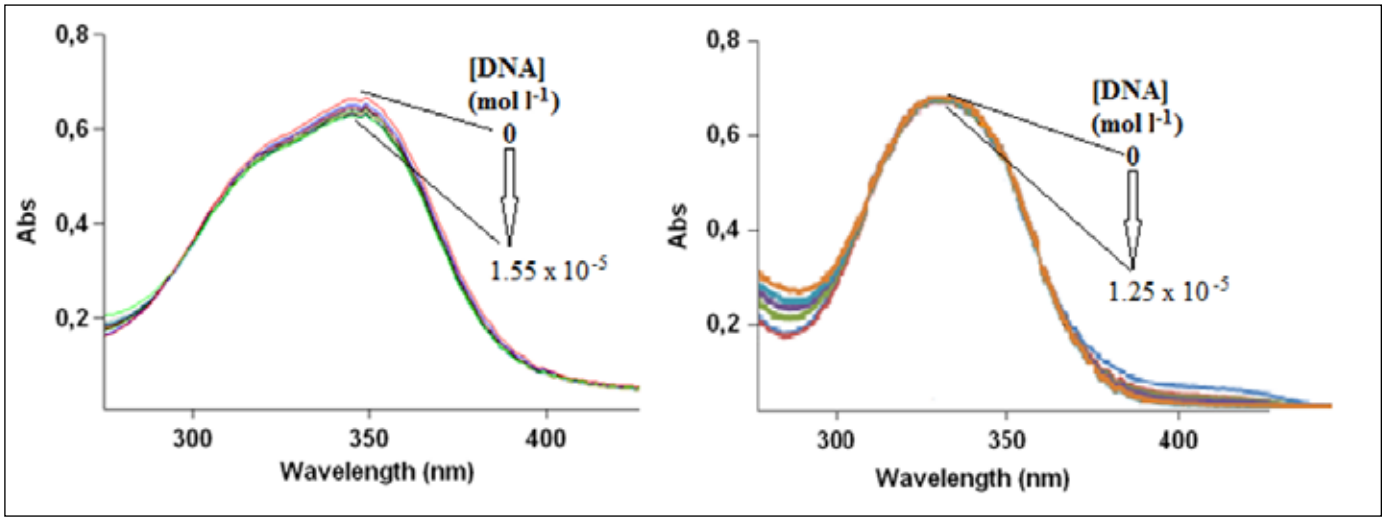

Figure 3. The absorption spectra of **K1** and **K2** in the absence and presence of CT-DNA ([**K**]= 20 μM; [DNA]= 0 – 20 μM).

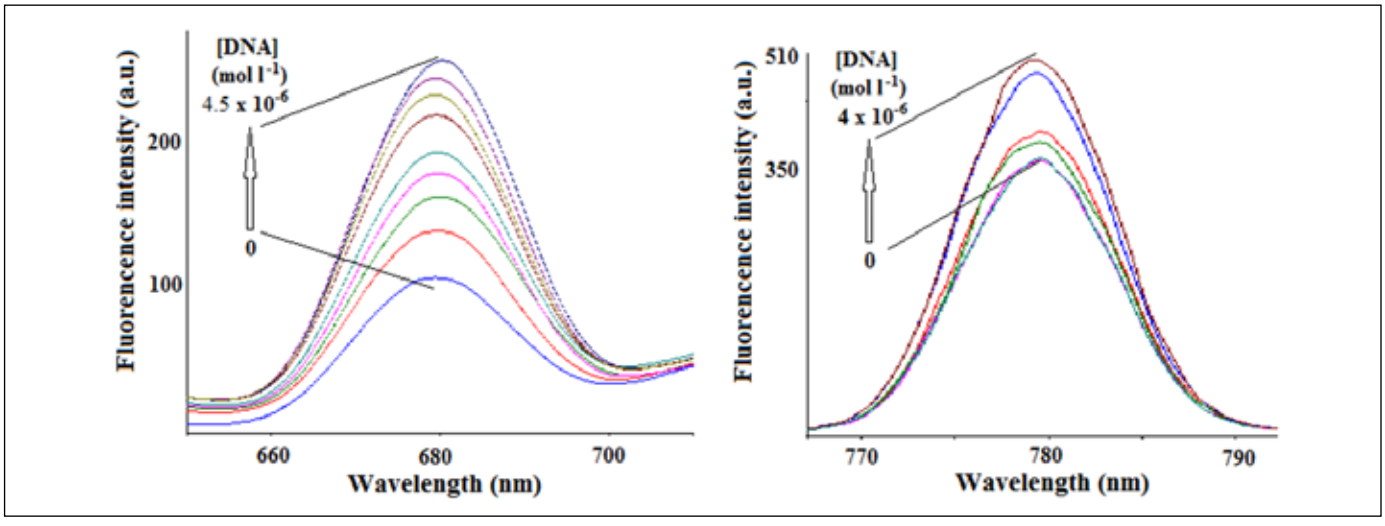

Figure 4. The emmission spectra of **K1** and **K2** in the absence and presence of CT-DNA ([**K**]= 2 μM; [DNA]= 0 – 5 μM).

3.3. Competitive Binding Experiments

Competitive binding experiments with a well-established quenching assay based on the displacement of the intercalating drug EB from CT-DNA may give further information regarding the DNA-binding properties of the complex. A complex which is involved in strong DNA intercalation would compete with the intercalatively bound EB for DNA binding and quench the EB emission. When a complex displaces EB from DNA-bound EB, the emission of the solution will be greatly quenched because the free EB molecules are readily quenched by the surrounding water molecules (Ortmans et al. 2004). DNA intercalators were reported to cause evident reduction in EB emission intensities (Kelly et al. 1985), but only moderate decays in emission intensities were reported for groove DNA binders. The results can be interpreted by the Stern–Volmer equation (6) (Eftink and Ghiron 1981), which is used to calculate the degree of binding of the complexes:

$$I_0/I = 1 + K_{SV}\,[Q], \qquad (6)$$

where I_0 and I represent the fluorescence intensities in the absence and presence of the complex, respectively, K_{SV} is a linear Stern–Volmer quenching constant and $[Q]$ is the concentration of the quencher. As shown in Fig. 6, the emission of EB is quenched by both complexes. Appreciable reduction in emission intensity was achieved on addition of both complexes to the EB–DNA system. The quenching plots shown in the insets in Fig. 5 are in good agreement with the linear Stern-Volmer equations with K_{SV} are 4.9 x 10^5 M^{-1} and 5.5 x 10^5 M^{-1} for **K1** and **K2**, respectively.

To compare the binding strengths of these two complexes to DNA, apparent DNA binding constants (K_{app}) were calculated as 1.9 x 10^5 M^{-1} and 2.53 x 10^5 M^{-1} for **K1** and **K2**, respectively, using equation 7:

$$K_{app} = K_{EB} \times C_{EB}/C_{50}, \qquad (7)$$

Where C_{50} is the concentration of the complex that causes 50 % reduction of the initial fluorescence of DNA-EB emission intensity, K_{EB} is the binding constant (K_b) of 4.54 x 10^5 M^{-1} for EB (Baguley and Falkenhaug 1978). These values are very similar to those derived by direct spectroscopic measurements. A comparable difference was obtained where **K2** was found to quench the EB emission more efficiently than **K1**. In addition, ligands **L1** and **L2** had no effect on the emission intensity of EB-DNA complex indicating that the ligands are not intercalating into DNA.

3.4. Cleavage of pBR322 DNA by Ni(II) Complexes

The potential of the present complexes to cleave DNA was studied by gel electrophoresis using supercoiled pBR322 DNA in ammonium acetate buffer (pH 7.5). When circular plasmid DNA is subjected to gel electrophoresis, relatively fast migration will be observed for the intact supercoiled form (form I). If scission occurs on one strand (nicked circular), the supercoiled form will relax to generate a slower-moving open circular form (form II). If both strands are cleaved, a linear form (form III) that migrates between forms I and II will be generated (Barton and Raphael 1984). Fig. 6 shows separation of pBR322 DNA by gel electrophoresis after incubation with **K1** and **K2**, respectively.

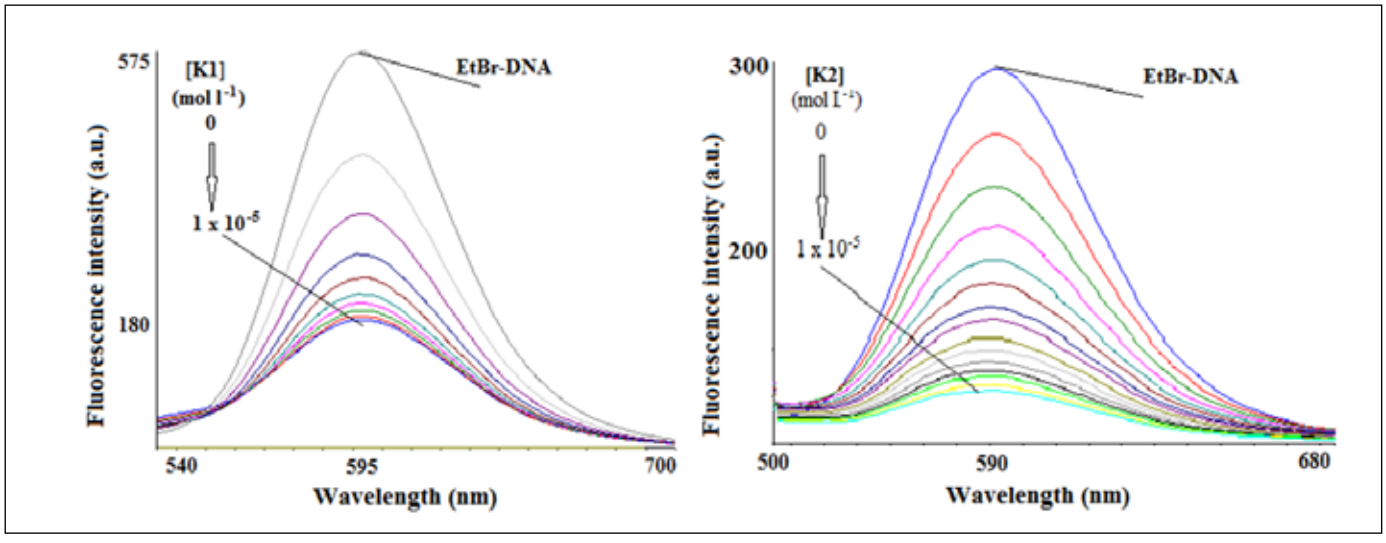

Figure 5. Emission spectra of EB (bound to DNA) in the presence of complexes **K1** and **K2**.

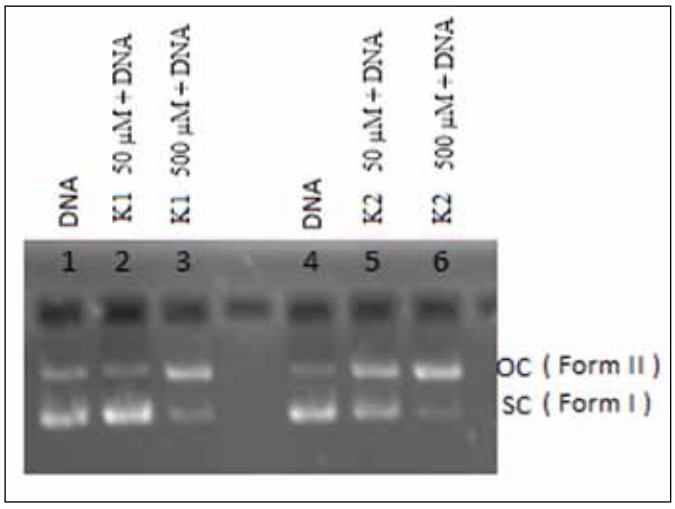

Figure 6. Cleavage of pBR322 DNA in the presence of increasing concentration of complexes **K1** and **K2.** Lanes 1 and 4, DNA alone; Lanes 2 and 3, **K1** in 50 and 500 µM concentrations; Lanes 2 and 3, **K2** in 50 and 500 µM concentrations.

As shown in Fig. 6, K**1** is unable to cleave DNA in low concentrations, whereas it exhibits a slight reduced mobility (travel less distance) in the high concentrations with respect to control (DNA alone), which may be attributed to a scission on a single strand (nicked circular; form II). In comparison, it is clear that K**2** changes the DNA conformation both in low and high concentrations.

4. Conclusion

The DNA binding abilities of two previously synthesized Ni(II) complexes **K1** and **K2** have been investigated and the results of three different methods (direct UV-spectrophotometric method, fluorescence and competitive emission quenching strength) show that both complexes can bind to DNA but with different affinities, resulting in intrinsic binding constants K_b = 5.1 (± 0.3) x 10^5 M^{-1} and 7.1 (± 0.6) x 10^5 M^{-1}, respectively for **K1** and **K2**, respectively, derived by direct spectroscopic measurements. The results also suggest that the complexes bind to DNA mainly through non-classical intercalation binding mode (partial intercalation). Fluorescence studies also support this result with similar binding values. Finally DNAse activities of the complexes through gel electrophoresis studies show that both complexes breaks single strand of DNA causing the relaxed form (Form II) of plasmid DNA and clearly **K2** has a higher DNAse activity producing slightly more relaxed form of DNA. The difference between the complexes can be explained by the elevated hydrophobic nature of the **K2** due to the diethylated nitrogen compare to the methyl derivative (**K1**) and this feature gives better binding ability to DNA's hydrophobic environment.

This is the first example of planar non-aromatic ligand partially-intercalating into DNA base pairs.

5. Acknowledgements

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