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Synthesis, characterization and DNA-binding studies of 1-cyclohexyl-3-tosylurea and its Ni(II), and Cd(II) complexes

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Abstract

1-Cyclohexyl-3-tosylurea (HL) and its two complexes, $ML_2 \cdot 2H_2O$ [M=Ni(**1**), and Cd(**2**)], have been synthesized and characterized on the basis of elemental analyses, molar conductivities, IR spectra and thermal analyses. In addition, the DNA-binding properties of the ligand and the two complexes have been investigated by electronic absorption, fluorescence, CD spectroscopy and viscosity measurements. The experiment results suggest that the ligand and its two complexes bind to DNA via a groove binding mode, and the binding affinity of the complex **2** is higher than that of the complex **1** and the ligand.

Keywords: 1-Cyclohexyl-3-tosylurea ligand; Sulfonylurea metal complexes; DNA-binding; Fluorescence spectroscopy; CD spectroscopy; Viscosity measurements

1. Introduction

One of the important drugs regulating biological functions of pancreatic islets is the sulfonylurea, which has been used for the treatment of type 2 diabetes mellitus because of its insulinotropic activity on pancreatic islets [1]. The design of small complexes that bind and react at specific sequences of DNA becomes important. Researching the co-operation between the metal ions and the more complete understanding of how to target DNA sites with specificity will lead to novel chemotherapeutics to probe DNA and to develop highly sensitive diagnostic agent [2]. The metal complexes also have many noticeable bioactivities [3]. The synthesis of the complexes of sulfonylurea plays an important role in exploring the mechanism of the molecular biology [4].

Transition metal complexes have attracted considerable attention as catalytic systems for use in the oxidation of organic compounds [5], probes in electron-transfer reactions involving metallo-proteins [6], and intercalators with DNA [7]. The interactions between Cd^{2+} ions and DNA have recently been reported by Hossain and Huq [8]. They believed that Cd^{2+} ions covalently bind into adenine and guanine in DNA. The coordination compounds of urea have been reported to act as enzyme inhibitors [9], nickel(II) complexes of relevance to the active site of the enzyme [10] some of which have a urea molecule bound to the nickel centre and are useful due to their pharmacological applications [11,12].

We have now extended our efforts and prepared the transition metal complexes with a sulfonylurea ligand such as 1-cyclohexyl-3-tosylurea. We have prepared and characterized 1-cyclohexyl-3-tosylurea and its complexes, we described a comparative study of the interaction of the ligand and its complexes with calf thymus DNA (CT-DNA), using electronic absorption, fluorescence, CD spectroscopy and viscosity measurements. Information obtained from this study will be helpful to understand the mechanism of the interaction between the sulfonylurea complexes and nucleic acids.

2. Experimental

2.1. Instrumentation

The melting points of the compounds were determined on a Beijing XT4-100× microscopic melting point apparatus (the thermometer was not corrected). Elemental analyses were carried out on an Elemental Vario EL analyzer. The metal contents of the complexes were determined by titration with EDTA

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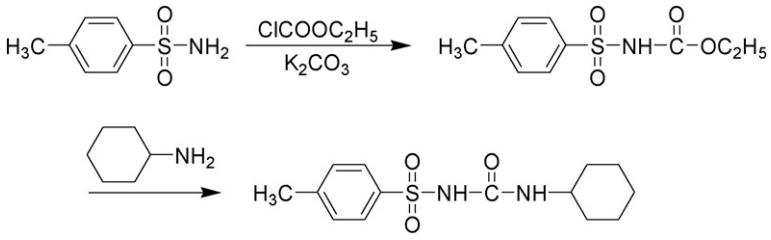


Fig. 1. The preparation of the ligand.

(xylenol orange tetrasodium salt used as an indicator and hexamethylidynetetraimine as buffer). The IR spectra were obtained in KBr disks on a Therrno Mattson FTIR spectrometer in the 4000–400 cm^{-1} region. ^1H NMR spectra were recorded on a Varian VR 300-MHz spectrometer in $CDCl_3$ or $(CD_3)_2SO$ with TMS as internal standard. All conductivity measurements were performed in DMF with a DDS-11A conductor at 25 °C. The UV spectra were recorded on a Varian Carry 100 UV–vis spectrophotometer. The thermal behaviour was monitored on a PCT-2 differential thermal analyzer. Mass spectra were performed on a VG ZAB-HS (FAB) instrument and electrospray mass spectra (ESI-MS) were recorded on a LQC system (Finngan MAT, USA) using CH_3OH as mobile phase. Fluorescence measurements were made on a Hitachi RF-4500 spectrofluorophotometer. The CD spectra were recorded on an Olos RSM 1000 at increasing complex/DNA ratio ($r = 0.0$ and 0.5). Each sample solution was scanned in the range of 220–320 nm. A CD spectrum was generated which represented the average of three scans from which the buffer background had been subtracted. The concentration of DNA was 1.0×10^{-4} M.

2.2. Materials and methods

Calf thymus DNA (CT-DNA) was purchased from Sigma without further purification. EDTA and transition metal nitrates were produced in China. All chemicals used were of analytical grade. All the experiments involving interaction of the ligand and the complexes with CT-DNA were carried out in doubly distilled water buffer containing 5 mM Tris and 50 mM NaCl and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm of about 1.8–1.9, indicating that the CT-DNA was sufficiently free of protein [13]. The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm. The ligand and the complexes were dissolved in a mixture solvent of 1% CH_3OH and 99% Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.2) at concentration 1.0×10^{-5} M. An absorption titration experiment was performed by maintaining 10 μM compounds and varying the concentration of nucleic acid. While measuring the absorption spectra, an equal amount of CT-DNA was added to both the compound solution and the reference solution to eliminate the absorbance of CT-DNA itself.

Viscosity experiments were conducted on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained at 25 ± 0.1 °C. Titrations were performed for the complexes (0.5–3 μM), and each compound was introduced into CT-DNA solution (5 μM) present in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound to CT-DNA, where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-DNA containing solution corrected from the flow time of buffer alone (t_0), $\eta = (t - t_0)/t_0$ [14].

Absorption titration experiment was performed with fixed concentrations of the drugs, while gradually increasing concentration of DNA. While measuring the absorption spectra, an equal amount of DNA was added to both compound solution and the reference solution to eliminate the absorbance of DNA itself. From the absorption titration data, the binding constant was determined using [15].

$$\frac{[\mathrm{DNA}]}{(\varepsilon_a - \varepsilon_f)} = \frac{[\mathrm{DNA}]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}$$

where [DNA] is the concentration of DNA in base pairs, ε_a corresponds to the extinction coefficient observed (A_{obsd}/[M]), ε_f corresponds to the extinction coefficient of the free compound, ε_b is the extinction coefficient of the compound when fully bound to DNA, and K_b is the intrinsic binding constant. The ratio of slope to intercept in the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] gives the values of K_b. Over a range of DNA concentrations from 50 to 350 μM.

To affirm quantitatively the affinity of the complexes bound to DNA, the intrinsic binding ability of the complexes to DNA were obtained by fluorescence titration method. Fixed amounts (10 μM) of the complexes were titrated with increasing amounts of CT-DNA. Excitation and emission wavelengths of the samples were 279 and 330 nm, slit width 5/5 nm. All experiments were conducted at 20 °C in a buffer containing 5 mM Tris–HCl (pH 7.2) and 50 mM NaCl concentrations. All experiments were conducted at 20 °C in a buffer containing 5 mM Tris–HCl (pH 7.2) and 50 mM NaCl concentrations.

2.3. Preparation of the ligand (HL)

The preparation of the ligand is shown in Fig. 1. A mixture of *p*-toluene sulfonamide (5.14 g, 0.03 mol) and finely pulverized K_2CO_3 (11.00 g, 0.078 mol) in 35 mL of acetone was stirred and heated to reflux for 30 min. Acetone solution (10 mL) of ethyl chloroformate (3.80 mL, 0.040 mol, 1.33 equiv.) was added to the mixture and heating continued for another 4 h. The mixture of the results were poured into 100 mL of H_2O, then the aqueous

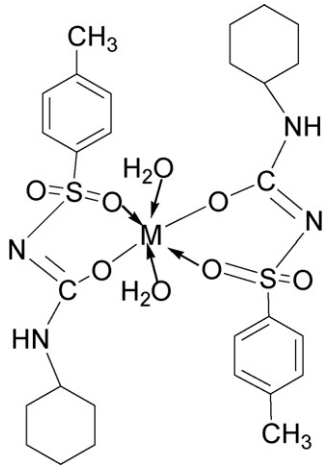


Fig. 2. The suggested structure of the complexes (M=Ni, Cd).

phases was acidified with 10 mL of 1.00 N aqueous HCl to get the solid, washed with 80 mL H_2O for several times. Recrystallization from 20 mL ethanol provided 6.35 g (87%) of ethyl *N*-(3-tossulfonyl) carbamate as a white crystalline solid: m.p. 76–78 °C.

A solution of ethyl *N*-(3-tossulfonyl) carbamate (0.73 g, 3.00 mmol) and cyclohexanamine (0.45 mL, 3.90 mmol, 1.30 equiv.) in 40 mL PhMe was heated to reflux for 6 h. After the solution stood overnight at room temperature, the resulting precipitate was collected, washed twice time with PhMe, and dried in vacuum to afford 0.67 g (75%) of a white solid: m.p. 156–158 °C; FAB-MS: $m/z = 297$ $[M+H]^+$. Anal. calcd for $C_{14}H_{20}N_2O_3S$: C, 56.73; H, 6.80; N, 9.45. Found: C, 56.80; H, 6.75; N, 9.56. IR ν_{max} (cm^{-1}): ν_{NH}(–CONH–): 3340, ν_{NH}(–SOONH–): 3047, ν_{CO}(–CO–): 1604, ν_{SO}(–SO_2NH–): 1130. U_{max} (nm): (CH_3OH) 203, 227.

2.4. Preparation of the complexes

The ligand (600 mg, 2.03 mmol) was added to an ethanol solution of $NaOC_2H_5$ (142.80 mg, 2.10 mmol), then the Ni $(NO_3)_2{\cdot}3H_2O$ (290.80 mg, 1 mmol) (10 ml ethanol) was added to the system. Immediately there was a green precipitate in the solution. After stirring for 4 h at room temperature, the precipitate was separated by the centrifugal and washed seven times with ethanol and one time with ether, and finally dried in vacuo. The Cd (II) complex was synthesized by the same way. Anal. calcd for complex **1** $C_{28}H_{42}N_4O_8S_2Ni$: C, 49.72; H, 6.47; N, 8.00; Ni, 8.38. Found: C, 50.03; H, 6.36; N, 7.91; Ni, 8.91. Λ_m($s\,cm^2\,mol^{-1}$): 10.8. ESI-MS [CH_3OH, m/z]: 649.2 ($\{[Ni(L)_2{\cdot}2H_2O] - 2H_2O + H\}^+$), 353.1($\{[Ni(L)_2{\cdot}2H_2O] - 2H_2O - L\}^+$), IR ν_{max} (cm^{-1}): ν_{SO}(SO_2NH): 1088 cm^{-1}, ν_{CO}(–CO–): 1520 cm^{-1}, ν(C=N): 1678 cm^{-1}. U_{max} (nm): (CH_3OH) 208, 229, 273. Anal. calcd for complex **2** $C_{28}H_{42}N_4O_8S_2Cd$: C, 46.18; H, 6.01; N, 7.43; Cd, 14.90. Found: C, 46.36; H, 5.86; N, 7.49; Cd, 14.77. Λ_m($s\,cm^2\,mol^{-1}$): 13.2. ESI-MS [CH_3OH, m/z]: 705.1 ($\{[Cd(L)_2{\cdot}2H_2O] - 2H_2O + H\}^+$), 409.0 ($\{[Cd(L)_2{\cdot}2H_2O] - 2H_2O - L\}^+$), IR ν_{max} (cm^{-1}): ν_{SO}(SO_2NH): 1092 cm^{-1}, ν_{CO}(–CO–): 1520 cm^{-1}, ν(C=N): 1678 cm^{-1}. U_{max} (nm): (CH_3OH) 208, 228, 274.

3. Results and discussion

All of the complexes are air stable for extended periods and remarkably soluble in DMSO and DMF; soluble in methanol and slightly soluble in ethanol, insoluble in benzene, water and diethyl ether. The molar conductivities in DMF solution indicate that the complex **1** and complex **2** (10.8 and 13.2 $S\,cm^2\,mol^{-1}$) are in the range expected for no electrolytes [16]. The elemental analyses, ESI-MS and molar conductivities show that the formulas of the complexes conform to $ML_2{\cdot}2H_2O$ (M=Ni, Cd).

3.1. Infrared spectra

The spectra of the ligand exhibit ν_{NH}(–CONH–) vibration bands at the 3340 cm^{-1} and ν_{NH}(–SOONH–) vibrations at the 3047 cm^{-1}, in the complex the ν_{NH}(–CONH–) and the ν_{NH}(–SOONH–) vibration were blue shifted to 3353 cm^{-1} and 3255 cm^{-1}, respectively. The band of ν_{CO}(–CO–) appeared at 1624 cm^{-1} in the ligand while the band of the complexes was exhibited at 1608 cm^{-1}; $\Delta\nu_{(ligand-complexes)}$ is to 16 cm^{-1}. This shift confirms that the group loses its original characteristics and forms coordinative bonds with the metal [17]. The band at the 1130 cm^{-1} was ν_{SO}(–SO_2NH–) vibration in the ligand. In the complexes these bands are presented at 1088 cm^{-1}. The band at about 1678 cm^{-1} in the complexes can be assigned to (C=N) which suggested that the H atom of the group (–SOONH–) had been substituted by the metal atom. Weak bands at 552 cm^{-1} are assigned to ν(M–O). These shifts demonstrate that the ligand coordinated Ni^{2+} and Cd^{2+} ions through the oxygen of carbonyl and sulphanilamide.

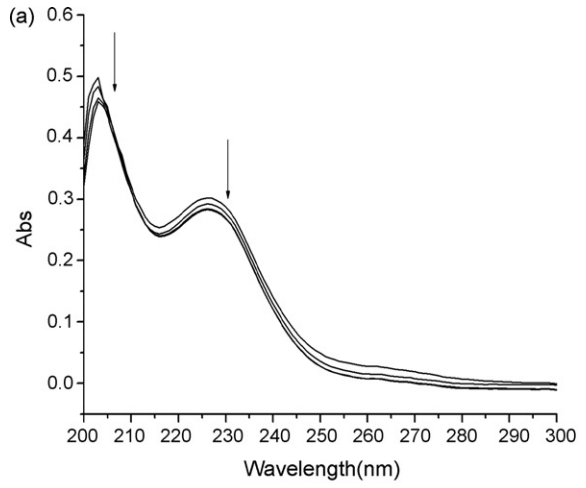
3.2. UV spectra

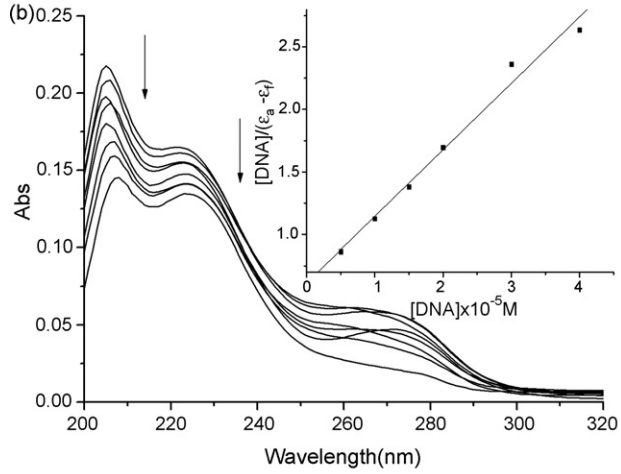
The study of the electronic spectra in the ultraviolet and visible ranges for the complexes and the ligand were carried out in the buffer solution. The electronic spectra of ligand had a strong band at $\lambda_{max} = 203$ nm, a medium band at $\lambda_{max} = 227$ nm. There are three bands at 208, 229 and 273 nm for complex **1** and complex **2**. These changes indicate that complexes are formed.

3.3. Thermal analyses

The complexes begin to decompose at 212 °C or so and there are two exothermic peaks appearing around 212–353 °C. The corresponding TG curves show a series of weight loss. Under 200 °C, there are no endothermic peak and no weight loss on corresponding TG curves. It indicates that there are no crystal or coordinate solvent molecules. While being heat to 800 °C, the complexes become their corresponding oxides, the residues are in accordance with calculation.

On the basis of above evidence and analyses, the possible structure of the complexes is shown in Fig. 2.





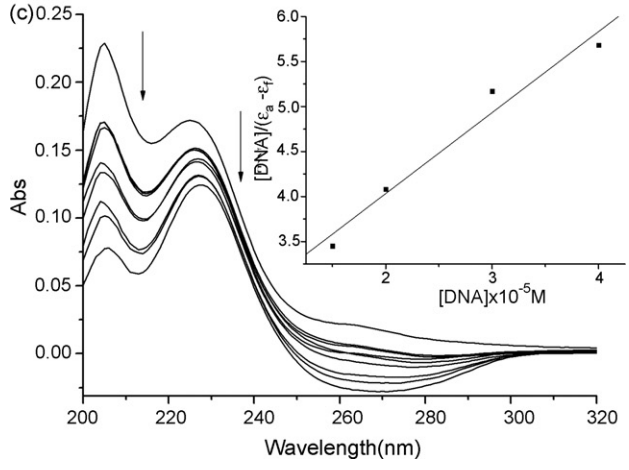
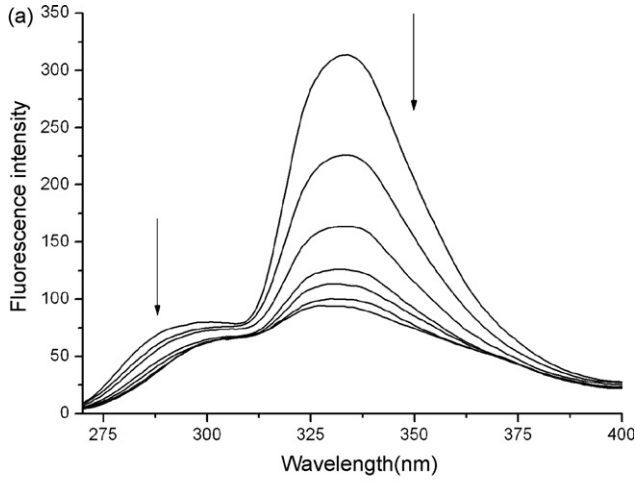
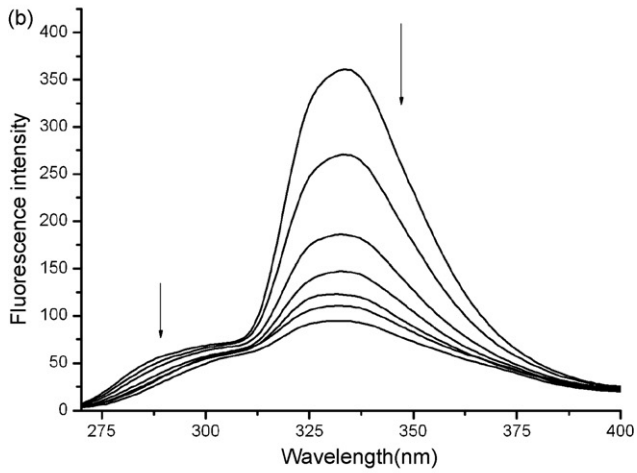


Fig. 3. (a) Electronic spectra of the ligand (10 μM) in the presence of 0, 10, 20, 30, 40, 60 and 80 μl 1.0×10^{-3} M CT-DNA. (b) Electronic spectra of the complex **1** (10 μM) in the presence of 0, 10, 20, 30, 40, 50, 60, 70 and 80 μl 1.0×10^{-3} M CT-DNA. (c) Electronic spectra of the complex **2** (10 μM) in the presence of 0, 10, 20, 30, 40, 50, 60, 70 and 80 μl 1.0×10^{-3} M CT-DNA. Arrow shows the absorbance changes upon increasing CT-DNA concentration. Inset: plots of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs. [DNA] for the titration of complexes with DNA; (■) experimental data points; (solid line) linear fitting of the data.





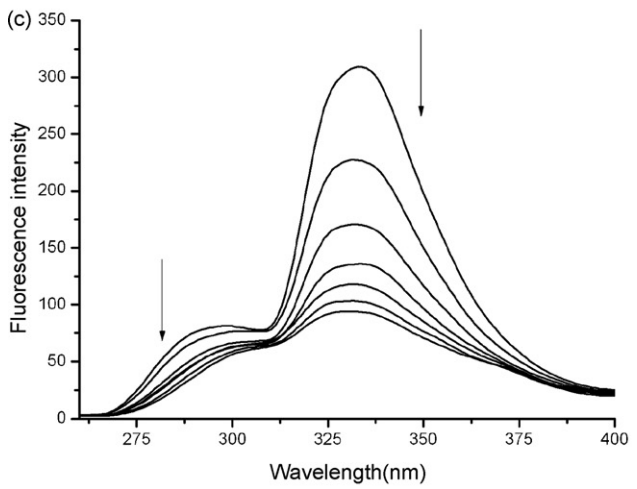


Fig. 4. (a) Electronic spectra of the ligand (10 μM) in the presence of 0, 5, 10, 15, 15, 20, 25, 30, 35, 40, 45 and 50 μl 1.0×10^{-3} M CT-DNA. (b) Electronic spectra of the complex **1** (10 μM) in the presence of 0, 5, 10, 15, 15, 20, 25, 30, 35, 40, 45 and 50 μl 1.0×10^{-3} M CT-DNA. (c) Electronic spectra of the complex **2** (10 μM) in the presence of 0, 5, 10, 15, 15, 20, 25, 30, 35, 40, 45 and 50 μl 1.0×10^{-3} M CT-DNA. Arrow shows the absorbance changes upon increasing CT-DNA concentration.

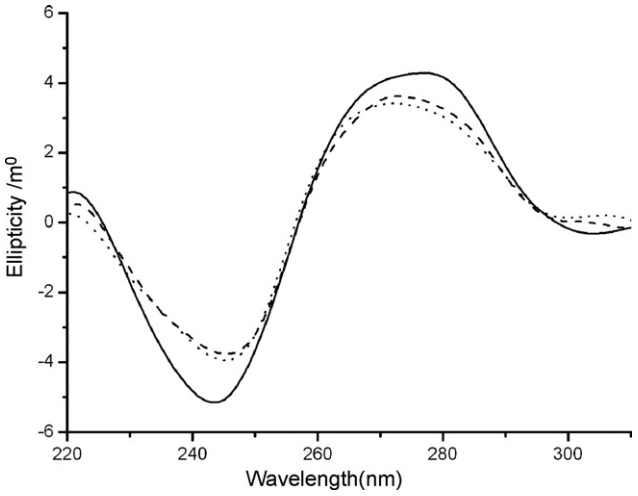


Fig. 5. CD spectrum of CT-DNA adduct with compound. Solid line: free CT-DNA; dash line: complex **1** with CT-DNA, $r_i = 0.5$; dot line: complex **2** with CT-DNA, $r_i = 0.5$ (r_i = molar ratio compound: CT-DNA).

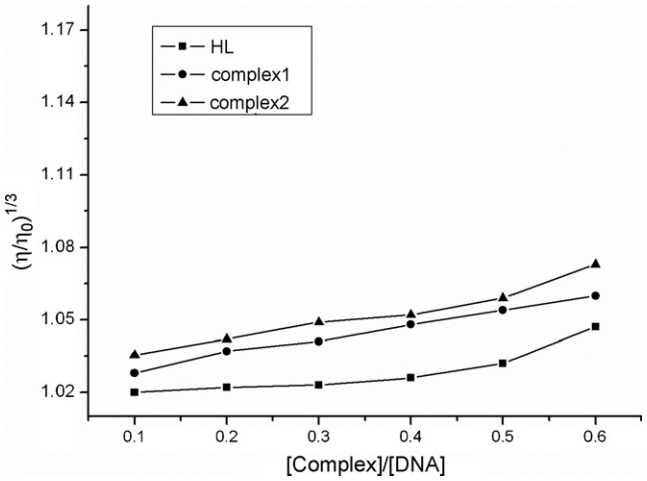


Fig. 6. Effect of increasing amounts of the complexes on the relative viscosity of CT-DNA at 25.0 ± 0.1 °C.

3.4. Electronic absorption titration

Electronic absorption spectroscopy is universally employed to determine the binding characteristics of metal complex with DNA [18–20]. The absorption spectra of the ligand, complex **1** and complex **2** in the absence and presence of CT-DNA are given in Fig. 3a, b and c, respectively. There exist in Fig. 3a two well-resolved bands at 203 and 227 nm for the ligand, and in Fig. 3b and c also have two well-resolved bands at about 208 and 229 nm for the complexes. With increasing DNA concentrations, the hypochromisms are 7.31% at 203 nm for ligand; 30.21% at 208 nm for complex **1**; 55.43% at 208 nm for complex **2**. The λ_{max} for the ligand increased from 203 to 204, for complex **1** increased from 208 to 210 nm and that for complex **2** increased from 208 to 211 nm. Such a small change in λ_{max} is more in keeping with groove binding, leading to small perturbations. Such small increases in the λ_{max} and the hypochromicity have been observed in the case of some porphyrin and copper complexes on their interaction with DNA [21,22].

The binding constants, K_b for the complexes **1** and **2** have been determined from the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] and found to be $4.03 \times 10^4\ M^{-1}$ and 8.72×10^4, respectively, and the K_b for the ligand ($0.23 \times 10^4\ M^{-1}$) is very small. The K_b value obtained here is lower than that reported for classical intercalator (for ethidium bromide and [Ru(phen)DPPZ] whose binding constants have been found to be in the order of 10^6–10^7 M) [23–26]. The results indicate that the binding strength of complex **2** is stronger than that of **1** and the ligand.

3.5. Fluorescence spectra

Fixed amounts (10 μM) of the complexes were titrated with increasing amounts of CT-DNA. The ligand and complexes emit luminescence in Tris buffer with a maximum appearing at 333 nm. The fluorescence titrations spectra of the ligand, complex **1** and complex **2** in the absence and presence of CT-DNA are given in Fig. 4a, b and c. Compared to the ligand and complexes alone, the fluorescence intensity decrease with the increase of CT-DNA concentration. As shown in Fig. 4, the fluorescence intensity of the complex **1** and **2** are quenched steadily with the increasing concentration of the CT-DNA. This phenomenon of the quenching of luminescence of the complex by DNA may be attributed to the photoelectron transfer from the guanine base of DNA to the excited MLCT state of the complex [25,27–33].

3.6. CD spectroscopy

CD spectral variations of CT-DNA were recorded by the respective addition of the ligand and complex **1** and **2** to CT-DNA. Fig. 5 shows the CD spectra of CT-DNA which was added with the ligand, complex **1** and **2**. The observed CD spectrum of calf thymus DNA consists of a positive band at 277 nm due to base stacking and a negative band at 245 nm due to helicity, which is characteristic of DNA in the right-handed B form. While groove binding interaction of small molecules with DNA show little or no perturbations on the base stacking and helicity bands, intercalation enhances the intensities of both the bands, stabilizing the right-handed B conformation of CT-DNA. In all two cases, the intensities of both the negative and positive bands decrease significantly. This suggests that the DNA-binding of the complexes induces certain conformational changes, such as the conversion from a more B-like to a more C-like structure within the DNA molecule [26,34]. These changes are indicative of a non-intercalative mode of binding of these complexes and offer support to their groove binding nature [29,35–36].

3.7. Viscosity studies

Hydrodynamic measurements that are sensitive to the length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data [14,37,38]. As a means for further clarifying the binding of these compounds with DNA, viscosity studies were carried out. Intercalating agents are expected to elongate the double helix

to accommodate the ligands in between the base leading to an increase in the viscosity of DNA. In contrast, complexes that binds exclusively in the DNA grooves by partial and/or non-classical intercalation, under the same conditions, typically cause less pronounced (positive or negative) or no change in DNA solution viscosity [39]. Fig. 6 shows the relative viscosity of DNA (50 μM) in the presence of varying amounts of the ligand, complex **1** and **2**. The results reveal that the complex **1** and **2** show relatively small changes in DNA viscosity, indicating that they bind weakly to DNA which is consistent with DNA groove binding suggested above [40,41]. The increased degree of viscosity which follows the order of **2** > **1** > ligand, may depend on its affinity to DNA. This is consistent with our foregoing hypothesis.

4. Conclusions

In this paper, we have investigated and characterized HL and its two transition metal complexes $ML_2{\cdot}2H_2O$ [M = Ni(**1**), and Cd(**2**)]. In addition, the DNA-binding properties were investigated by electronic absorption, fluorescence, CD spectroscopy and viscosity measurement. The results support the fact that the complexes **1**, **2** and the ligand can bond to CT-DNA by the mode of groove binding, and the complex **2** have stronger binding affinity than complex **1** and the ligand. Information obtained from the present work is helpful to the development of nucleic acids molecular probes and new therapeutic reagents for some diseases.

Acknowledgments

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