



ISSN: 0976-3376

Available Online at <http://www.journalajst.com>

ASIAN JOURNAL OF
SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 01, pp.4125-4132, January, 2017

RESEARCH ARTICLE

DNA BINDING, DNA CLEAVAGE AND ANTIBACTERIAL ACTIVITY OF NI(II) AND CU(II) COMPLEXES DERIVED FROM PYRIDOXAL THIOSEMICARBAZONE

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ARTICLE INFO

Article History:

Received 24th October, 2016
Received in revised form
17th November, 2016
Accepted 20th December, 2016
Published online 31st January, 2017

Key words:

Antibacterial activity,
DNA binding and cleavage activity,
Metal complexes,
Pyridoxal thiosemicarbazone.

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ABSTRACT

Ni(II) and Cu(II) complexes of Pyridoxal thiosemicarbazone (PLTSC) have been synthesized and characterized by ESI-MS, FTIR and UV-Vis spectral studies, elemental analyses, molar conductance, magnetic susceptibility measurements and thermal analysis. The spectral and analytical data suggest the tridentate nature of the ligand and formation of complexes in octahedral geometry. DNA-binding properties of these metal complexes with CT-DNA in a potassium phosphate buffer (pH 7.2) were investigated using UV-Vis absorption spectroscopy, fluorescence spectroscopy and viscosity measurements. Experimental studies suggest good DNA binding ability of the complexes. The cleavage of plasmid pBR322 DNA without any additives was evaluated using agarose gel electrophoresis and the complexes showed significant nuclease activity. The metal complexes are also screened for in vitro antibacterial activity and exhibited moderate activity against the tested organisms.

INTRODUCTION

Transition metal complexes of thiosemicarbazones have attracted much attention because of their good complexing and significant biological properties (Jesic *et al.*, 2011). They possess a broad spectrum of pharmacological activities such as antioxidant activity (Li *et al.*, 2010), antifungal, antibacterial, in vitro anti leukemic activity (Pahontu *et al.*, 2015), cytotoxic and antitumor activity (Priya *et al.*, 2015). One of the most explored compounds in this group with respect to synthesis, spectral properties and crystal structures is certainly Pyridoxal thiosemicarbazone (PLTSC). It is derived from pyridoxal (a form of vitamin B₆) and thiosemicarbazide. It is tridentate ligand with ONS donor atoms and is a good chelating agent (Sebastian *et al.*, 2009). In its metal complexes, PLTSC can exist in neutral (H₂L), singly-deprotonated (HL)⁻ or doubly deprotonated (L²⁻) forms (Leovac *et al.*, 2005). Few reports are made on biological activities of metal complexes of PLTSC like inductive effect on Friend erythroleukemia cells (FLC) (Marisa *et al.*, 1994), inhibition of reverse transcriptase (Jose *et al.*, 1998) and cytotoxicity (Marisa *et al.*, 2004). However, no studies were made on the DNA binding and DNA cleavage activities of the metal complexes of PLTSC. Investigation of metal complexes for their DNA binding properties and

developing them as artificial metallo-nucleases which cleave nucleic acids under physiological conditions has gained a lot of interest since two decades (Padmaja *et al.*, 2015). Transition metal complexes can bind to the various potential binding sites on DNA such as electron rich bases, the anionic phosphate backbone and the major or minor grooves. Metal complexes possessing heterocyclic planar ligands are known to act as intercalating agents with a stacking insertion between the DNA base pairs (Vamsikrishna *et al.*, 2016). Keeping in view the biological significance of pyridoxal based ligands, it was considered appropriate to study the DNA binding and cleavage properties and antibacterial activity of their metal complexes. Hence the present work is focused on synthesis and characterization of Cu (II) and Ni (II) complexes of PLTSC and to analyze their biological activity.

Experimental

Materials and reagents: All the chemicals were of analytical grade, procured from Sigma Aldrich and used without further purification. PLTSC (H₂L) was synthesized according to a reported procedure (Tido *et al.*, 2010).

Methods

Instrumentation

Elemental analyses (% CHN) were obtained using Thermo Finnigan 1112 elemental analyzer. ESI mass spectra of the

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complexes were recorded on LCMS 2010A, Shimadzu spectrometer. FT-IR spectra of the complexes were recorded using KBr pellets in the range of 4000–250 cm^{-1} on a Shimadzu IR Prestige-21 FTIR spectrophotometer. UV-Vis spectra in DMSO solution were recorded on Systronics UV-Vis Double beam spectrophotometer 2201 in the range of 200–1000 nm. The molar conductivity was measured with a Digisun digital conductivity bridge using a freshly prepared solution of the complexes in DMSO. Thermo gravimetric (TG) analyses were performed using Shimadzu TGA-50H in nitrogen atmosphere in the temperature range of 0°C to 1000°C with a heating rate of 20°C per min. Magnetic susceptibilities were measured at room temperature on Faraday balance model 7550 using $\text{Hg}[\text{Co}(\text{NCS})_4]$ as the internal standard. Diamagnetic corrections were made by using Pascal's constants (Figgis *et al.*, 1960). DNA cleavage experiments were performed with the help of Biotech electrophoresis system supported by Genei power supply over a potential range of 50-500 V, visualized and photographed by Biotech Transilluminator system.

DNA binding activity by Electronic absorption spectra

The binding of complexes with CT DNA was measured in potassium phosphate buffer solution (pH 7.2). A solution of DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of 1.85–1.9, indicating that the DNA was sufficiently free of protein. The concentration of DNA was determined from the UV absorbance at 260 nm using the extinction coefficient $\epsilon_{260} = 6600\text{M}^{-1}\text{cm}^{-1}$. The absorbance titrations were performed at a fixed concentration of complexes and varying the concentration of double stranded CT-DNA (2-20 μM). While measuring the absorption spectra, a proper amount of CT-DNA was added to both compound solution and the reference solution to eliminate the absorbance of CT DNA itself. Concentrated stock solutions of the complexes were prepared by dissolving the complexes in DMSO and diluting suitably with the corresponding buffer to the required concentration (20 μM) for all the experiments. After the addition of DNA to the metal complex, the resulting solution was allowed to equilibrate for 10 min, after which absorption readings were noted. The data were then fit to the following equation to obtain intrinsic binding constant K_b

$$[\text{DNA}]/[\epsilon_a - \epsilon_f] = [\text{DNA}]/[\epsilon_b - \epsilon_f] + 1/K_b[\epsilon_b - \epsilon_f] \dots\dots\dots (1)$$

Where [DNA] is the concentration of DNA in base pairs, ϵ_a is the extinction coefficient observed for the MLCT absorption band at the given DNA concentration, ϵ_f is the extinction coefficient of the complex free in solution, and ϵ_b is the extinction coefficient of the complex when fully bound to DNA. A plot of $[\text{DNA}]/[\epsilon_a - \epsilon_f]$ versus [DNA] gave a slope $1/[\epsilon_a - \epsilon_f]$ and Y intercept equal to $(1/K_b)[\epsilon_b - \epsilon_f]$, respectively. The intrinsic binding constant K_b is the ratio of the slope to the intercept (Patel *et al.*, 2011).

Competitive DNA binding fluorescence experiments

Relative binding of the complexes to CT DNA was studied using fluorescence spectroscopy, by the displacement of ethidium bromide (EB) bound to CT DNA in a potassium phosphate buffer solution (pH 7.2) (Srivastava *et al.*, 2013). In a typical experiment, 480 μl of CT DNA (20 μM) solution was

added to 2020 μl of EB in buffer solution $\{[\text{DNA}]/[\text{EB}] = 1\}$. The fluorescence intensity was measured upon excitation at $\lambda_{\text{max}} 520\text{nm}$; maximum emission was observed at $\lambda_{\text{max}} 605\text{nm}$. The changes in fluorescence intensities at 605nm of EB bound to DNA were recorded with an increasing amount of the complex concentration (from its 50 μM stock solution). Stern – Volmer quenching constants were calculated using the equation $I_0/I = 1 + K_{sv}.r$, where I_0 and I are the fluorescence intensities in the absence and presence of the complex respectively, K_{sv} is a linear Stern-Volmer quenching constant and r is the ratio of total concentration of complex to that of DNA. The value of K_{sv} is given by the ratio of slope to intercept in a plot of I_0/I Vs $[\text{complex}]/[\text{DNA}]$.

Viscosity measurements

Viscosity measurements were carried out with the Ostwald viscometer, maintained at 25°C in a thermostatic water bath. Each complex (50 μM) was introduced into CT-DNA solution (300 μM) in phosphate buffer (pH 7.2) present in the viscometer. Flow time of solutions was recorded in triplicate for each sample and an average flow time was calculated. Data were presented as $(\eta'_{sp}/\eta_{sp})^{1/3}$ versus the ratio of the concentration of the complex to CT-DNA, where η'_{sp} is the viscosity of CT-DNA in the presence of the complex and η_{sp} is the viscosity of CT-DNA alone (Bhat *et al.*, 2010).

DNA cleavage studies

Agarose gel electrophoresis technique was used to monitor the DNA cleavage ability of the metal complexes on super coiled pBR 322 DNA. Generally, plasmid DNA is converted from super coiled DNA (Form I) to nicked circular (Form II) and linear forms (Form III) (Anbu *et al.*, 2012). In the experiment, plasmid DNA (300ng/3 μl) was treated with the complexes in DMSO (20-60 μM) in 5mM Tris.HCl/50mM NaCl buffer (pH 7.2). The mixture was incubated for 1hr at 37°C. A loading buffer containing 1% bromophenol blue and 40% Sucrose (1 μl) was added and loaded onto a 0.8% agarose gel containing EB (1 $\mu\text{g}/\text{ml}$). The gel was run in TAE buffer (40mM Tris base, 20mM Acetic acid, 1mm EDTA, pH 8.3) at a constant voltage 60 V for 2 h until the bromophenol blue had traveled through 75% of the gel. The bands were visualized by viewing the gel on a transilluminator and photographed.

Antibacterial activity

The complexes are screened for their antibacterial activity using agar well diffusion method against two gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and two gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* at a concentration of 1000 $\mu\text{g}/\text{ml}$ (Balouiri *et al.*, 2016).

Synthesis of metal complexes:

Synthesis of Ni(II)-PLTSC

Ni(II)-PLTSC was prepared by the addition of 0.2138g (1mmol) $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (in 10ml distilled water) to a hot aqueous solution 0.5g (2mmol) of the Schiff base ligand (in 40ml distilled water) and refluxed for two hours. A red colored precipitate was formed on adjusting the pH to 7 using a

mixture of methanol and ammonia solution. The mixture was continued to reflux for another hour. Then the precipitate was filtered, washed several times with hot distilled water, finally with petroleum ether and air dried.

[Ni(HL)₂]: (Yield: 0.36g, 73%). IR: $\nu_{\max}/\text{cm}^{-1}$: 3437s, 3331m, 1577m, 1558m, 1500m, 1259m, 1145m, 520-550m(Ni-O), 450-470m(Ni-N). ESI-MS in MeOH: m/Z 539[M⁺+H]. Elemental analysis: Found (calc.): C, 40.03 (40.07); H, 4.10 (4.08); N, 20.73 (20.77) % $\mu_{\text{eff}} = 2.8835\text{B.M.}$ $\Lambda_{\text{M}}[\Omega^{-1}\text{cm}^2\text{mol}^{-1}, 10^{-3}, [\text{DMSO}]]: 005$

Synthesis of Cu(II)-PLTSC

Cu(II)-PLTSC was prepared by the by the addition of 0.154g (1mmol) CuCl₂ .2H₂O (in 10ml distilled water) to a hot aqueous solution 0.5g (2mmol) of the Schiff base ligand (in 40ml distilled water) and refluxed for two hours. A green colored precipitate was formed on adjusting the pH to 7 using a mixture of methanol and ammonia solution. The mixture was continued to reflux for another hour. Then the precipitate was filtered, washed several times with hot distilled water, finally with petroleum ether and air dried.

[Cu(HL)₂].2H₂O: (Yield: 0.34g, 71%). IR: $\nu_{\max}/\text{cm}^{-1}$: 3466s, 3377m, 2899m, 1585m, 1545m, 1500m, 1263m, 1132m, 515-570m(Cu-O), 430-440m(Cu-N). ESI-MS in MeOH: m/Z 578[M⁺-H]. Elemental analysis: Found (calc.): C, 37.33 (37.37); H, 4.53 (4.50); N, 19.35 (19.37) %. $\mu_{\text{eff}} = 1.6961\text{B.M.}$ $\Lambda_{\text{M}}[\Omega^{-1}\text{cm}^2\text{mol}^{-1}, 10^{-3}, [\text{DMSO}]]: 010$

RESULTS AND DISCUSSION

Characterization of metal complexes

Both the metal complexes synthesized were colored, amorphous, and stable to air, soluble only in DMSO and DMF. The complexes show a very low conductivity indicating non-electrolytic nature of the complexes.

Mass spectra

The electrospray ionization mass spectrometry (ESI-MS) studies confirmed the proposed molecular formulae of the metal complexes. [Ni(HL)₂] shows the molecular ion peak at m/z 539 (M⁺) and [Cu(HL)₂].2H₂O at 578 (M⁺-1). The m/z values of these complexes indicate the stoichiometric ratio of 1:2(ML₂) for both the complexes.

IR spectral analysis

Comparison of the IR spectra of the complexes with that of ligands indicates the formation of complexes by coordination through ONS atoms (phenolic oxygen, azomethine nitrogen and thioamide sulphur) in PLTSC. The IR data suggests the involvement of mono-anionic form of ligand in both the complexes. Bands in the region 3000-3500cm⁻¹ in the ligand and complexes are attributed to ν (N-H) and ν (O-H) vibrations of the CH₂OH group. A medium band in the range 2800-2900cm⁻¹ is assigned for ν (NH⁺) of the pyridine ring which is formed as a consequence of shifting of proton from the phenolic group to the pyridine nitrogen (Marisa *et al.*, 2004), is

missing in the complexes due to loss of proton. The frequencies of azomethine group in both the complexes have been shifted compared to ligand in the range of 1550-1600 cm⁻¹ suggesting coordination of nitrogen atom of this group to the metal ion. A medium intense band at 841cm⁻¹ attributed to C=S in PLTSC has been shifted to lower frequency in the complexes indicating coordination of this group to the metal ion. Shifting in the frequency of the Py-C-O vibration in the range of 1100-1200cm⁻¹ indicates the involvement of phenolic oxygen in coordination.

Thermal analysis

TGA and DTA analyses of the complexes indicate the presence of lattice water in copper complex of PLTSC, while no water molecules in [Ni(HL)₂]. Thermogram of [Cu(HL)₂].2H₂O shows a weight loss (6.5%) observed from 100-160°C along with two endothermic peaks in DTA curve which corresponds to loss of two lattice water molecules. Both the complexes show a similar behavior after 300°C in TGA with a loss in ligand moiety accompanied by an exothermic peak in DTA suggesting a rearrangement in the structure of these complexes at this temperature (Ljiljana *et al.*, 2016).

UV-Visible spectra

The electronic absorption spectra of PLTSC and its metal complexes were recorded in DMSO solution at room temperature. The UV-Vis spectrum of PLTSC shows a band at 30,211cm⁻¹ which is assigned to the n- π^* transition of the C=N chromophore. On complexation, this band is shifted to lower wavelength, indicating coordination of azomethine nitrogen to the metal ion. The number and position of d-d transitions in the electronic spectrum of a metal complex gives valuable information about the geometry of the complex. Three d-d transitions are observed in the electronic spectrum of [Ni(HL)₂] at 10,787cm⁻¹, 11,198cm⁻¹ and 25,000cm⁻¹ due to ³A_{2g} → ³T_{2g}, ³A_{2g} → ³T_{1g} (F) and ³A_{2g} → ³T_{1g} (P) respectively indicating an octahedral geometry. Similarly, three d-d transitions are observed in the electronic spectrum [Cu(HL)₂].2H₂O at 10,787cm⁻¹, 11,198 cm⁻¹ and 25,000cm⁻¹ due to ³A_{2g} → ³T_{2g}, ³A_{2g} → ³T_{1g}(F) and ³A_{2g} → ³T_{1g}(P) respectively indicating a distorted octahedral geometry (Lever, 1984).

Magnetic moments

The magnetic moment value μ_{eff} of [Ni(HL)₂] is found to be 3.0922BM indicating the presence of two unpaired electrons in the central metal ion. Whereas the magnetic moment value of [Cu(HL)₂].2H₂O is found to be 2.14BM indicating the presence of single unpaired electron. These values also suggest the paramagnetic nature of the complexes. Based on the spectral and analytical data the structures of the Ni(II) and Cu(II) complexes of PLTSC are proposed as given below.

DNA binding experiments

Absorption spectral titration

Various spectroscopic techniques are used to evaluate the binding modes of metal complexes to DNA. Among which absorption spectrum of a complex is the most widely employed method to monitor the changes in absorption of a

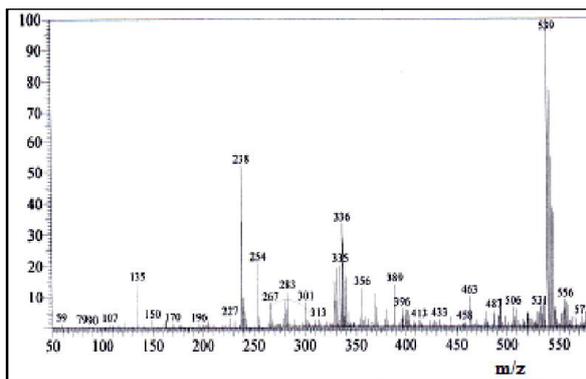


Figure 1. Mass spectrum of $[\text{Ni}(\text{HL})_2]$

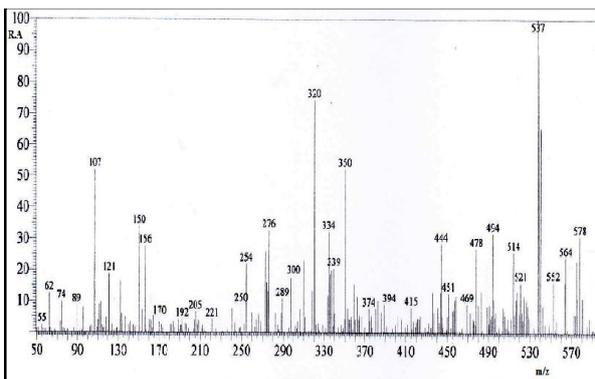


Figure 2. Mass Spectrum of $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$

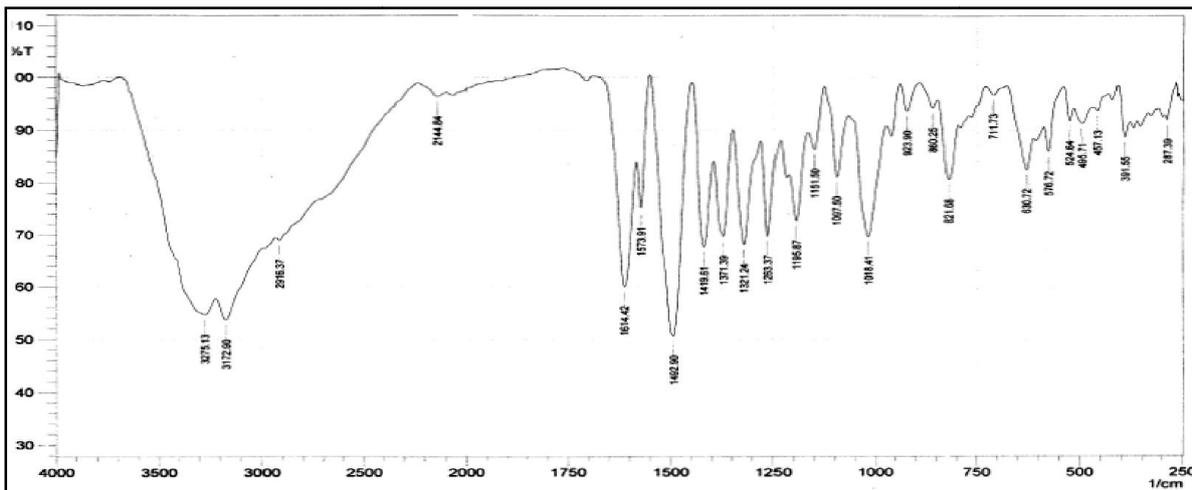


Figure 3. IR spectrum of $[\text{Ni}(\text{HL})_2]$

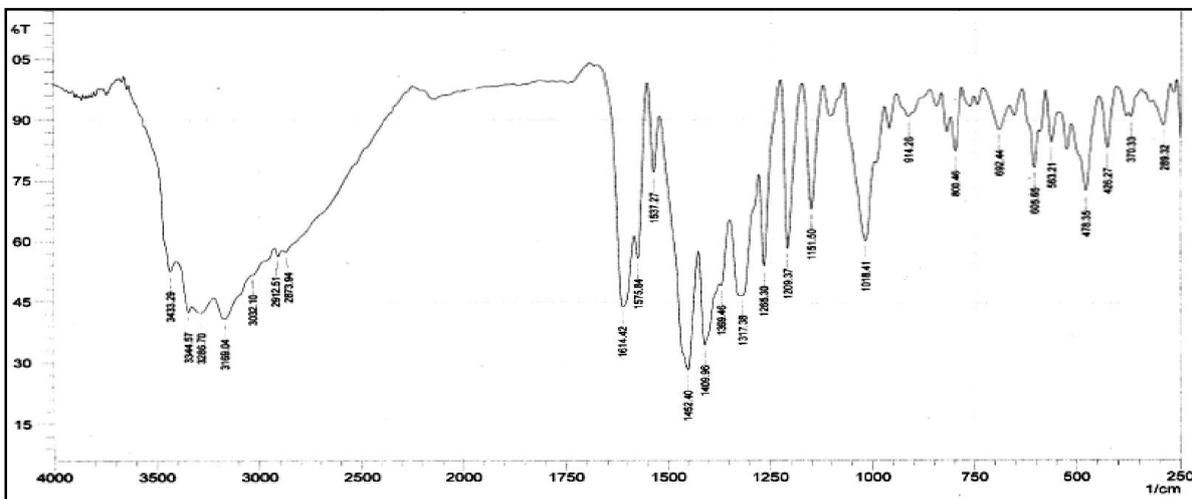


Figure 4. IR spectrum of $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$

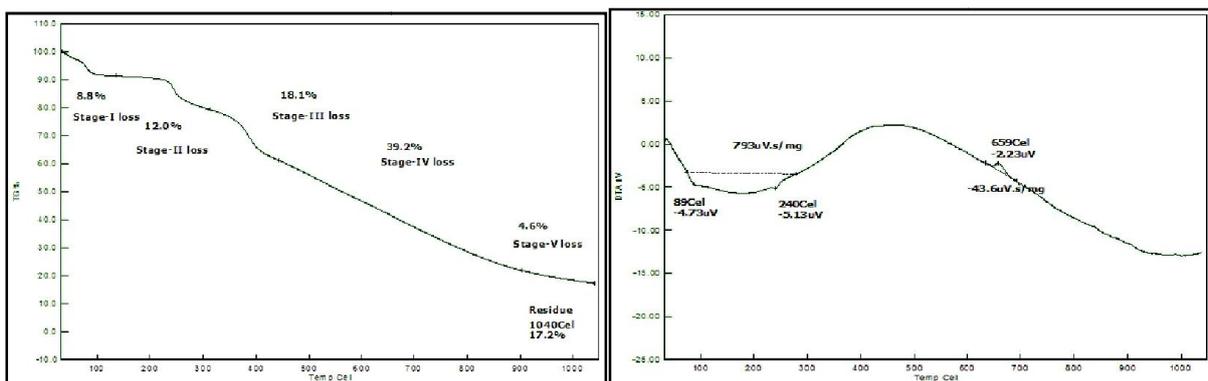
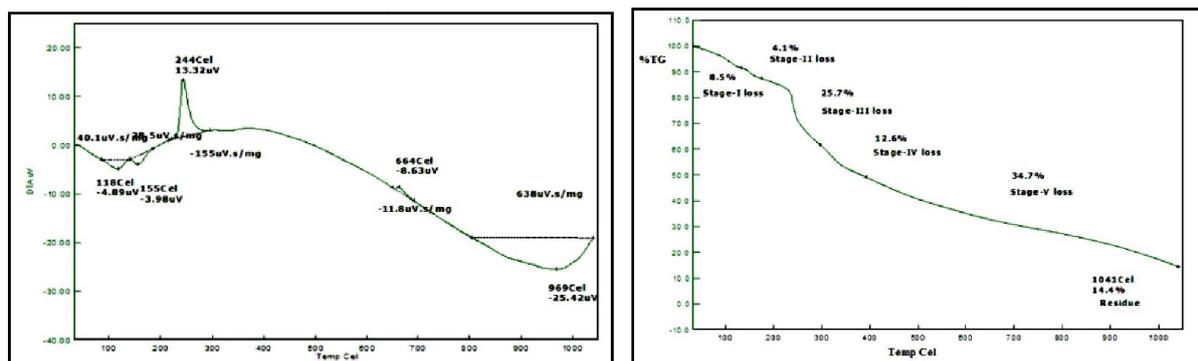
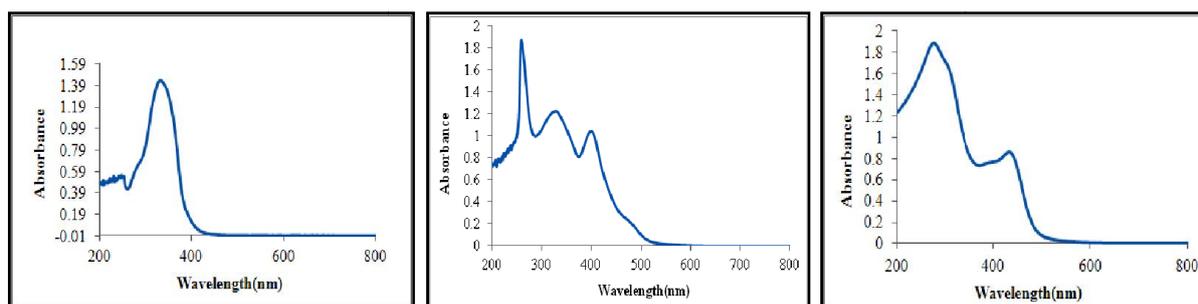
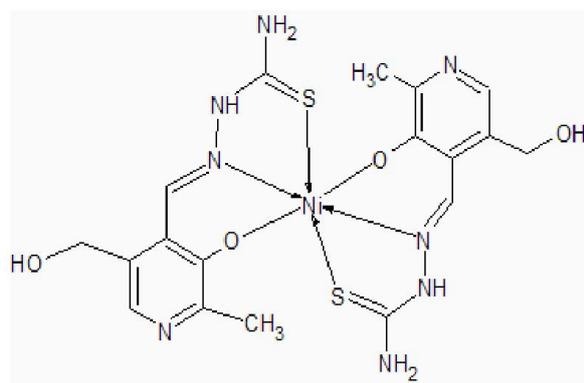
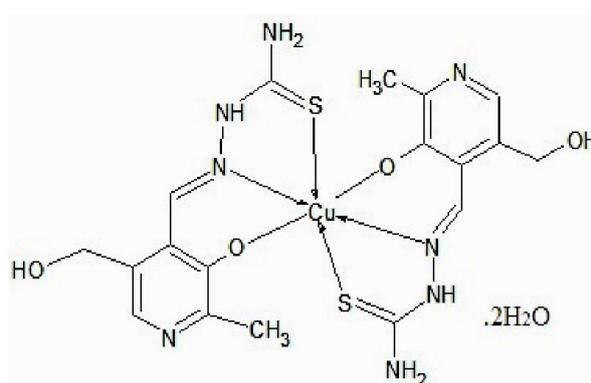


Figure 5. TGA and DTA curves of $[\text{Ni}(\text{HL})_2]$

Figure 6. TGA and DTA curves of $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$ Figure 7. Electronic spectra of PLTSC, $[\text{Ni}(\text{HL})_2]$ and $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$ Figure 8. Structure of $[\text{Ni}(\text{HL})_2]$ Figure 9: Structure of $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$

complex with the increasing concentration of DNA and also to determine the binding constant. Transition metal complexes bind to DNA either through covalent or non-covalent interactions. Covalent binding involves the replacement of the labile ligand by a nitrogen base of DNA, while the non-covalent includes three other modes like electrostatic, groove binding and intercalation (Raman *et al.*, 2010). In case of intercalation of a complex to DNA, hypochromism along with red or blue shift is observed which involves binding between the aromatic chromophore of the complex and the base pairs of DNA. The extent of hypochromism suggests the strength of intercalation (Selim *et al.*, 2012). A strong π - π interaction between the DNA base pairs and the hetero aromatic ring of the ligand results in hypochromism. Electronic absorption titrations were carried out to assess the binding ability of metal complexes to DNA and also to determine the overall binding constants. $[\text{Ni}(\text{HL})_2]$ showed hypochromism with red shift while complex $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$ showed hypochromism with blue shift indicating their binding through intercalation owing to the planar structure and extended π -electron system of the ligands.

Quantitative comparison of the complexes was made by the calculation of their intrinsic binding constants (K_b) with CT-DNA. The changes in the absorption were monitored by increasing the concentration of CT-DNA and using equation 1. K_b values were found to be 1.67×10^4 and 3.75×10^4 of $[\text{Ni}(\text{HL})_2]$ and $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$ respectively. It can be inferred from the K_b values that the Cu(II) complex of PLTSC is able to bind more effectively with DNA than $[\text{Ni}(\text{HL})_2]$.

Competitive DNA binding studies of the complexes and Ethidium bromide: Ethidium bromide (EB) is a well known intercalating agent. When a metal complex is added to an EB-DNA adduct, change in the emission intensity occurs indicating the displacement of EB from the EB-DNA adduct. Therefore monitoring the emission intensity of EB bound to CT-DNA with the increase in concentration of the complex gives important information of the DNA binding ability of the complex (Nagababu *et al.*, 2011). EB was non-emissive in phosphate buffer (pH 7.2) due to fluorescence quenching of free EB by the solvent molecules.

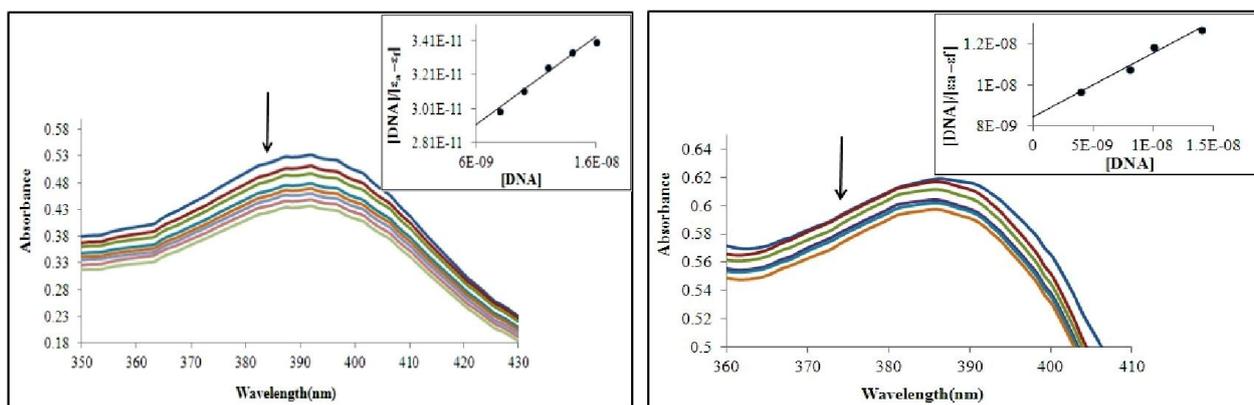


Fig. 10. Absorption spectra of Ni(II) and Cu(II) complexes of PLTSC(20 μ M) in phosphate (pH 7.2) buffer upon addition of CT-DNA (0-20 μ M). Arrow indicates hypochromism with increase in concentration of DNA. Inset: Plot of $[DNA]/(\epsilon_b - \epsilon_f)$ versus $[DNA]$ for absorption titration of CT DNA with the complex.

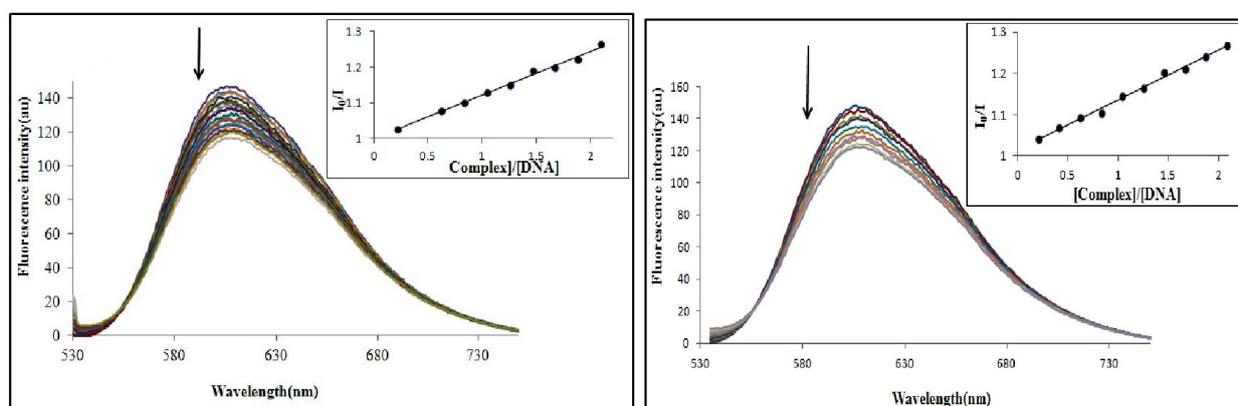


Fig. 11. Emission spectra of EB bound CT DNA in the absence and presence of complexes. Arrow shows the decrease in the emission intensity with the increase in complex concentration. Inset: Plot of I_0/I versus $[Complex]/[DNA]$

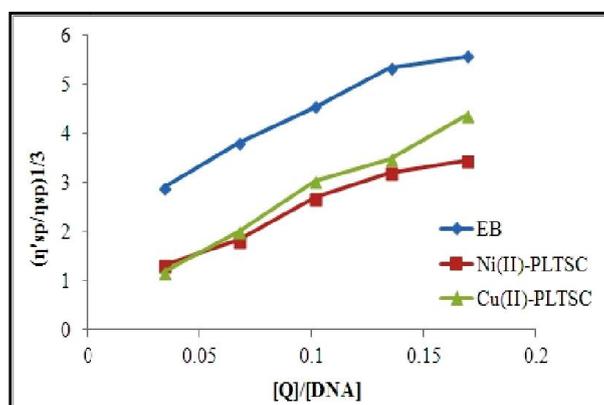


Fig. 12. Effect of increasing amounts of (◆) EB, (■) $[Ni(HL)_2]$ and (▲) $[Cu(HL)_2] \cdot 2H_2O$ on the relative viscosities of CT DNA at 25 $^{\circ}C$

In the presence of DNA, EB showed enhanced emission intensity due to its intercalative binding to DNA. When the complexes were added to DNA pretreated with EB, the DNA induced emission intensity of EB was decreased. As the complexes have planar ligands, they replace EB from intercalative binding sites due to which quenching of emission intensity of DNA-bound EB is observed (Bhat *et al.*, 2010). The quenching plots of I_0/I versus $[Complex]/[DNA]$ (insets of fig. 11) are in good agreement with the linear Stern-Volmer equation. Stern-Volmer quenching constants (K_{sv}) were calculated as 0.1274 and 0.1202 respectively for the complexes $[Ni(HL)_2]$ and $[Cu(HL)_2] \cdot 2H_2O$.

Viscometric measurements

When a metal complex binds to DNA through intercalation, it is known to cause a significant increase in the viscosity of a DNA solution. This is due to the increase in overall DNA contour length by the insertion of ligand in between the base pairs leading to increase in separation of base pairs at intercalation sites. Whereas no change or less change occurs in the viscosity of DNA solution when a complex binds in the DNA grooves (Prמודini *et al.*, 2012). The effect of EB and the metal complexes of PLTSC on the viscosity of DNA was studied to understand the binding mode of complexes with the

DNA. Fig. 12 shows the changes in the viscosity of DNA in the presence of EB and complexes. EB being a potential intercalator shows a significant increase in the viscosity, while the complexes showed a similar effect to a less extent indicating intercalative mode of binding of the metal complexes of PLTSC to CT DNA.

DNA cleavage without added reagents

Efficient hydrolytic agents of phosphodiester bonds in nucleic acids can be used as artificial restriction enzymes in molecular biology. They are valuable as they can be designed to cleave DNA with different sequence selectivity than that of natural enzymes (Sathiyaraj *et al.*, 2014). Hydrolysis of phosphodiester bond involves the nucleophilic attack of water or hydroxide oxygen. But this reaction is disfavored due to the negative charge on the phosphodiester bond. Therefore presence of a Lewis acid like a metal ion is required for the facilitation of the same reaction. Metal ions activate the nucleophilic attack of phosphate group by water or hydroxide and enhance the hydrolytic cleavage of DNA (Megha *et al.*, 2007). To evaluate the DNA cleavage ability of the complexes, pBR 322 plasmid DNA was incubated with three different concentrations of the complexes in 5mM Tris HCl and 50mM NaCl buffer (pH 7.2) for 1hour at 37°C without any additives. A concentration dependent DNA cleavage was observed upon the gel electrophoresis of the reaction mixture. Fig 13 shows no cleavage for the control without the added metal complex. While hydrolytic cleavage of plasmid DNA is observed in the presence of metal complexes. With the increase in the concentration of the complexes (lanes 2-4 for $[\text{Ni}(\text{HL})_2]$ and lanes 5-7 for $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$ the amount of Form-I of plasmid DNA diminished gradually and the amount of Form II of DNA has increased gradually. And Ni(II) complex exhibited a better chemical nuclease efficiency over Cu(II) complex.



Fig. 13. Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complexes. Lane 1, DNA control, Lanes 2-4 DNA + $[\text{Ni}(\text{HL})_2]$ (20,40,60 μM resp.) and Lanes 5-7 DNA + $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$ (20,40,60 μM resp.)

Antibacterial activity

In most cases, metal complexes are shown to be more effective anti bacterial agents than free ligands. Chelation involves partial sharing of the positive charge of the metal ion with the donor groups of the ligand and thereby reduces the polarity of the metal atom. Consequently, there is an increase in the lipophilic character of the metal chelates, favouring their permeation through the lipid bilayers of the bacterial cell membrane (Ikotun *et al.*, 2011). Antibacterial activity of the ligand PLTSC and its Ni(II) and Cu(II) complexes is assessed by well diffusion method on nutrient agar medium. The complexes exhibited better activity than the ligand against

gram positive bacteria which was indicated by the zone of inhibition, while they were inactive towards gram negative bacteria. The ligand showed a zone of inhibition of 11mm and 8mm, $[\text{Ni}(\text{HL})_2]$ showed 18mm and 15mm, while $[\text{Cu}(\text{HL})_2] \cdot 2\text{H}_2\text{O}$ showed 22mm and 17mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively indicating a moderate antibacterial activity of these metal complexes against the tested organisms.

Conclusion

Pyridoxal thiosemicarbazone forms complexes with Ni(II) and Cu(II) ions by coordination through ONs atoms. Both the metal ions formed complexes with the ligand in 1:2 (ML_2) ratio and they exhibited octahedral geometry. The complexes were evaluated for their DNA binding activity by absorption titrations, fluorescence spectra and viscometric measurements. The experimental observations suggested that both the complexes are able to bind DNA through intercalation. The complexes were able to cleave pBR 322 plasmid DNA without any additives suggesting a hydrolytic pathway. The complexes also showed a moderate antibacterial activity against gram positive bacteria.

Acknowledgements

The authors are thankful to University Grants Commission, New Delhi for financial support and also Osmania University, Hyderabad and St. Francis College for Women, Hyderabad for providing necessary facilities to carry out this work.

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