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DNA Binding Properties and Antibacterial Activity of Heterolyptic Transition Metal Complexes with 2,2-Bipyridyl and 2-Acetylthiophene Thiosemicarbazone

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Metal complexes having the composition $M(Bipy)Cl_2$ (where, M = Cu(II), Ni(II) and Co(II); Bipy = 2,2-bipyridyl) are reacted with 2-acetylthiophene thiosemicarbazone (ATT) to produce heteroleptic transition metal complexes with molecular formula [M(Bipy)ATT]. The complexes are characterized by mass spectra, molar conductivity, infrared and electronic spectra. Electrochemical behaviour of these metal complexes was investigated by cyclic voltammetric studies. The metal complexes show quasi reversible cyclic voltammetric responses for the Cu(II)/Cu(I) couple. The binding properties of these complexes with calf-thymus DNA have been investigated by using absorption spectrophotometry. Metal complexes are screened for their antibacterial activity by using agar well diffusion method against pathogenic bacterial strains *viz. Escherichia coli and Staphylococcus aureus.* Antibacterial activity of the present complexes are comparable with the activity of ciprofloxacin. The $Cu(Bipy)Cl_2$ complex inhibits bacteria more strongly than any other complex. The Ni(Bipy)ATT complex shows more activity than the parent complex, $Ni(Bipy)Cl_2$.

Keywords: Heterolyptic transition metal complexes, 2,2-Bipyridyl, ATT, DNA binding, Antibacterial activity.

INTRODUCTION

Thiosemicarbazones are prepared by the condensation of thiosemicarbazides with aldehydes or ketones in the presence of a few drops of glacial acetic acid. Thiosemicarbazones with general formula $R_1R_2C{=}N{-}NH{-}C(S){-}NR_3R_4$ usually react as chelate with transition metal ions by bonding through the sulfur and hydrazinic nitrogen atoms in ligands. Thiosemicarbazones are versatile ligands in both neutral (HL) and anionic forms (L–). They are extensively delocalized systems, especially when aromatic radicals are bound to the azomethine carbon atom. The R_1 and R_2 groups may provide additional donor atoms and R_3 and R_4 are the N^4 substituents. Thiosemicarbazones have emerged as an important class of sulfur containing ligands particularly for transition metal ions. The real impetus towards developing their coordination chemistry was due to their physicochemical properties [1,2] and significant biological activities [3,4].

Thiosemicarbazones are thiourea derivatives and the studies on their chemical and structural properties have received much attention due to the widespread application in the chemotherapeutic field. Thiosemicarbazones and their metal complexes are a broad class of biologically active compounds [3,4]. The group N–C=S is of considerable chemotherapeutic interest and is responsible for pharmacological activity. Thiosemicarbazones and their metal complexes present a wide range of applications that stretch from their use in analytical chemistry [5-8], through pharmacology [9-11] to nuclear medicine [12]. Transition metal complexes of thiosemicarbazone showed antibacterial [13,14], antimalarial [15], antitrypanosomal [16], antiviral [17], antitumor [18] and anticancer [19,20] activities.

Transition metal complexes with assorted ligands containing hetero donor atoms (N, S) are known to exhibit interesting stereochemical, electrochemical and electronic properties [21,22]. Mixed ligand metal complexes with 2,2-bipyridine and acetylacetone [23]; 2,2-bipyridine and maltolate [24] have been investigated. But no report is available on mixed ligand metal complexes with 2,2-bipyridine and thiosemicarbazone. Pharmacological activity of metal complexes is traced to their ability to bind/interact with DNA, ladder of life. The heterocyclic

ligand, 2,2-bipyridyl is known as potential intercalating compound due to its planar structure.

Bipyridine chelators act as potential antitumor agents [25]. The choice of bipyridine is mainly due to two factors. (i) The ligand is rigid, planar and provides two aromatic nitrogens whose unshared electron pairs can act co-operatively in binding cations (ii) The π-electron deficiency makes 2,2-bipyridyl an excellent π-acceptor ligand The metal complexes of bipyridine are of considerable due to their biological or pharmacological properties.

Structural and spectral studies on 2-acetylthiophene thiosemicarbazones (ATT) have been reported [26,27]. Platinum and palladium complexes of ATT) are also reported in the literature [28]. 2-Acetylthiophene thiosemicarbazones has been used for the spectrophotometric determination of copper(II) in alloys, edible oils and seeds [29]. However, transition metal complexes with polypyridyl ligands and thosemicarbazone have not been much investigated [30].

It is of interest to study mixed ligand transition metal complexes. This is because they are the most general and probable form of coordination compounds in the biological system. Therefore studies of mixed ligand complexes of biologically important compounds may serve as models for biochemical processes [31-33]. They are also characterized by their extreme stability and the properties of the central metal ion is more pronounced in these complexes. In the light of the above and in continuation of our ongoing research on transition metal complexes, herein we report synthesis, characterization and DNA binding properties of heterolyptic Cu(II), Ni(II) and Co(II) complexes with 2,2-bipyridyl and 2-acetylthiophene thiosemicarbazone (ATT).

EXPERIMENTAL

Thiosemicarbazide, 2-acetylthiophene, 2,2-bipyridyl, were purchased from Sigma-Aldrich. All other chemicals were of AR grade and used as provided. The solvents used for the synthesis were distilled before use. Calf-thymus DNA (CT-DNA) was purchased from Genio Bio labs, Bangalore, India. Elemental analyses were carried out on a Heraeus Vario EL III Carlo Erba 1108 instrument. Magnetic measurements were taken at 298K using lakeshore VSM 7410 instrument. Molar conductivity measurements at 298 ± 2 K in dry and purified DMF were carried out using a CM model 162 conductivity cell (ELICO). The electronic spectra were recorded in DMSO with a UV lamda50 (Perkin-Elmer) spectrophotometer. IR spectra were recorded in the range 4000–400 cm^{-1} with a Perkin-Elmer spectrum100 spectrometer on KBr discs. ESR spectra were recorded on a Varian E-112 X-band spectrophotometer at room temperature and liquid nitrogen temperature (LNT) in solution (DMF) state. Cyclic voltammetric measurements were taken on a CH instruments assembly equipped with an X–Y recorder. Measurements were taken on degassed (N_2 bubbling for 5 min) solutions (10^{-3} M) containing 0.1 M Bu_4NPF_6 as the supporting electrolyte. The three-electrode system consisted of glassy carbon (working), platinum wire (auxiliary) and Ag/AgCl (reference) electrodes.

Preparation of ATT: The ligand, 2-acetylthiophene thiosemicarbazone (ATT) was prepared using thiosemicarbazide and carbonyl compound *viz.* 2-acetylthiophene. A methanolic solution of thiosemicarbazide (5 mmol), 2-acetylthiophene (5 mmol) in methanol was mixed in a round bottom flask. Two drops of HCl/CH_3COOH were added to the reaction mixture. This reaction mixture was refluxed for 3 h and the reaction mixture was cooled to room temperature. The ligand ATT was obtained as yellow coloured crystalline product, which are subsequently used for the synthesis of metal complexes. Yield 65 %, m.p. 155-157 °C, IR spectra (cm^{-1}) 3404, 3207, 3126, 1605. 1232 cm^{-1} are assigned to ν(N-H asym), ν(N-H sym), ν(C-H thiophene), ν(C=N) and ν(C=S) stretching vibrations respectively. NMR spectra (δ) 8.773 (singlet 1H), 7.030-7.371 (multiplet 3H), 6.521 (singlet 2H broad), 2.311(singlet 3H) are assigned to >NH, thiophene H, $-NH_2$and CH_3 protons respectively. Mass spectrum (Fig. 1) of ATT shows molecular ion peak at 199. The structure of ligand is shown in Fig. 2.

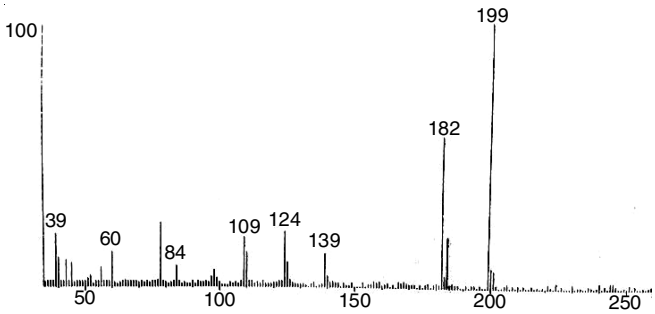


Fig. 1. Mass spectrum of ATT ligand

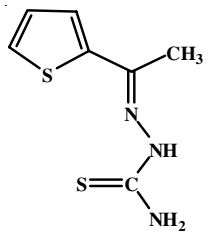


Fig. 2. Structure of ATT ligand

Preparation of complexes: The complexes, $Cu(Bipy)Cl_2$, $Ni(Bipy)Cl_2$, $Co(Bipy)Cl_2$ were prepared by mixing one equivalent of 2,2′-bipyridine in 30 mL of ethanol to one equivalent of respective metal chlorides in 15 mL of ethanol.

Preparation of Cu(Bipy)ATT, Ni(Bipy)ATT, Co(Bipy)ATT complexes: A 1.2 g of ATT ligand (0.006 mol) was dissolved in 15 mL of 0.05 N NaOH in ethanol solvent in 100 mL beaker. A 1.0 g $Cu(Bipy)Cl_2$ (0.003 mol) was dissolved in 15 mL of ethanol solvent in 100 mL beaker. Ligand solution and $Cu(Bipy)Cl_2$ solution were transferred into 100 mL round bottom flask and heated under reflux for 1 h. On cooling the contents of flask, dark green coloured complex was formed. It was collected by filtration, washed with small quantities of ethanol and dried in air. Ni(Bipy)ATT and Co(Bipy)ATT complexes are prepared similarly.

DNA binding experiments: The electronic spectra of metal complexes were monitored in the absence and presence of CT-DNA. The interaction of the complexes with DNA was carried out in *tris*-buffer. Solution of calf thymus DNA (CT-DNA) in (50 mM NaCl/5 mM Tris-HCl; pH =7.0) buffer gave absorbances ratio at 260 nm and 280 nm of 1.85, indicating

that the DNA was sufficiently free of proteins [34,35]. The DNA concentration per nucleotide was determined by absorption coefficient (6600 dm^3 mol^{-1} cm^{-1}) at 260 nm. Stock solutions stored at 4 °C were used after no more than 4 days. The electronic spectra of metal complexes were monitored in the absence and presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration 2×10^{-5} M and varying nucleic acid concentration. Absorption spectra were recorded after each successive addition of DNA solution. The intrinsic binding constant (K_b) was calculated by the equation:

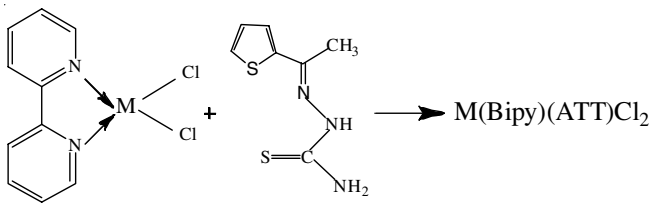
$$[DNA]/\varepsilon_a-\varepsilon_f = [DNA]/\varepsilon_a-\varepsilon_f + 1/K_b\ (\varepsilon_a-\varepsilon_f)$$

where [DNA] is the molar concentration of DNA in base pairs, ε_a, ε_b, ε_f are apparent extinction coefficient (A_{obs}/[M], the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively. A plot of [DNA]/(ε_a-ε_f) *versus* [DNA] gave a slope of 1/(ε_a-ε_f)x K_b is the ratio of the intercept.

Evaluation of antibacterial activity: The pathogenic bacterial strains were purchased from National Chemical Laboratory (NCL) Pune, India. Antibacterial activity of compounds such as Cu-Bipy-Cl_2, Cu-Bipy-ATT, Ni-Bipy-Cl_2, Ni-Bipy-ATT, Co-Bipy-Cl_2 and Co-Bipy-ATT were screened by Agar well diffusion method against bacterial strains such as Gram-negative bacteria such as *Escherichia coli* [NCIM-5051] and Gram-positive bacteria *Staphylococcus aureus* [NCIM-5022]. Nutrient agar (NA) plates were prepared using sterile nutrient agar medium was poured into sterile petri-dishes and allowed to solidify. After, 100 µL of 24 h mature broth culture of individual pathogenic bacterial strains while spreading all over the surface of agar plates using sterilized L-shaped glass rod. About 6 mm well are made in each nutrient agar plate using sterile cork borer. Different concentrations of compounds (100, 200 and 300 µg/well) were used to assess the dose dependent activity of the product. The metal complexes were dissolved in 10 % dimethyl sulfoxide and micropipettes were used for the addition of compounds into the wells. Simultaneously the standard antibiotics (ciprofloxacin used as a positive control) are tested against the pathogenic bacterial strains. Then the plates were incubated at 37 °C for 36 h. After incubation, the zone of inhibition of each well was measured and the values were noted. The experiments were carried out in triplicates with each compound and the average values were calculated for determining the zone of inhibition.

RESULTS AND DISCUSSION

Metal complexes having the composition M(Bipy)Cl_2 (where, M = Cu(II), Ni(II) and Co(II); Bipy = 2,2-bipyridyl) are reacted (**Scheme-I**) with 2-acetylthiophene thiosemicarbazone (ATT) to produce heteroleptic transition metal complexes with molecular formula [M(Bipy)ATT].



Scheme-I: Synthesis of mixed ligand metal complexes [where, M = Cu(II), Ni(II), Co(II)]

All the complexes are stable at room temperature, non-hygroscopic, insoluble in water, slightly soluble in methanol and ethanol but readily soluble in DMF and DMSO. The physico-chemical data for the complexes are summarized in Table-1.

Analytical data support the compositions of the complexes. For 1:1 electrolyte the molar conductivity values are in the range 65-90 Ω^{-1} cm^2 mol^{-1} in dimethylformamide. The observed values indicate non-electrolytic nature [36] of complexes.

Electronic spectra: The electronic spectra of the complexes are recorded in DMF. The significant bands obtained from electronic spectral data are presented in Table-2. A Strong sharp band is observed in the region of 33898-31847 cm^{-1} for the metal complexes is associated with $\pi \rightarrow \pi^*$ transition of aromatic chromophore [37]. Medium intensity band is obtained in the range of 30211-28089 cm^{-1} which corresponds to charge transfer spectra caused by ligand to the metal ion [38]. A weak band in the region of 13106-16806 cm^{-1} region may be assigned to *d-d* transition. The electronic spectrum of complex Co(Bipy)(ATT)Cl_2 is shown in Fig. 3.

IR spectra: IR spectral data of the 2-acetylthiophene thiosemicarbazone (ATT) and its metal complexes along with assignment of peaks are given in Table-3. A strong band is observed in the IR spectrum of ATT at 1605 cm^{-1}, which is assigned to ν(C=N) group. In all the complexes, this band is shifted to lower frequency (11-27 cm^{-1}) indicating the participation of azomethine nitrogen atom in coordination [39,40]. A medium band is appeared in the spectrum of ATT ligand at 1232 cm^{-1}, which is assigned to ν(C=S) group. In all the complexes, this peak disappears and a new band is formed in 768-759 cm^{-1} region due to ν(C-S). These changes suggest the enolization of >C=S to >C-SH. In the enolic form, subsequently the ligand (ATT) undergoes deprotonation and binds metal by forming covalent bond between sulphur and metal. In far IR region, new peaks are observed in 506-498 and 457-

TABLE-1
PHYSICO-CHEMICAL AND ANALYTICAL DATA OF Cu(II), Ni(II) AND Co(II) COMPLEXES

Complex	Colour	Yield (%)	ESI-MS	f.w.	Molar conductivity ($\Omega^{-1}cm^2$ mol^{-1})
Cu(Bipy)Cl_2	Light green	71.56	289.41	291	30.90
Cu(Bipy)(ATT)Cl_2	Dark green	89.03	487.60	490	15.64
Ni(Bipy)Cl_2	Parrot green	81.93	281.30	285	27.75
Ni(Bipy)(ATT)Cl_2·0.5H_2O	Dark brown	86.26	496.10	496	40.46
Co(Bipy)Cl_2	Blue	72.77	282.32	286	46.05
Co(Bipy)(ATT)Cl_2	Black	88.43	480.95	485	67.36

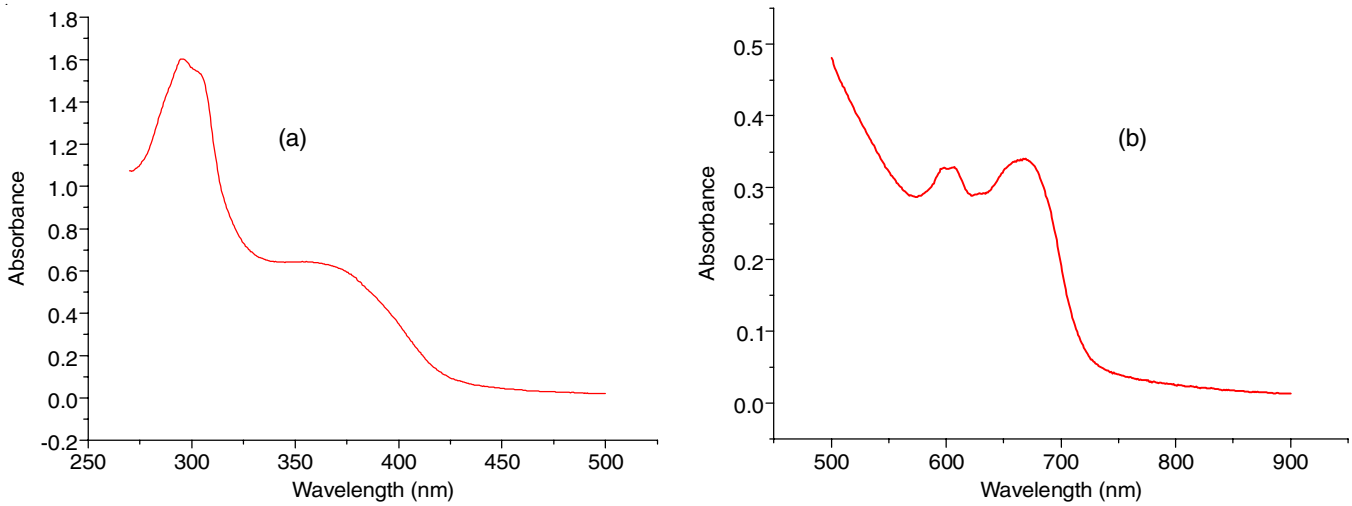


Fig. 3. Electronic spectrum of Co(Bipy)(ATT)Cl_2 complex (a) in lower concentration and (b) in higher concentration

TABLE-2
ELECTRONIC SPECTRAL DATA FOR
Cu(II), Ni(II) AND Co(II) COMPLEXES

Complex	λ_{max} (nm)	Frequency (cm^{-1})	Assignment
Cu(Bipy)Cl_2	301	33222	π-π* transition
	312	32015	CT transition
	763	13106	*d-d* transition
Cu(Bipy)(ATT)Cl_2	314	31847	π-π* transition
	348	28735	CT transition
	595	16806	*d-d* transition
Ni(Bipy)Cl_2	298	33557	π-π* transition
	305	32786	CT transition
	615	16260	*d-d* transition
Ni(Bipy)(ATT)Cl_2·0.5H_2O	306	32679	π-π* transition
	331	30211	CT transition
Co(Bipy)Cl_2	295	33898	π-π* transition
	518	19305	CT transition
	675	14814	*d-d* transition
	990	10101	*d-d* transition
Co(Bipy)(ATT)Cl_2	296	33783	π-π* transition
	356	28089	CT transition
	602	16611	*d-d* transition
	667	14992	*d-d* transition

449 cm^{-1} regions, which are assigned to ν(M-N) and ν(M-S) vibrations [41,42] respectively.

ESR spectra: The ESR spectra of copper complexes were recorded in DMF solution at room temperature and at liquid nitrogen temperature. A typical ESR spectrum of Cu(Bipy)(ATT)Cl_2 recorded at LNT is shown in Fig. 4. The spin Hamiltonian, orbital reduction and bonding parameters of complexes are

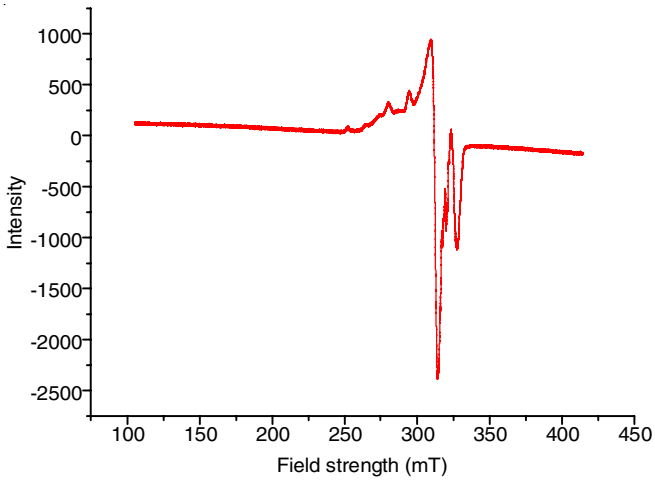


Fig. 4. ESR spectrum of Cu(Bipy)(ATT)Cl_2 complex (LNT)

given in Table-4. The $g_{\|}$ and g are computed from the spectra using TCNE free radicals as g marker. The observed $g_{\|}$ values for complexes are less than or equal to 2.3 suggesting signi-ficant covalent character of metal ligand bond in agreement with observation of Kivelson and Neiman [43]. The $g_{\|}$ and $g_{\perp}$ were more than 2, corresponding to an axial symmetry. The trend The $g_{\|} > g_{\perp} > g_e$ (2.0023) observed for these complexes suggests that the unpaired is localized in the $d_{x^2-y^2}$ orbital [43] of the copper ion. The axial symmetry parameter G is defined as [44]:

$$G = \frac{[g_{\|} - 2.0023]}{[g_{\perp} - 2.0023]}$$

The calculated G values for these complexes are less than 4.0, which indicates the presence of small exchange coupling

TABLE-3
IR THIOSEMICARBAZONE (ATT) SPECTRAL BANDS (cm^{-1}) OF Cu(II), Ni(II) AND Co(II)
COMPLEXES OF BIPYRIDINE AND 2-ACETYLTHIOPHENE

ATT	Cu(Bipy)(ATT)Cl_2	Ni(Bipy)(ATT)Cl_2·0.5H_2O	Co(Bipy)(ATT)Cl_2	Assignment
1605	1578, 1513	1594	1587	ν(C=N) Azomethine
	1448, 1284	1439, 1309	1423, 1301	ν(C-C) thiophene
1232	1095	1112, 989	1152, 1006, 850	ν(C=S) thione
	768	759	768	ν(C-S)
	498		506	ν(M-N)
	457	449	457	ν(M-S)

TABLE-4
ESR SPECTRAL DATA† OF COPPER COMPLEXES

Complex	In DMF at LNT								
	$g_{\parallel}$	$g_{\perp}$	g_{avg}	G	$A_{\parallel} \times 10^{-5}$	$A_{\perp} \times 10^{-5}$	$K_{\parallel}$	$K_{\perp}$	λ	α^2
$Cu(Bipy)Cl_2$	2.28 (2.14)	2.05 (2.08)	2.13 (2.10)	4.87 (1.74)	0.0016	–	0.995	1.027	463	0.2997
$Cu(Bipy)(ATT)Cl_2$	2.33 (2.13)	1.99 (2.06)	2.10 (2.08)	3.96 (2.21)	0.0013	0.00093	0.304	0.181	693	0.0495

†ESR data in DMF at room temperature are given in parenthesis.

and misalignment of molecular axes. The $g_{\parallel}$, $g_{\perp\parallel}$, $A_{\perp}$ of complexes and the energies of the *d-d* transitions are used to calculate the orbital reduction parameters ($K_{\perp}$, $K_{\perp}$), the bonding parameter (α^2). The factor α^2 which is usually taken as a measure of covalency and it is evaluated by the expression:

$$\alpha^2 = -A_{\parallel}/p + (g_{\parallel} - 2.0023) + 3/7(g_{\perp} - 2.0023) + 0.04$$

Hatchway pointed out that for pure σ bonding $K_{\parallel} \approx K_{\perp} \approx 0.77$, for in-plane π-bonding $K_{\parallel} < K_{\perp}$, while out of plane π-bonding $K_{\parallel} > K_{\perp}$ the following simplified expressions were used to calculate $K_{\parallel}$ and $K_{\perp}$:

$$K_{\parallel}^2 = \frac{(g_{\parallel} - 2.0023)}{8 \times \lambda_o} \times \text{d-d transition}$$

$$K_{\perp}^2 = \frac{(g_{\perp} - 2.0023)}{8 \times \lambda_o} \times \text{d-d transition}$$

The observed $K_{\parallel} < K_{\perp}$ relation for $Cu(Bipy)Cl_2$ complex indicates the significant out of plane π-bonding and $K_{\parallel} > K_{\perp}$ relation for $Cu(Bipy)(ATT)Cl_2$ complex indicates the significant out of plane π-bonding.

Based on analytical, physico-chemical and spectral data, a general structure (Fig. 5) is proposed for the complexes.

Cyclic voltammetry: The redox behaviour of the complexes has been investigated by cyclic voltammetry in DMF using 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. The cyclic voltammogram of $Ni(Bipy)(ATT)Cl_2$ complex is shown in Fig. 6 and the electrochemical data of complexes are summarized in Table-5.

The cathodic peak current function values were found to be independent of the scan rate. Repeated scans at various scan rates suggest that the presence of stable redox species in solution. It has been observed that cathodic (Ip_c) and anodic (Ip_a) peak currents were not equal. The $E_{1/2}$ values of copper(II) complexes are noticed at potential range of 0.179–0.298 V. It may be concluded that all the bivalent metal complexes undergo one electron reduction to their respective M(I) complexes. The non-equivalent current in cathodic and anodic peaks indicates

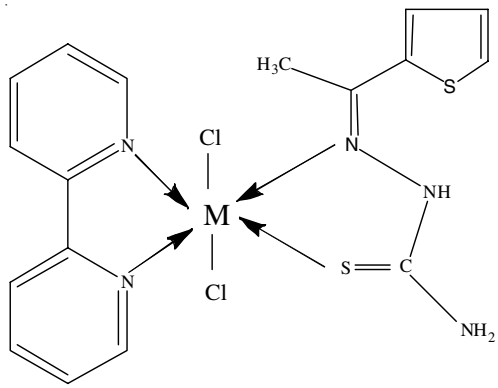


Fig. 5. Structure of $M(Bipy)(ATT)Cl_2$ complex

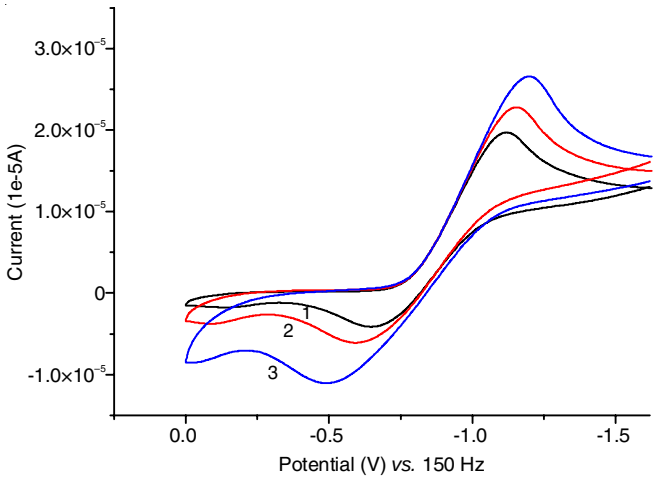


Fig. 6. Cyclic voltammogram of $Ni(Bipy)(ATT)Cl_2$ 0.5 H_2O, at different scan rates (1) 0.05 (2) 0.1 (3) 0.2 mV s^{-1}

quasi-reversible behaviour [45]. The difference, ΔEp in all the complexes be better than the Nerstian requirement 59/n mV (n = number of electrons involved in oxidation reduction), which demonstrate quasi-reversible character of electron transfer [46]. The complexes show large separation between anodic and cathodic peaks indicating quasi-reversible character.

TABLE-5
CYCLIC VOLTAMMETRIC DATA OF Cu(II), Ni(II) AND Co(II) COMPLEXES

Complex	Redox couple	C_v cathodic E_{pc}	C_v anodic E_{pa}	ΔEp (mV)	$E_{1/2}$	$-i_c/i_a$	log Kc[a]	$-\Delta G^b$
$Cu(Bipy)Cl_2$	II/I	-0.047	0.488	535	0.267	1.510	0.062	362
$Cu(Bipy)(ATT)Cl_2$	II/I	-0.072	0.523	595	0.297	1.432	0.056	323
$Ni(Bipy)Cl_2$	II/I	-1.291	-0.743	548	-1.017	2.740	0.061	354
$Ni(Bipy)(ATT)Cl_2 \cdot 0.5H_2O$	II/I	-1.196	-0.505	691	-0.850	2.381	0.048	280
$Co(Bipy)Cl_2$	II/I	-1.034	-0.726	308	-0.880	0.895	0.010	629
$Co(Bipy)(ATT)Cl_2$	II/I	-1.370	-0.677	693	-1.023	2.950	0.048	279

[a]$\log K_c = 0.434ZF/RT\Delta E_p$; [b]$\Delta G^o = -2.303RT \log K_c$

DNA binding studies: The binding interaction of the complexes with DNA was monitored by comparing their absorption spectra with and without CT-DNA. Typical absorption spectra of Ni(Bipy)(ATT)Cl_2 complex in the absence and in the presence of CT DNA are shown in Fig. 7. It has been observed that molar absorptivity of complexes decreases (hypochromism, Δε, +11.91 to +38.8 %, Table-6) for each addition of CT-DNA of the π-π^* absorption band as well as a hypsochromic shift in the case of Cu(II) complexes and bathochromic shift for Ni(II) and Co(II) complesxes of a few nanometers (0.5-2.0 nm). The intrinsic binding constants (K_b), were determined by using the equation. The intrinsic binding constants of copper complexes are given in Table-6.

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

Hyperchromic effect and hypochromic effect are the special features of DNA concerning its double helix structure. Hypochromism results from the contraction of DNA in the helix axis as well as from the change in conformation on DNA while hyperchromism emerges from the damage of the double helix structure [47]. Hypochromism was observed due to intercalative mode involving strong stacking interactions between aromatic chromophore of metal complexes and nitrogenous bases of DNA [47]. Where the hyperchromism due to the dissociation of ligand accumulated and the breakage of intermolecular hydrogen bonds when metal complex bound to DNA [34]. Hypochromism and bathochromic shift in case of the Ni(II) and Co(II) complexes suggest that these complexes bind DNA through intercalation involving a strong π-stacking interaction between the aromatic chromophore and base pairs of DNA [48].

Antibacterial activity: All the metal complexes are screened for their antibacterial activity by using agar well diffusion method against pathogenic bacterial strains such as *E. coli* and *S. aureus.* Inhibition zones are determined in the presence of different amounts (100, 200 and 300 µg/well) of complexes with reference to the positive control *viz.* ciprofloxacin. The diameters of inhibition of zone were measured with Vernier callipers in mm and its values are depicted in the Table-7. Antibacterial activity of present complexes is quite comparable to the standard compound (Fig. 8) as shown in bar graph. The zone of inhibition by Cu(Bipy)Cl_2 complexes is highly significant. The data indicates that the parent complexes {Cu(Bipy)Cl_2 and Co(Bipy)Cl_2} show more activity than the ternary complexes Cu(Bipy)(ATT)Cl_2 and Co(Bipy)(ATT)Cl_2. However, the ternary Ni(Bipy)(ATT)Cl_2 complex shows higher activity than the parent complexes possibly due to synergistic interactions of two organic ligands with bacteria.

Conclusion

Mixed ligand transition metal complexes with 2,2-bipyridyl (Bipy) and 2-acetylthiophene thiosemicarbazone (ATT) have been synthesized and characterized based on mass spectra, molar conductivity, infrared and electronic spectra. Electrochemical properties of these complexes are uncovered by using

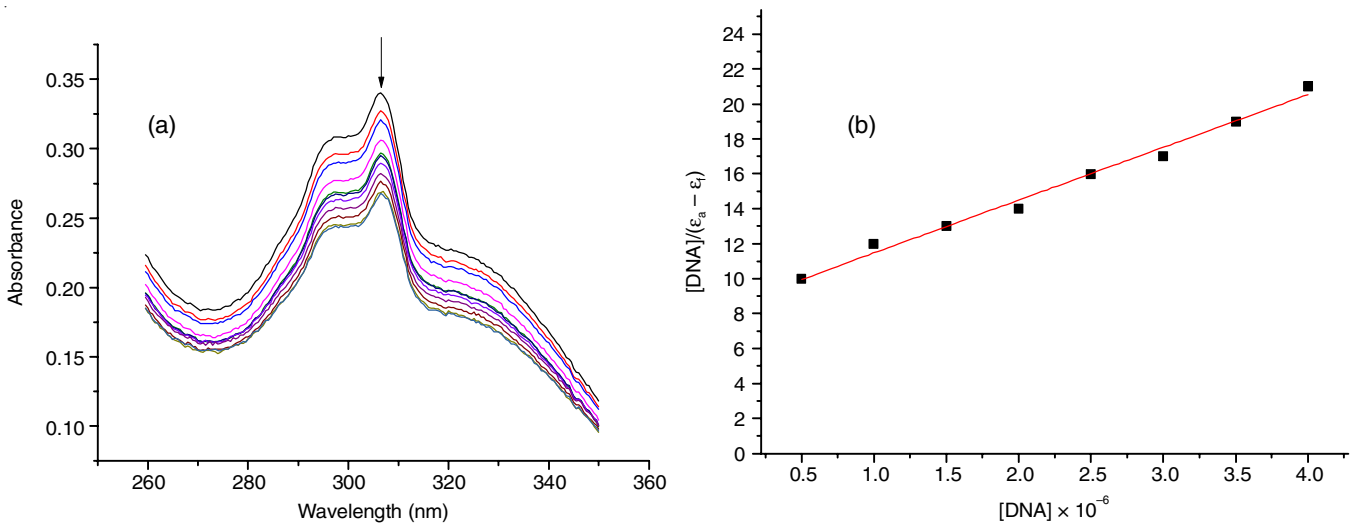


Fig. 7. Absorption spectra of Ni(Bipy)(ATT)Cl_2 0.5H_2O. In the absence and in the presence of increasing concentration of CT-DNA; [(a) most spectrum is recorded in the absence of CT-DNA and (b) spectra on addition 20 µL DNA each time] A plot [DNA]/(ε_a-ε_f) *vs.* [DNA] × 10^{-6} is shown

TABLE-6
ELECTRONIC ABSORPTION DATA UPON ADDITION OF CT-DNA TO THE COMPLEX

Complex	λ_{max} (nm)		Δλ	H (%)	K_b [M^{-1}]
	Free	Bound			
Cu(Bipy)Cl_2	300.5	300.0	0.5	38.80	3.50×10^5
Cu(Bipy)(ATT)Cl_2	302.0	300.0	2.0	11.91	4.00×10^5
Ni(Bipy)Cl_2	306.0	306.5	0.5	36.60	3.56×10^5
Ni(Bipy)(ATT)Cl_2·0.5H_2O	306.0	306.5	0.5	13.43	4.37×10^5
Co(Bipy)Cl_2	295.0	296.5	1.5	17.77	3.12×10^5
Co(Bipy)(ATT)Cl_2	302.0	303.5	1.5	11.91	4.34×10^5

TABLE-7
ANTIBACTERIAL ACTIVITY OF DIFFERENT METAL COMPLEXES AGAINST PATHOGENIC BACTERIAL STRAINS

Complex	Treatment conc. (µg/µL)	*E. coli* (Mean±SE)	*S. aureus* (Mean±SE)
Ciprofloxacin (S)	5	11.70±0.06	10.03±0.03
$Cu(Bipy)Cl_2$ (**1**)	100	4.17±0.17	4.27±0.15
	200	6.67±0.33	6.60±0.31
	300	8.17±0.17	8.53±0.09
$Cu(Bipy)(ATT)Cl_2$ (**2**)	100	1.17±0.00	1.00±0.00
	200	2.50±0.50	2.33±0.33
	300	4.17±0.17	3.50±0.29
$Ni(Bipy)Cl_2$ (**3**)	100	1.40±0.17	1.17±0.17
	200	2.67±0.17	2.83±0.17
	300	4.33±0.17	4.50±0.29
$Ni(Bipy)(ATT)Cl_2{\cdot}0.5H_2O$ (**4**)	100	1.07±0.07	1.40±0.31
	200	3.40±0.31	3.91±0.38
	300	4.50±0.29	5.83±0.44
$Co(Bipy)Cl_2$ (**5**)	100	2.30±0.15	2.63±0.19
	200	3.83±0.17	4.17±0.17
	300	5.50±0.29	7.17±0.17
$Co(Bipy)(ATT)Cl_2$ (**6**)	100	1.97±0.15	2.10±0.03
	200	2.80±0.12	3.10±0.21
	300	4.53±0.17	5.17±0.17

Values are the mean ± SE (Standard Error) of inhibition zone in mm.

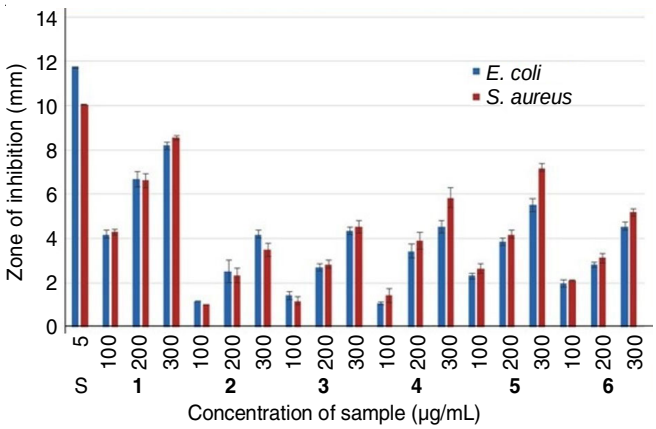


Fig. 8. Graphical representation of antibacterial activity of metal complexes against pathogenic bacterial strains

cyclic voltammetry. The complexes show quasi reversible cyclic voltammetric responses for the Cu(II)/Cu(I) couple. The binding properties of these complexes with calf-thymus DNA have been investigated by using absorption spectrophotometry. Mixed ligand metal complexes show high binding affinity towards DNA. Metal complexes are screened for their antibacterial activity by using agar well diffusion method against pathogenic bacterial strains *viz. Escherichia coli* and *Staphylococcus aureus*. Antibacterial activity of the present complexes are comparable with the activity of ciprofloxacin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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