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# DNA BINDING AND ANTIBACTERIAL ACTIVITY OF BIVALENT METAL COMPLEXES WITH 1,10- PHENANTHROLINE AND 2- ACETYLTHIOPHENE THIOSEMICARBAZONE

**Kummara Srinivasulu, D. Dhanalakshmi, K. Anuja and Katreddi Hussain Reddy***

Department of Chemistry, Sri Krishnadevraya University, Ananthapuramu-515 003, Andhra Pradesh, India.

***Corresponding Author: Prof. Katreddi Hussain Reddy**
Department of Chemistry, Sri Krishnadevraya University, Ananthapuramu-515 003, Andhra Pradesh, India.

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**ABSTRACT**
Bivalent metal complexes having the composition $M(Phen)_2Cl_2$ (where, M= Cu(II), Ni(II) and Co(II); Phen = 1,10-Phenonthroline) are reacted with 2-acetylthiophene thiosemicarbazone (ATT) to produce bivalent metal complexes with molecular formula $[M(Phen)_2ATT]Cl$. The complexes are characterized using physical (molar conductivity) and spectral (mass spectra, infrared and electronic spctroscopies) methods. Electrochemical behaviour of complexes was uncovered using cyclic voltammetry. The complexes show quasi reversible cyclic voltammetric responses for the Cu(II)/Cu(I) couple. The DNA binding properties of the complexes are determined by using UV-Visible absorption spectrophotometry. Metal complexes are screened for their antibacterial activity by using agar well diffusion method against pathogenic bacterial strains *viz.* Gram –ve such as Escherichia *coli, Klebsiella pneumonia and* Gram +ve such as *Staphylococcus aureus, Bacillus cereus.* Anti bacterial activity of the present complexes are comparable with the activity of Ciprofloxacin. The $[Cu(Phen)_2(ATT)]Cl$ $1.5\ H_2O$ complex inhibits bacteria more strongly than any other complex. The $[Co(Phen)_2(ATT)]Cl$ complex shows more activity than the parent complex, $Co(Phen)_2Cl_2$.

**KEYWORDS**: Bivalent metal complexes, 2-Acetylthiophene thiosemicarbazone, DNA binding, Antibacterial activity.

## 1. INTRODUCTION

Thiosemicarbazones are considered as pivot class of sulphur containing ligands particularly for transition metal ions. The real impetus towards developing their coordination chemistry was due to their physicochemical properties.[1,2] 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], anti-trypanosomal[16], antiviral[17], antitumor[18] and anticancer[19,20] activities.

Transition metal complexes with heterocyclic thiosemicarbazones are expected to exhibit interesting stereochemical, electrochemical, and electronic properties.[21,22] Metal complexes of 1,10-phenantrholine (Phen) are known to function as potential antitumour agents.[23] The choice of 1,10-phenantrholine 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 pi-electron deficiency makes Phen as an excellent π-acceptor ligand. The metal complexes of 1,10-phenantrholine are of considerable interest due to their biological or pharmacological properties. The pharmacological properties are mainly due to its planar structure which facilitates the *intercalative mode* of binding with DNA, the ladder of life.

Recently, mixed ligand metal complexes with Schiff bases as a main ligand 1,10-penanthroline as a co-ligand are reviewed.[24] But no report is available in the literature[25-27] on mixed ligand metal complexes with 1,10-phenantrholine and 2-acetylthiophene thiosemicarbazone(ATT). 2-Acetylthiophene thiosemicarbazone(ATT) was synthesized and characterized[28,29] based on spectral data. Platinum and palladium complexes of ATT were reported in the literature.[30] ATT has been used for the spectrophotometric determination of copper(II) in alloys, edible oils and seeds.[31] However, transition metal complexes with 1,10-phenathroline as primary ligand

and thiosemicarbazone as secondary ligand have not been much investigated.[33]

Mixed ligand complexes of biologically important compounds may serve as models for biochemical processes.[34,35] In the light of the above and in continuation of our ongoing research on transition metal complexes, herein we reported synthesis, characterization and DNA binding properties of heterolyptic Cu(II), Ni(II) and Co(II) complexes with 1,10-phenathroline and 2-acetylthiophene thiosemicarbazone(ATT).

## 2. EXPERIMENTAL

### 2.1 MATERIALS AND METHODS

Thiosemicarbazide, 2-acetylthiophene and 1,10-phenonthroline were purchased from Sigma-Aldrich. All other chemicals were of AR grade and used as provided. The solvents 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 ± 2K in dry and purified DMF were carried out using a CM model 162 conductivity cell (ELICO). The electronic spectra were recorded in DMF with a UV Lamda 50 (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 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.

### 2.2 Preparation of ATT

Methanolic solutions of thiosemicarbazide (5mmol) and 2-acetylthiophene (5mmol) were 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 hours and the reaction mixture was cooled to room temperature. The ligand ATT was obtained as yellow colored crystalline product, which are subsequently used in the synthesis of metal complexes. Yield 65%, M.Pt. 155-157 $^oC$, IR spectra ($cm^{-1}$) 3404, 3207, 3126, 1605. 1232 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 (multiplet3H), 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.**

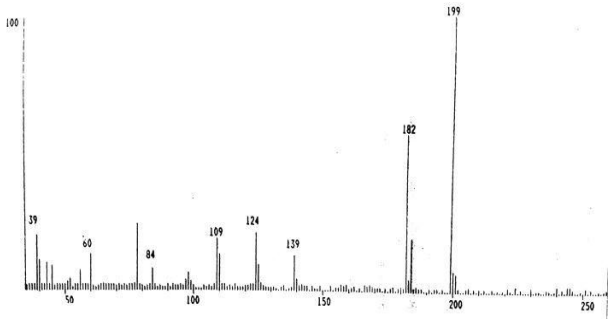



**Fig. 1: Mass Spectrum of ATT Ligand.**

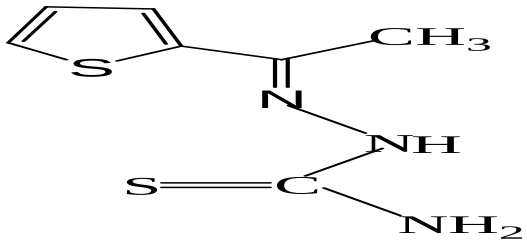



**Fig. 2: Structure of ATT Ligand.**

### 2.3 Preparation of mixed ligand metal complexes

A 1.2 g of ATT ligand (0.006 moles) was dissolved in 15 mL of 0.05 N NaOH in ethanol solvent in 100-mL beaker. A 1.0 g Cu $(Phen)_2$ $Cl_2$ (0.003 moles) was dissolved in 15 mL of ethanol solvent in 100 mL beaker. Ligand solution and Cu $(Phen)_2$ $Cl_2$ solution were transferred into 100- mL round bottom flask and heated under reflux for 1hr. On cooling the contents of flask, light green coloured complex was formed. It was collected by filtration, washed with small quantities of ethanol and dried in air. $Ni(Phen)_2$ATT and $Co(Phen)_2$ATT complexes were prepared similarly.

**3. DNA binding experiments:** The electronic spectra of metal complexes were monitored in the absence and in the 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 (50mM NaCl/5 mM Tris-HCl; pH =7.0) buffer gave absorbance ratio at 260 nm and 280 nm of 1.85, indicating that the DNA was sufficiently free of proteins. 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 $^oC$ were used after no more than four days. The electronic spectra of metal complexes were monitored in the absence and in the presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration 2 x $10^{-5}$M and varying nucleic acid concentration. Absorption spectra were recorded after each successive addition of DNA solution. The intrinsic binding constant (Kb) was calculated by the equation, [DNA]/εa-εf = [DNA]/εa-εf + 1/Kb (εa-εf), where [DNA] is the molar concentration of DNA in base pairs, εa, εb, εf are apparent extinction coefficient(Aobs/[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] / (εa-εf) versus [DNA] gave a slope of 1/(εa-εf)x Kb is the ratio of the intercept.

**4. Evaluation of Antibacterial activity**

The pathogenic bacterial strains were purchased from National Chemical Laboratory (NCL)Pune, India. Antibacterial activity of compounds such as $Co(Phen)_2Cl2$, $Ni(Phen)_2Cl2$, $Cu(Phen)_2Cl2$, $[Co(Phen)_2(ATT)]Cl$, $[Ni(Phen)_2(ATT)]Cl$, $[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$, were screened against bacterial strains such as Gram–ve bacteria such as *Escherichia coli, Klebsiella pneumoniae and* Gram +ve bacteria such as *Bacillus cereus* and *Staphylococcus aureus* by using Agar well diffusion method. Nutrient agar (NA) plates were prepared using sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. About 6mm wells 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 (DMSO) 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 antibacterial activity.

**5. RESULTS AND DISCUSSION**

Metal complexes having the composition $M(Phen)_2Cl2$ (where, M= Co(II), Ni(II) and Cu(II); Phen = 1,10-Phenonthroline) are reacted with 2-acetylthiophene thiosemicarbazone (ATT) to produce heteroleptic transition metal complexes with molecular formula $[M(Phen)_2$ ATT]Cl. 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 gave support the compositions of the complexes. For 1:1 electrolyte the molar conductivity values are in the range 65-90 $\Omega^{-1}$ $cm^2$ $mol^{-1}$ in dimethylformamide. The observed values for Complex 1 and other complexes(2-6) indicate non-electrolytic and electrolytic nature complexes.

**Table. 1: Physicochemical and Analytical data of Cu(II) Ni(II) and Co(II) complexes.**

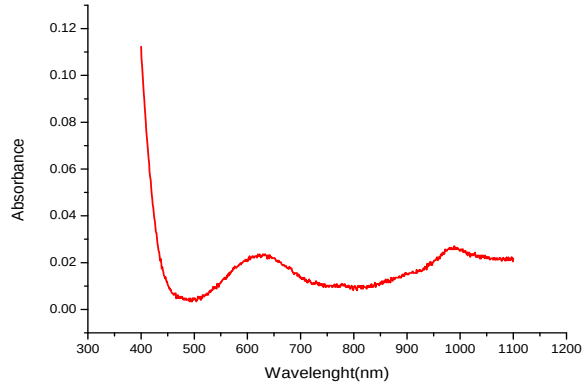
| S. No. | Complex | Colour (Yield %) | Molecular Weight | Molar Conductivity ($\Omega^{-1}cm^2mol^{-1}$) |
|---|---|---|---|---|
| 1. | **$Cu(Phen)_2Cl_2$** | Light Green (72.98%) | 496 | 1.84 |
| 2. | **$[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$** | Dark Green (86.00%) | 687 | 11.70 |
| 3 | **$Ni(Phen)_2Cl_2$** | Light Blue (73.72%) | 491 | 19.45 |
| 4. | **$[Ni(Phen)_2(ATT)]Cl$** | Dark Brown (75.07%) | 654 | 21.95 |
| 5 | **$Co(Phen)_2Cl_2$** | Light Blue (74.50%) | 492 | 17.85 |
| 6 | **$[Co(Phen)_2(ATT)]Cl$** | Brown (76.23%) | 655 | 18.47 |

**5.1 Electronic spectra**

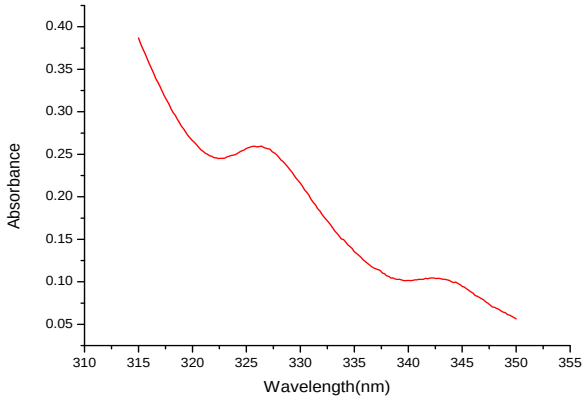
The electronic spectra of the complexes are recorded in DMF. The significant bands obtained from electronic spectral data are presented in **Table 2**. A strong sharp band is observed in the region of 36,496 - 30,581 $cm^{-1}$ for the metal complexes is associated with π →π* transition of aromatic chromophore.[37] Medium intensity band is obtained in the range of 30,120 - 28,490 $cm^{-1}$ which corresponds to charge transfer spectra caused by ligand to the metal ion.[38] A weak band in the region of 13,245-16,447 $cm^{-1}$ region may be assigned to d-d transition. The electronic spectrum of **$Ni(Phen)_2Cl_2$** complex is shown in **Fig. 3**.

**Table 2: Electronic Spectral Data for Cu(II), Ni(II) and Co(II) Complexes.**

| S. No | Complex | Wavelength λ max (nm) | Frequency($cm^{-1}$) | Assignment |
|---|---|---|---|---|
| **1** | **$Cu(Phen)_2Cl_2$** | 295 | 33,898 | π-→π* transition |
| | | 330 | 30,303 | M→LCT |
| | | 755 | 13,245 | d-d transition |
| **2** | **$[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$** | 298 | 33,557 | π-→π* transition |
| **3** | **$Ni(Phen)_2Cl_2$** | 327 | 30,581 | M→LCT |
| | | 345 | 28,985 | d-d transition |
| | | 627 | 15,948 | d-d transition |
| | | 990 | 10,101 | d-d transition |
| **4** | **$[Ni(Phen)_2(ATT)]Cl$** | 292 | 34,246 | π-→π* transition |
| | | 334 | 29,940 | M→LCT |
| **5** | **$Co(Phen)_2$ $Cl_2$** | 290 | 34,482 | π-→π* transition |
| | | 351 | 28,490 | d-d transition |
| | | 608 | 16,447 | d-d transition |
| | | 677 | 14,771 | d-d transition |
| **6** | **$[Co(Phen)_2(ATT)]Cl$** | 332 | 30,120 | M→LCT |




**(a)**




**(b)**

**Fig. 3: Electronic Spectra of $Ni(Phen)_2Cl_2$Complex (a) at Higher concentration and (b) at Lower concentration of the complex.**

**5.2 IR spectra**

IR spectral data of 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 (18-27 $cm^{-1}$) indicating the participation of azomethine nitrogen atom in coordination.[39,40] A medium band is appeared in the spectrum of ATT at 1232 $cm^{-1}$, which is assigned to ν(C=S) group. This peak disappears in spectra of all complexes and a new band is formed in 721-718 $cm^{-1}$ 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 539-596 and 439-463 $cm^{-1}$ regions which are assigned to υM-N and υM-S vibrations[41] respectively.

**Table. 3: IR spectral bands ($cm^{-1}$) of Cu(II), Ni(II) and Co(II) Complexes of 1,10 Phenonthroline And 2-acetylthiophene thiosemicarbazone (ATT).**

| ATT | $[Cu(Phen)_2(ATT)]Cl\ 1.5\ H_2O$ | $[Ni(Phen)_2(ATT)]Cl$ | $[Co(Phen)_2(ATT)]Cl$ | Assignment |
|---|---|---|---|---|
| 1605 | 1583 | 1587 | 1578 | υC=N (Azomethine) |
| | 1492<br>1224 | 1424<br>1326 | 1489<br>1341 | υC-C (thiophene) |
| 1232 | -<br>1112<br>1039<br>832 | -<br>1145<br>1096<br>850 | -<br>1147<br>1087<br>842 | υC=S (thione) |
| ---- | 721 | 718 | 718 | υC-S |
| | 615 | 637 | 637 | |
| | 584 | 539 | 596 | υM-N |
| | 463 | 457 | 439 | υM-S |

**5.3 ESR spectra:** ESR spectra of copper complexes were recorded in DMF solution at room temperature and at liquid nitrogen temperature. A typical ESR spectrum of $[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$ recorded at LNT is shown in **Fig. 4.** The spin Hamiltonian, orbital reduction and bonding parameters of complexes are given in **Table 4**. The $g\|$ and $g\perp$ 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 significant covalent character of metal ligand bond in agreement with observation of Kivelson[43]. The $g\|$ and $g\perp$ were more than 2, corresponding to an axial symmetry. The trend The $g\| > g^{\perp} >$ ge (2.0023) observed for these complexes suggests that the unpaired is localized in the $dx^2$- $y^2$ 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 and misalignment of molecular axes. The g||, g⊥, $A\|$, $A^{\perp}$ of complexes and the energies of the d–d transitions are used to calculate the orbital reduction Parameters ($K\|$, $K^{\perp}$), the bonding parameter ($\alpha^2$).The factor $\alpha^2$which is usually taken as a measure of covalency and it is evaluated by the expression

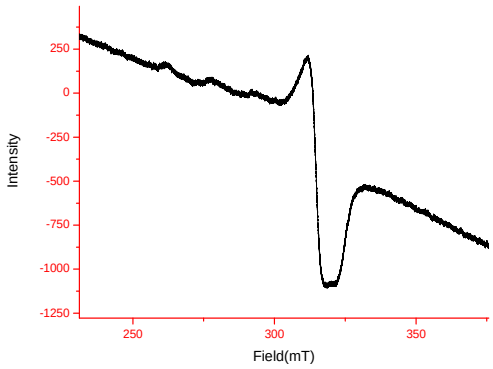
$\alpha^2 = -A\|/p + (g\| - 2.0023) + 3/7(g^{\perp} - 2.0023) + 0.04$

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

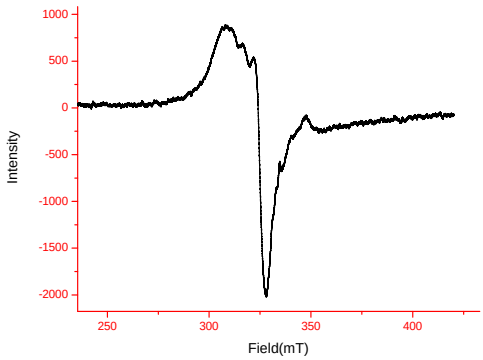
$K\|^2 = (g\| - 2.0023)/8x\ \lambda_0$ X d-d transition

$K^{\perp 2} = (g^{\perp} - 2.0023)/8x\ \lambda_0$ X d-d transition

The observed $K\| < K^{\perp}$ relation for $Cu(phen)_2Cl_2$complex indicates the significant out of plane π -bonding and $K\| > K^{\perp}$ relation for $[Cu(phen)_2(ATT)]Cl$ 1.5 $H_2O$ complex indicates the significant out of plane π-bonding. Based on analytical, physicochemical and spectral data, a general structure **Fig. 5.** is proposed for the complexes.




**(a)**




**(b)**

**Fig. 4: ESR Spectrum of $[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$ Complex ( a) at LNT (b) at RT.**

**Table. 4: ESR Spectral data† of copper complexes.**

| Complex | In DMF at LNT | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $g_{\|}$ | $g_{\perp}$ | $g_{avg}$ | G | $A_{\|} \times 10^{-5}$ | $A_{\perp} \times 10^{-5}$ | $K_{\|}$ | $K_{\perp}$ | λ | $\alpha^2$ |
| **$Cu(Phen)_2Cl_2$** | 2.26 (2.16) | 2.05 (2.23) | 2.12 (2.21) | 0.51 (0.69) | 0.00104 | - | 0.991 | 1.061 | 432 | 0.366 |
| **$[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$** | 2.08 (2.13) | 2.03 (2.05) | 2.04 (2.08) | 3.05 (2.44) | 0.00136 | 0.000371 | 0.982 | 0.775 | 562 | 0.091 |

†ESR data in DMF at Room Temperature are given in parenthesis

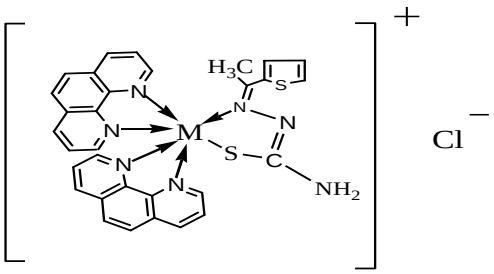



**Fig. 5: Structure of M (1, 10 - Phenonthroline) (ATT) Complex. [M = Cu, Ni and Co].**

**5.4 Cyclic voltammetry:** Electrochemical Properties of complexes are investigated by cyclic voltammetry in DMF using 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. The cyclic voltammogram of $[Co(Phen)_2(ATT)]Cl$ complex is shown in **Fig.6.** and the electrochemical data of complexes are summarized in **Table 5**.

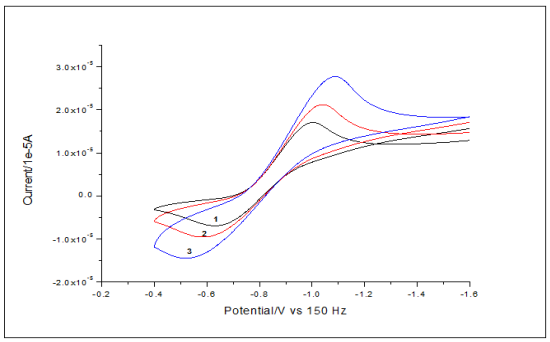



**Fig. 6**: **Cyclic voltammogram of [Co (Phen)$_2$(ATT)]Cl. At different scan rates (1) 0.05 (2) 0.1 (3) 0.2 mVs$^{-1}$.**

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 (Ipc) and anodic (Ipa) peak currents were not equal. The $E_{½}$ values of copper(II) complexes are noticed at potential range of 0.084–0.113 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 indicate quasi-reversible behaviour.[44] 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.[45] The complexes show large separation between anodic and cathodic peaks indicating quasi-reversible character.

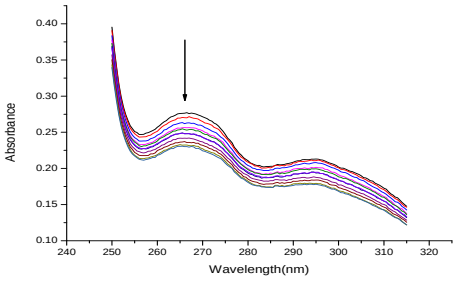
**Table. 5: CV data of Cu (II), Ni(II) and Co(II) complexes.**

| Complex | Redox couple | $E_{pc}$ | $E_{pa}$ | ΔEp (mV) | $E_{1/2}$ | $-i_c/i_a$ | LogKc[a] | -ΔG[b] |
|---|---|---|---|---|---|---|---|---|
| **Cu(Phen)$_2$Cl$_2$** | II/I | -0.086 | 0.140 | 226 | 0.113 | 1.461 | 0.0148 | 854 |
| **[Cu(Phen)$_2$(ATT)]Cl 1.5 H$_2$O** | II/I | -0.054 | 0.114 | 168 | 0.084 | 1.245 | 0.0199 | 682 |
| **Ni(Phen)$_2$Cl$_2$** | II/I | -1.83 | -0.68 | 115 | -1.25 | 1.317 | 0.0292 | 168 |
| **[Ni(Phen)$_2$(ATT)]Cl** | II/I | -1.22 | -0.53 | 690 | -0.875 | 1.812 | 0.0210 | 121 |
| **Co(Phen)$_2$Cl$_2$** | II/I | -1.21 | -0.55 | 660 | -0.880 | 1.261 | 0.0508 | 293 |
| **[Co(Phen)$_2$(ATT)]Cl** | II/I | -1.04 | -0.59 | 450 | -0.815 | 1.468 | 0.0746 | 430 |

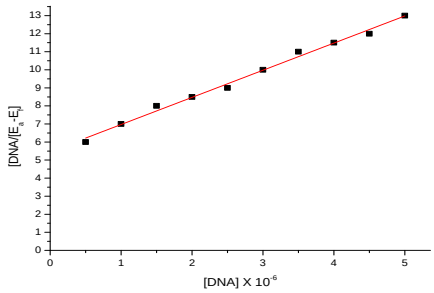
[a]log $K_c$ = 0.434ZF/RTΔ$E_p$; [b]ΔG° = -2.303RTlog $K_c$

**5.5 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 [Cu(Phen)$_2$(ATT)]Cl 1.5 $H_2O$ 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, +12.60 to +20.79%, **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) complexes of a few nanometres (0.5 – 1.0 nm).

The intrinsic binding constants (Kb) were determined by using the equation. The intrinsic binding constants of copper complexes are given in Table 6. Hyper chromic effect and hypo chromic 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[46]. Hypochromism was observed due to intercalative mode involving strong stacking interactions between aromatic chromophore of metal complexes and nitrogenous bases of DNA. Where the hyperchromism is due to the dissociation of ligand accumulated and the breakage of intermolecular hydrogen bonds when the complex bound to DNA. Hypochromism and bathochromic shift in the case of 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.[46]




**(a)**




**(b)**

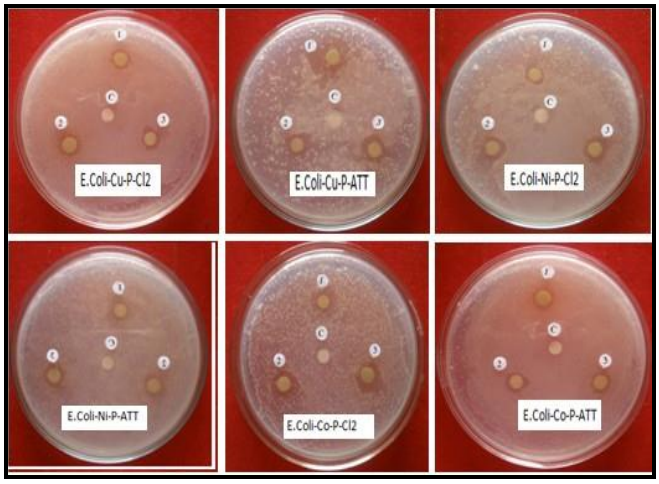
**Fig.7**: **(a) Absorption Spectra of [Cu(Phen)$_2$(ATT)]Cl 1.5 $H_2O$ In the absence and in the presence of increasing concentration of CT-DNA; [The Top most spectrum is recorded in the absence of CT-DNA and below spectra on addition 20µL DNA each time.] (b) A plot [DNA]/ ($E_a$-$E_f$) vs [DNA] X 10$^{-6}$ is shown.**

**Table 6: Electronic Absorption Data upon addition of C T –DNA to the Complex**.

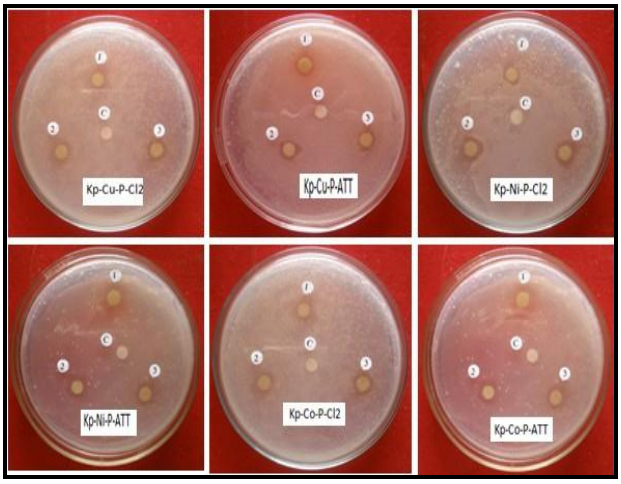
| S. No | Complexes | λ max (nm) | | Δ λ | H% | $K_b$ [$M^{-1}$] |
|---|---|---|---|---|---|---|
| | | Free | Bound | | | |
| 1 | $Cu(Phen)_2Cl_2$ | 269 | 270 | 1 | 20.79 | 1.44 $X10^5$ |
| 2 | $[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$ | 268 | 268.5 | 0.5 | 12.60 | 3.78 $X10^5$ |
| 3 | $Ni(Phen)_2Cl_2$ | 270 | 270.5 | 0.5 | 15.83 | 1.26 $X10^5$ |
| 4 | $[Ni(Phen)_2(ATT)]Cl$ | 269.5 | 270.5 | 1 | 07.04 | 9.18 $X10^5$ |
| 5 | $Co(Phen)_2Cl_2$ | 269.5 | 270.5 | 1 | 12.19 | 1.73 $X10^5$ |
| 6 | $[Co(Phen)_2(ATT)]Cl$ | 269 | 270 | 1 | 19.09 | 2.03 $X10^5$ |

**5.6 Antibacterial activity**

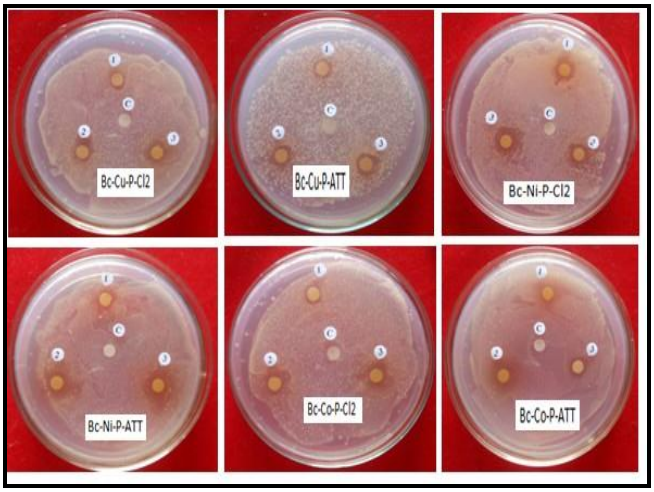
Metal complexes are screened for their antibacterial activity by using agar well diffusion method **Fig. 8.** shows photographs of inhibition zones the diameters of inhibition of Zone were measured with Verniercallipers in mm and its values are depicted in the **Table.7**. Antibacterial activity of present complexes are quite comparable to the standard compound **Fig.9.** The zone of inhibition by the [Cu $(Phen)_2$(ATT)]Cl 1.5 $H_2O$ complex inhibits bacteria more strongly than any other complex. The [Co $(Phen)_2$(ATT)]Cl complex shows more activity than the parent complex $Co(Phen)_2Cl_2$. The mixed ligand complexes show higher activity than the parent complexes possibly due to synergistic interactions of two organic ligands with bacteria.



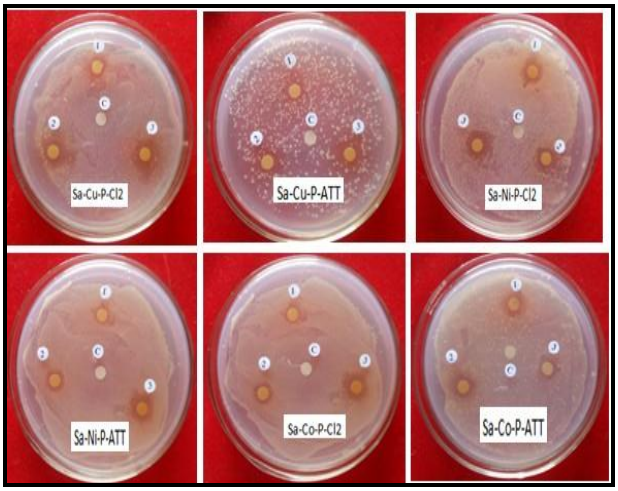

**(a)**




**(b)**




**(c)**




**(d)**

**Fig. 8: Photographs showing the Antibacterial activity of Metal complexes (a) *Escherichia coli* (b) *Klebsiella pneumonia* (c) *Bacillus cereus* (d) *Staphylococcus aureus* when compare to standard (C). (1=100µg/µL,2= 200µg/µL 3= 300µg/µL).**

**Table 7: Antibacterial activity of different Metal Complexes against pathogenic bacterial strains.**

| S. No | Sample | Treatment (Concentration) | *E. coli* (Mean±SE) | K.pneumoniae (Mean±SE) | *S. aureus* (Mean±SE) | B.cereus (Mean±SE) |
|---|---|---|---|---|---|---|
| 1 | S-Ciprofloxacin | (5µg/µL) | 10.50±0.02 | 09.80±0.09 | 10.03±0.03 | 12.16±0.05 |
| 2 | $Cu(Phen)_2Cl_2$ | 100µg/µL | 3.98±0.14 | 4.17±0.17 | 3.87±0.18 | 3.14±0.25 |
| | | 200µg/µL | 4.37±0.47 | 3.47±0.47 | 4.80±0.32 | 5.45±0.75 |
| | | 300µg/µL | 5.12±0.80 | 4.93±0.30 | 6.97±0.06 | 6.87±0.36 |
| 3 | $[Cu(Phen)_2(ATT)]Cl$ 1.5 $H_2O$ | 100µg/µL | 5.25±0.50 | 5.27±0.02 | 6.04±0.54 | 4.57±0.74 |
| | | 200µg/µL | 6.34±0.87 | 6.50±0.50 | 4.98±0.74 | 5.33±0.83 |
| | | 300µg/µL | 7.18±0.35 | 7.17±0.80 | 7.70±0.90 | 7.40±0.23 |
| 4 | $Ni(Phen)_2Cl_2$ | 100µg/µL | 2.40±0.11 | 2.40±0.17 | 1.50±0.28 | 2.87±0.16 |
| | | 200µg/µL | 2.67±0.09 | 3.67±0.17 | 2.34±0.15 | 1.93±0.26 |
| | | 300µg/µL | 1.98±0.06 | 5.33±0.17 | 3.50±0.63 | 1.40±0.32 |
| 5 | $[Ni(Phen)_2(ATT)]Cl$ | 100µg/µL | 2.42±0.12 | 2.07±0.07 | 1.40±0.74 | 1.80±0.81 |
| | | 200µg/µL | 1.80±0.27 | 2.13±0.31 | 1.91±0.78 | 1.71±0.12 |
| | | 300µg/µL | 1.15±0.14 | 3.25±0.98 | 1.73±0.94 | 1.83±0.62 |
| 6 | $Co(Phen)_2Cl_2$ | 100µg/µL | 1.30±0.15 | 1.20±0.18 | 1.53±0.30 | 1.83±0.01 |
| | | 200µg/µL | 2.83±0.17 | 1.93±0.13 | 1.87±0.87 | 2.01±0.34 |
| | | 300µg/µL | 1.50±0.29 | 2.01±0.15 | 1.69±0.23 | 1.57±0.36 |
| 7 | $[Co\ (Phen)_2(ATT)]Cl$ | 100µg/µL | 2.67±0.24 | 2.54±0.42 | 2.13±0.20 | 1.87±0.07 |
| | | 200µg/µL | 3.18±0.98 | 2.30±0.59 | 3.68±0.17 | 2.47±0.10 |
| | | 300µg/µL | 3.64 ±0.37 | 3.53 ±0.87 | 4.19±0.89 | 3.19±0.34 |

Values are the mean ± SE of inhibition zone in mm.

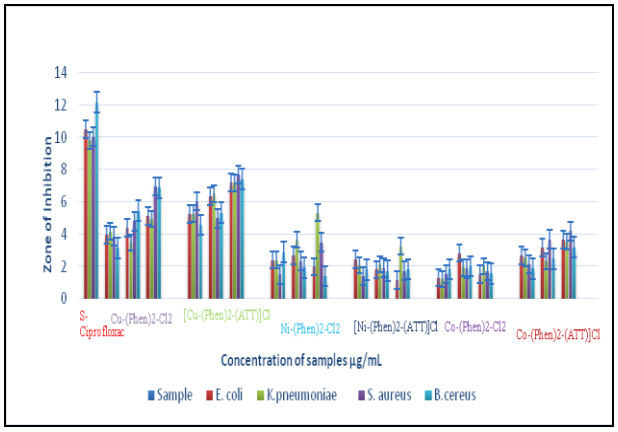



**Fig. 9: Graphical representation of Antibacterial activity of Metal Complexes against Pathogenic Bacterial Strains**

## 6. CONCLUSIONS

Mixed ligand transition metal complexes with 1,10-Phenonthroline (Phen) and 2-acetylthiophene thiosemicarbazone (ATT) are synthesized and characterized based on mass spectra, molar conductivity, infrared and electronic spectra. Electrochemical properties of these complexes are uncovered by using cyclic voltammetry. The complexes show quasi reversible cyclic voltammetric responses for the M(II)/M(I) couple. The binding properties of these complexes with calf-thymus DNA are 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 Anti bacterial activity of the present complexes are comparable with the activity of Ciprofloxacin.

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