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# Synthesis, Characterization, DNA Binding Studies & Biological Activities of Cobalt (III) Complexes Containing 1,10 Phenanthroline and Schiff Base N-Phenylsalicylaldimine

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**ABSTRACT**

The new cis-$[Co(Phen)_2(NPS)](ClO_4)_2$ . cobalt(II) complexes ( phen = 1,10-phenanthroline, NPS= N-phenylsalicylaldimine) have been synthesized and characterized by CHN analysis, molar conductance, electronic absorption, IR & NMR studies. They have been tested for their in vitro DNA binding activities by the spectroscopic methods such as UV-Visible, Emission, Cyclic volumetric and viscosity measurements. Further complex **1** was tested for their antimicrobial activities and it was found to have good antimicrobial activities.

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## INTRODUCTION

Several cobalt (III) complexes have been reported which bind DNA through intercalation and are effective nucleases. Ji and co-workers have shown that these complexes are avid intercalates of DNA. They also have investigated the effect of ligand containing N-N donor atom and hydrogen bonding ability on the DNA binding by cobalt (III) complexes[1-3]. Polypyridyl cobalt complexes have received considerable interest because of their good redox activity and strong affinity to biomolecules [4-6].

Schiff bases are an important class of ligands in coordination chemistry and are found to possess extensive applications in different fields, such as pharmacological field[7] which are able to inhibit the growth of several animal tumors[8]. Schiff bases are an important class of compounds in medicinal and pharmaceutical field. They show biological applications including antibacterial[9-14], antifungal[15-17] anti-viral agents[18,19], herbicidal activities[20] and antitumor activity[21,22]. In addition, Schiff bases are able to inhibit the growth of several animal tumors, and some metal chelates have shown good antitumor activities against animal tumors[23,24]. So, well-designed organic ligands enable a fine turning of special properties of the metal ions.

In this context, we focused our interest on the development of cobalt (III) complex of 1, 10-phenanthroline with Schiff's base, and investigated their DNA binding, antibacterial and antifungal activity. We report here the synthesis of cobalt (III) complex of 1, 10-phenanthroline, with Schiff's base, $[Co(phen)_2\ NPS](ClO_4)_2$ and characterization by IR, $^1$HNMR, molar conductance and elemental analysis. The binding properties of the complex to CT-DNA (calf thymus DNA) were carried out using UV–Visible absorption, fluorescence spectroscopic, cyclic Voltammetric and viscosity techniques. We have tested antimicrobial activities of this complex against Gram +ve and Gram - ve bacteria and fungi.

## EXPERIMENTAL

### MATERIALS

Cobaltous chloride hexahydrate and 1,10 phenanthroline were purchased from Merck, India. Calf thymus DNA, N phenyl salicylaldimine were obtained from Sigma–Aldrich, Germany, and were used as such. The spectroscopic titration was carried out in the buffer (50 mM NaCl–5 mM Tris–HCl, pH 7.1) at room temperature. A solution of calf thymus DNA in the

buffer gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free of protein[25]. Milli-Q water was used to prepare the solutions. The complex, *cis*-[Co (phen)$_2$Cl$_2$]Cl 3H$_2$O, was prepared as reported earlier[26]. An absorption spectral study was carried out by using UV–VIS–NIR Cary 300 spectrophotometer which is having cuvettes of 1 cm path length, and emission spectral study was carried out by using JASCO FP 770 spectrofluorimeter.

**Synthesis of cis-[Co(phen)$_2$Cl$_2$] Cl 3H$_2$O[25]**

4.76g of Cobaltous chloride (CoCl$_2$.6H$_2$O) was dissolved in 12 mL of water and 7.92g of 1, 10 phenonthroline was added and heated in a round bottomed flask until solution becomes partly then it was cooled rapidly with constant stirring to yield fine pink crystal of [Co(phen)$_2$Cl$_2$]Cl. 3H$_2$O. With frequent stacking, chlorine gas was passed and it was gradually converted into dirty violet paste within 60-90 min. The product was separated, washed several times with 2M HCl and dried in air yields 8.4g. The cherry red solution heated with 25 ml of Con. HCl on the water bath. Dark violet crystal with greyish tinge in the form of prism gradually separated, more than 80% of the product was recrystallised.

**Synthesis of cis-[Co(phen)$_2$NPS] (ClO$_4$)$_2$ (1)**

About 0.98 g of N-phenyl salicyl aldimine dissolved in 15 mL of ethanol was added a solution of 2.53 g of cis-[Co(phen)$_2$Cl$_2$]Cl 3H$_2$O in 15 mL of water. The mixture was heated at 50 - 60˚C for 3 hours. The solution changed from light brown to reddish brown. The mixture was cooled and thick crystalline precipitate of [Co(phen)$_2$NPS]$^{2+}$ (ClO$_4$)$_2$ was collected and recrystallized using distilled water. The yield is about 80%.

## RESULTS AND DISCUSSION

### General Aspects

The elemental analyses data were found to be in good agreement, with those of the calculated values. The $\Lambda_M$ value of the complex in water is 123 Ohm$^{-1}$ cm$^2$ mol$^{-1}$, which indicated that the complex is 1:2 electrolytes[27]. The values are given in table **1**. The synthetic strategy of the complex is outlined in Scheme **1**.

## UV Visible spectra

Electronic absorption spectra was initially employed to study the binding of cobalt(III) complex with CT-DNA. In the UV region, the complex presented (Fig. 1) bands at 225 and 267 nm which can be attributed to π→π* transition of the coordinated phenanthroline ligand. The UV-Visible spectal data was given in table 2.

**Table 1: The elemental analyses and molar conductivity of the complex 1**

| Complex Name | Carbon | Nitrogen | Hydrogen | Molar Conductance ($Sm^2mol^{-1}$) |
|---|---|---|---|---|
| $[Co(Phen)_2NPS](ClO_4)_2$ | 54.46 (54.56) | 8.39 (8.60) | 3.10 (3.22) | 123 |

**Table 2: UV-Visible spectral data of complex 1**

| Complex Name | $\lambda_{max(nm)}$ | $\varepsilon_{max}$ ($mol^{-1}$ $cm^{-1}$) |
|---|---|---|
| $[Co(Phen)_2NPS](ClO_4)_2$ | 225<br>267 | 139686<br>112464 |

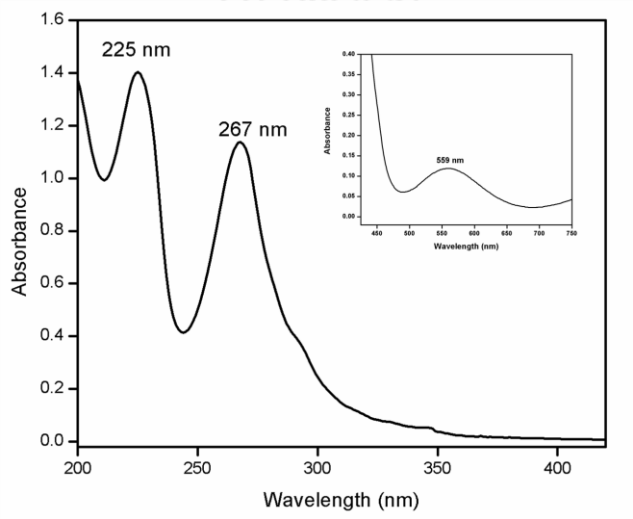
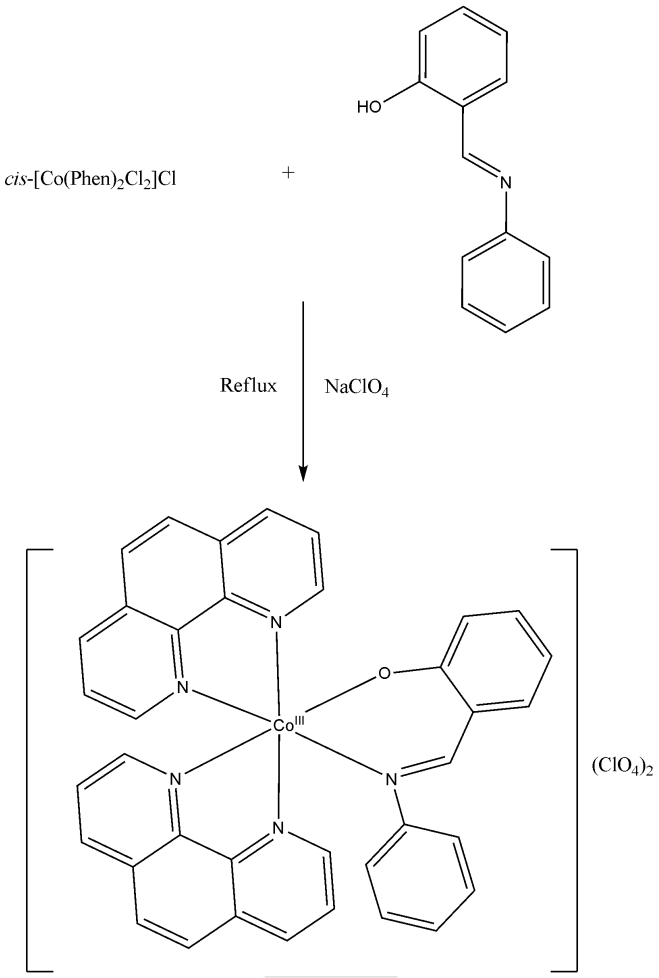



**Figure 1: UV Visible spectra of $[Co(Phen)_2NPS](ClO_4)_2$**




**Scheme 1: Synthesis of $[Co(Phen)_2NPS](ClO_4)_2$ (1)**

## IR spectra

In the IR region, the bands around 1607 $cm^{-1}$ and 1524 $cm^{-1}$ can be attributed to the ring stretching frequencies (ν (C=C) and ν (C=N)) of 1,10-phenanthroline[28]. The IR values, ν(C–H) 751$cm^{-1}$ and 718 $cm^{-1}$ observed for phenanthroline are shifted to 836 $cm^{-1}$ and 716$cm^{-1}$. These shifts can be explained by the fact that each of the two nitrogen atoms of phenanthroline ligands donates a pair of electrons to the central cobalt metal forming a coordinate covalent bond[29]. The coordination of the nitrogen and oxygen atoms is confirmed with the presence of new bands at ca. 622 $cm^{-1}$, assignable to ν (Co-O)[30,31]. Furthermore, the appearance of two new bands around 1078 $cm^{-1}$ is indicative of uncoordinated perchlorate ions.

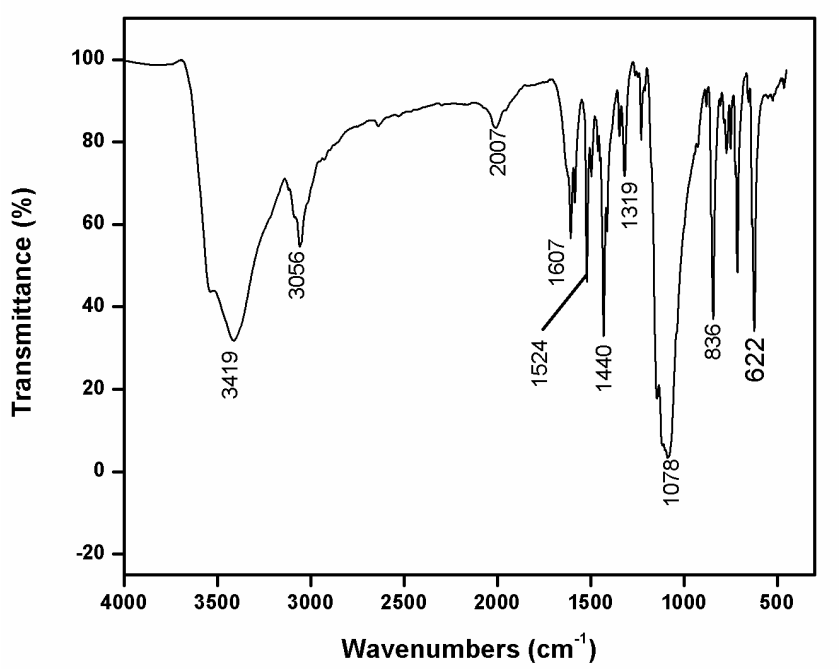



**Figure 2: IR spectra of $[Co(Phen)_2NPS](ClO_4)_2$**

**NMR spectra**

The electronic environment of many aromatic hydrogen atoms is similar and hence their $^1H$ NMR signals appear in a narrow chemical shift range. In fact the aromatic regions of the spectra of this complex complicated due to the overlapping of several signals, which have precluded the identification of individual resonance. However, from the direct comparison of the intensity of the aromatic protons with that of the observable azomethine proton (–CH=N–) in the downfield [d (–CH=N–), δ 9.9], the number of aromatic protons expected for these complex was confirmed. The singlet due to the azomethine proton in the complexes is considerably deshielded (δ > 9 ppm) relative to that of the free ligands, (δ 8.5 ppm), as a consequence of electron donation to the metal due to the coordination of the azomethine nitrogen[32].

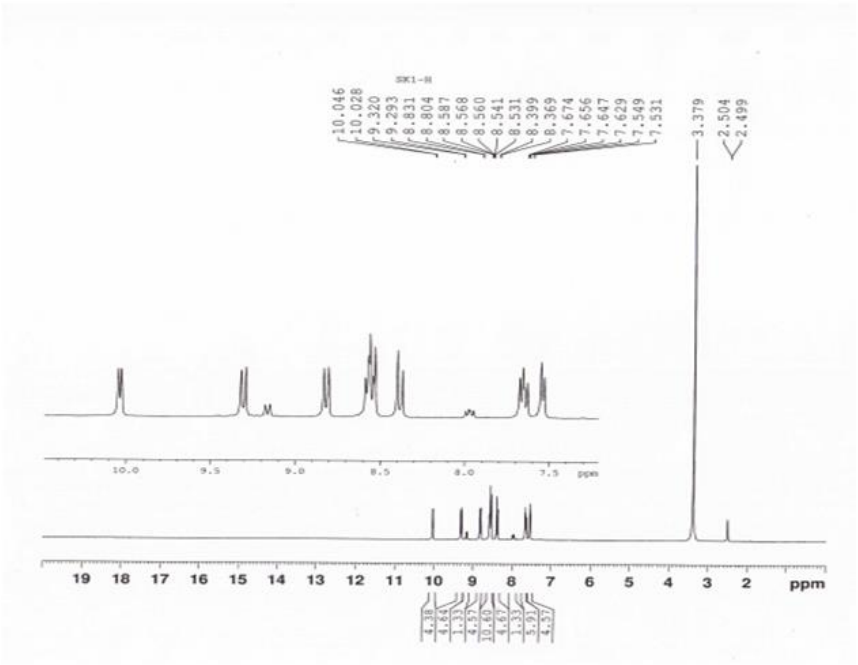



**Figure 3: NMR spectra of $[Co(Phen)_2NPS](ClO_4)_2$**

**DNA Binding Studies**

**Electronic Absorption Spectral Study**

Electronic absorption spectroscopy is often employed to ascertain the binding of the complexes with DNA. A complex bound to DNA through intercalation is characteristic of hypochromism and redshift, due to the strong stacking interaction between aromatic chromospheres of the complex and the base pairs of DNA. The absorption spectra of the cobalt(III) complex in the absence and presence of calf thymus DNA are shown in Fig. 4 with increasing concentration of calf thymus DNA, the absorption bands of the complex are affected, resulting in the obvious tendency of hypochromism and a redshift. An electrostatic interaction between the DNA and cobalt(III) complex could be observed through the hypochromism and redshift.

The cobalt(III) complexes can bind to the DNA in different binding modes on the basis of their structure and charge and type of ligands. Intercalation to DNA may be one of the binding patterns since the complex contains phenanthroline ligand, which should provide an aromatic moiety extending from the metal center through which overlapping occurs with base pairs of DNA by intercalation. However, the hypochromism effects observed in the present

study suggest that there is a strong intercalation between phenanthroline and an interior base pair of DNA.

The intrinsic binding constants $K_b$ of the two complexes with CT-DNA were determined according to the following equation (Olmsted et al., 1977)[33]:

$(DNA)/(\varepsilon_a-\varepsilon_f) = (DNA)/(\varepsilon_o-\varepsilon_f) + 1/Kb(\varepsilon_o-\varepsilon_f)$

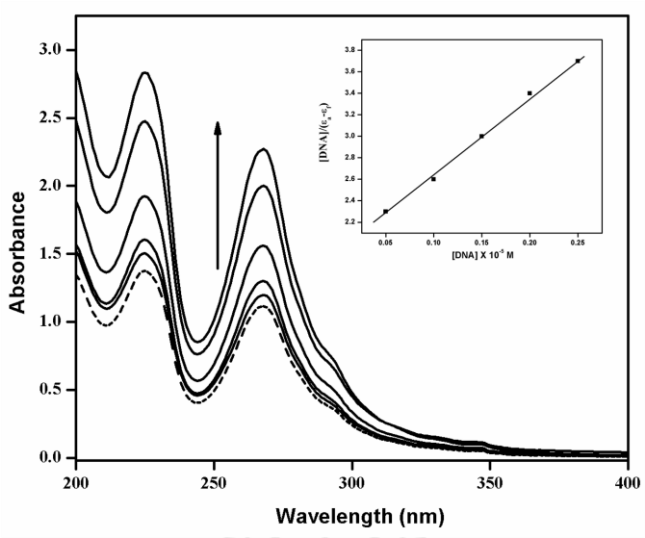



**Figure 4: Electronic Absorption spectra of $[Co(Phen)_2NPS](ClO_4)_2$. in the absence and in the presence of increasing amounts of DNA concentrations. [Complex] = 15 µM. [DNA] = (5,10,15,20,25) µM. The Arrow shows the absorbance changes upon increasing DNA concentrations.**

where [DNA] is the DNA concentration in the base pairs, the apparent absorption coefficients $\varepsilon_a$, $\varepsilon_f$ and εb correspond to [A]obsd/[Co], the extinction coefficient for the free cobalt complex and the extinction coefficient for the free cobalt complex in the fully bound form, respectively. In plots [DNA]/( εa - εf) versus [DNA], $K_b$ is given by the ratio of slope to the y-intercept. Intrinsic binding constants $K_b$ of 7.2 X $10^{-7}$ $M^{-1}$) 1 are determined for $[Co(phen)NPS]^{2+}$ using the absorption at 296 nm. This is expected since the phenanthroline possesses, the more extended planar system. These spectral characteristics also suggest a mode of binding that involves a stacking interaction between the aromatic chromophore and the DNA base pairs.

**Fluorescence studies**

No luminescence was observed for the cobalt(III) complexes at room temperature in aqueous solution or in any organic solvent examined or in the presence of calf thymus DNA. Therefore, the binding of cobalt(III) complexes and DNA can not be directly found out by the emission spectra. In such situations ethidium bromide (EB) was used, which fluorescences intensely in the presence of DNA, due to its strong intercalation between the adjacent DNA base pairs (Olmsted *et al.*, 1977)[34]. It is well known that fluorescence can be quenched by the addition of a second molecule (Baguley et al., 1984)[34] and the quenching extent of fluorescence of EB binding to DNA be used to determine the extent of binding between the second molecule and DNA. In our study, the addition of the solutions of cobalt (III) complexes to DNA solution pretreated with EB causes appreciable reduction in the emission intensity (Fig 2). These reduction in intensities are treated by the classical Stern–Volmer equation (Lakowiczet al., 1973)[35],

$$Io/I = 1 + Ksv\ r$$

Where $I_0$ and I are the fluorescence intensities in the absence and the presence of the complex, respectively. Ksv is a linear Stern–Volmer constant and r is the ratio of the total concentration of complex to that of DNA. These quenching plots (Fig. 5) illustrate that the quenching of EB bound to DNA by the cobalt(III) complexes was in good agreement with the linear Stern –Volmer equation, which also indicates that the complexes bind to DNA.

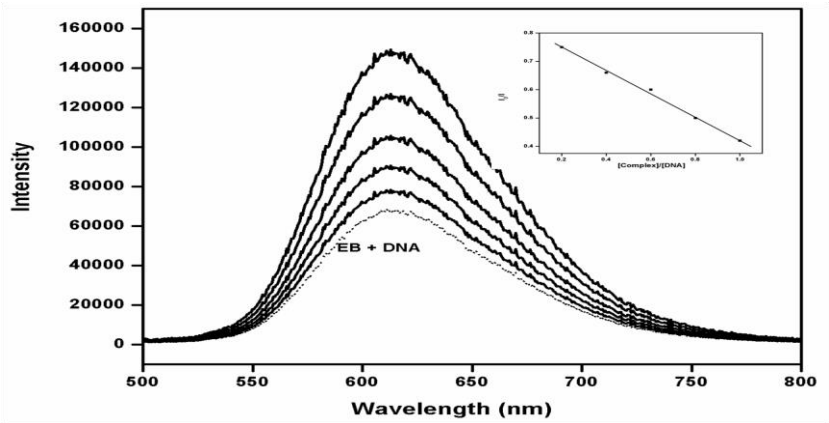



**Figure 5: Emission spectra of EB bound to DNA in the absence (a) and in the presence of $[Co(Phen)_2NPS](ClO_4)_2$. [complex] = 8,16,24,32,40 x $10^{-6}$ M. [DNA] = 3 x $10^{-5}$ M, [EB] = 3 x $10^{-5}$ M . The Arrow shows the intensity changing upon increasing complex concentrations.**

The Ksv value for the cobalt complex is 0.41. The data suggest that the $[Co(phen)_2NPS]^{2+}$ binds strongly with DNA, which is consistent with the absorption spectral results. It is generally agreed that strong fluorescence enhancement accompanies due to a gradual release of the free EB out of the EB-DNA complex. This must be due to the perturbation of DNA organization leading to dissociation of the EB in the EB-DNA system[36].

**Viscosity studies**

Mode of interaction between the metal complexes and DNA was clarified by viscosity measurements. Hydrodynamic measurements are sensitive to the length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and the most critical test of binding in solution. A classical intercalation mode demands that the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity. In contrast, a partial non-classical intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length and concomitantly its viscosity[37].

The effect complex on the viscosity of DNA is shown in Fig. 6. The viscosity of DNA is increased with the increase of the concentration of the complex. Based on the viscosity results, it was observed that complex bind with DNA through intercalation mode[38].

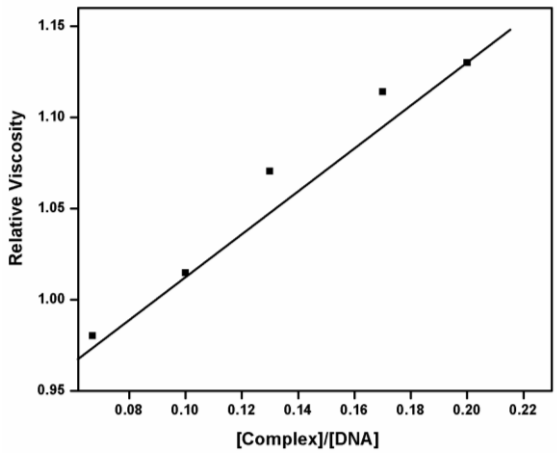



**Figure 6: Effect of increasing amount of $[Co(Phen)_2NPS](ClO_4)_2$ ( 1,15,20,25,30 µM) on the relative viscosity of calf thymus DNA (15 µM) in 5mM Tris-HCl/50mM NaCl buffer.**

**Cyclic Voltammetric Study**

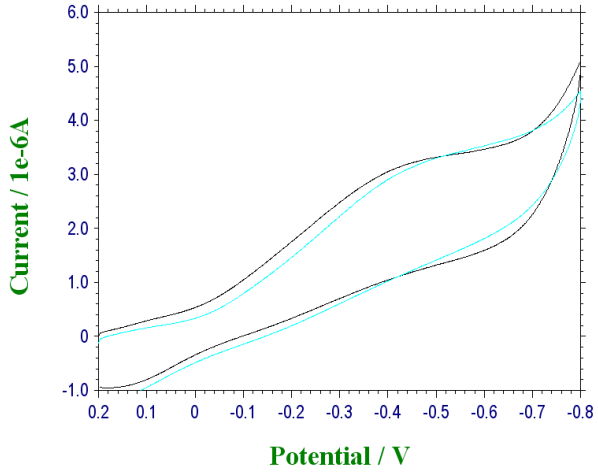



**Figure 7: Cyclic voltammogram of $[Co(Phen)_2NPS](ClO_4)_2$ (1 mM) complex in the absence (—) and in the presence (---) of CT-DNA (1.5 x $10^{-5}$ M). 5 mM in buffer containing 50 mM NaCl–5 mM Tris–HCl, pH 7.2. Scan rate: 100 mV $s^{-1}$**

Cyclic Voltammetric (CV) response for complex **1** in Tris-HCl buffer (pH 7.28) in the presence and absence of CT DNA is shown in Fig.7 When CT-DNA is added to a solution of complexes, marked decrease in the peak current and potential values were observed. The cyclic voltammetric behavior were not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complexes after the addition of DNA due to the binding of complex **1** to the DNA[39]. When the concentration of DNA increased, the changes in peak current and potential become slow. This reveals that the complexes were interact with CT-DNA.

**Antibacterial and antifungal screening**

The cobalt(III) complex was screened in vitro for their microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. This complex concentration of 0.5mm was found to exhibit considerable activity against Gram-positive (*Staphylococcus aureus* and *Bacillus Cereus*) and Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and the pathogenic yeast *Candida albicans*. The test solutions were prepared in dimethyl sulphoxide (1%) and the results of the antimicrobial activities are summarized in Table 3. The cobalt (III) complex showed significant microbial activity against Gram-positive, Gram negative bacteria and fungus. In our biological experiments, using cobalt (III) complex, we have observed high antibacterial activity against Gram-

positive bacteria (Staphylococcus aureus and Bacillus subtilis) than Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The cobalt (III) complexes are also very active against the yeast *Candida albicans*.

**Table 3: Biological activity of complex 1**

| Name of the Organism | Diameter Zone of Inhibition (mm) Ciproflaxacin/ Amphotericin-B |
|---|---|
| | Complex 1 |
| ***A. flavus*** | - |
| ***A. niger*** | 24 |
| ***B. cereus*** | 18 |
| ***C. albicans*** | 28 |
| ***E. coli*** | 09 |
| ***K. pneumoniae*** | 21 |
| ***M. luteus*** | 09 |
| ***P. aeruginosa*** | 08 |
| ***S.aureus*** | 11 |

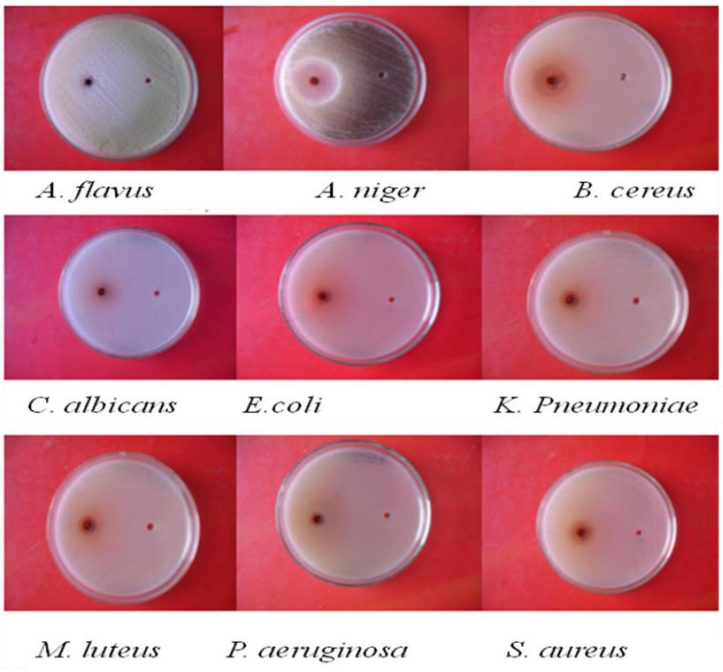



**Figure 8: Antimicrobial screening of complex 1**

The activity of cobalt (III) complex may be due to an efficient diffusion of the metal complexes into the bacterial/fungal cells and/or interaction with the bacterial/fungal cells (Chohan et al., 2006)[40-44]. The antimicrobial activity of this cobalt (III) complex was so compared with standard drugs Ciprofloxacin (for bacteria) and Fluconazole (for fungi). Cobalt (III) complex possessed very good activity against all the microorganisms may be due to higher hydrophobic character of the complex which can damage the bacterial/fungal cellular membrane/wall. It may be concluded that our cobalt (III) complex inhibit the growth of bacteria and fungi.

## CONCLUSION

We described here new cobalt(III) complex. Further characterization of the complex was achieved through Physico-chemical and spectroscopic methods. The effectiveness of binding of the complex is being confirmed by means of hypochromism in the electronic spectral studies and decrease in intensity of emission in the case of emission spectral studies. Besides that, the effectiveness of binding is also confirmed by the viscometric and cyclic voltammetric studies. This shows that the complex is intercalative binding mode with DNA base pairs effectively. The complex $[Co(phen)_2NPS]$ $(ClO_4)_2$ exhibit good antimicrobial activity.

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