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**Research Article**



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**Synthesis, Characterization, DNA Binding studies and  
Biological activities of Cobalt (III) Complexes containing  
1,10 Phenanthroline and 8- Hydroxy Quinoline &  
Orthophenyldiamine**

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**Abstract**

The new *cis*-[Co(Phen)<sub>2</sub>(8HQ)](ClO<sub>4</sub>)<sub>2</sub> & *cis*-[Co(Phen)<sub>2</sub>(OPD)](ClO<sub>4</sub>)<sub>3</sub>. cobalt(II) complexes (phen = 1,10-phenanthroline, HQ= 8-Hydroxy quinoline & OPD= orthophenyldiamine) 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 complexes **1** & **2** were tested for their antimicrobial activities and it was found to have good antimicrobial activities.

**Keywords:** Cobalt (III) Complexes, Orthophenyldiamine, DNA Binding & anti-microbial activity.

**1.1 Introduction**

Transition metal polypyridyl complexes have been used extensively as probes of DNA structure, for DNA dependent electron transfer, and for site specific cleavage of nucleic acids with the aim of developing novel therapeutic and diagnostic agents<sup>1</sup>. These complexes can bind to DNA in non-covalent modes such as electrostatic, intercalative and groove binding. The above applications require that the complex can bind to DNA through an intercalative mode wherein the planar aromatic heterocyclic group inserts and stacks between the base pairs of DNA. The majority of studies to date have been carried out on ruthenium-polypyridyl complexes due to large perturbations of their strong visible absorbance and luminescence characteristics on binding to DNA<sup>2-5</sup>. However, complexes of other metal ions such as cobalt(III), which have similar characteristics.. Liang-Nian Ji et al.<sup>6-10</sup> have shown that [Co(L)<sub>2</sub>(L)]<sup>3+</sup> (L = 1,10-phenanthroline or 2,2'-bipyridine; L' = 2-(2-chloro-5-

nitrophenyl) imidazo-[4,5-f] 1, 10-phenanthroline (CNOIP), 2-phenylimidazo-[4,5-f]-1, 10-phenanthroline (PIP) or 2-(2-hydroxy-5-nitrophenyl)imidazo-[4,5-f] 1,10-phenanthroline (HNOIP) bind to DNA through an intercalative mode

Cobalt is an essential trace element in human, exhibiting many useful biological functions. Numerous compounds, naturally occurring and man-made, contain the cobalt at two common oxidation states Co<sup>(II)</sup> and Co<sup>(III)</sup>. There is growing interest in investigating the cobalt and other transition metal complexes for their interaction with DNA<sup>11-15</sup>. Moreover, cobalt complexes appear to be very promising candidates for anticancer therapy; an idea supported by a considerable number of research articles describing the synthesis and cytotoxic activities of numerous cobalt complexes<sup>16-23</sup>.

Cancer is undoubtedly one of the main health concerns facing our society and one of the primary targets regarding medicinal chemistry. Even though platinum-based complexes had been in the primary focus of research on chemotherapy agents<sup>24-26</sup>, the interests in this field have shifted to non-platinum based agents<sup>27-28</sup>, in order to find different metal complexes with less side effects and similar, or better, cytotoxicity. Thus, a wide variety of metal complexes based on titanium, gallium, germanium, palladium, gold, copper, nickel, ruthenium and tin are being intensively studied as platinum replacements<sup>29, 30</sup>.

## 1.2 Experimental Section

### 1.2.1. Materials

Cobaltous chloride hexahydrate and 1, 10 phenanthroline were purchased from Merck, India. Calf thymus DNA, N phenyl salicylalimine 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<sup>27</sup>. Milli-Q water was used to prepare the solutions. The complex, cis-[Co(phen)<sub>2</sub>Cl<sub>2</sub>]Cl 3H<sub>2</sub>O, was prepared as reported earlier<sup>28</sup>. 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.

### 1.2.2 Synthesis of [Co(phen)<sub>2</sub>Cl<sub>2</sub>]Cl<sup>38</sup>

4.76g of Cobaltous chloride (CoCl<sub>2</sub>.6H<sub>2</sub>O) was dissolved in 12 mL of water and 7.92g of 1, 10 phenanthroline was added and heated in a round

bottomed flask until solution become partly then it was cooled rapidly with constant stirring to yield fine pink crystal of [Co(phen)<sub>2</sub>Cl<sub>2</sub>]Cl. 3H<sub>2</sub>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.

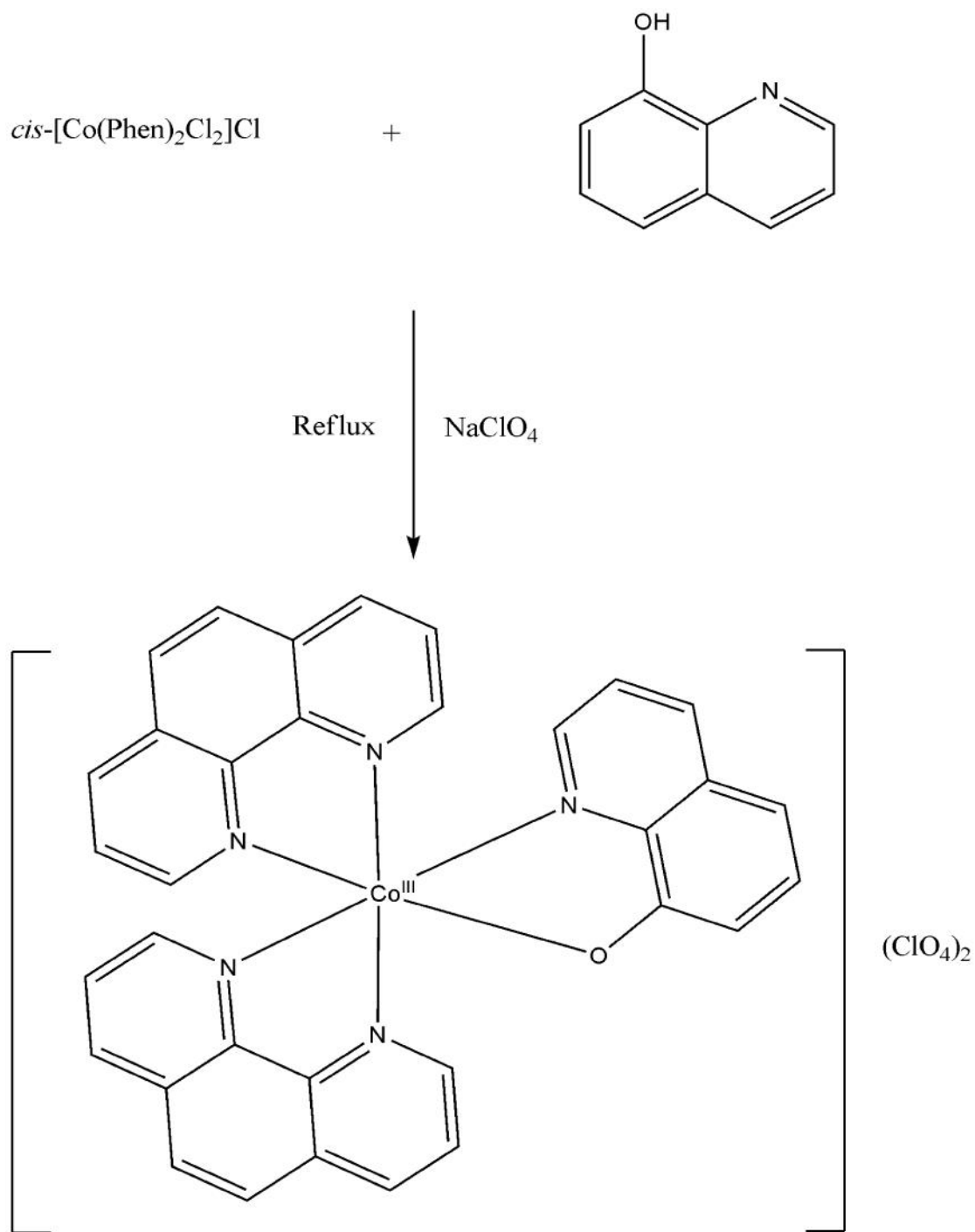
### 1.2.3 Synthesis of cis-[Co(Phen)<sub>2</sub>(8HQ)](ClO<sub>4</sub>)<sub>2</sub> (1)

2.9 g of [Co(phen)<sub>2</sub>Cl<sub>2</sub>]Cl complex was dissolved in equal ratio of 1:1mmol of (25ml) ethanol and water with constant stirring and added 0.72 g of 8- Hydroxy Quinoline. The mixture was refluxed for 5 hours. On cooling the solution to ambient temperature, an aqueous solution of sodium per chlorate (12.244 g) was added and the mixture was refluxed for 30 minutes until the formation of brown precipitate which was filtered and washed with ethanol. Yield: ~64%.

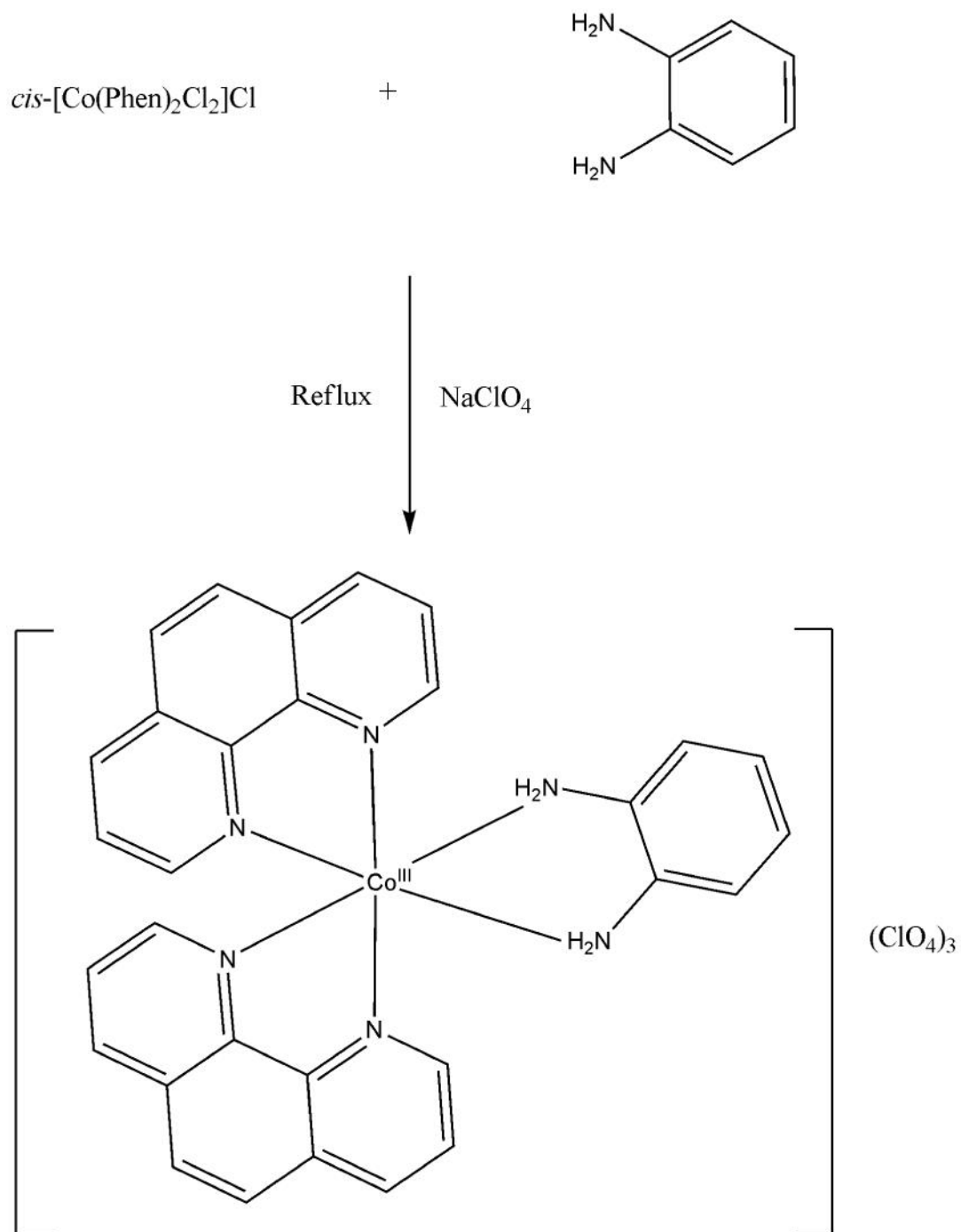
**Caution!** Although no problems were encountered in this work, per chlorate salts of transition metal complexes with organic ligands are potential explosives. Only a small amount of material should be prepared and handled with caution.

### 1.2.4 Synthesis of cis-[Co(Phen)<sub>2</sub>(OPD)](ClO<sub>4</sub>)<sub>3</sub> (2)

2.9 g of [Co(phen)<sub>2</sub>Cl<sub>2</sub>]Cl complex was dissolved in equal ratio of 1:1mmol of (25ml) ethanol and water with constant stirring and added 0.72 g of Orthophenyldiamine. The mixture was refluxed for 5 hours. On cooling the solution to ambient temperature, an aqueous solution of sodium per chlorate (12.244 g) was added and the mixture was refluxed for 30 minutes until the formation of brown precipitate which was filtered and washed with ethanol. Yield: ~67%.



**Scheme 2:** Synthesis of complex 1



Scheme 3: Synthesis of complex 2

### 1.3 Results and discussion

#### 1.3.1 General Aspects

These complexes are stable in solid state and soluble in water and common organic solvents. The elemental analysis data of the cobalt (III) complexes (Table 1.1)

agree with the theoretical values. The synthetic strategy of the complexes is outlined in Scheme 2.

The molar conductance values revealed<sup>29, 30</sup> that, the cobalt (III) complexes (2-3) behave as 1:3 electrolytes in aqueous medium.

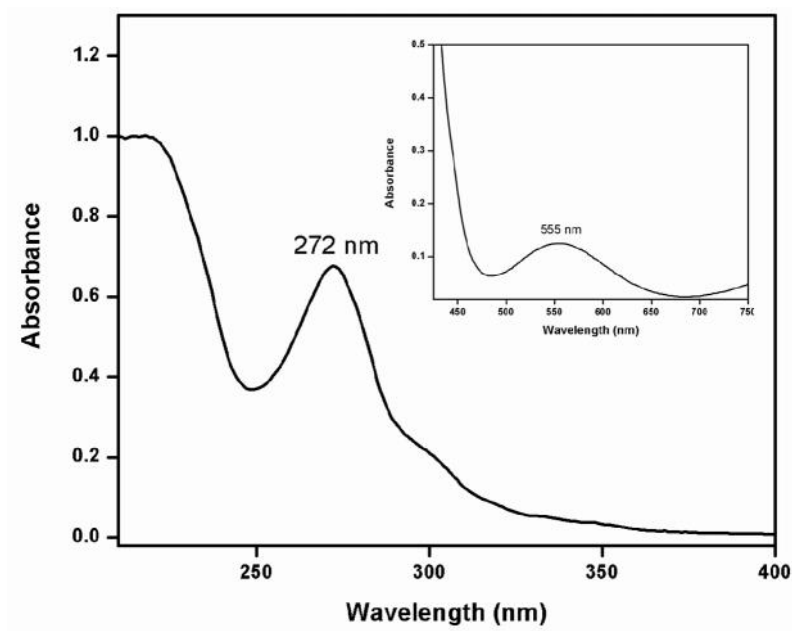
**Table 1.1:** Elemental analysis and molar conductance of the complexes **1** and **2**

Complexes	Calcd (Found)			Molar Conductance (Sm <sup>2</sup> mol <sup>-1</sup> )
	Carbon	Hydrogen	Nitrogen	
Complex 1	52.53 (52.34)	3.24 (3.14)	9.01 (8.86)	396
Complex 2	43.63 (43.46)	2.93 (2.74)	10.18 (10.01)	379

#### 1.3.2 Electronic Spectra

In the UV region, the complex presented (Fig 1.1) bands around 270 nm which can be attributed to \*

transition of the coordinated phenanthroline ligand<sup>31</sup>, and the complexes **1** and **2** exhibit d-d band at The UV-Visible spectral data was given in table 1.2



**Fig 1.1:** UV Visible spectra of complex **1**

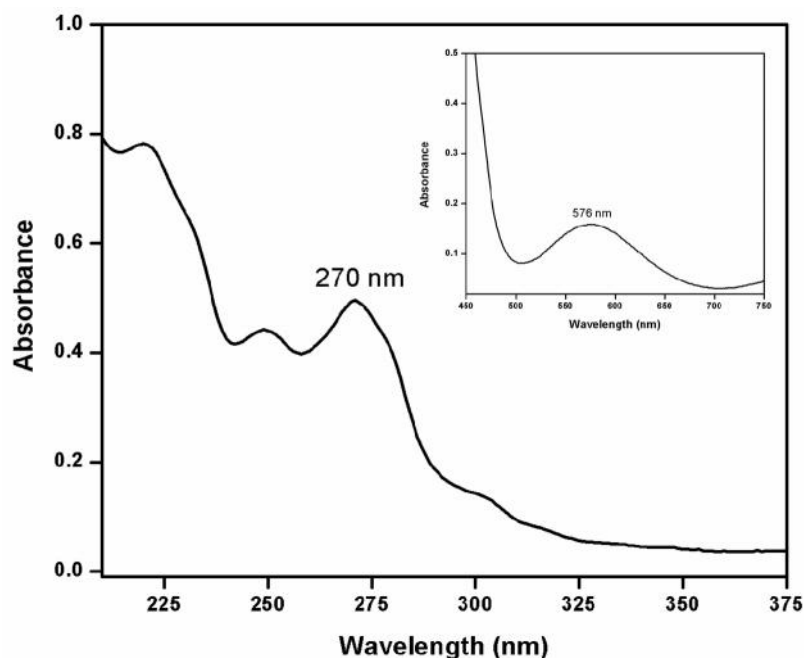


Fig 1.2: UV Visible spectra of complex 2

Table 1.2: UV-Visible spectral data of complex 1 and 2

Complex Name	max(nm)	max (mol <sup>-1</sup> cm <sup>-1</sup> )
Complex 1	272	67809
	555	125
	578	
Complex 2	249	44230
	270	49255
	576	160

### 1.3.3 Infrared spectra

Infrared spectrum of the complexes 1 and 2 was shown in Fig 1.2 and 1.3. The characteristic out-of-plane hydrogen bending modes of free phen observed at 853, 738 cm<sup>-1</sup>, are red shifted to 851 and 723 cm<sup>-1</sup> (1), 833 and 714 cm<sup>-1</sup> (2) upon metal complexation. This shift can be explained on the basis of the fact that the nitrogen atoms of phenanthroline ligand donate a pair of electrons each to the central cobalt metal, forming a coordinate covalent bond. Besides, it is also confirmed by the shift of (C–N) of phenanthroline from about 1670 cm<sup>-1</sup> in the free ligand to 1621cm<sup>-1</sup>(1) and 1594cm<sup>-1</sup>(2) after coordination<sup>31</sup>. For complex 1 a very strong band at 1080 cm<sup>-1</sup> have been assigned to (Cl–O) of per chlorate anions. Per chlorate bands at 622 cm<sup>-1</sup> belong to an ionic species; this means that this counter-ion is not involved in the cobalt–ligand coordination<sup>32</sup>.

### 1.3.4 NMR spectra

The electronic environment of many aromatic hydrogen atoms is similar and hence their <sup>1</sup>H 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–), 8.9], the number of aromatic protons expected for these complex was confirmed. The singlet due to the azomethine proton in the complexes is considerably deshielded (> 9 ppm) relative to that of the free ligands, (8.5 ppm) (Fig. 1.2, Supplementary material) as a consequence of electron donation to the metal due to the coordination of the azomethine nitrogen<sup>33,34</sup>.

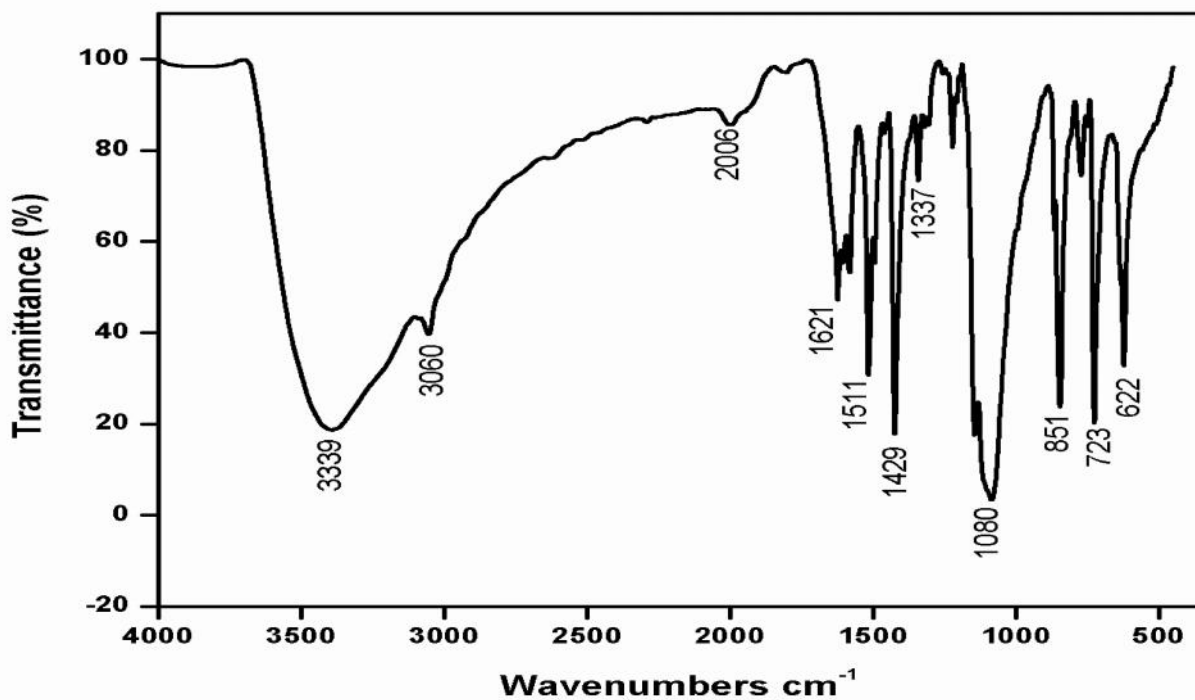


Fig 1.3: IR spectra of complex 1

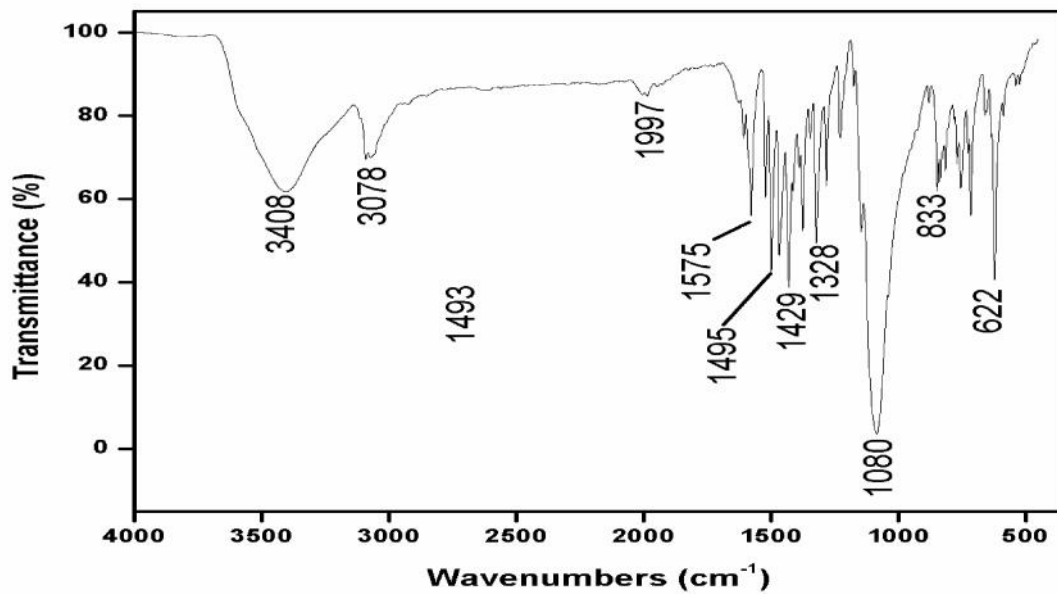


Fig 1.4: IR spectra of complex 2

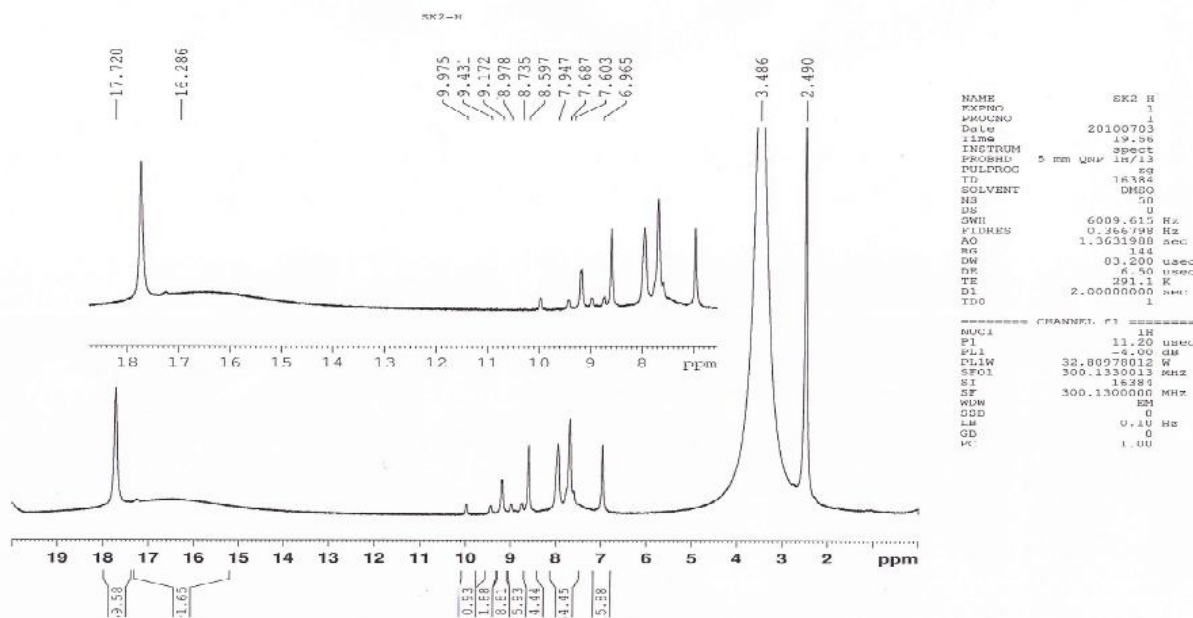


Fig 1.5: NMR spectra of complex 1

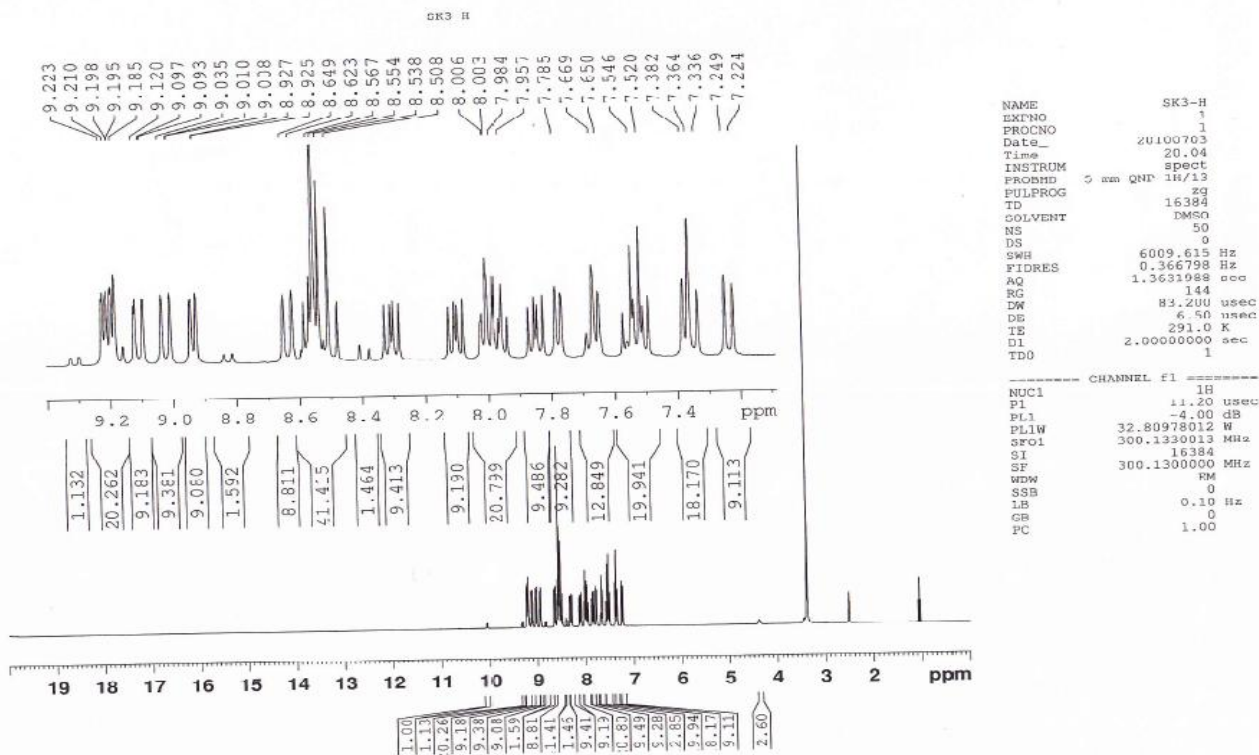


Fig 1.6: NMR spectra of complex 2



## 1.4 DNA binding studies

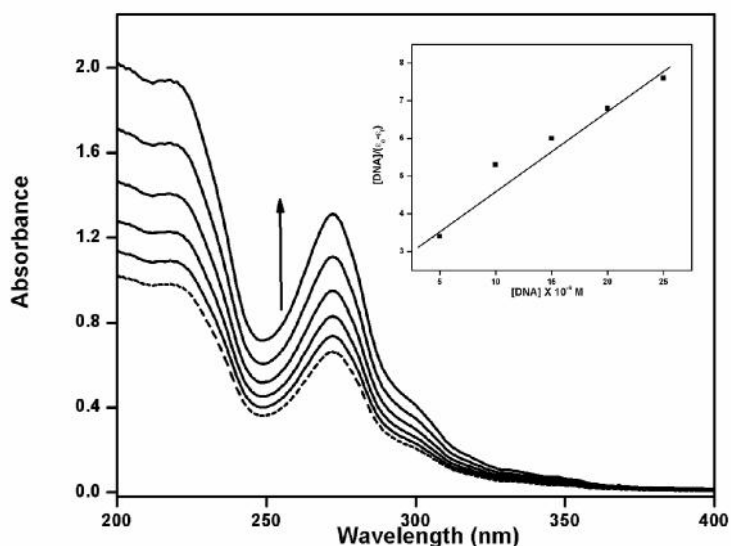
### 1.4.1 UV-Vis absorption spectra

Electronic absorption spectra were initially employed to study the binding of cobalt(III) complexes with CT-DNA. The absorption spectra in aqueous buffer media of cobalt(III) complexes (**1** and **2**) in the absence and in the presence of CT-DNA are given in Figure 1.5 and 1.6. As the concentration of DNA increased, the absorption band of cobalt(III) complex at 270 nm exhibits hyperchromism and blue shift. A similar hyperchromism has been observed for the Soret bands of certain porphyrins when interacted with DNA, but have not yet been clearly explained<sup>34, 35</sup>. The cobalt(III) complexes can bind to the double stranded DNA in different binding modes on the basis of their structure and charge and type of ligands. The cobalt(III) complexes containing phenanthroline ligands can bind to DNA by intercalation mode between these phen and thymine groups. The hyperchromism effect may be due to intercalation mode between positively charged complex and negatively charged phosphate backbone at the periphery of the double helix CT DNA<sup>17</sup>. Structurally, intercalation to DNA may be one of the binding patterns, since the cobalt(III) complexes contain phen ligands which

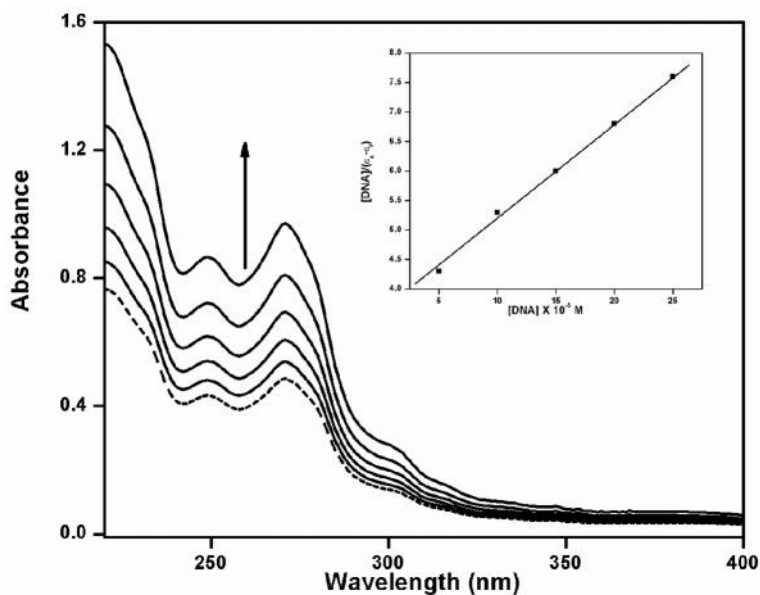
should provide aromatic moiety extending from the center through which overlapping occurs in base pairs of DNA by an electrostatic binding mode<sup>35</sup>. The intrinsic binding constant ( $K_b$ ) was determined from the following equation:

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

The apparent extinction coefficient ( $\epsilon_a$ ) was obtained by calculating  $A_{obsd}/[Co]$ . The terms  $\epsilon_f$  and  $\epsilon_a$  correspond to the extinction coefficients of free (unbound) and fully bound complex, respectively. A plot of  $[DNA]/(\epsilon_a - \epsilon_f)$  Vs  $K_b$  is the ratio of the slope and intercept. The intrinsic binding constant ( $K_b$ ) for the association of the complexes with CT DNA (inset of Fig. 1.7 and 1.8) was calculated as 1.5 and 1.62 respectively. The  $K_b$  value is classically higher than earlier reported. This indicates that complexes have an effective binding affinity with DNA<sup>47</sup>.



**Fig 1.7:** Electronic spectra of  $5.0 \times 10^{-5} M$  complexes **1** in the absence (---) and presence (—) of increasing amount of CT DNA at the ratio  $r = 0.3, 0.5, 0.7, 1.0$ . Arrow ( ) shows the absorbance changes upon increasing DNA concentration. Inset: linear plot for the calculation of the intrinsic DNA binding constant,  $K_b$ .

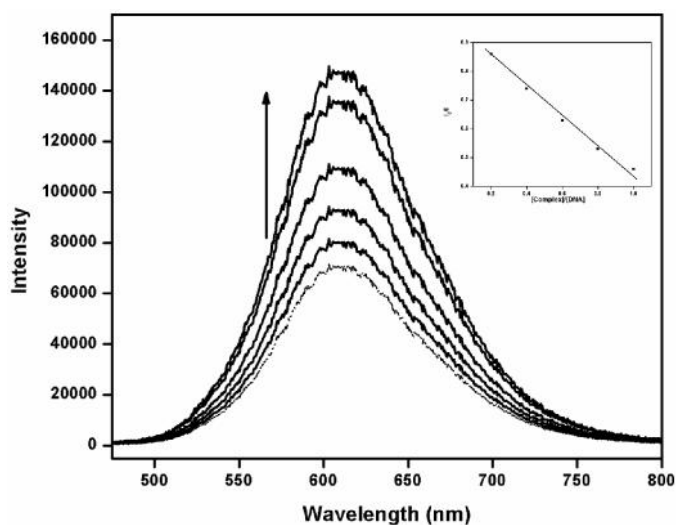


**Fig 1.8:** Electronic spectra of  $5.0 \times 10^{-5} \text{M}$  complexes **2** in the absence (---) and presence (—) of increasing amount of CT DNA at the ratio  $r = 0.3, 0.5, 0.7, 1.0$ . Arrow ( ) shows the absorbance changes upon increasing DNA concentration. Inset: linear plot for the calculation of the intrinsic DNA binding constant,  $K_b$ .

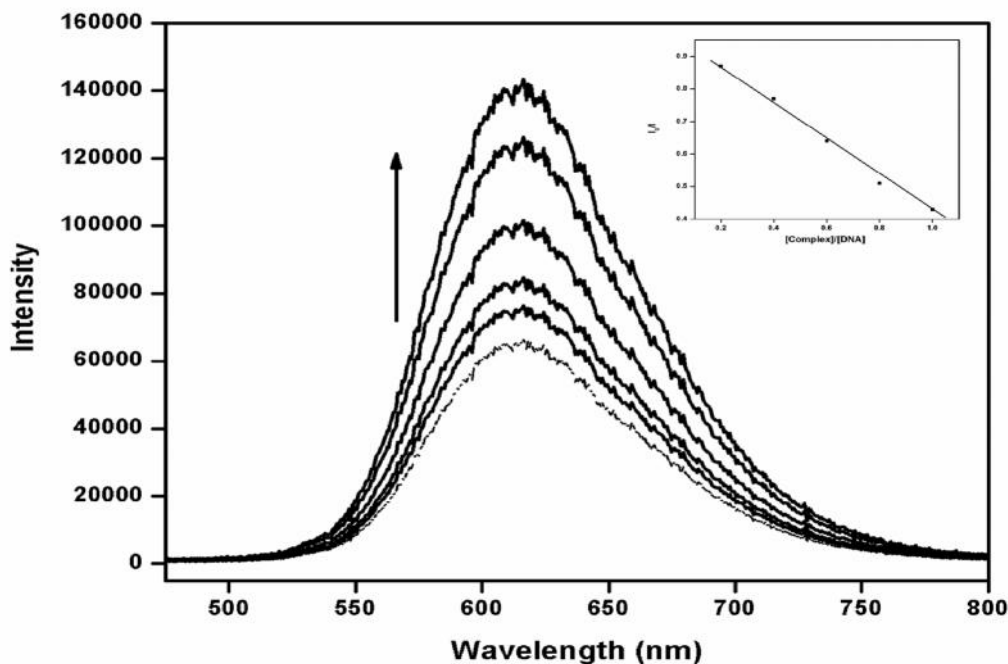
#### 1.4.2 Fluorescence spectra.

Competitive binding studies using DNA with bound ethidium bromide (EtBr) was carried out for the complex **1** and **2**. The extent of fluorescence quenching of ethidium bromide (EB) by competitive displacement from DNA is a measure of the strength of interaction between the second molecule and DNA. The results in (Fig. 1.9) showed that the fluorescence intensity of CT-DNA-EB decreased remarkably with the addition of cobalt complexes, which indicated that the complex can bind to DNA and replace EB from the CT-DNA-EB system. The above data was analyzed by

means of the Stern-Volmer equation<sup>47</sup>. The quenching plots (inset of Figure 1.9) illustrates that the fluorescence quenching of EB bound to DNA by the complex is in linear agreement with the Stern-Volmer relationship, which corroborates that the complex bind to DNA<sup>36</sup>. In the plot  $I_0/I$  vs  $[\text{complex}]/[\text{DNA}]$ ,  $K_{sq}$  value for the complexes are 0.5 (**1**) and 0.57 (**2**).



**Fig 1.9:** Emission spectra of EB bound to DNA. Complex **1**,  $[\text{EB}] = 40 \mu\text{M}$ ,  $[\text{DNA}] = 40 \mu\text{M}$ ,  $[\text{Complex}] = (0-50 \mu\text{M})$ . An arrow ( ) shows the intensity changes upon increasing the concentration of the complex. Inset: Stern-Volmer quenching curves.

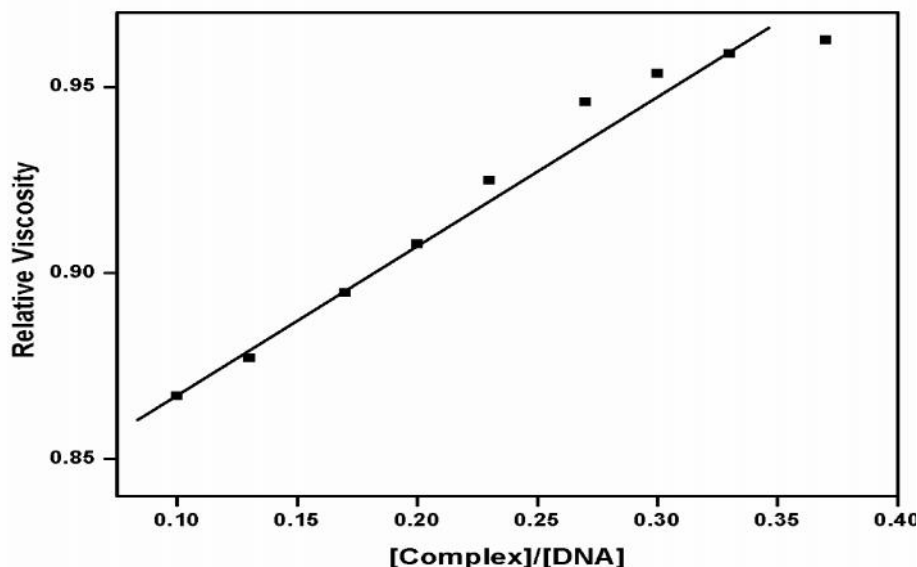


**Fig 1.10:** Emission spectra of EB bound to DNA. Complex 2, [EB]= 40  $\mu$ M, [DNA] = 40  $\mu$ M, [Complex] = (0-50  $\mu$ M). An arrow ( ) shows the intensity changes upon increasing the concentration of the complex. Inset: Stern-Volmer quenching curves.

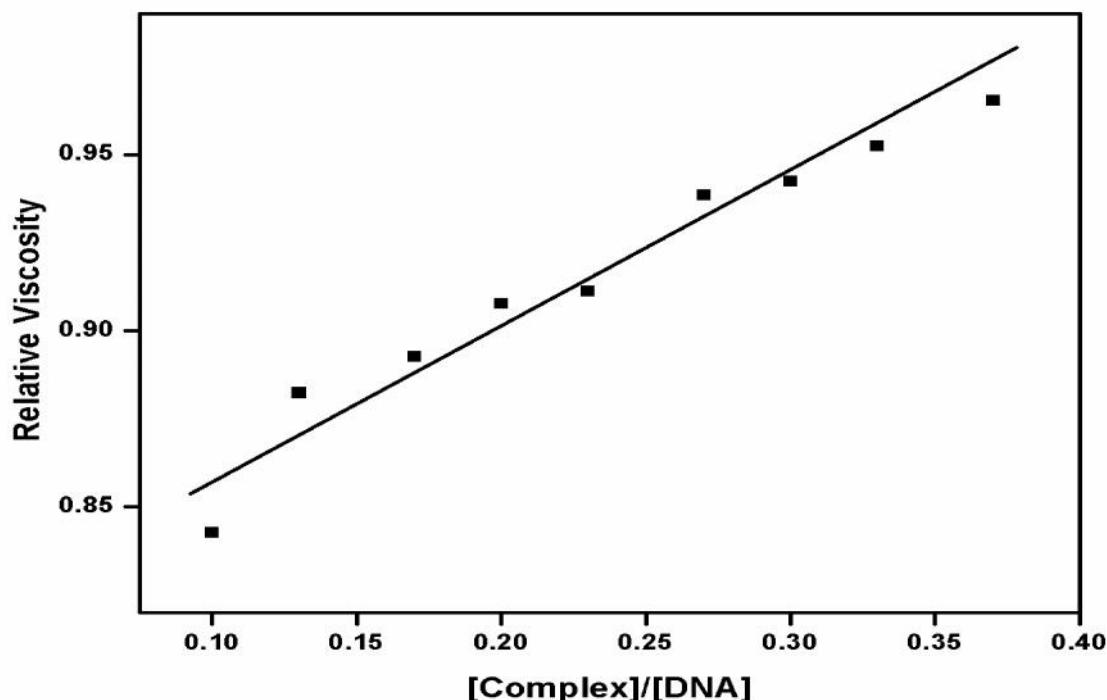
### 1.4.3 Viscosity studies

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

the DNA helix, reduce its effective length and concomitantly its viscosity<sup>37</sup>. The effect of the complexes 1 and 2 on the viscosity of rod like DNA is shown in the Fig 1.11 and 1.12. The viscosity of DNA is increased with the increase of the concentration of the complexes, in contrast to that of proven DNA intercalator EtBr. Based on the viscosity results, it was observed that these complexes bind with DNA through intercalation mode.



**Fig 1.11:** Effects of increasing amounts of complexes 1 on the relative viscosities of CT DNA at 25<sup>0</sup>C:

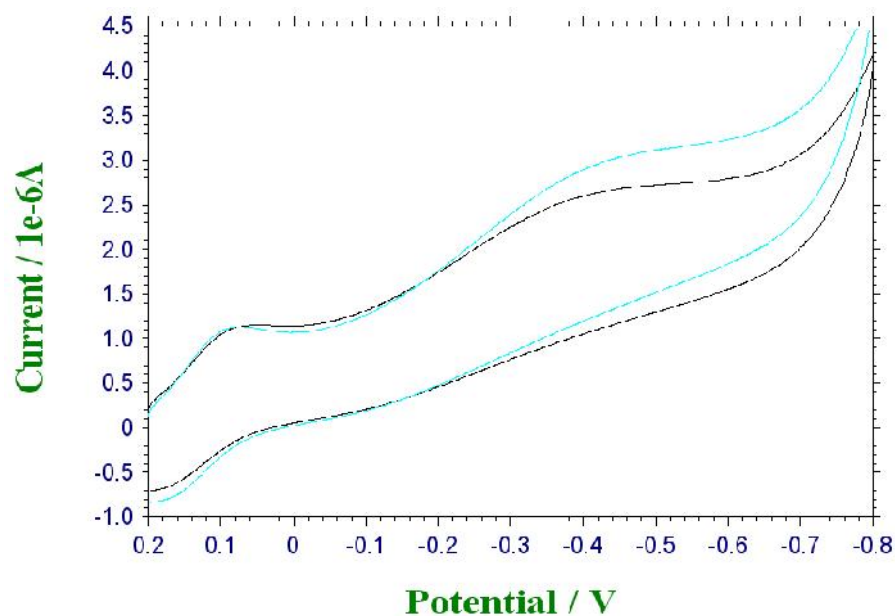


**Fig 1.12:** Effects of increasing amounts of complexes 2 on the relative viscosities of CT DNA at 25<sup>0</sup>C:

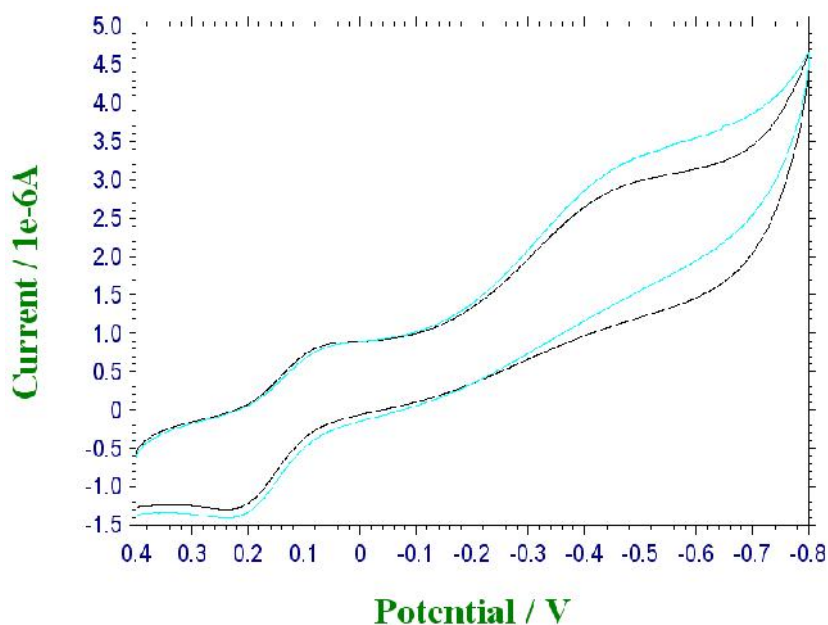
#### 1.4.4 Cyclic voltammetry studies.

The Cyclic Voltammetric (CV) response for complexes 1 and 2 Tris-HCl buffer (pH 7.28) in the presence and absence of CT DNA is shown in Fig 1.13 and 1.14. When CT-DNA is added to a solution complex, marked decrease in the peak current and potential values was observed. The cyclic voltammetric

behavior was not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complexes after addition of DNA due to the binding of complexes to the DNA<sup>38</sup>. When the concentration of DNA increased, the changes in peak current and potential become slow. This reveals that the complexes were interacting with CT-DNA.



**Fig 1.13:** Cyclic voltammogram of 0.5 mM complex 1 [complexes, in the absence (—) and presence (---) of 2.5 mM DNA.



**Fig 1.14:** Cyclic voltammogram of 0.5 mM complex 2 [complexes, in the absence (—) and presence (---) of 2.5 mM DNA.

When CT-DNA is added to a solution of complex, marked decrease in the peak current and potential values was observed. The cyclic voltammetric behavior was not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complex after the addition of DNA due to the binding of  $[\text{Co}(\text{phen})(\text{OPD})]^{3+}$  complex to the DNA. 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.

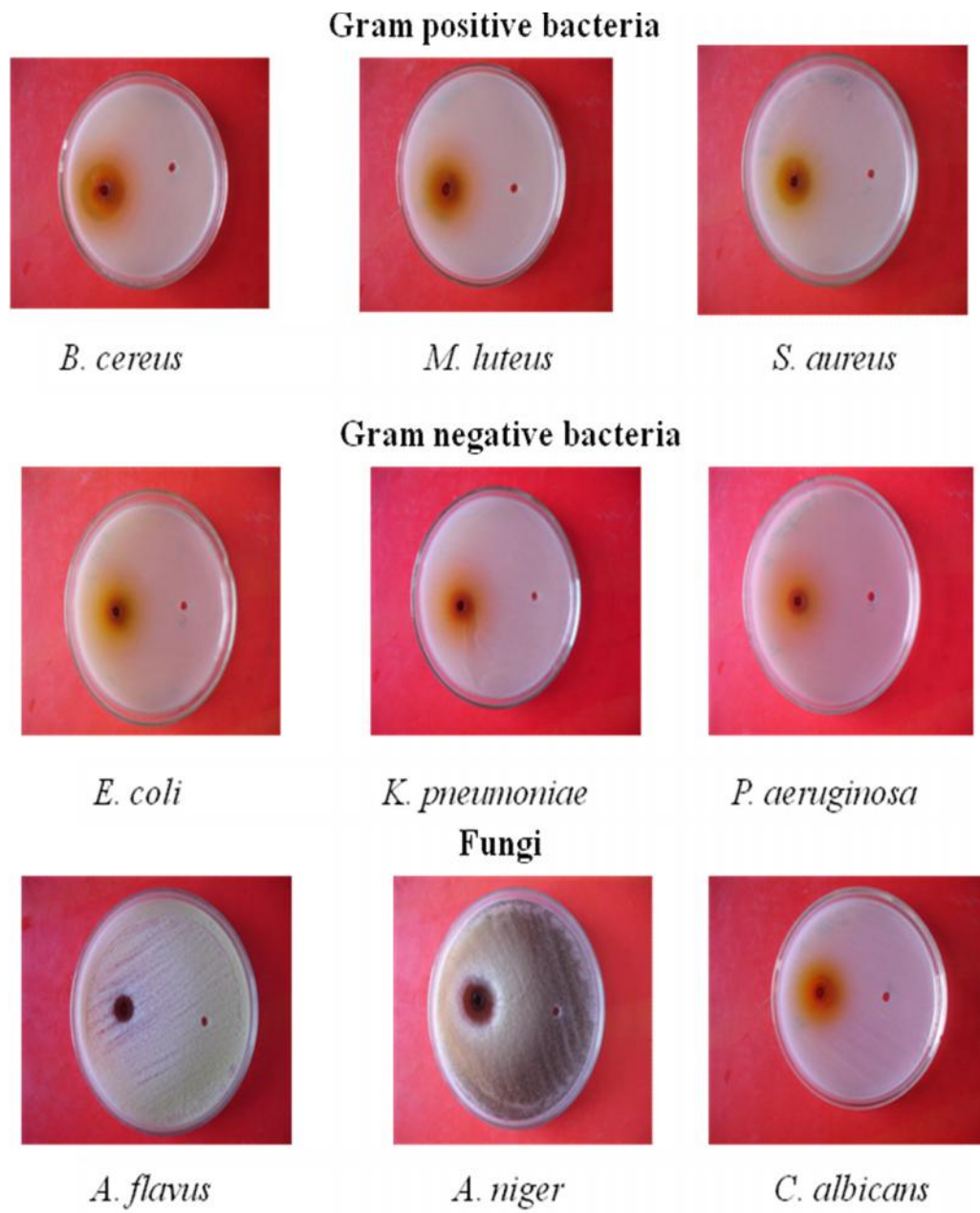
### 1.5 Antibacterial and antifungal screening

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 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 1. The cobalt (III) complex showed significant microbial activity against Gram positive, Gram negative bacteria and fungus<sup>39</sup>. In our biological experiments, using cobalt(III) complex, we have observed high antibacterial activity against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) than Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The cobalt (III) complexes are also very active against the yeast *Candida albicans*.

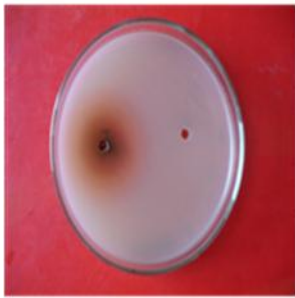
**Table 1.3:** Biological activity of complex 1 and 2

Name of the Organism	Diameter Zone of Inhibition (mm)	
	Complex 1	Complex 2
<i>A. flavus</i>	9	7
<i>A. niger</i>	14	14
<i>B. cereus</i>	22	20
<i>C. albicans</i>	23	13
<i>E. coli</i>	12	-
<i>K. pneumoniae</i>	14	-
<i>M. luteus</i>	13	5
<i>P. aeruginosa</i>	9	-
<i>S.aureus</i>	16	10



**Fig 1.14:** Antimicrobial activity of complex 1

**Gram positive bacteria**



*B. cereus*

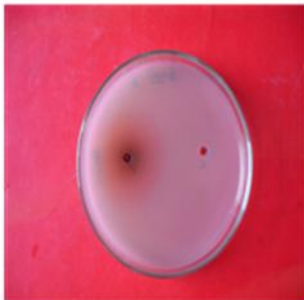


*M. luteus*



*S. aureus*

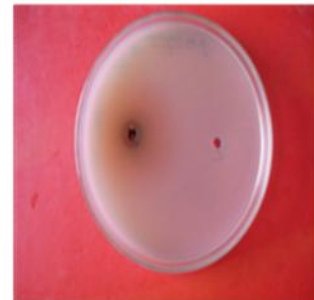
**Gram negative bacteria**



*E. coli*

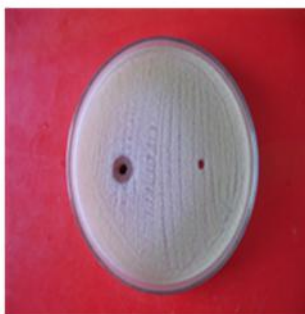


*K. pneumoniae*

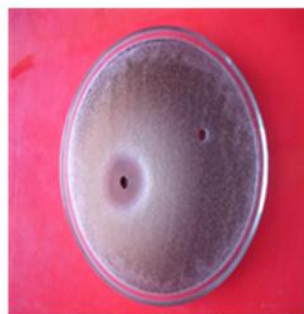


*P. aeruginosa*

**Fungi**



*A. flavus*



*A. niger*



*C. albicans*

**Fig 1.15: Antimicrobial activity of complex 2**

## 1.6 Conclusion


We described the new cobalt(III) complexes and 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 EPR 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 voltametric studies. This shows that the complex is intercalative binding mode with DNA base pairs effectively. The complex [Co(phen)<sub>2</sub>NPS] (ClO<sub>4</sub>)<sub>2</sub> exhibit good antimicrobial activity.

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