CHAPTER-IV

DNA INTERACTION AND MOLECULAR DOCKING STUDY OF A NOVEL SCHIFF BASE Co(II) COMPLEX

4.1. Introduction:

Schiff base metal complexes, recognised by azomethine or imine group (>C=N-), are the most comprehensively studied coordination compounds. They have been extensively involved as ligands in the preparation of transition metal complexes [1,2]. In neotric scientific studies an ample amount of research connected with Schiff base complexes showed remarkable biological activity and are of great significance in both chemical and biological field [2-4]. The most vital step for such investigation is the identification of target. For cancer, DNA has been marked as the principal target thus, the study of DNA binding and cleavage activity by metal complexes is the key for developing new metal-based drugs. Development of such molecules that can interact with DNA of cancerous cell and obstruct them is an example of new anticancer approach. This has paved way for an increase in anticancer therapeutics in search of metallo-nucleases [4-7]. Transition metals which are used largely in this area of research include platinum [8], ruthenium [9-10], titanium [11], rhodium [12], palladium [13], gold [14], Vanadium [15] and Nickel [16]. Among these transition metals, Platinum(II) based compounds and related drugs fascinated the inorganic chemists during a long period because of its anti-cancer activity, especially cis-platin [5,6]. But, these drugs have so many disadvantages too such as lesser solubility, dose-limiting side effects like nephrotoxicity and neurotoxicity and acquired resistance in most type of cancers [6] which necessitates the upgradation of anticancer approach [17].

Cobalt which is an integral constituent of cyanocobalamin (vitamin B12), is very essential in our circulatory system [18,19]. Cobalt also functions as an activating agent for diverse enzyme systems. But interaction of Cobalt complexes with DNA is rarely available in the literature. To explore the chemistry of Co(II) complex and assess its

biological activity, here we have prepared a new Co(II) complex and investigated its interaction with DNA using different physico-chemical and spectroscopic techniques.

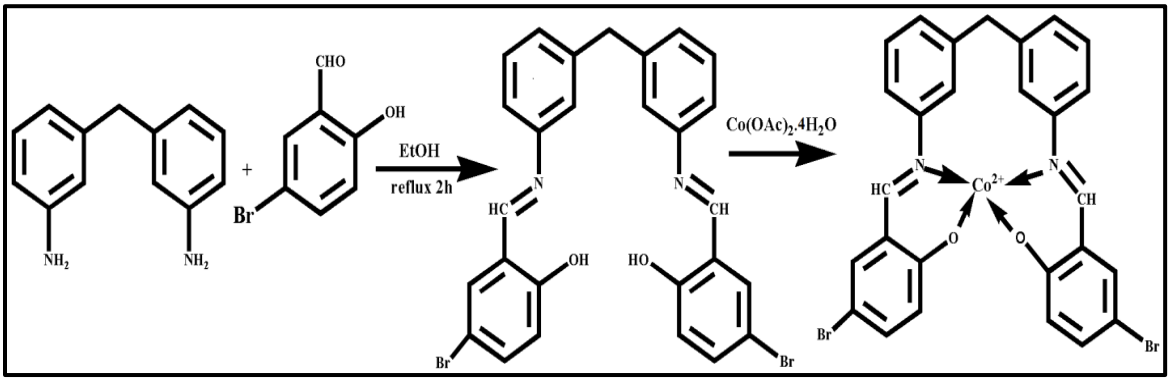
4.2. Experimental Section:

4.2.1. Synthesis of Schiff Base Ligand:

To a hot ethanolic solution of 3, 3' Methylenedianiline (0.1 mol), an ethanolic solution of 5- bromo salicylaldehyde (0.2 mol) was added in dropwise manner and refluxed it for 1h. The resultant orange colored ligand was separated by filtration, washed several times with ethanol and ether and dried in vacuum desiccator.

4.2.2. Synthesis of Metal Complex:

The metal complex was prepared by adding hot ethanolic solution of Co(II) acetate to the equimolar amount of hot ethanolic solution of synthesized ligand (Scheme.4.1.). The resulting reaction mixture was then refluxed for 2 h at 50°C whereupon the Co(II) metal complex was precipitated out. The blue colored precipitate was filtered off, washed with ethanol and dried in vacuum desiccator.



Scheme.4.1. Synthesis of schiff base ligand and its Co(II) complex.

4.3. Results and Discussion:

4.3.1. Characterization of the ligand and its Co(II) complex:

The synthesized compounds have been characterized by using different analytical and spectroscopic tools like IR, Proton NMR, Magnetic moment, UV-Visible, molar conductance *etc*. The analytical data of the synthesized compounds is listed in Table.4.1.

4.3.1.1. Molar Conductance:

The room temperature molar conductance of synthesized Co(II) complex (10^{-3} mol dm^{-3}) was measured in DMSO (Table1). The molar conductance value indicates that the metal complex is a non-electrolyte [20].

Table 4.1. Analytical and Physical data of Schiff base ligand and its Co(II) complex:

Compound	m.p. (◦C)	Colour (% yield)	Mol. Wt. (gm)	% Found (calcd.)			Λ_m (Ω^{-1} mol^{-1} cm^2)
				C	H	N	
H_2L ($C_{27}H_{20}N_2O_2Br_2$)	154	Orange (76)	564	57.31 (57.47)	3.62 (3.57)	4.43 (4.96)	_
[Co(L)] ($C_{27}H_{18}N_2O_2Br_2Co$)	>250	Blue (69)	621	51.06 (52.21)	2.98 (2.92)	4.26 (4.51)	13.83

4.3.1.2. Electronic Spectra and magnetic moment measurement:

The absorption spectra of the newly synthesized molecules were recorded in DMSO depicted in Fig.4.1. The ligand displayed bands at 375 nm and 432 nm, attributed to π-π* transition of phenyl ring and n-π* transition of azomethine (-CH=N-), respectively [22]. However, these absorption bands were undergo blue shift in the spectra of Co(II) complex, confirming the coordination of ligand to Co(II) ion. In addition the Co(II) complex showed a band at 598 nm due to $^4A_2 \rightarrow {}^4T_1$ (P) transition which is a typical one

for tetrahedral Co(II) complex [23]. The value of magnetic moment 4.56 BM also supports the tetrahedral geometry of the synthesized complex [23].

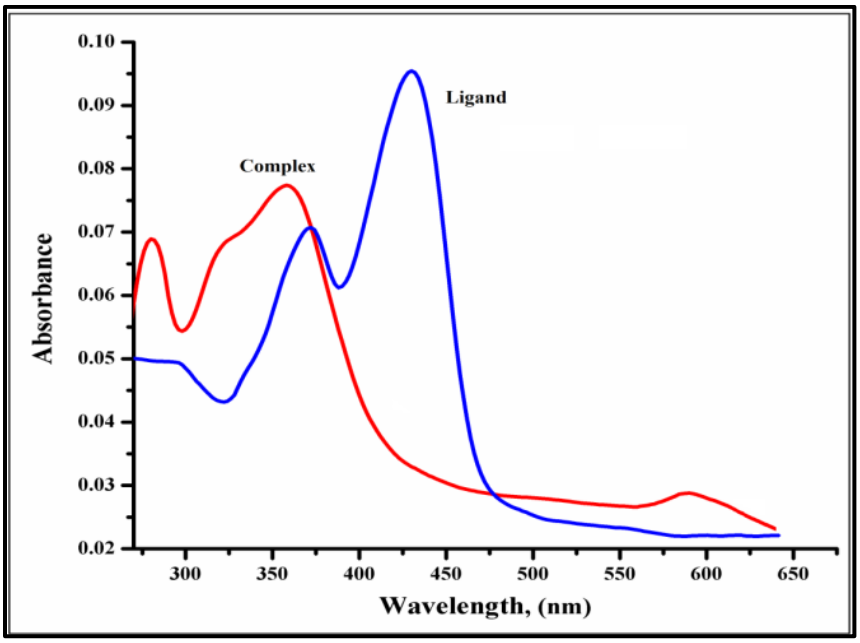


Fig.4.1. UV-visible absorption spectra of ligand and its Co(II) complex in DMSO.

4.3.1.3. Infrared Spectra:

IR spectra afford relevant clue regarding the coordination pattern of ligand to the metal ion. The IR spectrum of the Cobalt(II) complex was matched with the spectrum of ligand to identify the changes come about during complexation. The synthesized Schiff base ligand exhibits a characteristic band at 1594 cm^{-1} due to azomethine group ν(C=N) shown in Fig.4.2. This stretching vibration band is shifted to 1600 cm^{-1} in the Co(II) complex indicating the involvement of the nitrogen atom of (>C=N-) group in complexation [20]. In the spectra of synthesized ligand, a broad band was noticed at 3447 cm^{-1}due to phenolic -OH group of ligand which is found to disappear in the spectra of synthesized complex suggesting the participation of phenolic oxygen in bonding with Co(II) ion through deprotonation [21]. The coordination of phenolic oxygen to metal ion is further confirmed by the shift in ν(C-O) stretching band of ligand at 1199 cm^{-1} to 1169 cm^{-1} upon complexation. The appearance of two non-ligand bands in the IR spectra of synthesized complex at 541 cm^{-1} and 461 cm^{-1} due to ν(Co-O) and ν(Co-N) confirms the

participation of phenolic O and azomethine N in complexation. In conclusion, the above arguments suggest the dibasic tetradentate behavior of the synthesized compound.

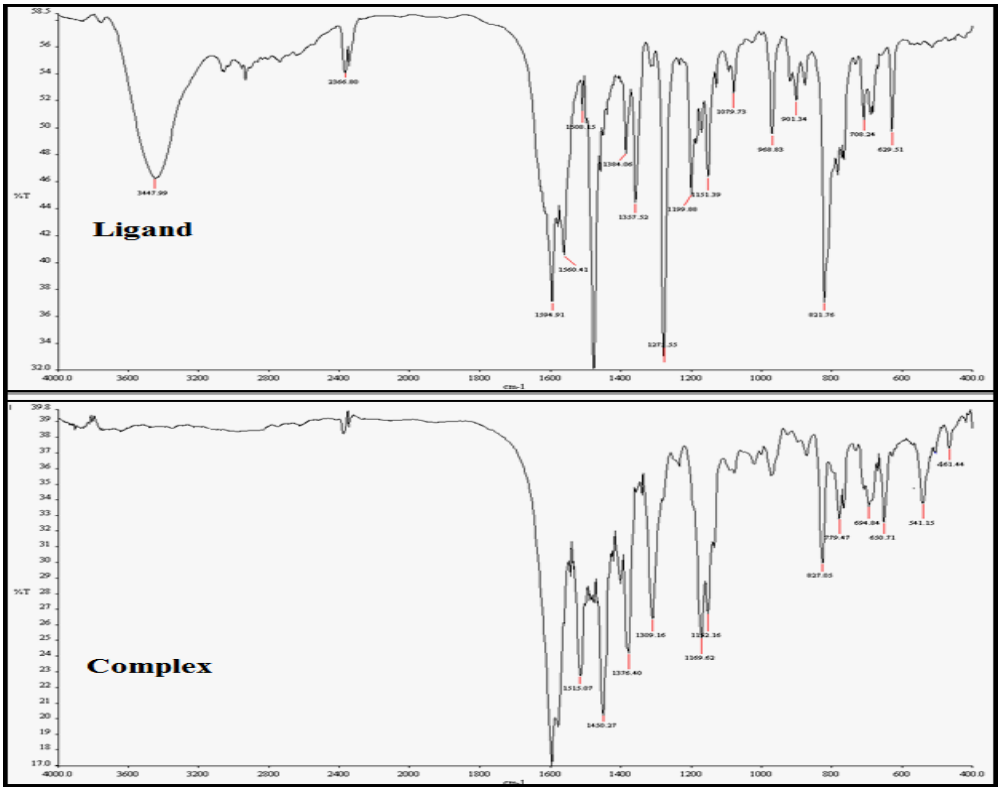


Fig.4.2. FTIR Spectra of the synthesized ligand and its Co(II) complex.

4.3.1.4. NMR Spectra:

The proton NMR spectra of synthesized ligand and was recorded in DMSO-d_6 solution (Shown in Fig.4.3.). The aromatic protons showed signals in the region 6.8-7.8 ppm. The formation of Schiff base is affirmed by the occurrence of azomethine (HC═N) proton signal at 9.20 ppm. Moreover, the ligand showed a hydroxyl (-OH) proton signal at 10.40 ppm [22].

The ^{13}C spectra of the ligand (Fig. 4.3.) showed peaks at 111.13-147.11 ppm for aromatic carbons and at 42.09 for -CH2. Peaks at 165.21 ppm and 168.23 ppm for –CH=N and –C–OH group also indicates the formation of Schiff base ligand.

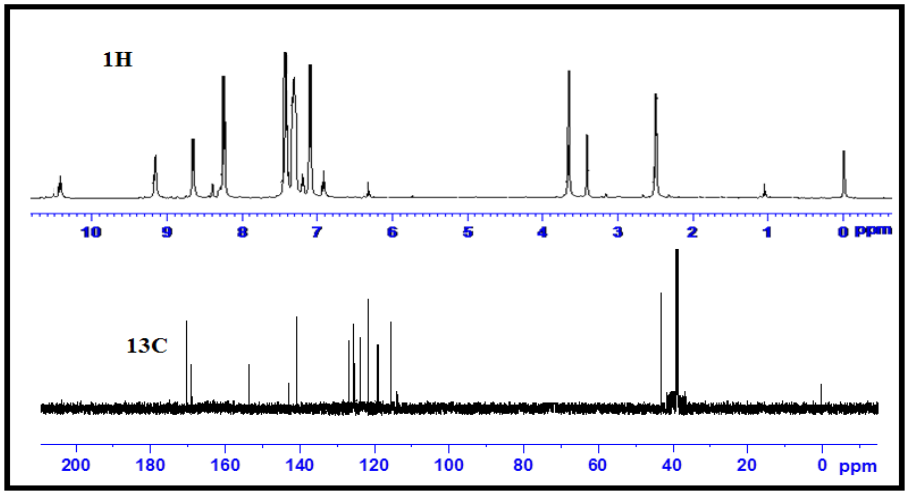


Fig.4.3. ^{1}H and ^{13}C NMR spectrum of Schiff base ligand.

4.3.1.5. Thermogravimetric analysis:

Thermogravimetric analysis (TGA) delivers a quantitative measurement of change in weight with temperature, and is commonly used to explore the thermal stability of synthetic molecules along with compositional analysis. Thermal stability of the Cobalt(II) complex was studied in the temperature range 50-1000 $^{\circ}C$ at the rate of 10 $^{\circ}C$/min. The gradual degradation of synthesized complex with temperature and formation of metal oxides is shown in Fig.4.4. The synthesized complex is quite stable up to 200 $^{\circ}C$ which indicates the absence of any counter ions in the complex molecule. The sharp weight loss of 26.25% (calcd. 26.76%) at the first decomposition stage at 281 $^{\circ}C$ was because of liberation of two bromine atoms. Thereafter, the Co(II) complex showed a gradual decomposition up to 850 $^{\circ}C$ due to the loss of the remaining ligand moiety and ultimately the sample decomposes to corresponding metal oxide. The weight of the residue corresponds to cobalt oxide.

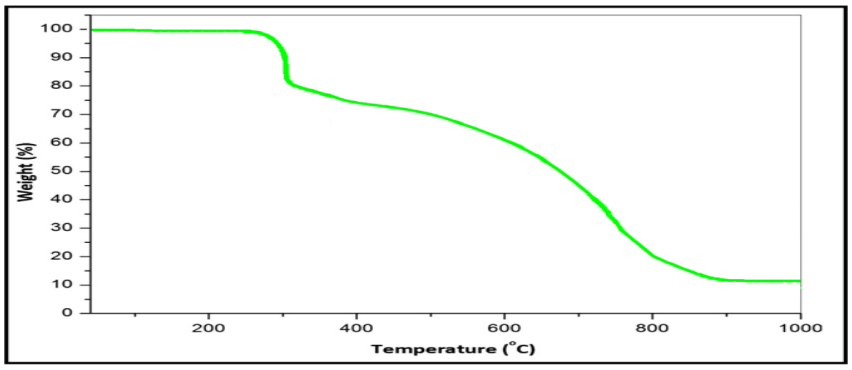


Fig.4.4. TGA curve of the Co(II) complex in the temperature range 50-1000 ^{o}C.

4.3.1.6. Powdered XRD spectra:

The powdered XRD investigation of ligand exhibited sharp peaks, showing its crystalline nature. But upon complexation the peak intensity was reduced and line broadening wanoticed (Fig.4.5). This suggests the change in crystalline nature of ligand upon complexation and showing amorphous nature of the synthesized complex. The crystalline sizes were computed using Debye Scherer's equation: $D= 0.9\ \lambda/\beta\ \cos\theta$, where constant 0.9 is the shape factor while λ, β, θ are the X-ray wavelengths (1.5406 Å), FWHM i.e., full width at half maximum, Bragg diffraction angle respectively. The experimental average grain size of the ligand molecule and its complex were found to be 25.71 nm and 11.76 nm respectively.

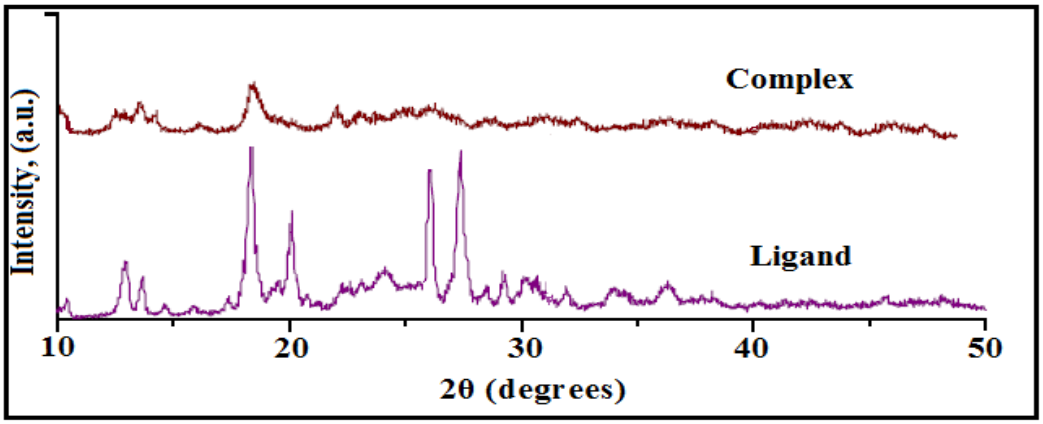


Fig.4.5. PXRD patterns of the ligand and its Co(II) complex.

4.3.1.7. Stability constant and stoichiometry of complex:

Stoichiometry of the synthesized complex has been determined by spectrophotometric titration using mole-ratio method. The changes in the electronic spectra of ligand (initially 1×10^{-5} mol · L^{-1}) were recorded by stepwise addition of 2.5 µL of $Co(OAc)_2$ (1×10^{-3} mol · L^{-1}) metal solution at each step until the complexation was completed. During the titration, a gradual rise in the intensity at 432 nm, stipulates the formation of complex as presented in Fig. 4.6. The spectrophotometric data was analyzed with the absorbance values at λ =432 nm as shown in Fig.4.6. (inset), which suggests the stoichiometry to be 1:1. Also using the ($c_M c_L$/A *versus* c_M) data, the stability constant of the complex was computed to be $\log K_f = 5.90 \pm 0.02$ at 25 °C.

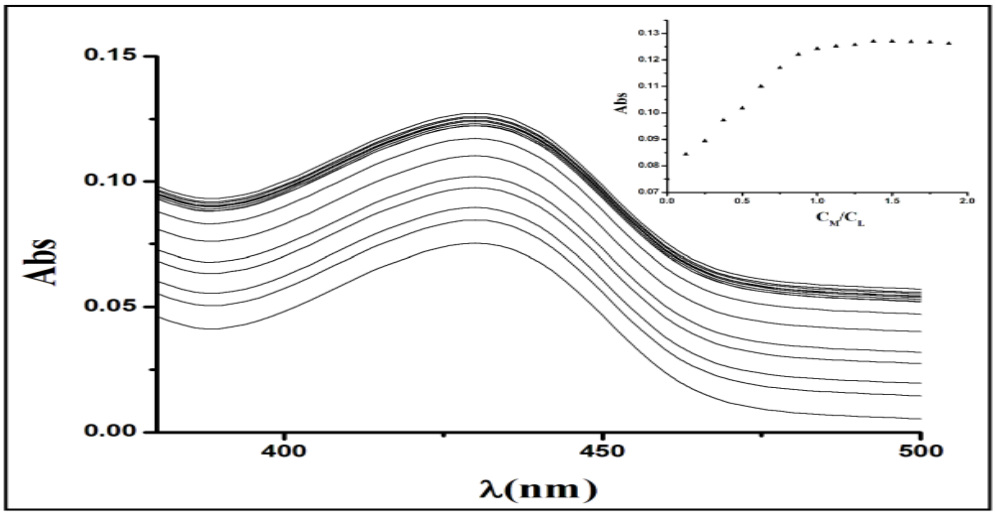


Fig.4.6. UV–Vis spectra of the ligand in presence of increasing concentrations of Co(II) ions. Inset: Absorbance plot for the ligand with Co(II) ion against *c*M:*c*L.

4.3.2. DNA interaction studies:

4.3.2.1. Electronic absorption titration:

Absorption spectroscopic titration functions as a vital tool to study the interaction of DNA with small molecules. If a metal complex binds to DNA through intercalative mode, the obtained results show changes in absorbance and wavelength. The binding affinity of the synthesized metal complex with CT-DNA was studied in a solvent mixture

of 1% DMSO and 99% Tris-HCl buffer employing a fixed concentration of complex to which increasing amount of CT-DNA solution was added. While recording the absorption spectra, equal increment of CT-DNA was added to both the complex solution chamber and the reference chamber to exclude the absorbance of free CT-DNA.

On successive addition of DNA in the complex solution chamber, change in both absorbance and wavelength (2.2 nm for n-π* band) were observed (shown in Fig.4.7.) because of strong stacking interaction between DNA base pairs and aromatic chromophore suggesting an intecalative mode of binding [24,25]. In order to calculate the binding ability of metal complex to CT-DNA, the intrinsic binding constant (K_b) was determined using Wolfe-Shimer equation:

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

and the value of K_b is $(2.5\pm0.2) \times 10^4\ M^{-1}$.

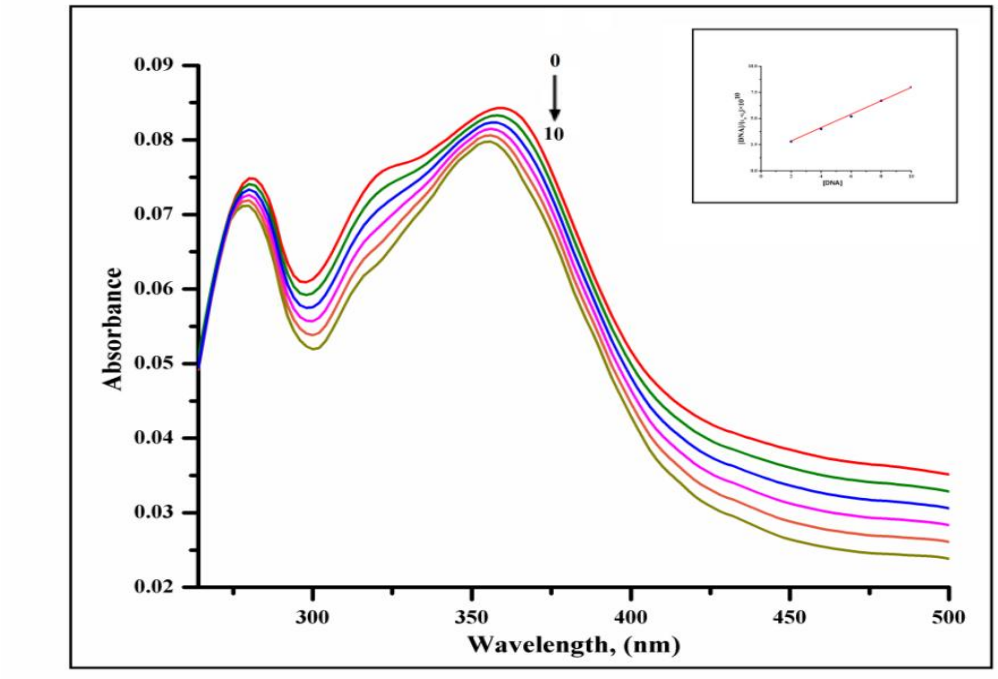


Fig.4.7. Absorption spectra of Co(II) complex (red line) in the absence and presence of increasing amount of CT DNA (0–10 µM), Inset: plot for binding constant (K_b).

4.3.2.2. Ethidium Bromide (EB) competitive study with fluorescence emission spectroscopy:

To affirm the mode of binding of synthesized Co(II) complex to CT-DNA, a competitive binding experiment was performed using Ethidium Bromide displacement strategy. EB-DNA couple shows an intense emission band at 592 nm because of intercalation of the planar phenanthridine ring of EB between adjacent DNA base pairs [26]. A noticeable quenching of the EB-DNA emission band may be seen if a foreign molecule having the ability to intercalate to DNA equally or stronger than EB, is added into EB-DNA solution [27].

The competitive binding experiment was performed in Tris-HCl buffer, keeping [DNA]/ [EB] =1.13 with increasing the concentration of the synthesized complex. The addition of Co(II) complex solution in EB-DNA solution resulted in significant quenching in the emission intensity (shown in Fig.4.8). This lowering in emission intensity of EB-DNA couple upon addition of Co(II) complex suggests displacement of EB which can be delineated as intercalative mode of binding. The quenching constant or quenching strength (K_{sv}) of synthesized complex toward EB-DNA conjugate was further calculated using the classical Stern‑Volmer equation [28]:

$I_0/I = 1 + K_{SV}r$, Where I and I_0 are the fluorescence intensities in the presence and absence of the quencher and r is the ratio of total concentration of complex to that of DNA. The calculated value of K_{sv} for the synthesized complex is $(1.1\pm0.3)\times10^4$ M^{-1}.

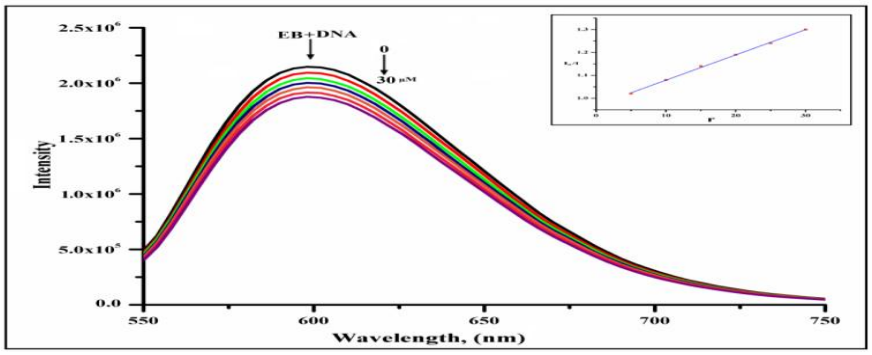


Fig.4.8. Emission spectra from EB bound to the DNA in the absence and presence of increasing amount of Co(II) complex (0–30 μM), Inset: plot for quenching constant (Ksv).

4.3.2.3. DNA melting study:

DNA melting study is an essential tool to measure the extent of intercalation [29]. Intercalation of foreign molecules into DNA base pairs increases the value of melting temperature (T_m). For performing this experiment, solutions of CT-DNA and complex was prepared in 1% DMSO and 99% Tris-HCl buffer solvent mixture. Fig.4.9. presents the melting curve of CT-DNA solution both in absence and in presence of Co(II) complex. The measured value of T_m for CT-DNA solution was 79.0^0C which increased noticeably (86.0^0C) in presence of Co(II) complex. These increase in T_m value supports intercalative binding of Co(II) complex [29].

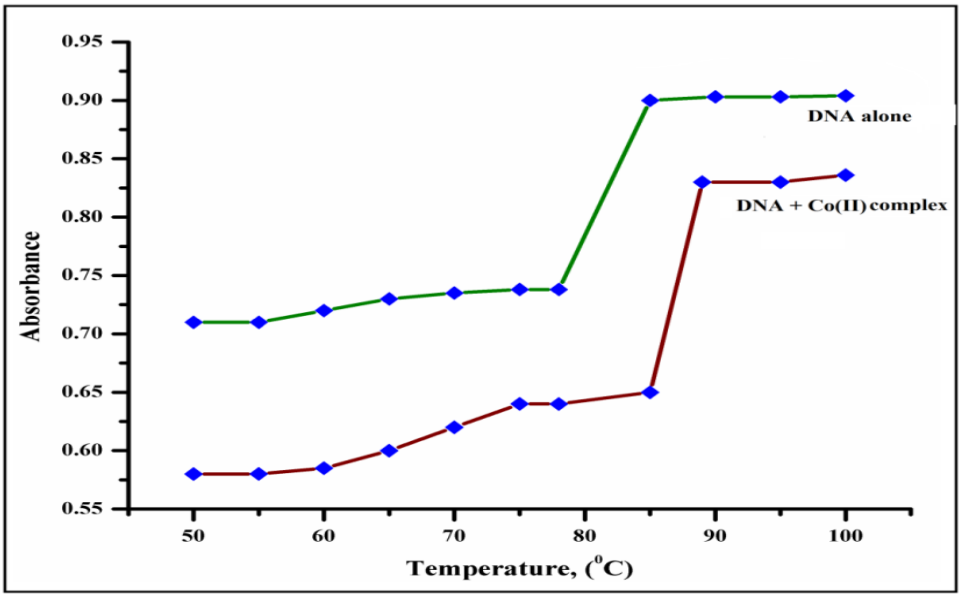


Fig.4.9. Plot of absorbance versus temperature (^{0}C) for the melting of 1) CT DNA alone 2) CT DNA + Co(II) complex.

4.3.2.4. Viscosity measurement:

Hydrodynamic tactics (like viscosity measurement), which are sensitive to length of DNA, are substantial tool to investigate the binding mode of metal complexes to DNA. An intercalative mode of interaction results in increase in viscosity values because of lengthening of DNA helix. Viscosity measurements were carried out using a capillary type viscometer (Ostwald viscometer) thermostated at 25±1 $^\circ$C keeping the concentration

of CT-DNA (100 μM) constant while varying the concentration of complex (10-100 μM). The relative viscosity of DNA in absence (η_o) and presence (η) of Co(II) complex was measured using the relation: $\eta = (t - t^o)/ t^o$, where t is the flow time of CT-DNA containing complex solution and t^o is the flow time of buffer alone in seconds [30]. The obtained results were represented as $(\eta/\eta_o)^{1/3}$ versus 1/ R (R = [DNA]/[Complex]).

Here with increasing the concentration of Co(II) complex, the relative viscosity of DNA increases sharply similar to the nature of well known intercalator EB (depicted in Fig.4.10.) suggesting intercalative binding.

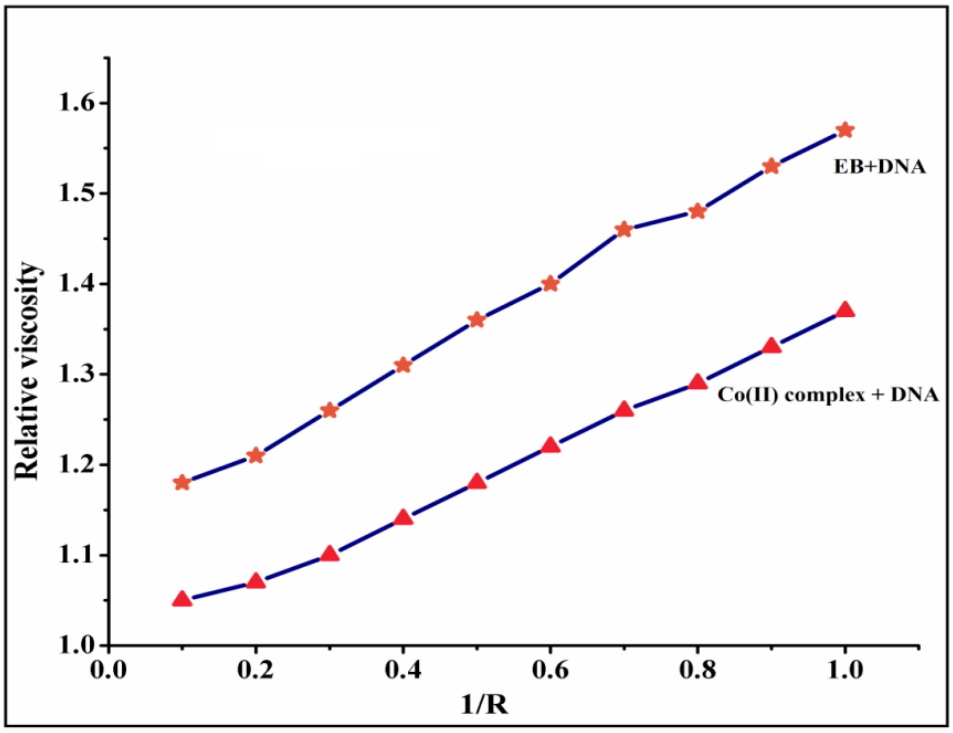


Fig.4.10. Effect of increasing amounts of (a) EB (b) Co(II) complex on the relative viscosity of CT-DNA.

4.3.3. Molecular Docking study:

The bioactive molecules and drugs have a tendency to bind with proteins and other important macromolecules to modulate their biological functions. The synthesized Co(II) complex is examined for possible bioactivity using Swiss targets prediction web server (http://www.swisstargetprediction.ch). The Swiss prediction report shows the probable

association of synthesized complex with biological network. The repot proposed that among the probable targets, 20% are enzymes, 27% are cytosolic proteins and there is a huge chunk of unclassified proteins of 27%. Among the series of predicted targets, some are very important compounds such as BCl-2 like protein 1, microtubule-associated protein tau, and several receptor proteins which are linked to various cellular activities [34]. The proteins like progesterone receptor and Mcl-1 has showed high binding affinity with the Co(II) complex (Table.4.2; Fig.4.11). The progesterone receptor has the maximum interaction with the complex having binding affinity -9.7 kcal/mol. So, it might have some implications in inhibition or expression of progesterone hormones in biological systems.

Table.4.2. Binding affinity of the Co(II) complex with different proteins:

Protein Name	Gene Name	PDB ID	Binding affinity (kcal/mol)
Progesterone receptor	PGR	1a28	-9.7
Dihydroorotate dehydrogenase (quinone), mitochondrial	DHODH	1d3g	-9.0
Tyrosyl-DNA phosphodiesterase 1	TDP1	1jy1	-9.4
Bcl-2-like protein 1	BCL2L1	1r2d	-8.6
Induced myeloid leukemia cell differentiation protein Mcl-1	MCL1	2nl9	-9.4
Microtubule-associated protein tau	MAPT	2on9	-6.7
Muscleblind-like protein 1	MBNL1	3d2q	-8.7
Bcl-2-like protein 2 (by homology)	BCL2L2	4cim	-9.4
Apoptosis regulator Bcl-2 (by homology)	BCL2	4lxd	-9.5

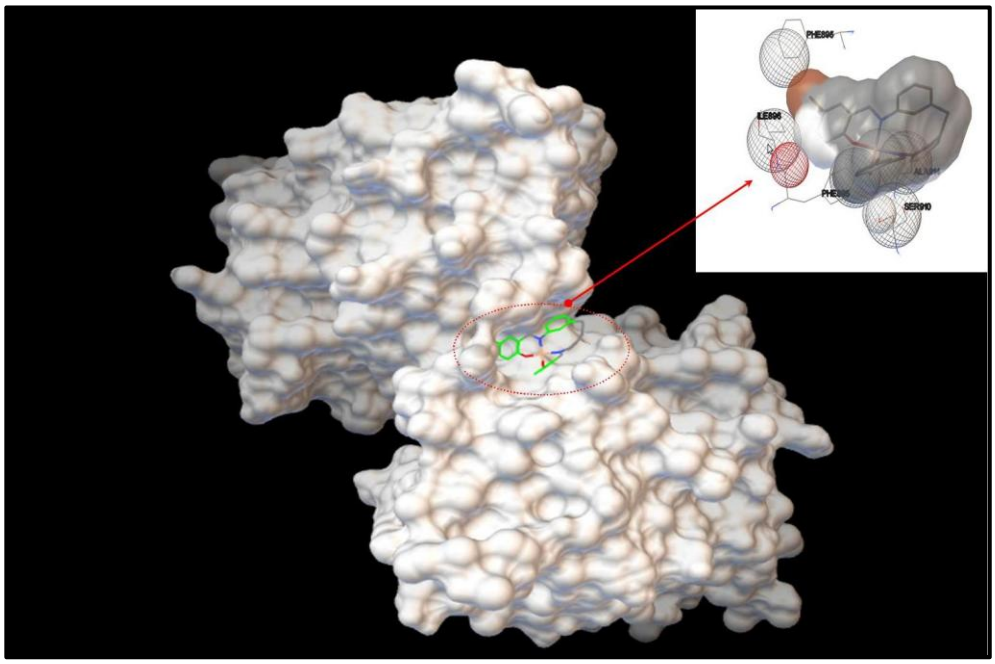


Fig.4.11. The molecular interaction of the proteins with Co(II) complex.

4.3.4. Antibacterial activity:

The synthesized compounds along with Cobalt acetate salt were examined for antibacterial activity following standard procedure of well diffusion method [35]**.** Two pathogenic gram positive bacteria *Bacillus subtilis* (MTCC-121), *Staphylococcus aureus* (MTCC-3160) and two pathogenic gram negative bacteria *Escherichia coli* (MTCC-1698), *Pseudomonas aeruginosa* (MTCC-1035) were used for assessing the antibacterial activities. The test solution of the compounds was prepared in DMSO at a concentration of 5, 7.5 and 10 mg/ml. The zone of inhibitions is expressed as mean values with standard deviation of mean (SD).

The Synthesized Co(II) complex shows significant antimicrobial activities (zone of inhibition, mm) against the *E. coli* and *P. aeruginosa* (Table.4.3; Fig.4.12) and moderate activity against *B. subtilis* and *S. aureus*. But such zone of inhibition is very low in case of ligand and Cobalt acetate salt. This implies that both gram positive and gram negative bacterial were susceptible to such metal complex and may provide a support to protect against bacterial disorder. So this Co(II) complex might prove beneficial as a novel drug candidate against bacterial infection in future.

Table 4. 3. Antibacterial activity of Co(II) complex (zone of inhibition in mm):

Sample	Concentration (mg/ml)	Diameter of zone of inhibition (mm.)			
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
Co(II) complex	5	11.5±0.7	12.5±1.41	13±1.41	13.5±0.7
	7.5	10.5±0.7	10.5±0.7	15±1.41	18.5±0.7
	10	14±1.41	14±1.41	16±2.82	12.5±0.7
DMSO		8.5±0.7	8.5±0.7	8.5±0.7	8.5±0.7

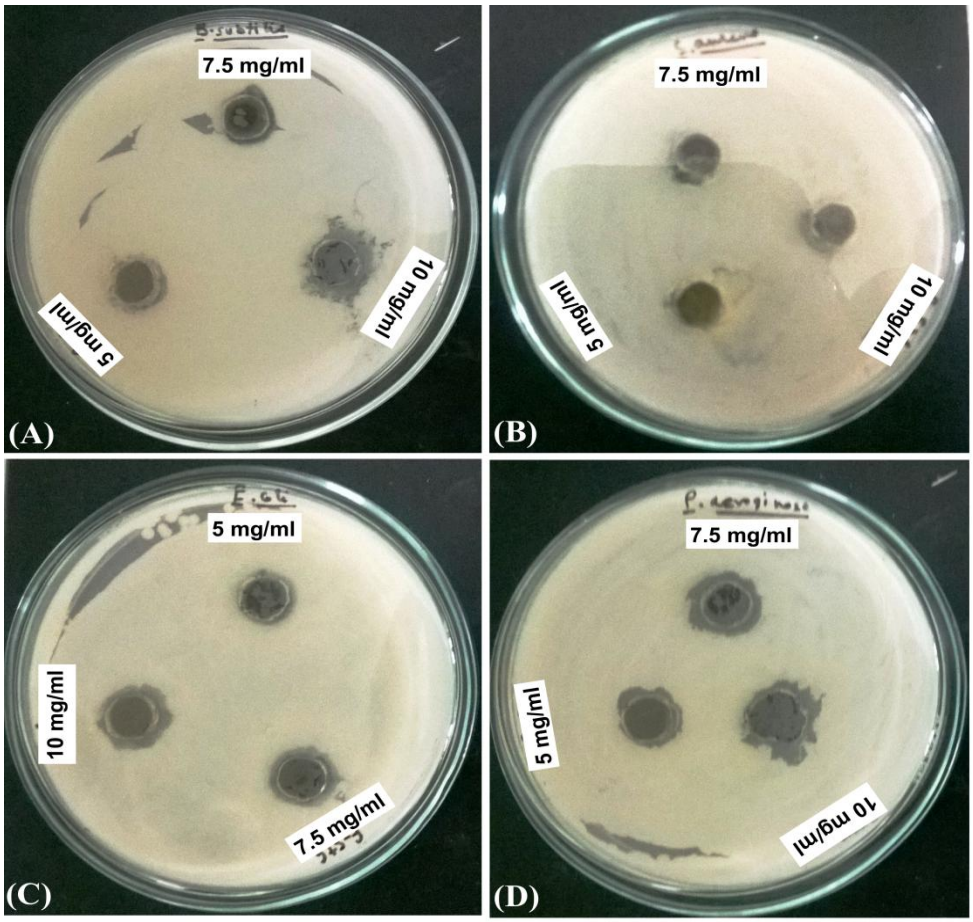


Fig.4.12. Anti- bacterial activity of Co(II) complex on (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli* and (D) *P. aeruginosa*

4.4. Conclusions

Here in this chapter, a biologically important ligand and its Co(II) complex have been prepared and characterized. The physico-chemical and spectroscopic results reveal that

the synthesized complex is mononuclear and posses tetrahedral geometry around the Co(II) ion. The results of DNA binding study suggest that the synthesized complex act as an efficient metallointercalators. The result of antibacterial activity showed that both gram positive and gram negative bacterial were susceptible to such metal complex and may provide a support to protect against bacterial disorder. According to molecular docking analysis, proteins like progesterone receptor and Induced myeloid leukemia cell differentiation protein Mcl-1 has high binding affinity with the Co(II) complex. Because of such benevolent biological activities, the synthesized complex may be productive for the design of new metal-based drugs.

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