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Research Article

Schiff Base Metal Complexes

Chemical Synthesis, Characterization and DNA Binding Studies of 2, 4-Dihydroxy Acetophenone Pramipexole Schiff Base Metal Complexes

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Abstract: Schiff and their complexes are versatile compounds synthesized from the condensation of an amino compound with carbonyl compounds and widely used for industrial purposes and also exhibit a broad range of biological activities, including antifungal, antibacterial, anti-malarial, ant proliferative, anti-inflammatory, antiviral, and antipyretic properties. Many Schiff base complexes show excellent activity in various reactions in the presence of moisture. Over the past few years, many reports have been on their applications in homogeneous and heterogeneous catalysis. The influence of certain metals on the biological activity of these compounds and their intrinsic chemical interest as multidentate ligands has prompted a considerable increase in the study of their coordination behavior. The development of new chemotherapeutic Schiff bases and their metal complexes is now attracting the attention of medicinal chemists. The present paper deals with synthesizing and characterizing metal complexes of new Schiff bases derived from Pramipexole (4, 5, 6, 7-Tetrahydro-N6-propyl-2, 6-benzothiazole diamine) and 2,4-dihydroxy acetophenone (2,4 DHAP) by using modified Sand Mayer's method. Synthesized complexes were characterized by several techniques using IR, ^{1}HNMR, UV-visible spectrometry, TG-DTA, and Conduct Metric Titrations. Infrared spectral data indicate the coordination between ligand and central metal ion through deprotonated phenolic oxygen and azomethine nitrogen. In addition, the authors have screened the compounds for DNA binding studies. It was found that these complexes bind DNA with high affinities.

Keywords: Synthesis, Characterization, Schiff base (2, 4-DHAPP), Pramipexole, DNA Binding studies.

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1. INTRODUCTION

Schiff bases area vast group of compounds characterized by the presence of a double bond linking carbon and nitrogen atoms, the versatility of which is generated in the many ways to combine a variety of alkyl or aryl substituents. Compounds of this type are found in nature and synthesized in the laboratory. Schiff bases are versatile organic compounds widely used and synthesized by the condensation reaction of different amino compounds with aldehydes or ketones known as imines. Schiff base ligands are considered privileged ligands as they are synthesized by condensation. They show a broad range of applications in medicine, pharmacy, coordination chemistry, biological activities, industries, food packages, dyes, and polymers and are also used as an O_2 detectors. Coordination complexes are gaining importance in recent years, especially in designing long-acting drugs in metabolism. The metal complexes from bidentate ligands have often been studied recently because of their technical applications [1,2] and applications in enhancing drug action [3,4]. Transition metals are essential for the normal functioning of living organisms, therefore, are of great interest as potential drugs[5]. The coordination chemistry of nitrogen donor ligands is an active area of research. Much attention has focused on the complexes formed by metals with bidentate ligands using oxygen and nitrogen[6,7]. The Schiff bases are an important class of ligands in coordination chemistry. The imine nitrogen is basic and exhibits pi-acceptor properties. The ligands are typically derived from alkyl diamines and aromatic aldehydes. Chiral Schiff bases were one of the first ligands used for asymmetric catalysis[8].
Studying structural and binding features of various Schiff base complexes can play an important role in better understanding the complex biological process[9]. Schiff bases derived from 2,4-dihydroxy acetophenone are well known for their interesting ligation properties and exclusive applications in different fields[,10]. Furthermore, it is well known from the literature that Schiff bases derived from Pramipexole have a strong ability to form metal complexes[11]. The interaction of these donor ligands and metal ions gives complexes of different geometries, and the literature survey reveals that these complexes are potentially more biologically active. Thus, in recent years Schiff bases and their metal complexes have attained much attraction because of their extensive biological activities[12,13]. Keeping the above fact in mind and continuing our earlier work on transition metal complexes with Schiff bases, the ligand DHAPP Schiff base (L) has been synthesized. The present paper reports synthesizing and characterizing the ligand and its complexes with Cu (II) and Ru (II).

2. MATERIALS AND METHODS

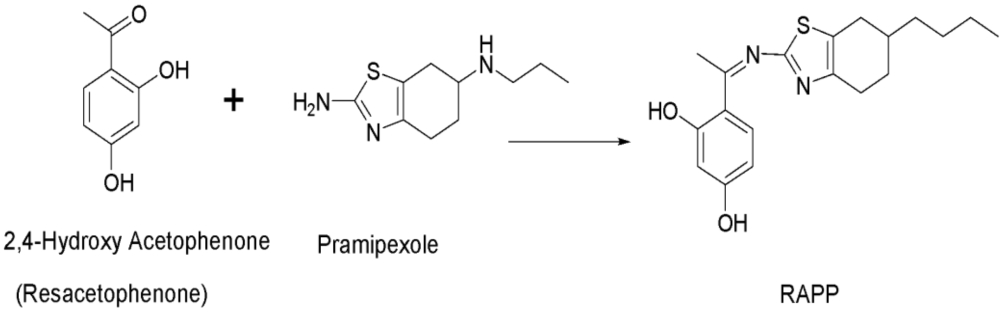
2.1. *Instrumentation*

The percentage compositions of the elements(CHNO) for the compounds were determined using an element analyzer[14] CHNO model Fison EA 1108.The Infrared spectra were recorded as potassium bromide (KBr) discs using a JASCO FT/IR-5300.The 1H (400Hz) nuclear magnetic resonance spectra were recorded using the ACF200 Broker Germany Spectrometer. Ultraviolet Spectra were recorded using Perkin-Elmer lab India UV-Vis Spectrometer. The Electron spin resonance spectra were recorded using the JES-FA Series, and TG-DTA spectra were recorded using the SPTQ600 PA. Thermo gravimetric analyses of the metal complexes[15] were carried out using the Perkin Elmer system in the thermal analysis center Stick Cochin, and ethyl alcohol was used as the solvent. All chemicals used in the present investigation were pure Aldrich chemicals.

2.2. *Preparation of the ligand and its metal complexes*

2.2.1. *Preparation of Pramipexole and 2, 4 - dihydroxyacetophenone Schiff base (2,4 DHAPP)*

Pramipexole 4.22g (0.02 mole) and 2,4 Di Hydroxy-acetophenone 3.04g (0.02mole) were dissolved in 25ml of methanol were taken in 250ml borosil reflection flask and 1 ml of tri ethyl amine. The mixture was refluxed for 3 hours on a water bath and then cooled to room temperature; light brown colored, sharp needles were separated and washed with methanol and dried in vacuum desiccators over CaCl2 anhydrous.



2.2.2. *Preparation of Pramipexole and 2, 4 - dihydroxyacetophenone Schiff base (2, 4 DHAPP) Metal Complexes*

Cu (II) and Ru (II) metal chloride salts were used to prepare. Dissolve 3.2947g (0.01 Mole) of newly synthesized ligand in adequate methanol. An aqueous solution of 1.3434 g (0.01Mole) and 1.718 g (0.01Mole) metal chlorides and 1 ml of Sodium acetate was added. The mixture was refluxed for 6 hours in a water bath and then cooled to room temperature; dark green colored, dark brown colored, sharp needles were separated. The colored metal complexes were washed with water and then methanol and were recrystallized from the ether and dried in vacuum desiccators over $CaCl_2$ anhydrous. The elemental analysis was carried out for the newly synthesized ligand metal complexes. The prepared metal complexes were in 1:2 ratios. Ligands and metal complexes analytical data were tabulated in Table-1.

Table 1: Analytical data of the ligand and their metal complexes.

			Complex		
			2,4 DHAPP	**$\mathrm{Cu(2,4DHAPP)_2X_2}$**	**$\mathrm{Ru(2,4DHAPP)_2X_2}$**
Molecular weight			329.479	789.488	828.01
Color			Light brown	Dark green	Dark brown
Yield			76	74	72
M.P			210-212	230-232	250-252
Elemental Analysis	C %	Calculated	65.52	54.71	52.17
		Found	62.00	54.05	52.00
	H%	Calculated	6.65	6.33	6.03
		Found	6.05	6.22	6.00
	N %	Calculated	12.15	10.63	10.14
		Found	12.01	10.34	10.01
	O%	Calculated	9.26	12.15	11.59
		Found	9.20	12.00	11.52
	M%	Calculated	-	8.04	11.20
		Found	-	7.99	10.98

3. RESULTS AND DISCUSSION

3.1. Infrared spectral analysis

Infrared spectra were recorded with a JASCO FT/IR-5300 Spectrometer (4000-400cm^{-1}) using KBr pellets. By utilizing this spectroscopy, the presence of important functional groups in the compound can be identified through light on the observation made in analyzing IR spectra of ligand and metal complexes. The typical IR spectra are presented in Fig.1, 2, and 3.

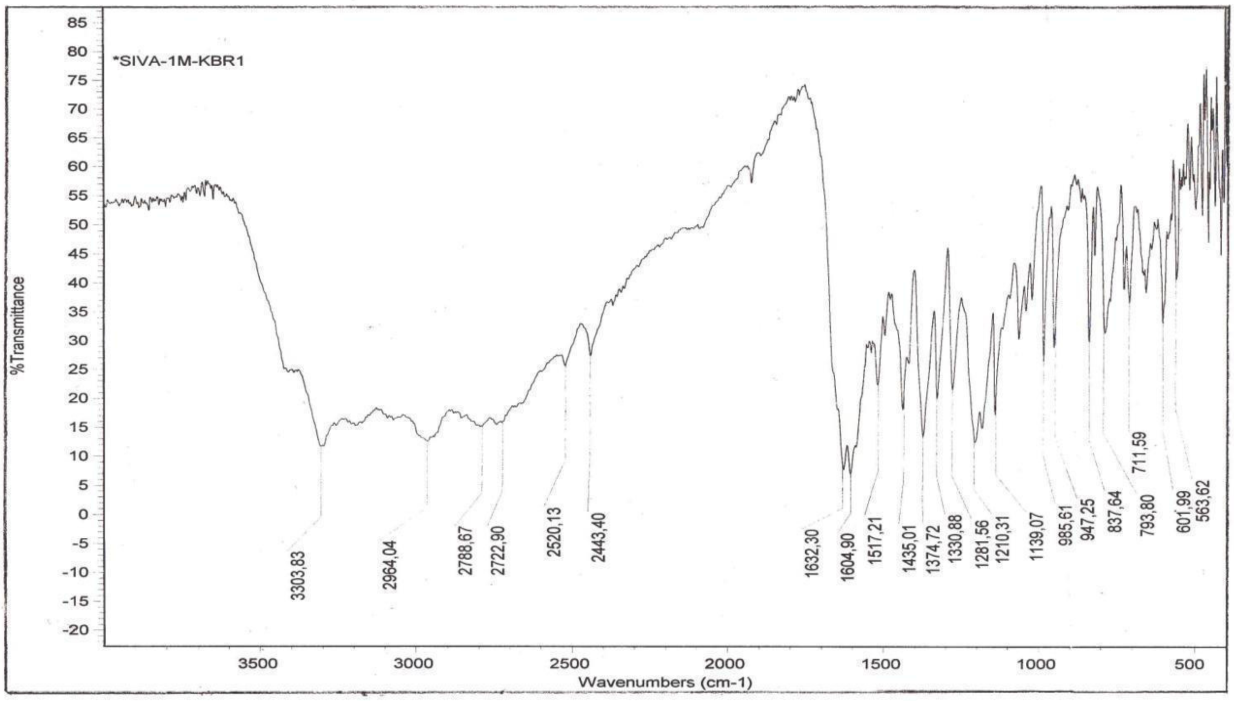


Fig-1: IR Spectrum of 2,4 DHAPP Ligand

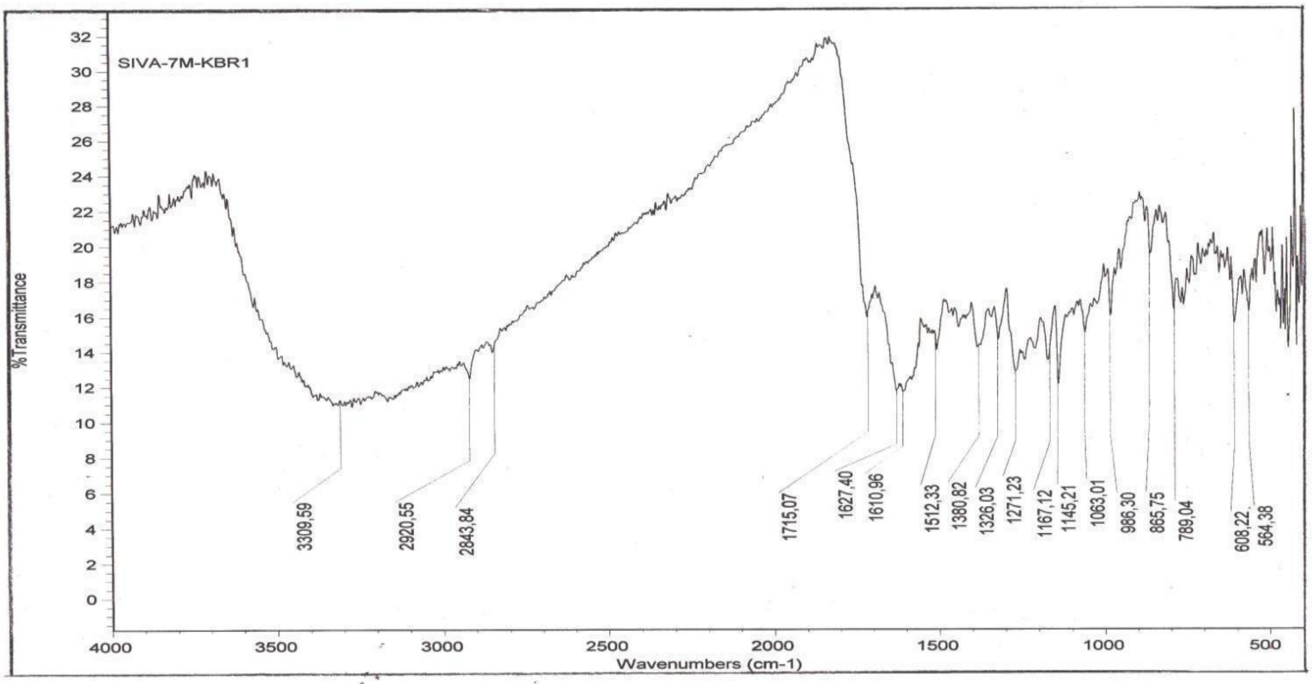


Fig-2: IR Spectrum of Cu (2,4 DHAPP)$_2$ complex.

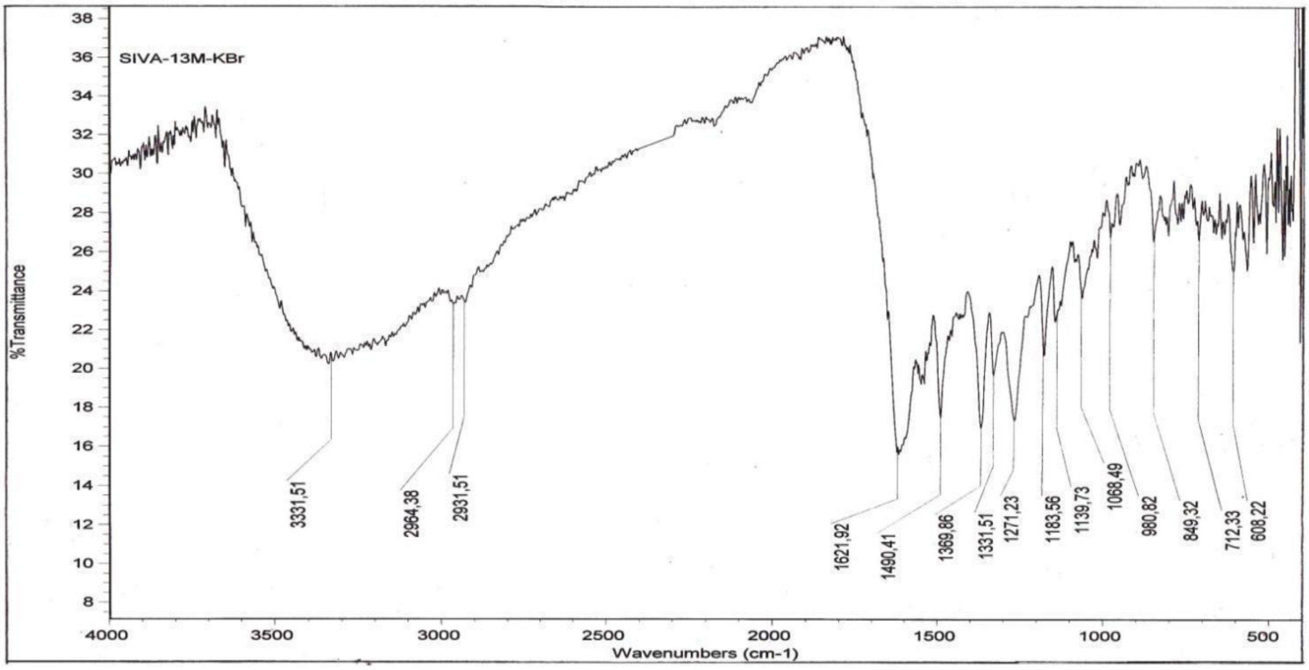


Fig3: IR spectrum of Ru (2,4DHAPP)$_2$ Complex.

3.2. *Interpretation of 2,4 DHAPP and Cu (II) and Ru (II)complexes*

The Infrared spectrum of the ligand was compared with the spectra of Cu (II) and Ru (II) complexes. The data are summarized in the Table2 along with their assignment.

Table2: The important IR bands of the Ligand and Their Metal Complexes

Compound	OH(Water)	υ OH (Phenolic)	υ C=N	υ N-H	υ M-O	υ M-N	υ C-H
2,4 DHAPP	-	3303	1632	3303	-	-	2964
Cu(2,4DHAPP)$_2$	3309	-	1627	3309	695	451	2912
Ru(2,4DHAPP)$_2$	3331	-	1621	3309	608	564	2900

Table-2illustrates that the IR spectrum of the ligand shows the broadband at 1632 cm$^{-1,}$ which was assigned due to υC=N stretching of the azomethine group. In complexes, this band was shifted to lower regions, 1627 cm^{-1} and 1621cm^{-1}for Cu (II) and Ru (II) complexes, respectively, suggesting the involvement of the azomethine group(>C=N) group in complexation. It was due to the reduction of electron density in Nitrogen. Thereby indicating the coordination of the metal through the nitrogen atoms. The IR spectra of metal chelates show the disappearance of the υ(OH)bond at 3303 cm^{-1}. It indicates the proton displacement from the phenolic (OH) group on complexation. Thus, the bonding of the metal ions to the ligands under investigation occurs through a covalent link with the oxygen of the phenolic group. The band at 2964 $cm^{-1}$is associated with the ligand's υ (C-H) stretching vibration.The IR spectra of Cu (II) and Ru (II) metal complexes exhibit a broad band around 3309 cm^{-1} and 3331, cm^{-1}respectively, Which can be assigned to the N-H and OH stretching vibration of the coordinated water molecules. These results indicate the formation of the complex. The weaker bands at 817.50 cm^{-1} and 807.20 cm^{-1} were attributed to OH rocking and wagging vibrations of coordinated water molecules.The complexes display a sharp band in the 946-968 cm^{-1} region due to the υ(V=O) mode. New bands were observed in the complexes, which were not in ligands. The bands at 695 cm^{-1} and 608 cm^{-1} were assigned to stretching frequencies of (M-O), and the band at 451cm-1 and 564cm^{-1} were assigned to the stretching frequencies (M-N), respectively. A weak band observed around 2900 cm^{-1} in both ligands and complexes could be assigned to the C-H stretching frequency.

3.3. NMR Spectrum of 2,4 DHAPP Ligand and its Metal complexes

The ^{1}H NMR spectra of ligand and metal complexes in DMSO-d6 as solvent were given in Fig.4,5, and 6. The chemical shift values of the ligand and metal complexes are shown in Table-3.

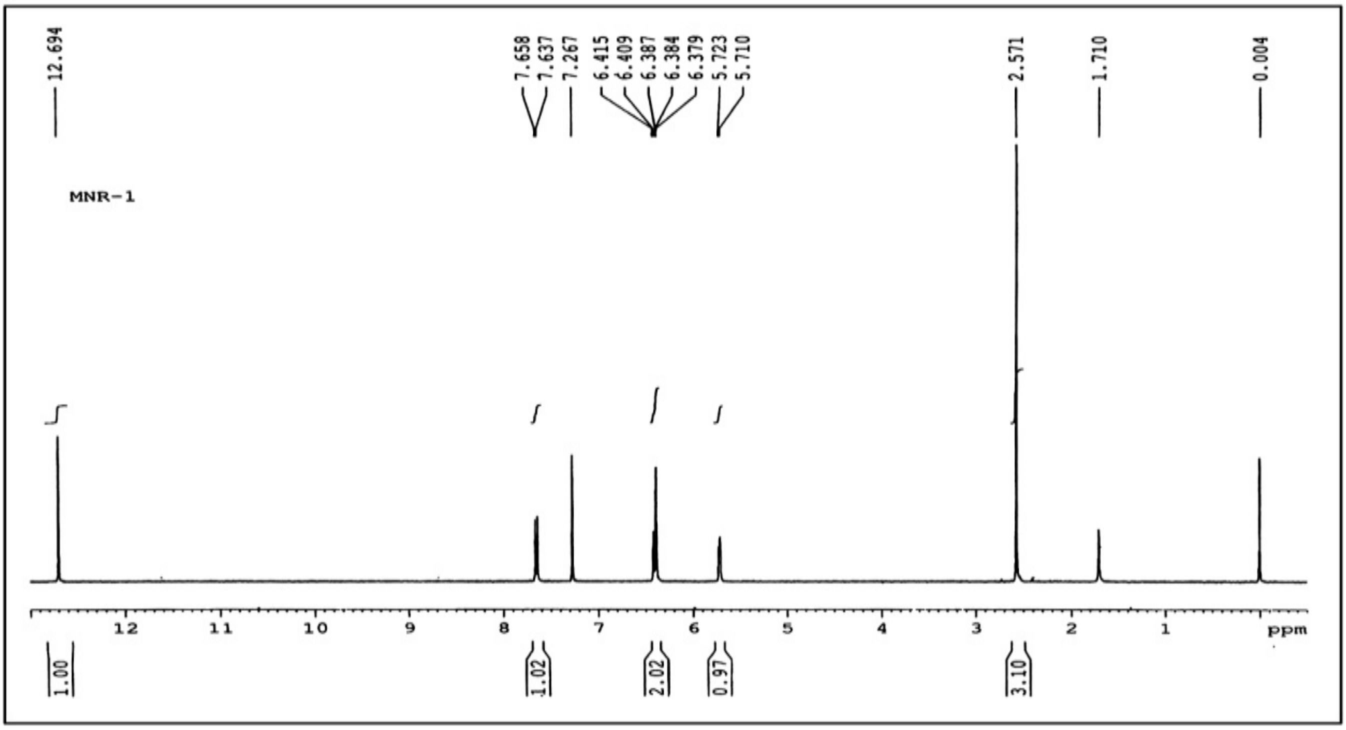


Fig4: NMR Spectrum of 2,4 DHAPP

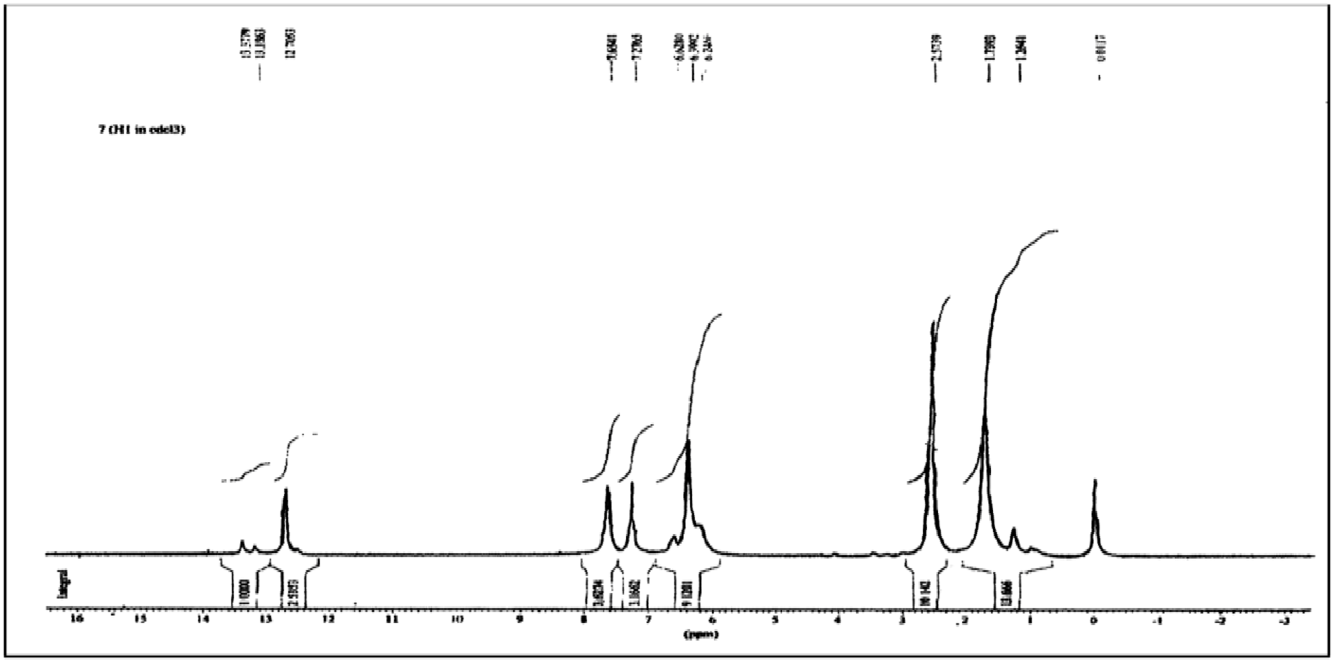


Fig5: NMR Spectrum of Cu (2,4 DHAPP) $_2$ complex

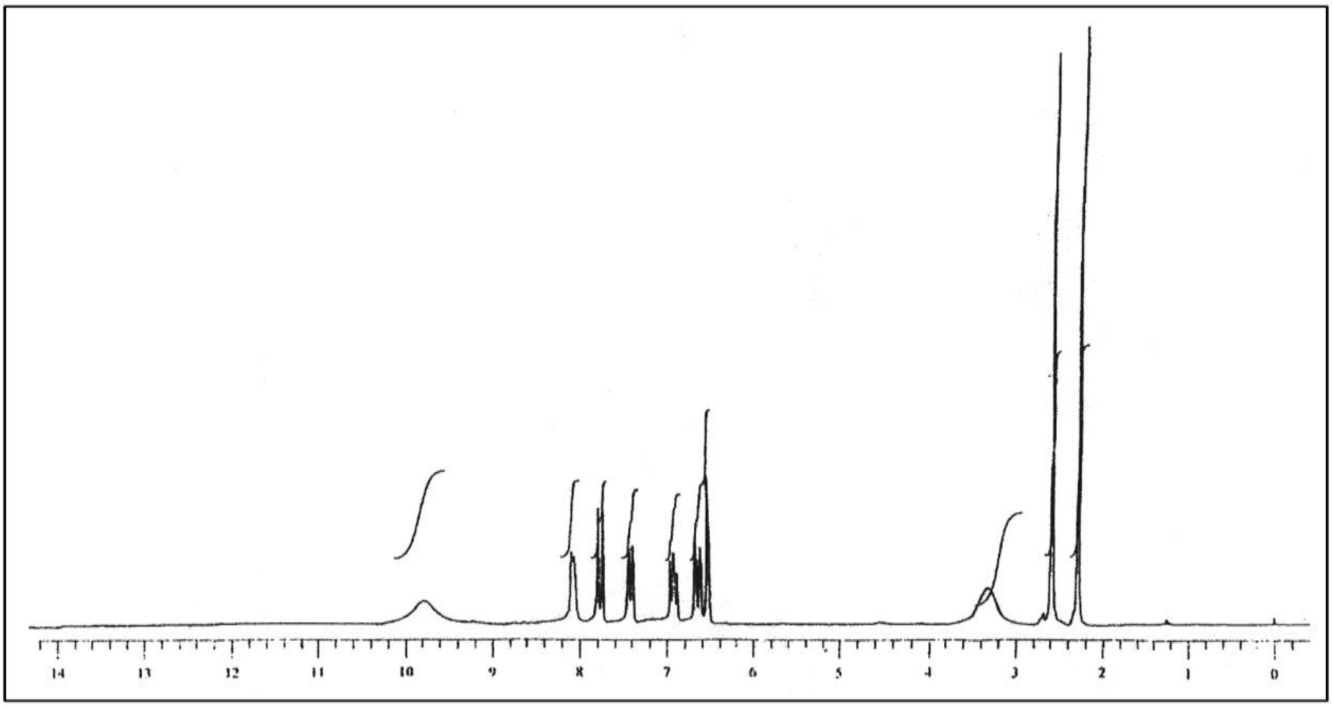


Fig 6: NMR Spectrum of Ru (2,4 DHAPP) $_2$ complex

Table-3: ^{1}H NMR Spectrum of the ligands and its metal complexes in DMSO-d6 in ppm

Compound	CH_3-C=N	Ar-H	OH-H2O	CH2	Ar-OH	N-H
2,4DHAPP	2.71	6.37-7.65	-	1.70	5.71	12.69
Cu(2,4DHAPP) $_2$	2.573	6.64-6.62	4.7	1.73	7.27	12.7
Ru(2,4 DHAPP) $_2$	2.4	6.41-7.59	4.9	1.81	7.2	8.35

Table -3 illustrates that Ligand showssingletat2.71ppm [21] due to protons bonded to the Schiff base group. On complexation, this band was shifted to low field regions 2.573ppm and 2.4ppm for Cu (II) and Ru (II) complexes, respectively. This shifting indicates the shielding of azomethine. The singlet that appeared at 1.70 ppm is attributed to the methylene protons of the cyclohexane ring. Another doublet was observed at 5.71-5.72 ppm for $^{\text{the}}$ ^{1}H NMR spectrum of the phenyl ring's O and P- hydroxyl[22] proton. A multiplet is observed in the region 6.37-7.65 due to the aromatic C-H protons of the phenyl ring. A singlet that appeared at 7.2 ppm is attributed to the C-H proton attached to the phenyl ring in the ligand. The doublet appeared at 7.63-7.65 ppm due to two C-H protons attached to the phenyl ring. The singlet appeared at 12.69 ppm due to the N-H proton of the ligand. In the ^{1}H NMR spectrum of the RAPP –Cu complex, a signal appeared due to methyl protons attached to the azomethine group having been shifted to 2.573 ppm compared to 2.71 ppm in the case of ligand. This downfield shift indicates the deshielding of azomethine proton on coordination through the nitrogen atom of the azomethine group. The signal observed at 1.73 ppm due to the methylene protons in the cyclohexane ring ligand is shifted to 1.26-1.73 ppm for the (Cu) complex. A singlet was observed at 7.27 ppm[23] due to the para hydroxyl proton shifting for the Cu complex. The signal disappeared at 5.71-5.72 ppm due to the phenolic hydroxyl proton being absent in the NMR spectrum of the (Cu) complex, indicating the hydroxyl group's deprotonation and the involvement of that oxygen in coordination. The multiplet observed in the region 6.37-7.65 ppm due to aromatic protons for the ligand showed a shift to 6.64-6.62 ppm for the (Cu) complex may be due to the drifting of a ring of electrons towards the metal ion. A signal observed at 12.69 ppm in ligand due to N-H proton is shifted to 12.7 ppm for Cu complex. A signal was observed at 13.18-13.37 ppm due to the O-H proton of water molecules present in the complex. In the ^{1}H NMR spectrum of the RAPP-Ru complex, a signal appeared due to methyl protons attached to the azomethine group having been shifted to 2.4 ppm in the case of ligand. This downfield shift indicates the deshielding of azomethine proton on coordination through the nitrogen atom of the azomethine group. The signal observed at 1.81 ppm was due to the cyclo hexane protons of the Ru complex. The signal disappeared at 5.71-5.72 ppm because the phenolic hydroxyl proton is absent in the Ru complex's NMR spectrum, indicating the deprotonation of the hydroxyl group and the involvement of that oxygen in coordination. A new signal is observed at 4.8-4.9 ppm in the case of the Ru complex, indicating the presence of water molecules coordinated with the metal atom. The multiplet observed in the region 6.37-7.59 ppm due to aromatic protons for the ligand showed a shift to 6.41 -7.59 ppm for the Ru complex may be due to the drifting of a ring of electrons towards the metal ion. A signal was observed at 8.35 ppm due to the N-H proton for the Ru complex.

3.4. *Conductivity measurements*

The molar conductance of complexes in DMF (~$10^{-}3$ M) was determined at 27+20C using Systronic 303 direct reading conductivity bridge. A known amount of solid complexes is transferred into a 25ml standard flask and dissolved in dimethyl form amide (DMF). The contents are made up to the mark with DMF. The complex solution is transferred into a clean and dry 100 ml beaker.

Table 4: Conductance data for Metal-2, 4 DHAPP Complexes: Cell constant: 1.00

S.No.	Metal Complex	Conductance Ohm-^{1}	Specific Conductance Ohm^{-}1 cm^{-1}	Molar Conductance Ohm^{-}1 cm2 mol^{-1}
1.	Cu(2,4DHAPP)$_2$	0.00017 x 10^{-3}	0.00017 x 10^{-3}	17
2.	Ru(2,4DHAPP)$_2$	0.00019 x 10^{-3}	0.00019 x 10^{-3}	19

Table-4 illustrates that the molar conductance of the complexes was less than 20 Ohm^{-1} cm^{2} mol^{-1}, indicating the Non-electrolytic nature. These values suggest the non-electrolytic nature of the present complexes.

3.5. *Electronic spectra*

In UV-Visible electromagnetic radiation, the transitions are associated with the electronic energy levels of the compound under investigation. The electronic spectra were recorded on a Thermo Spectronic Heylosa spectrophotometer. The transition metal ions occur in a variety of structural environments. Because of this, the electronic structures are extremely varied. The electronic structures have been identified with UV-Visible spectroscopy.

Table 5: Electronic spectral data

Complexes	λ max of the complex in nm	λ max of the ligand in nm
Cu(2,4 DHAPP)$_2$	316	292
Ru(2,4 DHAPP)$_2$	330	292

Table-5 illustratesthe electronic spectra of ligands and their metal complexes were reported. Ligand shows the signal band at 292 nm, assigned to π-π^{*}transitions. In complexes, this band was shifted to higher wavelength regions. New bands were observed in the complexes corresponding to the charge transfer transitions. In high-concentration spectra of complexes, d-d transitions were observed in the visible region.

3.6. *Electronic spin resonance spectra*

In the present study, the X-band (~9.3GHz) ESR spectra of Cu(II) complex in DMF were recorded at room temperature and liquid nitrogen temperature (LNT) on a JES-FA SERIES spectrometer. DPPH radical was used as a field maker. The ESR spectra of the complex are shown in Fig.7

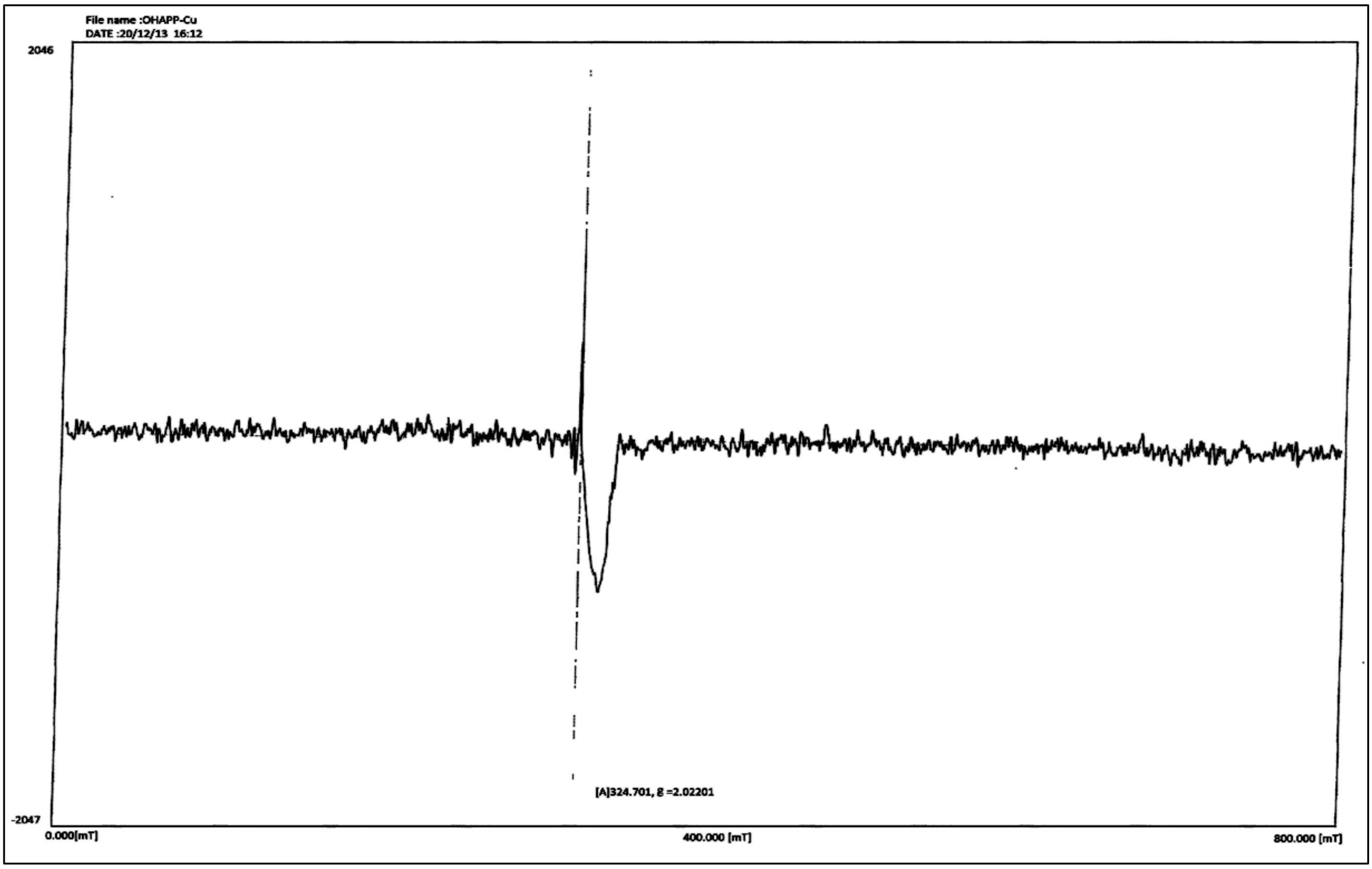


Fig7: ESR Spectra of Cu (2,4 DHAPP)$_2$

3.7. *Analysis of 2,4 DHAPP through ESR spectra of Cu (II) complex*

The ESR spectra of the complex in a polycrystalline state exhibit only one broad signal, which is attributed to dipolar broadening and enhanced spin-lattice relaxation. Anisotropic spectra obtained for these complexes in DMF at LNT is a low-temperature spectrum; four peaks of small intensity have been identified, which are considered to originate from the $g_{\parallel}$ component. The spin Hamiltonian, orbital reduction and bonding parameters of the Cu(II) complex are presented in Table- 6. The $g_{\parallel}$ and $g_{\perp}$ are computed from the spectrum using DPPH free radical as a g marker. Kvelson&Neimanhave reported that the $g_{\parallel}$ value is less than 2.3 for covalent character and is greater than2.3forioniccharacterofthe metal-ligand bond in complexes. Applying this criterion, the covalent bond character can be predicted to exist between the metal and the ligand complexes [24].

Table 6: Spin Hamiltonian and orbital reduction parameters of copper and Manganese complexes in DMF solution	
Parameters	**Cu(2,4DHAPP)$_2$**
$g_{\parallel}$	2.04559
$g_{\perp}$	1.99000
gave	2.00853
G	3.8201
$A_{\parallel}$ *	0.0182
$A_{\perp}$*	0.0022
Aave *	0.0081
d-d	13500
$K_{\parallel}$	0.8891
$K_{\perp}$	0.9824
P*	0.036
α 2	0.420

Table-6 illustrates that The trend $g_{\parallel}$>gave> $g_{\perp}$> 2.0023 observed for the complex, suggesting that the unpaired electron is localized in dx2- y2 and dz2 orbital of the copper (II) ions for the complex. It is observed that the G value for these complexes is greater than four and suggests that there are no interactions between metal-metal centers in the DMF medium. The ESR parameters $g_{\parallel}$, $g_{\perp}$, $A_{\parallel}$*, and $A_{\perp}$* of the complexes and the energies of d-d transitions are used to evaluate the orbital reduction parameters ($K_{\parallel}$,$K_{\perp}$), the bonding parameters (α^2), and the dipolar interaction(P)[25].The observed $K_{\parallel}<K_{\perp}$ indicates the presence of out-of-plane Pi-bonding. Theα^2valuesforthepresentchelateslieinthe range 0.42-0.48 and support the covalent nature of these complexes. Giordano and Berman suggested identifying bonding groups from the values of the dipolar term P. The reduction of P values from the ion value (0.036cm-^{1}) might be attributable to the strong covalent bonding. The P values obtained for the present complexes in between 0.029-0.036cm^{-1} and remain consistent with the bonding of metal ions to oxygen and nitrogen donor atoms, respectively. The shape of ESR lines, ESR data, and electronic spectral data suggest an octahedral geometry for these complexes [26].

3.8. *Magnetic susceptibility measurements of copper (II) and Ruthenium (II) complexes*

The effective magnetic moment values for all the complexes are represented in Table.7. There are considerable orbital contributions and effective magnetic moments for octahedral complexes at room temperature.

Table-7: Magnetic moments of cupper and Manganese

S.No.	Metal Complexes	Effect. In B.M.	
		Theoretical	Observed
1.	Cu(2,4 DHAPP)$_2$	4.90	4.82
2.	Ru(2,4 DHAPP)$_2$	1.68	1.79

Table-7 illustrates that The magnetic moments of the present (2,4 DHAPP)$_2$ Cu complex is 4.82 B.M., and the value is less than the spin-only value; it shows reduced Para magnetism, which suggests the formation of a low-spin complex having octahedral geometry. The magnetic moments of the present (2,4 DHAPP)$_2$ Ru complex is 1.79 B.M. This value is less than the spin-only value, showing reduced para magnetism, which suggests the formation of a low-spin complex having a square planar.

3.9. *Thermal analysis*

The thermal studies of these complexes are carried out to know the stability of the complexes on thermal decomposition, as well as to know the different final products obtained in thermal decomposition having novel catalytic properties. Thermo analytical data of metal complexes are given in Table.8. The representative thermo grams are shown in Fig.8 and 9.

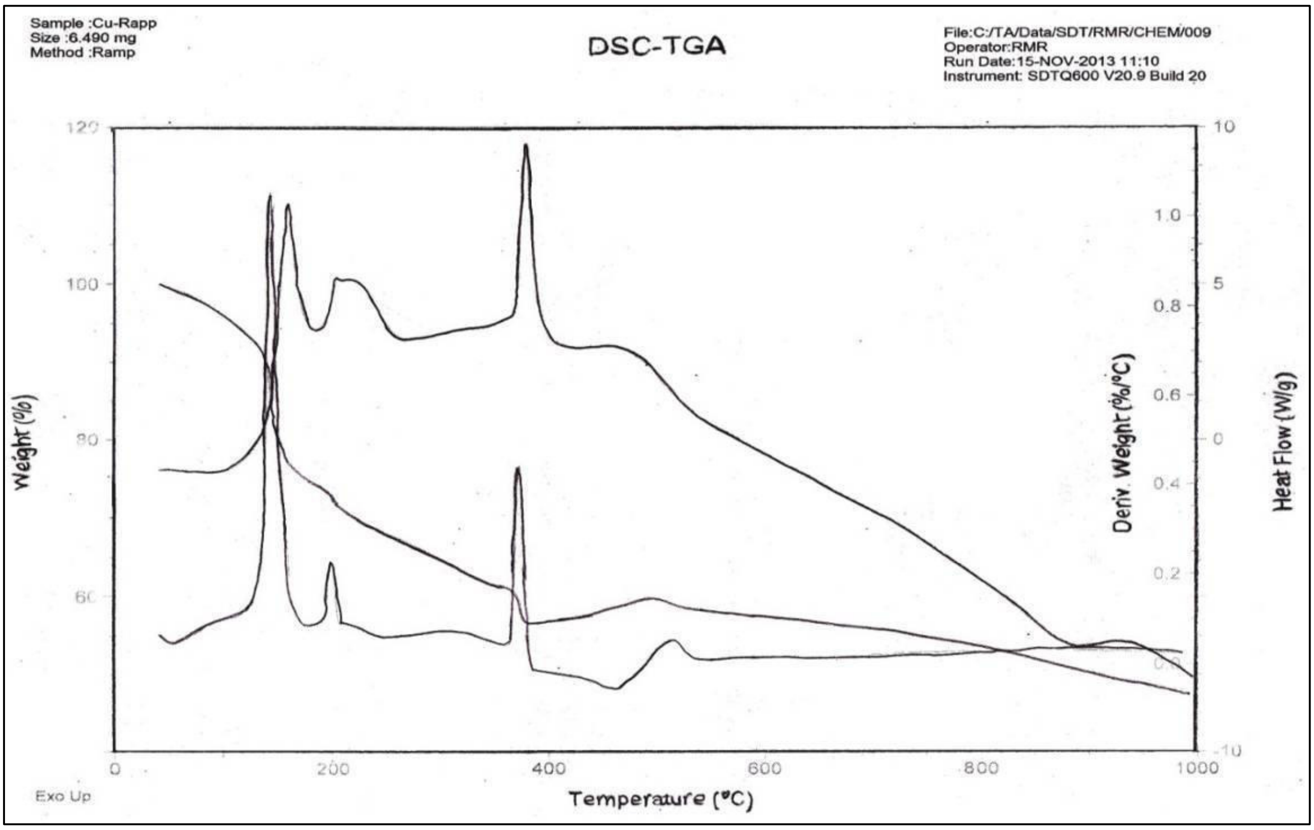


Fig 8: TG & DTA Spectrum of Cu (2,4 DHAPP)$_2$

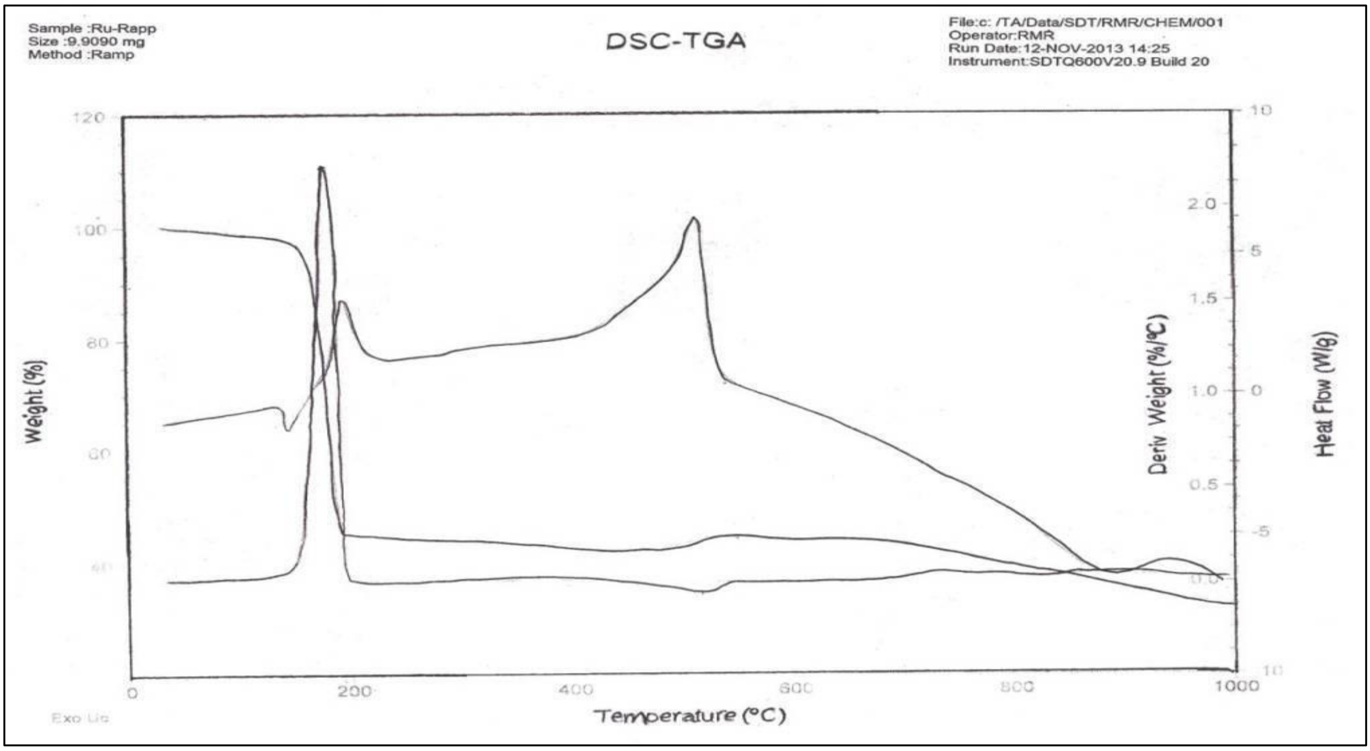


Fig 9: TG & DTA spectrum of Ru (2,4 DHAPP)$_2$

3.10. Study of 2,4 DHAPP and its Cu (II) and Ru (II)metal complexes by TGA-DTA spectra

Thermo analytical data of metal complexes were given in Table.8. Table-8 illustrates that the Cu complexes are thermally stable up to 160^{0}C. The first stage of the decomposition corresponds to endothermic dehydration of the complex, and the two lattice water molecules are lost in the temperature range 160-1700C to give an anhydrous complex[27].The second decomposition stage with two endothermic is known as a stable intermediate formed around 180-350 ^{0}C[28-30.] Finally, exothermic decomposition expresses to give the corresponding metal oxides as the final decomposition product at a high temperature, i.e., above 360-720^{0}C. The decomposition behavior of the complexes was observed in the nitrogen atmosphere.

Table 8: Thermal analytical data of the Ligand and their metal complexes

Complex X=H_2O	Molecular weight in gms	Weight of the complextake in mgs	Temperature Range during weight loss in ^{0}C	%offractionof weight loss	Probable assignment
[$Cu.L_2.X_2$] L=$C_{18}H_{23}N_3SO_2$	789.48	8.1910	140-160.46 200-380 500-850	12.95 42.89 7.6	Loss of $2H_2O$ molecule. Loss of two L molecules. Remaining residue Corresponds to CuO
[$Ru.L_2.X_2$] L= $C_{18}H_{23}N_3SO_2$	828.01	12.1630	150-170 180-350 Above 520	10.56 57.16 10.39	Loss of $2H_2O$ molecule. Loss of two L molecules. Remaining residue Corresponds to RuO

Table-8 illustrates that the Ru complexes are thermally stable up to 150^{0}C. The first stage of the decomposition corresponds to exothermic dehydration of the complex, and the two lattice water molecules are lost in the temperature range of 150-170^{0}C to give anhydrous complexes. The second decomposition stage with two exothermic is a stable intermediate formed between 180-350^{0}C.Exothermic decomposition expresses to give the corresponding metal oxides as the final decomposition product at a high temperature, i.e., above 520^{0}C. The decomposition behavior of the complexes was observed in the nitrogen atmosphere. All the experimental mass loss has shown in Table.8. At high temperatures, the corresponding metal oxides were formed as stable products. All the experimental percentage mass loss was compared with the calculated weights. Based on thermal data, it was shown that the stability order of the complexes was Cu (II)> Ru (II).

3.11. DNA Binding studies of newly synthesized Metal complexes

- ***Present Studies***

In the present study, DNA interactions were studied with newly synthesized Cu and Ru complexes of RAPP using absorption spectrophotometric titrations, and the results are presented below.

- ***Method Employed***

UV–Visible spectral studies monitored the interactions of all newly synthesized complexes. UV – Visible spectroscopy is the best tool for studying interactions of complexes[31] with DNA. Studies on DNA drug interactions are important for several purposes. If a metal complex acts as a DNA probe, its spectroscopic properties should change upon binding with DNA. These variations in spectroscopic properties provide excellent data about DNA conformation and structure. Hypochromicity in absorption, the appearance of isosbestic points, red–shift in the absorption maxima, and luminescence increase is the special characteristic features of DNA interactions. In some cases, the hyperchromic shift is also observed upon the successive addition of calf–thymus DNA to the complexes. Therefore, a change in absorbance upon the addition of DNA has been indicative of the binding of the complexes with DNA.

- ***Chemical required***

Calf thymus DNA, Buffer solution, DMSO, newly synthesized complexes

- ***Maintenance and Sterility***

All the required apparatus was sterilized before use, and every reasonable precaution was taken to avoid contamination throughout the operation.

- ***Procedure***

Disodium salt of calf thymus DNA was stored at 5°C. The solution of DNA in the buffer 45 mMNaCl/5 mMTris HCl (pH 7.1) in water gave a ratio of 1:9 of UV absorbance at 260 and 280 nm. A260/A280 indicating that the DNA was sufficiently free from protein [28]. The concentration of DNA was measured by using its coefficient at 260 nm (6600 Cm^{-1}) after 1:100 dilutions. Concentrated stock solutions of the complexes were prepared by dissolving in DMSO and diluting suitably with the corresponding buffer to the required concentrations for all the experiments. Absorption spectra were recorded in 240–400 ranges. The ranges were selected where maximum absorption change was observed on adding DNA.

- ***Experimental studies***

The absorption spectra of complexes were compared in the absence and presence of CT–DNA. In the presence of increasing amounts of DNA, the spectra of complexes showed a strong decrease (hypochromic) in intensity with a shift in absorption maxima towards higher (redshift) wavelengths, as shown in Fig.10 and Fig.11.

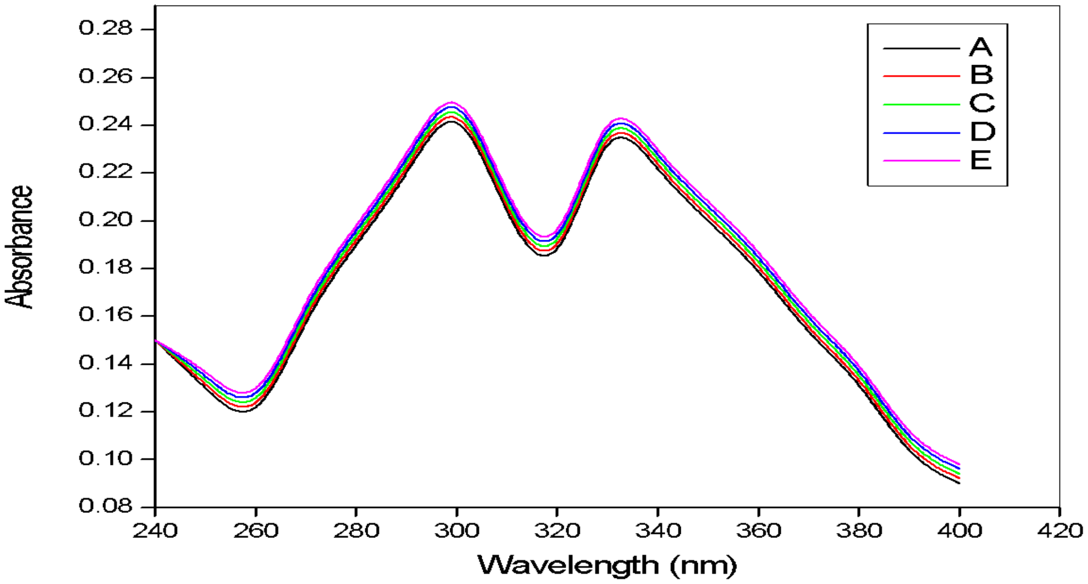


Fig 10: DNA Binding interaction of Cu (2,4 DHAPP)$_2$

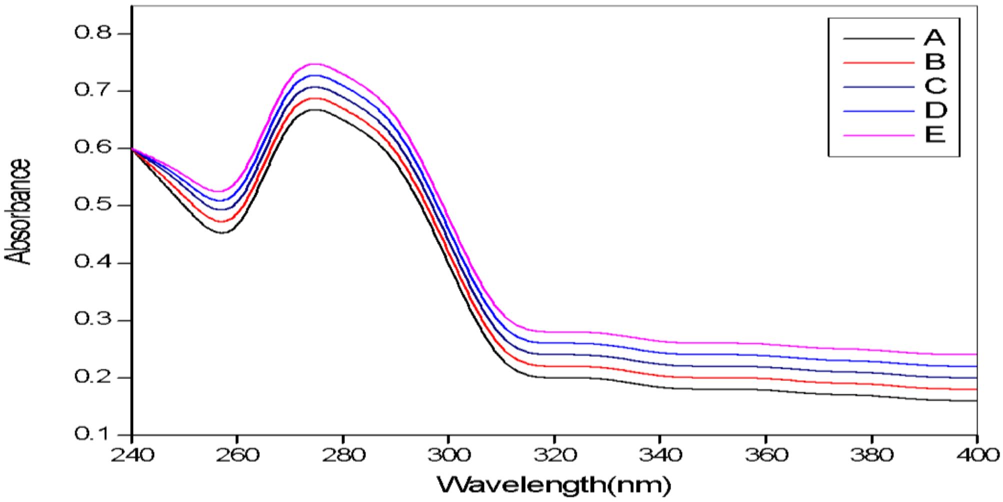


Fig.11. DNA Binding interaction of Ru (2,4 DHAPP)$_2$

The binding of intercalative molecules[32] to DNA has been well characterized by large hypochromic and significant red–shift due to strong stacking interaction between the aromatic chromophore of the ligand and DNA base pairs with the extent of hypochromic and redshift commonly consistent with the strength of interactive interaction. The intrinsic binding constants $K_b$of the complexes for binding with CT DNA were obtained using the equation to compare the DNA binding affinities quantitatively.

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

Where [DNA] is the concentration of DNA in base–pairs, ε_a is the apparent extinction coefficient obtained by calculating A_{obs}/[complex], ε_f corresponds to the extinction coefficient of the complex in its free form, and ε_b refers to the extinction coefficient of the complex in the bound form. Each set of data, when fitted to the above equation, gave a straight line with a slope of 1/ (ε_a–ε_f) and a y–intercept of 1/K_b (ε_b– ε_f), and K_b was determined from the ratio of the slope to intercept—the intrinsic binding constants K_b, obtained for the complexes. Electronic absorption spectral data upon the addition of CT–DNA and binding constants were given in Table.9.

Table.9. DNA binding constants of metal complexes

S.No	Complex (X= H_2O)	λ max nm		Δ λ nm	H%	$K_b(M^{-1})$
		Free	Bound			
1	[Cu (2,4 DHAPP)$_2$X$_2$]	291	298	7	6.33	2.00×10^5
2	[Ru (2,4 DHAPP)$_2$X$_2$]	272	278	6	6.03	2.50×10^5

Table -9 illustrates that it was evident that these complexes bind with DNA with high affinities, and the estimated binding constants are in the range of 10^5–10^6 M^{-1}. It may be due to pi–the stacking of the pyridine ring in the ligand moiety.

4. CONCLUSION

The above results' outcome confirms the complexes' stoichiometry to be 1:2 [M: L] as indicated by elemental analysis and Conduct metric measurements. Furthermore, IR spectra suggest that the ligand behaves as bidentate and coordinates to the central metal ion through azomethine nitrogen and phenolic –OH group. It has been further confirmed based on NMR spectral studies. Thus, based on the above physicochemical and spectral studies, the complexes have DNA binding properties compared to the respective ligand and the parent drug, which justifies the research work's purpose. The present work will extend to synthesizing metal complexes using other biologically active metals and evaluating their biological activities. All the metal complexes carry on charge and are thermally stable. As such, no single technique is independent of predicting the final structures of the complexes.

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6. AUTHORS CONTRIBUTION STATEMENT

T. Noorjahan Begum and A. Jayaraju conceptualized and gathered the data about this work. Then, Dr. K. Aruna gathered the data from DNA Binding studies, and necessary inputs were given towards the design of the manuscript. Finally, all authors discussed the methodology and results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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