

Spectral Analysis, Invitro Biological Evaluation and DNA Studies of OHAP-At Metal Complexes

Paper Submission: 14/08/2020, Date of Acceptance: 29/08/2020, Date of Publication: 30/00/2020

Abstract

The aim of the present article is spectral analysis, Biological evaluation (invitro conditions) and DNA binding activity of newly synthesized metal complexes of Atomoxetine and its Cu (II), Ru (III) metal complexes. Analysis results from various techniques (UV-VIS, ESR, FT-IR, $^1\text{H-NMR}$, VSM and CONDUCTOMETRY) supposed the complexes are non electrolytes with stoichiometric ratio of 1:2 and exhibit octahedral geometry. TG/DTA analysis results suggested the complexes are thermally stable at high temperatures. Ligands showed weak biological activity and the metal complexes more. By the presence of chromophores in the complexes they can participated in DNA binding activity, it was identified by UV-Visible spectrometry.

Keywords: OHAP (Ortho Hydroxy Acetophenone), Methanol, Biological Evaluation and DNA Binding Activity.

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Introduction

Imines are synthesized by Hugo Schiff in 1864; these are the key points for the synthesis of metal complexes. Schiff bases can also used as intermediates in polymer synthesis and drug designing. Imines can also be named as Azo methines or Schiff bases or Anils. The characteristic functional group is $>\text{C}=\text{N}$, an important to co-ordinate with electron deficient metal ions and to exhibit biological geometry. They have enormous applications like detection, determination and identification of carbonyl compounds in organic studies and also to check the purity of amino compounds and carbonyl compounds.

Review of Literature

Metal complexes have expansive applications in various fields like medical, electrochemistry, they possesses anti bacterial, antifungal and anti inflammatory activities and also used as catalysts [1- 9].

Hydroxy ketones and aldehydes play an important role in Co-ordination chemistry for the synthesis of metal complexes with Transition elements [10, 11]. Transition metal ions have biological activity, so one can use to enhance biological activity of the complexes by condensing with Imines. Metal complexes of OHAP have more applications in pharmaceutical and biological areas like anti pyritic, anti-cancer, inflammatory and the field of biochemical, analytical and clinical [12 – 17].

By insight these applications author's trust to synthesis, spectral investigation, biological evolution and DNA studies of the metal complexes of OHAP –AT metal complexes of Cu (II) and Ru (III) metal ions. The metal complexes are very new to the literature. Imine was prepared by condensing OHAP (Ortho hydroxy Acetophenone) with Atomoxetine by adding few drops of concHCl by conventional method.

The analysis has been exhausted by various instrumental techniques like FT-IR, $^1\text{H-NMR}$, ESR, UV-VIS, XRD, CONDUCTOMETRY, TG/DTA &VSM to know electrolytic nature, stoichiometry and mode of bonding, geometry and thermal stability of the complexes. The ligand and its metal complexes screened for biological activity with Escherichia coli, Bacillus Subtilis and Klebsiella. DNA binding mode of the complexes can be identified by UV-VIS spectroscopy.

Objective of the Study

1. Comparison of experimental Elemental analysis with Calculated Elemental analysis.
2. Calculation of diffractograms values.

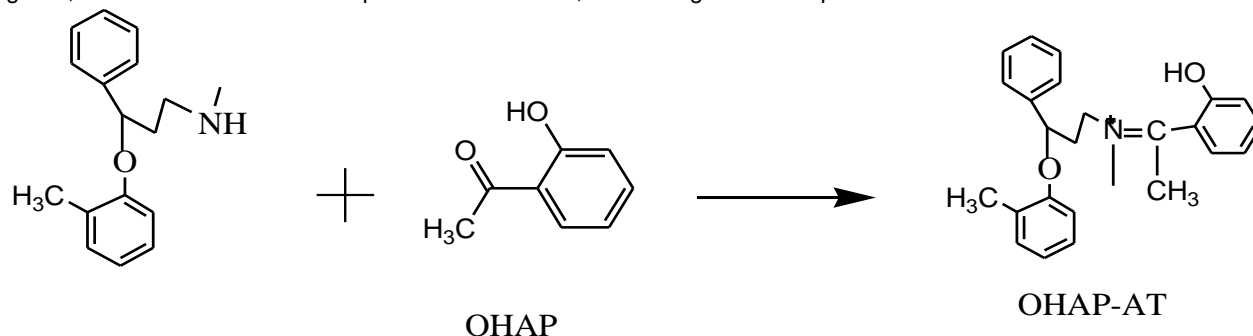
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- Finding thermal stability of metal complexes by Thermo gravimetric Analysis.
- Crystalline nature of the complexes by X-Ray Diffraction studies.
- Identification of biological activity of ligands and their corresponding metal complexes by using bacterium like Escherichia coli, Bacillus Subtilis and Klebsiella in invitro conditions.

Experimental

All the chemicals purchased are anala a grade; the chemicals used at present are OHAP,



Scheme 1:- synthesis of OHAP-AT ligand

Metal Complexes Synthesis

The metal complexes were synthesized by mixing 1: 2 ratio of metal ions and imines separately in methanol and refluxed for six hours by adding few drops of con HCl. The solutions are kept in ice bath for two hours, parrot green and greenish brown colored sharp needle like crystals were obtained. These were washed and recrystallized with 50% methanol.

Atomoxetine, Methanol, Copper Chloride and Ruthenium Chloride.

Synthesis of OHAP-AT:

The ligand was synthesized by mixing of OHAP and AT (Atomoxetine); and heated for two hours by adding few drops of con HCl. This solution was kept in ice bath; pink color sharp needle like crystals are obtained. The crystals were washed and recrystallized with 50 % Methanol. The purity of OHAP-AT was found to be 80%. The synthesis of ligand was represented in the scheme 1.

Table 1: Elemental analysis of the ligands and their metal complexes

S.No	Name of the Complex	Color	M.W	% yield	% of "C"		% of "H"		% of "N"		% of "O"		% of "M"	
1.	OHAP-AT	Light pink	389.53	72.5	80.17	80.15	8.02	8.00	3.60	3.59	8.21	8.19	-	-
2.	OHAP-AT-Cu	Greenish brown	908.71	75.3	75.34	75.29	7.54	7.46	3.08	2.99	7.04	6.98	6.99	6.91
9	OHAP-AT-Ru	Greenish brown	962.23	76	72.35	72.25	7.24	7.18	2.96	2.89	6.76	6.69	10.68	10.61

Bolded values are calculated values

IR Spectral Data

IR spectra of Imine and its metal complexes were performed with KBr pellets. To understand co-ordination, the spectra of complexes were compared with ligands. The stretching vibrational frequency of Imine was observed at 1626 cm^{-1} and for metal complexes the frequencies at 1614 cm^{-1} and 1622 cm^{-1} , this change in frequency values revealed a good co-ordination between Imine group and metal ions. The vibrational frequencies at 620 cm^{-1} and 630 cm^{-1} specified co-ordination between metal and nitrogen atom of Imine group, another band at 438 cm^{-1} and

450 cm^{-1} specified co-ordination between metal atom and Oxygen moiety of hydroxy group of Ortho Hydroxy Acetophenone [18]. A strong band at 3363 cm^{-1} indicated the presence of Phenolic hydroxy group of Imine; which was absent in complexes brought in to open the co-ordination between metal ion and Imine. A broad band at 3304 cm^{-1} & 3433 cm^{-1} specified the presence of water molecules of the metal complexes, which was not appeared in Imines. The characteristic frequencies of OHAPAT ligand and its metal complexes are represented in table 2 and graphs from fig 1 – 3.

Table 2: IR Spectral data of OHAPAT Ligand and Metal Complexes

compound	ν OH Water	ν OH Phenolic	ν C=N	ν M-O	ν M-N
OHAPAT	-	3363	1626	-	-
OHAPAT-Cu	3304	-	1614	620	438
OHAPAT-Ru	3433	-	1622	630	450

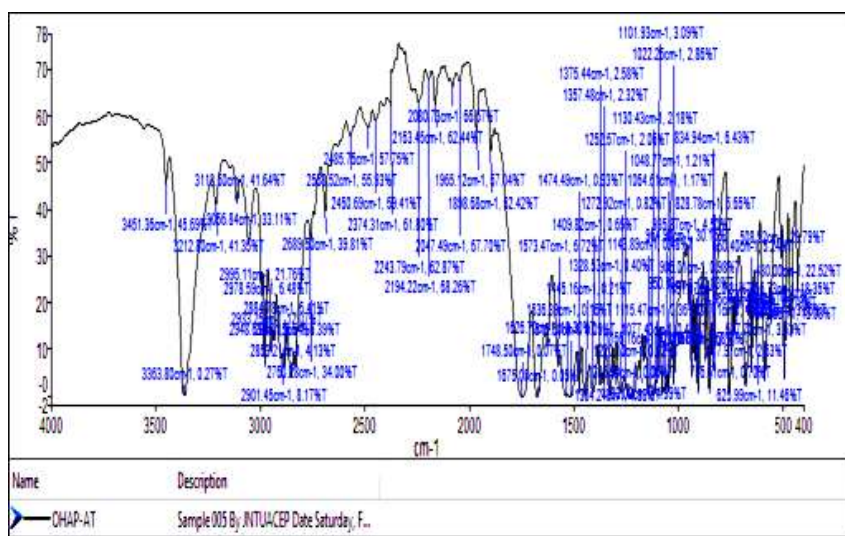


Fig 1: IR Spectra of OHAP-AT Ligand

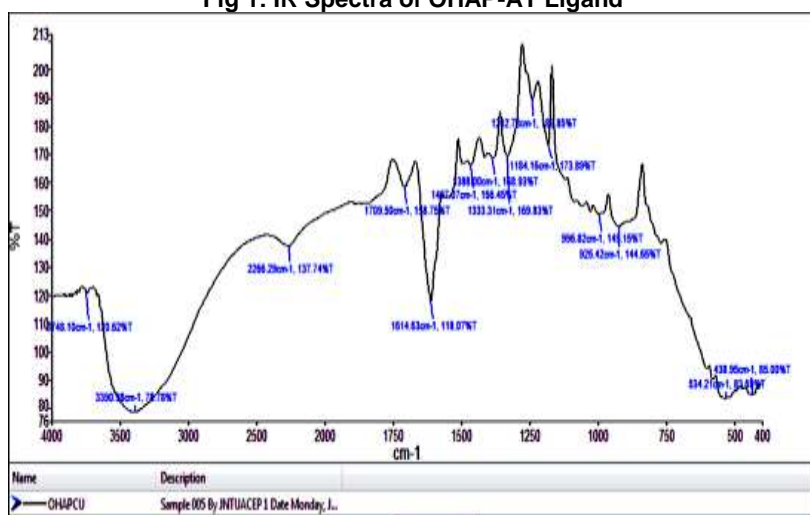


Fig 2: IR Spectra of OHAPAT-Cu metal complex

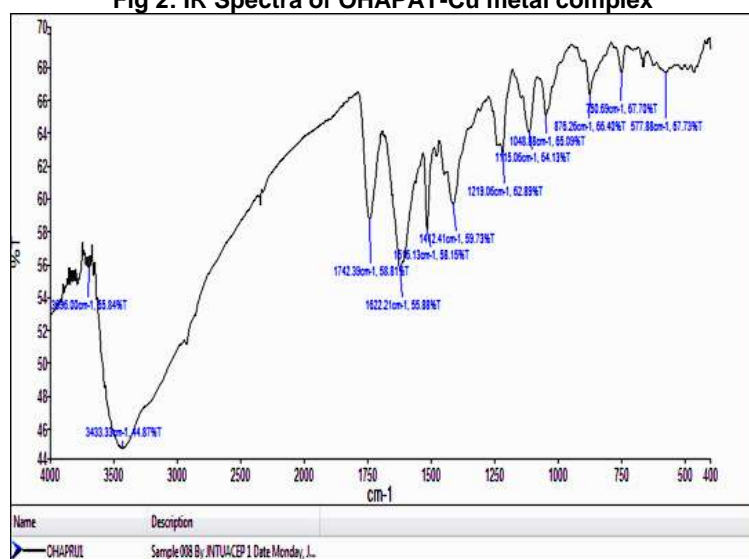


Fig 3: IR Spectra of OHAPAT-Ru metal complex

H¹NMR Spectral Data

An H¹-NMR spectrum was recorded at room temperature on BRUKER 400MHZ SUPERCON Spectrometer. The singlet at 4.8 ppm and 4.15 ppm disclosed the co-ordination of water molecule with metal ions of Copper and Ruthenium [19]. The singlet at 7.28 ppm specified the presence of hydroxy group of Imine, which was not developed in complexes confirmed the involvement of hydroxy group of ligand

in coordination with metal ions. The multiplet at 6.94-7.48 ppm clarified aromatic protons of Imine & which was changed to 7.5-7.8 ppm for the complex of Copper and Ruthenium [20]. The singlet signals at 2.0ppm, 2.10ppm and 2.1ppm for methyl protons of ligand and complexes of OHAPAT-Cu and OHAPAT-Ru. Chemical shift values of the ligands and their complexes were represented in the table 3 & graphs from 4 - 6.

Table – 3: H¹-NMR Spectral data of OHAPAT Ligand Its Cu & Ru Metal Complexes

S.No	Compound	eM-C=N	Ar-H	OH-Phenilic	OH-H ₂ O	-CH ₃
1	OHAPAT	2.0	6.94-7.48	7.28	-	2.5
2	OHAPAT-Cu	2.10	7.5-7.8	-	4.8	2.6
3	OHAPAT-Ru	2.1	7.5-7.6	-	4.15	2.0

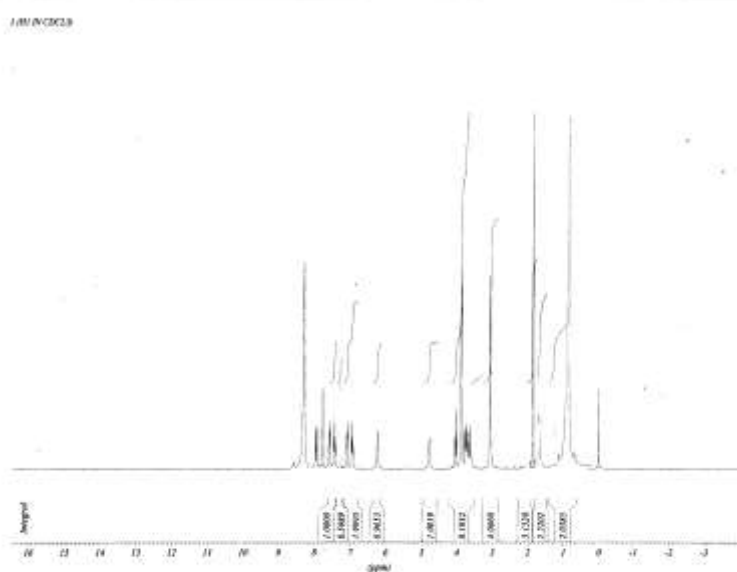


Fig – 4: NMR spectra of OHAPAT- Ligand

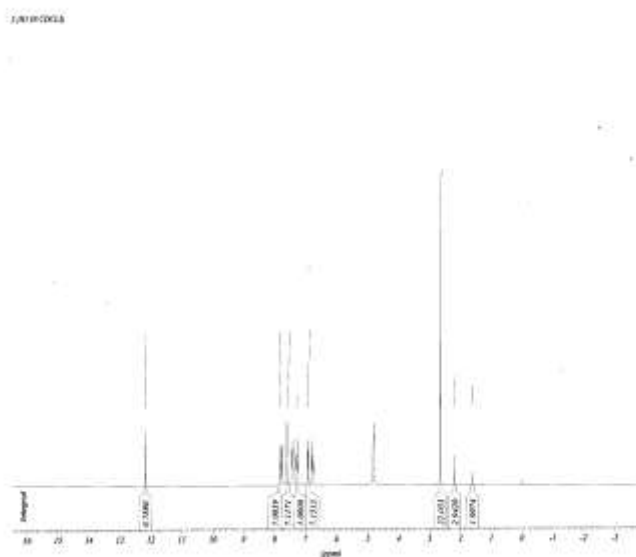


Fig – 5: NMR spectra of OHAPAT-Cu metal complex

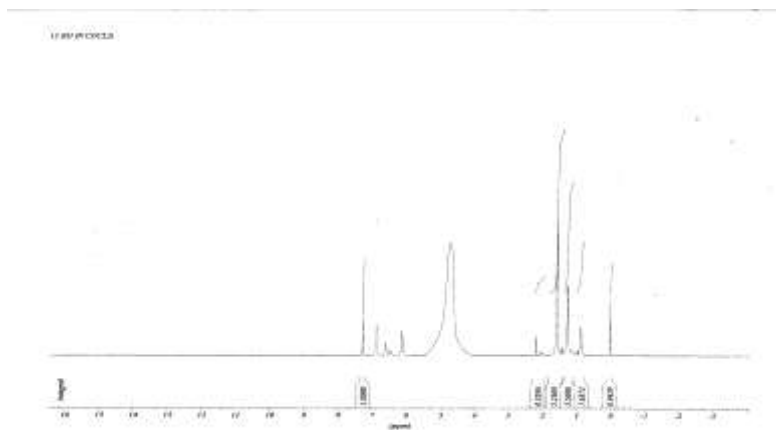


Fig – 6: NMR spectra of OHAPAT-Ru metal complex

ESR Spectral Studies

ESR spectroscopy gives about the presence of unpaired electrons of the complex. $g_{||} > g_{ave} > g_{\perp}$ values are greater than 2.0023 proposed $d_{x^2-y^2}$ & d_z^2 orbital unpaired electrons are delocalized for Cu & Ru ions respectively. “G” of the complexes at 4.5813 & 4.6123 revealed covalent nature of the complex [21], and it was supported by α^2 values [22]. $G > 4$ specified mono nuclear nature of the complex, out plane π -bonding can be stated by the values of $k_{||} < k_{\perp}$. Spin Hamiltonian and orbital reduction and bonding Parameters of OHAPAT-Cu and OHAPAT-Ru metal complexes are represented in the table 4 and Graphs from 7 - 8

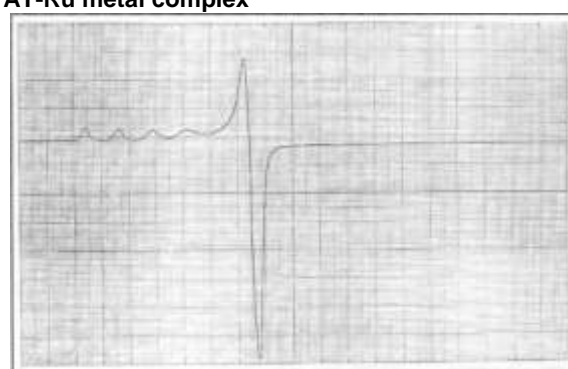


Fig 8: ESR spectra of OHAPAT- Ru
Electronic Spectral Data

Table 4: Spin Hamiltonian and Orbital Reduction And Bonding Parameters of OHAPAT-Cu & OHAPAT-Ru Metal Complexes

parameters	OHAPAT-Cu	OHAPAT-Ru
$g_{ }$	2.6860	2.8760
g_{\perp}	2.2613	2.3163
g_{ave}	1.702	1.8123
G	4.5813	4.6123
$A_{ }^*$	0.0089	0.0123
A_{\perp}^*	0.0094	0.0136
A_{ave}	0.0098	0.0142
$K_{ }$	0.792	0.842
K_{\perp}	0.813	0.913
P^*	0.0615	0.0765
α^2	0.3835	0.4036

The presence of an unsaturation and co-ordination between Imine and metal complexes can be identified by UV-Vis spectrophotometric technique. λ_{max} for Imine and metal complexes at 201nm, 250nm and 328nm specified the transfer of charge from ligand to metal suggested octahedral geometry of the complexes [23]. UV-Visible Spectral data of the ligand and its Cu (II), Ru (III) metal complexes is represented in table 5 and graphs from 9 – 11.

Table 5: UV-Visible Spectral data of the ligand & its Cu (II), Ru (III) metal complexes

S.No	Name of the compound	λ_{max}
1	OHAPAT	201
2	OHAPAT-Cu	250
3	OHAPAT-Ru	328

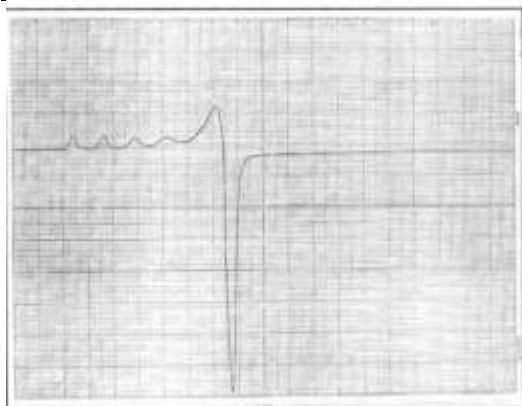


Fig 7: ESR spectra of OHAPAT- Cu

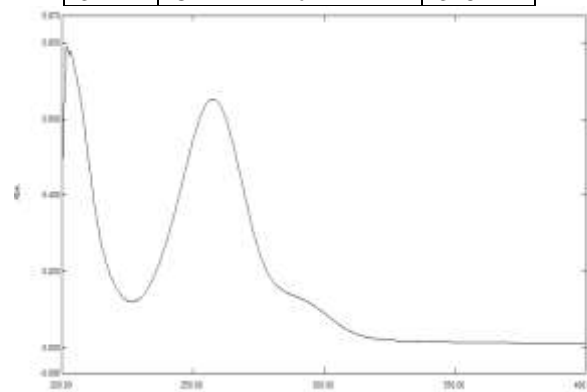


Fig 9: UV-Spectral data of OHAP-AT Ligand

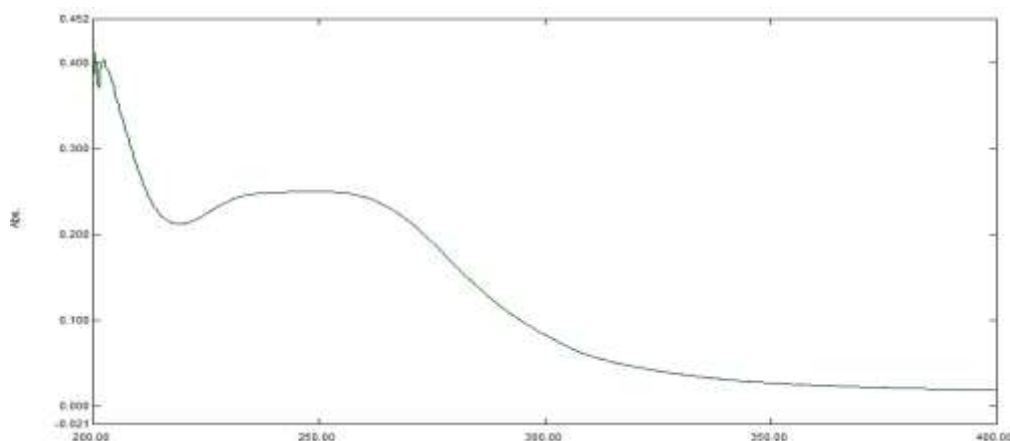


Fig 10: UV-Spectral data of OHAPAT-Cu metal complex

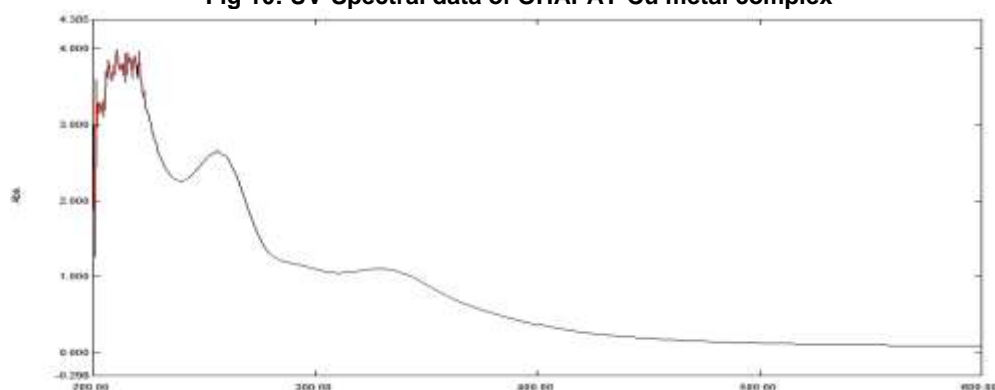


Fig 11: UV-Spectral data of OHAPAT-Ru metal complex

X-Ray Diffraction Studies

Crystalline nature of the complexes can be identified by X-Ray Diffraction studies. Diffractograms (3 – 86) from fig 12 & 13 considered 2θ values of the complexes of Cu (II) and Ru (III) ions suggested poor

crystallinity of the complexes [24]. Diffractograms and “D” values specified a good agreement between metal ions and Imine. The X-Ray Diffraction data of the complex was represented in table 6 - 7 and graphs from Fig 12 – 13.

Table 6: X-ray Diffraction study of OHAP AT-Cu metal complex

S.No	d exp	d cal	2θ exp	2θ cal	h k l
1	0.0272	0.0266	3.1213	3.1205	1 1 1
2	0.0396	0.0390	5.8971	5.8964	2 1 1
3	0.0535	0.0529	7.9790	7.9783	3 2 1
4	0.0671	0.0665	9.6523	9.6516	4 2 1
5	0.0813	0.0804	12.1229	12.1221	5 2 2
6	0.0887	0.0881	13.2280	13.2272	5 3 1
7	0.0955	0.0948	14.2473	14.2469	6 3 1
8	0.1079	0.1072	16.1108	16.1008	7 2 2
9	0.1199	0.1191	17.9105	17.9095	8 2 2
10	0.1259	0.1251	18.8193	18.8185	8 4 2
11	0.1374	0.1369	20.5558	20.5551	9 3 2
12	0.1416	0.1401	21.1892	21.1886	10 1 1
13	0.1459	0.1452	21.8460	21.8454	10 2 2
14	0.1609	0.1598	24.1295	24.1289	10 5 2
15	0.1862	0.1558	27.9885	27.9879	10 8 3
16	0.1924	0.1918	28.9297	28.9291	10 9 1
17	0.2092	0.2085	31.5319	31.5311	10 9 6
18	0.2175	0.2169	32.8807	32.8001	11 10 4
19	0.2344	0.2336	35.4361	35.4354	11 11 6
20	0.2691	0.2685	40.9110	40.9102	13 13 6
21	0.2939	0.2931	44.8734	44.8728	14 14 7
22	0.3720	0.3713	57.7687	57.7679	19 16 1
23	0.5225	0.5216	85.4457	85.4449	25 25 12
24	0.5263	0.5259	86.2063	86.2058	25 25 13

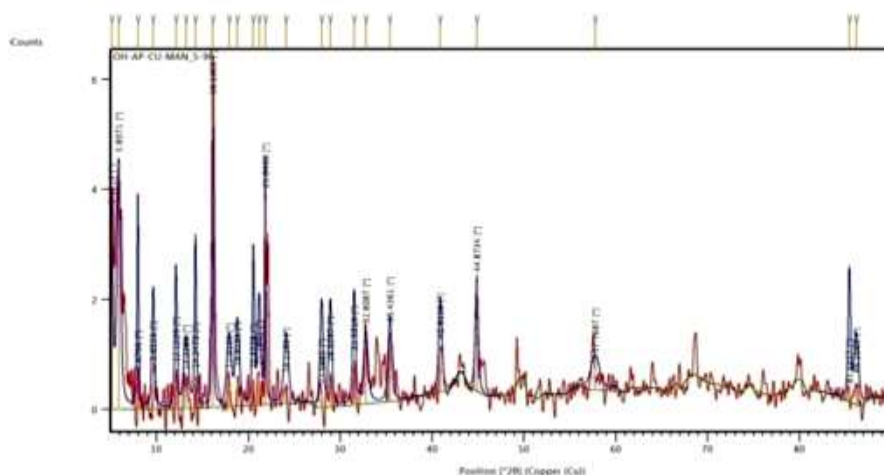


Fig 12: X-Ray Diffraction Spectrum of OHAPAT-Cu complex
Table 7: X-ray Diffraction study of OHAP AT-Ru metal complex

S.No.	d exp	d cal	2θ exp	2θ cal	h k l
1	0.0395	0.0389	5.8900	5.8895	1 1 1
2	0.0442	0.0436	6.5813	6.5809	2 2 2
3	0.0530	0.0524	7.8991	7.8986	3 2 2
4	0.0602	0.0598	8.9700	8.9693	4 1 1
5	0.0811	0.0806	12.0976	12.0971	5 3 1
6	0.1289	0.1283	19.2684	19.2679	6 6 4
7	0.1320	0.1316	19.7472	19.7468	7 6 1
8	0.1383	0.1376	20.6945	20.6940	8 5 3
9	0.1533	0.1529	22.9748	22.9744	8 7 3
10	0.1624	0.1619	24.3472	24.3468	8 8 2
11	0.1688	0.1681	25.3180	25.3176	9 7 3
12	0.1982	0.1976	29.8254	29.8249	10 9 3
13	0.2200	0.2196	33.1976	33.1969	12 9 4
14	0.2682	0.2676	40.7635	40.7629	12 12 9
15	0.2774	0.2769	42.2259	42.2255	12 12 10
16	0.4434	0.4429	70.3058	70.3053	19 18 17

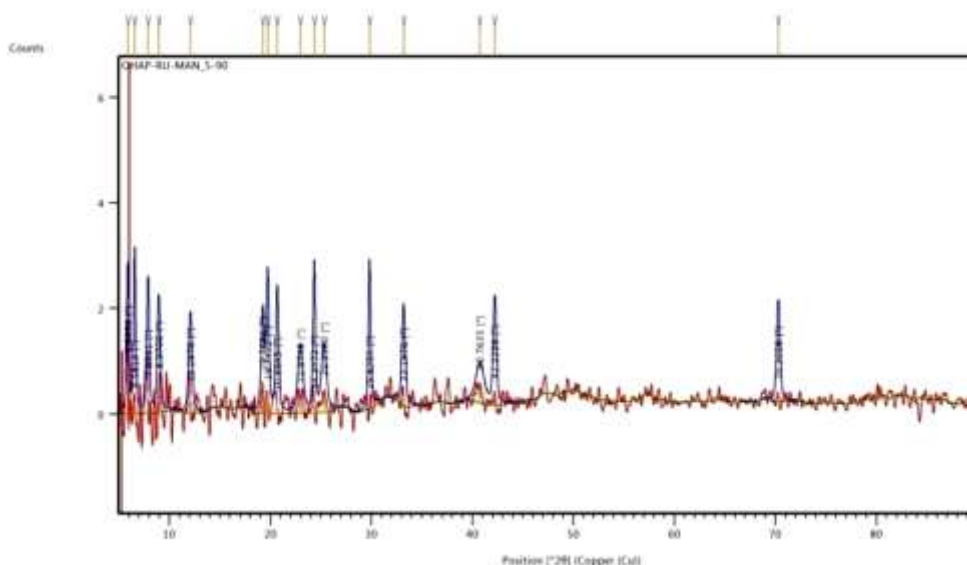


Fig 13: X-Ray Diffraction Spectrum of OHAPAT-Ru complex

Thermal Stability of the Complexes

Thermal stability of the complexes can be explained by change in the temperature range of three levels, first level decomposition at 87.47-

193.20^oC to 223.11^oC and 345.23-524.15^oC recommended dehydration due to the loss of water molecules from the complexes, second level decomposition at 262.91^oC and 345.23^oC suggested

the formation of intermediates (stable) and third level decomposition specified the loss of Imine moieties up to 500^oc and 544.57^oc; considerable temperature results confirmed the formation of stable oxides of Cu

and Ru ions by exothermic decomposition [25]. Thermal data of the complexes was represented in the table 8 and graphs from 14 - 15.

Table 8: Thermal Analytical data of OHAPAT-Cu & OHAPAT-Ru metal complexes

complex	Molecular weight (grams)	Temperature range in ^o c	Probable assignment	Mass loss (%)	Total mass loss (%)
OHAP AT-Cu	908.71	87.47-193.20 262.91-500 Above 500	Loss of two H ₂ O molecules Loss of two ligand molecules Formation of CuO	6.7 23.80 47.89	78.19
OHAP AT-Ru	962.23	225-290 345.23-524.15 Above 524.57	Loss of two H ₂ O molecules Loss of two ligand molecules Formation of RuO	5.12 28.69 49.16	82.97

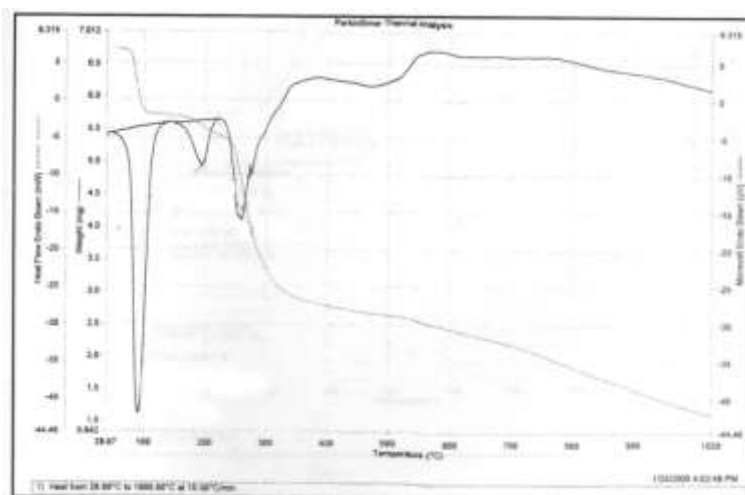


Fig 14: TG/DTA spectrum of OHAPAT-Cu metal complex

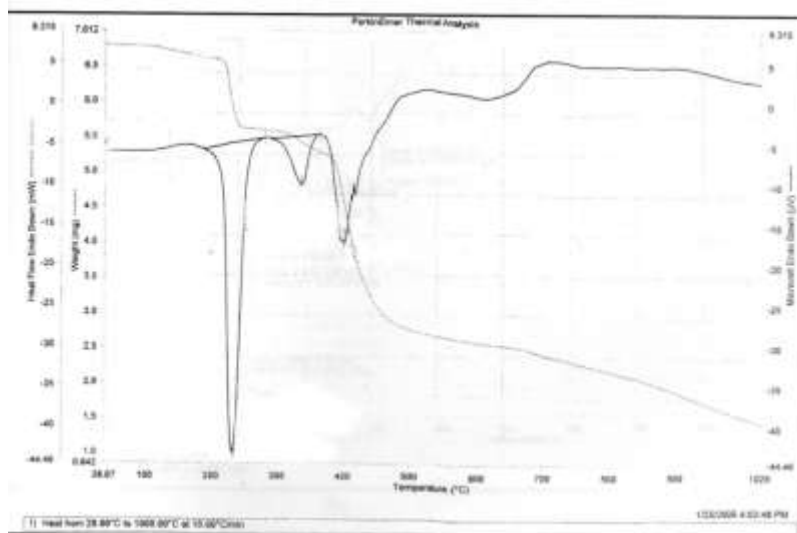


Fig 15: TG/DTA spectrum of OHAPAT-Ru metal complex

Conductometric Analysis

Conductometric analysis gives information regarding electrolytic nature of the complexes. Conductometric measurements of the complexes of

Copper and Ruthenium at 55 Ohm⁻¹cm²mol⁻¹ and 57 Ohm⁻¹cm²mol⁻¹ at 30^oc recommends the complexes were non-electrolytes [26]. The conductance values were represented in the table - 9

Table 9: Conductometric Analysis of OHAPAT-Cu and OHAPAT-Ru metal complexes

S.No	complex	Conductance Ohm ⁻¹ cm ² mol ⁻¹
1	OHAPAT-Cu	55
2	OHAPAT-Ru	57

Magnetic Susceptibility Measurements:

Magnetic momentum values at 4.13BM and 5.26BM confirmed that the complexes exhibit octahedral geometry by the presence of lone pair of electrons [27]. Magnetic momentum values are represented in the table 10.

Table 10: Magnetic momentum values of OHAPAT-Cu & OHAPAT-Ru complexes

S.No	complex	Magnetic momentum (BM)
1	OHAPAT-Cu	4.13
2	OHAPAT-Ru	5.26

Biological Evaluation

Biological activity of the complexes and ligands were performed by employing bacterium like Escherichia coli, Bacillus Subtilis and Klebsiella. As per Chelation theory, the growth of micro organisms is prohibited by increasing lipophilicity due to

delocalization of the charge. So, the complexes exhibit more anti bacterial activity than their corresponding ligands. Anti bacterial activity of the ligands and their metal complexes represented in the table 11.

Table 11: Anti Bacteria Activity of The Ligands and Their Metal Complexes

Compound	E-Coli	Klebsiella	Bacillus
OHAPAT	9	12	14
OHAPAT-Cu	11	13	15
OHAPAT-Ru	10	13	15

DNA Studies

UV-Vis spectroscopy can be used to identify DNA binding mode of the complexes. The complexes would show hypochromic shift in CT-DNA by the presence of chromophores, the reaction has changed to red shift. DNA binding constants were represented in the table 12 and graphs in 16 - 17.

Table 12: DNA Binding Constants Of Metal Complexes

S.No	Complex	λ_{max} nm		$\Delta\lambda$ nm	H%	$K_b(M^{-1})$
		Free	Bound			
1	OHAP-Cu	256	260	5	6.53	2.94×10^6
2	OHAP-Ru	334	339	5	6.35	2.89×10^6

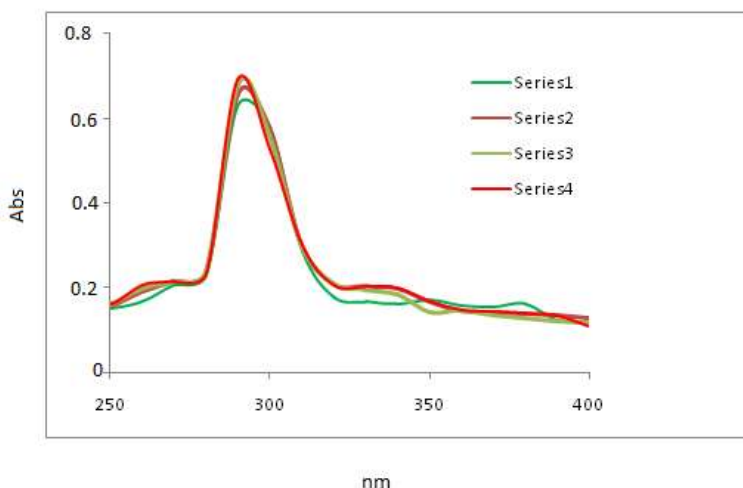


Fig 16: DNA binding mode of OHAPAT-Cu

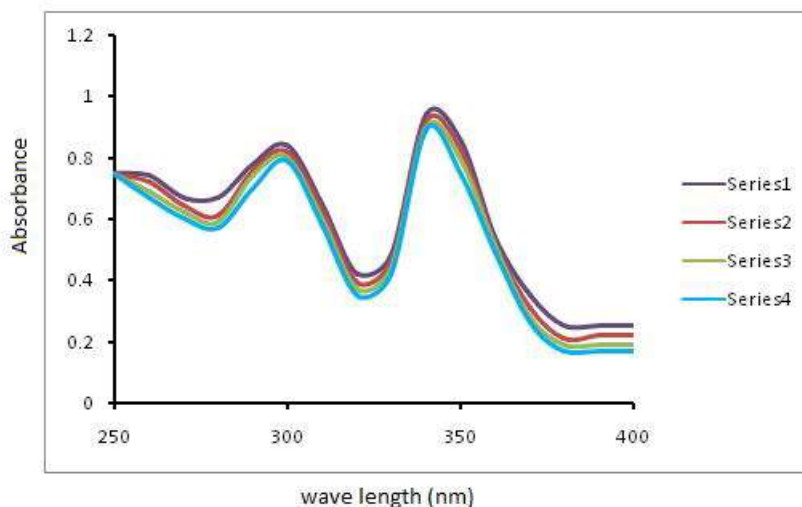


Fig 17: DNA binding mode of OHAPAT-Ru

Conclusion

Imine metal complexes were synthesized by condensing OHAP-AT ligand with Cu (II) and Ru (III), and characterized by employing elemental analysis, FT-IR, UV-Vis, $^1\text{H-NMR}$, ESR, Conductometry; characterization results suggested the complexes were non electrolytes and exhibit octahedral geometry. TG/DTA analysis specified the complexes were thermally stable at high temperature. Biological activity of the complexes in in-vitro conditions suggested the complexes exhibit more anti bacterial activity than corresponding ligands. DNA binding mode of the complexes confirmed by UV-Vis spectrophotometer suggested the complexes show DNA activity.

Acknowledgement

Author is very thankful to Professor J. Sreeramulu garu for his valuable guidance and would like to express her thanks to RGM college of Engineering & Technology for encouragement.

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