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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF RU (III) COMPLEX WITH MONODENTATE AZOMETHINE LIGAND

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Abstract

The present paper deals with the synthesis and characterization of metal complex of monodentate Azomethine ligand derived from the condensation of 4-Morpholinoaniline and vanillin in a 1: 1 molar ratio. The complex of Ru (III) has been synthesized by the reflexen of monodentate ligand and Ru (III) chloride in 2: 1 ratio. The synthesized Schiff base and complex were characterized by using different methods such as Elemental analysis , Molar conductance as well as spectral studies such as FTIR, NMR,UV-Visible , ESR,VSM, XRD and SEM . The analyzed data showed that the complex having six coordination number. The complex was colour and stable in air. The Synthesized ligand and complex were screened for in-vitro antibacterial activity against two bacterial pathogens and fungal activity against one antifungi.

Keywords: 4-Morpholinoaniline, Vanillin, Methanol, Ether and Hydrochloric acid.

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INTRODUCTION

Azomethine are the condensation products of aldehyde or ketone with primary amines and it was first reported by Hugo Schiff in 1864 [1]. Azomethine which contain alkyl substitutes are relatively unstable and readily polymerizable [2], While aryl substitutes having effective conjugation are more stable and readily synthesized. In general, aldehydes react faster than ketones in condensation reactions, leading to the formation of Azomethine as the reaction centre of aldehydes are sterically less hindered than that of ketone. The condensation process was increases when electron withdrawing group attached to aromatic aldehyde and electron donating group attached to aromatic amine. The general reaction was represented in Fig:1.

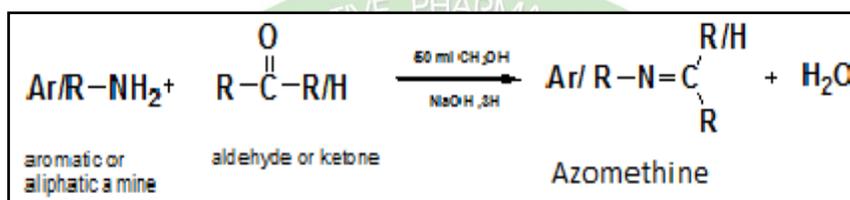


Fig.1

Azomethine ligands have eminent importance in chemistry; particularly in the evolution of Azomethine complexes, because Azomethine are potentially capable of forming stable complexes with metal ions. Many Azomethine complexes show eminent catalytic activity in the various reactions at high temperature and in the presence of moisture. Metal complexes of Azomethine have played a central role in the development of coordination chemistry [3-5]. Metal complexes of Azomethine have been extensively studied because of their biological applications including antimicrobial [6-7], antitumor [8-9] anti HIV [10-11] anticancer [12] and antioxidant [13] activities, antifungal activity [14-16]. They are also used as catalysts [17]. More Schiff bases are synthesized by using vanillin and various primary amines but no work had been done with 4-Morpholinoaniline and vanillin as the basic nucleus of Schiff base.

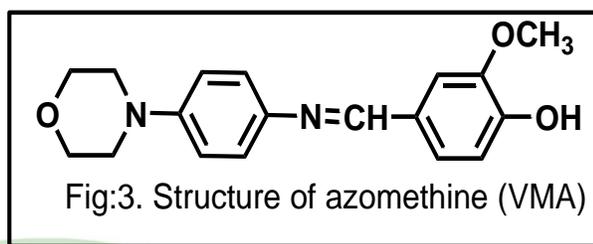
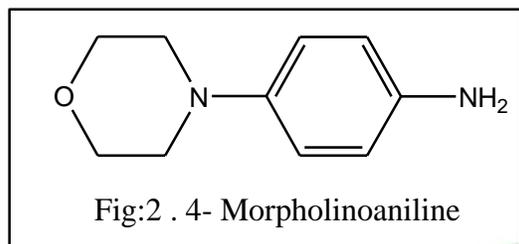
Experimental

Chemicals: All the chemicals and reagents used were analytical grade. The solvents were used without any purification. The pure sample of 4-Morpholinoaniline, molecular formula $\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}$, Molecular weight 178.23 g, melting point $132\text{-}135^\circ\text{C}$ was purchased from Aldrich pharmaceuticals Ltd. (Figure : 2). Metal salt RuCl_3 was of Merck chemicals.

Synthesis of Schiff base

The mixture of Vanillin (0.01mole) and 4-Morpholinoaniline (0.01mole) were dissolved in 50 ml methanol and added a few drops of hydrochloric acid. The mixture was transferred in to 250 ml

round bottom flask. The mixture was refluxed on water bath for about 3 hours at 60⁰c. The mixture was poured in to ice the yellow crystals were produced, which were filtered washed with the same solvent and recrystallized with methanol. The melting point was 175-178⁰c and yield was 90 %. The Azomethine ligand structure was represented in Fig:3



Preparation of metal complex

An Methanolic solution of Azomethine ligand (VMA) adding with the methanolic solution of Ruthenium (III) chloride and few drops of concentration of hydrochloric acid are refluxed on a water bath up to 6 hours. The mixture was poured in to ice light green crude product was obtained. The product was washed with water and re crystallized with methanol. The melting point was 320⁰ C and yield was 82%.

Physical Measurements

The melting point of the ligand and metal complex recoded in open capillaries on capillary melting point apparatus. The IR spectra were recorded In KBr medium and FTIR Affinity-I techniques on a shimadzu spectrometer in wave number region 4000-400 Cm⁻¹ in Annamacharya pharmacy college, Rajampet, Kadapa. The electronic spectra of the methanolic solutions of azomethine ligand (VMA) and VMA- Ru complexes are recorded in at IIIT Edupula paya Kadapa district. The NMR spectra of the ligand and metal complex were recorded on AV-400 M-HZ NMR spectrometer in IICT, Hyderabad in DMSO-D₆ and CDCl₃ solvents at room temperature . The Mass spectra of the ligand and metal complex were recorded at Hyderabad central university, Hyderabad. The ESR,VSM, XRD and TGA/DTA of metal complex and SEM of ligand and metal complex were recorded at IIT Madras .

In Vitro Anti –bacterial activity:

Qualitative analysis for screening of antibacterial activity and fungal activity of Schiff base ligand (VMA) and Ruthenium (III) Schiff base complex was carried out by agar well diffusion method under aseptic conditions. The Schiff base and metal complex were tested against one gram positive and one gram negative bacteria at 10⁻³ M concentration. Then the inoculated medium was

poured in to the pre labeled petridishes and allowed for solidification. After solidification, wells were made in the agar medium by using sterile glass bores. Amoxicillin was used as a positive reference to determine the sensitivity of bacteria and Amphotericin-B was used as a reference to determine the sensitivity of anti fungi. Tested sample solutions and standard drugs were introduced in to the corresponding wells of the petriplates and then plates were kept for incubation at 37⁰c for 24 hours. After incubation, cultures were examined for the bacteria and zones of inhibition formed by them.

RESULTS AND DISCUSSION

Ruthenium Azomethine metal complex is formed in the 2:1 stoichiometric ratio of Azomethine ligand (VMA) and Ruthenium (III) chloride. The Azomethine ligand is yellow in colour where as its Ruthenium (III) complex is light green in colour. The complex is stable at room temperature and insoluble in water and soluble in methanol and DMSO. The molar conductance value of the Azomethine Ruthenium (III) complex is 95 Ohm⁻¹ Cm²mole⁻¹. The analytical data and molar conductance values are represented in the Table:1

Table 1: Analytical data of Azomethine (VMA) and its Ruthenium(III) complex

Ligand /complex	Molecular weight	Percentage of elements analyzed (calculated)					Percentage of yield (colour)	Melting point
		C	H	N	O	M		
VMA	312.38	69.14 (69.20)	6.40 (6.41)	8.95 (8.97)	15.36 (15.38)	-	90 (Yellow)	174-175 ⁰ c
VMA-Ru	868.23	49.75 (49.79)	5.06 (5.11)	6.44 (6.45)	14.73 (14.74)	11.63 (11.64)	82 (Light green)	307-309 ⁰ c

FTIR structural study

The functional groups present in the Schiff base and metal complex were identified by using FTIR spectrometer. The Schiff base ligand and its Ruthenium (III) complex of FTIR comparative data was represented in the Table: 2. The IR spectrum of adsorption band of Schiff base was exhibited at 1667cm⁻¹, it was indicate the azomethine (- C=N-) group, which shifted to a lower value in the complex suggesting that the ligand of the nitrogen are coordinated with the metal ion [18]. A broad band appeared at 3056 Cm⁻¹ and 1379 Cm⁻¹ due to the stretching and bending vibrations of phenolic -OH respectively. In addition, The medium a broad band at 3328 Cm⁻¹ in the complex due to the stretching vibration of OH of water [19] and sharp band obtained at 1592 Cm⁻¹ may be assigned to bending vibration of water and water molecule are coordinated with metal, It was further conformed by occurrence of additional weak and sharp bands at 528 Cm⁻¹

due to OH rocking vibrations.[20] Further a new band appeared at 628 Cm^{-1} in the metal complex which indicate the azomethine group of nitrogen is involved in the coordination bond formation with metal, supporting IR structural study [21]. Further a new band appeared at 420 Cm^{-1} in the metal complex which indicates the chlorine is involved in the coordination bond formation with metal, supporting IR structural study [22]. Fig 4 and 5 are shows the FTIR specters of Schiff base (VMA) and Ru (III) complex.

Table 2: Important FTIR spectral values (Cm^{-1}) of the ligand and Ruthenium (III) complex

Ligand/ complex	$\nu(\text{O-H})$ phenolic	$\nu -\text{C}=\text{N}$	$\nu \text{ M-Cl}$	$\nu \text{ M-N}$	$\nu \text{ M-O}$
Ligand (VMA)	3056	1667	-	-	-
Ru (III) Complex	3228	1592	420	628	528

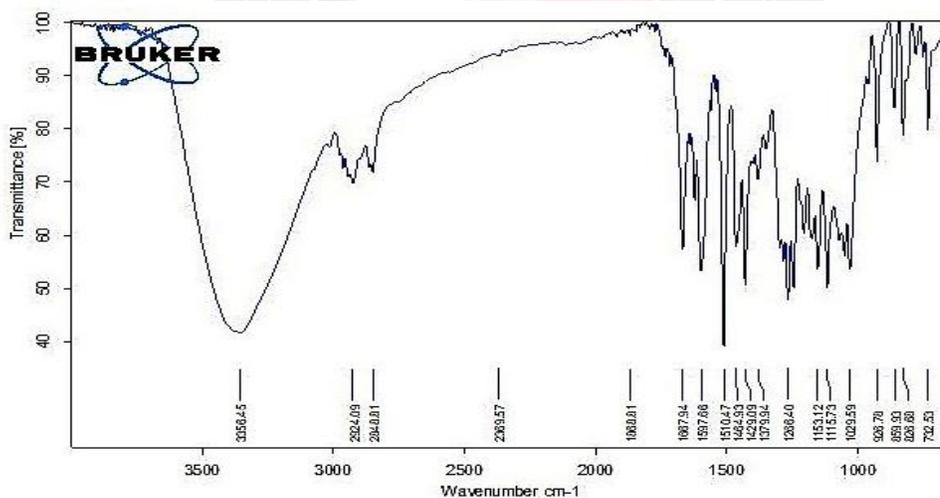


Fig. 4. FTIR spectra of VMA Azomethine ligand

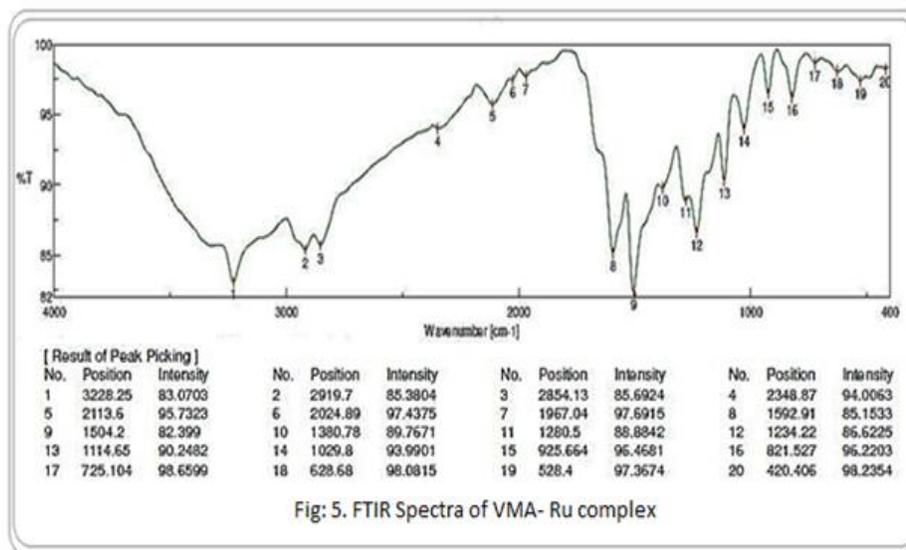


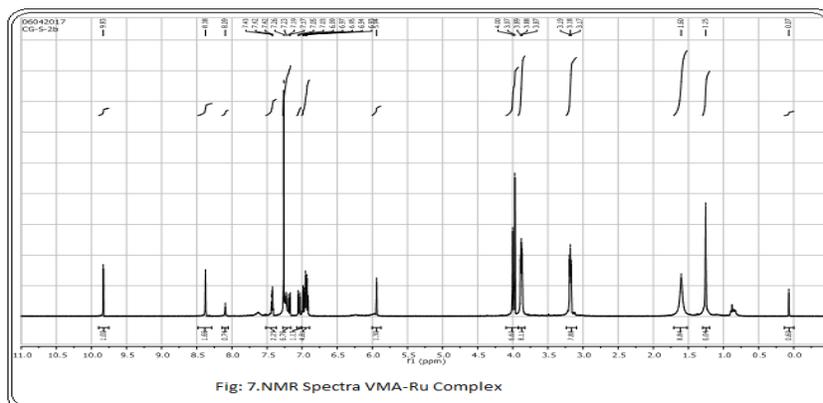
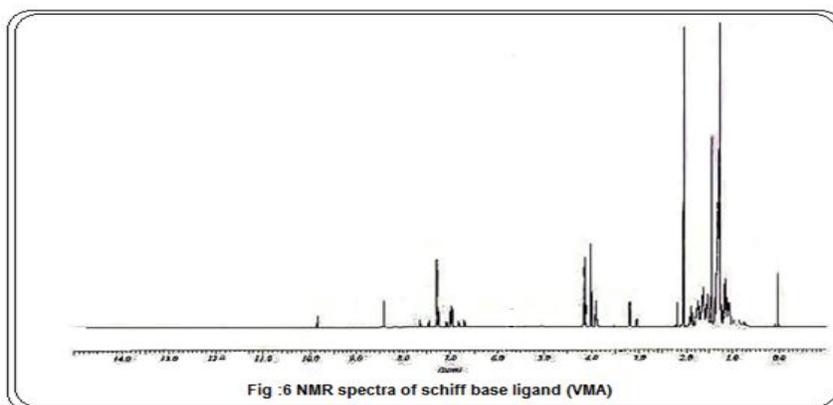
Fig. 5. FTIR Spectra of VMA- Ru complex

¹H NMR Spectra

The Proton NMR spectra values of the Schiff base ligand (VMA) and Ru (III) complex are represented in the Table:3. Fig: 6 and 7 are shows the NMR specters of Schiff base ligand and Ru(III) complex. The signal appeared at 8.52 ppm assigned to azomethine (-CH=N-) proton for the azomethine ligand, which is shifted to 8.38 ppm, indicate involved in the azomethine group of nitrogen in coordination with the metal. In azomethine ligand, methoxy proton signals appeared at 3.86 ppm which was shifted to 3.99 ppm in the complex. The signals observed in the region 6.68 -7.35 ppm, due to aromatic protons in azomethine ligand which was shifted to 6.92- 7.64 ppm in the complex. The singlet observed in the region 9.83 ppm in both azomethine ligand and Ru(III) complex indicate phenolic OH group of oxygen is not involved in the coordination with the metal. A new peak is observed in the metal complex at 5.94 ppm indicate the water molecule coordinated to Ruthenium metal ion.

Table 3: Important ¹H NMR Spectral data of the Azomethine (VMA) and its Ruthenium (III) complex

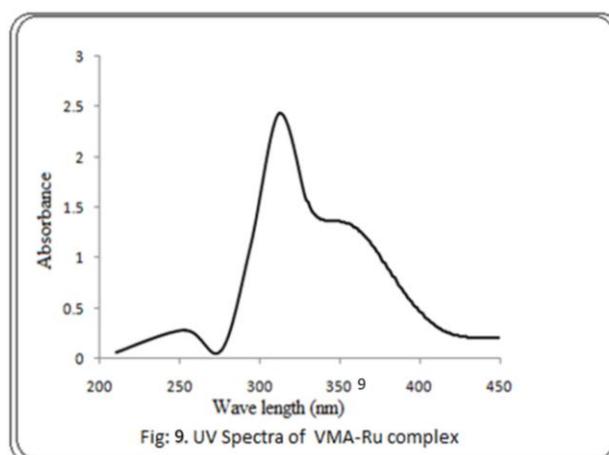
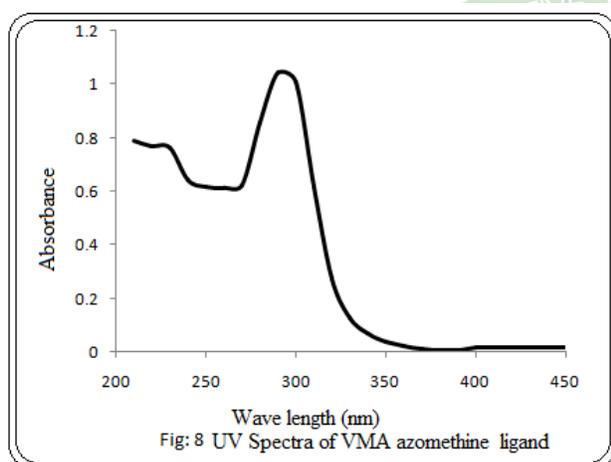
Compound	H-C=N	Ar-H	-OCH ₃	-OH	H-OH
Ligand (VMA)	8.5	6.68-7.35	3.86	9.83	-
Ru (III) Complex	3.37	6.92-7.64	3.99	9.83	5.94



UV Spectra

The UV-vis spectral data of ligand and VMA-La and VMA- Ru complexes are given in Table V.4 and spectra are Fig. (8-9). The electronic spectrum of the methanolic solutions of VMA azomethine ligand and VMA-Ru complex recorded in the 200-600 nm. The azomethine exhibit bands at 235nm and 295 nm assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ But on complexation with Ruthenium metal ions, new bands appeared at 342 nm corresponding to the transitional charge transfer from the ligand to metal ions [22].

Based on the results octahedral structure is proposed for Ru (III) Complex.



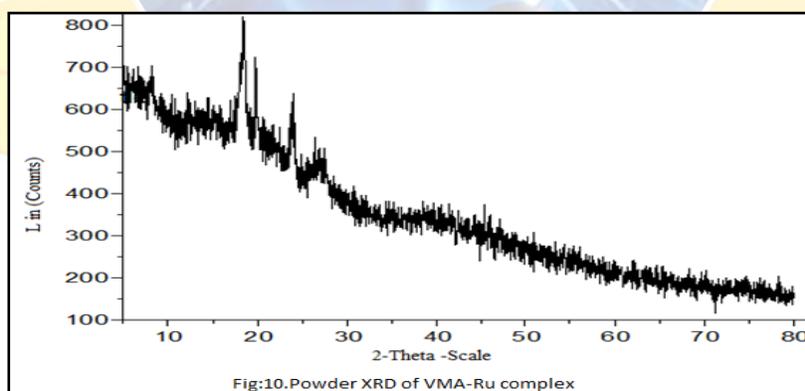
The powder XRD analysis

The powder X-ray diffraction data obtained for VMA –Ru complex with diffractograms using powder X-Ray diffractometer . The XRD patterns are used to explain qualitatively the degree of crystallinity. The diffractogram reflects is given in the Fig:10 between 5-80(2θ). Where θ is Brages angle all the main peaks are indicted and calculated values of Miller indices (h k l) along with observed d-specified and reveled intensities are specified in the Fig: 10. All the peaks have been indexed 2θ values compared in graph. Calculated 'd' values and ' 2θ ' values by using Bragg's equation: $n\lambda=2d\sin\theta$. Comparison values revels that there is good agreement between values of 2θ and d-values. The VMA-Ru complex do not form well- define crystalline peaks due to their very poor crystallinity [23].

The XRD diffraction data of VMA – Ru complex are represented in Table.4.

Table 4: X-ray Diffraction data of VMA -Ru complex

S.No.	d expt	d Calc	2θ expt	2θ Calc	h k l
1.	16.2624	16.2540	5.4297	5.4336	1 1 1
2.	13.6432	13.6429	6.4731	6.4744	2 1 0
3.	12.0795	12.0790	7.3121	7.3127	2 1 1
4.	10.0890	10.0888	8.7574	8.7582	2 2 1
5.	9.2375	9.2370	9.5664	9.5677	3 1 1
6.	8.5418	8.5415	10.3476	10.3482	2 2 2
7.	7.5003	7.50029	11.7893	11.7932	3 2 2
8.	6.98612	6.98603	12.6604	12.6610	3 3 1
9.	6.83062	6.83054	12.9498	12.9501	4 0 2
10.	6.1242	6.1235	14.4511	14.4533	4 2 2
11.	5.8675	5.8670	15.0870	15.6964	4 3 2
12.	5.21245	5.21234	16.9962	16.9974	4 3 3
13.	5.08367	5.08368	17.430	17.4310	4 4 2
14.	4.9618	4.96122	17.8616	17.8646	5 2 3
15.	4.8912	4.8906	18.1216	18.1248	6 1 1
16.	4.8090	4.8070	18.434	18.4428	4 4 3
17.	4.4872	4.4864	19.7688	19.7727	5 4 2
18.	3.7506	3.7505	23.7029	23.7043	5 5 4
19.	3.6932	3.6927	24.0767	24.080	7 4 2
20.	3.5230	3.5220	25.258	25.2654	5 5 5
21.	3.3776	3.3775	26.365	26.3640	5 5 6
22.	3.251	3.245	27.4115	27.4625	7 6 2
23.	3.145	3.1445	28.354	28.3587	6 7 3
24.	1.8962	1.8952	47.9355	47.9592	10 9 9



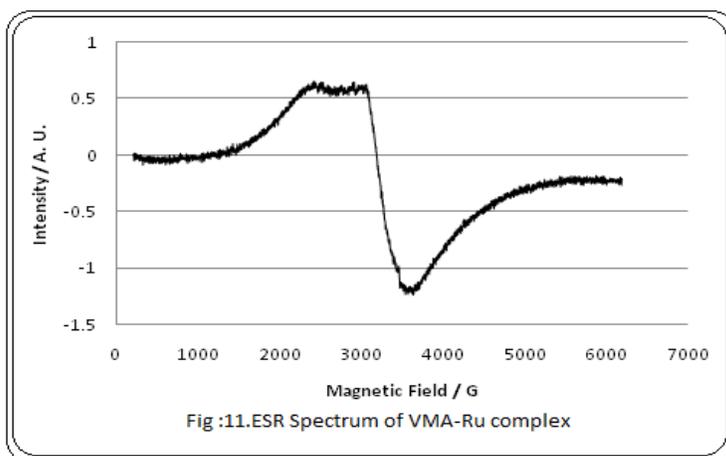
ESR Spectral analysis

ESR spectra of metal complexes give useful information regarding the stereochemistry and nature of metal–ligand bonding. The ESR spectra for VMA-Ru complexes were recorded at room temperature and at liquid nitrogen temperature (LNT). The ESR spectra for VMA –Ru complexes are presented in Fig.11. The VMA-Ru complex gives only one broad signal indicating anisotropy

in these systems The and the complex is in octahedral geometry. The VMA-Ru complex g_{\parallel} value 2.224 and g_{\perp} value 2.048. The trend $g_{\parallel} > g_{ave} > g_{\perp} > 2.0023$ observed for VMA-Ru complexes suggests that the unpaired electron is localised in $d_{x^2-y^2}$ or d_z^2 orbital [37] of the Ru(III) ions for the complexes. The G value of VMA-Ru complexes are given in Table 5. It is observed that the G value of present complex is greater than four and suggest that there are no interactions between Ruthenium – Ruthenium centers in DMF medium [23].

Table 5: ESR Spectral data of VMA-Ru complex

S.No.	Parameters	VMA-Ru
1	g_{\parallel}	2.0534
2	g_{\perp}	1.9889
3	g_{ave}	2.046
4	G	8.5879
5	A_{\parallel}^*	0.01276
6	A_{\perp}^*	0.007659
7	A_{ave}^*	0.00936
9	K_{\parallel}	0.618
10	K_{\perp}	0.724
11	P^*	0.0183
12	α^2	0.5549



Magnetic susceptibility

The magnetic property of Ruthenium complex helps to know the geometry of them. The magnetic property of Ruthenium is 6.28 B.M for VMA-Ru complex. This results indicate that the Ruthenium complex of ligands show octahedral geometry.

Molar conductivity

The molar conductance of the VMA-Ru complex was carried out in methanol. The molar conductance value of Ruthenium azomethine complex is $98 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ [24].The values suggest VMA-Ru complex is ionic in nature.

SEM Analysis:

The surface design of the complex has been examined using SEM [25] and the Azomethine ligand and its Ruthenium complex were shown in Fig: 12-13. The SEM image of azomethine ligand has shown a un uniform surface with different size pallets. The SEM image of VMA-Ru complex has shown with large voids with irregular agglomerated particles with different sizes.

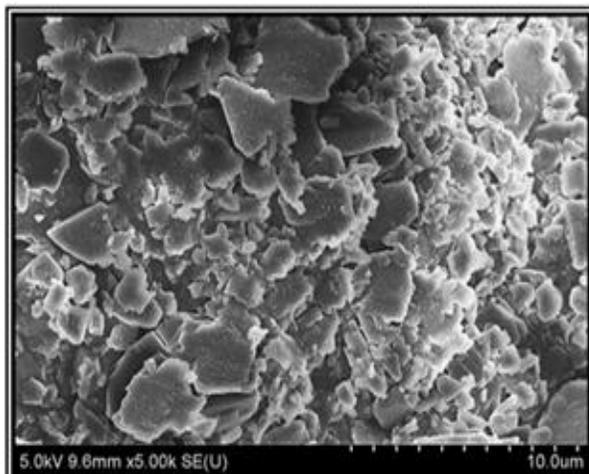


Fig.12.SEM Image of VMA Azomethine

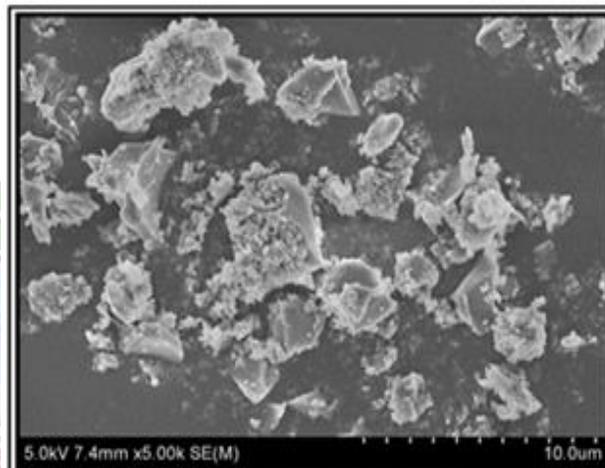


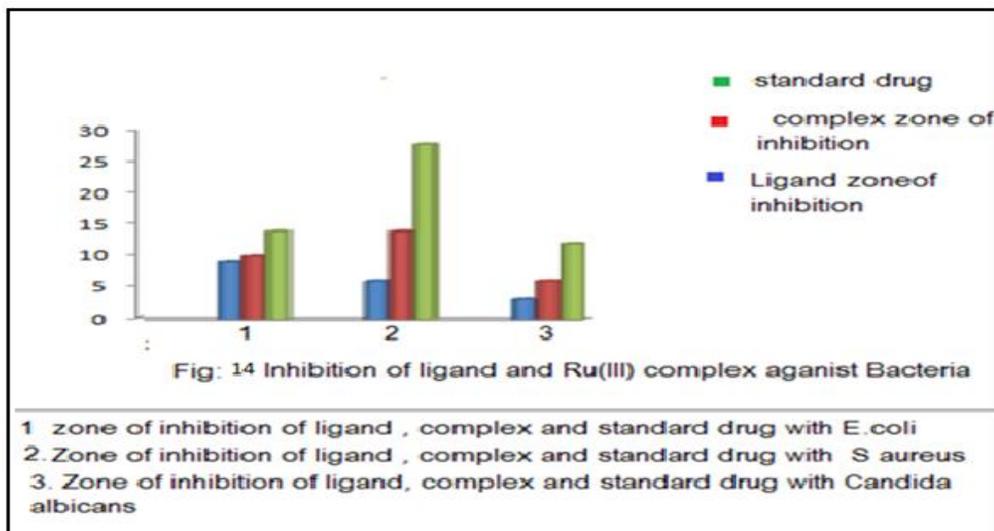
Fig.13. SEM Image of VMA- Ru complex

Anti biological and fungal activity

The zone of inhibition of the azomethine ligand and its ruthenium metal complexes against gram positive and gram negative bacterial strains was determined by using disc diffusion method. The Azomethine and its Ruthenium complex of Anti bacterial activities and anti fungal activity are reported in Table -6. In case of Gram positive *S.aureus*) showed good activity in both Azomethine and Ru(III) complex , Where as Gram negative((*E.Coli*) showed moderate activity with azomethine and good activity with its Ruthenium complex. In case of anti fungal activity, *Candida albicans* has zone of inhibition of parent drug is 12 mm and complex was more inhibition than the Schiff base ligand.

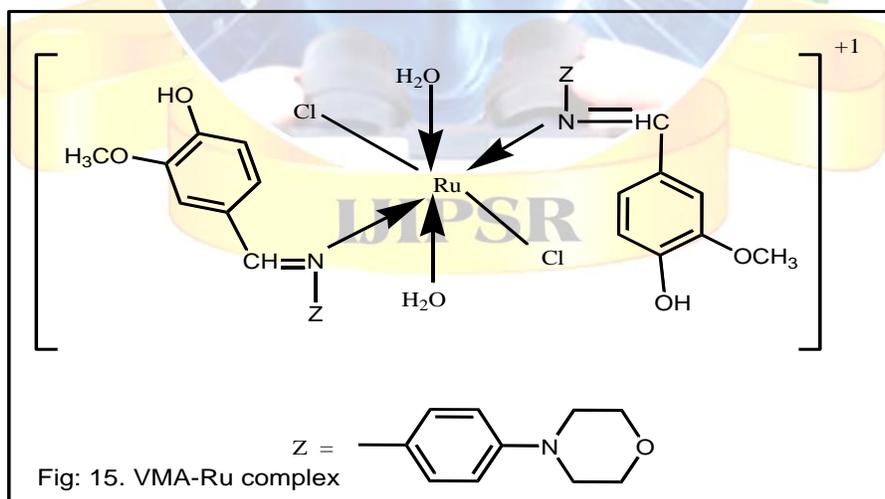
Table 6: Anti biological and fungal activity of Schiff base and Ru (III) complex

Compound	Diameter zone of inhibition (mm)		
	Gram negative((<i>E.Coli</i>)	Gram positive <i>S.aureus</i>)	Fungi (<i>C.albicans</i>)
Ligand	9	6	3
Complex	10	14	6
Streptocymine	14	28	-
Amphotericin-B	-	-	12



CONCLUSION

The mono dentate Azomethine were synthesized from 4-Morpholinoaniline with Vanillin and the ligand was conformed form FTIR,UV and NMR . VMA-Ru complex was prepared by reacting Schiff base with RuCl_3 salt in the ratio (2:1). The complex was conformed FTIR, UV, NMR, ESR XRD and SEM studies and analytical data. The complex is ionic and paramagnetic in nature. The anti bacterial and antifungal studies of azomethine and complex have been studied which indicate that activity increases with the chelation. The studies shows that VMA-Ru complex was octahedral. The structure was represented in Fig.15.



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