ON DONORS

Chapter -2: Synthesis, Characterization, crystal structure and biological evaluation of ON donor Schiff bases and their metal complexes

This chapter is divided into three parts.

PART-I

2.1: Synthesis, characterization, crystal structure and the biological evaluation of pyrazolone basedSchiff bases and their metal complexes

PART-II

2.2: Synthesis, characterization, crystal structureand the biological evaluationofo-vanillin-4-aminoantipyrine Schiff base and its metal complexes

PART-III

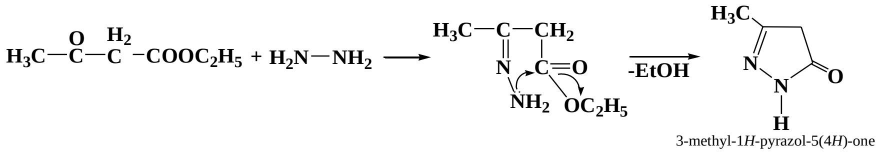
2.3: Synthesis, characterization and the biological evaluation of o-vanillin Schiff bases and their metal complexes.

2.1. Synthesis, characterization,crystal structure and the biological evaluation of aldehyde pyrazolone based Schiff bases and their metal complexes

2.1.1. INTRODUCTION

Brief history of pyrazolone

Pyrazolones are five membered heterocyclic compounds containing two adjacent nitrogen atoms. They have elicited interest among researchers due to their medicinal properties and their industrial applications [70, 71]. Knorr first synthesized such a system in 1883 by the reaction between ethyl aceto acetate and hydrazine yielding 3-methyl-1H-pyrazol-5(4H)-one[72, 73].

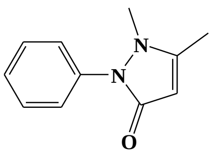


Knorr synthesis of pyrazolone

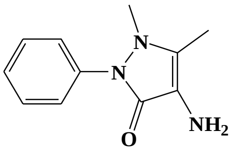
4-formyl and 4-acyl derivatives of pyrazolone are widely used in the formation of coordination complexes with various metal ions. Due to the presence of two oxygen donor atoms and a facile keto–enol tautomerism, they easily coordinate with metal ions after deprotonation of the enolic hydrogen and provide stable metal complexes with six-membered chelate rings. In addition, pyrazolone derivatives can form a variety of Schiff bases and are reported to be superior reagents in biological, clinical and analytical applications [74]. Pyrazolones are used in analytical chemistry for the determination and isolation of almost all metal ions due to their high extracting ability, intense color of the complex extracts and low solubility of the complex in some solvents [75].

Derivatives of pyrazolone are an important class of β-diketones. The Schiff base ligands (SB) produced from condensation of ortho-hydroxybenzaldehydes and β-diketones with 1,3-diaminopropan-2-ol can readily interact with two metal ions supplying the alkoxo-oxygen atom as endogenous bridge, followed by coordination of exogenous bridging group which can be widely varied between small bi-dentate molecules [76–87].

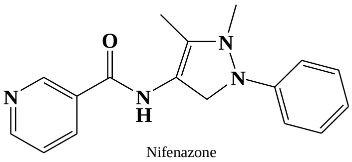
The term pyrazolone is sometimes also referred to anti-inflammatory agents. Pyrazolones and their analogues, e.g., antipyrine, are not much used now in medicine because of their toxicity on human beings [88-90]. At present research on the compounds are centered on their application as anti-inflammatory drugs [91] and the preparation of new compounds with antifungal [92], antitumor [93] and anti-hyperglycemic properties [94].Some of the pyrazolone based drugs are given below.



Antipyrine
(Analgesic and antipyratic)



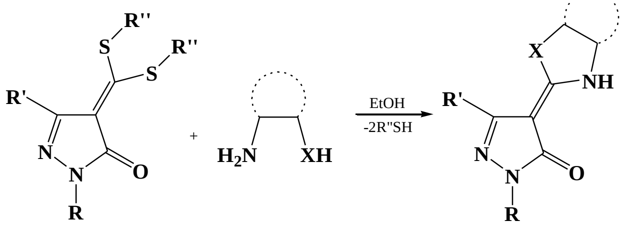
Ampyron
(Antiinflammatory)



Nifenazone
(Analgesic)

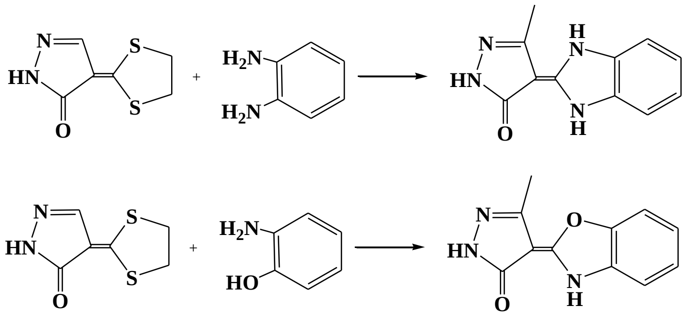
Also, they are used for extraction of metal ions [95], analytical purpose [96], and preparation of azo-dyes [97] and ligands for complexes with catalytic activity [98]. They are also used in synthesis of rare earth metal complexes with photo physical properties [99-102].

The basic character of pyrazolones is determined by different tautomers[103]. The hydrogen atom at C_4 can be easily deprotonated and a carbon nucleophile can be generated. Ketene di-thioacetals are very important synthetic compounds in organic chemistry. They are easily prepared by the reaction between a carbon nucleophile and carbon disulfide. The sulfur atom exercises a stabilizing effect on neighboring species.Hence the double bond in ketene dithioacetals are reactive to both nucleophilic and electrophilic reagents,which is an excellent condition for organic synthetic purposes. The pyrazolone dithioacetals are regio-specific toward bi nucleophiles. The synthesis of ketene of dithioacetals and applications in manipulations of functionality or in the synthesis of heterocyclic system has been extensively investigated [104-111]. The preparation of ketene dithioacetal and the synthesis of heterocyclic substituted pyrazolone are represented below:



X= NH / O

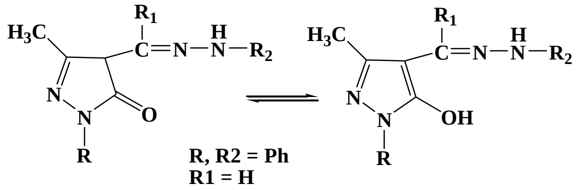
Examples of synthesis of heterocylic substituted pyrazolones from dithioacetals.



Heterocyclic substituted pyrazolones are found effective against central nervous system (CNS) disorders (Alzheimer diseases), inflammatory disorders (psoreasisis), cardiac diseases (arthrosclerosis, restenosis and thrombosis) and metabolic disorders (diabetes) and infectious diseases (viral and fungal infections) [112]. Of the diverse cordination compounds the Schiff bases are forming, aldehyde pyrazolones are a special class and they are dealt with in the following chapter. In this section, other pyrazolone analogues and their Schiff bases are considered.

Dabhi *et al.* [113] reported Formation constants and thermodynamic parameters of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} chelates derived from tetradentateSchiff-base of 4-substituted-2-pyrazolin-5 ones with diamines. They found stability constants to be Mn < Co < Ni < Cu > Zn for all systems.

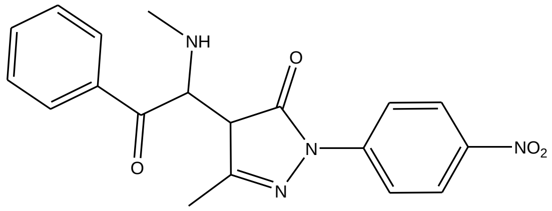
Pandya *et al.* [114] have synthesized and characterized Mn(II), Co(II), Ni(II), and Cu(II) complexes of 4-carboxaldehydephenylhydrazone-1-phenyl-3-methyl-2-pyrazolin-5-one and investigated their antibacterial activity.



These compound were found to be of the type [$ML_2(H_2O)_2$]. All the compound on experiment with bacteria showed antibacterial activity by inhibition of growth of these organism.

Dabhi *et al.* [115] studied Formation Constants and Thermodynamic Parameters of transition metal chelates derived from Schiff base of 4-Benzoyl-1-(*p*-

nitrophenyl)-3-methyl-2-pyrazolin-5-ones with some diamines. The stability constant of metal complexes were taken at 25 and 35 °C using Calvin-Bjerrum technique.



Schiff base of 4-Benzoyl-1-(p-nitrophenyl)-3-methyl-2-pyrazolin-5-ones

Niknam *et al.* [116] reported Silica-bonded S-sulfonic acid: an efficient and recyclable solid acid catalyst for the synthesis of 4,4’-(arylmethylene)bis(1H-pyrazol-5-ols)

Aamal *et al.* [117] studied microwave versus ultrasound assisted synthesis of some new heterocycles based on pyrazolone moiety. They were used for synthesis of some derivatives of spiropiperidine-4,40-pyrano[2,3-c]pyrazole, dihydropyrano[2,3-c]pyrazole, pyrazole-4-carbothioamide, 4-(2-oxo-1,2-diphenylethylidene)-1H-pyrazol-5(4H)-one, azopyrazole, arylmethylenebis-1Hpyrazol- 5-ol andaráylidene-1H-pyrazol-5(4H)-one via reactions with different reagents applying the ultrasound method in some cases.

Xing *et al.* [118] carried out multi-component domino reactions of acetylenedicarboxylates: divergent synthesis of multi-functionalized pyrazolones and C-tethered bispyrazol-5-ols. The study reveals that the electron-donating aryl groups (EDAG)-attached aldehydes resulted in the pyrazolone skeleton, whereas the electron-withdrawing aryl groups (EWAG) led to the C-tethered bispyrazol-5-ols with simultaneous formation of two new pyrazole rings.

Popov *et al.* [119] have synthesized binuclear Cu(II) complexes of Schiff base ligand derived from 1-phenyl-3-methyl-4-formyl-pyrazol-5-one and 1,3-diaminopropan-2-ol, and studied their structure and magnetic properties.

Kiran *et al.* [120] studied o-pheniline diamine and p-pheniline diamine bridged binuclear Mn(III) Schiff base complexes derived from pyrazolone based ligands.

The importance of Mn(III) complexes is derived from the role that Mn plays in biological systems. Mn(III) Schiff base complexes provide a large number of

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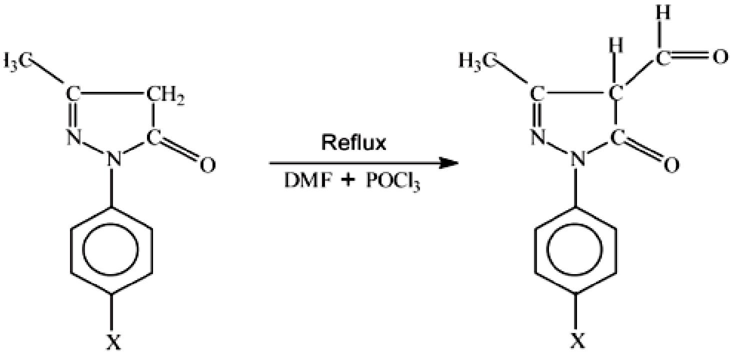
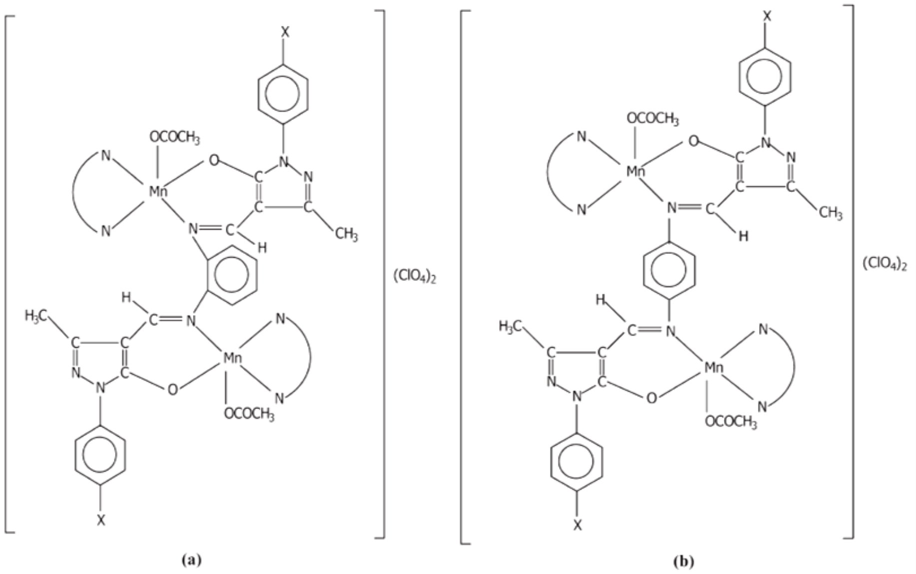
structural types that can be used as models for the magnetic and structural properties of mangano-enzymes [121-129]. The modeling of biological processes with artificial inorganic complexes has been an important approach of the inorganic chemist to understand the structural and mechanistic features of the biological processes [130-132].



For PMFP, X = H
PMFP-Me, X = CH_3



(a)

(b)

Where X= H = PMFP; X = CH_3 = PMFP-Me

(a)(I) $[Mn(PMFP)_2(o\text{-}Phd)(Bipy)_2(OAc)_2](ClO_4)_2$; (II) $[Mn(FMFP\text{-}Me)_2(o\text{-}Phd)(Bipy)_2(OAc)_2]\ (ClO_4)_2$
(b) (III) $[Mn(FMFP)_2(p\text{-}Phd)(Bipy)_2(OAc)_2](ClO_4)_2$; (IV) $[Mn(PMFP\text{-}Me)_2(p\text{-}Phd)(Bipy)_2(OAc)_2](ClO_4)_2$

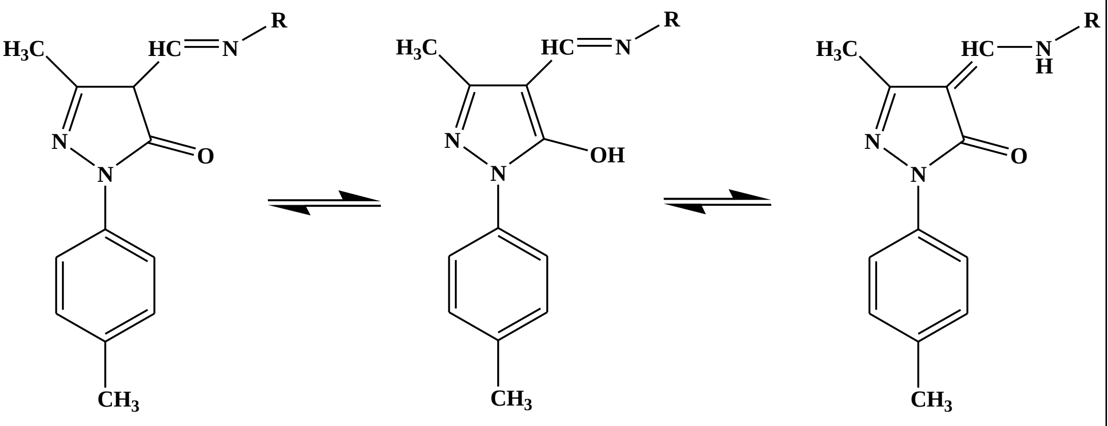
Azomethine metal complexes are used as additives to lubricants [133-141]. Bulov *et al.* [142] have prepared 1-phenyl-3-methyl-4-dodecyl-iminomethylene-pyrazol-5-one and its copper complex (CuL_2) as an effective additive to lubricants.

Mouchan *et al.* [143] studied the tautomerism and acid base properties of formyl derivatives of 1-phenyl-3-methyl-4,5-dihydropyrazol-5-one and its thio-analogue. The acidity and basicity constants of 1-phenyl-3-methyl-4-formyl-4,5-

dihydropyrazol-5-one and its analog 5–thione in 50% aqueous dioxane were measured.

A single crystal X-ray diffraction study has shown that 1-phenyl-3-methyl-4-formyl-4,5-dihydropyrazol-5-one occurs in solid phase as the conformers of N-H tautomers.The steady interest in the study of pyrazol-5-one with substituent in 4th position is due to their wide use as metal exractant, colour formation ingredients, drugs etc. as also due to their tautomerism [144,145]. It is known that in non polar solvents, the enol forms of the N^1 substituted pyrazolones are realized because of their stabilization by fairly strong intramolecular H-bonding with the substituent in the 4thposition. In proton donor solvents the forms N-H and or OH are stabilized by intermolecular H-bonding or dipole-dipole interaction may prevail [146-150].

The different tautomeric forms in the aldehydepyrazolone ligands



Imine-one
I

Imine-ol
II

Amine-one
III

However, the literature survey shows that the study of Schiff bases and their metal complexes derived from aldehyde pyrazolone is scant. In the following section, a work on this class of Schiff bases and their metal complexes is undertaken. The Schiff base ligands and the metal complexes are characterized by X-ray crystallography and different spectroscopic methods. To find an application of these compounds, a limited study of their biological activity with respect to a few bacterial species is also carried out.

2.1.2EXPERIMENTAL SECTION

2.1.2.1 Materials

The compound 3-methyl-1-p-tolyl-1H-pyrazol-5-ol(PTPMP) was obtained from Nutan Dye Chem., Sachin, Surat, India as free gift samples. Substituted amines were obtained from Merck &Loba chem. Calcium hydroxide, $Cu(OAc)_2{\cdot}H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$ and $Ni(NO_3)_2{\cdot}6H_2O$ were obtained form Merck. Absolute alcohol was obtained from Baroda Chem. Industry Ltd. Methanol was obtained from spectrochem Mumbai, India. All the chemicals used were of AR grade. Solvents used in this study were purified and dried following the standard procedures wherever required [151].

2.1.2.2. Single crystal X-ray structure determination

Crystals having good morphology were chosen for three-dimensional intensity data collection. X-ray intensity data of some of the complexes were collected at room temperature on Bruker CCD area-detector diffractometer equipped with graphite monochromated MoKα radiation (α=0.71073 Å). The crystals used for data collection was of suitable dimensions. The unit cell parameters were determined by least-square refinements of all reflections in both cases. All the structures were solved by direct method and refined by full-matrix least squares on F^2. Data were corrected for Lorentz, polarization and multi-scan absorption correction [152]. The structures were solved by direct methods using SHELXS97. All non-hydrogen atoms of the molecule were located in the best E-map. Full-matrix least-squares refinement was carried out using SHELXL97 [152]. Hydrogen atoms were placed at geometrically fixed positions and allowed to ride on the corresponding non-H atoms with C-H = 0.93-0.96Å, and Uiso=1.5 Ueq of the attached C atom for methyl H atoms and 1.2 Ueq for other H atoms. Atomic scattering factors were taken from International Tables for X-ray Crystallography (1992, Vol. C, Tables 4.2.6.8 and 6.1.1.4). An ORTEP [153] view of both the complexes with atomic labeling is shown in further section. The geometry of the molecule has been calculated using the software PLATON [154] and PARST [155].

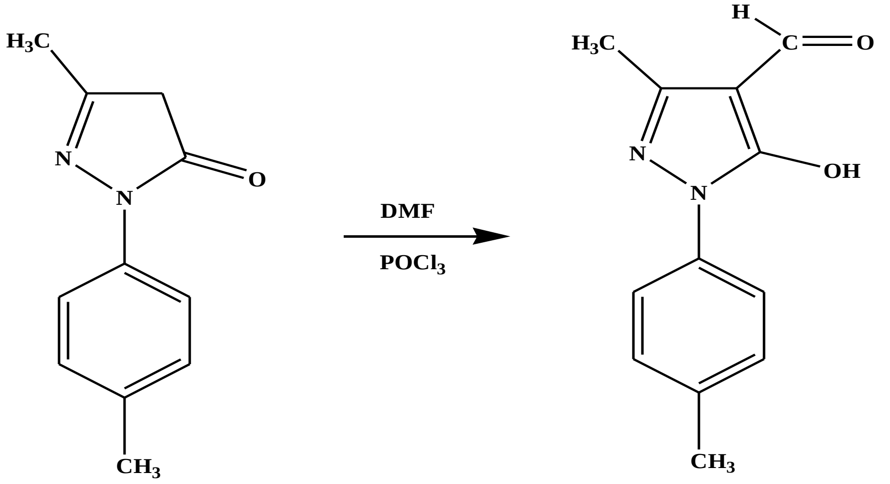
2.1.2.3. Antimicrobial screening

The test organism was activated by inoculating a loop full of the strain in 25 ml of Nutrient Broth and kept overnight on a rotary shaker. The assay was performed by agar well diffusion method [156]. 200 µl inoculums (1×10^{8} cfu/ml), was introduced into molten nutrient agar and poured into Petri dishes when temperature reached 40-42°C. The media was solidified and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). 100 µl of test compound was introduced into the well and the plates were incubated at 37°C for 24 hours.DMSO (dimethylsulphoxide) was taken as negative control.

2.1.3. Preparation of Schiff base ligands and metal complexes

2.1.3.1 Synthesis of 5-hydroxy-3-methyl-1-p-tolyl-1H-pyrazole-4-carbaldehyde[APTPMP]

3-methyl-1-p-tolyl-pyrazol-5-one (9.4 g, 0.05 mol) in DMF (10 cm^3, 0.05 mol) was cooled to 0-5 ^{o}C in ice bath. $POCl_3$(5.5cm^3, 0.06 mol) was added dropwise with constant stirring and maintaining temperature 10-20 ^{o}C of the reaction mixture. After the complete addition of $POCl_3$, reaction mixture was heated on a steam bath for 2.5 hours and poured into 1L ice water. The product was allowed to stand overnight, filtered and washed with water and dried[157]. (Scheme-2.1)



3-methyl-1-*p*-tolyl-pyrazol-5-one 5-hydroxy-3-methyl-1-*p*-tolyl-1*H*-pyrazole-4-carbaldehyde

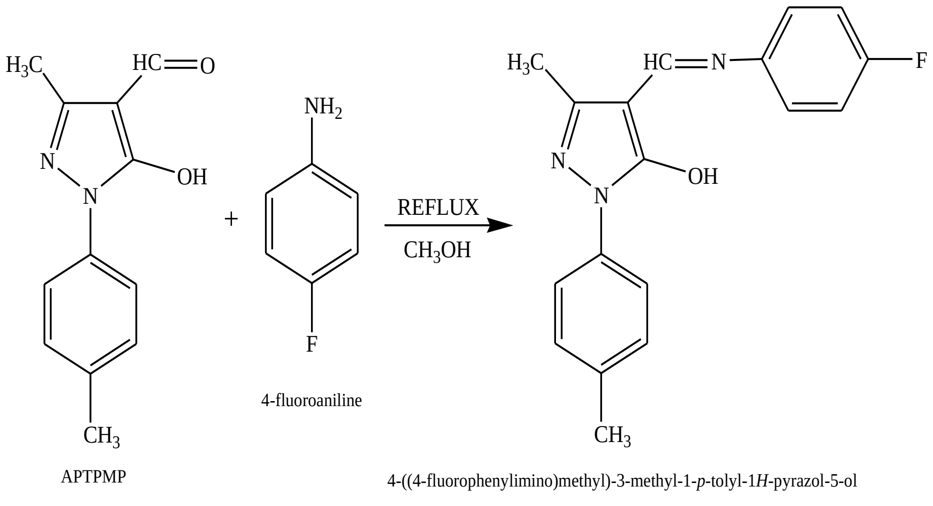
Scheme - 2.1: APTPMP

APTPMP is yellow compound. Yield: 85%, m.p.: 155°C.Anal. Calc. for $C_{12}H_{12}N_2O_2$ M.W.: 216.24, C (66.65%), H(5.59%), N(12.96%), found: C (66.58%), H(5.61%), N(13.01%).

IR (KBr, cm^{-1}): 3470-3350 (m) (O-H Hydrogen bonded/N-H Hydrogen bonded), 2810-2750 (s) (C-H aldehydic, doublet), 1672 (s) (C=O, pyrazolone ring), 1515 (s) (C=N cyclic).^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 2.39 (s, 3H), δ 2.82 (s, 3H), δ7.05-7.71 (m,Ar-H), δ 10.92 (s, 1H).MASS: m/z =217.17 [$C_{12}H_{12}N_2O_2$], 189.34 $[C_{10}H_8N_2O_2]^+$, 113.22 $[C_4H_4N_2O_2]^+$, 99.09 $[C_4H_6N_2O]^+$, 85.00 $[C_3H_4N_2O]^+$, 70.85 $[C_3H_6N_2]^+$, 56.60 $[C_4H_8]^+$.

2.1.3.2. Synthesis of 4-((4-fluorophenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol **[APTPMP-4F]**

The Schiff base ligand was synthesized by adding APTPMP (1.08 gm, 0.005M) dissolved in hot methanol (20 cm^3) to 4-fluoroaniline (0.7 ml, 0.005M) in methanol. The reaction mixture was refluxed for 12 hours. The microcrystalline compounds then filtered and washed with suitable solvent and dried. (Scheme –2.2)



Scheme - 2.2: APTPMP-4F

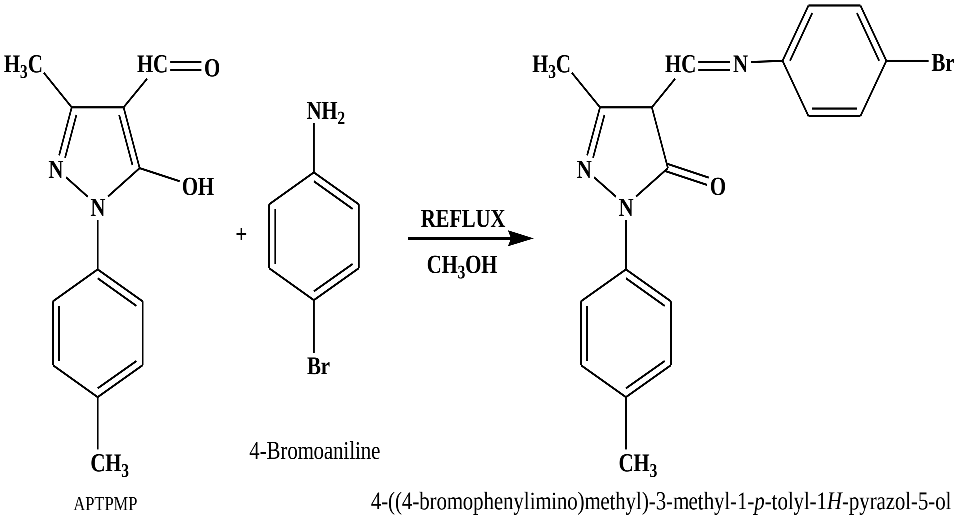
APTPMP-4F is yellowish brown crystalline compound. Yield: 76%, m.p.: 180°C.Anal. Calc. for $C_{18}H_{16}FN_3O$ M.W.: 309.34, C (69.89%), H(5.21%), N(13.58%), found: C (69.95%), H(5.17%), N(13.62%).

IR (KBr, cm^{-1}): 3260 (m) (O-H), 2910 (N-H), 1666 (s) (C=O, pyrazolone ring), 1575 (s) (C=N cyclic), 1215 (s) (C=N azomethine).^{1}H NMR ($CDCl_3$, 400 MHz, TMS):

δ2.28 (s, 3H, CH_3), δ 2.33 (s, 3H, CH_3), δ 7.07-7.85 (m, 8H, Ar-H),δ 7.79 (s, CH=N-), δ11.46 (s, 1H, O-H/N-H).MASS: m/z = 309.52 [$C_{18}H_{16}FN_3O$], 292.27 $[C_{18}H_{15}FN_3]^+$, 264.32 $[C_{16}H_{11}FN_3]^+$, 199.27 $[C_{12}H_{13}N_3]^+$, 189.14 $[C_{10}H_7FN_3]^+$,161.22 $[C_{10}H_7FN]^+$, 146.61 $[C_{10}H_7N]^+$, 121.82 $[C_7H_{10}N_2]^+$, 105.25$[C_8H_9N]^+$, 94.86 $[C_6H_7N]^+$, 77.10 $[C_6H_5]^+$, 64.90 $[C_5H_5]^+$.

2.1.3.3. Synthesis of 4-((4-bromophenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol **[APTPMP-4BR]**

The Schiff base ligand was synthesized by adding APTPMP (1.08 gm, 0.005M) dissolved in hot methanol (20 cm^3) to 4-bromoaniline (0.86 gm, 0.005M) in methanol. The reaction mixture was refluxed for 12 hours. The microcrystalline compounds then filtered and washed with suitable solvent and dried. (Scheme –2.3)



Scheme - 2.3: APTPMP-4BR

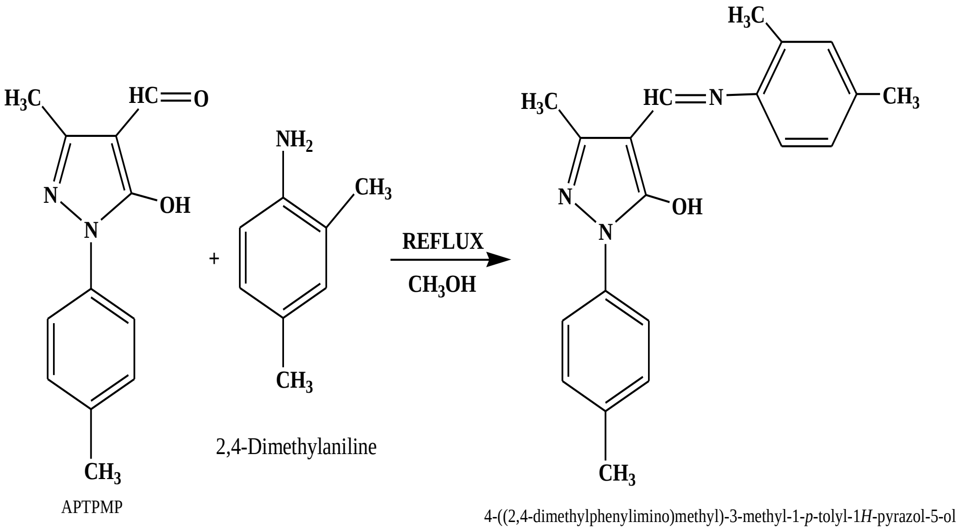
APTPMP-4BR is reddish brown crystalline compound. Yield: 72%, m.p.: 186°C.Anal. Calc. for $C_{18}H_{16}BrN_3O$ M.W.: 370.24, C (58.39%), H(4.36%), N(11.35%), found: C (58.43%), H(4.39%), N(11.29%).

IR (KBr, cm^{-1}): 3268 (m) (O-H), 2925 (N-H), 1665 (s) (C=O, pyrazolone ring), 1590 (s) (C=N cyclic), 1220 (s) (C=N azomethine).^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 2.31 (s, 3H, CH_3), δ 2.36 (s, 3H, CH_3), δ 7.08-7.87 (m, 8H, Ar-H),δ 7.86 (s, CH=N-), δ 11.61 (s, 1H, O-H/N-H).MASS: m/z = 370.50 [$C_{18}H_{16}BrN_3O$],354.70$[C_{18}H_{16}BrN_3]^+$, 326.10 $[C_{16}H_{12}BrN_3]^+$,249.70

$[C_{10}H_8BrN_3]^+$,224$[C_{10}H_{10}BrN]^+$, 144.80 $[C_{10}H_{11}N]^+$, 121.82 $[C_8H_9N]^+$, 94.86 $[C_6H_7N]^+$, 77.10 $[C_6H_5]^+$.

2.1.3.4. Synthesis of 4-((2,4-dimethylphenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol[APTPMP-2,4-ME]

The Schiff base ligand was synthesized by adding APTPMP (1.08 gm, 0.005M) dissolved in hot methanol (20 cm^3) to 2,4-dimethylaniline (0.8 ml, 0.005M) in methanol. The reaction mixture was refluxed for 12 hours. The microcrystalline compounds then filtered and washed with suitable solvent and dried. (Scheme –2.4)



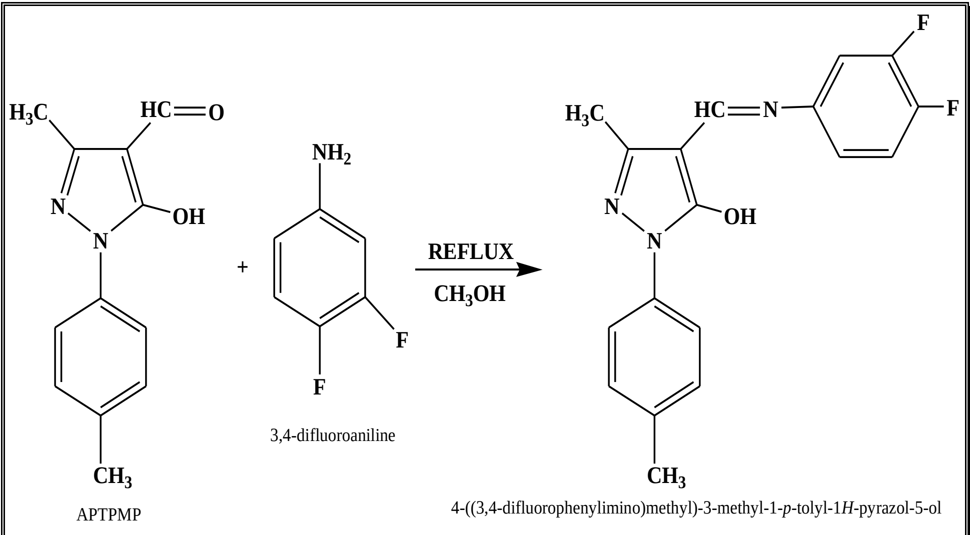
Scheme - 2.4: APTPMP-2,4ME

[APTPMP-2,4ME] is yellow crystalline compound. Yield: 80%, m.p.: 170°C.Anal. Calc. for $C_{20}H_{21}N_3O$ M.W.: 319.40, C (75.21%), H(6.63%), N(13.16%), found: C (75.18%), H(6.70%), N(13.20%).

IR (KBr, cm^{-1}): 3269 (m) (O-H), 2911 (N-H), 1668 (s) (C=O, pyrazolone ring), 1583 (s) (C=N cyclic), 1210 (s) (C=N azomethine).^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 2.31 (s, 3H, CH_3), δ 2.36 (s, 3H, CH_3), δ 7.08-7.87 (m, 8H, Ar-H),δ 7.86 (s, CH=N-), δ 11.61 (s, 1H, O-H/N-H).MASS: m/z = 320.12 $[C_{20}H_{21}N_3O]$, 202.60$[C_{12}H_{15}N_3]^+$, 201.20 $[C_{12}H_{12}N_2O]^+$, 175.09 $[C_{12}H_{15}N]^+$, 134.80 $[C_9H_{11}N]^+$, 114.20 $[C_5H_8N_2O]^+$, 107.80 $[C_7H_7N]^+$, 94.86 $[C_6H_7N]^+$, 77.10 $[C_6H_5]^+$

2.1.3.5. Synthesis of 4-((3,4-difluorophenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol[APTPMP-3,4F]

The Schiff base ligand was synthesized by adding APTPMP (1.08 gm, 0.005M) dissolved in hot methanol (20 cm^3) to 3,4-difluoroaniline (0.64 gm, 0.005M) in methanol. The reaction mixture was refluxed for 12 hours. The microcrystalline compounds then filtered and washed with suitable solvent and dried. (Scheme –2.5)



Scheme -2. 5: APTPMP-3,4 F

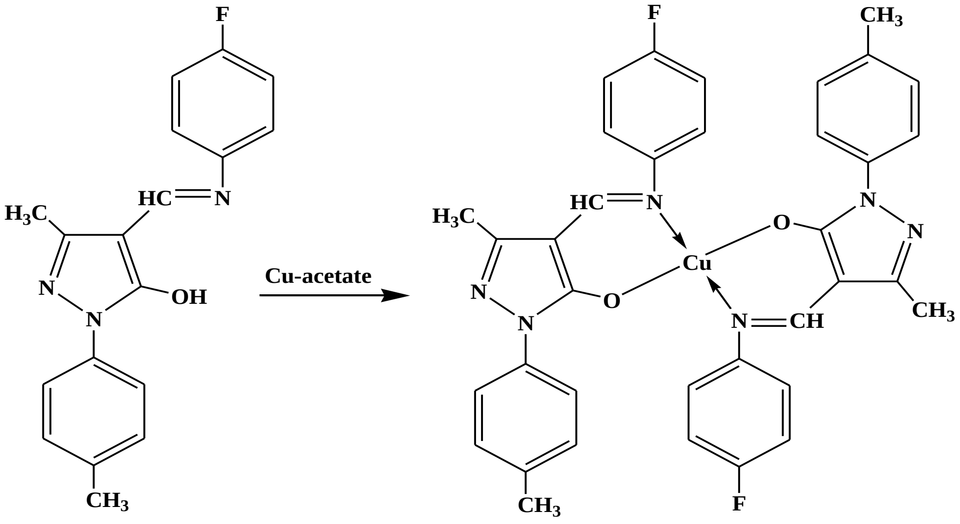
[APTPMP-3,4F] is yellow crystalline compound. Yield: 82%, m.p.: 186^{o}C.Anal. Calc. for $C_{18}H_{15}F_2N_3O$ M.W.: 327.33, C (66.05%), H(4.62%), N(12.84%), found: C (66.00%), H(4.65%), N(12.90%).

IR (KBr, cm^{-1}): 3265 (m) (O-H), 2919 (N-H), 1673 (s) (C=O, pyrazolone ring), 1607 (s) (C=N cyclic), 1210 (s) (C=N azomethine).^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 2.31 (s, 3H, CH_3), δ 2.36 (s, 3H, CH_3), δ 7.08-7.87 (m, 7H, Ar-H),δ 7.83 (s, CH=N-), δ 11.52 (s, 1H, O-H/N-H).MASS: m/z = 326.91 [$C_{18}H_{15}F_2N_3O$], 205.82 [$C_{10}H_7F_2N_3$]$^+$, 200.25 [$C_{12}H_{12}N_2O$]$^+$, 179.24 [$C_{10}H_9F_2N$]$^+$, 139.52 [$C_7H_5F_2N$]$^+$, 112.84 [$C_5H_8N_2O$]$^+$, 106.50 [C_7H_7N]$^+$, 90.85 [C_6H_7N]$^+$, 56.82 [C_4H_6]$^+$.

Preparation of Schiff base metal complexes

2.1.3.6. Synthesis of [Cu(APTPMP-4F)$_2$]

Copper nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolicsolution of the 4-(4-fluorophenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol (4 mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum (Scheme – 2.6).



APTPMP-4F

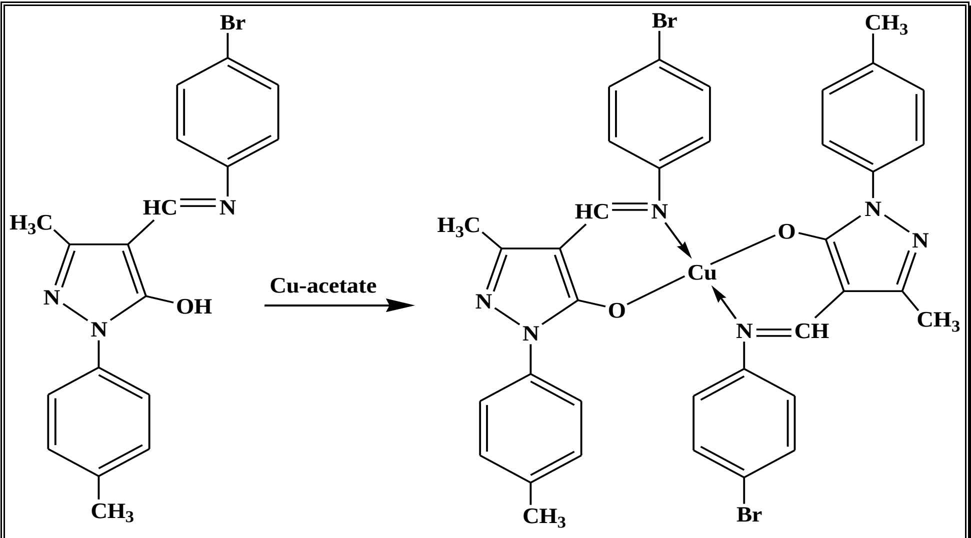
Scheme - 2.6: [Cu(APTPMP-4F)$_2$]

[Cu(APTPMP-4F)$_2$] is green colored crystalline compound. Yield: 72%, m.p.: >300^{o}C, Solubility: DMF. Anal. Calc. for $C_{36}H_{30}CuF_2N_6O_2$ M.W.: 680, C (63.57%), H(4.45%), N(12.36%), Cu (9.34%) found: C (63.61%), H(4.47%), N(12.31%), Cu (9.48%).

IR (KBr, cm^{-1}): 1549 (s) (C=N cyclic), 1211 (s) (C=N azomethine), 470 (Cu-O), 445 (Cu-N).ESI-MS: m/z = 681.0 [$C_{36}H_{30}CuF_2N_6O_2$], 650.0 [$C_{34}H_{25}CuF_2N_6O_2$]$^+$, 641.1 [$C_{36}H_{31}CuN_6O_2$]$^+$, 620.9 [$C_{32}H_{21}CuF_2N_6O_2$]$^+$, 613.0 [$C_{34}H_{27}CuN_6O_2$]$^+$, 588.0 [$C_{32}H_{23}CuN_6O_2$]$^+$, 372.0 [$C_{18}H_{15}CuFN_3O$]$^+$, 310.1 [$C_{18}H_{16}FN_3O$]$^+$, 217.1 [$C_{11}H_9FN_3O$]+, 189.1 [$C_{10}H_7N_3O$]+, 144.9 [$C_9H_8N_2$]$^+$, 105.9 [$C_6H_5N_2$]$^+$.

2.1.3.7. Synthesis of [Cu(APTPMP-4BR)$_2$]

Copper nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolicsolution of the 4-(-(4-bromophenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol(4 mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.7)



APTPMP-4BR

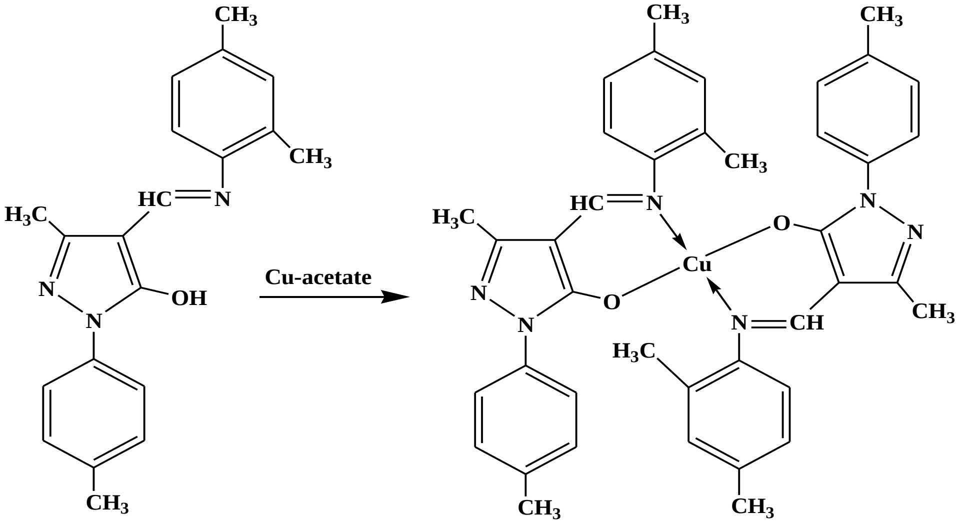
Scheme - 2.7: [Cu(APTPMP-4BR)$_2$]

[Cu(APTPMP-4BR)$_2$] is dark green crystalline compound. Yield: 83%, m.p.: >300^{o}C, Solubility: DMF. Anal. Calc. for $C_{36}H_{30}Br_2CuN_6O_2$ M.W.: 802, C (53.91%), H(3.77%), N(10.48%), Cu (7.92%) found: C (53.97%), H(3.73%), N(10.53%), Cu (8.00%).

IR (KBr, cm^{-1}): 1565 (s) (C=N cyclic), 1213 (s) (C=N azomethine), 472 (Cu-O), 440 (Cu-N).ESI-MS: m/z = 802.9 [$C_{36}H_{30}Br_2CuN_6O_2$], 743.0 [$C_{32}H_{22}Br_2CuN_6O_2$]$^+$,718.3 [$C_{36}H_{31}BrCuN_6O_2$]$^+$, 711.9 [$C_{29}H_{24}Br_2CuN_6O_2$]$^+$, 679.4 [$C_{33}H_{25}BrCuN_6O_2$]$^+$, 539.0 [$C_{22}H_{19}BrCuN_6O_2$]$^+$, 475.2 [$C_{23}H_{22}CuN_6O_2$]$^+$, 453.3 [$C_{21}H_{18}CuN_6O_2$]$^+$, 431.0 [$C_{18}H_{15}BrCuN_3O$]$^+$, 370.0 [$C_{18}H_{16}BrN_3O$]+, 338.3 [$C_{16}H_{12}BrN_3O$]$^+$, 279.0 [$C_{11}H_{10}BrN_3O$]$^+$, 217.1 [$C_{12}H_{13}N_3O$]$^+$, 189.1 [$C_{11}H_{12}N_2O$]$^+$

2.1.3.8. Synthesis of [Cu(APTPMP-2,4-ME)$_2$]

Copper nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolicsolution of the 4-(-(2,4-dimethyl-phenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol(4 mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.8)



APTPMP-2,4ME

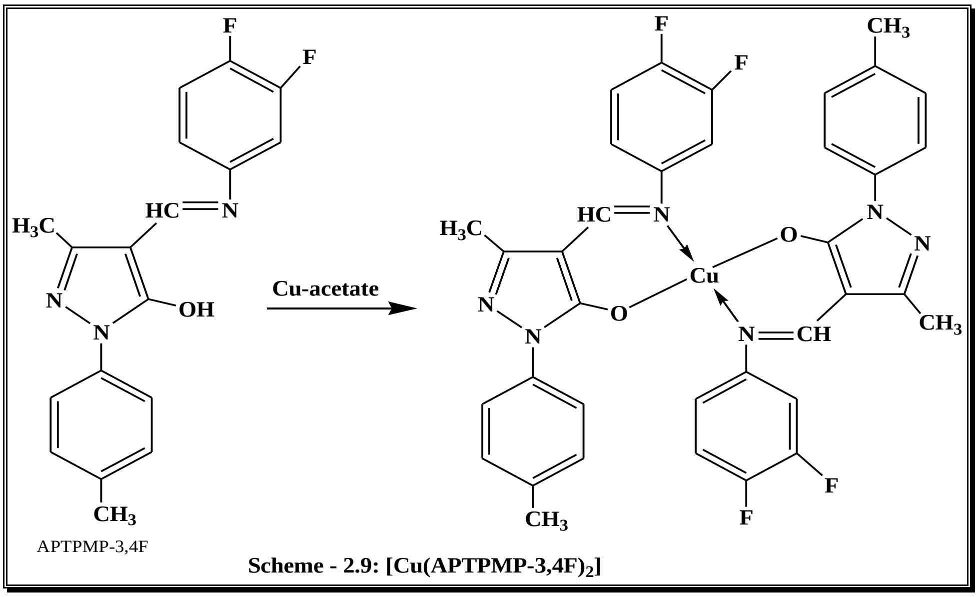
Scheme - 2.8: [Cu(APTPMP-2,4ME)$_2$]

[Cu(APTPMP-2,4-ME)$_2$] is dark green crystalline compound. Yield: 86%, m.p.: >300^{o}C, Solubility: DMF. Anal. Calc. for $C_{40}H_{40}CuN_6O_2$, M.W.: 700.33, C (68.60%), H(5.76%), N(12.00%), Cu (9.07%) found: C (68.58%), H(5.81%), N(12.12%), Cu (9.13%).

IR (KBr, cm^{-1}): 1578 (s) (C=N cyclic), 1214 (s) (C=N azomethine), 473 (Cu-O), 444 (Cu-N).

2.1.3.9. Synthesis of [Cu(APTPMP-3,4F)$_2$]

Copper nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolicsolution of the 4-((3,4-difluorophenylimino)methyl)-3-methyl-1-p-tolyl-1H-pyrazol-5-ol (4 mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.9)



Scheme - 2.9: [Cu(APTPMP-3,4F)$_2$]

[Cu(APTPMP-3,4F)$_2$] is dark green crystalline compound. Yield: 79%, m.p.: >300^{o}C, Solubility: DMF. Anal. Calc. for $C_{36}H_{28}CuF_4N_6O_2$, M.W.: 716.19, C (60.37%), H(3.94%), N(11.73%), Cu (8.87%) found: C (60.41%), H(3.97%), N(11.69%), Cu (8.92%)

IR (KBr, cm^{-1}): 1549 (s) (C=N cyclic), 1207 (s) (C=N azomethine), 467 (Cu-O), 440 (Cu-N).

2.1.4. Characterization of Schiff base ligands and metal complexes

2.1.4.1. Physico-chemical properties of synthesized Schiff base ligands and metal complexes

The Schiff base ligands and their metal complexes of aldehyde pyrazolone were synthesized in a very facile and essentially identical way. All these compounds are intensively colored, air and moisture free crystalline solids. They are soluble in common organic solvents. The structures of the ligands and metal complex areconfirmed by spectroscopic and crystallographic studies as well as some important analytical techniques.

IR spectra (4000–400 cm^{-1}) of the ligands and metal complexes were recorded using KBr discs on 8400 FT-IR Shimadzu spectrometer. The mass spectra of the ligands were recorded on a Trace GC ultra DSQ II. ^{1}H NMR spectra of ligands were recorded on Bruker Avance-II 400 MHz FT-NMR spectrometer using TMS as an internal standard and $CDCl_3$ as a solvent.X-ray intensity data were collected on

Bruker CCD area-detector diffractometer equipped with graphite monochromated Mo Kα radiation (λ= 0.71073 Å). ESI-Mass spectra of complexes were recorded VG-70S spectrometer. Electronic spectra of the metal complexes in DMF were recorded on a Perkin-Elmer Lambda 19 spectrophotometer. Molar conductance of the metal complexes was determined on systronics direct reading conductivity meter type CM-82T. Elemental analysis (C, H and N) was carried out on elemental analyzer Perkin-Elmer 2400, while analysis of copper was determined by EDTA after decomposing the complexes with HNO_3. Magnetic susceptibility measurements of the complexes were carried out by Gouy balance using Hg $[Co(SCN)_4]$ as calibrant. Purity of the ligands and their complexes was evaluated by thin layer chromatography. The analytical and physical data of ligands and their metal complexes are given in Table 2.1.

Table 2.1: The physical and analytical data of ligands and metal complexes

Complex	**Formula**	**M.W.**	**Color**	**% Yield**	**M.P.**	**Conductance Ω^{-1} cm^2 mol^{-1}**
APTPMP-4F	$C_{18}H_{16}FN_3O$	309.34	Yellow	76	180	20
APTPMP-4BR	$C_{18}H_{16}BrN_3O$	370.24	Reddish brown	72	186	22
APTPMP-2,4ME	$C_{20}H_{21}N_3O$	319.40	Yellow	80	170	19
APTPMP-3,4F	$C_{18}H_{15}F_2N_3O$	327.33	Yellow	82	186	24
[Cu(APTPMP-4F)$_2$]	$C_{36}H_{30}CuF_2N_6O_2$	680	Green	72	>300	28
[Cu(APTPMP-4BR)$_2$]	$C_{36}H_{30}Br_2CuN_6O_2$	802	Dark green	83	>300	26
[Cu(APTPMP-2,4ME)$_2$]	$C_{40}H_{40}CuN_6O_2$	700.33	Dark green	86	>300	28
[Cu(APTPMP-3,4F)$_2$]	$C_{36}H_{28}CuF_4N_6O_2$	716.19	Dark green	79	>300	25

2.1.4.2. Crystal structure of Schiff base ligands and metal complexes

2.1.4.2.1. *The Crystal structure of Schiff base ligand*

An ORTEP view of Schiff base ligand APTPMP-2,4-ME is shown in Figure-2.1. Single crystal data reveal that the Schiff base ligand is present in amine-one tautomeric form [158]in the solid state. Crystal structure data and important bond length & bond angle for the Schiff base ligand are given in Table- 2.2 and 2.3 respectively.

Figure- 2.1: Single Crystal ORTEP view of Schiff base ligand APTPMP-2,4-ME

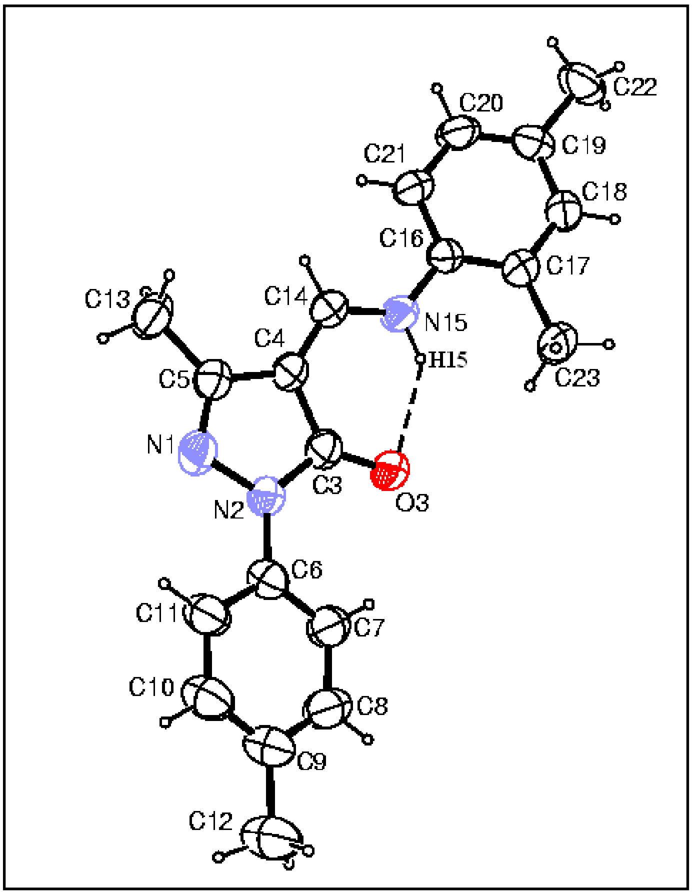


Table-2.2: Crystallographic data of Schiff base ligand [APTPMP-2,4ME]

Compound	**[APTPMP-2,4ME]**
Crystal color	White
Empirical formula	$C_{20}H_{21}N_3O$
Formula weight	319.40
Crystal system	triclinic

Space group	P -1
Crystal size	0.3 x 0.2 x 0.2 mm
a (Å)	8.2144(9)
b (Å)	10.4330(10)
c (Å)	11.5760(11)
α (°)	105.905(8)
β (°)	93.814(8)
Γ (°)	112.945(9)
V(Å^3)	861.61(15)
Z	2
$\rho_{calcd.}$(g cm^{-3})	1.231
Abs coeff, μ(cm^{-1})	0.078
F(000)	340
No. of parameters refined	220
Final R	0.0768
wR(F2)	0.1937
Goodness-of-fit	1.032

Table2.3: Selected bond lengths and bond angles

Bond distances (Å) with esd's in parentheses		**Bond angles (°) with esd's in parentheses**	
N1-C5	1.300(4)	C5 N1 N2	106.1(3)
N1-N2	1.410(4)	C3 N2 N1	111.7(3)
N2-C3	1.373(4)	N1 C5 C13	120.4(3)
N2-C6	1.414(4)	O3 C3 N2	126.7(3)
C3-O3	1.240(4)	O3 C3 C4	128.5(3)
C4-C14	1.362(4)	C14 C4 C3	123.0(3)
C5-C13	1.498(5)	C11 C6 N2	120.5(3)
C9-C12	1.517(6)	N15 C14 C4	122.8(3)

C14-N15	1.317(4)	C17 C16 N15	119.0(3)
N15-C16	1.407(4)	C14 N15 C16	128.9(3)
C19-C22	1.509(5)	C10 C9 C12	122.2(4)
C17-C23	1.518(5)	C16 C17 C23	120.8(3)

2.1.4.2.2.Crystal structure of Schiff base metal complex

The molecular structure and the atom labeling scheme of the copper complexes [Cu(APTPMP-2,4-ME)$_2$] and [Cu(APTPMP-3,4F)$_2$] are depicted in Fig. 2.2, 2.2Aand 2.3 respectively. The crystal structures of the complexes were solved by single-crystal XRD in the space group *P*-1 of the triclinic system and refined to give the formula [Cu(APTPMP-2,4-ME)$_2$] and [Cu(APTPMP-3,4F)$_2$] (DMF) for complexes. The crystallographic data of the complexes are listed in Table 2.4 and 2.6. Important bond lengths and bond angles of these complexes are given in Table- 2.5 and 2.7.

Crystal structure data revealed that the two N,O-chelating Schiff base ligands were coordinated to the copper metal centre to create a square planar geometry. Thus both the ligands form a six-membered chelate ring with the copper metal centre. The bite angles O14-Cu1-N16 and O38-Cu1-N40 are found to be 95.06(7) and 94.88(7), respectively, for complex **1**. The ligands were coordinated in an *anti* configuration to each other. In the complex **2** the bite angles O14-Cu1-N16 and O38-Cu1-N40 were found to be 94.64(9) and 94.62(10), respectively. One DMF molecule has also been trapped in the crystal lattice and the ligands were coordinated in an *anti* configuration to each other. In the complex **2**, F24 and F24’ have occupancies of 0.63 & 0.37, respectively.

Figure 2.2: ORTEP view of the copper complex [Cu(APTPMP-2,4-ME)$_2$]

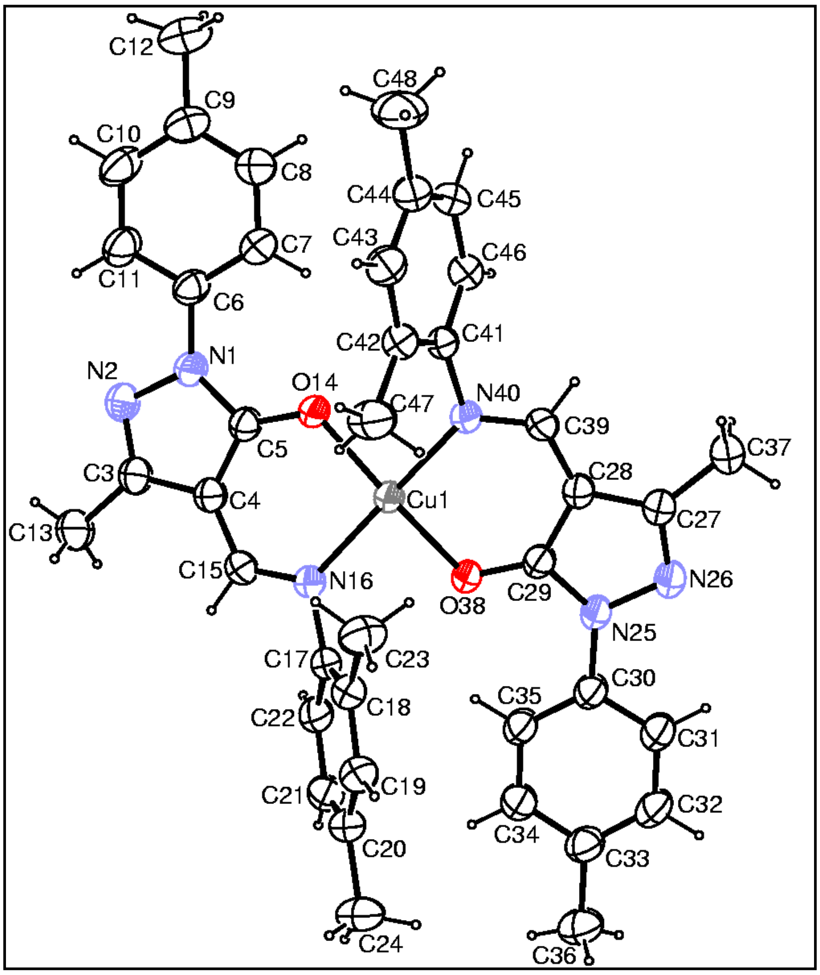


Figure 2.2A: Crystal packing of complex [Cu(PTPMP-ME)$_2$]. View down the *b* axis.

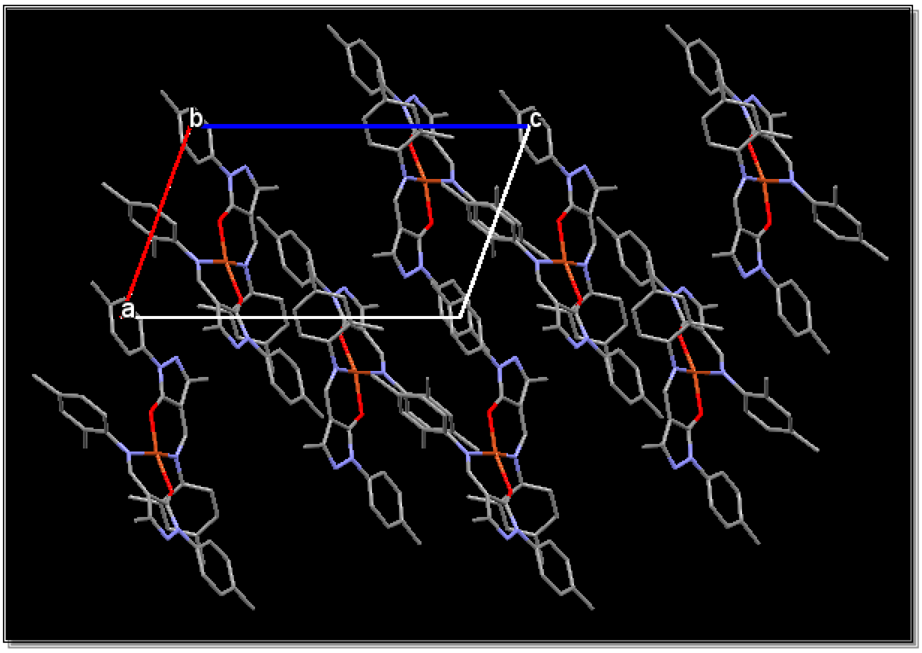


Table2.4: Crystal data and other experimental details for [Cu(APTPMP-2,4-ME)$_2$]

Compound	**[Cu(APTPMP-2,4ME)$_2$]**
Crystal description	Green Block
Empirical formula	$C_{40}H_{40}CuN_6O_2$
Formula weight	700.32
Crystal system	triclinic
Space group	P-1
a (Å)	9.9935(4)
b (Å)	12.4868(6)
c (Å)	15.8021(6)
α (º)	97.545(3)
β (º)	106.020(3)
Γ (º)	108.895(4)
V(Å^3)	1739.58(13)
Z	2
Crystal size	0.3 x 0.2 x 0.2 mm
Limiting indices	-11 ≤ h ≤ 12
	-15 ≤ k ≤ 14
	-15 ≤ l ≤ 19
Abs coeff, μ(cm^{-1})	1.235
F(000)	734
No. of parameters refined	450
Final R	0.0499
wR(F2)	0.1170
Goodness-of-fit	1.017

Table2.5: Selected bond lengths and bond angles for [Cu(APTPMP-2,4-ME)$_2$]

Bond distances (Å) with esd's in parentheses		**Bond angles (º) with esd's in parentheses**	
Cu1 O38	1.9361(14)	C15 N16 Cu1	121.93(15)
Cu1 O14	1.9593(14)	C17 N16 Cu1	123.49(13)
Cu1 N40	1.9989(17)	C29 N25 N26	111.56(18)
Cu1 N16	2.0020(18)	C39 N40 Cu1	122.35(14)
N1 N2	1.401(3)	C41 N40 Cu1	123.16(13)
N1 C6	1.425(3)	C5 O14 Cu1	121.41(14)
N16 C17	1.444(3)	C29 O38 Cu1	122.09(14)
C20 C24	1.519(4)	O38 Cu1 O14	165.00(7)
C18 C23	1.501(3)	O14 Cu1 N40	87.30(7)
C5 O14	1.280(3)	O38 Cu1 N40	94.88(7)
C15 N16	1.304(3)	O14 Cu1 N40	87.30(7)
N16 C17	1.444(3)	O38 Cu1 N16	86.51(7)
N25 N26	1.400(3)	N40 Cu1 N16	165.63(8)

Figure 2.3: ORTEP view of the copper complex [Cu(APTPMP-3,4F)$_2$]

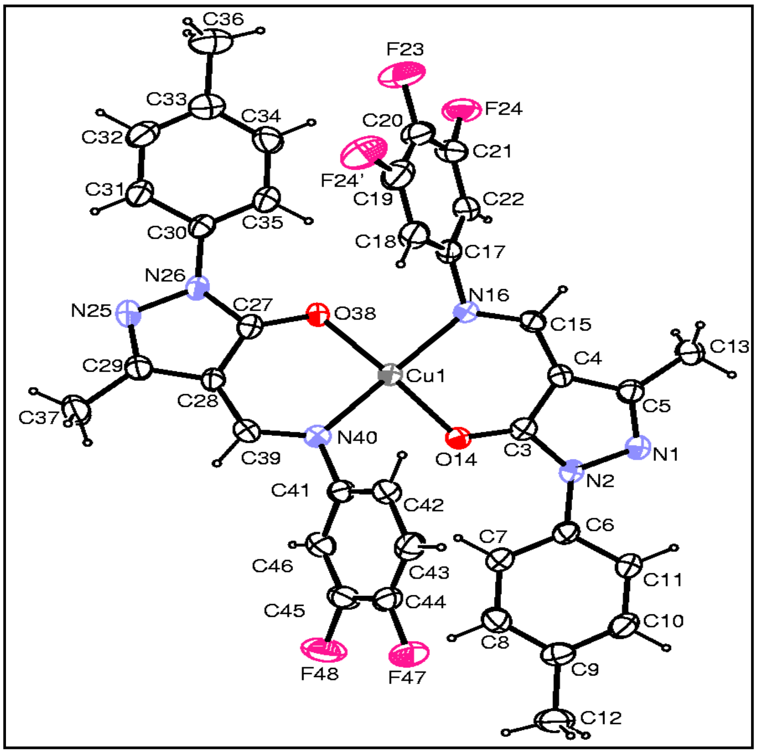


Figure 2.3A: Crystal packing of complex [Cu(PTPMP-3,4F)$_2$]. View down the *b* axis.

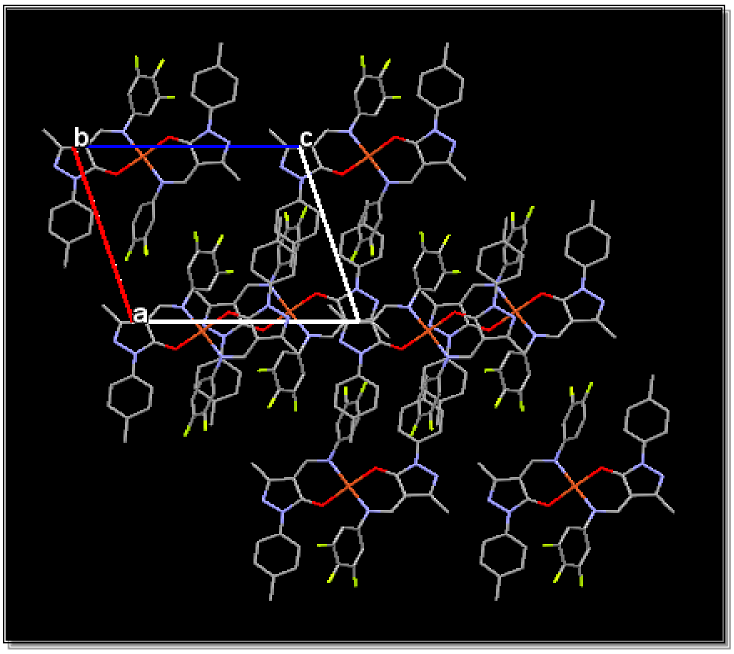


Table2.6: Crystal data and other experimental details for [Cu(APTPMP-3,4F)$_2$]

Compound	**[Cu(APTPMP-3,4F)$_2$]**
Crystal description	Green Block
Empirical formula	$C_{36}H_{27}CuF_4N_6O_2$
Formula weight	789.28
Crystal system	triclinic
Space group	P-1
a (Å)	11.6268(6)
b (Å)	13.4815(8)
c (Å)	14.1250(8)
α (°)	64.989(5)
β (°)	73.492(5)
Γ (°)	87.008(4)
V(Å^3)	1917.83(19)
Z	2

Crystal size	0.3 x 0.2 x 0.2 mm
Limiting indices	$-14 \leq h \leq 14$
	$-16 \leq k \leq 16$
	$-17 \leq l \leq 17$
Abs coeff, $\mu(cm^{-1})$	0.635
F(000)	814
No. of parameters refined	545
Final R	0.0877
$wR(F^2)$	0.1438
Goodness-of-fit	1.008

Table- 2.7: Selected bond lengths and bond angles for [Cu(APTPMP-3,4F)$_2$]

Bond distances (Å) with esd's in parentheses		**Bond angles (°) with esd's in parentheses**	
N16 Cu1	1.999(3)	C15 N16 Cu1	123.3(2)
N40 Cu1	1.997(3)	C17 N16 Cu1	121.4(2)
O14 Cu1	1.934(2)	C29 N25 N26	105.7(3)
O38 Cu1	1.940(2)	C39 N40 Cu1	123.7(2)
N1 N2	1.407(4)	C41 N40 Cu1	120.2(2)
C6 N2	1.420(4)	C3 O14 Cu1	122.2(2)
C17 N16	1.436(4)	C27 O38 Cu1	121.8(2)
C20 F23	1.357(4)	O14 Cu1 O38	162.81(11)
C21 F24	1.277(5)	O14 Cu1 N40	86.76(10)
C29 N25	1.307(4)	O38 Cu1 N40	94.62(10)
C30 N26	1.423(4)	O14 Cu1 N16	94.64(9)
C41 N40	1.427(4)	O38 Cu1 N16	88.19(10)
N25 N26	1.405(4)	N40 Cu1 N16	165.89(12)

2.1.4.3. IR spectral studies

The characteristic IR bands of Schiff base ligands and metal complexes of APTPMP recorded as KBr discs. IR spectral analysis confirms the presence of characteristic groups present in the ligands. Each of the Schiff base under discussion exhibits two characteristic bands in the ranges 3200-3300 cm^{-1} and 2900-2980 cm^{-1}, which can be assigned to $\upsilon_{O-H}$and υ_{N-H}respectively. The sharp bands observed at 1650-1675 cm^{-1} for $\upsilon_{C=O}$ and 1200-1225 for $\upsilon_{C=N}$, which can either be assigned to υ_{O-H} and $\upsilon_{C=N}$ respectively for the tautomeric imine-ol form or υ_{N-H} and$\upsilon_{C=O}$, respectively for the amine-one form of the ligands. We assigned this band to υ_{N-H} and $\upsilon_{C=O}$ for the later form based on the information obtained from crystallographic studies. The strong band at 1550-1610 cm^{-1} is observed in all ligands, is assigned to $\upsilon_{C=N}$ of the pyrazolone ring, which also suggests that ligands exist as the keto form in solid state, consisting with the crystal structure. [159,160](Figure:2.4-2.8).

Figure 2.4: IR spectrum of APTPMP

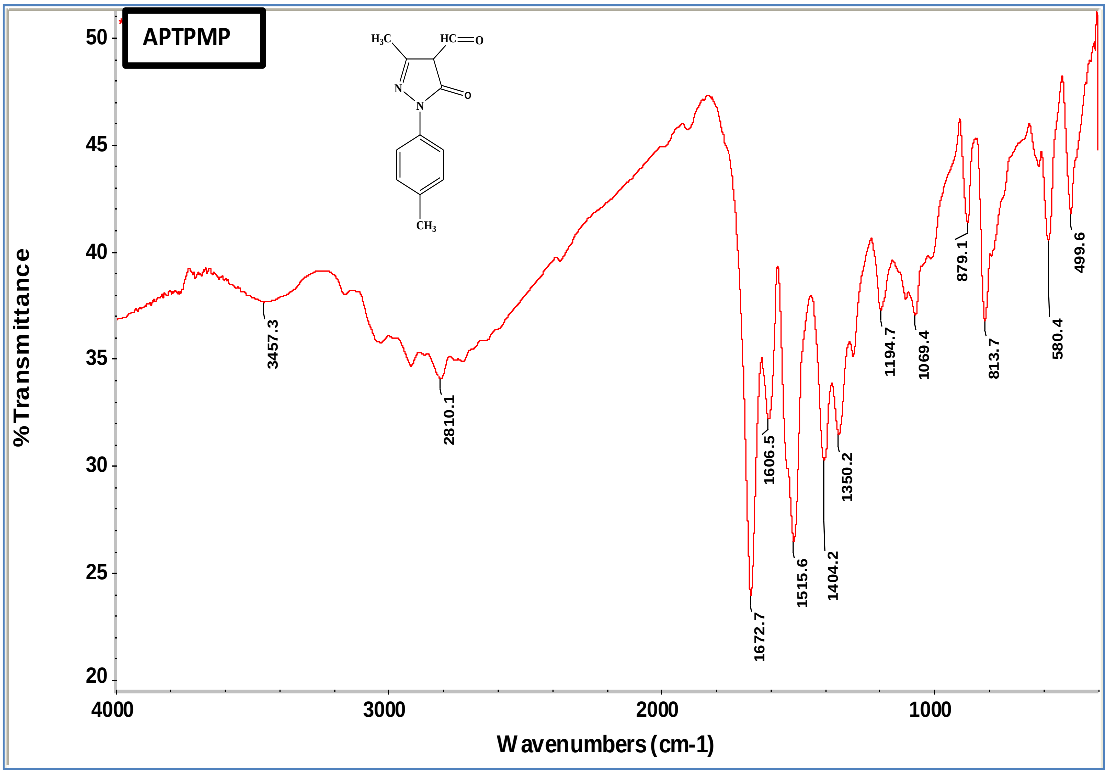


Figure 2.5: IR spectrum of Schiff base ligand APTPMP-2,4-ME

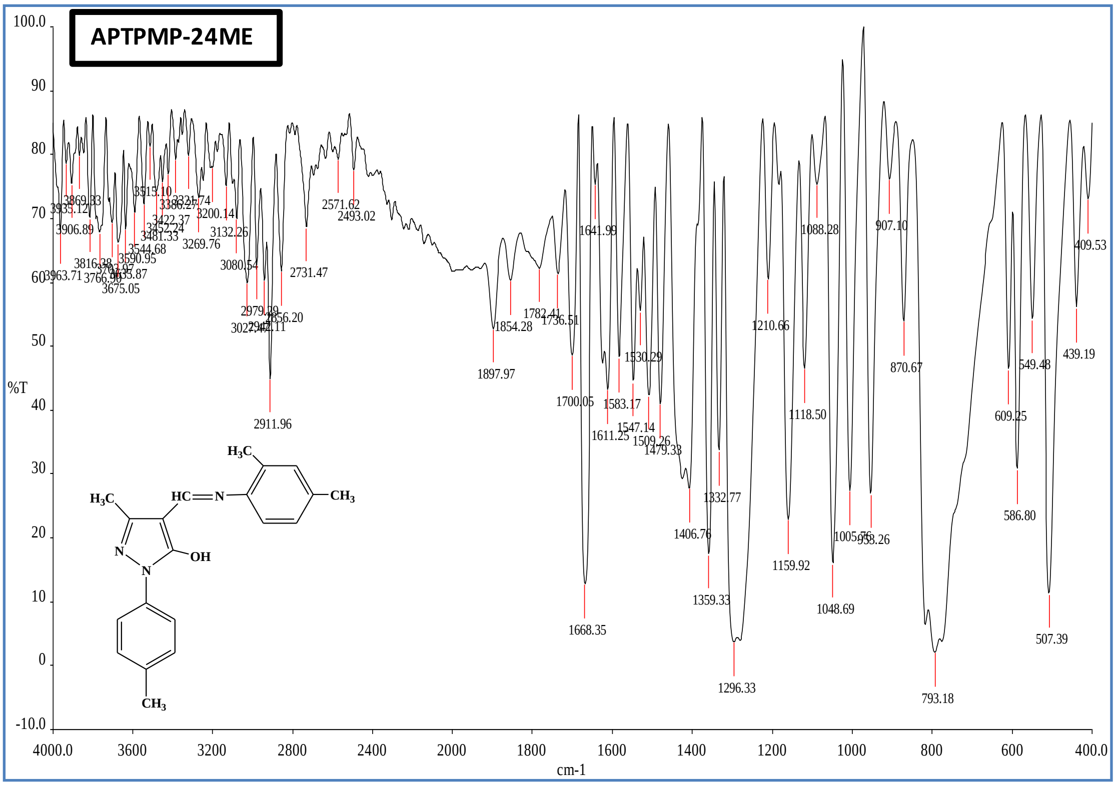


Figure 2.6: IR spectrum of Schiff base ligand APTPMP-3,4F

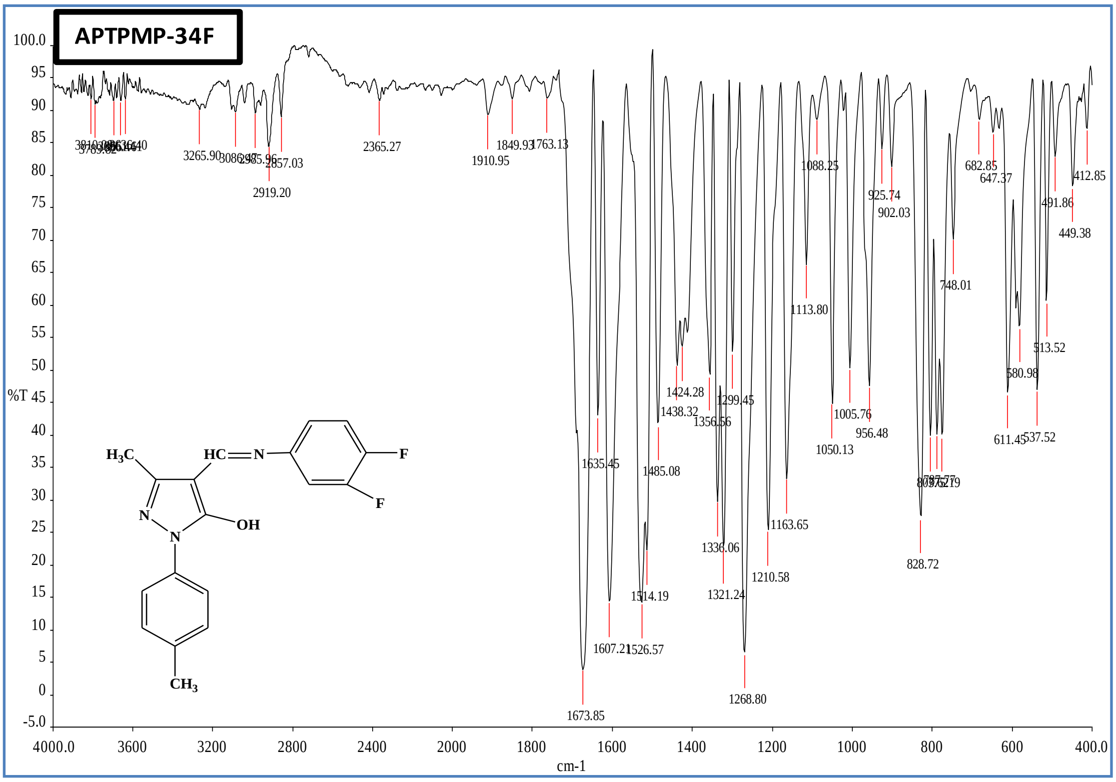


Figure 2.7: IR spectrum of Metal complex [Cu(APTPMP-2,4-ME)$_2$]

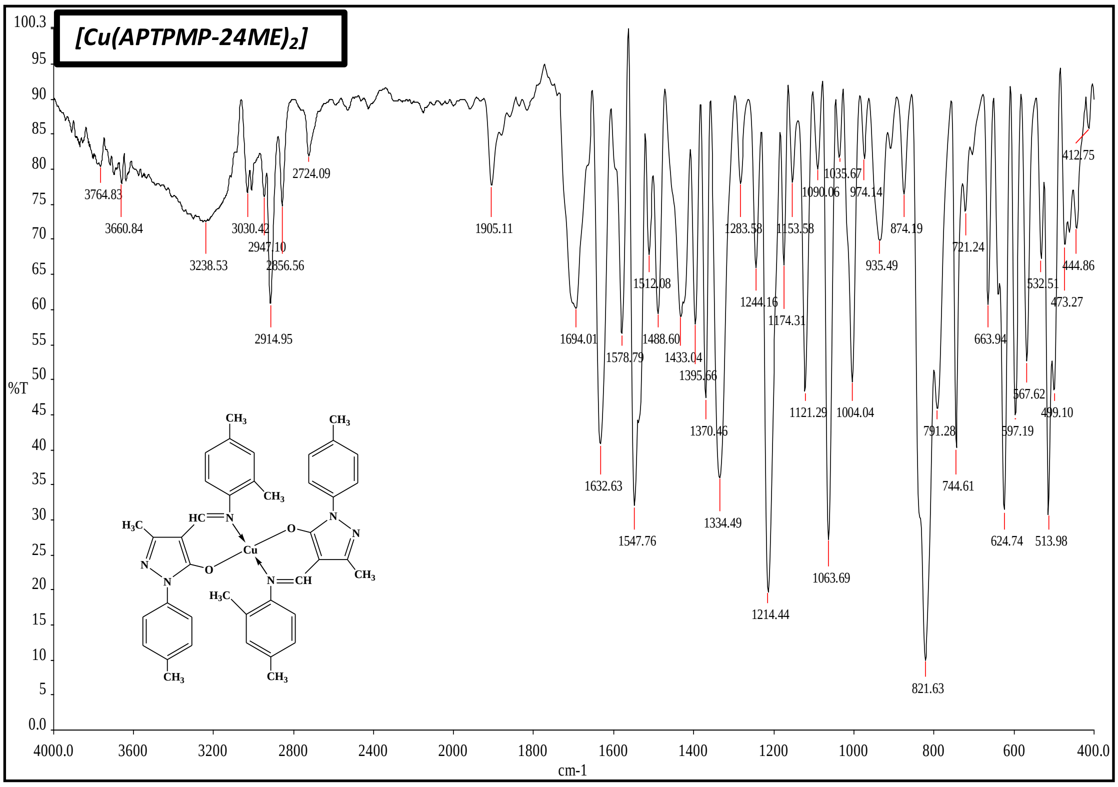
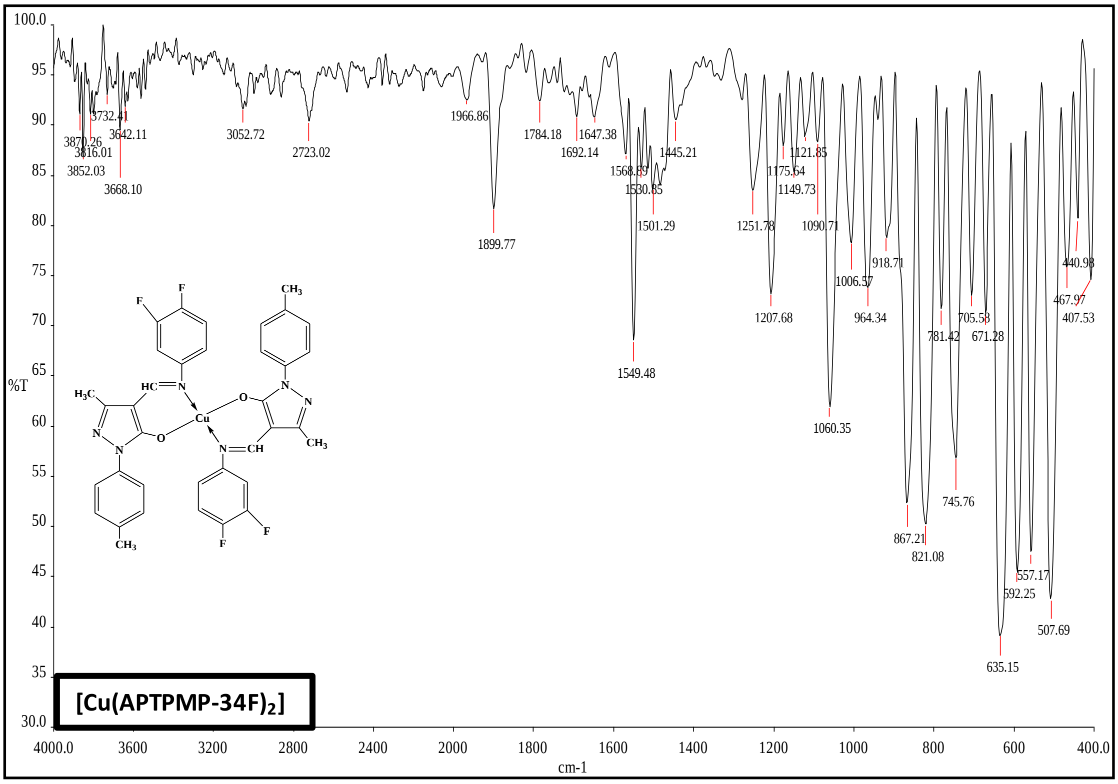


Figure 2.8: IR spectrum of Metal complex [Cu(APTPMP-3,4F)$_2$]



2.1.4.4. ^{1}H NMR spectral studies

The synthesized Schiff base ligands were characterized by the ^{1}H NMRspectra in $CDCl_3$. All the spectra are in good agreement with theproposed structure of the ligands. In the spectra of the ligands singlet appeared for the methyl group between 2.31-2.40 δppm. Due to the aryl protons of phenyl ringsdoublets, overlapped doublet and multiplet have been observed in theregion 6.9–8.0δppm.Another singlet corresponding to one proton for all compounds is observed in the range δ11.41-11.70δppm. This signal disappeared or weakened when a D_2O exchange experiment was carried out. It can be bonding with the other atom (N/O). It may be noted that the integration of this signal perfectly matches with one proton and there is no other fragment(s) of this signal, which suggests that only one tautomeric form of the ligands exist in solution under the experimental conditions. We have not done any temperature dependent experiments. Comparing with the crystal structure study, we prefer to assign this signal to –NH in the solid state. The ligands exist in the amine-one form in the solution state [158, 161-162] (Figure 2.9-2.10).

Figure 2.9: ^{1}H NMR spectrum of Schiff base ligand APTPMP-4F

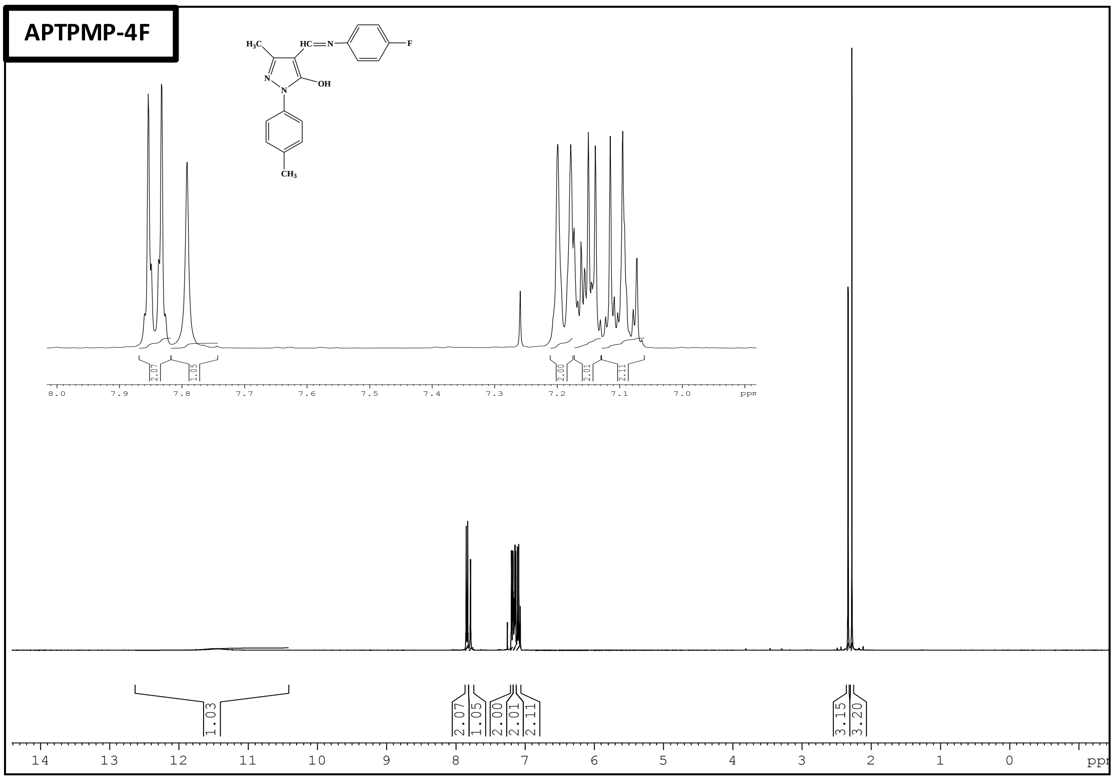
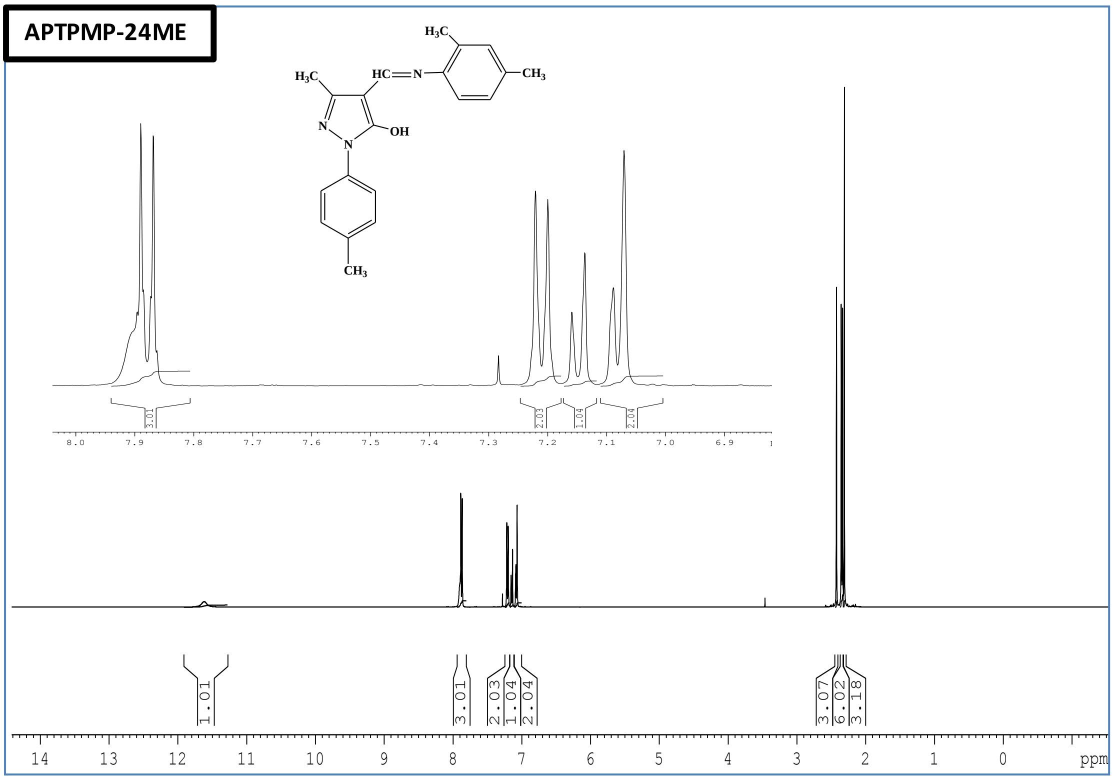


Figure 2.10: ^{1}H NMR spectrum of Schiff base ligand APTPMP-2,4-ME



2.1.4.5. Mass spectral studies

The mass spectra of Schiff base ligands were in good agreement with the proposed structures. Schiff base ligands show molecular ion peak corresponding to its molecular mass. The other peaks appeared in the mass spectrum (abundance range 1–100%) are attributed to the fragmentation of ligand molecule obtained from the rupture of different bonds inside the molecule. Melting point of each metal complex is very high, as a result of this; the mass spectra were carried out by ESI. The electron-impact mass spectra of pyrazolone derivatives, such as hydrazones, usually show an intense molecular ion peak.ESI–Mass spectra of complex shows molecular ion peakcorresponding to their molecular weight. Mass spectra of all the complexes are in good agreement with the structures of the complexes, which are further confirmed by X-ray crystallographic data [163, 164] (Figure 2.11-2.17).

Figure 2.11: Mass spectrum of APTPMP-4F

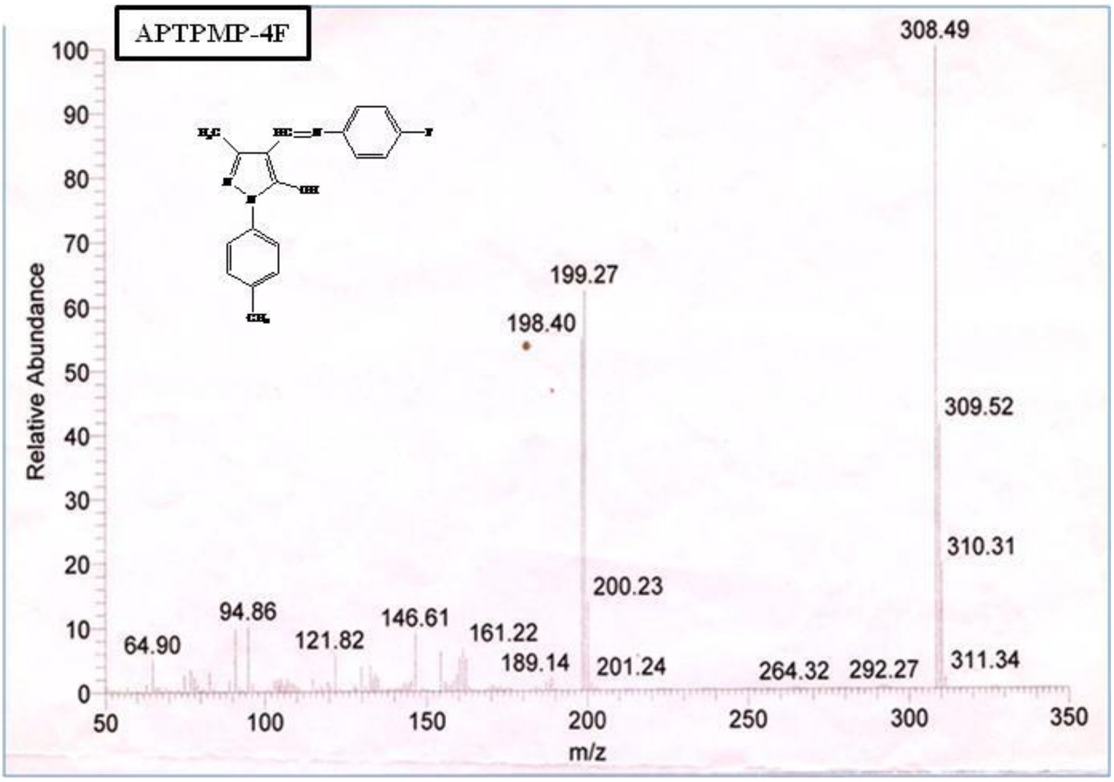


Figure 2.12: Mass spectrum of APTPMP-3,4F

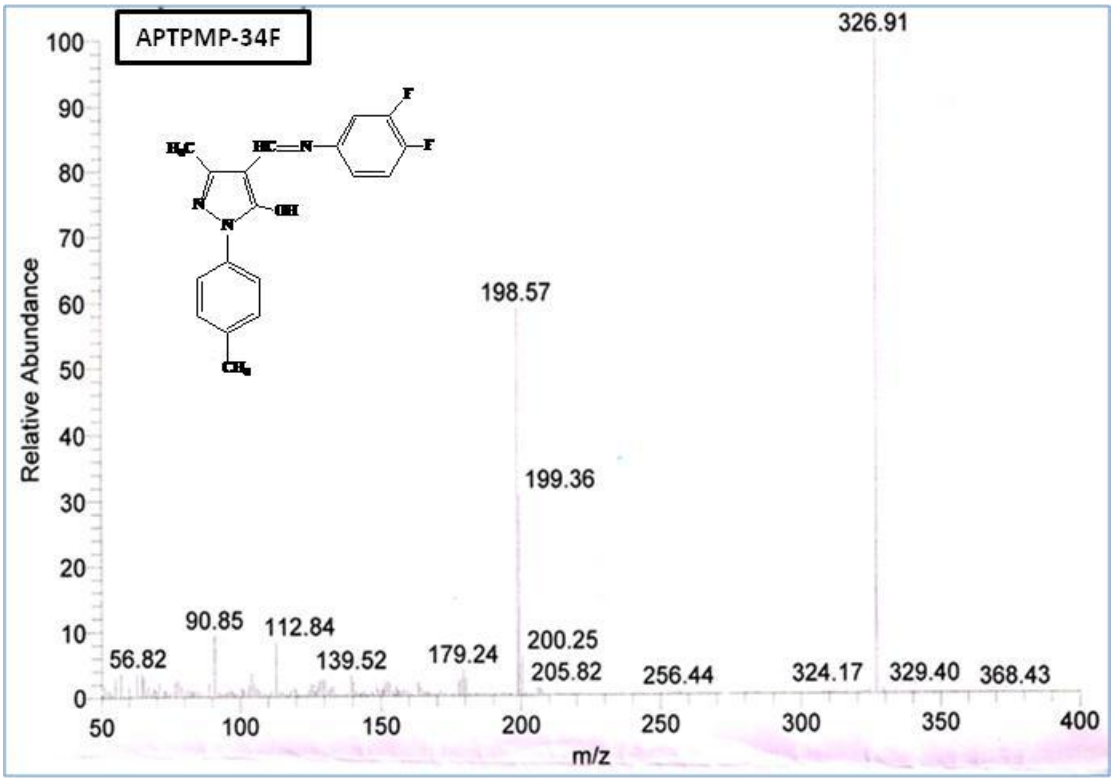


Figure 2.13: Mass spectrum of [Cu(APTPMP-4F)$_2$]

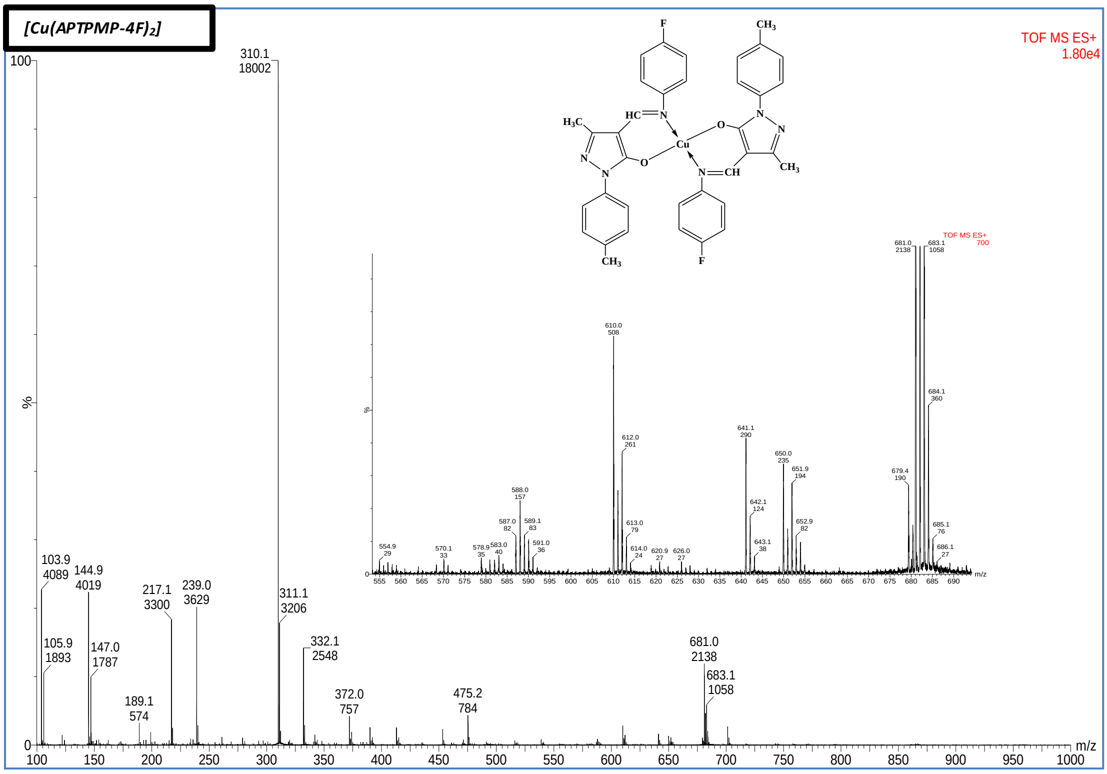


Figure 2.14: Mass fragmentation of APTPMP-4F

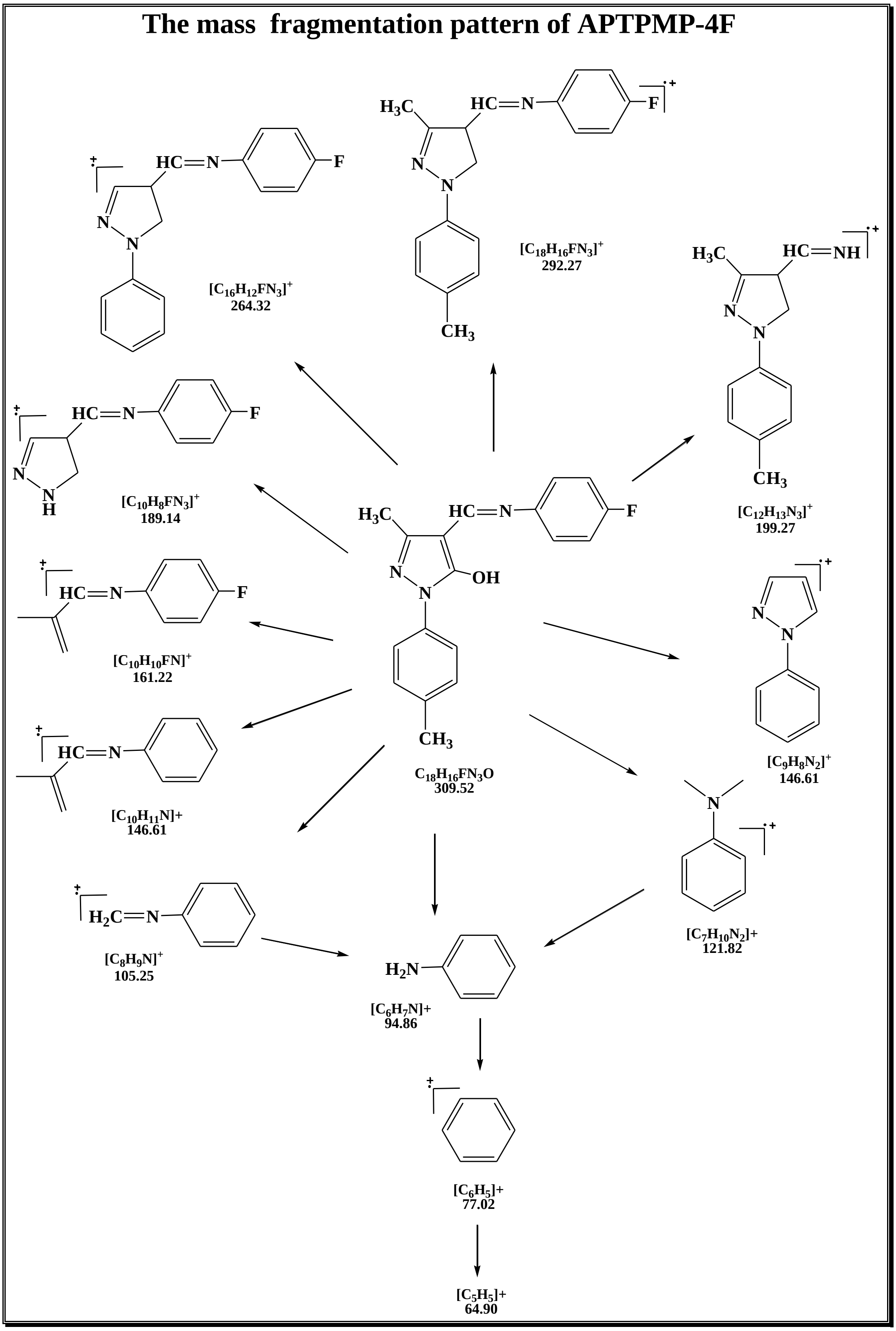


Figure 2.15: Mass spectrum of metal complex [Cu(APTPMP-4BR)$_2$]

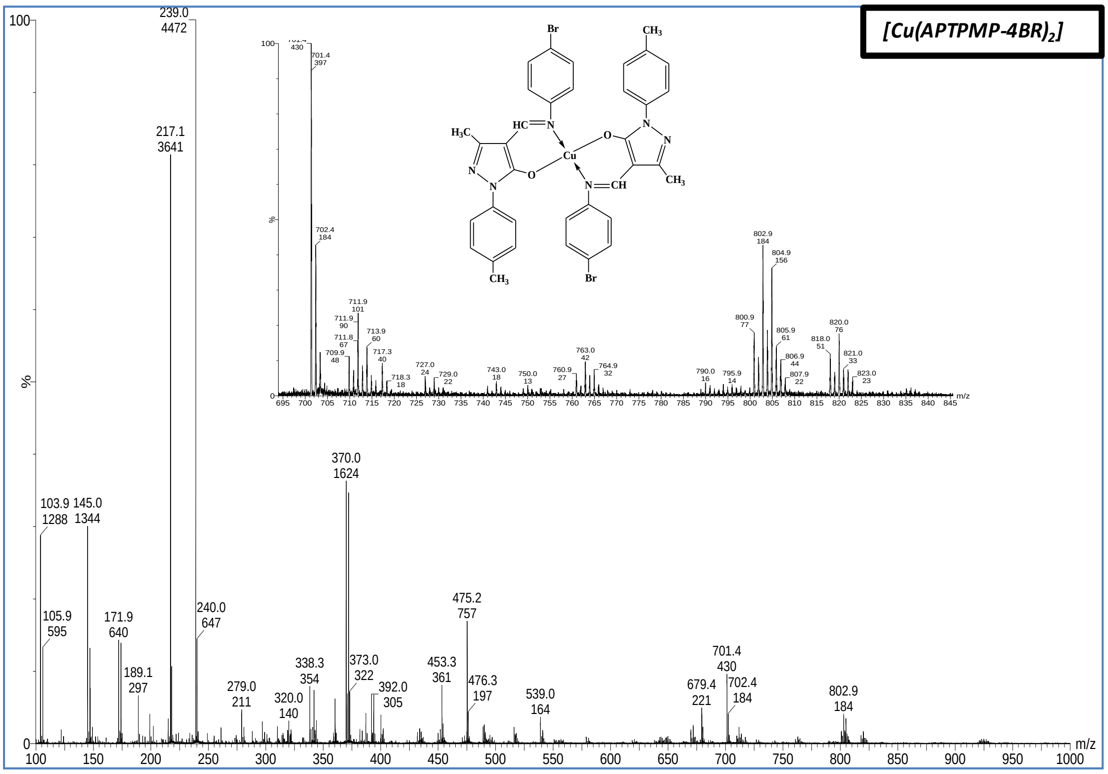


Figure 2.16: Mass spectrum of metal complex [Cu(APTPMP-3,4F)$_2$]

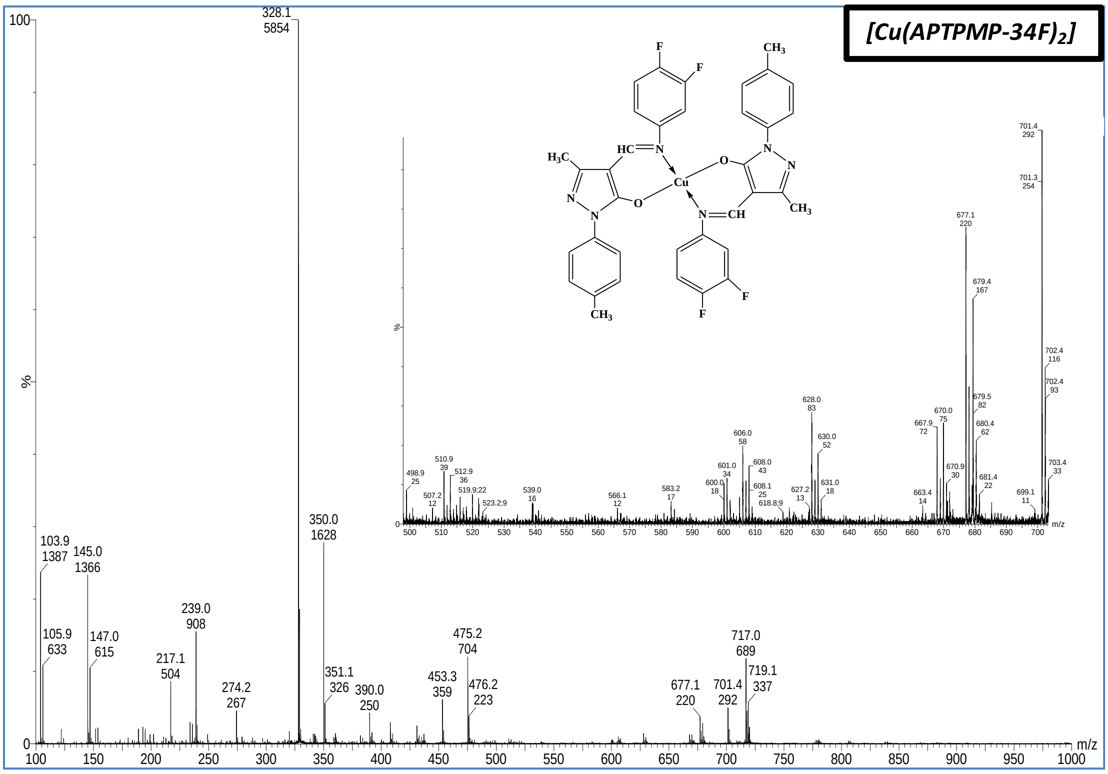
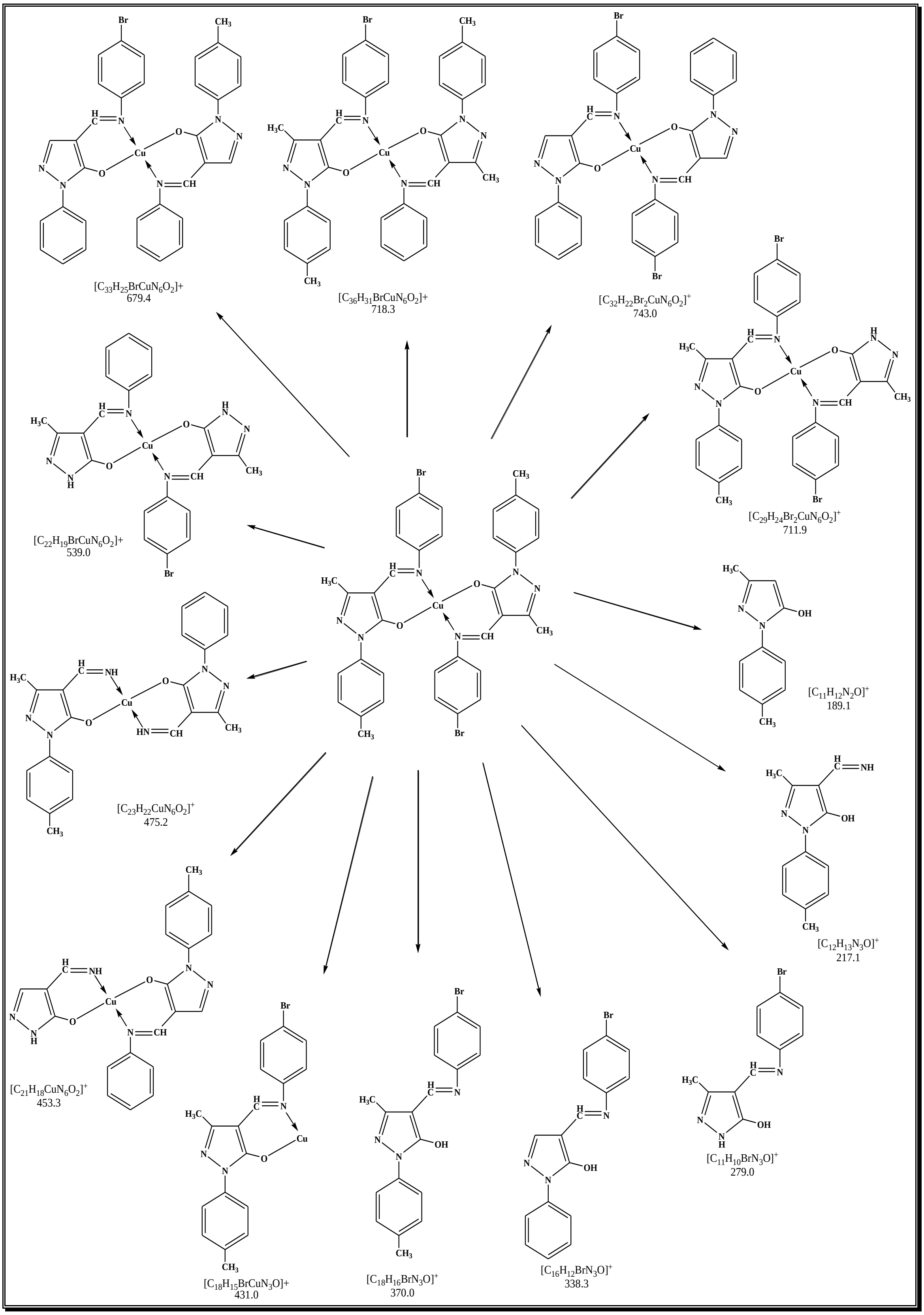


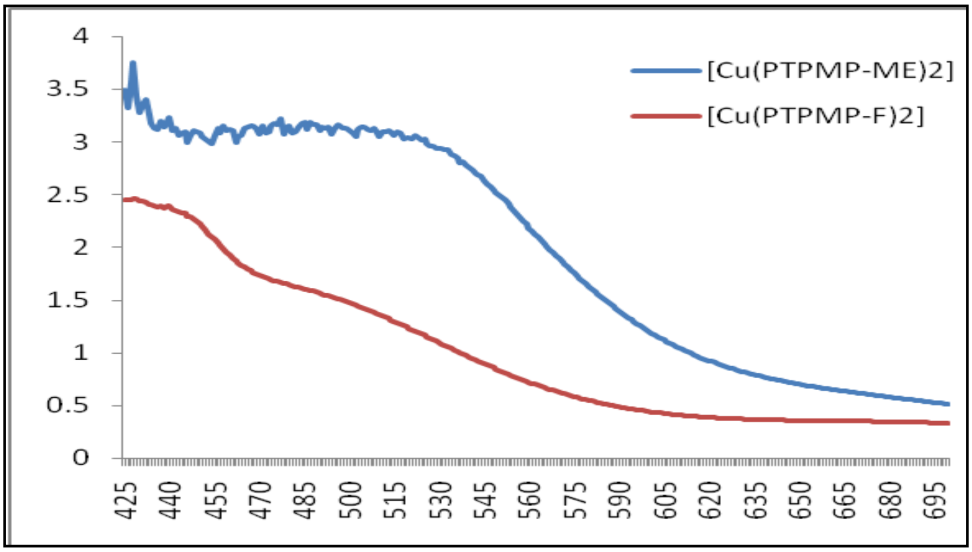
Figure 2.17: Mass fragmentation of metal complex [Cu(APTPMP-3,4F)$_2$]



2.1.4.6. UV – Visible spectral studies

Electronic spectra of all the copper complexes were recorded in Dimethylformamide (DMF), For square planar Cu(II) complex, the expected transitions are $^2B_{1g}\rightarrow{}^2A_{1g}$ and $^2B_{1g}\rightarrow{}^2E_g$ with respective absorptions at 505–520 and 665–650 nm. Due to Jahn–Teller (J-T) distortions, square planar Cu(II) complexes give a broad absorption between 600 and 700 nm and the peak at 505–520 nm merges with the broad band, and thus only one broad band is observed. All the Copper complexes under this study exhibit a broad band in the region of 530–600 nm [165,166]. (Figure 2.18).

Figure 2.18: UV-Visible spctrum of Copper complexes



2.1.4.7. Conductivity measurement

The observed molar conductance of the metal(II) complexes in $10^{-3}M$ DMF solution are in the range 5–17Ω^{-1} cm^2 mol^{-1}. The molar conductance values are consistent with the non-electrolytic nature for all metal complexes [167].

2.1.4.8. Magnetic measurement

Magnetic moment measurements of all the complexes show μ_{eff} of 1.71-1.80 BM, which corresponds to one unpaired electron and is expected from mononuclear Cu(II) complexes (d^9) with some orbital contribution. The magnetic moment values of the copper complexes correspond to the spin only value of 1.73 B.M. for the Cu(II) complexes [168].

2.1.5. Antimicrobial activity

Antimicrobial activity of the ligands and the metal complexes were evaluated against the *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. Metal complexesshow good inhibition against almost all bacterial species as compared to their respective ligands. It might be due to the complexation of metal ions with Schiff base ligand moreover such metal complexes might be inhibiting the enzyme activity of the bacterial system. Antibacterial activity of the ligands and complexes were quite comparable to the standard drugs e.g. Levofloxacin, Moxifloxacin, Amoxicillin, Cefprozil, Ciprofloxacin etc [169-172](Figure 2.19; Table 2.9-2.10).

Table 2.9: Antimicrobial activity of Schiff base ligands and copper complexes

Ligand/complex	**Microbial species (Zone of Inhibition in mm)**			
	S. aureus	***B. subtilis***	***E. Coli***	***P. aeruginosa***
APTPMP-4F	6	5	7	7
APTPMP-4BR	8	6	7	8
APTPMP-2,4-ME	7	7	5	9
APTPMP-3,4F	6	6	4	3
[Cu(APTPMP-4F)$_2$]	8	7	9	9
[Cu(APTPMP-4BR)$_2$]	10	8	10	10
[Cu(APTPMP-2,4-ME)$_2$]	9	6	9	4
[Cu(APTPMP-3,4F)$_2$]	9	9	4	7

Figure 2.19: Antimicrobial activity Chart

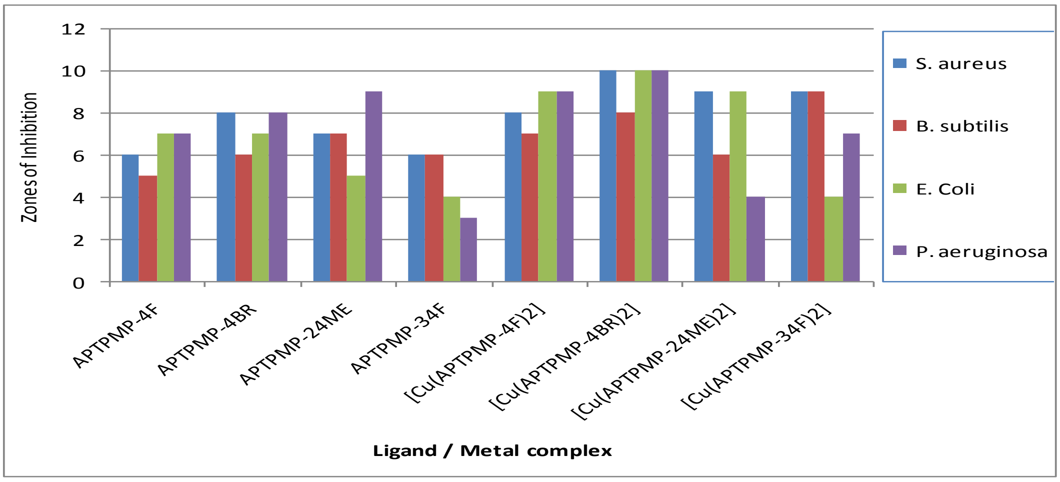


Table – 2.10: Standard drugs activity data

Standard Antibiotics	**Gram Negative Isolates** **Zone of inhibition (mm)**	
	E.coli	***P.aeruginosa***
Levofloxacin	6	12
Moxifloxacin	10	-
Cefprozil	6	-
Sparfloxacin	7	-
Gatifloxacin	10	-

Standard Antibiotics	**Gram Positive Isolates** **Zone Of Inhibition(mm)**	
	B.subtilis	***S.aureus***
Penicillin	-	15
Amoxicillin	6	15
Amoxicillin clavulanic acid	4	11
Co-trimoxazole	-	10
Cephalexin	20	14
Cefazolin	16	16
Cefuroxime	4	15
Erythromycin	12	4
Chloramphenicol	15	2
Ciprofloxacin	14	13
Ofloxacin	14	10
Piperacillin	11	20
Azithromycin	6	17
Tetracycline	13	18

2.1.6. Conclusion

Schiff base ligands of pyrazolone derivatives and their copper complexes were synthesized. Crystal and molecular structures of the complexes and one of the ligand was resolved by single crystal X-ray diffraction and other spectroscopic techniques. Schiff base ligands were found to exist in the amine-one form. All the copper complexes exist in the square planar geometry and two Schiff base ligands coordinated to copper metal centre in an *anti*-configuration. Antibacterial activities suggest that Schiff base ligands and their copper complexes have potential antibacterial activity. Further investigation is required to explore such ligands and metal complexes of pyrazolone derivatives for the industrial and pharmaceutical use.

2.1.7 References

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2.2: Synthesis, characterization, crystal structureand the biological evaluation of o-vanillin-4-aminoantipyrine Schiff base and its metal complexes

2.2.1 INTRODUCTION

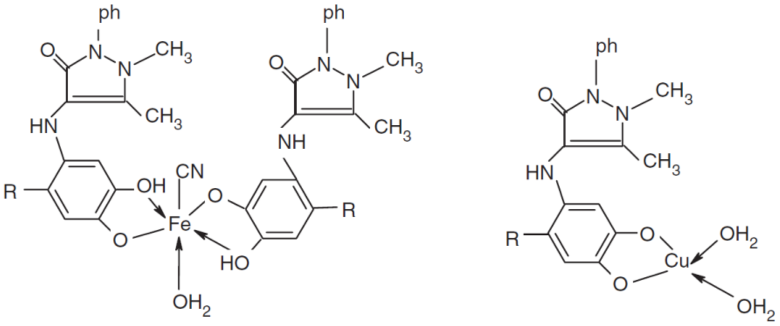
The metal complexes derived from N-heterocyclic ligands, largely based on derivatives of pyrazolone such as 4-aminoantipyrine and derivatives of pyridine, play an important role in pharmaceutical, clinical and analytical applications. Pyrazolone is an active moiety as a pharmaceutical ingredient, especially in the class of non-steroidal anti-inflammatory agents (NSAIDs) used in the treatment of arthritis and other musculoskeletal and joint disorders. The study reveals that drugs showed increased activity when administered as metal chelates rather than as organic compounds [173]. The coordinating 4-aminoantipyrine has been modified into a flexible ligand system by condensation with a variety of reagents like aldehyde, ketone etc.

In 1884, Knorr [174] discovered a derivative of pyrazole which showed an antipyretic action in humans and named the compound antipyrine. N. Raman *et al*. [175] have reported the DNA cleavage efficiency of Schiff bases and metal complexes derived from salicylidene-4-aminoantipyrine and 2-amino-3-hydroxypyridine. Transition metal complexes of pyrazolone derivatives are of importance for their biological activities. Among the pyrazolone derivatives, 4-amino antipyrine forms a variety of Schiff bases with aldehydes and ketones and they are reported to be superior reagents in biological, pharmacological and analytical applications [176]. With N-phenyl group attached adjacent to the carbonyl group 4-aminoantipyrine resembles a substituted amide and researchers have extensively studied this moiety. The carbonyl group in 4-aminoantipyrine group is a potential donor due to the large dipole moment (4.58 D) and strong basic character [177]. Since the 4-amino antipyrine system has 3 sites for binding with metal - the oxygen, the amino nitrogen and nitrogen of the 4-amino group- thecoordination chemists have taken interest to study the complexes of this compound.

There are reports of both octahedral and square planner geometry of Fe(III), Cu(II) and VO(IV) complexes formed from 4-aminoantipyrine Schiff base ligands.

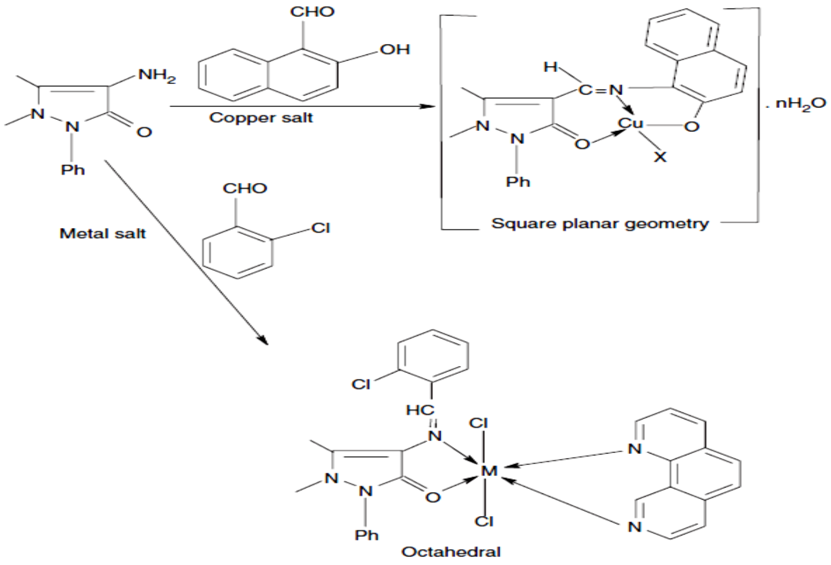
Mn(II), Co(II) and Ni(II) complexes with tridentate ligands derived from 4-aminoantipyrine and different aldehyde (2-hydroxy-1-nephthaldehyde and 2,4-dihydroxy benzaldehyde) were isolated and for all these complexes spectroscopic result suggested octahedral geometry [178].

Mohamad *et al.* [179] synthesized Fe(III) and Cu(II) complexes derived from catachol amine and 4 amino antipyrine. These were characterized by IR, UV-Vis, magnetic and thermal studies which showed Fe(III) forming 1:2 (M:catachol amine chelate) while Cu(II) formed 1:1 chelates. Magnetic moment measurements revealed Fe(III) chelates in octahedral geometry and Cu(II) in square planner structure.

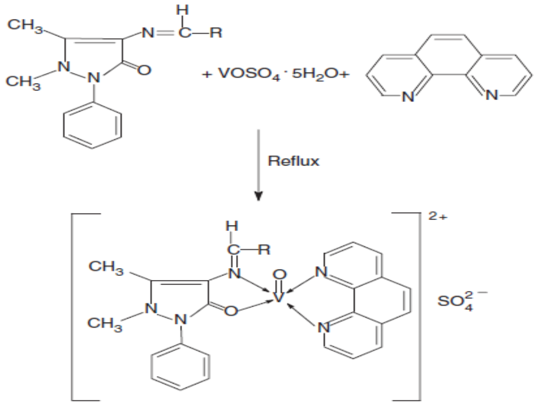


Isaa*et al.* [180] synthesized Schiff bases derived from 4-aminoantipyrine and benzaldehyde derivatives. Their UV-Vis, IR, H^1 NMR and fluorescent spectra were examined, supported by MO calculation and electron delocalization MO theory.

The square planner and octahedral geometry of 4-amino antipyrine metal complexes are given below.



Three oxovanadium(IV) Schiff-base complexes having the formula [VO(Phen)(L)]SO_4 (where L= 4[(benzylidene)amino]antipyrine, [(cinnamalidene)amino]antipyrine and 4[(2-chlorobenzylidene)amino]antipyrine) were designed using benzaldehyde/cinnamaldehyde/ 2-chlorobenzaldehyde with 4-aminoantipyrine, 1,10-phenanthroline, and vanadyl sulphate in the 1 : 1 : 1 molar ratio. Micro-analytical and spectral techniques were used to confirm the structures. Electronic spectral studies suggest square pyramidal geometry around vanadium [181].



A number of Schiff bases and metal complexes derived from 4-aminoanitpyrine and variety of aldehyde and ketones have been studied in the manner shown above together with their biological, medicinal and analytical applications [177]. A literature search shows however that o-vanillin, yet another type of aldehyde (3-hydorxy-3-methoxybenzaldehyde), itself possessing medicinal and biological properties,has not been so far used with 4-aminoantipyrine to prepare a Schiff base. It will be of interest to know what properties are altered when these two compounds form a Schiff base and its metal complexes.

In this section, the synthesis, characterization, single crystal x-ray study and the biological activity of 4-aminoantipyrine-o-vanillin Schiff base and its metal complexes are described.

2.2.2 EXPERIMENTAL SECTION

2.2.2.1 Materials

The compounds o-vanillin and 4-aminoantipyrine were obtained from Merck & Spectrochem Pvt. Ltd. Metal salts like$Cu(NO_3)_2{\cdot}3H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$ and

$VO(SO_4)_2$ were obtained form Merck. Absolute alcohol was obtained from Baroda Chem. Industry Ltd. Methanol was obtained from spectrochem Mumbai, India. All the chemicals used were of AR grade. Solvents used in this study were purified and dried following the standard procedures wherever required [151].

2.2.2.2 Single crystal X-ray structure determination

Single crystal X-Ray analysis was done by following method. The data collection was carried out on a Bruker Mart Apex CCD diffractometer using graphite-monochromated Mo-Kα (λ=71,073 Å) radiation at 293 K. An absorption correction based on SADABS [182] was also applied. The structure was solved by direct methods (SHELXTL) and refined by least-squares methods with atomic anisotropic thermal parameters for all non-hydrogen atoms[183]. All hydrogens were either generated geometrically or obtained from the Fourier difference map and were assigned fixed isotropic thermal parameters in the structure factor calculations. Conformational and H-bonding analysis was performed using PLATON[184].

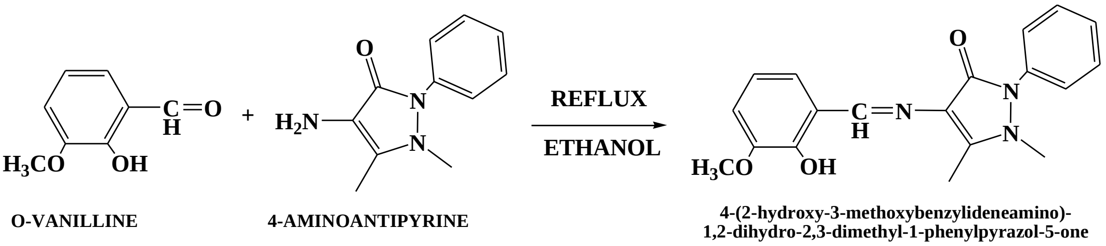
2.2.2.3 Antimicrobial screening

Antibacterial activity of all the ligands and complexes were evaluated against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli & Pseudomonas aeruginosa.* All strains were type strains. A young culture of each bacterial culture was prepared. Each bacterial culture added to the sterilized medium before solidification. The media with bacteria was poured into sterilized Petri dishes under aseptic condition. Different weights of Schiff base ligands and their metal complexes; (1mg, 5mg and 10mg) were placed on the surface of the culture and incubated at 37^{o}C for 24 hours. After incubation zone of inhibitions (mm) were recorded [185,186].

2.2.3 Preparation of Schiff base ligands and metal complexes

2.2.3.1 Synthesis of 4-(2-hydroxy-3-methoxybenzylideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one (V-4AAP)

Equimolar (10 mmol) ethanolic solution (50 mL) of 2-hydroxy-3-methoxybenzaldehyde and 4-aminoantipyrine was refluxed for 6 h in round bottom flask. During the reflux yellow crystal of V-4AAP was separated, which was isolated by filtration and dried in air. (Scheme- 2.10)



Scheme- 2.10: Synthesis of V-4AAP

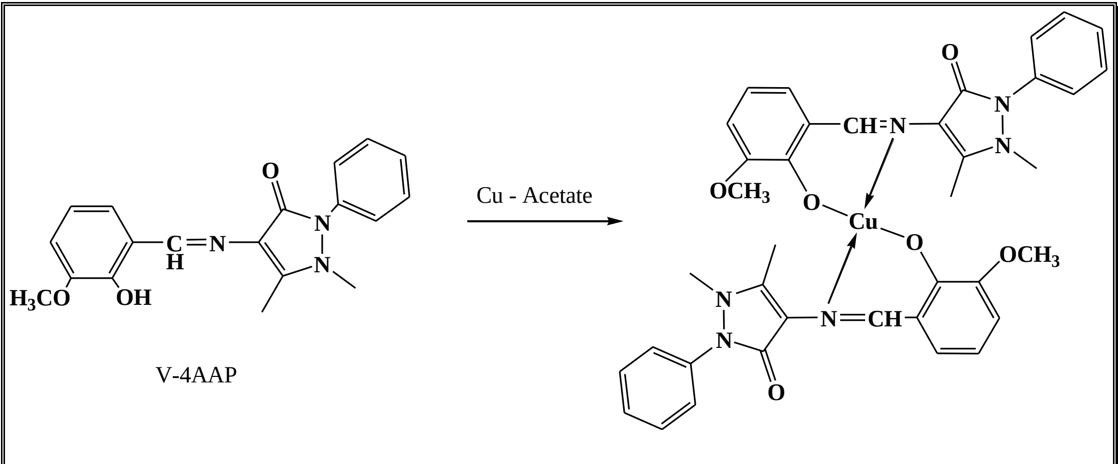
V-4AAP is yellow crystalline compound. Yield: 92%, m.p.: 190^{o}C. Anal. Calc. for $C_{19}H_{19}N_3O_3$ M.W.: 337.37, C (67.64%), H(5.68%), N(12.46%), found: C (67.69%), H(5.71%), N(12.52%).

IR (KBr, cm^{-1}): 3438 (O-H), 1591(C=N), 1663 (C=O) 2984(C-H), 1457 (N-H), 1600-1400 (Ar -C=C- ring skeletal), 1133 (C-O of -OH), 1097 (C-C), 846 (O=C-C). ^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 2.40 (3H singlet), δ 3.17 (3H singlet), δ 3.92 (3H singlet), δ 6.81-7.46 (Ar-H, multiplet), δ 9.81 (1H singlet), δ 13.92 (1H singlet). MASS: m/z = 337 [$C_{19}H_{19}N_3O_3$], 320 [$C_{19}H_{19}N_3O_2$]$^+$, 291 [$C_{18}H_{17}N_3O$] $^+$, 263 [$C_{16}H_{13}N_3O$]$^+$, 216 [$C_{12}H_{13}N_3O$]$^+$,201 [$C_{11}H_{13}N_3O$]$^+$, 188 [$C_{11}H_{12}N_2O$]$^+$, 169 [$C_{10}H_{11}N_3$]$^+$,137 [$C_6H_9N_3O$]$^+$, 124 [$C_5H_7N_3O$]$^+$, 84 [$C_3H_4N_2O$]$^+$, 77 [C_6H_6]$^+$, 66 [C_5H_6]$^+$.

Preparation of Schiff base metal complexes

2.2.3.2 Synthesis of [Cu(V-4AAP)$_2$]

Copper nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolicsolution of the 4-(2-hydroxy-3-methoxybenzylideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum.(Scheme – 2.11)



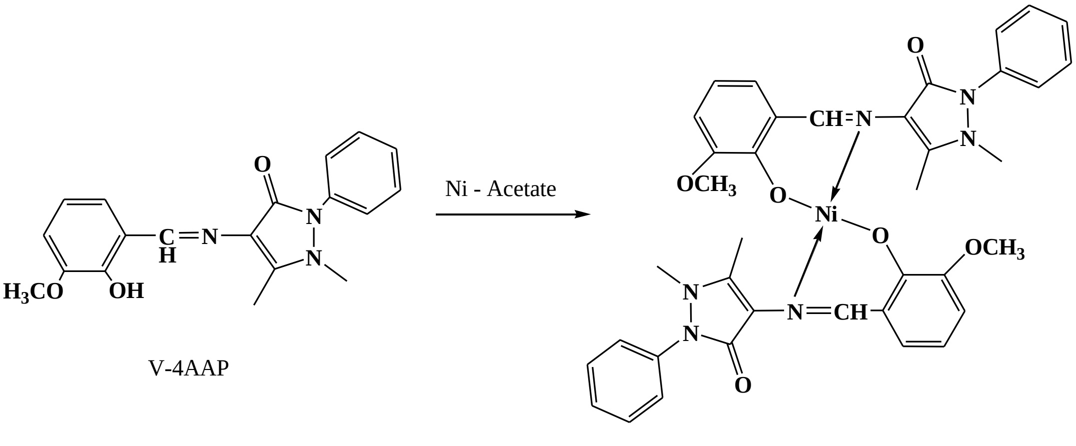
Scheme - 2.11: Synthesis of [Cu(4AAP)$_2$]

[Cu(V-4AAP)$_2$] is green crystalline compound. Yield: 85%, m.p.: >300^{o}C, Solubility: DMF. Anal. Calc. for $C_{38}H_{36}CuN_6O_6$ M.W.: 736, C (61.99%), H (4.93%), N (11.41%), Cu(8.63%) found: C (62.02%), H (4.89%), N (11.38%), Cu (8.71%).

ESI-MS: m/z = 736.2[$C_{38}H_{36}CuN_6O_6$], 701.3 [$C_{37}H_{34}CuN_6O_5$]$^+$, 605.5 [$C_{32}H_{26}CuN_6O_3$]$^+$, 419.1 [$C_{20}H_{21}CuN_3O_3$]$^+$, 399.0 [$C_{19}H_{18}CuN_3O_3$]$^+$, 338.1 [$C_{19}H_{19}N_3O_3$]$^+$, 307.1 [$C_{19}H_{19}N_3O_3$]$^+$, 263.1 [$C_{13}H_{15}N_3O_3$]$^+$, 245.1 [$C_{13}H_{15}N_3O_2$]$^+$, 214.1 [$C_{12}H_{13}N_3O$]$^+$, 151.1 [$C_7H_{11}N_3O$]$^+$.

2.2.3.3 Synthesis of [Ni(V-4AAP)$_2$]

Nickel Nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolicsolution of the 4-(2-hydroxy-3-methoxybenzylideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum.(Scheme – 2.12)



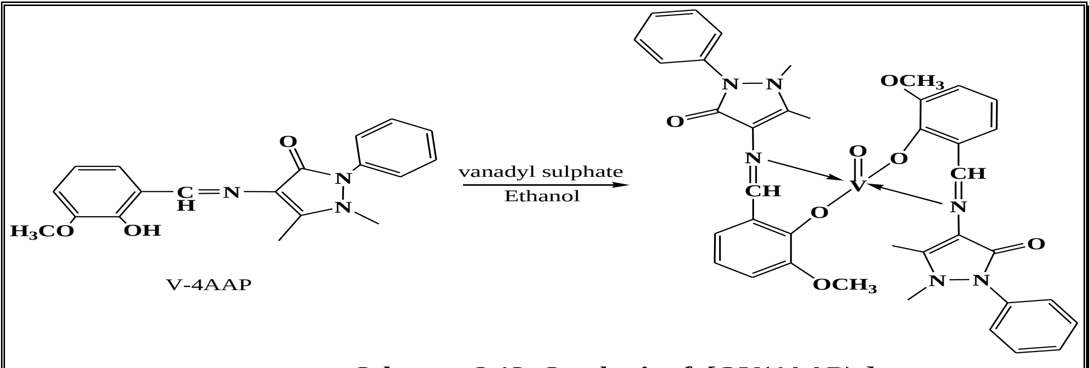
Scheme - 2.12: Synthesis of [Ni(4AAP)$_2$]

[Ni(V-4AAP)$_2$] is dark green crystalline compound. Yield: 81%, m.p.: >300°C, Solubility: DMF. Anal. Calc. for $C_{38}H_{36}N_6NiO_6$ M.W.: 731, C (62.40%), H (4.96%), N (11.49%), Ni(8.02%) found: C (62.47%), H (4.89%), N (11.52%), Ni(8.10%).

ESI-MS: m/z = 730.2 [$C_{38}H_{36}N_6NiO_6$], 719.2 [$C_{37}H_{34}N_6NiO_6$]$^+$, 713.2 [$C_{38}H_{36}N_6NiO_5$]$^+$, 701.5 [$C_{36}H_{32}N_6NiO_6$]$^+$, 475.3 [$C_{24}H_{21}N_3NiO_4$]$^+$, 360.1 [$C_{18}H_{16}N_3NiO_2$]$^+$, 338.1 [$C_{19}H_{19}N_3O_3$]$^+$, 307.1 [$C_{18}H_{17}N_3O_2$]$^+$, 263.1 [$C_{16}H_{13}N_3O$]$^+$, 159.1 [$C_9H_{11}N_3$]$^+$

2.2.3.4 Synthesis of [OV(V-4AAP)$_2$]

Vanadyl sulphate (2 mmol) was dissolved in minimumamount of distilledwater and the solution was added to hot ethanolicsolution of the 4-(2-hydroxy-3-methoxybenzylideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one (4mmol) with constant shaking.The reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum.(Scheme – 2.13)



Scheme - 2.13: Synthesis of [OV(4AAP)$_2$]

[OV(V-4AAP)$_2$] is light green crystalline compound. Yield: 72%, m.p.: >300^{o}C, Solubility: DMF. Anal. Calc. for $C_{38}H_{38}N_6O_8V$; M.W.: 737, C (60.24%), H (5.06%), N (11.09%), V(6.72%) found: C (60.30%), H (5.03%), N (11.04%), V(6.81%).

ESI-MS: m/z =736.2 [$C_{38}H_{36}N_6O_7V$], 713.3 [$C_{37}H_{34}N_6O_6V]^+$, 697.3 [$C_{36}H_{32}N_6O_6V]^+$, 417.1 [$C_{20}H_{21}N_3O_4V]^+$, 400.1 [$C_{19}H_{18}N_3O_4V]^+$, 376.1 [$C_{17}H_{13}N_3O_4V]^+$, 360.1 [$C_{17}H_{16}N_3O_3V]^+$, 338.1 [$C_{19}H_{19}N_3O_3]^+$, 307.1 [$C_{18}H_{17}N_3O_2]^+$, 263.1 [$C_{16}H_{13}N_3O]^+$, 245.1 [$C_{16}H_{15}N_3]^+$.

2.2.4 Characterization of Schiff base ligands and metal complexes

2.2.4.1 Physico-chemical properties of synthesized Schiff base ligand and metal complexes

The Schiff base ligand of o-vanillin-4-aminoantipyrine and its metal complexes have been synthesized in a very facile and essentially identical way, as in the case of other complexes in the study.All these compounds are intensely colored, air and moisture free crystalline solids. They are soluble in common organic solvents. The structure of the ligand and metal complexes were confirmed by spectroscopic and crystallographic studies as well as some important analytical techniques. The analytical and physical data of ligands and metal complexes are listed in Table 2.11.

IR spectra (4000-400 cm^{-1}) of the ligand and metal complexes were recorded using KBr discs on 8400 FT-IR SHIMADZU spectrometer. GC-Mass spectra of the ligands were recorded on QP 2010 SHIMADZU GCMS spectrometer. ^{1}H NMR spectra of ligands were recorded on Bruker Avance-II 400 MHz FT-NMR spectrometer using TMS as the internal standard and $CDCl_3$ as the solvent. X-ray intensity data were collected on Bruker Mart Apex CCD diffractometer using graphite-monochromatedMo-Kα (λ=71,073 Å) radiation at 293 K.ESI mass spectra of complexes were recorded VG-70S Spectrometer. Electronic spectra of the metal complexes in DMF were recorded on a Perkin Elmer Lambda 19 spectrophotometer. Molar conductance of the metal complexes was determined on Systronics direct reading conductivity meter type CM-82T. A simultaneous TG/DTA was recorded on Perkin Elmer Pyris-1 model. Elemental analysis (C, H and N) were carried out on elemental analyzer PERKIN ELMER 2400, while analysis of metals were determined by EDTA after decomposing the complexes with HNO_3.

Table 2.11: The physical and analytical data of metal complexes

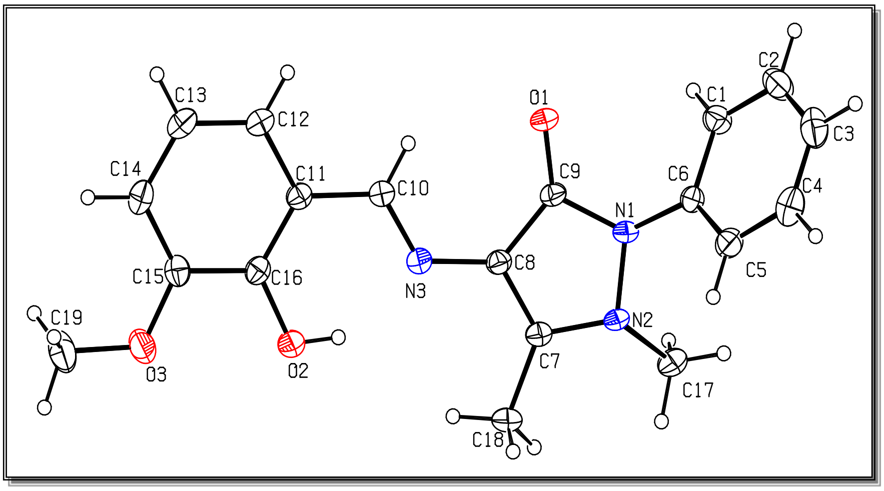
Complex	**Formula**	**M.W.**	**Color**	**% Yield**	**M.P.**	**Elemental analysis, % Found/(Calcd.)**				**Conductance Ω^{-1} cm^2 mol^{-1}**
						C	**H**	**N**	**M**	
[Cu(V-4AAP)$_2$]	$C_{38}H_{36}CuN_6O_6$	736	Greenish brown	86%	>300	62.08 (61.99)	4.85 (4.93)	11.35 (11.41)	8.90 (8.63)	12
[Ni(V-4AAP)$_2$]	$C_{38}H_{36}N_6NiO_6$	731	Green	75%	>300	62.33 (62.40)	5.02 (4.96)	11.41 (11.49)	8.11 (8.02)	15
[VO(V-4AAP)$_2$·H_2O]	$C_{38}H_{36}N_6O_7V$	737	Dark green	82%	>300	61.82 (61.70)	4.97 (4.91)	11.31 (11.36)	6.97 (6.89)	19

2.2.4.2 Crystal structure

The Crystal structure of Schiff base ligand [V-4AAP]

The molecular structures of Schiff base ligand V-4AAP was determined from single crystal X-ray studies. *ORTEP* view of the ligand with atom numbering scheme is shown in Fig.2.20. The crystallographic data are provided in the Table 2.12. Important bond lengths and angles for the compound are listed in Table 2.13 and H-bonding data are given in Table 2.14. The crystal structure data suggest that the ligand possesses strong intramolecular O-H---N hydrogen bond. The strong intramolecular O-H---N hydrogen bond is the evidence of the preference for the phenol-imine tautomeric form in the solid state [187].

Figure- 2.20: ORTEP view of Schiff base ligand V-4AAP



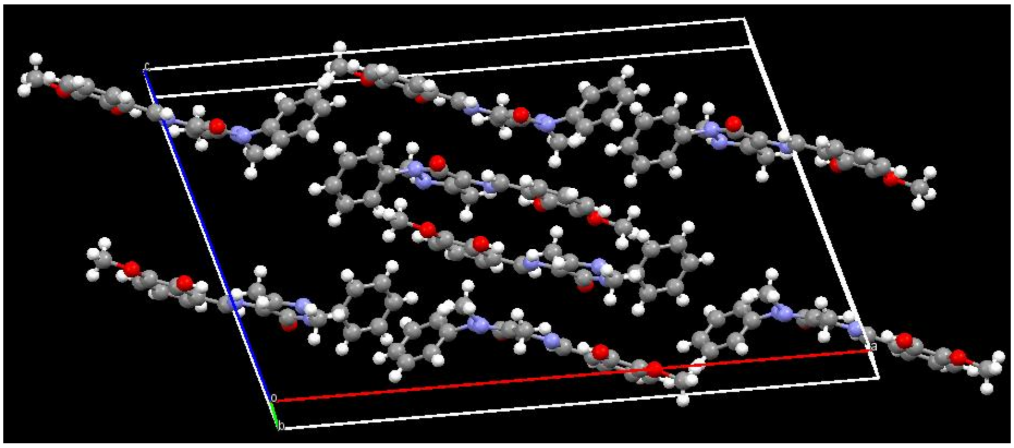


Table 2.12: Crystal data and other experimental details for V-4AAP

Identification code:	**Vaapfm**
Empirical formula:	C19 H19 N3 O3
Formula weight:	337.37
Temperature:	293(2) K
Wavelength:	0.71073 A
Crystal system:	Monoclinic
Space group:	C2/c
Unit cell dimensions:	a = 27.705(18) A alpha = 90 deg. b = 7.522(5) A beta = 106.168(10) deg. c = 16.565(10) A gamma = 90 deg
Volume:	3315(4) A^3
Z:	8

Density (calculated):	1.352 Mg/m^3
Absorption coefficient:	0.093 mm^-1
F(000):	1424
Crystal size:	0.43 x 0.32 x 0.12 mm
Theta range for data collection:	1.53 to 26.00 deg.
Index ranges:	-31<=h<=34, -4<=k<=9, -18<=l<=20
Reflections collected:	7485
Independent reflections:	3200 [R(int) = 0.0459]
Absorption correction:	Semi-empirical from equivalents
Max. and min. transmission:	0.9889 and 0.9610
Refinement method:	Full-matrix least-squares on F^2
Data / restraints / parameters:	3200 / 0 / 230
Goodness-of-fit on F^2:	1.108
Final R indices [I>2sigma(I)]:	R1 = 0.0710, wR2 = 0.2091
R indices (all data):	R1 = 0.0794, wR2 = 0.2172
Largest diff. peak and hole:	0.307 and -0.330 e.A^-3

Table 2.13: Selected bond lengths and angles in the ligand V-4AAP

Bond distances (Å) with esd’s in parentheses		**Bond angles(°)with esd’s in parentheses**	
O(1)-C(9)	1.229(3)	C(15)-O(3)-C(19)	117.5(2)
O(2)-C(16)	1.347(3)	C(9)-N(1)-N(2)	109.33(17)
O(3)-C(15)	1.359(3)	C(9)-N(1)-C(6)	121.02(18)
O(3)-C(19)	1.419(3)	C(7)-N(2)-N(1)	106.27(16)
N(1)-C(9)	1.399(3)	C(10)-N(3)-C(8)	122.40(19)
N(1)-N(2)	1.410(3)	C(5)-C(6)-N(1)	121.1(2)
N(2)-C(17)	1.464(3)	O(1)-C(9)-N(1)	123.4(2)
N(3)-C(10)	1.291(3)	O(3)-C(15)-C(14)	125.5(2)
N(3)-C(8)	1.383(3)	O(2)-C(16)-C(11)	122.6(2)

C(10)-C(11)	1.443(3)	C(11)-C(16)-C(15)	119.8(2)
C(7)-C(18)	1.489(3)	N(3)-C(10)-C(11)	120.5(2)

Table 2.14: Hydrogen coordinates(x 10^4) and isotropic displacement parameters (A^2 x 10^3) of V-4AAP

	X	Y	Z	U(eq)
H(2)	-380	6833	4073	69
H(2A)	2304	11703	2490	63
H(1)	1516	10444	2210	50
H(3)	2957	10578	3547	68
H(12)	-485	12701	4010	47
H(13)	-1149	13234	4547	54
H(10)	90	10665	3597	41

2.2.4.3 IR spectral studies

The characteristic IR bands of Schiff base ligand and metal complexes of V-4AAP recorded as KBr discs. IR spectral analysis confirms the presence of characteristic groups present in the ligands. The IR spectrum of the ligand shows a broad band at 3400–3460 cm^{-1} due to the stretching vibrations of phenolichydroxyl group. The broadness is due to intramolecularhydrogen bonding between the phenolic groupand the azomethine group. The strong band observed at 1591 cm^{-1} is assigned to the stretching vibrations of theazomethine (-C=N-) group. The sharp band at 1663 cm^{-1} is indicates the presence of C=O group. Two moderately intense bands observed at 3100 and 2921 cm^{-1} are due to aromatic and aliphaticν(C–H), respectively.Whereas band appeared in the rangeof 2927–2930 cm^{-1} is due to stretching vibrations of the–OCH_3group. The IR spectra of metal complexes show sharp bands in the range 1600–1550 cm^{-1}, which are then shifted to lower frequency as compared to ligand, suggesting coordination of the azomethine nitrogen to the metal ion. The disappearance of ν (O–H) shows the deprotonation of the -OH group and its subsequent coordination to the central metal atom. Two new bands observed at 578–

564 and 481-470 cm^{-1} are characteristic of M–O and M–N absorptions, respectively [188-192]. (Figure 2.21-2.24)

Figure 2.21: IR spectrum of Schiff base ligand V-4AAP

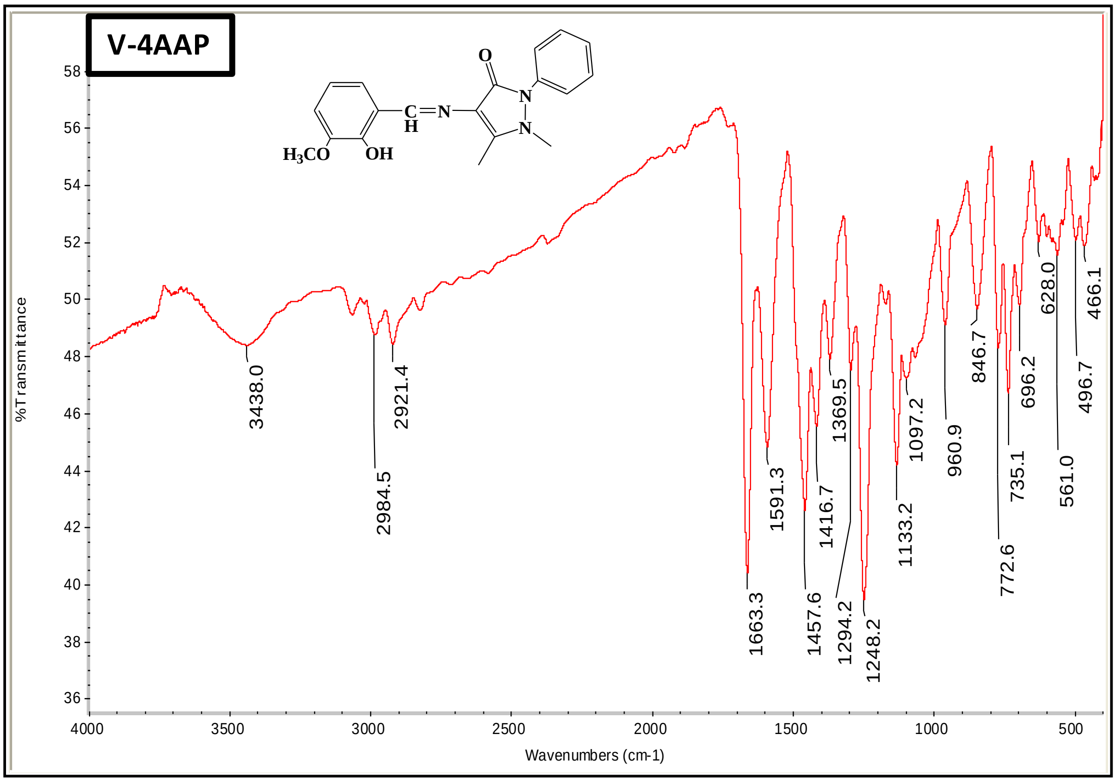


Figure 2.22: IR spectrum of metal complex [Cu(V-4AAP)$_2$]

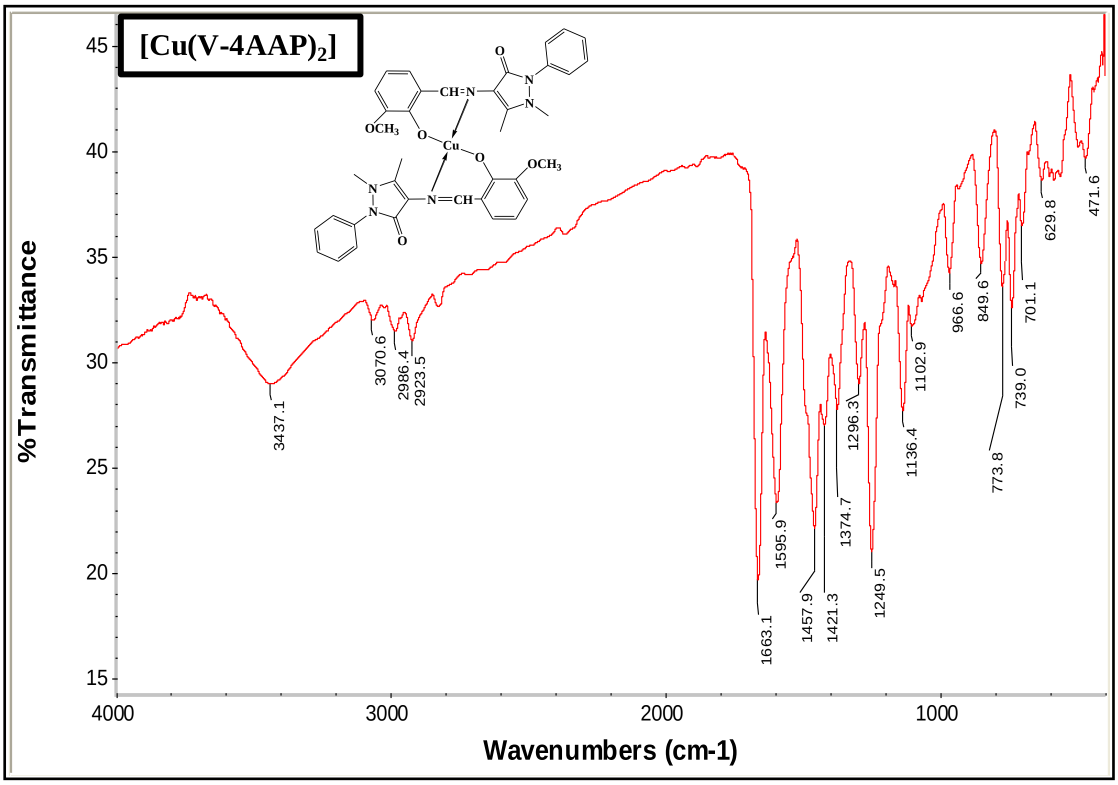


Figure 2.23: IR spectrum of metal complex [Ni(V-4AAP)$_2$]

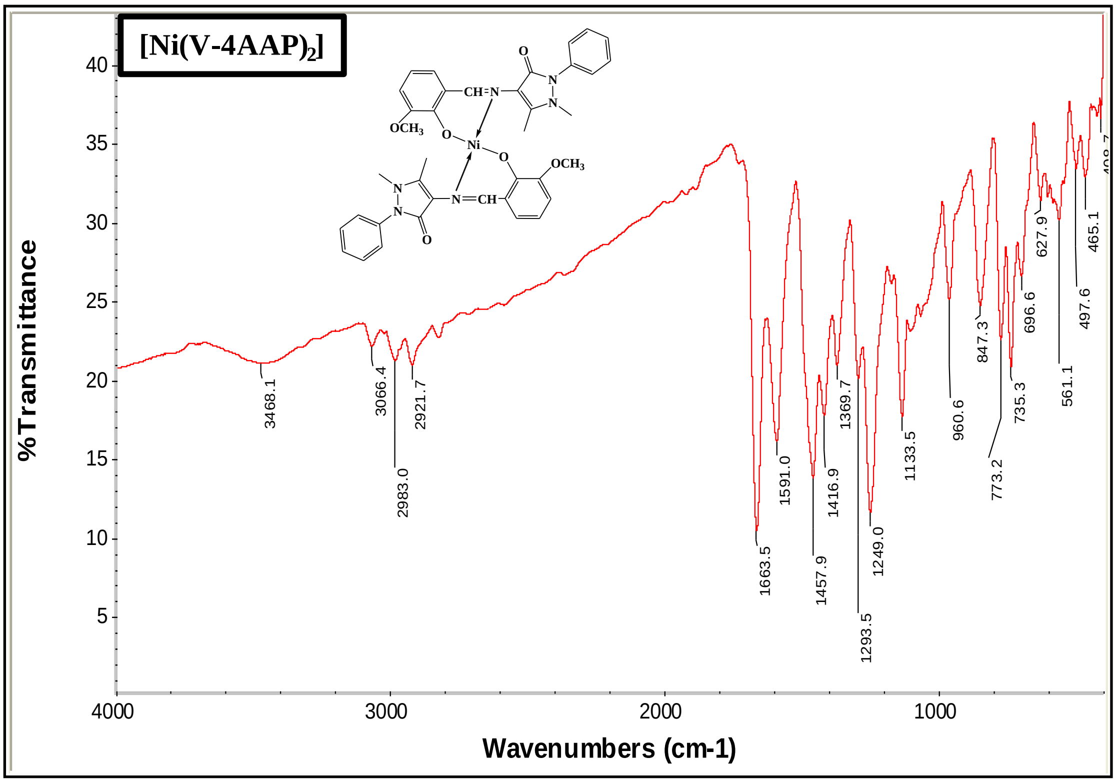
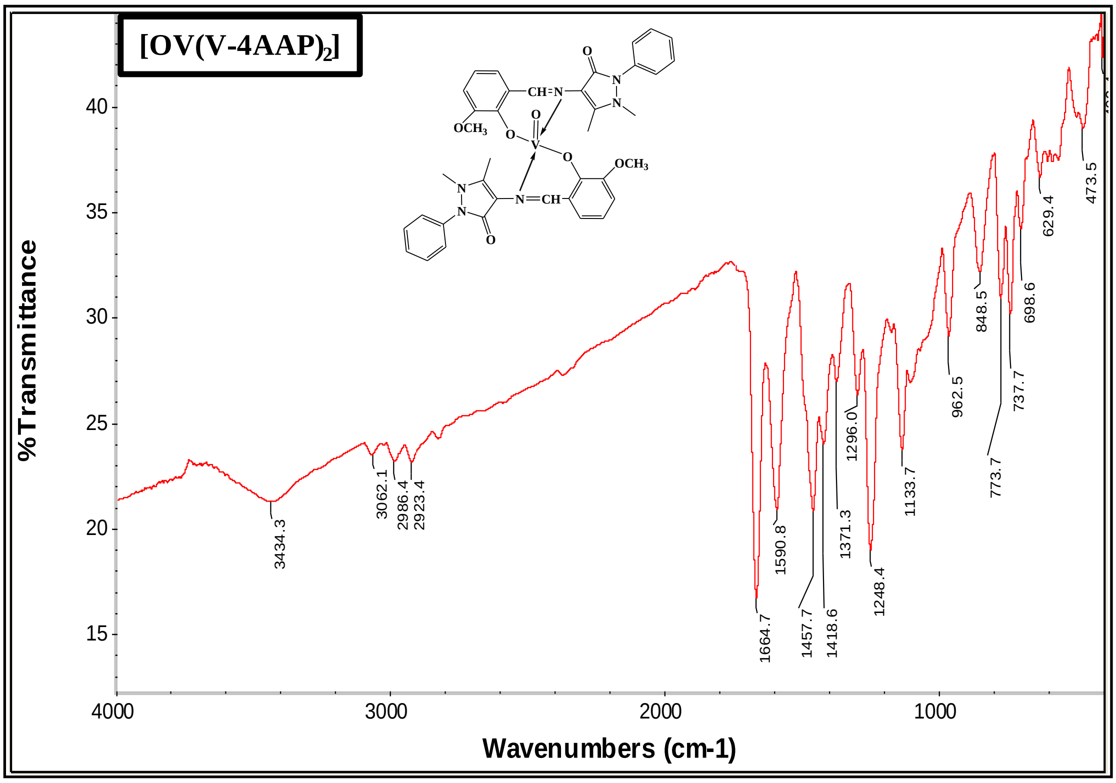


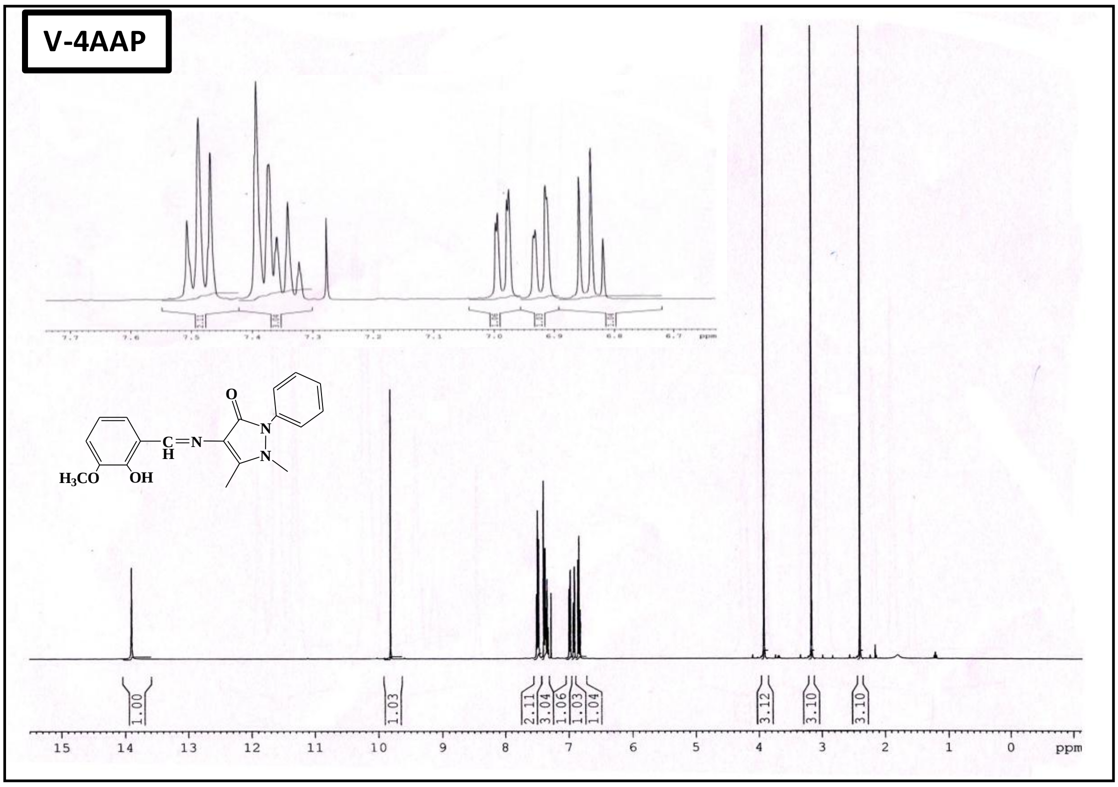
Figure 2.24: IR spectrum of metal complex [OV(V-4AAP)$_2$]



2.2.4.4 ^{1}HNMR spectral studies

The synthesized Schiff base ligands have been characterized by the ^{1}H NMRspectra in $CDCl_3$. The signals due to methyl protons appeared as singlet at2.36δppm (C-CH_3) as well as 3.17 δppm (N-CH_3), whereas signal dueto methoxy protons appeared as singlet at 3.92 δ ppm.Aromatic protons are observed between 6.81-7.48 δppm with few doublets/multiplets,which are due to aryl protons of benzene/pyridine rings. The signal of azomethine proton(-CH=N-) isobserved as singlet at 9.81δ ppm. Phenolic proton in the Schiff base ligand appeared as singlet at 13.90 δ ppm. During the complexation with metal ion phenolic proton signal disappeared. It can be bonding with the other atom (N/O). It may be noted that the integration of this signal perfectly matches with one proton and there is no other fragment(s) of this signal, which suggests that only one tautomeric form of the ligands exist in solution under the experimental conditions. We have not done any temperature dependent experiments [193-196]. (Figure 2.25)

Figure 2.25: ^{1}H NMR spectrum of Schiff base ligand V-4AAP



2.2.4.5 Mass spectral studies

The mass spectra of Schiff base ligands were in good agreement with the proposed structures. Schiff base ligands show molecular ion peak corresponding to its molecular mass. The other peaks appeared in the mass spectrum (abundance range 1-100%) are attributed to the fragmentation of ligand molecule obtained from the rupture of different bonds inside the molecule. Melting point of each metal complex is very high, as a result of this; the mass spectra were carried out by ESI. The electron-impact mass spectra of o-vanillin derivatives usually show an intense molecular ion peak.ESI–Mass spectra of complex shows molecular ion peak identical to their molecular weight. The mass spectra of all the complexes are in good agreement with the structures of the complexes [163,164]. (Figure 2.26-2.30)

Figure 2.26: Mass spectrum of Schiff base ligand V-4AAP

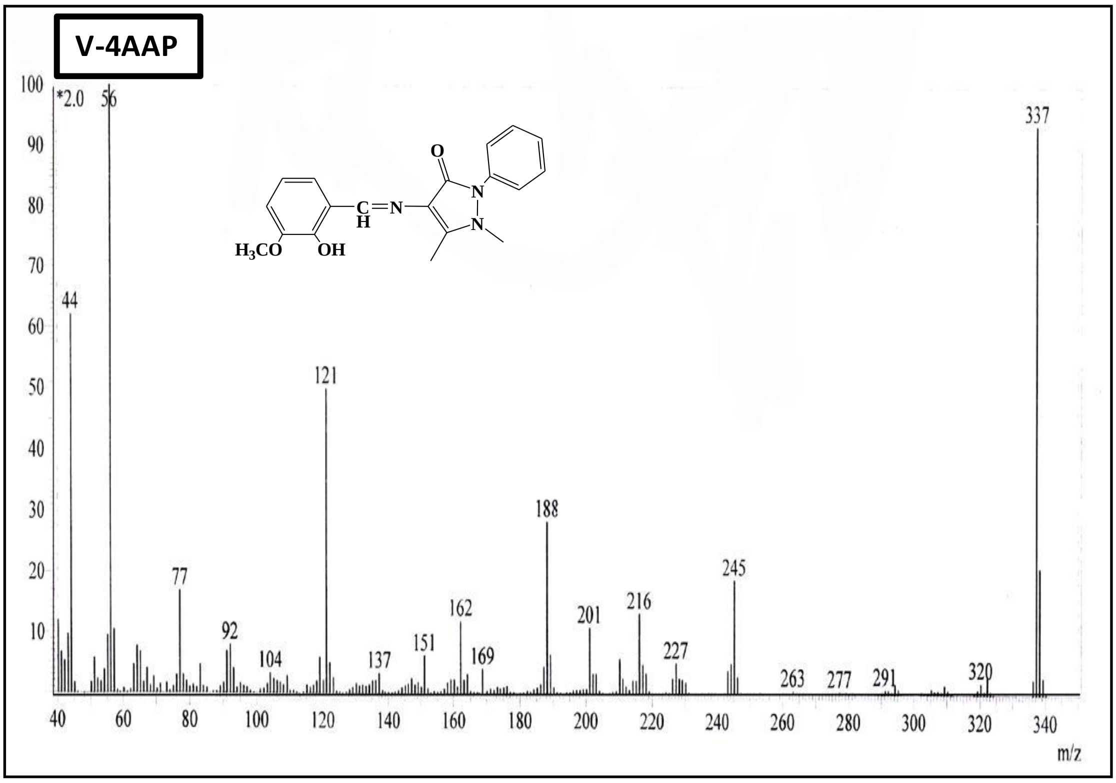


Figure 2.27: Mass fragmentation of Schiff base ligand V-4AAP

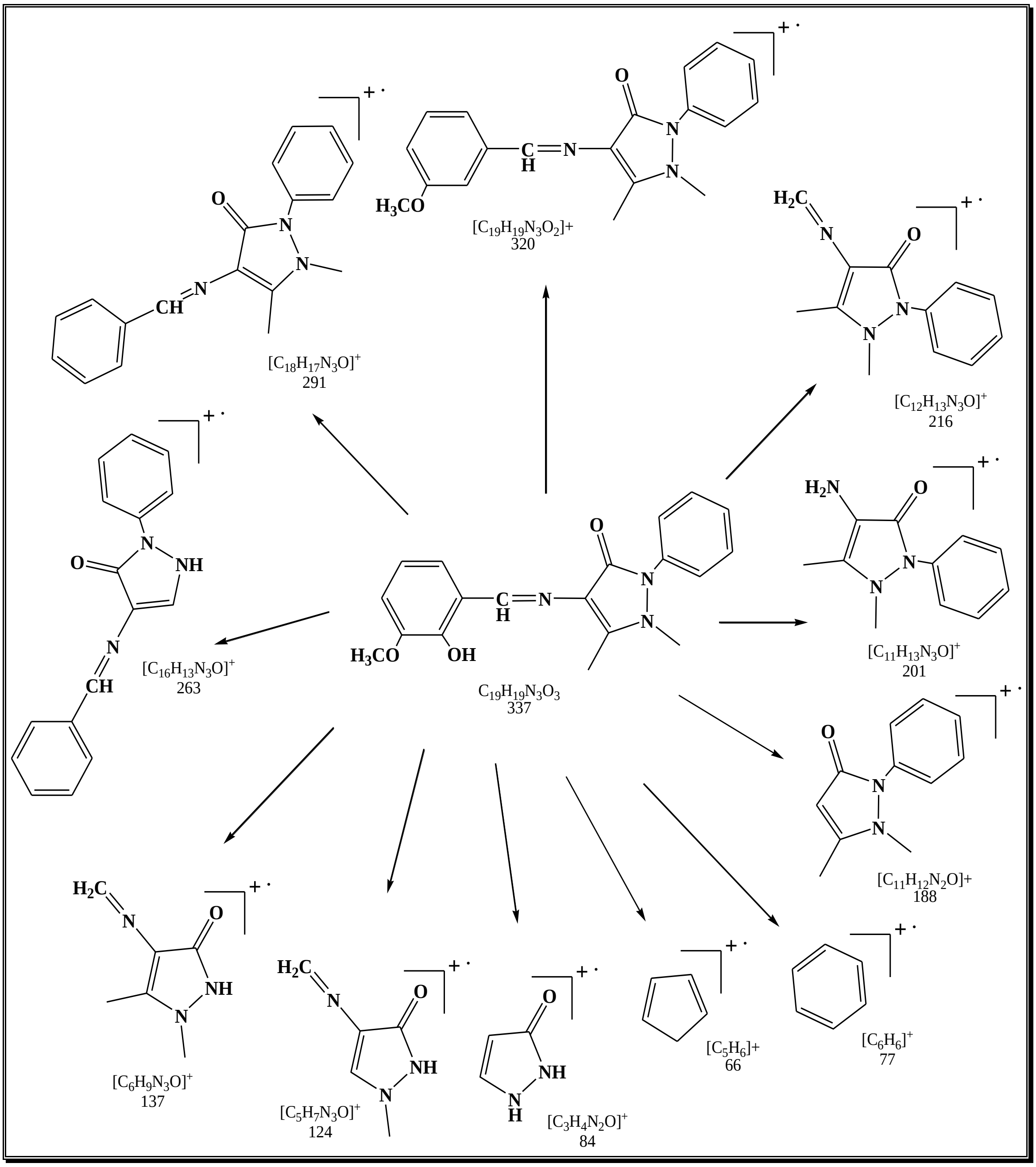


Figure 2.28: Mass spectrum of metal complex [Cu(V-4AAP)$_2$]

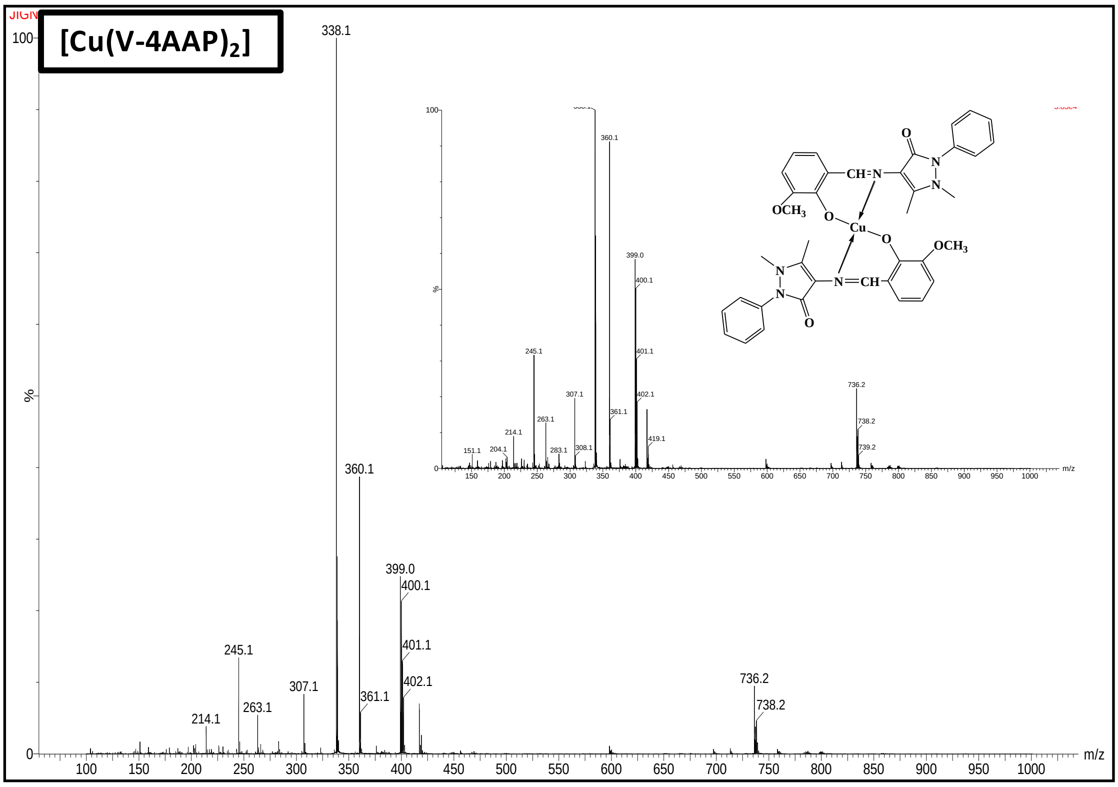


Figure 2.29: Mass spectrum of metal complex [OV(V-4AAP)$_2$]

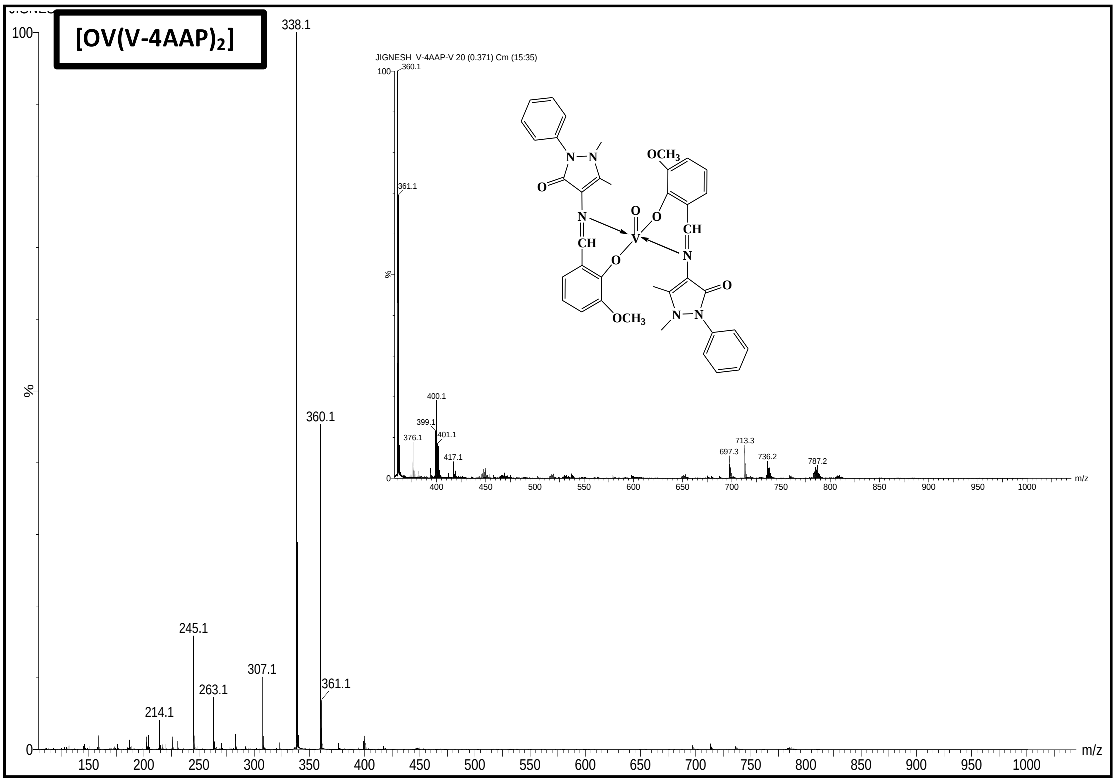
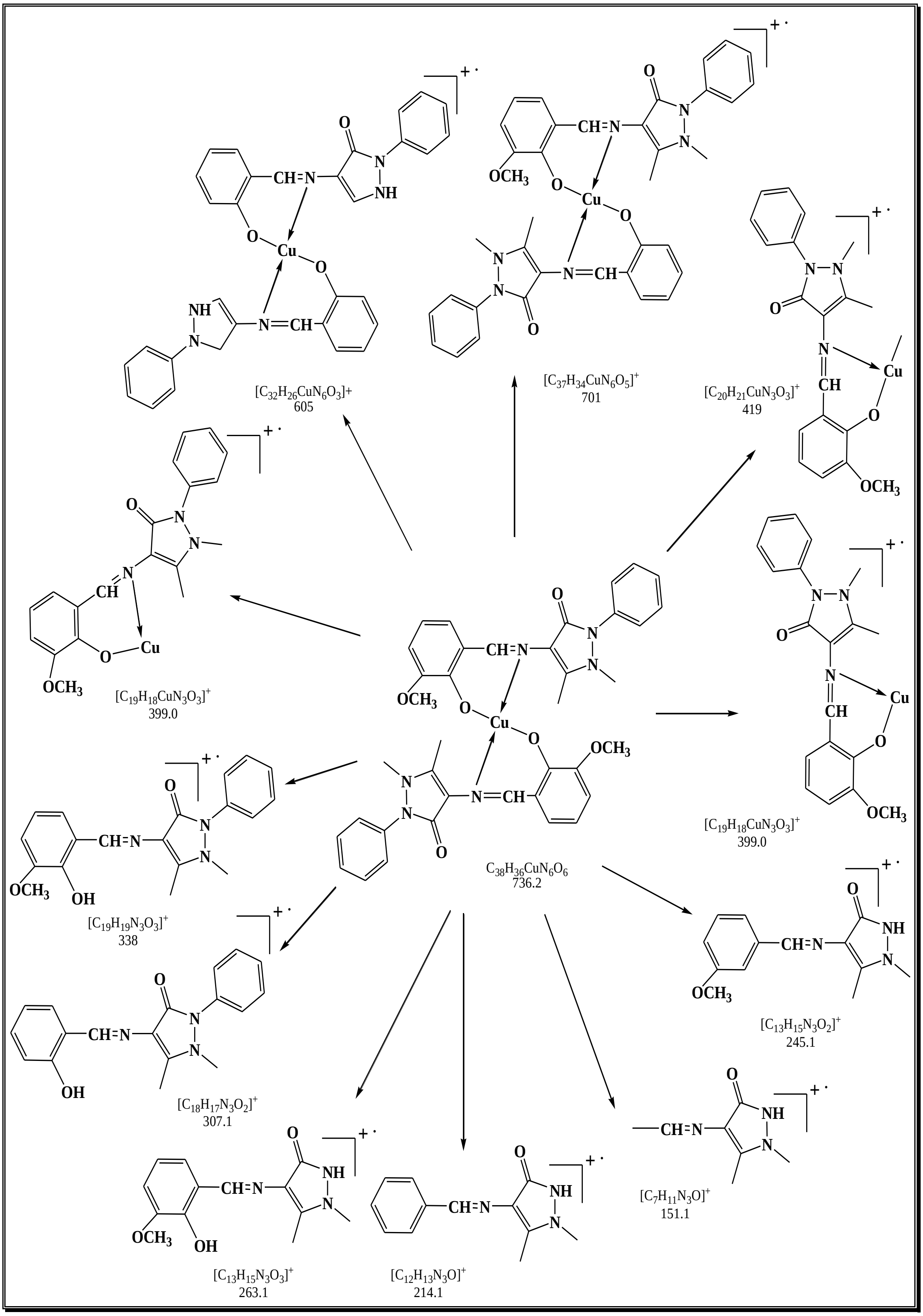


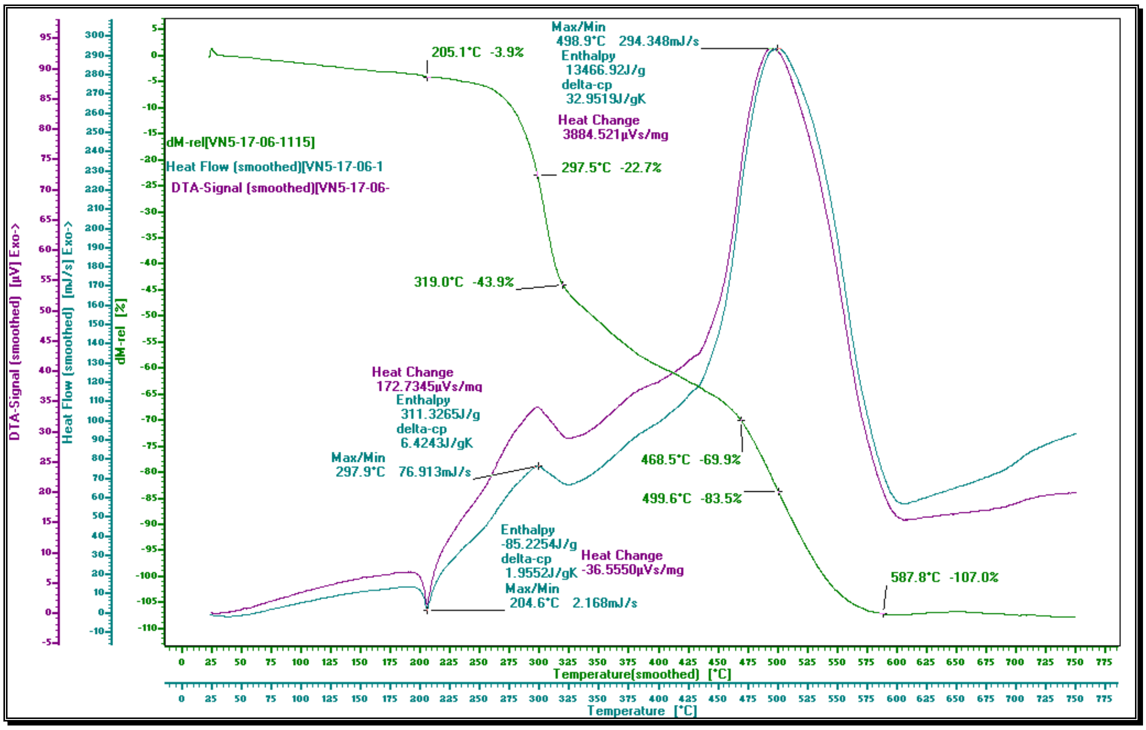
Figure 2.30: Mass fragmentation of metal complex [Cu(V-4AAP)$_2$]



2.2.4.6 Thermal studies

Thermal analysis of all the complexes were carried out by the TG-DTA-DSC techniques. The experimental results revealed that the degradation occurred in multiple stages, following a complex mechanism. The thermal behavior of all complexes is explained as follows. In the present investigation heating rates were suitably controlled at 5 ºC min^{-1} and mass loss followed up to 25–750 ºC.The TG curve follows the decrease in sample mass with increase in temperature. The decomposition of the complexes undergoes in three stages. The first stage involves degradation of two –OCH_3 molecules at 297^{o}C. The second stage between 319^{o}C-468^{o}C is due to decomposition of one ligand. The third stage corresponds to the decomposition of second ligand between 468^{o}C-575^{o}C and further formation of metal oxide at 587 ^{o}C. The maximum rate of mass loss is indicated by DTA peak at 499^{o}C [197-199]. (Figure 2.31)

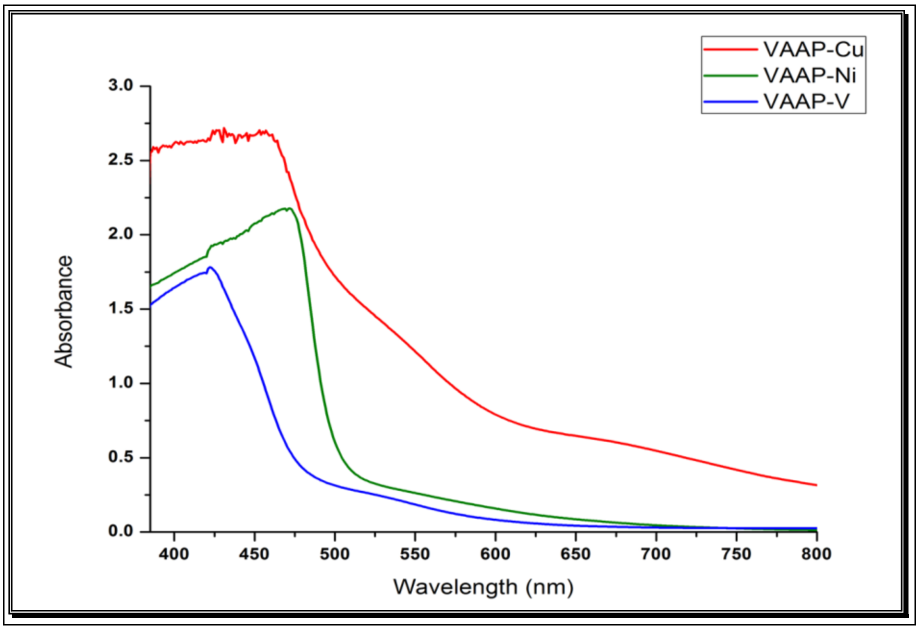
Figure 2.31: TG-DT analysis of metal complex



2.2.4.7 UV – Visible Spectral studies

Electronic spectra of all the complexes were recordedin dimethylformamide (DMF). For square planer Cu (II) complexes,the expected transition is $^{2}B_{1g}\rightarrow{}^{2}A_{1g}$ with absorption at460–475 nm. Due to Jahn-Teller (J-T) distortions, squareplanar Cu(II) complexes give a broad absorption between600 and 700 nm and the peak at 460–475 nm merges withthe broad band, and thus only one broad band is observed.The Ni(II) complexes showed one strong band at435–445 nm, which is assigned to the square planar $^{1}A_{1g}\rightarrow{}^{1}A_{2g}$ transition.The electronic spectra of oxovanadium(IV) complex shows three bands in the region 810–380nm. These bands may be assigned to three transitions $d_{xy}\rightarrow d_{xz}$& d_{yz}, $d_{xy}\rightarrow d_{x^2-y^2}$, $d_{xy}\rightarrow d_{z^2}$ following the Scheme. In addition, the complex shows a band at ~320 cm^{-1}, which may be due to the first spin forbidden charge transfer transition, confirm the tetragonal pyramidal geometry of complex [199-205].(Figure 2.32)

Figure 2.32: UV-Visible spectrum of metal complexes



2.2.4.8 Conductivity measurement

The observed molar conductance of the metal(II) complexesin 10^{-3}MDMF solution are in the range 11–25 Ω^{-1} cm^{2} mol^{-1}. The molar conductance values are consistentwith the non-electrolytic nature for all metal complexes [206].

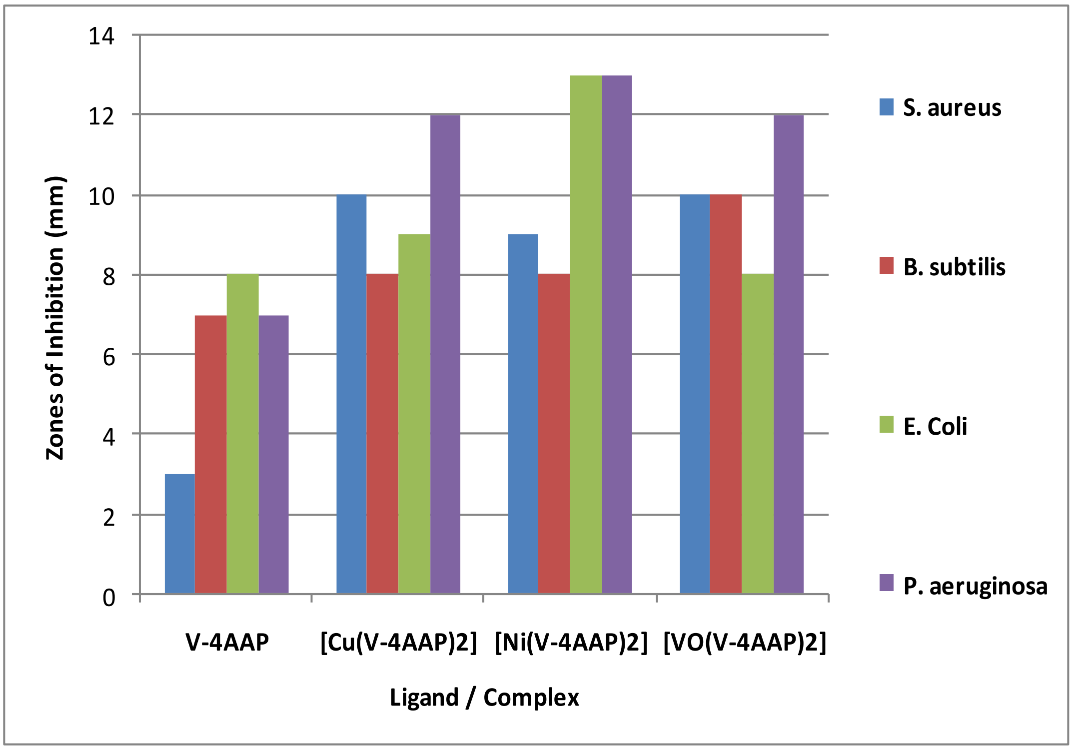
2.2.5 Antimicrobial activity

Antibacterial activity of the ligand and the metal complexes were evaluated against the *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* & *Pseudomonas aeruginosa*. Metal complexes show better inhibition as compared to their ligands. V-4AAP ligand does not show good antibacterial activity. While Cu(II) and Ni(II) and V(II) complexes of this ligand show remarkable antibacterial activity. It might be due to complexation of metal ions with Schiff base ligand. Such metal complexes might be inhibiting the enzyme activity of the bacterial system. Antibacterial activity of the ligands and complexes were quite comparable to the standard drugs. Antimicrobial activity data for the ligands and their metal complexes are shown below [172, 207]. (Figure 2.33 & Table 2.15)

Table 2.15: Antimicrobial activity of Schiff base ligand and metal complexes

Ligand/complex	**Microbial species (Zone of Inhibition in mm)**			
	S. aureus	***B. subtilis***	***E. Coli***	***P. aeruginosa***
V-4AAP	3	7	8	7
$[Cu(V\text{-}4AAP)_2]$	10	8	9	12
$[Ni(V\text{-}4AAP)_2]$	9	8	13	13
$[VO(V\text{-}4AAP)_2]$	10	10	8	12

Figure 2.33: Antimicrobial activity chart



2.2.6 Conclusion:

The Schiff base ligands of antipyrine derivatives and their metal complexes were synthesized. Crystal and molecular structures of the Schiff base ligand was resolved by single crystal X-ray diffraction and other spectroscopic techniques. Schiff base ligands were found to exist in the phenol-imine tautomeric forms. The copper and nickel complexes exist in the square planar geometry and vanadium complex exists in tetragonal pyramidal geometry. Antibacterial activities suggest that Schiff base ligands and their metal complexes have potential antibacterial activity. Further investigation is required to explore such ligands and metal complexes of antipyrine derivatives for the industrial and pharmaceutical use.

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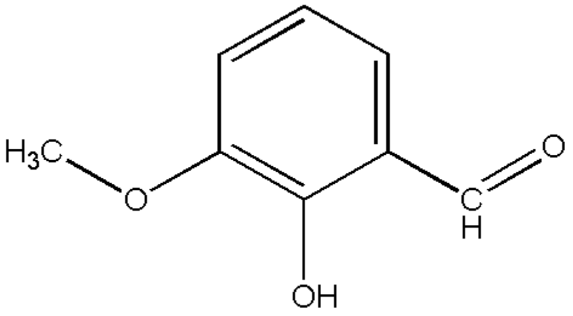
2.3: Synthesis, characterization and the biological evaluation of o-vanillin Schiff bases and their metal complexes

2.3.1 INTRODUCTION

2-hydroxy-3-methoxy-bezaldehyde (o-vanillin) is a naturally occurring compound found in *Andropogen Nardus.* It is a cure for stomach ache and used also in spicery [208]. In addition, the Schiff bases derived from this compound are found to exhibit antifungal, anti bacterial activities [209] and it possesses anti-mutagenic and commutageninc properties in E. coli [210]. Like any other aldehyde, o-vanillin also gives a variety of Schiff bases with a number of primary amines. As these compounds contain donor atoms like O, N and S, they bind with transition metal ions giving rise to a variety of metal complexes. A lot of researchhas gone into these types of Schiff bases and their metal complexes with particular emphasis on their biological activity.



2-hydroxy-3-methoxy-bezaldehyde (o-vanillin)

Schiff bases were prepared by Hann Raymon *et al*. [211] as early as 1929 from 5-chlorovanillin by heating its derivatives with amine in 95% ethanol.o-toluidine, p-toluidine, m-nitroanilin, p-chloroanillin, cymidine, p-anisidine, benzidine, m-aminobenzoic acid, o-dianisidine, bis-nitro-p-toluidine Schiff bases were prepared. No derivaties were obtained from ortho and para nitroanilin, 2,4 dichloroanilin and trinitroanilin.

Vavon and Montheard [212] reported Schiff bases from methyl amine, para and ortho hydroxyl benzaldehyde, vanillin and o-vanillin in the solvents benzyl alcohol, ethyl alcohol, dioxane, nitrobenzene, toluene with 3 % alcohol and chloroform.

Balog *et al*. [213] prepared Schiff bases using aldehydes like o-vanillin with aromatic amines in order to prepare chelates of Fe(III) with Schiff bases.

Cyba [214] has synthesized a Schiff base from the reaction of o-vanillin with p-sec-decyl-o-aminophenol. The Schiff base was synthesized to prepare transition metal chelates for their potential use as stabilizers for gasoline and polymers.

Csaszar [215] has prepared 1:1mol complexes of Schiff bases from o-vanillin with picric acid. Their UV, Visible and IR spectral behaviour was investigated. The results suggested that the molecular complexes were formed via charge transfer interactions. Csaszar[216] studied the UV-Visible and IR spectra of the Schiff bases formed from o-vanillin with aniline and substituted anilines. At 400-450 nm an intense band indicative of the benzenoid-quininoid tautomer equilibrium was observed. Correlations were found between the intensities of these bands, the tautomer equilibrium constants and the dielectric constants of the solvents.

Chongwu*et al.* [217] obtained Schiff bases by the reaction of o-vanillin with lot many compounds like ethylene diamine, benzidine, o-phenylene diamine, m-phenylenediamine using ethanol or benzene as solvents. When the similar reaction was carriedout between o-vanillin with 1, 8-diamino naphthalene under similar reaction conditions, it resulted in a cyclic compound.

Goudar *et al.*[218] have synthesized Tin(IV) complexes from Schiff bases derived from o-vanillin & 2-aminopyridine, 2-amino-3-methylpyridine and 2-aminothiazole. The complexes are non-electrolytes and the ligand-metal linkage was formed between the phenolic -OH and the azomethane N.

Vishwanathmurthi *et al.*[219] have synthesized Schiff bases by condensation of o-vanillin with aniline, o-toluidine or p-toluidine in benzene in the presence of N-Et_3. These Schiff bases were used to synthesize ruthenium complexes.The Schiff bases and the complexes were tested in vitro against the pathogenic fungi *Aspergillus niger* and *Fusarium Sp*.

Wang Guangbin *et al.*[220] synthesized a new dipeptide by the condensation of DL-alanine with o-vanillin.The copper, zinc, nickel and cobalt complexes were prepared and characterized.

Ming*et al.*[221] studied conformation of four Schiff bases using molecular dynamics and quantum chemical analytical approach and synthesized four new Schiff bases from o-vanillin and dl- amino acids.

Kam*et al*. [222] synthesized 1:1 and 1:2 Schiff bases derived from 2, 3-diaminopyridine (DAPY) and o-vanillin and eight transition metal complexes of theseSchiff bases and all were found to display antibacterial activity.

Jayabalakrishnan *et al*. [223] prepared Schiff bases by condensing o-vanillin with N(4)- phenylsemicarbazide and complexed with ruthenium. The complex was tested for *in-vitro* growth inhibitory activity against *Pseudomonas sp.,Bacillus sp.and Eschereria coli.*

Fe(III), Ni(II) and Cu(II) complexes of o-vanilin and L-histidine were synthesized and used for x-ray diffraction studies [224].

Schiff bases with o-vanillin and o-aminophenol and o-aminothiophenol were synthesized and ruthenium complexes were prepared by Jayabalakrishnan, *et al*., [225].

Singh*et al*. [226] have prepared Schiff bases by condensation from 4- amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine and o- vanillin and complexed with cobalt, nickel, copper and zinc in an attempt to prepare some new complexes.

Yanhong *et al*. [227] have synthesized schiff bases of o-vanillin with amino acids and their complexes. These complexes were used to study the inhibitory activity on the formation of peroxides.

Singh *et al*.[228] have synthesized new Schiff bases derived by nucleophilic addition of o-vanillin to 4-amino-5-mercapto-3-methyl-s-triazole and 4-amino-3-ethyl-5-mercapto-s-triazole. Bivalent metal complexes of Co,Ni,Cu,Zn were prepared using these Schiff bases.

Zhu*et al*.[229] have prepared Schiff bases of 2-amino-5-mercapto-1,3,4-thiadiazole with o-vanillin and their molybdenum complexes. Their effect on promoting cell survival rate of mung bean sprouts was investigated.

Babu *et al*. [230] reported the synthesis and characterization of a new Schiff base o-vanillin-Tris(hydroxymethyl) methylamine and made complexes with Lead ions.

Xu, Tao *et al*. [231] synthesized a novel Schiff base compound by thereaction of o-vanillin and hydroxylamine hydrochloride in absolute methanol.

In this section, the Schiff bases derived from o-vanillin and substituted anilines and their metal complexes are characterized by spectroscopic methods, conductivity measurement and elemental analysis and their biological activity is briefly investigated.

2.3.2 EXPERIMENTAL SECTION

2.3.2.1 Materials

The compounds o-vanillin and substituted anilines were obtained from Merck & Spectrochem Pvt. Ltd. Metal salts like$Cu(NO_3)_2{\cdot}3H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$ and $VO(SO_4)_2$ were obtained form Merck. Absolute alcohol was obtained from Baroda Chem. Industry Ltd. Methanol was obtained from spectrochem Mumbai, India. All the chemicals used were of AR grade. Solvents used in this study were purified and dried following the standard procedures wherever required [151].

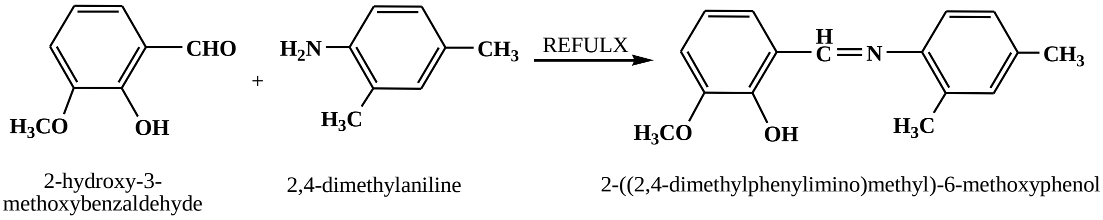
2.3.2.2 Antimicrobial screening

Antibacterial activity of all the ligands and complexes were evaluatedagainst *Staphylococcus aureus, Bacillus subtilis, Escherichia coli & Pseudomonasaeruginosa.* All strains were type strains. A young culture of each bacterial culturewas prepared. Each bacterial culture added to the sterilized medium beforesolidification. The media with bacteria was poured into sterilized Petri dishes underaseptic condition. Different weights of Schiff base ligands and their metal complexes;(1mg, 5mg and 10mg) were placed on the surface of the culture and incubated at 37^{o}Cfor 24 hours. After incubation, zones of inhibitions (mm) were recorded.

2.3.3 Preparation of Schiff base ligands and metal complexes

2.3.3.1 Synthesis of 2-((2,4-dimethylphenylimino)methyl)-6-methoxyphenol (V-2,4-ME)

The Schiff base ligand was synthesized by adding o-vanillin (3.8 g 25 mmol) dissolved in hot absolute EtOH (20 cm^3) to 2,4-dimethylaniline (3.0 g, 25 mmol) in absolute EtOH (20 cm^3). The reaction mixture was refluxed for 4 hours. The product obtained was filtered off and washed several times with a small amount of EtOH and ether. The product was kept in desiccators until use. Recrystallization was carried out in EtOH. The progress of the reaction was monitored by TLC. (Scheme – 2.14)



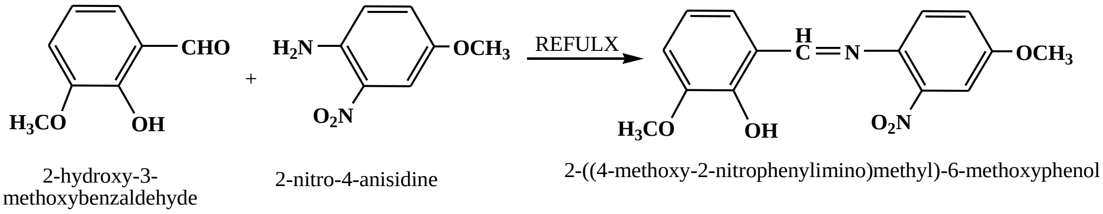
Scheme -2.14: Synthesis of V-24ME

V-2,4-ME is orange crystalline compound. Yield: 86%, m.p.: 130°C.Anal. Calc. for $C_{16}H_{17}NO_2$ M.W.: 255, C (75.27%), H (6.71%), N (5.49%), found: C (75.27%), H (6.71%), N (5.49%).

IR (KBr, cm^{-1}): 3449 (O-H), 1614(C=N), 3003(C-H), 1549-1330 (Ar -C=C- ring skeletal), 1208 (C-O of -OH), 1101 (C-C).^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 2.32-2.34 (6H singlet, $2CH_3$), δ 3.87 (3H singlet, $-OCH_3$), δ 6.85-7.21 (6H multiplet, Ar-H), δ 8.77 (1H singlet, -CH), δ 13.96 (1H singlet, -OH). MASS: m/z = 255 [$C_{16}H_{17}NO_2$], 237 $[C_{16}H_{17}NO]^+$, 212 $[C_{14}H_{13}NO]^+$, 208 $[C_{15}H_{15}N]^+$, 194 $[C_{14}H_{13}N]^+$, 150 $[C_8H_9NO_2]^+$, 132 $[C_9H_{11}N]^+$, 121 $[C_8H_{11}N]^+$, 103 $[C_7H_7N]^+$, 91$[C_7H_8]^+$, 77 $[C_6H_6]^+$, 65 $[C_5H_5]^+$.

2.3.3.2 Synthesis of 2-((4-methoxy-2-nitrophenylimino)methyl)-6-methoxyphenol (V-2N4A)

The Schiff base ligand was synthesized byadding o-vanillin (3.8 g 25 mmol) dissolved inhot absolute EtOH (20 cm^3) to 2-nitro-4-anisidine (4.2 g, 25 mmol)in absolute EtOH (20 cm^3). The reaction mixture was refluxed for 4 hours. The product obtained was filtered offand washed several times with a small amount of EtOH and ether. The product was kept in desiccators until use.Recrystallization was carried out in EtOH. The progress ofthe reaction was monitored by TLC. (Scheme – 2.15)



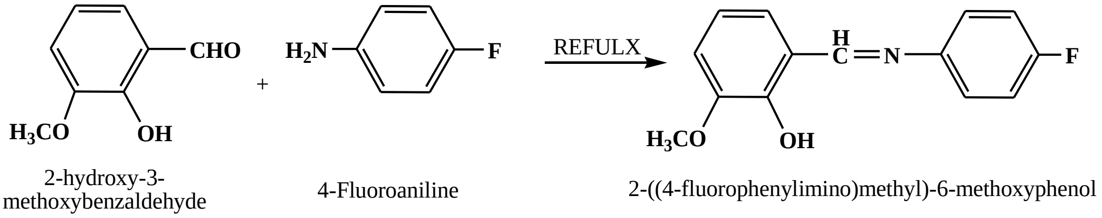
Scheme -2.15: Synthesis of V-2N4A

V-2N4A is yellow crystalline compound. Yield: 79%, m.p.: 121°C.Anal. Calc. for $C_{15}H_{14}N_2O_5$ M.W.: 302, C (59.60%), H (4.67%), N (9.27%), found: C (59.67%), H (4.71%), N (9.31%).

IR (KBr, cm^{-1}): 3489 (O-H), 1639(C=N), 3167(C-H), 1510-1338 (Ar -C=C- ring skeletal), 1210 (C-O of -OH), 1091 (C-C).^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 3.35 &δ 3.75 (6H singlet, -2OCH$_3$), δ 6.96-7.38 (6H multiplet, Ar-H), δ 8.91 (1H singlet, -CH), δ 10.35 (1H singlet, -OH). MASS: m/z = 303 [$C_{15}H_{14}N_2O_5$], 256 [$C_{15}H_{14}NO_3$]$^+$, 242 [$C_{15}H_{15}NO_2$]$^+$, 182 [$C_{13}H_{11}N$]$^+$, 119 [C_8H_9N]$^+$, 106 [C_7H_7N]$^+$, 92 [C_7H_8]$^+$, 78 [C_6H_6]$^+$.

2.3.3.3 Synthesis of 2-((4-fluorophenylimino)methyl)-6-methoxyphenol (V-4F)

The Schiff base ligand was synthesized by adding o-vanillin (3.8 g 25 mmol) dissolved in hot absolute EtOH (20 cm^3) to 4-fluoroaniline (3.0 g, 25 mmol) in absolute EtOH (20 cm^3). The reaction mixture was refluxed for 4 hours. The product obtained was filtered off and washed several times with a small amount of EtOH and ether. The product was kept in desiccators until use. Recrystallization was carried out in EtOH. The progress of the reaction was monitored by TLC. (Scheme – 2.16)



Scheme -2.16: Synthesis of V-4F

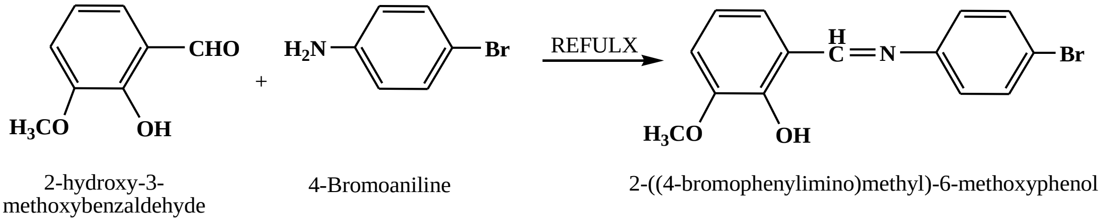
V-4F is brown crystalline compound. Yield: 88%, m.p.: 118°C.Anal. Calc. for $C_{14}H_{12}FNO_2$ M.W.: 245, C (68.56%), H (4.93%), N (5.71%), found: C (68.62%), H (4.89%), N (5.75%).

IR (KBr, cm^{-1}): 3496 (O-H), 1625(C=N), 3171(C-H), 1520-1340 (Ar -C=C- ring skeletal), 1208 (C-O of -OH), 1105 (C-C). ^{1}H NMR ($CDCl_3$, 400 MHz, TMS):

δ3.91(3H singlet, -OCH_3), δ 6.89-7.36 (7H multiplet, Ar-H), δ 8.70 (1H singlet, -CH=N), δ 10.04 (1H singlet, -OH). MASS: m/z = 245 [$C_{14}H_{12}FNO_2$], 227 [$C_{14}H_{13}NO_2$]$^+$, 214 [$C_{13}H_{10}FNO$]$^+$, 199 [$C_{13}H_{10}FN$]$^+$, 182 [$C_{13}H_{11}N$]$^+$, 150 [$C_9H_{10}O_2$]$^+$, 135 [$C_8H_{10}O_2$]$^+$, 122 [C_7H_6FN]$^+$, 111 [C_6H_6FN]$^+$, 95[C_6H_5F]$^+$, 75 [C_6H_4]$^+$, 63 [C_5H_3]$^+$.

2.3.3.4 Synthesis of 2-((4-bromophenylimino)methyl)-6-methoxyphenol (V-4BR)

The Schiff base ligand was synthesized by adding o-vanillin (3.8 g 25 mmol) dissolved in hot absolute EtOH (20 cm^3) to 4-bromoaniline (4.3 g, 25 mmol) in absolute EtOH (20 cm^3). The reaction mixture was refluxed for 4 hours. The product obtained was filtered off and washed several times with a small amount of EtOH and ether. The product was kept in desiccators until use. Recrystallization was carried out in EtOH. The progress of the reaction was monitored by TLC. (Scheme – 2.17)



Scheme -2.17: Synthesis of V-4BR

V-4BR is brown crystalline compound. Yield: 91%, m.p.: 132°C.Anal. Calc. for $C_{14}H_{12}BrNO_2$ M.W.: 306, C (54.92%), H (3.95%), N (4.58%), found: C (54.98%), H (4.00%), N (4.62%).

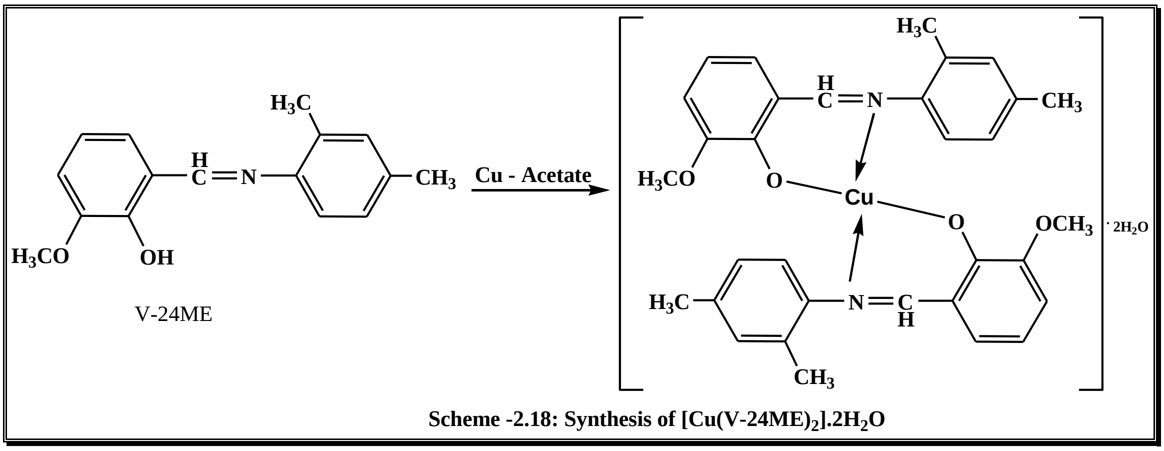
IR (KBr, cm^{-1}): 3510 (O-H), 1612(C=N), 3051(C-H), 1568-1365 (Ar -C=C- ring skeletal), 1255 (C-O of -OH), 1199 (C-C).^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ 3.89(3H singlet, -OCH_3), δ 6.92-7.40 (7H multiplet, Ar-H), δ 8.82 (1H singlet, -CH=N), δ 10.20 (1H singlet, -OH). MASS: m/z = 307 [$C_{14}H_{12}BrNO_2$], 289 [$C_{14}H_{12}BrNO$]$^+$, 276 [$C_{13}H_{10}BrNO$]$^+$, 260 [$C_{13}H_{10}BrN$]$^+$, 225 [$C_{14}H_{13}NO_2$]$^+$, 210 [$C_{14}H_{13}NO$]$^+$, 197 [C_8H_8BrN]$^+$, 182 [C_7H_6BrN]$^+$, 171 [C_6H_6BrN]$^+$, 154[C_6H_5Br]$^+$, 150 [$C_9H_{10}O_2$]$^+$, 135 [$C_8H_{10}O_2$]$^+$, 121 [$C_8H_{10}O$] $^+$, 106 [C_7H_8O]$^+$, 92 [C_6H_7N]$^+$.

Preparation of Schiff base metal complexes

2.3.3.5 Synthesis of [Cu(V-2,4-ME)$_2$].2H_2O

Cupric nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((2,4-

dimethylphenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.18)



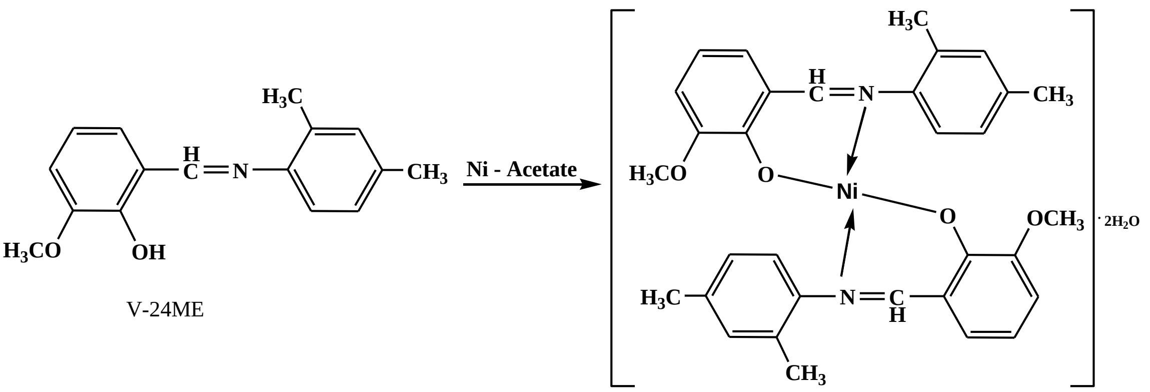
Scheme -2.18: Synthesis of [Cu(V-24ME)$_2$].2H$_2$O

[Cu(V-2,4-ME)$_2$].2H$_2$O is green crystalline compound. Yield: 78%, m.p.: >300^{o}C, Solubility: DMF/DMSO. Anal. Calc. for $C_{32}H_{32}CuN_2O_4$ M.W.: 572, C (67.17%), H (5.64%), N (4.90%), Cu(11.11%) found: C (67.25%), H (5.60%), N (4.96%), Cu (11.08%).

IR (KBr, cm^{-1}): 3440 (O-H of H_2O), 1590 (C=N azomethane), 445 (Cu-N), 468 (Cu-O); ESI-MS: m/z = 572 [$C_{32}H_{32}CuN_2O_4$], 530 [$C_{30}H_{28}CuN_2O_3$]$^+$, 507 [$C_{30}H_{28}CuN_2O_2$]$^+$, 498 [$C_{28}H_{24}CuN_2O_3$]$^+$, 468 [$C_{27}H_{22}CuN_2O_2$]$^+$, 386 [$C_{18}H_{20}CuN_2O_4$]$^+$, 379 [$C_{20}H_{16}CuN_2O_2$]$^+$, 317 [$C_{15}H_{14}CuN_2O_2$]$^+$, 317[$C_{16}H_{16}CuNO_2$]$^+$, 271 [$C_{14}H_{12}CuNO$]$^+$, 256 [$C_{16}H_{17}NO_2$]$^+$, 241 [$C_{15}H_{15}NO_2$]$^+$, 193 [$C_{14}H_{13}N$]$^+$, 106 [C_7H_9N]$^+$.

2.3.3.6 Synthesis of [Ni(V-2,4-ME)$_2$].2H$_2$O

Nickel nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((2,4-dimethylphenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.19)



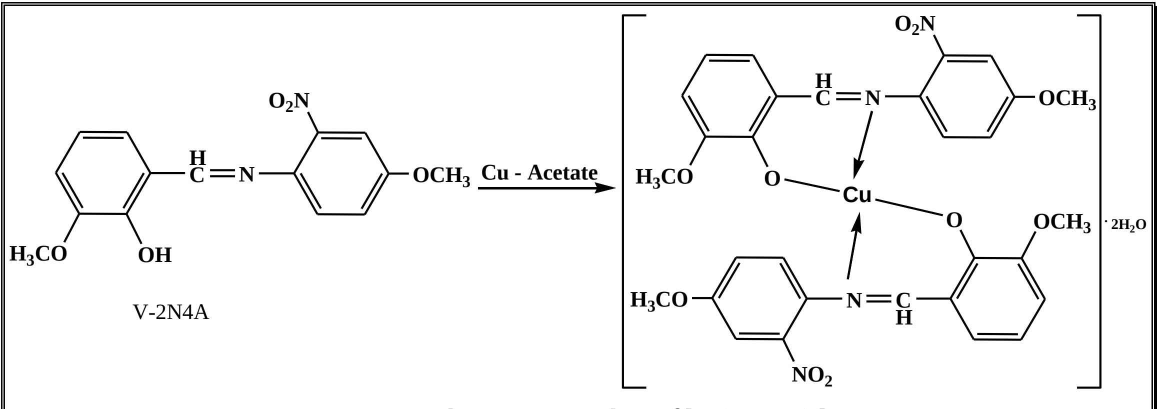
Scheme -2.19: Synthesis of $[Ni(V\text{-}24ME)_2].2H_2O$

$[Ni(V\text{-}2,4\text{-}ME)_2].2H_2O$ is Dark green crystalline compound. Yield: 74%, m.p.:>300°C, Solubility: DMF/DMSO. Anal. Calc. for $C_{32}H_{32}N_2NiO_4$M.W.: 567, C (67.75%), H (5.69%), N (4.94%), Ni (10.35%) found: C (67.82%), H (5.65%), N (5.02%), Ni (10.41%).

IR (KBr, cm^{-1}): 3460 (O-H of H_2O), 1575 (C=N azomethane), 460 (Ni-N), 472 (Ni-O);ESI-MS: m/z = 568 $[C_{32}H_{32}N_2NiO_4]$, 537 $[C_{31}H_{30}N_2NiO_3]^+$, 510 $[C_{29}H_{26}N_2NiO_3]^+$, 446 $[C_{24}H_{24}N_2NiO_3]^+$, 433 $[C_{23}H_{22}N_2NiO_3]^+$, 419 $[C_{23}H_{23}NNiO_3]^+$, 330 $[C_{16}H_{17}NNiO_3]$, 313 $[C_{16}H_{16}NNiO_2]^+$, 255 $[C_{16}H_{17}NO_2]^+$, 237 $[C_{16}H_{17}NO]^+$, 212 $[C_{14}H_{13}NO]^+$, 208 $[C_{15}H_{15}N]^+$, 194 $[C_{14}H_{13}N]^+$, 150 $[C_8H_9NO_2]^+$, 132 $[C_9H_{11}N]^+$, 121 $[C_8H_{11}N]^+$, 103 $[C_7H_7N]^+$, 91$[C_7H_8]^+$, 77 $[C_6H_6]^+$, 65 $[C_5H_5]^+$.

2.3.3.7 Synthesis of $[Cu(V\text{-}2N4A)_2].2H_2O$

Cupric nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((4-methoxy-2-nitrophenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.20)



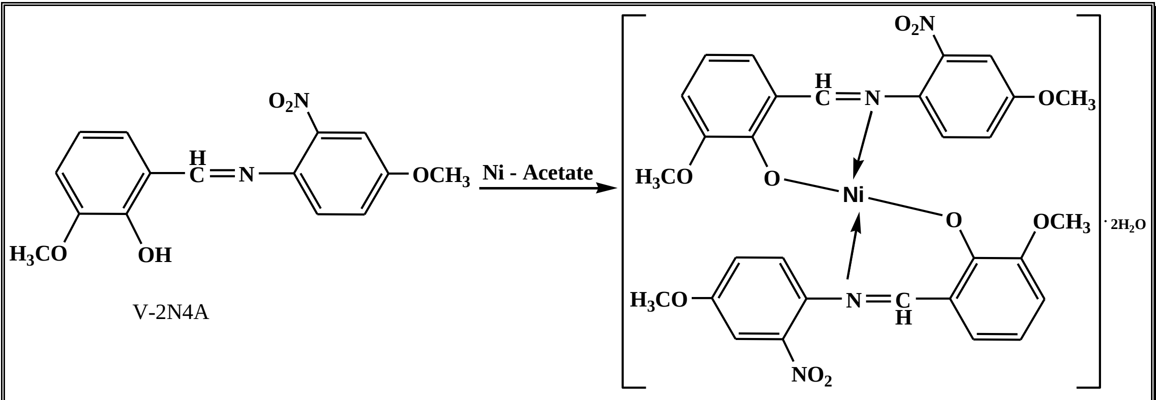
Scheme -2.20: Synthesis of [Cu(V-2N4A)$_2$].2H$_2$O

[Cu(V-2N4A)$_2$].2H$_2$O is greenish brown compound. Yield: 72%, m.p.: >300^{o}C, Solubility: DMF/DMSO. Anal. Calc. for $C_{30}H_{26}CuN_4O_{10}$ M.W.: 666, C (54.09%), H (3.93%), N (8.41%), Cu(9.54%) found: C (54.13%), H (3.89%), N (8.45%), Cu(9.62%).

IR (KBr, cm^{-1}): 3470 (O-H of H$_2$O), 1590 (C=N azomethane), 465 (Cu-N), 468 (Cu-O); ESI-MS: 666 [$C_{30}H_{26}CuN_4O_{10}$], 620 [$C_{30}H_{27}CuN_3O_8$]$^+$, 560 [$C_{28}H_{23}CuN_3O_6$]$^+$, 499 [$C_{23}H_{21}CuN_3O_6$]$_{+}$, 485 [$C_{22}H_{19}CuN_3O_6$]$^+$, 471 [$C_{22}H_{20}CuN_2O_6$]$^+$, 457 [$C_{21}H_{18}CuN_2O_6$]$^+$, 382 [$C_{15}H_{14}CuN_2O_6$]$^+$, 366 [$C_{15}H_{13}CuN_2O_5$]$^+$, 303 [$C_{15}H_{14}N_2O_5$]$^+$, 256 [$C_{15}H_{14}NO_3$]$^+$, 242 [$C_{15}H_{15}NO_2$]$^+$, 182 [$C_{13}H_{11}N$]$^+$, 119 [C_8H_9N]$^+$, 106 [C_7H_7N]$^+$, 92 [C_7H_8]$^+$, 78 [C_6H_6]$^+$.

2.3.3.8 Synthesis of [Ni(V-2N4A)$_2$].2H$_2$O

Nickel nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((4-methoxy-2-nitrophenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.21)



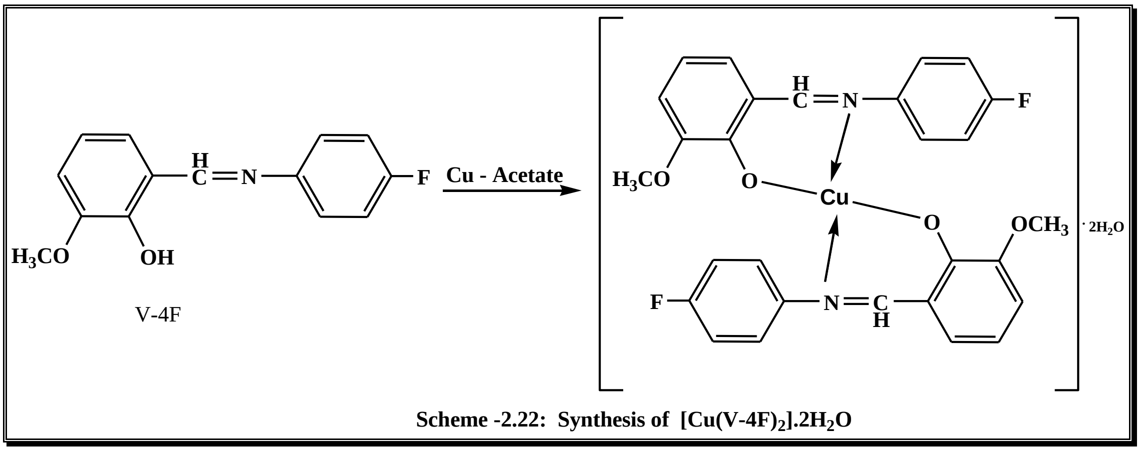
Scheme -2.21: Synthesis of $[Ni(V\text{-}2N4A)_2].2H_2O$

$[Ni(V\text{-}2N4A)_2].2H_2O$ is dark green crystalline compound. Yield: 62%, m.p.:>300^oC, Solubility: DMF/DMSO. Anal. Calc. for $C_{30}H_{26}N_4NiO_{10}$M.W.: 661, C (54.49%), H (3.96%), N (8.47%), Ni (8.88%) found: C (54.53%), H (4.00%), N (8.51%), Ni (8.95%).

IR (KBr, cm^{-1}): 3500 (O-H of H_2O), 1607 (C=N azomethane), 455 (Ni-N), 463 (Ni-O); ESI-MS: 661 $[C_{30}H_{26}N_4NiO_{10}]$, 615 $[C_{30}H_{27}N_3NiO_8]^+$, 556 $[C_{28}H_{23}N_3NiO_6]^+$, 480 $[C_{22}H_{19}N_3NiO_6]^+$, 466 $[C_{22}H_{20}N_2NiO_6]^+$, 453 $[C_{21}H_{18}N_2NiO_6]^+$, 390 $[C_{16}H_{16}N_2NiO_6]^+$, 376 $[C_{15}H_{14}N_2NiO_6]^+$, 360 $[C_{15}H_{13}N_2NiO_5]^+$, 302 $[C_{15}H_{14}N_2O_5]^+$, 256 $[C_{15}H_{14}NO_3]^+$, 242 $[C_{15}H_{15}NO_2]^+$, 182 $[C_{13}H_{11}N]^+$, 119 $[C_8H_9N]^+$, 106 $[C_7H_7N]^+$, 92 $[C_7H_8]^+$, 78 $[C_6H_6]^+$.

2.3.3.9 Synthesis of $[Cu(V\text{-}4F)_2].2H_2O$

Cupric nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((4-fluorophenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.22)



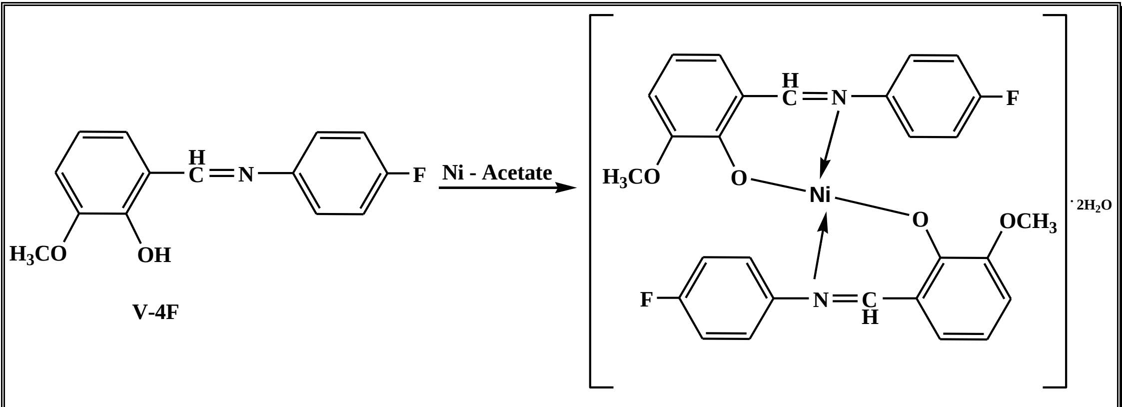
Scheme -2.22: Synthesis of $[Cu(V\text{-}4F)_2].2H_2O$

$[Cu(V\text{-}4F)_2].2H_2O$ is greenish brown compound. Yield: 80%, m.p.: >300°C, Solubility: DMF/DMSO. Anal. Calc. for $C_{28}H_{22}CuF_2N_2O_4$ M.W.: 552, C (60.92%), H (4.02%), N (5.07%), Cu(11.51%) found: C (60.98%), H (4.07%), N (5.00%), Cu(11.60%).

IR (KBr, cm^{-1}): 3485 (O-H of H_2O), 1600 (C=N), 452 (Cu-N), 470 (Cu-O).

2.3.3.10 Synthesis of $[Ni(V\text{-}4F)_2].2H_2O$

Nickel nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((4-fluorophenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.23)



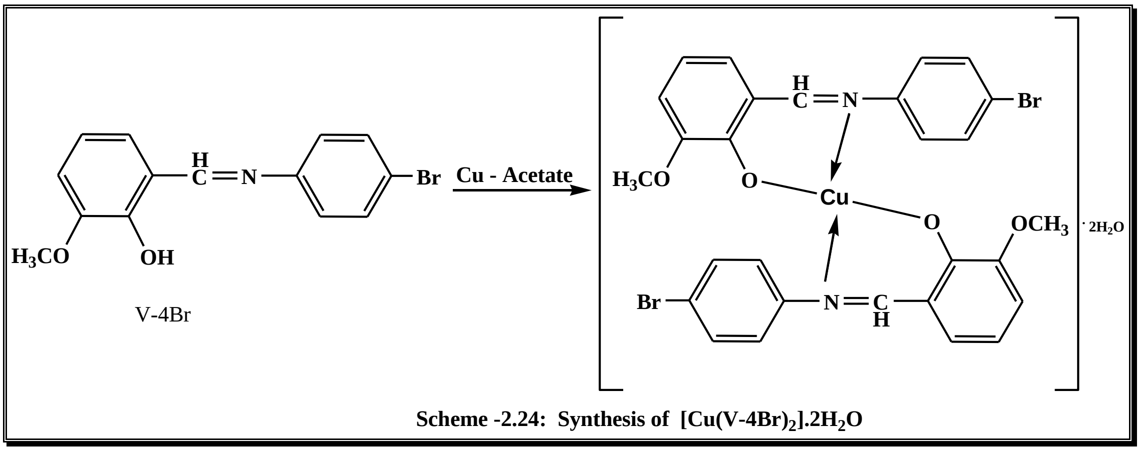
Scheme -2.23: Synthesis of $[Ni(V\text{-}4F)_2].2H_2O$

$[Ni(V\text{-}4F)_2].2H_2O$ is dark green crystalline compound. Yield: 73%, m.p.: >300°C, Solubility: DMF/DMSO. Anal. Calc. for $C_{28}H_{22}F_2N_2NiO_4$M.W.: 547, C (61.46%), H (4.05%), N (5.12%), Ni (10.73%) found: C (61.52%), H (4.00%), N (5.07%), Ni (10.80%).

IR (KBr, cm^{-1}): 3475 (O-H of H_2O), 1595 (C=N), 445 (Ni-N), 465 (Ni-O).

2.3.3.11 Synthesis of [Cu(V-4BR)$_2$].2H$_2$O

Cupric nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((4-bromophenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.24)



Scheme -2.24: Synthesis of $[Cu(V\text{-}4Br)_2].2H_2O$

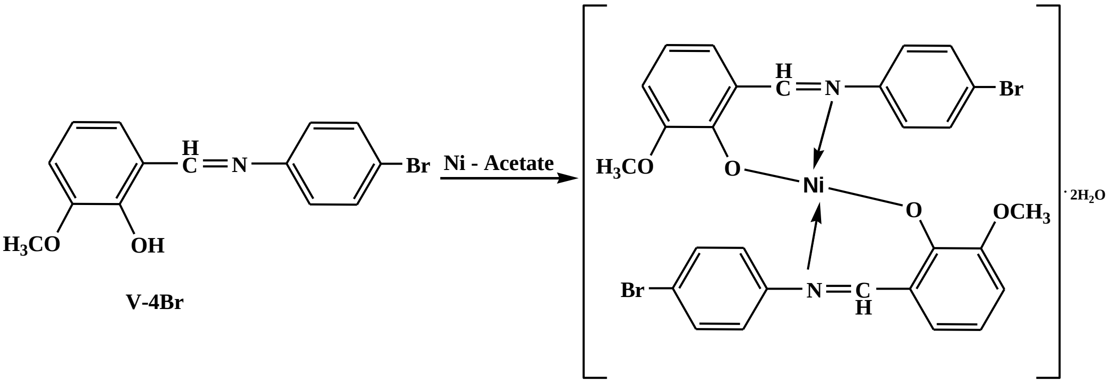
$[Cu(V\text{-}4Br)_2].2H_2O$ is dark green compound. Yield: 86%, m.p.: >300°C, Solubility: DMF/DMSO. Anal. Calc. for $C_{28}H_{22}Br_2CuN_2O_4$M.W.: 673, C (49.91%), H (3.29%), N (4.16%), Cu(9.43%) found: C (60.98%), H (4.07%), N (5.00%), Cu(11.60%).

IR (KBr, cm^{-1}): 3480 (O-H of H_2O), 1585 (C=N azomethane), 448 (Cu-N), 470 (Cu-O).

2.3.3.12 Synthesis of [Ni(V-4BR)$_2$].2H$_2$O

Nickel nitrate (2 mmol) was dissolved in minimumamount of distilled water and the solution was added to hot ethanolic solution of the 2-((4-

bromophenylimino)methyl)-6-methoxyphenol(4mmol) with constant shaking. Afterthe complete addition, small amount of sodium acetate was addedand the reaction mixture was refluxed for 4 hours. A crystalline solidwas formed which was filtered, washed with hot distilled water and then ethanol and dried under vacuum. (Scheme – 2.25)



Scheme -2.25: Synthesis of $[Ni(V\text{-}4Br)_2].2H_2O$

$[Ni(V\text{-}4Br)_2].2H_2O$ is Greenish brown crystalline compound. Yield: 79%, m.p.:>300^{o}C, Solubility: DMF/DMSO. Anal. Calc. for $C_{28}H_{22}Br_2N_2NiO_4$M.W.: 668, C (50.27%), H (3.31%), N (4.19%), Ni (8.77%) found: C (50.32%), H (3.25%), N (4.15%), Ni (8.85%).

IR (KBr, cm^{-1}): 3497 (O-H of H_2O), 1603 (C=N azomethane), 440 (Ni-N), 468 (Ni-O).

2.3.4 Characterization of Schiff base ligands and metal complexes

2.3.4.1 Physico-chemical properties of the synthesized Schiff base ligand and metal complexes

The Schiff base ligands of o-vanillin and substituted anilines and their metal complexes have been synthesized in a very facile and essentially identical way as the other compounds in the study. All these compounds are intensely colored, air and moisture free crystalline solids. They are soluble in common organic solvents. The analytical and physical data of ligands and metal complexes are listed in Table 2.16.

IR spectra (4000-400 cm^{-1}) of the ligands and metal complexes were recorded using KBr discs on 8400 FT-IR SHIMADZU spectrometer. GC-Mass spectra of the ligands were recorded on QP 2010 SHIMADZU GCMS spectrometer. ^{1}H NMR spectra of ligands were recorded on Bruker Avance-II 400 MHz FT-NMR spectrometer using TMS as an internal standard and $CDCl_3$ as a solvent. ESI mass spectra of complexes were recorded VG-70S Spectrometer. Electronic spectra of the metal complexes in DMF were recorded on a Perkin Elmer Lambda 19 spectrophotometer. Molar conductance of the metal complexes was determined on Systronics direct reading conductivity meter type CM-82T. A simultaneous TG/DTA was recorded on Perkin Elmer Pyris-1 model. Elemental analysis (C, H and N) were carried out on elemental analyzer PERKIN ELMER 2400, while analysis of metals were determined by EDTA after decomposing the complexes with HNO_3.

Table 2.16: Analytical and physical data of metal complexes

Complex	Formula	M.W.	Color	% Yield	Elemental analysis, % Found/(Calcd.)				Conductance Ω^{-1} cm^2 mol^{-1}
					C	H	N	M	
[Cu(V-2,4-ME)$_2$].2H$_2$O	$C_{32}H_{32}CuN_2O_4$	572	Green	78	67.25 (67.17)	5.60 (5.64)	4.96 (4.90)	11.08 (11.11)	24
[Ni(V-2,4-ME)$_2$].2H$_2$O	$C_{32}H_{32}N_2NiO_4$	567	Dark green	74	67.82 (67.75)	5.65 (5.69)	5.02 (4.94)	10.41 (10.35)	18
[Cu(V-2N4A)$_2$].2H$_2$O	$C_{30}H_{26}CuN_4O_{10}$	666	Greenish brown	72	54.13 (54.09)	3.89 (3.93)	8.45 (8.41)	9.62 (9.54)	20
[Ni(V-2N4A)$_2$].2H$_2$O	$C_{30}H_{26}N_4NiO_{10}$	661	Dark green	62	54.53 (54.49)	4.00 (3.96)	8.51 (8.47)	8.95 (8.88)	11
[Cu(V-4F)$_2$].2H$_2$O	$C_{28}H_{22}CuF_2N_2O_4$	552	Greenish brown	80	60.98 (60.92)	4.07 (4.02)	5.00 (5.07)	11.60 (11.51)	15
[Ni(V-4F)$_2$].2H$_2$O	$C_{28}H_{22}F_2N_2NiO_4$	547	Dark green	73	61.52 (61.46)	4.00 (4.05)	5.07 (5.12)	10.80 (10.73)	14
[Cu(V-4BR)$_2$].2H$_2$O	$C_{28}H_{22}Br_2CuN_2O_4$	673	Dark green	86	60.98 (49.91)	4.07 (3.29)	5.00 (4.16)	11.60 (9.43)	20
[Ni(V-4BR)$_2$].2H$_2$O	$C_{28}H_{22}Br_2N_2NiO_4$	668	Greenish brown	79	50.32 (50.27)	3.25 (3.31)	4.15 (4.19)	8.85 (8.77)	13

2.3.4.2 IR spectral studies

The characteristic IR bands of Schiff base ligands and metal complexes of O-vanillin-substituted anilines recorded as KBr discs. IR spectral analysis confirms the presence of characteristic groups present in the ligands. The IR spectrum of the ligand shows a broad band at 3380–3460 cm^{-1} due to the stretching vibrations of phenolichydroxyl group. The broadness is due to intramolecularhydrogen bonding between the phenolic group and the azomethane group. The strong band observed at around 1610-1640 cm^{-1} is assigned to the stretching vibrations of theazomethine group.Two moderately intense bands observed in the range of 3050-2900 cm^{-1} are due to aromatic and aliphaticν(C–H), respectively.Whereas band appeared in the rangeof 2900–2850 cm^{-1} is due to stretching vibrations of the–OCH_3 group.The IR spectra of the metal complexes show sharp band in the range 1600–1550 cm^{-1}, which is shifted to lowerfrequency as compared to ligand, suggesting coordinationof the azomethine nitrogen to the metal ion. Thedisappearance of ν(O–H) shows the deprotonation of the-OH group and its subsequent coordination to the centralmetal atom. Two new bands observed at 578–564 and481-470 cm^{-1} are characteristic of M–O and M–N absorptions,respectively [216,232-233]. (Figure 2.34-2.35)

Figure 2.34: IR spectrum of Schiff base ligand V-2,4-ME

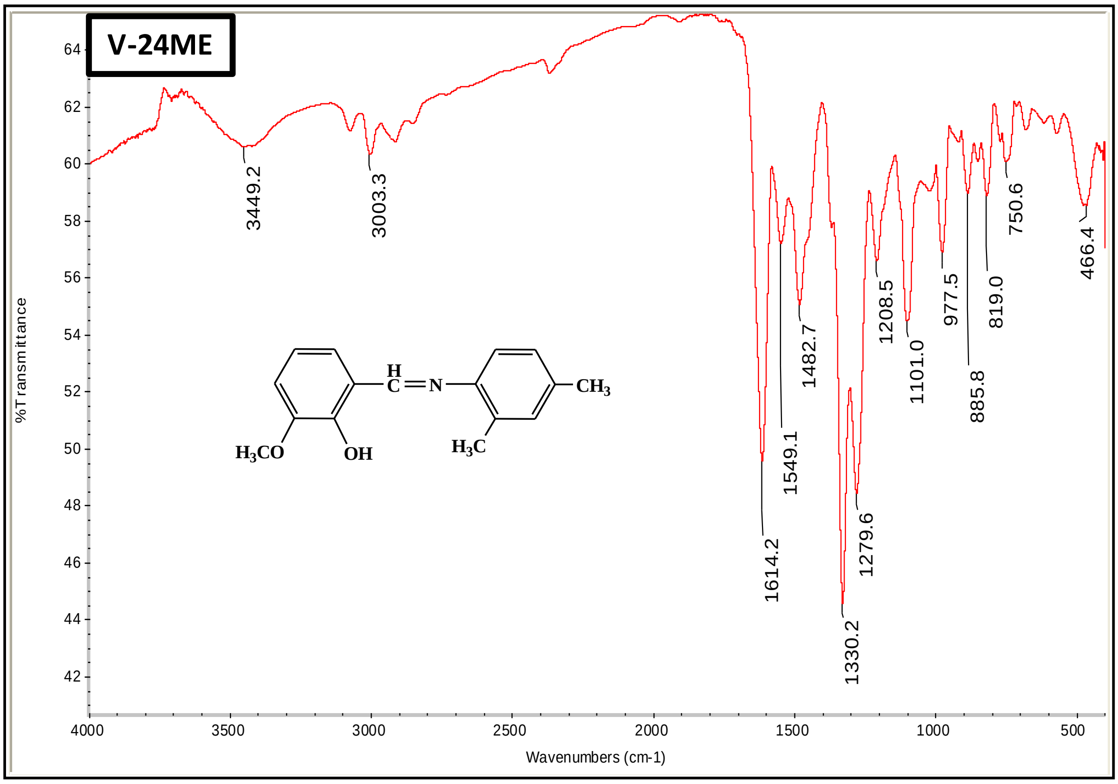
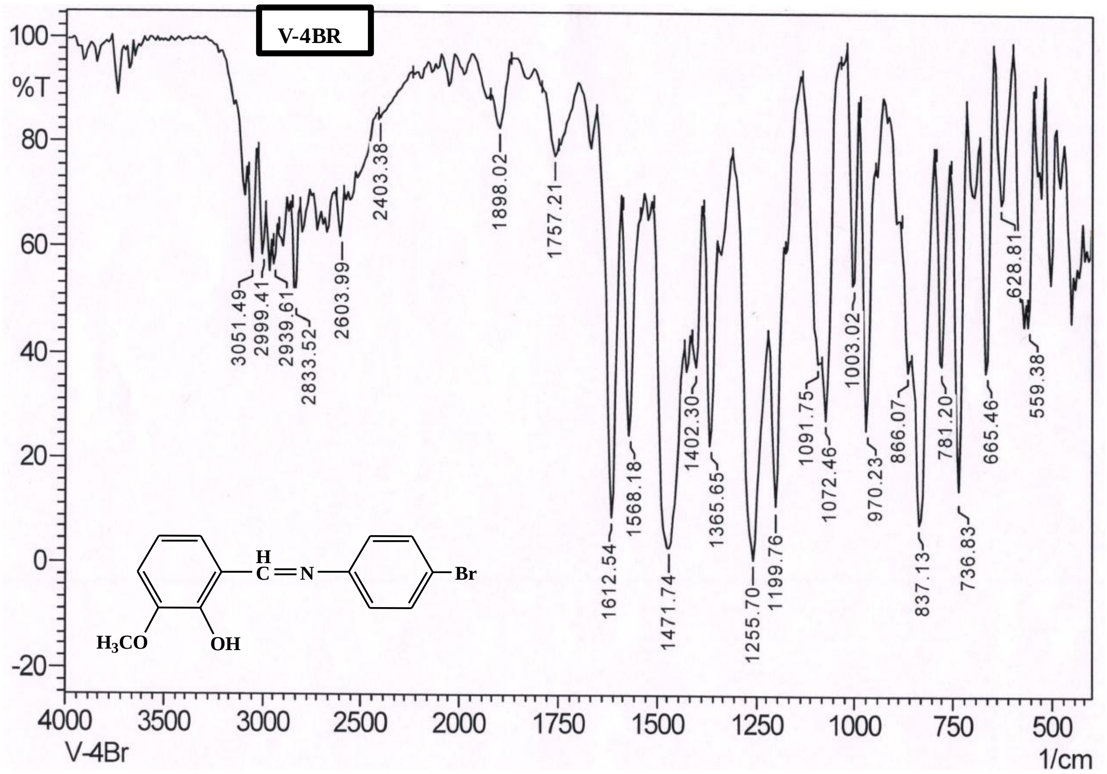


Figure 2.35: IR spectrum of Schiff base ligand V-4BR



2.3.4.3^{1}H NMRspectral studies

The synthesized Schiff base ligands have been characterized by the ^{1}H NMRspectra in $CDCl_3$. The signals due to methyl protons appeared as singlet in the range of 2.30-2.35 δ ppm, whereas signal dueto methoxy protons appeared as singlet at 3.35-3.90 δ ppm.Aromatic protons are observed between 6.85-7.40 δppm with few doublets/multiplets,which are due to aryl protons of substituted benzenerings. The signal of azomethine proton(-CH=N-) isobserved as singlet at 8.70-8.95 δ ppm. Phenolic proton in the Schiff base ligands appeared as singlet in the range 10.00-14.00 δ ppm. During the complexation with metal ion phenolic proton signal disappeared [234,235].(Figure 2.36-2.37)

Figure 2.36: ^{1}H NMR spectrum of Schiff base ligand V-2,4-ME

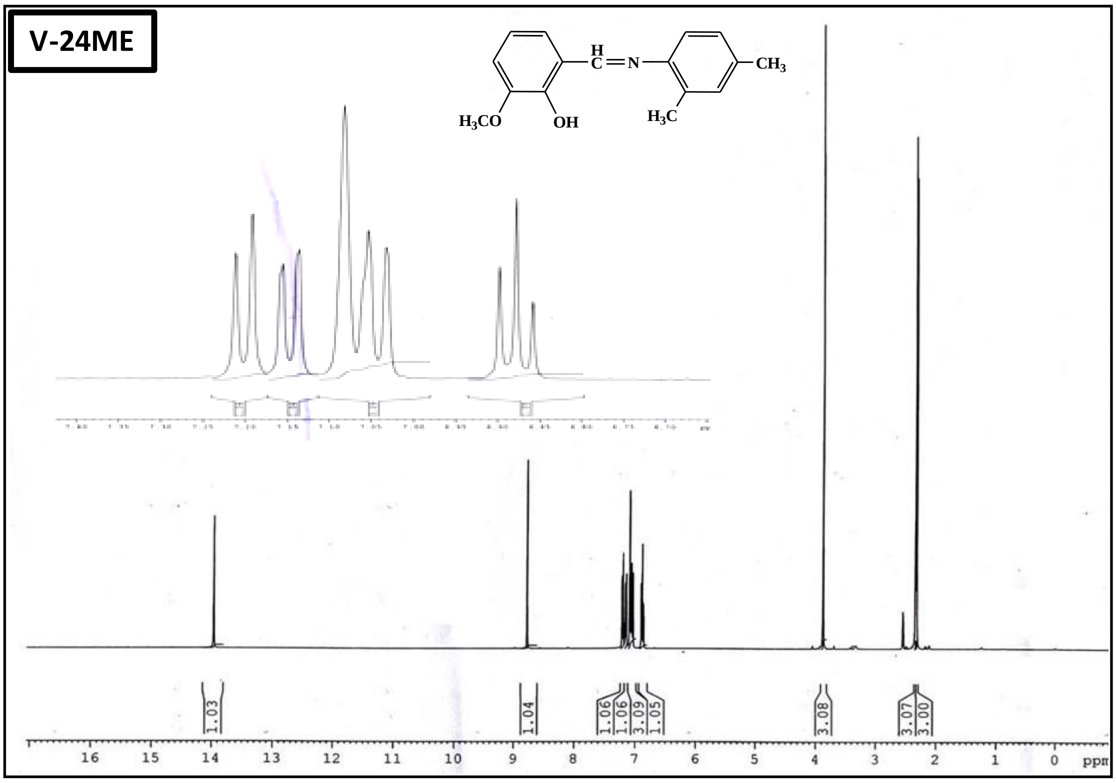
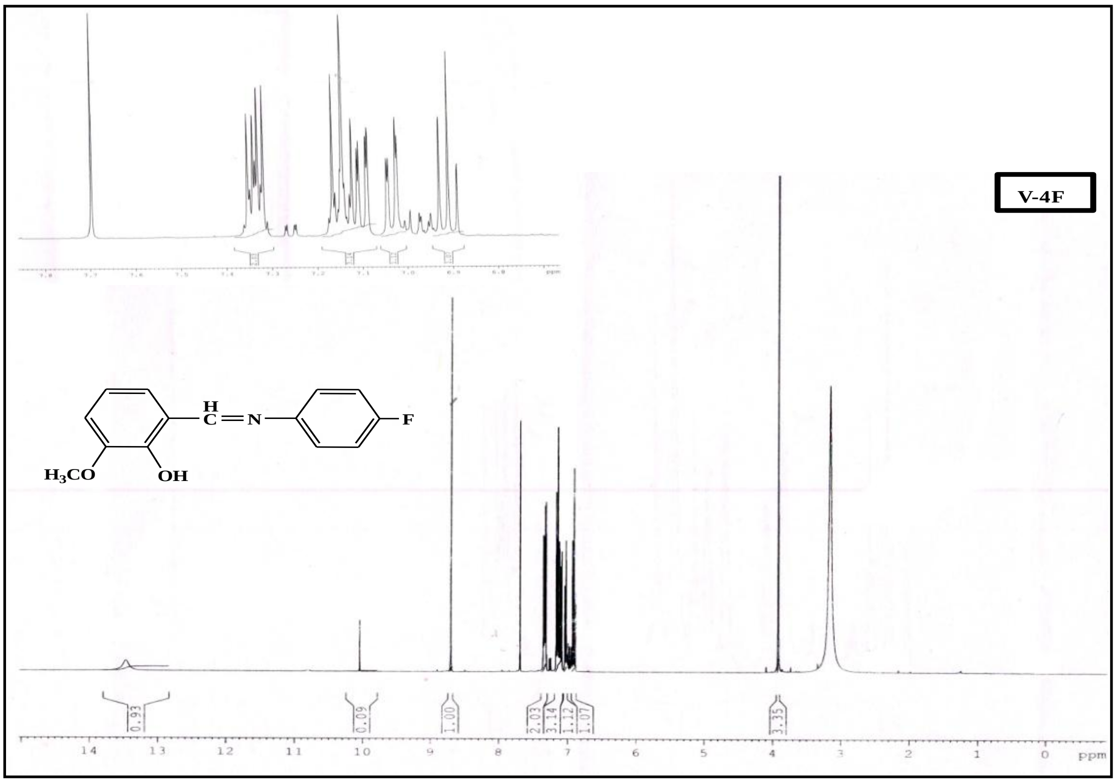


Figure 2.37: ^{1}H NMR spectrum of Schiff base ligand V-4F



2.3.4.4 Mass spectral studies

The mass spectra of Schiff base ligands were in good agreement with the proposed structures. Schiff base ligands show molecular ion peaks corresponding to their molecular masses. The other peaks appeared in the mass spectrum (abundance range 1-100%) are attributed to the fragmentation of ligand molecule obtained from the rupture of different bonds inside the molecule. Melting point of each metal complex is very high, as a result of this; the mass spectra were carried out by ESI. The electron-impact mass spectra of o-vanillin derivatives usually show an intense molecular ion peak.ESI–Mass spectra of the complexes show molecular ion peaks corresponding to their molecular weights. Mass spectra of all the complexes are in good agreement with the structures of the complexes [202] (Figure 2.38-2.41).

Figure 2.38: Mass spectrum of Schiff base ligand V-2,4-ME

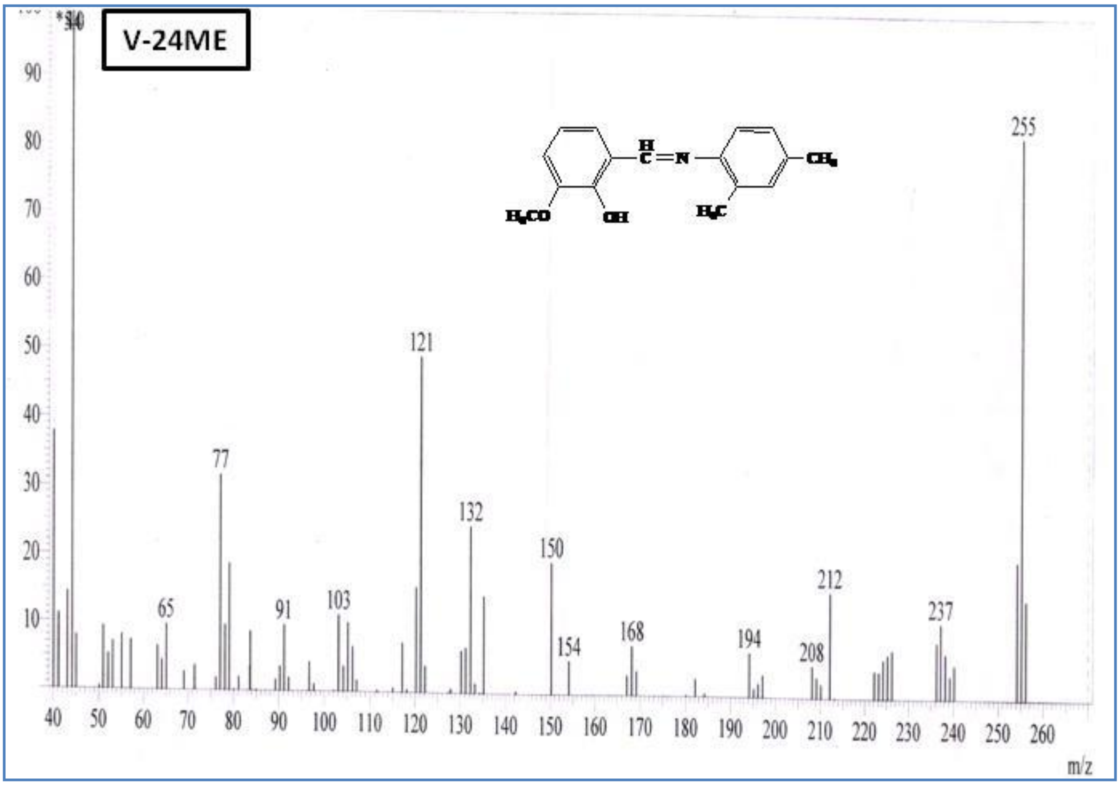


Figure 2.39: Mass spectrum of Schiff base ligand V-4F

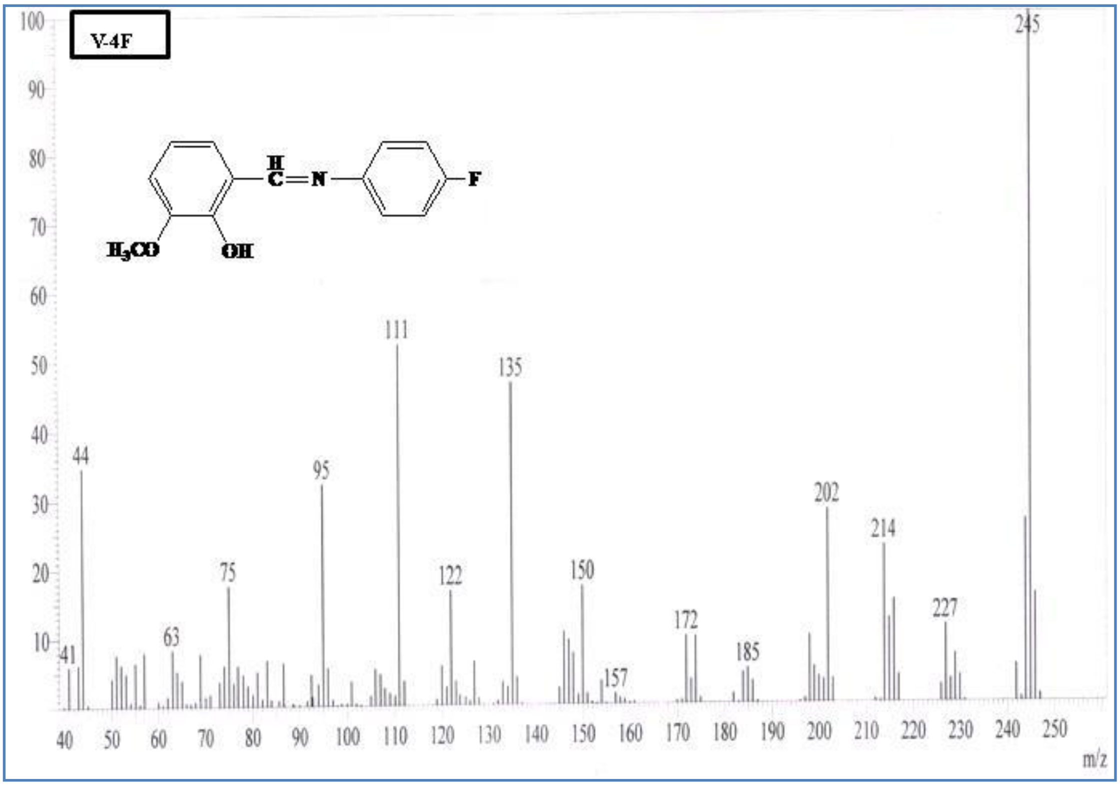


Figure 2.40: Mass spectrum of Schiff base ligand V-4BR

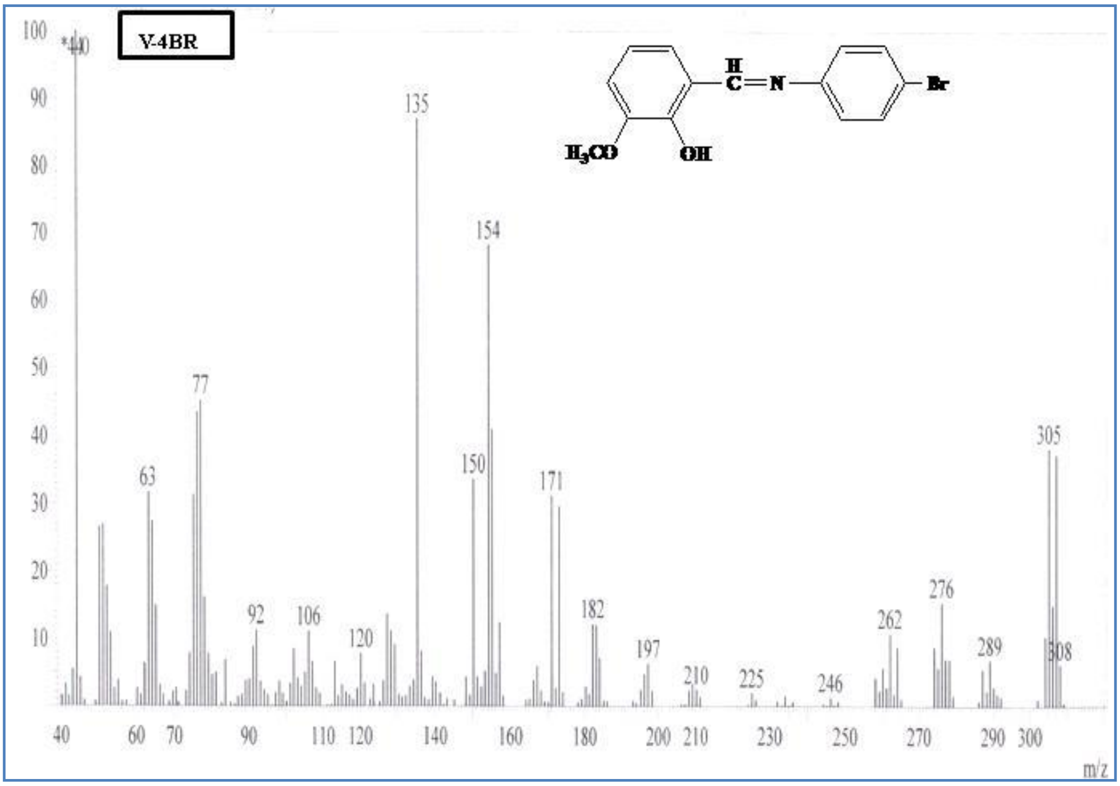
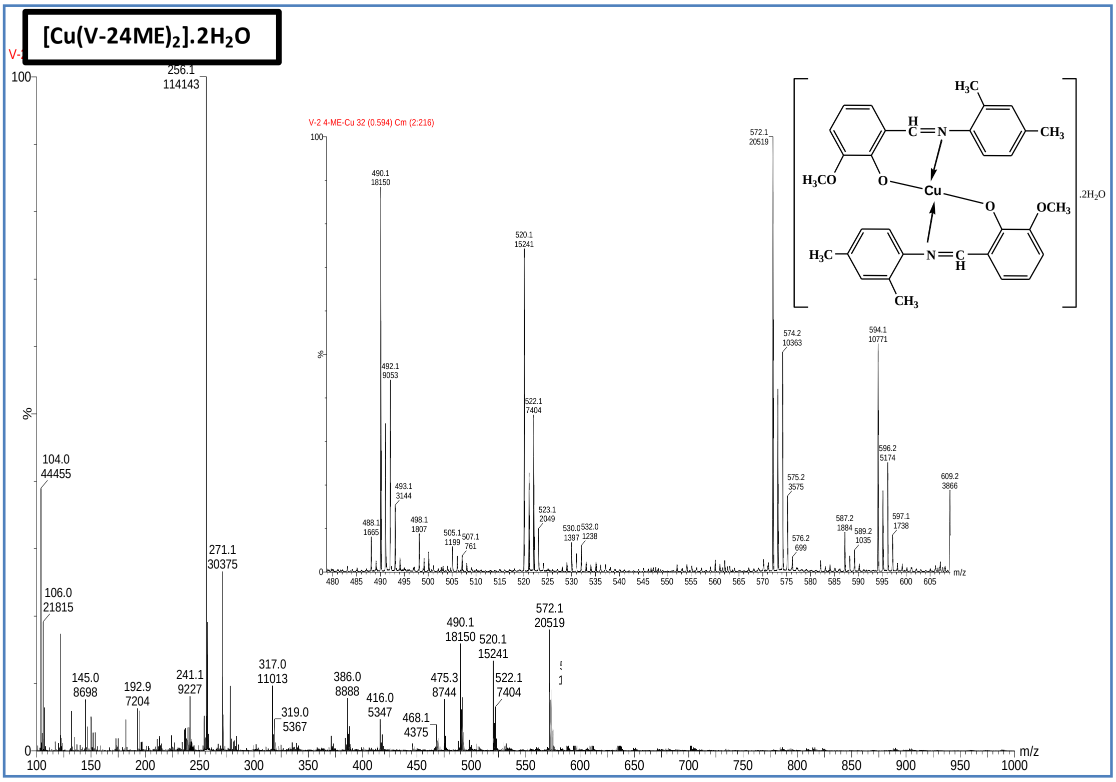


Figure 2.41: Mass spectrum of metal complex [Cu(V-2,4-ME)$_2$]$^{\cdot}$2H$_2$O



2.3.4.5 Thermal studies

The TG curve follows the decrease in sample mass with increase in temperature. In thepresent investigation, heating rates were suitably controlled at 10°C min^{-1} and mass loss followed up to 100-550°C.The simultaneous TG-DTG-DTA curves of the Cu(II) and Ni(II) complexes are presented in Figures. The TG curve follows the decrease in sample mass with increase in temperature. The decomposition of the complexes undergoes in two stages. The degradation of two mole –OCH$_3$ molecules takes place in the first stage at 231ºC and 229ºC with a mass loss of 10.85% (calcd.: 10.84%) and 10.95% (calcd.: 10.94%) respectively. The maximum rate of mass loss is indicated by the DTG peak at 268°C in case of Cu-complex and 323°C in case of Ni-complex. One endotherm is observed for both complexes in the DTA trace at 297°C and 343°C respectively.

For Copper complex, the mass loss occurred at 287°C corresponds to decomposition of one ligand molecule. The observed mass loss is 44.16% which is consistent with the theoretical value of 44.13%. For Ni-complex, the mass loss occurred at 475°C corresponds to decomposition of one ligand molecule. The observed mass loss is 44.83% which is consistent with the theoretical value of 44.82% [235,236].(Figure 2.42-2.43 & Table 2.17)

Figure 2.42: TG-DT analysis of metal complex $[Cu(V\text{-}2,4\text{-}ME)_2]^{\cdot}2H_2O$

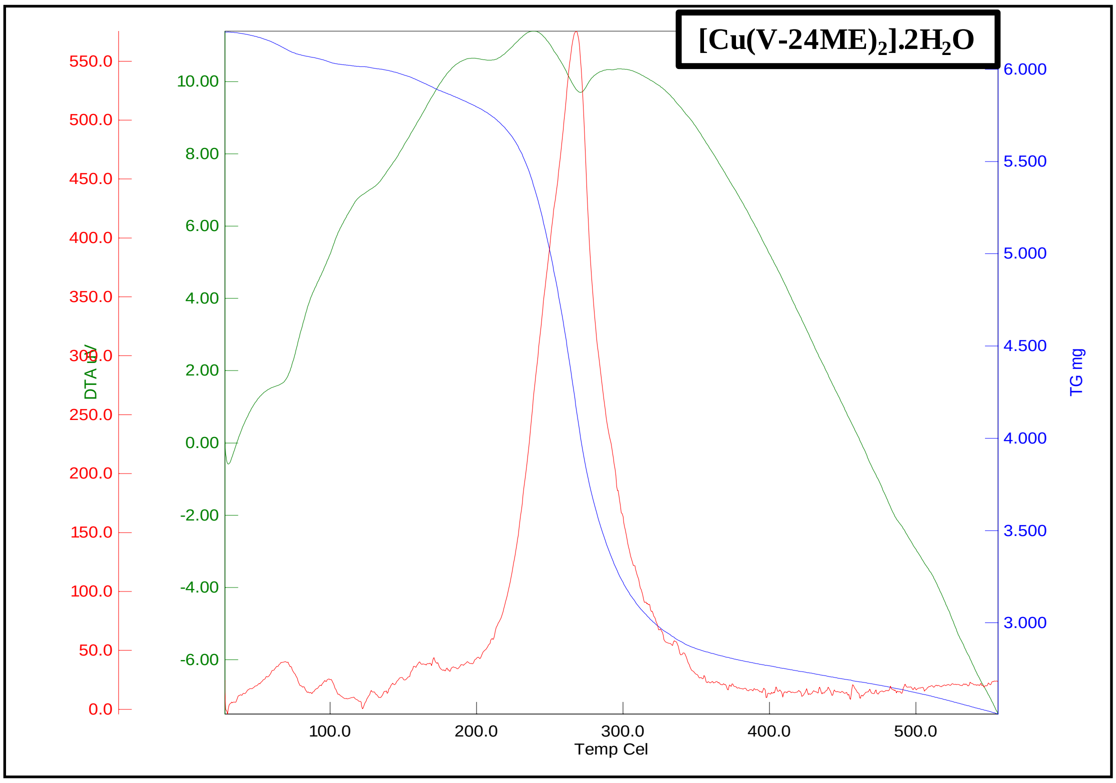


Figure 2.43: TG-DT analysis of metal complex $[Ni(V\text{-}2,4\text{-}ME)_2].2H_2O$

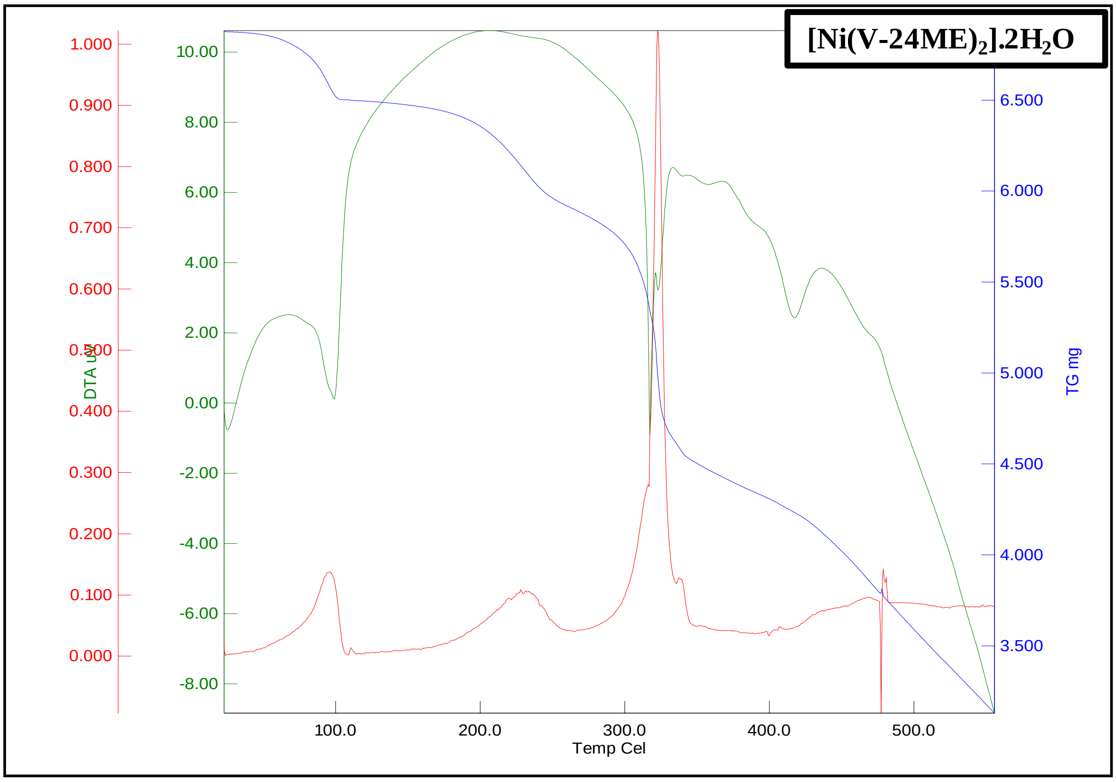
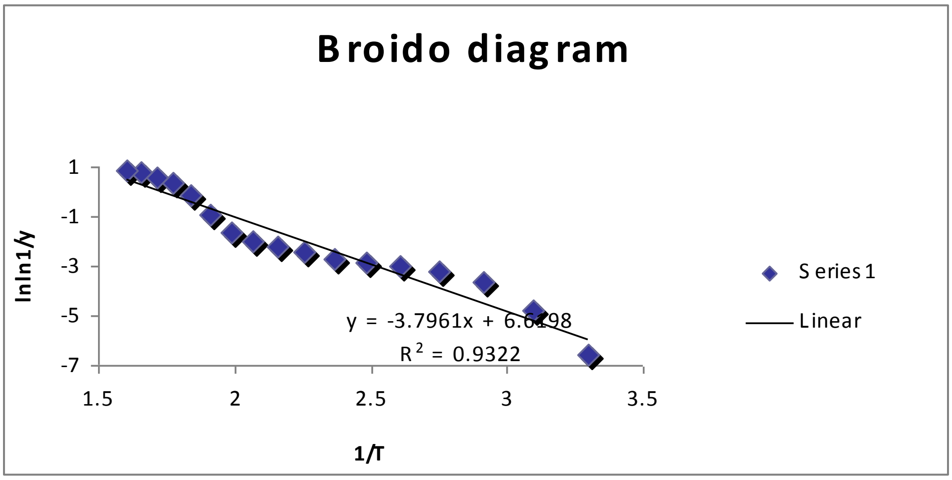


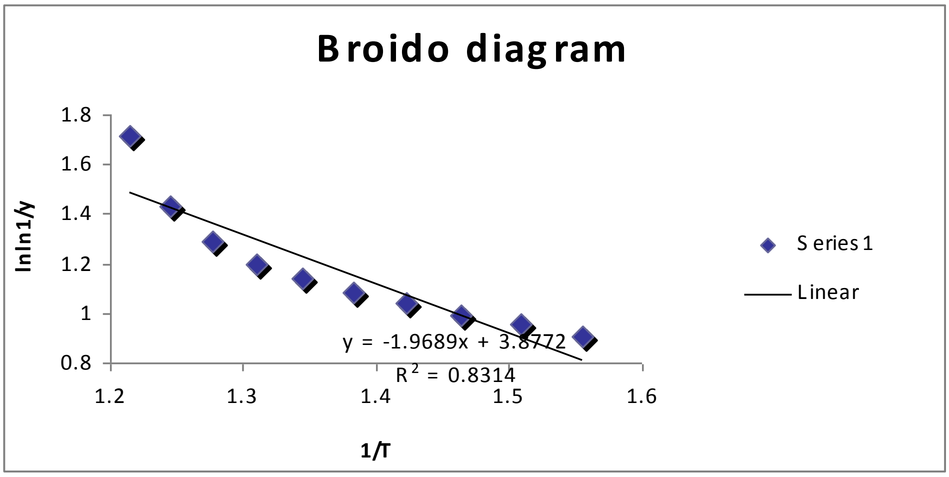
Table- 2.17: Thermo-analytical data of the complexes

Complexes	TG range (°C)	DTGmax (°C)	DTAmax (°C)	*ΔE* (kJ mol^{-1})*	Mass loss(%) obs.(calcd.)	Assignments
V-2,4-ME-Cu	50-250	268	297	0.932	10.85 (10.84)	Loss of two –OCH_3 molecules
	250-550	-	-	0.831	44.16 (44.13)	Loss of one Ligand molecule
V-2,4-ME-Ni	50-250	323	343	0.916	10.95 (10.94)	Loss of two –OCH_3 molecules
	250-550	-	-	0.873	44.83 (44.82)	Loss of one ligand molecule

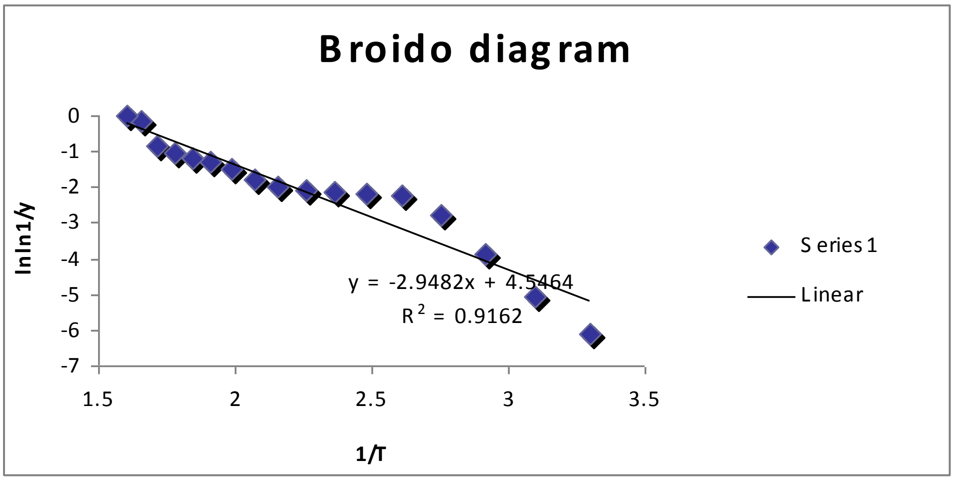
<u>For step-1 of Cu complex</u>



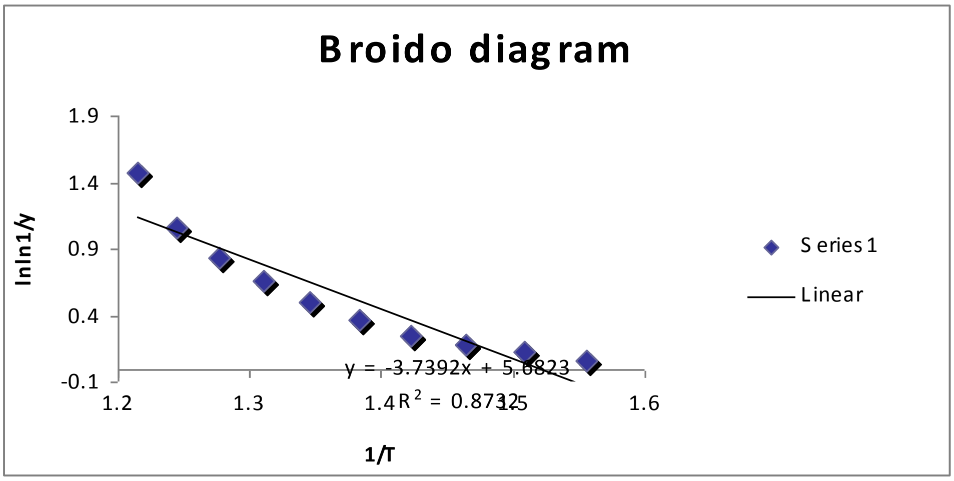
<u>For Step-2 of Cu complex</u>



For step-1 of Ni complex



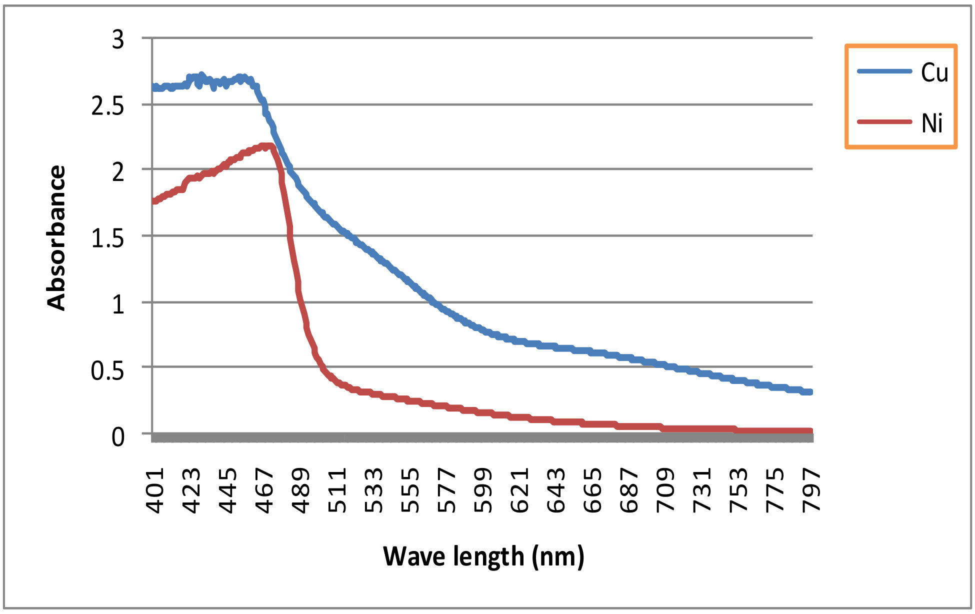
For step-2 of Ni complex



2.3.4.6 UV – Visible Spectral studies

Electronic spectra of all the complexes were recorded in dimethylformamide. For square planar Cu (II) complexes, the expected transition is $^2B_{1g}\rightarrow{}^2A_{1g}$ with absorption at 450–480 nm. Due to Jahn-Teller (J-T) distortions, square planar Cu(II) complexes give a broad absorption between 620 and 700 nm and the peak at 460–480 nm merges with the broad band, and thus only one broad band is observed. The Ni(II) complexes showed one strong band at 430–450 nm, which is assigned to the square planar $^1A_{g1}\rightarrow{}^1A_{g2}$ transition [218, 237, 238]. (Figure 2.44)

Figure 2.44: UV-Visible spectrum of metal complexes



2.3.4.7 Conductivity

The observed molar conductance of the metal(II) complexes in $10^{-3}M$ DMF solution are in the range 9–28 Ω^{-1} cm^2 mol^{-1}. The molar conductance values are consistent with the non-electrolytic nature for all metal complexes [235].

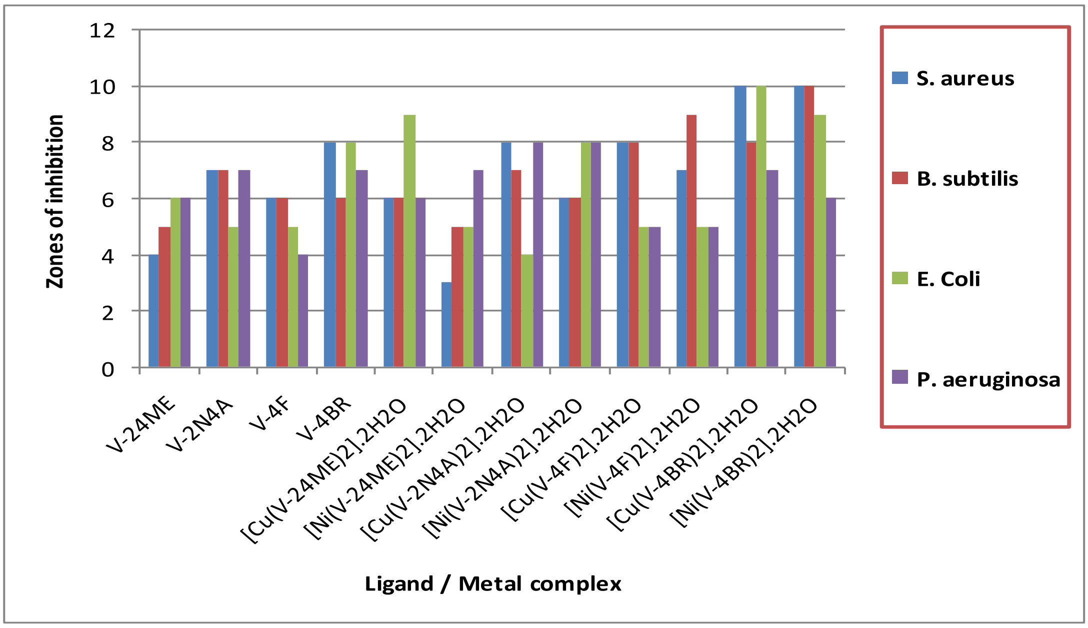
2.3.5 Antimicrobial activity

Antibacterial activity of the ligands and the metal complexes were evaluated against the *Staphylococcus aureus, Bacillus subtilis, Escherichia coli & Pseudomonas aeruginosa*. Metal complexes show better inhibition as compared to their ligands. It might be due to complexation of metal ions with Schiff base ligand. Such metal complexes might be inhibiting the enzyme activity of the bacterial system. Antibacterial activity of the ligands and complexes were quite comparable to the standard drugs. Antimicrobial activity data for the ligands and their metal complexes are shown below [196,239]. (Table 2.18 & Figure 2.45)

Table 2.18: Antimicrobial activity of Schiff base ligand and metal complexes

Ligand/complex	Microbial species (Zone of Inhibition in mm)			
	S. aureus	***B. subtilis***	***E. Coli***	***P. aeruginosa***
V-2,4-ME	4	5	6	6
V-2N4A	7	7	5	7
V-4F	6	6	5	4
V-4BR	8	6	8	7
$[Cu(V\text{-}2,4\text{-}ME)_2].2H_2O$	6	6	9	6
$[Ni(V\text{-}2,4\text{-}ME)_2].2H_2O$	3	5	5	7
$[Cu(V\text{-}2N4A)_2].2H_2O$	8	7	4	8
$[Ni(V\text{-}2N4A)_2].2H_2O$	6	6	8	8
$[Cu(V\text{-}4F)_2].2H_2O$	8	8	5	5
$[Ni(V\text{-}4F)_2].2H_2O$	7	9	5	5
$[Cu(V\text{-}4BR)_2].2H_2O$	10	8	10	7
$[Ni(V\text{-}4BR)_2].2H_2O$	10	10	9	6

Figure 2.45: Antimicrobial activity chart



2.3.6 Conclusion

O-vanillin Schiff base ligands derivatives and their metal complexes were synthesized. All the ligands and metal complexes were characterized by different spectroscopic techniques. The copper and nickel complexes exist in the square planar geometry. Antibacterial activities suggest that Schiff base ligands and their metal complexes have potential antibacterial activity. Further investigation is required to explore such ligands and metal complexes of o-vanillin derivatives for the industrial and pharmaceutical use.

2.3.7 References

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