

# Synthesis, Characterization and Antimicrobial Analysis of Vanadium (IV) And Nickel(II) Complexes with N-(3-Nitrobenzylidene)-N, N'-Dimethyl-4-Aminoantipyrine

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**Abstract:** The Schiff base ligand (3-nitrobenzylidene)-N, N'-dimethyl-4-amino antipyrine was prepared by the condensation between 3-nitrobenzaldehyde and N, N'-dimethyl-4-amino antipyrine. The Ni and V complexes of the corresponding ligand were prepared and was characterized by different methods like CHN analysis, IR and UV spectra. From CHN analysis and IR spectral data the structure of Ni complex is found to be tetrahedral and that of Vanadium complex is octahedral structure. The complexes were analysed for antimicrobial studies.

**Keywords:** 3-nitrobenzaldehyde, N, N'-dimethyl-4-aminoantipyrine, Antibacterial, Antifungal, Schiff base

## 1. Introduction

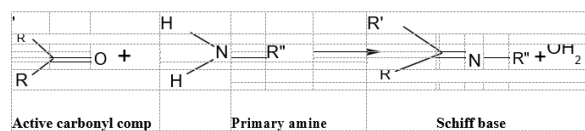
Coordination Chemistry is a branch of inorganic chemistry which encompasses a great diversity of structures. The term coordination is used to describe the nature of metal ligand bond. Coordination compounds of transition metals was discovered in 1798 by Tassart. The increased development in the field of spectroscopic analysis like IR, UV, Raman, NMR, EPR, Mass spectroscopy etc facilitate the progress of research in the field of coordination compounds.

According to Werners theory ligand donate electron pair to metal ion or atom to form co-ordinate linkage. This approach was first applied to co-ordination compounds by Linus Pauling and Slater (1931) [1]. In 1931 Pauling gave Valence bond theory which is based on revolutionary idea of hybridization of atomic orbitals [2]. According to this theory, the central metal atoms make available equal number of empty orbitals to it's coordination number.

Crystal field theory developed by Bethe [3] and Van Vleck [4] is another approach to study the complexes. According to this theory, the bond between metal and ligand is neither due to sharing of electron nor due to interaction of atomic orbitals. Crystal field theory involves electrostatic approach to the bonding in complexes.

Schiff bases are condensation products of amines with active carbonyl compounds. They were discovered by a German chemist, Nobel prize winner, Hugo Schiff in 1864<sup>[5]</sup>. The Schiff bases are also called imines, anils and azomethines [6] They contain azomethine (>C=N) group and hence can act as

effective ligand. The chemistry of the carbon–nitrogen double bond plays a vital role in the progress of chemical science. Solvent based synthesis of Schiff bases through classical condensation of aldehyde and amines require P<sup>H</sup> control, however, the yield of product, in high and in low P<sup>H</sup> range (3-4) depending upon the basicity of amines.



(R, R' and R'' may be acyclic, alicyclic, aromatic and heterocyclic etc.)

Schiff bases have active imine >(C=N) linkage which provide binding site for the metal ions through nonbonding electrons of nitrogen.

A number of Schiff bases exhibit the ability of capturing proton, good mechanical properties and excellent thermal stability. Schiff base compounds had been shown to exhibit a variety of applications including electronic applications, pH and ion sensors, molecular wires, organic lightemitting diodes (OLED), non-linear optical devices and organic photovoltaics [7].

### A. Applications

#### Application of schiff bases in various fields:

Application of Schiff base compounds in various fascinating area is current research interest to the inorganic chemist all over the world.

The importance of Schiff base complexes for, bioinorganic chemistry, biomedical application, supramolecular chemistry, catalysis, medical sciences, separation encapsulation processes and formation of compounds with unusual properties and structures has been well recognized and reviewed. Among the several classes of ligands, a new tetradentate Schiff base ligands derived from orthophenylenediamine, salicylaldehyde and isatin/aceyl acetone/2-hydroxy naphthalaldehyde containing oxygen and nitrogen donor sites have been recognized and synthesized. The different types of Schiff base ligands and their

applications have importance in generating new areas of fundamental chemistry and many opportunities of applied chemistry. The majority of Schiff base ligands represent creative and focused efforts to design molecules which will have particular uses.

## 2. Material and Methods

### A. Materials

The chemicals used for the synthesis in the present investigation are of commercial grade and they are directly used without further purification. 3-nitrobenzaldehyde is used as aldehyde and N, N' – dimethyl aminoantipyrine is used as the amine and the solvent used is methanol.

### B. Instruments

Instruments used in this investigation are given below:

1. Schimadzu IR prestige-20 spectrometer
2. Schimadzu UV-2450 A Spectrometer
3. Systronics conductivity meter 304
4. Gouy type magnetic balance
5. Vario-III CHN elemental analyser

### C. Methods

#### 1) Synthesis of N-(3-Nitrobenzylidene)-N, N'-Dimethyl-4-Aminoantipyrine

3-nitrobenzaldehyde (0.151g, 0.001M) dissolved in 20 ml methanol and N,N'-Dimethyl-4- Aminoantipyrine (.231g, 0.0001 M) in 20 ml methanol was mixed well. The resulting mixture was refluxed for about four hours. On cooling, yellow crystals were separated from the solution. These crystals were filtered and dried.

#### 2) Synthesis of metal complexes

##### a) Synthesis of Vanadium complex

Ammonium metavanadate has been used as a synthetic intermediate for the preparation of V(IV) complex. The methanolic solution of the ligand (0.001M) was just heated to dissolve the ligand and to this, methanolic solution of the metal (0.0005M) was added so that the ratio will be 1:2 and the mixture is refluxed for four hours. The pH is maintained between 6-7. Then the volume was reduced to half its initial volume. After concentration, the solution was cooled and the Dark Green complex formed is separated out. It is filtered, washed with methanol and dried in vacuum.

##### b) Synthesis of Nickel complex

Nickel Chloride has been used as a synthetic intermediate for the preparation of Ni(II) complex. The methanolic solution of the ligand (0.001M) was just heated to dissolve the ligand and to this, methanolic solution of the metal (0.001M) was added so that the ratio will be 1:1 and the mixture is refluxed for four hours. The pH is maintained between 6-7. Then the volume was reduced to half its initial volume. After concentration, the solution was cooled and the complex formed is separated out. It is filtered, washed with methanol and dried in vacuum.

Compound	Colour	Solubility	Yield(%)
Ligand	Yellow	Chloroform	76.3%
Ni Complex	Green	Chloroform	78%
V Complex	Dark Green	Chloroform	80%

### D. Infrared spectra

The IR spectra of the solid samples were recorded in Schimadzu IR prestige -20 spectrometer in the range of 4000-400 cm<sup>-1</sup>. Potassium bromide disc method was employed for sample preparation.

### E. Electronic absorption spectroscopy

Electronic absorption spectroscopy is used to study the stereochemistry and the geometry of the complexes.

The UV-Visible spectra of the samples in DMSO solution were recorded in Schimadzu UV-2450 A spectrometer in the range of 200-800 nm.

### F. Molar conductance

Molar conductance of transition metal complexes were determined in DMF and N,N'-dimethyl formamide at room temperature using a systolic conductivity Meter 304. The cell constant of the conductivity cell was 1 cm<sup>-1</sup>. The concentration of the solution was around 1x 10<sup>-3</sup> M. The molar conductance is measured by the equation,

$$M = 1000 \text{ k/c}$$

Where c = concentration of the solution in mol/L

k = conductivity (specific conductance)

### G. CHN analysis

CHN analysis was done in Vario-III CHN elemental analyser at the Saif, Cochin University of Science and Technology, Kochi.

### H. Magnetic susceptibility

Magnetic susceptibility measurements of the metal complexes were studied at room temperature (3000K) by using Magway MSB Mk1 magnetic susceptibility balance. Diamagnetic corrections were computed using Pascal's constant by adding the diamagnetic contribution of various atoms and structural units. Gram susceptibility was calculated using the formula,

$$X_g = (\alpha + \beta F) / W$$

Where  $\alpha$  = Air Displacement Constant

$\beta$  = Tube Constant

F = change in weight in milligram

W = weight of sample in gram

The effective magnetic moment  $\mu_{\text{eff}}$  was calculated using the formula,  $\mu_{\text{eff}} = 2.84\sqrt{X_m T}$

where  $X_m$  = molar susceptibility corrected for diamagnetism and T = Temperature, 293 K

### I. Estimation of vanadium

The most convenient method for the estimation of Vanadium

is a volumetric process. The Vanadium is first obtained in acid solution as vanadate, reduced to the tetravalent state by one of several reducing agents which are available. The solution is then titrated in the presence of sulphuric acid with potassium permanganate solution, which quantitatively oxidises the lower Vanadium salt to Vanadate. Diphenylamine sulfonic acid is used as indicator. Using sulfur dioxide to effect the reduction, the following reaction take place.

Excess of sulphur dioxide is removed before the titration by boiling the reduced solution in an atmosphere of CO<sub>2</sub>. The titration is completed when a pink end point stable atleast for one minute.

#### J. Estimation of nickel

The nickel is precipitated as nickel dimethyl glyoxime by adding alcoholic solution of dimethyl glyoxime and then adding a slight excess of aqueous ammonia solution.



The complex solution was first digested with HCl – H<sub>2</sub>SO<sub>4</sub> mixture and is made upto 25 mL and 10 mL is pipetted out into a 100 ml beaker. Then it is diluted to 50 mL The solution is heated to 80°C and 10 mL of 1% dimethyl glyoxime in rectified spirit is added immediately followed by dilute ammonia solution drop wise directly to the solution. The precipitate is heated for 20 – 30 minutes and the supernatant liquid is tested for complete precipitation. The precipitate is allowed to cool and then filtered through a previously weighed sintered crucible (IG<sub>4</sub>). The precipitate is washed with cold water and dried at 110-120°C. It is allowed to cool in desiccator and weighed.

### 3. Results and Discussion

Schiff base derived from 3-nitrobenzaldehyde and N,N'-dimethyl-4-aminoantipyrine and their vanadium and nickel complexes are synthesized.

#### A. General properties

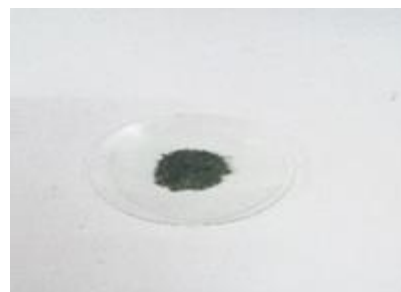
V(IV) is dark green in coloured and Ni(II) complex is green in coloured and are stable in air. Both the complexes are sensitive to light and decomposes when exposed to light. The two complexes are insoluble in water and readily soluble in chloroform and DMSO.

#### B. Analysis

Metal content in the complexes were determined by standard method after decomposing the complexes with hydrochloric acid-sulphuric acid mixture. The metal complexes of Schiff base ligand were prepared by the stoichiometric reaction of the corresponding metal and ligand in 1:2 ratio. The gravimetric analysis of Nickel complex and volumetric analysis of Vanadium complex are found to be successful and from these, the metal content in the complexes are determined.



Ligand



Vanadium complex



Nickel Complex

#### C. Molar conductance

Molar conductance of 10<sup>-3</sup> M solutions of the metal complexes at 25°C were measured in DMF and N,N'-dimethyl formamide. The molar conductance values of Ni(II) and V(IV) complexes under investigation are found to be 44.3 Ω<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup> and 3.8 Ω<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup> respectively. The molar conductance value shows that the Ni(II) and V(IV) complex was electrolytic in nature. Because there is no charged species in the complex to neutralize the charge of the central metal ion.

#### D. Magnetic measurements

Magnetic susceptibility of the complexes were determined using Magway MSB Mk1 magnetic susceptibility balance. The measurements were made at room temperature. Table shows the effective magnetic moments calculated from the magnetic susceptibility which is corrected for diamagnetic corrections. Some indications about the structure, geometry and coordination of the complexes can be obtained from magnetic moment values. The Ni(II) and V(IV) complexes are paramagnetic in nature.

The magnetic moment values of Ni(II) and V(IV) complexes are found to be 4.1 BM and 2.01 BM. From this, it is clear that the Ni(II) complex has tetrahedral and V(IV) complex has octahedral structure.

Complex	colour	Yield(%)	Molecular Weight	Magnetic moment (BM)	Molar conductance ( $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ )
Ni(II) complex	Green	78%	459.1134	4.1	44.3
V(IV) complex	Dark green	80%	813.7815	2.01	3.8

E. CHN analysis

The experimental percentage values of carbon, hydrogen and nitrogen in the two complexes found out by CHN analysis were similar to the percentage calculated from the theoretical aspects. So from CHN analysis, the structure and denticity of the ligand and complex can be confirmed.

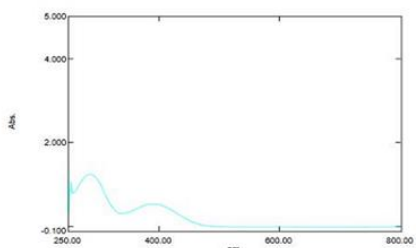
Here, the CHN analysis values are in good agreement with the calculated percentage of carbon, hydrogen and nitrogen in the complex. The ligand is bidentate. The structures of the complexes can also be confirmed from CHN analysis. The structure of Ni complex is tetrahedral and Vanadium complex is octahedral.

F. Electronic spectra

The electronic spectra are often helpful in the evaluation of results furnished by other methods of analysis. The electronic spectral bands of the ligand and complexes was recorded over the range of 200-800 nm in DMSO.

1) Electronic spectrum of ligand

DST F1ST UV Vis spectrophotometer Department of Chemistry



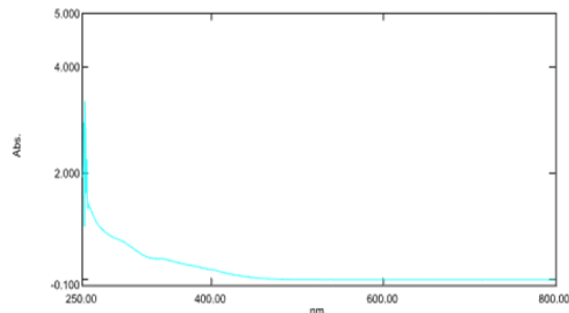
No.	P/V	Wavelength	Abs.	Description
1	●	390.00	0.542	
2	●	289.50	1.247	
3	●	228.50	1.204	
4	●	213.50	1.712	
5	●	204.50	2.858	
6	●	339.50	0.313	
7	●	237.50	0.137	
8	●	224.50	-4.050	
9	●	211.50	-0.142	

Ultraviolet spectra of the ligands recorded in DMSO showed strong bands around 286.5 nm and at 339.5 nm region which confirms the presence of benzenoid and azomethine linkages, which are characteristic of  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transition respectively.

Complex	C%		H%		N%	
	experimental	calculated	experimental	calculated	experimental	Calculated
Ni complex	48%	47.04%	6.29%	4.35%	11.35%	12.19%
V complex	46%	44.50%	5.29%	4.53%	11.98%	11.53%

2) Electronic spectra of complexes (Nickel Complex)

CHRISTIAN COLLEGE, KATTAKADA, Shimadzu Corp - 80282 UV - Visible Spectrophotometer UV Spectrum

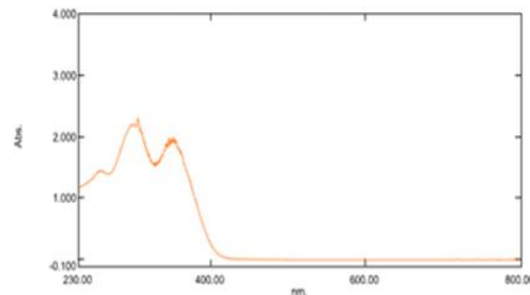


No.	P/V	Wavelength	Abs.	Description
1	●	340.50	0.390	
2	●	221.00	3.440	
3	●	338.50	0.385	

Ultraviolet spectra of the Nickel complex recorded in DMSO showed strong bands around 221 nm and at 338.5 nm region which confirms the presence of benzenoid and azomethine linkages, which are characteristic of  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transition respectively.

3) Vanadium complex

CHRISTIAN COLLEGE, KATTAKADA, Shimadzu Corp - 80282 UV - Visible Spectrophotometer UV Spectrum

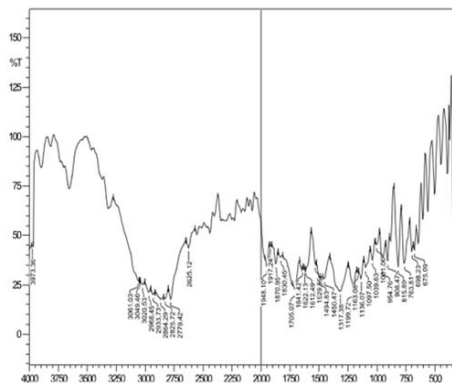


No.	P/V	Wavelength	Abs.	Description
1	●	763.00	-0.003	
2	●	346.00	1.982	
3	●	306.00	2.284	
4	●	258.50	1.439	
5	●	207.50	4.000	
6	●	328.50	1.533	
7	●	268.50	1.369	
8	●	230.50	1.175	

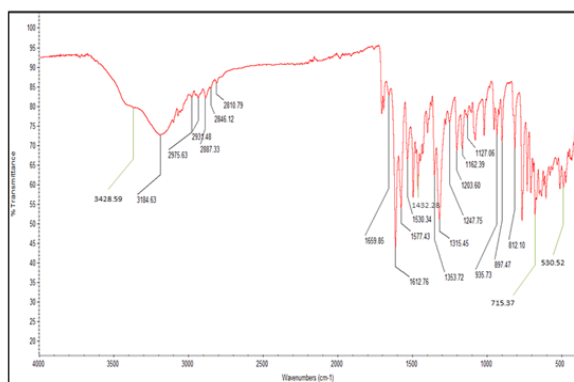
Ultraviolet spectra of the Vanadium complex recorded in DMSO showed strong bands around 268.5 nm and at 346 nm region which confirms the presence of benzenoid and azomethine linkages, which are characteristic of  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transition respectively.

G. Infrared spectra

The IR spectral data of the Schiff base ligand and its metal complexes are presented in tables. The spectra of the complexes were compared with the spectrum of the ligand.



IR spectrum of ligand

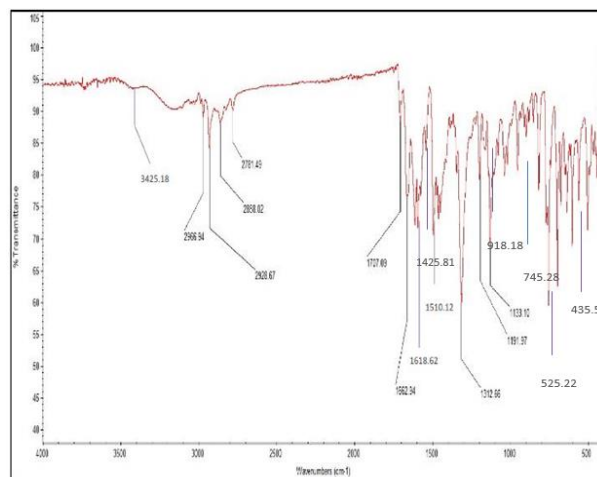


IR spectrum of nickel complex

Ligand	Ni Complex	Assignment
-	3428.59	Coordinated water molecule
3020.53	2975.63	=CH
1622.13	1612.76	C=N
1529.55	1530.34	C=C
1450.47	1432.28	N=O (Sym. Bending)
1317.38	1315.45	N=O (asym. Bending)
698.23	715.37	Monosubstituted
-	530.52	Ni-N

In the IR spectrum of the ligand, a medium strength band is observed at 3020.53 cm<sup>-1</sup> which corresponds to =CH group. In the complex, it is shifted to a lower frequency of 2975.63 cm<sup>-1</sup>. The intense band near 1622.13 cm<sup>-1</sup> can be attributed to ν (C=N) of the ligand. It is shifted to a lower frequency of 1612.76 cm<sup>-1</sup> which indicates the presence of coordination. Two peaks at 1432.28 cm<sup>-1</sup> and 1315.45 cm<sup>-1</sup> corresponds to the symmetric and asymmetric bending of NO<sub>2</sub> group. In Ni complex, a strong band is obtained in 3428.59 cm<sup>-1</sup> which indicates the presence of coordinated water molecule. The peak at 530.52 cm<sup>-1</sup> can be attributed to Ni-N bond which is absent in the free ligand. This

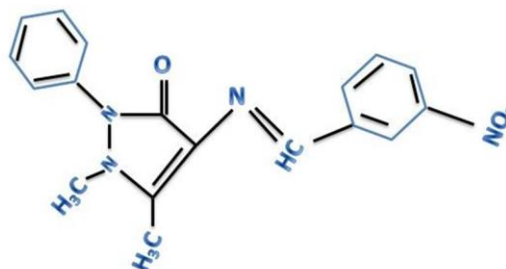
peak at 530.52 cm<sup>-1</sup> confirms the coordination in Ni complex.



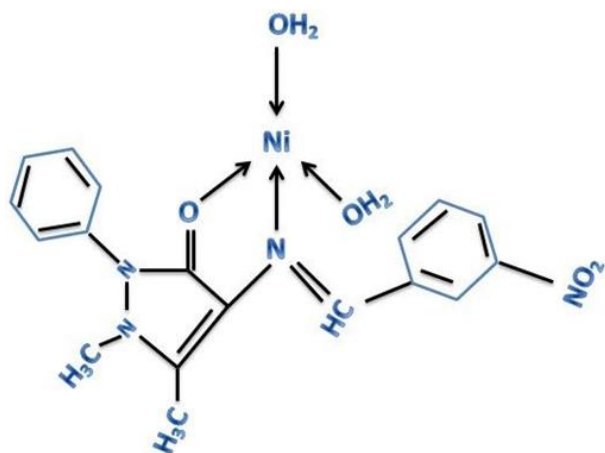
IR spectrum of vanadium complex

Ligand	V Complex	Assignment
-	3425.18	Coordinated water molecule
3020.53	2966.94	=CH
1622.13	1618.62	C=N
1529.55	1510.12	C=C
1317.38	1312.66	N=O (asym.bending)
698.23	745.28	Monosubstituted
-	435.51	V-N
-	918.18	V=O
	525.22	V-O

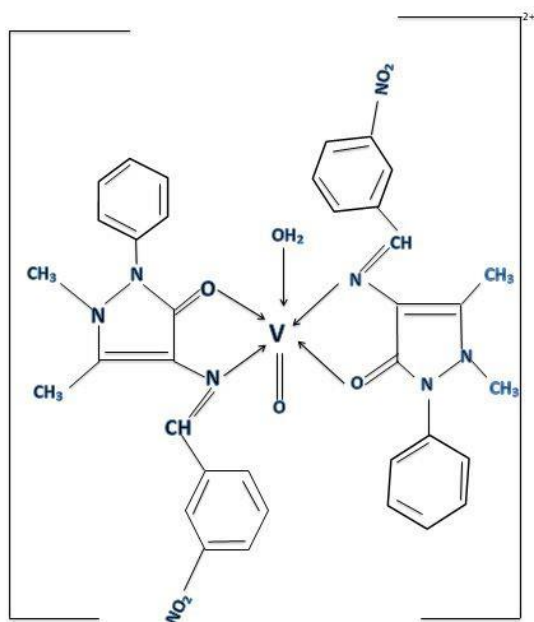
In the IR spectrum of the ligand, a medium strength band is observed at 3020.53cm<sup>-1</sup> which corresponds to =CH group. In the complex, it is shifted to a lower frequency of 2966.94 cm<sup>-1</sup>. The intense band near 1622.13 cm<sup>-1</sup> corresponds to ν (C=N) of the ligand. It is shifted to a lower frequency of 1618.62 cm<sup>-1</sup> which indicates the presence of coordination. The peak at 1312.66 cm<sup>-1</sup> indicates the asymmetric bending of NO<sub>2</sub> group. The peak at 3425.18 cm<sup>-1</sup> confirms the presence of coordinated water molecule. The strong bands at 435.51 cm<sup>-1</sup> and 918.18 cm<sup>-1</sup> attribute to V-N and V=O bonds which are absent in the free ligand. These peaks confirm the formation of metal complexes.



Structure of ligand



Structure of nickel complex



Structure of vanadium complex

### H. Antibacterial and antifungal studies

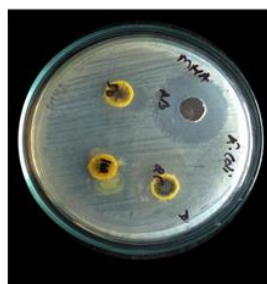
#### Antibacterial studies:

The antibacterial and antifungal studies were conducted by comparing the zone of inhibition of a standard bacterial agent with the complexes and their extend of antimicrobial activity is measured.

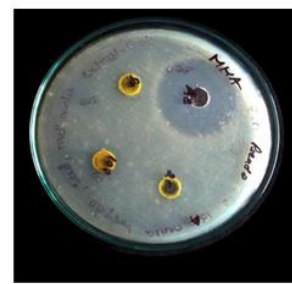
#### 1) Antibacterial studies of vanadium complexes

Vanadium complex is inactive for Gram negative Bacteria like E Coli and Pseudomonas aeruginosa. The vanadium complex is inactive for Gram positive stain Streptococcus mutans at the concentration of 250 and 500. But it is slightly active at the concentration of 1000. The complex shows slight activity for the Gram positive stain Staphylococcus aureus at 250, 500 and 1000 concentrations.

E COLI



PSEUDOMONAS AERGINOSA



STREPTOCOCCUS MUTANS



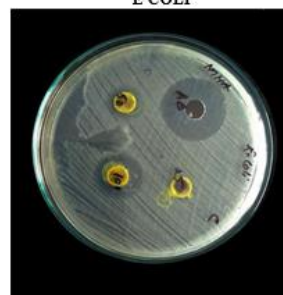
STAPHYLOCOCCUS AUREUS



Organism	Antibacterial Agent	Zone of inhibition			
		AGENT	250	500	1000
E Coli	Streptomycin	26	-	-	-
Pseudomonas aeruginosa	Streptomycin	30	-	-	-
Streptococcus mutans	Streptomycin	26	-	-	15
Staphylococcus Aureus	Streptomycin	27	12	15	17

#### 2) Antibacterial studies of nickel complexes

E COLI



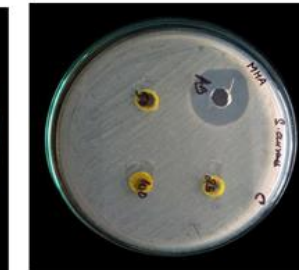
PSEUDOMONAS AERGINOSA



STREPTOCOCCUS MUTANS



STAPHYLOCOCCUS AUREUS



Organism	Antibacterial Agent	Zone of inhibition			
		AGENT	250	500	1000
E Coli	Streptomycin	20	-	12	18
Pseudomonas aerginosa	Streptomycin	30	-	10	16
Streptococcus mutans	Streptomycin	26	-	-	16
Staphylococcus Aureus	Streptomycin	27	-	-	16

Nickel complex is inactive for Gram negative Bacteria like E Coli at the concentration of 250 and shows moderate activity at the concentrations of 500 and 1000. The complex is inactive for Pseudomonas aerginosa at 250 concentration and shows slight activity at 500 and 1000 concentration. The nickel complex is inactive for Gram positive stain Streptococcus mutans at the concentration of 250 and 500. But it is slightly active at the concentration of 1000. The complex is inactive for the Gram positive stain Staphylococcus aureus at 250, 500 and shows slight activity at 1000 concentrations.

*Antifungal studies:*

3) *Antifungal results of vanadium complex*



Aspergillus Niger

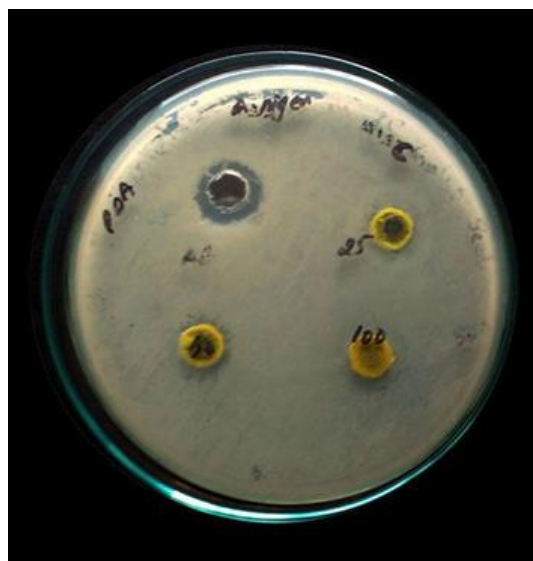


Candida Albicans

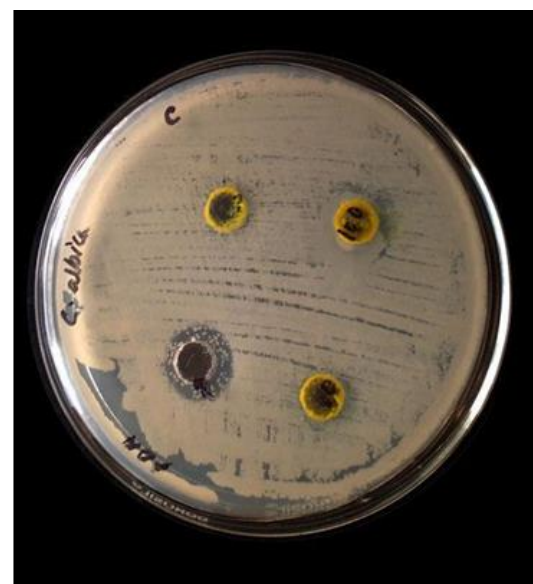
Organism	Antifungal Agent	Zone of inhibition			
		Agent	250	500	1000
Aspergillus niger	Clotrimazole	30	-	15	20
Candida albicans	Clotrimazole	15	-	12	18

The standard antifungal agent used for the antifungal studies is Clotrimazole. Vanadium complex is inactive for Aspergillus niger fungus at the concentration of 250. But the complex is slightly active at 500 and 1000 concentration.

4) *Antifungal results of nickel complex*



Aspergillus Niger



Candida Albicans

Organism	Antifungal Agent	Zone of inhibition			
		Agent	250	500	1000
Aspergillus niger	Clotrimazole	15	-	-	-
Candida albicans	Clotrimazole	15	-	-	-

The standard antifungal agent used for the antifungal studies is Clotrimazole. The Nickel complex is inactive for both *Aspergillus niger* and *Candida albicans* at all concentrations of 250, 500 and 1000.

#### 4. Summary and Conclusion

The Schiff base ligand, N-(3-nitrobenzylidene)-N,N'-dimethyl-4-aminoantipyrine has been synthesized by the condensation of 3-nitrobenzaldehyde and N,N'-dimethyl-4-aminoantipyrine. It is yellow in colour and having 76.3% yield and soluble in chloroform. The spectral data of the ligand concluded that the ligand is bidentate.

The Nickel complex of this ligand is green in colour and having 78% yield and soluble in chloroform. The molar conductance value of this complex were found to be  $44.3 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ . This value indicates that the complex is electrolytic in nature. The magnetic moment value of this complex is 4.1 BM which suggests that the complex has tetrahedral structure. The appearance of new bands at  $530.52 \text{ cm}^{-1}$  in the IR spectrum is assigned to Ni-N stretching vibration. The spectral data of the ligand concluded that the ligand is bidentate.

The oxovanadium complex of this ligand is dark green in colour and having 80% yield and soluble in chloroform. The molar conductance value of this complex were found to be  $3.8 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ . This value indicates that the complex is electrolytic in nature. The magnetic moment value of this complex is 2.01 BM which suggests that the complex has octahedral structure. The appearance of new bands at  $435.51 \text{ cm}^{-1}$  and  $918.18 \text{ cm}^{-1}$  in the IR spectrum are assigned to V-N and V=O stretching vibrations.

The UV spectra of ligand and complexes shows characteristic absorption at the range of 285 nm and at 340 nm region which confirms the presence of benzenoid and azomethine linkages, which are characteristic of  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transition respectively.

The experimental percentage values of carbon, hydrogen and nitrogen in the two complexes found out by CHN analysis were similar to the percentage calculated from the theoretical aspects. So from CHN analysis, the structure and denticity of the ligand and complex can be confirmed.

The antibacterial and antifungal studies of complexes were conducted by comparing the zone of inhibition of a standard bacterial agent with the complexes and their extend of antimicrobial activity is measured. Vanadium complex is

inactive for Gram negative Bacteria like *E. coli* and *Pseudomonas aeruginosa*. The vanadium complex is inactive for Gram positive stain *Streptococcus mutans* at the concentration of 250 and 500. But it is slightly active at the concentration of 1000. The complex shows slight activity for the Gram positive stain *Staphylococcus aureus* at 250, 500 and 1000 concentrations.

Nickel complex is inactive for Gram negative Bacteria like *E. coli* at the concentration of 250 and shows moderate activity at the concentrations of 500 and 1000. The complex is inactive for *Pseudomonas aeruginosa* at 250 concentration and shows slight activity at 500 and 1000 concentration. The nickel complex is inactive for Gram positive stain *Streptococcus mutans* at the concentration of 250 and 500. But it is slightly active at the concentration of 1000. The complex is inactive for the Gram positive stain *Staphylococcus aureus* at 250, 500 and shows slight activity at 1000 concentrations.

The standard antifungal agent used for the antifungal studies is Clotrimazole. Vanadium complex is inactive for *Aspergillus niger* fungus at the concentration of 250. But the complex is slightly active at 500 and 1000 concentration. The Nickel complex is inactive for both *Aspergillus niger* and *Candida albicans* at all concentrations of 250, 500 and 1000.

#### Acknowledgement

I acknowledge the Department of Science and Technology (DST) for providing fund under the scheme of "Fund for Improvement of S & T infrastructure in universities and higher education institutions (FIST)" for providing fund to purchase equipment in instrumentation lab and also for computer facilities in Computer and Networking Lab.

#### References

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