

PREPARATION AND ANTIMICROBIAL STUDIES OF Mn (II) AND Fe (II) COMPLEXES OF 2-(IMINE-2-ANTHRANILIC ACID)-3-HYDROXYIMINOBUTANE

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Abstract: The new schiff base ligand 2-(imine-2-anthranilic acid)-3-hydroxyiminobutane [L] and its Mn(II) and Fe(II) metal complexes were prepared by condensation of 2, 3-Butanedione 3-monoxime, anthranilic acid and metal salts. The ligand and metal complexes were characterized by elemental analysis, magnetic susceptibility measurements and spectral (I.R., UV-Visible, ¹H NMR) analysis. Based on electronic spectral data and magnetic susceptibility measurement, the octahedral geometry has proposed for both the metal complexes. The ligand and metal complexes were screened for their antimicrobial activity against microorganisms *S. aureus* (gm positive), *P. aeruginosa* (gm negative), *Aspergillus niger* and *Candida albicans* which shows moderate activity against all microorganisms as compared to the standard drugs. Metal complexes behave as good antimicrobial agent as compared to its ligand.

I. INTRODUCTION:

Metal complexes with various donar groups had attracted by many workers [1-3] due to their biological, pharmacological, clinical and analytical importance. Thiophene derivatives exhibit an array of biological activity such as antibacterial and antifungal activity, antioxidant and molecular docking study [4-7]. Piperonylamine Schiff bases metal complexes are most potent antimicrobial agents as compared to its Schiff bases [8]. Macrocyclic Schiff base metal complexes derived from 1,4-dicarbonylphenyl dihydrazide and pentane-2,4-dione also acts as growth inhibiting agents against some bacteria and fungi.[9]. There is enormous interest presently in the field of coordination chemistry of '3d' transition metals with Schiff bases. They have also been use as biological models [10], oxygen carriers and antifertile agents on male albino rats [11]. Some studies of metal complexes of diacetylmonoxime and their related ligands have been reported [12-13]. We have already reported the antibacterial and antifungal properties of 2-nitroaniline-2,3-Butanedione-3-monoxime and 3-nitroaniline-2,3-Butanedione-3-monoxime and their metal complexes [14]. In this paper we reported the study of 2-(imine 2--anthranilic acid)-3-hydroxyiminobutane L, its Mn(II) and Fe(II) complexes. The main interest in this ligand originates in its imine group containing -N-O- donar atoms.

II. Materials and methods:

All the chemicals used are of analytical grade such as 2,3-Butanedione 3-monoxime, anthranilic acid and metal chlorides etc. Ligand prepared according to the literature method [14, 16]. The solvents Ethanol, Petroleum ether, Ethyl acetate were use after distillation.

Synthesis of Schiff base ligands:

Scheme 1: The alcoholic solutions of anthranilic acid and 2,3-butanedione 3-monoxime were mix together in 1:1 molar concentration. After refluxing for about 3-4 hrs., solid crude product were form, after cooling, product were recrystallize and dried in desiccator over anhydrous CaCl_2 [4-5].

Synthesis of metal complexes

Scheme 2: A suitable ligand and metal chloride in 1:2 molar concentrations were reflux for 4-5hrs. Solid colored products were form on cooling. After recrystallization, the complexes dried over anhydrous CaCl_2 . The physical and analytical data listed in table 1.

Antibacterial activity and Antifungal activity:

The synthesized ligand and complexes tested for in vitro growth inhibitory activity against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Pseudomonas aeruginosa*. Nutrient agar plates made uniformly surface inoculated from the culture of the tested microorganisms. Well was prepared on the agar medium at the center by using cork borer. Normal saline water was use to prepare the inoculum of the bacteria to be used for the antibacterial study [10].

The synthesized ligand and complexes also screened for in vitro growth inhibitory activity against *Aspergillus niger* and *Candida albicans*. Sabouraud dextrose agar plates made uniformly and ditch made on the agar medium at the center. Two separate striking lines of two different fungi made on both the sides of ditch [6, 10]. Mueller Hint Agar Media (HI Media) used to subculture various strains of microorganisms. The lowest concentration of the compound which shows positive antibacterial and antifungal activity was subjected to dilution method for quantitative measurement of inhibitory (micro static) activity. The lowest concentration, which completely inhibited visible microbial growth, was record as the Minimum inhibitory concentration (MIC, $\mu\text{g/ml}$) [5, 9]. The stock solution of test compound (0.04mg/ml) was prepare in DMF and subsequent dilutions of stock solution made in the same solvent. The activity of DMF was check previously. Under aseptic conditions, the diluted test solutions with different concentrations added to the disc and ditch placed on the numbered plates. Then the plates were incubating at room temperature for 24 hr. During this period, the test solution diffused and the growth of the inoculated microorganisms affected. Antibacterial activity was indicating by the presence of clear inhibition zone around the well and antifungal activity indicated by the presence of inhibition zone nearer to the ditch.

III. Results and Discussion:

The complexes are stable in air but decomposed at high temperature. All compounds are easily soluble in dimethylformamide (DMF) and dimethylsulphoxide (DMSO). The physical and elemental analysis of ligand and metal complexes listed in table 1.

Table 1: Physical and Elemental analysis

Name of the compounds	% Yield Color	Melting point	μ_{eff} BM	Elemental Analysis found (calculated) %			
				C %	H %	N%	Mn%
L [$\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_3$]	90% colorless	383K	-	57.88 (60.00)	4.827 (5.45)	10.585 (12.72)	-
Mn[L ₂ .2H ₂ O]	65%	<390K	6.602	49.91	4.94	10.57	10.38
Mn[C ₂₂ H ₂₆ N ₂ O ₄]	Blue			(49.93)	(4.95)	(10.59)	(10.38)
Fe[L ₂ .2H ₂ O]	60%	<390K	4.634	49.85	4.94	10.56	10.52
Fe[C ₂₂ H ₂₆ N ₂ O ₄]	Reddish blue			(49.84)	(4.95)	(10.57)	(10.53)

Electronic Spectra and Magnetic moment:- The electronic spectrum of ligand and complexes helps to indicate the geometry. The important electronic spectral bands recorded in table 2. The spectra of strong absorption band in the range 250- 255nm in both ligand and metal complexes remain more or less at the same frequency [17] which are $\pi \rightarrow \pi^*$ charge transfer transitions. The azomethine group which is assigned as $n \rightarrow \pi^*$ transitions observed at 360 nm in the ligand shifted to lower frequencies in

both metal complexes indicating that the imine nitrogen atom is involved in coordination to the metal ion [18]. The low intensity absorption bands associated with d-d transition observed only in metal complexes supports the coordinated geometry of the metal complexes [19]. Fig. 1 indicate the electronic spectra of Fe[L₂.2H₂O] complex.

The room temperature magnetic moment values also help to indicate the octahedral geometry of metal complexes. The magnetic moment value of Mn^{II} at 6.0 BM is close to theoretical spin only value ($\mu_{\text{eff}} = 6.4$ to 6.5 BM) for Mn²⁺ (d⁵ system) and Fe^{II} at 4.636 BM is close to spin only value ($\mu_{\text{eff}} = 5.0$ -5.6 BM) for high spin d⁶ system with four unpaired electrons [20].

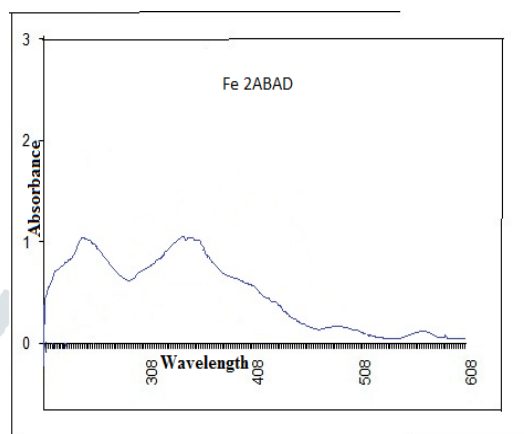


Fig.1: Electronic absorption spectra of Fe-complex

Table 2: Electronic spectral data of complexes.

Name of the compounds	$\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions and charge transitions (nm)	d-d transitions
L	254, 360	-
Mn[L ₂ .2H ₂ O]	255, 388	570, 624, 700
Fe[L ₂ .2H ₂ O]	250, 346	491, 587,

Infrared Spectra:- The I.R. spectra (Table 3) shows, complexes behave as bidentate coordinating ligand via the azomethine nitrogen (C=N*) and oxygen of oxime (-NOH) group by replacement of hydrogen ion forming six-membered ring around metal ion [13]. In the ligand azomethine (C=N*) group is at high wavenumber but in metal complexes it shifts to lower wavenumber, this indicates the imine nitrogen atom involved in coordination to the metal ion [17, 21] while oxime (C=N) remain more or less at the same position. The N-O band in ligand observed at 965cm⁻¹ but in metal complexes it shifts to higher wavenumber, this indicates the oxygen atom by replacing oxime proton involved in coordination to the metal ion [13, 19]. Acidic (O-H) in ligands and their metal complexes observed in rang 2898-2833cm⁻¹ means (-OH) of carboxylic acid does not involved in coordination or ligand formation. A broad band observed in all the complexes in rang ~3400 cm⁻¹ due to (-OH) of the coordinated H₂O. This is supported by the appearance of an additional band in rang 950-900 cm⁻¹ for (-OH) rocking deformation and 800-750 cm⁻¹ for (-OH) wagging mode of coordination which is not observed in the ligand spectrum [19]. Thus, H₂O is coordinated in metal complexes. New bands appear in the 530-520 cm⁻¹ and 450-460 cm⁻¹ assignable to the ν (M-O) and ν (M-N) resp. in metal complexes only [22, 23]. Figure 2 and 3 indicates IR spectra of ligand and its Mn^{II} complex.

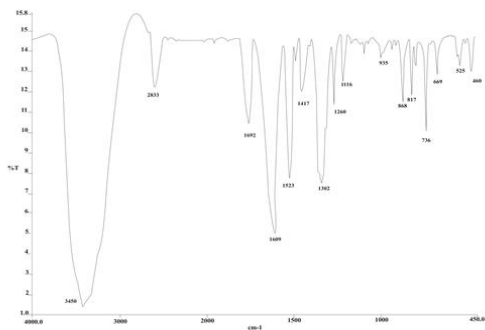


Fig.2: IR spectra of Ligand

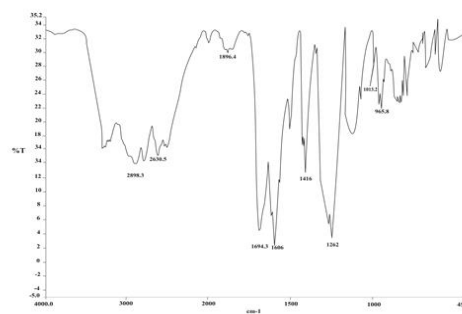
Fig. 3: IR spectra of Mn[L₂.2H₂O]

Table 3: I.R. bands of ligand and metal complexes.

Compound	$\nu(\text{C}=\text{N}^*)$	$\nu(\text{C}=\text{N})$	$\nu(\text{N}-\text{O})$	$\nu(\text{O}-\text{H})$ of (-COOH)	Coord. -H ₂ O	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{O})$
L	1602	1419	965	2898	-	-	-
Mn[L ₂ .2H ₂ O]	1609	1523	1116	2833	3450	460	523
Fe[L ₂ .2H ₂ O]	1602	1563	998	2834	3450	458	519

¹H N.M.R. analysis:- The proton nuclear magnetic resonances for ligands recorded in deuterated CDCl₃ as a solvent. In ligands a broad peak of -OH of oxime is not observed due to rapid exchange with the solvent CDCl₃ [12, 24]. There are two signals in between 1.90ppm due to presence of methyl protons in Schiff base. A peak of carboxylic -OH for L observed at 10.00ppm. Similarly, multiple signals in the region $\delta = 7.20-8.00$ ppm are assignable to aromatic protons.

Antibacterial and Antifungal activity:- The inhibitory zones are shown in Table 4. It has been observe that the metal complexes show moderate antibacterial and antifungal activity than the free ligand but less activity as compared to standard drugs. Only Mn[L₂.2H₂O] complex show inhibitory zone against for *Staphylococcus aureus* and moderate activity for *Pseudomonas aeruginosa* at higher concentrations. There is moderate to high activity of ligand and its complexes against *Aspergillus niger* as well as *Candida albicans*. Mn[L₂.2H₂O] complex shows good antibacterial and antifungal agent as compared to L and Fe[L₂.2H₂O] complex.

It has been suggest that the complexes having antimicrobial activity may act either by killing the microbes or by inhibiting multiplication of the microbes by blocking their active sites of enzymes [25]. This is also probably due to the greater lipophilic nature of the complexes. The lipid membrane that surrounds the cell favors the passage of only lipid soluble material, which controls the microbial activity. On chelation, the metal ion will be reduce largely due to the overlap of the ligand orbital and partial sharing of positive charge of the metal ion with donar groups. Further, it increases the delocalization of π -electron over the completely chelate ring and enhances the lipophilicity of the complex [26]. This lipophilicity enhances the penetration of the complex into lipid membrane and blocks the metal binding sites on enzymes of microorganisms.

Table 4: Antibacterial activity:

Name and Conc. of compound in %	<i>Pseudomonas aeruginosa</i> (mm)					<i>Staphylococcus aureus</i> (mm)				
	20	40	60	80	100	20	40	60	80	100
L	-	-	-	-	++(19)	-	-	-	-	-
Mn[L ₂ .2H ₂ O ₂]	-	+	-	++(19)	++(20)	-	-	+(16)	+(19)	+(20)
Fe[L ₂ .2H ₂ O]	-	-	-	-	++(20)	-	-	-	-	-
Ciprofloxacin	++	+	++	+++	+++	+	++	+++	+++	+++

-No activity, + 10 to 15mm (less), ++15 to 20mm (moderate), +++ above 25mm (highly active)										
Name and Conc. of compound in %	<i>Aspergillus niger</i>					<i>Candida albicans</i>				
	20	40	60	80	100	20	40	60	80	100
L	-	-	-	+	++	+	+	+	+	+
[MnL ₂ .2H ₂ O]	++	+	++	++	++	+	++	++	++	++
[FeL ₂ .2H ₂ O]	++	+	++	++	++	-	+	+	+	+
Fluconazole	++	+	++	+++	+++	+	++	+++	+++	+++

+ less activity, ++ moderate activity, +++ higher activity.

III. CONCLUSION

The ligands 2-(imine-2-anthranilic acid)-3-hydroxyiminobutane (L) and its Mn^{II} and Fe^{II} complexes had synthesized and characterized. Both the complexes exhibit octahedral geometry by involment of azomethine nitrogen and oxime oxygen. The ligand and metal complexes were screened for their antimicrobial activity against microorganisms *S. aureus* (gm positive), *P. aeruginosa* (gm negative), *Aspergillus niger* and *Candida albicans* which shows moderate activity against all microorganisms as compared to the standard drugs. Metal complexes behave as good antimicrobial agent as compared to its ligand.

IV. Acknowledgment: Authors are thankful to Department of Microbiology, Walchand College of Biotechnology, Solapur for providing biological activity facility and Chemical Science Department, IIT Mumbai, for providing elemental and spectral analysis facility.

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