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Synthesis and Bio-Spectral Studies of The Mn(II) Complex of 2´-Hydroxy-4´-Methoxyacetophenoneoxime (HMAOX)

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ABSTRACT

Mn(II) complex of 2´-hydroxy-4´-methoxyacetophenoneoxime (HMAOX) was synthesized from the paeonol oxime by using standard protocol and characterized .The stoichiometry of the complex was determined by spectrophotometric and potentiometric studies and mass spectral data which reveal a ML_2 type metal:ligand composition. The stability constant of the complex and the important thermodynamic parameters were computed from potentiometric and spectrophotometric studies, and thermal data respectively. Beer's law is obeyed in the concentration range 1-12 ppm of Mn. The value of molar extinction coefficient and sensitivity as per Sandell's scale are found to be 2.40 x 10^2 L.mol^{-1} $cm^{-1}$and 0.228 μg-Mn cm^{2-1} respectively. Limit of interference due to the presence of foreign ions in the spectrophotometric determination has also been determined. The IR studies reveal that the phenolic proton is lost on complexation and the oxygen of the phenolic (–OH) and nitrogen of the oximino (=NOH) groups coordinate with Mn(II)ion. The electronic spectra and magnetic susceptibility measurements indicate that the complex is para-magnetic and tetrahedral in nature. The antimicrobial activity of different concentrations of ligand and its Mn(II)-complex was measured by determining the growth of test fungi and bacteria by dry weight increase method and by agar diffusion method respectively against Aspergillus niger, Aspergillus flavus, Aspergillus nidulans and Alternaria alternata fungi and Staphylococcus, Streproproteus, Staph and Escherchia coli bacteria. The results indicated that this complex have good anti-microbial properties as compared to the standard drugs (fluconazole and ciproflaxacin). The activity index (AI) for the, bioactivity was also derived.

Keywords: Mn(II)-complex, Spectra, Thermodynamic parameters, Antimicrobial screening.

INTRODUCTION

Phenones and their oximes have been widely used as antiseptics, germicides, anthelmintics, analgesics[1], antituberculotis[2], against mycobacteria[3] and also show antimicrobial (antibacterial and antifungal) [4-5], antiviral[6] and antimutagenic activity[7]. Their use as herbicides is also reported [8]. Paeonol forms sulfated derivative when orally administered to rats which is excreted with urine[9]; so, no apparent toxicity was exhibited with numerous doses upto 50 mg kg^{-1}. In the last few years, there has been a great surge in the development of chelation chemistry and its use in medicine and related areas of life science research. Chelating agents containing oxygen, sulpher and nitrogen as donar atoms especially exhibit broad biological activity, and are of special interest due to their binding behaviour to metal ions[10].The presence of transition metals in human blood plasma indicates their importance in the mechanism for accumulation ,storage and transport of transition metals in living organisms and their key role in biological systems such as cell division, respiration, nitrogen fixation and photosynthesis[11]. A number of phenones have aroused considerable interest as regards to their chelating ability with transition metal ions[12-14], and their use as excellent analytical reagents[15]for gravimetric and spectrophotometric determination of transition metal ions[16-18]. The study of HMAOX and its Mn(II) complex has a peculiar importance from pharmacological point of view. So, the present communication deals with the synthesis of Mn(II)-HMAOX complex , its charecterization by elemental analyses and spectral data. and its investigation by potentiometric, spectrophotometric, mass spectral and thermal studies and magnetic susceptibility measurement. The antimicrobial activity of HMAOX and its Mn(II) complex has also been evaluated against selected bacteria and fungi by using standard protocols[22,23], and the results compared with standard drugs.

MATERIALS AND METHODS

Materials: Paeonol (PEE-ESS Aromatics, Chennai) and hydroxylamine hydrochloride (Glaxo) ltd respectively were used for the preparation of paeonol oxime. The chloride of manganese (II) ion was used as hydrated salt. Anhydrous sodium acetate, perchloric acid, sodium perchlorate and the required solvents (ethanol, dioxane, dimethylformamide, etc.) used in the work were of analytical grade, purchased commercially. The solvents used were purified / dried by recommended procedures[19].

Physical measurements: The elemental analyses were carried by Elementar Vario EL III Model, and the estimation of metal was performed by AA-640-13 Shimadzu flame atomic absorption spectrophotometer. A Systronic spectrocolorimeter (Type 103) was used for the absorbance measurements and pH measurements were made on a Systronic(335) digital pH- meter, and the values corrected by using Van Uitert and Hass equation[20]. The electronic spectrum of the complex was recorded on Beckman DU-64 spectrophotometer. The IR spectra of the ligand and its metal complex were recorded on Perkin Elmer FT-IR spectrophotometer in KBr; their NMR spectra were recorded by high performance FT-NMR spectrometer. The FAB mass spectrum of the complex was recorded at USIC facility at IIT, Roorkee. Magnetic susceptibility measurement was carried out at room temperature by using powdered sample on a vibrating sample magnetometer PAR 155 with 5000 G-field strength, using $Hg[Co(CNS)_4]$ as a calibrant.

TG curve was recorded by Rigaku Model 8150 thermo-analyzer at the heating rate of 5 ° min^{-1}. The instrument was calibrated by calcium oxalate for TG. The TG curve helped to identify the number of decomposition steps. The thermodynamic activation parameters such as E, ΔH, ΔS and ΔG were calculated from potentiometric data , using Coats and Redfern method[32].

Synthesis of paeonol oxime (HMAOX): HMAOX was prepared as reported earlier[21], and purified/dried by recommended method[19].

Isolation of Mn(II) complex of HMAOX:50 mL of 0.2M aqueous solution of $MnCl_2$ was added to 100 mL of 0.4M solution of 2´-hydroxy-4´-methoxyacetophenoneoxime in 50% ethanol, and the mixture was stirred for about an hour at room temperature. The Mn(II)-HMAOX complex separated as a dark brown precipitate within the pH range 8.0-9.0. The precipitated complex was digested, filtered and washed first with hot water and then with 25% ethanol, and finally dried at 105-110ºC in an air oven. The complex was analyzed for C,H,N and metal contents [C=53.21(53.00): H=4.93, (4.82): N=7.26(7.48) and Mn=12.75(12.99)], The results of elemental analyses revealed a 1:2 (metal:ligand) stoichiometry for the complex.The 1:2 (M:L) stoichiometry was also verified by spectrophotometric studies and FAB mass spectrum.The general composition of the complex could thus be formulated as $[Mn (C_{18}H_{20}N_2O_6)]$.

Potentiometric studies: Calvin and Bjerrum technique [27] was used to determine stability constant of the complex by evaluating $\bar{n}$, $\bar{n}$H and pL values at different temperatures, and concentrations by using standard formulae [28].

The following solutions were titrated against standard carbonate free sodium hydroxide (0.05 M) to carry out potentiometric studies:

A. 2.0 mL $HClO_4$ (0.05 M) + 4.0 mL $NaClO_4$ (1.0 M) + 4.0 mL H_2O + 30.0 mL dioxane.

B. 2.0 mL $HClO_4$ (0.05 M) + 4.0 mL $NaClO_4$ (1.0 M) + 4.0 mL H_2O + 10.0 mL ligand (0.01 M) + 20.0 mL dioxane.

C. 2.0 mL $HClO_4$ (0.05 M) + 4.0 mL $NaClO_4$ (1.0 M) + 1.5 mL H_2O + 2.5 mL metal solution (0.008 M) + 10.0 mL ligand (0.01M) + 20.0 mL dioxane.

Spectrophotometric studies: A Systronic spectrocolorimeter (Type 103) was used for the absorbance measurement while pH value was adjusted on a Systronic (335) digital pH-meter. The nature of complex was determined by Vosburg and Cooper method[30], and its composition was known by Mole ratio method.

Biological studies

Antibacterial screening: The antibacterial activitiy of the test compounds (HMAOX and its Mn-complex) was measured by paper disc diffusion method [22] using agar nutrient medium and 5mm diameter paper discs of Whatman No. 1 filter paper. The filter paper discs were soaked in a solution of known amount (0.05 to 0.40% w/v) of test compounds and a standard specimen (prepared in DMF), dried and laid on the surface of petri-plates which were already seeded with the test organism *Staphylococcus*, *Streproproteus* , *Staph or Escherichia coli*. All the agar dishes were then incubated in an incubator at 27±1ºC for about 48 hours. After incubation for the stated period, the growth of the micro-organism was studied in terms of inhibition zone (mm), formed in each disc in the form of a turbid layer, except in the region where the concentration of antibacterial agent is above the MIC. The size of the zone of inhibition depends upon sensitivity of the organism, nature of the culture medium, incubation conditions, rate of diffusion of the agent, and the concentration of the antibacterial agent.

Antifungal screening: The antifungal activity of different concentrations (0.05 to 0.40%w/v) of test compounds and a standard specimen (prepared in DMF), was measured by determining the growth of test fungi-*Aspergillus niger, Aspergillus flavus,Aspergillus nidulans and Alternaria alternata* by dry weight increase method. Richard liquid medium was used as culture medium[23] in the experiment. The test compounds of varying concentration (0.05 to 0.40% w/v) were directly added in to the Richard liquid medium carrying the test fungus in a sterilized chamber, and was kept for seven days in an incubation chamber at 27±1ºC . Media with test solution served as treated while without the test as check. The resultant mycelial mats in each set were carefully removed, washed, dried and then weighed separately. The percentage inhibition was calculated by the following formula:

$$\text{Percentage inhibition of fungal growth} = \frac{(\text{Cg} - \text{Tg}) \times 100}{\text{Cg}}$$

where, Cg = average growth in the check set, Tg = average growth in the treated set,Activity index (A.I.) was also calculated using the standard formula[5].

RESULTS AND DISCUSSION

The complexation reaction between metal ions and the ligand may be represented as

$$MnCl_2 + 2HL \rightarrow [MnL_2] + 2HCl$$

Spectrophotometric studies: Vosburg and Cooper method[30] shows that Mn(II) ion forms only one complex with HMAOX having $\lambda_{\max}$ at 410 nm in the pH range 7.0-11.0.

The absorbance was measured at room temperature at regular intervals of time up to two weeks, and also at different temperatures varying from 300 K to 325 K. The results showed that the complex is stable for one week at 318 K without any change in absorbance. The optimum pH range for the complexation was 10.0. The composition of the complex was found to be 1:2 (metal Ligand) by mole ratio method. The stability constant of the complex was calculated using the following equation:

$$K=c\ (1-\alpha)/(m\alpha c)^{m}(n\alpha c)^{n}, \alpha=E_m-E_s/E_m.$$

Absorbance measurements of a set of six solutions prepared in a similar way, and having the same concentration of all the reagents, show that the reproducibility of measurements is quite good with a standard deviation of 0.26%.

The stability constant of the complex is found to be 5.648×10^{7}, and the value of standard free energy of formation is 1.06 kcal mol^{-1} at 30ºC.

Beer's law is obeyed in the concentration range 1-12 ppm of Mn. The value of molar extinction coefficient and sensitivity as per Sandell's scale are 2.40×10^{2} L $mol^{-1}cm^{-1}$ and 0.228 µg Mn $cm^{2\ -1}$respectivly.

Effect of foreign ions: The effect of foreign ions on the spectrophotometric determination of manganese was studied by adding these ions in quantities ranging from 5 to 2000 ppm to a solution containing a known amount of manganese. After adjusting the pH of the solution at 10.0, manganese was extracted as Mn (II)-HMAOX complex in the usual manner, and the absorbance of the organic layer measured.

It was observed that 9 ppm of Mn(II) ion; 1500 ppm of Cl^{-}, SO_4^{2-} and CH_3COO^{-} ions; 1200 ppm of $NH_4^{+}, K^{+}, Na^{+}, NO_3^{-}, Br^{-}$ and I^{-} ions; 800 ppm of SO_3^{2-} and; NO_2^{-} ions ; 500 ppm of Ca^{2+}, Sr^{2+}, Ba^{2+}, citrate and tartarate ions; and 150 ppm of Zn^{2+}, Cd^{2+} and Be^{2+} ions could be tolerated. However Cu^{2+}, Pd^{2+}, Co^{2+}, Fe^{3+} and UO_2^{2+} ions interfered seriously. A limit of 2.0% change in the absorbance was observed as the limiting concentration.

Magnetic moment and Electronic spectrum: The observed magnetic moment value (5.92 B.M.) of Mn(II)-HMAOX complex indicates the presence of five unpaired electron and hence its paramagnetic nature. The d^5 configuration being spherically symmetrical, the ground state suffers no change in the stereochemistry which is a characteristic of mononuclear tetrahedral complex. The bands occurring at 17100, 18750, 20050, 21200, 22450 and 27500 cm^{-1} in the electronic spectrum of the complex correspond to the $^6A_1 \rightarrow {}^4T_1$ (G), $^6A_1 \rightarrow {}^4T_2$(G), $^6A_1 \rightarrow {}^4E$ (G), $^6A_1 \rightarrow {}^4T_2$(D), $^6A_1 \rightarrow {}^4E$(D) and $^6A_1 \rightarrow {}^4E$(P) transitions respectively. The tetrahedral nature of the complex has also been confirmed on the basis of its molar absorbance value [31].

Infrared spectra and mode of bonding: The IR spectra of metal chelate and of free ligand were recorded both in the high frequency region (650-4000cm^{-1}) and low frequency region (50-650cm^{-1}). In general, vibrations which occur in the high frequency region , originate due to the ligand itself whereas those in the lower frequency region originate due to the metal- ligand bonds (Table 1).In HMAOX, the broad band at 3280cm^{-1} has been assigned to the phenolic OH group.The band at 3240 cm^{-1} is due to =NOH group. The band at 2900cm^{-1} is due to C-H stretching vibrations, the band at 1630 cm^{-1} is due to C=N stretching, the bands at 1260 cm^{-1} and 1070 cm^{-1} are due to the presence of OCH_3 group in the benzene ring and the band at 1000 cm^{-1} is due to N-O stretching. The absence of band at 3280 cm^{-1} and a strong band of the free ligand at 1290 cm^{-1} is due to C-OH (phenolic) shift to higher frequency region in the complex which indicates deprotonation of the phenolic group, and coordination of the phenolic oxygen to Mn(II) ion. The shifting of broad and low intensity band due to ν(O-H) mode of N-OH group from 3240 cm^{-1} to 3140 cm^{-1} suggests weakening of N-OH bond due to the formation of Mn-N bond. The coordination of the oximino group through nitrogen is indicated by lowering of the C=N band from 1630 cm^{-1} in the ligand to 1600 cm^{-1} in the metal complex. Shifting of the N-O band at 1000 cm^{-1} in HMAOX to 1010 cm^{-1} in the metal complex further suggests the participation of nitrogen of the oximino group in the complexation with the formation of a Mn-N bond. In the IR spectrum of the complex, the bands observed at 610 cm^{-1} and 490 cm^{-1} are assigned to the Mn-N and Mn-O stretching vibrations [24]. A band at 1368 cm^{-1} belonging to the benzene ν(C=C) is affected on complexation showing that the ligand is coordinated to metal through the oxygen of hydroxyl group of benzene ring [25]. It is observed that the aliphatic protons are not greatly affected on complexation [26]. It is clear from the discussion that the free ligand interacts with Mn(II) ion resulting in the formation of a metal-ligand complex.

Table 1 Significant peaks of HMAOX and its Mn(II) complex in the IR spectra (value in cm-1)

HL	MnL_2	Tentative Assignment
3280	-	OH Stretching(hydrogen bonded)
3240	3140(w)	OH Stretching (N-OH)
2900(s)	2870	C-H Stretching
1630	1600(s)	C=N Stretching
1290	1298	C-O(phenolic) Stretching
1260(s),1070(m)	1205(s)	C-OCH_3 Stretching
1000	1010(s)	N-O Stretching
-	610(w)	Mn-N Stretching
-	490 (w)	Mn-O Stretching

^{1}H-NMR spectra: ^{1}H- NMR spectra of the ligand and its Mn(II) complex were recorded in $CDCl_3$. The absence of the phenolic OH proton signal (at 9.23δ) in the HMAOX)-Mn(II) complex indicates coordination of phenolic oxygen to the Mn(II) ion after deprotonation. The NMR spectral data of HMAOX and its Mn(II) complex are appended in table 2.

Table 2 ^{1}H-NMR spectral data of HMAOX and Mn(II) complex

Compound	^{1}H-NMR (ppm)	Complex	^{1}H-NMR (ppm)
HL	2.55[s,3H,-CH_3] 3.84[s,3H,-OCH_3] 6.44-7.7[m,3H,ArH] 6.54[s,1H,O-H oximino] 9.23[s, 1H,O-H phenolic]	ML_2	2.46[s,6H,-CH_3] 3.84[s,6H,-OCH_3] 6.39-7.94[m,6H,ArH] 7.20[s,2H,O-H oximino]

Mass spectra: The FAB mass spectra of Mn(II)-HMAOX complex(Fig.1) reveals its stoichiometric composition. The molecular $[M^+]$ ion peak of the complex is shown at M/Z=414/415, suggesting the stoichiometry of the complex as ML_2.

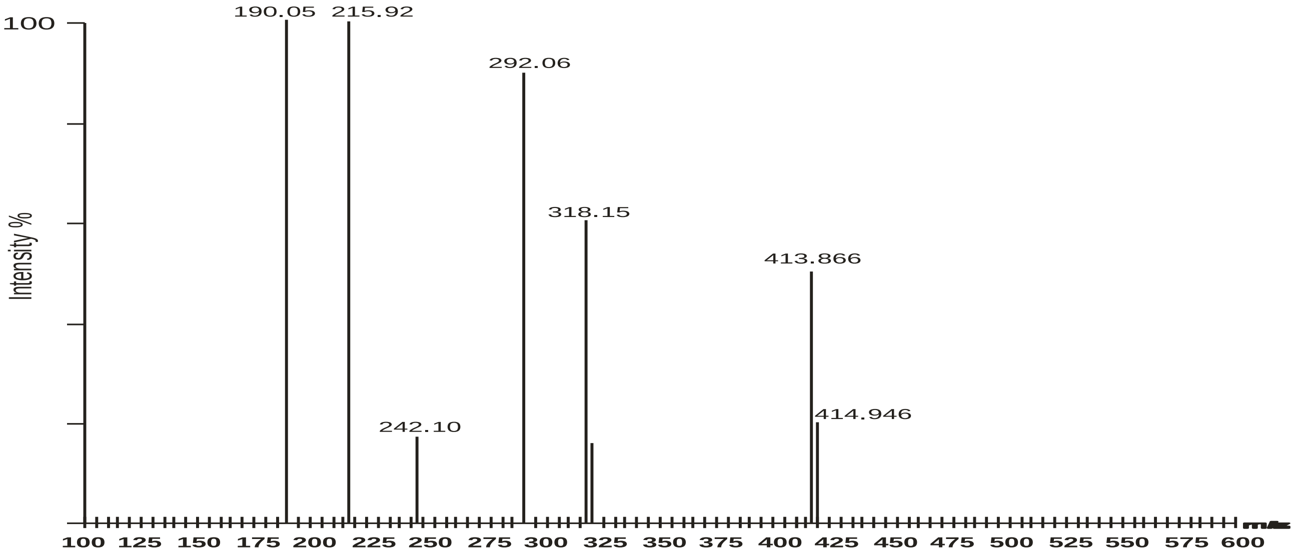


Fig.1. FAB Mass spectra of Mn(II)-HMAOX complex

Thermogravimetric studies: Thermo-gravimetric analysis (TGA) of Mn(II)-HMAOX suggests that complex is stable upto 318ºC. This indicates that the complex is not in the hydrated form. The initial decomposition shown in the TG curve was taken as a measure of the thermal stability of the complex. Sharp initial decomposition of the complex in the TG curve, is associated with a rapid loss in weight. The weight of Mn(II)-HMAOX complex decreases after decomposition, continuously upto 530ºC. On further heating, the weight of the residue remains constant and corresponds to MnO_2. The total mass loss is 79.00% (calculated value 79.04%) which is confirmed by comparing observed and calculated mass of the pyrolysis product.The kinetic parameters were calculated graphically by employing the Coats-Redfern equation[32]

$$\log[-\log(1-\alpha)/T_2]=\log[AR/\theta E^o (1-2RT/E^o)]-E^o/2.303RT$$

where, α is the mass loss up to temperature T, R is gas constant, E^o is the activation energy in J mol^{-1} , θ is the linear heating rate, and the term $(1- 2RT/E^o)=1$. A slope of the linear plot drawn between $-\log[-\log(1-\alpha)/T^2]$ and1/T gives the value of E^o as 12.54 J mol^{-1} while its intercept corresponds to A (the Arrhenius constant). Straight line of the graph confirms the first order kinetics for thermal decomposition of the complex.

Potentiometric study: Proton-ligand stability constant ($\log K_1$) was calculated from the proton-ligand formation curve (the plot between $\bar{n}$ H and pH), and metal ligand stability constant ($\log K_2$) was calculated from the formation curve (plot between $\bar{n}$ and pL)
The thermodynamic formation constants were obtained by extrapolation of the observed formation constants to zero ionic strength on the graph between log of the stability constant and $\sqrt{\mu}$.
The thermodynamic parameters ΔH, ΔS and ΔG were calculated at different temperatures (Table 3 and Table 4) using the following equations:

$$\Delta G = -2.303 RT \log K^{\mu=0}, \qquad \Delta H = 2.303 R \times (T_2 \times T_1/T_2-T_1) (\log K_2''/ K_1') \text{ and}$$

$$\Delta S = 2.303 \log K + \Delta H/T$$

Table 3 Protonation constants and the Thermodynamic parameters of HMAOX at different temperatures at μ=0.05M

Temperature K	pk_1^H	-ΔG Kcal/mol	-ΔH Kcal/mol	ΔS cal/deg/mole	θ°C	pk_m^H
293	11.46	15.28		21.60		
300	11.30	15.36	9.00	21.58	220	9.44
305	11.15	15.58		21.59		

It is noted from the data that the accrued ligand (HMAOX) behaved as a monoprotic acid due to deprotonation of the phenolic OH group ortho to the oximino group from which the proton was replaced by metal ion during complex formation. This was evident from the fact that metal titration curve was well separated from the ligand titration curve (Fig.2). The value of log β_n and log K^H decreases with the increase in ionic strength. It shows that the activity of metal ion for its interaction with other molecular species decreases with the increase in the ionic strength of the medium under consideration. The protonation constant of the ligand and stability constant of metal complex decreases with the increase in temperature. The complex has a negative entropy which indicates a more ordered activated state, compensated by enthalpies of activation leading to almost the same value for the free energy of activation[29].

The ionisation depends upon the dielectric constant ($\in$) of the medium. A solvent of low $\in$ value increases the electrostatic force between the ions, and hence facilitates the formation of molecular species resulting in the increase in pk_1^H value.

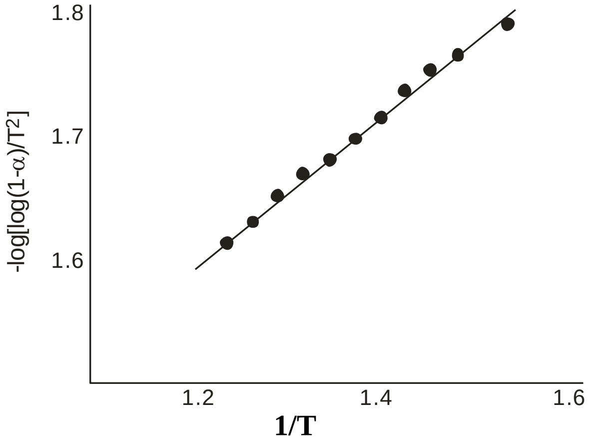


Fig.2. Kinetic linearization plot of the Mn(II)-HMAOX complex

Table 4 Stability constants and Thermodynamic parameters of Mn(II)-HMAOX complex at different temperatures in dioxane medium

	305 K			298 K			291 K			298 K		
	$Logk_1$	$Logk_2$	$Log\beta_n$	$Logk_1$	$Logk_2$	$Log\beta_n$	$Logk_1$	$Logk_2$	$Log\beta_n$	-ΔG KJ $mol^{-1}k^{-1}$	-ΔH $Jmol^{-1}$	ΔS J mol^{-1} K^{-1}
HL	11.15			11.30			11.48					
Mn(II) Complex	4.92	4.38	9.30	5.03	4.50	9.53	5.19	4.68	9.87	5.586	2965	-28. 690

APPLICATIONS

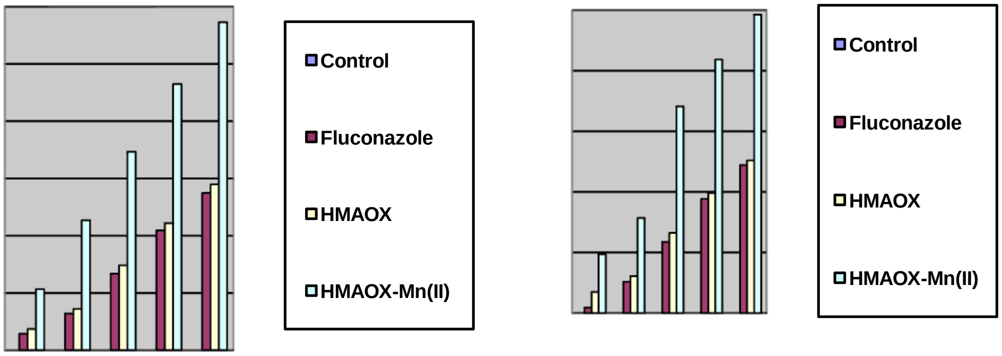
Antimicrobial activity: The fungicidal and bactericidal data of the graded concentrations (0.05 to 0.40%) of 2´-hydroxy-4´-methoxy acetophenoneoxime(HMAOX) and its Mn(II) complex against *Alternaria alternata, Aspergillus niger, Aspergillus nidulans, Aspergillus flavus* fungi *and Staphylo-coccus, Streproproteus, Staph and E.coli* bacteria are recorded in tables 5 and 6, and are displayed in the form of bar diagrams. The observed results reveal that antimicrobial activity of the compound is directly proportional to the concentration of the test compound. The activity for a given ligand or metal complex differs from fungus to fungus and from bacteria to bacteria. The Mn(II)-HMAOX complex has more antimicrobial activity as compared to the ligand (HMAOX) (Fig.3). The complex showed maximum fungicidal activity against *Alternaria 2493lternate* and the least against *Alternaria nidulans*, the over all order of fungicidal activity being *Aa > Ag > Af > An.* The complex showed maximum bactericidal activitiy against *Streproproteus* and the least against *Staph*, the overall order of antibacterial activity being *Sp > Sc > E.coli > St.*

Table 5 Antifungal activity data of HMAOX(HL) and Mn(II) complex ML_2 against Alternaria alternate, Aspergillus flavus, Aspergillus nidulans and Aspergillus niger**.**

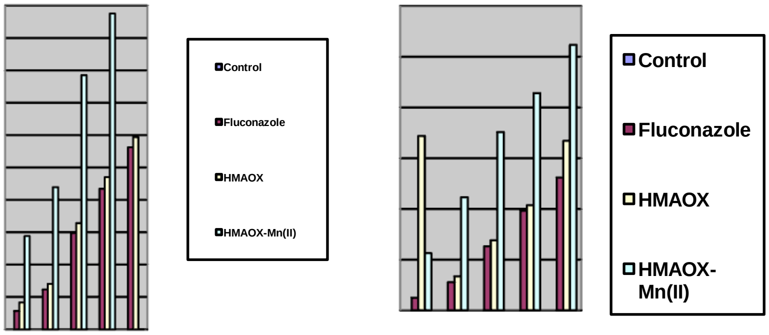
		Alternaria alternata				*Aspergillus flavus*				*Aspergillus nidulans*				*Aspergillus niger*			
Con.	% of Inhibition	Control	Drug Fluconazole	HL	ML_2	Control	Drug Fluconazole	HL	ML_2	Control	Drug Fluconazole	HL	ML_2	Control	Drug Fluconazole	HL	ML_2
0.05 %	Wt.	0.975	0.961	0.957	0.923	1.089	1.084	1.07	1.036	1.146	1.133	1.127	1.08	1.046	1.033	1.028	0.987
	%		1.435	1.846	5.33		0.459	1.744	4.86		1.134	1.658	5.76		1.24	1.72	5.64
	AI			1.286	3.71			3.80	10.58			1.46	5.08			1.39	4.55
0.10 %	Wt	0.970	0.939	0.935	0.860	1.082	1.054	1.049	0.997	1.138	1.11	1.106	1.038	1.041	1.0121	1.006	0.925
	%		3.195	3.608	11.34		2.587	3.05	7.85		2.46	2.81	8.78		2.78	3.36	11.14
	AI			1.129	3.55			1.179	3.034			1.142	3.57			1.21	4.0
0.20 %	Wt.	0.958	0.894	0.887	0.792	1.072	1.009	1.001	0.889	1.128	1.061	1.054	0.951	1.030	0.965	0.959	0.849
	%		6.68	7.41	17.327		5.87	6.62	17.07		5.94	6.56	15.70		6.31	6.90	17.57
	AI			1.109	2.59			1.128	2.91			1.104	2.64			1.09	2.78
0.30 %	Wt	0.946	0.847	0.841	0.726	1.060	0.960	0.955	0.838	1.117	1.02	1.012	0.899	1.018	0.918	0.913	0.800
	%		10.465	11.099	23.156		9.434	9.91	20.94		8.684	9.40	19.51		9.82	10.31	21.45
	AI			1.06	2.22			1.05	2.22			1.082	2.246			1.05	2.18
0.40 %	Wt	0.932	0.804	0.797	0.665	1.047	0.919	0.915	0.789	1.102	0.978	0.971	0.860	1.005	0.873	0.837	0.742
	%		13.734	14.485	28.65		12.22	12.61	24.64		11.25	11.88	21.96		13.15	16.72	26.17
	AI			1.054	2.086			1.03	2.02			1.056	1.95			1.27	1.56

Table 6. Antibacterial activity data of HMAOX [HL] and Mn(II) complex ML$_2$ against streproproteus,staph, staphylococcus and E. coli

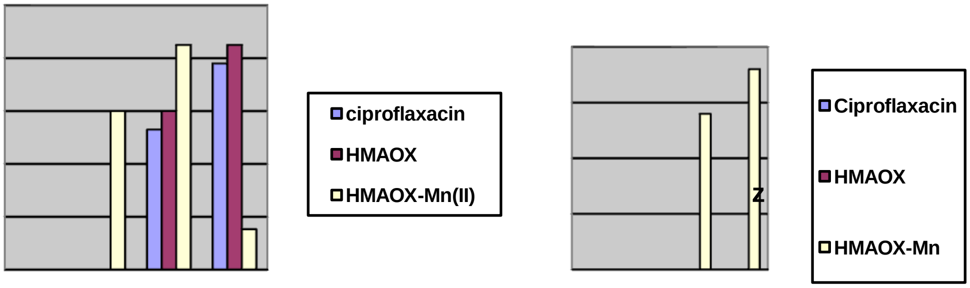
		Streproproteus [Sp]			Staph [St]			Staphylococcus [Sc]			E.coli		
Concentration	Zone of inhibition [nm]	Drug Ciproflaxin	HL	ML$_2$	Drug Ciproflaxin	HL	ML$_2$	Drug Ciproflaxin	HL	ML$_2$	Drug Ciproflaxin	HL	ML$_2$
0.05 %	Inhibition Zone	-	-	-	-	-	-	-					
	AI	-	-	-	-								
0.10 %	Inhibition Zone	-	-	6.0	-	-				5.8			6.2
	AI												
0.30 %	Inhibition Zone	5.3	6.0	8.5	-		5.6	7.10	7.8	8.2		5.3	7.0
	AI		1.13	1.060				1.09					
0.40 %	Inhibition Zone	7.8	8.5	12.0	-		7.2	7.50	8.4	11.6	6.20	7.0	9.4
	AI		1.089	1.53					1.12	1.55		1.13	1.52



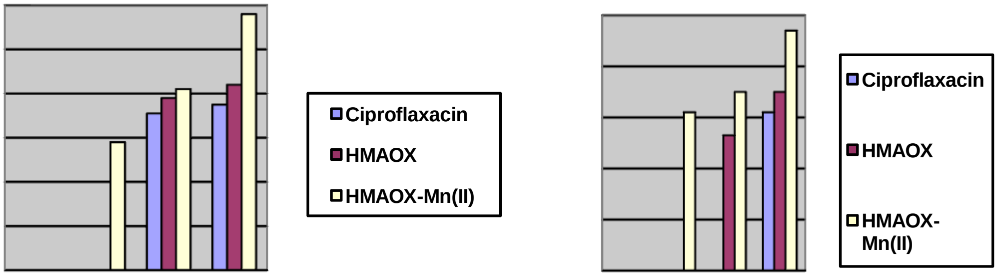
Antifungal activity of HMAOX and its Mn(II)- complex against a) Alternaria 2494lternate and b}Aspergillus flavus



Antifungal activity of HMAOX and its Mn(II)-complexagainst c)Aspergillusnidule and d)Aspergillus



Antibacterial activity of HMAOX and its Mn(II)- Complex against e) Streproproteus[Sp] and **f)** Staph



Antibacterial activity of HMAOX and Mn(II)- complex against g)Staphylococcus[Sc],h) E.coli.

Fig.3. Antibacterial activity of HMAOX and Mn(II)- complex against various Fungi

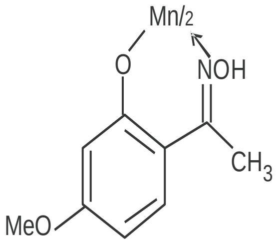
Mechanism of 'Bioactivity': The antimicrobial studies demonstrated that chelation increases antimicrobial activity. It has been suggested that metal chelation reduces polarity of metal ion mainly because of the partial sharing of its positive charge with the donar group, and the possibility of *d*-electron delocalization occurring within the chelate ring system formed on coordination. The process of chelation thus increases the lipophilic nature of the central metal atom which, in turn, favours its permeation through the lipoid layer of the membrane [33,34], and the mechanism of action is understood to be alkylation of essential cellular proteins.Thus, increase in antimicrobial activity is due to faster diffusion of the free ligand with electron withdrawing group, and metal complex as a whole through the cell membrane or due to combined activity of the ligand and metal [35]. This have been supported by the experimental findings, which suggest that the compounds having higher electron density have low antimicrobial activity.

Oxime has high antimicrobial activity as compared to semicarbazone, phenylhydrazone and phenone itself. This is attributed to the formation of dimeric and pseudomacrocyclic species by way of intermolecular hydrogen-bonding [36]. Antimicrobial properties are also found to be related to thermodynamic stability[37] and selectivity.

CONCLUSIONS

Spectrophotometric studies suggested that Mn(II) ion forms only one complex with the ligand HMAOX having the composition ML_2.The observed magnetic moment and electronic spectrum of the complex indicates the presence five unpaired electrons, pointing a mono nuclear tetrahedral geometry and paramagnetic nature.IR spectral studies indicate deprotonation of the phenolic group, and coordination of the phenolic oxygen,and participation of nitrogen of the oximino group in complexation of Mn (II) ion with HMAOX. The fact is also supported by NMR spectral data. FAB, mass spectrum of the complex reveals ML_2 stoichiometric composition for the complex

TGA of the complex reveals its thermal stability in a graded manner while potentiometric study on the complex provides data to evaluate the proton-ligand stability vis a vis metal-ligand stability. Finally, antimicrobial activity data suggest the complex to be more active than the ligand showing maximum activity against *Alternaria alternata* and *Streproproteus* and least against *Alternaria nidulans* and *Staph* respectively. Structurally, the Mn(II)-HMAOX complex can be represented as



Proposed structure of Mn(II)-HMAOX complex

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