

Research Article

Synthesis and Bio-Spectral Studies of Ni(II) Complex of 5-chloro-2,4-dihydroxy butyrophenoneoxime (CDHBOX)

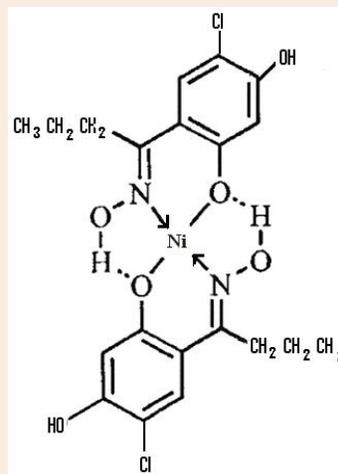
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Abstract

Ni(II) complex of 5-Chloro-2,4-dihydroxybutyrophenoneoxime(CDHBOX) was synthesized from 5-Chloro-2,4-dihydroxybutyrophenoneoxime by using standard protocol, and characterized by elemental analyses, melting point determination and spectral data. The ML2 (metal/ligand) stoichiometry of the complex was determined by spectrophotometric and potentiometric studies, and mass spectral data. The value of stability constant of the complex was found to be 6.39×10^7 while its standard free energy of formation is 10.731 kcal/mol at 27 °C. Beer's law is obeyed in the concentration range 25-150 ppm of Ni. The value of molar extinction coefficient and sensitivity as per Sandell's scale were found to be 1.8×10^2 L.mol⁻¹ cm⁻¹ and 0.450 µg Ni/cm² respectively. 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 Ni(II) ion. The electronic spectra and magnetic susceptibility measurement indicate that the complex is diamagnetic and square-planar in nature. The antimicrobial activity of different concentrations of ligand and its Ni (II)-complex has been evaluated against *Curvularia lunata*, *Fusarium oxysporum* and *Alternaria alternata* fungi and *Streptoproteus*, *Staph*, *Escherchia coli*, *Klebsella*, and *pseudomonas* bacteria. The results indicated that the ligand (CDHBOX) and its Ni(II) complex have good antimicrobial properties. The activity index (AI) for the, bioactivity was also derived.



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Introduction

Phenones and their oximes have been widely used as antiseptics, germicides, anthelmintics, analgesics[1], antituberculosics[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 up to 50 mg/kg. 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, sulphur and nitrogen as donor atoms especially exhibit broad biological activity, and are of special interest due to their binding behavior 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 CDHBOX and its Ni(II) complex has a peculiar importance from pharmacological point of view. So, the present communication deals with the synthesis of Ni(II)-CDHBOX complex, its characterization 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 CDHBOX and its Ni(II) complex has also been evaluated against selected bacteria and fungi by using standard protocols[22,23], and the results compared with standard drugs.

Experimental

Materials

Resorcinol, butyric acid, anhydrous zinc chloride, con.HCl, KMnO_4 and hydroxylamine hydrochloride (Glaxo Ltd) respectively were used for the preparation of CDHBOX. The sulphate of cobalt(II) ion was used as its 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 spectrophotometer (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 $\text{Hg}[\text{Co}(\text{CNS})_4]$ 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 2,4-dihydroxy butyrophenone(DHB)

It was prepared by condensing resorcinol (0.1 mol) with butyric acid (0.1 mol) in the presence of anhydrous zinc chloride (0.1mol). The reaction mixture was kept at $160\text{-}165^\circ\text{C}$ for half an hour. It was then cooled and poured in ice cold 50% hydrochloric acid. The precipitated product 2, 4-dihydroxy butyrophenone (DHB) was filtered, and crystallized from ethanol. The purity was checked by melting point (65°C) and TLC.

Synthesis of 5-chloro-2,4-dihydroxy butyrophenone (CDHB)

2,4-dihydroxy butyrophenone (0.1mol) was dissolved in minimum quantity of ethanol in around bottomed flask fitted with an inlet tube and a reflux condenser. Dry chlorine gas was then passed into the flask through the inlet tube maintaining the temperature of the solution between $70\text{-}80^\circ\text{C}$. The product was filtered, and crystallized from aqueous ethanol. The purity was checked by melting point (95°C) and TLC.

Synthesis of 5-chloro-2,4-dihydroxy butyrophenone oxime (CDHBOX)

CDHBOX was prepared as reported earlier [21], and purified/dried by the recommended procedure [19].

Isolation of Ni(II) complex of HMAOX

50 ml of 0.2M aqueous solution of NiSO₄ was added to 100 ml of 0.4M solution of 5-chloro-2,4-dihydroxy butyrophene oxime in 50% ethanol, and the mixture was stirred for about an hour at room temperature. The Ni (II)-CDHBOX complex separated as a green precipitate within the pH range 4.0-8.5. The precipitated complex was digested, filtered and washed first with hot water and then with 25% ethanol, and finally dried at 105-110°C in an air oven. The complex was analyzed for C, H, N and metal content [C=46.60(46.53), H=4.20(4.27), N=5.48(5.43), Cl=13.51(13.77) and Co=11.32(11.38)],

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 [C₂₀H₂₂N₂O₆Ni].

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:

- 2.0 ml HClO₄ (0.05 M) + 4.0 ml NaClO₄ (1.0 M) + 4.0 ml H₂O + 30.0 ml dioxane.
- 2.0 ml HClO₄ (0.05 M) + 4.0 ml NaClO₄ (1.0 M) + 4.0 ml H₂O + 10.0 ml ligand (0.01 M) + 20.0 ml dioxane.
- 2.0 ml HClO₄ (0.05 M) + 4.0 ml NaClO₄ (1.0 M) + 1.5 ml H₂O + 2.5 ml metal solution (0.008 M) + 10.0 ml ligand (0.01M) + 20.0 ml dioxane.

Spectrophotometric studies

A Systronic spectrophotometer (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 using standard protocol [31].

Biological studies

Antibacterial screening

The antibacterial activity of the test compounds (CDHBOX and its Ni-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 *Streptoproteus*, *Staph*, *Escherichia coli*, *Klebsella*, and *pseudomonas coccus*. All the agar dishes were then incubated in an incubator at 27±1°C for about 48 hours. After incubation for the stated period, the growth of the micro-organism was measured 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 *Curvularia lunata*, *Fusarium oxysporum* and *Alternaria alternata* by dry weight increase method. Richard liquid medium was used as culture medium [23] in

the experiment. The test compound of varying concentration (0.05 to 0.40% w/v) was 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 $27 \pm 1^\circ\text{C}$. Media with test solution served as treated while those without it 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:

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

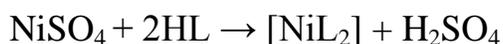
where, C_g = average growth in the check set

T_g = 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



Spectrophotometric studies

Vosburg and Cooper method[30] shows that Ni(II) ion forms only one complex with HMAOX having λ_{max} at 410 nm in the pH range 4.0-11.0. 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 8.0. It was also found that a four fold excess of the reagent was necessary to attain the maximum colour intensity.

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 value of stability constant of the complex was found to be 6.39×10^7 and the standard free energy of formation is 10.731 kcal/mol at 27°C . Beer's law is obeyed in the concentration range 25-150 ppm of Ni. The value of molar extinction coefficient and sensitivity as per Sandell's scale were found to be $1.8 \times 10^2 \text{ L.mol}^{-1} \text{ cm}^{-1}$ and $0.450 \mu\text{g Ni/cm}^2$ respectively.

3.1.1 Effect of foreign ions

The effect of foreign ions on the spectrophotometric determination of nickel was studied by adding these ions in quantities ranging from 50 to 2000 ppm to a solution containing a known amount of nickel. After adjusting the pH of the solution at 10.0, nickel was extracted as Ni (II)-CDHBOX complex in the usual manner, and the absorbance of the organic layer measured. It was observed that 50 ppm of Ni(II) ion; 2000 ppm of Cl^- , SO_4^{2-} and CH_3COO^- ions; 1500 ppm of NH_4^+ , K^+ , Na^+ , NO_3^- , Br^- and I^- ions; 850 ppm of SO_3^{2-} and NO_2^- ions; 500 Ca^{2+} , Sr^{2+} , Ba^{2+} , citrate and tartarate ions; and 200 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 2.5% change in the absorbance was observed as the limiting concentration.

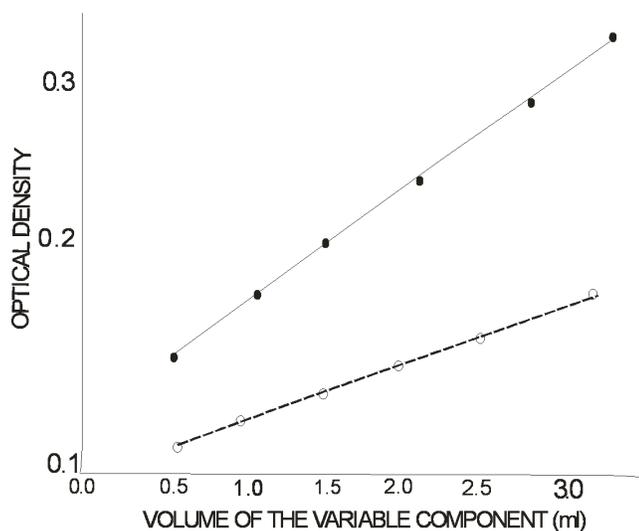


Figure 1 Composition of the Ni (II)–CHDBOX Complex by Slope Ratio Method

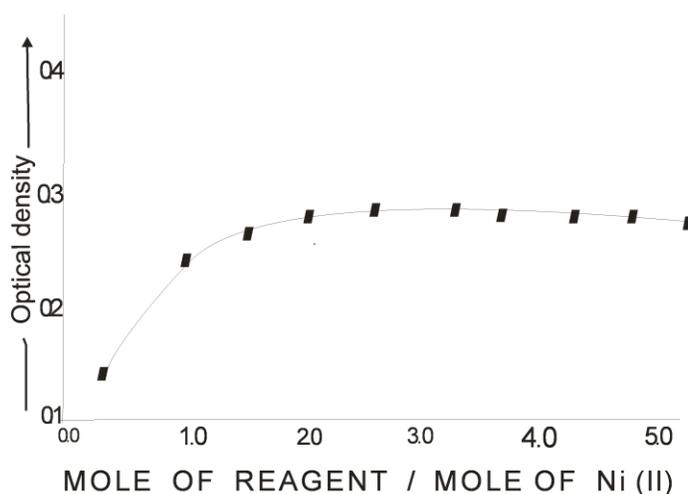


Figure 2 Composition of the Ni (II)–CHDBOX Complex by Mole Ratio Method

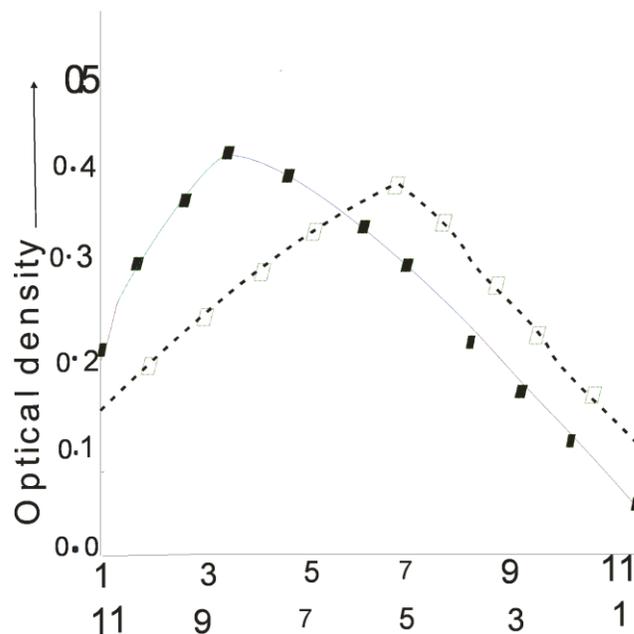


Figure 3 Composition of the Ni (II)–CHDBOX Complex by Job's Method

Effect of foreign ions

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Magnetic moment and Electronic spectrum

The observed magnetic moment value (2.42 B.M.) of Ni(II)-CDHBOX complex indicates the present complex is diamagnetic and has square-planer geometry. The bands occurring at 17070, 18070, and 29580 cm^{-1} in the electronic spectrum of the complex correspond to the ${}^1A_1 \rightarrow {}^1A_2 (G)$, ${}^1A_1 \rightarrow {}^1B_1(G)$, and ${}^1A_1 \rightarrow {}^1E_1 (G)$ transitions respectively. The square planar nature of complex could readily be distinguished from tetrahedral or octahedral geometry by the fact that no transition occurs below 10000 cm^{-1} . This situation arises as a consequence of the large separation between the $d_{x^2-y^2}$ orbital and next lower orbital which is invariably greater than 10000 cm^{-1} .

Infrared spectra and mode of bonding

Table 1 Significant peaks of CDHBOX and its Ni (II) complex in the IR spectra (value in cm^{-1})

HL	NiL ₂	Tentative Assignment
3320	-	OH Stretching(hydrogen bonded)
3270	3240(m) 3160(s)	OH Stretching (N-OH)
2970(s) , 2870(m)	2980(s)	C-H Stretching
1670	1650(s)	C=N Stretching
1290	1298	C-O(phenolic) Stretching
760(s)	790(s)	C-Cl Stretching
900	1010(s)	N-O Stretching
-	645(w)	Ni-N Stretching
-	4705 (w)	Ni-O Stretching

The IR spectra of metal chelate and of free ligand were recorded both in the high frequency region (650-4000 cm^{-1}) and low frequency region (50-650 cm^{-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 CDHBOX, the broad band at 3320 cm^{-1} has been assigned to the phenolic OH group. The band at 3270 cm^{-1} is due to =NOH group. The band at 2970 cm^{-1} and 2870 cm^{-1} are due to C-H stretching vibrations, the band at 1670 cm^{-1} is due to C=N stretching, the bands at 760 cm^{-1} are due to the presence of C-Cl stretching and the band at 990 cm^{-1} is due to N-O stretching. The band at 1190 cm^{-1} further confirms benzene ring substitution. The absence of band at 3320 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 Ni(II) ion. The shifting of broad and low intensity band due to $\nu(O-H)$ mode of N-OH group from 3270 cm^{-1} to 3240 cm^{-1} suggests weakening of N-OH bond due to the formation of Ni-N bond. The coordination of the oximino group through nitrogen is indicated by lowering of the C=N band from 1670 cm^{-1} in the ligand to 1650 cm^{-1} in the metal complex. Shifting of the N-O band at 990 cm^{-1} in CDHBOX 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 Ni-N bond. In the IR spectrum of the complex, the bands observed at 645 cm^{-1} and 475 cm^{-1} are assigned to the Ni-N and Ni-O stretching

vibrations [24]. A band at 1368 cm^{-1} belonging to the benzene $\nu(\text{C}=\text{C})$ 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 above discussion that the free ligand interacts with Ni(II) ion resulting in the formation of a metal-ligand complex.

¹H-NMR spectra

¹H-NMR spectra of the ligand and its Ni(II) complex were recorded in CDCl_3 . The absence of the phenolic OH proton signal (9.23δ) in the CDHBOX-Ni(II) complex indicates coordination of phenolic oxygen to the Ni(II) ion after deprotonation. The NMR spectral data of HMAOX and its Ni(II) complex are appended in **Table 2**.

Table 2 ¹H-NMR spectral data of CDHBOX and Ni(II) complex

Compound	¹ H-NMR (ppm)	Complex	¹ H-NMR (ppm)
HL	2.85[s,3H,-CH ₃] 6.64-7.9[m,3H,ArH] 6.67[s,1H,O-H oximino] 8.64[s, 1H,O-H phenolic]	ML ₂	2.78[s,6H,-CH ₃] 6.58-8.16[m,6H,ArH] 7.92[s,2H,O-H oximino]

Mass spectra

The FAB mass spectra of Ni(II)-CDHBOX complex reveals its stoichiometric composition. The molecular $[\text{M}^+]$ ion peak of the complex is shown at $\text{M}/\text{Z}=427/433$, suggesting the stoichiometry of the complex as ML_2 [**Figure 4**].

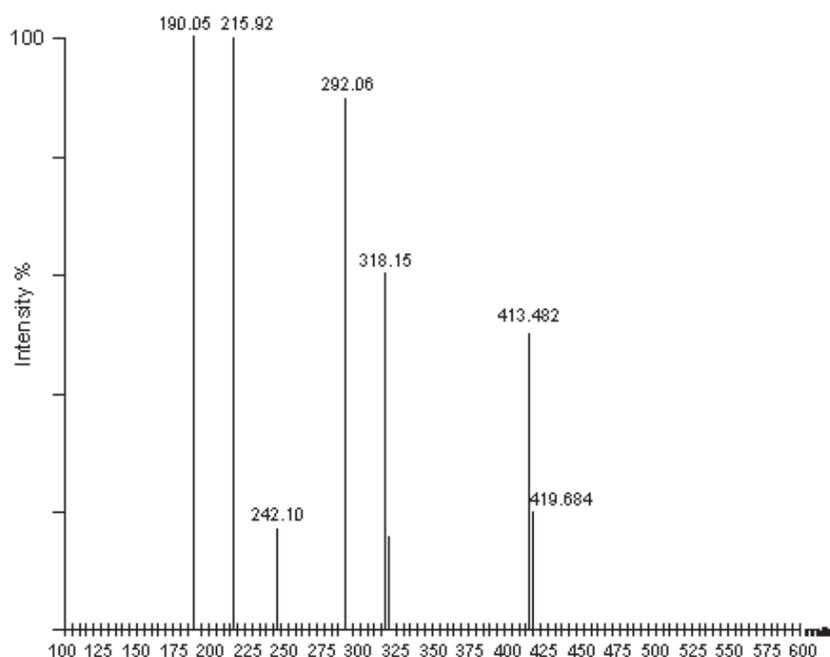


Figure 4 FAB Mass spectra of Ni(II)-CDHBOX complex

Thermogravimetric studies

Thermo-gravimetric analysis (TGA) of Ni (II)-CDHBOX suggests that complex is stable up to 300°C. 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 Ni (II)-CDHBOX complex decreases after decomposition, continuously up to 715°C. On further heating, the weight of the residue remains constant and corresponds to NiO. The total mass loss is 85.50% (calculated value 85.58%) 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]

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

where, α is the mass loss up to temperature T, R is gas constant, E° is the activation energy in Jmol^{-1} , θ is the linear heating rate, and the term $(1-2RT/E^\circ)=1$. A slope of the linear plot drawn between $-\log[-\log(1-\alpha)/T^2]$ and $1/T$ gives the value of E° while its intercept (the Arrhenius constant) gives the value of A (Arrhenius constant) as straight line of the graph confirms the first order kinetics for thermal decomposition of the complex(**Figure 5**)

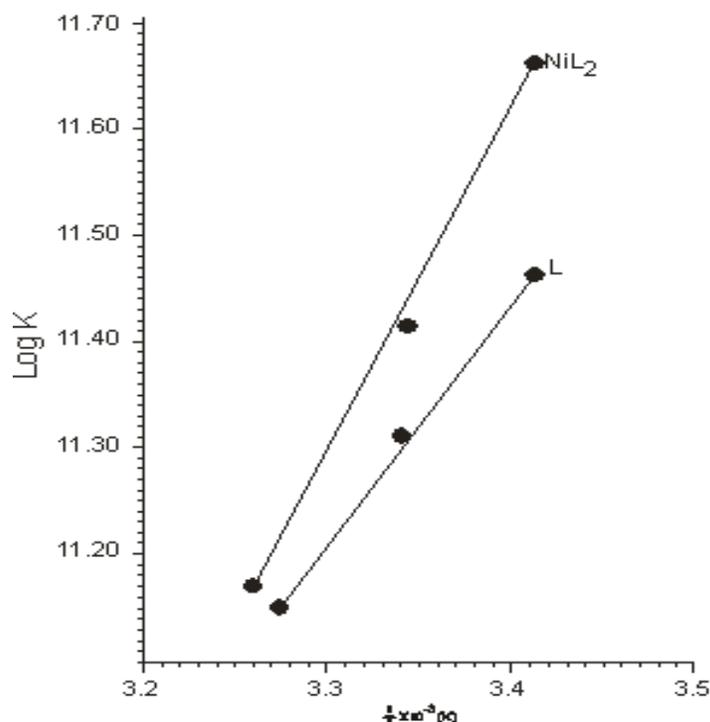


Figure 5 Kinetic linearization plot of the Ni (II)-CDHBOX complex

Potentiometric study

Proton-ligand stability constant ($\log K_1$) was calculated from the proton-ligand formation curve (the plot between \bar{n} and pH), and metal ligand stability constant ($\log K_2$) was calculated from the formation curve the 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 $\sqrt{\mu}$. [Figure 6]

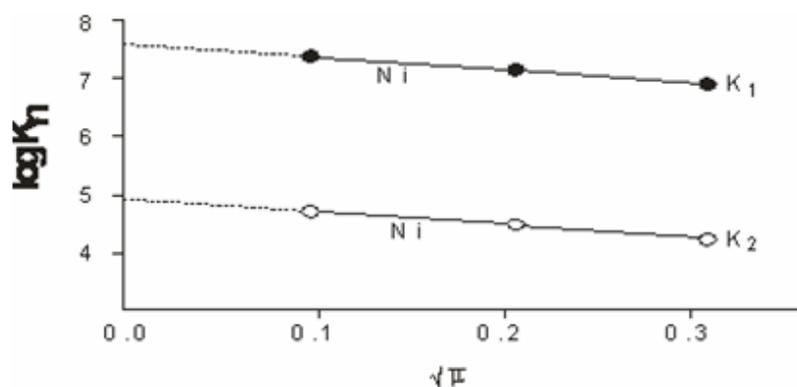


Figure 6 Extrapolation of $\log K_n$ to zero ionic strength for Ni (II) - CDHBOX interaction

The thermodynamic parameters ΔH , ΔS and ΔG were calculated at different temperatures (Table 3 and Table 4) using the following equations:

$$\Delta G = -2.303 RT \log K_{\mu=0}, \quad \Delta H = 2.303 R \times (T_2 \times T_1 / T_2 - T_1) (\log K_2'' / K_1'') \quad \text{and} \quad \Delta S = 2.303R \log K + \Delta H/T$$

Table 3 Protonation constants and the Thermodynamic parameters of CDHBOX at different temperatures at $\mu=0.1M$

Temperature K	pK_1^H	$-\Delta G$ Kcal/mol	$-\Delta H$ Kcal/mol	ΔS cal/deg/mole	$\theta^\circ C$	pK_m^H
293	12.00	16.11		26.80		
300	11.86	16.30	8.26	26.82	205	10.10
305	11.70	16.51		26.81		

Table 4 Stability constant and Thermodynamic Parameters of Ni(II) -CDHBOX complex in Dioxane medium at different Concentrations.

Conc.	$\mu = 0.1 M$			$\mu = 0.05 M$			$\mu = 0.01 M$		
	$\log K_1$	$\log K_2$	$\log \beta_n$	$\log K_1$	$\log K_2$	$\log \beta_n$	$\log k_1$	$\log K_2$	$\log \beta_n$
HL	11.86		11.86	11.92		11.92	12.13		12.13
Ni(II) complex	6.13	5.16	11.29	6.26	5.24	11.50	6.38	5.33	11.71

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. The value of $\log \beta_n$ 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 (ϵ) of the medium. A solvent of low ϵ 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.

Antimicrobial activity

The fungicidal and bactericidal data of the graded concentrations (0.05 to 0.40%) of CDHBOX and its Ni(II) complex against *Curvularia lunata*, *Fusarium oxysporum* and *Alternaria alternata* fungi and *Streptoproteus*, *Staph*, *Escherchia coli*, *Klebsella*, and *pseudomonas* bacteria, are recorded in **Tables 5 and 6**, and are displayed in the Bar diagram.

Table 5 Antifungal Activity data of CDHBOX(HL) and Ni(II) complex ML₂ against *Alternaria alternate*, *Curvularia lunata*, and *Fusarium oxysporium*

Con.	% of Inhibition	Alternaria alternate			Curvularia lunata			Fusarium oxysporium		
		Control	HL	ML ₂	Control	HL	ML ₂	Control	HL	ML ₂
0.05 %	Wt.	1.158	0.681		2.172	1.121		2.229	1.2	
	%		41.15	57.09		43.89	58.82		42.43	58.18
	AI									
0.10 %	Wt.	1.150	0.473	0.338	2.165	0.862		2.221	1.0	
	%		58.82	70.58		60.15	77.46		52.91	74.86
	AI			1.199						
0.20 %	Wt.	1.080	0.401	0.263	2.155	0.784		2.211	0.8	
	%		62.86	75.59		63.59	80.98		63.06	78.16
	AI			1.202						
0.30 %	Wt.	1.064	0.353		2.143	0.538		2.2	0.6	
	%		66.67	81.16		74.87	85.72		68.62	86.94
	AI									
0.40 %	Wt.	1.042	0.242		2.13	0.468		2.185	0.2	
	%		76.76	85.13		78.02	89.24		87.17	99.02
	AI									

Table 6 Antibacterial Activity data of CDHBOX and Ni(II) complex ML₂ against *Klebsella*, *Staph*, *Streproproteus*, *Pseudomonas coccus* & *E. Coli*.

Concentration	Zone of Inhibition [mm]	Klebsella		Staph		Streproproteus		Pseudomonas coccus		E. Coli	
		HL	ML	HL	ML ₂	HL	ML ₂	HL	ML ₂	HL	ML ₂
.05%	Inhibition Zone										
.10%	Inhibition Zone										
.15%	Inhibition Zone				7.4						
.20%	Inhibition Zone	9.0	8.0	8.0	11	6.0	7.2				
.30%	Inhibition Zone	10.	13.	10	12	8.0	11.0			6.0	8.0
.40%	Inhibition Zone	6	0								
		12	17	12	16	12.0	14.8	5.6	7.0	8.2	10.2

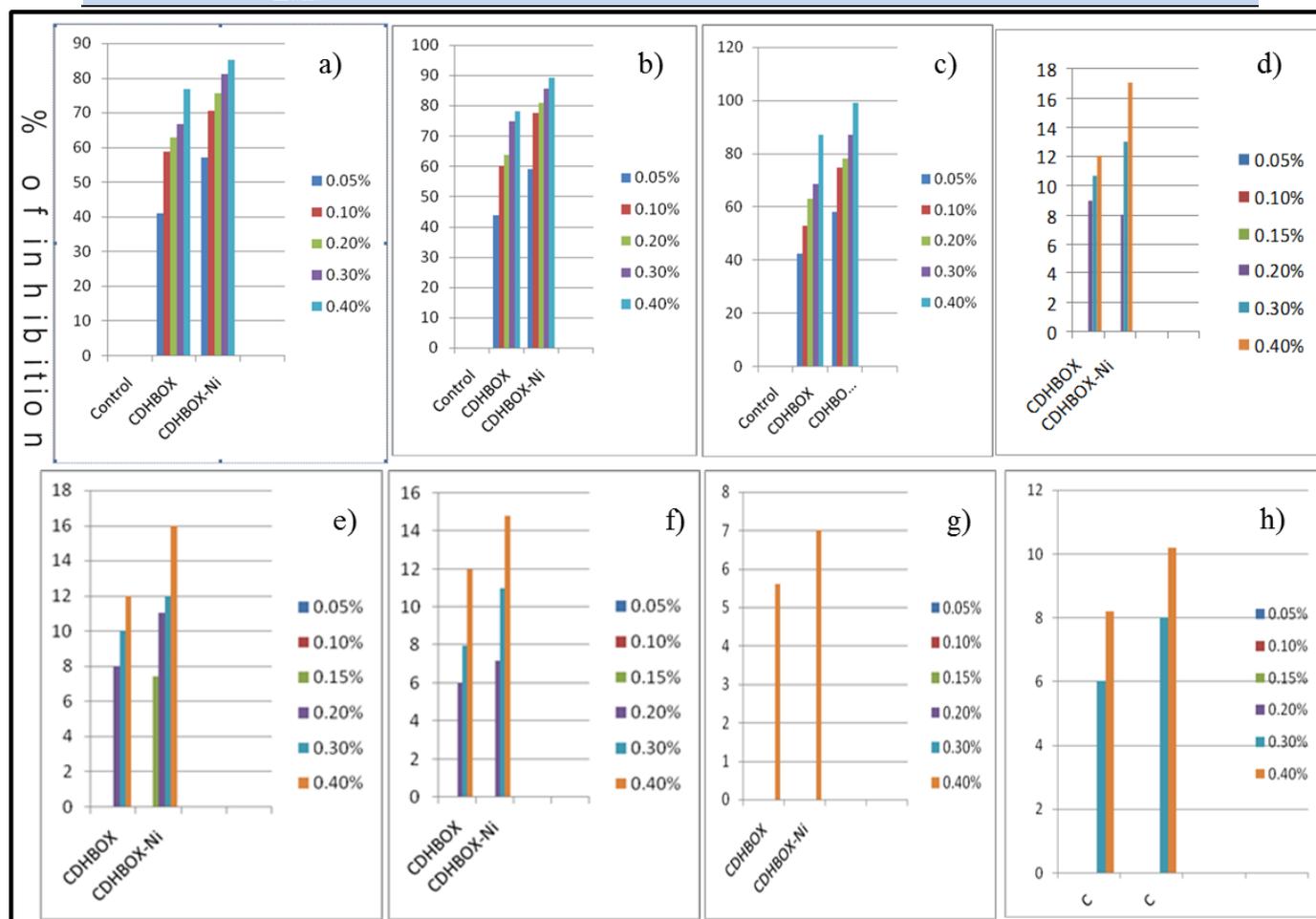
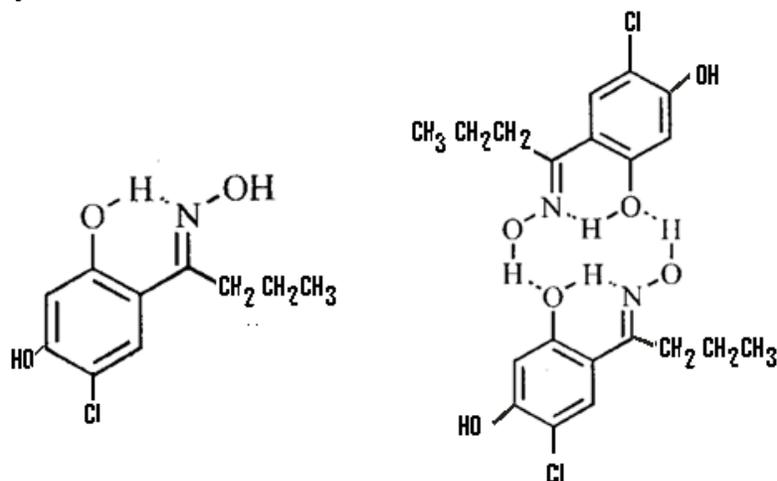


Figure 7 Antifungal Activity of CDHBOX and its Ni (II) Complex against (a) *Alternaria alternate*, (b) *Curvularia lunata*, (c) *Fusarium oxysporium*, (d) *Klebsella*, (e) *Staph*, (f) *Streproproteus*, (g) *E.Coli*, (h) *Pseudomonas coccus*.

The observed results (displayed in the form of bar diagrams **Figure 7-14**) 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 Ni (II)-CDHBOX complex has more antimicrobial activity as compared to the ligand (CDHBOX). The complex showed maximum fungicidal activity against *Fusarium oxysporum* and the least against *Alternaria alternata*, the over all order of fungicidal activity being $Aa > Ag > Af > An$. The complex showed maximum bactericidal activity against *Klebsella*, and the least against *Escherchia coli*, the over all order of antibacterial activity being $Kl > St > St > E.coli$.

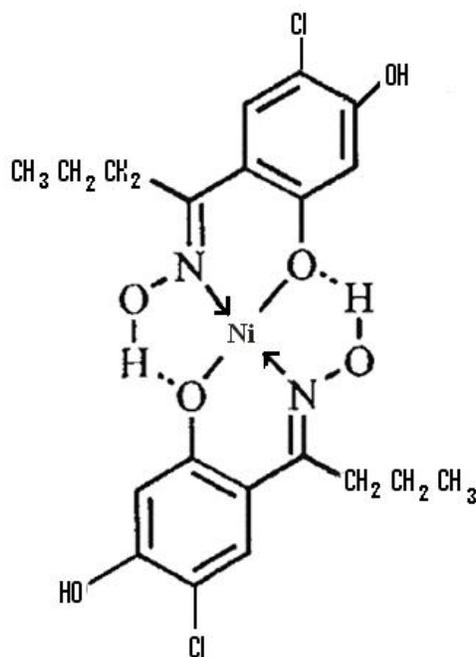
Mechanism of 'Bioactivity'



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 donor 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 lipid 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 has 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.

Conclusion

Spectrophotometric studies suggested that Ni(II) ion forms only one complex with the ligand CDHBOX having the composition ML_2 . The observed magnetic moment and electronic spectrum of the complex point to a mono nuclear square-planar geometry and diamagnetic 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 Ni (II) ion with CDHBOX. 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 *Fusarium oxysporum* and *Klebsella* and the least against *Alternaria alternate* and *E. Coli*. respectively. Structurally, the Mn(II)-CDHBOX complex can be represented as given below.



Proposed structure of Ni(II)-CDHBOX complex

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