세자리 리간드의 전이금속 착물에 대한 합성과 전위차 및 분광학적 확인
그리고 미생물학적 연구

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Synthesis, Potentiometric, Spectral Characterization and Microbial Studies of Transition Metal Complexes with Tridentate Ligand

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요약. o-phenylene diamines, dehydroacetic acid (DHA) 및 p-chloro benzaldehyde에서 유도된 세자리 Schiff 염기 리간드인 4-hydroxy-3(1-{2-(benzylideneamino)-phenylimino}-ethyl)-6-methyl-2H-pyran-2-one (HL)의 Cu(II), Ni(II), Co(II), Mn(II) 및 Fe(III) 착물의 형성상수와 항미생물 활성과의 관계를 연구하였다. 리간드와 착물은 원소분석, 전도도, 자기수자율, 열분석, X-선 회절, IR, ^{1}H-NMR, UV-vis 및 질량 스펙트럼으로 특성조사를 하였다. 분석데이터로부터 착물들의 화학량론비가 1:2 (금속:리간드)임을 알았다. 금속 착물들의 몰 전도도 값은 이들의 비전해질 성질을 의미한다. X-선 회절 데이터에서 Ni(II) 착물은 단사정계 그리고 Cu(II) 및 Co(II) 착물은 삼사정계 결정계임을 규명하였다. IR 스펙트럼 데이터로부터 리간드는 중심금속에 대해 ONN 주개원자 배열의 세자리 리간드로 행동함을 알았다. 열적 행동 (TG/DTA)과 Coats-Redfern 법에 의해 계산한 반응속도 파라메터는 착물형성 과정에서 좀 더 질서 있는 활성화 상태를 제안하고 있다. 착물의 양성자화 상수를 THF:물 (60:40) 용액, 25 ^{o}C 및 이온세기 μ = 0.1 M (NaClO$_4$)에서 전위차법으로 측정하였다. *Staphylococcus aureu* 및 *Escherichia coli.*에 대한 항박테리아 활성을 시험관에서 조사하였다. 또한 *Aspergillus Niger* 및 *Trichoderma*에 대한 항세균 활성도 조사하였다. 금속 이온 및 착물의 안정도가 항미생물학적 활성에 미치는 영향을 고찰하였다.

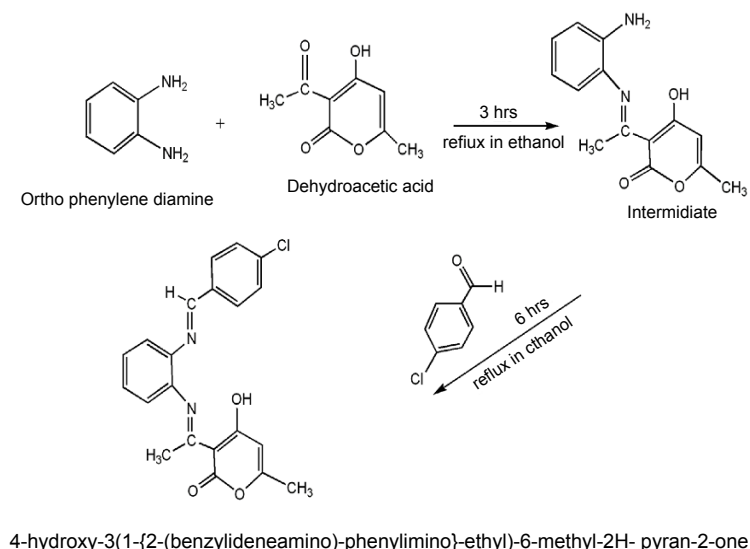**주제어:** 세자리 리간드, Schiff 염기, 금속 착물, 항미생물학적 활성, 전위차법

ABSTRACT. A relation between antimicrobial activities and the formation constants of solid complexes of Cu(II), Ni(II), Co(II), Mn(II) and Fe(III) with tridentate Schiff base ligand, 4-hydroxy-3(1-{2-(benzylideneamino)-phenylimino}-ethyl)-6-methyl-2H-pyran-2-one (HL) derived from o-phenylene diamines, dehydroacetic acid (DHA) and p-chloro benzaldehyde have been studied. The ligand and metal complexes were characterized by elemental analysis, conductivity, magnetic susceptibility, thermal analysis, X-ray diffraction, IR, ^{1}H-NMR, UV-vis and mass spectra. From the analytical data, the stiochiometry of the complexes was found to be 1:2 (metal:ligand) with octahedral geometry. The molar conductance values suggest the nonelectrolytic nature of metal complexes. The X-ray diffraction data suggests monoclinic crystal system for Ni(II) and orthorhombic crystal system for Cu(II) and Co(II) complexes. The IR spectral data suggest that the ligand behaves as tridentate ligand with ONN donor atoms sequence towards central metal ion. Thermal behavior (TG/DTA) and kinetic parameters calculated by Coats-Redfern method suggests more ordered activated state in complex formation. The protonation constants of the complexes were determined potentiometrically in THF:water (60:40) medium at 25 ^{o}C and ionic strength μ = 0.1 M (NaClO$_4$). Antibacterial activities in vitro were performed against *Staphylococcus aureu* and *Escherichia coli. A*ntifungal activities were studied against *Aspergillus Niger and Trichoderma*. The effect of the metal ions and stabilities of complexes on antimicrobial activities are discussed.

Keywords: Tridentate ligand, Schiff bases, Metal complexes, Antimicrobial activity, Potentiometry

INTRODUCTION

3-acetyl-4-hydroxy-6methyl-2H-pyran-2-one (dehydro-acetic acid or DHA) is widely used as fungicide,[1] herbicide and preservative which shows promising antimicrobial activities against bacteria, yeast and particularly molds.[2] The

4-hydroxy-3(1-{2-(benzylideneamino)-phenylimino}-ethyl)-6-methyl-2H- pyran-2-one

Synthesis Scheme of Ligand

copper complexes of dehydroacetic acid are more active than dehydroacetic acid or copper salts.[3] The structural and interesting biological activities of DHA appeals to inorganic chemists working in the field of coordination chemistry. Schiff bases and their metal complexes exhibit a wide range of biological activities and various structural features. The metal complexes of Schiff bases derived from diamines and heterocyclic compounds have been the centre of attraction for many workers in recent years.[4-7] Dehydroacetic acid is one of such heterocyclic compound. Literature survey reveals that little attention has been given to tridentate Schiff bases of DHA containing ONN donor systems. Considering the enormous importance of DHA, its metal complexes and in continuation of our earlier work,[8-11] we are reporting in this communication, synthesis and characterization of tridentate Schiff base ligand derived from o-phenylene diamines, dehydroacetic acid (DHA) and p-chloro benzaldehyde. The solid complexes of Cu(II), Ni(II), Co(II), Mn(II) and Fe(III) with this ligand have been prepared and characterized by different physicochemical methods. Stability constants of these complexes have also been determined potentiometrically. The structure- microbial activity correlation of Schiff base and its metal complexes is discussed on the basis of their stability constants.

EXPERIMENTAL

DHA (purity $\geq$ 99%) was purchased from Merck and used as supplied. o-Phenylenediamine and p-chloro benzaldehyde

of AR grade were used for synthesis of ligand. AR grade metal chlorides were used for complex preparation. AR grade solvents were used for spectral measurements. The carbon, hydrogen, nitrogen contents were determined on Perkin Elmer (2400) CHNS analyzer. IR spectra in the range of 4000 - 400 cm^{-1} were recorded on Jasco FT IR 4100 spectrometer using KBr pellets. ^{1}H-NMR spectra of the ligand was recorded in CDCl$_3$ using TMS as an internal standard. The TG/DTA and XRD were recorded on Perkin Elmer TA/SDT-2960 and Philips 3701 respectively. The UV-vis spectra of the complexes were recorded on Jasco UV-530 Spectrophotometer. Magnetic susceptibility measurements of the metal complexes were done on Guoy balance at room temperature using Hg[CO(SCN)$_4$] as calibrant. Molar conductance of complexes was measured on Elico CM-180 conductometer using 1 mM solution in dimethyl sulphoxide. Elico digital pH meter (model LI-127) equipped with a CL-51B combined electrode was used for pH measurements. The pH meter was calibrated against standard buffers (pH 4.02 and 9.18) before measurements. pH meter readings were corrected for organic-aqueous media. Titrations were performed in a double walled glass cell in an inert atmosphere (nitrogen) at ionic strength of 0.1 M (NaClO$_4$) and the solutions were titrated pH-metrically against (0.2 N) NaOH. The system used for potentiometric measurements and calculations has been described in our earlier publication.[12]

Synthesis of Ligand
The ligand was prepared by modification of reported

method.[13-15] 50 mL solution of 0.001 mol (0.168 g) of DHA, 0.001 mol (0.168 g) of o-phenylenediamine in ethanol was refluxed for 3 h and mono Schiff base thus obtained was refluxed for 6 h with 0.001 mol (0.112 mL) of p-chloro benzaldehyde in super dry ethanol. The precipitate formed was filtered, dried in vaccum over $CaCl_2$ and recrystallised in ethanol (yield: 72%).

Synthesis of Metal Complexes

To a hot methanolic solution (0.02 mol) of ligand in (25 mL) methanolic solution (25 mL) of metal chloride (0.01 mol) was added with constant stirring. The pH of reaction mixture was adjusted to 7.5 ~ 8.5 by adding 10% alcoholic ammonia solution and refluxed for about 3 h. The precipitated solid metal complex was filtered off in hot condition and washed with hot methanol, petroleum ether (40 ~ 60 ^oC) and dried over calcium chloride in vaccum dessicator (yield 65%).

Potentiometric Study

THF-water (60:40) solution was used as a solvent for potentiometric studies. THF has advantage of low dielectric constant (7.52 as compared to 78.30 for water at 25 ^oC) thus preventing decomposition of the metal chelates. THF used in the present investigation was obtained from Merck was further distilled by known literature method.[16] All metal ion solutions were prepared from their AR grade metal chlorides and standardized by known literature methods. The initial ionic strength of solutions was maintained at 0.1 M by adding sodium perchlorate solution. Initially, the pH meter was standardised at 25 ^oC using appropriate buffer solutions of pH 4.01 and 9.12, equilibrated for 30 minutes before measurements. NaOH solution of (0.2 M) was prepared in doubly distilled water and standardised against oxalic acid solution. $HClO_4$ solution (0.2 N) was prepared and standardised against NaOH solution. The ionic strength was maintained at (0.1 M) by adding appropriate amount of $NaClO_4$. Stock solution of ligand (0.1 M) and metal ion (0.01 M) were prepared in THF and water respectively.

RESULT AND DISCUSSION

The analytical data of ligand and metal complexes is given in *Table* 1. The elemental analysis show 1:2 (metal:ligand) stoicheometry for the complexes. It corresponds well with the general formula [ML$_2$] where M = Cu(II), Ni(II), Co(II), Mn(II) and Fe(III). The magnetic susceptibilities of all complexes at room temperature are found to be consistent with octahedral geometry. The metal complex solutions in DMSO show low conductance which support their non electrolyte nature.

^{1}H-NMR Spectrum of Ligand

The ^{1}H NMR spectrrm of the ligand was recorded in CDCl$_3$. It shows following signals at 2.12 δ, (s, C6-CH$_3$), 2.55 δ (s,3H,N=C-CH$_3$),5.84 δ (s,1H,C5-H),7.4 to 8.0 (m, 8H, phenyl), 8.8 δ (s, 1H, N=C-H) and 15.88 δ (s, 1H, enolic OH of DHA moiety).

FT IR Spectra

The IR spectrum of free ligand shows characteristic bands at 3059 ~ 3300, 1697, 1663, 1356 and 1213 cm^{-1} assignable to intramolecular hydrogen bonded (υ OH), lactone carbonyl (υ C=O), azomethine (υ C=N), arylazomethine (υ C-N) and phenolic (υ C-O) stretching modes respectively.[17,18] In the IR spectra of metal complexes, the absence of weak broad band in the range of 3059 - 3300 cm^{-1} region indicates deprotonation of the intramolecular hydrogen bonded OH group on complexation. This is supported by upward shift in phenolic (υ C-O).[19] On complexation the (υ C=N) band is shifted to lower wave number with respect to free ligand denoting that the nitrogen of azomethine group is coordinated to the metal ion. This is supported by upward shift in (υ C-N) values. The IR spectra of metal complexes showed new bands in 457 - 540 cm^{-1} region which can be assigned to υ (M-O) and (M-N) stretching vibrations respectively.[20] From the above facts it is evident that the coordination takes place *via* azomethine nitrogen and phenolic OH of the ligand molecule.

Electronic Absorption Spectra and Magnetic Measurements

The magnetic and electronic spectral data is given in *Table* 2. The electronic spectra of Cu(II) complexes in DMSO show bands at 13586, 16181 and 25380 cm^{-1} assignable to a $^2Eg \rightarrow ^2T_{2g}$ and charge transfer transition respectively. The electronic spectral data coupled with observed magnetic moment 1.87 B. M. suggest octahedral geometry for Cu(II) complex.[21] Ni (II) complex display three bands at 9398, 14836 and 25906 cm^{-1} assignable to $^3A_{2g} \rightarrow ^3T_{2g}(F)$, $^3A_{2g} \rightarrow ^3T_{1g}(F)$ and, $^3A_{2g} \rightarrow ^3T_{1g}(P)$ transitions respectively. These electronic transitions along with magnetic moment 3.1 B. M. suggest octahedral geometry for Ni (II) complex.[22,23] The Co(II) complex shows three transitions at 10834, 18796 and 27472 cm^{-1} assignable to $^4T_{1g}(F) \rightarrow ^4T_{1g}(P)$, $^4T_{1g}(F) \rightarrow ^4A_{2g}$ and $^4T_{1g}(F) \rightarrow ^4T_{1g}(P)$ transitions respectively. These transitions and observed magnetic moment 4.56 B. M. indicate high spin octahedral geometry of the complex.[24,25]

Table **1.** Analytical and physical data of the compounds

Compound	Empirical formula	Colour	Contents (found/calcd) %				M	$\Lambda\ \Omega^{-1}cm^{-1}\ cm^2$
			C	H	N	Cl		
HL	$C_{21}H_{17}N_2O_3Cl$	Cream white	66.20/66.23	4.40/4.49	7.30/7.35	9.40/9.30	-	-
CuL_2	$C_{42}H_{32}N_4O_6Cl_2Cu$	Green	66.90/66.87	3.70/3.91	6.50/6.80	8.70/8.61	7.60/7.71	13.02
NiL_2	$C_{42}H_{32}N_4O_6Cl_2Ni$	Yellow	62.50/61.64	3.70/3.94	6.90/6.84	8.70/8.66	7.20/7.17	26.05
CoL_2	$C_{42}H_{32}N_4O_6Cl_2CO$	Brown	61.70/61.62	3.90/3.94	6.75/6.84	8.60/8.66	7.20/7.19	21.04
FeL_2	$C_{42}H_{32}N_4O_6Cl_2Fe$	Brown	61.80/61.85	3.90/3.95	6.90/6.87	8.75/8.69	6.50/6.84	12.02
MnL_2	$C_{42}H_{32}N_4O_6Cl_2Mn$	Brown	61.80/61.95	3.70/3.95	6.90/6.87	8.50/8.70	6.50/6.74	10.02

Table **2.** Electronic absorption and magnetic susceptibility data of metal complexes

Compound	Magnetic moment (μ_{eff}) B. M.	Frequency in cm^{-1} (ϵ in L $mol^{-1}cm^{-1}$)	Band assignment	Geometry
HL	-	31347 (531)	-	-
		33670 (412)		
CuL_2	1.87	13586 (32)		Octahedral
		16181 (55)	$^2E_g \rightarrow\ ^2T_{2g}$	
		25380 (311)	Charge transfer	
NiL_2	3.10	9398 (11)	$^3A_{2g}(F) \rightarrow\ ^3T_{2g}(F)$	
		14836 (16)	$^3T_{1g}(F) \rightarrow\ ^3T_1g(F)$	Octahedral
		25906 (1454)	$^3A_{2g}(F) \rightarrow\ ^3T_{1g}(P)$	
CoL_2	4.56	10834 (19)	$^4T_{1g}(F) \rightarrow\ ^4T_2\ 1$	
		18796 (46)	$^4T_{1g}(F) \rightarrow\ ^4A_{2g}(F)$	Octahedral
		27472 (1269)	$^4T_{1g}(F) \rightarrow\ T_{1g}\ (P)$	
FeL_2	5.84	13227 (21)	$^6A_{1g} \rightarrow\ ^4T_1(D)$	
		19305 (31)	$^6A_{1g} \rightarrow\ ^4T_{1g}$	Octahedral
		32051 (1254)	$^6A_{1g} \rightarrow\ ^4T_{2g}$	
MnL_2	5.98	13812 (0.70)	$^6A_{1g} \rightarrow\ ^4T_1$	
		24154 (75)	$^6A_{1g} \rightarrow\ ^4T_{2g}$	Octahedral
		26315 (178)	$^6A_{1g} \rightarrow\ ^4Eg$	

Fe(III) complex exhibits bands at 13227, 19305 and 32051 cm^{-1} assignable to $^6A_{1g} \rightarrow\ ^4T_1(D)$, $^6A_{1g} \rightarrow\ ^4T_1$ and $^6A_1 \rightarrow\ ^4T_{2g}$, respectively. The observed magnetic moment 5.84 B. M along with electronic transitions corresponds to octahedral geometry.[26-29] In case of Mn(II) complex the observed magnetic moment 5.98 B. M and the spectral bands at 13812, 24154 and 26315 cm^{-1} assignable to $^6A_{1g} \rightarrow\ ^4T_1(G)$, $^6A_{1g} \rightarrow\ ^4T_{2g}$ (G) and $^6A_{1g} \rightarrow\ ^4E_g$ transitions respectively, indicate octahedral geometry of the complex.[30,31] The calculated values of ligand field splitting energy (10 Dq), Racah interelectronic repulsion parameter (B), covalent factor (β), ratio υ_2/υ_1 and ligand field stabilization energy (LFSE) data of metal complexes given in *Table* 3 supports the proposed geometry for
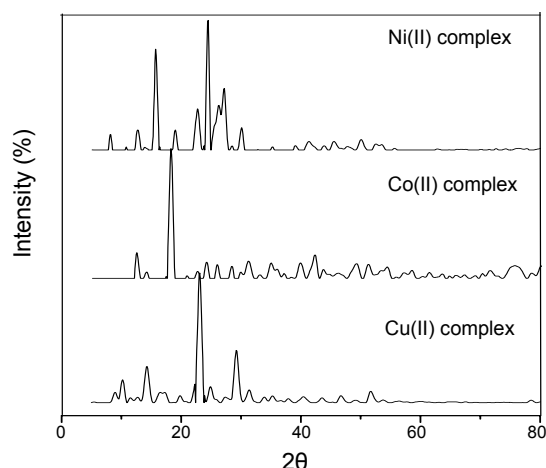
all the complexes.

Powder X-ray Diffraction

The X-ray diffractograms of Cu(II), Ni(II) and Co(II) complexes were scanned in the range 5 ~ 100° at wavelength 1.543 Å and are given in *Fig*. 1. The diffractogram and associated data depict 2θ values for each peak, relative intensity and interplanar spacing (d-values). The diffractogram of Cu(II) complex shows seven reflections with maxima at 2θ = 23.07 corresponding to d value 3.851 Å. The diffractogram of Co(II) complex had eleven reflections with maxima at 2θ = 18.11° corresponding to d value 4.89 Å ,where as the diffractogram of Ni(II) complex had thirteen reflections

Table **3.** Ligand field parameter of metal complexes

Compound	Ligand field splitting energy Dq cm^{-1}	Racah Interelectronic repulsion parameter B cm^{-1}	Covalent factor (β)	% β	ν_2/ν_1	Ligand field stabilization energy LFSE kcal mol^{-1}
CuL$_2$	1351.3	96.06	-	-	-	38.512
NiL$_2$	936.32	843.57	0.8103	23.40	1.584	26.685
CoL$_2$	1016.26	913.97	0.9412	6.23	1.832	28.963
FeL$_2$	126582	788.63	0.7769	22.30	1.496	36.075
MnL$_2$	1385.042	648.04	0.6743	32.56	1.760	39.473

Fig. **1.** X-ray diffractograms of Cu(II), Co(II) and Ni(II) complexes.

with maxima at 2θ = 24.35° corresponding to d value 3.651 Å. The X-ray diffraction pattern of these complexes with respect to major peaks having relative intensity grater than 10% have been indexed by using computer programme.[32] The above indexing method also yields Miller indices (hkl), unit cell parameters and unit cell volumes. The unit cell of Cu(II) complex yielded values of lattice constants a = 15.40 Å, b = 10.46 Å, c = 5.70 Å and unit cell volume V = 920.49 (Å).[3] Co (II) complex yielded values of lattice constants a = 11.34 Å, b = 7.12 Å, c = 4.88 Å and unit cell volume V = 394.25 (Å).[3] The unit cell of Ni(II) complex yielded values of lattice constants, a = 12.18 Å, b = 6.32 Å, c = 7.75 Å and unit cell volume V = 539.17 (Å).[3] In concurrence with these cell parameters of these complexes, the condition such as a ≠ b ≠ c and α = β = γ = 90° required for the compound to be orthorhombic were tested and found to be satisfactory for Cu(II) and Co(II) complexes. The condition a ≠ b ≠ c and α = γ = 0° ≠ β required for the compound to be monoclinic was tested and found to be satisfactory for Ni(II) complex. Hence it can be concluded that Cu(II), Co(II) shows orthorhombic crystal system whereas Ni(II) complex show monoclinic crystal system. Experimental density values of the complexes were determined by using specific gravity

method[33] and found to be 2.6637, 2.965 and 2.5374 gcm^{-3} for Cu(II), Co(II), and Ni(II) complexes respectively. By experimental density values, molecular weight of complexes, Avogadro's number, volume of the unit cell, the number of molecules per unit cell were calculated by using equation ρ = nM/NV and was found to be two, one, two for Cu(II), Co(II), and Ni(II) complexes respectively. With these values, theoretical density were computed and found to be 2.673, 2.956, 2.517 gcm^{-3} for respective complexes. Comparison of experimental and theoretical density value shows good agreement within the limits of experimental error.[11]

Thermal Analysis

The Cu(II), Ni(II) and Co(II) complexes were chosen for thermal study. The TG/DT analysis of metal complexes was carried from ambient temperature to 1000 °C in nitrogen atmosphere using α-Al$_2$O$_3$ as reference. The TG/DTA curves of these complexes show no weight loss up to 270 °C indicating the absence of coordinated water molecules.[34] In TG curve of copper complex, the first step decomposition in the range 280 to 375 °C with a mass loss of 51.20% (calcd. 51.60%) and an exothermic peak ΔT$_{max}$ = 350 °C in DTA may be attributed to decomposition of non coordinated part of ligand. The second step decomposition in the range 460 to 700 °C with mass loss 37.20% (calcd. 37.70%) corresponds to decomposition of coordinated part of ligand. finally stable metal oxide CuO corresponding to mass 9.62% (calcd. 9.80%) was obtained. In TG curve of cobalt complex first step decomposition in the range 280 to 350 °C with mass loss 60.95% (calcd. 61.07%) corresponds to decomposition of non coordinated part of ligand. In DTA, corresponding endothermic peak at 290 °C was obtained. Second step decomposition in the range 360 ~ 890 °C with mass loss of 38.80 (calcd. 38.92%) corresponds to the decomposition of non coordinated part of ligand .A broad exothermic peak in DTA was obtained at 398 °C. Finaly stable nickel oxide was formed which corresponds to 9.10% (calcd. 9.16%). In case of nickel complex, first step decomposition in the range 275 to 375 °C with mass loss of 51.60% (calcd. 51.90%)

Table **4.** Kinetic parameters of metal complexes

Complex	Steps	Decomposition. Temperature (°C)	Activation energy(E_a) kJmol^{-1}	Free energy change(ΔG), (kJmol^{-1})	Entropy change(ΔS) JK^{-1}mol^{-1}	Frequency factor (Z), S^{-1}	Correlation coefficient
CuL$_2$	I	325	27.66	42.40	-213.84	81.07	0.9807
	II	575	9.64	37.67	-274.89	7×10^{-2}	0.9942
NiL$_2$	I	350	9.66	9.66	-262.417	2.563×10^{-1}	0.9996
	II	625	18.33	18.53	-260.120	4.812×10^{-1}	0.9945
CoL$_2$	I	310	25.48	41.81	-223.34	27.47	0.9963
	II	650	18.14	47.43	-262.47	3.79×10^{-1}	0.9959

Table **5.** Sability constants of the complexes at 25 ± 0.1 °C, $\mu = 0.1$ NaClO$_4$ and protonation constants of Schiff base pk$_1$ = 3.01, pk$_2$ = 9.97

Compound	CuL$_2$	NiL$_2$	CoL$_2$	FeL$_2$	MnL$_2$
log k$_1$	9.97	9.94	9.93	9.75	9.90
log k$_2$	7.47	6.34	3.24	2.99	3.08
log β	17.44	16.28	13.17	12.74	12.98

corresponds to decomposition of non coordinated part of ligand. A sharp exotherm at 370 °C was obtained in the DTA curve. Second step decomposition from 390 to 820 °C with mass loss 38.02% (calcd. 38.93%) may be attributed to decomposition of coordinated part of ligand. The residue at the end of the decomposition of complex corresponds to stable metal oxides with mass 9.12% (calcd. 9.15%).
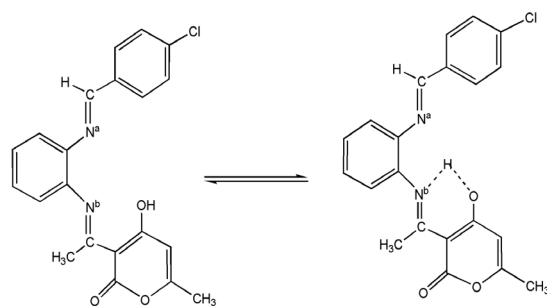
Kinetic Calculations

The kinetic and thermodynamic parameters for decomposition of metal complexes have been determined by Coats-Redfern method[35] and given in *Table* 4. The calculated free energy of activation is relatively low indicating the autocatalytic effect of metal ion on thermal decomposition of metal complexes.[36,37] Negative ΔS values indicate more ordered activated states that may be possible through chemisorptions of oxygen and other decomposition products. The more ordered nature may be due to the polarization of bonds in activated state which might happen through charge transfer electronic transitions.

Potentiometry

Calvin-Bjerrum pH titration technique as modified by Irving and Rossotti was used to determine proton- ligand and metal-ligand stability constants in THF-water mixture (60% v/v) at a constant temperature 25 °C. Irving and Rossotti method was used to calculate the $\overline{n}_A, \overline{n}$, pK and pL values from the pH titration curves. The constants of ligand and metal complexes are given in the *Table* 5.

In the present ligand, protonation takes place in the initial stages of titrations because of the presence of azomethine

Fig. 2. Resonance structure of the ligand.

nitrogen. The pK$_1$ and pK$_2$ were determined at $\overline{n}_A = 1.5$ and 0.5 respectively. The first stability constant pK$_1$ therefore refers to the imine nitrogen. However there are two imine nitrogens present in the ligand and only one pK value is displayed by it, suggesting that out of two nitrogens, one is not involved in protonation. This may be attributed to the intramolecular hydrogen bonding of nitrogen N^b (*Fig.* 2) which undergo chelation with enolic OH of dehydroacetic acid moiety and hence does not take part in protonation and deprotonation. Therefore the pK value shown by the ligand is due to the protonation of nitrogen N^a. The second pK observed as 9.97 in the ligand is due to the dissociation of enolic proton which is sufficiently acidic due to the resonance effect. All the metals form 1:1 and 1:2 chelates with this ligand. The 1:1 and 1:2 chelates are formed in a simultaneous process in all the systems. Hence, the method of least squares has been invariably used for getting accurate values of log k$_1$ and log k$_2$. These values are reported in *Table* 5.

Table **6.** Antibacterial activity of compounds (diameter of inhibition zone in mm) and antifungal activity weight in mg (% inhibition) of the ligand and complexes

Compound	Antibacterial activity				Antifungal activity			
	E.Coli		*Staphylococcus*		*Aspergillus Niger*		*Trichoderma*	
	500ppm	1000ppm	500ppm	1000ppm	500ppm	1000ppm	500ppm	1000ppm
Ciprofloxin/Control	30	35	32	34	75	72	65	60
HL	15	17	09	11	65 (13)	60 (17)	45 (31)	27 (55)
CuL$_2$	30	32	20	24	40 (47)	10 (86)	30 (54)	20 (67)
NiL$_2$	10	16	15	17	42 (44)	13 (82)	32 (51)	22 (63)
CoL$_2$	22	24	16	22	45 (40)	18 (75)	36 (45)	24 (60)
FeL$_2$	25	27	17	23	53 (29)	20 (72)	40 (38)	25 (58)
MnL$_2$	27	28	18	23	54 (28)	22 (69)	45 (31)	27 (55)

Coordination of metal ion with ligand takes place *via* protonated nitrogen and oxygen of the enolic group. The order of stability constants is Cu > Ni > Co > Fe > Mn which is in agreement with Irving -Williams order.[38]

Antimicrobial Activity

The antibacterial activity of ligand and metal complexes were tested in vitro against bacteria *staphylococcus* *aureus* and *Escheria coli* by paper disc method.[19] The compounds were tested at 500 and 1000 ppm concentrations in DMSO. The 10mm diameter Whatmann No.1 paper discs were soaked in different solutions of compounds, dried and then placed on the lawn cultures on nutrient agar plates. The plates were incubated for 24 h at 37 °C and the inhibition zone around each disc was measured. The results obtained were compared with known antibiotics, ciprofloxin. For antifungal activity, compounds were screened against *Aspergillus Niger* and *Trichoderma* by mycelia dry weight method[39] with glucose nitrate media. The compounds were tested at the concentration 500 to 1000 ppm in DMSO and compared with control. Three replicates were taken and the average values are given in *Table* 6. The results of antibacterial studies show that the metal complexes exhibit enhanced inhibitory effect than the free ligand against the same organisms under identical conditions. Increased activity of metal chelates can be explained on the basis of chelation theory.[40] The biological activity of free ligand is enhanced in presence of the metal.[41] This is a consequence of the increased lipid solubility of the metal complex as compared to the parent ligand. Transport of both metal and ligand across lipophilic membranes to vital intramolecular sites is favored by chelation. Once intracellular, the fully coordinated complex or one of its derivatives, including the dissociated metal or ligand may be the active entity.

For each class of microorganism, higher antibacterial activity shown by Cu(II) complex may be because of the higher stability of Cu(II) chelate than the other complexes. Other metal complexes, except Ni(II) complex, also show that activity is enhanced to approximately same extent irrespective of their stability constants. Activity was found to be increased with the increasing concentration of complexes which may be due to the effect of metal ions on normal process of cell. The toxicity of metal chelates follow the order Cu > Mn > Fe > Co > Ni. This indicates that the antibacterial activity is not in accordance with stability order of metal ions.

Investigation of antifungal activity of the ligand and its metal complexes revealed that all metal chelates are more fungi toxic than their parent ligand *Table* 6. The antifungal activity of the ligand is found to enhance several times on being coordinated with metal ions. Antifungal activity of these complexes is found to be increased as the stability of the complex increased. The activity of these complexes follow the order Cu > Ni > Co > Fe > Mn which is exactly same as the order of stability constants of these complexes. Comparison of the activities of ligand, its metal chelates shows that the copper complex is approximately four-five times active as ligand against *Aspergillus niger*. Activity of ligand against *Trichoderma* also increases after chelation with Cu(II), Ni(II), Co(II), Mn(II) and Fe(III). This high antifungal activity of ligand and its metal complexes may be attributed to the chloro substituent present in the ligand.[42,43]

CONCLUSION

On the basis of physicochemical and spectral data discussed above, octahedral geometry for Cu(II), Ni(II), Co(II), Mn(II) and Fe(III) complexes is proposed. From IR spectra it is assumed that the ligand behaves as ONN tridentate, coordinating *via* phenolic oxygen and imino nitrogen as illustrated in *Fig*. 2. Thermal study reveals that the complexes are thermally stable. The XRD study suggests monoclinic crystal

system for Ni(II) and orthorhombic crystal system for Cu(II) and Co(II) complexes. The antimicrobial activity of the complexes is more as compared to ligand. Antibacterial activity shows that the copper complex is more biologically active in all complexes. Antifungal activity of these complexes is found to be increased in the similar order of increase in the stability constants of metal complexes .

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