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Synthesis, Characterisation and Biological Activity of Chiral Mixed Ligand Ni(II) Complexes

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Abstract

Chiral Mixed Ligand (CML) metal complexes of the type [M(PMINAP)(aa)·2H$_2$O], where M is Ni(II), PMINAP is sodium salt of p-methoxyisonitrosoacetophenone and aa is a chiral amino acid have been synthesized. The present CML metal complexes could also be synthesized from racemic amino acids by in situ stereoselective complexation. The metal complexes have been characterized by elemental analysis and various physico-chemical techniques. The bonding and structure of the complexes are discussed in detail on the basis of the results of various studies. The metal complexes have been screened for their biological activity against selected microbial strains.

Keywords: Nickel, mixed ligand complexes, biological activity.

Introduction

Mixed ligand complexes plays an important role in numerous chemical and biological systems like water softening, ion exchange resin, electroplating, dying, antioxidant, photosynthesis in plants, removal of undesirable and harmful metals from living organisms. Many of these metal complexes shown good biological activity against pathogenic microorganisms[1-7]. The mixed ligand complexes are suitable for mimicking the role of metal ions, detoxification mechanism and drug designing. The ternary complexes play a decisive role in the activation of enzymes and also in the storage and transport of active substances. The ternary transition metal complexes have shown catalytic activity and have also shown biological activity[8-9]. Ternary complexes containing an amino acid as a secondary ligand are of significance as they are potential models for enzyme-metal ion substrate complexes. The present paper reports the synthesis, characterization and biological activity of chiral mixed ligand Ni(II) complexes of sodium salt of p-methoxyisonitrosoacetophenone as primary ligand and various chiral amino acids as secondary ligands.

Material and Methods

Most of the chemicals used were of Analytical Grade. The nickel(II) sulphate heptahydrate used without further purification while sodium salt of p-methoxyisonitrosoacetophenone was prepared by the method reported in the literature[10]. The chiral amino acids such as L-alanine, L-valine, L-leucine, L-methionine, L-phenylalanine and Racemic amino acids were obtained from THOMAS BAKER. Solvents like ethanol, DMF, DMSO whenever used were distilled and purified according to standard procedures[11]. The bacterial and fungal subcultures were obtained from the Haffkine Institute, Mumbai. The nickel content in the complexes was determined gravimetrically as [Ni(DMG)$_2$] as per standard methods[12]. The elemental analysis were carried out at the microanalytical laboratory Sophisticated Analytical Instrument Facility (S.A.I.F) I.I.T., Mumbai. The molar conductance values were measured in DMF solution of 10^{-3} M concentration on a CM-180 Elico digital conductivitymeter with a dip-type conductivity cell fitted with a platinum electrode (cell constant = 1.0 cm^{-1}). Room temperature magnetic susceptibilities were measured by a Gouy's method using [Ni(en)$_3$]SO$_4$ as a calibrant. Effective magnetic moments were calculated after applying diamagnetic corrections for the ligand components using Pascal's constants[13]. The specific optical rotation values, $[\alpha]_D$ at 25°C, for all the nickel complexes were measured in DMF solution (0.01%) using Jasco P-2000 Polarimeter. Electronic absorption spectra of the complexes were recorded in DMF on a Shimadzu UV-160A spectrophotometer. The reflectance spectra of solid complexes in the visible region were taken against BaSO$_4$ on a Shimadzu UV-2100 spectrophotometer fitted with a reflectance assembly. Infrared spectra of all the ligands and their metal complexes were recorded in KBr on a Perkin-Elmer Precisely Spectrum 100 FT-IR Spectrometer in the region 4000-400 cm^{-1}. Thermal Analysis (TG and DTA) of all the metal complexes were recorded on a Rigaku Thermo Plus-8120 TG-DTA instrument.

Preparation of Complexes: The mixed ligand Ni(II) complexes C-1 is [Ni(PMINAP)(Ala)·2H$_2$O], C-2 is [Ni(PMINAP)(Val)·2H$_2$O], C-3 is [Ni(PMINAP)(Leu)·2H$_2$O], C-4 is [Ni(PMINAP)(Met)·2H$_2$O] and C-5 is [Ni(PMINAP)(Phe)·2H$_2$O] were prepared by using L_1 is primary ligand PMINAP and L_2 is secondary ligand of various chiral amino acids and racemic amino acids respectively by following method.

Preparation of the CML complexes using Chiral Amino Acids: The CML Ni(II) complexes were prepared from aqueous solution (10 mL) of Ni(II) sulphate heptahydrate (280 mg, 1 mmol) and the aqueous solution (10 mL) of sodium salt of p-

methoxyisonitrosoacetophenone (201 mg, 1 mmol). This mixture was stirred and kept in a boiling water bath for 30 minutes. To this was added 1:1 an aqueous solution (10 mL) of the sodium salt of chiral amino acid (1 mmol) and the mixture (1:1:1 molar proportion) was heated in a hot water bath for three hours. The mixture was cooled and the solid was filtered, washed with ice-cold water followed by 1:1 ethanol: water. The complexes thus prepared were dried under vacuum.

Preparation of the CML complexes using Racemic Amino Acids: The CML metal complexes were also prepared using racemic amino acids *via* stereoselective complexation from Ni(II) sulphate heptahydrate, sodium salt of p-methoxyisonitrosoacetophenone and racemic amino acids such as (±)-alanine, (±)-valine, (±)-leucine, (±)-methionine and (±)-phenylalanine. The aqueous solution (10 mL) of sodium salt of p-methoxyisonitrosoacetophenone (201 mg, 1 mmol) was added to an aqueous solution (10 mL) of Ni(II) sulphate heptahydrate (280 mg,1 mmol). The mixture was stirred and kept in a boiling water bath for 30 minutes. To this mixture was added 1:2 an aqueous solution (10 mL) of the sodium salt of racemic amino acid (2 mmol). This reaction mixture (1:1:2 molar proportion) was heated in a hot water bath for three hours. The mixture was cooled and the solid was filtered, washed with ice-cold water followed by 1:1 ethanol:water. The complexes thus prepared were dried under vacuum. The products were not crystallized to avoid any possibility of isomer enrichment.

Antimicrobial Screening: Broth Dilution Method: This Method was used to determine the Minimum Inhibition Concentration (MIC) of Ni(II) complexes[14]. The Muller Hinton Broth was used for antibacterial activity and Sabouraud Broth was used for antifungal activity as the nutrient media. Initially, the DMSO solvent was used to prepare stock solution of 1000 µg per mL then furthur required dilutions was done by respective Broth medium. For each microbial species the concentration of the complexes used was 50,100 and 200 ppm.

Paper Disc Diffusion Method: This method was used to study the antibacterial activity of the complexes against *E.coli, S.typhi* and *S.aureus* pathogenic bacteria. In this method, 0.1 mL of inoculums of the test organism was spread uniformly on the surface of the agar medium in a petri plate by using a spreader. The sterilized Whatmann filter paper discs of 5 mm diameter were dipped into the 200 ppm solution of the complexes in DMSO and then were placed on the surface of the agar. Up to four discs in each plate were used. The plates were incubated at 37°C for 24 hours. During incubation, the complex diffuses from the filter paper into agar. The activity of the complexes was assessed by measuring the diameter of the inhibited zone in millimeters (mm). The results were compared against those of control (tetracycline), which was screened simultaneously. Solvent DMSO, used as blank, was also run to know its activity.

Tube Dilution Method: This method was used to study the antifungal activity of the complexes against *C.albicans* and *A.niger* pathogenic fungi. The fungus inoculums was prepared by inoculating the selected fungus into sterilized Sabouraud broth to which 0.1 mg per mL of streptomycin was added to prevent bacterial contamination. After sporulation the spores were harvested in the same media by gentle stirring using a magnetic stirrer and the spore suspension was poured into another sterile flask. To a 5 mL of Sabouraud broth contained in a 15 mL Corning test tube 0.1 mL of 200 ppm solution of the complexes in DMSO was added. It was autoclaved at 15 lb pressure for 15 minutes. The tubes were then kept on a rotary shaker and incubated at room temperature for 24 hours. The percentage growth of the fungus was calculated after determining the optical density (OD) of the solution on a spectrophotometer at 530 nm with inoculated Sabouraud broth as blank. The growth of the fungus in the tube, which contained none of the antifungal agent, was assumed as 100%. The results were compared against those of the control (amphotericin), which was screened simultaneously.

Results and Discussion

Characterization of Metal Complexes: The synthesis of mixed-ligand Ni(II) complexes using chiral amino acids may be represented by the following equation.

$$Ni(II) + Na\text{-}PMINAP + Na\text{-}aa \rightarrow (-)[Ni(PMINAP)(aa)] + 2Na^{+}$$

Where Na-PMINAP is sodium salt of p-methoxyisonitrosoacetophenone and Na-aa is sodium salt of L-amino acid. Some of the CML complexes were also prepared from the racemic amino acids such as(±)-alanine, (±)-valine, (±)-leucine, (±)-methionine and (±)-phenylalanine using Ni(II):Na-PMINAP:racemic amino acid in 1:1:2 proportion; the $[\alpha]_D$ values are all negative suggesting L-stereoselective complexation, which may be represented by the following equation.

$$Ni(II) + Na\text{-}PMINAP + (\pm)Na\text{-}aa \rightarrow (-)[Ni(PMINAP)(aa)] + (+)Na\text{-}aa + Na^{+}$$

All the complexes are non-hygroscopic, stable solids, insoluble in water and shows varying solubility in common organic solvents. All the complexes are light green in color and thermally stable indicating a strong metal ligand bond. The elemental analysis data table-1 of the metal complexes are consistent with their general formulation as mixed ligand complexes $[Ni(PMINAP)(aa)\cdot 2H_2O]$. The molar conductance values of the complexes in DMF solution at 10-3 M concentration are in the range 0.002-0.005 $mhos.cm^2mol^{-1}$, indicating their non-electrolytic nature[15]. The specific rotation values, $[\alpha]_D$ for all the complexes, in DMF solution (0.01%) were found to be negative and varied from -74.99 to -243.13°**.** This indicates that the specific rotation of the complexes is due to the corresponding chiral amino acid moiety. It appears that in the first step, the non-chiral ligand Na-PMINAP coordinates to Nickel(II) to yield a 1:1 intermediate which subsequently reacts stereoselectively with one of the optical isomers (L) from the racemic mixture (DL) of amino acid in second step, giving the CML NI(II) complex. The complexes of racemic amino acids

were identified by elemental analysis and standard physico-chemical techniques and were found to be identical to those prepared from chiral amino acids. The effect of steric hindrance on the enatioselective complexation and hence on the enantiomeric excess (ee) is visible when the R group of the amino acid is varied from the least hindered alanine to the most hindered valine as expected, the steric hindrance enhances % enantiomeric excess ee table-1. In case of the complex with methionine ee is reduced to zero, which possibly may be explained as due to some anti-enantioselective interaction of the sulfur atom in the amino acid. An important feature of the preparation of complexes is that they are prepared only in aqueous medium. Solvent free approach is non-polluting and does not employed any toxic materials qualifying it as a green approach. Therefore, the complexes are obtained by a green chemistry synthetic route without use of any solvent. Thus, apart from being a new simple route for synthesizing chiral mixed ligand metal complexes, the present method could be a new technique for optical resolution of amino acids. The possibility of application of this system for resolution of other racemic compounds can be employed.

Infrared Spectra: The FTIR spectra of the metal complexes were recorded in KBr over the range 4000-400 cm^{-1}. The important vibrational bands have been assigned on the basis of the reported assignments of infrared spectral bands of several carbonyl oxime, several amino acids and their metal complexes[16-18]. Abroad band observed in the region between 3570-3332 cm^{-1} due to asymmetric (asym) and symmetric (sym) O-H stretching modes are indicative of the presence of lattice water[19]. The N-H asym and N-H sym vibrations observed between 3037-3027 and 2981-2944 cm^{-1}, respectively, in the free amino acids are shifted to higher wave number i.e. in the range 3441-3266 cm^{-1} and 3172-3063 cm^{-1} respectively, in the spectra of the complexes, suggesting coordination of the amino group through nitrogen with the metal ion. The C-N sym stretching frequency observed in the region 978-913 cm^{-1} in the spectra of amino acids is found to be shifted to lower wave number i.e. 907-832 cm^{-1} in the spectra of the complexes, confirming coordination through the amino group of the amino acids. The $\nu_{asym}(COO^-)$ band of the free amino acids observed in the range 1596-1563 cm^{-1} is shifted to higher wave number, i.e. in the range 1643-1633 cm^{-1} and the $\nu_{sym}(COO^-)$ mode observed between 1425-1407 cm^{-1} in the spectra of free amino acids is found to be shifted to lower wave number i.e.1385-1348 cm^{-1}, in the spectra of the CML complexes indicating the coordination of the carboxylic acid group *via* oxygen with the metal ion. Nakamoto, Morimoto and Martell showed that for a given ligand, the difference (ν_{asym}-ν_{sym}) would increase as the M-O bond becomes more covalent, since the carboxylate stretching becomes correspondingly more asymmetrical[20]. In the present investigation, this difference being in the range 258-285 cm^{-1} indicates that the M-O bond have covalent character[21]. An important feature of the infrared spectra of the CML Ni(II) complexes is the absence of the band due to O-H stretching vibrations of the–COOH group of amino acid. This observation leads to the conclusion that the complex formation takes place by deprotonation of the carboxylic group of amino acid moiety. The C=N stretching frequency observed at 1543 cm^{-1} in the spectrum of PMINAP is found to be shifted to the range 1508-1517 cm^{-1} in the spectra of the complexes, indicating bonding through the nitrogen donor atom of the oxime group. This conclusion is furthur supported by the observation that a new band attributed to $\nu(N\rightarrow O)$ is observed in the range 1198-1207 cm^{-1} in the spectra of the complexes. The C=O stretching frequency observed at 1603 cm^{-1} in the spectrum of PMINAP is found to be shifted to the range 1570-1553 cm^{-1} in the spectra of the complexes, indicating coordination through the oxygen donor atom of the oxime group. This is confirmed by the appearance of some new bands of weak intensity observed in the regions around 695-627 and 473-436 cm^{-1} may be ascribed to the M-O and M-N vibrations, respectively[21]. It may be noted that these vibrational bands are absent in the infrared spectra of Na-PMINAP as well as the amino acids. Some of the important IR bands and their tentative assignment are shown in table-2 and the FTIR spectra of representative complexes [Ni(PMINAP)(Val)·$2H_2O$] is shown in figure-1.

Table - 1
Analytical data of the metal complexes prepared from chiral amino acids

Complex	Empirical formula (formula wt.)	Yield %	Color	Decomp .temp. (°C)	Elemental analysis, % found (calculated)					μ_{eff} B.M	$[\alpha]_D$		ee[c] %
					M	C	N	H	S		L[a]	DL[b]	
C-1	$C_{12}H_{18}NiN_2O_7$ (360.98)	79.56	Light green	258	16.22 (16.26)	39.95 (39.93)	7.74 (7.76)	5.07 (5.03)	-	2.69	-177.45	-19.70	11.10
C-2	$C_{14}H_{22}NiN_2O_7$ (389.03)	75.86	Light green	268	15.02 (15.09)	43.20 (43.22)	7.26 (7.20)	5.73 (5.70)	-	2.97	-154.70	-48.12	31.10
C-3	$C_{15}H_{24}NiN_2O_7$ (403.06)	79.79	Light green	250	14.53 (14.56)	44.74 (44.70)	6.90 (6.95)	6.04 (6.00)	-	2.99	-216.43	-33.10	15.29
C-4	$C_{14}H_{22}NiN_2O_7S$ (421.09)	73.54	Light green	265	13.90 (13.94)	39.96 (39.93)	6.68 (6.65)	5.21 (5.26)	7.64 (7.61)	3.08	-74.99	0	0
C-5	$C_{18}H_{22}NiN_2O_7$ (437.07)	77.81	Light green	243	13.47 (13.43)	49.40 (49.46)	6.48 (6.41)	5.02 (5.07)	-	3.02	-243.13	-32.26	13.26

a: Specific optical rotation for the complexes prepared from chiral L-amino acids; also assumed as authentic for % ee calculations.
b: Specific optical rotation for the complexes prepared from racemic amino acids.
c : Percentage ee for the complexes obtained from racemic amino acids.

Table- 2
Some important infrared spectral bands (cm^{-1}) of CML Ni(II) complexes

Complex	ν_{O-H} H_2O	ν_{N-H} asym. (aa)	ν_{N-H} sym. (aa)	ν_{COO^-} asym. (aa)	ν_{COO^-} sym. (aa)	ν_{C-N} sym. (aa)	$\nu_{C=O}$ PMINAP	$\nu_{C=N}$ PMINAP	$\nu_{N\rightarrow O}$ PMINAP	ν_{M-O}	ν_{M-N}
C-1	3570 w	3407 w	3074 w	1640 s	1379 m	890 s	1555 s	1509 m	1200 s	636[a]w 627[b] w	472[a] w 436[b] w
C-2	3332 w	3266 w	3172 w	1633 s	1364 w	894 s	1570 m	1514 m	1201 s	649[a] w 630[b]w	473[a] w 435[b] w
C-3	3357 w	3294 w	3071 w	1643 s	1348 w	832 s	1555 s	1508 s	1198 s	692[a] w 616[b] w	462[a] w 437[b] w
C-4	3540 w	3441 w	3063 w	1641 s	1380 w	894 s	1553 s	1517 m	1207 s	682[a] w 630[b] w	464[a] w 436[b] w
C-5	3355 w	3299 w	3084 w	1638 s	1385 s	907 s	1560 m	1514 w	1205 m	695[a] w 640[b] w	464[a] w 437[b] w

Where, aa :deprotonated chiral secondary ligands: alanine, valine, leucine, methionine and phenylalanine respectively, s: strong, m: medium, w: weak;. a :PMINAP; b : amino acid.

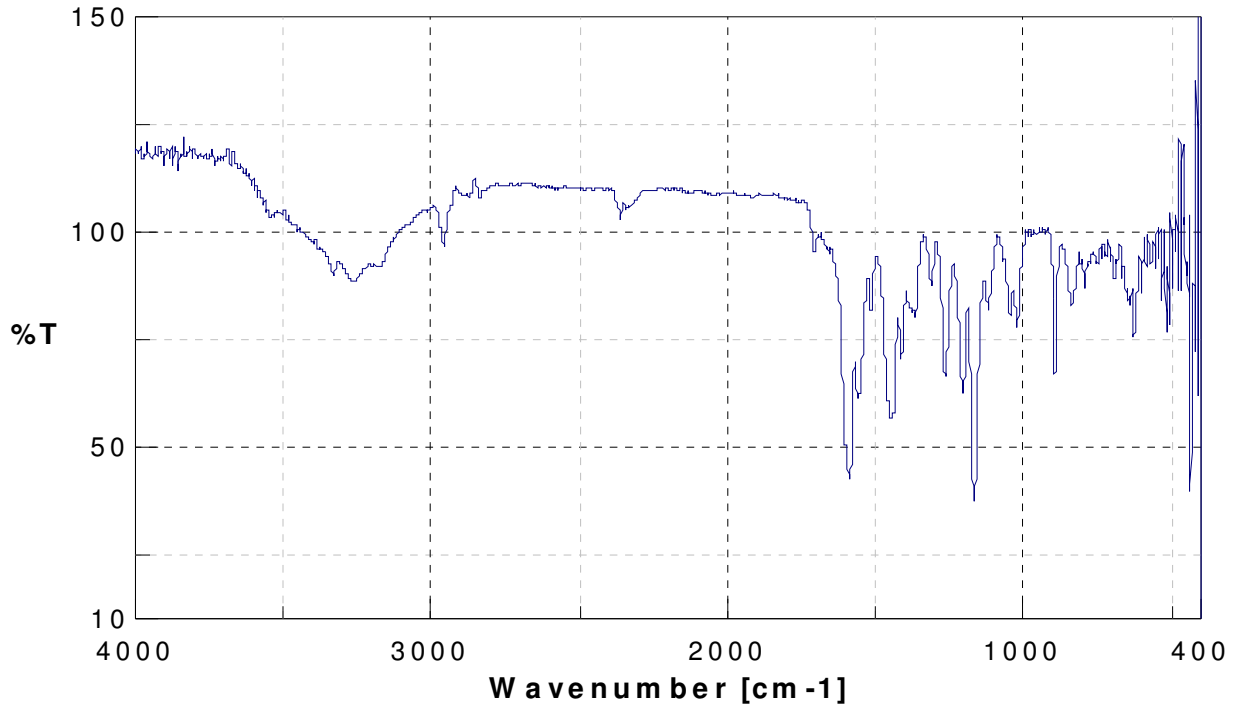

Figure-1
FTIR spectrum of [Ni(PMINAP)(Val)·2H_2O]

Room Temperature Magnetic Susceptibility Measurements: The room temperature magnetic susceptibility measurements for all the nickel complexes reported in the present study were made by the Gouy's method using $[Ni(en)_3]SO_4$ as a calibrant. Effective magnetic moments were calculated after applying diamagnetic corrections for the ligand components using Pascal's constants[13]. The μ_{eff} values for the Ni(II) complexes are in the range 2.69-3.08 B.M., which are well within the range expected for octahedral Ni(II) complexes[22]. The magnetic moments of the compounds investigated are in agreement with the findings of electronic absorption and reflectance spectra.

Electronic Spectra: The electronic spectra in the ultraviolet region of the metal complexes in DMF solution was recorded. The bands observed in the range 39,682-47,619 cm^{-1} are assigned to the $\pi\rightarrow\pi^*$ transitions of the aromatic chromophore. In addition, the band observed in the range 28,985-29,850 cm^{-1} can be attributed to the $n\rightarrow\pi^*$ transition. The bands in the range 25,974-26,315 cm^{-1} can be assigned to the ligand to metal charge transfer (LMCT) transitions. The electronic absorption spectra in the visible and near-infrared region of the Ni(II) complexes in DMF solution show two transition bands. The bands around 24,000 and 16,000 cm^{-1}, are attributed to d-d

transitions table-3. The ultraviolet and visible spectrum of the representative complex [Ni(PMINAP)(Val)·$2H_2O$] is shown in figure-2 and figure-3 respectively. The diffuse reflectance spectra of CML Ni(II) complexes exhibit two bands in the range of 15,290-15,772 and 22,935-24,570 cm^{-1}, which may be ascribed[23] to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2) and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3), respectively, in an octahedral field. For an octahedral d^8 configuration it is obvious that such a system should exhibit three transitions arising from the ground state $^3A_{2g}$ (F) to higher excited states. As the lower band occurs at low energy, usually in the range not accessible due to instrumental limitations, it is not observed in the present cases. Various spectral parameters have been calculated on the basis of the observed transitions, according to the equations of KÖnig[24].

Table- 3
Absorption spectral data for the CML Ni(II) complexes

Complex	Electronic spectral data in DMF		Proposed Assignments
	Peak Position ν(cm^{-1}) {ε $M^{-1}cm^{-1}$}		
	UV[a]	Visible[b]	
C-1	$47619(2.96\times10^4)$	-	Intra-ligand
	$40816(3.56\times10^4)$	-	Intra-ligand
	$29411(0.86\times10^4)$	-	Intra-ligand
	$25974(1.32\times10^4)$	-	Charge transfer
	-	$24096(1.5\times10^3)$	d-d transition
	-	$16806(0.3\times10^3)$	d-d transition
C-2	$46948(3\times10^4)$	-	Intra-ligand
	$40000(3.58\times10^4)$	-	Intra-ligand
	$29850(0.8\times10^4)$	-	Intra-ligand
	$26315(1.3\times10^4)$	-	Charge transfer
	-	$24271(1.6\times10^3)$	d-d transition
	-	$16528(0.5\times10^3)$	d-d transition
C-3	$47169(3.1\times10^4)$	-	Intra-ligand
	$40322(3.6\times10^4)$	-	Intra-ligand
	$29411(0.75\times10^4)$	-	Intra-ligand
	$26178(1.26\times10^4)$	-	Charge-transfer
	-	$24213(1.4\times10^3)$	d-d transition
	-	$16666(0.4\times10^3)$	d-d transition
C-4	$46511(3.4\times10^4)$	-	Intra-ligand
	$39840(3.8\times10^4)$	-	Intra-ligand
	$28985(0.9\times10^4)$	-	Intra-ligand
	$25974(1.4\times10^4)$	-	Charge transfer
	-	$24096(1.6\times10^3)$	d-d transition
	-	$16393(0.5\times10^3)$	d-d transition
C-5	$46948(3.6\times10^4)$	-	Intra-ligand
	$39682(4\times10^4)$	-	Intra-ligand
	$29850(0.96\times10^4)$	-	Intra-ligand
	$26041(1.46\times10^4)$	-	Charge transfer
	-	$24154(1.7\times10^3)$	d-d transition
	-	$16666(0.6\times10^3)$	d-d transition

Where[a]: at 10^{-4}M concentration; [b] : at 10^{-3}M concentration.

Table-4
Diffuse reflectance spectral data for the CML Ni(II) complexes in $BaSO_4$

Complex	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ $\nu_1(cm^{-1})$ [a]	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ $\nu_2(cm^{-1})$	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ $\nu_3(cm^{-1})$	Dq (cm^{-1})	B (cm^{-1})	β	ν_2/ν_1
C-1	8592	15290	23419	859.2	862.2	0.798	1.77
C-2	9017	15313	24271	901.7	835.5	0.773	1.69
C-3	8902	15698	24213	890.2	880.2	0.815	1.76
C-4	8336	15600	22935	833.6	901.8	0.835	1.87
C-5	9064	15772	24570	906.4	876.5	0.811	1.74

Where[a]: Calculated values.

Table-5
Thermal data for CML Ni(II) complexes

Complex	Temp. range (°C)	% Weight loss		Decomposition product
		Found	Calculated	
C-1	100-200	9.94	9.98	[Ni(PMINAP)(Ala)]
	200-305	24.37	24.40	[Ni(PMINAP)]
	305-490	49.42	49.35	[NiO]
C-2	100-205	9.22	9.26	[Ni(PMINAP)(Val)]
	205-320	29.93	29.85	[Ni(PMINAP)]
	320-500	45.76	45.80	[NiO]
C-3	100-210	8.89	8.94	[Ni(PMINAP)(Leu)]
	210-300	32.26	32.30	[Ni(PMINAP)]
	300-500	44.29	44.20	[NiO]
C-4	100-200	8.67	8.55	[Ni(PMINAP)(Met)]
	200-310	27.49	27.58	[Ni(PMINAP)]
	310-520	42.28	42.31	[NiS]
C-5	100-205	8.38	8.24	[Ni(PMINAP)(Phe)]
	205-305	37.48	37.56	[Ni(PMINAP)]
	305-490	40.70	40.76	[NiO]

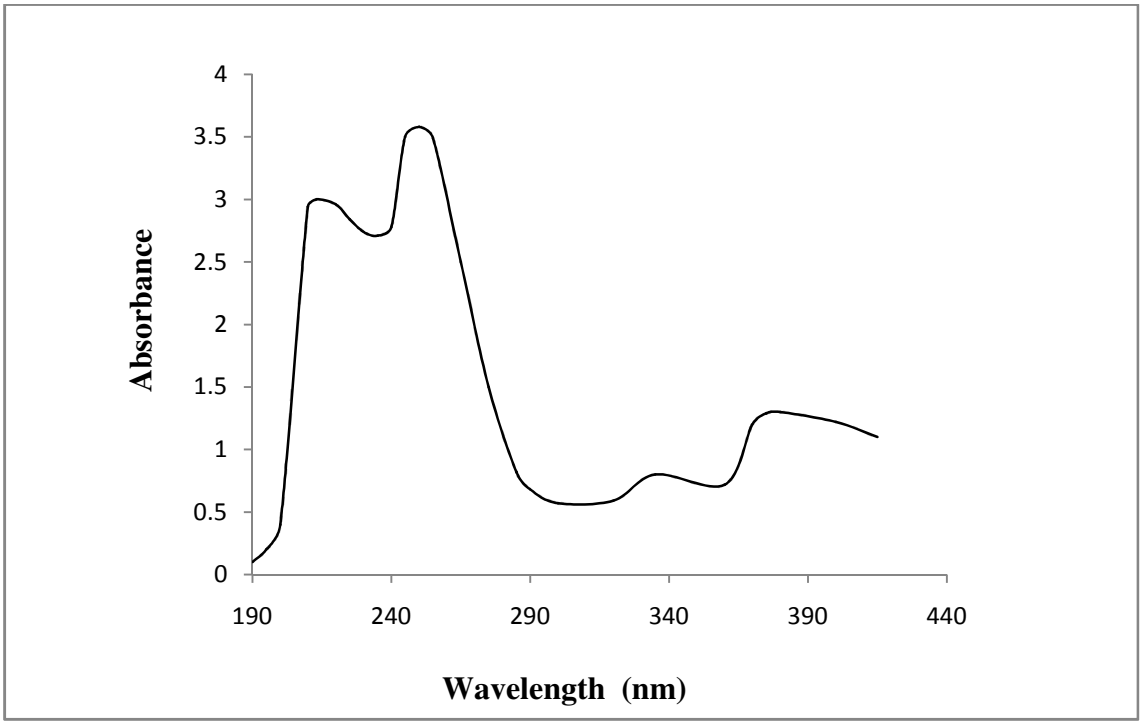

Figure-2
Ultraviolet spectrum of [Ni(PMINAP)(Val)·2H_2O]

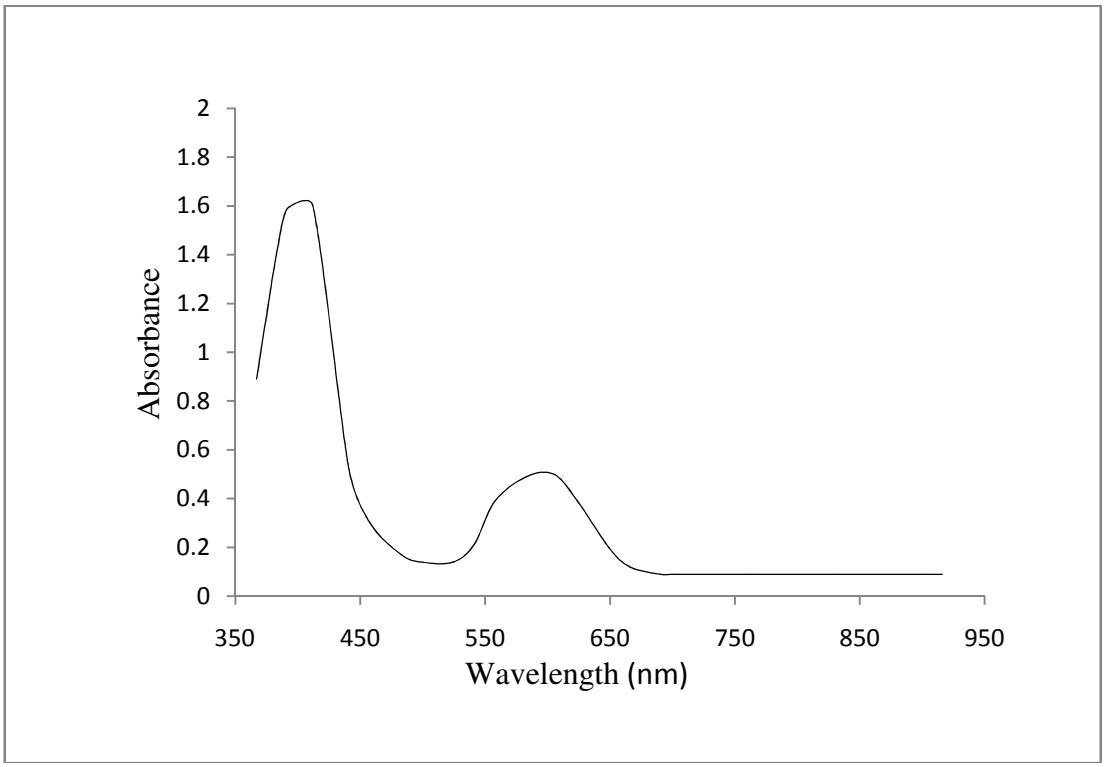

Figure-3
Visible spectrum of [Ni (PMINAP)(Val)·2H_2O]

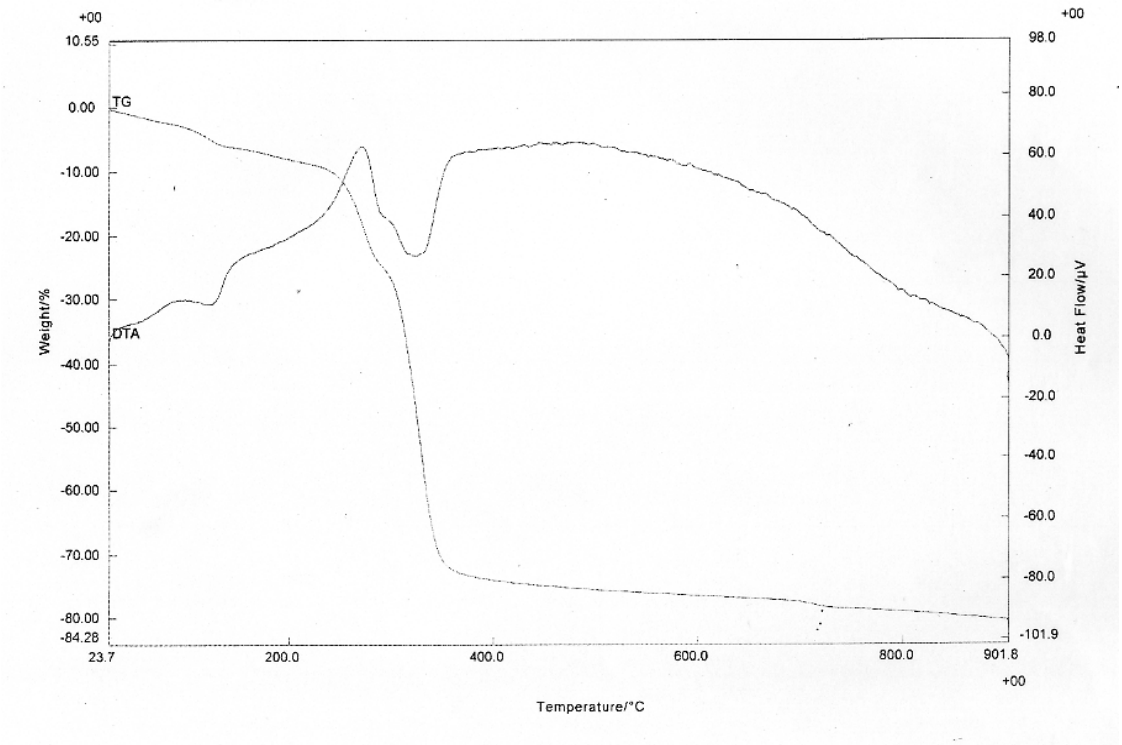

Figure-4
TG/DTA of [Ni(PMINAP)(Val)·2H_2O]

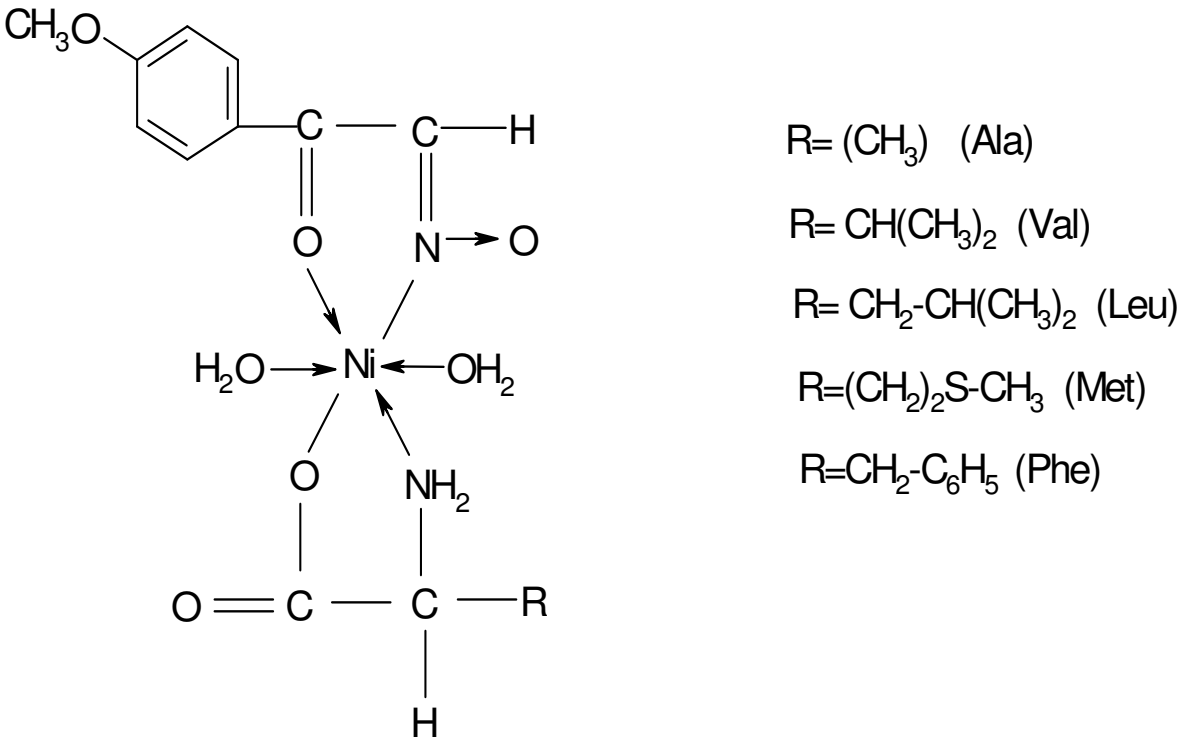

Figure-5
Proposed bonding and structure of the CML Ni(II) complexes

From the values of Dq and B, the transition ν_1 has been calculated Table-4. The parameter B, which measures Racah interelectronic repulsion (B'), is usually lower in complex than in the free ion[25-26], which is an indication of orbital overlap and delocalisation of d-orbitals. This reduction in B value on complex formation is a general phenomenon and indicates reduction of the inter-electron repulsion due to some degree of covalency of metal ligand bond. The value[27] of B for free Ni(II) ion is 1080 cm^{-1}. The nephelauxetic ratio (β) values are less than unity, suggesting an appreciable covalent character of the M-O bond[25]. For many octahedral Ni(II) complexes, the ratio ν_2/ν_1 lie in the range 1.6-1.8 and for the present complexes it is around 1.87. The value of Dq for the complexes are in the range 833.6-906.4 cm^{-1}, which lies well within the range reported for octahedral Ni(II) complexes (i.e. 640-1270 cm^{-1}). The observed spectral features of all the Ni(II) complexes are, therefore, in confirmity with the octahedral geometry proposed on the basis of their analytical data and observed magnetic moments.

Thermal Measurements: The simultaneous TG and DTA studies of the complexes was recorded in nitrogen atmosphere on increasing the temperature from room temperature upto 600ºC at the heating rate of 10ºC/min. All the complexes investigated shows similar behavior in their thermograms. The thermogram of the representative complex [Ni(PMINAP)(Val)·$2H_2O$], is shown in figure- 4 exhibit three steps. In the first step the complex losses two water molecule in the temperature range between 100°C to 200°C indicates that the complex is thermally stable up to nearly 100°C above which it loses the water molecule. The DTA curve of complex displays an endothermic peak at 100°C, which is attributed to the loss of two water molecules. The dehydrated product is stable up to 200°C above that temperature the second step starts. The complex losses some moiety in the temperature range between 210°C to 320°C which could be attributed due to loss of the amino acid ligand. Like most of the metal organic complexes, the CML complexes decomposes by the production of finely divided metal powder by virtue of the reducing gaseous environment, produced by the gaseous products such as CO, NH_3, etc. formed as a result of fragmentation of the ligands during the decomposition of complex. The third step involving the loss of PMINAP ligand in the temperature range between 300°C to 520°C table- 5. In thermogram the sudden decrease in the slope suggests a simultaneous loss of ligands from the complex which is also reflected by the strong exothermic peak by the DTA curve. There can also be significant contribution to this heat effect from the spontaneous oxidation of the final metal powder formed in the decomposition process into NiO which is confirmed by X-ray analysis[28]. The perusal of thermograms shows the presence of water molecules in the complexes which further corroborates the observation made on the basis of infrared spectral studies and is in good agreement with the elemental analysis presented in table-1. On the basis of the physico-chemical studies, the proposed bonding and structure in the metal complexes can be represented as shown in figure-5.

Table- 6
Antimicrobial activity of the CML Ni(II) complexes

Complex	Antibacterial activity at 200 ppm (zone of inhibition in mm)			Antifungal activity at 200 ppm (% Inhibition)	
	E.coli	***S.typhi***	***S.aureus***	***C. albicans***	***A. niger***
C-1	7	7	8	43	56
C-2	6	6	7	42	54
C-3	8	8	9	47	60
C-4	9	8	9	52	62
C-5	6	6	7	42	54
Na-PMINAP	4	3	4	18	16
$NiSO_4 \cdot 7H_2O$	3	2	4	32	30
Tetracycline	14	15	13	-	-
Amphotericin	-	-	-	97	98

Biological Activities: It has been found that a majority of the metal complexes showing biological activity are chelates[29-30]. The antibacterial and antifungal activities of the complexes have been studied against some pathogenic bacteria and fungi. The Broth dilution method was used to determine the Minimum Inhibition Concentration (MIC). The culture that shows no growth in the presence of lowest concentration of the complex represents the MIC of the complex against that particular bacteria and fungi. It has been found that at 200 ppm the culture does not show growth. Hence, 200 ppm concentration of the complex is assessed for antibacterial and antifungal activity. The paper disc diffusion method has been used to study the antibacterial activity against *E. coli, S. typhi* and *S. aureus*. The activity was assessed by measuring the diameter of the zone of inhibition in mm. The antibacterial activity data of sodium salt of p-methoxyisonitrosoacetophenone, nickel sulphate and the standard antibacterial compound tetracycline is shown in table-6. It has been observed that the amino acids used for current investigation do not show antibacterial and antifungal activity. The data shows that the antibacterial activity of the metal sulphate as well as that of the ligands is significantly enhanced on complexation. All the complexes show good antibacterial activity against *S. aureus.* The CML complexes with methionine shows good activity against all the organisms under study. A bacteriostatic effect has been observed in a number of cases, which show that the complexes inhibit protein synthesis and act by binding to the ribosome[31]. The binding, however, is not tight and when the concentration of the complex becomes free from the ribosome and growth is resumed. Chelation reduces considerably the polarity of the metal ions in the complexes[32]. This is mainly due to the partial sharing of its positive charge with the donor group and possible π-electron delocalisation over the whole chelate ring system through pπ-pπ or dπ-dπ interactions of the orbitals of the ligands and metal ions, which in turn increases the hydrophobic character of the chelate and thus enable its permeation through the lipid layer (cell membrane) of microorganisms. Compared to standard antibacterial compound tetracycline, the present CML complexes are less active. The tube dilution method[29] was studied for the antifungal activity of the complexes against *Candida albicans* and *Aspergillus niger.* The results have been expressed as percentage inhibition table-6. The data shows that the antifungal activity of the metal sulphate as well as that of the ligands is significantly enhanced on complexation. All the complexes show antifungal activity against both fungi. The complexes show higher activity against *A. niger* than against *C. albicans.* The present CML complexes are less active compared to standard antifungal compound amphotericin.

Conclusion

The higher decomposition temperature and electrical conductance studies show the presence of strong metal-ligand bonding and non-electrolytic nature of the complexes. Specific rotation measurement studies are indicative of the chirality of the complexes. Room temperature magnetic studies are indicative of an octahedral geometry of the nickel complexes which is confirmed by crystal field transitions shown by the electronic spectra. The IR spectra show bonding of the metal through N- and O- donor atoms of the two ligands. Thermal analysis confirms the presence of coordinated water molecules. The studies on antimicrobial activity indicate that the nickel sulphate as well as that of ligands is significantly enhanced on complexation. All the complexes show good antibacterial activity against *S.aureus*. The CML complexes with methionine shows good antibacterial activity against all the organisms under study. The complexes show higher antifungal activity *A.niger* than against *C.albicans.* Compared to the standard antibacterial and antifungal compound, the present complexes are less active against the representative strains.

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