Synthesis, characterization of some transition metal(II) complexes of acetone *p*-amino acetophenone salicyloyl hydrazone and their anti microbial activity

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Abstract Complexes of the type [M(apash)Cl] and [M(Hapash)(H$_2$O)SO$_4$], where M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II); Hapash = acetone *p*-amino acetophenone salicyloyl hydrazone have been synthesized and characterized by elemental analyses, molar conductance, magnetic moments, electronic, ESR and IR spectra, thermal studies (TGA & DTA) and X-ray diffraction studies. The ligand coordinates through two >C=N and a deprotonated enolate group in all the chloro complexes, whereas through two >C=N– and a >C=O group in all the sulfato complexes. The electronic spectra suggest a square planar geometry for Co(II), Ni(II) and Cu(II) chloride complexes and an octahedral geometry for the sulfate complexes. ESR data show an isotropic symmetry for [Cu(apash)Cl] and [Cu(Hapash)(H$_2$O)SO$_4$] in solid state. However, ESR spectra of both Cu(II) complexes indicate the presence of unpaired electron in d$_{x^2-y^2}$. The X-ray diffraction parameters for [Co(apash)Cl] and [Cu(Hapash)(H$_2$O)SO$_4$] complexes correspond to a tetragonal and an orthorhombic crystal lattices, respectively. Thermal studies of [Co(apash)Cl] complex shows a multi-step decomposition pattern. Most of the complexes show better antifungal activity than the standard miconazole against a number of pathogenic fungi. The antibacterial activity of these complexes has been evaluated against *E. coli* and *Clostridium* sp. which shows a moderate activity.

Keywords Metal(II) complexes · Synthesis and characterization · Salicyloyl hydrazone · Antifungal · Antibacterial activity

Introduction

Transition metal ions with different oxidation state have a strong role in bio-inorganic chemistry and redox enzyme systems and may provide the basis of models for active sites of biological systems. Copper is the third most abundant metallic element in the human body, following iron and zinc. It also occurs in all other form of life and plays a role in the action of a multitude of enzymes that catalyze a great variety of reactions (Karlin and Tyeklar 1993). Superoxide dismutase has normal Cu(II) enzyme active sites containing tetragonally coordinated Cu(II) ion. The "blue" copper sites (Chapman 1991) seem generally to be involved in electron transfer, with the copper cycling between +1 and +2 oxidation states (Kyritsis et al. 1994).

Nickel is an essential component in at least 4 types of enzymes viz. urease, carbon monoxide dehydrogenase (CODH) or acetyl coenzyme A synthase,

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hydrogenase and methyl-S-coenzyme M reductase. There is spectroscopic evidence for nickel-methyl complexes in CODH of anaerobic bacteria (Kumar et al. 1995). The interaction of Co(II) complexes in solution with O_2 has been the subject of intensive study. Some of these complexes behave as reversible carriers of O_2 and have been used as models for natural oxygen transport systems (Macke and Williams 1988). The best studied complexes are those of Schiff bases such as Co(II) (acacen)(DMF)O_2, which in solution (pyridine, DMF or similar solvents) will pick up O_2 (Cotton et al. 2003). Polydentate Co(II) complexes with ligands capable of intercalation into DNA strands are capable of inducing DNA cleavage under photochemical conditions (Bhattacharya and Mandal 1996). Since the recognition of Vitamin B_{12} and synthesis of cobalmines, responsible for erythrocytes (RBC) formation in human body, there has been much study of "model" systems.

Zinc is essential to all forms of life (Miles 1989) and a large number of diseases and congenital disorders have been traced to zinc deficiency. Several zinc enzymes have been reported viz. carbonic anhydrase, carboxy peptidase, alcohol dehydrogenases, aldoses, proteases, phosphatases, DNA- and RNA- polymerases etc. The most important natural role of manganese is in oxidation of water in green plant photosynthesis, where its presence in photosystem II (PS II) is essential (Greenwood and Earnshaw 1998).

The metal complexes formed by the combination of transition metal ion with a potent acylhydrazone ligand should be more biologically active than the metal salts or the ligand individually. Therefore, we report here the synthesis and characterization of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes with acetone p-amino acetophenone salicyloyl hydrazone. The antifungal and antibacterial properties of the ligand and its complexes were also tested.

Materials and methods

Chemicals

All the chemicals used were of BDH or equivalent grade. The precursor salicyloyl hydrazine, $C_6H_4(OH)CONHNH_2$ (sh) was prepared by the reported procedure (Narang et al. 1994) and was

characterized by its had melting point. sh, m. p. 146°C (lit. 147°C).

Preparation of p-amino acetophenone salicyloylhydrazone

p-Amino acetophenone salicyloylhydrazone (Hpash), $H_2NC_6H_4C(CH_3)=NNHCO$ C_6H_4OH was prepared by reacting p-amino acetophenone (10 mmol, 13.5 g) with salicyloyl hydrazine (10 mmol, 15.2 g) dissolved in 50 ml ethanol. After refluxing the reaction mixture for 5–6 h, the product was filtered on a suction pump, washed several times with aqueous ethanol (50%, v/v) and recrystallized from hot ethanol. The pure product Hpash was dried over anhydrous $CaCl_2$ in a desiccator.

Preparation of acetone p-amino acetophenone salicyloylhydrazone

Acetone p-amino acetophenone salicyloyl hydrazone (Hapash), $(CH_3)_2C=NC_6H_4$ $C(CH_3)=NNHCOC_6H_4OH$ was prepared by reacting Hpash (5 mmol, 13.45 g) with an excess of acetone (100 ml). The reaction mixture was refluxed for 2 h at 40°C and then it was transferred in a beaker. The excess of acetone was allowed to evaporate and the residue was recrystallized from hot ethanol.

The ligand was characterized by elemental analyses (C, H, N), melting points (Table 1), infrared spectra (Table 4) ^{1}H and 13C NMR spectra.

Hapash, ^{1}H NMR (ppm): three –CH$_3$ protons (1.2), >NH (6.9), aromatic ring protons (7.1–7.8), aromatic C–OH (11.0).

Hapash, ^{13}C NMR (ppm): three –CH$_3$ (11.2, 14.5 and 18.6), aromatic ring carbons (118.5–155.2), two >C=N– (158.3 and 162.4), >C=O (171.1).

Synthesis of the metal complexes

The metal(II) chloride complexes were synthesized by taking 50 ml solutions of each metal(II) chloride (10 mmol) in ethanol and 50 ml hot ethanolic solution of the ligand Hapash (10 mmol, 3.09 g) in a RB flask separately in 1:1 (M:L) molar ratio. After refluxing the reactants for 2–3 h and cooling to room temperature, the complexes precipitated and were filtered in a glass crucible. The products were washed several times with water, ethanol and finally with

Table 1 Analytical data of the ligand and its complexes

| Compounds (Colour) | Empirical formula (Formula wt.) | Melting point (°C) | Found (Calculated) % | | | | | Yield (%) | Λ_M (Ω^{-1} cm^2 mol^{-1}) |
			Metal	Cl$^-$/SO$_4^{2-}$	C	H	N		
Hapash	$C_{18}H_{19}N_3O_2$	142			69.65	6.24	13.37	90	6.15
(Light yellow)	(309)				(69.90)	(6.15)	(13.59)		
[Mn(apash)Cl]	$C_{18}H_{18}N_3O_2ClMn$	216	13.65	8.76	54.36	4.46	10.66	75	3.09
(Yellow)	(398.5)		(13.80)	(8.91)	(54.20)	(4.52)	(10.54)		
[Co(apash)Cl]	$C_{18}H_{18}N_3O_2ClCo$	273	14.80	8.63	53.78	4.39	10.38	82	8.11
(Light brown)	(402.5)		(14.66)	(8.82)	(53.66)	(4.47)	(10.43)		
[Ni(apash)Cl]	$C_{18}H_{18}N_3O_2ClNi$	195	14.55	8.89	53.53	4.56	10.58	86	6.35
(Light pink)	(402.5)		(14.66)	(8.82)	(53.66)	(4.47)	(10.43)		
[Cu(apash)Cl]	$C_{18}H_{18}N_3O_2ClCu$	180	15.50	8.70	52.89	4.34	10.41	85	5.72
(Greenish brown)	(407)		(15.60)	(8.72)	(53.07)	(4.42)	(10.32)		
[Zn(apash)Cl]	$C_{18}H_{18}N_3O_2ClZn$	199	15.76	8.55	52.67	4.46	10.16	75	7.81
(Yellow)	(408.5)		(15.91)	(8.69)	(52.88)	(4.41)	(10.28)		
[Mn(Hapash)(H$_2$O)SO$_4$]	$C_{18}H_{21}N_3O_7SMn$	186	11.37	20.00	45.32	4.44	8.64	79	4.32
(Light yellow)	(478)		(11.51)	(20.08)	(45.19)	(4.39)	(8.79)		
[Co(Hapash)(H$_2$O)SO$_4$]	$C_{18}H_{21}N_3O_7SCo$	222	12.10	20.11	44.97	4.42	8.76	82	2.33
(Pink)	(482)		(12.24)	(19.92)	(44.81)	(4.36)	(8.71)		
[Ni(Hapash)(H$_2$O)SO$_4$]	$C_{18}H_{21}N_3O_7SNi$	207	12.35	19.80	44.62	4.28	8.77	85	7.65
(Light green)	(482)		(12.24)	(19.92)	(44.81)	(4.36)	(8.71)		
[Cu(Hapash)(H$_2$O)SO$_4$]	$C_{18}H_{21}N_3O_7SCu$	245	13.20	19.60	44.24	4.39	8.50	85	3.22
(Light green)	(486.5)		(13.05)	(19.73)	(44.40)	(4.32)	(8.63)		
[Zn(Hapash)(H$_2$O)SO$_4$]	$C_{18}H_{21}N_3O_7SZn$	212	13.30	19.50	44.45	4.40	8.52	75	4.54
(Yellow)	(488)		(13.32)	(19.67)	(44.26)	(4.30)	(8.61)		

diethyl ether and the pure complexes thus obtained were dried in a desiccator over anhydrous CaCl$_2$.

The metal(II) sulfate complexes were prepared by refluxing 50 ml aqueous ethanolic solutions of each metal(II) sulfate (10 mmol) with 50 ml hot ethanolic solution of the ligand (10 mmol, 3.09 g) for 1–2 h. The complexes were precipitated after cooling the reaction solution at room temperature. They were filtered, washed with aqueous ethanol and diethyl ether and dried in a desiccator.

Analyses of the complexes

Elemental analyses

The complexes were analyzed for metal content gravimetrically by literature procedures (Vogel 1989) after decomposing the organic matter with a mixture of HNO$_3$ and HCl and evaporating the residue to

dryness with concentrated H$_2$SO$_4$. The chloride content in the complex was determined gravimetrically as AgCl and sulfate as BaSO$_4$.

Carbon, hydrogen and nitrogen were determined microanalytically on a Elementar Vario EL III Carlo Erba 1108 model, microanalyzer.

Physico-chemical measurements

The molar conductance of the complexes were determined by preparing 10^{-3} M solutions of the complexes in DMSO at room temperature and measured on a Systronic Conductivity meter model-306. Thermal studies (TGA and DTA) of some of the complexes were carried out on a Perkin-Elmer Thermal Analyzer between room temperature to 800°C. Room temperature magnetic susceptibility measurements were carried out on a Faraday balance using Hg[Co(SCN)$_4$] as calibrant and corrected for diamagnetism (Dutta and Syamal 1993). The

electronic spectra of the complexes were recorded in DMSO solution on a Perkin-Elmer Lambda-2 spectrophotometer in the range 1,100–200 nm. Infrared spectra of the complexes and parent ligand were recorded on Vector-22 spectrophotometer in the range 4,000–500 cm^{-1} in KBr medium. ^{1}H and ^{13}C NMR spectra of the ligand were recorded in DMSO on a JEOL AL 300 FT NMR Spectrometer. The X-band ESR spectra of copper(II) complexes were recorded on a EMX 1444 EPR spectrometer at room temperature (298 K) in solid state using DPPH as g marker (g = 2.0023). Powder X-ray diffraction patterns of a few complexes were recorded on Iso Debye Flex 2002 apparatus using CuKα radiation. The analytical and physico-chemical data are given in Tables 1–6.

Biological activity

Antifungal activity

The ligand as well as its complexes was screened for their antifungal activity against various fungi viz. *Aspergillus* sp., *Trichoderma* sp. and *Stemphylium* sp. These species were isolated from the infected organs of the host plants on potato dextrose agar (potato 250 g + dextrose 20 g + agar 20 g) medium. The

cultures of the fungi were purified by single spore isolation technique.

The solution in different concentrations 0.5, 1 and 1.5 mg/ml of each compound in DMSO were prepared for testing against spore germination. A drop of the solution of each concentration was kept separately on glass slides. The conidia, fungal reproducing spores (approximately 200) lifted with the help of an inoculating needle, were mixed in every drop of each compound separately. Each treatment was replicated thrice and a parallel DMSO solvent control set was run concurrently on separate glass slides. All the slides were incubated in humid chambers at 25 ± 2°C for 24 h. Each slide was observed under the microscope for spore germination and percent germination was finally calculated. The results were also compared with a standard antifungal drug Miconazole at the same concentrations.

Antibacterial activity

The antibacterial activity of the ligand and its complexes were studied against *Clostridium* sp. and *E. coli* bacteria. Each of the compounds was dissolved in DMSO and solutions of the concentration 2 mg/ml and 1 mg/ml were prepared separately. Paper discs of Whatman filter paper (No. 42) of uniform diameter (2 cm) were cut and sterilized in an

Table 2 Magnetic moments, electronic spectral data and ligand field parameters of the complexes

Complexes	μ_{eff} (B.M.)	v_1	v_2	v_3	10 Dq	B (cm^{-1})	β	β^0 (%)	LFSE (kcal/mol)
[Mn(apash)Cl]	5.96	19,230	23,255						
[Co(apash)Cl]	2.25	–	21,505	27,775					
[Ni(apash)Cl]	Diamagnetic	12,195	20,410	27,025					
[Cu(apash)Cl]	1.71	–	16,390	–					
[Mn(Hapash)(H$_2$O)SO$_4$]	5.91	19,725	25,125						
[Co(Hapash)(H$_2$O)SO$_4$]	4.80	9,360	–	20,430	10,575	819	0.843	15.70	24.10
[Ni(Hapash)(H$_2$O)SO$_4$]	2.94	10,515	16,720	28,280	10,515	897	0.861	13.90	35.95
[Cu(Hapash)(H$_2$O)SO$_4$]	1.78	9,900	14,810	–	–				

Table 3 ESR spectral parameters of Cu(II) complexes in solid state at room temperature (298 K)

Complex	g$_{||}$ (G)	g$_\perp$ (G)	g$_{av}$ (G)	A$_{||}$ (G)	A$_\perp$ (G)	g$_{av}$ (G)
[Cu(apash)Cl]			g$_{iso}$ = 2.0794			A$_{iso}$ = 52
[Cu(Hapash)(H$_2$O)SO$_4$]	2.2627	2.0409	2.1148	116	83	94

Table 4 Important IR spectral bands (cm^{-1}) and its assignments

Compounds	ν(OH/NH)	Amide I ν(C=O)	ν(C=N)	Amide II	Amide III	ν(N=C–O)	ν(C–O)	ν(N–N)	ν(M–O)	Water bands
Hapash	3438b, 3204b	1663s	1617s	1565m	1355s	–	–	977w	–	–
[Mn(apash)Cl]	3436b	–	1600s	–	–	1492s	1314s	995w	560w	–
[Co(apash)Cl]	3439b	–	1597s	–	–	1490s	1312s	998w	573w	–
[Ni(apash)Cl]	3435b	–	1599s	–	–	1491s	1312s	1005w	570w	–
[Cu(apash)Cl]	3436b	–	1592s	–	–	1494s	1313s	1002w	572w	–
[Zn(apash)Cl]	3440b	–	1595s	–	–	1492s	1311s	998w	568w	–
[Mn(Hapash)(H$_2$O)SO$_4$]	3436b, 3218s	1640s	1600s	1547m	1366s	–	–	999w	571w	941m, 755w, 625w
[Co(Hapash)(H$_2$O)SO$_4$]	3432b, 3219s	1645s	1602s	1552m	1361s	–	–	1006w	575w	940m, 756w, 653w
[Ni(Hapash)(H$_2$O)SO$_4$]	3430b, 3217s	1641s	1593s	1541s	1365s	–	–	1000w	567w	960m, 755w, 662w
[Cu(Hapash)(H$_2$O)SO$_4$]	3437b, 3208s	1648s	1597s	1544s	1370s	–	–	1008w	566w	967m, 47w, 665w
[Zn(Hapash)(H$_2$O)SO$_4$]	3430b, 3206b	1642s	1604s	1542m	1365s	–	–	1002w	570w	938m, 755w, 661w

b, road; s, strong; m, medium; w, weak

autoclave. The paper discs soaked in the desired concentration of the complex solutions were placed aseptically in the petridishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with *Clostridium* sp. and *E. coli* bacteria separately. The petridishes were incubated at 37°C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated 9 times.

The antibacterial activity of a common standard antibiotic Ampicillin was also recorded using the same procedure as above at the same concentrations and solvent. The % Activity Index for the complex was calculated by the formula as under:
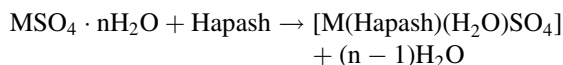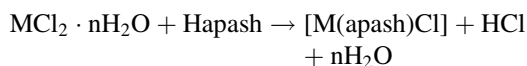
$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diamter)}}{\text{Zone of inhibition by standard (diamter)}} \times 100$$

Determination of minimum inhibitory concentration (MIC) value: The antibacterial screening concentrations of the compounds to be used were estimated from the minimum inhibitory concentration (MIC) value. The MIC was determined using the disc diffusion technique by preparing discs containing 0.1–1.0 mg/ml of each compound against both the bacteria and applying the protocol. All the compounds were more effective at 1.0 and 2.0 mg/ml concentrations. Consequently all the compounds were screened at these concentrations against both the bacteria. The results of MIC values (mg/ml) are given in Table 7B.
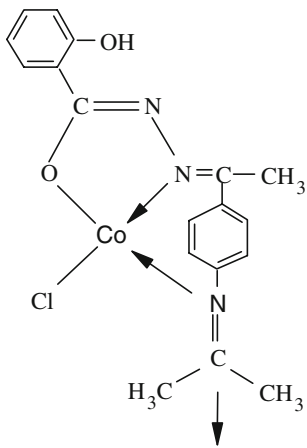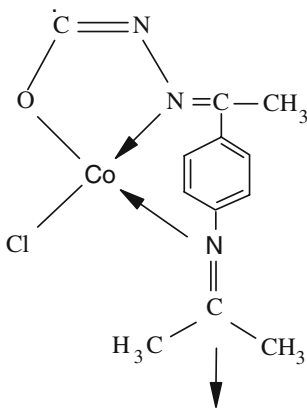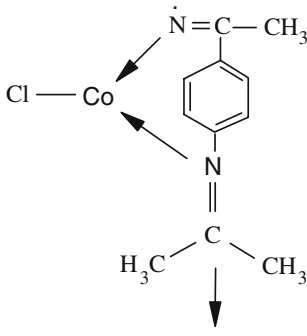
Results and discussion

It appears from the analytical data (Table 1) that the ligand Hapash, acetone *p*-amino acetophenone salicyloyl hydrazone reacts with metal salts in 1:1 (M:L) molar ratio to form the complexes of general compositions [M(apash)Cl] and [M(Hapash)(H$_2$O)SO$_4$]. The reactions may be written as:

$$\text{MCl}_2 \cdot n\text{H}_2\text{O} + \text{Hapash} \rightarrow [\text{M(apash)Cl}] + \text{HCl} + n\text{H}_2\text{O}$$

$$\text{MSO}_4 \cdot n\text{H}_2\text{O} + \text{Hapash} \rightarrow [\text{M(Hapash)(H}_2\text{O)SO}_4] + (n-1)\text{H}_2\text{O}$$

where, M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II).

It appears that the ligand Hapash enolizes and deprotonates during complexation with metal(II)

Table 5 Thermal decomposition of [Co(apash)Cl] complex

S. No.	Temp (°C)	Proposed decomposition pattern	% Loss of wt. Obs.	% Loss of wt. Calc.	Energy change	Remarks
1.	40–272	(structure)	0.00	0.00	Endothermic	No decomposition
2.	273	(structure)	22.00	22.11	Endothermic	Partial decomposition of ligand
3.	360	(structure)	32.80	32.54	Exothermic	Partial decomposition of ligand
4.	420	Co–Cl	44.0	43.73	Exothermic	Decomposition of organic ligand

chloride. Such deprotonation does not occur during the reaction with metal(II) sulfates. The complexes are powdery solids and their colour varies from light yellow, yellow, light green to pink. They are generally insoluble in water, ethanol, methanol, benzene, chloroform, carbon tetrachloride and diethyl ether but are soluble in more polar organic solvents DMF and DMSO. The complexes melt with decomposition

Table 6 Observed and calculated Q and hkl values

Powder pattern	Angle°	Intensity	d(Å)	Qobs (1/d²)	Qcalc	hkl
[Co(apash)Cl]						
1.	11.83	100.00	7.4835	0.0178	0.0178	100
2.	14.49	76.23	6.1128	0.0268	0.0268	101
3.	15.72	73.75	5.6359	0.0315	0.0356	110
4.	18.68	68.61	4.7490	0.0443	0.0446	111
5.	19.77	68.30	4.4906	0.0496	0.0538	102
6.	22.48	77.86	3.9543	0.0639	0.0712	200
7.	24.85	77.53	3.5826	0.0779	0.0802	201
8.	30.23	69.00	2.9563	0.1144	0.1166	113
[Cu(Hapash)(H₂O)SO₄]						
9.	10.65	59.88	8.3106	0.0145	0.0145	100
10.	11.44	56.04	7.7355	0.0167	0.0167	010
11.	13.77	59.81	6.4301	0.0242	0.0242	001
12.	17.54	56.89	5.0553	0.0391	0.0387	101
13.	18.29	56.96	4.8512	0.0425	0.0409	011
14.	21.32	59.80	4.1685	0.0575	0.0554	111
15.	23.20	72.35	3.8338	0.0680	0.0668	020
16.	25.88	86.90	3.4426	0.0844	0.0822	201
17.	27.27	98.68	3.2702	0.0935	0.0910	021
18.	28.41	69.68	3.1414	0.1013	0.0989	211
19.	29.06	63.88	3.0731	0.1059	0.1055	121
20.	29.75	66.23	3.0029	0.1109	0.1113	102
21.	31.29	57.87	2.8587	0.1224	0.1280	112
22.	31.79	56.83	2.8151	0.1262	0.1248	220
23.	32.58	61.56	2.7483	0.1324	0.1305	300
24.	37.84	56.58	2.3775	0.1769	0.1745	031
25.	39.18	58.66	2.2992	0.1892	0.1890	131
26.	40.07	57.34	2.2500	0.1975	0.1973	320
27.	40.87	56.64	2.2081	0.2051	0.2083	230
28.	47.82	58.63	1.9022	0.2763	0.2758	203
29.	57.10	56.66	1.6131	0.3843	0.3872	004

between 180 and 273°C. The low molar conductance values (2.333–8.11 $\Omega^{-1}cm^2$ mol^{-1}) of 10^{-3} M solutions of the complexes in DMSO at room temperature suggest that they are non-electrolytes (Geary 1971).

Magnetic moments

The magnetic moments of the Cu(II) complexes, in present study, correspond to μ_{eff} values for one unpaired electron (1.71 and 1.78 B.M.). Nickel(II) sulfate complex shows μ_{eff} value 2.94 B.M. corresponding to two unpaired electrons in an octahedral environment (Cotton et al. 2003), whereas, Nickel(II) chloride complex is diamagnetic suggesting square planar geometry for the complex.

Cobalt(II) sulfate complexes shows μ_{eff} value 4.80 B.M. is fairly close to those reported for three unpaired electrons in an octahedral environment. However, Co(II) chloride complex shows μ_{eff} value 2.25 B.M. corresponding to one unpaired electron and suggests square planar geometry for the complex (Dutta and Syamal 1993). Mn(II) complexes have their μ_{eff} value 5.96 and 5.91 B.M. corresponding to five unpaired electrons (Table 2).

Electronic spectra

[Cu(Hapash)(H₂O)SO₄] shows two bands at 9,900 cm^{-1} and 14,810 cm^{-1}, which may be assigned to $^2B_{1g} \rightarrow {}^2B_{2g}$ and $\rightarrow {}^2A_{1g}$ indicating a distorted octahedral geometry for the complex. [Cu(apash)Cl] shows a broad band near 16,390 cm^{-1} similar to $[Cu(NH_3)_4]^{2+}$ (16,600 cm^{-1}) suggesting a square planar geometry for the complex (Lever 1984).

The electronic spectra of [Ni(apash)Cl] shows weak bands at 12,195, 20,410 and 27,025 cm^{-1} corresponding to $^1A_{1g} \rightarrow {}^1B_{1g}$ and $\rightarrow {}^1B_{2g}$ and a charge transfer transition similar to that reported for Ni(II) acetylacetone bis-acylhydrazone (Narang and Singh 1993) and many other Ni(II) complexes with a square planar geometry (Bigoli et al. 2000). Nickel(II) complexes generally show three bands in octahedral environment corresponding to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(v_1)$, $\rightarrow {}^3T_{2g}(F)(v_2)$ and $\rightarrow {}^3T_{1g}(P)(v_3)$ (Cotton et al. 2003). [Ni(Hspash)(H₂O)SO₄] complex also shows above three transitions at 10,515 cm^{-1} (v_1), 16,720 cm^{-1} (v_2) and 28,280 cm^{-1} (v_3) suggesting an octahedral geometry for the complex. [Co(Hspash)(H₂O)SO₄] shows two bands at 9,360 cm^{-1} and 20,430 cm^{-1} indicating octahedral geometry for the complex and are assigned as $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(v_1)$ and $\rightarrow {}^4T_{1g}(P)(v_3)$. The v_2 transition was not observed due to very weak intensity (Lever 1984). Ligand field parameters (10 Dq, B, β, β^0 and LFSE) have also been calculated for both Ni(II) and Co(II) sulfate complexes (Table 2). These parameters indicate significant covalent character of the metal-ligand bonds. The bands observed for [Co(apash)Cl] are in good agreement with the bands reported for cobalt(II) square planar complexes (Nishida and Kida 1979). The weak intensity bands observed for

Table 7 Antifungal (A), Antibacterial (B) activity of the ligand and its complexes

Compound	% Inhibition of spore germination								
	Aspergillus sp. (mg/ml)			*Stemphylium* sp. (mg/ml)			*Trichoderma* sp. (mg/ml)		
	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
A. Antifunga activity of the ligand and its complexes									
Hapash	36	47	59	45	57	63	42	56	62
[Co(apash)Cl]	53	72	91	50	70	88	49	63	89
[Ni(apash)Cl]	52	78	92	52	78	92	53	75	88
[Cu(apash)Cl]	58	87	100	53	74	94	52	68	82
[Co(Hapash)(H$_2$O)SO$_4$]	77	88	96	59	72	89	65	78	91
[Ni(Hapash)(H$_2$O)SO$_4$]	56	79	92	59	76	86	50	70	85
[Cu(Hapash)(H$_2$O)SO$_4$]	66	78	95	55	77	88	68	80	95
Miconazole (standard)	62	80	90	52	70	90	55	70	92

Compound	*E. coli* (mg/ml)					*Clostridium* sp. (mg/ml)				
	MIC (mg/ml)	Diameter of inhibition zone (in mm)		% Activity Index		MIC (mg/ml)	Diameter of inhibition zone (in mm)		% Activity Index	
		1.0	2.0	1.0	2.0		1.0	2.0	1.0	2.0
B. Antibacterial activity of the ligand and its complexes										
Hapash	0.8	2	3	13	17	0.7	3	5	21	31
[Co(apash)Cl]	0.5	10	12	67	67	0.5	11	14	79	88
[Ni(apash)Cl]	0.4	12	15	80	83	0.5	11	13	79	81
[Cu(apash)Cl]	0.4	12	17	80	94	0.4	12	14	86	88
[Co(Hapash)(H$_2$O)SO$_4$]	0.3	14	15	93	83	0.3	13	15	93	94
[Ni(Hapash)(H$_2$O)SO$_4$]	0.3	14	16	93	89	0.4	11	13	79	81
[Cu(Hapash)(H$_2$O)SO$_4$]	0.4	13	16	87	89	0.4	12	15	86	94
Ampicillin (standard)	0.2	15	18	100	100	0.3	14	16	100	100

[Mn(apash)Cl] and [Mn(Hspash)(H$_2$O)SO$_4$] complexes suggest a tetrahedral and an octahedral geometry, respectively.

ESR spectra

ESR spectra of [Cu(apash)Cl] in solid state at 298 K show an intense broad signal having no hyperfine structure (Fig. 1). The g$_{iso}$ value of 2.0794 suggests a geometry involving grossly misaligned axes (Hathaway and Billing 1970). This may be due to dipolar exchange and unresolved hyperfine interactions in solid state (Narang and Singh 1996). [Cu(Hspash)(H$_2$O)SO$_4$] shows an axial signal with two g values. The axial signals were analyzed by using the procedure given by Hathaway and Billing (1970). The both g values (g$_{||}$ = 2.2627 and g$_\perp$ = 2.0409) are >2.04
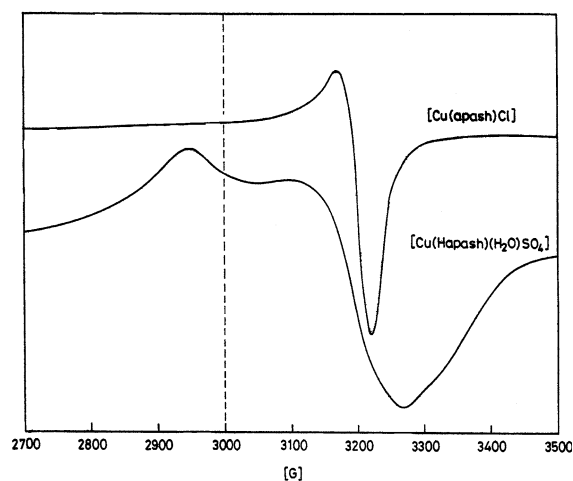
Fig. 1 ESR spectra of copper (II) complexes at 298 K in the solid state

(Table 3). These values indicate an elongated tetragonally distorted octahedral stereochemistry. The g_{av} and A_{av} values were, calculated using the equations $g_{av} = (g_{||} + 2g_\perp)/3$; $A_{av} = (A_{||} + 2A_\perp)/3$. The trend $g_{||} > g_\perp > g_e$ suggest that the unpaired electron is in the $d_{x^2-y^2}$ orbital of the copper(II) ion (Bindu et al. 1999; Singh et al. 2005).

IR spectra

The bonding of the ligand, Hapash to cobalt(II) has been judged by a careful comparison of the infrared spectra of the complexes with those of the free ligand. A few significant bands have been selected to observe the effect on ligand vibration in the complexes.

The ligand shows a broad band centered at 3,204 cm^{-1} due to v(N–H). In the metal(II) sulfate complexes these bands either occur at the same frequency as in the ligand or slightly shifted to higher frequency suggesting no participation of the >NH group in bonding. However, v(NH) is not observed in metal(II) chloride complexes indicating the involvement of >NH group in enolization during complexation. A broad band in the same region (3,430–3,440 cm^{-1}) assigned as v(OH) indicates the presence of –OH group or water molecules in the complexes (Nakamoto 1997).

The v(C=O) band observed at 1,663 cm^{-1} in the ligand is shifted to lower wave number by 15–23 cm^{-1} in its metal(II) sulfate complexes (Table 4) indicating coordination of the >C=O group to the metal ion (Shama and Omara 2001). In these complexes, amide II bands appear to have shifted considerably to lower frequency (13–14 cm^{-1}) compared to the ligand bands and a shift to higher frequency (6–15 cm^{-1}) in the amide III band in all the metal(II) sulfate complexes, further supporting the coordination through the >C=O. The disappearance of v(C=O) and appearance of v(N=C–O) and v(C–O) in the region 1,490–1,494 cm^{-1} and 1,311–1,314 cm^{-1}, respectively in all the metal(II) chloride complexes suggest bonding to the metal through deprotonated C–O group (Butcher et al. 1976).

The v(C=N) frequency observed at 1,627 cm^{-1} in the spectra of ligand shifted to lower frequency (17–25 cm^{-1}) in the metal complexes suggesting coordination through the two azomethine group (Narang and Singh 1993). v(N–N) observed at 977 cm^{-1} in the ligand shifts to higher frequency by 18–31 cm^{-1}

in the complexes, indicating the coordination of one of the nitrogen atom of the N–N group (Chohan et al. 2002). All the metal(II) sulfate complexes also show weak bands in the ranges 938–967 cm^{-1}, 747–756 cm^{-1} and 652–665 cm^{-1} due to coordinated water. The bands observed near 1,208–1,225 cm^{-1}, 1,152–1,165 cm^{-1} and 1,046–1,070 cm^{-1} indicate the presence of a bidentate chelating sulfate group in all the metal(II) sulfate complexes (Narang and Singh 1996). A non-ligand band appears in the region 560–575 cm^{-1} in all the complexes has been assigned to v(M–O).

On the basis of above discussion, general structures for all the metal complexes are proposed (Fig. 2).

Thermal analysis (TGA & DTA)

Thermal studies of [Co(apash)Cl] indicate that the complex is highly stable up to 272°C and shows no loss of weight. The partial decomposition of organic liquid in the complex starts at the temperature 273°C by an exothermic process. The complex shows various decomposition steps at different temperatures (273, 360 and 420°C), where significant heat liberation occurs as a result of ligand decomposition. The decomposition scheme for the complex has been given in Table 5.

X-ray diffraction studies

Since the complexes are insoluble in common organic solvents suitable for single crystal growth for X-ray analysis, X-ray powder diffraction patterns for two of the complexes were recorded and the

[M(apash)Cl]   [M(Hapash)(H$_2$O)SO$_4$]

where M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)
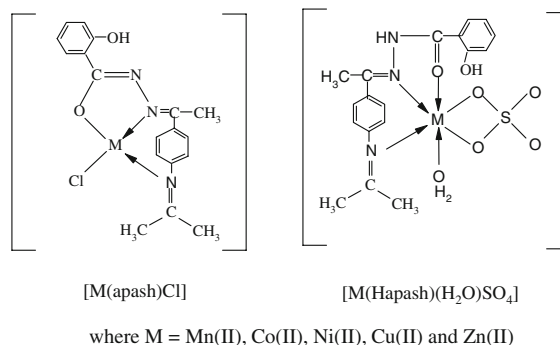
Fig. 2 Representative structures of the complexes

prominent line were indexed by Ito's method (Singh and Gupta 2006) (Table 6). The following lattice constants were calculated:

[Co(apash)Cl]
$a = 7.48, b = 7.48$ and $c = 10.54$ Å

[Cu(Hapash)(H$_2$O)SO$_4$]
$a = 8.31, b = 7.74$ and $c = 6.43$ Å

The above values of lattice constants indicate a tetragonal crystal lattice for the former and an orthorhombic crystal lattice for the latter complex.

Antifungal activity

The experimental antifungal activity data (Table 7A) indicate that the ligand as well as its complexes shows an appreciable activity against *Aspergillus* sp., *Stemphylium* sp. and *Trichoderma* sp. at 0.5, 1.0 and 1.5 mg/ml concentration. Their activity generally increases with increasing the concentration of the compounds. DMSO control has shown a negligible activity as compare to the metal complexes and ligands. The experimental results of the compounds were compared against DMSO as the control and are expressed as percentage inhibition versus control. The complexes are more effective against *Aspergillus* sp. than *Stemphylium* sp. and *Trichoderma* sp. [Cu(apash)Cl] shows the highest activity (100%) against *Aspergillus* sp. at the concentration of 1.5 mg/ml among all the metal complexes. The same complex also shows the highest activity (94%) against *Stemphylium* sp. The antifungal activity varies in the following order of fungal species.

Aspergillus sp. > *Stemphylium* sp. > *Trichoderma* sp.

All the metal complexes exhibited greater antifungal activity against *Aspergillus* sp. as compare to the standard drug Miconazole. The Ni(II) and Cu(II) chloride complexes show better activity against *Stemphylium* sp. than the standard, whereas, Cu(II) sulfate complex is more effective against *Trichoderma* sp.

From the data it has also been observed that the complexes are more active than the ligand (Thimmaiah et al. 1984). The toxicity of the complexes can be related to the strength of the metal-ligand bond, besides other factors such as size of the cation (Nagar 1990), receptor sites, diffusion and a combined effect of the metal and ligand (Johari and Sharma 1988) for inactivation of the biomolecules.

Antibacterial activity

The metal complexes, ligands, standard drug Ampicillin and DMSO solvent control were screened separately for their antibacterial activity against *E. coli* and *Clostridium* sp. at 1.0 and 2.0 mg/ml concentration. Their activity is greatly enhanced at the higher concentration (Abd El-Wahab and El-Sarrag 2004). The activity of the complexes has been compared with the activity of a common standard antibiotic Ampicillin and % Activity Index for the complexes has been calculated. The antibacterial results suggest that the ligands and their complexes (Table 7B) show a moderate activity against both the bacteria (Panchal et al. 2005; Deepa and Aravindakshan 2005) as compared to the standard drug (Ampicillin). The metal complexes show higher antibacterial activity than the ligands. The DMSO control showed no activity against any bacterial strain. The % Activity Index data show the highest activity (94%) for [Co(Hapash) (H$_2$O)SO$_4$] and [Cu(Hapash)(H$_2$O)SO$_4$] against *Clostridium* sp. at the concentration of 2.0 mg/ml. [Cu(apash)Cl] shows the highest activity (94%) against *E. coli* at 2.0 mg/ml concentration. [Cu(apash) Cl] shows the highest activity (94%) against *E. coli* at 2.0 mg/ml concentration. The complexes are more effective against *Clostridium* sp. than *E. coli* and show better antibacterial activity than the ligand.

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