Synthesis, spectral characterization and *in vitro* microbiological evaluation of novel glyoxal, biacetyl and benzil bis-hydrazone macrocyclic Schiff bases and their Co(II), Ni(II) and Cu(II) complexes

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ABSTRACT

A series of binuclear Co(II), Ni(II) and Cu(II) complexes were synthesized by the template condensation of glyoxal, biacetyl or benzil bis-hydrazide, 2,6-diformyl-4-methylphenol and Co(II), Ni(II) or Cu(II) chloride in a 2:2:2 M ratio in ethanol. These 22-membered macrocyclic complexes were characterized by elemental analyses, magnetic, molar conductance, spectral, thermal and fluorescence studies. Elemental analyses suggest the complexes have a 2:1 stoichiometry of the type $[M_2LX_2]\cdot nH_2O$ and $[Ni_2LX_22H_2O]\cdot nH_2O$ (where M = Co(II) and Cu(II); L = H_2L^1, H_2L^2 and H_2L^3; X = Cl; n = 2). From the spectroscopic and magnetic studies, it has been concluded that the Co(II) and Cu(II) complexes display a five coordinated square pyramidal geometry and the Ni(II) complexes have a six coordinated octahedral geometry. The Schiff bases and their metal complexes have also been screened for their antibacterial and antifungal activities by the MIC method.

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1. Introduction

Metal complexes of macrocyclic Schiff bases derived from 2,6-diformyl-4-methylphenol have great interest as potential compartmental ligands capable of forming mono and binuclear coordination compounds with various metals because of several donor centers in their molecules [1–10]. Currently, there is a growing interest in the design and synthesis of bis-hydrazones along with their metal complexes because of their applications as anticancer, antiviral, antibacterial and antifungal agents [11–13]. Our research has continued on bis-hydrazones with glyoxal, biacetyl and benzil with 2,6-diformyl-4-methylphenol. Many binuclear metal complexes have been studied extensively as phenoxo bridged acyclic and macrocyclic complexes of 2,6-diformyl-4-methylphenol, which have specific electric, magnetic, optical and catalytic properties [12,14–24].

A previous study has reported that the C2-symmetric dialkylamino substructure in glyoxal bis-hydrazones is the key design element resulting in the successful application of these compounds as novel N-ligands for the enantioselective copper(II)-catalyzed diels–alder cycloaddition reaction [25]. Bifunctional bis(thiosemicarbazone) ligands with arylcarboxylates appended at the exocyclic nitrogens have been developed and characterized by X-ray crystallography [26]. Very recently oxido-bridged copper benzoate, bis-hydroxido-bridged copper azide and copper thiocyanate complexes have been synthesized with 2,6-bis(benzyliminomethyl)-4-methylphenol. These complexes have been characterized by X-ray crystallography and their magnetic properties have been studied [19].

As a part of our continuing efforts to explore multidentate Schiff-base ligands for the building blocks of new supramolecular assemblies, we describe here the synthesis and spectral investigation of phenoxo-bridged binuclear Co(II), Ni(II) and Cu(II) transition metal ions with 2,6-diformyl-4-methylphenol and glyoxal, biacetyl and benzil bis-hydrazone Schiff bases. A survey of the literature reveal that no work has been carried out on the above mentioned metal complexes. These Schiff bases have donor sites with the NON NON sequence and varied coordination abilities. This nature of the Schiff bases (Scheme 1) have attracted our attention and aroused our interest in elucidating the structures of Co(II), Ni(II) and Cu(II) complexes. These complexes are evaluated for their antibacterial and antifungal properties against various pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Shigella dysentry*, *Micrococcus*, *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa*) and fungal strains (*Aspergillus niger*, *Penicillium* and *Candida albicans*) using the minimum inhibitory concentration (MIC) method.

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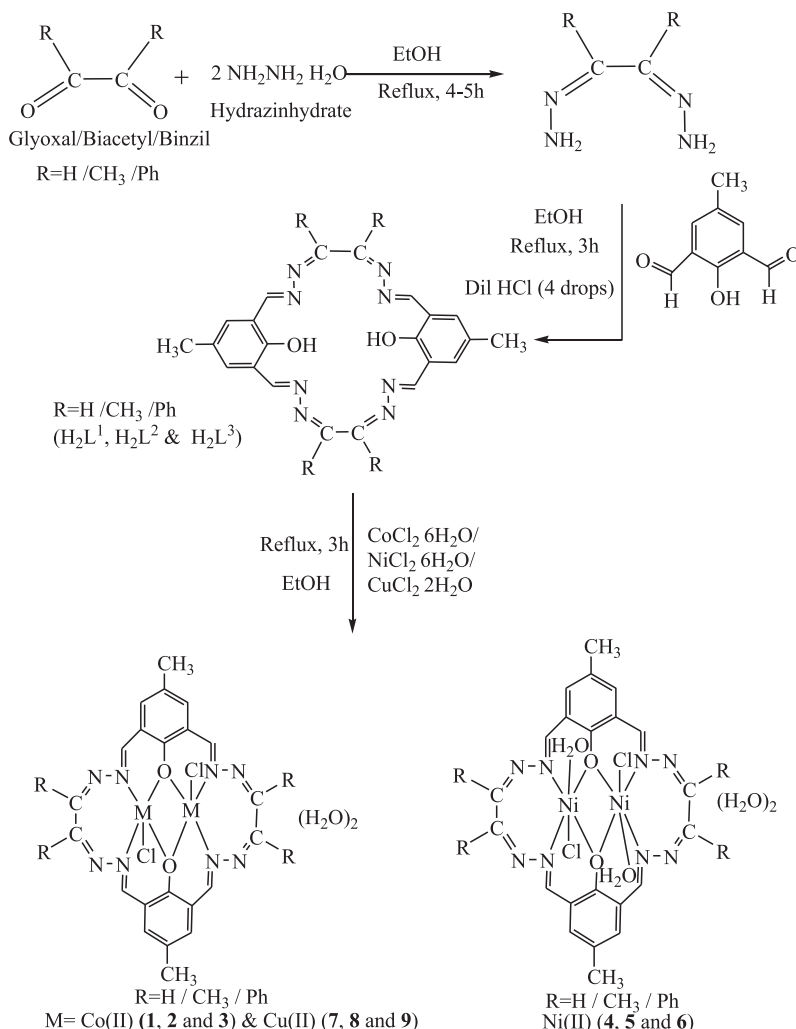
Scheme 1. Structures of the hexadentate Schiff bases H_2L^1, H_2L^2 and H_2L^3, five coordinated square pyramidal geometry of the Co(II) (**1**, **2** and **3**) and Cu(II) (**7**, **8** and **9**) complexes and six coordinated octahedral geometry of the Ni(II) (**4**, **5** and **6**) complexes.

2. Experimental

2.1. Physical measurements

The IR spectra of the Schiff base ligands and their Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000–250 cm^{-1} region as KBr disks. The electronic spectra of the complexes were recorded in DMF on a VARIAN CARY 50-BIO UV-spectrophotometer in the region 200–1100 nm. The ^{1}H NMR spectra of the Schiff bases were recorded in CDCl$_3$ on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10Am) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature, and *m*-nitrobenzyl alcohol was used as the matrix. The mass spectrometer was operated in the positive ion mode. Molar conductivity measurements were recorded on an ELICO-CM-82T conductivity bridge with a cell having a cell constant of 0.51, and magnetic moments were measured with a faraday balance. Thermogravimetric analysis data were measured from room temperature to 1000 °C at a heating rate of 10 °C/min. The data were obtained using a PERKIN-ELMER DIAMOND TG/DTG instrument.

2.2. Chemical and reagents

All chemicals used were of reagent grade. Solvents were distilled and dried before use according to standard procedures [27]. The remaining reagents were purchased from Ranbaxy chemicals and were used without further purification.

2.3. Analyses

The Co(II), Ni(II) and Cu(II) complexes were analyzed by a gravimetric method [28]. The results of elemental analyses (C, H and N), magnetic and molar conductance values agree well with the predicted structures.

2.4. Methods

2.4.1. Synthesis of the dihydrazides of glyoxal, biacetyl and benzyl

A mixture of glyoxal, biacetyl or benzil (0.1 mol) and hydrazine hydrate (2 cc) in ethanol was refluxed for 4–5 h. The reaction mixture was allowed to cool to room temperature, then the cooled solution was poured onto ice cold water. The dihydrazide of glyoxal, biacetyl or benzil thus obtained was filtered and recrystallized from ethanol and then dried in a vacuum over P$_2$O$_5$. Yield: 80%.

2.4.2. Preparation of 2,6-diformyl-4-methylphenol

2,6-Diformyl-4-methylphenol was prepared according to the reported method [29,30]. Note, skin contact with 2,6-diformyl-4-methylphenol causes persistent deep yellow stains.

2.4.3. Synthesis of glyoxal, biacetyl and benzil bis-hydrazone Schiff bases (H_2L^1, H_2L^2 and H_2L^3)

A mixture of glyoxal, biacetyl or benzil bis-hydrazide and 2,6-diformyl-4-methylphenol in 2:2 M proportions in an alcoholic medium containing a few drops of concentrated HCl were refluxed for 3–4 h. The product separated on evaporation of the alcohol and was recrystallized from ethanol. Yield: 75%.

2.4.4. Synthesis of $[M_2LX_2]·nH_2O$ and $[Ni_2LX_22H_2O]·nH_2O$ (where M = Co(II) and Cu(II); L = H_2L^1, H_2L^2 and H_2L^3; X = Cl; n = 2) complexes

To a hot solution of 2,6-diformyl-4-methylphenol (0.02 mol) in ethanol (20 ml), crystalline glyoxal, biacetyl or benzil bis-hydrazide (0.02 mol) was added, followed by the drop-wise addition of the metal salt (CoCl$_2$·6H$_2$O/NiCl$_2$·6H$_2$O/CuCl$_2$·2H$_2$O; (0.02 mol) in ethanol solution with constant stirring. The reaction mixture was refluxed for about an hour, then 2 g of sodium acetate (0.02 mol) in water was added and refluxing was continued for about 3 h. The metal(II) complex that separated was filtered and washed free of reagents with distilled water containing small quantity of ethanol, and then it was dried in a vacuum over fused CaCl$_2$.

3. Pharmacology

3.1. In vitro antibacterial and antifungal assay

The biological activities of the newly synthesized Schiff bases (H_2L^1, H_2L^2 and H_2L^3) and their Co(II), Ni(II) and Cu(II) metal complexes have been studied for their antibacterial and antifungal activities by the disk diffusion method [31,32]. The antibacterial activities were recorded for the following organisms: *E. coli*, *S. aureus*, *S. dysentry*, *Micrococcus* species, *B. subtilis*, *B. cereus* and *P. aeruginosa*. These bacterial strains were chosen as they are known pathogens of the human body, and *A. niger*, *Penicillium* species and *C. albicans* were used for antifungal activities at 10, 25, 50 and 100 μg/mL concentrations in the solvent DMF, where a DMF poured disk was used as a negative control. The bacteria were subcultured in agar medium. The petri dishes were incubated for 24 h at 37 °C. A standard antibacterial drug (Gentamycine) was also screened under similar conditions for comparison. The fungi were subcultured in potato dextrose agar medium. A standard antifungal drug (Fluconazole) was used for comparison. The petri dishes were incubated for 48 h at 37 °C (Figs. 1 and 2).

3.1.1. Minimum inhibitory concentration (MIC)

Compounds showing promising antibacterial/antifungal activity were selected for minimum inhibitory concentration studies.
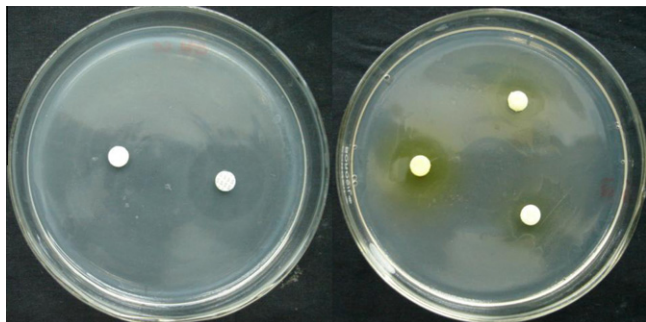

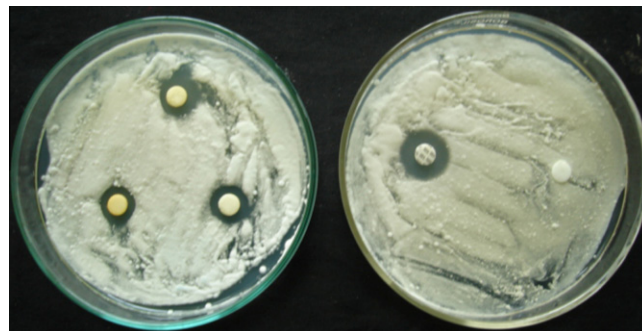**Fig. 1.** Photograph showing the antibacterial (*E. coli*) screening of gentamycine and the Ni(II) complex (**5**).

Fig. 2. Photograph showing the antifungal (*Candida albicana*) screening of the Schiff base H_2L^2 and flucanazole.

The MIC is the lowest concentration of an antibacterial compound that will inhibit the visible growth of microorganisms after 24 h incubation, and the minimum inhibitory concentrations are important in diagnostic laboratories to confirm the resistance of microorganisms to antimicrobial agents. The synthesized compounds were screened against bacterial and fungal strains through a macrodilution tube method [33]. The minimum inhibitory concentration was determined by assaying at 100, 50 and 25 μg ml^{-1} concentrations, along with standards at the same concentrations.

4. Results and discussion

The spectral and analytical data show the formula of the Co(II), Ni(II) and Cu(II) complexes as $[M_2LX_2]·nH_2O$ and $[Ni_2LX_22-H_2O]·nH_2O$ (where M = Co(II) and Cu(II); L = H_2L^1, H_2L^2 and H_2L^3; X = Cl; n = 2). All the Co(II), Ni(II) and Cu(II) complexes are colored, stable in air and non-hygroscopic solids. They are sparingly soluble in common organic solvents; however, these complexes are soluble in DMF and DMSO. The elemental analyses show that the Co(II), Ni(II) and Cu(II) complexes have a 2:1 stoichiometry of the type M$_2$:L. The molar conductance values at 10^{-3} M concentration are too low to account for any dissociation of the complexes in DMF. Hence, the Co(II), Ni(II) and Cu(II) complexes may be regarded as non-electrolytes. In order to establish whether the water molecules present in the complexes coordinated to the metal ion, weighed complexes were dried in over P$_2$O$_5$ in vacuum desiccators for 1 h and then weighed again. No loss in weight was observed. These observations suggest that the water molecules in the complexes are coordinated to the metal ion. The spectroscopic and magnetic studies conclude a five coordinated square pyramidal geometry for the Co(II) and Cu(II) complexes and a six coordinated octahedral geometry for the Ni(II) complexes. The elemental analytical data of the Schiff bases and their Co(II), Ni(II) and Cu(II) complexes are listed in Tables 1 and 2 respectively.

4.1. Infrared spectra

The important IR frequencies of the Schiff bases and their Co(II), Ni(II) and Cu(II) complexes are presented in Tables 3 and 4. In order to study the binding mode of the Schiff base to the metal ion in the complexes, the IR spectrum of the free Schiff base was compared with the spectra of the transition metal complexes. A strong band is observed for the free Schiff bases at around 1630 cm^{-1}, characteristic of the azomethine (C=N) stretching vibrations. In the metal chelates, the band characteristic of the azomethine group is shifted to 1610–1590 cm^{-1} from 1630 cm^{-1} in the ligand, suggesting coordination of the azomethine nitrogen atom to the metal ion [34,35]. In addition to this, a medium intensity broad band observed at around 3100 cm^{-1} in the Schiff bases is due to

Table 1
Analytical data of Schiff bases H_2L^1, H_2L^2 and H_2L^3.

Comp. No.	Empirical formula	C%		H%		N%		Yield (%)
		Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.	
H_2L^1	$(C_{22}H_{20}N_6O_2)$	61.57	61.68	4.75	4.67	19.56	19.62	64
H_2L^2	$(C_{26}H_{28}N_6O_2)$	64.41	64.46	5.69	5.78	17.28	17.35	67
H_2L^3	$(C_{46}H_{36}N_6O_2)$	75.36	75.4	4.85	4.91	11.41	11.47	70

Table 2
Elemental analysis, magnetic and molar conductance data of the Co(II), Ni(II) and Cu(II) complexes of the Schiff bases H_2L^1, H_2L^2 and H_2L^3.

Comp.No.	Empirical formula	M%		N%		Cl%		λ_m (cm^2 Ω^{-1} mol^{-1})	μ_{eff} (BM)
		Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.		
1	$[Co_2(H_2L^1)Cl_2]\cdot2H_2O$	18.12	18.15	17.18	17.23	10.72	10.76	22.16	4.23
2	$[Co_2(H_2L^2)Cl_2]\cdot2H_2O$	16.65	16.71	15.79	15.86	9.87	9.91	21.87	4.42
3	$[Co_2(H_2L^3)Cl_2]\cdot2H_2O$	12.30	12.36	11.68	11.74	7.25	7.33	20.65	4.84
4	$[Ni_2(H_2L^1)Cl_2(H_2O)_2]\cdot2H_2O$	16.89	16.95	16.31	16.37	10.18	10.23	24.57	2.52
5	$[Ni_2(H_2L^2)Cl_2(H_2O)_2]\cdot2H_2O$	15.61	15.67	15.09	15.13	9.37	9.45	25.32	3.24
6	$[Ni_2(H_2L^3)Cl_2(H_2O)_2]\cdot2H_2O$	11.68	11.74	11.25	11.33	6.92	7.08	26.54	3.53
7	$[Cu_2(H_2L^1)Cl_2]\cdot2H_2O$	19.11	19.17	16.96	17.04	10.61	10.65	23.32	1.42
8	$[Cu_2(H_2L^2)Cl_2]\cdot2H_2O$	17.58	17.64	15.59	15.68	9.78	9.80	22.76	1.40
9	$[Co_2(H_2L^3)Cl_2]\cdot2H_2O$	13.01	13.09	11.61	11.64	7.21	7.27	21.14	1.45

Table 3
The important infrared frequencies (in cm^{-1}) of the Schiff bases H_2L^1, H_2L^2 and H_2L^3.

Ligand No.	v(C=N)	H-bonded-OH	v_{CH_3}	Phenolic C–O
H_2L^1	1624	2744	2932	1291
H_2L^2	1626	2735	2927	1283
H_2L^3	1630	2726	2932	1285
H_2L^4	1620	2741	2924	1294

v(NH). A high intensity band is present in the 1295–1285 cm^{-1} region, with an additional band around 1500 cm^{-1}, which were assigned to phenolic v(C–O) vibrations [35,36].

In the Schiff bases it is generally observed that an intramolecular H-bonded-OH band occurs in the region 2700–2600 cm^{-1} as a broad weak band with fine structure [37]. These bands are missing in the metal complexes indicating that the phenol character of the ligand has been lost upon complexation. The bands present in the range 3010–3050 cm^{-1} may be assigned to v(C–H) stretching vibrations of benzil. The various absorption bands in the region 1450–1588 cm^{-1} may be assigned to v(C=C) aromatic stretching vibrations of the benzil ring. The bands in the region 740–785 cm^{-1} may be assigned to n(C–H) out of plane bending of the aromatic rings.

The presence of broad stretching vibrations in the 3440–3400 cm^{-1} region can be attributed to coordinated or lattice water molecules in all these complexes [38]. Broad bands in the regions 550–400, 474–400 and 340 cm^{-1} are assigned to v(M–O), v(M–N) and v(M–Cl) vibrations respectively [39–44].

4.2. ^{1}H NMR spectra

The ^{1}H NMR spectra of the Schiff bases H_2L^1, H_2L^2 and H_2L^3 exhibited a signal at 11.5 ppm due to the azomethine protons. The spectra of the ligands show the –N–H···O resonance at 13.72 ppm [45,46]. We also observed a multiplet at 7.5–7.8 ppm due to aromatic protons. All the compounds exhibited a signal at 10.50 ppm due to the phenolic OH proton. The compounds exhibited a signal at 2.5 ppm due to methyl protons. All these observations provide support for the IR inferences.

4.3. Electronic absorption spectra

The electronic absorption spectra of the Co(II), Ni(II) and Cu(II) complexes have been studied with a view to obtain more information on the configuration of the complexes. The electronic spectra of the Co(II) complexes exhibit absorption bands in the regions 10 965, 17 134 and 19 972 cm^{-1}, which can be assigned to the transitions $^4A_2 + {}^4E \rightarrow {}^4B_1$, $^4A_2 + {}^4E \rightarrow {}^4E$ (P) and $^4A_2 + {}^4E \rightarrow {}^4A_2$ (P) respectively and are characteristic of a square pyramidal geometry [47]. The Ni(II) complexes shown three bands at 10 992, 15 259 and 21 968 cm^{-1} which are assigned to $^3A_{2g}$ (F) $\rightarrow$ $^3T_{2g}$ (F) (v_1), $^3A_{2g}$ (F) $\rightarrow$ $^3T_{1g}$ (F) (v_2) and $^3A_{2g}$ (F) $\rightarrow$ $^3T_{1g}$ (P) (v_3) transitions respectively, indicating an octahedral geometry [47]. The Cu(II) complexes exhibited a high intensity band at 27 468 cm^{-1} in the UV-region. The appearance of this band is due to a $\pi \rightarrow \pi^*$ transition associated with the azomethine linkage and a L $\rightarrow$ M charge transfer transition. The electronic spectra of the Cu(II) complexes also show three bands at 10 972, 14 828 and 23 156 cm^{-1}, and

Table 4
The important infrared frequencies (in cm^{-1}) of the Co(II), Ni(II) and Cu(II) complexes of the Schiff bases H_2L^1, H_2L^2 and H_2L^3.

Complex No.	v(OH)	v(C=N)	v_{CH_3}	Phenolic C–O	v(M–N)	v(M–O)	v(M–Cl)
1	3430	1607	2926	1548	464	535	325
2	3425	1590	2918	1550	470	525	330
3	3436	1608	2922	1535	435	536	326
4	3440	1612	2930	1538	422	550	335
5	3421	1603	2915	1542	448	533	322
6	3434	1595	2921	1536	436	521	331
7	3426	1608	2930	1542	454	540	324
8	3438	1593	2917	1544	435	532	340
9	3434	1591	2930	1550	463	545	328

these bands have been assigned to the transition $^2B_1 \rightarrow {}^2A_{1\ (\nu1)}$, $^2B_1 \rightarrow {}^2B_2\ (\nu_2)$ and $^2B_1 \rightarrow {}^2E\ (\nu_3)$ respectively. These transitions are characteristic of a square pyramidal geometry [47,48].

4.4. Magnetic studies

The magnetic moments of the Co(II), Ni(II) and Cu(II) complexes were obtained at room temperature. The Co(II) (1–3) and Ni(II) (4–6) complexes shown magnetic moment values of around 4.23–4.84 and 2.52–3.53 BM respectively, which are lower than the respective spin only values and indicate weak antiferromagnetic coupling interactions between the metal ions, further confirming the dinuclear nature of the complexes. The Cu(II) complexes showed a magnetic moment value of around 1.45 MB, which is considerably lower than the spin only value. The low value of the magnetic moment is attributed to an antiferromagnetic coupling interaction between two metal ions. This fact suggests the dinuclear nature of the Cu(II) complexes [49].

4.5. FAB mass spectra of the Schiff base H_2L^1 and its Cu(II) complex (7)

The FAB spectrum of the Schiff base H_2L^1 showed a molecular ion peak at m/z 773 (Fig. 3) which is equivalent to its molecular weight. The fragments in the spectrum lead to the formation of the species $[C_{46}H_{36}N_8O_2]^+$. The FAB mass spectrum of the Cu(II) complex of the ligand H_2L^1 (7) showed a molecular ion peak M^+ at m/z 963, which is equivalent to the molecular weight of the Cu(II) complex (7) shown in Fig. 4. The molecular ion peak at m/z 926 is due to the loss of two water molecules.

4.6. ESR spectrum of Cu(II) complex (7)

The X-band ESR spectrum of the Cu(II) complex with the ligand H_2L^1 (7) was recorded at room temperature using DPPH as a reference standard. The $g_\parallel$ and $g_\perp$ values have been found to be 2.089 and 2.024 respectively. In general, dinuclear Cu(II) complexes give broad ESR peaks and the broadening is assigned to a dipolar inter-
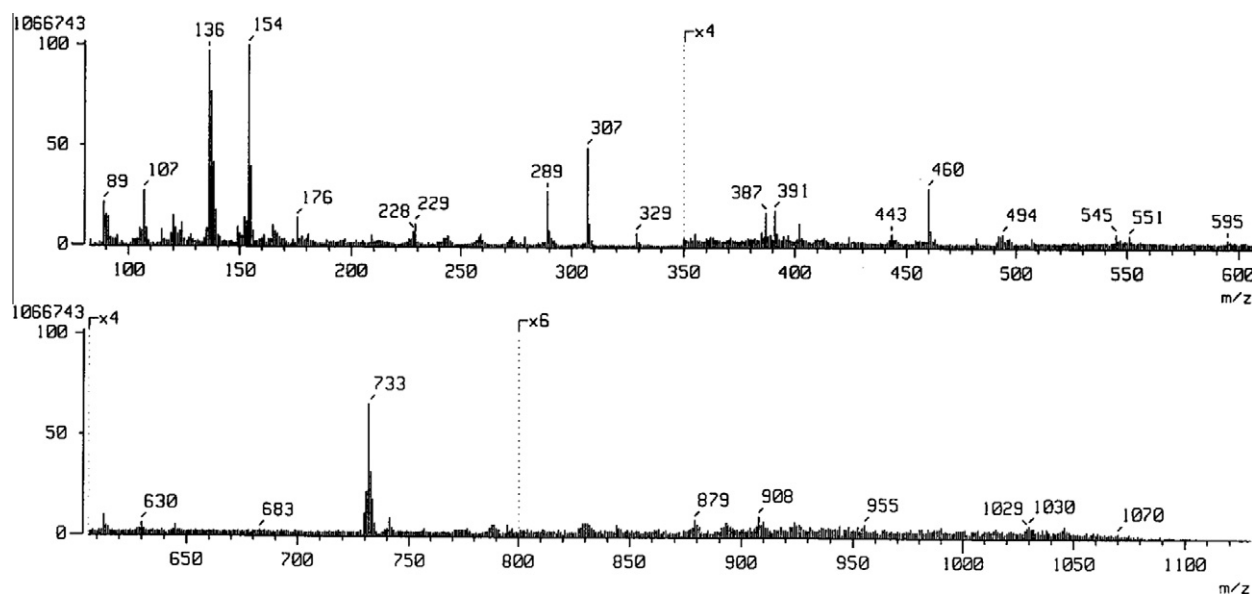
Fig. 3. FAB-mass spectrum of the Schiff base H_2L^1.
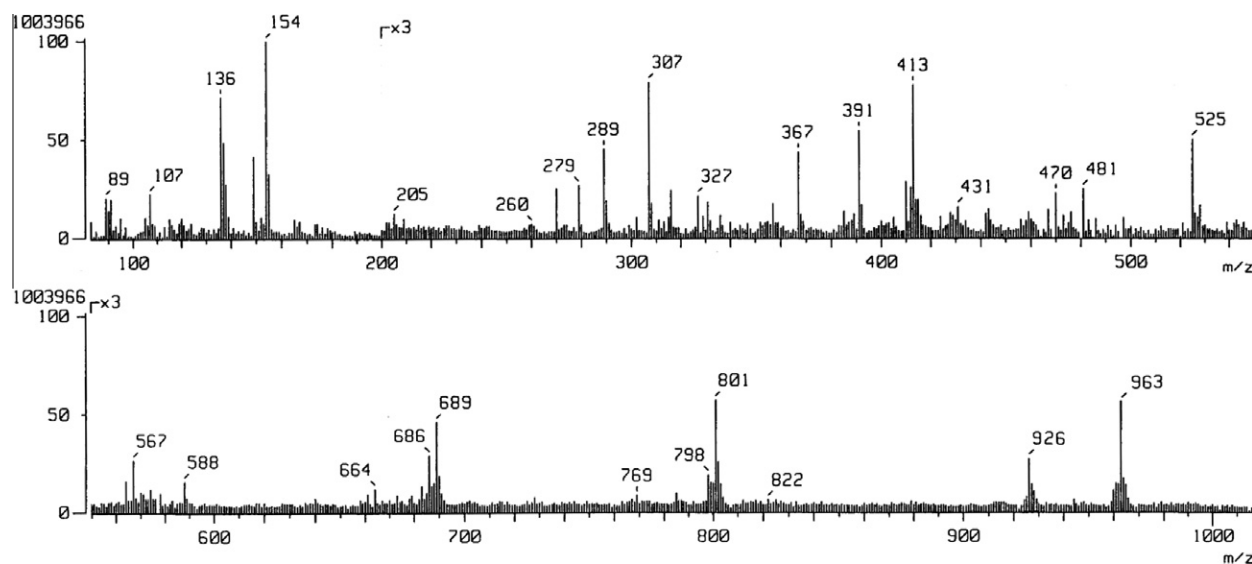
Fig. 4. FAB-mass spectrum of the Cu(II) complex (7).
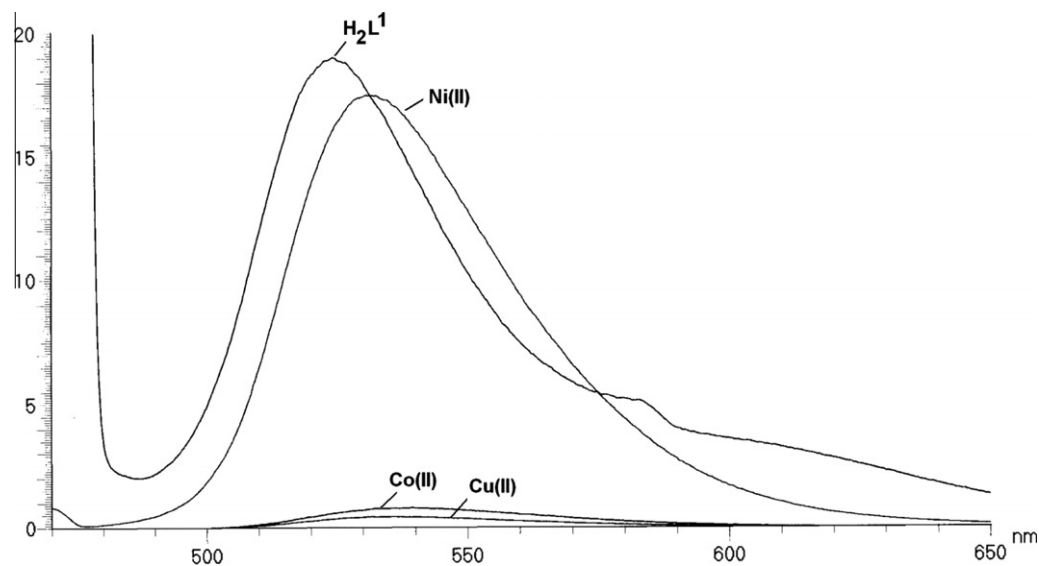
Fig. 5. Emission spectra of the Schiff base H_2L^1 and its Co(II) (**1**), Ni(II) (**4**) and Cu(II) (**7**) complexes in DMSO.

Table 5
Thermogravimetric data of the Co(II) (**2**), Ni(II) (**5**) and Cu(II) (**8**) complexes of the Schiff base H_2L^2.

Comp. No.	Empirical formula	Decomposition temperature (°C)	% Weight loss		Inference
			Obsd.	Calcd.	
2	$[Co_2(H_2L^2)Cl_2]\cdot 2H_2O$	125–130	5.02	5.09	Loss of water molecules
		242–253	44.96	45.08	Loss of aldehyde moieties
		315–332	31.12	31.16	Loss of bishydrazone moieties
5	$[Ni_2(H_2L^2)Cl_2(H_2O)_2]\cdot 2H_2O$	115–139	4.78	4.86	Loss of coordinated water molecules
		250–265	42.91	42.97	Loss of one aldehyde moieties
		342–362	29.69	29.72	Loss of one bishydrazone moieties
8	$[Cu_2(H_2L^2)Cl_2]\cdot 2H_2O$	110–120	4.98	5.04	Loss of water molecules
		237–240	44.48	44.53	Loss of aldehyde moieties
		310–320	30.76	30.81	Loss of bishydrazone moieties

Fig. 6. Thermogravimetric (TG/DTG) curves of the Ni(II) complex (**5**).

Table 6
Selected bond lengths (Å) and bond angles (°) of the Cu(II) complex (**7**).

Compound No. (**7**)	Bond lengths (Å)	Compound No. (**7**)	Bond angles (°)
C(7)–N(29)	1.49161	C(5)–C(7)–N(29)	128.048
C(16)–N(28)	1.29690	C(7)–N(29)–N(8)	113.210
C(6)–N(27)	1.29697	N(8)–C(10)–C(12)	131.831
C(17)–N(26)	1.29928	C(10)–C(12)–N(15)	132.921
N(29)–N(8)	1.41698	C(12)–N(15)–N(28)	119.985
N(28)–N(15)	1.38493	N(15)–N(28)–C(16)	112.392
N(26)–N(9)	1.39200	N(28)–C(16)–C(20)	126.759
N(27)–N(14)	1.38492	C(16)–C(20)–C(23)	117.650
N(8)–C(10)	1.27944	C(20)–C(23)–C(19)	122.922
N(15)–C(12)	1.28119	C(23)–C(19)–C(22)	116.572
N(9)–C(11)	1.27945	C(19)–C(22)–C(21)	122.878
N(14)–C(13)	1.28118	C(22)–C(21)–C(18)	119.632
C(1)–C(5)	1.42629	C(21)–C(18)–C(20)	117.188
C(5)–C(7)	1.43919	C(18)–C(20)–C(23)	119.732
C(10)–C(12)	1.47903	C(16)–C(20)–C(18)	121.877
C(16)–C(20)	1.44424	C(20)–C(18)–C(21)	117.188
C(20)–C(18)	1.42803	C(18)–C(21)–C(17)	123.019
C(18)–C(21)	1.42631	C(22)–C(21)–C(17)	116.960
C(21)–C(17)	1.43912	C(26)–C(17)–C(21)	128.095
C(11)–C(13)	1.47898	C(17)–N(26)–N(9)	113.182
C(6)–C(4)	1.44417	N(26)–N(9)–C(11)	119.472
C(4)–C(1)	1.42801	N(9)–C(11)–C(13)	131.862
C(4)–C(3)	1.40288	C(13)–N(14)–N(27)	119.991
C(4)–C(45)	1.39687	N(27)–C(6)–C(4)	126.750
C(45)–C(2)	1.39250	C(4)–C(1)–C(5)	117.190
C(2)–C(5)	1.40858	Cu(30)–O(24)–Cu(31)	106.289
C(45)–C(46)	1.50974	O(24)–Cu(31)–O(25)	73.727
C(20)–C(23)	1.40287	Cu(31)–O(25)–Cu(30)	106.278
C(23)–C(19)	1.39690	O(25)–Cu(30)–O(24)	73.707
C(19)–C(22)	1.39245	N(29)–Cu(31)–O(28)	96.538
C(22)–C(21)	1.40861		
C(19)–C(47)	1.50973		
C(1)–O(24)	1.30740		
C(18)–O(25)	1.30739		
O(24)–Cu(13)	2.02873		
O(25)–Cu(31)	1.96210		
O(25)–Cu(30)	2.02933		
O(24)–Cu(30)	1.96242		
Cl(32)–Cu(30)	2.34122		
Cl(33)–Cu(31)	2.34039		
N(29)–Cu(31)	1.75416		
N(28)–Cu(31)	2.09015		
N(26)–Cu(30)	1.93543		
N(27)–Cu(30)	2.08855		

action [50]. The observed ESR spectrum is characteristic of a square pyramidal geometry. The g_{av} value was calculated to be 2.046. The existence of $g_{\parallel} > g_{\perp}$ suggests that the $d_{x^2-y^2}$ orbital is in the ground state and the d^9 configuration is $(e_g)^4(a_{1g})^2(b_{2g})^2(b_{1g})^1$. The g values are related to the axial symmetry and $g_{\parallel} > g_{\perp}$ suggests a square

pyramidal geometry of the Cu(II) complex. The axial symmetry parameter $G = 3.70$, which being less than 4.0 indicates a considerable exchange interaction between the metal ions in the solid complex, which further supports the dinuclear nature of the Cu(II) complexes.

4.7. Fluorescence studies

The emission spectrum of the Schiff base H_2L^1 and its Co(II) (**1**), Ni(II) (**4**) and Cu (II) (**7**) complexes were investigated in various solvents, viz., DMF, DMSO, MeCN and THF. The Schiff base H_2L^1 shows a weak absorption band at 524 nm, Fig. 5, which is due to the 2,6-diformyl-4-methylphenolate ion. The Co(II) (**1**), Ni(II) (**4**) and Cu (II) (**7**) complexes were characterized by an emission band at around 535 nm in DMF and DMSO, and it is also observed that the emission band of the Schiff base, at around 524 nm, was blue shifted in the Co(II) (**1**), Ni(II) (**4**) and Cu(II) (**7**) complexes due to the interaction of the phenolic oxygen with the metal ions (Fig. 5). We also observed a fluorescence quenching interaction in the above mentioned complexes in DMF and DMSO solutions.

4.8. Thermal studies

TG and DTG studies were carried out for the Co(II) (**2**), Ni(II) (**5**) and Cu(II) (**8**) complexes. These complexes decomposed gradually with the formation of the respective metal oxide above 400 °C. The nature of the proposed chemical change with temperature range and the percentage loss of compounds obtained are given in Table 5. The thermal decomposition of the Co(II) (**2**), Ni(II) (**5**) and Cu(II) (**8**) complexes occurs in three steps, as indicated by DTG peaks corresponding to the mass loss of coordinated water molecules, aldehyde moieties and bis-hydrazone moieties respectively (Fig. 6).

4.9. Molecular modeling

In the absence of a crystal structure, to obtain the molecular conformation of a complex energy minimization studies were carried out. These studies were performed using GAUSSIAN 09 software. Optimization was performed using the B3LYP/6-31+G* basis set in the gas phase along with frequency to obtain a global minimum of the Cu(II) (**7**) complex as a representative example. The metal–ligand bond lengths and bond angles are given in Table 6.

4.10. Pharmacology results

The microbial results of the Schiff bases and their Co(II), Ni(II) and Cu(II) complexes are recorded in Table 7. The biological activ-

Table 7
Bacteriological results of the Schiff bases H_2L^1, H_2L^2 and H_2L^3 and their Co(II), Ni(II) and Cu(II) complexes at a concentration of 25 µg ml^{-1}.

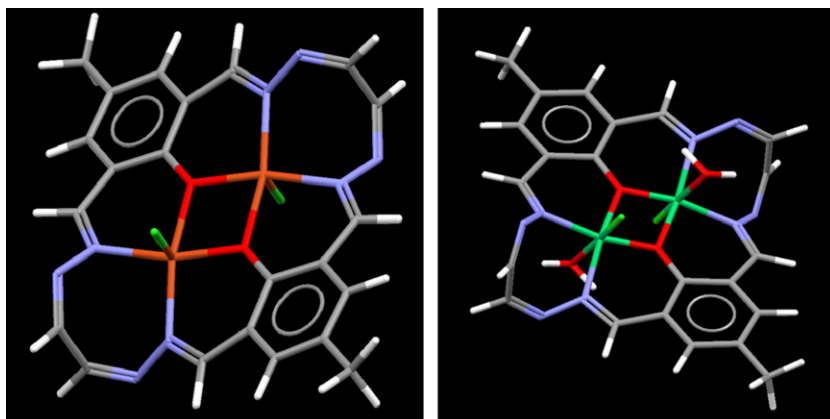| Empirical formula	Activity against bacteria (mm)							Activity against fungi (mm)		
	E. coli	P. aeruginosa	B. cereus	S. aureus	S. dysentry	Micrococcus	B. subtilis	A. niger	Penicillium	C. albicans
$(C_{22}H_{20}N_6O_2)$	08	10	12	14	08	08	06	14	10	12
$(C_{26}H_{28}N_6O_2)$	10	12	16	12	12	10	08	16	14	14
$(C_{46}H_{36}N_6O_2)$	12	10	14	12	10	12	08	14	12	12
$[Co_2(H_2L^1)Cl_2]\cdot 2H_2O$	08	12	10	14	10	10	10	14	12	10
$[Co_2(H_2L^2)Cl_2]\cdot 2H_2O$	12	16	14	14	12	12	08	16	12	12
$[Co_2(H_2L^3)Cl_2]\cdot 2H_2O$	10	14	12	12	12	10	10	12	10	10
$[Ni_2(H_2L^1)Cl_2(H_2O)_2]\cdot 2H_2O$	10	10	10	10	08	08	06	14	12	14
$[Ni_2(H_2L^2)Cl_2(H_2O)_2]\cdot 2H_2O$	14	12	12	12	10	10	10	16	10	10
$[Ni_2(H_2L^3)Cl_2(H_2O)_2]\cdot 2H_2O$	12	10	14	14	08	12	08	14	12	14
$[Cu_2(H_2L^1)Cl_2]\cdot 2H_2O$	12	12	10	12	12	10	10	14	12	12
$[Cu_2(H_2L^2)Cl_2]\cdot 2H_2O$	14	14	12	14	10	08	12	16	10	12
$[Co_2(H_2L^3)Cl_2]\cdot 2H_2O$	12	10	14	10	12	12	10	12	10	10
Gentamycine	20	20	20	20	20	20	20	–	–	–
Flucanazole	–	–	–	–	–	–	–	24	24	24
DMF	12	12	12	12	12	12	12	12	12	12

Fig. 7. Spartan minimized structures of the Cu(II) (**7**) and Ni(II) (**4**) complexes.

ity of the Schiff bases exhibited a considerable enhancement on coordination with the metal ions against all fungal strains, and the metal complexes showed good antifungal activity against *A. niger*, *Penicillium* species and *C. albicans*. It was evident from the data that this activity significantly increased on coordination. This enhancement in the activity may be rationalized on the basis that their structures mainly possess an additional C=N bond. It has been suggested that Schiff bases with nitrogen and oxygen donor systems inhibit enzyme activity since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. Moreover, coordination reduces the polarity [51,52] of the metal ion, mainly because of the partial sharing of its positive charge with the donor groups [53,54] within the chelate ring system formed during coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the microorganism [55–57], thus destroying them more aggressively.

Some important factors, such as the nature of the metal ion, nature of the ligand, coordinating sites, geometry of the complex, concentration, hydrophilicity, lipophilicity and presence of co-ligands, have considerable influence on the antibacterial activity. Certainly, steric and pharmacokinetic factors also play a decisive role in deciding the potency of an antimicrobial agent. Apart from this, the mode of action of these compounds may also invoke hydrogen bonds through the >C=N–N=CH– group with the active centers and thus interfere with the normal cell process. The presence of lipophilic and polar substituents is expected to enhance antibacterial activity. Bis-hydrazone ligands with multifunctionality have a greater chance of interaction either with nucleoside bases (even after complexation with metal ions) or with biologically essential metal ions present in the biosystem, and can be promising candidates as bactericides since they always tend to interact, especially with some enzymatic functional groups, in order to achieve higher coordination numbers [58].

5. Conclusion

The synthesized 22-membered macrocyclic glyoxal, biacetyl and benzil bis-hydrazone Schiff bases act as hexadentate ligands. The metal ion is coordinated through the azomethine nitrogen and phenolic oxygen atoms in all the Schiff bases. The bonding of the ligands to the metal ion was confirmed by the analytical, IR, NMR, ESR, electronic, magnetic, FAB mass and thermal data. All compounds are insoluble in water but soluble in DMF and DMSO solvents, and decompose at high temperatures. All these observations put together lead us to propose the following structures

shown in Fig. 7 (Scheme 1). Schiff bases and their Co(II), Ni(II) and Cu(II) complexes were found to be potentially active towards microbial strains.

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