

Research Article

Synthesis, Characterization and Antimicrobial Studies of a Few Mn^{II}, Fe^{II}, Zn^{II}, Cd^{II} and Hg^{II} Complexes Derived from N-[Morpholino(phenyl)methyl]acetamide as New Mannich Base Ligand

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Abstract

A new Mannich base ligand: N-[Morpholino(phenyl)methyl]acetamide(MBA) was synthesized and characterized by elemental analyses, IR, UV, NMR and mass spectral studies. A few complexes of this ligand with Mn(II), Fe(II), Zn(II), Cd(II) and Hg(II) were prepared and characterized by elemental and chemical analyses, IR, electronic absorption and ¹H NMR spectral studies and magnetic susceptibility measurements. The ligand behaved as a bidentate/tridentates chelating in

nature. Tetrahedral geometry was proposed for Cd^{II} sulphato complex. Octahedral geometry was assigned for the remaining complexes. Antimicrobial studies were carried out which showed that the Zn(II) complex was more active than the other complexes.

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Introduction

Mannich bases have often been used as chelating ligands in the field of coordination chemistry. Their metal complexes are found to be of great interest during recent years. It is well known that N, O and S atoms play an important role in the coordination of metals at the active sites of numerous metalloproteins [1]. These metal complexes have been widely studied, because they have industrial, antifungal, antibacterial, anticancer and herbicidal applications [2,3]. They serve as models for biologically important species and find applications in biomimetic catalytic reactions. They are of special interest because of the variety of ways in which they are bonded to metal ions. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities [4-6]. The possibility of synthesizing Mannich base metal complexes with a wide choice of ligands, and their coordination environments, has prompted us to undertake research in this area [7]. Zinc can function as an active site of hydrolytic enzymes, where it is coordinated to hard donors (N or O). It has long been recognized as an important co-factor in biological molecules, either as a structural template in protein folding or as a Lewis acid catalyst [8-10]. There is a substantial interest in the coordination chemistry of cadmium complexes, because of its toxic environmental impact. The mobilization and immobilization of cadmium in the environment, in organisms, and in some technical processes have been shown to depend

significantly on the complexation of the metal center by chelating nitrogen donor ligands [11,12].

As a part of our work on Mannich base complexes containing N, S and O donor atoms [13], and in light of the importance of Cd and Zn metals, we now report the synthesis and characterization of Mn^{II}, Fe^{II}, Zn^{II}, Cd^{II} and Hg^{II} complexes of the ligand N-[Morpholino(phenyl)methyl]acetamide(MBA), which can be a bi- or tridentate ligand. This is because, it contains three donor atoms: carbonyl oxygen, CNC nitrogen and amido nitrogen.

Experimental

High purity acetamide(Merck), benzaldehyde(Merck), morpholine(Merck) were used as supplied. All other solvents and metal salts used were of A.R. grade and used as received. Micro elemental (C, H and N) data were obtained with Carlo Erba 1108 elemental analyzer at RSIC, CDRI, Lucknow. Metal contents were estimated by usual procedure, after digesting the complexes with con. HNO₃. Sulphate was estimated gravimetrically as BaSO₄ and chlorides were estimated volumetrically by Volhard's method. The conductance data were obtained in ~10⁻³ M DMF solutions of the complexes at room temperature using a systronics direct reading digital conductivity meter-304 with dip type conductivity cell. IR spectra were recorded using

spectrum-one Perkin Elmer FT-IR spectrometer by using KBr pellets. The UV-Visible regions were recorded in DMF solutions using double beam UV-Visible spectrometer, Perkin EZ-301 of working range 1100-190 nm. The ^1H and ^{13}C NMR of the ligand and its d^{10} metal complexes were recorded on a bruker instrument and on a JEOL-GSX 400 spectrometer employing TMS as internal reference and DMSO- d_6 as solvent at ambient temperature. The FAB mass recorded for the ligand was carried out using a JEOL GC mate mass spectrometer. The room temperature magnetic susceptibility measurements of the complexes were made by using a Gouy magnetic balance calibrated using mercury (II)tetrathiocyanatocobaltate(II).

Antimicrobial Screening

Antibacterial activities of MBA and their sulphato metal complexes, such as Mn^{II} , Fe^{II} , Zn^{II} and Cd^{II} were tested *in vitro* against six bacterial species *E.coli*, *P.aeruginosa*, *S.typhi*, *B.subtilis*, *S.pyogenes* and *S.aureus* and the fungal species *A.niger* and *A.flavus* by disc diffusion method using agar nutrient as medium and gentamycin as control. The paper disc containing the compound (10, 20 and 30 $\mu\text{g}/\text{disc}$) was placed on the surface of the nutrient agar plate previously spread with 0.1 mL of sterilized culture of microorganism. The diameter of inhibition zone around the paper disc was measured after incubating this at 37°C for 24 hrs.

Synthesis of the Ligand

N-[Morpholino(phenyl)methyl]acetamide(MBA) was synthesized by employing the Mannich synthetic route[14]. Acetamide(5.90 g, 0.1 mol) was dissolved in minimum quantity of ethanol. To this solution, benzaldehyde(10 mL, 0.1 mol) followed by morpholine(9 mL, 0.1 mol) were added in small

quantities with constant stirring in an ice bath. After 28 days a yellow solid was obtained. It was then washed with water and with acetone. The compound was dried in air and then at 60°C in an air oven and recrystallised from ethanol.

Molecular formula: $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_2$, Yield: 73 %, MP: $148\text{-}150^\circ\text{C}$, Mol.wt: 234, FT-IR KBr in cm^{-1} : 3297(NH), 1647(C=O), 1493, 1449, 1206(methylene CH_2), 1116(C-N-C), 1049, 1034(C-O-C), 749(monosubstituted morpholine). ^1H NMR (300MHz, DMSO- d_6) δ 8.44(s, NH), 5.61(s, CH_2), 7.44 - 7.28(m, 5H phenyl ring), 2.51(m, CH_2), 3.58(m, $\text{O}(\text{CH}_2)_2$). ^{13}C NMR (300MHz, DMSO- d_6) 170.13(s, C=O), 139.71 - 127.76(m, 5C, benzene ring), 66.70(s, $\text{O}(\text{CH}_2)_2$), 48.98 (s, $\text{N}(\text{CH}_2)_2$). UV-Vis(DMF) 286($n \rightarrow \pi^*$), 242($\pi \rightarrow \pi^*$). FABMS $m/z = 234(\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_2)$, $m/z = 143(\text{C}_6\text{H}_{11}\text{N}_2\text{O}_2^+)$, $m/z = 114(\text{C}_5\text{H}_{10}\text{N}_2\text{O}^+)$, $m/z = 86(\text{C}_4\text{H}_8\text{NO}^+)$. Calculated; C 66.67 %, H 7.69 % and N 11.97 %. Found: C 66.41 %, H 7.74 % and N 11.65 %.

Synthesis of the Complexes

The hot methanolic solution of the metal salt was added slowly with constant stirring to the hot ethanolic solution of the ligand in 2:1 mol ratio. The insoluble complexes formed were filtered, washed with methanol/ethanol to remove the unreacted metal and ligand, dried in air and then in an air oven at 80°C .

Results and Discussion

To find out the stoichiometry of the complexes, the percentage of the metal ions, anions and CHN were determined. The molar conductance values are seen that all the complexes are non-electrolytes. The percentage of the CHN, metal ions & anions, molar conductance and magnetic susceptibility data of the complexes are given in Table 1.

Table 1 Analytical, molar conductance and magnetic susceptibility data for Mn^{II} , Fe^{II} , Zn^{II} , Cd^{II} and Hg^{II} Complexes of MBA

Complex	Colour	% C Obs. (Cal.)	% H Obs. (Cal.)	% N Obs. (Cal.)	%Metal Obs. (Cal.)	%Anion Obs. (Cal.)	Δ_M ohm^{-1} $\text{cm}^2\text{mol}^{-1}$	μ_{eff} (B.M)
$\text{MnSO}_4 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	Colour less	31.49 (31.64)	3.84 (3.65)	5.57 (5.68)	11.36 (11.14)	19.58 (19.47)	17.32	5.72
$\text{FeSO}_4 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	Orange brown	28.22 (28.47)	3.61 (3.28)	4.99 (5.11)	10.34 (10.19)	17.89 (17.52)	25.80	3.54
$\text{ZnSO}_4 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	Colour less	30.21 (29.89)	3.25 (3.45)	5.09 (5.36)	12.78 (12.52)	18.84 (18.39)	40.32	-
$\text{CdSO}_4 \cdot \text{MBA} \cdot \text{H}_2\text{O}$	Colour less	34.36 (33.91)	3.56 (3.91)	5.97 (6.09)	24.64 (24.44)	20.71 (20.87)	18.56	-
$\text{HgCl}_2 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	Colour less	28.64 (28.78)	3.54 (3.32)	5.34 (5.17)	36.92 (37.01)	13.64 (13.08)	31.07	-
$\text{HgSO}_4 \cdot \text{MBA} \cdot \text{H}_2\text{O}$	Colour less	29.10 (28.42)	3.48 (3.64)	4.75 (5.10)	37.23 (36.54)	17.11 (17.49)	20.81	-

Table 2 Important IR Absorption Bands (cm^{-1}) of MBA and of Mn^{II} , Fe^{II} , Zn^{II} , Cd^{II} and Hg^{II} Complexes

Compound	ν_{NH}	$\nu_{\text{C=O}}$	ν_{CNC}	ν_3	ν_4	ν_1	ν_2
MBA	3297	1647	1116	-	-	-	-
$\text{MnSO}_4 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	3435	1631	1071	1170 1119 990	633 619 604	828	543
$\text{FeSO}_4 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	3400	1631	1098	1142 1005 984	788 631 601	872	471
$\text{ZnSO}_4 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	3338	1637	1119	1119 991 920	671 657 606	790	512
$\text{CdSO}_4 \cdot \text{MBA} \cdot \text{H}_2\text{O}$	3544	1622	1116	982	617	750	470
$\text{HgCl}_2 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	3527	1614	1104	-	-	-	-
$\text{HgSO}_4 \cdot \text{MBA} \cdot \text{H}_2\text{O}$	3233	1615	1111	1148 1009 985	760 654 611	874	463

Table 3 ^1H NMR Signals (δppm) for MBA and its Zn^{II} , Cd^{II} and Hg^{II} Complexes

Compound	Amide NH	Methyl CH_3	Methylene $(\text{CH}_2)_2\text{N}$	Methine CH	Water
MBA	8.44	1.90	2.51	5.61	-
$\text{ZnSO}_4 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	-	2.37	2.51	-	3.35
$\text{CdSO}_4 \cdot \text{MBA} \cdot \text{H}_2\text{O}$	-	2.37	2.52	-	3.35
$\text{HgCl}_2 \cdot \text{MBA} \cdot 2\text{H}_2\text{O}$	-	2.51	3.02	6.77	3.35
$\text{HgSO}_4 \cdot \text{MBA} \cdot \text{H}_2\text{O}$	-	2.50	3.17	6.76	3.33

In the IR spectra of all the MBA complexes as shown in Table 2, the stretching frequencies of C=O and C-N-C bonds are found lowered showing that both carbonyl oxygen and CNC nitrogen atoms are coordinated to the metal ions. So the ligand acts as ON donor. The IR spectrum of the sulphato complexes shows the presence of coordinated sulphato group. The bands at the ranges of 1150, 1000 and 900 cm^{-1} are due

to 'SO' stretching mode, (ν_3) of sulphato group and the triply degenerate 'OSO' bending mode, (ν_4) and splits up into its components in the ranges of 650, 600 and 580 cm^{-1} for the complex. The frequencies 750(ν_1) and 500(ν_2) are also observed. These frequencies are due to coordinate sulphato group, which are normally associated with bidentate chelation[15]. The bands around 3300 - 3500, 1600 - 1650, 800 - 880, 600

- 690 and 460 - 530 cm^{-1} found in the spectra of all the complexes of MBA, except Co^{II} nitrate complex, indicate the presence of coordinated water molecule.

The UV-Visible spectrum of Mn^{II} complex shows three absorption bands, at 16013 cm^{-1} , due to ${}^5\text{E}_g \rightarrow {}^5\text{T}_{2g}$ transition and at 19597 & 26248 cm^{-1} are attributable to the charge transfer transitions. The room temperature magnetic moment value was found to be 5.72 B.M. These observations suggest a high spin octahedral geometry [16] of the complex.

The UV-Visible spectrum of Fe^{II} complex exhibit electronic transition bands at 14802, 17803 and 20404 cm^{-1} respectively due to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{G})$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$ and ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g(\text{G})$ transitions respectively. The highly intense CT bands at 25900 & 27964 cm^{-1} obscure the other very low intensity d-d absorption bands. The μ_{eff} value of 3.54 B.M. points to octahedral geometry around Fe^{II} ion.

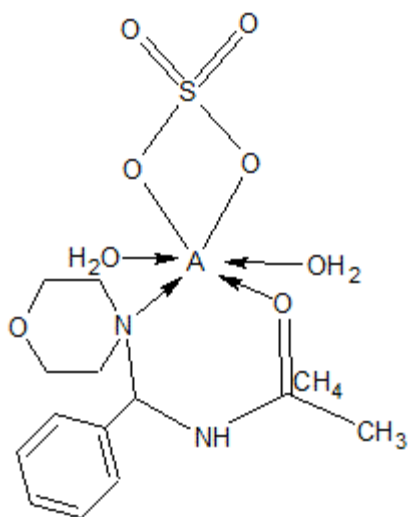


Figure 1 $\text{A.SO}_4.\text{MBA}.2\text{H}_2\text{O}$ ($\text{A}=\text{Mn}$, Fe and Zn)
(Tentative structures proposed for Mn/ Fe/ Zn sulphato complexes)

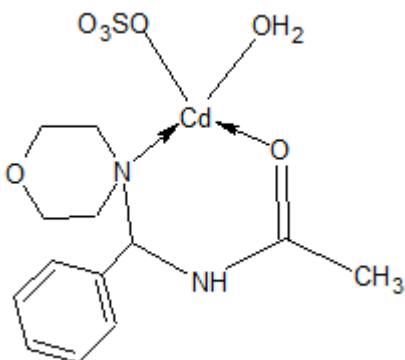


Figure 2 $\text{CdSO}_4.\text{MBA}.\text{H}_2\text{O}$
(Tentative structure proposed for CD sulphato complex)

To substantiate the type of bonding in Zn^{II} , Cd^{II} and Hg^{II} complexes, ${}^1\text{H}$ NMR spectra [17] were recorded [Table 3]. The complexes experienced a downfield shift in the cases of $-\text{CH}_3$ and $-\text{CH}_2$ protons, which are directly attached to CO and amino N. This can be explained on the basis of the reduction in electron density around the proton, which consequently leads to deshielding and moves the proton signal to higher frequency. The signals due to methylene $(\text{CH}_2)_2\text{N}$ and amide NH protons of the ligand are not affected much due to complexation. These support the nature of coordination suggested by IR data. The Far-IR absorption bands are observed around 591 & 522, 467, 400 & 360 and 240 cm^{-1} which are assignable to the M-O, M-N and M-Cl stretching modes respectively for Hg^{II} chloro complex.

Based on the above data obtained, the tentative structures proposed for the complexes are shown clearly in the following figures 1-4.

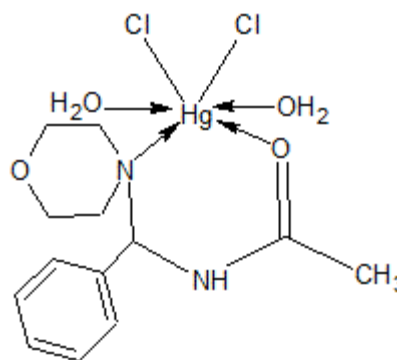


Figure 3 $\text{HgCl}_2.\text{MBA}.2\text{H}_2\text{O}$
(Tentative structure proposed for CD sulphato complex)

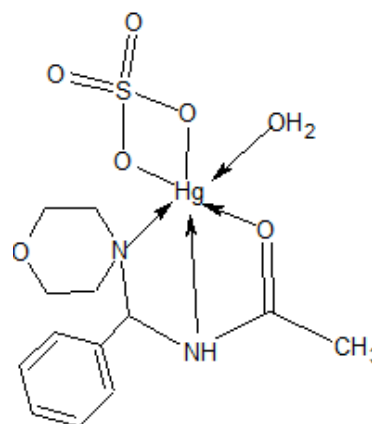


Figure 4 $\text{HgSO}_4.\text{MBA}.\text{H}_2\text{O}$
(Tentative structure proposed for CD sulphato complex)

Antimicrobial study:

Table 4 Antibacterial activity of ligand and its complexes

Compound	<i>E. coli</i>			<i>P. aeruginosa</i>			<i>S.typhi</i>			<i>B. subtilis</i>			<i>S. pyogenes</i>			<i>S. aureus</i>		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Conc. ($\mu\text{g}/\text{disc}$)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Control	12	15	20	10	13	18	14	17	22	11	14	18	10	12	16	12	17	20
MBA	-	-	08	04	08	09	04	06	06	-	06	09	04	04	06	-	07	10
MnSO ₄ .MBA.2H ₂ O	16	18	20	14	16	17	11	14	19	12	16	21	11	13	17	16	18	24
FeSO ₄ .MBA.2H ₂ O	15	19	21	14	18	21	11	18	24	14	19	26	13	16	22	16	19	25
ZnSO ₄ .MBA. 2H ₂ O	18	21	24	17	22	27	20	28	30	23	26	32	18	24	29	25	28	35
CdSO ₄ .MBA. H ₂ O	14	17	23	15	16	22	14	20	26	17	19	27	14	18	25	17	21	28

Table 5 Antifungal activity of ligand and its complexes

Compound	<i>A. niger</i>			<i>A. flavus</i>		
Conc. ($\mu\text{g}/\text{disc}$)	10	20	30	10	20	30
MBA	04	04	05	04	05	05
MnSO ₄ .MBA.2H ₂ O	08	09	12	05	10	12
FeSO ₄ .MBA.2H ₂ O	13	16	24	12	16	20
ZnSO ₄ .MBA.2H ₂ O	15	19	26	16	19	28
CdSO ₄ .MBA.H ₂ O	12	15	18	14	18	25

A comparison of diameters of the inhibition zones of the compounds investigated and listed in Table 4 and 5, shows that Zn^{II} sulphato complex exhibit highest antibacterial and antifungal activity against all the bacterial and fungal species studied. They have largest diameters of inhibition zones than even the control gentamycin at the same concentration and identical conditions. The complexes are more antibacterial and antifungal activities than the ligand against all the bacteria and fungi studied. This inhibition can be explained on the thermodynamic and kinetic aspects of complex-receptor site based on HSAB theory[18]. The Cd^{II} is soft metal and Zn^{II} is neither soft nor hard (border

line). It is noteworthy that both the hard acids are small in size and slightly polarisable. On the other hand, soft acids are larger in size and highly polarisable. The ligating sites are called as bases. The important endogenous ligating sites in the biological systems are O, N and S and their hardness sequence is O > N > S. Therefore, a hard metal prefers to bind to oxygen or nitrogen and soft metal prefers sulphur sites. The Zn^{II} complex is coordinately unsaturated. Hence it exhibits a tendency to bind with additional enzymatic -SH groups. This complex has two labile axial water molecule which can exchange its coordination sites with the enzymatic -SH group, because of the pronounced affinity of the Zn^{II}

to bind with soft bases such as “S” of enzymatic –SH and hence becomes more toxic to the bacterium[18].

A possible mechanism of toxicity may be speculated in the light of Chelation Theory[19]. Chelation reduces considerably the polarity of the metal ion mainly because of partial sharing of its positive charge with donor groups and possible π -delocalization of electron over the chelate ring. This increases the lipophilic character of the neutral chelate, which favors its permeation through lipid layers of fungus membranes. Furthermore, the mechanism of action of the complexes under study may involve the formation of hydrogen bond through the uncoordinated heteroatoms O, S and N with the active centers of the cell constituents. This results in the interference of the normal cell process. Presence of lipophilic and polar substituents like CO, NH, etc., is expected to enhance the fungi toxicity. These complexes have a greater chance of interaction with either the nucleotide bases or with biologically essential metal ions present in the biosystem or with some of the enzymatic functional groups.

Conclusion

Thus the conclusion is made with the ligand, *N*-[Morpholino(phenyl)methyl]acetamide(MBA) was synthesized and characterized. It was found to behave as a bidentate in the complexes of Mn(II), Fe(II), Zn(II), Cd(II) and tridentate in Hg(II) complex. Spectral and magnetic studies were carried out. Based on these measurements, tetrahedral geometry was proposed for Cd^{II} sulphato complex and octahedral geometry for the remaining complexes. Antibacterial and antifungal studies showed that the Zn(II) complex was more active than the other complexes.

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