

Synthesis, Characterization and *in vitro* Antimicrobial Studies of some Transition Metal Complexes with a New Mannich Base *N*-(1-Morpholinosalicylyl) Acetamide

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Abstract: The present study deals with the structure and antimicrobial properties of some complexes of Cu (II), Co (II), Ni (II) and Zn (II) with a new Mannich base N (1-morpholinosalicylyl) acetamide (MSA). The structure of each complex was characterized by spectroscopic methods (IR, UV-Visible, ¹H-NMR), conductometric, magnetic data, and elemental analysis. The cyclic voltammogram of copper complex in MeCN solution shows quasi-reversible peak. Based on these data, we propose an octahedral geometry for Cu (II), tetrahedral geometry for Co (II) and Zn (II) and square planar geometry for Ni (II) complexes. Antibacterial activity of the ligand and its complexes was tested against some select bacteria. The ligand and all the complexes showed antimicrobial activity.

Key Words: Mannich base, metal complexes, antimicrobial activity.

INTRODUCTION

Mannich base complexes have remained an important and popular area of research due to their simple synthesis, adaptability, and diverse range of applications. From the survey of existing literature, it appears that metal complexes of Mannich bases played a vital role in the development of coordination chemistry¹⁻⁵. Many potent antibacterial and antifungal compounds synthesized by the condensation of aldehyde, amine and amide have been reported. Organic chelating ligands containing amide moiety as a functional group have a strong ability to form metal complexes and exhibit a wide range of biological activities⁶⁻¹⁰. Literature studies revealed that during the past decades, there has been a great deal of interest in

the synthesis and structural elucidation of transition metal complexes containing amide moiety. However, there is no study on any metal complexes of N-(1-morpholinosalicylyl) acetamide. Earlier work reported that some drugs showed increased activity when administered as metal chelates rather than as organic compounds¹¹⁻¹³. In the present work, Mannich base derived from the condensation of salicylaldehyde, morpholine and acetamide, and its metal complexes with Cu (II), Co (II), Ni (II) and Zn (II), are synthesized and are characterized using different physicochemical techniques. The ligand and its metal complexes have biological activity against various microbes such as *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgaris* and *Candida albicans*.

EXPERIMENTAL

Chemicals

Reagents such as salicylaldehyde, morpholine, acetamide, various metal (II) chlorides were of Merck products. IR spectra were recorded on a Perkin Elmer 783 instrument in anhydrous KBr pellets. UV-Visible spectra of the complexes were recorded on Perkin Elmer Lambda EZ201 spectrophotometer in DMF solutions. ¹H-NMR spectra was recorded on a JEOL FX-90X instrument using CDCl₃ as a solvent and TMS as an internal standard. Molar conductivity was determined using Systronic Conductivity Bridge with a dip type cell using 10⁻³ M solution of complexes in DMSO. Electrochemical measurements were carried out with electrochemical analyzer mode BAS-27 voltammogram. Magnetic susceptibility was measured with a Guoy balance. Muller-Hinton agar was used for testing the susceptibility of microorganisms to antimicrobial agents using the *well-diffusion* technique. Tetracycline was used as standard for antibacterial activity and amphotericin for antifungal activity.

Synthesis of Mannich base

In a typical procedure⁵, an ethanolic solution of salicylaldehyde, morpholine and acetamide were taken in 1:1:1 mole ratio. Morpholine 8.7 mL (10 mM), acetamide 5.9 g (10 mM) and 10.4 mL of salicylaldehyde (10 mM) were mixed under ice-cold condition. The product obtained was a yellow solid

(Fig-1) and it was recrystallised from methanol. Thin layer chromatography was used to check the purity of the compound. Yield: 68%, m.p.163°C.

Synthesis of metal complexes

The Mannich base (dissolved in CHCl₃) and the metal chlorides in ethanol were mixed in 1:1 molar ratio. The reaction mixture was gently warmed on a water-bath for one hour. The resulting solid complex formed (Fig-2) was filtered washed with water and recrystallised in ethanol and dried in *vacuum*.

invitro Antibacterial and Antifungal Assay

The biological activities of synthesized Mannich base and its Cu (II), Co (II), Ni (II) and Zn (II) complexes have been studied for their antibacterial and antifungal activities by *well diffusion* test using Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA). The antibacterial and antifungal activities were done at 60 µg/mL concentrations in DMF solvent using bacteria (*S. aureus*, *B. subtilis*, *P. vulgaris*) and fungi (*C. albicans*) at the minimum inhibitory concentration (MIC) method. These bacterial strains were incubated for 24h at 37°C and fungi strains were incubated for 48h. at 37°C. Standard antibacterial (tetracycline) and antifungal drug (amphotericin) was used for comparison under similar conditions. Activity was determined by measuring the diameter of the zone showing complete inhibition (mm).

Figure 1. Formation of Mannich base

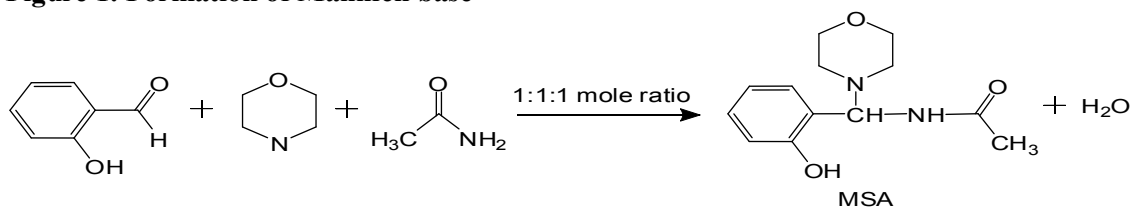


TABLE-1:Physical Characterization,Analytical, Molar conductance,Magnetic susceptibility data

Compound	Found/(Calculated)					Mol. W t	Yield %	λ_m $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$	μ_{eff} BM
	M%	C%	H%	N%	Cl%				
MSA	-	62.24(62.38)	7.11(7.25)	11.01(11.19)	-	250.3	68	-	-
Cu(II) complex	15.01(15.10)	37.01(37.11)	5.11(5.27)	6.52(6.66)	(16.85)	420.78	57	2.9	1.9
Co(II) complex	15.42(15.50)	40.92(41.08)	4.63(4.77)	7.28(7.37)	(18.65)	380.14	55	4.5	3.6
Ni(II) complex	15.34(15.45)	41.02(41.10)	4.63(4.78)	7.25(7.37)	(18.66)	379.92	58	2.1	-
Zn(II) complex	16.86(16.91)	40.27(40.39)	4.57(4.69)	7.18(7.25)	(18.34)	386.58	60	3.4	dia

RESULTS AND DISCUSSION

The complexes prepared are variously coloured microcrystalline powders; air stable, some of them are hygroscopic solids, insoluble in common organic solvents, but soluble in dimethylformamide. The results of elemental analysis (C, H, and N), along with molecular formulae are presented in Table 1. Molar conductivities show that all the complexes are non-electrolytes¹⁴ with $\lambda_M = 2.9 - 4.5 \Omega^{-1}\text{cm}^2\text{mol}^{-1}$ in 10^{-3} DMF solutions at room temperature.

Infrared spectra

The IR spectra of the Mannich base ligand (MSA) and its complexes are recorded. The IR spectra of the complexes were compared with those of the free ligand in order to determine the coordination sites that may be involved in coordination. In MSA, the infrared bands observed at 3255, 1649 and 1185 cm^{-1} have been assigned to $\nu_{\text{N-H}}$, amide $\nu_{\text{C=O}}$ and $\nu_{\text{C-N-C}}$ of morpholine group respectively. In IR spectra of all the complexes, the $\nu_{\text{N-H}}$ band remained at the same position as in the free ligand, indicating that the secondary nitrogen is not coordinated. A band due to $\nu_{\text{C-N-C}}$ stretching vibration of the morpholine ring appeared at 1185 cm^{-1} in the Mannich base. This band is shifted to 1110-1120 cm^{-1} in the metal complexes, suggesting the involvement of the nitrogen atom from the morpholine ring to the central metal ion. The oxygen atom from the amide to be involved in coordination of the metal ion. The new bands at 528 and 431 cm^{-1} in the spectra of the metal complexes were assigned to $\nu_{\text{M-O}}$ and $\nu_{\text{M-N}}$ stretching vibrations¹⁵⁻¹⁸. The presence of coordinated water molecules in Cu (II) complex is determined by the appearance of bands at 3300-3450 cm^{-1} and a peak at 850 cm^{-1} assignable to the OH stretching and rocking mode of coordinated water molecules. The rocking vibration of water is not observed in the IR spectra of Ni (II), Co (II) and Zn (II) complexes. This is confirmed that water molecule is not coordinated in these complexes.

Magnetic moment and UV-Visible spectra

Electronic spectra and magnetic measurements were recorded in order to obtain information about the geometry of the complexes. The μ_{eff} (1.9) value of the Cu (II) representing an octahedral geometry of the ligand around the central metal ion. The four coordinated Co (II) complex shows μ_{eff} value of 3.6 which indicates the presence of three unpaired electrons, supports tetrahedral geometry. The observed zero magnetic moment value confirms the square planar environment for the Ni (II). The Zn (II) complex is found to be diamagnetic as expected for d^{10} configuration.

The electronic spectra of the ligand and its complexes were recorded in DMSO solution. The Cu (II) ion with d^9 configuration in a complex can be either octahedral or tetrahedral or rarely square planar. The octahedral coordinated Cu (II) ion has the ground state ${}^2E_g (t_{2g})^6 (e_g)^3$. The only excited state should then be ${}^2T_g (t_{2g})^5 (e_g)^4$, the energy difference being 10 Dq. The Cu (II) complex under the present study exhibits a broad band in the region 12720 cm^{-1} . The broadness of the band may be due to Jahn-Teller distortion^{19, 20}. These observations indicate that the complexes have distorted octahedral structure. Co (II) complex displays a band at 15655 cm^{-1} which assigned to ${}^4A_2 \rightarrow {}^4T_1$ for tetrahedral geometry²¹. The Ni (II) complex is a diamagnetic suggesting a square planar geometry. It showed a broad band at 14588 cm^{-1} which is assigned to ${}^1A_{1g} \rightarrow {}^1B_{1g}$. The spectra of this complex consistent with this assignment. The spectra of Zn (II) complex exhibited band assigned to $L \rightarrow M$ charge transfer. It is diamagnetic as expected.

ESR and ¹H-NMR spectra

The EPR spectrum of the Cu (II) complex shows $g_{\parallel} > g > 2.0023$ and a g value within the range of 2.08-4.49 which are consistent with the $d_{x^2-y^2}$ ground state in an octahedral geometry.

Evidence for the bonding mode of the ligand is also provided by the ¹H-NMR spectra of the Mannich base and the diamagnetic Zn (II) complex, which were recorded in CDCl_3 . The ¹H-NMR spectrum of MSA displayed the following signals: a multiplet at 7-7.3 δ (Ar-H), 5.7 δ (d, 1H, NH), 6.2 δ (d, Ali-CH) 2.6 δ (morpholine N-CH₂), 3.7 δ (morpholine -OCH₂) and 2.1 δ (s, CH₃). The peak at 10.8 ppm is attributed to the phenolic -OH group present in salicylaldehyde. The presence of this peak noted for Zn (II) complex confirms that the -OH proton is free from complexation. In Zn (II) complex, the doublet of N-H proton shifted slightly down field to 5.9 δ , reveals the coordination of carbonyl oxygen to Zn (II) ion. The signal due to morpholine N-CH₂ protons is also shifted slightly downfield and appeared at 2.8 δ in the complex. This is an indication of the coordination of morpholine nitrogen. Therefore, it is concluded that the Mannich base behaves as a neutral bidentate ligand in all the complexes.

Electrochemical Study

The Cu (II) complex exhibited two quasi-reversible peaks. A cyclic voltammogram of Cu (II) displays two reduction peaks, first one at $E_{\text{pc}} = -0.65$ V with an associated oxidation peak at $E_{\text{pa}} = -0.5$ V and second reduction peak at $E_{\text{pc}} = -1.58$ V with an associated oxidation peak at $E_{\text{pa}} = -1.8$ V corresponding to the Cu (II) / Cu (I) and

Cu (I) / Cu (0) respectively at a scan rate of 0.2 V/s. The value of ΔE_p are 1.5 and 2.02 for first and second redox couples respectively and increase with scan rate giving evidence for quasi-reversible nature associated with one electron reduction.

In vitro antimicrobial assay

For *in vitro* antimicrobial activity, the synthesized compounds were tested against the bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris* and fungi *Candida albicans*^{22, 23}. The minimum inhibitory concentration (MIC) values of the investigated compounds with the standard drugs are presented in Table.2. Antimicrobial activity of metal

chelates can be explained on the basis of chelation theory²⁴. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π -electrons over the whole chelate ring and enhances the presentation of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganism. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

Figure-2 Proposed structure of metal complexes

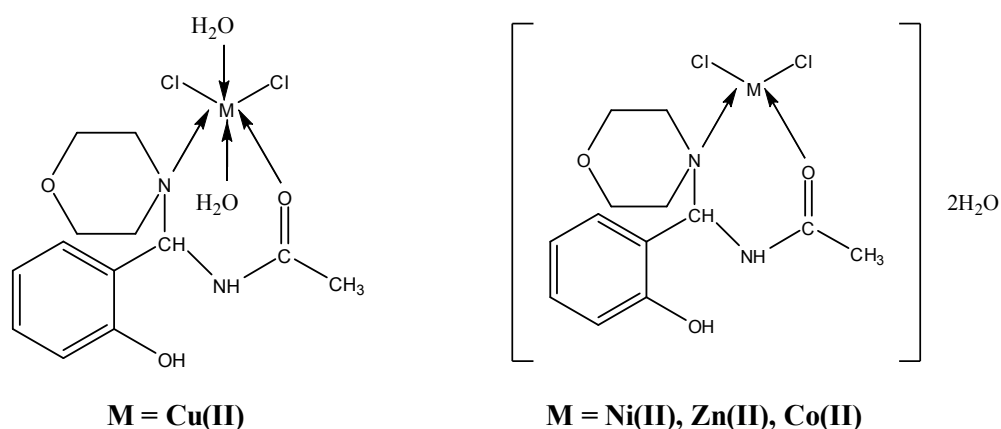


TABLE-2: Antimicrobial Activity of Metal Complexes of MSA

Compound	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>	<i>Candida albicans</i>
MSA	25	14	12	15
Cu (II)	11	11	11	13
Co (II)	11	11	13	13
Ni (II)	12	14	12	15
Zn (II)	13	12	9	15

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