

Synthesis, Characterization and Antimicrobial Activity of Metal Complexes of N-(4-methoxybenzylidene) isonicotinohydrazone Schiff base

ABSTRACT

Two complexes of Cu(II) and Ni(II) with the ligand N-(4-methoxybenzylidene) isonicotinohydrazide, having the formula $[M(La)_2]^{2+}$ (M = Cu(II) and Ni(II)) were synthesized. The ligand was synthesized by the condensation of isonicotinic acid hydrazide (isoniazid) with 4-methoxybenzaldehyde (p-anisaldehyde). The complexes were characterized by FTIR, UV-Vis spectroscopy, molar conductivity, magnetic susceptibility and evaluated for their in vitro antibacterial activity against human pathogens like gram negative *Escherichia coli* (E.coli) and gram positive *Bacillus cereus* (B. cereus) strains. The results indicate that all complexes consist of N and O coordination with metals have better antimicrobial activity.

Keywords: Schiff base ligands, Cu(ii) and Ni(II), Isoniazid, Antimicrobial activity.

1. INTRODUCTION

Developing metal-based drug is emerging as an active area of scientific research nowadays. Schiff base metal complexes have been amongst the most widely studied coordination compounds. This is because of the fact that Schiff bases offer opportunities for altering the metal-centered electronic factor, enhancing the stability and solubility [1-4] and making them active against a wide range of organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Mycobacteria*, *Plasmopora viticola* and *Trychophyton gypseum* [5].

There has been growing interest in the study of hydrazones because of their physiological activity, coordinative capability and applications in analytical chemistry [6- 8].

In comparison to the simple hydrazone Schiff bases, aryl or heteroaryl Schiff bases have additional donor sites. This introduces a wider range of properties like antibacterial activity [9-10], antimycobacterial, anticonvulsant, analgesic [11- 14], anti-inflammatory [15] and antitumor activities [16].

The transition metal complexes are far more biologically active than uncoordinated hydrazones. Their enhanced biological activity has been an active area of investigation among medicinal researchers [17-19]. These coordination compounds of

arylhydrazones have been also reported to act as an enzyme inhibitor [20].

In general, arylhydrazones $R-CO-NH-N=CHR$ act as chelating ligands with transition metal ions by binding through the N atom of azomethine group and O atom of the carbonyl group and hence their ability to coordinate in vivo to the metals.

Three new complexes of Ni(II), Zn(II) and Co(II) with the ligand N-(4-methoxybenzylidene)isonicotinohydrazide, having the formula $[M((LA))(ac)_2] \cdot xH_2O$ (M = Ni(II), Zn(II) and Co(II)) with their antibacterial properties have recently been reported. The result indicates that in all complexes, ligand was coordinated to metal via N and O donor atoms. On the other hand, CH_3COO^- satisfied the remaining coordination numbers as well as oxidation states of metal ions. Therefore, all obtained compounds were neutral with octahedral geometry [9].

Taking these antecedents into account, this work reported the synthesis of two new Schiff base complexes of Cu(II) and Ni(II) with the ligand N-(4-methoxybenzylidene) isonicotinohydrazone derived from Isoniazid (INH) and p-anisaldehyde along with their characterization and biological activity studies. Our obtained result is totally different with the result reported by G.S. Kulkarni [9]. We proposed square planar geometry of our prepared complexes on the basis of some physical and chemical properties. Reported

octahedral complexes are non-electrolytes whereas our synthesized complexes are 1:2 electrolytes.

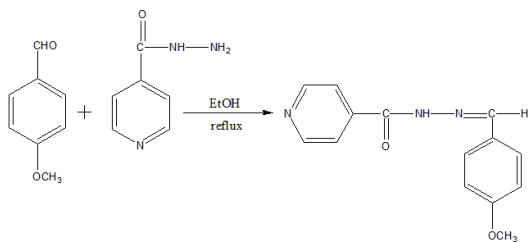
2. EXPERIMENTAL

All used chemicals were purchased from Merck and Loba chemicals. All the melting points were determined on a digital melting point apparatus. Products were characterized by comparison of spectroscopic data (UV-Visible and FT-IR) and melting points with authentic samples.

The wavelength of absorbance was determined by UV-Visible spectrophotometer [JASCO 503] using a quartz cuvette and ethanol as the reference. The IR spectrums were recorded on FT-IR spectrophotometer [JASCO, FT-IR/4100] Japan using dry KBr as the standard reference. The magnetic susceptibility of the complexes was measured at room temperature using a Gouy balance. All the human pathogenic bacteria experimented were obtained from Department of Pharmacy, University of Rajshahi, Bangladesh.

2.1 General Procedure to Synthesize N-(4-methoxybenzylidene) isonicotinohydrazone (La)

INH (1.37 g, 10.0 mmol) was mixed with absolute ethanol (15 mL) and the mixture brought to boiling to produce a slurry. Barely sufficient additional ethanol was then added to give a homogeneous solution at reflux. P-anisaldehyde (1.22 mL, 10 mmol) was added drop-wise over 5 minutes and washed with 5 mL of ethanol. The reaction mixture was refluxed for 4 hours, then allowed to cool slowly and to stand overnight. Finally a white crystalline solid produced was filtered off and dried.



3. RESULTS AND DISCUSSION

Scheme 1: Synthesis of Schiff base ligand, La

2.2 Experimental Procedure for Synthesis of Complexes

To the warm methanolic solution (10 mL) of ligand La (2 mmol), 10 mL warm methanolic solutions (1 mmol) of nitrate salts of metal Cu(II) and Ni(II) was added and the resulting mixture was refluxed for about 3-4 hours. The obtained precipitates were filtered, washed with methanol and dried under vacuum on anhydrous CaCl_2 .

2.3 Antimicrobial Activity

The ligand (La) and its metal complexes were screened for in vitro antimicrobial activity in DMSO against gram-negative *Escherichia coli* (*E. coli*) and gram-positive *Bacillus Cereus* (*B. cereus*) strains by Kirby Bauer's disc diffusion technique.

A uniform suspension of test organism of 24 hours old culture was prepared in a test tube containing the sterile saline solution. Sterile nutrient agar was then added in each of the Petri dishes. The dishes were related to ensuring the uniform mixing of the microorganism in the agar medium which was then allowed to solidify.

Sterile Whatmann filter paper discs were dipped in the solution of each compound and placed on the labelled plates. The DMSO was used as a control of the solvent. Kanamycin was used as a standard compound for comparison. Plates were kept in the refrigerator for half an hour for diffusion and incubated at 37°C for 24 hours.

The diameter of the zone of inhibition around each disc was measured by scale and results were recorded in terms of mm.

In the present study, it was observed that the reaction under refluxed at 80°C had significantly improved the yield of the product. By the reaction of Cu(II) and Ni(II) nitrate with ligand La, complexes of the type $[M(La)_2]^{2+}$ were obtained. All the complexes have a different color, stable at room temperature, insoluble in common polar solvent but soluble in DMSO and DMF, do not have the sharp melting point but decompose above 250°C.

The measurement of molar conductivity at 10^{-3} M concentration carried out in DMSO at room temperature. The molar conductivity values

show that the nitrate complexes were 1:2 electrolytes [21].

The analytical and physical data (color, melting point, molar conductivity and magnetic moment) of the complexes are given in **Table 1**. For the Cu(II) complexes the magnetic moments were 1.83 BM indicating paramagnetic nature. This value corresponds to a square planar geometry [22-23]. For the Ni(II) complex the value for the magnetic moments is 0.24 BM indicates the diamagnetic complex of Ni(II) with square-planar geometry [24].

Table 1: Analytical and Physical Properties Data of La and its Complexes

Symbol of Compounds	Complexes	M.P or De (Decomposition) Temp) / °C	Color	Solubility	Molar conductance $\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$	μ_{eff} in B.M
				DMSO & DMF		
Ligand (La)	$\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2$	143	White	(+) ve	5	
Cu-La	$[\text{Cu}(\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2)_2](\text{NO}_3)_2$	265 (De)	Brownish Purple	(+) ve	153	1.83
Ni-La	$[\text{Ni}(\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2)_2](\text{NO}_3)_2$	290 (De)	Darkish Yellow	(+) ve	150	Dia

3.1 IR Spectral Studies

The IR spectrum of ligand, La in **Table 2** exhibited characteristic bands at $1658.84.5 \text{ cm}^{-1}$ and 1598.69 cm^{-1} assigned to $\nu(\text{C=O})$ and $\nu(\text{C=N})$ respectively (*Figure 1.1*) [25].

The band at $1658.84.5 \text{ cm}^{-1}$, attributable to the $\nu(\text{C=O})$ stretching vibration of the Schiff base ligand is shifted to another region ranging $1626\text{--}1637 \text{ cm}^{-1}$ in the complexes of Cu and Ni indicating coordination of the carbonyl oxygen to the metal ions (*Figure 1.2 and 1.3 respectively*). The presence of bands at $528\text{--}596 \text{ cm}^{-1}$ in the IR spectra of complexes is due to M–O stretching vibrations [21, 26].

The azomethine band at 1598.69 cm^{-1} of Schiff base was shifted to lower frequencies ranging $1592\text{--}1595 \text{ cm}^{-1}$ in the spectra of all the complexes, confirming the participation of the azomethine nitrogen atom in the coordination of the metal ions. In the IR spectra of these complexes, the new bands which appear in the $421\text{--}435 \text{ cm}^{-1}$ region are assigned to the $\nu(\text{M-N})$ vibration [21-22, 26]. The strong sharp band observed at 1384 cm^{-1} in the complexes can be assigned to uncoordinated nitrate ion [24].

All of these IR data confirms that a bidentate ligand coordinated in Cu and Ni metal complex through its O and N atoms respectively.

Table 2: Key Infrared Bands (cm^{-1}) of La and its Complexes

Symbol of Compounds	Compounds	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$
Ligand (La)	$\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2$	1658.84	1598.69		

Cu-La	$[\text{Cu}(\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2)_2](\text{NO}_3)_2$	1636.38	1592.95	528.14	434.16
Ni-La	$[\text{Ni}(\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2)_2](\text{NO}_3)_2$	1626.92	1594.48	595.78	421.27

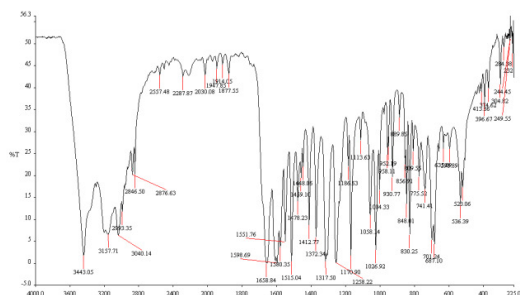


Figure 1.1: IR Spectrum of La

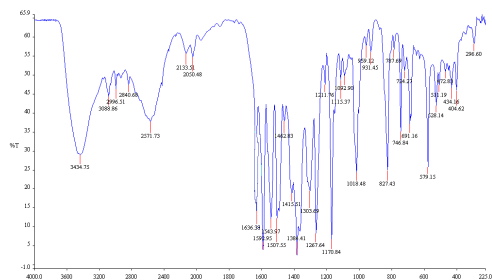


Figure 1.2: IR Spectrum of Cu-La

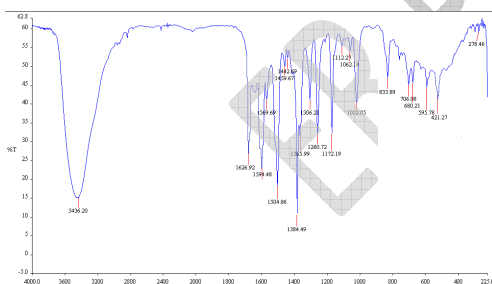


Figure 1.3: IR Spectrum of Ni-La

3.2 UV- Visible Spectra

The UV-Visible spectra of the ligand shows two bands at 281 and 347 nm which are assigned to $\pi-\pi^*$ and $n-\pi^*$ transition respectively.

All the complexes showed the charge transfer transitions which can be assigned to charge transfer from the ligand to metal (LMCT) and vice versa. For complexes, absorption bands at the range of 373–378 nm may be associated with $L \rightarrow M$ charge transfer and vice versa ($M \rightarrow L$) [22– 23].

In the UV-region, the complexes showed absorption band at 275–277 nm (Figure 1.4) which may be assigned to $\pi - \pi^*$ transition. The spectra of all the complexes exhibiting bands assigned to $\pi - \pi^*$ and $M \rightarrow L$ charge transfer and hence, the metals normally prefer square-planar geometry [22–24]. All observations were summarized in the Table 3.

Table 3: UV- Visible Spectrum the Ligand La and its Complexes

Symbol of Compounds	Compounds	λ in nm	Assignment
Ligand (La)	$\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2$	281	$\pi-\pi^*$
		347	$n-\pi^*$
Cu-La	$[\text{Cu}(\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2)_2](\text{NO}_3)_2$	277	$\pi-\pi^*$
		373	C.T ($M \rightarrow L$)
Ni-La	$[\text{Ni}(\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2)_2](\text{NO}_3)_2$	275	$\pi-\pi^*$
		378	C.T ($M \rightarrow L$)

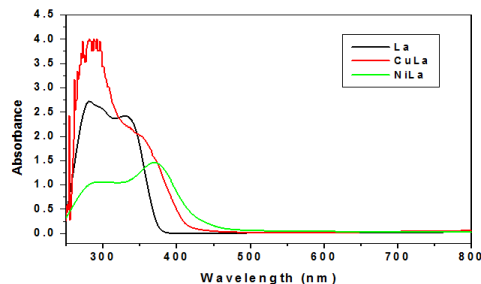


Figure 1.4: UV- Visible Spectrum of the Ligand and its Complexes

From the electronic and physical properties (i.e. FT-IR, UV-Vis, Magnetic Susceptibility, Melting Points and Molar Conductance), the structure as shown in Figure 2 can be obtained.

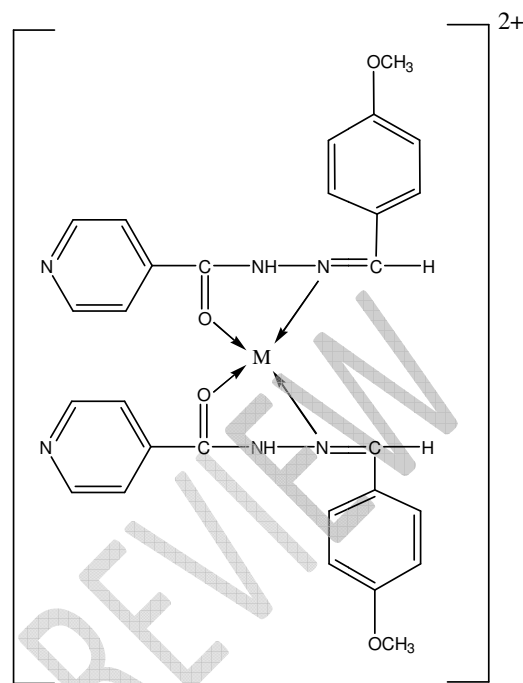


Figure 2: The proposed geometry of Cu(II) and Ni(II) Chelate complexes for La (M= Cu(II) and Ni(II))

3.3 Antimicrobial Screening Result

The Schiff-base ligand and its metal complexes reported here were evaluated for antibacterial activity against *Escherichia coli* and *Bacillus cereus*. The values of zone inhibition were measured in millimeter. The data of antibacterial activities of ligand and its complexes are given in **Table 4**.

The inhibitory zone data reveals that the ligand, as well as its metal complexes, shows good antibacterial activity. The biological activity of Schiff base ligand arises from the presence of imine group which imports in elucidating the mechanism of transformation reaction in biological systems. However, its metal complexes showed remarkable antibacterial activity as a result of chelation of metal with organic ligand synergistically increasing its effect [21, 25-27]. The concentration of standard compound, test ligand and complexes used was 30 µg/disc. The DMSO control did not show any antimicrobial activity against the tested bacterial strains whereas considering

with respect to the standard, all the tested compounds were found to be moderately active.

Such increased activity of the metal chelates can be explained on the basis of Overtone's cell permeability concept and Tweedy's chelation theory [28]. The former concerning cell permeability that the lipid membrane surrounding the cell favors the passage of only lipid soluble materials which in turn reflects that their liposolubility is an important factor controlling the antibacterial activity.

On chelation, there is a reduction in the polarity of the metal atom by the partial sharing of its positive charge with donor groups and possible π -electron delocalization over the whole ring. This, in turn, increases the lipophilic character of the metal chelate and favors its permeation through the lipid layers of the membranes of the micro-organism and thus blocks the metal binding sites on enzymes of microorganisms [29-30]. Apart from this, other factors such as conductivity, solubility and dipole moment

influenced by the presence of the metal ions
may also be the reasons for the increased

antimicrobial activity.

Table 4: Antibacterial Screening Results of Ligand La and its Complexes

Compounds	Antibacterial Zone of Inhibition (in mm)	
	Gram Negative <i>Escherichia coli</i>	Gram Positive <i>Bacillus cereus</i>
Kanamycin	32	35
La	4	5
Cu- La	11	10
Ni- La	12	10

4. CONCLUSIONS

The synthesis and characterization of isonicotinoyl Schiff base and its Cu(II) and Ni(II) complexes have been investigated by spectroscopy and some other techniques (i.e. FTIR, UV-Vis spectroscopy, molar conductivity and magnetic susceptibility). In all the complexes, the Schiff base is bound to the metal through the carbonyl oxygen and the azomethine nitrogen to result in a square-planar geometry around the metal centre.

The synthesized compounds are found to be active against all bacteria experimented and showed better antibacterial activity than the ligand.

5. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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