



Green Synthesis, Magnetic, Spectroscopic and *in-vitro* Antimicrobial Studies of Transition Metal Complexes with Biologically Active *O,O*-Donor Ligand Containing Amide Moiety

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Abstract The present study deals with transition metal complexes of an *O,O*-chelating ligand possessing amide moiety. The Ligand 2-Hydroxypyridine-3-carboxamide(L) and its new mononuclear complexes $[M(H_2O)_2L_2]Cl_2$ with Co(II), Ni(II) and Cu(II) have been prepared using microwave assisted synthesis, an environmentally benign method of synthesis. The ligand and the metal complexes have been characterized by elemental analyses, magnetic susceptibility measurements and spectroscopic methods like Fourier Transform-Infrared spectroscopy and UV-visible spectroscopy. The ligand and metal complexes were screened against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*.

Keywords Environmentally Benign Synthesis, Bioactive Ligands, 2-Hydroxypyridine-3-carboxamide, Microwave Assisted Synthesis, Biological evolution

Introduction

Nicotinamide, also known as niacin or vitamin-B₃, shows crucial bioactivities and used to cure neurodegenerative disorders and many cognitive diseases in humans (Depinte et al. (2006); Lin and Gaurent 2003). Various Nicotinamide derivatives are found to have antitumour activities and have been reported as effective anticancer agent in case of leukaemia, melanoma and other forms of cancer like cancer of lungs, brain, colon, kidney and ovary etc. (Hino and Ford 2001). Many transition metal complexes of derivatives of Nicotinamide are also reported to have significant biological activities (Maiese et al. 2000). Transition metal complexes containing amide moiety with *O,O*-chelation modes have unique structural features and biological significance (Wang et al. 2015). Hydroxy group present at 2-position of nicotinamide showing keto-enol tautomerism has been a subject of much interest in heterocyclic and coordination chemistry because of its significant biochemical relevance (Scanlan and MacDowell 1983; Suradi et al. 1982). In many cases biological activities of organic ligands is greatly affected when it under goes metal coordination. Such as, transition metal complexes of thioamide-based ligands have more significant biological activity than free ligands (Reiss et al. 2011). The four new complexes of 2-Hydroxypyridine-3-carboxamide(L) with Co(II), Ni(II) and Cu(II) are synthesized, characterized spectroscopically and their antibacterial studies are also carried out against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*.



Experimental:

Chemicals and Instruments: All the chemicals, metal salts, solvents used were of analytical grade and purchased from Sigma-Aldrich. Thin layer chromatography was used to check purity of prepared ligand and its complexes with various solvent systems. Vibrational spectra of all three complexes and ligand are recorded on FT-IR instrument (Bruker Optic Model Alpha) (Zn-Se Optics, ATR) using KBr pallets, in the range of 4000-500 cm^{-1} . Magnetic susceptibility measurements were taken on the magnetometer (vibrating sample, model 155) at field strength of 5500 Gauss. Environmentally benign synthesis was carried out by microwave irradiation. For this purpose, the used instruments are microwave oven (Model KENSTAR-OM20ACF, 2450MHz, 800W) and GMBR (Green Microwave Biochemical Reactor). Electronic spectra are recorded using a Double Beam UV-Visible Spectrophotometer, (ECIL, model UV 5704SS) with quartz cell of 10 mm light path. All antibacterial activities were carried out through disc diffusion method with horizontal laminar.

Synthesis of 2-Hydroxypyridine-3-carboxamide(L):

Ligand 2-Hydroxypyridine-3-carboxamide(L) was synthesized by mixing concentrated Ammonium hydroxide and solid Ammonium Chloride, as catalyst, with aqueous solution of 2-hydroxynicotinic acid (10 mmol), followed by microwave irradiation (at 200 watts, 3.8 minutes) in Green Microwave Biochemical Reactor. The solid product was recrystallized from ethanol. Continuous monitoring of reaction and purity of synthesized ligand was verified by thin layer chromatography.

Synthesis of the complexes:

Metal complexes of the bioactive ligand 2-Hydroxypyridine-3-carboxamide(L) with Co(II), Ni(II) and Cu(II) were also synthesized by the same method i.e. microwave assisted synthesis method. To synthesize complexes, metal chlorides of Co(II), Ni(II) and Cu(II) were added slowly, with constant stirring, into the saturated aqueous solution of 2-Hydroxypyridine-3-carboxamide(L) in 2:1 ratio. The reaction mixture was exposed to microwave irradiation in Green Microwave Biochemical Reactor (GMBR) for 2-4 minutes, at 600W, maintaining the occasional shaking. The progress of reaction was confirmed by TLC data. The solid precipitate was separated and crystallized. Crystals were purified and recrystallized with alcohol and dried under vacuum. (Verma et al. 2018). The physical and analytical data of ligand and the metal complexes are shown in Table 1.

Table 1: Physical and analytical data of ligand and the metal complexes.

Ligand/ Complexes	Colour	M.P. ($^{\circ}\text{C}$)	Reaction period (Min.)	R _f value	Yield (%)
2-Hydroxypyridine-3-carboxamide(L)	White	211	4.2	(0.73) ^b	69
[Co(H ₂ O) ₂ (L) ₂] Cl ₂	Light Purple	204	3.5	(0.59) ^c	70
[Ni(H ₂ O) ₂ (L) ₂] Cl ₂	Off white	219	2.5	(0.59) ^a	69
[Cu(H ₂ O) ₂ (L) ₂] Cl ₂	Light green	207	3.4	(0.61) ^a	68

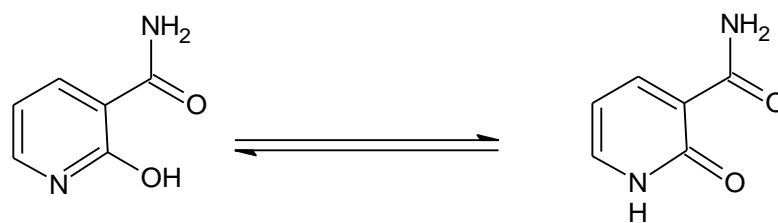
a = C₂H₅OH: C₆H₆ (3:7), b = C₂H₅OH: C₆H₆ (5:5), c = CH₃COOC₂H₅: CCl₄ (3:7), d = CH₃COOC₂H₅: CCl₄ (4:6), e = CH₃OH: Petroleum ether (2:8), f = CH₃COOC₂H₅: Petroleum ether (5:5), g = CH₃COCH₃: CCl₄ (4:6).

Results and Discussion**IR spectral data:**

IR spectra of ligands and metal complexes are shown in figure-2(a-d) and corresponding vibrational assignments, for comparison purpose, are presented in table-2.

Ligand 2-Hydroxypyridine-3-carboxamide(L) shows keto-enol tautomerism (figure-1) and in solid state, it exists as keto form rather enol form (Nakamoto 1965). Existence of keto form of ligand is confirmed by absence of characteristic peaks of enol form i.e., strong and broad band of O-H stretching around 3400 cm^{-1} , $\delta(\text{C}_2\text{-OH})$ bending vibrational peak, around 1280 cm^{-1} , and strong band for pyridine ring around 1023 cm^{-1} , in IR spectra of ligand (Wong et al. 1992).





2-Hydroxypyridine-3-carboxamide

2-Oxo-1H-pyridine-3-carboxamide

Figure 1: Keto – Enol tautomerism of the Ligand

Ligand, in keto form, have two amide groups, a free amide group at position-3 and a ring amide group involving ring nitrogen atom. Both these amide groups appear as mixed band, of $\nu_{C=O}$ ^a i.e. amide-I band. Position of this $\nu_{C=O}$ ^a band, in IR spectra of complexes, is found at lower wave number than ligand. This negative shifting of amide-I band is clear evidence of metal-ligand coordination through carbonyl oxygen of both amide groups i.e. *O,O* chelation mode and not through N atoms of amide groups (Xue J, Hua et al.2014). Non-participation of N atoms of amide groups in chelation was further suggested by a positive shift in the position of N-H stretching (ν_{N-H}) in case of metal complexes in comparison to ligand (Nakamoto 1963). Vibrational spectra of complexes showed strong band in the region 3365-3426 cm^{-1} , characteristic band for rocking and wagging modes of $\nu(O-H)$ stretch, accounts for the presence of coordinated water in metal complexes (Sigel and Martin 1982). *O,O* chelation mode in metal complexes was further evidenced by the presence of medium intensity band (at 450-550 cm^{-1}) which may be assigned to $\nu(M-O)$ stretching (Köse and Necefolu 2008).

Table 2: Characteristic vibrational assignments (cm^{-1}) of the ligand and complexes

Ligand/ Complexes	ν_{N-H}	$(\nu_{C=O})^x$	$(\nu_{C-N+\delta N-H})^y$	$(\nu_{N-H+\delta C-N})^z$	ν_{O-H}	ν_{M-O}
2HNICAM	3177	1660	1604	1555	-----	-----
[Co(H ₂ O) ₂ (L) ₂] Cl ₂	3224	1629	1604	1573	3366	527
[Ni(H ₂ O) ₂ (L) ₂] Cl ₂	3234	1640	1614	1572	3366	513
[Cu(H ₂ O) ₂ (L) ₂] Cl ₂	3225	1638	1613	1582	3426	503

x = amide - I band, y = amide - II band, z = amide - III band

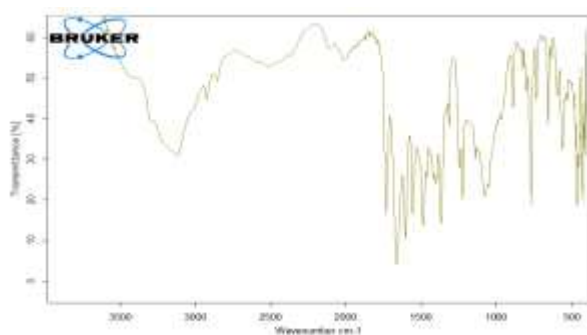
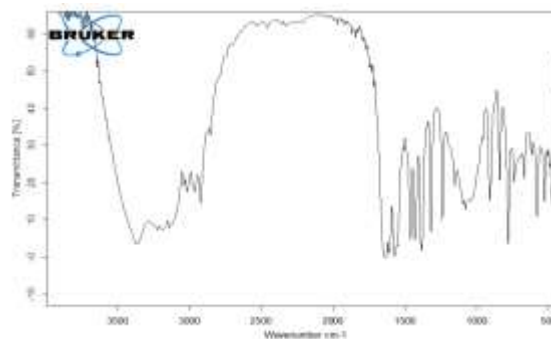
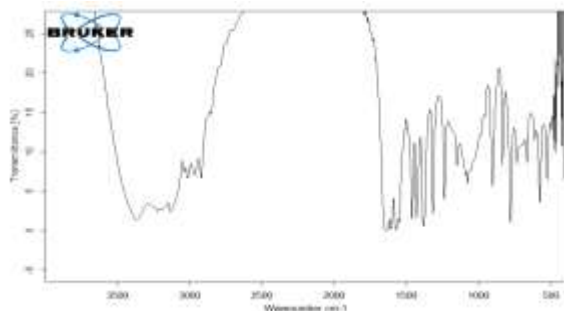
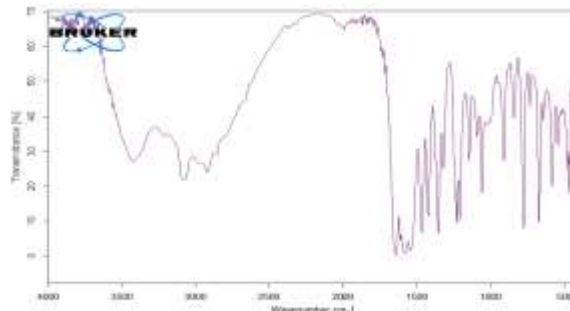


Figure 2(a): IR Spectrum of 2-Hydroxypyridine-3-carboxamide(L)

Figure 2(b): Vibrational Spectrum of [Co(H₂O)₂(L)₂]Cl₂Figure 2(c): Vibrational Spectrum of [Ni(H₂O)₂(L)₂]Cl₂Figure 2(d): Vibrational Spectrum of [Cu(H₂O)₂(L)₂]Cl₂

Electronic Spectral Bands and Magnetic Measurements

In order to finalize geometry of the complexes, UV-Visible spectra, presented in figure 3(a-d), and magnetic susceptibility measurements were recorded. For metal complexes μ_{eff} values, electronic spectral bands and corresponding assignments are presented in Table-3. Observed value of μ_{eff} for Co(II) and Ni(II) were found 4.96 BM, indicative of quartet ground state term $4F$ ($S = 3/2$) and 3.18 BM respectively (Kettle 1996), which indicates octahedral coordination environment around these bivalent metal ions. For Cu(II) complex, μ_{eff} is found in the range 1.87-1.92 BM, also indicating the octahedral geometry (Lewis and Wilkins 1960).

For Co(II) and Ni(II) the range (λ_{max}) of electronic spectral bands, and their corresponding assignments provides a clear indication for octahedral geometry of these complexes (Figgis and Hitchman 2000). For Cu(II) complex, a single asymmetric broad band is found in the region $10050-14124 \text{ cm}^{-1}$ which suggested the further splitting of octahedral free Cu(II) ion terms (2E_g and ${}^2T_{2g}$) to generate new terms (${}^2B_{1g}$, ${}^2A_{1g}$, 2E_g and ${}^2B_{2g}$) tetragonal distortion to the regular octahedral geometry (Purcell and Kotz 1977).

Table 3: Electronic spectral bands & Corresponding tentative assignments of complexes

Metal Complexes	μ_{eff} (BM)	Electronic Spectral bands $\lambda_{\text{max}}(\text{cm}^{-1})$	Electronic assignments	Expected Geometry
$[\text{Co}(\text{H}_2\text{O})_2(\text{L})_2] \text{Cl}_2$	4.96	11974, 16584 21739	${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{2g}(\text{F})$, ${}^4T_{1g}(\text{F}) \rightarrow {}^4A_{2g}(\text{F})$ ${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{1g}(\text{P})$	<i>Oh</i>
$[\text{Ni}(\text{H}_2\text{O})_2(\text{L})_2] \text{Cl}_2$	3.18	12165, 16575 25542	${}^3A_{2g}(\text{F}) \rightarrow {}^3T_{2g}(\text{F})$, ³ $A_{2g}(\text{F}) \rightarrow {}^3T_{1g}(\text{F})$ ${}^3A_{2g}(\text{F}) \rightarrow {}^3T_{1g}(\text{P})$	<i>Oh</i>
$[\text{Cu}(\text{H}_2\text{O})_2(\text{L})_2] \text{Cl}_2$	1.88	10050-14124 cm^{-1} (Asymmetric broad band)	${}^2B_{1g} \rightarrow {}^2A_{1g}$, ${}^2B_{1g} \rightarrow {}^2B_{2g}$ ${}^2B_{1g} \rightarrow {}^2E_g$,	<i>Distorted Oh</i>

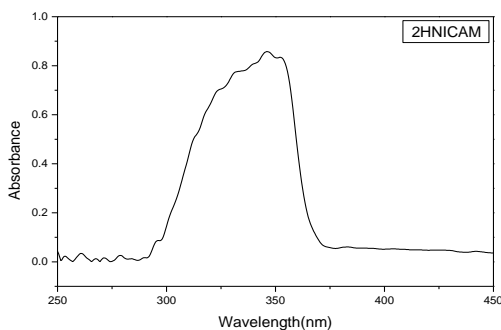


Figure 3(a): UV-Visible spectrum of Ligand

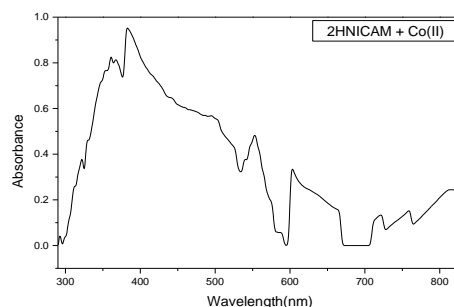
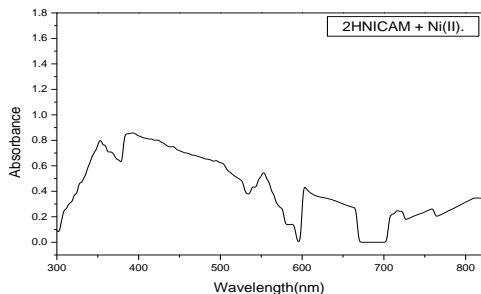
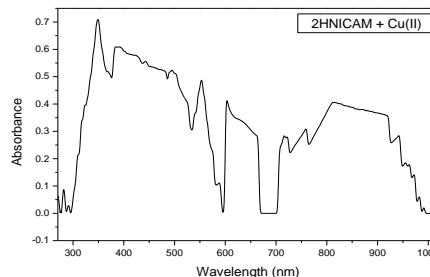


Figure 3(b): UV-Visible Spectrum of $[\text{Co}(\text{H}_2\text{O})_2(\text{L})_2]\text{Cl}_2$



Figure 3(c): UV-Visible Spectrum of $[\text{Ni}(\text{H}_2\text{O})_2(\text{L})_2]\text{Cl}_2$ Figure 3(d): UV-Visible Spectrum of $[\text{Cu}(\text{H}_2\text{O})_2(\text{L})_2]\text{Cl}_2$

Biological Studies

Antibacterial studies of the ligand 2-Hydroxypyridine-3-carboxamide(L) and its metal complexes with Co(II), Ni(II) and Cu(II) were screened *in-vitro*, against some pathogenic stains of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* using Muller Hinton Agar media and paper disc method. The result of these studies as zone of inhibition, are tabulated in Table 4 and graphical representation of the same is reported in figure 4. On Comparing the result between ligand and metal complexes it is concluded that antibacterial activities of metal complexes are found to be greater than the ligand with few exceptions. *E. coli*, and *B. subtilis* was most inhibited by Cu(II) complex and Ni(II) complexes, respectively whereas against *S. aureus* both Co(II) and Ni(II) complexes are found to have equal and highest activity. Co(II) complex showed no activity against *Bacillus subtilis*.

Table 4: Antimicrobial studies of ligand and metal complexes

S. No.	Compounds (100 ppm)	Zone of inhibition (in mm)		
		<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>
1	2-Hydroxypyridine-3-carboxamide(L)	4.5	5.5	4.5
3	$[\text{Co}(\text{H}_2\text{O})_2(\text{L})_2]\text{Cl}_2$	5.5	6.5	0.0
4	$[\text{Ni}(\text{H}_2\text{O})_2(\text{L})_2]\text{Cl}_2$	4.5	6.5	6.0
5	$[\text{Cu}(\text{H}_2\text{O})_2(\text{L})_2]\text{Cl}_2$	6.5	5.5	5.6

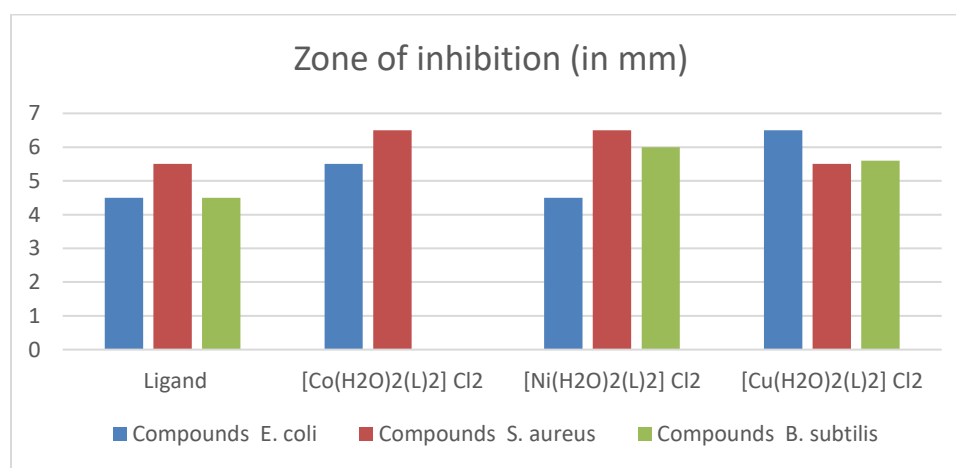


Figure 4: Graphical Representation of Antimicrobial Studies

Conclusion

In this research work we have successfully carried out environmentally benign synthesis i.e. microwave assistant synthesis of the bioactive ligand 2-Hydroxypyridine-3-carboxamide(L) and its metal complexes with Co(II), Ni(II)



and Cu(II) metal ions, In this study microwave radiation method appears as an efficient method of synthesis for ligand as well as metal complexes as it takes laser time and produces good yield. The complexes shows the molecular formula $[M(H_2O)_2L_2]Cl_2$. The electronic spectral bands and their respective tentative assignments suggested the structure of complex of Co(II), Ni(II) as octahedral and tetragonally distorted octahedral structure for Cu(II) complex. Vibrational spectroscopic analysis of ligand and complexes confirmed the *O,O*-chelation mode of ligand in the complexes and also the ligand, 2-Hydroxypyridine-3-carboxamide(L), is probably exists in keto form rather enol form.

The antibacterial activities of the ligands and its three complexes against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* concluded that the complexes possesses greater activities than free ligand. Tentative structure of the complexes is reported in figure 5.

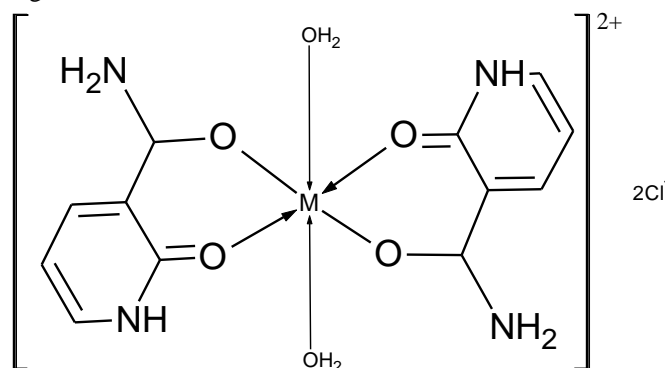


Figure 5: Tentative Structure of the complexes $[M(H_2O)_2L_2]Cl_2$ where $M = Co(II), Ni(II)$ and $Cu(II)$ *
(* $Cu(II)$ Complex has distorted octahedral geometry)

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