

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND ANTIMICROBIAL STUDY OF MIXED THIAMINE-NICOTINAMIDE METAL COMPLEXES.

A. Lawal*, and J.A. Obaleye

Department of Chemistry, University of Ilorin, P.M.B 1515, Ilorin, Nigeria.

E-mail: amudatlawal@yahoo.com; Tel: +2348077778518

ABSTRACT

Ni(II), Co(II), Mn(II) and Cu(II) mixed complexes of thiamine hydrochloride and nicotinamide have been synthesized and characterized using physical and spectroscopic methods such as melting point test, conductivity measurement, infrared, electronic and proton magnetic nuclear resonance techniques. The antimicrobial study of the complexes and the ligands was carried out against *Escherichia coli*, *Staphylococcus*, *Bacillus subtilis*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium species*. The result of the spectroscopic studies confirmed that both thiamine hydrochloride and nicotinamide act as monodentate ligands, coordinating through nitrogen of the pyridine (N-1) position in thiamine and that of pyridine ring in nicotinamide. The result of antibacterial and antifungal studies of mixed thiamine and nicotinamide showed that the mixed complexes are more effective antibacterial and antifungal against the tested species.

Keywords: Mixed complexes, Thiamine, Nicotinamide, Antimicrobial study.

INTRODUCTION

Some transition metals are essential for the normal function of living organism (Mishra *et al.*, 2009). Research has shown significant project in utilization of transition metals complexes as drugs to treat several human diseases (Warra, 2011)

The chemistry of thiamine and its derivatives with metal ions is very interesting since thiamine hydrochloride has a wide variety of coordination site. Many studies have been reported on the interaction of thiamine with some metal ions (Cramer *et al.*, 1984; Adeyemo, 1981; Casas *et al.*, 1995; Hadjiliadis and Yannopoulos, 1983).

Nicotinamide is known as a component of the vitamin B complex as well as a component of the coenzyme nicotinamide adenine dinucleotide (NAD). It is reported that heterocyclic compounds play a significant role in many biological systems, especially N- donor ligand systems being a component of several vitamins such as nicotinamide, thiamine, niacin, pyridoxine (Kato and Muto, 1980; Nagar, 1990). Nicotinamide itself plays an important role in the metabolism of living cells and some of its metal complexes are biologically active as antibacterial or insulin-mimetic agents (Eugen *et al.*, 1996). Therefore the structure of nicotinamide has been the subject of many studies (Kose *et al.*, 2007).

The structure of mixed- ligand complex formation have also a great deal of importance in the field of biological and inorganic chemistry. Studies on the mixed – ligand metal complexes of the salicylate and nicotinamide has been reported by Kose *et al.*, 2007. The structure of mixed ligand metal- nicotinamide-nitrobenzoate Complexes and their derivatives have also been extensively studied by Hokelek and Necefoglu (1997).

This paper report the synthesis, spectroscopic characterization and antimicrobial study of Ni(II), Co(II), (Mn(II) and Zn(II) complexes of mixed thiamine-nicotinamide.

MATERIALS AND METHODS

The metal salts used for the synthesis were obtained from British Drug House Chemical Limited, England and were used as supplied. Thiamine hydrochloride and nicotinamide were obtained from Swiss Chemical Limited Lagos. Cultures of the micro-organisms used were obtained from Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

Synthesis of Metal Complexes of Thiamine and Nicotinamide

The procedure described by Abd El-Wahab and El-Sarrag, 2004, with slight modification. 5 mmol of metal salts (CuCl_2 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) was dissolved in 10 ml of methanol followed by addition of 10 mmol (3.372g) of thiamine hydrochloride and 10 mmol (1.231g) of nicotinamide. The mixtures were refluxed for 3 h and left to stand overnight. The precipitate formed was filtered, washed with methanol and stored in a well-labelled container and dried over CaCl_2 in a dessicator.

Characterization of the compounds

Infrared spectra of the ligand and the complexes were recorded in KBr pellets in the range ($4000\text{--}400\text{cm}^{-1}$) on Buck Scientific M500 IR spectrometer. Electronic spectra were on Aquamate Scientific Spectrophotometer model V4.60. The metal content was done using an alpha 4 Atomic Absorption Spectrophotometer with PM 8251 simple-pen recorder. Conductivity measurements were carried out using HANNA conductivity meter with a cell constant of 0.83.

Antimicrobial studies

The antimicrobial activity of the ligand and the metal complexes were determined according to the procedure previously reported by Chohan *et al.*, 1994. The bacteria species used for this study include *Escherichia coli*, *Staphylococcus* and *Bacillus subtilis* while the fungi species used include *Aspergillus flavus*, *Aspergillus niger* and *penicillium species*.

RESULTS AND DISCUSSION

The physical properties and spectroscopic data are presented in Tables 1-4.

Table 1: The physical properties of mixed thiamine-nicotinamide metal complexes

Mixed complexes	Colour	M.pt(°C) Decomp. Temp	% yield	R _f	Conductivity scm ² mol ⁻¹	% Metal Calcd (found)
Mixedthiamine-nicotinamide						
Ni(Th)(Nic)Cl ₂	green	>360	65.6	0.87	152.3	10.5(10.8)
Co(Th)(Nic)Cl ₂	purple	>360	55.0	0.82	100.5	8.1(9.2)
Mn(Th)(Nic)Cl ₂	white	>360	40.1	0.90	90.8	11.6(11.9)
Cu(Th)(Nic)Cl ₂	blue	>360	57.5	0.94	103.0	13.2(13.7)

All the mixed ligand complexes decomposed above 360 °C. This high melting point confirms the formation of the mixed complexes. All the mixed ligand complexes are of various colour. The blue colour of the Cu (II) complex suggests a tetrahedral geometry.

The results of molar conductance in DMSO solutions showed that all the mixed ligand complexes are electrolytes. This is similar to the work done by Obaleye and Orjiekwe, 1993.

Table 2: Selected IR data of mixed Thiamine-Nicotinamide metal complexes.

Ligand/ complex	ν (N – H)	ν (C=O)	ν (C=N)	ν M - N	ν M-Cl
Thaimine HCl	3352(m) 3144(m)	-	1593(s)	773(s)	-
Nicotinamide	3351(m)	1685(s)	1600(s)	-	-
Ni(Th)(Nic)Cl ₂	3439(m,b)	1658(s)	1543(m)	674(s) 620(s)	425(m) 413(s)
Co(Th)(Nic)Cl ₂	3412(s,b) 3302(w,b)	1667(s) 1618(s)	1564(w)	694(s) 651(w)	460(w)
Mn(Th)(Nic)Cl ₂	3430(m) 3187(w,b)	1660(s)	1527(m)	718(s) 688(m)	561(w)
Cu(Th)(Nic)Cl ₂	3404(m) 3106(m)	1710(w) 1624(s)	1561(s) 1509(s)	689(s) 657(s)	547(s,b) 444(s)

S = strong, m = medium, w = weak, b = broad.

The infrared spectra of thiamine HCl, nicotinamide were compared with those of their mixed ligand complexes.

The N-H stretching frequency which occurs between 3429 cm^{-1} in thiamine appeared at 3351 cm^{-1} in the nicotinamide. This region has been slightly shifted in the mixed complexes (David, 2000).

The $\nu\text{ C} - \text{N}$ occurs at 1593 cm^{-1} in the thiamine HCl and 1600 cm^{-1} in the nicotinamide. This region has been shifted to lower frequencies in the mixed complexes it appeared at 1543 cm^{-1} in the Ni(II) complex and at 1527 cm^{-1} the Mn(II) complex showing that this position has been affected by coordination.

The two (M - N) bands can be assigned to 674 cm^{-1} (due to M - N of thiamine HCl) and another at 620 cm^{-1} (due to M-N of the nicotinamide). The $\nu\text{ M} - \text{Cl}$ occurred between 560 cm^{-1} and 410 cm^{-1} (Lever, 1984).

Table 3: UV/Visible spectra of Thiamine HCl, Nicotinamide and their mixed ligand complexes

Ligand/complex	Wavelength (nm)	Energies (cm^{-1})	Assignment
Thiamine HCl	234	42735	$\pi \rightarrow \pi^*$
	298	33557	$n \rightarrow \pi^*$
Nicotinamide	218	45872	$\pi \rightarrow \pi^*$
	280	35714	$n \rightarrow \pi^*$
Ni(Th)(Nic)Cl ₂	365	27397	$^3A_2 \rightarrow ^3T_1(P)$
	420	23810	$^3A_2 \rightarrow ^3T_1(P)$
	610	16393	$^3A_2 \rightarrow ^3T_2$
Co(Th)(Nic)Cl ₂	460	21739	Charge transfer
Mn(Th)(Nic)Cl ₂	602	16611	$d \rightarrow d^*$ transition
Cu(Th)(Nic)Cl ₂	618	16181	$d \rightarrow d^*$ transition

The uv/visible spectra of both thiamine HCl and nicotinamide were compared with those of its mixed complexes.

Both thiamine HCl and nicotinamide have two absorption bands each, at 234 nm (42735 cm^{-1}) and 298 nm (33557 cm^{-1}) in thiamine HCl, and at 218 nm (45892 cm^{-1}) and 280 nm (35714 cm^{-1}) in nicotinamide. These bands have been shifted to higher wavelengths in the mixed ligand complexes due to complexation. Additional bands was seen in the Ni (II) complex due to $d \rightarrow d$ transition.

The mixed Co (II), Mn (II) and Cu (II) complexes of thiamine HCl and nicotinamide show one absorption band each at 460 nm (21739 cm^{-1}), 602 nm (16611 cm^{-1}) and 618 nm (16181 cm^{-1})

which were assigned to metal- ligand charge transfer and $d \rightarrow d^*$ transitions respectively (Lever, 1984).

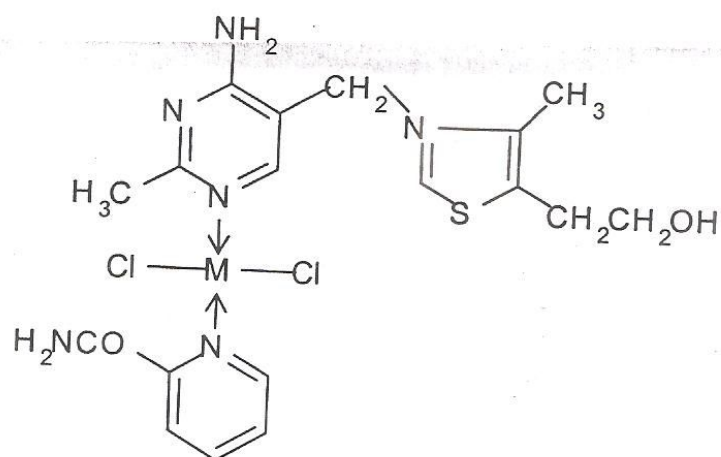
Table 4: ^1H nmr spectra assignment of mixed complex

Hnmr Thiamine	δ ppm Nicotinamide	δ ppm $\text{Co(Th)(Nic)}_2\text{Cl}_2$
2.56 (C-2 ¹ -CH ₃)	8.70 H(2)	2.49
8.37 (C-6 ¹ -H)	8.23 H(6)	8.31
9.26 (C-4 ¹ -NH ₂)	9.05 CONH ₂	8.62
		9.06
3.65 OCH ₂		9.21
		3.60

The peaks at 2.56 ppm and 8.37 ppm in free thiamine shifted to 2.49 ppm and 8.31 ppm in the mixed complex showing that these points have been affected by coordination. In the free Nicotinamide, the peak at 8.70 ppm due to H(2) has been shifted to 8.62 ppm in the mixed complex.

The result of X-ray analysis show that the Co(Th)(Nic)Cl_2 complex is polycrystalline. Hence no single – crystal was obtained. In the mixed Thiamine-Nicotinamide complex, both thiamine and nicotinamide act as monodentate ligands. Coordination occurs through the nitrogen (N-I¹) atom of the pyrimidine ring of thiamine and that of pyridine ring in the nicotinamide.

On the basis of the spectroscopic data obtained, the proposed structure below represent the structure tentatively proposed for the mixed Thiamine-Nicotinamide metal complex (as shown in figure 1).



M = Ni(II), Co(II), Mn(II), Cu(II)

Figure 1: Proposed Structure for Mixed Thiamine-Nicotinamide Metal Complex
Antibacterial activities of the Ligands and their complexes.

Table 5: Antibacterial activities of the ligands and their complexes showing The zone of inhibition against the organisms.

The zone of inhibition against the organisms:						
Ligands/ Complexes	<i>E coli</i>		<i>Staphylococcus</i>		<i>Bacillus subtilis</i>	
	Zone of inhibition (mm)					
Thiamine HCl (Th)	10±1.1	15±1.1	21±1.3	30±1.4	18±1.0	24±1.8
Nicotinamide(Nic)	14±1.2	20±1.1	15±1.6	17±1.3	19±1.2	21±1.1
Mixed complexes						
Cu(Th)(Nic)Cl ₂	25±1.3	28±1.8	17±1.1	20±1.3	25±1.2	32±2.1
Mn(Th)(Nic)Cl ₂	28±1.3	31±1.6	20±1.2	26±1.8	30±2.0	37±2.6

NA = No activity

Values are mean of 3 replicates ± SD.

The results of the antibacterial activities of the ligands and their complexes are presented in Table 5. The antibacterial activities test of the ligands and their complexes were tested against three bacteria species; which include *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*.

The antibacterial activity of the compounds was estimated on the basis of the average size of inhibition zone formed around the well on the seeded agar plate.

The result of antibacterial activities of both thiamine and nicotinamide metal complexes show that the metal complexes have higher antibacterial activities compared to their respective ligands.

In the mixed complexes, Mn(II) complex of mixed thiamine nicotinamide complex Mn(Th)(Nic)Cl₂ showed the highest inhibition against all the three bacteria species. The result of antibacterial test showed that the mixed ligand complexes are more effective antibacterial agent than the single vitamin complexes.

Antifungal Studies of the Ligands and their Complexes**Table 6:** Antifungal activities test of the ligands and their complexes showing the zone of inhibition against the organisms.

Ligands/ Complexes	<i>Aspergillus flavus</i>		<i>Aspergillus niger</i>		<i>Penicillium sp</i>	
	Zone of inhibition (mm)					
	50ppm	100ppm	50ppm	100ppm	50ppm	100ppm
Thiamine HCl (Th)	12±1.3	16±1.1	18±1.9	23±2.0	15±1.0	25±2.0
Nicotinamide (Nic)	15±1.1	19±1.2	10±0.8	17±1.4	12±1.2	20±1.6
Mixed complexes						
Cu(Th)(Nic)Cl ₂	22±1.9	30±2.5	23±1.7	28±1.9	23±1.3	36±1.4
Mn(Th)(Nic)Cl ₂	26±1.9	32±2.4	25±1.8	30±1.9	27±1.3	32±1.7

Values are mean of 3 replicates ± SD.

The results of the antifungal activities test of the ligands and their complexes are presented in Table 6.

The antifungal activities test of the ligands and their complexes are tested against three fungi species: *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* species Cu(II) complex of mixed thiamine-nicotinamide complex Zn(Th)(Nic)Cl showed the lowest antifungal activity against *Aspergillus flavus* while Mn(II) complex of mixed Thiamine-nicotinamide complex, Mn(Th)(Nic)Cl showed the lowest antifungal activity against *Penicillium* species.

Hence, the result of these studies showed that all the mixed thiamine-nicotinamide-metal complexes are more effective antibacterial and antifungal against the tested species (Chohan, 2004).

CONCLUSION

The results of the physical and spectroscopic data confirmed the formation of the complexes. The two ligands were monodentate in their mode of coordination with the metal ions. The complexes were of tetrahedral geometry.

The antimicrobial study of the mixed ligand complexes of thiamine-nicotinamide showed that the mixed complexes have higher activities against the tested bacteria and fungi species.

ACKNOWLEDGEMENTS

The authors wish to thank Swiss Chemical Limited, Lagos, Nigeria for the supply of raw vitamin samples used for this study and Mr. Olabanji of department of Microbiology, University of Ilorin for the antimicrobial study.

REFERENCES

- Abd El-wahab, A. and El-sarrag, M. R. (2004). Derivatives of phosphate Schiff based transition metal complexes: Synthesis, studies and biological activity" *Spect.Acta.* 60 (1-2): 271-277.
- Casas, J.S., Castellano, E.E., Couce, M.O., Sanctez, A., Sordo, J., Vereela, J.M. and Zukerman-schpector, J. (1995). "Vitamin B1 –Chemical interaction with CdCl_2 And in-vivo effect on cadmium toxicity in rats. Crystal structure of $\text{Cd}(\text{thiamine})\text{Cl}_3 \cdot 2\text{H}_2\text{O}$ ". *Inorg.Chem.* 34:2430-2437.
- Chohan, Z.H., Afri, M., Akhtar, M.A and Supiran, C.T. (2006) Metal-based Antibacterial and antifungal agents: Synthesis, characterization and in-vivo Evaluation of Co(II) , Cu(II) , Ni(II) and Zn(II) complexes with aminoacids - Derived compounds". *Bioinorg.Chem.* 83:131-141.
- David, W. (2000). Interpreting Organic Spectra. *Royal Society of Chemistry.* 1-7: 59-169.
- Eugen, J., Marian, K.J., Milan, M. and Jerzy, M. (1996) *J. Coord. Chem.* 40:167.
- Hokelek, T. and Necefoglu (1997) Caesium 5- (2- cyanoethylthio) -2- thioxo-1,3-dithioic-4-thiolate. *Acta. Cryst.* C53, 187.
- Kose, D.A, Lcbudak, H. and Necefoglu, H. (2007). Synthesis and characterization of Nicotinamide-Acetylsalicylato complexes of Co(II) , Ni(II) , Cu(II) and Zn(II) " *haceltepe J.B.ol & Chem.* 35(2): 123-128.
- Kato, M. and Muto, Y. (1988) *Coord. Chem. Rev.* 92:45.
- Lawal, A. (2010). "Synthesis, Characterization and Biological studies of mixed vitamin-metal complexes", Ph.D thesis, University of Ilorin, Ilorin, Nigeria.
- Lever, A.B.P. (1984). *Inorganic Electronic Spectroscopy* Elsevier, Amsterdam.
- Mishra, A.P., Mishra, R.K. and Shrivastava (2009). Structural and antimicrobial studies of coordination compounds of Vo(II) , Co(II) , Ni(II) and Cu(II) with some Schiff bases involving 2-amino-4-chlorophenol" *J. Serb. Chem. Soc.* 74(5): 523-535.
- Nagar, J. (1990). Selective cell death by water- soluble Fe-porphyrins with superoxide dismutase (SOD) activity" *J. Inorg. Biochem.* 40:349-355.
- Obaleye, J.A. and Orjiekwe, C.L. (1993). Synthesis and Characterization of copper (II) and zinc(II) complexes of Ascorbic acid" *Int. J. Chem.* 4(2): 37-51.
- Warra, A.A. (2011). Transition metal complexes and their application in drugs and Cosmetics" – A Review. *J. Chem. Pharm. Res.*, 3(4): 951-958.